Synthesis of Thiosugars and Disaccharides

Thesis submitted to

Indian Institute of Science Education and Research Pune, Pune

in partial fulfilment for the BS-MS dual degree programme

by

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Certificate

This is to certify that this dissertation entitled '**Synthesis of Thiosugars and Disaccharides**' towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research Pune, Pune represents study/work carried out by Lokesh Godavarthi at Indian Institute of Science Education and Research Pune under the supervision of Prof. Srinivas Hotha, Department of Chemistry during the academic year 2023-2024.

Prof. Srinivas Hotha

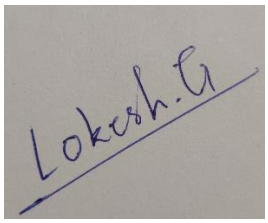
Committee

Prof. Srinivas Hotha

This thesis is dedicated to my beloved brother

Declaration

I hereby declare that the matter embodied in the report entitled '**Synthesis of Thiosugars and Disaccharides**' are the results of the work carried out by me at the Department of Chemistry, Indian Institute of Science Education and Research, Pune, under the supervision of Prof. Srinivas Hotha and the same has not been submitted elsewhere for any other degree



Lokesh Godavarthi

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ABSTRACT

Thioglycosylation, a versatile synthetic method for the formation of glycosidic bonds using thioglycosides as donors, has emerged as a powerful tool in carbohydrate chemistry. This abstract provides an overview of thioglycosylation reactions, their underlying chemistry, diverse applications, and future perspectives. Thioglycosylation reactions involve the activation of a glycosyl acceptor and a thioglycoside donor, followed by the formation of a glycosidic bond through nucleophilic substitution or activation of the glycosyl acceptor. The choice of activation method, protecting groups, and reaction conditions significantly influence the efficiency and regioselectivity of thioglycosylation reactions. Thioglycosylation has found widespread applications in the synthesis of complex carbohydrates, glycoconjugates, and glycomimetics with tailored properties. These include the preparation of oligosaccharides, glycopeptides, glycolipids, and glycoconjugate vaccines for biological studies and therapeutic interventions. Moreover, thioglycosylation plays a pivotal role in the synthesis of glycosylated natural products, pharmaceuticals, and materials with diverse functionalities. Recent advances in thioglycosylation methodologies, such as the development of novel glycosyl donors, catalysts, and reaction conditions, have expanded its synthetic scope and improved its efficiency and selectivity. Furthermore, the integration of thioglycosylation with other synthetic strategies, including enzymatic glycosylation and chemical ligation, offers exciting opportunities for the rapid assembly of complex glycostructures and glycoconjugates. Looking ahead, thioglycosylation holds great promise for addressing key challenges in glycobiology, drug discovery, and materials science. Future research directions may focus on the development of more efficient and sustainable thioglycosylation methodologies, the exploration of new glycosyl donors and acceptors, and the application of thioglycosylation in emerging fields such as glycoengineering and glycotherapy. In conclusion, thioglycosylation represents a valuable synthetic tool for constructing glycosidic linkages and synthesizing diverse glycoconjugates with biological and material applications. Continued research efforts in thioglycosylation chemistry are poised to yield novel insights, methodologies, and applications, driving innovation and discovery in carbohydrate science and beyond.

In this project I have aimed to synthesize Thio glycosides with different furanose sugars and decode the underlying mechanism of the glycosylation reaction by

identifying the side products. For this I have used a pyridine sugar carbonate donor system of different Furanose sugars and a Thio-acceptors. The thioglycosylation reaction was carried out using TMSOTf as a Lewis acid to activate the donor system. Different conditions were tested to optimise the reaction and side product was identified to give insights into the reaction mechanism. In this project, the aim is to devise a reliable and enduring synthetic method for producing a range of Thio-glycosides derived from furanose.

Abbreviations

Å: Angstrom

Ac: Acetate

Bn: Benzyl

cat.: Catalytic

$CDCl_3$: Chloroform-d

$CHCl_3$: Chloroform

TMSOTf: Trimethylsilyl trifluoromethanesulfonate

DCM: Dichloromethane

DEPT: Distortion less Enhancement by Polarization Transfer

DMAP: 4-Dimethyl aminopyridine

DMF: N, N'-Dimethyl formamide

eq.: Equivalents

Et_3N: Triethyl amine

Et_2O: Diethyl Ether

EtOAc: Ethyl Acetate

G: gram

HRMS: High Resolution Mass Spectroscopy

Hz: Hertz

J: Coupling Constant

MeI: Methyl Iodide

mg: milligram

min.: minutes

MHz: Mega hertz

mL: milli litre

MS: Molecular Sieves

NaH: Sodium Hydride

NMR: Nuclear Magnetic Resonance

HF: hydrogen fluoride

HCl: Hydrogen chloride

Contents

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Introduction

Carbohydrates, naturally existing organic substances, are ubiquitously found on Earth and represent some of the earliest organic compounds whose structures were elucidated. Consequently, carbohydrates played a pivotal role in connecting the fields of organic chemistry and biochemistry. They also played a crucial role in shaping and advancing life on our planet by establishing a direct connection between solar energy and chemical energy. Modern studies have explored the role of carbohydrates (glycans) in biological processes. Aberrations in carbohydrates are linked to various diseases, including cancer, infectious diseases, and congenital disorders. Understanding carbohydrates helps develop therapies and diagnostics, advancing our ability to manage and treat these diseases more effectively.

And the simplest form of a carbohydrate or sugar is a saccharide. It is a fundamental class of organic compounds consisting of carbon, hydrogen, and oxygen atoms arranged in a specific ratio. Saccharides can be categorized into three main groups: monosaccharides (single sugar molecules like glucose and fructose), disaccharides (composed of two monosaccharides, e.g., sucrose and lactose), and polysaccharides (long chains of monosaccharides, such as starch and cellulose). Saccharides serve as a primary source of energy for living organisms and play vital roles in various biological processes. Saccharide-based therapeutics involve using carbohydrates, specifically saccharides (sugars), for medical and therapeutic purposes. This field of research and application has gained importance in recent years due to the recognition of the diverse and critical roles that carbohydrates play in various biological processes. Saccharide-based therapies hold great potential for addressing various illnesses and medical conditions by harnessing the intricate and varied functions of carbohydrates in the realm of biology*(Kulkarni, S et al. 2018)*. Nevertheless, their advancement necessitates a profound comprehension of carbohydrate biochemistry and the challenges associated with synthesizing and delivering these molecules effectively in medical applications.

Thioglycosides

Thioglycosides are organic compounds characterized by the presence of a sugar molecule linked to a thiol group (-SH) instead of the typical hydroxyl group (-OH). This

substitution imparts unique chemical properties to thioglycosides, making them valuable tools in carbohydrate chemistry and bioconjugation studies. One of the key advantages of thioglycosides is their stability, both in isolation and during glycosylation reactions. Compared to glycosyl halides or trichloroacetimidates, thioglycosides are less prone to hydrolysis and can be stored for extended periods without degradation. This stability allows for easier handling and storage in laboratory settings. Thioglycosides also exhibit high regioselectivity, meaning that glycosylation reactions typically occur at the thiol-bearing carbon atom rather than other hydroxyl groups present in the sugar molecule. This regioselectivity simplifies the synthesis of complex carbohydrates and glycoconjugates by ensuring the desired glycosidic bond formation at specific positions within the molecule *(Gingras, M et, al 2013).*

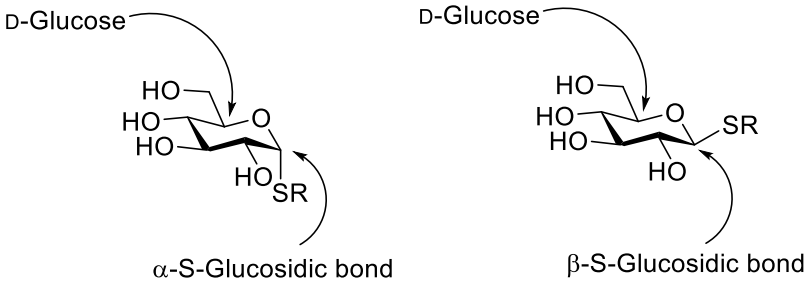


Fig. 1.1 : Configuration of thioglycosides

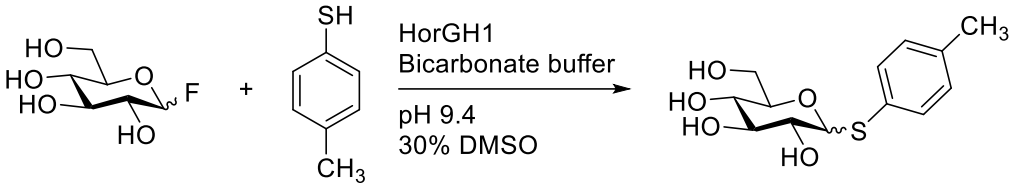
Additionally, thioglycosides are readily activated under mild conditions, making them versatile glycosyl donors for use in glycosylation reactions. Common activation methods include the use of Lewis acids or thiophilic reagents, which facilitate the transfer of the sugar moiety to an acceptor molecule. This ease of activation enables efficient synthesis of glycoconjugates and glycomimetics with precise control over stereochemistry and Regio chemistry. Overall, thioglycosides play a crucial role in carbohydrate chemistry, enabling the synthesis of diverse glycoconjugates and providing insights into glycan-mediated biological processes. Their stability, regioselectivity, and ease of activation make them indispensable tools for researchers in fields ranging from chemical biology to drug discovery.

Different methods to synthesize thioglycosides

Enzymatic thioglycosylation

Enzymatic thioglycosylation is a biochemically mediated process wherein sugar molecules, known as glycans, are attached to acceptor molecules via enzymatic

catalysis. Thioglycosyltransferases are the key enzymes involved in this process, facilitating the transfer of glycan moieties from activated donor substrates (thioglycosides) to specific sites on acceptor molecules



HorGH1: Glycosyl hydrolase from *Halothermothrix orenii*

Fig. 1.2: Enzymatic synthesis of aromatic thioglycosides

Enzymatic thioglycosylation has arisen as a substitute for conventional approaches in glycoside synthesis, aided by advancements in enzyme engineering and artificial synthesis. The advantage lies in its complete kinetic control, contrasting with the thermodynamic control characteristic of other synthetic methods.

Thioglycosyltransferases are a class of enzymes involved in catalysing the transfer of glycan moieties from activated donor substrates, known as thioglycosides to acceptor molecules(*Namdjou D.j et al. 2008*). These enzymes play a crucial role in glycan biosynthesis and modification in living organisms.

Free Radical-mediated thioglcosylation

Free radical-mediated thioglycosylation is a synthetic method in which thioglycosides are formed through the activation of glycosyl donors by free radical initiators. This approach typically involves the generation of radical intermediates from glycosyl donors, which then react with thiols to yield thioglycosides. The use of free radicals as initiators allows for the formation of thioglycosides under mild reaction conditions and without the need for transition metal catalysts.

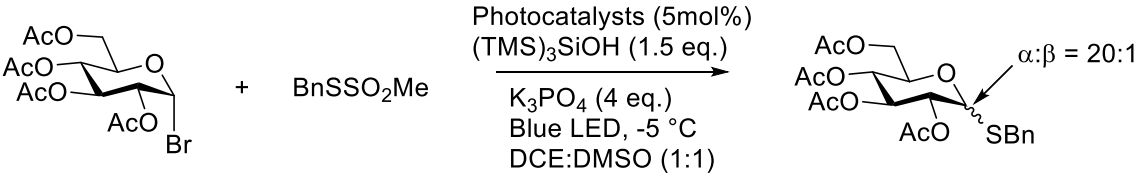


Fig. 1.3: Photoredox catalyzed thioglycosylation *(*Kumar, R. *et al.* 2006*)*

One common method for free radical-mediated thioglycosylation involves the use of radical initiators such as azo compounds, peroxides, or photoinitiators. These initiators generate radicals upon activation, which can abstract hydrogen atoms from glycosyl donors to form radical intermediates. These radicals can then undergo addition reactions with thiols to produce thioglycosides.

Free radical-mediated thioglycosylation offers several advantages, including mild reaction conditions, broad substrate compatibility, and the absence of transition metal catalysts, which may simplify purification procedures and reduce costs. Additionally, this approach enables the synthesis of thioglycosides under environmentally friendly conditions, as many radical initiators are non-toxic and easily removed from reaction mixtures.

However, free radical-mediated thioglycosylation may also present challenges, such as potential side reactions and issues related to selectivity and efficiency. Optimization of reaction conditions, choice of radical initiator, and careful selection of reaction parameters are essential for achieving high yields and regioselectivity in these reactions.

Overall, free radical-mediated thioglycosylation represents a valuable strategy for the synthesis of thioglycosides and has found applications in carbohydrate chemistry, drug discovery, and materials science. Continued research in this area aims to develop new radical initiators, expand the scope of thioglycosylation reactions, and improve the efficiency and selectivity of these processes.

Transition metal-mediated thioglycosylation

Transition metal-catalysed thioglycosylation refers to a synthetic method in which transition metal complexes are employed to facilitate the formation of thioglycosides from glycosyl donors and thiols. These reactions typically involve the activation of glycosyl donors by transition metal catalysts, leading to the formation of reactive intermediates that can react with thiols to generate thioglycosides. Various transition metals, such as gold, silver, palladium, and copper, have been utilized in catalysing thioglycosylation reactions, each offering unique reactivity and selectivity profiles.

Gold-catalyzed thioglycosylation, for example, has gained significant attention due to the mild reaction conditions and high efficiency it offers. Gold catalysts promote the activation of glycosyl donors, facilitating glycosylation with thiols to produce thioglycosides. Silver catalysts have also been employed in thioglycosylation reactions, often in the form of silver triflate (AgOTf), which activates glycosyl donors through coordination and subsequent departure of the leaving group.

Palladium and copper catalysts have been utilized in thioglycosylation reactions as well, although to a lesser extent compared to gold and silver catalysts. These transition metals can activate glycosyl donors and mediate thioglycoside formation through various mechanisms, such as oxidative addition and reductive elimination.

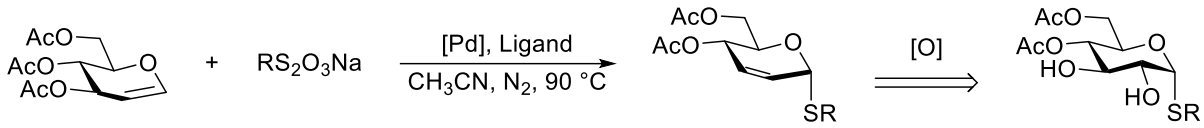


Fig. 1.4: Transition metal catalyzed thioglycosylation (Ding, Y. N et al. 2021)

Transition metal-catalyzed thioglycosylation offers several advantages, including high efficiency, mild reaction conditions, and broad substrate compatibility *(Li, J., Wang et al. 2021)*. These reactions have found applications in the synthesis of complex carbohydrates, glycoconjugates, and natural product derivatives, among others. Additionally, the development of novel transition metal catalysts and reaction methodologies continues to expand the scope and utility of transition metal-catalysed thioglycosylation in organic synthesis.

Nucleophilic substitution of Anomeric leaving group

The simplest approach to synthesizing thioglycoside analogs involves connecting the thiobase acceptor to the sugar, which is activated with a leaving group at the anomeric position. These reactions can lead to the formation of various intermediate products, such as a 1'-2 dioxolenium ion or an oxocarbenium ion, or they may involve direct attack by a nucleophile *(Yip, V. L et al. 2006)*. The stereochemistry of these reactions is primarily influenced by the neighbouring group participation of the C-2' protecting group.

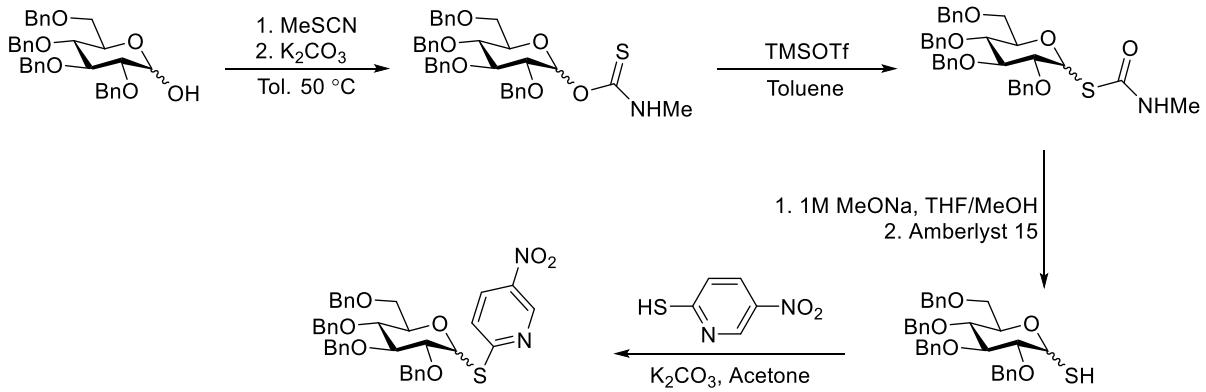


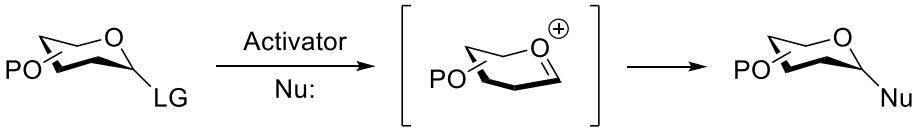
Fig. 1.5: Nucleophilic substitution based thioglycosylation

Glycosylation

Glycosylation is a biological process where sugar molecules, or glycans, are attached to proteins, lipids, or other molecules. This modification is crucial for protein folding, stability, and function. It influences cell-cell recognition, signaling, and immune responses. Dysregulation of glycosylation is implicated in various diseases, including cancer and autoimmune disorders. Glycosylation is studied using techniques like mass spectrometry and glycan microarrays, offering insights into disease mechanisms and potential therapeutic targets.

Chemical Glycosylation

Glycosylation, from a chemistry perspective, involves the chemical attachment of sugar molecules (glycans) to other molecules such as proteins, lipids, or carbohydrates. This process plays a vital role in various biological functions and cellular processes.



LG = Leaving group; Nu = Nucleophile

Fig. 1.5: Chemical glycosylation representation

At its core, glycosylation is a type of chemical reaction where the hydroxyl group of a sugar molecule acts as a nucleophile, attacking an electrophilic centre on the acceptor molecule. The reaction typically proceeds through the formation of a glycosidic bond, linking the sugar moiety to the acceptor molecule. Glycosylation reactions can occur via different mechanisms, depending on the nature of the glycosyl donor and acceptor.

These mechanisms include SN1-like, SN2-like, and associative processes. The stereochemistry of the resulting glycosidic bond is influenced by the configuration of the sugar molecule and the orientation of the reacting groups. Various factors influence the regioselectivity and stereoselectivity of glycosylation reactions, including the protecting groups used to mask specific functional groups on the sugar molecule and the choice of catalysts or enzymes involved.

Thioglycosylation

Thioglycosylation, a key process in carbohydrate chemistry, involves the chemical reactivity of thiol (-SH) groups with carbohydrate molecules. This reaction pathway typically proceeds via the activation of the thiol group, often through the use of activating agents such as iodine or Lewis acids. Activation facilitates the nucleophilic attack of the thiol on the electrophilic center of the carbohydrate, leading to the formation of a thioglycoside linkage. The reactivity of thioglycosylation reactions is influenced by various factors, including the nature of the carbohydrate substrate, the type of thiol reagent, and the reaction conditions. Importantly, thioglycosylation can occur selectively at specific hydroxyl groups within the carbohydrate structure, allowing for precise control over regioselectivity. Moreover, the mild reaction conditions commonly employed in thioglycosylation reactions ensure compatibility with sensitive functional groups, thus enabling the synthesis of complex glycoconjugates and natural products. This versatile reactivity has found applications in diverse fields, ranging from the synthesis of pharmaceuticals to the study of carbohydrate-mediated biological processes *(Mukherjee, S. 2012).* Overall, understanding the chemical reactivity of thioglycosylation provides valuable insights for designing efficient synthetic routes and probing the functional roles of carbohydrates in biological systems.

In summary, thioglycosylation is a versatile chemical reaction used in carbohydrate chemistry for the synthesis of diverse glycoconjugates and glycomimetics. Its broad applications and recent advancements make it a valuable tool in glycobiology, drug discovery, and materials science.

Gold catalysed glycosylation

Inspired by Hashmi's research on the gold-catalysed synthesis of arenes, Hotha and Kashyap pioneered activating anomeric propargyl glycosyl donors using gold catalysis

in 2006. Before their work, the use of gold catalysis in glycosylation had been largely unexplored, despite reports on the use of other transition metals. Their development of $AuCl_3$-mediated activation of glycosyl 1-2 propargyl ortho ester as a donor marked a significant advancement in this area.

Further progress came when it was discovered that 1-ethynyl cyclohexanol glycosyl donors exhibited higher reactivity and were more readily activated compared to propargyl glycosides when using AuCl3 and AgOTf as catalysts. Subsequently, Mishra and colleagues established 1-ethynylcyclohexyl glycosyl carbonate donors catalysed by Au(I)/Ag(I) as a mild and reactive glycosylation platform.

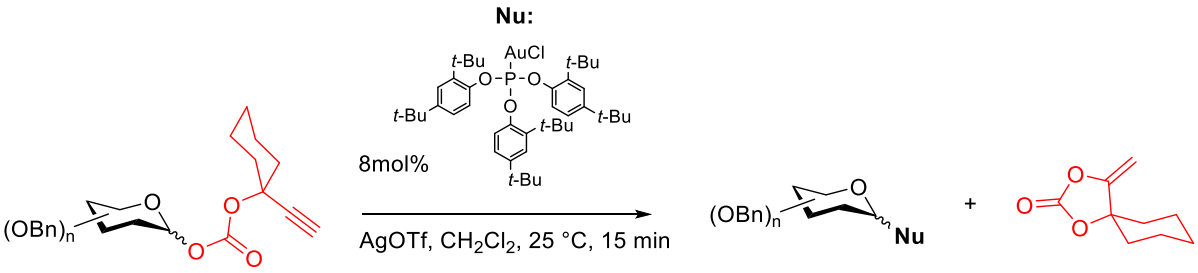


Fig. 1.6: Au/Ag catalysed glycosylation of cyclohexyl carbonate donor

These Au(I)-catalysed glycosylation methods represent notable improvements over previous approaches due to their higher yields and milder reaction conditions, making them significant contributions to the field of glycosylation chemistry.

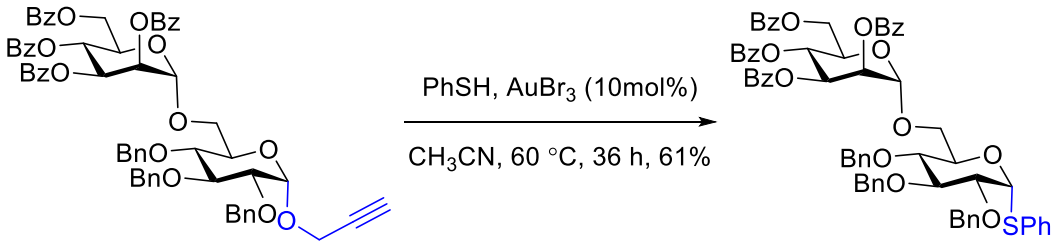


Fig. 1.7: Gold catalysed thioglycosylation *(Hotha, S. et al. 2012)*

TMSOTf mediated glycosylation

TMSOTf (trimethylsilyl trifluoromethanesulfonate) mediated glycosylation is a widely used method for the synthesis of glycosides, including thioglycosides. TMSOTf acts as a Lewis acid catalyst, facilitating the activation of glycosyl donors and promoting glycosylation reactions.

In this process, the glycosyl donor, typically a glycosyl chloride or a trichloroacetimidate, is activated by TMSOTf to form a reactive intermediate. This

activation involves the coordination of TMSOTf to the oxygen atom adjacent to the anomeric carbon of the glycosyl donor, leading to the departure of a leaving group (such as chloride or trichloroacetamide) and the formation of a cationic intermediate.

Subsequently, the activated glycosyl donor undergoes nucleophilic attack by an acceptor molecule, which may be a hydroxyl group, thiol group, or other nucleophile. The nucleophilic substitution reaction results in the formation of a glycosidic bond between the sugar moiety of the glycosyl donor and the nucleophile, yielding the desired glycoside product.

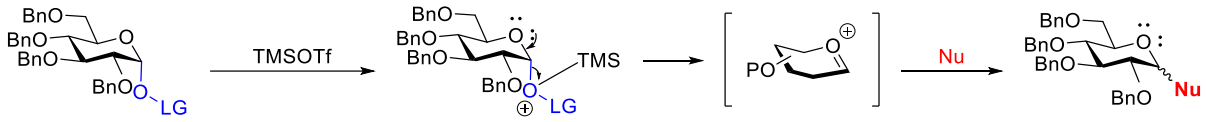


Fig. 1.8: TMSOTf catalysed glycosylation representation

TMSOTf-mediated glycosylation offers several advantages, including high efficiency, regioselectivity, and compatibility with a wide range of glycosyl donors and acceptors. Additionally, TMSOTf is relatively easy to handle and is commercially available, making it a popular choice for glycosylation reactions in both academic and industrial settings.

Overall, TMSOTf-mediated glycosylation is a powerful and versatile tool in carbohydrate chemistry, enabling the efficient synthesis of glycosides for applications in drug discovery, chemical biology, and materials science. Continued research in this area aims to further improve the efficiency, selectivity, and scope of TMSOTf-mediated glycosylation reactions.

Thioglycosides as antibacterial drugs

The glycans that envelop bacterial surfaces serve as promising targets for antibiotics due to their distinct monosaccharides, which are associated with pathogenesis and are absent in human cells. By disrupting glycan biosynthesis, it becomes possible to impede a bacterium's ability to infect its host. Prior research has demonstrated the efficacy of O-glycosides as metabolic inhibitors, effectively disrupting bacterial glycan biosynthesis*(Quintana, I. D, et al 2023)*. Motivated by recent findings indicating that thioglycosides (S-glycosides) are notably more potent than O-glycosides in inhibiting glycan biosynthesis in mammalian cells, we developed a range of S-glycosides based

on rare bacterial monosaccharides. These newly synthesized thioglycosides induced alterations in glycan biosynthesis and impacted the fitness of pathogenic bacteria, while displaying minimal effects on glycosylation or growth in beneficial bacteria or mammalian cells.

In contrast to observations in mammalian cells, our study found that both S-glycosides and O-glycosides exhibited similar potency against bacteria. However, S-glycosides demonstrated heightened selectivity compared to O-glycosides. These novel metabolic inhibitors offer the opportunity for selective manipulation of bacterial glycocalyx for functional investigations and pave the way for broadening our antibiotic arsenal.

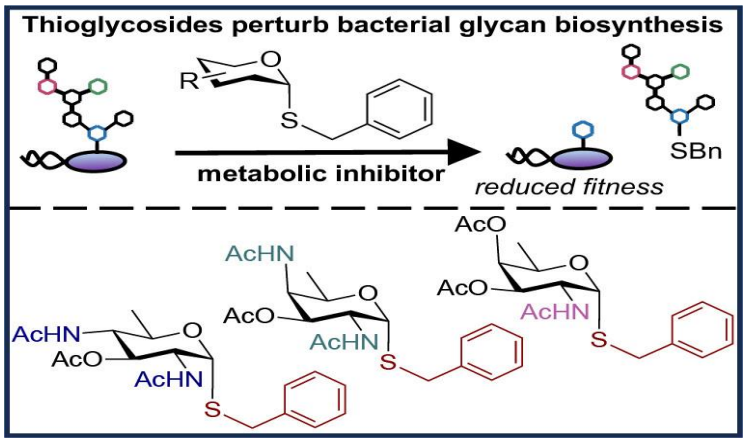


Fig1.9: Mechanism of action of thiglycoside antibacterial drugs

Overall, thioglycoside analogs represent an exciting area of research in medicinal chemistry, with the potential to address unmet medical needs and improve patient outcomes across various diseases and conditions.

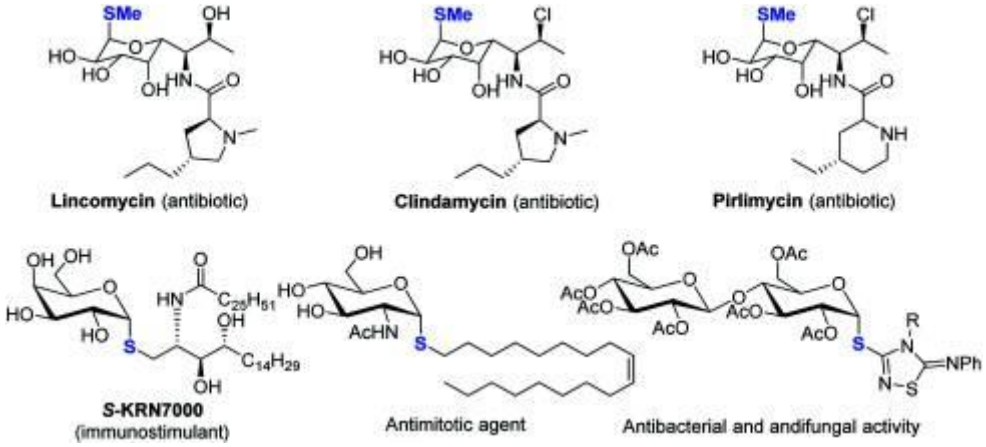


Fig. 1.10: Some selected examples of thioglycoside drugs

Modification in S-glycosides analogues

In medicinal chemistry, thioglycoside analogs represent a promising class of compounds with potential therapeutic applications. These analogs are structurally related to natural thioglycosides but are modified to enhance their pharmacological properties, such as bioavailability, selectivity, and efficacy. Thioglycoside analogs are designed and synthesized to develop novel drugs or drug candidates. These analogs may be modified at various positions on the sugar moiety or the thiol group to optimize their pharmacokinetic and pharmacodynamic properties.(Das, A. et al 2020)

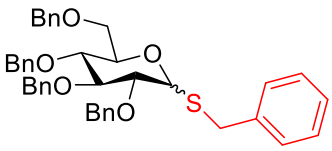
Sugar modification: Different sugars with different substitutions and protection groups can be chosen based on the tailored needs and properties of the molecule

Position of the Sulphur atom: the position of the sulfur atom concerning the sugar backbone can widely dictate the bioactivity of the compound, as compounded with the anomeric effect present in the sugars the stability and half-life of the molecule can be varied based on the position of the sulphur atom

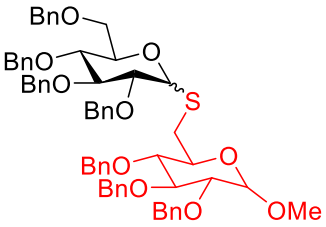
Substrate attached with the sulphur atom: Different substrates can be chosen to be covalently linked with the sulphur atom to generate a wide array of compounds that can be screened for the bioactivity

Present work

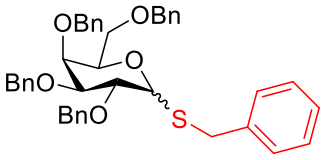
Having already discussed the importance and techniques in the synthesis of thioglycosides, currently through the tenure of my project I have tried to synthesize thioglycosides using different donors and acceptors.The aim was to develop a methodology using a simple donor system and readily available cost-efficient Lewis acid catalyst suiting a wide array of donor systems containing different sugar moieties in mild conditions and to elucidate the mechanism by identifying the key intermediates of the glycosylation reaction and further optimize the reaction conditions



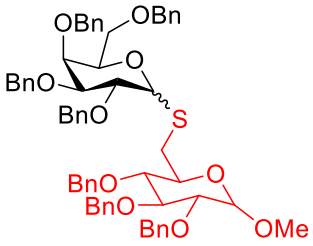
Thioglycoside of glucose 11



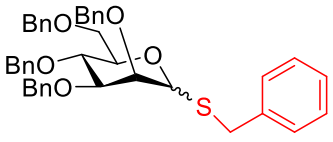
thio disaccaharide of glucose with glucose 14



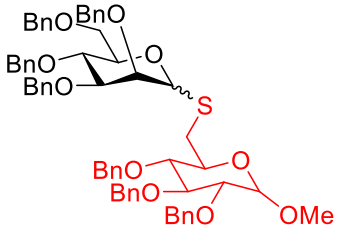
Thioglycoside of galactose 12



thio disaccaharide of glucose with galactose 15



Thioglycoside of mannose 13



thio disaccaharide of glucose with mannose 16

Fig. 1.12: Target Compounds

Retrosynthesis

The desired compound can be retrosynthetically dissected into donor and acceptor components, as illustrated in the following scheme. The pyridine sugar carbonate donor as well as the Thio sugar acceptor can be synthesized from methyl-a-D-glucopyranoside, which in detail will be discussed in the next chapter.

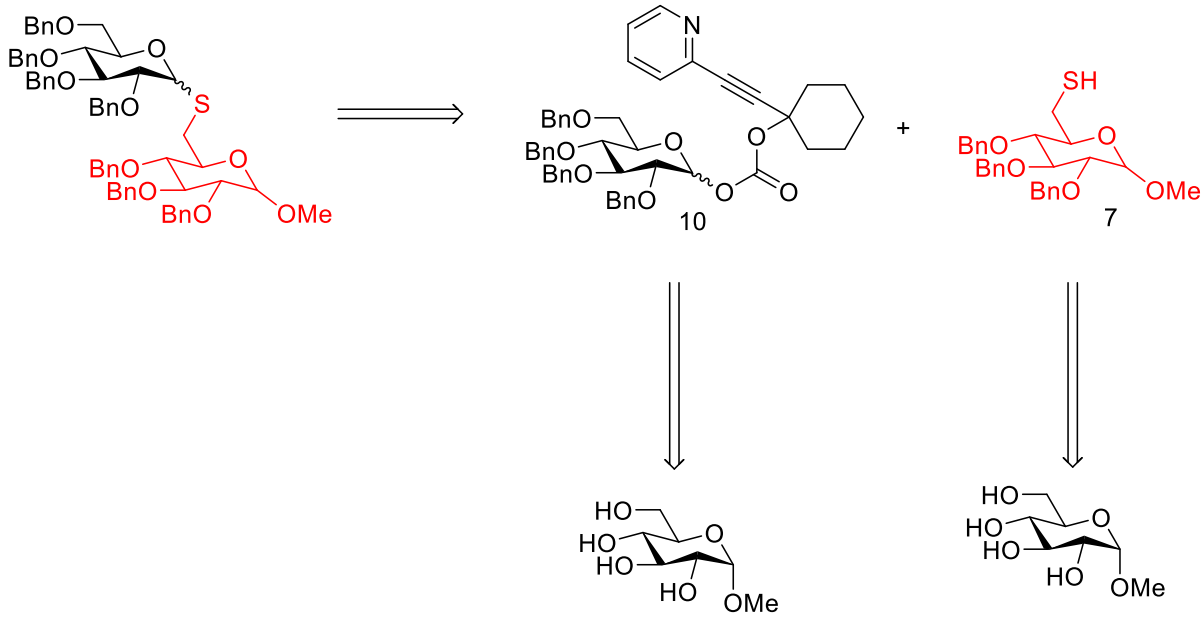


Fig. 1.13 : Retrosynthesis scheme for the synthesis of thioglycosides

Results and discussion

Key reactions and mechanisms

I will commence by providing a concise overview of the reaction mechanism underlying Glycosylation catalyzed by Au/Ag, which constitutes a pivotal step in synthesizing numerous glycosides. Typically, the choice of donor involves utilizing the 1-ethynyl cyclohexyl carbonate donor pioneered by the Hotha group, which undergoes nucleophilic displacement in the presence of Au/Ag catalysts. The proposed mechanism, as elucidated by Mishra et al., is delineated below along with accompanying schematics:

The silver ion Ag+ derived from AgN(Tf)2 acts as a chloride scavenger, resulting in the formation of an active Au-Ag complex (LAuN(Tf)2) upon interaction with the gold catalyst. This complex readily engages with the alkyne group of the donor, forming a gold-alkyne complex.

Activation of the triple bond in donor A facilitates the cyclization of the 1-ethynyl cyclohexyl carbonate donor. This phenomenon is attributed to the lone pair on the endocyclic oxygen moiety, which directs electron density toward the electrophilic center. The presence of -OBn on C-2 facilitates the formation of a trioxolenium ion, thereby stabilizing the intermediate.

The free O-H of nucleobase derivative B acts as an acceptor, initiating an attack on the electrophilic anomeric center, leading to the formation of Vinyl gold cyclic carbonate, which captures a proton, resulting in the generation of spirocyclic compound Y as a by-product, subsequently regenerating the catalyst.

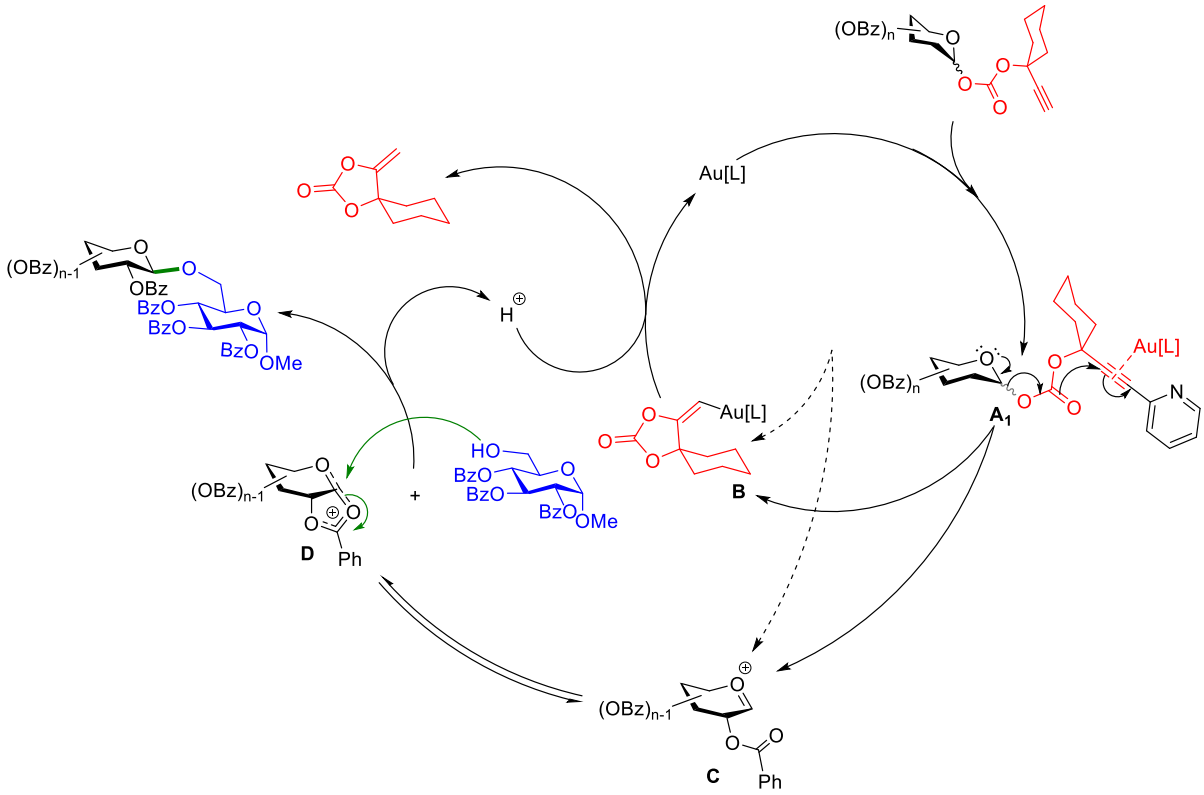


Fig2.1: Mechanism for gold catalysed glycosylation

Plausible reaction mechanism for TMSOTf [1]mediated thioglycosylation

Comparable to the mechanism elucidated for the gold-catalysed glycosylation reaction pioneered by Dr. Hotha's laboratory, a similar mechanism was proposed for the thioglycosylation protocol. Initially, TMSOTf coordinates with the triple bond of the glycosyl donor, facilitating electron push from the anomeric oxygen and subsequent decarboxylation. This process generates the oxocarbenium ion and the envisioned spiro side product within the medium, culminating in the formation of the desired products

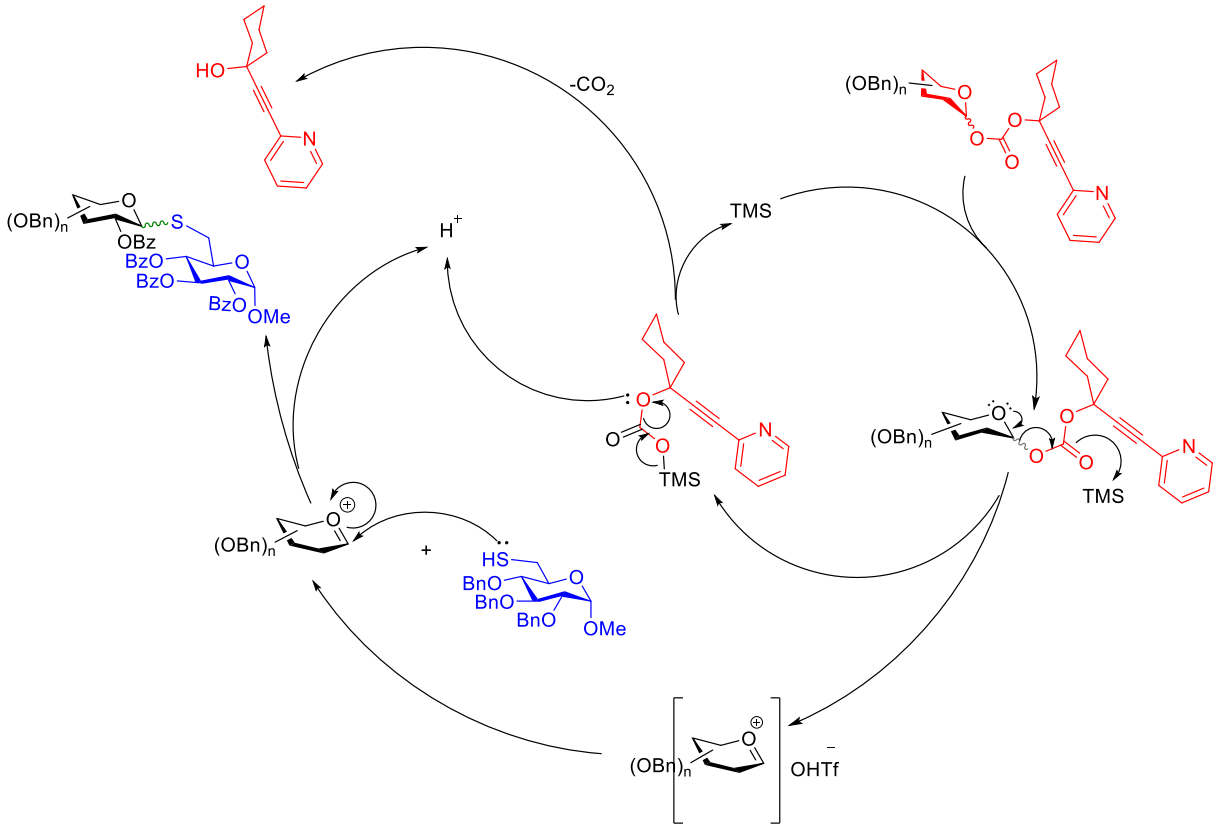


Fig. 2.2: Hypothesized mechanism for TMSOTf-catalysed glycosylation

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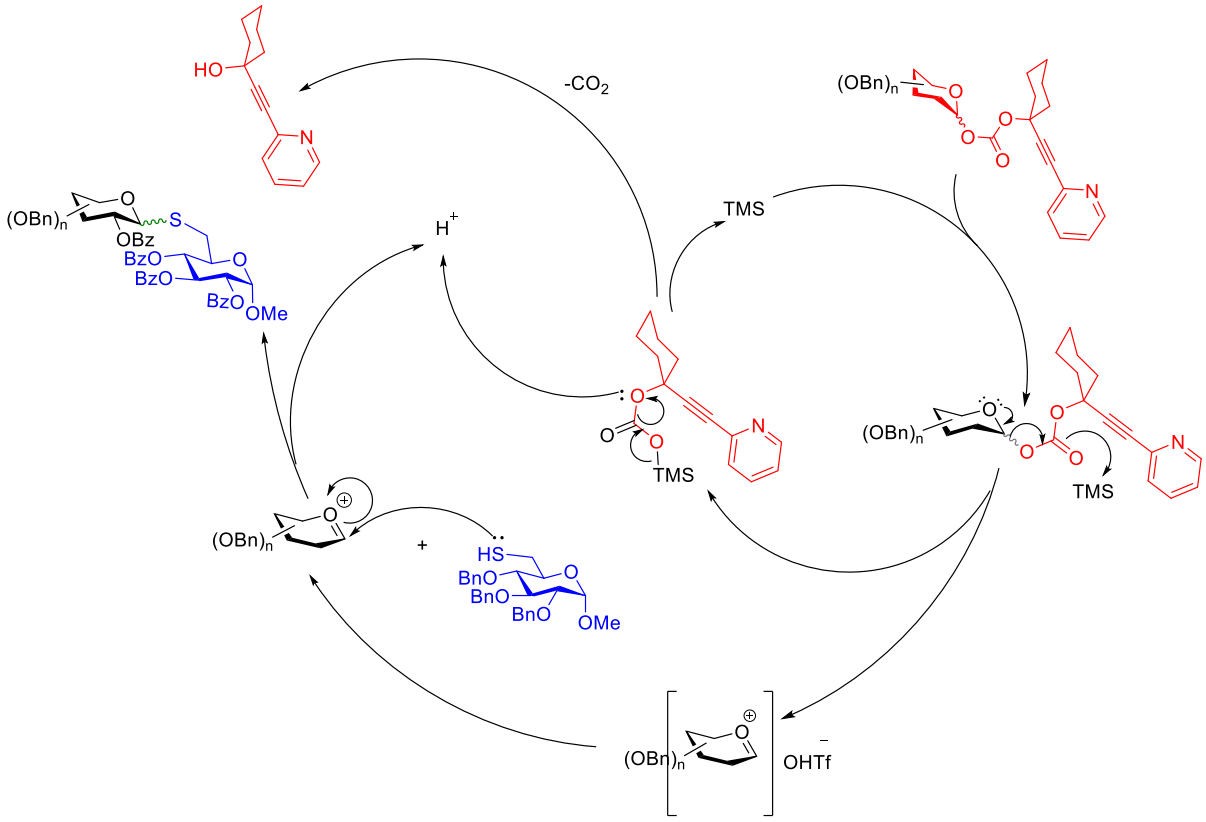
Proposed mechanism

As depicted in the mechanism above, the side product remained unidentified, and upon isolation, the characterized side product exhibited a distinct structure. Consequently, this discrepancy prompted us to formulate an alternative mechanism.



ORTEP DIAGRAM OF COMPOUND 8

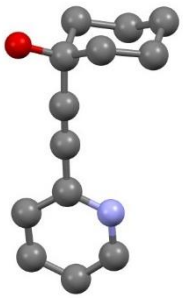


Fig. 2.3: Observed side product from TMSOTf catalysed thioglycosylation

The initial observation gleaned from the structure suggested that the alkyne was not activated. This prompted us to theorize that TMSOTf directly interacted with the carboxy ester, thereby inducing a direct decarboxylation, which is shown in the mechanistic scheme above.

Synthesis of lactol

The synthesis begins with alpha-methoxy-D-glucopyranoside. The protection of the free alcohol was done using benzyl bromide in sodium hydride to afford the per-benzylated D-glucopyranoside. The anomeric position is then selectively deprotected in acidic conditions using 2M sulphuric acid in glacial acetic acid.

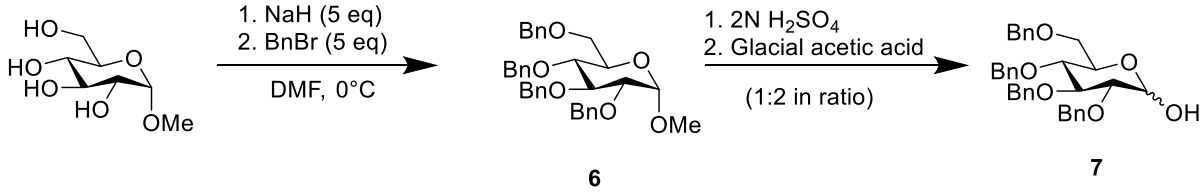


Fig. 2.4: Scheme for the synthesis of lactol

Synthesis of the Glycosyl Donor

Using sonogashira coupling cycohexyl propargyl alcohol has been coupled to 2 bromopyridine to yield the coupled product. Then the free hydroxy group of the coupled product has been capped with carbonochloridate to form the carbonate. The carbonate donor is prepared by coupling the carbonate with the lactol.

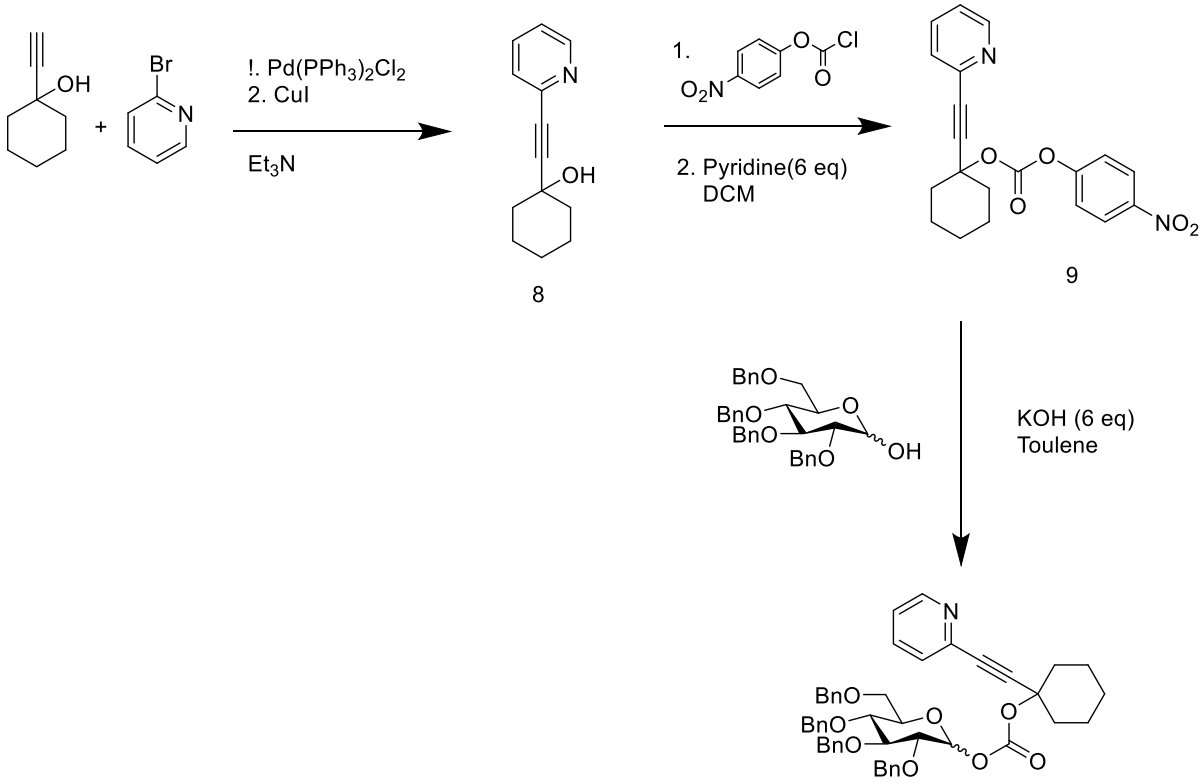


Fig. 2.5: Scheme for the synthesis of the donor system

Synthesis of the Thioglycosyl acceptor:

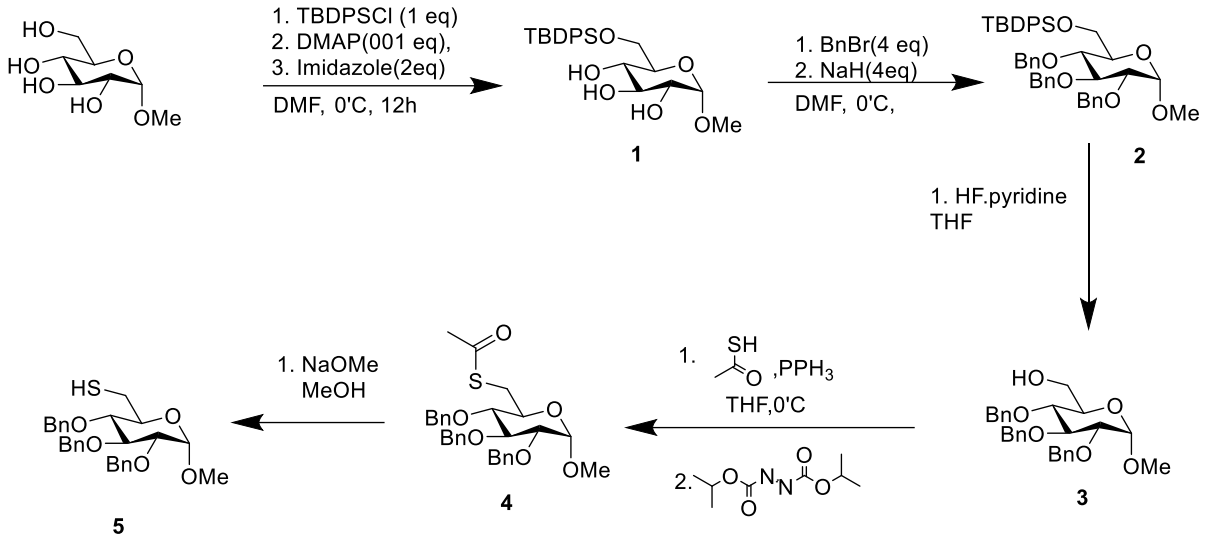


Fig. 2.6 : Synthesis of thioglycosyl acceptor

The synthesis of the thio acceptor begins with the TBDPS protection of the c6 protection of methoxy-alpha-D glucopyranose, followed by the benzylation of the free oh groups. The TBDPS is then orthogonally deprotected using HF-pridine to afford the primary alcohol. Mitsunobu reaction is then performed on the primary alcohol to afford the thio-acetate. Further, the acetyl cap on the sugar is removed using sodium methoxide to afford the thioacceptor.

Methods

The NMR spectra were recorded using Bruker 400 MHz, JEOL ECX 400, and Bruker 600 MHz instruments. Tetramethyl silane (TMS) was employed as an internal reference for calibration, with chemical shifts measured in ppm relative to TMS. All chemicals used in synthesis were procured from Sigma-Aldrich, TCI, or Spectro Chem.

High-resolution mass Spectroscopy was performed using a Waters Synapt G2 or a MALDI-TOF spectrometer.

X-ray crystallography was performed using a crystal X-ray diffractometer System with Mo and Cu by KAPPA APEX II DUO

Reactions were monitored via Thin Layer Chromatography on E-Merck silica plates (0.25mm, F-254) pre-coated with UV-sensitive compounds. Visualization was

achieved under UV light, and chemical staining was conducted using an anisaldehyde stain.

Silica Gel column chromatography was conducted using 100-200 mesh silica gel.

All reactions were conducted under inert argon or nitrogen atmospheres, employing anhydrous solvents sourced from Merck and Finar.

Evaporations were carried out using Heidolph rotary evaporators at temperatures below 50°C.

Transitional metal salts were sourced from multinational commercial suppliers.

General procedures

Thioglycosylation reaction

To an oven-dried round bottom flask evacuated and refilled with nitrogen was added pyridine sugar carbonate (1 equiv) followed by DCM (anhydrous) and thiosugar acceptor (0.9 equiv). The reaction was cooled to 0 degrees Celsius. To this cold reaction mixture, TMSOTf (1 equiv) was added and allowed to stir at the same temperature for 1 h. Later the reaction was neutralized using triethylamine (1 equiv) and then the residue was directly subjected to silica gel column chromatography to afford the products in moderate to excellent yields in colorless liquids

Preparation of pyridine sugar carbonate

In an oven-dried round bottom flask evacuated and refilled with nitrogen, 4-nitrophenyl carbonate (2 equiv) was taken and Toulene was added to it. Later, KOH (4 equiv) was added to it followed by the benzyl protected D-pyranose(1 equiv). The solution was allowed to stir at 65 degrees Celsius for 4 h. Thereafter, the reaction was cooled at rt and washed with saturated sodium bicarbonate

The solution to remove the unreacted 4-nitrophenol. Later the organic layer was evaporated in a vacuum and the residue was purified using column chromatography to afford the product in a colorless liquid.

Preparation of 4-nitrophenol-derived sugar carbonate

To a stirred solution of pyridine substituted cyclopropyl alcohol at 0 degrees Celsius in DCM, pyridine was added and stirred for 15 minutes. Later 4-nitrophenylcarbonate chloride was dissolved in DCM and added to it at 0 degrees Celsius and the stirring was continued further at the same temperature for 30 minutes and later at room temperature for 3 h. Subsequently, the reaction mixture was quenched using 1N HCL

and extracted in DCM. The combined organic layer was washed with saturated sodium bicarbonate solution and concentrated in a vacuum. The crude was further purified using column chromatography to afford the product as a colorless liquid

Coupling of cyclohexyl propargyl alcohol and 2 bromo-pyridine

To a dry round bottom flask, copper iodide, Bis (triphenylphosphine)palladium (II) chloride, triethylamine,2-bromopyridine, and cyclohexyl propargyl alcohol was successively added, and the reaction mixture at room temperate till the reaction could complete

Benzylation of methyl-D-glycopyranoside

To an oven-dried round bottom flask, alpha-D-glycopyranose(1 equiv) was added followed by the addition of DMF followed by cooling it to 0 degrees Celsius using an ice bath followed by the addition of Sodium hydride (5 equiv) leading to a cake formation followed by addition of benzyl bromide (5 equiv) and TBAI(0.05 equiv). After the completion of the reaction, the reaction was quenched with brine solution. Followed by a workup using ethyl acetate and brine solution. The organic layer was concentrated in a vacuum followed by silica gel column chromatography

TBDPS protection of primary alcohol of alpha-d-methoxyglucopyranose

In an open round bottom flask, alpha-d-methoxyglucopyranose (1 equiv) was taken followed by dissolving it in DMF. To this reaction mixture imidazole (2 equiv) was added followed by 4-dimethyl aminopyridine (0.1 equiv) and finally TBDPS chloride (1.1 equiv) was added. After the reaction was completed. The compound was extracted using ethyl acetate and brine workup followed by a concentration of organic layer in a vacuum

TBDPS deprotection

The compound was taken in a round-bottomed flask, followed by the addition of THF, and to this solvent mixture HF.pyridine was added (1ml per gram of starting). Once the reaction was complete, the compound was extracted by ethyl acetate and sodium bicarbonate workup followed by vacuum evaporation followed by silica gel chromatography using hexane and ethyl acetate

Thioacetate nucleophilic substitution

In a round-bottomed flask, Diisopropyl azodicarboxylate was added to a solution of triphenyl phosphine dissolved in THF at zero degrees Celsius, followed by the formation of a precipitate. To this mixture, the deprotected alcohol was added to facilitate the nucleophilic substitution. Workup was done using ethyl acetate and brine

solution. The compound was separated by column chromatography in hexane and ethyl acetate system

Deprotection of thioacetate

The Acetyl-protected thiol compound was dissolved in methanol in a round-bottomed flask. To this system, Sodium methoxide was added dropwise to facilitate the reaction. Prior to the workup, the solvent was evaporated in a rota bath in a vacuum. Then followed by a workup using 1 N HCl and Ethylacetate. The organic layer was collected and concentrated. Following making a slurry in the silica gel and separating the compound in column chromatography

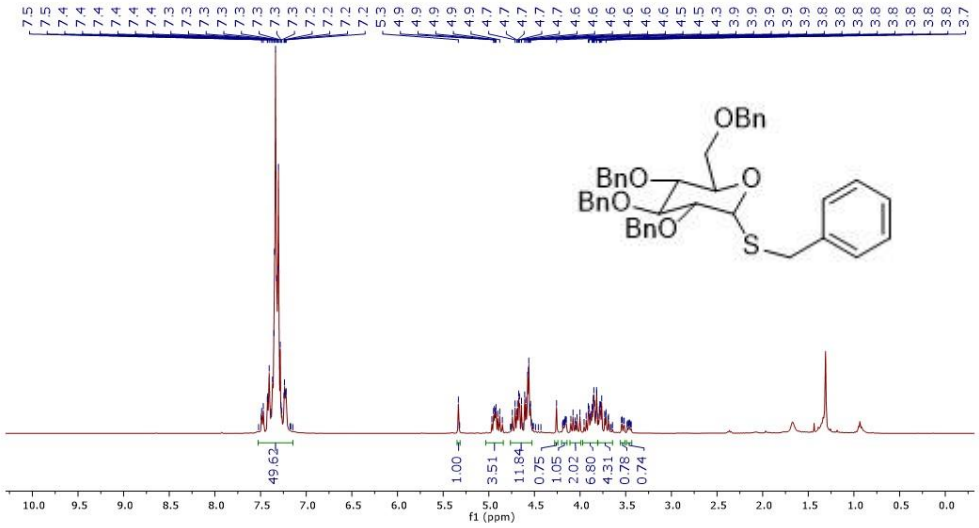
Conclusions

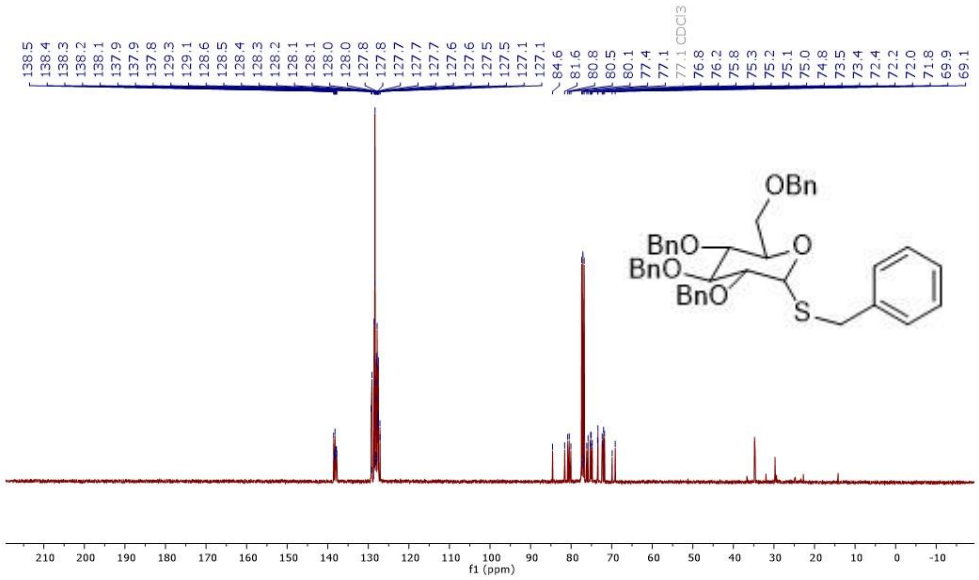
The synthetic pathway to efficiently produce thioglycosides from cheap starting material in a sustainable way with reproducible and scalable steps has been a challenge for several years. The reaction involves pyridine sugar cyclohexyl carbonate which undergoes glycosylation efficiently under the presence of TMSOTf. The main challenges involved in the process were to identify the side product and to determine the plausible mechanism. Further optimization will be required to improve the yields and to perform the glycosylation reaction in ambient conditions.

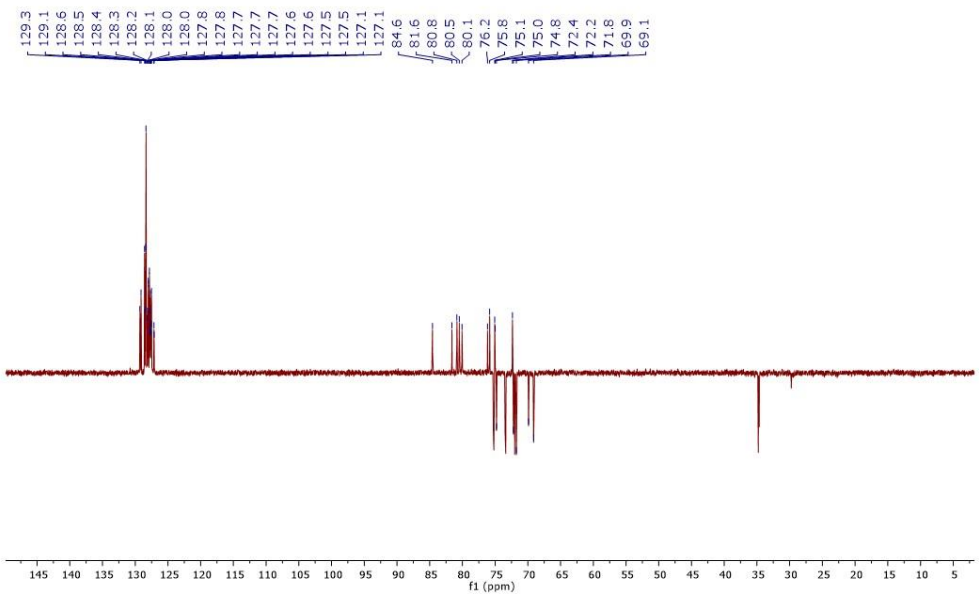
In conclusion, a successful efficient decarboxylative thioglycosylation under transition metal-free conditions has been developed with insights into the reaction mechanism and the parent glycosyl donor can be easily prepared from the commercially available starting materials. The reaction has a wide range of substrate scope and can be employed for the synthesis of disaccharides. Further elaboration on the scope of the reaction and mechanistic studies are in progress.

NMR spectra

NMR ^{1}H, ^{13}C and DEPT of the thioglycosides **xx**







NMR ^{1}H, ^{13}C and DEPT of the thioglycosides **xx**

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