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Factors and Strategies to AchieveStereoselectivity of Glycosylation Reaction: A Review

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I. Introduction

Glycosylation, the formation of glycosidic bonds between carbohydrates, is a crucial reaction in carbohydrate biochemistry and is important for the synthesis of complex carbohydrates and glycoconjugates such as glycoproteins, glycolipids, and proteoglycans[1-3]. It also plays a critical role in determining the structure and function of many proteins[4,5]. For example, the antibody molecules that are involved in the immune response are heavily glycosylated. The glycans on these antibodies are essential for their function because they allow the antibodies to bind to pathogens and initiate the immune response[6]. The glycans also help to stabilize the antibody molecules and protect them from degradation[7,8]. The stereoselectivity of glycosylation reactions is also evident in the structure of carbohydrates and glycoconjugates found in nature. For example, the α-anomer is often found in glycoproteins, whereas the β-anomer is often found in bacterial polysaccharides[9].

The stereoselectivity of glycosylation reactions has been studied extensively, and various factors that affect the stereochemical outcome of these reactions have been identified[10]. A comprehensive understanding of these factors can facilitate the design and synthesis of glycosylated molecules with specific stereochemical properties that are relevant for their biological activity. In this review, we will discuss some of the key factors that influence the stereoselectivity of glycosylation reactions.

The importance of Glycosylation

Glycosylation is important for the function of many enzymes. For example, the enzyme lactase, which is involved in the digestion of lactose, is heavily glycosylated. The glycans on lactase are essential for its stability and activity. Without glycosylation, lactase would be unable to function properly, leading to lactose intolerance[11].

Glycosylation also plays an important role in cell recognition[12]. The glycans on the surface of cells act as signals that allow cells to recognize and interact with each other[13]. This is essential for many biological processes, including development, tissue regeneration, and immune responses[14]. For example, the ABO blood type system is based on the presence of specific glycans on the surface of red blood cells. These glycans are recognized by antibodies in the blood, which can cause an immune response if they are not compatible with the recipient's blood type[15]. This is why it is important to match blood types before transfusions.

Glycosylation can also play a role in the development and progression of diseases[16]. For example, alterations in glycosylation patterns have been observed in many types of cancer[17]. These alterations can affect the function of proteins involved in cell signaling, proliferation, and metastasis, leading to the development of cancer[13]. One example is the protein Epidermal Growth Factor Receptor (EGFR) as the role of glycosylation in cancer[18,19]. EGFR is a transmembrane protein that is involved in cell signaling and is often overexpressed in cancer cells[20]. The glycans on EGFR have been shown to affect its activity and localization, and alterations in glycosylation patterns have been linked to the development of drug resistance in cancer cells[21].

Glycosylation also plays a role in the development of autoimmune diseases[22]. In autoimmune diseases, the immune system mistakenly attacks the body's own cells and tissues[23]. The glycans on the surface of cells can act as targets for autoantibodies, leading to tissue damage and inflammation[24-26]. For example, in

rheumatoid arthritis, antibodies against glycans on the surface of cartilage cells can lead to the destruction of the joints[27].

Therefore, the glycosylation has been used in the development of therapeutics[28，29]. Many biologic drugs, such as monoclonal antibodies, are heavily glycosylated. The glycans on these drugs can affect their pharmacokinetics, immunogenicity, and efficacy[30]. For example, the drug erythropoietin (EPO) is a hormone that stimulates the production of red blood cells and is used to treat anemia. The glycans on EPO are critical for its activity and stability. Recombinant EPO that is produced in mammalian cells is heavily glycosylated, and the glycans on the molecule affect its pharmacokinetics and biological activity[31].

Another example of the use of glycosylation in therapeutics is the development of glycoengineered antibodies. Glycoengineering is the manipulation of the glycans on antibody molecules to enhance their pharmacokinetics and efficacy. For example, the removal of certain glycans from antibodies can increase their half-life in the bloodstream and reduce their immunogenicity. The addition of specific glycans can also enhance the antibody's ability to activate the immune system and kill cancer cells[32].

Factors and Strategies for Setereoselectivity

The stereochemistry of the glycosidic bond, i.e., the spatial orientation of the anomeric carbon atom with respect to the neighboring substituent, plays a critical role in the biological activity of these molecules[33]. The stereochemical outcome of glycosylation reactions is also dependent on the mechanism of the reaction. The two primary mechanisms of glycosylation reactions are the S_N2 and S_Ni mechanisms. The S_N2 mechanism involves the attack of the acceptor molecule on the anomeric carbon of the donor molecule, resulting in inversion of configuration. In contrast, the S_Ni mechanism involves the formation of an oxocarbenium intermediate, which can undergo either retention or inversion of configuration. The choice of mechanism can influence the stereochemical outcome of the reaction, with the S_Ni mechanism often leading to a higher degree of stereoselectivity.[34]

1. Anomeric Effect: The anomeric effect is a well-known stereoelectronic effect that arises due to the interaction of the lone pair of electrons on the oxygen atom of the glycosyl donor with the antibonding orbital of the C1-O1 bond[35]. This interaction stabilizes the axial conformation of the glycosyl donor and leads to the formation of the α-anomer. In contrast, the equatorial conformation of the glycosyl donor leads to the formation of the β-anomer[36]. Therefore, the anomeric effect plays a critical role in determining the stereoselectivity of glycosylation reactions.(Fig. 1)

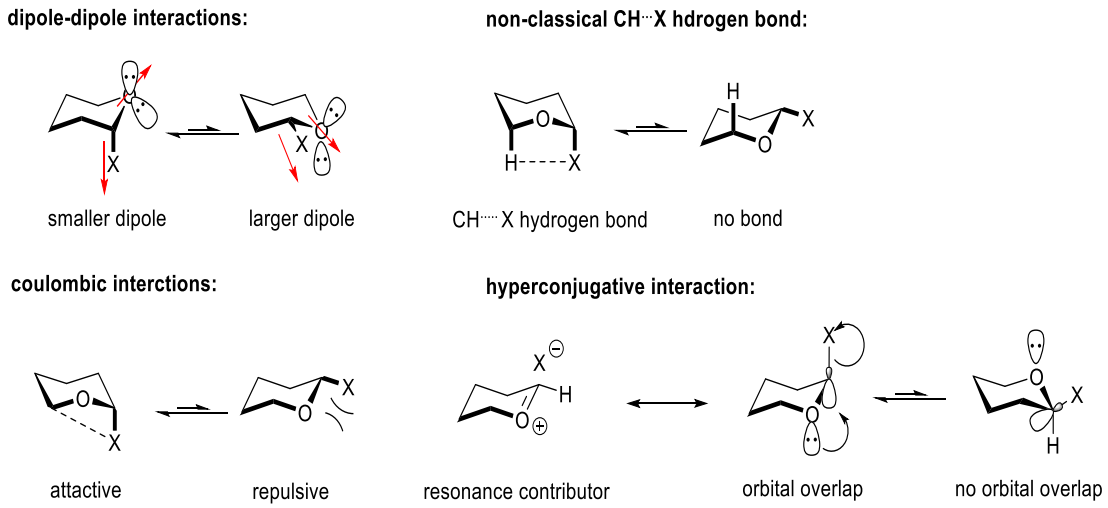

Fig. 1 Hypotheses advanced to account for the anomeric effect.

2. Steric Hindrance: Steric hindrance can also affect the stereochemical outcome of glycosylation reactions. For example, bulky substituents on the acceptor or donor molecule can hinder the approach of the other molecule, leading to reduced reactivity and stereoselectivity[37].(Fig. 2)

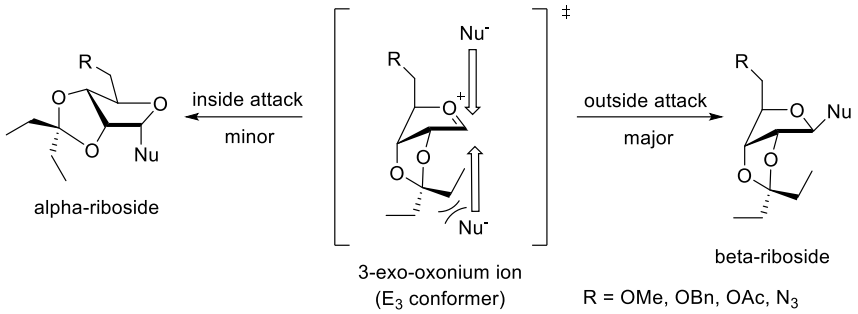

Fig. 2 Schematic representation of the β-stereoselectivity of the nucleophilic attack to oxocarbenium ions of 3-pentylidene-protected ribofuranoside.

3. Solvent Effects: The solvent can also influence the stereochemical outcome of glycosylation reactions. For example, polar solvents such as water can favor the formation of the α-anomer, whereas nonpolar solvents can favor the formation of the β-anomer[38].(Fig. 3)

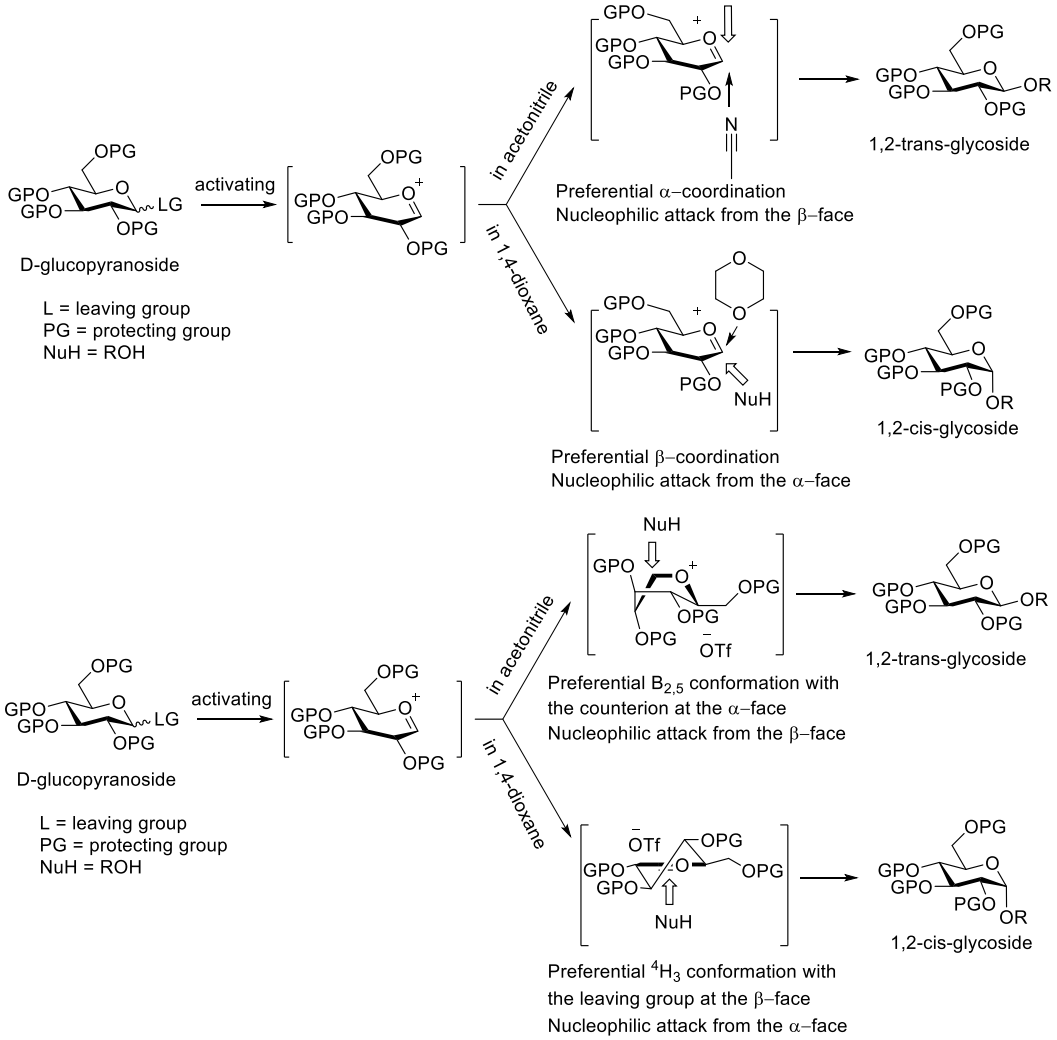

Fig. 3 Two alternative hypotheses concerning solvent effects in glycosylation reactions

4. Lewis Acidity: Lewis acids such as boron trifluoride (BF_3) and Trimethylsilyl trifluoromethanesulfonate(TMSOTf) can act as catalysts in glycosylation reactions and influence the stereoselectivity of the reaction. For example, BF_3 can promote the formation of theβ-anomer, whereas TMSOTf can promote the formation of the α/β-anomer[39].(Fig. 4)

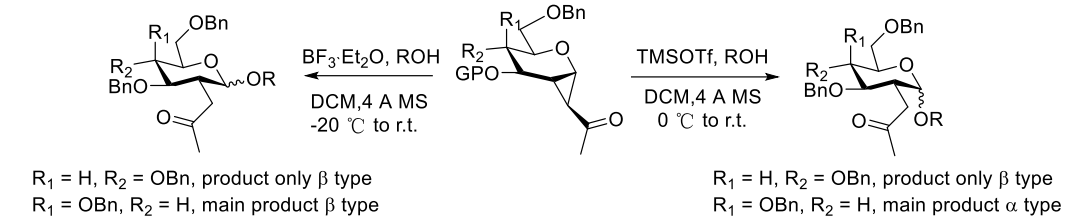

Fig. 4 1,2-Cyclopropaneacetylated sugars as glycosyl donors reacted with a series of glycosyl acceptors in the presence of Lewis acid to produce oligosaccharides and glycoconjugates containing 2-C-acetylmethylsugars.

5. Temperature: Temperature can also affect the stereochemical outcome of glycosylation reactions. For example, lower temperatures can favor the formation of the α-anomer, whereas higher temperatures can favor the formation of the β-anomer[40].

6. Protecting Groups: Protecting groups are often used in glycosylation reactions to protect certain functional groups and prevent unwanted reactions. The choice of protecting group can also affect the stereochemical outcome of the reaction. For example, the use of a benzylidene protecting group can favor the formation of theβ-anomer[41,42], and the use of a acetyl protecting group can favor the formation of the β-anomerwhenitisonthe C2.(Fig. 5)

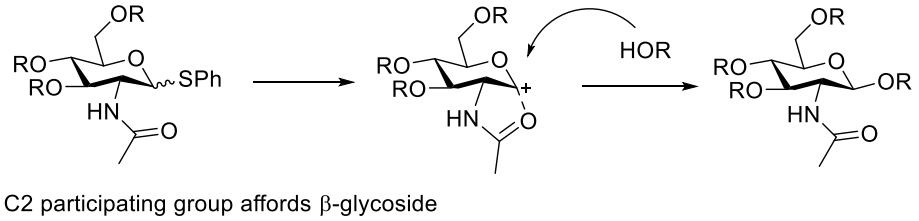

Fig.5 Protective group effects the glycosylation reaction.

7. Enzymatic Catalysis: Enzymatic catalysis has emerged as a powerful tool for the synthesis of complex carbohydrates and glycoconjugates with high stereoselectivity. Enzymes such as glycosyltransferases and transglycosidases can catalyze glycosylation reactions with high efficiency and selectivity, and can be used to synthesize glycosylated molecules with specific stereochemical properties[43].(Fig. 6)

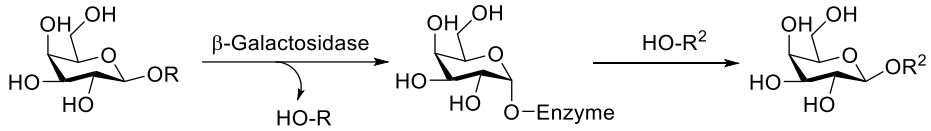

Fig. 6 The reaction catalyzed by β-galactosidase

8. Stereoselective Synthesis: The development of stereoselective synthetic methodologies has also contributed significantly to the synthesis of glycosylated molecules with specific stereochemical properties. For example, the use of chiral auxiliaries and chiral ligands in glycosylation reactions can promote the formation of specific stereoisomers[44].(Fig. 7)

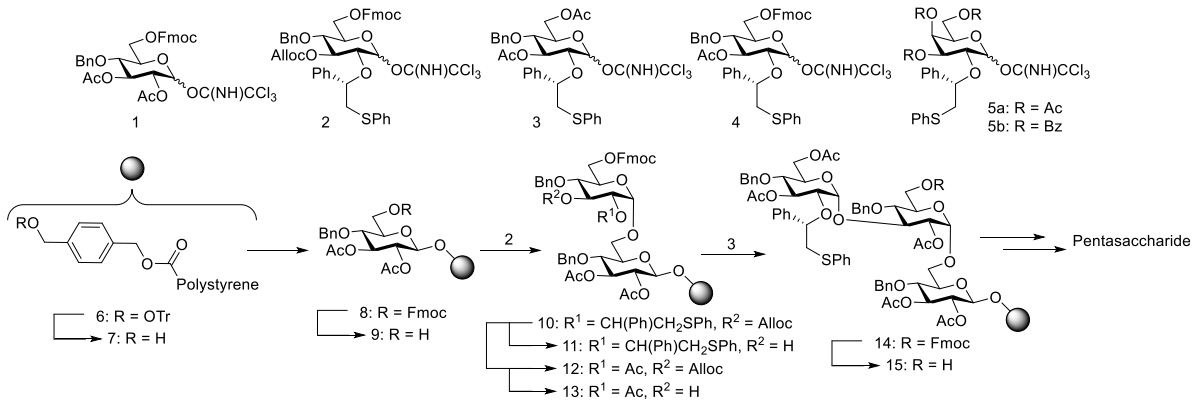

Fig. 7 Stereoselective solid-supported synthesis of pentasaccharide

9. Glycosylation Regioselectivity: The regioselectivity of glycosylation reactions, i.e., the specific site of glycosylation, can also influence the stereochemistry of the glycosidic bond. For example, glycosylation at a secondary hydroxyl group can result in the formation of the α-anomer, whereas glycosylation at a primary hydroxyl group can result in the formation of the β-anomer[45].(Fig. 8)

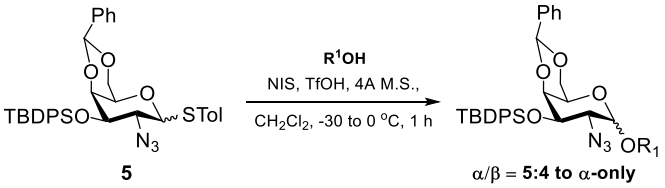

Fig. 8 The regioselectivity of glycosylation reactions

10. Glycosylation Linkage: The linkage between carbohydrates can also affect the stereochemical outcome of glycosylation reactions. For example, the formation of α,α-linkages can be more challenging than the formation of α,β-linkages due to steric hindrance[46].(Fig. 9)

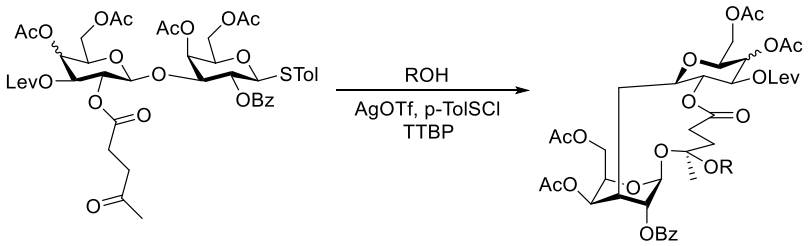

Fig. 9 The long-range participation of a levulinoyl group across a glycosidic linkage was observed in glycosylation reactions.

II. Conclusions

Therefore, several glycosylation strategies have been developed to promote the formation of specific stereoisomers. For example, the use of neighboring group participation (NGP) can promote the formation of specific anomers by stabilizing the transition state of the reaction. In addition, the use of chiral auxiliaries and chiral ligands can facilitate the formation of specific stereoisomers.In conclusion, the stereochemistry of the glycosidic bond plays a critical role in the biological activity of carbohydrates and glycoconjugates. The stereoselectivity of glycosylation reactions is influenced by a variety of factors, including the anomeric effect, steric hindrance, solvent effects, Lewis acidity, temperature, protecting groups, reaction conditions, substrate configuration, enzymatic catalysis, and stereoselective synthesis. A deeper understanding of these factors can enable the design and synthesis of glycosylated molecules with specific stereochemical properties that are relevant for their biological activity.

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