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Synthesis of modular building blocks using glycosyl phosphate donors for the construction of asymmetric N-glycans

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ABSTRACT

Glycosyl phosphates are known as versatile donors for the synthesis of complex oligosaccharides both chemically and enzymatically. Herein, we report the stereoselective construction of modular building blocks for the synthesis of N-glycan using glycosyl phosphates as donors. We have synthesized four trisaccharide building blocks with orthogonal protecting groups, namely, Manβ2GlcNAc(OAc)$_3$β6GlcNAc (**9**), Manβ2GlcNAc-β6GlcNAc(OAc)$_3$ (**15**), Manβ2GlcNAc(OAc)$_3$β4GlcNAc (**18**) and Manβ2GlcNAcβ4GlcNAc(OAc) (**22**) for further selective elongation using glycosyltransferases. The glycosylation reaction using glycosyl phosphate was found to be high yielding with shorter reaction time. Initially, The phthalimide protected glucosamine donor was exploited to ensure the formation of β-glycosidic linkage and later converted to the N-acetyl group before the enzymatic synthesis. The selective deprotection of O-benzyl group was performed prior to enzymatic synthesis to avoid its negative interference.

1. Introduction

Glycans are composed of a number of monosaccharides linked together *via* glycosidic bond(s) and constitute the carbohydrate portion of glycoproteins, glycolipids or other glycoconjugates. In glycoproteins, glycans are broadly divided into O-glycans and N-glycans. They affect several biological processes in the cell such as protein folding, cell signaling, embryogenesis etc. [1] N-Glycans are assembled during protein biosynthesis through attachment to the amide nitrogen of asparagine in a defined peptide chain. The synthesis and processing of N-glycans generally takes place in the endoplasmic reticulum and Golgi complex involving different stages of glycosylation and deglycosylation [2]. The glycan precursor $Glc_3Man_9GlcNAc_2$ is first transferred from a lipid pyrophosphate derivative to a growing polypeptide in the endoplasmic reticulum (ER) and further trimmed by ER glucosidases before travelling to the Golgi complex for further processing down to $Man_5GlcNAc_2$, which is further glycosylated under the catalysis of N-acetylglucosamine transferase (GnT) to convert it to a complex or hybrid-type glycan [3]. Next, a series of enzymes of the GnT family modify the glycoform with the addition of GlcNAc moieties to the D1 arm of the glycan [4]. Then, the Golgi-generated glycoforms are converted to highly diverse glycan species through a series of sequential galactosylation, sialylation and fucosylation. The naturally occurring glycans can only be isolated in minor quantities and as complex mixtures, difficult to separate, and cannot provide a reliable source for detailed biological studies. More than twenty thousand structures of N-glycans exist depending on the linkages of monosaccharides, and many of which are difficult to sequence due to their isomeric nature. In order, to study the effect of individual N-glycans on the function of a particular glycoprotein would ideally require a structurally diverse collection of synthetic N-glycans with systematic variations in compositions and branching, terminal sugars, and core sugar modifications. The chemical synthesis to access diverse and complex N-glycan structures is challenging due to step-wise synthesis and multiple purification processes. However, over the last two decades, chemical and chemo-enzymatic synthesis methods have been reported for the synthesis of complex and hybrid type N-glycans [5,6]. In 2013, we reported a convergent synthesis of multi-antennary N-glycans and their

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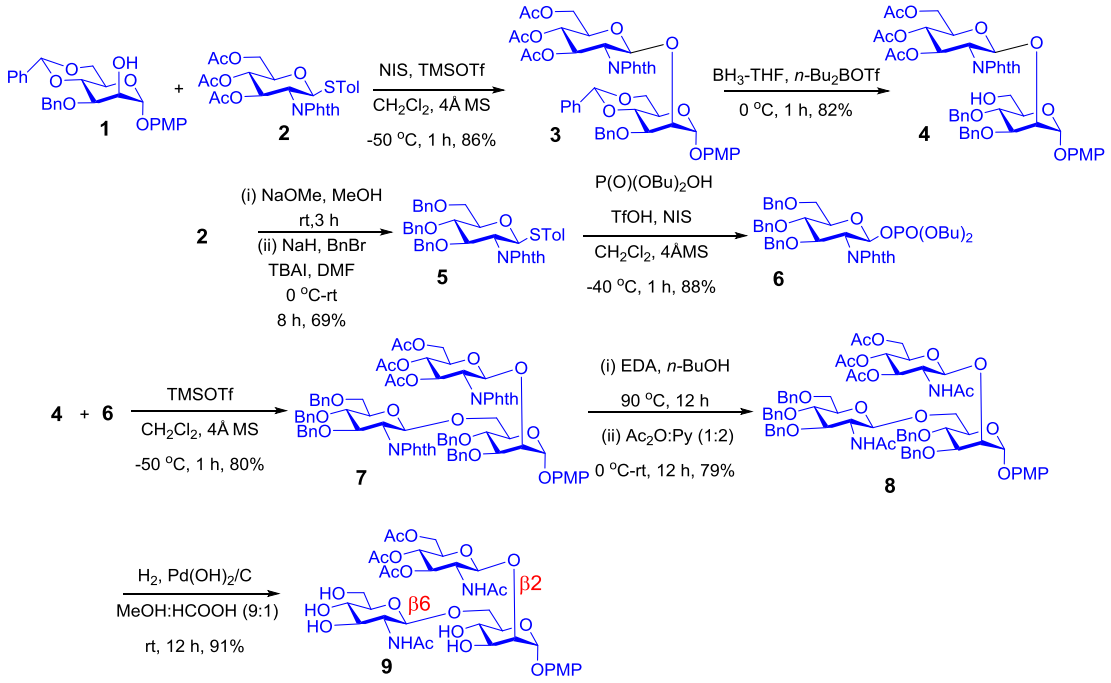
application to the study of HIV-antigenicity [7a]. Recently, we also developed a modular chemo-enzymatic approach for the synthesis of high-mannose, hybrid- and complex-type N-glycan structures for the development of glycan arrays for the rapid screening and identification of epitopes on HIV-1 recognized by the broadly neutralizing antibodies isolated from HIV-1 patients [7b].

In the last 100 years, synthetic chemists have been developing efficient glycosyl donors for the synthesis of complex oligosaccharides. Numerous versatile glycosyl donors have been developed, among which glycosyl trichloroacetimidates [8] and thioglycosides [9] are more commonly utilized. However, the application of glycosyl donors, such as glycosyl sulfoxides [10], *n*-pentenyl glycosides [11], glycosyl phosphites [12], glycosyl halides [13], anhydrosugars [14], glucals [15], has been also widely documented for the synthesis of complex oligosaccharide. Since mid-1990's, glycosyl phosphates have become an attractive class of glycosylating donors due to their stability, ease of activation and high reactivity at low temperature. The use of glycosyl phosphate donors for enzymatic syntheses is also well known. It is known that glycosyltransferases use nucleotide 5′-phosphosugars (NDPs) for the biosynthesis of complex oligosaccharides [16]. Seeberger and coworkers reported the synthesis of glycosyl phosphates for the synthesis of O-glycosides [17], C-glycosides [18], and complex oligosaccharides [19]. Previously, we have reported the synthesis of glycosyl phosphates and their application in the chemical and chemo-enzymatic synthesis of oligosaccharides [12]. In the current context, we extend our chemo-enzymatic modular approach using glycosyl phosphates and glycosyltransferases to construct several modular building blocks which are useful for the assembly of symmetric and asymmetric N-glycans.

2. Result and discussion

The synthesis of the targeted trisaccharide **9** was initiated from orthogonally protected glycosyl donor **2** and acceptor **1**, which were synthesized from commercially available D-mannose and D-glucosamine, respectively, using known procedures [7b]. Initially, glucosamine donor **2** and acceptor **1** were coupled using NIS/TMSOTf to furnish disaccharide **3** with 1,2-β-glycosidic linkage in 86% yield. Selective opening of the 4,6-*O*-benzylidene ring in the presence of BH_3-THF/*n*-Bu_2BOTf led to 6-hydroxy mannosyl acceptor **4** in 82%. On the other hand, the glycosyl phosphate donor **6** was prepared from thioglycoside donor **2**, which upon deacetylation under Zemplén condition followed by benzyl protection using BnBr/NaH provided *O*-benzyl protected thioglycoside **5**. The anomeric phosphate group was installed using dibutyl phosphate in the presence of NIS/TfOH to generate **6** with excellent yield. Next, disaccharide **4** and phosphate donor **6** were coupled using TMSOTf at −50 °C to obtain trisaccharide **7** in 80% yield. Later, removal of phthalimide protecting groups using ethylenediamine/*n*-BuOH followed by N-acetylation using Ac_2O/Py generated trisaccharide **8**. In order to avoid any negative interference of the bulky -OBn groups during the enzymatic reaction, we planned to remove the benzyl groups using $Pd(OH)_2/C-H_2$ to furnish **9** (Scheme 1). The C4 hydroxyl group at the GlcNAc (β6 arm) of trisaccharide **9** can be elongated by enzymatic glycosylation to form the desired N-glycan structure.

To further investigate the application of the phosphate donor towards the synthesis of diverse branching units, we installed tri-*O*-acetyl glucosamine entity on the β6 arm of the trisaccharide motif. Glycosyl phosphate **6** was coupled with mannosyl acceptor **1** using TMSOTf to furnish disaccharide **10** in good yield. The presence of doublet at 5.27 ppm with a coupling constant ($J = 8.4$ Hz) in ^{1}H NMR spectrum along with the signal at 96.9 ppm in ^{13}C NMR indicated the formation of 1,2-β-linked disaccharide **10**. Selective opening of the 4,6-*O*-benzylidene ring in the presence of BH_3-THF/*n*-Bu_2BOTf led to the 6-hydroxy disaccharide acceptor **11** in 78% yield. The absence of the signal at 5.42 ppm and 101.5 ppm in ^{1}H and ^{13}C NMR spectrum, respectively, indicated the cleavage of 4,6-*O*-benzylidene ring. Next, the disaccharide acceptor **11** was coupled with phosphate donor **12** using TMSOTf to furnish the β2-β6 linked trisaccharide **13**. The presence of two doublet signals at 5.20 (d, $J = 8.5$ Hz, 1H) and 5.10 (d, $J = 8.4$ Hz, 1H) in ^{1}H NMR confirmed that the two glucosamine units are β2-β6 linked with mannose in **13**.



Scheme 1. Synthesis of Manβ2GlcNAc(OAc)$_3$β6GlcNAc.