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Alkoxyamino glycoside acceptors for the regioselective synthesis of oligosaccharides using glycosynthases and transglycosidases

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ABSTRACT

Alkoxyamino derivatives of oligosaccharides have been synthesized by enzymatic synthesis using a glycosynthase and a transglycosidase. The chemoselective assembly of unprotected oligosaccharides bearing glucose at the reducing end with *N*-alkyl-*O*-benzylhydroxylamine provides sugar derivatives that are good acceptors for enzymatic synthesis using either glycosynthase or transglycosidase. Furthermore, this method affords the possibility of controlling the regioselectivity of coupling depending on the nature of the alkoxyamino substituent and provides high-yield coupling of sugars without the need for complex protecting group chemistry.

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The important role of oligosaccharides and their conjugates in biology has been increasingly recognized in recent years. Unfortunately, despite the considerable development of efficient methods in this field, the assembly of oligosaccharides remains a substantial challenge. Glycosidic bond formation requires meticulous control of both regio- and stereoselectivity. The regioselectivity is extremely challenging because of the similar reactivities of the hydroxyl groups. In order to circumvent these difficulties, it is often necessary to employ extensive protecting group chemistry with all its attendant problems.

Chemo-enzymatic glycosylation has become a powerful tool for the stereoselective synthesis of various glycosidic compounds. In this way, complete control over both stereo- and regioselectivity can be attained. Glycosyl transferases are useful in this regard as they catalyze the formation of glycosidic bonds with both high yield and selectivity. Unfortunately, glycosyl transferase methodology is limited for the large-scale production of oligosaccharides because of the difficulty in accessing nucleotide sugar donors. Engineered retaining glycosidases have provided an efficient approach for the synthesis of oligosaccharides. A first methodology, using site-directed mutagenesis, was developed by Withers[1–7] and co-workers on exoglycosidases and Planas and co-workers on endoglycosidases.[8–12] This approach was based on the mutation of the catalytic nucleophile (aspartate or glutamate) by a neutral amino acid (glycine, serine or alanine) and the use of donors with an anomeric configuration opposite to that of the original substrate (such as α-glycosyl fluorides), which yielded new enzymes called glycosynthases.[13] The mutant glycosidases provided high transglycosylation yields because of the inability to hydrolyze the transglycosylation product. A second approach, which was developed recently in our laboratory, was based on the directed evolution of glycosidases into transglycosidases.[14–16] The mutant enzymes have almost lost their hydrolytic activity while keeping their transglycosidase activity.

One of the main drawbacks of the glycosynthase and transglycosidase approaches applied to exoglycosidases is often the need for a phenyl ring at the (+2) subsite to ensure the correct positioning of acceptors in the active site.[14] Such an aromatic group provides stacking interaction with aromatic active-site residues and leads to improved regioselectivity and yield in the oligosaccharide synthesis. However, these substituents need to be eliminated in order to functionalize the anomeric position for the subsequent synthesis of glycoconjugates. Deprotection of aryl groups is difficult without partial hydrolysis of glycosidic bonds, whereas alternative phenyl thio acceptors have been shown to be excellent substrates which can be easily deprotected by *N*-bromosuccinimide. Furthermore, all these approaches rely on the organic synthesis of aromatic substrate acceptors, which limits the applicability of enzyme synthesis and its use in sustainable chemistry.

Recently, anomeric modification, such as oxime ligation, which enables the formation of glycosyl derivatives without using protecting groups, has been exploited for the synthesis of various glycoconjugates.[18–20] This chemoselective conjugation provides predominantly a β-pyranoside for reducing terminal gluco-configuration, which can be subsequently cleaved in weakly acidic water

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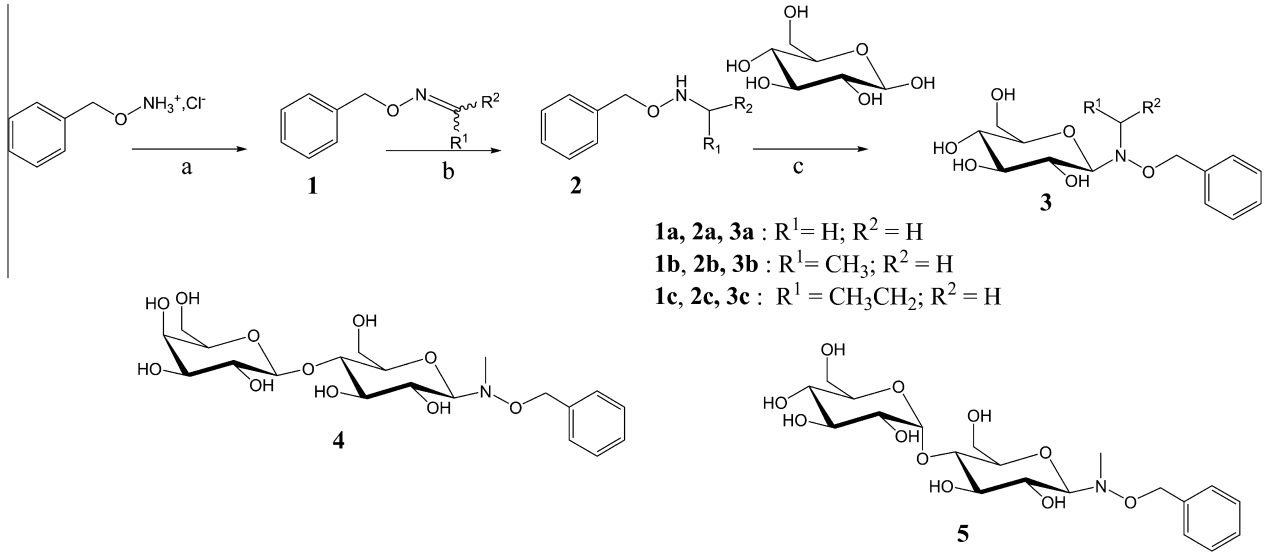


Scheme 1. Chemoselective synthesis of acceptors **3a–c**, **4** and **5**. Reagents and conditions: (a) AcONa, H_2O/MeOH 4/1, aldehyde or ketone (3 equiv), 95–97%; (b) $NaCNBH_3$, EtOH, HCl, 80–89%; (c) THF/AcOH 75/25, 43–86%.

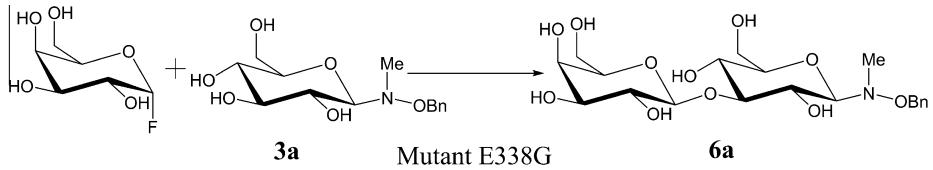
solutions. This Letter presents the synthesis of aromatic glycoconjugates using oxime ligation and their use as acceptors in the enzymatic synthesis of oligosaccharides. This approach is validated with TtβGly, for which both glycosynthase and transglycosidase mutants have been cloned and over-expressed in our laboratory.[14–17]

Mutant TtβGly efficiently catalyzes the synthesis of β-(1 → 3)-disaccharides. Previous work has shown that the glycosynthase E338G can act as an efficient catalyst for the regioselective synthesis of the β-(1 → 3)-disaccharides when the acceptor has a β-*O*-aryl group and, more precisely, an aromatic aglycon group.[17,21] For example, the E338G mutant can produce a nearly quantitative yield (96%) of phenyl β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranoside with the most favorable β-D-GlcPh acceptor.[17] We have previously shown that the replacement of β-D-GlcPh by β-D-GlcBn as an acceptor results in a drop in the regioselectivity of the enzyme leading to a mixture of (1 → 3) and (1 → 6)-disaccharides (ratio 55/45).[17] However, substitution of the benzyl aglycon group by a biphenyl group allows partial recovery of the β-(1 → 3) regioselectivity, suggesting that modulation of the size of the aglycon group can control the regioselectivity of the disaccharide synthesis.[17] Following these observations, we present here an alternative that replaces the aglycon of the sugar acceptor by an alkoxyamino group, which can be easily introduced at the anomeric position and subsequently cleaved. Furthermore, this aglycon modification can accommodate various bulky modifications (**1a–c**, Scheme 1).

The choice of an *N*-alkyl-*O*-benzylhydroxylamine group at the anomeric position for the acceptor was made since we have already shown that the presence of a β-*O*-aryl anomeric group is necessary for either the catalytic activity of wild glycosynthase from *Thermus thermophilus* or for the derived glycosynthases to ensure a correct positioning of the acceptor in the active site.[17] Oxyamino groups have usually been described in the literature for their use in the synthesis of neoglycopeptides by chemoselective ligation with native reducing sugars.[20,22–24] The most attractive of these chemoselective reactions involves aminooxy-derivatized peptides with natural, unmodified reducing sugars. It has also been shown that the use of the oxy-methylamino group allows the preservation of the pyranose cyclic form of the linked sugar.[25,26]

For the synthesis of acceptor compounds **3a–c**, commercially available formaldehyde, acetaldehyde or propionaldehyde was reacted with *O*-benzylhydroxylamine hydrochloride in the presence of sodium acetate in H_2O/methanol (4/1) at rt for 2 h, affording the corresponding *O*-benzyl-oximes **1a–c** with 95–97% yield. The oximes were reduced to the corresponding *N*-methyl-*O*-benzylhydroxylamines **2a–c** by treatment with $NaCNBH_3$ and hydrochloric acid at rt for 30 min (80–89% yield). The hydroxylamines **2** and D-glucose were then reacted in THF/AcOH (75/25) at rt for 20 h affording compounds **3a–c** in 89%, 43% and 86% yield, respectively.[25,26] Using the same procedure, compounds **4** and **5** were synthesized from the *N*-methyl-*O*-benzylhydroxylamine **2a** using D-lactose and D-maltose, respectively, in an aqueous acetate buffer (0.1 M, pH 4.5) at rt for 24 h, in 98% and 95% yield, respectively.

Compounds **3a–c, 4** and **5** (50 mM) were then tested for their ability to react with α-galactosyl fluoride[27,28] (50 mM) as donor in the presence of the mutant glycosynthase, E338G, at 55 °C and pH 7.8 (0.15 M $NaHCO_3$) as shown in Scheme 2. The kinetics of the transglycosylation reaction were followed by TLC until the fluoride donor was completely consumed (16–20 h) and the reaction was stopped. The components were separated by silica gel column chromatography and the structures of the products were determined by standard one- and two-dimensional ^1H and ^{13}C



Scheme 2. Transglycosylation reaction of compound **3a** with α-galactosyl fluoride as donor. Reagents and conditions: aqueous ammonium hydrogen carbonate buffer at pH 7.8, 55 °C, glycosynthase E338G, 87%.

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