Enzymatic Building-Block Synthesis for Solid-Phase Automated Glycan Assembly
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Abstract: Automated chemical oligosaccharide synthesis is an attractive concept that has been successfully applied to a large number of target structures, but requires excess quantities of suitably protected and activated building blocks. Herein we demonstrate the use of biocatalysis to supply such reagents for automated synthesis. By using the promiscuous NmlGtB-B β1-4 galactosyltransferase from Neisseria meningitidis we demonstrate fast and robust access to the LacNAc motif, common to many cell-surface glycans, starting from either lactose or sucrose as glycosyl donors. The enzymatic product was shown to be successfully incorporated as a complete unit into a tetrasaccharide target by automated assembly.

Oligo- and polysaccharides and their conjugates are important targets for chemical synthesis and a number of automated approaches have recently been developed in this field, including solid-phase synthesis, HPLC-assisted synthesis, fluorous-tag solvent-phase synthesis and artificial Golgi apparatus.[1–9] The formation of glycosidic linkages can either be achieved through biocatalysis using carbohydrate active enzymes or by chemical methods, which require selective protection and activation. The main strength of the enzymatic approach is scalability due to low numbers of steps, whereas chemical methods give access to a much broader range of natural and unnatural targets, while limited by the availability of complex, differentially protected building blocks. There have been some efforts to combine chemical and enzymatic methods, in particular incorporating chemically synthesized building blocks as starting materials for enzymatic strategies.[10–12]

Here we extend the chemoenzymatic toolbox, by demonstrating that biocatalysis can also be used to provide robust and short methods to access a building block for automated glycan assembly (AGA), thus combining the efficiency and scalability of biocatalysis where it is needed with the flexibility of chemical synthesis. We demonstrate this strategy for N-acetyllactosamine (LacNAc), which is a widely recurrent motif in biologically relevant glycoconjugates, including human milk glycans, Lewis X antigens, N-glycans, O-glycans and bacterial polysaccharide capsules.[13–16]

The target motif (Figure 1) was chosen as an example of a di-LacNAc sequence that is isomeric to the common type 2 poly-LacNAc oligomer motif in glycoproteins and glycolipids. The target sequence has been reported in naturally derived oligosaccharide sequences[17–20] but its structural and functional characteristics have not been explored as of yet. Compared to the more common motifs bearing a central GlcNAcβ1-3Gal sequence the β1-4 linkage is synthetically more challenging, because of the reduced reactivity of the axial hydroxyl group.[21] In addition, whilst Type 1 (β1-3) and 2 (β1-4) polyLacNAc sequence can be generated enzymatically,[22,23] the required enzyme with β1-4 linkage selectivity is currently not available and hence this linkage has to be generated chemically. Due to its biological relevance, a variety of chemical synthetic strategies for the LacNAc motif 2 have

Figure 1. General strategy for combining biocatalysis with AGA. (A = anomeric activating group, for example, S-Tol; P = activating and β-directing protecting group, for example, N-trifluoroacetyl).

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been investigated,[24–26] often multistep with modest overall yield,[24–26] as exemplified by a nine-step synthesis with an overall yield < 30%.[26] Another approach to a multigram synthesis of LacNAc moiety is the convenient Heyns rearrangement starting from lactulose proposed by Wrodnigg et al.[27] The enzymatic strategy we propose present some advantages in respect to the Heyns rearrangement, such as operating at mild conditions, without the use of metal catalyst and with reduced organic solvents, affording the desired LacNAc motif with a higher yield and without sub products. Furthermore lactulose is a relatively expensive chemical compared to lactose. A second important example of LacNAc building block application has been provided by Broder et al.[28] with an more specific investigation of different protective groups. Also in this case the advantages of the enzymatic strategy we propose compared to this approach are the same as the above mentioned. Enzymatic syntheses of the LacNAc moiety using carbohydrate active enzymes such as galactosyltransferases, glycosynthases and glycosidases have been reported, however lack of selective protection and activation of products has limited applications in automated chemical synthesis.[29,30] Herein, we explore the promiscuity of carbohydrate active enzymes that tolerate protecting and activating groups which are needed for subsequent automated chemical glycan assembly (Figure 1).

For the LacNAc motif 2, an activating and β-directing C2 protecting group (such as N-trichloroacetyl) and anomeric activation as a thioglycoside (such as S-Tol) are required to ensure select chemical incorporation of the building block into target tetrasaccharide 3 by automated assembly. This strategy requires an enzyme that can generate 2 from easily available N-protected glucosamine 1 using cheaply available galactosyl donors, such as lactose or sucrose, rather than expensive sugar nucleotides. We have recently identified and investigated the transgalactosylation activity of NmLgtB-B[31] a recombinant bacterial galactosyltransferase (GalT) from Neisseria meningitidis. This enzyme has some interesting promiscuous activities and, importantly, it is able to use lactose as an inexpensive galactosyl donor (Figure 2) rather than the expensive UDP-Gal, thus enabling economically feasible scale-up. The enzymatic transformation of 1 (reaction Figure 2a) was carried out in the presence of excess of lactose and UDP to push the reaction towards the formation of the desired product 2. The product could easily be extracted from the reaction mixture after the removal of the enzyme and evaporation of the reaction media using a mixture of acetone, methanol, ethanol and water (4/2/2/0.5 v/v) followed by chromatographic purification in 83% yield with robust reproducibility. The starting material could also be easily recovered leading to 97% of total moles recovered. The purified compound 2 (Figure 2) was quantitatively acetylated with pyridine and acetic anhydride to afford the final building block 3 (Scheme 1) with 82% of total yield. Interestingly the

![Scheme 1. Protection of the enzymatically prepared building block.](image)

![Figure 2. Enzymatic galactosylation of 1 using either lactose (a) or sucrose (b) as inexpensive sources for activated sugar donors.](image)
concentration of UDP was varied, with a positive result obtained using a concentration of 7.5 mM. The amount of enzymes was found to be a key parameter for a successful reaction with 17.5% w/w the minimum amount required to achieve a conversion of over 80%. Once robust and reproducible reactions towards the synthesis of product 3 (Scheme 1) were established, we tested it as a building block for automated glycosylation using the Glyconeer 2.1. Synthesis of tetramer 7 was chosen as the model system to display the compatibility of 3 as a starting material in the automated synthesizer (Scheme 2). The reactor was loaded with photo-cleavable resin (4) bearing a hydroxyl group which acted as an acceptor for the first glycosylation cycle using glucoside building block 5. After the removal of the Fmoc group, exposing the free hydroxyl group in position 4, the second cycle employed galactoside building block 6. Once again position 4 of the terminal unit was deprotected enabling the glycosylation using the enzymatically prepared building block 3. The resin was collected and loaded into a UV photoreactor and exposed to UV light to cleave the amino linker, followed by preparative HPLC purification to afford the fully protected compound 7 with an isolated yield of 56%. In order to obtain good yields every glycosylation was performed using an excess of building blocks 3, 5 and 6 (two glycosylation reactions using 6.5 equivalents each). The large quantity of starting material for automated synthesis highlights the importance of developing green and economical strategies to access these building blocks.

In summary, we have demonstrated that selectively protected and activated building blocks can be generated by biocatalysis at gram scale, exploiting the promiscuity of carbohydrate-active enzymes. The short and efficient synthesis can be scaled up to provide reagents for automated solid-phase glycan assembly.[1,3,6] As microbial glycosyltransferases are becoming more accessible and are increasingly shown to be tolerant to protecting and activating groups, this chemoenzymatic strategy should be a useful addition to modern carbohydrate synthesis.

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Conflict of interest

The authors declare no conflict of interest.

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