Carbohydrates play important roles in biological systems. They exist on cell surfaces as glycoprotein or glycolipid conjugates with crucial structural and functional roles in numerous biological recognition processes, including viral and bacterial infection, cancer metastasis, inflammatory response, innate and adaptive immune recognition processes, including viral and bacterial infection, and innate and adaptive immunity and many other receptor-mediated signaling processes.[1-4]

For detailed structural and functional analysis, larger amounts of different, specific carbohydrate structures generally are needed. Carbohydrates and glycosylated natural products can often not be obtained in an homogeneous form from natural sources. The various combinations of different sugars and numerous possible linkages give nature the opportunity to provide an enormous spectrum of molecular information. The synthesis of specific glycosidic linkages is difficult, as carbohydrates are highly functionalized with hydroxyl groups of similar electronic properties.[5] Apart from polysaccharide synthesis, it has been known for a long time that short-chain products can also be obtained. In the presence of suitable (saccharide) acceptor substrates, glucan synthesis is directed towards oligosaccharide synthesis.[6] A broad range of acceptor reactions with small acceptor molecules has already been reported and characterized,[10,11] and gave access to different, more or less complex glycostructures.[5,7,12,13,14] Nevertheless, the acceptor products synthesized by glucansucrases with small sugars are limited, since mostly α-1,6 glucosidic linkages are formed. In some cases, an alteration of linkage specificity was observed,[15] but in order to use these enzymes for distinct sugar modifications, a way to control their reaction specificity has to be found. Different approaches were used to change the regioselectivity of different enzymatic condensations, altering both the enzyme and the structure of the acceptor, mostly by using glycosidases.[16,17] MacManus and Vulfson have demonstrated a change in regioselectivity of glycosidases by blocking hydroxyl groups.[18]

Very recently, Lairson et al. presented a similar approach with a Leloir glycosyltransferase.[19] We wanted to change and direct the specificity of wild-type enzymes by chemical engineering the substrate. In our strategy we designed acceptor substrates that allow the control of linkage specificity by the enzyme and further chemical reactions to enhance glycodiversity. We would like to expand this system to transglycosidases and non-Leloir glycosyltransferases, to give access to a toolkit that provides different linkages and offers another way to synthesis complex glycostructures.

Enzymatic glucosylation

According to CAZY,[26] glucansucrases belong to family 70 of the glycoside hydrolase enzymes (GH70), containing a permuted (β/β8) barrel structure, compared to α-amylase superfamilies (GH13) proteins. We recently demonstrated that the GTFR glucansucrase from Streptococcus oralis converts sucrose (1) into a glucan with mainly α-1,6-bonds and a minor amount of α-1,3-bonds.[21] Previous investigations showed the ability of GTFR to glycosylate different unnatural non-saccharide acceptors, such as alcohols of different chain length and amino acids, thus demonstrating the diversity of acceptor reactions catalyzed by this enzyme.[21]

We observed that GTFR glucosylated the acceptor substrate maltose 2 with an α-1,6-linkage, to form panose 3.[21] When lactose 4 is used as an acceptor, GTFR forms an α-1,2 glucosidic bond to yield 5 (Scheme 1). The chemoselectivity, and thus the reaction specificity, of the GTFR enzyme can be directed by the right choice of the acceptor.

When α-glucose 6 is used as GTFR acceptor substrate, iso-maltose 7 is formed, a disaccharide of glucose linked with α-1,6-linkage. To block position 6 of α-glucose, we synthesized the acceptor molecule 6-O-tosyl-glucopyranose 10.

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[a] H. Hellmuth,* L. Hillringhaus,* S. Höbbel, Dr. J. Seibel
Technical Chemistry, Department for Carbohydrate Technology
Technical University Braunschweig
Hans-Sommer Strasse 10, 38106 Braunschweig (Germany)
Fax: (+49) 531-391-5357
E-mail:j.seibel@tu-bs.de
[b] S. Kralj, L. Dijkhuizen
Centre for Carbohydrate Bioprocessing
TNO-University of Groningen and Department of Microbiology
Groningen Biomolecular Sciences and Biotechnology Institute
University of Groningen
Kerklaan 30, 9751 NN, Haren (The Netherlands)
[1] These authors contributed equally to this work.
[2] Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

COMMUNICATIONS

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Highly Efficient Chemoenzymatic Synthesis of Novel Branched Thiooligosaccharides by Substrate Direction with Glucansucrases

Hendrik Hellmuth,[a] Lars Hillringhaus,[a] Sven Höbbel,[a] Slavko Kralj,[b] Lubbert Dijkhuizen,[b] and Jürgen Seibel[a]

The reaction of GTFR with sucrose and this new acceptor substrate 10 resulted in the formation of an acceptor product, 11, in good yield (48%; Scheme 2). Isolation and characterisation of 11 by correlation spectroscopy showed that transglycosylation took place at position 3 to form an α-1,3 glucosidic bond, in line with the minor α-1,3-forming activity of the GTFR enzyme. So, blockage of the preferred transglycosylation site switched GTFR glycosylation activity from the major α-1,6 to the minor α-1,3 activity. With various acceptor substrate to donor substrate (sucrose) concentrations, the yields of the glycosylated products were in the range of 50%. Thus, we further varied the acceptors substrates by using methyl 6-O-tosyl-α-d-glucopyranoside 12 and allyl 6-O-tosyl-α-d-glucopyranoside 14 with the effect that the yields of the corresponding products 13 (95%) and 15 (62%) increased dramatically up to nearly full conversion (Table 1).

To further enhance the structural diversity of the products, the reuteransucrase (GTFA) enzyme from Lactobacillus reuteri 121 was used. This enzyme synthesizes a glucan that contains mainly α-1,4-linked glucose units, together with a lower amount of α-1,6-linked glucose units. Nevertheless, the acceptor products of α-glucose (6) and maltose (2) synthesized by this enzyme are mostly isomaltose (7) and panose (3), the α-1,6-linked products, also synthesized by GTFR. When 7 is used as acceptor substrate, the resulting product predominantly shows an α-1,4-linkage, followed by oligosaccharides with increasing degrees of polymerization (DP) that contain mostly α-1,6-linkages. This diversity reflects the variability of the linkage formation by GTFA and indicates the possibility, similar to GTFR, of interchanging the position of glycosylation from 6 to 4. As expected, GTFA synthesized the disaccharides 16 (17%) and 17 (17%) with α-1,4-linkages from sucrose and methyl 6-O-tosyl-α-glucopyranoside (12) and allyl 6-O-tosyl-α-glucopyranoside (14) acceptor substrates, but hydrolysis was also observed as a dominant reaction. In summary, the data show that it is possible to direct the enzyme chemoselectivity and reaction specificity from a major glycosylation point to a side activity by proper choice of acceptor substrates.

**Chemical glycosylation**

Both the 6-tosyl and 1-allyl groups provide the possibility of further chemical modifications to giving branched oligosaccharides and even glycopeptides that can be used in biological applications or as building blocks in glycochemistry.

Driguez has reported a convergent approach for the synthesis of α-1,6-thio-linked linear and branched

| Table 1. | Glucosylation of tosylated monosaccharides by different glucansucrases. |
|-----------|------------------|------------------|
| Acceptor | GTFR | Products (yields in %)[a] | GTFA |
| α-Glc6Ts 10 | α-α-Glc(1→3)-α-Glc6Ts 11 (47) | n.t.[b] |
| α-α-Glc6Ts1Me 12 | α-α-Glc(1→3)-α-α-Glc6Ts1Me 13 (95) | α-α-Glc(1→4)-α-α-Glc6Ts1Me 16 (17) |
| α-α-Glc6Ts1allyl 14 | α-α-Glc(1→3)-α-α-Glc6Ts1allyl 15 (62) | α-α-Glc(1→4)-α-α-Glc6Ts1allyl 17 (17) |

[a] Isolated yields. [b] Not tested.

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Scheme 1. α1,6 and α1,2 glucosylation by GTFR glucansucrase enzyme.

Scheme 2. Acceptor–substrate-directed synthesis by GTFR and GTFA enzymes.
oligosaccharides by anomic S-alkylation by S_2 displacement of 6-halides from S-acetyl-protected 1-thiosugars. In our first attempts after acetylation of the disaccharide 11, the tosylate was substituted under basic conditions by the peracetylated glucose thiolate 22 in the presence of kryptofix and sodium hydride in DMF to yield the peracetylated trisaccharide 23 in 31% (Table 2). However, the high nucleophilicity of thiolate group compared with the hydroxyl group should enable a

Table 2. Chemical synthesis of thiosugars.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Acceptor</th>
<th>Product</th>
<th>Yield [%]</th>
</tr>
</thead>
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<tr>
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<tr>
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<td><img src="image6" alt="Product 23" /></td>
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<td><img src="image8" alt="Acceptor 22" /></td>
<td><img src="image9" alt="Product 24" /></td>
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</tr>
<tr>
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<td><img src="image12" alt="Product 26" /></td>
<td>90</td>
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<tr>
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<tr>
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</tr>
<tr>
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<td><img src="image23" alt="Acceptor 28" /></td>
<td><img src="image24" alt="Product 32" /></td>
<td>90</td>
</tr>
</tbody>
</table>

[a] Obtained in situ by treatment of the acetylated thiols with sodium methanolate solution.
direct and selective substitution of the tosylate without using protecting groups. Thus, we investigated further reaction conditions for this reaction. We found that the unprotected mono- and disaccharides can be directly substituted by thiosugars in DMF at 80 °C.

Treatment of 1-thio sugars 25 and 28, obtained by treatment of 19 and 22 with sodium methanolate solution, with 13 in DMF at 80 °C afforded the corresponding oligosaccharides 27 and 29 in excellent yields (91 and 87%, respectively). A similar oligosaccharide, but with a α-1,6 linkage, has been published by Koro et al., whose synthesis requires more than 15 steps with a yield of less than 2%.25

Sialic acids 2,6-linked to different motifs are of special interest as they are a part of mucins26 and diverse structures from human milk27,28. The state of sialylation is a critical determinant in the cell-surface recognition of glycoproteins.29 Here we further demonstrate that 2-thionoamidic acid 30 can also be incorporated with this strategy to yield interesting sialylated structures like 31 that are related to mucin core structures and might be helpful for the elucidation of carbohydrate functions.

In conclusion, we have reported a two-step chemoenzymatic approach in which the chemoselectivity of the used glucosur- cses can be guided from α-1,6- to α-1,2-, α-1,3- or α-1,4- linked glucose by changing the acceptor for the successful construction of various complex glycoconjugates containing thioglycosidic linkages with the glycopyranosides (galactose, glucose, neuraminic acid) of choice. Thioglycosides are tolerat- ed by most biological systems, but are less susceptible to acid/ base or enzyme-mediated hydrolysis, contrary to the naturally occurring O-glycosides.30,31 In addition the allyl group may be modified by ozonolysis into an aldehyde group. The modified sugar thus obtained can now be attached to aglycons, such as peptides, natural products, drugs or solid supports, such as microtiter plates for example, by reductive amination32 to investi- gate, say, protein–carbohydrate interactions. The synthesized molecules may then be used in further experiments to identify carbohydrate–lectin and –selectin interactions of biological relevance. Taken together, the choice of distinct acceptors and enzymes in combination with chemical synthesis could lead to a powerful set of tools for glycosynthesis.

Those studies will be expanded in future to other glucansur- cses and glycosyltransferases for the synthesis of novel struc- tures.

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