Natural Product Synthesis

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Reversible Sugar Transfer by Glycosyltransferases as a Tool for Natural Product (Bio)synthesis**

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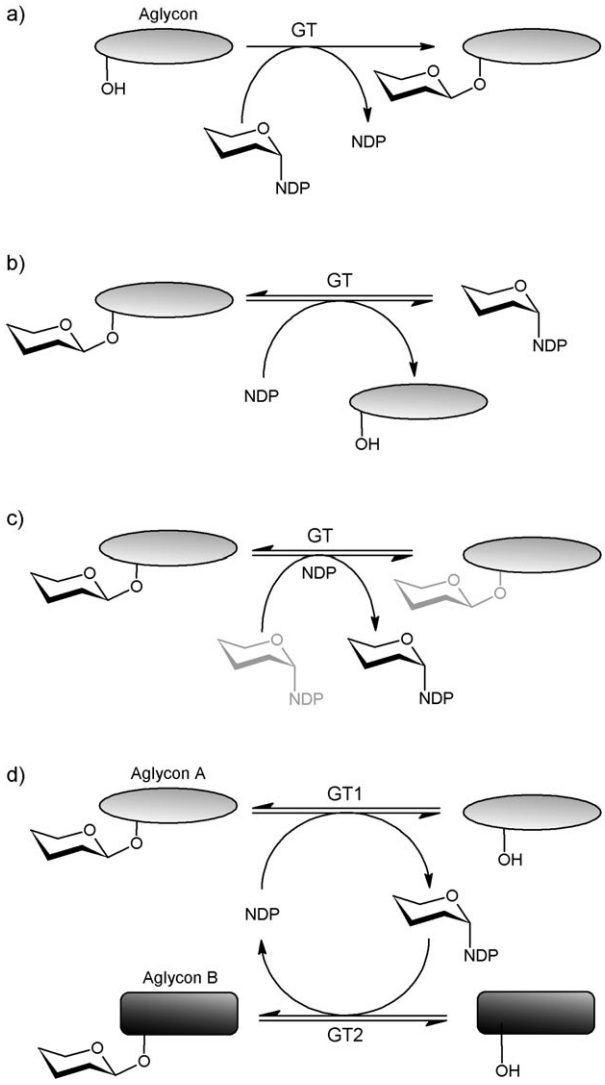
Since the beginning of medicine, sugars have played an important role. Without the sugar cube, the eradication of polio in Europe and the US would have been much more difficult as it made the oral vaccination procedure easier, especially for children. However, the vaccine would have done the job without the sugar, which is not true for most glycosylated natural products: Many of these clinically used bioactive compounds or their semisynthetic derivatives represent glycosylated polyketides, non-ribosomally made peptides, or terpenoids, which often show as severely a reduced activity (if any) as aglycons.[1,2] As the attached sugar moieties often strongly influence the compound's specificity, substrate binding, and pharmacology, there is a strong interest to deliberately change and diversify natural product glycosylation patterns.[3]

Biochemically, glycosyltransferases catalyze the enzymatic connection between an aglycon and a nucleoside diphosphate (NDP)-activated sugar leading to glycosylated compounds (Scheme 1 a).[4,5] In general, three strategies have been employed to create compounds with "non-natural" glycosylation patterns or libraries of glycosylated natural products that are required to study structure–activity relationships and to optimize the compound's activity.

1) The total synthesis or semisynthesis, which was used to show the essential nature of sugar residues for bioactivity as shown by, for example,



Scheme 1. Examples of glycosyltransferase (GT) catalysis. a) The classical GT- catalyzed sugar transfer, b) NDP-sugar biosynthesis, c) sugar exchange (the different sugar molecules are colored gray and black), and d) aglycon exchange.

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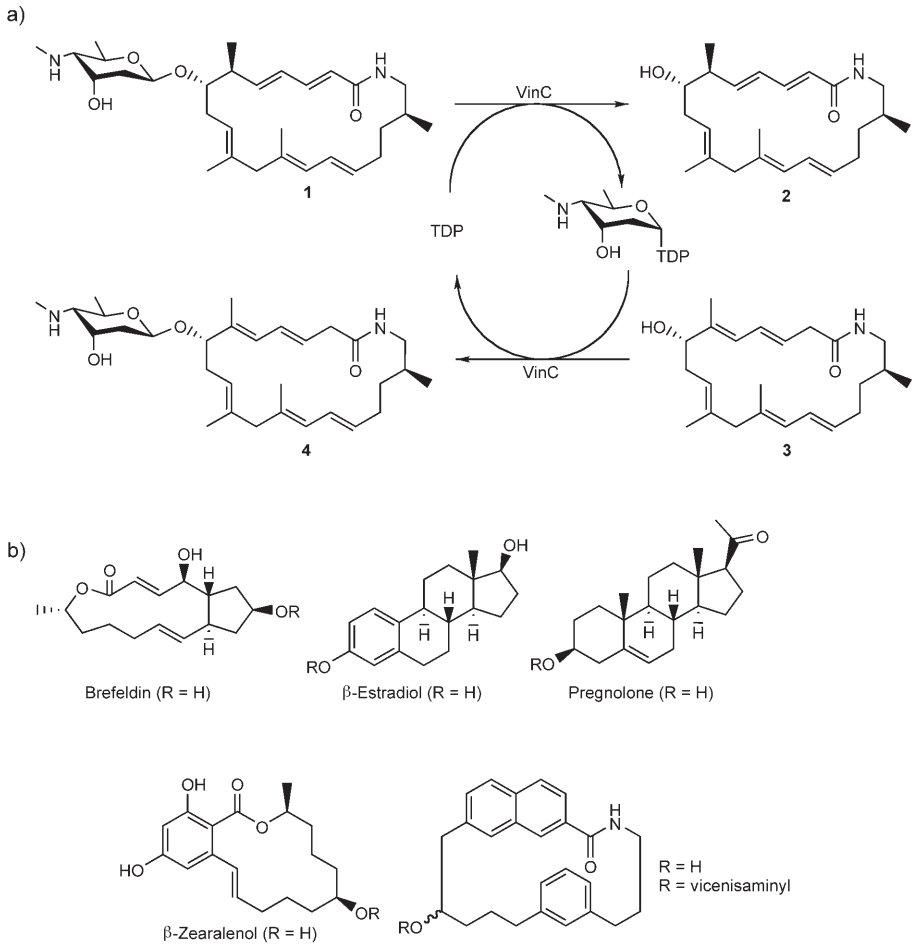
rebeccamycin.[6] Furthermore, progress has even been made in the last few years in automated synthesis of sugar oligomers.[7]

2) Pathway engineering in vivo (often called combinatorial biosynthesis). In this case, the biosynthesis and the attachment of the natural sugar moiety is manipulated by using additional enzymes or complete pathways. These modifications may lead to new or modified sugar moieties that can be attached to the aglycon. By using this approach, libraries of natural products have been obtained that not only differ in the sugar moiety but also in the additional follow-up modifications that first emerge after the glycoside forms. This is exemplified by the formation of several indolocarbazoles.[8] The prerequisite for the pathway engineering is a detailed understanding of the biosynthesis of activated sugars and the glycosyltransferases involved.[9] Numerous different sugar biosynthesis pathways have been investigated in detail after the analysis of corresponding biosynthesis gene clusters of glycosylated compounds. From this work, several unusual enzymatic activities such as N- or C-glycosylation, as well as iteratively acting glycosyltransferases, have been identified and can now be used to expand the possibilities of combinatorial biosynthesis.[10–12]
3) Glycorandomization: This process basically involves two steps: First, the activation of a variety of sugars (natural or synthetic) by kinases, which have a promiscuous substrate specificity, and the subsequent use of nonspecific nucleotidyltransferases. These enzymes are used to generate a library of nucleotide diphosphate (NDP) sugars that can then be employed as substrates for the glycosylation of different aglyca, finally leading to libraries of glycosylated natural products.[13–15] The power of this approach has been demonstrated by the synthesis of 11 known and 39 novel vancomycin derivatives, some of which show superior bioactivity when compared with the natural compound.[13] The overall size of the produced library can be expanded even more if the starting sugar bears functional groups that can be modified specifically by chemical methods (e.g. by click chemistry).

The latest and probably most elegant addition to the biochemical and chemical glycosylation tool box is the use of glycosylated natural products as the source of activated sugars (Scheme 1b). Furthermore, the interdependent exchange of sugar moieties (Scheme 1c) or aglyca (Scheme 1d) is possible. The biochemical basis of these



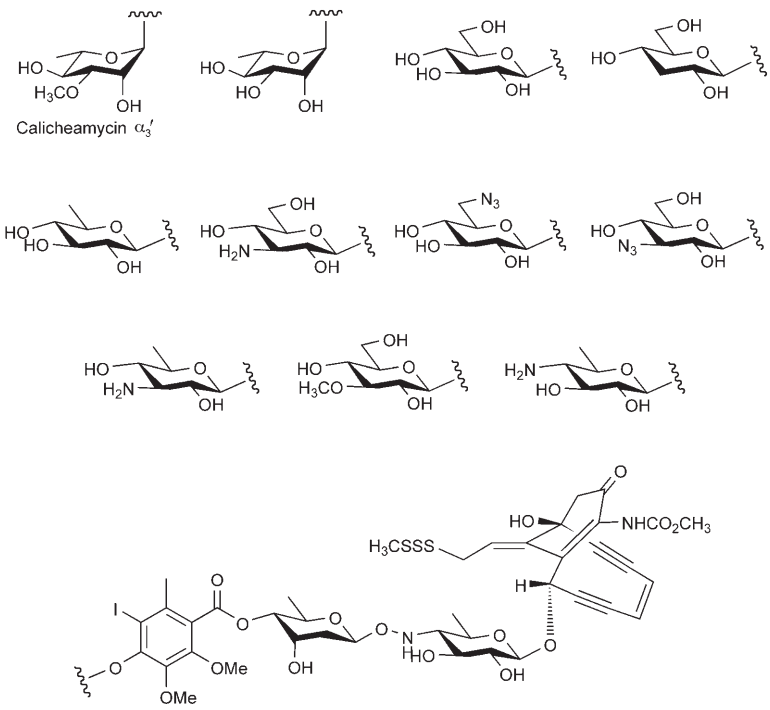
Scheme 2. a) Deglycosylation of vicenistatin (**1**; top), glycosylation of neovicenilactam (**3**; bottom), and transcglycosylation of vicenisamine from **1** to give **4** (both reactions together). b) Further aglyca and their glycosylated derivatives obtained in similar aglycon exchange reactions.

breakthrough transformations in natural product chemistry of glycosylated compounds is the finding that most glycosyltransferases act in a reversible manner. Until very recently, glycosyltransferase were mostly regarded as unidirectional catalysts that drive the formation of glycosidic bonds between NDP-sugar donors and aglycon acceptors. Nevertheless, it was known that sucrose synthase can also catalyze the reverse reaction to afford NDP-glucose and fructose from sucrose and NDP.[16]

In 2005, Eguchi and co-workers published the chemoenzymatic application of a reversibly acting glycosyltransferase involved in natural product biosynthesis for the first time.[17] They used VinC, a glycosyltransferase that attaches NDP-vicenisamine to vicenilactam (**2**) as the last step in the biosynthesis of vicenistatin (**1**), an antitumor compound from *Streptomyces halstedii* HC 34.[18,19] These authors could show that VinC also catalyzes the thymidinediphosphate (TDP)-dependent deglycosylation and coupled formation of TDP-vicenisamine from **1** in the presence of TDP (Scheme 2a, top).[17] The addition of neovicenilactam (**3**), a double bond isomer of **1**, to the reaction mixture led to the formation of neovicenistatin (**4**) in 42% yield in a one-pot reaction (Scheme 2a). In further experiments, Eguchi and co-workers expanded the applicability of **1** as a TDP-vicenisamine donor in order to glycosylate five other aglyca. This resulted in the formation of the corresponding glycosylated compounds in yields between 7–24% (Scheme 2b). Although these aglyca look quite different at first glance, molecular-modeling data (to determine their three-dimensional structure) revealed a very similar molecular size with the hydroxy group occupying almost the identical position as in **2**.

Recently, Thorson and co-workers expanded this approach by using CalG1 and CalG4 and GtfD and GtfE, which are glycosyltransferases from the calichemycin and vancomycin biosynthetic pathways, respectively.[20] Further to the deglycosylation process described above, the broad substrate promiscuity of CalG1 allowed the generation of 10 calicheamycin glycosides that differ in the sugar residue attached to the benzoic acid moiety (Scheme 3). Furthermore, these authors observed a sugar exchange during incubation of calicheamycin with CalG1 when TDP-3-deoxy-α-D-glucose is in excess. Expanding this strategy to eight different natural or semisynthetic calicheamycin derivatives in combination with 10 TDP sugars led to the formation of more than 70 new calicheamycins. This highlights the power of the combinatorial sugar exchange. Similar results were also obtained with CalG4. Expanding these studies beyond endyine compounds, Thorson and co-workers next investigated the applicability of GtfD and GtfE, glycosyltransferases involved in vancomycin biosynthesis. This approach also led to the predicted sugar exchanges. Finally, a combination of CalG1 and GtfD together with the vancomycin derivative **5** and the calicheamycin aglycon **6** enabled the formation of the new calicheamycin derivative **7** in a one-pot reaction with an overall conversion yield of 48% (Scheme 4).

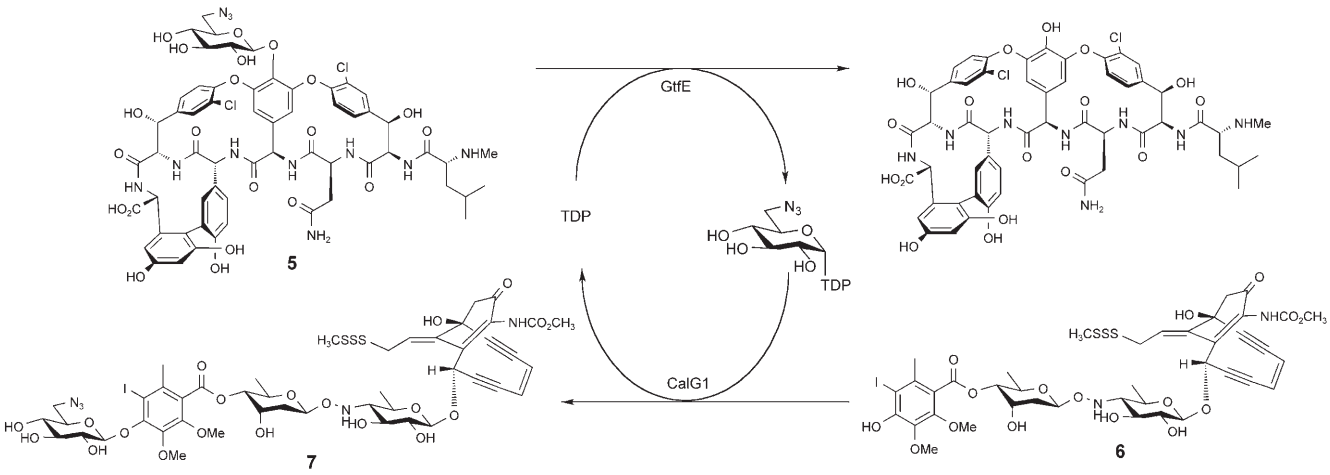
By using this approach, rare NDP-sugars can simply be harvested from glycosylated natural products by using the corresponding glycosyltransferase and then attached to a second natural product aglycon by a different glycosyltransferase. This is of significant importance as numerous activated sugars involved in natural product biosynthesis are very difficult to obtain synthetically or by degradation of natural products bearing these sugar moieties.



Scheme 3. Calicheamycin derivatives obtained by sugar exchange starting from calicheamycin α_3' by using CalG1 and 10 different TDP-activated sugars.

The pioneering work of Eguchi and co-workers,[17] which was significantly extended by Thorson and co-workers,[20] has greatly expanded our view on glycosyltransferases and their role and use in natural product (bio)synthesis in general. However, in both cases, the required amount of enzyme in the sugar transfer reactions was in such a high range that it hardly qualifies as actual catalysis. Therefore, a lot of improvement is required to make the process biotechnologically feasible (the synthesis of preparative amounts). In principle, such improvements should be possible as an impressive recent example of large-scale (bio)synthesis of TDP sugars demonstrates. In this case, TDP-6-deoxy-4-keto-α-D-glucose could be obtained in a three-step enzymatic one-pot reaction with 72% overall yield on a 0.2-g scale by using highly efficient enzyme catalysis.[21]

Once the required optimization is achieved, a large variety of biochemically characterized glycosyltransferases can already be used for glycosylation



Scheme 4. An example of a one-pot aglycon exchange between a vancomycin derivative (**5**) and a calicheamycin aglycon (**6**) by using the two glycosyltransferases GtfE and CalG1 for deglycosylation and glycosylation, respectively.

and sugar or aglycon exchange. In light of the opportunities to employ the enormous number of such enzymes identified in sequencing projects of various secondary metabolite biosynthesis gene clusters or whole genomes,[22] a new era of sweet natural product chemistry is clearly developing. This is especially true as late biosynthesis steps, including glycosylations, often have a remarkable influence on the biological activity of the natural product.[1]

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