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Aglycon switch approach toward unnatural glycosides from natural glycoside with glycosyltransferase VinC

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Abstract—New aglycon switch approach using glycosyltransferase VinC was explored to create unnatural glycosides from natural glycoside in one-pot reaction. This aglycon switch comprises from two reactions, where NDP-vicenisamine generated in situ from natural glycoside vicenistatin and NDP by the reverse reaction is transferred to the targeted additional aglycons to form unnatural vicenisaminides by the forward reaction.

Glycosides, consisting of a major class of natural products, are important for medicinal use.[1] From intense desire to create unnatural glycoside libraries based on natural glycosides, enzymatic approach has been explored to alter the sugar and the aglycon parts of natural glycosides by in vitro transglycosylation, and a successful fruit was exemplified by glycorandomization to form unnatural glycosides especially exchanged in sugar part.[2–5] But as to the aglycon part exchange, little is known as successful example to form unnatural natural glycosides using structurally unrelated aglycons as a glycosyl acceptor.[5,6] Because construction of glycoside libraries composed of diverse scaffolds appears to promise high potential for discovery of biologically significant compounds, a new approach has long been sought to particularly diversify the aglycon part of natural glycosides. To this end, we envisioned a new aglycon switch approach from natural glycosides.

Recently, we identified the biosynthetic gene cluster (*vin*) for vicenistatin (**1**), an antitumor antibiotic glycoside produced by *Streptomyces halstedii* HC 34,[7] and confirmed that VinC is a glycosyltransferase catalyzing the transfer of dTDP-vicenisamine (**3**) to vicenilactam (**2**) in the last step of vicenistatin biosynthesis (Fig. 1).[8] More recently, we have illustrated that, with its broad substrate specificity to the aglycon part, VinC catalyzes the transfer of vicenisamine toward both structurally related synthetic aglycons and structurally unrelated aglycons.[6] These results indicated high potential of VinC as a useful glycosyltransferase for the construction of glycoside library by altering aglycon part. But one difficulty, always encountered in the standard transglycosylation approach, is preparation of nucleoside diphosphosugar (NDP-sugar), dTDP-vicenisamine in this case. Chemical or biochemical preparation of NDP-sugar, especially of 2-deoxysugars and aminosugars, is problematic. Therefore, exploitation of alternative transglycosylation approach without the need for previous preparation of an activated substrate such as NDP-sugar has been strongly desired.

One solution to this problem was the unusual enzymatic method described by Withers and co-workers, where contribution of glycosyl fluoride and a nucleoside diphosphate was used as a glycosyl donor instead of NDP-sugar in the reaction of lipopolysaccharide galactosyltransferase C.[9] We attempted this method for the VinC reaction without success (data not shown). So we turned our attention to explore a different method, hopefully applicable generally to glycosyltransferases.

It is well known that glycosyltransferase catalyzes a glycosyl transfer reaction between NDP-sugar and an aglycon, and generate the corresponding glycoside and NDP. Since enzyme reactions are generally reversible, we envisioned a possibility of in situ generation of NDP-sugar from glycoside having a desirable sugar by

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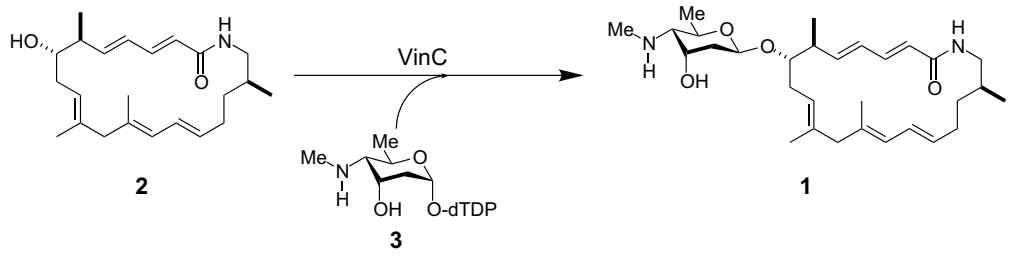

Figure 1. Glycosyl transfer reaction catalyzed by VinC in vicenistatin biosynthesis.

reverse catalysis of glycosyltransferase. Actually, it is known that sucrose synthase can catalyze the reverse reaction with sucrose and NDP to afford NDP-glucose and fructose.[10] According to this line, we envisioned a new aglycon switch approach as shown in Figure 2. This approach is composed of two reactions; in situ generation of NDP-sugar from natural glycoside by reverse reaction, which subsequently transfer simultaneously the transient NDP-sugar to a targeted acceptor by itself. In this scenario, supplementation of an appropriate NDP is necessary as a chemical initiator as shown in Figure 2. In oligosaccharide synthesis, glycosidase mediated glycoside synthesis based on aglycon switch approach has already been known.[11] However, the present aglycon switch approach has differences from the glycosidase approach as follows: (1) strict stereo- and regio-selectivity, (2) synthesis of structurally complex unnatural molecules.

Prior to pursue our approach, the reverse reaction by VinC was first examined by incubating **1**, dTDP and VinC at 28 °C for 2 h. After extraction of the reaction mixture with ethyl acetate, the crude extract was analyzed by HPLC, and the formation of vicenilactam **2** was clearly confirmed (Fig. 3A). Control assays in the absence of either VinC or dTDP gave no vicenilactam formation. The formation of dTDP-vicenisamine **3** was also confirmed separately by HPLC analysis of the reaction mixture (Fig. 3B). These results clearly demonstrated that VinC was able to catalyze the reverse reaction to form the corresponding activated donor **3**. Substrate specificity to NDP was as follows: 7% and 6% conversion yields from **1** to **2** were observed with dTDP and UDP, respectively, but no reaction occurred with ADP, CDP, or GDP.

The next stage of this new method described in Figure 2 was the forward reaction catalyzed by VinC between dTDP-vicenisamine generated in situ and an additional aglycon. The transfer of transient **3** to a different aglycon was first confirmed with neovicenilactam (**4**) as an alternative glycosyl acceptor. Thus, a mixture of **1**, dTDP, **4**, and VinC was incubated at 28 °C for 2 h, and the reaction was chased again by HPLC, which clearly indicated the formation of **2** and neovicenistatin (**5**) as the corresponding vicenisaminide from **4**, as shown in Figure 4. In the control experiments, in the absence of either VinC, dTDP, or **1**, the formation of neovicenistatin was not detected at all. Neovicenistatin **5** thus formed was further identified by ESI-MS and ^{1}H NMR analyses, which allowed to confirm that the obtained neovicenistatin was a β-anomer. These results indicated that VinC in fact transferred dTDP-vicenisamine generated in situ to a supplemented aglycon to yield the corresponding vicenisaminide in strictly stereochemical control.

It should be pointed out from these results that natural **1** can be a source of NDP-vicenisamine and prior preparation of an activated sugar is not required for the glycosyl transfer reaction by VinC catalysis to give a new glycoside in one-pot synthesis. Since VinC was able to transfer **3** to both structurally related and structurally unrelated aglycons,[6] this new in situ approach was further attempted using various aglycons such as vicenistatin mimic aglycon (**6**), brefeldin A (**7**), α- and β-zearalenol (**8** and **9**), β-estradiol (**10**), and pregnenolone (**11**) as a glycosyl acceptor (Fig. 5). Namely, one-pot reactions incubating VinC with **1**, dTDP, and aglycon were examined separately, and production of β-glycosidic vicenisaminide **12**–**17**[6] generated from aglycons **6** to **11**, respectively, was monitored by HPLC analysis.[12] The final conversion yields to respective vicenisaminides were 42% with **4**, 24% with **6**, 27% with **7**, 16% with **8**, 10% with **9**, 16% with **10**, and 7% with **11**. These results indicate that one-pot glycoside synthesis using VinC is

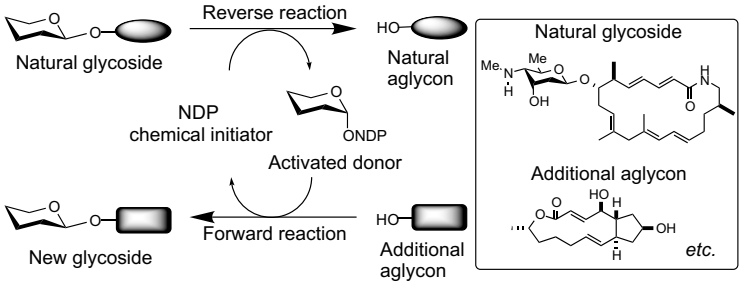

Figure 2. New enzymatic aglycon switch approach using natural glycoside, NDP, and additional aglycon.

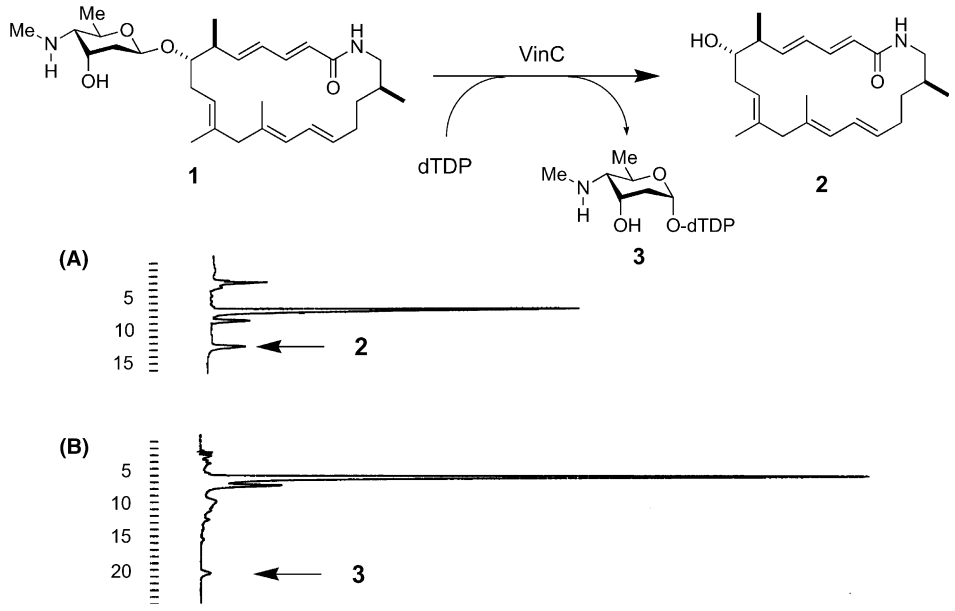

Figure 3. HPLC profiles of (A) the organic extracts and (B) the enzyme reaction mixture of the reverse reaction catalyzed by VinC.

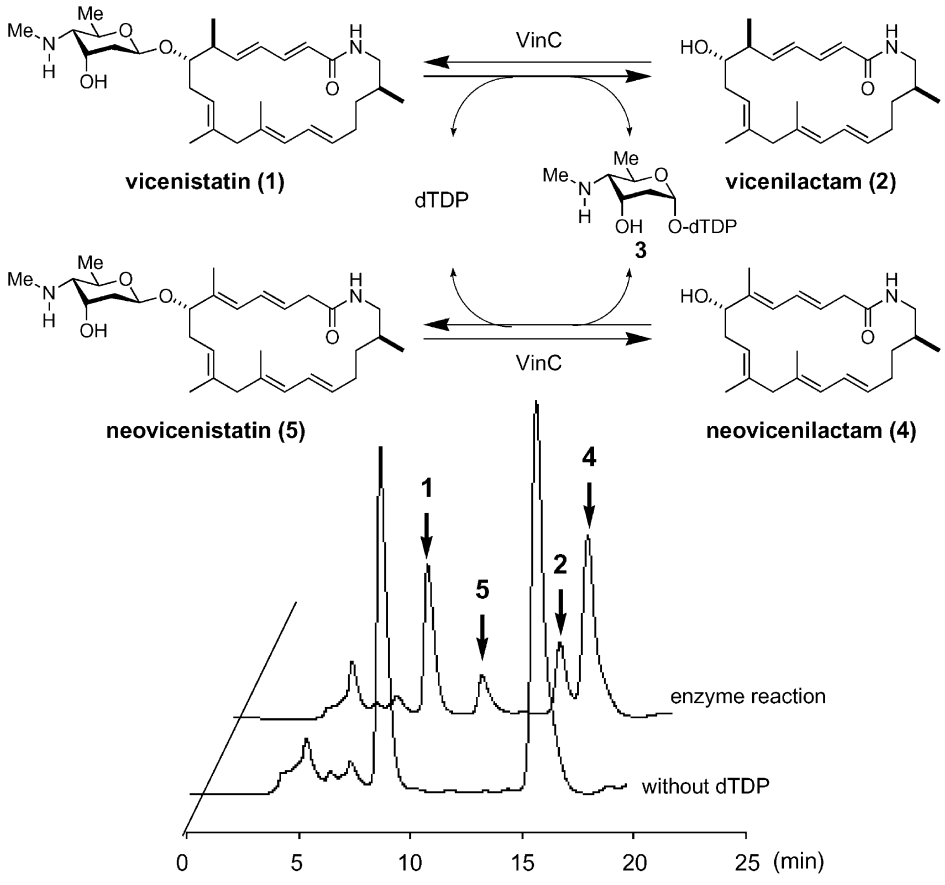

Figure 4. HPLC charts of the enzymatic aglycon switch reaction with vicenistatin (**1**), dTDP, and neovicenilactam (**4**).

apparently applicable to the preparation of new vicenisaminides. This new *enzymatic aglycon switch* from natural glycoside promises general applicability to glycosyltransferases, and is more advantageous than conventionally accepted method; (1) prior synthesis of NDP-sugar is unnecessary, which in some cases is difficult and tedious, and (2) a glycosyl donor, natural glycoside in this case, can be recovered. Even though, low conversion yields to the targeted vicenisaminides and necessity of a large amount of the enzyme,[12] this

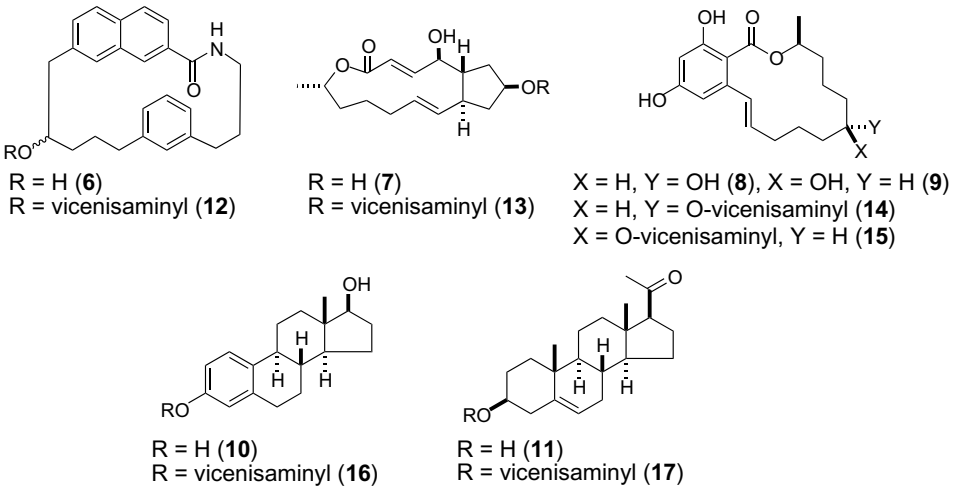

Figure 5. Structures of the additional substrates and the enzyme reaction products.

approach is simple and easy in order to create unnatural glycosides with different aglycons, once a glycosyltransferase of interest has been observed.

In summary, we successfully demonstrated in situ formation of activated glycosyl donor from NDP and glycoside, and the aglycon switch from a glycoside to another by adding the alternative aglycon to the reverse reaction mode. This *enzymatic aglycon switch* appears to have significant potential of application to many glycosyltransferases to develop glycoside diversities.

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12. Typical conditions of one-pot enzyme reaction are as follows: A reaction mixture (final volume, 50 μL in Tris–HCl buffer, pH 8) containing 2 μL of dTDP (2 mM final concentration), 2 μL of vicenistatin in DMSO (0.2 mM final concentration), 2 μL of aglycon in DMSO (0.2 mM final concentration), and 23 μL of VinC (0.6 mM final concentration) in Tris–HCl buffer (50 mM, pH 8) was incubated at 28 °C. The enzyme reaction was terminated by the addition of ethyl acetate, and then extracted with ethyl acetate. The combined organic layer was concentrated, and redissolved in 30 μL of MeOH. The samples were analyzed by the same procedure described in Ref. 6.