Transition-state analog inhibitors for *N*-acetylglucosaminyltransfer enzymes

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Transition-state analog inhibitors which contain both the donor and the acceptor molecules involved in the enzymatic glycosylation are believed to have a higher inhibitory activity and specificity to the enzyme than acceptor-like or donor-like monosubstrate inhibitors. A transition-state analog inhibitor of *N*-acetylglucosaminyltransferase V, whose increase in the activity is associated with the metastatic potential of tumor cells, has been designed. The inhibitor includes aza-*N*-acetylglucosamine as a mimic of the donor sugar in the transition state, and three azasugars with five-membered and six-membered rings are chemoenzymatically synthesized by using FDP-aldolase catalyzed reaction, dephospholyration with phosphatase and reductive amination on Pd-C. The detailed synthesis of these azasugars as key intermediates for the synthesis of transition-state analog inhibitors of glycosyltransferases on cell surfaces is described in this review.

Many potent inhibitors of key enzymes in mammalians, bacteria and viruses have been developed and utilized as medicines. In order to design effective inhibitors having a high specificity for the target enzymes, detailed characterization of the substrate-enzyme complex or the transition-state structure is necessary. Inhibitors of the enzyme which catalyzes a reaction involving two different substrates should have a structure feature that contains the binding sites for the two substrates or the transition state to ensure a specific and effective inhibition. For instance, tunicamycin (**1**) isolated from *Streptomyces lysosuperficus* is considered to mimic the transition-state structure of the enzymatic transfer of GlcNAc-1-phosphate from UDP-GlcNAc to dolichol phosphate (Fig. 1).[1] Glycosyltransferases also accept two substrates: a sugar nucleotide as donor and another molecule as acceptor.

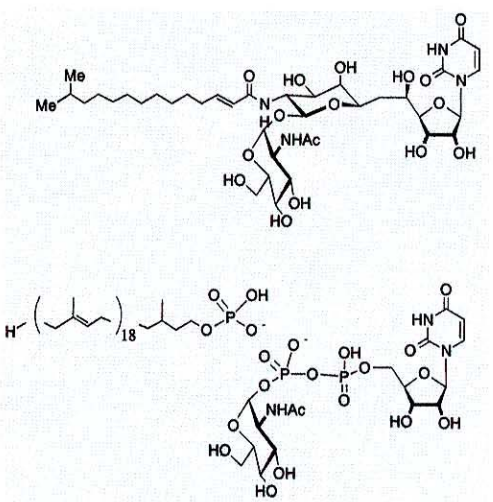

Fig. 1. The similarity of tunicamycin (the upper) to the transition-state of GlcNAc-1-phosphate transfer (the lower).

The first glycosyltransferase inhibitor which contains both donor and acceptor moiety is the inhibitor of α-(1-2)-fucosyltransferase developed by Hindsgaul and Palcic in 1985. However, the fucose moiety was just roughly mimicked by a phenyl group.[2]

The biosynthetic pathways of *N*-linked type glycoproteins have been investigated in detail, and the biological functions of these glycochains are better understood than other types of glycoconjugates. In the biosynthetic process, many glycosyltransferases and glycosidases act systematically to build three different types of oligosaccharides, i.e. high mannose, complex, and hybrid types. The activity of each *N*-acetylglucosaminyltransferase (GnTase) shown in Fig. 2 determines the diversity of the oligosaccharides of complex and hybrid type *N*-linked glycoproteins. Among them, GnTase V is the most interesting target for inhibition, because the increased activity of this enzyme causes the production of highly branched oligosaccharides which are related to the high metastatic potential of tumors.[3] Therefore, inhibitors specific for GnTase V could be useful for cancer chemotherapy.

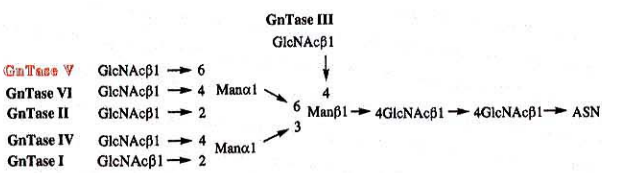

Fig. 2. N-acetylglucosaminyltransferases I-VI.

Before we embarked on the synthesis of *transition-state analogs* of GnTase V, two *monosubstrate analog inhibitors* had already been reported.[4] These are the trisaccharide acceptor analogs in which the nucleophilic hydroxy group of the acceptor was masked. However, the inhibitory activities were not strong enough (*Ki* values were still in the range of 70 μM).

We have therefore designed an inhibitor as shown in Fig. 3 to improve the potency and increase the specificity for GnTase V.

Glycosyltransferase-catalyzed reactions are believed to proceed through a transition-state with a positive charge

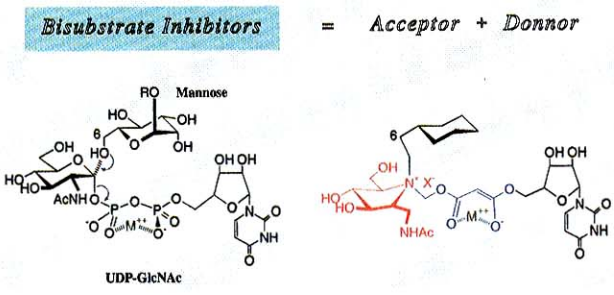

Fig. 3. Transition-state of the reaction catalyzed by GnTase V (left) and the designed inhibitor (right).

on the anomeric center of donor sugars. According to this hypothesis, aza-*N*-acetylglucosamines may replace the *N*-acetylglucosamine part of the transition-state in *N*-acetylglucosaminyltransferase catalyzed reactions, since the positive charge on the nitrogen can mimic the positive charge in the transition state. Two five-membered aza-GlcNAc (**2**, **3**) and a six-membered one (**4**) shown below were prepared from azido aldehydes (**5**, **6**, and **7**) and dihydroxyacetone phosphate by taking advantage of the FDP (fructose-1,6-diphosphate)-aldolase catalyzed reaction (refer the previous review by Dr. Wong).[5] The former (**5**, **6**) and the latter (**7**) aldehydes were respectively prepared from cinnamic aldehyde and acrolein *via* lipase resolution as shown in Fig. 4. It is worthwhile to notice the success in the resolution of amine (**7**) with *Pseudomonas* lipase (Amano P), which represents the first resolution of simple amines by esterases. After the enzymatic aldol reaction, each of the condensed products is dephosphorylated with acid phosphatase followed by hydrogenation on palladium-charcol.

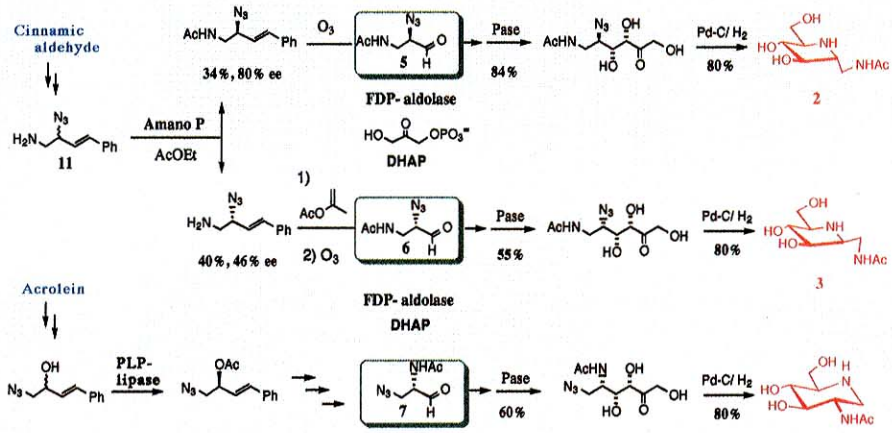

Fig. 4. Synthesis of Aza-*N*-acetylglucosamines.

The conjugation of these azasugars, uridine, and mannose mimic with malonate, which is incorporated into the inhibitor as a mimic of the diphosphate moiety, is now in progress.

There still remains another important problem to be addressed, i.e. the desired conformation of the acceptor saccharide which is recognized by GnTase-V. Two conformationally restricted saccharide analogs (**8**, **9**) of β-D-glucopyranosyl-α-(1-2)-D-mannopyranosyl-β-(1-6)-D-gluco-pyranose, one of the excellent substrates for GnTase V, were synthesized to investigate which conformation of C-5-C-6 bond in the reducing end of glucose is the preferred substrate for the enzyme (Fig. 5).[6]

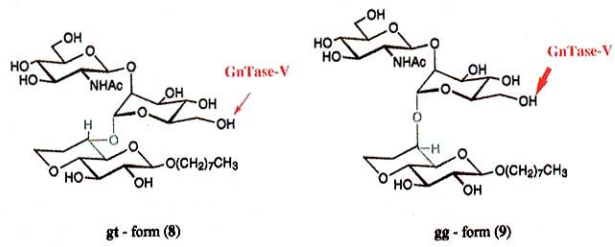

Fig. 5. Two conformationally restricted saccharide analogs as the substrates of GnTase V.

It was concluded that the *gt* rotamer (**12**) was a 50 times better substrate than the *gg* (**13**) rotamer, and a similar result is expected to be true in the case of the natural substrate, β-D-N-acetylglucopyranosaminyl-(1-2)-α-D-mannopyranosyl-(1-6)-β-D-mannopyranose. Hopefully, the transition-state analog inhibitors carrying the mimic of the conformationally rigid acceptor and donor UDP-GlcNAc would be synthesized and could have much higher inhibitory activity than those designed so far. The ultimate goal is to design and synthesize simpler molecules which act as potent and selective inhibitors of glycosyltransferases.

References

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