A Rationally Designed Inhibitor of α-1,3-Galactosyltransferase

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Because cell-surface glycoconjugates are known to play an important role in a variety of cell−cell interactions,[1] inhibitors of the oligosaccharide-synthesizing enzymes, glycosyltransferases, have been actively studied. A number of designed inhibitors have been described: these are analogues of an acceptor molecule (oligosaccharide[2] or peptide),[3] a donor molecule (sugar nucleotide),[4] and a transition-state mimic (mono-[5] or bisubstrate).[6] Although a few successful examples of acceptor and bisubstrate analogues have been reported, they are complex molecules and are difficult to synthesize and unstable due to the incorporated nucleoside diphosphate moiety.

We have chosen α-1,3-galactosyltransferase (α1,3-GalT) as the target enzyme for our inhibitor design[7] because of its biochemical importance in xenotransplant rejection caused by the recipient's anti-Gal antibody reaction to the donor's α-Gal epitope.[8] Since the α1,3-GalT reaction proceeds with overall retention of the configuration of the galactose anomeric position via double displacement, we assumed that a general base group (a carboxylate-containing amino acid residue) could be transiently involved in forming a glycosyl−enzyme complex,[9] as shown in Figure 1, in a manner similar to that of a retaining glycosidase reaction.[10] If this is the case, we hypothesized that a derivative of our newly developed galactose-type 1-*N*-iminosugar **2** would be able to efficiently inhibit the enzyme activity by forming a rigid inhibitor−enzyme complex via a favorable electrostatic interaction (Figure 1), as it does with β-galactosidase (β-Gal'ase).[11] In this paper we describe the rational design of an 1-*N*-iminosugar-based UDP-galactose analogue **1**, which was found to be a selective potent inhibitor of α1,3-GalT but not of β1,4-GalT and shows a significantly reduced inhibitory activity against β-Gal'ase because of its incorporation of a neutral analogue of the UDP moiety.

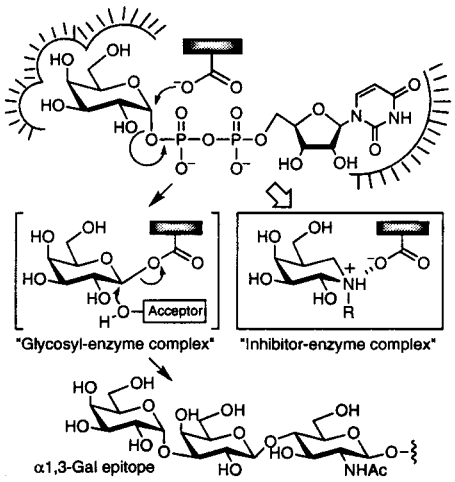

Figure 1. Proposed reaction mechanism of α1,3-GalT and possible interaction between the 1-*N*-iminosugar-based inhibitor and the enzyme.

In a preliminary screening of inhibitors of α1,3-GalT, we confirmed that the iminosugar **2** alone could inhibit the α1,3-GalT reaction with a K_i of 70 μM but did not inhibit β1,4-GalT, whereas deoxygalactonojirimycin inhibited neither of the galactosyltransferases effectively.[12] Although we have identified the 1-*N*-iminosugar **2** as a novel inhibitor of α1,3-GalT, this iminosugar was also a very potent inhibitor of β-Gal'ase, with a K_i of 4 nM.[11] Our objective in the present study was to improve the potency of iminosugar **2** in inhibiting α1,3-GalT and to reduce its inhibitory activity against β-Gal'ase by introducing various groups on its nitrogen atom.

We have divided the UDP-galactose molecule into three segments: a galactose residue (segment A), a pyrophosphate moiety (segment B), and a uridine residue (segment C). Our strategy was to replace these three segments with a galactose-type 1-*N*-iminosugar, a vicinal diol derived from L-tartaric acid, and a 5′-thio-uridine, respectively, to avoid an unnecessary negative charge on the molecule and to increase the stability of the designed inhibitor.

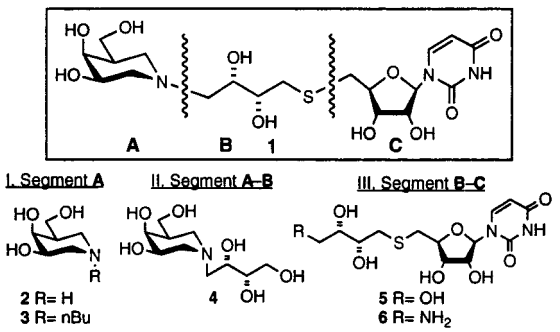

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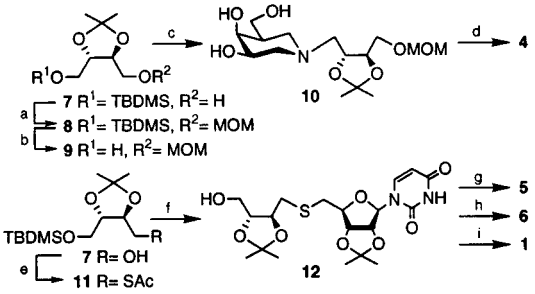
Table 1. Inhibitory Potency of the Compounds against α- and β-Galactosyltransferases and α- and β-Galactosidases

	inhibitory activity (IC_{50}) against			
compd	α-GalTase[a]	β-GalTase[b]	β-Gal'ase[c]	α-Gal'ase[d]
1	7.5 μM[e]	MI[f]	17 μM	20 μM
2	15 μM[g]	NI	10 nM[h]	200 μM
3	45% at 5 mM	NI	5 μM	45 μM
4	70 μM[i]	NI	7 μM	50 μM
5	20% at 5 mM	NI	NI	NI
6	5 mM	NI	NI	NI

[a] Recombinant porcine α-1,3-galactosyltransferase from Calbiochem (San Diego, CA). [b] Bovine β-1,4-galactosyltransferase from Sigma (St. Louis, MO). [c] β-Galactosidase (*Aspergillus oryzae*) from Sigma. [d] α-Galactosidase (coffee beans) from Sigma. [e] $K_{is} = 4.4 \pm 1.3$ μM. [f] No inhibition at 5 mM. [g] $K_{is} = 10 \pm 1$ μM. [h] $K_i = 4$ nm. [j] $K_{is} = 55 \pm 14$ μM.

Scheme 1[a]



[a] Reagents and conditions: (a) MOMCl/iPr_2NEt/CH_2Cl_2; (b) Bu_4NF/THF; (c) (i) Dess−Martin periodinane/CH_2Cl_2, (ii) **2**/BH_3·pyr/MeOH−phosphate buffer, pH 6.8; (d) 1 N HCl; (e) (i) TsCl/pyr, (ii) KSAc/DMF; (f) (i) K_2CO_3/MeOH, (ii) 2′,3′-*O*-isopropylidene-5′-*O*-tosyl-uridine/DMF, (iii) Bu_4NF/THF; (g) CF_3CO_2H; (h) (i) TsCl/pyr, (ii) NaN_3/DMSO, (iii) H_2/Lindlar catalyst/MeOH, (iii) CF_3CO_2H; (i) Dess−Martin oxidation/CH_2Cl_2, (ii) **2**/BH_3·pyr/MeOH−phosphate buffer, pH 6.8, (iii) CF_3CO_2H.

When a simple alkyl group was introduced into **2** (segment A), the inhibitory activity of the resulting *N*-butyl iminosugar **3** against α1,3-GalT was decreased by >500-fold (45% inhibition at 5 mM, Table 1),[13] and that for β-Gal'ase fell by >400-fold ($IC_{50} = 5$ μM). Incorporation of segment B, a triol group (a pyrophosphate-like diol moiety), into **2** was examined with the expectation that the diol moiety might fit into the enzyme binding site to which the pyrophosphate moiety normally binds.

The synthesis was accomplished by reductive amination of **2** with the aldehyde derived from L-tartaric acid (Scheme 1).[14] A TBDMS derivative of L-threitol **7**[15] was converted to an MOM derivative **9**, which was oxidized[16] and reductively aminated with **2** to afford **4**. Compound **4**, with the triol moiety as the pyrophosphate mimetics, was found to restore the inhibitory activity against α1,3-GalT to some extent, with a K_i of 70 μM (Table 1).

Uridine (segment C) was not an inhibitor of α1,3-GalT; therefore, we next prepared a neutral analogue of UDP **5** to see whether this UDP mimetic could inhibit the enzyme activity. For this conjugation we used a sulfur atom (sulfide) in order to confer flexibility upon the linkage beween the diol moiety (segment B) and the uridine residue. The threitol derivative **7** was converted to the thio derivative **11**, which was then coupled[17] with a 5′-*O*-tosyl derivative of uridine[18] to give **5**. The neutral UDP mimetics **5** only weakly inhibited α1,3-GalT activity (20% inhibition at 5 mM).[19] We next introduced an amino group into **5** to determine whether the amino group could interact with the putative general base in the enzyme's active site, and the resulting UDP mimetic was equivalent to **1** except for the fact that it lacked the galactose framework. For this synthesis, the primary OH group of **12** was sequentially converted to an amino group (Scheme 2). The resulting amino compound **6** showed an improved inhibitory potency, with an IC_{50} of 5 mM for α1,3-GalT. This result suggested the presence of a general base (either an Asp or Glu residue) in the active site, a situation which had previously been inferred from the inhibition data obtained for the 1-*N*-iminosugar **2** in our earlier study[12] and others.[7]

Finally, we combined all three segments and synthesized a neutral UDP-galactose analogue **1** (Scheme 2). Oxidation of **12**, followed by reductive amination[20] with the galactose-type 1-*N*-iminosugar **2**, afforded the conjugate **1** after acidic deprotection. This conjugate showed a potent inhibitory activity against α1,3-GalT, with a K_i of 4.4 μM, and a diminished inhibitory potency for β-Gal'ase ($IC_{50} = 17$ μM). Since the β1,4-GalT reaction involves inversion of the stereochemistry of the UDP-Gal, there would be no glycosyl−enzyme complex formation with a nearby carboxylate such as shown in Figure 1, and as we expected, compounds **1**−**6** did not inhibit β1,4-GalT at concentrations up to 5 mM (Table 1). We have also examined the inhibitory potencies of compounds **1**−**6** against α-Gal'ase and found a moderate increase in inhibitory activity ($IC_{50} = 20$ μM for **1**) with increasing size of the substituent on the C-1 amino group (Table 1), a finding that is consistent with previous observations concerning N-modification of those 1-*N*-iminosugars.[11] An analysis of the inhibition kinetics data[21] indicated that, to our surprise, neither **1** nor **2** showed a competitive mode of inhibition against UDP-Gal, even though **1** is a UDP-Gal analogue. Similar observations have been reported in inhibition studies of other glycosyltransferases in which acceptor oligosaccharide analogues were used as inhibitors.[10] The type of inhibition we observed for **1** and **2** suggests that they interact not only with the free enzyme but also with the enzyme−substrate complex.[21]

In conclusion, we have demonstrated the rational design of an inhibitor of α1,3-GalT by introducing an additional neutral UDP analogue into the relatively simple motif of an 1-*N*-iminosugar. The resulting conjugate showed an improvement in the desired specific inhibitory potency against α1,3-GalT and suppressed the unwanted inhibitory activity against the glycosidase. Although we do not yet completely understand the structure−inhibitory activity relationship determined by the stereochemistry of the diol moiety of inhibitor **1**, our approach may indicate a general strategy for designing effective inhibitors of glycosyltransferases. We are currently extending this design strategy to other 1-*N*-iminosugars for potential inhibitor of glycosyltransferases.

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Supporting Information Available: Experimental procedures for the synthesis of **1**, **4**, **5**, and **6** and kinetic data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) In the present study we used a recombinant porcine α-1,3-galactosyltransferase obtained from Calbiochem (San Diego, CA). The inhibition assay was carried out as described in ref 12, except for the use of pH 7.0 and LacNAc as the acceptor.

(14) Synthesis of compounds **1**, **4**, **5**, and **6** is described in Supporting Information.

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