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Reports

Two Galactosyltransferases' Selection of Different Binders from the Same Uridine-Based Dynamic Combinatorial Library

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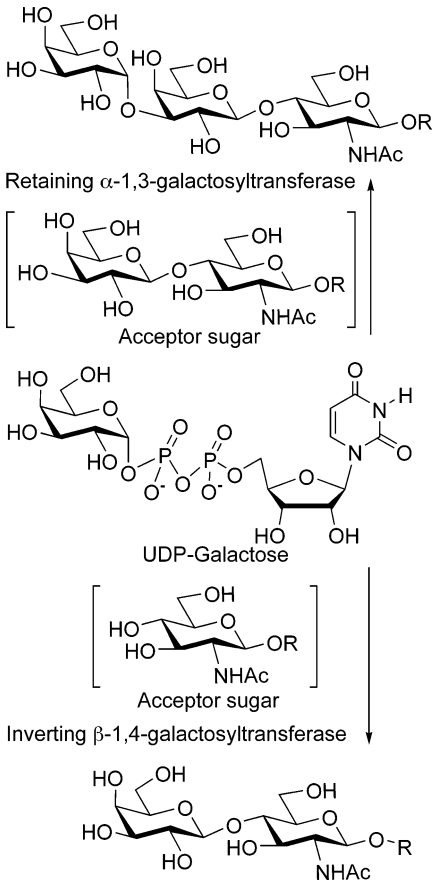
Introduction. An appealing and intrinsic attribute of the recently developed dynamic combinatorial libraries (DCL)[1] is their adaptability. Any changes by molecular recognition events that influence the thermodynamic stability of the members will generate shifts in the equilibria, thus changing the composition of the library. This approach has been used as a tool to identify protein binders or enzyme inhibitors,[2] even when the enzyme has poor binding properties.[3] Furthermore, it does not need any prior knowledge of the enzyme structure or of the precise molecular mechanism of action. Dynamic combinatorial chemistry (DCC) seems, therefore, to be a good choice in the search for glycosyltransferase (GT) binders because most of the 3D structures of GTs are not yet available, making rather difficult the rational design of good binders.[4,5]

Results and Discussion. We report here simple experiments with two galactosyltransferases utilizing this concept. Both enzymes are Leloir-type GTs that catalyze the transfer of a D-galactose residue from the same sugar nucleotide donor (UDP−Gal) to the hydroxyl group of a specific acceptor with retention (the α-1,3-galactosyltransferase enzyme, α1,3GalT) or inversion (the β-1,4-galactosyltransferase enzyme, β1,4GalT) of the anomeric configuration (Scheme 1).[6] The design of GT inhibitors has often been focused on producing donor analogues,[4,7] including, in the case of the galactosyltransferases, the search for selective compounds that target different enzymes.[4c] Very recent synthetic work has also provided access to hybrid compounds linking uridine and sugar derivatives.[7b,8] We report preliminary results showing that the two enzymes utilizing the same

Scheme 1. D-Galactose Transfer onto the Acceptor Sugars by the Two Galactosyltransferases.



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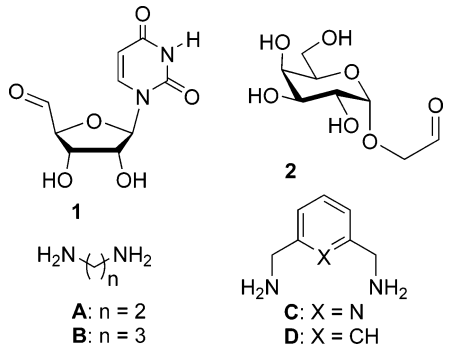

Figure 1. Structure of the building blocks for a DCL designed to generate possible UDP−galactose mimics.

substrate are able to select different binders when they are exposed to the same dynamic combinatorial library based on starting building blocks intended to self-assemble in members mimicking UDP−Gal.

We designed a DCL using the generation of imines to introduce diversity whereby uridinal **1** was used as a scaffold for two reasons (Figure 1):[9] First, this motif would hopefully direct members of the library toward the active site because it has been shown in several instances that an important part of the binding energy of GTs to their donor substrates lies in the nucleoside moiety.[4,10] Second, the uracyl portion would allow an easy HPLC-UV detection of the different library products. The distribution of the imine DCL members was "fixed" by a reductive step with sodium cyanoborohydride,[2a] leading to a stable library of the amine products that was analyzed by HPLC.

The following simple mixture of starting building blocks was thus set up: uridinal **1**, prepared via the corresponding aminal according to Moffatt's conditions,[11] and aldehyde **2**, derived from D-galactose following Lee's procedure (allyl glycoside formation, ozonolysis, and reduction) (Figure 1).[12] All amines were commercially available except for amine **C**, which was prepared according to a known procedure.[13] A mixture of amines **A**−**D** (540 or 600 μM each) was equilibrated for 9 or 13 days with aldehydes **1** and **2** (80 μM each) at room temperature in two different experiments: in either a 17 mM Tris-acetate buffer (pH 7.9), 6.8 mM $MgCl_2$ containing 34% glycerol (conditions for the α1,3GalT) or in a 32 mM MOPS buffer (pH 7.4), 12.8 mM $MgCl_2$ (conditions for the β1,4GalT).[14] After the required incubation time in the presence of sodium cyanoborohydride, the distribution of the reduced products was analyzed by reversed-phase HPLC. Five members of the library (**A1**, **B1**, **C1**, **C2**, and **D1**) are shown in Figure 2a (experiments α and β).[15] Equilibration of the same library in the presence of the α1,3GalT (1.6 μM, experiment α, Figure 2c) or the β1,4GalT (16 μM, experiment β, Figure 2c) induced a notable change in the distribution of the amines with selected enrichments in amines *depending on the nature of the enzyme*.[16] Hence, the α1,3GalT enzyme triggered amplification of these five members with the enrichment of mostly amine reduction products **C1** and **D1** (7- to 10-fold amplification), whereas the β1,4GalT gave rise to modest amplifications and only amines **A1**/**B1** (1.7-fold amplification) and, to a lesser extent, amine **C2**. Without the help of HPLC/MS analysis, amplification of the active components may become difficult to evaluate if a strong overlap occurs (see, for instance, amines **A1** and **B1** in Figure 2). Assembly and analysis of sublibraries is a fast solution to circumvent such a difficulty. In the case of β1,4GalT, for example, two sublibraries containing the same components except for diamine **A** or **B** were easily constructed and tested. No amplification was detected in the **A1**/**B1** peak area of the chromatogram in the presence of the enzyme in the sublibrary containing 1,2-diaminopropane **B**, whereas a clear amplification in this area was seen in the sublibrary containing 1,2-diaminoethane **A** (results not shown). This clearly indicates that the enzyme selected the imine precursor of **A1** rather than the one leading to amine **B1**.

To verify these amplification effects resulting from specific interactions with both templates, the two enzymes were replaced by bovine serum albumine (BSA, Figure 2b, experiments α and β). These experiments afforded results very close to those without the enzymes (compare with Figure 2a, experiments α and β), indicating that the DCLs selectively sense both target enzymes. Incubations were also performed in the presence of UDP (80 μM), a good competitive inhibitor of both enzymes. As shown in Figure 2d, the addition of UDP did not influence the distribution of the "fixed" library products, suggesting that the imines derived from the nucleoside motif may not bind to the active site. This result is surprising, since the building blocks of the DCL were chosen for their similarity to the donor sugar. The selected members may preferably sense other binding sites at the surface of the enzymes that are possibly unknown regulating sites. These results correlate literature antecedents reporting that α1,3GalT inhibitors based on the sugar-nucleotide structure are remarkably noncompetitive against UDP−Gal.[4a,b,d]

Amplification of the preferred components by the enzymes may result from both thermodynamic and kinetic control. Moreover, the amines are the final stabilized library members only produced for analytical purposes that may not maintain the binding properties of the transient imine components. This was readily seen in preliminary inhibition tests carried out against both enzymes using an assay with radiolabeled UDP−Gal as donor and *N*-acetyllactosamine as acceptor. In this assay with the α1,3GalT enzyme, the K_m value of UDP−Gal was 75 μM, and an IC_{50} value of 40 μM was determined for UDP. The amines **A1**−**D1** (Figure 2) were synthesized separately and yielded IC_{50} values superior or equal to 5 mM. The analogous amide **3**, "mimic" of the imine leading to amine **B1** (a nonamplified component) showed an IC_{50} value of 5 mM, whereas amide **4**, "mimic" of the imine leading to amine **C1** (a 7-fold amplified component) exhibited an IC_{50} value of 0.4 mM. These results suggest that, in our system, the amines have lost the binding properties of the imines that are best mimicked by the corresponding neutral amides.

The same amide **4** did not bind to the β1,4GalT (IC_{50} value superior to 5 mM). This also correlates with the selection process induced by this enzyme that did not exhibit any tendency to pick out the aromatic amines **C** and **D** (see

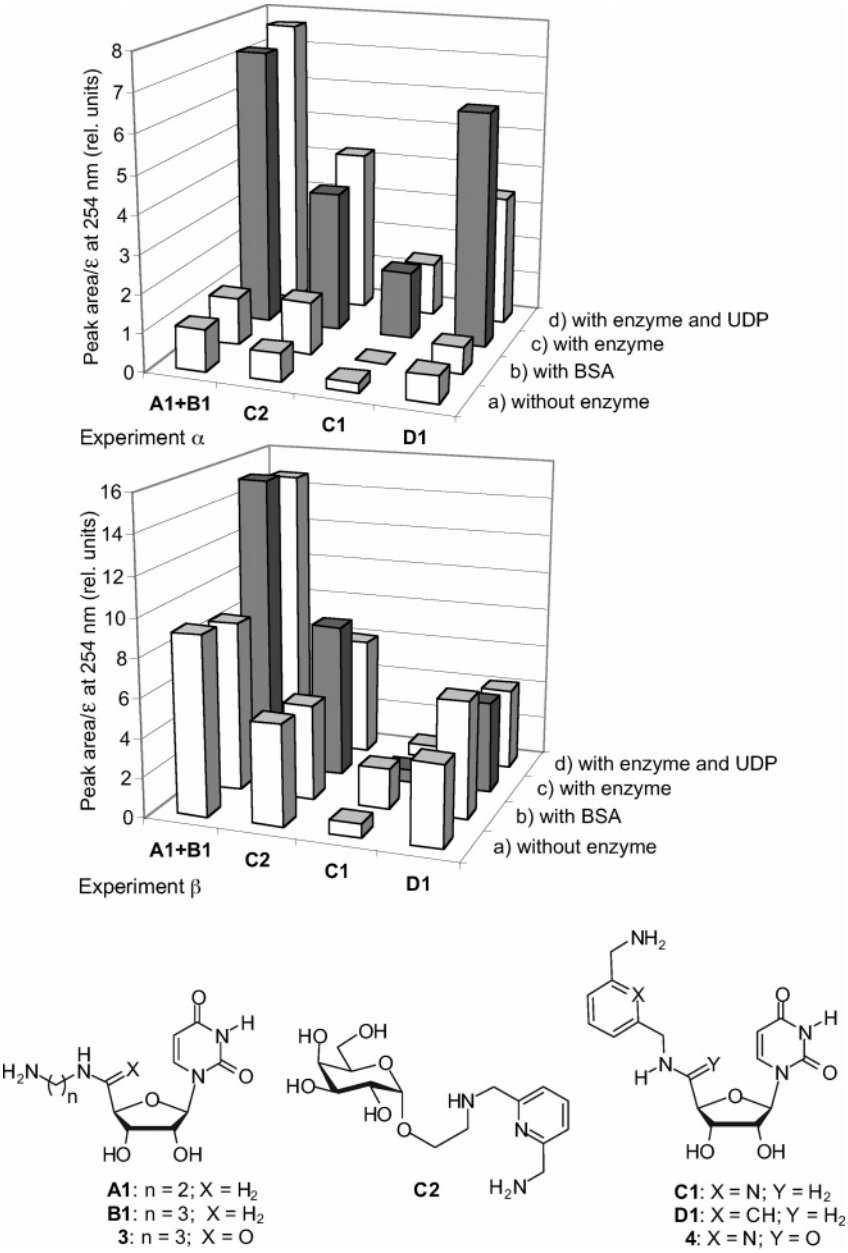

Figure 2. Library components of the "fixed" DCL made from aldehydes **1** and **2** with diamines **A**−**D**. Experiment α, with the α1,3GalT enzyme over 13 days; experiment *β*, with the *β*1,4GalT enzyme over 9 days for both sets of experiments (a) in the absence of the enzyme, (b) in the presence of BSA, (c) in the presence of the enzyme, and (d) in the presence of the enzyme and UDP. The sequence order of the bars corresponds to the elution order of the HPLC chromatograms. Compounds are numbered according to the amine (**A**, **B**, ...) followed by the aldehyde number (**1** or **2**).

Figure 2c, experiment *β*). Other constructs based on the reported findings are currently being elaborated and tested.[17]

The work presented here demonstrates the potential of DCLs to search for selective binders to enzymes using the same substrate. Despite the simplicity of the DCL composition, this adaptive system is able to differentiate the two enzymes and identify very simple binders that may serve as starting points for the elaboration of selective inhibitors with novel motifs.

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Supporting Information Available. Experimental and analytical chromatography procedures, assay procedures, preparation, and spectral data of diamine **C** and amide **3**. This information is available free of charge via the Internet at http://pubs.acs.org.

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(14) We have previously checked that each enzyme was still active after 9 or 13 days only under these buffer conditions, and for the study with the DCL members, we have kept those conditions (buffer, pH) that are optimal for the activity of each enzyme.

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(16) Although the α1,3GalT was able to induce amplifications with an unusually small concentration (1.6 μM), 10 times more of the β1,4GalT (16 μM) was needed to detect amplifications.

(17) Setup of the DCLs: Recombinant bovine α1,3GalT was purchased from Calbiochem. A solution of bovine β1,4GalT (Calbiochem) was prepared by dissolving the lyophilized recombinant enzyme (0.54 mg) in 500 μL of MOPS buffer (50 mM, pH 7.4) containing $MnCl_2$ (20 mM). Stock solutions of amines **A**−**D** (10 mM each), aldehydes **1** and **2** (hydrated form, 2 mM each), and sodium cyanoborohydride (50 mM) were prepared in distilled water. Conditions for the α1,3GalT enzyme: Solutions of amines **A**−**D** (12 μL), aldehydes **1** and **2** (12 μL, and sodium cyanoborohydride (7 μL) were introduced into an Eppendorf tube containing a solution of α1,3GalT in a Tris-acetate buffer (25 mM, pH 7.9), $MgCl_2$ (10 mM,) and 50% v/v of glycerol (200 μL). A control experiment was performed in a Tris-acetate buffer (25 mM, pH 7.9), $MgCl_2$ (10 mM), and 50% v/v of glycerol without the enzyme. The resulting mixtures (295 μL) were equilibrated at ambient temperature for 13 days. Aliquots (20 μL) were removed and diluted with acetic acid (10 μL). Conditions for the β1,4GalT enzyme: Solutions of amines **A**−**D** (6 μL), aldehydes **1** and **2** (4 μL), and sodium cyanoborohydride (2 μL) were introduced into an Eppendorf tube containing a solution of β1,4GalT in a MOPS buffer (64 μL) and water (2 μL). A control experiment was performed in a MOPS buffer (50 mM, pH 7.4), $MnCl_2$ (20 mM) without the enzyme. The resulting mixtures (100 μL) were equilibrated at ambient temperature for 9 days. Aliquots (20 μL) were removed and diluted with acetic acid (10 μL).

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