Glycosyltransferases (GTs) of the Leloir pathway (1) catalyze the transfer of a monosaccharide unit from an activated nucleotide sugar donor to the hydroxyl group of an acceptor with complete regio- and stereoselectivity. The molecules to which GTs transfer monosaccharide units include oligosaccharides, proteins or lipids. GTs are involved in several metabolic pathways and modulation of their activities by efficient inhibitors has potential for the control of certain cellular functions (2). Compounds that can modulate the biosynthesis of glycoconjugates are of great interest as novel therapeutic agents. They may also find applications in the study of biological pathways and can be valuable tools in the preparative stereoselective synthesis of oligosaccharides (3, 4).

Different strategies have been used to design potent inhibitors of GTs (5-8). Identification of potent inhibitors has been developing very rapidly during the last two decades since the 3D structures of several GTs were found (9-11) and catalytic mechanism proposed (12). The use of computer-aided structure-based approach have been very useful for the optimization and de novo discovering inhibitors of GTs.

Most GTs utilize donors containing uridine pyrophosphate leaving group (UDP). N-acetylglucosaminyltransferase I (GnT I) (10) and β-1,4-galactosyltransferase I (β4GalT I) (13) are typical examples. They belong to the GT-A superfamily and employ a DxD motif, which is present on the N-terminal domain, to bind a divalent metal, most commonly Mn⁺ cation. The metal ion is essential for catalysis since it interacts with the pyrophosphate group of the UDP-sugar donor in the enzyme active site. A variety of pyrophosphate analogues that can mimic pyrophosphate-metal interactions have been developed. It has been reported that monosaccharide units might act as pyrophosphate-metal ion complex mimetics (14-16). It is likely that complexation with Mn⁺ is feasible with two hydroxyl group of the sugar.

Recently, we have synthesized 2-deoxy-hexopyranosyl derivatives of uridine as donor substrate analogues of glucosyltransferases and galactosyltransferases (Figure 1) (17). Compounds A–E are composed of uridine and one or two residues of 2-deoxy-hexopyranose and can be synthesized in a totally stereoselective manner using the Falck-Mioskowski protocol (18). In our study the central 2-deoxy-α-D-glucopyranose moiety replacing the key pyrophosphate unit can be linked with terminal 2-deoxygalactosyl moiety through α-(1→3)-, α-(1→4)- and α-(1→6)-linked glycosidic linkages.

The stereoselective synthesis of these compounds requires several steps and purification after each step by column chromatography. The synthesized compounds A–E possessed isopropylidene block as a protection of two hydroxyl groups on ribose part. There is a worry that such compounds

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INTERACTIONS BETWEEN GLYCOSYLTRANSFERASES AND 2-DEOXY GLYCOSYL DERIVATIVES OF URIDINE SIMULATED BY MOLECULAR DOCKING

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Abstract: The focus of the present work was to investigate interactions between 2-deoxy sugar derivatives of uridine and active sites of N-acetylglucosaminyltransferase I and β-1,4-galactosyltransferase I. The ligand-protein interactions were simulated with a docking software (GOLD). The results suggest that the synthesized compounds bind the enzyme by a similar mode as the natural substrate.

Keywords: inhibitors of glycosyltransferases, molecular docking, structure-based drug design

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Figure 1. 2-Deoxy-O-glycosyl rings as pyrophosphate linkage mimetics.

Figure 2. Set of ligands for docking simulations and selected ligands for synthesis.
will not fit into the active site of the protein because two polar domains are excluded. Removal of iso-proplidene groups can be performed in the presence of trifluoroacetic acid or acetic acid. Unfortunately, yield of deprotection was only 25-30% due to small stability of 2-deoxyglycosidic linkage under acidic conditions. Therefore, as a rational design of potent inhibitors of GTs this report focused on comparison studies by molecular docking of two set of ligands (Figure 2): set A containing totally deprotected derivatives and set B containing structures that can be synthesized according to the described procedure (17).

The proposed structures are combinations of several 2-deoxy-hexopyranosyl moieties, connected with uridine. The main objective was to investigate interactions between 2-deoxy sugar derivatives of uridine and active sites of GnT I and β4GalT I. The structures with the best affinity will be synthesized and subjected to biological studies.

**Methodology**

X-ray structure of GnT I complexed with UDP-GlcNAC (PDB 1FOA) (10) and β4GalT I (PDB 1O0R) (13), in which all the active site amino acid residues were resolved, were chosen for docking purpose. Before docking simulations ligands were removed from the active sites. Hydrogen atoms were added to protein, all the water molecules present in protein were removed and proper protonation states were assigned for acidic and basic residues of protein using molecular graphics pro-

![Figure 3. a/ The binding interactions of UDP-GlcNAC, b/ the putative interactions of 2-deoxy-α-D-galactopyranosyl(1→6)-2-deoxy-α-D-glucopyranosyl(1→5)-uridine (9) in the active site of GnT I.](image-url)
gram – the Chimera software (UCSF Chimera, for detailed information, see http://www.cgl.ucsf.edu/chimera). The Mn\(^{2+}\) ion was placed in the active sites of each protein considering octahedral geometry of this ion.

Possible geometries of analyzed ligands 1-28 were found using MOPAC’s AM1 quantum semi-empirical method implemented in Chem3D system.

Docking simulations were performed using GOLD 3.2 software (19), which does flexible docking using a genetic algorithm. A 10 Å radius active site was defined considering the points: (1.49, 18.82, 11.01) and (10.70, 25.79, 33.14) as the center of the active site for GnT I and β4GalT I, respectively. All docking runs were carried out using standard default settings with a population size of 100, a maximum of 100 000 operations, number of islands as 5, a niche size of 2, and a mutation and crossover rate of 95. Ten orientations of the highest score for each compound were then selected using the score function GoldScore. The resulted top scored orientations of docked ligands 1-28 were inspected visually. Visual inspection of the structures was performed with GoldMine 1.0.

RESULTS AND DISCUSSION

A small but diverse library containing 28 compounds was assembled (Figure 2). Structures in the set possess common uridine motif and two moieties

![Diagram](image-url)
Table 1. H-bond interactions and the active site amino acid residues interacting with highly scored ligands.

Docking into the active site of GnT I (PDB 1FOA)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ligand</th>
<th>Ranking (^b)</th>
<th>Hbound count</th>
<th>His190 (Å) (^d)</th>
<th>Asp144 (Å) (^d)</th>
<th>Other interacting amino acid residues</th>
<th>Distance to Mn (Å)</th>
<th>Goldscore Fitness</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>2.4 O2</td>
<td>2.5 N3</td>
<td>Val321, Asp212, Ala114, Cys115</td>
<td>2.3 O4(^+) 2.1 O6(^+)</td>
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</tr>
<tr>
<td>2</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>3.0 O2</td>
<td>-</td>
<td>Ile113(^a), Asp212, Arg117, Gly317, Arg295, Asp291, Leu269</td>
<td>1.9 O3(^+)</td>
<td>65.18</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>2.6 O2</td>
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<td>N3 Ile113(^a), Ala114, Arg117, Gly320(^a), Arg318(^a)</td>
<td>2.1 O3(^+)</td>
<td>64.97</td>
</tr>
<tr>
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<td>9</td>
<td>11</td>
<td>7</td>
<td>2.9 O2</td>
<td>-</td>
<td>Asp212, Asp212, Arg117, Asp213, Asp291, Asp291</td>
<td>2.0 O3(^+) 2.1 O4(^+)</td>
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</tr>
<tr>
<td>5</td>
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<td>12</td>
<td>5</td>
<td>3.0 O2</td>
<td>-</td>
<td>Asp212, Asp212, Gly317, Asp291</td>
<td>1.6 O3(^+) 2.9 O4(^+)</td>
<td>60.69</td>
</tr>
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</table>

Docking into the active site of b4GalT I (PDB 1O0R)

<table>
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<tr>
<th>Entry</th>
<th>Ligand</th>
<th>Ranking (^c)</th>
<th>Hbound count</th>
<th>Arg189(^a) (Å) (^d)</th>
<th>Arg189(^a) (Å) (^d)</th>
<th>Other interacting amino acid residues</th>
<th>Distance to Mn (Å)</th>
<th>Goldscore Fitness</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>6</td>
<td>1</td>
<td>7</td>
<td>2.7 O2</td>
<td>2.4 N3</td>
<td>Pro187(^a), Tyr289, Glu317, Gly316(^a), Asp318,</td>
<td>2.2 O3(^+)</td>
<td>65.93</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>2</td>
<td>8</td>
<td>2.8 O2</td>
<td>2.7 N3</td>
<td>Pro187(^a), Tyr289, Val253(^a), Asp252, Gly315(^a), Glu317,</td>
<td>2.0 O3(^+)</td>
<td>63.54</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>6</td>
<td>7</td>
<td>2.7 O2</td>
<td>2.3 N3</td>
<td>Pro187(^a), Asp252, Lys279, Tyr289, Asp118</td>
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<td>61.22</td>
</tr>
<tr>
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<td>8</td>
<td>8</td>
<td>2.6 O2</td>
<td>1.9 N3</td>
<td>Asn353, Gly315(^a), Asn353, Asn318, Tyr289, Trp314</td>
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</tr>
<tr>
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<td>13</td>
<td>9</td>
<td>9</td>
<td>2.4 O2</td>
<td>2.4 N3</td>
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<td>2.5 O6(^+)</td>
<td>60.29</td>
</tr>
</tbody>
</table>

\(^a\) backbone; \(^b\) from total 256 solutions; \(^c\) from total 248 solutions; \(^d\) hydrogen bond as distance between donor and acceptor.
of 2-deoxy sugar connected by α-(1→3), α-(1→4) or α-(1→6)-linked glycosidic linkages. The uridine fragment is supposed to ensure reasonable interactions with enzyme, similar to that of the natural substrate.

As a first macromolecular object rabbit GnT I was chosen. It transfers the first N-acetylgalacosamine (GlcNAc) residue onto the oligomannose core, all other enzymes in the hybrid and complex pathway depend on its prior action (20). This enzyme plays a fundamental role in mammalian development. In Figure 3a key residues of GnT I that interact with natural substrate UDP-GlcNAc described by Unlilig et al. (10) are shown. Manganese ion interacts only with Asp213 and two oxygen atom of pyrophosphate moiety.

β4GalT I was the second target. Human β4GalT I plays a role in the biosynthesis of blood groups antigens and is involved in some pathologies such as arthritis and cancer (21). In Figure 4a key residues of b4GalT I that interact with natural substrate UDP-Gal are shown (13).

In the first experiment, all ligands 1-28 were docked into the active site of GnT I and the bound conformations inside the active site of GnT I were visually examined. From the obtained complexes only those with proper ligand binding mode of uridine moiety were selected for further analysis. The most important criterion was the possibility of an interaction between:

- uracil nitrogen (N3) of the ligand and Asp144 in the enzyme active site,
- uracil oxygen (O2) of the ligand and His190 in the enzyme active site,

To further validate these models, binding energies of each docked ligand were evaluated. Goldscore fitness function is composed of four components: protein-ligand hydrogen bond energy, protein-ligand van der Waals energy, ligand internal energy. The fitness score is taken as the negative of the sum of the components energy terms, so that the larger fitness scores are better. The 30 highest scores from 248 solutions were between 56.82 and 65.93. None of ligands from set B was correctly docked in this range.

Similarly to solutions in the first experiment, the highest scored ligands possess α-(1-6) glycosidic linkage between two 2-deoxy sugar rings (6, 9, 12) or α-(1-4) glycosidic linkage as in the case of ligand 8.

In the second experiment, ligands 1-28 were docked into the active site of b4GalT I. Only ligands that formed interactions between the uracil heteroatoms and backbone oxygen and nitrogen of Arg189 were taken into considerations. The 30 highest scores from 248 solutions were between 56.82 and 65.93. None of ligands from set B was correctly docked in this range.

Visual analysis of the simulated binding modes of ligands 1-28 into active sites of Gnt I and β4GalT I suggests that two hydroxyl groups 2-OH and 3-OH in the ribose moiety were crucial for interactions for both targets. Only the ligands from set A could bind the enzymes active site by a similar mode as its natural substrate. The ligands from set B most often were docked “tail to head”. Currently, five ligands: 6, 7, 9, 12 and 13 are being synthesized and biolog-
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critical evaluations toward commercially available bovine milk b4GalT I will be carried out soon.

REFERENCES