**Glycosidases**

**Multivalency To Inhibit and Discriminate Hexosaminidases**

Dimitri Alvarez-Dorta, Dustin T. King, Thibaut Legigan, Daisuke Ide, Isao Adachi, David Deniaud, Jérôme Désiré, Atushi Kato, David Vocadlo, Sébastien G. Gouin, and Yves Blériot

Dedicated to Professor Charles Tellier on the occasion of his retirement

**Abstract:** A set of multivalent polyhydroxylated acetamidozepanes based on ethylene glycol, glucoside, or cyclo-dextrin scaffolds was prepared. The compounds were assessed against plant, mammalian, and therapeutically relevant hexosaminidases. Multimerization was shown to improve the inhibitory potency with synergy, and to fine tune the selectivity profile between related hexosaminidases.

**Introduction**

exo-N-Acetyl-β-glucosaminidases are found in diverse organisms ranging from bacteria to humans. These enzymes catalyze the removal of terminal N-acetylglucosamine residues (GlcNAc) from a wide range of glycoconjugates and saccharides. Humans express three exo-N-acetyl-β-glucosaminidases, namely the isoenzymes β-hexosaminidase A (HexA) and β-hexosaminidase B (HexB), as well as O-GlcNAcase (OGA), and considerable attention has been given to these enzymes because of their involvement in various diseases. HexA and HexB share high sequence similarity and belong to glycoside hydrolase (GH) family 20 (GH20) of the CAzy classification system. Heritable deficiencies in HexA activity cause GM2-ganglioside to accumulate in the nervous system, resulting in lethal neurodegenerative disorders known as Tay-Sachs and Sandhoff diseases.

O-GlcNAcase (OGA) belongs to GH family 84 (GH84) and removes O-GlcNAc residues from particular hydroxyls of serine and threonine residues of nuclear and cytoplasmic proteins. O-GlcNAc has been implicated in a range of cellular processes and inhibitors of OGA have been proposed as a potential therapeutic strategy to treat neurodegenerative diseases. Although of nonhuman origin, NagZ, a fourth functionally related exo-N-acetyl-β-glucosaminidase from family 3 (GH3), has also been implicated in human diseases. NagZ is involved in the highly conserved Gram-negative peptidoglycan cell wall recycling pathway. NagZ releases GlcNAc from the cytosolic GlcNAc-1,6-anhydroMurNAcpeptidoglycan recycling intermediates to yield 1,6-anhydroMurNAcpeptides that act as signalling molecules to promote resistance of Gram-negative bacteria to a wide range of β-lactam antibiotics. Noteworthy GH3 enzymes, including NagZ, use a catalytic mechanism that differs from that used by GH20 and GH84 enzymes, which use substrate-assisted catalysis. Furthermore, significant differences in the active site structures of all of these enzymes have been noted. These various differences have enabled the generation of selective inhibitors of each enzyme. Accordingly, specific inhibitors have been designed, including potent cyclic derivatives such as Thiamet-G that mimic the oxazolinium-like transition state of the substrate-assisted mechanism. Similarly, modification of the acetamido group has resulted in potent hexosaminidase inhibitors derived from PUGNAc, nagstatin, and DNJNAc, and these have also been reported to yield selective NagZ inhibitors (Figure 1). Furthermore, the N-alkylation of DNJNAc with elaborated pharmacophores have afforded potent HexA and B (HexAB) inhibitors. Other structural modifications on GlcNAc mimics have been recently explored to target hexosaminidases.

An alternative promising approach has recently emerged for developing potent and selective inhibitors of glycosidases and glycosyltransferases. Carbohydrate-binding proteins, lectins,
are generally multimeric and interact in a multivalent manner with their sugar ligands, which enables high avidity despite their generally weak affinity for monomeric ligands. This highly synergistic multivalent effect inspired the development of synthetic glyoclusters bearing multiple copies of sugar epitopes on a single scaffold, leading to affinity enhancements of several orders of magnitude over the corresponding monovalent binding interaction. Although this so-called “glyocluster effect” was coined more than twenty years ago, this concept only progressed recently from carbohydrate-binding lectins to carbohydrate-processing enzymes. In 2009, conducting a systematic evaluation of multivalent iminosugars based on the deoxymannojirimycin (DMJ) moiety against commercial glycosidases, we observed a significant multivalent effect on the α-mannosidase from jack bean (JbMan). Since then, higher avidities have been reached by using multivalent DNJ constructs with higher valency, and much effort was dedicated to unravel the JbMan binding mechanism. The concept was then extended to other targets, including biologically relevant classes of glycosidases and glycosyltransferases. Interestingly, the initial ligand specificity of lectin and glycosidases may also fade out with multivalency, as recently probed with multivalent constructs based on carbohydrates and iminosugars that bind/inhibit the mismatching proteins. Here, we assess the potential sensitivity of various hexosaminidases of biological interest to the effect of multivalent inhibitor clusters.

Results and Discussion

We have contributed to the development of a new class of seven-membered iminosugars, the polyhydroxylated azepanes, that inhibit glycosidases in a competitive manner. Introduction of a NHAc group on the azepane ring as in AzeNaC 5 led to potent and broadly effective inhibitors of hexosaminidases, including OGA and NagZ (Figure 1). AzeNaC 5 was exploited here to synthesize a set of multivalent iminosugar clusters with varied valencies that are based on the trihydroxylated acetamidoazepane moiety. Copper-catalyzed azide alkyne cyclization (CuAAC) was used as a robust methodology to construct the multivalent entities. The azido-functionalized azepane 6 was first designed (Scheme 1) as a protected epitope to be grafted onto alkynyl-armed scaffolds.

Starting from acetamido azepane 8, which is available in five steps from known azidolactol 7, N-alkylation with 1-azido-9-bromononane in EtOAc/H2O in the presence of K2CO3 furnished azepane 10 in 85% yield. For ease of deprotection, this derivative was then debenzylated using BCl3 in CH2Cl2 at −78 °C and subsequently per-O-acetylated (Ac2O, pyr) to produce azepane 6. The CuAAC protocol was first implemented with 11 and propargyl alcohol 11 to form protected cycloaduct 15 with 90% yield (Scheme 1). This protocol was successfully repeated with previously described alkyne-derived ethylene glycol 12, methyl glucoside 13, and γ-cyclodextrin 14 to form the corresponding di-, tetra-, and octavalent cycloaducts 16–18. Acetates were removed under Zemplén conditions to furnish monovalent 19 alongside multivalent iminosugar clusters 20–22 in quantitative yields.

In preliminary screening, compounds 19–22 were assayed as inhibitors of three hexosaminidases isolated from jack bean, bovine kidney, and HL60 (Table 1). The relative potency (Rp) of the multivalent derivatives can be obtained by dividing the measured IC50 values by the value obtained for monovalent reference 19. Dividing the Rp by the valency (n) of the cluster enables one to estimate if the enhancements in binding are truly synergistic or only statistical. A true multivalent effect is

Multivalent iminosugars 20–22 showed low, moderate, and strong multivalent effects against NagZ, hOGA, and HexAB, respectively. The most significant effect was obtained with HexAB with Rp/n values of 131, 32, and 75 observed for compounds 20, 21, and 22 with increasing valency of 2, 4, and 8, respectively. Previously, several studies have found that higher valency does not necessarily correlate with improved multivalent binding avidity for targeted carbohydrate-binding or carbohydrate-processing proteins. Inhibitory activities of PUGNAc and iminosugars multivalency can be used to discriminate between related hexosaminidases.

Table 1. Inhibitory activities of 19–22 against plant and mammalian hexosaminidases.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>Val.</th>
<th>IC_{50} [μM] Hexosaminidases (Rp/n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>0.24 (–)</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>0.14 (15)</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>0.43 (2.4)</td>
</tr>
<tr>
<td>22</td>
<td>8</td>
<td>0.32 (1.6)</td>
</tr>
</tbody>
</table>

Table 2. Inhibitory activities of PUGNAc and iminosugars 19–22 against relevant human and bacterial hexosaminidases.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>Val.</th>
<th>IC_{50} [μM] Hexosaminidases (Rp/n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>1</td>
<td>0.24 (–)</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>0.14 (15)</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>0.43 (2.4)</td>
</tr>
<tr>
<td>22</td>
<td>8</td>
<td>0.32 (1.6)</td>
</tr>
</tbody>
</table>

In conclusion, we developed a set of multivalent polyhydroxylated acetamidoazepane clusters based on hydrophilic and biocompatible scaffolds. Multivalent inhibitory effects were observed for the first time on plant, mammalian, and therapeutical...
cally relevant hexosaminidase targets. The strategy proved effective in designing nanomolar inhibitors of HexAB with a high selectivity profile and without the need of intensive structure–activity relationship studies. These results further expand the scope of multivalent iminosugars able to interfere with glycosidase activity.

Acknowledgements

This work was carried out with financial support from the Centre National de la Recherche Scientifique (CNRS), the Ministère de l’Enseignement Supérieur et de la Recherche in France.

Conflict of interest

The authors declare no conflict of interest.

Keywords: exo-N-acetyl-β-glucosaminidases · glyoclusters · glycosidases · iminosugars · multivalency


Manuscript received: April 19, 2017
Accepted manuscript online: May 26, 2017
Version of record online: June 20, 2017