Piperidine homoazasugars: natural occurrence, synthetic aspects and biological activity study

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Dedicated to Dr. A. V. Rama Rao on the occasion of his 70th birthday
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Abstract
A number of natural and synthetic analogues of homoazasugars, known in the literature, are promising glycosidase inhibitors. The methodologies used for the synthesis of piperidine homoazasugars are: (i) intramolecular reductive amination, (ii) intermolecular double reductive amination, (iii) amino/amido mercuration, (iv) intramolecular nucleophilic substitution, (v) synthesis from non-carbohydrate building block and aza-heterocycles and (vi) enzyme catalyzed intramolecular reductive amination. Homoazasugars showed higher selectivity and potency in the glycosidase inhibitory activity. In this report, natural occurrence, synthetic methodologies and potential application to glycosidase inhibitory activity of homoazasugars will be reviewed.

Keywords: Piperidine, azasugar, homoazasugar, glycosidase inhibitor

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1. Introduction

The search for selective and effective inhibitors of oligosaccharide processing enzymes has promoted intense research over the last 20 years in the synthesis of stereochemically well-defined polyhydroxylated piperidines. This class of compounds, commonly called as azasugars or iminosugars, are known to be endowed with a remarkable therapeutic potential in the treatment of diabetes, viral infections (including HIV) and tumor metastasis\textsuperscript{1b} due to their action as glycosidase inhibitors.\textsuperscript{1} The polyhydroxylated piperidine namely nojirimycin (NJ 1, Figure 1) was first isolated from a fermentation broth of \textit{Streptomyces roseochromogenes} R-468.\textsuperscript{2a} The more stable form of NJ is the 1-deoxynojirimycin (DNJ, 2), also called as moranoline, was first prepared\textsuperscript{2b} by catalytic hydrogenation of NJ and later on isolated from different species of the plant \textit{Morus}.\textsuperscript{2c}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{Figure 1}
\end{figure}

The search for promising glycosidase inhibitors led to the discovery of homoazasugars. In general, the homoazasugars are classified into two categories. In the first type, hydroxymethyl group/polyhydroxylated carbon chain is present at both the carbons (C-1 and C-5) adjacent to the ring nitrogen (type I, Figure 2) and is also known as aza-C-glycoside. While, in other the type carbon homologation is present at C-6 of the piperidine (type II).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{Figure 2}
\end{figure}

The homoazasugars are found to be more stable towards chemical and enzymatic degradation than azasugars, while retaining the powerful biological activity of the parent azasugars, the homoazasugars having substituents recognizing the aglycon-binding site of the enzyme are expected to further increase the selectivity. Due to their higher selectivity and potency in the glycosidase inhibitory activity, the homoazasugars are now gaining their own independent identity. A brief review of natural occurrence, synthetic aspects and biological activity of piperidine homoazasugars is described in this article.
2. Natural occurrence

Homoazasugars are natural alkaloids widely diffused in plants and microorganisms. Interestingly, $\alpha$-homonojirimycin ($\alpha$-HNJ 3, Figure 3) was first synthesized in the protected form just before its isolation in 1988. The naturally occurring homoazasugars are listed in Figure 3 and their natural sources are summarized in Table 1.

![Structures of naturally occurring piperidine homoazasugars.](image)

**Figure 3.** Structures of naturally occurring piperidine homoazasugars.

**Table 1.** Polyhydroxylated piperidine homoazasugars isolated from natural sources

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Chemical/Common name</th>
<th>Plant/Organism name</th>
<th>Plant part</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>$\alpha$-homonojirimycin (3)</td>
<td><em>Omphalea diandra</em>, <em>Urania Fulgens</em>, <em>Aglaonema treubii</em>, <em>Hyacinthus orientalis</em></td>
<td>leaves</td>
<td>5, 6</td>
</tr>
<tr>
<td>2.</td>
<td>$\beta$-homonojirimycin (4)</td>
<td><em>Aglaonema treubii</em></td>
<td>whole plant bulbs</td>
<td>8, 11</td>
</tr>
<tr>
<td>3.</td>
<td>$\alpha$-homomannonojirimycin (5)</td>
<td><em>Aglaonema treubii</em></td>
<td>whole plant</td>
<td>8, 11</td>
</tr>
<tr>
<td>4.</td>
<td>$\beta$-homomannonojirimycin (6)</td>
<td><em>Aglaonema treubii</em></td>
<td>whole plant</td>
<td>8, 11</td>
</tr>
<tr>
<td>5.</td>
<td>$\beta$-homoaltronojirimycin (7)</td>
<td><em>Hyacinthus orientalis</em></td>
<td>bulbs</td>
<td>10</td>
</tr>
<tr>
<td>6.</td>
<td>$\alpha$-homoallonojirimycin (8)</td>
<td><em>Aglaonema treubii</em></td>
<td>whole plant</td>
<td>11</td>
</tr>
<tr>
<td>7.</td>
<td>$\alpha$-3,4-di-epi-homonojirimycin (9)</td>
<td><em>Aglaonema treubii</em></td>
<td>whole plant</td>
<td>8</td>
</tr>
<tr>
<td>8.</td>
<td>7-$\beta$-D-glucopyranosyl-$\alpha$-homonojirimycin (MDL 25637) (10)</td>
<td><em>Aglaonema treubii</em></td>
<td>whole plant</td>
<td>8</td>
</tr>
<tr>
<td>9.</td>
<td>5-$\alpha$-D-galactopyranosyl-$\alpha$-homonojirimycin (11)</td>
<td><em>Aglaonema treubii</em></td>
<td>whole plant</td>
<td>8</td>
</tr>
<tr>
<td>10.</td>
<td>$\beta$-L-homofuconojirimycin (12)</td>
<td><em>Anglyocalyx pynaertii</em></td>
<td>bark</td>
<td>12</td>
</tr>
</tbody>
</table>
3. Synthesis of piperidine homoazasugars

A number of piperidine homoazasugars are known in the literature and are described in the review articles related to azasugars. As the homoazasugars have a close resemblance with five or six carbon sugars, most of the synthetic strategies make use of sugars as the starting materials. The common methodologies used for the synthesis of piperidine homoazasugars are: (i) intramolecular reductive amination, (ii) intermolecular double reductive amination, (iii) amino/amido mercuration, (iv) intramolecular nucleophilic substitution, (v) synthesis from non-carbohydrate building block and aza-heterocycles and (vi) enzyme catalyzed intramolecular reductive amination.

(i) Intramolecular reductive amination strategy
Cipolla and co-workers have synthesized the protected α-allyl-C-glycoside of nojirimycin by sequential reductive amination (Scheme 1). Thus, reaction of perbenzylated D-glucose with benzylamine afforded N,2,3,4,6-pentabenzyl-D-glucopyranosylamine, that on allylation gave stereoselectively the open chain amino alcohol. Protection of the amino functionality by Fmoc, oxidation of the free hydroxy group to ketone, hydrolysis of the Fmoc group and final intramolecular reductive amination with Na(OAc)BH afforded the α-allyl-C-glycoside nojirimycin.

\[ \text{Scheme 1. Reagents and conditions: } (a) \text{PhCH}_2\text{NH}_2, p-\text{TSA, CH}_2\text{Cl}_2, \text{MS, 5 d, 80\%; (b) CH}_2=\text{CHCH}_2\text{MgBr, Et}_2\text{O, 81\%, 90\% de; (c) FmocCl, dioxane-10\% aq. Na}_2\text{CO}_3, 89\%; (d) PCC, CH}_2\text{Cl}_2, \text{MS, 90\%; (e) piperidine, DMF; (f) NaHB(OAc)}_3, \text{AcOH, Na}_2\text{SO}_4, 1,2\text{-dichloroethane, -35°C, 78\%, 90\% de; (g) H}_2, \text{Pd(OH)}_2, \text{AcOH, AcOEt-EtOH (1:1).} \]

We have recently reported two different strategies for the synthesis of 6-homoazasugars and 20. The first method relies on the reductive amination followed by diastereoselective intramolecular conjugate addition of the in situ generated benzylamine in the formation of desired piperidine ring (Scheme 2). Thus, D-glucose was converted to α,β-unsaturated ester that...
on 1,2-acetonide cleavage afforded hemiacetal 18. The reaction of 18 with benzylamine forms imine that was reduced to sugar benzylamine. The concomitant in situ addition of amine to α,β-unsaturated ester followed by domino lactonization gave lactone 19a with the required homoazasugars ring skeleton. Reduction of the lactone functionality and removal of the protecting group by hydrogenation afforded 1-deoxy-L-ido-homonojirimycin 17.

**Scheme 2.** Reagents and conditions: (a) ref. 19b (b) PPh3CHCO2Et, MeCN, reflux, 2 h; (c) TFA-H2O (3:2), rt, 2 h; (d) BnNH2, NaCNBH3, AcOH, MeOH, -78°C, 2 h, rt, 24 h; (e) Ac2O, Py, DMAP, rt, 24 h; (f) LAH, THF, 0°C-rt, 2 h; (g) MeOH, 10% Pd/C, HCO2NH4, reflux, 1 h.

The second method20 described the synthesis of homoazasugars 17 and 20. The key step involved TMSOTf catalyzed 1,3-addition of silyl ketene acetal 21 to D-glucose derived nitrone 2221 which afforded a diastereomeric mixture of D-gluco- and L-ido-configured N-benzyl hydroxylamines 23a and 23b in a good diastereoselectivity in favor of required D-gluco isomer. Column chromatographic separation and NO bond reductive cleavage afforded D-gluco- and L-ido-β-amino esters 24a and 24b. Reduction of the ester group in 24a,b followed by hydrogenolysis gave amino alcohol 25a,b that on selective N-Cbz protection gave 26a,b. The hydrolysis of 1,2-acetonide functionality in 26a,b and intramolecular reductive amination afforded D-gluco-homo-1-deoxynojirimycin 20 and L-ido-homo-1-deoxynojirimycin 17, respectively (Scheme 3).
Scheme 3. Reagents and conditions: (a) ref. 21. (b) CH₂Cl₂, Lewis acid, -78°C, CH₂=C(OEt)OTBDMS (21). (c) Cu(OAc)₂, Zn dust, glacial AcOH, 70°C, EDTA, 1 h. (d) THF, LAH, 0°C-rt, 2 h; (e) MeOH, 10% Pd/C, HCOONH₄, reflux, 1 h. (f) EtOH-H₂O, NaHCO₃, CbzCl, 6 h. (g) TFA-H₂O (3:2), 25°C, 2 h. (h) MeOH, 10% Pd/C, H₂, 70 psi, 16 h.

In the next report, we have described an efficient and practical strategy for the synthesis of N-hydroxyethyl-1-deoxy-homonojirimycins 27 and 28 with full stereocontrol (Scheme 4). The key step involved is the intermolecular conjugate addition of benzylamine to D-glucose derived α,β-unsaturated ester 29 to afford D-gluco- and L-ido- configured β-amino esters 30a and 30b. The sequential N-alkylation of 30a and 30b with ethyl bromoacetate, reduction with LAH, acetylation, hydrogenation and selective protection with -Cbz group afforded 31a and 31b, respectively. Separate removal of 1,2-acetonide functionality, hydrogenation and deacetylation of 30a and 30b afforded N-hydroxyethyl-D-gluco-1-deoxyhomonojirimycin (27) and N-hydroxyethyl-L-ido-1-deoxyhomonojirimycin (28), respectively. The glycosidase inhibition activity of compounds 27 and 28 was evaluated using sweet almond seed as a rich source of different glycosidases.
Scheme 4. Reagents and conditions: (a) BnNH2, rt, 20 h (b) i. BrCH2COOEt, K2CO3, DMF, rt, 24-30 h; ii. LAH, THF, 0°C, 2 h; iii. Ac2O, Py, 0-25°C, 43 h; iv. 10% Pd-C, H2, 80 psi, MeOH, 12 h; v. CbzCl, NaHCO3, EtOH-H2O (8:2), 0°C-rt, 4 h. (c) i. TFA-H2O (3:2), 0-30°C, 3 h; ii. 10% Pd-C, HCOONH4, MeOH, reflux, 45 min; iii. NaOMe, MeOH, 0-30°C, 2 h.

Transmetalation of the hydroxy protected stannylmethanol derivative 32 with butyllithium is an effective source of the hydroxymethyl carbonion, which undergoes efficient nucleophilic addition to carbonyl compounds and has been utilized for one carbon chain extension of carbohydrate lactones.23 Shilvock and co-workers have exploited this strategy for the synthesis of β-homogalactonojirimycin (β-HGJ) 33 (Scheme 5).24,25 Thus, hydroxy-methylation of protected 5-azido-L-manno-1,4-lactone 34 gave 35, which on hydrogenation afforded the piperidine derivative 36 as a single diastereomer. De-protection of the acetonide and MOM groups yielded 33 in good yield. The same strategy was extended for the synthesis of a variety of homoazasugars by changing the sugar lactones. For example, 5-azido-d-gulono-1,4-lactone 37 furnished α-HGJ 38 and C-5 epimeric homoazasugar 39, whereas 6-deoxy-5-azido-d-gulono-1,4-lactone 40 gave 41 and C-5 epimeric homoazasugar 42 (Scheme 5).
Scheme 5. Reagents and conditions: (a) Bu₃SnCH₂OMOM (32), n-BuLi, THF, -78°C. (b) TBAF, THF, 81%. (c) H₂, 10% Pd/C, EtOAc, 94%. (d) HCl, MeOH, 85%.

(ii) Intermolecular double reductive amination methodology
Saavedra and Martin²⁶ used two different approaches for the synthesis of β-homonojirimycin (4). In the first approach (Scheme 6), tetra-O-benzyl-D-glucono-1,5-lactoe 43 was treated with (methoxymethoxy)methylthium and the resulting heptulopyranose derivative 44 was reduced to alcohols 45a and 45b (~1:1). The oxidation of the mixture of 45a and 45b using DMSO-TFAA gave heptodiulose 46, which was immediately subjected to double reductive amination using HCOONH₄ in the presence of NaBH₃CN to give 47 as a single stereoisomer. The high degree of stereoselectivity observed in this double reductive amination reaction, probably involves cyclic intermediate and the stereoselective hydride addition (axial attack) appeared due to torsional effects.²⁷,²⁸ In the subsequent step, removal of the benzyl and MOM protecting groups provided 4.
Scheme 6. Reagents and conditions: (a) LiCH$_2$OMOM, THF, -78°C, 70%. (b) LAH, THF, 97%. (c) TFAA, DMSO-CH$_2$Cl$_2$, Et$_3$N. (d) HCOONH$_4$, NaBH$_3$CN, MeOH, 50%. (e) aq. HCl, THF, 93%. (f) i. TMSI; ii. H$_2$O, 70%.

In the second approach (Scheme 7),$^{26}$ one carbon extension by Wittig reaction of perbenzylated α-D-glucopyranose 14 followed by dihydroxylation afforded 49a and 49b (10:1, the major isomer was predicted from Kishi’s empirical rule$^{29}$) in which the primary hydroxyl group was protected as silyl ether. Oxidation of secondary alcohol functionality in 50a/50b afforded 1,5-diketone. Subsequently, double reductive amination using ammonium formate and sodium cyanoborohydride gave β-homonojirimycin derivative 57 that on removal of the protecting groups yielded 4.

Scheme 7. Reagents and conditions: (a) Ph$_3$P=CH$_2$; (b) OsO$_4$. NMO, 98%. (c) TBDMSCl, Et$_3$N, CH$_2$Cl$_2$, 70%. (d) TFAA, DMSO, Et$_3$N. (e) NCOONH$_4$, NaBH$_3$CN, MeOH. (f) TBAF, THF, 88%. (g) i. TMSI; ii. H$_2$O.

β-Homogalactonojirimycin 33 was also synthesized in six steps from heptenitol 52 by Martin and co-workers.$^{30}$ The synthetic route consisted in forming the piperidine ring by way of double reductive amination process of diketone 53 using ammonium formate and sodium cyanoborohydride (Scheme 8).
Scheme 8. Reagents and conditions: (a) i. OsO₄, NMO (cat), 98% (de~90%). ii. TBDMSCl, 82%. (b) Swern oxidation. (c) HCOONH₄, NaBH₃CN, 44%. (d) AcOH-H₂O, THF, 78%. (e) TMSI, then H₂O.

(iii) Amino/amido mercuration route
Martin and co-workers³⁰ synthesized α-homogalactonojirimycin 38 in 10 steps from D-galactose derivative 54 by way of a chain extension-amination-cyclization sequence (Scheme 9). The key step involved the cyclization of D-galacto-configured aminohexenitol 55 by intramolecular amidomercuration, which proceeded with 6-exo-trig cyclization with a high degree of diastereoselectivity (Scheme 9).

Scheme 9. Reagents and conditions: (a) [Ph₃P=CH₂], PhMe, 84%. (b) pO₂NC₆H₄CO₂H, Ph₃P, DEAD, 78%. (c) MeONA, 77%. (d) Phthalimide, Ph₃P, DEAD, 79%. (e) NH₂NH₂H₂O, MeOH. (f) CbzCl, THF, 84%. (g) i. (CF₃CO₂)₂Hg; ii. I₂, THF, 71%. (h) H₂, 10% Pd/C, 75%. (i) KOH, MeOH-H₂O, 81%.

The intramolecular amido mercuration strategy was also exploited by P.S. Liu in the total synthesis of 7-O-β-D-glucopyranosyl-α-homonojirimycin 10 (MDL 25637).⁴ The 2,3,4,6-tetra-O-benzyl-D-glucopyranose 14 was converted to the oxime 56 (via Wittig olefination and oxidation of secondary hydroxyl group to ketone), which on reduction with LAH and Cbz protection gave D and L amino sugars in the ratio 6:1, respectively. The major D-gluco-isomer 57 on treatment with mercuric acetate in THF underwent stereospecific cyclization to give exclusively α-
mercuriomethyl 48 that on demercuration oxygenation\textsuperscript{31} gave 58. The high stereoselectivity of the cyclization can be accounted for by the chelation effect of mercury with the vicinal α-benzyloxy group, resulting in the preferential addition of the carbamate (nucleophile) from the opposite side of the olefin. The coupling with 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl bromide furnished 59a (19\%) and 59b (24\%). Separation and de-protection in 59b afforded β-glucoside 10 (Scheme 10).

\textbf{Scheme 10. Reagents and conditions:} (a) Ph\textsubscript{3}P=CH\textsubscript{2}. (b) i. DCC, DMSO. ii. NH\textsubscript{2}OH.HCl, KHCO\textsubscript{3}, 75\%. (c) i. LAH; ii. CbzCl, K\textsubscript{2}CO\textsubscript{3}, 67\%. (d) i. Hg(OAc)\textsubscript{2}; ii. KCl/H\textsubscript{2}O. (e) NaBH\textsubscript{4}-DMF-O\textsubscript{2}, 71\%. (f) CH\textsubscript{2}Cl\textsubscript{2}, HgBr\textsubscript{2}, rt; (g) CHCl\textsubscript{3}, EtOH, 5 NHCl, 10\% Pd/C, H\textsubscript{2}, 3 d, 74\%.

(iv) Intramolecular nucleophilic substitution method

Cipolla and co-workers\textsuperscript{32} have exploited the intramolecular nucleophilic displacement of O-triflate with amine to give piperidine ring skeleton in the synthesis of N-butyl homoazasugars 60a and 60b (Scheme 11). The D-gluco- and D-manno-pyranosylamine 15 and 61, respectively, were reacted separately with allylmagnesium chloride to give allylated derivatives, which on treatment with Tf\textsubscript{2}O led to the formation of –OTf derivative that undergoes intramolecular displacement of –OTf by amine giving piperidine ring that on removal of O-benzyl groups gave homoazasugars 60a and 60b, respectively.
Scheme 11. Reagents and conditions: (a) RNH₂, CH₂Cl₂, MS. (b) R’MgX, THF, rt; (c) Tf₂O, Py. (d) H₂, 10% Pd/C, EtOH, H⁺.

(v) Synthesis from non-carbohydrate building block and aza-heterocycles
The first synthesis of (+)-α-homonojirimycin (4) was reported by S. Aoyagi et al.³³ via a non-carbohydrate based approach utilizing suitably protected allylic alcohol 62 (Scheme 12). The allylic alcohol 62 was converted to syn epoxide 63 by the Sharpless asymmetric epoxidation. Regio- and diastereoselective ring opening of the epoxide was effected by using dialkylaluminium amide followed by selective amino group protection to give amino alcohol 64 as a single diastereomer, which on MOM protection and desilylation furnished 65. Swern oxidation of 65 gave the aldehyde 66 that on Wittig reaction followed by hydroxylation provided 2.5:1 diastereoselectivity in favor of the desired anti-diol 67. Compound 67 was then converted to homonojirimycin 4 (Scheme 12) by conversion of secondary alcohol to mesylate and hydrogenation wherein de-protection of N-Cbz lead to an amine that subsequently undergoes intramolecular nucleophilic substitution to give piperidine ring skeleton.
Scheme 12. Reagents and conditions: (a) (+)-DET, Ti(OPr)i4, TBHP. (b) i. Et2AlNHCH2Ph, CH2Cl2; ii. CbzCl, aq. Na2CO3, CH2Cl2. (c) MOMCl, (iPr)2Net, CHCl3, then TBAF, THF; (d) (COCl)2, DMSO, Et3N, CH2Cl2; (e) Ph3PCH3Br, n-BuLi, THF; (f) NMO, OsO4, aq. Me2CO. (g) TBDMSCl, imidazole, DMF, then MsCl, Et3N, CH2Cl2. (h) H2, Pd(OH)2, MeOH; (i) Et3N, MeOH, reflux; (j) conc. HCl, MeOH, reflux.

Johnson and Johns34 synthesized a number of β-1-C-aryl-mannonojirimycin analogues of type 68 using asymmetric strategy. The polyhydroxylated piperidine ring was constructed using vinyl bromide 70, which was synthesized in six steps from bromobenzene by microbial oxidation to get bromo diol 69. A palladium-catalyzed Suzuki cross-coupling of vinyl bromide 70 and the corresponding arylboronic acid reaction served as the key pseudoanomeric C-C bond forming step. Ozonolysis and selective reduction of the resultant carbonyl functions followed by reductive amination produced the azasugar ring (Scheme 13). The stereochemical outcome of the reductive amination resulted into β-oriented aryl group as the hydrogen delivery resulted from the α-face opposite to that of the adjacent β-oriented acetonide group.
Scheme 13. Reagents and conditions: (a) Arb(OH)$_2$, PdCl$_2$(PPh$_3$)$_2$, 2 M aq. Na$_2$CO$_3$, THF, reflux, 2-24 h. (b) O$_3$, DMS; (c) NaBH$_3$CN, pH 4 buffer, THF. (d) 10% Pd/C, H$_2$, MeOH/H$_2$O. (e) 6 N HCl, THF, rt, 12 h, 82-99%.

The homoaminoazasugars 71 and 72 were synthesized by C.-H. Wong and co-workers$^{36}$ from commercially available nojirimycin bisulfite and mannonojirimycin bisulfite, respectively. The reaction of bisulfite adducts 73 and 74 with potassium cyanide in the presence of Ba(OH)$_2$ and ethanolic HCl afforded the corresponding α-nitriles 75 and 76, which were converted to the homoaminoazasugars 71 and 72 by a palladium-catalyzed reduction under acidic conditions (Scheme 14).

Scheme 14. Reagents and conditions: (a) Ba(OH)$_2$, KCN, HCl (>75%). (b) H$_2$, 10% Pd/C, HCl (71, 100%; 72, 78%).
Tri-O-acetyl imino glucal 78, prepared from D-glucal 77 was utilized for the synthesis of iminosugar C-glycosides. Imino glucal 78 easily undergoes a variety of Lewis acid mediated C-C bond forming reactions at C-1 of the piperidine ring (e.g. 79 and 80) giving major β-anomeric homoazasugars (Scheme 15).

![Scheme 15. Reagents and conditions: (a) BF₃·Et₂O, Et₂Zn, CH₂Cl₂, -20°C-rt. (b) OsO₄ (cat.), NMO, actone-H₂O, 5 d. (c) Ac₂O, Py, 2 h. (d) piperidine, CH₂Cl₂, 1 h.](image)

(vi) Enzyme catalyzed intramolecular reductive amination strategy
Enzymes are increasingly recognized as useful catalyst for the organic syntheses. The synthesis of monosaccharides and related compounds via enzymatic aldol addition reaction, catalyzed by aldolases, has been proven to be very useful and several successful examples have been described in recent years. In addition, the use of enzyme aldolases with azido substrate followed by hydrogenation of the azido-sugar produces various five-, six- and seven-membered azasugars followed by hydrogenation of the azido-sugar produces various five-, six- and seven-membered azasugars (Figure 4). The same strategy is also extended for homoazasugars.

![Figure 4. Preparation of azasugars using aldolases.](image)

For example, Wong and co-workers prepared β-L-homofuconojirimycin (β-HFJ) 12 by an aldolase-based strategy. The acceptor substrate (±)-threo-azidoaldehyde 82 was synthesized from commercially available 2-butyn-1-al diethyl acetal 81 (Scheme 16). Aldolase catalyzed aldol condensation of 82 with dihydroxyacetone phosphate (DHAP) followed by dephosphorylation with acid phosphatase afforded the desired enantiomerically pure azidoketose 83. Hydrogenation of 83 in the presence of 10% Pd/C produced β-HFJ 12 as the only product. The complete diastereoselectivity is due to the delivery of hydrogen from the less hindered side of the possible cyclic imine intermediate during the reductive amination process.
**Scheme 16.** Reagents and conditions: (a) Ni(OAc)₄, 4H₂O, NaBH₄, NH₂(CH₂)₂NH₂, then H₂, 1 atm, 81%. (b) mCPBA, NaHCO₃, CH₂Cl₂, 71%. (c) NaN₃, NH₄Cl, EtOH-H₂O, 90°C, 44%. (d) 0.1 NHC₂, 50°C, 5 h. (e) i. DHAP, FDP aldolase, pH 6.7, 25°C; ii. acid phosphatase, pH 4.7, 37°C, 66%. (f) H₂, (50 psi), Pd/C, 94%.

The same group also utilized the azidoketose 83 for the synthesis of 2-aminomethyl-HFJ 84. Amino-homoazasugar was prepared by a novel chemoenzymatic strategy in which azido sugar 83 was constructed by enzymatic aldol reaction under acidic hydrogenation condition (Scheme 17).

**Scheme 17.** Reagents and conditions: (a) H₂, (1 atm), Pd/C, aq. HCl, quant. (b) KCN, dioxane, H₂O, 77%. (c) H₂, PtO₂, conc. HCl, EtOH, 99%.

Homoazasugars 85, 86 and 87 were prepared by A. Straub et al. by using rabbit muscle aldolase (RAMA). Aldol addition of DHAP to 3-azido-2hydroxybutanal 82 (erythro:threo = 92:8) afforded a mixture of 88 and 83 with major 83 (88%). After anion exchange chromatography 83 was isolated as a 18:82 mixture of the β/α anomers. Reductive amination of 83 gave a mixture of homoazasugars 85 and 86 (3:2), whereas hydrogenation of a mixture of 88 and 83 provided 85, 86 and 87 (Scheme 18).
Scheme 18. Reagents and conditions: (a) i. DHAP, rabbit muscle aldolase, BaCl₂. (b) Wolfatit H⁺, Pase (EC 3.1.3.2), Dowex 1 x 8 HCO₃⁻. (c) PtO₂/H₂.

K. E. Holt et al.⁴⁴ and C.- H. Wong et al.⁴⁵ used RAMA for the synthesis of a number of naturally occurring homoazasugars. The four stereoisomers of the four-carbon azido sugar ⁸⁹ have been stereoselectively synthesized by a route involving Sharpless epoxidation and these compounds were considered as substrates for rabbit muscle fructose 1,6-bisphosphate aldolase, giving (after treatment with phosphatase) 6-azido-6-deoxyheptuloses ⁹⁰a-d, respectively. Further hydrogenation gave corresponding homoazasugars ⁶, ⁴, ³ and ⁵ respectively (Scheme 19).
Scheme 19. Reagents and conditions: (a) BuLi, BDPSiCl. (b) DET, Ti(OPr)i4, Bu’OOH. (c) PCC. (d) RAMA, DHAP. (e) acid phosphatase. (f) 10% Pd/C, H2, 40 psi.

4. Biological activity

Glycosidases are involved in several important biological processes such as digestion, biosynthesis of glycoproteins and the lysosomal catabolism of glycoconjugates. Therefore, glycosidase inhibitors have many potential medical applications, for example, diabetes type 2,46 cancers,47 viral infection,48 and hereditary lysosomal storage diseases.49 Homoaazasugars are selective and in some cases better glycosidase inhibitors50 and thus have been used for the treatment of a number of carbohydrate mediated diseases.

4.1. Glycosidase inhibition

The IC50 values of a number of piperidine homoaazasugars, shown in Table 2, was evaluated by Fleet and co-workers.9 α-HNJ 3 inhibited α-glucosidases and trehalase to a similar extent as DNJ 2, failing to have any activity toward other glycosidases tested. Thus, the hydroxymethyl group at the anomeric position of 2 contributed to a greater selectivity. β-HNJ 4 was very specific inhibitor of α-glucosidase (IC50 = ~10 µM). Similar selectivity and potency was found in case of α-HMJ (5) against human liver α-mannosidases.51 β-HMJ 6 is a potent inhibitor of rice and rat α-
glucosidases and human α-L-fucosidase ($K_i = 4.5$ mM). $\beta$-4,5-Di-epi-HNJ (7) showed potent inhibitory activity toward all α-glucosidases tested and was found much better α-galactosidase inhibitor than that of β-galactosidase. Thus, the epimerization at C-4 of 6 definitely enhances its inhibition toward α-glucosidases and α-galactosidase. N-alkylation of 3 enhances the potency and selectivity against the glycosidases tested. It has been proposed that the C-6 OH axial conformation of the N-alkyl derivatives of DNJ best fit the active site of ER α-glucosidase $^{52}$ or glucoamylase from Aspergillus awamori $^{53}$ and is responsible for strong inhibitory activity. In fact Me-HNJ 91 (Figure 5) with this preferred conformation is more potent inhibitor of α-glucosidase 1 than 3.

![Diagram of compounds](image)

**Table 2.** IC$_{50}$ of piperidine homoazasugars

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>91</th>
<th>92</th>
</tr>
</thead>
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<tr>
<td>α-glucosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rice</td>
<td>0.04</td>
<td>8.4</td>
<td>110</td>
<td>3.2</td>
<td>0.7</td>
<td>NI</td>
<td>0.06</td>
<td>4.2</td>
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<td>rat intestinal maltase</td>
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<td>15</td>
<td>46</td>
<td>4.6</td>
<td>1.6</td>
<td>NI</td>
<td>0.17</td>
<td>3.0</td>
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<tr>
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<td>27</td>
<td>3.0</td>
<td>0.8</td>
<td>410</td>
<td>0.02</td>
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<td>8.2</td>
<td>NI</td>
<td>5.0</td>
<td>2.7</td>
<td>NI</td>
<td>1.0</td>
<td>2.2</td>
</tr>
<tr>
<td>trehalase (porcine kidney)</td>
<td>34</td>
<td>NI</td>
<td>460</td>
<td>360</td>
<td>140</td>
<td>NI</td>
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<td>α-galactosidase (coffee bean)</td>
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<td>NI</td>
<td>NI</td>
<td>6.4</td>
<td>80</td>
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<tr>
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<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>86</td>
<td>160</td>
<td>-</td>
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<td>NI</td>
<td>NI</td>
<td>130</td>
<td>290</td>
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<td>α-L-fucosidase</td>
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<tr>
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<td>2.6</td>
<td>38</td>
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<tr>
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<td>4.4</td>
<td>20</td>
<td>NI</td>
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</table>

*NI = less than 50% inhibition at 1000 µM.*

Homoazasugars, due to the remarkable glycosidase inhibitory activity, were studied against diabetes. For example, compound 10 (MDL 25637) was reported to effectively reduce...
postprandial elevations of blood glucose and plasma insulin in animals when administered 30-60 min before a sucrose load.\textsuperscript{54}

4.2. Antiviral activity
Glycoproteins are often essential proteins in that they are required in the viral life cycle, either in viron assembly and secretion and/or infectivity. As processing of these glycoprotein occurs through the cellular machinery, processing glycosidase inhibitors have been used to study the role of N-linked oligosaccharides in several viral systems including human immunodeficiency virus (HIV),\textsuperscript{55} human hepatitis B virus (HBV),\textsuperscript{56} human cytomegalovirus (HCMV),\textsuperscript{57} influenza virus,\textsuperscript{58} Sinvis virus\textsuperscript{59} and VSV.\textsuperscript{60} α-Glucosidase inhibitors are potent inhibitors of HIV replication and HIV-mediated syncytium formation in vitro.\textsuperscript{55,61,62} Whereas, N-linked oligosaccharide processing inhibitors have no effect on the secretion of infectious virus.\textsuperscript{62,63} α-HNJ 3 and its N-methyl derivative 91 are more potent against α-glucosidase I both in vitro and in cell culture.\textsuperscript{64} So it is expected that these homonojirimycins would show excellent anti-HIV activity. Surprisingly, both HNJ 3 and Me-HNJ 91 showed no significant anti-HIV activity even at concentrations of 500 µg/mL.\textsuperscript{9}

5. Conclusions
In conclusion, we have described the natural occurrence of piperidine homoazasugars. The synthetic aspects of homoazasugars using chiron, asymmetric approaches including chemo-enzymatic methods have been discussed. The structural basis for the specificity of glycosidase inhibition and biological applications are also discussed.

6. References
51. Bruce, I.; Fleet, G. W. J.; de Bello, C. I.; Winchester, B. Tetrahedron 1992, 48, 10191.