REVIEW

Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt

# Glycosidase inhibitors and their chemotherapeutic value, part 2*

E. S. H. EL ASHRY, N. RASHED and A. H. S. SHOBIER

The various compounds that have been investigated as glycosidase inhibitors are reviewed. The second of three parts of this review article covers the following classes of compounds: sugars with nitrogen in the ring, e.g. azepine analogues, piperidine analogues and pyrrolidine analogues and fused rings with a bridgehead nitrogen.

## 16. Sugars with nitrogen in the ring

Many mono- and bicyclic polyhydroxylated pyrrolidines, piperidines and azepines (referred to as iminosugars or azasugars) are strong glycosidase inhibitors [13, 24, 46, 181–185] and some of them showed promising chemotherapeutic effects against viral diseases [186–188], and as potential antidiabetic [189], as well as antitumor [190] agents. The mechanisms of these effects have been studied extensively [24, 25, 191]. Some of these compounds are naturally occurring and owing to the pronounced biological activity of this class of compounds various synthetic efforts were designed for the synthesis of many of them. In this review they were classified according to the size of the ring.

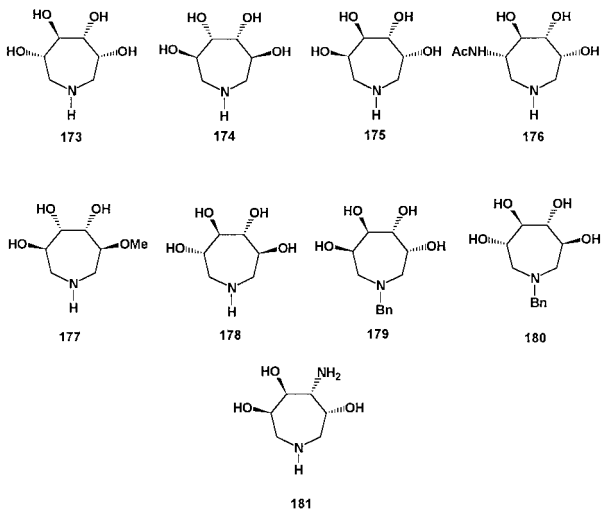
### *16.1. Azepine analogues*

Several polyhydroxyperhydroazepines **173**–**181** have been obtained either by chemoenzymatic or chemical synthesis. Some of these compounds display significant activity as glycosidase inhibitors with $K_i$ values from moderate to low in the micromolar range. The higher inhibition of both green coffee bean α-galactosidase and bovine kidney α-fucosidase by **173** ($K_i \approx 5.0 \times 10^{-6}$ M) was due to the higher degree of ring flexibility than smaller ring heterocycles. Interestingly, compound **175** ($K_i = 4.6 \times 10^{-6}$ M) was better than 1-deoxy-*N*-acetylglucojirimycin ($K_i = 9.8 \times 10^{-6}$ M) [192] as an inhibitor of β-*N*-acetylglucosaminidase. Compound **178** ($K_i = 6.5 \times 10^{-6}$ M) was better than 1-deoxygalactojirimycin ($K_i > 1.0 \times 10^{-3}$ M) [193] as an inhibitor of β-galactosidase, and it was better ($K_i = 2.6 \times 10^{-5}$ M) than 1-deoxymannojirimycin ($K_i = 1.5 \times 10^{-4}$ M) [194] as an inhibitor of α-mannosidase. The N-benzyl derivatives **179** and **180** did not improve inhibition activity except in the case of α-fucosidase and β-glucosidase, respectively (**179**: $K_i = 2.3 \times 10^{-5}$ M and **180**: $K_i = 3.1 \times 10^{-5}$ M) [195–197]. The inactivity of aminotrihydroxyhexahydroazepine **181** against mannosidases can be explained in terms of the relative energies of the axial versus the equatorial conformations of the critical hexahydroazepine ring substituents [198]. The X-ray crystal structure of a representative compound **179** was determined and shown to adopt a pseudochair conformation which clarified how these seven-membered iminocyclitols act as inhibitors of glycosidases.

### *16.2. Piperidine analogues*

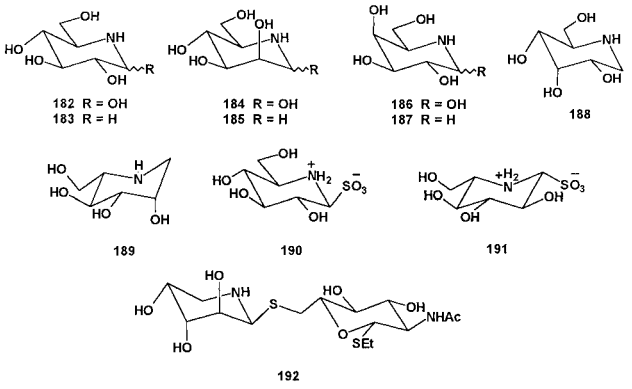
The antibiotics nojirimycin (**182**) and 1-deoxynojirimycin (**183**) are the first naturally occurring azahexoses [199 to 202]. The D-manno analogues [52, 203] mannojirimycin (**184**) and 1-deoxymannojirimycin (**185**) as well as the D-galacto analogues galactostatin (**186**) and 1-deoxy galactostatin (**187**) [204, 205] are also naturally occurring. All these analogues have been shown to be potent and specific inhibitors of the hydrolysis of the corresponding glycosides by specific glycosidases. Azasugars like **183, 185** and **187** are competitive inhibitors of gluco, manno- and galactosidases, respectively [206–209]. This competitive inhibition was due to the H-bonding and electrostatic interactions with a nearby carboxylate group in the enzymes. Both bovine lysosomal and cytosolic β-glucosidases were inhibited by **182** with $K_i$ values of $8.0 \times 10^{-7}$ M at pH 5.0 and $4.2 \times 10^{-5}$ M at pH 7.0. [210].

1-Deoxynojirimycin (**183**) is a potent inhibitor for all types of mammalian α-glucosidases [201, 211–214]. It is a potent competitive inhibitor of ER α-glucosidase II, involved in N-linked oligosaccharide processing, with a $K_i$ value of $1.3 \times 10^{-6}$ M. The $K_i$ values of (**183**) towards hydrolysis of maltose, maltotriose, maltotetraose and maltoheptaose by human small glucoamylase-maltase are $6.7 \pm 0.3$, $3.6 \pm 0.7$, $2.4 \pm 0.5$ and $3.2 \pm 0.4 \times 10^{-7}$ M, respectively [215]. It is a better inhibitor of α-glucosidase II than of α-glucosidase I. It competitively inhibited pig kidney trehalase ($K_i = 8.5 \times 10^{-6}$ M) [216], calf liver α-glucosidase ($K_i = 1.0 \times 10^{-6}$ M) [217], yeast α-glucosidase ($K_i = 8.7 \times 10^{-6}$ M) [116], α-D-mannosidase from jack bean ($K_i = 4.0 \times 10^{-4}$ M) and β-glucosidase from sweet almond ($K_i = 1.8 \times 10^{-5}$ M) [63, 116]. It exhibited a powerful inhibition of rice α-glucosidase, with an $IC_{50}$ value of $5.0 \times 10^{-8}$ M [218]. The introduction of the α-glucopyranosyl residue to the C-3 position enhanced the inhibition activity towards this enzyme, whereas the β-glucosylation of 1-deoxynojirimycin at C-2 or C-4 markedly lowered



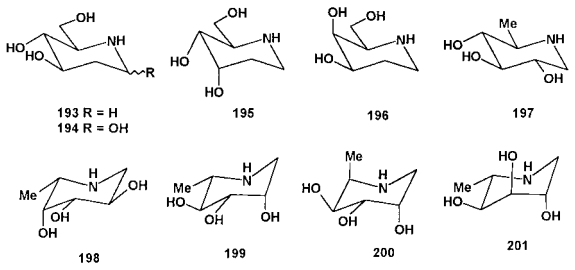
its inhibition. It inhibited α-L-rhamnosidase effectively ($K_i = 3.2 \times 10^{-5}$ M) [219]. *Exo*-glucanase was inhibited by **183** ($K_i = 9.1 \times 10^{-5}$ M) from *C. albicans* ($K_i = 7.0 \times 10^{-7}$ M) and from a basidiomycete in an uncompetitive manner [220]. Deoxymannojirimycin (**185**) is a specific inhibitor of α-mannosidases ($K_i = 6.8$ and $8.3 \times 10^{-5}$ M for jack bean and calf liver, respectively), but not almond β-glucosidase ($K_i = 5.3 \times 10^{-3}$ M) at pH 5.0 [52]. It is a moderate competitive inhibitor of pig kidney trehalase ($K_i = 3.9 \times 10^{-4}$ M) [216]. It retained a potency towards rat and bovine liver lysosomal α-glucosidases I. It is a good inhibitor of Golgi α-mannosidase I. It inhibited also Golgi α-mannosidase II in a competitive manner ($K_i = 4.1 \times 10^{-4}$ M). It is a moderate inhibitor of lysosomal and epididymis α-mannosidases but not an inhibitor of endoplasmic reticulum (ER) α-glucosidase or soluble rat liver α-mannosidases. Compound **185** is a good inhibitor of bovine epididymis α-L-fucosidase in addition to bovine liver lysosomal α-glucosidase, and a fairly poor inhibitor [221] of the plant mannosidase I. It is a potent inhibitor of yeast α-glucosidase ($IC_{50} = 6.5 \times 10^{-6}$ M), emulsin β-glucosidase ($IC_{50} = 7.3 \times 10^{-6}$ M) in a competitive manner and insect trehalase ($IC_{50} = 5.5 \times 10^{-5}$ M) [68]. It did not show any inhibition of *Canavalia ensiformis* α-mannosidase, *Aspergillus niger* α- and β-galactosidase and *Helix pomatia* β-glucuronidase (at concentrations up to $10^{-2}$ M) [68]. Sufficient flexibility is inherent in the ring of **185** to allow the ring to flip and provide optimum alignment of the topographically equivalent hydroxyl groups of the inhibitor and cation. The natural and synthetic galactonojirimycin (**186**) and galacto-1-deoxynojirimycin (**187**) are powerful and specific inhibitors of several α- and β-galactosidases, and glucosidases. The latter competitively inhibited the hydrolysis of *p*-nitrophenyl α-D-galactopyranoside ($K_m = 1.1 \times 10^{-3}$ M) by green coffee bean α-galactosidase ($K_i = 1.6 \times 10^{-9}$ M, $IC_{50} = 4.0 \times 10^{-7}$ M) [193]. High concentrations of **187** ($1.0 \times 10^{-3}$ M) caused no inhibition of bovine β-D-galactosidase. It is a potent inhibitor of human placental ceramide trihexosidase ($IC_{50} = 4.0 \times 10^{-9}$ M) [193]. It showed promise in developing a mouse model of Fabry's disease, a lysosomal storage disease for which no animal model exists. 1,5-Dideoxy-1,5-imino-L-mannitol (**189**) inhibited α-galactosidase competitively with a $K_i$ value of $8.2 \times 10^{-6}$ M. α-L-Fucosidase (bovine epididymis) and α-L-rhamnosidase in (naringinase) were also competitively inhibited by **189** with $K_i$ values of 4.7 and $2.9 \times 10^{-4}$ M, respectively [219]. The biological activities of the (+)- and (−)- nojirimycin bisulfite adducts **190** and **191** indicated that **191** possessed high inhibitory activity against almond β-D-glucosidase ($IC_{50} = 4.5 \times 10^{-6}$ g $ml^{-1}$, I = 91.7%), whereas it has almost no inhibitory activity against yeast α-D-glucosidase [222]. The synthetic (+)-nojirimycin bisulfite adduct **190** showed an excellent inhibitory activity against almond β-D-glucosidase ($IC_{50} = 9.4 \times 10^{-6}$ g $ml^{-1}$, I = 85.8%) and a very good activity against yeast α-D-glucosidase ($IC_{50} = 1.7 \times 10^{-5}$ g $ml^{-1}$, I = 76.1%) [222]. The deoxyallo isomer **188** fairly retained a potency towards intestinal isomaltase ($IC_{50} = 3.4 \times 10^{-5}$ M) [23]. The azapyranosyl thioglycoside **192** was synthesized as a potential *endo*-glycosidase inhibitor [223].

Fagomine (**193**) is a very weak competitive inhibitor of pig kidney trehalase ($K_i = 6.8 \times 10^{-4}$ M) [216], and inactive against α- and β-glucosidase as well as jack bean mannosidase [116, 224] with a concentration up to $10^{-3}$ M due to the lack of an OH group. A moderate inhibitory activity against α-L-fucosidase was observed for fagomine




[23]. It has some activity against mammalian gut α-glucosidase [225]. Although, fagomine (**193**) exhibited no inhibition for β-glucosidase, 3-*epi*-fagomine (**195**) was a moderately good inhibitor of *Caldocellum saccharoliticum* β-glucosidase ($IC_{50} = 6.8 \times 10^{-5}$ M) [226]. In contrast, fagomine showed an $IC_{50}$ of $5.6 \times 10^{-5}$ M against green coffee bean α-galactosidase, while no inhibition was seen for 3-*epi*-fagomine. Similar inhibition activity of the rice α-glucosidase and almond β-glucosidase was observed for **195** ($IC_{50} = 1.2 \times 10^{-4}$ M, for each) [226]. The dideoxy-D-allo isomer **195** fairly retained a potency towards intestinal isomaltase ($IC_{50} = 6.4 \times 10^{-6}$ M) [23]. It potently inhibited rat intestinal lactase and bovine liver cytosolic β-galactosidase in a competitive manner ($K_i = 1.9$ and $1.5 \times 10^{-6}$ M, respectively) [23]. It exhibited no significant inhibition towards lysosomal and epididymal enzymes which is optimally active in acidic conditions. However, **193** is a good inhibitor of isomaltase and certain α- and β-galactosidases, its 3-epimer **195** is a more potent inhibitor of isomaltase and β-galactosidase than **193** and does not inhibit α-galactosidase [227]. The α-glucosidase inhibitor, 2-deoxynojirimycin (2DN, **194**) significantly decreased blood glucose levels in sucrose loading model mice [228]. The dideoxy analogue **196** exhibited no inhibition towards α- or β-glucosidase, α-mannosidase, α-L-fucosidase, trehalase or β-galactosidase [23]. The 1,6-dideoxynojirimycin (**197**) exhibited competitive inhibition against α-glucosidase from brewer's yeast ($K_i = 1.6 \times 10^{-3}$ M), and β-glucosidase from sweet almond ($K_i = 7.8 \times 10^{-4}$ M) [116].

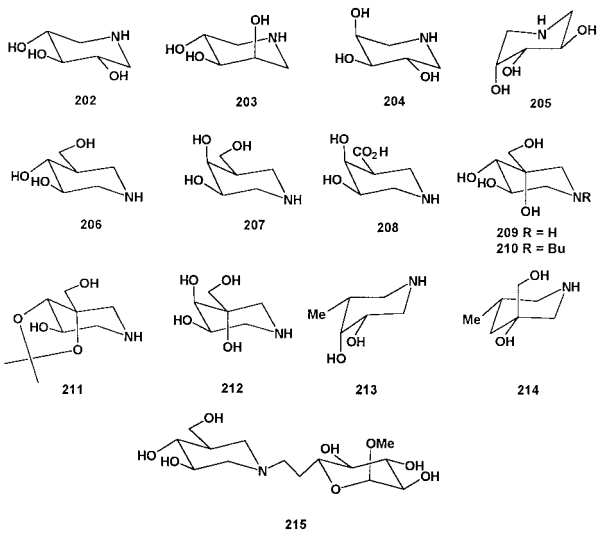
The iminofucitol **198** is an exceptionally powerful inhibitor of human α-L-fucosidases ($K_i = 1.0 \times 10^{-8}$ M) [229 to 232], a potent competitive inhibitor of canine α-L-fucosidase ($K_i = 4.0 \times 10^{-11}$ M) [233] and bovine epididymis α-L-fucosidase ($K_i = 4.8 \times 10^{-9}$ M, $IC_{50} = 2.5 \times 10^{-8}$ M) [229]. In contrast, no other enzymes such as yeast α-glucosidase, almonds β-glucosidase, green coffee beans α-galactosidase, *Aspergillus niger* β-galactosidase, jack bean α-mannosidase, and *Aspergillus niger* β-xylosidase was inhibited at a concentration of $5.0 \times 10^{-4}$ M [229]. 1-Deoxyrhamnojirimycin (**199**) is a moderate inhibitor of α-L-fucosidase ($K_i = 4.9 \times 10^{-4}$ M) [234] and may have a potential value as selective antimicrobial agent or as herbicide, as rhamnose is often found in microorganisms or plants, but not in animal or humans [235, 236]. 1,5-Dideoxy-1,5-imino-L-rhamnitol (**199**) exhibited moderate inhibition of amyloglucosidase as well as the α-L-rhamnosidase activity in hesperidinase. It is a competitive inhibitor of α-L-fucosidase from bovine epididymis and bovine kidney with $K_i$ values of 1.8 and $2.2 \times 10^{-6}$ M, respectively. The more potent inhibition by fucodeoxynojirimycin **198**, ($K_i = 4.8 \times 10^{-9}$ M) [229], compared to **199**, of α-L-fucosidase from bovine kidney suggested the importance of




the proper stereochemistry at C-2 and C-4 in these inhibitors in relation to L-fucose. Compound **199** also inhibited α-galactosidase and α-L-rhamnosidase in naringinase competitively with $K_i$ values of $2.0 \times 10^{-4}$ and $3.4 \times 10^{-5}$ M, respectively [219]. 5-*Epi*-L-rhamnojirimycin (5-*epi*-LRJ, **200**) was found to be a strong competitive inhibitor of naringinase (L-rhamnosidase) from *Penicillium decumbens* ($K_i = 1.0 \times 10^{-6}$ M, $IC_{50} = 5.0 \times 10^{-6}$ M) and a mild inhibitor of almond emulsin β-glucosidase ($I = 60\%$ at $9.7 \times 10^{-4}$ M) [237]. 1,6-Dideoxy-L-altrojirimycin (**201**) is a potent fucosidase inhibitor ($K_i \leq 1.0 \times 10^{-8}$ M) [238].

The investigation of the iminoalditols **202**–**204** showed that des(hydroxymethyl)nojirimycin **202** inhibited sweet almond β-glucosidase (35% of control activity at $1.0 \times 10^{-3}$ M) at pH 5.0, but had no effect on yeast α-glucosidase, jack bean α-mannosidase, coffee bean α-galactosidase, β-galactosidase, β-glucuronidase, or β-hexosaminidase (all bovine) [239]. Its inhibition against almond β-glucosidase was competitive with a $K_i$ value of $4.3 \pm 1.0 \times 10^{-4}$ M similar to that for deoxynojirimycin (**183**) ($3.7 \times 10^{-4}$ M at pH 5.0) [63]. At very low concentrations ($\leq 3.0 \times 10^{-5}$ M), **202** behaved as a mild activator of β-glucosidase [239]. These data indicated that the hydroxymethyl side chain of **183** is relatively unimportant for inhibitor binding. The mannose analogue **203** competitively inhibited only jack bean α-mannosidase. Its effect was comparable to that of 1-deoxymannojirimycin (40% of control activity at $1.0 \times 10^{-3}$ M). However, the galactose analogue **204** had only marginal effects on α- or β-galactosidase, suggesting that the $-CH_2OH$ group of galactose may serve as a much more important recognition unit for catalyzed glycoside hydrolysis.

The 5-nor iminofucitol **205** has a 1500-fold larger $K_i$ against α-L-fucosidase, compared to the iminofucitol **198**, *i.e.* replacement of the methyl group in **198** by a hydrogen caused a decrease in the standard free energy of binding amounting to 4.3 Kcal $mol^{-1}$ and might give **205** a greater flexibility within the binding site, thus making hydrogen bonds less effective. The inhibition constants of **205** ranged from $2.4 \times 10^{-5}$ M (pH 5.0) to $2.2 \times 10^{-6}$ M (pH 7.0), and its inhibitory potency was greatly lowered by *N*,*N*-dimethylation ($K_i = 3.6 \times 10^{-3}$ M at pH 5.0 to $3.1 \times 10^{-4}$ M at pH 7.0) [240]. It has been discovered that isofagomine (**206**) is a powerful inhibitor of glucoside hydrolases, particularly β-glucosidase ($K_i = 1.1 \times 10^{-7}$ M) [241, 242] as a good transition state analogue. Galacto-isofagomine (**207**) was a very potent inhibitor of *Aspergillus aryzae* β-galactosidase ($K_i = 4.0 \times 10^{-9}$ M) [243], but did not inhibit coffee bean α-galactosidase ($IC_{50} = 2.0 \times 10^{-4}$ M) as strongly as β-galactosidase. It was a powerful inhibitor for almond β-glucosidase ($IC_{50} = 1.9 \times 10^{-7}$ M) [243]. The carboxylic acid analogue **208** inhibited β-D-glucuronidase ($K_i = 7.9 \times 10^{-6}$ M) [244]. The iminosugar **209** selectively inhibited α-glucosidase from yeast ($IC_{50} = 3.8 \times 10^{-4}$ M), and β-glucosidase from almond ($IC_{50} = 2.7 \times 10^{-4}$ M) [245]. Its inhibition against β-glucosidase from almond was pH dependent *i.e,* the $K_i$ of **209** was $2.6 \times 10^{-4}$ M at pH 5.0, whereas it became $4.3 \times 10^{-6}$ M at the pH 6.8 [241, 245]. It weakly inhibited α-mannosidase from jack beans ($IC_{50} > 2.0 \times 10^{-3}$ M) [241], although it is structurally similar to the potent mannosidase inhibitor deoxymannojirimycin (dMJ). The inhibition studies of compounds **209** and **211** against α-glucosidase from baker's yeast and β-glucosidase from almonds [147] showed that only 5-hydroxyisofagomine (**209**) had significant effects against α-glucosidase ($K_i = 2.3 \times 10^{-4}$ M) and β-glucosidase ($K_i = 1.3 \times 10^{-5}$ M) [246]. Compound **209** inhibited β-glucosidase better than α-glucosidase but it was 100 fold less potent than isofagomine [241]. The acetonide **211** was a much weaker inhibitor than **209** towards both α- and β-glucosidases ($K_i = 2.0 \times 10^{-3}$ and $5.6 \times 10^{-4}$ M, respectively). This indicated that the extra hydroxyl group at the C-branch as in **209** decreased the inhibition and a better mimic would be obtained by removing this OH-group. The N-butyl iminosugar **210** was a poor inhibitor for α-glucosidase from yeast ($IC_{50} = 1.5 \times 10^{-3}$ M), and β-glucosidase from almonds ($IC_{50} > 1.0 \times 10^{-2}$ M) [245]. The galactose-type iminosugar **212** was a less potent inhibitor of *Aspergillus oryzae* β-galactosidase ($IC_{50} = 1.8 \times 10^{-5}$ M) than the galacto-isofagomine (**207**). It inhibited weakly coffee bean α-galactosidase with $IC_{50} = 6.1 \times 10^{-4}$ M [243]. Iso-fuco-fagomine **213** exhibited a potent competitive inhibition of α-fucosidase (human placenta) with an inhibition constant $6.4 \times 10^{-6}$ M, less than 1-deoxy-fuconojirimycin which has been found to inhibit α-fucosidase from other sources up to $10^5$ times more strongly [238, 247]. A similar trend was observed for the inhibition of α-galactosidase by galactostatin and isogalacto-fagomine. The former compound was a strong inhibitor [207], while the latter was much weaker [243]. In general, α-glucosidases are more potently inhibited by hydroxypiperidines of the nojirimycin type while β-glycosidases were more potently inhibited by hydroxypiperidines of isofagomine type [248]. The aza sugars **213** and **214** showed moderate inhibition of almond β-glucosidase ($K_i = 1.2$ and $2.2 \times 10^{-4}$ M, respectively), and weakly inhibited both baker's yeast α-glucosidase and *E. coli* β-galactosidase ($K_i > 1.0 \times 10^{-3}$ M) [248]. Compound **215** showed a remarkably improved inhibition ($K_i = 2.3 \times 10^{-6}$ M) of β-glucosidase as compared to dNJ [249]. Various *N*-alkyl derivatives of aza sugars were shown to have different inhibition properties [116]. *N*-Methyl-1-deoxynojirimycin (**216**) inhibited *exo*-glucanase I and II with $K_i$ 1.2 and $1.5 \times 10^{-5}$ M, respectively. These values

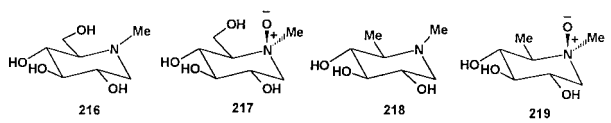


were 7- and 3-fold higher, respectively, for 1-deoxynojirimycin (**183**). *Exo*-glucanase from a Basidiomycete and *C. albicans* were inhibited by **216** ($K_i = 1.6 \times 10^{-4}$ M uncompetitively and $6.0 \times 10^{-5}$ M competitively, respectively) [220]. It inhibited α-glucosidase from brewer's yeast ($K_i = 3.7 \times 10^{-4}$ M), α-glucosidase I from calf liver ($K_i = 7.0 \times 10^{-8}$ M) [217], and β-glucosidase from sweet almond ($K_i = 4.3 \times 10^{-5}$ M), whereas its *N*-oxide **217** inhibited both α- and β-glucosidase from brewer's yeast and sweet almond with $K_i$ values $> 1.0 \times 10^{-2}$ and $8.0 \times 10^{-5}$ M, respectively. The deoxy analogue **218** inhibited the latter two enzymes with $K_i$ values $1.8 \times 10^{-3}$ and $1.4 \times 10^{-4}$ M, respectively. The *N*-oxide **219** also inhibited these two enzymes with $K_i$ values of 7.0 and $1.5 \times 10^{-3}$ M, respectively. These data indicated that **219** was a less potent inhibitor than **218** for β-glucosidase from sweet almond by one order of magnitude, although it was similar to **218** for the inhibition of α-glucosidase from brewer's yeast.

The *N*-oxide **217** was slightly less effective than **216** as a β-glucosidase inhibitor and no significant inhibition was observed for α-glucosidase indicating that the addition of an oxygen atom to the N perturbs the binding to the enzyme, resulting in a weaker complex. The results for compounds **217** and **219** referred to their zwitter ionic character, which may have a stronger electrostatic interaction with the putative active site carboxylate and carboxylic acid residues of glycosidases [154, 162, 250, 251]. For *in vivo* inhibition, *N*-alkylation, may facilitate transport of the inhibitor across the cell membrane, thereby increasing the effectiveness of the inhibition [252]. A comparison of the $K_i$ values of **197**, **216** and **218** indicated that the 6-OH group was important for binding, presumably through interaction with a hydrogen bond acceptor. In recent years, deoxynojirimycin and especially its *N*-butyl derivative **220** have attracted much attention as an α-glucosidase I inhibitors and agents against the HIV-virus [253]. The *N*-butyl-dNM **220** was shown to be a more potent lipophilic derivative than the *N*-methyl analogue **216** that showed antiviral activity both *in vitro* and in animal models. It was a potent inhibitor of porcine liver and calf liver α-glucosidase I ($IC_{50} = 5.7 \times 10^{-7}$ and $K_i$ $9.0 \times 10^{-8}$ M, respectively) [217, 254] and it was used to prevent lysosomal storage in Tay-Sachs mice [255]. The *N*-decyl derivative **221** has a potent glucosidase inhibitory activity in human hepatoma $HepG_2$ cells at $1.0 \times 10^{-3}$ M, but it was cell toxic at higher concentrations [253, 256], which was postulated to be due to the amphiphilic character of the molecule. 1,5-Dideoxy-1,5-(2-hydroxyethylimino)-D-glucitol (Miglitol) (**222**) was an effective α-glucosidase inhibitor, and it was used in clinical studies of type II diabetics. No biotransformation of Miglitol has been observed in rats, dogs or humans, and it was quantitatively excreted unchanged via urine [257].

From compounds containing an oxygen was inserted in the side chain (**223**–**228**), the *N*-(7-oxadecyl) **225** only showed complete inhibition of α-glucosidase I in $HepG_2$ cells as well as potent inhibition of HIV-1 virus with no toxic effect. *N*-7-Oxadecyl-dNM (**225**) inhibited purified porcine liver α-glucosidase I ($IC_{50} = 2.8 \times 10^{-7}$ M). It reduced adjuvant-induced arthritis in rats. The inhibition of intestinal α-glucohydrolase activity was a method for reducing the glycemic response from dietary carbohydrates and may be used for the treatment of diabetes mellitus [253]. The position of the oxygen atom in the *N*-decyl side chain was of importance since *N*-3-oxadecyl-dNM (**224**) was less active and, moreover, toxic to $HepG_2$ cells. It inhibited HIV-indused syncytia formation and lymphocyte proliferation *in vitro* and it was considered as a potential candidate for treating atoimmune diseases like rheumatoid arthritis [119]. New *N*-alkyl, alkenyl and benzyl substituted deoxynojirimycin derivatives bearing a silicon atom in their side chain appear to be potent and selective inhibitors of intestinal disaccharidases as well as of human lysosomal α-glucosidases. The inhibition was of "slow-tightbinding" type, anticipating a long-lasting inhibition of the enzymes *in vivo* [258]. Branching the *N*-alkyl side chain led to a remarkable reduction in the activity against α-glucosidase inhibition in $HepG_2$ cells and antiviral activity in HIV-1 infected cells compared to the straight alkyl chain, while elongation of the *N*-alkyl group caused high activity [37]. It has been shown that 1,5-dideoxy-1,5-[(6-deoxy-1-*O*-methyl-6-α-D-glucopyranosyl)imino]-D-glucitol (MDL 73945, **229**, a time dependent intestinal α-glucohydrolase inhibitor) acted nearly irreversible and was a potent inhibitor of sucrase ($IC_{50} = 2.0 \times 10^{-7}$ M), maltase ($IC_{50} = 1.0 \times 10^{-6}$ M), glucoamylase ($IC_{50} = 5.0 \times 10^{-6}$ M), isomaltase ($IC_{50} = 8.0 \times 10^{-6}$ M). The reduction in the glycemic response to a sucrose was accompanied by a reduced insulin secretion. MDL 73945 was slightly less effective against a starch load, but it was more effective against a sucrose load in streptozocin-treated rats than in control rats. It effectively reduced the glycemic and insulin responses to sucrose in cynomolgus monkeys and had a long duration of action [259]. Doses that reduced the glycemic response to carbohydrate did not inhibit liver lysosomal α-glucosidase activity or cause lysosomal glycogen accumulation.

2-Deoxy-2-fluoro derivative **230** showed no significant inhibition against α- and β-glucosidase and α-mannosidase [116] ($K_i > 1.0 \times 10^{-3}$ M) at the concentration of $1.0 \times 10^{-3}$ M. These results indicated that the 2-OH group



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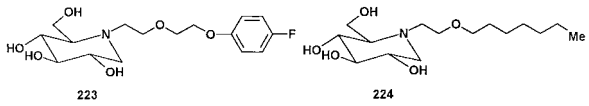

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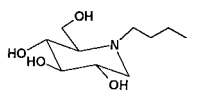

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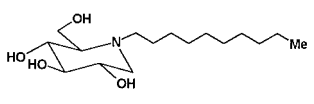

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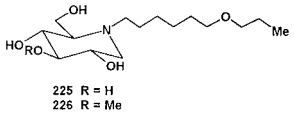
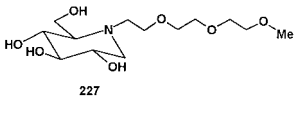


225 R = H
226 R = Me



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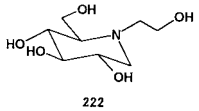

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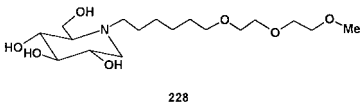

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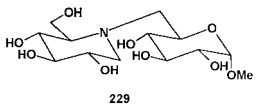


229

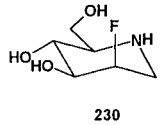
may interact with a hydrogen-bond acceptor in the active site. 1,2,5-Trideoxy-2-fluoro-1,5-imino-D-glucitol (**231**) weakly inhibited yeast α-glucosidase ($K_i = 2.0 \times 10^{-3}$ M) [260]. It was a very weak inhibitor of almonds β-glucosidase ($K_i > 1.0 \times 10^{-2}$ M), and *Asp. wentii* β-glucosidase ($K_i = 2.8 \times 10^{-3}$ M). It competitively inhibited *Agrobacterium faecalis* ($K_i = 3.5 \times 10^{-4}$ M) and bovine kidney, lysosomal β-glucosidase ($K_i = 1.8 \times 10^{-4}$ M). 1,3,5-Trideoxy-3-fluoro-1,5-imino-D-glucitol (**232**) and 1,5,6-trideoxy-6-fluoro1,5-imino-D-glucitol (**233**) were competitive inhibitors against yeast α-glucosidase. Glucosidase inhibitory activity was drastically reduced when the C-6 hydroxyl group was replaced by a fluorine substituent [261]. Compound **233** inhibited this enzyme ($K_i = 1.9 \times 10^{-5}$ M) strongly and more than **232** ($K_i = 2.5 \times 10^{-3}$ M). Compounds **232** ($K_i = 3.5 \times 10^{-7}$ M) and **233** ($K_i = 4.0 \times 10^{-7}$ M) could be bind strongly to rice α-glucosidase, but they were very weak inhibitors of almonds β-glucosidase ($K_i > 1.0 \times 10^{-2}$ and $6.0 \times 10^{-4}$ M, respectively). Compound **232** was a good inhibitor against bovine kidney, lysosomal β-glucosidase ($K_i = 2.9 \times 10^{-5}$ M, pH 6.0). It competitively inhibited *Asp. wentii* β-glucosidase ($K_i = 1.6 \times 10^{-4}$ M) at pH 6.0. Compounds **232** and **233** have relatively similar inhibition activities against *Asp. wentii* β-glucosidase at pH 5.0 ($K_i = 3.8$ and $2.5 \times 10^{-4}$ M, respectively) [260]. These results showed that the replacement of a hydroxyl function by fluorine caused an impairment of the inhibitory potency. This effect was smallest for the hydroxyl group at C-6 and up to four orders of magnitude larger for replacement at C-2 and C-3. The *in vitro* evaluation of 1,5,6-trideoxy-6,6-difluoronojirimycin (**234**) with yeast α-glucosidase and almond β-glucosidase indicated that they were inhibited weakly by **234** ($K_i = 7.5$ and $8.7 \times 10^{-3}$ M in a competitive and non-competitive manner, respectively) [262]. Since the difluoromethyl group is electron-withdrawing, it may destabilize the positive charge that was formed upon protonation, thereby possibly accounting for the weak inhibition against yeast α-glucosidase.

2-Acetamido-1,2-dideoxynojirimycin (**235**) inhibits β-N-acetyl-D-glucosaminidases from bovine kidney, *Helix pomatia,* and jack beans ($K_i = 6.0 \times 10^{-4}$, $8.0 \times 10^{-2}$, and $1.4 \times 10^{-4}$ M, respectively). Replacement the acetyl moiety by a fluoroacetyl group caused a dramatic impairment of affinity ($K_i = 2.0 \times 10^{-2}$ and $4.3 \times 10^{-3}$ M) with bovine kidney and jack beans *N*-acetyl glucosaminidases, respectively [263]. Previously, **235** was reported to be an efficient inhibitor of bovine β-*N*-acetyl-D-glucosaminidase ($K_i = 6.0 \times 10^{-7}$ M) [118] and ($K_i = 3.8 \times 10^{-7}$ M) [116]. **235** and **236** were also considered as potent inhibitors of jack bean *N*-acetyl-hexosaminidase ($K_i = 1.4–2.3 \times 10^{-7}$ and $1.2 \times 10^{-9}$ M, respectively) [118], while analogue **237** was relatively poorer ($IC_{50} = 1.0 \times 10^{-4}$ M) [264]. 2-Acetamido-1,2-dideoxymannojirimycin (**238**) exhibited no inhibition against α-D-mannosidase (jack been) and β-N-acetyl-D-glucosaminidase (bovine kidney) [265]. Inhibition studies of the 1-β-amino-1-deoxynojirimycins **239** and **240** indicated that the *gem*-diamine **239** exhibited competitive inhibition against almond β-glucosidase ($K_i = 4.0 \pm 0.3 \times 10^{-5}$ M) [266]. The inhibition of β-glucosidase by **239** was more potent than with 1-deoxynojirimycin ($K_i = 7.6 \times 10^{-5}$ M at pH 5.6) [63]. The *N*-benzyl derivative **240** also inhibited β-glucosidase ($K_i = 2.0 \pm 0.5 \times 10^{-5}$ M), although the increase in activity was due to the binding in the hydrophobic aglycon pocket of the enzyme. Neither diamine **239** nor **240** inhibited yeast α-glucosidase, jack bean α-mannosidase, green coffee bean α-galactosidase or bovine liver β-galactosidase. 3-Hydroxymethyl-3-decarboxy-siastatin B (**241**) showed $IC_{50}$ values of $4.2 \times 10^{-8}$ and $2.7 \times 10^{-7}$ g ml$^{-1}$ against β-*N*-acetylglucosaminidase and α-N-acetylgalactosaminidase, respectively [267]. The piperidine carboxylic acid **242** has been shown to have a marked inhibitory activity against β-glucosiduronase and exhibited a potent inhibition of an experimental pulmonary metastatic B16 line (B16 BL6) [268, 269].

The trihydroxypipecolic acids such as the glucuronic acid analogue BR1 (**243**) and the mannuronic acid equivalent **244** were found to be glycosidase inhibitors [270]. D-Gluconolactam (**245**) displayed significant competitive inhibition against β-glucosidase. It exhibited a certain degree of inhibition against sweet almond β-glucosidase ($K_i = 3.7 \pm 0.6 \times 10^{-5}$ M at 27 °C, pH 6.2) [63], and *Aspergillus wentii* β-glucosidase ($K_i = 3.6 \times 10^{-5}$ M at 25 °C, pH 4.0) [129]. Recently, it was evaluated as a moderatly strong inhibitor of almond β-glucosidase ($K_i = 1.3 \times 10^{-4}$ M at pH 6.8), bovine lysosomal and cytosolic β-glucosidases ($K_i = 1.2 \times 10^{-4}$ and $2.6 \times 10^{-6}$ M, respectively) [210], while it strongly inhibited *A. faecalis* β-glucosidase ($K_i = 5.2 \times 10^{-6}$ M) in neutral medium. A weak inhibition was also observed by **245** against rabbit intestinal sucrase (α-glucosidase) with a $K_i$ value of $2.3 \times 10^{-2}$ M [271], and yeast α-glucosidase ($K_i = 1.1 \times 10^{-3}$ M). The NH function of **245** led to the interaction with this enzyme. Fuconolactam (**246**) was a glycosidase inhibitor [270]. The neutral molecule **247** exhibited only moderate inhibition ($K_i = 8.0 \times 10^{-4}$ M) against sweet almond β-glucosidase [116].

Some of the 1,5-dideoxy-1,5-iminoheptitols with L-glycero-D-manno, D-glycero-L-gulo and L-glycero-D-altro configurations **248, 249** and **250** have been synthesized [272] to be tested and used as glycosidase inhibitors. Inhibition studies of the 1,5-dideoxy-1,5-iminooctitols **251**–**254** showed that the iminooctitols (+)-**253** and (+)-**254** were weak inhibitors of β-glucosidase from almond and β-galactosidases from *Aspergillus niger, Aspergillus orizae* and jack bean [273], while (−)-**251** and (−)-**252** did not show any significant inhibition towards fifteen commercially available glycosidases [274]. Compound **255** was a potent inhibitor of yeast α-glucosidase ($K_i = 3.0 \times 10^{-6}$ M) [275].

Glycosidase inhibition measurements [276] of (+)-3,7,8-trideoxy-3,7-imino-D-threo-L-galactooctitol (+)-**256** showed that this compound did not inhibit bovine epididymis α-L-fucosidase, *Aspergillus niger* and *Escherichia coli* α-galactosidases, coffee beans, *Aspergillus niger, Escherichia coli,* bovine liver and *Aspergillus orizae* β-galactosidases, yeast and rice maltases, isomaltase from baker's yeast, *Aspergillus niger* and *Rhizopus* mold amyloglucosidase, jack beans and almond α-mannosidases, *Helix pomatia* β-mannosidase, *Aspergillus niger* β-xylosidase and α-*N*-acetylhexosaminidases from chicken liver, from jack beans and from bovine epididymis. A weak activity was



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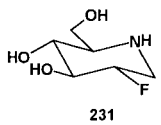

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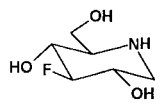

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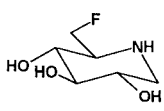

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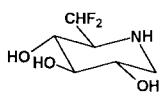

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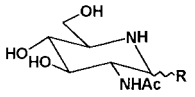
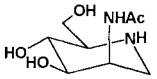


235 R = H
236 R = OH

237



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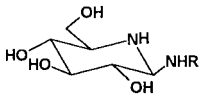
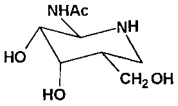


239 R = H
240 R = Bn



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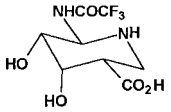

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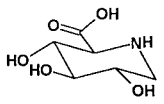

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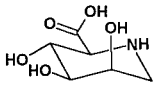

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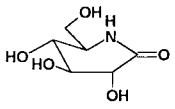

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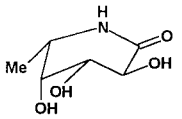

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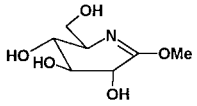

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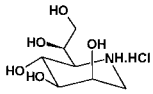

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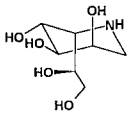

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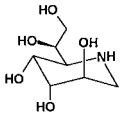

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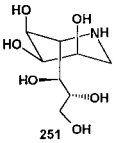

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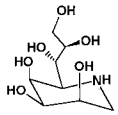

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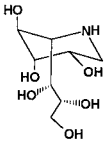

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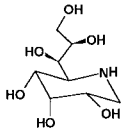

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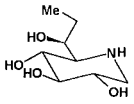
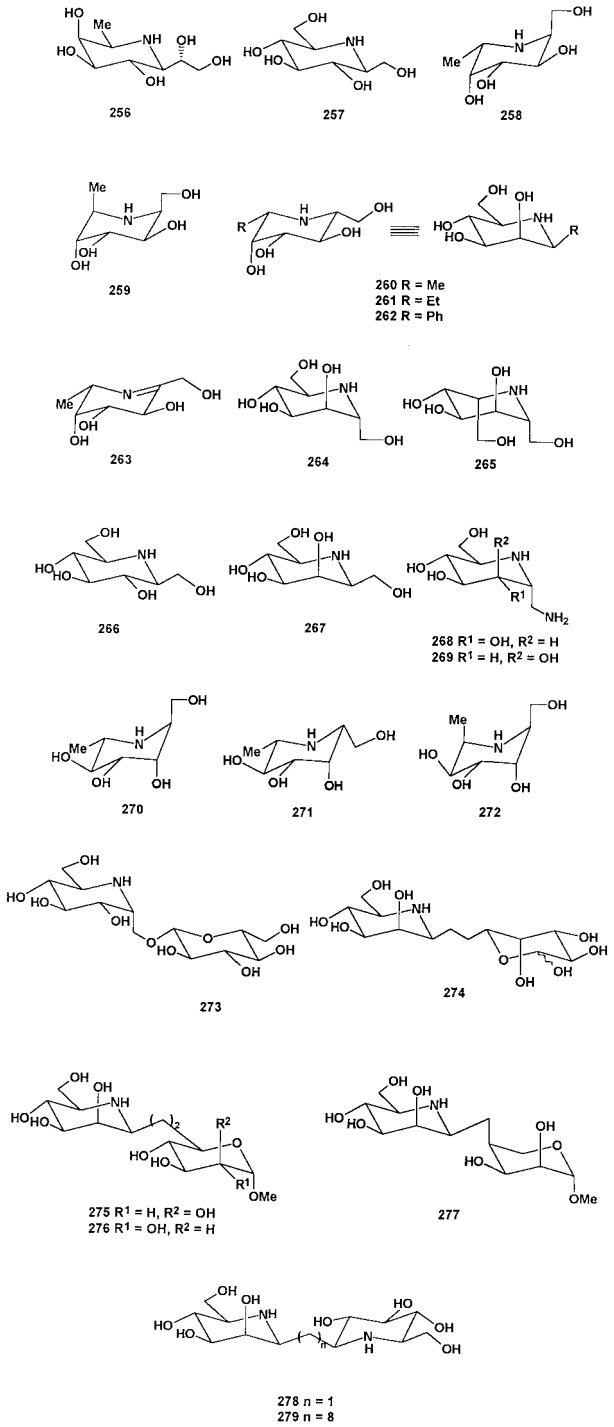


255

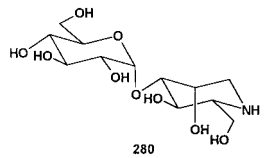
detected for the α-galactosidase from coffee beans (34% inhibition at $1.0 \times 10^{-3}$ M concentration of (+)-**256**). However for the hydrolysis of *p*-nitrophenyl β-**D-**glucopyranoside catalyzed by β-glucosidase from almond (pH 4.5, 37 °C) ($IC_{50} = 9.8 \times 10^{-5}$ M) was found to have a $K_i$ of $1.5 \times 10^{-5}$ M. Under similar conditions, the β-glucosidase from *Caldocellum saccharolyticum* was inhibited ($IC_{50} = 1.1 \times 10^{-4}$ M and $K_i = 4.1 \times 10^{-5}$ M). The two latter glycosidases accepted (+)-**256** as a reversible inhibitor. Compound **257** showed a preference for inhibiting β-glucosidase [277]. The α-L-homofuconojirimycin (**258**) was a powerful inhibitor against a number of α-fucosidases ($K_i \approx 1.0 \times 10^{-8}$ M) [278]. 6-*Epi*-α-L-homofuconojirimycin (**259**) as well as β-L-homofuconojirimycin (**260**), which may also be considered as β-methyl deoxymannojirimycin, together with β-ethyl **261** and β-phenyl **262** analogues were found to be potent and specific competitive inhibitors of human liver α-L-fucosidase with inhibition constants of $5.0 \times 10^{-6}$, $1.0 \times 10^{-8}$, $7.0 \times 10^{-8}$ and $1.0 \times 10^{-6}$ M, respectively [279]. None of these compounds caused any significant inhibition of mannosidase activity. The six-membered ring ketimine **263** exhibited the most potent inhibitory activity against α-L-fucosidase from bovine epididymis with a $K_i$ of $6.9 \times 10^{-9}$ M [280] as its parent iminosugar **260** ($K_i = 5.6 \times 10^{-9}$ M) [281]. The inhibition studies of α-homomannojirimycin (HMJ, **264**), 6-*epi*-HMJ (**265**) and DMJ (**185**) showed that, **265** did not inhibit any lysosomal, Golgi II and neutral α-mannosidase, indicating that the correct configuration at C-5 is essential for the inhibition of α-mannosidase [230, 282]. In contrast, DMJ (**185**) and 6-*epi*-HMJ (**265**) were powerful inhibitors of human liver α-fucosidase ($K_i = 5.0$ and $4.5 \times 10^{-6}$ M, respectively), whereas HMJ (**264**) was a weak inhibitor for this enzyme (29% at $1.0 \times 10^{-3}$ M). The specificity and potency of inhibition of human α-mannosidases by HMJ (**264**) and DMJ (**185**) were very similar where their inhibition ranged from 21 to 58%. Neither compound **264** nor **265** inhibited β-mannosidase. β-1-Homonojirimycin (HNJ, **266**) inhibited α- and β-glucosidases ($K_i = 9.0$ and $4.3 \times 10^{-4}$ M, respectively) more than α- and β-mannosidases, whereas β-1-homomannojirimycin (**267**) inhibited β-mannosidase strongly ($K_i = 8.0 \times 10^{-5}$ M) and β-glucosidase much less [277]. *N*-Methylhomonojirimycin (MHNJ) was found to be a good inhibitor of glucosidase I ($K_i = 1.0 \times 10^{-6}$ M) and was about three times as effective on this enzyme as was homonojirimycin (HNJ). On the other hand, HNJ inhibited glucosidase II with a $K_i$ of about $1.0 \times 10^{-6}$ M, whereas MHNJ was three times less effective ($K_i = 1.0 \times 10^{-5}$ M). However, the butyl derivative of HNJ had very low activity towards these two processing glucosidases [283]. Compound **268** was an inhibitor of α-glucosidase ($K_i = 3.1 \times 10^{-5}$ M), while its analogue **269** was an inhibitor of α-mannosidase ($K_i = 5.0 \times 10^{-4}$ M) [234]. α-Homorhamnojirimycin (**270**) was a potent inhibitor ($K_i = 5.3 \times 10^{-6}$ M, $IC_{50} = 1.5 \times 10^{-5}$ M), while β-homo anomer **271** and the homo-*epi*-isomer **272** were much weaker inhibitors ($IC_{50} = 7.3$ and $8.5 \times 10^{-4}$ M, respectively) against naringinase with a competitive manner. However, **271** was found to be a powerful inhibitor of coffee bean α-galactosidase with $IC_{50} = 4.0 \times 10^{-6}$ M [237].

2,6-Dideoxy-2,6-imino-7-*O*-β-D-glucopyranosyl-D-glycero-L-gulo-heptitol (**273**) was a transition-state inhibitor of rat intestinal α-glucohydrolases *in vitro*. The order of inhibition was trehalase ($IC_{50} = 2.5 \times 10^{-7}$ M) > isomaltase ($IC_{50} = 7.0 \times 10^{-7}$ M) > sucrase ($IC_{50} = 3.5 \times 10^{-6}$ M) > glucoamylase ($IC_{50} = 6.3 \times 10^{-6}$ M) > maltase ($IC_{50} = 9.6 \times 10^{-6}$ M). It was a much less effective inhibitor of α-amylase and lactase ($IC_{50} = 1.0 \times 10^{-4}$ M for both enzymes) [284]. It inhibited the rise in serum glucose after a sucrose or starch load but not after a glucose load. It also reduced the glycemic response to sucrose in rats and has been identified as a drug candidate for antidiabetic therapy [284]. Its hydrochloride derivative was found to be a potent competitive inhibitor for intestinal sucrase ($K_i = 2.0 \times 10^{-6}$ M), and also inhibited maltase, trehalase, glucoamylase and α-amylase [7]. The aza-C-disaccharide **274** mimics the disaccharide Man(β-1,6)Gal and it was proposed to be inert towards acidic or enzymatic hydrolysis while presenting the strong binding properties of the parent azasugar dNM. The synthesis of oligosaccharide analogues containing various glycoside-linked dNMs and related moieties at the reducing end have also been reported [285]. The aza-C-disaccharides **275**–**278** inhibited amyloglucosidase, where **276** exhibiting the best activity ($IC_{50} = 1.2 \times 10^{-5}$ M) followed by **275** ($IC_{50} = 2.5 \times 10^{-5}$ M) and **278** ($IC_{50} = 2.6 \times 10^{-5}$ M) and the weakest one was **277**




($IC_{50} = 1.5 \times 10^{-4}$ M). Interestingly, derivative **277** exhibited the weakest amyloglucosidase inhibition but it was the only compound that inhibited both yeast α-glucosidase and α-galactosidase ($IC_{50} = 2.0$ and $3.3 \times 10^{-4}$ M, respectively) [286]. The *bis*-azasugar **279** inhibited both amyloglucosidase and α-mannosidase with $IC_{50}$ values of 2.0 and $4.9 \times 10^{-5}$ M, respectively [287].

1-Deoxy-3-O-(α-D-glucopyranosyl)mannojirimycin (**280**) effectively inhibited glycoprotein-processing hydrolase, *endo*-mannosidase ($IC_{50} = 1.7 \times 10^{-6}$ M), *in vitro*. All the hydroxyl groups of the deoxymannojirimycin unit of **280**, namely, OH-2, OH-4, OH-6, and the NH-5 group, interact with the charged and polar groups of the enzyme, since the deoxygenation and alkylation reduced the activity [288–292]. It was proved to be 90 times stronger as an inhibitor of *endo*-mannosidase than its 5-oxygen analogue **98**, which was derived from α-D-Glc(1 → 3) D-Man by deoxygenation at the 1-position [290]. Apparently, the interaction of NH-5 from **280** with the protein induced a different conformational change in the catalytic region of the protein than when O-5 was at its place [137]. It was found that 4-O-α-D-glucopyranosylmoranoline (**281**) and its *N*-substituted derivatives **282**–**290** had strong inhibitory activity against rabbit sucrase but only weak activity against rabbit maltase. The $IC_{50}$ values of sucrase inhibition ranged from 1.0 to $7.2 \times 10^{-5}$ M. Lengthening the chain moiety of alkyl derivatives **282**–**285**, aralkyl derivatives **288** or phenoxyalkyl derivatives **289** did not considerably change the inhibitory activity. The introduction of a bromine atom or a methyl group at the *para* position of **288** (n = 1) did not change the inhibitory activity as seen in **286** and **287**. The introduction of an unsaturated bond into **288** (n = 3) did not affect the activity as shown in **290**. The $IC_{50}$ values for maltase inhibition varied much more than those for sucrase inhibition. The inhibitory activity against maltase of **282**, **284** and **286** was more potent than that of the other derivatives. The $K_i$ values of **281** for rabbit maltase and sucrase (competitive inhibition) were $2.6 \times 10^{-5}$ and $3.8 \times 10^{-6}$ M, respectively. All these compounds had potent postprandial hypoglycemic activity in the sucrose loaded rat, and compound **281** has potent hypoglycemic activity in starch-loaded dogs [293]. The 4-O-α-D-glucopyranosylmoranoline (**281**) and its N-substituted derivatives (**282**–**287**) are specific inhibitors of glucohydrolases and have a potential antidiabetic activity [293]. Compound **281** was a potent inhibitor against rice α-glucosidase ($IC_{50} = 6.1 \times 10^{-7}$ M), but it weakly inhibited rat liver lysosomal α-glucosidase ($IC_{50} = 4.4 \times 10^{-4}$ M) [218]. It inhibited β-glucosidase at nearly the same concentration ($IC_{50} = 8.0$ and $5.0 \times 10^{-5}$ M) for almond and *Caldocellum saccharolyticum*, respectively [218]. Simple aza sugars like 1-deoxynojirimycin were considered to be too small to inhibit *endo*-glycosidases, which possess 4–6 subsites for binding with individual sugar rings in a long polysaccharide chain. So the incorporation of an aza sugar moiety into an oligosaccharide framework proved to be a useful approach to the design of *endo*-cellulase inhibitors. The inhibition studies of some α- and β-D-glucosides of 1-deoxynojirimycin against various α-, β-glucosidases and trehalase showed that 2-O-α-D-glucopyranosyl-1-deoxynojirimycin (**291**) potently inhibited rice α-glucosidase and porcine kidney trehalase ($IC_{50} = 1.6$ and $5.6 \times 10^{-6}$ M, respectively). It moderately inhibited *Caldocellum saccharolyticum* β-glucosidase ($IC_{50} = 2.3 \times 10^{-4}$ M), whereas it weakly and similarly inhibited rat liver lysosomal α-glucosidase and almond β-glucosidase ($IC_{50} = 1.0 \times 10^{-3}$M) [218]. 3-*O*-α-D-Glucopyranosyl-1-deoxynojirimycin (**292**) exhibited very potent inhibition of rice α-glucosidase ($IC_{50} = 3.4 \times 10^{-8}$ M), but its inhibition of rat liver lysosomal α-glucosidase was less pronounced ($IC_{50} = 2.5 \times 10^{-5}$ M) [218]. 2-*O*-β-D-Glucopyranosyl-1-deoxynojirimycin (**293**) inhibited only rice α-glucosidase ($IC_{50} = 2.3 \times 10^{-4}$ M) [218]. 3-*O*-β-D-Glucopyranosyl-1-deoxynojirimycin (**294**) inhibited the rice α-glucosidase ($IC_{50} = 3.0 \times 10^{-5}$ M) [226], but it did not inhibit baker's yeast α-glucosidase. 4-*O*-β-D-Glucopyranosyl-1-deoxynojirimycin (**295**) was a good inhibitor of rice α-glucosidase ($IC_{50} = 2.2 \times 10^{-5}$ M), but it weakly inhibited rat liver lysosomal α-glucosidase ($IC_{50} = 1.0 \times 10^{-3}$ M). It also inhibited *Caldocellum saccharolyticum* β-glucosidase and porcine kidney trehalase ($IC_{50} = 5.6$ and $6.0 \times 10^{-4}$ M, respectively) [218]. 6-*O*-β-D-Glucopyranosyl-1-deoxynojirimycin (**296**) moderately inhibited



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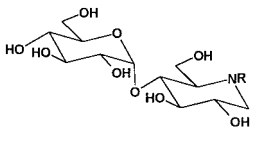
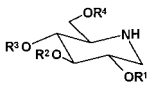
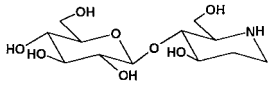


281 R = H 282 R = Me
283 R = Et 284 R = Pr
285 R = Bu 286 R = *p*-Br Bn
287 R = *p*-MeBn 288 R= -$(CH_2)_n$Ph, n = 1,2,3
289 R = -$(CH_2)_n$OPh, n = 2,3,4
290 R = - $CH_2CH$=CHPh



| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| 291 | α-D-Glc | H | H | H |
| 292 | H | α-D-Glc | H | H |
| 293 | β-D-Glc | H | H | H |
| 294 | H | β-D-Glc | H | H |
| 295 | H | H | β-D-Glc | H |
| 296 | H | H | H | β-D-Glc |
| 297 | α-D-Gal | H | H | H |
| 298 | H | H | H | α-D-Gal |

Glc = glucopyranosyl
Gal = galactopyranosyl



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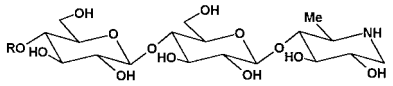
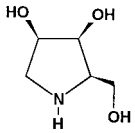


300 R = H
301 R = β-1,4-glucopyranosyl

the rice α-glucosidase ($IC_{50} = 5.4 \times 10^{-4}$ M), but it did not inhibit baker's yeast α-glucosidase [226]. The glucoside derivatives **281**, **291**–**293** and **295** exhibited no significant inhibitory activity against baker's yeast α-glucosidase [218]. These results indicated that the addition of a glucosyl residue to 1-deoxynojirimycin resulted in a significanty decreased of inhibitory activity against rat liver lysosomal α-glucosidase, but the presence of a α-glucopyranosyl residue at C-2 and C-4 of 1-deoxynojirimycin as in **291** and **281** led to an increase activity against porcine kidney trehalase and β-glucosidase, respectively [218]. 2-*O*-(α-D-Galactopyranosyl)-1-deoxynojirimycin (**297**) potently inhibited rice α-glucosidase ($IC_{50} = 9.5 \times 10^{-7}$ M), but no inhibition of baker's yeast α-glucosidase was observed. On the other hand, it showed an $IC_{50}$ of $5.2 \times 10^{-5}$ M against porcine kidney trehalase [226]. 6-*O*-(α-D-Galactopyranosyl)-1-deoxynojirimycin (**298**) was a potent inhibitor of rice α-glucosidase ($IC_{50} = 6.0 \times 10^{-6}$ M), but it was a moderate inhibitor of baker's yeast α-glucosidase ($IC_{50} = 2.3 \times 10^{-4}$ M) [226]. 4-O-(β-D-Glucopyranosyl)fagomine (**299**) failed to show inhibition up to $1.0 \times 10^{-3}$ M against α- and β-glucosidases (yeast and apricot emulsin), α-mannosidase (*Canavalia ensiformis*), α- and β-galactosidases (*Aspergillus niger*), β-glucuronidase (*Helix pomatia*), α-fucosidase (bovine epididymis) and β-xylosidase (*Aspergillus niger*) [224]. The oligomers **300** and **301** were powerful mixed-competitive inhibitors of several *endo*-cellulases from the aerobic, thermophilic soil bacterium *Thermomonospora fusca* [46].

### *16.3. Pyrrolidine analogues*

Some 1,4-iminopentitols have been found to be powerful glycosidase inhibitors. Thus, 1,4-dideoxy-1,4-imino-D-lyxitol (**302**) showed a highly potent competitive inhibition of α-D-galactosidase from green coffee beans ($IC_{50} = 2.0 \times 10^{-7}$ M) [182] and a moderate inhibition of α-D-mannosidase from jack bean ($IC_{50} = 1.4 \times 10^{-5}$ M). The naturally occurring 1,4-dideoxy-1,4-imino-D-arabinitol (**303**) was found to be a strong inhibitor of yeast α-D-glucosidase ($IC_{50} = 1.8 \times 10^{-7}$ M) [182], rather than the synthetic L-isomer **305** ($IC_{50} = 1.0 \times 10^{-5}$ M) [182]. Compound **303** was a potent competitive inhibitor of ER α-



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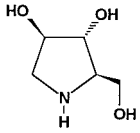

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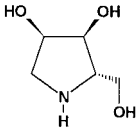

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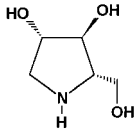

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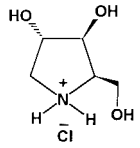

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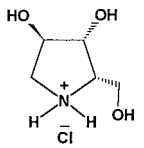
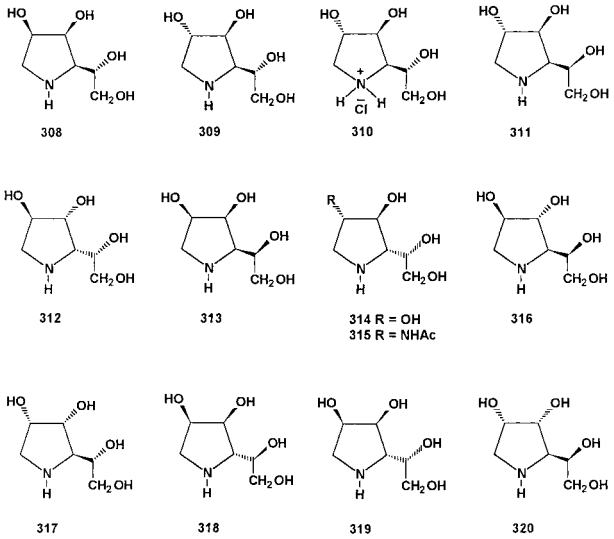


307

glucosidase II ($K_i = 9.7 \times 10^{-6}$ M) [23]. It was a good inhibitor of Golgi α-mannosidase II in a competitive manner ($K_i = 3.5 \times 10^{-5}$ M) [23], but it was a weak inhibitor of lysosomal and epididymal α-mannosidases as well as jack bean α-mannosidase ($IC_{50} = 1.1 \times 10^{-4}$, $8.4 \times 10^{-5}$ and $1.0 \times 10^{-4}$ M, respectively), and did not inhibit soluble (or ER) α-mannosidases [294]. Furthermore, **302** has no significant anti-HIV activity [187, 295]. The inhibition of a number of mouse gut disaccharidases has shown that the L-isomer **305** was a more potent inhibitor than the D-isomer **303**. The concentrations required from **305** to cause 50% inhibition of the hydrolysis of the 6-O-α-glucopyranosyl disaccharides isomaltose and palatinose were $6.6 \times 10^{-8}$ and $2.4 \times 10^{-7}$ M, in comparison to the values of $4.0 \times 10^{-6}$ and $1.3 \times 10^{-5}$ M, respectively for the D-isomer **303** [225]. 1,4-Dideoxy-1,4-imino-L-ribitol (**304**) was a glycosidase inhibitor [296] and it was evaluated as immunostimulatory agent [297]. 1,4-Dideoxy-1,4-imino-D-xylitol hydrochloride (**306**) and its enantiomer **307** showed similar inhibition of almond emulsin β-glucosidase with $K_i$ values of 7.1 and $7.3 \times 10^{-3}$ M, respectively and with various other glucosidases, low non-specific inhibition was found for both enantiomers [298].

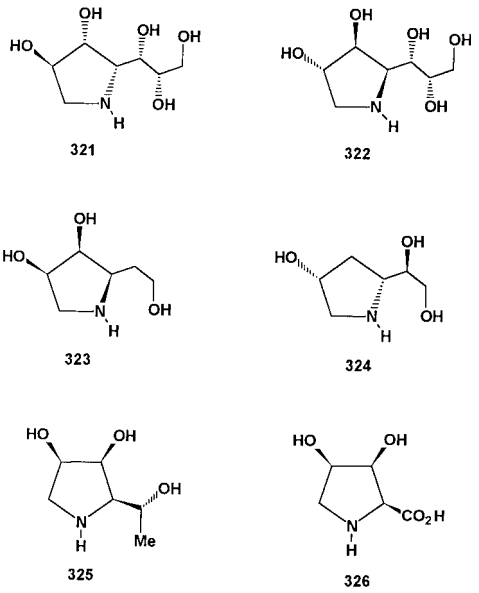
The azafuranose analogue of mannose, 1,4-dideoxy-1,4-imino-D-mannitol (**308**) was a powerful inhibitor of mannosidase, both *in vitro* [299] and *in vivo* [300]. It inhibited glycoprotein processing mannosidase. It was a potent competitive inhibitor of jack bean α-mannosidase ($K_i = 7.6 \times 10^{-7}$ M, $IC_{50} = 5.0 \times 10^{-7}$ M) [182, 299]. It exhibited a weak inhibition of yeast α-glucosidase ($IC_{50} = 5.0 \times 10^{-4}$ M) and almond emulsin β-glucosidase ($IC_{50} = 4.5 \times 10^{-4}$ M) [182, 299]. It also inhibited green coffee beans α-galactosidase ($IC_{50} = 4.0 \times 10^{-4}$ M) and *Asp. niger* β-galactosidase ($IC_{50} = 1.6 \times 10^{-4}$ M) [182]. 1,4-Dideoxy-1,4-imino-D-glucitol (**309**) was found to be a glycosidase inhibitor [270], and it had been expected that its hydrochloride salt **310** might well be a powerful glucosidase inhibitor, on the basis of molecular modeling studies. However it was found to be a weak inhibitor of almond emulsin β-glucosidase ($K_i = 1.3 \times 10^{-3}$ M) and *Helix pomatia* β-gluco- and galactosidase [298], and it was a slight activator of *Bacillus* α-glucosidase. 1,4-Dideoxy-1,4-imino-L-iditol (**311**) was a potent inhibitor of α-D-galactosidase and a weak inhibitor of α-D-arabinosidase (95 and 62%, respectively at $1.0 \times 10^{-3}$ M) [301]. The enantiomer 1,4-dideoxy-1,4-imino-D-iditol (**312**) was a moderate inhi-

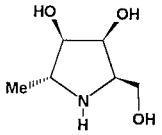



bitor of α-L-fucosidase (69% at $1.0 \times 10^{-3}$ M). The L-gulitol analogue **313** did not inhibit any of the glycosidases [302]. Although the configurations of 1,4-dideoxy-1,4-imino-D-galactitol (**314**) and its L-galactitol enantiomer **316** were similar to the 1,4-dideoxy-1,4-imino-L-and D-arabinitol, respectively [303], compound **314** weakly inhibited α-D-glucosidase ($IC_{50} = 1.0 \times 10^{-3}$ M), and **316** inhibited it very slightly [301], in spite of the strong inhibition that was exhibited by the imino arabinitol analogues [182]. They exhibited no inhibition against α- or β-galactosidases, and this lack of inhibition suggested that these enzymes were not particularly susceptible to aza furanose analogues of galactose [301]. The 1,4-dideoxy-1,4-imino-D-allitol (**317**) was a weak inhibitor of β-D-glucosidase ($IC_{50} = 1.0 \times 10^{-3}$ M) [304], whereas 1,4-dideoxy-1,4-imino-L-allitol (**318**) was a moderate competitive inhibitor of lysosomal α-D-mannosidase ($K_i = 1.2 \times 10^{-4}$ M) [304, 305] and a weak inhibitor of neutral cytosolic α-D-mannosidase, β-D-glucosidase, as well as N-acetyl-β-D-hexosaminidase and α-L-fucosidase [301]. Neither of the 1,4-dideoxy-1,4-imino-allitols had any effect on the human immunodeficiency virus (HIV) [187]. 1,4-Dideoxy-1,4-imino-D-talitol (**319**) has a similar specificity of inhibition to **318** against human liver lysosomal α-D-mannosidase ($K_i = 1.2 \times 10^{-4}$ M) [296, 304], and a weak/moderate inhibition of α-L-fucosidase [301]. At a concentration of $1.0 \times 10^{-3}$ M of **319**, 80% inhibition of α-mannosidase activity was observed. Human liver neutral α-mannosidase was also inhibited by **319** ($IC_{50} = 1.0 \times 10^{-3}$ M at pH 6.5) [296]. It has blocked the lysosomal catabolism of asparagine-linked glycans of glycoproteins. *In vivo*, it was not a strong inhibitor of Golgi α-mannosidase I, of endoplasmic reticulum α-mannosidase, or of Golgi α-mannosidase II [296]. These observations were consistent with those of the *in vitro* specificity of the iminotalitol **319**. 1,4-Dideoxy-1,4-imino-L-talitol (**320**) did not inhibit any of the glycosidases appreciably [298]. The immunostimulating activities of **302**, **304**, **308** and **319** were determined in terms of the capacity to restore the depression of mitogenic responses of mouse spleen cells by immunosuppressive factors in tumor-bearing mouse serum [306]. The minimal effective concentrations of these derivatives were $1.6 \times 10^{-5}$, $1.6 \times 10^{-5}$, $1.3 \times 10^{-5}$ and $4.0 \times 10^{-6}$ g ml$^{-1}$, respectively and it was less than that of swainsonine ($1.0 \times 10^{-8}$ g ml$^{-1}$) [297]. It was found that the configuration at the 2-position in the pyrrolidine compounds seemed to be important for the immunostimulant activity. Thus, the 2*R* derivatives **304** and **319** were more active than their 2*S* counterparts **302** and **308**. This result suggested that the *R*-configuration at C-8a of swainsonine was important for the activity. The inhibition study of human liver glycosidase showed that only N-acetyl-β-D-glucosaminidase (82%) and N-acetyl-β-D-galactos-aminidase (59%) activities were inhibited by the pyrrolidine **315** at a concentration of $1.0 \times 10^{-3}$ M. It was a reversible competitive inhibitor of hexosaminidase towards 4-methylumbelliferyl-*N*-acetylglucosaminide and *N*-acetylgalactosaminide substrates with $IC_{50}$ values of 2.5 and $6.0 \times 10^{-4}$ M and $K_i$ values of 1.0 and $2.0 \times 10^{-4}$ M, respectively at pH 4.0 [307].

The 1,4-iminoheptitol **321** was found to be a powerful inhibitor of yeast α-glucosidase ($K_i = 1.6 \times 10^{-6}$ M) [24] and almond β-glucosidase ($K_i = 1.8 \times 10^{-5}$ M) [24]. Its isomeric analogue **322** showed a strong inhibition against α- and β-glucosidases ($K_i = 1.0$ and $1.2 \times 10^{-5}$ M, respectively). The hydroxypyrrolidine **323** exhibited no significant inhibition against jack bean α-mannosidase [308], whereas **324** exhibited a moderate inhibition of this enzyme ($K_i = 2.5 \times 10^{-4}$ M). No inhibition of α- or β-galactosidase was observed with **324** [308]. 1,4,6-Trideoxy-1,4-imino-D-mannitol (**325**) was found to be a potent competitive inhibitor of jack bean α-mannosidase ($K_i = 5.0 \times 10^{-7}$ M, $IC_{50} = 6.0 \times 10^{-7}$ M) [309]. It weakly inhibited almond β-glucosidase at $1.0 \times 10^{-3}$ M, but it did not exhibit inhibition of all other enzymes tested (green coffee α-galactosidase, bovine β-galactosidase, bovine liver β-glucosidase and bovine β-*N*-acetylhexosaminidase) at $1.0 \times 10^{-3}$ M [309]. Dihydroxypyrrolidine **326** was synthesized as a glycosidase inhibitor [270].

The pyrrolidine **327** was a strong competitive inhibitor of α-D-mannosidase and α-L-fucosidase (83% and 95% inhibition at $1.0 \times 10^{-3}$ M, $K_i = 5.3 \times 10^{-5}$ M and $9.0 \times 10^{-6}$ M, respectively), but a weak inhibitor of β-D-glucosidase (40% inhibition at $1.0 \times 10^{-3}$ M) and has no effect on α-D-glucosidase. It was as potent as 1-deoxymannojirimycin. The isomer **328** seemed to be a specific α-L-fucosidase inhibitor (85% inhibition at $1.0 \times 10^{-3}$ M). The activity of **327** was similar to that of the unmethylated pyrrolidine compound **329**, except that the latter has no effect on α-L-fucosidase [182]. The 5-methyl substituent seemed to be responsible for the α-L-fucosidase inhibitory activity, but has a marginal effect on α- and β-D-glucosidase and α-D-mannosidase [310,311]. The monocyclic L-rhamnitol **330** was a good inhibitor of naringinase with



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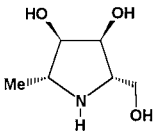


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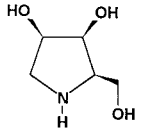


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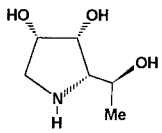


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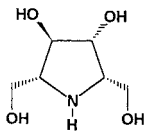


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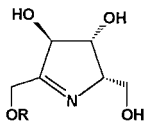
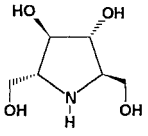



332 R = H
333 R = $CO(CH_2)_2CH_3$

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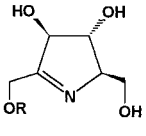
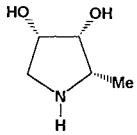



335 R = H
336 R = $CO(CH_2)_2CH_3$

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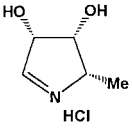


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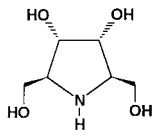
$K_i$ of $1.0 \times 10^{-6}$ M, and exhibited a weak inhibition of *E. coli* β-galactosidase (I = 49% at $1.0 \times 10^{-3}$ M) [312].

Cyclic aldimine sugars were known to be potent inhibitors of glycosidases and as immunomodulators [302, 313], whereas cyclic ketimine sugars were comparable to or better than the fully hydrogenated iminocyclitols [182, 281], except for the inhibition of α-glucosidase by compound **332** ($K_i = 2.8 \pm 0.3 \times 10^{-4}$ M) where the imino sugar **331** has a better $K_i$ value of $2.8 \times 10^{-6}$ M. Most notable was the potent inhibition of α-mannosidase by **335** ($K_i \approx 1.7 \times 10^{-5}$ M), since the fully hydrogenated version **334** showed no inhibitory activity against this enzyme. Also, the ketimine **338** showed an inhibition against α-L-fucosidase from bovine kidney ($K_i = 1.6 \times 10^{-7}$ M) more potently than its reduced form **337** ($K_i = 2.2 \times 10^{-6}$ M) [234]. The presence of a butyrate moiety in such compound greatly influenced the inhibition properties, perhaps due to masking of a hydroxyl group and an additional bulk [280]. Thus, the butyrate **333** inhibited *E. coli* β-galactosidase moderatly ($K_i = 3.2 \pm 0.4 \times 10^{-4}$ M) whereas its debutyrate derivative **332** as well as the imino sugar **331** has no activity against this enzyme. The butyrate **336** inhibits α-glucosidase (brewer's yeast), β-galactosidase (*E. coli*) and α-fucosidase (bovine epididymis) with $K_i$ values of 1.7 ± 0.3, 2.5 ± 0.4 and 3.4 ± $0.5 \times 10^{-4}$ M, respectively. The 5-aminofructose analogue **334** strongly inhibited α- and β-glucosidase ($IC_{50}$ about $2.0 \times 10^{-7}$ M) and invertase ($IC_{50} \approx 1.5 \times 10^{-6}$ M) at pH above 6.5 [314]. The inhibition was competitive in all cases and was dependent upon pH in a manner which suggested that only the protonated form of **334** was active as an inhibitor.

2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine (**339**) was a reasonable inhibitor of α-glucosidase and also inhibited glycoprotein processing at the glucosidase I stage [315]. The analogue **340** was a potent competitive inhibitor of brewer's yeast α-glucosidase ($K_i = 2.8 \times 10^{-6}$ M), almond β-glucosidase ($K_i = 1.9 \times 10^{-5}$ M), green coffee bean α-galactosidase ($K_i = 5.0 \times 10^{-5}$ M), and jack bean α-mannosidase ($K_i = 3.1 \times 10^{-3}$ M), but no inhibition (up to $1.0 \times 10^{-3}$ M) of *E. coli* β-galactosidase was observed [182, 235, 236]. This broad spectrum of inhibition was found to be similar to that observed for a glucose-based amidinium ion, and can be explained on the basis of the active-site model [116], which indicated that compounds mimicking the flattened-chair transition state of glycosidic cleavage and with a positive charge character will have broad spectrum of inhibition. Compound **340** may be protonated and possesses an envelope conformation for binding to the active site. 2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine (**341**) inhibited the processing of the viral hemagglutinin [315] by inhibiting glucosidase I and II. 2,5-Dideoxy-2,5-imino-D-mannitol (**342**) was a powerful inhibitor of *Agrobacterium faecalis* β-glucosidase ($K_i = 2.0 \times 10^{-7}$ M) [260], and intestinal β-glucosidase. It strongly inhibited yeast α-glucosidase ($K_i = 7.3 \times 10^{-7}$ M). Its inhibition activities against almonds β-glucosidase and invertase yeast β-D-fructofuranosidase was almost the same at different pH values ($K_i = 1.7 \times 10^{-6}$ M at pH 5.0 and $1.1 \times 10^{-6}$ at pH 7.0, respectively) [314]. It bound to *Asp. wentii* and bovine kidney lysosomal β-glucosidase in a competitive mode. Its inhibition constants were relatively high (5.7 and $4.4 \times 10^{-5}$ M, respectively). Its inhibition activities against β-D-fructofuranosidase at different pH values were similar ($K_i = 6.8$, 3.5 and $1.1 \times 10^{-6}$ M at pH 5.0, 6.0 and 7.0, respectively). The aza sugar **342** was a potent inhibitor of rat intestinal lactase and bovine liver cytosolic β-galactosidase [23], but it exhibited no significant inhibition towards lysosomal and epididymal enzymes which are optimally active in acidic conditions. It was a good inhibitor of insect trehalase and almond β-glucosidase ($IC_{50}$ = 5.5 and $7.8 \times 10^{-5}$ M, respectively) [181]. It was a moderate inhibitor against mammalian trehalases and soluble α-mannosidases, but it had no effect on processing α-glucosidase II at $1.0 \times 10^{-3}$ M. It shows an anti-HIV activity [187, 295, 316]. 2,5-Dideoxy-2,5-imino-1-*O*-methyl-D-mannitol (**343**) competitively inhi-

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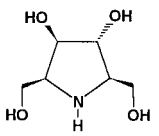


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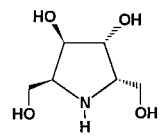


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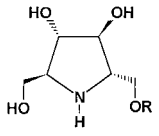
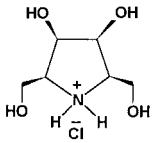



342 R = H
343 R = Me

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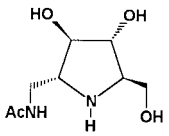


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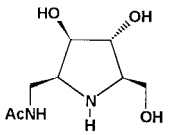


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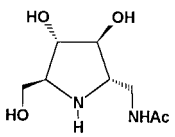


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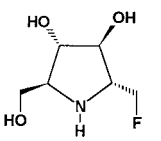


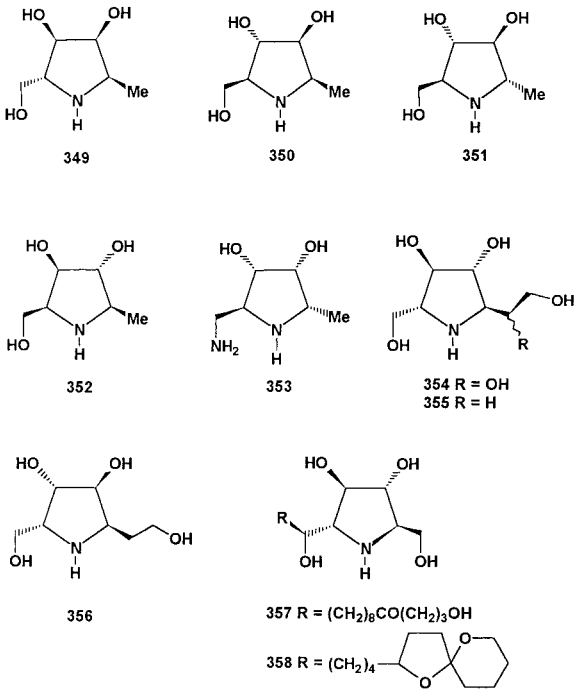

348

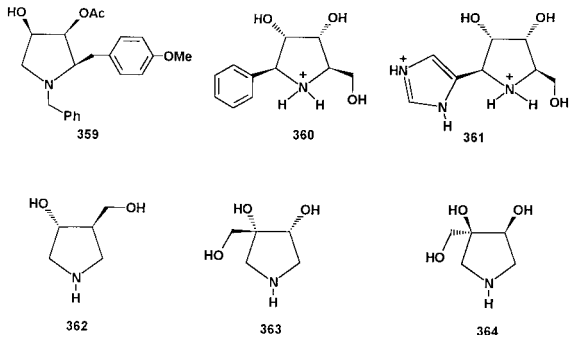
bited *Agrobacterium faecalis* β-glucosidase [260] ($K_i = 1.0 \times 10^{-5}$ M at pH 7.0). The aza sugar **344** which mimis the galactosyl cation inhibited both green coffee bean α-galactosidase and bovine β-galactosidase with $K_i$ values of $5.0 \times 10^{-8}$ and $4.1 \times 10^{-4}$ M, respectively [317]. Compound **345** was found to be a potent competitive inhibitor of β-*N*-acetylglucosaminidase from both bovine kidney ($K_i = 9.8 \times 10^{-6}$ M) and from jack beans ($K_i = 1.9 \times 10^{-6}$ M). Compound **346** was also an inhibitor of the enzyme from bovine kidney ($K_i = 6.9 \times 10^{-5}$ M) and from jack beans ($K_i = 3.6 \times 10^{-6}$ M) [318]. The 1-acetamido-l-deoxy derivative **347** was a good inhibitor of hexosaminidases, but it was not as efficient as its 6-membered ring analogue, 2-acetamido-1,2,5-trideoxy-2,5-imino-D-glucitol [319]. 1,2,5-Trideoxy-l-fluoro-2,5-imino-D-mannitol (**348**) was not a powerful as its parent compound 2,5-dideoxy-2,5-imino-D-mannitol (**342**) but it showed interesting activities and unexpected selectivities against a set of α- and β-glucosidases and invertase [260, 319]. The pyrrolidine derivative **348** was a competitive inhibitor of yeast α-glucosidase and *Agrobacterium faecalis* β-glucosidase ($K_i = 5.7 \times 10^{-5}$ M at pH 6.5 and $3.0 \times 10^{-5}$ M at pH 7.0, respectively). It inhibited almond and *Asp. wentii* β-glucosidase ($K_i = 2.6$ and $1.9 \times 10^{-4}$ M, respectively) as well as β-D-fructofuranosidase competitively ($K_i = 8.5 \times 10^{-6}$ M at pH 6.0) [260].

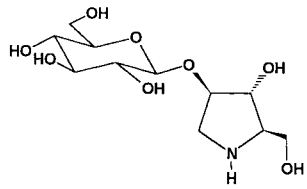
The deoxy analogues **349**–**352** were competitive inhibitors of α-fucosidase from bovine kidney at pH 5.5 with $K_i$ values of 1.4, 8.0, 2.2 and $4.0 \times 10^{-6}$ M, respectively [320, 321]. Compound **349** was a slightly better inhibitor, perhaps because its shape was closer to the transition state of the fucosidic cleavage. Compound **351** was found to be β-mannosidase inhibitor [322]. The deoxyamino derivative **353** also inhibited α-L-fucosidase from bovine kidney with a $K_i$ value of $1.9 \times 10^{-6}$ M [323]. The aza sugar DL-glycero-D-manno analogue **354** was a more potent inhibitor ($K_i = 1.5 \times 10^{-6}$ M) of almond β-glucosidase than **334** ($K_i = 1.0 \times 10^{-5}$ M) and **303** ($K_i = 2.8 \times 10^{-4}$ M). However, only **303** inhibited rabbit gut β-glucosidase (less than 50% inhibition). **334** and **354** were found to be inhibitory to *Phleumpratense* invertase with $K_i$ values of 7.8 and $7.7 \times 10^{-5}$ M, respectively; **303** was a weaker inhibitor with a $K_i$ of $1.1 \times 10^{-3}$ M. All the three alkaloids inhibited sucrase but only **354** inhibited lactase and maltase, whereas **334** and **354** inhibited trehalase [181]. The pyrrolidine **334** has been shown to be inhibitory to several plant parasitic nematode species [324], in addition **334** and **303** have been reported to be antifeedants to insects [325]. The presence of a number of glycosidase inhibitors in bluebell leaves and bulbs may act as a defence to a number of classes of potential predators [326]. The aza sugar heptitol **354** was a potent inhibitor of bacterial β-glucosidase, mammalian β-galactosidases, and mamalian trehalases, while the 6-deoxy analogue **355** was a potent inhibitor of rice α-glucosidase and rat intestinal maltase. The gulo analogue **356** was found to be a good inhibitor of α-L-fucosidase [327]. The pyrrolidine alkaloids of *Broussonetia kazinoki* Sieb., **357** and **358**, possessed potent glycosidase-inhibiting activity and were useful for treating and preventing diseases caused by excess blood sugar in humans and animals. Thus, compound **357** showed $IC_{50}$ values of $3.0 \times 10^{-5}$, $2.0 \times 10^{-7}$ and $5.0 \times 10^{-8}$ g ml$^{-1}$ against α-glucosidase, β-glucosidase, and β-galactosidase, respectively [328].

*N*-Benzyl-4-*epi*-(−)-anisomycin (**359**) has been prepared to be used as glycosidase inhibitor [329]. 1,4-Dideoxy-1,4-imino-1-(*S*)-phenyl-D-ribitol (**360**) and its (4-imidazolyl) analogue **361** were potent competitive inhibitors with dissociation constants of $3.0 \times 10^{-8}$ and $2.5 \times 10^{-6}$ M, respectively, for nucleoside hydrolase from the trypanosome *Crithidia fasciculate*. The observed highly effective inhibition demonstrated the importance of including both charge and aglycon mimicry in a glycosylase transition state analogue [330]. Compound **360** has a $K_i$ value of $1.7 \times 10^{-7}$ M. The isoiminosugar **362** inhibited baker's yeast α-D-, almond β-D-glucosidase and bovine kidney α-L-fucosidase with $K_i$ values of $8.0 \times 10^{-4}$, $1.0 \times 10^{-3}$ and $9.0 \times 10^{-4}$ M, respectively [331]. Both of **363** and its analogue **364** exhibited a weak and unspecific inhibition for α-glucosidases from baker's yeast ($K_i = 3.8$ and $2.0 \times 10^{-3}$ M, respectively) and β-glucosidases from almond ($K_i = 1.4$ and $1.1 \times 10^{-3}$ M, respectively).

1,4-Dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol (**365**) inhibited baker's yeast and rice α-glucosidases ($IC_{50} = 4.6$ and $7.3 \times 10^{-4}$ M, respectively), as well as *Caldocellum saccharolyticum* β-glucosidase ($IC_{50} = 9.0 \times 10^{-4}$ M) [226]. The imino-C-disaccharide **366** was a moderate α-mannosidase inhibitor [I = 43% (jack bean) and 27% (almond) at $1.0 \times 10^{-3}$ M], but on the contrary to the simpler analogues it was much more specific [332 to 335]. The $K_i$ values of some new imino sugar derivatives **367**–**370** of both baker's yeast α-glucosidase and almond β-glucosidase were **367**: $6.0 \times 10^{-4}$ and $3.2 \times 10^{-3}$; **368**: 1.4 and $6.9 \times 10^{-3}$; **369**: $2.1 \times 10^{-3}$ and no inhibition; **370**: 9.6 and $5.8 \times 10^{-4}$ M, respectively. The very high $K_i$ value of **368** compared with that of its parent pyrrolidine **340** (300–500 fold difference) indicated that





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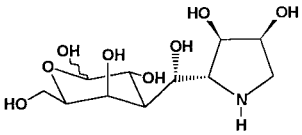

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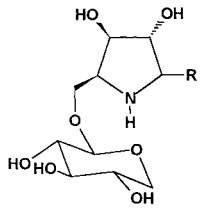
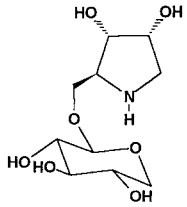


367 R = H
368 R = $CH_2OH$(*R*)
369 R = $CH_2OH$(*S*)

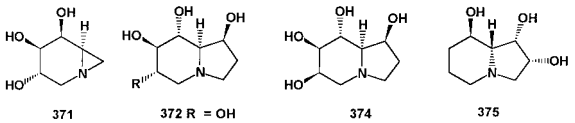


370

**368** does not fit into enzymes in the expected manner and thus it was a poor transition state analogue. It was suggested that hydroxypyrrolidines of an unnatural sugar, L-xylose, might not be very good glycon mimics for this type of analogues [315].

### 17. Fused rings with bridgehead nitrogen

Several new saccharide derived irreversible inactivators capable of alkylating selectively the active site of glycosidases were prepared. Thus the aziridinyl triol **371** displayed potent, time dependent inactivation of green coffee bean α-galactosidase, at $1.0 \times 10^{-3}$ M but had no effect on yeast α-glucosidase, jack bean α-mannosidase, or bovine β-galactosidase [46]. The apparent second-order rate constant for the association of **371** with α-galactosidases ($K_{inact}/K_M = 2540\ min^{-1}\ M^{-1}$), indicated that it represented the most potent and specific green coffee bean α-galactosidase inactivator [336]. In the presence of the competitive inhibitor galacto-1-deoxynojirimycin (**187**), α-galactosidase was protected against irreversible inactivation by **371**. The natural product (+)-castanospermine (**372**) was a potent inhibitor of β-glucosidase from almond ($K_i = 1.5 \times 10^{-6}$ M), and effective against glucosidase II [337] as well as lysosomal α-glucosidase [338] and intestinal sucrase [339]. It inhibited the processing of the viral hemagglutinin by inhibiting glucosidase I [315], and exhibited activity against HIV [20, 22]. However, it was unsuitable for therapeutic use in diabetes, but it was a drug candidate for the treatment of cancer and viral infections [14, 340, 341]. Castanospermine (**372**) was found to be the best competitive *exo*-glucanase inhibitor. When the substrate was laminarin, it produced an almost 50% inhibition of the *Saccharomyces exo*-glucanase at a concentration of $3.0 \times 10^{-5}$ M ($K_i$ ranging from 2.0 to $5.0 \times 10^{-5}$ M), but it has no effect on *exo*-glucanase from a basidiomycete. It was a potent inhibitor of *C. albicans exo*-glucans ($K_i = 2.0 \times 10^{-7}$ M) [220]. The presence of castanospermine in the culture medium of growing yeasts did not have any effect on yeast growth in spite of the fact that, under the conditions used, the external *exo*-glucanase was fully inhibited. It was a slowly acting inhibitor of glucose-induced insulin release and inhibited islet lysosomal acid glucan-1,4-α-glucosidase activity ($EC_{50} \approx 1.0 \times 10^{-7}$ M) [342]. The inhibition studies of several derivatives of castanospermine modified at C-1 or C-7 showed that the structural criteria for inhibition of β-D-glucosidase were more rigorous than for α-D-glucosidase. An unsubstituted C-1 hydroxy group of the configuration found in castanospermine was essential for the optimal glucosidase activity [343]. The alteration of the configuration of the other hydroxy groups led to a significantly weaker inhibition of α- and β-glucosidases. Although the correct configuration of the C-7 hydroxy group is essential for an inhibition of β-glucosidase, α-glucosidase was weakly inhibited by several analogues modified at C-7. The hexose corresponding to 7-*epi*-castanospermine is D-altrose, which did not occur in mammalian cells. This may explain the lack of specificity at C-7 of castanospermine derivatives for inhibition of α-glucosidase [343]. It was found that 6-acetamido-6-deoxy castanospermine (**373**) was a very powerful inhibitor of β-*N*-acetylglucosaminidase from human placenta ($IC_{50} = 5 \times 10^{-7}$ M), bovine kidney ($IC_{50} = 1.5 \times 10^{-6}$ M), jack bean ($IC_{50} = 1.6 \times 10^{-6}$ M), porcine placenta ($IC_{50} = 4.0 \times 10^{-7}$ M), and bovine epididymis ($IC_{50} = 7.0 \times 10^{-7}$ M) [344]. 6-*Epi*-castanospermine (**374**) was inactive against jack bean α-mannosidase ($IC_{50} > 1.0 \times 10^{-3}$ M) [345]. Its inactivity was due to the incorrect ring conformation for good superimposition [191]. D-(−)-Swainsonine (**375**) prevented the processing of the oligosaccharide chains of the influenza viral hemagglutinin [346], and other cellular glycoproteins [347–352] by inhibiting the Golgi and immunomodulatory processing enzyme, mannosidase II [194, 353]. It has shown antitumor and immunomodulatory activity [306, 354, 355]. Swainsonine (**375**) and its epimers **376**–**379** exhibited potent α-D-mannosidase activity [194, 299, 356–358], but they have no any structural similarity to α-D-mannose. Swainsonine (**375**) inhibited competitively jack bean α-mannosidase with a $K_i$ value $1.8–9.5 \times 10^{-6}$ M and $IC_{50} = 1.0 \times 10^{-7}$ M [359]. It completely inhibited acid α-mannosidase at pH 4.0 and a concentration of $2.0 \times 10^{-5}$ M from all mammalian tissues tested, and acid α-mannosidase from the liver of the lampreyeel [357]. It did not inhibit α-glucosidase, β-galactosidase, hexosaminidase or β-glucuronidase from mouse liver by 10 times its concentration for total inhibition of α-mannosidase at pH 4.0. The neutral form of α-mannosidase (pH 6.5) was inhibited (60%) by swainsonine at a concentration of $2.0 \times 10^{-5}$ M. It was a reversible active-site-directed inhibitor of lysosomal α-mannosidase [357]. L-Swainsonine (**380**) was a very potent inhibitor of naringinase ($K_i = 4.5 \times 10^{-7}$ M, $IC_{50} = 3.0 \times 10^{-7}$ M) and weak



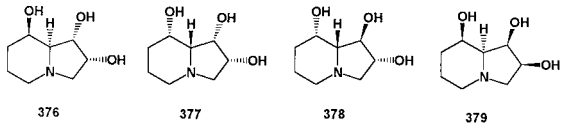
371



372 R = OH
373 R = NHAc



374 375



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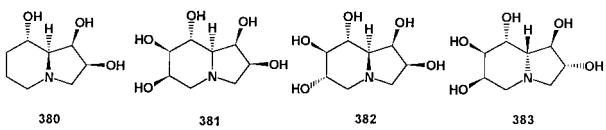

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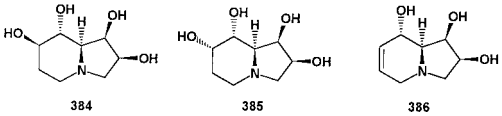
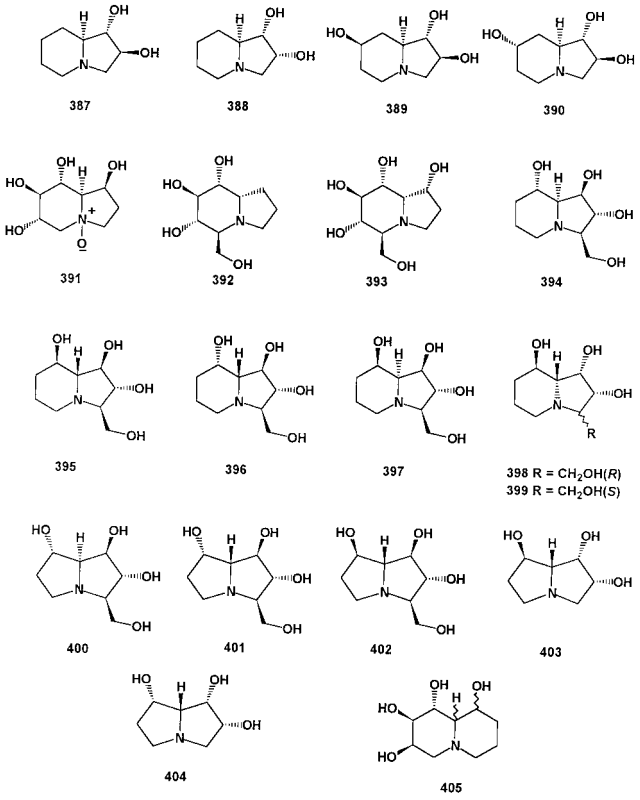


384 385 386

inhibitor of jack bean α-mannosidase ($K_i = 2.5 \times 10^{-3}$ M). In contrast, D-swainsonine (**375**) showed no inhibition of the L-rhamnosidase [360]. L-(+)-Swainsonine (**380**) is potential useful for the prevention of metastasis of cancer [354, 361, 362]. 2-Hydroxy-6-*epi*-castanospermine (**381**) ($IC_{50} = 5.3 \times 10^{-4}$ M) and 2-hydroxycastanospermine (**382**) ($IC_{50} = 6.1 \times 10^{-4}$ M) possessing a *cis*-diol unit in the pyrrolidine moiety were moderate inhibitors of naringinase (L-rhamnosidase) due to their structural resemblance to L-(+)-swainsonine (**380**), where no such inhibition was found for **383** with the corresponding *trans*-diol unit. Niether castanospermine (**372**) nor 6-*epi*-castanospermine (**374**) caused any inhibition of naringinase. Although, castanospermine (**372**) was a very powerful inhibitor of intestinal sucrase, the 2-hydroxy analogue **382** was only a very weak inhibitor of the rabbit gut disaccharidases. It thus appeared that the pyrrolidine aza furanose mimic predominated over the piperidine azapyranose mimic and so **381** and **382** may be better described as dihydroxy-L-swainsonines [312]. Removal of the hydroxyl groups at C-6 in **381** and **382** to give **384** resulted in a ten-fold increase in the naringinase inhibition ($IC_{50} = 5.0 \times 10^{-5}$ M). However, the hydroxy-L-swainsonine (**385**) was a significantly weaker inhibitor ($IC_{50} = 2.6 \times 10^{-4}$ M). Dehydro-L-swainsonine (**386**) was a very weak inhibitor of L-rhamnosidase (I = 28% at $8.3 \times 10^{-4}$ M) and almond β-glucosidase (I = 42% at $8.3 \times 10^{-4}$ M).

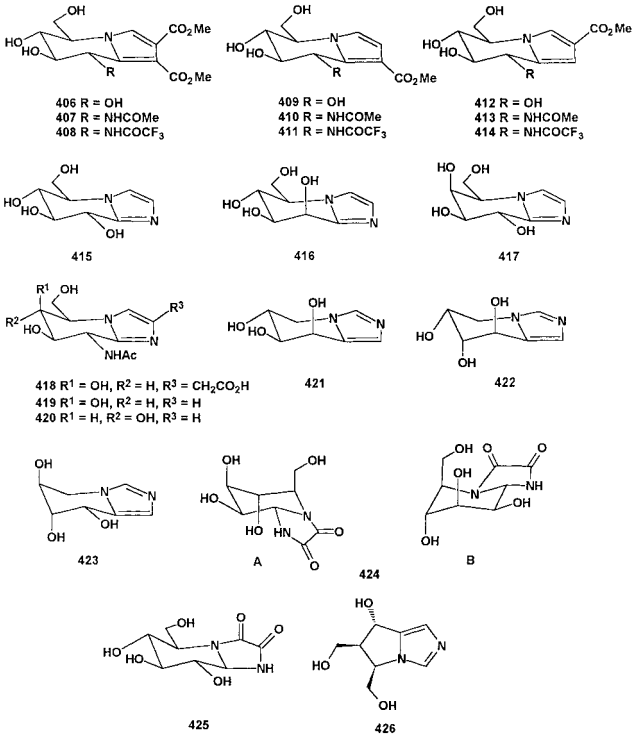
The (+)-enantiomer lentiginosine (**387**) displayed inhibition specificity for *Aspergillus niger* amyloglucosidase ($K_i = 2.0 \times 10^{-6}$ M) 5 times stronger than that reported for natural lentiginosine ($K_i = 1.0 \times 10^{-5}$ M), 35 times than that measured for its enantiomer ($K_i = 7.0 \times 10^{-5}$ M) [360]. Pure (+) and (−)-enantiomers of **387** showed $K_i$ values against *Rhizopus* mold amyloglucosidase equal to $3.0 \times 10^{-6}$ and $9.8 \times 10^{-5}$ M, respectively. Thus, the alkaloide (+)-**387** was the most potent and specific competitive inhibitor of amyloglucosidases among the aza sugars and their analogues [363]. Lentiginosine (**387**) was much less effective as an inhibitor of amyloglucosidase than castanospermine (**372**) [364]. *Cis*-indolizidinediol (**388**), which has only two hydroxyl groups and the ring heteroatom to bind to the active site, exhibited weak inhibition against rat liver α-mannosidase ($IC_{50} = 7.5 \times 10^{-3}$ M) [365]. (7*R*)- and (7*S*)-7-Hydroxylentiginosine (**389** and **390**) are specific inhibitors of amyloglucosidases from *Aspergillus niger* ($K_i = 4.4 \times 10^{-6}$ and $6.9 \times 10^{-5}$ M, respectively) and from *Rhizopus* mold ($K_i = 7.2 \times 10^{-6}$ and $8.0 \times 10^{-5}$ M, respectively). The trihydroxy derivatives **389** and **390** were less powerful inhibitors than lentiginosine (**387**) but in contrast **389** possessed a weak inhibiting activity toward α-L-fucosidase from bovine epididymis (I = 60%) [366]. The addition of an oxygen atom to N perturbs the binding to the enzyme, resulting in a weaker complex. This situation was observed for castanospermine *N*-oxide (**391**) where its $K_i$ value for the inhibition of β-glucosidase (sweet almond) was $2.5 \times 10^{-3}$ M [116] and $7.6 \times 10^{-3}$ M [367]. The polyhydroxylated indolizidines **392** and **393** were found to be potent inhibitors of the glycoprotein processing enzyme α-glucosidase I (pig kidney) ($IC_{50} = 3.0 \times 10^{-7}$ M and $IC_{50} = 1.5 \times 10^{-7}$ M, respectively) [368, 369] comparable to castanospermine (**372**) ($IC_{50} = 1.0 \times 10^{-7}$ M). The indolizidine analogues homoalexine (**394**), 8-*epi*-homoaustraline (**395**), homoaustraline (**396**) and 8-*epi*-homoalexine (**397**) were found to be good inhibitors of amyloglucosidase (*Aspergillus niger*) with the $IC_{50}$ values $7.5 \times 10^{-5}$, $1.2 \times 10^{-5}$, $9.5 \times 10^{-5}$ and $4.5 \times 10^{-6}$ M, respectively. Both (3*R*)- and (3*S*)-3-(hydroxymethyl)swainsonine (**389**



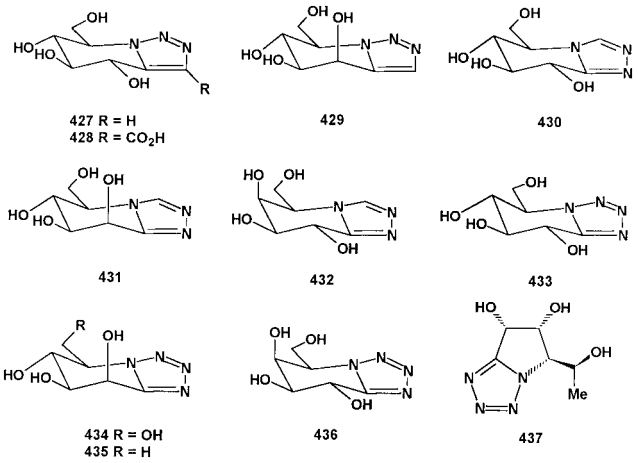

and **399**) were found to be effective inhibitors of α-mannosidase (jack bean) ($IC_{50} = 1.2 \times 10^{-6}$ and $4.5 \times 10^{-5}$ M, respectively) [359]. The inhibition of amyloglucosidase by alexine (**400**) ($IC_{50} = 1.1 \times 10^{-5}$ M), australine (**401**) ($IC_{50} = 5.8 \times 10^{-6}$ [370], $1.5 \times 10^{-6}$ M [183]) and 7-*epi*-australine (**402**) ($IC_{50} = 1.3 \times 10^{-6}$ M) [183], was roughly ten-fold stronger than that exhibited by the corresponding indolizidine homologues **394**–**396**. In contrast to the pyrrolizidine inhibitors, which did not possess mannosidase inhibitory activity [370, 371], the indolizidines **394**–**397** were found to inhibit α-mannosidase (jack bean) albeit weakly ($IC_{50}$ = 5.3, 1.5, 1.9 and $4.8 \times 10^{-4}$ M, respectively) [372]. Contraction of the piperidine ring in swainsonine to pyrrolidine ring abolished the potent and specific inhibition of α-mannosidases and also decreased the inhibition of other glycosidases. Thus, the pyrrolizidine analogue **403** was a poor inhibitor of lysosomal α-mannosidase ($IC_{50} = 1.5 \times 10^{-3}$ M) and less effective in inhibiting the Golgi II or neutral processing mannosidases. At a concentration of $1.0 \times 10^{-3}$ M, **403** inhibited lysosomal β-galactosidase by 69%, the broad specificity β-galactosidase/β-glucosidase moderatly (25%) and α-fucosidase by (33%). It inhibited jack bean α-mannosidase very weakly ($K_i = 1.7 \times 10^{-3}$ M) in comparison to the inhibition by swainsonine. The 7*S*-epimer analogue **404** did not inhibit human liver α-mannosidase, but was a weak inhibitor of the broad specificity β-galactosidase/β-glucosidase (40%) [373]. None of the quinolizidine analogues of D-manno-tetrahydroxyquinolizidine, (9*S*, 9a*R*), (9*R*, 9a*S*), (9*S*, 9a*S*) and (9*R*, 9a*R*)-**405** showed significant glycosidase activity against mannosidases, glucosidases or fucosidases which was due to their *trans*-decalin-like structures. It was clear that the enzyme binding site did not tolerate the added steric bulk imposed by the second ring of **405** [355].

The pyrroles **406, 409** and **412** were tested against sweet almonds β-glucosidase ($K_i = 2.5 \times 10^{-2}$, $3.0 \times 10^{-4}$, and $6.0 \times 10^{-3}$ M, respectively) [374]. The deoxyamino analogues **407**, **408**, **410**, **411**, **413** and **414** were tested against

the *N*-acetylglucosaminidase from bovine kidney ($K_i$ values between 1.0 and $7.5 \times 10^{-5}$ M) and **408**, **413** and **414** also against *N*-acetylglucosaminidase from jack bean ($K_i$ = 1.4, 1.6, and $1.0 \times 10^{-4}$ M, respectively). The $K_i$ values of these pyrroles, as compared to their tetrazole and imidazole analogues, indicate that the heteroatom, corresponding to the glycosidic O-atom, is required for strong inhibition. The tested enzymes were inhibited about twice as strongly, by the trifluoroacetamido analogues, than by the acetamides. The slightly stronger inhibition observed may be the result of the compensating influences of H-donation, H-acceptance, and hydrophobic interaction [375]. The imidazole **415** shows a strong competitive inhibition of sweet almond β-glucosidase ($K_i = 1.0 \times 10^{-7}$ M) [76], and the β-glucosidase from *Caldocellum saccharolyticum* was inhibited in a mixed fashion ($K_i = 2.0 \times 10^{-8}$ M). These results prove that the lateral protonation, and not the interaction of the azole ring with the cation-stabilizing carboxylate group was the dominating factor for the inhibition [376]. The pyranoimidazole **415** inhibited brewer's yeast α-glucosidase competitively with a $K_i$ value of $5.9 \times 10^{-5}$ M. Imidazomanno- and imidazogalacto-jirimycin **416** and **417** were potent inhibitors for β-mannosidase (snail) and β-galactosidase (*E. coli*) ($K_i = 5.5 \times 10^{-8}$ and $4.0 \times 10^{-9}$ M, respectively) [76]. The natural product nagstatin (**418**) was a strong inhibitor of an *N*-acetyl-β-D-glucosaminidase from bovine kidney ($IC_{50} = 1.2 \times 10^{-9}$ g ml$^{-1}$ [377], $1.3 \times 10^{-8}$ M [375]) and the de-branched nagstatin **419**, lacking the acetic acid fragment, shows on $IC_{50}$ value of $1.5 \times 10^{-9}$ g ml$^{-1}$ although the pyranose sugar moiety possesses *N*-acetyl-β-D-galactosamine configuration [378]. Also its gluco analogue **420** showed a strong inhibiting activity against the same enzyme ($IC_{50} = 1.7 \times 10^{-9}$ g ml$^{-1}$) [378]. The imidazo analogues **421** and **426** were evaluated as glycosidase inhibitors. Both analogues showed a Weaker antiviral activity than castanospermine (**372**), which was used as a reference substance [379]. Imidazo-D-arabino-piperidinose (**422**) was a specific and potent inhibitor of jack bean α-mannosidase ($K_i = 5.0 \times 10^{-5}$ M), while imidazo-L-lyxo-piperidinose (**423**) showed weak and moderate inhibition of jack bean β-galactosidase and baker's yeast α-glucosidase (isomaltase) ($K_i = 1.3 \times 10^{-3}$ and $6.0 \times 10^{-4}$ M, respectively). This poor glycosidase inhibitory activity may be due to the lack of the $CH_2OH$ group at C-5 in addition to the lyxo configuration of the sugar which does do not seem to occur in nature, neither in the D- nor in the L-series [380]. Kifunensine (**424**) has an annulated oxamide ring to the 1-aminodeoxymannojirimycin [381], which forced the sugar ring to be in $_4C^1$ **424B** rather than $_1C^4$ **424A** conformation. It may be concluded from the structure and activity of kifunensine that the more structural information a molecule contains the more likely is its strong activity as inhibitor, as long as it contains the basic essentials for inhibition, such as the presence of nitrogen atoms, in the structure [364]. Kifunensine **424B** was more effective than deoxymannojirimycin and it was a very potent inhibitor of plant mannosidase I ($IC_{50}$ = 2.0 to $5.0 \times 10^{-8}$ M) [364], but it was inactive towards plant mannosidase II. Kifunensine had a strong inhibitory effect on the release of mannose from [$^3$H]mannose-labeled $Man_9$-GlcNAc. The results showed that there was a very marked inhibition of the membrane processing mannosidase activity at low concentrations of kifunensine ($IC_{50} \approx 1.0 \times 10^{-7}$ M). This inhibition leveled off at about 20% of control values (i.e. about 80% inhibition), even at high concentrations of this inhibitor ($1.0 \times 10^{-5}$ g ml$^{-1}$). It was assumed that this residual 20% activity was due to the presence of the ER α-mannosidase, which has been shown to be resistant to inhibition by deoxymannojirimycin [382]. The ER α-mannosidase was also resistant to kifunensine [364]. The soluble rat liver mannosidase activity which has been shown to be similar immunologically to the ER α-mannosidase was also not inhibited by kifunensine [294]. Kifunensine was a potent inhibitor of the animal Golgi mannosidase I but not the ER processing mannosidase [364]. It was also an excellent inhibitor of glycoprotein processing in cell culture studies and it was reported to be a moderate inhibitor of jack bean α-mannosidase ($IC_{50} = 1.2 \times 10^{-4}$ M) [381]. It was a poor inhibitor of mung bean aryl α-mannosidase [364]. 8-*Epi*-kifunensine (**425**) showed an inhibitory activity against α-glucosidase from yeast ($IC_{50} = 2.2 \times 10^{-4}$ M) [383].

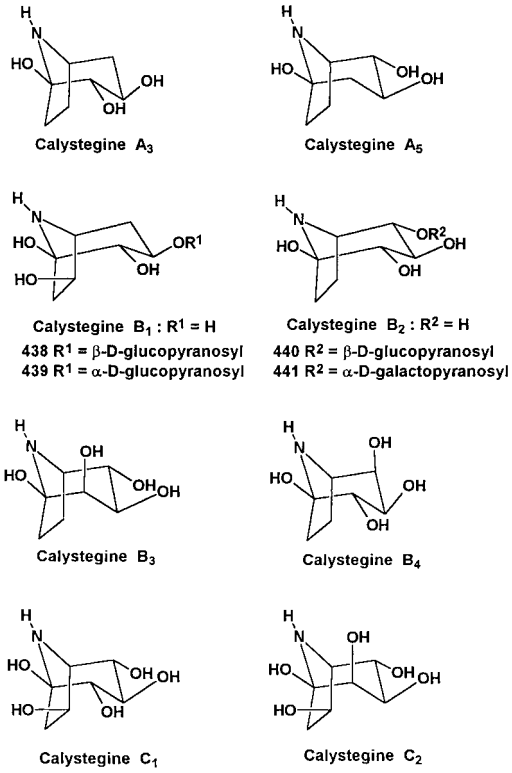



The triazole **427** was a very weak inhibitor of β-glucosidases. It weakly inhibited almond β-glucosidase ($K_i > 8.0 \times 10^{-3}$ M at pH 6.8) as well as *C. saccharolyticum* β-glucosidase ($IC_{50} = 2.0 \times 10^{-3}$ M at pH 6.8) [384]. The mannotriazole **429** exhibited also weak inhibition of snail β-mannosidase ($K_i > 8.0 \times 10^{-3}$ M) [384]. The D-glucotriazole carboxylic acid **428** showed weak but competitive inhibition of glycogen phosphorylase b (GPb) at pH 6.8. It would be completely ionized as the corresponding carboxylate anion with a $K_i$ value of $7.4 \times 10^{-3}$ M. It was 140 times less effective than the nojirimycin tetrazole analogue **433** ($K_i = 5.3 \times 10^{-5}$ M). In contrast, the tetrazole **433** showed uncompetitive inhibition [385]. In contrast to the weak inhibition by the 1,2,3-triazole analogue, the isomeric 1,2,4-triazole isomers proved to be a good inhibitor of retaining β-glycosidases. Thus, the triazole **430** competitively inhibited β-glucosidases from sweet almond and *Caldocellum saccharolyticum* ($K_i = 1.9 \times 10^{-5}$ and $1.7 \times 10^{-7}$ M, respectively). The inhibition of brewer's yeast α-glucosidase by **430** ($K_i = 8.7 \times 10^{-4}$ M) was 15 times less than the inhibition of the same enzyme by imidazole **415**. Both triazoles **431** and **432** had the same $K_i$ value of $2.0 \times 10^{-7}$ M against snail β-mannosidase and *E. coli* β-galactosidase, respectively [386]. The tetrazole and the triazole analogues differed only in the presence or




absence of an anomeric heteroatom. The formation of a hydrogen bond to this heteroatom may be required for a strong inhibition of β-glucosidases [384]. Sweet almond β-glucosidase was inhibited to a moderate extent by compound **433** which was termed "nojiritetrazole" ($IC_{50} = 8.0 \times 10^{-5}$ M at pH 4.5; $K_i = 1.5 \times 10^{-4}$ M at pH 6.8) [387]. It has been shown that it possessed a half-chair conformation. It was observed that nojiritetrazole (**433**) was bound to *Agrobacterium* β-glucosidase some 4000 times more tightly than D-glucose [388]. The inhibition of the Glu358A*sp* mutant of *Agrobacterium* β-glucosidase by nojiritetrazole (**433**) afforded a $K_i$ value of $2.0 \times 10^{-4}$ M [389]. The inhibition of yeast α-glucosidase by **433** was neither competitive nor noncompetitive. It competitively inhibited α-glucosidase II in porcine liver extract ($K_i = 1.8 \times 10^{-2}$ M, $IC_{50} = 3.0 \times 10^{-2}$ M) [387]. It was a potent inhibitor of *A. faecalis* β-glucosidase ($K_i = 1.4 \times 10^{-6}$ M at pH 7.0) [65]. Nojiritetrazole (**433**) bound well to bovine liver β-galactosidase ($K_i = 1.5 \times 10^{-6}$ M), while mannonojiritetrazole (**434**) did not ($K_i = 1.4 \times 10^{-2}$ M) [389]. The azole **434** inhibited snail-β-mannosidase moderately at pH 6.8 ($K_i = 1.6 \times 10^{-4}$ M) [489], and human liver α-mannosidase ($I = 56\%$ at a concentration of $1.0 \times 10^{-3}$ M), but it exhibited no significant inhibition of α-fucosidase and poor inhibition of human liver β-mannosidase ($I = 28\%$ at same concentration). The rhamnotetrazole **435** was a significantly better inhibitor than the mannotetrazole **434**, indicating that C-6 OH was detrimental to the binding of the inhibitor to the enzyme. It showed inhibition of human liver α-mannosidase ($I = 92\%$ at $1.0 \times 10^{-3}$ M) and no inhibition of human liver β-mannosidase or α-fucosidase [390]. Very weak inhibition of naringinase was observed for the pyranotetrazole **435** ($I = 25\%$ at $7.7 \times 10^{-4}$ M) [237] in contrast to the furanotetrazole analogue **437** ($K_i = 5.6 \times 10^{-5}$ M, $IC_{50} = 7.0 \times 10^{-5}$ M) [312, 368]. The tetrazole **435** was also a weak inhibitor of almond emulsin β-glucosidase ($I = 44\%$ at $7.7 \times 10^{-4}$ M). *E. coli* β-galactosidase was inhibited by the galactotetrahydropyridotetrazole **436** with a $K_i$ value of $1.0 \times 10^{-6}$ M [384].

Although the calystegine $B_2$ and deoxynojirimycin (**183**) have structural similarities, their biological properties were different, that is, **183** was a potent α-glucosidase inhibitor, while calystegine $B_2$ was a potent competitive inhibitor with $K_i = 1.2 \times 10^{-6}$ M, $IC_{50} = 2.6 \times 10^{-6}$ M for almond β-glucosidase and $K_i = 2.3 \times 10^{-6}$ M, $IC_{50} = 3.9 \times 10^{-6}$ M for *Aspergillus niger* α-galactosidase [226, 391, 392]. The calystegine B complex exhibited potent inhibitory activity against the latter two enzymes with $K_i$ values of 3.0 and $7.0 \times 10^{-6}$ M, respectively [393]. Calystegine $B_2$ potently inhibited *C. saccharoliticum* β-glucosidase ($K_i = 5.5 \times 10^{-7}$ M, $IC_{50} = 2.4 \times 10^{-6}$ M), and green coffee bean α-galactosidase ($K_i = 8.6 \times 10^{-7}$ M, $IC_{50} = 1.9 \times 10^{-6}$ M) in a competitive manner. It also inhibited moderately rice α-glucosidase ($IC_{50} = 7.5 \times 10^{-5}$ M) and porcine kidney trehalase ($IC_{50} = 1.0 \times 10^{-5}$ M) [226]. The deoxygenation or epimerization at C-4 of calystegine $B_2$ had no effect on rat intestinal trehalase, but it decreased the potency towards other glycosidases. The catabolism by *Rhizobium meliloti*, glycosidase inhibition, and allelopathic activities were uniquely associated with natural calystegine $B_2$. Furthermore, the *N*-methyl derivative of calystegine $B_2$ was not catabolized by *Rhizobium meliloti*, and it inhibited α-galactosidase, but not β-glucosidase, whereas the parent alkaloid inhibited both enzymes. Therefore the *N*-methyl derivative of calystegine $B_2$ could serve to construct a cellular or animal model for Fabry's disease, which was caused by a lack of α-galactosidase activity [394]. Calystegine $B_3$ showed a weak or no inhibitory activity toward α- and β-galactosidases although it was good superimposed onto 1,5-dideoxy-1,5-imino-D-galactitol which was a potent inhibitor of coffee bean α-galactosidase ($K_i = 1.6 \times 10^{-9}$ M) [208]. Although calystegine $B_4$ superimposed well on manno-dNM, it was quite inactive against α- and β-mannosidases and α-L-fucosidase. Calystegine $B_4$ appeared to be more effective for big kidney trehalase ($K_i = 1.2 \times 10^{-6}$ M, $IC_{50} = 4.8 \times 10^{-6}$ M) than for rat intestinal trehalase ($IC_{50} = 9.8 \times 10^{-6}$ M) and had a four-fold stronger affinity to the enzyme than calystegine $B_2$. The order of sensitivity [216] of various trehalases to calystegine $B_4$ was mammal > insect > fungus > yeast, where calystegines $B_2$ and $B_4$ had a very weak inhibitory activity towards the enzyme from the pathogenic fungus *Rhizoctonia solani* ($IC_{50} = 7.0$ and $5.4 \times 10^{-4}$ M, respectively). None of the calystegines exhibited any appreciable inhibition toward baker's yeast trehalase. Calystegines $A_3$, $B_2$, and $B_4$ exhibited good inhibitory activities towards trehalase of *Bombyx mori* and *Spodoptera litura* [216]. Calystegine $B_1$ was a potent competitive inhibitor



of almond β-glucosidase ($K_i = 1.8 \times 10^{-6}$ M), and bovine liver β-galactosidase ($K_i = 1.6 \times 10^{-6}$ M) [391]. Calystegine $A_5$ showed no inhibition against trehalases of various origins [216]. Calystegine $C_1$ which was the first naturally occurring pentahydroxy-nor-tropane alkaloid was a more powerful competitive inhibitor against almond β-glucosidase ($K_i = 4.5 \times 10^{-7}$ M, $IC_{50} = 8.2 \times 10^{-7}$ M) and *C. saccharolyticum* β-glucosidase ($K_i = 2.9 \times 10^{-7}$ M, $IC_{50} = 8.6 \times 10^{-7}$ M) than calystegine $B_2$. However calystegine $C_1$ was a much weaker inhibitor than calystegine $B_2$ of green coffee bean α-galactosidase ($IC_{50} = 3.6 \times 10^{-4}$ M) and *Aspergillus niger* α-galactosidase ($IC_{50} = 4.4 \times 10^{-4}$ M) [226]. Its inhibition of rice α-glucosidase and porcine kidney trehalase ($IC_{50} = 4.2$ and $2.7 \times 10^{-4}$ M, respectively), was less than calystegine $B_2$ by an order of magnitude. Calystegines $B_1$ and $C_1$ were potent competitive inhibitors of the bovine, human, rate liver β-glucosidase activities with $K_i$ values of $1.5 \times 10^{-4}$, $1.0 \times 10^{-5}$ and $1.9 \times 10^{-6}$ M, respectively for $B_1$ and $1.5 \times 10^{-5}$, $1.5 \times 10^{-6}$ and $1.0 \times 10^{-6}$ M, respectively for $C_1$. Calystegines $B_2$ was a strong competitive inhibitor of the α-galactosidase activity in all the livers. Human β-xylosidase was inhibited with calystegine $C_1$ with a $K_i$ of $1.3 \times 10^{-7}$ M [395]. Calystegine $C_2$, the 2-epimer of calystegine $C_1$, was over 100-fold weaker as an inhibitor of β-glucosidase [$IC_{50} = 1.7 \times 10^{-4}$ M (almond), $IC_{50} = 9.0 \times 10^{-5}$ M (*Caldocellum saccharolyticum*)] than calystegine $C_1$ and had no inhibitory activity against α- and β-galactosidase. Only calystegine $C_2$ exhibited a moderate inhibitory activity against all α-mannosidases [$IC_{50} = 6.8 \times 10^{-4}$ M (almond), $4.6 \times 10^{-4}$ M (jack beans), $6.0 \times 10^{-5}$ M (rat liver soluble), $2.4 \times 10^{-4}$ M (rat liver lysosome) and $1.0 \times 10^{-4}$ M (rat epididymis)] and it was not an inhibitor of β-mannosidases. The structural basis of the inhibition of glycosidases by the calystegines was not obvious. The C-2, C-3, C-4 OH groups and ring heteroatom of calystegine $B_2$ lie in the same region of space as the C-4, C-3, C-2 OH groups and ring heteroatom of 1-deoxynojirimycin, which means that calystegine $B_2$ superimpose well on 1-deoxynojirimycin. Calystegines $B_3$ and $B_4$ superimpose well on 1,5-dideoxy-1,5-imino-D-galactitol and 1-deoxymannojirimycin, respectively. However, the biological properties of calystegines and piperidine alkaloids are quite different [396]. The introduction of a glycosyl residue to calystegines $B_1$ and $B_2$ resulted in a significant decrease in activity against β-glucosidase, α- or β-galactosidase, and β-xylosidase. Since calystegines $B_1$ and $B_2$ were competitive inhibitors of these enzymes [391] and can be considered to interact with their glycon binding site, this was to be expected because the glycosyl groups were likely to interfere with this interaction in *exo*-glycosidases, as seen in castanospermine nucleosides [397]. Calystegine glycosides might be an inhibitor of some *endo*-glycanases because 4-*O*-β-D-glucopyranosyl-1,6-dideoxynojirimycin was active against some cellulases from the cellulolytic bacterium *Thermomonospora fusca* [398]. Interestingly, the 3-*O*-β-D-glucoside (**438**) of calystegine $B_1$, but not the 3-*O*-α-D-glucoside of calystegine $B_1$ (**439**) nor the 4-*O*-β-D-glucoside of calystegine $B_2$ (**440**), exhibited a potent inhibitory activity against rice α-glucosidase, with an $IC_{50}$ value of $1.9 \times 10^{-6}$ M and $K_i$ value of $9.0 \pm 1.0 \times 10^{-7}$ M in a noncompetitive manner. The 4-O-α-D-galactoside of calystegine $B_2$ (**441**) retained potency against trehalase [399].

* For part 1 including chapters 1–15 and references 1–180 see PHARMAZIE **55**, 251 (2000).

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Prof. El Sayed H El-Ashry
Chemistry Department
Faculty of Science
Alexandria University
Alexandria
Egypt