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ScienceDirect
Tetrahedron
Tetrahedron 62 (2006) 10277–10302

Tetrahedron report number 773

α- and β-Glucosidase inhibitors: chemical structure and biological activity

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Received 14 August 2006
Available online 7 September 2006

Abstract—Glycoside trimming enzymes are crucially important in a broad range of metabolic pathways, including glycoprotein and glycolipid processing and carbohydrate digestion in the intestinal tract. Amongst the large array of enzymes, glucosidases are postulated to be a powerful therapeutic target since they catalyze the cleavage of glycosidic bonds releasing glucose from the non-reducing end of an oligo- or polysaccharide chain involved in glycoprotein biosynthesis. Glucosidase inhibitors are currently of interest owing to their promising therapeutic potential in the treatment of disorders such as diabetes, human immunodeficiency virus (HIV) infection, metastatic cancer, and lysosomal storage diseases. Glucosidase inhibitors have also been useful in probing biochemical pathways and understanding structure–activity relationship patterns required for mimicking the enzyme transition state. Amongst the various types of glucosidase inhibitors, disaccharides, iminosugars, carbasugars, thiosugars, and non-sugar derivatives have received great attention. This review is aimed at highlighting the main chemical classes of glucosidase inhibitors, as well as their biological activities toward α- and β-glucosidases, but it is not intended to be an exhaustive review on the subject. Inhibition data on the compounds covered in this review are included in a tabular form as an Appendix, where the type of each glucosidase associated with a specific inhibitor is also given.
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Contents

1. Introduction

Glucosidases are enzymes that catalyze the cleavage of glycosidic bonds in oligosaccharides or glycoconjugates. Several glucosidases are specific for the cleavage of glycosidic bonds depending on the number, position, or configuration of the hydroxyl groups in the sugar molecule. Thus, α- and β-glucosidases are able to catalyze the cleavage of glycosidic bonds involving terminal glucose connected at the site of cleavage, respectively, through α- or β-linkages at the anomeric center. The transition state structure for the substrates of these enzymes has a pseudoaxial orientation of the C–O bond and a skew conformation, suggesting that the main differences between α- and β-glucosidases are concerned with positioning of the catalytic nucleophile and the

Keywords: α-Glucosidase; β-Glucosidase; Glucosidase inhibitor; Disaccharide; Iminosugar; Carbasugar; Thiosugar; Non-glycosidic bond.
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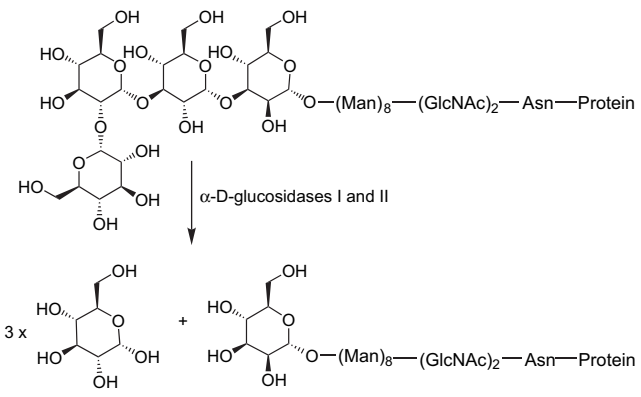
0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.08.055

catalytic proton donor, represented by two carboxylic acids units.[1]

The activity of glucosidases is fundamental to several biochemical processes such as (i) degradation of diet polysaccharides to furnish monosaccharide units, which are then able to be metabolically absorbed and used by the organism, (ii) lysosomal glycoconjugate catabolism and glycoprotein processing, and (iii) biosynthesis of oligosaccharide units in glycoproteins or glycolipids.[2]

The generation of glycoproteins involves the cotranslational transference of the tetradeca-oligosaccharide $Glc_3Man_9GlcNAc_2$ from the dolichyl diphosphate (DolPP) to the *N*-asparagine of the nascent protein, by the action of the oligosaccharyl-transferase (OT) in the lumen of the reticulum endoplasmatic membrane.[3] The enzymes glucosidases I and II are involved in the key steps of trimming of this *N*-linked oligosaccharide by cleaving Glc(1-2)Glc and Glc(1-3)Glc linkages, respectively, liberating the three glucose terminal residues of the $Glc_3Man_9GlcNAc_2$ glycoprotein (Scheme 1).[4] Subsequently, this immature glycoprotein is processed by the concomitant action of glycosidases and transferases to give specific glycoconjugates, which play fundamental roles in the biological processes, such as the immune response, intercellular recognition (including fertilization), cellular differentiation, the stability and solubility of proteins, and in pathological processes, such as inflammation and cancer, since α-glucosidase may play a role in tumor cells for the metastatic process.[5]

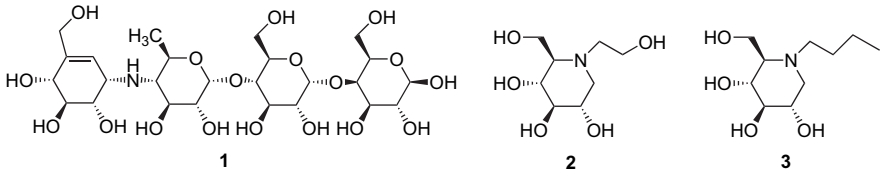


Scheme 1. Processing of the oligosaccharide ($Glc_3Man_9GlcNAc_2$) portion of the immature *N*-glycoprotein by the action of glucosidases I and II.

Inhibitors of glucosidases I and II have also been studied as potential anti-HIV agents.[6] The HIV viral envelope is composed of a bilipidic layer and a complex protein known as *env* that consists of glycoproteins gp41 (transmembrane) and gp120, the latter being displayed on the viral surface and anchored to gp41.[7] Glucosidases I and II participate in the formation of gp120, through processing of the $Glc_3Man_9GlcNAc_2$ *N*-linked glycoprotein, which is responsible for the recognition of the virus by CD4 receptors from T4 lymphocytes, in the initial process of viral infection. The modulation of the antigenicity of gp120 is dependent on the extension and variability of surface glycosylation and represents an interesting target to be explored in drug design.

The multiple functions of glucosidases in the organism warrant the search for potential therapeutic inhibitors to be used in diabetes,[8] obesity,[9] glycosphingolipid lysosomal storage disease,[10] HIV infections,[11] and tumors in general.[12]

Currently, three drugs are therapeutically used as anti-glucosidases: acarbose (**1**) (Precose®), miglitol (**2**) (Glyset®), and *N*-butyl-1-deoxynojirimycin (**3**) (Zavesca®). Drugs **1** and **2** are used in the treatment of non-insulin-dependent diabetes, type II, since they reduce the postprandial hyperglycemia by interfering with the digestion of dietary carbohydrates, while drug **3** is employed for the control of Gaucher's disease, related to disturbed lysosomal storage.



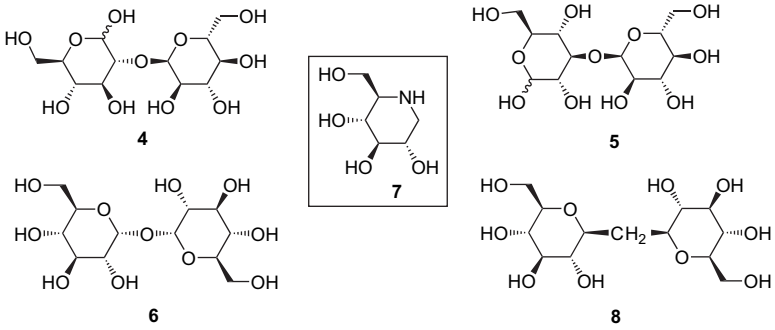
In support of the increasing interest in synthetic and natural glucosidase inhibitors as important tools for understanding biochemical processes and also as prospective therapeutic agents, this review describes the chemical structural diversity of the main α- and β-glucosidase inhibitors that comprise disaccharides, iminosugars, carbasugars, thiosugars, and non-glycosidic inhibitors, in addition to their corresponding biological activities.

2. Disaccharides

The isolation of kojibiose (**4**) and nigerose (**5**), inhibitors of α-D-glucosidase I and α-D-glucosidase II,[13] respectively, opened up new perspectives for the development of novel drugs, especially of the pseudodisaccharide class, for the treatment of HIV infections. Kojibiose (**4**) containing α-(1→2) glycosidic bonds was isolated in 1953 from sake extracts and also from its primary source, koji, a product related to rice fermentation by *Aspergillus oryzae*.[14] On the other hand, acid hydrolysis of amylopectin produced nigerose (**5**), which was shown to have an α-(1→3) linkage.[15] The importance of nigerose and nigerosylmaltooligosaccharides has also been shown to influence the immune function and quality of life in the healthy elderly person as a supplemental syrup on food.[16]

Extracts of *Mormodica charantia* seeds and of *Grifola frondosa* fruits showing α-glucosidase inhibitory activity have also been investigated and D-(+)-trehalose (**6**) was identified as the active component.[17] Trehalose, constituted by two units of glucose linked by an α-(1→1) bond, is employed in the preparation of foods and manufacture of cosmetics and

was recently suggested as a drug to be used in the treatment of osteoporosis, since it was shown to increase trabecular density in rat tibias. Its inhibitory capacity compared with the model 1-deoxynojirimycin (**7**) indicated that, while a concentration of 1×10^{-7} M of **7** showed a 52% inhibition of α-glucosidase, trehalose at 2×10^{-3} M had only 42% inhibitory activity.[17]



C-Disaccharides constitute another class of glycosidic analogues with potential enzymic inhibitory activity. In these compounds, a *C*-glycosidic bond is substituted for the usual *O*-bond, but they are similar to disaccharides, and assume conformations similar to these natural substrates. Postema et al.[18] synthesized several of these compounds, one with an α-1,1 glucosidic linkage (**8**), moderately inhibiting β-glycosidases (K_i 126 μM) and this chemistry has been reviewed in a research monograph.[19]

3. Iminosugars

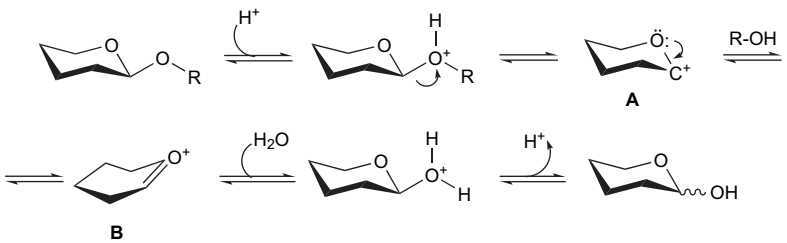
Iminosugars, isolated from plants or microorganisms, are considered to have a high potential therapeutic value and are of interest to be applied in the elucidation of biological recognition processes, due to their glucosidase inhibition properties.[20] The great potency and specificity of these inhibitors are related to their ability to mimic transition state pyranosidic or furanosidic units of natural glucosidase substrates. Significant competitive inhibition is observed with many inhibitors, suggesting that both conformational (shape) and electrostatic (charge) influences may be important in the active site binding.[1]

Iminosugars or polyhydroxylated alkaloids are low-molecular-weight compounds, able to inhibit glucosidases because they may mimic the conformation and charge of the oxycarbenium ion intermediate (**B**), but not of the carbocation (**A**) normally generated in the transition state with sp^2 character during the glycosidic bond cleavage (Scheme 2). The subsequent reaction results in overall retention or inversion of glucoside anomeric configuration, respectively, through double or single nucleophilic displacement.[1,21]

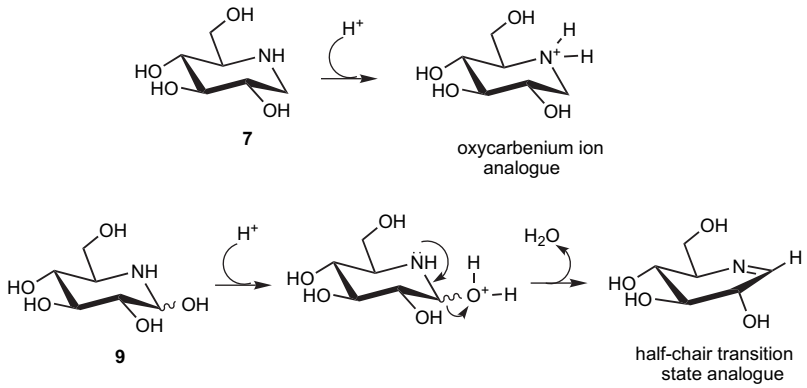
Considering that partial cleavage of the glycosidic bond intensifies the positive charge generated in the oxygen or anomeric carbon of the natural glycoside, substitution of one of the two atoms by protonated nitrogen will mimic, in the transition state, the charge in these centers.[22] In fact, the main characteristics consisting of stabilization of the positive charge on the nitrogen atom, trigonal anomeric center, half-chair conformation, and specific configuration of the hydroxyl ions are crucial for activity in these alkaloids.[23] Thus, relevant structural factors for glucosidase inhibition may be related to the charge and/or shape, defined by the hybridization and conformation of the pyranose ring in natural substrates or piperidine ring in inhibitors, like **7** and **9**. There is evidence suggesting that inhibitors in their natural basic form must be protonated to interact through ionic bonds with the carboxyl group in the active site, like **7** (Scheme 3).

Polyhydroxylated alkaloid structures containing at least two hydroxyl groups and one heterocyclic nitrogen atom may be mono or bicyclic and represented by rings consisting of piperidines, pyrrolidines, pyrrolizidines, indolizidines, or nor-tropanes.[24]

Nojirimycin (**9**) was discovered in 1966 as the first glucose analogue, which comprises endocyclic nitrogen in place of the oxygen pyranosidic atom. The polyhydroxypiperidine, initially isolated as an antibiotic from several strains of



Scheme 2. Carbocation (**A**) and oxycarbenium ion (**B**) intermediate formation during the cleavage of glycosidic bond catalyzed by β-glucosidases.



Scheme 3. Proposed mechanism for α-glucosidase inhibition by iminosugars, 1-deoxynojirimycin (**7**), and nojirimycin (**9**).

Bacillus, *Streptomyces*, and mulberry tree leaves, was shown to be a potent inhibitor of both α- and β-glucosidases of different origins. However, the presence of a hydroxyl group at C-1 adds instability that harms the biological assays. Reduction of **9** by catalytic hydrogenation or sodium borohydride provides analogue **7**, a more stable and a potent glucosidase inhibitor in vitro. Based on experiments involving cultured cells, compound **7** inhibited the formation of complex-type oligosaccharides, leading to the accumulation of $Glc_{1\text{-}3}Man_{7\text{-}9}GlcNAc_2$. However, its in vivo activity is only moderate, besides producing side effects.[25]

The search for more potent iminosugars has led to the preparation of *N*-alkylated analogues, like compounds **2** and **3** with anti-HIV activity, for the treatment of diabetes and Gaucher's disease and even with antiviral effects against B[26] and C hepatitis, bovine diarrhea (BVDV),[27] and dengue virus.[28] Chemical synthesis of *N*-alkylated compounds commonly involves reductive amination reactions between iminosugars and alkyl aldehydes in the presence of a reducing agent like sodium cyanoborohydride. The activity of 1-deoxynojirimycin (**7**) is pH dependent and inhibits glucosidase II more strongly than glucosidase I, while N-alkylation gives the opposite result, either for experiments involving purified enzymes or cell culture.[29] Interestingly, inversion of the configuration at C-5 in **7** to give 1-deoxy-L-idonojirimycin (**10**) leads to loss of the inhibitory activity toward yeast α-glucosidase shown by the former compound, which is a potent competitive inhibitor of this enzyme. Compound **10** shows only a non-competitive inhibition of the enzyme[30] and the fact that 1,5-dideoxy-1,5-iminoxylitol (**11**) has no effect on the activity of yeast α-glucosidase indicates that the 5-CH_2OH group in **7** plays an important role in promoting effective binding.[31]

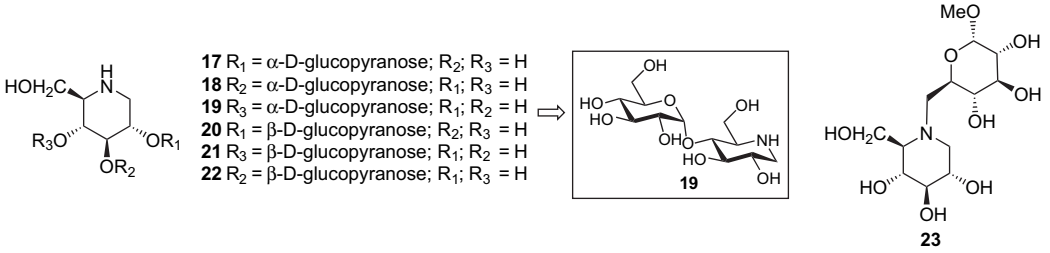
Polyhydroxypiperidine derivatives comprise the main class of glucosidase inhibitors, with a great variety of compounds of natural origin, isolated from fungi, bacteria, and plants, besides the synthetic derivatives, and several of them show high inhibitory constants for both α- and β-glucosidases. Recently, this class of compounds has attracted more attention owing to the inhibition of other enzymes such as glycosyltransferases, glycogen phosphorylase,[32] nucleoside phosphorylases,[33] and sugar-nucleotide mutases (UDP-Gal*p* mutase).[34]

α-Glucosidase inhibition studies in human hepatoblastoma cells (HepG2) using a series of *N*-alkylated iminosugars showed increasing activity with extension of the length of the linear alkyl chain and decreasing activity with the introduction of branching. However, in vitro assays with purified pork α-glucosidase I showed a decrease in the inhibitory activity, suggesting that these lipophilic alkylated groups may have a relevant role in cellular uptake.[35] Better results from the *N*-alkyl variations relied on a less lipophilic group instead of a linear long alkyl chain, revealing that derivatives incorporating an oxygen atom in an *N*-decyl side chain might be less cytotoxic and as potent as **3** against α-glucosidase. For instance, the *N*-7-oxadecyl-DNJ **12** had a strong effect toward α-glucosidase I, even though it was a weak inhibitor in cellular assays. Furthermore, compound **12** was able to inhibit HIV-1-induced syncytia formation and lymphocyte proliferation in vitro, along with a reduction profile in arthritis in rats.[36] Abolishing the basicity of the nitrogen by N-oxidation or N-acylation afforded less active products.[4]

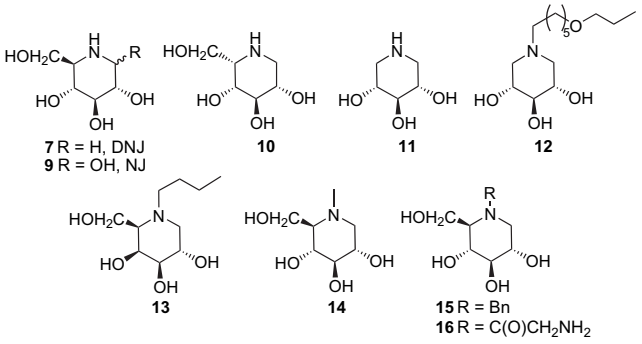
Ceramide glycosyltransferase inhibition occurred only with the *N*-alkyl derivatives of **7** containing at least three carbon atoms. In relation to the stereochemistry and functional groups of compounds similar to **7**, C-3 modifications induced loss of activity against α-glucosidases I and II, and ceramide glycosyltransferase, suggesting the common structural features for inhibition of these enzymes. The exclusive inhibition of a transferase by *N*-butyl-1-deoxygalactonojirimycin (NB-DGJ, **13**) is the only exception to this rule. In fact, compounds **3** and **13** are good inhibitors for ceramide-specific glucosyltransferase, either in vitro or in vivo, suggesting that they mimic the ceramide substrate, since there are similar stereochemical features for both α-glucosidase and ceramide glucosyltransferase inhibitors.[10]

The activity of compound **3** is extremely dependent on the positions and functions of groups in the piperidine ring, which should be adequately positioned to mimic the oxycarbenium ion intermediate in the enzyme active site. Thus, epimerization at C-2 or C-4 or methylation of the hydroxyl at C-3 decreases the activity, while modifications at C-1 and C-6 or at the *N*-alkyl group are tolerated.[10] Additionally, an investigation of the epimerization, deoxygenation, and conformation contributions of a series of seven polyhydroxylated piperidines toward their inhibitory activity was performed, leading to some structure-based evidence that 2-deoxygenation and/or 3-epimerization of **3** enhance the activity of rat intestinal lactase and bovine liver cytosolic β-galactosidase.[36]

Comparing compounds **3**, **7**, and *N*-methyl-1-deoxynojirimycin (**14**), there is no significant difference in their ring conformation, but a slight difference was detected in their conformations about the C-5–C-6 bond that is predominantly tg (trans/*gauche* H–H relationship) or gt for compound **7** and gg for **3** and **14**.[37] Moreover, the *N*-methyl derivative **14** is a more potent inhibitor of α-glucosidase I than is **7** and is also more active against the replication of the HIV virus.[6] An interesting activity was demonstrated by compound **14** and miglitol (**2**), owing to their protective effect against post-ischemia dysfunction of the left ventricle, by altering glycogenolysis through α-1,6-glucosidase



inhibition.[38] On the other hand, studying the interactions of the enzyme bound to *N*-^{13}C-methyl-deoxynojirimycin as a chemical probe, Hines et al.[39] prepared analogues **15** and **16** to explore the π–π stacking or cation–π interactions with the aromatic residues of the glucosidase active site. Although compound **15** showed no activity up to 3.0 mM, the *N*-glycyl derivative **16** was more potent than **14**.



The use of native or immobilized glucosidases in transglucosylation reactions with *N*-benzyloxycarbonyl-1-deoxynojirimycin as an acceptor molecule, led Asano et al.[40] to develop a strategy to prepare *N*-containing sugars illustrated by 2-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (**17**), 3-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (**18**) and 4-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (also named 4-*O*-α-D-glucopyranosylmoranoline, **19**),[41] and the corresponding anomers 2-*O*-β-D-glucopyranosyl-1-deoxynojirimycin (**20**) and 4-*O*-β-D-glucopyranosyl-1-deoxynojirimycin (**21**), by incubating with α-glucosidase and β-glucosidase, respectively. After cleavage of the *N*-benzyloxycarbonyl group of the product glycosides, the *N*-protective group being used to prevent complete loss of glucosidase activity during the enzymic synthesis, compounds **18** and **19** retained the activity of **7** toward rat digestive glucosidase, including sucrase and isomaltase, while **18** was a much stronger inhibitor in rice α-glucosidase experiments than **7** and the same effect was observed for **17** against trehalase. A series of disaccharides related to lactosamine and chitobiose also comprising a 1-deoxynojirimycin moiety were synthesized, but their activity toward glucosidase was not reported.[42]

Studies on 1-deoxynojirimycin-containing glycans isolated from *Morus alba* also gave the potent glucosidase inhibitors described above, along with 3-*O*-β-D-glucopyranosyl-1-deoxynojirimycin (**22**) and the corresponding inactive 6-*O*-β-derivative against maltase and weak inhibitor of sucrase (IC_{50} 940 μM). Isomer **19** was also isolated from natural sources such as *Scilla sibirica*.[43]

From this series of glycosylated deoxynojirimycins, it is worth mentioning compound **23**, namely MDL 73945, which showed potent inhibition against α-glucosidases, such as sucrase, maltase, and isomaltase, and which was approved for clinical trials to treat postprandial hyperglycemia in diabetes mellitus patients.[44] Despite the convenient introduction of a sugar residue into many positions of **7**, the results with compound **23** provide evidence that glycosylation on the nitrogen gives a more effective inhibitor.

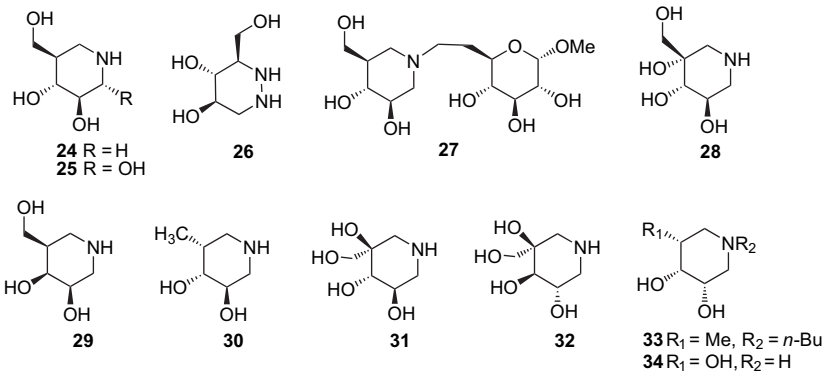
Some piperidinyl analogues are able to mimic the anomeric carbon's positive charge in the transition state, since the presence of the hydroxymethylene group is not essential to β-glucosidase inhibition, as demonstrated for dehydroxymethyl-1-deoxy-nojirimycin (**11**).[36]

These substances are called 1-azasugars and their main representative is isofagomine (**24**), which has the anomeric carbon and the ring oxygen of glucose replaced by nitrogen and carbon, respectively; the 2-OH group is also absent, but the remaining hydroxyl groups preserve the D-glucose configuration.[45] Compound **24** and derivatives, like **26**–**31**, lacking substituents on the α-carbon atom's neighboring nitrogen, are potent inhibitors, **24** being about 440 times more potent than **7** toward β-glucosidases, but only a moderate inhibitor of α-glucosidase.[46] Stereochemical alterations in ring-substituent groups may reduce activity, as illustrated by product **32**. Introducing a hydroxyl group at C-2, as shown in compound **25**, namely noeuromycin, afforded a stronger β-glucosidase inhibitor, probably due to an additional hydrogen bond interaction in the active site. Although compound **25** was prepared as a mixture of stereoisomers at C-2 (α/β 1:2), the activity was stronger than that of compounds **7**, **9**, and also **24**, presumably caused by the equatorial β-isomer. In contrast to isofagomine, compound **25** was also a potent inhibitor of α-glucosidase.[47]

Crystallographic studies on isofagomine derivatives in the endocellulase active site have provided evidence for the

presence of a protonated center, in the nitrogen-containing inhibitor bound with the active center, with a conformation similar to that of the ground state. Other studies, however, have revealed a variety of conformational distortions in the iminosugar target interactions. Although it is difficult to establish the true conformation assumed by these rings in an enzyme site, it seems clear that the binding of these derivatives is enthalpy favored and that protonation of the amino group contributes exothermically to this process.[22]

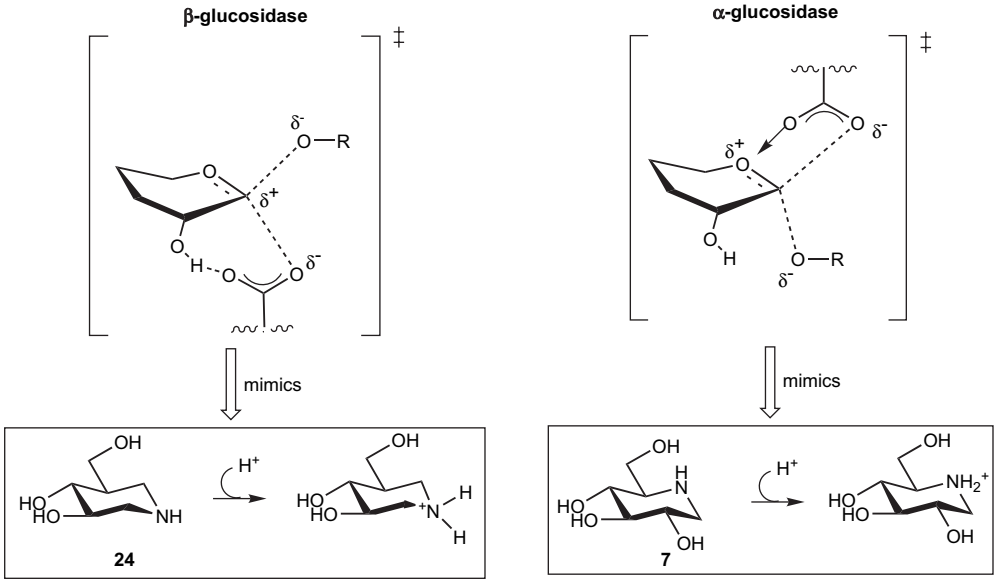
a *syn* interaction of the carboxyl nucleophile with the anomeric carbon, but, on β-glucosidase, the nucleophile interacts additionally with the hydroxyl group at C-2, favoring the carbocation formation, while, with α-glucosidase, this interaction occurs with the endocyclic oxygen, developing an oxycarbenium ion-like transition state.[21] Comparatively, deoxynojirimycin (**7**) may mimic the charge of the oxycarbenium ion, having potent anti-α-glucosidase activity, while isofagomine (**24**) mimics the charge developed on the anomeric carbon, leading to a more potent β-glucosidase



Concerning the specificities of the iminosugar inhibitor **7** and the azasugar, isofagomine (**24**), for α- and β-glucosidases, respectively, it is worth noting the similarities in mechanism of these inhibitors with that involving the corresponding natural substrate of each enzyme. According to kinetics and three-dimensional studies involving α- and β-glucosidases in the transition state, there is a difference in the oxycarbenium ion character between these enzymes. As shown in Scheme 4, the differing orientation at C-1 of the α- and β-anomeric group in the enzymic substrates will direct the best fit for interaction with the nucleophilic carboxyl oxygens in the active site. In both enzymes, there is

inhibitor. An interesting activity was observed for **24** consisting of potential inhibition of hepatic glycogen phosphorylase with an IC_{50} value of 0.7 μM, since this enzyme is involved in the suppression of the increased basal and glucagon-stimulated glycogenolyses in type II diabetes patients.[12]

Based on the assumption that the carbocation transition state might be more important for equatorial hydrolysis (β-glucosidase), while the oxycarbenium transition state is favored for axial hydrolysis (α-glucosidase), Bols[45] synthesized the hydrazine analogue of isofagomine, compound **26**, comprising both mimic functions. Comparatively, the inhibitory



Scheme 4. Comparison of mechanism of action of isofagomine (**24**) and deoxynojirimycin (**7**) with the corresponding substrates of β- and α-glucosidases, respectively, in their preferential transition state.

effect of **26** on α-glucosidase was stronger than that of compounds **7** and **24**, whereas on β-glucosidase, it was weaker than **24**, but more potent than **7**.

1-Azasugar analogues bearing an *N*-linked glucose residue, such as **27**, led to a stronger glucoamylase inhibitor than **24**, preserving high β-glucosidase activity. Alkylation at nitrogen in **27** afforded the ammonium derivative, which was not as active as the corresponding *N*-oxide compound.[45]

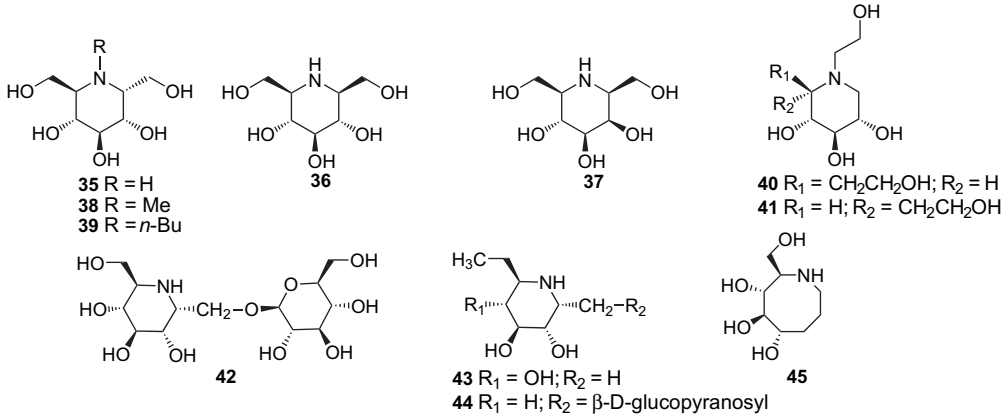
Some iminosugars that are not configurationally derived from glucose may also have high anti-glucosidase activity. Igarashi et al.,[48] in a search for new α-fucosidase inhibitors, synthesized compounds **33** and **34**, respectively, with reasonable and potent anti-glucosidase activity.

Nojirimycin homologues with a hydroxymethylene group at C-1 are named as α-homonojirimycin (**35**) and β-homonojirimycin (**36**) and were isolated from leaves of *Omphalea diandra*[49] and *Aglaonema treubii*,[50] respectively. The ability of **35** to inhibit digestive α-glucosidase was reported by Kite et al.[49] and compared to that of deoxynojirimycin (**7**), which suggested that the attachment of a hydroxymethyl group at the anomeric position of **7** gives better enzyme selectivity. Nevertheless, compound **36** was inactive as an inhibitor of almond β-glucosidase, but showed reasonable activity toward rice α-glucosidase.[51] In order to investigate the contribution of the chiral centers and conformation on glycosidase inhibition, Asano et al.[52] prepared a series of natural epimers of α-homonojirimycin and its *N*-alkylated derivatives and achieved some interesting results with β-homomannojirimycin (**37**) against α-glucosidases and α-L-fucosidase. Concerning the *N*-alkyl derivatives, *N*-methyl-α-homonojirimycin (**38**) was a better inhibitor of glucosidase I than **35** and the corresponding *N*-butyl-α-homonojirimycin (**39**), but, in contrast to **7**, all of them were inactive against HIV-1 replication. An approach to perform some modifications on miglitol (**2**) was also described involving the preparation of *N*-hydroxyethyl-D-*gluco*-1-deoxyhomonojirimycin (**40**) and *N*-hydroxyethyl-L-*ido*-1-deoxyhomonojirimycin (**41**), which differs in the configuration at C-5. Thus, the inhibition assays provide evidence that the introduction of an extended hydroxyethyl group in the α-position to the amino group, instead of a hydroxymethyl as observed in **7**, afforded better β-glucosidase inhibitors, as compared to α-glucosidases. The corresponding peracetylated derivative of **41** was also potent against β-glucosidase.[53]

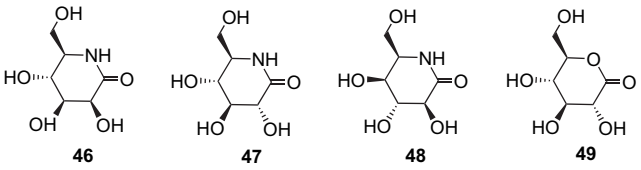
An analogue of homonojirimycin, 7-*O*-β-D-glucopyranosyl-α-homonojirimycin (**42**), was isolated from a methanolic extract of *Lobelia sessifolia*, and is a hybrid containing a polyhydroxylated piperidine system connected via an oxymethylenic bridge to a glucose unit. This compound has high α-glucosidase and trehalase inhibitory activities. Several other homonojirimycin derivatives were isolated from the roots of *Adenophora* spp., another member of the same family. Among the polyhydroxylated alkaloids obtained, such as adenophorine (**43**), the glucoside derivative of 5-deoxyadenophorine (**44**), along with compound **42**, showed potent inhibitory activity of α-glucosidases and, additionally, α-galactosidase.[54]

Godin et al.[55] recently described an expansion of the ring in piperidine derivatives to an eight-membered iminoalditol (**45**). ^{1}H NMR studies showed that this compound exists mainly in the boat–chair conformation, with the substituents assuming a pseudo-equatorial conformation. However, compound **45** was weakly inhibitory to α- and β-glucosidases (22.1 and 37%, respectively), probably due to the absence of OH substituents at C-6 and C-7, which would correspond to the OH groups at C-3 and C-2 in natural glycosides.

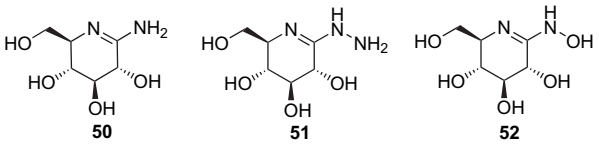
Glycono-δ-lactams, which also belong to the iminosugar class, were one of the first glycosidic groups found to have potent anti-β-glucosidase activity. Studies based on X-ray crystallographic analysis and molecular modeling of eight D-glycono-δ-lactams have been described by Nishimura et al.[56] These compounds follow some of the general rules for β-glucosidase inhibition, which are the half-chair conformation, simulation of the oxycarbenium ion intermediate, and the positive charge around the anomeric carbon. However, the *gluco*-configuration does not seem to be a requisite for high-potency glucosidase inhibition, since D-mannono-δ-lactam (**46**) was more active against β-glucosidase than D-glucono-δ-lactam (**47**), while D-idono-δ-lactam (**48**) showed a similar activity to **47**. It is possible that the carbonyl group in these compounds is topologically equivalent to the glycosidic oxygen atom in the high-energy transition state observed in β-glucosidases. The parent compound, D-glucono-δ-lactone (**49**), has also been described as a strong inhibitor of β-glucosidase, similar to the piperidine **9** and lactams **46**–**48**, and is more effective by a factor of 100 when compared to its inhibition of α-glucosidase.[57] The relative importance of the stereochemical and conformational similarities between **49** and the transition state in the



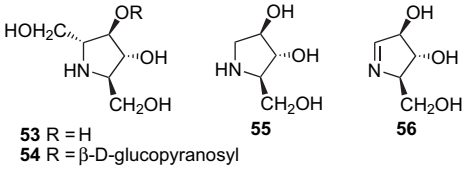
enzyme-catalyzed reaction has been argued about since 1968,[58] besides the influence of the polar oxy group that partially mimics the oxycarbenium ion intermediate, which may be stabilized by a carboxylate group at the active site involving charge–charge interactions.[57] Nevertheless, the true character of the transition state analogue is still under investigation.



Ganem et al.[59] prepared a variety of monosaccharide-like alkaloids containing anomeric centers hybridized to sp^2 carbon consisting of amidine (**50**), amidrazone (**51**), and amidoxime (**52**), obtained from nojirimycin (**9**), showing potent β-glucosidase inhibitory activity. The presence of nitrogen atoms in the *exo* and *endo* positions, 'circling' the anomeric carbon, combines the structural features of both families of iminosugars and glycosylamines. Owing to the common structural elements, these compounds may interact in the active site by similar H-bonding and electrostatic forces, even though they have different basicity properties. The authors concluded that the broad-spectrum of inhibition of **50**–**52** can be attributed largely to the flattened anomeric conformation adopted in the transition state binding, rather than through achieving the formal charge that mimics the oxycarbenium ion (**B**) (Scheme 2). On the other hand, Heightman and Vasella[1] suggested that the neutral inhibitor **52**, the inhibition of which is pH dependent, undergoes an enzymic protonation within the binding site and the basic derivatives **50** and **51**, which are protonated at pH values up to 7, interact most probably by electrostatic interaction.



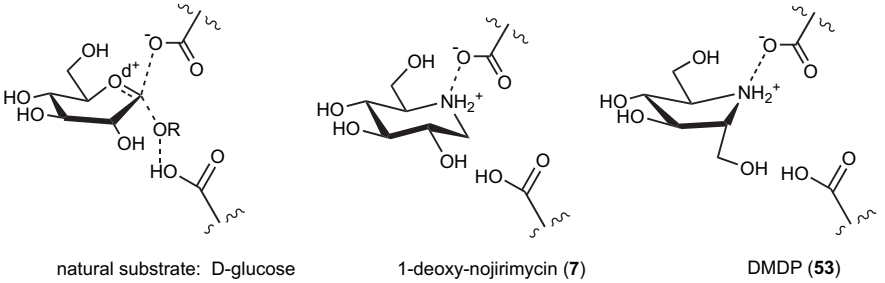
Analogues of pyrrolidine alkaloids include 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidines, for example, or 2,5-dideoxy-2,5-imino-D-mannitol (DMDP, **53**) having the all *R*-configuration, which can be regarded as a mimic of a natural β-D-fructofuranose unit. Compound **53** was first isolated from *Derris elliptica* and also from several plants and microorganisms, suggesting that it is a common metabolite in several species.[54] The relatively flat five-membered ring with a unique C_2 axis of symmetry makes compound **53** similar to the transition state observed during substrate hydrolyses. Additionally, its high affinity to glucosidases probably results from the spatial arrangement of the hydroxyl groups, similar to the natural substrate molecule, glucose (Scheme 5).[60] Standard substitution of the hydroxyl function of the hydroxymethylene group at C-1, exemplified by adding fluoro, amino, or methoxyl groups, decreases the activity, while introduction of *O*- or *N*-alkylated chains or an aryl group produces lipophilic derivatives with a potency similar or higher than that of the prototype **53**.[61] Introduction of methyl or hydroxymethylene groups at the hydroxymethylene carbon produces derivatives of reduced potency.[21]



The iminoalditol **53** is known as a potent reversible inhibitor of α-glucosidases I and II, almond β-glucosidase, bovine liver β-galactosidase, invertase, and PFP (pyrophosphate-D-fructose-phosphate-1-phosphotransferase), along with an interesting property, its ability to neutralize the activity of compound **7**. The ability of **53** to promote accumulation of $Glc_3Man_9GlcNAc_2$ *N*-linked oligosaccharides in the endoplasmic reticulum is regarded as a result of its strong inhibitory activity of processing by glucosidase I, and it showed significant anti-HIV activity.[37] An extra biological activity shown by DMDP is its toxicity observed toward several insects, demonstrating an antifeedant protector effect.[60]

Recently, 3-*O*-β-D-glucopyranosyl-DMDP (**54**) was isolated from the roots of *Stemona tuberosa* Lour, as a component of Thai traditional drugs used in China and Japan for various medicinal purposes. Despite the high activity shown by glycosylated-DNJ, including **18** and **19**, this derivative proved to be a weaker inhibitor of β-galactosidase than the parent compound **53**, and it was inactive toward β-glucosidase.[62]

Another active natural product comprising a pyrrolidine ring is illustrated by 1,4-dideoxy-1,4-imino-D-arabinitol (DAB-1, **55**), which was first isolated from *Angylocalyx boutiquenus* and showed a potent inhibitory activity along with a broad inhibitory spectrum toward mammalian

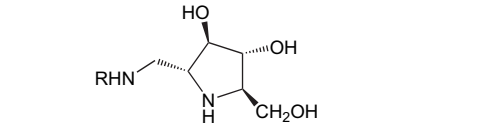


Scheme 5. Transition state in reaction of D-glucose with glucosidase, compared to those with inhibitors DNJ (**7**) and DMDP (**53**).

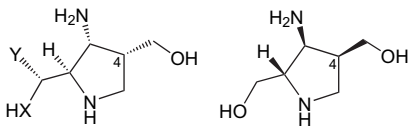
glucosidases, including α-glucosidase II, α-mannosidases I and II, intestinal isomaltase, and trehalase.[60] As proposed for **24**, compound **55** was also found to be a potent inhibitor of hepatic glycogen phosphorylase that could be used as a new antihyperglycemic agent for the treatment of type II diabetes (IC_{50}=1 μM).[12] However, the five-membered analogue **55** was less potent than compounds **7** and **53** as an anti-HIV agent, since the inhibition of α-glucosidase II is less correlated with anti-HIV activity.[37] Another pyrrolidine compound, namely nectrisine (**56**), isolated from the fungus *Nectria lucida* as an immunomodulator, has also received much attention, owing to its potent α-glucosidase inhibitory activity in vitro and in cultured cells by acting as a competitive inhibitor.[63]

Structure–activity relationship studies demonstrated that the introduction of amide groups comprising aromatic or aliphatic lipophilic chains at C-1 produces β-glucosidase inhibitors active in the nanomolar range. The coumarinic derivative bound by an amide bridge to the nucleus (**53**), exemplified by compound **57**, was the most potent pyrrolidine inhibitor of β-glucosidase (K_i=1.2 nM), while naphthyl derivatives bound by amide **58** and sulfonamide **59** bridges showed inhibition constants of 550 and 100 nM, respectively. On the other hand, introducing the *N*-dimethylamino group into the aromatic ring in **59** gave a sulfonamide derivative with strong inhibitory activity (K_i=2.4 nM). Unlike piperidine derivatives, introduction of the *N*-alkyl group did not increase the relative potency of **57** against the HIV virus.[61]

Falb et al.,[64] exploring intramolecular cycloaddition reactions between oximes and alkenes, from L- and D-amino acids as starting materials, synthesized compounds **60–62** and **63**, respectively. In these analogues, the hydroxyl group at C-4 in compounds such as **53** is replaced by a hydroxymethyl group, providing branched-chain azasugars. Additionally, they have a C-3 amino group introduced as an isostere of the hydroxyl group to keep the hydrogen bonding in the active site. These structural characteristics provide the necessary pattern for anti-glucosidase activity, as illustrated by the planar ring conformation and the possibility to stabilize a positive charge generated in the transition state. Among these derivatives, compound **63** was the best inhibitor of α-glucosidase.



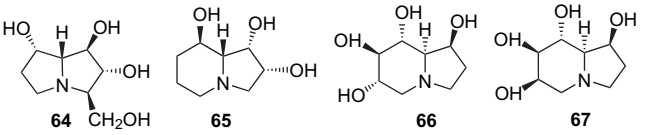
57 **58** **59**



60 X= O; Y= H
61 X= O; Y= Me
62 X= S; Y= H

63

Polyhydroxylated pyrrolizidine alkaloids, exemplified by australine (**64**, isolated from *Castanospermum australe*), are structurally related to the fusion of two pyrrolidine rings, with a common nitrogen atom at the junction. Unlike the symmetry of pyrrolidine derivative **53**, australine does not have a C_2 axis of symmetry, but it does provide a good pattern of competitive inhibition toward processing α-glucosidase and amyloglucosidase.[65]



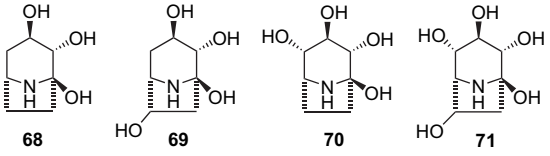
64 **65** **66** **67**

Fusion of the piperidine and pyrrolidine rings results in the indolizidine class of compounds comprising (−)-swainsonine (**65**), isolated from *Swainsona canescens*, *Astragalus lentiginosus*, *Ipomoea carnea*,[66] etc., and castanospermine (**66**), extracted initially from the seeds of *Castanospermum australe*,[67] the first alkaloids to exhibit strong inhibition toward α-mannosidases and α-glucosidases, respectively. Compound **66** may be regarded as a derivative of 1-deoxynojirimycin (**7**) on account of its similar structural and biological properties. The hydroxymethyl group of **7** is spatially locked by an ethylene bridge, which is linked to the nitrogen, generating a pyrrolidine ring. This rigidity could explain the preferred anti-glucosidase activity exhibited by **66** and its derivatives, as the ethylenic bridge helps to stabilize the transition state conformation, with participation of the enzyme acidic group.[11] The basic nitrogen in the indolizine class plays an important role in the inhibition, since experiments performed with castanospermine *N*-oxide involving almond β-glucosidase gave K_i values 500-fold larger than **66**.[21]

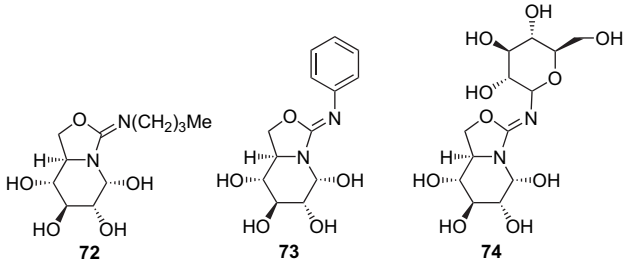
The octahydroindolizidine alkaloid **66** is one of the derivatives with the highest inhibitory effects on glucosidases, inhibiting both mammalian and plant α- and β-glucosidases, β-glucocerebrosidase, and rat intestinal sucrase. However, **58** has no effect on yeast glucosidase or β-*N*-acetyl-hexosaminidase. The corresponding diastereomer (6*R*), (+)-6-epicastanospermine (**67**), does not have the wide range of activity as **66**, even though it was a potent inhibitor of amyloglucosidases.[68] Structure–activity studies suggest that the bicyclic system of compound **66**, able to lock the bond corresponding to C-5–C-6 in hexopyranoses, provides a high specific enzymic activity when compared to the monocyclic compound **9**.[69] It seems that compound **66** may also interfere in the transport of free oligosaccharides (FOS) performed in the endoplasmic reticulum and cytosol during glycoprotein biosynthesis. The progressive pathology in patients with glucosidase I congenital deficiency may be included in this anomaly.[70] Furthermore, the use of castanospermine (**66**),[71] swainsonine (**65**),[72] and glucono-lactone (**49**)[73] screened on B16 melanoma cells led to the inhibition of experimental metastasis by blockage of protein glycosylation or oligosaccharide processing, whereas **66**[74] and **7**[75] inhibited virus

syncytium formation and HIV replication of human immunodeficiency virus.

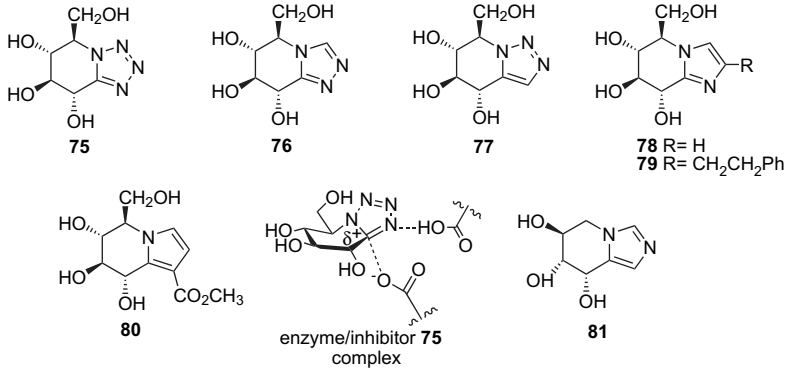
Another class of natural products contains the bicyclic system of nor-tropane, and these compounds exhibit potential β-glucosidase-inhibiting properties. Such compounds with the nor-tropane structure are called calystegins (**68–71**) and are isolated from many plants, such as *Calystegia sepium*, *Ipomoea carnea*, and *Physalis alkekengi* var. *francheti*. They are classified according to the number of hydroxyl groups present in the bicyclic system, as illustrated by calystegins A_3 (**68**), B_1 (**69**), B_2 (**70**), and C_1 (**71**). Despite the structural variation, all calystegins have a hydroxyl group at the ring junction, in the α-position relative to the nitrogen atom, generating an amino-hemiacetal function and an ethano bridge across the 1,5-positions.[76] Initially known by their unique involvement in plant–bacterium relationships, the potential use of calystegins B complex (27% of **69** and 73% of **70**) has been extended by Molyneaux et al.[77] as a competitive inhibitor of β-glucosidase and α-galactosidase, while **71** was shown to exhibit strong inhibition only on β-glucosidase.



Recently, Garcia-Moreno et al.[69] reported a new class of hybrid compounds, derivatives of indolizidines, such as castanospermine and trehazolins. The structure of this new class of azasugars was designed to replace the sp^3 amino nitrogen by the pseudo-amide nitrogen (as in urea, thiourea, and carbamate) with higher sp^2 character. This structural change should increase the anomeric effect in the proposed aminoacetal center and keep the original conformation and configuration in aqueous media that is not observed in classical iminosugars. The isourea-type products synthesized include compounds **72–74** comprising structural characteristics of polyhydroxyindolizidines and, in **74** (trehazolin), which is a natural inhibitor of trehalase, comprising exocyclic nitrogen susceptible to further modifications that should include the introduction of different substituents to modulate enzyme specificity. In fact, the inhibitory activity was greatly influenced by the nature of these substituents and pH, leading to an increase in activity or preferred inhibition between α- or β-glucosidase.



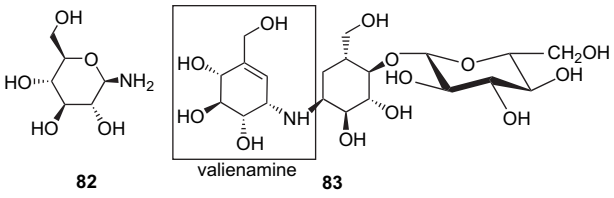
Other bicyclic systems with the piperidine nucleus fused to a tetrazole ring **75**, a triazole ring **76**, **77**, a pyrazole ring **78**, **79**, and a pyrrole ring **80** have been synthesized to afford a half-chair conformation of the six-membered ring as a result of its fusion with the heterocycle, thereby mimicking the transition state of glucosidases. A higher degree of selective anti-β-glucosidase activity was achieved in derivatives containing a nitrogen atom adjacent to the anomeric center in the iminosugar, as in compounds **76**, **78**, and **79**. This suggests that this nitrogen atom, corresponding to the oxygen of the *O*-glycosidic linkage, is important for interaction in the active site by hydrogen bonding with a carboxylic acid group, which in turn intensifies the anomeric center's positive charge, as illustrated in the enzyme/inhibitor complex for **75**, favoring interaction with a carboxylate group. This model requires a coplanar ring arrangement of the tetrazole nucleus and the carboxylate group in addition to the presence of flexible hydrophobic aglycon mimics to provide unspecific interactions in the binding site. These factors could explain, at a molecular level, the strong activity of compound **79**, a tetrahydroimidazo[1,2-*a*]pyridine, the most potent β-glucosidase inhibitor of this group, which contains an extra hydrophobic 2-phenylethyl group.[1,78] It is worth mentioning that changes in the nitrogen position and in the number and conformation of the hydroxyl groups to give compound **81** provided a weak inhibitor of yeast α-glucosidase.[79]



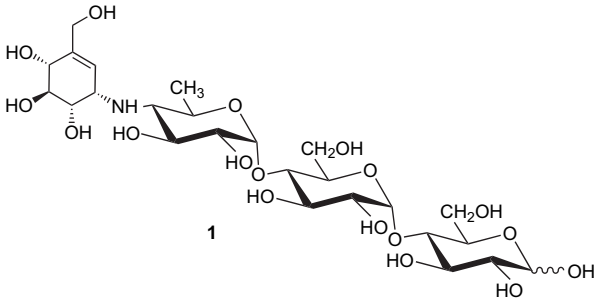
4. Carbasugars and pseudoaminosugars

The aminoglycoside, 1-amino-1-deoxy-D-glucose (**82**), having an unstable hemi-aminal structure, was the first glucosidase inhibitor to be described having a basic nitrogen group.[80] Its inhibitory activity was enhanced by N-alkylation, due to an additional interaction with a hydrophobic

pocket of the enzyme.[81] However, the greater interest in this class of compounds lies with the pseudoaminosugars, in which the oxygen atom of the pyranose ring is substituted by carbon, the oxygen of the glycosidic bond is replaced by nitrogen, and the overall configuration is similar to D-glucose. They exist in a monomeric form or condensed to other cyclic units, as illustrated by the main representatives, acarbose (**1**) and validamycin A (**83**).[82] The more complex structures consist of an unsaturated cyclitol linked via a nitrogen bridge to the other cyclic units. The aminocyclitol, valienamine (**84**), is probably biosynthesized from 2-*epi*-5-*epi*-valiolone (**85**) by *Streptomyces hygroscopicus* var. *limoneus*.[83] Valienamine (**84**) and validamine (**86**) were obtained by microbiological degradation of validoxylamine A (**87**) by *Pseudomonas denitrificans* and *Flavobacterium saccharophilum* or chemically by treatment with *N*-bromosuccinimide (NBS), as well as being synthesized in both racemic and enantiomerically pure forms.[84]



82 **83**



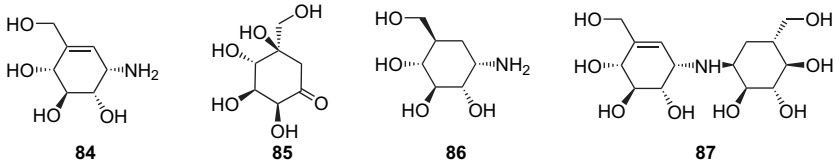
1

Validamycin A (**83**) is the main component isolated from *S. hygroscopicus* var. *limoneus* and it represents a pseudotrisaccharide consisting of a unit of **84** connected by a nitrogen bridge to validamine (**86**), as in validoxylamine A (**87**),[85] which is itself linked to D-glucose. However, instead of being a potent α-glucosidase inhibitor, **83** showed antibiotic activity against *Rhizoctonia solani* and *Pellicularia sasakii*, and is also used in agriculture to control sheath blight disease of rice plants caused by the fungus *R. solani*.[82]

sp. SE-50.[88] Catalytic hydrogenation of **1** afforded fragments consisting of trisaccharide derivatives, which are devoid of inhibitory activity on α-amylase or sucrase, suggesting the importance of the valienamine unit (**84**). Besides the valienyl residue, the pseudotetrasaccharide (**1**) contains a 4-amino-4,6-dideoxy-glucose unit and two glucose residues (forming maltose). The combined first two residues form a pseudodisaccharide called acarviosin and, connected by an *N*-glycosidic bond, are able to mimic the *O*-glycosidic bond of natural substrates.[82]

Valiolamine (**88**), hydroxyvalidamine (**89**), and voglibose (**90**) have similar absolute configurations to α-D-glucose, and these derivatives are strong α- and β-glucosidase inhibitors having a considerable potential for use in the treatment of cancer or AIDS.[24] Compound **84** exhibited strong inhibitory activity against α-glucosidase and α-glucoamylase from *Rhizopus* and a moderate to weak effect on almond β- and α-amylases, respectively. Extension of this work by Kameda et al.[89] showed that **84** is also an antibiotic against *Bacillus* sp. On the other hand, assays involving porcine intestinal sucrase, maltase, and isomaltase revealed that **88** was a more effective compound than the other aminocyclitols.[82]

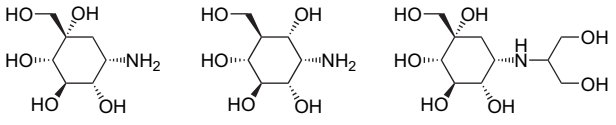
Voglibose (Basen®, **90**), which also has a high inhibitory activity against sucrase and maltase, has been employed in Japan for the treatment of diabetes since 1994. In recent studies based on α-glucosidase inhibitory activity, it was shown to be 20 to 30 times more potent than **1**, thus increasing glucose tolerance by inhibiting its digestion and absorption in the intestine, especially after meals.[90] Additionally, the use of **90** led to less adverse effects including flatulency and abdominal distention, as shown in a random comparative study.[91]



84 **85** **86** **87**

Amongst the members of the aminocyclitol family of natural products, acarbose (**1**)[86] is one of the most important clinical derivatives, being effective in the micromolar range against bacterial, fungal, and plant glucosidases, including sucrase, maltase, dextrinase, and glucoamylase, but to a lesser extent against α-amylase.[77] Treatment of diabetic patients with compound **1** to decrease postprandial hyperglycemia has been related to a reduction in frequency of myocardial infarction (49%) and hypertension (34%) and to an interference in physiopathological and atherothrombotic processes by mechanisms probably involving fibrinogen and a positive modulation of the hemostatic balance.[87]

Acarbose (**1**) has been produced as a secondary metabolite on a large scale from fermentation cultures of *Actinoplanes*



88 **89** **90**

Other compounds related to **1** are adiposin-1 (**91**) and trestatin B (**92**). Compound **91** and adiposin-2 (which has an additional D-glucose unit at the reducing end of adiposin) were isolated from *Streptomyces calvus* TM-521, and **91** can be regarded as a pseudotrisaccharide, differing from acarbose by replacing its 4-amino-4,6-dideoxy-glucose unit by a group derived from 4-amino-4-deoxy-glucose. Along with its inhibitory effect against human α-amylase and disaccharidases from porcine small intestine, this derivative exhibited antibacterial and anti-phytopathogenic fungal activities.[92] As pointed out for compound **1**, compound **92**, isolated as a complex mixture along with trestatins A and C from *Streptomyces dimorphogenes*, provides the pharmacophore with high affinity for the glucosidase active center, being primarily responsible for the main interactions leading to inhibition.[93] Other natural products of C_7N aminocyclitols comprise amilostatins, oligostatins, oxirane pseudooligosaccharides, salbostatin, piralomycin, and epoxyquinomicins.[82]

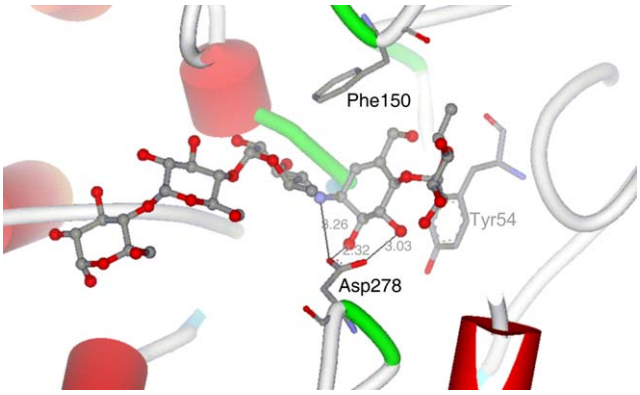
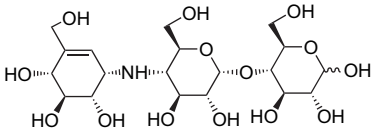


Figure 1. Complex of modified acarbose (**1**) in the catalytic site of *T. maritima* 4-α-glucantransferase (code PDB 1LWJ), showing interactions of the cyclitol unit and the bridge nitrogen of modified **1** with residues in the active site of the enzyme.[95]



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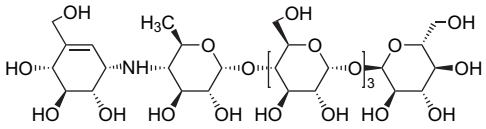
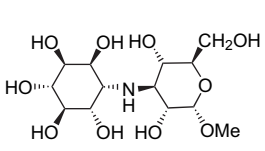


92

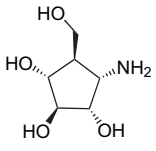
Because of the non-availability in the Protein Data Bank (PDB) of three-dimensional structures of α-glucosidase enzymes commonly used in biological assays, such as the native or complexed protein of *Saccharomyces cerevisiae*, we recently constructed a model of a glucosidase homologue based on a 4-α-glucanotransferase of *Thermotoga maritima* (PDB code 1LWJ), the latter showing a high sequential identity with the glucosidase of *S. cerevisiae*.[94] In the *T. maritima* 4-α-glucantransferase complex,[95] containing modified acarbose (a pentasaccharide form), the glycosidic nitrogen bridge interacts strongly with the conserved residue Asp278 by hydrogen bonding (corresponding to Asp349 in the glucosidase model of *S. cerevisiae*) through a distance of 2.73 Å. Based on the overview of this acarbose complex, it is also possible to visualize the main interactions of the cyclitol unit of modified **1** comprising the hydrogen bonding between the C-2 hydroxyl group with the Asp349 residue, and a similar linkage involving the hydroxyl at C-3 and His 348, close to Asp 349. Additionally, the cyclitol primary hydroxyl interacts with residues Asp 214 and His 111 (close to the ε nitrogen of imidazole). It is also possible to observe the flanking of the carbasugar system by aromatic residues Phe 150 and Tyr 54, corresponding to residues Phe 177 and Tyr 71, respectively, in the *S. cerevisiae* glucosidase model (Fig. 1).

In order to provide additional information about the structural requirements for anti-glucosidase activity, a pseudodisaccharide **93** was synthesized, which mimics the valienamine unit **84** and simultaneously preserves the structural similarity of two glycosidic units, cleaved by glucosidase II, of the natural oligosaccharide [$Glc_3Man_9GlcNAc_2$] of the immature glycoprotein (Scheme 1). However, assays performed with baker's yeast α-glucosidase demonstrated that inhibition promoted by **93** was only 20% when compared to the activity of 1-deoxynojirimycin (**7**) and it showed no inhibition activity against glucosidases I and II using rat liver microsome possessing activity in these enzymes.[96]

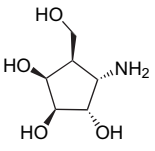
A series of amino-(hydroxymethyl)cyclopentanetriols, illustrated by compounds **94** and **95**, showed strong inhibition against glucosidase, the strength of which depends on the configuration of the amino group, since, in a protonated form, it may mimic the exocyclic oxygen of the protonated substrate. Compound **94** preserving the *gluco*-configuration exhibited similar K_i values for both α- and β-glucosidases,[97] while, surprisingly, the corresponding *galacto*-configured **95** was a more potent inhibitor toward β-glucosidase.[98]



93



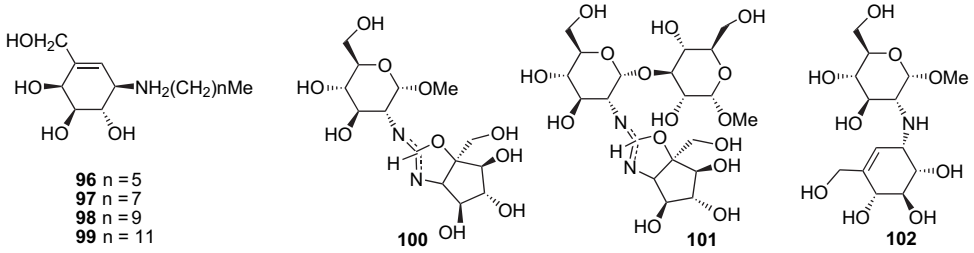
94



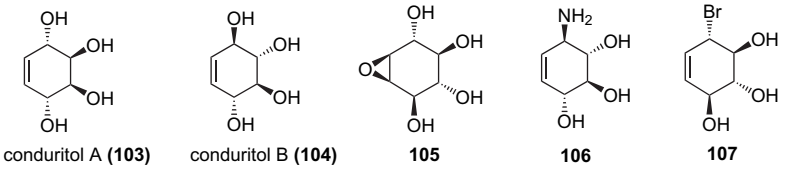
95

Ogawa et al.[99] synthesized β-galactose analogues of valienamine **96**–**99**, which exhibited considerable anti-β-glucosidase activity and anti-α- and β-galactosidase effects, due to the introduction of an *N*-alkyl group. The longest *N*-alkyl chain in compound **99** provided the best β-glucosidase inhibitor. However, compound **97** has received much attention as a potential drug for the treatment of GM_1-gangliosidosis and β-galactosidosis. The same group obtained two kojibiose-type analogues, pseudodisaccharide (**100**) and pseudotrisaccharide (**101**), both containing a 5-amino-1-hydroxymethyl-1,2,3,4-cyclopentanetetrol residue. A further analogue **102**, which contained valienamine linked to C-2 glucose via nitrogen, was also synthesized. Compounds **100** and **101** were potent inhibitors of baker's yeast α-glucosidase with IC_{50} values of 0.012 and 0.18 μM, respectively.

However, products **100**–**102** did not possess activity against rat sucrase and isomaltase or rat liver processing α-glucosidase I.[100]



Unsaturated cyclitols, the conduritols, comprise a 1,2,3,4-cyclohexenetetrol unit, differing from each other by the configurations of the hydroxyl groups around the ring. These polyhydroxylated cyclohexenoid derivatives have attracted great synthetic interest, due to their potential use as therapeutic agents in diabetes, viral infection, cancer, and other diseases. Several conduritol derivatives exhibited antifeedant, antibiotic, antileukemic, and growth-modulation activity.[101] The first representative of the conduritols was isolated by Kübler[102] in 1908 from the bark of the vine *Marsdenia condurango*, and investigation of its structure by Dangschat and Fisher,[103] and again by Kern et al.,[104] showed it to be conduritol A (**103**).



The conduritol family consisting of conduritols A–F includes 10 isomeric forms, since two are *meso* (A and D) and four are D,L pairs (B, C, E, and F).[105] Of these, only conduritol F is found in small quantities in green plants, while **103** is restricted to specific tropical plant families and, notably, it can be isolated from *Gymnena sylvestre*, a shrub, which is the basis of a popular medicine used in India and Asia against diabetes for 2000 years.[106] The four other conduritols are isomeric products obtained by synthesis and are not found in nature.[101,107] These compounds are also synthetic precursors for the preparation of cyclitols of biological interest, exemplified by inositol phosphate, quercitols, cyclophellitol, pseudosugars, pancratistatine, licoridinei, aminoglycosidic antibiotics, sugar amino acid analogues, etc.[101]

Conduritol A (**103**) is particularly important in this class of compounds because of its ability to inhibit intestinal glucose absorption and its potential use as an agent in the treatment of obesity and diabetes. Although the mechanism of its action remains unclear, Miyatake et al.[108] showed that the hypoglycemic effect of **103** prevented diabetic rats developing cataracts as a consequence of the inhibition of lens aldose reductase, the enzyme that converts aldoses to sugar alcohols. Furthermore, the hypoglycemic activity of **103** was also shown by conduritol B (**104**) having the all trans hydroxyl configuration and it was also able to modulate insulin release from isolated pancreatic islets.[109] The preferred half-chair conformation adopted by the cyclohexene ring may favor interaction at the active site. Reduction of the double bond considerably decreased the enzymic activity, but changes in the hydroxyl configuration also had an effect.

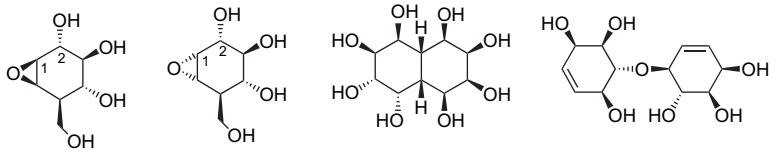
Conduritol B epoxide (1L- and 1D-1,2-anhydroinositols, **105**) is a potent glucosidase inhibitor. For instance, the lower reactivity of the oxirane of **105**, due to the electron-withdrawing effect of the hydroxyl group, makes it more resistant to hydration, allowing an effective interaction that requires protonation and nucleophilic attack by two distinct carboxyl groups at the enzyme active site. Exploring the glucocerebrosidase inhibitory activity, Kanfer et al.[110] and Das et al.[111] studied the effect of aminoconduritol (**106**) in animal models and cells for Gaucher's disease. Compound **106** was able to completely inhibit the glucocerebrosidase from rat peritoneal macrophages in a concentration of 10 and 100 μM for 16 and 2 h of incubation, respectively. However, **106** showed no inhibition against yeast and rice α-glucosidases, amyloglucosidase or almond, and *Caldocellum saccharolyticum* β-glucosidases.[112]

Based on the studies of active site-directed inactivation, it was demonstrated that conduritol epoxide is a potent inhibitor against many different sources of β-glucosidase, while its activity toward α-glucosidase is remarkably lower, suggesting that the trans-diaxial orientation may play a role in the selective inhibition. Even though both conduritol B epoxide (**105**) and bromoconduritol B (**107**)[113] are irreversible glucosidase inhibitors, when covalently linked to the enzyme, **107** has the ability to promote the accumulation of $Glc_1Man_9GlcNAc_2$ by the inhibition of α-glucosidase I or II from rat liver. The same result was observed when virus-infected cells were treated with **107**. Thus, while the position of the acidic group in the β-enzyme favors interaction with **105** (Scheme 6), in the α-enzyme an initial

intermediary epoxide is formed by a hydroxyl intramolecular attack on the neighboring carbon, releasing the halogen leaving group (Scheme 7), and the resulting epoxide then reacts, inactivating the enzyme.[114]

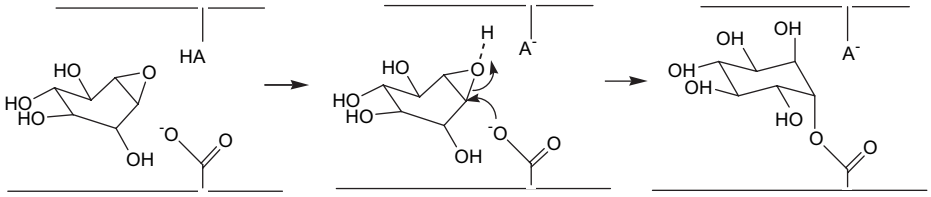
Cyclophellitol (**108**), (1*S*,2*R*,3*S*,4*R*,5*R*,6*R*)-5-(hydroxymethyl)-7-oxabicyclo[4.1.0]heptane-2,3,4-triol, a conduritol B epoxide analogue, was isolated from a culture filtrate of the fungus *Phellinus* sp. and can be regarded as a carbasugar analogue of D-glucopyranose.[115] Compound **108** is one of the most potent and specific competitive and irreversible inhibitors of almond and Molt-4 lysate β-glucosidases, being around 3, 50, and 14 times more active than nojirimycin (**9**), 1-deoxynojirimicyn (**7**), and castanospermine (**66**), respectively.[116] Moreover, the activity toward Molt-4 β-glucocerebrosidase was higher than that of **66**, **7**, and **9**, and **108** did not show cytotoxicity in cultured cells, both properties being required for the treatment of Gaucher's disease. However, compound **108** has little chance of being used as an anti-HIV agent, since Atsumi et al.[116] showed that it had no effect on infected CEM cell lines and MT-4 cells, suggesting that the anti-HIV activity observed for **7** and **66** may be a result of their α-glucosidase inhibition. As an extension of this work, performed by the same group,[117] the non-natural epoxide diastereomer of **108**, (1*R*,6*S*)-cyclophellitol (**109**), was synthesized and it showed potent inhibition only against baker's yeast α-glucosidase. An explanation might be that the orientation of the C1–O positions, that is recognized by both enzymes, involves a pseudo-equatorial orientation in **108** and pseudoaxial in **109** mimicking the natural substrate of the corresponding β- and α-glucosidases, respectively.

In a search for potential glycosidase inhibitors, Mehta and Ramesh[118] prepared new types of cyclitols consisting of two conduritols fused in the double-bond position, thereby simulating the conformational restriction of conduritols. The octahydroxydecahydronaphthalene (**110**) exhibited selective inhibitory activity against α-glucosidase that was higher than **7**, but was inactive against β-glucosidase. Further work involving a diastereomer of **110** had revealed that the substituent configurations is an important feature for enzyme inhibition, since this diastereomer showed no significant inhibition up to millimolar concentrations.

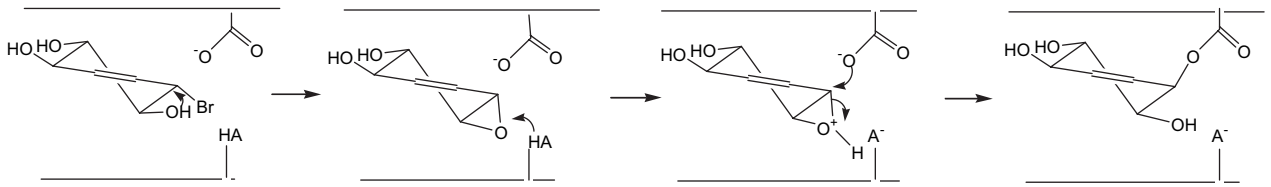


A new class of derivatives that may be used as α-glucosidase inhibitors is the oligoinositols. Work by Freeman and Hudlicky[119] with the oligoinositol of conduritol F (**111**) showed that this compound is active because it mimics the enzyme transition state. In contrast, the corresponding oligomer of *muco*-inositol did not exhibit the same effect when evaluated against many glycosidases.

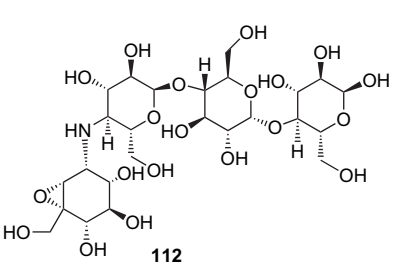
CKD-711 (**112**) is a novel pseudotetrasaccharide inhibitor of α-glucosidase that was isolated from culture of *Streptomyces* sp. as a component of CK4416 (**113**). According to Kwon et al.,[120] its structure consists of an epoxide C_7N aminocyclitol unit connected to three linear glucose molecules. Based on a comparative screening, compound **112** was as active as acarbose (**1**) against intestinal sucrase and maltase, showed a two-fold lower activity than acarbose (**1**) against α-amylase, and exhibited no toxicity. Consequently, **112** might cause less side effects such as flatulence, abdominal pain, and diarrhea that are observed on strong α-amylase inhibition.[121]

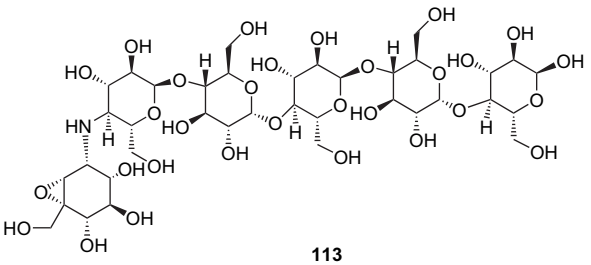


Scheme 6. Proposed mechanism for the reaction of conduritol epoxides like **105** in the active site of β-glucosidases.



Scheme 7. Proposed mechanism for the reaction of bromoconduritol (**107**) in the active site of α-glucosidase.


112


113

5. Thiosugars

Replacement of the oxygen atom in the ring of a carbohydrate by sulfur affords thiosugars, a class of compounds comprising derivatives with strong anti-α-glucosidase activity. The naturally occurring salacinol (**114**) is a remarkable member of this class, consisting of a thiocyclopentane with a trivalent sulfur atom in the ring (sulfonium ion) and an *O*-sulfate as part of an erythritol side chain, which is able to act as a counterion. Its structure was established by X-ray crystallographic analysis, showing it to have a spiro-like configuration with the 1-deoxy-4-thioarabinofuranosyl cation, bearing a resemblance with the iminoalditol **55**, linked through sulfur to a 1′-deoxyerythrosyl-3′-sulfate anion. Compound **114** was isolated by Yoshikawa et al.[122] from an aqueous extract of the roots and stems of *Salacia reticulata* Wight, traditionally used in India and Sri Lanka for the treatment of diabetes, and it has been synthesized.[123] Salacinol (**114**) produced a strong inhibition for the increase of serum glucose levels in in vivo screening, along with competitive inhibition against intestinal α-glucosidases such as maltase, sucrase, and isomaltase, in which the activity against isomaltase was higher than that of acarbose (**1**). Kotalanol (**115**), a derivative of 1,2,3-trihydroxy-propyl-salacinol (**114**), also consists of an inner salt sulfonium-sulfate structure and has been isolated from *Salacia reticulate*, and showed more potent inhibitory activity against sucrase than **114** and **1**.[124]

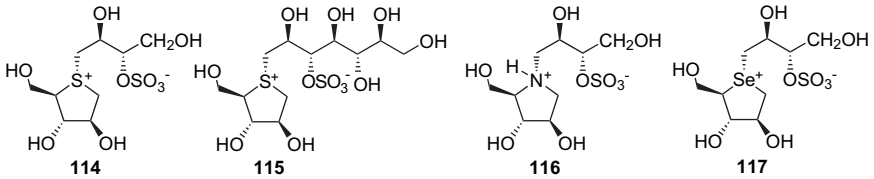
With the aim of gaining information on structure–activity relationships, the azacyclic version of **114** was synthesized in which the thiosugar sulfur was replaced by nitrogen to give compound **116**. Inhibition assays using intestinal α-glucosidase indicated that it was less potent against maltase and isomaltase, compared to **114** and **55**, but was as potent as **55** against sucrase.[125] Despite the low activity of the natural sulfonium ion toward glucoamylase, product **116** exhibited a 10-fold higher inhibition value than **114**.[126] Furthermore, substitution of the sulfonium ion of **114** by selenium produced a derivative **117** with activity against glucoamylase G2 and with a K_i of 0.7 mM, but there was no activity against pancreatic α-amylase.[127]

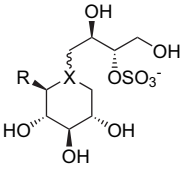
Several salacinol analogues have been synthesized with varying stereochemistry at one or more stereogenic centers by modifying substituents and ring size or by replacing the sulfur in the sulfonium ion by nitrogen or selenium. Pinto et al.[22] synthesized a series of pyranosil compounds (**118a–122a**) and the corresponding isomers (**118b–122b**), in which an L-erythritol sulfated side chain was attached to the ring N, S, or Se atom. The objective was to investigate if changes to this group, acting as a counterion to ammonium or selenium salt analogues, might increase the in vivo stability or biomembrane permeability. However, the activity of these salacinol analogues with six-membered rings against glucoamylase G2 was weak or absent, indicating the importance of the five-membered ring incorporated in salacinol.

The synthesis of heteroanalogues of disaccharides was also described using the isostere strategy of changing the pyranose oxygen and/or the glycosidic oxygen atoms for carbon, sulfur, selenium, or nitrogen. From this series, compounds **123–126** are worthy of mention, owing to their competitive inhibition of glucoamylase G2. The activity of **126** was due, apparently, to the α-anomer, but the compound was, in fact, isolated as an α/β mixture.[128]

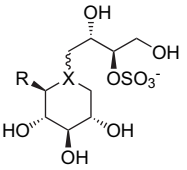
A bicyclic analogue of castanospermine (**66**), carrying a positively charged sulfonium salt in place of the nitrogen, led to **127** with a permanent and stable positive charge that might improve interaction at the active site.[129] Screening of inhibition with three glucosidase enzymes, glucoamylase G2, porcine pancreatic α-amylase, and barley α-amylase, showed that **127** was a slightly better inhibitor than **114** against the former enzyme.[130]

Expanded-ring derivatives containing ring sulfur or nitrogen, the tetra-hydroxythiepanes (**128–133**)[131] and the tetra-hydroxyazepane (**134**),[132] showed competitive anti-α- and β-glucosidase activities. This property has been attributed to the flexibility of the seven-membered ring, which allows mimicking of the natural substrate transition state. Thiepane derivatives **130** and **132** were moderately active against α-glucosidase, but inactive against β-glucosidases.

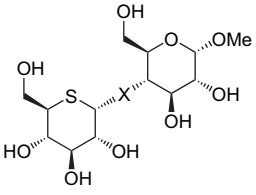

114 115 116 117



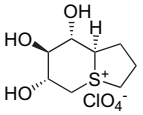
118a X= NH, R= H
119a X= NH, R= CH_2OH
120a X= S, R= H
121a X= S, R= CH_2OH
122a X= Se, R= H



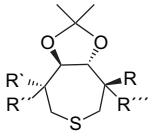
118b X= NH, R= H
119b X= NH, R= CH_2OH
120b X= S, R= H
121b X= S, R= CH_2OH
122b X= Se, R= H



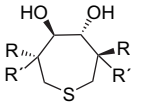
123 X= O
124 X= S
125 X= Se
126 X= N



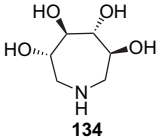
127



128 R, R′= N_3, R′′, R′′′= H
129 R= N_3, R′= H, R′′= N_3, R′′′= H
130 R, R′= NH_2, R′′, R′′′= H
131 R= NH_2, R′= H, R′′= NH_2, R′′′= H



132 R= N_3, R′= H
133 R= NH_2, R′= H

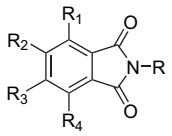


134

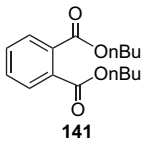
6. Non-glycosidic derivatives

There is great structural diversity among glucosidase inhibitors, which are not based on a sugar scaffold.

Based on pharmacological studies involving thalidomide, it was found that tetrachlorophthalimide derivatives (**135**–**140**) exhibited potent α-glucosidase inhibition. The compounds have hydrophobic groups such as halogen and *N*-alkyl side chains, and structure–activity relationship studies revealed the importance of hydrophobic *N*-substituents and the positive influence of electron-withdrawing groups attached to the aromatic ring, as exemplified by **135** and **137**, which were stronger inhibitors than **7** toward α-glucosidase (from Wako Pure Chemical Industries).[133] Interestingly, dibutyl phthalate (**141**) (isolated from *Streptomyces melanosporofaciens*) is also described as a non-competitive α-glucosidase inhibitor.[134]

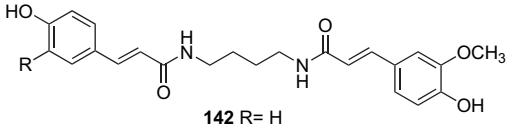


135 R= $(CH_2)_4Ph$, R_1 to R_4= Cl
136 R= $(CH_2)_2Ph$, R_1 to R_4= Cl
137 R= Ph, R_1 to R_4= Cl
138 R= $(CH_2)_3Ph$, R_1 to R_4= Cl
139 R= Ph, R_1= NO_2, R_3 to R_4= H
140 R= Ph, R_2= NO_2, R_1, R_3, R_4= H



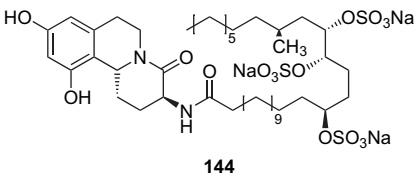
141

Bisamides were recently isolated as side products of corn starch processing. The compounds were active in reducing glucose levels occurring after meals. This class is represented by *N*-*p*-coumaroyl-*N*′-feruloylputrescine (**142**) and *N*-*N*′-diferuloylputrescine (**143**). Compound **142** is more potent in reversible α-glucosidase inhibition assays. Studies on structure–activity relationship indicated that the phenyl hydroxyl group is fundamental for the activity of these compounds.[135]

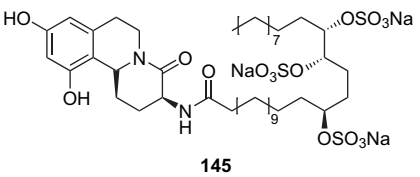


142 R= H
143 R= OCH_3

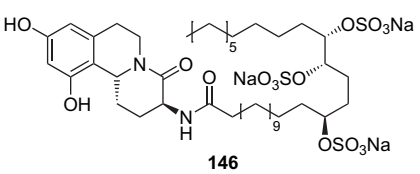
Currently, several natural compounds from marine sources have been described as potent inhibitors of α-glucosidase. Takada et al.,[136] isolated from a hydrophilic extract of the sea sponge *Penares schulzei* three tetrahydroisoquinolinic alkaloids containing two amido functions and a C_{28} sulfated fatty acid, which belong to a new class of compounds named schulzeines, the three compounds being schulzeine A (**144**), B (**145**), and C (**146**). The IC_{50} values of these compounds against yeast α-glucosidase varied from 48 to 170 nM and those analogues devoid of the sulfate group still had inhibitory activity, indicated that these groups are not fundamental for their activity. Schulzeines are structurally similar to another class of compounds, also isolated from the *Penaria* sp. family, the penarolide sulfates A_1 (**147**) and A_2 (**148**), bearing a proline unit inserted into a macrolide trisulfate system and having potent anti-yeast α-glucosidase activity (IC_{50}=1.2 and 1.5 μg/mM, respectively). Recently, Nakao et al.[137] have isolated from the same sponge penasulfate A (**149**), a compound 10 times more potent than **147** and **148** against yeast α-glucosidase.



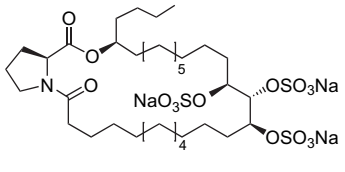
144



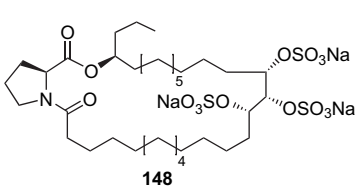
145



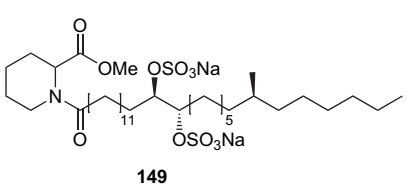
146



147

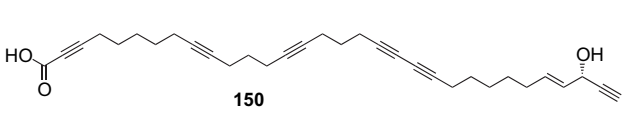


148

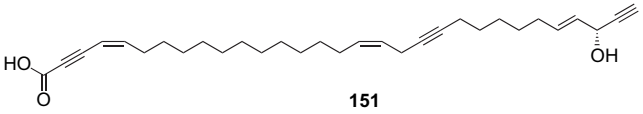


149

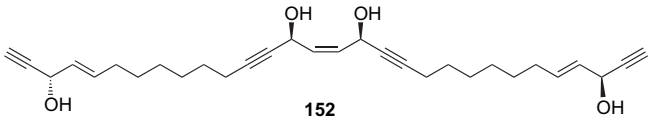
Polyacetylenic compounds (**150**–**152**), isolated from marine sponges on the coast of Japan, showed potent anti-α-glucosidase activity, besides other interesting biological activities. Callyspongynic acid (**150**) is a carboxylic derivative in this class of substances isolated from the sponge *Callyspongia truncata* by Nakao et al.[138] Other compounds were also active, such as corticatic acid A (**151**) from *Petrosia corticata* and petrosynol (**152**) from *Petrosia* sp. The inactivity of the polyacetylene, callytetrayne (**153**) (also isolated from *C. truncata*), and the products of methylation of the carboxylic acid groups of **150** and **151**, suggests the importance of the carboxylic acid group and the allyl/propargyl alcohol functionality in generating the inhibitory properties of these compounds.[139]



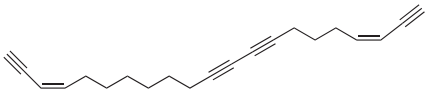
150



151

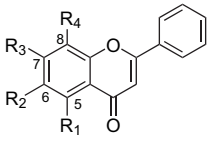


152



153

Baicalein (**154**), a 5,6,7-trihydroxyflavone isolated from marjoram leaves of *Origanum majorana*, is a potent α-glucosidase inhibitor. Compound **154** is a unique flavone bearing a 6-hydroxy group together with the 5,7-dihydroxy substituents. Structure–activity relationship studies with different derivatives **155**–**160** indicated that loss of hydroxyls from positions 5, 6, and 7 significantly reduced the activity, and introduction of an electron-withdrawing or -donating group in R suggests an unfavorable steric influence in the enzyme interaction (Appendix).[140]



154 R_1, R_2, R_3= OH; R_4= H
155 R_1, R_3= OH; R_2, R_4= H
156 R_1, R_2, R_3= OH; R_4= F
157 R_1, R_2, R_3, R_4= OH
158 R_1, R_2, R_3= OH; R_4= NH_2
159 R_1= OH; R_2, R_3, R_4= H
160 R_1, R_3, R_4= H; R_2= OH

7. Concluding remarks

Glucosidase inhibitors have proved useful to reduce postprandial hyperglycemia by suppressing the absorption of glucose, being effective for the treatment of type II diabetes and obesity. Current interest in these compounds has been extended to a diverse range of diseases including lysosomal storage disorders and cancer, and special attention has been given to those compounds with anti-HIV activity. Isolation of suitable glucosidase inhibitors from natural sources or their chemical synthesis provides biochemical tools for the elucidation of enzyme mechanistic activity through the use of kinetic data combined with variations in potential inhibitor structural information. Such knowledge is fundamental to the discovery of lead compounds, because of their promising therapeutic potential.

The polyhydroxy glucosidase inhibitors are a widely diverse class of compounds often isolated from plants and microorganisms and they have significant therapeutic use or potential. Some carbocyclic compounds include potent HIV inhibitors, such as conduritol epoxides and aminoconduritols, while conduritol A analogues modulate the release of insulin. Thiosugars, either synthesized or isolated from natural sources, have also been investigated as inhibitors and have widened the structural diversity of compounds available from natural sources. Compounds with no obvious structural similarity to a carbohydrate skeleton are a new class of inhibitors and the elucidation of their mechanism of action may add new insights in the search for new therapeutic agents.

Acknowledgements

The authors are grateful to Professor Zuleika Rotschield and Dr. Alan H. Haines for valuable comments on the manuscript.

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Appendix. Data on the inhibition of activity produced in various α- and β-glucosidases by compounds 1–160

	α-Glucosidase inhibition (μM)				β-Glucosidase inhibition (μM)			
Compound	IC_{50}		K_i	% Inhibition-concentration	IC_{50}	K_i	% Inhibition-concentration	Ref.
1	0.47[a] 0.06[c] 108.5[f] 5.9[g]		0.99[b] 1.1×10^{-6}[c] 9.0×10^{-3}[d] 46.3[e]	38–1.0[h]		NI		8, 12, 21, 22, 82
2			0.086[b] 0.36[e] 0.21[h]	61–1.0[b] 40–1.0[d] 0–200.0[h] 43–2300[q]		NI		8
3	15.0[i] 5.3[j] 0.57[k]		3.7[i]					10, 29
4				12–1000[i] 91–1000[k]				13
5				53–2000[i] 7–2000[k]				13
6				45–2000[b]				17
7	0.22[b] 0.096[d] 0.13[e] 9.6[f] 4.6[i]	0.4[j] 0.36[l] 0.05[m] 12.6[n] 330[o]	1.3[i] 12.6[f] 0.01[m] 14.6[f]	52–0.1[b]	153[a′] 81[c′] 520[d′]	300 (pH 5.0)[a′] 47 (pH 6.8)[a′] 2.7[b′] 210[e′] 180[f′] 25[g′]		1, 17, 21, 29, 30, 36, 40, 60, 82
8			NI			126[a′]		18
9	0.56[b] 0.76[d] 0.25[e] 6.3[f] 1.7[l]		6.3[f] 0.01[m] 330[n]			0.89[a′] 0.36[b′] 4.5[f′]		1, 21, 47, 82
10								
11			NI[f]			430[a′]	35–1000[a′]	31
12	0.28[k]							36
13	940[e] 1000[j]		NI[f,i]			(540 as DGJ)[a′]		36, 59
14	20[i] 1.5[j]		5.8[i] 54[f]					29, 39
15			NI					39
16			23[n]					39
17	2.4[b] 24[l]				1000[a′] 230[h′]			40
18	0.35[b] 5.2[l]				NI[a′] NI[h′]			40
19	22[b] 2.3[l]				80[a′] 50[h′]			40, 41
20	0.79[b] 4.0[l]				NI[a′] NI[h′]			40
21	2.5[b] 24[l]				NI[a′] 560[h′]			40
22	0.31[b] 1.7[l] 30[m]				NI[a′] NI[h′]			43
23	0.2[b] 5.0[d] 8.0[e] 1.0[l]							44
24			86[f] 3.7[d] 7.2[p]			0.11[a′]		45, 47
25			0.022[f] 0.025[p]			0.069[a′]		47
26	3.9[f] 1.06[p]				0.65 (pH 6.8)[a′] 0.76 (5.0)[a′] 1.09 (pH 7.5)[a′]			45
27	59[f] 100[p] 0.063[c]				2.3[a′]			45
28	NI[f]				420[a′]			45
29	NI[f]				0.19[a′]			45
30						30[i′]		46
31						96[i′]		46

(*continued*)

Appendix. (*continued*)

	α-Glucosidase inhibition (μM)				β-Glucosidase inhibition (μM)			
Compound	IC_{50}		K_i	% Inhibition-concentration	IC_{50}	K_i	% Inhibition-concentration	Ref.
32						580[i′]		46
33					80[a′]			48
34					8.8[a′]			48
35	0.17[b]	8.4[i]						52
	0.04[m]	0.26[j]						
	0.70[e]	0.34[l]						
36	7.2[b]	15[l]			NI			52
	8.2[j]	8.4[m]						
37	3.0[b]	4.6[l]						52
	5.0[j]	3.2[m]						
38	0.02[b]	1.0[j]						52
	2.8[e]	0.17[l]						
	50[i]	0.06[m]						
39	3.0[b]	2.2[j]						52
	100[e]	4.8[l]						
	100[i]	4.2[m]						
40					4.25[a′]			53
41					2.11[a′]			53
42	3.3[b]	2.3[l]			NI			54
	0.27[e]	0.25[m]						
43	110[b]	280[l]			NI			54
	110[e]	21[m]						
44	2.4[b]	6.1[l]			NI			54
	2.1[e]	0.49[m]						
45				NI[b]			37–1×10[3a′]	55
				22.1–1000[f]				
				33.9–1000[e]				
				28.8–1000[l]				
				33–1000[m]				
46	NI[o]				0.79[a′]	0.51[a′]		56
47	NI[o]				62[a′]	51[a′]		56
48	NI[o]				160[a′]	85[a′]		56
49			3×10[3c]			0.20[a′]		21, 57
						9.5×10[−3b′]		
						0.015[j′]		
50						10±2[a′]		12, 59
51						8.4±0.9[a′]		12, 59
52						13.8±3 (pH 5.6)[a′]		12, 59
53	20.1[a]		0.73–7[f]		2.4–13[a′]	1.7–50[a′]		60, 61
	NI[b]				7.8[c′]	57[b′]		
	91[c]				11[h′]	44[g′]		
	3.3[f]				34[d′]			
	NI[i]				25[k′]			
	92[j]							
	290[l]							
	200–300[m]							
	0.059[n]							
	3.6–15[o]							
54	NI[r]							62
55	0.18[f]	20[i]			965[a′]	280[a′]		60
	7.0[o]	100[j]			200[c′]			
	5.8[e]	55[l]			NI[d′]			
56	0.048[f]				41[a′]			63
57					1.2×10[−3k′]			
58					0.55[k′]			61
59					0.1[k′]			61
60			150[o]		NI[a′]			64
61			240[o]		1220[a′]			64
62			NI[o]		1120[a′]			64
63			18[o]					64
64	5.8[d]							1, 65
65 IC_{50}=0.001 Jack beans α-mannosidase					NI[l′]			66
66			≥1500[f]			1.5[a′]		21, 60
			0.015[m]			0.9[b′]		
			0.1[j]			25[g′]		
			0.55×10[−3b]					
67 (+)-6-Epicastanospermine	20[c]							68

(*continued*)

Appendix. (*continued*)

	α-Glucosidase inhibition (μM)			β-Glucosidase inhibition (μM)			
Compound	IC_{50}	K_i	% Inhibition-concentration	IC_{50}	K_i	% Inhibition-concentration	Ref.
68	NI[o] NI[m]			37[h′] 26[a′]	20[a′] 12[h′]		76
69	NI[o] NI[r]			4[a′] 1[h′]	1.8[a′] 0.43[h′]		76
70	NI[o] 75[m]			2.6[a′] 2.4[h′]	1.2[a′] 0.55[h′]		76
71	NI[o] 420[m]			0.82[a′] 0.86[h′]	0.45[a′] 0.29[h′]		76
72		57[f] NI[c]			30 (pH 5.5)[a′] 15 (pH 7.3)[a′] 27[m′]		69
73		57[f] NI[c]			23 (pH 5.5)[a′] 23 (pH 7.3)[a′] 244[m′]		69
74		17[f] NI[c]			212 (pH 5.5)[a′] 157[m′]		69
75					150[a′]		1
76					19[a′]		1
77					>8000[a′]		1
78					0.1[a′]		1
79					0.1×10^{-3}[h′]		78
80					300[a′] 410 (pH 6.8)[h′]		1
81		580[o]					79
82		32[f]			310[a′] 240[e′] 65[f′] 2000[c′] 1.7[b′]		21
83 Anti-fungal antibiotic		NI			NI		82
84	18[f] 53[a] 340[r] 1000[s] $>10\times10^{3}$[g] 6800[t]	300[b] 960[l] 890[d] 760[e]		8800[a′] $>10\times10^{3}$[n′]			84
85 2-*epi*-5-*epi*-Valiolone	NG			NG			83, 88
86	7.5[a] 580[f] 110[r] $>10\times10^{3}$[t] $>10\times10^{3}$[g] 130[s]	32[b] 180[l] 160[d] 88[e]		1500[a′] $>10\times10^{3}$[n′]			84
87 Validoxylamine A IC_{50}=0.0024 (pig kidney trehalase)		NI[b] NI[l]					85
88	0.049[a] 190[f] 2.2[r] $>10\times10^{3}$[t] $>10\times10^{3}$[g] 2.7[s]	0.32[b] 2.9[l] 1.2[d] 0.91[e]		8100[a′] $>10\times10^{3}$[n′]			84
89	420[a] 360[f] 8300[r] $>10\times10^{3}$[t] $>10\times10^{3}$[g] $>10\times10^{3}$[s]			7400[a′] $>10\times10^{3}$[n′]			84
90	0.0046[a] 0.015[r]						12, 90
91			2.0 Unit/mg[a] 265 Unit/mg[g] 123 Unit/mg[q]				92
92	0.61[g] 31[u] NI[f]			NI[n′] NI[a′]			93
93		~1500[f] NI[k] NI[i]					96

(*continued*)

Appendix. (*continued*)

	α-Glucosidase inhibition (μM)				β-Glucosidase inhibition (μM)			
Compound	IC_{50}		K_i	% Inhibition-concentration	IC_{50}	K_i	% Inhibition-concentration	Ref.
94			1.6[v] 0.6[w] 85[m]			6.5[a′] 1.5[h′]		97
95						2.2[a′] 0.17[h′]		98
96					1.2[a′]			99
97					3.1[a′]			99
98					2.5[a′]			99
99					0.87[a′]			99
100	0.012[o] NI[b]	NI[e] NI[k]						100
101	0.18[o] NI[b]	NI[e] NI[k]						100
102	3.1[o] NI[b]	NI[e] NI[k]						100
103				45–100[x]				109
104				41–100[x]				109
105			25×10^{3}[f] $>50\times10^{3}$[y] $>10\times10^{3}$[z]			17×10^{3}[f′] 4×10^{3}[b′] $\geq0.6\times10^{3}$[f′] 4.1×10^{3}[e′]		21
106			NI[f] NI[m] NI[c]			NI[a′] NI[h′]		112
107				$48.4–5\times10^{3}$[z1]				113
108				15–568[f]	4.5[a′]		99–568[a′]	115, 116
109	5.7[o]				NI[a′]			117
110			12[o]			NI[a′]		118
111			60[z]			NI[i′]		119
112	2.5[r] 0.5[a] 78[g]							121
113	NG					NG		82
114	2.5[b] 9.6[l] 1.76[e]		0.92[l] 0.95[b] 1.40[e] 10±2[g] 15±1[z] 1700[z2]					122, 125, 130
115								124
116	306[l] 44[b] 136[e]							125, 126
117			NI[g] 720[z2] NI[z3]					127
118			**118b**: 70×10^{3}[z2]					22
119			NI[z2]					22
120			NI[z2]					22
121			**121a**: 70×10^{3}[z2]					22
122			NI[z2]					22
123			1340±0.06[z2]					128
124			2040±0.42[z2]					128
125			796±0.03[z2]					128
126			4±0.3[z2]					128
127			1320[c] NI[z3] NI[g]					130
128			35[o]			NI[a′]		131
129			170[o]			NI[a′]		131
130			410[o]			190[a′]		131
131			280[o]			800[a′]		131
132				10–0.5[o]		NI[a′]		131
133			760[o]			NI[a′]		131
134			4.8[z4]	97–1000[z4]		17[a′]	86–1000[a′]	132
135	2.0[z5]							133
136	6.0[z5]							133
137	2.6[z5]				17.9[a′]			133
138	4.5[z5]							133
139	25.9[z5]							133

(*continued*)

Appendix. (*continued*)

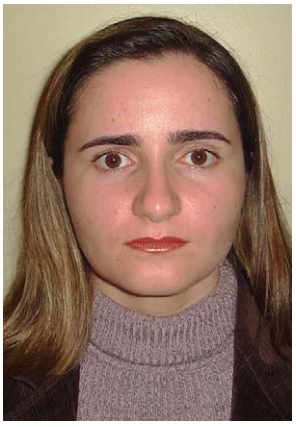
	α-Glucosidase inhibition (μM)			β-Glucosidase inhibition (μM)			
Compound	IC_{50}	K_i	% Inhibition-concentration	IC_{50}	K_i	% Inhibition-concentration	Ref.
140	23.7[z5]						133
141	4.0[f]	3.9[f]					134
142	~2000[f]						135
143			17–2000[f]				135
144	0.048–0.17[f]						136
145	0.048–0.17[f]						136
146	0.048–0.17[f]						136
147	1.33[f]						137
148	1.66[f]						137
149	4.4[o]						138
150	0.53[z5]				NI[a′]		139
151	0.34[z5]						139
152	8.8[z5]						139
153	NI[z5]						139
154	45[b]						140
155	NI[b]						140
156	86[b]						140
157	960[b]						140
158	1000[b]						140
159	NI[b]						140
160	NI[b]						140

NI: no inhibition; NG: not given.
[a] *α-Glucosidase*: porcine intestinal sucrase.
[b] *α-Glucosidase*: rat intestinal sucrase.
[c] *α-Glucosidase*: *Aspergillus niger* glucoamylase.
[d] *α-Glucosidase*: rat intestinal glucoamylase.
[e] *α-Glucosidase*: rat intestinal isomaltase.
[f] *α-Glucosidase*: yeast α-glucosidase.
[g] *α-Glucosidase*: porcine pancreas α-amylase.
[h] *α-Glucosidase*: rat pancreas α-amylase.
[i] *α-Glucosidase*: glucosidase II rat liver ERII.
[j] *α-Glucosidase*: rat liver lysosomal α-glucosidase.
[k] *α-Glucosidase*: rat liver α-glucosidase I.
[l] *α-Glucosidase*: rat intestinal maltase.
[m] *α-Glucosidase*: rice α-glucosidase.
[n] *α-Glucosidase*: brewers' yeast α-glucosidase.
[o] *α-Glucosidase*: baker's yeast α-glucosidase.
[p] *α-Glucosidase*: yeast isomaltase.
[q] *α-Glucosidase*: human pancreatic α-amylase.
[r] *α-Glucosidase*: porcine maltase.
[s] *α-Glucosidase*: porcine isomaltase.
[t] *α-Glucosidase*: glucoamylase (*Rhizopus* sp.).
[u] *α-Glucosidase*: *A. oryzae* α-amylase.
[v] *α-Glucosidase*: yeast maltase.
[w] *α-Glucosidase*: bakers yeast isomaltase.
[x] *α-Glucosidase*: modulation of insulin release from pancreatic islets.
[y] *α-Glucosidase*: rabbit intestine sucrase.
[z] *α-Glucosidase*: rabbit intestine isomaltase.
[z1] *α-Glucosidase*: whitefly α-glucosidase.
[z2] *α-Glucosidase*: glucoamylase G2.
[z3] *α-Glucosidase*: barley α-amylase (AMY1).
[z4] *α-Glucosidase*: *Bacillus stearothermophilus*.
[z5] *α-Glucosidase*: not specified.
[a′] *β-Glucosidase*: sweet almond.
[b′] *β-Glucosidase*: *Aspergillus wentii*.
[c′] *β-Glucosidase*: bitter almond.
[d′] *β-Glucosidase*: rat intestine cellobiose.
[e′] *β-Glucosidase*: calf liver cytosol.
[f′] *β-Glucosidase*: calf spleen lysosome.
[g′] *β-Glucosidase*: bovine kidney.
[h′] *β-Glucosidase*: *Caldocellum saccharolyticum*.
[i′] *β-Glucosidase*: not specified.
[j′] *β-Glucosidase*: human liver cytosolic.
[k′] *β-Glucosidase*: *Agrobacterium* sp.
[l′] *β-Glucosidase*: rat epididymis.
[m′] *β-Glucosidase*: bovine liver.
[n′] *β-Glucosidase*: β-amylase (sweet potato).

Biographical sketch

Ivone Carvalho received her B.Sc., Master, and Ph.D degrees from the University of São Paulo (USP), completing her doctoral thesis on synthesis of natural products in 1991 under the guidance of Professor Maurício Gomes Constantino. In 1995, she joined Professor A. H. Haines's group at University of East Anglia as a postdoctoral researcher to work on the synthesis of pseudodisaccharides as potential α-glucosidase inhibitors. She then returned to Great Britain in 2000 to complete another postdoctoral period in Professor R. A. Field's group at the University of St Andrews in Scotland, where she worked on the synthesis of glycopeptides to study the mechanism and function of parasite enzymes. She is currently working as an Associate Professor of Medicinal Chemistry at Faculty of Pharmaceutical Sciences of Ribeirão Preto/USP and her research interests concentrate on the design and synthesis of potential bioactive compounds.

Eduardo Borges de Melo was born in Riolândia, State of São Paulo, Brazil. He completed his Master Degree in 2001 under the supervision of Professor I. Carvalho at the Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, where he worked on the synthesis of ketoconduritols with potential glucosidase inhibitors. In 2002, he became assistant teacher of Pharmaceutical Chemistry at Pharmacy School of State University of Western Parana (UNIOESTE). In 2005, he joined Professor M. M. C. Ferreira's research group at State University of Campinas (UNICAMP) as a Ph.D student to work with molecular modeling and structure–activity relationship methods applied to design of HIV-integrase inhibitors.



Adriane da Silveira Gomes was born in Iporá, State of Goiás, Brazil. She graduated from University of Goiás in 2002 before moving to Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, where she is carrying out her Ph.D studies under the supervision of Professor Dr Ivone Carvalho since 2003. Her research has focused on the synthesis of glucosidase inhibitors of biological interest.