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Review Article

ALKALOIDAL SUGAR MIMETICS: BIOLOGICAL ACTIVITIES AND THERAPEUTIC APPLICATIONS

NAOKI ASANO*

Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3 Kanagawa-machi, Kanazawa 920-1181, Japan

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Alkaloids mimicking the structures of sugars inhibit glycosidases because of a structural resemblance to the sugar moiety of the natural substrate. Glycosidases are involved in a wide range of important biological processes, such as intestinal digestion, post-translational processing of glycoproteins and the lysosomal catabolism of glycoconjugates. The realization that alkaloidal sugar mimics might have enormous therapeutic potential in many diseases such as viral infection, cancer and diabetes led to increasing interest and demand for these compounds. In this review, the structural basis of the specificity of alkaloidal sugar mimics and their current and potential applications to biomedical problems are reviewed.

Keywords: Alkaloidal sugar mimics; Glycosidase inhibition; Antidiabetes; Antivirus; Anticancer; Lysosomal storage disease; Chemical chaperone therapy

1. INTRODUCTION

Alkaloids mimicking the structures of sugars are now believed to be widespread in plants and microorganisms and inhibit glycosidases because of a structural resemblance to the sugar moiety of the natural substrate. Such

* Fax: +81 76 229 6196. E-mail: naoki22@po.incl.ne.jp.

sugar mimics must be one of the most interesting discoveries in the field of natural products of recent years and certain sugar mimics have aroused increasing interest as potential antiviral, anticancer and antidiabetic agents and agrochemicals. Most of these effects can be shown to result from the direct or indirect inhibition of glycosidases. Glycosidases are involved in a wide range of important biological processes, such as intestinal digestion, post-translational processing of glycoproteins and the lysosomal catabolism of glycoconjugates. The possibility of modifying or blocking these processes by using glycosidase-inhibiting sugar mimics for cell biological and therapeutic applications has attracted a lot of attention. Inhibitors are also being used to study the mechanism of action, topography of the active site and purification of glycosidases.

2. GLYCOSIDASE INHIBITORS

2.1. α-Glucosidase Inhibitors

2.1.1. Natural Occurrence

In 1966 nojirimycin (NJ) (**1**; Figure 1) was discovered as the first natural glucose mimic, with a nitrogen atom in place of the ring oxygen.[1] NJ was first described as an antibiotic produced by *Streptomyces roseochromogenes* R-468 and *S. lavendulae* SF-425, and was shown to be a potent inhibitor of α- and β-glucosidases from various sources.[1,2] However, because this iminosugar with the hydroxyl group at C-1 is fairly unstable, it is usually stored as the bisulfite adduct or it may be reduced by catalytic hydrogenation using a platinum catalyst or by $NaBH_4$ to 1-deoxynojirimycin (DNJ) (**2**). DNJ was first prepared by the reduction of NJ as described above but later isolated from the roots of mulberry trees and called molanoline.[3] DNJ was also produced by many strains of the genera *Bacillus* and *Streptomyces*.[4] The first naturally occurring *N*-methyl derivative of DNJ was isolated from the leaves and roots of *Morus* spp. (mulberry trees) and, furthermore, the genus *Morus* has been shown to co-produce many kind of the glycosides of DNJ such as 2-*O*-, 3-*O*-, 4-*O*-α-D-glucosides, 2-*O*-, 3-*O*-, 4-*O*-, 6-*O*-β-D-glucosides, and 2-*O*- and 6-*O*-α-D-galactosides.[5] 1,2-Dideoxynojirimycin, fagomine (**3**), was isolated from the seeds of Japanese buckwheat (*Fagopyrum esculentum*) and the Moreton Bay chestnut (black bean), *Castanospermum australe*, and it also occurred as the 4-*O*-β-D-glucoside in the seeds of legume *Xanthocercis zambesiaca*.[6] In 1988, α-homonojirimycin

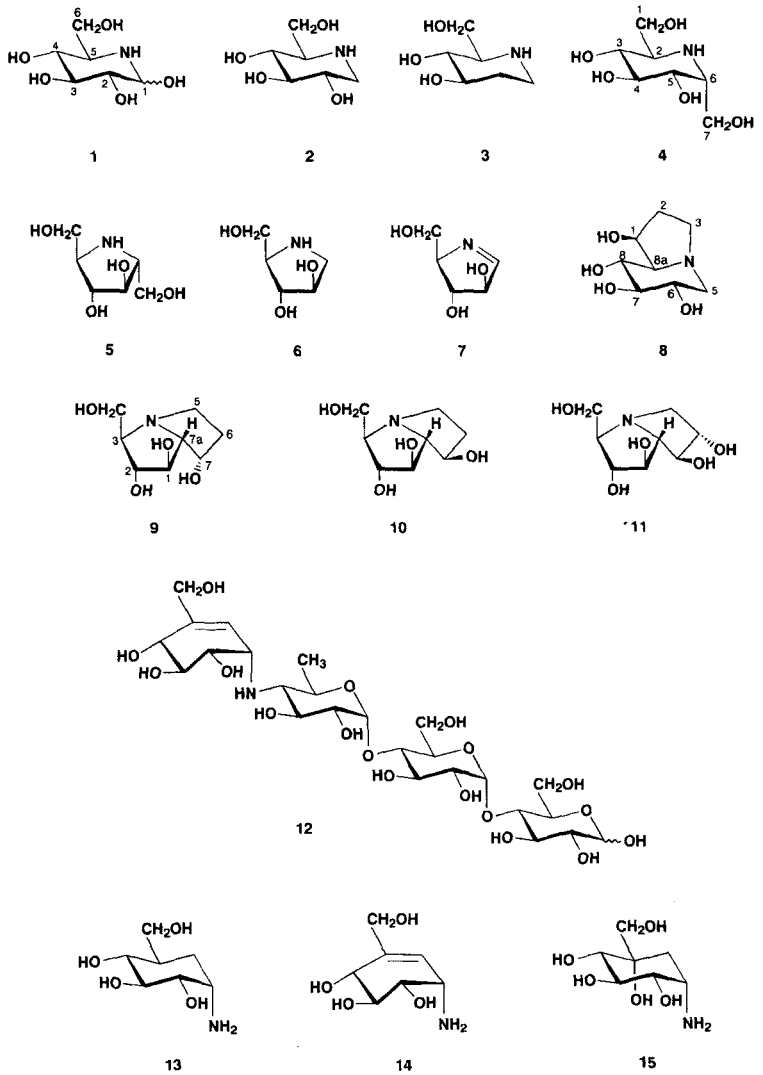


FIGURE 1 Structures of naturally occurring α-glucosidase inhibitors.

(α-HNJ) (**4**) was isolated from the neotropical liana *Omphalea diandra*.[7] This isolation is the first report of the naturally occurring C-1 branched DNJ derivative. However, before the isolation of the natural product, its 7-*O*-β-D-glucoside (MDL 25637) had been designed as a potential drug for the treatment of diabetes mellitus.[8] α-HNJ recently has been also isolated

from the whole plants of *Aglaonema treubii* (Araceae) and the bulbs of *Hyacinthus orientalis*.[9,10]

In 1976, 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP) (**5**) mimicking β-D-fructofuranose was found in the leaves of the legume *Derris elliptica*.[11] DMDP is now being reported from many disparate species of plants and microorganisms, which would indicate that this is a common metabolite.[12] Removal of one hydroxymethyl group from DMDP leads to 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1) (**6**), which was first found in the fruits of *Angylocalyx boutiqueanus* and is now being reported from many disparate species of plants as well as DMDP.[12,13] The polyhydroxypyrroline nectrisine (FR-900483) (**7**) was isolated as an immunomodulator from the culture broth of the fungus *Nectricia ludica*.[14]

Castanospermine (**8**) was isolated in 1981 from the seeds of *C. australe*,[15] and later from the dried pods of *Alexa leiopetala*.[16] Castanospermine may be regarded as a bicyclic derivative of DNJ, with an ethylene bridge between the hydroxymethyl group and the ring nitrogen. X-ray crystallography showed that the chirality of the four substituents at C-6, C-7, C-8 and C-8a on the six-membered ring of castanospermine is the same as that on C-2, C-3, C-4 and C-5 of the pyranose ring of D-glucose.[15] *C. australe* also produced polyhydroxylated pyrrolizidine alkaloids, australine (**9**) and 7-*epi*-australine (**10**), other than indolizidine alkaloids. Australine can be regarded as a ring-contracted form of castanospermine or as a derivative of DMDP with an ethylene bridge between the hydroxymethyl group and the ring nitrogen. Casurine (**11**) and its 6-*O*-α-D-glucoside occurred in the bark of *Casuarina equisetifolia* (Casuarinaceae) which has been prescribed in Western Samoa for the treatment of breast cancer.[17] Both compounds were also isolated from the leaves of *Eugenia jambolana*,[18] which is a well known tree in India for the therapeutic value of its seeds, leaves and fruits against diabetes and bacterial infections.

The Bayer group had searched for inhibitors of intestinal sucrase since the end of the 1960s for clinical development for the treatment of diabetes and found the pseudotetrasaccharide acarbose (**12**) from the fermentation broth of the *Actinoplanes* strain SE 50.[19] The characteristic core-structure for inhibition is composed of a trihydroxy(hydroxymethyl)cyclohexene moiety and a 4-amino-4,6-dideoxy-D-glucopyranose moiety, bonded by way on an imino linkage at the allylic position. A similar structural unit is found in the antibiotic validamycin.[20] Later carba-glucosylamines, validamine (**13**), valienamine (**14**), and valiolamine (**15**), were isolated as α-glucosidase inhibitors from the culture broth of validamycin-producing organism, *Streptomyces hygroscopicus* var. *limoneus*.[21]

2.1.2. Biological Activities and Therapeutic Application

The intestinal oligo- and disaccharidases are fixed components of the cell membrane of the brush border region of the wall of the small intestine. These enzymes digest dietary carbohydrate to monosaccharides which are absorbed through the intestinal wall. They include sucrase, maltase, isomaltase, lactase, trehalase and hetero-β-glucosidase. Inhibition of all or some of these activities by inhibitors could regulate the absorption of carbohydrate. Mulberry leaves have traditionally been used to cure "Xiao-ke" (diabetes) in traditional Chinese medicine. The original isolation of DNJ was prompted by the knowledge that extracts of mulberry were able to suppress the rise in blood glucose that follows eating and that this component might be beneficial to diabetes. The discovery of the inhibitory effect on mammalian α-glucosidases opened the possibility of a therapeutic application for DNJ. However, despite the excellent α-glucosidase inhibitory activity *in vitro*, its efficacy *in vivo* was only moderate.[22] Therefore, a large number of DNJ derivatives were prepared in the hope of increasing the *in vivo* activity. Thus, miglitol (BAY m 1099) (**16**), emiglitate (BAY o 1248) (**17**), MDL 25637 (**18**) and MDL 73945 (**19**) were selected as the favorable inhibitors out of a large number of *in vitro* active agents (Figure 2). They have been reported to effectively reduce postprandial elevations of blood glucose and plasma insulin in animals in loading tests with starch and sucrose.[8b,23] The inhibitor dose (mg/kg body weight) which reduces the postprandial increase in blood glucose by 50% (ED_{50}) after the administration of a defined amount of sucrose on rats was calculated for miglitol and emiglitate.[24] The ED_{50} values of miglitol and emiglitate were 0.24 mg and 0.16 mg/kg body weight, respectively. Both compounds are also characterized by a long lasting effect *in vivo*. MDL 25637 and MDL 73945 appear to be more effective on postprandial glucose and insulin response when administrated 30–60 min before a sucrose load than when given simultaneously with sucrose. The long lasting effect of MDL 73945 has been reported to be caused by quasi-irreversible binding to α-glucosidases. Acarbose (**12**) is a potent inhibitor of pig intestinal sucrase, with an IC_{50} value of 0.5 μM and is also effective in carbohydrate loading tests on rats and healthy volunteers, as was indicated by a reduction of postprandial blood glucose and an insulin increase.[24] Acarbose was introduced onto the market (GLUCOBAY^{TM}) in 1990 for the treatment of diabetes. Horii *et al.* synthesized numerous *N*-substituted valiolamine derivatives in order to enhance its α-glucosidase inhibitory activity *in vitro* to select the very simple derivative AO-128 (the genetic name voglibose).[25] Voglibose (**20**) is obtained by reductive

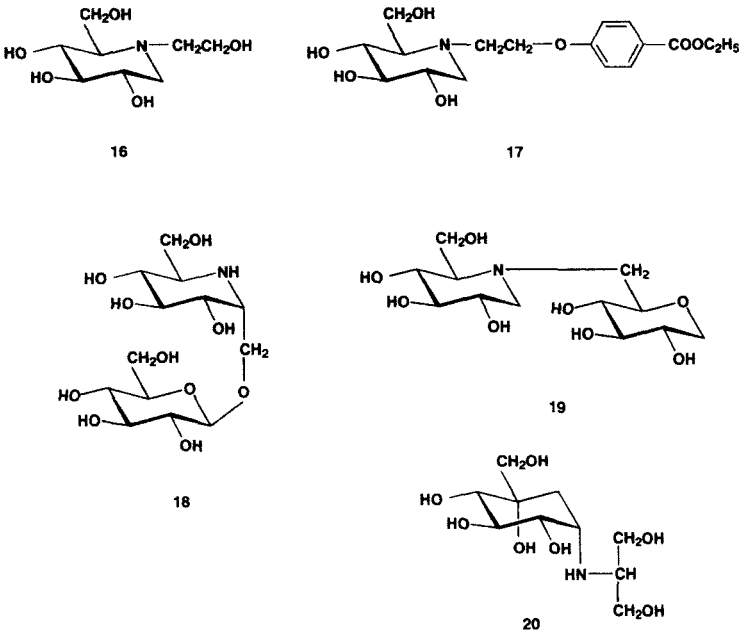


FIGURE 2 Structures of chemically synthesized antidiabetic agents.

amination of valiolamine with dihydroxyacetone. Valiolamine is a potent inhibitor of pig intestinal maltase and sucrase, with IC_{50} values of 2.2 and 0.049 μM, respectively, while voglibose is a more potent inhibitor of both enzymes, with IC_{50} values of 0.015 (toward maltase) and 0.0046 μM (toward sucrase).[25] Voglibose (BASEN™) is now on the market in Japan.

A reversible deficiency of a lysosomal glycosidase can be induced in cells in culture or in animals by inclusion of a certain glycosidase-inhibiting sugar mimic in the culture medium or diet, respectively. The resultant intralysosomal accumulation of partially catabolized glycoconjugates leads to the cellular changes and clinical symptoms associated with a genetic lysosomal storage disease. Castanospermine has been reported to be toxic to animals and to cause various symptoms, including gastrointestinal upset. It has been found that castanospermine is a potent inhibitor of lysosomal α- and β-glucosidases but has no effect on a number of other glycosidases.[26] Castanospermine also disturbs the lysosomal catabolism of glycogen and glycolipids by inhibiting lysosomal α-glucosidase in animals or β-glucosidase in cells, and these resemble the genetic disorders Pompe and Gaucher diseases, respectively.[27] Miglitol induces the accumulation of glycogen in normal human fibroblasts and polarized HepG2 cells.[28] The difference in potency

between emiglitate and miglitol (the former is 1.5-fold more effective than the latter in sucrose loading tests on rats) can be explained by their difference in lipophilicity. Emiglitate with its lipophilic properties reaches the lysosomes much more easily than does miglitol and is therefore much more potent.[29] α-Glucosidase inhibition at the lysosomal levels leads to glycogen storage within the cellular organelles. Although the morphological picture resembles that of human glycogen storage disease (Pompe disease), deleterious effects which could be attributed to this glycogen storage have not been seen so far even after chronic high-dose administration.[29]

Several reviews have nicely covered various aspects of the structure and biosynthesis of the oligosaccharide chains of the *N*-linked glycoprotein.[30] The oligosaccharide chains of the *N*-linked glycoproteins are believed to confer biological specificity at the cell surface where they may be involved in cell–cell adhesion, differentiation, recognition, regulation, modulation of protein receptors, and so on. Thus, it is not surprising that there is such great interest in compounds that can prevent the glycosylation of *N*-linked glycoproteins or cause alterations in the structure of the carbohydrate chains. Certain sugar mimics have been effective and valuable tools to study how alterations in oligosaccharide structure affect the function of specific *N*-linked glycoproteins, and whether such alterations in turn affect cell function. In the endoplasmic reticulum, α-glucosidase I specifically removes the outermost α-1,2-linked glucose to give a $Glc_2Man_9(GlcNAc)_2$-protein, and then α-glucosidase II removes the next two α-1,3-linked glucose residues (Figure 3). Although DNJ, α-HNJ and castanospermine inhibit processing α-glucosidases I and II, DNJ and α-HNJ inhibit α-glucosidase II more strongly, whereas castanospermine has a greater affect on α-glucosidase I.[31,37] *N*-Alkylation of DNJ and α-HNJ induces a shift in specific inhibition of purified glucosidases from α-glucosidase II to α-glucosidase I.[31a,32] Castanospermine has been found to be more effective against α-glucosidase I than DNJ.[31b] This greater activity of castanospermine than DNJ has been suggested to be due to the fixed positioning of the C-1 OH group (corresponding to the C-6 OH group in DNJ) oriented axially to the piperidine ring by the five-membered ring closure.[33] From X-ray diffraction studies, the C-6 OH group of DNJ or the C-1 OH group of α-HNJ is equatorial to the piperidine ring in the crystal structure and the NH $\cdots$ O-6 in DNJ or the NH $\cdots$ O-1 in α-HNJ intramolecular interaction stabilizes the C-6–O-6 or C-1–O-1 conformation.[33,34] The substitution at the nitrogen atom in DNJ or α-HNJ, which would disrupt this intramolecular interaction, might be expected to increase the potency of the *N*-alkyl derivatives, by favoring the C-6 OH or C-1 OH axial conformation (Figure 4).[34,35]

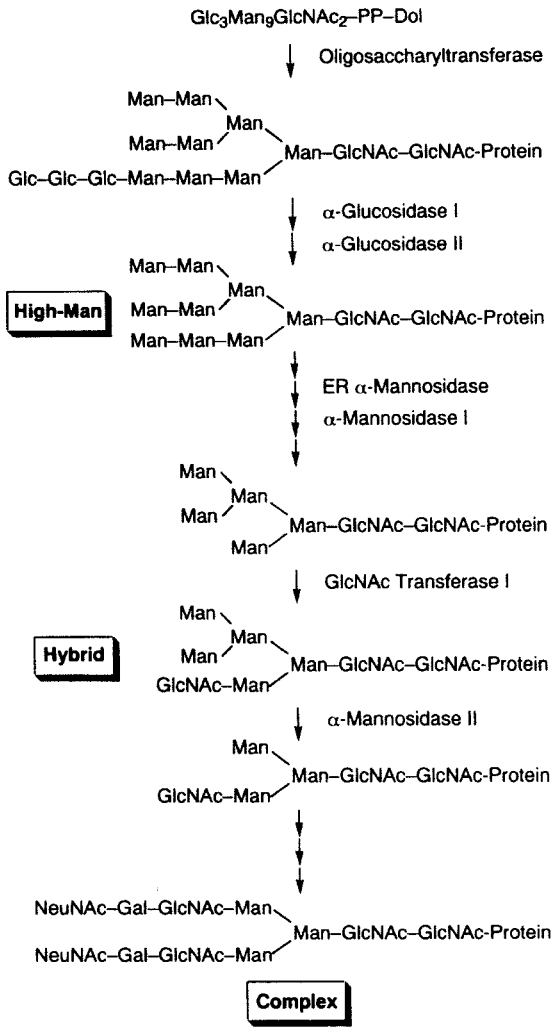


FIGURE 3 Reactions involved in the processing of the oligosaccharide chains of the *N*-linked glycoproteins. Glc = glucose, Man = mannose, Gal = galactose, NeuNAc = *N*-acetylneuraminic acid (sialic acid), GlcNAc = *N*-acetylglucosamine.

When administered to various types of animal cells in culture, castanospermine prevented glycoprotein processing and therefore caused the production of *N*-linked glycoproteins having oligosaccharides mostly of the $Glc_3Man_{7-9}(GlcNAc)_2$ type.[36] In IEC-6 intestinal cells, DNJ (at 5 mM) caused a decrease in complex types of oligosaccharides and an increase in high-mannose types. Since these high-mannose structures were less

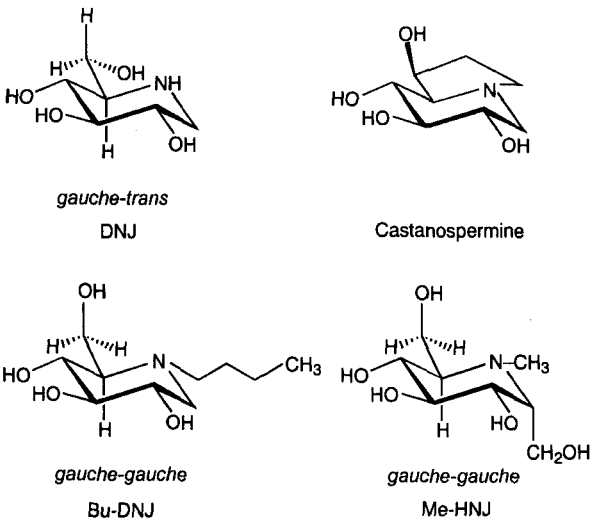


FIGURE 4 Preferred conformation of DNJ, castanospermine, Bu-DNJ, and Me-HNJ.

susceptible to α-mannosidase, they were thought to contain glucose residues.[37] In influenza virus-infected Madin–Darby canine kidney (MDCK) cells, DMDP inhibited mostly the synthesis of the complex types of *N*-linked oligosaccharides at 250 μg/ml to cause the accumulation of high-mannose structures with $Glc_3Man_{7-9}(GlcNAc)_2$ types, indicating that DMDP also inhibits processing α-glucosidase I.[38] α-HNJ and its *N*-methyl derivative (Me-HNJ) were also tested *in vivo* using influenza virus-infected MDCK cells, and measuring the inhibition of *N*-linked oligosaccharide processing of the viral envelope glycoproteins. With 100 μg/ml of Me-HNJ in the medium, essentially all of the *N*-linked oligosaccharide chains of the virus were of the high-mannose type with the major structure being characterized as $Glc_3Man_9(GlcNAc)_2$ (Figure 3).[31a] Similar results were obtained with HNJ although this compound was less effective *in vivo* as well as *in vitro*. In contrast, the 7-*O*-β-D-glucoside of α-HNJ (MDL 25637) is a much better inhibitor of α-glucosidase II than of α-glucosidase I. At 250 μg/ml of MDL 25637, the major *N*-linked oligosaccharide from the viral hemagglutinin was characterized as $Glc_2Man_{7-9}(GlcNAc)_2$ on the basis of several criteria.[39] DNJ has been reported to inhibit the formation of $Glc_3Man_9(GlcNAc)_2$-PP-dolichol.[40] The major lipid-linked oligosaccharide found in IEC-6 cells in the presence of DNJ (5 mM) was $Man_9(GlcNAc)_2$-PP-dolichol, whereas cells treated with 2 mM of the *N*-methyl derivative of

DNJ and control cells synthesized similar amount of $Glc_3Man_9(GlcNAc)_2$-PP-dolichol.

α-Glucosidase inhibitors, such as DNJ, Bu-DNJ and castanospermine, are potent inhibitors of HIV replication and HIV-mediated syncytium formation *in vitro*.[41] All sugar mimics showing anti-HIV activity have the common property that they are potent inhibitors of processing α-glucosidase I. It is presumed, although not proven, that the anti-HIV activity results from the inhibition of processing α-glucosidase I since there is a good correlation between potency of anti-HIV activity and that of α-glucosidase I.[42] However, inhibition of HIV by this class of compounds is not necessarily correlated with inhibition of α-glucosidase I. The potency of inhibition for α-glucosidase I in Me-DNJ and *N*-butyl-DNJ (Bu-DNJ) are almost the same, but the anti-HIV activity of Bu-DNJ is obviously more potent than that of Me-DNJ.[35,41a,43] α-HNJ, and especially its Me-HNJ, is more potent against α-glucosidase I than is either DNJ or castanospermine both *in vitro* and in cell culture.[31a] Surprisingly, both α-HNJ and Me-HNJ showed no significant anti-HIV activity even at concentrations of 500 μg/ml in MT-4 cells, whereas the 50% effective concentration (EC_{50}) value for Bu-DNJ was 37 μg/ml.[34] It has been reported that a major mechanism of action of Bu-DNJ as an inhibitor of HIV replication is the impairment of viral entry at the level of post-CD4 binding, due to an effect on viral envelope components.[41b] Furthermore, analysis of gp120 by a panel of conformation-dependent antibodies revealed structural changes within the V1/V2 loop region of gp120.[44] However, problems in achieving the therapeutic serum concentrations of Bu-DNJ needed to inhibit α-glucosidase sufficiently in humans may limit the practical use of this drug as an anti-HIV agent.[45]

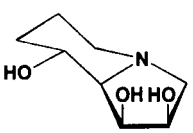
Growing evidence suggests that carbohydrate residues on cell-surface glycoconjugates play an important role in the metastatic spread of tumor cells. The use of biologically active sugar mimics to prevent the formation of the aberrant *N*-linked oligosaccharides and to inhibit catabolic glycosidases is being actively pursued as a therapeutic strategy for cancer.[46] Castanospermine and Me-DNJ have been shown to exhibit antimetastatic activity by inhibiting platelet aggregation of metastatic cells as well as reducing tumor cell adhesion.[47] Furthermore, these compounds have been shown to inhibit cellular transformation by altering oncogene glycosylation.[48] The effect of castanospermine on tumor growth in nude mice and on angiogenesis, the formation of new capillaries, *in vivo* in C57/BL mice was investigated.[49] When castanospermine (2.5 mg/day/mouse) was administered i.p. over a 5-day period beginning on day 29 after injection of tumor cells EHS-BAM, tumor growth inhibition in the treated animals was about 50% of control

when measured 13 days after treatment. Angiogenesis to basic fibroblast growth factor in castanospermine-treated C57/BL mice was similarly reduced. Nectrisine is a new immunoactive substance produced by a fungus, *Nectria lucida* F-4490.[14a] This compound restored Concanavalin A-stimulated lymphocyte proliferation to a normal level and the capacity of immunosuppressed mice to produce antibody against sheep red blood cells.

2.2. α-Mannosidase Inhibitors

2.2.1. Natural Occurrence

In 1979 the toxicity to livestock of the legume *Swainsona canescens* in Australia led to the isolation of the toxic principle swainsonine (**21**; Figure 5)[50] and resulted in a great impitus to research on sugar mimics with a nitrogen in the ring and their applications. Swainsonine is also present in locoweeds (*Astragalus* and *Oxytropis* species), which cause the disorder "locoism" in the western United States.[51] *C. australe* produces 6-*epi*-castanospermine (**22**), together with castanospermine.[52] 6-*Epi*-castanospermine has the *manno* configuration in the piperidine ring and is a good inhibitor of human neutral α-mannosidase.[53] 1-Deoxymannojirimycin (DMJ) (**23**) was first isolated from the seeds of the legume *Lonchocarpus sericeus*, a native of the West Indies and tropical America[54] and later isolated from the neotropical liana, *Omphalea diandra* (Euphorbiaceae),[7] and the legume *Angylocalyx pynaertii* growing in tropical African forests.[55] DMJ was also isolated from the culture broth of *Streptomyces lavendurae* GC-148,[56] which has



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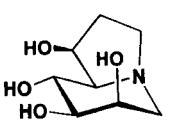

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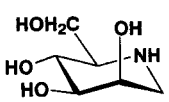

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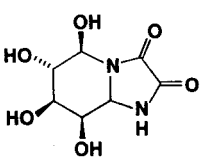

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FIGURE 5 Structures of naturally occurring α-mannosidase inhibitors.

been already reported as a high-yielding strain of DNJ.[4c] *Streptomyces subrutilus* ATCC 27467 produces both DNJ and DMJ in its culture broth, and mannojirimycin (MJ) has been suggested as an intermediate in the biosynthesis of DMJ.[57] In this organism, it has also been suggested that epimerization of MJ can occur at C-2 to give NJ which is then dehydrated and reduced to DNJ. *Agrobacterium* spp strain 19-1 has been shown to be able to epimerize DNJ to DMJ.[58] A new inhibitor of Golgi α-mannosidase I, kifunensine (**24**), was initially isolated as a weak inhibitor of jack bean α-mannosidase ($IC_{50} = 100\,\mu M$) from the culture broth of the actinomycete, *Kitasatosporia kifunense* 9482.[59] Kifunensine can be regarded as the cyclic oxamide of 1-amino-deoxymannojirimycin and its structure is quite different from those of other α-mannosidase inhibitors, although it would still be classified as a sugar mimic with a nitrogen in the ring.

2.2.2. *Biological Activities and Therapeutic Application*

The indolizidine alkaloid swainsonine is a potent inhibitor of lysosomal α-mannosidase and prolonged ingestion of swainsonine by animals leads to a neurological disorder that is a phenocopy of the genetically induced lysosomal storage disease mannosidosis.[60] Mannosidosis is characterized by accumulation in cells, and excretion in the urine, of mannose-rich oligosaccharides resulting from a deficiency of lysosomal α-mannosidase. Swainsonine is a lysosomotropic compound and accumulates rapidly in lysosomes of normal human fibroblasts in culture to produce inhibition of intracellular lysosomal α-mannosidase,[61] and it is assumed that this is its mode of action *in vivo*. The low concentration of swainsonine ingested is made effective by its ability to permeate the plasma membrane freely, but once inside lysosomes it becomes protonated due to the low pH and becomes concentrated.[61]

Swainsonine is the first compound that was found to inhibit glycoprotein processing. Swainsonine was shown to be a potent inhibitor ($IC_{50} = 0.2\,\mu M$) of rat liver Golgi α-mannosidase II, but was without effect on Golgi α-mannosidase I.[62] Swainsonine also did not inhibit ER α-mannosidase, nor the corresponding soluble α-mannosidase.[63] When swainsonine was placed in the culture medium of various animal cells, it caused the formation of hybrid types of oligosaccharides having an oligomannosyl core [Man_5-$(GlcNAc)_2$] characteristic of neutral oligosaccharides, and a (or several) NeuNAc-Gal-GlcNAc sequence(s) characteristic of complex chains. Thus, the oligosaccharide chains of the G protein of vesicular stomatitis virus (VSV) were altered from complex to hybrid structures,[64] as was the *N*-linked carbohydrate of fibronectin.[65] Swainsonine has been used in a number of

studies in order to determine whether changes in the structure of the *N*-linked oligosaccharides affect glycoprotein function. In most cases, it has little effect on the glycoprotein in question, which may indicate that a partial complex chain is sufficient for activity, and that protein conformation is not altered.[30c] On the other hand, DMJ is a fairly potent inhibitor of rat liver Golgi α-mannosidase I, with an IC_{50} value of 1 μM, but not Golgi α-mannosidase II and ER α-mannosidase.[66] In intact cells, DMJ blocked the synthesis of complex types of *N*-linked oligosaccharides and caused the accumulation of glycoprotein having $Man_{7-9}(GlcNAc)_2$ structures, with predominant $Man_9(GlcNAc)_2$ oligosaccharides.[67] Kifunensine proved to be an effective inhibitor of plant α-mannosidase I ($IC_{50} = 0.02\,\mu M$) but had no activity against plant α-mannosidase II.[68] When kifunensine was added to the culture medium of mammalian cells at concentrations of 1 μg/ml or higher, it caused a complete shift in the structure of the *N*-linked oligosaccharides from complex structures to $Man_9(GlcNAc)_2$ structures.[68] The inhibition of the processing α-mannosidase I in cell culture did not affect the secretion of the antibodies, IgD and IgM, by hybridoma cells,[66] nor did it affect the secretion of α_1-acid glycoprotein or α_1-antitripsin by primary cultures of hepatocytes.[69]

When B16-F10 murine melanoma cells were treated with swainsonine at 3 μg/ml in growth medium and then injected intravenously into syngeneic C57BL/6 mice, greater than 80% inhibition of colony formation were consistently observed in multiple experiments.[70] Despite this dramatic inhibition of colonization, this treatment had no effect on B16-F10 viability or on cellular tumorigenicity after subcutaneous implantation. Swainsonine-treated radiolabeled B16-F10 cells were cleared from the lungs at a greater rate than control cells, suggesting that one effect of treatment is to alter tumor cell retention in the target organ. Similarly, both the lymphoid tumor line MDAY-D2 and B16-F10 melanoma cells were less metastatic when grown in swainsonine (0.3 μg/ml) for 48 h prior to injection of the cells into the lateral tail veins of mice, and the addition of swainsonine (2.5 μg/ml) to the drinking water of the mice further reduced the incidence of lung colonization by B16-F10 melanoma cells.[71] Swainsonine alone did not inhibit tumor cell growth *in vitro*, but it enhanced the antiproliferative effect of α/β-interferon *in vitro* and *in vivo*.[71] Swainsonine and interferon α-2 also have a synergistic effect on the growth of the human H29 colon carcinoma *in vitro* and *in vivo*.[72] There is much evidence that swainsonine augments the natural tumoricidal activity of the immune system *in vitro*[73] and *in vivo*.[74] Swainsonine elicited a 32% increase in spleen cell number 2 days after administration and induced a concomitant 2- to 3-fold increase in splenic natural killer (NK) cell activity, but had no antimetastatic effect in

mice depleted of NK cells.[74] Treatment with swainsonine enhanced the proliferative response of the T-cell clone to antigen and to the mitogen concanavalin A, whereas treatment with castanospermine had the opposite effect and inhibited the proliferative response of the T cell to antigen.[75] Swainsonine also enhances lymphocyte interleukin-2 (IL-2) receptor expression,[76] and induces tumoricidal activity and secretion of IL-1 in macrophages.[77]

3. NEW THERAPEUTIC PROSPECTS FOR LYSOSOMAL STORAGE DISEASES

The glycosphingolipid (GSL) storage diseases are relatively rare hereditary disorders that are severe in nature and frequently fatal.[78] Possible strategies for the treatment of these lysosomal storage diseases include enzyme replacement therapy, gene therapy, substrate deprivation and bone marrow transplantation. Only successful therapy for the diseases to date is the enzyme replacement for the patients with type 1 Gaucher disease.[79] This disease results from mutations in the glucocerebrosidase gene, which leads to the storage of glucosylceramide (GlcCer). The placentally derived glucocerebrosidase (CEREDASE™), which normally carries complex oligosaccharide chains, was modified after purification to expose terminal mannose residues. These allow the enzyme to bind to the mannose receptors present on the surface of reticulo-endothelial cells,[80] which are specifically affected in type 1 Gaucher disease. However, it would be to an extremely limited number of patients that long-life treatment with this "world's most expensive drug" (The Wall Street Journal) becomes available. Furthermore, as lysosomal enzymes do not cross the blood–brain barrier, enzyme replacement therapy is only useful in diseases in the absence of neuropathology such as type 1 Gaucher disease and Fabry disease (Figure 6).

There is a new approach that may be generally applicable to the GSL storage disorders. As long as the biosynthesis of substrates continues, the pathological accumulation of substrates in the lysosomes proceeds. If substrate influx into the lysosomes should be reduced by inhibition of GSL biosynthesis, it should be possible to positively influence the severity as well as the onset of these diseases with the aid of synthetic inhibitors. This approach assumes that a definite residual activity of the enzyme is present in the lysosomes, which is especially the case in juvenile and adult patients. Bu-DNJ, which had been developed as an anti-HIV agent, was discovered to be a specific inhibitor of the glucosyltransferase-catalyzed biosynthesis of

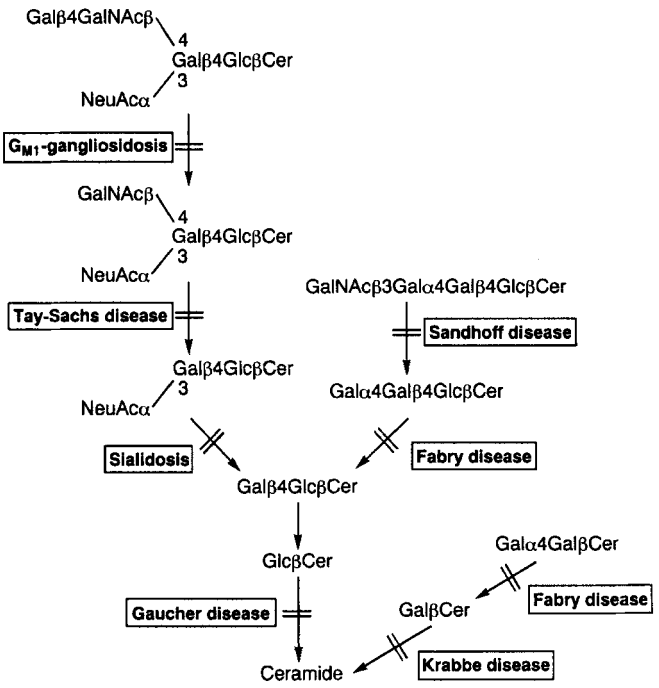

FIGURE 6 Degradation of selected glycosphingolipids (GSLs) in the lysosomes of the cells. The names of individual hereditary diseases are given in boldface (in box). Cer = ceramide, GalNAc = *N*-acetylgalactosamine.

GlcCer, the first step in the biosynthetic pathway of GlcCer-based GSLs.[81] Oral administration of Bu-DNJ to healthy mice resulted in GSL depletion in multiple organ, without causing any overt pathology in the treated animals.[82] When Tay–Sachs mice were treated with Bu-DNJ, the accumulation of G_{M2} ganglioside in the brain was prevented, with the number of storage neurons and the quantity of ganglioside stored per cell markedly reduced.[83] *N*-Butyl-1-deoxygalactonojirimycin (Bu-DGJ) was also found to be a potent inhibitor of GSL biosynthesis but in contrast to Bu-DNJ had no effect on the maturation of *N*-linked oligosaccharides or on lysosomal glucocerebrosidase.[84] Although Bu-DNJ and Bu-DGJ are moderate inhibitors of the transferase (IC_{50} of 20 and 41 μM, respectively), owing to their oral availability (no report on Bu-DGJ) and low toxicity, the discovery of these compounds as inhibitors of GlcCer-based GSLs certainly constitutes great progress toward the treatment of this group of severe diseases.

There is one more quite interesting approach to a molecular therapy that may be extensively applicable to all lysosomal storage diseases.

Fabry disease is a disorder of GSL metabolism caused by deficiency of lysosomal α-galactosidase A (α-Gal A), resulting in renal failure along with premature myocardial infarction and strokes.[85] No effective treatment of this disorder is available at present. In general, a complete deficiency leads to an early onset and a severe course of the disease. Many mutations only lead to a partial loss of enzyme activity. A residual activity of only a few percent can be sufficient to delay the onset of the disease and cause a mild course of the disease.[86] Chemically synthesized 1-deoxygalactonojirimycin (DGJ) is a powerful inhibitor of coffee bean α-galactosidase, with a K_i value of 1.6 nM.[87] This compound was also a powerful inhibitor of α-Gal A, with an IC_{50} value of 4.7 nM, and surprisingly enhanced α-Gal A activity in lymphoblasts derived from hemizygous Fabry patients with the R301Q or Q279E mutations identified in cardiac Fabry patients, a late-onset form of the disorder.[88] The enzyme activity increased 8- or 7-fold (up to 48% or 45% of normal) after cultivation of R301Q or Q279E lymphoblasts, respectively, with DGJ at 20 μM for 4 days. When DGJ was administered to the R301Q transgenic mice as a 0.05 or 0.5 mM DGJ solution available *ad libitum* in their drinking water for a week, the enzyme activity was elevated 4.8- and 18-fold respectively in the heart. The rationale for these observations is that in some Fabry patients, a mutation in α-Gal A causes incomplete but flexible folding of the enzyme, whereas the catalytic center remains intact. It is known that the ER has an efficient quality control system to ensure that transport to the Golgi complex is limited to properly folded and assembled proteins, and the main process of the quality control is enforced by a variety of chaperones. Therefore, the mutant enzyme would be retarded in the normal transport pathway, resulting in a deficiency of the enzyme activity. A competitive inhibitor DGJ might specifically protect the catalytic center and reduce the flexibility of folding *in situ*, thus leading the mutant α-Gal A to a proper conformation. Consequently, the mutant enzyme would be allowed to pass through the quality control system and be transported to the Golgi complex to achieve molecular maturation. After the mutant enzyme has been transported to the lysosome, it remains stable in this acidic condition. DGJ acts as a chaperone to force the mutant enzyme to assume the proper conformation. Thus, the concept of "chemical chaperone" was proposed to describe a small molecule whose function is to assist a protein to properly fold and enter normal processing pathway. The bioavailability of DGJ is crucial in the possible clinical applications for Fabry disease. Furthermore, this means that inhibitors of lysosomal enzymes have the therapeutic potential for corresponding lysosomal storage diseases.

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