Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 16 (2008) 2734–2740

Effect of five-membered sugar mimics on mammalian glycogen-degrading enzymes and various glucosidases

Yasuhiro Minami,[a] Chinami Kuriyama,[a] Kyoko Ikeda,[a] Atsushi Kato,[b] Kenji Takebayashi,[b] Isao Adachi,[b] George W. J. Fleet,[c] Aikkarach Kettawan,[d] Tadashi Okamoto[e,f] and Naoki Asano[a,f,*]

[a]*Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3 Kanagawa-machi, Kanazawa 920-1181, Japan*
[b]*Department of Hospital Pharmacy, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan*
[c]*Oxford Glycobiology Institute, University of Oxford, South Parks Road, Oxford OX1 3QU, UK*
[d]*The Food Chemistry Division, Institute of Nutrition, Mahidol University, Thailand*
[e]*Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Kobe 651-2180, Japan*
[f]*Cooperative Research Center of Life Science, Kobe Gakuin University, Chuo-ku, Kobe 650-8586, Japan*

Received 24 October 2007; revised 7 January 2008; accepted 8 January 2008
Available online 26 January 2008

Abstract—We investigated inhibitory activities of five-membered sugar mimics toward glycogen-degrading enzymes and a variety of glucosidases. 1,4-Dideoxy-1,4-imino-D-arabinitol (D-AB1) is known to be a potent inhibitor of glycogen phosphorylase. However, the structural modification of D-AB1, such as its enantiomerization, epimerization at C-2 and/or C-3, introduction of a substituent to C-1, and replacement of the ring nitrogen by sulfur, markedly lowered or abolished its inhibition toward the enzyme. The present work elucidated that D-AB1 was also a good inhibitor of the de-branching enzyme of glycogen, amylo-1,6-glucosidase, with a IC_{50} value of 8.4 μM. In the present work, the de-sulfonated derivative of salacinol was isolated from the roots of *Salacia oblonga* and found to be a potent inhibitor of rat intestinal isomaltase with an IC_{50} value of 0.64 μM. On the other hand, salacinol showed a much more potent inhibitory activity toward maltase in Caco-2 cell model system than its de-sulfonated derivative, with an IC_{50} value of 0.5 μM, and was further a stronger inhibitor of human lysosomal α-glucosidase than the derivative (IC_{50} = 0.34 μM). This indicates that the sulfate in the side chain plays an important role in the specificity of enzyme inhibition.

1. Introduction

Recently, glycosidase-inhibiting sugar mimics are arousing great interest as potential therapeutic agents such as antidiabetics, antiobesities, antivirals, and therapeutic agents for some genetic disorders.[1] Acarbose (Glucobay), voglibose (Basen), and miglitol (Glyset) are on the market for the treatment of type 2 diabetes, and zanamivir (Relenza) and oseltamivir (Tamiflu), both approved in 1999 by the U.S. Food and Drug Administration, are members of a new class of antiviral agents that selectively inhibit the neuraminidase of both influenza A and B viruses. From the success of α-glucosidase inhibitors as antidiabetics and neuraminidase inhibitors as antiinfluenza drugs, the practical use of glycosidase inhibitors appears to be limited to diabetes and viral infection. However, since glycosidases are involved in a wide range of anabolic and catabolic processes of carbohydrates, glycosidase inhibitors could have many kinds of beneficial effects as therapeutic agents.

Glycogen in mammals exists both as cytosolic and lysosomal forms, and is broken down by different pathways. The cytosolic form is cleaved by glycogen phosphorylase (GP) and the debranching enzyme amylo-1,6-glucosidase (amylo-1,6-Glu), while the lysosomal form is degraded by a single enzyme acid (lysosomal) α-glucosidase. The liver is a predominant source of blood glucose. It is generally recognized that the hepatic glucose output in type 2 diabetes is elevated and thus significantly contributes to hyperglycemia.[2–4] A possible way to suppress hepatic glucose production and lower blood glucose in

Keywords: Glycogen phosphorylase; Amylo-1,6-glucosidase; Glucosidase; Inhibitor; Caco-2 cell.
* Corresponding author. Fax: +81 76 229 2781; e-mail: n-asano@hokuriku-u.ac.jp

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.01.032

type 2 diabetes may be through inhibition of GP.[5] In enzyme assay, Fosgerau et al. reported that a five-membered iminosugar 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1, **1**) is a potent inhibitor of hepatic GP and is the most potent inhibitor of basal and glucagon-stimulated glycogenolysis ever reported in primary rat hepatocytes, with an IC_{50} value of 1 μM.[6,7] 1,4-Dideoxy-1,4-imino-L-arabinitol (L-AB1, **2**), a synthetic L-enantiomer of **1**, is a much more potent inhibitor of mammalian isomaltase than **1**, with an IC_{50} value of 0.08 μM.[8] Introduction of the hydroxymethyl group to C-1 in **1** leads to 2,5-dideoxy-2,5-imino-D-mannitol (D-DMDP, **3**), which was found in leaves of the legume *Derris elliptica*.[9] In 2004, the L-enantiomer (L-DMDP, **4**) of **3** was synthesized from D-gulonolactone and found to be a more powerful and more specific α-glucosidases than the natural product D-DMDP.[10] These D-enantiomers **1** and **3** inhibit α-glucosidases in a competitive manner, whereas their L-enantiomers **2** and **4** were found to be non-competitive inhibitors of the enzymes.[8] It would thus be of interest to investigate the inhibitory properties of five-membered sugar mimics. We here report the effect of natural and synthetic five-membered sugar mimics toward mammalian glycogen-degrading enzymes and various glucosidases.

2. Results

2.1. Preparation of five-membered sugar mimics

Naturally occurring iminosugars D-AB1 (**1**), 1,4-dideoxy-1,4-imino-D-ribitol (D-DRB, **5**), 1,4-dideoxy-1,4-imino-D-xylitol (D-DIX, 6), and *N*-hydroxyethyl-D-AB1 (**11**) were isolated from *Angylocalyx pynaertii* (Leguminosae).[11,12] D-DMDP (**3**) from *Derris malaccensis* (Leguminosae),[13] 2-deoxy-D-AB1 (CYB-3, **7**) from *Castanospermum australe* (Leguminosae),[14] 2,5-dideoxy-2,5-imino-D-glucitol (D-DIG, **8**) from *Albizia myriophylla* (Leguminosae),[15] and 2,5-dideoxy-2,5-imino-D-*glycero*-D-*manno*-heptitol (homoDMDP, **9**) and 2,5-dideoxy-2,5-imino-D-*manno*-heptitol (Deoxy-homoDMDP, **10**) from *Scilla sibirica*[16] were prepared according to the literature. Synthetic L-AB1 (**2**) and L-DMDP (**4**) were prepared from D-xylose and D-gulonolactone, respectively, according to the literature.[10,17]

In 1997, in the course of search for α-glucosidase inhibitors from *Salacia reticulata* Yoshikawa et al. isolated a novel five-membered sugar analogue salacinol (**12**), which is the inner salt composed of 1,4-anhydro-4-thio-arabinofuranosyl cation and 1-deoxy-erythritol-3-sulfate anion.[18] Its absolute configuration of salacinol was unequivocally established as a 1,4-anhydro-4-thio-D-arabinitol (D-ATA, **13**) unit linked to an L-erythritol unit by the synthesis of both enantiomers.[19] Yoshikawa et al. reported the isolation of salacinol by silica gel chromatography and repeated HPLC from the water-soluble fraction of the roots of *S. reticulata*.[18] In the present work, we developed a conventional purification method with ion-exchange resins (see Experimental). The 50% aqueous ethanol extract of *S. oblonga* roots was subjected to an Amberlite IR-120B (H^+ form) and the effluent was applied to an Amberlite IR-400J (OH^- form), followed by elution with M AcOH. The AcOH eluate was concentrated and further applied to a Dowex 1-X2 (OH^- form). Two components in the AcOH eluate of this resin were detected on TLC. We were able to separate two components by a Dowex 1-X2 ($HCOO^-$ form) column chromatography using water as eluant. The latter component eluted from this column was determined to be salacinol from its NMR spectroscopic data, while the NMR spectroscopic data of another component (**14**) were different from those of another known compound kotaranol from *S. reticulata*.[20] D-ATA (**13**) was prepared by heating salacinol under reflux in 1% CH_3ONa methanol for 6 h (see Section 5).

2.2. Structural determination of compound 14

Compound **14** was determined to have the molecular formula $C_9H_{18}O_6S$ ($C_9H_{18}O_9S_2$ for **12**) by HRMS (FAB). The complete connectivity of carbon and hydrogen atoms was defined by 2D ^1H–^1H and ^1H–^{13}C COSY, and HMBC spectroscopic data. The ^1H NMR (Table 1) and ^{13}C NMR spectroscopic data of **14** were closely related to those of **12**, except for a 1.08-ppm upfield shift of H-3′ and a 4.8-ppm upfield shift of C-3′. Yoshikawa et al. have reported that the positive-ion FABMS and liquid SIMS of **12** show a fragment ion peak at *m/z* 255 $[M–SO_3 + H]^+$, together with quasimolecular ion peaks at *m/z* 335 $[M+H]^+$ and *m/z* 357 $[M+Na]^+$.[18] On the other hand, **14** showed a quasimolecular ion peak at *m/z* 255 $[M+H]^+$. Hence, from the MS and NMR spectroscopic data described above, compound **14** was presumed to be the derivative de-sulfonated at C-3′ of **12**. Therefore, we attempted the chemical conversion of **12** to **14**. A small amount of **12** was heated with 5% HCl in MeOH under reflux and the reaction mixture was concentrated. The residue was subjected to a short column of Dowex 1-X2

Table 1. Concentration of five-membered sugar mimics giving 50% inhibition of glycogen phosphorylase b (GP b) and amylo-1,6-glucosidase (amylo-1,6-Glu)

Compounds	IC_{50} (μM)	
	GP b	Amylo-1,6-Glu
D-AB1 (**1**)	0.43	8.4
L-AB1 (**2**)	—[a]	200
D-DMDP (**3**)	—[a]	9.8
L-DMDP (**4**)	—[a]	nd[c]
D-DRB (**5**)	27	—[b]
D-DIX (**6**)	100	—[b]
CYB-1 (**7**)	—[a]	48
D-DIG (**8**)	—[a]	11
homoDMDP (**9**)	150	100
Deoxy-homoDMDP (**10**)	—[a]	11
N-Hydroxyethyl-D-AB1 (**11**)	100	12
Salacinol (**12**)	—[a]	48
D-ATA (**13**)	—[a]	—[b]
Compound **14**	—[a]	16

[a] No inhibition (less than 50% inhibition at 400 μM).
[b] No inhibition (less than 50% inhibition at 1000 μM).
[c] Not determined.

($HCOO^-$ form) and eluted with water to give **14**. The optical rotation value and NMR spectroscopic data of the chemically prepared compound were completely identical with those of naturally occurring compound **14**. Hence, the structure of **14** was tentatively determined to be 1,4-dideoxy-1,4-{(*S*)-[(2S,3S)-2,4-dihydroxy-3-butyl]episulfoniumylidene}-D-arabinitol inner salt. With respect to the structure of **14**, further investigation would be needed. Whether compound **14** is an artifact or a natural product is now under examination.

2.3. Effects of five-membered sugar mimics on glycogen-degrading enzymes

We investigated the inhibitory activity of five-membered sugar mimics toward glycogen degrading enzymes, GP and amylo-1,6-Glu. The results are shown in Table 1. D-AB1 (**1**) potently inhibited rabbit muscle GP b with an IC_{50} value of 0.43 μM, whereas its enantiomer **2** showed no inhibition toward the enzyme. Introduction of the hydroxymethyl group to the C-1 position of **1** to give **3** and **8** abolished its inhibition. The epimerization at C-2 to give **5** lowered its inhibition 60-fold and the removal of the 2-OH group of **1** to give **7** showed null inhibition of GP. Replacement of the ring nitrogen by a sulfur atom to give **13** completely abolished its inhibition of GP, and the derivatives, salacinol (**12**) and the de-sulfonated compound (**14**) of salacinol, also showed no inhibition of the enzyme.

D-AB1 (**1**) was also a good inhibitor of another glycogen-degrading enzyme, amylo-1,6-Glu, with an IC_{50} value of 8.4 μM, and, interestingly, its enantiomer **2** also inhibited weakly this enzyme. Introduction of the hydroxymethyl or hydroxyethyl group to the C-1 position of **1** to give **3**, **8**, and **10** retained its inhibitory potential, and the *N*-hydroxyethyl derivative **11** also retained its potency toward amylo-1,6-Glu. Compound **14** was a threefold better inhibitor of amylo-1,6-Glu than salacinol **12**.

2.4. Effects of five-membered sugar mimics on various glucosidases

The IC_{50} values of five-membered sugar mimics toward various glucosidases are shown in Table 2. We recently reported that L-AB1 (**2**) and L-DMDP (**4**) are much more potent inhibitors of α-glucosidases (other than yeast α-glucosidase) than their D-enantiomers, and that D-enantiomers inhibit D-glucohydrolases in a competitive manner, whereas L-enantiomers are their non-competitive inhibitors.[8] In particular, L-AB1 (**2**) and L-DMDP (**4**) are very potent inhibitors of rat intestinal isomaltase, with IC_{50} values of 0.08 and 0.05 μM, respectively. D-AB1 is known to be a potent inhibitor of yeast α-glucosidase,[17] whereas its 2-deoxy derivative **7** abolished its inhibitory activity toward the enzyme. Introduction of the hydroxymethyl group to the β-orientation at C-1 of **1** to give **8** markedly lowered or abolished its inhibition toward α-glucosidases. Replacement of the imino group of **1** by a sulfur atom to give **13** led to marked decrease of inhibition toward glucosidases, while the *S*-1-deoxyerythritol-3-sulfate anion derivative **12** is known to be a potent inhibitor of α-glucosidases.[18,21] In the present work, salacinol (**12**) was found to be a potent inhibitor of human lysosomal α-glucosidase, with an IC_{50} value of 0.34 μM, and the de-sulfonated compound **14** was found to potently inhibit rat intestinal isomaltase (IC_{50} = 0.64 μM). With respect to rice α-glucosidase and rat intestinal maltase and sucrase, compound **12** was a more potent inhibitor than **14**.

3. Discussion

Fosgerau et al. have reported that D-AB1 (**1**) is a potent inhibitor of GP with anti-hyperglycemic effect in ob/ob mice and the mode of inhibition is uncompetitive or non-competitive, with respect to glycogen and phosphate, respectively.[6] However, Oikonomacos et al. recently have reported X-ray crystallographic investigation of a GP–D-AB1 complex that, in the presence of phosphate, D-AB1 binds at the catalytic site of GP b, with the three hydroxyl groups mimicking the hydroxymethyl and the hydroxyl groups in the C-6, C-3, and C-4 positions in a glucopyranose moiety.[22] We were not able to find better inhibitors of GP than D-AB1 in the present study but showed that GP b has a strict structure requirement for inhibitors at the catalytic site. Furthermore, the present study suggests that the anti-hyperglycemic effect in ob/ob mice reported may be due to a combination of GP b and amylo-1, 6-Glu inhibition since D-AB1 was also a good inhibitor

Table 2. Concentration of five-membered sugar mimics giving 50% inhibition of various glucosidases

Enzyme	IC_{50} (μM)									
	1	**2**	**3**	**4**	**7**	**8**	**11**	**12**	**13**	**14**
α-Glucosidase										
Rice	250	1.7	370	1.5	—	—	—	2.6	220	8.6
Yeast	0.18	10	1.1	—[a]	—	28	290	—	—	900
Rat maltase	55	1.3	290	1.4	160	—	720	2.4	290	10
Rat isomaltase	5.8	0.08	90	0.05	730	150	70	5.7	1000	0.64
Rat sucrase	16	1.7	64	0.10	300	320	120	2.2	—	5.2
Human lysosome	900	31	—	70	—	—	—	0.34	—	4.4
β-Glucosidase										
Human lysosome	120	—	150	—	—	—	—	—	—	—

[a] Less than 50% inhibition at 1000 μM.

of amylo-1,6-Glu. L-AB1 (**2**) and L-DMDP (**4**) are much more potent inhibitors of rat digestive α-glucosidases than their D-enantiomers, whereas these L-enantiomers showed no significant inhibition toward glycogen-degrading enzymes (Fig. 1).

In the course of preparation of salacinol (**12**) from *S. oblanga*, we found its de-sulfonated compound (**14**) and were also able to chemically prepare it from **12**. Very recently, Tanabe et al. have reported biological evaluation of **12** and **14** on α-glucosidases.[23] They concluded that the *O*-sulfonated anion moiety of **12** is not essential for the inhibitory activity since the de-sulfonated derivative of **12** showed a potent inhibitory activity equal to that of **12** toward rat intestinal maltase and sucrase. However, the present study elucidated that the de-sulfonated compound is a ninefold stronger inhibitor of rat intestinal isomaltase than **12**, while compound **12** is a 13-fold stronger inhibitor of human lysosomal α-glucosidase than **14**. In addition, we estimated inhibitory activities of compounds **12** and **14** toward maltase using Caco-2 cell model system. This experiment is based on the assumption that maltose, which was added to the apical side of Caco-2 monolayer, is hydrolyzed by maltase expressed in the apical side of Caco-2 cells to give D-glucose. As shown in Figure 2, salacinol (**12**) gave an IC_{50} value of 0.5 μM toward maltase in this model system, while its de-sulfonated compound **14** showed only 30% inhibition of maltase even at 1 μM. This indicates that the sulfate in the side chain plays an important role in the specificity of enzyme inhibition.

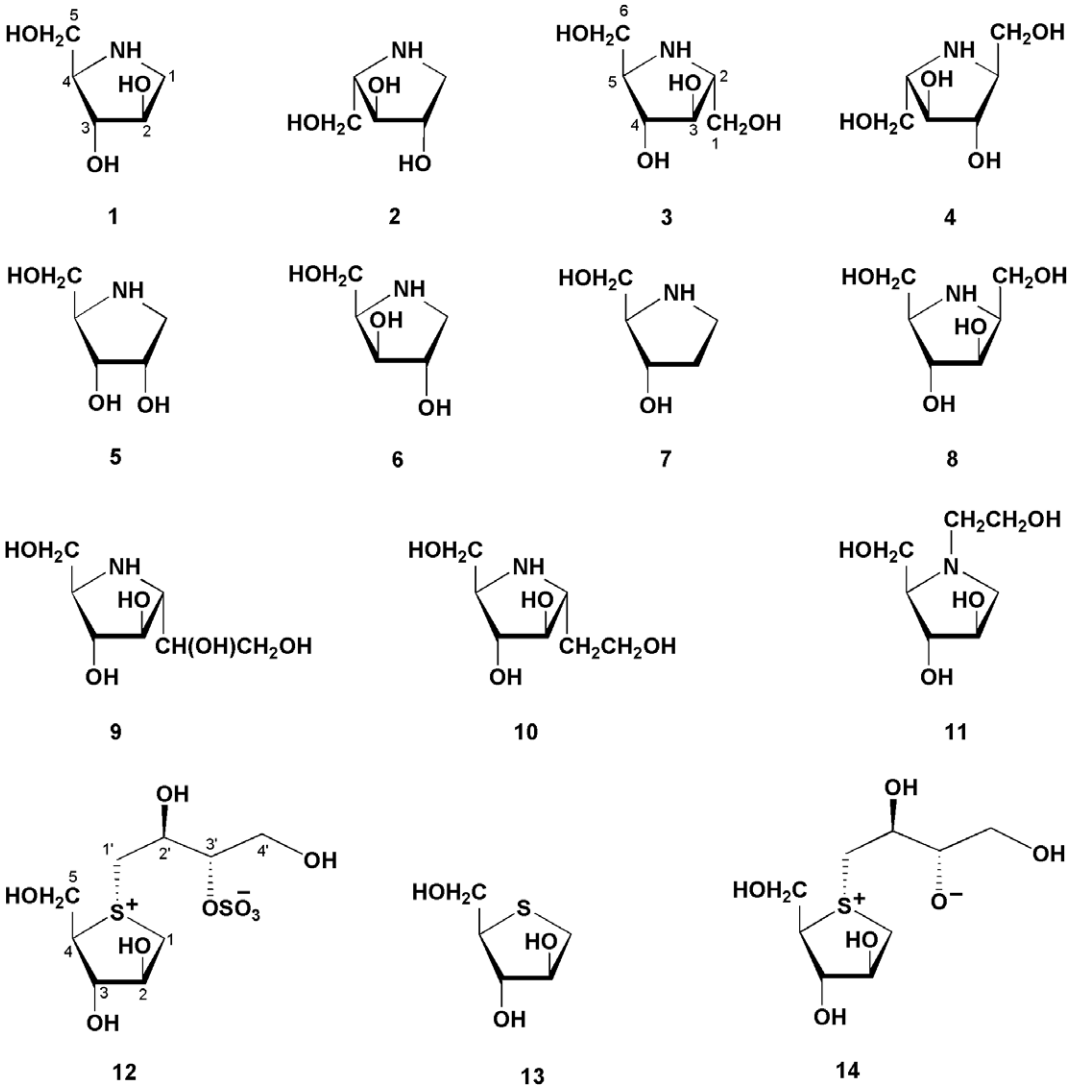

Figure 1. Structures of five-membered sugar mimics. **1**, 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1); **2**, L-AB1; **3**, 2,5-dideoxy-2,5-imino-D-mannitol (D-DMDP); **4**, L-DMDP; **5**, 1,4-dideoxy-1,4-imino-D-ribitol (D-DRB); **6**, 1,4-dideoxy-1,4-imino-D-xylitol (D-DIX); **7**, 1,4-imino-1,2,4-trideoxy-D-arabinitol (CYB-1); **8**, 2,5-dideoxy-2,5-imino-D-glucitol (D-DIG); **9**, 2,5-dideoxy-2,5-imino-D-*glycero*-D-*manno*-heptitol (homoDMDP); **10**, 2,5-imino-2,5,6-trideoxy-D-*manno*-heptitol (Deoxy-homoDMDP); **11**, 1,4-dideoxy-1,4-(hydroxyethyliminiumyl)-D-arabinitol (*N*-Hydroxyethyl-D-DB1); **12**, 1,4-dideoxy-1,4-{(*S*)-[(2S,3S)-2,4-dihydroxy-3-(sulfooxy)butyl]episulfoniumylidene}-D-arabinitol inner salt (salacinol); **13**, 1,4-anhydro-4-thio-D-arabinitol (D-ATA); **14**, 1,4-dideoxy-1,4-{(*S*)-[(2S,3S)-2,4-dihydroxy-3-butyl]episulfoniumylidene}-D-arabinitol inner salt (neosalacinol).

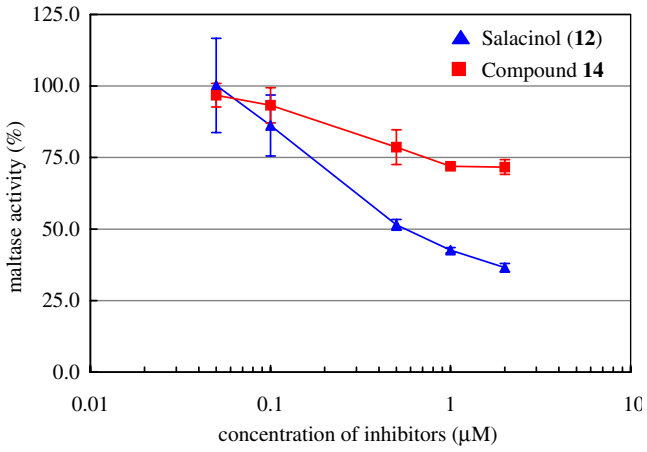

Figure 2. Maltase inhibitory activity of salacinol (**12**) and its de-sulfonated compound **14** at the apical side of Caco-2 monolayer.

Recent experimental data show that some human genetic diseases are due to mutations in proteins that influence their folding and lead to retaining of mutant proteins in the endoplasmic reticulum (ER) and successive degradation.[24] Lysosomes are membrane-bound cytoplasmic organelles that serve as a major degradative compartment in eukaryotic cells. The degradative function of lysosomes is carried out by more than 50 acid hydrolases contained within the lumen.[25] The glycosphingolipid (GSL) storage diseases are genetic disorders in which a mutation of one of GSL glycohydrolases blocks GSL degradation, leading to lysosomal accumulation of undegraded GSL.[26] In recent years, remarkable progress has been made in developing a molecular therapy for GSL storage disorders.[1,24,27] One of novel approaches is pharmacological (or chemical) chaperone therapy. The concept of pharmacological chaperone therapy is that an intracellular activity of misfolded mutant enzymes can be restored by administering competitive inhibitors that serve as pharmacological chaperones. These inhibitors appear to act as a template that stabilizes the native folding state in the ER by occupying the active site of the mutant enzyme, thus allowing its maturation and trafficking to the lysosome.[27] Lysosomal glycosidase inhibitors are candidates as pharmacological chaperones for GSL storage disorders. Pompe disease (glycogen storage disease type II) is a fatal genetic muscle disorder caused by deficiency of lysosomal α-glucosidase, a glycogen-degrading enzyme. The present study found that salacinol (**12**) is a very potent inhibitor of human lysosomal α-glucosidase, with an IC_{50} value of 0.34 μM. We very recently reported that 1-deoxynojirimycin is a potent inhibitor of lysosomal α-glucosidase, with an IC_{50} value of 1 μM.[28] In 2006, Myozyme has been approved for the treatment of patients with Pompe disease, which is glycogen storage disease type II caused by a deficiency of lysosomal α-glucosidase. Salacinol was also a potent inhibitor of this therapeutic enzyme Myozyme, with an IC_{50} value of 0.52 μM, while its de-sulfonated compound **14** was a 36-fold weaker inhibitor of the enzyme than salacinol. We are now investigating the chaperoning activity of salacinol using cells derived from Pompe disease patients.

4. Materials

4.1. Preparation of five-membered sugar mimics

Naturally occurring iminosugars **1**, **5**, **6**, and **11** were isolated from the bark and pods of *A. pynaertii*,[11,12] **3** from the leaves of *D. malaccensis*,[13] **7** from the beans of *C. australe*,[14] **8** from the wood of *A. myriophylla*,[15] and **9** and **10** from the bulbs of *S. sibirica* were according to the literature. These iminosugars were extracted with 50% aqueous MeOH or 50% aqueous EtOH and isolated by chromatography, using a variety of ion-exchange resins such as Amberlite IR-120B (H^+ form), Amberlite CG-50 (NH_4^+ form), CM-Sephadex C-25 (NH_4^+ form), and Dowex 1-X2 (OH^- form). Synthetic compounds **2** and **4** were prepared from D-xylose and D-gulonolactone, according to the literature.[10,17]

The roots (10 kg) of *S. oblonga* were extracted three times with 50% aqueous EtOH (10 L). The extract was applied to a column of Amberlite IR-120B (1.2 L, H^+ form), and the effluent and washings with water were applied to an Amberlite IR-400 J (500 mL, OH^- form) column. After washing with water, the column was eluted with M AcOH and the eluate was concentrated to give a brown syrup (18.8 g). This syrup was further applied to a Dowex 1-X2 (200 mL, OH^- form) and eluted with M AcOH to give a colorless syrup (1.96 g). The syrup was chromatographed over a Dowex 1-X2 (1.5 cm ×90 cm, $HCOO^-$ form) column with water as eluant (fraction size 5 mL). The water eluate was divided into two pools: I (fractions 11–18, 50 mg) and II (fractions 44–55, 58 mg). Each pool was further chromatographed with DEAE-Bio-Gel A (1.5 cm × 90 cm) with water as eluent to give compound **14** (42 mg) from pool I and salacinol (**12**, 15 mg) from pool II. Chemical conversion of salacinol to compound **14** was carried out as follows. Salacinol (5 mg) in 5% HCl methanol solution (1 mL) was heated under reflux for 3 h. The reaction mixture was concentrated and applied to a Dowex 1-X2 (1.0 cm × 19 cm, $HCOO^-$ form) column with water as eluant (fraction size 2 mL). Fractions 2–5 were concentrated to give compound **14** (3.5 mg). 1,4-Anhydro-4-thio-D-arabinitol (ATA, **13**) was prepared in the following way. Salacinol or compound **14** (10 mg) in 1% CH_3ONa methanol solution (2 mL) was heated under reflux for 6 h. The reaction mixture was neutralized with Amberlyst 15 (H^+ form) and then filtered off. The filtrate was concentrated and applied to a short column of Dowex 1-X2 (1.0 cm × 9 cm, OH^- form). The column was washed with water (10 mL) and then eluted with M AcOH (12 mL). The eluate was concentrated to give **13** (4.8 mg). Compound **13** has already been chemically synthesized from D-xylose.[29]

4.1.1. 1,4-Dideoxy-1,4-imino-D-arabinitol (D-AB1) Hydrochloride (1-HCl). $[\alpha]_D$ +34.9° (*c* 1.00, H_2O). HRMS (FAB): *m/z* 134.0817 $[M+H]^+$ ($C_5H_{12}NO_3$ requires 134.0817).

4.1.2. 1,4-Dideoxy-1,4-imino-L-arabinitol (L-AB1) Hydrochloride (2-HCl). $[\alpha]_D$ − 34.6° (*c* 0.37, H_2O). HRMS (FAB): *m/z* 134.0815 $[M+H]^+$ ($C_5H_{12}NO_3$ requires 134.0817).

4.1.3. 2,5-Dideoxy-2,5-imino-D-mannitol (D-DMDP) (3). $[\alpha]_D$ +53.8° (*c* 0.32, H_2O). HRMS (FAB): *m/z* 164.0924 $[M+H]^+$ ($C_5H_{12}NO_3$ requires 164.0923).

4.1.4. 2,5-Dideoxy-2,5-imino-L-mannitol (L-DMDP) (4). $[\alpha]_D$ − 52.7° (*c* 0.28, H_2O). HRMS (FAB): *m/z* 164.0924 $[M+H]^+$ ($C_5H_{12}NO_3$ requires 164.0923).

4.1.5. 1,4-Dideoxy-1,4-imino-D-ribitol (D-DRB) (5). $[\alpha]_D$ +42.0° (*c* 0.53, H_2O). HRMS (FAB): *m/z* 134.0816 $[M+H]^+$ ($C_5H_{12}NO_3$ requires 134.0817).

4.1.6. 1,4-Dideoxy-1,4-imino-D-xylitol (D-DIX) (6). $[\alpha]_D$ +9.4° (*c* 0.35, H_2O). HRMS (FAB): *m/z* 134.0812 $[M+H]^+$ ($C_5H_{12}NO_3$ requires 134.0817).

4.1.7. 1,4-Imino-1,2,4-trideoxy-D-arabinitol (CYB-3) Hydrochloride (7-HCl). $[\alpha]_D$ +25.0° (*c* 1.26, H_2O). HRMS (FAB): *m/z* 118.0867 $[M+H]^+$ ($C_5H_{12}NO_2$ requires 118.0868).

4.1.8. 2,5-Dideoxy-2,5-imino-D-glucitol (D-DIG) (8). $[\alpha]_D$ +26.1° (*c* 0.84, H_2O). HRMS (FAB): *m/z* 164.0923 $[M+H]^+$ ($C_5H_{12}NO_3$ requires 164.0923).

4.1.9. 2,5-Dideoxy-2,5-imino-D-*glycero*-D-*manno*-heptitol (homoDMDP) (9). $[\alpha]_D$ +28.8° (*c* 3.75, H_2O). MS (FAB): *m/z* 194 $[M+H]^+$.

4.1.10. 2,5-Imino-2,5,6-trideoxy-D-*manno*-heptitol (Deoxy-homoDMDP) (10). $[\alpha]_D$ +59.2° (*c* 1.77, H_2O). MS (FAB): *m/z* 178 $[M+H]^+$.

4.1.11. 1,4-Dideoxy-1,4-(hydroxyethyliminiumyl)-D-arabinitol (*N*-hydroxyethyl-D-DB1) (11). $[\alpha]_D$ +26.1° (*c* 0.84, H_2O). HRMS (FAB): *m/z* 164.0923 $[M+H]^+$ ($C_5H_{12}NO_3$ requires 164.0923).

4.1.12. 1,4-Dideoxy-1,4-{(*S*)-[(2S,3S)-2,4-dihydroxy-3-(sulfooxy)butyl]episulfoniumylidene}-D-arabinitol inner salt (Salacinol) (12). $[\alpha]_D$ +5.6° (*c* 0.62, H_2O). NMR data (500 MHz, D_2O): ^1H, δ 5.27 (dt, 1H, *J* 3.7, 7.7 Hz, H-3′), 5.12 (s, 2H, H-2, H-3), 5.01 (br dt, 1H, H-2′), 4.80 (dd, 1H, *J* 4.6, 12.8 Hz, H-1′b), 4.70 (br t, 1H, H-4), 4.63 (dd, 1H, *J* 4.1, 12.8 Hz, H-1′a), 4.62 (dd, 1H, *J* 3.7, 11.9 Hz, H-4′b), 4.55 (dd, 1H, *J* 6.4, 11.9 Hz, H-5b), 4.53 (dd, 1H, *J* 7.3, 11.9 Hz, H-5a), 4.38 (dd, 1H, *J* 3.7, 11.9 Hz, H-4′a), 4.32 (br d, 2H, H-1a, H-1b); ^{13}C, δ 79.3 (C-3′), 79.2 (C-3), 78.4 (C-2), 72.4 (C-4), 67.6 (C-2′), 62.3 (C-4′), 60.2 (C-5), 52.8 (C-1′), 50.5 (C-1). HRMS (FAB): *m/z* 335.0470 $[M+H]^+$ ($C_9H_{19}O_9S_2$ requires 335.0471).

4.1.13. 1,4-Anhydro-4-thio-D-arabinitol (D-ATA) (13). $[\alpha]_D$ +37.6° (*c* 0.22, H_2O). NMR data (500 MHz, D_2O): ^1H, δ 4.23 (ddd, 1H, *J* 6.0, 6.4, 6.9 Hz, H-2), 3.92 (t, 1H, *J* 6.4 Hz, H-3), 3.86 (dd, 1H, *J* 5.0, 11.5 Hz, H-5b), 3.67 (dd, 1H, *J* 6.9, 11.5 Hz, H-5a), 3.30 (ddd, 1H, *J* 5.0, 6.4, 6.9 Hz), 3.05 (dd, 1H, *J* 6.0, 11.0 Hz, H-1b), 2.77 (dd, 1H, *J* 6.9, 11.0 Hz, H-1a); ^{13}C, δ 81.8 (C-3), 79.2 (C-2), 66.1 (C-5), 53.6 (C-4), 34.8 (C-1). MS (FAB): *m/z* 151 $[M+H]^+$.

4.1.14. Compound 14 (1,4-Dideoxy-1,4-{(*S*)-[(2S,3S)-2,4-dihydroxy-3-butyl]episulfoniumylidene}-D-arabinitol inner salt). $[\alpha]_D$ +7.3° (*c* 2.06, H_2O). NMR data (500 MHz, D_2O): ^1H, δ 5.06 (br ddd, 1H, H-2), 4.87 (dd, 1H, *J* 1.6, 2.5 Hz, H-3), 4.68 (ddd, 1H, J 3.4, 6.2, 8.7 Hz, H-2′), 4.60 (br ddd, 1H, H-4), 4.42 (dd, 1H, J 3.4, 13.0 Hz, H-1′b), 4.38 (dd, 1H, *J* 5.5, 11.9 Hz, H-5b), 4.30 (dd, 1H, *J* 9.6, 11.9 Hz, H-5a), 4.27 (dd, 1H, *J* 8.7, 13.0 Hz, H-1′a), 4.21 (br d, 2H, H-1a, H-1b), 4.19 (ddd, 1H, *J* 4.4, 5.0, 6.2 Hz, H-4′a), 4.16 (dd, 1H, *J* 4.4, 11.2, H-4′b), 4.10 (dd, 1H, *J* 5.0, 11.2 Hz, H-4′a); ^{13}C, δ 78.3 (C-3), 78.1 (C-2), 74.5 (C-3′), 72.5 (C-4), 68.4 (C-2′), 63.0 (C-4′), 59.9 (C-5), 51.1 (C-1′), 50.9 (C-1). HRMS (FAB): *m/z* 255.0900 $[M+H]^+$ ($C_9H_{19}O_9S_2$ requires 255.0902).

5. Experimental

5.1. General experimental procedures

The purity of samples was checked by HPTLC on Silica Gel 60 F_{254} (E. Merck) using the solvent systems PrOH–AcOH–H_2O (4:1:1), and a chlorine–*o*-tolidine reagent for iminosugars **1**–**11** and a phosphomolybdate reagent for thiosugars **12**–**14** were used for detection. Optical rotations were measured with a Jasco DIP-370 digital polarimeter (Tokyo, Japan). ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a JEOL ECP-500 spectrometer (Tokyo, Japan). Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP) in D_2O or tetramethylsilane (TMS) in C_5D_5N or C_5D_5N–D_2O (5:1) as internal standard. FABMS were measured using glycerol as a matrix on a JEOL JMS-700 spectrometer.

5.2. Biological assays

α-Glucosidases (from rice, assayed at pH 5.0; from yeast, pH 6.8), *p*-nitrophenyl α-D-glucoside, and disaccharides were purchased from Sigma Chemical Co. Brush border membranes prepared from rat small intestine according to the method of Kessler et al.[30] were used as the source of rat intestinal glucosidases. The activities of rice α-glucosidase and rat intestinal α-glucosidases were determined using the appropriate disaccharides as substrates. The released D-glucose was determined colorimetrically using the Glucose CII-test Wako (Wako Pure Chemical Ind.). The activity of yeast α-glucosidase was determined using *p*-nitrophenyl α-D-glucoside as substrate. The reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm. The cell lysate of normal human fibroblasts (GM00498B) was used as the source of lysosomal α-glucosidase. The activity was determined using 4-methylumbelliferyl α-D-glucoside (Sigma Chemical Co.) as substrate. Liberated 4-methylumbelliferone was measured (excitation 362 nm, emission 450 nm) with a F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Glycogen phosphorylase activity was assayed in the direction of glycogen breakdown from the rate of NADPH formation in an assay coupled to phosphoglucomutase and

glucose 6-phosphate dehydrogenase.[31] Amylo-1,6-glucosidase was prepared from rabbit skeletal muscle according to the literature[31] and assayed using 6-*O*-α-D-glucosyl-α-cyclodextrin (Wako Pure Chemical Ind.) as substrate.[32,33] The released D-glucose was determined colorimetrically using the Glucose CII-test Wako.

Maltase inhibitory activity using Caco-2 cell model system was measured according to the literature.[34] Caco-2 cells were cultured on polyethylene terephthalate membranes (Falcon, pore size: 0.4 μm, pore density 1.6 in a 6-well plate. After cells reached 100% confluence (5 days), cells were washed three times 2 mL of phosphate-buffered saline (PBS). The culture medium in the apical chamber was replaced with a reaction mixture consisting of inhibitor solution (0.2 mL) and 28 mM maltose solution in PBS (0.8 mL). To the basal chamber, 1 mL of PBS instead of the culture medium was added. The assay plate was then incubated at 37 °C in 5% CO_2 atmosphere for 2 h. After incubation, the released D-glucose in the apical chamber was determined colorimetrically using the Glucose CII-test Wako.

References and notes

1. Asano, N. *Glycobiology* **2003**, *13*, 93R.
2. Firth, R. G.; Bell, P. M.; Hansen, H. M.; Rizza, R. A. *J. Clin. Invest.* **1986**, *77*, 1525.
3. Ferrannini, E.; Simonson, D. C.; Katz, L. D.; Reichars, G., Jr.; Bevilacqua, S.; Barrett, E. J.; Olsson, M.; DeFronzo, R. A. *Metab. Clin. Exp.* **1988**, *37*, 79.
4. Mitrakou, A.; Kelley, D.; Veneman, T.; Jenssen, T.; Pangburn, T.; Reilly, J.; Gerich, J. *Diabetes* **1990**, *39*, 1381.
5. Martin, J. L.; Veluraja, K.; Ross, K.; Johnson, L. N.; Fleet, G. W. J.; Ramsden, N. G.; Bruce, I.; Orchard, M. G.; Oikonomakos, N. G.; Papageorgiou, A. C.; Leonidas, D. D.; Tsitoura, H. S. *Biochemistry* **1991**, *30*, 10101.
6. Fosgerau, K.; Westergaard, N.; Quistorff, B.; Grunner, N.; Kristiansen, M.; Lundgren, K. *Arch. Biochem. Biophys.* **2000**, *380*, 274.
7. Andersen, B.; Rassov, A.; Westergaard, N.; Lundgren, K. *Biochem. J.* **1999**, *342*, 545.
8. Asano, N.; Ikeda, K.; Yu, L.; Kato, A.; Takebayashi, K.; Adachi, I.; Kato, I.; Ouchi, H.; Takahata, H.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2005**, *16*, 223.
9. Welter, A.; Jadot, J.; Dardenne, G.; Marlier, M.; Casimir, J. *Phytochemistry* **1976**, *15*, 747.
10. Yu, C.-Y.; Asano, N.; Ikeda, K.; Wang, M.-X.; Butters, T. D.; Wormald, M. R.; Dwek, R. A.; Winters, A. L.; Nash, R. J.; Fleet, G. W. J. *Chem. Commun.* **2004**, 1936.
11. Asano, N.; Yasuda, K.; Kizu, H.; Kato, A.; Fan, J.-Q.; Nash, R. J.; Fleet, G. W. J.; Molyneux, R. J. *Eur. J. Biochem.* **2001**, *268*, 35.
12. Yasuda, K.; Kizu, H.; Yamashita, T.; Kameda, Y.; Kato, A.; Nash, R. J.; Fleet, G. W. J.; Molyneux, R. J.; Asano, N. *J. Nat. Prod.* **2002**, *65*, 198.
13. Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. *J. Med. Chem.* **1994**, *37*, 3701.
14. Kato, A.; Kano, E.; Adachi, I.; Molyneux, R. J.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J.; Wormald, M. R.; Kizu, H.; Ikeda, K.; Asano, N. *Tetrahedron: Asymmetry* **2003**, *14*, 325.
15. Asano, N.; Yamauchi, T.; Kagamifuchi, K.; Shimizu, N.; Takahashi, S.; Takatsuka, H.; Ikeda, K.; Kizu, H.; Chuakul, W.; Kettawan, A.; Okamoto, T. *J. Nat. Prod.* **2005**, *68*, 1238.
16. Yamashita, T.; Yasuda, K.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J.; Asano, N. *J. Nat. Prod.* **2002**, *65*, 1875.
17. Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. *Tetrahedron Lett.* **1985**, *26*, 3127.
18. Yoshikawa, M.; Murakami, T.; Shimada, H.; Matsuda, H.; Yamahara, J.; Tanabe, G.; Muraoka, O. *Tetrahedron Lett.* **1997**, *38*, 8367.
19. Ghavami, A.; Johnston, B. D.; Pinto, B. M. *J. Org. Chem.* **2001**, *66*, 2312.
20. Yoshikawa, M.; Murakami, T.; Yashiro, K.; Matsuda, H. *Chem. Pharm. Bull.* **1998**, *46*, 1339.
21. Rossi, E. J.; Sim, L.; Kuntz, D. A.; Hahn, D.; Johnston, B. D.; Ghavami, A.; Szczepina, M. G.; Kumar, N. S.; Sterchi, E. E.; Nichols, B.; Pinto, B. M.; Rose, D. R. *FEBS J.* **2006**, *273*, 2673.
22. Oikonomakos, N. G.; Tiraidis, C.; Leonidas, D. D.; Zographos, S. E.; Kristiansen, M.; Jessen, C. U.; Nørskov-Lauritsen, L.; Agius, L. *J. Med. Chem.* **2006**, *49*, 5687.
23. Tanabe, G.; Yoshikai, K.; Hatanaka, T.; Yamamoto, M.; Shao, Y.; Minematsu, T.; Muraoka, O.; Wang, T.; Matsuda, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2007**, *15*, 3926.
24. Cohen, F. E.; Kelly, J. W. *Nature* **2003**, *426*, 905.
25. de Duve, C. In *Lysosome*; de Reuck, A. V. S., Cameron, M. P., Eds.; Chuchill: London, 1963; p 1.
26. Kornfeld, S.; Mellman, I. *Annu. Rev. Cell Biol.* **1989**, *5*, 483.
27. Fan, J.-Q. *Trends Pharmacol. Sci.* **2003**, *24*, 355.
28. Yu, L.; Ikeda, K.; Kato, A.; Adachi, I.; Godin, G.; Compain, P.; Martin, O. R.; Asano, N. *Bioorg. Med. Chem.* **2006**, *14*, 7736.
29. Yuasa, H.; Kajimoto, T.; Wong, C.-H. *Tetrahedron Lett.* **1994**, *35*, 8243.
30. Kessler, M.; Acuto, O.; Strelli, C.; Murer, H.; Semenza, G. A. *Biochem. Biophys. Acta* **1978**, *506*, 136.
31. Taylor, C.; Cox, A. J.; Kernohan, J. C.; Cohen, P. *Eur. J. Biochem.* **1975**, *51*, 105.
32. Tayler, P. M.; Whelan, W. J. *Arch. Biochem. Biophys.* **1966**, *113*, 500.
33. Tabata, S.; Hizukuri, S. *Eur. J. Biochem.* **1992**, *206*, 345.
34. Hansawasdi, C.; Kawabata, J. *Fitoterapia* **2006**, *77*, 568.