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Docking and SAR studies of D- and L-isofagomine isomers as human β-glucocerebrosidase inhibitors

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ABSTRACT

We report the structure–activity relationship of a series of D-, and L-isofagomine and fagomine isomers as glycosidase inhibitors. Our study revealed that a positive charge at the anomeric position of D-isofagomines enhanced the potency toward β-glycosidases, while the epimerization at the C3 OH group drastically reduced their inhibitory potency by over three orders of magnitude. Furthermore, D-3,4-di-*epi*-isofagomine abolished their inhibition activities against all enzymes. L-Isofagomine was also a fairly potent inhibitor of human β-glucocerebrosidase, with an IC_{50} value of 8.7 μM. A molecular docking study revealed that the positions and orientations of the piperidine ring of D-3-*epi*-isofagomine in the binding site was similar to that of D-isofagomine, while D-3-*epi*-isofagomine missed the hydrogen bond interactions between Asp127 and the 3-OH group and between Trp179 and the 3-OH group. Furthermore, the top 10 docking models ranked by IFDscore suggested that D-3,4-di-*epi*-isofagomine can not bind to β-glucocerebrosidase at a stable interaction mode. These results provide an insight into the structural requirements of isofagomine isomers for developing a new type of pharmacological chaperone for Gaucher disease.

1. Introduction

Iminosugars are sugar mimics with a nitrogen atom in place of the ring oxygen of monosaccharides. The biological properties can be explained by their structural resemblance to the terminal sugar moiety in the natural substrates. Glycosidases are involved in several important biological processes, such as intestinal digestion, post-translational processing of the glycoproteins, lysosomal catabolism of glycoconjugates, and quality-control systems in the endoplasmic reticulum (ER) and the ER-associated degradation (ERAD) mechanism. Furthermore, the scope of their biological activity has been extended to the inhibition of a number of enzymes such as glycosyltransferases and glycogen phosphorylases. Hence, there is great interest in utilizing these inhibitors to discover novel therapeutic agents. To date, around 200 natural products are known that may be described as carbohydrate mimics in which the ring oxygen of a sugar is replaced by nitrogen.[1] Recently, modifications of the core naturally-occurring iminosugars have been developed to give sub-nanomolar inhibition of α-L-fucosidases,[2] α-L-rhamnosidases,[3] α-*N*-acetyl-galactosaminidases.[4] These designed inhibitors are mainly focused on the enantiomers of the natural D-iminosugars. We have previously reported that these synthetic enantiomers were frequently powerful glycosidase inhibitors.[5,6] For example, the unnatural enantiomer 1,4-dideoxy-1,4-imino-L-arabinitol (LAB) is a more potent and more specific inhibitor of α-glucosidases than the natural product 1,4-dideoxy-1,4-imino-D-arabinitol (DAB). Furthermore, in the course of designing unnatural iminosugars, we found 1,4-dideoxy-2-hydroxymethyl-1,4-imino-D-threitol (isoDAB), which is a pyrrolidine bearing a branched carbon chain, is a potent and specific inhibitor of rice α-glucosidase and rat intestinal maltase, with K_i value of 4 and 6 μM, respectively.[7] It is therefore necessary to elucidate the structural requirements for the design of selective glycosidase inhibitors involving both enantiomers.

In this paper, we focused on the 1-*N*-iminosugar isofagomine in which a nitrogen atom is at the anomeric position of a monosaccharide. There are some reports and reviews on in vitro glycosidase inhibition by D-isofagomine and its derivatives.[8–10] D-Isofagomine was reported as the most potent inhibitor of β-glucocerebrosidase in vitro and the most effective pharmacological chaperone capable of increasing residual β-glucocerebrosidase activity in N370S fibroblasts. Intracellular β-glucocerebrosidase activity increased approximately twofold when cells were incubated with D-isofagomine. D-Isofagomine (Plicera) has proceeded to Phase II clinical tri-

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als for the treatment of Gaucher disease but it has been finally withdrawn from clinical trials due to lack of efficacy. To get closer to therapeutic application, we decided to further systematically study the biological properties of the enantiomers of isofagomine and other diastereomers of isofagomine. Herein, we report the syntheses of both enantiomers of isofagomine, 3-*epi*-isofagomine, 4-*epi*-isofagomine, 3,4-di-*epi*-isofagomine and systematic studies of their glycosidase inhibitory activities. An additional aim of ours was to synthesize both enantiomers of fagomine isomers and investigated the structure–activity relationships of isofagomine and fagomine isomers from the viewpoint of the position of the nitrogen atom and different configurations of OH groups. Furthermore, we investigate the molecular docking properties of all D-isofagomine isomers toward β-glucocerebrosidase by using Induced Fit Docking score.

2. Results and discussion

2.1. Preparation of D- and L-isofagomine isomers

In general, most syntheses of iminosugars are performed by transforming carbohydrate derivatives as the chiral starting materials. However, this procedure is not always suited for obtaining all stereoisomers. Accordingly, our synthesis began with an appropriate common chiral building block readily available from starting materials.

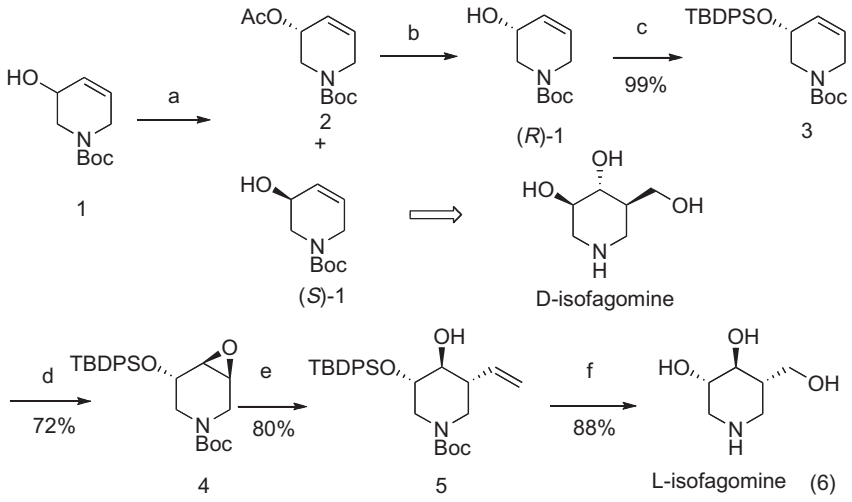
Both enantiomers of isofagomine (**6**) were synthesized in a highly stereocontrolled mode from *N*-Boc-5-hydroxy-3-piperidene **1** as a common chiral building block according to our previous report.[11] The lipase-catalyzed trans-esterification of **1** with vinyl acetate gave the acetate **2** along with the unreacted alcohol (*S*)-**1**, The enzymatic hydrolysis of the acetate **2** with the same lipase in 0.1 M phosphate buffer afforded the enantiometic alcohol (*R*)-**1**. With both enantiomers **1** in hand, (*R*)-**1** was transformed to L-isofagomine (**6**) via stereoselective epoxidation and regioselective ring-cleavage with higher ordered cuprates in a highly stereocontrolled manner as shown in Scheme 1. Other isomers L-3-*epi*-isofagomine (**10**), L-4-*epi*-isofagomine (**11**), and L-3,4-di-*epi*-isofagomine (**14**) were prepared from 5-hydroxymethyl-3-piperidene (**8**), which was obtained by [2,3]-Stille–Wittig rearrangement of *O*-tributylstannylmethyl-3-piperidene **7** derived from readily available (*R*)-**1**. This rearrangement of **7** by transmetallation using *n*-BuLi proceeded smoothly in a nonpolar solvent such as *n*-pentane with no racemization. Thus, the prepared hydroxymethylpiperidine **8** was transformed by dihydroxylation, and epoxidation into stereoisomers **10**, **11**, and **14** of isofagomine as exhibited in Scheme 2.[12] D-Forms of **6**, **10**, **11**, and **14** were prepared from (*S*)-**1** according to procedures similar to those described above.

2.2. Inhibition effects of D- and L-isofagomine isomers on various glycosidases

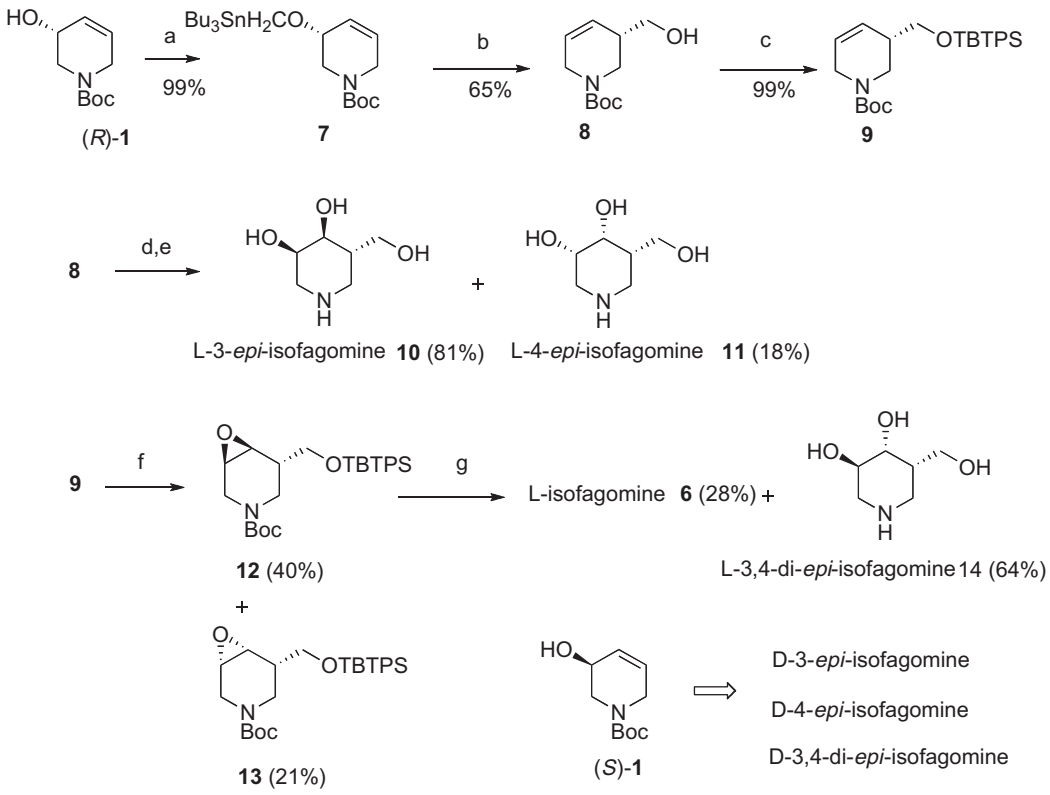
The IC_{50} values of the D- and L-enantiomers of isofagomine isomers toward a variety of glycosidases are shown in Table 1. D-Isofagomine is known to be a potent inhibitor of all types of β-glucosidases. Especially, D-isofagomine was an extremely powerful inhibitor of human lysosomal β-glucosidase (β-glucocerebrosidase), with an IC_{50} value of 0.063 μM. Furthermore, its enantiomer L-isofagomine was also a fairly potent inhibitor of this enzyme, with IC_{50} value of 8.7 μM. L-Isofagomine is a better inhibitor than D-isofagomine of rat intestinal sucrase. In the present study, D-isofagomine was also found to be a potent inhibitor of bovine liver β-galactosidase and rat intestinal lactase, with IC_{50} values of 3.6 and 0.12 μM, respectively. In addition, D-4-*epi*-isofagomine as a D-galactose-type 1-*N*-iminosugar was a potent inhibitor of both rat intestinal cellobiase and lactase, with IC_{50} values of 4.5 and 0.51 μM, respectively. These results suggested that the stereochemistry at the C4 OH group of 1-*N*-iminosugars was not strictly distinguished by β-glucosidases and β-galactosidases. To date, around 200 natural products are known that may be described as carbohydrate mimics. While these naturally-occurring iminosugars are analogues of monosaccharides in which the ring oxygen has been replaced by nitrogen atom, synthesized 1-*N*-iminosugars have a nitrogen atom at the anomeric position of a monosaccharide ring. Therefore, we compared the inhibition spectrum and potency of isofagomine and fagomine isomers against various glycosidases from the viewpoint of the position of the nitrogen atom and different configurations of OH groups (Fig. 1).

2.3. Preparation of D- and L-fagomine isomers

Fagomine was first isolated from buckwheat seeds (*Fagopyrum esulentum* Moenchi) and subsequently isolated from seeds of *Castanospermum australe* (Leguminosae).[13,14] We have previously



Scheme 1. Reagents and conditions: (a) vinyl acetate, lipase PS (immobilized on ceramic particles), *t*-BuOMe; (b) lipase PS (immobilized on ceramic particles), 0.1 M phosphate buffer (pH 7), acetone; (c) TBDPSCl, imidazole, cat. DMAP, CH_2Cl_2; (d) Oxone, aq Na_2EDTA, $NaHCO_3$, CF_3COCH_3, CH_3CN; (e) $(CH_2{=}CH)_2CuCNLi_2$), BF_3, OEt_2, Et_2O; (f) (1) cat. OsO_4, $NaIO_4$, 50% EtOH; (2) $NaBH_4$, 50% EtOH; (3) 10% HCl, dioxane; (4) NH_4OH.



Scheme 2. Reagents and conditions: (a) Bu_3SnCH_2Cl, KH, THF; (b) *n*-BuLi, *n*-pentane; (c) TBDPSCl, imidazole, cat. DMAP, CH_2Cl_2; (d) cat. OsO_4, NMO; (e) (1) concd HCl, CH_3OH, H; (2) NH_4OH; (f) Oxone, CF_3COCH_3, Na_2EDTA, $NaHCO_3$, CH_3CN; (g) (1) 0.3 M KOH, 1,4-dioxane; (2) 6 N HCl; (3) NH_4OH.

Table 1
Concentration of D- and L-isofagomine isomers giving 50% inhibition of various glycosidases

Enzyme	IC_{50} (μM)							
	Isofagomine		3-*epi*-Isofagomine		3,4-di-*epi*-Isofagomine		4-*epi*-Isofagomine	
	D	L	D	L	D	L	D	L
α-Glucosidase								
Rice	NI[a]	NI	NI	NI	NI	NI	NI	NI
Rat intestinal maltase	653	NI	NI	NI	NI	NI	NI	NI
Rat intestinal isomaltase	290	NI	NI	NI	NI	NI	NI	NI
Rat intestinal sucrase	531	34	NI	NI	NI	NI	NI	NI
β-Glucosidase								
Almond	0.98	236	443	NI	NI	NI	3.2	983
Rat intestinal cellobiase	0.30	110	334	NI	912	NI	4.5	607
Bovine liver	31	611	NI	NI	NI	676	63	NI
Human lysosome	0.063	8.7	113	NI	NI	NI	54	NI
α-Galactosidase								
Coffee beans	NI	NI	NI	NI	NI	NI	144	450
β-Galactosidase								
Bovine liver	3.6	821	NI	NI	NI	389	21	NI
Rat intestinal lactase	0.12	20	52	469	211	646	0.51	90
α-Mannosidase								
Jack beans	NI	NI	NI	NI	NI	NI	NI	NI
β-Mannosidase								
snail	NI	NI	NI	NI	NI	NI	NI	NI
α-L-Fucosidase								
Bovine epididymis	NI	NI	NI	NI	NI	NI	NI	NI
α-L-Rhamnosidase								
P. decumbens	NI	NI	NI	NI	NI	NI	NI	NI

[a] NI: no inhibition (less than 50% inhibition at 1000 μM).

reported that fagomine and its 3-OH epimer occurs abundantly in the leaves and roots of *Xanthocercis zambesiaca*, growing in dry forest and woodland of southern Africa.[15] Fagomine and its 4-*O*-β-D-glucopyranoside were found to have a potent antihyperglycemic

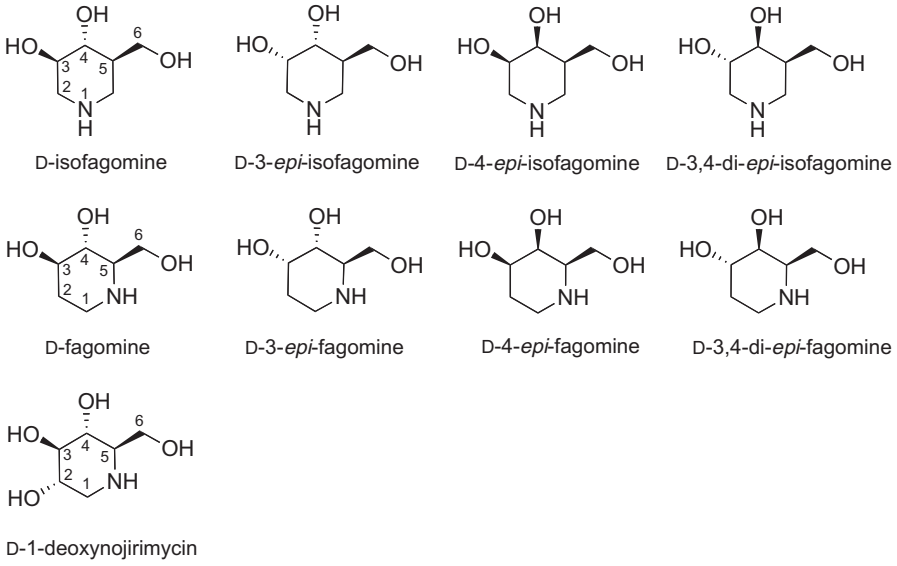


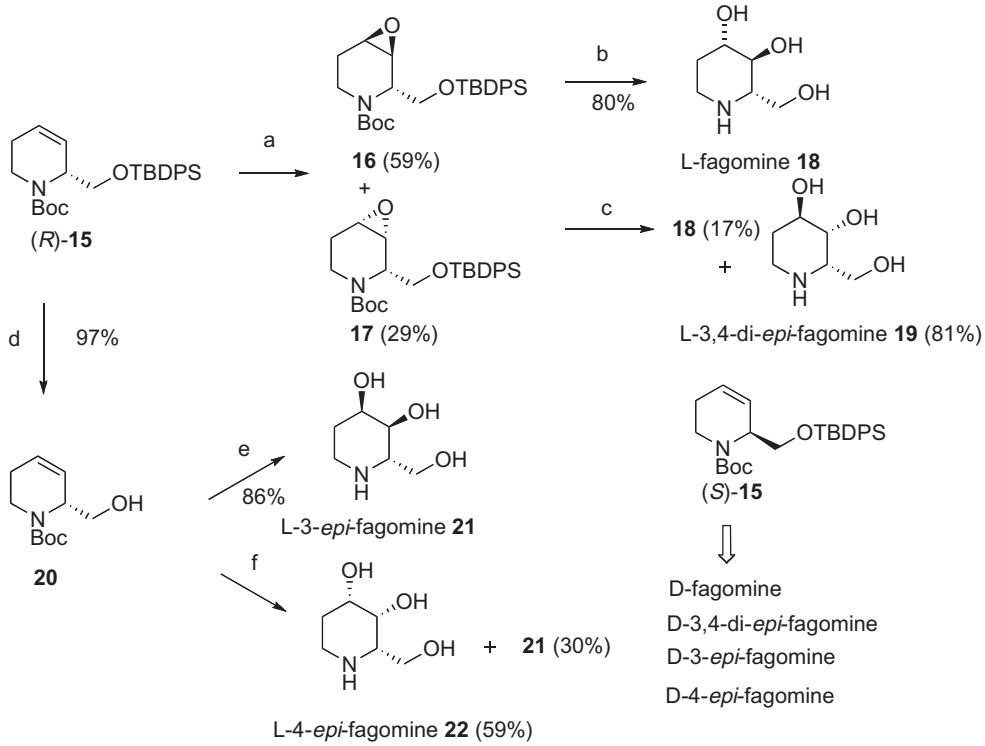
Figure 1. Structural relationship of D-isofagomine isomers, D-fagomine isomers, and D-1-deoxynojirimycin.

effect in streptozotocin-induced diabetic mice and to potentiate markedly insulin release.[16]

The synthesis of D-forms of fagomine and its stereoisomers from 6-silyloxymethylpiperidines (*S*)-**15** as Garner aldehyde-derived chiral building blocks was reported by us.[17] The preparation of L-forms was conducted by almost the same procedure as that reported as shown in Scheme 3. Epoxidation of (*R*)-**15** gave two stereoisomers **16** and **17**. *trans*-Epoxide **16** was hydrolyzed with acid to provide L-fagomine (**18**) as a single product. On the other hand, hydrolysis of *cis*-epoxide **17** with base provided L-3,4-di-*epi*-fagomine **19** as a major product together with a small amount of **18**. Desilylation of **15** afforded hydroxymethylpiperidine **20**. Dihydroxylation of **20** with a modified Upjohn condition gave L-3-*epi*-fagomine **21** as a single diastereomer, while the homoallylic alcohol-directed of **20** with OsO_4–TMEDA complex[18] provided L-4-*epi*-fagomine **22** as a major diastereomer accompanied by **21**.

2.4. Inhibition effects of D- and L-fagomine isomers on various glycosidases

As shown in Table 2, D-fagomine is a moderate inhibitor of rat intestinal isomaltase and sucrase with IC_{50} values of 37 and 34 μM, respectively. However, the potency of inhibition against



Scheme 3. Reagents and conditions: (a) Oxone, CF_3COCH_3, $NaHCO_3$, aq Na_2, EDTA, CH_3CN; (b) (1) H_2SO_4, 1,4-dioxane, H_2O; (2) Amberlite IRA-410; (c) KOH, 1,4-dioxane, H_2O; (2) HCl, CH_3OH; (3) Amberlite IRA-410;(d) TBAF, THF; (e) (1) cat. $K_2OsO_4{\cdot}2H_2O$, NMO, H_2O; (2) 10% HCl; (3) Amberlite IRA-410; (f) (1) OsO_4, TMEDA, CH_2Cl_2; (2) 35% HCl, MeOH; (3) Amberlite IRA-410.

these enzymes was much weaker than the conventional glucose-type iminosugar D-1-deoxynojirimycin, which showed IC_{50} values of 0.65 and 0.51 μM. This behavior is similar to that of D-4-*epi*-fagomine which was found to be a potent and selective inhibitor of coffee bean α-galactosidase with IC_{50} values of 8 μM but this value is over three orders of magnitude weaker than the galactose-type iminosugar D-1-deoxygalactonojirimycin. We have previously reported that L-1-deoxygalactonojirimycin, which can be regarded as the C6 hydroxylated derivative of L-1-deoxyfuconojirimycin, was a potent competitive inhibitor of bovine epididymis α-L-fucosidase with IC_{50} and K_i values of 0.63 and 0.080 μM, respectively.[6] However, this study revealed that L-4-*epi*-fagomine, which can be regarded as the 2-deoxy derivative of L-1-deoxygalactonojirimycin was completely inactive against this enzyme. These results suggested that the presence of the C2 OH group in the iminosugar piperidine ring is an essential feature for recognition and strong binding by the active site of glycosidases. Interestingly, while the structure of the 1-*N*-iminosugars does not have a C2 OH group, isofagomine showed potent inhibition against β-glucosidases and β-galactosidases. However, the epimerization at the C3 OH group in D-isofagomine drastically reduced its inhibitory potency by over three orders of magnitude against β-glucosidases and β-galactosidases (Table 1). In contrast, the epimerization at the C3 OH group in D-fagomine enhanced the inhibitory potency toward these enzymes and abolished inhibition toward α-glucosidases. From the results of these structure–activity relationships, we concluded that a positive charge at the anomeric position shifts the inhibition spectrum from α-glycosidases to β-glycosidases. Stereochemistry at the C4 OH group of 1-*N*-iminosugars was not strictly distinguished by β-glucosidases and β-galactosidases. Moreover, the C3 OH group in isofagomine is a dominant factor for the inhibition of these enzymes. In addition, 3,4-di-*epi*-isofagomine abolished their inhibition activities against all enzymes.

2.5. Induced Fit Docking study of D-isofagomine isomers against human β-glucocerebrosidase

Docking models generated from Induced Fit Docking (Schrödinger Suite 2009, Schrödinger, LLC) were ranked using IFDscore. The top 10 docking models ranked by IFDscore were examined. Interestingly, the variety of poses of ligands, which were extracted from the top 10 ranked docking models for D-isofagomine and D-isofagomine isomers, became increased as the activities of those compounds became weaker. The averaged value of RMSD (Root Mean Square Deviation) between top 1 pose and the other pose was 0.54 Å for D-isofagomine, 1.07 Å for D-4-*epi*-isofagomine, 1.26 Å for D-3-*epi*-isofagomine, and 2.23 Å for D-3,4-di-*epi*-isofagomine) (Fig. 2). The variety of poses for D-3,4-di-*epi*-isofagomine was very large so that a definite binding mode could not be decided. This result suggests that D-3,4-di-*epi*-isofagomine can not bind to β-glucocerebrosidase in a stable interaction mode so that D-3, 4-di-*epi*-isofagomine hardly remains in the binding site of β-glucocerebrosidase.

Top 10 ranked docking models of D-isofagomine, D-4-*epi*-isofagomine and D-3-*epi*-isofagomine were minimized by an OPLS2005 force field and then interaction energies between β-glucocerebrosidase and the ligand were calculated. The best docking model of D-isofagomine was selected based on the interaction energy and was compared with the crystal structure of the β-glucocerebrosidase–D-isofagomine complex. The binding pose in the best docking model of D-isofagomine was almost identical to the binding conformation in the crystal structure of β-glucocerebrosidase–D-isofagomine complex (PDB code: 2NSX) (RMSD: 0.36 Å). In the crystal structure of β-glucocerebrosidase–D-isofagomine complex, the hydroxyl groups of D-isofagomine interact with Asp127, Trp179, Trp381, and Asn396 and the imino group is stabilized by Glu235 and Glu340.[19] The hydrogen bonds formed in the crystal structure of β-glucocerebrosidase–D-isofagomine complex were reproduced successfully in our docking model. These results showed that the

Table 2
Concentration of D- and L-fagomine isomers giving 50% inhibition of various glycosidases

Enzyme	IC_{50} (μM)									
	Fagomine		3-*epi*-Fagomine		3,4-di-*epi*-Fagomine		4-*epi*-Fagomine		1-Deoxynojirimycin	
	D	L	D	L	D	L	D	L	D	L
α-Glucosidase										
Rice	320	NI[a]	120	188	NI	980	NI	NI	0.03	4.3
Rat intestinal maltase	113	NI	NI	357	NI	668	NI	NI	0.65	28
Rat intestinal isomaltase	37	NI	NI	NI	NI	NI	NI	NI	0.65	150
Rat intestinal sucrase	34	NI	NI	225	NI	NI	NI	NI	0.51	18
β-Glucosidase										
Almond	NI	NI	120	NI	NI	NI	NI	NI	80	980
Rat intestinal cellobiase	NI	NI	39	809	NI	NI	NI	NI	327	NI
Bovine liver	250	NI	5.6	NI	NI	NI	NI	NI	NI	NI
Human lysosome	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
α-Galactosidase										
Coffee beans	NI	NI	NI	NI	NI	NI	8.0	NI	880	NI
β-Galactosidase										
Bovine liver	38	NI	3.0	960	NI	NI	NI	NI	NI	560
Rat intestinal lactase	NI	NI	4.3	122	NI	NI	NI	NI	49	317
α-Mannosidase										
Jack beans	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
β-Mannosidase										
Snail	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
α-L-Fucosidase										
Bovine epididymis	NI	NI	NI	260	NI	NI	NI	NI	NI	NI
α-L-Rhamnosidase										
P. decumbens	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

[a] NI: no inhibition (less than 50% inhibition at 1000 μM).

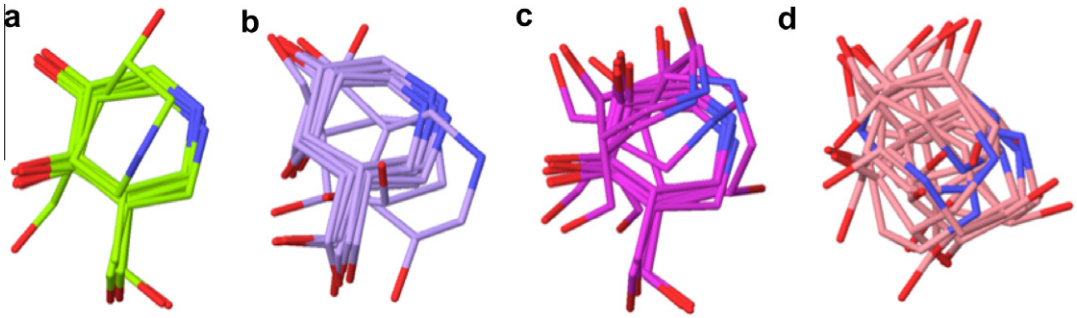


Figure 2. Ligand poses extracted from top 10 docking models ranked by IFDscore. (a) D-isofagomine; (b) D-4-*epi*-isofagomine; (c) D-3-*epi*-isofagomine; (d) D-3,4-di-*epi*-isofagomine.

method using Induced Fit Docking could reproduce the native structure of the β-glucocerebrosidase–ligand complex.

The best docking models for D-3-*epi*-isofagomine and D-4-*epi*-isofagomine were selected and decided as the native docking models that are close to the crystal structures based on the interaction energy. The binding poses were compared between D-isofagomine, D-3-*epi*-isofagomine, and D-4-*epi*-isofagomine. Positions and orientations of the piperidine rings of D-3-*epi*-isofagomine and D-4-*epi*-isofagomine in the binding site were similar to that of D-isofagomine. Orientations of the hydroxyl group of the chiral inversions were only different between D-isofagomine, D-3-*epi*-isofagomine, and D-4-*epi*-isofagomine (Fig. 3). Hydrogen bonds interactions of D-3-*epi*-isofagomine and D-4-*epi*-isofagomine with β-glucocerebrosidase were observed. For D-4-*epi*-isofagomine, one of seven hydrogen bonds, that is, a hydrogen bond interaction between Trp381 of β-glucocerebrosidase and 4-OH group of D-4-*epi*-isofagomine, was absent. For D-3-*epi*-isofagomine, two of seven hydrogen bonds, that is, the hydrogen bond interactions between Asp127 and 3-OH group and between Trp179 and 3-OH group, were missed (Fig. 4). It is likely that one of the reasons for the low activities of D-3-*epi*-isofagomine and D-3,4-di-*epi*-isofagomine is due to a lack of the hydrogen bond interaction between the 3-OH group of each compound and β-glucocerebrosidase (Asp127 and Trp179) caused by the chirality inversion. These results suggested the importance of interactions between the 3-OH group of D-isofagomine and β-glucocerebrosidase.

3. Conclusion

A large number of iminosugars have been isolated from plants and microorganisms.[1] Such sugar mimics have attracted considerable interest because of their ability to effectively and specifically inhibit various glycosidases that are integral to a wide range of important biological processes.[20] Consequently, extensive efforts have been made in recent years to developing methodologies for the asymmetric syntheses of iminosugars. However, the focus of many of these syntheses has been on D-type iminosugar derivatives. On the other hand, there are few reports of systematic studies of the biological properties of the L-enantiomers of iminosugars. In this study, we have synthesized both enantiomers of isofagomine and fagomine isomers in a highly stereocontrolled manner. D-Isofagomine was an extremely powerful inhibitor of human lysosomal β-glucocerebrosidase, with an IC_{50} value of 0.063 μM. Interestingly, its enantiomer L-isofagomine was also a fairly potent inhibitor of this enzyme, with IC_{50} value of 8.7 μM. However, the inhibition potency of the L-form was 10–100 times weaker than its miller image D-form. This behavior is similar to that observed for D-, and L-1-deoxynojirimycin isomers.[6] From the structure–activity relationships of D-isofagomine isomers, the stereochemistry at the C4 OH group was not strictly distinguished by β-glucosidases and β-galactosidases. In contrast, the epimerization at the C3 OH group in D-isofagomine drastically reduced its inhibitory potency over three orders of magnitude on these enzymes (Table 1). Furthermore, D-3,4-di-*epi*-isofagomine abolished their inhibition activities against all enzymes. To understand the structural basis of the interaction of D-isofagomine isomers with β-glucocerebrosidase, we built a docking model generated from Induced Fit Docking by using IFDscore. As a result, we found the averaged values of RMSD between top 1 poses and the other poses for D-3,4-di-*epi*-isofagomine (2.23 Å) was four times larger than for D-isofagomine (0.54 Å) (Fig. 2). Positions and orientations of the piperidine rings of D-3-*epi*-isofagomine in the binding site were similar to that of D-isofagomine. However, D-3-*epi*-isofagomine missed the hydrogen bond interaction between 3-OH group and β-glucocerebrosidase (Asp127 and Trp179) (Figs. 3 and 4). These results support that the C3 OH group in the isofagomine is a dominant factor for the inhibition of β-glucocerebrosidase. Gaucher disease is caused by mutations in the gene encoding β-glucocerebrosidase, the lysosomal enzyme responsible for the metabolism of glucosylceramide into D-glucose and ceramide.[21] Deficiency of the enzyme activity results in the progressive accumulation of glucosylceramide in the macrophages, leading to various clinical manifestations that include hepatosplenomegaly, secondary hypersplenism, anemia, skeletal abnormality, neurologic dysfunctions and Type II and III patients have central nervous system impairment.[22] Recently, pharmacological chaperone therapy has been attempted as a new potential treatment for Gaucher disease. These pharmacological chaperones appear to act as a template that stabilizes the native folding state in the endoplasmic reticulum (ER) by occupying the active site of the mutant β-glucocerebrosidase, thereby facilitating correct folding and trafficking to

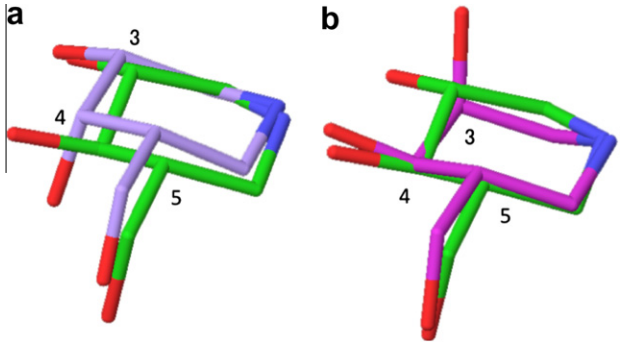


Figure 3. The comparison of the best docking pose of D-isofagomine isomers with the binding conformation of D-isofagomine determined by X-ray crystallography. (a) D-Isofagomine (green) and D-4-*epi*-isofagomine (light purple); (b) D-isofagomine (green) and D-3-*epi*-isofagomine (magenta).

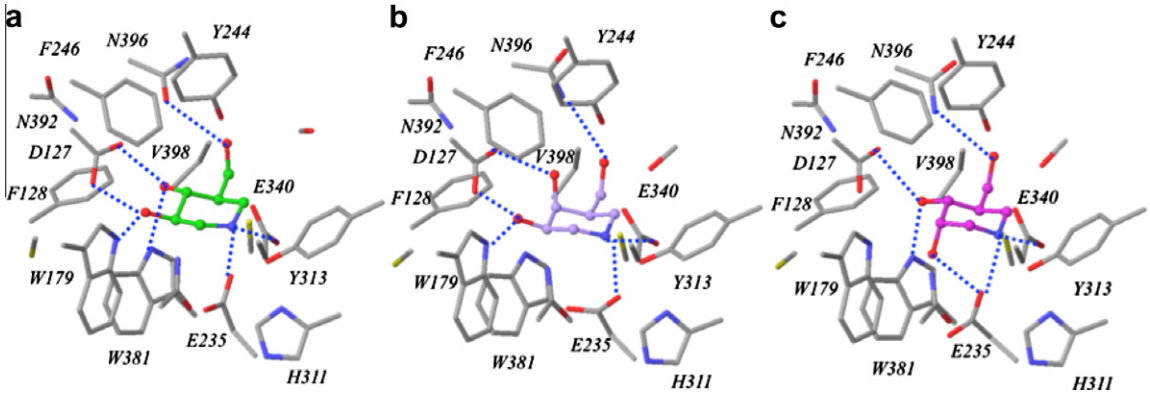


Figure 4. The hydrogen bonds between β-glucocerebrosidase and D-isofagomine isomers. (a) D-Isofagomine; (b) D-4-*epi*-isofagomine; (c) D-3-*epi*-isofagomine.

lysosomes, and increasing total cellular β-glucocerebrosidase activity.[23–25] D-Isofagomine has proceeded to Phase II clinical trials for the treatment of Gaucher disease but it has been finally withdrawn from clinical trials due to lack of efficacy. We expect that the results reported here could help to guide further structural modifications of isofagomines and 1-*N*-iminosugars to improve the permeability and chaperone effects.

4. Experimental

4.1. Chemistry

Infrared (IR) spectra were recorded on a Perkin–Elmer 1600 series FT-IR spectrometer. Mass spectra (MS) were recorded on a JEOL JMN-DX 303/JMA-DA 5000 spectrometer. Microanalyses were performed on a Perkin–Elmer CHN 2400 Elemental Analyzer. Optical rotations were measured with a JASCO DIP-360 or JASCO P-1020 digital polarimeter. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on JEOL JNM-EX 270 (270 MHz) or 300 MHz on a Varian Gemini-300 or JEOL JNM-AL 400 (400 MHz) 500 MHz on a Varian Unity-500 or JNM-LA (600 MHz) spectrometer, using tetramethylsilane as an internal standard. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Column chromatography was carried out on Merck Silica Gel 60 (230–400 mesh) or KANTO Silica Gel 60N (40–50 μm) for flash chromatography. Purification of products via ion-exchange resin chromatography was performed with Dowex 1×2 OH^- form, using water as eluent. D-forms of isofagomine, 3-*epi*-isofagomine, 4-*epi*-isofagomine, and 3,4-di-*epi*-isofagomine were prepared according to our previous reports[11,12] and their spectral data were described in their literatures.[11,12] In addition, D-forms of fagomine, 3,4-di-*epi*-fagomine, 3-*epi*-fagomine, and 4-*epi*-fagomine were available from D-serine-derived Garner aldehyde with the same procedures as previously described.[17]

4.1.1. (*R*)-*N*-*tert*-Butoxycarbonyl-5-(*tert*-butyldiphenylsilyloxy)-3-piperidene (3)

To a solution of (*R*)-**1**[11] (897 mg, 4.5 mmol) in CH_2Cl_2 (10 mL) was added imidazole (511 mg, 7.5 mmol), DMAP (122 mg, 1 mmol), and *tert*-butylchlorodiphenylsilane (1.374 g, 5.0 mmol). The mixture was stirred at room temperature for 3 h. The reaction mixture was filtered through a Celite pad. The filtrate was washed with brine (10 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (*n*-hexane/AcOEt = 8:1) to give **3** (1.96 g, 99%) as a colorless oil. $[\alpha]_D^{29}$ −22.3 (*c* 1.40, $CHCl_3$). [lit.[11] $[\alpha]_D^{27}$ +22.6 (*c* 1.80, $CHCl_3$) for *ent*-**3**]. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.08 (s, 9H), 1.40 (s, 9H), 3.21 (br s, 1H), 3.62–3.96 (m, 3H), 4.25 (br s, 1H), 5.58–5.72 (m, 2H), 7.36–7.44 (m, 6H), 7.67–7.70 (m, 4H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 19.2, 26.9, 28.4, 42.7, 47.9, 65.1, 79.5, 126.0, 127.4, 129.2, 129.5, 133.7, 135.5, 154.4. IR (neat) (cm^{-1}) 1703. EI-MS (*m*/*z*) 438 (M^++1).

4.1.2. (3*S*,4*S*,5*S*)-*N*-*tert*-Butoxycarbonyl-3-(*tert*-butyldiphenylsilyloxy)-4,5-epoxypiperidine (4)

To a cooled (0 °C) solution of **3** (1400 mg, 3.2 mmol) in CH_3CN (35 mL) was added 4 mmol/L of aq Na_2EDTA (17.5 mL, 0.07 mmol) and 1,1,1-trifluoroacetone (3.5 mL). The mixture of $NaHCO_3$ (2.21 g, 26.3 mmol) and Oxone (10.73 g, 17.5 mmol) as a solid was added slowly over a period of 1 h at 0 °C. After being stirred at 0 °C overnight, the solution was quenched by adding water (100 mL) and extracted with $CHCl_3$ (3 × 100 mL). The organic extracts were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (*n*-hexane/AcOEt = 8:1) to give **4** (1040 mg, 72%) as colorless prisms and (3*S*,4*R*,5*R*)-*N*-*tert*-butoxycarbonyl-3-(*tert*-butyldiphenylsilyloxy)-4,5-epoxypiperidine (272 mg, 19%) as colorless needles. Mp 70–72 °C (*n*-pentane). $[\alpha]_D^{31}$ −29.7 (*c* 1.82, $CHCl_3$). [lit.[11] $[\alpha]_D^{30}$ +29.2 (*c* 1.11, $CHCl_3$) for *ent*-**4**]. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.10 (s, 9H), 1.37 and 1.46 (2s, 9H), 3.07–3.41 (m, 4H), 3.07–3.88 (m, 2H), 4.09–4.15 (m, 1H), 7.36–7.45 (m, 6H), 7.63–7.69 (m, 4H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 19.3, 27.0, 28.4, 41.4, 45.3, 50.6, 53.7, 65.2, 79.8, 127.7, 129.8, 133.1, 135.5, 154.9. IR (KBr) (cm^{-1}) 1701. EI-MS (*m*/*z*) 453 (M^+).

4.1.3. (3*S*,4*S*,5*R*)-*N*-*tert*-Butoxycarbonyl-3-(*tert*-butyldiphenylsilyloxy)-4-hydroxy-5-vinylpiperidine (5)

A solution of *n*-BuLi (3.125 mL, 1.6 M in *n*-hexane, 5 mmol) was added to a solution of tetravinyltin (1.14 g, 5 mmol) in Et_2O (7 mL) at room temperature and the mixture was stirred for 1 h at the same temperature. The solution was added to a suspension of CuCN (224 mg, 2.5 mmol) in dry Et_2O (20 mL) at −78 °C and the mixture was stirred under argon atmosphere at −78 °C for 10 min. The resulting solution was warmed to −10 °C and stirred for 30 min. The reaction mixture was cooled to −78 °C and a solution of **4** (454 mg, 1 mmol) in dry Et_2O (20 mL) was added to the reaction mixture dropwise via syringe at −78 °C. The mixture was stirred at −78 °C for 10 min. $BF_3{\cdot}OEt_2$ (4 mol/L in Et_2O, 5 mL, 2 mmol) was added to the reaction mixture at −78 °C and the solution was stirred for 2 h. The reaction mixture was quenched with 28% NH_4OH/satd NH_4Cl (1:9) solution (50 mL) and stirred overnight at room temperature. The organic layer was separated and the aqueous layer was extracted with Et_2O (2 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica

gel (*n*-hexane/AcOEt = 8:1) to give **5** (383 mg, 80%) as a colorless oil. $[\alpha]_D^{30}$ +10.6 (*c* 1.00, $CHCl_3$). [lit.[11] $[\alpha]_D^{24}$ −10.5 (*c* 1.16, $CHCl_3$) for *ent*-**5**]. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.09 (s, 9H), 1.31 (s, 9H), 2.12 (br s, 1H), 2.23 (br s, 1H), 2.50 (br s, 1H), 2.65 (dd, *J* = 11.0, 12.5 Hz, 1H), 3.42 (t, *J* = 9.0 Hz, 1H), 3.51–3.55 (m, 1H), 3.82–4.23 (m, 2H), 5.16–5.20 (m, 2H), 5.66–5.72 (m, 1H), 7.38–7.45 (m, 6H), 7.68–7.71 (m, 4H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 19.4, 27.0, 28.3, 45.8, 46.5, 48.7, 73.6, 77.7, 79.8, 117.7, 127.7, 129.8, 133.3, 135.7, 135.9, 154.0. IR (neat) (cm^{-1}) 3472, 1698. EI-MS (*m*/*z*) 482 (M^++1).

4.1.4. (3*S*,4*S*,5*S*)-3,4-Dihydroxy-5-hydroxymethylpiperidine (L-isofagomine) (6)

To a solution of **5** (145 mg, 0.3 mmol) in EtOH (3 mL) and H_2O (3 mL) was added an aqueous solution of 4% OsO_4 (98 μL, 0.015 mmol) and the solution was stirred at room temperature for 10 min. $NaIO_4$ (142 mg, 0.66 mmol) as a powder was added slowly over a period of 30 min, and the mixture was stirred at room temperature for 24 h. $NaBH_4$ (57 mg, 0.15 mmol) as a powder was added, and the reaction mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo. The residue was diluted with $CHCl_3$ (60 mL) and successively washed with 10% $Na_2S_2O_3$ (15 mL), satd $NaHCO_3$ (15 mL), and brine (7 mL). The organic solution was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (*n*-hexane/AcOEt = 1:1) to give triol as a colorless oil. To a solution of the prepared triol (122 mg) in dioxane (2.0 mL) was added a solution of 10% HCl (10 mL), and the solution was refluxed for 1 h. After the reaction mixture was cooled to room temperature and diluted with H_2O (40 mL), the resulting dilute solution was washed with Et_2O. The aqueous layer was concentrated under reduced pressure. The residue was dissolved in 28% NH_4OH and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (28% NH_4OH/EtOH = 1:10) to give **6** (30.9 mg, 88%) as a white powder. $[\alpha]_D^{24}$ −23.8 (*c* 1.10, EtOH). [lit.[11] $[\alpha]_D^{27}$ +25.4 (*c* 1.30, EtOH) for *ent*-**6**]. ^{1}H NMR (600 MHz, D_2O) δ: 1.61–1.68 (m, 1H), 2.33–2.38 (m, 2H), 3.05 (dd, *J* = 13.0, 3.5 Hz, 1H), 3.10 (dd, *J* = 12.1, 4.8 Hz, 1H), 3.27 (t, *J* = 9.9 Hz, 1H), 3.43–3.48 (m, 1H), 3.59 (dd, *J* = 11.4, 7.0 Hz, 1H), 3.78 (dd, *J* = 11.4, 3.3 Hz, 1H). ^{13}C NMR (67.8 MHz, D_2O) δ: 43.9, 45.7, 48.8, 59.8, 71.4, 73.1. EI-MS (*m*/*z*): 147 (M^+).

4.1.5. (*R*)-*N*-*tert*-Butoxycarbonyl-5-(tributylstannyl)methoxy-3-piperidene (7)

A solution of (*R*)-**1** (995 mg, 5 mmol) in THF (5 mL) was added to a suspension of potassium hydride (860 mg, 35 wt % in oil, 7.5 mmol) in THF (20 mL) and DMF (5 mL) at 0 °C and it was stirred for 1 h. A solution of Bu_3SnCH_2I (3.235 g, 7.5 mmol) in THF (5 mL) was added to the reaction mixture at 0 °C and it was stirred overnight. Ice water was added to the reaction mixture and it was extracted with ether (50 mL) three times. The extracts were washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by flash column chromatography on silica gel (*n*-hexane/AcOEt = 15:1) to give **7** (2.49 g, 99%) as an oil. $[\alpha]_D^{26}$ −43.9 (*c* 1.08, $CHCl_3$). [lit.[11] $[\alpha]_D^{25}$ +45.7 (*c* 5.10, $CHCl_3$) for *ent*-**7**]. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.86–0.94 (m, 15H), 1.25–1.35 (m, 6H), 1.46–1.56 (m, 15H), 3.11 (br s, 0.5H), 3.31 (br s, 0.5H), 3.63–4.00 (m, 6H), 5.71–5.92 (m, 2H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 9.0, 13.7, 27.2, 28.4, 29.1, 43.6, 44.5, 59.4, 74.6, 79.5, 125.8, 126.9, 154.5. IR (neat) (cm^{-1}) 1703, 758. EI-MS (*m*/*z*): 502 (M^+−1).

4.1.6. (*S*)-*N*-*tert*-Butoxycarbonyl-5-(hydroxymethyl)-3-piperidene (8)

A solution of *n*-BuLi (6.3 mL, 1.6 M in *n*-hexane, 9.77 mmol) was added dropwise to a solution of **7** (2.97 g, 5.92 mmol) in dry pentane (120 mL) at −80 °C and stirred for 1 h at the same temperature, and subsequently stirred at −40 °C for 2 h. satd NH_4Cl (30 mL) was added to the reaction mixture and this was then separated. The organic layer was dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography on silica gel (*n*-hexane/AcOEt = 15:1–2:1) to give **8** (815 mg, 65%) as an oil. $[\alpha]_D^{22}$ +89.1 (*c* 1.50, $CHCl_3$). [lit.[11] $[\alpha]_D^{24}$ −92.1 (*c* 1.10, $CHCl_3$) for *ent*-**8**]. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.46 (s, 9H), 2.11 (br s, 0.5H), 2.38 (br s, 1H), 2.96 (br s, 0.5H), 3.21–3.83 (m, 5H), 4.03 (d, *J* = 18.3 Hz, 1H), 5.73 (br s, 2H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 28.4, 38.1, 41.3, 44.2, 63.1, 79.9, 125.8, 126.2, 154.9. IR (neat) (cm^{-1}) 3435, 1698. EI-MS (*m*/*z*): 213 (M^+).

4.1.7. (*S*)-*N*-*tert*-Butoxycarbonyl-5-((*tert*-butyldiphenylsilyloxy)methyl)-3-piperidene (9)

To a solution of **8** (608 mg, 2.86 mmol) in CH_2Cl_2 (18 mL) was added imidazole (292 mg, 4.28 mmol), DMAP (7.0 mg, 0.06 mmol), and *tert*-butylchlorodiphenylsilane (864 mg, 3.14 mmol). The mixture was stirred at room temperature for 3 h. The reaction mixture was filtered through a Celite pad. The filtrate was washed with brine (12 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (*n*-hexane/AcOEt = 15:1) to give **9** (1.28 g, 99%) as a colorless oil. $[\alpha]_D^{21}$ +85.4 (*c* 1.30, $CHCl_3$). [lit.[11] $[\alpha]_D^{21}$ −82.9 (*c* 1.20, $CHCl_3$) for *ent*-**9**]. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.08 (s, 9H), 1.49 (s, 9H), 2.44–2.54 (m, 1H), 3.30– 3.59 (m, 3H), 3.70–3.89 (m, 2H), 3.94 (dd, *J* = 18.7, 2.0 Hz, 1H), 5.61 (br s, 1H), 5.71 (br s, 1H), 7.38–7.45 (m, 6H), 7.67–7.70 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 19.2, 26.8, 28.4, 38.4, 43.3, 64.9, 79.4, 126.0, 127.6, 129.6, 133.6, 134.8, 135.5, 155.1. IR (KBr) (cm^{-1}) 1699. EI-MS (*m*/*z*): 452 (M+1).

4.1.8. (3*R*,4*S*,5*S*)-3,4-Dihydroxy-5-(hydroxymethyl)piperidine (L-3-*epi*-isofagomine) (10) and (3*S*,4*R*,5*S*)-3,4-dihydroxy-5-(hydroxymethyl)piperidine (L-4-*epi*-isofagomine) (11)

To a solution of (*S*)-**8**[12] (100 mg, 0.47 mmol) in acetone (3 mL) was added an aqueous 4% OsO_4 solution (65 μL, 0.01 mmol). After 10 min, an aqueous 50% NMO solution (176 mg, 0.75 mmol) was added and the mixture was stirred overnight. To the solution were added Na_2SO_3 and Na_2SO_4. The mixture was filtered through a Celite pad and the filtrate was evaporated. The residue was chromatographed on silica gel ($CHCl_3$/MeOH = 10:1) to give a triol. To a solution of the above triol in 1,4-dioxane (3 mL) was added 10% HCl (8 mL). The reaction mixture was refluxed for 1 h and evaporated. NH_4OH (28%) was added to the residue and evaporated. The residue was chromatographed on silica gel (MeOH/10% NH_4OH = 10:1) to give **10** (51 mg, 81%) and **11** (11 mg, 18%) as oils. For **10**: $[\alpha]_D^{25}$ −83.7 (*c* 1.06, EtOH). [lit.[12] $[\alpha]_D^{24}$ +85.2 (*c* 1.04, EtOH) for *ent*-**10**]. ^{1}H NMR (270 MHz, D_2O) δ: 1.72–1.88 (m, 1H), 2.22 (t, *J* = 12.1 Hz, 1H), 2.54 (d, *J* = 14.2 Hz, 1H), 2.80–2.98 (m, 2H), 3.42–3.54 (m, 2H), 3.61 (dd, *J* = 11.4, 3.8 Hz, 1H), 3.73 (s, 1H). ^{13}C NMR (67.8 MHz, D_2O) δ: 39.0, 44.9, 48.1, 60.3, 66.8, 69.2. EI-MS (*m*/*z*): 147 (M^+). HRMS calcd for $C_6H_{13}NO_3$: 147.0895, found: 147.0894. For **11**: $[\alpha]_D^{25}$ +3.5 (*c* 1.02, EtOH). [lit.[12] $[\alpha]_D^{25}$ −5.1 (*c* 0.98, EtOH) for *ent*-**11**]. ^{1}H NMR (270 MHz, D_2O) δ: 1.64–1.78 (m, 1H), 2.33 (t, *J* = 12.3 Hz, 1H), 2.53 (t, *J* = 11.6 Hz, 1H), 2.63 (dd, *J* = 12.9, 4.5 Hz, 1H), 2.72 (dd, *J* = 12.1, 4.9 Hz, 1H), 3.36–3.57 (m, 3H), 4.61 (s, 1H). ^{13}C NMR (67.8 MHz, D_2O) δ: 39.9, 41.5, 43.5, 60.1, 67.1, 68.1. EI-MS (*m*/*z*): 147 (M^+).

4.1.9. (3*R*,4*S*,5*R*)-*N*-*tert*-Butoxycarbonyl-3-((*tert*-butyldiphenylsilyloxy)methyl)-4,5-epoxypiperidine (12) and (3*R*,4*R*,5*S*)-*N*-*tert*-butoxycarbonyl-3-((*tert*-butyldiphenylsilyloxy)methyl)-4,5-epoxypiperidine (13)

To a cooled (0 °C) solution of **9** (346 mg, 0.77 mmol) in CH_3CN (8 mL) was added 4 mmol/L aq Na_2EDTA (4 mL, 0.016 mmol) and 1,1,1-trifluoroacetone (0.8 mL). The mixture of $NaHCO_3$ (504 mg,

6 mmol) and Oxone® ((2.46 g, 4.0 mmol) as a solid were added slowly over a period of 1 h at 0 °C. After being stirred at 0 °C overnight, the solution was quenched by adding water (25 mL), and extracted with $CHCl_3$ (25 mL × 3). The organic extracts were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (*n*-hexane/AcOEt = 11:1) to give **12** (142 mg, 40%) and **13** (75 mg, 21%) as colorless oils. **12**: $[\alpha]_D^{22}$ +41.3 (*c* 1.26, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.06 (s, 9H), 1.41 (br d, *J* = 28.9 Hz, 9H), 2.33 (br d, *J* = 25.0 Hz, 1H), 3.06–3.28 (m, 3H), 3.37–3.77 (m, 4H), 3.93 (dd, *J* = 52.4, 14.5 Hz, 1H), 7.30–7.45 (m, 6H), 7.65 (d, *J* = 7.7 Hz, 4H). ^{1}H NMR (400 MHz, C_6D_6, 60 °C) δ: 1.17 (s, 9H), 1.45 (s, 9H), 2.31 (t, *J* = 5.9 Hz, 1H), 2.68 (t, *J* = 3.2 Hz, 1H), 2.96 (s, 1H), 3.10 (dd, *J* = 13.4, 6.8 Hz, 1H), 3.35 (br s, 1H), 3.57–3.67 (m, 3H), 3.89 (d, *J* = 15.1 Hz, 1H), 7.26–7.30 (m, 6H), 7.72–7.75 (m, 4H). ^{13}C NMR (100 MHz, C_6D_6, 60 °C) δ: 19.5, 27.1, 28.5, 37.1, 40.9, 42.9, 49.8, 52.3, 63.9, 79.2, 127.8, 128.1, 128.3, 128.5, 130.1, 133.9, 133.9, 135.9, 136.0, 155.1. IR (neat) cm^{-1}: 2962, 2931, 2859, 1696, 1473, 1461, 1428, 1392, 1366, 1248, 1174, 1113. EI-MS (*m*/*z*): 468 (M^++1). HRMS calcd for $C_{27}H_{37}NO_4Si$: 467.2492, found: 467.2465.

Compound **13**: $[\alpha]_D^{22}$ +30.5 (*c* 1.33, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.07 (s, 9H), 1.44 (s, 9H), 2.27–2.32 (m, 1H), 2.70–2.72 (m, 1H), 3.25 (br s, 1H), 3.25–3.30 (m, 2H), 3.43–3.51 (m, 1H), 3.64–3.80 (m, 3H), 3.92–4.06 (m, 1H), 7.38–7.45 (m, 6H), 7.66–7.69 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 19.2, 26.8, 28.4, 38.2, 40.1, 40.7, 41.7, 50.7, 51.9, 63.7, 79.8, 127.7, 129.7, 133.4, 133.4, 135.5, 135.6, 154.7. IR (neat) cm^{-1}: 2962, 2932, 2859, 1695, 1473, 1462, 1428, 1392, 1366, 1247, 1174, 1152, 1113, 1083. EI-MS (*m*/*z*): 468 (M^++1). HRMS calcd for $C_{27}H_{37}NO_4Si$: 467.2492, found: 467.2580.

4.1.10. (3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-hydroxymethylpiperidine (L-3,4-di-*epi*-isofagomine) (14)

A mixture of **12**[12] (140 mg, 0.30 mmol), 1,4-dioxane (7.6 mL), and 3 M KOH (15.3 mL) was refluxed overnight. After evaporation, methanol (3 mL) and 6 N HCl (9.1 mL) were added to the residue. The mixture was heated at 60 °C for 1 h and evaporated. The residue was treated with 28% NH_4OH and evaporated. The residue was chromatographed on silica gel (MeOH/10% NH_4OH = 10:1) to give **6** (12 mg, 28%) and **14** (28 mg, 64%). $[\alpha]_D^{24}$ −18.7 (*c* 1.28, EtOH). [lit.[12] $[\alpha]_D^{19}$ +18.4 (*c* 0.96, EtOH) for *ent*-**14**]. ^{1}H NMR (270 MHz, D_2O) δ: 1.90–2.02 (m, 1H), 2.47–2.65 (m, 3H), 2.84 (dd, *J* = 13.8, 2.9 Hz, 1H), 3.42 (dd, *J* = 11.1, 8.0 Hz, 1H), 3.48–3.68 (m, 2H), 3.69 (dd, *J* = 5.6, 3.6 Hz, 1H). ^{13}C NMR (67.8 MHz, D_2O) δ: 38.2, 41.4, 45.7, 59.3, 66.8, 68.7. EI-MS (*m*/*z*): 147 (M^+).

4.1.11. (2*S*,6*R*,7*S*)-2-(*tert*-Butyldiphenylsilanyloxymethyl)-7-oxa-3-azabicyclo[4.1.0]heptane-3-carboxylic acid *tert*-butyl ester (17) and (2*S*,6*S*,7*R*)-2-(*tert*-butyldiphenylsilanyloxymethyl)-7-oxa-3-azabicyclo[4.1.0]heptane-3-carboxylic acid *tert*-butyl ester (16)

To a solution of (*R*)-**15**[17] (377 mg, 0.83 mmol) in CH_3CN (6.4 mL) was successively added 4×10^{-4} M Na_2·EDTA (4.2 mL) and CF_3COCH_3 (0.78 mL) at 0 °C. A mixture of $NaHCO_3$ (545 mg) and Oxone (2.56 g) was added to the reaction mixture over 1 h at 0 °C and the whole mixture was stirred at the same temperature for 30 min. H_2O (7 mL) was added to the reaction mixture and the mixture was extracted with CH_2Cl_2. The extract was dried and evaporated. The residue was purified by chromatography (hexane/AcOEt = 5:1) to yield a diastereomeric mixture. The mixture was purified by medium-pressure chromatography (hexane/AcOEt = 15:1) to yield **17** (115 mg, 29%) and **16** (231 mg, 59%) as oils. **17**: $[\alpha]_D^{24}$ +79.8 (*c* 2.41, $CHCl_3$). [lit.[17] $[\alpha]_D^{24}$ −71.2 (*c* 1.27, $CHCl_3$) for *ent*-**17**]. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.07 (s, 9H), 1.38 (s, 6H), 1.44 (s, 3H), 1.86–2.01 (m, 2H), 2.63–2.67 (m, 1H), 3.34–3.71 (m, 3H), 3.79–3.83 (m, 2H), 4.44–4.45 (m, 0.75H), 4.75 (br s, 0.25H), 7.36–7.41 (m, 6H), 7.69–7.73 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 19.4, 25.3, 26.9, 28.4, 33.6, 51.5, 51.6, 51.9, 61.6, 80.4, 127.8, 129.8, 133.6, 135.6, 135.7, 135.8, 154.3; IR (neat) 2931.7, 1696.0, 1416.1, 1110.8, 1003.5 cm^{-1}; HRMS calcd for $C_{27}H_{37}NO_4Si$ (M^+) 467.2422, found 467.2399. Compound **16**: $[\alpha]_D^{26}$ +57.8 (*c* 1.75, $CHCl_3$); [lit.[17] $[\alpha]_D^{26}$ −50.3 (*c* 1.42, $CHCl_3$) for *ent*-**16**]. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.06 (s, 9H), 1.40 (s, 9H), 1.88–1.93 (m, 1H), 1.99 (br s, 1H), 3.00 (br s, 1H), 3.28 (br s, 1H), 3.31 (br s, 1H), 3.40–3.88 (m, 3H), 4.50 (br s, 1H), 7.36–7.45 (m, 6H), 7.63−7.67 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 19.3, 22.8, 26.9, 28.5, 50.5, 51.8, 63.8, 80.3, 127.95, 127.96, 129.9, 130.0, 133.109, 135.6, 135.7, 155.3; IR (neat) (cm^{-1}) 2932.3, 1695.4, 1421.5, 1172.1, 1109.9 ; HRMS calcd for $C_{27}H_{37}NO_4Si$ (M^+) 467.2422, found 467.2452.

4.1.12. (2*S*,3*S*,4*S*)-2-Hydroxymethylpiperidine-3,4-diol (L-fagomine) (18)

A mixture of **16** (136 mg, 0.285 mmol), 1,4-dioxane (1.8 mL), H_2O (1.17 mL), and H_2SO_4 (0.12 mL) was heated at 95 °C for 3 h. After evaporation of the reaction mixture, the residue was treated with ion-exchange resin (Amberlite IRA-410) to yield L-fagomine (**18**) (34 mg, 80%) as a solid. MP 184–185 °C; $[\alpha]_D^{26}$ −20.8 (*c* 1.13, H_2O). [lit.[17] $[\alpha]_D^{24}$ +18.0 (*c* 0.92, H_2O) for *ent*-**18**]. ^{1}H NMR (500 MHz, D_2O) δ: 1.31 (ddd, *J* = 20.0, 12.8, 4.2 Hz, 1H), 1.83–1.87 (m, 1H), 2.36–2.40 (m, 1H), 2.46 (td, *J* = 12.8, 2.56 Hz, 1H), 2.84–2.88 (m, 1H), 3.02 (t, *J* = 9.4 Hz, 1H), 3.47–3.43 (m, 1H), 3.50 (dd, *J* = 11.5, 6.4 Hz, 1H), 3.72 (dd, *J* = 11.5, 2.9 Hz, 1H). ^{13}C NMR (125 MHz, D_2O) δ: 33.5, 43.4, 61.7, 62.5, 74.0, 74.0.

4.1.13. (2*S*,3*R*,4*R*)-2-Hydroxymethylpiperidine-3,4-diol (L-3,4-di-*epi*-fagomine) (19)

A mixture of **17** (200 mg, 0.42 mmol), 1,4-dioxane (10.7 mL), and 0.3 M KOH (21.5 mL) was refluxed overnight. After evaporation, MeOH (4.1 mL) and 6 N HCl (11.7 mL) were added to the residue. The mixture was heated at 60 °C for 1 h and then evaporated to give an oil. The residue was separated by ion-exchange resin chromatography to give L-fagomine (**18**) (10.5 mg, 17%) and L-3,4-di-*epi*-fagomine (**19**) (51.2 mg, 81%). For **19**. $[\alpha]_D^{25}$ −14.0 (*c* 1.96, H_2O). [lit.[17] $[\alpha]_D^{25}$ +13.4 (*c* 0.32, H_2O) for *ent*-**19**]. ^{1}H NMR (500 MHz, D_2O) δ: 1.43–1.50 (m, 1H), 1.86 (m, 1H), 2.78 (m, 2H), 3.02–3.06 (m, 1H), 3.56–3.57 (m, 2H), 3.61 (br s, 1H), 3.80 (br s, 1H). ^{13}C NMR (125 MHz, D_2O) δ: 28.2, 39.3, 56.0, 61.3, 68.2, 69.1.

4.1.14. (*R*)-6-Hydroxymethyl-3,6-dihydro-2*H*-pyridine-1-carboxylic acid *tert*-butyl ester (20)

A solution of TBAF (1.59 mL, 1.59 mmol) was added to a solution of **15** (597 mg, 1.32 mmol) in THF (15 mL) with ice cooling and the mixture was stirred at room temperature for 2.5 h. After being diluted with H_2O, the mixture was extracted with AcOEt three times. The extracts were washed with brine, dried, and evaporated. The residue was purified by chromatography (hexane/AcOEt = 3:1) to yield **20** (274 mg, 97%) as an oil. $[\alpha]_D^{24}$ +230.7 (*c* 1.75, $CHCl_3$). [lit.[17] $[\alpha]_D^{24}$ −227.5 (*c* 1.02, $CHCl_3$) for *ent*-**20**]. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.42 (s, 9H), 1.91–1.99 (m, 1H), 2.12–2.21 (m, 1H), 2.52 (br s, 0.5H), 2.83–2.90 (m, 1H), 3.17 (br s, 0.5H), 3.57–3.63 (m, 2H), 4.05 (br s, 1H), 4.49 (br s, 1H), 5.57–5.63 (m, 1H), 5.88–5.93 (1H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.0, 26.7, 28.6, 39.3, 54.2, 64.8, 80.2, 124.7, 127.5, 134.7; IR (neat) (cm^{-1}) 3440.0, 2975.8, 2930.1, 1693.0, 1674.3, 1423.6 . HRMS calcd for $C_{11}H_{19}NO_3$ (M^+) 213.1652, found 213.1638.

4.1.15. (2*S*,3*S*,4*R*)-2-Hydroxymethylpiperidine-3,4-diol (L-3-*epi*-fagomine) (21)

A solution of NMO (559 mg, 2.39 mmol) and a solution of K_2OsO_4·$2H_2O$ (14.7 mg, 39.7 μmol) in water (0.7 mL) were succes-

sively added to a solution of **20** (170 mg, 0.80 mmol) in acetone (7 mL) and the mixture was stirred at room temperature overnight. Na_2SO_3 was added to the mixture and the mixture was stirred. The insoluble materials were filtered off and the filtrate was evaporated. The residue was dissolved in 1,4-dioxane (4.8 mL), 10% HCl (13.9 mL) was added, and the mixture was heated at 60 °C for 1 h, and then evaporated. The residue was washed with ether and then purified by ion-exchange resin chromatography to give L-3-*epi*-fagomine (**21**) (100 mg, 86%) as a white solid. Mp 220–222 °C. $[\alpha]_D^{26}$ −72.0 (*c* 1.19, H_2O). [lit.[17] $[\alpha]_D^{26}$ +74.4 (*c* 0.95, H_2O)]. ^{1}H NMR (500 MHz, D_2O) δ:1.56–1.63 (m, 1H), 1.69–1.73 (m, 1H), 2.61–2.68 (m, 2H), 2.70–2.75 (m, 1H), 3.33 (dd, *J* = 10.2, 2.9 Hz, 1H), 3.49 (dd, *J* = 11.63, 6.5 Hz, 1H), 3.69 (dd, *J* = 11.5, 2.9 Hz, 1H), 3.95 (q, *J* = 2.9 Hz, 1H). ^{13}C NMR (125 MHz, D_2O) δ: 31.8, 39.1, 56.5, 62.9, 68.7, 70.3.

4.1.16. (2*S*,3*R*,4*S*)-2-Hydroxymethylpiperidine-3,4-diol (L-4-*epi*-fagomine) (22)

A solution of OsO_4 (120 mg, 0.47 mmol) in CH_2Cl_2 (1.2 mL) was added to a solution of **20** (98.5 mg, 0.46 mmol) and TMEDA (58.6 mg, 0.50 mmol) in CH_2Cl_2 (12 mL) at −78 °C. The mixture was stirred at the same temperature for 2 h, warmed to room temperature, and stirred for 1 h. The reaction mixture was evaporated. The residue was dissolved in MeOH (12 mL), 35% HCl (four drops) was added, and the mixture was stirred at room temperature for 2 h, and then evaporated. The residue was treated with ion-exchange resin (Amberlite IRA-410) and then separated by ion-exchange resin chromatography to give L-4-*epi*-fagomine (**22**) (39.7 mg, 59%) and L-3-*epi*-fagomine (**21**) (20.2 mg, 30%) as solids. **22**: mp 220–222 °C. $[\alpha]_D^{23}$ −10.4 (*c* 1.02, H_2O). [lit.[17] $[\alpha]_D^{22}$ +10.2 (*c* 1.42, H_2O)]. ^{1}H NMR (300 MHz, D_2O) δ: 1.49–1.56 (m, 2H), 2.39–2.49 (m, 1H), 2.57 (td, *J* = 6.9, 5.2 Hz, 1H), 2.91 (td, *J* = 12.9, 3.3 Hz, 1H), 3.47 (dd, *J* = 6.7, 3.4 Hz, 2H), 3.50–3.60 (m, 1H), 3.74 (br s, 1H). ^{13}C NMR (75 MHz, D_2O) δ: 28.4, 43.6, 59.7, 62.5, 68.5, 70.7.

4.2. Biological assays

The enzymes α-glucosidase (from rice, assayed at pH 5.0), β-glucosidases (from almond, pH 5.0; from bovine liver, pH 6.8), α-galactosidase (from coffee bean, pH 6.5), β-galactosidase (from bovine liver, pH 6.8), α-mannosidase (from jack beans, pH 4.5), β-mannosidase (from snail, pH 4.5), α-L-fucosidase (from bovine epididymis, pH 5.5), α-L-rhamnosidase (from *Penicillium decumbens*, pH 4.0), *p*-nitrophenyl glycosides, and various disaccharides were purchased from Sigma–Aldrich Co. Brush border membranes were prepared from the rat small intestine according to the method of Kessler et al,[26] and were assayed at pH 5.8 for rat intestinal maltase, isomaltase, sucrase, cellobiase, and lactase using the appropriate disaccharides as substrates. For rice α-glucosidase and rat intestinal maltase activities, the reaction mixture contained 25 mM maltose and the appropriate amount of enzyme, and the incubations were performed for 10–30 min at 37 °C. The reaction was stopped by heating at 100 °C for 3 min. After centrifugation (600*g*; 10 min), 0.05 mL of the resulting reaction mixture were added to 3 mL of the Glucose CII-test Wako (Wako Pure Chemical Ind., Osaka, Japan). The absorbance at 505 nm was measured to determine the amount of the released D-glucose. Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate at the optimum pH of each enzyme. The reaction mixture contained 2 mM of the substrate and the appropriate amount of enzyme. The reaction was stopped by adding 2 mL of 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm. Human lysosomal α-galactosidase and β-glucosidase were obtained from Genzyme (Boston, MA) and their activities were determined using 4-methylumbelliferyl α-D-galactoside and β-D-glucoside (Sigma–Aldrich Co.), respectively, as substrates. Liberated 4-methylumbelliferone was measured (excitation 362 nm, emission 450 nm) with a F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan).

4.3. Docking studies

We modeled the three-dimensional structures of human β-glucocerebrosidase complexed with D-isofagomine and three D-isofagomine isomers using Induced Fit Docking protocol[27] in Schrödinger software package. The three-dimensional coordinate of the crystal structure of human β-glucocerebrosidase–D-isofagomine complex (PDB code: 2NSX B chain) was used in the docking analyses, because this crystal structure was determined at pH 5.5 (an acidic-pH reflective of the lysosome environment). Water molecules were removed from the structure and H atoms were added using the Protein Preparation Wizard in Maestro 9.0. The docking site was defined as an enclosing box 26 × 26 × 26 Å centered at the centroid of the co-crystallized ligand. First, the docking of D-isofagomine to β-glucocerebrosidase was performed for the purpose of showing that the ligand docking by Induced Fit Docking protocol could reproduce the crystal structure of β-glucocerebrosidase–D-isofagomine complex. In addition the result of the docking analysis for D-isofagomine was used to compare with the results of the docking analyses for D-isofagomine isomers.

The chemical structures of D-isofagomine and D-isofagomine isomers were sketched with ChemDraw (ChemDraw Ultra, Versions 8.0.3, Cambridge Soft Corporation, Cambridge, MA) and were imported into Chem3D (Chem3D Ultra, Versions 8.0.3, Cambridge Soft Corporation, Cambridge, MA) to generate reasonable 3D structures. Then, the conformational analyses using CAMDAS (Conformational Analyzer with Molecular Dynamics and Sampling) program[28] were carried out to generate initial conformers for the docking analyses.

Standard-precision (SP) mode was used in the docking analysis. For the initial docking process of the Induced Fit Docking protocol, the van der Waals radii of both the ligand and the receptor were scaled to 0.5 to reduce the effect of steric clashes and to soften the surfaces of both the protein and the ligand so that a wider variety of poses could be generated. Observation of more than one crystal structure of human β-glucocerebrosidase obtained from the Protein Data Bank database presented that the side chain of Asn396 within the binding site could rotate. Therefore, Asn396 was mutated to alanine temporarily to generate more than one binding mode in the initial docking process. In the final docking process, the original residue type for Asn396 mutated to alanine was restored and ligands were redocked back into all new receptor conformations using the default vdW radii scaling (1.0 receptor, 0.8 ligand).

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