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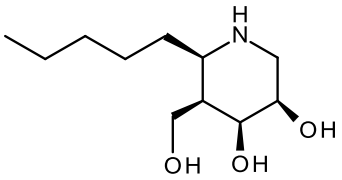
Graphical abstract



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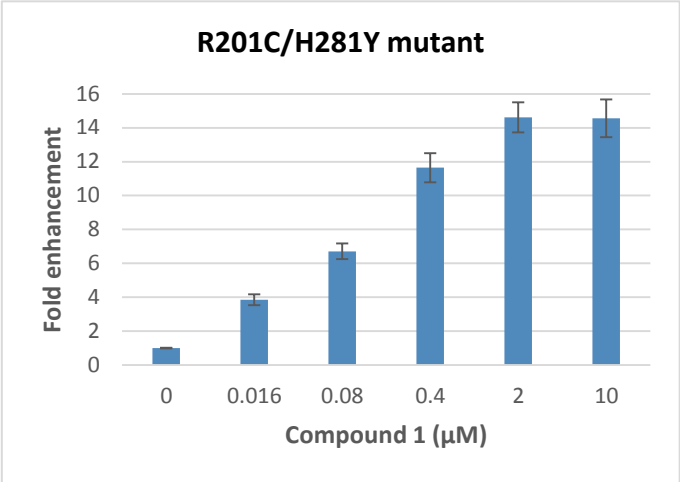
A potent inhibitor of hβGal (IC_{50} = 8 nM)
An effective chaperone for
several mutant hβGal



(5a*R*)-5a-*C*-Pentyl-4-Epi-Isofagomine: a Powerful Inhibitor of Lysosomal β-Galactosidase and a Remarkable Chaperone for Mutations Associated with GM1-Gangliosidosis and Morquio Disease Type B

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ABSTRACT

This report is about the identification, synthesis and initial biological characterization of derivatives of 4-epi-isofagomine as pharmacological chaperones (PC) for human lysosomal β-galactosidase. The two epimers of 4-epi-isofagomine carrying a pentyl group at C-5a, namely (5a*R*)- and (5a*S*)-5a-*C*-pentyl-4-epi-isofagomine, were prepared by an innovative procedure involving in the key step the addition of nitrohexane to a keto-pentopyranoside. Both epimers were evaluated as inhibitors of the human β-galactosidase: the (5a*R*)-stereoisomer (compound **1**) was found to be a very potent inhibitor of the enzyme (IC_{50} = 8 nM, 30x more potent than 4-epi-isofagomine at pH 7.3) with a high selectivity for this glycosidase whereas the (5a*S*) epimer was a much weaker inhibitor. In addition, compound **1** showed a remarkable activity as a PC. It significantly enhanced the residual activity of mutant β-galactosidase in 15 patient cell lines out of 23, with enhancement factors greater than 3.5 in 10 cell lines and activity restoration up to 91% of normal. Altogether, these results indicated that (5a*R*)-5a-*C*-pentyl-4-epi-isofagomine constitutes a promising PC-based drug candidate for the treatment of GM1-gangliosidosis and Morquio disease type B.

Keywords: galactosidase inhibitors, GM1-gangliosidosis, Morquio disease type B, iminosugars, pharmacological chaperones

1. Introduction

GM1-gangliosidosis [1] and Morquio disease type B [2] are two clinically distinct lysosomal storage disorders due to deficiency of the lysosomal enzyme β-galactosidase, which hydrolyzes terminal galactose residues from cell constituents, including GM1-gangliosides, glycoproteins, oligosaccharides, and the glycosaminoglycan keratan sulfate. Both diseases are inherited in an autosomal recessive manner. A defective lysosomal enzyme results in the accumulation of its physiological substrates in lysosomes, and more largely in cells, tissues, organs and body fluids. Depending on the mutations, degradation of one or the other of the β-galactosidase substrates is more or less impaired. If degradation of GM1-gangliosides is predominantly defective, the patients develop the symptomatology of GM1-gangliosidosis, while accumulation of keratan sulfate is an indication for Morquio disease type B. GM1-gangliosidosis is a neurodegenerative disorder with three clinical forms based on the age of onset of symptoms, severity, and residual β-galactosidase activity. Morquio disease type B belongs to the mucopolysaccharidoses, a group of inherited inborn errors of metabolism caused by defects in the degradation of glycosaminoglycans. This disease presents clinical features overlapping those of Morquio disease type A, but is the result of a different deficient enzyme (β-galactosidase *vs.* *N*-acetyl-galactosamine-6-sulfatase).

Morquio disease type B is characterized by normal intelligence and skeletal dysplasia that results in short stature and skeletal abnormalities. GM1-gangliosidosis and Morquio disease type B are very rare diseases, with a prevalence in the range of 1 in 100’000 and 1 in 250’000 births, respectively [2]. For GM1-gangliosidosis, this value is reported to be higher in some geographical regions, especially in Brazil, with 6 new cases per 100’000 births [3]. As of today, both diseases remain orphan pathologies. Patients are only offered palliative treatments, such as orthopedic surgery in the case of Morquio disease type B.

Therapies relying on enzyme substitution, substrate reduction, gene replacement and pharmacological chaperones constitute options for the treatment of lysosomal diseases [4].

Pharmacological chaperones (PCs) offer the advantages that they can be designed to distribute into the target organs, in particular the brain and the bones, and to be orally available [5]. In addition, as they are low molecular weight compounds, they should be devoid of immunogenic properties and the cost of manufacturing might be kept affordable. Finally, the recent development of 1-deoxygalactonojirimycin (DGJ) as a pharmacological chaperone for α-galactosidase (Fabry disease) validates the clinical and commercial viability of the pharmacological chaperone-based therapies for lysosomal storage diseases [6].

Over the last decade, a few investigators reported the development of β-galactosidase PCs, the first example being galactose [7]. One of the most investigated compounds, *N*-octyl-4-epi-β-valienamine (NOEV), was already reported in 2003 by Suzuki and coworkers [8]. Importantly, their study showed that this compound was therapeutically active in β-galactosidase-deficient transgenic mice with a mutated form of the human β-galactosidase. This was the first *in vivo* evidence of the therapeutic potential of PCs for the treatment of these two pathologies. Other β-galactosidase PCs were reported, which were mainly exploiting the DGJ scaffold [9-12]. A bicyclic derivative of DGJ developed by Garcia-Fernandez, Higaki and coworkers was also evaluated in the humanized GM1-gangliosidosis mouse model. As observed with NOEV, this new compound was therapeutically active, albeit to a lesser extent, which likely reflects the high concentrations required to restore β-galactosidase activity in patient cells [12]. To our knowledge, none of the other β-galactosidase PCs was evaluated in disease animal models, and none is currently undergoing further preclinical development.

Our report focus on the identification, synthesis and initial biological characterization of a derivative of 4-epi-isofagomine (4-epi-IFG) as a potent and specific PC for the human lysosomal β-galactosidase [13]. Previous studies by our group [14] and others [15] have shown that the substitution of an iminosugar moiety with a group mimicking an aglycone, substantially improved the potency and the specificity of iminosugar-based glycosidase inhibitors. Following this approach, 4-epi-IFG (Fig. 1) [16]

was modified with a C_5-alkyl chain at the C-5a position in either of the two possible configurations. In one of them, the modification considerably increased the potency and the selectivity of this compound, previously known as a moderate inhibitor of the human β-galactosidase, but lacking the desired selectivity for development as a drug candidate. In addition, the resulting compound **1** (Fig. 1) increased β-galactosidase enzyme activity in two-thirds of the tested GM1-gangliosidosis and Morquio disease type B patient cell lines. Taken together, the results presented in this report indicate that compound **1** constitutes a potential PC-based drug candidate for the treatment of GM1-gangliosidosis and Morquio disease type B.

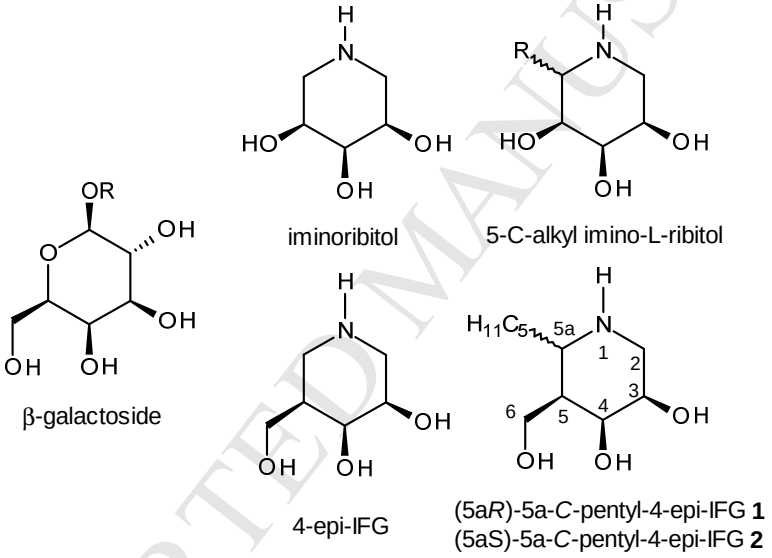


Fig. 1. Structures of galactoside mimics

2. Results and discussion

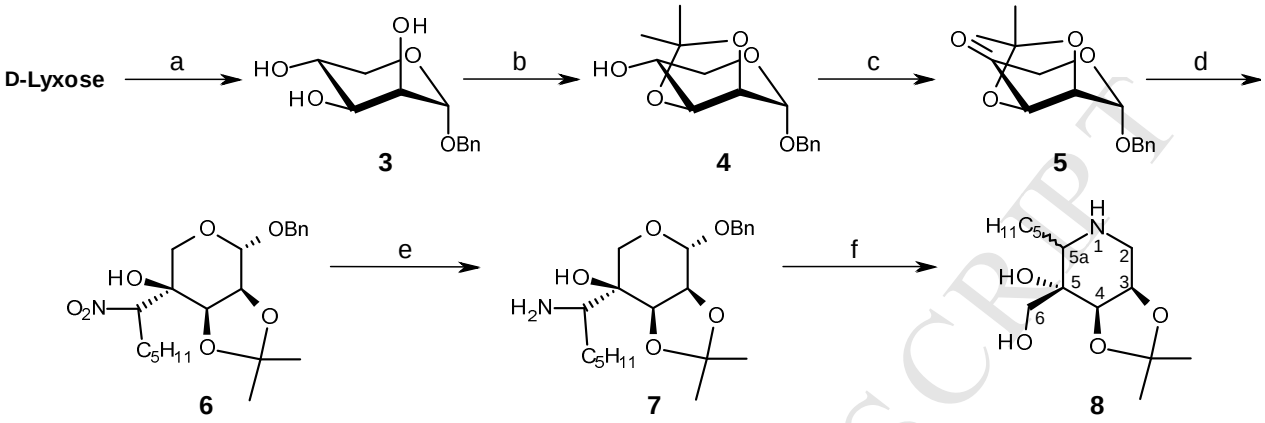
2.1. Synthesis of iminosugars 1 and 2

We recently reported iminoribitol derivatives (Figure 1) as β-galactosidase inhibitors [17]. SAR studies showed that adding an alkyl group next to the nitrogen atom in the imino-L-ribitol structure leads to a 400-fold improvement of its inhibitory activity on bovine galactosidase. No similar effect was observed on the human enzyme. In these investigations we also observed that 4-epi-IFG was a

much better inhibitor than iminoribitol. We therefore decided to investigate 4-epi-IFG derivatives carrying a substituent at C-5a, hypothesizing that this modification would improve the activity of the parent compound, as it was observed in the iminoribitol series. We therefore embarked on the synthesis of (5a*R*)- and (5a*S*)-5a-*C*-pentyl-4-epi-IFG **1** and **2** (strictly (2*R*, 3*R*, 4*S*, 5*R*)- and (2*S*, 3*R*, 4*S*, 5*R*)-3-hydroxymethyl-2-pentylpiperidine-4,5-diol). To our knowledge, there is only one example of a related compound in the literature: 5a-*C*-methyl-4-epi-IFG was prepared from a bicyclic lactam in the course of synthetic studies [18]. Very recently, preliminary results on 4-epi-IFG derivatives carrying a functionalized alkyl group at C-5a were reported and the compounds were shown to be good β-galactosidase inhibitors [19]. 5a-*C*-Alkyl-isofagomine derivatives (as glucoside mimics) have also been synthesized and found to be potent inhibitors of β-glucocerebrosidase [20], the enzyme responsible for Gaucher disease, and good candidates as PCs [21]. Our synthesis differs significantly from these studies, as it is inspired from the concise synthesis of IFG developed by Andersch and Bols, which used the addition of nitromethane to a pentopyranose-derived ketone as the key step [22]. Using a longer chain nitroalkane, we planned to simultaneously introduce the nitrogen atom and the C-C_5 fragment of the final molecule. A C_5-chain was introduced as a good compromise to provide some lipophilic character to the molecule without risking problems of toxicity that are common with longer alkyl chains [23].

The synthesis started from D-lyxose (Scheme 1), which was converted to ketone **5** by adaptation of known procedures [24,25]. The reaction of this ketone in neat nitrohexane in the presence of NEt_3 gave the branched-chain sugar derivative **6** in 52% yield; use of a co-solvent resulted in a significant drop in yield. Interestingly the addition of nitrohexane was quite stereoselective, as it led to a 7:3 mixture of the epimers at the nitromethine group, and a single configuration at the ring carbon atom. The C-4(R) configuration was indeed favored by both kinetic (addition to the less hindered face of the ketone) and thermodynamic factors (equatorial disposition of the nitrohexyl group). Two consecutive catalytic hydrogenations promoted the reduction of the nitro function, the cleavage of the benzyl group, and an

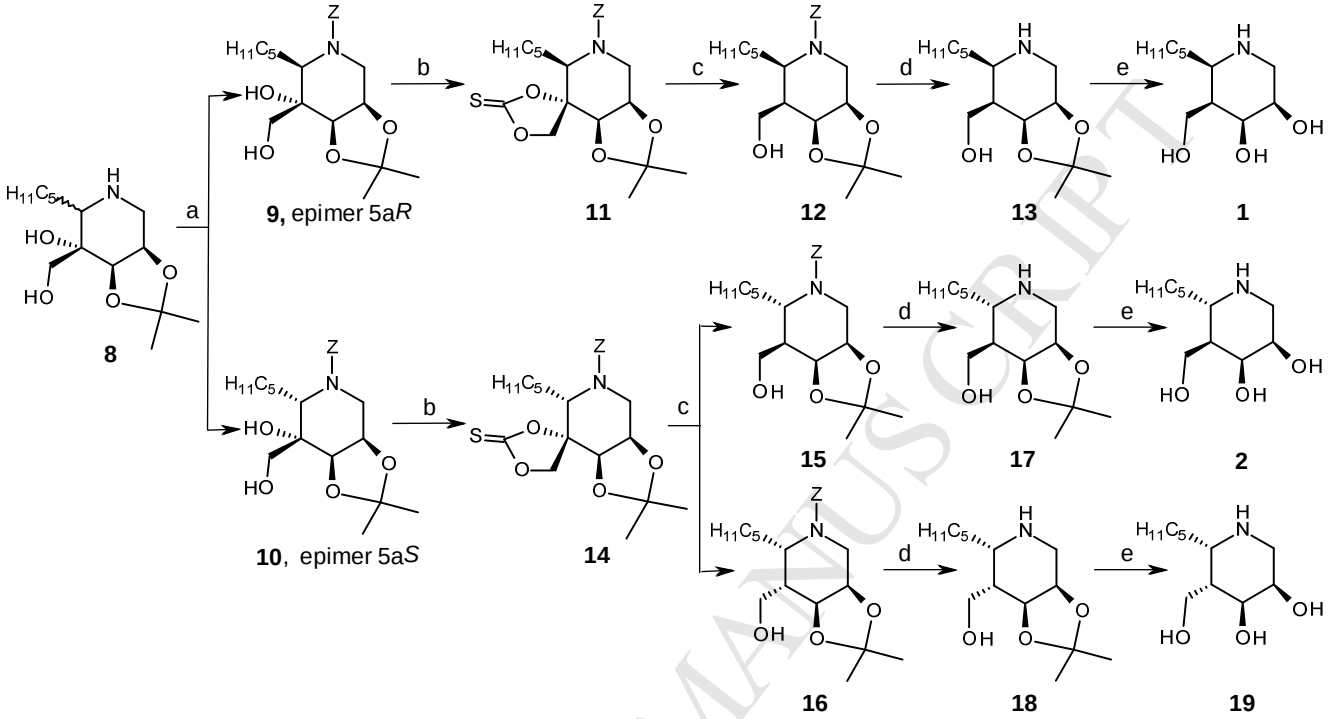
intramolecular reductive amination, leading to an inseparable mixture of two iminosugars, epimeric at C-5a (**8**) and still bearing a hydroxyl group at position C-5.



Scheme 1. Reagents and conditions: (a) BnOH, PTSA, 60 °C, 78 h, 78%; (b) 2,2-dimethoxypropane, acetone, PTSA, rt, 18 h, 64%; (c) Dess-Martin periodinane, CH_2Cl_2, rt, 16 h, 99%; (d) 1-nitrohexane, NEt_3, rt, 4 d, 52%; (e) H_2, Raney Ni, AcOH, iPrOH, rt, 5 d, 85%; (f) H_2, $Pd(OH)_2/C$, AcOH, iPrOH, rt, 3 d, quant.

In order to remove this group, dehydration of branched nitroaldol **6** was attempted under various conditions, including acetylation/elimination according to literature procedures [26], but these reactions were unsuccessful. Finally, reductive cleavage of the tertiary alcohol function of **8** could be achieved under radical conditions by way of a cyclic thiocarbonate (Scheme 2). Preliminary protection of the nitrogen atom in **8** as a benzyl carbamate allowed the separation of the isomers **9** and **10**. On both isomers, the cyclic thiocarbonate was prepared efficiently and radical deoxygenation was realized with Bu_3SnH, which gave the expected product containing a tertiary C-H bond [27]. Interestingly, the reaction of thiocarbonate **11** led to a single compound, **12**, precursor of the desired (5a*R*)-5a-*C*-pentyl-4-epi-IFG **1**, whereas compound **14** gave a separable mixture of the two iminosugars **15** and **16**, which were later identified as the precursors of (5a*S*)-5a-*C*-pentyl-4-epi-IFG **2** and its epimer at C-5 (**19**). Hydrogen transfer at C-5 anti with respect to the substituents at C-4 and C-5a appeared to be highly favored in the case of **11**, whereas the transfer became less stereoselective when the substituents at C-4 and C-5a were trans as in **14**. Final deprotection steps were conducted on each epimer, providing thus

the three isomeric products **1**, **2** and **19**. NMR analyses of the three compounds unambiguously confirmed their configuration at C-5 and C-5a.



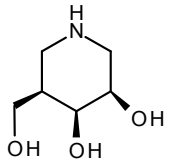
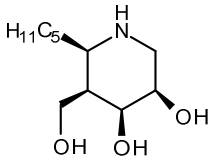
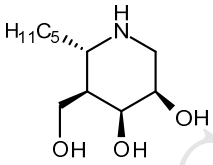
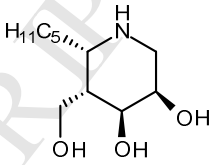
Scheme 2. Reagents and conditions: (a) benzyl chloroformate, DIPEA, EtOH, rt, 16 h, **9**: 18%, **10**: 31%; (b) thiocarbonyldiimidazole, THF, reflux, 4 h, **11**: 86%, **14**: 91%; (c) Bu_3SnH, AIBN, toluene, reflux, 16 h, **12**: 60%, **15**: 38%, **16**: 43%; (d) H_2, 10% Pd/C, iPrOH, rt, **13**: 96%, **17**: 49%, **18**: 67%; (e) Dowex 50WX8, dioxane/H_2O (5:3), rt, 18h, **1**: 84%, **2**: 86%, **19**: quant.

2.2. Inhibition studies on human β-galactosidase

Compound **1** was first evaluated for its capacity to inhibit the human β-galactosidase activity. Using lysate of human peripheral blood mononuclear cells (PBMC) as a source of enzyme, it was found that the IC_{50} of **1** was 8 nM at pH 7.3 (Table 1). By comparison, the parent compound, 4-epi-IFG, had an IC_{50} of 240 nM, indicating that the introduction of a pentyl chain at position C-5a resulted in a 30-fold increase in potency. Epimers **2** and **19** were also tested for their inhibitory capacity of the human β-galactosidase. Remarkably, 5,5a-trans isomer **2** was found to be 1600-fold less active than all-cis isomer **1** and the double epimer of **1** at C-5 and C-5a, compound **19**, was only weakly active on the

enzyme (IC_{50} >150 µM), illustrating the exquisite specificity of the human β-galactosidase for structure **1**.

Table 1. Inhibitory activities of 4-epi-IFG derivatives towards human β-galactosidase

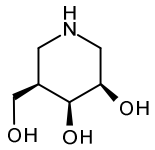
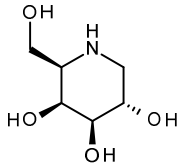
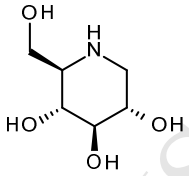
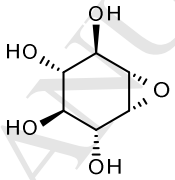
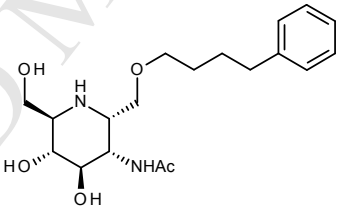
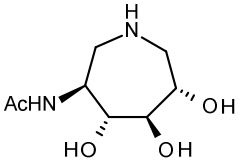
	IC_{50} (µM)			
				
	4-epi-IFG	**1**	**2**	**19**
pH 7.3	0.24 ± 0.015	0.008 ± 0.0004	13 ± 4.7	>150
pH 4.3	12 ± 2.8	0.18 ± 0.019	nd[a]	nd[a]

[a] not determined

The selectivity of **1** was next evaluated on a set of human glycosidases. As for the β-galactosidase, assays were established using PBMC lysate, as a source of human enzyme, and a corresponding chromogenic or fluorogenic substrate. A compound blocking one of the glycosidase activities was included in each assay, as a validation of the different enzymatic tests. Five of the 6 glycosidases tested were not inhibited by compound **1** up to 160 µM. The β-glucosidase was the only glycosidase weakly blocked by this compound, with an IC_{50} of 100 µM, indicating that compound **1** was considerably less active on the β-glucosidase than on the β-galactosidase (Table 2). Overall, compound **1** was found exquisitely selective for human β-galactosidase, as it was not active on a set of closely related glycosidases.

Table 2. Inhibitory activities towards a set of human glycosidases

Human enzymes[a]	IC_{50} (µM) compound **1**	IC_{50} (µM) reference compound	Structure reference compound

β-galactosidase	0.01	0.24		4-epi-IFG
α-galactosidase	> 160	0.08		DGJ
α-glucosidase	> 160	0.05		DNJ
β-glucosidase	100	30		Conduritol B epoxide
α-hexosaminidase	> 160	0.1		[b]
β-hexosaminidase	> 160	15		[c]

[a] Human PBMC lysates were used as the source of glycosidase activities.

[b] DORPHAN's proprietary inhibitor [28].

[c] Ref. [29]

2.3. Heat denaturation of β-galactosidase

The capacity of a compound to protect an enzyme from denaturation by heat and chemical treatments is regarded as predictive of its pharmacological chaperone activity [30]. Compound **1** was evaluated for

its capacity to protect β-galactosidase from heat-induced inactivation. β-Galactosidase activity in human PBMC lysates was reduced by half following heat exposition at 48 °C for 3 min. In the presence of 20 and 200 nM of compound **1**, the same effect was obtained after 4 and 17 min at 48°C, respectively. At higher concentrations of compound **1**, the level of β-galactosidase activity remained above 50% over at least 40 min at 48 °C, illustrating the remarkable capacity of this compound to stabilize the native structure of the β-galactosidase protein (Fig. 2).

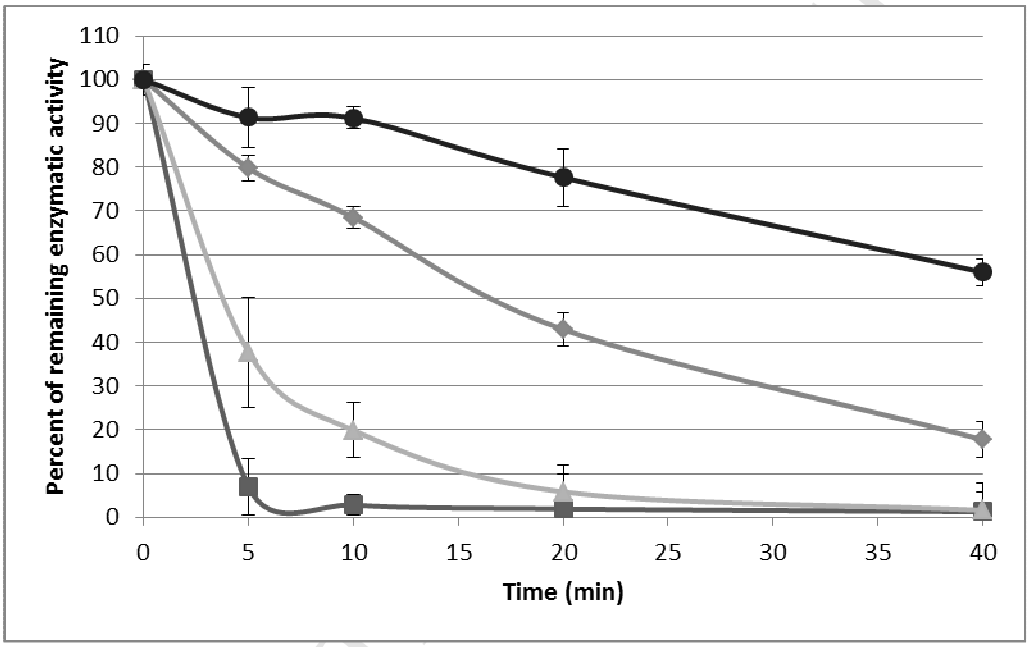


Fig. 2. Protection of β-galactosidase against heat denaturation by compound **1**. The concentrations of compound **1** were 0 (squares), 20 nM (triangles), 200 nM (diamonds) and 2 µM (circles).

2.4. Evaluation of chaperone activity in treated fibroblasts from GM1-gangliosidosis and Morquio disease type B patients

It was next investigated whether compound **1** had the capacity to restore β-galactosidase activity in cells from GM1-gangliosidosis and Morquio disease type B patients. Following an experimental design applied in several studies [7,9,11,31], patient fibroblasts were first cultured for 5 days in the presence of increasing concentrations of compound **1**. β-Galactosidase and β-hexosaminidase activities were

then determined in cell lysates. β-Hexosaminidase activity was used as a means to assess cell viability and cell number between cultures. Figure 3 presents the results obtained with fibroblasts derived from a GM1-gangliosidosis patient bearing the R201C/H281Y mutations. The β-galactosidase activity was enhanced with increasing concentrations of compound **1**; exposure to 0.08, 0.4 and 2 µM of compound **1** resulted in a 7, 12 and 15-fold activity increase of β-galactosidase respectively. By contrast, the β-hexosaminidase activity remained stable over the range of concentrations tested (Fig. 3). These observations indicated that compound **1** had the capacity to specifically induce the recovery of β-galactosidase activity in patient cells.

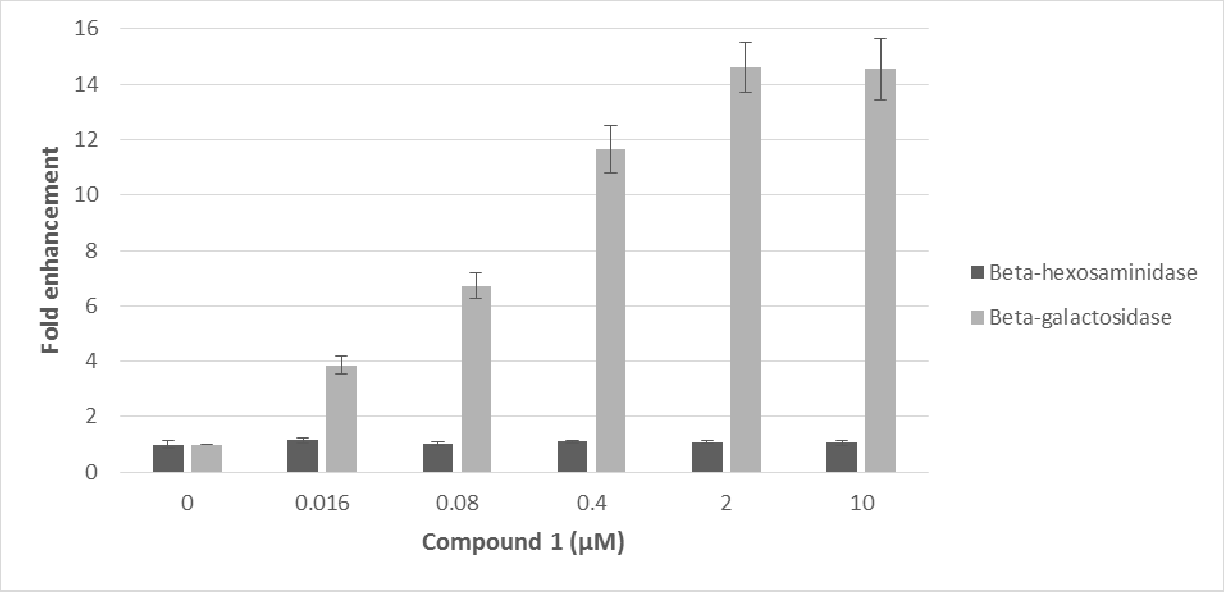


Fig. 3. Chaperone activity on fibroblasts from a GM1-gangliosidosis patient. Results presented are means of 4 values obtained from independent cultures.

These studies were then extended to a panel of 22 patient cell lines. As the chaperone effect was observed in numerous tests over a broad concentration range, Table 3 presents a compilation of the β-galactosidase activity values induced by concentrations of compound **1** ranging between 2 and 10 µM, concentrations at which maximal fold enhancements were usually observed. Although the set of cell lines represents only a limited fraction of the mutations reported thus far, our observations indicate that

mutations associated with the different forms of GM1-gangliosidosis (infantile, juvenile and late onset), as well as with Morquio disease type B, were responsive to compound **1**. Overall, increased β-galactosidase activity was detected in the cell lines carrying missense mutations while, as expected, no rescue of activity was detected in the cell lines carrying a null allele at homozygous level (patients 1 and 17). The chaperone effect was heterogeneous across different genetic backgrounds, from a modest 1.5-fold up to a 35-fold enhancement of the β-galactosidase activity. As residual enzyme activity in the patient cells is very variable, from less than 1% up to 5-10%, it was expected that the resulting induced recovery of glycosidase activity would span a broad range, from a few percent to more than 50% of the level found in cells from healthy individuals. Remarkably, in half of the cell lines, the recovery reached at least 10% of the level found in normal cells, a value believed to represent a threshold above which a therapeutic benefit might be obtained [32,33].

Table 3. Enhancement of β-galactosidase activity in fibroblasts of GM1-gangliosidosis and Morquio disease type B patients following treatment with compound **1**

Patient	Disease/Phenotype	Mutation	Maximal fold enhancement[a]	Residual activity[b]	Restored activity[c]
1	GM1-gangliosidosis/infantile	Homozygous insertion 733+2T>C	1.1	0.25	0.28
2	GM1-gangliosidosis/adult	I51T/I51T	1.6	4[d]	6.4
3	GM1-gangliosidosis/infantile	R59H/R59H	1.4	0.5	0.7
4	GM1-gangliosidosis/infantile	R59H/R59H	1.1	nd	nd
5	GM1-gangliosidosis/infantile	C127Y/W161G	1.1	0.8	0.9
6	GM1-gangliosidosis/infantile	R148S/S532G/L305F	1	0.4	0.4
7	GM1-gangliosidosis/late infantile	S191N/R351Term	11	1	11
8	GM1-gangliosidosis/juvenile	R201C/R201C	15	5	75
9	GM1-gangliosidosis/juvenile	R201C/R201C	5.4	2[d]	11
10	GM1-gangliosidosis/juvenile	R201C/H281Y	18	nd	nd
11	GM1-gangliosidosis/juvenile	R201H/c.247dup1	3.5	6.7	23
12	GM1-gangliosidosis/juvenile	R201H/G76E	6.7	2.3	15
13	GM1-gangliosidosis/juvenile	R201H/H281Y	4.7	4.6	22
14	GM1-gangliosidosis/infantile	R208C/IVS10+1 G>A	4.4	nd	nd
15	GM1-gangliosidosis/infantile	Q255H/K578R	20	2	40
16	GM1-gangliosidosis/infantile	H281Y/splicing	35	2.6	91
17	GM1-gangliosidosis/infantile	R351Ter/R351Ter	1	0.3	0.3
18	GM1-gangliosidosis/juvenile/adult	G438E/G438E	2	3.4	6.8
19	GM1-gangliosidosis/adult	R442Q/W92Term	1.5	3.2	4.8
20	GM1-gangliosidosis/adult	R457Q/R457Q	7.3	4[d]	29

21	GM1-gangliosidosis/infantile	P549L/P549L	1.3	0.8	1
22	Morquio B	W273L/R482H	1.5	6.5	10
23	Morquio B	W273L/W509C	1.5	6.5	10

[a] Fold enhancement was determined following treatment of the cells for up to 5 days in the presence or the absence of compound **1**. The maximal enhancement obtained, up to the maximal concentration of compound tested (10 μM), is presented.

[b] The values indicate the β-galactosidase activity (in %) in fibroblasts of untreated patient cells as compared with the activity in cells from healthy individuals

[c] The values are calculated by multiplying the fold enhancement factor with the residual activity

[d] Data from [31]

nd: not determined

2.5. pH dependence of β-galactosidase inhibition

Compound **1** was found to have significant chaperone activity on an array of β-galactosidase mutants. The tight binding of compound **1** to β-galactosidase is believed to induce proper folding of the newly synthesized protein in the endoplasmic reticulum, and to rescue it from early degradation before trafficking to the lysosomes. Once the protein has reached the lysosome, it is desirable that the inhibitory activity would be reduced in the acidic environment of this organelle. Compound **1** was found indeed to have appropriate pH dependence as a galactosidase inhibitor (Table 1). At a pH = 7.3, mimicking the endoplasmic reticulum medium, the compound was a strong inhibitor of human β-galactosidase, with an IC_{50} = 8 nM. The IC_{50} value was found to increase progressively with decreasing pH values, to reach an IC_{50} = 180 nM at pH 4.3, a value 22-fold higher than at pH 7.3 (Table 1); the catalytic rate of the enzyme was 4-fold higher than at neutral pH. As a result of this pH dependence, compound **1** dissociates readily from the enzyme in the acidic environment of the lysosomes and thus does not prevent its action on lysosomal substrates.

3. Conclusions

(5a*R*)-5a-*C*-Pentyl-4-epi-IFG **1** and its (5a*S*)-epimer **2** were synthesized from D-lyxose and evaluated as inhibitors of human lysosomal β-galactosidase. The all-cis, (5a*R*)-epimer was found to be a very potent

inhibitor of this enzyme (IC_{50} = 8 nM), much more potent than the (5aS)-epimer and the parent 4-epi-isofagomine (1600x and 30x respectively), thus revealing fine structure-activity relationship in this family of 1-*N*-iminosugar derivatives. Compound **1** behaved as a remarkable PC, considering it is capable of restoring β-galactosidase activity and expression in two-thirds of the tested GM1-gangliosidosis and Morquio disease type B patient cell lines, with enhancement of residual activities up to 35-fold, and restoration of enzyme activity up to 91%. In addition its capacity to dissociate readily from the enzyme in an acidic environment indicated that compound **1** constitutes a promising PC-based drug candidate for the treatment of these two severe lysosomal storage disorders. Further investigations in this direction are in progress and will be reported in due course.

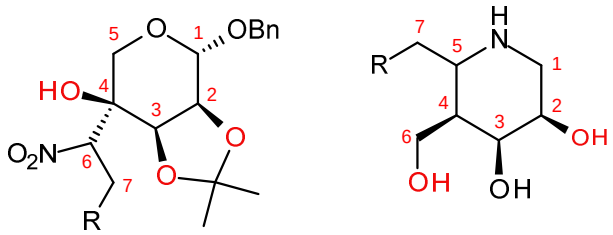
4. Experimental section

4.1. Chemistry.

Materials and methods: All reactions requiring anhydrous conditions were carried out using oven-dried glassware under an atmosphere of dry Ar. THF was dried through a column containing activated alumina under nitrogen pressure (Dry Solvent Station GT S100, GlassTechnology, Geneva, Switzerland). CH_2Cl_2 was distilled from P_2O_5. All reagent-grade chemicals and anhydrous toluene were obtained from commercial suppliers and were used as received. High resolution mass spectra were recorded on a Bruker Q-TOF MaXis spectrometer. ^1H and ^{13}C NMR spectra were recorded on Bruker Avance II (250 MHz), Bruker Avance III HD nanobay (400 MHz) or Bruker Avance III (600 MHz) spectrometers. Chemical shifts are given in ppm and are referenced to the residual solvent signal or to TMS as internal standard. Carbon multiplicities were assigned by DEPT experiments. ^1H and ^{13}C signals were attributed on the basis of H-H and C-H correlations. Spectral splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; br, broad. The purity of tested iminosugars was shown to be ≥96% by HPLC (VWR Hitachi LaChrom Elite system) using a Nucleodur Sphinx RP column (Macherey-Nagel, 150 mm x 4.6 mm, particle size: 5 µm) and a SEDEX

85 LT-ELSD detector (SEDERE). A gradient of solvent from a 98:2 mixture of H_2O/MeOH containing 0.5% of formic acid to pure MeOH containing 0.5% of formic acid in 30 min was used (flow rate: 1 mL/min). Specific rotations were measured in a 1-dm cell with a Perkin-Elmer 341 polarimeter. Melting points were determined in capillary tubes with a Büchi apparatus. Analytical thin layer chromatography was performed using Silica Gel $60F_{254}$ precoated plates (Merck) with visualization by UV light and ceric sulfate/ammonium molybdate solution (0.5 g/0.24 g in 500 mL of 1M aqueous H_2SO_4). Flash chromatography was performed on Silica Gel 60 (40-63 µm, Merck).

Numbering schemes for NMR data:



Benzyl α-D-lyxopyranoside (3). To a solution of D-lyxose (20.0 g, 0.133 mol) in benzyl alcohol (37 mL) was added PTSA (210 mg, 1.10 mmol, 0.008 eq). The mixture was heated at 60 °C for 78 h. Toluene (10 mL) was added and the resulting mixture was concentrated under vacuum and co-evaporated with toluene (3 x 30 mL) to give a soft white solid, which was then suspended in a mixture of hexane/CH_2Cl_2 (2:1) (180 mL). The solid was filtered and washed thoroughly with cold Et_2O. The filtrate was concentrated under vacuum and the procedure was repeated three times to give the desired compound **3** (24.8 g, 78%) as a white solid. ^{1}H NMR spectrum is in accordance with literature data [24].

Benzyl 2,3-*O*-isopropylidene-α-D-lyxopyranoside (4). To a suspension of compound **3** (10.1 g, 0.042 mol) in acetone (140 mL) were added 2,2-dimethoxypropane (17.5 mL, 0.142 mol, 3.4 eq) and PTSA (178 mg, 0.936 mmol, 0.02 eq). The mixture was stirred at rt for 18 h. A 1:1 mixture of hexane/Et_2O

(120 mL) was added and the solution was washed with saturated aqueous $NaHCO_3$ (60 mL), water (2 x 60 mL) and dried over $MgSO_4$. Concentration under vacuum gave crude **4** as a colorless oil, which was purified by flash column chromatography on silica gel (petroleum ether/EtOAc 85:15, then 7:3, then 6:4) to afford compound **4** (7.55 g, 64%) as a white solid. ^{1}H NMR spectrum is in accordance with literature data [24].

Benzyl 2,3-*O*-isopropylidene-β-L-*erythro*-pent-4-uloside (5). To a solution of alcohol **4** (1.0 g, 3.57 mmol) in anhydrous CH_2Cl_2 (36 mL) under Ar was added Dess-Martin periodinane (1.82 g, 4.29 mmol, 1.2 eq). The mixture was stirred for 16 h at rt. The solvent was evaporated under vacuum. The residue was suspended in cold Et_2O and the solid was removed by filtration through Celite. The filtrate was concentrated under vacuum to give the clean desired ketone **5** (0.98 g, 99%) as a pale yellow solid. ^{1}H NMR spectrum is in accordance with literature data [25].

Benzyl 2,3-*O*-isopropylidene-4-*C*-[(1*R*,*S*)(1-nitrohex-1-yl)]-β-L-ribopyranosides (6a) and (6b). To a solution of ketone **5** (1.98 g, 7.11 mmol) in 1-nitrohexane (5.5 mL) was added NEt_3 (2.8 mL, 20.1 mmol, 2.8 eq). The mixture was stirred for 4 d at rt. The reaction was quenched with saturated aqueous NH_4Cl (40 mL). The aqueous phase was extracted with EtOAc (80 mL). The organic phase was then washed with brine (40 mL), dried over $MgSO_4$ and concentrated under vacuum to give the crude product. Flash column chromatography on silica gel (petroleum ether/EtOAc, 92:8) afforded an inseparable mixture of the two diastereoisomers **6a** and **6b** (1.5 g, 52%) in a 7:3 ratio as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47-7.28 (m, 5H, H_{arom}), 5.05 (br s, 1H, H1), 4.75 (d, 1H, CH_2Ph, J = 11.6 Hz), 4.60-4.51 (m, 1H, H6), 4.54 (d, 1H, CH_2Ph, J = 11.6 Hz), 4.26 (d, 0.7H, $H3_{MAJ}$, J = 6.4 Hz), 4.17-4.09 (m, 1.3H, $H2_{MAJ}$, $H3_{min}$, $H2_{min}$), 3.88 (d, 0.3H, $H5b_{min}$, J = 12.4 Hz), 3.81 (d, 0.7H, $H5b_{MAJ}$, J = 12.0 Hz), 3.73 (d, 0.7H, $H5a_{MAJ}$, J = 12.0 Hz), 3.58 (d, 0.3H, $H5a_{min}$, J = 12.4 Hz), 3.04 (s, 0.7H, OH_{MAJ}), 2.96 (s, 0.3H, OH_{min}), 2.16-1.95 (m, 1.3H, $H7b_{MAJ}$, $2H7_{min}$), 1.72-1.65 (m, 0.7H, $H7a_{MAJ}$), 1.55 (s, 0.9H, CH_3iPr_{min}), 1.54 (s, 2.1H, CH_3iPr_{MAJ}), 1.37 (s, 0.9H, CH_3iPr_{min}), 1.36 (s, 2.1H,

CH_3iPr_{MAJ}), 1.30-1.29 (m, 6H, CH_2hex), 0.95-0.82 (m, 3H, CH_3hex). ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.65 (C_{arom}), 128.69-128.28 (CH_{arom}), 110.24 ($CiPr_{MAJ}$), 110.15 ($CiPr_{min}$), 96.60 ($C1_{MAJ}$), 96.40 ($C1_{min}$), 92.71 ($C6_{min}$), 92.68 ($C6_{MAJ}$), 74.35 ($C2_{MAJ}$), 74.32 ($C2_{min}$), 72.52 ($C3_{min}$), 72.03 ($C3_{MAJ}$), 69.74 (CH_2Ph_{MAJ}), 69.66 (CH_2Ph_{min}), 69.49 ($C4_{MAJ}$), 69.05 ($C4_{min}$), 61.26 ($C5_{min}$), 60.79 ($C5_{MAJ}$), 31.12, 28.02, 25.99, 22.41 (CH_2hex_{MAJ}), 31.17, 27.23, 25.93, 22.45 (CH_2hex_{min}), 26.17, 25.36 (CH_3iPr_{MAJ}), 26.13, 25.36 (CH_3iPr_{min}), 14.00 (CH_3hex_{min}), 13.98 (CH_3hex_{MAJ}). HRMS (ESI): *m/z* $[M+NH_4]^+$ calcd for $C_{21}H_{35}NO_7$: 427.2439, found: 427.2438; *m/z* $[M+Na]^+$ calcd for $C_{21}H_{31}NNaO_7$: 432.1993, found: 432.1992.

Benzyl 2,3-*O*-isopropylidene-4-*C*-[(1*R*,*S*)(1-aminohex-1-yl)]-β-L-ribopyranosides (7a) and (7b). To a solution of the mixture of isomers **6a** and **6b** (289 mg, 0.706 mmol) in iPrOH (7.1 mL) were added glacial AcOH (0.71 mL) and Raney Ni (excess, 2800, slurry in H_2O). The mixture was stirred under a H_2 atmosphere for 5 d at rt. Then the catalyst was filtered through a membrane, washed with MeOH and the filtrate was concentrated under vacuum to give the crude product as a green oil. Flash column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) gave an inseparable mixture of the two diastereoisomers **7a** and **7b** (228 mg, 85%) in a 7:3 ratio as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD): δ 7.43-7.28 (m, 5H, H_{arom}), 4.90 (d, 1H, H1, *J* = 3.3 Hz), 4.79 (d, 1H, CH_2Ph, *J* = 11.9 Hz), 4.61 (d, 1H, CH_2Ph, *J* = 11.9 Hz), 4.36 (d, 1H, H3, *J* = 5.8 Hz), 4.13 (dd, 1H, H2, *J* = 3.3, 5.8 Hz), 3.78 (d, 1H, H5b, *J* = 12.0 Hz), 3.57 (d, 1H, H5a, *J* = 12.0 Hz), 2.96 (dd, 0.7H, $H6_{MAJ}$, *J* = 2.4, 9.6 Hz), 2.81-2.79 (m, 0.3H, $H6_{min}$), 1.69-1.26 (m, 14H, CH_2hex, CH_3iPr), 0.95 (t, 3H, CH_3hex, *J* = 6.6 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 138.63 (C_{arom}), 129.41, 129.19, 128.90 (CH_{arom}), 110.96 ($CiPr_{MAJ}$), 110.63 ($CiPr_{min}$), 99.73 ($C1_{min}$), 99.56 ($C1_{MAJ}$), 76.33 (C2), 76.09 (C3), 71.20 (C4), 70.76 (CH_2Ph), 64.84 ($C5_{min}$), 63.52 ($C5_{MAJ}$), 58.39 ($C6_{min}$), 57.59 ($C6_{MAJ}$), 32.95, 30.66, 27.24, 23.60 (CH_2hex_{MAJ}), 31.66, 27.48 (CH_2hex_{min}), 26.92 (CH_3iPr_{min}), 26.84, 25.96 (CH_3iPr_{MAJ}), 14.39 (CH_3hex). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{34}NO_5$: 380.2432, found: 380.2433.

(2*R*,*S*, 3*S*, 4*R*, 5*R*)-3-Hydroxymethyl-4,5-*O*-isopropylidene-2-pentylpiperidine-3,4,5-triol (8a) and (8b). To a solution of the mixture of isomers **7a** and **7b** (367 mg, 0.967 mmol) in iPrOH (10 mL) were added glacial AcOH (1 mL) and 20% $Pd(OH)_2/C$ (150 mg). The mixture was stirred under a H_2 atmosphere for 3 d at rt. Then the catalyst was filtered through a membrane, washed with iPrOH and the filtrate was concentrated under vacuum to give the crude product as a clean mixture of epimers **8a** and **8b** (264 mg, quant.) in a 7:3 ratio as a colorless oil. ^{1}H NMR (600 MHz, CD_3OD): δ 4.55 (dt, 0.7H, $H2_{MAJ}$, *J* = 3.9, 3.9, 7.2 Hz), 4.48 (dt, 0.3H, $H2_{min}$, *J* = 4.8, 4.8, 6.6 Hz), 4.29 (d, 0.7H, $H3_{MAJ}$, *J* = 7.2 Hz), 4.21 (dd, 0.3H, $H3_{min}$, *J* = 1.2, 6.6 Hz), 3.72 (d, 0.3H, $H6b_{min}$, *J* = 11.4 Hz), 3.66 (d, 0.3H, $H6a_{min}$, *J* = 11.4 Hz), 3.64 (d, 0.7H, $H6b_{MAJ}$, *J* = 11.4 Hz), 3.61 (d, 0.7H, $H6a_{MAJ}$, *J* = 11.4 Hz), 3.51 (dd, 0.7H, $H1b_{MAJ}$, *J* = 3.9, 13.5 Hz), 3.34 (dd, 0.3H, $H1b_{min}$, *J* = 5.4, 13.2 Hz), 3.23 (dd, 0.7H, $H5_{MAJ}$, *J* = 3.6, 13.2 Hz), 3.21-3.18 (m, 0.3H, $H5_{min}$), 3.18 (dd, 0.7H, $H1a_{MAJ}$, *J* = 3.9, 13.5 Hz), 2.92 (dd, 0.3H, $H1a_{min}$, *J* = 6.0 Hz, 13.2 Hz), 1.93-1.83 (m, 1H, CH_2pent), 1.76-1.60 (m, 2H, CH_2pent), 1.51 (s, 3H, CH_3iPr), 1.40-1.27 (m, 5H, CH_2pent), 1.38 (s, 3H, CH_3iPr), 0.94 (t, 3H, CH_3pent, *J* = 6.6 Hz). ^{13}C NMR (62.5 MHz, CD_3OD): δ 111.20 ($CiPr_{min}$), 110.68 ($CiPr_{MAJ}$), 75.52 ($C3_{min}$), 75.20 ($C3_{MAJ}$), 72.82 ($C4_{min}$), 72.74 ($C4_{MAJ}$), 70.82 ($C2_{min}$), 70.79 ($C2_{MAJ}$), 65.27 ($C6_{min}$), 64.70 ($C6_{MAJ}$), 60.01 ($C5_{min}$), 54.83 ($C5_{MAJ}$), 42.43 ($C1_{MAJ}$), 41.61 ($C1_{min}$), 32.84, 28.41, 26.53, 23.45 (CH_2pent_{MAJ}), 32.67, 28.60, 27.40 (CH_2pent_{min}), 27.18, 24.86 (CH_3iPr_{min}), 27.08, 24.58 (CH_3iPr_{MAJ}), 14.35 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{28}NO_4$: 274.2013, found: 274.2015.

(2*R*, 3*S*, 4*R*, 5*R*)-*N*-Benzyloxycarbonyl-3-hydroxymethyl-4,5-*O*-isopropylidene-2-pentylpiperidine-3,4,5-triol (9) and (2*S*, 3*S*, 4*R*, 5*R*)-*N*-Benzyloxycarbonyl-3-hydroxymethyl-4,5-*O*-isopropylidene-2-pentylpiperidine-3,4,5-triol (10). The mixture of epimers **8a** and **8b** (500 mg, 1.83 mmol) was dissolved in EtOH (15 mL). At 0 °C, DIPEA (956 µL, 5.49 mmol, 3 eq) and benzyl chloroformate (392 µL, 2.75 mmol, 1.5 eq) were added. The mixture was stirred for 30 min at 0 °C and then 16 h at rt. The solvent was removed under vacuum and the residue was purified by flash column chromatography

on silica gel (toluene/acetone 7:1) to give the major epimer **10** (228 mg, 31%) and the minor one **9** (132 mg, 18%), both as white solids and mixtures of rotamers (65:35).

9: mp = 140-143°C. $[\alpha]_D^{20}$ = + 6.4 (*c* = 1.01, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44-7.22 (m, 5H, H_{arom}), 5.25-5.03 (m, 2H, CH_2Ph), 4.40-4.23 (m, 2H, $H1b_{min}$, H2, $H5_{MAJ}$), 4.22-4.16 (m, 0.35H, $H5_{min}$), 4.12 (dd, 0.65H, $H1b_{MAJ}$, *J* = 7.2, 13.6 Hz), 4.04-3.97 (m, 1H, H3), 3.77-3.64 (m, 2H, H6), 3.22 (br s, 0.65H, OH_{MAJ}), 2.81-2.58 (m, 1.65H, H1a, OH_{MAJ}), 2.58 (s, 0.35H, OH_{min}), 2.17 (br s, 0.35H, OH_{min}), 2.10-1.93 (m, 1H, H7b), 1.47 (s, 3H, CH_3iPr), 1.31 (s, 3H, CH_3iPr), 1.41-1.02 (m, 7H, $3CH_2$pent, H7a), 0.95-0.78 (m, 3H, CH_3pent). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.29 (CO_{MAJ}), 156.64 (CO_{min}), 136.67 ($C_{arommin}$), 136.56 ($C_{aromMAJ}$), 129.10-127.70 (CH_{arom}), 110.04 ($CiPr_{min}$), 109.95 ($CiPr_{MAJ}$), 76.34 (C3), 74.31 ($C4_{MAJ}$), 73.57 ($C4_{min}$), 70.03 ($C2_{MAJ}$), 69.85 ($C2_{min}$), 67.70 (CH_2Ph_{MAJ}), 67.62 (CH_2Ph_{min}), 65.49 ($C6_{MAJ}$), 65.06 ($C6_{min}$), 57.45 ($C5_{min}$), 57.10 ($C5_{MAJ}$), 40.27 ($C1_{MAJ}$), 39.87 ($C1_{min}$), 31.59 (CH_2pent_{min}), 31.54 (CH_2pent_{MAJ}), 28.53, 26.20 (CH_3iPr_{MAJ}), 28.48, 26.27 (CH_3iPr_{min}), 25.99-25.68, 22.63 (CH_2pent), 14.14 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{34}NO_6$: 408.2381, found: 408.2377; *m/z* $[M+Na]^+$ calcd for $C_{22}H_{33}NNaO_6$: 430.2200, found: 430.2209.

10: mp = 84-86°C. $[\alpha]_D^{20}$ = + 52.2 (*c* = 1.38, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42-7.22 (m, 5H, H_{arom}), 5.21-5.04 (m, 2H, CH_2Ph), 4.40-4.25 (m, 2H, H2, H3), 4.25-4.09 (m, 1H, H1b), 4.09-4.00 (m, 0.65H, $H5_{MAJ}$), 3.96-3.87 (m, 0.35H, $H5_{min}$), 3.78 (br d, 1H, H6b, *J* = 11.2 Hz), 3.57-3.44 (m, 1H, H6a), 3.35-3.20 (m, 1H, H1a), 2.82-2.72 (m, 1H, OH), 2.58 (br s, 0.65H, OH_{MAJ}), 2.41 (br s, 0.35H, OH_{min}),1.69-1.41 (m, 2H, H_7), 1.40-1.11 (m, 12H, $3CH_2$pent, $2CH_3$iPr), 0.97-0.76 (m, 3H, CH_3pent). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.92 (CO_{MAJ}), 156.58 (CO_{min}), 138.00 ($C_{arommin}$), 136.92 ($C_{aromMAJ}$), 129.17-127.90 (CH_{arom}), 109.11 ($CiPr_{min}$), 109.00 ($CiPr_{MAJ}$), 75.47, 73.46 ($C2_{min}$, $C3_{min}$), 75.27, 73.15 ($C2_{MAJ}$, $C3_{MAJ}$), 72.84 ($C4_{MAJ}$), 72.42 ($C4_{min}$), 67.58 (CH_2Ph_{min}), 67.24 (CH_2Ph_{MAJ}), 65.59 ($C6_{min}$), 65.22 ($C6_{MAJ}$), 54.38 ($C5_{min}$), 54.06 ($C5_{MAJ}$), 41.90 ($C1_{min}$), 41.80 ($C1_{MAJ}$), 32.13 (CH_2pent), 28.76 ($C7_{min}$), 28.09 ($C7_{MAJ}$), 26.24 (CH_2pent_{min}), 26.08 (CH_2pent_{MAJ}), 26.01 (CH_3iPr_{min}), 25.88 (CH_3iPr_{MAJ}),

24.53 (CH_3iPr), 22.72 (CH_2pent), 14.19 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{34}NO_6$: 408.2381, found: 408.2377; *m/z* $[M+Na]^+$ calcd for $C_{22}H_{33}NNaO_6$: 430.2200, found: 430.2204.

General procedure for the formation of cyclic thiocarbonate: The diol was dissolved in anhydrous THF (0.1 M) under Ar. Thiocarbonyldiimidazole (1.5 eq) was added. The mixture was heated under reflux for 4 h, then cooled to rt. The solvent was removed under vacuum and the residue was purified by flash column chromatography on silica gel to give the cyclic thionocarbonate as a mixture of rotamers (6:4).

(2*R*, 3*S*, 4*R*, 5*R*)-*N*-Benzyloxycarbonyl-3-hydroxymethyl-4,5-*O*-isopropylidene-2-pentylpiperidine-3,4,5-triol 3,3'-thiocarbonate (11). Compound **11** (74 mg, 86%) as a colorless syrup was obtained from diol **9** (78 mg, 0.191 mmol) following general procedure, after purification using petroleum ether/EtOAc (7.5:1) as the eluent. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46-7.28 (m, 5H, H_{arom}), 5.25-5.07 (m, 2H, CH_2Ph), 4.85-4.72 (m, 1H, H6b), 4.65-4.54 (m, 0.4H, $H5_{min}$), 4.50-4.32 (m, 3.2H, H6a, H3, $H5_{MAJ}$, $H1b_{MAJ}$), 4.24-4.14 (m, 1.4H, H2, $H1b_{min}$), 2.94-2.82 (m, 0.4H, $H1a_{min}$), 2.80-2.70 (m, 0.6H, $H1a_{MAJ}$), 2.05-1.91 (m, 1H, H7b), 1.51 (s, 3H, CH_3iPr), 1.36 (s, 3H, CH_3iPr), 1.32-1.10 (m, 7H, 3CH_2pent, H7a), 0.95-0.80 (m, 3H, CH_3pent). ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.92 (CS_{min}), 189.78 (CS_{MAJ}), 155.52 (CO_{MAJ}), 155.47 (CO_{min}), 136.31 ($C_{arommin}$), 136.20 ($C_{aromMAJ}$), 128.70-127.92 (CH_{arom}), 111.12 (CiPr), 87.71 ($C4_{min}$), 87.52 ($C4_{MAJ}$), 74.60 ($C2_{MAJ}$), 74.35 ($C2_{min}$), 72.95 (C6), 69.83 ($C3_{min}$), 69.55 ($C3_{MAJ}$), 68.08 (CH_2Ph_{MAJ}), 67.89 (CH_2Ph_{min}), 55.62 ($C5_{MAJ}$), 55.17 ($C5_{min}$), 39.73 ($C1_{min}$), 39.07 ($C1_{MAJ}$), 31.45, 26.32 (CH_2pent_{min}), 31.32, 25.96, 25.45 (CH_2pent_{MAJ}), 28.01 (CH_3iPr_{MAJ}), 27.81 (CH_3iPr_{min}), 26.14 (CH_3iPr), 22.48 (CH_2pent), 14.03 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{32}NO_6S$: 450.1945, found: 450.1943; *m/z* $[M+NH_4]^+$ calcd for $C_{23}H_{35}N_2O_6S$: 467.2210, found: 467.2210; *m/z* $[M+Na]^+$ calcd for $C_{23}H_{31}NNaO_6S$: 472.1764, found: 472.1763.

(2*S*, 3*S*, 4*R*, 5*R*)-*N*-Benzyloxycarbonyl-3-hydroxymethyl-4,5-*O*-isopropylidene-2-pentylpiperidine-3,4,5-triol 3,3'-thiocarbonate (14). Compound **14** (200 mg, 91%) as a colorless syrup was

obtained from diol **10** (199 mg, 0.488 mmol) following general procedure, after purification using petroleum ether/EtOAc (6:1) as the eluent. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49-7.26 (m, 5H, H_{arom}), 5.23-5.05 (m, 2H, CH_2Ph), 4.89-4.78 (m, 1H, H6b), 4.49-4.29 (m, 4H, H6a, $H5_{MAJ}$, H2, H3, $H1b_{min}$), 4.28-4.16 (m, 1H, $H1b_{MAJ}$, $H5_{min}$), 3.29-3.13 (m, 1H, H1a), 1.65-1.51 (m, 2H, H7), 1.46-1.11 (m, 12H, $3CH_2$pent, $2CH_3$iPr), 0.95-0.78 (m, 3H, CH_3pent). ^{13}C NMR (100 MHz, $CDCl_3$): δ 190.53 (CS_{MAJ}), 190.40 (CS_{min}), 156.78 (CO_{MAJ}), 156.04 (CO_{min}), 136.42 ($C_{aromMAJ}$), 135.89 ($C_{arommin}$), 128.97-128.10 (CH_{arom}), 110.69 ($CiPr_{MAJ}$), 110.54 ($CiPr_{min}$), 87.95 ($C4_{min}$), 87.71 ($C4_{MAJ}$), 75.53 ($C6_{MAJ}$), 75.43 ($C6_{min}$), 74.56, 72.79 ($C2_{MAJ}$, $C3_{MAJ}$), 74.47, 72.91 ($C2_{min}$, $C3_{min}$), 68.11 (CH_2Ph_{min}), 67.58 (CH_2Ph_{MAJ}), 55.14 ($C5_{min}$), 54.58 ($C5_{MAJ}$), 42.07 ($C1_{MAJ}$), 41.61 ($C1_{min}$), 31.93, 25.37 (CH_2pent_{MAJ}), 31.86, 25.50 (CH_2pent_{min}), 28.36 ($C7_{MAJ}$), 28.08 ($C7_{min}$), 25.68, 24.37 (CH_3iPr_{min}), 25.54, 24.20 (CH_3iPr_{MAJ}), 22.55 (CH_2pent), 14.08 (CH_3pent_{MAJ}), 14.05 (CH_3pent_{min}). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{32}NO_6S$: 450.1945, found: 450.1944; *m/z* $[M+Na]^+$ calcd for $C_{23}H_{31}NNaO_6S$: 472.1764, found: 472.1765.

General procedure for deoxygenation: Half of a degassed solution of AIBN (0.12 eq, 4 mM) in anhydrous toluene was added to a degassed solution of the cyclic thiocarbonate (0.05 M) and Bu_3SnH (2 eq) in anhydrous toluene. The mixture was stirred for 2 h at rt, and then the second half of the solution of AIBN was added. The mixture was stirred for 16 h under reflux. After cooling to rt, the reaction mixture was washed twice with 10% aqueous KF (v mL = 1.5 x total volume of toluene) and saturated aqueous $NaHCO_3$ (v mL). The combined aqueous phases were extracted 3 times with EtOAc (3v mL). The combined organic phases were dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by flash column chromatography on silica gel (petroleum ether/EtOAc 7:3) to give the protected iminosugar as a mixture of rotamers (1:1) containing traces of impurities coming from Bu_3SnH.

(2*R*, 3*R*, 4*S*, 5*R*)-*N*-Benzyloxycarbonyl-3-hydroxymethyl-4,5-*O*-isopropylidene-2-pentylpiperidine-4,5-diol (12). Compound **12** (38 mg, 60%) as a colorless syrup was obtained from cyclic thionocarbonate **11** (72 mg, 0.160 mmol) following general procedure. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43-7.20 (m, 5H, H_{arom}), 5.20 (d, 0.5H, CH_2Ph, *J* = 12.4 Hz), 5.12 (s, 1H, CH_2Ph), 5.05 (d, 0.5H, CH_2Ph, *J* = 12.4 Hz), 4.42-4.26 (m, 2H, H3, H5), 4.22 (dd, 0.5H, 0.5H1b, *J* = 7.2, 13.6 Hz), 4.17-4.00 (m, 1.5H, H2, 0.5H1b), 3.87-3.73 (m, 2H, 2H6), 2.82-2.64 (m, 1H, H1a), 2.26-2.13 (m, 1H, H4), 2.03-1.81 (m, 2H, H7b, OH), 1.49 (s, 3H, CH_3iPr), 1.33 (s, 3H, CH_3iPr), 1.43-1.05 (m, 7H, 3CH_2pent, H7a), 0.90-0.78 (m, 3H, CH_3pent). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.76, 155.48 (CO), 136.78, 136.74 (C_{arom}), 128.61-127.76 (CH_{arom}), 109.54 (CiPr), 73.41, 73.31 (C3), 71.22, 70.98 (C2), 67.45, 67.31 (CH_2Ph), 61.82, 61.75 (C6), 51.17, 50.99 (C5), 42.24, 41.86 (C4), 39.84, 39.49 (C1), 31.70, 25.90, 25.81, 22.66 (CH_2pent), 28.77, 26.29, 26.24 (CH_3iPr), 26.93, 26.78 (C7), 14.15, 14.14 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{34}NO_5$: 392.2432, found: 392.2432; *m/z* $[M+Na]^+$ calcd for $C_{22}H_{33}NNaO_5$: 414.2251, found: 414.2250.

(2*S*, 3*R*, 4*S*, 5*R*)-*N*-Benzyloxycarbonyl-3-hydroxymethyl-4,5-*O*-isopropylidene-2-pentylpiperidine-4,5-diol (15) and (2*S*, 3*S*, 4*S*, 5*R*)-*N*-Benzyloxycarbonyl-3-hydroxymethyl-4,5-*O*-isopropylidene-2-pentylpiperidine-4,5-diol (16). Compound **16** (71 mg, 43%) and compound **15** (63 mg, 38%) as colorless syrups were obtained from cyclic thionocarbonate **14** (189 mg, 0.420 mmol) following general procedure.

15: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45-7.23 (m, 5H, H_{arom}), 5.22-5.04 (m, 2H, CH_2Ph), 4.61-4.51 (m, 1H, H3), 4.37-4.21 (m, 1.5H, H2, 0.5H1b), 4.21-4.08 (m, 1.5H, 0.5H1b, H5), 3.90-3.72 (m, 2H, 2H6), 2.91-2.78 (m, 1H, H1a), 2.54-2.29 (m, 1H, OH), 1.77-1.58 (m, 2H, H4, H7b), 1.48-1.09 (m, 13H, H7a, 3CH_2pent, 2CH_3iPr), 0.91-0.74 (m, 3H, CH_3pent). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.99, 156.57 (CO), 137.07, 136.74 (C_{arom}), 128.46, 128.02, 127.88 (CH_{arom}), 109.01, 108.96 (CiPr), 74.37, 74.01, 73.64, 73.56 (C2, C3), 67.32, 66.93 (CH_2Ph), 62.92, 62.78 (C6), 49.11, 48.82 (C5), 42.93, 42.79 (C1),

40.85, 40.76 (C4), 33.50, 33.10 (C7), 32.20, 24.79, 22.70 (CH_2pent), 25.97, 24.32 (CH_3iPr), 14.15 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{34}NO_5$: 392.2432, found: 392.2429; *m/z* $[M+NH_4]^+$ calcd for $C_{22}H_{37}N_2O_5$: 409.2697, found: 409.2692; *m/z* $[M+Na]^+$ calcd for $C_{22}H_{33}NNaO_5$: 414.2251, found: 414.2249.

16: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44-7.22 (m, 5H, H_{arom}), 5.28-5.03 (m, 2H, CH_2Ph), 4.61-4.45 (m, 1H, H1b), 4.45-4.26 (m, 1H, H5), 4.18-4.02 (m, 2H, H3, H2), 3.83 (dd, 1H, H6b, *J* = 7.2, 10.4 Hz), 3.61 (br s, 1H, H6a), 3.26-3.10 (m, 1H, H1a), 2.30 (br s, 1H, OH), 2.03-2.15 (m, 1H, H4), 1.46 (s, 3H, CH_3iPr), 1.42-1.10 (m, 11H, 4CH_2pent, CH_3iPr), 0.90-0.76 (m, 3H, CH_3pent). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.11 (CO), 137.05 (C_{arom}), 128.49, 127.92, 127.46 (CH_{arom}), 109.02 (CiPr), 74.58, 71.80 (C2, C3), 67.19 (CH_2Ph), 63.62 (C6), 51.93 (C5), 44.14 (C4), 39.44 (C1), 31.59, 26.93, 25.51, 22.65 (CH_2pent), 28.43, 26.49 (CH_3iPr), 14.08 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{34}NO_5$: 392.2432, found: 392.2430; *m/z* $[M+NH_4]^+$ calcd for $C_{22}H_{37}N_2O_5$: 409.2697, found: 409.2694; *m/z* $[M+Na]^+$ calcd for $C_{22}H_{33}NNaO_5$: 414.2251, found: 414.2250.

General procedure for benzyloxycarbonyl deprotection: To a solution of the *N*-protected compound in iPrOH (0.02 M) was added 10% Pd/C (500 mg/mmol). The mixture was stirred under a H_2 atmosphere at rt until completion of the reaction (TLC). More catalyst was added if needed. Then the catalyst was filtered through a membrane, washed with iPrOH and the filtrate was concentrated under vacuum to give the deprotected compound free of Sn-containing impurities.

(2*R*, 3*R*, 4*S*, 5*R*)-3-Hydroxymethyl-4,5-*O*-isopropylidene-2-pentylpiperidine-4,5-diol (13). Compound **13** (24 mg, 96%) as a yellow syrup was obtained from *N*-protected compound **12** (38 mg, 0.097 mmol) following general procedure. $[\alpha]_D^{20}$ = + 31.7 (*c* = 1.00, MeOH). ^{1}H NMR (250 MHz, CD_3OD): δ 4.38 (dd, 1H, H3, *J* = 3.3, 5.8 Hz), 4.14 (dt, 1H, H2, *J* = 5.8, 5.8, 7.0 Hz), 3.68 (d, 2H, 2H6, *J* = 7.5 Hz), 2.99 (ddd, 1H, H5, *J* = 2.9, 6.3, 10.9 Hz), 2.79 (dd, 1H, H1b, *J* = 5.8, 13.0 Hz), 2.68 (dd,

1H, H1a, *J* = 7.0, 13.0 Hz), 2.16 (ddt, 1H, H4, *J* = 3.3, 6.3, 7.5, 7.5 Hz), 1.92-1.74 (m, 1H, H7b), 1.46 (s, 3H, CH_3iPr), 1.31 (s, 3H, CH_3iPr), 1.56-1.14 (m, 7H, 3CH_2pent, H7a), 0.92 (t, 3H, CH_3pent, *J* = 6.6 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 109.81 (CiPr), 73.90 (C3), 73.32 (C2), 61.76 (C6), 52.70 (C5), 42.84 (C1), 42.46 (C4), 33.09, 27.46, 23.62 (CH_2pent), 29.26 (C7), 28.06, 25.45 (CH_3iPr), 14.40 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{28}NO_3$: 258.2064, found: 258.2066.

(2*S*, 3*R*, 4*S*, 5*R*)-3-Hydroxymethyl-4,5-*O*-isopropylidene-2-pentylpiperidine-4,5-diol (17). Compound **17** (17 mg, 49%) as a colorless syrup was obtained from *N*-protected compound **15** (54 mg, 0.138 mmol) following general procedure, after purification using CH_2Cl_2/MeOH (9:1) as the eluent. $[\alpha]_D^{20}$ = - 28.3 (*c* = 1.00, MeOH). ^{1}H NMR (250 MHz, CD_3OD): δ 4.47 (dd, 1H, H3, *J* = 3.5, 5.5 Hz), 4.11 (dt, 1H, H2, *J* = 5.5, 5.5, 7.9 Hz), 3.33-3.29 (m, 2H, 2H6), 2.93 (dd, 1H, H1b, *J* = 5.5, 12.6 Hz), 2.58 (dd, 1H, H1a, *J* = 7.9, 12.6 Hz), 2.64-2.51 (m, 1H, H5), 1.72 (dddd, 1H, H4, *J* = 3.5, 5.3, 7.5, 11.0 Hz), 1.66-1.58 (m, 1H, H7b), 1.47 (s, 3H, CH_3iPr), 1.58-1.42 (m, 1H, H7a), 1.34 (s, 3H, CH_3iPr), 1.42-1.23 (m, 6H, 3CH_2pent), 0.92 (t, 3H, CH_3pent, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 110.62 (CiPr), 73.90 (C3), 72.50 (C2), 61.64 (C6), 53.44 (C5), 46.08 (C1), 43.58 (C4), 33.57 (C7), 33.06, 26.02, 23.58 (CH_2pent), 27.72, 25.46 (CH_3iPr), 14.37 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{28}NO_3$: 258.2064, found: 258.2064.

(2*S*, 3*S*, 4*S*, 5*R*)-3-Hydroxymethyl-4,5-*O*-isopropylidene-2-pentylpiperidine-4,5-diol (18). Compound **18** (26 mg, 67%) as a yellow syrup was obtained from *N*-protected compound **16** (60 mg, 0.153 mmol) following general procedure. $[\alpha]_D^{20}$ = + 22.4 (*c* = 1.00, MeOH). ^{1}H NMR (250 MHz, CD_3OD): δ 4.33-4.22 (m, 2H, H2, H3), 3.90 (dd, 1H, H6b, *J* = 5.0, 11.1 Hz), 3.69 (dd, 1H, H6a, *J* = 4.3, 11.1 Hz), 3.15 (dd, 1H, H1b, *J* = 6.1, 12.9 Hz), 3.10 (dt, 1H, H5, *J* = 3.8, 7.4, 7.4 Hz), 2.82 (dd, 1H, H1a, *J* = 7.0, 12.9 Hz), 2.11-2.05 (m, 1H, H4), 1.64-1.52 (m, 2H, 2H7), 1.50 (s, 3H, CH_3iPr), 1.46-1.28 (m, 9H, 3CH_2pent, CH_3iPr), 0.98-0.87 (m, 3H, CH_3pent). ^{13}C NMR (100 MHz, CD_3OD): δ 109.43 (CiPr), 76.72 (C3), 71.95 (C2), 61.56 (C6), 54.94 (C5), 47.02 (C1), 41.80 (C4), 32.94, 26.90, 23.56

(CH_2pent), 30.98 (C7), 28.53, 26.45 (CH_3iPr), 14.36 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{28}NO_3$: 258.2064, found: 258.2064.

General procedure for isopropylidene deprotection: The protected compound was dissolved in a mixture of dioxane/H_2O (5:3; 0.01 M) and Dowex 50WX8 ion-exchange resin (H^+ form, 10 mL/mmol), previously washed with dioxane/H_2O (1:1), was added. The mixture was stirred for 18 h at rt, then poured into a column and washed with dioxane/H_2O (1:1) and with H_2O. Elution with aqueous 0.5 N NH_4OH (800 mL/mmol), followed by concentration under vacuum gave the expected iminosugar.

(2*R*, 3*R*, 4*S*, 5*R*)-3-Hydroxymethyl-2-pentylpiperidine-4,5-diol or (5a*R*)-5a-*C*-pentyl-4-epi-isofagomine (1). Compound **1** (16 mg, 84%) as a colorless syrup was obtained from protected iminosugar **13** (23 mg, 0.089 mmol) following general procedure. $[\alpha]_D^{20}$ = - 9.5 (*c* = 1.00, MeOH). ^{1}H NMR (250 MHz, CD_3OD): δ 3.90 (dd, 1H, H6b, *J* = 3.5, 11.5 Hz), 3.87-3.84 (m, 1H, H3), 3.80 (dd, 1H, H6a, *J* = 4.5, 11.5 Hz), 3.75-3.68 (m, 1H, H2), 3.00 (dd, 1H, H1b, *J* = 2.8, 13.4 Hz), 2.73 (d, 1H, H1a, *J* = 13.4 Hz), 2.70-2.64 (m, 1H, H5), 1.89-1.82 (m, 1H, H4), 1.72-1.53 (m, 2H, 2H7), 1.49-1.26 (m, 6H, CH_2pent), 0.97-0.87 (m, 3H, CH_3pent). ^{13}C NMR (100 MHz, CD_3OD): δ 72.04 (C3), 68.92 (C2), 58.63 (C5), 58.15 (C6), 50.76 (C1), 44.08 (C4), 33.11, 27.39, 23.63 (CH_2pent), 32.96 (C7), 14.39 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_{24}NO_3$: 218.1751, found: 218.1753.

(2*S*, 3*R*, 4*S*, 5*R*)-3-Hydroxymethyl-2-pentylpiperidine-4,5-diol or (5a*S*)-5a-*C*-pentyl-4-epi-isofagomine (2). Compound **2** (12 mg, 86%) as a colorless syrup was obtained from protected iminosugar **17** (17 mg, 0.067 mmol) following general procedure. $[\alpha]_D^{20}$ = - 34.5 (*c* = 1.05, MeOH). ^{1}H NMR (250 MHz, CD_3OD): δ 4.10 (t, 1H, H3, *J* = 2.5 Hz), 3.65 (d, 2H, 2H6, *J* = 6.0 Hz), 2.53 (ddd, 1H, H2, *J* = 2.5, 6.6, 9.3 Hz), 2.92-2.71 (m, 3H, H5, 2H1), 1.73-1.55 (m, 1H, H7b), 1.53-1.40 (m, 1H, H4), 1.53-1.22 (m, 7H, H7a, 3CH_2pent), 0.98-0.85 (m, 3H, CH_3pent). ^{13}C NMR (100 MHz, CD_3OD): δ

70.91 (C2), 70.69 (C3), 62.14 (C6), 52.06 (C5), 48.02 (C4), 46.91 (C1), 33.64 (C7), 33.32, 25.92, 23.63 (CH_2pent), 14.40 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_{24}NO_3$: 218.1751, found: 218.1752.

(2*S*, 3*S*, 4*S*, 5*R*)-3-Hydroxymethyl-2-pentylpiperidine-4,5-diol (19). Compound **19** (22 mg, quant.) as a colorless syrup was obtained from protected iminosugar **18** (26 mg, 0.101 mmol) following general procedure. $[\alpha]_D^{20}$ = + 9.7 (*c* = 1.00, MeOH). ^{1}H NMR (250 MHz, CD_3OD): δ 4.02 (t, 1H, H3, *J* = 3.2 Hz), 3.87 (dt, 1H, H2, *J* = 3.2, 7.4, 7.4 Hz), 3.78 (dd, 1H, H6b, J_{6B-4}= 7.0, 11.3 Hz), 3.69 (dd, 1H, H6a, J_{6A-4}= 4.6, 11.3 Hz), 3.13 (dt, 1H, H5, *J* = 3.8, 7.0, 7.0 Hz), 2.87 (d, 2H, 2H1, *J* = 7.4 Hz), 2.02-1.94 (m, 1H, H4), 1.58-1.44 (m, 2H, 2H7), 1.44-1.26 (m, 6H, 3CH_2pent), 0.97-0.86 (m, 3H, CH_3pent). ^{13}C NMR (100 MHz, CD_3OD): δ 71.15 (C3), 67.38 (C2), 60.54 (C6), 53.50 (C5), 46.84 (C1), 45.72 (C4), 33.01, 27.19, 23.59 (CH_2pent), 31.60 (C7), 14.36 (CH_3pent). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_{24}NO_3$: 218.1751, found: 218.1752.

4.2. Enzymatic assays

Human PBMC lysates were used as a source of human glycosidases. Each assay was conducted using lysate from the indicated number of cells with the appropriate buffer and substrate supplemented with protease inhibitors (cOmplete™ Protease Inhibitor Cocktail Tablets, Roche) and 1% Triton X-100. Otherwise indicated, the β-galactosidase assays were conducted with the equivalent of 2 x 10^6 cells per reaction in 50 mM sodium phosphate, pH 7.3, using, as substrate, 4-methylumbelliferyl β-D-galactopyranoside at 0.8 mM. The α-galactosidase assays were conducted with the equivalent of 2 x 10^6 cells per reaction in 100 mM sodium citrate, pH 4.3, using, as substrate, 4-methylumbelliferyl α-D-galactopyranoside at 1 mM. The α-glucosidase assays were conducted with the equivalent of 2 x 10^6 cells per reaction in 100 mM sodium citrate, pH 4.3, using, as substrate, 4-methylumbelliferyl α-D-glucopyranoside at 1 mM. The β-glucosidase assays were conducted with the equivalent of 2 x 10^6 cells per reaction in 100 mM sodium citrate, pH 6.0, supplemented with 10 mM taurocholic acid and 5

mM 2-mercaptoethanol, using, as substrate, 4-methylumbelliferyl β-D-glucopyranoside at 1 mM. The *N*-acetyl-α-D-glucosaminidase assays were conducted with the equivalent of 2.5 x 10^6 cells per reaction in 50 mM sodium citrate, pH 4.3, using, as substrate, 4-methylumbelliferyl 2-acetamido-2-deoxy-α-D-glucopyranoside at 0.25 mM. The β-hexosaminidase assays were conducted with the equivalent of 0.4 x 10^6 cells per reaction in 50 mM sodium citrate, pH 4.3, using, as substrate, 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside at 2 mM. The enzymatic reactions were run for 2 hours at 37°C, with the exception of the *N*-acetyl-α-D-glucosaminidase assays, which were incubated for 17 hours. The fluorogenic reactions were stopped by the addition of an equal volume of glycine 0.15 M, pH 10.2 and fluorescence emission was determined at 445 nm using an excitation wavelength at 365 nm. The colorimetric reactions were stopped by the addition of an equal volume of NaOH 0.2 M and absorbance was determined at 405 nm.

4.3. Heat denaturation assays

The tests were conducted essentially as described in the literature [34]. Samples of human PBMC lysates (equivalent of 2 x 10^6 cells/reaction) in 50 mM sodium phosphate, pH 7.3, supplemented with protease inhibitors and 1% Triton X-100 were incubated at 48 °C for 0, 5, 10, 20, and 40 minutes in the presence of graded concentrations of compound **1**. The samples were then diluted in citrate buffer, pH 4.3, and 0.25 mM of 4-methylumbelliferyl β-D-galactopyranoside was added. After 17 hours at 37 °C, the reactions were stopped by the addition of an equal volume of glycine 0.15 M, pH 10.2. Fluorescence was then determined at 445 nm using an excitation wavelength at 365 nm.

4.4. Cell-based chaperone assays

Patient fibroblasts were purchased from the Coriell Institute (Camden, NJ) or were obtained directly from patients according to the ethical committee requirements of the respective institutions in which the cell lines were generated. The GM03348 fibroblast cell line, from a healthy donor, was from the Coriell Institute. The chaperone assays were conducted following the method described in Rigat *et al*

[11]. Fibroblasts were cultured in 25 cm^2 flasks placed in a humidified incubator (37 ^oC and 5% CO_2) using DMEM complete medium (DMEM-c) made of DMEM medium supplemented with non-essential amino acids, penicillin, streptomycin and 10% fetal calf serum (Invitrogen Thermo Fisher Scientific). For the chaperone assays, the cells were harvested by trypsinization, seeded in triplicates at 2 x 10^4 cells per well of 24 well plates in 2 ml of DMEM-c with adequate concentrations of compound. After 3 days in culture, 1 ml of medium was removed from each well and replaced by 1 mL of fresh DMEM-c medium containing the corresponding concentration of compound. Following another 2 days in culture, the medium of each well was discarded, the cells were washed twice with phosphate buffer saline, 125 µL of lysis buffer were added per well (sodium citrate 100 mM, pH 4.3, supplemented with protease inhibitors and 1% Triton X-100) and the plates were frozen at -80 ^oC. The lysates were then thawed and clarified by centrifugation (5 minutes at 15'000 rpm in an Eppendorf microfuge). For the determination of the β-galactosidase activity, 80 µL of lysate were distributed in wells of black wall fluorometric 96 well plates and 20 µL of substrate (4-methylumbelliferyl β-D-galactopyranoside at 5 mM in lysis buffer) were added per well. For the determination of the β-hexosaminidase activity, 15 µL of lysate were distributed in wells of black wall fluorometric 96 well plates, 65 µL of lysis buffer and 20 µL of substrate (4-methylumbelliferyl 2-acetamido-2-deoxy-α-D-glucopyranoside at 5 mM in lysis buffer) were added per well. The plates were then incubated for 2 hours at 37 ^oC. The enzymatic reactions were stopped by the addition of an equal volume of glycine 0.15 M, pH 10.2 and fluorescence emission was determined at 445 nm using an excitation wavelength at 365 nm.

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Abbreviations

PCs, Pharmacological chaperones; DGJ, 1-deoxygalactonojirimycin; NOEV, *N*-octyl-4-epi-β-valienamine; IFG, isofagomine; PBMC, peripheral blood mononuclear cells; DMEM, Dulbecco's Modified Eagle's Medium.

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Highlights:

- The synthesis of 3 new *C*-alkylated derivatives of 4-epi-isofagomine is described.
- Their inhibitory activities and selectivity towards human β-galactosidase were evaluated.
- Compound **1** behaved as an effective pharmacological chaperone towards several mutations.