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Synthesis of 1,5-Dideoxy-1,5-iminoribitol *C*-Glycosides through a Nitrone–Olefin Cycloaddition Domino Strategy: Identification of Pharmacological Chaperones of Mutant Human Lysosomal β-Galactosidase

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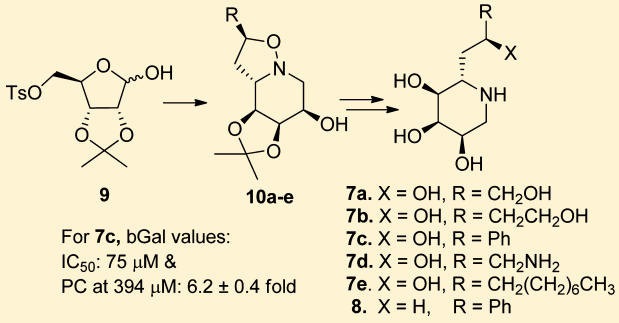
Supporting Information

ABSTRACT: We report herein a newly developed domino reaction that facilitates the synthesis of new 1,5-dideoxy-1,5-iminoribitol iminosugar *C*-glycosides **7a–e** and **8**. The key intermediate in this approach is a six-membered cyclic sugar nitrone that is generated in situ and trapped by an alkene dipolarophile via a [2 + 3] cycloaddition reaction to give the corresponding isooxazolidines **10a–e** in a "one-pot" protocol. The iminoribitol *C*-glycosides **7a–e** and **8** were found to be modest β-galactosidase (bGal) inhibitors. However, compounds **7c** and **7e** showed "pharmacological chaperone" activity for mutant lysosomal bGal activity and facilitated its recovery in GM1 gangliosidosis patient fibroblasts by 2–6-fold.



INTRODUCTION

Natural products such as 1-deoxynojirimycin (DNJ, **1**; Figure 1) and its analogues have been found to inhibit various carbohydrate-processing enzymes such as glycosidases[1] and glycosyltransferases.[2] These promising activities have led to them being developed as potential drugs for the treatment of various diseases such as viral infections, diabetes, and lysosomal storage disorders (LSDs). The LSDs result in GM1 gangliosidosis and Gaucher diseases[3] due to the 10–20% reduction of the acid β-galactosidase (bGal) and β-glucocerebrosidase (GCase) activity, respectively, in the lysosome.[4] It has been demonstrated that the intracellular activity of a given defective enzyme, leading to an LSD, can be increased using an appropriate "pharmacological chaperone" (PC).[5] Small molecules such as iminosugar motifs continue to attract considerable interest as potential PCs for the treatment for LSDs. For example, *N*-butyl-1-deoxynojirimycin (**2**) (marketed under the trade name Zavesca) is used in substrate reduction therapy of Gaucher disease.[5] A number of *N*-alkylated iminosugars such as 1-deoxygalactonojirimycin (DGJ, **3a**) and *N*-nonyl-DGJ (**3b**) are known as PCs able to rescue the intracellular activity of mutant bGal in GM1 gangliosidosis patient fibroblasts.[4a] The *N*-octyl-4-*epi*-β-valienamine (**4**) and a bicyclic DGJ analogue, **5a**, which are competitive inhibitors of bGal, also function as PCs in a mouse model of GM1 gangliosidosis (featuring the R201C point mutation).[4a,6]

A popular strategy for the design of a potential PC is to take a natural inhibitor of the compromised enzyme of interest and make a rational chemical modification to its structure, with a view to accentuate its inhibitory activity and selectivity toward its target enzyme. However, the inhibitory potency of an iminosugar does not always correlate in a simple way with its performance as a PC, and even rather poor inhibitors of a particular mutant glycosidase have proved in several instances to behave as PCs.[6b] Such findings underline the difficulties in formulating reliable structure–activity relationships (SARs) for PCs. Recently, our group as well as others have described iminosugar *C*-glycosides **6a–c** with interesting PC activities for mutant GCase.[7] In the continuation of our interest in this area, we were curious to know how compounds analogous to those described for GCase would behave as PCs toward mutant bGal. In this respect, we report here a new domino strategy[8] for the synthesis of a small library of iminosugar *C*-glycosides **7a–e**

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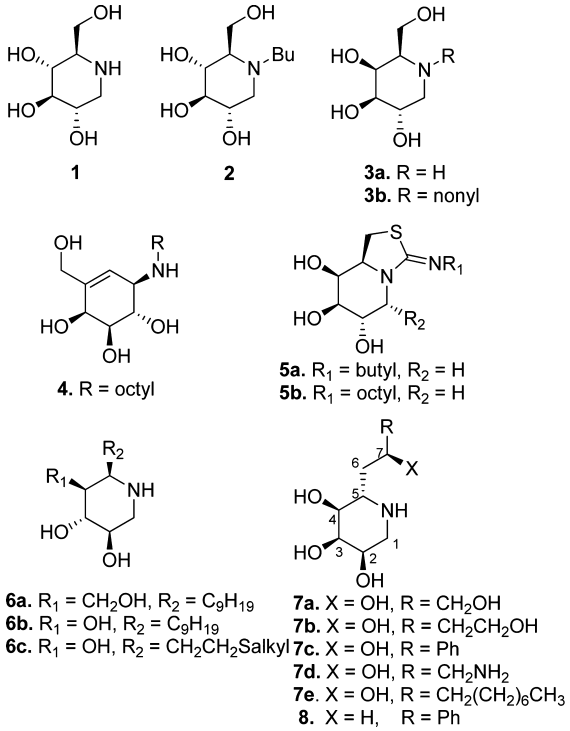


Figure 1. Iminosugars and iminosugar *C*-glycosides **7a–e** and **8**.

and **8** and a study of their glycosidase inhibition activities as well as their effect as PCs of mutant bGal in GM1 gangliosidosis patient fibroblasts.

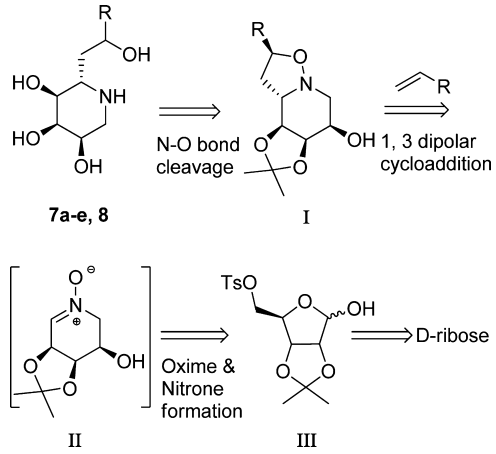
RESULTS AND DISCUSSION

Among the various synthetic approaches described to iminosugars and their analogues,[9] several that figure prominently exploit sugar-derived nitrones and five-membered cyclic sugar nitrone[10] intermediates.[11] Fewer reports exist however in which the analogous six-membered nitrones are intermediates in the syntheses of the corresponding piperidine, indolizidine, and pyrrolidizine skeletons.[12] Indeed, the use of a six-membered sugar nitrone as a precursor in the synthesis of iminosugar *C*-glycosides via a domino sequence,[8] to the best of our knowledge, is not known.

A retrosynthetic analysis (Scheme 1) suggests that the targeted iminosugar *C*-glycosides **7** and **8** could be obtained from the isooxazolidine **I** upon deprotection of the acetonide group and N–O bond cleavage. The key intermediate **I** might in turn be obtained via an intermolecular 1,3-dipolar cycloaddition of an in situ generated six-membered sugar nitrone, **II**, and an alkene. We anticipated that a D-ribose-derived hemiacetal, **III**, bearing a 5-*O*-tosyl group as a leaving group would give the required nitrone **II** in a single step.[13] Furthermore, if conditions could be developed such that the in situ formed sugar nitrone **II** were to undergo a [2 + 3] cycloaddition with a preadded dipolarophile in a "one-pot" domino pathway, then the need for isolation or purification of the relatively unstable six-membered ring sugar nitrone intermediate **II** would be obviated.

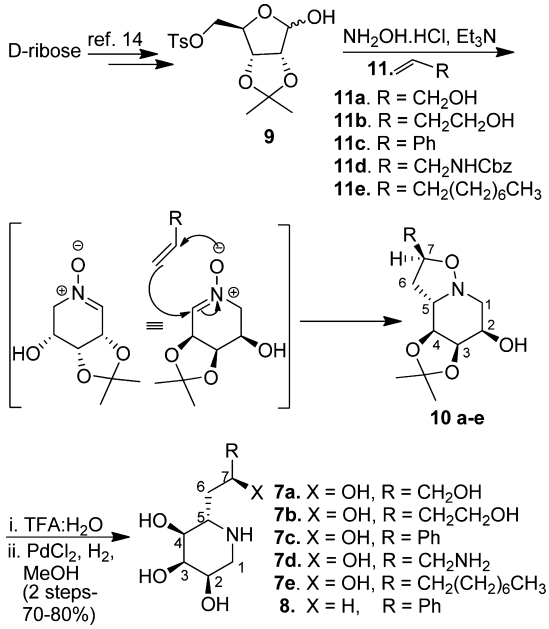
The required key precursor, 2,3-*O*-isopropylidene-5-*O*-tosyl-α-D-ribofuranose (**9**), was obtained from D-ribose in two steps in an overall 86% yield as described earlier.[14] In a trial reaction, precursor **9** was mixed with hydroxylamine hydrochloride and allyl alcohol (**11a**) (10 equiv) in the presence of triethylamine,

Scheme 1. Retro-Synthetic Analysis of Iminosugar *C*-Glycosides



and upon being heated at 100 °C, the resulting mixture afforded the ring-fused isooxazolidine **10a** as the major isolable product in 68% yield (Scheme 2 and Table 1, entry i). We

Scheme 2. Synthesis of Iminosugars 7a–e and 8



believe that the domino pathway involves first the formation of a sugar oxime (from D-ribose) followed by its intramolecular *N*-alkylation with departure of the *O*-tosyl group to give the corresponding six-membered cyclic nitrone, which concomitantly undergoes an intermolecular 1,3-dipolar cycloaddition

Table 1. Domino Reaction of 9 with Different Alkenes 11a–e

entry	alkene	solvent	time (h)/temp (°C)	product	yield (%)
i	**11a**	neat	4/100	**10a**	68
ii	**11b**	neat	4/100	**10b**	73
iii	**11c** (3.5 M)	ethanol	8/70	**10c**	69
iv	**11d** (3.5 M)	ethanol	10/70	**10d**	67
v	**11e** (3.5 M)	toluene	11/80	**10e**	66

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with allyl alcohol present to give the expected isooxazolidine **10a** in a one-pot transformation.

The [2 + 3] cycloaddition step in this domino sequence proceeded with high stereoselectivity to give only one diastereoisomeric isooxazolidine. In principle, four diastereomers would be possible due to the *exo*/*endo*-selectivity as well as the regioselective addition of allyl alcohol to nitrone in the 1,3-dipolar cycloaddition. Tufariello and co-workers[15] reported that the [2 + 3] cycloaddition reaction of allyl alcohol with a six-membered cyclic nitrone occurs regioselectively and that the nitrone oxygen attacks the internal carbon of the alkene moiety, with an *exo*-selective addition of allyl alcohol, and moreover explained that the stereochemical identity at the ring junction is governed by the π-facial selectivity at the nitrone carbon. We expected that the orientation of H-5 (axial or equatorial) at the fused carbon C-5 in **10a** with respect to H-4 (as shown in stereostructures **A** and **B**, Figure 2) could in principle be

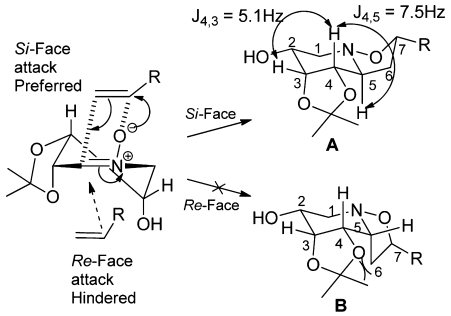

Figure 2. Facial approach of the alkene to the nitrone.

established from an exhaustive analysis of the NMR coupling constant data for **10a**. However, the ^{1}H NMR spectrum of compound **10a** in $CDCl_3$ features broad, overlapping sets of signals due to equivalence of protons that appear at nearly identical δ values.[16] In an attempt to overcome this problem, the ^{1}H NMR spectrum of **10a** was recorded in alternative deuterated solvents in the hope that a solvent shift effect[17] might come into play and give the appropriately resolved spectrum. Although, in the case of acetone-d_6 and MeOH-d_4, the signals for H-4 and H-5 remained overlapped (merged with signals for H-2 and H-7), the spectrum of **10a** in benzene-d_6 at 30 °C was found to be better resolved. The resolution was further improved when the spectrum was recorded at 50 °C in benzene-d_6 (see the Supporting Information, Figures 23S, 23.1S, and 23.2S), giving signals with good multiplicity from which the assignments were made conveniently. In the decoupling experiments, irradiation of the signal at δ 3.91 (H-3) converts the doublet of doublets at 3.69 (H-4) into a doublet ($J_{4,5}$ = 7.5 Hz) while irradiation of the signal at δ 2.85 (H-5) led to collapse of the multiplet at δ 3.69 (H-4) into a doublet ($J_{4,3}$ = 5.1 Hz). The large coupling observed for H-4 ($J_{4,5}$ = 7.5 Hz) requires an axial−axial orientation of H-4 and H-5. As H-4 is axially oriented in both stereostructures **A** and **B** (Figure 2), H-5 must necessarily also be axially orientated, thus placing the alkyl side chain equatorial with an absolute configuration of 5*R*. The value of the axial−equatorial coupling constant for H-4 ($J_{4,3}$ = 5.1 Hz) suggests that the dihedral angle between H-4 and H-3 is close to 10°. We ascribe this to a distortion of the piperidine ring conformation possibly provoked by its being part of a rather strained tricyclic framework. The assignments are consistent with a preferred "*si*-facial" attack of the dipolarophile at the nitrone carbon, the alternative "*re*-facial" attack expected to be disfavored due to steric hindrance by the isopropylidene functionality as depicted in Figure 2.

The assignment of the relative stereochemistry at the newly generated carbon centers C-5 and C-7 was established using the ^{1}H NMR chemical shift difference (δ) of H-6 methylene protons wherein $\Delta\delta$ for *cis*-isooxazolidine is higher than for *trans*-isooxazolidine.[18] The negligible $\Delta\delta$ value observed for the H-6 methylene protons is consistent with conformation **A** formed via the *exo*-selective addition of the alkene at the nitrone carbon to circumvent the unfavorable steric interactions between the ring hydrogens with the alkyl/aryl group of alkene existing in the "*endo*" addition mode.[15] Thus, the overall 1,3-dipolar cycloaddition of the alkene to the six-membered cyclic nitrone was found to be *exo* with *si*-facial selectivity at the nitrone carbon to give isooxazolidine **10a**.

To establish the scope of the domino reaction, we next varied the alkene component. Thus, homoallyl alcohol was reacted with precursor **9** under the same reaction conditions that afforded isooxazolidine **10b** as a single diastereoisomer in high yield (Table 1, entry ii). The application of the domino sequence to alternate alkenes, including styrene (**11c**), Cbz-protected allylamine (**11d**), and 1-decene (**11e**), required their prior solution in mixtures of ethanol/toluene as a solvent. The change in operational conditions to include a cosolvent rather than a neat alkene component afforded the corresponding cycloadducts **10c**−**e** with high selectivity and good yields (Table 1, entries iii−v).

Targeting the synthesis of iminosugar *C*-glycosides, the individual isooxazolidines **10a**−**e** were separately treated with TFA−H_2O for the hydrolysis of the acetonide group, affording on reductive cleavage of the N−O bond using $PdCl_2$ under hydrogen pressure the corresponding 1,5-dideoxy-1,5-iminoribitol *C*-glycosides **7a**−**e** in 70−80% yield. In contrast, treatment of **10c** under the same conditions afforded two compounds, one arising from the N−O bond cleavage (**7c**, 34%) and a second arising from both the N−O bond rupture and the O−CHPh bond cleavage (**8**, 44%). No optimization was attempted, and instead both analogues were subjected to biological assays.

Conformational Assignments of 7a−e and 8. It is known that the biological activity of iminosugar *C*-glycosides is dependent on their preferred solution conformation ($^4C_1$ or $^1C_4$), and it is the relative orientation of the *C*-alkyl substituent that has a major influence on the conformational preference.[19] Conformations adopted by **7a**−**e** and **8** were established using their ^{1}H NMR data. Of particular significance was the value of the coupling constant between the H-1 and H-2 protons (^{1}H NMR in D_2O), which were in some cases evaluated with recourse to decoupling experiments. The large coupling constant values for 1Ha ($J_{1a,2a}$ = 11−12 Hz and $J_{1a,1e}$ = 11−12 Hz) suggested that compounds **7a**−**e** and **8** exist predominantly in their $^1C_4$ conformation (Figure 3). This observation is in agreement with the previously reported *C*-glycoside iminosugars.[7b,d]

Inhibitory Activity toward Selected Glycosidases. The inhibitory activity of iminosugar *C*-glycosides **7a**−**e** and **8** was studied against lysosomal bGal, human α-galactosidase (haGal), human α-glucosidase (haGlu), α-glucosidase (aGlu), α-galactosidase (aGal), and α-mannosidase (aMan). The observed values are shown in Table 2 (see also the Experimental Section and Supporting Information). None of

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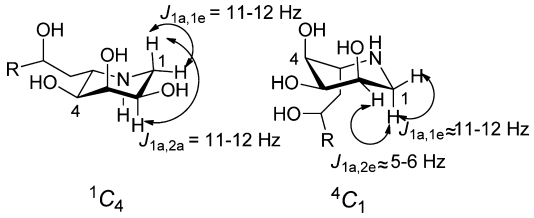

Figure 3. Conformations of **7a–e** and **8**.

Table 2. Inhibitory Activity (IC_{50}, mM) of Synthetic Compounds against Screened Glycosidases

compd	bGal[a]	haGal[b]	haGlu[c]	aGlu[d]	aGal[e]	aMan[f]
7a	0.66	NI[g]	NI	0.18	0.21	0.17
7b	0.66	NI	NI	0.44	0.20	0.18
7c	0.075	NI	NI	0.20	0.15	0.29
7d	0.38	NI	NI	1.1	0.27	0.22
7e	0.044	NI	NI	0.16	1.8	0.15
8	0.11	NI	NI	0.20	0.20	0.15

[a]Lysosomal β-galactosidase. [b]Human α-galactosidase. [c]Human α-glucosidase. [d]α-Glucosidase (*Bacillus stearothermophilus*). [e]α-Galactosidase (green coffee beans). [f]α-Mannosidase (*Canavalia ensiformis*). [g]NI = no inhibition observed at the highest concentration tested (1 mM).

the analogues **7a–e** and **8** showed any inhibition of haGal and haGlu even at high concentration (1 mM), although weak inhibition of bGal, aGlu, aGal, and aMan was observed. This β-selective recognition of bGal over haGal and haGlu, displayed by the iminosugars, is considered a favorable characteristic for compounds intended as PCs.[6b,20] The IC_{50} values against lysosomal enzyme bGal for compounds **7c** and **7e** were found to be 75 and 44 μM, respectively. These values are large relative to those reported for bicyclic analogue **5a**/**5b** or DGJ (**3a**) (3.1 μM (pH 7.0), 0.5 μM (pH 7.0), and 25 μM (pH 5.0), respectively) and much higher than those observed for **3b** and **4** (0.12 and 0.02 μM, respectively), which are both reported as PCs for bGal.[6b] No significant inhibition was observed with compounds **7a–e** and **8** when screened for the nonmammalian orthologues aGlu, aGal, and aMan.

Treatment of GM1 Gangliosidosis Patient Fibroblasts. We were curious to ascertain if any of the new compounds behave as PCs and enhance the activity of mutant bGal in patient cells, with the knowledge that relatively poor inhibitors of glycosidase had nevertheless shown significant activity as PCs in the past.[6b] Fibroblasts from a patient with GM1 gangliosidosis heterozygous for the mutations (p.R201H/IVS14-2A>G) were used to test the efficacy of the compounds on increasing the intracellular activity of the mutant bGal enzyme (see the Experimental Section).

Only compounds **7c**, **7e**, and **8** with IC_{50} values of <100 μM were examined against patient fibroblasts and were found to enhance mutant bGal activity after 5 days of incubation (Figure 4A). Intracellular β-hexosaminidase (Hex) activity was used as a control for cell number as well as to monitor toxicity. Although compound **7e** increased intracellular bGal activity approximately 2-fold, it has a significant negative effect on the viability of cells at concentrations greater than 100 μM as indicated by the parallel decrease in bGal and Hex activity (Figure 4B). On the other hand, compound **7c** elicited a 6-fold increase in bGal activity, an increase greater than the 4-fold increase recently

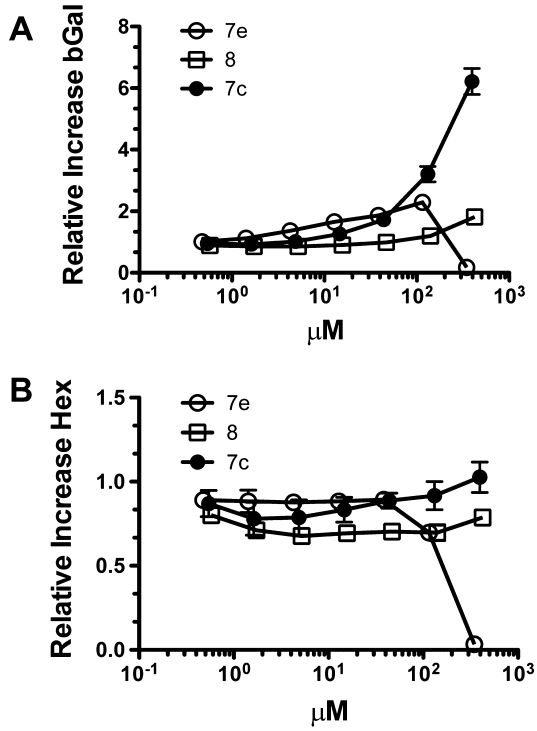

Figure 4. Intracellular bGal activity in compound-treated GM1 gangliosidosis patient fibroblasts: (A) mutant bGal activity, (B) wild-type Hex activity controlling for cell number and toxicity. Average values (N = 3) and standard deviations are shown.

reported for *N*-nonyl-DGJ (**3b**) against the same bGal mutant.[4a]

The difference in chaperoning efficacy of compound **7c** compared to **7e** suggests that the nature of the *C*-aglycon is important in dictating the PC activities of such iminosugar-based analogues. The origin of the effect might relate to the physicochemical properties of the pseudoaglycon function that are known to have consequences on the bioavailability of the compound, i.e., the ability of the PC to enter the cell and gain access to the endoplasmic reticulum (ER) or other off-target effects. This has been suggested previously to explain variations observed in PC efficacy of a number of analogues directed against GCase.[7a–c,21,22]

A comparison of the maximal increase in mutant bGal PC activity vs bGal inhibitory activity (IC_{50}) of compound **7c** (IC_{50} = 75 μM; PC at 394 μM, (6.2 ± 0.4)-fold) with corresponding data for *N*-nonyl-DGJ (**3b**) (IC_{50} = 0.12 μM; PC at 1.1 μM, 4.5-fold) against the same patient fibroblasts (p.R201H/IVS14-2A>G)[4a] is pertinent. IC_{50} values were determined using the same substrate concentration (MU-bGal, 0.45 mM) and similar enzyme preparation (human bGal (K_m = 0.3 mM) concanavalin A-enriched lysosomal enzyme preparation). The bicyclic DGJ isothiourea analogue **5b** maximally increases the intracellular activity of bGal featuring the R201C mutation more than 6-fold (at 240 μM) as compared to that achieved with analogue **4** (at 0.2 μM).[6b] That the PC activity of any iminosugar analogue, toward a given mutant enzyme, often correlates very poorly with its inhibitory potency of the wild-type enzyme has been remarked upon in the past in the case of both Gaucher and gangliosidosis GM1.[6b,20] A notable example is a series of *N*-substituted δ-lactams that were reported to be weak inhibitors of human GCase.[21] For all but one member of the latter series, IC_{50} values of >100 μM were recorded. Nonetheless, several of

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the latter *N*-substituted δ-lactams were reported to display significant PC activity.

CONCLUSION

In conclusion, we have devised a new domino strategy for the synthesis of 1,5-dideoxy-1,5-iminoribitol *C*-glycosides **7a−e** and **8**. Our methodology involves treatment of 2,3-*O*-isopropylidine-5-*O*-tosyl-α-D-ribofuranose with hydroxylamine hydrochloride to give a six-membered cyclic nitrone trapped by an alkene dipolarophile in a [2 + 3] cycloaddition reaction to give the corresponding isooxazolidines **10a−e** in one pot. Two of these analogues, **7c** and **7e**, have been shown to inhibit only modestly the target lysosomal bGal activity (IC_{50} < 100 μM), but none of the analogues **7a−e** and **8** inhibited either the haGal or the corresponding haGlu activities. The high β/α-selectivity shown by these new analogues is notable. Although weak inhibitors of bGal, compounds **7c** and **7e** were found to facilitate the recovery of mutant lysosomal bGal activity in GM1 gangliosidosis patient fibroblasts by 2−6-fold.

EXPERIMENTAL SECTION

General Methods. Melting points were recorded with a melting point apparatus and are uncorrected. IR spectra were recorded with an FTIR instrument as a thin film or using KBr pellets, and the data are expressed in inverse centimeters. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded using $CDCl_3$, acetone-d_6, or D_2O as the solvent(s). Chemical shifts are reported in δ (parts per million) with reference to TMS as an internal standard, and *J* values are given in hertz. Optical rotations were measured using a polarimeter. High-resolution mass spectrometry (HRMS) was performed on an electron spray ionization time-of-flight (ESI-TOF) instrument. Thin-layer chromatography was performed on precoated plates (0.25 mm, silica gel 60 F254). Column chromatography was carried out with silica gel (100−200 mesh). The reactions were carried out in oven-dried glassware under a dry N_2 atmosphere. Methanol, acetone, and ethanol were purified and dried with the method reported in Vogel's textbook of practical organic chemistry. The petroleum ether (PE) that was used was a distillation fraction between 40 and 60 °C. $PdCl_2$ was purchased from a chemical supplier. The purity of the compounds was determined to be >95% by elemental microanalysis.

2,3-*O*-Isopropylidene-5-*O*-tosyl-α-D-ribofuranose (9).[14] To a suspension of D-ribose (10 g, 66.64 mmol) in dry acetone (60 mL) was added dropwise concd H_2SO_4 (6 mL) at room temperature. The mixture was stirred at rt for 3 h. The reaction mixture was neutralized using solid $NaHCO_3$ and filtered, and the filtrate was evaporated in vacuo. The residue was purified by column chromatography by eluting with petroleum ether−EtOAc (55:45) to give a thick liquid (11.7 g, 93%).

A solution of TsCl (3.31 g, 17.35 mmol, 1.1 equiv) in pyridine (5 mL) was added dropwise to a mixture of the above compound (3.0 g, 15.77 mmol, 1 equiv) in pyridine (5 mL) at −10 °C. The resulting mixture was stirred at 0 °C for 1/2 h and then at 25 °C for 4 h. Ethyl acetate (50 mL) was added, and the resulting solution was washed with 10% H_2SO_4 (4 × 10 mL). The organic layer was further washed with $NaHCO_3$ (3 × 8 mL) and dried (Na_2SO_4), and the solvent was evaporated under vacuum. The residue was purified by column chromatography by eluting with petroleum ether−EtOAc (85:15) to give **9** (4.3 g, 80%) as a white solid, mp =95−96 °C. The spectroscopic data of **9** were identical to those previously reported.[14]

General Procedure for Compounds 10a and 10b. To mixtures of compound **9** (1.0 g, 2.91 mmol, 1 equiv) and alkenes **11a,b** (29.1 mmol, 10 equiv) as the solvent for reaction were added $NH_2OH{\cdot}HCl$ (0.8 g, 11.64 mmol, 4 equiv) and Et_3N (2.03 mL, 14.55 mmol, 5 equiv) simultaneously. The reaction mixtures were stirred for 30 min and on TLC analysis showed consumption of starting material. Then the reaction mixtures were refluxed for 4 h, and the solvent was evaporated at reduced pressure. The crude masses were subjected to column chromatography to obtain compounds **10a** and **10b**.

*Data for (3aR,4R,8S,9aS,9bS)-8-(hydroxymethyl)-2,2-dimethylhexahydro-3aH-[1,3]dioxolo[4,5-c]isoxazolo[2,3-a]pyridin-4-ol (**10a**):* R_f = 0.3 in EtOAc:acetone = 9:1; column purification in EtOAc:acetone = 94:6; yield 0.48 g, 68%; mp 121−123 °C; $[\alpha]_D^{27.1}$ = −54.4 (*c* = 0.10 $CHCl_3$); IR (neat; ν, cm^{-1}) 3252 (br), 2980, 1640, 1448, 1375, 1219, 1030; ^{1}H NMR (300 MHz, $CDCl_3$ + 1 drop of D_2O) δ 1.38 (s, 3H), 1.53 (s, 3H), 2.04−2.24 (m, 1H), 2.35 (ddd, *J* = 4.8, 7.2, 11.7 Hz, 1H), 2.50−2.64 (m, 2H), 3.32−3.46 (m, 1H), 3.56 (dd, *J* = 4.8, 12 Hz, 1H), 3.76 (dd, *J* = 1.8, 12 Hz, 1H), 4.02−4.30 (m, 3H), 4.33 (t, *J* = 4.8 Hz, 1H); ^{13}C NMR (75 MHz, acetone-d_6) δ 26.1, 28.1, 35.9, 56.4 (w), 64.0, 66.4 (w), 76.2, 77.4, 78.0, 78.4, 109.6; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{11}H_{20}NO_5$ 246.1342, found 246.1341.

*Data for (3aR,4R,8S,9aS,9bS)-8-(2-hydroxyethyl)-2,2-dimethylhexahydro-3aH-[1,3]dioxolo[4,5-c]isoxazolo[2,3-a]pyridin-4-ol (**10b**):* R_f = 0.4 in EtOAc:acetone = 9:1; column purification in EtOAc:acetone = 95:5; yield 0.55 g, 73%; $[\alpha]_D^{28.7}$ = −155.2 (*c* = 0.139 $CHCl_3$); IR (neat; ν, cm^{-1}) 3367 (br), 2937, 1647, 1382, 1244, 1039; ^{1}H NMR (300 MHz, $CDCl_3$ + 1 drop of D_2O) δ 1.39 (s, 3H), 1.53 (s, 3H), 1.78−1.90 (m, 2H), 2.14−2.24 (m, 2H), 2.72−2.92 (br m, 2H), 3.35−3.5 (m, 1H), 3.75 (t, *J* = 5.7 Hz, 2H), 4.05−4.17 (m, 2H), 4.28−4.34 (m, 2H); ^{13}C NMR (75 MHz, acetone-d_6) δ 25.7, 27.7, 38.5, 38.8, 56.1 (w), 58.9, 65.6, 66.0 (w), 74.4, 75.7, 77.1, 109.3; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{12}H_{22}NO_5$ 260.1499; found 260.1499.

General Procedure for Compounds 10c−e. To mixtures of compound **9** (1 g, 2.91 mmol, 1 equiv) and alkenes **11c−e** (3.5 M solution in ethanol/toluene, 29.1 mmol, 10 equiv) were added $NH_2OH{\cdot}HCl$ (0.8 g, 11.64 mmol, 4 equiv) and Et_3N (2.03 mL, 14.55 mmol, 5 equiv) simultaneously. The reaction mixtures were stirred for 45 min and on TLC analysis showed consumption of starting material. Then the reaction mixtures were refluxed for 8−11 h, and the solvent was evaporated at reduced pressure. The crude masses were subjected to column chromatography to obtain compounds **10c−e**.

*Data for (3aR,4R,8S,9aS,9bS)-2,2-dimethyl-8-phenylhexahydro-3aH-[1,3]dioxolo[4,5-c]isoxazolo[2,3-a]pyridin-4-ol (**10c**):* R_f = 0.5 in petroleum ether:EtOAc = 1:1; column purification in petroleum ether:EtOAc = 76:24; yield 0.58 g, 69%; $[\alpha]_D^{27.3}$ = −85.2 (*c* = 0.10 $CHCl_3$); IR (neat; ν, cm^{-1}) 3392 (br), 2986, 1647, 1455, 1381, 1219, 1048, 866, 699; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 3H), 1.54 (s, 3H), 2.39−2.58 (m, 3H; after D_2O exchange this multiplet integrated for 2 protons), 2.82−3.10 (m, 2H), 3.57−3.65 (m, 1H), 4.13−4.30 (m, 2H), 4.38 (t, *J* = 4.8 Hz, 1H), 5.07 (dd, *J* = 6, 8.7 Hz, 1H), 7.20−7.42 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 27.8, 41.4, 55.5 (w), 65.9 (w), 66.0, 74.7, 76.7, 78.6, 109.8, 126.4 (s), 127.9, 128.5 (s), 140.9; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{16}H_{22}NO_4$ 292.1550, found 292.1546.

*Data for benzyl (((3aR,4R,8S,9aS,9bS)-4-hydroxy-2,2-dimethylhexahydro-3aH-[1,3]dioxolo[4,5-c]isoxazolo[2,3-a]pyridin-8-yl)methyl)carbamate (**10d**):* R_f = 0.5 in EtOAc; column purification in petroleum ether:EtOAc = 52:48; yield 0.74 g, 67%; $[\alpha]_D^{28.7}$ = +97.5 (*c* = 0.096 $CHCl_3$); IR (neat; ν, cm^{-1}) 3347 (br), 2925, 2854, 1705, 1539, 1455, 1381, 1253, 1055, 866, 698; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (s, 3H), 1.51 (s, 3H), 2.08−2.24 (m, 2H), 2.65−2.95 (m, 2H), 3.22−3.50 (m, 3H), δ 3.95−4.22 (m, 3H), 4.28 (t, *J* = 4.8 Hz, 1H), 5.07 (br s, 2H), 7.14−7.25 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.8, 27.6, 29.7, 36.0, 44.1, 56.1 (w), 65.1 (w), 66.0, 66.8, 74.5, 75.7, 77.2, 109.8, 128.11, 128.16, 128.5, 136.3, 156.5; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{19}H_{27}N_2O_6$ 379.1879, found 379.1867.

*Data for (3aR,4R,8R,9aS,9bS)-2,2-dimethyl-8-octylhexahydro-3aH-[1,3]dioxolo[4,5-c]isoxazolo[2,3-a]pyridin-4-ol (**10e**):* R_f = 0.6 in petroleum ether:EtOAc = 1:1; column purification in petroleum ether:EtOAc = 78:22; yield 0.63 g, 66%; $[\alpha]_D^{28.4}$ = −68.0 (*c* = 0.112 $CHCl_3$); IR (neat; ν, cm^{-1}) 3360 (br), 2926, 2855, 1650, 1378, 1246, 1071; ^{1}H NMR (300 MHz, $CDCl_3$ + 1 drop of D_2O) δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.15−1.70 (m, 20H), 2.0−2.17 (m, 2H), 2.55−2.85 (br m, 2H), 3.40−3.52 (m, 1H), 4.02−4.20 (m, 3H), 4.32 (t, *J* = 4.8 Hz, 1H); ^{13}C NMR (75 MHz, acetone-d_6) δ 14.3, 23.2, 26.2, 26.6, 28.3, 29.0, 29.9, 30.2, 32.5, 35.9, 39.4, 56.1 (w), 66.1, 66.2 (w), 76.3, 77.4, 77.7,

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109.7; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{18}H_{34}NO_4$ 328.2489, found 328.2500.

General Procedure for Compounds 7a−e and 8. Solutions of compounds **10a−e** (0.2 g, 1 equiv) in 4 mL of TFA−water (3:2) were stirred for 2 h at 0 °C to rt. TFA was coevaporated with toluene at reduced pressure. The crude reaction masses were taken in methanol, and $PdCl_2$ (0.2 equiv) was added. The reaction mixtures were hydrogenated at balloon pressure for 6 h. The reaction mixtures were filtered through Celite and washed with methanol, and the solvent was evaporated at reduced pressure. Purification by column chromatography (chloroform−methanol−25% aq NH_3) gave compounds **7a−e** and **8**.

*Data for (2S,3S,4R,5R)-2-((S)-2,3-dihydroxypropyl)piperidine-3,4,5-triol (**7a**):* R_f = 0.4 in $CHCl_3$:MeOH (1 drop of 25% aq NH_3) = 2:3; column purification in $CHCl_3$:MeOH:aq NH_3 (25% solution) = 71:28:1; yield 0.13 g, 77%; $[\alpha]_D^{28.4}$ = −88.0 (c = 0.1 MeOH); IR (neat; ν, cm^{-1}) 3389 (br); ^{1}H NMR (300 MHz, D_2O) δ 1.42 (ddd, J = 2.4, 9.6, 13.0 Hz, 1H), 1.90 (ddd, J = 3.0, 10.4, 13.0 Hz, 1H), 2.73 (t, J = 11.9 Hz, 1H), 2.80−2.98 (m, 2H), 3.36 (dd, J = 1.9, 10.0 Hz, 1H), 3.53 (dd, J = 6.6, 11.7 Hz, 1H), 3.61 (dd, J = 4.5, 11.7 Hz, 1H), 3.70−3.82 (m, 1H), 3.86−3.98 (m, 1H), 4.12 (br s, 1H); ^{13}C NMR (75 MHz, D_2O) δ 34.5, 43.7, 50.3, 65.8, 68.0, 68.3, 71.2, 71.9; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_8H_{18}NO_5$ 208.1186, found 208.1184. Anal. Calcd for $C_8H_{17}NO_5$: C, 46.37; H, 8.27; N, 6.76. Found: C, 46.43; H, 8.24; N, 6.69.

*Data for (2S,3S,4R,5R)-2-((S)-2,4-dihydroxybutyl)piperidine-3,4,5-triol (**7b**):* R_f = 0.5 in $CHCl_3$:MeOH (1 drop of 25% aq NH_3) = 2:3; column purification in $CHCl_3$:MeOH:aq NH_3 (25% solution) = 74:25:1; yield 0.14 g, 80%; $[\alpha]_D^{28.4}$ = −285.9 (c = 0.154 MeOH); IR (neat; ν, cm^{-1}) 3100 (br); ^{1}H NMR (300 MHz, D_2O) δ 1.45 (ddd, J = 1.8, 9.0, 11.8 Hz, 1H), 1.67−1.82 (m, 2H), 1.90 (ddd, J = 2.4, 9.6, 11.8 Hz, 1H), 2.70 (t, J = 11.1 Hz, 1H), 2.78−2.99 (m, 2H), 3.33 (dd, J = 2.4, 9.9 Hz, 1H), 3.63−3.82 (m, 3H), 3.90−4.02 (m, 1H), 4.08 (br s, 1H); ^{13}C NMR (75 MHz, D_2O) δ 38.4, 39.1, 43.6, 50.5, 58.4, 65.0, 67.8, 71.1, 71.8; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_9H_{20}NO_5$ 222.1342, found 222.1342. Anal. Calcd for $C_9H_{19}NO_5$: C, 48.86; H, 8.66; N, 6.33. Found: C, 48.82; H, 8.89; N, 6.23.

*Data for (2S,3S,4R,5R)-2-((S)-2-hydroxy-2-phenylethyl)piperidine-3,4,5-triol (**7c**):* R_f = 0.4 in $CHCl_3$:MeOH (1 drop of 25% aq NH_3) = 4:1; column purification in $CHCl_3$:MeOH:aq NH_3 (25% solution) = 88:11:1; yield 0.06 g, 34%; mp 158−160 °C; $[\alpha]_D^{28.3}$ = −47.2 (c = 0.121 MeOH); IR (neat; ν, cm^{-1}) 3321 (br), 883, 697; ^{1}H NMR (300 MHz, D_2O) δ 1.68 (ddd, J = 3.4, 9.1, 13.2 Hz, 1H), 2.24 (ddd, J = 3.0, 9.5, 13.2 Hz, 1H), 2.68 (t, J = 11.8 Hz, 1H), 2.82 (dd, J = 5.1, 11.8 Hz, 1H), 2.85−2.92 (td, J = 2.4, 9.8 Hz, 1H), 3.34 (dd, J = 2.4, 9.8 Hz, 1H), 3.65−3.76 (m, 1H), 4.09 (br s, 1H), 4.95 (dd, J = 3.4, 9.5 Hz, 1H), 7.30−7.55 (m, 5H); ^{13}C NMR (75 MHz, D_2O) δ 40.3, 43.8, 50.7, 68.0, 70.5, 71.3, 72.0, 125.8, 127.8 (s), 128.7 (s), 144.0; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{13}H_{20}NO_4$ 254.1393, found 254.1393. Anal. Calcd for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.66; H, 7.42; N, 5.52.

*Data for (2S,3S,4R,5R)-2-((S)-3-amino-2-hydroxypropyl)-piperidine-3,4,5-triol (**7d**):* R_f = 0.2 in MeOH (1 drop of 25% aq NH_3); column purification in MeOH:aq NH_3 (25% solution) = 99:1; yield 0.085 g, 78%; $[\alpha]_D^{27.7}$ = −8.7 (c = 0.19 MeOH); IR (neat; ν, cm^{-1}) 3593−3300 (br); ^{1}H NMR (300 MHz, D_2O) δ 1.32−1.44 (m, 1H), 1.78−1.95 (m, 1H), 2.60−2.94 (m, 5H), 3.30 (dd, J = 2.1, 10.2 Hz, 1H), 3.66−3.78 (m, 1H), 3.78−3.92 (m, 1H), 4.09 (br s, 1H); ^{13}C NMR (75 MHz, D_2O) δ 36.1, 43.8, 46.3, 50.2, 67.6, 68.3, 71.3, 72.2; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_8H_{19}N_2O_4$ 207.1346, found 207.1340. Anal. Calcd for $C_8H_{18}N_2O_4$: C, 46.59; H, 8.80; N, 13.58. Found: C, 46.45; H, 9.00; N, 13.27.

*Data for (2S,3S,4R,5R)-2-((R)-2-hydroxydecyl)piperidine-3,4,5-triol (**7e**):* R_f = 0.8 in $CHCl_3$:MeOH (1 drop of 25% aq NH_3) = 4:1; column purification in $CHCl_3$:MeOH:aq NH_3 (25% solution) = 95:4:1; yield 0.13 g, 73%; mp 136−138 °C; $[\alpha]_D^{26.6}$ = −36.5 (c = 0.12 MeOH); IR (neat; ν, cm^{-1}) 3277 (br); ^{1}H NMR (300 MHz, D_2O) δ 0.87 (br t, J = 6.0 Hz, 3H), 1.30−1.60 (m, 15H), 1.77−1.94 (m, 1H), 2.72 (t, J = 11.4 Hz, 1H), 2.80−2.97 (m, 2H), 3.35 (dd, J = 2.4, 10 Hz, 1H), 3.68−3.78 (m, 1H), 3.78−3.92 (m, 1H), 4.09 (br s, 1H); ^{13}C NMR (75 MHz, D_2O) δ 14.4, 23.7, 26.8, 30.4, 30.7, 30.8, 33.0, 39.2, 39.8, 46.0, 53.1, 69.6, 69.8, 72.9, 73.7; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{15}H_{32}NO_4$ 290.2332, found 290.2332. Anal. Calcd for $C_{15}H_{31}NO_4$: C, 62.25; H, 10.80; N, 4.84. Found: C, 62.48; H, 10.84; N, 4.94.

*Data for (2S,3S,4R,5R)-2-Phenethylpiperidine-3,4,5-triol (**8**):* R_f = 0.6 in $CHCl_3$:MeOH (1 drop of 25% aq NH_3) = 4:1; column purification in $CHCl_3$:MeOH:aq NH_3 (25% solution) = 92:7:1; yield 0.07 g, 44%; mp 151−153 °C; $[\alpha]_D^{26.8}$ = −46.5 (c = 0.11 MeOH); IR (neat; ν, cm^{-1}) 3728, 3271 (br), 841, 707; ^{1}H NMR (300 MHz, D_2O) δ 1.51−1.66 (m, 1H), 2.02−2.18 (m, 1H), 2.70 (t, J = 11.4 Hz, 1H), 2.58−2.92 (m, 4H), 3.34 (dd, J = 3.0, 10.2 Hz, 1H), 3.69−3.78 (ddd, J = 2.4, 4.8, 10.8 Hz, 1H), 4.08 (br s, 1H), 7.22−7.52 (m, 5H); ^{13}C NMR (75 MHz, D_2O) δ 31.1, 32.9, 43.8, 52.9, 68.0, 71.3, 71.8, 126.0, 128.4 (s), 128.6 (s), 142.4; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{13}H_{20}NO_3$ 238.1444, found 238.1445. Anal. Calcd for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.61; H, 7.83; N, 5.91.

Glycosidase Inhibition Assay. Glycosidase inhibition assay of the derivatives was carried out by mixing a 0.1 unit/mL concentration each of α-galactosidase, α-mannosidase, and α-glucosidase with the samples, and the resulting mixtures were incubated for 1 h at 37 °C. Enzyme action for α-galactosidase was initiated by addition of 10 mM p-nitrophenyl α-D-galactopyranoside (pNPG) as a substrate in 200 mM sodium acetate buffer followed by incubation for 10 min at 37 °C and stopped by adding 2 mL of 200 mM borate buffer of pH 9.8. α-Mannosidase activity was initiated by addition of 10 mM p-nitrophenyl α-D-mannopyranoside as a substrate in 100 mM citrate buffer of pH 4.5. The reaction was incubated at 37 °C for 10 min and stopped by adding 2 mL of 200 mM borate buffer of pH 9.8. Initiation of α-glucosidase activity was carried out by addition of 10 mM p-nitrophenyl α-D-glucopyranoside in 100 mM phosphate buffer of pH 6.8 and stopped by adding 2 mL of 0.1 M Na_2CO_3 after incubation for 10 min at 37 °C. α-Glycosidase activity was determined by measuring the absorbance of the p-nitrophenol released from pNPG at 420 nm using a spectrophotometer.

Purified recombinant preparations of the lysosomal enzyme haGlu were utilized. For bGal and haGlu a concanavalin binding fraction from human placenta lysate enriched in lysosomal enzymes was used as the enzyme source.[7d] The enzyme activities of bGal, haGlu, and haGal were monitored using the fluorogenic substrates [final concentration] 4-methylumbelliferyl β-D-galactopyranoside (MU-bGal) [0.45 mM], 4-methylumbelliferyl α-D-glucopyranoside (MU-aGlu) [0.7 mM], and 4-methylumbelliferyl α-D-galactopyranoside (MU-aGal) [0.5 mM], respectively, as previously described.[4a,7d,e] The substrates were purchased from a chemical supplier. All enzyme reactions were performed at 37 °C in McIlvaine citrate phosphate buffer (100 mM), pH 4.5. The reactions were terminated with a 5-fold excess of 0.1 M MAP, pH 10.5, and fluorescence was detected using an excitation/emission wavelength of 365 nm/450 nm on a spectrofluorometer. To determine the inhibitory activity of the compounds, serial 3-fold dilutions of the compounds in DMSO in triplicate were incubated with the diluted lysosomal enzymes prior to initiation of the reaction with addition of the appropriate fluorogenic substrate. IC_{50} values were determined using built-in equations within PRISM Graphpad v5.2.

Treatment of Patient Fibroblasts. Unaffected human skin fibroblasts or those derived from GM1 gangliosidosis (p.R201H/IVS14-2A>G)[4a] prior to treatment were seeded at (1−2) × 10^4 cells/well into 96-well tissue culture plates and grown in αMEM medium with 10% fetal calf serum at 37 °C in a CO_2 humidified incubator. Serially diluted compounds were evaluated in triplicate and diluted 100-fold into the medium to ensure that the DMSO solvent was maintained at 1%. Following 5 days of treatment, the growth medium was removed, and adherent cells were washed with two changes of PBS. The cells were lysed using 75 μL of McIlvaine citrate phosphate buffer containing 0.4% Triton X-100 at 4 °C for a minimum of 30 min. Lysosomal enzyme activities in the lysates (25 μL) were evaluated using the 4-methylumbelliferone-based fluorogenic substrates described above. Human bGal and β-N-acetylhexosaminidase activities

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were monitored using 0.45 mM MU-bGal and 1.6 mM 4-methylumbelliferyl β-*N*-acetylglucosaminide, respectively. Following 1 h of incubation at 37 °C, the reaction was terminated with a 5-fold excess of 0.1 M MAP, pH 10.5, and fluorescence was detected using an excitation/emission wavelength of 365 nm/450 nm on a spectrofluorometer. Activity values obtained for compound-treated cells were normalized to those of cells treated with solvent (DMSO, 1%) only.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Characterization data of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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