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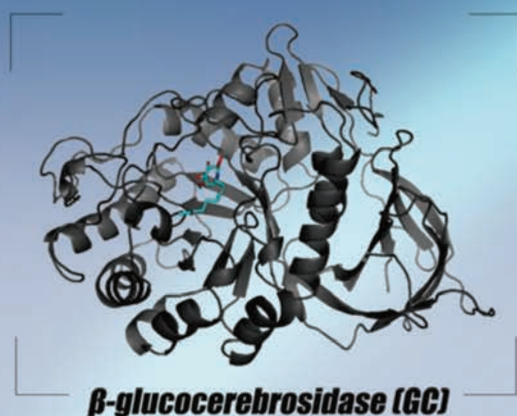

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COMMUNICATION

Synthesis of *N*-substituted ε-hexonolactams as pharmacological chaperones for the treatment of N370S mutant Gaucher disease†

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A series of *N*-substituted ε-hexonolactams have been designed and prepared by a concise route with a tandem ring-expansion reaction as the key step. Some of the *N*-substituted ε-hexonolactams show better enhancements to N370S mutant β-glucocerebrosidase activity than NB-DNJ and NN-DNJ. Both the experimental results and computational studies highlight the importance of the carbonyl group for stabilizing protein folds in the mutant enzyme. The structure–activity relationships are also discussed. These novel *N*-alkylated iminosugars are promising pharmacological chaperones for the treatment of N370S mutant Gaucher disease.

Gaucher disease (GD), caused by deficient activity of β-glucocerebrosidase (GC), is the most prevalent lysosomal storage disorder. The estimated incidence of GD is about 1 : 60 000 in the general population[1] and 1 : 800 in the Ashkenazi Jewish population.[2] The deficiency in enzyme activity results in the accumulation of glucosylceramide in the lysosomes, leading to various clinical manifestations that include hepatomegaly, splenomegaly, anemia, bone lesions, and in some cases, central nervous system disease.[3] Enzyme replacement therapy (ERT) is clinically used for the treatment of type 1 GD;[4] however, it is costly and the enzyme is unable to cross the blood–brain barrier to treat central nervous system symptoms of the disease. As an alternative approach, substrate reduction therapy (SRT) works by inhibiting the biosynthesis of glucosylceramide. In SRT, *N*-butyl-deoxynojirimycin (NB-DNJ, miglustat)[5] has reached the furthest stage in clinical development. However, due to the side effects miglustat has been recommended only for adults with mild-to-moderate type 1 GD for which ERT is not an option. The fact that this compound is also a potent inhibitor of α-glucosidase I, a cellular glycosidase involved in the elaboration of the glycan structure of glycoproteins, is a major concern, particularly for long-term treatment.[6] Chaperon mediated therapy (CMT)[7] is the currently emerging strategy for treating GD in which small molecules selectively bind to the active site of the enzyme and stabilize the mutant GC in the endoplasmic reticulum, thereby facilitating proper folding and intracellular enzyme trafficking to the lysosome.[8–10]

There have been numerous point mutations identified in the glucocerebrosidase gene associated with Gaucher disease.[11] The most common mutation is known as N370S, accounting for 77% of the cases among Ashkenazi Jewish patients and about 30% of the cases in the non-Jewish population.[12,13] This mutation acts by destabilizing the native conformation, and therefore, renders the protein more susceptible to mistrafficking and degradation.[14,15] It was revealed that GC contains two substrate-binding sites in the catalytic domain: the hydrophilic site that recognizes the glucosyl residue, and the hydrophobic site that recognizes the ceramide moiety.[16] *N*-Alkylated iminosugars containing both a hydrophilic iminosugar part and a hydrophobic alkyl chain have received considerable attention in the search for chaperones.[17–20] In a previous study on δ-lactams, we found that the carbonyl group provides an additional hydrogen bond with mutant GC, which stabilizes the substrate-bound conformations of the mutant enzyme.[21] *N*-Substituted ε-hexonolactams with an additional carbon and a more flexible scaffold are envisaged to have a similar function to *N*-substituted δ-lactams. However, there are few investigations on ε-hexonolactams since it was reported that this type of compound has no significant inhibitory effect on a number of glycosidases.[22] There are many studies showing that good pharmacological chaperones for mutant N370S GC do not have to be potent inhibitors of normal GC.[23] Thus, to some extent, if ε-hexonolactams could stabilize the mutant N370S GC as pharmacological chaperones, the fact that ε-hexonolactams are not good inhibitors for glycosidases may suggest high selectivity with minimal side effects. Based on this assumption, a series of novel *N*-substituted ε-hexonolactams have been synthesized by a concise synthetic route with a one-pot tandem reaction as a key step. These *N*-substituted ε-hexonolactams have been evaluated as chaperones for the N370S mutant GC.

The synthesis of *N*-substituted ε-gluconolactams started from methyl α-D-glucopyranoside (**1**) (Scheme 1). The perbenzylation of **1** which was followed by a convenient and selective acetolysis

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† Electronic supplementary information (ESI) available: Experimental procedures, NMR spectra for new compounds, and data plot for NN-DNJ. See DOI: 10.1039/c2ob06987c

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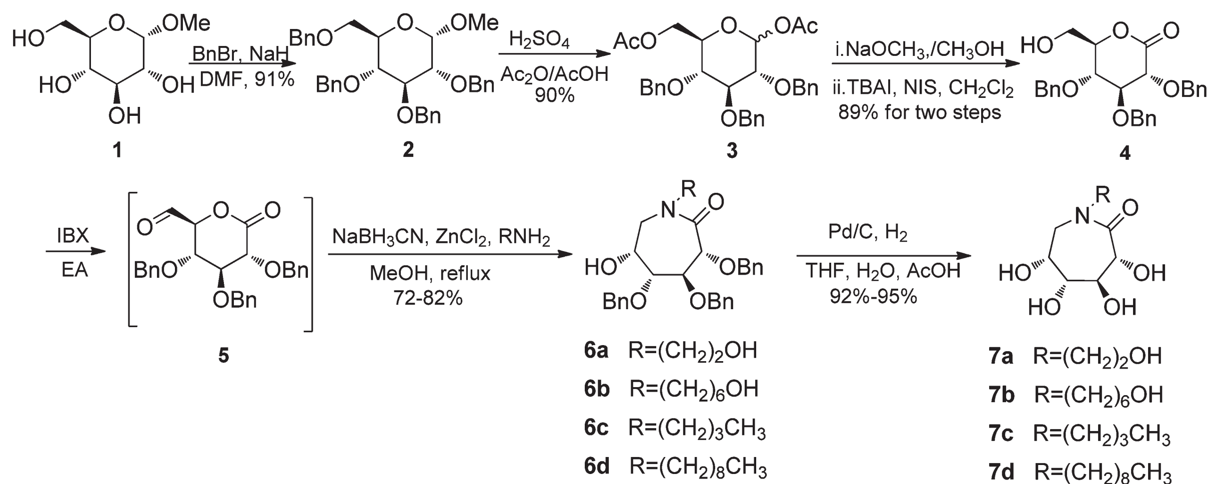

Scheme 1 Synthesis of *N*-substituted ε-gluconolactams.

afforded the 1,6-acetylated compound **3** in good yield.[24] The acetyl groups in **3** were removed and a stepwise oxidation was carried out: the hemiacetal was oxidized to the lactone **4** by *N*-iodosuccinimide (NIS)/tetrabutylammonium iodide (TBAI) and the 6-OH was then oxidized to the aldehyde **5** by 2-iodoxybenzoic acid (IBX). It is noteworthy that the direct oxidation of both hydroxyl groups under various conditions such as pyridinium chlorochromate (PCC), (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO), IBX, and Swern oxidation, led to either incomplete oxidation or a side product of 1,6-lactone. After the oxidation by IBX was finished, the insoluble IBX was filtered off and without further purification, the aldehyde **5** underwent a one-pot tandem reaction with different amines to generate the ε-gluconolactams **6a–d** smoothly. It is believed that in this key reaction a reductive amination between the aldehyde and amine occurs, which is followed by an intramolecular exchange from lactone to lactam, thus resulting in ring expansion to afford *N*-substituted ε-gluconolactams. Finally, the benzyl groups in **6a–d** were cleaved by catalytic hydrogenolysis to yield the target *N*-substituted ε-gluconolactams **7a–d**.

Generally, iminosugar syntheses starting from commercially available monosaccharides involve the introduction of an amino function in the sugar skeleton and subsequent aminocyclization in order to generate the imino ring. Moreover, at least one more step is required to introduce substituted groups on the nitrogen atom. The approach described here combines amination, cyclization and introduction of substituted groups on the nitrogen atom in a one-pot manner. Correspondingly, *N*-substituted ε-galactonolactams **10a–d** and *N*-substituted ε-mannonolactams **13a**, **13c–e** were prepared following the same procedure as the

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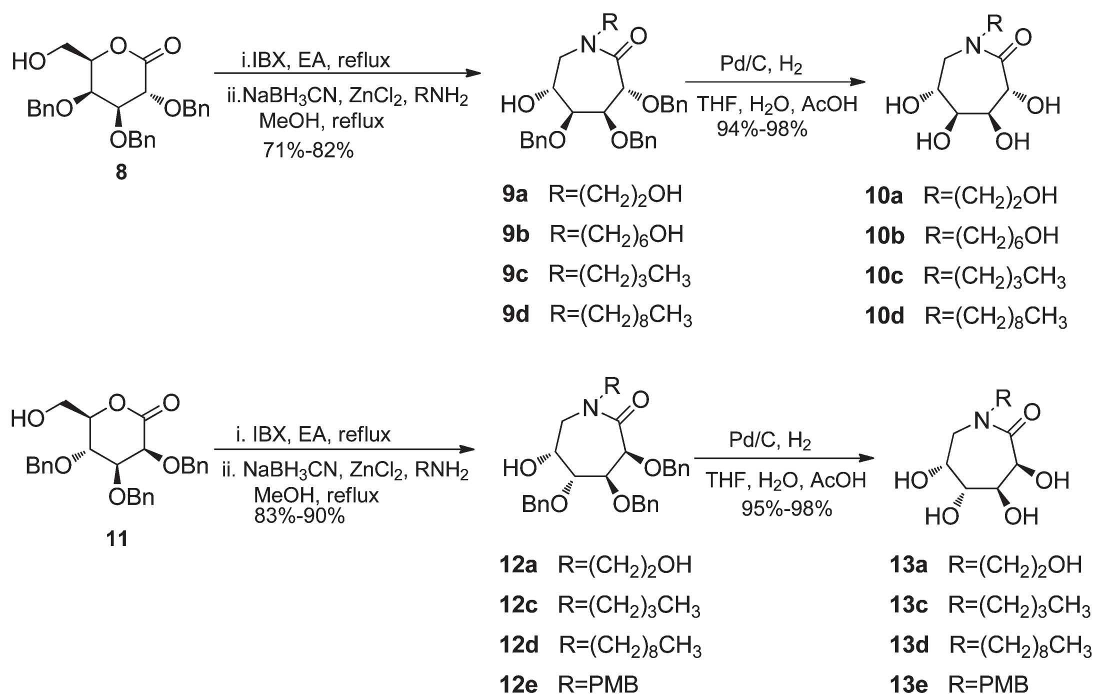

Scheme 2 Synthesis of *N*-substituted ε-galactonolactams and ε-mannonolactams.

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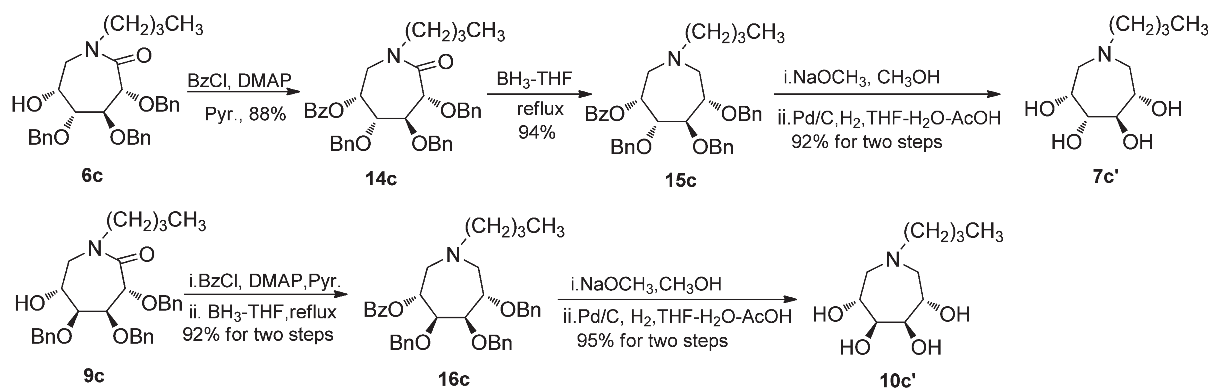

Scheme 3 Synthesis of *N*-butyl tetrahydroxyazepanes.

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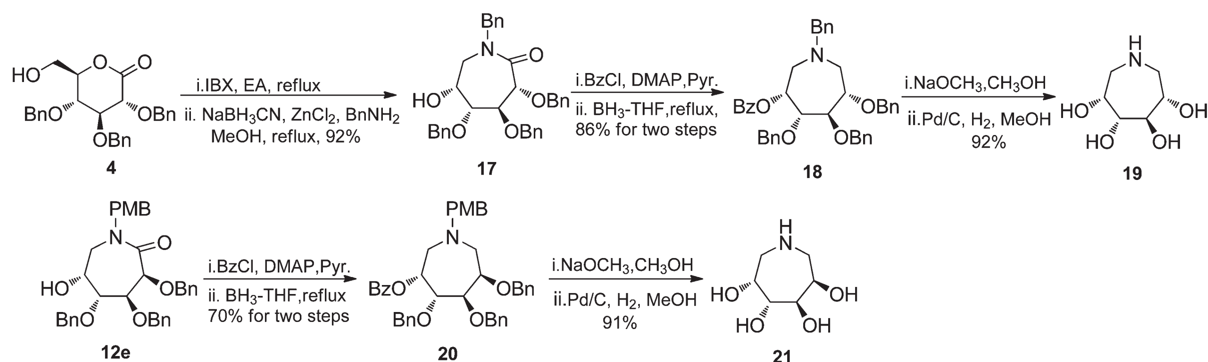
preparation of compounds **7a–d** from compound **8** and compound **11**,[25] respectively (Scheme 2).

To clarify the biological function of the carbonyl group, *N*-butyl tetrahydroxyazepanes **7c′** and **10c′** were designed and synthesized. BH_3–THF was used as reductant for the reduction of the lactam.[26,27] As shown in Scheme 3, the nascent hydroxyl group was protected prior to the reduction of the carbonyl group in the lactam, since direct reduction led to the desired product in low yield with the formation of by-products due to the interaction of the OH and carbonyl group. Although two more steps including benzoylation and debenzoylation were added, they were straightforward and the global yield was higher than direct lactam reduction. Finally, the benzyl groups were removed by hydrogenolysis to produce the products **7c′** and **10c′**. Moreover, tetrahydroxyazepanes **19** and **21** were obtained when benzylamine or 4-methoxybenzylamine was used in the one-pot tandem reaction followed by the treatment as described in the preparation of compound **7c′** (Scheme 4). Tetrahydroxyazepanes have been shown to be potent inhibitors of glycosidases, and therefore have received considerable attention on their synthesis.[28–30] The route we presented here provides an optional strategy for the synthesis of tetrahydroxyazepanes.

Therefore, this one-pot tandem reaction allows for the concise and economical construction of *N*-substituted ε-hexonolactams with various chains on the nitrogen and can be applied to obtain *N*-substituted ε-hexonolactams with various stereochemistry. Additionally, this approach also represents a general strategy for the synthesis of tetrahydroxyazepane derivatives containing substituents or no substituents on the nitrogen.

With a small library of *N*-substituted ε-hexonolactams in hand, the inhibition of all the synthesized compounds towards human placental GC was determined and the results are summarized in Table 1. The apparent IC_{50} data showed that only *N*-nonyl ε-gluconotonolactam (**7d**) and *N*-nonyl ε-galacotonolactam (**10d**) are weak inhibitors of human GC. Cytotoxicity assays were evaluated before performing a cellular assay in Gaucher mutant cells. All the compounds, including those with long alkyl chains, have no apparent cytotoxicity at 100 μM in HL60 cells after three days.

Next, the *N*-substituted ε-hexonolactams and tetrahydroxyazepanes were added to the growth media of Gaucher lymphoblasts homozygous for the N370S at 50 μM for three days. NB-DNJ and *N*-nonyldeoxynojirimycin (NN-DNJ), which are known pharmacological chaperones for GC, were used as positive control. Their activation achieved in our experiments compared favorably with the published data.[18,31] Compounds **7c**, **7d**, **10a**, and **10d** showed better enhancements to mutant GC than NB-DNJ, and compound **10c** exhibited a similar fold increase to NB-DNJ. Schueler and colleagues have reported that the threshold of lysosomal GC activity was 11–15% of wild-type physiological levels; levels below this are thought to result in Gaucher disease.[32] For patients receiving enzyme replacement therapy, a 1.7- to 9.6-fold increase of GC activity was observed after intravenous infusion of enzyme, which is sufficient to reduce hepatosplenomegaly, bone crises, and improve blood counts.[33] Based on these data, it is believed that an approximately 2-fold increase in the N370S lysosomal GC activity would be sufficient to ameliorate Gaucher disease.[20] Therefore,



Scheme 4 Synthesis of tetrahydroxyazepanes **19** and **21**.

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Table 1 Results of inhibition and activation assays

Compound	App. IC_{50} (μM)[a]	Cytotoxicity,[b] % dead	Fold increase[c]
NB-DNJ	259 ± 22	2.69	1.7 ± 0.2
NN-DNJ	—	—	2.1 ± 0.4[d]
Control	—	2.32 (4.24)[e]	
7a	>0.5 mM	2.45	ND[f]
7b	>0.5 mM	2.23	ND
7c	>0.5 mM	2.43	2.0 ± 0.4
7d	150	2.99	2.4 ± 0.5
7c′	>0.5 mM	2.17	ND
10a	>0.5 mM	2.57	1.8 ± 0.5
10b	>0.5 mM	2.40	ND
10c	>0.5 mM	2.88	1.7 ± 0.3
10d	230	2.97	2.2 ± 0.6
10c′	>0.5 mM	2.34	ND
13a	>0.5 mM	2.86	ND
13c	>0.5 mM	2.65	ND
13d	>0.5 mM	4.03	ND
13e	>0.5 mM	2.39	ND

[a] The app. IC_{50} were determined with human placental GC (Km for 4-MU-β-glucoside, 1.9 ± 0.3 mM). Experiments were performed in triplicate and the mean ± standard deviation (SD) is shown. [b] HL60 cells were treated with 100 μM of compounds for three days, and the cytotoxicity was evaluated as described in the experimental procedures. [c] Gaucher lymphoblasts (N370S) were cultured in the presence of compounds (50 μM) for 3 days before GC activity was measured. The fold increase in enzyme activity is compared to untreated cells, *i.e.*, normalized value = 1. The mean ± standard error of the mean (SEM) obtained from 3 separate experiments is shown. [d] Gaucher lymphoblasts (N370S) were cultured in the presence of various concentrations of NN-DNJ for 3 days before GC activity was measured. The maximal fold increase as shown in the table appeared at 1 μM (see Supporting Information). The fold increase in enzyme activity is compared to untreated cells, *i.e.*, normalized value = 1, and shown as relative enzyme activity. Experiments were performed in triplicate, and the mean ± SD is shown. [e] Control cells (no compound addition) were analyzed following addition of water or 0.01% DMSO. [f] ND = not detected.

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compounds **7c**, **7d**, **10a**, **10c**, and **10d** are potential pharmacological chaperones for the treatment of Gaucher disease.

It is consistent with the "hydrophobic–hydrophilic two substrate-binding sites" theory[19] that in the ε-gluconolactam series, the structures containing hydrophobic chains (**7c**, **7d**) provided greater enzyme enhancement than the structures with hydrophilic chains (**7a**, **7b**), and the activation of *N*-nonyl ε-hexonolactams are better than *N*-butyl ε-hexonolactams. The phenomenon that *N*-nonyl ε-gluconolactam and ε-galactonolactam **7d** and **10d** are potent chaperones while *N*-nonyl ε-mannonolactam **13d** has no activation to mutant GC indicates that some structural and spatial features are essential for activation. Kefurt *et al.*[34,35] have done considerable research on conformations of ε-hexonolactams. Their research indicated that the seven-membered lactam ring exists in the chair-like conformation $^{1,N}C_4$ or $^4C_{1,N}$. The decisive conformation-determining requirement is the equatorial position of the substituent on the carbon next to the carbonyl group. It was proved that ε-gluconolactam and ε-galactonolactam adopt the $^4C_{1,N}$ conformation whereas ε-mannonolactam adopts the $^{1,N}C_4$ conformation (Fig. 1). The difference in their predominant conformation may provide an explanation for their different behaviors in binding mutant GC.

When the carbonyl groups of **7c** and **10c** were reduced to compounds **7c′** and **10c′** respectively, the enhancement of mutant GC activity was lost. This observation highlights the importance of the carbonyl group for stabilizing protein folds in

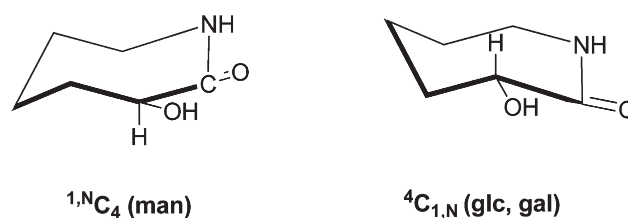

Fig. 1 The predominant conformations of ε-hexonolactams.

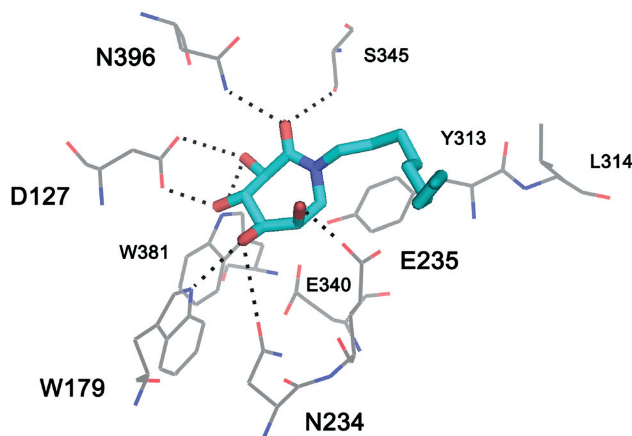

Fig. 2 The optimized docking structure of **7d** (cyan sticks) bound to the active site of GC. Key residues involved in the binding process are shown as gray lines. Hydrogen bonds are represented by black dashed lines.

N370S GC. Computational studies were carried out in which compound **7d** was flexibly docked into the binding site of GC (Fig. 2, PDB code 2V3E, complexed with NN-DNJ).[36] The optimized docking shows that compound **7d** interacts with most of the residues that have a hydrogen bond interaction with NN-DNJ. However, there are two different residue interactions between the binding of these two structures: instead of the hydrogen bond between 2-OH of NN-DNJ and E340, the 5-OH of compound **7d** forms a hydrogen bond with E235; whereas a single hydrogen bond is found between 6-OH and N396-Oδ1 in the NN-DNJ-GC complex, the carbonyl group of **7d** forms hydrogen bonds with both N396-Nδ2 and S345-Oγ. Based on the crystal structure of the isofagomine–GC complex, it has been hypothesized that the interaction between E349 and D315 will promote the movement of W312 and Y313, and the change in conformation will reduce substrate binding in the N370S GC.[8] In this respect, the electrostatic contact between the carbonyl group and S345 will be good for stabilizing the substrate-bound conformations of GC, since it can stabilize loop 2 which is at the opening of the GC active site and avoid the close interaction between E349 and D315.

In conclusion, we have demonstrated a concise and efficient methodology for the synthesis of *N*-substituted ε-hexonolactams by virtue of the one-pot tandem reaction as the key step starting from sugar building blocks. This strategy facilitates the construction of *N*-substituted ε-hexonolactams with a variety of different chains on the nitrogen and with various ring stereochemistries. Moreover, this approach provides a new avenue to make tetrahydroxyazepane compounds with either substituents or

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non-substituents on the nitrogen atom. Among the small library of *N*-substituted ε-hexonolactams, *N*-alkyl ε-gluconolactams and ε-galactonolactams represent potent chaperones for N370S GC. To the best of our knowledge, these are the first reported seven-membered iminosugars that show activation of N370S mutant enzyme. The carbonyl group of this seven-membered lactam is important to stabilize the mutant GC in the substrate–binding conformation. Additionally, it seems that these *N*-substituted ε-hexonolactams have no cytotoxity even though they contain a long chain on the nitrogen. As a consequence, *N*-alkyl ε-hexonolactams may be potentially active pharmacological chaperones for the treatment of N370S mutant Gaucher disease.

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