

Piperidine Azasugars Bearing Lipophilic Chains: Stereoselective Synthesis and Biological Activity as Inhibitors of Glucocerebrosidase (GCCase)

Francesca Clemente, Camilla Matassini, Sara Giachetti, Andrea Goti, Amelia Morrone, Macarena Martínez-Bailén, Sara Orta, Pedro Merino,* and Francesca Cardona*



Cite This: *J. Org. Chem.* 2021, 86, 12745–12761



Read Online

ACCESS |



Metrics & More

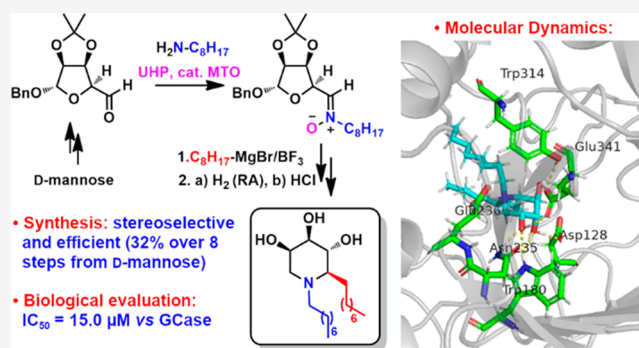


Article Recommendations



Supporting Information

ABSTRACT: We report a straightforward synthetic strategy for the preparation of trihydroxypiperidine azasugars decorated with lipophilic chains at both the nitrogen and the adjacent carbon as potential inhibitors of the lysosomal enzyme glucocerebrosidase (GCCase), which is involved in Gaucher disease. The procedure relies on the preparation of C-erythrosl *N*-alkylated nitrones **10** through reaction of aldehyde **8** and primary amines **13** followed by oxidation of the imines formed *in situ* with the methyltrioxorhenium catalyst and urea hydrogen peroxide. The addition of octylmagnesium bromide to nitron **10e** provided access to both epimeric hydroxylamines **21** and **22** with opposite configuration at the newly created stereocenter in a stereodivergent and completely stereoselective way, depending on the absence or presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$. Final reductive amination and acetonide deprotection provided compounds **14** and **15** from low-cost D-mannose in remarkable 43 and 32% overall yields, respectively, over eight steps. The C-2 *R*-configured bis-alkylated trihydroxypiperidine **15** was the best ligand for GCCase ($\text{IC}_{50} = 15 \mu\text{M}$), in agreement with MD simulations that allowed us to identify the chair conformation corresponding to the best binding affinity.



1. INTRODUCTION

Iminosugars [e.g., deoxynojirimycin, DNJ (**1**), Figure 1] are among the most fascinating monosaccharide analogues in

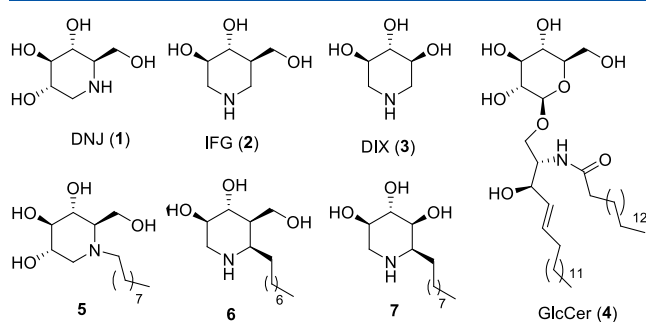


Figure 1. Some imino- and azasugars, their *N*- and *C*-alkyl derivatives, and glucosylceramide (GlcCer), the natural substrate of GCCase.

which a nitrogen atom replaces the endocyclic oxygen.¹ Together with azasugars, [e.g. isofagomine, IFG (**2**), or 1,5-dideoxy-1,5-iminoxylitol, DIX (**3**), Figure 1], which are characterized by a nitrogen atom replacing the anomeric carbon of monosaccharides, iminosugars have been extensively

investigated in the last thirty years as glycosidase² and glycosyltransferase inhibitors.³

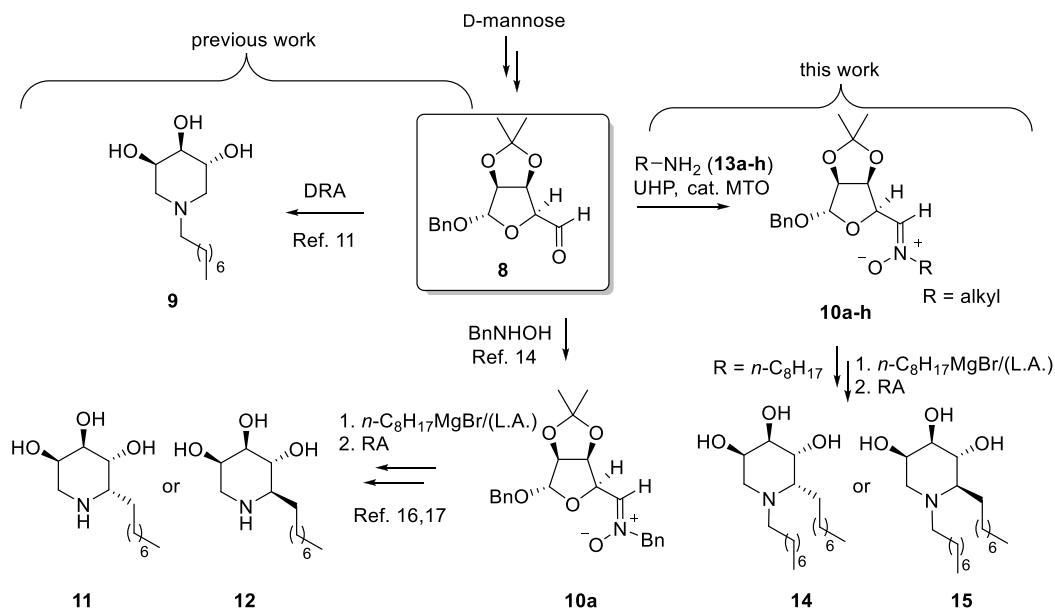
More recently, imino- and azasugar-based glycomimetics became attractive as potential therapeutic agents toward lysosomal storage disorders (LSDs), following the observation of their counter-intuitive effect in enhancing the enzyme activity, thus acting as chaperones. In the pharmacological chaperone therapy (PTC) of LSDs, these glycomimetics are employed at sub-inhibitory concentration to favor the mutated enzyme correct folding in the endoplasmic reticulum (ER), facilitate its translocation to the lysosomes, and recover some hydrolytic activity, compromised as a consequence of diverse genetic mutations.⁴

Gaucher disease (GD), the most common LSD, is determined by mutations in the *GBA* gene, which encodes for the lysosomal enzyme glucocerebrosidase (GCCase). The

Received: June 4, 2021

Published: September 1, 2021



Scheme 1. Previous Results and Scope of This Work^a

^aGeneral strategies employed to access C- or N-alkyl trihydroxypiperidines from the “masked dialdehyde” **8** and nitrone **10a** derived thereof. *This work*: “one-pot” synthesis of C-erythrolyl N-alkyl nitrones **10** and their application to the synthesis of C,N-dialkylated azasugars **14** and **15**.

GBA mutations provoke partial deficit of GCCase, with consequent loss or reduction of its hydrolytic activity [*i.e.*, hydrolysis of glucosyl ceramide, GlcCer (**4**), **Figure 1**, to ceramide and glucose] in the lysosomes. Accumulation of glucosylceramide then leads to organ dysfunctions and severe impairment. Several good candidates behaving as pharmacological chaperones (PC) for GCCase have been found, but no drugs are on the market yet.⁵

The presence of an alkyl chain either at the nitrogen atom or at the adjacent carbon of imino- and azasugars was found to improve their pharmacokinetic properties, furnishing better PCs for GD. For instance, contrary to DNJ (**1**), N-nonyl-DNJ (**5**) (**Figure 1**) is a potent inhibitor of lysosomal GCCase ($IC_{50} = 1 \mu\text{M}$) which showed a twofold increase in the activity of the N370S mutant enzyme in fibroblasts at $10 \mu\text{M}$ concentration. However, it did not enhance the intracellular activity of the L444P variant.⁶

With IFG and DIX derivatives, better results in terms of PC properties were obtained by shifting the alkyl chain from nitrogen to the adjacent carbon. Indeed, 6-nonyl IFG (**6**) (**Figure 1**) displayed a remarkable GCCase inhibition and PC activity ($IC_{50} = 0.6 \text{ nM}$, 1.5-fold enzyme activity enhancement at 3 nM in N370S GD fibroblasts⁷ and α -1-C-nonyl-DIX (**7**) showed an IC_{50} of 6.8 nM toward GCCase and a 1.8-fold enzyme activity increase in N370S mutated fibroblasts at 10 nM .⁸

Among the most straightforward synthetic strategies to afford polyhydroxylated piperidine imino- and azasugars⁹ are the reductive amination (RA) ring-closure starting from nitrogen-containing carbohydrate-based precursors or the double reductive amination (DRA) of dicarbonyl carbohydrate derivatives with an external nitrogen source.¹⁰ By applying the latter strategy on the “masked” dialdehyde **8** derived from D-mannose, we have synthesized the N-alkyl trihydroxypiperidine **9** (**Scheme 1**),¹¹ which showed $IC_{50} = 30 \mu\text{M}$ toward GCCase and 1.25-fold activity increase in N370S-mutated fibroblasts at $100 \mu\text{M}$.¹²

The former RA strategy, instead, was applied to the C-erythrolyl nitrone **10a**,¹³ obtained in turn by condensation of the same “masked” dialdehyde **8** with N-benzyl hydroxylamine.¹⁴ Stereoselective Grignard additions to this key nitrone in the presence or absence of a suitable Lewis acid^{14,15} afforded, in a stereodivergent manner, both epimers of a series of 2-alkylated trihydroxypiperidines, among which the octyl derivatives **11** and **12** (**Scheme 1**) showed the most promising biological properties.¹⁶ In particular, the (2R) diastereoisomer **12** showed remarkable PC properties toward fibroblasts bearing the N370S/RecNcil mutation (1.9-fold enzyme recovery at $100 \mu\text{M}$) and, more importantly, proved to be responsive with the homozygous L444P mutation (1.80-fold enzyme recovery at $100 \mu\text{M}$), which is refractory to most PCs. Remarkably, both compounds **9** and **12** performed PC tasks toward wild-type fibroblasts (1.5-fold at $50 \mu\text{M}$ and 1.4-fold at $100 \mu\text{M}$, respectively), which is an important factor for targeting sporadic forms of Parkinson disease.¹⁷

Given the relevance of the presence of a long alkyl chain for inducing good enzyme recognition, and in consideration of the structure of the natural substrate GlcCer (**4**), we speculated that compounds possessing two alkyl chains might show improved biological properties.

We therefore addressed the synthesis of 1,2-dialkyltrihydroxypiperidines and envisaged that a general synthetic strategy could involve the Grignard addition to C-erythrolyl N-alkyl nitrones **10** followed by RA (**Scheme 1**).

In this work, we report our results on this subject, which include a docking study of the putative 1,2-dialkyltrihydroxypiperidines in the GCCase catalytic site; a general direct synthesis of nitrones **10** and their conversion to target compounds **14** and **15** and to derivatives containing three alkyl tails; the biological evaluation of the dialkylated trihydroxypiperidines toward commercial glycosidases and human lysosomal enzymes and their *in vitro* activity on cell lines; a molecular dynamics (MD) simulation of the new compounds within the GCCase enzyme active cavity.

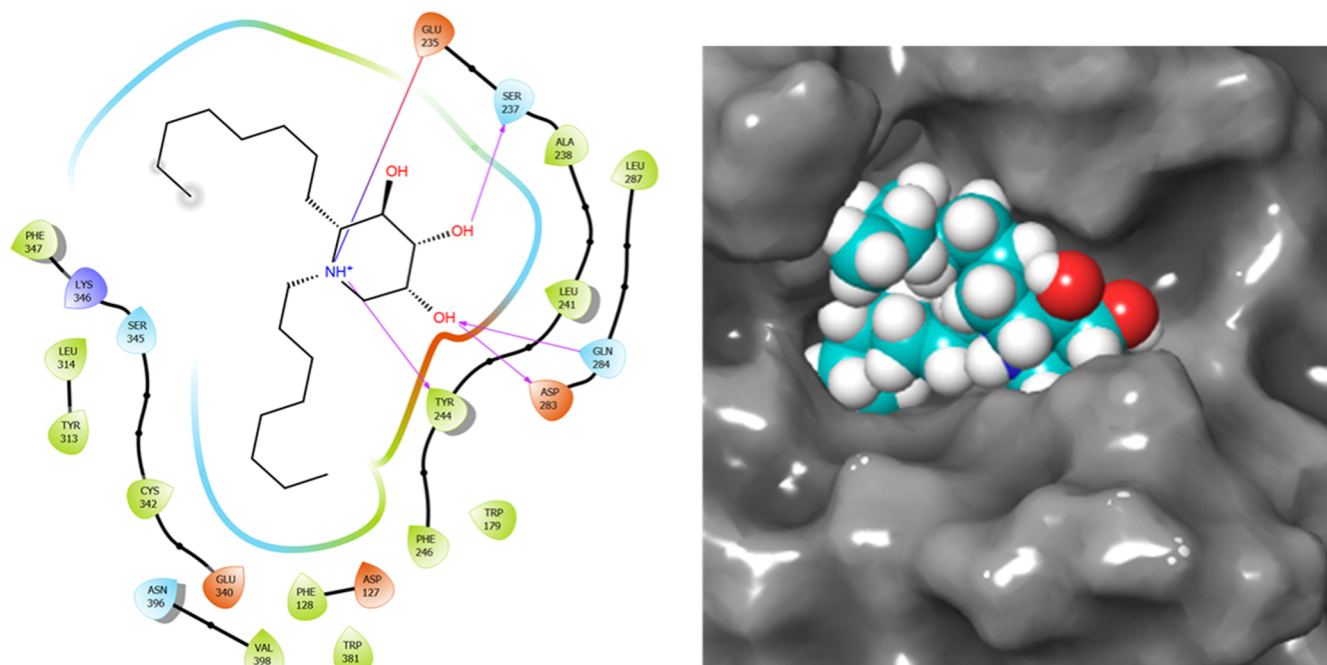


Figure 2. Molecular docking for protonated **15** with an (*S*)-configuration at the nitrogen atom. Left: 2D schematic view of interaction in the binding site. Right: Visualization of the ligand, represented as cyan CPK at the binding site.

2. RESULTS AND DISCUSSION

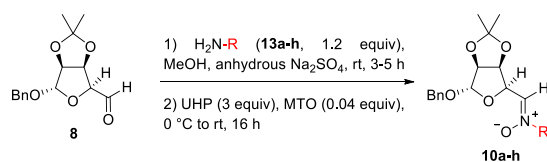
2.1. Preliminary Docking Studies. With the aim of assessing the viability of target compounds **14** and **15** in GCCase catalytic site, we carried out preliminary docking studies with acid-beta-glucosidase (PDB ID 2NSX).¹⁸ The protonated forms of compounds **14** and **15** were considered, taking into account that a stereogenic nitrogen atom is formed (for details, see the [Supporting Information](#) section). For compound **14**, the best pose corresponds to an (*R*)-configuration at the nitrogen atom adopting a ¹C₄ conformation (score −6.332). The observed orientation for the piperidine ring is different with respect to that observed in a known chaperone [*S*-hydroxymethyl-3,4-dihydropiperidine, IFG (**2**), [Figure 1](#)], thus losing interactions of hydroxyl groups with Asp127, Trp179, and Asn396 that now form new interactions with Ser237, Asp283, and Gln284. Although the aliphatic chain linked to the nitrogen atom shows hydrophobic interactions with Phe128, Trp179, Tyr313, Leu314, and Ala238, the other aliphatic chain is clearly exposed to the solvent (see the [Supporting Information](#) section). The (*S*)-isomer shows a close value in the docking score (−6.071) for the same conformation and, also in this case, the piperidine ring is oriented in the wrong way with respect to IFG. A similar situation was found for protonated **15**. In this case, the (*S*)-isomer in a ¹C₄ conformation showed the best docking value (−6.614) due to hydrophobic interactions of the aliphatic chains in the binding site, the (*R*)-isomer in the same conformation being very close (docking score = −6.354). Again, in both cases, the piperidine ring is oriented in the wrong way when compared with the original structure bound to IFG. For the (*R*)-isomer, the chain bonded to the nitrogen atom gives interactions with Phe128, Trp179, Phe246, and Trp381. The similar orientation of the piperidine ring to that observed for **14** gives rise to the same interactions of the hydroxyl groups with Ser237, Asp283, and Gln284 ([Figure 2](#)). These preliminary results should be considered with caution

due to the predicted orientations of the piperidine ring in a rigid protein. Even though the calculations predict a good binding to the enzyme, the particular interactions and orientations of the ligands will be confirmed (or rejected) through further molecular dynamics studies that will allow conformational changes in the protein (*vide infra*).

2.2. Synthesis and Structural Assignment. The aldehyde **8** was synthesized in four steps from D-mannose on gram scale as reported.^{11,19}

The synthesis of nitrones **10** from **8** by condensation in analogy to the reported **10a** would require the corresponding *N*-monosubstituted hydroxylamines, compounds not readily available in general. However, nitrones can also be obtained in several alternative ways,²⁰ among which the oxidation of imines developed by some of us²¹ was selected as the most appropriate for our purposes. This procedure was established first on preformed imines employing methyltrioxorhenium (MTO) as the catalyst and urea hydrogen peroxide complex (UHP) as a mild stoichiometric oxidant. The methodology has the great advantage over other oxidation methods (on secondary amines²² or *N,N*-disubstituted hydroxylamines)^{20a} of forming a single nitrone, since the double bond is previously installed during the imine formation. Moreover, a convenient one-pot version has been subsequently implemented, where imines are formed *in situ* from inexpensive or readily available primary amines and aldehydes.²³

In this work, we employ the one-pot condensation/oxidation strategy to our carbohydrate-derived “masked” dialdehyde **8** with primary amines **13a–h** for the synthesis of several new *C*-carbohydrate *N*-alkyl nitrones **10** ([Scheme 1](#)). We also demonstrate that such nitrones undergo stereodivergent and highly stereoselective Grignard reagent additions in the presence or absence of a suitable Lewis acid. The following reductive amination (RA) of the formed adducts directly gives access to new *C,N*-dialkyl trihydroxypiperidines, as demonstrated with the synthesis of compounds **14** and **15** ([Scheme](#)

Table 1. Condensation/Oxidation Strategy for the Synthesis of Nitrones 10a–h from Aldehyde 8 and Primary Amines 13a–h^a

Entry	Amine 13 (1.2 equiv)	Nitron 10	Yield
1	13a	10a	80%
2	13b	10b	80%
3	13c	10c	75%
4	13d	10d	80%
5	13e	10e	80%
6	13f	10f	70%
7	13g	10g	65%
8	13h	10h	60%

^aConcerning the synthesis of nitron 10a (Table 1, entry 1), this method compares very well with the previously reported condensation procedure of aldehyde 8 with *N*-benzyl hydroxylamine (85%),¹⁴ especially considering the difference of costs with benzylamine (13a).

1). The target compounds were designed as to better mimic the two chains of the GlcCer (4) natural substrate.

The one-pot condensation/oxidation was investigated starting from aldehyde 8 with structurally diversified primary

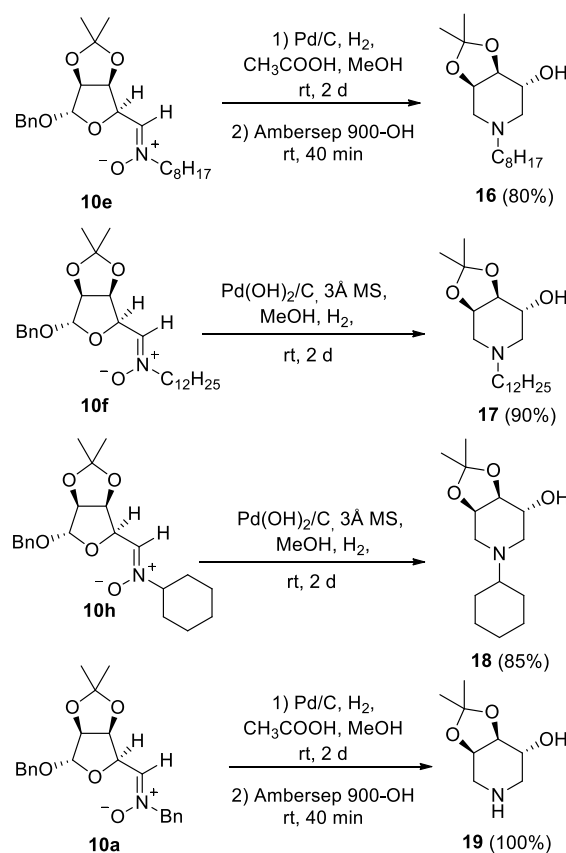
amines **13a–h** followed by addition of UHP and catalytic MTO, and the results are shown in Table 1. The reaction afforded the corresponding *N*-alkyl nitrones **10a–h** with good yields and in an operationally very simple manner. The procedure was optimized using benzylamine (**13a**) for the preparation of the known nitrone **10a**. The best results were obtained by stirring a solution of the aldehyde **8** with 1.2 equiv of the appropriate amine **13** in MeOH at room temperature in the presence of anhydrous Na₂SO₄, until disappearance of the starting aldehyde **8** at an ¹H NMR control (3–5 h, depending on the amine), which attested the formation of the corresponding imine. After cooling of the reaction mixture at 0 °C, addition of UHP (3 equiv) and MTO (4 mol %) caused the solution to turn yellow, indicating the formation of the catalytically active peroxorhenium species.²⁴ Upon completion of the oxidation at room temperature, a simple work-up consisting of solvent removal under reduced pressure followed by CH₂Cl₂ addition allowed filtering off the undissolved urea and Na₂SO₄ to give, after evaporation of the solvent, the crude nitrones which were purified by flash column chromatography on silica gel. Alternative addition of UHP and MTO since the beginning of the reaction (prior to imine formation) was found to be unpractical and afforded complex mixtures of products deriving from competitive oxidation of the primary amines.

The scope listed in Table 1 demonstrates the versatility of this method, which gives comparable results with complete conversions and good yields (70–80%) for benzylamines **13a–c** (entries 1–3), homobenzylamine **13d** (entry 4), and linear aliphatic amines **13e–f** (entries 5–6). Decrease in reaction yields with the α -branched (methyl)benzyl **13c** (entry 3) and aliphatic amines **13g** and **13h** (entries 7–8) suggests that the oxidation reaction is somewhat sensitive to steric effects. However, the corresponding nitrones **10c** and **10g–h** were still obtained in overall satisfactory yields (60–75%) through the two-step process. All the nitrones **10** were obtained exclusively as the more stable *Z*-diastereoisomer, in agreement with previous results (see also the Supporting Information section for 1D NOESY experiments on nitrone **10f**).²¹

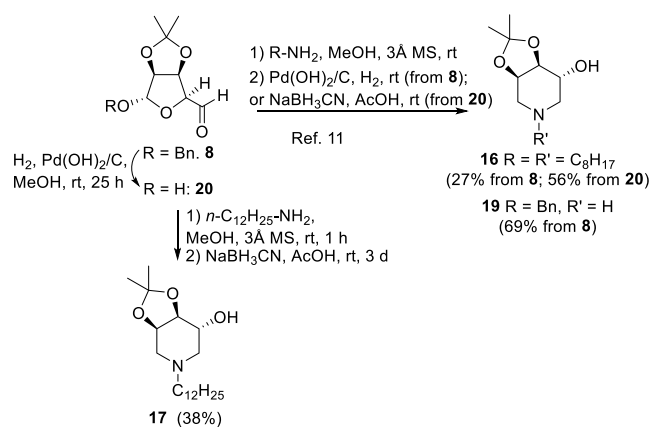
Nitrones **10** turned out to be excellent starting materials for the straightforward synthesis of *N*-substituted trihydroxy piperidines. Indeed, treatment of the nitrones **10e**, **10f**, and **10h** in MeOH under H₂ atmosphere (balloon) over Pd/C or Pd(OH)₂/C as the catalyst afforded the *N*-substituted piperidines **16**,¹¹ **17**, and **18**, respectively, in excellent yields (Scheme 2). The overall efficiency of this one-pot process is remarkable, considering that it results from a cascade of several reactions, consisting of nitrone reduction, debenzoylation, condensation with sugar aldehyde and iminium or enamine reduction (RA). Application of the same procedure to nitrone **10a** afforded the *N*-unsubstituted piperidine **19**¹¹ in quantitative yield.

It is worth to note that the yields obtained using this strategy were by far superior to those previously reported for the synthesis of piperidines **16** and **19** employing condensation of aldehyde **8** with primary amines and subsequent reduction to amine and RA-cyclization step, or, in alternative, employing a DRA strategy with NaBH₃CN as the reducing agent on aldehyde **20**, in turn derived from **8**.¹¹ (Scheme 3). For comparison, *N*-dodecyl trihydroxypiperidine **17**, obtained in 90% yield from nitrone **10f** (Scheme 2) was obtained also *via* DRA of **20** with dodecylamine in a modest 38% yield (Scheme 3).

Scheme 2. Reduction/Ring-Closure/RA Sequence to Trihydroxy Piperidines 16–19 from Nitrones 10



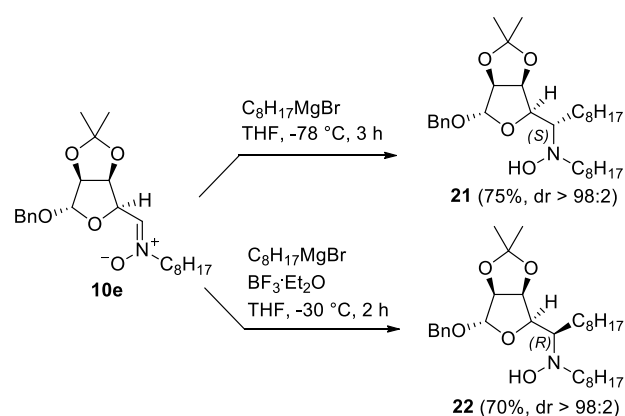
Scheme 3. Previously Reported Strategies for the Synthesis of 16 and 19 Starting from the Aldehyde 8 and Alternative Synthesis of the Novel *N*-Dodecyl Trihydroxypiperidine 17



The synthetic strategy based on the addition of Grignard reagents to nitrone **10e** followed by intramolecular RA provided access to the desired 1,2-octyl trihydroxypiperidines **14** and **15** (Scheme 1). The length of the chains (C8) was chosen on the basis of the data previously obtained from biological assays. We had previously observed that the addition of Grignard reagents to the carbohydrate-derived nitrone **10a** proceeded with opposite diastereofacial preference in the presence or absence of a suitable Lewis acid. The addition reaction, followed by RA, allowed the introduction of different alkyl groups with opposite configurations at the piperidine C-2,

affording two diastereomeric series of 2-alkyl trihydroxy piperidines.^{14,16,17} The addition of octylMgBr to nitrone **10e** was first carried out in THF at $-78\text{ }^{\circ}\text{C}$ for 3 h without any Lewis acid. The reaction gave smoothly the hydroxylamine **21**, with the *S* absolute configuration at the newly formed stereocenter accordingly to our prior work in good yield (75%) and excellent stereoselectivity (>98%) (Scheme 4). The

Scheme 4. Addition of Octyl Magnesium Bromide (1.8 equiv) to Nitron **10e** in the Presence or Absence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (1 equiv)



configuration at the newly created stereocenter was unambiguously confirmed by ^1H NMR and 1D NOESY spectra at a later stage of the synthesis (vide infra). The addition of octylMgBr to nitron **10e** in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ at $-30\text{ }^{\circ}\text{C}$ for 2 h resulted in a complete reversal of the diastereoselectivity in favor of the epimeric hydroxylamine **22**, which was obtained with excellent stereoselectivity (>98%) and good yield (70%) with an *R* configuration at the newly formed stereocenter (Scheme 4).

Albeit the observed stereochemical outcome of the additions of octylMgBr to the nitron **10e** in the absence or presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ is in agreement with the results obtained previously in the additions of several Grignard reagents to nitron **10a**, we were delighted to see that in this case a complete diastereoselectivity was reached. Instead, in the Grignard additions to **10a**, we had observed diastereomeric ratios in the range 1.4–5.6:1 without Lewis acids and 3–9:1 with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ in favor of the (*S*) and (*R*) configured adducts, respectively.^{16,17}

A magnesium Cram-chelate transition state (TS) may account for the preferred nucleophilic attack to the *Si* diastereoface of nitron **10e** in the absence of Lewis acid, resulting in the exclusive formation of the hydroxylamine **21** with the *S* configuration at the newly formed stereocenter (Figure 3A). Conversely, when an equimolar amount of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ is added, chelation is prevented, thus favoring the nucleophilic attack to the more accessible *Re* face of nitron **10e**, which leads to the hydroxylamine **22** with the *R* configuration at the newly formed stereocenter (Figure 3B).^{14,17}

The hydroxylamines **21** and **22** showed high tendency to oxidize spontaneously in air to the corresponding aldonitrones **23** and **24** (Scheme 5), according to the behavior observed for other hydroxylamines obtained by addition of Grignard reagents to the *N*-benzyl nitron **10a**; notwithstanding the $\text{C}=\text{N}$ bond does not benefit of conjugation in the present

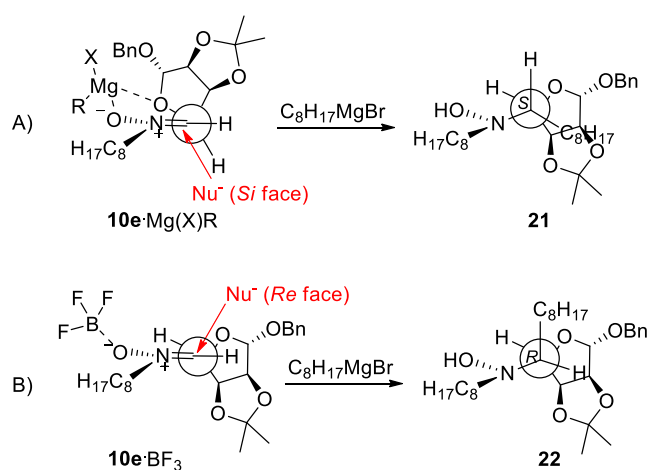


Figure 3. Transition state models proposed for the nucleophilic attack to nitron **10e**: (A) Cram-chelate TS in the absence of Lewis acid; (B) TS in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

case.^{16,17} In particular, spontaneous partial oxidation of **21** and **22** occurred with 50% conversion, as evaluated by ^1H NMR spectroscopy. The exclusive formation of aldonitrones instead of ketonitrones was providential, since it did not trigger any loss of configurational integrity at the newly formed stereocenter and was not detrimental for our synthetic purposes (see below).

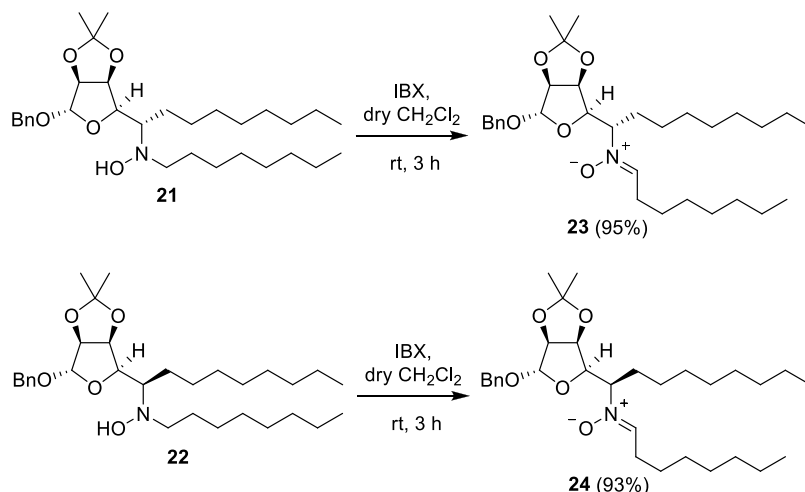
However, a certain regioselectivity in favor of the aldonitrones was expected based on our previous studies.

Due to the low stability of hydroxylamines **21** and **22**, they were characterized only by ^1H NMR and MS analyses immediately after purification by column chromatography. Characterization of pure nitrones **23** and **24** was carried out after complete oxidation of the hydroxylamines **21** and **22** with the hypervalent iodine reagent 2-iodoxy benzoic acid (IBX), which is the reagent of choice to promote regioselective oxidation of *N,N*-disubstituted hydroxylamines in favor of the corresponding aldonitrones.^{13,25} In this case, it provided the corresponding nitrones **23** and **24** in excellent yields and with complete regioselectivity (Scheme 5).

The final reductive amination step was performed on the hydroxylamine/nitron mixtures by employing 2 equiv of acetic acid under an H_2 atmosphere (balloon) and Pd/C as the catalyst in MeOH (0.015 M solution), followed by treatment with a strongly basic resin to give the free amines **25** and **26** in excellent 90 and 80% yield, respectively (Scheme 6).

Careful analysis of the ^1H NMR, 2D NMR (COSY, HSQC), and 1D NOESY spectra of piperidines **25** and **26** allowed us to establish unambiguously their configuration, thus validating the stereochemical outcome of the additions of octylMgBr reported in Scheme 4. The two chair conformations of each compound are reported in Figure 4. The ^1H NMR spectrum of (*2S*) piperidine **25** showed a small coupling constant between 3-H and 4-H ($^3J_{3-4} = 4.0\text{ Hz}$), suggesting a high preference for its $^1\text{C}_4$ conformation where both 3-H and 4-H are equatorial. The $^1\text{C}_4$ is expected to be the absolute minimum energy conformation for the *S*-configured piperidine at C-2, with the octyl chain lying in equatorial position (Figure 4). Accordingly, the 1D NOESY spectrum of **25** did not show the nOe correlation peak between 2-H and 4-H. For the diastereomeric piperidine **26**, the opposite *R* configuration at C-2 shifted the equilibrium to a preferred $^4\text{C}_1$ conformation in order to accommodate again the octyl chain in an equatorial position, as

Scheme 5. Oxidation of Hydroxylamines 21 and 22 with IBX: Synthesis of Nitrones 23 and 24



Scheme 6. Ring-Closure/Reductive Amination to 25 and 26 and Their Final Deprotection to 1,2-Dioctyl Trihydroxypiperidines 14 and 15

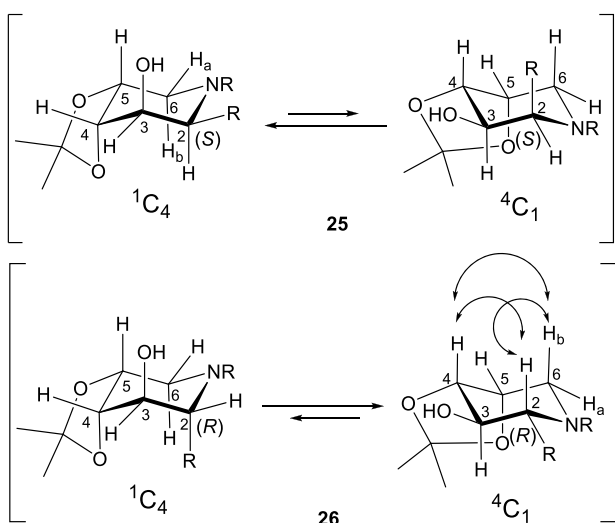
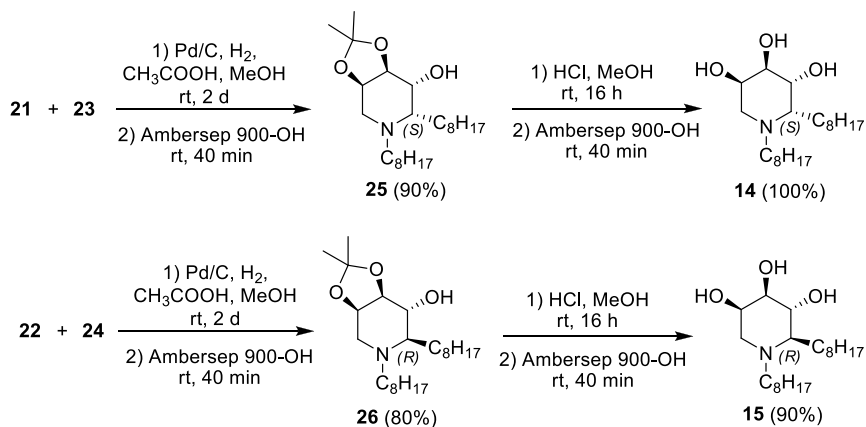


Figure 4. Chair conformations of piperidines 25 and 26 ($R = nC_8H_{17}$). Double-ended arrows show the observed diagnostic nOe correlation peaks.

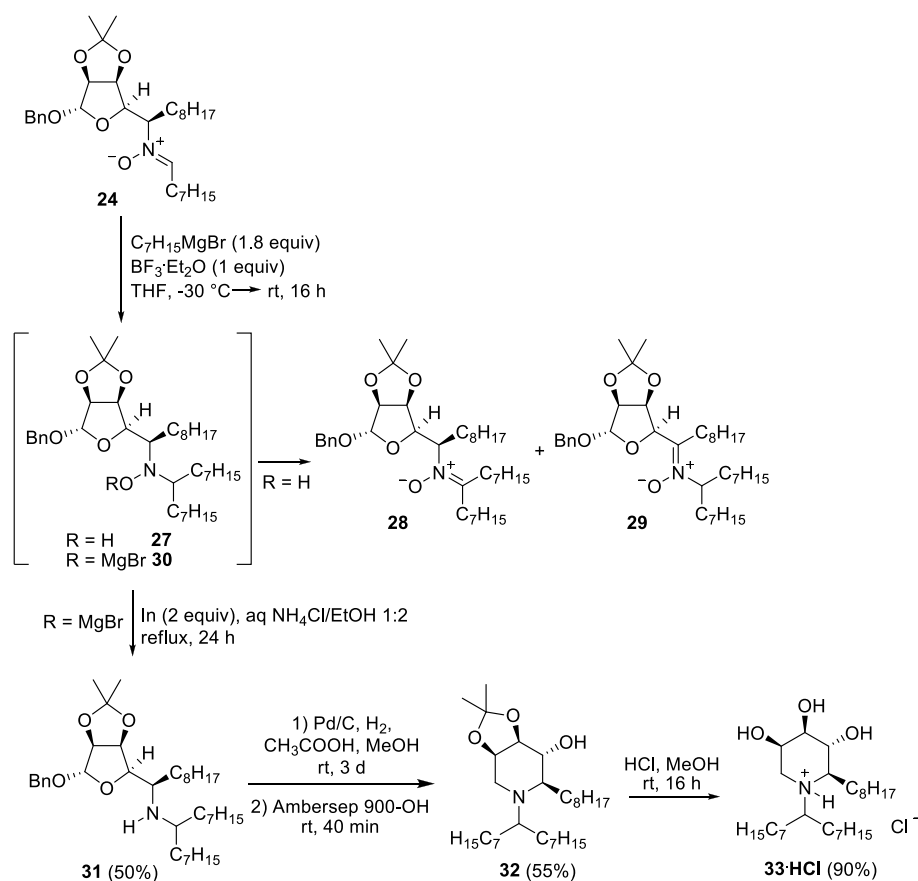
attested by the increase of the coupling constant between 3-H and 4-H ($^3J_{3-4} = 8.0$ Hz) in agreement with their *ax-ax* relationship (Figure 4). The set of strong nOe correlation

peaks observed in the 1D-NOESY spectrum of 26 among all axial protons (2-H with 4-H and 6-H_b and 4-H with 6-H_b, 2-H, see the Supporting Information section) substantiated this assignment.

Final deprotection of the acetonide protecting groups under acidic conditions (aqueous HCl in MeOH) followed by treatment with the strongly basic resin Ambersep 900-OH gave the target 1,2-dioctyl trihydroxypiperidines 14 and 15 as free amines in excellent yields (Scheme 6).

We envisaged that the intriguing formation of the aldonitrones 23 and 24 by easy and regioselective oxidation of hydroxylamines 21 and 22 would furnish the chance for further extending our study. Indeed, another alkyl chain might be introduced *via* iteration of the Grignard addition to these nitrones to provide final piperidines possessing three lipophilic tails. This hypothesis was proven with nitrone 24. In order to avoid formation of a further stereogenic center resulting in a mixture of diastereoisomers, heptyl magnesium bromide was chosen as the appropriate Grignard reagent, but the addition turned out to be sluggish. In the absence of the promoter at different temperatures (-30 °C, 0 °C, rt), in THF, it failed to give any product. Addition of $BF_3 \cdot Et_2O$ (1.0 equiv) resulted in the formation of the desired hydroxylamine 27 after 16 h (Scheme 7), as attested by the peak at m/z : 618.31 ($[M + H]^+$) at an ESI-MS analysis. The hydroxylamine 27 was

Scheme 7. Addition of heptylMgBr to Nitron 24, One-Pot Addition/Reduction to Amine 31, and Synthesis of the Three-Tailed Trihydroxypiperidine 33·HCl



unstable and presumably underwent mainly rapid oxidation to the corresponding ketonitrone 28 and 29, as suggested by the presence of two TLC spots with very similar R_f and a peak at 638.55, corresponding to $[\text{M} + \text{Na}]^+$, in the ESI-MS spectrum (Scheme 7).

The lack of regioselectivity in the oxidation of 27 is a major drawback in view of our synthetic target, since the ketonitrone 29 has lost the stereochemical information to be installed at C-2 in the final piperidine. In order to prevent the rapid oxidation of the hydroxylamine 27, the adduct 30 was reduced *in situ* to the corresponding amine 31 by direct addition to the crude reaction mixture of indium powder in slightly acidic aqueous ethanol.²⁶ This one-pot Grignard addition/reduction afforded the amine 31 in 50% yield without any loss of stereochemical integrity (Scheme 7). The RA of amine 31 with H_2 as the reducing agent in the presence of catalytic Pd/C and acetic acid (2 equiv) in MeOH provided the partially protected trihydroxypiperidine 32 in 55% yield (Scheme 7). Final deprotection of the acetonide performed under acidic conditions (aqueous HCl in MeOH) led to the desired trihydroxypiperidine 33 as the hydrochloride salt (Scheme 7).²⁷

2.3. Biology and Molecular Dynamics. **2.3.1. Preliminary Biological Evaluation toward Commercial Glycosidases.** Preliminary biological evaluation of compounds 14 and 15 (at 100 μM inhibitor concentration) was performed toward a panel of 12 commercial glycosidases (α -L-fucosidase EC 3.2.1.51 from *Homo sapiens*, α -galactosidase EC 3.2.1.22 from coffee beans, β -galactosidases EC 3.2.1.23 from *Escherichia coli*

and *Aspergillus oryzae*, α -glucosidases EC 3.2.1.20 from yeast and rice, amyloglucosidase EC 3.2.1.3 from *Aspergillus niger*, β -glucosidase EC 3.2.1.3 from almonds, α -mannosidase EC 3.2.1.24 from Jack beans, β -mannosidase EC 3.2.1.25 from snail, and β -N-acetylglucosaminidases EC 3.2.1.52 from Jack beans and bovine kidney).

No remarkable inhibitory activity was found at this concentration for compounds 14 and 15 toward any of these enzymes apart from a 30% inhibition of β -glucosidase from almonds by compound 14 (see the Supporting Information section). Even if moderate, this value was encouraging, since we have previously noticed compounds with a low inhibitory activity toward this commercial enzyme that turned out to be stronger inhibitors of human lysosomal GCase.^{12,17}

2.3.2. Inhibitory Activity of GCase. Compounds 14, 15, and 33·HCl were then tested at 1 mM for GCase inhibition in human leukocyte homogenates. The percentages of inhibition, together with the corresponding IC_{50} values, are shown in Table 2. The results obtained were compared with data of previously reported compounds 9, 11, and 12.^{12,16,17}

Our results show that both the newly synthesized trihydroxypiperidines 14 and 15 were able to strongly inhibit GCase, imparting 100% inhibition of the enzyme at 1 mM concentration (Table 2, entries 4–5). However, a remarkable difference emerged between the two compounds from measurement of their IC_{50} , which followed the same trend observed for the C-2 mono-alkylated azasugars 11 and 12. Indeed, the 1,2-dioctyl azasugar 15 with the R configuration at C-2 was a stronger inhibitor than its S-configured diaster-

Table 2. GCase Inhibition and IC₅₀ Values in Human Leukocytes from Healthy Donors

entry	compound	GCase inhibition [%] ^a	IC ₅₀ (μM) ^b
1	9	98	30.0 ± 1.0 ^c
2	11	80	93.5 ± 5.3 ^d
3	12	100	29.3 ± 1.8 ^d
4	14	100	100.0 ± 9.0
5	15	100	15.0 ± 4.0
6	33·HCl	75	130 ± 15

^aPercentage inhibition of GCase in human leukocytes extracts incubated with azasugars (1 mM). ^bIC₅₀ values were determined by measuring GCase activity at different concentrations of each inhibitor. ^cRef 12 (IC₅₀ value obtained using the same experimental protocol and substrate concentration of this manuscript). ^dRefs 16 and 17 (IC₅₀ value obtained using the same experimental protocol and substrate concentration of this manuscript).

eoisomer 14 (IC₅₀ = 15.0 μM vs IC₅₀ = 100 μM, Table 2 entry 5 vs 4). In this latter case, the difference was even more pronounced than for the corresponding monoalkylated congeners 12 and 11. It is also worth to note that the dialkylated piperidine 15 is twofold more active than its monoalkylated counterpart 12. Apparently, the presence of a second octyl chain at the nitrogen atom imparted beneficial interactions within the enzyme active site only in the case of the 2*R*-configured pair. Conversely, the 1,2-dialkylated azasugar 33·HCl, although maintaining the octyl chain at C-2 with *R* configuration is a much less potent GCase inhibitor than 15 (IC₅₀ = 130.0 μM vs IC₅₀ = 15.0 μM, Table 2 entry 6 vs 5), suggesting that the presence of a further alkyl chain is detrimental.

2.3.3. Molecular Dynamic Studies. We carried out MD simulations for (*R*) and (*S*) protonated forms of compounds 14 and 15, considering both conformations ⁴C₁ and ¹C₄ as starting points and taken into consideration the best poses obtained from the docking studies and also the best ones in which the piperidine ring is correctly oriented as IFG. In all cases, MD converged to stable complexes which were reached after 10 ns of simulation and continued to be stable for 250 ns (all MD simulations were replicated four times). The complexes showed the ligand in the binding site establishing different interactions with key residues Asp128, Trp180, Asn235, Glu236, Tyr314, and Glu341 and with the correct orientation of the piperidine ring (see the Supporting Information section).²⁸ The overall RMSD for the protein system appeared to have reached equilibrium in the first ns, and the stabilization of the protein–ligand complex after 20 ns keeping the interactions of the complexes constant during the rest of the simulation. The ligands showed a complete stability of chair conformations and tend to arrange the aliphatic chains toward the external part, exposed to the solvent, in order to keep interactions of the hydroxyl groups of the piperidine ring. The presence of the alkyl chains induces some visible conformational change of the protein in the area close to the binding site. Interestingly, similar protein conformations were observed for (*R*)-14H⁺ ⁴C₁ and (*S*)-14H⁺ ¹C₄, whereas a loop formed by residues 345–349 is partially opened with (*R*)-14H⁺ ¹C₄ and completely opened with (*S*)-14H⁺ ⁴C₁ (see the Supporting Information section). In the case of compound 15H⁺, the protein shows a higher flexibility with the loop formed by residues 345–349 adopting different orientations. The mobility of the loop, rich in hydrophobic residues

(Met347, Phe348, and Trp349) can be attributable to hydrophobic interactions with alkyl chains that stabilizes the complex as expected. The sole inspection of the interactions in the binding site shows that (*R*)-15H⁺ ⁴C₁ is the complex having the highest stability (Figure 5).

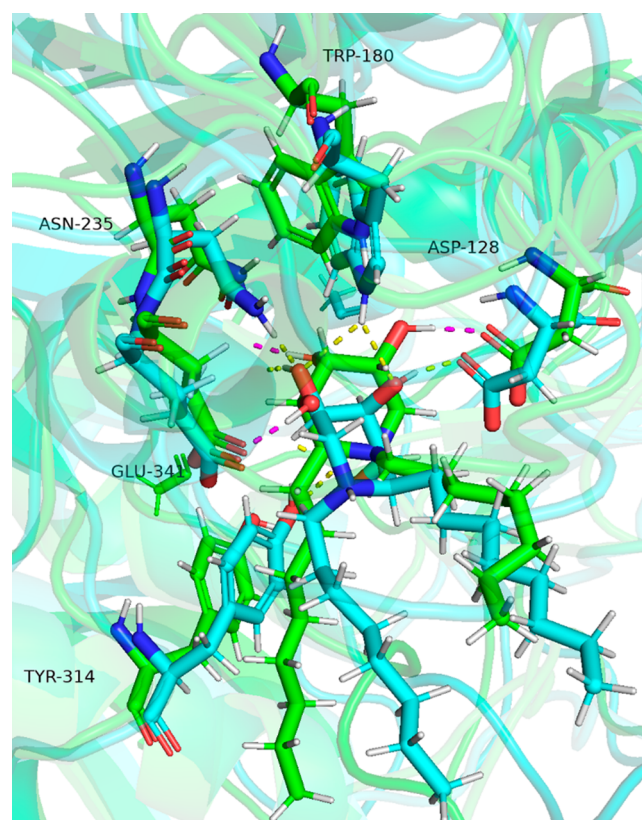


Figure 5. Close-up view of GCase in complex with compounds (*R*)-15H⁺ ⁴C₁ (cyan) with (*R*) configuration at C-2 and (*R*)-14H⁺ ⁴C₁ (green) with (*S*) configuration at C-2. Dashed yellow and magenta lines indicate H-bond interactions of (*R*)-15H⁺ ⁴C₁ and (*R*)-14H⁺ ⁴C₁, respectively. It can be appreciated that the former has more polar contacts with the residues of the binding site than the latter.

With the aim of obtaining more accurate information on the stability of the complexes (rather than the qualitative observation of the interactions), we carried out additional calculations to evaluate the binding energy. For the purpose of comparison both MM/GBSA and MM/PSBA calculations were performed.²⁹ In both cases, higher values were obtained for compound 15, the best values being found for (*R*)-15H⁺ ⁴C₁ ($E_{\text{binding}} = -57.9$ kcal/mol with MM/GBSA and -49.4 kcal/mol with MM/PBSA) corroborating the observed interactions and in good agreement with experimental results (see the Supporting Information section).

2.3.4. Preliminary Biological Screening toward Human Lysosomal Glycosidases. In order to avoid undesired inhibitory side effects of a potential new drug, it has to be selective for a given target. The selectivity of the newly synthesized compounds 14, 15, and 33·HCl toward GCase was then investigated, evaluating their inhibition at 1 mM concentration toward six other lysosomal glycosidases (namely, α - and β -mannosidases, α - and β -galactosidases, α -fucosidase, and α -glucosidase) in cell homogenates (leucocytes or lymphocytes) isolated from healthy donors. The results,

reported in the Supporting Information section, clearly show that these compounds are selective inhibitors of GCCase. Indeed, only moderate inhibitory activity (*ca.* 50%) was found toward human β -galactosidase for both piperidines **14** and **15**, while negligible inhibitory activity was detected in all other cases.

2.3.5. Pharmacological Chaperoning Activity. The ability of compounds **14** and **15** to enhance the activity of GCCase after incubation (4 days) with fibroblasts bearing the selected mutations was evaluated. The experiments were performed using fibroblasts derived from Gaucher patients bearing the N370S/RecNcil and L444P/L444P mutations (see the Supporting Information section). Unfortunately, no enzymatic activity rescue was observed after incubation with increasing concentrations of compounds **14** and **15** from 5 nM to 10 μ M. The assay could not be performed at higher concentration (50 or 100 μ M), since the low cell viability observed hampered the measurement of the enzymatic activity.

These data show that the simultaneous presence of an octyl chain at both the endocyclic nitrogen and C-2 of the piperidine skeleton makes these compounds too cytotoxic at the highest concentrations, while at the lowest concentrations, no activity rescue was observed.

The low viability detected above 50 μ M concentration may be ascribed to interactions of **14** and **15** with lipids and proteins of the cell membranes which lead to cell lysis, as it was previously reported for other amphiphilic compounds when tested *in vitro*.³⁰

Expecting an even higher impact on cell viability from compound **33**•HCl, which has an additional lipophilic chain, a preliminary MTT test was carried out on wild-type fibroblasts (see Supporting Information). Since a remarkable cytotoxicity was observed for prolonged incubation at 100 and 50 μ M concentrations, compound **33**•HCl was co-incubated in Gaucher patients' fibroblasts (bearing the N370S/RecNcil mutation) in lower concentrations, ranging from 5 nM to 30 μ M. Unfortunately, also in this case, no enzymatic activity rescue was observed after 4 days of incubation.

3. CONCLUSIONS

Following our interest in the discovery of new inhibitors and/or pharmacological chaperones for the GCCase enzyme, we investigated the condensation/oxidation reaction of carbohydrate-derived aldehyde **8** with several primary amines employing urea hydrogen peroxide as the stoichiometric oxidant and methyltrioxorhenium as the catalyst. This strategy afforded in a simple and straightforward way several new C-carbohydrate N-alkyl nitrones **10a–h**. Further ring-closure reductive amination provided a series of new precursors of trihydroxypiperidine azasugars bearing different substituents at the nitrogen atom much more effectively than previously reported.

The reaction of the C-erythrosyl N-octyl nitrone **10e** with octylMgBr in the presence or absence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ provided access to both epimeric hydroxylamines with opposite configuration at the newly created stereocenter in a stereodivergent and completely stereoselective way. Final reductive amination and acetonide deprotection provided compounds **14** and **15** from low-cost D-mannose in remarkable 43 and 32% overall yields, respectively, over eight steps. A third alkyl chain was introduced by iteration of the organometal addition to a nitrone easily obtained by oxidation with complete regioselectivity after the first addition, followed by the one-pot hydroxylamine/amine reduction mediated by indium metal.

This strategy provided the azasugar **33**•HCl bearing three alkyl chains.

The new compounds, assayed toward commercial and human lysosomal glucosidases, proved to be good and selective GCCase (human lysosomal β -glucosidase) inhibitors. In particular, the C-2 R-configured bis-alkylated trihydroxypiperidine **15** showed a lower IC_{50} than the corresponding mono-alkylated analogue **12** ($\text{IC}_{50} = 15 \mu\text{M}$ vs $\text{IC}_{50} = 29.3 \mu\text{M}$). Conversely, the presence of a third alkyl tail as in compound **33**•HCl affected negatively the GCCase inhibitory activity ($\text{IC}_{50} = 130 \mu\text{M}$).

However, once tested in fibroblasts derived from Gaucher patients bearing the N370S/RecNcil and L444P/L444P mutations, no GCCase activity enhancements were observed, demonstrating that the addition of one (**14** and **15**) or two alkyl chains (**33**•HCl) strongly increases the cytotoxicity of the compounds and it is detrimental for the pharmacological chaperoning activity.

Preliminary docking studies suggested compound **15** as the best ligand although the preferred conformation and orientation were not envisaged. Further MD simulations identified the protonated derivative of **15** with (R) configuration at the nitrogen atom and adopting a ${}^4\text{C}_1$ conformation as the best ligand. The different isomers and the corresponding conformers induce a substantial change in the conformation of a loop, composed by residues 345–349, to accommodate the aliphatic alkyl chains. MM/GBSA calculations corroborated the preference by (R)-**15H**⁺ ${}^4\text{C}_1$ with a binding energy of -57.9 kcal/mol.

4. EXPERIMENTAL SECTION

4.1. Chemistry. **4.1.1. General Methods.** Commercial reagents were used as received. All reactions were carried out under magnetic stirring and monitored by TLC on 0.25 mm silica gel plates (Merck F254). Column chromatographies were carried out on Silica Gel 60 (32–63 μm) or on silica gel (230–400 mesh, Merck). Yields refer to spectroscopically and analytically pure compounds unless otherwise stated. ${}^1\text{H}$ NMR spectra were recorded on a Varian Gemini 200 MHz, a Varian Mercury 400 MHz, or on a Varian INOVA 400 MHz instruments at 25 °C. ${}^{13}\text{C}$ NMR spectra were recorded at 50 MHz or at 100 MHz. Chemical shifts are reported relative to CDCl_3 (${}^1\text{H}$: $\delta = 7.27$ ppm, ${}^{13}\text{C}$: $\delta = 77.0$ ppm). Integrals are in accordance with assignments, coupling constants are given in Hz. For detailed peak assignments, 2D spectra were measured (g-COSY, g-HSQC) and 1D-NOESY. The following abbreviations were used to designate multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, quin = quintuplet, sext = sextet, sept = septet, br s = broad singlet, and dd = double-doublet. IR spectra were recorded with an IRAffinity-1S Shimadzu spectrophotometer. ESI-MS spectra were recorded with a Thermo Scientific LCQ fleet ion trap mass spectrometer. Elemental analyses were performed with a Thermo Finnigan FLASH EA 1112 CHN/S analyzer. Optical rotation measurements were performed on a JASCO DIP-370 polarimeter.

4.1.2. General Procedure for the Synthesis of Nitrones. Amines **13a–h** were purchased from Merck (**13a**, **13c**, **13f**, and **13h**), Fluka (**13d**, **13g**), TCI (**13e**), Janssen (**13b**) and used without further purification.

To a stirred solution of aldehyde **8** (200 mg, 0.72 mmol) and anhydrous Na_2SO_4 (380 mg) in MeOH (9 mL) at room temperature, the appropriate amine **13a–h** (1.2 equivalents) was added and the resulting mixture was stirred at room temperature under nitrogen atmosphere until ${}^1\text{H}$ NMR control attested the formation of imine intermediates (3 h). The reaction mixture was cooled at 0 °C and urea–hydrogen peroxide (UHP, 203 mg, 2.16 mmol), and methyltrioxorhenium (MTO, 7.2 mg, 0.29 mmol) were added sequentially. The reaction mixture was stirred at room temperature

for 16 h, when a TLC control (PEt/EtOAc 1:1) attested the disappearance of the starting material and the presence of a new UV visible spot; then, the solvent was removed under reduced pressure. CH₂Cl₂ was added to the crude mixture, and the undissolved urea was filtered off. Solvent removal under reduced pressure afforded the crude product, which was purified by flash column chromatography on silica gel.

4.1.3. Synthesis of (Z)-Benzyl 2,3-O-(1-methylethylidene)-5-deoxy-N-benzyl-D-lyxofuranosylamine N-Oxide (10a).¹⁴ Application of the general procedure to 200 mg (0.72 mmol) of **8** with 94 μ L (0.86 mmol) of benzylamine (**13a**) furnished, after purification by column chromatography (PEt/EtOAc 3:1), 221 mg (0.56 mmol, 80%) of **10a** as a white solid ($R_f = 0.40$, PEt/EtOAc 2:1).

10a: white solid. mp 118–120 °C. $[\alpha]_D^{25} + 55.9$ (c 0.65, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.37–7.19 (m, 10H, Ar), 6.75 (dd, $J = 4.4, 2.4$ Hz, 1H), 5.11–5.07 (m, 2H), 5.04 (s, 1H), 4.89 (s, 2H), 4.61–4.58 (m, 2H), 4.37 (d, $J = 11.7$ Hz, 1H), 1.30 (s, 3H), 1.21 (s, 3H) ppm. ¹³C{¹H} NMR (50 MHz, CDCl₃): δ 137.0, 135.6, 132.4, 129.4–127.9 (10 C), 112.6, 105.0, 84.5, 79.7, 76.7, 69.1, 68.9, 26.0, 24.7 ppm.

4.1.4. Synthesis of (Z)-Benzyl 2,3-O-(1-methylethylidene)-5-deoxy-N-(2-methoxybenzyl)-D-lyxofuranosylamine N-Oxide (10b). Application of the general procedure to 200 mg (0.72 mmol) of **8** with 112 μ L (0.86 mmol) of 2-methoxybenzylamine (**13b**) furnished, after purification by column chromatography (PEt/EtOAc 2:1), 238 mg (0.58 mmol, 80%) of **10b** as a white solid ($R_f = 0.20$, PEt/EtOAc 2:1).

10b: white solid. mp 118–120 °C. $[\alpha]_D^{25} + 55.9$ (c 0.65, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.42–7.26 (m, 7H, Ar), 6.99 (t, $J = 6.6$ Hz, 1H, Ar), 6.92 (d, $J = 8.0$ Hz, 1H, Ar), 6.79 (d, $J = 4.0$ Hz, 1H, HC=N), 5.17–5.10 (m, 2H, 4-H and 3-H), 5.09 (s, 1H, 1-H), 4.99 AB system, $J = 12.0$ Hz, 2H NCH₂Ar, 4.67 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 4.66–4.65 (m, 1H, 2-H), 4.43 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 3.85 (s, 3H, OCH₃), 1.35 (s, 3H, C(CH₃)₂), 1.27 (s, 3H, C(CH₃)₂) ppm. ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 157.9 (Ar-OCH₃), 137.1 (Ar), 135.3 (C=N), 132.0 (Ar), 130.9 (Ar), 128.6–128.0 (5C, Ar), 121.0 (Ar), 120.8 (Ar), 112.6 (C(CH₃)₂), 110.8 (Ar), 105.0 (C-1), 84.6 (C-2), 79.8 (C-3), 76.8 (C-4), 69.0 (OCH₂Ar), 64.0 (NCH₂Ar), 55.2 (OCH₃), 26.2 (C(CH₃)₂), 24.8 (C(CH₃)₂) ppm. MS (ESI) m/z (%): 849.0 (100) [2M + Na]⁺, 436.1 (82) [M + Na]⁺. IR (CDCl₃): ν : 976, 1159, 1254, 1379, 1462, 1496, 1606, 2241, 2843, 2941, 3034 cm⁻¹. Anal. Calcd for C₂₃H₂₇NO₆: C, 66.81; H, 6.58; N, 3.39. Found: C, 67.01; H, 6.50; N, 3.20.

4.1.5. Synthesis of (Z)-Benzyl 2,3-O-(1-methylethylidene)-5-deoxy-N-(1-phenylethyl)-D-lyxofuranosylamine N-Oxide (10c). Application of the general procedure to 200 mg (0.72 mmol) of **8** with 110 μ L (0.86 mmol) of (S)-(-)-1-phenylethylamine (**13c**) furnished, after purification by column chromatography (PEt/EtOAc 2:1), 214 mg (0.34 mmol, 75%) of **10c** as a white solid ($R_f = 0.20$, PEt/EtOAc 2:1).

10c: white solid. mp 97–99 °C. $[\alpha]_D^{25} - 5.4$ ($c = 0.86$, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.50 (d, $J = 8.0$ Hz, 2H, Ar), 7.38–7.26 (m, 8H, Ar), 6.79 (d, $J = 4.0$ Hz, 1H, HC=N), 5.16–5.15 (m, 2H, 4-H and 3-H), 5.13 (s, 1H, 1-H), 5.10 (q, $J = 6.8$ Hz, 1H, (CH₃)CHAr), 4.69 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 4.66–4.65 (m, 1H, 2-H), 4.45 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 1.84 (d, $J = 6.8$ Hz, 3H, (CH₃)CHAr), 1.35 (s, 3H, C(CH₃)₂), 1.25 (s, 3H, C(CH₃)₂) ppm. ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 137.8 (Ar), 136.9 (Ar), 134.2 (HC=N), 128.7–127.3 (10C, Ar), 112.4 (C(CH₃)₂), 104.8 (C-1), 84.4 (C-2), 79.6 (C-3), 76.7 (C-4), 73.2, ((CH₃)CHAr), 68.9 (OCH₂Ar), 26.0 (C(CH₃)₂), 24.7 (C(CH₃)₂), 18.7 ((CH₃)CHAr) ppm. MS (ESI) m/z (%): 817.0 (100) [2M + Na]⁺, 420.12 (61) [M + Na]⁺. IR (CDCl₃): ν : 970, 1382, 1456, 1496, 1608, 2249, 2873, 2938, 2983, 3034, 3406 cm⁻¹. Anal. Calcd for C₂₃H₂₇NO₆: C, 69.50; H, 6.85; N, 3.52. Found: C, 69.45; H, 6.96; N, 3.65.

4.1.6. Synthesis of (Z)-Benzyl 2,3-O-(1-methylethylidene)-5-deoxy-N-[2-(3-methoxyphenyl)ethyl]-D-lyxofuranosylamine N-Oxide (10d). Application of the general procedure to 200 mg (0.72 mmol) of **8** with 120 μ L (0.86 mmol) of 2-(3-methoxyphenyl)-ethylamine (**13d**) furnished, after purification by column chromatog-

raphy (PEt/EtOAc 2:1), 246 mg (0.58 mmol, 80%) of **10d** as a white solid ($R_f = 0.20$, PEt/EtOAc 2:1).

10d: white solid. mp 87–89 °C. $[\alpha]_D^{25} - 18.0$ (c 0.90, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.32–7.21 (m, 5H, Ar), 7.17 (t, $J = 7.4$ Hz, 1H, Ar), 6.77 (d, $J = 8.0$ Hz, 1H, Ar), 6.74 (d, $J = 8.0$ Hz, 1H, Ar), 6.73 (s, 1H, Ar), 6.67 (d, $J = 5.2$ Hz, 1H, HC=N), 5.10 (pseudo t, $J = 4.2$ Hz, 1H, 4-H), 5.06–5.04 (m, 2H, 3-H and 1-H), 4.63 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 4.62–4.60 (m, 1H, 2-H), 4.40 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 4.01–3.95 (m, CH₂N), 3.73 (s, 3H, OCH₃), 3.18 (t, $J = 7.2$ Hz, 2H, NCH₂CH₂Ar), 1.30 (s, 3H, C(CH₃)₂), 1.23 (s, 3H, C(CH₃)₂) ppm. ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 159.9 (Ar-OCH₃), 139.0 (Ar), 137.0 (Ar), 135.5 (HC=N), 129.8 (Ar), 128.5–128.0 (5C, Ar), 121.0 (Ar), 114.4 (Ar), 112.7 (C(CH₃)₂), 112.5 (Ar), 105.1 (C-1), 84.7 (C-2), 79.8 (C-3), 76.3 (C-4), 68.9 (OCH₂Ar), 66.1 (NCH₂CH₂Ar), 55.2 (OCH₃), 33.8 (NCH₂CH₂Ar), 26.1 (C(CH₃)₂), 24.7 (C(CH₃)₂) ppm. MS (ESI) m/z (%): 450.13 (100) [M + Na]⁺. IR (CDCl₃): ν : 976, 1078, 1159, 1211, 1379, 1460, 1490, 1604, 2241, 2839, 2876, 2941, 3032 cm⁻¹. Anal. Calcd for C₂₄H₂₉NO₆: C, 67.43; H, 6.84; N, 3.28. Found: C, 67.40; H, 6.85; N, 3.22.

4.1.7. Synthesis of (Z)-Benzyl 2,3-O-(1-methylethylidene)-5-deoxy-N-octyl-D-lyxofuranosylamine N-Oxide (10e). Application of the general procedure to 300 mg (1.08 mmol) of **8** with 215 μ L (1.30 mmol) of octylamine (**13e**) furnished, after purification by column chromatography (Hex/EtOAc 2:1), 350 mg (0.86 mmol, 80%) of **10e** as a white solid ($R_f = 0.34$, Hex/EtOAc 2:1).

10e: white solid. mp 64–66 °C. $[\alpha]_D^{25} - 8.2$ (c 0.65, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.32–7.24 (m, 5H, Ar), 6.83 (d, $J = 4.4$ Hz, 1H, HC=N), 5.14–5.11 (m, 2H, 4-H and 3-H), 5.09 (s, 1H, 1-H), 4.65 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 4.64–4.63 (m, 1H, 2-H), 4.43 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 3.78 (t, $J = 6.0$ Hz, 2H, CH₂N), 1.95–1.91 (m, 1H), 1.85–1.83 (m, 1H), 1.38 (s, 3H, C(CH₃)₂), 1.34–1.26 (m, 10H), 1.25 (s, 3H, C(CH₃)₂), 0.85 (t, $J = 6.0$ Hz, 3H) ppm. ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 137.0 (Ar), 135.1 (HC=N), 128.5–127.9 (5C, Ar), 112.6 (C(CH₃)₂), 105.0 (C-1), 85.4 (C-2), 79.7 (C-3), 76.4 (C-4), 69.0 (OCH₂Ar), 65.3 (CH₂N), 31.8–22.7 (6C and 2C, C(CH₃)₂), 14.1 ppm. MS (ESI) m/z (%): 833.0 (100) [2M + Na]⁺, 428.23 (20) [M + Na]⁺. IR (CDCl₃): ν : 970, 1028, 1101, 1161, 1209, 1267, 1356, 1456, 2232, 2928, 3034, 3066 cm⁻¹. Anal. Calcd for C₂₃H₃₅NO₆: C, 68.12; H, 8.70; N, 3.45. Found: C, 68.34; H, 8.40; N, 3.50.

4.1.8. Synthesis of (Z)-Benzyl 2,3-O-(1-methylethylidene)-5-deoxy-N-dodecyl-D-lyxofuranosylamine N-Oxide (10f). Application of the general procedure to 200 mg (0.72 mmol) of **8** with 160 mg (0.86 mmol) of dodecylamine (**13f**) furnished, after purification by column chromatography (PEt/EtOAc 2:1), 233 mg (0.50 mmol, 70%) of **10f** as a white solid ($R_f = 0.31$, PEt/EtOAc 2:1).

10f: white solid. mp 95–97 °C. $[\alpha]_D^{20} - 3.2$ (c 0.81, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.34–7.26 (m, 5H, Ar), 6.86 (d, $J = 4.8$ Hz, 1H, HC=N), 5.18–5.14 (m, 2H, 4-H and 3-H), 5.13 (s, 1H, 1-H), 4.70 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 4.69–4.66 (m, 1H, 2-H), 4.47 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 3.82 (t, $J = 7.0$ Hz, 2H, CH₂N), 1.98–1.95 (m, 1H), 1.88–1.85 (m, 1H), 1.41 (s, 3H), 1.32–1.26 (m, 21H), 0.88 (t, $J = 6.6$ Hz, 3H) ppm. ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 137.1 (Ar), 135.2 (HC=N), 128.6–128.0 (5C, Ar), 112.7 (C(CH₃)₂), 105.1 (C-1), 84.7 (C-2), 79.8 (C-3), 76.5 (C-4), 69.1 (OCH₂Ar), 65.5 (CH₂N), 32.0–24.8 (10C and 2C, C(CH₃)₂), 14.2 ppm. MS (ESI) m/z (%): 944.73 (100) [2M + Na]⁺, 484.32 (30) [M + Na]⁺. IR (CDCl₃): ν : 972, 1026, 1087, 1161, 1211, 1263, 1356, 1456, 2232, 2928, 3034 cm⁻¹. Anal. Calcd for C₂₇H₄₃NO₆: C, 70.25; H, 9.39; N, 3.03. Found: C, 70.35; H, 9.25; N, 3.00.

4.1.9. Synthesis of (Z)-Benzyl 2,3-O-(1-methylethylidene)-5-deoxy-N-isopropyl-D-lyxofuranosylamine N-Oxide (10g). Application of the general procedure to 200 mg (0.72 mmol) of **8** with 74 μ L (0.86 mmol) of isopropylamine (**13g**) furnished, after purification by column chromatography (Hex/EtOAc 2:1), 157 mg (0.47 mmol, 65%) of **10g** as a colorless oil ($R_f = 0.20$, Hex/EtOAc 2:1).

10g: colorless oil. $[\alpha]_D^{24} - 15.9$ (c 1.00, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.36–7.26 (m, 5H, Ar), 6.93 (d, $J = 6.8$ Hz, 1H, HC=N), 5.20–5.13 (m, 2H, 4-H and 3-H), 5.12 (s, 1H, 1-H), 4.71

(d, $J = 12.0$ Hz, 1H, OCH₂Ar), 4.70–4.67 (m, 1H, 2-H), 4.46 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 4.13 (sept, $J = 6.0$ Hz, 1H, CH(CH₃)₂), 1.47–1.43 (m, 6H, –CH(CH₃)₂), 1.41 (s, 3H, C(CH₃)₂), 1.28 (s, 3H, C(CH₃)₂) ppm. ¹³C{¹H} NMR (50 MHz, CDCl₃): δ 137.0 (Ar), 132.8 (HC=N), 128.6–128.1 (5C, Ar), 112.7 (C(CH₃)₂), 105.0 (C-1), 84.6 (C-2), 79.8 (C-3), 76.3 (C-4), 69.1 (OCH₂Ar), 66.3 (CH(CH₃)₂), 26.2 (C(CH₃)₂), 24.8 (C(CH₃)₂), 21.2 (CH(CH₃)₂), 20.7 (CH(CH₃)₂) ppm. MS (ESI) m/z (%): 358.12 (100) [M + Na]⁺. IR (CDCl₃) ν : 972, 1082, 1161, 1377, 1456, 1737, 2234, 2875, 2933, 2985, 3606, 3689 cm⁻¹. Anal. Calcd for C₁₈H₂₅NO₃: C, 64.46; H, 7.51; N, 4.18. Found: C, 64.50; H, 7.71; N, 3.98.

4.1.10. Synthesis of (Z)-Benzyl 2,3-O-(1-methylethylidene)-5-deoxy-N-cyclohexyl-D-lyxofuranosylamine N-Oxide (10h). Application of the general procedure to 200 mg (0.72 mmol) of **8** with 99 μ L (0.86 mmol) of cyclohexylamine (**13h**) furnished, after purification by column chromatography (Hex/EtOAc 2:1), 162 mg (0.43 mmol, 60%) of **10h** as a white solid ($R_f = 0.30$, Hex/EtOAc 2:1).

10h: white solid. mp 113–115 °C. $[\alpha]_D^{24} = 11.1$ (c 1.00, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.36–7.25 (m, 5H, Ar), 6.90 (d, $J = 8.0$ Hz, 1H, HC=N), 5.19 (pseudo t, $J = 4.2$ Hz, 1H, 4-H), 5.14–5.12 (m, 2H, 1-H and 3-H), 4.70 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 4.67 (dd, $J = 6.0, 1.2$ Hz, 1H, 2-H), 4.46 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 3.78–3.72 (m, 1H, CHN), 2.09–2.06 (m, 2H), 1.91–1.77 (m, 4H), 1.70–1.67 (m, 2H), 1.41 (s, 3H, C(CH₃)₂), 1.28 (s, 3H, C(CH₃)₂), 1.36–1.20 (m, 3H) ppm. ¹³C{¹H} NMR (50 MHz, CDCl₃): δ 137.1 (Ar), 133.0 (HC=N), 128.6–128.1 (5C, Ar), 112.7 (C(CH₃)₂), 105.0 (C-1), 84.7 (C-2), 79.8 (C-3), 76.4 (C-4), 73.9 (CHN), 69.0 (OCH₂Ar), 31.4–31.0 (2C), 26.2–24.8 (3C, and 2C, C(CH₃)₂) ppm. MS (ESI) m/z (%): 772.64 (100) [2M + Na]⁺, 398.13 (98) [M + Na]⁺. IR (CDCl₃) ν : 968, 1026, 1088, 1161, 1211, 1359, 1382, 1454, 2231, 2939 cm⁻¹. Anal. Calcd for C₂₁H₂₉NO₃: C, 67.18; H, 7.79; N, 3.73. Found: C, 67.00; H, 7.80; N, 3.75.

4.1.11. Synthesis of (3R,4S,5R)-5-Hydroxy-3,4-O-(1-methylethylidene)-N-octyl-piperidine (16).¹¹ To a mixture of nitron **10a** (75 mg, 0.18 mmol) in dry MeOH (16 mL), acetic acid (2 equivalents) and Pd/C (40 mg) were added under nitrogen atmosphere. The mixture was stirred at room temperature under hydrogen atmosphere (balloon) for 2 days, until a control by ¹H NMR spectroscopy attested the presence of the acetate salt of (3R,4S,5R)-3-hydroxy-4,5-O-(1-methylethylidene)-N-octyl-piperidine (**16**). The mixture was filtered through Celite, and the solvent was removed under reduced pressure. The corresponding free amine was obtained by dissolving the residue in MeOH; then, the strongly basic resin Ambersep 900-OH was added, and the mixture was stirred for 40 min. The resin was removed by filtration, and the solvent evaporated to afford compound **16** (41 mg, 0.14 mmol) as a white solid in 80% yield.

16: white solid. ¹H NMR (400 MHz, CDCl₃): δ 4.27 (dt, $J = 7.6, 5.1$ Hz, 1H), 4.08 (dd, $J = 4.9, 3.9$ Hz, 1H), 3.95 (dd, $J = 7.6, 3.8$ Hz, 1H), 2.82 (ddd, $J = 7.7, 6.3, 1.5$ Hz, 1H), 2.56 (d, $J = 2.4$ Hz, 2H), 2.43–2.34 (m, 3H), 1.50 (s, 3H), 1.48–1.41 (m, 4H), 1.35 (s, 3H), 1.33–1.20 (m, 8H), 0.88 (t, $J = 6.8$ Hz, 3H) ppm.

4.1.12. Synthesis of (3R,4S,5R)-5-Hydroxy-3,4-O-(1-methylethylidene)-N-dodecyl-piperidine (17). To a mixture of nitron **10b** (65 mg, 0.14 mmol) in dry MeOH (15 mL), Pd(OH)₂ (40 mg) was added under nitrogen atmosphere. The mixture was stirred at room temperature under hydrogen atmosphere (balloon) for 2 days, until a control by ¹H NMR spectroscopy attested the presence of (3R,4S,5R)-3-hydroxy-4,5-O-(1-methylethylidene)-N-dodecyl-piperidine (**17**). The mixture was filtered through Celite, and the solvent was removed under reduced pressure to afford compound **17** (38 mg, 0.13 mmol) as a colorless waxy solid in 90% yield.

17: colorless waxy solid. $[\alpha]_D^{22} + 12.0$ (c 0.31 in CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 4.31 (pseudo q, $J = 6.2$ Hz, 1H, 3-H), 4.08 (t, $J = 4.4$ Hz, 1H, 4-H), 3.97–3.94 (m, 1H, 5-H), 2.82 (dd, $J = 12.0, 6.0$ Hz, 1H, 2-Ha), 2.58 (d, $J = 2.9$ Hz, 2H, 6-H), 2.42–2.35 (m, 3H, 2-Hb and CH₂N), 1.60 (br s, OH), 1.51 (s, 3H, C(CH₃)₂), 1.50–1.46 (m, 2H), 1.36 (s, 3H, C(CH₃)₂), 1.31–1.25 (m, 18H), 0.87 (t, $J = 6.8$ Hz, 3H) ppm. ¹³C{¹H} NMR (50 MHz, CDCl₃): δ 109.5 (C(CH₃)₂), 77.3 (C-4), 72.4 (C-3), 67.8 (C-5), 58.1 (CH₂N), 56.1 (C-2), 55.8 (C-6), 32.1–22.9 (10C and 2C, C(CH₃)₂), 14.3 ppm. MS

(ESI) m/z (%): 342.33 (100) [M + H]⁺. IR (CHCl₃) ν : 3458, 2928, 2854, 1468, 1456, 1404, 1383, 1246, 1144 cm⁻¹. Anal. Calcd for C₂₀H₃₉NO₃: C, 70.33; H, 11.51; N, 4.10. Found: C, 70.51; H, 11.72; N, 4.22.

4.1.13. Synthesis of (3R,4S,5R)-5-Hydroxy-3,4-O-(1-methylethylidene)-N-cyclohexyl-piperidine (18). To a solution of nitron **10d** (117 mg, 0.31 mmol) in dry MeOH (30 mL), Pd(OH)₂ (70 mg) was added under nitrogen atmosphere. The mixture was stirred at room temperature under hydrogen atmosphere (balloon) for 2 days, until a control by ¹H NMR spectroscopy attested the presence of (3R,4S,5R)-5-hydroxy-3,4-O-(1-methylethylidene)-N-cyclohexylpiperidine (**18**). The mixture was filtered through Celite, and the solvent was removed under reduced pressure to afford compound **18** (67 mg, 0.26 mmol) as a colorless oil in 85% yield.

18: colorless oil. $[\alpha]_D^{24} + 22.9$ (c 1.00, CHCl₃). ¹H NMR (400 MHz, CD₃OD): δ 4.31–4.29 (m, 1H, 3-H), 3.87–3.84 (m, 1H, 4-H), 3.83–3.78 (m, 1H, 5-H), 3.04 (d, $J = 12.0$ Hz, 1H, 2-H_a), 2.83 (d, $J = 12.0$ Hz, 1H, 6-H_a), 2.76 (d, $J = 12.0$ Hz, 1H, 2-H_b), 2.52–2.41 (m, 1H, CHN), 2.30–2.25 (m, 1H, 6-H_b), 1.91–1.89 (m, 2H), 1.85–1.82 (m, 2H), 1.68–1.65 (m, 1H), 1.50 (s, 3H, C(CH₃)₂), 1.34 (s, 3H, C(CH₃)₂), 1.43–1.10 (m, 5H) ppm. ¹³C{¹H} NMR (100 MHz, CD₃OD): δ 110.2 (C(CH₃)₂), 80.0 (C-4), 74.4 (C-3), 70.5 (C-5), 64.8 (CHN), 53.3 (C-6), 50.7 (C-2), 29.6–26.5 (5C, and 2C, C(CH₃)₂) ppm. MS (ESI) m/z (%): 256.16 (100) [M + H]⁺, 278.11 (36) [2M + Na]⁺. IR (CD₃OD) ν : 1073, 1110, 1220, 1380, 2065, 2640, 2858, 2930, 3340 cm⁻¹. Anal. Calcd for C₁₄H₂₅NO₃: C, 65.85; H, 9.87; N, 5.49. Found: C, 65.87; H, 9.77; N, 5.53.

4.1.14. Synthesis of (3R,4S,5R)-5-Hydroxy-3,4-O-(1-methylethylidene)-piperidine (19).¹¹ To a solution of nitron **10e** (915 mg, 2.39 mmol) in dry MeOH (150 mL), acetic acid (2 equivalents) and Pd/C (458 mg) were added under nitrogen atmosphere. The mixture was stirred at room temperature under hydrogen atmosphere (balloon) for 2 days, until a control by ¹H NMR spectroscopy attested the presence of the acetate salt of (3R,4S,5R)-5-hydroxy-3,4-O-(1-methylethylidene)-piperidine (**19**). The mixture was filtered through Celite, and the solvent was removed under reduced pressure. The corresponding free amine was obtained by dissolving the residue in MeOH; then, the strongly basic resin Ambersep 900-OH was added, and the mixture was stirred for 40 min. The resin was removed by filtration, and the solvent evaporated under reduced pressure to afford (3R,4S,5R)-5-hydroxy-3,4-O-(1-methylethylidene)-piperidine (**19**, 414 mg, 2.39 mmol) as a white solid in 100% yield.

19: white solid. ¹H NMR (400 MHz, CD₃OD): δ 4.26–4.16 (m, 1H), 3.91 (t, $J = 6.1$ Hz, 1H), 3.75–3.62 (m, 1H), 3.13 (dd, $J = 14.5, 2.5$ Hz, 1H), 3.01–2.94 (m, 1H), 2.94–2.86 (m, 1H), 2.38 (dd, $J = 13.1, 9.2$ Hz, 1H), 1.50 (s, 3H), 1.35 (s, 3H) ppm.

4.1.15. Synthesis of Hydroxylamines: (5S)-Benzyl-2,3-O-(1-methylethylidene)-5-deoxy-5-octyl-D-lyxofuranosyl N-octyl-hydroxylamine (21) and (5R)-Benzyl-2,3-O-(1-methylethylidene)-5-deoxy-5-octyl-D-lyxofuranosyl N-octyl-hydroxylamine (22).

4.1.15.1. Procedure without Lewis Acid. A solution of nitron **10a** in dry THF (0.03 M) was stirred at –78 °C under nitrogen atmosphere and 2.0 M solution of octylmagnesium bromide in diethyl ether (1.13 mmol, 600 μ L) was slowly added. The reaction mixture was stirred at –78 °C under nitrogen atmosphere for 3 h, when a TLC control (Hex/EtOAc 2:1) attested the disappearance of the starting material. A saturated ammonium chloride solution (10 mL) and Et₂O (10 mL) were added to the mixture at 0 °C and stirred for 15 min. The two layers were separated, and the aqueous layer was extracted with Et₂O (2 \times 10 mL). The combined organic layers were washed with brine (2 \times 30 mL), dried with Na₂SO₄, and concentrated under reduced pressure to give a mixture of hydroxylamines **21** and **22** (**21** > 98%). The crude mixture was purified by silica gel column chromatography (gradient eluent from Hex/EtOAc 12:1 to 10:1) to give 246 mg (0.47 mmol, 75%) of **21** ($R_f = 0.36$, Hex/EtOAc 12:1) as a colorless oil.

21: colorless oil. ¹H NMR (400 MHz, CDCl₃): δ 7.37–7.26 (m, 5H, Ar), 5.13 (s, 1H, 1-H), 5.11 (br s, OH), 4.69–4.66 (m, 2H, 3-H and OCH₂Ar), 4.61 (d, $J = 4.0$ Hz, 1H, 2-H), 4.52 (d, $J = 12.0$ Hz, 1H, OCH₂Ar), 4.24 (dd, $J = 9.2, 3.0$ Hz, 1H, 4-H), 3.31–3.26 (m,

1H, 5-H), 2.91–2.86 (m, 1H, CH₂N), 2.77–2.72 (m, 1H, CH₂N), 1.64–1.54 (m, 2H), 1.45–1.27 (m, 30H), 0.88 (t, J = 8.0 Hz, 6H) ppm. MS (ESI) *m/z* (%): 542.45 (100) [M + Na]⁺, 520.37 (34) [M + H]⁺.

The secondary hydroxylamine **21** spontaneously oxidizes to the corresponding nitron **23** so we could only perform ¹H NMR and MS-ESI spectra immediately after their purification by column chromatography.

4.1.15.2. Procedure with Lewis Acid. To a stirred solution of nitron **10a** in dry THF (0.03 M) at room temperature, boron trifluoride diethyl etherate (0.82 mmol, 100 μL) was added, and the resulting mixture was stirred at room temperature under nitrogen atmosphere for 15 min. The reaction mixture was cooled at –30 °C and 2.0 M solution of octylmagnesium bromide in diethyl ether (1.48 mmol, 800 μL) was slowly added. The reaction mixture was stirred at –30 °C under nitrogen atmosphere for 2 h, when a TLC control (Hex/EtOAc 2:1) attested the disappearance of the starting material. A saturated ammonium chloride solution (10 mL) and Et₂O (10 mL) were added to the mixture at 0 °C and stirred for 20 min. The two layers were separated, and the aqueous layer was extracted with Et₂O (2 × 10 mL). The combined organic layers were washed with brine (2 × 30 mL), dried with Na₂SO₄, and concentrated under reduced pressure to give a mixture of hydroxylamines **21** and **22** (**22** > 98%). The crude mixture was purified by silica gel column chromatography (gradient eluent from Hex/EtOAc 12:1 to 10:1) to give 298 mg (0.57 mmol, 70%) of **22** (R_f = 0.20, Hex/EtOAc 12:1) as a colorless oil.

22: colorless oil. ¹H NMR (400 MHz, CDCl₃): δ 7.34–7.25 (m, 5H, Ar), 6.54 (br s, OH), 5.07 (s, 1H, 1-H), 4.77–4.76 (m, 1H, 3-H), 4.66 (d, J = 12.0 Hz, 1H, OCH₂Ar), 4.61 (d, J = 4.0 Hz, 1H, 2-H), 4.48 (d, J = 12.0 Hz, 1H, OCH₂Ar), 4.32–4.29 (m, 1H, 4-H), 3.30 (q, J = 8.0 Hz, 1H, 5-H), 2.77–2.69 (m, 2H, CH₂N), 1.65–1.58 (m, 2H), 1.48–1.28 (m, 30H), 0.89 (t, J = 6.0 Hz, 6H) ppm. MS (ESI) *m/z* (%): 520.36 (100) [M + H]⁺, 542.31 (46) [M + Na]⁺.

The secondary hydroxylamine **22** spontaneously oxidizes to the corresponding nitron **24**, so we could only perform the ¹H NMR and MS-ESI spectra immediately after their purification by column chromatography.

4.1.16. Synthesis of (5*S,Z*)-Benzyl-2,3-*O*-(1-methylethylidene)-5-deoxy-5-octyl-*D*-lyxofuranosyl 5-(*N*-octylidene *N*-oxide) (23**).** To a stirred solution of hydroxylamine **21** (213 mg, 0.41 mmol) in dry CH₂Cl₂ (6 mL), IBX [2-iodoxybenzoic acid contains stabilizer (45 wt %)] (382 mg, 0.62 mmol) was added, and the resulting mixture was stirred under nitrogen atmosphere at room temperature for 3 h, when a TLC control (Hex/EtOAc 12:1) attested the disappearance of the starting material. Saturated solution of NaHCO₃ (10 mL) was added, and the aqueous layer was extracted with CH₂Cl₂ (3 × 10 mL). The combined organic layers were washed with brine (2 × 15 mL) and evaporated under reduced pressure after drying with Na₂SO₄. The residue was purified by silica gel flash column chromatography (Hex/EtOAc from 2:1) to give 202 mg (0.39 mmol, 95%) of nitron **23** (R_f = 0.34, Hex/EtOAc from 2:1) as a straw yellow oil.

23: straw yellow oil. [α]_D²⁴ + 80.0 (c 0.80, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.32–7.26 (m, 5H, Ar), 6.71 (t, J = 6.0 Hz, 1H, HC=N), 5.00 (s, 1H, 1-H), 4.71–4.70 (m, 1H, 3-H), 4.65–4.62 (m, 3H, 2-H and OCH₂Ar), 4.53 (dd, J = 8.0, 4.0 Hz, 1H, 4-H), 4.39 (d, J = 12.0 Hz, 1H, OCH₂Ar), 3.83 (t, J = 8.0 Hz, 1H, 5-H), 2.55 (q, J = 6.7 Hz, 2H, CH₂CHN), 2.10–2.02 (m, 1H), 1.60–1.54 (m, 3H), 1.45 (s, 3H, C(CH₃)₂), 1.40–1.25 (m, 23H), 0.89–0.84 (m, 6H) ppm. ¹³C{¹H} NMR (50 MHz, CDCl₃): δ 139.8 (HC=N), 137.4 (Ar), 128.6–127.9 (5C, Ar), 112.8 (C(CH₃)₂), 104.5 (C-1), 85.4 (C-2), 79.7 (C-3), 78.8 (C-4), 75.1 (C-5), 68.7 (OCH₂Ar), 32.0–22.8 (13C, and 2C, C(CH₃)₂), 14.2 (2C) ppm. MS (ESI) *m/z* (%): 1057.16 (100) [2M + Na]⁺, 540.43 (23) [M + Na]⁺. IR (CDCl₃) ν: 957, 1080, 1107, 1161, 1209, 1496, 1595, 2857, 2928, 3032, 3067 cm⁻¹. Anal. Calcd for C₃₁H₅₁NO₅: C, 71.92; H, 9.93; N, 2.71. Found: C, 71.90; H, 10.05; N, 3.01.

4.1.17. Synthesis of (5*R,Z*)-Benzyl-2,3-*O*-(1-methylethylidene)-5-deoxy-5-octyl-*D*-lyxofuranosyl 5-(*N*-Octylidene *N*-oxide) (24**).** To a stirred solution of hydroxylamine **22** (150 mg, 0.29 mmol) in dry CH₂Cl₂ (4.5 mL), IBX [2-iodoxybenzoic acid contains stabilizer (45

wt %)] (273 mg, 0.44 mmol) was added, and the resulting mixture was stirred under nitrogen atmosphere at room temperature for 3 h, when a TLC control (Hex/EtOAc 12:1) attested the disappearance of the starting material. Saturated solution of NaHCO₃ (8 mL) was added, and the aqueous layer was extracted with CH₂Cl₂ (3 × 8 mL). The combined organic layers were washed with brine (2 × 13 mL) and evaporated under reduced pressure after drying with Na₂SO₄. The residue was purified by silica gel flash column chromatography (Hex/EtOAc from 2:1) to give 140 mg (0.27 mmol, 93%) of nitron **24** (R_f = 0.35, Hex/EtOAc from 2:1) as a straw yellow oil.

24: straw yellow oil. [α]_D²⁴ – 47.5 (c 0.72, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.35–7.26 (m, 5H, Ar), 6.71 (t, J = 6.0 Hz, 1H, HC=N), 5.03 (s, 1H, 1-H), 4.69–4.67 (m, 1H, 3-H), 4.64 (d, J = 12.0 Hz, 1H, OCH₂Ar), 4.60 (d, J = 8.0 Hz, 1H, 2-H), 4.48 (d, J = 12.0 Hz, 1H, OCH₂Ar), 4.40 (dd, J = 8.0, 4.0 Hz, 1H, 4-H), 3.96–3.90 (m, 1H, 5-H), 2.60–2.41 (m, 2H, CH₂CHN), 2.10–2.01 (m, 1H), 1.75–1.69 (m, 1H), 1.59–1.49 (m, 2H), 1.43 (s, 3H, C(CH₃)₂), 1.39–1.24 (m, 23H), 0.87 (t, J = 8 Hz, 6H) ppm. ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 141.9 (HC=N), 137.4 (Ar), 128.6–127.9 (5C, Ar), 112.4 (C(CH₃)₂), 105.5 (C-1), 85.3 (C-2), 79.9 (C-4), 79.4 (C-3), 73.2 (C-5), 69.2 (OCH₂Ar), 32.0–22.7 (13C and 2C, C(CH₃)₂), 14.2 (2C) ppm. MS (ESI) *m/z* (%): 1057.02 (100) [2M + Na]⁺, 540.42 (32) [M + Na]⁺. IR (CDCl₃) ν: 961, 1012, 1080, 1161, 1209, 1261, 1496, 1597, 2237, 2856, 2927, 3032, 3066 cm⁻¹. Anal. Calcd for C₃₁H₅₁NO₅ (517.38): C, 71.92; H, 9.93; N, 2.71. Found: C, 71.80; H, 10.00; N, 2.63.

4.1.18. Synthesis of (2*S,3*R,4*S,5*R*)-3-Hydroxy-4,5-*O*-(1-methylethylidene)-2-octyl-*N*-octyl-piperidine (**25**).*** To a mixture of nitron **23** and hydroxylamine **21** in dry MeOH (0.015 M), acetic acid (2 equivalents) and Pd/C (107 mg) were added under nitrogen atmosphere. The mixture was stirred at room temperature under hydrogen atmosphere (balloon) for 2 days, until a control by ¹H NMR spectroscopy attested the presence of the acetate salt of compound **25**. The mixture was filtered through Celite, and the solvent was removed under reduced pressure. The corresponding free amine was obtained by dissolving the residue in MeOH; then, the strongly basic resin Ambersep 900-OH was added, and the mixture was stirred for 40 min. The resin was removed by filtration, and the crude product was purified on silica gel by flash column chromatography (CH₂Cl₂/MeOH/NH₄OH (6%) 10:1:0.1) to afford 150 mg (0.38 mmol, 90%) of **25** (R_f = 0.50, CH₂Cl₂/MeOH/NH₄OH (6%) 10:1:0.1) as a white solid.

25: white solid. mp 50–52 °C. [α]_D²⁵ + 22.7 (c 0.77, CHCl₃). ¹H NMR (400 MHz, CD₃OD): δ 4.26–4.23 (m, 1H, 5-H), 4.04 (t, J = 6.0 Hz, 1H, 4-H), 3.87 (dd, J = 6.4, 4.0 Hz, 1H, 3-H), 2.94 (dd, J = 14.0, 4.0 Hz, 1H, 6-H_a), 2.82 (dd, J = 14.0, 3.9 Hz, 1H, 6-H_b), 2.75–2.68 (m, 2H, 2-H and CH₂N), 2.64–2.57 (m, 1H, CH₂N), 1.55–1.43 (m, 3H), 1.47 (s, 3H, C(CH₃)₂), 1.38–1.32 (m, 26H), 0.91 (t, J = 6.0 Hz, 6H) ppm. ¹³C{¹H} NMR (50 MHz, CD₃OD): δ 109.8 (C(CH₃)₂), 78.3 (C-4), 74.6 (C-5), 70.1 (C-3), 61.8 (C-2), 55.8 (CH₂N), 49.2 (C-6), 30.7–23.7 (13C and 2C, C(CH₃)₂), 14.4 (2C) ppm. MS (ESI) *m/z* (%): 817.04 (100) [2M + Na]⁺, 398.43 (75) [M + H]⁺. IR (CD₃OD) ν: 1072, 1109, 1219, 1246, 1379, 1462, 2250, 2295, 2637, 2859, 2930, 3339 cm⁻¹. Anal. Calcd for C₂₄H₄₇NO₃: C, 72.49; H, 11.91; N, 3.52. Found: C, 72.50; H, 12.05; N, 3.85.

4.1.19. Synthesis (2*R,3*R,4*S,5*R*)-3-Hydroxy-4,5-*O*-(1-methylethylidene)-2-octyl-*N*-octyl-piperidine (**26**).*** To a mixture of nitron **24** and hydroxylamine **22** in dry MeOH (0.015 M), acetic acid (2 equivalents) and Pd/C (125 mg) were added under nitrogen atmosphere. The mixture was stirred at room temperature under hydrogen atmosphere (balloon) for 2 days, until a control by ¹H NMR spectroscopy attested the presence of the acetate salt of compound **26**. The mixture was filtered through Celite, and the solvent was removed under reduced pressure. The corresponding free amine was obtained by dissolving the residue in MeOH; then, the strongly basic resin Ambersep 900-OH was added, and the mixture was stirred for 40 min. The resin was removed by filtration, and the crude product was purified on silica gel by flash column chromatography (CH₂Cl₂/MeOH/NH₄OH (6%) 10:1:0.1) to afford

150 mg (0.38 mmol, 80%) of **26** ($R_f = 0.50$, $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ (6%) 10:1:0.1) as a straw yellow oil.

26: straw yellow oil. $[\alpha]_D^{25} - 27.0$ (c 0.92, CHCl_3). ^1H NMR (400 MHz, CD_3OD): δ 4.28 (q, $J = 4.6$ Hz, 1H, 5-H), 3.90 (t, $J = 6.0$ Hz, 1H, 4-H), 3.58 (t, $J = 7.5$ Hz, 1H, 3-H), 3.08 (dd, $J = 13.6, 3.8$ Hz, 1H, 6- H_a), 2.71–2.63 (m, 2H, 6- H_b and CH_2N), 2.46–2.39 (m, 1H, CH_2N), 2.24–2.19 (m, 1H, 2-H), 1.71–1.63 (m, 1H), 1.60–1.53 (m, 1H), 1.47 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.44–1.32 (m, 27H), 0.91 (t, $J = 6.0$ Hz, 6H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3OD): δ 110.0 ($\text{C}(\text{CH}_3)_2$), 80.7 (C-4), 74.2 (C-5), 72.0 (C-3), 64.2 (C-2), 53.3 (CH_2N), 51.3 (C-6), 33.1–23.7 (13C and 2C, $\text{C}(\text{CH}_3)_2$), 14.4 (2C) ppm. 1D-NOESY: irradiation of 2-H gave a NOE at 4-H and 6- H_b , and irradiation of 4-H gave a NOE at 2-H and 6- H_b . MS (ESI) m/z (%): 398.40 (100) $[\text{M} + \text{H}]^+$. IR (CD_3OD) ν : 1084, 1117, 1219, 1246, 1379, 1464, 2293, 2640, 2857, 2930, 3343 cm^{-1} . Anal. Calcd for $\text{C}_{24}\text{H}_{47}\text{NO}_3$: C, 72.49; H, 11.91; N, 3.52. Found: C, 72.40; H, 11.89; N, 3.50.

4.1.20. Synthesis of (2S,3R,4R,5R)-1,2-Dioctylpiperidine-3,4,5-triol (14). A solution of **25** (150 mg, 0.38 mmol) in MeOH (24 mL) was stirred with 12 M HCl (750 μL) at room temperature for 16 h. The crude mixture was concentrated to yield the hydrochloride salt of compound **14**. The corresponding free amine was obtained by dissolving the residue in MeOH; then, the strongly basic resin Ambersep 900-OH was added, and the mixture was stirred for 40 min. The resin was removed by filtration, and the solvent evaporated to afford 134 mg (0.38 mmol, 100%) of **14** as an orange oil.

14: orange oil. $[\alpha]_D^{25} + 11.9$ (c 0.91, CH_3OH). ^1H NMR (400 MHz, CD_3OD): δ 3.95–3.93 (m, 1H, 5-H), 3.77–3.74 (m, 2H, 4-H and 3-H), 2.67–2.53 (m, 5H, 2-H, 6-H and CH_2N), 1.55–1.51 (m, 2H), 1.49–1.31 (m, 24H), 0.90 (t, $J = 6.0$ Hz, 6H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (50 MHz, CD_3OD): δ 72.5 (C-4), 71.3 (C-3), 67.4 (C-5), 59.4 (C-2), 54.2 (CH_2N), 53.2 (C-6), 33.1–23.7 (13C), 14.4 (2C) ppm. MS (ESI) m/z (%): 358.42 (100) $[\text{M} + \text{H}]^+$. Anal. Calcd for $\text{C}_{21}\text{H}_{43}\text{NO}_3$: C, 70.54; H, 12.12; N, 3.92. Found: C, 70.54; H, 12.15; N, 4.10.

4.1.21. Synthesis of (2R,3R,4R,5R)-1,2-Dioctylpiperidine-3,4,5-triol (15). A solution of **26** (131 mg, 0.32 mmol) in MeOH (20 mL) was stirred with 12 M HCl (650 μL) at room temperature for 16 h. The crude mixture was concentrated to yield the hydrochloride salt of compound **15**. The corresponding free amine was obtained by dissolving the residue in MeOH; then, the strongly basic resin Ambersep 900-OH was added, and the mixture was stirred for 40 min. The resin was removed by filtration, and the solvent evaporated to afford 107 mg (0.30 mmol, 90%) of **15** as a yellow oil.

15: yellow oil. $[\alpha]_D^{25} - 14.9$ (c 0.84, CH_3OH). ^1H NMR (400 MHz, CD_3OD): δ 3.84 (br s, 1H, 5-H), 3.53 (t, $J = 8.0$ Hz, 1H, 3-H), 3.29–3.24 (m, 1H, 4-H), 2.98 (dd, $J = 12.0, 4.0$ Hz, 1H, 6- H_a), 2.71–2.65 (m, 1H, CH_2N), 2.39–2.32 (m, 2H, 6- H_b and CH_2N), 2.08–2.06 (m, 1H, 2-H), 1.78–1.73 (m, 1H), 1.69–1.62 (m, 1H), 1.50–1.32 (m, 24H), 0.90 (t, $J = 6.0$ Hz, 6H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3OD): δ 76.6 (C-4), 71.5 (C-3), 69.5 (C-5), 66.0 (C-2), 54.2 (C-6), 53.2 (CH_2N), 33.1–23.8 (13C), 14.5 (2C) ppm. MS (ESI) m/z (%): 358.42 (100) $[\text{M} + \text{H}]^+$. Anal. Calcd for $\text{C}_{21}\text{H}_{43}\text{NO}_3$: C, 70.54; H, 12.12; N, 3.92. Found: C, 70.35; H, 11.99; N, 4.02.

4.1.22. Synthesis of Benzyl-2,3-O-(1-methylethylidene)- α -D-lyxofuranosyl-5-(octyl)-5-(N-pentadecan-8-amine) (31). To a stirred solution of nitron **24** (190 mg, 0.37 mmol) in dry THF (7 mL) at room temperature, boron trifluoride diethyl etherate (0.37 mmol, 46 μL) was added and the resulting mixture was stirred at room temperature under nitrogen atmosphere for 15 min. The reaction mixture was cooled at -30 $^\circ\text{C}$, and a solution of heptylmagnesium bromide in THF (0.74 mmol, 1.00 mL) was slowly added. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 16 h, when a TLC control (Hex/EtOAc 2:1) attested the disappearance of the starting material. A 2:1 solution of EtOH and sat. aq. NH_4Cl (3 mL) and indium powder (85 mg, 0.74 mmol) were added, and this mixture was heated to reflux (oil bath).

After completion of the reaction (TLC control), the mixture was cooled, filtered through Celite, and the solvent evaporated under reduced pressure. Then, a sat. aq. Na_2CO_3 solution (15 mL) was

added, and the product was extracted with ethyl acetate (3×15 mL). The organic phase was dried over Na_2SO_4 , then filtered, and the solvent evaporated under reduced pressure. The crude mixture was purified by silica gel column chromatography (gradient eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ (6%) 20:1:0.1) to give 100 mg (0.17 mmol, 50%) of **31** ($R_f = 0.50$, $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ (6%) 20:1:0.1) as a colorless oil.

31: colorless oil. $[\alpha]_D^{24} + 9.14$ (c 1.20, CHCl_3). ^1H NMR (400 MHz, CDCl_3): δ 7.34–7.23 (m, 5H, Ar), 5.04 (s, 1H, 1-H), 4.77–4.75 (m, 1H, 3-H), 4.66 (d, $J = 12.0$ Hz, 1H, OCH_2Ar), 4.62–4.59 (m, 1H, 2-H), 4.46 (d, $J = 12.0$ Hz, 1H, OCH_2Ar), 3.83–3.81 (m, 1H, 4-H), 3.06–3.05 (m, 1H, 5-H), 2.65 (br s, 1H, CHN), 1.62–1.27 (m, 44H), 0.89–0.87 (m, 9H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (50 MHz, CDCl_3): δ 137.8 (Ar), 128.5–127.8 (5C, Ar), 112.2 ($\text{C}(\text{CH}_3)_2$), 105.3 (C-1), 85.3 (C-2), 82.4 (C-4), 80.1 (C-3), 68.9 (OCH_2Ar), 54.8 (CHN), 52.9 (C-5), 32.1–22.8 (19C and 2C, $\text{C}(\text{CH}_3)_2$), 14.2 (3C) ppm. MS (ESI) m/z (%): 602.41 (100) $[\text{M} + \text{Na}]^+$. IR (CDCl_3) ν : 957, 1080, 1107, 1161, 1209, 1496, 1595, 2857, 2928, 3032, 3067 cm^{-1} . Anal. Calcd for $\text{C}_{38}\text{H}_{67}\text{NO}_4$: C, 75.82; H, 11.22; N, 2.33. Found: C, 76.01; H, 11.02; N, 2.02.

4.1.23. Synthesis of (2R,3R,4S,5R)-3-Hydroxy-4,5-O-(1-methylethylidene)-2-octyl-N-pentadecan-8-yl-piperidine (32). To a solution of amine **31** (100 mg, 0.17 mmol) in dry MeOH (20 mL), acetic acid (2 equivalents) and Pd/C (50 mg) were added under nitrogen atmosphere. The mixture was stirred at room temperature under hydrogen atmosphere (balloon) for 2 days, until a control by ^1H NMR spectroscopy attested the presence of the acetate salt of compound **32**. The mixture was filtered through Celite, and the solvent was removed under reduced pressure. The corresponding free amine was obtained by dissolving the residue in MeOH; then, the strongly basic resin Ambersep 900-OH was added, and the mixture was stirred for 40 min. The resin was removed by filtration, and the solvent evaporated to afford **32** (46 mg, 0.093 mmol) as a straw yellow oil in 55% yield.

32: straw yellow oil. $[\alpha]_D^{24} - 16.4$ (c 0.40, CHCl_3). ^1H NMR (400 MHz, CD_3OD): δ 4.23–4.21 (m, 1H, 5-H), 3.91 (t, $J = 6.5$ Hz, 1H, 4-H), 3.62 (t, $J = 7.2$ Hz, 1H, 3-H), 3.08 (dd, $J = 14.0, 4.0$ Hz, 1H, 6- H_a), 2.62–2.59 (m, 2H, 6- H_b and CHN), 2.37 (br s, 1H, 2-H), 1.66–1.59 (m, 4H), 1.40 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.32–1.20 (m, 37H), 0.91 (t, $J = 6.0$ Hz, 9H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3OD): δ 110.0 (s, $\text{C}(\text{CH}_3)_2$), 80.8 (C-4), 74.8 (C-5), 72.7 (C-3), 62.4 (C-4), 58.3 (CHN), 44.9 (C-6), 33.1–30.5 (19C and 2C, $\text{C}(\text{CH}_3)_2$), 14.4 (3C) ppm. MS (ESI) m/z (%): 496.20 (100) $[\text{M} + \text{H}]^+$. IR (CD_3OD) ν : 1084, 1246, 1380, 1463, 2293, 2640, 2857, 2930, 3342 cm^{-1} . Anal. Calcd for $\text{C}_{31}\text{H}_{61}\text{NO}_3$: C, 75.09; H, 12.40; N, 2.82. Found: C, 75.03; H, 12.76; N, 2.53.

4.1.24. Synthesis of (2R,3R,4R,5R)-2-Octyl-1-(pentadecan-8-yl)-piperidine-3,4,5-triol Hydrochloride (33-HCl). A solution of **32** (60 mg, 0.12 mmol) in MeOH (10 mL) was stirred with 12 M HCl (300 μL) at room temperature for 16 h. The crude mixture was concentrated to yield the hydrochloride salt of compound **33** (53 mg, 0.11 mmol) as a yellow waxy solid in 90% yield.

33-HCl: yellow waxy solid. $[\alpha]_D^{23} - 31.4$ (c 0.50, MeOH). ^1H NMR (400 MHz, CD_3OD): δ 4.14 (br s, 1H, 5-H), 3.81 (t, $J = 9.0$ Hz, 1H, 3-H), 3.56 (dd, $J = 9.2, 3.2$ Hz, 1H, 4-H), 3.42–3.38 (m, 2H, 6- H_a and CHN), 3.23 (d, $J = 12.0$ Hz, 1H, 6- H_b), 3.18–3.16 (m, 1H, 2-H), 1.92–1.89 (m, 2H), 1.84–1.79 (m, 2H), 1.75–1.70 (m, 2H), 1.61–1.29 (m, 32H), 0.91–0.89 (m, 9H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3OD): δ 74.2 (C-4), 71.0 (C-3), 66.9 (C-5), 66.4 (C-2), 63.0 (CHN), 51.6 (C-6), 33.9–23.7 (19C), 14.4 (3C) ppm. MS (ESI) m/z (%): 456.35 (100) $[\text{M} + \text{H}]^+$. Anal. Calcd for $\text{C}_{28}\text{H}_{58}\text{ClNO}_3$: C, 68.32; H, 11.88; N, 2.85. Found: C, 68.30; H, 11.90; N, 2.93.

4.2. Biology. **4.2.1. Preliminary Biological Screening toward Commercial Glycosidases.** The percentage (%) of inhibition for compounds **14** and **15** toward the corresponding glycosidase was determined by quadruplicate in the presence of 100 μM of the inhibitor on the well. Each enzymatic assay (final volume 0.12 mL) contains 0.01–0.5 units/mL of the enzyme (with previous calibration) and 4.2 mM aqueous solution of the appropriate *p*-nitrophenyl glycopyranoside (substrate) buffered to the optimal pH

of the enzyme. Enzyme and inhibitor were pre-incubated for 5 min at rt, and the reaction started by addition of the substrate. After 20 min of incubation at 37 °C, the reaction was stopped by addition of 0.1 mL of sodium borate solution (pH 9.8). The *p*-nitrophenolate formed was measured by visible absorption spectroscopy at 405 nm (Asys Expert 96 spectrophotometer). Under these conditions, the *p*-nitrophenolate released led to optical densities linear with both reaction time and concentration of the enzyme.

4.2.2. Enzymatic Assays with Human GCCase. **4.2.2.1. Biochemical Characterization with Human GCCase.** The compounds **14**, **15**, and **33·HCl** were screened toward GCCase in leukocytes isolated from healthy donors (controls). Isolated leukocytes were disrupted by sonication, and a micro BCA protein assay kit (Sigma-Aldrich) was used to determine the total protein amount for the enzymatic assay, according to the manufacturer instructions. Enzyme activity was measured in a flat-bottomed 96-well plate. Compound solution (3 μL), 4.29 μg/μL leukocytes homogenate (7 μL), and substrate 4-methylumbelliferyl-β-D-glucoside (3.33 mM, 20 μL, Sigma-Aldrich) in citrate/phosphate buffer (0.1:0.2, M/M, pH 5.8) containing sodium taurocholate (0.3%) and Triton X-100 (0.15%) at 37 °C were incubated for 1 h. The reaction was stopped by addition of sodium carbonate (200 μL; 0.5 M, pH 10.7) containing Triton X-100 (0.0025%), and the fluorescence of 4-methylumbelliferone released by β-glucosidase activity was measured in SpectraMax M2 microplate reader ($\lambda_{\text{ex}} = 365$ nm, $\lambda_{\text{em}} = 435$ nm; Molecular Devices). Percentage GCCase inhibition is given with respect to the control (without compound). Data are mean SD ($n = 3$).

4.2.2.2. IC₅₀ Determination. The IC₅₀ values of inhibitors against GCCase were determined by measuring the initial hydrolysis rate with 4-methylumbelliferyl-β-D-glucoside (3.33 mM). Data obtained were fitted by using the appropriate Equation (for more details, see the Supporting Information section).

4.2.3. Preliminary Biological Screening toward Human Lysosomal Glycosidases. The effect of 1 mM concentration of **14**, **15**, and **33·HCl** was assayed toward six lysosomal glycosidases other than GCCase, namely, α-mannosidase, β-mannosidase, α-galactosidase, β-galactosidase, α-fucosidase from leukocytes isolated from healthy donors (controls) and α-glucosidase from lymphocytes isolated from healthy donors' flesh blood (controls). Isolated leukocytes or lymphocytes were disrupted by sonication, and a micro BCA protein assay kit (Sigma-Aldrich) was used to determine the total protein amount for the enzymatic assay, according to the manufacturer's instructions (for more details, see the Supporting Information section).

4.2.4. Pharmacological Chaperoning Activity. Fibroblasts with the N370S/RecNcil (or L444P/L444P) mutation from Gaucher disease patients were obtained from the "Cell line and DNA Biobank from patients affected by Genetic Diseases" (Gaslini Hospital, Genova, Italy). Fibroblast cells (15.0×10^4) were seeded in T25 flasks with DMEM supplemented with fetal bovine serum (10%), penicillin/streptomycin (1%), and glutamine (1%) and incubated at 37 °C with 5% CO₂ for 24 h. The medium was removed, and fresh medium containing the compounds (**14**, **15** and **33·HCl**) was added to the cells and incubated for 4 days. The medium was removed, and the cells were washed with PBS and detached with trypsin to obtain cell pellets, which were washed four times with PBS, frozen, and lysed by sonication in water. Enzyme activity was measured as reported above. Reported data are mean S.D. ($n = 2$).

4.2.5. Cytotoxicity Test. The MTT test was carried out using the human fibroblasts wild type at different concentrations of compound **33·HCl**. Fibroblasts were grown in the presence of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 1% glutamine, and 1% penicillin–streptomycin, at 37 °C in controlled atmosphere with 5% CO₂. For the experiments, cells were seeded at a density of 20,000 cells per well in 24-well plates and grown for 24 h (or 48 h) before adding the compound. The compound was dissolved in water; then, aliquots of these were diluted in the growth medium. To preserve sterility of solutions, these were filtered with 0.22 μm filters before adding to the dishes containing fibroblasts. Then, cells were incubated at 37 °C in 5% CO₂ for 24 h

(or 48 h). After this time, the media were replaced with medium containing 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); the cells were incubated for an additional 1 h at 37 °C in 5% CO₂. Finally, the number of viable cells was quantified by the estimation of their dehydrogenase activity, which reduces MTT to water-insoluble formazan. Growth medium was removed and substituted with 300 μL of DMSO to dissolve the formazan produced. The quantitation was carried out measuring the absorbance of samples at 570 nm with the iMark microplate absorbance reader (BIO RAD) in a 96-well format.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.joc.1c01308>.

Copies of ¹H NMR and ¹³C NMR spectra of all new compounds, 1D NOESY spectra for compounds **25** and **26**; biological screening toward commercial glycosidases; percentage of GCCase inhibition of compounds **14**, **15**, and **33·HCl** toward human GCCase. IC₅₀ for compounds **14**, **15**, and **33·HCl** toward GCCase; biological screening toward human lysosomal glycosidases; chaperoning activity assays on fibroblasts with the N370S/RecNcil and L444P/L444P mutations from Gaucher disease patients; and computational methods (docking and MD simulations), docked poses, final snapshots of MD simulations and full data of MMGBSA and MMPBSA data (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Pedro Merino – Unidad de Glicobiología, Instituto de Biocomputación y Física de Sistemas Complejos (BIFI), Universidad de Zaragoza, 50009 Zaragoza, Spain; orcid.org/0000-0002-2202-3460; Email: pmerino@unizar.es

Francesca Cardona – Dipartimento di Chimica "Ugo Schiff" (DICUS), University of Firenze, 50019 Sesto Fiorentino (FI), Italy; orcid.org/0000-0002-6766-4624; Email: francesca.cardona@unifi.it

Authors

Francesca Clemente – Dipartimento di Chimica "Ugo Schiff" (DICUS), University of Firenze, 50019 Sesto Fiorentino (FI), Italy; orcid.org/0000-0002-9899-0836

Camilla Matassini – Dipartimento di Chimica "Ugo Schiff" (DICUS), University of Firenze, 50019 Sesto Fiorentino (FI), Italy; orcid.org/0000-0002-8336-383X

Sara Giachetti – Dipartimento di Chimica "Ugo Schiff" (DICUS), University of Firenze, 50019 Sesto Fiorentino (FI), Italy

Andrea Goti – Dipartimento di Chimica "Ugo Schiff" (DICUS), University of Firenze, 50019 Sesto Fiorentino (FI), Italy; orcid.org/0000-0002-1081-533X

Amelia Morrone – Paediatric Neurology Unit and Laboratories, Neuroscience Department, Meyer Children's Hospital, and Department of Neurosciences, Pharmacology and Child Health, University of Florence, 50139 Firenze, Italy

Macarena Martínez-Bailén – Dipartimento di Chimica "Ugo Schiff" (DICUS), University of Firenze, 50019 Sesto Fiorentino (FI), Italy; Departamento de Química Orgánica,

Facultad de Química, Universidad de Sevilla, E-41012 Sevilla, Spain

Sara Orta – Unidad de Glicobiología, Instituto de Biocomputación y Física de Sistemas Complejos (BIFI), Universidad de Zaragoza, 50009 Zaragoza, Spain

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acs.joc.1c01308>

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Fondazione Cassa di Risparmio di Pistoia e Pescia (Bando Giovani@Ricerca scientifica 2017) for a fellowship to C.M. and financial support. We thank MIUR-Italy (“Progetto Dipartimenti di Eccellenza 2018-2022” allocated to the Department of Chemistry “Ugo Schiff”), Università di Firenze and Fondazione CR Firenze for financial support (“Bando congiunto per il finanziamento di progetti competitivi sulle malattie neurodegenerative”. Project: A multidisciplinary approach to target Parkinson’s disease in Gaucher related population, Acronym: MuTaParGa). Fondazione CR Firenze (grant no 2018.0942) is also acknowledged for financial support. F.Ca also thanks Regione Toscana (Bando Salute 2018) for the project: Late onset Lysosomal Storage Disorders (LSDs) in the differential diagnosis of neurodegenerative diseases: development of new diagnostic procedures and focus on potential pharmacological chaperones (PCs), Acronym: LysoLate. M.M.-B. thanks The Spanish MINECO (CTQ2016-77270-R) for financial support. P.M. and S.O. thank the Spanish MINECO (PID2019-104090RB-I00) and Government of Aragón (Grupos Consolidados, E34_20R) for financial support. S.O. thanks also Government of Aragón for a pre-doctoral contract. The authors thankfully acknowledge the resources from the supercomputers “Memento” and “Cierzo”, technical expertise and assistance provided by BIFI-ZCAM (Universidad de Zaragoza, Spain), and the Biological and Computational Research Group (BIFI, Universidad de Zaragoza) for GPU facilities. We also thank Prof. Paolo Paoli (Dipartimento di Scienze Biochimiche, Sperimentali e Cliniche Mario Serio, Università di Firenze) for the help in the analysis of biological data.

DEDICATION

Dedicated to Prof. Alberto Brandi on the occasion of his 70th birthday.

REFERENCES

(1) Compain, P.; Martin, O. R. *Iminosugars: From Synthesis to Therapeutic Applications*; Wiley VCH: New York, 2007.

(2) (a) Brás, N. F.; Cerqueira, N. M.; Ramos, M. J.; Fernandes, P. A. Glycosidase inhibitors: a patent review (2008 - 2013). *Expert Opin. Ther. Pat.* **2014**, *24*, 857–874. (b) López, Ó.; Merino-Montiel, P.; Martos, S.; González-Benjumea, A. Glycosidase inhibitors: versatile tools in glycobiology. *Carbohydr. Chem.* **2012**, *38*, 215–262.

(3) (a) Conforti, I.; Marra, A. Iminosugars as glycosyltransferase inhibitors. *Org. Biomol. Chem.* **2021**, *19*, 5439–5475. (b) Merino, P.; Delso, I.; Marca, E.; Tejero, T.; Matute, R. Chemistry and Biology of Iminosugar Di- and Oligosaccharides. *Curr. Chem. Biol.* **2009**, *3*, 253–271. (c) Butters, T. D.; Dwek, R. A.; Platt, F. M. Imino sugar inhibitors for treating the lysosomal glycosphingolipidoses. *Glycobiology* **2005**, *15*, 43R–52R. (d) Compain, P.; Martin, O. Design, Synthesis and Biological Evaluation of Iminosugar-Based Glycosyltransferase Inhibitors. *Curr. Top. Med. Chem.* **2003**, *3*, 541–560.

(e) Butters, T. D.; Dwek, R. A.; Platt, F. M. Inhibition of glycosphingolipid biosynthesis: application to lysosomal storage disorders. *Chem. Rev.* **2000**, *100*, 4683–4696.

(4) (a) Boyd, R. E.; Lee, G.; Rybczynski, P.; Benjamin, E. R.; Khanna, R.; Wustman, B. A.; Valenzano, K. J. Pharmacological chaperones as therapeutics for lysosomal storage diseases. *J. Med. Chem.* **2013**, *56*, 2705–2725. (b) Pereira, D. M.; Valentão, P.; Andrade, P. B. Tuning protein folding in lysosomal storage diseases: the chemistry behind pharmacological chaperones. *Chem. Sci.* **2018**, *9*, 1740–1752.

(5) Sánchez-Fernández, E. M.; García Fernández, J. M.; Mellet, C. O. Glycomimetic-based pharmacological chaperones for lysosomal storage disorders: Lessons from Gaucher, GM1-gangliosidosis and Fabry diseases. *Chem. Commun.* **2016**, *52*, 5497–5515.

(6) Sawkar, A. R.; Cheng, W.-C.; Beutler, E.; Wong, C.-H.; Balch, W. E.; Kelly, J. W. Nonlinear partial differential equations and applications: Chemical chaperones increase the cellular activity of N370S -glucosidase: A therapeutic strategy for Gaucher disease. *Proc. Natl. Acad. Sci.* **2002**, *99*, 15428–15433.

(7) Zhu, X.; Sheth, K. A.; Li, S.; Chang, H.-H.; Fan, J.-Q. Rational Design and Synthesis of Highly Potent β -Glucocerebrosidase Inhibitors. *Angew. Chem., Int. Ed. Engl.* **2005**, *44*, 7450–7453.

(8) Compain, P.; Martin, O. R.; Boucheron, C.; Godin, G.; Yu, L.; Ikeda, K.; Asano, N. Design and synthesis of highly potent and selective pharmacological chaperones for the treatment of Gaucher’s disease. *ChemBioChem* **2006**, *7*, 1356–1359.

(9) For some reviews see: (a) Sousa, C.; Mendes, R.; Costa, F.; Duarte, V.; Fortes, A.; Alves, M. Synthesis of Iminosugars from Tetroses. *Curr. Org. Synth.* **2014**, *11*, 182–203. (b) Horne, G.; Wilson, F. X. Therapeutic Applications of Iminosugars: Current Perspectives and Future Opportunities. *Prog. Med. Chem.* **2011**, *50*, 135–176. (c) Davis, B. G. A silver-lined anniversary of Fleet iminosugars: 1984-2009, from DIM to DRAM to LABNAC. *Tetrahedron: Asymmetry* **2009**, *20*, 652–671. (d) Winchester, B. G. Iminosugars: from botanical curiosities to licensed drugs. *Tetrahedron: Asymmetry* **2009**, *20*, 645–651. (e) Ayad, T.; Genisson, Y.; Baltas, M. Chemical approaches towards synthesis of some naturally occurring iminosugars. *Curr. Org. Chem.* **2004**, *8*, 1211–1233. (f) Yoda, H. Recent advances in the synthesis of naturally occurring polyhydroxylated alkaloids. *Curr. Org. Chem.* **2002**, *6*, 223–243. (g) Dragutan, I.; Dragutan, V.; Demonceau, A. Targeted drugs by olefin metathesis: piperidine-based iminosugars. *RSC Adv.* **2012**, *2*, 719–736. (h) Pandit, U. K.; Overkleeft, H. S.; Borer, B. C.; Bieräugel, H. Synthesis Mediated by Ring-Closing Metathesis - Applications in the Synthesis of Azasugars and Alkaloids. *Eur. J. Org. Chem.* **1999**, 959–968. (i) van der Pijl, F.; van Delft, F. L.; Rutjes, F. P. J. T. The Aza-Achmatowicz Reaction: Facile Entry into Functionalized Piperidinones. *Eur. J. Org. Chem.* **2015**, 4811–4829.

(10) Clemente, F.; Matassini, C.; Cardona, F. Reductive Amination Routes in the Synthesis of Piperidine Imino Sugars. *Eur. J. Org. Chem.* **2020**, 4447–4462.

(11) Matassini, C.; Mirabella, S.; Goti, A.; Cardona, F. Double Reductive Amination and Selective Strecker Reaction of a d-Lyxaric Aldehyde: Synthesis of Diversely Functionalized 3,4,5-Trihydroxypiperidines. *Eur. J. Org. Chem.* **2012**, 3920–3924.

(12) Parmeggiani, C.; Catarzi, S.; Matassini, C.; D’Adamo, G.; Morrone, A.; Goti, A.; Paoli, P.; Cardona, F. Human Acid β -Glucosidase Inhibition by Carbohydrate Derived Iminosugars: Towards New Pharmacological Chaperones for Gaucher Disease. *ChemBioChem* **2015**, *16*, 2054–2064.

(13) Matassini, C.; Goti, A.; Parmeggiani, C.; Cardona, F. On the Oxidation of Hydroxylamines with o-Iodoxybenzoic Acid (IBX). *Synthesis* **2017**, *49*, 2890–2900.

(14) Mirabella, S.; Fibbi, G.; Matassini, C.; Faggi, C.; Goti, A.; Cardona, F. Accessing 2-substituted piperidine iminosugars by organometallic addition/intramolecular reductive amination: aldehyde vs. nitron route. *Org. Biomol. Chem.* **2017**, *15*, 9121–9126.

(15) For reviews on organometallic additions to other carbohydrate-derived nitrones see: (a) Lombardo, M.; Trombini, C. Nucleophilic

additions to nitrones. *Synthesis* **2000**, 759–774. (b) Merino, P. New developments in nucleophilic additions to nitrones. *C. R. Chim.* **2005**, *8*, 775–788. (c) Merino, P.; Franco, S.; Merchan, F. L.; Tejero, T. Nucleophilic additions to chiral nitrones: new approaches to nitrogenated compounds. *Synlett* **2000**, 442–454.

(16) Clemente, F.; Matassini, C.; Goti, A.; Morrone, A.; Paoli, P.; Cardona, F. Stereoselective Synthesis of C-2 Alkylated Trihydroxypiperidines: Novel Pharmacological Chaperones for Gaucher Disease. *ACS Med. Chem. Lett.* **2019**, *10*, 621–626.

(17) Clemente, F.; Matassini, C.; Faggi, C.; Giachetti, S.; Cresti, C.; Morrone, A.; Paoli, P.; Goti, A.; Martínez-Bailén, M.; Cardona, F. Glucocerebrosidase (GCase) activity modulation by 2-alkyl trihydroxypiperidines: Inhibition and pharmacological chaperoning. *Bioorg. Chem.* **2020**, *98*, 103740.

(18) Lieberman, R. L.; Wustman, B. A.; Huertas, P.; Powe, A. C.; Pine, C. W.; Khanna, R.; Schlossmacher, M. G.; Ringe, D.; Petsko, G. A. Structure of acid β -glucosidase with pharmacological chaperone provides insight into Gaucher disease. *Nat. Chem. Biol.* **2007**, *3*, 101–107.

(19) Chen, F.-E.; Zhao, J.-F.; Xiong, F.-J.; Xie, B.; Zhang, P. An improved synthesis of a key intermediate for (+)-biotin from D-mannose. *Carbohydr. Res.* **2007**, *342*, 2461–2464.

(20) (a) Murahashi, S.-I.; Imada, Y. Synthesis and Transformations of Nitrones for Organic Synthesis. *Chem. Rev.* **2019**, *119*, 4684–4716. (b) Merino, P. *Nitrones and its Cyclic Analogues*; Bellus, D., Padwa, A., Eds.; George Thieme: Stuttgart, 2004; Vol. 27, pp 511–580. (c) Merino, P. *Nitrones and cyclic analogues*. An update; Schaumann, E., Ed.; George Thieme: Stuttgart, 2011; Vol. 2010/4, pp 325–403.

(21) Soldaini, G.; Cardona, F.; Goti, A. Catalytic Oxidation of Imines Based on Methyltrioxorhenium/Urea Hydrogen Peroxide: A Mild and Easy Chemo- and Regioselective Entry to Nitrones. *Org. Lett.* **2007**, *9*, 473–476.

(22) (a) Murahashi, S.; Mitsui, H.; Shiota, T.; Tsuda, T.; Watanabe, S. Tungstate-catalyzed oxidation of secondary amines to nitrones. α -Substitution of secondary amines via nitrones. *J. Org. Chem.* **1990**, *55*, 1736–1744. (b) Murahashi, S.-I.; Shiota, T. Selenium dioxide catalyzed oxidation of secondary amines with hydrogen peroxide. Simple synthesis of nitrones from secondary amines. *Tetrahedron Lett.* **1987**, *28*, 2383–2386. (c) Goti, A.; Nannelli, L. Synthesis of nitrones by methyltrioxorhenium catalyzed direct oxidation of secondary amines. *Tetrahedron Lett.* **1996**, *37*, 6025–6028.

(23) Cardona, F.; Bonanni, M.; Soldaini, G.; Goti, A. One-Pot Synthesis of Nitrones from Primary Amines and Aldehydes Catalyzed by Methyltrioxorhenium. *ChemSusChem* **2008**, *1*, 327–332.

(24) (a) Adolfsson, H. *Modern Oxidation Methods*; Wiley-VCH: Weinheim, 2004; pp 32–43. and references therein. (b) Herrmann, W. A.; Fischer, R. W.; Marz, D. W. Methyltrioxorhenium als Katalysator für die Olefin-Oxidation. *Angew. Chem.* **1991**, *103*, 1706–1709. Methyltrioxorhenium as catalyst for olefin oxidation. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1638–1641. (c) Herrmann, W. A.; Fischer, R. W.; Rauch, M. U.; Scherer, W. Alkylrhenium oxides as homogeneous epoxidation catalysts: activity, selectivity, stability, deactivation. *J. Mol. Catal.* **1994**, *86*, 243–266. (d) Herrmann, W. A.; Kühn, F. E. Organorhenium oxides. *Acc. Chem. Res.* **1997**, *30*, 169–180. (e) Espenson, J. H. Atom-transfer reactions catalyzed by methyltrioxorhenium(VII)-mechanisms and applications. *Chem. Commun.* **1999**, 479–488.

(25) Matassini, C.; Parmeggiani, C.; Cardona, F.; Goti, A. Oxidation of *N,N*-Disubstituted Hydroxylamines to Nitrones with Hypervalent Iodine Reagents. *Org. Lett.* **2015**, *17*, 4082–4085.

(26) Matassini, C.; Bonanni, M.; Marradi, M.; Cicchi, S.; Goti, A. On the Virtue of Indium in Reduction Reactions. A Comparison of Reductions Mediated by Indium and Zinc: Is Indium Metal an Effective Catalyst for Zinc Induced Reductions? *Eur. J. Inorg. Chem.* **2020**, 1106–1113.

(27) Attempted isolation of the free amine **33** (by FCC purification using a basic eluent, or treatment with the strongly basic resin Ambersep 900-OH, or extraction from a basic aqueous solution)

allowed to recover only minute amount of **33**, likely due to its amphiphilic properties.

(28) Numbering of residues is increased in one unit with respect to docking due to the presence of the cap termini counted as residue 1 in MD simulations.

(29) Wang, E.; Sun, H.; Wang, J.; Wang, Z.; Liu, H.; Zhang, J. Z. H.; Hou, T. End-Point Binding Free Energy Calculation with MM/PBSA and MM/GBSA: Strategies and Applications in Drug Design. *Chem. Rev.* **2019**, *119*, 9478–9508.

(30) (a) Kragh-Hansen, U.; Le Maire, M.; Møller, J. V. The Mechanism of Detergent Solubilization of Liposomes and Protein-Containing Membranes. *Biophys. J.* **1998**, *75*, 2932–2946. (b) Lichtenberg, D.; Robson, R. J.; Dennis, E. A. Solubilization of phospholipids by detergents structural and kinetic aspects. *Biophys. Acta* **1983**, *737*, 285–304. (c) Womack, M. D.; Kendall, D. A.; MacDonald, R. C. Detergent effects on enzyme activity and solubilization of lipid bilayer membranes. *Biochim. Biophys. Acta* **1983**, *733*, 210–215. (d) Mellor, H. R.; Nolan, J.; Pickering, L.; Wormald, M. R.; Platt, F. M.; Dwek, R. A.; Fleet, G. W. J.; Butters, T. D. Preparation, biochemical characterization and biological properties of radiolabelled N-alkylated deoxynojirimycins. *Biochem. J.* **2002**, *366*, 225–233. (e) Mellor, H. R.; Platt, F. M.; Dwek, R. A.; Butters, T. D. Membrane disruption and cytotoxicity of hydrophobic N-alkylated imino sugars is independent of the inhibition of protein and lipid glycosylation. *Biochem. J.* **2003**, *374*, 307–314.