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pH-Responsive Trihydroxylated Piperidines Rescue The Glucocerebrosidase Activity in Human Fibroblasts Bearing The Neuronopathic Gaucher-Related L444P/L444P Mutations in *GBA1* Gene

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Engineering bioactive iminosugars with pH-responsive groups is an emerging approach to develop pharmacological chaperones (PCs) able to improve lysosomal trafficking and enzymatic activity rescue of mutated enzymes. The use of inexpensive L-malic acid allowed introduction of orthoester units into the lipophilic chain of an enantiomerically pure iminosugar affording only two diastereoisomers contrary to previous related studies. The iminosugar was prepared stereoselectively from the chiral pool (D-mannose) and chosen as the lead bioactive compound, to develop novel candidates for restoring the lysosomal enzyme glucocerebrosidase (GCase) activity. The stability of orthoester-appended iminosugars was studied by ^{1}H NMR spectroscopy both in neutral and acidic environments, and the loss of inhibitory activity with time in acid medium was demonstrated on cell lysates. Moreover, the ability to rescue GCase activity in the lysosomes as the result of a chaperoning effect was explored. A remarkable pharmacological chaperone activity was measured in fibroblasts hosting the homozygous L444P/L444P mutation, a cell line resistant to most PCs, besides the more commonly responding N370S mutation.

Introduction

GBA1 is the gene encoding the lysosomal enzyme glucocerebrosidase (GCase). It has recently attracted increasing attention since its mutations are among the most commonly known genetic risk factors for the development of Parkinson disease and related synucleinopathies.[1] Recent studies showed that the loss of glucocerebrosidase activity may contribute to the pathogenesis of Parkinson disease even in the absence of gene mutations.[2,3]

Mutations in the *GBA1* genes are widely known to be responsible for the most common of the rare inherited lysosomal storage disorders, Gaucher disease (GD).[4] GD arises from a deficiency in the activity of GCase, which brings to a harmful accumulation of undegraded glucosylceramide entrapped in the lysosomes, with onset of several severe symptoms.

Gaucher disease is classified into three major clinical forms on the basis of the presence and the severity of neurological manifestations. The most common form (GD1) lacks an early onset involvement of the central nervous system (CNS), while GD2 and GD3 are both neuropathic forms of Gaucher Disease (nGD), with GD3 presenting a much slower neurological progression than the one observed in GD2.[5] The non-neurological GD1 can be treated with the enzyme replacement therapy.[6,7] The substrate reduction therapy is also used for some forms of GD.[8]

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One strategy that is emerging for the treatment of nGD employs pharmacological chaperones (PCs),[9] small molecules that bind to the misfolded mutated proteins in the endoplasmic reticulum (ER) and promote their correct folding, which in turn induces their proper trafficking into the lysosomes. Once in the lysosomes, a re-establishment of the enzymatic activity will be observed by replacement of the pharmacological chaperone with the exceedingly abundant substrate.[10,11]

The relevance of the emerging PC approach is attested by the recent commercialization in Europe of Migalastat (Galafold, Amicus Therapeutics), the first oral drug for the therapy of Fabry disease, another inherited lysosomal storage disease. Conversely, no PC for Gaucher disease has yet reached the market. The above-mentioned involvement of GCase not only in GD, but also in the pathogenesis of Parkinson disease and other related synucleinopathies, calls for concerted efforts to develop effective PCs for this enzyme.[12]

Such a task appears quite challenging, since the pharmacological chaperoning ability cannot be investigated in silico but has to be demonstrated case by case in cell lines experiments. Nitrogen-containing glycomimetics, such as iminosugars (e.g. carbohydrate mimics with the nitrogen atom replacing the endocyclic oxygen), have been proposed as PCs for the GCase enzyme.[13] However, most compounds are active towards the most common N370S non-neuronopathic mutation, while the cell lines bearing the L444P mutations (which are more relevant in the context of neuronopathic forms of the disease) are insensitive to the majority of PCs. In this context, relevant examples have been reported by Ortiz Mellet and co-workers, who proposed sp^2-fluorinated iminosugars encapsulated into ß-cyclodextrins[14] and by us, with the development of trihydroxypiperidine-based iminosugars alkylated at the carbon adjacent to nitrogen.[15]

One main drawback of the therapeutic strategy based on pharmacological chaperones is represented by the binding strength of the PC-enzyme complex, since a too strong binding may hamper the displacement of the PC by the natural substrate into the lysosomes. Besides, a slow but progressive accumulation of the free PC in the lysosome will interfere with the equilibrium, affecting the substrate-enzyme complex and the entire enzymatic process. To address these issues, an emerging and still scarcely investigated approach consists of inserting a pH labile unit in the lipophilic chain of the PC, which allows a degree of control based on the local pH difference between the ER (pH = 7.0–7.2) and the lysosomes (pH = 4.5–5.5). Once entered in the lysosome, the more acidic pH should promote hydrolysis of the PC chain and thereby favor the dissociation of the PC-enzyme complex, restoring the enzymatic activity, provided that the hydrolyzed PC possesses a considerably lower affinity towards GCase.

Inspired by the pioneering results reported by C. Ortiz Mellet *et al.*[16] and N. I. Martin *et al.*[17] on a set of 1-deoxynojirimycin and 1,5-dideoxy-1,5-imino-D-xylitol derivatives connected via an exocyclic *N*-thioureidic or *N*-guanidinium moiety to an orthoester containing a lipophilic chain, we report here our results on the use of the orthoester functionality linked to a trihydroxypiperidine iminosugar that has shown promising results as PC for GCase in our studies.[18,19,20]

Both Ortiz Mellet's and Martin's groups employed a racemic aminodiol to build the orthoester moiety, thus forming mixtures of four diastereoisomers. We envisaged that inexpensive enantiopure L-malic acid could be employed to reduce the number of stereoisomers to two epimers, with clear advantage, in the future, for the identification of the more active isomer. Moreover, they limited their investigation to saturated lipophilic chains, while we extended the study to an aromatic moiety, which we have recently found to shift the type of inhibition from competitive to non-competitive.[21]

In this work, we disclose our results on compounds **1a–d**, where the original basic character of the endocyclic sp^3 nitrogen was maintained, the L-malic frame was introduced as linker, and both lipophilic and aromatic tags have been introduced (Figure 1).

Results and Discussion

Synthesis

Methyl orthoester **10** was identified as a common intermediate to access a small set of orthoester-appended piperidines **1a–d** (Figure 1), and the L-malic acid-derived tosylate **7** envisaged as the suitable linker for the construction of the orthoester moiety thereby reducing the number of stereoisomers that can be formed.

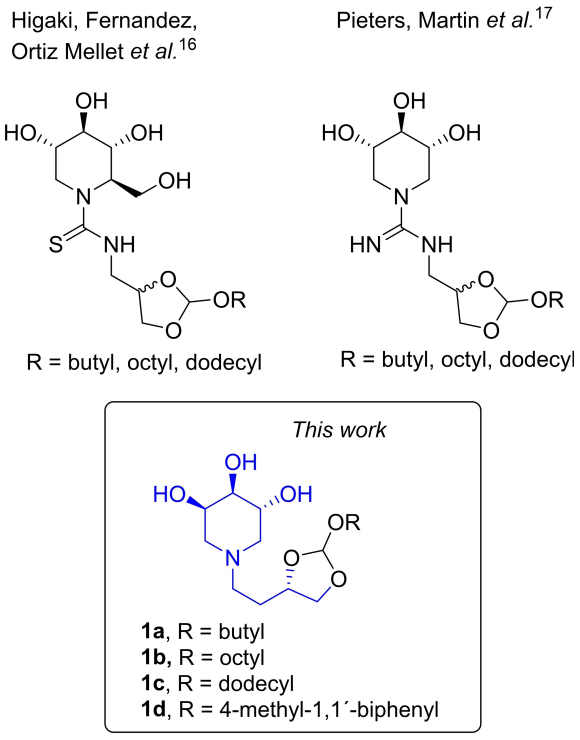
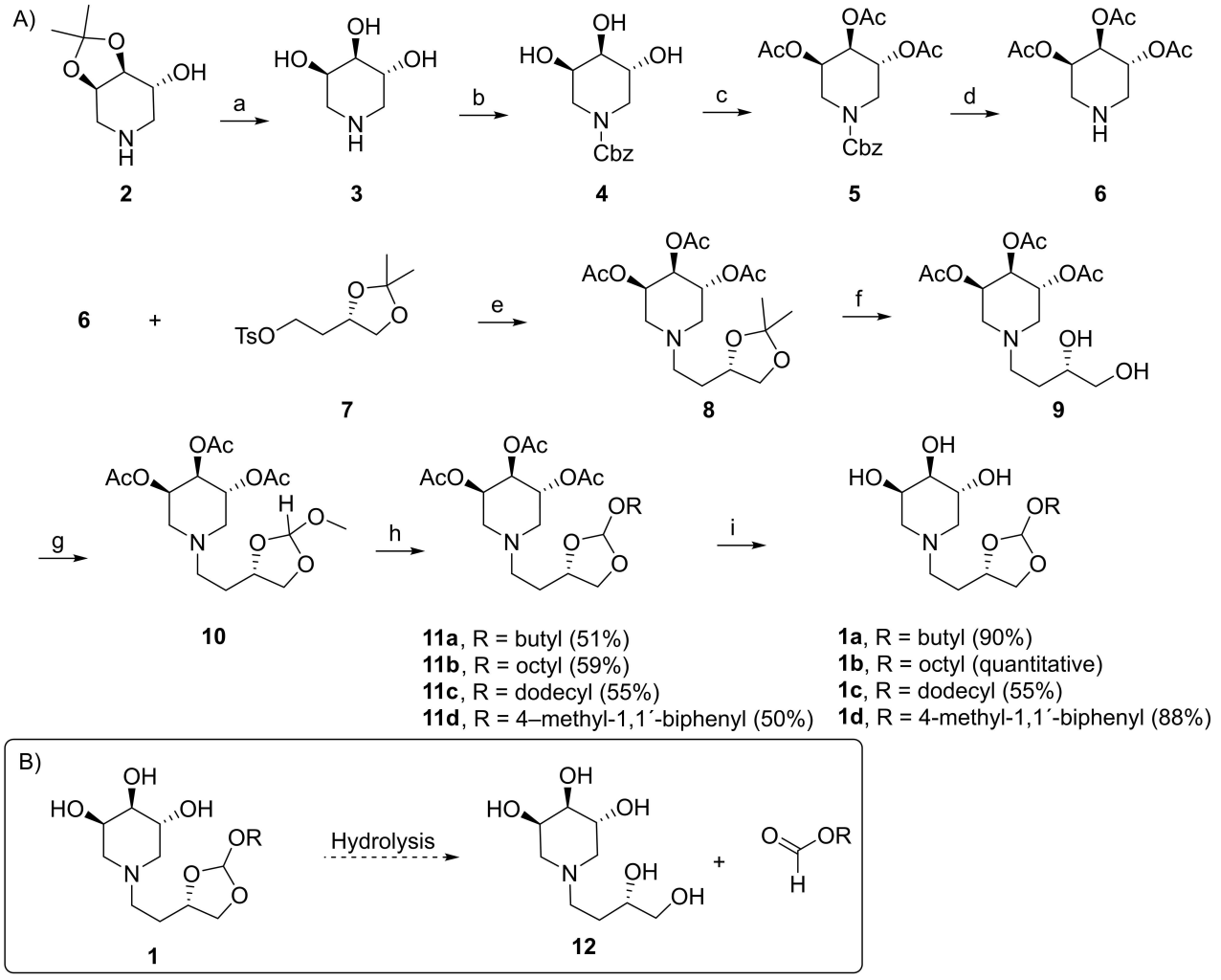


Figure 1. Orthoester-thiourea and orthoester-guanidinium compounds previously reported and orthoester-iminosugars **1a–d** synthesized and evaluated in this work.

The key intermediate **10** was synthesized starting from the trihydroxy piperidine **2** (Scheme 1, part A), which can be prepared from D-mannose in 80% yield over 6 steps.[22] The three hydroxy groups on the piperidine skeleton required to be protected with functionalities resistant to acid treatment. Therefore, the acid-labile acetonide protecting group on **2** was removed and all hydroxyl groups acetylated via a deprotection/protection sequence to give the triacetylated piperidine **6**. The acetonide on piperidine **2** was removed by concentrated HCl in MeOH to afford **3**,[21a] which was protected at nitrogen with a benzyloxycarbonyl (Cbz) group by reaction with CbzCl in EtOH/H_2O in the presence of $NaHCO_3$ for 3 h to give **4** (90%). Acetylation with acetic anhydride in dry pyridine yielded **5** (93%), which furnished the desired free amine **6** in 89% yield upon deprotection by catalytic hydrogenation with $Pd(OH)_2/C$ in EtOH for 18 h.

Based on our previous results,[23] which showed that the presence of a short carbon atom chain at nitrogen of the trihydroxypiperidine (e.g. *N*-butyl trihydroxypiperidine) resulted in negligible interaction with GCase, we envisaged that the C_4-tosylate **7** would serve as an appropriate linker for connecting the trihydroxypiperidine to the aliphatic chains through an orthoester moiety also for this reason, expecting a low affinity towards GCase of the product arising by acidic cleavage of the orthoester. The tosylate **7** was conveniently prepared from L-malic acid in four steps and 45% overall yield following literature procedures.[24–27] Hence, **7** was reacted with the amine **6** in the presence of NEt_3 in CH_3CN at 80 °C to afford the ketal **8** (66%). The acetonide group was subsequently removed with trifluoroacetic acid (TFA) in dry CH_2Cl_2 to give the diol **9** in 98% yield, which was in turn converted to the key methyl orthoester intermediate **10** in 88% yield by treatment with catalytic *p*-toluenesulfonic acid (*p*-TSA) and trimethyl orthoformate in dry CH_2Cl_2 for 3 h. Compound **10** was converted into the butyl (**11a**, 51%), octyl (**11b**, 59%), dodecyl (**11c**, 55%) and biphenyl-4-methyl (**11d**, 50%) orthoesters by reaction with the corresponding alcohols (1-butanol, 1-octanol, 1-dodecanol, or biphenyl-4-methanol, respectively) in the presence of pyridinium *p*-

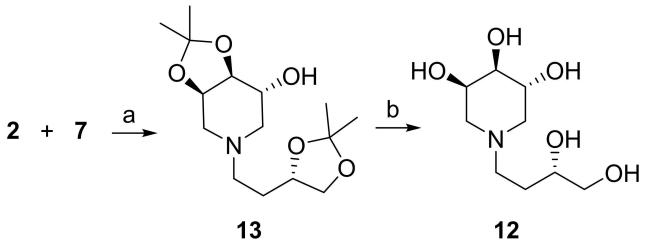


Scheme 1. Synthetic route for the preparation of orthoester-iminosugars **1a**–**d** (A) and their hydrolysis to pentahydroxypiperidine **12** and the corresponding formic esters (B). Reaction conditions: a) HCl 12 M, MeOH, r.t., 18 h, quantitative; b) $NaHCO_3$, CbzCl, EtOH, H_2O 4:1, r.t., 3 h, 90%; c) Ac_2O, dry pyridine, r.t., 24 h, 93%; d) H_2, $Pd(OH)_2/C$, EtOH, r.t., 18 h, 89%; e) CH_3CN, NEt_3, 80 °C, 2 d, 66%; f) TFA, dry DCM, r.t., 2 h, 98%; g) *p*-TSA, trimethyl orthoformate, dry DCM, r.t., 3 h, 88%; h) ROH, PPTS, dry toluene, reflux, 3 h; i) Na_2CO_3, MeOH or Ambersep 900-OH, MeOH, r.t., 18 h.

toluenesulfonate (PPTS) in dry toluene at reflux. Deacetylation of **11a–d** under basic conditions (Na_2CO_3 or Ambersep 900-OH in MeOH) furnished the target trihydroxypiperidine orthoesters **1a–d** (**1a**: 90%, **1b**: quantitative, **1c**: 55%, **1d**: 88%, Scheme 1).

Since the hydrolysis of all the orthoesters **1a–d** will produce a common pentahydroxy piperidine **12** together with the corresponding formates (Scheme 1, part B), compound **12** was also synthesized independently as reference compound and used both for the NMR stability assessment and the biological test.

The protected trihydroxypiperidine **2** was alkylated with the tosylate **7** in the presence of NEt_3 in CH_3CN at 80 °C to give **13** in 90% yield. Acidic treatment of **13** with HCl in MeOH for 18 h furnished the reference piperidine **12** in 90% yield (Scheme 2).



Scheme 2. Synthesis of the reference compound **12**. Reaction conditions: a) CH_3CN, NEt_3, 80 °C, 2 d, 90%; b) HCl 12 M, MeOH, r.t., 15 h, 90%.

Stability assessment

A qualitative stability test for orthoesters **1a–d** was carried out by means of ^{1}H NMR spectroscopy to get information on the hydrolysis rate at different pH and give insight into the stability of the compounds under the biological test conditions. The NMR samples were prepared in D_2O or in a D_2O/dmso-d6 mixture at pH* 7 and pH* 5.8, where pH* is the pH measured in a D_2O solution of the H_2O-calibrated pH-meter,[28] and the spectra were recorded on freshly prepared samples and then monitored for 4 days at 37 °C. pH values, time frame and temperature were chosen to mimic the conditions of the PC biological test, where the value of 5.8 is chosen since it is the optimal activity pH of the GCase enzyme.[15,18] Considering that the conversion of pH* to pD can be accomplished by adding a constant of ca. 0.4,[28] we were quite confident that if hydrolysis occurred in the NMR experiments, it would have occurred even faster in the acidic environment of the lysosomes (pH 4–5).

As an example, we illustrate here the hydrolysis reaction of orthoester **1d**. The stability of **1d** at neutral pH within 4 days was verified and no change was detected in the ^{1}H NMR spectra (data not shown). Then, **1d** was dissolved in a D_2O solution with deuterated citrate-phosphate buffer at pH* 5.8 and the hydrolysis was followed by ^{1}H NMR spectroscopy (Figure 2). In the first hour no hydrolysis was observed (spectra a *vs* b, Figure 2) and after 2.5 h the hydrolysis was negligible (spectrum c, Figure 2). After 26 h the partial hydrolysis of **1d** was detected by the formation of the typical peaks of orthoformate at 8–8.5 ppm and by the decrease of the characteristic orthoester signals of **1d** around 6 ppm. After 6 days, almost complete

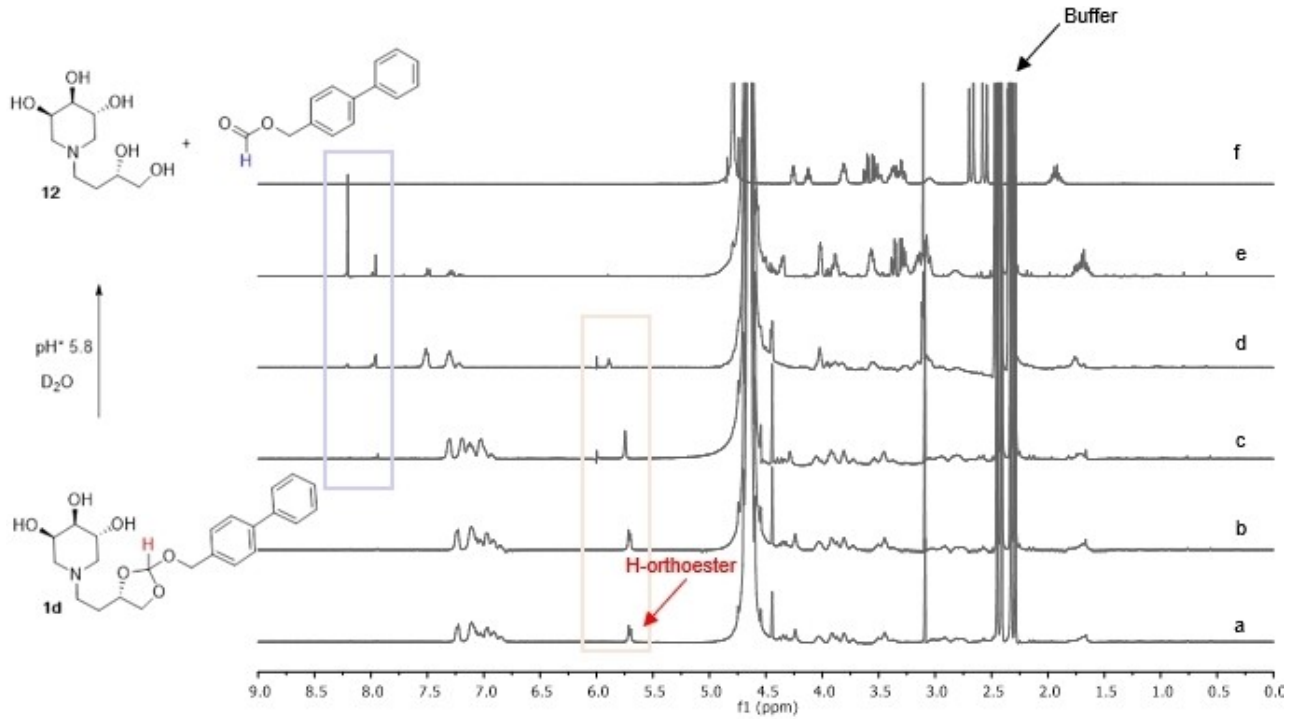


Figure 2. ^{1}H NMR- spectra at 400 MHz in D_2O and citrate-phosphate buffer at pH* 5.8 and 37 °C. From bottom to top (spectra a-e): hydrolysis of **1d** (t = 0, 1 h, 2.5 h, 26 h, 6 d); f: compound **12** in buffer at pH* 5.8.

hydrolysis had occurred, with formation of the polyalcohol **12** and 4-methyl-1,1'-biphenyl formate. The stability results for the three orthoesters **1a**–**c** were analogous (See the Supporting Information).

Biological studies

Compounds **1a**–**d** and **12** were first screened as human lysosomal GCase inhibitors at 1 mM in human leukocyte homogenates derived from healthy donors and then evaluated as pharmacological chaperones towards two cell lines bearing different mutations (N370S/RecNcil and L444P/L444P), following a protocol well established in our group,[18] where the compounds are incubated with the enzyme at pH 5.8 for 1 h, a timeframe in which the orthoester is still stable (see Figure 2b). The results of this screening are reported in Table 1, in terms of GCase inhibition percentage at 1 mM as well as IC_{50}, which was calculated when the GCase inhibition percentage at 1 mM was higher than 80% (Table 1).

The inhibition properties of **1a**–**c** improved upon increasing the alkyl chain length, showing that a longer chain is beneficial for inhibition as expected from previous findings. The *n*-butyl orthoester **1a** exhibited only 37% inhibition, while *n*-octyl **1b** and *n*-dodecyl **1c** orthoester derivatives were excellent GCase inhibitors (97 and 98% inhibition at 1 mM, respectively) with IC_{50} values in the low micromolar range (30 μM and 15 μM, respectively). The *n*-dodecyl orthoester **1c** was the best inhibitor of the series. The methyl-biphenyl orthoester **1d**, the only orthoester bearing an aromatic tag, also showed good inhibition at 1 mM (92%) with an IC_{50} value of 70 μM. Kinetic analyses were performed to determine the mechanism of action of **1c**, the best inhibitor of the series, and **1d**, the inhibitor with the aromatic portion, by analyzing the dependence of the main kinetic parameters (Km and Vmax) upon the inhibitor concentration (See the Supporting Information). It was found that compound **1c** bearing an aliphatic chain behaves as a competitive GCase inhibitor, with a K_i value of 15 μM (Table 1), which is in agreement with our previous results obtained with trihydroxy piperidines decorated with alkyl chains.[15,18] Conversely, a non-competitive behaviour was observed for the aryl substituted inhibitor **1d**, with a K_i value of 59 μM (See Table 1 and the Supporting Information), which is in line with our recent findings which showed a relationship between the presence of an aromatic moiety and a non-competitive inhibition mode.[21]

It is noteworthy that piperidine **12** exhibited negligible inhibition towards GCase, indicating that upon hydrolysis of the orthoesters at the local pH of the lysosome, the enzyme-inhibitor complex would be disrupted after translocation, promoting the rescue of the enzyme activity.

To better mimic what may happen in the environment of the lysosome and in light of the progressive hydrolysis observed in time by NMR studies (Figure 2), we also tested the inhibitory activity of orthoesters **1c** and **1d** after preincubation at acidic pH. For comparison, compound **14** was also tested in the same conditions. Thus, compounds **14**, **1c** and **1d** were preincubated at 37 °C in a pH 5.8 buffer solution for increasing time intervals, and then tested under general GCase inhibition assay conditions. Gratifyingly, upon incubation at acidic pH, both pH-sensitive compounds **1c** and **1d** showed a progressive loss of inhibitory activity after 1, 2, and 3 days of incubation, according to the ongoing progressive hydrolysis. Conversely, the inhibitory activity of compound **14** (Table 2) *vs* GCase enzyme was not affected by the acidic environment (Figure 3, for experimental details see the Supporting Information).

Next, the ability of the strongest GCase inhibitors **1b**–**d** as pharmacological chaperones was investigated. The compounds were assayed in human fibroblasts derived from Gaucher patients bearing the N370S mutation, one of the most common GCase mutations and known to be responsive to PCs, or bearing the homozygous L444P/L444P mutation, responsible for neuropathic form of GD and known to be a mutation that is hard to rescue.[12] Compound **1a** was also screened for N370S mutation despite its low inhibition properties, to verify if other factors such as easier dissociation from the target enzyme and/or compound bioavailability can have a role in the chaperoning test. The results of the chaperoning tests towards the two cell lines are reported in Table 2 as fold-increase of mutant GCase activity, together with the PC concentration (in brackets).

Orthoesters **1a**–**c** showed GCase activity rescue of 1.5-to-2.0-fold towards mutated N370S/RecNcil fibroblasts. The enhancement increased upon elongation of the lipophilic alkyl chain from **1a** to **1c**, with the best pharmacological chaperoning properties observed for **1c**, which is also the best inhibitor.

Table 1. β-Glucosidase (GCase) inhibition percentage of compounds **1a**–**d** and **12** in human leukocytes homogenate derived from healthy donors with selected IC_{50} [μM] and K_i values [μM].

Compound	GCase inhibition [%]	IC_{50} [μM]
1a	37	>1000
1b	97	30 ± 0.3
1c	98	15 ± 0.2 Competitive inhibitor (K_i = 15.0 ± 5.25 μM)
1d	92	70 ± 1.0 Non-competitive inhibitor (K_i = 59.0 ± 2.75 μM)
12	6	>1000

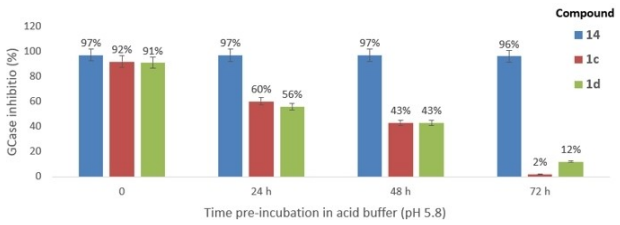
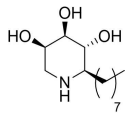
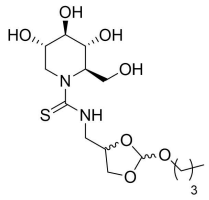
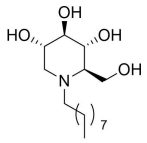


Figure 3. Inhibition percentage of lysosomal GCase in an extract from human leukocytes isolated from healthy donors incubated with 1 mM concentration of compounds **14**, **1c** and **1d** after immediate evaluation (t = 0 h) and different incubation times (24 h, 48 h and 72 h) in a 5.8 pH buffer at 37 °C.

Table 2. GCase rescue on mutated fibroblasts bearing the N370S/RecNcil or L444P/L444P mutations for novel compounds **1a–d** and literature compounds **14**, **15** and **16**. GCase activity was determined in lysates from mutated fibroblasts incubated for 4 days with or without (control) different concentrations of the inhibitor. The values reported in the table are given as activity ratio *vs* the control. The inhibitor concentrations (μM) are indicated in brackets.

Compound	N370S/RecNcil GCase rescue [μM]	L444P/L444P GCase rescue [μM]
1a	1.50 (100) 1.50 (0.1)	Not determined
1b	1.70 (100) 1.67 (50)	1.11 (50) 1.11 (0.01)
1c	1.92 (50) **2.06 (10)**	**2.15 (50)** 1.24 (10)
1d	1.61 (100)	**2.11 (50)** **2.03 (10)**
 **14**	1.86 (50)[15,18]	1.80 (100)[15,18]
 **15**	1.50 (20)[16]	No rescue
 **16**	2.00 (10)[29]	No rescue

Compound **1a**, albeit showing a modest inhibitory activity (37% inhibition), was also able to rescue the enzymatic activity of GCase up to 1.50-fold in N370S/RecNcil fibroblasts, confirming that PCs can be also found among weak inhibitors. A remarkable 2.1-fold maximal increase at 10 μM was found for the orthoester **1c**, which was effective at a much lower concentration with respect to **1b** (2.1-fold rescue at 10 μM *vs* 1.7-fold rescue at 50 or 100 μM, Table 2) in fibroblasts bearing N370S mutations. Similar enhancement (1.6-fold rescue) at 100 μM concentration was found for the aromatic tagged orthoester **1d**. Overall, the enhancements on mutated N370S/RecNcil fibroblasts are in line with those previously measured for known compound **14**, previously synthesized in our group,[15][18] and for compounds, **15**[16] and **16**[28] (Table 2). These latter were chosen for comparison as they are examples of an orthoester-appended sp^2-iminosugar (**15**) and of an *N*-alkylated iminosugar (**16**) known to be highly effective on the non-neuropathic mutation N370S.

Then, we evaluated the GCase activity rescue of orthoesters **1b-d** on the more challenging fibroblasts bearing the homozygous neuropathic L444P/L444P mutations, which are often resistant to most PCs. Only a 10% increase (1.1-fold) was found at 10 nM and 50 μM for compound **1b**; however, we were delighted to measure a rescue of ca. 2.1-fold in mutant GCase activity for compounds **1c** and **1d** at 50 μM. Remarkably, compound **1d** gave a similar rescue (2.0-fold) even at a lower concentration (10 μM).

It is worth noting that these results exceed those obtained with compound **14**, which gave a 1.80-fold rescue at a double concentration (100 μM), thus clearly highlighting the advantage of using these pH-sensitive iminosugars with respect to classical ones, and that compounds **15** and **16** did not give any rescue on these neuropathic mutations. More relevant rescues (up to 2.8-fold at 20 μM) on these cell lines were obtained only with much more complex systems (sp^2-fluorinated iminosugars complexed with ß-cyclodextrins.[14]

The chaperoning assay also showed a very different impact on cell viability of compounds **1c** and **1d**. Indeed, the measurement of the enzymatic activity in both cell lines was hampered by low cell viability at the highest concentration (100 μM) only in case of **1c**, while **1d** did not suffer from this drawback (See the Supporting Information). Moreover, it is worth noting that only few examples of non-competitive inhibitors, such as **1d**, are reported to behave as good PCs for the GCase enzyme.[12]

Conclusions

A pH labile orthoester functionality has been explored as linker to connect an enantiomerically pure trihydroxypiperidine to a lipophilic chain or to an aromatic moiety with the aim of rescuing the activity of mutated GCase. The use of L-malic acid as linker to build the orthoester moiety allowed to reduce the number of formed stereoisomers to only two epimers, thus simplifying the NMR spectra and allowing, in the future, an easier identification of the more active isomer.

The orthoesters **1a–d** were synthesized from a common intermediate **10** (obtained in 7 steps and 42% overall yield from the known trihydroxypiperidine **2**), and their hydrolysis rate was studied by ^{1}H NMR spectroscopy, showing high stability at neutral pH* and complete hydrolysis at acidic pH* within hours.

The inhibitory data and kinetic measurements after 1 h incubation indicated that the presence of a long alkyl chain length is critical to ensure a competitive inhibitory activity towards GCase, while the aromatic motif imparted non-competitive inhibitory behavior, indicating that this inhibitor binds to an allosteric site.

The piperidine **12**, expected to form in the lysosome at acidic pH by hydrolysis of the orthoesters, showed negligible inhibition against the GCase enzyme, thus endorsing the rational design of our study. Further confirmation was provided by preincubating compounds **1c** and **1d** at pH 5.8 and 37 °C and then running the inhibition test. Upon increasing incubation times, we observed a progressive loss of ability to bind GCase, indicating a progressive hydrolysis, while the pH-stable compound **14** maintained a constant inhibition.

When assayed as pharmacological chaperones on mutated fibroblasts bearing the N370S/RecNcil mutations, compounds **1a**–**d** were able to achieve a 1.5-to-2.0-fold increase of the GCase activity, in analogy with other iminosugars reported by us and by other groups.

Remarkably, the studied orthoesters showed the ability to rescue the activity of GCase also on the L444P neuronopathic mutations, which are often resistant to most PCs and that are typically present in the neuropathic forms of Gaucher disease. In particular, orthoesters **1c** and **1d** remarkably promoted the recovery of mutated GCase activity in fibroblasts bearing L444P/L444P mutations up to 2.2 and 2.1-fold at a lower concentration (50 μM *vs* 100 μM) with respect to the pH-stable compound **14**. The rescue activities described herein are the highest among the few examples of pH-sensitive PCs reported to date (even at a lower compound concentration), and among the highest in general.

Genetic risk factors leading to neurodegenerative pathologies such as Parkinson disease have recently attracted growing interest, and among them *GBA1* mutations are currently the most investigated. Therefore, the development of new PCs able to increase the activity of the GCase enzyme, particularly on the neuronopathic mutations, is becoming a cutting-edge objective. Considering that even a little increase in the enzyme activity may be considered clinically useful, in particular for fibroblasts with a very small basal level of functioning GCase enzyme such as L444P fibroblasts, the results reported herein are worthy to be further investigated and encourage further exploration of the pH sensitive functional groups chemical space. Studies are ongoing in our laboratories along these lines and the results will be reported in due time.

Experimental Section

General Procedures

Reagents were purchased from commercial suppliers and used without purification. All reactions were carried out under magnetic stirring and monitored by TLC on 0.25 mm silica gel plates with fluorescent indicator. Flash Column Chromatography (FCC) was carried out on Silica Gel 60 (32–63 μm) or (230–400 mesh). Yields refer to spectroscopically and analytically pure compounds unless otherwise stated. Melting points were obtained with a Stuart Scientific melting point apparatus and are uncorrected. Elemental analyses were performed with a Thermoscientific FlashSmart Elemental Analyzer CHNS/O. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 200 MHz, a Varian Mercury 400 MHz or on a Varian INOVA 400 MHz instrument at 25 °C. ^{1}H NMR and ^{13}C NMR spectra were referenced against the residual solvent signal.[30] Integrals are in accordance with assignments, coupling constants are given in Hz. For detailed peak assignments 2D spectra were measured (COSY, HSQC). For practical reasons the assignment of H and C atoms in NMR characterizations reflects the numbering of chemical structures in the Supporting Information. A signal at 110 ppm was present in ^{13}C spectra recorded at the Varian Inova spectrometer due to FM radio frequency interference and is indicated in the corresponding spectra. IR spectra were recorded with IRAffinity-1S SHIMADZU or IRAffinity-1 SHIMADZU system spectrophotometers. Optical rotation measurements were performed on a JASCO DIP-370 polarimeter. High Resolution Mass spectrometry (HRMS) were recorded with an ESP-MALDI-FT-ICR spectrometer equipped with a 7 T magnet (calibration of the instrument was done with Na trifluoroacetic acid (TFA) cluster ions) using Electrospray Ionization (ESI). Compounds **3** and **7** were synthesized following previously reported procedures.[21a,23–26]

Synthetic Procedures

Compound 4: To a solution of trihydroxypiperidine **3**[21] (50 mg, 0.376 mmol) in H_2O (0.3 mL) and EtOH (1.30 mL), $NaHCO_3$ (88 mg, 1.05 mmol) and then CbzCl (64 μL, 0.446 mmol) were added. The reaction mixture was stirred at room temperature for 3 h until the disappearance of **3** was assessed by a TLC control (CH_2Cl_2:MeOH:NH_4OH (6%) 10:1:0.1). The mixture was concentrated under vacuum and then the crude was purified by FCC on silica gel (CH_2Cl_2:MeOH 10:1) to give 90 mg of **4** (0.337 mmol, 90%) as a white solid. M.p. = 149.5–151.3 °C. *Rf* = 0.23 (CH_2Cl_2:MeOH 10:1). $[\alpha]_D^{27} = -15.67$ (c = 0.6, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 7.39–7.26 (m, 5H, Ar), 5.15–5.06 (m, 2H, CH_2Ph), 3.92–3.86 (m, 1H, H-3), 3.86–3.60 (m, 3H, H-5, Ha-2, Ha-6), 3.59–3.52 (m, 1H, H-4), 3.44–3.33 (m, 1H, Hb-2), 3.27–3.03 (m, 1H, Hb-6). ^{13}C NMR (50 MHz, CD_3OD) δ ppm: 157.8, 138.1, 129.5 (2C), 129.0, 128.7 (2C), 74.6, 74.3, 69.0, 68.8, 68.3, 68.3, 68.0, 47.9, 47.5 (mixture of rotamers). HRMS (ESP+): m/z calcd for $C_{13}H_{17}NO_5$: 268.11795 $[M+H]^+$; found: 268.11810.

Compound 5: A solution of **4** (77 mg, 0.288 mmol) in dry pyridine (1 mL) was stirred with acetic anhydride (0.3 mL) at room temperature for 24 h until a TLC control attested the disappearance of **4** (CH_2Cl_2:MeOH 10:1). The crude mixture was diluted with toluene and then concentrated under vacuum. The crude was purified by FCC on silica gel (Hexane:AcOEt 2:1) to give 104 mg of the acetylated compound **5** (0.264 mmol, 93%) as a colorless oil. *Rf* = 0.29 (Hexane:AcOEt 2:1). $[\alpha]_D^{24} = -19.17$ (c = 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.39–7.27 (m, 5H, Ar), 5.23–5.15 (m, 2H, H-3, H-4), 5.14–4.96 (m, 3H, CH_2Ph, H-5), 4.07–3.93 (m, 1H, Ha-6), 3.92–3.80 (m, 1H, Ha-2), 3.46 (d, *J* = 14.0 Hz, 1H, Hb-2), 3.38–3.22 (m, 1H, Hb-6), 2.05 (s, 3H, CH_3CO), 2.02–1.90 (m, 6H, CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.0, 169.9, 169.8, 155.5, 136.4, 128.6, 128.3, 128.0 (5C), 70.4, 70.1, 67.6, 67.3, 67.1, 66.9, 44.6 (2C), 44,5, 44.3, 20.8 (mixture of rotamers). HRMS (ESP+): m/z calcd for: $C_{19}H_{23}NO_8$ 394.14964 $[M+H]^+$; found: 394.14992. IR ($CHCl_3$) $\tilde{\nu}$ = 3871, 3759, 3649, 3020, 1746, 1706, 1436, 1372, 1259, 1233, 1056 cm^{-1}.

Compound 6: To a solution of the protected amine **5** (330 mg, 0.839 mmol) in EtOH (11 mL), $Pd(OH)_2$/C (165 mg) was added under nitrogen atmosphere. Then nitrogen was replaced by hydrogen gas, bubbling hydrogen with a balloon and the reaction mixture was stirred at room temperature for 18 h until ^{1}H NMR analysis assessed the disappearance of benzyl and aromatic protons. The catalyst was removed by filtration on a short pad of Celite and the solvent was evaporated under vacuum. The crude product was purified by FCC on silica gel (CH_2Cl_2:MeOH:NH_4OH (6%) 10:1:0.1) to afford 195 mg of the corresponding amine **6** (0.752 mmol, 89%) as a white waxy solid. *Rf* = 0.27 (CH_2Cl_2:MeOH:NH_4OH (6%) 10:1:0.1). $[\alpha]_D^{24} = -41.0$ (c = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.26–5.22 (m, 1H, H-3), 5.05–4.92 (m, 2H, H-4, H-5), 3.27 (dd, *J* = 14.1, 4.1 Hz, 1H, Ha-6), 3.15 (dd, *J* = 14.7, 4.4 Hz, 1H, Ha-2), 2.84 (dd, *J* = 14.5, 2.1 Hz, 1H, Hb-2), 2.61–2.53 (m, 1H, Hb-6), 2.09 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.3 (O=$\underline{C}CH_3$), 170.2 (O=$\underline{C}CH_3$), 170.1 (O=$\underline{C}CH_3$), 71.5 (C-4), 69.4 (C-3), 69.2 (C-5), 47.6 (C-6), 47.5 (C-2), 21.1 ($\underline{C}H_3CO$), 21.1 ($\underline{C}H_3CO$), 20.9 ($\underline{C}H_3CO$). HRMS (ESP+): m/z calcd for $C_{11}H_{17}NO_6$: 260.11286 $[M+H]^+$; found: 260.11301. IR ($CHCl_3$) $\tilde{\nu}$ = 3749, 3673, 3566, 3030, 2966, 1745, 1650, 1457, 1373, 1237, 1124, 1050 cm^{-1}.

Compound 8: A solution of **6** (135 mg, 0.521 mmol), NEt_3 (95 μL, 0.682 mmol) and tosylate **7** (203 mg, 0.676 mmol) in CH_3CN (7 mL) was stirred at 80 °C for 2 days until the disappearance of **6** was assessed by a TLC control (CH_2Cl_2:MeOH:NH_4OH (6%) 7:1:0.1). The mixture was concentrated under vacuum and then the crude was purified by FCC on silica gel (Hexane:AcOEt 1:1) to give 133 mg of **8** (0.343 mmol, 66%) as a pale-yellow oil. *Rf* = 0.17 (Hexane:AcOEt 1:1). $[\alpha]_D^{24} = -36.8$ (c = 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.31–5.25 (m, 1H, H-3), 5.14 (td, *J* = 8.4, 4.4 Hz, 1H, H-5), 4.94 (dd, *J* = 8.6, 3.4 Hz, 1H, H-4), 4.12–4.03 (m, 2H, H-3′, Ha-4′), 3.58–3.48 (m, 1H, Hb-4′), 3.03–2.93 (m, 1H, Ha-6), 2.90–2.81 (m, 1H, Ha-2), 2.56–2.43 (m, 2H, H-1′), 2.38 (br d, *J* = 12.1 Hz, 1H, Hb-2), 2.30–2.19 (m, 1H, Hb-6), 2.09 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.83–1.73 (m, 1H, Ha-2′), 1.71–1.62 (m, 1H, Hb-2′), 1.38 (s, 3H, $OC(\underline{C}H_3)_2$), 1.34 (s, 3H, $OC(\underline{C}H_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.5 (C=O), 170.2 (2C, C=O), 108.7 ($O\underline{C}(CH_3)_2$), 74.8 (C-3′), 71.1 (C-4), 69.6 (C-4′), 68.3 (C-5), 68.0 (C-3), 54.6 (C-6), 54.1 (C-1′), 53.6 (C-2), 30.9 (C-2′), 27.0 ($OC(\underline{C}H_3)_2$), 25.9 ($OC(\underline{C}H_3)_2$), 21.2 ($\underline{C}H_3CO$), 21.1 ($\underline{C}H_3CO$), 21.0 ($\underline{C}H_3CO$). HRMS (ESP+): m/z calcd for $C_{18}H_{29}NO_8$: 388.19659 $[M+H]^+$; found: 388.19698. IR ($CHCl_3$) $\tilde{\nu}$ = 3749, 3674, 3650, 3030, 2963, 1743, 1372, 1261, 1234, 1048 cm^{-1}.

Compound 9: A solution of **8** (138 mg, 0.356 mmol) in dry CH_2Cl_2 (6 mL) was left stirring with trifluoroacetic acid (604 μL, 7.84 mmol) at room temperature for 2 h until ^{1}H NMR analysis assessed the disappearance of **8**. Then, the crude mixture was concentrated and the crude residue was purified by FCC on silica gel (CH_2Cl_2:MeOH:NH_4OH (6%) 20:1:0.2) to give 122 mg of **9** (0.351 mmol, 98%) as a colourless oil. *Rf* = 0.17 (CH_2Cl_2:MeOH:NH_4OH (6%) 20:1:0.1). $[\alpha]_D^{24} = -15.5$ (c = 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.29–5.24 (m, 1H, H-3), 5.16–5.06 (m, 1H, H-5), 5.01–4.91 (m, 1H, H-4), 3.84–3.79 (m, 1H, H-3′), 3.58 (dd, *J* = 11.3, 3.9 Hz, 1H, Ha-4′), 3.49 (dd, *J* = 11.3, 5.1 Hz, 1H, Hb-4′), 3.11 (br s, 1H, Ha-6), 2.83 (br s, 1H, Ha-2), 2.70-2.50 (m, 3H, Hb-2, H-1′), 2.32–2.11 (m, 1H, Hb-6), 2.08 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 1.78–1.66 (m, 1H, Ha-2′), 1.60–1.50 (m, 1H, Hb-2′). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm: 170.3 (C=O), 170.1 (C=O), 170.0 (C=O), 72.6 (C-3′), 70.6 (C-4), 67.9 (C-5), 67.6 (C-3), 66.3 (C-4′), 55.2 (C-1′), 54.2, 53.8 (C-2, C-6), 28.6 (C-2′), 21.0 (2C, $\underline{C}H_3CO$), 20.9 ($\underline{C}H_3CO$). HRMS (ESP+): m/z calcd for $C_{15}H_{25}NO_8$: 348.16529 $[M+H]^+$; found: 348.16643. IR ($CHCl_3$) $\tilde{\nu}$ = 3760, 3640, 3560, 2938, 2840, 1750, 1372, 1253, 1065 cm^{-1}.

Compound 10: To a solution of diol **9** (37 mg, 0.107 mmol) in dry CH_2Cl_2 (36 mL), trimethyl orthoformate (52 μL, 0.471 mmol) and *p*-TSA (4 mg, 0.0232 mmol) were added. The reaction mixture was stirred at room temperature for 3 h until the disappearance of starting material **9** was assessed by a TLC control (CH_2Cl_2:MeOH:NH_4OH (6%) 10:1:0.1). The solution was washed successively with saturated aqueous $NaHCO_3$ and brine. The reunited organic layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude mixture was purified by FCC on silica gel (CH_2Cl_2:MeOH 20:1) to give 37 mg of **10** (0.0950 mmol, 88%) as a 1.2:1 mixture, as attested by ^{1}H NMR, of two diastereoisomers A and B, as a colourless oil. Rf = 0.29 (CH_2Cl_2:MeOH 20:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.69 (s, 1H, HCOMe, isomer A or B), 5.68 (s, 1H, HCOMe, isomer A or B), 5.28–5.23 (m, 2H, H-3, isomer A and B), 5.15–5.08 (m, 2H, H-4, isomer A and B), 4.93 (dd, *J* = 8.6, 3.4 Hz, 2H, H-5, isomer A and B), 4.29 (quint, *J* = 6.4 Hz, 1H, H-3′, isomer A or B), 4.18–4.03 (m, 3H, H-3′, isomer A or B, Ha-4′, isomer A and B), 3.65 (t, *J* = 7.7 Hz, 1H, Hb-4′, isomer A or B), 3.58 (t, *J* = 6.8 Hz, 1H, Hb-4′, isomer A or B), 3.29 (s, 6H, OCH_3, isomer A and B), 3.00–2.93 (m, 2H, Ha-6, isomer A and B), 2.88–2.81 (m, 2H, Ha-2, isomer A and B), 2.59-2.43 (m, 4H, H-1′, isomer A and B), 2.38 (br d, J = 12.4 Hz, 2H, Hb-2, isomer A and B), 2.30–2.21 (m, 2H, Hb-6, isomer A and B), 2.07 (s, 6H, 2xCH_3CO, isomer A and B), 2.03 (s, 6H, 2xCH_3CO, isomer A and B), 2.01 (s, 6H, 2xOAc, isomer A and B), 1.92–1.60 (m, 4H, H-2′, isomer A and B). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.4 (C=O, 2C isomer A and 2C isomer B), 170.1 (C=O, isomer A and B), 116.1 (C-5′, isomer A or B), 115.7 (C-5′, isomer A or B), 75.7 (C-3′, isomer A or B), 74.1 (C-3′, isomer A or B), 71.0 (C-4, isomer A and B), 69.1 (C- 4′, isomer A or B), 68.6 (C-4′, isomer A or B), 68.3 (C-5, isomer A and B), 67.9 (C-3, isomer A and B), 54.6 (C-6, isomer A and B), 53.9 (C-1′, isomer A or B), 53.7 (C-1′, isomer A or B), 53.6 (C-2, isomer A and B), 51.8 (OCH_3, isomer A or B), 51.7 (OCH_3, isomer A or B), 31.1 (C-2′, isomer A or B), 30.7 (C-2′, isomer A or B), 21.1 (CH_3CO, isomer A and B), 21.0 (CH_3CO, isomer A and B), 20.9 (CH_3CO, isomer A and B). HRMS (ESP+): m/z calcd for $C_{17}H_{27}NO_9$: 390.17586 $[M+H]^+$; found: 390.17618. IR ($CHCl_3$) $\tilde{\nu}$ = 3854, 3735, 3674, 2941, 2836, 2261, 1743, 1372, 1232, 1132, 1067, 946 cm^{-1}.

Orthoester 11a: To a solution of compound **10** (35 mg, 0.0899 mmol) in dry toluene (1 mL), 1-butanol (8 μL, 0.0899 mmol) and pyridinium *p*-toluenesulfonate (1 mg, 0.00398 mmol) were added. The resulting solution was stirred under reflux for 3 h until the disappearance of starting material **10** was assessed by a TLC control (Hexane:AcOEt 1:1). After addition of cyclohexane (1 mL), the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by FCC on silica gel (Hexane:AcOEt 1:1) to give 20 mg of **11a** (0.0464 mmol, 51%) as a 1.3:1 mixture, as attested by ^{1}H NMR, of two diastereoisomers A and B, as a colourless oil. *Rf* = 0.25 (Hexane:AcOEt 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.77 (s, 1H, $\underline{H}COBu$, isomer A or B), 5.76 (s, 1H, $\underline{H}COBu$, isomer A or B), 5.31–5.25 (m, 2H, H-3, isomer A and B), 5.18–5.09 (m, 2H, H-5, isomer A and B), 4.94 (dd, *J* = 8.6, 3.2 Hz, 2H, H-4, isomer A and B), 4.30 (quint, *J* = 6.4 Hz, 1H, H-3′, isomer A or B), 4.20–4.04 (m, 3H, H-3′, isomer A or B, Ha-4′, isomer A and B), 3.66 (t, *J* = 7.7 Hz, 1H, Hb-4′, isomer A or B), 3.58 (t, *J* = 7.0 Hz, 1H, Hb-4′, isomer A or B), 3.51 (t, *J* = 6.5 Hz, 2H, H-6′, isomer A or B), 3.50 (t, *J* = 6.6 Hz, 2H, H-6′, isomer A or B), 3.02-2-96 (m, 2H, Ha-6, isomer A and B), 2.90–2.81 (m, 2H, Ha-2, isomer A and B), 2.61–2.43 (m, 4H, H-1′, isomer A and B), 2.38 (br d, *J* = 12.2 Hz, 2H, Hb-2, isomer A and B), 2.31–2.21 (m, 2H, Hb-6, isomer A and B), 2.09 (s, 6H, 2xCH_3CO, isomer A and B), 2.05 (s, 6H, 2xCH_3CO, isomer A and B), 2.03 (s, 6H, 2xCH_3CO, isomer A and B), 1.95–1.63 (m, 4H, H-2′, isomer A and B), 1.56 (quint, *J* = 6.8 Hz, 4H, H-7′, isomer A and B), 1.42–1.31 (m, 4H, H-8′, isomer A and B), 0.91 (t, *J* = 7.4 Hz, 6H, H-9′, isomer A and B). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm: 170.5 (C=O, isomer A and B), 170.2 (C=O, 2C isomer A and 2C isomer B), 115.6 (C-5′, isomer A or B), 115.2 (C-5′, isomer A or B), 75.7 (C-3′, isomer A or B), 74.1 (C-3′, isomer A or B), 71.1 (C-4, isomer A and B), 69.0 (C-4′, isomer A or B), 68.6 (C-4′, isomer A or B), 68.3 (C-5, isomer A and B), 68.0 (C-3, isomer A and B), 64.7 (C-6′, isomer A and B), 54.6 (C-6, isomer A and B), 54.0 (C-1′, isomer A or B), 53.8 (C-1′, isomer A or B), 53.6 (C-2, isomer A and B), 31.7 (C-7', isomer A and B), 31.1 (C-2′, isomer A or B), 30.7 (C-2′, isomer A or B), 21.1 ($\underline{C}H_3CO$, 2C isomer A and 2C isomer B), 20.9 ($\underline{C}H_3CO$, isomer A and B), 19.4 (C-8', isomer A and B), 13.9 (C-9′, isomer A and B). HRMS (ESP+): m/z calcd for $C_{20}H_{33}NO_9$: 432.22281 $[M+H]^+$; found: 432.22289. IR ($CHCl_3$) $\tilde{\nu}$ = 3854, 3749, 3674, 3617, 2961, 2261, 1743, 1372, 1233, 1144, 1063, 952 cm^{-1}.

Orthoester 11b: To a solution of compound **10** (62 mg, 0.159 mmol) in toluene (1.8 mL), 1-octanol (25 μL, 0.159 mmol) and pyridinium *p*-toluenesulfonate (1 mg, 0.00398 mmol) were added. The resulting solution was stirred under reflux for 4 h until the disappearance of starting material **10** was assessed by a TLC control (Hexane:AcOEt 1:1). After addition of cyclohexane (2 mL), the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by FCC on silica gel (Hexane:AcOEt 2:1) to give 46 mg of **11b** (0.0943 mmol, 59%) as a 1.3:1 mixture, as attested by ^{1}H NMR, of two diastereoisomers A and B, as a

colourless oil. *Rf* = 0.21 (Hexane : AcOEt 2 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.77 (s, 1H, HCO-octyl, isomer A or B), 5.76 (s, 1H, HCO-octyl, isomer A or B), 5.31–5.25 (m, 2H, H-3, isomer A and B), 5.18–5.09 (m, 2H, H-5, isomer A and B), 4.98–4.91 (m, 2H, H-4, isomer A and B), 4.30 (quint, *J* = 6.3 Hz, 1H, H-3', isomer A or B), 4.20–4.03 (m, 3H, H-3', isomer A or B, Ha-4', isomer A and B), 3.66 (t, *J* = 7.8 Hz, 1H, Hb-4', isomer A or B), 3.58 (t, *J* = 7.0 Hz, 1H, Hb-4', isomer A or B), 3.50 (t, *J* = 5.9 Hz, 2H, H-6', isomer A or B), 3.48 (t, *J* = 6.4 Hz, 2H, H-6', isomer A or B), 3.03-2.93 (m, 2H, Ha-6, isomer A and B), 2.91–2.81 (m, 2H, Ha-2, isomer A and B), 2.60–2.46 (m, 4H, H-1', isomer A and B), 2.39 (br d, *J* = 12.0 Hz, 2H, Hb-2, isomer A and B), 2.32–2.21 (m, 2H, Hb-6, isomer A and B), 2.09 (s, 6H, CH_3CO, isomer A and B), 2.05 (s, 6H, CH_3CO, isomer A and B), 2.03 (s, 6H, CH_3CO, isomer A and B), 1.96–1.63 (m, 4H, H-2', isomer A and B), 1.57 (quint, *J* = 6.4 Hz, 4H, H-7', isomer A and B), 1.35-1.21 (m, 20H, H-8', H-9', H-10', H-11', H-12', isomer A and B), 0.87 (t, *J* = 6.0 Hz, 6H, H-13', isomer A and B). ^{13}C NMR (50 MHz, $CDCl_3$) δ ppm: 170.5 (C=O, isomer A and B), 170.2 (C=O, 2C isomer A and 2C isomer B), 115.6 (C-5', isomer A or B), 115.2 (C-5', isomer A or B), 75.7 (C-3', isomer A or B), 74.1 (C-3', isomer A or B), 71.1 (C-4, isomer A and B), 69.0 (C-4', isomer A or B), 68.6 (C-4', isomer A or B), 68.3 (C-5, isomer A and B), 68.0 (C-3, isomer A and B), 65.1 (C-6', isomer A and B), 54.6 (C-6, isomer A and B), 54.0 (C-1', isomer A or B), 53.8 (C-1', isomer A or B), 53.6 (C-2, isomer A and B), 32.0 (C-7', isomer A and B), 31.1 (C-2', isomer A or B), 30.7 (C-2', isomer A or B), 29.7, 29.5, 29.4, 26.2, 22.8 (C-8', C-9', C-10', C-11', C-12', 5C isomer A and 5C isomer B), 21.1 (CH_3CO, 2C isomer A and 2C isomer B), 20.9 (CH_3CO, isomer A and B), 14.2 (C-13', isomer A and B). HRMS (ESP+): m/z calcd for $C_{24}H_{41}NO_9$: 488.28541 $[M+H]^+$; found: 488.28654. IR ($CHCl_3$) $\tilde{\nu}$ = 3689, 2929, 2857, 2263, 1743, 1467, 1372, 1233, 1143, 1053 cm^{-1}.

Orthoester 11c: To a solution of compound **10** (62 mg, 0.159 mmol) in toluene (1 mL), 1-dodecanol (36 μL, 0.159 mmol) and pyridinium *p*-toluenesulfonate (1 mg, 0.00398 mmol) were added. The resulting solution was stirred under reflux for 6 h until the disappearance of starting material **10** was assessed by a TLC control (Petroleum ether : AcOEt 2 : 1). After addition of cyclohexane (1 mL), the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by FCC on silica gel (gradient eluent from Petroleum ether : AcOEt 4 : 1 to 2 : 1) to give 47 mg of **11c** (0.0864 mmol, 55%) as a 1.2, :1 mixture as attested by ^{1}H NMR, of two diastereoisomers A and B, as a colourless oil. *Rf* = 0.34 (Petroleum ether : AcOEt 4 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.76 (s, 1H, HCO-dodecyl, isomer A or B), 5.74 (s, 1H, HCO-dodecyl, isomer A or B), 5.26 (br s, 2H, H-3, isomer A and B), 5.16–5.07 (m, 2H, H-5, isomer A and B), 4.96–4.88 (m, 2H, H-4, isomer A and B), 4.28 (quint, *J* = 6.4 Hz, 1H, H-3', isomer A or B), 4.17–4.00 (m, 3H, H-3', isomer A or B, Ha-4', isomer A and B), 3.64 (t, *J* = 7.8 Hz, 1H, Hb-4', isomer A or B), 3.56 (t, *J* = 7.3 Hz, 1H, Hb-4', isomer A or B), 3.48 (t, *J* = 6.4 Hz, 2H, H-6', isomer A or B), 3.46 (t, *J* = 6.1 Hz, 2H, H-6', isomer A or B), 3.00–2.91 (m, 2H, Ha-6, isomer A and B), 2.90–2.79 (m, 2H, Ha-2, isomer A and B), 2.58–2.42 (m, 4H, H-1', isomer A and B), 2.36 (br d, *J* = 12.0 Hz, 2H, Hb-2, isomer A and B), 2.30–2.20 (m, 2H, Hb-6, isomer A and B), 2.07 (s, 6H, CH_3CO, isomer A and B), 2.03 (s, 6H, CH_3CO, isomer A and B), 2.01 (s, 6H, CH_3CO, isomer A and B), 1.91–1.61 (m, 4H, H-2', isomer A and B), 1.55 (quint, *J* = 6.2 Hz, 4H, H-7', isomer A and B), 1.32–1.19 (m, 36H, H-8', H-9', H-10', H-11', H-12', H-13', H-14', H-15', H-16', isomer A and B), 0.85 (t, *J* = 5.8 Hz, 6H, H-17', isomer A and B). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.3 (C=O, isomer A and B), 170.0 (C=O, 2C isomer A and 2C isomer B), 115.5 (C-5', isomer A or B), 115.1 (C-5', isomer A or B), 75.6 (C-3', isomer A or B), 74.0 (C-3', isomer A or B), 71.0 (C-4, isomer A and B), 68.9 (C-4', isomer A or B), 68.6 (C-4', isomer A or B), 68.2 (C-5, isomer A and B), 67.9 (C-3, isomer A and B), 65.0 (C-6', isomer A and B), 54.6 (C-6, isomer A and B), 53.9 (C-1', isomer A or B), 53.8 (C-1', isomer A or B), 53.5 (C-2, isomer A and B), 31.0 (C-2', isomer A or B), 30.7 (C-2', isomer A or B), 32.0, 29.7, 29.6, 29.5, 29.4, 26.2, 22.8 (C-7', C-8', C-9', C-10', C-11', C-12', C-13', C-14', C-15', C-16', 10C isomer A and 10C isomer B), 21.1 (CH_3CO, isomer A and B), 21.1 (CH_3CO, isomer A and B), 20.9 (CH_3CO, isomer A and B), 14.2 (C-17', isomer A and B). HRMS (ESP+): m/z calcd for $C_{28}H_{49}NO_9$: 544.34801 $[M+H]^+$; found: 544.34976. IR ($CHCl_3$) $\tilde{\nu}$ = 3689, 2928, 2856, 2262, 1744, 1435, 1372, 1259, 1230, 1142, 1062, 1025 cm^{-1}.

Orthoester 11d: To a solution of compound **10** (70 mg, 0.180 mmol) in toluene (2 mL), biphenyl-4-methanol (33 mg, 0.180 mmol) and pyridinium *p*-toluenesulfonate (5 mg, 0.0199 mmol) were added. The resulting solution was stirred under reflux for 24 h. After addition of cyclohexane (5 mL), the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by FCC on silica gel (Petroleum ether : AcOEt 3 : 1) to give 34 mg of **11d** (0.0628 mmol, 35% yield, 50% yield calculated on the converted starting material) as a 1.8 : 1 mixture, as attested by ^{1}H NMR, of two diastereoisomers A and B, as a colourless oil and 10 mg of biphenyl-4-methanol (0.0543 mmol). *Rf* = 0.1 (Petroleum ether : AcOEt 4 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.60–7.55 (m, 8H, Ar, isomer A and B), 7.47–7.40 (m, 8H, Ar, isomer A and B), 7.37–7.31 (m, 2H, Ar, isomer A and B), 5.93 (s, 1H, $HCOCH_2Ph$, isomer A or B), 5.92 (s, 1H, $HCOCH_2Ph$, isomer A o B), 5.31–5.26 (m, 2H, H-3, isomer A and B), 5.19–5.10 (m, 2H, H-5, isomer A and B), 4.99–4.91 (m, 2H, H-4, isomer A and B), 4.65 (s, 2H, CH_2Ph, isomer A or B), 4.64 (s, 2H, CH_2Ph, isomer A or B), 4.37 (quint, *J* = 6.4 Hz, 1H, H-3', isomer A or B), 4.27–4.09 (m, 3H, H-3', isomer A or B, Ha-4', isomer A and B), 3.74 (t, *J* = 7.7 Hz, 1H, Hb-4', isomer A or B), 3.64 (t, *J* = 7.2 Hz, 1H, Hb-4', isomer A or B), 3.06-2.94 (m, 2H, Ha-6, isomer A and B), 2.91-2.80 (m, 2H, Ha-2, isomer A and B), 2.60–2.45 (m, 4H, H-1', isomer A and B), 2.38 (br d, *J* = 11.8 Hz, 2H, Hb-2, isomer A and B), 2.31–2.19 (m, 2H, Hb-6, isomer A and B), 2.10–2.07 (m, 6H, 2xCH_3CO, isomer A and B), 2.05 (m, 6H, 2xCH_3CO, isomer A and B) 2.04 (m, 6H, 2xCH_3CO, isomer A and B), 1.99-1.67 (m, 4H, H-2', isomer A and B). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.5 (C=O, isomer A and B), 170.2 (C=O, 2C isomer A and 2C isomer B), 141.0 (isomer A and B), 140.8 (isomer A and B), 136.6 (isomer A and B), 128.9 (2C isomer A and 2C isomer B), 128.4 (2C isomer A and 2C isomer B), 127.4 (isomer A and B), 127.3 (2C isomer A and 2C isomer B), 127.2 (2C isomer A and 2C isomer B), 115.2 (C-5', isomer A or B), 114.8 (C-5', isomer A or B), 75.9 (C-3', isomer A or B), 74.3 (C-3', isomer A or B), 71.1 (C-4, isomer A and B), 69.1 (C-4', isomer A or B), 68.8 (C-4', isomer A or B), 68.3 (C-5, isomer A and B), 68.0 (C-3, isomer A and B), 66.6 (CH_2Ph, isomer A or B), 66.5 (CH_2Ph, isomer A or B), 54.6 (C-6, isomer A and B), 54.0 (C-1', isomer A or B), 53.8 (C-1', isomer A or B), 53.6 (C-2, isomer A and B), 31.2 (C-2', isomer A or B), 30.8 (C-2', isomer A or B), 21.1 (CH_3CO, 2C isomer A and 2C isomer B), 20.9 (CH_3CO, isomer A and B). HRMS (ESP+): m/z calcd for $C_{29}H_{35}NO_9$: 564.22040 $[M+Na]^+$; found: 564.21946. IR ($CHCl_3$) $\tilde{\nu}$ = 3021, 2961, 2920, 2361, 1742, 1371, 1260, 1236, 1049 cm^{-1}.

Orthoester 1a: Sodium carbonate (37 mg, 0.349 mmol) was added to a solution of **11a** (30 mg, 0.0695 mmol) in MeOH (5 mL) and the mixture was stirred at room temperature for 16 h, until a TLC control attested the disappearance of the starting material **11a** (CH_2Cl_2 : MeOH : NH_4OH (6%) 10 : 1 : 0.1). Then, the mixture was filtered, and the solvent was removed under vacuum. The crude was purified by FCC on silica gel (CH_2Cl_2 : MeOH 4 : 1) to afford 19 mg of pure **1a** (0.0622 mmol, 90%) as a colourless oil. *Rf* = 0.39 (CH_2Cl_2 : MeOH 4 : 1). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 5.78 (s, 1H, HCOBu, isomer A or isomer B), 5.75 (s, 1H, HCOBu, isomer A or isomer B), 4.31 (quint, *J* = 6.4 Hz, 1H, H-3', isomer A or B), 4.18–4.05 (m, 3H, H-3', isomer A or B, Ha-4', isomer A and B), 3.92–3.87 (m, 2H, H-3, isomer A and B), 3.79 (td, *J* = 7.9, 4.0 Hz, 2H, H-5, isomer A and B), 3.63 (t, *J* = 7.8 Hz, 1H, Hb-4', isomer A or B), 3.58 (t, *J* = 7.1 Hz, 1H, Hb-4', isomer A or B), 3.52 (t, *J* = 6.9 Hz, 2H, H-6', isomer A or B),

3.51 (t, $J=6.7$ Hz, 2H, H-6′, isomer A or B), 3.42–3.36 (m, 2H, H-4, isomer A and B), 2.88–2.70 (m, 4H, Ha-2, Ha-6, isomer A and B), 2.57–2.40 (m, 4H, H-1′, isomer A and B), 2.28 (m, 2H, Hb-2, isomer A and B), 2.09 (m, 2H, Hb-6, isomer A and B), 1.93–1.71 (m, 4H, H-2′, isomer A and isomer B), 1.59–1.50 (m, 4H, H-7′, isomer A and B), 1.44–1.33 (m, 4H, H-8′, isomer A and B), 0.96–0.90 (m, 6H, H-9′, isomer A and B). ^{13}C NMR (100 MHz, CD_3OD) δ ppm: 116.8 (C-5′, isomer A or B), 116.4 (C-5′, isomer A or B), 76.7 (C-3′, isomer A or B), 75.3 (C-3′, isomer A or B and C-4, isomer A and B), 69.8, 69.5, 69.2 (C-3, C-5 and C-4', isomer A and B), 65.1 (C-6′, isomer A and B), 58.4 (C-6, isomer A and B), 57.6 (C-2, isomer A and B), 55.6 (C-1′, isomer A or B), 55.3 (C-1′, isomer A or B), 32.8 (C-7′, isomer A or B), 32.7 (C-7′, isomer A or B), 32.0 (C-2′, isomer A or B), 31.6 (C-2′, isomer A or B), 20.4 (C-8′, isomer A or B), 20.3 (C-8′, isomer A or B), 14.2 (C-9′, isomer A or B), 14.1 (C-9′, isomer A or B). HRMS (ESP+): m/z calcd for $C_{14}H_{27}NO_6$: 306.19111 $[M+H]^+$; found: 306.19119.

Orthoester 1b: Ambersep 900 OH (500 mg) was added to a solution of **11b** (45 mg, 0.0923 mmol) in MeOH (3 mL) and the mixture was stirred at room temperature for 18 h, until a TLC control attested the disappearance of the starting material **11b** (CH_2Cl_2:MeOH:NH_4OH (6%) 10:1:0.1). Then, the mixture was filtered, and the solvent was removed under vacuum to afford 33 mg of pure **1b** (0.0913 mmol, quantitative) as white waxy solid. ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 5.78 (s, 1H, HCO-octyl, isomer A or isomer B), 5.75 (s, 1H, HCO-octyl, isomer A or isomer B), 4.31 (quint, $J=6.4$ Hz, 1H, H-3′, isomer A or B), 4.20–4.05 (m, 3H, H-3′, isomer A or B, Ha-4′, isomer A and B), 3.89 (br s, 2H, H-3, isomer A and B), 3.83–3.75 (m, 2H, H-5, isomer A and B), 3.64 (t, $J=7.7$ Hz, 1H, Hb-4′, isomer A or B), 3.58 (t, $J=7.1$ Hz, 1H, Hb-4′, isomer A or B), 3.52 (t, $J=7.0$ Hz, 2H, H-6′, isomer A or B), 3.50 (t, $J=6.8$, Hz, 2H, H-6′, isomer A or B), 3.43–3.36 (m, 2H, H-4, isomer A and B), 2.86–2.70 (m, 4H, Ha-2, Ha-6, isomer A and B), 2.59–2.40 (m, 4H, H-1′, isomer A and B), 2.29 (br d, $J=11.0$ Hz, 2H, Hb-2, isomer A and B), 2.18–2.06 (m, 2H, Hb-6, isomer A and B), 1.93–1.70 (m, 4H, H-2′, isomer A and B), 1.62–1.49 (m, 4H, H-7′, isomer A and B), 1.38-1.27 (m, 20H, H-8′, H-9′, H-10′, H-11′, H-12′, isomer A and B), 0.94–0.87 (m, 6H, H-13′, isomer A and B). ^{13}C NMR (100 MHz, CD_3OD) δ ppm: 116.8 (C-5′, isomer A or B), 116.4 (C-5′, isomer A or B), 76.7 (C-3′, isomer A or B), 75.3 (C-3′, isomer A or B and C-4, isomer A and B), 69.8 (C-5, isomer A and B), 69.6 (C-4′, isomer A or B), 69.5 (C-4′, isomer A or B), 69.1 (C-3, isomer A and B), 65.4 (C-6′, isomer A and B), 58.2 (C-6, isomer A and B), 57.5 (C-2, isomer A and B), 55.5 (C-1′, isomer A or B), 55.2 (C-1′, isomer A or B), 33.0, 32.0, 31.6, 30.6, 33.0, 30.4, 27.2, 23.7 (C-2′, C-7′, C-8′, C-9′, C-10′, C-11′, C-12, isomer A and B′), 14.4 (C-13′, isomer A and B). HRMS (ESP+): m/z calcd for $C_{18}H_{35}NO_6$: 362.25371 $[M+H]^+$; found: 362.25428.

Orthoester 1c: Sodium carbonate (40 mg, 0.377 mmol) was added to a solution of **11c** (41 mg, 0.0754 mmol) in MeOH (5 mL) and the mixture was stirred at room temperature for 18 h, until a TLC control attested the disappearance of the starting material **11c** (CH_2Cl_2:MeOH:NH_4OH (6%) 10:1:0.1). Then, the mixture was filtered, and the solvent was removed under vacuum. The crude was purified by FCC on silica gel (CH_2Cl_2:MeOH:NH_4OH (6%) 5:1:0.1) to afford 17 mg of pure **1c** (0.0407 mmol, 55%) as a colourless oil. $Rf=0.25$ (CH_2Cl_2:MeOH:NH_4OH (6%) 5:1:0.1). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 5.78 (s, 1H, HCO-dodecyl, isomer A or B), 5.75 (s, 1H, HCO-dodecyl, isomer A or B), 4.31 (quint, $J=6.3$ Hz, 1H, H-3′, isomer A or B), 4.21–4.05 (m, 3H, H-3′, isomer A or B, Ha-4′, isomer A and B), 3.93–3.88 (m, 2H, H-3, isomer A and B), 3.84–3.77 (m, 2H, H-5, isomer A and B), 3.65 (t, $J=7.8$ Hz, 1H, Hb-4′, isomer A or B), 3.59 (t, $J=7.0$ Hz, 1H, Hb-4′, isomer A or B), 3.52 (t, $J=6.6$ Hz, 2H, H-6′, isomer A or B), 3.50 (t, $J=6.6$ Hz, 2H, H-6′, isomer A or B), 3.45–3.39 (m, 2H, H-4, isomer A and B), 2.89–2.73 (m, 4H, Ha-2, Ha-6, isomer A and B), 2.64–2.45 (m, 4H, H-1′, isomer A and B), 2.41–2.30 (m, 2H, Ha-2, isomer A and B), 2.26–2.12 (m, 2H, Hb-6, isomer A and B), 1.94–1.72 (m, 4H, H-2′, isomer A and B), 1.61–1.51 (m, 4H, H-7′, isomer A and B), 1.38–1.26 (m, 36H, H-8′, H-9′, H-10′, H-11′, H-12′, H-13′, H-14′, H-15′, H-16′, isomer A and B), 0.90 (t, $J=7.0$ Hz, 6H, H-17′, isomer A and B). ^{13}C NMR (50 MHz, CD_3OD) δ ppm: 116.9 (C-5′, isomer A or B), 116.4 (C-5′, isomer A or B), 76.6 (C-3′, isomer A or B), 75.2 (C-3′, isomer A or B), 75.0 (C-4, isomer A and B), 69.7, 69.5 (C-5 and C-4′, isomer A and B) 68.9 (C-3, isomer A and B), 65.5 (C-6′, isomer A and B), 58.1 (C-6, isomer A and B), 57.4 (C-2, isomer A and B), 55.6 (C-1′, isomer A or B), 55.3 (C-1′, isomer A or B), 31.8 (C-2′, isomer A or B), 31.4 (C-2′, isomer A or B), 33.1, 30.7, 30.6, 30.5, 27.3, 27.2, 23.7 (C-7′, C-8′, C-9′, C-10′, C-11′, C-12′, C-13′, C-14′, C-15′, C-16′, isomer A and B), 14.5 (C-17′, isomer A and B). HRMS (ESP+): m/z calcd for $C_{22}H_{43}NO_6$: 418.31631 $[M+H]^+$; found: 418.31667.

Orthoester 1d: Ambersep 900 OH (500 mg) was added to a solution of **11d** (23 mg, 0.0425 mmol) in MeOH (5 mL) and the mixture was stirred at room temperature for 18 h, until a TLC control attested the disappearance of the starting material **11d** (CH_2Cl_2:MeOH:NH_4OH (6%) 10:1:0.1). Then, the mixture was filtered, and the solvent was removed under vacuum to afford 15 mg of pure **1d** (0.0361 mmol, 88%) as white waxy solid. $Rf=0.14$ (CH_2Cl_2:MeOH:NH_4OH (6%) 10:1:0.1). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 7.63–7.57 (m, 8H, Ar, isomer A and B), 7.46–7.39 (m, 8H, Ar, isomer A and B), 7.36–7.29 (m, 2H, Ar, isomer A and B), 5.94 (s, 1H, $HCOCH_2Ph$, isomer A or isomer B), 5.92 (s, 1H, $HCOCH_2Ph$, isomer A or isomer B), 4.64 (s, 2H, CH_2Ph, isomer A or B), 4.61 (s, 2H, CH_2Ph, isomer A or B), 4.37 (quint, $J=6.4$ Hz, 1H, H-3′, isomer A or B), 4.25–4.11 (m, 3H, H-3′, isomer A or B, Ha-4′, isomer A and B), 3.92–3.86 (m, 2H, H-3, isomer A and B), 3.83–3.76 (m, 2H, H-5, isomer A and B), 3.72 (t, $J=7.8$ Hz, 1H, Hb-4′, isomer A or B), 3.62 (t, $J=7.1$ Hz, 1H, Hb-4′, isomer A or B), 3.44–3.35 (m, 2H, H-4, isomer A and B), 2.89–2.69 (m, 4H, Ha-2, Ha-6, isomer A and B), 2.60–2.40 (m, 4H, H-1′, isomer A and B), 2.35–2.21 (m, 2H, Hb-2, isomer A and B), 2.20–2.02 (m, 2H, Hb-6, isomer A and B), 1.97–1.71 (m, 4H, H-2′, isomer A and B). ^{13}C NMR (50 MHz, CD_3OD) δ ppm: 142.0 (isomer A and B), 141.8 (isomer A and B), 138.2 (isomer A and B), 129.9 (2C, isomer A and B), 129.4 (2C, isomer A and B), 128.4 (4C, isomer A and B), 127.9 (4C, isomer A and B), 116.5 (C-5′, isomer A or B), 116.1 (C-5′, isomer A or B), 76.9 (C-3′, isomer A or B), 75.4 (C-3′, isomer A or B), 75.3 (C-4, isomer A and B), 69.9 (C-4′, isomer A or B), 69.7 (C-5, isomer A and B), 69.5 (C-4′, isomer A or B), 69.1 (C-3, isomer A and B), 67.0 (CH_2Ph, isomer A and B), 58.3 (C-6, isomer A and B), 57.6 (C-2, isomer A and B), 55.6 (C-1′, isomer A or B), 55.3 (C-1′, isomer A or B), 32.0 (C-2′, isomer A and B), 31.5 (C-2′, isomer A and B). HRMS (ESP+): m/z calcd for $C_{23}H_{29}NO_6$: 438.18871 $[M+Na]^+$; found: 438.18841. IR (neat): $\tilde{\nu}=3348$, 2920, 2824, 2767, 1489, 1436, 1407, 1342, 1275, 1144, 1109, 1071, 1047, 1019, 1007, 967 cm^{-1}.

Compound 13: To a solution of piperidine **2** (108 mg, 0.624 mmol) in CH_3CN (3.5 mL), NEt_3 (113 μL, 0.811 mmol) and tosylate **7** (244 mg, 0.812 mmol) were added. The reaction mixture was stirred at 80 °C for 2 days until the disappearance of **2** was assessed by a TLC control (CH_2Cl_2:MeOH:NH_4OH (6%) 5:1:0.1). The mixture was concentrated under vacuum and then the crude was purified by FCC on silica gel (CH_2Cl_2:MeOH:NH_4OH (6%) 20:1:0.1) to give 170 mg of **13** (0.564 mmol, 90%) as a pale-orange oil. $Rf=0.14$ (CH_2Cl_2:MeOH:NH_4OH (6%) 20:1:0.1). $[\alpha]_D^{24}=+6.33$ (c=0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.25 (m, 1H, H-3), 4.10–3.98 (m, 3H, Ha-4′, H-3′, H-4), 3.91–3.88 (m, 1H, H-5), 3.51 (t, $J=7.2$ Hz, 1H, Hb-4′), 2.69 (dd, $J=12.0$, 5.6 Hz, 1H, Ha-2), 2.60 (dd, $J=11.7$, 2.7 Hz, 1H, Ha-6), 2.57–2.40 (m, 4H, H-1′, Hb-2, Hb-6), 1.80–1.72 (m, 2H, H-2′), 1.47 (s, 3H, $OC(CH_3)_2$), 1.36 (s, 3H, $OC(CH_3)_2$), 1.32 (s, 3H, $OC(CH_3)_2$, 1.31 (s, 3H, $OC(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 109.4 ($OC(CH_3)_2$), 108.9 ($OC(CH_3)_2$), 77.1 (C-4), 74.6 (C-3′), 72.2 (C-3), 69.5 (C-4′), 67.8 (C-5), 55.7, 55.3 (C-2 and C-6), 54.4 (C-1′), 31.1 (C-2′), 28.3 ($OC(CH_3)_2$), 27.0 ($OC(CH_3)_2$), 26.4 ($OC(CH_3)_2$), 25.8 ($OC(CH_3)_2$). MS-ESI

m/z (%) = 324.17 (100) $[M+Na]^+$. IR ($CHCl_3$): $\tilde{\nu}$ = 3688, 3025, 2940, 2830, 1455, 1380, 1227, 1206, 1157, 1059 cm^{-1}.

Compound 12: A solution of **13** (70 mg, 0.232 mmol) in MeOH (5 mL) was left stirring with 12 M HCl (60 μL) at room temperature for 15 h. The crude mixture was concentrated to yield **12** as hydrochloride salt. The crude mixture was purified by FCC on silica gel (CH_2Cl_2:MeOH:NH_4OH (6%) 2:1:0.1) to give 46 mg of **12** (0.208 mmol, 90%) as a colourless oil. *Rf* = 0.13 (CH_2Cl_2:MeOH:NH_4OH (6%) 2:1:0.1). $[\alpha]_D^{22} = -51.88$ (c = 0.85, MeOH). ^{1}H NMR (400 MHz, D_2O) δ ppm: 3.97–3.90 (m, 1H, H-3), 3.79 (td, *J* = 8.8, 4.3 Hz, 1H, H-5), 3.69–3.61 (m, 1H, H-3′), 3.49 (dd, *J* = 11.7, 4.1 Hz, 1H, Ha-4′), 3.46–3.38 (m, 2H, H-4, Hb-4′), 2.95–2.72 (m, 2H, Ha-2, Ha-6), 2.61–2.43 (m, 2H, H-1′), 2.37–2.27 (m, 1H, Hb-2), 2.08 (br s, 1H, Hb-6), 1.68–1.49 (m, 2H, H-2′). ^{13}C NMR (100 MHz, D_2O) δ ppm: 73.2 (C-4), 70.7 (C-3′), 67.2 (C-3, C-5), 65.1 (C-4′), 56.1, 55.4 (C-2, C-6), 53.7 (C-1′), 28.5 (C-2′). HRMS (ESP+): m/z calcd for $C_9H_{19}NO_5$: 222.13360 $[M+H]^+$; found: 222.13395.

Biochemical Characterization

Inhibitory activity towards human GCase from leukocyte homogenates

All experiments on biological materials were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. In keeping with ethical guidelines, all blood and cell samples were obtained for storage and analysed only after written informed consent of the patients (and/or their family members) was obtained, using a form approved by the local Ethics Committee (Codice Protocollo: Lysolate "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). Project ID code:16774_bio, 5 May 2020, Comitato Etico Regionale per la Sperimentazione Clinica della Regione Toscana, Area Vasta Centro, Florence, Italy). Control and patient samples were anonymized and used only for research purposes.

Compounds **1a**–**d** and **12** were screened towards GCase from 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's instructions. Enzyme activity was measured in a flat-bottomed 96-well plate. Iminosugar 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 the 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 (λex = 365 nm, λem = 435 nm; Molecular Devices). Percentage GCase inhibition is given with respect to the control (without iminosugar). Data are mean + SD (n = 3).

-IC_{50} determination: The IC_{50} values of inhibitors against GCase 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.

-Inhibitory activity towards human GCase from leukocyte homogenates measured on compounds 14, 1c and 1d after preincubation for different times in a 5.8 pH buffer: Compounds **14**, **1c** and **1d** were dissolved in citrate/phosphate buffer (0.1:0.2, M/M, pH 5.8) and 37 °C at different hours prior to (17 h, 24 h, 48 h and 72 h) or right before the biological evaluation for GCase inhibition at 1 mM (as described above).

-Kinetic Analysis for compounds 1c and 1d: The action mechanism of both compounds was determined by studying the dependence of the main kinetic parameters (Km and Vmax) from the inhibitor concentration. Kinetic data were analyzed using the Lineweaver–Burk plot (for more details, See the Supporting Information).

Chaperoning activity assays

The effect of compounds **1a**–**d** on mutated GCase activity was evaluated in Gaucher patients' cells fibroblasts with the N370S/RecNcil or L444P/L444P mutations. Gaucher disease patients' cells were obtained from the "Cell line and DNA Biobank from patients affected by Genetic Diseases" (Gaslini Hospital, Genova, Italy). Fibroblasts cells (30×10^4) 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% CO_2 for 24 h. The medium was removed, and fresh medium containing the compounds was added to the cells and left 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).

Supporting Information

The Supporting Information File contains ^{1}H NMR and ^{13}C NMR spectra of compounds **4**, **5**, **6**, **8**, **9**, **10**, **11a**–**d**, **1a**–**d**, **13**, **12**, stability assessment for compounds **1b**–**d**, Inhibitory activity towards human GCase, IC_{50} graphs, Kinetic Analysis and Chaperoning activity assays.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: glycomimetic · iminosugar · inhibitor · pharmacological chaperones · pH-responsive

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