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New Glucocerebrosidase Inhibitors by Exploration of Chemical Diversity of *N*-Substituted Aminocyclitols Using Click Chemistry and in Situ Screening

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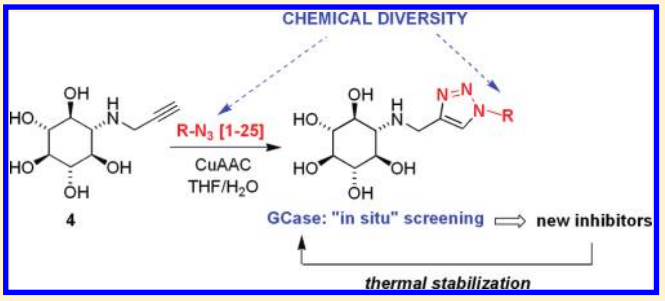
Supporting Information

ABSTRACT: A library of aminocyclitols derived from CuAAC reaction between *N*-propargylaminocyclitol **4** and a series of azides [**1**−**25**] is described and tested against GCase. Azides have been chosen from a large collection of potential candidates that has been filtered according to physical and reactivity constraints. A synthetic methodology has been optimized in order to avoid the use of protecting groups on the aminocyclitol scaffold. Because the reaction can be carried out in an aqueous system, the resulting library members can be screened in situ with minimal manipulation. From the preliminary GCase inhibition data, the most potent library members have been individually resynthesized for further biological screening and complete characterization. Some of the library members have shown biochemical data (IC_{50}, K_i, and stabilization ratio) similar or superior to those reported for **NNDNJ**. Docking studies have been used to postulate ligand−enzyme interactions to account for the experimental results.



INTRODUCTION

Gaucher disease is one of the most prevalent lysosomal storage disorders characterized by the accumulation of the sphingolipid glucosylceramide (GC) in the lysosomes.[1] The disease is caused by the deficient activity elicited by several mutated forms of the enzyme glucocerebrosidase (GCase, EC 3.2.1.45), a β-glucosidase that hydrolyzes GC into glucose and ceramide (Cer).[2] Cellular levels of the mutated, misfolded enzyme are abnormally low due to its ER-associated degradation (ERAD) mediated by the proteasome instead of proper folding and trafficking to the lysosome.[3,4] Several therapeutic strategies focused on Gaucher disease have been developed over the last years. Classical approaches rely on enzyme replacement and substrate reduction therapies.[5] However, new and promising approaches are starting to emerge in order to circumvent some of the drawbacks associated to the above strategies. Among them, the use of pharmacological chaperones, small-molecule competitive inhibitors of the target enzyme that raise the folded population of the mutant enzyme at subinhibitory concentration by increasing the stability of the enzyme−ligand complex,[6−9] has become an active field of research. This strategy combines the specificity of an enzyme replacement approach with the oral bioavailability and the potential to cross the blood−brain barrier of a small-molecule approach, such as substrate reduction. This is especially important to target CNS variants of the disease for which enzyme replacement approaches are not suitable.[10]

Over recent years, we have worked actively on the development of new aminocyclitols with potential applicability as pharmacological chaperones of GCase. In particular, some symmetric *N*-alkyl substituted *scyllo*-aminoinositols (compounds **28**, **29**, and **30**), closely related to the iminocyclitol family of GCase inhibitors such as *N*-nonyldeoxynojirimycin (**NNDNJ**)[11] (Figure 1), behaved as efficient competitive inhibitors of recombinant GCase[12] and were able to stabilize the enzyme against thermal denaturation.[13] Moreover, some of them were able to increase the activity of GCase in stably transfected cell lines and in patient fibroblasts showing different enzyme mutations.[14]

However, attempts to explore the chemical diversity of the above aminocyclitols by the systematic variation of the amino group functionalization afforded modest results.[15] In addition, an inherent limitation of our reported approach to *N*-substituted

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aminocyclitols lay on the required deprotection (by massive *O*-benzyl removal) of each library member at the last synthetic step, which rendered the process cumbersome. In this context, the Huisgen Cu(I)-catalyzed alkyne−azide cycloaddition (CuAAC, a paradigm of click chemistry) has become a widely used strategy for chemical space exploration in drug design[16,17] Because this Cu-catalyzed process is extremely fast at room temperature and compatible with water or water-rich solvent systems, the use of protecting groups in most synthetic transformations is not required. Interestingly, as the above process can be carried out in water-containing solvent mixtures, it is possible to rapidly assemble combinatorial libraries for direct in situ screening without the need to carry out tedious isolation and purification protocols.[18−20] Moreover, the process can be performed, if required, in a 96-well microtiter plate format for higher flexibility. On the basis of these premises, we started a program aimed at the exploration of the chemical diversity in *N*-substituted aminocyclitols based on the CuAAC click reaction. Preliminary results with a set of aliphatic azides and a series of *N*-(*ω*-alkynyl)alkylaminocyclitols, differing on the length of the alkynyl spacer, unravelled the importance of the correct placement of the 1,2,3-triazole moiety along the aliphatic chain in the resulting systems.[21] The above work set the stage for the development of the protocol here reported, which allowed the construction of a small demonstration library of *N*-substituted aminocyclitols by CuAAC reaction between propargylamino derivative **4** (Scheme 1) and a series of azides [**1**−**25**] (Figure 2). Interestingly, the process does not require the use of protecting groups on the aminocyclitol scaffold. Moreover, because the reaction can be carried out in an aqueous system, the resulting library members can be screened in situ with minimal manipulation. From the preliminary GCase inhibition data, the most potent library members were individually resynthesized for further biological screening and complete characterization.

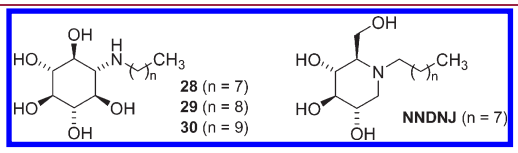

Figure 1. Reported *scyllo*-aminocyclitols **28**−**30** as potential pharmacological chaperones and **NNDNJ**.

Finally, docking studies have been used to postulate ligand−enzyme interactions to account for the experimental results.

LIBRARY DESIGN

Reactants were selected from commercial databases based on diversity criteria as well as synthetic suitability and economic cost. Thus, starting from a collection of 528 commercial alkyl alcohols and bromides, after applying an initial filter to remove duplicates (compounds that would give the same product after conversion to the corresponding azide) as well as highly expensive compounds and/or those with unfavorable properties (for example, unsuitable molecular weight, incompatibility of functional groups with biological matrices or the synthetic sequence), we ended up with a collection of 343 potential reactants. Diversity selection was carried out by enumerating the virtual library of aminocyclitols, calculating a set of standard molecular descriptors, and selecting a diverse subset using an algorithm based on the calculation of the Euclidean distance among compounds in normalized descriptor space and selection of those which are farther apart (see Experimental Section and Supporting Information). To this end, a subset of 25 diverse reactants[22] was selected, which were converted to the corresponding azides [**1**]−[**25**] (Figure 2) following standard protocols.

LIBRARY SYNTHESIS

The aminocyclitols described in this study were obtained by the CuAAC of *N*-(2-propynyl)aminocyclitol **4** with azides [**1**−**25**] in H_2O−THF as solvent system (Scheme 1).

Aminocyclitol **4** was obtained by a straightforward protocol from tetra-*O*-benzyl conduritol B epoxide (**2**), as shown in Scheme 1. Thus, the regio- and diastereoselective opening of epoxide **2** with propargylamine, following our previously reported protocol,[23] afforded aminocyclitol **3**.[21,24] Removal of the *O*-benzyl groups was carried out with BCl_3 at −78 °C similar to or superior than for 3 h, followed by MeOH quench at the same temperature. Low temperature was crucial in this step to obtain the required aminocyclitol **4** in high yields (higher than 90%) and enough purity to be used in the CuAAC click reaction without further manipulation. Initial deprotection attempts following our previously reported conditions (typically, addition BCl_3 acid at −78 °C and further stirring at rt for 24 h)[12] led to complex

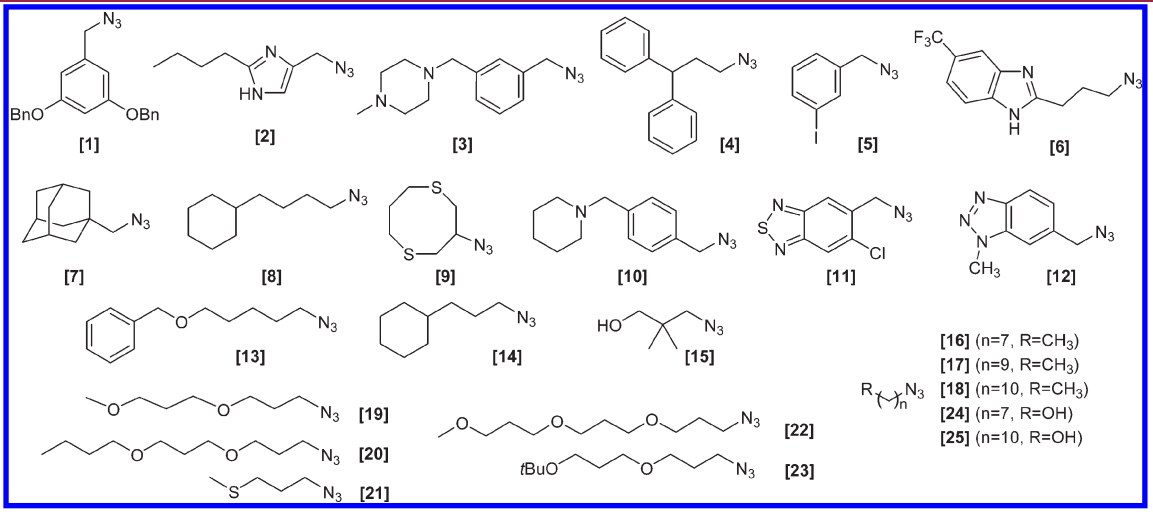

Figure 2. Azides used for CuAAC with aminocyclitol **4**.

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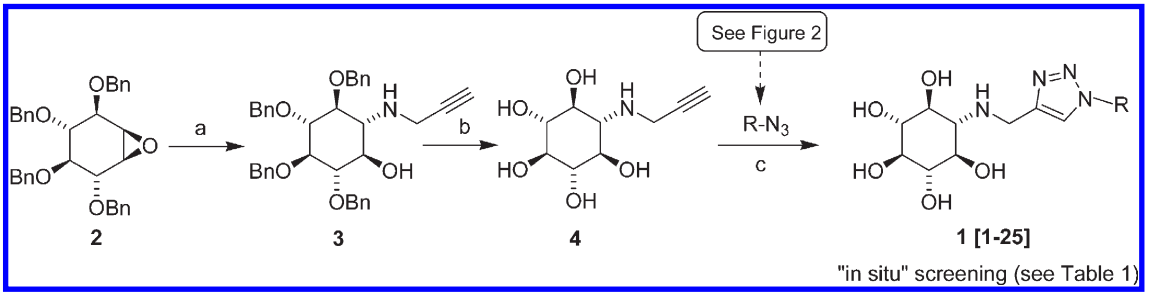
Scheme 1[a]



[a] Reagents and conditions: (a) propargylamine, $LiClO_4$, CH_3CN; (b) BCl_3, CH_2Cl_2 (−78 °C); (c) RN_3 ([**1**−**25**], see Figure 2), $CuSO_4$, sodium ascorbate, H_2O/THF (1:1).

reaction mixtures, probably due to the formation of byproduct arising from insertion of the C−C triple bond into the B−Cl bond of the Lewis acid reagent.[25] Thus, a strict control of the reaction temperature and time was required in order to obtain aminocyclitol **4** efficiently.

Azides [**1**−**25**] were obtained either from commercially available alcohols (by reaction of the corresponding mesylates with NaN_3) or bromides (by direct azide displacement), as described in the Experimental Section.

To set up a protocol amenable to in situ screening, CuAAC were carried out at small scale (typically 10 mg of **4**) and 1.2 equiv/mol of the required azide in 1 mL of a (1:1) H_2O/THF mixture for 24 h at rt to ensure total consumption of the starting alkyne. The identity of the resulting aminocyclitols **1**[**1**−**25**] was confirmed by UPLC-MS analysis of the crude reaction mixtures. These were directly screened as GCase inhibitors and IC_{50} values were determined, as shown in Table 1.

Aminocyclitols with IC_{50} below 3.5 μM were resynthesized independently for complete characterization and further enzymatic studies (see below). In this case, CuAAC reactions with the required azides were carried out from tetra-*O*-benzyl aminocyclitol **3**, followed by massive *O*-benzyl removal by hydrogenolysis over Pd/C catalyst under acidic conditions (Scheme 2).[26] This approach was not suitable for the synthesis of the dibenzyloxy derivative **1**[**1**], which was obtained by reductive alkylation of unprotected *scyllo*-aminocyclitol **6**[12] with aldehyde **27**, following our previously reported protocol.[27] The required aldehyde **27** was obtained, in turn, by Swern oxidation of alcohol **26**, arising from CuAAC reaction of propargyl alcohol with azide [**1**] (Scheme 2).

Table 1. IC_{50} Values (pH = 5.2) of Library Members by in Situ Screening from Crude CuAAC Reaction Mixtures

aminocyclitol[a]	IC_{50} (μM)
1[**1**]	0.10
1[**2**]	237
1[**3**]	66.5
1[**4**]	1.90
1[**5**]	10.3
1[**6**]	12.2
1[**7**]	3.41
1[**8**]	2.30
1[**9**]	35.9
1[**10**]	30.9
1[**11**]	17.3
1[**12**]	114
1[**13**]	10.0
1[**14**]	4.32
1[**15**]	134
1[**16**]	2.25
1[**17**]	0.15
1[**18**]	0.06
1[**19**]	466
1[**20**]	181
1[**21**]	44.5
1[**22**]	453
1[**23**]	325
1[**24**]	5.80
1[**25**]	2.19

[a] See Scheme 1 and Figure 2 for structural details. Compounds with IC_{50} below 3.5 μM were individually synthesized for further studies (see Table 2).

RESULTS AND DISCUSSION

The crude reaction mixtures were tested as inhibitors of recombinant GCase (Imiglucerase, from Genzyme) at the optimum pH for enzyme activity (pH = 5.2), as described in the Experimental Section. Independent blank experiments with the CuAAC reagents ($CuSO_4$ and sodium ascorbate) and each of the reaction partners (azides [**1**−**25**] and aminocyclitol **4**) were also carried out to rule out unspecific effects (See Supporting Information). Results in Table 1 show the corresponding IC_{50} values for the 25 library members. These results were used as criteria for preliminary screening and compound selection.

In agreement with our previous results,[21] compounds bearing a long aliphatic chain at the *N*-triazolyl moiety (**1**[**17**] and **1**[**18**]) showed low IC_{50} values as GCase inhibitors (below 1 μM). Chain length seemed crucial for enzyme inhibition, as evidenced by the around 10 times higher IC_{50} value found for the *n*-octyl derivative **1**[**16**]. In addition, the *N*-alkyl substituent on the triazolyl moiety seems quite sensitive to the presence of polar groups. Thus, both the presence of oxygen or sulfur atoms along the aliphatic chain (compounds **1**[**19**]−**1**[**23**]), as well as the introduction of a terminal hydroxyl group, proved detrimental for enzyme inhibition in comparison with the corresponding hydrocarbon counterparts (see compound **1**[**15**] and compare the pairs **1**[**16**] vs **1**[**24**] and **1**[**18**] vs **1**[**25**]). Among analogues

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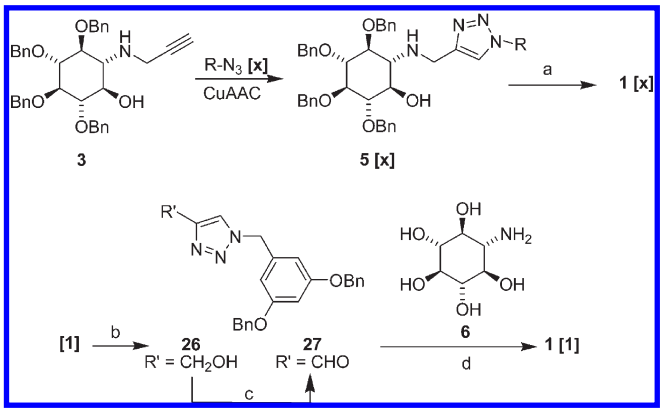
Scheme 2[a]



[a] Reagents and conditions: (a) H_2, Pd/C, MeOH, HCl; (b) propargyl alcohol, CuAAC; (c) Swern oxidation; (d) supported borohydride (see text).

bearing a cycloaliphatic or an aromatic substituent, only **1**[**1**], **1**[**4**], **1**[**7**], and **1**[**8**] showed IC_{50} values below 3.5 μM, which was set as the threshold for further studies. The high inhibitory activity of **1**[**1**] (bearing a bulky aromatic 3,5-dibenzyloxybenzyl group) is remarkable, because this compound turned out to be the most potent of the library, not only as GCase inhibitor (K_i = 0.05 μM, Table 2) but also as in vitro pharmacological chaperone by its ability to stabilize GCase after thermal denaturation (see below). A still remarkable activity profile was found for the related analogue **1**[**4**] (K_i = 0.77 μM, Table 2), whose 3,3-diphenylpropyl group adopts a spatial disposition when bound to GCase close to that observed for **1**[**1**] (see Supporting Information). A similar activity profile was found for **1**[**8**] (R = 4-cyclohexylbutyl), whereas IC_{50} for the adamantylmethyl derivative **1**[**7**] was only slightly below the established threshold.

As in the aliphatic series, compound **1**[**14**], a lower homologue of **1**[**8**], showed a reduced inhibitory activity (IC_{50} around 4 μM) and was thus disregarded. Library members arising from CuAAC between azides [**3**], [**5**], [**6**], [**9**], [**10**], [**11**], and [**13**], and alkyne **4** (Scheme 1) showed IC_{50} values between 10 and 70 μM and were also disregarded, as well as those arising from azides [**2**], [**12**], and [**15**], with IC_{50} values above 100 μM. From a structural standpoint, this set of compounds is characterized by possessing a short aliphatic chain connecting the triazole moiety with a densely heteroatom-substituted terminal aromatic or cycloaliphatic group (see Computational Studies).

Compounds with IC_{50} values below 3.5 μM were individually synthesized, as depicted in Scheme 2. To our delight, IC_{50} values (determined at the enzyme optimal pH = 5.2) were consistent with those determined from the crude CuAAC reaction mixtures, confirming the reliability of this approach as a tool for preliminary screening of GCase inhibitors. Interestingly, IC_{50} values at pH = 7.4 (Table 2) were similar to, or even better (as for **1**[**8**]) to those found at pH = 5.2, which indicates the ability of these compounds to interact with the target enzyme at the neutral pH of the ER.

Inhibition constant (K_i) and GCase stabilization after in vitro thermal denaturation were also determined for the aminocyclitols shown in Table 2. This property is generally used as an in vitro model to evaluate the potential of the tested compounds as pharmacological chaperones.[11] For comparative purposes, the stabilization ratio (SR) has been used as a measure of GCase stabilization. This parameter is defined as the ratio of the relative enzymatic activities (with vs without inhibitor) at a given inhibitor concentration and incubation time. The relative enzymatic activity is obtained, in turn, by comparing the enzyme activity at 48 °C for a given aminocyclitol concentration and incubation time with that of a control assay at 37 °C (a nondenaturalizing temperature) under otherwise identical conditions. Relative activities for selected aminocyclitols (those with K_i values around or lower than 1.0 μM) at different concentrations and incubation times are plotted in Figure 3, while SR values at 100 μM aminocyclitol concentration and 60 min incubation time are recorded in Table 2. For the matter of comparison, **NNDNJ** and **30**[13] (Figure 1) were also tested under the same experimental conditions.

Inspection of Figure 3 reveals that the 3,3-diphenylpropyl and the adamantylmethyl derivatives (compounds **1**[**4**] and **1**[**7**], respectively) behave as weak thermal stabilizers at high concentration (150 μM), while they act mainly as enzyme inhibitors at lower concentrations. According to our previous findings, the lipophilicity of the *N*-alkyltriazolyl moiety seems crucial in this kind of compound, as evidenced by comparison of the activity profiles of the *N*-octyl (**1**[**16**]), the *N*-decyl (**1**[**17**]),[21] and the *N*-undecyl (**1**[**18**]) derivatives as GCase and GCS inhibitors (Table 2). In agreement with this observation, the increase of two methylene units (moving from **1**[**16**] to **1**[**18**]) led to a

Table 2. GCase Inhibitory Activity, Thermal Stabilization, and GCS Inhibition of Selected Compounds

aminocyclitol	IC_{50} (μM)[a]	IC_{50} (μM)[b]	K_i (μM)[c]	stabilization ratio (SR)[d]	% inhibition GCase[e]	% inhibition GCS[f]
1[**1**]	0.05	0.06	0.05	31.2	93	9
1[**4**]	1.5	1.8	0.77	0.5	73	18
1[**7**]	2.2	1.9	1.05	0.7	63	32
1[**8**]	1.5	0.30	0.29	7.5	30	8
1[**16**][g]	1.0	1.1	1.84	1.0	20	0
1[**17**][g]	0.10	0.09	0.09	21.0	90	0
1[**18**]	0.12	0.10	0.05	30.5	97	29
1[**25**]	1.8	1.3	0.89	6.9	28	28
30	1.8[h]	(nd)	0.30[h]	4.9	nd[l]	nd
NNDNJ	1.30	0.30	0.30[i]	9.2	nd	30[j] 40[k]

[a] GCase inhibition at pH = 5.2. [b] GCase inhibition at pH = 7.4. [c] GCase competitive inhibitors (pH = 5.2). [d] Relative enzymatic activities (inhibitor vs control) at 100 μM inhibitor and 60 min incubation time. For values at different inhibitor concentration, see Figure 3 and Supporting Information. [e] In intact fibroblasts at 50 μM aminocyclitol. [f] Determined in cell lysates using CerNBD as substrate at 250 μM inhibitor, see Supporting Information for details. [g] Data from ref 21. [h] See ref 13. [i] See ref 32. [j] 10 μM inhibitor. [k] 50 μM inhibitor. [l] not determined.

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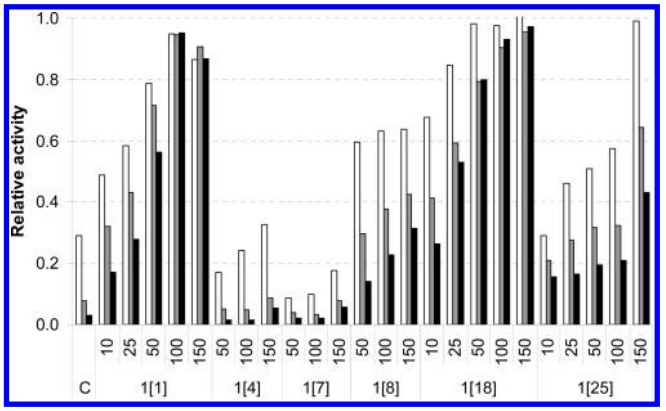

Figure 3. Relative enzymatic activity after thermal denaturation (48 °C) for: 20 min (white bar), 40 min (gray bar), and 60 min (black bar) at the indicated aminocyclitol concentrations (μM), compared to the corresponding assay at 37 °C. C: control without inhibitor.

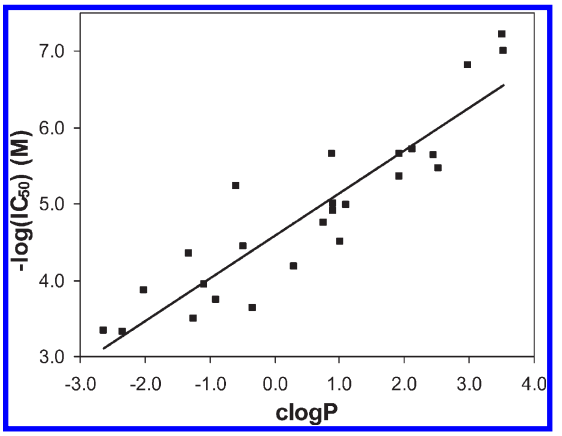

Figure 4. GCase inhibitory activities from Table 1, expressed as $-\log(IC_{50})$, vs predicted clogP values (see Supporting Information) for aminocyclitols **1**[**1**]−[**1**[**25**].

significant SR enhancement (Table 2 and Figure 3). On the other hand, introduction of a polar substituent in the aliphatic chain, as in the *N*-(10-hydroxydecyl)triazolyl derivative **1**[**25**], led to a substantial decrease of both the relative enzyme activity and the SR in comparison with the nonhydroxylated counterpart **1**[**17**]. Very interestingly, the *N*-[3,5-bis(benzyolxy)benzyl] derivative **1**[**1**] turned out to be the best compound of the library according to its relative enzymatic activity and enzyme thermal stabilization. In addition, it is a weak GCS inhibitor, a strong in vivo GCase inhibitor (intact human wt fibroblasts) (Table 2), noncytotoxic (MTT test),[28] and totally devoid of activity against a panel of commercial glycosidases.[29]

COMPUTATIONAL STUDIES

We have previously reported that the GCase inhibition potency of a related series of aminocyclitols showed a clear dependence on their hydrophobicity, expressed as clogP.[21] A similar correlation is also observed for aminocyclitols **1**[**1**]−**1**[**25**] (Figure 4), supporting our previous hypothesis about the importance of hydrophobicity for GCase inhibitory activity. However, other factors like the relative position of the triazole ring with respect to the aminocyclitol moiety appeared to influence the potency of these compounds because analogues differing only on the length of the alkyl chain linker between the amino and triazole groups showed large differences in activity, greater than 100-fold in some cases, despite their very similar clogP values.

Docking studies with our previous collection of compounds allowed us to propose two possible binding modes into the active center of GCase, which arise from two slightly different structures for the protein (PDB codes 2V3E and 2NSX).[21] These two structures differ on their surface topology close to the entrance to the active site. Thus, on 2V3E the access to a hydrophobic groove between loops 1 and 3 (L1 and L3) is blocked while on 2NSX it is open (Figure 5). Docking studies with the above two GCase structures have now been extended to aminocyclitols **1**[**1**]−**1**[**25**] to determine their potential binding modes.

Parts A and B of Figure 5 show the best docked poses obtained for compounds **1**[**1**]−**1**[**25**] using GCase structures 2V3E and 2NSX, respectively, as targets. As determined for the previous collection of analogue aminocyclitols, most of the compounds in this work showed different binding modes depending on which structure was used as docking target. Hence, all the compounds could place their cyclitol moiety inside the active site cavity of both GCase structures, establishing hydrogen bond interactions mainly with residues D127, W179, E235, W381, and N396, and most of them exhibit an additional hydrogen bond between the protonated secondary amino group and residue E235. Poses obtained from docking to the 2V3E structure show the triazole-attached substituent occupying the valley between loops L1−L2 and L3−L4, while those from docking to 2NSX show that the substituent of the triazole is oriented toward or inside the narrow groove between loops L1 and L3. These differences are more noticeable for the compounds with linear chains, ie. **1**[**16**]−**1**[**25**], than for those with smaller or more rigid substituents on the triazole ring, such as **1**[**1**]. Thus, the docked poses obtained for compound **1**[**1**] from the two GCase targets (Figure 5C,D) suggest that the two aromatic "arms" of the 3,5-dibenzyloxybenzyl substituent can establish hydrophobic interactions with the side chains of residues in loops L1 (F347, W348) and L3 (W312, L314, and F316). Similar arrangements were observed for the less active compounds **1**[**4**], **1**[**7**], and **1**[**8**] (see Supporting Information), while the long and linear chains of the also active **1**[**16**], **1**[**17**], **1**[**18**], and **1**[**25**] were located in the valleys between loops L3 and L4 or L1 and L3, depending on which target structure was used (see Supporting Information and ref 21). Although the docking results for the rest of the less, or much less, active compounds suggest that these could adopt bound conformations which are similar to those of the active compounds, most of them exhibit a lower hydrophobicity (clogP < 1.0) that probably reduces their capacity to efficiently interact with the above-mentioned hydrophobic residues on loops L1 and L3, as well as others (L240, L241, L286, L287) located near the entrance to the catalytic site. Concerning the role of the triazole ring on the binding to GCase, the present docking results do not provide a clear answer. However, as it was previously observed,[21] the docked poses obtained for some of the compounds with structure 2NSX (i.e. **1**[**7**], see Figure S2D of Supporting Information) show the triazole ring in an orientation in which one of the nitrogen atoms forms a hydrogen bond with residue Q284, while other poses suggest that it could establish a stacking interaction with Y313 (i.e. **1**[**1**], Figure 5F). Alternatively, from docking with 2V3E, a hydrogen bond with Y244 can be inferred (i.e. compounds **1**[**8**] and **1**[**18**], see Figure S2E,G of Supporting Information). Hence, the present results obtained from a larger variety of compounds, suggest that the contribution of the triazole ring, being located just outside of the tight cyclitol

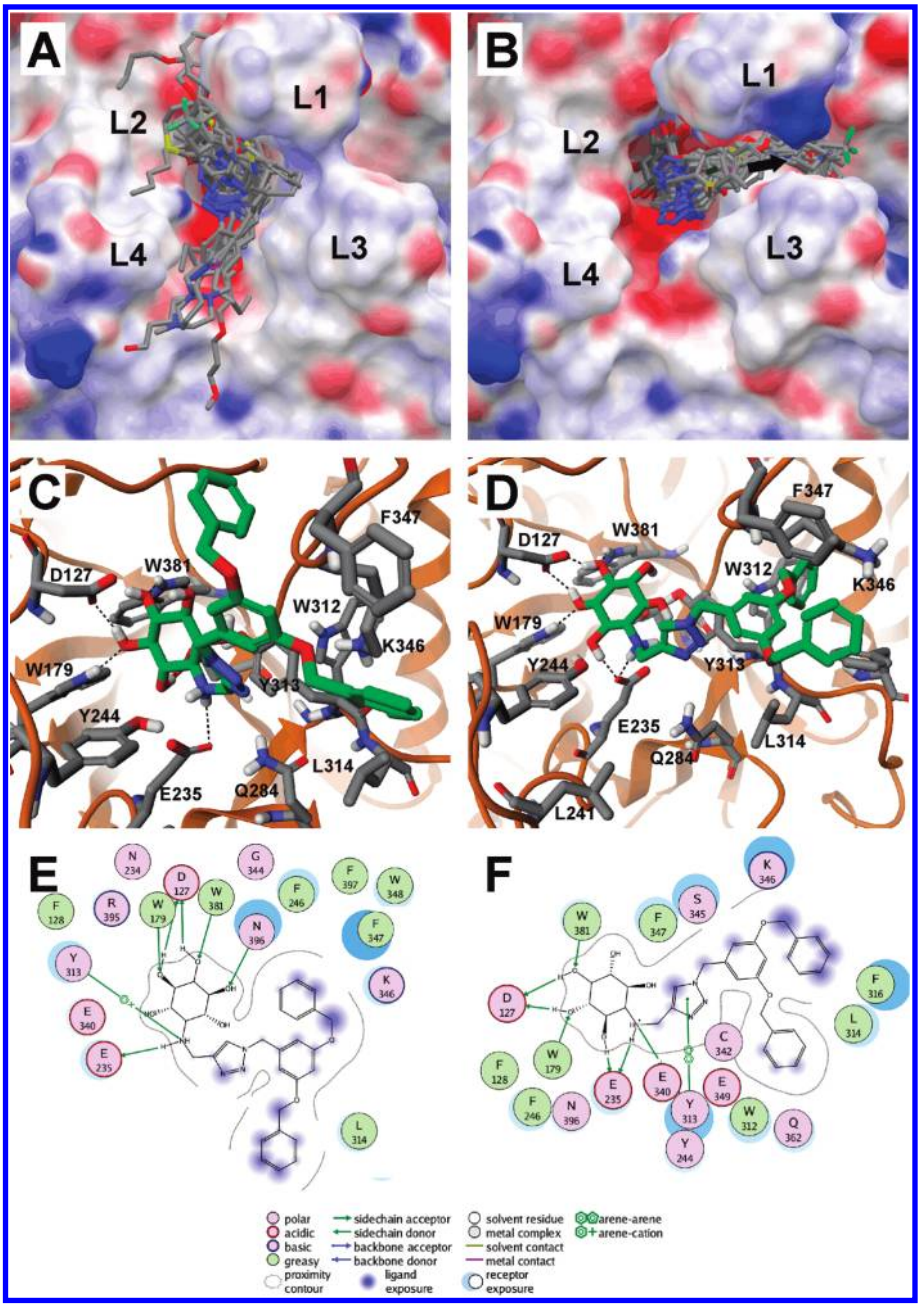

Figure 5. Best poses obtained for aminocyclitols **1**[**1**]−**1**[**25**] docked against GCase structures 2V3E (A) and 2NSX (B). L1−L4 denote the position of the four loops that constitute the entrance to the GCase active site.[21] In (B), the black arrow shows the accessible groove between loops L1 and L3. Detail of the best poses obtained for compound **1**[**1**] docked against 2V3E (C) and 2NSX (D), and corresponding interaction diagrams (E and F, respectively) that show strong hydrogen bond interactions between the cyclitol hydroxyl groups and the side chains of residues D127, W179, E235, W381, and N396, as well as between the protonated amino group and residue E235. The positive charge on this group is also stabilized by a π−cation interaction with the aromatic ring of residue Y313.

binding cavity, might depend on the nature of the substituent and its capacity to interact with one or another region on the enzyme surface. Studies to further elucidate the role of the triazole moiety in this kind of aminocyclitols are currently underway and will be reported in due course.

CONCLUSION

A click chemistry approach based on the CuAAC reaction between *N*-propargyl aminocyclitol **4** and a series of azides has been optimized for the synthesis and in situ screening of a variety of *N*-substituted aminocyclitols as GCase inhibitors. The highly aqueous content of the reaction media makes possible the direct screening of the products by simple dilution of the crude reaction mixtures in the adequate buffer. The process is suitable for its implementation into a microtiter plate format and, hence, amenable to combinatorial protocols. In this work, a small demonstration library of 25 compounds has been produced in order to test the potential of this approach. Maximum diversity among the library members has been secured by application of a proper selection algorithm from an initial collection of 343 compounds. Library members with IC_{50} values below 3.5 μM

were individually synthesized and tested as GCase inhibitors and also for their ability to induce enzyme thermal stabilization, an in vitro indication of their potential as pharmacological chaperones. Activity values of individually synthesized compounds were consistent with those determined from the crude CuAAC reaction mixtures, confirming the reliability of this approach as a tool for preliminary screening of GCerase inhibitors. Noteworthy, the highly lipophilic compounds **1**[**1**], with two aromatic "arms", and **1**[**18**], with a linear aliphatic side chain, showed the highest enzyme stabilization ratios and are promising candidates for further development. In summary, this approach has shown to be very effective and simple for the rapid identification of selective, potent GCase inhibitors within the aminocyclitol family of compounds.

EXPERIMENTAL SECTION

Library Design. Electronic collections of alkyl alcohols and bromides (R-OH and R-Br) were obtained as sdf formatted files from the web pages of commercial brands. The initial collection of reactants contained 528 compounds, which were processed in steps with the program MOE (version 2008.10, Chemical Computing Group, Montreal) by (1) converting them into 3D structures, (2) clipping the leaving groups (OH or Br) and keeping the R-groups, (3) removing duplicate R-groups, and (4) filtering undesired R-groups according to a predefined set of rules (see Supporting Information). An additional filtering step was manually carried out to remove compounds unsuitable from the synthetic point of view or with too high price. In this way, a library of 343 R-groups (reactants) was obtained which was used to enumerate the virtual library of aminocyclitols. The structures of the compounds in this aminocyclitol library were energy minimized using the implemented MMFF94x force field, a modified version of the MMFF94s force field.[30,31] The library was characterized by calculating a set of 47 standard molecular descriptors including different physicochemical properties, as well as spatial, topological, and information content indices (see Supporting Information). Principal component analysis (PCA) showed that the first three most important components accounted for 73% of the variance, while up to nine components were required to account for more than 90% of the variance (see Supporting Information). The Diverse Subset module implemented in MOE, which relies on the calculation of the Euclidean distance among compounds in normalized descriptor space, was used to carry a diverse selection of 30 compounds from the virtual library of aminocyclitols. However, synthetic problems with some of them forced us to modify the original selection to the final collection of reactants that was used to obtain azides [**1**]−[**25**]. The diversity of this final subset was assessed by cell-based methods, both in terms of space coverage (number of cells covered by selected subset/total number of cells) and population coverage (total number of compounds included in represented cells/total number of molecules in library). Hence, binning the diversity space into 27 clusters (closest possible number of bins to the number of selected compounds) that arise from subdividing the space into three equiprobable regions for each of the three most important principal components, the 25 aminocyclitols resulting from the selected subset of reactants, representing only a 7.3% of the possible products, achieved a 52% space coverage (14 clusters occupied) and 53% population coverage (183 compounds represented).

Docking. Computational docking of compounds **1**[**1**]−**1**[**25**] against GCase structures 2V3E and 2NSX was performed as previously described.[21]

Chemistry. Solvents were distilled prior to use and dried by standard methods. FT-IR spectra are reported in cm^{-1}. ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ solutions at 500 MHz (for ^1H) and 100 MHz (for ^{13}C), respectively, unless otherwise indicated. Chemical shifts (δ) are reported in ppm relative to the solvent ($CDCl_3$) signal. In cases where triazole carbon atoms in compounds 1[x] were not apparent, they were indirectly assigned by a combination of 2D experiments (gHSQC and gHMBC) by means of long-range scalar coupling through two or three chemical bonds of the CH_2 groups adjacent to the triazole ring. ESI/HRMS spectra were recorded on a Waters LCT Premier mass spectrometer. Parallel syntheses of azides were carried out in a 12-position Carousel reaction station under inert atmosphere at low temperature, when required. Azide purifications were carried out in a 12-position parallel purification system. Parallel click chemistry reactions were carried out in 5 mL screw cap vials, provided with a small magnetic bar, and placed in 24 position reaction blocks. Parallel evaporations were carried out in a centrifugal evaporator. For complete characterization of azides [**1**]−[**25**], see Supporting Information. Purity of compounds used for enzyme assays was ≥95%, as judged by HPLC analysis (Gemini C18 column, 4.6 mm × 250 mm, 5 μm) under the following chromatographic conditions: mobile phase A, water containing 0.1% v/v trifluoroacetic acid; mobile phase B, ACN containing 0.1% v/v trifluoroacetic acid; flow rate of 1.0 mL/min; detection, SATIN-ELS (evaporative light scattering) (λ = 254 nm); gradient elution, 0 min, from 80% A/20% B to 0% A/100% B over next 20 min. Each run was followed by a 3 min wash with 80% ACN, 20% water.

*(1R,2S,3r,4R,5S,6s)-6-(Prop-2-ynylamino)cyclohexane-1,2,3,4,5-pentaol (**4**).* A solution of aminocyclitol **3**[21] (100 mg, 0.17 mmol) in CH_2Cl_2 (20 mL) is cooled to −78 °C under Ar and treated with 1 M BCl_3 solution in heptane (1.8 mL, equivalent to 1.8 mmol). After stirring for 3 h at −78 °C, the reaction was quenched by addition of MeOH (4 mL), previously cooled to −78 °C, and allowed to warm to rt with stirring. Solvents were removed under reduced pressure in a centrifugal evaporator. EtOAc (3 mL) was next added to the residue, and the resulting mixture was sonicated until precipitation of a white solid. This was collected by filtration, thoroughly washed with additional EtOAc (3 × 2 mL), and dried to afford the final aminocyclitol **4**·**HCl** (35 mg, 93% yield). ^1H NMR (500 MHz, CD_3OD), δ (ppm) = 4.20 (d, 2H, J = 2.4 Hz) 3.56 (dd, 2H, J = 9.1 Hz, J = 10.4 Hz) 3.29 (t, 2H, J = 9.2 Hz) 3.23 (m, 2H) 3.15 (t, 1H, J = 10.7 Hz). ^{13}C NMR (100 MHz, CD_3OD), δ (ppm) = 79.05, 76.62, 76.55, 75.18, 75.13, 71.00, 70.50, 62.41, 36.94. IR: 2128 cm^{-1} (C≡C). HRMS: calcd for $C_9H_{16}NO_5$ ($M + H^+$), 218.1028; found, 218.1045

General Method for Parallel Click Chemistry and in Situ Screening. A solution of alkyne **4** (10 mg, 0.046 mmol) and azide [**1**]−[**25**] (0.055 mmol) in a 1:1 mixture of H_2O/THF (1 mL) in a 5 mL screw cap vial was treated with a catalytic amount of $CuSO_4 \cdot 5H_2O$ (around 250 μg) followed by sodium ascorbate (5 μmol, around 1 mg). After stirring for 24 h at rt, an aliquot was analyzed (UPLC-HRMS) to confirm click adduct formation (see Supporting Information). The crude reaction mixtures containing compounds **1**[**1**−**25**] were directly used as mother solutions (46 mM) for in vitro IC_{50} calculation of GCase inhibition, as described above (for results, see Table 1).

Synthesis of Individual Aminocyclitols. *(1RS,2SR,3RS,4RS,5SR,6RS)-2,3,4,5-Tetrakis(benzyloxy)-6-(prop-2-ynylamino)cyclohexanol (**3**).* A solution of the starting epoxide **2** (500 mg, 0.96 mmol) in CH_3CN (10 mL) was slowly added dropwise under argon at rt over previously dried $LiClO_4$ (2.47 g, 23.2 mmol). A solution of 9.6 mmol (10 equiv/mol) of the required amine in CH_3CN (2 mL) was next added and the reaction mixture was stirred at 80 °C under Ar. After 18 h, the reaction mixture was cooled to rt, quenched with H_2O (10 mL), extracted with CH_2Cl_2 (3 × 20 mL), and dried over anhydrous Mg_2SO_4. Filtration and evaporation afforded crude aminocyclitols (3:1 mixture of diastereomers), which were purified by filtration through a plug of silica on elution with hexanes/EtOAc (6:4) to afford aminoalcohol **5** (435 mg, 79% yield). ^1H NMR: 7.36 (m, 20H), 4.95 (m, 5H), 4.82 (dd, 1H, J = 3.5 Hz, J = 11.1 Hz), 4.76 (d, 1H, J = 11.2 Hz), 3.62 (m, 2H), 3.46 (m, 3H), 2.71 (dt, 1H,

J = 1.8 Hz, J = 9.9 Hz), 2.28 (d, 1H, J = 2.1 Hz). ^{13}C NMR: 127.6−128.5, 84.52, 83.67, 83.04, 81.91, 75.24−75.78, 73.13, 71.51, 60.08, 37.53. HRMS: calcd for $C_{37}H_{40}NO_5$ (M + H^+), 578.2906; found, 578.2929.

Click Chemistry Reaction of ω-Alkynyl Aminocyclitol 3 with Selected Azides. To a solution of **3** (50 mg, 0.23 mmol) and the selected azide (0.28 mmol) in 1:1 H_2O/THF (3 mL) in a screw cap glass vial, a catalytic amount of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate (0.023 mmol, 4.6 mg) were added. After stirring at rt for 10 min, the reaction mixture was diluted with EtOAc (10 mL). The organic phase was collected, dried over $MgSO_4$, and worked up as usual to give a residue which was purified by flash chromatography on elution with hexanes−EtOAc (from 1:1 to 0:1) to afford the corresponding cycloaddition adducts **5**.

*(1RS,2SR,3RS,4RS,5SR,6RS)-2,3,4,5-Tetrakis(benzyloxy)-6-[[1-(3,3-diphenylpropyl)-1H-1,2,3-triazol-4-yl]methylamino]cyclohexanol (**5**[**4**]).* Obtained in 76% yield. ^{1}H NMR: 7.33 (m, 20H, 10H, 1H) 4.94 (m, 8H) 4.27 (s, 2H) 4.09 (m, AB system, 2H) 3.93 (t, 1H, J = 7.6 Hz) 3.67 (m, 5H) 2.68 (m, 2H, 1H). ^{13}C NMR: 143.24, 138.71−138.36, 126.88−128.84, 121.60, 80.72- 84.70, 73.01, 62.49, 48.25, 35.90. HRMS: calcd for $C_{52}H_{55}N_4O_5$ (M + H^+), 815.4172; found, 815.4197.

*(1RS,2SR,3RS,4RS,5SR,6RS)-2,3,4,5-Tetrakis(benzyloxy)-6-[[1-adamantyl-1H-1,2,3-triazol-4-yl]methylamino]cyclohexanol (**5**[**7**]).* Obtained in 92% yield. ^{1}H NMR: 7.34 (m, 20H, 1H) 4.90 (m, 8H) 4.05 (m, 2H, 2H) 3.57 (m, 5H) 2.66 (m, 1H) 1.99 (m, 3H) 1.64 (m, 6H,) 1.49 (m, 6H). ^{13}C NMR: 138.76−138.37, 128.63−127.76, 121.32, 84.82, 84.20, 83.11, 81.88, 75.99, 75.59, 73.05, 62.33, 40.32, 36.60, 28.16. HRMS: calcd for $C_{48}H_{57}N_4O_5$ (M + H^+), 769.4330; found, 769.4352.

*(1RS,2SR,3RS,4RS,5SR,6RS)-2,3,4,5-Tetrakis(benzyloxy)-6-[[1-(4-cyclohexylbutyl)-1H-1,2,3-triazol-4-yl]methylamino]cyclohexanol (**5**[**8**]).* Obtained in 87% yield. ^{1}H NMR: 7.41 (m, 20H, 1H) 4.93 (m, 8H) 4.31 (t, 2H, J = 6.7 Hz) 4.13 (m, AB system, 2H) 3.63 (m, 5H) 2.71 (m, 1H) 1.87 (m, 2H) 1.69 (m, 4H) 1.24 (m, 9H) 0.89 (m, 2H). ^{13}C NMR: 146.52, 138.70−138.35, 127.61−128.74, 121.47, 84.71, 84.06, 83.05, 81.62, 75.90, 75.58, 72.92, 61.53, 50.41, 43.15, 37.48, 36.88, 33.37, 30.64, 26.71, 26.41, 23.87. HRMS: calcd for $C_{47}H_{59}N_4O_5$ (M + H^+), 759.44850; found, 759.4548.

*(1RS,2SR,3RS,4RS,5SR,6RS)-2,3,4,5-Tetrakis(benzyloxy)-6-[[1-undecyl-1H-1,2,3-triazol-4-yl]methylamino]cyclohexanol (**5**[**18**]).* Obtained in 83% yield. ^{1}H NMR: 7.38 (m, 20H, 1H) 4.94 (m, 8H) 4.30 (t, 2H, J = 7.2 Hz) 4.08 (m, AB system, 2H, J = 13.6 Hz) 3.57 (m, 5H) 2.67 (m, 1H) 1.87 (m, 2H) 1.21 (m, 16H) 0.90 (t, 3H, J = 6.8 Hz) ^{13}C NMR: 147.20, 138.87−138.54, 128.77−127.91, 121.32, 84.91, 84.29, 83.26, 82.03, 76.11, 76.08, 75.71, 73.21, 61.72, 50.54, 43.28, 32.14, 30.54, 29.81, 29.80, 29.66, 29.56, 29.28, 26.76, 22.93, 14.40. HRMS: calcd for $C_{48}H_{63}N_4O_5$ (M + H^+), 775.4798; found, 775.4825.

*(1RS,2SR,3RS,4RS,5SR,6RS)-2,3,4,5-Tetrakis(benzyloxy)-6- [[1-(10-hydroxydecyl)-1H-1,2,3-triazol-4-yl]methylamino]cyclohexanol (**5**[**25**]).* Obtained in 85% yield. ^{1}H NMR: 7.38 (m, 20H, 1H) 4.91 (m, 8H) 4.30 (t, 2H, J = 7.2 Hz) 4.08 (m, AB system, 2H, J = 14.1 Hz) 3.59 (m, 7H) 2.67 (m, 1H) 1.87 (m, 2H) 1.56 (m, 2H) 1.21 (m, 12H). ^{13}C NMR: 147.06, 138.40−138.73, 127.77−128.64, 121.21, 84.77, 84.15, 83.12, 81.90, 75.98, 75.94, 75.88, 73.06, 63.01, 61.62, 50.36, 43.18, 32.81, 30.32, 29.42, 29.36, 29.29, 28.95, 26.46, 25.78. HRMS: calcd for $C_{47}H_{61}N_4O_6$ (M + H^+), 777.4591; found, 777.4620.

Synthesis of Aminocyclitol 1[1]. *a. Huisgen Cycloaddition between Azide [**1**] and Propargyl Alcohol: Synthesis of [1-[3,5-Bis-(benzyloxy)benzyl]-1H-1,2,3-triazol-4-yl]methanol (**26**).* To a solution of propargyl alcohol (65 mg, 1 mmol) and azide [1] (400 mg, 1.16 mmol) in 1:1 H_2O/THF (4 mL) in a screw cap glass vial, a catalytic amount of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate (20 mg, 0.1 mmol) were added. After stirring at rt for 24 h, the reaction mixture was diluted with EtOAc (10 mL). The organic phase was collected, dried over $MgSO_4$, and evaporated to give alcohol **26** in quantitative yield as an orange solid, which was used in the next step without further purification. ^{1}H NMR: 7.41 (m, 10H, 1H) 6.61 (t, 1H, J = 2.1 Hz) 6.50 (d, 2H, J = 2.0 Hz) 5.44 (s, 2H) 5.02 (s, 4H) 4.79 (s, 2H). ^{13}C NMR: 160.54, 136.51, 128.79, 128.28, 127.68, 107.37, 102.26, 70.30. HRMS: calcd for $C_{24}H_{23}N_3O_3Na$ (M + H^+), 424.1637; found, 424.1627.

*b. Swern Oxidation of **26**: Synthesis of 1-[3,5-Bis(benzyloxy)benzyl]-1H-1,2,3-triazole-4-carbaldehyde (**27**).* A solution of alcohol **26** (100 mg, 0.25 mmol) in EtOAc (10 mL) was treated with IBX (203 mg, 0.75 mmol). After stirring at reflux temperature for 4 h, the reaction mixture was allowed to cool to rt and filtered over a pad of Celite, which was thoroughly washed with EtOAc. The combined organic phases were evaporated to dryness to give an oil, which, after hexane washings, afforded aldehyde **27** as a white solid in quantitative yield. ^{1}H NMR: 10.14 (s, 1H) 7.99 (s, 1H) 7.37 (m, 10H) 6.64 (t, 1H, J = 2.2 Hz) 6.51 (d, 2H, J = 2.2 Hz) 5.49 (s, 2H) 5.02 (s, 4H). ^{13}C NMR: 185.14, 160.68, 148.09, 136.34, 135.51, 128.79, 128.33, 127.64, 125.31, 107.59, 102.60, 70.34, 54.68.

*c. Reductive Alkylation of Aminocyclitol **6** with Aldehyde **27**: Synthesis of (1R,2S,3r,4R,5S,6s)-6-[(1-(3,5-Bis(benzyloxy)benzyl)-1H-1,2,3-triazol-4-yl)methylamino]cyclohexane-1,2,3,4,5-pentaol (**1**[**1**]).* To a screw cap glass vial, a solution of aminocyclitol **6**[12] (15 mg, 84 μmol) in DMSO (3.5 mL), followed by AcOH (70 μL) and 22 mg (55 μmol) of aldehyde **27** at rt, was added. After shaking for 1 h, supported cyanoborohydride (150 mg, equivalent to 300 μmol) was added portionwise and the mixture was shaken for additional 12 h, followed by addition of Wang resin (15 mg, equivalent to 45 μmol) and further shaking of the resulting slurry for 12 h. Resins were filtrated and thoroughly washed with MeOH/H_2O (9/1 mixture). The combined filtrates were evaporated to dryness and the residue taken up in 9/1 MeOH/H_2O (3 mL), treated with carbonate resin (500 mg, equivalent to 1.75 mmol) and Wang resin (15 mg, equivalent to 45 μmol), and stirred for 4 h. Resins were filtrated, washed with MeOH/H_2O (9/1 mixture), and evaporated to dryness to give a residue, which was purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH from 94:5:1 to 83:17:1). Aminocyclitol **1**[**1**] (12 mg, 25% yield) was obtained. ^{1}H NMR (500 MHz, CD_3OD): 8.02 (s, 1H) 7.40 (m, 10H) 6.63 (m, 1H) 6.59 (d, 2H, J = 1.8 Hz) 5.54 (s, 2H) 5.04 (s, 4H) 4.42 (m, AB system, 2H) 3.48 (t, 2H, J = 9.6 Hz) 3.24 (m, 3H,) 2.88 (t, 1H, J = 10.5 Hz). ^{13}C NMR (100 MHz, CD_3OD): 161.79, 151.35 (C_q triazole, 2D assignment), 138.70, 138.35 (CH triazole, 2D assigment), 129.54, 128.98, 128.64, 125.93, 108.43, 102.93, 76.69, 75.31, 71.98, 71.12, 62.93, 54.96, 42.31. HRMS: calcd for $C_{30}H_{35}N_4O_7$ (M + H^+), 563.2506; found, 563.2511.

Debenzylation of Aminocyclitols 5 by Catalytic Hydrogenation. In a typical reaction, each of the above aminoalcohols (0.2 mmol) was dissolved in a mixture of THF (5 mL), concentrated HCl (few drops), and 50 mg of 10% Pd/C in a glass pressure flask. The flask was repeatedly filled and evacuated with hydrogen and vigorously stirred at room temperature for 24 h under H_2 (1.5 atm). The reaction mixture was next filtered through a plug of Celite, which was washed with 3 × 2 mL THF/MeOH (1:1). The combined filtrates and washings were concentrated to give aminocyclitols **1** as the corresponding hydrochloride salts.

*(1R,2S,3r,4R,5S,6s)-6-[[1-(3,3-Diphenylpropyl)-1H -1,2,3-triazol-4-yl]methylamino]cyclohexane-1,2,3,4,5-pentaol (**1**[**4**]).* Obtained from **5**[4] in quantitative yield. ^{1}H NMR (500 MHz, CD_3OD): 8.07 (s, 1H) 7.29 (d, 8H, J = 4.3 Hz) 7.19 (m, 2H) 4.57 (s, 2H) 4.38 (m, AB system, 2H) 3.94 (t, 1H, J = 7.9 Hz) 3.58 (t, 2H, J = 9.7 Hz) 3.23 (m, 3H) 3.08 (t, 1H, J = 10.7 Hz) 2.71 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): 151.52 (C_q triazole, 2D assignment), 144.97, 140.61 (CH triazole, 2D assigment), 139.2, 129.73, 128.84, 127.65, 76.58, 75.16, 70.67, 62.58, 50.16, 41.51, 36.80 (one carbon around 50, obscured by the solvent signal). HRMS: calcd for $C_{24}H_{31}N_4O_5$ (M + H^+), 455.2294; found, 455.2306.

*(1R,2S,3r,4R,5S,6s)-6-[(1-Adamantyl-1H-1,2,3-triazol-4-yl)methylamino]cyclohexane-1,2,3,4,5-pentaol (**1**[**7**]).* Obtained from **5**[7] in quantitative yield. ^{1}H NMR (500 MHz, CD_3OD): 8.10 (s, 1H) 4.62 (s, 2H) 4.15 (m, AB system, 2H) 3.61 (t, 2H, J = 9.5 Hz) 3.28 (m, 3H) 3.09

(t, 1H, J = 10.6 Hz) 2.01 (m, 3H) 1.58 (m, 6H) 1.71 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD): 152.52 (C_q triazole, 2D assignment), 138.41 (CH triazole, 2D assigment), 76.63, 75.14, 70.67, 62.52, 41.15, 37.62, 29.61. HRMS: calcd for $C_{20}H_{33}N_4O_5$ ($M + H^+$), 409.2452; found, 409.2451.

*(1R,2S,3r,4R,5S,6s)-6-[[1-(4-Cyclohexylbutyl)-1H-1,2,3-triazol-4-yl]methylamino]cyclohexane-1,2,3,4,5-pentaol (**1**[**8**]).* Obtained from **5**[**8**] in quantitative yield. ^{1}H NMR (500 MHz, CD_3OD): 8.26 (s, 1H) 4.63 (s, 2H) 4.47 (m, AB system, 2H) 3.64 (m, 2H) 3.25 (m,3H) 3.12 (t, 1H, J = 10.1 Hz) 1.91 (s, 2H) 1.68 (m,4H) 1.25 (m, 9H) 0.90 (m, 2H).

^{13}C NMR (100 MHz, CD_3OD): 139.57, 127.62, 76.49, 75.02, 70.60, 62.66, 51.81, 41.5, 38.74, 37.91, 34.40, 31.54, 27.69, 27.40, 24.71. HRMS: calcd for $C_{19}H_{35}N_4O_5$ ($M + H^+$), 399.2607; found, 399.2599.

*(1R,2S,3r,4R,5S,6s)-6-[[1-Undecyl-1H-1,2,3-triazol-4-yl]methylamino]-cyclohexane-1,2,3,4,5-pentaol (**1**[**18**]).* Obtained from **5**[**18**] in quantitative yield. ^{1}H NMR (500 MHz, CD_3OD): 8,18 (s, 1H) 4,59 (s, 2H) 4,45 (m, AB system, 2H) 3.61 (t, 2H, J = 9.3 Hz) 3.28 (m, 3H) 3.10 (t, 1H, J = 10.2 Hz) 1.93 (m, 2H) 1.28 (m, 16H) 0.90 (t, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, CD_3OD): 152.43 (C_q triazole, 2D assignment), 139.44 (CH triazole, 2D assigment), 76.54, 75.09, 70.59, 62.65, 51.66, 41.56, 33.01, 31.33, 30.67, 30.65, 30.55, 30.41, 30.11, 27.46, 23.69, 14.41. HRMS: calcd for $C_{20}H_{39}N_4O_5$ ($M + H^+$), 415.2920; found, 415.2931.

*(1R,2S,3r,4R,5S,6s)-6-[[1-(10-Hydroxydecyl)-1H-1,2,3-triazol-4-yl]methylamino]cyclohexane-1,2,3,4,5-pentaol(**1**[**25**]).* Obtained from **5**[**25**] in quantitative yield. ^{1}H NMR (500 MHz, CD_3OD): 8.17 (s,1 H) 4.60 (s, 2H) 4.46 (m, AB system, 2H) 3.60 (t, 2H) 3.55 (t, 2H, J = 6.4 Hz) 3.27 (m, 3H) 3.10 (t, 1H, J = 10.4 Hz) 1.94 (m, 2H) 1.53 (m, 2H) 1.33 (m, 12H). ^{13}C NMR (100 MHz, CD_3OD): 153.25 (C_q triazole, 2D assignment), 138.23 (CH triazole, 2D assigment), 76.57, 75.12, 70.61, 62.95, 62.64, 61.52, 51.62, 41.53, 33.61, 31.31, 30.59, 30.51, 30.08, 27.44, 26.90. HRMS: calcd for $C_{19}H_{37}N_4O_6$ ($M + H^+$), 417.2713; found, 417.2718.

Cells and Cultures. Fibroblasts were from Eucellbank and grown in Eagle's minimal essential medium (MEM) with 15% inactivated fetal calf serum at 37 °C in a humidified, 5% CO_2 incubator. Cells used were between the 14th and the 30th passage.

Enzyme Assays. 4-Methylumbelliferyl-β-D-glucopyranoside was obtained from Sigma. GlucCerase (Imiglucerase) was kindly provided by Genzyme Corporation (USA). HPLC measurements were performed using a Waters 2690 Alliance system coupled to a Waters 2475 fluorescence detector (Milford, MA). Empower Software (Waters Corporation) was utilized for data acquisition and processing.

GCase Activity. In vitro activity was determined with 4 mM 4-methylumbelliferyl-β-D-glucopyranoside in McIlvaine buffer (pH = 5.2 or 7.4). Enzyme solutions (25 μL from a stock solution containing 0.1 mg protein/mL) in the presence of 0.2% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100 in McIlvaine buffer (pH = 5.2 or 7.4) were incubated at 37 °C without (control) or with inhibitor (variable concentrations) during 30 min and, after addition of corresponding substrate solution (60 μL), incubations were maintained at 37 °C for 10 min. Enzymatic reactions were stopped by the addition of 150 μL of 100 mM glycine/NaOH buffer (pH 10.6). The amount of 4-methylumbelliferone formed was determined with a fluorometer at 355 nm (excitation) and 460 nm (emission).

IC_{50} values were determined by plotting percent activity versus log [I], using at least five different inhibitor concentrations. Type of inhibition and K_i values for more active inhibitors were determined by Lineweaver−Burk or Dixon plots of assays performed with different concentrations of inhibitor and substrate. IC_{50} data from crude click chemistry mixtures are reported in Table 1. Data from individually synthesized compounds are reported in Table 2.

GCase Inhibition in Intact Fibroblasts. Fibroblasts were plated into 24-well assay plates with DMEM containing aminocyclitol (50 μM, final concentration) dissolved in DMSO and incubated as described above for 24 h. Then, medium supplemented with aminocyclitols was removed and cells were washed with PBS. The enzymatic assay was performed as follows: substrate (100 μL, 5 mM 4-methylumbelliferyl β-D-glucoside) in 0.1 M acetate buffer (pH = 4.0) was added to each well up to a total volume of 260 μL; incubation was for 1 h at 37 °C. Enzyme reaction was stopped with 2 mL of 100 mM glycine−NaOH buffer (pH = 10.6) and fluorescence measured at 355 nm (excitation) and 460 nm (emission). For each experiment, untreated (no compound added) and treated cells were plated in quadruplicate. Data are reported in Table 2.

Thermal Stabilization Assay. Following a modification of a reported method,[11] GCase aliquots (48 μL, 2 mg/mL) were incubated at pH 7.4 at 48 °C with 0 (2 μL H_2O, control) or 2 μL test compound to reach a final concentration of 10, 25, 50, 100, or 150 μM. Subsequently, 150 μL of 0.1 M acetate−phosphate buffer (pH 5.0) and 100 μL of substrate (4 mM 4-methyllumbelliferyl β-D-glucoside) in McIlvaine buffer (pH 5.2) were added at different times (20, 40, 60 min) and incubated for 10 min at 37 °C. Then, 300 μL of glycine buffer (200 mM, pH 10.6) were added and liberated 4-methylumbelliferone was measured. Enzyme activity was reported relative to that of the enzyme at 37 °C. Data are reported in Table 2, Figure 3, and Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information. Experimental protocols for the parallel synthesis and characterization of azides **1**−**25**, UPLC-HRMS of adducts **1**[**1**−**25**], evaluation of click chemistry reagents in GCase assay, full SR data for compounds shown in Figure 3, GCS activity and cytotoxicity data, MOE filter definitions, molecular descriptors, diversity coverage of the virtual library, pIC_{50} values derived from Table 1, and predicted clogP values, Best poses for aminocyclitols **1**[**4**], **1**[**7**], **1**[**8**], **1**[**18**], and **1**[**25**] against GCase structures 2V3E and 2NSX and NMR spectra of the synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

clogP, calculated logarithm of the partition coefficient; CNS, central nervous system; CuAAC, copper-catalyzed alkyne azide cycloaddition; GCase, β-glucocerebrosidase; GCS, glucosyl ceramide synthase.; CerNBD, 6-[(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]hexanoylsphingosine

■ REFERENCES

(1) Grabowski, G. A. Phenotype, diagnosis, and treatment of Gaucher's disease. *Lancet* **2008**, *372*, 1263–1271.

(2) Brady, R. O. Gaucher's disease: past, present and future. *Baillieres Clin. Haematol.* **1997**, *10*, 621–634.
(3) Ron, I.; Horowitz, M. ER retention and degradation as the molecular basis underlying Gaucher disease heterogeneity. *Hum. Mol. Genet.* **2005**, *14*, 2387–2398.
(4) Schmitz, M.; Alfalah, M.; Aerts, J. M.; Naim, H. Y.; Zimmer, K. P. Impaired trafficking of mutants of lysosomal glucocereb rosidase in Gaucher's disease. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 2310–2320.
(5) Platt, F. M.; Lachmann, R. H. Treating lysosomal storage disorders: current practice and future prospects. *Biochim. Biophys. Acta, Mol. Cell Res.* **2009**, *1793*, 737–745.
(6) Morello, J.-P.; Bouvier, M.; Petaja-Repo, U. E.; Bichet, D. G. Pharmacological chaperones: a new twist on receptor folding. *Trends Pharmacol. Sci.* **2000**, *21*, 466–469.
(7) Fan, J.-Q. A contradictory treatment for lysosomal storage disorders: inhibitors enhance mutant enzyme activity. *Trends Pharmacol. Sci.* **2003**, *24*, 355–360.
(8) Butters, T. D. Pharmacotherapeutic strategies using small molecules for the treatment of glycolipid lysosomal storage disorders. *Expert Opin. Pharmacother.* **2007**, *8*, 427–435.
(9) Ringe, D.; Petsko, G. A. What are pharmacological chaperones and why are they interesting? *J. Biol.* **2009**, *8*, 80.
(10) Barton, N. W.; Brady, R. O.; Dambrosia, J. M.; Di Bisceglie, A. M.; Doppelt, S. H.; Hill, S. C.; Mankin, H. J.; Murray, G. J.; Parker, R. I.; Argoff, C. E.; et al. Replacement therapy for inherited enzyme deficiency—macrophage-targeted glucocerebrosidase for Gaucher's disease. *N. Engl. J. Med.* **1991**, *324*, 1464–1470.
(11) Sawkar, A. R.; Cheng, W. C.; Beutler, E.; Wong, C. H.; Balch, W. E.; Kelly, J. W. Chemical chaperones increase the cellular activity of N370S beta-glucosidase: a therapeutic strategy for Gaucher disease. *Proc. Natl. Acad. Sci. U.S.A* **2002**, *99*, 15428–15433.
(12) Egido-Gabas, M.; Serrano, P.; Casas, J.; Llebaria, A.; Delgado, A. New aminocyclitols as modulators of glucosylceramide metabolism. *Org. Biomol. Chem.* **2005**, *3*, 1195–1201.
(13) Egido-Gabas, M.; Canals, D.; Casas, J.; Llebaria, A.; Delgado, A. Aminocyclitols as Pharmacological Chaperones for Glucocerebrosidase, a Defective Enzyme in Gaucher Disease. *ChemMedChem* **2007**, *2*, 992–994.
(14) Sanchez-Olle, G.; Duque, J.; Egido-Gabas, M.; Casas, J.; Lluch, M.; Chabas, A.; Grinberg, D.; Vilageliu, L. Promising results of the chaperone effect caused by iminosugars and aminocyclitol derivatives on mutant glucocerebrosidases causing Gaucher disease. *Blood Cells, Mol., Dis.* **2009**, *42*, 159–166.
(15) Serrano, P.; Casas, J.; Zucco, M.; Emeric, G.; Egido-Gabas, M.; Llebaria, A.; Delgado, A. Combinatorial Approach to N-Substituted Aminocyclitol Libraries by Solution-Phase Parallel Synthesis and Preliminary Evaluation as Glucocerebrosidase Inhibitors. *J. Comb. Chem* **2007**, *9*, 43–52.
(16) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
(17) Gil, M. V.; Arévalo, M. J.; López, Ó. Click Chemistry—What's in a Name? Triazole Synthesis and Beyond. *Synthesis* **2007**, 1589–1620.
(18) Brik, A.; Wu, C. Y.; Wong, C. H. Microtiter plate based chemistry and in situ screening: a useful approach for rapid inhibitor discovery. *Org. Biomol. Chem.* **2006**, *4*, 1446–1457.
(19) Wu, C. Y.; Chang, C. F.; Chen, J. S.; Wong, C. H.; Lin, C. H. Rapid diversity-oriented synthesis in microtiter plates for in situ screening: discovery of potent and selective alpha-fucosidase inhibitors. *Angew. Chem., Int. Ed.* **2003**, *42*, 4661–4664.
(20) Liang, F. S.; Brik, A.; Lin, Y. C.; Elder, J. H.; Wong, C. H. Epoxide opening in water and screening in situ for rapid discovery of enzyme inhibitors in microtiter plates. *Bioorg. Med. Chem.* **2006**, *14*, 1058–1062.
(21) Diaz, L.; Bujons, J.; Casas, J.; Llebaria, A.; Delgado, A. Click Chemistry Approach to New N-Substituted Aminocyclitols as Potential Pharmacological Chaperones for Gaucher Disease. *J. Med. Chem.* **2010**, *53*, 5248–5255.
(22) Some of the resulting fragments are also present in a variety of iminocyclitol-derived modulators of glucosylceramide metabolism. Adamantyl:(a) Wennekes, T.; van den Berg, R.; Boltje, T. J.; Donker-Koopman, W. E.; Kuijper, B.; van der Marel, G. A.; Strijland, A.; Verhagen, C. P.; Aerts, J.; Overkleeft, H. S. Synthesis and Evaluation of Lipophilic Aza-C-glycosides as Inhibitors of Glucosylceramide Metabolism. *Eur. J. Org. Chem.* **2010**, 1258–1283. (b) Wennekes, T.; Meijer, A. J.; Groen, A. K.; Boot, R. G.; Groener, J. E.; van Eijk, M.; Ottenhoff, R.; Bijl, N.; Ghauharali, K.; Song, H.; O'Shea, T. J.; Liu, H. L.; Yew, N.; Copeland, D.; van den Berg, R. J.; van der Marel, G. A.; Overkleeft, H. S.; Aerts, J. M. Dual-Action Lipophilic Iminosugar Improves Glycemic Control in Obese Rodents by Reduction of Visceral Glycosphingolipids and Buffering of Carbohydrate Assimilation. *J. Med. Chem.* **2010**, *53*, 689–698. (c) Risseeuw, M. D. P.; van den Berg, R.; Donker-Koopman, W. E.; van der Marel, G. A.; Aerts, J.; Overhand, M.; Overkleeft, H. S. Synthesis and evaluation of D-gluco-pyranocyclopropyl amines as potential glucosidase inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6600–6603. (d) Wennekes, T.; van den Berg, R. J. B. H. N.; Bonger, K. M.; Donker-Koopman, W. E.; Ghisaidoobe, A.; van der Marel, G. A.; Strijland, A.; Aerts, J. M. F. G.; Overkleeft, H. S. Synthesis and evaluation of dimeric lipophilic iminosugars as inhibitors of glucosylceramide metabolism. *Tetrahedron: Asymmetry* **2009**, *20*, 836–846. (e) Bijl, N.; Scheij, S.; Houten, S.; Boot, R. G.; Groen, A. K.; Aerts, J. M. The glucosylceramide synthase inhibitor *N*-(5-adamantane-1-yl-methoxy-pentyl)-deoxynojirimycin induces sterol regulatory element-binding protein-regulated gene expression and cholesterol synthesis in HepG2 cells. *J. Pharmacol. Exp. Ther.* **2008**, *326*, 849–855. (f) Wennekes, T.; Berg, R. J.; Donker, W.; Marel, G. A.; Strijland, A.; Aerts, J. M.; Overkleeft, H. S. Development of Adamantan-1-yl-methoxy-Functionalized 1-Deoxynojirimycin Derivatives as Selective Inhibitors of Glucosylceramide Metabolism in Man. *J. Org. Chem.* **2007**, *72*, 1088–1097. (g) Boot, R. G.; Verhoek, M.; Donker-Koopman, W.; Strijland, A.; van Marle, J.; Overkleeft, H. S.; Wennekes, T.; Aerts, J. M. Identification of the non-lysosomal glucosylceramidase as beta-glucosidase 2. *J. Biol. Chem.* **2007**, *282*, 1305–1312. (h) Overkleeft, H. S.; Renkema, G. H.; Neele, J.; Vianello, P.; Hung, I. O.; Strijland, A.; van der Burg, A. M.; Koomen, G. J.; Pandit, U. K.; Aerts, J. M. Generation of specific deoxynojirimycin-type inhibitors of the non-lysosomal glucosylceramidase. *J. Biol. Chem.* **1998**, *273*, 26522–26527. Cyclohexylalkyl or phenylalkyl: (i) Alonzi, D. S.; Dwek, R. A.; Butters, T. D. Improved cellular inhibitors for glycoprotein processing alpha-glucosidases: biological characterisation of alkyl- and arylalkyl-N-substituted deoxynojirimycins. *Tetrahedron: Asymmetry* **2009**, *20*, 897–901. (j) Rawlings, A. J.; Lomas, H.; Pilling, A. W.; Lee, M. J.; Alonzi, D. S.; Rountree, J. S.; Jenkinson, S. F.; Fleet, G. W. J.; Dwek, R. A.; Jones, J. H.; Butters, T. D. Synthesis and Biological Characterisation of Novel *N*-Alkyl-Deoxynojirimycin alpha-Glucosidase Inhibitors. *ChemBioChem* **2009**, *10*, 1101–1105. *N*-Alkoxyalkyl: (k) 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. (l) Butters, T. D.; van den Broek, L. A. G. M.; Fleet, G. W. J.; Krulle, T. M.; Wormald, M. R.; Dwek, R. A.; Platt, F. M. Molecular requirements of imino sugars for the selective control of N-linked glycosylation and glycosphingolipid biosynthesis. *Tetrahedron: Asymmetry* **2000**, *11*, 113–124. *N*-alkyl: (m) Suzuki, Y.; Ogawa, S.; Sakakibara, Y. Chaperone therapy for neuronopathic lysosomal diseases: competitive inhibitors as chemical chaperones for enhancement of mutant enzyme activities. *Perspect. Med. Chem.* **2009**, *3*, 7–19. (n) Delgado, A.; Casas, J.; Llebaria, A.; Abad, J. L.; Fabrias, G. Chemical Tools to Investigate Sphingolipid Metabolism and Functions. *ChemMedChem* **2007**, *2*, 580–606.
(23) Serrano, P.; Llebaria, A.; Delgado, A. Regio- and stereoselective synthesis of aminoinositols and 1,2-diaminoinositols from conduritol B epoxide. *J. Org. Chem.* **2005**, *70*, 7829–7840.
(24) Exploratory attempts to carry out the nucleophilic opening of fully deprotected conduritol B epoxide with with the highly nucleophilic

NaN_3 were unsuccessful and unreacted material or complex reaction mixtures were obtained in all cases.

(25) Meller, A.; Hirninger, F. J.; Noltemeyer, M.; Maringgele, W. Umsetzungen von Metall- und Metalloidverbindungen mit mehrfunktionellen Molekülen, XXXIII. Zur Reaktion von Propargylaminen bzw. *N*-Organyl-*N*-(tri-methylsilyl)propargylaminen mit Bor-Halogen- bzw. Bor-Schwefel-Verbindungen. *Chem. Ber* **1981**, *114*, 2519–2535.

(26) Gonzalez-Bulnes, P.; Casas, J.; Delgado, A.; Llebaria, A. Practical synthesis of (−)-1-amino-1-deoxy-myo-inositol from achiral precursors. *Carbohydr. Res.* **2007**, *342*, 1947–1952.

(27) Sisa, M.; Trapero, A.; Llebaria, A.; Delgado, A. Small-Scale One-Pot Reductive Alkylation of Unprotected Aminocyclitols with Supported Reagents. *Synthesis* **2008**, 3167–3170.

(28) Compounds shown in Table 2 were not cytotoxic at concentrations up to 300 μM . For details of the MTT test, see the Experimental Section.

(29) Compounds shown in Table 2 were tested as inhibitors of the following commercial glycosidases: α-glucosidase (yeast), α-glucosidase (rice), α-galactosidase (coffee beans), and β-galactosidase (bovine liver). All of them showed IC_{50} values above 100 μM, with the exception of **1**[**25**], which behaved as a weak β-galactosidase (bovine liver) inhibitor (IC_{50} = 43 μM).

(30) Halgren, T. A. MMFF VII. Characterization of MMFF94, MMFF94s, and other widely available force fields for conformational energies and for intermolecular-interaction energies and geometries. *J. Comput. Chem.* **1999**, *20*, 730–748.

(31) Halgren, T. A.; MMFF, V. I. MMFF94s option for energy minimization studies. *J. Comput. Chem.* **1999**, *20*, 720–729.

(32) Compain, P.; Martin, O. R.; Boucheron, C.; Godin, G.; Yu, L.; Ikeda, K.; Asano, N. *ChemBioChem* **2006**, *7*, 1356–1359.