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Synthesis and Evaluation of Hydroxymethylaminocyclitols as Glycosidase Inhibitors

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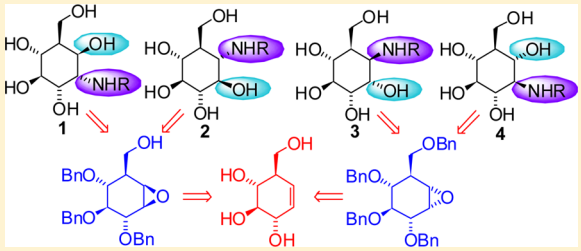
Supporting Information

ABSTRACT: Four series of C_7N aminocyclitol analogues of glucose were synthesized by stereocontrolled epoxide opening of hydroxyl protected forms of the cyclohexane epoxides cyclophellitol and 1,6-*epi*-cyclophellitol. The resulting hydroxymethyl substituted aminocyclitols were tested as glycosidase inhibitors. Cyclitols having an amino group in an α configuration at a position equivalent to the anomeric in the sugar were found to be low micromolar inhibitors of the α-glucosidase from baker's yeast with K_i's near to 2 μM. On the other hand, *N*-octyl aminocyclitols having the nitrogen substituents in an α or β configuration were found to be good inhibitors of recombinant β-glucocerebrosidase with K_i values between 8.3 and 17 μM, and also inhibited lysosomal β-glucosidase activity in live cells at low-micromolar concentrations. A computational docking study suggests a differential binding among the different series of β-glucocerebrosidase inhibitors. In agreement with the experimental results, the binding poses obtained indicate that the presence of an alkyl lipid substituent in the inhibitor mimicking one of the lipid chains in the substrate is critical for potency. In contrast, the matching of hydroxymethyl substituents in the aminocyclitols and the parent glucosylceramide does not seem to be strictly necessary for potent inhibition, indicating the risk of simplifying structural analogies in sugar mimetic design.



INTRODUCTION

Glycosidases are involved in a large range of important biological processes. These enzymes are attractive targets for the design of therapies for numerous diseases such as diabetes, viral infections, lysosomal storage disorders, and cancer.[1] Therefore, glycosidase inhibitors are currently of great interest as promising candidates for new drug development.

Aminocyclitols, which have been demonstrated as effective mimics of natural carbohydrates, are amino polyhydroxy cycloalkanes and comprise an important group of compounds for drug development. The amino functionality can modulate biological activity with respect to the parent carbohydrate, and the replacement of the endocyclic oxygen confers hydrolytic stability.[2−4] Different families of aminocyclitol analogues have been synthesized over the last decades, and their biological activities have been studied. These include the C_7N aminocyclitols,[2] polyhydroxylated aminocyclohexanes with an exocyclic methyl or hydroxymethyl substituent. C_7N compounds have found applications in medicinal chemistry as antifungal agents, such as salbostatin,[5] and antibiotics, such as validamycin A,[6] the most active of the validamycin family, which contain a valienamine[4] unit, linked to either validamine, valiolamine, or hydroxyvalidamine[7] (Figure 1).

Other C_7N aminocyclitols such as voglibose,[8] a derivative of valiolamine,[9] or acarbose[10] are α-glucosidase inhibitors used to treat type II diabetes. C_7N aminocyclitols have therapeutic potential for the treatment of lysosomal storage disorders, such as 4-epi-*N*-octyl-β-valienamine for G_{M1}-gangliosidosis or *N*-octyl-β-valienamine for Gaucher disease.[11]

Over the past decade, applications of some *N*-alkylated aminocyclitols as β-glucocerebrosidase (GCase) inhibitors with therapeutic potential for Gaucher disease have been described.[12−17] In this context, we reported on a series of inosamine derivatives (*scyllo*- and *chiro*-aminocyclitols, Figure 1), as selective inhibitors of GCase.[12,13] In continuation of our efforts in the design and synthesis of new glycosidase inhibitors, we became interested in four series of C_7N aminocyclitols (**1**−**4**, Figure 2) to study their glycosidase inhibitory activities. These compounds were designed taking into account the transformation of the *scyllo*-aminocyclitols or *chiro*-aminocyclitols previously reported[12,13] to the C_7N aminocyclitols by replacing a hydroxyl group by hydroxymethyl substituents (Figure 2). As far as the configuration of the D-glucose secondary hydroxyl groups is maintained in the new

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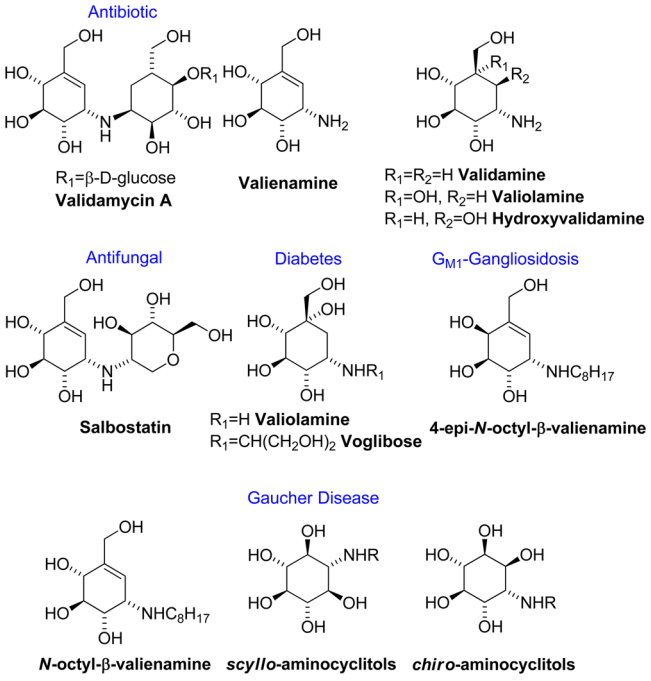


Figure 1. Structures of biologically active aminocyclitol derivatives.

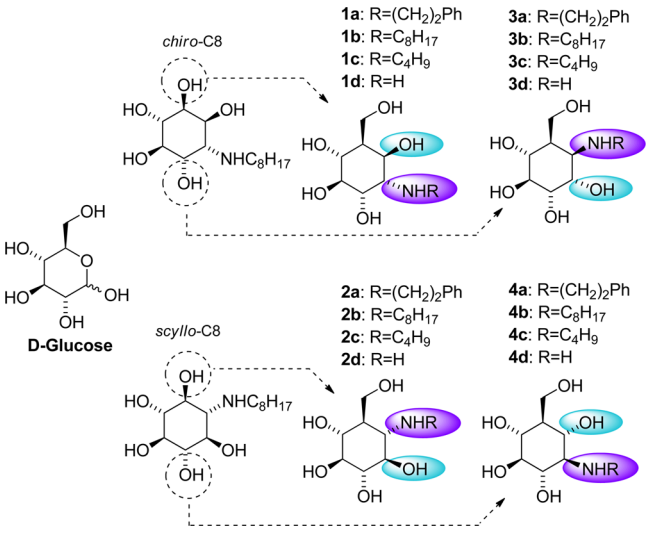


Figure 2. Chemical structures of C_7N aminocyclitols evaluated in this work and their relationship with the previously reported aminocyclitols.

compounds, two different hydroxyl groups (encircled in Figure 2) can be replaced by the hydroxymethyl substituents, and then four series are possible. We considered compounds differing not only in the configuration of the cyclohexane substituents but also in the nature of nitrogen substituents, and R = H, *n*-butyl, *n*-octyl, and 2-phenylethyl derivatives were synthesized.

RESULTS AND DISCUSSION

Design and Synthesis of C_7N Aminocyclitols. The retrosynthetic approach for the synthesis of the C_7N aminocyclitols is outlined in Figure 3. The four series of C_7N aminocyclitols could be prepared from cyclophellitol (+)-**5** and *epi*-cyclophellitol (+)-**6** derivatives based on the regioselective opening of epoxides with suitable nucleophiles, followed by hydroxyl group deprotection. This scheme has been previously

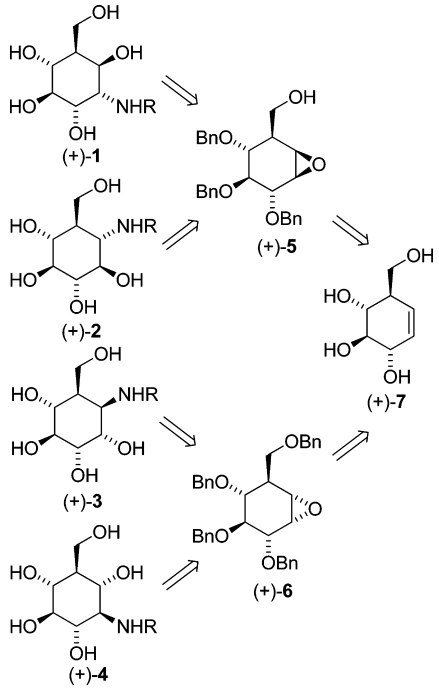


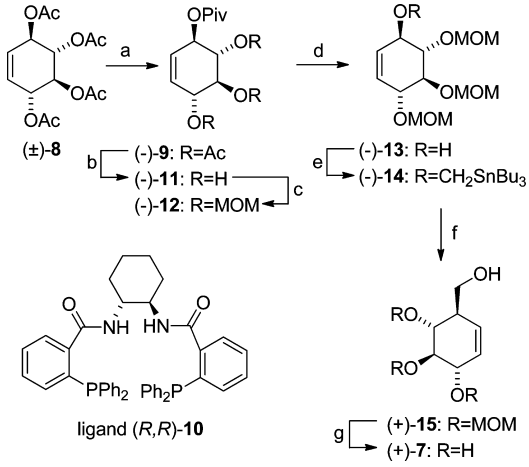
Figure 3. Retrosynthetic analysis of C_7N aminocyclitols.

described for the stereocontrolled synthesis of aminocyclitols and 1,2-diaminocyclitols from conduritol B.[13,16,18,19] The selective access to key epoxides could be achieved from a common cyclohexene intermediate (+)-**7** by choosing the appropriate conditions or protecting groups for a hydroxyl directed epoxidation reaction.[20]

Compound (+)-**7** was prepared as shown in Scheme 1, following a modified sequence described by Trost et al.[21] from tetraacetate (±)-**8**, which was synthesized in three steps from *p*-benzoquinone according to the literature procedure.[22]

In a previous work, we reported[23] the synthesis of pivalate (+)-**9** from tetraacetate (±)-**8** by a simple modification of

Scheme 1. Synthesis of (1*R*,2*R*,3*S*,6*R*)-6-(Hydroxymethyl)cyclohex-4-ene-1,2,3-triol (7)[a]



[a]Reagents and conditions: (a) ligand (*R*,*R*)-**10**, $[\eta^3\text{-}C_3H_5PdCl]_2$, THAB, $^tC_4H_9CO_2H$, NaOH, H_2O, DCM, 30 °C, 44%, >96% *ee*; (b) NH_3, MeOH, rt, 96%; (c) MOMBr, iPr_2NEt, DMF, 75 °C, 73%; (d) NaOMe, MeOH, rt, 94%; (e) KH, Bu_3SnCH_2I, DME, 0 °C, 79%; (f) *n*-BuLi, THF, −78 °C, 86%; (g) AcCl, MeOH, 40 °C, 99%.

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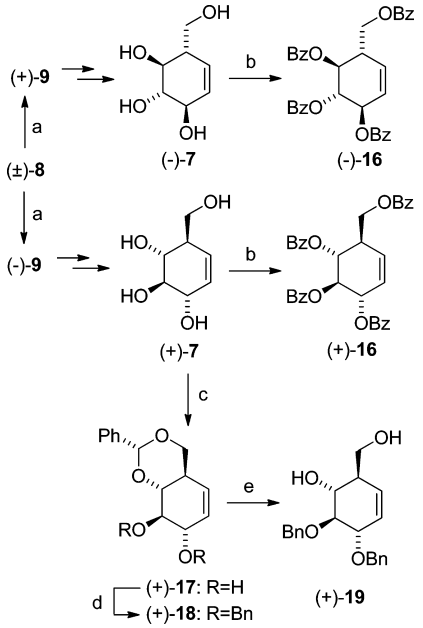
Trost's original procedure,[21,24] which consisted of the *in situ* preparation of the catalyst precursor, bis(π-allyl)-palladium dichloride, by reacting allyltrimethylsilane and $PdCl_2(CH_3CN)_2$ in acetonitrile.[25] These conditions gave more reproducible results and eliminated the problems associated with the manipulation of the palladium sensitive compound. Therefore, tetraacetate (±)-**8** was subjected to palladium-catalyzed kinetic resolution using chiral phosphine ligand (*R*,*R*)-**10**. Under these conditions, the (−)-enantiomer of **8** was converted to pivalate (−)-**9**,[21,24] whereas the (+)-enantiomer of the tetraacetate remained unreacted. The cleavage of the *O*-acetyl groups of (−)-**9** in the presence of the pivalate was performed using methanolic ammonia to give triol (−)-**11**.[21,24] Unfortunately, the clean protection of the triol (−)-**11** as a benzyl ether[21,24] was not possible at our work scale due to the lability of the pivalate group under the conditions described for this reaction. Therefore, we decided to use the methoxymethyl ether (MOM) group instead of benzyl ether as hydroxyl protecting group. With this modification, this reaction proceeded uneventfully to give (−)-**12**, followed by cleavage of the pivalate ester to alcohol (−)-**13**, which was converted to its corresponding stannylmethyl ether (−)-**14** (Scheme 1). Intermediate (−)-**14** was used as precursor for a 2,3-sigmatropic rearrangement, which was accomplished by treatment with 1.5 equiv of *n*-BuLi at −78 °C for 5 min to put the hydroxylmethyl group in place with regio- and stereochemically control to give (+)-**15** according to the Still−Wittig procedure. The deprotection of MOM ethers under acidic conditions gave the compound (+)-**7** in good yield (Scheme 1).

The spectroscopic data obtained for cyclohexene (+)-**7** were in agreement with those described,[26,27] but the magnitude and sign of the optical rotation found for this compound ($[\alpha]_D^{25}$ +120.9 (*c* 1.1, MeOH)) were different from those reported in the literature ($[\alpha]_D$ −13.4 (*c* 1.1, MeOH)).[26] The stereochemistry of (+)-**7** was unambiguously confirmed by the synthesis of compounds (+)-**16**,[21] (−)-**16**,[21] (+)-**17**,[28] (+)-**18**,[28] and (+)-**19**[26,28] (see Scheme 2), and comparison of their spectroscopic and analytical data that matched those reported in the literature for the same compounds obtained by different synthetic sequences. These results confirmed the structure and enantiomeric purity of intermediate (+)-**7**.

With the key intermediate (+)-**7** in hand, cyclophellitol and *epi*-cyclophellitol benzylated derivatives (+)-**5** and (+)-**6** were obtained as shown in Scheme 3. The epoxidation of the tetraol (+)-**7** with *m*-CPBA was directed[20] by the free allylic hydroxyl group to furnish exclusively 1,6-*epi*-cyclophellitol (**20**),[29,30] which was then benzylated to afford tetra-*O*-benzyl derivative (+)-**6**.[31]

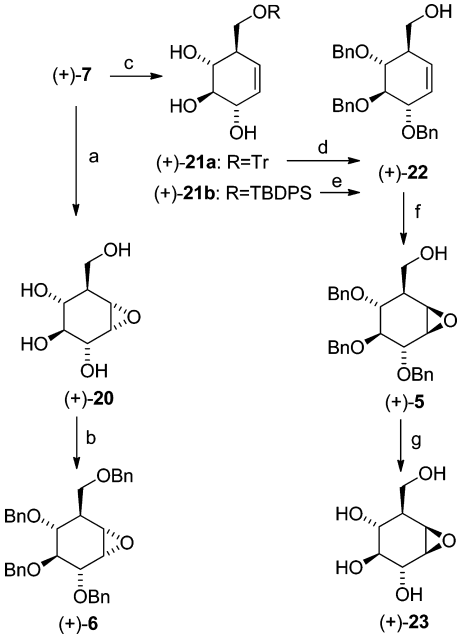
To reverse the facial selectivity of the epoxidation step, a protection−deprotection sequence of secondary hydroxyl groups was developed (Scheme 3). This strategy has been used in several previous synthesis[21,32−34] of cyclophellitol, but the epoxidation only occurred with good stereocontrol when it is directed by the primary hydroxyl group.[35] Selective protection of the primary alcohol in (+)-**7** as a TBDPS or trityl ether and benzylation yielded fully protected derivatives, which were subjected to detritylation or desilylation to afford the primary alcohol (+)-**22** (see Scheme 3).[21,32,33] The epoxidation with *m*-CPBA was stereochemically directed by the free homoallylic alcohol, giving the cyclophellitol derivative (+)-**5**.[21,24,33] Although this sequence involved additional protection/deprotection steps, it was high yielding, scalable, and was consistently used several times. Both trityl and TBDPS groups were useful to protect the primary hydroxyl group in this sequence.

Scheme 2. Synthesis of Compounds 16−19[a]



[a]Reagents and conditions: (a) ref 23, ligand (*R*,*R*)-**10** for (−)-**9** and ligand (*S*,*S*)-**10** for (+)-**9**; (b) BzCl, py, DMAP, DCM, rt, 64% for (+)-**16**, 66% for (−)-**16**; (c) $PhCH(OMe)_2$, *p*-TsOH, DMF, 60 °C/15−20 mmHg, 78%; (d) NaH, BnBr, DMF, rt, 77%; (e) 80% aq. AcOH, 40 °C, 74%.

Scheme 3. Synthesis of Epoxides 5 and 6[a]



[a]Reagents and conditions: (a) *m*-CPBA, AcOH, rt, 78%; (b) BnBr, NaH, DMF, rt, 52%; (c) $(Ph_3C\text{-pyr})BF_4$, CH_3CN/DMF (20/1), rt, 77% for **21a**; TBDPSCl, imidazole, DMAP, DMF, 45 °C, 83% for **21b**; (d) 1. BnBr, NaH, DMF, 0 °C; 2. *p*-TsOH, DCM/MeOH, (1/4), rt, 88%; (e) 1. BnBr, NaH, DMF, 0 °C; 2. TBAF, THF, rt, 70%; (f) *m*-CPBA, DCM, rt, 70%; (g) H_2 (1 atm), Pd/C, THF, 91%.

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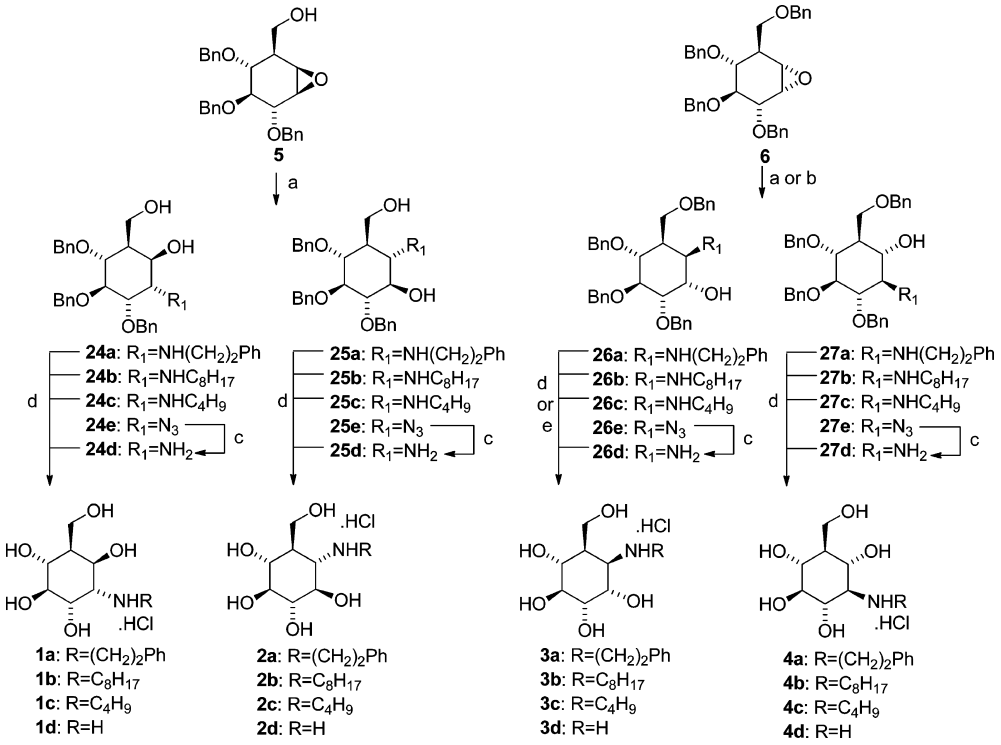
Scheme 4. Synthesis of C_7N Aminocyclitols 1−4 from Epoxides 5−6[a]



[a]Reagents and conditions: (a) nucleophile (10 equiv), 2 N $LiClO_4$, CH_3CN, 80 °C; (b) nucleophile (10 equiv), NH_4Cl, MeOH:H_2O (8:1), 100 °C; (c) $LiAlH_4$, THF (78−89%); (d) BCl_3, DCM (77%−94%); (e) H_2 (2 atm), Pd/C, HCl, THF (92−93%).

Table 1. Reaction of Epoxides 5 and 6 with Amines and NaN_3

entry	substrate	nucleophile	react. cond.[a]	products (ratio)[b]	yield[c] (%)
1	5	$Ph(CH_2)_2NH_2$	A	**25a**/**24a** (91:9)	83
2	5	$C_8H_{17}NH_2$	A	**25b**/**24b** (83:17)	82
3	5	$C_4H_9NH_2$	A	**25c**/**24c** (87:13)	85
4	5	NaN_3	A	**25e**/**24e** (75:25)	82
5	6	$Ph(CH_2)_2NH_2$	A	**27a**	74
6	6	$C_8H_{17}NH_2$	A	**27b**	76
7	6	$C_4H_9NH_2$	A	**27c**	72
8	6	NaN_3	A	**26e**/**27e** (56:44)	85
9	6	$Ph(CH_2)_2NH_2$	B	**26a**/**27a** (59:41)	82
10	6	$C_8H_{17}NH_2$	B	**26b**/**27b** (59:41)	85
11	6	$C_4H_9NH_2$	B	**26c**/**27c** (55:45)	79

[a]Reaction conditions A: nucleophile (10 equiv), 2 N $LiClO_4$, CH_3CN, 80 °C. Conditions B: nucleophile (10 equiv), NH_4Cl, MeOH:H_2O (8:1), 100 °C. [b]Determined by ^1H NMR analysis of the crude reaction mixture. [c]Isolated yields.

The stereochemistry of the intermediate (+)-**7** was also confirmed by the synthesis of the reported cyclophellitol (+)-**23**[21,36] from epoxide (+)-**5** (Scheme 3).

We planned the synthesis of the series of new aminocyclitols by a regio- and stereocontrolled epoxide opening of compounds (+)-**5** and (+)-**6** with suitable nitrogen nucleophiles (Scheme 4). The stereoselectivity of cyclohexane epoxide opening is controlled by the well-established Fürst−Plattner rule,[37] which predicts *trans*-diaxial opening from the most stable chair conformation. The *trans*-diequatorial opening can be obtained by a lithium salt promoted reaction.[18] Interestingly, the other regioisomers were obtained by epoxide opening under nonchelating conditions.[13]

Epoxides (+)-**5** and (+)-**6** were subjected to ring-opening reaction using an excess $LiClO_4$ and sodium azide or primary amines (10 equiv/mol) at 80 °C in acetonitrile as solvent (condition A, Table 1) or using primary amines (10 equiv/mol) under acidic conditions (excess ammonium chloride in methanol−water mixture, condition B, Table 1) at 100 °C. The obtained results are shown in Table 1.

Opening of epoxide **5** with NaN_3 afforded the corresponding azidoalcohol derivatives with moderate regioselectivity (entry 4, condition A). However, the use of primary amines as nucleophiles for the opening of the epoxide **5** under chelating conditions gave the corresponding amino alcohols with high regioselectivity (entries 1−3, condition A) favoring the regioisomers **25a−c**. Since the minor regioisomers **24a−c** could also be isolated in high purity and characterized after column chromatography, no attempts to perform the opening of epoxide **5** in acidic conditions (condition B) were done.

On the other hand, the reaction of epoxide **6** with primary amines under lithium chelating conditions afforded a single

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amino alcohol with total regioselectivity (entries 5−8, condition A), which corresponds to the expected all-equatorial substituents. In contrast, nearly 1:1 regioisomeric mixtures were obtained on reaction with NaN_3 as nucleophile (entry 8, condition A) or under acidic conditions (entries 9−11, condition B). Epoxide opening showed remarkable stereo- and regiochemical control when using amines as nucleophiles and $LiClO_4$. Again, the use of sodium azide as nucleophile resulted in lower regioselectivities than when amines were used, in contrast with our previous experience in related reactions, where azide opening was highly selective.[18] The reasons for the low regioselectivity obtained with an azide nucleophile in these reactions are at present unclear.

Alternatively, the opening of epoxides in mild acidic aqueous media (entries 9−11, condition B) showed a low regioselectivity. Reduction of azides **24e−26e**[31] and **27e**[31] with $LiAlH_4$ provided the corresponding primary amines **24d−27d** in good yields. The final products were obtained after *O*-benzyl deprotection with BCl_3,[13] but deprotection on compounds **26a−c** under these conditions afforded complex reaction mixtures. Gratifying, the alternative *O*-benzyl removal of these compounds by catalytic hydrogenation over Pd/C under acidic conditions afforded the desired aminocyclitols **3a−c** in good yields.

The stereochemistry of compounds **1−4** was established to be as shown in Figure 4, using ^{1}H NMR, gDQCOSY, and

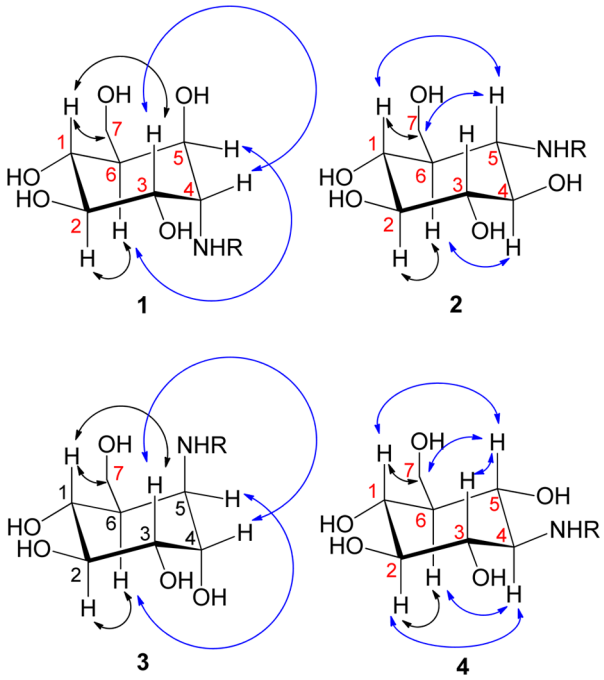

Figure 4. NOE correlations of compounds **1−4**.

NOESY spectral studies (see the Supporting Information). The NOESY spectra of compounds **1** and **3** showed strong NOE interactions between H5/H6 and H4/H3. On the other hand, the NOESY spectrum of compound **4** showed strong NOE interactions between H5/H1, H5/H3, H5/H7, H4/H6, and H4/H2. Similarly, the characterization of compound **2** was also confirmed by NOE interactions between H5/H1, H5/H7, and H4/H6. The NOE interactions between H5/H3, H4/H2 in compound **2** were not able to be determined due to the overlapped signals.

Enzyme Inhibition Studies. The four series of C_7N aminocyclitols **1−4** were tested as inhibitors against a series of commercial glycosidases, including α-glucosidase (baker's yeast and rice), β-glucosidase (sweet almond), α-galactosidase (green coffee beans), and β-galactosidase (bovine liver). Compounds **1−4** were further evaluated as inhibitors of glucosylceramide synthase and recombinant human GCase (imiglucerase, Cerezyme from Genzyme). Inhibitory activity against α-glucosidases, β-glucosidase (from sweet almond), β-galactosidase (from bovine liver), and imiglucerase is shown in Table 2 (see also the Supporting Information, Table S1).

All C_7N aminocyclitols were inactive when tested at 1.1 mM against green coffee beans α-galactosidase, and only compounds **1a**, **1b**, and **3b** displayed weak inhibition of the glycosyltransferase glucosylceramide synthase (between 52% and 67% inhibition at 250 μM).

Of the 16 aminocyclitols obtained, only **3a** (IC_{50} = 63 μM) and **3b** (IC_{50} = 147 μM) exhibited moderate inhibitory activity against almond β-glucosidase and only aminocyclitols **3b** (IC_{50} = 60 μM) and **4b** (IC_{50} = 114 μM) displayed moderate inhibition of bovine liver β-galactosidase. Moreover, only the free amino derivatives (**1d−4d**) showed weak to moderate inhibition against rice α-glucosidase (IC_{50} = 21−773 μM), indicating that the presence of lipophilic chains in the inhibitor is unfavorable for the activity of this enzyme.

Activity data reflect that the configurations of amino groups and hydroxyl groups (encircled in Figure 2) have an important effect on baker's yeast α-glycosidase inhibition. Compounds **1a−c** exhibited significant baker's yeast α-glucosidase inhibitory activity (IC_{50} = 2.6−27 μM), whereas the corresponding free amino derivative **1d** and aminocyclitols **2−4** showed little or no inhibitory activity toward this enzyme. Inhibition constants (K_i) were determined for compounds **1a** and **1b**, and in both cases, a competitive inhibition was found with K_i values of 2.1 and 1.9 μM, respectively. It has been reported[4,9] that validamine (Figure 1), structurally related to **1**, also inhibited baker's yeast α-glucosidase using maltose as substrate with an IC_{50} of 580 μM. For comparative purposes, DNJ (deoxynojirimycin) was also tested, being less potent than compounds **1a−c**.

Although initially designed to improve the activity as GCase inhibitors, the data obtained with C_7N aminocyclitols clearly indicate that some of the compounds showed no significant inhibition against imiglucerase. We found that compound **1b** is a low micromolar inhibitor of imiglucerase with an IC_{50} of 44 μM, whereas **1a** and **1d** were only weak inhibitors. The fact that several members of family **1** were inhibitors of the β-glucosidase imiglucerase is remarkable, since these compounds have an amino group in an axial position, resembling more an α- than a β-glucoside. To try to understand these unexpected results, we studied the inhibition mode of **1b**. Mixed inhibition was found for this aminocyclitol (K_i = 17 μM), suggesting the existence of an independent binding site for the inhibitor different from the active site. This result contrasts with the *chiro*-C8 derivative (Figure 2) that is a competitive inhibitor with a K_i value of 158 μM.[13]

In general, it seems that the main factor for imiglucerase inhibition is the presence of a liphophilic chain. The *N*-octyl derivatives **1b−4b** were found to be better inhibitors for this enzyme than the *N*-butyl or free amino derivatives, a fact that is in agreement with the correlation between lipophilicity (chain length) and the inhibitory activity that was observed in other glycomimetic families[12,42] with this enzyme. Compounds **3a** and **3b**, which contain the hydroxymethyl and amino

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Table 2. Inhibition of α-Glucosidases (from Baker's Yeast and Rice), β-Glucosidase (from Sweet Almond), β-Galactosidase (from Bovine Liver), and Imiglucerase by Aminocyclitols 1−4[k]

	α-glucosidase (baker's yeast)		α-glucosidase (rice)		β-glucosidase (almond)	β-galactosidase (bovine liver)	imiglucerase	
compound	IC_{50}	K_i	IC_{50}	K_i	IC_{50}	IC_{50}	IC_{50}	K_i
1a	2.6	2.1[a]	NI[b]		NI	NI	258	
1b	7.0	1.9[a]	NI		NI	NI	44	17[c]
1c	27		NI		NI	NI	NI	
1d	NI		773		NI	NI	43%[d]	
2a	962		NI		NI	NI	NI	
2b	383		NI		NI	NI	400	
2c	44%[e]		NI		NI	NI	NI	
2d	NI		166		NI	NI	NI	
3a	NI		58%[e]		63	45%[d]	138	162[c]
3b	NI		61%[e]		147	60	17	8.3[a]
3c	NI		NI		56%[e]	NI	NI	
3d	NI		21	26[a]	NI	NI	NI	
4a	NI		NI		NI	NI	NI	
4b	NI		NI		NI	114	23	9.0[c]
4c	NI		NI		NI	NI	NI	
4d	NI		167		NI	NI	NI	
DNJ	190[f]	8.7[a,g]	0.05[h]		81[f]	NI	506[i]	88[a,j]

[a]Type of inhibition: competitive. [b]NI: No significant inhibition at 800 μM (for baker's yeast α-glucosidase, rice α-glucosidase, and almond β-glucosidase) or 200 μM (for imiglucerase and bovine liver β-galactosidase). [c]Type of inhibition: mixed. [d]% inhibition at 200 μM. [e]% inhibition at 800 μM. [f]Reference 38. [g]Reference 39. [h]Reference 40. [i]Reference 41. [j]Reference 13. [k]IC_{50} and K_i values are given in μM.

substituents in contiguous carbon atoms, behaved as mixed (K_i = 162 μM) and competitive (K_i = 8.3 μM) inhibitors (see Figure S1, Supporting Information), respectively. It is remarkable that the *N*-octyl aminocyclitol **3b** is significantly more potent than the competitive *chiro*-C8 inhibitor (K_i = 158 μM). The *N*-octyl aminocyclitol **4b** was also an effective and selective inhibitor of imiglucerase (K_i = 9.0 μM). Surprisingly, mixed inhibition was found for aminocyclitol **4b**, which apparently can be considered structurally closer to the *scyllo*-C8 (K_i = 2.4 μM)[13] and to the glucosylceramide (GlcCer) enzyme substrate. Therefore, the introduction of a hydroxymethyl substituent as a hydroxyl replacement in the aminocyclitol inhibitors is not increasing the potency of *scyllo*-C8 for imiglucerase inhibition, in spite of having the cyclohexane nitrogen substituents in an equatorial position (compounds **2** and **4**), and apparently having a better structural match to the GlcCer substrate.

Overall, the data collected suggest a different recognition of the C_7N inhibitors at the imiglucerase active site, and would explain the relatively lower inhibitory activity of the hydroxymethyl aminocyclitols when compared with the *scyllo* series counterparts.

The C_7N aminocyclitols **1a−c**, showing the strongest inhibition potency for baker's yeast α-glucosidase, were selected for further evaluation of the lysosomal α-glucosidase inhibition in intact human fibroblasts. Similarly, aminocyclitols **1a−b**, **3a−b**, and **4b**, showing the strongest inhibition potency for imiglucerase, were also selected for further evaluation of the lysosomal β-glucosidase inhibition in wild-type human fibroblasts. Cytotoxicity assays were done to ensure that the enzyme activity was not affected by the possible toxicity of the compounds. The tested compounds did not show signs of cellular toxicity at concentrations up to 300 μM. We initially studied the enzyme activity incubating the compounds at 100 μM concentration, and we lowered the concentration of the compound when the inhibition was significant.

The *N*-phenylethyl derivative **1a** displayed a moderate inhibitory activity against lysosomal α-glucosidase (68% and 15% at 100 and 50 μM, respectively), whereas compounds **1b−c** showed less than 13% of lysosomal α-glucosidase inhibition at 100 μM concentration.

Compound **3a** showed 22% of lysosomal β-glucosidase inhibition at 100 μM, whereas the *N*-phenylethyl derivative **1a** and the *N*-octyl derivatives **1b**, **3b**, and **4b** showed more than 73% of lysosomal β-glucosidase inhibition at the same concentration (see Figure 5). The high potency of compounds

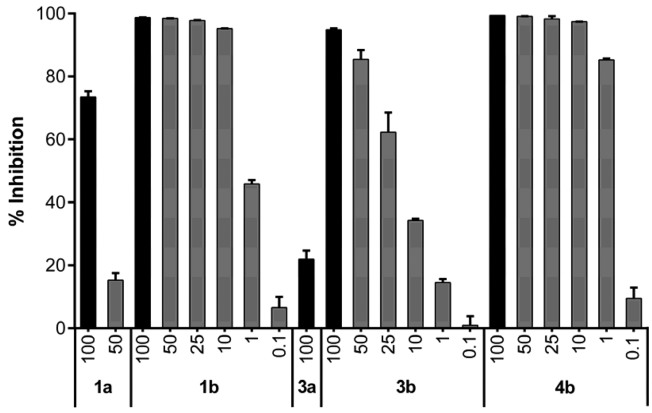

Figure 5. Inhibition of lysosomal human β-glucosidase in intact wild-type human fibroblasts after 24 h incubation time at the indicated inhibitor concentrations (μM).

1a, **1b**, **3b**, and **4b** incited analysis at lower concentrations. Interestingly, the *N*-octyl derivatives **1b**, **3b**, and **4b** behaved as lysosomal β-glucosidase inhibitors at low-micromolar concentrations in cellular assays, lower than required for inhibition in isolated enzyme experiments. These results show that the compounds are powerful lysosomal human β-glucosidase

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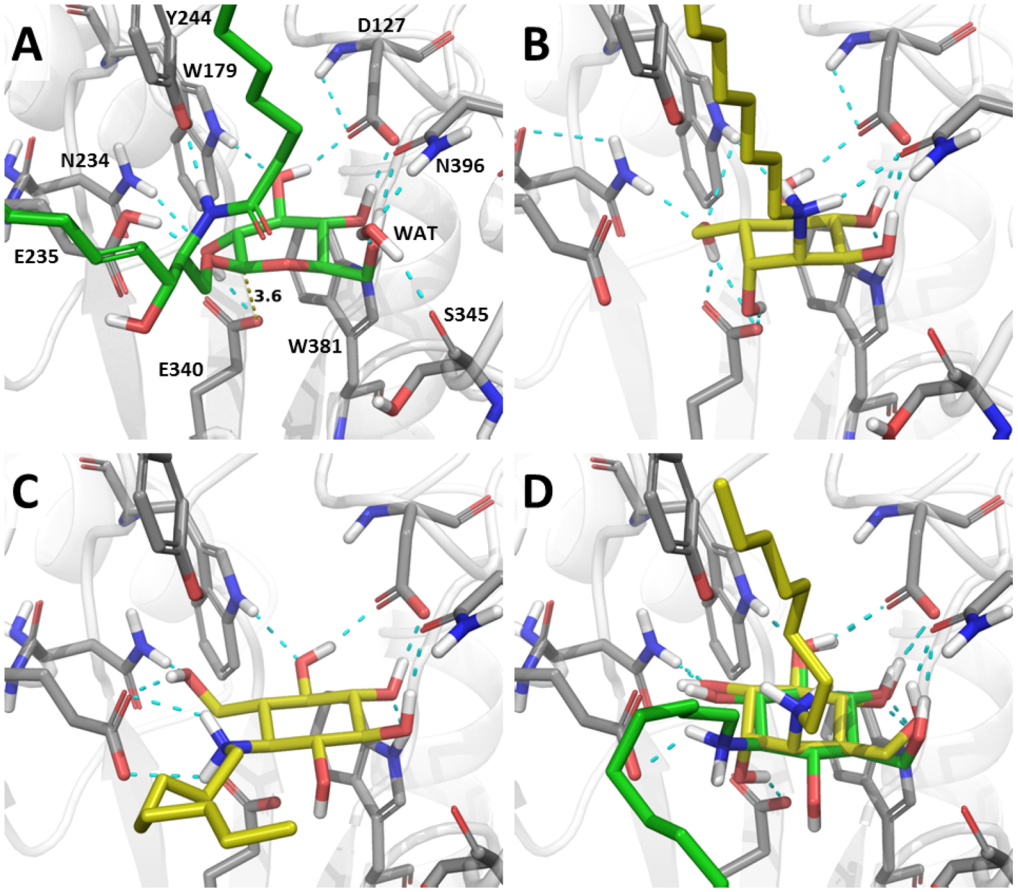

Figure 6. Best docked poses against the structure of human β-glucocerebrosidase[43] (PDB code: 2V3E) obtained for a truncated model of the natural substrate glucosylceramide (A), and aminocyclitols **1b** (B), **2b** (C), and **3b**/**4b** (superposed in yellow and green, respectively) (D). Panel A shows the carboxylate of residue E235 in its protonated form, which is postulated as the acid catalyst that protonates the ceramide hydroxyl group, once the scissile ketal bond is cleaved. Panels B–D, on the contrary, show the aminocyclitols bound to the E235 unprotonated form of the enzyme. This was based on the assumption that the presence of a charged amino group (expected at pH 5.2) in these ligands, at close distance from the carboxylate of E235, would stabilize the anionic form of the amino acid, as has been observed in the crystal structure of the isofagomine-bound GCase.[44]

inhibitors in live cells, reflecting good membrane permeability and cellular stability properties to inhibit the cellular enzyme.

Computational Docking. To shed further light on the binding mode of the compounds that showed inhibition of imiglucerase, computational docking against known structures of GCase[43,44] was carried out. For comparison purposes, a truncated model of the natural substrate GlcCer was also docked against the target enzyme.

Figure 6A shows the best docked pose obtained for GlcCer bound in a plausible prereactive conformation, where the anomeric carbon lies at 3.6 Å of the carboxylate of the essential nucleophile residue E340 and the oxygen of the scissile ketal bond accepts a hydrogen bond from the protonated catalytic residue E235. In this conformation, the hydroxymethyl group of GlcCer is disposed, donating a hydrogen bond to the carbonyl oxygen of the amide group of residue N396 and accepting another one from a crystallographic water, which is also hydrogen bonded to N396 and S345. In contrast, the best pose for **1b** shows its cyclohexyl moiety in an inverted conformation relative to GlcCer, in which the C6-hydroxymethyl group is oriented toward the opposite side of the catalytic cavity, forming hydrogen bonds with residues N234 and E340 (Figure 6B). This is due to the steric requirements of the axial octylamino substituent, which precludes binding in a GlcCer-like manner. Compound **2b** shows a similar orientation to **1b**, although, in this case, the determined binding pose suggests that the polar interactions between the charged amino group of the ligand and the E235 carboxylate are the main determinants of this orientation (Figure 6C). On the contrary, compounds **3b** and **4b** show binding poses with their cyclohexyl moieties disposed in a more GlcCer-like orientation, with the corresponding hydroxymethyl substituents interacting with residue N396 (Figure 6D). Noteworthy, these two compounds were the best GCase inhibitors found in this study. Comparison with the predicted binding modes for the parent C_6N analogues *chiro*-C8 and *scyllo*-C8 (Figure 2) reveals some similarities (Figure S2, Supporting Information). The best pose obtained for the *chiro*-configured compound resembles those of compounds **1b** and **3b**, while the best pose for the *scyllo*-C8 analogue resembles those of **2b** and **4b**. Thus, replacement of a hydroxyl in the parent *chiro*-C8 or *scyllo*-C8 by a hydroxymethyl group did not result in large differences in the ways of interaction with the target enzyme and most of the hydrogen bonds with the receptor (i.e., with residues D127, W179, or N234) are maintained in all the compounds. Similarly, the hydrophobic interactions between the axial alkyl substituent of compounds **1b**, **3b**, or *chiro*-C8 and residues L241, Y244, P245, and F246, as well as those between the equatorial alkyl chain of compounds **2b**, **4b**, or *scyllo*-C8 and residues Y313, L314, and K346, are mostly maintained.

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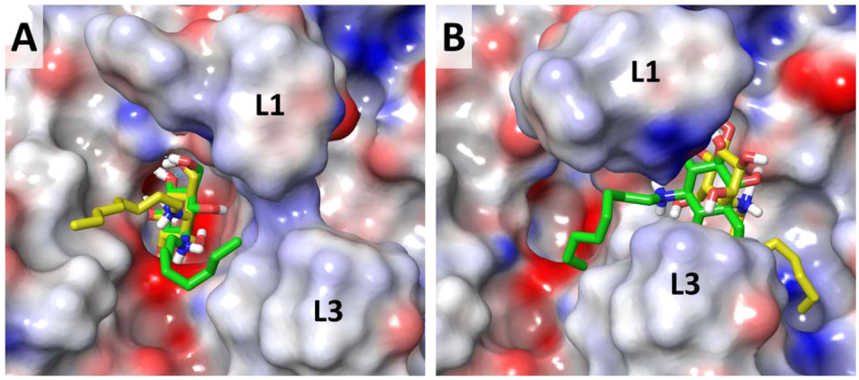

Figure 7. Comparison of the surface topologies of the active sites of the GCase structures 2V3E (A) and 2NSX[44] (B) and their surroundings. Docked poses obtained for compounds **3b** (yellow) and **4b** (green) bound into the GCase active site (A), and into the adjacent groove (B), which is only accessible in structure 2NSX, are shown. Surfaces are colored according to electrostatic potential (blue: positive; red: negative). Labels denoting the location of loops L1 and L3 are also shown.

The presence of both axial and equatorial amino groups among the imiglucerase active inhibitors group is somewhat surprising, since the mimetism of the anomeric β-oxygen of GluCer with the nitrogen atom in the aminocyclitol inhibitor was initially believed to play a critical role on inhibitor potency.[45] The results of the docking of those inhibitors having equatorial amino groups (**2b**, **4b**, or *scyllo*-C8) show that this is the case and hydrogen bonding of the nitrogen and the catalytic acid E235 is obtained. The lipidic chain in this series would be located in the same pocket where the sphingosine chain of the ceramide substrate is found. For the axial alkylamino compounds, **1b**, **3b**, or *chiro*-C8, no hydrogen bonding interaction of the nitrogen with residues of the active site is observed. In this case, the six-membered ring is found rotated about 60°, placing the *N*-alkyl chain in the pocket where the alkyl fatty acid group of the GluCer substrate is found; this hydrophobic interaction would account for the unexpected recognition of the axial aminocyclitols in the imiglucerase active site, which is believed to be dominated by the lipidic interactions, as shown for the importance of the alkyl length in the inhibitory potency. The presence of two long alkyl chains in the GluCer substrate would facilitate the duality of axial and equatorial structures in the *N*-alkyl active inhibitors found. The binding modes shown by all of these compounds are compatible with a competitive mechanism of inhibition; however, analysis of the inhibition mode showed that some of them (**1b** and **4b**) exhibit a mixed mechanism. This suggests that there could be alternative binding modes in addition to those predicted by these docking studies.

In this sense, it is worth mentioning that, among all the crystal structures reported for human GCase, several of them (PDB codes 2NSX,[44] 2J25,[46] 2WKL,[47] 2XWE,[48] 3GXI,[49] 3GXM,[49] and 3RIL[50]) present a narrow groove between loops L1 (residues 341−350) and L3 (residues 312−319), two of the loops that conform the entrance to the enzyme active site.[43,51] We and others have previously proposed that this groove, which is not accessible in other GCase structures (Figure 7), could be occupied by one of the alkyl chains of glucosylceramide or by the hydrophobic moieties of some inhibitors bound into the active center of GCase.[14,44,51] Using structure 2NSX as a target for docking of the C_7N GCase inhibitors, we observed that, in addition to the detection of poses in the active site cavity, which were similar to those shown in Figure 6 (results not shown), poses with the aminocyclitol moieties bound into that adjacent groove were also detected (Figure S3, Supporting Information). This is illustrated in Figure 7B for compounds **3b** and **4b**. Although binding of inhibitors at this site might not have as large an effect as direct binding at the active site of GCase, it is tempting to propose that this could be an alternative site for modulation of the GCase activity, particularly if part of the inhibitor projects toward the entrance to the active site, as it is shown for compound **4b** in Figure 7B. Binding in an opposite orientation, as that shown for **3b**, could have a lower effect, although it would still hamper the binding of any substrate moiety in that location. It is well-known that occurrence of different binding modes like these could translate into mixed-type inhibition properties.[52] Thus, it seems reasonable to propose that inhibitors such as aminocyclitols C_7N could explore these different binding modes, and that the relative populations of each one would determine their behavior as purely competitive, mixed-type, or other type of inhibition.

■ CONCLUSIONS

In conclusion, we have developed a strategy for the synthesis of four series of C_7N aminocyclitols from cyclohexane epoxides (+)-**5** and (+)-**6** by stereocontrolled ring opening with nitrogen nucleophiles. The final compounds have been tested as α-glycosidase and recombinant GCase inhibitors, and other glycosidases. Activity data reflect that the configurations of amino groups and hydroxyl groups have an important effect on glycosidase inhibition. Among the four series of C_7N aminocyclitols, *N*-alkyl aminocyclitols **1a** and **1b** were found to be low micromolar inhibitors of baker's yeast α-glucosidase with K_i values of 2.1 and 1.9 μM, respectively. Furthermore, *N*-octyl compounds **1b**, **3b**, and **4b** were the most potent recombinant GCase inhibitors of the series of hydroxymethylaminocyclitols described in this work with K_i values between 8.3 and 17 μM. The presence of both axial and equatorial alkylamino groups among this group is somewhat surprising, since the mimetism of the anomeric β-oxygen of GluCer by the nitrogen atom was initially believed to exert a critical influence on inhibitor potency. The docking studies suggest that the presence of a lipidic chain is a main structural determinant for high potency and some inhibitors having an axial alkylamino substituent have also favorable interactions with several residues in the active site that would account for its inhibitory potency. These results show that design of glycoanalogues based on

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simple structural correlations involving hydroxyl matching or anomeric configuration with the sugar substrates is not as direct as one could expect, and that other less evident effects need also to be considered. The *N*-octyl substituted most active compounds (**1b**, **3b**, and **4b**) also behaved as inhibitors of lysosomal β-glucosidase in intact wild-type human fibroblasts at low-micromolar concentrations, showing a good permeability and stability under cell culture conditions. This is a positive trend for their potential use as pharmacological chaperones,[17,53] as the activity shown in isolated enzyme is maintained in live cells.

EXPERIMENTAL SECTION

General Experimental Methods. Solvents were distilled prior to use and dried by standard methods. FT-IR spectra are reported in cm^{-1}. Unless otherwise stated, ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$, D_2O, or CD_3OD solutions at 500 MHz (for ^1H) and 100 MHz (for ^{13}C). Chemical shifts (δ) are given in ppm relative to the residual solvent peak ($CDCl_3$: ^1H, δ = 7.26 ppm; ^{13}C, δ = 77.16 ppm), and the coupling constants (J) are reported in hertz (Hz). Optical rotations were measured at the sodium D line (589 nm), and specific rotations are reported in 10^{-1} deg cm^2 g^{-1}. HRMS were recorded in a time-of-flight (TOF) mass spectrometer with electrospray ionization (ESI).

(1*RS*,2*SR*,3*SR*,4*RS*)-Cyclohex-5-ene-1,2,3,4-tetrayl Tetraacetate ((±)-8). The title compound was synthesized according to a literature procedure.[22]

(1*S*,2*S*,3*R*,6*R*)-6-(Pivaloyloxy)cyclohex-4-ene-1,2,3-triyl Triacetate (9). The title compound was synthesized according to a literature procedure[23] using the ligand (*R*,*R*)-**10**.

(1*R*,4*R*,5*S*,6*R*)-4,5,6-Trihydroxycyclohex-2-en-1-yl Pivalate (11). The title compound was synthesized according to a literature procedure.[21,24]

(1*R*,4*R*,5*S*,6*S*)-4,5,6-Tris(methoxymethoxy)cyclohex-2-enyl Pivalate (12). To a stirred mixture of (−)-**11**[21,24] (2.38 g, 10.3 mmol) and *N*,*N*-diisopropylethylamine (12 mL, 68.9 mmol) in anhydrous DMF (20 mL) was added slowly bromomethyl methyl ether (6 mL, 73.5 mmol) at 0 °C. The mixture was stirred at 75 °C for 3 h, and then poured into ice−water. The resulting mixture was extracted with ether. The extracts were washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure to give a brown oil (3.5 g), which was purified by *flash* chromatography (silica gel, hexane/EtOAc, 4:1). Product **12** was obtained as a yellow oil (2.76 g, 7.6 mmol, 73%).

Data for **12**: $[\alpha]_D^{25}$ −130.8 (*c* 1.0, $CHCl_3$); IR (film): υ = 2960, 2894, 2825, 1729, 1480, 1280, 1156, 1038, 921 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.21 (s, 9H), 3.38 (s, 3H), 3.41 (s, 3H), 3.44 (s, 3H), 3.74−3.83 (m, 2H), 4.21−4.23 (m, 1H), 4.74−4.89 (m, 6H), 5.40−5.44 (m, 1H), 5.48 (dt, 1H, J = 10.3, 2.3 Hz), 5.77 (dt, 1H, J = 10.3, 2.0 Hz); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 27.2, 38.9, 55.8, 56.3, 56.4, 74.4, 78.2, 79.0, 79.3, 97.4, 98.0, 98.2, 125.9, 130.2, 178.0. HRMS calculated for $C_{17}H_{30}O_8Na$: 385.1838 $[M + Na]^+$. Found: 385.1850.

(1*R*,4*R*,5*S*,6*S*)-4,5,6-Tris(methoxymethoxy)cyclohex-2-enol (13). A solution of pivalate (−)-**12** (5.68 g, 15.6 mmol) in MeOH (10 mL) was added dropwise to a solution of NaOMe (1.4 g, 24.6 mmol) in MeOH (50 mL) at 0 °C. The reaction mixture was stirred at rt for 14 h, and then the solvent was evaporated. The resulting residue was dissolved with DCM (100 mL) and washed with saturated aqueous NH_4Cl (60 mL) and brine (60 mL). The organic layer was dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure to give a yellow oil (5.8 g), which was purified by *flash* chromatography (silica gel, hexane/EtOAc, 2:1). Product **13** was obtained as a colorless oil (4.1 g, 14.6 mmol, 94%).

Data for **13**: $[\alpha]_D^{25}$ −108 (*c* 1.0, $CHCl_3$); IR (film): υ = 3442, 3039, 2894, 2825, 1470, 1384, 1125, 916, 774 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 3.41 (s, 3H), 3.43 (s, 3H), 3.48 (s, 3H), 3.73 (dd, 1H, J = 10.4, 7.7 Hz), 4.17 (d, 1H, J = 2.0 Hz), 4.20−4.22 (m, 2H), 4.74−4.85 (m, 6H), 5.66−5.72 (m, 2H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 55.7, 56.08, 56.09, 56.1, 71.5, 78.5, 78.6, 87.3, 97.2, 98.0, 98.6, 127.8, 128.8. HRMS calculated for $C_{12}H_{22}O_7Na$: 301.1263 $[M + Na]^+$. Found: 301.1250.

Tributyl(((1*R*,4*R*,5*S*,6*S*)-4,5,6-tris(methoxymethoxy)cyclohex-2-enyloxy)methyl)stannane (14). A solution of alcohol **13** (810 mg, 2.9 mmol) in DME (10 mL) was added dropwise to a suspension of potassium hydride (760 mg, 30% suspension in mineral oil, 5.6 mmol) in DME (10 mL) at 0 °C. The reaction mixture was stirred at rt for 30 min. After cooling to 0 °C, iodomethyltributyltin (1.9 g, 4.5 mmol) was added, and the mixture was stirred at rt for an additional 2 h. Then, 30 mL of saturated aqueous NH_4Cl was added and the mixture was extracted with Et_2O (3 × 50 mL). The organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification of the residue by *flash* chromatography (silica gel, hexane/EtOAc, 6:1) provided the tributylstannylmethyl ether **14** (1.3 g, 79%) as a colorless oil.

Data for **14**: $[\alpha]_D^{25}$ −80 (*c* 2.0, $CHCl_3$); IR (film): υ = 3039, 2925, 2851, 2822, 1465, 1388, 1153, 1034, 921, 774 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.87−0.92 (m, 15H), 1.27−1.31 (m, 6H), 1.45−1.52 (m, 6H), 3.41 (s, 3H), 3.44 (s, 6H), 3.59 (d, 1H, J = 9.8 Hz), 3.65−3.72 (m, 2H), 3.83−3.88 (m, 2H), 4.17−4.19 (m, 1H), 4.74−4.91 (m, 6H), 5.70−5.75 (m, 2H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 9.0, 13.7, 13.9, 17.7, 27.0, 27.5, 28.0, 29.2, 55.7, 56.2, 56.2, 58.8, 78.1, 79.0, 79.4, 84.6, 97.4, 97.9, 98.2, 127.2, 128.7. HRMS calculated for $C_{25}H_{50}O_7NaSn$: 605.2476 $[M + Na]^+$. Found: 605.2450.

(1*R*,4*S*,5*R*,6*R*)-4,5,6-Tris(methoxymethoxy)cyclohex-2-enyl)methanol (15). The stannylmethyl eher **14** (0.51 g, 0.86 mmol) was dissolved in anhydrous THF (15 mL), and the solution was cooled to −78 °C. After 30 min, *n*-BuLi (0.8 mL of a 1.6 M solution in hexanes, 1.29 mmol) was added slowly. The reaction mixture was stirred at −78 °C for 5 min, and then the reaction was quenched with saturated aqueous NH_4Cl (15 mL) at −78 °C, and extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with brine and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was purified by *flash* chromatography (silica gel, hexane/EtOAc, 2:1 to 1:1) to afford the desidered homoallylic alcohol **15** (216 mg, 0.74 mmol, 86%) as a colorless oil.

Data for **15**: $[\alpha]_D^{25}$ +113 (*c* 1, $CHCl_3$); IR (film): υ = 3454, 2892, 2826, 1651, 1469, 1444, 1392, 1151, 1105, 1025, 918, 771 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.42−2.46 (m, 1H), 3.40 (s, 3H), 3.43 (s, 3H), 3.44 (s, 3H), 3.66−3.70 (m, 2H), 3.79 (dd, 1H, J = 10.1, 7.7 Hz), 3.88 (dd, 1H, J = 11.5, 3.8 Hz), 4.18−4.21 (m, 1H), 4.71−4.94 (m, 6H), 5.59 (dt, 1H, J = 10.1, 2.0 Hz), 5.73 (dt, 1H, J = 10.1, 2.6 Hz); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 45.5, 55.7, 56.2, 56.3, 63.0, 77.8, 79.5, 81.6, 97.1, 98.0, 99.0, 129.0, 129.1; HRMS calculated for $C_{13}H_{24}O_7Na$: 315.1420 $[M + Na]^+$. Found: 315.1431.

(1*R*,2*R*,3*S*,6*R*)-6-(Hydroxymethyl)cyclohex-4-ene-1,2,3-triol (7). To a solution of AcCl (0.6 mL, 7.9 mmol) in MeOH (20 mL) was added dropwise a solution of **15** (0.38 g, 1.32 mmol) in MeOH (10 mL) at 0 °C. The reaction mixture was stirred at 40 °C for 1 h, and then the solvent was removed under reduced pressure to give the expected product as a white amorphous solid (0.21 g, 1.31 mmol, 99%).

Data for **7**: mp 134−136 °C; $[\alpha]_D^{25}$ +120.9 (*c* 1.1, MeOH); lit[26] $[\alpha]_D$ −13.4 (*c* 1.1, MeOH); IR (KBr): υ = 3340, 1652, 1081, 723 cm^{-1}; ^1H NMR (δ, 500 MHz, CD_3OD): 2.21−2.30 (br s, 1H), 3.4−3.47 (m, 2H), 3.59 (dd, 1H, J = 10.6, 6.1 Hz), 3.79 (dd, 1H, J = 10.6, 4.1 Hz), 3.99−4.06 (m, 1H), 5.59−5.64 (m, 2H); ^{13}C NMR (δ, 100 MHz, CD_3OD): 47.8, 63.5, 72.0, 73.7, 78.9, 128.6, 131.1; HRMS calculated for $C_7H_{12}O_4Na$: 183.0633 $[M + Na]^+$. Found: 183.0629. NMR data are in accordance with literature values.[26−28]

(1*R*,2*R*,3*S*,6*R*)-6-(Benzoyloxymethyl)cyclohex-4-ene-1,2,3-triyl Tribenzoate (16). To a solution of **7** (17 mg, 0.1 mmol), dry py (0.3 mL, 3.7 mmol) and DMAP (7.2 mg, 0.06 mmol) in DCM (1.5 mL) was added BzCl (0.2 mL, 1.7 mmol). After stirring overnight at rt, the reaction mixture was diluted with DCM (25 mL) and washed with H_2O (10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product obtained was purified by *flash* chromatography (silica gel, hexane/EtOAc, 5:1)). Product **16** was obtained as a white powder (39 mg, 0.07 mmol, 64%).

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Enantiomeric excess > 96% determined by HPLC [Chiralpak IA column, eluting with 90:10 hexane/*i*PrOH, 0.7 mL/min, 240 and 210 nm]. (−)-enantiomer t_R 25.69 min, (+)-enantiomer t_R 35.33 min.

Data for (+)-**16**: mp 101−104 °C; $[\alpha]_D^{25}$ +159.7 (*c* 0.7, DCM); IR (film): υ = 3063, 3034, 2955, 2928, 2854, 1736, 1601, 1451, 1315, 1275, 1178, 1113 707 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 3.25−3.30 (m, 1H), 4.40 (dd, 1H, *J* = 11.3, 4.9 Hz), 4.61 (dd, 1H, *J* = 11.3, 4.5 Hz), 5.88−5.92 (m, 1H), 5.94−6.0 (m, 2H), 6.02−6.08 (m, 2H), 7.26−7.33 (m, 4H), 7.40−7.47 (m, 6H), 7.54−7.57 (m, 2H), 7.85−7.91 (m, 4H), 8.03 (d, 4H, *J* = 7.5 Hz); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 42.2, 64.0, 70.2, 73.0, 127.0−129.9, 133.2, 133.3, 133.32, 133.4, 166.0 (2), 166.1, 166.4; ^{13}C NMR (δ, 100 MHz, CD_3OD): 43.3, 65.5, 71.9, 74.2, 74.7, 127.7−134.5, 167.3, 167.3, 167.4, 167.7; HRMS calculated for $C_{35}H_{28}O_8Na$: 599.1682 $[M + Na]^+$. Found: 599.1674.

Data for (−)-**16**: mp 112−115 °C; $[\alpha]_D^{25}$ −147.2 (*c* 0.7, DCM, 86% *ee*); lit[21] $[\alpha]_D^{25}$ −76.9 (*c* 1.1, DCM, 88% *ee*).

(1*R*,3*R*,6*R*,9*S*,10*S*)-9,10-Dihydroxy-3-phenyl-2,4-dioxabicyclo[4.4.0]dec-7-ene (17). To a solution of **7** (25 mg, 0.15 mmol) in DMF (2 mL) were added $PhCH(OMe)_2$ (0.03 mL, 0.2 mmol) and *p*-TsOH (2 mg, 0.01 mmol) at rt. The mixture was immersed into a 60 °C preheated water bath and stirred under reduced pressure (20 mmHg) for 5 h. After the solution was cooled to rt, solid $NaHCO_3$ (10 mg) was added and the solvent was removed under reduced pressure. The resulting residue was purified by *flash* chromatography (silica gel, hexane/EtOAc, 1:1) to give **17** (30 mg, 0.12 mmol, 78%) as a white amorphous solid.

Data for **17**: mp 170−172 °C; lit[28] mp 165−167 °C; $[\alpha]_D^{25}$ +32 (*c* 1.8, EtOH); lit[28] $[\alpha]_D^{25}$ +28 (*c* 2.5, EtOH); IR (film): υ = 3333, 2957, 2922, 2851, 1462, 1453, 1369, 1360, 1261, 1165, 1095, 1025 cm^{-1}; ^1H NMR (δ, 500 MHz, CD_3OD): 2.55−2.60 (br s, 1H), 3.62 (t, 1H, *J* = 13 Hz), 3.66 (t, 1H, *J* = 14 Hz), 3.78 (dd, 1H, *J* = 13.0, 9.2 Hz), 4.16−4.2 (m, 1H), 4.25 (dd, 1H, *J* = 10.7, 4.5 Hz), 5.40 (dt, 1H, *J* = 12.0, 2.0 Hz), 5.59−5.62 (m, 1H), 5.67 (dt, 1H, *J* = 12.5, 3.5 Hz), 7.30−7.42 (m, 3H), 7.51 (t, 2H, *J* = 12.6 Hz); ^{13}C NMR (δ, 100 MHz, CD_3OD): 40.2, 71.1, 75.2, 76.6, 82.3, 103.3, 125.1, 127.5, 129.0, 129.8, 132.3, 139.8; HRMS calculated for $C_{14}H_{17}O_4$: 249.1127 $[M + H]^+$. Found: 249.1129.

(1*R*,3*R*,6*R*,9*S*,10*S*)-9,10-Dibenzyloxy-3-phenyl-2,4-dioxabicyclo[4.4.0]dec-7-ene (18). To a suspension of NaH (20 mg, 60% dispersion in mineral oil, 0.5 mmol) in DMF (4 mL) at 0 °C was added a solution of diol **17** (30 mg, 0.12 mmol) in DMF (2 mL), followed by BnBr (0.06 mL, 0.5 mmol). The reaction mixture was stirred for 1 h at rt, and then H_2O (1 mL) was added at 0 °C. The reaction mixture was diluted with H_2O (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (2 × 50 mL), dried over $MgSO_4$, and filtered, and the solvent was removed under reduced pressure to give a yellow oil, which was purified by *flash* chromatography (silica gel, hexane/EtOAc, 10:1). Product **18** was obtained as a colorless oil (39.5 mg, 0.09 mmol, 77%).

Data for **18**: $[\alpha]_D^{25}$ +40.6 (*c* 1.85, $CHCl_3$); lit[28] $[\alpha]_D^{20}$ +34 (*c* 2.6, $CHCl_3$); IR (film): υ = 3406, 2922, 2852, 1627, 1599, 1573, 1495, 1462, 1453, 1434, 1371, 1084, 1069, 1025 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.58−2.82 (m, 1H), 3.66 (t, 1H, *J* = 11.2 Hz), 3.83 (t, 1H, *J* = 9.9 Hz), 4.02 (ddd, 1H, *J* = 10.3, 7.0, 0.9 Hz), 4.24−4.41 (m, 2H), 4.55−4.98 (m, 3H), 5.04 (d, 1H, *J* = 11.3 Hz), 5.41 (dt, 1H, *J* = 9.9, 1.8 Hz), 5.63−5.68 (m, 1H), 5.77 (dtd, 1H, *J* = 9.9, 3.0, 0.7 Hz), 7.26−7.56 (m, 15H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 38.7, 70.1, 72.4, 74.8, 80.8, 82.0, 82.3, 101.6, 125.2, 126.1, 127.7−129.0, 138.3, 138.5, 138.8; HRMS calculated for $C_{28}H_{29}O_4$: 429.2066 $[M + H]^+$. Found: 429.2062. HRMS calculated for $C_{28}H_{28}O_4Na$: 451.1885 $[M + Na]^+$. Found: 451.1885. NMR data are in accordance with literature values.[28]

(1*R*,2*R*,5*S*,6*S*)-5,6-Bis(benzyloxy)-2-(hydroxymethyl)cyclohex-3-enol (19). A solution of **18** (32.2 mg, 0.07 mmol) in 80% aqueous AcOH (2 mL) was stirred for 90 min at 40 °C and then concentrated to dryness. The resulting crude was purified by *flash* chromatography (silica gel, hexane/EtOAc, 1:1) to give **19** (19 mg, 0.05 mmol, 74%) as a pale yellow oil.

Data for **19**: $[\alpha]_D^{25}$ +34 (*c* 2.6, $CHCl_3$); $[\alpha]_D^{25}$ +158 (*c* 1.1, acetone); lit[28] $[\alpha]_D^{23}$ +147 (*c* 1.1, acetone); lit[26] $[\alpha]_D$ +104.7 (*c* 1.0, $CHCl_3$); IR (film): υ = 3415, 3088, 3063, 3031, 1649, 1454, 1098, 1052 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.48−2.53 (m, 1H), 3.64−3.72 (m, 3H), 3.79 (dd, 1H, *J* = 10.7, 4.2 Hz), 4.20−4.24 (m, 1H), 4.64 (d, 1H, *J* = 11.5 Hz), 4.72 (t, 2H, *J* = 11.5 Hz), 5.05 (d, 1H, *J* = 11.3 Hz), 5.53 (dt, 1H, *J* = 10.2, 1.9 Hz), 5.80 (dt, 1H, *J* = 10.2, 2.5 Hz), 7.29−7.39 (m, 10H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 45.3, 65.5, 71.6, 72.8, 75.0, 80.3, 83.4, 127.5−128.7, 138.1, 138.5; HRMS calculated for $C_{21}H_{24}O_4Na$: 363.1572 $[M + Na]^+$. Found: 363.1583. NMR data are in accordance with literature values.[26,28]

(1*R*,2*R*,3*S*,4*R*,5*R*,6*S*)-5-Hydroxymethyl-7-oxa-bicyclo[4.1.0]-heptane-2,3,4-triol (20). *m*-CPBA (1.0 g, 4.05 mmol) was added to a solution of (+)-**7** (300 mg, 1.87 mmol) in AcOH (15 mL) at 0 °C. The reaction mixture was stirred at rt overnight. The solvent was removed under reduced pressure, and the residue was triturated with Et_2O to dissolve excess *m*-CPBA and 3-chlorobenzoic acid. The remaining white solid was washed with Et_2O several times and dried at vacuum to give **20** (255 mg, 1.45 mmol, 78%) as a white powder.

Data for **20**: mp 143−145 °C; lit[29] mp 150−152 °C; $[\alpha]_D^{25}$ +79 (*c* 0.4, H_2O); lit[29] $[\alpha]_D^{25}$ +80 (*c* 0.36, H_2O); ^1H NMR (δ, 500 MHz, CD_3OD): 1.92−1.96 (m, 1H), 3.18 (d, 1H, *J* = 4.0 Hz), 3.22 (t, 1H, *J* = 9.9 Hz), 3.27 (dd, 1H, *J* = 3.7, 1.5 Hz), 3.33−3.38 (m, 1H), 3.69−3.74 (m, 2H), 3.88 (dd, 1H, *J* = 10.8, 3.5 Hz); ^{13}C NMR (δ, 100 MHz, CD_3OD): 46.4, 55.3, 58.1, 62.1, 71.3, 73.3, 74.8; HRMS calculated for $C_7H_{12}O_5Na$: 199.0582 $[M + Na]^+$. Found: 199.0575.

(1*S*,2*R*,3*S*,4*R*,5*R*,6*S*)-2,3,4-tris(benzyloxy)-5-(Benzyloxymethyl)-7-oxabicyclo[4.1.0]heptane (6). To a suspension of NaH (350 mg, 60% dispersion in mineral oil, 8.75 mmol) in DMF (15 mL) at 0 °C was added a solution of epoxide **20** (200 mg, 1.13 mmol) in DMF (5 mL), followed by BnBr (0.9 mL, 7.6 mmol). The reaction mixture was stirred for 6 h at rt, and then H_2O (1 mL) was added at 0 °C. The reaction mixture was diluted with H_2O (30 mL) and extracted with Et_2O (3 × 40 mL). The combined organic layers were washed with brine (2 × 100 mL), dried over $MgSO_4$, and filtered, and the solvent was removed under reduced pressure to give a yellow oil, which was purified by *flash* chromatography (silica gel, hexane/EtOAc, 20:1). Product **6** was obtained as a white powder (315 mg, 0.59 mmol, 52%).

Data for (+)-**6**: mp 97−99 °C; lit[31] mp 103−104 °C; $[\alpha]_D^{25}$ +53.5 (*c* 0.50, $CHCl_3$); lit[31] $[\alpha]_D^{25}$ +60 (*c* 0.50, $CHCl_3$); IR (film): υ = 3062, 3029, 2923, 2852, 1737, 1496, 1454, 1363, 1158, 1072, 1027, 735, 697 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.22 (dt, 1H, *J* = 9.7, 3.2 Hz, H-5), 3.17 (d, 1H, *J* = 4.0 Hz, H-6), 3.34 (dd, 1H, *J* = 3.75, 1.4 Hz, H-1), 3.48 (t, 1H, *J* = 9.7 Hz, H-4), 3.56 (d, 2H, *J* = 3.5 Hz, H-8, H-8′), 3.73 (t, 1H, *J* = 9.3 Hz, H-3), 3.89 (dd, 1H, *J* = 8.5, 1.3 Hz, H-2), 4.34−4.88 (m, 8H, 4 × $PhCH_2O$), 7.16−7.42 (m, 20H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 43.0 (C5), 55.0 (C6), 55.2 (C1), 68.4 (C8), 73.0 ($PhCH_2O$), 73.3 ($PhCH_2O$), 75.4 ($PhCH_2O$), 75.9 ($PhCH_2O$), 77.7 (C4), 79.9 (C2), 82.4 (C3), 127.7−128.5 (CHar), 138.0 (Car), 138.5 (Car), 138.6 (Car), 138.8 (Car); HRMS calculated for $C_{35}H_{36}O_5Na$: 559.2460 $[M + Na]^+$. Found: 559.2479.

(1*R*,2*R*,3*S*,6*R*)-6-(Trityloxymethyl)cyclohex-4-ene-1,2,3-triol (21a). To a solution of compound (+)-**7** (0.36 g, 2.25 mmol) in CH_3CN/DMF (20:1, 15 mL) was added tritylpyridinium tetrafluoroborate (1.01 g, 2.47 mmol). The reaction was stirred for 12 h, and then the solvent was removed *in vacuo*. The residue was taken in EtOAc (20 mL) and filtered, and the solid was washed several times with EtOAc. The filtrate and washings were evaporated to dryness under reduced pressure. The resulting residue was purified by flash chromatography (silica gel, hexane/EtOAc, 1:1) to give compound **21a** (702 mg, 1.74 mmol, 77%) as a white solid.

Data for **21a**: mp 118−120 °C; $[\alpha]_D^{25}$ +85.6 (*c* 1.0, $CHCl_3$); ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.58−2.59 (br s, 1H), 3.12−3.16 (m, 1H), 3.42−3.46 (m, 1H), 3.58−3.65 (m, 2H), 4.21−4.22 (m, 1H), 5.35−5.37 (m, 1H), 5.61- 5.64 (m, 1H), 7.27−7.45 (m, 15H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 44.2, 66.5, 72.3, 73.6, 77.6, 87.6, 126.5, 127.4, 128.1, 128.7, 129.5, 143.6; HRMS calculated for $C_{26}H_{26}O_4Na$: 425.1729 $[M + Na]^+$. Found: 425.1728.

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(1*R*,2*R*,3*S*,5*R*,6*R*)-6-((*tert*-Butyldiphenylsilyloxy)methyl)cyclohex-4-ene-1,2,3-triol (21b). Imidazole (148 mg, 2.17 mmol) was added to a stirred solution of (+)-**7** (163 mg, 1.01 mmol) in DMF (10 mL) at 0 °C. After 30 min, DMAP (12 mg, 0.1 mmol) and *tert*-butyldiphenylsilyl chloride (0.34 mL, 1.3 mmol) were added, and the resulting mixture was stirred at 45 °C. After 2 h, the mixture was cooled to 0 °C, quenched by the addition of MeOH (2 mL), and solvents were removed under reduced pressure. The compound **21b** (347 mg, 0.84 mmol, 83%) was isolated as a white solid after *flash* chromatography (silica gel, DCM/MeOH, 20:1).

Data for **21b**: mp 125−127 °C; $[\alpha]_D^{25}$ +92.6 (*c* 0.56, MeOH); IR (film): υ = 3405, 2929, 2857, 1659, 1589, 1472, 1427, 1112, 823, 701 cm^{-1}; ^1H NMR (δ, 500 MHz, CD_3OD): 1.04 (s, 9H), 2.30−2.34 (br s, 1H), 3.41 (dd, 1H, *J* = 10.1, 8.0 Hz), 3.59 (t, 1H, *J* = 9.7), 3.79−3.89 (m, 2H), 4.03−4.06 (m, 1H), 5.58−5.66 (m, 2H), 7.38−7.44 (m, 6H), 7.66−7.68 (m, 4H); ^{13}C NMR (δ, 100 MHz, CD_3OD): 20.2, 27.3, 48.0, 65.0, 71.5, 73.7, 79.0, 128.8, 129.3, 130.8, 130.9, 136.7, 136.7. HRMS calculated for $C_{23}H_{30}O_4SiNa$: 421.1811 $[M + Na]^+$. Found: 421.1808.

(1*S*,2*R*,3*R*,4*R*)-4-Hydroxymethyl-1,2,3-tribenzyloxy-5-cyclohexene (22). (a) To a suspension of NaH (304 mg, 60% dispersion in mineral oil, 7.6 mmol) in DMF (10 mL) at 0 °C was added a solution of **21a** (690 mg, 1.71 mmol) in DMF (10 mL), followed by BnBr (0.9 mL, 7.6 mmol). The reaction mixture was stirred for 30 min at 0 °C, and the mixture was warmed to rt over a period of 30 min, and stirred at rt for an additional 2 h. The mixture was then cooled back to 0 °C and quenched by the addition of H_2O (0.5 mL) The reaction mixture was diluted with H_2O (40 mL) and extracted with Et_2O (3 × 60 mL). The combined organic layers were washed with brine (2 × 100 mL), dried over $MgSO_4$, and filtered, and the solvent was removed under reduced pressure to give a yellow oil. This material was used without further purification. The residue was dissolved in DCM/MeOH (1:4, 10 mL), *p*-toluenesulfonic acid monohydrate (33 mg, 0.17 mmol) was added, and the reaction mixture was stirred at rt. After 3 h, the reaction was stopped by addition of Et_3N (0.05 mL), and then concentrated and purified by *flash* chromatography (silica gel, hexane/EtOAc, 5:1) to give **22** (0.65 g, 1.50 mmol, 88%) as a colorless oil.

(b) To a suspension of NaH (144 mg, 60% dispersion in mineral oil, 3.6 mmol) in DMF (15 mL) at 0 °C was added a solution of **21b** (287 mg, 0.72 mmol) in DMF (5 mL), followed by BnBr (0.5 mL, 4.2 mmol). The reaction mixture was stirred for 2 h at 0 °C, and then H_2O (2 mL) was added at 0 °C. The reaction mixture was diluted with H_2O (30 mL) and extracted with Et_2O (3 × 40 mL). The combined organic layers were washed with brine (2 × 100 mL), dried over $MgSO_4$, and filtered, and the solvent was removed under reduced pressure to give a yellow oil, which was purified by *flash* chromatography (silica gel, hexane/EtOAc, 40:1). The resulting oil (376 mg, 0.56 mmol) was redissolved in THF (15 mL), and TBAF (1.0 M soln. in THF, 1.2 mL, 1.2 mmol) was added at 0 °C. After 30 min at 0 °C, the reaction was stirred at 40 °C for an additional 2 h. The solution was then cooled to 0 °C, and MeOH (0.5 mL) was added. The solvents were removed under reduced pressure, and the crude product was purified by *flash* chromatography (silica gel, hexane/EtOAc, 10:3 to 2:1) to give **22** (217 mg, 70%) as a colorless oil.

Data for **22**: $[\alpha]_D^{25}$ +67.3 (*c* 0.4, $CHCl_3$); lit[21] $[\alpha]_D^{23}$ +104.5 (*c* 1.92, $CHCl_3$); IR (film): υ = 3442, 3063, 3030, 2923, 2853, 2360, 2343, 1496, 1454, 1359 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.47−2.50 (br s, 1H), 3.64−3.71 (m, 3H), 3.85 (dd, 1H, *J* = 10.0, 7.8 Hz), 4.24−4.28 (m, 1H), 4.69 (d, 1H, *J* = 11.2 Hz), 4.73 (s, 2H), 4.94−4.99 (m, 2H), 5.0 (d, 1H, *J* = 11.2 Hz), 5.55 (dt, 1H, *J* = 10.0, 1.8 Hz), 5.76 (dt, 1H, *J* = 10.2, 2.1 Hz), 7.28−7.39 (m, 15H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 45.9, 63.4, 72.3, 75.2, 75.4, 78.7, 80.9, 85.3, 127.7−128.7, 138.4, 138.5, 138.9; HRMS calculated for $C_{28}H_{30}O_4Na$: 453.2042 $[M + Na]^+$. Found: 453.2051. NMR data are consistent with those reported in the literature.[21,32,33]

((1*R*,2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-Tris(benzyloxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methanol ((+)-2,3,4-Tri-*O*-benzylcyclophellitol, 5). *m*-CPBA (370 mg, 70%, 1.5 mmol) was added to a cooled (0 °C) solution of the alkene **23** (219 mg, 0.5 mmol) in DCM (30 mL). The mixture was warmed to rt. After 18 h, the reaction mixture was washed with saturated aqueous $NaHCO_3$ (30 mL) and the aqueous layer extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting residue was purified by *flash* chromatography (silica gel, hexane/EtOAc, 4:1 to 3:1) to afford the desired epoxide **5** (160 mg, 0.36 mmol, 70%) as a white solid, followed by the diastereomeric epoxide (27 mg, 0.06 mmol, 12%).

Data for epoxide **5**: mp 98−100 °C; lit[21] mp 101 °C; lit[36] mp 92−93 °C; $[\alpha]_D^{25}$ +65.8 (*c* 0.9, $CHCl_3$); lit[36] $[\alpha]_D$ +71.0 (*c* 0.9, $CHCl_3$); IR (film): υ = 3356, 2957, 2924, 2854, 1463, 1454, 1070, 736, 694 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.18−2.22 (m, 1H), 3.19 (d, 1H, *J* = 3.7 Hz), 3.36 (d, 1H, *J* = 3.4 Hz), 3.49 (t, 1H, *J* = 10.0 Hz), 3.59 (dd, 1H, *J* = 10.0, 8.0 Hz), 3.89 (dd, 2H, *J* = 11.8, 4.5 Hz), 3.98 (dd, 1H, *J* = 10.8, 4.5 Hz), 4.57 (d, 1H, *J* = 10.9 Hz), 4.74−4.94 (m, 5H), 7.28−7.37 (m, 15H); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 44.1, 53.1, 56.0, 63.0, 73.3, 75.5, 75.7, 75.7, 79.9, 85.1, 127.7−128.7, 137.7, 138.2, 138.7; HRMS calculated for $C_{28}H_{30}O_5Na$: 469.1991 $[M + Na]^+$. Found: 469.1992. ^1H and ^{13}C NMR data are completely consistent with those reported.[21,33,36]

(1*S*,2*R*,3*S*,4*R*,5*R*,6*R*)-5-Hydroxymethyl-7-oxa-bicyclo[4.1.0]heptane-2,3,4-triol (23). In a glass pressure flask, epoxide **5** (60 mg, 0.13 mmol) was dissolved in THF (3 mL) and Pd/C (50 mg, 5−15% Pd on activated C, water-wet) was then added. The flask was repeatedly filled and evacuated with hydrogen and vigorously stirred at room temperature for 14 h under H_2 (1 atm). The reaction mixture was next filtered through a plug of Celite to separate the catalyst, and the filter was washed three times with MeOH. The combined filtrates and washings were concentrated to give **23** (21.5 mg, 0.12 mmol, 91%) as a white solid.

Data for epoxide **23**: mp 132−134 °C; lit[21] mp 145 °C; $[\alpha]_D^{25}$ +101 (*c* 0.5, H_2O); lit[21] $[\alpha]_D^{23}$ +102 (*c* 0.7, H_2O); lit[36] $[\alpha]_D^{27}$ +103 (*c* 0.5, H_2O); ^1H NMR (δ, 500 MHz, CD_3OD): 1.94−2.01 (m, 1H), 3.04−3.08 (m, 2H), 3.20 (dd, 1H, *J* = 9.9, 8.3 Hz), 3.40−3.42 (m, 1H), 3.64 (d, 1H, *J* = 8.2 Hz), 3.68 (dd, 1H, *J* = 10.1, 9.3 Hz), 4.01 (dd, 1H, *J* = 10.5, 4.3 Hz); ^{13}C NMR (δ, 100 MHz, CD_3OD): 45.9, 56.0, 57.4, 62.4, 68.8, 72.8, 78.5; HRMS calculated for $C_7H_{12}O_5Na$: 199.0582 $[M + Na]^+$. Found: 199.0578. NMR data are in accordance with literature values.[21,36]

General Method A: Reaction of epoxides 5 and 6 with nucleophiles in the presence of $LiClO_4$. A 2 N suspension of $LiClO_4$ in anhydrous CH_3CN (3 mL) was added dropwise under an argon atmosphere to the starting epoxides **5** or **6** (0.3 mmol). NaN_3 or the corresponding amine (3.0 mmol) was added next, and the reaction mixture was stirred at 80 °C. After 18 h, the mixture was cooled to rt, quenched by the addition of H_2O (0.5 mL) and concentrated *in vacuo*. The residue was taken up in 30 mL of Et_2O and washed with H_2O (15 mL), and the aqueous layer was extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with brine and dried over $MgSO_4$. Filtration and evaporation afforded crude aminoalcohols, which were purified as indicated below.

*(1R,2S,3S,4S,5R,6S)-3,4,5-Tris(benzyloxy)-6-(hydroxymethyl)-2-(phenethylamino)cyclohexanol (**24a**) and (1R,2S,3S,4R,5R,6S)-2,3,4-Tris(benzyloxy)-5-(hydroxymethyl)-6-(phenethylamino)-cyclohexanol (**25a**).* According to the general method A, the ring-opening of epoxide **5** (141 mg, 0.32 mmol) with 2-phenylethylamine (0.4 mL, 3.2 mmol) afforded a mixture of regioisomers **25a** and **24a** in a ratio of 91:9. Chromatography on silica gel (hexane/EtOAc, 1:2) provided **24a** (14 mg, 0.02 mmol, 8%), followed by **25a** (135 mg, 0.24 mmol, 75%).

Data for **24a**: Pale yellow oil; $[\alpha]_D^{25}$ +41.2 (*c* 1.00, $CHCl_3$); IR (film): υ = 3320, 3103, 3072, 2925, 2851, 1499, 1445, 1348, 1061, 693 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.12−2.20 (m, 1H, H-6), 2.73−2.84 (m, 4H, PhCH_2, NCH_2), 3.07 (t, 1H, *J* = 3.9 Hz, H-2), 3.73 (dd, 1H, *J* = 11.0, 2.5 Hz, H-7), 3.87 (t, 1H, *J* = 8.4 Hz, H-5), 3.91 (t, 1H, *J* = 8.4 Hz, H-4), 3.98 (dd, 1H, *J* = 8.7, 4.2 Hz, H-3), 4.05 (dd, 1H, *J* = 11.0, 3.5 Hz, H-7′), 4.15−4.17 (m, 1H, H-1), 4.50−4.92 (m, 6H, 3 × PhCH_2O), 7.20−7.38 (m, 20 H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 36.6 (PhCH_2), 41.5 (C6), 49.8 (NCH_2), 60.0 (C2), 63.5 (C7), 72.3 (C1), 72.5 (PhCH_2O), 74.7 (PhCH_2O), 75.4 (PhCH_2O), 75.7 (C5), 79.9 (C3), 83.7 (C4), 126.3−128.7 (CHar), 138.6 (Car),

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138.9 (Car), 139.1 (Car), 140.0 (Car); HRMS calculated for $C_{36}H_{42}NO_5$: 568.3063 $[M + H]^+$. Found: 568.3069.

Data for **25a**: Pale yellow oil; $[\alpha]_D^{25}$ +6.8 (*c* 1.00, $CHCl_3$); IR (film): υ = 3389, 3063, 3030, 2924, 2854, 1666, 1496, 1454, 1360, 1066, 1028, 750, 698 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.84−1.90 (m, 1H, H-5), 2.64 (t, 1H, *J* = 10.9 Hz, H-6), 2.72−2.89 (m, 2H, $PhCH_2$), 2.95−3.08 (m, 2H, NCH_2), 3.24 (t, 1H, *J* = 9.9 Hz, H-4), 3.30 (t, 1H, *J* = 9.3 Hz, H-2), 3.54−3.62 (m, 2H, H-1, H-3), 3.66 (dd, 1H, *J* = 10.6, 7.8 Hz, H-7), 3.99 (dd, 1H, *J* = 10.6, 3.0 Hz, H-7′), 4.55−4.98 (m, 6H, 3 × $PhCH_2O$), 7.20−7.38 (m, 20H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 36.3 ($PhCH_2$), 42.6 (C5), 45.6 (NCH_2), 60.5 (C6), 63.9 (C7), 72.4 (C1), 75.5 ($PhCH_2O$), 75.6 ($PhCH_2O$), 75.7 ($PhCH_2O$), 79.5 (C4), 83.8 (C2), 85.6 (C3), 126.6−128.8 (CHar), 138.0 (Car), 138.36 (Car), 138.42 (Car), 138.9 (Car); HRMS calculated for $C_{36}H_{42}NO_5$: 568.3063 $[M + H]^+$. Found: 568.3045.

*(1R,2S,3S,4S,5R,6S)-3,4,5-Tris(benzyloxy)-6-(hydroxymethyl)-2-(octylamino)cyclohexanol (**24b**) and (1R,2S,3S,4R,5R,6S)-2,3,4-Tris(benzyloxy)-5-(hydroxymethyl)-6-(octylamino)cyclohexanol (**25b**).* According to the general method A, the ring-opening of epoxide **5** (131 mg, 0.29 mmol) with octylamine (0.48 mL, 2.9 mmol) afforded a mixture of regioisomers **25b** and **24b** in a ratio of 83:17. Chromatography on silica gel (hexane/EtOAc, 1:2) provided **24b** (22 mg, 0.04 mmol, 13%), followed by **25b** (117 mg, 0.20 mmol, 69%).

Data for **24b**: Pale yellow oil; $[\alpha]_D^{25}$ +38.7 (*c* 0.64, $CHCl_3$); IR (film): υ = 3389, 3081, 3061, 3021, 2954, 2926, 2855, 1471, 1451, 1060, 1023, 661 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.88 (t, 3H, *J* = 6.9 Hz, CH_3), 1.18−1.42 (m, 12H, 6 × CH_2), 2.12−2.20 (m, 1H, H-6), 2.39−2.54 (m, 2H, NCH_2), 3.04 (t, 1H, *J* = 3.8 Hz, H-2), 3.75 (dd, 1H, *J* = 10.9, 2.1 Hz, H-7), 3.87 (t, 1H, *J* = 8.5 Hz, H-5), 3.90 (t, 1H, *J* = 8.5 Hz, H-4), 3.98 (dd, 1H, *J* = 8.5, 4.1 Hz, H-3), 4.06 (dd, 1H, *J* = 10.9, 3.2 Hz, H-7′), 4.15−4.18 (m, 1H, H-1), 4.59−4.91 (m, 6H, 3 × $PhCH_2O$), 7.26−7.38 (m, 15H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 14.3 (CH_3), 22.8 (CH_2), 27.4 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 29.8 (CH_2), 32.0 (CH_2), 41.6 (C6), 48.7 (NCH_2), 60.3 (C2), 63.5 (C7), 72.4 (C1), 72.5 ($PhCH_2O$), 74.7 ($PhCH_2O$), 75.4 ($PhCH_2O$), 75.8 (C5), 80.3 (C3), 83.8 (C4), 127.7−128.7 (CHar), 138.6 (Car), 138.8 (Car), 139.1 (Car); HRMS calculated for $C_{36}H_{50}NO_5$: 576.3689 $[M + H]^+$. Found: 576.3693.

Data for **25b**: Pale yellow oil; $[\alpha]_D^{25}$ +5.1 (*c* 1.00, $CHCl_3$); IR (film): υ = 3389, 3084, 3056, 3026, 2958, 2927, 2852, 1478, 1458, 1081, 1024, 893, 651 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.87 (t, 3H, *J* = 7.1 Hz, CH_3), 1.15−1.55 (m, 12H, 6 × CH_2), 1.95−2.01 (m, 1H, H-5), 2.74−2.88 (m, 3H, H-6, NCH_2), 3.24 (t, 1H, *J* = 9.8 Hz, H-4), 3.33 (t, 1H, *J* = 9.3 Hz, H-2), 3.58 (t, 1H, *J* = 9.3 Hz, H-3), 3.65 (t, 1H, *J* = 9.8 Hz, H-1), 3.70 (dd, 1H, *J* = 10.7, 8.2 Hz, H-7), 4.07 (dd, 1H, *J* = 10.5, 2.7 Hz, H-7′), 4.54−4.97 (m, 6H, 3 × $PhCH_2O$), 7.26−7.36 (m, 15H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 14.2 (CH_3), 22.8 (CH_2), 27.0 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.9 (CH_2), 31.9 (CH_2), 41.8 (C5), 45.0 (NCH_2), 61.0 (C6), 63.7 (C7), 71.8 (C1), 75.6 ($PhCH_2O$), 75.70 ($PhCH_2O$), 75.73 ($PhCH_2O$), 79.0 (C4), 83.4 (C2), 85.3 (C3), 127.9−128.8 (CHar), 137.8 (Car), 138.2 (Car), 138.3 (Car); HRMS calculated for $C_{36}H_{50}NO_5$: 576.3689 $[M + H]^+$. Found: 576.3702.

*(1R,2S,3S,4S,5R,6S)-3,4,5-Tris(benzyloxy)-2-(butylamino)-6-(hydroxymethyl)cyclohexanol (**24c**) and (1R,2S,3S,4R,5R,6S)-2,3,4-tris(benzyloxy)-6-(butylamino)-5-(hydroxymethyl)cyclohexanol (**25c**).* According to the general method A, the ring-opening of epoxide **5** (141 mg, 0.32 mmol) with butylamine (0.32 mL, 3.2 mmol) afforded a mixture of regioisomers **25c** and **24c** in a ratio of 87:13. Chromatography on silica gel (hexane/EtOAc, 1:2) provided **24c** (19 mg, 0.04 mmol, 11%), followed by **25c** (121 mg, 0.23 mmol, 74%).

Data for **24c**: Pale yellow oil; $[\alpha]_D^{25}$ +41.4 (*c* 0.88, $CHCl_3$); IR (film): υ = 3389, 3088, 3063, 3030, 2956, 2924, 2854, 1496, 1454, 1360, 1094, 1067, 1028, 733, 697, 665 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.90 (t, 3H, *J* = 7.3 Hz, CH_3), 1.23−1.54 (m, 4H, 2 × CH_2), 2.14−2.19 (m, 1H, H-6), 2.44−2.57 (m, 2H, CH_2), 3.07 (t, 1H, *J* = 3.6 Hz, H-2), 3.77 (dd, 1H, *J* = 11.0, 2.5 Hz, H-7), 3.89 (t, 1H, *J* = 8.5 Hz, H-5), 3.92 (t, 1H, *J* = 8.4 Hz, H-4), 4.00 (dd, 1H, *J* = 8.6, 4.1 Hz, H-3), 4.07 (dd, 1H, *J* = 11.0, 4.1 Hz, H-7′), 4.17−4.21 (m, 1H, H-1), 4.62−4.93 (m, 6H, 3 × $PhCH_2O$), 7.28−7.38 (m, 15H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 14.1 (CH_3), 20.5 (CH_2), 29.8 (CH_2), 41.6 (C6), 48.4 (NCH_2), 60.3 (C2), 63.5 (C7), 72.3 (C1), 72.5 ($PhCH_2O$), 74.7 ($PhCH_2O$), 75.4 ($PhCH_2O$), 75.8 (C5), 79.8 (C3), 83.7 (C4), 127.6−128.7 (CHar), 138.6 (Car), 138.8 (Car), 139.1 (Car); HRMS calculated for $C_{32}H_{42}NO_5$: 520.3063 $[M + H]^+$. Found: 520.3061.

Data for **25c**: Pale yellow oil; $[\alpha]_D^{25}$ +12.4 (*c* 0.84, $CHCl_3$); IR (film): υ = 3309, 3078, 3051, 3028, 2851, 1493, 1458, 1090, 1065, 1025, 668 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.83−1.54 (m, 7H, CH_3, 2 × CH_2), 2.12−2.18 (m, 1H, H-5), 2.95−3.15 (m, 3H, CH_2, H-6), 3.27 (t, 1H, *J* = 9.7 Hz, H-4), 3.39 (t, 1H, *J* = 9.2 Hz, H-2), 3.61 (t, 1H, *J* = 9.2 Hz, H-3), 3.74 (dd, 1H, *J* = 10.6, 8.9 Hz, H-7), 3.80 (t, 1H, *J* = 9.8 Hz, H-1), 4.16 (dd, 1H, *J* = 10.8, 2.9 Hz, H-7′), 4.53−4.96 (m, 6H, 3 × $PhCH_2O$), 7.26−7.40 (m, 15H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 13.6 (CH_3), 19.7 (CH_2), 29.8 (CH_2), 40.7 (C5), 45.8 (NCH_2), 61.8 (C6), 63.3 (C7), 71.3 (C1), 75.5 ($PhCH_2O$), 75.7 ($PhCH_2O$), 75.8 ($PhCH_2O$), 78.3 (C4), 82.7 (C2), 84.7 (C3), 127.8−128.8 (CHar), 137.6 (Car), 138.0 (Car), 138.1 (Car); HRMS calculated for $C_{32}H_{42}NO_5$: 520.3063 $[M + H]^+$. Found: 520.3080.

*(1R,2S,3S,4S,5R,6S)-2-Azido-3,4,5-tris(benzyloxy)-6-(hydroxymethyl)cyclohexanol (**24e**) and (1R,2S,3S,4R,5R,6S)-6-Azido-2,3,4-tris(benzyloxy)-5-(hydroxymethyl)cyclohexanol (**25e**).* According to the general method A, the ring-opening of epoxide **5** (171 mg, 0.38 mmol) with NaN_3 (0.25 g, 3.8 mmol) afforded a mixture of regioisomers **25e** and **24e** in a ratio of 75:25. Chromatography on silica gel (hexane/EtOAc, 2:1) provided **25e** (115 mg, 0.23 mmol, 61%), followed by **24e** (39 mg, 0.08 mmol, 21%).

Data for **24e**: Colorless oil; $[\alpha]_D^{25}$ +22 (*c* 1.00, $CHCl_3$); IR (film): υ = 3440, 3049, 3022, 2909, 2104, 1714, 1496, 1452 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.71−1.76 (m, 1H, H-6), 3.70−3.74 (m, 1H, H-7), 3.82−3.92 (m, 3H, H-2, H-4, H-5), 4.00 (dd, 1H, *J* = 10.9, 2.3 Hz, H-7′), 4.06−4.08 (m, 2H, H-1, H-3), 4.70−5.00 (m, 6H, 3 × $PhCH_2O$), 7.29−7.40 (m, 15H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 41.6 (C6), 62.8 (C2), 63.0 (C7), 73.1 (C1), 73.5 ($PhCH_2O$), 74.7 (C5), 75.0 ($PhCH_2O$), 76.0 ($PhCH_2O$), 80.3 (C3), 84.3 (C4), 127.7−128.8 (CHar), 138.2 (Car), 138.6 (Car), 138.8 (Car); HRMS calculated for $C_{28}H_{31}N_3O_5Na$: 512.2161 $[M + Na]^+$. Found: 512.2159.

Data for **25e**: Colorless oil; $[\alpha]_D^{25}$ −8.8 (*c* 1.00, $CHCl_3$); IR (film): υ = 3439, 3054, 3026, 2903, 2106, 1953, 1721, 1493, 1455 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.41−1.47 (m, 1H, H-5), 2.57−2.75 (brs, 1H, OH), 3.38−3.45 (m, 2H, H-2, H-6), 3.56−3.62 (m, 3H, H-1, H-3, H-4), 3.79 (dd, 1H, *J* = 10.8, 2.4 Hz, H-7), 3.85 (dd, 1H, *J* = 10.8, 1.6 Hz, H-7′), 4.72−5.02 (m, 6H, 3 × $PhCH_2O$), 7.28−7.40 (m, 15H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 45.2 (C5), 58.3 (C7), 60.9 (C6), 75.3 ($PhCH_2O$), 75.7 ($PhCH_2O$), 75.9 ($PhCH_2O$), 76.3, 76.8, 83.2 (C2), 85.7 (C3), 127.9−128.8 (CHar), 138.1 (Car), 138.27 (Car), 138.35 (Car); HRMS calculated for $C_{28}H_{31}N_3O_5Na$: 512.2161 $[M + Na]^+$. Found: 512.2153.

*(1S,2R,3S,4S,5R,6S)-3,4,5-Tris(benzyloxy)-6-(benzyloxymethyl)-2-(phenethylamino)cyclohexanol (**27a**).* According to the general method A, the ring-opening of epoxide **6** (56 mg, 0.10 mmol) with 2-phenylethylamine (0.13 mL, 1.0 mmol) afforded 51 mg (0.08 mmol, 74%) of **27a** after chromatography on silica gel (hexane/EtOAc, 2:1 to 1:1).

Data for **27a**: Pale yellow oil; $[\alpha]_D^{25}$ +11.6 (*c* 0.43, $CHCl_3$); IR (film): υ = 3331, 3062, 3028, 2923, 2853, 1658, 1495, 1453, 1069, 1028, 876, 750 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.68 (t, 1H, *J* = 10.2 Hz, H-6), 2.49 (t, 1H, *J* = 10.2 Hz, H-2), 2.68−2.77 (m, 2H, $PhCH_2$), 2.91 (dt, 1H, *J* = 11.2, 7.1 Hz, NCH_2), 3.13 (dt, 1H, *J* = 11.3, 7.1 Hz, NCH_2), 3.43 (t, 1H, *J* = 9.2 Hz, H-3), 3.49 (t, 1H, *J* = 10.3 Hz, H-1), 3.60−3.67 (m, 2H, H-4, H-5), 3.80 (dd, 1H, *J* = 8.9, 3.0 Hz, H-7), 3.88 (dd, 1H, *J* = 8.9, 1.9 Hz, H-7′), 4.54−4.99 (m, 8H, 4 × $PhCH_2O$), 7.14−7.34 (m, 25H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 37.1 ($PhCH_2$), 47.2 (C6), 47.9 (NCH_2), 65.2 (C2), 65.9 (C7), 66.9 (C1), 73.4 ($PhCH_2O$), 75.3 ($PhCH_2O$), 75.60 ($PhCH_2O$), 75.62 ($PhCH_2O$), 77.9, 80.7 (C3), 88.0, 126.3−128.9 (CHar), 138.4 (Car), 138.51 (Car), 138.53 (Car), 138.7 (Car), 139.8 (Car); HRMS calculated for $C_{43}H_{48}NO_5$: 658.3532 $[M + H]^+$. Found: 658.3545.

*(1S,2R,3S,4S,5R,6S)-3,4,5-Tris(benzyloxy)-6-(benzyloxymethyl)-2-(octylamino)cyclohexanol (**27b**).* According to the general method A,

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the ring-opening of epoxide **6** (38 mg, 0.07 mmol) with octylamine (0.12 mL, 0.7 mmol) afforded 36 mg (0.05 mmol, 76%) of **27b** after chromatography on silica gel (hexane/EtOAc, 1:1).

Data for **27b**: Pale yellow oil; $[\alpha]_D^{25}$ +9.5 (*c* 0.72, $CHCl_3$); IR (film): υ = 3344, 3088, 3064, 3030, 2924, 2854, 1496, 1454, 1360, 1148, 1068, 1028, 734, 697, 666 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.91 (t, 3H, *J* = 7.0 Hz, CH_3), 1.17−1.38 (m, 12H, 6 × CH_2), 1.70 (t, 1H, *J* = 10.0 Hz, H-6), 2.47 (t, 1H, *J* = 10.2 Hz, H-2), 2.57 (dt, 1H, *J* = 11.0, 7.1 Hz, NCH_2), 2.76 (dt, 1H, *J* = 11.0, 7.2 Hz, NCH_2), 3.47 (t, 1H, *J* = 9.2 Hz, H-3), 3.50 (t, 1H, *J* = 10.2 Hz, H-1), 3.63−3.69 (m, 2H, H-4, H-5), 3.79 (dd, 1H, *J* = 8.9, 2.9 Hz, H-7), 3.90 (d, 1H, *J* = 8.8 Hz, H-7′), 4.48−5.03 (m, 8H, 4 × $PhCH_2O$), 7.21−7.37 (m, 20H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 14.3 (CH_3), 22.8 (CH_2), 27.4 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 30.9 (CH_2), 31.9 (CH_2), 46.6 (NCH_2), 47.3 (C6), 65.1 (C2), 65.9 (C7), 66.8 (C1), 73.4 ($PhCH_2O$), 75.4 ($PhCH_2O$), 75.6 (2 × $PhCH_2O$), 77.9, 80.5 (C3), 88.2, 127.7−128.7 (CHar), 138.50 (Car), 138.53 (Car), 138.55 (Car), 138.72 (Car); HRMS calculated for $C_{43}H_{56}NO_5$: 666.4158 $[M + H]^+$. Found: 666.4181.

*(1S,2R,3S,4S,5R,6S)-3,4,5-Tris(benzyloxy)-6-(benzyloxymethyl)-2-(butylamino)cyclohexanol (**27c**).* According to the general method A, the ring-opening of epoxide **6** (39 mg, 0.07 mmol) with butylamine (0.08 mL, 0.7 mmol) afforded 32 mg (0.05 mmol, 72%) of **27c** after chromatography on silica gel (hexane/EtOAc, 1:1).

Data for **27c**: Pale yellow oil; $[\alpha]_D^{25}$ +8.7 (*c* 0.90, $CHCl_3$); IR (film): υ = 3349, 3088, 3063, 3030, 2956, 2924, 2870, 1496, 1454, 1360, 1145, 1089, 1068, 1028, 734, 697, 666 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.88 (t, 3H, *J* = 7.3 Hz, CH_3), 1.20−1.40 (m, 4H, 2 × CH_2), 1.69 (t, 1H, *J* = 9.0 Hz, H-6), 2.49 (t, 1H, *J* = 10.2 Hz, H-2), 2.60 (dt, 1H, *J* = 11.2, 7.1 Hz, NCH_2), 2.78 (dt, 1H, *J* = 11.0, 7.2 Hz, NCH_2), 3.47−3.55 (m, 2H, H-1, H-3), 3.62−3.67 (m, 2H, H-4, H-5), 3.79 (dd, 1H, *J* = 9.0, 3.2 Hz, H-7), 3.88 (dd, 1H, *J* = 9.0, 1.9 Hz, H-7′), 4.48−5.02 (m, 8H, 4 × $PhCH_2O$), 7.19−7.37 (m, 20H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 14.1 (CH_3), 20.4 (CH_2), 29.8 (CH_2), 46.5 (NCH_2), 47.3 (C6), 65.2 (C2), 66.0 (C7), 66.8 (C1), 73.4 ($PhCH_2O$), 75.4 ($PhCH_2O$), 75.6 (2 × $PhCH_2O$), 77.9, 80.3 (C3), 88.1, 127.7−128.7 (CHar), 138.4 (Car), 138.47 (Car), 138.50 (Car), 138.7 (Car); HRMS calculated for $C_{39}H_{48}NO_5$: 610.3532 $[M + H]^+$. Found: 610.3553.

*(1S,2S,3S,4R,5R,6R)-6-Azido-2,3,4-tris(benzyloxy)-5-(benzyloxymethyl)cyclohexanol (**26e**) and (1S,2R,3S,4S,5R,6S)-2-Azido-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)cyclohexanol (**27e**).* According to the general method A, the ring-opening of epoxide **6** (161 mg, 0.30 mmol) with NaN_3 (0.20 g, 3.0 mmol) afforded a mixture of regioisomers **26e** and **27e** in a ratio of 56:44. Chromatography on silica gel ($CHCl_3$/EtOAc, 40:1) provided **26e** (79 mg, 0.14 mmol, 46%), followed by **27e** (68 mg, 0.12 mmol, 39%).

Data for **26e**: Colorless oil; $[\alpha]_D^{25}$ +27.9 (*c* 0.49, $CHCl_3$); lit[31] $[\alpha]_D^{25}$ +32 (*c* 0.44, $CHCl_3$); IR (film): υ = 3393, 3063, 3030, 2919, 2106, 1454, 1361, 1063, 1027, 735, 698 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.51−2.65 (m, 1H, H-5), 3.46−3.50 (m, 2H, H-4, H-7), 3.72 (dd, 1H, *J* = 9.4, 2.9 Hz, H-2), 3.80−3.84 (m, 2H, H-3, H-7′), 4.10 (t, 1H, *J* = 3.4 Hz, H-1), 4.20 (t, 1H, *J* = 3.4 Hz, H-6), 4.47−4.91 (m, 8H, 4 × $PhCH_2O$), 7.19−7.37 (m, 20H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 40.4 (C5), 61.0 (C6), 67.8 (C7), 68.9 (C1), 73.27 ($PhCH_2O$), 73.30 ($PhCH_2O$), 75.4 ($PhCH_2O$), 75.9 ($PhCH_2O$), 78.4 (C4), 80.3 (C2), 83.4 (C3), 127.7−128.7 (CHar), 137.9 (Car), 138.2 (Car), 138.3 (Car), 138.8 (Car); HRMS calculated for $C_{35}H_{37}N_3O_5Na$: 602.2631 $[M + Na]^+$. Found: 602.2655.

Data for **27e**: Colorless oil; $[\alpha]_D^{25}$ +54.0 (*c* 0.52, $CHCl_3$); lit[31] $[\alpha]_D^{25}$ +61 (*c* 0.4, $CHCl_3$); IR (film): υ = 3390, 3062, 3030, 2922, 2857, 2108, 1452, 1360, 1147, 1063, 1025, 992, 696 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.77−1.82 (m, 1H, H-6), 3.38 (t, 1H, *J* = 9.8 Hz, H-3), 3.41 (t, 1H, *J* = 9.9 Hz, H-2), 3.51 (t, 1H, *J* = 9.6 Hz, H-5), 3.56 (t, 1H, *J* = 9.7 Hz, H-1), 3.64 (t, 1H, *J* = 9.2 Hz, H-4), 3.68 (dd, 1H, *J* = 9.1, 4.6 Hz, H-7), 3.86 (dd, 1H, *J* = 9.0, 2.5 Hz, H-7′), 4.47−4.94 (m, 8H, 4 × $PhCH_2O$), 7.19−7.38 (m, 20H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 46.2 (C6), 66.8 (C7), 69.7 (C1), 70.0 (C2), 73.4 ($PhCH_2O$), 75.7 ($PhCH_2O$), 75.8 ($PhCH_2O$), 76.0 ($PhCH_2O$), 77.3 (C5), 81.3 (C3), 86.3 (C4), 127.7−128.6 (CHar), 137.8 (Car), 137.9 (Car), 138.2 (Car), 138.5 (Car); HRMS calculated for $C_{35}H_{37}N_3O_5Na$: 602.2631 $[M + Na]^+$. Found: 602.2617.

General Method B: Reaction of Epoxide 6 with Nucleophiles under Acidic Conditions. To a solution of the epoxide **6** (100 mg, 0.18 mmol) in a 8:1 MeOH−H_2O mixture (4.5 mL) was added NH_4Cl (59 mg, 1.1 mmol) and the corresponding amine (1.8 mmol). After stirring at 100 °C (48−72 h), the reaction mixture was cooled to rt and quenched with H_2O, and the solvent was then removed *in vacuo*. The residue was taken up in Et_2O and washed with H_2O (10 mL). The aqueous layer was extracted with Et_2O (3 × 20 mL), the combined organic layers were dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure to give a yellow oil.

*(1S,2S,3S,4R,5R,6R)-2,3,4-Tris(benzyloxy)-5-(benzyloxymethyl)-6-(phenethylamino)cyclohexanol (**26a**).* Following the general method B, the ring-opening of epoxide **6** (80 mg, 0.15 mmol) with 2-phenylethylamine (0.19 mL, 1.5 mmol) afforded a mixture of regioisomers **26a** and **27a** in a ratio of 59:41. Chromatography on silica gel (hexane/EtOAc, 4:1 to 1:2) provided **26a** (49 mg, 0.08 mmol, 50%), followed by **27a** (32 mg, 0.05 mmol, 32%).

Data for **26a**: Pale yellow oil; $[\alpha]_D^{25}$ +14.8 (*c* 1.00, $CHCl_3$); IR (film): υ = 3328, 3051, 3027, 2961, 2841, 2095, 1645, 1493, 1058, 1025, 743 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.31−2.33 (m, 1H, H-5), 2.62−2.79 (m, 4H, $PhCH_2$, NCH_2), 3.11−3.13 (m, 1H, H-6), 3.58 (d, 1H, *J* = 9.3 Hz, H-7), 3.85 (d, 1H, *J* = 8.8 Hz, H-3), 3.91−4.0 (m, 4H, H-1, H-2, H-4, H-7′), 4.32−4.95 (m, 8H, 4 × $PhCH_2O$), 7.18−7.37 (m, 25H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 36.7 ($PhCH_2$), 39.9 (C5), 50.1 (NCH_2), 61.1 (C6), 68.6, 70.0 (C7), 73.2 ($PhCH_2O$), 73.3 ($PhCH_2O$), 75.1 ($PhCH_2O$), 75.6 ($PhCH_2O$), 77.8, 80.9, 84.4 (C3), 126.1−128.8 (CHar), 138.2 (Car), 138.5 (Car), 139.1 (Car), 139.2 (Car), 140.3 (Car); HRMS calculated for $C_{43}H_{48}NO_5$: 658.3532 $[M + H]^+$. Found: 658.3533.

*(1S,2S,3S,4R,5R,6R)-2,3,4-Tris(benzyloxy)-5-(benzyloxymethyl)-6-(octylamino)cyclohexanol (**26b**).* Following the general method B, the ring-opening of epoxide **6** (100 mg, 0.18 mmol) with octylamine (0.3 mL, 1.8 mmol) afforded a mixture of regioisomers **26b** and **27b** in a ratio of 59:41. Chromatography on silica gel (hexane/EtOAc, 4:1 to 1:2) provided **26b** (64 mg, 0.09 mmol, 52%), followed by **27b** (41 mg, 0.06 mmol, 33%).

Data for **26b**: Pale yellow oil; $[\alpha]_D^{25}$ +19.4 (*c* 0.72, $CHCl_3$); IR (film): υ = 3341, 3098, 3061, 3025, 2951, 2862, 1483, 1446, 1357, 1134, 1052, 661 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.88 (t, 3H, *J* = 7.1 Hz, CH_3), 1.20−1.33 (m, 12H, 6 × CH_2), 2.27−2.30 (m, 1H, H-5), 2.45 (t, 2H, *J* = 6.8 Hz, NCH_2), 3.05 (t, 1H, *J* = 2.5 Hz, H-6), 3.65 (dd, 1H, *J* = 9.1, 1.1 Hz, H-7), 3.83 (t, 1H, *J* = 9.1 Hz, H-3), 3.97−4.00 (m, 4H, H-1, H-2, H-4, H-7′), 4.46−4.91 (m, 8H, 4 × $PhCH_2O$), 7.22−7.38 (m, 20H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 14.3 (CH_3), 22.8 (CH_2), 27.5 (CH_2), 29.4 (CH_2), 29.7 (CH_2), 30.6 (CH_2), 32.1 (CH_2), 40.0 (C5), 49.1 (NCH_2), 61.7 (C6), 68.7, 70.4 (C7), 73.2 ($PhCH_2O$), 73.4 ($PhCH_2O$), 75.2 ($PhCH_2O$), 75.7 ($PhCH_2O$), 77.9, 81.0, 84.6 (C3), 127.5−129.2 (CHar), 138.2 (Car), 138.5 (Car), 139.2 (Car), 139.3 (Car); HRMS calculated for $C_{43}H_{56}NO_5$: 666.4158 $[M + H]^+$. Found: 666.4164.

*(1S,2S,3S,4R,5R,6R)-2,3,4-Tris(benzyloxy)-5-(benzyloxymethyl)-6-(butylamino)cyclohexanol (**26c**).* Following the general method B, the ring-opening of epoxide **6** (90 mg, 0.17 mmol) with butylamine (0.17 mL, 1.7 mmol) afforded a mixture of regioisomers **26c** and **27c** in a ratio of 55:45. Chromatography on silica gel (hexane/EtOAc, 4:1 to 1:2) provided **26c** (45 mg, 0.07 mmol, 43%), followed by **27c** (36 mg, 0.06 mmol, 36%).

Data for **26c**: Pale yellow oil; $[\alpha]_D^{25}$ +12.5 (*c* 1.00, $CHCl_3$); IR (film): υ = 3394, 3321, 3085, 3068, 3033, 2954, 2864, 1493, 1456, 1066, 1024, 663 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 0.87 (t, 3H, *J* = 6.5 Hz, CH_3), 1.22−1.34 (m, 4H, 2 × CH_2), 2.31−2.34 (m, 1H, H-5), 2.47 (t, 2H, *J* = 6.1 Hz, NCH_2), 3.07−3.09 (m, 1H, H-6), 3.66 (d, 1H, *J* = 9.3 Hz, H-7), 3.85 (t, 1H, *J* = 9.0 Hz, H-3), 4.00−4.05 (m, 4H, H-1, H-2, H-4, H-7′), 4.46−4.95 (m, 8H, 4 × $PhCH_2O$), 7.26−7.39 (m, 20H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 14.1 (CH_3), 20.5 (CH_2), 32.6 (CH_2), 40.0 (C5), 48.6 (NCH_2), 61.7 (C6), 68.7, 70.4 (C7), 73.2 ($PhCH_2O$), 73.5 ($PhCH_2O$), 75.2 ($PhCH_2O$), 75.6 ($PhCH_2O$), 77.9, 80.9, 84.5 (C3), 127.5−128.6 (CHar), 138.2

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(Car), 138.5 (Car), 139.2 (Car), 139.3 (Car); HRMS calculated for $C_{39}H_{48}NO_5$: 610.3532 $[M + H]^+$. Found: 610.3550.

General Method C: Reduction of Azides 24e/25e and 26e/27e: Synthesis of 24d/25d and 26d/27d. $LiAlH_4$ (0.13 mmol) was added in one portion to a solution of the starting azide (0.07 mmol) in anhydrous THF (5 mL) at 0 °C and stirred at this temperature for 1 h. The reaction mixture was then stirred for 1 h at rt. After cooling to 0 °C, the reaction was carefully quenched with dropwise addition of saturated aqueous Na_2SO_4 and the aluminum salts were removed by filtration through Celite. The plug of Celite was washed several times with Et_2O. The combined washings and filtrate were evaporated *in vacuo* to give the desired products.

*(1R,2S,3S,4S,5R,6S)-2-Amino-3,4,5-tris(benzyloxy)-6-(hydroxymethyl)cyclohexanol (**24d**).* According to general method C, amine **24d** was obtained in 87% yield (30 mg, 0.06 mmol) from 36 mg (0.07 mmol) of azide **24e**.

Data for **24d**: Colorless oil; $[\alpha]_D^{25}$ +40.4 (*c* 1.00, $CHCl_3$); IR (film): υ = 3362, 2928, 2924, 2853, 1598, 1461, 1452, 1049, 1027, 696 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.07−2.10 (m, 1H, H-6), 3.39−3.43 (m, 1H, H-2), 3.67−3.71 (brs, 3H, NH_2, OH), 3.77−3.81 (m, 1H, H-7), 3.85−3.93 (m, 3H, H-3, H-4, H-5), 4.04−4.09 (m, 2H, H-1, H-7′), 4.66−4.96 (m, 6H, 3 × $PhCH_2O$), 7.28−7.36 (m, 15H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 41.6 (C6), 53.3 (C2), 63.1 (C7), 72.8 ($PhCH_2O$), 74.8 (C1), 74.9 ($PhCH_2O$), 75.6 ($PhCH_2O$), 76.1 (C5), 80.5 (C3), 83.6 (C4), 127.6−128.7 (CHar), 138.7 (Car), 138.8 (Car), 139.0 (Car); HRMS calculated for $C_{28}H_{34}NO_5$: 464.2437 $[M + H]^+$. Found: 464.2438.

*(1R,2S,3S,4R,5R,6S)-6-Amino-2,3,4-tris(benzyloxy)-5-(hydroxymethyl)cyclohexanol (**25d**).* According to general method C, amine **25d** was obtained in 89% yield (84 mg, 0.18 mmol) from 100 mg (0.20 mmol) of azide **25e**.

Data for **25d**: Colorless oil; $[\alpha]_D^{25}$ +20.6 (*c* 1.00, $CHCl_3$); IR (film): υ = 3356, 3030, 2921, 1586, 1496, 1453 1359, 1065, 1027, 734, 697, 665 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.50−1.61 (m, 1H, H-5), 2.58 (t, 1H, *J* = 10.3 Hz, H-6), 2.70−3.00 (brs, 3H, OH, NH_2), 3.20 (t, 1H, *J* = 9.3 Hz, H-1), 3.31 (t, 1H, *J* = 9.3 Hz, H-2), 3.35 (t, 1H, *J* = 9.8 Hz, H-4), 3.63 (t, 1H, *J* = 9.3 Hz, H-3), 3.76 (dd, 1H, *J* = 10.6, 6.3 Hz, H-7), 4.01 (d, 1H, *J* = 10.5 Hz, H-7′), 4.61−5.00 (m, 6H, 3 × $PhCH_2O$), 7.28−7.40 (m, 15H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 46.4 (C5), 54.4 (C6), 62.5 (C7), 75.5 ($PhCH_2O$), 75.6 ($PhCH_2O$), 75.7 ($PhCH_2O$), 77.2 (C1), 79.2 (C4), 83.6 (C2), 85.9 (C3), 127.8−128.8 (CHar), 138.12 (Car), 138.14 (Car), 138.46 (Car), 138.51 (Car); HRMS calculated for $C_{28}H_{34}NO_5$: 464.2437 $[M + H]^+$. Found: 464.2434.

*(1S,2S,3S,4R,5R,6R)-6-Amino-2,3,4-tris(benzyloxy)-5-(benzyloxymethyl)cyclohexanol (**26d**).* According to general method C, amine **26d** was obtained in 84% yield (56 mg, 0.10 mmol) from 70 mg (0.12 mmol) of azide **26e**.

Data for **26d**: Colorless oil; $[\alpha]_D^{25}$ +30.7 (*c* 0.43, $CHCl_3$); IR (film): υ = 3331, 3062, 3034, 2907, 2863, 1581, 1458, 1149, 683 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 2.30−2.33 (m, 1H, H-5), 3.52−3.54 (m, 1H, H-6), 3.69 (dd, 1H, *J* = 2.4, 9.4 Hz, H-7), 3.83 (t, 1H, *J* = 9.0 Hz, H-3), 3.88−3.91 (m, 2H, H1, H-7′), 3.97 (t, 1H, *J* = 10.0 Hz, H-4), 4.00 (dd, 1H, *J* = 3.0, 9.2 Hz, H-2), 4.45−4.90 (m, 8H, 4 × $PhCH_2O$), 7.21−7.34 (m, 20H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 40.3 (C5), 53.3 (C6), 69.7 (C7), 71.6 (C1), 73.0 ($PhCH_2O$), 73.4 ($PhCH_2O$), 75.2 ($PhCH_2O$), 75.6 ($PhCH_2O$), 77.5 (C4), 80.8 (C2), 84.3 (C3), 127.8−128.6 (CHar), 138.26 (Car), 138.32 (Car), 138.9 (Car), 139.2 (Car); HRMS calculated for $C_{35}H_{40}NO_5$: 554.2906 $[M + H]^+$. Found: 554.2922.

*(1S,2R,3S,4S,5R,6S)-2-Amino-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)cyclohexanol (**27d**).* According to general method C, amine **27d** was obtained in 78% yield (47 mg, 0.08 mmol) from 63 mg (0.11 mmol) of azide **27e**.

Data for **27d**: Colorless oil; $[\alpha]_D^{25}$ +9.4 (*c* 0.18, $CHCl_3$); IR (film): υ = 3366, 3090, 3030, 2917, 2863, 1584, 1481, 1454, 1059, 693 cm^{-1}; ^1H NMR (δ, 500 MHz, $CDCl_3$): 1.77 (t, 1H, *J* = 1 0.7 Hz, H-6), 2.20−2.60 (br s, 3H, OH, NH_2), 2.67 (t, 1H, *J* = 9.8 Hz, H-2), 3.24 (t, 1H, *J* = 9.5 Hz, H-3), 3.46 (t, 1H, *J* = 10.0 Hz, H-1), 3.54 (t, 1H, *J* = 10.1 Hz, H-5), 3.62 (t, 1H, *J* = 9.3 Hz, H-4), 3.69 (dd, 1H, *J* = 4.3, 8.8 Hz, H-7), 3.86 (dd, 1H, *J* = 1.3, 9.0 Hz, H-7′), 4.45−4.99 (m, 8H, 4 × $PhCH_2O$), 7.18−7.37 (m, 20H, Ph); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 47.0 (C6), 59.2 (C2), 67.1 (C7), 70.5 (C1), 73.4 ($PhCH_2O$), 75.6 (2 × $PhCH_2O$), 75.7 ($PhCH_2O$), 78.0 (C5), 83.0 (C3), 87.1 (C4), 127.8−128.7 (CHar), 138.2 (Car), 138.42 (Car), 138.47 (Car), 138.6 (Car); HRMS calculated for $C_{35}H_{40}NO_5$: 554.2906 $[M + H]^+$. Found: 554.2899.

General Method D: Synthesis of Amino Alcohols 1, 2, 4a−c,d, and 3d by Debenzylation with BCl_3. A solution of the corresponding amine (0.21 mmol) in anhydrous DCM (10 mL) at −78 °C was treated with 1 M BCl_3 in heptane (2.5 equiv for OBn group). The reaction mixture was allowed to rt and stirred for an additional 16 h. The mixture was then cooled to −78 °C and quenched with MeOH (0.5 mL). Solvents were then removed under reduced pressure, and EtOAc (3 mL) was added next to the oily residue. After sonication in an ultrasonic bath for 1 min, the suspended solid was collected by filtration and dried at vacuum. Following this protocol, **1**, **2**, **4**, and **3d** were obtained as the corresponding hydrochloride salts.

*(1R,2S,3S,4S,5R,6S)-6-(Hydroxymethyl)-4-(phenethylamino)-cyclohexane-1,2,3,5-tetraol Hydrochloride (**1a**).* Following the general procedure D, from **24a** (13 mg, 0.02 mmol) was obtained **1a** (7 mg, 0.02 mmol, 90%) as a white amorphous solid.

Data for **1a**: mp 89−91 °C; $[\alpha]_D^{25}$ +6.5 (*c* 0.70, CH_3OH); ^1H NMR (δ, 500 MHz, CD_3OD): 1.93−2.05 (m, 1H, H-6), 3.04−3.08 (m, 2H, $PhCH_2$), 3.33−3.36 (m, 2H, NCH_2), 3.54 (t, 1H, *J* = 4.9 Hz, H-4), 3.61 (t, 1H, *J* = 7.3 Hz, H-2), 3.72 (t, 1H, *J* = 7.5 Hz, H-1), 3.85 (dd, 1H, *J* = 10.6, 7.5 Hz, H-7), 3.96 (dd, 1H, *J* = 10.6, 4.8 Hz, H-7′), 4.07 (dd, 1H, *J* = 7.6, 4.5 Hz, H-3), 4.41 (dd, 1H, *J* = 4.7, 4.0 Hz, H-5), 7.26−7.36 (m, 5H, Ph); ^{13}C NMR (δ, 100 MHz, CD_3OD): 32.9 ($PhCH_2$), 46.7 (C6), 49.1 (NCH_2), 60.6 (C7), 62.5 (C4), 65.4 (C5), 69.6 (C3), 71.6 (C1), 75.0 (C2), 128.3 (CHar), 129.7 (CHar), 130.0 (CHar), 137.8 (Car); HRMS calculated for $C_{15}H_{24}NO_5$: 298.1654 $[M + H]^+$. Found: 298.1656.

*(1R,2S,3S,4S,5R,6S)-6-(Hydroxymethyl)-4-(octylamino)-cyclohexane-1,2,3,5-tetraol Hydrochloride (**1b**).* Following the general procedure D, from **24b** (20 mg, 0.03 mmol) was obtained **1b** (11 mg, 0.03 mmol, 91%) as a white amorphous semisolid.

Data for **1b**: $[\alpha]_D^{25}$ +5.4 (*c* 1.00, CH_3OH); ^1H NMR (δ, 500 MHz, CD_3OD): 0.91 (t, 3H, *J* = 6.9 Hz, CH_3), 1.23−1.76 (m, 12H, 6 × CH_2), 1.91−1.95 (m, 1H, H-6), 3.00−3.10 (m, 2H, NCH_2), 3.45 (t, 1H, *J* = 4.9 Hz, H-4), 3.58 (t, 1H, *J* = 7.6 Hz, H-2), 3.71 (t, 1H, *J* = 7.8 Hz, H-1), 3.85 (dd, 1H, *J* = 10.6, 7.4 Hz, H-7), 3.95 (dd, 1H, *J* = 10.6, 4.8 Hz; H-7′), 4.05 (dd, 1H, *J* = 7.9, 4.4 Hz, H-3), 4.33 (t, 1H, *J* = 4.2 Hz, H-5); ^{13}C NMR (δ, 100 MHz, CD_3OD): 14.4 (CH_3), 23.7 (CH_2), 26.6 (CH_2), 27.7 (CH_2), 30.2 (CH_2), 30.22 (CH_2), 32.9 (CH_2), 46.5 (C6), 48.1 (NCH_2), 60.6 (C7), 62.2 (C4), 65.3 (C5), 69.6 (C3), 71.6 (C1), 75.0 (C2); HRMS calculated for $C_{15}H_{32}NO_5$: 306.2280 $[M + H]^+$. Found: 306.2280.

*(1R,2S,3S,4S,5R,6S)-4-(Butylamino)-6-(hydroxymethyl)-cyclohexane-1,2,3,5-tetraol Hydrochloride (**1c**).* Following the general procedure D, from **24c** (17 mg, 0.03 mmol) was obtained **1c** (9 mg, 0.03 mmol, 94%) as a white amorphous solid.

Data for **1c**: mp 87−90 °C; $[\alpha]_D^{25}$ +7.6 (*c* 0.90, CH_3OH); ^1H NMR (δ, 500 MHz, CD_3OD): 1.00 (t, 3H, *J* = 7.4 Hz, CH_3), 1.39−1.75 (m, 4H, 2 × CH_2), 1.92−1.99 (m, 1H, H-6), 3.11 (dd, 2H, *J* = 9.5, 6.9 Hz, NCH_2), 3.46 (t, 1H, *J* = 5.0 Hz, H-4), 3.58 (t, 1H, *J* = 7.4 Hz, H-2), 3.71 (t, 1H, *J* = 7.1 Hz, H-1), 3.84 (dd, 1H, *J* = 10.6, 7.5 Hz, H-7), 3.95 (dd, 1H, *J* = 10.7, 4.8 Hz, H-7′), 4.05 (dd, 1H, *J* = 7.8, 4.5 Hz, H-3), 4.37 (dd, 1H, *J* = 5.2, 4.0 Hz, H-5); ^{13}C NMR (δ, 100 MHz, CD_3OD): 14.0 (CH_3), 21.0 (CH_2), 28.6 (CH_2), 46.5 (C6), 48.0 (NCH_2), 60.7 (C7), 62.2 (C4), 65.4 (C5), 69.6 (C3), 71.6 (C1), 75.1 (C2); HRMS calculated for $C_{11}H_{24}NO_5$: 250.1654 $[M + H]^+$. Found: 250.1647.

*(1R,2S,3S,4S,5R,6S)-4-Amino-6-(hydroxymethyl)cyclohexane-1,2,3,5-tetraol Hydrochloride (**1d**).* Following the general procedure D, from **24d** (25 mg, 0.05 mmol) was obtained **1d** (11 mg, 0.05 mmol, 87%) as a hygroscopic white amorphous solid.

Data for **1d**: $[\alpha]_D^{25}$ +11.1 (*c* 0.60, CH_3OH); ^1H NMR (δ, 500 MHz, CD_3OD): 1.78−1.86 (m, 1H, H-6), 3.45−3.49 (m, 2H, H-2, H-4), 3.62 (t, 1H, *J* = 9.1 Hz, H-1), 3.83 (dd, 1H, *J* = 7.9, 10.5 Hz, H-7),

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3.93−3.97 (m, 2H, H-3, H-7′), 4.24 (t, 1H, J = 3.5 Hz, H-5); ^{13}C NMR (δ, 100 MHz, CD_3OD): 45.4 (C6), 56.9 (C4), 60.8 (C7), 67.2 (C5), 69.5 (C3), 71.2 (C1), 75.6 (C2); HRMS calculated for $C_7H_{16}NO_5$: 194.1028 $[M + H]^+$. Found: 194.1022.

*(1R,2S,3S,4R,5S,6R)-6-(Hydroxymethyl)-5-(phenethylamino)-cyclohexane-1,2,3,4-tetraol Hydrochloride (**2a**).* Following the general procedure D, from **25a** (120 mg, 0.21 mmol) was obtained **2a** (54 mg, 0.18 mmol, 77%) as a white amorphous solid.

Data for **2a**: mp 77−80 °C; $[\alpha]_D^{25}$ +9.6 (c 1.00, CH_3OH); ^{1}H NMR (δ, 500 MHz, CD_3OD): 1.83−1.90 (m, 1H, H-6), 3.00 (t, 2H, J = 7.7 Hz, PhCH_2), 3.14 (t, 1H, J = 9.4 Hz, H-1), 3.16−3.23 (m, 3H, H-2, H-3, H-5), 3.33−3.40 (m, 1H, NCH_2), 3.54−3.60 (m, 2H, NCH_2, H-4), 3.70 (t, 1H, J = 9.8 Hz, H-7), 4.13 (dd, 1H, J = 10.8, 3.3 Hz, H-7′), 7.25−7.36 (m, 5H, Ph); ^{13}C NMR (δ, 100 MHz, CD_3OD): 33.8 (PhCH_2), 42.8 (C6), 48.0 (NCH_2), 63.3 (C5), 63.5 (C7), 71.7 (C1), 72.7 (C4), 76.0, 77.4, 128.4 (CHar), 129.9 (CHar), 130.0 (CHar), 137.6 (Car); HRMS calculated for $C_{15}H_{24}NO_5$: 298.1654 $[M + H]^+$. Found: 298.1646.

*(1R,2S,3S,4R,5S,6R)-6-(Hydroxymethyl)-5-(octylamino)cyclohexane-1,2,3,4-tetraol Hydrochloride (**2b**).* Following the general procedure D, from **25b** (100 mg, 0.17 mmol) was obtained **2b** (55 mg, 0.16 mmol, 92%) as a white amorphous solid.

Data for **2b**: mp 82−85 °C; $[\alpha]_D^{25}$ +11.5 (c 1.00, CH_3OH); ^{1}H NMR (δ, 500 MHz, CD_3OD): 0.91 (t, 3H, J = 6.6 Hz, CH_3), 1.22−1.73 (m, 12H, 6 × CH_2), 1.88−1.94 (m, 1H, H-6), 3.09−3.31 (m, 6H, NCH_2, H-1, H-2, H-3, H-5), 3.57 (dd, 1H, J = 10.2, 8.9 Hz, H-4), 3.77 (dd, 1H, J = 10.4, 8.9 Hz, H-7), 4.16 (dd, 1H, J = 10.8, 3.0 Hz, H-7′); ^{1}H NMR (δ, 400 MHz, D_2O): 0.87 (t, 3H, J = 6.8 Hz, CH_3), 1.21−1.47 (m, 12H, 5 × CH_2), 1.72 (dt, J = 15.1, 7.5 Hz, 2H, NCH$_2$CH_2),1.97−2.08 (m, 1H, H-6), 3.08−3.25 (m, 2H, NCH_2), 3.26−3.42 (H-1, H-2, H-3, H-5), 3.71 (dd, 1H, J = 10.4, 8.9 Hz, H-4), 3.86 (dd, 1H, J = 11.6, 7.4 Hz, H-7), 4.07 (dd, 1H, J = 11.6, 2.9 Hz, H-7′); ^{13}C NMR (δ, 100 MHz, CD_3OD): 14.4 (CH_3), 23.7 (CH_2), 27.5 (CH_2), 27.7 (CH_2), 30.2 (CH_2), 30.2 (CH_2), 32.9 (CH_2), 42.8 (C6), 46.6 (NCH_2), 63.0 (C5), 63.2 (C7), 71.6 (C1), 72.5 (C4), 76.0, 77.4; ^{13}C NMR (δ, 100 MHz, D_2O): 13.3 (CH_3), 21.9 (CH_2), 25.5 (CH_2), 25.8 (CH_2), 28.0 (CH_2), 28.04 (CH_2), 30.9 (CH_2), 40.5 (C6), 44.3 (NCH_2), 59.7 (C5), 60.2 (C7), 69.6 (C1), 70.1 (C4), 73.8, 75.2; HRMS calculated for $C_{15}H_{32}NO_5$: 306.2280 $[M + H]^+$. Found: 306.2268.

*(1R,2S,3S,4R,5S,6R)-5-(Butylamino)-6-(hydroxymethyl)cyclohexane-1,2,3,4-tetraol Hydrochloride (**2c**).* Following the general procedure D, from **25c** (115 mg, 0.22 mmol) was obtained **2c** (56 mg, 0.19 mmol, 88%) as a white amorphous semisolid.

Data for **2c**: $[\alpha]_D^{25}$ +13.9 (c 0.48, CH_3OH); ^{1}H NMR (δ, 500 MHz, CD_3OD): 0.99 (t, 3H, J = 7.4 Hz, CH_3), 1.40−1.71 (m, 4H, 2 × CH_2), 1.86−1.93 (m, 1H, H-6), 3.10−3.30 (m, 6H, NCH_2, H-1, H-2, H-3, H-5), 3.55 (dd, 1H, J = 10.4, 8.8 Hz, H-4), 3.77 (dd, 1H, J = 10.7, 8.7 Hz, H-7), 4.17 (dd, 1H, J = 10.8, 3.2 Hz, H-7′); ^{13}C NMR (δ, 100 MHz, CD_3OD): 13.9 (CH_3), 20.7 (CH_2), 29.7 (CH_2), 42.7 (C6), 46.3 (NCH_2), 63.0 (C5), 63.3 (C7), 71.6, 72.5 (C4), 76.0, 77.5; HRMS calculated for $C_{11}H_{24}NO_5$: 250.1654 $[M + H]^+$. Found: 250.1657.

*(1R,2S,3S,4R,5S,6R)-5-Amino-6-(hydroxymethyl)cyclohexane-1,2,3,4-tetraol Hydrochloride (**2d**).* Following the general procedure D, from **25d** (75 mg, 0.16 mmol) was obtained **2d** (34 mg, 0.15 mmol, 91%) as a white amorphous solid.

Data for **2d**: mp 108−111 °C; $[\alpha]_D^{25}$ +7.2 (c 0.80, CH_3OH); ^{1}H NMR (δ, 500 MHz, CD_3OD): 1.66−1.73 (m, 1H, H-6), 3.05 (t, 1H, J = 10.9 Hz, H-5), 3.16−3.23 (m, 3H, H-1, H-2, H-3), 3.37 (dd, 1H, J = 10.4, 9.3 Hz, H-4), 3.80 (dd, 1H, J = 6.9, 10.9 Hz, H-7), 4.03 (dd, 1H, J = 3.3, 10.9 Hz, H-7′); ^{13}C NMR (δ, 100 MHz, CD_3OD): 44.7 (C6), 56.1 (C5), 62.0 (C7), 71.5, 74.2 (C4), 76.0, 77.8; HRMS calculated for $C_7H_{16}NO_5$: 194.1028 $[M + H]^+$. Found: 194.1021.

*(1R,2S,3S,4S,5R,6R)-5-Amino-6-(hydroxymethyl)cyclohexane-1,2,3,4-tetraol Hydrochloride (**3d**).* Following the general procedure D, from **26d** (50 mg, 0.09 mmol) was obtained **3d** (18 mg, 0.08 mmol, 85%) as a white amorphous solid.

Data for **3d**: mp 98−101 °C; $[\alpha]_D^{25}$ +7.8 (c 1.00, CH_3OH); ^{1}H NMR (δ, 500 MHz, CD_3OD): 2.22−2.26 (m, 1H, H-6), 3.61−3.73 (m, 4H, H-1, H-2, H-3, H-5,), 3.82 (dd, 1H, J = 11.2, 3.2 Hz, H-7), 4.01−4.04 (m, 2H, H-4, H-7′); ^{13}C NMR (δ, 100 MHz, CD_3OD): 42.8 (C6), 61.3 (2) (C5, C7), 67.7 (C4), 71.3, 73.6, 74.2 (C1, C2, C3); HRMS calculated for $C_7H_{16}NO_5$: 194.1028 $[M + H]^+$. Found: 194.1023.

*(1R,2S,3S,4R,5S,6S)-6-(Hydroxymethyl)-4-(phenethylamino)-cyclohexane-1,2,3,5-tetraol Hydrochloride (**4a**).* Following the general procedure D, from **27a** (42 mg, 0.06 mmol) was obtained **4a** (18 mg, 0.05 mmol, 87%) as a white amorphous semisolid.

Data for **4a**: $[\alpha]_D^{25}$ +4.2 (c 1.00, CH_3OH); ^{1}H NMR (δ, 500 MHz, CD_3OD): 1.50 (tt, 1H, J = 10.5, 3.2 Hz, H-6), 3.04−3.10 (m, 3H, PhCH_2, H-4), 3.27−3.38 (m, 3H, H-1, H-2, H-3), 3.39−3.46 (m, 2H, NCH_2), 3.73 (t, 1H, J = 10.5 Hz, H-5), 3.88−3.94 (m, 2H, H-7, H-7′), 7.27−7.36 (m, 5H, Ph); ^{13}C NMR (δ, 100 MHz, CD_3OD): 33.4 (PhCH_2), 47.4 (NCH_2), 50.1 (C6), 58.4 (C7), 65.8 (C5), 66.2 (C4), 70.09 (C3), 70.11 (C1), 79.2 (C2), 128.3 (CHar), 129.8 (CHar), 130.0 (CHar), 137.9 (Car); HRMS calculated for $C_{15}H_{24}NO_5$: 298.1654 $[M + H]^+$. Found: 298.1640.

*(1R,2S,3S,4R,5S,6S)-6-(Hydroxymethyl)-4-(octylamino)-cyclohexane-1,2,3,5-tetraol Hydrochloride (**4b**).* Following the general procedure D, from **27b** (34 mg, 0.05 mmol) was obtained **4b** (16 mg, 0.05 mmol, 92%) as a white amorphous solid.

Data for **4b**: mp 84−86 °C; $[\alpha]_D^{25}$ +7.1 (c 1.00, CH_3OH); ^{1}H NMR (δ, 500 MHz, CD_3OD): 0.91 (t, 3H, J = 6.9 Hz, CH_3), 1.24−1.51 (m, 11H, 5 × CH_2, H-6), 1.71−1.79 (m, 2H, NCH$_2$CH_2), 2.95 (t, 1H, J = 10.6 Hz, H-4), 3.12−3.20 (m, 2H, NCH_2), 3.27−3.46 (m, 3H, H-1, H-2, H-3), 3.71 (t, 1H, J = 10.5 Hz, H-5), 3.89−3.95 (m, 2H, H-7, H-7′); ^{13}C NMR (δ, 100 MHz, CD_3OD): 14.4 (CH_3), 23.7 (CH_2), 27.3 (CH_2), 27.6 (CH_2), 30.2 (CH_2), 33.0 (CH_2), 46.2 (NCH_2), 50.2 (C6), 58.4 (C7), 65.7 (C5), 66.1 (C4), 70.0 (C3), 70.1 (C1), 79.2 (C2); HRMS calculated for $C_{15}H_{32}NO_5$: 306.2280 $[M + H]^+$. Found: 306.2268.

*(1R,2S,3S,4R,5S,6S)-4-(Butylamino)-6-(hydroxymethyl)-cyclohexane-1,2,3,5-tetraol Hydrochloride (**4c**).* Following the general procedure D, from **27c** (25 mg, 0.04 mmol) was obtained **4c** (11 mg, 0.03 mmol, 86%) as a white amorphous semisolid.

Data for **4c**: $[\alpha]_D^{25}$ +4.6 (c 0.72, CH_3OH); ^{1}H NMR (δ, 500 MHz, CD_3OD): 1.00 (t, 3H, J = 7.4 Hz, CH_3), 1.39−1.75 (m, 5H, 2 × CH_2, H-6), 2.99 (t, 1H, J = 10.7 Hz, H-4), 3.13−3.20 (m, 2H, NCH_2), 3.25−3.44 (m, 3H, H-1, H-2, H-3), 3.71 (t, 1H, J = 10.5 Hz, H-5), 3.88−3.93 (m, 2H, H-7, H-7′); ^{13}C NMR (δ, 100 MHz, CD_3OD): 13.9 (CH_3), 20.9 (CH_2), 29.3 (CH_2), 45.8 (NCH_2), 50.2 (C6), 58.4 (C7), 65.7 (C5), 66.1 (C4), 69.9 (C3), 70.1 (C1), 79.2 (C2); HRMS calculated for $C_{11}H_{24}NO_5$: 250.1654 $[M + H]^+$. Found: 250.1646.

*(1R,2S,3S,4R,5S,6S)-4-Amino-6-(hydroxymethyl)cyclohexane-1,2,3,5-tetraol Hydrochloride (**4d**).* Following the general procedure D, from **27d** (45 mg, 0.08 mmol) was obtained **4d** (17 mg, 0.07 mmol, 90%) as a white amorphous solid.

Data for **4d**: mp 110−112 °C; $[\alpha]_D^{25}$ +10.1 (c 0.54, CH_3OH); ^{1}H NMR (δ, 500 MHz, CD_3OD): 1.46 (tt, 1H, J = 10.6, 2.8 Hz, H-6), 2.87 (t, 1H, J = 10.1 Hz, H-4), 3.24−3.27 (m, 2H, H-2, H-3), 3.34−3.39 (m, 1H, H-1), 3.55 (t, 1H, J = 10.2 Hz, H-5), 3.87−3.94 (m, 2H, H-7, H-7′); ^{13}C NMR (δ, 100 MHz, CD_3OD): 50.1 (C6), 58.3 (C7), 60.7 (C4), 67.3 (C5), 70.3 (C1), 71.6, 79.0 (C2, C3); HRMS calculated for $C_7H_{16}NO_5$: 194.1028 $[M + H]^+$. Found: 194.1022.

General Method E: Synthesis of Amino Alcohols 3a−3c by Hydrogenolysis Using Pd/C Catalyst. In a glass pressure flask, the benzylated amino alcohol (0.1 mmol) was dissolved in a mixture of THF (3 mL) and concentrated HCl (4 drops). Pd/C (50 mg, 5−15% Pd on activated C, water-wet) was then added. The flask was repeatedly filled and evacuated with hydrogen and vigorously stirred at rt for 14 h under H_2 (2 atm). After this period, the reaction mixture was filtered through a plug of Celite to separate the catalyst, and the filter was washed three times with MeOH. The filtrate and combined washings were concentrated to give the required amino alcohols.

*(1R,2S,3S,4S,5R,6R)-6-(Hydroxymethyl)-5-(phenethylamino)-cyclohexane-1,2,3,4-tetraol Hydrochloride (**3a**).* Following the general procedure E, compound **3a** was obtained in 92% yield (21 mg, 0.06 mmol) as a white amorphous semisolid from 45 mg (0.07 mmol) of **26a**.

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Data for **3a**: $[\alpha]_D^{25}$ +7.2 (*c* 0.90, CH_3OH); ^1H NMR (*δ*, 500 MHz, CD_3OD): 2.38−2.41 (m, 1H, H-6), 2.97−3.12 (m, 2H, $PhCH_2$), 3.37−3.48 (m, 2H, NCH_2), 3.67−3.72 (m, 1H, H-5), 3.73−3.87 (m, 4H, H-1, H-2, H-3, H-7), 4.17 (dd, 1H, *J* = 11.1, 7.1 Hz, H-7′), 4.20−4.22 (m, 1H, H-4), 7.25−7.36 (m, 5H); ^{13}C NMR (*δ*, 100 MHz, CD_3OD): 33.3 ($PhCH_2$), 42.8 (C6), 50.1 (NCH_2), 61.2 (2) (C5, C7), 67.6 (C4), 71.3, 73.6, 74.1 (C1, C2, C3), 128.3 (CHar), 129.8 (CHar), 130.0 (CHar), 137.7 (Car); HRMS calculated for $C_{15}H_{24}NO_5$: 298.1654 $[M + H]^+$. Found: 298.1630.

*(1R,2S,3S,4S,5R,6R)-6-(Hydroxymethyl)-5-(octylamino)cyclohexane-1,2,3,4-tetraol Hydrochloride (**3b**).* Following the general procedure E, compound **3b** was obtained in 93% yield (26 mg, 0.08 mmol) as a white amorphous semisolid from 55 mg (0.08 mmol) of **26b**.

Data for **3b**: $[\alpha]_D^{25}$ +11.6 (*c* 1.00, CH_3OH); ^1H NMR (*δ*, 500 MHz, CD_3OD): 0.91 (t, 3H, *J* = 6.9 Hz, CH_3), 1.29−1.40 (m, 10H, 5 × CH_2), 1.70−1.76 (m, 2H, NCH_2CH_2), 2.41−2.42 (m, 1H, H-6), 3.11−3.22 (m, 2H, NCH_2), 3.64 (dd, 1H, *J* = 9.1, 4.6 Hz, H-5), 3.74−3.81 (m, 4H, H-1, H-2, H-3, H-7), 4.15−4.19 (m, 2H, H-4, H-7′); ^1H NMR (*δ*, 400 MHz, D_2O): 0.87 (t, 3H, *J* = 5.8 Hz, CH_3), 1.21−1.46 (m, 10H, 5 × CH_2), 1.67−1.79 (m, 2H, NCH_2CH_2), 2.23−2.34 (m, 1H, H-6), 3.12−3.32 (m, 2H, NCH_2), 3.67−3.87 (m, 4H, H-1, H-2, H-3, H5), 3.94 (dd, 1H, *J* = 11.7, 2.3 Hz, H-7), 4.17 (dd, 1H, *J* = 11.6, 5.1 Hz, H-7′), 4.23−4.28 (m, 1H, H-4); ^{13}C NMR (*δ*, 100 MHz, CD_3OD): 14.4 (CH_3), 23.7 (CH_2), 27.2 (CH_2), 27.6 (CH_2), 30.2 (CH_2), 32.9 (CH_2), 42.8 (C6), 49.0 (NCH_2), 61.3 (2) (C5, C7), 67.6 (C4), 71.3, 73.7, 74.1 (C1, C2, C3); ^{13}C NMR (*δ*, 100 MHz, D_2O): 13.3 (CH_3), 21.9 (CH_2), 25.3 (CH_2), 25.4 (CH_2), 28.0 (CH_2), 28.05 (CH_2), 30.9 (CH_2), 39.1 (C6), 48.4 (NCH_2), 59.1 (C7), 60.9 (C5), 66.9 (C4), 68.1, 70.2, 73.5 (C1, C2, C3); HRMS calculated for $C_{15}H_{32}NO_5$: 306.2280 $[M + H]^+$. Found: 306.2276.

*(1R,2S,3S,4S,5R,6R)-5-(Butylamino)-6-(hydroxymethyl)cyclohexane-1,2,3,4-tetraol Hydrochloride (**3c**).* Following the general procedure E, compound **3c** was obtained in 92% yield (17 mg, 0.06 mmol) as a white amorphous solid from 40 mg (0.06 mmol) of **26c**.

Data for **3c**: mp 85−88 °C; $[\alpha]_D^{25}$ +5.0 (*c* 1.00, CH_3OH); ^1H NMR (*δ*, 500 MHz, CD_3OD): 0.99 (t, 3H, *J* = 6.8 Hz, CH_3), 1.40−1.45 (m, 2H, CH_2), 1.67−1.73 (m, 2H, NCH_2CH_2), 2.39−2.41 (m, 1H, H-6), 3.16−3.18 (m, 2H, NCH_2), 3.61−3.65 (m, 1H, H-5), 3.75−3.82 (m, 4H, H-1, H-2, H-3, H-7), 4.16−4.18 (m, 2H, H-4, H-7′); ^{13}C NMR (*δ*, 100 MHz, CD_3OD): 13.9 (CH_3), 20.8 (CH_2), 29.2 (CH_2), 42.7 (C6), 49.0 (NCH_2), 61.3 (2) (C5, C7), 67.6 (C4), 71.2, 73.5, 74.2 (C1, C2, C3); HRMS calculated for $C_{11}H_{24}NO_5$: 250.1654 $[M + H]^+$. Found: 250.1650.

Biological Assays. The glycosidases *α*-glucosidase (from baker's yeast and rice), *β*-glucosidase (from crude almond), *β*-galactosidase (from bovine liver), and *α*-galactosidase (from green coffee beans) that were used in the inhibition studies, as well as 4-methylumbelliferyl-*β*-D-glucoside and the corresponding *p*-nitrophenyl glycoside substrates, were purchased from Sigma. Imiglucerase (Cerezyme; recombinant human GCase analogue) was kindly provided by Genzyme.

General Procedure for the Inhibition Assay against Commercial Enzymes.[13] Commercial glycosidase solutions were prepared with the appropriated buffer and incubated in 96-well plates at 37 °C without (control) or with inhibitor for 5 min. After addition of the corresponding substrate solution, incubations were prolonged for different time periods: 3 min for *β*-glucosidase (from almond) and *α*-glucosidase (from baker's yeast), 5 min for *β*-galactosidase, 10 min for *α*-glucosidase (from rice), and 13 min for *α*-galactosidase and stopped by addition of Tris solution (50 *μ*L, 1 M) or Na_2CO_3 (180 *μ*L, 1 M), depending on the enzymatic inhibition assay. The amount of *p*-nitrophenol formed was determined at 405 nm with a Spectramax M5 (Molecular Devices Corporation) spectrophotometer. For *α*-glucosidase (from rice), the activity was determined with *p*-nitrophenyl-*α*-D-glucopyranoside (1 mM) in sodium acetate buffer (50 mM, pH 5.0). For *α*-glucosidase (from baker'yeast), the activity was determined with *p*-nitrophenyl-*α*-D-glucopyranoside (1 mM) in sodium phosphate buffer (100 mM, pH 7.2). For *β*-glucosidase (from almond), the activity was determined with *p*-nitrophenyl-*β*-D-glucopyranoside (1 mM) in sodium acetate buffer (100 mM, pH 5.0). *β*-Galactosidase activity was determined with *p*-nitrophenyl-*β*-D-galactopyranoside (1 mM) in sodium phosphate buffer (100 mM, 0.1 mM $MgCl_2$, pH 7.2). *α*-Galactosidase activity was determined with *p*-nitrophenyl-*α*-D-galactopyranoside (1 mM) in sodium phosphate buffer (100 mM, pH 6.8). The commercial glycosidase solutions were prepared as follows: *α*-glucosidase (from rice): $(NH_4)_2SO_4$ suspension (30 *μ*L) in buffer (1.8 mL); *α*-glucosidase (from baker's yeast): (0.1 mg mL^{-1} buffer); *β*-glucosidase (from crude almond): (0.1 mg mL^{-1} buffer); *β*-galactosidase from bovine liver (0.5 mg mL^{-1} buffer); *α*-galactosidase (from green coffee beans): 7.4 *μ*L in buffer (1.99 mL).

Glucosylceramide Synthase Assay.[54] A549 Cells were washed with sodium phosphate (PBS) (10 mM, 137 mM NaCl, pH 7.4) and collected by brief trypsinization. The cells were then washed twice with PBS and resuspended in 50 mM TRIS-HCl buffer (pH 7.4) and 10 mM $MgCl_2$ by sonication (three times, 30 s). The cell lysate (100 *μ*L) was incubated with inhibitor (0.25 mM final concentration) for 10 min at 37 °C. Then, 25 *μ*L of NAD (16 mM in TRIS-HCl, pH 7.4 and 10 mM $MgCl_2$), 25 *μ*L of UDP-Glucose (2 mM in TRIS-HCl 50 mM, pH 7.4 and 10 mM $MgCl_2$), and 52 *μ*L of NBD C_6-ceramide complexed to BSA at a 1:1 ratio (20 *μ*M in 50 mM TRIS-HCl buffer, pH = 7.4, 10 mM $MgCl_2$)) were added. After 15 min incubation at 37 °C, the reactions were stopped by adding 800 *μ*L of MeOH and centrifuged at 10 000 rpm for 3 min. The supernatant was transferred to HPLC vials. HPLC analyses were performed with a Waters 2690 Alliance System coupled to a Waters 2475 Fluorescence detector (Milford, MA) using a C18-Kromasil column and eluted with 15% H_2O and 85% CH_3CN, both with a 0.1% of trifluoroacetic acid, flowing at 1 mL/min. The detector was set at an excitation wavelength of 465 nm and measured the emission wavelength at 530 nm. Empower Software (Waters Corporation) was utilized for data acquisition and processing.

Imiglucerase Inhibition Assay. Imiglucerase (Genzyme) activity was determined with 4-methylumbelliferyl-*β*-D-glucopyranoside as previously reported.[13] Briefly, enzyme solutions (25 *μ*L from a stock solution containing 0.1 mg mL^{-1}) in the presence of 0.25% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100 in McIlvaine buffer (100 mM sodium citrate and 200 mM sodium phosphate buffer, pH 5.2) were incubated at 37 °C without (control) or with inhibitor at a final volume of 40 *μ*L for 30 min. After addition of 60 *μ*L of substrate (4 mM, McIlvaine buffer, pH 5.2), the samples were incubated at 37 °C for 10 min. Enzymatic reactions were stopped by the addition of aliquots (150 *μ*L) of glycine/NaOH buffer (100 mM, pH 10.6). The amount of 4-methylumbelliferone formed was determined with a SpectraMax M5 fluorometer (Molecular Devices Corporation) 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. The 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.

Cell Lines and Culture. Wild-type fibroblasts were obtained from Eucellbank (University of Barcelona). Fibroblast cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Invitrogen), 100 U mL^{-1} of penicillin, and 100 *μ*g mL^{-1} of streptomycin at 37 °C in 5% CO_2. Culture medium was replaced every 3−4 days, and all cells used in this study were between the 14th and 30th passages.

Cytotoxicity Assay in Wild-Type Human Fibroblasts.[54] Wild-type fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM; D5796; Sigma-aldrich) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin−streptomycin at 37 °C in 5% CO_2/95% air. Cells used were between the 14th and 30th passage. At the time of the experiments, cells were seeded at a density of 25 000 cells per well in 96-well plates. Media were renewed after 24 h, and compounds were added to give final concentrations of 300−18 *μ*M. All compounds were dissolved in DMSO, and control experiments were performed with DMSO. Cells were incubated at 37 °C in 5% CO_2 for 24 h. Then, the media were replaced with 100 *μ*L of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution, and the mixture was incubated for an additional 3 h at 37 °C in

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5% CO_2/95% air. The number of viable cells was quantified by the estimation of its dehydrogenase activity, which reduces MTT to water-insoluble formazan, which was dissolved in 100 μL of DMSO and measured at 570 nm with a SpectraMax M5 (Molecular Devices Corporation) in 96-well format.

Inhibition Assay of Human Lysosomal Glycosidases.[54,55] Fibroblasts were seeded at a density of 10^5 cells per well in 24-well plates. After 24 h, the media were replaced with fresh media with or without a test compound and incubated at 37 °C in 5% CO_2 for 24 h. The enzyme activity assay was performed after removing media supplemented with the corresponding compound. The monolayers were washed with 100 μL of PBS solution. Then, 80 μL of PBS and 80 μL of 200 mM acetate buffer (pH 4.0) were added to each well. The reactions were started by the addition of 100 μL of substrates (200 mM acetate buffer, pH 4.0) to each well, followed by incubation at 37 °C for 2 h. The substrates were 4-methylumbelliferyl-β-D-glucopyranoside (5 mM, for lysosomal human β-glucosidase) and 4-methylumbelliferyl-α-D-glucopyranoside (5 mM, for lysosomal human α-glucosidase). Enzymatic reactions were stopped by lysing the cells with 1.8 mL of glycine/NaOH buffer (100 mM, pH 10.6). Liberated 4-methylumbelliferone was measured (excitation 355 nm, emission 460 nm) with a SpectraMax M5 fluorometer (Molecular Devices Corporation) in 24-well format. All determinations were performed in triplicate. Cells used were between the 14th and 30th passages.

Computational Methods. Protein complexes were modeled with the package Schrödinger Suite 2014,[56] through its graphical interface Maestro.[57] The program MacroModel[58] with its default force field OPLS 2005,[59] a modified version of the OPLS-AA force field, and GB/SA water solvation conditions[60] were used for all energy calculations. Coordinates of GCase (PDB codes 2V3E[43] and 2NSX[44]) were obtained from the Protein Data Bank[61] at Brookhaven National Laboratory. The program Glide[62] was used for the docking calculations using the default XP precision settings except for a setting of 500 000 poses per ligand for the initial phase of docking, a scoring window of 200 for keeping initial poses, and a limit of 1000 poses per ligand for energy minimization.

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^1H, ^{13}C, and 2D NMR spectra of all new compounds, representative Lineweaver−Burk plots against imiglucerase, % glycosidase inhibition, best docked poses against the structure of human GCase (PDB code: 2V3E) obtained for aminocyclitols **1b**−**4b**, *chiro*-C8, and *scyllo*-C8, as well as docked poses against structure 2NSX. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Asano, N. *Glycobiology* **2003**, *13*, 93R−104R.
(2) Mahmud, T. *Nat. Prod. Rep.* **2003**, *20*, 137−166.
(3) Arjona, O.; Gomez, A. M.; Lopez, J. C.; Plumet, J. *Chem. Rev.* **2007**, *107*, 1919−2036.
(4) Chen, X.; Fan, Y.; Zheng, Y.; Shen, Y. *Chem. Rev.* **2003**, *103*, 1955−1977.
(5) Vértsey, L.; Fehlhaber, H.-W.; Schulz, A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1844−1847.
(6) Horii, S.; Iwasa, T.; Mizuta, E.; Kameda, Y. *J. Antibiot.* **1971**, *24*, 59−63.
(7) Horii, S.; Iwasa, T.; Kameda, Y. *J. Antibiot.* **1971**, *24*, 57−58.
(8) Horii, S.; Fukase, H.; Matsuo, T.; Kameda, Y.; Asano, N.; Matsui, K. *J. Med. Chem.* **1986**, *29*, 1038−1046.
(9) Kameda, Y.; Asano, N.; Yoshikawa, M.; Takeuchi, M.; Yamaguchi, T.; Matsui, K.; Horii, S.; Fukase, H. *J. Antibiot.* **1984**, *37*, 1301−1307.
(10) Schmidt, D. D.; Frommer, W.; Junge, B.; Müller, L.; Wingender, W.; Truscheit, E.; Schafer, D. *Naturwissenschaften* **1977**, *64*, 535−536.
(11) Suzuki, Y.; Ogawa, S.; Sakakibara, Y. *Perspect. Med. Chem.* **2009**, *3*, 7−19.
(12) Egido-Gabás, M.; Canals, D.; Casas, J.; Llebaria, A.; Delgado, A. *ChemMedChem* **2007**, *2*, 992−994.
(13) Egido-Gabás, M.; Serrano, P.; Casas, J.; Llebaria, A.; Delgado, A. *Org. Biomol. Chem.* **2005**, *3*, 1195−1201.
(14) Díaz, L.; Casas, J.; Bujons, J.; Llebaria, A.; Delgado, A. *J. Med. Chem.* **2011**, *54*, 2069−2079.
(15) Trapero, A.; Egido-Gabás, M.; Llebaria, A. *Med. Chem. Commun.* **2013**, *4*, 1584−1589.
(16) Trapero, A.; González-Bulnes, P.; Butters, T. D.; Llebaria, A. *J. Med. Chem.* **2012**, *55*, 4479−4488.
(17) Trapero, A.; Llebaria, A. *Future Med. Chem.* **2013**, *5*, 573−590.
(18) Serrano, P.; Llebaria, A.; Vazquez, J.; de Pablo, J.; Anglada, J. M.; Delgado, A. *Chem.—Eur. J.* **2005**, *11*, 4465−4472.
(19) Trapero, A.; Llebaria, A. *ACS Med. Chem. Lett.* **2011**, *2*, 614−619.
(20) Hoveyda, A. H. *Chem. Rev.* **1993**, *93*, 1307−1370.
(21) Trost, B. M.; Patterson, D. E.; Hembre, E. J. *Chem.—Eur. J.* **2001**, *7*, 3768−3775.
(22) Guo, Z.-X.; Haines, A. H.; Pyke, S. M.; Pyke, S. G.; Taylor, R. J. K. *Carbohydr. Res.* **1994**, *264*, 147−153.
(23) González-Bulnes, P.; Casas, J.; Delgado, A.; Llebaria, A. *Carbohydr. Res.* **2007**, *342*, 1947−1952.
(24) Trost, B. M.; Hembre, E. J. *Tetrahedron Lett.* **1999**, *40*, 219−222.
(25) Ogoshi, S.; Yoshida, W.; Ohe, K.; Murai, S. *Organometallics* **1993**, *12*, 578−579.
(26) Hansen, F. G.; Bundgaard, E.; Madsen, R. *J. Org. Chem.* **2005**, *70*, 10139−10142.
(27) Toyokuni, T.; Ogawa, S.; Suami, T. *Bull. Chem. Soc.* **1983**, *56*, 1161−1170.
(28) Ogawa, S.; Miyamoto, Y.; Nose, T. *J. Chem. Soc., Perkin Trans. 1* **1988**, *9*, 2675−2680.
(29) Tatsuta, K.; Niwata, Y.; Umezawa, K.; Toshima, K.; Nakata, M. *J. Antibiot.* **1991**, *44*, 456−458.
(30) Ohba, K.; Suzuki, K.; Nakata, M. *Carbohydr. Lett.* **1996**, *2*, 175−181.
(31) Tatsuta, K.; Niwata, Y.; Umezawa, K.; Toshima, K.; Nakata, M. *J. Antibiot.* **1991**, *44*, 912−914.
(32) Kireev, A. S.; Breithaupt, A. T.; Collins, W.; Nadein, O. N.; Kornienko, A. *J. Org. Chem.* **2005**, *70*, 742−745.
(33) Ishikawa, T.; Shimizu, Y.; Kudoh, T.; Saito, S. *Org. Lett.* **2003**, *5*, 3879−3882.
(34) (a) Sato, K.; Bokura, M.; Moriyama, H.; Igarashi, T. *Chem. Lett.* **1994**, *23*, 37−40. (b) Shing, T. K. M.; Tai, V. W. F. *J. Chem. Soc., Chem. Commun.* **1994**, *14*, 2017−2025.
(35) Marco-Contelles, J. *Eur. J. Org. Chem.* **2001**, *2001*, 1607−1618.
(36) Ziegler, F. E.; Wang, Y. *J. Org. Chem.* **1998**, *63*, 426−427.
(37) Fürst, A.; Plattner, P. A. *Helv. Chim. Acta* **1949**, *32*, 275−283.
(38) Evans, S. V.; Fellows, L. E.; Shing, T. K. M.; Fleet, G. W. J. *Phytochemistry* **1985**, *24*, 1953−1955.
(39) Kajimoto, T.; Liu, K. K. C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco, J. A.; Wong, C. H. *J. Am. Chem. Soc.* **1991**, *113*, 6187−6196.

DOI: 10.1021/acs.joc.5b00133
J. Org. Chem. 2015, 80, 3512−3529

(40) Asano, N.; Oseki, K.; Kaneko, E.; Matsui, K. *Carbohydr. Res.* **1994**, *258*, 255−266.
(41) 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. *J. Biol. Chem.* **1998**, *273*, 26522−26527.
(42) Godin, G.; Compain, P.; Martin, O. R.; Ikeda, K.; Yu, L.; Asano, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5991−5995.
(43) Brumshtein, B.; Greenblatt, H. M.; Butters, T. D.; Shaaltiel, Y.; Aviezer, D.; Silman, I.; Futerman, A. H.; Sussman, J. L. *J. Biol. Chem.* **2007**, *282*, 29052−29058.
(44) Lieberman, R. L.; Wustman, B. A.; Huertas, P.; Powe, A. C., Jr.; Pine, C. W.; Khanna, R.; Schlossmacher, M. G.; Ringe, D.; Petsko, G. A. *Nat. Chem. Biol.* **2007**, *3*, 101−107.
(45) (a) Ogawa, S.; Kanto, M. *Curr. Top. Med. Chem.* **2009**, *9*, 58−75. (b) Borges de Melo, E.; da Silveira Gomes, A.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277−10302. (c) Dickson, L. G.; Leroy, E.; Reymond, J. L. *Org. Biomol. Chem.* **2004**, *2*, 1217−1226.
(46) Brumshtein, B.; Wormald, M. R.; Silman, I.; Futerman, A. H.; Sussman, J. L. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2006**, *62*, 1458−1465.
(47) Brumshtein, B.; Salinas, P.; Peterson, B.; Chan, V.; Silman, I.; Sussman, J. L.; Savickas, P. J.; Robinson, G. S.; Futerman, A. H. *Glycobiology* **2010**, *20*, 24−32.
(48) Brumshtein, B.; Aguilar-Moncayo, M.; Benito, J. M.; Garcia Fernandez, J. M.; Silman, I.; Shaaltiel, Y.; Aviezer, D.; Sussman, J. L.; Futerman, A. H.; Ortiz Mellet, C. *Org. Biomol. Chem.* **2011**, *9*, 4160−4167.
(49) Lieberman, R. L.; D'Aquino, J. A.; Ringe, D.; Petsko, G. A. *Biochemistry* **2009**, *48*, 4816−4827.
(50) Orwig, S. D.; Tan, Y. L.; Grimster, N. P.; Yu, Z.; Powers, E. T.; Kelly, J. W.; Lieberman, R. L. *Biochemistry* **2011**, *50*, 10647−10657.
(51) Díaz, L.; Bujons, J.; Casas, J.; Llebaria, A.; Delgado, A. *J. Med. Chem.* **2010**, *53*, 5248−5255.
(52) Segel, I. H. *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems*; Wiley: New York, 1975.
(53) Trapero, A.; Llebaria, A. *Future Med. Chem.* **2014**, *6*, 975−978.
(54) Trapero, A.; Alfonso, I.; Butters, T. D.; Llebaria, A. *J. Am. Chem. Soc.* **2011**, *133*, 5474−5484.
(55) Sawkar, A. R.; Cheng, W. C.; Beutler, E.; Wong, C. H.; Balch, W. E.; Kelly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15428−15433.
(56) *Suite 2014 Update 3*; Schrödinger, LLC: New York, NY, 2014.
(57) *Maestro*, version 9.9; Schrödinger, LLC: New York, NY, 2014.
(58) *MacroModel*, version 10.5; Schrödinger, LLC: New York, NY, 2014.
(59) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225−11236.
(60) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127−6129.
(61) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000**, *28*, 235−242.
(62) (a) *Glide*, version 6.4; Schrödinger, LLC: New York, NY, 2014. (b) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. *J. Med. Chem.* **2004**, *47*, 1739−1749. (c) Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. *J. Med. Chem.* **2006**, *49*, 6177−6196. (d) Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. *J. Med. Chem.* **2004**, *47*, 1750−1759.

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J. Org. Chem. 2015, 80, 3512−3529