Structure–Activity Studies of N-Butyl-1-deoxynojirimycin (NB-DNJ) Analogues: Discovery of Potent and Selective Aminocyclopentitol Inhibitors of GBA1 and GBA2

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Introduction

Found widespread in plants and microorganisms, iminosugars were first isolated and synthesized in the 1960s.[1, 2] They differ from monosaccharides in that the endocyclic oxygen atom is replaced by a basic nitrogen and that they lack the anomeric hydroxy group (Figure 1). Iminosugars mimic carbohydrates or their hydrolysis transition state[3] (the intermediate glycosyl (oxycarbenium) cation or the protonated glycoside) and therefore often inhibit carbohydrate-processing enzymes such as glucosidases. With the development of better insight into glycosidases, researchers have synthesized various iminosugars with the hope of finding new inhibitors for these enzymes. In this study, we report the synthesis of N-butyl-1-deoxynojirimycin (NB-DNJ) analogues and their evaluation as inhibitors of ceramide-specific glucosyltransferase (CGT), non-lysosomal β-glucosidase 2 (GBA2) and the lysosomal β-glucosidase 1 (GBA1). Compounds 5a–6f, which carry sterically demanding nitrogen substituents, and compound 13, devoid of the C3 and C5 hydroxy groups present in DNJ/NB-DGJ (N-butyldeoxygalactojirimycin) showed no inhibitory activity for CGT or GBA2. Inversion of stereochemistry at C4 of N-(n-butyl)- and N-(n-nonyl)-DGJ (compounds 24) also led to a loss of activity in these assays. The aminocyclopentitols N-(n-butyl)-, N-(n-nonyl)-4-amino-5-(hydroxymethyl)cyclopentane- and N-(1-(pentyloxy)methyl)adamantan-1-yl)-1,2,3-triol, were found to be selective inhibitors of GBA1 and GBA2 that did not inhibit CGT (>1 mM), with the exception of 35f, which inhibited CGT with an IC$_{50}$ value of 1 μM. The N-butyl analogue 35a was 100-fold selective for inhibiting GBA1 over GBA2 ($K_i$ values of 32 nM and 3.3 μM for GBA1 and GBA2, respectively). The N-nonyl analogue 35b displayed a $K_i$ value of 14 nM for GBA1 inhibition and a $K_i$ of 43 nM for GBA2. The N-(1-(pentyloxy)methyl)adamantan-1-yl) derivative 35f had $K_i$ values of 16 and 14 nM for GBA1 and GBA2, respectively. The related N-bis-substituted aminocyclopentitols were found to be significantly less potent inhibitors than their mono-substituted analogues. The aminocyclopentitol scaffold should hold promise for further inhibitor development.

Figure 1. Structures of iminosugars.
cobiology, an increasing number of new targets have been identified in the search for new therapeutic interventions, and as a result iminosugars have been proposed as source of compounds from which therapeutic agents can be discovered. Based on the natural product deoxynojirimycin (DNJ, Figure 1), two marketed drugs have been developed: miglitol for the treatment of type 2 diabetes and miglustat (N-buty1-1-deoxynojirimycin, NB-DNJ, Figure 1) for the treatment of the lysosomal storage disorders Gaucher’s and Nieman–Pick type 2 diseases. Other iminosugars are in clinical trials for rare diseases such as Pompe’s and Fabry’s diseases. They also hold promise for the treatment of cancer, cystic fibrosis, Parkinson’s and Alzheimer’s diseases, as antibacterial agents, and for dengue viral infections.

The glycosphingolipid metabolic pathway is regulated by several key enzymes that can be inhibited by NB-DNJ: CGT (ceramide-specific glucosyltransferase, Figure 2a), GBA1 (GBA, β-glucocerebrosidase) a lysosomal glucosylceramidase (Figure 2b), GBA2 (β-glucosidase 2, non-lysosomal glucosylceramidase, and bile acid β-glucosidase, Figure 2b), which is membrane bound. These enzymes catalyze the synthesis and degradation of glucosylceramide. In Gaucher’s disease an inherited deficiency of GBA1 leads to lysosomal accumulation of glycosphingolipids, which can be treated by inhibition of CGT with miglustat. Mutation of the GBA2 gene is the cause of hereditary spastic paraplegia and cerebellar ataxia. Miglitol is an α-glycosidase inhibitor that is used for the treatment of type 2 diabetes by preventing the digestion of carbohydrates into monosaccharides. In 2002, it was reported that NB-DNJ and N-butyldexoygalactonojirimycin (NB-DGJ, Figure 1) cause reversible infertility in male C57BL/6 mice. Subsequent studies, however, revealed that these specific compounds were not active in other species, including humans.

Inhibition of these enzymes was initially believed to cause unstable cellular glucosylceramide levels in the testes, hence a dysfunction in glycosphingolipid biosynthesis, to which NB-DNJ’s male contraceptive effect in mice was attributed. It was later reported that NB-DNJ and NB-DGJ inhibit GBA2 more potently than CGT, indicating that inhibition of GBA2 was most likely responsible for the observed contraceptive effect rather than the inhibition of CGT.

The search for inhibitors of the glycosphingolipids metabolic pathway enzymes relies largely on structure–activity relationship studies by synthesizing and screening iminosugar analogues (Figure 3). As documented in the literature, analogues of NB-DNJ showed low potency (micromolar inhibition) when tested as inhibitors of CGT. These studies revealed that an alkyl group on the nitrogen atom is crucial for inhibitory activity; the inhibition increases with the increase of the chain length. The glucose- and galactose-derived iminosugars NB-DNJ and NB-DGJ are active compounds; therefore, the configuration at C5 appears not to be critical. Methyl ether formation at the C4 hydroxy group is detrimental, suggesting that a free hydroxy group at that position is necessary for enzyme inhibition. Removal or acetylation of the hydroxy group of the C6 hydroxymethyl moiety results in a large decrease in activity. The effect of ring size modification of NB-DNJ analogues has also been studied. Synthetic seven- and eight-membered aza sugars were reported as inhibitors of CGT, along with some members of ring contracted 4-membered analogues, and a deoxygenated, C-alkylated five-membered iminosugar. A hydrophobic nojirimycin derivative, N-(5-(adamantan-1-ylmethoxy)pentyl)-1-deoxynojirimycin (AMP-DNJ, AMP-DNM, Figure 1) possesses enhanced potency (>100-fold) relative to NB-DNJ in inhibiting CGT. The highly lipophilic AMP-DNJ was also tested as an inhibitor of GBA1 and GBA2 and dis-
played selective inhibitory activity of 1.7 nm for GBA2 and about 50–200 nm for GBA2. AMP-DNJ has several promising biological effects. AMP-DNJ enhances insulin sensitivity in mice and rats, thus represents a lead for the development of a type 2 diabetes treatment. Miglustat and AMP-DNJ also hold promise for the treatment of cystic fibrosis patients that carry the mutant delF508-CTRF protein, the most common mutation in cystic fibrosis patients. In one study, the effect of miglustat was attributed to the rescue of the delF508-CTRF protein by inhibiting its deglucosylation (inhibition of ER α1,2-glucosidase) and the subsequent del508-CTRF/calnexin interaction, a process that leads to protein degradation. In other structure–activity studies, hydrophobic groups were tethered to six- and seven-membered DNJ analogues, which functioned as pharmacological chaperones to improve folding of mutant GBA1 in Gaucher’s disease and to promote GBA1 trafficking from the endoplasmic reticulum to the lysosome.

Results and Discussion

The sterically demanding hydrophobic adamantyl group is located at the distal position in the AMP-DNJ molecule and related derivatives; however, the effect of such a group proximal to the nojirimycin core is not known. In our research, we therefore appended increasingly bulkier substituents (5a–c, Scheme 1) to the nitrogen to analyze the effect of proximal sterically demanding substituents on inhibitory activity. In addition, an electron-withdrawing N-phenyl group (5d and 5e, Scheme 1) was introduced designed to probe the effect of decreasing basicity of the nitrogen atom and potential interactions with π moieties in the enzyme active site. Additionally, an analogue containing a N-morpholinopiperidine group (5f, Scheme 1) was prepared as a proximal sterically demanding group that is also electronically different. DNJ analogues with modified N-substituents were prepared in four steps as outlined in Scheme 1. Diole 2 was prepared by LAH reduction from tetra-O-benzyl-α-glucopyranose (1) and then was oxidized under Swern conditions to yield an unstable keto aldehyde 3. The crude keto aldehyde intermediate 3 was subjected to a double reductive alkylation to yield tertiary amines 4. The O-benzyl protecting groups were removed via hydrogenolysis to afford the analogues 5a–f.

To generate additional structure–activity relationship information that had not yet been explored, we prepared an analogue of NB-DNJ in which the C3 and C5 hydroxy groups were deleted (compound 13, Scheme 2) and the 4-epi derivatives of NB-DGJ (compounds 24a and 24b, Scheme 3).

For the synthesis of 3,5-dideoxy analogue 13 (Method A in Scheme 2), we applied a synthetic method developed in our group. β-Amino acid 7 was prepared from amino acid derivative 6 via Arndt–Eistert homologation and was then converted into Weinreb amide 8. Because the direct alkylation of 8 was slow and low yielding, we removed the Boc protecting groups.

Scheme 1. Syntheses of N-substituted DNJ analogues 5a–5f.

Scheme 2. Synthesis of DNJ 3,5-dideoxy analogue 13.
The syntheses of N-alkyl-4-epi-DGJ analogues 24a and 24b are shown in Scheme 3. N,O-Protected o-serine 6 was converted into the corresponding Weinreb amide 15, followed by Boc deprotection, and N-allylation of intermediate 16 to furnish 17. Boc re-protection of 17 provided 18, which was subjected to treatment with vinylmagnesium bromide at low temperature to furnish ring-closing metathesis precursor 19. Cyclic enone 20 was obtained using the second-generation Grubbs catalyst in a ring-closing metathesis reaction. 1,2-Reduction of 20 under Luche conditions afforded allylic alcohol 21 as the exclusive product. Allylic alcohol 21 was next subjected to dihydroxylation under Upjohn conditions to yield triol 22 stereoselectively. Deprotection of the Boc group and subsequent reductive alkylation gave N-alkyl triols 23 and hydrogenolysis provided desired products 24a and 24b.

As described below, the novel analogues did not lead to potent inhibition of tested enzymes. We therefore decided to investigate a structurally related but different scaffold. Aminocyclopentitols are known inhibitors of glucosidas and are considered carbohydrate mimics that are missing an oxygen atom in the furanose ring and that carry an exocyclic amino group. While the mechanism of inhibition remains unclear, it has been proposed that, similar to the deoxyajirimycins, they are mimicking either the protonated glycoside or the intermediate glycosyl (oxycarbenium) cation. N-Alkylated aminocyclopentitol analogues generally showed stronger inhibition than primary amines. Regardless, no aminocyclopentitols have been tested as inhibitors of CGT, GBA1, and GBA2. Thus, we designed and prepared aminocyclopentitols that stereochemically resemble NB-DNJ (Scheme 4).

The synthesis of the aminocyclopentitol is shown in Scheme 4. The primary alcohol of methyl-α-D-glucopyranoside (25) was protected as a silyl ether and the three secondary hydroxy groups were protected as benzyl ethers to generate intermediate 27. The TBS group was removed under acidic conditions, and then the unmasked primary alcohol intermediate 28 was converted into iodomethyl derivative 29. Fragmentation of 29 was performed under ultrasonic conditions to afford aldehyde 30. Treatment of aldehyde 30 with hydroxylamine hydrochloride in warm methanol in the presence of sodium bicarbonate provided the corresponding oxime 31 as a mixture of E and Z isomers. An intramolecular 1,3-dipolar cycloaddition of the crude oxime 31 in toluene at reflux furnished cis-fused isoxazolidine 32. The N-O bond was cleaved with activated zinc under acidic aqueous conditions to provide amine 33. The direct reductive alkylation reaction of free amine 33 with butyraldehyde and nonyl aldehyde was low yielding and the reaction products were difficult to purify. Better yields and only mono-alkylated products were obtained when the HCl salt of 33 (Et₃N was used to neutralize the reaction medium) was used, providing improved yields (49–73% and 41–70%, respectively) of 34a and 34b. Subsequently, compounds 34c–34d were prepared using the same protocol. Following that, the benzyl protecting groups of the N-butylnaminocyclopentitols 34 were removed under hydrogenolysis conditions to yield 35a and 35c, while Birch conditions were needed to obtain the N-nonyl derivatives 35b and 35d. For
the synthesis of compound 35f, amino alcohol 33 was subject-
ed to hydrogenolysis to furnish intermediate aminotriol 35e, which was then reacted with 5-(adamantan-1-yl)methoxy)pen-
tenal following a reported procedure[46] and subjected to re-
ductive amination to generate the targeted adamantyl 35f.

Enzyme inhibition studies

The iminosugar analogues were assayed for CGT and GBA2 in-
hibitory activities as described in our previous publications (concentrations up to 1000 μM for both assays) and for other glycosidases with concentrations up to 100 μM.[19] The GBA1 enzymatic inhibition studies are detailed in the experimental section. NB-DNJ was used as the positive control in all assays.

NB-DNJ analogues 5a–5f (Scheme 1), bearing proximal steri-
cally demanding substituents showed no inhibition of CGT or GBA2 at 1000 μM, indicating that such groups are detrimental to inhibitory activity (data not shown). The 3,5-dideoxy ana-
logue 13 (Scheme 2) prepared in this report also lacks inhibitory-
activity for CGT and GBA2, thus supporting earlier findings that tetra-hydroxy substitution is critical for the effective inhibi-
tory activity of these iminosugar analogues.[47] Inversion of con-
figuration of the 3-hydroxy group (analogues 24a and 24b, Scheme 3) also resulted in loss of activity in our assays (1000 μM, data not shown). The aminocyclitols 35 did not inhib-
it CGT at concentrations up to 1000 μM, with the exception of 35f, which inhibited CGT with an IC\textsubscript{50} of 1000 μM. Most of the aminocyclitols 35, however, significantly inhibited GBA2 (Table 1). The N-butyl analogue 35a exhibited 2- and 15-fold increases in potency relative to NB-DGJ and NB-DNJ, respec-
tively. This potency was enhanced with the increase in exocyclic N-alkyl chain length. Compound 35b, in which the nitro-
gen carries a nonyl group, provided approximately 200- and 1000-fold increases in potency compared with control com-
ounds NB-DGJ and NB-DNJ, respectively. Notably, 35b pos-
sesses >10000-fold selectivity for GBA2 (K\textsubscript{I} = 0.043 μM) over CGT, as it did not inhibit this enzyme at 1000 μM (data not shown). Compound 35b is not as potent in inhibiting GBA2 as AMP-DNJ (IC\textsubscript{50} = 1.0 nM) but it is a comparatively more selec-
tive inhibitor for GBA2 than for CGT[30] AMP-DNJ is 200 times more active against GBA2 than CGT.[30] However, several other adamantyl-functionalized DNJ analogues are known that are also highly potent and highly selective for GBA2 over CGT.[48] Bis-substitution at the nitrogen significantly decreased inhibitory-
activity. The di-butyl analogue 35c and the di-nonyl ana-
logue 35d inhibited GBA2 with K\textsubscript{I} values of 95 and 0.89 μM, respectively. The most potent inhibitor was 35f, carrying the 1-
((pentyloxy)methyl)adamantan-1-yl moiety at the nitrogen. Compound 35f inhibited GBA2 with a K\textsubscript{I} of 0.014 μM and the IC\textsubscript{50} for CGT was 1000 μM (data not shown). This compound did not inhibit α-galactosidase, β-galactosidase, α-mannosi-
dase and β-mannosidase at 100 μM. Thus, aminocyclitols 35b and 35f are among the most selective inhibitors of GBA2 over CGT reported to date. We further evaluated the inhibitory properties of the new iminosugars toward other readily available carbohydrate-processing enzymes, α-glucosidase (Saccha-
romyces cerevisiae) and β-glucosidase (almond), α-galactosi-
dase (green coffee beans), β-galactosidase (Escherichia coli), α-
mannosidase (jack bean), and β-mannosidase (Roman snail) as described previously.[19] We found that aminoclopentitols 35a, 35b and 35f showed activity in the glucosidase assays (Table 1) that was an improvement over the standards NB-DNJ and castanospermine. Derivatives 35a and 35b are compar-
able or better inhibitors of both α- and β-glucosidase relative to the controls with a slight anomeric selectivity, favoring β-glucosi-
dase. Compounds 35a and 35b are 4- and 2-fold more potent, respectively, in inhibiting β-glucosidase over α-glucosi-
dase. Compound 35f, the most potent GBA2 inhibitor, was
also the most potent compound in these assays and inhibited α-glucosidase with a $K_i$ of 0.30 μM and β-glucosidase with a $K_i$ of 4.3 μM. However, all three compounds (35a, 35b, and 35f) are more selective for GBA2 inhibition. The three derivatives did not show inhibitory activity for α-galactosidase, α-mannosidase, and β-mannosidase at a concentration of 100 μM (data not shown).

Aminocyclitols 35a and 35f were next tested for inhibition of recombinant human lysosomal β-glucosidase 1 (GBA1) to determine compound selectivity. NB-DNJ and NB-DGJ were used as reference compounds and had $K_i$ values of 34 μM and >1 μM, respectively (Table 1). Under the assay conditions used, NB-DNJ (IC$_{50}$ = 74 μM) was a $\approx$5- to 7-fold more potent inhibitor of GBA1 compared to previously reported IC$_{50}$ values, which ranged from 400 to 520 μM. NB-DGJ (1 mM) did not inhibit GBA1, which is consistent with two of the three previously reported IC$_{50}$ values of 320 μM, $>1$ mM, and $>3$ mM. The N-butyl iminosugar 35a was $>1000$-fold more potent than NB-DNJ and was $\approx$100-fold more potent than GBA1 than GBA2 with a $K_i$ of 32 nM. This finding compares favorably to a recently reported selective GBA1 inhibitor based on a hydroxylated pyrrolidine scaffold, which was a 45-fold selective 2.0 μM inhibitor of GBA1. The nonyl iminosugar 35b appears to titrate the GBA1 enzyme present in the assay, producing complete inhibition of the enzyme at its nominal concentration of 14 nM. Similarly, the adamantyl iminosugar 35f has a measured IC$_{50}$ value of $\approx16$ nM, approximating the nominal GBA1 concentration. Therefore, the inhibitory potencies of both 35b and 35f cannot be accurately determined in this assay. In addition, both iminosugars 35b and 35f produce bi-phasic enzyme inhibition curves, in contrast to NB-DNJ and 35a, which both inhibit GBA1 monophasically. Although the reason for the biphasic 35b and 35f behavior is not known, it is possible that 35b and 35f discriminate between two forms of the enzyme present in approximately equal proportions in the enzyme preparation. For example, 35a and 35f

| Compound | GBA1 (μM) | GBA2 (μM) | α-Glucosidase (yeast) | β-Glucosidase (almond) |
|----------|-----------|-----------|----------------------|-----------------------|
| 35a      | 0.032 ± 0.004 | 3.3 ± 0.4 | 42 ± 2               | 10.6 ± 0.4            |
| 35b      | <0.014$^c$ | 0.043 ± 0.023 | 15 ± 1              | 6.7 ± 0.2            |
| 35c      | n.d. | 95 | n.d. | n.d. |
| 35d      | n.d. | 0.89 | n.d. | n.d. |
| 35f      | ≤0.016$^{b,c}$ | 0.014 ± 0.005 | 0.30 ± 0.02 | 4.3 ± 0.2 |

[a] The details of the enzyme inhibition study for GBA2, α-glycosidase, and β-glycosidase are the same as described in reference [19]. [b] Control experiments were conducted for all assays using the following positive control compounds: $K_i$ (μM) for GBA1: NB-DNJ: 34 ± 3, NB-DGJ: >1000; $K_i$ (μM) for GBA2: NB-DNJ: 48.3 ± 0.2, NB-DGJ: 8.2 ± 1.3; $K_i$ (μM) for α-glucosidase: DNJ: 40 ± 1, NB-DNJ: 515 ± 19, NB-DGJ: 5.0 ± 0.3; $K_i$ (μM) for β-glucosidase: castanospermine: 60 ± 2. Values are the mean ± SEM; n.d.: not determined. [c] Low-affinity component from biphasic curve fit; high-affinity component $K_i$ = 0.20 ± 0.02 μM.
may discriminate between two populations of recombinant GBA1 protein with different levels of glycosylation near the inhibitor binding site. In support of this possible explanation, a Coomassie stain of an SDS-PAGE gel of the recombinant GBA1 reveals two bands of \( m \approx 60-70 \text{ kDa} \) (data not shown). Alternatively, the biphasic behavior for 35b and 35f may somehow be related to the high inhibitory potency of these iminosugars relative to the GBA1 enzyme concentration. Resolution will require a more sensitive activity assay, as a decrease in the GBA1 concentration in the current assay will diminish the signal window to an unacceptable level. The apparent \( K \) values for 35b and 35f in Table 1 reflect the low affinity component of the bi-phasic concentration response.

### Conclusions

In summary, iminosugar analogues with different structural modifications were prepared and assayed for a selection of carbohydrate-processing enzymes. The DNJ analogues 5a–5f with sterically demanding substituents at the nitrogen atom, the 3,4-dideoxy analogue 13, and the 4-epi-DGJ analogues 24 did not inhibit CGT and GBA2 (tested up to 1000 \( \mu \text{M} \)). Analogues 35a, 35b and 35f showed potent inhibition of GBA1 and GBA2 with \( K \) values of 69 nM, \( < 14 \text{ nM} \), and \( \leq 16 \text{ nM} \), respectively. Compound 35a is a 32 nM inhibitor of GBA1 and 100-fold more potent for GBA1 inhibition than for GBA2. Compound 35a is \( \approx 1000\)-fold more potent than NB-DNJ for inhibition of GBA1. The aminocyclitol analogues 35d did not inhibit CGT (tested up to 1000 \( \mu \text{M} \)) with the exception of 35f, which inhibited CGT with an \( IC_{50} \) of 1 mM. The results from this study suggest that the aminocyclitol scaffold may hold promise for future inhibitor development.

### Experimental Section

**Chemistry:** \(^1\text{H}\) NMR spectra were recorded using 400 MHz or 500 MHz spectrometers. \(^13\text{C}\) NMR spectra were recorded using a 100 MHz or a 125 MHz spectrometer. All chemical shifts were recorded as parts per million (ppm), and all samples were dissolved in CDCl\(_3\) using the residual solvent peak as standard internal unless otherwise noted. Mass spectra were obtained from a ZAB HS mass spectrometer equipped with an 11/250 data system. Fast-atom bombardment mass spectrometry (FAB-MS) experiments were performed with a Xenon gun operated at 8 keV energy and 0.8 mA emission. Fast-atom bombardment high-resolution mass spectrometry (FAB-HRMS) data were recorded at 1:10000 resolution using linear voltage scans under data system control and collected in a multi-channel analyzer mode (MCA). A recording infrared spectrophotometer or an FTIR was used to record IR spectra. Optical rotations were obtained using a polarimeter at room temperature. Melting points are uncorrected. All moisture-sensitive reactions were performed using either oven or flame dried glassware under argon or dried by passing the solvent through a drying column. Silica gel (230–400 mesh) was used for flash column chromatography. All compounds were concentrated using a standard rotary evaporator and high-vacuum techniques. The purity of compounds 35a (95%), 35b (> 95%), 35c (> 95%), 35d (> 95%), and 35f (> 95%) was determined by HPLC using an evaporative light scattering detector (ELSD).

**Enzyme assays:** The enzyme preparation and enzymatic assays for GBA2 and CGT were carried out as described in detail previously by us.\(^{15}\) GBA1 activity was measured using recombinant GBA1 enzyme (R&D Systems) and 2.5 mM 4-methylumbelliferyl-\( \beta \)-glucoside (4-MU-\( \beta \)-glucoside) (Sigma) as substrate in 32.5 mM MES, 4 mM CaCl\(_2\) buffer, pH 6.5. The substrate mix and inhibitors were pre-incubated on ice in a 96-well plate and the assay was started with GBA1 (10 ng per well) addition (final volume 100 \( \mu \text{L} \)). The plate was incubated for 20 min at 37 \( ^\circ \text{C} \). The enzymatic reactions were stopped with the addition of 0.3 mM glycine/NaOH buffer, pH 10.1. The amount of liberated 4-methylumbelliferone was determined with a Spectromax fluorimeter (\( \lambda_{\text{ex}} = 365 \text{ nm} / \lambda_{\text{em}} = 445 \text{ nm} \)). A standard curve of 4-methylumbelliferone (Sigma) was determined under the same experimental conditions. A concentration-response of NB-DNJ was included in every experiment as positive control. Iminosugar \( IC_{50} \) values were determined using Prism 6 (GraphPad) from concentration–response curves generated using at least eight concentrations in triplicate. For the biphasic inhibitor 35f, \( IC_{50} \) values were calculated for the high and low concentrations using the biphasic function from 16 concentrations in triplicate. \( K \) values were calculated using the Cheng–Prusoff equation.

**Supporting information:** Synthesis procedures, spectroscopic data, and copies of \(^1\text{H} \) and \(^13\text{C} \) NMR spectra of all new compounds.

**Abbreviations:** AMP-DNJ: N-(S-(adamantan-1-ylmethyl)pentyl)-1-deoxynojirimycin, DNJ: 1-deoxynojirimycin, GBA2: \( \beta \)-glucosidase 2, GBA1: \( \beta \)-glucosidase 1, CGT: ceramide-specific glucosyltransferase, ELSD: evaporative light scattering detector, MCA: multi-channel analyzer mode, NB-DGJ: N-butyl-deoxycagalactojirimycin, NB-DNJ: N-butyl-1-deoxynojirimycin, FAB-HRMS: fast-atom bombardment high-resolution mass spectrometry, FAB-MS: fast-atom bombardment mass spectrometry.

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### Conflict of interest

*The authors declare no conflict of interest.*

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