ChemPubSoc Europe

DOI: 10.1002/cmdc.201700558

CHEMMEDCHEM Full Papers

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

Xingxian Gu,[a, b] Vijayalaxmi Gupta,[c] Yan Yang,[d] Jin-Yi Zhu,[d] Erick J. Carlson,[b] Carolyn Kingsley,[b] Joseph S. Tash,[c] Ernst Schönbrunn,[d] Jon Hawkinson,[b] and Gunda I. Georg*[b]

Analogues of *N*-butyl-1-deoxynojirimycin (*N*B-DNJ) were prepared and assayed for inhibition 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/*N*B-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)- (**35a**), *N*-(*n*-nonyl)-4-amino-5-(hydroxymethyl)cyclopentane- (**35b**), and *N*-(1-(pentyloxy)methyl)adamantan-1-yl)-1,2,3-triol (**35f**), 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 mM. 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.

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 gly-

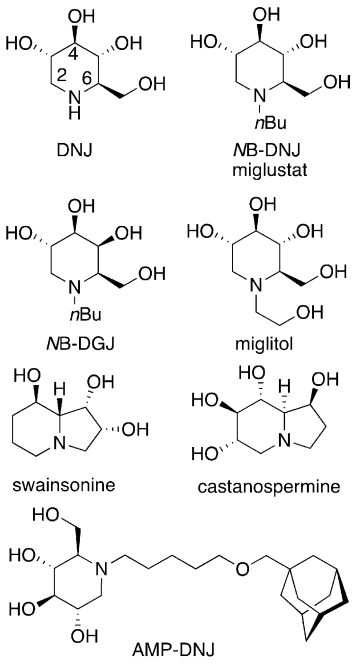


Figure 1. Structures of iminosugars.

[a] *Dr. X. Gu*
Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045 (USA)

[b] *Dr. X. Gu, E. J. Carlson, Dr. C. Kingsley, Prof. Dr. J. Hawkinson, Prof. Dr. G. I. Georg*
Department of Medicinal Chemistry and Institute for Therapeutics Discovery and Development, University of Minnesota, Minneapolis, MN 55414 (USA)
E-mail: georg@umn.edu

[c] *Dr. V. Gupta, Prof. J. S. Tash*
University of Kansas Medical Center, Kansas City, KS 66160 (USA)

[d] *Dr. Y. Yang, Dr. J.-Y. Zhu, Prof. Dr. E. Schönbrunn*
H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL 33612 (USA)

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/cmdc.201700558.

cobiology, an increasing number of new targets have been identified in the search for new therapeutic interventions,[4,5] and as a result iminosugars have been proposed as source of compounds from which therapeutic agents can be discovered.[5,6] 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*-butyl-1-deoxynojirimycin, *N*B-DNJ, Figure 1) for the treatment of the lysosomal storage disorders Gaucher's and Nieman–Pick type 2 diseases.[7] 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.[7,8]

The glycosphingolipid metabolic pathway is regulated by several key enzymes that can be inhibited by *N*B-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.[9,10] 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 *N*B-DNJ and *N*-butyldeoxygalactonojirimycin (*N*B-DGJ, Figure 1) cause reversible infertility in male C57BL/6 mice.[11] Subsequent studies, however, revealed that these specific compounds were not active in other species, including humans.[12,13]

Inhibition of these enzymes was initially believed to cause unstable cellular glucosylceramide levels in the testes, hence a dysfunction in glycosphingolipids biosynthesis, to which *N*B-DNJ's male contraceptive effect in mice was attributed. It was later reported that *N*B-DNJ and *N*B-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.[10]

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

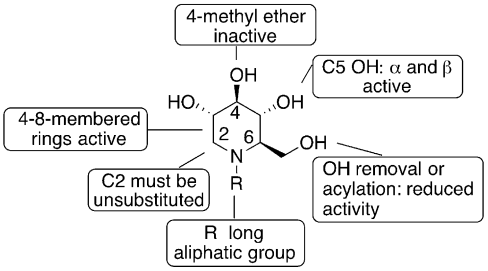


Figure 3. Known SAR for CGT inhibition with iminosugars.

of *N*B-DNJ showed low potency (micromolar inhibition) when tested as inhibitors of CGT.[14,15] 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.[15] The glucose- and galactose-derived iminosugars *N*B-DNJ and *N*B-DGJ are active compounds; therefore, the configuration at C5 appears not to be critical.[15,16] *N*-Butyl-α-homo-nojirimycin and other C2 substituted analogues are inactive, indicating that the C2 position needs to remain unsubstituted.[16] Methyl ether formation at the C4 hydroxy group is detrimental, suggesting that a free hydroxy group at that position is necessary for enzyme inhibition.[15] Removal or acetylation of the hydroxy group of the C6 hydroxymethyl moiety results in a large decrease in activity.[14] The effect of ring size modification of *N*B-DNJ analogues has also been studied. Synthetic seven-[17,18] and eight-membered[19] aza sugars were reported as inhibitors of CGT, along with some members of ring contracted 4-membered[19] analogues, and a deoxygenated, C-alkylated five-membered iminosugar.[20]

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 *N*B-DNJ in inhibiting CGT.[21,22] The highly lipophilic AMP-DNJ was also tested as an inhibitor of GBA1 and GBA2 and dis-

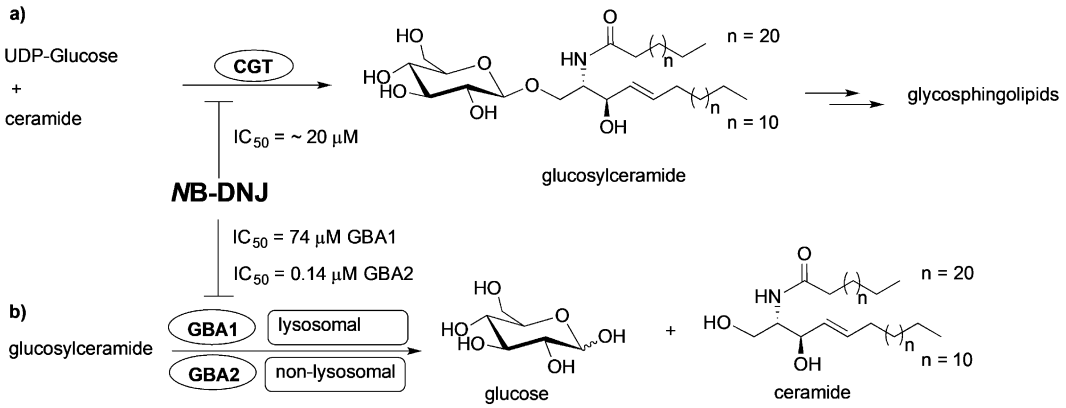


Figure 2. Inhibitory effects of *N*B-DNJ for CGT, GBA1, and GBA2.

played selective inhibitory activity of 1.7 nM for GBA2 and about 50–200 nM for GBA2.[23,24] 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.[25] Miglustat[26] 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 α-1,2-glucosidase) and the subsequent del508-CFTR/calnexin interaction, a process that leads to protein degradation.[26] In other structure–activity studies, hydrophobic groups were tethered to six- and seven-membered DNJ analogues,[27,28] 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.[27–29]

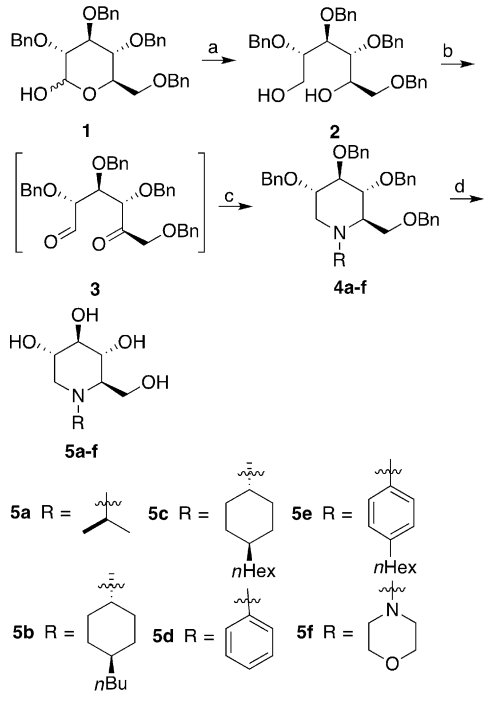
Results and Discussion

The sterically demanding hydrophobic adamantyl group is located at the distal position in the AMP-DNJ molecule and related derivatives;[30] 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.[31] Diol **2** was prepared by LAH reduction from tetra-*O*-benzyl-D-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**.

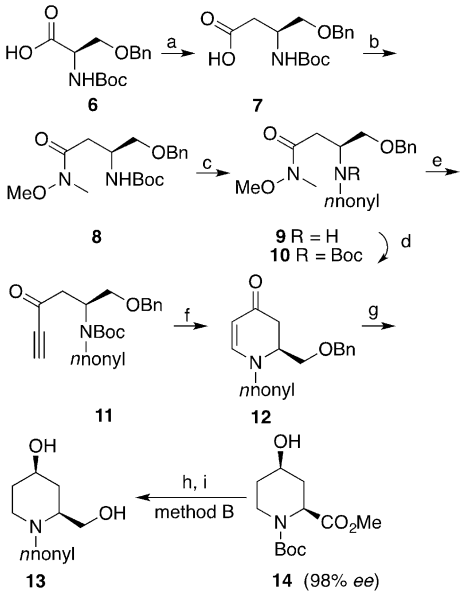
To generate additional structure–activity relationship information that had not yet been explored, we prepared an analogue of *N*B-DNJ in which the C3 and C5 hydroxy groups were deleted (compound **13**, Scheme 2) and the 4-epi derivatives of *N*B-DGJ (compounds **24a** and **24b**, Scheme 3).

For the synthesis of 3,5-dideoxy analogue **13** (Method A in Scheme 2), we applied a synthetic method developed in our group.[32] β-Amino acid **7** was prepared from amino acid derivative **6** via Arndt–Eistert homologation[33,34] and was then converted into Weinreb amide **8**. Because the direct alkylation of **8** was slow and low yielding, we removed the Boc protecting



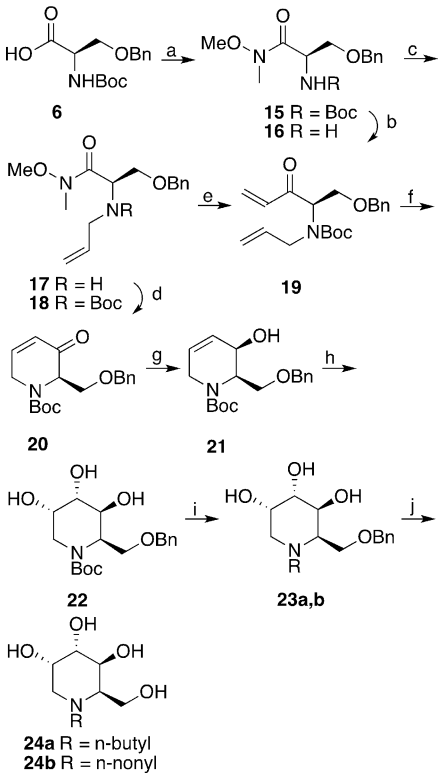
[a]Reagents and conditions: (a) LAH, THF, 0 °C to rt, overnight, 95%; (b) oxalyl chloride, TEA, DMSO, CH_2Cl_2, -78 °C; (c) RNH_2, $NaBH_3CN$, MeOH, molecular sieves 4Å, 44 - 65%; (d) H_2, $PdCl_2$, 37 - 78%.

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



[a]Reagents and conditions: **Method A** (a) 1. $ClCO_2Et$, 2. CH_2N_2, 3. CF_3CO_2Ag, 87%; (b) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, MeNHOMe•HCl, NMM, 91%; (c) 1. HCl, dioxane, 2. $NaBH(OAc)_3$, nonylaldehyde, DCE, 78%; (d) Boc_2O, DMAP, MeCN, 68%; (e) HCCMgBr, 82%; (f) 1. HCl, dioxane, 2. K_2CO_3, MeOH, 92%; (g) 1. $NaBH_4$, EtOH, 2. H_2, Pd/C, 45%; **Method B** (h) 1. HCl, dioxane, 2. *n*-nonylaldehyde, $NaB(OAc)_3H$, Et_3N, DCE, 80%; (i) DIBALH, toluene, 40%.

Scheme 2. Synthesis of DNJ 3,5-dideoxy analogue **13**.[a]



[a]Reagents and conditions: (a) MeNHOMe•HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, *N*-methylmorpholine, 0 °C to rt, 90%; (b) HCl, dioxane, (c) DIPEA, allyl bromide, MeOH, 50 °C, 50%; (d) $(Boc)_2O$, EtOAC, 94%; (e) $CH_2CHMgBr$, THF, - 78 to 0 °C, 90%; (f) Grubbs catalyst, 2nd generation, CH_2Cl_2, 40 °C, 75%; (g) $NaBH_4$, $CeCl_3$, MeOH, -78 °C to rt, 15 min, 92%; (h) OsO_4, NMO, CH_2Cl_2, H_2O, 0 °C to rt, 78%; (i) 1. HCO_2H, 2. $NaB(OAc)_3H$, butyraldehyde/nonylaldehyde, 73%, 71%,respectively; (j) Pd/C, HCO_2NH_4, MeOH, reflux, 66%, 88%, respectively.

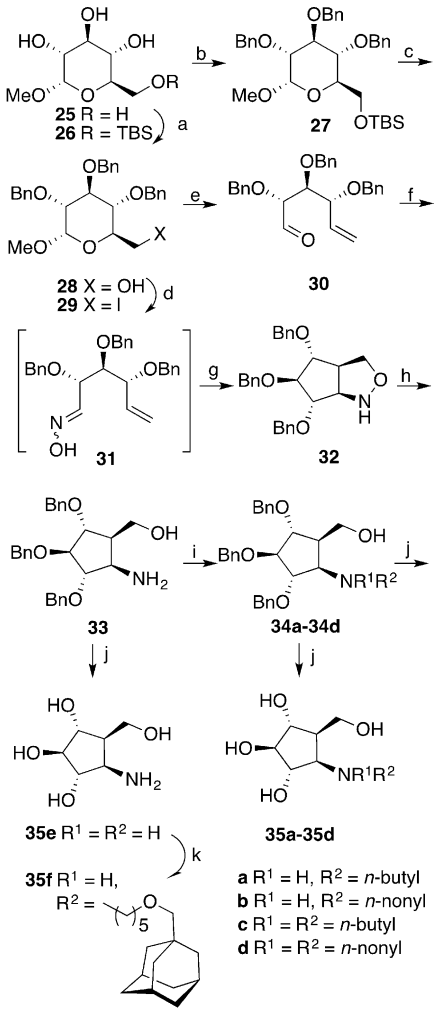
Scheme 3. Syntheses of *N*-alkyl-4-*epi*-DGJ analogues **24a** and **24b**.[a]

group to obtain the free amine, which was then subjected to reductive alkylation to yield **9**. Boc protection then furnished intermediate **10**. Ynone **11** was obtained upon treatment of **10** with ethynylmagnesium bromide. After removal of the *N*-Boc protecting group of **11** and subsequent treatment with K_2CO_3, a rapid ring closure occurred to yield key intermediate enaminone **12**. A two-step, one-flask reduction (sodium borohydride, followed by hydrogenolysis) of **12** completed the synthesis of the desired analogue **13**. The configuration of the 3-OH group was confirmed by comparing the NMR spectra of **13** with a sample derived from the commercially available pipecolic acid derivative **14** (Method B in Scheme 2). Although enantiopurity can be compromised occasionally during the cyclization step to form the enaminone,[35] it was well preserved in intermediate **12**. The specific rotations of **13** prepared by Method A was compared with that of **13** prepared by Method B, and they were $[\alpha] = -22$, $c = 0.75$, MeOH, and $[\alpha] = -21$, $c = 0.97$, MeOH, respectively, suggesting that no racemization took place during the cyclization reaction.

The syntheses of *N*-alkyl-4-*epi*-DGJ analogues **24a** and **24b** are shown in Scheme 3. *N*,*O*-Protected-D-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 glucosidases and are considered carbohydrate mimics that are missing an oxygen atom in the furanose ring and that carry an exocyclic amino group.[36] While the mechanism of inhibition remains unclear, it has been proposed that, similar to the deoxynojirimycins, they are mimicking either the protonated glycoside[37,38] or the intermediate glycosyl (oxycarbenium) cation.[39] N-Alkylated aminocyclopentitol analogues generally showed stronger inhibition than primary amines.[40] Regardless, no aminocyclopentitols have been tested as inhibitors of CGT, GBA1, and GBA2. Thus, we designed and prepared aminocyclopentitols **35** that stereochemically resemble *N*B-DNJ (Scheme 4).

The synthesis of the aminocyclopentitols is shown in Scheme 4. The primary alcohol of methyl-α-D-glucopyranoside (**25**) was protected as a silyl ether[41] **26** 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**.[42] 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[43,44] 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[45] 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** (Et_3N 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*-butyl-aminocyclopentitols **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



[a]Reagents and conditions: (a) TBSCl, imidazole, DMF, 0 °C to rt, 90%; (b) NaH, BnBr, DMF, 0 °C to rt, 85%; (c) TsOH, MeCN/H_2O, 65%; (d) PPh_3, imidazole, I_2, THF, reflux, 97%; (e) Zn, THF/H_2O, sonication, 94%; (f) NH_2OH•HCl, $NaHCO_3$, MeOH, reflux, 85%; (g) toluene, reflux, 70%; (h) 1. Zn, HOAc/H_2O, 2. HCl in ether, then NaOH, 87%; (i)1. HCl, 2. Et_3N, 3. butyraldehyde / nonyl aldehyde, $NaB(OAc)_3H$, DCE, 73% for **34a,** 70% for **34b,** 60% for **34c,** 35% for **34d**; (j) Pd/C, HCO_2NH_4, MeOH, 42% for **35a**; 79% for **35c;** 87% for **35e** from **33.** Li, NH_3, -78 °C, 52% for **35b**; 98% for **35d**. (k) 1. HCl, 2. Et_3N, 3. 5-(adamantan-1-yl)methoxy)pentanal, $NaB(OAc)_3H$, DCE, 92%.

Scheme 4. Syntheses of aminocyclitols **35a–35f**.[a]

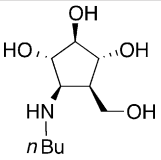
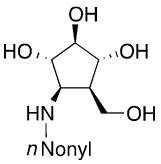
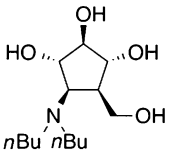
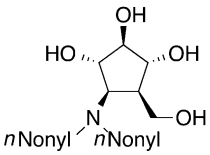
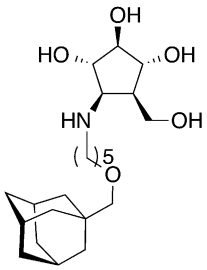
the synthesis of compound **35f**, amino alcohol **33** was subjected to hydrogenolysis to furnish intermediate aminotriol **35e**, which was then reacted with 5-(adamantan-1-yl)methoxy)pentanal following a reported procedure[46] and subjected to reductive amination to generate the targeted adamantyl **35f**.

Enzyme inhibition studies

The iminosugar analogues were assayed for CGT and GBA2 inhibitory 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. *N*B-DNJ was used as the positive control in all assays.

*N*B-DNJ analogues **5a–5f** (Scheme 1), bearing proximal sterically 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 analogue **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 inhibitory activity of these iminosugar analogues.[47] Inversion of configuration 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 inhibit CGT at concentrations up to 1000 μM, with the exception of **35f**, which inhibited CGT with an IC_{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 *N*B-DGJ and *N*B-DNJ, respectively. This potency was enhanced with the increase in exocyclic *N*-alkyl chain length. Compound **35b**, in which the nitrogen carries a nonyl group, provided approximately 200- and 1000-fold increases in potency compared with control compounds *N*B-DGJ and *N*B-DNJ, respectively. Notably, **35b** possesses >10000-fold selectivity for GBA2 (K_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_{50}=1.0 nM) but it is a comparatively a more selective 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 analogue **35d** inhibited GBA2 with K_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_i of 0.014 μM and the IC_{50} for CGT was 1000 μM (data not shown). This compound did not inhibit α-galactosidase, β-galactosidase, α-mannosidase 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 (*Saccharomyces cerevisiae*) and β-glucosidase (almond), α-galactosidase (green coffee beans), β-galactosidase (*Escherichia coli*), α-mannosidase (jack bean), and β-mannosidase (Roman snail) as described previously.[19] We found that aminocyclopentitols **35a**, **35b** and **35f** showed activity in the glucosidase assays (Table 1) that was an improvement over the standards *N*B-DNJ and castanospermine. Derivatives **35a** and **35b** are comparable or better inhibitors of both α- and β-glucosidase relative to the controls with a slight anomeric selectivity, favoring β-glucosidase. Compounds **35a** and **35b** are 4- and 2-fold more potent, respectively, in inhibiting β-glucosidase over α-glucosidase. Compound **35f**, the most potent GBA2 inhibitor, was

Table 1. Inhibitory activity of iminosugar analogues on human recombinant GBA1, LE rat testicular GBA2, and α- and β-glucosidases.[a,b]

Compound		K_i [μM] GBA1	GBA2	α-Glucosidase (yeast)	β-Glucosidase (almond)
	**35 a**	0.032 ± 0.004	3.3 ± 0.4	42 ± 2	10.6 ± 0.4
	**35 b**	≪0.014[c]	0.043 ± 0.023	15 ± 1	6.7 ± 0.2
	**35 c**	n.d.	95	n.d.	n.d.
	**35 d**	n.d.	0.89	n.d.	n.d.
	**35 f**	≤0.016[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: *N*B-DNJ: 34 ± 3, *N*B-DGJ: > 1000; K_i (μM) for GBA2: *N*B-DNJ: 48.3 ± 0.2, *N*B-DGJ: 8.2 ± 1.3; K_i (μM) for α-glucosidase: DNJ: 40 ± 1, *N*B-DNJ: 515 ± 19, *N*B-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 pM.

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 (**35 a**, **35 b**, and **35 f**) 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 **35 a** and **35 f** were next tested for inhibition of recombinant human lysosomal β-glucosidase 1 (GBA1) to determine compound selectivity. *N*B-DNJ and *N*B-DGJ were used as reference compounds and had K_i values of 34 μM and > 1 mM, respectively (Table 1). Under the assay conditions used, *N*B-DNJ (IC_{50} = 74 μM) was a ≈5- to 7-fold more potent inhibitor of GBA1 compared to previously reported IC_{50} values, which ranged from 400 to 520 μM.[23,49,50] *N*B-DGJ (1 mM) did not inhibit GBA1, which is consistent with two of the three previously reported IC_{50} values of 320 μM,[50] > 1 mM,[49] and > 3 mM.[24] The *N*-butyl iminosugar **35 a** was > 1000-fold more potent than *N*B-DNJ and was ≈100-fold more potent an inhibitor of 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.[51] The nonyl iminosugar **35 b** 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 **35 f** has a measured IC_{50} value of ≈16 nM, approximating the nominal GBA1 concentration. Therefore, the inhibitory potencies of both **35 b** and **35 f** cannot be accurately determined in this assay. In addition, both iminosugars **35 b** and **35 f** produce bi-phasic enzyme inhibition curves, in contrast to *N*B-DNJ and **35 a**, which both inhibit GBA1 monophasically. Although the reason for the biphasic **35 b** and **35 f** behavior is not known, it is possible that **35 b** and **35 f** discriminate between two forms of the enzyme present in approximately equal proportions in the enzyme preparation. For example, **35 a** and **35 f**

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 ≈60–70 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_i 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 μM). Analogues **35a**, **35b** and **35f** showed potent inhibition of GBA1 and GBA2 with K_i values of 69 nM, <14 nM, and ≤16 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 ≈1000-fold more potent than *N*B-DNJ for inhibition of GBA1. The aminocyclopentitol analogues **35** did not inhibit CGT (tested up to 1000 μM) with the exception of **35f**, which inhibited CGT with an IC_{50} of 1 mM. The results from this study suggest that the aminocyclopentitol scaffold may hold promise for future inhibitor development.

Experimental Section

Chemistry: ^{1}H NMR spectra were recorded using 400 MHz or 500 MHz spectrometers. ^{13}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 internal standard 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 a positive pressure of argon unless otherwise noted. Solvents and reagents that are commercially available were used without further purification unless otherwise noted. Tetrahydrofuran and diethyl ether were freshly distilled from sodium benzophenone ketyl under argon or dried by passing the solvent through a drying column. Methylene chloride was distilled freshly from calcium hydride 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.[19] GBA1 activity was measured using recombinant GBA1 enzyme (R&D Systems) and 2.5 mM 4-methlyumbelliferyl-β-glucoside (4-MU-β-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 μL). The plate was incubated for 20 min at 37 °C. The enzymatic reactions were stopped with the addition of 0.3 M glycine/NaOH buffer, pH 10.1. The amount of liberated 4-methylumbelliferone was determined with a Spectromax fluorimeter (λ_{ex} = 365 nm/λ_{em} = 445 nm). A standard curve of 4-methylumbelliferone (Sigma) was determined under the same experimental conditions. A concentration-response of *N*B-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 components using the biphasic function from 16 concentrations in triplicate. K_i values were calculated using the Cheng–Prusoff equation.

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

Abbreviations: AMP-DNJ: *N*-[5-(adamantan-1-ylmethoxy)pentyl]-1-deoxynojirimycin, DNJ: 1-deoxynojirimycin, GBA2: β-glucosidase 2, GBA1: β-glucosidase 1, CGT: ceramide-specific glucosyltransferase, ELSD: evaporative light scattering detector, MCA: multi-channel analyzer mode, *N*B-DGJ: *N*-butyl-deoxygalactojirimycin, *N*B-DNJ: *N*-butyl-1-deoxynojirimycin, FAB-HRMS: fast-atom bombardment high-resolution mass spectrometry, FAB-MS: fast-atom bombardment mass spectrometry.

Acknowledgements

This work was supported by the Contraception Research Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development through contracts HHSN275200503400C and HHSN275201300017C. We thank NICHD program officers Dr. H. K. Kim, Dr. Diana Blithe and Dr. Min Lee for their support of this program.

Conflict of interest

The authors declare no conflict of interest.

Keywords: aminocyclitols · enzyme inhibition · lysosomal glucosidase 1 · *N*-butyl-1-deoxynojirimycin · non-lysosomal glucosidase 2

[1] H. Paulsen, *Angew. Chem. Int. Ed. Engl.* **1966**, *5*, 495; *Angew. Chem.* **1966**, *78*, 501.
[2] S. Inouye, T. Tsuruoka, T. Ito, T. Niida, *Tetrahedron* **1968**, *24*, 2125.
[3] M. Bollen, W. Stalmans, *Eur. J. Biochem.* **2005**, *181*, 775.

[4] G. Horne, F. X. Wilson, *Prog. Med. Chem.* **2011**, *50*, 135.
[5] G. Horne, F. X. Wilson, J. Tinsley, D. H. Williams, R. Storer, *Drug Discovery Today* **2011**, *16*, 107.
[6] P. Compain, O. R. Martin, *Iminosugars: From Synthesis to Therapeutic Applications*, Wiley-VCH, Weinheim, **2007**.
[7] G. Horne, *Chem. Ind.* **2011**, *13*, 24.
[8] W. Yu, T. Gill, L. Wang, Y. Du, H. Ye, X. Qu, J.-T. Guo, A. Cuconati, K. Zhao, T. M. Block, X. Xu, J. Chang, *J. Med. Chem.* **2012**, *55*, 6061.
[9] Y. Yildiz, H. Matern, B. Thompson, J. C. Allegood, R. L. Warren, D. M. O. Ramirez, R. E. Hammer, F. K. Hamra, S. Matern, D. W. Russell, *J. Clin. Invest.* **2006**, *116*, 2985.
[10] C. M. Walden, R. Sandhoff, C.-C. Chuang, Y. Yildiz, T. D. Butters, R. A. Dwek, F. M. Platt, A. C. van der Spoel, *J. Biol. Chem.* **2007**, *282*, 32655.
[11] A. C. van der Spoel, M. Jeyakumar, T. D. Butters, H. M. Charlton, H. D. Moore, R. A. Dwek, F. M. Platt, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 17173.
[12] A. van der Spoel, R. Mott, F. Platt, *Pharmacogenomics* **2008**, *9*, 717.
[13] J. K. Amory, C. H. Muller, S. T. Page, E. Leifke, E. R. Pagel, B. Subramanyam, W. Bone, A. Radlmaier, W. J. Bremner, *Hum. Reprod.* **2007**, *22*, 702.
[14] H. R. Mellor, F. M. Platt, R. A. Dwek, T. D. Butters, *Biochem. J.* **2003**, *374*, 307.
[15] T. D. Butters, R. A. Dwek, F. M. Platt, *Chem. Rev.* **2000**, *100*, 4683.
[16] T. D. Butters, L. A. G. M. van den Broek, G. W. J. Fleet, T. M. Krulle, M. R. Wormald, R. A. Dwek, F. M. Platt, *Tetrahedron: Asymmetry* **2000**, *11*, 113.
[17] H. Li, T. Liu, Y. Zhang, S. Favre, C. Bello, P. Vogel, T. D. Butters, N. G. Oikonomakos, J. Marrot, Y. Blériot, *ChemBioChem* **2008**, *9*, 253.
[18] J. Deschamp, M. Mondon, S. Nakagawa, A. Kato, D. S. Alonzi, T. D. Butters, Y. Zhang, M. Sollogoub, Y. Blériot, *Bioorg. Med. Chem.* **2012**, *20*, 641.
[19] J. C. Lee, S. Francis, D. Dutta, V. Gupta, Y. Yang, J. Y. Zhu, J. S. Tash, E. Schönbrunn, G. I. Georg, *J. Org. Chem.* **2012**, *77*, 3082.
[20] V. Faugeroux, Y. Genisson, Andrieu-N. Abadie, S. Colie, T. Levade, M. Baltas, *Org. Biomol. Chem.* **2006**, *4*, 4437.
[21] N. Ardes-Guisot, D. S. Alonzi, G. Reinkensmeier, T. D. Butters, C. Norez, F. Becq, Y. Shimada, S. Nakagawa, A. Kato, Y. Bleriot, M. Sollogoub, B. Vauzeilles, *Org. Biomol. Chem.* **2011**, *9*, 5373.
[22] T. Wennekes, R. J. B. H. N. van den Berg, W. Donker, G. A. van der Marel, A. Strijland, J. M. F. G. Aerts, H. S. Overkleeft, *J. Org. Chem.* **2007**, *72*, 1088.
[23] H. S. Overkleeft, G. H. Renkema, J. Neele, P. Vianello, I. O. Hung, A. Strijland, A. M. van der Burg, G.-J. Koomen, U. K. Pandit, J. M. F. G. Aerts, *J. Biol. Chem.* **1998**, *273*, 26522.
[24] C. M. Ridley, K. E. Thur, J. Shanahan, N. B. Thillaiappan, A. Shen, K. Uhl, C. M. Walden, A. A. Rahim, S. N. Waddington, F. M. Platt, A. C. van der Spoel, *J. Biol. Chem.* **2013**, *288*, 26052.
[25] J. M. Aerts, R. Ottenhoff, A. S. Powlson, A. Grefhorst, M. van Eijk, P. F. Dubbelhuis, J. Aten, F. Kuipers, M. J. Serlie, T. Wennekes, J. K. Sethi, S. O'Rahilly, H. S. Overkleeft, *Diabetes* **2007**, *56*, 1341.
[26] C. Norez, S. Noel, M. Wilke, M. Bijvelds, H. Jorna, P. Melin, H. DeJonge, F. Becq, *FEBS Lett.* **2006**, *580*, 2081.
[27] J. D. Diot, I. G. Moreno, G. Twigg, C. O. Mellet, K. Haupt, T. D. Butters, J. Kovensky, S. G. Gouin, *J. Org. Chem.* **2011**, *76*, 7757.
[28] S. D. Orwig, Y. L. Tan, N. P. Grimster, Z. Yu, E. T. Powers, J. W. Kelly, R. L. Lieberman, *Biochemistry* **2011**, *50*, 10647.
[29] F. B. Bdira, W. W. Kallemeijn, S. V. Oussoren, S. Scheij, B. Bleijlevens, B. I. Florea, C. P. A. A. van Roomen, R. Ottenhoff, M. J. F. M. van Kooten, M. T. C. Walvoort, M. D. Witte, R. G. Boot, M. Ubbink, H. S. Overkleeft, J. M. F. G. Aerts, *ACS Chem. Biol.* **2017**, *12*, 1830.
[30] T. Wennekes, R. J. B. H. N. van den Berg, T. J. Boltje, W. E. Donker-Koopman, B. Kuijper, G. A. van der Marel, A. Strijland, C. P. Verhagen, J. M. F. G. Aerts, H. S. Overkleeft, *Eur. J. Org. Chem.* **2010**, 1258.
[31] C. R. R. Matos, R. S. C. Lopes, C. C. Lopes, *Synthesis* **1999**, 571.
[32] B. J. Turunen, G. I. Georg, *J. Am. Chem. Soc.* **2006**, *128*, 8702.
[33] M. R. Linder, S. Steure, J. Podlech, *Org. Synth.* **2003**, *79*, 154.
[34] G.-R. Vasanthakumar, B. S. Patil, V. V. S. Babu, *J. Chem. Soc. Perkin Trans. 1* **2002**, 2087.
[35] M. J. Niphakis, B. J. Turunen, G. I. Georg, *J. Org. Chem.* **2010**, *75*, 6793.
[36] M. Bøjstrup, I. Lundt, *Org. Biomol. Chem.* **2005**, *3*, 1738.
[37] E. Leroy, J.-L. Reymond, *Org. Lett.* **1999**, *1*, 775.
[38] O. Boss, E. Leroy, A. Blaser, J.-L. Reymond, *Org. Lett.* **2000**, *2*, 151.
[39] S. Ogawa, T. Morikawa, *Eur. J. Org. Chem.* **2005**, 4065.
[40] L. G. Dickson, E. Leroy, J.-L. Reymond, *Org. Biomol. Chem.* **2004**, *2*, 1217.
[41] D. J. Chambers, G. R. Evans, A. J. Fairbanks, *Tetrahedron* **2005**, *61*, 7184.
[42] B. Bernet, A. Vasella, *Helv. Chim. Acta* **1979**, *62*, 1990.
[43] S. Moutel, M. Shipman, *Synlett* **1998**, 1333.
[44] P. Dransfield, S. Moutel, M. Shipman, V. Sik, *J. Chem. Soc. Perkin Trans. 1* **1999**, 3349.
[45] M. C. Whisler, P. Beak, *J. Org. Chem.* **2003**, *68*, 1207.
[46] T. Wennekes, B. Lang, M. Leeman, G. A. van der Marel, E. Smits, M. Weber, J. van Wiltenburg, M. Wolberg, J. M. F. G. Aerts, H. S. Overkleeft, *Org. Process Res. Dev.* **2008**, *12*, 414.
[47] R. J. B. H. N. van den Berg, T. Wennekes, A. Ghisaidoobe, W. E. Donker-Koopman, A. Strijland, R. G. Boot, G. A. van der Marel, J. M. F. G. Aerts, H. S. Overkleeft, *ACS Med. Chem. Lett.* **2011**, *2*, 519.
[48] A. T. Ghisaidoobe, R. J. B. H. N. van den Berg, S. S. Butt, A. Strijland, W. E. Donker-Koopman, S. Scheij, A. M. C. H. van den Nieuwendijk, G.-J. Koomen, A. van Loevezijn, M. Leemhuis, T. Wennekes, M. van der Stelt, G. A. van der Marel, C. A. A. van Boeckel, J. M. F. G. Aerts, H. S. Overkleeft, *J. Med. Chem.* **2014**, *57*, 9096.
[49] F. M. Platt, G. R. Neises, G. B. Karlsson, R. A. Dwek, T. D. Butters, *J. Biol. Chem.* **1994**, *269*, 27108.
[50] T. Wennekes, A. J. Meijer, A. K. Groen, R. G. Boot, J. E. Groener, M. van Eijk, R. Ottenhoff, N. Bijl, K. Ghauharali, H. Song, T. J. O'Shea, H. Liu, N. Yew, D. Copeland, R. J. van den Berg, G. A. van der Marel, H. S. Overkleeft, J. M. Aerts, *J. Med. Chem.* **2010**, *53*, 689.
[51] R. J. B. H. N. van den Berg, E. R. van Rijssel, M. J. Ferraz, J. Houben, A. Strijland, W. E. Donker-Koopman, T. Wennekes, K. M. Bonger, A. B. T. Ghisaidoobe, S. Hoogendoorn, G. A. van der Marel, J. D. C. Codée, H. S. Overkleeft, J. M. F. G. Aerts, *ChemMedChem* **2015**, *10*, 2042.

Manuscript received: September 14, 2017
Accepted manuscript online: October 3, 2017
Version of record online: November 24, 2017

