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Assessment of Partially Deoxygenated Deoxynojirimycin Derivatives as Glucosylceramide Synthase Inhibitors

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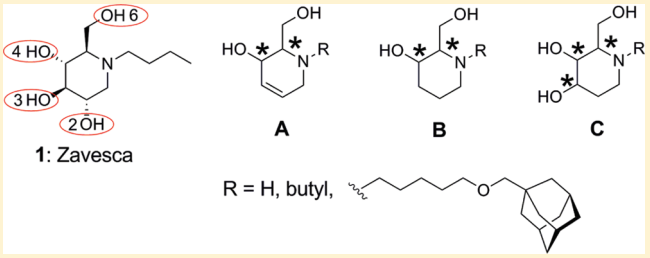
Supporting Information

ABSTRACT: Glucosylceramide synthase (GCS) is an approved drug target for the treatment of Gaucher disease and is considered as a valid target for combating other human pathologies, including type 2 diabetes. The clinical drug *N*-butyldeoxynojirimycin (Zavesca) is thought to inhibit through mimicry of its substrate, ceramide. In this work we demonstrate that, in contrast to what is proposed in this model, the C2-hydroxyl of the deoxynojirimycin core is important for GCS inhibition. Here we show that C6-OH appears of less important, which may set guidelines for the development of GCS inhibitors that have less affinity (in comparison with Zavesca) for other glycoprocessing enzymes, in particular those hydrolases that act on glucosylceramide.



KEYWORDS: Glucosylceramide synthase, iminosugar, Gaucher disease, ceramide, deoxynojirimycin

Glucosylceramide synthase (GCS) is a noncanonical glycosyl transferase in several aspects. Situated at the outer membrane of the Golgi apparatus, GCS catalyzes the transfer of glucose from UDP-glucose to ceramide to give glucosylceramide.[1] As such, and with the exception of the enzyme O-GlcNAc transferase,[2] GCS is the only mammalian glycosyltransferase known to act in cytoplasm. GCS appears evolutionary distinct from most other glycosyltransferases, that all act either in the ER or the Golgi apparatus. Although little structural data on GCS exists (the enzyme has, to date, defied attempts to gather Röntgen diffraction data), alignment of the primary sequence reveals large differences between GCS and other glycosyl transferases.[1] Perhaps thanks to these differences, potent GCS inhibitors of various signatures have been described, whereas for most other glycosyltransferases no effective inhibitors are available. In fact, the discovery that GCS is susceptible to inhibition by small compounds has led to the development of *N*-butyl-deoxynojirimycin as a drug (Zavesca (**1**); Figure 1) for the treatment of the lysosomal glycolipid storage disorder Gaucher disease.[3−5] Compound **1** is a moderately potent and selective GCS inhibitor. Treatment of mildly affected type 1 Gaucher patients with this agent results in sufficient pharmacological lowering of glucosylceramide to levels that the residual mutant glucosylceramidase (GBA1) can deal with.[6] We have reported on the development of much more potent (for instance, **2**) and also more selective with respect to other glycoprocessing enzymes (for instance, **3**) *N*-alkylated deoxynojirimycin derivatives.[7,8] Coinciding with these discoveries, recent studies strongly point toward GCS as a therapeutic target in several diseases areas: next to lysosomal glycolipid storage disorders other than Gaucher, also type 2 diabetes, hepatosteatosis, artherosclerosis, inflammatory diseases, and polycystic kidney disease.[9−14] The development of GCS inhibitors with improved properties therefore remains a valid research objective.

Perhaps most remarkably from an enzymology point of view is not so much that GCS inhibitors exist but that these include iminosugar-type piperidines. Iminosugars are widely pursued and versatile glycosidase inhibitors, and iminosugars acting on exoglycosidases processing many different glycosides (varying for instance in preference for the configuration of the substrate glycoside: glucosidases, mannosidases, galactosidases, etc) are known.[15−18] In contrast, and with the said exception of GCS, glycosyltransferases are normally insensitive toward this class of compounds.[19,20] Deoxynojirimycin-type iminosugars are thought to inhibit glycosidases through substrate-or-transition state mimicry. In such models, the basic ring nitrogen in its protonated form is thought to mimic the developing oxocarbenium ion through which glycoside hydrolysis occurs. The configuration of the iminosugar emulates that of the parent sugar and guides both glycosidase inhibitory potency and selectivity. In general, glycosidases are rather particular with respect to their substrate glycoside. Remarkably, GCS appears much less biased with

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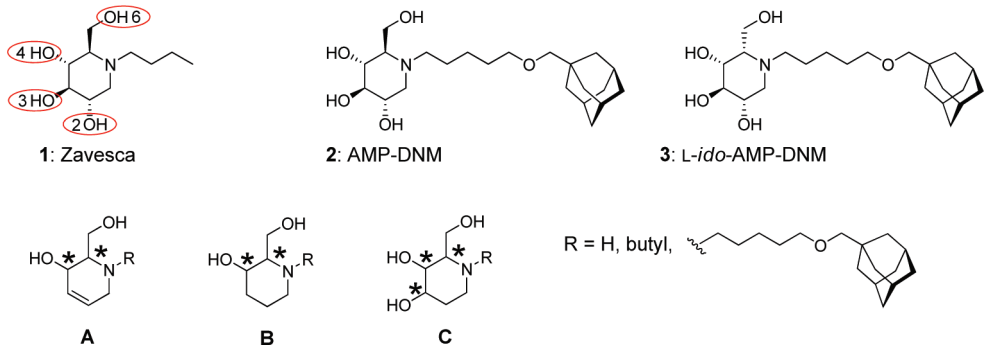

Figure 1. Known glucosylceramide synthase inhibitors (**1**−**3**) and the target compounds discussed in this study.

respect to the configuration of the (N-alkylated) iminosugar. We, and others, have demonstrated that at least three configurational deoxynojirimycin isomers are recognized by GCS: next to the D-*gluco*- and L-*ido* configurations (as in **2** and **3**, respectively), also the D-*galacto* configuration.[2,21−24]

The relative lack of configurational specificity of GCS toward iminosugars invites the hypothesis that N-alkylated deoxynojirimycin derivatives act not by glucose (or UDP-glucose) mimicry but by another molecular mechanism. Indeed, Butters and co-workers in their work on the development of Zavesca proposed a model in which *N*-butyldeoxynojirimycin binds to GCS as a mimic of its other substrate, namely, ceramide.[25] In this model the ring-nitrogen in **1** superimposes the ceramide nitrogen, with the alkyl (butyl) chain situated at the ceramide N-acyl side. The piperidine O3 and O4 and possibly O6 (glucopyranose numbering) would occupy the positions occupied by the primary and secondary hydroxyls of the ceramide structure. The experimental finding that compound **1** is competitive for ceramide but not UDP-glucose, binding to GCS, supports this model.[25] However, no structural evidence for the model has been obtained yet. We decided to put the model to the test in an attempt to design new and potentially selective GCS inhibitors. The model as proposed by Butters and co-workers suggests that, of the deoxynojirimycin substituents, C3-OH and C4-OH and possibly C6-OH are important contributors to GCS binding, whereas the presence of C2-OH is not required. In other words, 2-deoxy-DNM derivatives **C** should potentially inhibit GCS whereas 2,3-dideoxy derivatives **B** should not. In order to validate this reasoning, we set out to synthesize all configurational isomers of scaffolds **B** and **C**. We either left the secondary nitrogen unmodified (to obtain the respective deoxygenated analogues of the natural product, deoxynojirimycin), introduced a butyl group (enabling comparison with the clinically applied drug, Zavesca), or introduced an adamantanemethyloxypentyl group (to allow a head-to-head comparison with our lead structures, **2** and **3**). The synthetic schemes through which the compounds are prepared follow well-established literature procedures (see the experimental section for full experimental and analytical details[26−30]). These routes proceed through partially unsaturated piperidine intermediates, and we capitalized on this by also preparing piperidine derivatives **A**. In all, we assembled a 48 compound library, which we assessed on inhibitory potential against GCS,[31] GBA1,[32] the nonlysosomal glucosylceramidase GBA2,[33] as well as a selection of intestinal glycosidases.[24] The results are summarized in the below Table.

As can be seen (Table 1), none of the 48 compounds possessed any inhibitory potency against GCS at concentrations up to 20 μM. This result stands in contrast to the strong (IC_{50} 0.1−0.2 μM) inhibitory potency as exerted by lead compounds **2** and **3** toward the target enzyme. While these results do not necessarily negate the proposed mode of action by which *N*-butyldeoxynojirimycin inhibits GCS, they do indicate that one should take care in using the model as a guideline for designing new GCS inhibitors. It should be noted that one might see differences when comparing compounds from the series at higher (millimolar) concentrations; however, we decided to refrain from these studies for the dual reason that such measurements are complicated and that millimolar inhibitors hold no therapeutic value. When looking at the two glucosylceramidases, it is immediately apparent that removing both C2-OH and C3-OH, as in series **A** and **B**, is detrimental for all activity irrespective of the configuration of the remaining chiral carbon centers. Selected compounds from the **C** sublibrary, however, retain some activity toward GBA1 or GBA2, especially in those cases where R is adamantanemethyloxypentyl. For instance, D-*gluco*-configured derivative **30** inhibits GBA2 at an IC_{50} of 1 μM, whereas its L-*ido*-congerer **39** does so at 0.5 μM (IC_{50}); this compound, in fact, appears to be a rather selective GBA2 inhibitor that may find application as such. In general, however, deoxygenation at C2 appears to considerably impair inhibitory potency of deoxynojirimycin-type iminosugars, also against glycosidases.

Returning to the original research objective, our results strongly suggest that C2-OH does contribute to a large extent to GCS inhibition, this in contrast to the model proposed by Butters.[25] As a preliminary study, we probed the importance of the C6-hydroxyl to GCS inhibitory potency. To this end, we prepared both 6-deoxy-*N*-(adamantanemethoxyethyl)deoxynojiimycin **52** and its *xylo*-congener (lacking the C5-hydroxymethyl substituent, **53**) and established their IC_{50} values against the same panel of enzymes. Interestingly, the 6-deoxy-compound turned out to be a quite potent (IC_{50} 0.8 μM) GCS inhibitor, in fact, only 4-fold less potent than our lead compounds. It thus appears that the C6-OH is less important than C3-OH and C4-OH, which is suggested by the ceramide mimic model. Compound **53** retains some inhibitory potency against GBA1 and GBA2 as well, which is unfortunate when considering the need for selective GCS inhibitors. A substituent at C5 does appear of importance, as *xylo*-piperidine **53** turned out to have no GCS inhibitory activity.

In conclusion, we have added to the growing list of iminosugar inhibitor studies through the preparation and evaluation of a comprehensive library of *N*-alkyl C2-deoxy- and C2/C3-dideoxy deoxynojirimycin derivatives. We focused our inhibitory studies on enzymes involved in mammalian glucosylceramide metabolism

Table 1. Enzyme Inhibition Assay Results: Apparent IC_{50} Values in μM

Compound	R =	GCS[a] *in situ*	GBA1	GBA2	Compound	R =	GCS[a] *in situ*	GBA1	GBA2
AMP-DNM	**1**	50	400	0.23	**L-*ido*-AMP-DNM**	**3**	0.1	2.0	0.03
	2	0.2	0.2	0.001					
	**4:** H	> 10	> 1000	> 1000		**28:** H	> 10	> 1000	> 1000
	5: Butyl	> 10	> 1000	250		**29:** Butyl	> 10	> 1000	> 1000
	6: AMP	> 10	50	30		**30:** AMP	> 10	10	1.0
	**7:** H	> 10	> 1000	> 1000		**31:** H	> 10	> 1000	> 1000
	8: Butyl	> 10	> 1000	> 1000		**32:** Butyl	> 10	> 1000	> 1000
	9: AMP	> 10	65	130		**33:** AMP	> 10	110	6.5
	**10:** H	> 10	> 1000	> 500		**34:** H	> 10	> 1000	30
	11: Butyl	> 10	80	> 500		**35:** Butyl	> 10	> 1000	30
	12: AMP	> 10	400	> 500		**36:** AMP	> 10	200	100
	**13:** H	> 10	> 1000	> 1000		**37:** H	> 10	> 1000	> 1000
	14: Butyl	> 10	> 1000	25		**38:** Butyl	> 10	> 1000	20
	15: AMP	> 10	100	10		**39:** AMP	> 10	500	0.5
	**16:** H	> 10	> 1000	> 1000		**40:** H	> 10	700	> 1000
	17: Butyl	> 10	> 1000	> 1000		**41:** Butyl	> 10	> 1000	1000
	18: AMP	> 10	175	100		**42:** AMP	> 10	20	25
	**19:** H	> 10	> 1000	> 1000		**43:** H	> 10	> 1000	> 1000
	20: Butyl	> 10	> 1000	> 1000		**44:** Butyl	> 10	> 1000	> 1000
	21: AMP	> 10	350	> 1000		**45:** AMP	> 10	80	3.0
	**22:** H	> 10	400	> 1000		**46:** H	> 10	> 1000	> 1000
	23: Butyl	> 10	100	> 500		**47:** Butyl	> 10	> 1000	> 1000
	24: AMP	> 10	1000	> 1000		**48:** AMP	> 10	200	20
	**25:** H	> 10	80	> 1000		**49:** H	> 10	> 1000	> 1000
	26: Butyl	> 10	> 1000	> 500		**50:** Butyl	> 10	> 1000	> 1000
	27: AMP	> 10	500	500		**51:** AMP	> 10	200	75
	**52:** AMP	0.8	0.33	0.03		**53:** AMP	> 10	2.2	0.8

[a] Other enzyme assays are *in vitro*; AMP = 5-(adamantan-1-yl-methoxy)pentyl.

and did not identify a new attractive lead toward either GCS or GBA1. We do note the selectivity of compound **39** toward inhibition of GBA2,[34] which might be of interest for studies in which the physiological role of this enzyme is questioned. Moreover, our library encompasses all possible configurational isomers at C3, C4, and C5 and it is not excluded that individual compounds possess strong affinity toward glycoprocessing enzymes not included in our screening panel. With respect to the mode of action by which deoxynojirimycin-type iminosugars can inhibit GCS, we have strong, but indirect, evidence that a C2-OH is important but that the C6-OH is amenable for modification. We focus our current research on the development of potent and selective GCS inhibitors on preparing some configurational deoxynojirimycin isomers (other than those already known) and on modifying the nature of the N-alkyl substituent.

■ ASSOCIATED CONTENT

Supporting Information. Full details on the synthesis, purification, and analysis of the compound library and the enzyme assays used. This material is available free of charge via the Internet at http://pubs.acs.org.

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