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Identification of Potent and Selective Glucosylceramide Synthase Inhibitors from a Library of N-Alkylated Iminosugars

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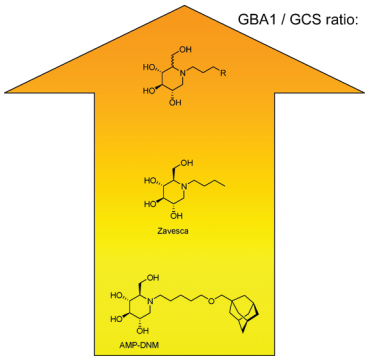
ABSTRACT Glucosylceramide synthase (GCS) is an important target for clinical drug development for the treatment of lysosomal storage disorders and a promising target for combating type 2 diabetes. Iminosugars are useful leads for the development of GCS inhibitors; however, the effective iminosugar type GCS inhibitors reported have some unwanted cross-reactivity toward other glyco-processing enzymes. In particular, iminosugar type GCS inhibitors often also inhibit to some extent human acid glucosylceramidase (GBA1) and the nonlysosomal glucosylceramidase (GBA2), the two enzymes known to process glucosylceramide. Of these, GBA1 itself is a potential drug target for the treatment of the lysosomal storage disorder, Gaucher disease, and selective GBA1 inhibitors are sought after as potential chemical chaperones. The physiological importance of GBA2 in glucosylceramide processing in relation to disease states is less clear, and here, selective inhibitors can be of use as chemical knockout entities. In this communication, we report our identification of a highly potent and selective N-alkylated L-*ido*-configured iminosugar. In particular, the selectivity of **27** for GCS over GBA1 is striking.

KEYWORDS Gaucher, type 2 diabetes, glucosylceramide synthase, acid glucosylceramidase, deoxynojirimycin



Glucosylceramide synthase (GCS) is an established drug target in the lysosomal storage disorder, Gaucher disease, and has received considerable attention in recent years as a potential target for the treatment of various other diseases. The glycolipid produced by GCS, glucosylceramide, accumulates in Gaucher cells due to inherited deficiency in the enzyme responsible for glucosylceramide hydrolysis, glucocerebrosidase (acid glucosylceramidase, GBA1).[1] Administration of the GCS inhibitor, *N*-butyl-1-deoxynojirimycin[2] (Miglitol, Zavesca, **1**, Figure 1), results in lowering glucosylceramide concentrations to levels that can be dealt with by genetically impaired GBA1 as expressed in mildly affected type 1 Gaucher patients.[3] This frequently called substrate reduction therapy is now widely applied in the clinic as an alternative for enzyme replacement therapy, in which recombinant GBA1 is administered to the patients.[4−6] Glucosylceramide is also the starting point in the biosynthesis of a wide variety of glycosphingolipids, of which many are found as the storage material in lysosomal glycolipid storage disorders related to Gaucher disease (Fabry, Tay-Sachs, etc.) and in which a specific lysosomal glycohydrolase is genetically impaired. Pharmacological lowering of levels of the glycosphingolipids underlying these diseases holds therapeutic potential, and from this, it follows that GCS is a potential clinical target in these areas as well.[5,7,8]

In quite another therapeutic arena, we and others have recently obtained compelling evidence that lowering excessive glycolipid levels through partial inhibition of GCS results in improved insulin sensitivity in type 2 diabetes models.[9−13] Other therapeutic areas currently under investigation and in which GCS may be implicated include hepatosteatosis, artherosclerosis, inflammatory diseases, and polycystic kidney disease.[14−19] Potent and selective GCS inhibitors thus hold considerable potential therapeutic value, and the development of such compounds is pursued by an increasing number of researchers worldwide.[20−30] In the search for selective GCS inhibitors, we give particular attention to the two known hydrolytic activities capable of hydrolyzing glucosylceramide, next to the aforementioned GBA1 and also the nonlysosomal glucosylceramidase or GBA2. As said, partially dysfunctional GBA1 is at the basis of Gaucher

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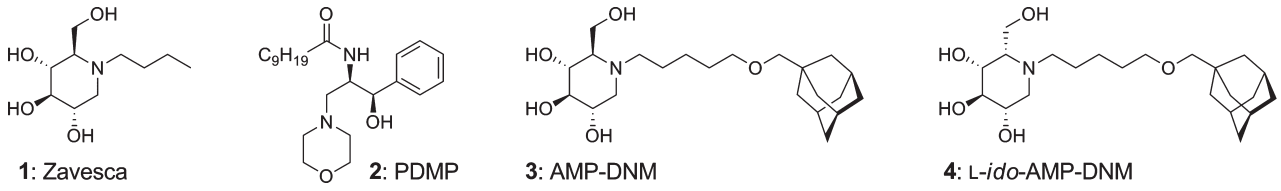

Figure 1. Reported GCS inhibitors.

disease, and further impairing this activity through partial inhibition might well offset the beneficial effect in reducing its substrate through GCS inhibition. At the same time, GBA1 inhibitors are extensively studied as chemical chaperone entities in an attempt to increase the number of lysosomal GBA1 copies in what is called chemical chaperone therapy.[31] Compounds that selectively target GBA1, and preferably do so in the neutral environment of the endoplasmic reticulum, are of interest in their own right in the context of this potential therapeutic application. With respect to GBA2, its physiological relevance to Gaucher disease or other (patho)-physiological processes is much less clear, and here, selective inhibitors may well serve to aid in unraveling its role in a chemical knockout strategy. Thus, whereas our main objective is to identify selective GCS inhibitors, we are aware of the potential of selective GBA1 and GBA2 inhibitors and pay attention to the evaluation of our compounds also in assays involving these glycosidases.

The two archetypal GCS inhibitors are 1-phenyl-2-decanoylamino-3-morpholino-1-propanol[32] (PDMP, **2**) and the aforementioned 1-butyldeoxynojirimycin **1**,[2] and most GCS inhibitors reported to date are based on these two scaffolds.[9] Over the past decade, we have focused on the latter class of compounds in our development and evaluation of iminosugar type inhibitors of enzymes involved in glucosylceramide metabolism. In the course of these studies, we found that increasing the size and hydrophobic character of the *N*-alkyl substituent, as in *N*-(adamantanemethyloxypentyl)-deoxynojirimycin (AMP-DNM, **3**), results in a drastic increase in GCS inhibitory potency (**1**, IC_{50} = 50 μM; **3**, IC_{50} = 0.2 μM).[33,34] This increased activity is accompanied by an increased inhibitory potency against both human glucosylceramide-processing enzymes GBA1 and nonlysosomal glucosylceramidase (GBA2), as well as several intestinal digestive glycosidases (sucrase, isomaltase). We found that AMP-DNM **3** can be used to improve glycemic control in obese rodents thanks to its dual action to both buffer carbohydrate assimilation (through inhibition of intestinal glycosidases) and reduce visceral glycolipid levels (inhibition of GCS).[35] At the same time, we recognized that to realize effective therapeutic applications based on modulating GCS as the single target, compounds need be developed that combine GCS inhibitory potency at least equal to that of AMP-DNM **3** with a much-reduced activity toward other glyco-processing enzyme. It should be noted here that 1-butyldeoxynojirimycin **1** is in fact a rather moderate GCS inhibitor with quite some activity against other enzymes. We identified the C-5-epimer of **3**, L-*ido*-AMP-DNM **4** as a much-improved compound in this respect.[35] Its GCS inhibitory activity is equal to, or even slightly better than, AMP-DNM **3**, whereas its activity toward GBA1 and GBA2 is about a 10-fold lower and its activity toward intestinal glycosidases next to nonexistent. Dividing GBA1 inhibition potency by that found for GCS gives insight in enzyme selectivity, and doing so for the three iminosugars mentioned reveals that replacing the butyl group in **1** for the bulky, hydrophobic adamantanemethyloxypentyl group as in **3** gives a selectivity drop (from 8 to 1). The L-*ido*-configured analogue **4** in contrast has a GBA1/GCS ratio of 20, with the larger number indicating elevated GCS selectivity (see for inhibitory data of all compounds toward all enzymes mentioned Table 1). Apparently altering the iminosugar configuration from D-*gluco* to L-*ido* holds no consequence, as corroborated by early findings,[36,37] for GCS recognition, where the hydrolases are more particular to the nature of the carbohydrate or carbohydrate mimetic. Indeed, we and others found that D-galacto-configured N-alkylated iminosugars are quite potent GCS inhibitors, and one strategy toward iminosugars with exclusive selectivity for GCS would be to explore each of the 16 possible deoxynojirimycin stereomers, in particular those that do not emulate in configuration a hexopyranose naturally occurring in man.[35,38] An alternative and perhaps less elaborate strategy entails altering the nature of the *N*-alkyl substituent. We here present our first results in this direction in a study in which we systematically introduced linear alkyl and alkyloxyalkyl chains onto both D-*gluco*- and L-*ido*-configured deoxynojirimycins. Head-to-head comparison of a 24 compound library with compounds **1**, **3**, and **4** revealed several compounds that match or even surpass the GCS inhibitory potency of **3** and **4** while at the same time being (much) more selective with respect to GBA1 and the intestinal glycosidases.

The preparation of the iminosugar library follows well-established routes of synthesis previously reported by us[35,39] and others.[40,41] Details on the preparation and characterization of all compounds are provided in the Supporting Information. Briefly, we prepared large quantities of 1-deoxynojirimycin[39] and its L-*ido* congener[35] and N-alkylated these using the appropriate alkyl bromide in DMF and potassium carbonate as the base. In this fashion, linear aliphatic alkyl substituents ranging from butyl to nonyl were introduced onto both piperidine cores, leading to D-*gluco* iminosugars **1** and **5**−**9** (Table 1) and L-*ido* derivatives **10**−**15**. Previous studies have indicated that iminosugars equipped with large alkyl chains are cellularly toxic and that introducing an ether functionality at a strategic position may prevent this undesired effect.[42−44] With this rationale in mind, and considering that our two leads **3** and **4** encompass a five-carbon spacer, we prepared the series of *N*-(alkyloxypentyl)-deoxynojirimycin **16**−**21**, with the alkyl group ranging from butyl (**16**) to nonyl (**21**), along with the related L-*ido* series **22**−**27**.

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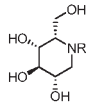
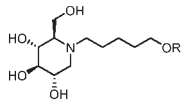
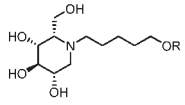
Table 1. Enzyme Inhibition Assay Results: Apparent IC_{50} Values in Micromolar

Compound	R =	GCS[a] *in situ*	GBA1	GBA2	Sucrase	Lactase	Maltase	GBA1/GCS ratio
AMP-DNM	**3**	0.2	0.2	0.001	0.5	35	4	1
L-*ido*-AMP-DNM	**4**	0.1	2	0.03	> 1000	> 1000	1000	20
	**1:** Butyl	50	400	0.23	0.6	750	9	8
	5: Pentyl	> 20	500	0.4	2	> 1000	10	< 25
	6: Hexyl	> 20	80	0.11	1	700	10	< 4
	7: Heptyl	40	18.5	0.045	0.5	500	10	0.5
	8: Octyl	4	4	0.020	0.75	300	8	1
	9: Nonyl	~ 4	1.5	0.007	0.4	200	8	0.4
	**10:** Butyl	20	> 1000	1	> 1000	> 1000	> 1000	> 20
	11: Pentyl	15	> 1000	0.25	> 1000	> 1000	> 1000	> 66
	12: Hexyl	40	> 1000	0.14	600	500	> 1000	> 25
	13: Heptyl	4	700	0.04	> 1000	200	> 1000	175
	14: Octyl	4	25	0.02	60	100	600	6.3
	15: Nonyl	2	50	0.01	> 1000	> 1000	> 1000	25
	**16:** Butyl	4	30	0.06	2	300	8	7.5
	17: Pentyl	2	705	0.04	1.2	300	4	3.8
	18: Hexyl	1	205	0.008	0.8	200	5	2.5
	19: Heptyl	0.3	1.75	0.015	1	250	7	5.8
	20: Octyl	0.2	0.5	0.010	1	200	10	2.5
	21: Nonyl	0.1	0.5	0.040	2.5	350	25	5
	**22:** Butyl	2	> 1000	0.09	> 1000	400	> 1000	500
	23: Pentyl	0.15	100	0.015	> 1000	200	> 1000	666
	24: Hexyl	0.1	95	0.025	> 1000	200	> 1000	950
	25: Heptyl	0.05	40	0.015	> 1000	250	> 1000	800
	26: Octyl	0.05	15	0.015	> 1000	300	> 1000	300
	27: Nonyl	< 0.05	12	0.045	> 1000	350	> 1000	> 240

[a] Other enzyme assays are in vitro.

We next assessed the inhibitory potency of the newly synthesized compounds against GCS, GBA1, GBA2, sucrase, lactase, and maltase using inhibitory assays previously reported.[35,45] All results are given in Table 1, with the last column the GBA1/GCS ratio. The first three entries depict the results obtained by leads **1**, **3**, and **4** data, which corroborates our previous results.[35] In general, extension of the *N*-alkyl chain results in more potent GCS inhibitors.[46] Within the D-*gluco* series, the *N*-alkyl derivatives (**1** and **5**−**9**) are moderate GCS inhibitors, with octyl and nonyl deoxynojirimycin derivatives **8** and **9** being the most potent. Interestingly, the dialkyl ether-substituted deoxynojirimycin derivatives (**16**−**21**) are in general quite a bit more potent, again with the compounds having the larger substituents being the most potent. The most potent GCS inhibitor from this series, compound **21**, emulates leads **3** and **4** in potency. A similar trend is seen in the L-*ido*-configured series. Again, GCS inhibitory potency increases with increasing *N*-alkyl substituent, and again, the *N*-alkyloxyalkyl derivatives (**22**−**27**) outperform the *N*-alkyl species (**10**−**15**). Head-to-head comparison of two stereoisomers from the two series reveals that in general the L-*ido* congener is the most potent GCS inhibitor. This trend is most obvious when considering that the *N*-alkyloxyalkyl series, wherein **27** (IC_{50} at or below 50 nM), the most potent compound of the series presented here, outperforms its D-*gluco* isomer (**21**, IC_{50} is 100 nM) by at least 2-fold.

The GBA1 inhibitory data reveal a related trend, wherein larger substituents give more potent inhibitors, with the important difference that the increase in inhibitory activity within the L-*ido* series is less pronounced than that observed in the D-*gluco* series. Without exception, the D-*gluco* compound is the more potent GBA inhibitor when directly compared with the respective L-*ido* diastereomer. The improved GCS selectivity of the L-*ido* compounds is most apparent when looking at the GBA1/GCS ratios. The most promising compounds are found in the *N*-alkyloxyalkyl-L-*ido* series, with **24** equally potent GCS inhibitor as leads **3** and **4**, but much more selective (GBA1/GCS ratio of 950 as opposed to 1 and 20, respectively), and **27** much more potent and selective.

The inhibitory data on the other enzymes reveal a trend that we had already observed for leads **3** and **4**. GBA2 appears sensitive to most compounds. Indeed, we have found this enzyme to be sensitive to almost all iminosugar type inhibitors that we have screened over the years.[47,48] With respect to the intestinal enzymes, these are inhibited to various extents by the D-*gluco* compounds but are hardly targeted by the L-*ido* compounds.

In conclusion, we have described the development of potent and selective GCS inhibitors based on an L-*ido* deoxynojirimyicin core, equipped this with a hydrophobic *N*-alkyl substituent of appropriate size and nature. Obviously, the nature of the *N*-alkyl group can be altered in other than demonstrated here, and current research is directed in this direction. We do note the apparently ideally positioned ether oxygen at a position five carbons removed from the ring nitrogen, already present in our leads (**3** and **4**) and leading to (much) more potent GCS inhibitors when compared to the

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N-alkyl series, of which the clinical drug **1** is the most prominent member. Compound **27** is to the best of our knowledge the most potent and selective iminosugar-based GCS inhibitor described to date and might be considered for further development for treating lysosomal storage disorders in which glucosylceramide or its glycosylated metabolites are the accumulating lipids, in particular Gaucher disease.

SUPPORTING INFORMATION AVAILABLE 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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