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Article

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Identification and development of biphenyl substituted iminosugars as improved dual glucosylceramide synthase/neutral glucosylceramidase inhibitors

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Keywords

Glucosylceramide synthase, glucosylceramide metabolism, neutral glucosylceramidase, deoxynojirimycin, Gaucher disease, type 2 diabetes

ABSTRACT

This work details the evaluation of a number of N-alkylated deoxynojirimycin derivatives on their merits as dual glucosylceramide synthase/neutral glucosylceramidase inhibitors. Building on our previous work we synthesised a series of D-*gluco* and L-*ido*-configured iminosugars N-modified with a variety of hydrophobic functional groups. We found that iminosugars featuring N-pentyloxymethylaryl substituents are considerably more potent inhibitors of glucosylceramide synthase than their aliphatic counterparts. In a next optimization round we explored a series of biphenyl-substituted iminosugars of both configurations (D-*gluco* and L-*ido*) with the aim to introduce structural features known to confer metabolic stability to drug-like molecules. From these series two sets of molecules emerge as lead series for further profiling. Biphenyl-substituted L-*ido*-configured deoxynojirimycin derivatives are selective for glucosylceramidase and the non-lysosomal glucosylceramidase and we consider these as leads for the treatment of neuropathological lysosomal storage disorders. Their D-*gluco*-counterparts are also potent inhibitors of intestinal glycosidases and by this virtue we regard these as leads for type 2 diabetes therapeutics.

INTRODUCTION

N-Substituted deoxynojirimycin derivatives are an important class of molecules in medicinal chemistry and drug discovery.[1] N-(Hydroxyethyl)-deoxynojirimycin **1** (Figure 1B) is marketed as Miglitol as an anti-diabetic agent (type 2 diabetes) and acts as a broad-spectrum inhibitor of several intestinal glycosidases (maltase, sucrase, lactase).[2] N-butyl-deoxynojirimycin (Zavesca, **2**) was developed as a glucosylceramide synthase inhibitor and is used in the clinic to treat the lysosomal storage disorder, Gaucher disease.[3] The therapeutic success of these molecules serves as an inspiration for many researchers and several reports on deoxynojirimycin derivatives bearing various N-substituents have appeared in recent years.[4]

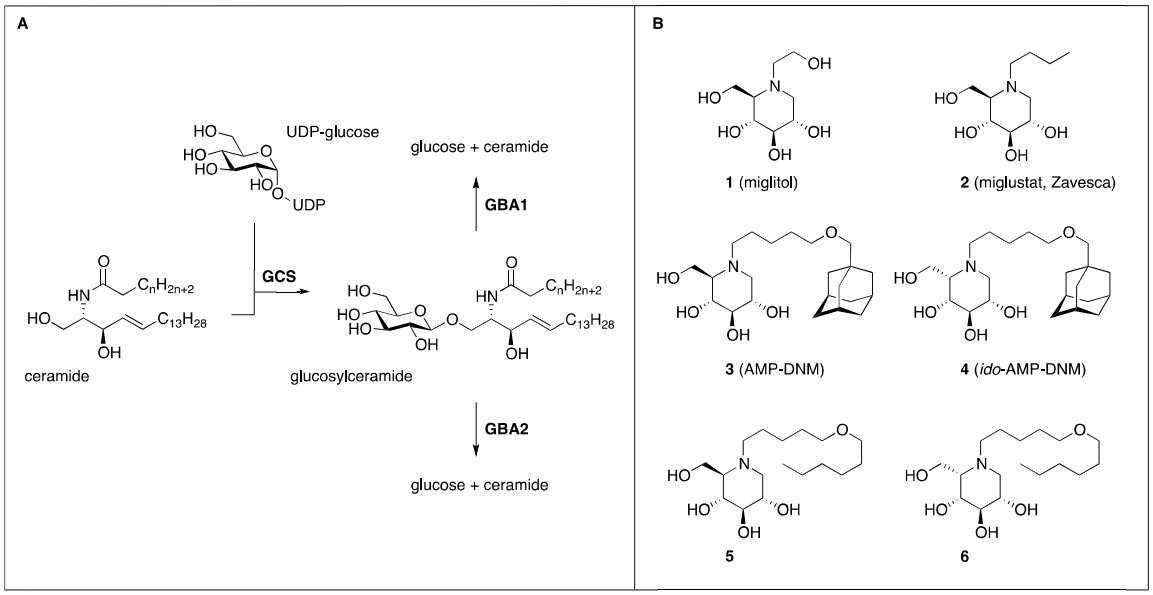


Figure 1. A) Glucosylceramide metabolism (**GCS** = glucosylceramide synthase, **GBA1** = acid glucosylceramidase, **GBA2** = neutral glucosylceramidase). B) Clinical drugs miglitol (**1**) and miglustat (Zavesca, **2**) based on deoxynojirimycin and lead structures **3**-**6** that form the basis of the here presented studies.

Our research on deoxynojirimycin derivatives focuses on compounds able to interfere with glucosylceramide metabolism (Figure 1A).[5] Glucosylceramide is the condensation product of

UDP-glucose and ceramide and its formation is catalysed by glucosylceramide synthase (GCS). Glucosylceramide is the starting point of a considerable part of all human glycosphingolipids and pharmacological interference with glucosylceramide metabolism also affects these glycoconjugates. Under normal circumstances glucosylceramide is degraded in lysosomes by acid glucosylceramidase (glucocerebrosidase, GBA1), however the neutral glucosylceramidase (GBA2) residing in the cytoplasm is also capable of processing glucosylceramide. Although its physiological role remains unclear, GBA2 is involved in neuropathological effects observed in several lysosomal storage disorders.[6] In Gaucher disease and Niemann-Pick type B disease (caused by inherited deficiency in acid sphingomyelinase), elevated lysosomal glucosylceramide levels lead to partial leakage to the cytosol, where it is processed by GBA2 to produce cytosolic ceramide. It may well be that the thus produced cytosolic ceramide itself in neuronal cells is at the basis of the observed neuropathology.[7] We have however indications that GBA2, like GBA1[8a] acts also as a retaining transglucosylase by transferring glucose from ceramide to cholesterol, and that the thus formed unusual (in man) glycolipid, cholesterol-beta-glucoside, might be causative in neuropathological lysosomal storage disorders (LSDs).[8]

In recent years the realisation has grown that each of the enzymes involved in glucosylceramide metabolism are relevant targets for drug development.[5] Moreover, and in contrast to the clinical dogma of compound selectivity, it may well be that the most effective compounds should inhibit a combination of glucosylceramide processing enzymes, or alternatively one of these in combination with unrelated glycoprocessing enzymes. The clinical drug used in substrate reduction therapy for type 2 Gaucher patients, Miglustat **2**, inhibits GCS at relatively high concentrations, but is a rather potent GBA2 inhibitor.[9] The observed clinical effect of this drug may therefore be based on this dual inhibition effect. It should be noted that the beneficial effect of Miglustat is accompanied by gastrointestinal- and metabolic side effects as a result of its strong inhibitory activity on intestinal glycosidases. In the same vein, a pharmacological chaperone for GBA1 – pharmacological

chaperone therapy being a new yet clinically unproven concept aimed at stabilizing the proper fold of mutant GBA1 in the endoplasmic reticulum (ER)[10] – that acts by active site binding may turn out to be a GCS inhibitor as well. In such a strategy, abnormal glucosylceramide levels may be restored by, at once reducing its biosynthesis (through GCS inhibition) and enhancing its turnover (by increasing lysosomal GBA1 activity). Finally we demonstrated recently that dual inhibition of GCS and intestinal glycosidases results in restoration of insulin sensitivity as well as reduction of glucose levels in type 2 diabetes animal models.[11] In this concept, AMP-DNM **3** emulates (and does so at lower concentrations) the action of the established drug Miglitol **1** – known to act by reducing blood glucose levels by blocking intestinal glycosidases – and complements this by reduction of cell surface glycolipids. Of these, ganglioside GM3 can interfere with insulin signalling through binding at the outer cell surface to the insulin receptor, thereby preventing insulin receptor dimerisation – a prerequisite for insulin signalling.[12] Ganglioside GM3 is a downstream metabolite of glucosylceramide and GCS inhibition therefore also affects the production of this glycosphingolipid.[13]

Our work on N-alkylated iminosugars in the past years has focused on N-(adamantanemethyloxypentyl)-deoxynojirimycin (AMP-DNM, **3**, Figure 1) and its C5-epimer, (adamantanemethyloxypentyl)-L-*ido*-deoxynojirimycin (*ido*-AMP-DNM, **4**). In our first studies, we found AMP-DNM **3** to be a superior GCS inhibitor compared to Zavesca **2** (**2**: IC_{50} = 25 μM, **3**: IC_{50} = 200 nM).[9] Compound **3** also strongly inhibits GBA1, GBA2 as well as intestinal lactase, sucrase and isomaltase. Though not a selective compound we could show that co-inhibition of GCS and intestinal glycosidases through application of **3** has a beneficial effect in the aforementioned studies on type 2 diabetes models.[11] Inversion of configuration at C5 gave *ido*-AMP-DNM **4**, which inhibits GCS and GBA2 with about equal potency as **3** but with little to no activity against the intestinal glycosidases, and we proposed this compound as a suitable lead for further development towards LSD therapeutics.[14] Therapeutics directed against type 2 diabetes and Gaucher disease should ideally not interfere with GBA1

activity. Importantly, L-*ido* congener **4** is comparatively the weaker GBA1 inhibitor (**3**: IC_{50} = 200 nM, **4**: IC_{50} = 2 μM). In an initial study[15] we found that replacing the adamantyl group in **4**, but not in **3**, with linear alkyl substituents (propyl up to nonyl, exemplified by structures **5** and **6**, respectively) led to a considerably improved selectivity. Whereas GCS activity of both D-*gluco* and L-*ido* congeners remained essentially the same (with the L-*ido*-based inhibitors being consistently slightly more potent), activity against GBA1 dropped significantly for the L-*ido*-compounds, but not in the D-*gluco*-series. We here report an in-depth study based on these initial findings. In a first set of compounds we explore a comprehensive series of aliphatic, unsaturated and aromatic alkyl side chains grafted onto the nitrogen of both deoxynojirimycin and L-*ido*-deoxynojirimycin. Replacement of the adamantane moiety with any of a series of aromatic substituents gives rise to compounds with remarkably improved GCS inhibitory potency. Of these series, we selected those compounds equipped with a methylbiphenyl substituent for further profiling since this particular moiety has proven its merits as a bioisostere for fatty acids with clear guidelines for structural modification to deliver drug-like compounds, which not only elicit strong inhibitory activity, but for which we may expect enhanced metabolic stability, bioavailability and reduced toxicity compared to the adamantyl functionality. In a second series of compounds we demonstrate that such alterations can be introduced without hampering enzyme inhibitory activity and selectivity, yielding a number of improved leads for development towards neuropathological LSDs and type 2 diabetes.

RESULTS

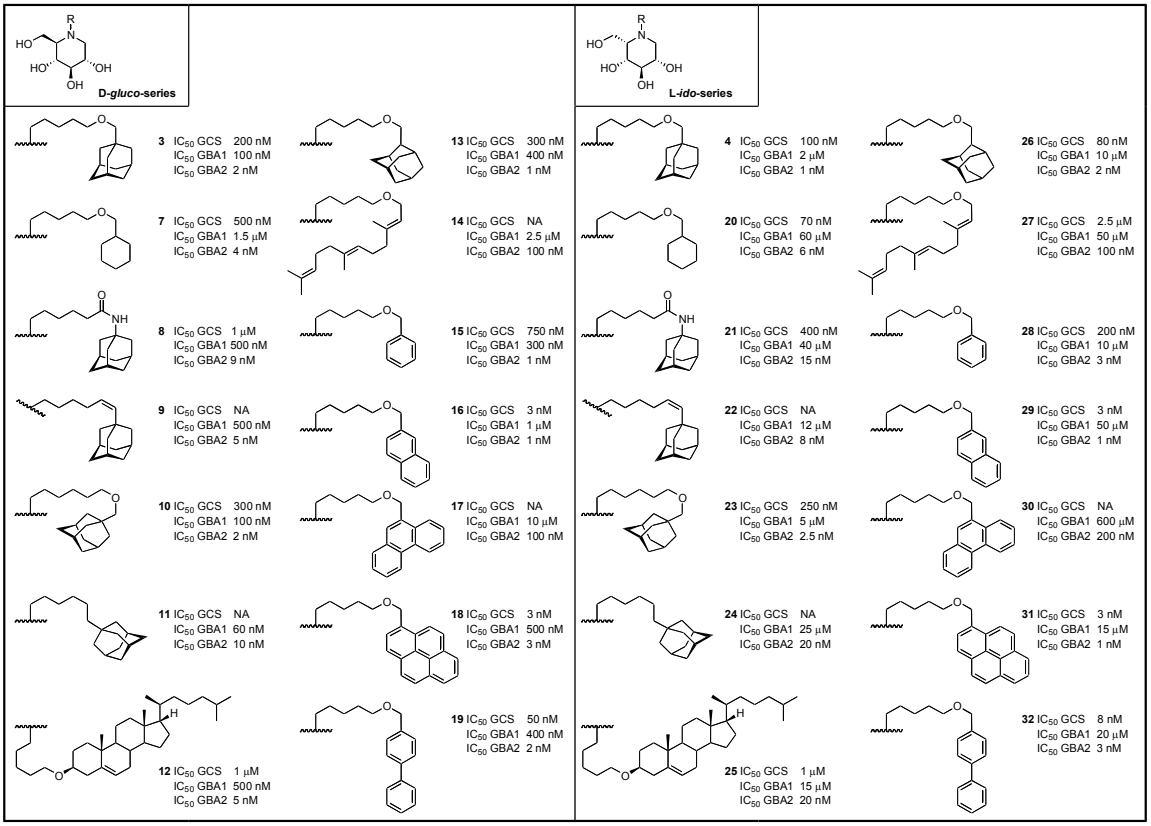


Figure 2. Inhibition of GCS, GBA1 and GBA2 by D-*gluco*-deoxynojirimycin and L-*ido*-deoxynojirimycin derivatives featuring a variety of hydrophobic N-substituents. NA: IC_{50} > 20 μM.

Figure 2 depicts the inhibitory potency of 26 N-alkylated iminosugars (**7**-**32**) towards the three enzymes involved in glucosylceramide metabolism: GCS, GBA1 and GBA2, in comparison to the lead structures, AMP-DNM **3** and *ido*-AMP-DNM **4**. The compounds were synthesised following established procedures. Briefly, we prepared gram quantities of deoxynojirimycin[16] and L-*ido*-deoxynojirimycin.[14] These were N-alkylated with the halide of the respective N-substituents, which in turn were prepared by standard chemical transformations (see Supporting Information). In this fashion, we prepared a series of close analogues of the two lead compounds in which the nature of the adamantane moiety is altered, as well as compounds bearing comparatively more different apolar substituents

ranging from farnesyl over cholesteryl to a number of aromatic moieties. Comparison of the inhibition data of this first set of compounds allows for several interesting observations.

Within the series of adamantane analogues, it appears that the nature of the linkage between the spacer moiety and the bulky head group is important for GCS inhibition. Whereas replacement of the ether linkage in D-*gluco*/L-*ido* derivatives **3**/**4** by an amide, as in **8**[17]/**21**, has limited influence on GCS (IC_{50} four-five fold lower), elimination of the oxygen (**11**/**24**, also **9**/**22**) has a drastic negative effect on GCS inhibition. Interestingly, the effect on GBA1 with a 20 to 50-fold decrease is much more pronounced. Linking the adamantane through a secondary carbon, instead of a tertiary carbon, yields compounds with an inhibitory profile essentially the same as that of the parent compounds (compare **13**/**26** with **3**/**4**). The same holds true when substituting the adamantane for either the more moderately sized hexyl or bulky cholesteryl moiety (compare **13**/**26** and **12**/**25**, respectively, with **3**/**4**). Compounds **14**/**27**, having farnesyl ether as a branched and partially unsaturated bulky head group, are poor GCS inhibitors. Substitution of the aliphatic head group in **3**/**4** by aromatic moieties however provided drastically improved GCS inhibitors. Benzyl derivatives **15**/**28** inhibit GCS in the range also observed for adamantane leads **3**/**4**. In contrast, enlarging the size of the aromatic moiety to probe the size of the hydrophobic binding cavity, as in the D-*gluco*/L-*ido* pairs **16**/**29**, **18**/**31** and **19**/**32**, afforded a series of low nanomolar GCS inhibitors. Interestingly, whereas naphthyl derivatives **16**/**29** and pyrenyl derivatives **18**/**31** proved to be single digit nanomolar GCS inhibitors, the corresponding phenanthryl derivatives **17**/**30** did not inhibit GCS at the measured concentrations (up to 10 μM). Apparently, branched hydrophobic groups at this side are not well accommodated in the GCS active site (it should be noted that no GCS structure is available for structure-activity studies). When comparing the D-*gluco* series with the L-*ido* series in general on GCS inhibition potency we observe a trend we have witnessed before, namely that the inhibition potency of a D-*gluco*-deoxynojirimycin derivative is at least matched (for instance, **18** and **31**) and often surpassed (for instance, compare **13**

with **26**) by that of the corresponding L-*ido*-congener. With respect to GBA1 inhibition without exception the D-*gluco* compounds are the stronger inhibitors. Therefore, and as we observed before, the L-*ido* configuration is preferable when aiming for correcting for glucosylceramide levels in Gaucher disease (in which as said GBA1 activity is already impaired) in substrate reduction therapy strategies by inhibition of GCS. We finally observe that, with IC_{50} values ranging from 1 to 200 nM, all compounds are potent GBA2 inhibitors, and that the most potent GCS inhibitors without exception also belong to the most potent GBA2 inhibitors.

Arguably and based on these initial studies, the most attractive selective dual GCS/GBA2 inhibitors are compounds **29**, **31** and **32**, which are single digit nanomolar GCS/GBA2 inhibitors that inhibit GBA1 only in the micromolar range. Of the three aromatic moieties we reasoned that the biphenyl moiety, present in **19**/**32**, would be the most suitable for further development for several reasons. Naphthyl- and pyrenyl moieties are prone to metabolic oxidation to deliver reactive aromatic epoxides that are mutagenic. Biphenyl moieties are less prone to the formation of such toxic metabolites, have better drug-like properties and are more attractive from a medicinal chemistry point of view to make various structural modifications. We therefore selected the biphenyl-methyl moiety for further optimization and identified a number of positions for chemical modification to arrive at a series comprising leads with not only different selectivity and potency profiles, but also improved DMPK properties. Chemical modifications are both in the external and internal ring, in the benzylic CH_2 connecting the biphenyl to the spacer, and in the spacer pentane itself. In our optimization program we decided on D-*gluco*-derivative **19** as our starting point. This for practical reasons since the starting iminosugar, deoxynojirimycin, is comparatively easier to access on a suitable scale and also gives better yields in the N-alkylation step than the corresponding L-*ido* congener. This also for intrinsic reasons since **19** can be considered as an improved lead structure compared to **3** for development towards type 2 diabetes therapeutics.

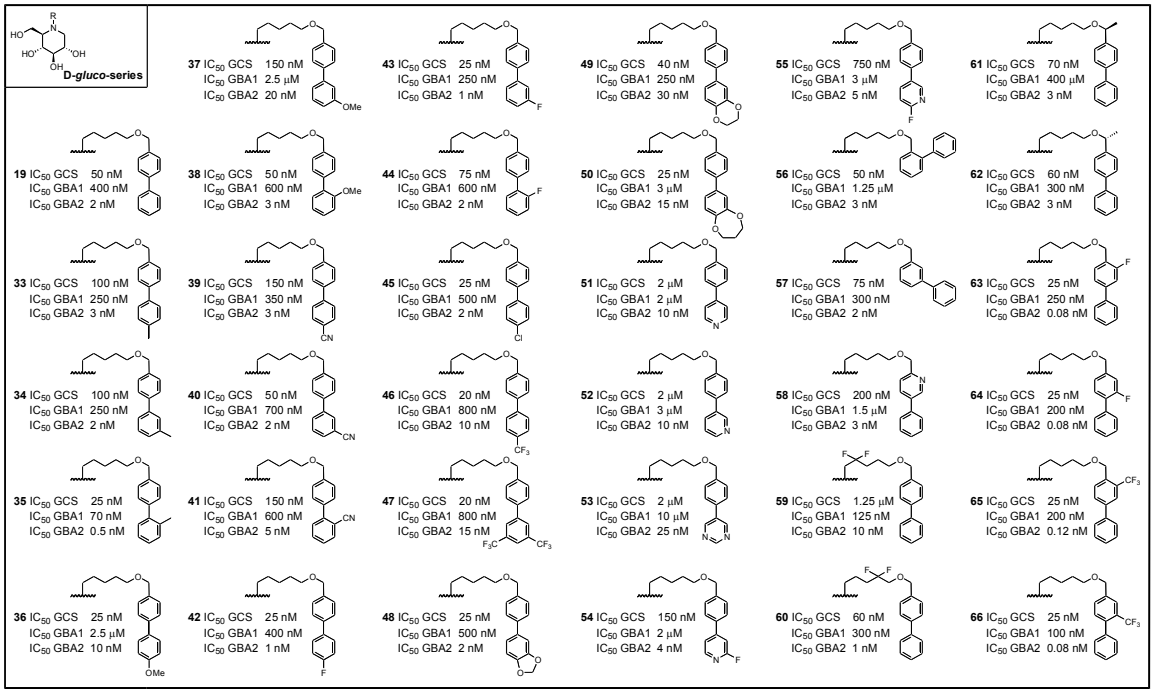


Figure 3. Inhibition of GCS, GBA1 and GBA2 by a variety of biphenyl-substituted D-*gluco*-deoxynojirimycin derivatives.

We thus assembled a comprehensive number of analogues of biphenyl-DNM derivative **19** and assessed their inhibitory potency on GCS, GBA1 and GBA2. The most attractive modifications were transferred to the L-*ido*-deoxynojirimycin scaffold to afford a list of compounds that were again tested on GCS, GBA1 and GBA2. Finally we selected from both series a number of compounds that we tested on a panel of intestinal glycosidases. Figure 3 depicts our focused library of biphenyl-substituted deoxynojirimycin derivatives and their inhibitory potency towards GCS, GBA1 and GBA2. Compounds **33**-**50** are biphenyl analogues with the terminal phenyl substituted at the ortho, meta- or para position, and carrying varying substitutions on the terminal phenyl ring. We selected both carbon substituents and heteroatom substituents and included both electron-donating and electron-withdrawing functional groups in our studies. As can be seen, the effect of these

modifications on GCS inhibition is modest, and all compounds inhibit GCS with an IC_{50} between 20 nM and 150 nM. Essentially the same holds true for GBA2 (IC_{50} 0.7-10 nM) whereas the nature of the functional group appears to have a somewhat more profound effect on GBA1 inhibition. Ortho-methyl derivative **35** inhibits GBA1 with an IC_{50} of 70 nM and is the most potent GBA1 inhibitor of this series. In contrast, the weakest inhibitor of this series is dioxetane derivative **50** with an IC_{50} of 3 μM. Overall however the effect of the substituents in **33**-**50** is limited when looking at inhibitory potency and selectivity. All modifications are tolerated, which holds promise for stabilizing the external phenyl ring at for instance the ortho- or para position, positions susceptible to oxidation by cytochrome P450 enzymes. Substitution of the terminal phenyl for pyridine (**51**, **52**) or pyrazine (**53**) has a more pronounced effect on GCS inhibition, with a drop in IC_{50} from 40 nM (**19**) to 2 μM. Apparently, a weak basic character at this position is poorly tolerated by the GCS active site, an observation underscored by the finding that inhibition is moderately (**55**) to largely (**54**) restored by decreasing the basicity through instalment of a fluorine substituent onto the pyridine moiety. Essentially the same trend within this series is observed for GBA1 whereas as before the nature of the modification appears to have limited influence on GBA2 inhibition. Ortho-biphenyl derivative **56** and meta-biphenyl derivative **57** resemble para-biphenyl derivative **19** where it comes to GCS and GBA2 inhibition, but interestingly the meta-analogue appears to fit better within the GBA1 active site. Substitution of the internal phenyl for pyridyl, as in **58**, has some detrimental effect on GCS inhibition, but not on GBA1/2 inhibition. The same holds true for analogue **59**, with the hydrogens on the spacer carbon beta to the deoxynojirimycin nitrogen substituted for fluorine. Arguably, this modification, designed to prohibit metabolic oxidation at this site, lowers the basic character of the ring nitrogen such that the molecule is accommodated slightly more poorly within the GCS active site. Substitution of the spacer methylene beta to the ether oxygen (for the same reason to increase metabolic stability) as in **60** has the opposite effect: stronger GCS inhibitor and weaker GBA1 inhibitor. Introduction of a branching methyl group at the

benzylic carbon (enantiomeric pair **61** and **62**) – a modification known to confer stability to this oxidation-prone position – yields compounds with an attractive GCS/GBA1 selectivity window: good GCS inhibitors and weak GBA1 inhibitors. Interestingly, diastereomers **61** and **62** appear equally potent inhibitors for all three enzymes and thus no enantiospecificity is at play with respect to the chiral benzyllic positions. Although we have no explanation for this finding, it might be that the benzyllic position is not directly interacting with the enzyme active sites but rather embedded within a membrane (both GCS and GBA2 being membrane-associated enzymes). Compounds **63**-**66** finally bear either a fluorine or trifluormethyl substituent at either the ortho- or meta-position of the inner phenyl ring. These compounds are GCS inhibitors (IC_{50} of 25 nM) and are highly potent GBA2 inhibitors (sub nanomolar activity). They are also amongst the strongest GBA1 inhibitors of this series.

Figure 4 depicts a small focused library of L-*ido*-derivatives **67**-**76** containing biphenyl modifications we selected from the D-*gluco*-series. We made this selection based on both inhibitor potency and selectivity and putative stabilizing effect on metabolic enzymes. In general and as before (Figure 2) the L-*ido*-isomers when compared with their respective D-*gluco*-congeners are more potent GCS inhibitors, are (considerably) less potent GBA1 inhibitors and inhibit GBA2 in about equal potency. We consider compounds **73**-**76** especially interesting from these series, as they emulate the beneficial properties of parent compound **32** (single digit nanomolar GCS inhibitor, subnanomolar GBA2 inhibitor offset by only micromolar GBA1 inhibition) and combine this with stabilizing substituents at the inner phenyl ring. We note that these compounds may not yet contain the most optimal combination of substitutions, and for instance including the distinguishing features in compounds **42** and **60** (Figure 3) may result in even more optimal compounds. We also conclude that, for optimal GCS inhibition, para-configured biphenyl moieties are the more attractive substituents (compare **32** with **69** and **70**).

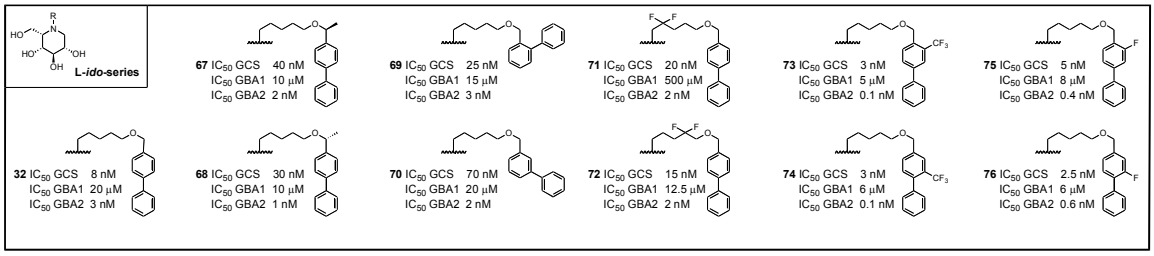


Figure 4. Inhibition of GCS, GBA1 and GBA2 by a variety of biphenyl-substituted L-*ido*-deoxynojirimycin derivatives.

From the set of L-*ido*-deoxynojirimycin derivatives depicted in Figure 4 we consider compounds **73**-**76** as the most suitable leads for further profiling as dual GCS/GBA2 inhibitors for the treatment of neuropathological lysosomal storage disorders. For this the compounds preferably should not display inhibition of intestinal glycosidases. For this purpose, but also to establish whether the analogous D-*gluco*-deoxynojirimycin derivatives would be viable leads for development towards anti-type 2 diabetics (co-inhibition of GCS and intestinal glycosidases) we assessed inhibition of intestinal sucrase, maltase and lactase with a selected number of compounds: all biphenyl modified L-*ido*-deoxynojirimycin derivatives alongside their D-*gluco*-congeners (Table 1). Without exception the hydrophobic D-*gluco*-deoxynojirimycin derivatives inhibit all three enzymes measured, whereas the L-*ido*-deoxynojirimycin derivatives do not. In this sense both series emulate the properties of their respective parent compounds, AMP-DNM **3** and L-*ido*-AMP-DNM **4**, respectively, and reflect the general trend that the intestinal glycosidases tested prefer glucose as the (natural) substrate over the non-natural (in mammals) L-idose configuration.

D-*gluco*-series				**L-*ido*-series**			
	sucrase	lactase	maltase		sucrase	lactase	Maltase
3	0.6	35	3	**4**	>100	200	>500
19	0.25	20	2	**32**	70	80	>100

61	0.25	9	2.5	**67**	>100	>100	>100
62	0.5	35	4	**68**	>100	45	>100
56	0.2	17	1.5	**69**	95	25	>100
57	0.2	20	1.5	**70**	100	60	>100
59	35	>100	>100	**71**	>100	>100	>100
60	0.4	27	9	**72**	>100	80	>100
65	1.5	50	8	**73**	>100	125	>100
66	1.25	45	7	**74**	>100	100	>100
63	0.25	30	2.5	**75**	>100	60	>100
64	0.4	20	3	**76**	>100	50	>100

Table 1. Inhibition of intestinal glycosidases by a selection of D-*gluco*- and L-*ido*-deoxynojirimycin derivatives bearing biphenyl substituents. Inhibition values are given as IC_{50} (μM) values.

DISCUSSION

In this paper we have described an in-depth study on 72 deoxynojirimycin derivatives differing in both configuration at C5 and the nature of the hydrophobic moiety attached to the ring-nitrogen, and their inhibitory profile towards the enzymes involved in glucosylceramide metabolism; GCS, GBA1 and GBA2. At the onset of our studies we set out to identify compounds with improved properties with respect to inhibition profile (potency, selectivity, compounds that would target two enzymes/enzyme classes simultaneously). Addition of hydrophobic moieties to the iminosugar in general increases potency, however, it may also lead to a deterioration of the physico-chemical properties required for oral bioavailability.

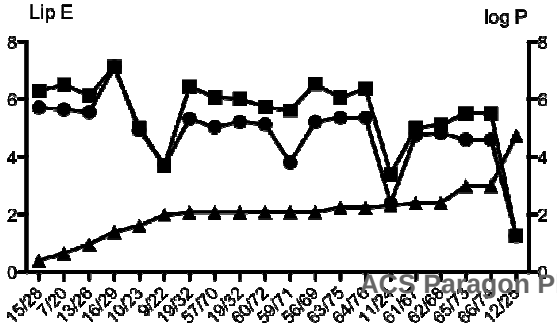


Figure 5. Lipophilic ligand efficiency (LipE) values of matched molecular pairs from the L-*ido* and D-*gluco* series for GCS activity (square = L-*ido* series; circle = D-*gluco* series; triangle = log P).

To gain insight in these issues, the lipophilic ligand efficiency index (LipE), which is defined as pIC50-LogP, is often used as a composite parameter to guide the optimization of compounds. A high LipE index indicates that the affinity of a compound for a target is likely driven by favourable inhibitor-protein interactions, rather than an aspecific entropy-driven effect.[18] In Figure 5, we have plotted the LipE values of matched molecular pairs from the L-*ido* and D-*gluco* series (for this purpose, LogP values were calculated using the ChemDraw version 13 software). It should be noted that most iminosugars have high LipE values (> 10.000-fold selectivity for GCS than for a generic hydrophobic surrounding). The L-*ido* series appear to outperform the D-*gluco* series in this evaluation, indicating that they exhibit a more specific interaction with GCS. In general, the LipE value of both compound series decreases with more hydrophobic substituents indicative of an entropy-driven binding to GCS.

Based on previous results we consider both neuropathological lysosomal storage disorders and type 2 diabetes as therapeutic application areas. GCS is an attractive target for both (established for Gaucher disease and indicated for type 2 diabetes) but according to our previous results is ideally targeted in combination with either GBA2 (LSDs) or intestinal glycosidases (type 2 diabetes). Our results reveal that subtle modification of the biphenyl core influences inhibitory potency towards GCS and GBA1, while having less effect on GBA2 inhibition. Our results also confirm our previous finding that D-*gluco*-configured deoxynojirimycin derivatives bearing a hydrophobic N-substituent are relatively good

inhibitors of the intestinal glycosidases maltase, sucrase and lactase, whereas the corresponding L-*ido*-configured iminosugars are inactive towards these enzymes. This work thus presents a number of improved lead structures for further development. We propose biphenyl-substituted N-alkylated *D*-*gluco*-deoxynojirimycin derivatives as type 2 diabetes leads based on their dual GCS/intestinal glycosidase inhibitory capacity. We note that these compounds also target GBA1 and GBA2. Our focused libraries delivered leads such as compound **61** with low GBA1 inhibitory activity. On the other hand it is difficult to steer away from GBA2 inhibitory activity. Interestingly though, a recent paper by Summers and co-workers indicate that the culprit (glyco)sphingolipid in type 2 diabetes varies between tissues.[19] They indicate that in adipocytes elevated levels of ganglioside GM3 are causative of insulin resistance. This confirms our previous study showing that downregulation of ganglioside GM3 through GCS inhibition restores insulin signalling in obese diabetic mice and rats. They however also show compelling evidence that in myocetes ceramide is the actual disease-inducing lipid. If true, then co-inhibition of GCS and GBA2, alongside inhibition of intestinal glycosidases (prevention of glucose assimilation) may actually turn out to be beneficial. Our L-*ido*-configured, N-alkylated deoxynojirimycin derivatives we propose as leads for neuronopathic lysosomal storage disorders based on their dual GCS/GBA2 inhibitory activity. Compounds **73**-**76** are single digit nanomolar GCS inhibitors and as such equal or even surpass the activity of non-iminosugar GCS inhibitors reported in recent years.[20] The latter compounds are based on ceramide and as far as we know do not target GBA2.

Finally, next to D-*gluco*- and L-*ido*-configured iminosugars, N-alkylated deoxygalactonojirimycin derivatives have been studied as compounds able to interfere with glucosylceramide metabolism. Originally[21] studied as GCS inhibitors, N-alkyl-deoxygalactonojirimycin derivatives were recently[22] put forward as inhibitors selective for GBA2 over GBA1. We have previously reported[14] a head-to-head study comparing, amongst others, N-butyl and N-adamantanemethyloxypentyl substituted iminosugars differing in

configuration at C4 and C5. The data obtained for the D-*gluco*, L-*ido* and D-*galacto*-configured iminosugars bearing these substituents are summarised in Figure 6 and to complete the list we prepared and evaluated biphenyl-modified galactonojirimycin **80**. Though our values deviate from those reported by van der Spoel and co-workers for compound **78**[22], the trend as observed from the N-butyl series (**2**, **77**, **78**) that GBA2 is considerably more sensitive to inhibitors of the deoxynojirimycin class is confirmed in the adamantane-substituted (**3**, **4**, **79**) and biphenyl-substituted (**19**, **32**, **80**) series. The D-*galacto*-configured compounds indeed favour GBA2 over GBA1 when compared to the D-*gluco*-configured compounds, though the differences are less pronounced when comparing with the L-*ido*-configured compounds.

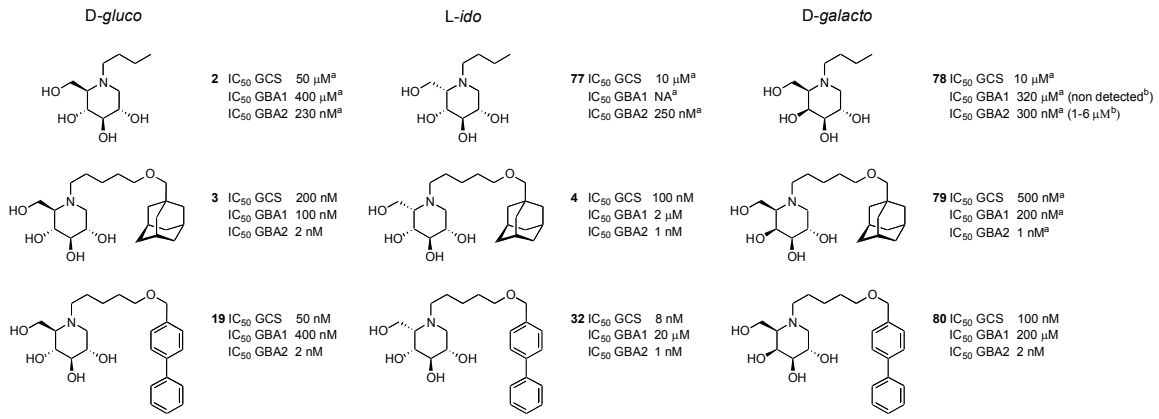
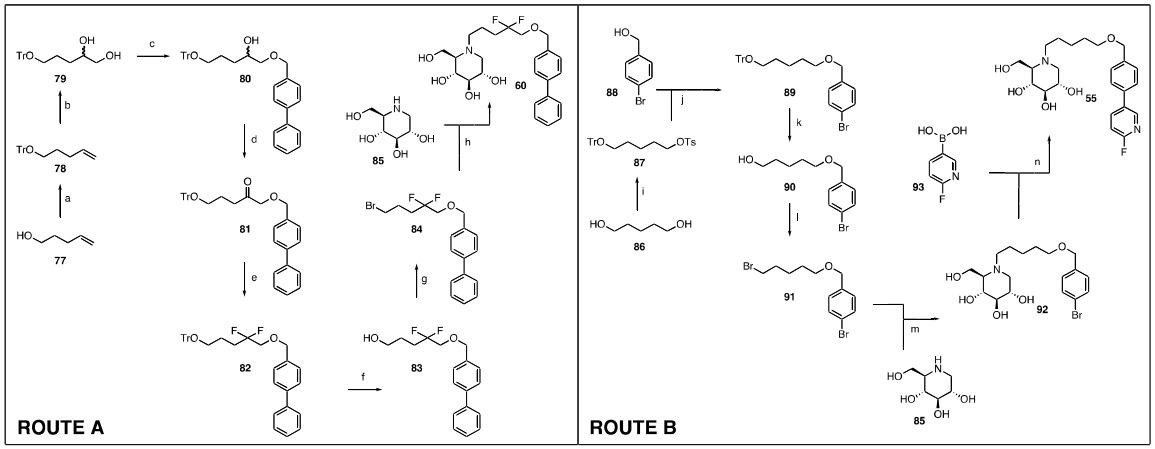


Figure 6. Comparison of inhibitory potency differently substituted D-*gluco*, L-*ido* and D-*galacto*-configured deoxynojirimycins on GCS, GBA1 and GBA2 ([a]values taken from reference 14, [b]values taken from reference 22).

In conclusion, our in-depth study on modifications on the biphenyl core, how to prepare these (as shown in detail in the experimental section) and their effect on enzyme inhibition potency and selectivity, will help the identification, after further studies on *in vivo* efficacy and pharmacochemical properties, of lead compounds for development in the direction of both human disease areas.

EXPERIMENTAL SECTION

Synthetic procedures. The ^{1}H and ^{13}C-NMR spectra were recorded on a 200/50, 300/75, 400/100, 500/125, or 600/150 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard for all ^{1}H NMR measurements in $CDCl_3$ and the deuterated solvent signal for all other NMR measurements. Coupling constants (J) are given in Hz. High resolution mass spectra were recorded on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode with resolution R = 60 000 at m/z 400 (mass range m/z = 150-2000). All tested iminosugars were analyzed via a combination of HPLC and LC/MS that showed a purity of >95%. All iminosugars were tested as their trifluoroacetic acid salts. AMP-DNM **3** and L-*ido*-DNM **4** were prepared as described previously.[14] Compounds **7**-**76** were prepared following either of two general schemes as demonstrated in the experimental scheme for two representative examples.



Experimental Scheme. Reagents and conditions: **ROUTE A**: (a) TrCl, Et_3N, CH_2Cl_2, 48h, 94%; (b) OsO_4, NMO, H_2O, acetone, *t*BuOH, 85%; (c) Bu_2SnO, toluene, reflux (Dean-Stark), 18h, then 4-bromomethylbiphenyl, TBABr, 90 °C, 3h, 69%; (d) DMSO, oxalyl

chloride, CH_2Cl_2, -78 °C, 10 min, then add **80**, -70 °C, 10 min, then Et_3N, -60 °C to rt, 92%; (e) DAST, CH_2Cl_2, 54 h, 94 %; (f) $BF_3{\cdot}OEt_2$, MeOH/toluene 1:1, 6h, 99%; (g) CBr_4, PPh_3, CH_2Cl_2, 0 °C, 73%; (h) K_2CO_3, DMF, 80 °C, 24h, 47%; **ROUTE B**: (i) 1) TrCl, TEA, EtOAc, 85 °C, 99% 2) TsCl, TEA, CH_2Cl_2, 20h, 96%; (j) NaH, DMF, **88**, 4h, 80%; (k) $BF_3{\cdot}OEt_2$, MeOH/toluene 1:1, 6h, 84%; (l) CBr_4, PPh_3, CH_2Cl_2, 0 °C, 99%; (m) K_2CO_3, DMF, 80 °C, 24h, 67%; (n) NaOMe, **92**, **93**, $Pd(PPh_3)_4$, EtOH, 65 °C, 31%.

In **ROUTE A**, key step is the direct alkylation of the unprotected iminosugar to the functionalized alkyl substituent under alkaline conditions. The route is exemplified for the synthesis of fluorinated derivative **60**. Alkylation of deoxynojirimycin **85**[16] with alkyl bromide **84** is accomplished in DMF at 0.2 M substrate concentration. Amine **85** and bromide **84** are reacted in stoichiometric amounts in the presence of a three-fold excess of potassium carbonate. The requisite alkyl bromides are prepared individually following established procedures, as is outlined here fore **84**. In **ROUTE B**, terminally modified phenyl substituents are attached to benzyl bromide core **92** using Suzuki cross-coupling chemistry. The synthesis of the required building block **92** follows chemistry related tot that described in **ROUTE A**, and as an individual example the cross-coupling of **92** with **93** to give library member **55** is depicted. The library members are prepared using the following general strategies: **ROUTE A** starting from deoxynojirimycin **85**: compounds **7**-**19** and **56**-**66**. **ROUTE A** starting from L-*ido*-deoxynojirimycin: compounds **20**-**32** and **67**-**76**. **ROUTE B** starting from deoxynojirimycin **85**: compounds **33**-**55**. All compounds are purified to >95% purity by preparative HPLC-MS. See for full experimental and analytical details on all intermediates and end-products the Supplementary Information.

Enzyme inhibition assays

The enzyme assays used for determining the inhibition of activity of glucosylceramide synthase (GCS)[24], glucocerebrosidase (GBA1)[25], β-glucosidase 2 (GBA2)[26], sucrase, lactase and maltase[16b] were carried out as described previously.

ASSOCIATED CONTENT

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Notes

The authors declare no competing financial interest

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ABBREVIATIONS USED

GCS, glucosylceramide synthase; GBA1, acid glucosylceramidase, GBA2; neutral glucosylceramidase

Supporting information: details on the synthesis and analysis of all new compounds described in this work. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Compain, P.; Martin, O. R. Iminosugars: from synthesis to therapeutic applications. Wiley: New York, **2007**. (b) Butters, T. D.; Dwek, R. A.; Platt, F. M. Therapeutic applications of imino sugars in lysosomal storage disorders. *Curr. Top. Med. Chem*. **2003**, *3*, 561-574.

(2) (a) Hillebrand, I.; Englert, R.; Schulz, H. Efficacy and tolerability of a 3-month treatment with the glucosidase inhibitor Miglitol (Bay +M 1099). *Diabetes* **1986**, *35*, A93-A93. (b) Scott, L. J.; Spencer, C. M. Miglitol. A review of its therapeutic potential in type 2 diabetes mellitus. *Drugs* **2000**, *59*, 521-549.

(3) (a) Platt, F. M.; Neises, G. R.; Dwek, R. A.; Butters, T. D. N-butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthsis. *J. Biol. Chem.* **1994**, *269*, 8362-8365. (b) Cox, T.; Lachmann, R.; Hollak, C.; Aerts, J.; van Weely, S.; Hrebicek, M.; Platt, F.; Butters, T.; Dwek, R.; Moyses, C.; Gow, I.; Elstein, D.; Zimran, A. Novel oral treatment of Gaucher's disease with N-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. *Lancet* **2000**, *355*, 1481-1485.

(4) (a) Diot, J. D.; Moreno, I. G.; Twigg, G.; Mellet, C. O.; Haupt, C. O.; Butters, T. D.; Kovensky, J.; Gouin, S. G. Amphiphilic 1-deoxynojirimycin derivatives through click strategies for chemical chaperoning in N370S Gaucher cells. *J. Org. Chem.* **2011**, *76*, 7757-7768. (b) Yu, W.; Gill, T.; Du, Y.; Ye, H.; Qu, X.; Guo, J.-T.; Cuconati, A.; Zhao, K.; Block, T. M.; Xu, X.; Chang, J. Design, synthesis and

biological evaluation of *N*-alkylated deoxynojirimycin (DNJ) derivatives for the treatment of dengue virus infection. *J. Med. Chem.* **2012**, *55*, 6061-6075. (c) Boucheron, C.; Desvergnes, V.; Compain, P.; Martin, O. R.; Lavi, A.; Mackeen, M.; Wormald, M.; Dwek, R.; Butters, T. D. Design and synthesis of iminosugar-based inhibitors of glucosylceramide synthase: The search for new therapeutic agents against Gaucher disease. *Tetrahedron Asymm.* **2005**, *16*, 1747-1756. (d) Aedes-Guisot, N.; Alonzi, D. S.; Reinkensmeier, G.; Butters, T. D.; Norez, C.; Becq, F.; Shimada, Y.; Nakagawa, S.; Kato, A.; Bleriot, Y.; Sollogoub, M.; Vauzeilles, B. Selection of the biological activity of DNJ neoglycoconjugates through click length variation of the side chain. *Org. Biomol. Chem.* **2011**, *9*, 5373-5388. (e) van den Broek, L. A. G. M.; Vermaas, D. J.; van Kemenade, F. J.; Tan, M. C. C. A.; Rotteveel, F. T. M.; Zandberg, P.; Butters, T. D.; Miedema, F.; Ploegh, H. L.; van Boeckel, C. A. A. Synthesis of oxygen-substituted N-alkyl 1-deoxynojirimycin derivatives: Aza sugar-glucosidase inhibitors showing antiviral (HIV-1) and immunosuppressive activity. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 507-516.

(5) Wennekes, T.; van den Berg, R. J. B. H. N.; Boot, R. G.; van der Marel, G. A.; Overkleeft, H. S.; Aerts, J. M. F. G. Glycosphingolipids - nature, function, and pharmacological modulation. *Angew. Chem. Int. Ed.* **2009**, *48*, 8848-8869.

(6) Aerts, J. M.; Hollak, C.; Boot, R.; Groener, A. Biochemistry of glycosphingolipid storage disorders: implications for therapeutic intervention. *Philos. Trans. R. Lond. B Biol. Sci*. **2003**, *358*, 905-914.

(7) Ashe, K. M.; Bangaari, D.; Li, L.; Cabreza-Salazar, M. A.; Bercury, S. D.; Nietupski, J. B.; Cooper, C. G.; Aerts, J. M.; Lee, E. R.; Copeland, D. P.; Cheng, S. H.; Scheule, R. K.; Marshall, J. Iminosugar-based inhibitors of glucosylceramide synthase prolong survival but paradoxically increase brain glucosylceramide levels in Niemann-Pick C mice. *Mol. Genet. Metab.* **2012**, *105*, 621-628.

(8) (a) Akiyama, H.; Kobayashi, S.; Hirabayashi, Y.; Murakami-Murofushi, K. Cholesterol glucosylation is catalysed by transglucosylation reaction of β-glucosidase-1. *Biochem. Biophys. Res. Commun.* **2013**, *441*, 838-843. (b) Mirzaian, M.; Gaspar, P.; Blommaart, E.; Boot, R. G.; Margues, A.; Wisse, P.; Overkleeft, H. S.; Aerts, J. M. F. G. manuscript in preparation.

(9) Overkleeft, H. S.; Renkema, G. H.; Neele, J.; Vianello, P.; Hung, I. O.; Strijland, A.; van der Burg, A. M.; Koomen, G. J.; Pandit, U. K.; Aerts, J. M. F. G. Generation of specific deoxynojirimycin-type inhibitors of the non-lysosomal glucosylceramidase. *J. Biol. Chem*. **1998**, *273*, 26522-26527.

(10) (a) Babajani, G.; Tropak, M. B.; Mahuran, D. J.; Kermode, A. R. Pharmacological chaperones facilitate the post-ER transport of recombinant N370S mutant beta-glucocerebrosidase in plant cells: evidence that N370S is a folding mutant. *Mol. Genet. Metab.* **2012**, *106*, 323-329. (b) Brumshtein, B.; Greenblatt, H. M.; Butters, T. D.; Shaaltiel, Y.; Aviezer, D.; Silman, I.; Futerman, A. H.; Sussman, J. L. Crystal structures of complexes of N-butyl- and N-nonyl-deoxynojirimycin bound to acid beta-glucosidase – Insights into the mechanism of chemical chaperone action in Gaucher disease. *J. Biol. Chem.* **2007**, *282*, 29052-29058.

(11) Aerts, J. M.; Ottenhoff, R.; Powlson, A. S.; Grefhorst, A.; van Eijk, M.; Dubbelhuis, P. F.; Aten, J.; Kuipers, F.; Serlie, M. J.; Wennekes, T.; Sethi, J. K.; O'Rahilly, S.; Overkleeft, H. S. Pharmacological inhibition of glucosylceramide synthase enhances insulin sensitivity. *Diabetes* **2007**, *56*, 1341-1349.

(12) (a) Kabayama, K.; Sato, T.; Saito, K.; Loberto, N.; Prinetti, A.; Sonnino, S.; Kinjo, M.; Igarashi, Y.; Inokuchi, J. I. Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 13678-13683. (b) Yamashita, T.; Hashiramoto, A.; Haluzik, M.; Mizukami, H.; Beck, S.; Norton, A.; Kono, M.; Tsuji, S.; Daniotti, J. L.; Werth, N.; Sandhoff, R.; Sandhoff, K.; Proia, R. L. Enhanced insulin sensitivity in mice lacking

ganglioside GM3. *Proc. Natl. Acad. Sci. U.S.A*. **2003**, *100*, 3445-3449. (c)Kabayama, K.; Sato, T.; Kitamura, F.; Uemura, S.; Kang, B. W.; Igarashi, Y.; Inokuchi, J. TNFalpha-induced insulin resistance in adipocytes as a membrane microdomain disorder: involvement of ganglioside GM3. *Glycobiology* **2005**, *15*, 21-29.

(13) van Eijk, M.; Aten, J.; Bijl, N.; Ottenhoff, R.; van Roomen, C. P. A. A.; Dubbelhuis, P. F.; Seeman, I.; Ghauharali-van der Vlugt, K.; Overkleeft, H. S.; Arbeeny, C.; Groen, A. K.; Aerts, J. M. F. G. Reducing glycosphingolipid content in adipose tissue of obese mice restores insulin sensitivity, adipogenesis and reduces inflammation. *PLoS One* **2009**, *4*, e4723.

(14) Wennekes, T.; Meijer, A. J.; Groen, A. K.; Boot, R. G.; Groener, J. E.; van Eijk, M.; Ottenhoff, R.;Bijl, N.; Ghauharali, K.; Song, H.; O'Shea, T. J.; Liu, H.; Yew, N.; Copeland, D.; van den Berg, R. J.; van der Marel, G. A.; Overkleeft, H. S.; Aerts, J. M. Dual-action lipophilic iminosugar improves glycemic control in obese rodents by reduction of visceralglycosphingolipids and buffering of carbohydrate assimilation. *J. Med. Chem.* **2010**, *53*, 689-698.

(15) Ghisaidoobe, A.; Bikker, P.; de Bruijn, A. C. J.; Godschalk, F. D.; Rogaar, E.; Guijt, M. C.; Hagens, P.; Halma, J. M.; van't Hart, S. M.; Luitjens, S. B.; van Rixel, V. H. S.; Wijzenbroek, M.; Zweegers, T.; Donker-Koopman, W. E.; Strijland, A.; Boot, R.; van der Marel, G.; Overkleeft, H. S.; Aerts, J. M. F. G.; van den Berg, R. J. B. H. N. Identification of potent and selective glucosylceramide synthase inhibitors from a library of N-alkylated iminosugars, *ACS Med. Chem. Lett.* **2011**, *2*, 119-123.

(16) (a) Wennekes, T.; Lang, B.; Leeman, M.; van der Marel, G. A.; Smits, E.; Weber, M.; van Wiltenburg, J.; Wolberg, M.; Aerts, J. M. F. G.; Overkleeft, H. S. Large-scale synthesis of the glucosylceramide synthase inhibitor N-[5-(adamantan-1yl-methoxy)-pentyl]-1-deoxynojirimycin. *Org. Process Res. Dev.* **2008**, *12*, 414-423. (b) Wennekes, T.; van den Berg, R. J. B. H. N.; Donker, W.; van der Marel, G. A.; Strijland, A.; Aerts, J. M. F. G.; Overkleeft, H. S. Development of adamantan-1-yl-

methoxy-functionalized 1-deoxynojirimycin derivatives as selective inhibitors of glucosylceramide metabolism in man. *J. Org. Chem.* **2007**, *72*, 1088-1097.

(17) Compound **8** has been proposed as a pharmacological GBA1 chaperone: see: Sawkar, A. R.; Adamski-Werner, S. L.; Cheng, W. C.; Wong, C. H.; Beutler, E.; Zimmer, K. P.; Kelly, J. W. Gaucher disease-associated glucocerebrosidases show mutation-dependent chemical chaperone profiles. *Chem. Biol.* **2005**, *11*, 1235-1244.

(18) Shultz, M. D. Setting expectations in molecular optimizations: strengths and limitations of commonly used composite parameters. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5980-5991.

(19) Chavez, J. A.; Siddique, M. M.; Wang, S. T.; Ching, J.; Shayman, J. A.; Summers, S. A. Ceramides and glucosylceramides are independent agonists of insulin signaling, *J. Biol. Chem.* **2014**, *289*, 723-734.

(20) (a) Vunnan, R. R.; Radin, N. S. Analogs of ceramide that inhibit glucocerebroside synthetase in mouse brain. *Chem. Phys. Lipids* **1980**, *26*, 265-278. (b) Zhao, H. M.; Przybylska, M.; Wu, I. H.; Zhang, J. H.; Siegel, C.; Komarnitsky, S.; Yew, N. S.; Cheng, S. H. Inhibiting glycosphingolipid synthesis improves glycemic control and insulin sensitivity in animal models of type 2 diabetes. *Diabetes* **2007**, *56*, 1210-1218. (c) Richards, S.; Larson, C. J.; Koltun, E. S.; Hanel, A.; Chan, V.; Nachtigall, J.; Harrison, A.; Aay, N.; Du, H.; Arcalas, A.; Galan, A.; Zhang, J.; Zhang, W.; Won, K.-A.; Tam, D.; Qian, F.; Wang, T.; Finn, P.; Ogilvie, K.; Rosen, J.; Aoyama, R.; Plonowski, A.; Cancilla, B.; Bentzien, F.; Yakes, M.; Mohan, R.; Lamb, P.; Nuss, J.; Kearney, P. Discovery and characterization of an inhibitor of glucosylceramide synthase. *J. Med. Chem.* **2012**, *55*, 4322-4335. (d) Koltun, E.; Richards, S.; Chan, V.; Nachtigall, J.; Du, H.; Noson, K.; Galan, A.; Aay, N.; Hanel, A.; Harrison, A.; Zhang, J.; Won, K.; Tam, D.; Qian, F.; Wang, T.; Finn, P.; Ogilvie, K.; Rosen, J.; Mohan, R.; Larson, C.; Lamb, P.; Nuss, J.; Kearney, P. Discovery of a new class of glucosylceramide synthase inhibitors. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6773-

6777. (e) Larsen, S. D.; Wilson, M. W.; Abe, A.; Shu, C. H.; Kirchhoff, P.; Showalter, H. D. H.; Xiang, J.; Keep, R. F., Shayman, J. A. Property-based design of a glucosylceramide synthase inhibitor that reduces glucosylceramide in the brain. *J. Lipid. Res.* **2012**, *53*, 282-291. (f) Arthur, J. R.; Wilson, M. W.; Larsen, S. D.; Rockwell, H. E.; Shayman, J. A.; Seyfried, T. N. Ethylenedioxy-PIP2 oxalate reduces ganglioside storage in juvenile Sandhoff disease mice. *Neurochem. Res.* **2013**, *38*, 866-875. (g) Cabrera-Salazar, M. A.; DeRiso, M.; Bercury, S. D.; Li, L.; Lydon, J. T.; Weber, W.; Pande, N.; Cromwell, M. A.; Copeland, D.; Leonard, J.; Cheng, S. H.; Scheule, R. K. Systemic delivery of a glucosylceramide synthase inhibitor reduces CNSA substrates and increases lifespan in a mouse model of type 2 Gaucher disease. *PLoS One* **2012**, *7*, e43310.

(21) Platt, F. M.; Neises, G. R.; Karlsson, G. B.; Dwek, R. A.; Butters, T. D. *N*-Butyldeoxygalactonojirimycin inhibits glycolipid biosynthesis but does not affect *N*-linked oligosaccharide processing. *J. Biol. Chem.* **1994**, *269*, 27108-27144.

(22) Ridley, C. M.; Thur, K. E.; Shanahan, J.; Thillaiappan, N. B.; Shen, A.; Uhl, K.; Walden, C. M.; Rahim, A. A.; Waddington, S. N.; Platt, F. M.; van der Spoel, A. C. β-Glucosidase 2 (GBA2) activity and iminosugar pharmacology. *J. Biol. Chem.* **2013**, *288*, 26052-26066.

(23) Andersson, U.; Butters, T. B.; Dwek, R. A.; Platt, F. M. N-Butyldeoxygalactonojirimycin: a more selective inhibitor of glycosphingolipid biosynthesis than N-butyldeoxynojirimycin, in vitro and in vivo. *Biochem. Pharmacol.* **2000**, *59*, 821-829.

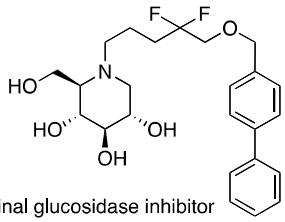
(24) van Weely, S.; van Leeuwen, M. B.; Jansen, I. D. C.; de Bruijn, M. A. C.; Brouwerkelder, E. M.; Schram, A. W.; Clarasamiranda, M.; Barranger, J. A.; Petersen, E. M.; Goldblatt, J.; Stotz, H.; Schwarzmann, G.; Sandhoff, K.; Svennerholm, L.; Erikson, A.; Tager, J. M.; Aerts, J. M. Clinical phenotype of

Gaucher disease in relation to properties of mutant glucocerebrosidase in cultured fibroblasts. *Biochim. Biophys. Acta* **1991**, *1096*, 301-311.

(25) Aerts, J. M.; Donker-Koopman, W. E.; van der Vliet, M. K.; Jonsson, L. M. V.; Ginns, E. I.; Murray, G. J.; Barranger, J. A.; Tager, J. M.; Schram, A. W. The occurrence of two immunologically distinguishable beta-glucocerebrosidases in human spleen. *Eur. J. Biochem.* **1985**, *150*, 565-574.

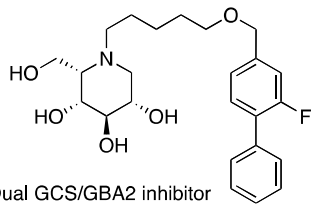
(26) van Weely, S.; Brandsma, M.; Strijland, A.; Tager, J. M.; Aerts, J. M. F. G. Demonstration of the existence of a second, nonlysosomal glucocerebrosidase that is not deficient in Gaucher disease. *Biochim. Biophys. Acta* **1993**, *1181*, 55-62.

GRAPHICAL ABSTRACT



Dual GCS/intestinal glucosidase inhibitor

Lead for type 2 diabetes



Dual GCS/GBA2 inhibitor

Lead for neuropathological LSDs