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Lipophilic iminosugars : synthesis and evaluation as inhibitors of glucosylceramide metabolism

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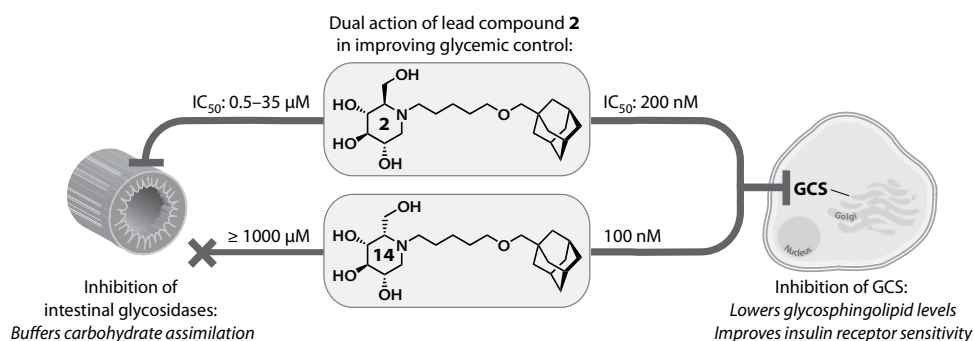
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Improving Glycemic Control with Lipophilic Iminosugars

Influence of Iminosugar Stereochemistry on the Mode of Action

Abstract

This chapter presents analogues of lead lipophilic iminosugar **2** that vary in C-4/C-5 stereochemistry and functionalization of the nitrogen atom. From these analogues, **14** was identified as an equally potent but much more selective inhibitor of glucosylceramide synthase (GCS). The obtained GCS-selective inhibitor **14** was used to study the mechanism by which **2** improves glycemic control in type 2 diabetes rodent models. It was found that **2** exerts its beneficial effects on glycemic control via a dual action by both lowering of glycosphingolipids in tissues and buffering carbohydrate assimilation in the small intestine.

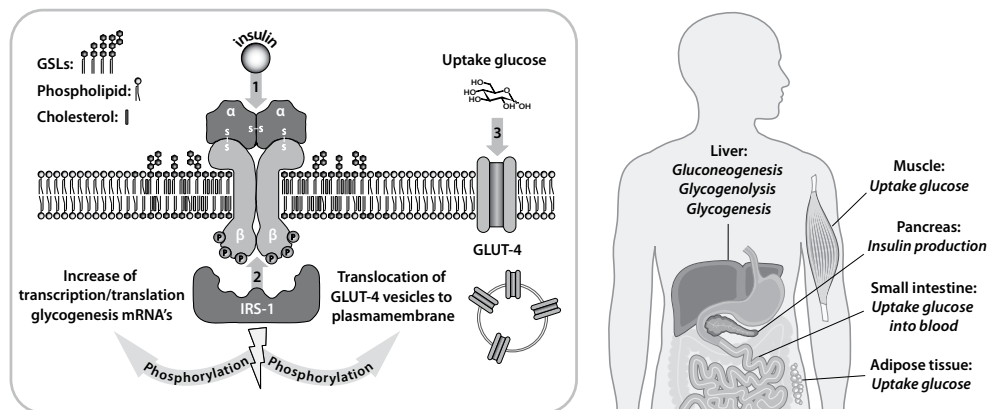


Introduction

Insulin Signaling and Glucose Metabolism.

Human energy metabolism starts with the extraction of carbohydrates (mainly glucose), amino acids and fats from food by the digestive system. An increase of blood glucose levels after eating triggers the increased release of insulin by β -cells of the pancreatic islets of Langerhans. The insulin hormone – a 51 amino acid polypeptide – binds the insulin receptor of insulin-responsive tissues mainly skeletal muscle and fat tissue but also hepatocytes of the liver. The insulin receptor is a dimeric transmembrane polypeptide with tyrosine kinase activity (Figure 1). Upon binding of insulin, the β -domains of the receptor inside the cell phosphorylate themselves, which is recognized by the insulin receptor substrate (IRS-1). IRS-1 binds the β -domains after which it is also phosphorylated and starts a signaling cascade. Amongst others, the signaling cascade may result in the translocation of the glucose transporter, GLUT-4, to the cell surface enabling the active uptake of glucose and thereby lowering/normalizing blood glucose levels – this is called glycemic control. Once inside the cell glucose is trapped by phosphorylation and is subsequently converted into energy by glycolysis. Insulin signaling also upregulates the cellular machinery for glycogenesis that results in the storage of glucose as glycogen – a process that occurs mainly in the liver. Glucose is also converted into fatty acids such as palmitate in the liver that are subsequently released as lipoproteins and absorbed by the fat tissues. Adipocytes in the fat tissues transform absorbed glucose into glycerol-1-phosphate that is esterified with absorbed fatty acids into triglycerides – also known as fat.¹

Figure 1. Overview of cellular insulin signaling and its effect in the human body.



Obesity and Type 2 Diabetes.

An unhealthy diet with excessive intake of sugar and fat has become commonplace in many developed and developing countries. Combined with the often sedentary lifestyle (e.g. low physical activity) of many people nowadays this has led to a rapid increase in

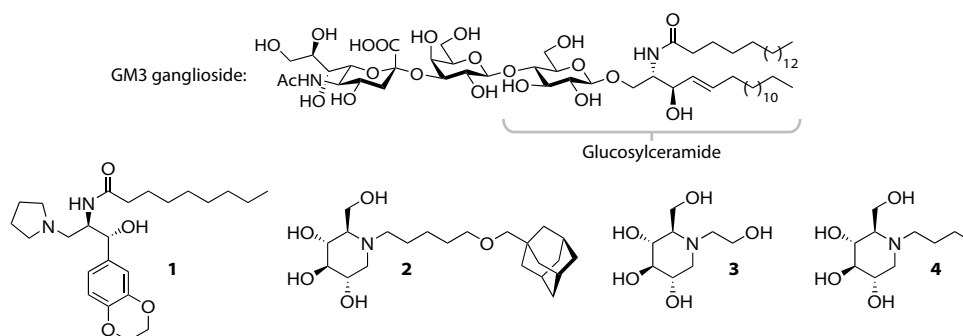
the occurrence of obesity. Coinciding with obesity, type 2 diabetes has reached epidemic proportions worldwide. Currently, 200 million people worldwide are believed to suffer from diabetes² – 23.6 million in the USA³ and 1 million in the Netherlands⁴ – and these figures are expected to have doubled in 2050.⁵ Type 2 diabetes – also called non-insulin dependent diabetes – is the most common type of diabetes and accounts for ~90% of all cases. Insensitivity of the insulin receptor to insulin signaling, called insulin resistance, is one of the earliest detectable abnormalities during the development of type 2 diabetes. The lower responsiveness to insulin results in its overproduction and an increased glucose concentration in the blood (hyperglycemia) and a diminished ability of the liver, muscles and fat tissues to absorb this glucose. When the blood glucose rises above a certain level the kidneys are unable to resorb it back into the blood and glucose is secreted via excessive urination – hence the original Greek name *diabetes mellitus* meaning honeysweet excretion. The inability of the tissues to absorb glucose has side effects that in the liver cause glycogen degradation (glycolysis) and *de novo* glucose synthesis (gluconeogenesis). In the muscles protein synthesis is inhibited. Adipocytes start to degrade stored fat by oxidation which produces ketone bodies that cause the pH of the blood to drop (acidosis). In the long term these chronic symptoms will lead to damage of the β -cells (resulting in type 1 diabetes), blood vessels, nervous system, kidneys and retinas.⁶

Role of Glycosphingolipids in Insulin Resistance.

The precise cause for the rapidly increasing occurrence of insulin resistance has not been firmly established, but there is growing evidence that obesity and the associated lipotoxicity of excess fat (hyperlipidemia) play a crucial role.⁷ Recent literature links insulin resistance to the presence of excessive amounts of a particular group of lipids, the so-called glycosphingolipids (GSLs). GSLs are located on the cell surface and together with cholesterol and specific proteins they congregate to form (detergent resistant) membrane microdomains also called lipid rafts. Recent reports indicate a close physical proximity of the insulin receptor to these microdomains.⁸⁻¹⁰ A regulatory role for glycosphingolipids, in particular the ganglioside GM3 (see Figure 2), in insulin sensitivity is substantiated by a rapidly growing body of experimental evidence. Interaction of GSLs and the insulin receptor was originally described by Nojiri *et al.*, demonstrating the ganglioside-mediated inhibition of insulin-dependent cell growth of leukemic cell lines.¹¹ Tagami and co-workers were the first to demonstrate that addition of GM3 to cultured adipocytes suppresses phosphorylation of the insulin receptor and IRS-1, resulting in reduced glucose uptake.¹² Inokuchi and co-workers reported that exposure of cultured adipocytes to TNF- α increases the levels of GM3 and inhibits insulin receptor and IRS-1 phosphorylation. This was found to be counteracted by 1-phenyl-2-decanoylamino-3-morpholinopropanol (PDMP), an inhibitor of GSL biosynthesis.¹³ Mutant mice lacking the capacity to synthesize GM3 have been reported to show an enhanced phosphorylation of the skeletal muscle insulin receptor after insulin binding and to be protected from

high-fat diet induced insulin resistance.¹⁴ Consistent with this is the recent report on improved insulin sensitivity and glucose tolerance in mice with increased expression of the sialidase Neu3 that can degrade the terminal sialic acid of GM3.¹⁵ Conversely, GM3 levels are elevated in the adipose tissue of certain obese, insulin resistant mouse and rat models.¹² Altered sphingolipid metabolism, reflected by increased GSL levels, has recently also been documented in relation to neuronal pathology in diabetic retinopathy.¹⁶ Very recently Kabayami *et al.* provided evidence that the interaction of negatively charged sialic acid residues of GM3 with the insulin receptor is mediated by a specific lysine residue located just above the transmembrane domain of the receptor. Excess levels of GM3 appear to promote the dissociation of the insulin receptor from its membrane microdomain, a location which is essential for insulin signal transduction.¹⁰

Figure 2. Structure of GM3, glucosylceramide, **1**, **2**, miglitol (**3**) and miglustat (**4**).



Effect of Pharmacological Inhibition of Glucosylceramide Synthase.

The value of pharmacological lowering of excessive GSL levels to improve insulin sensitivity has recently been demonstrated.¹⁷⁻¹⁹ Holland and co-workers reported that inhibition of the synthesis of ceramide, the precursor of GSLs, markedly improves glucose tolerance and prevents the onset of overt diabetes in obese rodents.¹⁸ Zhao *et al.* demonstrated that inhibition of the first step in the biosynthesis of GSLs – catalyzed by glucosylceramide synthase (GCS) at the Golgi apparatus – improves insulin sensitivity. The GCS inhibitor and PDMP analogue **1** lowered blood glucose and HbA1c-hemoglobin^{20,21} levels and improved glucose tolerance in insulin resistant rodents (*in vitro* IC₅₀ of **1** for GCS is 14 nM).¹⁹ Finally, it was recently shown that treatment of various rodent models of insulin resistance with the lipophilic iminosugar **2**, a well tolerated and potent inhibitor of GCS, very markedly lowered circulating glucose levels, improved oral glucose tolerance, reduced HbA1c, and improved insulin sensitivity in muscle and liver (description for HbA1c can be found in references section).¹⁷

The interpretation of the beneficial effect of **2** on glycemic control is hampered by the fact that **2** has a dual action and not only reduces GSL levels in tissues, but also reduces carbohydrate assimilation from food by inhibition of intestinal glycosidases.^{22,23}

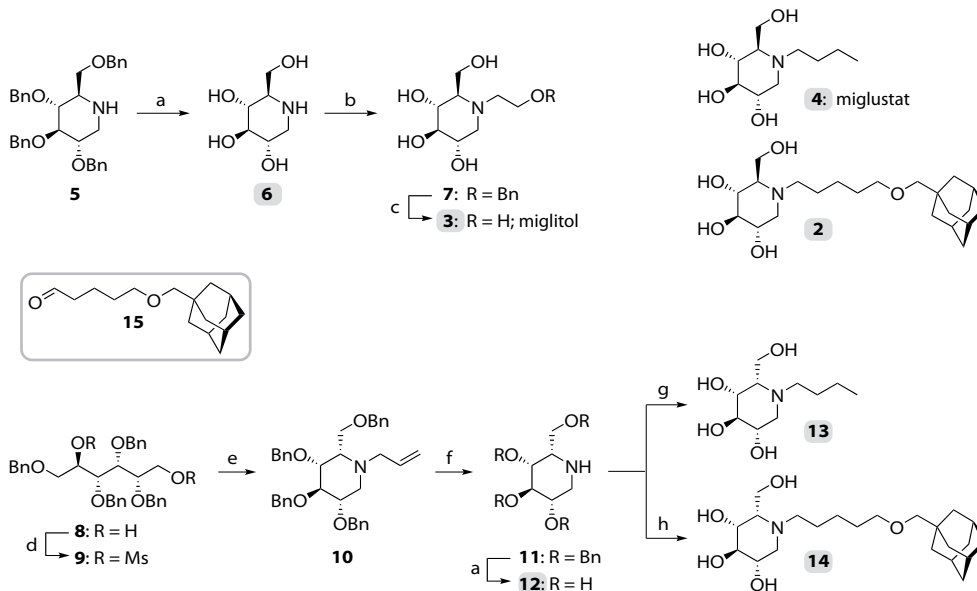
The latter effect is similar to the mode of action of registered anti-diabetics, including the iminosugar miglitol (**3**; Figure 2).²⁴ Furthermore, **2** also inhibits several other glycosidases among which glucocerebrosidase (GBA1) and β -glucosidase 2 (GBA2)²⁵ that are associated with glucosylceramide catabolism. To establish the relative importance of GSL lowering by GCS inhibition and reduction of carbohydrate assimilation in the small intestine with regard to insulin sensitivity, an analogue of **2** was needed that inhibits GCS more selectively.

There is literature precedence for C-4/C-5 epimerized *N*-alkylated derivatives of 1-deoxynojirimycin as viable GCS inhibitors. For the research described in this chapter this fact was used as a guide for developing a more selective inhibitor of GCS. The design and synthesis of a panel of nine **2** derivatives is reported in which the *D*-*gluco*-stereochemistry at C-4 and C-5 of **2** and the type of substitution on the nitrogen atom were varied. All three C-4/C-5 epimers of 1-deoxynojirimycin (*L*-*ido*, *D*-*galacto* and *L*-*altro*) were prepared and the nitrogen in each case was either left unsubstituted, substituted with a butyl for analogues of miglustat (**4**)²⁶ – a commercial GCS inhibitor – or substituted with a 5-(adamantan-1-yl-methoxy)-pentyl (AMP) group. The selectivity profile of the synthesized inhibitors was assessed for a panel of enzymes that included GCS, GBA1, GBA2 and the intestinal glycosidases. This assay showed that *L*-*ido*-AMP derivative **14** (the C-5 epimer of **2**) was a more potent and selective inhibitor of GCS. With this result, the relative contributions of the dual action of **2** were further investigated in a subsequent animal study. Compound **2**, **14**, miglitol (**3**), miglustat (**4**) and **1** were compared head-to-head in *ob/ob* mouse and ZDF rat models of insulin resistance and type 2 diabetes.

Results

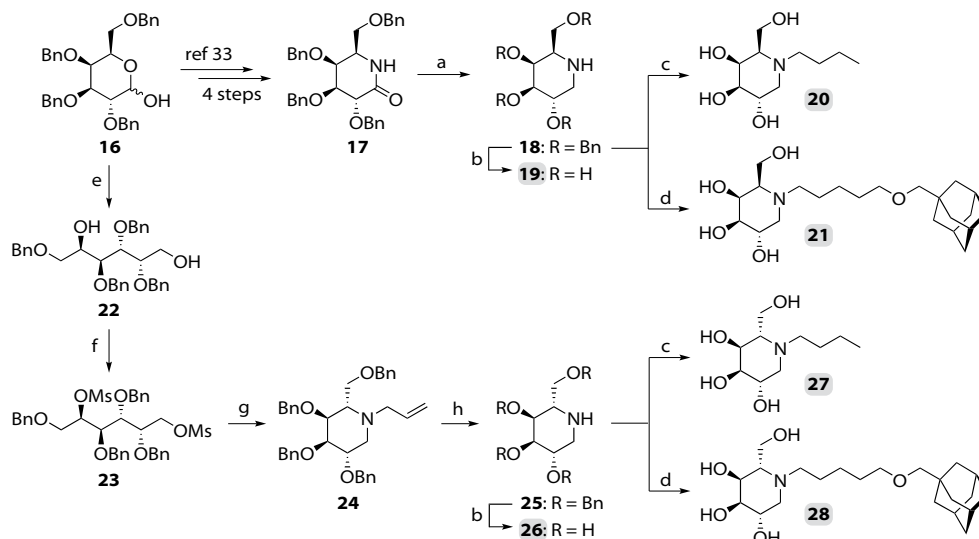
Synthesis of the Iminosugar Inhibitors.

1-Deoxynojirimycin (**6**) was obtained by Pd/C catalyzed hydrogenolysis of 2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin (**5**; from Chapter 2). The synthesis of miglitol (**3**) started with the alkylation of the nitrogen atom in **6**, for which two methods were evaluated (see Scheme 1 on the next page). Chemoselective alkylation of **6** with 2-benzyloxy-1-bromoethane under the agency of potassium carbonate provided **7** in 65% yield. Reductive amination of **6** with commercially available 2-benzyloxyacetaldehyde with sodium cyanoborohydride provided **7** in 86% yield. Hydrogenolysis of **7** with catalytic Pd/C provided **3** in 81% yield over two steps. Formation of the hydrochloric acid salt of miglitol, **3**·HCl, provided a stable compound that was used as such in enzyme assay and animal studies. Lead compound **2** was synthesized starting from commercially available 2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranose according to the procedure reported in Chapter 2.²⁷ Miglustat (**4**) is commercially available and was used in enzyme assay and animal studies as received.

Scheme 1. Synthesis of iminosugars based on *D*-gluco and *L*-ido stereochemistry.

Reagents and conditions: [a] Pd/C, 4 bar H₂, EtOH, aq HCl, 20h, **6**: 93%; **12**: 86%. [b] Method A: 2-benzyloxy-1-bromoethane, K₂CO₃, DMF, 90 °C, 5h, 65%; Method B: 2-benzyloxyacetaldehyde, NaCNBH₃, MeOH/AcOH, 48h, 86%. [c] Pd/C, 4 bar H₂, MeOH/H₂O, aq HCl, 20h, 95%. [d] MsCl, pyridine, 0 °C, 2h, used crude. [e] Allylamine, reflux, 20h, 67% two steps. [f] i: KOtBu, DMSO, 100 °C, 30 min; ii: 1M aq HCl, 15 min, 81%. [g] i: Butyraldehyde, NaCNBH₃, CH₃CN/AcOH, 20h; ii: BCl₃, DCM, 0 °C, 20h, 82% two steps. [h] i: **15**, Pd/C, 4 bar H₂, EtOH/AcOH, 20h; ii: Pd/C, 4 bar H₂, EtOH, aq HCl, 20h, 77% two steps.

The three *L*-ido-iminosugars (**12**, **13** and **14**) were synthesized starting from 2,3,4,6-tetra-O-benzyl-D-glucitol (**8**). Transformation of the two hydroxyl functions of **8** into their methanesulfonic esters and refluxing the obtained crude dimesylate **9** in allylamine provided **10** (Scheme 1).²⁸ Isomerization and cleavage of the allyl function in **10** by treatment with potassium *tert*-butoxide and subsequent acidic work-up provided **11**. Benzylamine was also evaluated in this protocol.²⁹ Although efficiently producing the *L*-ido-iminosugar, the excess benzylamine proved difficult to remove and the subsequent chemoselective cleavage of the *N*-benzyl group proved challenging. Hydrogenolysis of **11** with catalytic Pd/C provided *L*-ido-1-deoxynojirimycin (**12**). Reductive amination of **11** with butyraldehyde under the agency of NaCNBH₃ and subsequent deprotection of the crude intermediate with boron trichloride provided **13**.³⁰ Debenzylation with boron trichloride as opposed to Pd/C catalyzed hydrogenation proved more reproducible for the smaller scale synthesis of certain iminosugars. Synthesis of **14** was achieved by a selective Pd/C catalyzed hydrogenolysis of the intermediate imine between **11** and aldehyde **15** (from Chapter 2) in the presence of acetic acid. Deprotection of the crude intermediate by Pd/C catalyzed hydrogenolysis in the presence of hydrochloric acid produced **14** in a yield of 77% over two steps.³¹

Scheme 2. Synthesis of iminosugars with D-galacto and L-altro stereochemistry.

Reagents and conditions: [a] LiAlH_4 , THF, reflux, 3h, 71%. [b] BCl_3 , DCM, 0 °C, 20h, **19**: 96%; **26**: 77%. [c] i: Butyraldehyde, NaCNBH_3 , $\text{CH}_3\text{CN}/\text{AcOH}$, 20h; ii: BCl_3 , DCM, 0 °C, 20h, **20**: 74%; **27**: 91% two steps. [d] i: **15**, NaCNBH_3 , $\text{CH}_3\text{CN}/\text{AcOH}$, 20h; ii: Pd/C , 4 bar H_2 , EtOH, aq HCl, 20h, **21**: 61%; **28**: 89% two steps. [e] NaBH_4 , MeOH, 20h, 81%. [f] MsCl , pyridine, 0 °C, 2h, used crude. [g] Allylamine, reflux, 20h, 82% two steps. [h] i: KOtBu , DMSO, 100 °C, 30 min; ii: 1M aq HCl, 15 min, 73%.

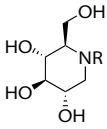
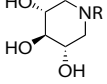
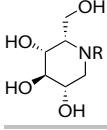
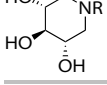
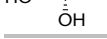
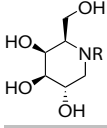
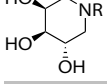
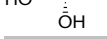
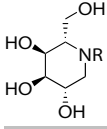
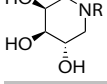
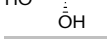
The synthesis of the three D-galacto-iminosugars (**19**, **20** and **21**) commenced with the preparation and of 2,3,4,6-tetra-O-benzyl-D-galacto-pyranose **16**³² and its transformation into **17** via a four step procedure as reported by Pandit *et al.* (Scheme 2).³³ Lactam **17** was reduced to **18** with LiAlH_4 in refluxing THF. Initially, an attempt was made to synthesize **18** from **22** via the three-step hemiacetal reduction/Swern oxidation/double reductive amination procedure described in Chapter 2. The first two steps of this procedure were successful, but the reductive amination yielded a complex mixture of products containing only trace amounts of **18**.³⁴ Next, deprotection of the benzyl ethers of **18** with boron trichloride provided **19**. Reductive amination of **18** with either butyraldehyde or 5-(adamantane-1-yl-methoxy)-1-pentanal (**15**) under the agency of NaCNBH_3 and subsequent deprotection of the crude N-alkylated intermediates produced **20** and **21**. The three L-altro-iminosugars (**26**, **27** and **28**) were synthesized starting from 2,3,4,6-tetra-O-benzyl-D-galactitol **22** via the same route as described for the L-ido-iminosugars **12**, **13** and **14** (Scheme 2).

Inhibition Profile of **2**, **3**, **4** and **6** and Effect on Glycemic Control in *ob/ob* Mice.

The three existing 1-deoxynojirimycin-based iminosugars, lead compound **2**, miglitol (**3**), miglustat (**4**), and 1-deoxynojirimycin (**6**) itself were comparatively investigated with respect to their ability to inhibit three intestinal glycosidases and GCS (see Table 1). To

further investigate their selectivity profile they were also assayed for the glycosidases GBA1, GBA2, lysosomal α -glucosidase and debranching enzyme. The lysosomal α -glucosidase was tested as it is known to be strongly inhibited by **2** and **4** and plays a critical role in lysosomal glycogen degradation during cellular turnover – also known as acid maltase it is the deficient enzyme in Pompe disease. The debranching enzyme is critical for cytosolic glycogen degradation (glycogenolysis) and it possesses both an α -1,4-transferase and α -1,6-glucosidase catalytic site for its substrate.

Table 1. Enzyme inhibition assay results: apparent IC_{50} values in μM (for GCS: % inhibition at μM).

Compound ^a	GCS ^b <i>in vivo</i>		GBA1	GBA2	Lysosomal α -gluco- sidase	Sucrase	Lactase	Maltase	De- branching enzyme
	%	μM							
6 : R = H	0	100	250	21	1.5	2	62	2	10
 3 : R = (CH ₂) ₂ OH	0	100	200	9	2.0	0.5	50	6	-
 7 : R = (CH ₂) ₂ OBn	50	360	11	-	3.7	-	-	-	> 10
4 : R = Butyl	50	50	400	0.230	0.1	0.5	> 100	9	10
2 : R = AMP	50	0.2	0.2	0.001	0.4	0.5	35	4	10
 12 : R = H	44	10	> 1000	400	> 1000	1000	> 1000	> 1000	> 100
 13 : R = Butyl	62	10	> 1000	0.25	> 1000	1000	> 1000	1000	> 100
 14 : R = AMP	75	0.1	2	0.03	> 1000	> 1000	> 1000	1000	> 100
 19 : R = H	0	10	350	100	6	0.26	1.5	50	10
 20 : R = Butyl	48	10	320	0.3	1.5	0.46	10	1000	10
 21 : R = AMP	50	0.5	0.2	< 0.001	0.5	3.5	15	375	4
 26 : R = H	0	10	> 1000	100	> 1000	220	> 1000	400	> 100
 27 : R = Butyl	< 10	10	> 1000	9	500	450	> 1000	> 1000	> 100
 28 : R = AMP	< 10	10	30	0.5	500	1000	> 1000	> 1000	> 100

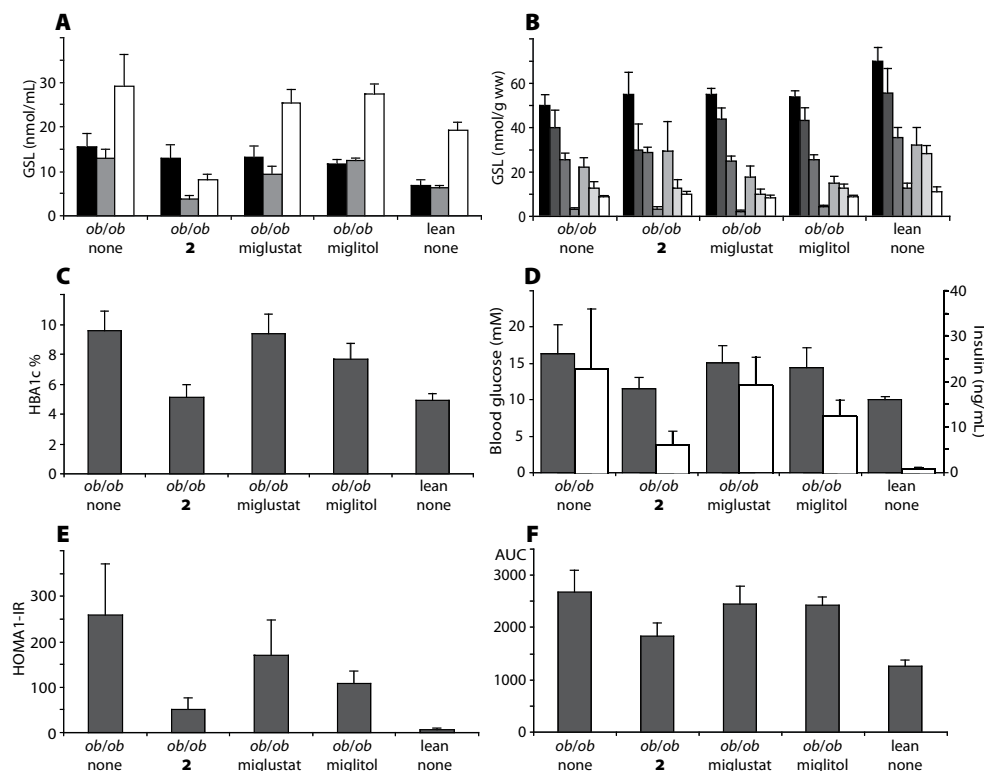
^aAMP = 5-(adamantan-1-yl-methoxy)-pentyl; ^bOther enzyme assays are *in vitro*.

As expected, the anti-diabetic **3** is a potent inhibitor of the intestinal glycosidases maltase and sucrase. Compounds **2**, **4** and **6** also inhibit these enzymes at μM concentrations. Lead compound **2** in particular is a potent inhibitor of GCS (IC_{50} 0.2 μM), **4** is a weaker inhibitor (IC_{50} 50 μM), whilst **3** and **6** do not inhibit GSL biosynthesis at all. All compounds inhibited the lysosomal α -glucosidase. Debranching enzyme was only inhibited in the high μM range.

Next, the effects of **2**, miglitol (**3**) and miglustat (**4**) on obese, insulin resistant *ob/ob* mice were studied. *Ob/ob* mice lack the ability to produce the hormone leptin, which is excreted by adipocytes after consumption of sufficient food and suppresses appetite (satiety) and upregulates energy metabolism. This deficiency causes these mice to

become polyphagic (eat too much food), lethargic, obese and eventually develop insulin resistance and other type 2 diabetes-like symptoms. For this part of the study, 7-week old, C57Bl/6J mice (control group) and *ob/ob* mice were treated for 4 weeks with 100 mg/kg/day of **2**, **3** or **4**. Only in the case of **2** a significant lowering of plasma and liver GSLs was observed, without concomitant changes in ceramide content (Figure 3 A, B). Mice treated with **2** also showed a markedly lowered circulating blood glucose and insulin, improved HOMA and oral glucose tolerance (OGT), and reduced HbA1c (Figure 3 C-F). Treatment with **4** had marginal positive effects. Treatment with **3** resulted in some reduction of blood glucose and HbA1c, but did not, as expected, improve oral glucose tolerance (a description of Homeostatic Model Assessment (HOMA) can be found in references section).³⁵⁻³⁷

Figure 3. Effects of lead compound **2**, miglitol (**3**) and miglustat (**4**) treatment on GSLs and glycemic control in *ob/ob* mice and comparative values in untreated normal C57Bl/6J mice.



Animals were treated for 4 weeks daily with 100 mg compound per kg bodyweight. Panel **A**: plasma content (nmol/mL) of GSLs: ceramide (black); glucosylceramide (grey); total gangliosides (white). Panel **B**: liver content (nmol/g) of GSLs: (left to right) ceramide/10; glucosylceramide; lactosylceramide; GM3; GM2; GM2-glycol/10; GD1a. Panel **C**: HbA1c. Panel **D**: Blood glucose (grey) and insulin (white). Panel **E**: HOMA1-IR index. Panel **F**: Oral glucose tolerance (OGT; area under curve).

L-Ido 14, a Potent and More Selective Inhibitor of Glucosylceramide Synthase.

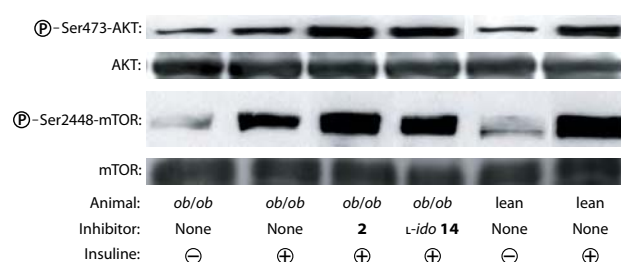
The piperidine rings of 1-deoxynojirimycin (**6**), miglitol (**3**), miglustat (**4**) and **2** possess D-*gluco* stereochemistry. It is a well established fact that structural mimicry of D-glucose is one of the causes for inhibition of intestinal glucosidases by these types of iminosugars. Therefore, changing the iminosugar stereochemistry could result in more selective GCS inhibitors. There is literature evidence that GCS allows manipulation of iminosugar inhibitors at the C-4 and C-5 position of the piperidine ring. Platt, Butters and co-workers have demonstrated previously that *N*-butyl-D-*galacto*-1-deoxynojirimycin (**20**), a C-4 epimer of **4**, still inhibits GCS.³⁸ The same has also been reported for *N*-pentyl-L-*ido*-1-deoxynojirimycin, a *N*-pentyl substituted C-5 epimer of **4**.³⁹ The synthesized nine C-4/C-5 analogues of **2** were analyzed for their inhibitory capacity towards the three intestinal glycosidases, GCS and the four other glycosidases (see Table 1).

Of the nine compounds only the three D-*galacto*-iminosugars (C-4 epimer; **19**, **20** and **21**) still substantially inhibit the intestinal glycosidases. This shows that D-glucose stereochemistry at the C-5 position is critical for binding of the active site of sucrase and maltase. Lactase binds and hydrolyzes a D-galactose from lactose and correspondingly **19–21** display potent inhibition of this enzyme. All three L-*altro*-iminosugars (C-4 and C-5 epimer; **26**, **27** and **28**) showed very weak to no inhibition of GCS or any of the enzymes except GBA2 – in fact **28** may be regarded as a lead towards the development of GBA2-selective inhibitors. In line with the literature reports, **20**, **21** and the L-*ido*-iminosugars (C-5 epimer; **13** and **14**) do inhibit GCS. The unsubstituted L-*ido*-iminosugar **12**, interestingly, also inhibits GCS to some extent and the D-*galacto*- and L-*ido*-miglustat analogues (**13** and **20**) are inhibitors of GCS in the micromolar range. For the D-*galacto*- and L-*ido*-iminosugars, a great increase in inhibitory potency for GCS is observed when switching from *N*-butyl substitution to *N*-AMP substitution, and a similar trend is seen for GBA1. Compared to **2**, the AMP-substituted D-*galacto*-iminosugar (**21**) is an only slightly less potent inhibitor of GCS, but it inhibits the debranching enzyme and as mentioned above the intestinal glycosidases. However, of particular interest was **14**, which did exhibit the required profile for a potent GCS-selective inhibitor. L-*Ido*-analogue **14** is slightly better than **2** with regard to inhibition of GCS ($IC_{50} = 100$ nM), but sharply contrasts from **2** in its much reduced capacity to inhibit intestinal glycosidases. Also, **14** is a much poorer inhibitor of GBA1 when compared to lead compound **2** (IC_{50} 1.0 μ M vs 0.2 μ M), acid α -glucosidase ($IC_{50} > 1$ mM vs 0.4 μ M), and debranching enzyme ($IC_{50} > 1$ mM vs 10 μ M), which further emphasizes its specificity in GCS inhibition. Exposure of various types of cultured cells to **2** and **14** resulted in comparable lowering of GSLs without concomitant increases in ceramide (data not shown). The pharmacokinetic properties of **2** and **14** were also found to be very similar (see Figure 8 in Experimental section).

Comparison of Effects of **2** and L-ido-Analogue **14** in *ob/ob* Mice and ZDF Rats.

The effect of L-ido **14** and **2** on *ob/ob* mice was comparatively investigated. For this purpose, 7-week old animals were treated for 4 weeks with 100 mg/kg/day of inhibitor. Insulin signaling in the liver of **14** or **2** treated and untreated *ob/ob* mice and untreated lean mice was visualized on immunoblots. These showed comparable significant improvements in phosphorylation of mTor and AKT after insulin stimulation for both **14** and **2** treated *ob/ob* mice (Figure 4). mTor and AKT are kinases downstream from IRS-1 in the intracellular insulin initiated phosphorylation cascade. Both treatment with **14** and **2** resulted in significant reduction of GSLs in plasma and liver without affecting ceramide levels (Figure 5 A, B; page 92). No differences were noted in body weight gain or food intake between animals treated with both compounds (data not shown). Although, clear improvements in blood glucose concentration, insulin levels, HOMA and HbA1c were observed in L-ido **14** treated animals, these were significantly smaller than those detected in animals treated with **2** (Figure 5 C-F). Of note, oral glucose tolerance was strongly improved in animals treated with L-ido **14** (Figure 5 G) – an effect not observed for the intestinal glycosidase targeting miglitol **3** (data not shown).

Figure 4. Effect of **2** and L-ido **14** on phosphorylation of liver Ser473-AKT and Ser 2448-mTOR following insulin stimulus.



Next, the impact of treatment of obese ZDF rats with **2** and **14** was comparatively investigated. In addition, the effect of **1**¹⁹, a (glucosyl)ceramide analogue and PDMP derivative that specifically inhibits GCS and not intestinal glycosidases (data not shown), was studied in this head-to-head comparison. The ZDF rats lack a receptor for leptin making them polyphagic (eating too much food), become obese and develop type 2 diabetes-like symptoms. All compounds resulted in significant lowering of plasma and liver GSL levels without changes in ceramide content (Figure 6 A, B; page 93). Similar to the findings with *ob/ob* mice, treatment of ZDF rats with **2** resulted in more prominent improvements in blood glucose concentration and HbA1c compared to treatment with **14** (Figure 6 C, D). Overall **1** was better in improving glycemic control compared to the lower dosage of **14**, but was outperformed by **2** and the higher dosage of **14**.

Figure 5. Effects of lead compound **2** and L-ido **14** on GSLs and glycemic control in *ob/ob* mice and comparative values in untreated normal C57Bl/6J mice.

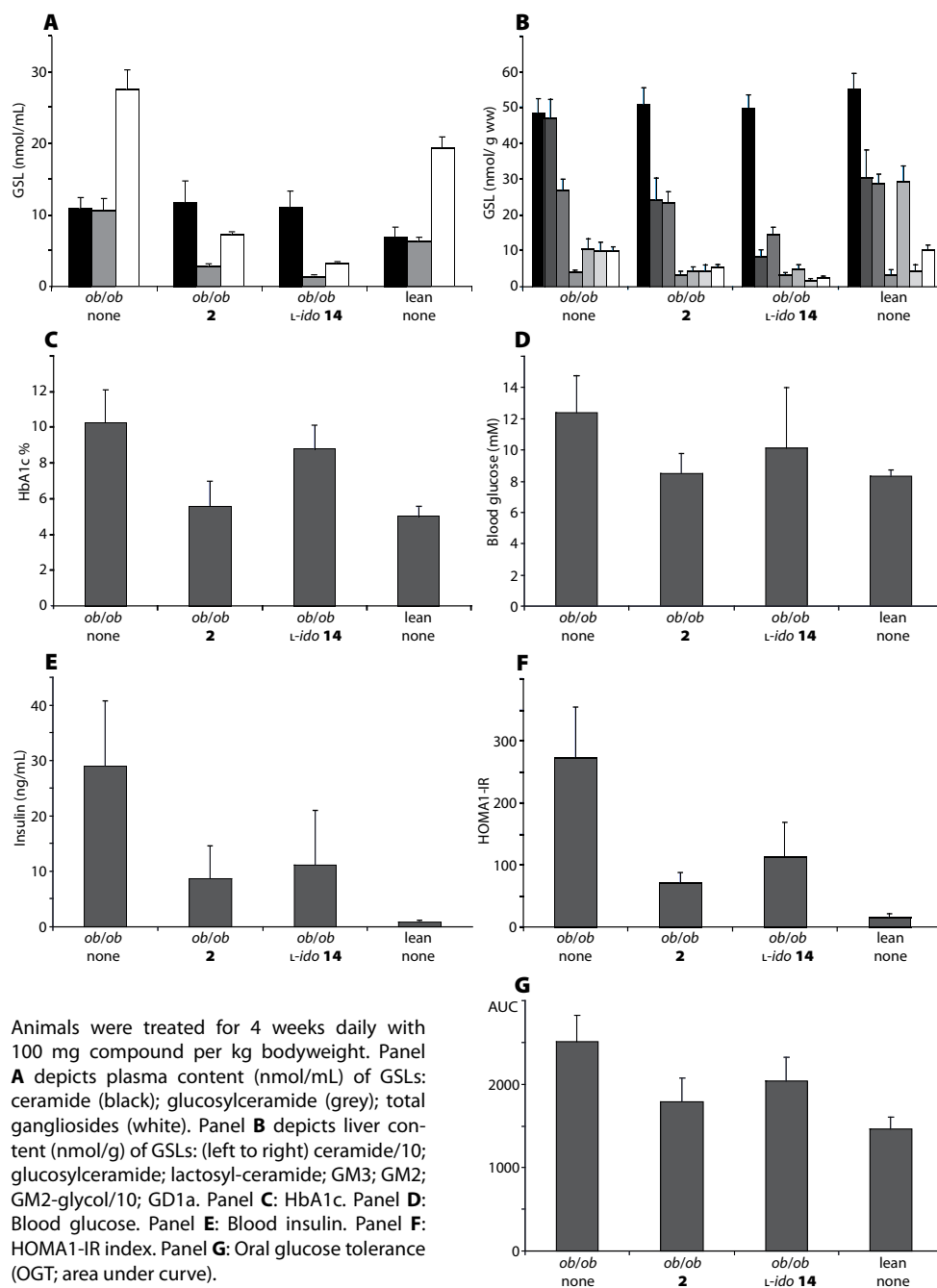
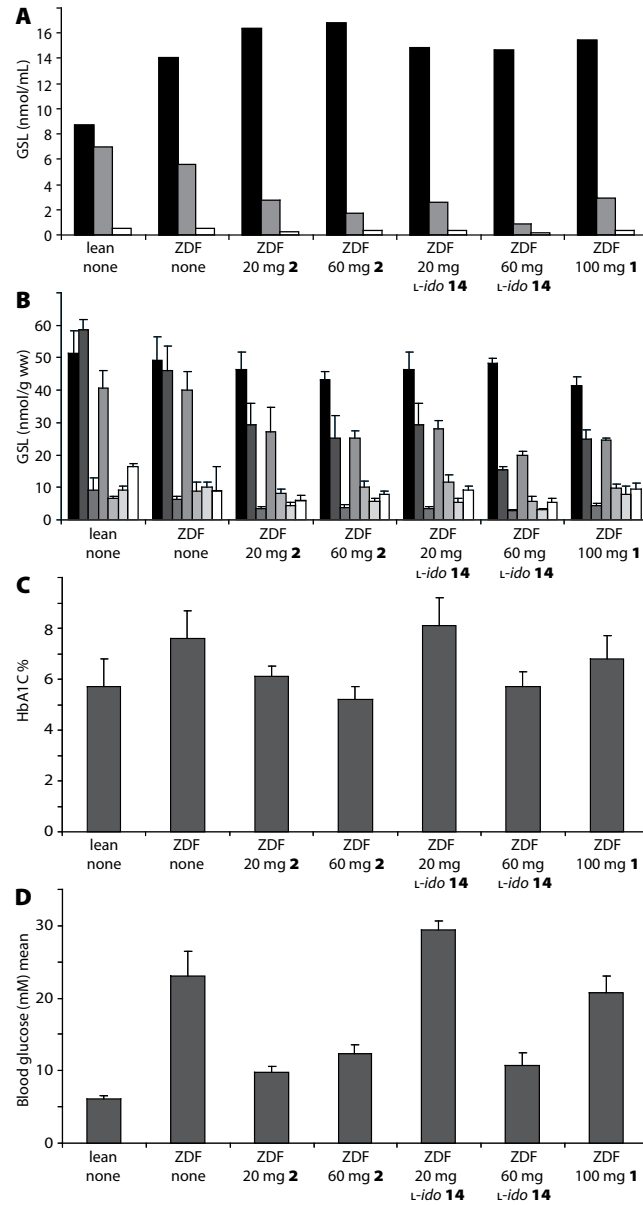
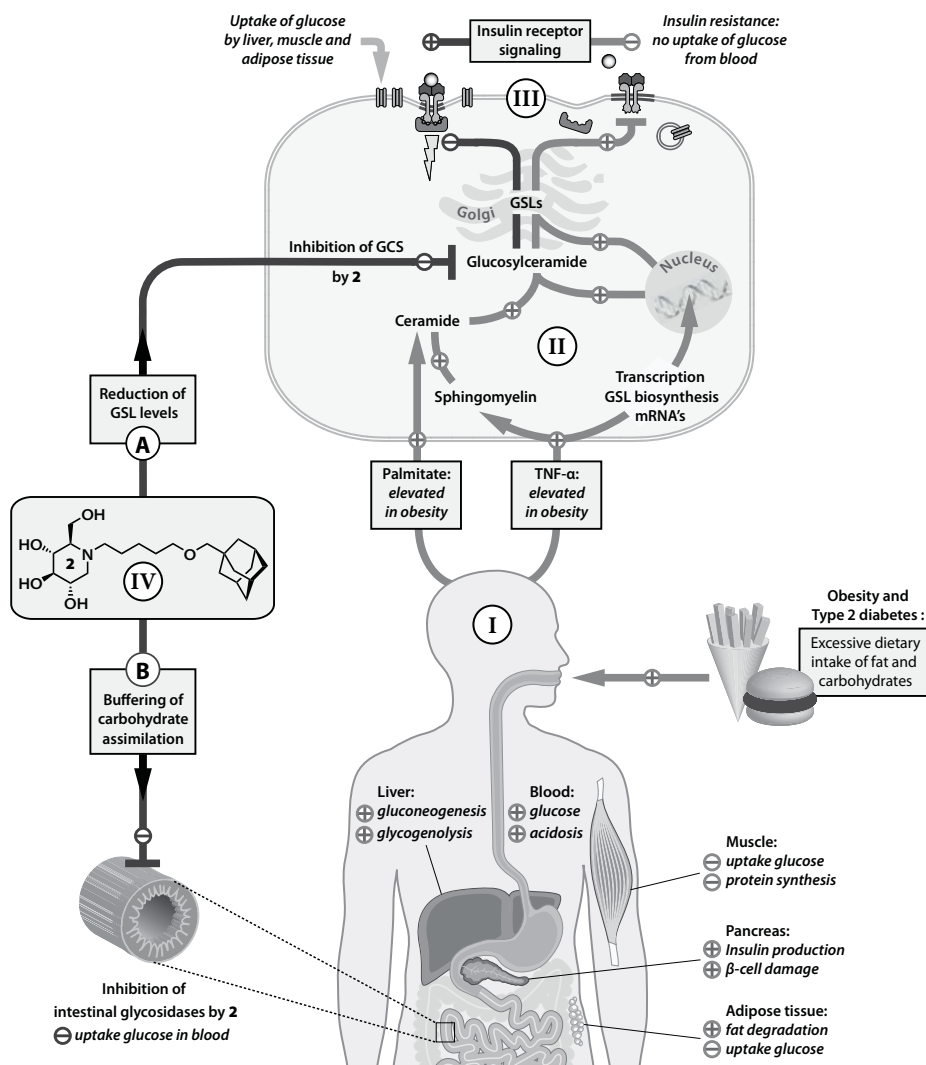


Figure 6. Effects of **2**, *L*-ido **14** and **1** on GSLs and glycemic control in ZDF rats and comparative values for lean littermates.



Animals were treated for 4 weeks daily with indicated amount of compound per kg bodyweight. Panel **A** depicts plasma content (nmol/mL) of GSLs: ceramide (black); glucosylceramide (grey); total gangliosides (white). Panel **B** depicts liver content (nmol/g) of GSLs: (left to right) ceramide/10; glucosylceramide; lactosylceramide; GM3; GM2; GM2-glycol/10; GD1a. Panel **C**: HbA1c. Panel **D**: Blood glucose.

Figure 7. Proposed model for improved glycemic control by the dual action (A/B) of the lipophilic iminosugar **2** in type 2 diabetes.



Excessive dietary consumption of fats and carbohydrates leads to obesity and type 2 diabetes with their associated damaging symptoms (I). Obese individuals have elevated palmitate levels and are in a chronic inflammatory state⁴⁷ (TNF- α production). These factors together with heightened glucose levels stimulate GSL biosynthesis (II). The insulin receptor resides in lipid microdomains that also harbor most GSLs. Elevated GSL levels have been implicated in insulin receptor insensitivity and evidence exists that they bind with and dissociate the receptor from the microdomain, which renders it inactive (III). Inhibitor **2** downregulates GSL levels by inhibiting the enzyme GCS (IV; action A) at the base of GSL biosynthesis. Normalization of GSL levels in the microdomain results in a return of normal receptor functioning. Secondly, **2** improves glycemic control in type 2 diabetes by lowering blood glucose levels through inhibition of intestinal glycosidases (IV; action B), which limits the amount of glucose generated in the intestine from food. — : effects of obesity/diabetes type 2; — : effects of lipophilic iminosugar **2**; $\oplus$: increase of level or activity; $\ominus$: decrease of level or activity.

Discussion

In an earlier study with **2** it was demonstrated that it has dramatic beneficial effects on the insulin resistance and hyperglycemia seen in ZDF rats, *ob/ob* mice and high-fat diet-induced glucose-intolerant mice via a mechanism that does not require a reduction in food intake or loss of bodyweight.¹⁷ In the ZDF type 2 diabetes model, protection of the pancreas by **2** was also observed. Given the ability of **2** to reduce GSLs in tissues as well as to buffer intestinal carbohydrate assimilation, it remained unclear how **2** was impacting glucose homeostasis. The study presented in this chapter dissects the two actions of **2** by the design, synthesis and use of *L-ido*-analogue **14** that inhibits GCS comparably to **2**, but shows greatly decreased inhibition of the intestinal glycosidases. Treatment of *ob/ob* mice and ZDF rats with **14** demonstrated that sole reduction of GSLs in tissues prominently contributes to improvements in blood glucose, HbA1c, oral glucose tolerance and insulin signaling in the liver. Improved pancreatic function in the ZDF diabetes model was also observed. These findings are consistent with observations made with **1**. This (glucosyl)ceramide-mimicking GCS inhibitor does not affect intestinal glycosidases and carbohydrate assimilation, but nevertheless helps to control hyperglycemia.

This study also reveals that the concomitant inhibition of intestinal carbohydrate assimilation by **2**, particularly at low concentrations, adds to its prominent beneficial effect on glycemic control in obesity induced type 2 diabetes models (see Figure 7 on page 94). The dual action of **2** is not surprising since the structurally related miglitol (**3**) positively affects glucose homeostasis via selective inhibition of intestinal glycosidases. Based on this mechanism of action, **3** is registered as anti-diabetic drug.²⁴ Miglitol itself does not inhibit glucosylceramide synthase as measured with cultured cells. Although its more lipophilic monobenzylated analogue (**7**) does slightly inhibit GCS (see Table 1). The present investigation rendered no indication that some metabolite of **3** is formed that causes reduction of GSLs. The development of **3** as anti-diabetic drug was stimulated by the ancient use in the Far East of iminosugar-rich mulberry leaves to control hyperglycemia.⁴⁰ Very recently, it has indeed been demonstrated that, compared with a placebo, co-ingestion of mulberry extract – containing 1-deoxynojirmycin (**6**) – with 75 g sucrose reduced the increase in blood glucose observed over the initial two hours of testing in control and type 2 diabetic subjects.^{41,42}

Evaluation of known iminosugar-based inhibitors combined with the design and preparation of novel analogues has shown that the C-4/C-5 configuration of the iminosugar and the type of substitution on the nitrogen atom are critical for potent inhibition of GCS (Table 1). In general, iminosugars with *D-gluco*-, *D-galacto* and *L-ido*-stereochemistry in combination with a hydrophobic substituent on the nitrogen atom inhibit GCS. This finding is in agreement with recent unpublished data by Butters.⁴³ Substitution with a *N*-5-(adamantane-1-yl-methoxy)-pentyl (AMP) group provides the most potent inhibitors of GCS and this trend is also observed for GBA2 and to a lesser extent GBA1. This trend might reflect the lipophilicity of the natural substrates

and products (ceramide/glucosylceramide) of these enzymes. Epimerization of the C-5 position of **2** greatly reduced inhibition of intestinal glycosidases and slightly increased the inhibitory potency for GCS, making *L-ido* **14** the most potent iminosugar-based GCS-selective inhibitor reported to date.

A considerable drawback of compounds that buffer carbohydrate assimilation by virtue of inhibition of intestinal glycosidases is the associated intestinal complaints that lower drug compliance. Compound **14** does not affect intestinal glycosidases. In this respect, the compound is an appealing drug, particularly for conditions in which the exclusive lowering of GSL levels in tissues is desirable without the need for buffering of carbohydrate assimilation. Examples in this respect are the inherited glycosphingolipidoses, such as Gaucher disease, Sandhoff disease, Tay-Sachs disease and Fabry disease.²³ In all these disorders, a particular GSL accumulates in the lysosomes due to an inherited deficiency in a catabolic lysosomal glycosidase. Reduction of GSL biosynthesis by inhibition of GCS is envisioned to be beneficial in all these conditions.^{23,44,45} Miglustat (**4**) has been registered as orphan drug for the treatment of mild to moderate type 1 Gaucher disease, and has proven to be efficacious.^{26,46} Given the significantly improved features of **14** as compared to **4**, such as better bioavailability, specificity and potency of inhibition of GCS, it seems warranted to investigate its potential as therapeutic agent for inherited glycosphingolipidoses (*e.g.* Gaucher disease).

Conclusion

The analogues of lead lipophilic iminosugar **2** described in this chapter show that C-4 stereochemistry and the 5-(adamantane-1-yl-methoxy)-pentyl group in **2** are critical for potent inhibition of GCS. Epimerization of C-5 retains GCS inhibition and reduces or abolishes inhibition of most other enzymes except for GBA2. The more selective GCS inhibitor (**14**) obtained in this way was used together with **2** to study the mechanism by which **2** improves glycemic control in type 2 diabetes models. It was found that **2** exerts beneficial effects on glycemic control by virtue of its lowering of GSLs in tissues and buffering of carbohydrate assimilation in the small intestine. This dual action is desirable for control of hyperglycemia, a hallmark of type 2 diabetes. *L-Ido* analogue **14** specifically and potently inhibits GSL biosynthesis and may be of interest as an agent to intervene in inherited glycosphingolipidoses.

Experimental section

Animals. Experimental procedures were all approved by the appropriate Ethics Committee for Animal Experiments. C57Bl/6J and *ob/ob* mice (C57Bl/6J background) were obtained from Harlan (Horst, the Netherlands) and ZDF (ZDF/GMi-*fa/fa*) rats and lean litter mates from Charles River Laboratories (Wilmington, USA). Animals were housed in a light- and temperature controlled facility. Animals were fed a commercially available lab chow (RMH-B, Hope Farms BV, Woerden, the Netherlands) containing about 6% fat and ~0.01% cholesterol (w/w). To induce glucose intolerance in C57Bl/6J mice, animals were fed with a high fat diet (16.4 % protein, 25.5 % carbohydrates and 58.0% fat) for 4 weeks and glucose intolerant mice were selected using an intraperitoneal glucose tolerance test. The iminosugars were mixed in the food. In the case of experiments with ZDF rats the compounds were administered by oral gavage two times daily.

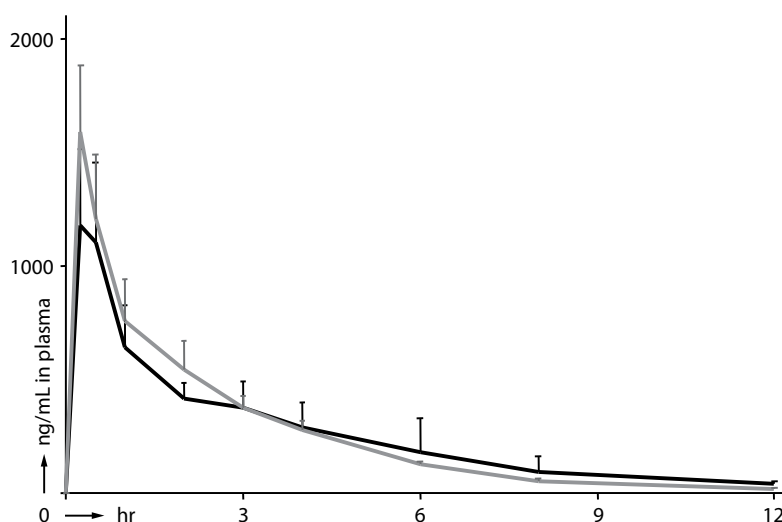
Plasma and tissue sampling. Blood samples were collected by either tail vein or retro orbital plexus puncture. Animals were sacrificed under isoflurane anaesthesia. A large blood sample was collected by cardiac puncture. Tissues were quickly removed and frozen for further analysis.

Analysis of lipids and measurement of enzyme activities. Lipids were extracted according to Folch *et al.*⁴⁸ Neutral GSLs were analyzed as described previously.⁴⁹ Ganglioside composition was determined as described previously.⁵⁰ IC₅₀ values of the iminosugars for the various enzyme activities were determined by exposing cells or enzyme preparations to an appropriate range of iminosugar concentrations (DMSO stock solutions diluted with RPMI medium). *In vivo* glucosylceramide synthase assay: The mouse macrophage cell line RAW-267 was grown to 90-100 % confluence in growth medium at 37 °C in a 5% CO₂ incubator. The growth medium consisted of RPMI-1640 + 10% FCS + penicillin (150 µg/mL) and streptomycin (250 µg/mL) + 50 mM Hepes buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). The incubation flask was washed with RPMI medium without serum (3×5 mL) to remove serum. Cells were taken up in 3 mL RPMI + 50 mM Hepes + 300 µM conduritol B epoxide (10 mM stock in RPMI) + the inhibitor (0.1, 1, 10 µM (20 mM stock in DMSO diluted in RPMI) and 5 nmol C₆-NBD-ceramide/ BSA complex (*N*-[7-(4-nitrobenzo-2-oxa-1,3-diazole)]-6-aminocaproyl-D-erythro-sphingosine) were added successively to the cell culture. The cells were incubated for 1 hour at 37 °C in a 5% CO₂ incubator. At the end of incubation, the flask was inspected for potential cytotoxicity of the inhibitor and washed with medium without serum (5×5 mL). A 50 mM potassium phosphate buffer (KPi buffer, pH 5.8, 0.75 mL) was added and the flask was placed in ice. Cells were removed from the flask by scraping and the harvested cells were collected in a capped plastic vial and immediately immersed in liquid nitrogen. The frozen cell lysate (~0.75 mL) was suspended in methanol (3 mL) and extracted with chloroform (3 mL). After extraction a 0.73% NaCl solution (2.0 mL) was added to the biphasic. The aqueous phase was extracted once more with chloroform (1 mL). The combined chloroform layers were isolated and concentrated at 30-40 °C under a nitrogen flow. Lipids were separated by thin-layer chromatography (HP-TLC plates 20×10 silicagel 60 van Merck) using chloroform/methanol/15 mM aq CaCl₂ (60:35:8, v/v/v) as the developing solvent. The C₆-NBD-labeled (glyco)sphingolipids were identified using standards, visualized with a Typhoon Trio Variable Mode Imager (λ_{ex} 488 nM, λ_{em} 520 nM) and quantified with ImageQuant TL software. Glucocerebrosidase activity was measured using recombinant enzyme and 4-methylumbelliferyl-beta-glucose as substrate.⁵¹ GBA2 was measured using enzyme-containing membrane preparations from Gaucher spleen and 4-methylumbelliferyl- β -glucoside as substrate.⁵¹ Lysosomal α -glucosidase was measured using purified enzyme from human urine and 4-methylumbelliferyl- α -glucoside as substrate.⁵¹ Lactase, maltase and sucrase activities were determined with homogenates of freshly isolated rat intestine by measuring liberated glucose from the corresponding disaccharides.⁵² The activity of debranching enzyme (α -1,6-glucosidase activity) was measured by determining liberated glucose from dextrin with an erythrocyte preparation as enzyme source.⁵³

Pharmokinetic (PK) profiles. Plasma levels of **2** and **14** were determined by mass spectrometry following high pressure liquid chromatography (Xendo, Groningen, the Netherlands). $C_{\max}$ = maximum reached concentration in blood; Dose = mg compound divided by weight of animal in kg; $t_{1/2}$ = half life of compound in blood; AUC_{∞} = Area under the concentration time curve extrapolated to infinity; MRT = mean retention time in blood; F = % oral bioavailability of compound.

Figure 8. Pharmokinetic (PK) profiles of **2** and **14** in ZDF rats

Pharmokinetics	Lead compound 2	L-ido compound 14	units
$C_{\max}$	1200 ± 400	1600 ± 300	ng-mL
$C_{\max}/\text{dose}$	120 ± 40	160 ± 30	ng-mL/(mg/kg)
$t_{1/2}$	4.2 ± 1.8	2.9 ± 2.0	hr
AUC_{∞}	3400 ± 500	3300 ± 500	ng-hr/mL
AUC_{∞}/dose	340 ± 50	330 ± 50	ng-hr/mL/(mg/kg)
MRT	4.2 ± 0.9	3.0 ± 0.8	hr
F	41 ± 7	52 ± 7	%



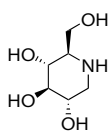
Analysis of insulin signaling in liver. Animals were treated for 4 weeks daily with 100 mg compound per kg bodyweight. Mice (*ob/ob* mice and untreated normal C57BL/6J mice) were fasted for 4 h, and insulin (0.75 U/kg body weight) was administered intravenously (1 U = 6.00 pmol insulin). After 5 min, animals were killed and livers were quickly collected and lysed in modified radioimmunoprecipitation assay buffer. Lysates were clarified by centrifugation (13,000 rpm for 10min). Equal amounts of lysates were separated by SDS-PAGE, and immunoblots were performed in parallel using the appropriate antibodies; anti-pTyr1446 IR- β , anti-P-Ser473 AKT, anti-P-Ser2448 mTOR and anti-p-70S8K (Cell Signaling Technology Inc., US).

Oral Glucose Tolerance (OGT) test. The tolerance test was performed in fasted animals (> 6 h) with oral gavage of glucose (1 or 2 g of glucose per kg of body weight). Blood glucose values were measured immediately before and 10, 20, 30, 60, 90 and 120 min after glucose injection. AUCs (areas under the curve; arbitrary units per minute) were determined for individual animals.

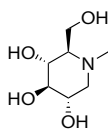
Blood glucose, insulin and HbA1c analysis. The concentrations of glucose, insulin and HbA1c levels in blood samples were determined as described previously.¹⁷

Statistical testing. Values presented in figures represent mean $\pm$ SEM. Statistical analysis of two groups was assessed by Student's t-test (two-tailed) or ANOVA for repeated measurement. Level of significance was set at $p < 0.05$.

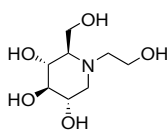
Synthesis of the iminosugars. *General methods and materials:* Lead compound **2** was synthesized as described in Chapter 2 and was used in enzyme assays and animal feeding experiments as its methanesulfonic acid salt (**2*MSA**). Miglustat (**4**) was obtained from Sigma (St Louis, USA). Compound **1** was obtained from Genzyme (Boston, USA) and used as its L-tartaric acid salt (Genz-123346). Reactions were executed at ambient temperature unless stated otherwise. All moisture sensitive reactions were performed under an argon atmosphere and residual water was removed from the starting material by coevaporation with dioxane, toluene or dichloroethane. All solvents were removed by evaporation under reduced pressure. All chemicals and solvents, unless indicated, were acquired from commercial sources and used as received. THF was distilled prior to use from LiAlH₄. EtOH was purged of acetaldehyde contamination by distillation from zinc/KOH. CH₂Cl₂ was distilled prior to use from P₂O₅. Reaction grade acetonitrile, dimethylsulfoxide, isopropanol and methanol were stored on 3 Å molsieves. Other reaction grade solvents were stored on 4 Å molsieves. Reactions were monitored by TLC analysis using silica gel coated aluminum plates (Schleicher & Schuell, F1500, LS254) and technical grade solvents. Compounds were detected during TLC analysis by UV absorption (254 nm) where applicable and/ or by spraying with a solution of (NH₄)₆Mo₇O₂₄×4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄×2H₂O (10 g/L) in 10% sulfuric acid followed by charring at ~150 °C. Visualization of olefins was achieved by spraying with a solution of KMnO₄ (5 g/L) and K₂CO₃ (25 g/L) in water. Visualization of hemiacetals and glycosides was achieved by spraying with a solution of 20% H₂SO₄ in ethanol followed by charring at ~150 °C. Visualization of deprotected iminosugar compounds during TLC analysis was accomplished by exposure to molecular iodine vapor. Column chromatography was performed on silica gel (particle size: 40–63 µm) for all compounds. The ¹H and ¹³C NMR, ¹H–¹H COSY and ¹H–¹³C HSQC experiments were recorded on a 200/50 MHz, 300/75 MHz, 400/100 MHz, 500/125 MHz or 600/150 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard for all ¹H NMR measurements in CDCl₃ and the deuterated solvent signal for all other NMR measurements. Coupling constants (*J*) are given in Hz. Where indicated, NMR peak assignments were made using COSY and HSQC experiments. All presented ¹³C NMR spectra are proton decoupled ¹³C-APT measurements. IR spectra were recorded on an apparatus fitted with a single bounce diamond crystal ATR-element and are reported in cm⁻¹. Optical rotations were measured on an automatic polarimeter (Sodium D-line, λ = 589 nm). Mass spectra were recorded on an electrospray interface apparatus. High resolution mass spectra were recorded on a mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 %, capillary temperature 275 °C) with resolution *R* = 100000 at *m/z* 400. The high resolution mass spectrometer was calibrated prior to measurements with a calibration solution (caffeine, MRFA, Ultramark 1621). Or high resolution mass spectra were recorded by direct injection (2 µL of a 2 µM solution in H₂O/CH₃CN; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution *R* = 60000 at *m/z* 400 (mass range *m/z* = 150–2000) and dioctylphthalate (*m/z* = 391.28428) as a "lock mass". The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).



1-Deoxynojirimycin (6). A solution of **5** (1.06 g, 1.91 mmol) in EtOH (50 mL) was acidified to pH ~2 with 1M aq HCl and purged of oxygen by bubbling argon through the solution for 15 minutes. Pd/C (10 wt%, 100 mg) was added and the mixture was exposed to 4 bar of hydrogen for 20 hours. The reaction mixture was filtered over a glass microfiber filter and the filter cake was rinsed successively with MeOH (4×20 mL) and H₂O (2×20 mL). The combined filtrate was concentrated and coevaporated with MeOH (3×50 mL). The residue was purified by column chromatography with aluminum oxide (isocratic 16:3.7:0.3, *n*-propanol:H₂O:NH₄OH) to provide **6** (290 mg, 1.78 mmol) in 93% yield as a white foam. *R*_f = 0.24 (10:12:3; MeOH:EtOAc:NH₄OH). ¹H NMR (400 MHz, D₂O) δ 3.79 (dd, *J* = 3.0, 11.7, 1H, H-6a), 3.59 (dd, *J* = 6.3, 11.7, 1H, H-6b), 3.45 (ddd, *J* = 5.2, 9.1, 10.8, 1H, H-2), 3.28 (dd, *J* = 9.1, 1H, H-3), 3.22 – 3.16 (dd, *J* = 9.1, 9.4, 1H, H-4), 3.08 (dd, *J* = 5.2, 12.3, 1H, H-1a), 2.50 (ddd, *J* = 3.0, 6.2, 9.5, 1H, H-5), 2.42 (dd, *J* = 10.8, 12.3, 1H, H-1b). ¹³C NMR (100 MHz, D₂O) δ 78.3 (C-3), 71.4 (C-4), 70.8 (C-2), 61.3 (C-6), 60.4 (C-5), 48.6 (C-1). IR *v*_{max} (thin film)/ cm⁻¹: 3370, 2890, 1453, 1361, 1090, 1039, 1019, 841. [α]_D²⁰: 49.5 (c 1.3, H₂O). HRMS: found 164.0918 [M+H]⁺, calculated for [C₆H₁₃O₄N₁+H]⁺ 164.0917.

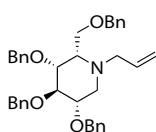


N-Benzyloxyethyl-1-deoxynojirimycin (7). *Method A:* A dry suspension of potassium carbonate (995 mg, 7.2 mmol), **6** (782 mg, 4.8 mmol) and 2-benzyloxy-1-bromoethane (1.24 g, 5.3 mmol; prepared via a literature procedure⁵⁴) in DMF was heated to 90 °C for 5 h. The reaction mixture was filtered over a glass microfiber filter and the filtrate was concentrated. The residue was purified by silicagel column chromatography (0% » 20% MeOH in CHCl₃ + 1% NH₄OH) to provide **7** (929 mg, 3.1 mmol, 3.12 mmol) in 65% yield. *Method B:* Commercially available 2-benzyloxyacetaldehyde (1.18 mL, 8.4 mmol) and NaCNBH₃ (703 mg, 11.2 mmol) were successively added to a solution of the HCl salt of 1-deoxynojirimycin (**6**) (1.125 g, 5.6 mmol) in MeOH/AcOH (56 mL, 150/1 v/v). The resulting brown solution was stirred for 48 h and subsequently the pH was adjusted to 8 with saturated aq 1M NaOH. The reaction mixture was concentrated and coevaporated with toluene. The residue was dissolved in methanol, celite was added, and the resulting suspension was concentrated and purified by silicagel column chromatography (0 » 20% MeOH in CHCl₃ + 1% NH₄OH) to produce **7** (1.431 g, 4.8 mmol) in 86% yield as a white foam. *R*_f = 0.15 (1:4; MeOH:CHCl₃ + 0.5% NH₄OH). ¹H NMR (200 MHz, MeOD) δ 7.43 – 7.11 (m, 5H), 4.51 (s, 2H), 3.93 (dd, *J* = 1.9, 12.0, 1H), 3.83 (dd, *J* = 1.7, 12.1, 1H), 3.61 (t, *J* = 5.2, 2H), 3.54 – 3.33 (m, 2H), 3.25 – 2.98 (m, 3H), 2.74 (dt, *J* = 4.7, 14.4, 1H), 2.39 – 2.14 (m, 2H). ¹³C NMR (50 MHz, MeOD) δ 137.8, 129.2, 128.9, 128.8, 78.2, 73.3, 69.8, 68.7, 66.1, 66.0, 57.2, 56.1, 51.5. IR *v*_{max} (thin film)/ cm⁻¹: 3341, 2955, 2716, 1636, 1458, 1396, 1366, 1080, 1026, 748. [α]_D²⁰: -0.6 (c 3.2, MeOH). HRMS: found 298.1664 [M+H]⁺, calculated for [C₁₅H₂₃O₅N₁+H]⁺ 298.1649.



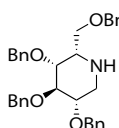
N-Hydroxyethyl-1-deoxynojirimycin (3). A solution of **7** (2.39 g, 8.0 mmol) in MeOH/H₂O (100 mL/15 mL) was acidified to pH ~3 with 36% aq HCl. The solution was purged of oxygen by bubbling argon through the solution after which Pd/C (10 wt%, 400 mg) was added. The mixture was exposed to 4 bar of hydrogen for 20 h. The reaction mixture was filtered over a glass microfiber filter and the filter cake was rinsed successively with MeOH (4×20 mL) and H₂O (2×20 mL). The combined filtrate was concentrated and coevaporated with MeOH (3×50 mL). The residue was purified by silica gel column chromatography (8:2:0.05 » 6:4:0.05, *n*-propanol:H₂O:NH₄OH) to provide **3** (1.583 g, 7.6 mmol) in 95% yield as an off-white foam. *R*_f = 0.50 (6:4:0.05; *n*-propanol:H₂O:Et₃N). ¹H NMR (300 MHz, D₂O) δ 3.94 (dd, *J* = 2.5, 12.8, 1H, H-6a), 3.83 (dd, *J* = 3.0, 12.8, 1H, H-6b), 3.75 (t, *J* = 6.4, 2H, HOCH₂-2'), 3.55 (ddd, *J* = 3.8, 7.7, 14.0, 1H, H-2), 3.43 – 3.33 (dd, *J* = 9.1, 9.1, 1H), 3.27 (dd, *J* = 9.1, 9.1, 1H), 3.09 (dd, *J* = 4.8, 11.6, 1H, H-1a), 2.95 (dt, *J* = 6.5, 14.0, 1H, NCHH-1'), 2.72 (dt, *J* = 5.8, 14.0, 1H, NCHH-1'), 2.43 – 2.29 (m, 2H, H-1b, H-5). ¹³C NMR (50 MHz, D₂O) δ 81.1, 72.9, 71.7, 68.5, 60.9, 60.5, 58.9, 55.8. IR *v*_{max} (thin film)/ cm⁻¹: 3333, 2924, 1636, 1420, 1358, 1265, 1080, 1026. [α]_D²⁰: -6.5 (c 0.56, H₂O). HRMS: found 208.1182 [M+H]⁺, calculated for [C₈H₁₇O₅N₁+H]⁺ 208.1185. **N-Hydroxyethyl-1-**

deoxynojirimycin hydrochloric acid salt (3·HCl). A solution of **3** (1.58 g, 7.6 mmol) in water (50 mL) was treated with Dowex OH[−] resin for one hour. The resin was removed by filtration and the filtrate was coevaporated with water (3×50 mL). The residue was dissolved in water (50 mL) and treated with 1M aq HCl (8.4 mL, 8.4 mmol). The solution was concentrated, coevaporated with water (3×50 mL) and lyophilized to quantitatively yield **3** (1.865 g, 7.6 mmol) as hygroscopic white foam. ¹H NMR (300 MHz, D₂O/ MeOD 1/1) δ 4.15 – 4.04 (m, 2H, CH₂-6), 4.00 (t, *J* = 4.9, 2H, OCH₂-2'), 3.91 – 3.78 (m, 1H, H-2), 3.77 – 3.65 (m, 2H, H-1a, H-4), 3.65 – 3.57 (m, 1H, NCHH-1'), 3.53 (dd, *J* = 9.4, 1H, H-3), 3.45 – 3.36 (m, 1H, NCHH-1'), 3.33 – 3.25 (m, 1H, H-5), 3.16 (dd, *J* = 11.8, 1H, H-1b).

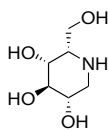


N-Allyl-2,3,4,6-tetra-O-benzyl-L-ido-1-deoxynojirimycin (10). Methanesulfonyl chloride (1.45 mL, 18.75 mmol) was added dropwise to a cooled (0 °C) solution of 2,3,4,6-tetra-O-benzyl-D-glucitol **8** (4.070 g, 7.50 mmol; for preparation see Chapter 2) in pyridine (30 mL).

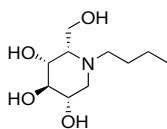
After TLC analysis indicated complete consumption of starting material (2 hours; *R_F* **8** = 0.25 in 2:1; PE:EtOAc), water (20 mL) was added and the reaction mixture was concentrated. The residue was dissolved in EtOAc (100 mL) and washed successively with 1M aq HCl (2×100 mL), sat aq NaHCO₃ (100 mL) and sat aq NaCl (100 mL). The organic phase was isolated, dried (Na₂SO₄) and concentrated to yield crude **9** (4.790g, 6.86 mmol) in ~91% yield as a yellow oil. *R_F* = 0.55 (2:1; PE:EtOAc). Crude **9** (4.790g, 6.86 mmol) was coevaporated with toluene, dissolved in allylamine (34 mL) and refluxed for 20 hours. The reaction mixture was concentrated, dissolved in EtOAc (100 mL) and washed successively with sat aq NaHCO₃ (2×100 mL) and sat aq NaCl (100 mL). The organic phase was isolated, dried (Na₂SO₄) and concentrated. The residue was purified by silicagel column chromatography (isocratic 15% EtOAc in PE) to produce **10** (2.591 g, 4.60 mmol) in 67% yield as an orange oil. *R_F* = 0.8 (2:1; PE:EtOAc). ¹H NMR (400 MHz, CDCl₃) δ 7.38 – 7.15 (m, 20H, H_{Ar} Bn), 5.84 – 5.70 (m, 1H, =CH allyl), 5.12 (dd, *J* = 1.3, 17.2, 1H, =CHH allyl), 5.07 (d, *J* = 10.2, 1H, =CHH allyl), 4.85 (d, *J* = 11.1, 1H, CHH Bn), 4.80 (d, *J* = 11.1, 1H, CHH Bn), 4.68 – 4.55 (m, 4H, 2×CH₂ Bn), 4.46 (s, 2H, CH₂ Bn), 3.83 (dd, *J* = 6.6, 10.2, 1H, H-6a), 3.76 – 3.66 (m, 2H, H-4, H-6b), 3.61 – 3.51 (m, 2H, H-2, H-3), 3.45 – 3.33 (m, 2H, H-5, CHH allyl), 3.18 (dd, *J* = 6.9, 14.1, 1H, CHH allyl), 2.92 (dd, *J* = 4.5, 11.8, 1H, H-1a), 2.58 – 2.53 (m, 1H, H-1b). ¹³C NMR (100 MHz, CDCl₃) δ 139.2, 138.7, 138.6, 138.5 (4×C_q Bn), 136.2 (=CH allyl), 129.6, 129.2, 128.6, 128.3, 128.2, 127.9, 127.7, 127.7, 127.5, 127.5, 127.4, 127.3, 127.1, 126.7 (CH_{Ar} Bn), 117.1 (=CH₂ allyl), 82.9, 80.1 (C-4), 78.7, 75.3, 73.2, 72.8, 72.6 (4×CH₂ Bn), 64.6 (C-6), 59.9 (C-5), 57.9 (NCH₂ allyl), 49.1 (C-1). IR ν_{max}(thin film)/ cm^{−1}: 3064, 3030, 2862, 1496, 1453, 1364, 1090, 1068, 914, 733. [α]_D²⁰: −19.8 (c 18.8, CHCl₃). HRMS: found 564.3090 [M+H]⁺, calculated for [C₃₇H₄₁O₄N₁+H]⁺ 564.3108.



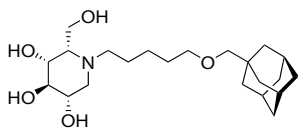
2,3,4,6-Tetra-O-benzyl-L-ido-1-deoxynojirimycin (11). Potassium *tert*-butoxide (259 mg, 2.3 mmol) was added to a solution of **10** (2.60 g, 4.61 mmol) in DMSO (9.2 mL) and the resulting brown reaction mixture was heated at 100 °C for 30 minutes. The reaction mixture was charged with 1M aq HCl (9 mL) and stirred vigorously for 15 minutes. The mixture was poured into sat aq NaHCO₃ (100 mL) and extracted with Et₂O (3×100 mL). The organic phase was isolated, dried (Na₂SO₄) and concentrated. The residue was purified by silicagel column chromatography (33% » 66% EtOAc in PE) to furnish **11** (1.954 g, 3.73 mmol) in 81% yield as a yellow oil. *R_F* = 0.2 (1:1; PE:EtOAc). ¹H NMR (400 MHz, CDCl₃) δ 7.38 – 7.20 (m, 20H, H_{Ar} Bn), 4.67 – 4.50 (m, 8H, 4×CH₂ Bn), 3.67 (dd, *J* = 9.5, 9.5, 1H, H-6a), 3.64 – 3.59 (m, 2H, H-3, H-4), 3.55 (dd, *J* = 5.2, 9.5, 1H, H-6b), 3.44 (dd, *J* = 6.3, 10.5, 1H, H-2), 3.41 – 3.35 (m, 1H, H-5), 3.00 (dd, *J* = 4.1, 12.9, 1H, H-1a), 2.86 (dd, *J* = 6.7, 12.9, 1H, H-1b), 2.00 (s, 1H, NH). ¹³C NMR (100 MHz, CDCl₃) δ 138.8, 138.7, 138.6, 138.5 (4×C_q Bn), 128.6, 128.5, 128.5, 128.5, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7 (CH_{Ar} Bn), 78.1, 77.3, 77.1 (C-2), 74.2, 73.5, 72.8, 72.2 (4×CH₂ Bn), 67.4 (C-6), 54.8 (C-5), 44.4 (C-1). IR ν_{max}(thin film)/ cm^{−1}: 3064, 3030, 2863, 1496, 1453, 1364, 1092, 1068, 734. [α]_D²⁰: −7.6 (c 3.3, CHCl₃). HRMS: found 524.2776 [M+H]⁺, calculated for [C₃₄H₃₇O₄N₁+H]⁺ 524.2795.



L-Ido-1-deoxyojirimycin (12). A solution of **11** (329 mg, 0.62 mmol) in EtOH (15 mL) was acidified to pH ~2 with 1M aq HCl and purged of oxygen by bubbling argon through the solution for 15 minutes. Pd/C (10 wt%, 50 mg) was added and the mixture was exposed to 4 bar of hydrogen for 20 hours. The reaction mixture was filtered over a glass microfiber filter and the filter cake was rinsed successively with MeOH (4×20 mL) and H₂O (2×20 mL). The combined filtrate was concentrated and coevaporated with MeOH (3×50 mL). The residue was purified by column chromatography with aluminum oxide (isocratic 16:3.7:0.3, *n*-propanol:H₂O:NH₄OH) to provide **12** (88 mg, 0.54 mmol) in 86% yield as a colorless oil. *R*_f = 0.18 (10:12:3; MeOH:EtOAc:NH₄OH). ¹H NMR (600 MHz, D₂O) δ 4.06 – 4.03 (m, 1H, H-2), 4.02 – 3.98 (m, 2H, H-3, H-4), 3.90 (dd, *J* = 5.0, 12.3, 1H, H-6a), 3.86 (dd, *J* = 9.0, 12.3, 1H, H-6b), 3.58 (ddd, *J* = 2.2, 4.9, 8.9, 1H, H-5), 3.42 (dd, *J* = 2.6, 13.6, 1H, H-1a), 3.34 (dd, *J* = 3.4, 13.6, 1H, H-1b). ¹³C NMR (150 MHz, D₂O) δ 66.7 (C-4), 66.5 (C-3), 65.7 (C-2), 58.1 (C-6), 55.9 (C-5), 44.4 (C-1). IR *v*_{max}(thin film)/ cm⁻¹: 3362, 3200, 2924, 2853, 1653, 1635, 1419, 1055, 963. [α]_D²⁰: –2.8 (c 2.3, H₂O). HRMS: found 164.0912 [M+H]⁺, calculated for [C₆H₁₃O₄N₁+H]⁺ 164.0917.



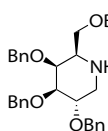
N-Butyl-L-ido-1-deoxyojirimycin (13). Butyraldehyde (66 μL, 0.73 mmol) and NaCNBH₃ (27 mg, 0.44 mmol) were successively added to a solution of **10** (76 mg, 0.145 mmol) in CH₃CN/AcOH (1.45 mL, 30/1, v/v). The reaction mixture was stirred for 20 hours after which sat aq NaHCO₃ (5 mL) was added and the resulting mixture was extracted with Et₂O (3×5 mL). The combined organic phases were dried (Na₂SO₄) and concentrated to provide the crude *N*-butylated intermediate (*R*_f = 0.5 in 4:1; PE:EtOAc). The crude intermediate was coevaporated with dichloroethane and dissolved in DCM (1.5 mL). The solution was cooled to 0 °C and BCl₃ (1.74 mL, 1M in DCM) was added. After stirring for 20 hours at 0 °C MeOH (2 mL) was carefully added. The mixture was concentrated and coevaporated with toluene. The residue was purified by silicagel column chromatography (0% » 20% MeOH in CHCl₃ + 1% NH₄OH) to produce **13** (26 mg, 0.12 mmol) in 82% yield over two steps as a colorless oil. *R*_f = 0.18 (3:1; CHCl₃:MeOH + 1% NH₄OH). ¹H NMR (400 MHz, MeOD) δ 4.06 – 3.96 (m, 3H, CH₂-6, H-4), 3.94 (d, *J* = 7.4, 1H, H-2), 3.83 (s, 1H, H-3), 3.51 (s, 1H, H-5), 3.44 (d, *J* = 12.8, 1H, H-1a), 3.36 – 3.28 (m, 3H, H-1b, CH₂-1 butyl), 1.87 – 1.65 (m, 2H, CH₂-2 butyl), 1.48 – 1.38 (m, 2H, CH₂-3 butyl), 1.01 (t, *J* = 7.4, 3H, CH₃-4 butyl). ¹³C NMR (100 MHz, MeOD) δ 69.2, 63.8, 55.0, 21.1 (CH₂-3 butyl), 14.1 (CH₃-4 butyl). IR *v*_{max}(thin film)/ cm⁻¹: 3279, 2963, 2876, 1637, 1653, 1445, 1063, 979.4. [α]_D²⁰: 12.2 (c 0.7, MeOH). HRMS: found 220.1538 [M+H]⁺, calculated for [C₁₀H₂₁O₄N₁+H]⁺ 220.1543.



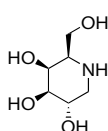
N-[5-(Adamantan-1-yl-methoxy)-pentyl]-L-ido-1-deoxyojirimycin (14). A solution of **10** (1.954 g, 3.73 mmol) and 5-(adamantan-1-yl-methoxy)-1-pentanol **15** (1.029 g, 4.11 mmol) in EtOH/AcOH (93 mL, 9/1, v/v) was purged of oxygen by bubbling argon through the solution. Pd/C (10 wt%, 329 mg) was added to the solution and the reaction mixture was exposed to 4 bar of hydrogen for 20 hours. Removal of Pd/C by filtration over a glass microfiber filter and concentration of the filtrate provided the crude *N*-alkylated intermediate (*R*_f = 0.6 in 4:1; PE:EtOAc) as a light yellow oil. A solution of the crude intermediate in EtOH (50 mL) was acidified with 2M aq HCl (12 mL) and purged of oxygen by bubbling argon through the solution. Pd/C (10 wt%, 500 mg) was added to the solution and the reaction mixture was exposed to 4 bar of hydrogen for 20 hours. After removal of Pd/C and concentration, the residue was purified by silicagel column chromatography (0% » 10% MeOH in CHCl₃ + 1% NH₄OH) to afford **14** (1.146 g, 2.88 mmol) in 77% yield over two steps as white foam. *R*_f = 0.3 (3:1; CHCl₃:MeOH + 1% NH₄OH). ¹H NMR (400 MHz, MeOD, major conformation) δ 3.91 (d, *J* = 5.1, 2H, CH₂-6), 3.85 (s, 1H, H-2/H-3/H-4), 3.74 (s, 1H, H-2/H-3/H-4), 3.63 (s, 1H, H-2/H-3/H-4), 3.40 (t, *J* = 6.2, 2H, OCH₂-5 pentyl), 3.31 – 3.27 (m, 1H, H-5), 3.23 – 2.97 (m, 4H, CH₂-1, NCH₂-1 pentyl), 2.97 (s, 2H, OCH₂), 1.95 (s, 3H, 3×CH Ada), 1.80 – 1.59 (m, 10H, CH₂-2' CH₂-4' pentyl, 3×CH₂ Ada), 1.56 (d, *J* = 2.0, 6H, 3×CH₂ Ada), 1.47 – 1.38 (m, 2H, CH₂-3 pentyl). ¹³C NMR (100 MHz, MeOD) δ 83.2 (OCH₂), 72.5 (C-2/C-3/C-4), 72.5 (OCH₂-5 pentyl), 70.2 (C-2/C-

3/C-4), 64.1 (C-5), 59.2 (C-6), 55.4 (NCH₂-1 pentyl), 53.6 (C-1), 41.0 (3×CH₂ Ada), 38.5 (3×CH₂ Ada), 35.3 (C_q Ada), 30.5 (CH₂ pentyl), 29.9 (3×CH Ada, CH₂ pentyl), 25.0 (CH₂ pentyl). IR ν_{max} (thin film)/ cm⁻¹: 3358, 2902, 2848, 1637, 1451, 1071. $[\alpha]_{\text{D}}^{20}$: 11.0 (c 0.4, MeOH). HRMS: found 398.2889 [M+H]⁺, calculated for [C₂₂H₃₉O₃N₁+H]⁺ 398.2901.

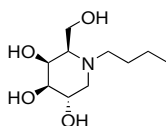
N-[5-(Adamantan-1-yl-methoxy)-pentyl]-L-ido-1-deoxynojirimycin hydrochloric acid salt (14*HCl). A solution of compound **14** in dioxane/H₂O (~0.3M, 9/1, v/v) was treated with 1M aq. HCl (1.1 eq) and lyophilized to produce **14*HCl** as a white foam. ¹H NMR (600 MHz, MeOD, major conformation) δ 4.03 (s, 1H, H-2/H-3/H-4), 4.02 – 3.90 (m, 3H, CH₂-6, H-2/H-3/H-4), 3.87 (s, 1H, H-2/H-3/H-4), 3.52 (s, 1H, H-5), 3.51 – 3.32 (m, 6H, CH₂-1, NCH₂-1 pentyl, OCH₂-5 pentyl), 2.98 (s, 2H, OCH₂), 1.94 (s, 3H, 3×CH Ada), 1.92 – 1.59 (m, 10H, CH₂-2' CH₂-4 pentyl, 3×CH₂ Ada), 1.56 (s, 6H, 3×CH₂ Ada), 1.47 (s, 2H, CH₂-3 pentyl). ¹³C NMR (100 MHz, MeOD, major conformation) δ 83.2 (OCH₂), 72.4 (C-2/C-3/C-4), 72.3 (OCH₂-5' pentyl), 69.1 4 (C-2/C-3/C-4), 68.2 4 (C-2/C-3/C-4), 63.9 (C-5), 61.5 (C-6), 55.3 (NCH₂-1' pentyl), 54.6 (C-1), 41.0 (3×CH₂ Ada), 38.5 (3×CH₂ Ada), 35.3(C_q Ada), 30.3 (CH₂-4' pentyl), 29.9 (3×CH Ada), 24.8 (CH₂-3' pentyl), 23.5 (CH₂-2' pentyl).



2,3,4,6-Tetra-O-benzyl-D-galacto-1-deoxynojirimycin (18). Lithium aluminumhydride (75 mg, 1.98 mmol) was added to a solution of 2,3,4,6-tetra-O-benzyl-D-galactono- δ -lactam **17** (353 mg, 0.66 mmol, prepared via a literature procedure³³) in THF (13 mL). The reaction mixture was refluxed for 3 hours or until TLC analysis indicated complete consumption of **17** (R_f = 0.25 in 2:1; PE:EtOAc). The reaction mixture was cooled to ambient temperature and EtOAc (6 mL) was carefully added. The mixture was poured into 1M aq NaOH (50 mL) and the aqueous layer was extracted with Et₂O (3×50 mL). The combined organic layers were dried (Na₂SO₄) and concentrated. The residue was purified by silicagel column chromatography (20% » 66% EtOAc in PE) to furnish **18** (247 g, 0.47 mmol) in 71% yield as an orange crystalline solid. R_f = 0.1 (2:1; PE:EtOAc). ¹H NMR (400 MHz, CDCl₃) δ 7.35 – 7.18 (m, 20H, H_{Ar} Bn), 4.97 (d, J = 10.9, 1H, CHH Bn), 4.88 – 4.81 (m, 2H, CHH Bn, CHH Bn), 4.71 – 4.63 (m, 2H, CH₂ Bn), 4.51 – 4.38 (m, 3H, CHH Bn, CH₂ Bn), 3.66 (dd, J = 2.6, 9.0, 1H, H-6a), 3.58 – 3.45 (m, 3H, H-2, H-3, H-6b), 3.35 (dd, J = 8.9, 9.6, 1H, H-4), 3.24 (dd, J = 4.8, 12.3, 1H, H-1ax), 2.72 (ddd, J = 2.5, 5.9, 9.6, 1H, H-5), 2.50 (dd + br. s, J = 10.2, 12.2, 2H, H-1eq, NH). ¹³C NMR (100 MHz, CDCl₃) δ 139.1, 138.6, 138.6, 138.1 (4×C_q Bn), 128.6, 128.5, 128.5, 128.5, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7 (CH_{Ar} Bn), 87.4 (C-3), 80.7 (C-2), 80.2 (C-4), 75.8, 75.3, 73.5, 72.9 (4× CH₂ Bn), 70.3 (C-6), 59.9 (C-5), 48.2 (C-1). IR ν_{max} (thin film)/ cm⁻¹: 3032, 2862, 1450, 1358, 1088, 1065, 1026, 841, 733. $[\alpha]_{\text{D}}^{20}$: 24.4 (c 13.6, CHCl₃). HRMS: found 524.2776 [M+H]⁺, calculated for [C₃₄H₃₇O₄N₁+H]⁺ 524.2795.

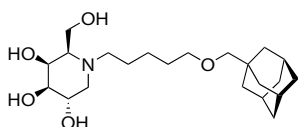


D-Galacto-1-deoxynojirimycin (19). Boron trichloride (1.2 mL, 1M in DCM) was added to a cooled (0 °C) solution of **18** (62 mg, 0.118 mmol) in DCM (2 mL). The reaction mixture was stirred for 20 hours at 0 °C after which MeOH (0.5 mL) was carefully added. The reaction mixture was concentrated and coevaporated with toluene. Column chromatography of the residue over aluminumoxide (1:2, MeOH:EtOAc » MeOH » 1:3, H₂O:MeOH) provided **19** (19 mg, 0.116 mmol) in 96% yield as a colorless oil. R_f = 0.24 (10:12:3; MeOH:EtOAc:NH₄OH). ¹H NMR (300 MHz, D₂O) δ 3.90 (dd, J = 3.2, 12.7, 1H, H-6a), 3.82 (dd, J = 5.2, 12.7, 1H, H-6b), 3.74 (ddd, J = 5.1, 9.1, 11.5, 1H, H-2), 3.59 – 3.42 (m, 3H, H-1a, H-3, H-4), 3.15 (ddd, J = 3.3, 5.1, 10.2, 1H, H-5), 2.92 (dd, J = 11.5, 12.3, 1H, H-1b). ¹³C NMR (75 MHz, D₂O) δ 76.9, 68.6, 67.7, 60.6, 58.5, 46.6. IR ν_{max} (thin film)/ cm⁻¹: 3242, 1651, 1405, 1094, 1022. $[\alpha]_{\text{D}}^{20}$: 24.7 (c 0.4, H₂O). HRMS: found 164.0912 [M+H]⁺, calculated for [C₆H₁₃O₄N₁+H]⁺ 164.0917.



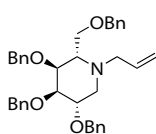
N-Butyl-D-galacto-1-deoxynojirimycin (20). Compound **20** (31 mg, 0.14 mmol) was synthesized from **18** (100 mg, 0.19 mmol) in 74% yield as a colorless oil following the procedure as described for **13**. R_f N-butylated intermediate = 0.78 (1:1; PE:EtOAc); R_f **20** = 0.13 (3:1; EtOAc:MeOH + 1% NH₄OH). ¹H NMR (300 MHz, MeOD) δ 4.02 (dd, J = 2.0, 12.4, 1H,

H-6a), 3.89 (dd, $J = 3.0, 12.4$, 1H, H6b), 3.65 (ddd, $J = 4.9, 9.2, 11.0$, 1H, H-2), 3.58 – 3.49 (m, 1H, H4), 3.35 – 3.27 (m, 2H, H-1a, H-3), 3.26 – 3.13 (m, 1H, NCHH-1 butyl), 3.08 – 3.00 (m, 1H, NCHH-1 butyl), 2.86 – 2.72 (m, 2H, H-1b, H-5), 1.73 – 1.59 (m, 2H, CH₂-2 butyl), 1.46 – 1.32 (m, 2H, CH₂-3 butyl), 0.98 (t, $J = 7.3$, 3H, CH₃-4 butyl). ¹³C NMR (75 MHz, MeOD) δ 78.8 (C-3), 69.8 (C-4), 68.7 (C-2), 67.5 (C-5), 56.4 (C-6), 55.6 (C-1), 53.9 (NCH₂-1 butyl), 26.6 (CH₂-2 butyl), 21.3 (CH₂-3 butyl), 14.2 (CH₃-4 butyl). IR $\nu_{\max}$ (thin film)/ cm^{-1} : 3312, 2962, 2876, 1654, 1458, 1380, 1085, 1050, 1029. $[\alpha]_{\text{D}}^{20}$: –4.5 (c 0.6, MeOH). HRMS: found 220.1539 $[\text{M}+\text{H}]^+$, calculated for $[\text{C}_{10}\text{H}_{21}\text{O}_4\text{N}_1+\text{H}]^+$ 220.1543.



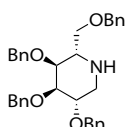
***N*-[5-(Adamantan-1-yl-methoxy)-pentyl]-D-galacto-1-deoxynojirimycin**

(21). Compound **21** (45 mg, 0.11 mmol) was synthesized from **18** (94 mg, 0.18 mmol) and **15** (90 mg, 0.36 mmol) in 61% yield as a colorless oil following the reductive amination procedure as described for **13** and deprotected as described for compound **14**. Silica gel column chromatography (0% » 10% MeOH in CHCl_3 + 1% NH_4OH). R_f *N*-alkylated intermediate = 0.68 (2:1; PE:EtOAc); R_f **21** = 0.19 (3:1; EtOAc:MeOH + 1% NH_4OH). ¹H NMR (400 MHz, MeOD, COSY) δ 3.97 (d, $J = 12.0$, 1H, H-6a), 3.88 (d, $J = 12.0$, 1H, H-6b), 3.63 – 3.54 (m, 1H, H-2), 3.50 – 3.46 (m, 1H, H-4), 3.41 (t, $J = 6.1$, 2H, CH₂-5 pentyl), 3.27 – 3.21 (m, 2H, H-1a, H-3), 3.12 – 3.04 (m, 1H, NCHH-1 pentyl), 2.98 (s, 2H, OCH₂), 2.95 – 2.86 (m, 1H, NCHH-1 pentyl), 2.65 – 2.54 (m, 2H, H-1b, H-5), 1.95 (s, 3H, 3 $\times$ CH Ada), 1.80 – 1.59 (m, 10H, CH₂-2 CH₂-4 pentyl, 3 $\times$ CH₂ Ada), 1.56 (s, 6H, 3 $\times$ CH₂ Ada), 1.47 – 1.39 (m, 2H, CH₂-3 pentyl). ¹³C NMR (100 MHz, MeOD) δ 83.3 (OCH₂), 79.5 (C-3), 72.5 (OCH₂-5 pentyl), 70.6 (C-4), 69.4 (C-2), 67.5 (C-5), 57.7 (C-6), 56.4 (C-1), 54.1 (NCH₂-1 pentyl), 41.0 (3 $\times$ CH₂ Ada), 38.5 (3 $\times$ CH₂ Ada), 35.3 (C_q Ada), 30.5 (CH₂-4 pentyl), 29.9 (3 $\times$ CH Ada), 25.1 (CH₂-2 pentyl), 24.7 (CH₂-3 pentyl). IR $\nu_{\max}$ (thin film)/ cm^{-1} : 3349, 2900, 2848, 1735, 1652, 1452, 1093, 1030. $[\alpha]_{\text{D}}^{20}$: 10.7 (c 0.28, MeOH). HRMS: found 398.2892 $[\text{M}+\text{H}]^+$, calculated for $[\text{C}_{22}\text{H}_{39}\text{O}_5\text{N}_1+\text{H}]^+$ 398.2901.



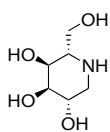
***N*-Allyl-2,3,4,6-tetra-O-benzyl-L-altro-1-deoxynojirimycin (24).**

Compound **24** (1.970 g, 3.50 mmol) was synthesized starting from 2,3,4,6-tetra-O-benzyl-D-galactitol **22** (2.324 g, 4.28 mmol, prepared via a literature procedure³²) in 82% yield over two steps following the procedure as described for **10**. R_f intermediate dimesylate **23** = 0.67 (1:1; PE:EtOAc); R_f **24** = 0.48 (5:1; PE:EtOAc). ¹H NMR (400 MHz, CDCl_3 , major conformation) δ 7.34 – 7.21 (m, 20H, H_{Ar} Bn), 5.83 (dddd, $J = 4.8, 7.7, 10.2, 15.0$, 1H, =CH allyl), 5.10 (m, 2H, =CH₂ allyl), 4.73 (d, $J = 11.9$, 1H, CHH Bn), 4.60 – 4.41 (m, 7H, CHH Bn, 3 $\times$ CH₂ Bn), 4.07 (dd, $J = 3.0, 6.7$, 1H, H-3), 4.01 – 3.91 (m, 3H, H-2, H-5, H-6a), 3.75 (dd, $J = 6.4, 10.6$, 1H, H-6b), 3.69 – 3.60 (m, 1H, CHH allyl), 3.31 (dd, $J = 5.3, 10.7$, 1H, H-1a), 3.24 (dd, $J = 4.6, 6.7$, 1H, H-4), 2.99 (dd, $J = 7.7, 14.2$, 1H, CHH allyl), 2.39 (dd, $J = 4.9, 10.7$, 1H, H-1b). ¹³C NMR (100 MHz, CDCl_3 , major conformation) δ 139.2, 138.8, 138.2, 138.1 (4 $\times$ C_q Bn), 136.0 (=CH allyl), 128.3, 128.2, 128.2, 128.1, 128.1, 127.6, 127.4, 127.4, 127.2, 127.1 (CH_{Ar} Bn), 116.4 (=CH₂ allyl), 83.9 (C-3), 82.1 (C-2), 78.6 (C-5), 73.0 (CH₂ Bn), 72.3 (C-6), 72.1 (CH₂ Bn), 71.3 (CH₂ Bn), 66.4 (C-4), 59.3 (NCH₂ allyl), 56.0 (C-1). IR $\nu_{\max}$ (thin film)/ cm^{-1} : 3064, 3031, 2861, 1496, 1453, 1362, 1205, 1090, 1073, 1026, 914, 733. $[\alpha]_{\text{D}}^{20}$: –13.6 (c 3.1, CHCl_3). HRMS: found 564.3090 $[\text{M}+\text{H}]^+$, calculated for $[\text{C}_{37}\text{H}_{41}\text{O}_4\text{N}_1+\text{H}]^+$ 564.3108.

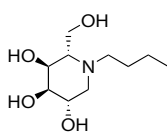


2,3,4,6-Tetra-O-benzyl-L-altro-1-deoxynojirimycin (25).

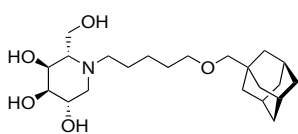
Compound **25** (1.211 g, 2.31 mmol) was synthesized from **24** (1.793 g, 3.18 mmol) in 73% yield following the procedure as described for **11**. R_f = 0.10 (1:1; PE:EtOAc). ¹H NMR (300 MHz, CDCl_3) δ 7.35 – 7.22 (m, 20H, H_{Ar} Bn), 4.75 (d, $J = 11.5$, 1H, CHH Bn), 4.59 – 4.43 (m, 7H, CHH Bn, 3 $\times$ CH₂ Bn), 4.10 (d, $J = 3.9$, 1H, H-5), 3.96 (dd, $J = 2.2, 5.3$, 1H, H-2), 3.94 – 3.83 (m, 2H, H-3, H-6a), 3.68 (dd, $J = 4.8, 10.5$, 1H, H-6b), 3.48 (dd, $J = 3.9, 9.3$, 1H, H-4), 3.33 (dd, $J = 5.4, 12.2$, 1H, H-1a), 2.92 (dd, $J = 2.3, 12.2$, 1H, H-1b), 1.95 (s, 1H, NH). ¹³C NMR (75 MHz, CDCl_3) δ 139.2, 138.6, 138.4, 138.3, 128.6, 128.5, 128.5, 128.4, 127.9, 127.8, 127.7, 127.5, 82.4, 82.3, 77.6, 73.5, 72.4, 71.8, 71.4, 71.3, 62.2, 51.6. IR $\nu_{\max}$ (thin film)/ cm^{-1} : 3064, 3030, 2862, 1486, 1453, 1359, 1206, 1092, 1026, 733. $[\alpha]_{\text{D}}^{20}$: –15.8 (c 2.0, CHCl_3). HRMS: found 524.2777 $[\text{M}+\text{H}]^+$, calculated for $[\text{C}_{34}\text{H}_{37}\text{O}_4\text{N}_1+\text{H}]^+$ 524.2795.



L-Altro-1-deoxynojirimycin (26). Compound **26** (449 mg, 0.86 mmol) was synthesized from **25** (108 mg, 0.66 mmol) in 77% yield following the procedure as described for **12**. R_f = 0.09 (10:12:3; MeOH:EtOAc:NH₄OH). ¹H NMR (400 MHz, D₂O) δ 4.40 – 4.38 (m, 2H, H-2, H-3), 4.13 (dt, J = 5.2, 8.8, 1H, H-5), 3.80 – 3.69 (m, 4H, H-1a, H-4, CH₂-6), 3.31 (d, J = 13.2, 1H, H-1b). ¹³C NMR (100 MHz, D₂O) δ 75.0 (C-2/C-3), 74.1 (C-2/C-3), 67.3 (C-5), 63.7 (C-6), 62.6 (C-4), 51.9 (C-1). IR $\nu_{\max}$ (thin film)/ cm⁻¹: 3326, 2925, 1590, 1454, 1088, 1043. $[\alpha]_D^{20}$: -27.9 (c 0.4, H₂O). HRMS: found 164.0913 [M+H]⁺, calculated for [C₆H₁₃O₄N₁+H]⁺ 164.0917.



N-Butyl-L-altro-1-deoxynojirimycin (27). Compound **27** (19 mg, 87 μ mol) was synthesized from **25** (50 mg, 96 μ mol) in 91% yield as a colorless oil following the procedure as described for **13**. R_f N-butylated intermediate = 0.45 (4:1; PE:EtOAc); R_f **27** = 0.18 (3:1; EtOAc:MeOH + 1% NH₄OH). ¹H NMR (400 MHz, MeOD) δ 4.25 (dd, J = 2.4, 4.4, 1H, H-3), 4.12 – 4.09 (m, 1H, H-2), 4.03 (dd, J = 10.0, 14.8, 1H, H-5), 3.76 (ddd, J = 5.4, 11.2, 16.6, 2H, CH₂-6), 3.65 (dd, J = 4.8, 11.6, 1H, H-1a), 3.29 – 3.22 (m, 1H, H-4), 3.19 – 3.12 (m, 1H, NCHH-1 butyl), 2.74 – 2.72 (m, 2H, H-1b, NCHH-1 butyl), 1.65 – 1.58 (m, 2H, CH₂-2 butyl), 1.45 – 1.31 (m, 2H, CH₂-3 butyl), 0.96 (t, J = 7.3, 3H, CH₃-4 butyl). ¹³C NMR (100 MHz, MeOD) δ 78.7 (C-3), 76.4 (C-2), 70.6 (C-5), 70.0 (C-4), 65.1 (C-6), 60.0 (C-1), 57.5 (NCH₂-1 butyl), 30.0 (CH₂-2 butyl), 21.3 (CH₂-3 butyl), 14.2 (CH₃-4 butyl). IR $\nu_{\max}$ (thin film)/ cm⁻¹: 3338, 2933, 1652, 1459, 1035. $[\alpha]_D^{20}$: -44.5 (c 0.2, MeOH). HRMS: found 220.1539 [M+H]⁺, calculated for [C₁₀H₂₁O₄N₁+H]⁺ 220.1543.

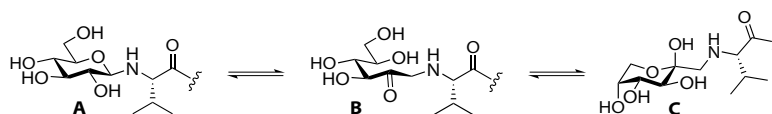


N-[5-(Adamantan-1-yl-methoxy)-pentyl]-L-altro-1-deoxynojirimycin (28). Compound **28** (34 mg, 85 μ mol) was synthesized from **25** (50 mg, 96 μ mol) in 89% yield as a colorless oil following the reductive amination procedure as described for **13**, but now with 5-(adamantan-1-yl-methoxy)-1-pentanal **15** (48 mg, 0.19 mmol) and the deprotection procedure as described for **14**. Silica gel column chromatography (0% » 10% MeOH in CHCl₃ + 1% NH₄OH). R_f N-alkylated intermediate = 0.60 (4:1; PE:EtOAc); R_f **28** = 0.27 (3:1; EtOAc:MeOH + 1% NH₄OH). ¹H NMR (400 MHz, MeOD) δ 4.21 (dd, J = 2.7, 4.8, 1H, H-3), 4.11 – 4.07 (m, 1H, H-2), 4.03 – 3.98 (m, 1H, H-5), 3.76 (d, J = 5.3, 2H, CH₂-6), 3.59 (dd, J = 5.1, 11.1, 1H, H-1a), 3.40 (t, J = 6.3, 2H, OCH₂-5 pentyl), 3.15 – 3.10 (m, 1H, H-4), 3.10 – 3.02 (m, 1H, NCHH-1 pentyl), 2.97 (s, 2H, OCH₂), 2.64–2.58 (m, 2H, H-1b, NCHH-1 pentyl), 1.95 (s, 3H, 3 $\times$ CH Ada), 1.79 – 1.67 (m, 6H, 3 $\times$ CH₂ Ada), 1.66 – 1.58 (m, 4H, CH₂-2, CH₂-4 pentyl), 1.56 (d, J = 2.5, 6H, 3 $\times$ CH₂ Ada), 1.46 – 1.38 (m, 2H, CH₂-3 pentyl). ¹³C NMR (75 MHz, D₂O) δ 83.2 (OCH₂), 79.2 (C-3), 76.8 (C-2), 72.5 (OCH₂-5 pentyl), 71.3 (C-5), 69.6 (C-4), 65.3 (C-6), 60.0 (C-1), 57.7 (NCH₂-1 pentyl), 41.0 (3 $\times$ CH₂ Ada), 38.5 (3 $\times$ CH₂ Ada), 35.3 (C_q Ada), 30.6 (CH₂-4 pentyl), 29.9 (3 $\times$ CH Ada), 28.2 (CH₂-2 pentyl), 25.1 (CH₂-3 pentyl). IR $\nu_{\max}$ (thin film)/ cm⁻¹: 3360, 2902, 2849, 1652, 1452, 1110. $[\alpha]_D^{20}$: -28.8 (c 0.3, MeOH). HRMS: found 398.2888 [M+H]⁺, calculated for [C₂₂H₃₉O₅N₁+H]⁺ 398.2901.

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- (20) *Note on HbA1c values:* Hemoglobin is a tetrameric protein and in the HbA1c-subtype glucose has reacted non-enzymatically with one or more of the N-terminal valines. First, hemiaminal **A** is formed and undergoes an Amadori rearrangement to D-fructose derivative **B**, which cyclizes to pyranoside **C**.²¹ This type of hemoglobin forms slowly during the life span of the erythrocyte (~120 days) at a predictable rate that is solely dependant on the blood glucose levels during this time. The excessive formation of HbA1c is also implicated in the pathology of diabetes. The HbA1c level in (diabetic) individuals is an important indicator for normal or defective glycemic control.



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- (34) *Note on synthesis of 18:* Double reductive aminations of C-1/C-5 diketone intermediates selectively provide β-aza-C-D-galactosides in good yields as reported by van Boom⁵⁵ and Mootoo.⁵⁶
- (35) *Note on HOMA values:* The homeostatic model assessment (HOMA)³⁶ of β-cell function and insulin resistance (IR) was first described in 1985. The physiological basis of the HOMA model is a predictable feedback loop between the liver and the β-cell during a fasting period. The blood glucose concentration in a fastening state is regulated by the rate of *de novo* glucose synthesis by the liver (gluconeogenesis), which is insulin and insulin receptor sensitivity dependent. The blood insulin concentration is dependent on β-cell response to the blood glucose concentration. Insulin levels regulate the uptake of glucose by fat tissue and skeletal muscles, which is dependant on insulin receptor sensitivity in these tissues. Uptake of glucose in the brain (~50% turnover of total fastening glucose) and disposal by the kidneys is only dependant on glucose concentration. In this study the adapted model of Matthews (HOMA1-IR)³⁶ was used; the HOMA1-IR = fasting blood insulin concentration (mU/L) × fasting blood glucose concentration (mmol/L) / 22.5 – a lower value means less insulin resistance and better glycemic control.
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