CHEMMEDCHEM

DOI: 10.1002/cmdc.200800350

Design, Synthesis, and Biological Evaluation of Enantiomeric β-*N*-Acetylhexosaminidase Inhibitors LABNAc and DABNAc as Potential Agents against Tay-Sachs and Sandhoff Disease

J. S. Shane Rountree,*[a, b] Terry D. Butters,*[b] Mark R. Wormald,[b] Stephanie D. Boomkamp,[b] Raymond A. Dwek,[b] Naoki Asano,[c] Kyoko Ikeda,[c] Emma L. Evinson,[d] Robert J. Nash,[d] and George W. J. Fleet*[a]

N-Acetylhexosaminidases are of considerable importance in mammals and are involved in various significant biological processes. In humans, deficiencies of these enzymes in the lysosome, resulting from inherited genetic defects, cause the glycolipid storage disorders Tay-Sachs and Sandhoff diseases. One promising therapy for these diseases involves the use of β-N-acetylhexosaminidase inhibitors as chemical chaperones to enhance the enzyme activity above sub-critical levels. Herein we describe the synthesis and biological evaluation of a potent inhibitor, 2-acetamido-1,4-imino-1,2,4-trideoxy-L-arabinitol (LABNAc), in a high-yielding 11-step procedure from D-lyxonolactone. The N-benzyl and N-butyl analogues were also prepared and found to be potent inhibitors. The enantiomers DABNAc and NBn-DABNAc were synthesised from L-lyxonolactone, and were also evaluated. The L-iminosugar LABNAc and its derivatives were found to be potent noncompetitive inhibitors of some β-N-acetylhexosaminidases, while the D-iminosugar DABNAc and its derivatives were found to be weaker competitive inhibitors. These results support previous work postulating that D-iminosugar mimics inhibit D-glycohydrolases competitively, and that their corresponding L-enantiomers show noncompetitive inhibition of these enzymes. Molecular modelling studies confirm that the spatial organisation in enantiomeric inhibitors leads to a different overlay with the monosaccharide substrate. Initial cell-based studies suggest that NBn-LABNAc can act as a chemical chaperone to enhance the deficient enzyme's activity to levels that may cause a positive pharmacological effect. LABNAc, NBn-LABNAc, and NBu-LABNAc are potent and selective inhibitors of β-N-acetylhexosaminidase and may be useful as therapeutic agents for treating adult Tay-Sachs and Sandhoff diseases.

Introduction

Tay-Sachs and Sandhoff diseases are glycosphingolipid (GSL) storage disorders that result from inherited defects in genes that encode the enzymes required to break down the GM2 ganglioside.[1] It is for this reason that these diseases may be collectively referred to as the GM2 gangliosidoses. The inability to break down GSLs results in the accumulation of undegraded materials, leading to lysosomal engorgement and vacuolisation of cells. In infants, this leads to rapid mental and motor deterioration, and in severe cases, death. Sub-acute (juvenile) and chronic (adult) forms may also occur, characterised by later onset and milder clinical course. Disease phenotype is only observed for cases in which β-*N*-acetylhexosaminidase activity has fallen to $<5\%$ of normal levels. When levels of the accumulated GM2 ganglioside (compound **8**, Figure 1) reach a critical value, cells are destroyed, possibly through apoptosis.[2] It has been hypothesised that a critical threshold level of between 5 and 10% of normal β-*N*-acetylhexosaminidase activity is required to maintain the rate of GM2 hydrolysis above or equal to the rate of ganglioside transport and incorporation into the lysosome.[3]

Tay-Sachs disease (TSD) is caused by mutations in the *HEXA* gene, which encodes the α subunit of dimeric β-hexosaminidases, resulting in a deficiency in the lysosomal enzyme, β-hexosaminidase A (αβ).[4] It has been found in a diverse range of ethnic groups globally, however, the Ashkenazi Jewish population has the highest incidence of this disease, with a carrier rate of approximately 1 in 30, compared with about 1 in 250 for the general population.[5] Sandhoff disease (SD) is less prev-

[a] *Dr. J. S. S. Rountree, Prof. G. W. J. Fleet*
Chemistry Research Laboratory, Department of Chemistry
University of Oxford, Mansfield Road, Oxford, OX1 3TA (UK)
Fax (G.W.J.F.): (+44) 1865-277386
E-mail: j.s.shane.rountree@sjc.oxon.org
george.fleet@chem.ox.ac.uk

[b] *Dr. J. S. S. Rountree, Dr. T. D. Butters, Dr. M. R. Wormald, S. D. Boomkamp, Prof. R. A. Dwek*
Oxford Glycobiology Institute, Department of Biochemistry
University of Oxford, South Parks Road, Oxford, OX1 3QU (UK)
Fax (T.D.B.): (+44) 1865-275216
E-mail: terry.butters@bioch.ox.ac.uk

[c] *Prof. N. Asano, Dr. K. Ikeda*
Department of Biochemistry, Faculty of Pharmaceutical Sciences
Hokuriku University, Kanazawa, Ishikawa 9201181 (Japan)

[d] *Dr. E. L. Evinson, Dr. R. J. Nash*
Institute of Grassland and Environmental Research
Plas Gogerddan, Aberystwyth SY23 3EB, Dyfed, Wales (UK)

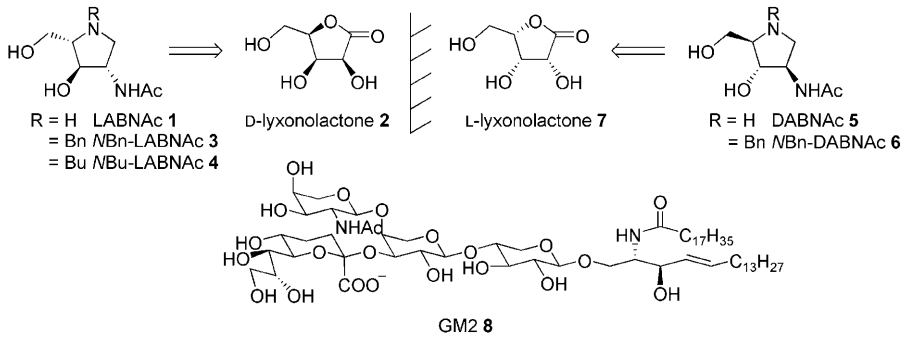


Figure 1. The GM2 ganglioside **8** and prepared *N*-acetylhexosaminidase inhibitors.

alent than TSD and is caused by mutations in the *HEXB* gene, which encodes the β subunit of the enzyme, resulting in a deficiency in both β-hexosaminidase A (αβ) and β-hexosaminidase B (ββ) activities.[6] In contrast to TSD, there is no ethnic group that has shown a predominantly high incidence of Sandhoff disease, although a few geographic isolates have been found to possess a high disease incidence. One study has shown that over a seven-year period, 36 cases of acute Sandhoff disease were diagnosed from 27 families, many of whom had ancestors from the same northwestern region of the province of Cordoba in Argentina.[7] Currently, there are no approved treatments for any of the GSL storage disorders that are characterised by deficiencies in lysosomal *N*-acetylhexosaminidases. Enzyme-replacement therapy (ERT) usually involves biweekly infusions of the deficient enzymes, and although it has been approved in the US for some other GSL storage disorders, including type 1 Gaucher and Fabry disease, it suffers from the difficulty of delivering the enzyme across the blood–brain barrier and is very expensive (US$ 150 000–200 000 per patient annually).[8] Substrate-reduction therapy is another approach that involves decreasing the levels of substrate for the deficient enzyme by using compounds that inhibit ceramide glucosyltransferase, which is the first enzyme in the biosynthetic pathway for the glycosylation of sphingolipids.[9] After clinical trials, the *N*-(*n*-butyl) analogue of 1-deoxynojirimycin (DNJ) **10**, *N*B-DNJ (Zavesca®) **11** was approved for use in Europe, Israel, and the US for the treatment of mild to moderate type 1 Gaucher disease (Figure 2).[10] One promising therapy, which initially showed potential for Fabry disease, involves the use of the *galacto*-analogue of DNJ, 1-deoxygalactonojirimycin (DGJ, Amigal™) **12**, a competitive inhibitor of the deficient enzyme α-galactosidase A, as a chemical chaperone.[11]

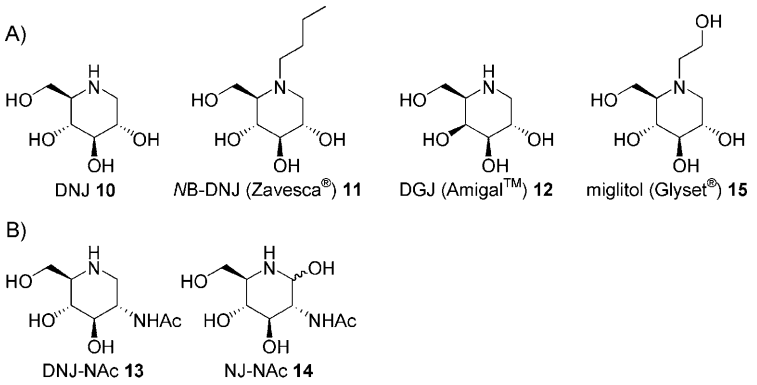


Figure 2. A) DNJ and some current approved iminosugar therapeutics; B) 2-acetamido-iminosugars used as chemical chaperones for TSD and SD.

Chemical chaperone therapy has been reviewed extensively in some recent articles.[12] It is thought that inherited genetic defects cause incorrect folding of the protein precursors of lysosomal enzymes, which results in their retention within the endoplasmic reticulum (ER).[13] This "quality control" mechanism prevents transport to the Golgi apparatus for further maturation and results in ER-associated degradation and consequently lysosomal deficiency. Competitive inhibitors bind to the active site of the misfolded enzyme and increase the chance of correct folding, enabling more of the mutant enzyme to proceed to the Golgi.[12a] Once the mutant enzyme–inhibitor complex reaches the lysosome, the high concentration of accumulated substrate will replace the inhibitor and allow the enzyme to function normally. At higher concentrations, potent inhibitors will act as an inhibitor of the enzyme; therefore, for enzyme enhancement, sub-inhibitory concentrations of inhibitors are required. Because only modest increases of the deficient enzyme activities are usually required to prevent accumulation of lysosomal GSLs, this therapy is highly suitable for the treatment of GSL storage disorders. Recently, Tropak et al. reported that both adult Tay-Sachs and Sandhoff fibroblasts grown in culture medium containing some β-hexosaminidase inhibitors (including the iminosugars DNJ-NAc **13** and NJ-NAc **14**) showed elevated β-hexosaminidase activity above critical levels.[14] Hence, more specific and potent inhibitors of human lysosomal β-hexosaminidase are needed to further investigate the potential of this therapy for the treatment of TSD and SD.

Iminosugars (or azasugars) are sugar analogues in which the ring oxygen has been replaced by a nitrogen atom. Over 100 of these compounds are naturally occurring alkaloids that have been isolated from plants and microorganisms, but many have also been produced synthetically.[15] Their ability to inhibit glycosidases that are involved in many important biological processes has made them ideal candidates for use as mechanistic probes and therapeutic agents for numerous diseases.[15,16] Recent studies suggest that iminosugars play an important role as major components in many of the traditional remedies in Chinese, Japanese, and Thai medicines for various ailments and diseases.[17] There are several glycosidase inhibitors that are currently in clinical trials or that have been approved for the treatment of diabetes,[18] Gaucher's disease,[19] HIV infec-

tion,[20] other viral infections,[21] or cancer.[22] DNJ-derived miglitol **15** (Glyset®) has been approved for the treatment of type 2 diabetes.[18] *N*B-DNJ **11** (Zavesca®) has been approved as a therapeutic agent for patients with type 1 Gaucher disease.[10] DGJ (Amigal™) **12** is currently in clinical trials for the treatment of Fabry disease.[11] Phase 2 clinical trials of DGJ **12** have been completed recently, and positive results were announced. Therefore, iminosugars and derivatives were chosen as our desired target compounds for the inhibition of *N*-acetylhexosaminidases, specifically DABNAc **5** and LABNAc **1**, the syntheses and preliminary biological data for which were described in a recent communication.[23]

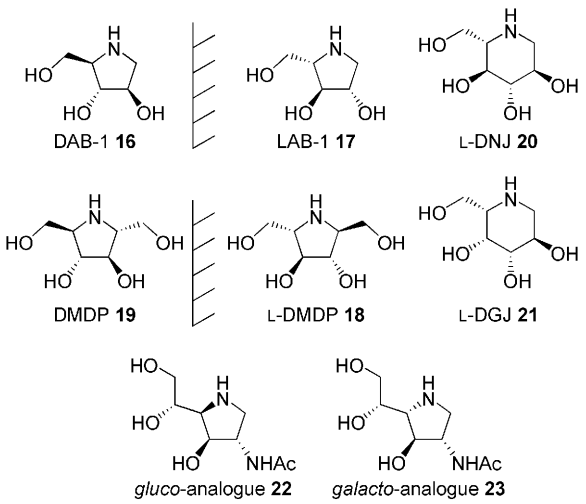


Figure 3. Various naturally occurring (**16** and **19**) and unnatural iminosugars (**17**, **18**, **20**, **21**), along with the 2-acetamidoiminofuranose analogues of glucose and galactose (**22** and **23**).

Results and Discussion

Design

The 2-acetamido analogue of DNJ, 2-acetamido-1,2-dideoxy-1,5-imino-D-glucitol (DNJ-NAc) **13**, is a good inhibitor of several hexosaminidases.[24] It is thought that this observation may be related to the fact that DNJ **10** itself is an excellent inhibitor of several α-glucosidases.[25] These inhibitors are structurally very similar to the substrate that the corresponding enzyme cleaves, and as the nitrogen atom in the ring is protonated at physiological pH, this also resembles the glycosyl cation that is considered to develop during hydrolysis of the glycosidic bond.[26] In this respect, these compounds may be considered both partially substrate-based and partially transition-state-based inhibitors, as they can be rationalised as either. There are several other hexosaminidase inhibitors that exhibit good inhibitory activity, but they are mostly based on iminopyranose sugar mimics and contain either an acetamido group at C2 or some other analogous functionality.[24,27] Due to the excellent inhibition of α-glucosidases by the naturally occurring iminofuranose DAB-1 **16** and its synthetic enantiomer LAB-1 **17**,[28] it was anticipated that the 2-acetamido analogues of these compounds could be potent inhibitors of hexosaminidases (Figure 3). Interestingly, the L-iminosugar LAB-1 **17** is a much more potent and selective inhibitor of α-glucosidases than the D-iminosugar DAB-1 **16**.[28b] Similarly, another unnatural L-iminofuranose, L-DMDP **18**, was found to be a more powerful and specific inhibitor of α-glucosidase than its naturally occurring D-iminosugar enantiomer DMDP **19**.[29] In both these cases, the L-iminosugars were noncompetitive inhibitors relative to the D-iminosugars that were competitive inhibitors. In another study, the L-iminosugar enantiomers of DNJ **10** and DGJ **12**, L-DNJ **20** and L-DGJ **21**, were synthesised and confirmed to be noncompetitive inhibitors of α-glucosidase and α-galactosidase, respectively.[30] These collective results led to the hypothesis that whereas D-iminosugar mimics inhibit D-glycohydrolases competitively, their L-enantiomers show noncompetitive inhibition.[31] Our target compounds were chosen as the enantiomeric 2-acetamido analogues of DAB-1 and LAB-1, DABNAc **5** and LABNAc **1**. The only other 2-acetamido-1,4-iminofuranoses known are the pyrrolidine analogues of glucose **22**[32] and galactose **23**.[33] These compounds, containing one more methylene group than our target compounds, were found to be relatively weak inhibitors of *N*-acetylhexosaminidases.

Synthesis

The methodology chosen for the synthesis of the pyrrolidine ring was based on a double S_N2 displacement of suitable leaving groups at C1 and C4 of a suitable sugar backbone by benzylamine to give a convenient *N*-benzyl-protected iminosugar. This type of synthetic procedure has been used successfully to prepare several other iminosugar targets.[34] Excellent starting materials for such syntheses are the sugar lactones, which are generally inexpensive and readily available or accessible from other sugars in a few steps.[35] Their advantage over normal sugars is that the oxygen atom at C1 does not need to be protected, and therefore anomerisation problems are avoided.

The initial retrosynthetic analysis for target compound DABNAc **5** is shown in Figure 4. Acetamide **6** was expected to be synthesised from azide **24** via an intermediate amine. The azide functionality is relatively stable and therefore may be introduced at an early stage of the synthetic route via S_N2 displacement of a suitable triflate. The pyrrolidine ring of azide **24** was thought to be accessible by one of the two routes shown in Figure 4; however, cyclisation to the *trans*-fused bicyclic compound **25** from dimesylate **26** may be thermodynamically disfavoured due to strain in its ring system (Route A). Therefore, it was anticipated that the pyrrolidine would have to be synthesised from the deprotected dimesylate **27** (Route B). Dimesylate **26** was expected to be accessible from lactone **28** via diol **29**. Azidolactone **28** was known to be available from hydroxylactone **30** via reaction of triflate **31** with sodium azide under kinetically controlled reaction conditions based on previous experience with the enantiomeric compounds.[36] Working back from the target compound **5**, the required starting material is of L-*lyxo* stereochemistry following inversions of configuration at C2 and C4. The 2,3-isopropyli-

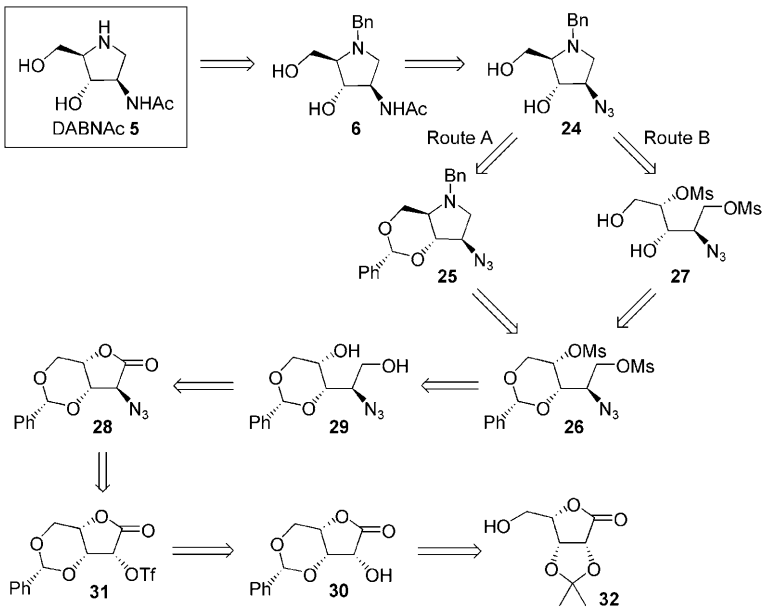
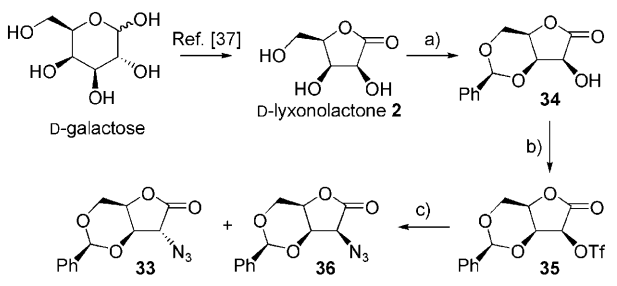


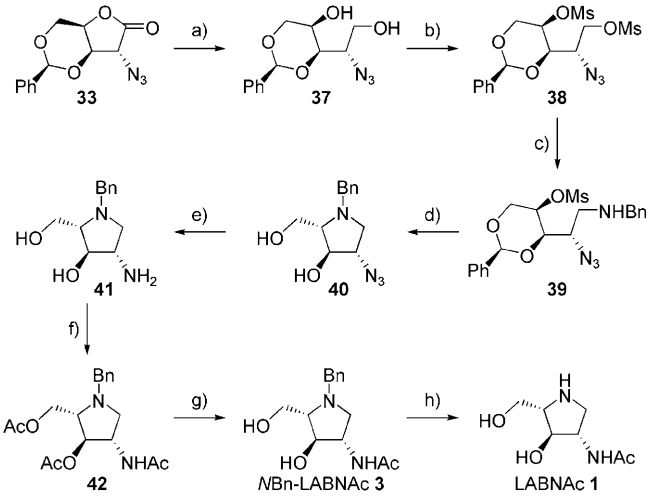
Figure 4. Retrosynthetic analysis of the DABNAc target.

dene-protected lactone **32** was chosen rather than the unprotected sugar lactone owing to its better availability (as the precursor in the synthesis of L-lyxonolactone **7**).[37] The conversion of 2,3-isopropylidene acetal **32** to 3,5-benzylidene acetal **30** is known to occur in excellent yield, and the subsequent triflation and azide displacement have also been described.[36] However, because we wanted to prepare both enantiomers of the target compound, we decided to develop the synthesis initially for LABNAc **1** using the more readily available D-lyxonolactone **2**, which can be obtained by oxygenation of an alkaline solution of D-galactose.[38] The required 3,5-benzylidene-protected azidolactone **33** of *xylo* configuration had been synthesised previously (Scheme 1). 3,5-Benzylidene-D-lyxonolactone **34** was prepared in excellent yield (90%) using benzaldehyde and hydrochloric acid.[39,28d] Triflation was achieved by using triflic anhydride in pyridine to give triflate **35** in quantitative yield.[40] Previous work had shown that depending on the reaction conditions employed during the azidation step, either the *xylo*-azidolactone **33** or the *lyxo*-azidolactone **36** could be obtained in high yields.[36] Under thermodynamic control [using excess sodium azide (2.5 equiv) and a longer reaction time (20 h)] and in the presence of pyridinium *p*-toluenesulfonate (to buffer the basicity of the excess azide), the *lyxo*-azide **36** with overall retention of configuration at C2 can be formed in excellent yield. However, for the present purposes, kinetic control was employed using 0.97 equivalents of sodium azide and a shorter reaction time (60 min). Under these conditions, the required *xylo*-azidolactone **33** was prepared in excellent yield (83%), along with a small amount of the *lyxo*-epimer **36** (9%).



Scheme 1. *Reagents and conditions*: a) PhCHO, HCl (concd), room temperature, 24 h, 90%; b) Tf_2O, pyridine, −30 °C, 3 h, 100%; c) NaN_3, DMF, room temperature, 60 min, 83% for **33**, 9% for **36**.

Reduction of the lactone **33** to the corresponding diol **37** was achieved using lithium borohydride in THF, resulting in near quantitative yield (97%; Scheme 2). The diol **37** was converted easily to the dimesylate **38** by using mesyl chloride and triethylamine in CH_2Cl_2 (81%). Subsequent reaction of the dimesylate **38** with benzylamine at 95 °C resulted in only single displacement to give the monomesylate compound **39** in good yield (81%). This observation confirmed our initial thoughts on the unfavourability of the *trans*-fused bicyclic compound **25**. Amberlyst® 15 acidic resin was used to hydrolyse the 3,5-benzylidene acetal in aqueous dioxane at 85 °C. The



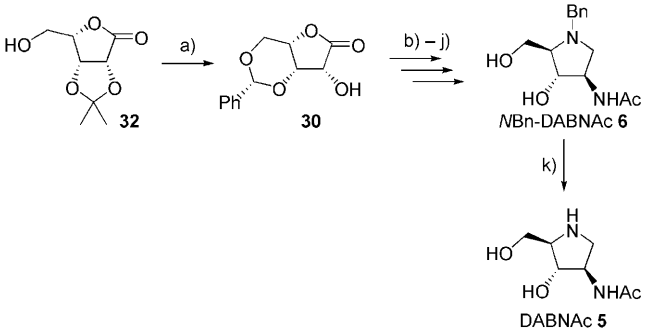
Scheme 2. *Reagents and conditions*: a) $LiBH_4$, THF, −30 °C, 3 h, 97%; b) MsCl, Et_3N, CH_2Cl_2, 0 °C, 2.5 h, 81%; c) $BnNH_2$, 95 °C, 14 h, 81%; d) Amberlyst® 15 beads, 1,4-dioxane/H_2O (50% *v/v*), 85 °C, 20 h, 89%; e) H_2/Pd-C (10% wt), THF, room temperature, 3 h, 100%; f) Ac_2O, pyridine, room temperature, 16 h, 73%; g) NaOMe, MeOH, room temperature, 1 h, 84%; h) H_2/Pd black, 1,4-dioxane, H_2O, room temperature, 20 h, 100%.

product of this reaction was the cyclised pyrrolidine **40**, obtained in excellent yield (89%). Intramolecular ring closure had occurred spontaneously after hydrolysis of the benzylidene, most likely due to the increased flexibility of the compound, as well as the basicity of the reaction mixture due to the presence of the amino group in the compound (autocatalysis), and the use of solid acid did not buffer this effect. The azide group of **40** was reduced selectively in the presence of the *N*-benzyl group by using hydrogen and 10% palladium on carbon in THF to give the aminodiol **41** in quantitative yield.

Subsequent acetylation using acetic anhydride and pyridine resulted in the per-*O*-acetylated compound **42** in good yield

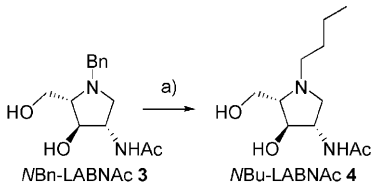
(75%). De-*O*-acetylation using NaOMe in methanol gave the *N*-benzyl-protected LABNAc precursor *N*Bn-LABNAc **3** in excellent yield (84%). This compound was crystallised from acetonitrile by competitive diffusion with cyclohexane, and the resulting crystals were proven to be of the correct stereochemistry by X-ray crystallography.[41] Finally, hydrogenation in aqueous dioxane using palladium black afforded LABNAc **1** in quantitative yield. The overall yield of LABNAc **1** from D-lyxonolactone was 26% over 11 steps.

Because a robust and high-yielding synthesis of LABNAc had been achieved, the same synthetic steps were applied for the synthesis of DABNAc **5** from L-lyxonolactone. In this case, the 2,3-isopropylidene-protected material was used as the starting material, as it is an intermediate in the syntheses of L-lyxonolactone from either D-gulonolactone[42] or D-ribose[37] and may be subjected to transacetylation to the 3,5-benzylidene in high yields. In fact, under the same conditions used for the benzylidenation of the unprotected D-lyxonolactone to give **34**, the 2,3-isopropylidene acetal **32** was converted into the 3,5-benzylidene acetal **30** in excellent yield using benzaldehyde and hydrochloric acid (88%). Each of the subsequent steps was repeated using the same procedures that were used for the corresponding enantiomeric steps to obtain DABNAc **5** in 22% yield over the 11 steps (Scheme 3).



Scheme 3. *Reagents and conditions*: a) PhCHO, HCl (concd), room temperature, 24 h, 95%; b) Tf_2O, pyridine, −30 °C, 3 h, 100%; c) NaN_3, DMF, room temperature, 50 min, 78% for *xylo*-azide, 14% for *lyxo*-azide; d) $LiBH_4$, THF, −30 °C, 3 h, 79%; e) MsCl, Et_3N, CH_2Cl_2, 0 °C, 4 h, 90%, f) $BnNH_2$, 1,4-dioxane, 95 °C, 5 days, 75%; g) Amberlyst® 15 beads, 1,4-dioxane/H_2O (50% *v/v*), 85 °C, 8 h, 75%; h) H_2/Pd-C (10% wt), THF, room temperature, 3 h, 89%; i) Ac_2O, pyridine, room temperature, 16 h, 90%; j) NaOMe, MeOH, room temperature, 3 h, 89%; k) H_2/Pd black, 1,4-dioxane, H_2O, room temperature, 20 h, 100%.

The *N*-butyl analogue of LABNAc **1**, *N*Bu-LABNAc **4**, was prepared directly from the *N*-benzyl compound **3** in one step by using hydrogen in the presence of palladium black and an excess of butyraldehyde (Scheme 4). When these conditions were applied for the conversion of *N*Bn-LABNAc **3** into *N*Bu-LABNAc **4**, the reaction proceeded smoothly and furnished the *N*-butyl compound in excellent yield (87%). The *N*-benzylated pyrrolidine structures **3** and **6** are relatively stable and have been shown to be accessible in high-yielding steps from sugar lactones. The use of an excess of aldehyde in the reductive transamination of **3** and **6** (such as in the preparation of **4**), provides excellent potential for the synthesis of various *N*-alkylated analogues.



Scheme 4. *Reagents and conditions*: a) H_2/Pd black, butyraldehyde, 1,4-dioxane, H_2O, 16 h, 87%.

Biological evaluation

In the initial biological evaluation, LABNAc **1**, DABNAc **5**, and their *N*-benzyl precursors were screened for inhibitory activity against various glycosidases including some hexosaminidases. No significant inhibitory activity (i.e. <50% inhibition at an inhibitor concentration of 1 mM) was observed against a variety of *p*-nitrophenylglycopyranoside (PNP-glycopyranoside) substrates for the corresponding glycosidases of various α- and β-processing enzymes (Table 1).

*N*Bn-DABNAc **6** and DABNAc **5** exhibited weak inhibition toward some *N*-acetylhexosaminidases, but interestingly, *N*Bn-LABNAc **3** and LABNAc **1** were found to be quite potent inhibitors of these enzymes, with IC_{50} values in the micromolar to sub-micromolar range. None of the compounds showed inhibition toward α-*N*-acetylgalactosaminidases, suggesting specific inhibition of β-hexosaminidase. The K_i values give a similar view of the inhibitory activities of these compounds against β-*N*-acetylhexosaminidases (Table 2). *N*Bn-DABNAc **6** appears to be a relatively potent inhibitor of the bovine kidney form (K_i = 16.9 μM). This result is interesting, as **6** is only a weak inhibitor of the plant and human forms of this enzyme and also because DABNAc **5** is a poor inhibitor of this enzyme in general. LABNAc **1** appears to bind with slightly higher affinity than *N*Bn-LABNAc **3** for the jack bean and bovine kidney enzyme forms. For the human placenta form, *N*Bn-LABNAc **3** (K_i = 3.7 μM) appears to be a better inhibitor than LABNAc **1** (K_i = 15 μM). These values were compared with that for a known inhibitor of this enzyme, DNJ-NAc **13**, the inhibition constant for which (K_i = 9.5 μM) was obtained for the same enzyme in the same study. *N*Bn-LABNAc **3** appears to inhibit human placenta β-*N*-acetylhexosaminidase 2.5-fold better than DNJ-NAc **13**, confirming that *N*Bn-LABNAc **3** is a potent inhibitor. LABNAc **1** appears to be 1.5-fold weaker than DNJ-NAc **13**, but is still a potent inhibitor. Relative to their known hexofuranose analogue, 2-acetamido-1,4-iminogalactitol **23**,[33] *N*Bn-LABNAc **3** and LABNAc **1** appear to be much better inhibitors of human β-hexosaminidases.[43]

The *N*-butyl analogue of LABNAc **1**, *N*Bu-LABNAc **4** (the synthesis of which is described above) was prepared subsequent to the confirmation of LABNAc **1** and *N*Bn-LABNAc **3** as potent and selective inhibitors of β-*N*-acetylhexosaminidases. Because the *N*-butyl analogue of DNJ had proved a useful modification for the delivery of DNJ into cells, it was thought that the increased lipophilicity of such a compound could have potential for the inhibition of cellular β-*N*-acetylhexosaminidases.[44] The IC_{50} and K_i values were obtained for *N*Bu-LABNAc **4** using jack

Table 1. Inhibition of various glycosidases by *N*Bn-LABNAc **3**, LABNAc **1**, *N*Bn-DABNAc **6**, and DABNAc **5**.

Enzyme and Source	IC_{50} [μM]			
	3	**1**	**6**	**5**
α-D-glucosidase:				
yeast[a]	–[d]	–[d]	–[d]	–[d]
Bacillus stearothermophilus[b]	–[e,g]	–[e,h]	–[e,g]	–[e,h]
rice (*Oryzae sativa*)[b]	–[e,g]	–[e,h]	–[e,g]	–[e,h]
rat intestinal maltase[c]	–[d]	–[d]	–[d]	–[d]
rat intestinal isomaltase[c]	–[d]	–[d]	–[d]	–[d]
β-D-glucosidase:				
almond[a]	–[d]	–[d]	–[d]	–[d]
human placenta[c]	–[d]	–[d]	–[d]	–[d]
α-D-mannosidase:				
jack bean[a]	–[d]	–[d]	–[d]	–[d]
β-D-mannosidase:				
snail[a]	–[d]	–[d]	–[d]	–[d]
α-D-galactosidase:				
coffee bean[a]	–[d]	–[d]	–[d]	–[d]
green coffee bean[b]	–[e,g]	–[e,h]	(41)[f,g]	–[e,h]
β-D-galactosidase:				
jack bean[a]	–[d]	–[d]	–[d]	–[d]
bovine liver[b]	–[e,g]	–[e,h]	–[e,g]	–[e,h]
α-L-fucosidase:				
bovine[a]	–[d]	–[d]	–[d]	–[d]
β-D-xylosidase:				
Aspergillus niger[a]	–[d]	–[d]	–[d]	–[d]
amyloglucosidase:				
Aspergillus niger[b]	–[e,g]	–[e,h]	–[e,g]	–[e,h]
glycogen phosphorylase b:				
rabbit muscle[c]	–[d]	–[d]	–[d]	–[d]
naringinase:				
Penecillium decumbens[b]	–[e,g]	–[e,h]	–[e,g]	–[e,h]
β-*N*-acetyl-D-hexosaminidase:				
jack bean[a]	5.2	3.4	446	–[d]
bovine kidney[b]	0.36	0.64	41.2	326
human placenta[c]	2.8	13	320	(46)[i]
Aspergillus oryzae[b]	(40)[f,g]	(37)[f,h]	(32)[f,g]	–[e,h]
α-*N*-acetyl-D-galactosaminidase:				
Charonia lampas[a]	–[d]	–[d]	–[d]	–[d]
human lysosomal[c]	–[d]	–[d]	–[d]	–[d]

–: No significant inhibition observed under the corresponding experimental conditions. [a] Carried out by J.S.S.R. and T.D.B. [b] Carried out by E.L.E. and R.J.N. [c] Carried out by K.I. and N.A. [d] Less than 30% inhibition at 1000 μM. [e] Less than 10% inhibition at the corresponding concentration. [f] Inhibition (%) at the corresponding concentration. [g] Inhibitor concentration: 540 μM. [h] Inhibitor concentration: 820 μM. [i] Inhibitor concentration: 1000 μM.

bean β-*N*-acetylhexosaminidase (Table 3). These values are very similar to those obtained for LABNAc **1** and show that the presence of the alkyl chain does not diminish activity against the enzyme. In contrast, the *N*-benzyl analogue **3** was found to be a slightly worse inhibitor than LABNAc **1** and *N*Bu-LABNAc **4**. The presence of the alkyl chain may improve cellular uptake, and therefore *N*Bu-LABNAc **4** is likely to have an improved pharmacological profile relative to LABNAc **1** and may be a suitable alternative for cell-based studies.

Table 2. Inhibitory activity of *N*Bn-LABNAc **3**, LABNAc **1**, *N*Bn-DABNAc **6**, and DABNAc **5** against some β-*N*-acetyl-D-hexosaminidases.

Enzyme and Source	K_i [μM]			
	3	**1**	**6**	**5**
β-*N*-acetyl-D-hexosaminidase:				
jack bean[a]	3.4†, 8.7‡	1.9†, 6.5‡	ND[d]	ND[d]
bovine kidney[b]	0.28	0.095	16.9	104
human placenta[c]	3.7	15	180	ND[d]

[a] Carried out independently by †J.S.S.R. and T.D.B. and ‡K.I. and N.A. [b] Carried out by E.L.E. and R.J.N. [c] Carried out by K.I. and N.A. [d] Not determined due to weak inhibition.

Table 3. Inhibition of jack bean β-*N*-acetyl-D-hexosaminidase by *N*Bu-LABNAc **4**.

Enzyme	*N*Bu-LABNAc **4**	
	IC_{50} [μM]	K_i [μM]
β-*N*-acetyl-D-hexosaminidase:		
jack bean[a]	3.9	1.9

[a] Carried out by J.S.S.R. and T.D.B.

Kinetic analysis of β-N-acetylhexosaminidase inhibitors

The kinetic analyses of these inhibitors provided some interesting results about their modes of inhibition. *N*Bn-LABNAc **3** and LABNAc **1** appear to be noncompetitive inhibitors of both jack bean β-*N*-acetylhexosaminidase and human placenta β-*N*-acetylhexosaminidase by analysis of the Lineweaver–Burk plots of the data obtained to determine the inhibition constant (K_i) (Figure 5 A–D). In each case, a further analysis of the data using Cornish–Bowden plots revealed the presence of some mixed type of inhibition (results not shown). In contrast, DNJ-NAc **13** (Figure 5 E) and *N*Bn-DABNAc **6** (Figure 5 F) were found to inhibit the enzyme in a competitive manner. The noncompetitive character of the inhibitors *N*Bn-LABNAc **3** and LABNAc **1** appears to suggest similarity with LAB-1 **17** and L-DMDP **18**; that is, they are better noncompetitive inhibitors than their corresponding enantiomers that are competitive inhibitors, although it is possible that they act in a different way due to the mixed type of inhibition.

Noncompetitive inhibition for compounds such as *N*Bn-LABNAc **3** can sometimes be explained by the interaction of a hydrophobic moiety such as the *N*-benzyl group with a lipophilic binding pocket at a binding site other than the active site (regulatory site). We might have expected LABNAc **1** to act in a noncompetitive manner in the same way that LAB-1 **17** inhibits α-glucosidases. One major difference, however, is that DABNAc **5** does not appear to be a significant inhibitor of β-*N*-

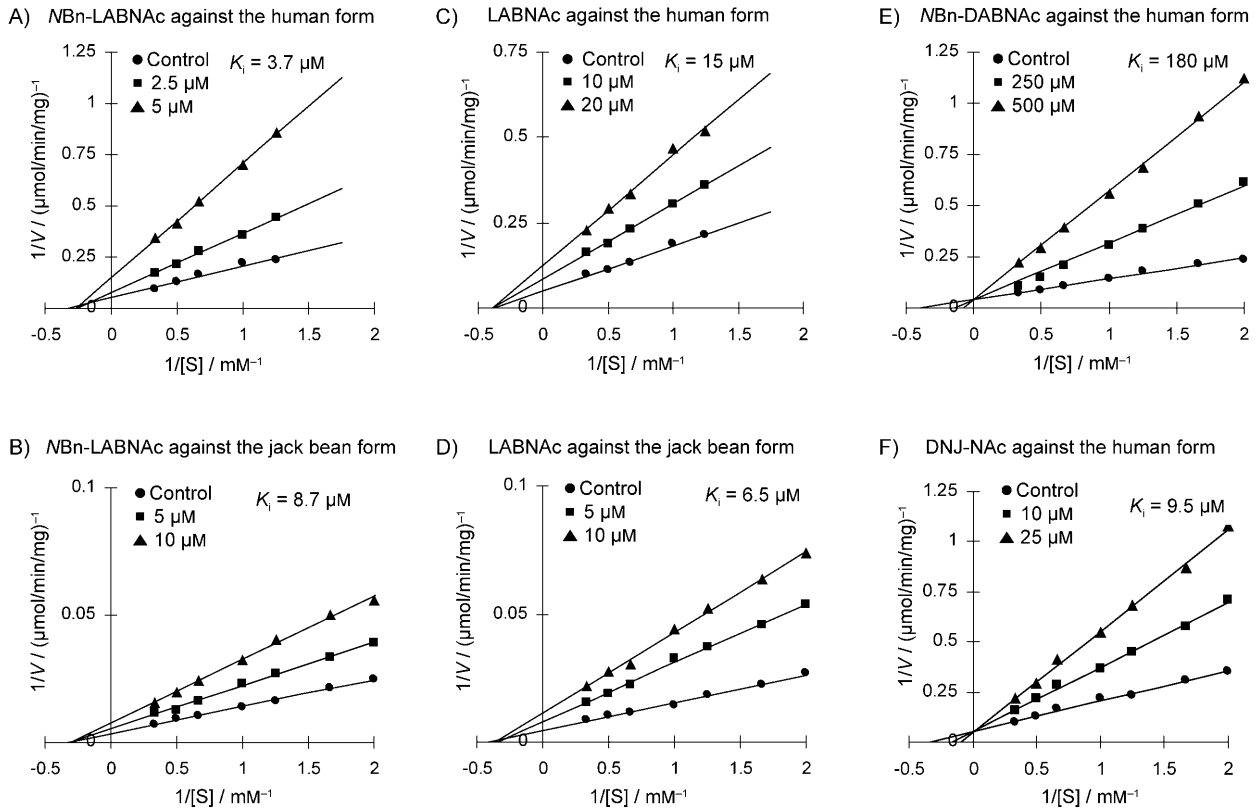


Figure 5. Lineweaver–Burk plots: A) *N*Bn-LABNAc **3** against human placenta β-*N*-acetylhexosaminidase, B) *N*Bn-LABNAc **3** against jack bean β-*N*-acetylhexosaminidase, C) LABNAc **1** against human placenta β-*N*-acetylhexosaminidase, D) LABNAc **1** against jack bean β-*N*-acetylhexosaminidase, E) DNJ-NAc **13** against human placenta β-*N*-acetylhexosaminidase, and F) *N*Bn-DABNAc **6** against human placenta β-*N*-acetylhexosaminidase. (Work carried out by K.I. and N.A.)

acetylhexosaminidases, whereas DAB-1 **16** is a significant competitive inhibitor of α-glucosidases.

Molecular modelling

A molecular model of the structure of LABNAc **1** was constructed to visualise how these compounds might bind to β-*N*-acetylhexosaminidases. Initially, a molecular model of LABNAc **1** was generated from the ^{1}H NMR data of *N*Bn-LABNAc **3** in water at pH 4.2. The orientation of the *N*-acetyl group relative to the ring was the major ambiguity that needed to be solved. The NHAc–H-2 coupling constant of 6.6 Hz is consistent with an H-*N*–H-2 torsion angle of around either $\pm 30^\circ$ or $\pm 145^\circ$. However, the incidence of a nuclear Overhauser effect from NHAc to H-3 is only consistent with a torsion angle of $\pm 145^\circ$. The equivalent torsion angle for GlcNAc (NHAc–H-2) has been shown to be approximately 180°.[45]

Molecular models were generated for β-D-GlcNAc, LABNAc **1**, DABNAc **5**, and DNJ-NAc **13**. The overlay of β-D-GlcNAc with DNJ-NAc **13** is almost perfect, differing only at the anomeric position and endocyclic heteroatom for β-D-GlcNAc. The ring nitrogen of DNJ-NAc **13** overlays perfectly with the ring oxygen of β-D-GlcNAc, which is consistent with the DNJ-NAc protonated nitrogen atom mimicking the partial positive charge developed on the β-D-GlcNAc ring oxygen during the transition state of the glycosidase mechanism. LABNAc **1** and DABNAc **5** were overlayed with β-D-GlcNAc based on the *N*-acetyl groups (Figure 6).

The DABNAc **5** C3-OH group overlays with β-D-GlcNAc C1-OH. However, this match would be poor for the α-anomer, suggesting that any inhibitory activity of DABNAc **5** would be greater for enzymes specific to β rather than α linkages. The DABNAc **5** ring nitrogen does not overlay with the β-D-GlcNAc ring oxygen, and the DABNAc **5** C5-OH group occupies space that would be expected to be occupied by enzyme amino acid side chains that interact with the β-D-GlcNAc ring oxygen atom. Thus, DABNAc **5** is predicted to be a poor transition-state mimic of β-GlcNAc or β-GalNAc type substrates for β-*N*-acetylhexosaminidases.

The LABNAc **1** C3-OH group overlays well with β-D-GlcNAc C3-OH. The ring nitrogen of the iminosugar overlays well with the β-D-GlcNAc ring oxygen atom, suggesting that it would be a good transition-state mimic. As LABNAc **1** is 1-deoxy, it has no substituent at C1 that could overlay with the β-D-GlcNAc C1-OH group in either the α or β positions and therefore is not likely to show any preference between enzymes specific for α or β linkages. The LABNAc **1** C5-OH group occupies space where it may be able to hydrogen bond with enzyme groups that would normally interact with either GlcNAc C4-OH or C6-OH. However, the LABNAc **1** C5-OH group would not be able to interact with enzyme groups specific for C4-OH of GalNAc. Thus, LABNAc **1** was predicted to be a good transition-state mimic for both α- and β-*N*-acetylhexosaminidases, and possi-

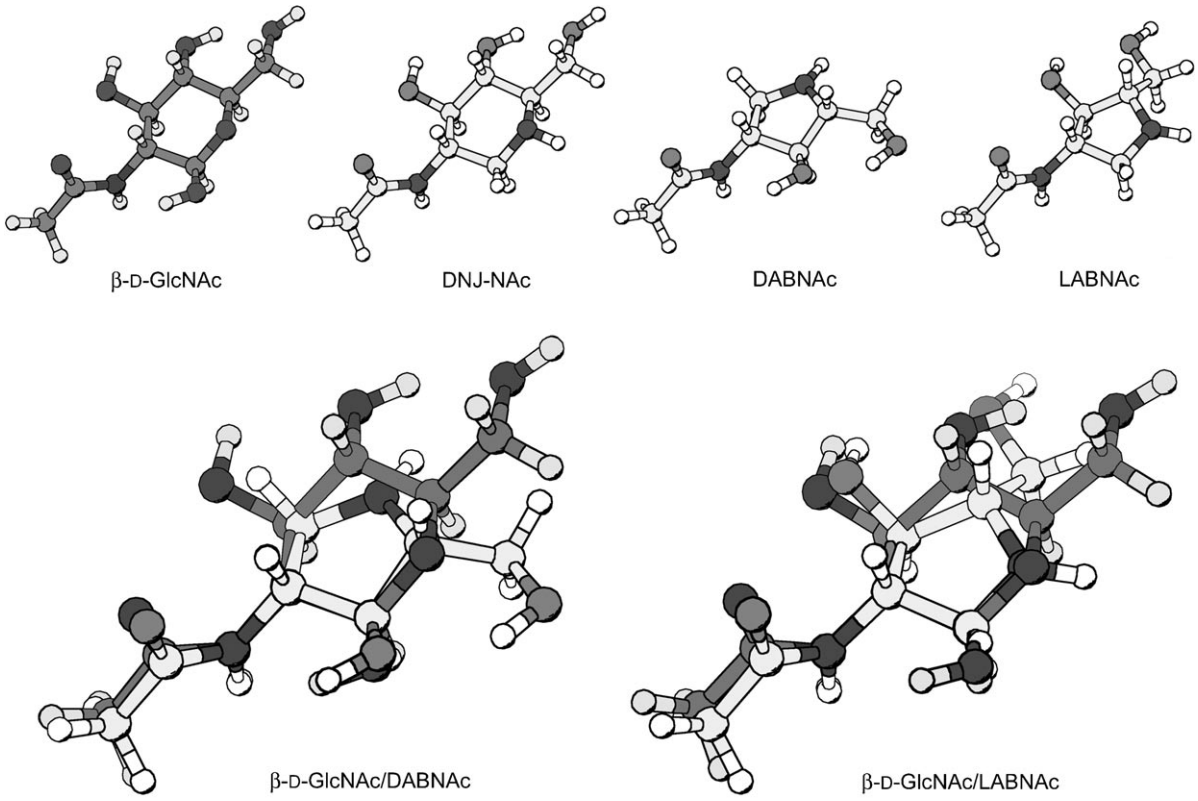


Figure 6. The overlay of DABNAc **5** and LABNAc **1** with β-D-GlcNAc (produced by M.R.W. using Insight II and Discover software, Accelrys Inc.).

bly a better inhibitor of GlcNAc-specific than GalNAc-specific enzymes.

Evaluation of the enzyme kinetics and molecular modelling

DABNAc **5** was predicted to be a poor inhibitor of β-*N*-acetylhexosaminidases due to its poor overlay with β-D-GlcNAc. The enzyme inhibitory data support this prediction, exhibiting only very weak interactions with the enzyme. LABNAc **1**, which was predicted to be a good inhibitor of β-*N*-acetylhexosaminidases, was found to be a potent inhibitor for this enzyme. Because LABNAc **1** and *N*Bn-LABNAc **3** mimic β-D-GlcNAc, it might have been expected that the mode of inhibition would be competitive, but kinetic analysis by Lineweaver–Burk plots implies non-competitive inhibition. However, Cornish–Bowden plots suggest some mixed type inhibition, and therefore the good overlay with β-D-GlcNAc suggests that even though LABNAc **1** and *N*Bn-LABNAc **3** may prefer to bind at the regulatory site (non-competitive inhibition), they can also interact with the binding site (competitive inhibition), leading to this observation.

Evaluation of N*Bn-LABNAc as a chemical chaperone*

The cell line GM3461, obtained from a Tay-Sachs patient with the adult-onset form of the disease, was used for the delineation of the potential chaperoning effects of *N*Bn-LABNAc **3** on the α subunit. This cell line is heterozygous for the *HEXA* gene and therefore may be used as a cellular model for adult-onset Tay-Sachs disease. The cells were grown in the presence or absence of up to 50 μM *N*Bn-LABNAc **3**, and hexosaminidase activity was assayed with 3.2 mM fluorescently tagged artificial substrates, 4-methylumbelliferyl-β-*N*-acetylglucosamine (MUG) or 4-methylumbelliferyl-β-*N*-acetylglucosamine-6-sulfate (MUGS) after 5 days. The use of MUG gives an estimate of the total β-hexosaminidase (A, B and S) activity, whereas MUGS gives an estimate of β-*N*-acetylhexosaminidase A and/or β-*N*-acetylhexosaminidase S activity.[46] *N*Bn-LABNAc **3** at a concentration of 50 μM showed a twofold increase in β-hexosaminidase activity using MUG; at 5 μM it resulted in a similar increase using MUGS (Figure 7). These data support the enhancement of β-*N*-acetylhexosaminidase B activity at higher concentrations of inhibitor, whereas β-*N*-acetylhexosaminidase A and/or β-*N*-acetylhexosaminidase S activity are increased at much lower concentrations. Hence, 2-acetamidoiminofuranose inhibitors of β-hexosaminidases, such as *N*Bn-LABNAc **3**, have been shown to be effective in raising β-hexosaminidase activity to levels that might be of therapeutic value to adult TSD and SD patients.

Conclusions

LABNAc **1**, the 2-acetamido analogue of unnatural compound LAB-1 **17**, was synthesised from D-lyxonolactone in a convenient 11-step procedure in 26% yield. The enantiomeric compound, DABNAc **5**, the 2-acetamido analogue of naturally occurring compound DAB-1 **16**, was prepared in a similar fashion

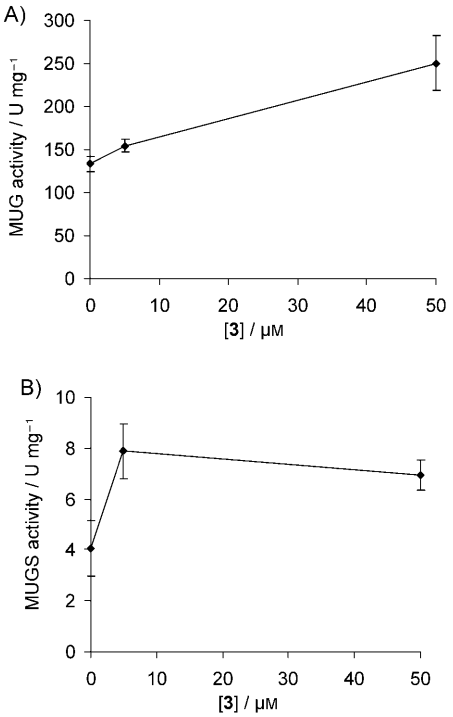


Figure 7. Enhancement of β-hexosaminidase activity when GM3461 cells were grown in the presence of 50 μM *N*Bn-LABNAc **3** over 5 days. The β-hexosaminidase activities, using A) MUG or B) MUGS substrates in the assay, are shown as the mean value ±SD. (Work carried out by S.D.B. and T.D.B.)

from L-lyxonolactone. Both of these pyrrolidine iminosugars and their *N*-benzyl precursors were tested for inhibitory activity against a wide range of glycosidases. Interestingly, LABNAc **1** and *N*Bn-LABNAc **3** were found to be potent and selective inhibitors of some β-*N*-acetylhexosaminidases, whereas their enantiomers, DABNAc **5** and *N*Bn-DABNAc **6**, were found to be much weaker inhibitors of the same enzymes. The *N*-butyl analogue of LABNAc **1**, *N*Bu-LABNAc **4**, was also prepared and found to be as good an inhibitor of β-*N*-acetylhexosaminidases as LABNAc **1** itself. Lineweaver–Burk plots suggest that LABNAc **1** and *N*Bn-LABNAc **3** are noncompetitive inhibitors, while DABNAc **5** and *N*Bn-DABNAc **6** are competitive inhibitors. This compares favourably with the L-iminosugars, LAB-1 **17** and L-DMDP **18**, which have been shown to be noncompetitive inhibitors of α-glucosidases, while their enantiomers, DAB-1 **16** and DMDP **19**, are competitive inhibitors of the same enzyme. However, Cornish–Bowden plots suggest some mixed type inhibition for LABNAc **1** and *N*Bn-LABNAc **3**. Molecular modelling studies show that LABNAc **1** overlays extremely well with β-D-GlcNAc (the usual substrate for these enzymes) which rationalises the possibility of interaction at the binding site and therefore the observation of some mixed type of inhibition. Some preliminary cellular data showed that *N*Bn-LABNAc **3** can raise β-hexosaminidase activity in cells of adult-onset TSD patients to levels that could reverse the progress of the disease. Our data support and compare favourably to those reported by Tropak et al., with which some other β-hexosaminidase inhibitors were shown to act as chemical chaperones.[14]

Due to their potent but relatively modest (sub-micromolar) inhibition of β-*N*-acetylhexosaminidases, it is possible that LABNAc **1**, *N*Bn-LABNAc **3**, and *N*Bu-LABNAc **4** could all be suitable agents to act as chemical chaperones (at sub-inhibitory concentrations) to enhance the deficient enzyme activity caused by protein misfolding in patients with adult-onset Tay-Sachs or Sandhoff diseases. If β-*N*-acetylhexosaminidase activity levels can be increased above the critical threshold, it may be possible to treat these diseases, or at least decrease their severity, using such compounds. Because *N*-butyl-DNJ (Zavesca®) **11**, is an approved drug for the treatment of type 1 Gaucher disease, it is possible that, by analogy, *N*Bu-LABNAc **4** may have an improved pharmacological profile relative to LABNAc **1** or *N*Bn-LABNAc **3** and therefore may be more suitable for potential clinical trials.

Experimental Section

General: *Solvents and reagents*: THF was distilled from sodium benzophenone ketyl or purchased dry from Aldrich in Sure/Seal™ bottles and recapped with Oxford Sure/Seal™ valve-caps. *N*,*N*-Dimethylformamide (DMF) and pyridine were purchased dry from Aldrich in Sure/Seal™ bottles and were recapped with Oxford Sure/Seal™ valve caps. Water was distilled. All other solvents were used as supplied (analytical or HPLC grade) without prior purification. Reactions performed under an atmosphere of nitrogen, argon, or hydrogen gas were maintained by an inflated balloon. Imidazole was recrystallised from dichloromethane. All other reagents were used as supplied without prior purification. *Chromatography*: Thin-layer chromatography (TLC) was performed on aluminium-backed sheets coated with 60 F_{254} silica. Sheets were developed by dipping in a solution of cerium(IV) sulfate (0.2% *w/v*) and ammonium molybdate (5%) in 2 M sulfuric acid and heating with a heat gun. Flash column chromatography was performed using Sorbosil® C_{60} 40/60 silica. Ion-exchange chromatography was performed using either Amberlite® CG-150 or Dowex® 50WX8–200, as stated. *NMR spectroscopy*: NMR spectra were recorded on a Bruker DQX 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) spectrometer at room temperature in the deuterated solvent stated. All chemical shifts (δ) are quoted in ppm. Coupling constants (^3J) are quoted in Hz. Residual signals from the solvents were used as an internal reference. The ^{13}C resonances were assigned using either DEPT or APT sequences or from ^{1}H–^{13}C HMQC and HMBC spectra. *Melting points*: Melting points were recorded on a Kofler hot block and are uncorrected. *Infrared spectroscopy*: IR spectra were recorded at room temperature on a PerkinElmer 1750 IR Fourier transform spectrophotometer or a Bruker Tensor 27 FTIR spectrometer, using thin films on NaCl or Ge plates, as stated, and were recorded over 16 or 32 scans at a resolution of 4 cm^{-1}. Only the characteristic peaks are quoted. *Mass spectrometry*: High-resolution mass spectra (HRMS) were recorded on a micromass Autospec 500 OAT spectrometer using the technique of chemical ionisation (CI), or on a Waters 2790-Micromass LCT mass spectrometer using the technique of electrospray ionisation (ESI). *Polarimetry*: Optical rotations were recorded on a PerkinElmer 241 polarimeter with a path length of 1 dm. Concentrations are quoted in g(100 mL)$^{-1}$. The wavelength at which the rotations were measured corresponds to the sodium D line (ca. 589 nm). *Elemental analysis*: Elemental analyses were performed by the microanalysis service of the Inorganic Chemistry Laboratory, University of Oxford.

Enzyme inhibition assays: (Typical experimental materials and methods used by J.S.S.R. and T.D.B., Oxford Glycobiology Institute.) All enzymes and corresponding PNP substrates were obtained from Sigma, apart from coffee bean α-D-galactosidase, jack bean β-D-galactosidase, α-D-mannosidase and β-*N*-acetyl-D-hexosaminidase, and *Charonia lampas* α-*N*-acetyl-D-galactosaminidase, which were purified from the natural sources in the Oxford Glycobiology Institute. *Enzyme solutions*: For the purposes of this investigation, all enzyme solutions were made up using 50 mM citrate–phosphate buffer (anhydrous disodium hydrogen phosphate dissolved in citric acid) at pH 5 containing 1 mg mL^{-1} bovine serum albumin (BSA) and 0.02% NaN_3, except α-D-glucosidase, for which Dulbecco's phosphate buffered saline (PBS) buffer at pH 7.4 was used. These solutions were stored on ice whilst in use and at −20 °C when not in use. *Inhibitor solutions*: *N*Bn-LABNAc **3**, *N*Bu-LABNAc **4**, LABNAc **1**, *N*Bn-DABNAc **6**, and DABNAc **5** were dissolved in distilled H_2O at a concentration of 10 mg mL^{-1}. These solutions were stored on ice whilst in use and at −20 °C when not in use. *Enzyme substrate solutions*: Substrate solutions were made from a stock solution (3 mM) in 10 mL batches, as required, by dissolving the appropriate mass of substrate in the appropriate buffer solution for the enzyme. These were kept at 4 °C when not in use. *Method*: Assays were carried out in triplicate, using H_2O as a blank in place of the inhibitor. The concentrations of the enzyme were adjusted so that the reading for the final absorbance was in the range of 0.5–1.5 units over a reaction time of 20 min. For example, β-*N*-acetyl-D-hexosaminidase was diluted from 6 U mL^{-1} to 0.4 U mL^{-1} by experiment. Linearity over the time course of the reaction was checked using a series of incubation times. The following were combined in the well of a flat-bottomed 96-well (300 μL) microtitre plate: 5 μL enzyme solution, 5 μL inhibitor solution, and 40 μL substrate solution. The reaction mix was incubated at 37 °C for 20 min (Denley Wellwarm 1) and was quenched by the addition of 200 μL of 0.5 M Na_2CO_3. Absorbance at 405 nm was measured immediately using a microtitre plate reader (Molecular Devices UVmax kinetic microplate reader and SOFTmax 2.35 software). *Determination of IC_{50} values*: Percentage inhibition was plotted against the log of the inhibitor concentration, and a trend line was fitted using Microsoft Excel. The IC_{50} value for each compound was calculated from the value of the log of the inhibitor concentration at 50% inhibition of enzyme activity. *Determination of K_i values*: The inhibition constants were determined for compounds that showed significant inhibition (>60% at 1 mM [*I*]) in the initial screening. K_i values were calculated from Lineweaver–Burk plots (1/*v* against 1/[S]) using a suitable range of substrate solutions and inhibitor concentrations. Values were plotted and subjected to linear regression using Microsoft Excel.

Molecular modelling: One- and two-dimensional ^{1}H NMR spectra of *N*Bn-LABNAc **3** (D_2O, pH 4.2, $T = 30$ °C) were run at 500 MHz on a Varian Unity Inova spectrometer. The mixing time used for the 2D NOESY spectrum was 200 ms. Molecular modelling was performed on a Silicon Graphics Fuel workstation, using the programs Insight II and Discover (Accelrys Inc., San Diego, CA, USA).

Cell-based assay for chaperone evaluation: (Materials and methods used by S.D.B. and T.D.B., Oxford Glycobiology Institute). β-Hexosaminidase activity was determined by incubating homogenates from GM3461 cells, derived from a patient of adult-onset Tay-Sachs disease (Coriell Cell Repositories), grown in the presence or absence of up to 50 μM *N*Bn-LABNAc **3**, with 3.2 mM fluorescently tagged artificial substrates, 4-methylumbelliferyl-β-*N*-acetylglucosamine (MUG) or 4-methylumbelliferyl-β-*N*-acetylglucosamine-6-sulfate (MUGS) in 100 mM citrate–phosphate buffer, pH 5.0, at 37 °C for 1 h under gentle agitation. The reaction was terminated by addition of 0.5 M Na_2CO_3, and the fluorescence was measured using an excitation wavelength of 365 nm and an emission wavelength of 450 nm. A 4-MU standard was used to generate a calibration curve for calculation of the total β-hexosaminidase activity or β-hexosaminidase A and β-hexosaminidase S activity using MUG or MUGS, respectively, and normalised to protein amount, as determined by the bicinchoninic acid (BCA) assay.

3,5-*O*-Benzylidene-D-lyxono-1,4-lactone 34: Concentrated HCl (8 mL) was added dropwise to a stirred solution of D-lyxono-1,4-lactone (10.0 g, 67.5 mmol) in benzaldehyde (120 mL) at room temperature under an atmosphere of N_2. After 24 h, analysis by TLC (EtOAc) indicated complete conversion of starting material (R_f = 0.06) to one product (R_f = 0.32). The solution was concentrated to ~100 mL in vacuo. The white precipitate that formed was collected and washed with Et_2O (3×40 mL). Recrystallisation (MeOH) yielded 3,5-*O*-benzylidene-D-lyxono-1,4-lactone **34** as a white crystalline solid (14.4 g, 90%): R_f = 0.32 (EtOAc); mp: 203–205 °C (MeOH) [Lit.: 203.5–206 °C (MeOH)[39]]; $[\alpha]_D^{20} = +27.6$ (c = 1.005, DMF) [Lit.: $[\alpha]_D^{24} = +31.6$ (c = 2.31, DMF)[39]]; ^{1}H NMR (400 MHz, $(CD_3)_2CO$): $\delta = 3.46$ (br s, 1H, O*H*), 4.33 (dd, $J_{5,5'} = 13.7$ Hz, $J_{5,4} = 2.0$ Hz, 1H, H5), 4.41 (dd, $J_{5',5} = 13.7$ Hz, $J_{5',4} = 1.3$ Hz, 1H, H5′), 4.47 (dt, $J_{4,5'} = 1.3$ Hz, $J = 2.1$ Hz, 1H, H4), 4.75 (d, $J_{2,3} = 4.2$ Hz, 1H, H2), 4.87 (ddd, $J_{3,2} = 4.2$ Hz, $J_{3,4} = 2.2$ Hz, $J = 0.5$ Hz, 1H, H3), 5.72 (1H, s, C*H*Ph), 7.33–7.56 ppm (5H, m, C*H*(Ar)).

3,5-*O*-Benzylidene-2-*O*-trifluoromethanesulfonyl-D-lyxono-1,4-lactone 35: Trifluoromethanesulfonic anhydride (1.71 mL, 10.2 mmol, 1.6 equiv) was added to a stirred solution of 3,5-*O*-benzylidene-D-lyxono-1,4-lactone **36** (1.50 g, 6.35 mmol) in pyridine (15 mL) at −30 °C under an atmosphere of N_2. After 3 h, analysis by TLC (EtOAc) showed no residual starting material (R_f = 0.07) and a major spot due to the product (R_f = 0.55). The solvents were removed in vacuo (high vacuum) using toluene to aid by co-evaporation. The remaining residue was dissolved in EtOAc (60 mL) and was washed successively with 0.1 M HCl (40 mL) and brine (40 mL). The aqueous phases were extracted with EtOAc (2×10 mL). The combined organics were dried ($MgSO_4$), and the solvents were removed in vacuo to yield 3,5-*O*-benzylidene-2-*O*-trifluoromethanesulfonyl-D-lyxono-1,4-lactone **35** (2.34 g, 100%) as a pale-yellow solid that was carried through to the next experiment without further purification.

2-Azido-3,5-*O*-benzylidene-2-deoxy-D-xylono-1,4-lactone 33 and 2-azido-3,5-*O*-benzylidene-2-deoxy-D-lyxono-1,4-lactone 36: NaN_3 (401 mg, 6.16 mmol, 0.97 equiv) was added to a stirred solution of 3,5-*O*-benzylidene-2-*O*-trifluoromethanesulfonyl-D-lyxono-1,4-lactone **35** (2.34 g, 6.35 mmol) in DMF (25 mL) at room temperature under an atmosphere of N_2. After 1 h, analysis by TLC (1:1, EtOAc/cyclohexane) showed no residual starting material (R_f = 0.24), a minor spot (R_f = 0.17), and a large spot due to the product (R_f = 0.49). The solvents were removed in vacuo (high vacuum) using toluene to aid by co-evaporation, and the remaining residue was purified by flash chromatography (1:1, EtOAc/cyclohexane) to yield 2-azido-3,5-*O*-benzylidene-2-deoxy-D-xylono-1,4-lactone **33** as a white solid (1.37 g, 83% over two steps): R_f = 0.49 (1:1, EtOAc/cyclohexane); mp: 119–121 °C; $[\alpha]_D^{24} = +143.4$ (c = 0.99, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.20$ (app dt, $J_{5,5'} = 13.9$ Hz, $J = 1.9$ Hz, 1H, H5), 4.23 (s, 1H, H2), 4.46–4.51 (m, 2H, H3, H4), 4.62 (app d, $J_{5',5} = 13.9$ Hz, 1H, H5′), 5.54 (d, $J = 1.1$ Hz, 1H, C*H*Ph), 7.37–7.47 ppm (m, 5H, C*H*(Ar)); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 63.2$ (C2), 66.0 (C5), 73.1 (C3), 75.6 (C4), 99.5 (*C*HPh), 126.1, 128.4, 129.6 (*C*H(Ar)), 136.4 (*C*(Ar)), 171.1 ppm (C1); IR (NaCl): $\tilde{\nu} = 2117$ (N_3), 1776 cm^{-1} (C=O); HRMS (ESI$^+$): calcd for $C_{12}H_{12}N_3O_4^+$ ([*M*+H]$^+$):

262.0828, found: 262.0825; and 2-azido-3,5-*O*-benzylidene-2-deoxy-D-lyxono-1,4-lactone **36** as a white solid (149 mg, 9% over two steps): $R_f = 0.17$ (1:1, EtOAc/cyclohexane); mp: 122–123 °C, 128–130 °C (recrystallised from EtOAc/cyclohexane) [Lit.: 121–122 °C (acetone)[36b]]; $[\alpha]_D^{23} = +37.5$ ($c = 0.985$, acetone) [Lit.: $[\alpha]_D^{24} = +37.4$ ($c = 1.00$, acetone)[36b]]; ^{1}H NMR (400 MHz, $(CD_3)_2CO$): $\delta = 4.40$ (dd, $J_{5,5'} = 13.8$ Hz, $J_{5,4} = 2.0$ Hz, 1H, H5), 4.49 (dd, $J_{5',5} = 13.8$ Hz, $J_{5',4} = 1.3$ Hz, 1H, H5′), 4.60 (d, $J_{2,3} = 4.1$ Hz, 1H, H2), 4.65 (dt, $J_{4,5'} = 1.3$ Hz, $J_{4,5} = 2.0$ Hz, $J_{4,3} = 2.0$ Hz, 1H, H4), 5.13 (dd, $J_{3,4} = 2.0$ Hz, $J_{3,2} = 4.1$ Hz, 1H, H3), 5.80 (s, 1H, C*H*Ph), 7.36–7.52 ppm (m, 5H, C*H*(Ar)); ^{13}C NMR (100 MHz, $(CD_3)_2CO$): $\delta = 62.4$ (C2), 66.5 (C5), 71.9 (C4), 75.3 (C3), 99.1 (*C*HPh), 126.5, 128.5, 129.4 (*C*H(Ar)), 138.1 (*C*(Ar)), 171.7 ppm (C=O); IR (NaCl): $\tilde{\nu} = 2119$ (N_3), 1796 cm^{-1} (C=O); elemental analysis calcd (%) for $C_{12}H_{11}N_3O_4$: C 55.17, H 4.24, N 16.09, found: C 55.13, H 4.33, N 16.08.

2-Azido-3,5-*O*-benzylidene-2-deoxy-D-xylitol 37: A 2 M solution of $LiBH_4$ in THF (5.74 mL, 11.5 mmol, 3.0 equiv) was added dropwise to a stirred solution of 2-azido-3,5-*O*-benzylidene-2-deoxy-D-xylono-1,4-lactone **33** (1.00 g, 3.83 mmol) in THF (15 mL) at −30 °C under an atmosphere of N_2. After 2 h, analysis by TLC (1:1, EtOAc/cyclohexane) showed no residual starting material ($R_f = 0.49$). A saturated solution of NH_4Cl (0.5 mL) was added dropwise to destroy the excess hydride, and after 15 min a further portion (1.5 mL) was added. After a further 45 min, the solvents were removed in vacuo, and the remaining residue was purified by flash chromatography (acetone) to yield 2-azido-3,5-*O*-benzylidene-2-deoxy-D-xylitol **37** as a colourless oil (998 mg, 98%): $R_f = 0.34$ (EtOAc); $[\alpha]_D^{24} = -14.5$ ($c = 1.04$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.20$ (s, 1H, O*H*), 3.01 (s, 1H, O*H*), 3.68 (s, 1H, H2), 3.75 (dd, $J_{5,5'} = 12.0$ Hz, $J_{5,4} = 4.3$ Hz, 1H, H5), 3.82 (dd, $J_{5',5} = 12.0$ Hz, $J_{5',4} = 3.3$ Hz, 1H, H5′), 3.86–3.92 (ddd, $J_{4,5} = 4.3$ Hz, $J_{4,3} = 8.6$ Hz, $J_{4,5'} = 3.3$ Hz, 1H, H4), 4.11 (dd, $J_{1,1'} = 12.1$ Hz, $J_{1,2} = 1.2$ Hz, 1H, H1), 4.15 (dd, $J_{3,4} = 8.6$ Hz, $J_{3,2} = 0.8$ Hz, 1H, H3), 4.25 (dd, $J_{1',1} = 12.1$ Hz, $J_{1',2} = 1.9$ Hz, 1H, H1′) 5.67 (s, 1H, C*H*Ph), 7.35–7.75 ppm (m, 5H, C*H*(Ar)); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 60.9$ (C5), 63.7 (C2), 63.9 (C3), 72.3 (C1), 80.5 (C4), 101.3 (*C*HPh), 125.7, 128.3, 129.1 (*C*H(Ar)), 137.2 ppm (*C*(Ar)); IR (NaCl): $\tilde{\nu} = 3410$ (OH), 2105 cm^{-1} (N_3); HRMS (ESI$^-$): calcd for $C_{12}H_{14}N_3O_4^-$ ($[M-H]^-$): 264.0984, found: 264.0985.

2-Azido-3,5-*O*-benzylidene-2-deoxy-1,4-di-*O*-methanesulfonyl-D-xylitol 38: Triethylamine (TEA, 2.0 mL, 14.6 mmol) was added to a stirred solution of 2-azido-3,5-*O*-benzylidene-2-deoxy-D-xylitol **37** (967 mg, 3.64 mmol) in CH_2Cl_2 (10 mL) at 0 °C under an atmosphere of N_2. After 20 min, methanesulfonyl chloride (1.13 mL, 14.5 mmol, 4.0 equiv) was added dropwise. After 2.5 h, analysis by TLC (EtOAc) showed no residual starting material ($R_f = 0.34$) and a major spot due to the product ($R_f = 0.64$). The solvents were removed in vacuo, and the remaining residue was purified by flash chromatography (1:3, EtOAc/cyclohexane) to yield 2-azido-3,5-*O*-benzylidene-2-deoxy-1,4-di-*O*-methanesulfonyl-D-xylitol **38** as a white solid (1.24 g, 81%): $R_f = 0.64$ (EtOAc); mp: 121–123 °C; $[\alpha]_D^{24} = -15.8$ ($c = 0.89$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.11$, 3.20 (2×s, 6H, 2×SO_2CH_3), 4.05–4.11 (m, 1H, H2), 4.17 (app d, $J_{5,5'} = 13.5$ Hz, 1H, H5), 4.31 (app d, $J = 8.7$ Hz, 1H, H3), 4.45 (dd, $J_{1,1'} = 11.7$ Hz, $J_{1,2} = 3.1$ Hz, 1H, H1), 4.50 (dd, $J_{1',1} = 11.6$ Hz, $J_{1',2} = 4.7$ Hz, 1H, H1′), 4.59 (dd, $J_{5',5} = 13.5$ Hz, $J_{5',4} = 1.3$ Hz, 1H, H5′), 4.80 (s, 1H, H4), 5.71 (s, 1H, C*H*Ph), 7.36–7.56 ppm (m, 5H, C*H*(Ar)); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 37.6$, 39.3 (2×SO_2CH_3), 60.1 (C2), 67.1 (C1), 69.1 (C5), 70.1 (C4), 77.3 (C3), 101.5 (*C*HPh), 125.9, 128.3, 129.3 (*C*H(Ar)), 136.6 ppm (*C*(Ar)); IR (NaCl): $\tilde{\nu} = 2107$ (N_3), 1355, 1334 and 1174 cm^{-1} (S=O); HRMS (ESI$^+$): calcd for $C_{14}H_{23}N_4O_8S_2^+$ ($[M+NH_4]^+$): 439.0957, found: 439.0956; elemental analysis calcd (%) for $C_{14}H_{19}N_3O_8S_2$: C 39.90, H 4.54, N 9.97, found: C 39.87, H 4.44, N 9.76.

1-Aminobenzyl-2-azido-3,5-*O*-benzylidene-1,2-dideoxy-4-*O*-methanesulfonyl-D-xylitol 39: 2-Azido-3,5-*O*-benzylidene-2-deoxy-1,4-di-*O*-methanesulfonyl-D-xylitol **38** (12.7 g, 30.1 mmol) was stirred in benzylamine (70 mL), and the resulting solution was heated at 95 °C. After 14 h, analysis by TLC (EtOAc) showed one major spot ($R_f = 0.56$). The solvents were removed in vacuo (high vacuum) using toluene to aid by co-evaporation, and the resulting orange residue was purified by flash chromatography (9:1→1:1, cyclohexane/EtOAc) to yield 1-aminobenzyl-2-azido-3,5-*O*-benzylidene-1,2-dideoxy-4-*O*-methanesulfonyl-D-xylitol **39** as an orange oil (10.6 g, 81%): $R_f = 0.56$ (EtOAc); $[\alpha]_D^{25} = -16.3$ ($c = 0.95$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.78$ (dd, $J_{1,1'} = 12.8$ Hz, $J_{1,2} = 5.5$ Hz, 1H, H1), 2.95 (dd, $J_{1,1'} = 12.8$ Hz, $J_{1',2} = 3.2$ Hz, 1H, H1′), 3.11, (s, 3H, SO_2CH_3), 3.77 (app d, $J = 13.2$ Hz, 1H, NC*H*HPh), 3.83 (app d, $J = 13.2$ Hz, 1H, NCH*H*Ph), 3.93 (ddd, $J_{2,1'} = 3.2$ Hz, $J_{2,3} = 8.8$ Hz, $J_{2,1} = 5.5$ Hz, 1H, H2), 4.10 (dd, $J_{5,5'} = 13.4$ Hz, $J_{5,4} = 1.2$ Hz, 1H, H5), 4.33 (dd, $J_{3,2} = 8.8$ Hz, $J_{3,4} = 1.4$ Hz, 1H, H3), 4.59 (dd, $J_{5',5} = 13.4$ Hz, $J_{5',4} = 1.7$ Hz, 1H, H5′), 4.65 (app q, $J = 1.5$ Hz, 1H, H4), 5.69 (s, 1H, CHPh), 7.26–7.58 ppm (m, 10H, CH(Ar)); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 39.4$ (SO_2CH_3), 47.2 (C1), 53.8 (NCH_2Ph), 62.1 (C2), 69.2 (C5), 70.7 (C4), 79.2 (C3), 101.2 (*C*HPh), 125.9, 127.2, 128.2, 128.3, 128.5, 129.1 (*C*H(Ar)), 136.9, 139.8 ppm (*C*(Ar)); IR (NaCl): $\tilde{\nu} = 2104$ (N_3), 1334 and 1174 cm^{-1} (S=O); HRMS (ESI$^+$): calcd for $C_{20}H_{25}N_4O_5S^+$ ($[M+H]^+$): 433.1546, found: 433.1549.

***N*-Benzyl-2-azido-1,2,4-trideoxy-1,4-imino-L-arabinitol 40**: Amberlyst® 15 (wet) beads were added to a stirred solution of 1-aminobenzyl-2-azido-3,5-*O*-benzylidene-1,2-dideoxy-4-*O*-methanesulfonyl-D-xylitol **39** (130 mg, 0.229 mmol) in 1,4-dioxane (2.0 mL) and H_2O (2.0 mL), and the resulting mixture was heated at 85 °C. After 20 h, analysis by TLC (EtOAc) showed no residual starting material ($R_f = 0.56$), and the beads were filtered off and washed thoroughly with MeOH and 1 M NH_4OH. The solvents were removed in vacuo, and the remaining residue was purified by ion-exchange chromatography (Amberlite® CG-150) and freeze-dried to yield *N*-benzyl-2-azido-1,2,4-trideoxy-1,4-imino-L-arabinitol **40** as a pale-yellow oil (66.4 mg, 89%): $R_f = 0.47$ (EtOAc); $[\alpha]_D^{25} = +97.3$ ($c = 0.985$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.46$ (br s, 2H, 3-O*H*, 5-O*H*), 2.63 (ddd, $J_{4,5'} = 3.8$ Hz, $J_{4,3} = 5.7$ Hz, $J_{4,5} = 2.4$ Hz, 1H, H4), 2.82 (dd, $J_{1,1'} = 10.9$ Hz, $J_{1,2} = 6.6$ Hz, 1H, H1), 3.00 (dd, $J_{1',1} = 10.9$ Hz, $J_{1',2} = 2.5$ Hz, 1H, H1′), 3.42 (d, $J = 13.2$ Hz, 1H, NC*H*HPh), 3.71–3.76 (m, 2H, H5, H2), 3.78 (dd, $J_{5',5} = 11.4$ Hz, $J_{5',4} = 3.8$ Hz, 1H, H5′), 3.99 (d, $J = 13.2$ Hz, 1H, NCH*H*Ph), 4.28 (dd, $J_{3,4} = 5.7$ Hz, $J_{3,2} = 3.4$ Hz, 1H, H3), 7.25–7.39 ppm (m, 5H, CH(Ar)); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 56.1$ (C1), 57.7 (NCH_2Ph), 59.4 (C5), 65.4 (C2), 71.6 (C4), 78.4 (C3), 127.5, 128.5, 128.6 (*C*H(Ar)), 137.7 ppm (*C*(Ar)); IR (NaCl): $\tilde{\nu} = 3370$ (OH), 2100 cm^{-1} (N_3); HRMS (ESI$^+$): calcd for $C_{12}H_{17}N_4O_2^+$ ($[M+H]^+$): 249.1352, found: 249.1357.

***N*-Benzyl-2-amino-1,2,4-trideoxy-1,4-imino-L-arabinitol 41**: Pd (10% wt on C, 100 mg) was added to a stirred solution of *N*-benzyl-2-azido-1,2,4-trideoxy-1,4-imino-L-arabinitol **40** (316 mg, 1.27 mmol) in THF (7.5 mL) at room temperature under an atmosphere of H_2. After 3 h, analysis by TLC (EtOAc) showed no residual starting material ($R_f = 0.47$) and a spot due to the product on the baseline. Pd/C was removed by filtering over Celite®, which was rinsed with MeOH, and the solvents were removed in vacuo to yield *N*-benzyl-2-amino-1,2,4-trideoxy-1,4-imino-L-arabinitol **41** as a white solid (282 mg, quant.): $R_f = 0.60$ (60:45:20, $CHCl_3$/MeOH/NH_4OH); mp: 143–145 °C; $[\alpha]_D^{25} = +58.3$ ($c = 0.855$, MeOH); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 2.52$ (app dt, $J_{4,3} = 4.5$ Hz, $J = 3.7$ Hz, 1H, H4), 2.65–2.70 (m, 2H, H1, H1′), 2.98–3.03 (m, 1H, H2), 3.40 (d, $J = 13.1$ Hz, 1H, NC*H*HPh), 3.68 (dd, $J_{5,5'} = 11.5$ Hz, $J_{5,4} = 3.5$ Hz, 1H, H5), 3.73 (dd, $J_{5',5} = 11.5$ Hz, $J_{5',4} = 3.9$ Hz, 1H, H5′), 3.84 (dd, $J_{3,2} = 2.6$ Hz,

$J_{3,4}$ = 4.5 Hz, 1H, H3), 4.08 (d, J = 13.1 Hz, 1H, NCH*H*Ph), 7.20–7.39 ppm (m, 5H, CH(Ar)); ^{13}C NMR (100 MHz, CD_3OD): δ = 57.3 (C2), 58.7 (NCH_2Ph), 59.0 (C1), 61.0 (C5), 73.2 (C4), 80.8 (C3), 127.1, 128.3, 129.1 (*C*H(Ar)), 139.2 ppm (*C*(Ar)); IR (Ge): $\tilde{\nu}$ = 3356 (OH, NH_2), 1584 cm^{-1} (NH_2); HRMS (ESI$^+$): calcd for $C_{12}H_{19}N_2O_2^+$ ([*M*+H]$^+$): 223.1447, found: 223.1447.

***N*-Benzyl-2-acetamido-3,5-di-*O*-acetyl-1,2,4-trideoxy-1,4-imino-L-arabinitol 42**: Acetic anhydride (11.3 mL, 1.59 mmol) was added dropwise to a stirred solution of *N*-benzyl-2-amino-1,2,4-trideoxy-1,4-imino-L-arabinitol **41** (4.45 g, 20.0 mmol) in pyridine (50.0 mL) at room temperature under an atmosphere of N_2. After 16 h, analysis by TLC (EtOAc) showed no residual starting material and a spot due to the product (R_f = 0.26). The solvents were removed in vacuo (high vacuum) using toluene to aid by co-evaporation. The remaining residue was purified by flash chromatography (EtOAc) to yield *N*-benzyl-2-acetamido-3,5-di-*O*-acetyl-1,2,4-trideoxy-1,4-imino-L-arabinitol **42** as a yellow oil (5.12 g, 73%): R_f = 0.26 (EtOAc); $[\alpha]_D^{25}$ = +22.7 (c = 1.02, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): δ = 1.56 (d, $J_{Me,NH}$ = 1.5 Hz, 3H, NHCOC*H*$_3$), 1.68, 1.80 (2×s, 6H, 2×OCOC*H*$_3$), 2.62 (dd, $J_{1,1'}$ = 10.1 Hz, $J_{1,2}$ = 5.4 Hz, 1H, H1), 2.65–2.70 (m, 2H, H4, H1′), 3.13 (d, J = 12.9 Hz, 1H, NC*H*HPh), 3.90 (d, J = 12.9 Hz, 1H, NCH*H*Ph), 4.33 (dd, $J_{5,5'}$ = 11.7 Hz, $J_{5,4}$ = 4.1 Hz, 1H, H5), 4.50 (dd, $J_{5',5}$ = 11.7 Hz, $J_{5',4}$ = 4.5 Hz, 1H, H5′), 4.56–4.61 (m, 1H, H2), 5.17 (app t, J = 2.8 Hz, 1H, H3), 5.75 (app t, J = 7.5 Hz, 1H, N*H*Ac), 7.16–7.30 ppm (m, 5H, C*H*(Ar)); ^{13}C NMR (100 MHz, C_6D_6): δ = 20.5, 20.7 (2×OCO*C*H$_3$), 22.8 (NHCO*C*H$_3$), 53.5 (C2), 57.5 (C1), 58.8 (NCH_2Ph), 63.1 (C5), 68.8 (C4), 80.6 (C3), 127.8, 128.8, 129.4 (*C*H(Ar)), 138.8 ppm (*C*(Ar)); IR (NaCl): $\tilde{\nu}$ = 1740 (C=O ester), 1653 (C=O amide band I), 1539 cm^{-1} (N–H amide band II); HRMS (ESI$^+$): calcd for $C_{18}H_{24}N_2O_5Na^+$ ([*M*+Na]$^+$): 371.1583, found: 371.1581.

***N*-Benzyl-2-acetamido-1,2,4-trideoxy-1,4-imino-L-arabinitol 3**: NaOMe (15.0 mg) was added to a stirred solution of *N*-benzyl-2-acetamido-3,5-di-*O*-acetyl-1,2,4-trideoxy-1,4-imino-L-arabinitol **42** (188 mg, 0.538 mmol) in MeOH (2.0 mL) at room temperature under an atmosphere of N_2. After 1 h, analysis by TLC (EtOAc) showed no residual starting material (R_f = 0.26) with the product on the baseline. Analysis by TLC (acetone) showed the single spot of the product (R_f = 0.22). The solvents were removed in vacuo, and the resulting residue was purified by flash chromatography (acetone) to yield *N*-benzyl-2-acetamido-1,2,4-trideoxy-1,4-imino-L-arabinitol **3** as a colourless oil that changed to an off-white solid on leaving under vacuum overnight (120 mg, 84%): R_f = 0.22 (acetone); mp: 126–128 °C; $[\alpha]_D^{22}$ = +82.1 (c = 0.96, MeOH); ^{1}H NMR (400 MHz, D_2O): δ = 1.82 (s, 3H, NHCOC*H*$_3$), 2.49 (app dt, $J_{4,3}$ = 5.7 Hz, J = 4.3 Hz, 1H, H4), 2.55 (dd, $J_{1,1'}$ = 11.0 Hz, $J_{1,2}$ = 2.9 Hz, 1H, H1), 2.76 (dd, $J_{1',1}$ = 11.0 Hz, $J_{1',2}$ = 7.2 Hz, 1H, H1′), 3.36 (d, J = 12.7 Hz, 1H, NC*H*HPh), 3.59 (dd, $J_{5,5'}$ = 12.0 Hz, $J_{5,4}$ = 4.0 Hz, 1H, H5), 3.64 (dd, $J_{5',5}$ = 12.0 Hz, $J_{5',4}$ = 4.6 Hz, 1H, H5′), 3.82–3.94 (m, 3H, H3, H2, NCH*H*Ph), 7.21–7.32 ppm (m, 5H, CH(Ar)); ^{13}C NMR (100 MHz, D_2O): δ = 22.1 (NHCO*C*H$_3$), 55.5 (C2), 56.3 (C1), 58.5 (NCH_2Ph), 60.3 (C5), 71.1 (C4), 77.9 (C3), 128.1, 128.9, 130.2 (*C*H(Ar)), 137.3 (*C*(Ar)), 174.2 ppm (C=O); IR (Ge): $\tilde{\nu}$ = 3312 (OH), 1651 (C=O amide band I), 1551 cm^{-1} (N–H amide band II); HRMS (ESI$^+$): calcd for $C_{14}H_{21}N_2O_3^+$ ([*M*+H]$^+$): 265.1552, found: 265.1548; elemental analysis calcd (%) for $C_{14}H_{20}N_2O_3$: C 63.62, H 7.63, N 10.60, found: C 63.21, H 7.78, N 10.26.

2-Acetamido-1,2,4-trideoxy-1,4-imino-L-arabinitol 1: Pd black (25.0 mg) was added to a stirred solution of *N*-benzyl-2-acetamido-1,2,4-trideoxy-1,4-imino-L-arabinitol **3** (80.0 mg, 0.303 mmol) in 1,4-dioxane (1.0 mL) and H_2O (1.0 mL) at room temperature under an atmosphere of H_2. After 20 h, analysis by TLC (acetone) showed no residual starting material (R_f = 0.22) and a spot due to the product on the baseline. The Pd black was filtered off over Celite®, washing with MeOH. The solvents were removed in vacuo, and the remaining residue was purified by ion-exchange chromatography (Dowex® 50WX8–200) to yield 2-acetamido-1,2,4-trideoxy-1,4-imino-L-arabinitol **1** as a colourless oil (52.7 mg, quant.): R_f = baseline (acetone); $[\alpha]_D^{22}$ = +3.9 (c = 0.765, H_2O); ^{1}H NMR (400 MHz, D_2O): δ = 1.87 (s, 3H, NHCOC*H*$_3$), 2.62 (dd, $J_{1,1'}$ = 12.0 Hz, $J_{1,2}$ = 6.3 Hz, 1H, H1), 2.89 (dt, $J_{4,5'}$ = 4.5 Hz, J = 6.3 Hz, 1H, H4), 3.10 (dd, $J_{1',1}$ = 12.0 Hz, $J_{1',2}$ = 7.5 Hz, 1H, H1′), 3.51 (dd, $J_{5,5'}$ = 11.7 Hz, $J_{5,4}$ = 6.1 Hz, 1H, H5), 3.60 (dd, $J_{5',5}$ = 11.7 Hz, $J_{5',4}$ = 4.5 Hz, 1H, H5′), 3.75 (t, J = 6.3 Hz, 1H, H3), 3.97 ppm (dt, $J_{2,1'}$ = 7.5 Hz, J = 6.3 Hz, 1H, H2); ^{13}C NMR (100 MHz, D_2O): δ = 22.2 (NHCO*C*H$_3$), 48.3 (C1), 57.5 (C2), 61.4 (C5), 64.7 (C4), 77.1 (C3), 174.7 ppm (C=O); IR (Ge): $\tilde{\nu}$ = 3284 (OH), 1652 (C=O amide band I), 1558 cm^{-1} (N–H amide band II); HRMS (ESI$^+$): calcd for $C_7H_{15}N_2O_3^+$ ([*M*+H]$^+$): 175.1083, found: 175.1087.

***N*-Butyl-2-acetamido-1,2,4-trideoxy-1,4-imino-L-arabinitol 4**: Pd black (15.0 mg) was added to a stirred solution of *N*-benzyl-2-acetamido-1,2,4-trideoxy-1,4-imino-L-arabinitol **3** (50.0 mg, 0.189 mmol) and butyraldehyde (0.17 mL, 1.89 mmol) in 1,4-dioxane (1.0 mL) and H_2O (1.0 mL). The solution was degassed (3×) and allowed to stir at room temperature under an atmosphere of H_2. After 16 h, analysis by TLC (acetone) showed a spot due to the product (R_f = 0.24). The Pd black was filtered off over Celite®, rinsing with MeOH. The solvents were removed in vacuo, and the remaining residue was purified by flash chromatography (EtOAc, then acetone) to yield *N*-butyl-2-acetamido-1,2,4-trideoxy-1,4-imino-L-arabinitol **4** as a white crystalline solid (38.0 mg, 87%): R_f = 0.24 (acetone); mp: 79–81 °C; $[\alpha]_D^{20}$ = +63.8 (c = 0.96, MeOH); ^{1}H NMR (400 MHz, D_2O): δ = 0.76 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.12–1.22 (m, 2H, CH_2CH_3), 1.23–1.45 (br m, 2H, NCH_2CH_2), 1.87 (s, 3H, NHCOC*H*$_3$), 2.17–2.26 (dt, J = 5.0 Hz, J = 11.3 Hz, 1H, NC*H*HCH$_2$), 2.46–2.51 (dt, $J_{4,3}$ = 6.2 Hz, $J_{4,5}$ = 4.5 Hz, 1H, H4), 2.69–2.78 (m, 2H, H1, NCH*H*CH$_2$), 2.81 (dd, $J_{1,1'}$ = 11.1 Hz, $J_{1,2}$ = 3.4 Hz, 1H, H1′), 3.60 (d, $J_{5,4}$ = 4.5 Hz, 2H, H5, H5′), 3.82 (dd, $J_{3,4}$ = 6.2 Hz, $J_{3,2}$ = 4.7 Hz, 1H, H3), 3.93 ppm (app pentet, J = 3.8 Hz, 1H, H2); ^{13}C NMR (100 MHz, D_2O): δ = 13.5 (CH_2CH_3), 20.5 (NCH_2CH_2), 22.2 (NHCO*C*H$_3$), 29.1 (CH_2CH_3), 54.9 (NCH_2CH_2), 55.6 (C2), 56.5 (C1), 60.4 (C5), 71.7 (C4), 77.6 ppm (C3); IR (Ge): $\tilde{\nu}$ = 3330 (OH), 1652 (C=O amide band I), 1556 cm^{-1} (N–H amide band II); HRMS (ESI$^+$): calcd for $C_{11}H_{23}N_2O_3^+$ ([*M*+H]$^+$): 231.1703, found: 231.1708.

3,5-*O*-Benzylidene-L-lyxono-1,4-lactone 30: Concentrated HCl (4.2 mL) was added to a stirred solution of 2,3-*O*-isopropylidene-L-lyxono-1,4-lactone **32** (3.50 g, 18.6 mmol) in benzaldehyde (45 mL) at room temperature under an atmosphere of N_2. After 20 h, analysis by TLC (EtOAc) showed a major product spot (R_f = 0.32) and no residual starting material (R_f = 0.45). The solvents were removed in vacuo (high vacuum) using toluene to aid by co-evaporation. The remaining residue was crystallised from hot MeOH (~100 mL) by allowing to cool to room temperature before cooling to 0 °C. The solid was collected and washed with Et_2O (3×30 mL) to yield 3,5-*O*-benzylidene-L-lyxono-1,4-lactone **30** as a white crystalline solid (4.17 g, 95%): R_f = 0.32 (EtOAc); mp: 203–205 °C (MeOH) [Lit.: 201.5–204 °C (acetone)[47]]; $[\alpha]_D^{20}$ = −31.2 (c = 1.005, DMF) [Lit.: $[\alpha]_D^{20}$ = −30.9 (c = 1.00, DMF)[47]]; ^{1}H NMR (400 MHz, $(CD_3)_2CO$): δ = 4.33 (dd, $J_{5,5'}$ = 13.6 Hz, $J_{5,4}$ = 2.0 Hz, 1H, H5), 4.41 (dd, $J_{5',5}$ = 13.6 Hz, $J_{5',4}$ = 1.3 Hz, 1H, H5′), 4.47 (dt, $J_{4,5'}$ = 1.3 Hz, J = 2.1 Hz, 1H, H4), 4.74 (d, $J_{2,3}$ = 4.2 Hz, 1H, H2), 4.87 (dd, $J_{3,2}$ = 4.2 Hz, $J_{3,4}$ = 2.2 Hz, 1H, H3), 5.06 (br s, 1H, OH), 5.72 (s, 1H, C*H*Ph), 7.34–7.52 ppm (m, 5H, CH(Ar)).

3,5-*O*-Benzylidene-2-*O*-trifluoromethanesulfonyl-L-lyxono-1,4-lactone 31: Trifluoromethanesulfonic anhydride (1.98 mL,

11.8 mmol, 1.6 equiv) was added to a stirred solution of 3,5-*O*-benzylidene-L-lyxono-1,4-lactone **30** (1.74 g, 7.35 mmol) in pyridine (16 mL) at −50 °C under an atmosphere of N_2. After 3 h, analysis by TLC (EtOAc) showed no residual starting material (R_f = 0.07) and a major spot due to the product (R_f = 0.55). The solvents were removed in vacuo (high vacuum), using toluene to aid by co-evaporation. The remaining residue was dissolved in EtOAc (80 mL) and was washed successively with 0.1 M HCl (40 mL) and brine (40 mL). The aqueous phases were extracted using EtOAc (2×10 mL). The combined organics were dried ($MgSO_4$), and the solvents were removed in vacuo to yield 3,5-*O*-benzylidene-2-*O*-trifluoromethanesulfonyl-L-lyxono-1,4-lactone **31** (2.71 g, 100%) as a white solid that was carried through to the next experiment without further purification.

2-Azido-3,5-*O*-benzylidene-2-deoxy-L-xylono-1,4-lactone 28 and 2-azido-3,5-*O*-benzylidene-2-deoxy-L-lyxono-1,4-lactone: NaN_3 (464 mg, 7.13 mmol) was added to a stirred solution of 3,5-*O*-benzylidene-2-*O*-trifluoromethanesulfonyl-L-lyxono-1,4-lactone **31** (2.71 g, 7.35 mmol) in DMF (30 mL) at room temperature under an atmosphere of N_2. After 50 min, analysis by TLC (1:1, EtOAc/cyclohexane) showed no residual starting material (R_f = 0.24) and a large spot due to the product (R_f = 0.49). The solvents were removed in vacuo (high vacuum), using toluene to aid by co-evaporation, and the remaining residue was purified by flash chromatography (2:1, cyclohexane/EtOAc) to yield 2-azido-3,5-*O*-benzylidene-2-deoxy-L-xylono-1,4-lactone **28** as a white crystalline solid (1.50 g, 78% over two steps): R_f = 0.49 (1:1, EtOAc/cyclohexane); mp: 120–122 °C; $[\alpha]_D^{25} = -173.2$ (c = 0.99, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.20 (dd, $J_{5,5'}$ = 13.9 Hz, $J_{5,4}$ = 2.0 Hz, 1H, H5), 4.24 (s, 1H, H2), 4.47–4.51 (m, 2H, H3, H4), 4.62 (d, $J_{5',5}$ = 13.9 Hz, 1H, H5′), 5.54 (d, J = 1.1 Hz, 1H, C*H*Ph), 7.37–7.47 ppm (m, 5H, C*H*(Ar)); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 63.2 (C2), 66.0 (C5), 73.1 (C3), 75.6 (C4), 99.5 (*C*HPh), 126.1, 128.4, 129.6 (*C*H(Ar)), 136.4 (*C*(Ar)), 171.1 ppm (C1); IR (NaCl): $\tilde{\nu}$ = 2115 (N_3), 1789 cm^{-1} (C=O); HRMS (CI$^+$): calcd for $C_{12}H_{12}N_3O_4^+$ ($[M+H]^+$): 262.0828, found: 262.0838; elemental analysis calcd (%) for $C_{12}H_{11}N_3O_4$: C 55.17, H 4.24, N 16.09, found: C 55.21, H 4.33, N 15.93; and 2-azido-3,5-*O*-benzylidene-2-deoxy-L-lyxono-1,4-lactone as a white solid that was recrystallised from EtOAc/cyclohexane (269 mg, 14% over two steps): R_f = 0.17 (1:1, EtOAc/cyclohexane); mp: 128–130 °C; $[\alpha]_D^{25} = -41.3$ (c = 0.99, $CHCl_3$); ^{1}H NMR (400 MHz, $(CD_3)_2CO$): δ = 4.40 (dd, $J_{5,5'}$ = 13.8 Hz, $J_{5,4}$ = 2.0 Hz, 1H, H5), 4.49 (dd, $J_{5',5}$ = 13.8 Hz, $J_{5',4}$ = 1.3 Hz, 1H, H5′), 4.59 (d, $J_{2,3}$ = 4.1 Hz, 1H, H2), 4.64 (dt, $J_{4,5'}$ = 1.3 Hz, J = 2.0 Hz, 1H, H4), 5.12 (dd, $J_{3,4}$ = 2.0 Hz, $J_{3,2}$ = 4.1 Hz, 1H, H3), 5.79 (s, 1H, C*H*Ph), 7.36–7.52 ppm (m, 5H, C*H*(Ar)); ^{13}C NMR (100 MHz, $(CD_3)_2CO$): δ = 62.4 (C2), 66.5 (C5), 71.9 (C4), 75.3 (C3), 99.1 (*C*HPh), 126.5, 128.5, 129.4 (*C*H(Ar)), 138.1 (*C*(Ar)), 171.7 ppm (C=O); IR (NaCl): $\tilde{\nu}$ = 2118 (N_3), 1794 cm^{-1} (C=O); HRMS (CI$^+$): calcd for $C_{12}H_{12}N_3O_4^+$ ($[M+H]^+$): 262.0828, found: 262.0832.

2-Azido-3,5-*O*-benzylidene-2-deoxy-L-xylitol 29: A 2 M solution of $LiBH_4$ in THF (7.22 mL, 14.4 mmol, 1.0 equiv) was added dropwise to a stirred solution of 2-azido-3,5-*O*-benzylidene-2-deoxy-L-xylono-1,4-lactone **28** (3.78 g, 14.4 mmol) in THF (38.0 mL) at −50 °C under an atmosphere of Ar. After 3 h, analysis by TLC (1:1, EtOAc/cyclohexane) showed no residual starting material (R_f = 0.49). Ten drops of saturated NH_4Cl solution were added to destroy the excess hydride and after 15 min, a further 1 mL was added. After stirring for 15 min, the solvents were removed in vacuo, and the remaining residue was dissolved in EtOAc/MeOH (9:1) and passed through a silica plug. The solvents were removed in vacuo, and the remaining residue was purified by flash chromatography (1:1, EtOAc/cyclohexane) to yield 2-azido-3,5-*O*-benzylidene-2-deoxy-L-xylitol **29** as a colourless oil (3.02 g, 79%): R_f = 0.34 (EtOAc); $[\alpha]_D^{25}$ = +15.0 (c = 0.95, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.20 (s, 1H, O*H*), 3.01 (s, 1H, O*H*), 3.68 (s, 1H, H2), 3.75 (dd, $J_{5,5'}$ = 12.0 Hz, $J_{5,4}$ = 4.3 Hz, 1H, H5), 3.82 (dd, $J_{5',5}$ = 12.0 Hz, $J_{5',4}$ = 3.3 Hz, 1H, H5′), 3.86–3.92 (ddd, $J_{4,5}$ = 4.3 Hz, $J_{4,3}$ = 8.6 Hz, $J_{4,5'}$ = 3.3 Hz, 1H, H4), 4.11 (dd, $J_{1,1'}$ = 12.1 Hz, $J_{1,2}$ = 1.2 Hz, 1H, H1), 4.15 (dd, $J_{3,4}$ = 8.6 Hz, J = 0.8 Hz, 1H, H3), 4.25 (dd, $J_{1',1}$ = 12.1 Hz, $J_{1',2}$ = 1.9 Hz, 1H, H1′) 5.67 (s, 1H, CHPh), 7.35–7.75 ppm (m, 5H, C*H*(Ar)); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 60.9 (C5), 63.7 (C2), 63.9 (C3), 72.3 (C1), 80.5 (C4), 101.3 (*C*HPh), 125.7, 128.3, 129.1 (*C*H(Ar)), 137.2 ppm (*C*(Ar)); IR (NaCl): $\tilde{\nu}$ = 3400 (OH), 2105 cm^{-1} (N_3); HRMS (ESI$^-$): calcd for $C_{12}H_{14}N_3O_4^-$ ($[M-H]^-$): 264.0984, found: 264.0989; elemental analysis calcd (%) for $C_{12}H_{15}N_3O_4$: C 54.33, H 5.70, N 15.84, found: C 54.27, H 5.86, N 15.73.

2-Azido-3,5-*O*-benzylidene-2-deoxy-1,4-di-*O*-methanesulfonyl-L-xylitol 26: TEA (5.49 mL, 39.4 mmol, 4.0 equiv) was added to a stirred solution of 2-azido-3,5-*O*-benzylidene-2-deoxy-D-xylitol **29** (2.61 g, 9.84 mmol) in CH_2Cl_2 (26 mL) at 0 °C under an atmosphere of N_2. After 30 min, methanesulfonyl chloride (3.05 mL, 39.4 mmol, 4.0 equiv) was added dropwise. After 4 h, analysis by TLC (EtOAc) showed no residual starting material (R_f = 0.34) and a major spot due to the product (R_f = 0.64). The solvents were removed in vacuo, and the remaining residue was purified by flash chromatography (3:1, cyclohexane/EtOAc) to yield 2-azido-3,5-*O*-benzylidene-2-deoxy-1,4-di-*O*-methanesulfonyl-L-xylitol **26** as a white crystalline solid (3.73 g, 90%): R_f = 0.64 (EtOAc); mp: 121–123 °C; $[\alpha]_D^{25} = +16.0$ (c = 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.12, 3.20 (2×s, 6H, 2×SO_2CH_3), 4.05–4.11 (m, 1H, H2), 4.18 (dd, $J_{5,5'}$ = 13.5 Hz, $J_{5,4}$ = 1.3 Hz, 1H, H5), 4.31 (dd, $J_{3,2}$ = 7.2 Hz, $J_{3,4}$ = 1.5 Hz, 1H, H3), 4.46 (dd, $J_{1,1'}$ = 11.6 Hz, $J_{1,2}$ = 3.1 Hz, 1H, H1), 4.51 (dd, $J_{1',1}$ = 11.6 Hz, $J_{1,2}$ = 4.5 Hz, 1H, H1′), 4.60 (dd, $J_{5',5}$ = 13.5 Hz, $J_{5',4}$ = 1.7 Hz, 1H, H5′), 4.81 (app q, J = 1.5 Hz, 1H, H4), 5.72 (s, 1H, C*H*Ph), 7.36–7.56 ppm (m, 5H, CH(Ar)); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 37.6, 39.4 (2×SO_2CH_3), 60.0 (C2), 67.1 (C1), 69.1 (C5), 70.0 (C4), 77.3 (C3), 101.5 (*C*HPh), 125.9, 128.4, 129.4 (*C*H(Ar)), 136.5 ppm (*C*(Ar)); IR (NaCl): $\tilde{\nu}$ = 2107 (N_3), 1354, 1334 and 1174 cm^{-1} (S=O); HRMS (ESI$^+$): calcd for $C_{14}H_{19}N_3NaO_8S_2^+$ ($[M+Na]^+$): 444.0511, found: 444.0513; elemental analysis calcd (%) for $C_{14}H_{19}N_3O_8S_2$: C 39.90, H 4.54, N 9.97, found: C 39.93, H 4.70, N 9.84.

1-Aminobenzyl-2-azido-3,5-*O*-benzylidene-1,2-dideoxy-4-*O*-methanesulfonyl-L-xylitol: Benzylamine (2.84 mL, 26.0 mmol, 3.0 equiv) was added to a solution of 2-azido-3,5-*O*-benzylidene-2-deoxy-1,4-di-*O*-methanesulfonyl-L-xylitol **26** (3.65 g, 8.66 mmol) in 1,4-dioxane (65 mL), and the resulting solution was heated at reflux (95 °C) under an atmosphere of Ar. After five days, analysis by TLC (EtOAc) showed one major spot (R_f = 0.56). The solvents were removed in vacuo, and the resulting orange residue was purified by flash chromatography (3:1, cyclohexane/acetone) to yield 1-aminobenzyl-2-azido-3,5-*O*-benzylidene-1,2-dideoxy-4-*O*-methanesulfonyl-L-xylitol as a pale-orange oil (3.17 g, 75%): R_f = 0.56 (EtOAc); $[\alpha]_D^{22} = +14.9$ (c = 0.905, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.78 (dd, $J_{1,1'}$ = 12.8 Hz, $J_{1,2}$ = 5.5 Hz, 1H, H1), 2.95 (dd, $J_{1',1}$ = 12.8 Hz, $J_{1',2}$ = 3.2 Hz, 1H, H1′), 3.11, (s, 3H, SO_2CH_3), 3.77 (d, J = 13.2 Hz, 1H, NC*H*HPh), 3.83 (d, J = 13.2 Hz, 1H, NCH*H*Ph), 3.93 (ddd, $J_{2,1'}$ = 3.2 Hz, $J_{2,3}$ = 8.8 Hz, $J_{2,1}$ = 5.5 Hz, 1H, H2), 4.10 (dd, $J_{5,5'}$ = 13.4 Hz, $J_{5,4}$ = 1.4 Hz, 1H, H5), 4.33 (dd, $J_{3,2}$ = 8.8 Hz, $J_{3,4}$ = 1.5 Hz, 1H, H3), 4.59 (dd, $J_{5',5}$ = 13.4 Hz, $J_{5',4}$ = 1.8 Hz, 1H, H5′), 4.65 (app q, J = 1.5 Hz, 1H, H4), 5.69 (s, 1H, C*H*Ph), 7.26–7.58 ppm (m, 10H, C*H*(Ar)); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 39.4 (SO_2CH_3), 47.2 (C1), 53.8 (N*C*H_2Ph), 62.1 (C2), 69.2 (C5), 70.7 (C4), 79.2 (C3), 101.2 (*C*HPh), 125.9, 127.2, 128.2, 128.3, 128.5, 129.1 (*C*H(Ar)), 136.9, 139.7 ppm (*C*(Ar)); IR (NaCl): $\tilde{\nu}$ = 2103 (N_3), 1334 and 1174 cm^{-1} (S=O); HRMS (ESI$^+$): calcd for $C_{20}H_{25}N_4O_5S^+$ ($[M+H]^+$): 433.1546,

found: 433.1553; elemental analysis calcd (%) for $C_{20}H_{24}N_4O_5S$: C 55.54, H 5.59, N 12.95, found: C 55.64, H 5.51, N 12.86.

***N*-Benzyl-2-azido-1,2,4-trideoxy-1,4-imino-D-arabinitol 24**: Amberlyst® 15 (wet) beads were added to a stirred solution of 1-aminobenzyl-2-azido-3,5-*O*-benzylidene-1,2-dideoxy-4-*O*-methanesulfonyl-L-xylitol (2.35 g, 5.43 mmol) in 1,4-dioxane (35 mL) and H_2O (35 mL), and the resulting mixture was heated at 85 °C under an atmosphere of N_2. After 8 h, analysis by TLC (EtOAc) indicated no residual starting material ($R_f = 0.56$). The beads were filtered off and washed thoroughly with 2 M NH_4OH (3×30 mL) and 1,4-dioxane (30 mL). The solvents were removed in vacuo, and the remaining residue was purified by flash chromatography (1:1, EtOAc/cyclohexane) to yield *N*-benzyl-2-azido-1,2,4-trideoxy-1,4-imino-D-arabinitol **24** as a colourless oil (1.00 g, 75%): $R_f = 0.47$ (EtOAc); $[\alpha]_D^{25} = -97.7$ ($c = 0.70$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.63$ (ddd, $J_{4,5'} = 3.8$ Hz, $J_{4,3} = 5.7$ Hz, $J_{4,5} = 2.4$ Hz, 1H, H4), 2.82 (dd, $J_{1,1'} = 10.9$ Hz, $J_{1,2} = 6.6$ Hz, 1H, H1), 3.00 (dd, $J_{1',1} = 10.9$ Hz, $J_{1',2} = 2.5$ Hz, 1H, H1′), 3.42 (d, $J = 13.2$ Hz, 1H, NC*H*HPh), 3.71–3.76 (m, 2H, H5, H2), 3.78 (dd, $J_{5',5} = 11.4$ Hz, $J_{5',4} = 3.8$ Hz, 1H, H5′), 3.99 (d, $J = 13.2$ Hz, 1H, NCH*H*Ph), 4.28 (dd, $J_{3,4} = 5.7$ Hz, $J_{3,2} = 3.4$ Hz, 1H, H3), 7.25–7.39 ppm (m, 5H, C*H*(Ar)); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 56.1$ (C1), 57.7 (NCH_2Ph), 59.4 (C5), 65.4 (C2), 71.6 (C4), 78.4 (C3), 127.5, 128.5, 128.6 (*C*H(Ar)), 137.7 ppm (*C*(Ar)); IR (NaCl): $\tilde{\nu} = 3360$ (OH), 2100 cm^{-1} (N_3); HRMS (ESI^+): calcd for $C_{12}H_{17}N_4O_2^+$ ($[M+H]^+$): 249.1352, found: 249.1357.

***N*-Benzyl-2-amino-1,2,4-trideoxy-1,4-imino-D-arabinitol**: Pd (10% wt on C, 200 mg) was added to a stirred solution of *N*-benzyl-2-azido-1,2,4-trideoxy-1,4-imino-D-arabinitol **24** (885 mg, 3.56 mmol) in THF (18 mL) at room temperature under an atmosphere of H_2. After 6 h, analysis by TLC (EtOAc) showed no residual starting material ($R_f = 0.47$) and a spot due to the product on the baseline. The Pd/C was removed over Celite®, which was rinsed with MeOH. The solvents were removed in vacuo, and the remaining residue was purified by ion-exchange chromatography (Dowex® 50WX8–200) to yield *N*-benzyl-2-amino-1,2,4-trideoxy-1,4-imino-D-arabinitol as a white solid that turned brown on standing (705 mg, 89%): $R_f = 0.60$ (60:45:20, $CHCl_3$/MeOH/NH_4OH); mp: 137–139 °C; $[\alpha]_D^{22} = -68.4$ ($c = 1.015$, MeOH); ^{1}H NMR (400 MHz, D_2O): $\delta = 2.45$–2.52 (m, 2H, H1, H4), 2.72 (dd, $J_{1',1} = 10.8$ Hz, $J_{1',2} = 7.2$ Hz, 1H, H1′), 3.00 (app pentet, $J = 3.7$ Hz, 1H, H2), 3.39 (d, $J = 12.7$ Hz, 1H, NC*H*HPh), 3.55 (dd, $J_{5,5'} = 12.0$ Hz, $J_{5,4} = 4.3$ Hz, 1H, H5), 3.59 (dd, $J_{5',5} = 12.0$ Hz, $J_{5',4} = 4.6$ Hz, 1H, H5′), 3.66 (dd, $J_{3,4} = 5.9$ Hz, $J_{3,2} = 4.1$ Hz, 1H, H3), 3.86 (d, $J = 12.7$ Hz, 1H, NCH*H*Ph), 7.20–7.39 ppm (m, 5H, CH(Ar)); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 57.3$ (C2), 58.7 (NCH_2Ph), 59.0 (C1), 61.0 (C5), 73.2 (C4), 80.8 (C3), 127.1, 128.3, 129.1 (*C*H(Ar)), 139.2 ppm (*C*(Ar)); IR (Ge): $\tilde{\nu} = 3346$ (OH, NH_2), 1576 cm^{-1} (NH_2); HRMS (ESI^+): calcd for $C_{12}H_{19}N_2O_2^+$ ($[M+H]^+$): 223.1447, found: 223.1447.

***N*-Benzyl-2-acetamido-3,5-di-*O*-acetyl-1,2,4-trideoxy-1,4-imino-D-arabinitol**: Acetic anhydride (1.23 mL, 13.0 mmol) was added dropwise to a stirred solution of *N*-benzyl-2-amino-1,2,4-trideoxy-1,4-imino-D-arabinitol (483 mg, 2.17 mmol) in pyridine (16 mL) at room temperature under an atmosphere of N_2. After 16 h, analysis by TLC (EtOAc) showed no residual starting material and a spot due to the product ($R_f = 0.26$). The solvents were removed in vacuo (high vacuum), using toluene to aid by co-evaporation, and the remaining residue was purified by flash chromatography (98:2, EtOAc/TEA) to yield *N*-benzyl-2-acetamido-3,5-di-*O*-acetyl-1,2,4-trideoxy-1,4-imino-D-arabinitol as a pale-yellow oil (681 mg, 90%): $R_f = 0.26$ (EtOAc); $[\alpha]_D^{25} = -25.8$ ($c = 0.85$, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta = 1.55$ (s, 3H, NHCOCH_3), 1.68, 1.80 (2×s, 6H, 2×OCOCH_3), 2.62 (dd, $J_{1,1'} = 10.1$ Hz, $J_{1,2} = 5.4$ Hz, 1H, H1), 2.65–2.70 (m, 2H, H4, H1′), 3.12 (d, $J = 12.9$ Hz, 1H, NC*H*HPh), 3.90 (d, $J = 12.9$ Hz, 1H, NCH*H*Ph), 4.33 (dd, $J_{5,5'} = 11.6$ Hz, $J_{5,4} = 4.1$ Hz, 1H, H5), 4.50 (dd, $J_{5',5} = 11.7$ Hz, $J_{5',4} = 4.5$ Hz, 1H, H5′), 4.56–4.62 (m, 1H, H2), 5.17 (app t, $J = 2.8$ Hz, 1H, H3), 5.75 (d, $J = 7.8$ Hz, 1H, N*H*), 7.15–7.30 ppm (m, 5H, C*H*(Ar)); ^{13}C NMR (100 MHz, C_6D_6): $\delta = 20.6$, 20.7 (2×OCO*C*H_3), 22.8 (NHCO*C*H_3), 53.4 (C2), 57.5 (C1), 58.8 (NCH_2Ph), 63.1 (C5), 68.9 (C4), 80.6 (C3), 127.8, 128.8, 129.4 (*C*H(Ar)), 138.8 (*C*(Ar)), 168.7, 169.9, 170.2 ppm (3×C=O); IR (NaCl): $\tilde{\nu} = 1741$ (C=O ester), 1654 (C=O amide band I), 1542 cm^{-1} (N–H amide band I); HRMS (ESI^+): calcd for $C_{18}H_{25}N_2O_5^+$ ($[M+H]^+$): 349.1763, found: 349.1763.

***N*-Benzyl-2-acetamido-1,2,4-trideoxy-1,4-imino-D-arabinitol 6**: NaOMe (15 mg) was added to a stirred solution of *N*-benzyl-2-acetamido-3,5-di-*O*-acetyl-1,2,4-trideoxy-imino-D-arabinitol (667 mg, 1.91 mmol) in MeOH (15 mL) at room temperature under an atmosphere of N_2. After 3 h, analysis by TLC (EtOAc) showed no residual starting material ($R_f = 0.26$) and a product spot on the baseline. Analysis by TLC (acetone) showed the single spot of the product ($R_f = 0.22$). The solvents were removed in vacuo, and the resulting residue was purified by flash chromatography (98:2, acetone/TEA) to yield *N*-benzyl-2-acetamido-1,2,4-trideoxy-1,4-imino-D-arabinitol **6** as a colourless oil that changed to a white solid on leaving on the vacuum line overnight (451 mg, 89%): $R_f = 0.22$ (acetone); mp: 126–128 °C; $[\alpha]_D^{22} = -76.2$ ($c = 0.98$, MeOH); ^{1}H NMR (400 MHz, D_2O): $\delta = 1.82$ (s, 3H, NHCOCH_3), 2.49 (dt, $J_{4,3} = 5.8$ Hz, $J = 4.3$ Hz, 1H, H4), 2.55 (dd, $J_{1,1'} = 11.0$ Hz, $J_{1,2} = 2.9$ Hz, 1H, H1), 2.76 (dd, $J_{1',1} = 11.0$ Hz, $J_{1',2} = 7.2$ Hz, 1H, H1′), 3.36 (d, $J = 12.7$ Hz, 1H, NC*H*HPh), 3.59 (dd, $J_{5,5'} = 12.0$ Hz, $J_{5,4} = 4.0$ Hz, 1H, H5), 3.64 (dd, $J_{5',5} = 12.0$ Hz, $J_{5',4} = 4.6$ Hz, 1H, H5′), 3.82–3.94 (m, 3H, H3, H2, NCH*H*Ph), 7.21–7.32 ppm (m, 5H, C*H*(Ar)); ^{13}C NMR (100 MHz, D_2O): $\delta = 22.1$ (NHCO*C*H_3), 55.5 (C2), 56.3 (C1), 58.5 (NCH_2Ph), 60.3 (C5), 71.1 (C4), 77.9 (C3), 128.1, 128.9, 130.2 (*C*H(Ar)), 137.3 (*C*(Ar)), 174.2 ppm (C=O); IR (Ge): $\tilde{\nu} = 3318$ (OH), 1652 (C=O amide band I), 1554 cm^{-1} (N–H amide band II); HRMS (ESI^+): calcd for $C_{14}H_{21}N_2O_3^+$ ($[M+H]^+$): 265.1552, found: 265.1547; elemental analysis calcd (%) for $C_{14}H_{20}N_2O_3$: C 63.62, H 7.63, N 10.60, found: C 63.62, H 7.64, N 10.39.

2-Acetamido-1,2,4-trideoxy-imino-D-arabinitol 5: Pd black (25.0 mg) was added to a stirred solution of *N*-benzyl-2-acetamido-1,2,4-trideoxy-imino-L-arabinitol **6** (200 mg, 0.757 mmol) in 1,4-dioxane (4.0 mL) and H_2O (4.0 mL) at room temperature under an atmosphere of H_2. After 20 h, analysis by TLC (acetone) showed no residual starting material ($R_f = 0.22$) and a spot due to the product on the baseline. The Pd black was removed over Celite®, washing with MeOH. The solvents were removed in vacuo, and the remaining residue was purified by ion-exchange chromatography (Dowex® 50WX8–200) to yield 2-acetamido-1,2,4-trideoxy-imino-D-arabinitol **5** as a pale-brown oil (132 mg, quant.): $R_f =$ baseline (acetone); $[\alpha]_D^{22} = -4.2$ ($c = 0.84$, H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 1.88$ (s, 3H, NHCOCH_3), 2.68 (dd, $J_{1,1'} = 11.9$ Hz, $J_{1,2} = 6.2$ Hz, 1H, H1), 2.97 (dt, $J_{4,5'} = 4.4$ Hz, $J = 6.3$ Hz, 1H, H4), 3.18 (dd, $J_{1',1} = 11.9$ Hz, $J_{1',2} = 7.5$ Hz, 1H, H1′), 3.54 (dd, $J_{5,5'} = 11.8$ Hz, $J_{5,4} = 6.1$ Hz, 1H, H5), 3.63 (dd, $J_{5',5} = 11.8$ Hz, $J_{5',4} = 4.4$ Hz, 1H, H5′), 3.79 (t, $J = 6.3$ Hz, 1H, H3), 4.00 ppm (dt, $J_{2,1'} = 7.5$ Hz, $J = 6.3$ Hz, 1H, H2); ^{13}C NMR (100 MHz, D_2O): $\delta = 22.2$ (NHCO*C*H_3), 48.3 (C1), 57.5 (C2), 61.4 (C5), 64.7 (C4), 77.1 ppm (C3); IR (Ge): $\tilde{\nu} = 3285$ (OH, NH), 1652 (C=O amide band I), 1558 cm^{-1} (N–H amide band II); HRMS (ESI^+): calcd for $C_7H_{15}N_2O_3^+$ ($[M+H]^+$): 175.1083, found: 175.1083.

Acknowledgements

J.S.S.R. gratefully acknowledges the Oxford Glycobiology Institute Scholarship award, which funded the research entirely. Thanks also go to United Therapeutics, who kindly donated 2,3-isopropylidene-L-lyxonolactone.

Keywords: azasugars · chaperone proteins · GM2 gangliosidoses · inhibitors · sugar lactones

[1] R. A. Gravel, J. T. R. Clarke, M. M. Kaback, D. J. Mahuran, K. Sandhoff, K. Suzuki in *The GM2 Gangliosidoses in The Metabolic and Molecular Bases of Inherited Disease, 7th Ed.* (Eds.: C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Valle), McGraw-Hill, New York, NY, **1995**, pp. 2839–2879.
[2] J.-Q. Huang, J. M. Trasler, S. Igdoura, J. Michaud, N. Hanai, R. A. Gravel, *Hum. Mol. Genet.* **1997**, *6*, 1879–1885.
[3] P. Leinekugel, S. Michel, E. Conzelmann, K. Sandhoff, *Hum. Genet.* **1992**, *88*, 513–523.
[4] a) R. Myerowitz, F. C. Costigan, *J. Biol. Chem.* **1998**, *263*, 18587–18589; b) R. Myerowitz, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 3955–3959; c) E. Arpaia, A. Dumbrille-Ross, T. Maler, K. Neote, M. Tropak, C. Troxel, J. L. Stirling, J. S. Pitts, B. Bapat, A.-M. Lamhonwah, D. J. Mahuran, S. M. Schuster, J. T. R. Clarke, J. A. Lowden, R. A. Gravel, *Nature* **1988**, *333*, 85–86.
[5] D. J. Mahuran, *Biochim. Biophys. Acta* **1999**, *1455*, 105–138.
[6] S. K. Srivastava, E. Beutler, *Nature* **1973**, *241*, 463–463.
[7] R. D. Kremer, M. I. Levstein, *Medicina* **1980**, *40*, 55–73.
[8] R. J. Desnick, E. H. Schuchman, *Nat. Rev. Genet.* **2002**, *3*, 954–966.
[9] F. M. Platt, T. D. Butters, *Biochem. Pharmacol.* **1998**, *56*, 421–430.
[10] T. Cox, R. Lachmann, C. Hollak, J. Aerts, S. van Weely, M. Hrebicek, F. Platt, T. Butters, R. Dwek, C. Moyses, I. Gow, D. Elstein, A. Zimran, *Lancet* **2000**, *355*, 1481–1485.
[11] a) J.-Q. Fan, S. Ishii, N. Asano, Y. Suzuki, *Nat. Med.* **1999**, *5*, 112–115; b) N. Asano, S. Ishii, H. Kizu, K. Ikeda, K. Yasuda, A. Kato, O. R. Martin, J.-Q. Fan, *Eur. J. Biochem.* **2000**, *267*, 4179–4186.
[12] a) J.-Q. Fan, *Trends Pharmacol. Sci.* **2003**, *24*, 355–360; b) T. D. Butters, R. A. Dwek, F. M. Platt, *Glycobiology* **2005**, *15*, R43–R52.
[13] L. Ellgaard, M. Molinari, A. Helenius, *Science* **1999**, *286*, 1882–1888.
[14] M. B. Tropak, S. P. Reid, M. Guiral, S. G. Withers, D. Mahuran, *J. Biol. Chem.* **2004**, *279*, 13478–13487.
[15] A. A. Watson, G. W. J. Fleet, N. Asano, R. J. Molyneux, R. J. Nash, *Phytochemistry* **2001**, *56*, 265–295.
[16] N. Asano, R. J. Nash, R. J. Molyneux, G. W. J. Fleet, *Tetrahedron: Asymmetry* **2000**, *11*, 1645–1680.
[17] N. Asano, T. Yamauchi, K. Kagamifuchi, N. Shimizu, S. Takahashi, H. Takatsuka, K. Ikeda, H. Kizu, W. Chuakul, A. Kettawan, T. Okamoto, *J. Nat. Prod.* **2005**, *68*, 1238–1242.
[18] A. Mitrakou, N. Tountas, A. E. Raptis, R. J. Bauer, H. Schulz, S. A. Raptis, *Diabetic Med.* **1998**, *15*, 657–660.
[19] T. D. Butters, R. A. Dwek, F. M. Platt, *Curr. Top. Med. Chem.* **2003**, *3*, 561–574.
[20] I. Robina, A. J. Moreno-Vargas, A. T. Carmona, P. Vogel, *Curr. Drug Metab.* **2004**, *5*, 329–361.
[21] a) P. Greimel, J. Spreitz, A. E. Stutz, T. M. Wrodnigg, *Curr. Top. Med. Chem.* **2003**, *3*, 513–523; b) N. Zitzmann, A. S. Mehta, S. Carrouee, T. D. Butters, F. M. Platt, J. McCauley, B. S. Blumberg, R. A. Dwek, T. M. Block, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 11878–11882.
[22] P. E. Goss, M. A. Baker, J. P. Carver, J. W. Dennis, *Clin. Cancer Res.* **1995**, *1*, 935–944.
[23] J. S. S. Rountree, T. D. Butters, M. R. Wormald, R. A. Dwek, N. Asano, K. Ikeda, E. L. Evinson, R. J. Nash, G. W. J. Fleet, *Tetrahedron Lett.* **2007**, *48*, 4287–4291.
[24] a) G. W. J. Fleet, P. W. Smith, R. J. Nash, L. E. Fellows, R. B. Parekh, T. W. Rademacher, *Chem. Lett.* **1986**, 1051–1054; b) H. Boshagen, F. Heiker, A. Schuller, *Carbohydr. Res.* **1987**, *164*, 141–148; c) G. W. J. Fleet, L. E. Fellows, P. W. Smith, *Tetrahedron* **1987**, *43*, 979–988.
[25] T. D. Butters, L. A. G. M. van den Broek, G. W. J. Fleet, T. M. Krulle, M. R. Wormald, R. A. Dwek, and F. M. Platt, *Tetrahedron: Asymmetry* **2000**, *11*, 113–124.
[26] D. L. Zechel, S. G. Withers, *Acc. Chem. Res.* **2000**, *33*, 11–18.
[27] a) Y. Nishimura, T. Satoh, T. Kudo, S. Kondo, T. Takeuchi, *Bioorg. Med. Chem.* **1996**, *4*, 91–96; b) S. Knapp, D. Vocadlo, Z. N. Gao, B. Kirk, J. P. Lou, S. G. Withers, *J. Am. Chem. Soc.* **1996**, *118*, 6804–6805.
[28] a) G. W. J. Fleet, S. J. Nicholas, P. W. Smith, S. V. Evans, L. E. Fellows, R. J. Nash, *Tetrahedron Lett.* **1985**, *26*, 3127–3130; b) A. M. Scofield, L. E. Fellows, R. J. Nash, G. W. J. Fleet, *Life Sci.* **1986**, *39*, 645–650; c) G. W. J. Fleet, P. W. Smith, *Tetrahedron* **1986**, *42*, 5685–5692; d) J. R. Behling, A. L. Campbell, K. A. Babiak, J. S. Ng, J. Medich, P. Farid, G. W. J. Fleet, *Tetrahedron* **1993**, *49*, 3359–3366.
[29] C.-Y. Yu, N. Asano, K. Ikeda, M. X. Wang, T. D. Butters, M. R. Wormald, R. A. Dwek, A. L. Winters, R. J. Nash, G. W. J. Fleet, *Chem. Commun.* **2004**, 1936–1937.
[30] A. Kato, N. Kato, E. Kano, I. Adachi, K. Ikeda, L. Yu, T. Okamoto, Y. Banba, H. Ouchi, H. Takahata, N. Asano, *J. Med. Chem.* **2005**, *48*, 2036–2044.
[31] N. Asano, K. Ikeda, L. Yu, A. Kato, K. Takebayashi, I. Adachi, I. Kato, H. Ouchi, H. Takahata, G. W. J. Fleet, *Tetrahedron: Asymmetry* **2005**, *16*, 223–229.
[32] P. D. Croucher, R. H. Furneaux, G. P. Lynch, *Tetrahedron* **1994**, *50*, 13299–13312.
[33] a) R. H. Furneaux, G. P. Lynch, G. Way, B. Winchester, *Tetrahedron Lett.* **1993**, *34*, 3477–3480; b) B. Liessem, A. Giannis, K. Sandhoff, M. Nieger, *Carbohydr. Res.* **1993**, *250*, 19–30.
[34] a) G. W. J. Fleet, J. C. Son, *Tetrahedron* **1988**, *44*, 2637–2647; b) G. W. J. Fleet, J. C. Son, D. S. Green, I. C. Dibello, B. Winchester, *Tetrahedron* **1988**, *44*, 2649–2655; c) A. A. Bell, L. Pickering, A. A. Watson, R. J. Nash, R. C. Griffiths, M. G. Jones, G. W. J. Fleet, *Tetrahedron Lett.* **1996**, *37*, 8561–8564; d) A. E. Håkansson, J. van Ameijde, L. Guglielmini, G. Horne, R. J. Nash, E. L. Evinson, A. Kato, G. W. J. Fleet, *Tetrahedron: Asymmetry* **2007**, *18*, 282–289.
[35] I. Lundt, *Top. Curr. Chem.* **1997**, *187*, 117–156.
[36] a) I. Bruce, G. W. J. Fleet, A. Girdhar, M. Haraldsson, J. M. Peach, D. J. Watkin, *Tetrahedron* **1990**, *46*, 19–32; b) T. M. Krulle, B. Davis, H. Ardron, D. D. Long, N. A. Hindle, C. Smith, D. Brown, A. L. Lane, D. J. Watkin, D. G. Marquess, G. W. J. Fleet, *J. Chem. Soc. Chem. Commun.* **1996**, 1271–1272.
[37] H. Batra, R. M. Moriarty, R. Penmasta, V. Sharma, G. Stanciuc, J. P. Staszewski, S. M. Tuladhar, D. A. Walsh, *Org. Process Res. Dev.* **2006**, *10*, 484–486.
[38] W. J. Humphlett, *Carbohydr. Res.* **1967**, *4*, 157–164.
[39] H. Zinner, H. Voigt, J. Voigt, *Carbohydr. Res.* **1968**, *7*, 38–55.
[40] Extra care must be taken in the handling of triflate **35**, as a co-worker has experienced a strong allergic response to this compound or its decomposition products.
[41] C. C. Harding, J. S. S. Rountree, D. J. Watkin, A. R. Cowley, T. D. Butters, M. R. Wormald, R. A. Dwek, G. W. J. Fleet, *Acta Crystallogr.* **2005**, *E61*, o1683–o1685.
[42] O. Varela, P. A. Zunszain, *J. Org. Chem.* **1993**, *58*, 7860–7864.
[43] Furneaux et al. report inhibitory data for **23** against human liver β-*N*-acetylglucosaminidase (K_i = 100 μM) and β-*N*-acetylgalactosaminidase (K_i = 200 μM), Ref. [33a]; Liessem et al. report inhibitory data for **23** against the human lysosomal β-*N*-acetylhexosaminidase A α subunit (K_i = 220 μM) and β subunit (K_i = 18 μM), Ref. [33b].
[44] a) H. R. Mellor, J. Nolan, L. Pickering, M. R. Wormald, F. M. Platt, R. A. Dwek, G. W. J. Fleet, T. D. Butters, *Biochem. J.* **2002**, *366*, 225–233; b) H. R. Mellor, F. M. Platt, R. A. Dwek, T. D. Butters, *Biochem. J.* **2003**, *374*, 307–314.
[45] a) M. R. Wormald, E. W. Wooten, R. Bazzo, C. J. Edge, A. Feinstein, T. W. Rademacher, R. A. Dwek, *Eur. J. Biochem.* **1991**, *198*, 131–139; b) A. Imberty, S. Perez, *Protein Eng.* **1995**, *8*, 699–709.
[46] The homodimeric isozyme β-*N*-acetylhexosaminidase S (αα) is considered to be minor and unstable, with insignificant activity.
[47] H. Kold, I. Lundt, C. Pederson, **1994**, *48*, 675–678.

Received: October 19, 2008
Revised: December 3, 2008
Published online on January 14, 2009

