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Scalable Syntheses of Both Enantiomers of DNJNAc and DGJNAc from Glucuronolactone: The Effect of *N*-Alkylation on Hexosaminidase Inhibition

Andreas F. G. Glawar,[a, b] Daniel Best,[b] Benjamin J. Ayers,[b] Saori Miyauchi,[c] Shinpei Nakagawa,[c] Matilde Aguilar-Moncayo,[d] José M. García Fernández,[e] Carmen Ortiz Mellet,[d] Elizabeth V. Crabtree,[a, b] Terry D. Butters,[a] Francis X. Wilson,[f] Atsushi Kato,[c] and George W. J. Fleet*[a, b]

Abstract: The efficient scalable syntheses of 2-acetamido-1,2-dideoxy-D-*galacto*-nojirimycin (DGJNAc) and 2-acetamido-1,2-dideoxy-D-*gluco*-nojirimycin (DNJNAc) from D-glucuronolactone, as well as of their enantiomers from L-glucuronolactone, are reported. The evaluation of both enantiomers of DNJNAc and DGJNAc, along with their *N*-alkyl derivatives, as glycosidase inhibitors showed that DGJNAc and its *N*-alkyl derivatives were all inhibitors of α-GalNAcase but that none of the epimeric DNJNAc derivatives inhibited this enzyme. In contrast, both DGJNAc and DNJNAc, as well as their alkyl derivatives, were potent inhibitors of β-GlcNAcases and β-GalNAcases. Neither of the L-enantiomers showed any significant inhibition of any of the enzymes tested. Correlation of the in vitro inhibition with the cellular data, by using a free oligosaccharide analysis of the lysosomal enzyme inhibition, revealed the following structure–property relationship: hydrophobic side-chains preferentially promoted the intracellular access of iminosugars to those inhibitors with more-hydrophilic side-chain characteristics.

Keywords: carbohydrates · cell penetration · inhibitors · iminosugars · oligosaccharides

Introduction

Although there are many naturally occurring hydroxylated piperidines that are related to sugars in which the oxygen atom of the pyranose ring has been replaced by a nitrogen atom,[1] neither DGJNAc (2-acetamido-1,2-dideoxy-D-*galacto*-nojirimycin, **1D**) nor DNJNAc (2-acetamido-1,2-dideoxy-D-*gluco*-nojirimycin, **2D**), which are analogues of GalNAc and GlcNAc, respectively, have yet been isolated as natural products (Figure 1). Nagstatin (**3**),[2] which is isolated from the fermentation broth of *Streptomyces amakusaensis* MG846-fF3,[3] and pochonicine (**4**),[4] which is isolated from *Pochonia suchlasporia* var. *suchlasporia* TAMA 87, are potent inhibitors of β-hexosaminidases but show no inhibition of α-GalNAcases. Although synthetic piperidines are common,[5] LABNAc (2-acetamido-1,4-imino-1,2,4-trideoxy-

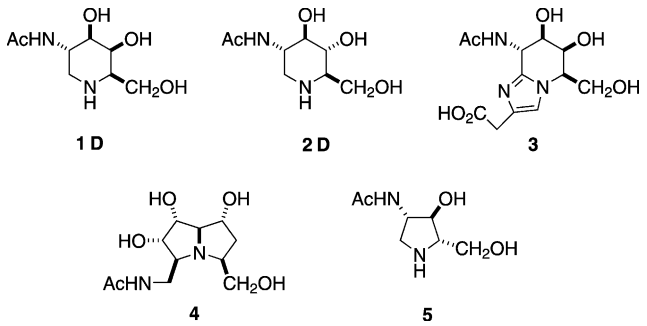

Figure 1. Hexosaminidase inhibitors.

[a] A. F. G. Glawar,[+] Dr. E. V. Crabtree, Dr. T. D. Butters, Prof. G. W. J. Fleet
Oxford Glycobiology Institute, University of Oxford
South Parks Road, Oxford, OX1 3QU (UK)
Fax: (+44) 1865 285002
E-mail: george.fleet@chem.ox.ac.uk

[b] A. F. G. Glawar,[+] Dr. D. Best,[+] B. J. Ayers, Dr. E. V. Crabtree, Prof. G. W. J. Fleet
Chemistry Research Laboratory, Department of Chemistry
University of Oxford Mansfield Road, Oxford, OX1 3TA (UK)

[c] S. Miyauchi, S. Nakagawa, Prof. A. Kato
Department of Hospital Pharmacy, University of Toyama
2630 Sugitani, Toyama 930-0194 (Japan)

[d] M. Aguilar-Moncayo, Prof. C. Ortiz Mellet
Departamento de Química Orgánica, Facultad de Química
Universidad de Sevilla, Profesor García González 1
41012 Sevilla (Spain)

[e] Prof. J. M. García Fernández
Instituto de Investigaciones Químicas
CSIC-Universidad de Sevilla
Américo Vespucio 49, Isla de la Cartuja
41092 Sevilla (Spain)

[f] Dr. F. X. Wilson
Summit PLC, 91, Milton Park
Abingdon, Oxon OX14 4RY (UK)

[+] These authors contributed equally to this work.

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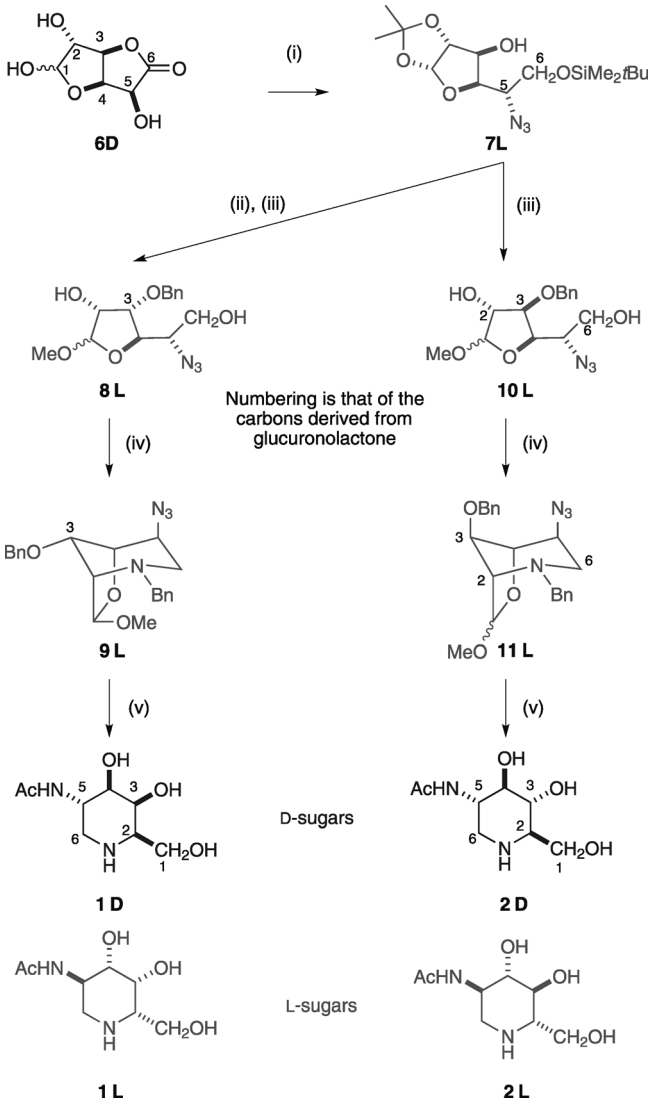
L-arabinitol, **5**) is a rare example of a pyrrolidine hexosaminidase inhibitor[6] that has promise as a chaperone in Sandhoff[7] and Tay–Sachs diseases.[8] The interest in selective inhibitors of hexosaminidases is due to their potential use in the chemotherapy of a wide range of diseases,[9] including *O*-GlcNAcase inhibition[10] and cancer metastasis.[11] DGJNAc is rare example of a potent inhibitor of α-GalNAcases.[12] Specific α-GalNAcase inhibition is of interest in the studies of Schindler–Kanzaki disease,[13] the treatment of cancer by the protection of macrophage-activating factor,[14] and the modification of a number of pathogens by the inhibition of *endo*-GalNAcases.[15] DNJNAc[16] and its *N*-alkyl derivatives[17] are highly potent and specific inhibitors of β-hexosaminidases but show no significant inhibition of α-GalNAcases. Herein, we report scalable syntheses of DGJNAc (**1D**) and DNJNAc (**2D**) from D-glucuronolactone (**6D**), as well as of their enantiomers (**1L** and **2L**, respectively) from L-glucuronolactone (**6L**), which is readily available from D-glucoheptonolactone.[18] A comparison of the inhibition profiles of both enantiomers of compounds **1** and **2**, together with a number of *N*-alkyl derivatives, is reported; the alkylation of DGJNAc (**1D**) decreases the inhibition of α-GalNAcases but not of β-hexosaminidases. The cellular lysosomal inhibition of β-hexosaminidases by compounds **1D**, **1Da**–**1Dc**, and **1De** by using protein-derived carbohydrate analysis was performed, thereby revealing enhanced cellular penetration of hydrophobic side-chain derivatives.

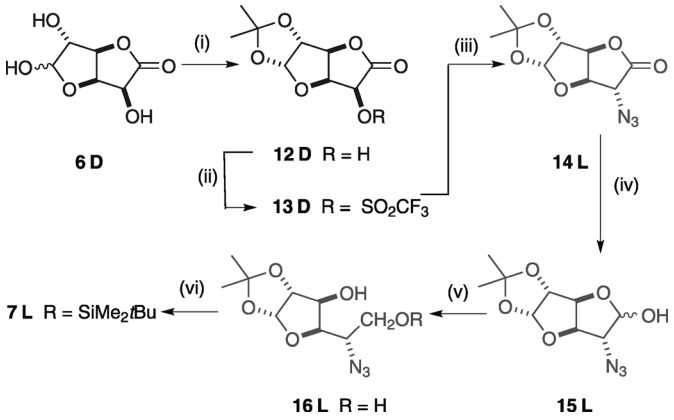
Results and Discussion

Synthesis: D-Glucuronolactone (**6D**) has been used for the efficient synthesis of many iminosugars and amino acids, wherein a nitrogen moiety is introduced at the C5 position and then joined to the C1 atom to form the nitrogen center in the piperidine ring.[19] For the preparation of both DGJNAc (**1D**) and DNJNAc (**2D**) from D-glucuronolactone (**6D**, Scheme 1): i) an azide group was introduced with inversion of the configuration at the C5 position as a precursor to the exocyclic acetamido group. Sequential reduction of the lactone at the C6 position and protection of the C6 primary alcohol gave silyl ether **7L** as a divergent intermediate. For the synthesis of DGJNAc (**1D**): ii) inversion of the configuration at the C3 position was necessary. iii) Protection as the benzyl ether and subsequent change in the protecting groups gave diol **8L**. iv) Activation of diol **8L** allowed double nucleophilic displacement of the leaving groups on the C2 and C6 positions by benzylamine to form the bicyclic piperidine (**9L**). v) Subsequent functional-group manipulation gave DGJNAc (**1D**). Performing analogous transformations (iii), (iv), and (v) without inversion of the C3 OH group in compound **7L** afforded access to DNJNAc (**2D**). The enantiomers (**1L** and **2L**, respectively) were prepared in an identical manner from L-glucuronolactone (**6L**); L-enantiomers of many iminosugars have surprising biological activities compared to their D-natural analogues.[8,20]



Scheme 1. (i) Introduction of a N atom with inversion of the stereochemistry at the C5 position and reduction of the C6 atom; (ii) inversion of the stereochemistry at the C3 position; (iii) protection of the oxygen atom at the C3 position; (iv) ring-closure between the C2 and C6 positions; (v) functional-group manipulation.

The synthesis of the divergent silyl ether in the D-series (**7L**) is shown in Scheme 2. D-Glucuronolactone (**6D**) was condensed with acetone in the presence of concentrated sulfuric acid to give the known acetonide (**12D**) in 90% yield. Esterification of the free alcohol group at the C5 position in compound **12D** with trifluoromethanesulfonic (triflic) anhydride in CH_2Cl_2 in the presence of pyridine gave the corresponding triflate (**13D**), which, on treatment with sodium azide in DMF, afforded azide **14L** with inversion of the configuration at the C5 position (92% yield). Compound **14L** was very sensitive to base, such that direct reduction with sodium borohydride gave a very low yield of the required diol (**16L**). A two-step procedure was much more efficient: initial reduction of lactone **14L** with diisobutylaluminum hydride (DIBAL-H) in CH_2Cl_2 gave lactol **15L** (84% yield),

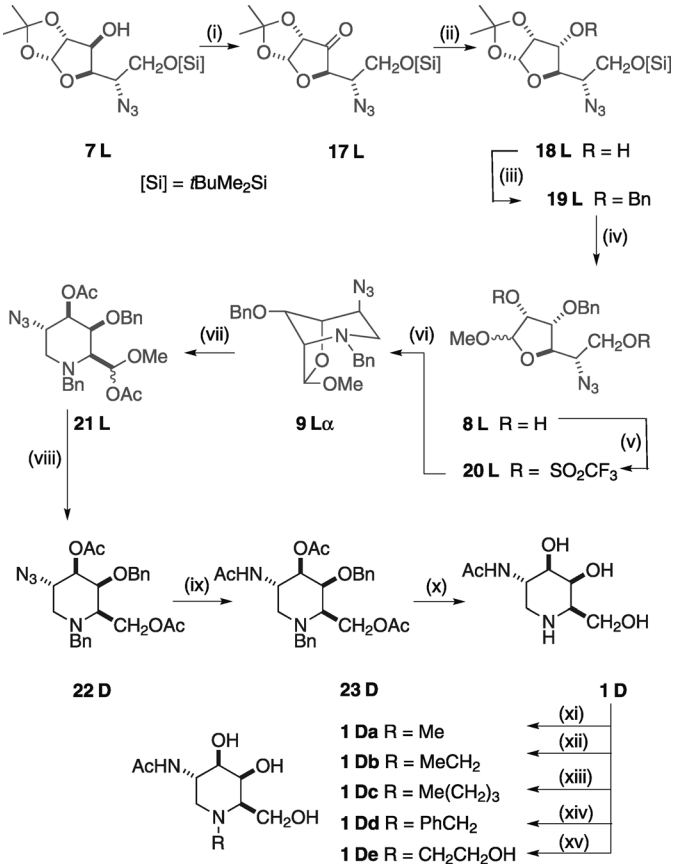


Scheme 2. (i) Me_2CO, conc. H_2SO_4, 90%; (ii) $(CF_3SO_2)_2O$, CH_2Cl_2, pyridine; (iii) NaN_3, DMF, 92% over two steps; (iv) DIBAL-H, CH_2Cl_2, 84%; (v) $NaBH_4$, MeOH, 90%; (vi) *t*$BuMe_2SiCl$, pyridine, 88%.

which, on treatment with sodium borohydride in MeOH, provided compound **16L** in 90% yield. Selective protection of the primary alcohol in compound **16L** with *tert*-butyldimethylsilyl chloride in pyridine afforded silyl ether **7L** (88% yield). Over 30 g of this key intermediate was prepared in one batch and 55% overall yield from D-glucuronolactone. The corresponding enantiomer (**7D**) was also prepared in an identical manner starting from L-glucuronolactone (**6L**).

The synthesis of DGJNAc (**1D**) required the inversion of the configuration of the alcohol at the C3 position in compound **7L**. Oxidation of compound **7L** with pyridinium chlorochromate (PCC) in CH_2Cl_2 in the presence of molecular sieves gave ketone **17L** (81% yield), which, on treatment with sodium borohydride in aqueous EtOH, underwent a highly diastereoselective reduction to give the more-hindered alcohol (**18L**) in 91% yield (Scheme 3). Treatment of compound **18L** with benzyl bromide and sodium hydride in DMF afforded the completely protected azide (**19L**, 89%). Treatment of compound **19L** with HCl in MeOH caused the concomitant removal of the silyl ether and the acetonide protecting groups to form an anomeric mixture of methyl furanosides **8L** (93% yield; α/β ratio: 7:1), which contained unprotected alcohol functionalities at the C2 and C6 positions. Esterification of both OH groups in compounds **8L** with triflic anhydride in CH_2Cl_2 in the presence of pyridine gave stable ditriflate **20L**. Reaction of ditriflate **20L** with benzylamine in THF proceeded in a stepwise manner (as inferred by monitoring the reaction by TLC) that probably involved initial nucleophilic substitution of the more reactive primary triflate group. Only the major α-anomer underwent subsequent displacement of the secondary triflate group at the C2 position to afford bicyclic piperidine **9Lα** (69% from **8L**), whilst the minor anomer remained unreacted.

All attempts to hydrolyze methyl furanoside **9Lα** by acid treatment were unsuccessful, instead leading to complex mixtures. Alternatively, acetolysis of compound **9Lα** could be achieved by conducting the reaction with over one equivalent of boron trifluoride etherate in acetic anhydride, there-



Scheme 3. (i) PCC, CH_2Cl_2, molecular sieves, 81%; (ii) $NaBH_4$, EtOH, H_2O, 91%; (iii) $PhCH_2Br$, NaH, DMF, 89%; (iv) AcCl, MeOH, 93%; (v) $(CF_3SO_2)_2O$, CH_2Cl_2, pyridine; (vi) $PhCH_2NH_2$, THF, 69% from **8L**; (vii) $Et_2O{\cdot}BF_3$, Ac_2O, 97%; (viii) DIBAL-H, CH_2Cl_2; then $NaBH_4$, MeOH; then Ac_2O, pyridine, 72% from **21L**; (ix) Zn, $CuSO_4$ (aq), THF/AcOH/Ac_2O 3:2:1, 91%; (x) MeONa, MeOH; then H_2, Pd/C (10%), HCl, 1,4-dioxane, H_2O, 99%; (xi) HCHO, H_2, Pd/C (10%), 1,4-dioxane, H_2O, 100%; (xii) MeCHO, H_2, Pd/C (10%), EtOH, 100%; (xiii) $MeCH_2CH_2CHO$, $NaBH_3CN$, AcOH, EtOH, 100%; (xiv) PhCHO, $NaBH_3CN$, AcOH, EtOH, 68%; (xv) $HOCH_2CHO$, H_2, Pd/C (10%), 1,4-dioxane, H_2O, 95%.

by leading to a mixture of the acetylated hemiacetals (**21L**, 97% yield); the first equivalent of the Lewis acid was needed to complex the amine, thereby allowing subsequent catalytic acetolysis of the glycosidic bond. A two-step reduction of compound **21L** by sequential treatment with DIBAL-H and sodium borohydride in MeOH gave the corresponding diol, which was transformed into diacetate **22D** by conventional acetylation with acetic anhydride in pyridine (72% yield). Reduction of azide **22D** with zinc in a mixture of THF/acetic-acid/acetic-anhydride in the presence of aqueous copper(II) sulfate[21] produced di-*O*-acetylated acetamide **23D** in 91% yield. Selective removal of the acetate esters in compound **23D** with sodium methoxide in MeOH, followed by hydrogenolysis of the benzyl protecting groups in the presence of 10% palladium/HCl, gave the target iminosugar DGJNAc (**1D**) in 99% yield, which corresponded to an overall yield of 27% from silyl ether **7L**. This procedure was suitable for the synthesis of DGJNAc (**1D**)

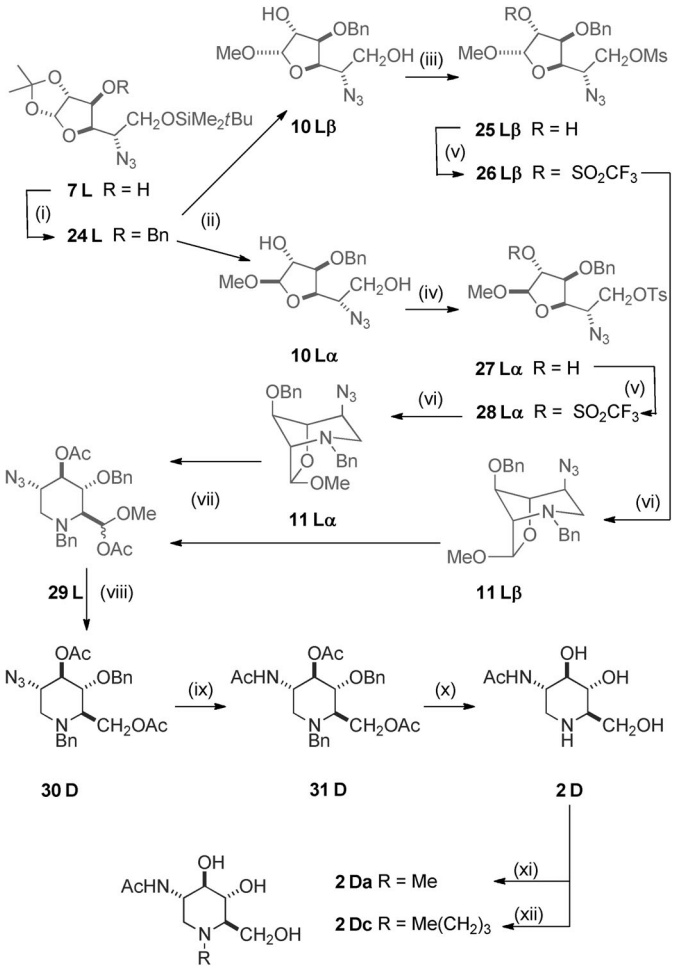
on a multigram scale in 15% yield from D-glucuronolactone (**6D**); the enantiomer L-DGJNAc (**1L**) was made by a similar sequence from L-glucuronolactone (**6L**, Scheme 3).

A series of *N*-alkylated derivatives of compound **1D** was prepared to determine the effect on hexosaminidase inhibition. Reductive amination of compound **1D** by hydrogenation with formaldehyde and glycoaldehyde in 1,4-dioxane/H_2O (1:1) in the presence of palladium gave *N*-methyl-DGJNAc (**1Da**) and *N*-(2-hydroxyethyl)-DGJNAc (**1De**) in yields of 100% and 95%, respectively. Hydrogenation of compound **1D** in EtOH in the presence of ethanal and palladium formed *N*-ethyl-DGJNAc (**1Db**) in 100% yield. Treatment of compound **1D** in EtOH with butyraldehyde and benzaldehyde in the presence of sodium cyanoborohydride gave the *N*-butyl- (**1Dc**) and *N*-benzyl analogues (**1Dd**) in yields of 100% and 68%, respectively.

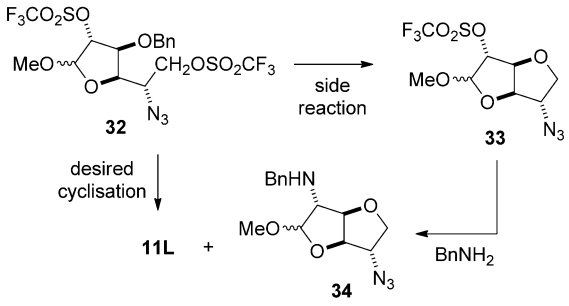
For the synthesis of DNJNAc (**2D**), silyl ether **7L** was first treated with benzyl bromide and sodium hydride in DMF to give the benzyl ether derivative (**24L**, 100%), which, on treatment with HCl in MeOH, afforded an easily separable mixture of approximately equal amounts of furanoside anomers **10Lβ** (48%) and **10Lα** (43%, Scheme 4).

Treatment of anomers **10Lα** or **10Lβ** with triflic anhydride in CH_2Cl_2 in the presence of pyridine gave ditriflate **32**, which was found to be much less stable than its C3 epimer (**20L**). The benzyl ether at the C3 position, which was in a *cis* disposition with respect to the primary triflate at the C6 position in compound **32**, spontaneously began to form 3,6-anhydro derivative **33** (Scheme 5); subsequent treatment with benzylamine in the next step gave monoamine **34**, which was not readily separable from the required bicycle (**11L**). All attempts to generate the 2,6-di-*O*-trifyl ester and react it in situ with benzylamine for the isolation of compound **11L** in good yield were unsuccessful. Replacing the triflyl groups by methanesulfonyl (mesyl) groups led to a stable disulfonate ester; however, following displacement at the C6 position with benzylamine, the compound was insufficiently reactive at the C2 position to allow ring closure. Therefore, it was necessary to introduce a less-reactive leaving group at the C6 position to suppress the formation of a 3,6-anhydro derivative whilst keeping a reactive triflate at the C2 position to facilitate ring closure.

In the case of the β-anomer (**10Lβ**), reaction with mesyl chloride in CH_2Cl_2 in the presence of 2,4,6-collidine allowed selective esterification of the primary alcohol group to produce the corresponding monomesylate (**25Lβ**, 86% yield; Scheme 4). Subsequent activation of the secondary alcohol in compound **25Lβ** by reaction with triflic anhydride formed mixed disulfonate **26Lβ**, which, on treatment with benzylamine, gave the bicyclic piperidine (**11Lβ**) in 70% yield. The α-anomer (**10Lα**) did not undergo selective mesylation under the above conditions. However, the reaction of compound **10Lα** with *p*-toluenesulfonyl (tosyl) chloride afforded the primary tosylate (**27Lα**) in satisfactory yield (74%). Esterification of compound **27Lα** with triflic anhydride formed the triflate (**28Lα**), which underwent double nucleophilic displacement with benzylamine to give the fully protected piperidine (**11Lα**, 68%). Acetolysis of bicyclic amines **11Lα** and **11Lβ** with boron trifluoride etherate in



Scheme 4. (i) $PhCH_2Br$, NaH, DMF, 100%; (ii) AcCl, MeOH, 48% **10Lβ**, 43% **10Lα**; (iii) $MeSO_2Cl$, 2,4,6-collidine, CH_2Cl_2, 86%; (iv) TsCl, 2,4,6-collidine, CH_2Cl_2, 74%; Ts = 4-toluenesulfonyl; (v) $(CF_3SO_2)_2O$, CH_2Cl_2, pyridine; (vi) $PhCH_2NH_2$, THF, 70% from **25Lβ**, 68% from **27Lα**; (vii) $Et_2O{\cdot}BF_3$, Ac_2O, 75% from **11Lβ**, 71% from **11Lα**; (viii) DIBAL-H, CH_2Cl_2, pyridine; then $NaBH_4$, MeOH; then Ac_2O, pyridine, 78%; (ix) Zn, $CuSO_4$ (aq), THF/AcOH/Ac_2O 3:2:1, 79%; (x) MeONa, MeOH; then H_2, Pd/C (10%), HCl, 1,4-dioxane, H_2O, 94%; (xi) HCHO, H_2, Pd/C (10%), H_2O, 94%; (xii) $MeCH_2CH_2CHO$, H_2, Pd/C (10%), 1,4-dioxane, H_2O, 96%.



Scheme 5. Unwanted formation of THF.

acetic anhydride led to the same epimeric mixture of the diacetates (**29L**) in 71% and 75% yield, respectively. A two-step reduction of compound **29L** by sequential treatment with DIBAL-H and sodium borohydride in MeOH, followed by acetylation with acetic anhydride in pyridine, gave the diacetate (**30D**) in 78% yield. Reductive acetylation of the azide group in compound **30D** with zinc/copper-sulfate in THF/acetic-acid/acetic-anhydride provided amide **31D** (79% yield), which, on deprotection by conventional catalytic transesterification and hydrogenolysis, formed DNJNAc (**2D**) in 94% yield. Enantiomer L-DNJNAc (**2L**) was prepared in a similar sequence from L-glucuronolactone (**6L**). Reductive alkylation of compound **2D** by hydrogenation with formaldehyde in water and butyraldehyde in aqueous 1,4-dioxane gave *N*-methyl-DNJNAc (**2Da**, 94%) and *N*-butyl-DNJNAc (**2Dc**, 96%), respectively.

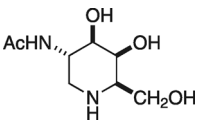
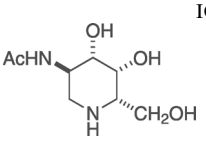
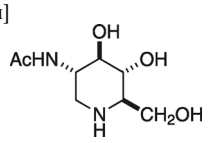
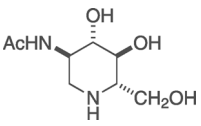
Biological evaluation: Both enantiomers of DGJNAc and DNJNAc, as well as their *N*-alkyl derivatives, were tested in vitro for their glycosidase-inhibiting potential against the following enzymes: β-*N*-acetyl-glucosaminidase (β-GlcNAcase), β-*N*-acetyl-galactosaminidase (β-GalNAcase), and α-*N*-acetyl-galactosaminidase (α-GalNAcase; Tables 1–3); the influence of DGJNAc (**1D**) and its derivatives **1Da**, **1Db**, **1Dc**, and **1De** on lysosomal glycosidase inhibition was investigated by using a free oligosaccharide (FOS) analysis in HL60 cells (Figure 2, Table 4). This set of glycosidases has been implicated in a range of pathological disorders. In cancer patients that carry pancreatic adenocarcinoma[22] or gliomas,[23] β-HexNAcase activity is elevated in the blood in comparison to healthy individuals. α-GalNAcase activity was identified as a biomarker for patients that suffer from melanoma.[14a] Furthermore, inhibitors of these enzymes could be employed to treat lysosomal-storage disorders, such as Tay–Sachs, Sandhoff, and Schindler–Kanzaki diseases, by improving mutant-enzyme activity through a chaperone-mediated pathway.[24] *N*-Butyl deoxynorjirimycin (*N*Bu-DNJ, Zavesca), which is structurally related to *N*-butyl-DGJNAc (**1Dc**), has been approved for the treatment of the lysosomal-storage disorder Type 1 Gaucher disease by substrate-reduction therapy. The *N*-hydroxyethyl derivative of DNJ (Glyset, Miglitol), which is analogous to compound **1De**, is used for the treatment of non-insulin-dependent diabetes mellitus.

Comparison of DGJNAc (1D) versus DNJNAc (2D): Table 1 shows the inhibitory data of the enantiomers of DGJNAc (**1**) and DNJNAc (**2**); the L-enantiomers of D-iminosugar competitive glycosidase inhibitors are often non-competitive inhibitors of the same enzyme.[25] The syntheses reported herein from the enantiomers of glucuronolactone ensured the enantiomeric purity of inhibitors **1** and **2**. Neither of the L-enantiomers (**1L** and **2L**) significantly inhibited any of the glycosidases at the highest concentration tested; L-DGJNAc (**1L**) showed very weak inhibition (IC_{50} = 830 μM) of human placenta β-GlcNAcase. In contrast, both compounds **1D** and **2D** were good inhibitors of β-GlcNAcases, with the exception of enzyme from *Aspergillus oryzae*. Therefore, β-GlcNAcases were inhibited by both epimers at the C4 position with either gluco- or galacto-stereochemistry; the same was true for β-GalNAcase inhibition against the enzyme that was derived from HL60 cell lysate. These results suggested that the stereochemistry at the C4 OH group was not strictly distinguished by β-GalNAcases and β-GlcNAcases.

DGJNAc (**1D**) was a potent inhibitor of α-GalNAcase with an IC_{50} value of 0.32 μM against chicken liver and a K_i value of 0.17 μM against *Charonia lampas*.[12] None of the other three NH iminosugars (compounds **1L**, **2D**, or **2L**) significantly inhibited α-GalNAcase from chicken liver (Table 1).

***N*-Alkyl derivatives of DNJNAc (2D)**: The *N*-alkylated derivatives of DNJNAc **2D** (**2Da** and **2Dc**) showed similar in-

Table 1. Concentrations of the iminosugars that give 50% inhibition of various glycosidases.

	IC_{50} [μM]			
				
Enzyme β-*N*-acetyl-glucosaminidase	DGJNAc (**1D**)	L-DGJNAc (**1L**)	DNJNAc (**2D**)	L-DNJNAc (**2L**)
human placenta	8.3	830	7.0	NI (31.2)
bovine kidney	4.2	NI (46.8)	7.4	NI (24.4)
Aspergillus oryzae	NI[a] (8.8)[b]	NI (0.8)	891	NI (0.7)
HL60	7.1	NI (31.3)	9.8	NI (25.9)
jack beans	1.8	NI (46.3)	2.9	NI (28.8)
α-*N*-acetyl-galactosaminidase				
chicken liver	0.32	NI (22.6)	NI (5.0)	NI (36.9)
β-*N*-acetyl-galactosaminidase				
Aspergillus oryzae	NI (9.6)	NI (0)	998	NI (0)
HL60	23	NI (12.9)	17	NI (14.8)

[a] NI = No inhibition (less than 50% inhibition at 1000 μM). [b] Inhibition (%) at 1000 μM are given in parentheses.

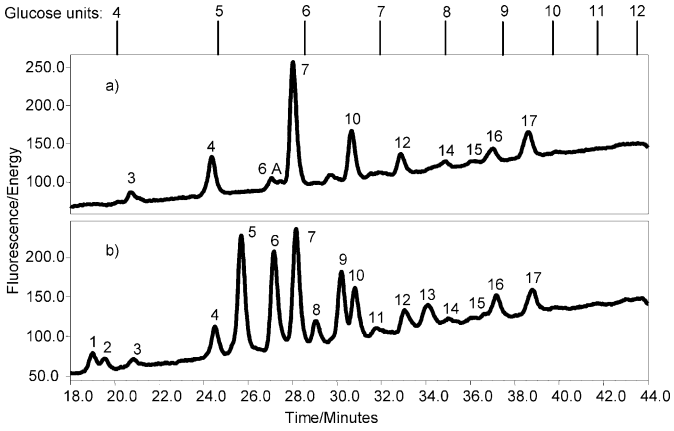


Figure 2. HL60 cells were homogenized and the FOS was extracted as described in the Experimental Section. After labeling with 2-AA, the FOS were separated by using HPLC. a) Control cells; b) cells that were treated with DGJNAc (**1D**, 500 μM). The peaks are numbered and their corresponding structures are given in Table 4.

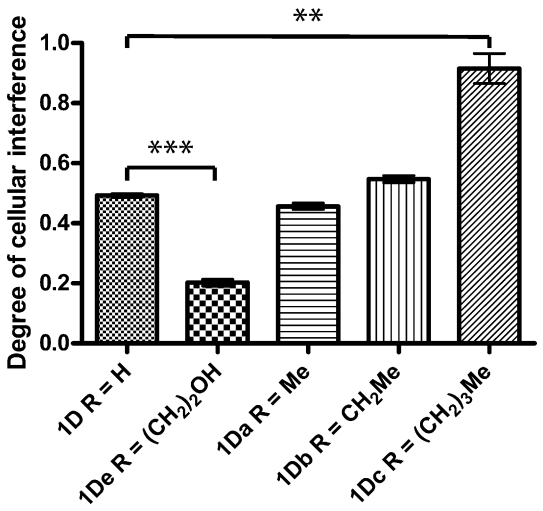


Figure 3. Ratio of the integration of peak 5 to the control peak 7 is shown for the derivatives of compound **1D** (50 μM). Because all of the derivatives have very comparable in vitro inhibition, the variation can be attributed to the differences in cell- and organelle penetration. To test for significance, a student t-test was performed (99% confidence interval).

hibition of β-GlcNAcase or β-GalNAcase to that of the NH parent compound (Table 2). In general, *N*-alkylation of compound **2D** had little effect on enzyme inhibition, in contrast to the observed increased inhibition for the L-lysine-linked dansyl derivatives of DNJNAc that were generated by Steiner et al.;[17b] however, inhibition of the *Aspergillus oryzae* enzyme was significantly increased by alkylation, notably for *N*-methyl-DNJNAc (**2Da**). None of the derivatives of compound **2D** inhibited α-GalNAcase.

***N*-Alkyl derivatives of DGJNAc (1D)**: All of the derivatives of DGJNAc (**1D**) were good inhibitors of β-GlcNAcases (except for *Aspergillus oryzae*, Table 3), although *N*-benzyl-DGJNAc (**1Dd**) was significantly weaker. *N*-Ethyl- (**1Db**), *N*-benzyl- (**1Dd**), and *N*-hydroxyethyl-DGJNAc (**1De**) were all good inhibitors of α-GalNAcase that was derived from chicken liver. However, the methyl and butyl groups seemed to be less-well-tolerated by the same enzyme. This trend was reflected in the K_i values against the enzyme that was derived from *Charonia lampas*, with the exception of the *N*-methyl derivative.

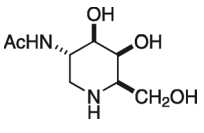
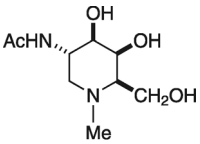
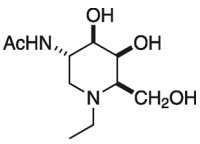
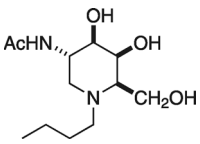
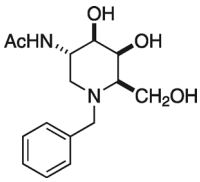
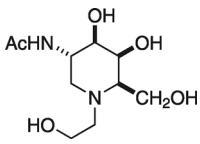
Free oligosaccharide (FOS) analysis[26] was performed on HL60 cells after treatment for 24 h with the *N*-alkyl derivatives of DGJNAc (**1Da**, **1Db**, **1Dc**, and **1De**), which gave additional free glycans (FOS) in the HPLC profiles that were derived from HL60 homogenates (Figure 2b) in comparison to the untreated control reactions (Figure 2a). Subsequent treatment of these FOSs with jack bean β-GlcNAcase identified the newly produced glycans as non-reducing terminal GlcNAc-containing species; their identity was verified by comparison with the retention times in glucose units

Table 2. Concentrations of DNJNAc and its *N*-alkyl derivatives that give 50% inhibition of various glycosidases.

	IC_{50} [μM]		
Enzyme	DNJNAc (**2D**)	*N*-Me-DNJNAc (**2Da**)	*N*-Bu-DNJNAc (**2Dc**)
β-*N*-acetyl-glucosaminidase			
human placenta	7.0	18	10
bovine kidney	7.4	12	8.1
Aspergillus oryzae	891	225	478
HL60	9.8	22	16
jack beans	2.9	17	24
α-*N*-acetyl-galactosaminidase			
chicken liver	NI[a] (5.0)[b]	NI (0.8)	NI (9.6)
β-*N*-acetyl-galactosaminidase			
Aspergillus oryzae	998	167	381
HL60	17	74	62

[a] NI = No inhibition (less than 50% inhibition at 1000 μM). [b] Inhibition (%) at 1000 μM are given in parentheses.

Table 3. Concentrations of DGJNAc and its *N*-alkyl derivatives that give 50% inhibition of various glycosidases.

	IC_{50} [μM]					
						
Enzyme	DGJNAc (**1D**)	*N*-Me-DGJNAc (**1Da**)	*N*-Et-DGJNAc (**1Db**)	*N*-Bu-DGJNAc (**1Dc**)	*N*-Bn-DGJNAc (**1Dd**)	*N*-(2-Hydroxyethyl)-DGJNAc (**1De**)
β-*N*-acetyl-glucosaminidase						
human placenta	8.3	5.7	7.3	2.7	45	8.2
bovine kidney	4.2	2.1	3.1	1.2	40	3.7
Aspergillus oryzae	NI[a] (8.8)[b]	NI (22.1)	NI (26.5)	NI (22.6)	NI (11.4)	NI (46.9)
HL60	7.1	5.4	7.9	1.7	52	8.3
jack beans	1.8	1.5	3.4	2.7	22	1.9
α-*N*-acetyl-galactosaminidase						
chicken liver	0.32	107	5.6	166	11	6.3
Charonia lampas	[0.17][c]	[0.97]	[7.9]	[148]	[14]	[3.9]
β-*N*-acetyl-galactosaminidase						
Aspergillus oryzae	NI (9.6)	NI (29.4)	NI (26.3)	NI (29.8)	NI (5.8)	926
HL60	23	26	44	14	187	35

[a] NI = No inhibition (less than 50% inhibition at 1000 μM). [b] Inhibition (%) at 1000 μM are given in parentheses. [c] K_i values are given in square brackets.

(GUs) reported by Boomkamp et al. (Table 4).[26] A structure of the glycans (peaks 8, 11, and 13) was proposed based on their retention times and the β-GlcNAcase-digestion results. The appearance of non-reducing terminal GlcNAc-containing glycans was dose-dependent for all of the inhibitors tested over the range 500 μM, 100 μM, and 50 μM; moreover, their appearance was due to inhibition of lysosomal β-HexNAcase, thus leading to incomplete degradation of the glycans.

Quantitative comparison of glycan 5 ($GlcNAc_2Man_3GlcNAc_1$) with control glycan 7 ($Man_5GlcNAc_1$) allowed an estimation of the degree of lysosomal β-GlcNAcase inhibition. At a concentration of 50 μM, the levels of inhibition that were displayed by the derivatives showed strong differences depending on the side-chain that was present on the inhibitor. Increasing the length of the alkyl side-chain in comparison to parent compound **1D** led to stronger inhibition in this cell-based assay, whilst the hydroxyethyl variant showed lower levels of inhibition (Figure 3). With the in vitro inhibition activity against β-GlcNAcase of all of the inhibitors being of comparable strength (HL60 data, Table 3), the apparent differences between the inhibition of lysosomal β-GlcNAcase in the cells could be attributed to differential penetration of the cells or organelles by the inhibitors. Accordingly, the *N*-butyl derivative (**1Dc**) was approximately 5-fold more-effective at inhibiting lysosomal β-GlcNAcase than the *N*-hydroxyethyl derivative (**1De**). This result correlated with the bio-distribution of two iminosugar inhibitors of DNJ that are already used clinically. Miglustat (Zavesca), the *N*-butyl derivative of DNJ, shows a volume of distribution (V_D) of 1.04–1.31 $L\,kg^{-1}$ (calculated for an 80 kg individual),[27] whilst Miglitol (Glyset), which is the *N*-hydroxyethyl derivative of DNJ, has a value of $V_D = 0.18\,L\,kg^{-1}$, which is 5.7–7.3-fold lower.[28] Therefore, whilst Miglustat is able to penetrate tissues beyond the bloodstream, Miglitol is primarily restricted to the extracellular fluid. Previous results have also demonstrated that the *N*-alk(en)yl derivatives (C_4, C_9, C_{18}) of DNJ are taken up by cells in less than 1 min.[29]

In the design of a potential therapeutic agent, a polar *N*-alkyl group, such as *N*-hydroxyethyl **1De**, on the ring nitrogen atom could restrict the iminosugar to the extracellular space, whilst a hydrophobic side-chain derivative, such as *N*-butyl, would assist in the cell-membrane-penetration of the inhibitor. Therefore, the correlation of the in vitro data with those in cells could be used to estimate how well the inhibitor is able to reach an intracellular therapeutic target. If the target is secreted into extracellular space, this method could be used to estimate the side-effects of intracellular enzyme inhibition. The iminosugar derivatives presented herein all showed this inhibition to some degree, albeit only at very non-physiological high concentrations of 50 μM (the maximum plasma concentration of Miglustat in a multiple dose regime is 12.7 μM; the maximum plasma concentration of Miglitol in a 100 mg oral dose is 8.4 μM).[30,31]

Table 4. Structures, number of glucose units (GUs), and percentage proportion of isolated FOS peaks from the control experiment and 500 μM DGJNAc-treated HL60 cells.[a]

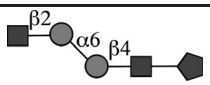
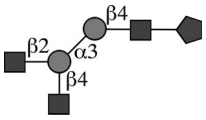
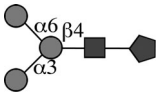
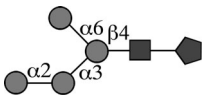
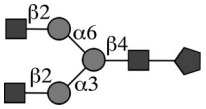
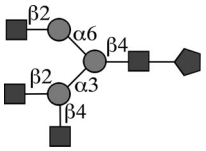
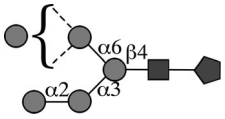
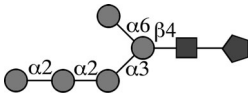
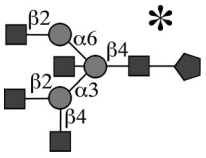
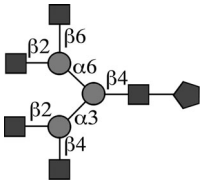
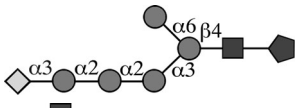
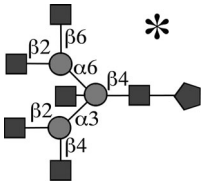
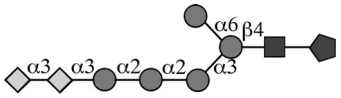
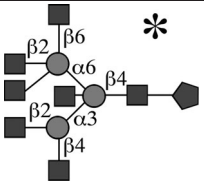
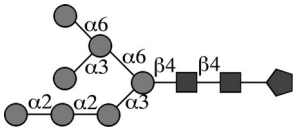
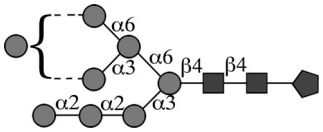
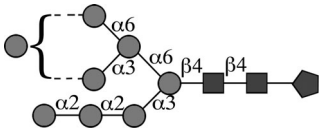
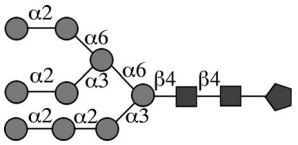
ID	Untreated GUs	Treated GUs	Structure name	Structure	In control cells [%]	In treated cells [%]
1	–	3.77	$GlcNAc_1Man_2GlcNAc_1$		–	2.45(±0.10)
2	–	3.88	$GlcNAc_2Man_2GlcNAc_1$		–	1.62(±0.21)
3	4.13	4.16	$Man_3GlcNAc_1$		3.44(±1.16)	1.66(±0.88)
4	4.95	4.99	$Man_4GlcNAc_1$		14.37(±1.67)	5.20(±0.93)
5	–	5.28	$GlcNAc_2Man_3GlcNAc_1$		–	17.98(±0.68)
6	–	5.65	$GlcNAc_3Man_3GlcNAc_1$		–	15.08(±0.11)
6A	5.62	–	$Man_5GlcNAc_1$		4.22(±0.45)	–
7	5.87	5.91	$Man_5GlcNAc_1$		38.76(±1.06)	16.81(±0.37)
8	–	6.16	$GlcNAc_4Man_3GlcNAc_1$		–	2.92(±0.29)
9	–	6.49	$GlcNAc_4Man_3GlcNAc_1$		–	10.00(±0.24)
10	6.62	6.67	$Glc_1Man_5GlcNAc_1$		14.92(±0.13)	7.37(±0.36)
11	–	6.96	$GlcNAc_5Man_3GlcNAc_1$		–	1.35(±0.16)
12	7.31	7.37	$Glc_2Man_5GlcNAc_1$		6.17(±0.35)	3.24(±0.57)

Table 4. (Continued)

ID	Untreated GUs	Treated GUs	Structure name	Structure	In control cells [%]	In treated cells [%]
13	–	7.73	$GlcNAc_6Man_3GlcNAc_1$		–	4.51(±0.12)
14	7.99	8.04	$Man_7GlcNAc_2$		2.25(±0.81)	0.88(±0.20)
15	8.47	8.42	$Man_8GlcNAc_2$		1.25(±0.12)	1.03(±0.60)
16	8.79	8.87	$Man_8GlcNAc_2$		5.39(±0.58)	3.88(±0.29)
17	9.46	9.55	$Man_9GlcNAc_2$		9.24(±0.54)	4.05(±0.23)

[a] The peak IDs correspond to the labels in Figure 2. The mean values were each taken from three independent measurements (±S.D.). The species were assigned in reference to the GU units in ref. [26]. The labels are as follows:

◇ Glucose ● Mannose ⬟ 2-aminobenzoic acid ■ *N*-acetylglucosamine ✻ Proposed structure

Conclusion

This paper describes efficient scalable syntheses of both enantiomers of DGJNAc (**1**) and DNJNAc (**2**) from glucuronolactone, thereby allowing easy access to this class of hexosaminidase inhibitor. DGJNAc (**1D**) and its *N*-alkyl derivatives (**1Da**–**1De**) were all inhibitors of α-GalNAcase but none of the epimeric DNJNAc (**2**) derivatives inhibited the same enzyme. In contrast, both DGJNAc (**1D**) and DNJNAc (**2D**), as well as their alkyl derivatives, were potent inhibitors of β-hexosaminidases. Neither of their enantiomers (**1L** and **2L**, respectively) showed any significant inhibition against this enzyme. The correlation of the in vitro inhibition data against β-GlcNAcase with the cellular lysosomal inhibition data of this enzyme by using protein-derived carbohydrate analysis revealed a side-chain-dependent variation in cell- and organelle penetration. Hydrophobic side-chains of increasing length promote cellular uptake whilst hydrophilic variants restrict access of the inhibitor to intracellular organelles.

Experimental Section

All commercial reagents were used as supplied. Dry pyridine and DMF were purchased from Aldrich in Sure-Seal™ bottles. All other solvents were used as supplied (analytical or HPLC grade). The reactions that were performed under an atmosphere of argon or hydrogen were maintained by an inflated balloon. All water sensitive reactions were performed under an inert atmosphere of argon/nitrogen unless otherwise stated. All solutions are saturated unless otherwise stated. Thin layer chromatography (TLC) analysis was performed on aluminum sheets (Merck) that were coated with 60 F_{254} silica. The sheets were either visualized by using a dip of 0.2% w/v cerium(IV) sulfate and 5% ammonium molybdate in 2 M sulfuric acid or potassium permanganate (0.5% in 1 M NaOH). Flash chromatography was either performed on Sorbsil C60 40/60 silica or Merck grade 9385, 230–400 mesh, 60 Å. Where chromatographic techniques that involve triethylamine-containing mobile phases were employed, silica was doped with triethylamine before use. Melting points were recorded on a Kofler hot block and are uncorrected. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are quoted in g 100 mL^{-1}. IR spectra were recorded on a Bruker Tensor 27 FTIR spectrophotometer by using thin films on either NaCl or Ge plates. Only the characteristic peaks are quoted. NMR spectra were record on a Bruker AMX500 (^{1}H: 500 MHz and ^{13}C: 125.7 MHz) or on a Bruker AV400 spectrometer (^{1}H: 400.2 MHz and ^{13}C: 100.6 MHz) in the stated deuterated solvent. Chemical shifts (δ) are quoted in ppm and coupling constants (J) are given in Hz. Residual signals from the solvents were used as an internal reference. MeCN or MeOH were used as an internal reference when D_2O was used as the solvent. MS (ESI) was recorded on a Micromass LCT spectrometer. HRMS (ESI) was performed on a Bruker MicroTof (resolution = 10000 FWHM). Assays for the inhibition of glycosidases were performed according to literature procedures.[32]

Synthesis of divergent protected silyl ether 7

*1,2-O-Isopropylidene-α-*D*-glucurono-3,6-lactone* (***12D***): D-Glucurono-3,6-lactone (**6D**, 30.0 g, 170 mmol) was dissolved in acetone (250 mL) and

concentrated sulfuric acid (8.3 mL). The mixture was stirred at RT for 4 h, after which time TLC analysis (EtOAc) indicated the complete consumption of the starting material (R_f = 0.40, streaks to baseline) and the formation of a major product (R_f = 0.88). The reaction mixture was neutralized by the addition of solid sodium bicarbonate, filtered, and concentrated in vacuo. The crude residue was recrystallized (toluene) to afford acetonide **12D** as a white crystalline solid (33.0 g, 90%). M.p. 118–120°C (toluene) [lit. 120.5–121.5°C[19c] (toluene)]; $[\alpha]_D^{25}$ = +59.2 (*c* 2.00, $CHCl_3$) [lit. $[\alpha]_D^{20}$ = +52.5[19c] (*c* 1.95, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.36, 1.53 (s, 2×3H; $C(CH_3)_2$), 3.02 (s, 1H; OH5), 4.53 (d, *J*(5,4) = 4.4 Hz, 1H; H5), 4.83 (d, *J*(2,1) = 3.4 Hz, 1H; H2), 4.84 (d, *J*(3,4) = 2.7 Hz, 1H; H3), 4.95 (dd, *J*(4,3) = 3.1 Hz, *J*(4,5) = 4.4 Hz, 1H; H4), 6.00 ppm (d, *J*(1,2) = 3.4 Hz, 1H; H1); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 26.5, 26.9 ($C(CH_3)_2$), 70.6 (C5), 78.1 (C4), 81.3 (C3), 82.9 (C2), 106.6 (C1), 113.6 ($C(CH_3)_2$), 173.7 ppm (C6); IR (thin film): $\tilde{\nu}$ = 1790 (s; C=O), 3441 cm^{-1} (s; OH); LRMS (ESI+): *m*/*z* (%): 239 (54) $[M+Na]^+$, 271 (30) $[M+MeOH+Na]^+$, 455 cm^{-1} (100) $[2M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_9H_{12}NaO_6$: 239.0526 $[M+Na]^+$; found: 239.0526.

For enantiomer **12L**: M.p. 117–119°C (toluene); $[\alpha]_D^{25}$ = −62.3 (*c* 2.00, $CHCl_3$).

*5-Azido-5-deoxy-1,2-O-isopropylidene-β-*L*-idurono-3,6-lactone* (***14L***): Trifluoromethanesulfonic anhydride (39.1 mL, 232 mmol) was added dropwise to a solution of lactone **12D** (38.65 g, 178.8 mmol) in CH_2Cl_2 (400 mL) and pyridine (43.4 mL, 536 mmol) at −40°C. The reaction mixture was stirred for 1 h at between −35°C and −25°C. After this time, TLC analysis (cyclohexane/EtOAc, 1:1) indicated the complete consumption of the starting material (R_f = 0.32) and the formation of a major product (R_f = 0.80). The reaction mixture was diluted with CH_2Cl_2 (150 mL), washed with 2M HCl (3×600 mL), and the organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to afford crude triflate **13D**. M.p. 160–162°C [lit. 160°C[19c] (EtOH)]; $[\alpha]_D^{25}$ = +55.1 (*c* 1.01, $CHCl_3$) [lit. $[\alpha]_D^{20}$ = +49.2[19c] (*c* 1.28, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.37, 1.54 (s, 2×3H; $C(CH_3)_2$), 4.87 (d, *J*(2,1) = 3.6 Hz, 1H; H2), 4.94 (d, *J*(3,4) = 2.7 Hz, 1H; H3), 5.08 (dd, *J*(4,3) = 2.9 Hz, *J*(4,5) = 4.3 Hz, 1H; H4), 5.42 (d, *J*(5,4) = 4.3 Hz, 1H; H5), 6.06 ppm (d, *J*(1,2) = 3.6 Hz, 1H; H1).

Sodium azide (15.20 g, 232.4 mmol) was added in one portion to a solution of crude triflate **13D** in DMF (400 mL) at −30°C. After 90 min, TLC analysis (cyclohexane/EtOAc, 2:1) indicated the complete consumption of the starting material (R_f = 0.58) and formation of a major product (R_f = 0.72). The reaction mixture was partitioned between EtOAc (600 mL) and a water/sat. brine (4:1) solution (300 mL), separated, and the organic phase was washed with water/sat. brine (4:1, 2×300 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (cyclohexane/EtOAc, 5:1) to afford the azide (**14L**) as a white crystalline solid (39.7 g, 92%). M.p. 110–112°C [lit. 114–116°C];[19c] $[\alpha]_D^{25}$ = +240.1 (*c* 1.00, $CHCl_3$) [lit. $[\alpha]_D^{20}$ = +243[19c] (*c* 1.1, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.35, 1.51 (s, 2×3H; $C(CH_3)_2$), 4.24 (s, 1H; H5), 4.66 (d, *J*(4,3) = 3.1 Hz, 1H; H4), 4.84 (d, *J*(2,1) = 3.8 Hz, 1H; H2), 4.95 (d, *J*(3,4) = 3.1 Hz, 1H; H3), 5.94 ppm (d, *J*(1,2) = 3.8 Hz, 1H; H1); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 26.5, 27.0 ($C(CH_3)_2$), 61.2 (C5), 81.1 (C4), 81.8 (C2), 84.9 (C3), 106.2 (C1), 113.4 ($C(CH_3)_2$), 170.7 ppm (C6); IR (thin film): $\tilde{\nu}$ = 1792 (s; C=O), 2124 cm^{-1} (s; N_3); LRMS (ESI+): *m*/*z* (%): 264 (25) $[M+Na]^+$, 296 (100) $[M+MeOH+Na]^+$, 328 (15) $[M+2MeOH+Na]^+$, 569 (85) $[2M+2MeOH+Na]^+$; (ESI−): 276 (100) $[M+^{35}Cl]^-$, 278 (40) $[M+^{37}Cl]^-$; HRMS (ESI+): *m*/*z* calcd for $C_{10}H_{15}N_3NaO_6$: 296.0853 $[M+MeOH+Na]^+$; found: 296.0855.

For enantiomer **14D**: M.p. 111–113°C; $[\alpha]_D^{25}$ = −213.5 (*c* 0.96, $CHCl_3$).

*5-Azido-5-deoxy-1,2-O-isopropylidene-β-*L*-ido-hexodialdo-1,4:3,6-difuranose* (***15L***): Diisobutylaluminum hydride (1.5M in toluene, 91.0 mL, 137 mmol) was added dropwise to a solution of azide **14L** (21.9 g, 90.8 mmol) in CH_2Cl_2 (200 mL) under an argon atmosphere at −78°C. The reaction mixture was stirred at this temperature for 90 min. After this time, TLC analysis (cyclohexane/EtOAc, 2:1) indicated the complete consumption of the starting material (R_f = 0.72) and formation of two products (major R_f = 0.40, minor R_f = 0.39). A saturated solution of sodium potassium tartrate (500 mL) and CH_2Cl_2 (600 mL) were added and the reaction mixture was stirred vigorously at RT for 18 h. The organic phase was collected and the aqueous layer was extracted with CH_2Cl_2 (8×300 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated in vacuo to afford crude lactol **15L** as a pale-yellow crystalline solid (18.6 g, 84%) in a 2:3 (A/B) ratio of the anomers. This crude product was reacted on without further purification. M.p. 53–55°C; $[\alpha]_D^{25}$ = −21.4 (*c* 1.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.33 (s, 3H; CH_3^B), 1.35 (s, 3H; CH_3^A), 1.50 (s, 6H; CH_3^A and CH_3^B), 3.45 (br s, 1H; $OH6^B$), 4.08 (s, 1H; $H5^A$), 4.08 (d, *J*(5,6) = 4.1 Hz, 1H; $H5^B$), 4.64 (d, *J*(2,1) = 3.8 Hz, 1H; $H2^B$), 4.72 (d, *J*(3,4) = 3.4 Hz, 1H; $H3^B$ or $H4^B$), 4.75 (app t, *J* = 3.1 Hz, 2H; $H2^A$, $H3^A$ or $H4^A$), 4.79 (d, *J*(3,4) = 3.8 Hz, 1H; $H3^B$ or $H4^B$), 4.81 (d, *J*(3,4) = 4.1 Hz, 1H; $H3^A$ or $H4^A$), 5.34 (s, 1H; $H6^A$), 5.52 (d, *J*(5,6) = 4.1 Hz, 1H; $H6^B$), 5.88 (d, *J*(1,2) = 3.4 Hz, 1H; $H1^B$), 6.00 ppm (d, *J*(1,2) = 3.8 Hz, 1H; $H1^A$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 26.5, 27.2 ($C(CH_3)_2{}^B$), 26.7, 27.3 ($C(CH_3)_2{}^A$), 66.4 ($C5^B$), 69.1 ($C5^A$), 83.5 ($C2^B$), 83.6, 85.0 (2C; $C3^B$ and $C4^B$), 84.7, 85.2, 86.9 (3C; $C2^A$, $C3^A$, and $C4^A$), 98.9 ($C6^B$), 102.5 ($C6^A$), 106.5 ($C1^B$), 106.8 ($C1^A$), 112.7 ($C(CH_3)_2{}^B$), 113.0 ppm ($C(CH_3)_2{}^A$); IR (thin film): $\tilde{\nu}$ = 2113 (s, N_3), 3425 cm^{-1} (br s; OH); LRMS (ESI+): *m*/*z* (%): 298 (100) $[M+MeOH+Na]^+$, 573 (83) $[2M+2MeOH+Na]^+$, 589 (48) $[2M+2MeOH+K]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{10}H_{17}N_3NaO_6$: 298.1010 $[M+MeOH+Na]^+$; found: 298.1010.

For enantiomer **15D**: M.p. 57–59°C; $[\alpha]_D^{25}$ = +27.4 (*c* 1.05, $CHCl_3$).

*5-Azido-5-deoxy-1,2-O-isopropylidene-β-*L*-idofuranose* (***16L***): Sodium borohydride (1.16 g, 30.6 mmol) was added slowly to a solution of lactol **15L** (18.6 g, 76.5 mmol) in MeOH (200 mL) at −30°C. The reaction mixture was stirred for 90 min, with an internal temperature maintained between −20°C and −10°C; after which time, TLC analysis (cyclohexane/EtOAc, 2:1) indicated the consumption of the starting material (major R_f = 0.40, minor R_f = 0.39) and the formation of a major product (R_f = 0.15). The reaction was neutralized with glacial acetic acid and concentrated in vacuo. The crude residue was dissolved in EtOAc (600 mL), washed with a saturated solution of sodium bicarbonate (600 mL), and the aqueous phase was extracted with EtOAc (2×800 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by column chromatography on silica gel (cyclohexane/EtOAc, 2:1 to 1:9) to afford diol **16L** as a white crystalline solid (16.9 g, 90%); M.p. 122–124°C [lit. 120–122°C];[12] $[\alpha]_D^{25}$ = −75.4 (*c* 1.00, $CHCl_3$) [lit. $[\alpha]_D^{25}$ = −69.6[12] (*c* 0.94, $CHCl_3$)]; ^{1}H NMR ([D_6]acetone, 400 MHz): δ = 1.27, 1.41 (s, 2×3H; $C(CH_3)_2$), 3.66 (app dt, *J*(6,5) = *J*(6,OH6) = 5.8 Hz, *J*(6,6′) = 10.6 Hz, 1H; H6), 3.70–3.75 (1H, m; H5), 3.78 (ddd, *J*(6′,5) = 3.4 Hz, *J*(6′,OH6) = 5.1 Hz, *J*(6′,6) = 10.6 Hz, 1H; H6′), 4.16–4.18 (m, 2H; H3 and H4), 4.27 (app t, *J*(OH6,6) = *J*(OH6,6′) = 5.5 Hz, 1H; OH6), 4.52 (d, *J*(OH3,3) = 4.8 Hz, 1H; OH3), 4.54 (d, *J*(2,1) = 3.8 Hz, 1H; H2), 5.91 ppm (d, *J*(1,2) = 3.8 Hz, 1H; H1); ^{13}C NMR ([D_6]acetone, 100 MHz): δ = 26.8, 27.4 ($C(CH_3)_2$), 62.8 (C6), 65.0 (C5), 75.5, 81.9 (C3, C4), 87.0 (C2), 105.9 (C1), 112.3 ppm ($C(CH_3)_2$); IR (thin film): $\tilde{\nu}$ = 2108 (s; N_3), 3335 cm^{-1} (br s; OH); LRMS (ESI+): *m*/*z* (%): 268 (93) $[M+Na]^+$, 513 cm^{-1} (100) $[2M+Na]^+$; (ESI−): 244 (25) $[M-H]^-$, 280 (100) $[M+^{35}Cl]^-$, 282 (70) $[M+^{37}Cl]^-$, 489 (55) $[2M-H]^-$; HRMS (ESI+): *m*/*z* calcd for $C_9H_{15}N_3NaO_5$: 268.0904 $[M+Na]^+$; found: 268.0903.

For enantiomer **16D**: M.p. 119–120°C; $[\alpha]_D^{25}$ = +63.1 (*c* 1.15, MeOH).

*5-Azido-6-O-tert-butyldimethylsilyl-5-deoxy-1,2-O-isopropylidene-β-*L*-idofuranose* (***7L***): *tert*-Butyldimethylsilyl chloride (19.5 g, 129 mmol) was added to a solution of diol **16L** (21.1 g, 86.0 mmol) in pyridine (210 mL). The reaction mixture was stirred at RT for 18 h, after which time TLC analysis (cyclohexane/EtOAc, 2:1) indicated the complete consumption of the starting material (R_f = 0.15) and the formation of a major product (R_f = 0.60). The reaction was quenched by the addition of MeOH (4 mL) and concentrated in vacuo. The crude residue was dissolved in EtOAc (750 mL), washed with 2M HCl (3×450 mL), dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by column chromatography on silica gel (cyclohexane/EtOAc, 20:1 to 3:1) to afford the silyl ether **7L** as a colorless oil (27.2 g, 88%); $[\alpha]_D^{25}$ = −13.9 (*c* 1.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.13 (s, 6H; $Si(CH_3)_2$), 0.91 (s, 9H; $SiC(CH_3)_3$), 1.32, 1.50 (s, 2×3H; $C(CH_3)_2$), 3.22 (d, *J*(OH3,3) = 3.5 Hz, 1H; OH3), 3.64–3.67 (m, 1H; H5), 3.77–3.82 (m, 2H; H6 and H6′), 4.12 (dd, *J*(4,3) = 2.5 Hz, *J*-

(4,5) = 7.8 Hz, 1H; H4), 4.21 (app t, $J(3,4)=J(3,OH3)=3.0$ Hz, 1H; H3), 4.55 (d, $J(2,1)=3.5$ Hz, 1H; H2), 5.97 ppm (d, $J(1,2)=3.5$ Hz, 1H; H1); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=-5.7$, -5.7 (Si(CH_3)$_2$), 18.1 (SiC(CH$_3$)$_3$), 25.7 (SiC(CH_3)$_3$), 26.2, 26.8 (C(CH_3)$_2$), 61.9 (C5), 63.3 (C6), 75.2 (C3), 82.4 (C4), 85.0 (C2), 104.5 (C1), 111.7 ppm (C(CH$_3$)$_2$); IR (thin film): $\tilde{\nu}=$ 2099 (s; N_3), 3464 cm^{-1} (br s; OH); LRMS (ESI+): m/z (%): 382 (100) [M+Na]$^+$, 741 (98) [2M+Na]$^+$; (ESI−): 358 (76) [M−H]$^-$, 394 (100) [M+^{35}Cl]$^-$, 396 (74) [M+^{37}Cl]$^-$, 404 (80) [M+HCOO]$^-$, 717 (98) [2M−H]$^-$; HRMS (ESI+): m/z calcd for $C_{15}H_{29}N_3NaO_5Si$: 382.1769 [M+Na]$^+$; found: 382.1769.

For enantiomer **7D**: $[\alpha]_D^{25}=+15.1$ (c 1.2, $CHCl_3$).

Synthesis of DGJNAc and derivatives

*5-Azido-6-O-tert-butyldimethylsilyl-5-deoxy-1,2-O-isopropylidene-β-*L*-lyxo-hexofuranos-3-ulose* (***17L***): Pyridinium chlorochromate (110.1 g, 510.7 mmol) was added to a solution of alcohol **7L** (30.6 g, 85.1 mmol) in CH_2Cl_2 (450 mL) over 3 Å molecular sieves. The reaction mixture was stirred under a nitrogen atmosphere at RT for 18 h, after which time TLC analysis (cyclohexane/EtOAc, 2:1) indicated almost complete consumption of the starting material ($R_f=0.60$) and the formation of a major product ($R_f=0.30$ streaking to 0.80). The reaction mixture was filtered through Celite and concentrated in vacuo. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc, 5:1 to 3:1) to afford the ketone (**17L**) as a colorless oil (24.6 g, 81%); $[\alpha]_D^{25}=+130.8$ (c 1.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=0.10$, 0.11 (s, 2×3H; Si-(CH$_3$)$_2$), 0.91 (s, 9H; SiC(CH$_3$)$_3$), 1.43, 1.46 (s, 2×3H; C(CH$_3$)$_2$), 3.80 (ddd, $J(5,4)=2.0$ Hz, $J(5,6)=5.5$ Hz, $J(5,6')=7.7$ Hz, 1H; H5), 3.87 (dd, $J(6,5)=5.5$ Hz, $J(6,6')=10.2$ Hz, 1H; H6), 3.94 (dd, $J(6',5)=8.0$ Hz, J-(6′,6) = 10.2 Hz, 1H; H6′), 4.39 (dd, $J(2,4)=1.0$ Hz, $J(2,1)=4.3$ Hz, 1H; H2), 4.41 (d, $J(4,2)=1.0$ Hz, $J(4,5)=2.0$ Hz, 1H; H4), 6.10 ppm (d, J-(1,2) = 4.3 Hz, 1H; H1); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=-5.6$, -5.6 (Si-(CH_3)$_2$), 18.2 (SiC(CH$_3$)$_3$), 25.7 (SiC(CH_3)$_3$), 27.1, 27.5 (C(CH_3)$_2$), 62.8 (C6), 63.4 (C5), 76.4 (C2), 77.9 (C4), 103.6 (C1), 114.6 (C(CH$_3$)$_2$), 208.9 ppm (C3); IR (thin film): $\tilde{\nu}=1777$ (s; C=O), 2111 cm^{-1} (s; N_3); LRMS (ESI+): m/z (%): 380 (100) [M+Na]$^+$, 412 (95) [M+MeOH+Na]$^+$, 737 (15) [2M+Na]$^+$; (ESI−): 424 (100) [M+MeOH+^{35}Cl]$^-$, 426 (40) [M+MeOH+^{37}Cl]$^-$; HRMS (ESI+): m/z calcd for $C_{16}H_{31}N_3NaO_6Si$: 412.1874 [M+MeOH+Na]$^+$; found: 412.1873.

For enantiomer **17D**: $[\alpha]_D^{25}=-140.1$ (c 1.11, $CHCl_3$).

*5-Azido-6-O-tert-butyldimethylsilyl-5-deoxy-1,2-O-isopropylidene-β-*L*-talofuranose* (***18L***): A solution of sodium borohydride (2.59 g, 68.4 mmol) in water (50 mL) was added dropwise to a solution of ketone **17L** (24.6 g, 68.4 mmol) in EtOH (200 mL) at 0°C. The reaction mixture was allowed to warm to RT and stirred for 90 min. After this time, TLC analysis (cyclohexane/EtOAc, 2:1) indicated the complete consumption of the starting material ($R_f=0.30$ streaking to 0.80) and the formation of a major product ($R_f=0.59$). The reaction was neutralized with glacial acetic acid, concentrated in vacuo, dissolved in EtOAc (400 mL), and washed with saturated solutions of sodium bicarbonate (2×400 mL) and brine (400 mL). The organic phase was dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by column chromatography on silica gel (cyclohexane/EtOAc, 50:1 to 3:1) to afford alcohol **18L** as a colorless oil (22.4 g, 91%); $[\alpha]_D^{25}=+65.3$ (c 1.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=0.11$ (s, 6H; Si(CH$_3$)$_2$), 0.92 (s, 9H; SiC(CH$_3$)$_3$), 1.37, 1.56 (s, 2×3H; C(CH$_3$)$_2$), 2.58 (d, J(OH3,3) = 9.9 Hz, 1H; OH3), 3.64 (ddd, J-(5,4) = 3.4 Hz, $J(5,6)=5.5$ Hz, $J(5,6')=7.9$ Hz, 1H; H5), 3.81 (dd, $J(4,5)=$ 3.4 Hz, $J(4,3)=8.5$ Hz, 1H; H4), 3.87 (dd, $J(6,5)=5.5$ Hz, $J(6,6')=$ 10.6 Hz, 1H; H6), 3.91 (dd, $J(6',5)=7.9$ Hz, $J(6',6)=10.6$ Hz, 1H; H6′), 4.06 (ddd, $J(3,2)=5.1$ Hz, $J(3,4)=8.9$ Hz, J(3,OH3) = 9.9 Hz, 1H; H3), 4.59 (dd, $J(2,1)=3.8$ Hz, $J(2,3)=5.1$ Hz, 1H; H2), 5.82 ppm (dd, $J(1,2)=$ 3.8 Hz, 1H; H1); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=-5.5$, -5.5 (Si(CH_3)$_2$), 18.1 (SiC(CH$_3$)$_3$), 25.7 (SiC(CH_3)$_3$), 26.5, 26.5 (C(CH_3)$_2$), 62.4 (C5), 63.9 (C6), 72.2 (C3), 78.4 (C2), 79.3 (C4), 104.1 (C1), 112.9 ppm (C(CH$_3$)$_2$); IR (thin film): $\tilde{\nu}=2099$ (s; N_3), 3473 cm^{-1} (br s; OH); LRMS (ESI+): m/z (%): 382 (100) [M+Na]$^+$, 741 (70) [2M+Na]$^+$; (ESI−): 358 (100) [M−H]$^-$, 394 (48) [M+^{35}Cl]$^-$, 396 (15) [M+^{37}Cl]$^-$, 717 (64) [2M−H]$^-$; HRMS (ESI+): m/z calcd for $C_{15}H_{29}N_3NaO_5Si$: 382.1769 [M+Na]$^+$;found: 382.1768.

For enantiomer **18D**: $[\alpha]_D^{25}=-70.7$ (c 1.08, $CHCl_3$).

*5-Azido-3-O-benzyl-6-O-tert-butyldimethylsilyl-5-deoxy-1,2-O-isopropylidene-β-*L*-talofuranose* (***19L***): Sodium hydride (60% min. oil suspension, 3.74 g, 93.4 mmol) was added to a solution of alcohol **18L** (22.4 g, 62.2 mmol) and benzyl bromide (11.1 mL, 93.4 mmol) in DMF (200 mL) at 0°C. The reaction mixture was allowed to warm to RT and stirred for 1 h. TLC analysis (cyclohexane/EtOAc, 2:1) indicated the complete consumption of the starting material ($R_f=0.58$) and the formation of a major product ($R_f=0.70$). The reaction mixture was quenched with MeOH (2 mL) at 0°C, diluted with EtOAc (500 mL), washed with water/sat. brine (1:1) solution (3×200 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (cyclohexane/EtOAc, 50:1 to 20:1) to afford the benzyl ether (**19L**) as a colorless oil (24.9 g, 89%); $[\alpha]_D^{25}=+110.0$ (c 0.90, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=0.09$, 0.10 (s, 2×3H; Si(CH$_3$)$_2$), 0.92 (s, 9H; SiC(CH$_3$)$_3$), 1.36, 1.58 (s, 2×3H; C(CH$_3$)$_2$), 3.58 (ddd, $J(5,4)=$ 2.0 Hz, $J(5,6)=4.8$ Hz, $J(5,6')=8.9$ Hz, 1H; H5), 3.85 (dd, $J(6,5)=4.4$ Hz, $J(6,6')=10.6$ Hz, 1H; H6), 3.88 (dd, $J(3,2)=5.1$ Hz, $J(3,4)=8.9$ Hz, 1H; H3), 3.90 (dd, $J(6',5)=8.9$ Hz, $J(6',6)=10.6$ Hz, 1H; H6′), 4.04 (dd, J-(4,5) = 2.1 Hz, $J(4,3)=8.9$ Hz, 1H; H4), 4.56 (app t, $J(2,1)=J(2,3)=$ 4.1 Hz, 1H; H2), 4.57 (d, $J_{gem}=12.1$ Hz, 1H; OCH_2Ph), 4.80 (d, $J_{gem}=$ 12.1 Hz, 1H; OCH_2Ph), 5.72 (d, $J(1,2)=3.4$ Hz, 1H; H1), 7.34–7.40 ppm (5H, m; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=-5.5$, -5.5 (Si(CH_3)$_2$), 18.1 (SiC(CH$_3$)$_3$), 25.8 (SiC(CH_3)$_3$), 26.5, 26.8 (C(CH_3)$_2$), 62.1 (C5), 64.4 (C6), 72.3 (OCH_2Ph), 76.9 (C2), 76.9 (C4), 77.8 (C3), 104.3 (C1), 113.1 (C(CH$_3$)$_2$), 128.0, 128.2, 128.5 (ArCH), 137.3 ppm (ArCC); IR (thin film): $\tilde{\nu}=2098$ cm^{-1} (s; N_3); LRMS (ESI+): m/z (%): 472 (97) [M+Na]$^+$, 921 (100) [2M+Na]$^+$; HRMS (ESI+): m/z calcd for $C_{22}H_{35}N_3NaO_5Si$: 472.2238 [M+Na]$^+$; found: 472.2239.

For enantiomer **19D**: $[\alpha]_D^{25}=-95.2$ (c 0.59, $CHCl_3$).

*Methyl-5-azido-3-O-benzyl-5-deoxy-*L*-talofuranoside* (***8L***): A solution of benzyl ether **19L** (19.2 g, 42.7 mmol) in a mixture of MeOH (200 mL) and acetyl chloride (20 mL) was stirred at 55°C for 2 h. TLC analysis (cyclohexane/EtOAc, 2:1) indicated the complete consumption of the starting material ($R_f=0.72$) and the formation of a major product ($R_f=0.20$). The reaction was neutralized by the addition of solid sodium bicarbonate, filtered, and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (cyclohexane/EtOAc, 9:1 to 2:3) to afford the methyl talofuranoside (**8L**) as a pale-yellow oil (12.3 g, 93%) as a 7:1 ratio of the anomers; ^{1}H NMR ($CDCl_3$, 400 MHz, major anomer only): 2.19 (dd, J(OH6,6) = 5.2 Hz, J(OH6,6′) = 7.6 Hz, 1H; OH6), 2.54 (d, J(OH2,2) = 2.6 Hz, 1H; OH2), 3.39 (s, 3H; OCH$_3$), 3.43 (td, $J(5,6')=$ 4.3 Hz, $J(5,4)=J(5,6)=6.1$ Hz, 1H; H5), 3.71 (ddd, J(6,OH6) = 5.2 Hz, J-(6,5) = 6.1 Hz, $J(6,6')=12.0$ Hz, 1H; H6), 3.77 (ddd, $J(6',5)=4.3$ Hz, J-(6′,OH6) = 7.6 Hz, $J(6',6)=12.0$ Hz, 1H; H6′), 4.04 (dd, J(2,OH2) = 2.6 Hz, $J(2,3)=4.3$ Hz, 1H; H2), 4.13 (dd, $J(4,5)=6.1$ Hz, $J(4,3)=7.5$ Hz, 1H; H4), 4.20 (dd, $J(3,2)=4.3$ Hz, $J(3,4)=7.5$ Hz, 1H; H3), 4.55 (d, $J_{gem}=11.4$ Hz, 1H; OCH_2Ph), 4.62 (d, $J_{gem}=11.4$ Hz, 1H; OCH_2Ph), 4.89 (s, 1H; H1), 7.32–7.42 ppm (m, 5H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz, major anomer only): $\delta=55.6$ (OCH$_3$), 62.5 (C6), 65.4 (C5), 72.5 (C2), 73.1 (OCH_2Ph), 79.6 (C3), 81.0 (C4), 108.6 (C1), 128.2, 128.7, 128.7, 128.8 (ArCH), 136.4 ppm (ArCC); IR (thin film): $\tilde{\nu}=2116$ (s; N_3), 3427 cm^{-1} (br s; OH); LRMS (ESI+): m/z (%): 332 (100) [M+Na]$^+$, 641 (41) [2M+Na]$^+$; HRMS (ESI+): m/z calcd for $C_{14}H_{19}N_3NaO_5$: 332.1217 [M+Na]$^+$; found: 332.1217.

*Methyl-5-azido-2-N,3-O-dibenzyl-2,5,6-trideoxy-2,6-imino-*L*-galactofuranoside* (***9Lα***): Trifluoromethanesulfonic anhydride (10.6 mL, 62.8 mmol) and pyridine (10.2 mL, 126 mmol) were added to a solution of diol **8L** (7.2 g, 23 mmol) in CH_2Cl_2 (75 mL) at −40°C under an argon atmosphere. After 1 h, with the internal temperature rising to −20°C, TLC analysis (cyclohexane/EtOAc, 2:1) indicated the complete consumption of the starting material ($R_f=0.19$) and the formation of two products (major $R_f=0.77$, minor $R_f=0.65$), which were thought to be a mixture of anomeric ditriflates. The reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with 2M HCl (3×60 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude ditriflate (**20L**) was dissolved in THF (75 mL), cooled to −30°C under an argon atmosphere, and benzylamine (7.6 mL, 70 mmol) was added dropwise. The reaction mixture was allowed to warm to RT and stirred for 2 h, after which time TLC analysis

(cyclohexane/EtOAc/triethylamine, 79:20:1) indicated the consumption of both ditriflates and the formation of the two anomeric monosubstituted triflates (major $R_f = 0.42$, minor $R_f = 0.30$). The reaction was heated to 60 °C for 16 h. TLC analysis indicated the complete consumption of the major component ($R_f = 0.42$), the formation of a new major product ($R_f = 0.67$), and the persistence of the minor component ($R_f = 0.30$). The reaction mixture was concentrated in vacuo and purified by column chromatography on silica gel (cyclohexane/EtOAc/triethylamine, 99:0:1 to 96:3:1) to afford the bicyclic compound (**9Lα**) as a pale-yellow oil and a single anomer (6.12 g, 69%). $[\alpha]_D^{25} = +16.2$ (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 2.74$ (d, $J(6,6') = 12.8$ Hz, 1H; H6), 3.21 (br s, 1H; H2), 3.52 (dd, $J(6',5) = 4.1$ Hz, $J(6',6) = 12.8$ Hz, 1H; H6′), 3.54 (s, 3H; OCH_3), 3.87 (app t, $J(5,4) = J(5,6') = 4.4$ Hz, 1H; H5), 3.90 (d, $J_{gem} = 13.8$ Hz, 1H; NCH_2Ph), 4.01 (d, $J_{gem} = 13.8$ Hz, 1H; NCH_2Ph), 4.11 (d, $J(3,2) = 1.0$ Hz, 1H; H3), 4.30 (d, $J(4,5) = 5.1$ Hz, 1H; H4), 4.52 (d, $J_{gem} = 11.8$ Hz, 1H; OCH_2Ph), 4.58 (d, $J_{gem} = 11.8$ Hz, 1H; OCH_2Ph), 5.32 (d, $J(1,2) = 2.7$ Hz, 1H; H1), 7.26–7.37 ppm (m, 10H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 48.6$ (C6), 57.6 (OCH_3), 59.7 (C5), 59.9 (NCH_2Ph), 61.4 (C2), 71.0 (OCH_2Ph), 78.7 (C4), 81.5 (C3), 109.7 (C1), 127.0, 127.7, 127.8, 128.3, 128.4 (Ar*C*H), 137.7, 139.1 ppm (Ar*C*C); IR (thin film): $\tilde{\nu} = 2109$ cm^{-1} (s; N_3); LRMS (ESI+): *m*/*z* (%): 381 (100) $[M+H]^+$, 403 (80) $[M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{21}H_{24}N_4NaO_3$: 381.1921 $[M+H]^+$; found: 381.1920.

For enantiomer **9Dα**: $[\alpha]_D^{25} = -21.4$ (*c* 1.20, $CHCl_3$).

*4-O-Acetyl-5-azido-2-N,3-O-dibenzyl-2,5,6-trideoxy-2,6-imino-*L*-galactose acetyl methyl acetal* (***21L***): Boron trifluoride diethyl etherate (5.1 mL, 40 mmol) was added dropwise to a solution of bicyclic acetal **9Lα** (6.12 g, 16.1 mmol) in acetic anhydride (60 mL) at −30 °C. The reaction was allowed to warm to RT and stirred for 2.5 h, after which time TLC analysis (cyclohexane/EtOAc/triethylamine, 69:30:1) indicated the complete consumption of the starting material ($R_f = 0.72$) and the formation of major products ($R_f = 0.47$, 0.56). The reaction mixture was concentrated in vacuo and the residue was dissolved in EtOAc (200 mL), washed with a saturated solution of sodium bicarbonate (3 × 100 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (cyclohexane/EtOAc/triethylamine, 97:2:1 to 79:20:1) to afford the mixed-acetal piperidine (**21L**) as a pale-yellow oil (7.54 g, 97%), in a 3:2 (A/B) mixture of diastereoisomers. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 2.05$ (s, 3H; $COCH_3^B$), 2.10 (s, 3H; $COCH_3^A$), 2.13 (s, 3H; $COCH_3^B$), 2.14 (s, 3H; $COCH_3^A$), 2.38 (dd, $J(6,5) = 10.6$ Hz, $J(6,6') = 13.8$ Hz, 1H; H6^A), 2.41 (dd, $J(6,5) = 10.8$ Hz, $J(6,6') = 14.0$ Hz, 1H; H6^B), 3.04 (dd, $J(2,3) = 1.5$ Hz, $J(2,1) = 7.9$ Hz, 1H; H2^B), 3.05 (dd, $J(6',5) = 4.6$ Hz, $J(6',6) = 14.0$ Hz, 1H; H6′^{B}), 3.07 (dd, $J(2,3) = 1.8$ Hz, $J(2,1) = 7.5$ Hz, 1H; H2^A), 3.10 (dd, $J(6',5) = 4.6$ Hz, $J(6',6) = 13.8$ Hz, 1H; H6′^{A}), 3.31 (s, 3H; OCH_3^B), 3.48 (s, 3H; OCH_3^A), 3.90 (s, 2H; NCH_2Ph^A), 3.94 (s, 2H; NCH_2Ph^B), 4.05 (dd, $J(3,2) = 1.8$ Hz, $J(3,4) = 2.9$ Hz, 1H; H3^A), 4.11 (ddd, $J(5,6') = 4.6$ Hz, $J(5,4) = 9.9$ Hz, $J(5,6) = 10.8$ Hz, 1H; H5^B), 4.13 (ddd, $J(5,6') = 4.6$ Hz, $J(5,4) = 10.0$ Hz, $J(5,6) = 10.6$ Hz, 1H; H5^A), 4.29 (dd, $J(3,2) = 1.5$ Hz, $J(3,4) = 2.9$ Hz, 1H; H3^B), 4.50 (d, $J_{gem} = 11.0$ Hz, 1H; OCH_2Ph^A), 4.59 (d, $J_{gem} = 11.6$ Hz, 1H; OCH_2Ph^B), 4.67 (d, $J_{gem} = 11.0$ Hz, 1H; OCH_2Ph^A), 4.80 (d, $J_{gem} = 11.6$ Hz, 1H; OCH_2Ph^B), 4.86 (dd, $J(4,3) = 2.9$ Hz, $J(4,5) = 10.2$ Hz, 1H; H4^B), 4.88 (dd, $J(4,3) = 2.9$ Hz, $J(4,5) = 10.0$ Hz, 1H; H4^A), 6.16 (d, $J(1,2) = 7.9$ Hz, 1H; H1^B), 6.31 (d, $J(1,2) = 7.5$ Hz, 1H; H1^A), 7.22–7.43 ppm (m, 20H; ArH^A and ArH^B); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta =$ 20.9, 21.0 ($COCH_3^B$), 20.0, 21.1 ($COCH_3^A$), 51.6 (C6^B), 51.9 (C6^A), 53.9 (NCH_2Ph^B), 53.9 (C5^B), 54.4 (C5^A), 54.7 (NCH_2Ph^A), 56.4 (OCH_3^A), 56.9 (OCH_3^B), 63.2 (C2^A), 64.2 (C2^B), 74.9 (OCH_2Ph^A), 75.2 (OCH_2Ph^B), 76.0 (C3^A), 76.8 (C3^B), 77.2 (C4^B), 77.6 (C4^A), 95.1 (C1^A, C1^B), 126.7, 127.0, 127.1, 127.4, 127.5, 127.7, 127.8, 128.3, 128.4, 128.5, 128.6 (Ar*C*H^A and Ar*C*H^B), 138.0, 138.2, 139.3, 139.6 (Ar*C*C^A and Ar*C*C^B), 170.2, 170.2, 170.6, 171.0 ppm ($COCH_3^A$ and $COCH_3^B$); IR (thin film): $\tilde{\nu} = 1743$ (s; C=O), 2106 cm^{-1} (s; N_3); LRMS (ESI+): *m*/*z* (%): 423, (95) $[M-OAc]^+$, 483 (98) $[M+H]^+$, 505 (100) $[M+Na]^+$, 521 (83) $[M+K]^+$, 955 (20) $[2M+H]^+$, 987 (95) $[2M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{25}H_{30}N_4NaO_6$: 505.2058 $[M+Na]^+$; found: 505.2058.

*3,6-Di-O-acetyl-2-azido-1-N,4-O-dibenzyl-1,2,5-trideoxy-1,5-imino-*D*-galactitol* (***22D***): Diisobutylaluminium hydride (1.5 M in toluene, 52.0 mL, 78.0 mmol) was added dropwise to a solution of mixed acetal **21L** (7.54 g, 15.6 mmol) in CH_2Cl_2 (75 mL) at −78 °C. After 45 min, TLC analysis (cyclohexane/EtOAc/triethylamine, 59:40:1) indicated the consumption of the starting material ($R_f = 0.70$, 0.73) and the appearance of three new spots ($R_f = 0.20$, 0.60, and 0.80). The reaction mixture was quenched and diluted with EtOAc (400 mL) and allowed to warm to RT. A saturated solution of sodium potassium tartrate (400 mL) was added and the biphasic mixture was stirred vigorously for 90 min. The organic layer was collected and the aqueous layer was extracted with EtOAc (3 × 300 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated in vacuo. TLC analysis (cyclohexane/EtOAc/triethylamine, 59:40:1) indicated the disappearance of the spot at $R_f = 0.80$ during the work-up procedure. The crude residue was dissolved in MeOH (100 mL) and sodium borohydride (590 mg, 15.6 mmol) was added at 0 °C. The reaction mixture was allowed to warm to RT and stirred for 3 h, after which time, TLC analysis (cyclohexane/EtOAc/triethylamine, 59:40:1) indicated the formation of one major product ($R_f = 0.25$) with the disappearance of all previous spots. The reaction mixture was concentrated in vacuo, co-evaporated with MeOH (3 × 50 mL), and dissolved in pyridine (75 mL) and acetic anhydride (75 mL). The reaction mixture was stirred at RT for 16 h, after which time, TLC analysis (cyclohexane/EtOAc/triethylamine, 59:40:1) indicated the consumption of the starting material ($R_f = 0.25$) and the formation of a major product ($R_f = 0.65$). The reaction mixture was concentrated in vacuo and purified by column chromatography on silica gel (cyclohexane/EtOAc/triethylamine, 97:2:1 to 89:10:1) to afford the diacetyl piperidine (**22D**) as a light-yellow oil (5.08 g, 72% over 3 steps). $[\alpha]_D^{25} = +65.7$ (*c* 0.60, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 2.04$, 2.14 (s, 2 × 3H; $COCH_3$), 2.26 (dd, $J(1,2) = 8.9$ Hz, $J(1,1') = 12.6$ Hz, 1H; H1), 2.95–3.00 (m, 1H; H5), 3.02 (dd, $J(1',2) = 4.4$ Hz, $J(1',1) = 12.7$ Hz, 1H; H1′), 3.64 (d, $J_{gem} = 14.1$ Hz, 1H; NCH_2Ph), 3.88 (d, $J_{gem} = 14.1$ Hz, 1H; NCH_2Ph), 3.96 (app td, $J(2,1') = 4.1$ Hz, $J(2,1) = J(2,3) = 8.5$ Hz, 1H; H2), 4.04 (app t, $J(4,3) = J(4,5) = 2.7$ Hz, 1H; H4), 4.23 (dd, $J(6,5) = 6.1$ Hz, $J(6,6') = 11.6$ Hz, 1H; H6), 4.55 (dd, $J(6',5) = 6.1$ Hz, $J(6',6) = 11.6$ Hz, 1H; H6′), 4.57 (d, $J_{gem} = 11.4$ Hz, 1H; OCH_2Ph), 4.75 (d, $J_{gem} = 11.4$ Hz, 1H; OCH_2Ph), 4.90 (dd, $J(3,4) = 2.7$ Hz, $J(3,2) = 8.5$ Hz, 1H; H3), 7.26–7.40 ppm (m, 10H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 21.0$, 21.0 ($COCH_3$), 51.4 (C1), 56.5 (C2), 56.9 (NCH_2Ph), 60.7 (C5), 61.9 (C6), 74.3 (OCH_2Ph), 74.8 (C4), 75.1 (C3), 127.3, 127.8, 127.9, 128.4, 128.5, 128.7 (Ar*C*H), 137.8, 138.0 (Ar*C*C), 170.1, 170.6 ppm ($COCH_3$); IR (thin film): $\tilde{\nu} = 1740$ (s; C=O), 2108 cm^{-1} (s; N_3); LRMS (ESI+): *m*/*z* (%): 475 (100) $[M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{24}H_{28}N_4NaO_5$: 475.1952 $[M+Na]^+$; found: 475.1950.

For enantiomer **22L**: $[\alpha]_D^{25} = -75.6$ (*c* 0.87, $CHCl_3$).

*2-Acetamido-3,6-di-O-acetyl-1-N,4-O-dibenzyl-1,2,5-trideoxy-1,5-imino-*D*-galactitol* (***23D***): Powdered zinc (9.0 g, 130 mmol) was added to a solution of azide **22D** (3.0 g, 6.6 mmol) in THF/glacial-acetic-acid/acetic-anhydride (3:2:1, 72 mL) and the mixture was stirred vigorously before the dropwise addition of a saturated copper(II) sulfate solution (9 mL) to initiate the reaction. After 30 min, TLC analysis (cyclohexane/EtOAc/triethylamine, 49:50:1) indicated the complete consumption of the starting material ($R_f = 0.78$) and the formation of a major product ($R_f = 0.15$). The reaction mixture was filtered (GF/A glass microfiber), concentrated in vacuo, and the crude residue was purified by column chromatography on silica gel (cyclohexane/EtOAc/triethylamine, 94:5:1 to 29:70:1) to afford the acetamide (**23D**) as a white crystalline solid (2.83 g, 91%). M.p. 112–114 °C; $[\alpha]_D^{25} = +20.3$ (*c* 1.02, acetone); ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta =$ 1.87 (s, 3H; $NHCOCH_3$), 2.05, 2.11 (s, 2 × 3H; $COCH_3$), 2.20 (dd, $J(1,2) = 5.2$ Hz, $J(1,1') = 12.6$ Hz, 1H; H1), 3.04 (dd, $J(1',2) = 3.1$ Hz, $J(1',1) = 12.6$ Hz, 1H; H1′), 3.33 (br s, 1H; H5), 3.72 (d, $J_{gem} = 13.8$ Hz, 1H; NCH_2Ph), 3.82 (d, $J_{gem} = 13.8$ Hz, 1H; NCH_2Ph), 3.86 (dd, $J =$ 2.9 Hz, $J = 4.3$ Hz, 1H; H4), 4.20 (br s, 1H; H2), 4.31 (dd, $J(6,5) = 3.6$ Hz, $J(6,6') = 11.9$ Hz, 1H; H6), 4.54 (d, $J_{gem} = 11.8$ Hz, 1H; OCH_2Ph), 4.66 (dd, $J(6',5) = 7.6$ Hz, $J(6',6) = 11.9$ Hz, 1H; H6′), 4.76 (d, $J_{gem} = 11.8$ Hz, 1H; OCH_2Ph), 5.19 (br s, 1H; H3), 5.70 (d, $J(NH,2) = 8.0$ Hz, 1H; $NHCOCH_3$), 7.25–7.38 ppm (m, 10H; ArH); ^{13}C NMR ([D$_6$]acetone, 126 MHz): $\delta = 21.0$, 21.0 ($COCH_3$), 22.9 ($NHCOCH_3$), 46.6 (C2), 50.1 (C1), 58.2 (NCH_2Ph), 61.5 (C5), 61.6 (C6), 72.5 (C3), 73.4 (OCH_2Ph), 75.5 (C4), 127.6, 128.1, 128.2, 128.9, 128.9, 129.3 (Ar*C*H), 139.4, 140.1

(Ar*C*C), 169.6, 170.2, 170.7 ppm (*C*OCH$_3$; NH*C*OCH$_3$); IR (thin film): $\tilde{\nu}$=1541 (br s; amide II), 1655 (s; amide I), 1739 (s; C=O), 3296 cm^{-1} (br s; OH); LRMS (ESI+): *m*/*z* (%): 469 (100) [*M*+H]$^+$, 491 (55) [*M*+Na]$^+$; (ESI−): 503 (55) [*M*+^{35}Cl]$^-$, 505 (15) [*M*+^{37}Cl]$^-$, 513 (100) [*M*+HCOO]$^-$; HRMS (ESI+): *m*/*z* calcd for $C_{26}H_{33}N_2O_6$: 491.2153 [*M*+H]$^+$; found: 491.2151.

For enantiomer **23L**: M.p. 113–115 °C; $[\alpha]_D^{25}=-28.6$ (*c* 1.06, $CHCl_3$).

2-Acetamido-1,2,5-trideoxy-1,5-imino-D-*galactitol* (*DGJNAc,* ***1D***): Sodium methoxide (10 mg, cat.) was added to a solution of the protected piperidine (**23D**, 2.83 g, 6.03 mmol) in MeOH (40 mL) at RT and the mixture was stirred for 16 h. TLC analysis (EtOAc/triethylamine, 99:1) indicated the complete consumption of the starting material (R_f=0.50) and the formation of a major product (R_f=0.14 streaking to 0.0). The reaction mixture was neutralized with glacial acetic acid and concentrated in vacuo. The crude residue was dissolved in water/2 M HCl/1,4-dioxane (10:2:1, 78 mL) and palladium (10% on carbon, 600 mg) was added. The vessel was degassed, charged with hydrogen, and the reaction mixture was stirred at RT for 24 h. LRMS and ^{1}H NMR indicated that a single product had been formed, with no trace of any benzylated intermediates observed. The reaction mixture was filtered (GF/A glass microfiber) and concentrated in vacuo to afford crude DGJNAc (**1D**) as its hydrochloride salt.

Selected data for DGJNAc·HCl (**1D**): ^{1}H NMR (D_2O, 400 MHz): δ= 1.96 (s, 3H; NHCOCH_3), 2.82 (app t, *J*(1,1′)=*J*(1,2)=12.5 Hz, 1H; H1), 3.34 (br s, 1H; H5), 3.41 (dd, *J*(1′,2)=5.0 Hz, *J*(1′,1)=12.9 Hz, 1H; H1′), 3.72 (dd, *J*(3,4)=2.6 Hz, *J*(3,2)=10.7 Hz, 1H; H3), 3.75 (dd, *J*(6,5)= 7.5 Hz, *J*(6,6′)=12.3 Hz, 1H; H6), 3.82 (dd, *J*(6′,5)=5.5 Hz, *J*(6′,6)= 12.3 Hz, 1H; H6′), 4.11 (dd, *J*(4,5)=1.0 Hz, *J*(4,3)=2.6 Hz, 1H; H4), 4.26 ppm (ddd, *J*(2,1′)=5.1 Hz, *J*(2,3)=11.0 Hz, *J*(2,1)=11.9 Hz, 1H; H2); LRMS (ESI+): *m*/*z* (%): 205 (100) [*M*+H]$^+$, 409 (35) [2*M*+H]$^+$; (ESI−): 203 (100) [*M*−H]$^-$.

The crude salt of compound **1D** was loaded onto a short column of Dowex (50W-X8, H$^+$) and the resin was washed with water until neutral fractions were obtained. The product was liberated with aqueous ammonia (2 M) and the ammoniacal fractions were concentrated in vacuo. The residue was co-evaporated with EtOH (3×50 mL) to give DGJNAc (**1D**) as a pale-yellow crystalline solid (1.22 g, 99%). M.p. 153–156 °C (EtOH); $[\alpha]_D^{25}=+49.3$ (*c* 0.65, H_2O); ^{1}H NMR (D_2O, 400 MHz): δ=1.88 (s, 3H; NHCOCH_3), 2.24 (app t, *J*(1,1′)=*J*(1,2)=12.2 Hz, 1H; H1), 2.63 (app t, *J*(5,6)=*J*(5,6′)=6.5 Hz, 1H; H5), 2.96 (dd, *J*(1′,2)=4.9 Hz, *J*(1′,1)= 13.1 Hz, 1H; H1′), 3.45–3.53 (m, 3H; H3, H6, and H6′), 3.83 (app td, *J*-(2,1′)=4.9 Hz, *J*(2,1)=*J*(2,3)=10.9 Hz, 1H; H2), 3.89 ppm (s, 1H; H4); ^{13}C NMR (D_2O, 100 MHz): δ=22.6 (NHCO*C*H$_3$), 47.7 (C1), 49.1 (C2), 59.3 (C5), 61.8 (C6), 68.9 (C4), 73.2 (C3), 175.1 ppm (NH*C*OCH$_3$); IR (thin film, Ge): $\tilde{\nu}$=1563 (s; amide II), 1638 (s; amide I), 3285 cm^{-1} (br s; OH/NH); LRMS (ESI+): *m*/*z* (%): 205 (100) [*M*+H]$^+$, 409 (30) [2*M*+H]$^+$; (ESI−): 203 (100) [*M*−H]$^-$; HRMS (ESI+): *m*/*z* calcd for $C_8H_{17}N_2O_4$: 205.1183 [*M*+H]$^+$; found: 205.1184; elemental analysis calcd (%) for $C_8H_{16}N_2O_4$: C 47.05, H 7.90, N 13.72; found: C 46.88, H 7.86, N 13.55.

For enantiomer **1L**: M.p. 152–156 °C (EtOH); $[\alpha]_D^{25}=-46.6$ (*c* 0.73, H_2O).*2-Acetamido-1,2,5-trideoxy-1,5-imino-1-N-methyl*-D-*galactitol,* (*N-methyl-DGJNAc,* ***1Da***): Formaldehyde (37% in water, 0.03 mL, 0.3 mmol) and palladium (10% on carbon, 6 mg) were added to a solution of DGJNAc (**1D**, 30 mg, 0.15 mmol) in 1,4-dioxane/water (1:1, 2 mL). The vessel was degassed, charged with hydrogen, and the reaction mixture was stirred at RT for 18 h. The reaction mixture was filtered (GF/A glass microfiber) and concentrated in vacuo. The residue was loaded onto a short column of Dowex (50W-X8, H$^+$) and the resin washed with water until neutral fractions were obtained. The product was liberated with aqueous ammonia (2 M) and the ammoniacal fractions were concentrated in vacuo to afford *N*-methyl-DGJNAc (**1Da**) as a brown, crystalline solid (35 mg, quant.). M.p. 203–206 °C; $[\alpha]_D^{22}=+23.6$ (*c* 0.65, H_2O); ^{1}H NMR (D_2O, 400 MHz): δ=1.88 (s, 3H; NHCOCH_3), 2.04 (app t, *J*-(1,1′)=*J*(1,2)=11.6 Hz, 1H; H1), 2.20 (app s, 4H; NCH_3, H5), 2.81 (dd, *J*(1′,2)=4.5 Hz, *J*(1′,1)=11.7 Hz, 1H; H1′), 3.41 (dd, *J*(3,2)=2.1 Hz, *J*-(3,4)=10.6 Hz, 1H; H3), 3.68 (dd, *J*(6,5)=6.1 Hz, *J*(6,6′)=11.6 Hz, 1H; H6), 3.74 (dd, *J*(6′,5)=4.5 Hz, *J*(6′,6)=11.6 Hz, 1H; H6′), 3.92–4.10 ppm (m, 2H; H2 and H4); ^{13}C NMR (D_2O, 126 MHz): δ=22.6 (NHCO*C*H$_3$), 41.5 (NCH$_3$), 47.8 (C2), 58.4 (C1), 61.3 (C6), 65.9, 70.0, 73.1 (C3, C4 and C5), 174.9 ppm (NH*C*OCH$_3$); IR (thin film): $\tilde{\nu}$=1561 (s; amide II), 1639 (s; amide I), 3289 cm^{-1} (br s; OH); LRMS (ESI+): *m*/*z* (%): 459 (100) [2*M*+Na]$^+$; HRMS (ESI+): *m*/*z* calcd for $C_9H_{18}N_2NaO_4$: 241.1159 [*M*+Na]$^+$; found: 241.1158.

2-Acetamido-1,2,5-trideoxy-1-N-ethyl-1,5-imino-D-*galactitol,* (*N-ethyl-DGJNAc,* ***1Db***): Ethanal (0.01 mL, 0.2 mmol) and palladium (10% on carbon, 8 mg) were added to a solution of DGJNAc (**1D**, 20.9 mg, 0.10 mmol) in EtOH (1 mL). The vessel was degassed, charged with hydrogen, and the reaction mixture was stirred at RT for 4.5 h, after which LRMS indicated the absence of the starting material. The reaction mixture was filtered (GF/A glass microfiber) and concentrated in vacuo. The residue was loaded onto a short column of Dowex (50W-X8, H$^+$) and the resin was washed with water. The product was liberated with aqueous ammonia (2 M) and the ammoniacal fractions were concentrated in vacuo to afford *N*-ethyl-DGJNAc (**1Db**) as a colorless oil (24 mg, quant.); $[\alpha]_D^{25}=+18.1$ (*c* 1.00, MeOH); ^{1}H NMR (D_2O, 400 MHz): δ=1.02 (t, *J*= 6.9 Hz, 3H; NCH$_2$CH_3), 2.01 (s, 3H; NHCOCH_3), 2.23 (app t, *J*(1,1′)=*J*-(1,2)=11.4 Hz, 1H; H1), 2.58 (app s, 1H; H5), 2.63 (dd, *J*=7.0 Hz, J_{gem}= 13.6 Hz, 1H; NC*H*H′CH$_3$), 2.85 (dd, *J*=6.9 Hz, J_{gem}=13.6 Hz, 1H; NCH*H*′CH$_3$), 2.98 (dd, *J*(1′,2)=4.0 Hz, *J*(1′,1)=11.5 Hz, 1H; H1′), 3.51 (br d, *J*=10.3 Hz, 1H; H3), 3.80 (dd, *J*(6,5)=6.4 Hz, *J*(6,6′)=11.4 Hz, 1H; H6), 3.87 (dd, *J*(6′,5)=3.9 Hz, *J*(6′,6)=11.4 Hz, 1H; H6′), 4.04–4.20 ppm (m, 2H; H2 and H4); ^{13}C NMR (D_2O, 100 MHz): δ=8.6 (NCH$_2$*C*H$_3$), 22.7 (NHCO*C*H$_3$), 46.6 (N*C*H$_2$CH$_3$), 47.8 (C2), 53.3 (C1), 60.8 (C6), 62.8 (C5), 70.0 (C4), 73.0 (C3), 175.0 ppm (NH*C*OCH$_3$); IR (thin film): $\tilde{\nu}$=1560 (s; amide II), 1637 (s; amide I), 3317 cm^{-1} (br s; OH); LRMS (ESI+): *m*/*z* (%): 487 (100) [2*M*+Na]$^+$; HRMS (ESI+): *m*/*z* calcd for $C_{10}H_{21}N_2O_4$: 233.1496 [*M*+H]$^+$; found: 233.1495.

2-Acetamido-1-N-butyl-1,2,5-trideoxy-1,5-imino-D-*galactitol,* (*N-butyl-DGJNAc,* ***1Dc***): Butyraldehyde (0.01 mL, 0.1 mmol) and sodium cyanoborohydride (6 mg, 0.1 mmol) were added to a solution of DGJNAc (**1D**, 22.4 mg, 0.110 mmol) in EtOH/acetic-acid (200:1, 10 mL). The mixture was stirred at RT for 2.5 h after which LRMS indicated the absence of the starting material. The reaction mixture was concentrated in vacuo and the crude residue was purified by cation-exchange chromatography (Bond Elut SCX 1 cc cartridge, residue loaded in 50 mM HCl and eluted with 3.5% ammonium hydroxide in MeOH/water 9:1) and reverse-phase chromatography (SepPak C_{18} 1 cc cartridge, residue loaded in water and eluted with MeOH) to afford *N*-butyl-DGJNAc (**1Dc**) as a white crystalline solid (32 mg, quant.). M.p. 180–184 °C; $[\alpha]_D^{25}=+$ 14.4 (*c* 0.62 in MeOH); ^{1}H NMR (D_2O, 400 MHz): δ=0.89 (t, *J*=7.3 Hz, 3H; NCH$_2$CH$_2$CH$_2$CH_3), 1.20–1.32 (m, 2H; NCH$_2$CH$_2$CH_2CH$_3$), 1.35–1.52 (m, 2H; NCH$_2$CH_2CH$_2$CH$_3$), 2.01 (s, 3H; NHCOCH_3), 2.18 (app t, *J*-(1,1′)=*J*(1,2)=11.5 Hz, 1H; H1), 2.43–2.52 (m, 2H; H5 and NC*H*H′CH$_2$CH$_2$CH$_3$), 2.67 (ddd, *J*=6.8 Hz, *J*=9.7 Hz, J_{gem}=13.5 Hz, 1H; NCH*H*′CH$_2$CH$_2$CH$_3$), 2.95 (dd, *J*(1′,2)=4.6 Hz, *J*(1′,1)=11.7 Hz, 1H; H1′), 3.49 (dd, *J*(3,4)=3.7 Hz, *J*(3,2)=10.8 Hz, 1H; H3), 3.78 (dd, *J*-(6,5)=6.7 Hz, *J*(6,6′)=11.4 Hz, 1H; H6), 3.86 (dd, *J*(6′,5)=4.6 Hz, *J*-(6′,6)=11.4 Hz, 1H; H6′), 4.07 (dd, *J*(4,3)=3.7 Hz, *J*(4,5)=5.7 Hz, 1H; H4), 4.07–4.13 ppm (m, 1H; H2); ^{13}C NMR (D_2O, 126 MHz): δ=13.8 (NCH$_2$CH$_2$CH$_2$*C*H$_3$), 20.7 (NCH$_2$CH$_2$*C*H$_2$CH$_3$), 22.7 (NHCO*C*H$_3$), 25.9 (NCH$_2$CH$_2$CH$_2$CH$_3$), 48.0 (C2), 52.4 (NCH$_2$CH$_2$CH$_2$CH$_3$), 54.4 (C1), 61.0 (C6), 63.1 (C5), 70.1 (C4), 73.4 (C3), 175.0 ppm (NH*C*OCH$_3$); IR (thin film, Ge): $\tilde{\nu}$=1559 (s; amide II), 1644 (s; amide I), 3286 cm^{-1} (br s; OH); LRMS (ESI+): *m*/*z* (%): 261 (100) [*M*+H]$^+$; HRMS (ESI+): *m*/*z* calcd for $C_{12}H_{25}N_2O_4$: 261.1809 [*M*+H]$^+$; found: 260.1809.

Data for the HCl salt: ^{1}H NMR (D_2O, 400 MHz): δ=0.93 (t, *J*=7.4 Hz, 3H; NCH$_2$CH$_2$CH$_2$CH_3), 1.31–1.45 (m, 2H; NCH$_2$CH$_2$CH_2CH$_3$), 1.58–1.81 (m, 2H; NCH$_2$CH_2CH$_2$CH$_3$), 2.03 (s, 3H; NHCOCH_3), 3.01 (app t, *J*(1,2)=*J*(1,1′)=11.9 Hz, 1H; H1), 3.16–3.38 (m, 2H; NCH_2CH$_2$CH$_2$CH$_3$), 3.45–3.57 (m, 2H; H1′ and H5), 3.78 (dd, *J*(3,4)= 2.8 Hz, *J*(3,2)=10.8 Hz, 1H; H3), 4.01 (app d, *J*=4.3 Hz, 2H; H6 and H6′), 4.31 (app s, 1H; H4), 4.37 ppm (td, *J*(2,1)=4.8 Hz, *J*(2,1′)=*J*(2,3)= 11.9 Hz, 1H; H2); ^{13}C NMR (D_2O, 100 MHz): δ=13.4 (NCH$_2$CH$_2$CH$_2$*C*H$_3$), 19.9 (NCH$_2$CH$_2$*C*H$_2$CH$_3$), 22.6 (NHCO*C*H$_3$), 24.4

($NCH_2CH_2CH_2CH_3$), 46.0 (C2), 52.1 (C1), 53.8 ($NCH_2CH_2CH_2CH_3$), 59.8 (C6), 64.5 (C5), 70.1 (C4), 70.6 (C3), 175.3 ppm ($NHCOCH_3$).

*2-Acetamido-1-N-benzyl-1,2,5-trideoxy-1,5-imino-*D*-galactitol,* (*N-benzyl-DGJNAc,* ***1Dd***): Benzaldehyde (0.01 mL, 0.1 mmol) and sodium cyanoborohydride (6 mg, 0.1 mmol) were added to a solution of DGJNAc (**1D**, 20 mg, 0.10 mmol) in EtOH/acetic-acid (200:1, 10 mL). The reaction was stirred at RT for 3 h, after which time LRMS indicated the absence of the starting material. The reaction mixture was concentrated in vacuo and the crude residue was purified by cation-exchange chromatography (Bond Elut SCX 1 cc cartridge, residue loaded in 50 mM HCl and eluted with 3.5% ammonium hydroxide in MeOH/water 9:1). A large proportion of the product was eluted during the wash fractions, which were concentrated and purified by column chromatography on Dowex (50W-X8, H^+, washed with water). The product was liberated with aqueous ammonia (2 M) and the ammoniacal fractions were concentrated in vacuo. The products of both columns were combined to afford *N*-benzyl-DGJNAc (**1Dd**) as a colorless oil (20 mg, 68%); $[\alpha]_D^{25} = +17.6$ (*c* 0.25, MeOH); ^{1}H NMR (D_2O, 400 MHz): $\delta = 1.95$ (s, 3H; $NHCOCH_3$), 2.30 (app t, $J(1,1') = J(1,2) = 11.7$ Hz, 1H; H1), 2.96 (app s, 1H; H5), 3.02 (dd, $J(1',2) = 4.2$ Hz, $J(1',1) = 11.7$ Hz, 1H; H1′), 3.58 (dd, $J = 2.9$ Hz, $J = 10.7$ Hz, 1H; H3), 3.83 (d, $J_{gem} = 13.1$ Hz, 1H; NCHH′Ph), 4.06 (dd, $J(5,6') = 5.9$ Hz, $J(6,6') = 12.1$ Hz, 1H; H6), 4.11–4.25 (m, 3H; H2, H4 and H6′), 4.30 (d, $J_{gem} = 13.1$ Hz, 1H; NCHH′Ph), 7.41–7.48 ppm (m, 5H; ArH); ^{13}C NMR (D_2O, 126 MHz): $\delta = 22.5$ ($NHCOCH_3$), 46.5 (C2), 52.7 (C1), 56.7 (NCH_2Ph), 60.5 (C6), 64.6 (C5), 70.2 (C4), 72.3 (C3), 128.9, 129.4, 131.3, 131.8 (ArCH), 136.8 (ArCC), 175.0 ppm ($NHCOCH_3$); IR (thin film, Ge): $\tilde{\nu} = 1558$ (s; amide II), 1635 (s; amide I), 3385 cm^{-1} (br s; OH); LRMS (ESI+): *m*/*z* (%): 295 (100) $[M+H]^+$, 317 (75) $[M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{15}H_{23}N_2O_4$: 295.1652 $[M+H]^+$; found: 295.1648.

*2-Acetamido-1,2,5-trideoxy-1-N-hydroxyethyl-1,5-imino-*D*-galactitol,* (*N-hydroxyethyl-DGJNAc,* ***1De***): Glycoaldehyde dimer (180 mg, 1.50 mmol) and palladium (10% on carbon, 12 mg) were added to a solution of DGJNAc (**1D**, 30 mg, 0.15 mmol) in 1,4-dioxane/water (1:1, 4 mL). The vessel was degassed, charged with hydrogen, and the reaction mixture was stirred at RT for 16 h. The reaction mixture was filtered (GF/A glass microfiber) and concentrated in vacuo. The residue was loaded onto a short column of Dowex (50W-X8, H^+) and the resin was washed with water. The product was liberated with aqueous ammonia (2 M) and the ammoniacal fractions were concentrated in vacuo to afford *N*-hydroxyethyl-DGJNAc (**1De**) as an off-white, viscous gum (35 mg, 95%); $[\alpha]_D^{25} = +14.2$ (*c* 0.96, MeOH); ^{1}H NMR (D_2O, 400 MHz): $\delta = 2.00$ (s, 3H; $NHCOCH_3$), 2.21 (app t, $J(1,1') = J(1,2) = 11.4$ Hz, 1H; H1), 2.52–2.71 (m, 2H; H5 and NCHH′CH_2OH), 2.85–2.96 (m, 1H; NCHH′CH_2OH), 3.02 (dd, $J(1',2) = 4.5$ Hz, $J(1',1) = 11.8$ Hz, 1H; H1′), 3.50 (dd, $J = 2.8$ Hz, $J = 10.5$ Hz, 1H; H3), 3.68 (app dt, $J = 5.7$ Hz, $J_{gem} = 11.8$ Hz, 2H; NCH_2CH_2OH), 3.79 (dd, $J(6,5) = 5.9$ Hz, $J(6,6') = 11.5$ Hz, 1H; H6), 3.85 (dd, $J(6',5) = 5.1$ Hz, $J(6',6) = 11.5$ Hz, 1H; H6′), 3.96–4.12 ppm (m, 2H; H2 and H4); ^{13}C NMR (D_2O, 100 MHz): $\delta = 22.6$ ($NHCOCH_3$), 48.2 (C2), 53.5 (NCH_2CH_2OH), 54.8 (C1), 58.7 (NCH_2CH_2OH), 61.4 (C6), 64.1 (C5), 70.6 (C4), 73.2 (C3), 175.0 ppm ($NHCOCH_3$); IR (thin film, Ge): $\tilde{\nu} = 1561$ (s; amide II), 1638 (s; amide I), 3318 cm^{-1} (br s; OH); LRMS (ESI+): *m*/*z* (%): 249 (27) $[M+H]^+$, 271 (66) $[M+Na]^+$, 519 (100) $[2M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{10}H_{20}N_2NaO_5$: 271.1264 $[M+Na]^+$; found: 271.1260.

Synthesis of DNJNAc and derivatives

*5-Azido-3-O-benzyl-6-O-tert-butyldimethylsilyl-5-deoxy-1,2-O-isopropylidene-β-*L*-idofuranose* (***24L***): Sodium hydride (60% min. oil suspension, 1.00 g, 25.0 mmol) was added to a solution of alcohol **7L** (5.90 g, 16.4 mmol) and benzyl bromide (3.0 mL, 25 mmol) in DMF (50 mL) at 0°C. The reaction mixture was allowed to warm to RT and stirred for 1 h. TLC analysis (cyclohexane/EtOAc, 3:1) indicated the complete consumption of the starting material ($R_f = 0.30$) and the formation of a single product ($R_f = 0.60$). The reaction mixture was diluted with EtOAc (150 mL), washed with a water/sat. brine solution (1:1, 3×150 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (cyclohexane/EtOAc, 49:1 to 9:1) to afford benzyl ether **24L** as a colorless oil (7.36 g, 100%). $[\alpha]_D^{25} = -39.4$ (*c* 1.78, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.01$, 0.03 (s, 2×3H; $Si(CH_3)_2$), 0.87 (s, 9H; $SiC(CH_3)_3$), 1.33, 1.49 (s, 2×3H; $C(CH_3)_2$), 3.53 (dd, $J(6,5) = 6.3$ Hz, $J(6,6') = 11.4$ Hz, 1H; H6), 3.68–3.72 (m, 2H; H5 and H6′), 3.89 (d, $J(3,4) = 3.0$ Hz, 1H; H3), 4.28 (dd, $J(4,3) = 3.0$ Hz, $J(4,5) = 8.3$ Hz, 1H; H4), 4.44 (d, $J_{gem} = 11.9$ Hz, 1H; OCH_2Ph), 4.67 (d, $J(2,1) = 3.8$ Hz, 1H; H2), 4.71 (d, $J_{gem} = 11.9$ Hz, 1H; OCH_2Ph), 5.97 (d, $J(1,2) = 3.8$ Hz, 1H; H1), 7.30–7.39 ppm (m, 5H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = -5.7$, -5.6 ($Si(CH_3)_2$), 18.1 ($SiC(CH_3)_3$), 25.7 ($SiC(CH_3)_3$), 26.4, 26.7 ($C(CH_3)_2$), 61.9 (C5), 63.1 (C6), 71.5 (OCH_2Ph), 78.8 (C4), 81.3 (C3), 81.8 (C2), 104.7 (C1), 111.9 ($C(CH_3)_2$), 128.0, 128.2, 128.6 (ArCH), 136.8 ppm (ArCC); IR (thin film): $\tilde{\nu} = 2100$ (s; N_3); LRMS (ESI+): *m*/*z* (%): 472 (67) $[M+Na]^+$, 551 (56), 916 (93) $[2M+NH_4]^+$, 921 (100) $[2M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{22}H_{35}N_3NaO_5Si$: 472.2238 $[M+Na]^+$; found: 472.2235.

For enantiomer **24D**: $[\alpha]_D^{25} = +30.1$ (*c* 1.21, $CHCl_3$).

*Methyl 5-azido-3-O-benzyl-5-deoxy-*L*-idofuranosides* (***10L***): A solution of benzyl ether **24L** (7.36 g, 16.4 mmol) in a mixture of MeOH (50 mL) and acetyl chloride (2.5 mL) was stirred at 50°C for 1 h. TLC analysis (cyclohexane/EtOAc, 3:1) indicated the complete consumption of the starting material ($R_f = 0.60$) and the formation of two products ($R_f = 0.00$; toluene/acetone (4:1): $R_f = 0.25$, 0.35). The reaction mixture was neutralized by the addition of solid sodium bicarbonate, filtered, and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (toluene/acetone, 49:1 to 3:1) to afford anomeric methyl furanoside **10Lβ** as a white crystalline solid ($R_f = 0.35$, 2.42 g, 48%) and **10Lα** as a colorless oil ($R_f = 0.25$, 2.19 g, 43%).

Data for compound **10Lβ**: M.p. 46–50°C; $[\alpha]_D^{25} = +79.1$ (*c* 1.39, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 2.04$ (t, $J(OH6,6) = J(OH6,6') = 6.3$ Hz, 1H; OH6), 2.85 (d, $J(OH2,2) = 6.8$ Hz, 1H; OH2), 3.51 (s, 3H; OCH_3), 3.57 (app dt, $J(6,5) = J(6,OH6) = 6.1$ Hz, $J(6,6') = 11.6$ Hz, 1H; H6), 3.66 (ddd, $J(6',5) = 4.0$ Hz, $J(6',OH6) = 6.6$ Hz, $J(6',6) = 11.6$ Hz, 1H; H6′), 3.82 (app dt, $J(5,6') = 4.0$ Hz, $J(5,4) = J(5,6) = 6.4$ Hz, 1H; H5), 3.99 (dd, $J(3,2) = 3.8$ Hz, $J(3,4) = 5.6$ Hz, 1H; H3), 4.28 (app t, $J(4,3) = J(4,5) = 6.0$ Hz, 1H; H4), 4.37 (ddd, $J(2,3) = 3.8$ Hz, $J(2,1) = 4.5$ Hz, $J(2,OH2) = 7.1$ Hz, 1H; H2), 4.55 (d, $J_{gem} = 11.9$ Hz, 1H; OCH_2Ph), 4.81 (d, $J_{gem} = 11.9$ Hz, 1H; OCH_2Ph), 5.05 (d, $J(1,2) = 4.8$ Hz, 1H; H1), 7.30–7.39 ppm (m, 5H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 55.8$ (OCH_3), 62.4 (C6), 62.9 (C5), 71.7 (OCH_2Ph), 76.4 (C2), 77.9 (C4), 83.1 (C3), 101.6 (C1), 127.9, 128.0, 129.5 (ArCH), 137.2 ppm (ArCC); IR (thin film): $\tilde{\nu} = 2105$ (s; N_3), 3424 (br s; OH); LRMS (ESI+): *m*/*z* (%): 332 (91) $[M+Na]^+$, 348 (78) $[M+K]^+$, 641 (100) $[2M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{14}H_{19}N_3NaO_5$: 332.1217 $[M+Na]^+$; found: 332.1215.

For enantiomer **10Dβ**: M.p. 43–44°C; $[\alpha]_D^{25} = -73.2$ (*c* 1.32, $CHCl_3$).

Data for compound **10Lα**: $[\alpha]_D^{25} = -41.4$ (*c* 0.92, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 2.20$ (t, $J(OH6,6) = J(OH6,6') = 6.5$ Hz, 1H; OH6), 2.51 (d, $J(OH2,2) = 4.3$ Hz, 1H; OH2), 3.47 (s, 3H; OCH_3), 3.50 (app dt, $J(6,5) = J(6,OH6) = 6.1$ Hz, $J(6,6') = 11.6$ Hz, 1H; H6), 3.65 (ddd, $J(6',5) = 3.5$ Hz, $J(6',OH6) = 6.1$ Hz, $J(6',6) = 11.6$ Hz, 1H; H6′), 3.91 (ddd, $J(5,6') = 3.8$ Hz, $J(5,6) = 5.8$ Hz, $J(5,4) = 8.1$ Hz, 1H; H5), 3.94 (dd, $J(3,2) = 2.3$ Hz, $J(3,4) = 5.6$ Hz, 1H; H3), 4.33 (app dt, $J(2,1) = J(2,3) = 2.0$ Hz, $J(2,OH2) = 4.0$ Hz, 1H; H2), 4.35 (dd, $J(4,3) = 5.6$ Hz, $J(4,5) = 8.3$ Hz, 1H; H4), 4.49 (d, $J_{gem} = 11.9$ Hz, 1H; OCH_2Ph), 4.73 (d, $J_{gem} = 11.9$ Hz, 1H; OCH_2Ph), 4.86 (d, $J(1,2) = 1.5$ Hz, 1H; H1), 7.30–7.39 ppm (m, 5H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 56.2$ (OCH_3), 62.2 (C6), 64.2 (C5), 72.1 (OCH_2Ph), 78.3 (C2), 80.7 (C4), 82.3 (C3), 109.6 (C1), 128.1, 128.1, 129.6 (ArCH), 137.0 ppm (ArCC); IR (thin film): $\tilde{\nu} = 2106$ (s; N_3), 3417 (br s; OH); LRMS (ESI+): *m*/*z* (%): 332 (74) $[M+Na]^+$, 348 (57), $[M+K]^+$, 641 (100) $[2M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{14}H_{19}N_3NaO_5$: 332.1217 $[M+Na]^+$; found: 332.1214.

For enantiomer **10Dα**: $[\alpha]_D^{25} = +33.6$ (*c* 1.03, $CHCl_3$).

*Methyl-5-azido-3-O-benzyl-5-deoxy-6-O-methanesulfonyl-β-*L*-idofuranoside* (***25Lβ***): Methanesulfonyl chloride (0.29 mL, 3.8 mmol) was added dropwise to a solution of diol **10Lβ** (982 mg, 3.17 mmol) and pyridine (0.61 mL, 7.6 mmol) in CH_2Cl_2 (9 mL) at 0°C. After 3 h, TLC analysis (toluene/acetone, 4:1) indicated the complete consumption of the starting material ($R_f = 0.35$) and the formation of a major product ($R_f = 0.45$) and a minor product ($R_f = 0.50$). The reaction mixture was diluted with CH_2Cl_2 (15 mL), washed with 2 M HCl (3×20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was purified by

column chromatography on silica gel (CH_2Cl_2/MeOH, 60:1) to afford the primary mesylate (**25Lβ**) as a colorless oil (582 mg, 86%); $[\alpha]_D^{25}=+72.6$ (*c* 0.97, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=2.74$ (d, J(OH2,2)= 7.6 Hz, 1H; OH2), 3.02 (s, 3H; SO_2CH_3), 3.49 (s, 3H; OCH_3), 3.95 (app dt, $J(5,6')=4.3$ Hz, $J(5,4)=J(5,6)=6.3$ Hz, 1H; H5), 4.05 (dd, $J(3,2)=$ 4.3 Hz, $J(3,4)=6.1$ Hz, 1H; H3), 4.20 (dd, $J(6,5)=6.7$ Hz, $J(6,6')=$ 10.6 Hz, 1H; H6), 4.25 (dd, $J(6',5)=4.3$ Hz, $J(6',6)=10.6$ Hz, 1H; H6′), 4.26 (app t, $J(4,3)=J(4,5)=6.0$ Hz, 1H; H4), 4.37 (app dt, $J(2,1)=J$-(2,3)=4.5 Hz, J(2,OH2)=7.6 Hz, 1H; H2), 4.57 (d, $J_{gem}=11.6$ Hz, 1H; OCH_2Ph), 4.83 (d, $J_{gem}=11.6$ Hz, 1H; OCH_2Ph), 5.01 (d, $J(1,2)=4.8$ Hz, 1H; H1), 7.30–7.40 ppm (m, 5H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=37.5$ (SO_2CH_3), 55.9 (OCH_3), 60.0 (C5), 68.5 (C6), 71.9 (OCH_2Ph), 76.6 (C2), 76.7 (C4), 82.8 (C3), 101.7 (C1), 128.1, 128.6 (Ar*C*H), 137.1 ppm (Ar*C*C); IR (thin film): $\tilde{\nu}=2110$ (s; N_3), 3518 (br s; OH); LRMS (ESI+): *m*/*z* (%): 410 (100) $[M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{15}H_{21}N_3NaO_7S$: 410.0992 $[M+Na]^+$; found: 410.0991.

For enantiomer **25Dβ**: $[\alpha]_D^{25}=-65.5$ (*c* 0.87, $CHCl_3$)

*Methyl-5-azido-2-N-benzyl-3-O-benzyl-2,6-imino-2,5,6-trideoxy-β-*L*-gulofuranoside* (***11Lβ***): Trifluoromethanesulfonic anhydride (0.69 mL, 4.2 mmol) and pyridine (0.68 mL, 8.4 mmol) were added to a solution of mesylate **25Lβ** (1.28 g, 3.38 mmol) in CH_2Cl_2 (14 mL) at −40°C. After 1 h, with the internal temperature rising to −30°C, TLC analysis (CH_2Cl_2/MeOH, 30:1) indicated the complete consumption of the starting material ($R_f=0.45$) and the formation of a single product ($R_f=0.65$). The reaction mixture was diluted with CH_2Cl_2 (25 mL), washed with 2M HCl (3×20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude triflate (**26Lβ**) was dissolved in benzylamine (5.8 mL) and stirred at 100°C for 18 h. TLC analysis (cyclohexane/EtOAc/triethylamine, 66:33:1) indicated the complete consumption of the triflate ($R_f=0.30$) and the formation of a major product ($R_f=0.70$). The reaction mixture was pre-adsorbed onto silica gel and purified by column chromatography on silica gel (cyclohexane/EtOAc/triethylamine, 99:0:1 to 94:5:1) to afford the bicyclic piperidine (**11Lβ**) as a pale-yellow crystalline solid (1.09 g, 70%). M.p. 50–54°C; $[\alpha]_D^{25}=+120.3$ (*c* 0.46, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=3.02$ (dd, $J(6,5)=4.3$ Hz, $J(6,6')=13.1$ Hz, 1H; H6), 3.06 (d, $J(6',6)=13.1$ Hz, 1H; H6′), 3.18 (d, $J(2,3)=4.3$ Hz, 1H; H2), 3.35 (s, 3H; OCH_3), 3.49–3.52 (m, 1H; H5), 3.73 (s, 2H; NCH_2Ph), 4.16 (app t, $J(3,2)=J(3,4)=4.6$ Hz, 1H; H3), 4.26 (t, $J(4,3)=J(4,5)=$ 4.6 Hz, 1H; H4), 4.51 (d, $J_{gem}=11.9$ Hz, 1H; OCH_2Ph), 4.63 (d, $J_{gem}=$ 11.9 Hz, 1H; OCH_2Ph), 4.94 (s, 1H; H1), 7.28–7.47 ppm (m, 10H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=49.4$ (C6), 55.4 (OCH_3), 56.3 (C5), 60.1 (NCH_2Ph), 61.5 (C2), 70.7 (OCH_2Ph), 72.2 (C4), 73.1 (C3), 101.4 (C1), 127.5, 127.6, 127.7, 128.3, 128.4, 129.1 (Ar*C*H), 137.5, 137.9 ppm (Ar*C*C); IR (thin film): $\tilde{\nu}=2114$ (s; N_3); LRMS (ESI+): *m*/*z* (%): 381 (75) $[M+H]^+$, 403 (77) $[M+Na]^+$, 783 (100) $[M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{21}H_{24}N_4NaO_3$: 403.1741 $[M+Na]^+$; found: 403.1737.

For enantiomer **11Dβ**: M.p. 48–50°C; $[\alpha]_D^{25}=-109.2$ (*c* 0.67, $CHCl_3$).

*Methyl-5-azido-3-O-benzyl-5-deoxy-6-O-p-toluenesulfonyl-α-*L*-idofuranoside* (***27Lα***): *p*-Toluenesulfonyl chloride (450 mg, 2.36 mmol) was added to a solution of diol **10Lα** (358 mg, 1.16 mmol) and 2,4,6-collidine (0.60 mL, 4.5 mmol) in CH_2Cl_2 (5 mL) at RT. After 24 h, TLC analysis (toluene/acetone, 4:1) indicated a trace of starting material ($R_f=0.25$), as well as the formation of a major product ($R_f=0.50$) and a minor product ($R_f=0.75$). The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with 2M HCl (3×10 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was purified by flash chromatography (toluene/acetone, 99:1 to 19:1) to afford the primary tosylate (**27Lα**) as a colorless oil (397 mg, 74%). $[\alpha]_D^{25}=-7.0$ (*c* 1.32, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=2.07$ (br s, 1H; OH2), 2.44 (s, 3H; $SO_2C_6H_4CH_3$), 3.40 (s, 3H; OCH_3), 3.90–3.95 (m, 2H; H3 and H5), 4.05 (dd, $J(6,5)=$ 5.8 Hz, $J(6,6')=10.4$ Hz, 1H; H6), 4.09 (dd, $J(6',5)=3.8$ Hz, $J(6',6)=$ 10.4 Hz, 1H; H6′), 4.28–4.29 (m, 1H; H2), 4.30 (dd, $J=6.1$ Hz, $J=$ 7.6 Hz, 1H; H4), 4.46 (d, $J_{gem}=11.6$ Hz, 1H; OCH_2Ph), 4.67 (d, $J_{gem}=$ 11.6 Hz, 1H; OCH_2Ph), 4.81 (d, $J(1,2)=1.5$ Hz, 1H; H1), 7.30–7.39 (m, 7H; ArH), 7.74–7.77 ppm (m, 2H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=21.7$ ($SO_2C_6H_4CH_3$), 56.1 (OCH_3), 60.8 (C5), 68.9 (C6), 72.3 (OCH_2Ph), 78.3 (C2), 79.7 (C4), 82.5 (C3), 109.6 (C1), 128.0, 128.1, 128.2, 128.6, 129.9 (Ar*C*H), 132.5, 137.0, 145.1 ppm (Ar*C*C); IR (thin film): $\tilde{\nu}=$ 2105 (s; N_3), 3443 (br s; OH); LRMS (ESI+): *m*/*z* (%): 486 (100) $[M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{21}H_{25}N_3NaO_7S$: 486.1305 $[M+Na]^+$; found: 486.1303.

For enantiomer **27Dα**: $[\alpha]_D^{25}=+11.0$ (*c* 1.15, $CHCl_3$).

*Methyl-5-azido-2-N,3-O-dibenzyl-2,5,6-trideoxy-2,6-imino-α-*L*-gulofuranoside* (***11Lα***): Trifluoromethanesulfonic anhydride (0.20 mL, 1.2 mmol) and pyridine (0.20 mL, 2.5 mmol) were added to a solution of tosylate **27Lα** (483 mg, 1.04 mmol) in CH_2Cl_2 (5 mL) at −40°C. After 1 h, with the internal temperature rising to −30°C, TLC analysis (cyclohexane/EtOAc, 2:1) indicated the complete consumption of the starting material ($R_f=0.15$) and the formation of a single product ($R_f=0.55$). The reaction mixture was diluted with CH_2Cl_2 (15 mL), washed with 2M HCl (3× 20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude triflate (**28Lα**) was dissolved in benzylamine (2.5 mL) and the mixture was stirred at 100°C for 18 h. TLC analysis (cyclohexane/EtOAc, 80:19:1) indicated the complete consumption of the triflate ($R_f=0.35$) and the formation of a major product ($R_f=0.60$). The reaction mixture was preadsorbed onto silica gel and purified by column chromatography on silica gel (cyclohexane/EtOAc/triethylamine, 99:0:1 to 94:5:1) to afford the bicyclic piperidine (**11Lα**) as a pale-yellow oil (267 mg, 68%). $[\alpha]_D^{25}=+39.2$ (*c* 1.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=3.00$ (d, J-(6,6′)=12.9 Hz, 1H; H6), 3.27 (app t, $J(2,1)=J(2,3)=3.7$ Hz, 1H; H2), 3.50 (s, 3H; OCH_3), 3.56 (app t, $J(5,4)=J(5,6')=4.7$ Hz, 1H; H5), 3.77 (dd, $J(6',5)=5.6$ Hz, $J(6',6)=12.9$ Hz, 1H; H6′), 3.83 (app t, $J(3,2)=J$-(3,4)=4.5 Hz, 1H; H3), 3.92 (d, $J_{gem}=13.4$ Hz, 1H; NCH_2Ph), 4.06 (d, $J_{gem}=13.4$ Hz, 1H; NCH_2Ph), 4.24 (app t, $J(4,3)=J(4,5)=4.5$ Hz, 1H; H4), 4.47 (d, $J_{gem}=12.1$ Hz, 1H; OCH_2Ph), 4.64 (d, $J_{gem}=12.1$ Hz, 1H; OCH_2Ph), 5.02 (d, $J(1,2)=2.8$ Hz, 1H; H1), 7.25–7.37 ppm (m, 10H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=49.0$ (C6), 56.4 (C5), 56.8 (OCH_3), 57.6 (C2), 60.1 (NCH_2Ph), 71.5 (OCH_2Ph), 74.9 (C4), 75.1 (C3), 107.3 (C1), 127.1, 127.6, 127.7, 128.2, 128.3, 129.1 (Ar*C*H), 137.7, 138.4 ppm (Ar*C*C); IR (thin film): $\tilde{\nu}=2114$ (s; N_3); LRMS (ESI+): *m*/*z* (%): 381 (97) $[M+H]^+$, 403 (100) $[M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{21}H_{25}N_4O_3$: 381.1921 $[M+H]^+$; found: 381.1921.

For enantiomer **11Dα**: $[\alpha]_D^{25}=-40.0$ (*c* 1.58, $CHCl_3$).

*4-O-Acetyl-5-azido-2-N,3-O-dibenzyl-2,5,6-trideoxy-2,6-imino-*L*-gulose acetyl methyl acetal* (***29L***): Boron trifluoride diethyl etherate (0.82 mL, 6.6 mmol) was added dropwise to a solution of bicyclic acetal **11Lβ** (797 mg, 2.09 mmol) in acetic anhydride (8 mL) at 0°C. The reaction mixture was allowed to warm to RT and stirred for 36 h, after which time TLC analysis (cyclohexane/EtOAc/triethylamine, 80:19:1) indicated the complete consumption of the starting material ($R_f=0.60$) and the formation of a major product ($R_f=0.50$). The reaction mixture was concentrated in vacuo and the residue was dissolved in EtOAc (40 mL), washed with a saturated solution of sodium bicarbonate (3×20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was purified by column chromatography on silica gel (cyclohexane/EtOAc/triethylamine, 99:0:1 to 90:9:1) to afford the mixed-acetal piperidine (**29L**) as a pale-yellow oil (951 mg, 94%), in a 2:1 (A/B) ratio of diastereoisomers.

Similar treatment of the epimer (**11Lα**, 96 mg, 0.25 mmol, $R_f=0.55$ in cyclohexane/EtOAc/triethylamine, 80:19:1) with boron trifluoride etherate for 3 h afforded compound **29L** as a pale-yellow oil (86 mg, 71%), as a 2:1 (A/B) ratio of diastereoisomers.

Data for compound **29L**: ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=2.06$ (s, 3H; $COCH_3{}^B$), 2.07, 2.09 (s, 2×3H; $COCH_3{}^A$), 2.14 (s, 3H; $COCH_3{}^B$), 2.16–2.23 (m, 2H; H6^A and H6^B), 2.82–2.85 (m, 2H; H2^A and H2^B), 2.96 (dd, $J(6',5)=4.8$ Hz, $J(6',6)=12.4$ Hz, 1H; H6′^{A}), 2.96 (dd, $J(6',5)=4.5$ Hz, J-(6′,6)=11.9 Hz, 1H; H6′^{B}), 3.35 (s, 3H; $OCH_3{}^B$), 3.39 (s, 3H; $OCH_3{}^A$), 3.42 (d, $J_{gem}=14.1$ Hz, 1H; NCH_2Ph^B), 3.45–3.52 (m, 2H; H5^A and H5^B), 3.57 (d, $J_{gem}=13.4$ Hz, 1H; NCH_2Ph^A), 3.64 (t, $J(3,2)=J(3,4)=8.1$ Hz, 1H; H3^B), 3.70 (t, $J(3,2)=J(3,4)=8.8$ Hz, 1H; H3^A), 4.29 (d, $J_{gem}=$ 13.4 Hz, 1H; NCH_2Ph^A), 4.38 (d, $J_{gem}=14.1$ Hz, 1H; NCH_2Ph^B), 4.63 (d, $J_{gem}=11.1$ Hz, 1H; OCH_2Ph^A), 4.64 (d, $J_{gem}=10.9$ Hz, 1H; OCH_2Ph^B), 4.68 (d, $J_{gem}=11.1$ Hz, 1H; OCH_2Ph^A), 4.73 (d, $J_{gem}=10.9$ Hz, 1H; OCH_2Ph^B), 5.08–5.13 (m, 2H; H4^A and H4^B), 6.14 (d, $J(1,2)=1.8$ Hz, 1H; H1^B), 6.23 (d, $J(1,2)=2.3$ Hz, 1H; H1^A), 7.24–7.39 ppm (m, 20H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=21.0$, 21.0 ($CO\mathit{C}H_3{}^A$), 21.1, 21.1

($CO\underline{C}H_3^B$), 52.0 ($C6^A$), 52.4 ($C6^B$), 57.0 ($N\underline{C}H_2Ph^A$), 57.2 ($O\underline{C}H_3^B$), 57.6 ($O\underline{C}H_3^A$), 58.3 ($N\underline{C}H_2Ph^B$), 58.7 ($C5^A$), 58.9 ($C5^B$), 66.2 ($C2^A$), 66.8 ($C2^B$), 74.2 ($O\underline{C}H_2Ph^A$), 74.3 ($O\underline{C}H_2Ph^B$), 76.6, 76.8, 76.9, 76.9 ($C3^A$, $C3^B$, $C4^A$, $C4^B$), 96.9 ($C1^A$), 97.2 ($C1^B$), 127.1, 127.2, 127.5, 127.7, 127.7, 127.7, 128.0, 128.1, 128.4, 128.4, 128.6, 128.9 (Ar*C*H), 137.9, 138.7, 138.7, 139.0 (Ar*C*C), 170.0, 170.2, 170.2, 171.0 ppm ($\underline{C}OCH_3$); IR (thin film): $\tilde{\nu}$ = 1746 (s, C=O), 2104 (s, N_3); LRMS (ESI+): *m*/*z* (%): 483 (97) $[M+H]^+$, 505 (100) $[M+Na]^+$, 987 (92) $[2M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{25}H_{31}N_4O_6$: 483.2238 $[M+H]^+$; found: 483.2242.

3,6-Di-O-acetyl-2-azido-1-N,4-O-dibenzyl-1,2,5-trideoxy-1,5-imino-D-*glucitol* (***30D***): Diisobutylaluminium hydride (16.8 mL, 16.8 mmol) was added dropwise to a solution of mixed acetal **29L** (1.6 g, 3.3 mmol) in CH_2Cl_2 (13 mL) at −78 °C. After 40 min, TLC analysis (cyclohexane/EtOAc/triethylamine, 79:20:1) indicated the complete consumption of the starting material (R_f = 0.40) and the formation of a major product (R_f = 0.25). The reaction mixture was quenched and diluted with EtOAc (88 mL) and allowed to warm to RT. A saturated solution of sodium potassium tartrate (88 mL) was added and the biphasic mixture was stirred vigorously for 1 h. The organic layer was collected and the aqueous layer was extracted with EtOAc (3 × 20 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was dissolved in MeOH (18 mL) and sodium borohydride (125 mg, 3.30 mmol) was added at −10 °C. After 2 h, TLC analysis (cyclohexane/EtOAc/triethylamine, 59:40:1) indicated the complete consumption of the intermediate (R_f = 0.55) and the formation of a major product (R_f = 0.25). The reaction mixture was neutralized with glacial acetic acid, concentrated in vacuo, and dissolved in pyridine (9 mL) and acetic anhydride (9 mL). The reaction was stirred at RT for 16 h, after which time TLC analysis (cyclohexane/EtOAc/triethylamine, 59:40:1) indicated the complete consumption of the starting material (R_f = 0.25) and the formation of a major product (R_f = 0.40). The reaction mixture was concentrated in vacuo and purified by column chromatography on silica gel (cyclohexane/EtOAc/triethylamine, 96:3:1 to 84:15:1) to afford the diacetyl piperidine (**30D**) as a white crystalline solid (1.315 g, 88% over 3 steps). M.p. 89–91 °C; $[\alpha]_D^{22}$ = +3.3 (*c* 1.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.06 (app t, $J(1,1')=J(1,2)$ = 11.4 Hz, 1H; H1), 2.07, 2.08 (s, 2 × 3H; $COCH_3$), 2.55 (app dt, $J(5,6)=J(5,6')$ = 2.7 Hz, $J(5,4)$ = 9.6 Hz, 1H; H5), 2.98 (dd, $J(1',2)$ = 4.8 Hz, $J(1',1)$ = 11.6 Hz, 1H; H1′), 3.27 (d, J_{gem} = 13.3 Hz, 1H; $NC\underline{H}_2Ph$), 3.47 (app dt, $J(2,1')$ = 4.8 Hz, $J(2,1)=J(2,3)$ = 10.4 Hz, 1H; H2), 3.66 (app t, $J(4,3)=J(4,5)$ = 9.4 Hz, 1H; H4), 4.09 (d, J_{gem} = 13.3 Hz, 1H; $NC\underline{H}_2Ph$), 4.31 (dd, $J(6,5)$ = 2.7 Hz, $J(6,6')$ = 12.6 Hz, 1H; H6), 4.59 (d, J_{gem} = 10.9 Hz, 1H; $OC\underline{H}_2Ph$), 4.64 (dd, $J(6',5)$ = 2.4 Hz, $J(6',6)$ = 12.8 Hz, 1H; H6′), 4.66 (d, J_{gem} = 10.9 Hz, 1H; $OC\underline{H}_2Ph$), 5.08 (app t, $J(3,2)=J(3,4)$ = 9.6 Hz, 1H; H3), 7.26–7.38 ppm (m, 10H; ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 20.9, 20.9 ($CO\underline{C}H_3$), 53.6 (C1), 56.5 ($N\underline{C}H_2Ph$), 59.3 (C2), 59.9 (C6), 63.9 (C5), 75.0 ($O\underline{C}H_2Ph$), 76.9 (C4), 77.4 (C3), 127.4, 128.0, 128.0, 128.5, 128.5, 128.7 (Ar*C*H), 137.3, 137.6 (Ar*C*C), 170.0, 170.6 ppm ($\underline{C}OCH_3$); IR (thin film): $\tilde{\nu}$ = 1743 (s, C=O), 2104 (s, N_3); LRMS (ESI+): *m*/*z* (%): 453 (94) $[M+H]^+$, 475 (100) $[M+Na]^+$, 927 (99) $[2M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{24}H_{28}N_4NaO_5$: 475.1952 $[M+H]^+$; found: 475.1954.

For enantiomer **30L**: M.p. 90–92 °C; $[\alpha]_D^{25}$ = −4.3 (*c* 2.2, $CHCl_3$).

2-Acetamido-3,6-di-O-acetyl-1-N,4-O-dibenzyl-1,2,5-trideoxy-1,5-imino-D-*glucitol* (***31D***): Powdered zinc (3.8 g, 58 mmol) was added to a solution of azide **30D** (1.31 g, 2.91 mmol) in THF/glacial-acetic-acid/acetic-anhydride (3:2:1, 35 mL) and the mixture was stirred vigorously before the dropwise addition of a saturated solution of copper(II) sulfate (9.2 mL) to initiate the reaction. After 20 min, TLC analysis (cyclohexane/EtOAc/triethylamine, 79:20:1) indicated the complete consumption of the starting material (R_f = 0.50) and the formation of a major product (R_f = 0.10). The reaction mixture was filtered through Celite, concentrated in vacuo, and the crude residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 40:1) to afford the acetamide (**31D**) as a white crystalline solid (1.11 g, 81%). M.p. 149–151 °C; $[\alpha]_D^{25}$ = +0.9 (*c* 0.92, acetone); ^{1}H NMR ([D_6]acetone), 400 MHz): δ = 1.74 (s, 3H; $NHCOC\underline{H}_3$), 1.98, 2.06 (s, 2 × 3H; $COCH_3$), 2.14 (dd, $J(1,2)$ = 10.4 Hz, $J(1,1')$ = 11.6 Hz, 1H; H1), 2.63 (app dt, $J(5,6)=J(5,6')$ = 3.0 Hz, $J(5,4)$ = 8.8 Hz, 1H; H5), 2.85 (dd, $J(1',2)$ = 4.5 Hz, $J(1',1)$ = 11.6 Hz, 1H; H1′), 3.34 (d, J_{gem} = 13.6 Hz, 1H; $NC\underline{H}_2Ph$), 3.72 (app t, $J(4,3)=J(4,5)$ = 8.8 Hz, 1H; H4), 4.05 (app dq, $J(2,1')$ = 4.5 Hz, $J(2,1)=J(2,3)=J(2,NH)$ = 9.9 Hz, 1H; H2), 4.13 (d, J_{gem} = 13.6 Hz, 1H; $NC\underline{H}_2Ph$), 4.35 (dd, $J(6,5)$ = 3.3 Hz, $J(6,6')$ = 12.6 Hz, 1H; H6), 4.64 (dd, $J(6',5)$ = 2.8 Hz, $J(6',6)$ = 12.6 Hz, 1H; H6), 4.65 (d, J_{gem} = 11.1 Hz, 1H; $OC\underline{H}_2Ph$), 4.72 (d, J_{gem} = 11.1 Hz, 1H; $OC\underline{H}_2Ph$), 4.90 (dd, $J(3,4)$ = 8.6 Hz, $J(3,2)$ = 9.6 Hz, 1H; H3), 6.77 (d, $J(NH,2)$ = 9.3 Hz, 1H; $N\underline{H}COCH_3$), 7.21–7.37 ppm (m, 10H; ArH); ^{13}C NMR ([D_6]acetone), 100 MHz): δ = 20.8, 21.0 ($CO\underline{C}H_3$), 22.9 ($NHCO\underline{C}H_3$), 49.0 (C2), 54.6 (C1), 57.3 ($N\underline{C}H_2Ph$), 60.6 (C6), 64.7 (C5), 75.2 ($O\underline{C}H_2Ph$), 77.7 (C3), 77.9 (C4), 127.8, 128.4, 128.7, 128.1, 128.1, 128.5 (Ar*C*H), 139.3, 140.0 (Ar*C*C), 169.5, 170.9, 170.9 ppm ($\underline{C}OCH_3$; $NHCOCH_3$); IR (thin film, Ge): $\tilde{\nu}$ = 1546 (s; amide II), 1660 (s; amide I), 1739 (s; C=O); LRMS (ESI+): *m*/*z* (%): 469 (100) $[M+H]^+$, 491 (64) $[M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{26}H_{33}N_2O_6$: 469.2333 $[M+H]^+$; found: 469.2334.

For enantiomer **31L**: M.p. 144–148 °C; $[\alpha]_D^{25}$ = −2.6 (*c* 1.18, acetone).

2-Acetamido-1,2,5-trideoxy-1,5-imino-D-*glucitol* (*DNJNAc,* ***2D***). Sodium methoxide (60 mg, cat.) was added to a solution of the protected piperidine (**31D**, 1.11 g, 2.63 mmol) in MeOH (25 mL) at RT and the mixture was stirred for 16 h. TLC analysis (EtOAc/triethylamine, 99:1) indicated the complete consumption of the starting material (R_f = 0.70) and the formation of a major product (R_f = 0.25). The reaction mixture was neutralized with solid CO_2, concentrated in vacuo, and purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 20:1 to 9:1). The crude residue was dissolved in water/2 M HCl/1,4-dioxane (13:3:4, 35 mL) and palladium (10% on carbon, 408 mg) was added. The vessel was degassed, charged with hydrogen, and the reaction mixture was stirred at RT for 22 h. LRMS and ^{1}H NMR spectroscopy indicated that a single product had been formed, with no trace of benzylated intermediates observed. The reaction mixture was filtered through Celite and concentrated in vacuo to afford crude DNJNAc (**2D**) as its hydrochloride salt.

Selected data for DNJNAc·HCl (**2D**): ^{1}H NMR (D_2O, 400 MHz): δ = 2.03 (s, 3H; $NHCOC\underline{H}_3$), 2.99 (app t, 1H, $J(1,2)=J(1,1')$ = 12.5 Hz; H1), 3.23 (ddd, $J(5,6')$ = 3.2 Hz, $J(5,6)$ = 5.0 Hz, $J(5,4)$ = 10.1 Hz, 1H; H5), 3.50 (dd, $J(1',2)$ = 5.0 Hz, $J(1',1)$ = 12.6 Hz, 1H; H1′), 3.63 (app t, $J(3,2)=J(3,4)$ = 9.6 Hz, 1H; H3), 3.67 (dd, $J(4,3)$ = 9.1 Hz, $J(4,5)$ = 10.2 Hz, 1H; H4), 3.90 (dd, $J(6,5)$ = 5.1 Hz, $J(6,6')$ = 12.8 Hz, 1H; H6), 3.96 (dd, $J(6',5)$ = 3.2 Hz, $J(6',6)$ = 12.8 Hz, 1H; H6′), 3.72 ppm (ddd, $J(2,1')$ = 5.0 Hz, $J(2,3)$ = 10.1 Hz, $J(2,1)$ = 12.3 Hz, 1H; H2).

The crude salt of compound **2D** was loaded onto a short column of Dowex (50W-X8, H^+) and the resin washed with water until neutral fractions were obtained. The product was liberated with aqueous ammonia (2 M) and the ammoniacal fractions were concentrated in vacuo to afford DNJNAc (**2D**) as a white crystalline solid (510 mg, 95%). M.p. 224–226 °C [lit. 227–228 °C];[16c] $[\alpha]_D^{25}$ = +14.6 (*c* 0.86, H_2O) [lit. $[\alpha]_D^{20}$ = +16.4[16c] (*c* 1.00, H_2O)]; ^{1}H NMR (D_2O, 400 MHz): δ = 2.00 (s, 3H; $NHCOC\underline{H}_3$), 2.43 (dd, $J(1,2)$ = 11.5 Hz, $J(1,1')$ = 12.6 Hz, 1H; H1), 2.54 (ddd, $J(5,6')$ = 3.0 Hz, $J(5,6)$ = 6.1 Hz, $J(5,4)$ = 9.7 Hz, 1H; H5), 3.06 (dd, $J(1',2)$ = 4.9 Hz, $J(1',1)$ = 12.6 Hz, 1H; H1′), 3.30 (app t, $J(3,2)=J(3,4)$ = 9.7 Hz, 1H; H3), 3.39 (dd, $J(4,3)$ = 9.1 Hz, $J(4,5)$ = 10.0 Hz, 1H; H4), 3.66 (dd, $J(6,5)$ = 6.1 Hz, $J(6,6')$ = 11.6 Hz, 1H; H6), 3.72 (ddd, $J(2,1')$ = 4.9 Hz, $J(2,3)$ = 10.2 Hz, $J(2,1)$ = 11.4 Hz, 1H; H2), 3.82 ppm (dd, $J(6',5)$ = 2.9 Hz, $J(6',6)$ = 11.6 Hz, 1H; H6′); ^{13}C NMR (D_2O, 100 MHz): δ = 22.6 ($NHCOC\underline{H}_3$), 47.5 (C1), 52.8 (C2), 61.0 (C5), 61.8 (C6), 72.5 (C3), 76.4 (C4), 174.9 ppm ($NH\underline{C}OCH_3$); IR (thin film, Ge): $\tilde{\nu}$ = 1561 (s, amide II), 1638 (s, amide I), 3286 (br s; OH/NH); LRMS (ESI+): *m*/*z* (%): 205 (100) $[M+H]^+$, 227 (90) $[M+Na]^+$, 431 (91) $[2M+Na]^+$; HRMS (ESI+): *m*/*z* calcd for $C_8H_{17}N_2O_4$: 205.1183 $[M+H]^+$; found: 205.1183.

For enantiomer **2L**: M.p. 220 °C (dec.); $[\alpha]_D^{25}$ = −18.8 (*c* 0.57, H_2O).

2-Acetamido-1,2,5-trideoxy-1,5-imino-1-N-methyl-D-*glucitol,* (*N-methyl-DNJNAc,* ***2Da***): Formaldehyde (37% in water, 0.10 mL) and palladium (10% on carbon, 5 mg) were added to a solution of DNJNAc (**2D**, 11.5 mg, 56.9 μmol) in water (1 mL). The vessel was degassed, charged with hydrogen, and the reaction mixture was stirred at RT for 24 h. The reaction mixture was filtered (GF/A glass microfiber) and concentrated in vacuo. The residue was loaded onto a short column of Dowex (50W-X8, H^+) and the resin washed with water until neutral fractions were obtained. The product was liberated with aqueous ammonia (2 M) and the

ammoniacal fractions were concentrated in vacuo to afford *N*-methyl-DNJNAc (**2Da**) as a white crystalline solid (11.6 mg, 94%). M.p. 248°C (dec.); $[\alpha]_D^{25}=+20.3$ (*c* 0.53, H_2O); ^1H NMR (D_2O, 400 MHz): $\delta=1.99$ (app dt, $J(5,6)=J(5,6')=2.8$ Hz, $J(5,4)=9.9$ Hz, 1H; H5), 2.01 (s, 3H; $NHCOCH_3$), 2.17 (t, $J(1,1')=J(1,2)=11.6$ Hz, 1H; H1), 2.33 (s, 3H; NCH_3), 2.89 (dd, $J(1',2)=4.8$ Hz, $J(1',1)=11.6$ Hz, 1H; H1′), 3.34 (dd, $J(3,4)=9.3$ Hz, $J(3,2)=10.1$ Hz, 1H; H3), 3.46 (app t, $J(4,3)=J(4,5)=9.6$ Hz, 1H; H4), 3.78–3.85 (m, 2H; H2 and H6), 3.82 ppm (dd, $J(6',5)=2.5$ Hz, $J(6',6)=12.9$ Hz, 1H; H6′); ^{13}C NMR (D_2O, 100 MHz): $\delta=22.6$ ($NHCOCH_3$), 41.3 (NCH_3), 50.5 (C2), 58.1 (C1), 58.1 (C6), 68.5 (C5), 70.9 (C4), 76.3 (C3), 174.9 ppm ($NHCOCH_3$); IR (thin film, Ge): $\tilde{\nu}=1565$ (s; amide II), 1635 (s; amide I), 3300 (br s; OH); LRMS (ESI+): *m/z* (%): 219 (70) $[M+H]^+$, 459 (100) $[2M+Na]^+$; HRMS (ESI+): *m/z* calcd for $C_9H_{19}N_2O_4$: 219.1339 $[M+H]^+$; found: 219.1340.

*2-Acetamido-1-N-butyl-1,2,5-trideoxy-1,5-imino-*D*-glucitol,* (*N-Butyl-DNJNAc,* ***2Dc***): Butyraldehyde (0.10 mL, 1.1 mmol) and palladium (10% on carbon, 5 mg) were added to a solution of DNJNAc (**2D**, 11.0 mg, 53.9 μmol) in water/1,4-dioxane (1:1, 2 mL). The vessel was degassed, charged with hydrogen, and the reaction mixture was stirred for 24 h. The reaction mixture was filtered (GF/A glass microfiber) and concentrated in vacuo. The residue was loaded onto a short column of Dowex (50W-X8, H^+) and the resin washed with water until neutral fractions were obtained. The product was liberated with 25% aqueous ammonia/MeOH (3:17) and the ammoniacal fractions were concentrated in vacuo to afford *N*-butyl-DNJNAc (**2Dc**) as a white crystalline solid (13.4 mg, 96%). M.p. 192–196°C; $[\alpha]_D^{25}=+13.3$ (*c* 0.56, H_2O); ^1H NMR (D_2O, 400 MHz): $\delta=0.89$ (t, $J=7.3$ Hz, 3H; $NCH_2CH_2CH_2CH_3$), 1.27 (sext, $J=7.3$ Hz, 2H; $NCH_2CH_2CH_2CH_3$), 1.40–1.48 (m, 2H; $NCH_2CH_2CH_2CH_3$), 2.01 (s, 3H; $NHCOCH_3$), 2.25 (app t, $J(1,1')=J(1,2)=11.6$ Hz, 1H; H1), 2.25 (app dt, $J(5,6)=J(5,6')=2.5$ Hz, $J(5,4)=9.6$ Hz, 1H; H5), 2.55–2.62 (m, 1H; $NCHH'CH_2CH_2CH_3$), 2.71–2.79 (m, 1H; $NCHH'CH_2CH_2CH_3$), 2.98 (dd, $J(1',2)=4.5$ Hz, $J(1',1)=11.9$ Hz, 1H; H1′), 3.32 (dd, $J(3,4)=9.3$ Hz, $J(3,2)=10.1$ Hz, 1H; H3), 3.45 (app t, $J(4,3)=J(4,5)=9.3$ Hz, 1H; H4), 3.79 (app dt, $J(2,1')=4.5$ Hz, $J(2,1)=J(2,3)=10.9$ Hz, 1H; H2), 3.84 (dd, $J(6,5)=2.8$ Hz, $J(6,6')=12.6$ Hz, 1H; H6), 3.92 ppm (dd, $J(6',5)=2.3$ Hz, $J(6',6)=12.6$ Hz, 1H; H6′); ^{13}C NMR (D_2O, 100 MHz): $\delta=13.8$ ($NCH_2CH_2CH_2CH_3$), 20.8 ($NCH_2CH_2CH_2CH_3$), 22.6 ($NHCOCH_3$), 25.9 ($NCH_2CH_2CH_2CH_3$), 50.6 (C2), 52.2 ($NCH_2CH_2CH_2CH_3$), 53.9 (C1), 58.2 (C6), 65.7 (C5), 71.2 (C4), 76.4 (C3), 174.9 ppm ($NHCOCH_3$); IR (thin film, Ge): $\tilde{\nu}=1562$ (s; amide II), 1640 (s; amide I), 3296 (br s; OH); LRMS (ESI+): *m/z* (%): 261 (94) $[M+H]^+$, 331 (74) $[M+MeOH+K]^+$, 543 (100) $[2M+Na]^+$; HRMS (ESI+): *m/z* calcd for $C_{12}H_{25}N_2O_4$: 261.1809 $[M+H]^+$; found: 261.1816.

Biological experiments

In vitro enzyme inhibition, IC_{50} determination: The β-*N*-acetyl-glucosaminidases (from human placenta, bovine kidney, *Aspergillus oryzae*, and jack beans), α-*N*-acetyl-galactosaminidase (from chicken liver), β-*N*-acetyl-galactosaminidase (from *Aspergillus oryzae*), and *p*-nitrophenyl glycosides were purchased from Sigma–Aldrich. The cell lysate of the human acute amyloid leukemia cell-line HL60 (RBRC), which was cultured in RPMI 1640 medium that contained 10% fetal calf serum, 100 units mL^{-1} of penicillin, and 100 μg mL^{-1} streptomycin (Invitrogen) at 37°C under 5% CO_2, was used as the source of β-*N*-acetyl-glucosaminidase and β-*N*-acetyl-galactosaminidase. The glycosidase activities were determined by using an appropriate *p*-nitrophenyl glycoside as the substrate at the optimum pH value of each enzyme. The reaction mixture (1 mL) contained 2 mM of the substrate and the appropriate amount of enzyme. The reaction was stopped by adding of 400 mM Na_2CO_3 (2 mL). The released *p*-nitrophenol was measured spectrometrically at 400 nm.

*α-N-Acetyl-*D*-galactosaminidase assay, K_i determination*: Before use, the enzyme solutions were thawed at RT then put on ice. The solutions were stored at −20°C. The inhibitors were dissolved in deionized H_2O to a stock concentration of 50 mM. These solutions were stored at RT. The enzyme substrate solutions were 4 mM in 50 mM citrate/citric acid buffer, pH 5.0. These solutions were stored at 4°C. Assays were carried out in triplicate/duplicate by using H_2O in place of the inhibitor as a positive control; replacing both the enzyme and inhibitor with H_2O was used as a blank to determine the background of the experiment. Linearity over the time course of the reaction was confirmed by using a series of incubation times.

Materials: Enzyme α-*N*-acetyl-D-galactosaminidase (*Charonia lampas*) was purified from the natural sources (Oxford Glycobiology Institute) in 50 mM citrate-phosphate buffer at pH 5 that contained 1 mg mL^{-1} bovine serum albumin (BSA) and 0.02% NaN_3. Substrate: *p*-nitrophenyl-2-acetamido-2-deoxy-α-D-galactopyranoside (Koch-Light Ltd.). Method: Solutions of the enzyme (5 μL) and the inhibitor (5 μL) were added separately to a 96-well microtitre plate that was pre-incubated at 37°C for 5 min before starting the reaction by addition of the substrate solution (40 μL). After a further 40 min of incubation at 37°C, the reaction was quenched by the addition of 0.5 M aq. Na_2CO_3 (200 μL). Absorbance at 405 nm was measured immediately by using a microtitre plate reader (Molecular Devices UVmax kinetic microplate reader and SOFTmax 2.35 software).

Data analysis

K_i values: Data sets were analyzed on Prism 4.0a by using a Lineweaver–Burk plot (1/rate versus 1/[substrate concentration]) for 4–7 different substrate concentrations that were selected from a suitable range (4, 2, 1, 0.75, 0.5, 0.2, 0.1 mM) and 3–5 inhibitor concentrations. The slopes were plotted against the inhibitor concentration and the K_i value was obtained from the intercept on the *x* axis.

Free oligosaccharide assay

Materials: Tissue culture media and supplies were purchased from PAA (Pashing, Austria); sodium cyanoborohydride, bicinchoninic acid/copper(-II) sulfate reagent, sodium acetate trihydrate, and anthranilic acid (2-AA) were purchased from Sigma–Aldrich (Dorset, UK); water was Milli-Q™ grade; MeCN (HPLC grade) was purchased from Merk (Darmstadt, Germany); boric acid was purchased from BDH; MeOH was purchased from VWR (Lutterworth, Leicestershire, UK); ion-exchange resins AG50-X12 and AG4-X4 were purchased from Biorad (Hemel Hampstead, Hertfordshire, UK), Spe-ed amide 2 was purchased from Applied Separations (Allentown, Pennsylvania, U.S.); an Amicon Ultra centrifugal filter was purchased from Millipore (Croxley, Watford, UK).

Cell culture HL60: The human acute amyloid leukemia cell-line HL60 (ATCC) was cultured in RPMI 1640 medium that contained 10% fetal calf serum, 100 units mL^{-1} of penicillin, and 100 μg mL^{-1} streptomycin (PAA) at 37°C and 5% CO_2. Cell viability was checked by using Trypan Blue (Sigma Aldrich).

Inhibition treatment of HL60 cells and FOS isolation: Having been propagated at a higher density, HL60 cells were seeded in a 6 well plate at a concentration of 5×10^5 cells mL^{-1} and treated with a fresh medium (2 mL) that contained the derivatives of DGJNAc (**1D**) at a concentration of 500 μM, 100 μM, and 50 μM for 24 h. NB-DNJ (500 μM) and untreated wells were used as controls. Following incubation, the cells were centrifuged at 350 g, 10°C, for 7 min and the pellet was washed three times with PBS. The washed cell pellets were stored at −20°C until use. Following thawing at RT, the samples were resuspended in water (650 μL) and Dounce homogenized. An aliquot (50 μL) was removed and treated with 0.25 M NaOH solution for 16 h prior to determination of the protein concentration in comparison to a standard by using bicinchoninic acid/copper(II) sulfate reagent (Sigma Aldrich). For desalting and deproteination, the homogenate (500 μL) was subjected to a mixed-bed ion-exchange column [AG50-X12 (0.2 mL), (H^+, 100–200 mesh), AG4-X4 (0.4 mL), (OH^-, 100–200 mesh)], which had been preequilibrated with water (5×1 mL). The homogenate was added to the column and eluted with water (4×1 mL). The eluent that contained the free oligosaccharides (FOS) was collected and dried by lyophilization.

Carbohydrate fluorescent labeling: The lyophilized samples were resuspended in water (300 μL), transferred to an Eppendorf tube, and evaporated to dryness by using a Thermo Savant (SPD121P) SpeedVac system. As previously described,[33] anthranilic acid (2-AA) was dissolved at a concentration of 30 mg mL^{-1} in a MeOH solution that contained 4% sodium acetate trihydrate (w/v) and 2% boric acid (w/v). To this mixture was added sodium cyanoborohydride to a final concentration of 45 mg mL^{-1} (labeling buffer). The dried samples were dissolved in water

(30 μL) and 2-AA labeling buffer (80 μL) was added, followed by incubation at 80 °C for 1 h.

Purification of fluorescently labeled FOS: After cooling, MeCN/water (97:3, 1 mL) was added to the labeled FOS samples and the samples were vortexed and added to a spe-ed amide-2 column that had been pre-equilibrated as follows: MeCN (1 mL), water (2 mL), MeCN (2 mL). The eluent was discarded and the column was washed with MeCN/water (95:5, 2 mL) and discarded. The purified 2-AA-labeled FOS was eluted by gravity by using water (2× 0.75 mL). The samples were stored at −20 °C until they were analyzed by using HPLC.

Carbohydrate analysis by normal-phase HPLC: As previously described, the chromatography system that was used consisted of a Water Alliance 2695 separations module and an in-line Waters 474 fluorescence detector that was set at Ex_λ 360 nm and Em_λ 425 nm.[34] The gain for the detector was set to 1000 and the emission bandwidth was set to 40 nm. Chromatography was performed at 30 °C. Solvent A was MeCN; solvent B was water; solvent C consisted of ammonium hydroxide (800 mM) that was titrated to pH 3.85 with acetic acid in water and was prepared by using a standard 5 M ammonium hydroxide solution (Sigma Aldrich). Data collection and -processing were performed by using Waters Empower software. Glucose units were derived by comparison to a 2-AA-labeled glucose oligomer ladder (which was obtained by the partial digestion of dextran) as an external standard. A sample/MeCN mixture (1:1, 50 μL) was injected for each run. Separation was performed on a 4.6×250 mm TSK gel-Amide 80 column (5 μM; Anachem, Luton, UK) and the following gradient conditions were used for the analysis of the FOS samples: time = 0 min (t=0), 71.6% A, 25.9% B, 2.5% C (0.8 mL min^{-1}); t=6, 71.6% A, 25.9% B, 2.5% C (0.8 mL min^{-1}); t=45, 46.2% A, 51.3% B, 2.5% C (0.8 mL min^{-1}); t=46, 35% A, 62.5% B, 2.5% C (0.8 mL min^{-1}); t=48, 35% A, 62.5% B, 2.5% C (0.8 mL min^{-1}); t=49, 71.6% A, 25.9% B, 2.5% C (0.8 mL min^{-1}); t=51, 71.6% A, 25.9% B, 2.5% C (1.2 mL min^{-1}); t=64, 71.6% A, 25.9% B, 2.5% C (1.2 mL min^{-1}); t=65, 71.6% A, 25.9% B, 2.5% C (0.8 mL min^{-1}).

Enzyme (*jack bean β-HexNAcase) digest*: Glycosidase digest using jack bean β-HexNAcase (10 μL of 6 U mL^{-1} in 50 mM citrate buffer (pH 5.0) that contained 1 mg mL^{-1} BSA and 0.02% sodium azide, purified in house) was performed on a representative sample (100 μL) of the complete FOS population from 500 μM DGJNAc-treated HL60 cells. After 16 h incubation at 37 °C, the enzyme digest was diluted with water (90 μL). An Amicon Ultra centrifugal filter (Millipore, 10000 MWCO) was pretreated with water (150 μL) and used to remove proteins following centrifugation at 13000 g for 15 min at 4 °C. The filter was washed with water (100 μL) and the combined eluate was evaporated to dryness before being reconstituted in MeCN/water (1:1, 100 μL) for HPLC analysis.

Statistical analysis: Experiments were performed in triplicate. To test for significance, Prism 4.0a software was used to perform a student t-test by using a 99% confidence interval.

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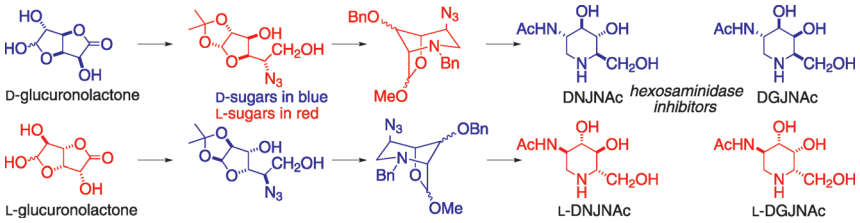
Enzyme Inhibitors

*A. F. G. Glawar, D. Best, B. J. Ayers, S. Miyauchi, S. Nakagawa, M. Aguilar-Moncayo, J. M. García Fernández, C. Ortiz Mellet, E. V. Crabtree, T. D. Butters, F. X. Wilson, A. Kato, G. W. J. Fleet** ..... ▮▮▮▮–▮▮▮▮

Scalable Syntheses of Both Enantiomers of DNJNAc and DGJNAc from Glucuronolactone: The Effect of *N*-Alkylation on Hexosaminidase Inhibition



Getting the NAc of it: Scalable syntheses of DGJNAc and DNJNAc from D-glucuronolactone are reported. DGJNAc and its N-alkyl derivatives were inhibitors of α-GalNAcase and both DGJNAc and DNJNAc were potent inhibitors of β-GlcNAcases and β-GalNAcases.

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