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Stereoselective Synthesis of 2-Acetamido-1,2-dideoxynojirimycin (DNJNAc) and Ureido-DNJNAc Derivatives as New Hexosaminidase Inhibitors

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2-Acetamido-1,2-dideoxyiminosugars are selective and potent inhibitors of hexosaminidases and therefore show high therapeutic potential for the treatment of various diseases, including several lysosomal storage disorders. A stereoselective synthesis of 2-acetamido-1,2-dideoxynojirimycin (DNJNAc), the iminosugar analog of *N*-acetylglucosamine, with high overall yield is here described. This novel procedure further allowed accessing ureido-DNJNAc conjugates through derivatization of the endocyclic amine on a key pivotal intermediate. Remarkably, some of the ureido-DNJNAc representatives behaved as potent and selective inhibitors of β-hexosaminidases, including the human enzyme, being the first examples of neutral sp^2-iminosugar-type inhibitors reported for these enzymes. Moreover, the amphiphilic character of the new ureido-DNJNAc is expected to confer better drug-like properties.

Introduction.

Since the isolation of nojirimycin in 1966, iminosugars —sugar analogs where the oxygen ring atom has been replaced by a nitrogen— have attracted an exponential interest as mimics of the transition state of the enzymatic hydrolysis of glycosidic substrates.[1,2] Their ability to act as inhibitors of a great diversity of carbohydrate processing enzymes, including glycosidases, glycosyl transferases, nucleoside-processing enzymes and glycogen phosphorylases, and the broad variety of biological and pathological processes in which carbohydrates are involved make iminosugars invaluable tools in glycobiology and promising candidates for the development of glycotherapies.[3–5] In fact, some iminosugars are already marketed drugs, such as miglitol (Glyset) and *N*-butyl-1-deoxynojirimycin (Zavesca), used for the treatment of type II diabetes mellitus and type 1 Gaucher disease respectively.[6]

Iminosugars reduced at C-1 and bearing an acetamido group at the position equivalent to C-2 in the parent monosaccharides, namely 2-acetamido-1,2-dideoxyiminosugars, have been the focus of considerable attention in recent years. Several representatives of acetamido iminosugars, for instance pochonicine (**1**)[7], siastatine B (**2**),[8] or nagstatine (**3**)[9,10] have been isolated from natural sources while derivatives from those and other compounds have been obtained by chemical synthesis[11,12]. Most of these representatives are piperidine derivatives, such as 2-acetamido-1,2-dideoxynojirimycin (DNJNAc, **4**)[13–16] and its *manno* (DMJNAc, **5**)[14,17] or *galacto* epimers (DGJNAc, **6**),[18,19] although acetamido iminosugars with five- (e.g. **7**)[20,21] or seven-membered ring skeletons (e.g. **8**)[22] have also been described. Several of these compounds have proven to be highly selective inhibitors of hexosaminidases —the enzymes cleaving off amino sugar residues from oligosaccharides and glycoconjugates— with inhibition constant (K_i) values in the low micromolar to nanomolar range. This property makes them potentially useful in the treatment of several diseases involving abnormal levels of *O*-linked glucosamine (GlcNAc) in glycoproteins, including diabetes, Parkinson's, osteoarthritis, and some cancers.[23–27] Furthermore, at subinhibitory concentrations competitive inhibitors of the hexosaminidases are able to promote the correct folding of mutant disease-associated lysosomal enzymes, thus bearing promise for the development of pharmacological chaperone therapies[28] against some lysosomal storage disorders.[20,29–31] Many studies have addressed the mechanism of action of these compounds, showing that the acetamido group is essential for their activity and selectivity.[32–34]

Most synthetic approaches to iminosugars are based on the *chiral pool* thus making these processes rather long.[35–39] This is also the case for acetamido iminosugars,[13,16,40,41] with a few exceptions limited to the stereoselective synthesis of 2-acetamido-1,2-dideoxyallonojirimycin (DAJNAc, **9**)[42] and the *manno* diastereomer DMJNAc (**5**).[17] Herein, we report a new stereoselective total synthesis of the *gluco* counterpart DNJNAc (**4**) and its regioisomer 3-acetamido-1,3-dideoxyaltronojirimycin (**29**). Moreover, the preparation of a series of ureido-DNJAc derivatives as examples of 2-acetamido sp^2-iminosugars, has also been accomplished. Characterized by the incorporation of a pseudoamide-type nitrogen atom with high sp^2-hybridation character in the ring [43–45], this subtype of glycomimetics, from which nagstatine **3** can be considered a natural representative, has previously shown an unprecedented potential for fine tuning the inhibitory potency and selectivity towards glycosidases by modulating the basicity of the *N*-functionality and the nature of the exocyclic moiety.[46] In our case, the evaluation of the new ureido-DNJNAc against a panel of glycosidases allowed the identification of hexosaminidase inhibitors with an amphiphilic character and a greatly reduced basicity, features that make these compounds better suited as drug candidates.

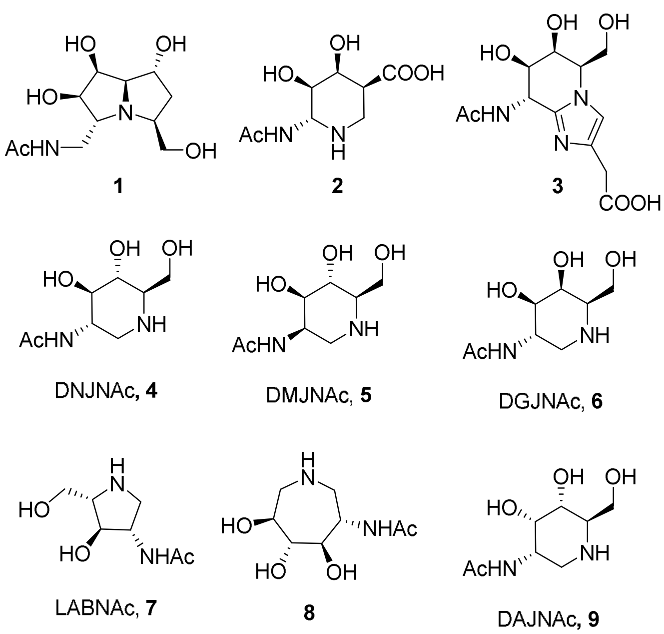
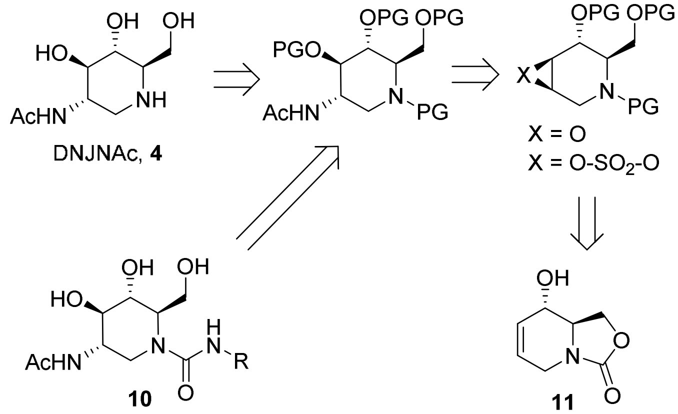


Figure 1. Structure of some acetamido iminosugars.

Results and discussion

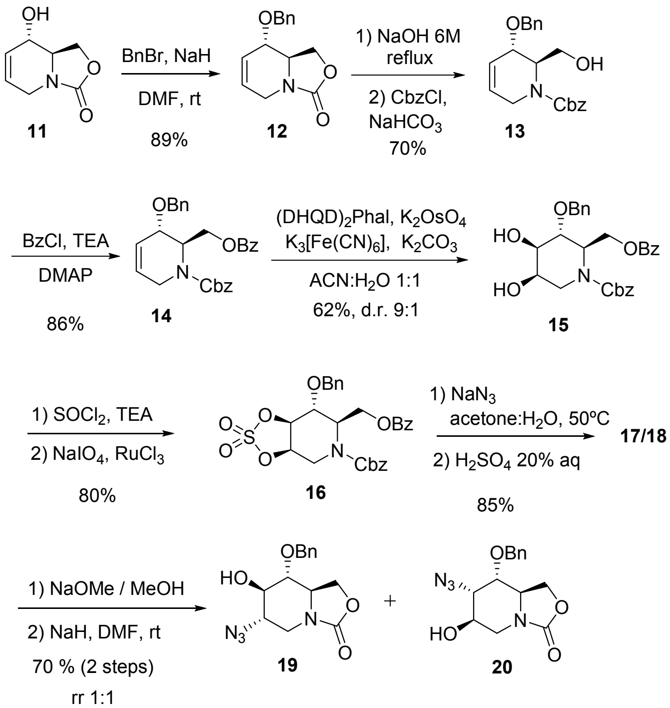
Our approach to DNJNAc, **4** and the ureido-DNJNAc derivatives **10** is shown in Scheme 1. An appropriate protecting group scheme was needed to introduce the urea fragment in the last steps. The protected compounds were prepared by introducing the amino function by nucleophilic ring opening of an epoxide or a cyclic sulfate obtained from the key intermediate **11**, which is readily accessible by Sharpless asymmetric epoxidation of 2,4-pentadien-1-ol.[47] This intermediate has been widely used for the synthesis of various iminosugars[17,48–50] including our recent synthesis of DAJNAc (**9**).[42]



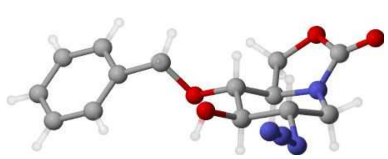
Scheme 1. Retrosynthetic analysis for the preparation of DNJNAc (**4**) and ureido-DNJNAc conjugates (**10**) from the common bicyclic precursor **11**.

Optically pure carbamate **11** was prepared in multigram scale from penta-1,4-dien-3-ol, and the allylic alcohol group was subsequently protected as the corresponding benzyl ether **12**.[48] We first considered the epoxidation of the double bond in **12** followed by regioselectively ring-opening by azide anion to introduce the amino substituent. Deceivingly, classical methodologies using *m*-chloroperoxybenzoic acid (MCPBA) or H_2O_2 proved inefficient while harsher oxidant methods such as CF_3CO_3H[51,52] or oxone[49] generated inseparable 1:1 mixtures of the corresponding epoxides in moderate yields. We hypothesized that the rigid bicyclic skeleton of **12** was probably responsible for the low reactivity observed. However, although hydrolysis of the cyclic carbamate by treatment with 6M NaOH at reflux, followed by *in situ* Cbz-protection of the endocyclic amine afforded the monocylic derivative **13** in satisfactory yield, all attempts at diastereoselective epoxidation of **13** failed, regardless of the epoxidation methodology used. Various combinations of *N*-carbamate and *O*-ester/ether protecting groups were also assayed without success. In view of these results, we explored the use of cyclic sulfates as an alternative to epoxides,[53,54] an approach that has been applied successfully in other iminosugar syntheses.[55,56]
The protection of the primary alcohol of **13** as a benzoate, followed by Sharpless asymmetric dihydroxylation of the intermediate ester (**14**), yielded a 90:10 mixture (HPLC) of diastereomeric diols, from which the major isomer **15** was isolated in 62% yield (Scheme 2). Treatment of **15** with thionyl chloride gave a mixture of sulfites that was oxidized without further purification with $RuCl_3/NaIO_4$ to the corresponding cyclic sulfate **16**, which was obtained as a single diasteroisomer in 80% overall yield (two steps). Treatment of **16** with NaN_3 at 50ºC gave an inseparable mixture of the azidoalcohols **17** and **18**. Attempts to quantify the relative proportion of the two compounds at this stage by NMR or HPLC failed. The two azidoalcohols were hypothesized to be the result of the

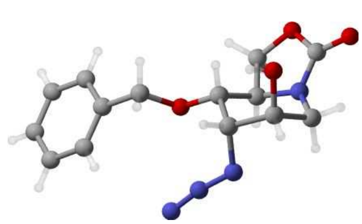
nuclephilic attack of the azide anion at C2 (*gluco*-configuration) and C3 (*altro*-configuration) positions. Sequential treatment of this mixture with NaOMe, in order to cleave the benzoate group, and NaH regenerated the 2-oxazolidinone ring, affording a 1:1 mixture of the bicyclic azidoalcohols **19** and **20** (Scheme 2). Although no selectivity was achieved during the cyclic sulfate opening reaction, both carbamates **19** and **20** were easily separated by column chromatography, which yielded crystalline compounds that could be analyzed by X-ray diffraction,† thus confirming the proposed stereochemistry (Figure 2).



Scheme 2. Synthesis of the azido intermediates **19** and **20**.



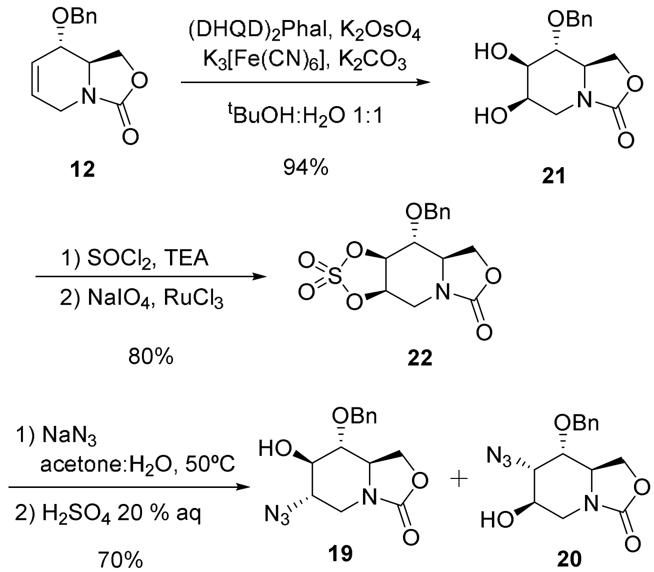
19



20

Figure 2. X-ray analysis of azido alcohols **19** and **20**.†

We envisaged that carbamate **19** would be an excellent precursor in the synthesis of DNJNAc (**4**) and ureido-DNJAc derivatives **10.** The straightforward purification and facile separation of the two regioisomers encouraged us to look for a shorter route to synthetize the mixture of **19** and **20**. Direct dihydroxylation of carbamate **12** using $K_2OsO_4 \cdot 2H_2O$/NMO afforded **21** in satisfactory yield and diastereoselectivity (78%, 85:15) (Scheme 3). Sharpless asymmetric dihydroxylation conditions increased both the yield and diasteroselectivity, affording diol **21** in 94% yield and nearly complete diasteroselectivity, as observed by ^{1}H-NMR.[17] The corresponding cyclic sulfate **22** was obtained in 80% yield by reaction of diol **21** with $SOCl_2$/TEA followed by *in situ* oxidation with $NaIO_4/RuCl_3$, as in the previous case. Attempts to perform direct sulfation of **21** using SO_2Cl_2/TEA[57] also afforded **22** but in lower yields.



Scheme 3. Synthesis of cyclic sulfate from **12** followed by ring-opening with sodium azide.

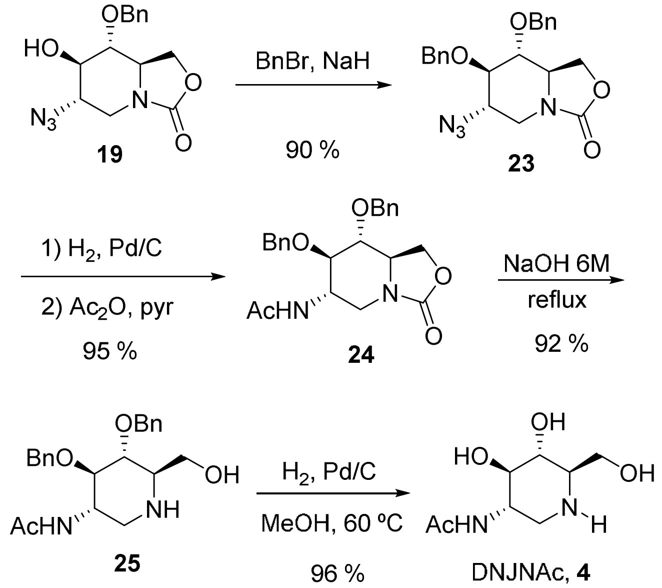
Regioselective ring-opening reactions of the key precursor **22** using NaN_3 as the nucleophile were extensively studied and are summarized in Table 1. We expected that the presence of the benzyl group at the C4 position would sterically hinder approaching of the azide anion nucleophile to C3, directing the attack to the C2 position (iminosugar numbering). The reaction did not take place in acetonitrile (entry 1) but proceeded in *N,N*-dimethylformamide (entries 2 and 3). Thus, treatment of sulfate **22** with sodium azide in DMF, followed by acidic hydrolysis (to cleave the intermediate residual sulfate), gave a 2:1 mixture of azidoalcohols in 70% yield (entry 2). However, increasing the temperature and the equivalents of NaN_3, led to a dramatic decrease in yield and a total loss of selectivity (entry 3). In an attempt to improve the regioselectivity, the reaction was performed in acetone/water (entry 4 and 5), observing that fewer equivalents of azide allowed similar ratios. The use of lower temperatures (40ºC), even fewer equivalents of azide (1.2) and a longer reaction time (16 h), afforded higher yields, but also at the expenses of a total loss of regioselectivity (entry 6). Conversely, portion-wise addition of sodium azide increased the regioselectivity, but with a significant decrease in yield (entry 7). According to our objective of obtaining derivatives of **4**, conditions of entry 5 were chosen for scaling up purposes.

Table 1. Optimization of the ring-opening reaction of sulfate **22** with sodium azide.

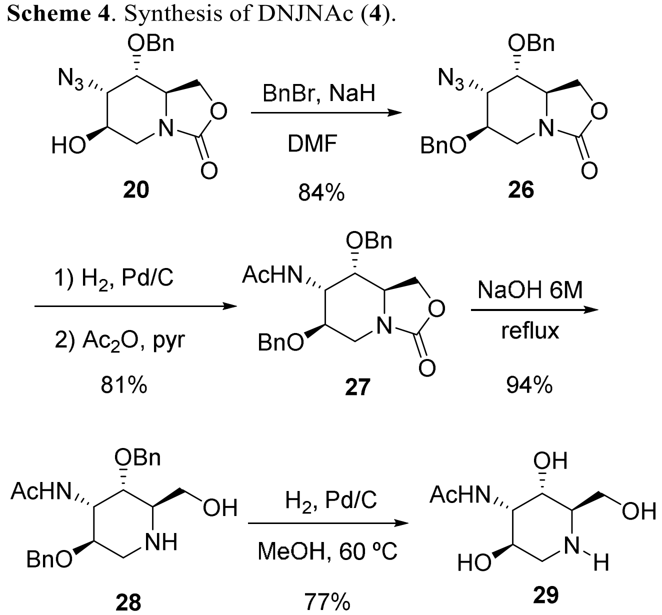
Entry	Solvent	T/ºC	t/h	NaN_3 Eq.	Yield /%	19/20 [a]
1	ACN	50	3	3	-	-
2	DMF	50	3	4	70	1.9:1
3	DMF	120	1	4	30	0.9:1
4	Acetone/ H_2O 2:1	50	3	3	68	1:1
5[b]	Acetone/ H_2O 2:1	50	6	2	62	1.8:1
6	Acetone/ H_2O 2:1	40	16	1.2	81	1:1
7[c]	Acetone/ H_2O 2:1	50	7	3	43	3:1

[a] Relation determined w/w after purification
[b] The reaction was performed at multigram scale using these conditions.
[c] Portionwise addition of NaN_3

The synthesis of DNJNAc **4** from azidoalcohol **19** is depicted in Scheme 4. Protection of the secondary alcohol by treatment with BnBr/NaH gave **23** in 90% yield. Azide reduction with H_2 and Pd/C followed by *in situ* acetylation with Ac_2O/pyridine afforded acetamide **24** in almost quantitative yield. This compound was then subjected to basic hydrolysis of the 2-oxazolidinone ring to give **25** in 92% yield. Final hydrogenolysis of the benzyl protecting groups gave DNJNAc (**4**) in 96% yield. The spectroscopic data of this compound were consistent with previously reported data.[18] The total synthesis of DNJNAc (**4**) from **11** was thus accomplished in 10 synthetic steps achieving a 23% overall yield.
Although some 3-acetamido iminosugar derivatives have been reported we could not find precedents of evaluation of their properties as glycosidase inhibitors.[41,58,59] We thus considered it of interest to apply the above synthetic sequence to azido alcohol **20**, i.e. benzylation (→**26**), azide reduction and acetylation of the resulting amine (→**27**), basic hydrolysis of the cyclic carbamate group (→**28**) and final hydrogenolysis of the benzyl protecting groups. In this manner, 3-acetamido-1,3-dideoxyaltronojirimycin **29** was prepared in excellent overall yield (Scheme 5).



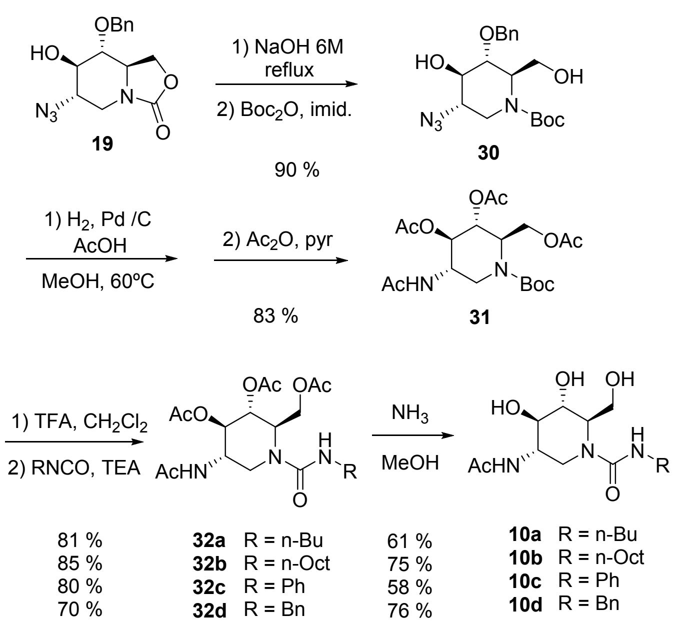
Scheme 4. Synthesis of DNJNAc (**4**).



Scheme 5. Synthesis of 3-acetamido-1,3-dideoxyaltronojirimycin (**29**).

It has been described that modifications of the acetamide moiety in DNJNAc lead to a dramatic decrease in the inhibitory activity against hexosaminidases,[40] while modifications at the endocyclic amine are well tolerated. Indeed, the incorporation of hydrophobic *N*-alkyl substituents has been previously investigated,[18,60] and found to lead to an improvement of the inhibitory potency which is consistent with the presence of a hydrophobic pocket in the vicinity of the active site of the enzyme.[61] All DNJNAc analogs reported to date keep the basic character of the piperidine glycone-like skeleton, generally considered a favorable structural feature to promote strong binding to the enzyme. However, it has been demonstrated that higher glycosidase affinities and, especially, improved selectivities can be achieved by the interplay of neutral glycone-type cores and substituents that provide additional non-glycone interactions.[62,63] Transmuting the endocyclic sp^3-amine nitrogen into a sp^2-hybridized pseudoamide functionality by introduction of amide, urea, thiourea or guanidinium moieties has proven particularly successful in this respect.[64–68] For instance, *N*-(*N'*-butylaminocarbamoyl)-1-deoxynojirimycin, the urea analog of the marketed drug Zavesca, was found to be a very selective inhibitor of bovine liver β-galactosidase.[67] The sp^2-hybridized character is also observed in some natural products such as kifunensine, a potent inhibitor of class I α-mannosidase.[69,70] To check this strategy for the particular case of hexosaminidases, we synthesized a series of ureido-DNJNAc derivatives (**10**). In addition to a much lower basic character at the endocyclic nitrogen, conversion of an amine into a urea offers flexibility in the choice of substituents, which can be taken advantage of to optimize the inhibitory capacity and the pharmacokinetic behavior.

The oxazolidinone ring of azido alcohol **19** was hydrolyzed under the usual conditions and the endocyclic amine was protected *in situ* using $Boc_2O/NaHCO_3$ to give azidoalcohol **30**. Concomitant azide reduction and cleavage of the benzyl group were accomplished by hydrogenation in methanol/acetic acid. The resulting *vic*-aminoalcohol was acetylated without further purification by treatment with Ac_2O in pyridine to afford acetamide **31** in 83% yield (2 steps). Next, the *N*-Boc group was selectively cleaved using TFA, and the resulting cyclic amine was reacted *in situ* with *n*-butyl, *n*-octyl, phenyl or benzyl isocyanate in the presence of triethylamine (TEA) to give the corresponding urea adducts **32a-d** in 70-85% yield. Final deacetylation using a saturated solution of ammonia in MeOH gave the target ureido-DNJNAc derivatives **10a-d** (Scheme 6).



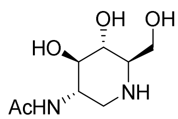
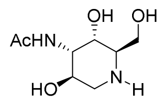
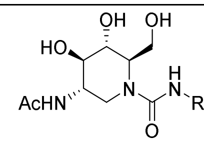
Scheme 6. Synthesis of ureido-DNJNAc derivatives **10a-d**.

Evaluation of the glycosidase inhibitory activity of the DNJNAc regioisomer **29** and the ureido-DNJNAc derivatives **10a-d**, in comparison with the parent acetamido iminosugar **4,** confirmed their total selectivity towards hexosaminidases among a panel that included the following: β-glucosidases (almonds and bovine liver), α-glucosidase (yeast), α-mannosidase (jack bean), β-mannosidase (*Helix pomatia*), trehalase (pig kidney), amyloglucosidase (*Aspergillus niger*), α-rhamnosidase (naringinasa; *Penicillium decumbens*), α-galactosidase (green coffee), β-galactosidase (*E. coli*), and isomaltase (yeast). Compound **29** was a much weaker inhibitor than DNJNAc, confirming that even when hexosaminidases are relatively promiscuous regarding the configurational pattern of iminosugar-type ligands, the location of the acetamido group next to the anomeric position is critical to ensure strong enzyme binding. Gratifyingly, all ureido-DNJNAc derivatives **10a-d** behaved as μM inhibitors of the three hexosaminidases assayed in this work, namely those from human placenta, bovine kidney, and jack beans. *N*'-alkyl substituents (*n*-butyl, *n*-octyl or benzyl; **10a**, **10b** and **10d**) led to a slight decrease in the inhibitory potency as compared with **4**, with inhibition constant (K_i) values in the 56-20 μM range for the human enzyme. The *N*'-phenyl derivative **10c** was an about one order of magnitude stronger inhibitor of the hexosaminidases as compred with the *N*'alkyl counterparts. Notably, the inhibition potency against the human enzyme surpassed that of **4** by over 3-fold. This result is remarkable considering the much lower basicity of **10c** as compared with **4**. The data suggest the involvement of the urea NH proton in hydrogen bonding in the complex of ureido-DNJNAc with the hexosaminidases, compensating the electrostatic interactions operating in the case of the basic iminosugar, as previously demonstrated for other sp^2-iminosugar:glycosidase complexes.[71] The higher hydrogen bond donor capability of arylureas as compared with alkylureas, due to the electron withdrawing character of the aromatic ring, is consistent with the observed activity trend. Most interestingly, the amphiphilic character of the compounds is expected to confer better drug-like properties. Altogether, the results reported herein are promising for the further development of therapeutic agents for β-GlcNAcase-related diseases.

Conclusions

Here we have described a new stereoselective synthesis of 2-acetamido-1,2-dideoxynojirimycin (DNJNAc), the iminosugar analog of *N*-acetylglucosamine, with high overall yield. The strategy is based on the stereoselective ring-opening of cyclic sulfates derived from the key intermediate **11**, which was conveniently prepared by a multigram procedure based on Sharpless epoxidation. This novel procedure gave access to the advanced intermediate **19** which provided us with the necessary protecting group arrangement to synthesize sp^2-iminosugar conjugates through derivatization of the endocyclic amine by reaction with isocyanates. These new ureido-DNJNAc derivatives are the first neutral inhibitors of hexosaminidases described to date. These compounds were potent inhibitors of β-GlcNAcase and, given their amphiphlic character, they are expected to show acceptable drug-like properties.

Table 2. Inhibition constants (K_i, μM)[a] against commercial β-*N*-acetylglucosaminidases **10a-d** and **29** determined from thee slope of Lineweaver-Burk plots and double reciprocal analysis compared with previously reported values for DNJNAc (**4**).[18]

						
Enzyme origin	**4**	**29**	**10a** R = *n*-Bu	**10b** R = *n*-Oct	**10c** R = Ph	**10d** R = Bn
Human placenta	7.0 ± 0.3	427 ± 20	56 ± 5	33 ± 3	2.1 ± 0.1	20 ± 1
Bovine kidney	7.4 ± 0.3	524 ± 40	138 ± 10	82 ± 5	4.1 ± 0.2	24 ± 2
Jack Bean	2.9 ± 0.2	130 ± 10	26 ± 3	19 ± 2	1.1 ± 0.1	10 ± 1

[a]Inhibition was competitive in all cases.

Experimental

General

All commercial reagents were used without further purification. Non-aqueous reactions were performed out under nitrogen atmosphere. Dry tetrahydrofuran, dichloromethane, and diethyl ether were obtained using a Solvent Purification System (SPS). Other solvents were used with no further purification. All reactions were monitored by TLC analysis using Merck 60 F254 silica gel on aluminum sheets. Silica gel chromatography was performed by using 35-70 mm silica or an automated chromatography system. NMR spectra were recorded at room temperature on a 400 MHz instrument. ^{1}H and ^{13}C-NMR spectra were referenced to the residual peaks of the deuterated solvent. The following abbreviations were used to define the multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; and br, broad signal. The chemical shifts (δ) are expressed in ppm and the coupling constants (J), in Hertz (Hz). IR spectra were recorded either by preparing a KBr pastille or by depositing a film of the product on a NaCl window. Absorptions are given in wavenumbers (cm^{-1}). Melting points were recorded in a Büchi M-540 apparatus without recrystallization of the final solids. Optical rotations were measured at room temperature (25°C). Concentration is expressed in g/100 mL and solvent is expressed for each case in brackets. The cell was 10 cm long and had 1 mL of capacity. Measuring λ was 589 nm, which corresponds to a sodium lamp. High Resolution Mass Spectrometry were conducted using nanoelectrospray technique..

Preparation of **11**[47], **12**[48] and **21**[17,48] was done following literature procedures. Starting material **11** was 99% ee.

Syntheses and characterizations of compounds in Scheme 5, and derivatives **32b-d** and **10b-d** can be found in the supporting information.

(2*R*,3*S*)-3-Benzyloxy-*N*-benzyloxycarbonyl-2-hydroxymethyl-3,6-dihydropyridine (13)

NaOH 6M (2.86 mL, 17.10 mmol) was added to a solution of **12** (421 mg, 1.71 mmol) in MeOH : H_2O 9:1 (18 mL), and the reaction was heated at reflux for 20 h. Solvents were removed at low pressure. The resulting white solid was dissolved in THF (30 mL) and H_2O (3 mL) and cooled at 0 ºC. $NaHCO_3$ (432 mg, 5.14 mmol) and CbzCl (0.39 mL, 2.57 mmol) were added and the reaction was stirred at 0 ºC for 4 h. H_2O (10 mL) was then added, and the crude product was extracted with EtOAc (3x 15 mL), dried over $MgSO_4$ and purified on SiO_2 using hexane/EtOAc to yield **13** (427 mg, 70%) as a colorless oil. $[\alpha]^{20}_D$ = +50.3 (c=1.05, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.32-7.25 (m, 10H), 5.91 (m, 2H), 5.18 (m, 2H), 4.78 (m, 2H), 4.52 (m, 2H), 4.40 (m, 2H), 3.96 (m, 1H), 3.55 (m, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 157.2 (CO), 138.1 (C), 137.1 (C), 128.5 (CH), 128.4 (CH), 127.9 (CH), 127.8 (CH), 127.6 (CH), 122.8 (CH), 70.4 (CH_2), 69.3 (CH), 67.4 (CH_2), 61.5 (CH), 54.6 (CH_2), 40.9 (CH_2). IR (film, ν_{max} / cm^{-1}): 3442, 1700, 1412, 1344, 1236. HRMS (ES): calcd. for $C_{21}H_{25}NO_4$: 354.1705 found 354.1703.

(2*R*,3*S*)-2-Benzoyloxymethyl-3-benzyloxy-*N*-benzyloxycarbonyl-3,6-dihydropyridine (14)

DMAP (10 mg, 0.08 mmol), TEA (0.29 mL, 2.04 mmol), and benzoyl chloride (0.19 mL, 1.63 mmol) were added to a solution of **13** (288 mg, 0.82 mmol) in CH_2Cl_2 (10 mL) and the reaction was stirred at r.t. until no starting material was observed by TLC. Solvent was removed under low pressure and the crude was purified by chromatography on silica gel using hexane/ethyl acetate to give **14** (316 mg, 86%) as a colorless oil. $[\alpha]^{20}_D$ = +15.6 (c=1.05, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$, 55 ºC, δ/ppm): 7.93 (d, J = 7.5 Hz, 2H), 7.55 (tt, J = 7.5, 1.5 Hz, 1H), 7.39 (tt, J = 7.5, 1.5 Hz, 2H), 7.33 – 7.17 (m, 10H), 6.00 (br, 1H), 5.94 (m, 1H), 5.17 (d, J = 12.5 Hz, 1H), 5.02 (br, 2H), 4.65 (br, 1H), 4.54 (m, 1H), 4.46 (m, J = 14.0, 8.0 Hz, 1H), 4.26 (s, 2H), 3.95 (s, 1H), 3.71 (d, J = 19.0 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 166.1 (CO), 166.0* (CO), 156.0 (CO), 155.8* (CO), 137.8 (C), 136.4 (C), 133.1 (C), 133.1* (C), 129.6 (CH), 129.6 (CH), 129.1 (CH), 128.4 (CH), 128.0 (CH), 127.9 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 122.6 (CH), 122.3* (CH), 70.4 (CH_2), 70.3* (CH_2), 69.3 (CH), 69.1* (CH), 67.3 (CH_2), 67.2* (CH_2), 62.5 (CH_2), 62.2* (CH_2), 52.0 (CH), 51.0* (CH), 40.9 (CH_2), 40.5* (CH_2) *Rotamers. IR (film, ν_{max} / cm^{-1}): 3039, 2943, 1720, 1702, 1421, 1414, 1272, 1068. HRMS (ES): calcd. for $C_{28}H_{27}NO_5Na$: 480.1781, found 480.1781.

6-*O*-Benzoyl-4-*O*-benzyl-5-*N*-benzyloxycarbonyl-1-deoxymannojirimycin (15)

$(DHQD)_2Phal$ (46 mg, 0.06 mmol), K_2OsO_4 (10 mg, 0.03 mmol), K_2CO_3 (288 mg, 2.08 mmol) and $K_3[Fe(CN)_6]$ (693 g, 2.08 mmol) were dissolved in ACN:H_2O 1:1 (6 mL). The reaction was cooled to 0 ºC and $CH_3SO_2NH_2$ (69 mg, 0.69 mmol) was then added. After 10 min,a solution of **14** (318 mg, 0.69 mmol) in ACN:H_2O 1:1 (6 mL) was added and the mixture was left to warm to r.t. and stirred until no starting material was observed by TLC. The reaction was treated with Na_2SO_3 (400 mg) and stirred for 60 min. It was then extracted with EtOAc (3x 10mL) and the organic phase was washed with brine (1x 10mL), dried with $MgSO_4$, and purified by chromatography on silica gel using hexane:EtOAc to give **15** (212 mg, 62%) as one diastereomer as a sticky white foam. $[\alpha]^{20}_D$ = -20.0 (c=0.76, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.93 (d, J = 7.5 Hz, 2H), 7.54 (t, J = 7.5 Hz, 1H), 7.38 (t, J = 7.5 Hz, 2H), 7.32 – 7.11 (m, 10H), 5.07 (d, J = 11.5 Hz, 1H), 4.94 (br, 1H), 4.86 (br, 1H), 4.76 (dd, J = 11.5, 9.5 Hz, 1H), 4.59 (br, 1H), 4.47 (d, , J = 12.0 Hz, 1H), 4.41 (dd, , J = 12.0, 5.0 Hz, 1H), 4.17 (br, 1H), 4.07 (s, 1H), 4.03 (br, 1H), 3.75 (t, J = 2.5 Hz, 1H), 3.14 (dd, J = 13.0, 11.0 Hz, 1H), 2.95 (br, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 166.4 (CO), 156.2 (CO), 137.4 (C), 136.2 (C), 133.0 (C), 129.8 (CH), 129.6 (CH), 128.4 (CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 127.9 (CH), 127.8 (CH), 127.6 (CH), 75.5 (CH), 71.3 (CH_2), 69.5 (CH), 67.5 (CH_2), 64.7 (CH), 61.7 (CH_2), 52.3 (CH), 39.4 (CH_2). IR (film, ν_{max} / cm^{-1}): 3428, 2911, 1719, 1699, 1450, 1429, 1274, 1068. HRMS (ES): calcd. for $C_{28}H_{30}NO_7$: 492.2017, found 492.2024.

6-*O*-Benzoyl-4-*O*-benzyl-5-*N*-benzyloxycarbonyl-2,3-*O*-(cyclic sulfate)-1-deoxymannojirimycin (16)

TEA (0.25 mL, 1.80 mmol) was added to a solution of **15** (211 mg, 0.43 mmol) in THF (12 mL) cooled at 0ºC and $SOCl_2$ (100 µL, 1.54 mmol) was then added dropwise. The reaction was stirred at 0ºC until no starting material was observed by TLC. H_2O (5 mL) was then added and the crude was extracted with CH_2Cl_2 (3x 5 mL) and dried over $MgSO_4$. The solvent was removed under low pressure and the obtained oil was dissolved in ACN:CCl_4:H_2O 1:1:1 mixture (7.5 mL) and cooled at 0ºC. $RuCl_3$ (9 mg, 0.04 mmol) and $NaIO_4$ (184 mg, 0.86 mmol) were added and the reaction was allowed to stir at 0ºC until no starting material was observed by TLC. H_2O (5mL) and Et_2O (5 mL) were added, and the reaction was stirred 10 min. The organic phase was separated and washed with $NaHCO_3$ (1x 5mL) and brine (1x 5mL), and dried with $MgSO_4$. The solvent was removed under low pressure. Purification by chromatography on silica gel using hexane/ethyl acetate gave **16** (190 mg, 75%) as one diastereomer as colorless oil. $[\alpha]^{20}_D$ = -16.3 (c=0.63, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.87 (dd, J = 7.5, 1.0 Hz, 2H), 7.58 (tt, J = 7.5, 1.0 Hz, 1H), 7.44 (t, J = 7.5 Hz, 2H), 7.34 – 7.24 (m, 9H), 7.19 (tt, J = 6.0, 1.5 Hz, 1H), 5.14 (d, J = 11.5 Hz, 1H), 5.08 (br, 2H), 5.02 (t, J = 7.0 Hz, 1H), 4.79 (d, J = 11.5 Hz, 1H), 4.65 (m, 2H), 4.55 (d, J = 11.0 Hz, 1H), 4.40 (br, 1H), 4.32 (t, J = 7.0 Hz, 1H), 3.41 (br, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 165.8 (CO), 154.9 (CO), 135.9 (C), 135.3 (C), 133.4 (CH), 129.6 (CH), 129.2 (C), 128.7 (CH), 128.6 (CH), 128.5 (CH), 128.4 (CH), 128.2 (CH), 81.7 (CH), 74.5 (CH), 73.7 (CH_2), 70.8 (CH), 68.4 (CH_2), 61.7 (CH_2), 53.9 (CH), 40.1 (CH_2). IR (film, ν_{max} / cm^{-1}): 1714, 1397, 1271, 1212, 1112, 1098, 982, 712, 699. HRMS (ES): calcd. for $C_{28}H_{31}N_2O_9S$: 571.1745, found 571.1747.

4-*O*-Benzyl-2,3-*O*-(cyclic sulfate)-5*N*,6*O*-(cyclic carbamate)-1-deoxymannojirimycin (22)

Diol **21** (3.18 g, 11.38 mmol) was dissolved in THF (120 mL) and cooled to 0ºC. Triethyl amine (6.66 mL, 47.81 mmol) was added and after 10 min $SOCl_2$ (2.5 mL, 38.72 mmol) was added dropwise. The reaction was stirred at 0ºC for 1 h, then treated with water (9 mL) and extracted with CH_2Cl_2 (3x15 mL). The solvent was removed under reduced pressure to give an orange oil which was dissolved in a 1:1:1 ACN:CCl_4:H_2O mixture (90 mL) and cooled to 0ºC. $RuCl_3$ (35 mg, 0.17 mmol) and $NaIO_4$ (4.87 g, 22.77 mmol) were added and the reaction was stirred at 0ºC vigorously for 4h. Treatment consisted of the addition of Et_2O (20 mL) and H_2O (20 mL). The organic phase was washed with $NaHCO_3$ (1x20 mL) and brine (1x10 mL), dried over $MgSO_4$, and purified by chromatography on silica gel using hexane/ethyl acetate and increasing the polarity ratio to give **22** as a white foam (3.12 g, 80%). $[\alpha]^{20}_D$ = +47.6 (c=0.5, $CHCl_3$). Mp: 157-158 ºC. ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.45 – 7.31 (m, 5H), 5.18 (m, 1H), 4.95 (dd, J = 8.0, 4.5 Hz, 1H), 4.91 (d, J = 11.5 Hz, 1H), 4.65 (d, J = 11.5 Hz, 1H), 4.45 (d, J = 16.0 Hz, 1H), 4.36 (dd, J = 9.5, 8.0 Hz, 1H), 3.98 (dd, J = 9.5, 8.0 Hz, 1H), 3.84 (dd, J = 9.5, 4.5 Hz, 1H), 3.59 – 3.50 (m, 1H), 3.36 (dd, J = 16.0, 3.0 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 156.1 (CO), 136.1 (C), 128.9 (CH), 128.8 (CH), 128.6 (CH), 86.7 (CH), 79.1 (CH), 75.2 (CH), 74.4 (CH_2), 65.4 (CH_2), 54.3 (CH), 39.9 (CH_2). IR (film, ν_{max} / cm^{-1}): 2902, 1755, 1439, 1389, 1214, 1070, 1014. HRMS (ES): calcd. for $C_{14}H_{16}NO_7S$: 342.06420, found 342.06516. EA: Anal. calcd. for $C_{14}H_{15}NO_7S$: C, 49.26%; H, 4.43%; N, 4.10%; S, 9.39%; found C, 48.92%; H, 4.48%; N, 4.25%; S, 9.41%

2-Azido-4-*O*-benzyl-5*N*,6*O*-(cyclic carbamate)-1,2-dideoxynojirimycin (19) and 3-Azido-4-*O*-benzyl-5*N*,6*O*-(cyclic carbamate)-1,3-dideoxyallonojirimycin (20)

NaN_3 (1.06 g, 16.31 mmol) and **22** (2.78 g, 8.16 mmol) were dissolved in acetone: H_2O 2:1 (135 mL) and heated at 50 ºC for 6 h. After removal of the acetone at low pressure, Et_2O (80 mL) and 20% aq H_2SO_4 (60 mL) were added and the mixture was stirred at r.t. for 24 h. The reaction was diluted with H_2O (30 mL), extracted with EtOAc (3x 50 mL), washed with aqueous $NaHCO_3$ (2x 15 mL), dried over $MgSO_4$, and purified by chromatography on silica gel using hexane/ethyl acetate and increasing the polarity ratio to obtain **19** (979 mg, 39%) and **20** (554 mg, 22%) as white solids.

19: $[\alpha]^{20}_D$ = +74.0 (c=0.37, $CHCl_3$). Mp: 194-197 ºC. ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.44 – 7.29 (m, 5H), 4.89 (d, J = 11.6 Hz, 1H), 4.72 (d, J = 11.6 Hz, 1H), 4.30 (dd, J = 9.0, 8.0 Hz, 1H), 4.06 (dd, J = 13.5, 6.0 Hz, 1H), 3.87 (dd, J = 9.0, 4.5 Hz, 1H), 3.59 (dt, J = 9.5, 3.5 Hz, 1H), 3.53 (ddd, J = 9.5, 8.0, 4.5 Hz, 1H), 3.44 (ddd, J = 11.0, 9.5, 6.0 Hz, 1H), 3.29 (t, J = 9.4 Hz, 1H), 2.76 (d, J = 3.5 Hz, 1H), 2.71 (dd, J = 13.5, 11.0 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 156.4 (CO), 137.3 (C), 128.8 (CH), 128.6 (CH), 128.2 (CH), 80.1 (CH), 77.6 (CH), 75.1 (CH_2), 65.6 (CH_2), 60.6 (CH), 56.5 (CH), 42.6 (CH_2). IR (film, ν_{max} / cm^{-1}): 3366, 2919, 2118, 1709, 1438, 1253, 1108, 1085. HRMS (ES): calcd. for $C_{14}H_{17}N_4O_4$: 305.12443, found 305.12464. EA: Anal. calcd. for $C_{14}H_{16}N_4O_4$:

C, 55.26%; H, 5.30%; N, 18.41%; found C, 55.35%; H, 5.35%; N, 18.51%.

20: $[\alpha]^{20}_D$ = +51.2 (c=0.43, $CHCl_3$). Mp: 148-152 ºC. ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.44 – 7.32 (m, 5H), 4.74 (d, *J* = 11.5 Hz, 1H), 4.52 (d, *J* = 11.5 Hz, 1H), 4.35 (dd, *J* = 8.5, 6.5 Hz, 1H), 4.17 (t, *J* = 3.0, 1H), 4.03 (m, 2H), 3.92 (m, 2H), 3.68 (d, *J* = 14.5 Hz, 2H), 3.25 (dd, *J* = 14.5, 1.5 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 158.7 (CO), 136.8 (C), 128.7 (CH), 128.4 (CH), 128.3 (CH), 74.8 (CH), 71.8 (CH_2), 67.5 (CH), 65.9 (CH_2), 59.9 (CH), 53.2 (CH), 42.7 (CH_2). IR (film, ν_{max} / cm^{-1}): 3294, 2919, 2099, 1727, 1447, 1088, 1067. HRMS (ES): calcd. for $C_{14}H_{17}N_4O_4$: 305.12443, found 305.12450. EA: Anal. calcd. for $C_{14}H_{16}N_4O_4$: C, 55.26%; H, 5.30%; N, 18.41%; found C, 55.48%; H, 5.40%; N, 18.42%.

2-Azido-3,4-di-*O*-benzyl-5*N*,6*O*-(cyclic carbamate)-1,2-dideoxynojirimycin (23)

A solution of **19** (330 mg, 1.08 mmol) in DMF (8 mL) was added via cannula to a suspension of NaH (40 mg, 1.62 mmol) in DMF (8.5 mL) cooled at 0ºC. After 10 min, benzyl bromide (0.18 mL, 1.52 mmol) was added drop wise and the reaction was allowed to stir at r.t. until no starting material was observed by TLC. H_2O (5 mL) was then added and the reaction was extracted with CH_2Cl_2 (3x 5 mL), dried over $MgSO_4$, and purified by chromatography on silica gel using hexane/EtOAc to give **23** (384 mg, 90%) as a white solid. $[\alpha]^{20}_D$ = +53.4 (c=0.49, $CHCl_3$). Mp: 110-112 ºC. ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.43 – 7.22 (m, 10H), 4.91 (dt, *J* = 10.5, 9.5 Hz, 3H), 4.61 (d, *J* = 11.5 Hz, 1H), 4.23 (dd, *J* = 9.0, 8.0 Hz, 1H), 4.04 (dd, *J* = 13.5, 5.5 Hz, 1H), 3.70 (dd, *J* = 9.0, 4.5 Hz, 1H), 3.60 – 3.40 (m, 3H), 3.34 (t, *J* = 9.0 Hz, 1H), 2.67 (dd, *J* = 13.5, 10.5 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 156.4 (CO), 137.3 (C), 137.2 (C), 128.7 (CH), 128.5 (CH), 128.5 (CH), 128.2 (CH), 128.2 (CH), 85.1 (CH), 80.0 (CH), 76.1 (CH_2), 75.1 (CH_2), 65.4 (CH_2), 60.7 (CH), 56.8 (CH), 43.0 (CH_2). IR (film, ν_{max} / cm^{-1}): 2917, 2110, 1761, 1425, 1091. HRMS (ES): calcd. for $C_{21}H_{23}N_4O_4$: 395.1714, found 395.1706. EA: Anal. calcd. for $C_{21}H_{22}N_4O_4$: C, 63.95%; H, 5.62%; N, 14.20%; found C, 63.85%; H, 5.50%; N, 14.06%.

2-Acetamido-3,4-di-*O*-benzyl-5*N*,6*O*-(cyclic carbamate)-1,2-dideoxynojirimycin (24)

Pd/C (18 mg, 0.02 mmol) was added to a solution of **23** (111 mg, 0.28 mmol) in EtOAc (5 mL) and the reaction was charged with H_2 (5 barg) and stirred at r.t. for 20h. Palladium was filtered with MeOH over Celite and solvents were removed under low pressure. The obtained colorless oil was dissolved in pyridine (2 mL) and Ac_2O (48 µL, 0.39 mmol) was added. The reaction was stirred at r.t. for 16h. H_2O (5 mL) was then added and the reaction was extracted with EtOAc (3x 5 mL), dried over $MgSO_4$, and purified by chromatography on silica gel using hexane/EtOAc to give **24** (110 mg, 95%) as a white solid. $[\alpha]^{20}_D$ = +106.5 (c=0.31, $CHCl_3$). Mp: 213 – 215 ºC. ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.44 – 7.28 (m, 10H), 5.33 (d, *J* = 5.0 Hz, 1H), 4.92 (d, *J* = 11.5 Hz, 2H), 4.66 (dd, *J* = 13.0, 11.5 Hz, 2H), 4.25 (dd, *J* = 9.0, 8.0 Hz, 1H), 4.04 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.76 – 3.52 (m, 4H), 3.39 (m, 1H), 2.82 (m, 1H), 1.78 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 170.4 (CO), 156.4 (CO), 137.9 (C), 137.4 (C), 128.8 (CH), 128.7 (CH), 128.4 (CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 81.9 (CH), 81.2 (CH), 75.0 (CH_2), 74.9 (CH_2), 65.6 (CH_2), 56.9 (CH), 50.5 (CH), 42.7 (CH_2), 23.3 (CH_3). IR (film, ν_{max} / cm^{-1}): 3299, 2946, 1749, 1652, 1521, 1088. HRMS (ES): calcd. for $C_{23}H_{27}N_2O_5$: 411.19145, found 411.19214. EA: Anal. calcd. for $C_{23}H_{26}N_2O_5$+ ½H_2O: C, 65.86%; H, 6.49%; N, 6.68%; found C, 65.85%; H, 6.13%; N, 6.50%.

2-Acetamido-3,4-di-*O*-benzyl-1,2-dideoxynojirimycin (25)

NaOH 6M (0.35 mL, 2.11 mmol) was added to a solution of **24** (87 mg, 0.21 mmol) in MeOH : H_2O 9:1 (8 mL) and the reaction was stirred at reflux for 4 h. H_2O (5 mL) was then added and the reaction was extracted with EtOAc (3x 5 mL), dried over $MgSO_4$, and purified by chromatography on silica gel using CH_2Cl_2/MeOH to give **25** (75 mg, 92%) as a white solid. $[\alpha]^{20}_D$ = -19.7 (c=0.08, CH_3OH). Mp: 210-212 ºC. ^{1}H-NMR (400 MHz, CD_3OD, δ/ppm): 7.34 – 7.24 (m, 10H), 4.81 (dd, *J* = 11.0, 1.5 Hz, 2H), 4.73 (d, *J* = 18.0 Hz, 1H), 4.67 (d, *J* = 18.0 Hz, 1H), 3.97 (m, 1H), 3.79 (dd, *J* = 11.0, 2.5 Hz, 1H), 3.68 (dd, *J* = 11.0, 5.0 Hz, 1H), 3.44 (m, 2H), 3.04 (dd, *J* = 12.0, 5.0 Hz, 1H), 2.55 (m, 1H), 2.44 (t, *J* = 12.0 Hz, 1H), 1.88 (s, 3H). ^{13}C-NMR (100 MHz, CD_3OD, δ/ppm): 173.1 (CO), 140.2 (C), 139.8 (C), 129.4 (CH), 129.3 (CH), 128.9 (CH), 128.7 (CH), 128.7 (CH), 128.6 (CH), 86.4 (CH), 81.5 (CH), 76.0 (CH_2), 62.6 (CH), 62.2 (CH_2), 53.3 (CH), 49.3 (CH_2), 22.9 (CH_3). IR (film, ν_{max} / cm^{-1}): 3275, 2933, 1650, 1554, 1072, 1027. HRMS (ES): calcd. for $C_{22}H_{29}N_2O_4$: 385.21218, found 385.21223. EA: Anal. calcd. for $C_{22}H_{28}N_2O_4$+ 3/2 H_2O: C, 64.21%; H, 7.59%; N, 6.81%; found C, 64.52%; H, 7.08%; N, 6.42%.

2-Acetamido-1,2-dideoxynojirimycin (DNJNAc, 4)

To a solution of **25** (20 mg, 0.05 mmol) in MeOH (4 mL) was added Pd/C (9 mg, 0.008 mmol) and the reaction was charged with H_2 (55 barg) and stirred at 60 ºC for 20 h. Palladium was then filtrated over Celite and the crude was purified by chromatography on silica gel using CH_2Cl_2/MeOH/NH_3 72.5:25:2.5 to give **4** (12 mg, 96%) as a white solid. $[\alpha]^{20}_D$ = +7.9 (c=0.15, H_2O). Mp: 210-212 ºC. ^{1}H-NMR (400 MHz, CD_3OD, δ/ppm): 3.81 (dd, *J* = 11.0, 3.0 Hz, 1H), 3.73 (m, 1H), 3.63 (dd, *J* = 11.0, 6.0 Hz, 1H), 3.24 (m, 2H), 3.11 (dd, *J* = 12.5, 5.0 Hz, 1H), 2.46 (ddd, *J* = 9.5, 6.0, 3.0 Hz, 1H), 2.38 (dd, *J* = 12.5, 11.0 Hz, 1H), 1.96 (s, 3H). ^{13}C-NMR (100 MHz, CD_3OD, δ/ppm): 173.6 (CO), 77.7 (CH), 73.9 (CH), 62.8 (CH_2), 62.7 (CH), 53.9 (CH), 49.1 (CH_2), 22.7 (CH_3). IR (film, ν_{max}/cm^{-1}): 3287, 2917, 1638, 1559, 1437, 1373, 1096, 1040. HRMS (ES): calcd. for $C_8H_{17}N_2O_4$: 205.11828, found 205.11784.

2-Azido-4-*O*-benzyl-5-*N*-benzyloxycarbonyl-1,2-dideoxynojirimycin (30)

NaOH 6M (2 mL, 11.89 mmol) was added to a solution of compound **19** (301 mg, 0.99 mmol) in MeOH:H_2O 9:1 (20 mL) and the reaction was heated at reflux for 15 h. Solvent was then removed under low pressure and the crude was redissolved in EtOAc:$NaHCO_3$ saturated aqueous 1:1 (14 mL). After 30 min of stirring, Boc_2O (436 mg, 1.91 mmol) was added and the crude was allowed to stir for 24 h. The crude was treated with water (6 mL), extracted with EtOAc (3x5 mL), dried with $MgSO_4$, and purified by chromatography on silica gel using hexane/ethyl acetate to give **30** (334 mg, 90%) as a colorless oil. $[\alpha]^{20}_D$ = -36.1 (c=0.67, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.38 – 7.28 (m, 5H), 4.83 (d, *J* = 11.5 Hz, 1H), 4.72 (d, *J* = 11.5 Hz, 1H), 4.00 (d, *J* = 11.0 Hz, 1H), 3.97 - 3.87 (br, 2H), 3.84 (dd, *J* = 14.0, 4.5 Hz, 1H), 3.68 (t, *J* = 7.0 Hz, 1H), 3.57 (t, *J* = 7.0 Hz, 1H), 3.53 (br, 1H), 3.39 (td, *J* = 7.5, 4.5 Hz, 1H), 3.29 (s, 1H), 3.18 (dd, *J* = 14.0, 7.5 Hz, 1H), 1.47 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 154.9 (CO), 138.0 (C), 128.6 (CH), 128.0 (CH), 128.0 (CH), 81.5 (s), 77.7 (C), 75.4 (CH), 74.1 (CH_2), 60.8 (CH), 60.7 (CH_2), 60.6 (CH), 45.7 (CH_2), 28.3 (CH_3). IR (film, ν_{max} / cm^{-1}): 3404, 2968, 2107, 1668, 1422, 1367, 1250, 1162. HRMS (ES): calcd. for $C_{18}H_{27}N_4O_5$: 379.19760, found 379.19777.

2-Acetamido-3,4-6-tri-*O*-acetyl-5-*N*-benzyloxycarbonyl-1,2-dideoxynojirimycin (31)

Pd/C (160 mg, 0.15 mmol) and acetic acid (0.43 mL, 7.55 mmol) were added to a solution of **30** (571 mg, 1.51 mmol) in degassed MeOH (15 mL) and the reaction was charged with H_2 (15 barg) and stirred at 60ºC for 16h. Palladium was filtered over Celite and solvents were removed under low pressure. The colorless obtained oil was redissolved in pyridine (10 mL) and Ac_2O (1.58 mL, 15.02 mmol) was added. The reaction was stirred at r.t. for 16h. H_2O (5 mL) was then added and the reaction was extracted with CH_2Cl_2 (3x 5 mL), dried over $MgSO_4$ and purified by chromatography on silica gel using hexane/EtOAc to give **31** (538 mg, 83%) as a colorless oil. $[\alpha]^{20}_D$ = -7.2 (c=2.3, $CHCl_3$). ^{1}H-NMR (400 MHz, CD_3OD, δ/ppm): 6.12 (d, *J* = 8.5 Hz, 1H), 4.94 (m, 1H), 4.92 (m, 1H), 4.61 (t, *J* = 7.5 Hz, 1H), 4.38 (dd, *J* = 11.5, 8.5 Hz, 1H), 4.23 (dd, *J* = 11.5, 6.5 Hz, 1H), 4.14 – 4.05 (m, 2H), 3.32 (dd, *J* = 15.0, 3.0 Hz, 1H), 2.11 (s, 6H), 2.06 (s, 3H), 1.99 (s, 3H), 1.47 (s, 9H). ^{13}C-NMR (100 MHz, CD_3OD, δ/ppm): 170.4 (CO), 168.9 (CO), 168.6 (CO), 168.2 (CO), 155.5 (CO), 80.8 (C), 67.8 (CH), 67.0 (CH), 59.9 (CH_2), 52.9 (CH), 46.1 (CH), 39.3 (CH_2), 28.2 (CH_3), 23.3 (CH_3), 20.8 (CH_3), 20.8 (CH_3), 20.7 (CH_3). IR (film, ν_{max} / cm^{-1}): 3333, 2975, 1745, 1687, 1369, 1223, 1046. HRMS (ES): calcd. for $C_{19}H_{31}N_2O$: 431.20241, found 431.20239.

2-Acetamido-3,4-6-tri-*O*-acetyl-5-*N*-(*N'*-butylaminocarbonyl)-1,2-dideoxynojirimycin (32a)

TFA (0.48 mL, 6.31 mmol) was added to a solution of **31** (90 mg, 0.21 mmol) in CH_2Cl_2 (8 mL) and the reaction was stirred at r.t. until no starting material was observed by TLC. Solvent was removed under reduced pressure and the resulting oil was dissolved in CH_2Cl_2 (8 mL). TEA (0.23 mL, 1.64 mmol) and butyl isocyanate (71 µl, 0.63mmol) were added and the reaction was heated at reflux for 4h. H_2O (5 mL) was then added and the reaction was extracted with CH_2Cl_2 (3x 5 mL), dried over $MgSO_4$, and purified by chromatography on silica gel using CH_2Cl_2/MeOH to give **32a** (73 mg, 81%) as a colorless oil. $[\alpha]^{20}_D$ = -63.5 (c=2.31, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 6.52 (d, *J* = 8.0 Hz 1H), 5.04 (t, *J* = 5.5 Hz 1H), 4.99 (t, *J* = 3.0 Hz 1H),4.88 (m, 1H), 4.43 (dd, *J* = 11.0, 7.5 Hz, 1H), 4.24 (t, *J* = 6.5Hz, 1H), 4.12 (dd, *J* = 11.0, 7.5 Hz, 1H) 4.03 (q, *J* = 3.5 Hz, 1H), 3.95 (d, *J* =5.0 Hz, 1H), 3.26 (dd, *J* = 15.0, 3.0 Hz, 1H) , 3.20 (m, 2H), 2.09 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.95 (s, 3H), 1.47 (p, *J* = 7.0 Hz, 2H), (h, *J* = 7.0 Hz, 2H), 0.90 (t, *J* = 7.5 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 171.0 (CO), 169.6 (CO), 168.8 (CO), 168.7 (CO), 159.0 (CO), 68.0 (CH), 67.0 (CH), 61.0 (CH_2), 54.0 (CH), 47.0 (CH), 40.8 (CH_2), 39.1 (CH_2), 32.1 (CH_2), 23.2 (CH_3), 20.8 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 20.0 (CH_2), 13.7 (CH_3). IR (film, ν_{max} / cm^{-1}): 3364, 2958, 2932, 1749, 1652, 1539, 1370, 1225, 1043. HRMS (ES): calcd. for $C_{19}H_{32}N_3O_8$: 430.21819, found 430.21839.

2-Acetamido-5-*N*-(*N'*-butylaminocarbonyl)-1,2-dideoxynojirimycin (10a)

Compound **32a** (73 mg, 0.17 mmol) was dissolved in a NH_3 saturated MeOH solution (4 mL) and the reaction was stirred at r.t. for 18h. Solvent was removed under low pressure, and the crude was purified by chromatography on silica gel using CH_2Cl_2/MeOH to give **10a** (32 mg, 61%) as a slightly yellow solid. $[\alpha]^{20}_D$ = +25.0 (c=1.63, CH_3OH). Mp: 70-72 ºC. ^{1}H-NMR (400 MHz, CD_3OD, δ/ppm): 3.94 (m, 1H), 3.91 – 3.81 (m, 3H), 3.76 (dd, *J* = 11.0, 4.5 Hz, 1H), 3.72 (t, *J* = 4.5 Hz, 1H), 3.60 (t, *J* = 4.5 Hz, 1H), 3.35 (dd, *J* = 13.5, 3.0 Hz, 1H), 3.24 – 3.07 (m, 2H), 1.95 (s, 3H), 1.48 (m, 2H), 1.37 (m, 2H), 0.93 (t, *J* = 7.5 Hz, 3H). ^{13}C-NMR (100 MHz, CD_3OD, δ/ppm): 172.7 (CO), 161.8 (CO), 71.7 (CH), 70.3 (CH), 61.9 (CH), 61.8 (CH_2), 51.6 (CH), 41.6 (CH_2), 41.0 (CH_2), 33.3 (CH_2), 22.9 (CH_3), 21.1 (CH_2), 14.2 (CH_3). IR (film, ν_{max} / cm^{-1}): 3353, 2923, 1623, 1540, 1469, 1418, 1021. HRMS (ES): calcd. for $C_{13}H_{26}N_3O_5$: 304.18670, found 304.18727.

General Procedures for Inhibition Assay

The glycosidases α-glucosidase (from yeast), amyloglucosidase (from *Aspergillus niger*), isomaltase (from yeast), β-glucosidases (from almond and bovine liver), naringinase (*Penicillium decumbes*), α-galactosidase (from green coffee beans), β-galactosidase (from *Escherichia coli*), α-mannosidase (from jack bean), β-mannosidase (from *Helix pomatia*), β-*N*-acetylglucosaminidases (from human placenta, bovine kidney and jack bean) used in the inhibition studies, as well as the corresponding *o*- or *p*-nitrophenyl glycoside substrates, were purchased from Sigma Chemical Co. Inhibitory potencies were determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the respective *o*- (for β-galactosidases) or *p*-nitrophenyl α- or β-D-glycopyranoside (for α-glucosidases, β-glucosidases, α-galactosidases, α-mannosidases and β-mannosidases) or *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide/galactosaminide (for hexosaminidases), in the presence of corresponding iminosugars. Each assay was performed in phosphate-citrate (for α- or β-mannosidase, amyloglucosidase or β-*N*-acetylglucosaminidase at pH 5.5 or 3.5) or in phosphate buffer (at pH 7.3 or 6.8 for the other glycosidases) at the optimal pH for each enzyme. The K_m values for the different glycosidases used in the tests and the corresponding working pHs are listed herein: α-glucosidase (yeast), K_m = 0.35 mM (pH 6.8); amyloglucosidase (*Aspergillus niger*), K_m = 3.0 mM (pH 5.5); isomaltase (from yeast), K_m = 1.0 mM (pH 6.8); β-glucosidase (almond), *K*m = 3.5 mM (pH 7.3); β-glucosidase (bovine liver), K_m = 1.0 mM (pH 7.3); naringinase (*Penicillium decumbes*), K_m = 2.7 mM (pH 6.8); α-galactosidase (coffee beans), K_m = 2.0 mM (pH 6.8); β-galactosidase (from *Escherichia coli*), *K*m = 0.12 mM (pH 7.3); α-mannosidase (jack bean), K_m = 2.0 mM (pH 5.5); β-mannosidase (*Helix pomatia*), K_m = 0.6 mM (pH 5.5); β-*N*-acetylglucosaminidase (from human placenta), K_m = 0.34 mM (pH 5.5); β-*N*-acetylglucosaminidase (from bovine kidney), K_m = 0.48 mM (pH 5.5); β-*N*-acetylglucosaminidase (from jack bean), K_m = 0.49 mM (pH 5.5). The reactions were initiated by addition of enzyme to a solution of the substrate in the absence or presence of various concentrations of inhibitor. After the mixture was incubated for 10–30 min at 37 ºC (or 55 ºC for amyloglucosidase), the reaction was quenched by addition of 1 M Na_2CO_3. The absorbance of the resulting mixture was determined at 405 nm. The K_i value and enzyme inhibition mode were determined from the slope of Lineweaver-Burk plots and double reciprocal analysis using Microsoft Office Excel 2007 program. Inhibition mode was competitive in all cases.

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Electronic Supplementary Information (ESI) available: Experimental procedures for the synthesis of 3-acetamido-1,3-dideoxyaltronojirimycin (**29**), and derivatives **32b-d** and **10b-d.** ^{1}H and ^{13}C spectra of all new compounds and selected Lineweaver-Burk plots of ureido-DNJNAc derivative **10c**. See DOI: 10.1039/b000000x/

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