Stereoselective Synthesis of 2-Acetamido-1,2-dideoxyojirimycin (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-dideoxyojirimycin (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 sp²-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Since the isolation of nojirimycin in 1966, iminosugars—sugar analogs where the oxygen ring atom has been replaced by a nitrogen—have attracted an exponential interest as mimics of the transition state of the enzymatic hydrolysis of glycosidic substrates.1,2 Their ability to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-buty1-1-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 synthesis11,12. Most of these representatives are piperidine derivatives, such as 2-acetamido-1,2-dideoxyojirimycin (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 therapies28 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) and the manno diastereomer DMJNAc (5). 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-DNJNAc derivatives as examples of 2-acetamido sp2-imosugars, has also been accomplished. Characterized by the incorporation of a pseudoamide-type nitrogen atom with high sp2-hybridation character in the ring, 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. 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.

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. This intermediate has been widely used for the synthesis of various iminosugars including our recent synthesis of DAJNAc (9).

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. 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 H2O2 proved inefficient while harsher oxidant methods such as CF3CO3H or oxone 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 monocyclic 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, an approach that has been applied successfully in other iminosugar syntheses.

The protection of the primary alcohol of 13 as a benzoate, followed by Sharpless asymmetric dibydroxylatation 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 RuCl3/NaIO4 to the corresponding cyclic sulfate 16, which was obtained as a single diastereoisomer in 80% overall yield (two steps). Treatment of 16 with NaI at 50°C 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
nucleophilic 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).

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 synthesise the mixture of 19 and 20. Direct dihydroxylation of carbamate 12 using K₂OsO₄·2H₂O/NMO afforded 21 in satisfactory yield and diastereoselectivity (78%, 85:15) (Scheme 3). Sharpless asymmetric dihydroxylation conditions increased both the yield and diastereoselectivity, affording diol 21 in 94% yield and nearly complete diastereoselectivity, as observed by ¹H-NMR.¹⁷ The corresponding cyclic sulfate 22 was obtained in 80% yield by reaction of diol 21 with SOCl₂/TEA followed by in situ oxidation with NaIO₄/RuCl₃, as in the previous case. Attempts to perform direct sulfation of 21 using SO₂Cl₂/TEA²² also afforded 22 but in lower yields.

Regioselective ring-opening reactions of the key precursor 22 using NaN₃ 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 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 NaN₃, 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

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

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>T/ºC</th>
<th>t/h</th>
<th>NaN₃ Eq.</th>
<th>Yield %/</th>
<th>19/20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ACN</td>
<td>50</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>DMF</td>
<td>50</td>
<td>3</td>
<td>4</td>
<td>70</td>
<td>1.9:1</td>
</tr>
<tr>
<td>3</td>
<td>DMF</td>
<td>120</td>
<td>1</td>
<td>4</td>
<td>30</td>
<td>0.9:1</td>
</tr>
<tr>
<td>4</td>
<td>Acetone/H₂O 2:1</td>
<td>50</td>
<td>3</td>
<td>3</td>
<td>68</td>
<td>1:1</td>
</tr>
<tr>
<td>5</td>
<td>Acetone/H₂O 2:1</td>
<td>50</td>
<td>6</td>
<td>2</td>
<td>62</td>
<td>1.8:1</td>
</tr>
<tr>
<td>6</td>
<td>Acetone/H₂O 2:1</td>
<td>40</td>
<td>16</td>
<td>1.2</td>
<td>81</td>
<td>1:1</td>
</tr>
<tr>
<td>7</td>
<td>Acetone/H₂O 2:1</td>
<td>50</td>
<td>7</td>
<td>3</td>
<td>43</td>
<td>3:1</td>
</tr>
</tbody>
</table>

* Relation determined w/w after purification
* The reaction was performed at multigram scale using these conditions.
* Portionwise addition of NaN₃

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 H₂ and Pd/C followed by in situ acetylation with Ac₂O/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).

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.82,83 Transmuting the endocyclic sp2-amine nitrogen into a sp3-hybridized pseudoamide functionality by introduction of amide, urea, thiourea or guanidinium moieties has proven particularly successful in this respect.84–86 For instance, N-(N′-butylaminocarbamoyl)-1-deoxyojirimycin, the urea analog of the marketed drug Zavesca, was found to be a very selective inhibitor of bovine liver β-galactosidase.67 The sp2-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 Boc2O/NaHCO3 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 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).

Evaluation of the glycosidase inhibitory activity of the DJNNAc 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), β-galactosidase (green coffee), β-galactosidase (E. coli), and isomaltase (yeast). Compound 29 was a much weaker inhibitor than DJNNAc, 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 (Ki) 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 compared 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 sp2-iminosugar:glycosidase complexes.71 The higher hydrogen bond donor capability of ary lureas 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-dideoxyojirimycin (DJNNAc), 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 sp²-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 amphiphilic character, they are expected to show acceptable drug-like properties.

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

<table>
<thead>
<tr>
<th>Enzyme origin</th>
<th>4</th>
<th>29</th>
<th>10a</th>
<th>10b</th>
<th>10c</th>
<th>10d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human placenta</td>
<td>7.0 ± 0.3</td>
<td>427 ± 20</td>
<td>56 ± 5</td>
<td>33 ± 3</td>
<td>2.1 ± 0.1</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Bovine kidney</td>
<td>7.4 ± 0.3</td>
<td>524 ± 40</td>
<td>138 ± 10</td>
<td>82 ± 5</td>
<td>4.1 ± 0.2</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>Jack Bean</td>
<td>2.9 ± 0.2</td>
<td>130 ± 10</td>
<td>26 ± 3</td>
<td>19 ± 2</td>
<td>1.1 ± 0.1</td>
<td>10 ± 1</td>
</tr>
</tbody>
</table>

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). All other solvents were used with no further purification. All commercial reagents were used without further purification. General procedures for the syntheses and characterizations of compounds in Scheme 5, preparation of nanoelectrospray technique, High Resolution Mass Spectrometry were conducted using a Büchi M-540 apparatus without brackets. The cell was 10 cm long and had 1 mL of capacity. Measuring brackets. The cell was 10 cm long and had 1 mL of capacity. Measuring brackets. The cell was 10 cm long and had 1 mL of capacity. Measuring brackets. The cell was 10 cm long and had 1 mL of capacity.

Preparation of 1147, 1249 and 2117,48 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.

(2R,3S)-3-Benzoyloxy-N-benzyloxycarbonyl-2-hydroxy methyl-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 : H2O 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 H2O (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. H2O (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.

13C-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, 1H).

1H-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, 1H).
6-O-Benzoyl-4-O-benzyl-5-N-benzyloxy carbonyl-1-deoxynojirimycin (15)

(DH2O)2Phal (46 mg, 0.06 mmol), K2OsO4 (10 mg, 0.03 mmol), K2CO3 (288 mg, 2.08 mmol) and K2[Fe(CN)6] (693 mg, 2.08 mmol) were dissolved in ACN:H2O 1:1 (6 mL). The reaction was cooled to 0 °C and CH3SO2NH (69 mg, 0.69 mmol) was then added. After 10 min, a solution of 14 (318 mg, 0.69 mmol) in ACN:H2O 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 Na2SO4 (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 MgSO4, and purified by chromatography on silica gel using hexane:EtOAc to give 15 (212 mg, 62%) as one diastereomer as a sticky white foam. [α]D20 = -20.0 (c=0.76, CHCl3).19HNMR (400 MHz, CDCl3, δ/ppm): 7.92 (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). 13C-NMR (100 MHz, CDCl3, δ/ppm): 166.4 (CO), 156.2 (CO), 137.4 (C), 136.2 (C), 133.0 (C), 129.8 (CH2), 129.6 (CH2), 128.4 (CH2), 128.4 (CH), 128.3 (CH2), 127.9 (CH2), 127.8 (CH2), 127.6 (CH2), 75.5 (CH2), 71.3 (CH2), 69.5 (CH2), 67.5 (CH2), 64.7 (CH), 61.7 (CH2), 52.3 (CH), 39.4 (CH2). IR (film, vmax / cm⁻¹): 3248, 2911, 1719, 1699, 1450, 1429, 1274, 1068. HRMS (ES): calculated for C16H13O6N: 304.0767, found 304.0767.

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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 0°C. 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. H2O (5 mL) was then added and the reaction was extracted with CHCl3 (3x 5 mL), dried over MgSO4, and purified by chromatography on silica gel using hexane/EtOAc to give 23 (384 mg, 90%) as a white solid. [α]D20 = +53.4 (c=0.49, CHCl3). Mp: 110-112°C. 1H-NMR (400 MHz, CDCl3, 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). 13C-NMR (100 MHz, CDCl3, δ/ppm): 156.4 (CO), 137.3 (C), 137.2 (C), 128.7 (CH), 128.5 (CH), 128.2 (CH), 128.2 (CH), 85.1 (CH), 80.0 (CH), 76.1 (CH2), 75.1 (CH), 65.4 (CH2), 60.7 (CH), 56.8 (CH), 43.0 (CH3). IR (film, vmax / cm⁻¹): 2971, 2110, 1761, 1425, 1091. HRMS (ES): calcd. for C23H22N2O4: 395.1714, found 395.1706. Anal. calcd. for C23H22N2O4: C, 65.58%; H, 5.40%; N, 14.82%. 2-Azido-3,4-di-O-benzyl-5N,6O-(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 H2 (5 barg) and stirred at r.t. for 20h. Palladium was filtered with MeOH over Celite and solvents were removed under reduced pressure. To the crude, pyridine (2 mL) and Ac2O (48 μL, 0.39 mmol) were added. The reaction was stirred at r.t. for 16h. H2O (5 mL) was then added and the reaction was extracted with EtOAc (3x 5 mL), dried over MgSO4, and purified by chromatography on silica gel using hexane/EtOAc to give 24 (110 mg, 95%) as a white solid. [α]D20 = +106.5 (c=0.31, CHCl3). Mp: 213 – 215°C. 1H-NMR (400 MHz, CDCl3, 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), 3.59 (m, 1H), 2.82 (m, 1H), 1.78 (s, 3H). 13C-NMR (100 MHz, CDCl3, δ/ppm): 156.0 (CO), 137.9 (C), 137.4 (C), 128.8 (CH), 128.7 (CH), 128.4 (CH), 128.4 (CH), 128.3 (CH), 81.9 (CH), 81.2 (CH), 75.0 (CH), 74.9 (CH), 65.6 (CH), 56.9 (CH), 50.5 (CH), 42.7 (CH3), 23.3 (CH3). IR (film, vmax / cm⁻¹): 3299, 2946, 1749, 1652, 1521, 1088. HRMS (ES): calcd. for C22H20N4O5: 395.11945, found 395.12142. Anal. calcd. for C22H20N4O5: C, 65.86%; H, 6.49%; N, 6.68%; found C, 65.83%; 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 : H2O 9:1 (8 mL) and the reaction was stirred at reflux for 4 h. H2O (5 mL) was then added and the reaction was extracted with EtOAc (3x 5 mL), dried over MgSO4, and purified by chromatography on silica gel using CH2Cl2/MeOH to give 25 (75 mg, 92%) as a white solid. [α]D20 = -19.7 (c=0.08, CH2OH). Mp: 210-212°C. 1H-NMR (400 MHz, CD2OD, 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). 13C-NMR (100 MHz, CD2OD, δ/ppm): 173.1 (CO), 140.2 (C), 139.8 (C), 129.4 (CH), 129.3 (CH), 128.9 (CH), 128.7 (CH), 128.6 (CH), 86.4 (CH), 81.5 (CH), 76.0 (CH2), 62.6 (CH2), 53.3 (CH), 49.3 (CH2), 22.9 (CH3). IR (film, vmax / cm⁻¹): 3275, 2933, 1650, 1554, 1072. HRMS (ES): calcd. for C22H22N2O5: 385.21218, found 385.21223. Anal. calcd. for C22H22N2O5: 3/2 H2O: 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 H2 (52 barg) and stirred at 60 °C for 20 h. Palladium was then filtered over Celite and the crude was purified by chromatography on silica gel using CH2Cl2/MeOH/NH3 72.5:25:2.5 to give 4 (12 mg, 96%) as a white solid. [α]D20 = +7.9 (c=0.15, H2O). Mp: 210-212°C. 1H-NMR (400 MHz, CD2OD, 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 (dd, J = 9.5, 6.0, 3.0 Hz, 1H), 2.38 (dd, J = 12.5, 11.0 Hz, 1H), 1.86 (s, 3H). 13C-NMR (100 MHz, CD2OD, δ/ppm): 173.6 (CO), 77.7 (CH), 73.9 (CH), 62.8 (CH2), 62.7 (CH2), 53.9 (CH), 49.1 (CH2), 22.7 (CH3). IR (film, vmax / cm⁻¹): 3287, 2971, 1638, 1559, 1437, 1373, 1096, 1040. HRMS (ES): calcd. for C18H19N3O4: 305.11828, found 305.11784.
2-Acetamido-3,4,6-tri-O-acetyl-5-N-benzoyloxy carbonyl-1,2-dideoxyxyojirimycin (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 H₂ (15 barg) and stirred at 60°C for 16 h. Palladium was filtered over Celite and solvents were removed under low pressure. The colorless obtained oil was redissolved in pyridine (10 mL) and Ac₂O (1.58 mL, 15.02 mmol) was added. The reaction was stirred at r.t. for 16 h. H₂O (5 mL) was then added and the reaction was extracted with CHCl₃ (3x 5 mL), dried over MgSO₄ and purified by chromatography on silica gel using hexane/EtOAc to give 31 (538 mg, 83%) as a colorless oil.

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

TFA (0.48 mL, 6.31 mmol) was added to a solution of 31 (90 mg, 0.21 mmol) in CH₂Cl₂ (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 CH₂Cl₂ (8 mL). TEA (0.23 mL, 1.64 mmol) and butyl isocyanate (71 μL, 0.63 mmol) were added and the reaction was heated at reflux for 4 h. H₂O (5 mL) was then added and the reaction was extracted with CHCl₃ (3x 5 mL), dried over MgSO₄, and purified by chromatography on silica gel using CH₂Cl₂/MeOH to give 32a (73 mg, 81%) as a colorless oil.

2-Acetamido-5-N- (V'-butylaminocarbonyl) -1,2-dideoxyxyojirimycin (10a)

Compound 32a (73 mg, 0.17 mmol) was dissolved in a NH₃ saturated MeOH solution (4 mL) and the reaction was stirred at r.t. for 18 h. Solvent was removed under low pressure, and the crude was purified by chromatography on silica gel using CH₂Cl₂/MeOH to give 10a (32 mg, 61%) as a slightly yellow solid.

General Procedures for Inhibition Assay

The glycosidases α-glucosidase (from yeast), amylglucosidase (from Aspergillus niger), isomaltase (from yeast), β-glucosidases (from almond and bovine liver), naringinase (Penicillium demicum), β-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 α- or β-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 α- (for β-galactosidases) or β-nitrophenyl α- or β-D-glycopyranoside (for α-glucosidases, β-glucosidases, α-galactosidases, α-mannosidases and β-mannosidases) or β-nitrophenyl-N-acetyl-D-glucosaminidase/galactosaminidase (for hexosaminidases), in the presence of corresponding iminosugars. Each assay was performed in phosphate-citrate (for α- or β-mannosidase, amylglucosidase 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 Km values for the different glycosidases used in the tests and the corresponding working pHs are listed herein: α-glucosidase (yeast), Km = 0.35 mM (pH 6.8); amylglucosidase (Aspergillus niger), Km = 3.0 mM (pH 5.5); isomaltase (from yeast), Km = 1.0 mM (pH 6.8); β-glucosidase (almond), Km = 3.5 mM (pH 7.3); β-glucosidase (bovine liver), Km = 1.0 mM (pH 7.3); naringinase (Penicillium demicum), Km = 2.7 mM (pH 6.8); α-galactosidase (coffee beans), Km = 2.0 mM (pH 6.8); β-galactosidase (from Escherichia coli), Km = 0.12 mM (pH 7.3); α-mannosidase (jack bean), Km = 2.0 mM (pH 5.5); β-mannosidase (Helix pomatia), Km = 0.6 mM (pH 5.5); β-N-acetylglucosaminidase (from human placenta), Km = 0.34 mM (pH 5.5); β-N-acetylglucosaminidase (from bovine kidney), Km = 0.48 mM (pH 5.5); β-N-acetylglucosaminidase (from jack bean), Km = 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 °C (or 55 °C for amylglucosidase), the reaction was quenched by addition of 1 M NaClO₄. The absorbance of the resulting mixture was determined at 405 nm. The Ki 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.
Notes and references


