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Cyanodeoxy-Glycosyl Derivatives as Substrates for Enzymatic Reactions

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Synthetic routes for the preparation of new sugar nitriles **8**–**10** derived from 2-acetamido-2-deoxy-β-D-glucopyranosides bearing a cyano group at the C-5 or C-6 position are presented. In an attempt to prepare the glycosyl azide **10** by treatment of tosylate **23** with KCN/DMF at 60 °C, an intramolecular 1,3-dipolar cycloaddition reaction occurred to give the highly constrained nonisolable tetrazole **24**, which was readily converted into the imino-azido compound **25** through an azido-tetrazole tautomerism. Compounds **8** and **10** were found to be poorer substrates of fungal β-*N*-acetylhexosaminidases than compound **9** and none of these compounds was accepted as substrates of the nitrilase or nitrile hydratase. Docking of the nitriles **8**–**10** in the active site of the β-*N*-acetylhexosaminidase from *Aspergillus oryzae* gave interaction energies comparable with the natural substrate. Based on these data, which indicate strong binding of these compounds (**8** > **9** > **10**) to the active site, it has been proposed that some cyano derivatives may act as competitive inhibitors of β-*N*-acetylhexosaminidases. This hypothesis is consistent with enzyme inhibition experiments which showed strong inhibitory properties of compound **9** (K_I = 0.37 mM) and in particular of compound **8** (K_I = 7.6 μM).

Introduction

Glycoscience covers a multifaceted range of activities and applications of carbohydrate research. Glycostructures themselves play a highly diverse and crucial role in a myriad of organisms and in important systems in biology, physiology, medicine, bioengineering and technology. Yet it is only in recent years that the tools have been developed that lead to an understanding of their highly complex functions and chemical background.

In the synthesis of complex carbohydrate structures, enzymatic methods, which are simple, selective and mild alternatives to synthetic chemistry, are often applied.[1] Glycosidases (EC, 3.2) are widely used for this purpose owing to their stability, low cost and virtually absolute stereoselectivity.[2] The main requirement of their substrates is the presence of a good leaving group, and recently, in addition to the traditional nitrophenyl glycosides,[3] glycosyl azides[4] have been demonstrated as efficient substrates.

Glycosidases are known to accept structurally modified substrates, which enables researchers to investigate various aspects of their substrate specificity. As a result, new competitive inhibitors are being found that can be used against glycosidase-induced infections in human and veterinary medicine and in agriculture.[5] Furthermore, structure–function-relationship studies form the basis for targeted mutagenesis.[6]

A large group of well-accepted structural modifications in the substrate pyranose ring are those at the C-6 position.[7] The introduction of a highly versatile functionality such as a nitrile group brings about the possibility of further modifications,[8] for example, reduction to an amino group possibly followed by conjugation with aldehydes or isothiocyanates or hydrolysis (chemical or enzymatic) to the respective carboxylic acid.

Several procedures for attaching a cyano group to different carbon atoms of the sugar backbone have been reported,[9] yielding, for example, methyl 3-cyano-3-deoxyaltropyranoside (**1**),[10] methyl 2-cyano-2-deoxy-α-D-altropyranoside (**2**),[11] and isopropyl 2-cyano-2-deoxy-α-D-glucopyranoside (**3**) (Figure 1).[12]

As for compounds carrying a cyano group at the C-5 position, several methods have been described that yield, for example, methyl α-D-galactopyranuronitrile (**4**) or methyl α-D-glucopyranuronitrile (**5**) (Figure 1).[13–15] 2,3,6-Trideoxy-6-cyano derivative **6** and the unsaturated 6-deoxy-6-cyano

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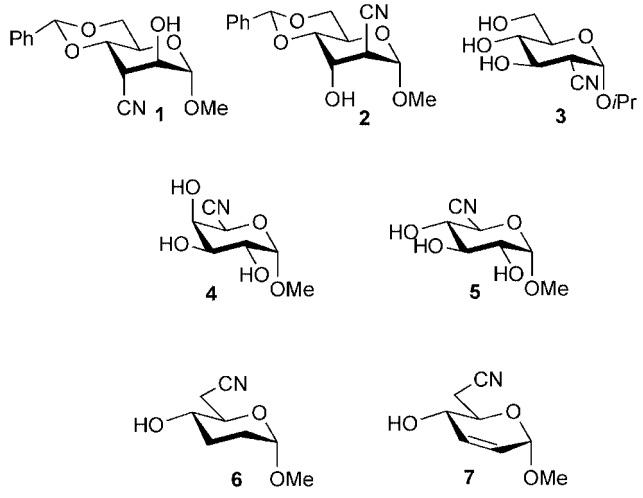


Figure 1. Cyanopyranosyl derivatives **1**–**7**.

derivative **7** were prepared by displacement of the corresponding 6-*O*-triflate with tetrabutylammonium cyanide.[16]

This paper presents the synthesis of nitriles derived from *N*-acetyl-D-glucosamine, that is, *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranosiduronitrile (**8**), *p*-nitrophenyl 2-acetamido-2,6-dideoxy-β-D-*gluco*-heptopyranosyluronononitrile (**9**) and 2-acetamido-2,6-dideoxy-β-D-*gluco*-heptopyranosyluronitrile azide (**10**) (Figure 2). Furthermore, the potential of these compounds as substrates of glycosidases and nitrilases and as inhibitors of glycosidases has been studied.

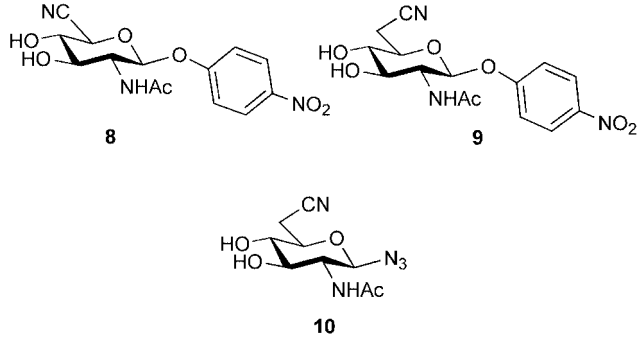


Figure 2. Glucopyranosiduronitrile **8** and *gluco*-heptopyranosyluronononitriles **9** and **10**.

Results and Discussion

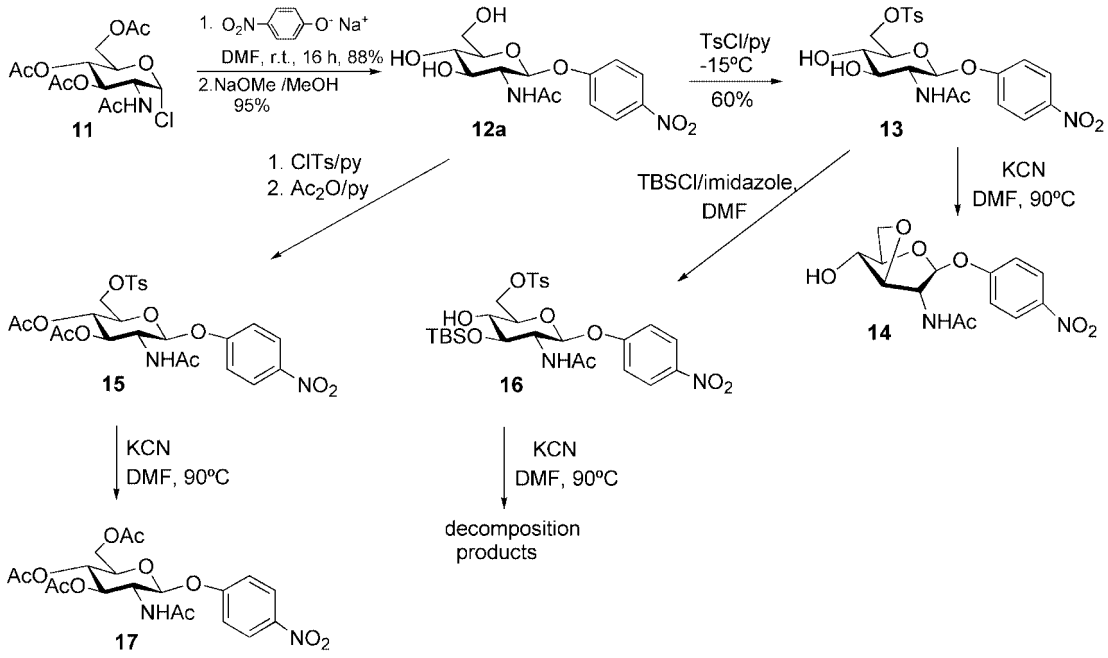
The synthesis of the *p*-nitrophenyl derivatives starts from *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**12a**),[17] which was prepared from the readily available 2-acetamido-2-deoxy-α-D-glucopyranosyl chloride (**11**) by nucleophilic displacement of the chloride with sodium *p*-nitrophenolate in DMF followed by methanolysis. The simplest route for the introduction of the cyano group at the C-6 position, which implies the regioselective introduction of a bulky leaving group at C-6, such as tosylate, followed by displacement was unsuccessful (Scheme 1). Although compound **13** was obtained in 60% yield, treatment with KCN and other cyanides in DMF did not give the desired compound. Instead the basic medium promoted an internal displacement of the tosylate by the OH group at the C-3 position to give the bicyclic compound **14**. In the ^{1}H NMR spectra of **13** and **14**, a remarkable change in the signals of 1-H (δ =5.13 ppm, d, $J_{1,2}$ = 8.4 Hz for **13** to 5.60 ppm, s for **14**) and of 2-H (δ = 3.86 ppm, dd, $J_{2,3}$ = 10.3 Hz, $J_{1,2}$ = 8.4 Hz for **13** to 4.43 ppm, d, $J_{2,3}$ = 3.0 Hz for **14**) was observed, indicating a semi-boat conformation of the pyranose ring. Protection of the hydroxy groups with acetates or TBS groups (compounds **15** and **16**, respectively) also failed to give the expected results after treatment with KCN. In the case of **15**, the *trans*-esterified peracetylated compound **17** was obtained and in the case of **16**, decomposition took place. The use of other reagents containing cyanide anion (TMSCN/TBAF, Bu_4NCN) were also unsuccessful.

To avoid internal displacements, we carried out another synthetic strategy that implies the protection of the O-3-H and O-4-H atoms in one step using a butanediacetal (BDA) protecting group. This synthetic scheme was applied to *p*-nitrophenyl and azido derivatives **12a** and **12b** (Scheme 2). The bis-acetal **18** was obtained in 78–80% yield by reaction with butanedione and methyl orthoformate applying Ley's methodology.[18] Compounds **18a** and **18b** were obtained as a mixture of diastereoisomers at the acetalic carbon atoms and were used directly in the following steps. Singlets due to 3 H atoms at δ = 3.29 and 3.22 ppm and to 6 H atoms at δ = 1.32 ppm corresponding to the OMe and Me groups, respectively, indicated the presence of the BDA protecting group.

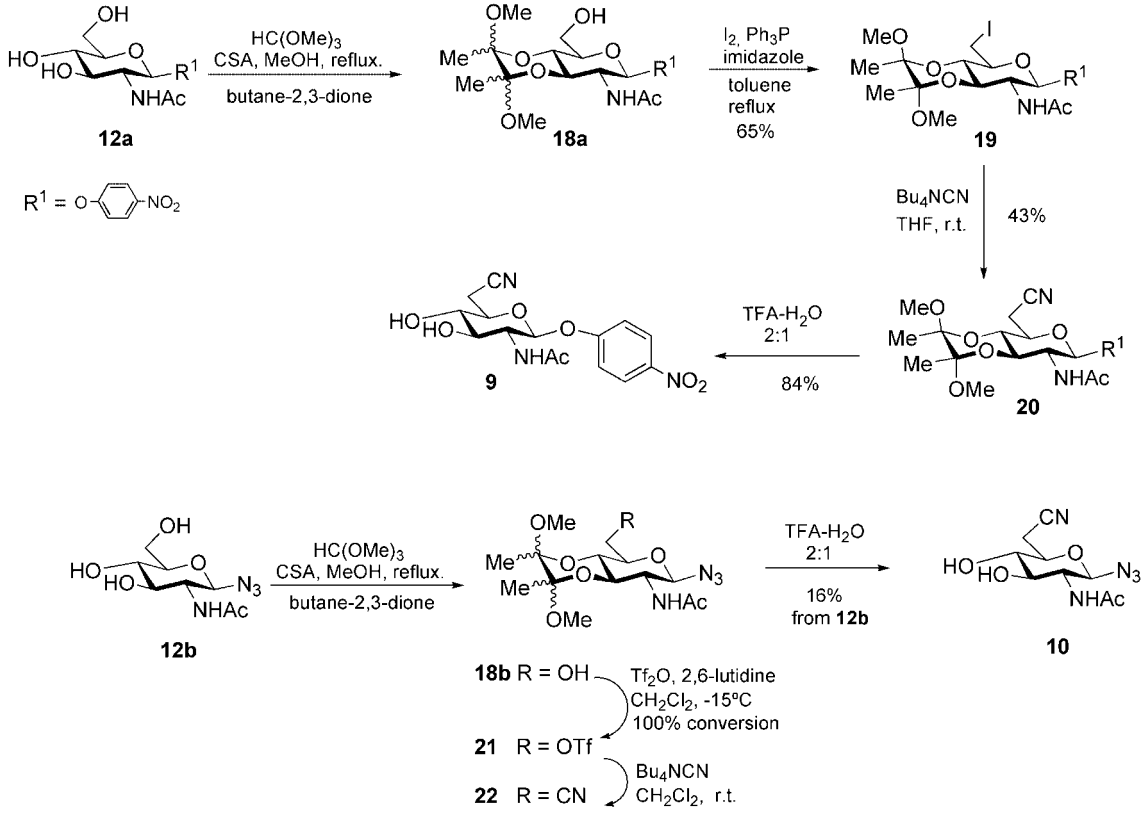
Tosylation of **18a** at the C-6 position followed by displacement with a cyanide anion led to decomposition products. However, iodination (I_2/PPh_3, 65% yield) of the mixture of *p*-nitrophenyl derivatives **18a** gave **19**, which was isolated as a single diastereoisomer. The iodo substituent in **19** was confirmed by the resonances at δ = 3.59 and 3.18 ppm for 6-H and 6′-H ($J_{6,5}$ = 2.4 Hz, $J_{6',5}$ = 8.1 Hz, $J_{6,6}$ = 10.8 Hz) in its ^{1}H NMR spectrum. Displacement with tetrabutylammonium cyanide (Bu_4NCN/THF) afforded the cyano compound **20** in 43% yield, which was finally deprotected in aqueous TFA to give **9**. In the ^{1}H NMR spectrum of **20**, deshielding of the 6-H and 6′-H signals to δ = 2.85 and 2.69 ppm with respect to the same protons as in **19** confirmed the substitution. The signal at δ = 116.2 ppm in the ^{13}C NMR spectrum of **20** verified the presence of the cyano group.

The cyano derivative **10** was obtained by 6-*O*-triflation of the bis-acetal **18b** and subsequent displacement at room temperature with tetrabutylammonium cyanide followed by acidic deprotection (15% overall yield from **12b**). Compounds **18b** were obtained from the known 2-acetamido-2-deoxy-β-D-glucopyranosyl azide **12b**[19] under BDA conditions.

With the idea of improving the yield of the preparation of the azido-derivative **10**, we changed the starting material from the highly reactive triflate **21** to the isolable tosylate **23** (Scheme 3), which was readily obtained from **18b** in 80%



Scheme 1.

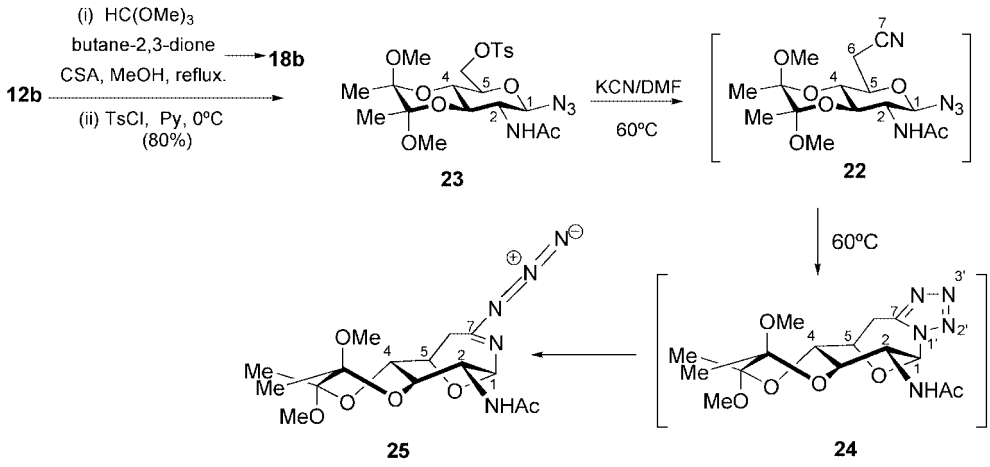


Scheme 2.

yield. At room temperature, only a partial reaction of **23** with cyanide anions occurred.

However, when the reaction was carried out with KCN/DMF at 60 °C, derivative **25** was obtained. Its formation can be explained by a 1,3-dipolar cycloaddition[20] between the 6-cyano and the 1-azido groups of **22**, which affords derivative **24**, followed by a tetrazole ring-opening[21] process that involves breakage of the N-1–N-2 bond in the tetrazole moiety. This process is highly favourable as a result of the high conformational strain of **24**.

Compound **25** exhibits a UV absorption at 274 nm, a strong band in its IR spectrum at 2191 cm^{-1} and a signal in



Scheme 3.

its ^{13}C NMR spectrum at δ = 115.7 ppm which has been assigned to the C-7 atom based upon its HSQC spectrum. The fact that the tetrazole carbon atom resonates at δ = 143–154 ppm[22] and that the tetrazole ring exhibits no IR absorption at around 2000–2200 cm^{-1} [23] confirms the tetrazole ring-opening. Additionally, hydrogenation (H_2/Pd/C, 1 atm) of **25** provokes the disappearance of the band at 2191 cm^{-1} from the IR spectrum and the UV absorption. This is further evidence of tetrazole ring-opening after the cycloaddition reaction. The ^1H NMR spectroscopic data of **25** also indicate that the sugar moiety adopts a boat conformation, which was unequivocally assigned by the change in $J_{1,2}$ and $J_{4,5}$ from 8.9 and 10.0 Hz, respectively, in **23** to $J_{1,2}$ = 0 Hz and $J_{4,5}$ = 2.3 Hz. The other coupling constants remain practically unaltered as a result of the torsional requirements of the bis-acetal.

Nitrile **8** was synthesized by oxidation of the 6-OH group in **18a** under Swern conditions to afford carbaldehyde **26** in an almost quantitative yield. The resonance at δ = 9.6 ppm in its ^1H NMR spectrum confirmed the presence of the formyl group. The nitrile group was formed under very mild conditions with iodine and ammonia in THF at room temperature.[24] This transformation took place through the imine and diiodo derivative to give the triple bond after dehydrohalogenation. Finally, deprotection with aqueous TFA yielded **8** in 93% yield (Scheme 4).

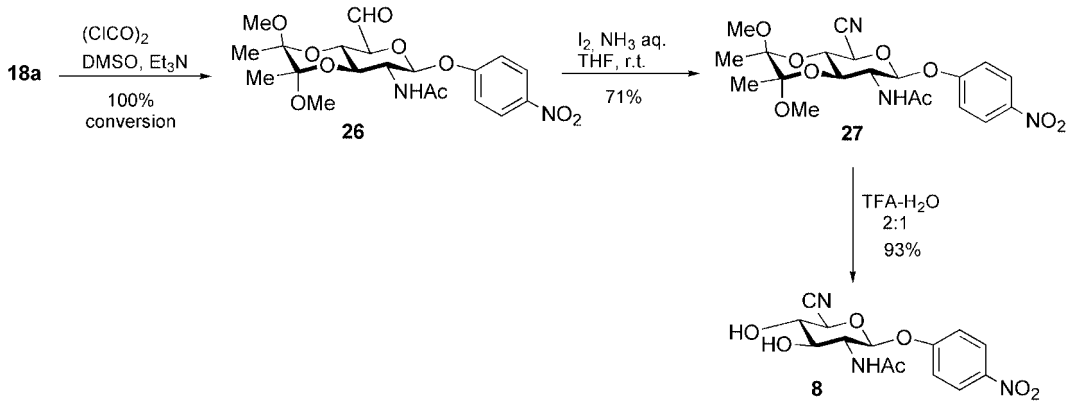
Compounds **8**–**10** were then subjected to an enzymatic hydrolysis screening comprising 33 fungal β-*N*-acetylhexosaminidases mainly from the *Aspergillus*, *Penicillium* and *Talaromyces* genera. Owing to the lack of a chromophore moiety, the hydrolysis of compound **10** was analyzed by TLC. The hydrolytic rates were measured relative to the hydrolysis of the standard substrate **12a**. None of the compounds proved to be a good substrate for the enzymes tested − the best results were obtained with compound **9** and the β-*N*-acetylhexosaminidases derived from *Talaromyces flavus* CCF 2686 (3.3% relative to **12a**), *Penicillium pittii* CCF 2277 (3.0%) and *Hamigera avellanea* CCF 2923 (2.9%). With compound **8**, *Fusarium oxysporum* CCF 377 (3.4%) proved to be the best source. In other cases, negligible or no hydrolysis was observed (lower than 1% relative to **12a**). With such a low hydrolytic potential, transglycosylation reactions are not feasible due to very high enzyme consumption. None of the compounds were accepted by nitrile-converting enzymes, a nitrile hydratase from *Rhodococcus equi* A4[25] and a nitrilase from *Aspergillus niger* K10.[26] This is not surprising in view of the previous observations that bulky nitriles are hardly accepted by these enzymes (see ref.[27] for a review).

However, compounds **8**–**10** could dock into the active site of the β-*N*-acetylhexosaminidase derived from *Aspergillus oryzae* CCF 1066, one of the most frequently studied representatives of the eukaryotic β-*N*-acetylhexosaminidases (Figure 3 and Figure 4).

The interaction energies of these compounds with the enzyme (steric and electrostatic contributions, Table 1) were compared with the corresponding substrates without the cyano group which are hydrolyzed well by the enzyme[4] (that is **8** and **9** are compared with **12a** and **10** with **12b**).

With respect to the above results from hydrolytic screening, the results of molecular modeling were rather surprising. The interaction energy of a poor substrate with this enzyme is typically lower than –150 kJ/mol.[7d] Thus, our results clearly indicate that the substrates bind well to the active site, although the sum of the steric and electrostatic energy contributions are smaller than those of the standard substrate. Azido derivatives **12b** and **10** show similar but slightly weaker binding than the nitrophenyl glycosides, which agrees with the experimental results for the hydrolysis of azides.[4] As a result, we have proposed a hypothesis that compounds **8**–**10** are competitive inhibitors of the β-*N*-acetylhexosaminidase from *A. oryzae*.

To verify this assumption, we determined the residual activities of this enzyme towards the standard substrate **12a** in the presence of different concentrations of compounds **8**–**10**. The results summarized in Table 2 as well as the inhibition studies show that compound **8** is a strong competitive inhibitor of the β-*N*-acetylhexosaminidase from *A. oryzae* (K_I = 7.6 μM with K_M for standard substrate **12a** being 0.75 mM).[4] Inhibitory properties were also demonstrated



Scheme 4.

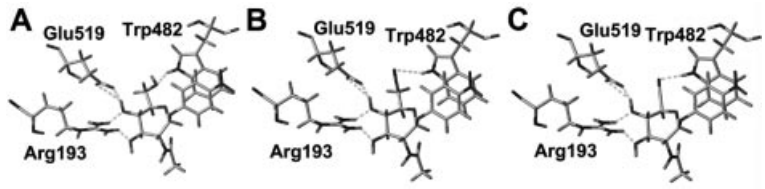


Figure 3. Substrates A) **12a**, B) **9** and C) **8** docked into the active site of the β-*N*-acetylhexosaminidase from *A. oryzae* CCF 1066. Hydrogen bonding (in green) to glutamic acid 519, arginine 193 and tryptophan 482 fixes the glycosides. The π-electron system of the aromatic ring of the *p*-nitrophenyl group is stabilized by aromatic stacking with tryptophan 482.

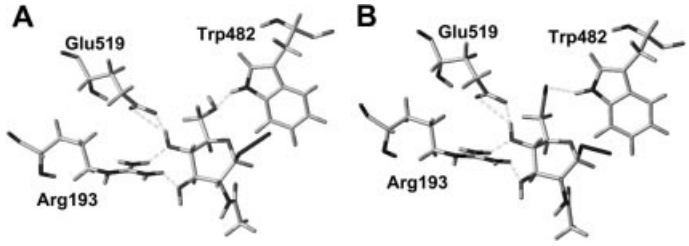


Figure 4. Substrates A) **12b** and B) **10** docked into the active site of the β-*N*-acetylhexosaminidase from *A. oryzae* CCF 1066. Hydrogen bonding (in green) to glutamic acid 519, arginine 193 and tryptophan 482 fixes the glycosides. The azido group is depicted as a blue stick.

Table 1. Interaction energies of substrates **8**–**10**, **12a** and **12b** with the β-*N*-acetylhexosaminidase isolated from *A. oryzae*.

Substrate	Interaction energy [kJ/mol][a]		
	Total	Steric	Electrostatic
12a	–300	–84	–216
12b	–257	–57	–200
8	–249	–86	–163
9	–238	–96	–142
10	–208	–65	–143

[a] Drop in the enzyme–substrate complex interaction energy reflects better binding of the substrate to the enzymatic active site.

by compound **9** (K_I = 0.37 mM). Thus, compound **8**, though a structural analogue of **9**, exhibits a 50-fold stronger inhibition effect, which can be explained by comparison of interaction energies at the enzymatic active site (Figure 1, Table 1). Additionally, the better cleavage of compound **9** in comparison with compound **8** may be influenced by a slight shift in the position of compound **8** in the active site compared with that of **12a** and **9** (the distance between the glycosidic oxygen atom and glutamic acid 519 is 2.8 Å for the latter, however, 2.9 Å for **8**; Figure 3). These results imply that modifications analogous to compound **8**, that is, conversion of C-6-OH into the CN group, could be a promising route to other glycosidase inhibitors. Compound **10** is not an inhibitor, which is probably due to weaker binding to the enzymatic active site relative to the nitrophenyl derivatives (Table 1).

Conclusions

New nitrile-substituted derivatives of 2-acetamido-2-deoxy-β-D-glucopyranosides **8**–**10** were prepared for enzymatic studies with respective glycosidases, both as potential substrates and inhibitors. A panel of 33 fungal β-*N*-acetylhexosaminidases was tested and slight cleavage by several enzymes was observed (ca. 3% hydrolytic rate relative to

Table 2. Residual activities of the β-*N*-acetylhexosaminidase from *A. oryzae* in the presence of **8**–**10**.

Inhibitor concentration [mM]	Substrate/inhibitor ratio	Residual activity towards **12a** (2 mM) [%] Compound **8**	Compound **9**	Compound **10**
0	–	100	100	100
0.2	10	16	88	100
0.4	5	10	82	100
1	2	3	60	100
2	1	1	44	100

the standard substrate **12a**). Compound **9** proved to be best accepted whereas glycosyl azide **10** was cleaved by none of the enzymes tested. Neither was hydrolysis of any of the compounds by a nitrile hydratase or a nitrilase observed, probably due to steric factors.

Molecular modeling and docking of the respective compounds into the active site of the β-*N*-acetylhexosaminidase from *A. oryzae* revealed interaction energies that suggest strong binding to the active site. Correspondingly, experiments showed competitive inhibition by compound **9** (K_I = 0.37 mM) and in particular by compound **8** (K_I = 7.6 μM). As a result, compound **8** appears to be a good lead structure for the further design of efficient glycosidase inhibitors.

An intramolecular 1,3-dipolar cycloaddition reaction was observed during the preparation of cyano-glycosyl azide **22**, giving the nonisolable tetrazole **24**, which, owing to the high conformational strain of its structure, was converted into the imino-azido compound **25** through a tetrazole/azido tautomerism.

As far as we are aware, this is the first example of this type of tautomerism in nonheteroaromatic compounds.

Experimental Section

General: Optical rotations were measured in a 1.0 cm tube with a Perkin–Elmer 241 MC spectropolarimeter. ^1H and ^{13}C NMR spectra were obtained for solutions in $CDCl_3$, [D_6]DMSO, CD_3OD and D_2O; *J* values are given in Hz and δ in ppm. All the assignments were confirmed by two-dimensional NMR experiments. The FAB mass spectra were obtained using glycerol or 3-nitrobenzyl alcohol as the matrix. TLC was performed on silica gel HF_{254} (Merck), with detection by UV light and Pancaldi reagent [$(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O]. Silica gel 60 (Merck, 230 mesh) was used for preparative chromatography. Anhydrous solvents and reagents were freshly distilled under N_2 prior to use.

Enzymes: All assays with β-*N*-acetylhexosaminidases were performed in citrate/phosphate buffer (0.05 M, pH 5.0). The fungal strains producing β-*N*-acetylhexosaminidases (EC, 3.2.1.52) originated from the Culture Collection of Fungi (CCF), Department of Botany, Charles University Prague, or from the Culture Collection of the Institute of Microbiology (CCIM), Prague, and were cultivated as described previously.[28] The screening comprised 33 enzymes: *Acremonium persicinum* CCF 1850, *Aspergillus awamori* CCF 763, *A. flavipes* CCF 1895, *A. flavipes* CCF 3067, *A. flavofurcatis* CCF 3061, *A. flavus* CCF 3056, *A. niger* CCIM K2, *A. niveus* CCF 3057, *A. nomius* CCF 3086, *A. oryzae* CCF 147, *A. oryzae* CCF 1066, *A. parasiticus* CCF 1298, *A. sojae* CCF 3060, *A. tamarii* CCF 3085, *A. terreus* CCF 2539, *A. terreus* USA, *Fusarium oxysporum* CCF 377, *Hamigera avellanea* CCF 2923, *Chaetomium globosum* CCF 430, *Penicillium brasilianum* CCF 2155, *P. brasilianum* CCF 2171, *P. chrysogenum* CCF 1269, *P. funiculosum* CCF 2985, *P. multicolor* CCF 2244, *P. oxalicum* CCF 1959, *P. oxalicum* CCF 2315, *P. oxalicum* CCF 2430, *P. pittii* CCF 2277, *P. spinulosum* CCF 2159, *Talaromyces flavus* CCF 2573, *T. flavus* CCF 2686, *T. ohiensis* CCF 2229, *Trichoderma harzianum* CCF 2687.

All assays with the nitrile hydratase from *Rhodococcus equi* A4 were performed in Na/K phosphate buffer (0.05 M, pH 7.5) with 0.5 mM substrate. The enzyme was prepared and purified as described previously.[28]

Activity Assay for β-*N*-Acetylhexosaminidases:[29] The reaction mixture containing compound **8**, **9** or the standard substrate **12a** (2 mM, starting concentration) and β-*N*-acetylhexosaminidase (0.15–0.3 U/mL for **8** and **9**; 0.01–0.02 U/mL for **12a**) in buffer (assay volume 50 μL) was incubated in microplates at 35 °C for 10 min. The reaction was stopped by adding aq. Na_2CO_3 (0.1 M, 150 μL). Liberated *p*-nitrophenol was determined spectrophotometrically (414 nm) on Titertek Multiscan® MCC/340 (Flow Laboratories, McLean, U.S.A.) microplate reader. One unit of enzymatic activity is defined as the amount of enzyme that releases 1 μmol of *p*-nitrophenol per minute under the above conditions. Enzymes were classified according to the ratio of hydrolysis rates of compound **8** or **9** and of the standard substrate **12a**, extrapolated to the same amount of enzyme. The activity towards compound **10** was estimated as follows. Compound **10** or the standard substrate **12a** (10 mM, starting concentration) and β-*N*-acetylhexosaminidase (1.79 U/mL for **10**, 0.09 U/mL for the standard substrate) in buffer were incubated at 35 °C under shaking. Samples were taken at regular intervals and analyzed by TLC (2-propanol/H_2O/aq. NH_3, 7:2:1). The spots were visualized by UV light and by charring with 5% sulfuric acid in ethanol. Enzymes were classified according to the ratio of total hydrolysis times for compound **10** and the standard substrate **12a**, extrapolated to the same amount of enzyme.

Inhibition Assay for β-*N*-Acetylhexosaminidases: Residual enzymatic activities in the presence of compounds **8**–**10** were determined as follows. The reaction mixture containing the standard substrate **12a** (2 mM), compound **8**, **9** or **10** (0–3 mM) and the β-*N*-acetylhexosaminidase from *A. oryzae* CCF 1066 (0.11 U/mL) was incubated at 35 °C under shaking. Samples were taken, quenched by adding aq. Na_2CO_3 (0.1 M) and analyzed spectrophotometrically as above. The residual activity is defined as the ratio of initial rates of standard substrate hydrolysis in the presence and absence of inhibitor **8**, **9** or **10**. Kinetic constants (K_M, K_I, V_{max}) were determined from initial reaction rates at different starting concentrations of the standard substrate **12a** (0.5–2.0 mM) in the presence of inhibitor **8** or **10** (0–3 mM) using SigmaPlot 2001 (SPSS Science, U.S.A.). Hydrolysis rates were determined as described above.

Activity Assay for Nitrile Hydratase: The reaction mixture containing 0.5 mM of compounds **8**–**10** and the purified nitrile hydratase from *Rhodococcus equi* A4[25] (0.06 mg of protein/mL, that is, approx. 3 U/mL, as assayed with benzonitrile) in 50 mM Na/K phosphate buffer, pH 7.5, (assay volume 0.5 mL) was incubated at 28 °C with shaking. At intervals (1, 3, 5 and 20 h) 0.05 mL of the reaction

mixture was withdrawn, mixed with 0.05 mL methanol and centrifuged. The supernatants were analyzed by HPLC using a system which consisted of the solvent delivery system 600 and the PDA detector 996 (Waters) and the Nova-Pak C_{18} column (5 μm, 3.9 × 150 mm; Waters). Nitriles **8** and **9** were eluted with a mobile phase consisting of 25% (v/v) acetonitrile and 0.1% (v/v) H_3PO_4 at a flow rate of 0.9 mL/min and detected at 296 nm. Nitrile **9** was eluted with 10% (v/v) acetonitrile and 0.1% (v/v) H_3PO_4 at the same flow rate and detected at 210 nm. Nitriles **8**–**10** remained unreacted.

Activity Assay for Nitrilase: The reaction mixture containing 0.5 mM of compounds **8**–**10** and the cell-free extract from *Aspergillus niger* A4 possessing nitrilase activity [26] (1 mg of protein/mL, that is, approx. 2.8 U/mL, as assayed with benzonitrile) in 50 mM Tris/HCl buffer, pH 8.0 (assay volume 0.5 mL), was incubated at 35 °C with shaking. Work up of the samples and HPLC analysis were performed as described for the nitrile hydratase assay. The unreacted nitriles **8**, **9** and **10** but no reaction products were detected.

Molecular Modeling: The primary sequence of the β-*N*-acetylhexosaminidase from *A. oryzae* CCF 1066 was aligned with the known X-ray structures of the β-*N*-acetylhexosaminidases from *Serratia marcescens* and *Streptomyces plicatus*, extracted from the Brookhaven Protein Database (PDB entry: 1QBA and 1HP4, respectively). The sequence data are available from the DDBJ/EMBL/GeneBank databases (http://www.ncbi.nlm.nih.gov/) under the access number AY091636. Three-dimensional models were generated using the Modeller6 package.[30] For model refinement and minimization, the SYBYL package with the TRIPOS force field (TRIPOS Associates Inc.) was used. The complete modeling, including the alignment and energy minimization, was performed exactly as described previously.[3b] The docking of ligands was performed as described earlier.[3b] The positioning of the ligands in the arbitrary site was carried out using the DOCK module included in SYBYL/MAXIMIN2, which calculates interaction energies based on steric contributions from the TRIPOS force field and on electrostatic contributions from any atomic charges present in the ligand. Exact positioning of the ligand was achieved by a two-step procedure, that is, energy minimization followed by molecular dynamics, in exactly the same manner for all ligands described. The ligand–protein system was minimized by 1000 interactions with the Powell minimizer and the TRIPOS force field including electrostatic interactions based on Gasteiger–Hückel partial charge distributions using a dielectric constant with a distance-dependent function $\varepsilon = 4r$ and a nonbonded interaction cut off of 8 Å. A molecular dynamics simulation at 290 K followed the minimization with the NTV ensemble over 15 ps. The resulting structure was then minimized with the same parameters as above to a convergence of the energy gradient less than 0.04 kJ/mol. The nonbinding interaction energy between the model and the ligands within the optimized complex was calculated using the TRIPOS force field. This estimation of real interaction energy neglects solvation and desolvation effects.

***p*-Nitrophenyl 2-Acetamido-2,6-dideoxy-6-iodo-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-β-D-glucopyranoside (19):** *p*-Nitrophenyl β-D-glucopyranoside **12a**[17] (1.5 g, 4.38 mmol), butane-2,3-dione (1.91 mL, 21.9 mmol), $CH(OMe)_3$ (7.2 mL, 65.7 mmol) and CSA (274 mg, 1.18 mmol) were stirred under reflux in MeOH (50 mL) for 6 h. Et_3N was added and the solution concentrated. Column chromatography on silica gel (CH_2Cl_2/MeOH, 35:1) afforded *p*-nitrophenyl 2-acetamido-2-deoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-β-D-glucopyranoside (**18a**) (1.58 g, 79%) as a solid, which was used directly in the next step. A mixture of **18a** (270 mg, 0.592 mmol), triphenylphosphane (435 mg, 1.660 mmol), imidazole (113 mg, 1.660 mmol) and iodine (281 mg, 1.106 mmol) in toluene (20 mL) was stirred under reflux for 2 h. The reaction mixture was cooled, an equal volume of saturated aqueous sodium hydrogencarbonate was added and the mixture was stirred for 5 min. Iodine was added in portions until the toluene phase remained iodine-colored. It was then stirred for an additional 10 min and then the excess of iodine was removed by addition of aqueous thiosulfate. The mixture was transferred to a separating funnel; the organic layer was diluted with toluene, and, after separation, the toluene layer was washed with water, dried, filtered and concentrated. Triphenylphosphane oxide was then precipitated in diethyl ether and the filtrate concentrated. The residue was purified by column chromatography on silica gel (diethyl ether/petroleum ether, 5:1) to give **19** (218 mg, 65%). $[\alpha]_D^{28} = +28$ ($c = 0.75$, CH_2Cl_2). IR: $\tilde{\nu}_{max}$ = 3264, 3086, 165, 1524, 1344, 1111, 1071, 1036, 887, 745, 596 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.21–8.17 (m, 2 H, Ph), 7.21–7.16 (m, 2 H, Ph), 5.88 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1-H), 5.70 (d, $J_{NH,2}$ = 6.9 Hz, 1 H, NH), 4.55 (dd, $J_{3,2}$ = 10.8, $J_{3,4}$ = 9.6 Hz, 1 H, 3-H), 3.71 (m, 1 H, 5-H), 3.59 (dd, $J_{6,5}$ = 2.4, $^2J_{6,6'}$ = 10.8 Hz, 1 H, 6-H), 3.50 (t, $J_{4,5}$ = 9.6 Hz, 1 H, 4-H), 3.48 (m, 1 H, 2-H), 3.18 (dd, $J_{6',5}$ = 8.1 Hz, $J_{6,6'}$ = 10.8 Hz, 1 H, 6′-H), 3.29 and 3.22 (2s, 3 H each, OMe), 1.98 (s, 3 H, CH_3CO), 1.32 (s, 6 H, CH_3) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 170.5 (C=O), 161.4 (C-1 Ph), 142.8 (C-4 Ph), 125.6, 116.8 (Ph), 99.9, 99.8 (C_q BDA), 96.9 (C-1), 70.0 (C-5), 69.7 (C-4), 66.2 (C-3), 56.2 (C-2), 48.3, 47.8 (2 OMe), 20.2 (C-6), 23.4 (NHCOCH_3), 17.5, 17.4 (2 CH_3) ppm. FABMS: *m*/*z* (%) = 589 (100) $[M + Na]^+$, 535 (70) $[M - OMe]^+$. CIMSHR: calcd. for $C_{20}H_{27}N_2O_9I$ + H: 567.0840; found 567.0795.

***p*-Nitrophenyl 2-Acetamido-2,6-dideoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-β-D-*gluco*-heptopyranosyluronononitrile (20):** Tetrabutylammonium cyanide (135 mg, 0.504 mmol) dissolved in THF (1 mL) was added to a stirred solution of **19** (190 mg, 0.336 mmol) in dry THF (3 mL). The mixture was stirred at room temp. for 16 h and then concentrated. The residue was dissolved in CH_2Cl_2, washed with sodium hypochlorite and water, dried (Na_2SO_4) and the solvents evaporated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 25:1→18:1) to give **20** (67 mg, 43%). $[\alpha]_D^{28} = +25$ ($c = 0.8$, CH_2Cl_2). IR: $\tilde{\nu}_{max}$ = 3268, 3082, 1661, 1593, 1346, 1113, 1071, 1042, 889, 746 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.22–8.17 (m, 2 H, Ph), 7.20–7.07 (m, 2 H, Ph), 5.96 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1-H), 5.82 (d, $J_{NH,2}$ = 7.1 Hz, 1 H, NH), 4.58 (dd, $J_{3,4}$ = 9.7, $J_{3,2}$ = 11.0 Hz, 1 H, 3-H), 3.94 (ddd, $J_{5,6}$ = 3.4 Hz, $J_{5,6'}$ = 6.5 Hz, $J_{5,4}$ = 9.7 Hz, 1 H, 5-H), 3.62 (t, $J_{4,5}$ = 9.7 Hz, 1 H, 4-H), 3.47 (ddd, $J_{2,NH}$ = 7.1 Hz, $J_{2,1}$ = 8.0 Hz, $J_{2,3}$ = 11.0 Hz, 1 H, 2-H), 3.29 and 3.22 (2s, 3 H each, OMe), 2.85 (dd, $^2J_{6,6'}$ = 16.9, $J_{6,5}$ = 3.4 Hz, 1 H, 6-H), 2.69 (dd, $J_{6',5}$ = 6.5 Hz, $J_{6',6}$ = 16.9 Hz, 1 H, 6′-H), 1.98 (s, 3 H, MeCO), 1.34 and 1.33 (2s, 3 H each, Me) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 170.9 (C=O), 161.6 (C-1 Ph), 143.1 (C-4 Ph), 125.9, 116.8 (Ph), 116.2 (CN), 100.2, 100.1 (C_q BDA), 97.0 (C-1), 70.0 (C-5), 69.7 (C-4), 66.2 (C-3), 56.2 (C-2), 48.4, 48.1 (2 OMe), 23.6 (NHCOCH_3), 20.2 (C-6), 17.7, 17.6 (2 CH_3) ppm. FABMS: *m*/*z* = 488 (30) $[M + Na]^+$. CIMSHR: calcd. for $C_{21}H_{27}N_3O_9$ + H: 466.1826; found 466.1802.

***p*-Nitrophenyl 2-Acetamido-2,6-dideoxy-β-D-*gluco*-heptopyranosyluronononitrile (9):** Compound **20** (50 mg, 0.108 mmol) was stirred in a solution of aqueous TFA (2:1) (2 mL) for 2 h at 40 °C. The mixture was concentrated and the residue purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 12:1) to give **9** (32 mg, 84%) as a white solid. $[\alpha]_D^{28} = -57$ ($c = 0.5$, MeOH). ^1H NMR (300 MHz, MeOD): δ = 8.24–8.18 (m, 2 H, Ph), 7.24–7.17 (m, 2 H, Ph), 5.28 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1-H), 3.95 (dd, $J_{2,3}$ = 10.4 Hz, $J_{2,1}$ = 8.5 Hz,

1 H, 2-H), 3.76 (ddd, $J_{5,6}$ = 3.3 Hz, $J_{5,6'}$ = 7.4 Hz, $J_{5,4}$ = 9.7 Hz, 1 H, 5-H), 3.61 (dd, $J_{3,4}$ = 8.7 Hz, $J_{3,2}$ = 10.4 Hz, 1 H, 3-H), 3.33 (dd, $J_{4,3}$ = 8.7 Hz, $J_{4,5}$ = 9.7 Hz, 1 H, 4-H), 3.01 (dd, $J_{6,5}$ = 3.3, $^2J_{6,6'}$ = 17.1 Hz, 1 H, 6-H), 2.80 (dd, $J_{6',5}$ = 7.4 Hz, 1 H, 6′-H), 1.97 (s, 3 H, Me-CO) ppm. ^{13}C NMR (75.4 MHz, MeOD): δ = 173.9 (C=O), 163.4 (C-1 Ph), 144.2 (C-4 Ph), 126.7, 117.7 (Ph), 118.5 (CN), 99.6 (C-1), 74.9 (C-3), 74.5 (C-4), 73.3 (C-5), 57.1 (C-2), 22.9 (NHCO*C*H$_3$), 21.4 (C-6) ppm. CIHRMS: calcd. for $C_{15}H_{17}N_3O_7$ + H: 352.1145; found 352.1173.

2-Acetamido-2,6-dideoxy-β-D-*gluco*-heptopyranosylurononitrile Azide (10): Compound **18b** (762 mg, 2.12 mmol) was suspended in dry CH_2Cl_2 (40 mL) under Ar. The reaction mixture was cooled to –20 °C and 2,6-lutidine (0.626 mL, 5.29 mmol) was added dropwise, followed by triflic acid anhydride (1.071 mL, 6.35 mmol). The reaction mixture was stirred at –15 °C and monitored by TLC (CH_2Cl_2/MeOH, 12:1). After 4 h, the reaction was quenched by adding sat. aq. $NaHCO_3$ (40 mL), then CH_2Cl_2 (50 mL) was added and the organic phase was separated. The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases were washed with water. All washing procedures were completed within 15 min at 0 °C. The organic phase was dried with Na_2SO_4 and the solvents evaporated under vacuum. The crude 2-acetamido-2-deoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-6-*O*-trifluoromethylsulfonyl-β-D-glucopyranosyl azide (**21**) was immediately used in the next step without further purification.

The crude compound **21** was dissolved in dry CH_2Cl_2 (10 mL) under argon and a solution of Bu_4NCN (1.7 g, 6.33 mmol) in dry CH_2Cl_2 (9 mL) was added at 0 °C whilst stirring. The reaction mixture was allowed to react for 4 h at room temp. Then the reaction mixture was diluted with CH_2Cl_2 (80 mL) and the organic phase was washed with $NaClO_4$ (2×40 mL) and water, dried (Na_2SO_4) and the solvent evaporated under vacuum to dryness to give 2-acetamido-6-cyano-2,6-dideoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-β-D-glucopyranosyl azide (**22**) which was used without purification in the next step.

Compound **22** (506 mg, 1.37 mmol) was dissolved in TFA/H_2O, 2:1 (20 mL) at 0 °C and stirred at room temp. for 5 h (TLC: CH_2Cl_2/MeOH, 8:1). The solvent was evaporated under vacuum. Purification by flash chromatography on silica gel (CH_2Cl_2/MeOH, 10:1) afforded **10** (75 mg, 0.294 mmol; 16% overall from **18b**). $[\alpha]_D^{28}$ = –17.9 (*c* = 0.67, MeOH). IR: $\tilde{\nu}_{max}$ = 3407, 2122, 1649, 1559, 1377, 1202, 1130, 1071, 1020, 721 cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ = 4.62 (d, $J_{1,2}$ = 9.3 Hz, 1 H, 1-H), 3.72 (dd, $J_{2,3}$ = 10.2 Hz, 1 H, 2-H), 3.62 (ddd, $J_{5,4}$ = 9.0, $J_{5,6}$ = 3.6, $J_{5,6'}$ = 6.3 Hz, 1 H, 5-H), 3.50 (dd, $J_{3,4}$ = 8.7 Hz, 1 H, 3-H), 3.61 (t, 1 H, 4-H), 3.02 (dd, $^2J_{6,6'}$ = 17.1 Hz, 1 H, 6-H), 2.86 (dd, 1 H, 6′-H), 2.02 (s, 3 H, Me-CO) ppm. ^{13}C NMR (75.4 MHz, MeOD): δ = 174.1 (C=O), 118.4 (CN), 90.2 (C-1), 75.4 (C-3), 75.1 (C-5), 74.6 (C-4), 56.9 (C-2), 23.1 (NHCO*C*H$_3$), 21.6 (C-6) ppm. CIHRMS: calcd. for $C_9H_{13}N_5O_4$ + H: 256.1046; found 256.1060.

2-Acetamido-2-deoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-6-*O*-tosyl-β-D-glucopyranosyl Azide (23): 2-Acetamido-2-deoxy-β-D-glucopyranosyl azide[19] (**12b**) (1 g, 4.06 mmol) was dissolved in dry methanol (46 mL) and butane-2,3-dione (1.77 mL; 20.3 mmol), $CH(OMe)_3$ (6.67 mL, 60.9 mmol) and CSA (255 mg; 1.096 mmol) were added under argon. The reaction mixture was refluxed for 8 h and then concentrated under vacuum to dryness. The crude product, 2-acetamido-2-deoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-β-D-glucopyranosyl azide (**18b**), was used without purification in the next step.

A solution of alcohol **18b** (350 mg, 0.97 mmol) in dry pyridine (3 mL) was cooled to 0 °C and a solution of TsCl (277 mg, 1.46 mmol) in dry pyridine (3 mL) was added dropwise. The mixture was stirred at room temp. for 2 h. Then H_2O (0.5 mL) was added, the solution stirred for 15 min and the mixture concentrated under vacuum. The corresponding residue was dissolved in dichloromethane and washed with HCl (1 M), sat. aq. $NaHCO_3$ and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The corresponding residue was purified by column chromatography (diethyl ether) to give **23** (399 mg, 80%) as a white foam. $[\alpha]_D^{28}$ = +25 (*c* = = 0.8, CH_2Cl_2). IR: $\tilde{\nu}_{max}$ = 3268, 3082, 1661, 1593, 1346, 1113, 1071, 1042, 889, 746 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.82 (m, 2 H, Ph), 7.36 (m, 2 H, Ph), 5.59 (br. d, $J_{NH,2}$ = 7.5 Hz, 1 H, NH), 5.18 (d, $J_{1,2}$ = 8.9 Hz, 1 H, 1-H), 4.32 (dd, $J_{3,4}$ = 9.7, $J_{3,2}$ = 10.9 Hz, 1 H, 3-H), 4.27 (d, $J_{6,5}$ = 3.1 Hz, 1 H, 6-H), 3.76 (dt, $J_{4,5}$ = 10.0 Hz, 1 H, 5-H), 3.62 (t, 1 H, 4-H), 3.26 and 3.18 (2s, 3 H each, OMe), 3.15 (ddd, 1 H, 2-H), 2.46 (s, 3 H, CH_3 of tosyl), 2.00 (s, 3 H, Me-CO), 1.29 (2s, 3 H each, Me) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 170.7 (C=O), 145.1 (C-1 of Ph), 132.5 (C-4 of Ph), 129.8, 128.0 (Ph), 100.0, 99.8 (C_q BDA), 87.4 (C-1), 73.4 (C-5), 67.2, 67.1 (C-3, C-6), 65.9 (C-4), 55.2 (C-2), 48.3, 48.0 (2 OMe), 23.5 (NHCO*C*H$_3$), 21.7 (CH_3 of tosyl), 17.6, 17.5 (2 CH_3) ppm.

(1*R*,2*R*,4*R*,5*R*,7*R*,8*R*,9*R*)-*N*-(11-Azido-4,5-dimethoxy-4,5-dimethyl-3,6,13-trioxa-10-azatricyclo[7.3.1.0^{2,7}]tridec-10-en-8-yl)acetamide (25): KCN (160 mg, 2.46 mmol) was added in one portion to a stirred solution of **23** (350 mg, 0.681 mmol) in DMF (10 mL). The mixture was stirred at 60 °C for 2 h. Then the solution was concentrated under vacuum and the resulting residue was purified by column chromatography (diethyl ether/acetone, 20:1) to give **25** (145 mg, 58%) as a white solid. $[\alpha]_D^{28}$ = +25 (*c* = 0.8, CH_2Cl_2). IR: $\tilde{\nu}_{max}$ = 3289, 2949, 2191, 1665, 1549, 1425, 1140 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.77 (br. d, $J_{NH,2}$ = 5.1 Hz, 1 H, NH), 5.71 (s, 1 H, 1-H), 4.73 (dd, $J_{5,4}$ = 2.3, $J_{5,6b}$ = 5.2 Hz, 1 H, 5-H), 3.98 (dd, $J_{3,4}$ = 9.0, $J_{3,2}$ = 9.9 Hz, 1 H, 3-H), 3.92 (dd, 1 H, 2-H), 3.74 (d, 1 H, 6a-H), 3.64 (dd, 1 H, 4-H), 3.54 (dd, $^2J_{6b,5}$ = 12.6, 1 H, 6b-H), 3.30 and 3.25 (2s, 3 H each, OMe), 2.10 (s, 3 H, Me-CO), 1.33 and 1.30 (2s, 3 H each, Me) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 170.4 (C=O), 100.7, (C_q BDA), 93.2 (C-1), 76.4 (C-5), 71.6 (C-4), 66.4 (C-3), 54.6 (C-2), 52.8 (C-6), 48.1 (2 OMe), 23.2 (NHCO*C*H$_3$), 17.6 (2 CH_3) ppm. FABMS: *m*/*z* = 488 (30) [M + Na]$^+$. CIMSHR: calcd. for $C_{21}H_{27}N_3O_9$ + H: 466.1826; found 466.1802.

***p*-Nitrophenyl 2-Acetamido-2-deoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-β-D-glucopyranosiduronitrile (27):** A solution of DMSO (121 μL, 1.71 mmol) in anhydrous CH_2Cl_2 (0.5 mL) was added dropwise to a stirred solution of oxalyl chloride (75 μL, 0.855 mmol) in anhydrous CH_2Cl_2 (2 mL) at –70 °C. The mixture was stirred at –70 °C for 15 min and then a solution of alcohol **18a** (300 mg, 0.658 mmol) in anhydrous CH_2Cl_2 (2 mL) was added dropwise. The mixture was stirred for 1 h at –70 °C and then Et_3N (0.5 mL, 3.29 mmol) was added. The mixture was allowed to reach room temp. and then was diluted with CH_2Cl_2 and washed with water and brine. The organic phase was dried (Na_2SO_4) and evaporated to give crude *p*-nitrophenyl 2-acetamido-2-deoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-β-D-*gluco*-hexodialdo-1,5-pyranoside (**26**), which was used in the following step without further purification. CIMSHR: *m*/*z* = 455.1688 (calcd. for $C_{20}H_{26}N_2O_{10}$ + H: 455.1666).

Iodine (31 mg, 0.121 mmol) was added to a solution of aldehyde **26** (50 mg, 0.110 mmol) in a solution of ammonia (1.1 mL) and THF (0.15 mL). The mixture was stirred at room temp. for 5 h and then a 5% aqueous solution of $Na_2S_2O_3$ was added and the mixture was stirred for 10 min. The mixture was extracted with CH_2Cl_2

(3 × 15 mL) and the combined organic phases were then dried and the solvents evaporated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 50:1) to give **27** (35 mg, 71%). $[\alpha]_D^{25}$ = +25 (*c* = 1, CH_2Cl_2). IR: $\tilde{\nu}_{max}$ = 3283, 3082, 1667, 1593, 1346, 1111, 1067, 1058, 887, 745 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.22–8.20 (m, 2 H, Ph), 7.12–7.10 (m, 2 H, Ph), 5.96 (d, $J_{1,2}$ = 6.9 Hz, 1 H, 1-H), 5.76 (d, $J_{NH,2}$ = 6.9 Hz, 1 H, NH), 4.51 (d, $J_{5,4}$ = 10.2 Hz, 1 H, 5-H), 4.48 (t, $J_{4,3}$ = 10.3 Hz, 1 H, 4-H), 3.97 (t, $J_{3,2}$ = 9.9 Hz,1 H, 3-H), 3.48 (dt, 1 H, 2-H), 3.35 and 3.23 (2s, 3 H each, OMe), 2.00 (s, 3 H, Me-CO), 1.35 and 1.34 (2s, 3 H each, Me) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 171.1 (C=O), 161.2 (C-1 Ph), 143.7 (C-4 Ph), 126.0, 117.1 (Ph), 115.2 (CN), 100.6, 100.5 (C_q BDA), 98.3 (C-1), 68.9 (C-3), 66.0 (C-4), 63.5 (C-5), 56.2 (C-2), 48.5, 48.3 (2 OMe), 23.6 (NHCO*C*H_3), 17.7, 17.5 (2 CH_3) ppm. FABMS: *m*/*z* = 474 (40) $[M + Na]^+$, 420 (80) $[M - OMe]^+$. CIMSHR: calcd. for $C_{20}H_{25}N_3O_9$ + H: 452.1669; found 452.1660.

***p*-Nitrophenyl 2-Acetamido-2-deoxy-β-D-glucopyranosiduronitrile (8):** Compound **27** (33 mg, 0.073 mmol) was stirred in a solution of TFA/H_2O, 2:1 (1.5 mL) for 3 h at 40 °C. The mixture was concentrated to give pure **8** (23 mg, 93%) as a white solid. $[\alpha]_D^{25}$ = –64 (*c* = 0.45, MeOH). ^1H NMR (300 MHz, MeOD): δ = 8.25–8.20 (m, 2 H, Ph), 7.25–7.16 (m, 2 H, Ph), 4.58 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1-H), 4.58 (d, $J_{5,4}$ = 8.7 Hz, 1 H, 5-H), 3.94 (dd, $J_{2,3}$ = 9.6 Hz, 1 H, 2-H), 3.68 (dd, $J_{4,3}$ = 8.7 Hz, 1 H, 4-H), 3.61 (dd, 1 H, 3-H), 1.97 (s, 3 H, CH_3CO) ppm. ^{13}C NMR (75.4 MHz, MeOD): δ = 173.9 (C=O), 163.0 (C-1 Ph), 144.7 (C-4 Ph), 126.7, 117.9 (Ph), 117.5 (CN), 100.0 (C-1), 74.0, 73.4 (C-3, C-4), 67.0 (C-5), 56.9 (C-2), 22.8 (NHCO*C*H_3) ppm. CIMSHR: calcd. for $C_{14}H_{15}N_3O_7$ + H: 338.0988; found 338.0978.

Supporting Information (for details see the footnote on the first page of this article): ^{13}C NMR spectra for compounds **8**–**10**, **19**, **20**, **23**, **25** and **27**.

Acknowledgments

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