Cyanodeoxy-Glycosyl Derivatives as Substrates for Enzymatic Reactions


Keywords: Sugar nitriles / Azido-tetrazole tautomerism / Glycosidase / Inhibitors / Enzymes / Carbohydrates

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 (Kᵢ = 0.37 mM) and in particular of compound 8 (Kᵢ = 7.6 µM).

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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 nitrophenyloxyl 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...
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 1H NMR spectra of 13 and 14, a remarkable change in the signals of 1-H (δ = 5.13 ppm, d, J = 2.4 Hz for 13 to 5.60 ppm, s for 14) and of 2-H (δ = 3.86 ppm, dd, J = 10.3 Hz, J = 8.4 Hz for 13 to 4.43 ppm, d, J = 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 peracylated compound 17 was obtained and in the case of 16, decomposition took place. The use of other reagents containing cyanide anion (TMSCN/TBAF, Bu4NCN) 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 butanediol 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 (I2/PPh3, 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 (J6,6′ = 2.85 Hz, J6,5 = 8.1 Hz, J5,6′ = 10.8 Hz) in its 1H NMR spectrum. Displacement with tetrahydrofuran cyanide (Bu4NCN/TFA) afforded the cyano compound 20 in 43% yield, which was finally deprotected in aqueous TFA to give 9. In the 1H 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 13C 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%
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
its $^{13}$C NMR spectrum at $\delta = 115.7$ ppm which has been assigned to the C-7 atom based on its HSQC spectrum. The fact that the tetrazole carbon atom resonates at $\delta = 143$–154 ppm[28] and that the tetrazole ring exhibits no IR absorption at around 2000–2200 cm$^{-1}$[29] 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 $^1$H 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 $\delta = 9.6$ ppm in its $^1$H 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 diodo 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 $\beta$-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 $\beta$-N-acetylhexosaminidases derived from Talaromyces flavus CCF 2686 (3.3% relative to 12a), Penicillium pittii CCF 2277 (3.0%) and Hanigera avelanea 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 Rhododendron equi A4[29] and a nitratase from Aspergillus niger K10.[126] 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 $\beta$-N-acetylhexosaminidase derived from Aspergillus oryzae CCF 1066, one of the most frequently studied representatives of the eukaryotic $\beta$-N-acetylhexosaminidases (Figure 3 and Figure 4).

The interaction energies of these compounds with the enzyme (stereic 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 $\sim 150$ kJ/mol.[24] 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 $\beta$-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 $\beta$-N-acetylhexosaminidase from A. oryzae ($K_I = 7.6$ µM with $K_S$ for standard substrate 12a being 0.75 mM). Inhibitory properties were also demonstrated.
Figure 3. Substrates A) 12a, B) 9 and C) 8 docked into the active site of the \(\beta\)-N-acetylhexosaminidase from \textit{A. oryzae} CCF 1066. Hydrogen bonding (in green) to glutamic acid 519, arginine 193 and tryptophan 482 fixes the glycosides. The \(\pi\)-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 \(\beta\)-N-acetylhexosaminidase from \textit{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 \(\beta\)-N-acetylhexosaminidase isolated from \textit{A. oryzae}.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Interaction energy [kJ/mol][a]</th>
<th>Total</th>
<th>Steric</th>
<th>Electrostatic</th>
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<tr>
<td>12a</td>
<td>–300</td>
<td>–84</td>
<td>–216</td>
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<tr>
<td>12b</td>
<td>–257</td>
<td>–57</td>
<td>–200</td>
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<td>8</td>
<td>–249</td>
<td>–86</td>
<td>–163</td>
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<td>9</td>
<td>–238</td>
<td>–96</td>
<td>–142</td>
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<tr>
<td>10</td>
<td>–208</td>
<td>–65</td>
<td>–143</td>
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[a] Drop in the enzyme–substrate complex interaction energy reflects better binding of the substrate to the enzymatic active site.

Conclusions

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

**Experimental Section**

**General:** Optical rotations were measured in a 1.0 cm tube with a Perkin-Elmer 241 MC spectropolarimeter. \(^1H\) and \(^13C\) NMR spectra were obtained for solutions in CDCl\(_3\), D\(_2\)JDMSEO, CD\(_2\)OD and D\(_2\)O. J values are given in Hz and \(\delta\) 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\(_254\) (Merek), with detection by UV light and Panalid reagent [(NH\(_4\))\(_2\)MoO\(_4\), Ce(SO\(_4\))\(_2\), H\(_2\)SO\(_4\), H\(_2\)O]. Silica gel 60 (Merek, 230 mesh) was used for preparative chromatography. Anhydrous solvents and reagents were freshly distilled under N\(_2\) prior to use.

**Enzymes:** All assays with \(\beta\)-N-acetylhexosaminidases were performed in citrate/phosphate buffer (0.05 \(\text{m}, \text{pH} 5.0\)). The fungal strains producing \(\beta\)-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 (CIM), Prague, and were cultivated as described previously.[23] The screening comprised 33 enzymes: Acetosidium pasteurianum CCF 1850, Aspergillus awamori CCF 1248, Aspergillus flavus CCF 1895, A. flavipes CCF 3067, A. flavovar- catis CCF 3061, A. flavus CCF 3056, A. nigro CCIM K2, A. niveus CCF 3057, A. novius CCF 3086, A. oryzae CCF 147, A. oryzae CCF 1066, A. parastigmatis CCF 1298, A. sojae CCF 3060, A. tamarii CCF 3085, A. terreus CCF 2539, A. terreus USA, Fusarium oxyspo- rum CCF 377, Hamigera avellanea CCF 2923, Chaetomium glo- bosum CCF 430, Penicillium brasiliense CCF 2155, P. brasiliense CCF 2171, P. chrysogenum CCF 1269, P. functionosum CCF 2985, P. multicolor CCF 2244, P. oxalicum CCF 1959, P. oxalacia CCF 2315, P. oxalacia CCF 2430, P. pittii CCF 2277, P. spinulosum CCF 2159, Talarnomyces flavus CCF 2573, T. flavus CCF 2686, T. ohiensis CCF 2229, Trichoderma harzianum CCF 2687.

All assays with the nitrile hydratase from *Rhododoccus equi* A4 were performed in Na/K phosphate buffer (0.05 \(\text{m}, \text{pH} 7.5\)) with 0.5 \(\text{m}\) substrate. The enzyme was prepared and purified as described previously.[23]

**Activity Assay for \(\beta\)-N-Acetylhexosaminidases:**[29] The reaction mixture containing compound 8, 9 or the standard substrate 12a (2 \text{mM}, starting concentration) and \(\beta\)-N-acetylhexosaminidase (0.15–0.3 \text{U/mL} for 8 and 9, 0.01–0.02 \text{U/mL} for 12a) in buffer (assay volume 50 \(\text{\muL}\)) was incubated in microplates at 35°C for 10 min. The reaction was stopped by adding eq. Na\(_2\)CO\(_3\) (0.1 \(\text{m}, 150 \text{\muL}\)). Liberated p-nitrophenol was determined spectrophotometrically (414 nm) on Titertek Multiscan. 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 \text{mM}, starting concentration) and \(\beta\)-N-acetylhexosaminidase (1.79 \text{U/mL} for 10, 0.09 \text{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\(_2\)O/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 \(\beta\)-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 \text{mM}), compound 8, 9 or 10 (0–3 \text{mM}) and the \(\beta\)-N-acetylhexosaminidase from *A. oryzae* CCF 1066 (0.11 \text{U/mL}) was incubated at 35°C under shaking. Samples were taken, quenched by adding eq. Na\(_2\)CO\(_3\) (0.1 \(\text{m}\)) 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 (\(K_{i, 8}, K_{i, 9}\)) were determined from initial reaction rates at different starting concentrations of the standard substrate 12a (0.5–2.0 \text{mM}) in the presence of inhibitor 8 or 10 (0–3 \text{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 \(\text{mM}\) of compounds 8–10 and the purified nitrile hydratase from *Rhododoccus equi* A4[25] (0.06 mg of protein/mL, that is, approx. 3 \text{U/mL}, as assayed with benzoylacetone) in 50 \text{mM} Na/K phosphate buffer, pH 7.5, (assay volume 0.5 \text{mL}) was incubated at 28°C with shaking. At intervals (1, 3, 5 and 20 h) 0.05 \text{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 C18 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) H3PO4 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) H3PO4 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 [28] (1 mg of protein/mL, that is, approx. 2.8 μM/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 nitrilase 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-acetyhexosaminidases from *Serratia marcescens* and *Streptomyces pilocatus*, 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 AA091636. Three-dimensional models were generated using the Modeller6 package.[40] 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.[38] The docking of ligands was performed as described earlier.[38] 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 ε = 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 nonbonding 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-ido-3,4-O-(2',3'-dimethoxybutane-2,3'-diyl)-β-D-glucopyranosylurononitrite (Tetrabutyrammonium cyanide (135 mg, 0.594 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 CH2Cl2, washed with sodium hypochlorite and water, dried (Na2SO4) and the solvents evaporated. The residue was purified by column chromatography on silica gel (CH2Cl2/MeOH, 25:1–18:1) to give 20 (67 mg, 43%). [α]26 = +25 (c = 0.8, CH2Cl2). IR: 961, 538, 3264, 3086, 1524, 1344, 1111, 1071, 887, 745 cm⁻¹. 1H NMR (500 MHz, CDCl3): δ = 8.28–8.17 (m, 2 H, Ph), 7.20–7.07 (m, 2 H, Ph), 6.02 (d, J = 4.0 Hz, 2 H, CH2OAc), 2.40 (s, 6 H, CH3). FABMS: m/z (%) = 589 (100 [M + Na]+), 535 (70 [M + MeO]+). C31H27N2O9Cl: calculated for C31H27N2O9Cl: + 567.0840; found 567.0795.

**p-Nitrophenyl 2-Acetamido-2,6-dideoxy-6-ido-3,4-O-(2',3'-dimethoxybutane-2,3'-diyl)-β-D-glucopyranosylurononitrite (Tetrabutyrammonium cyanide (135 mg, 0.594 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 CH2Cl2, washed with sodium hypochlorite and water, dried (Na2SO4) and the solvents evaporated. The residue was purified by column chromatography on silica gel (CH2Cl2/MeOH, 25:1–18:1) to give 20 (67 mg, 43%). [α]26 = +25 (c = 0.8, CH2Cl2). IR: 961, 538, 3264, 3086, 1524, 1344, 1111, 1071, 887, 745 cm⁻¹. 1H NMR (500 MHz, CDCl3): δ = 8.28–8.17 (m, 2 H, Ph), 7.20–7.07 (m, 2 H, Ph), 6.02 (d, J = 4.0 Hz, 2 H, CH2OAc), 2.40 (s, 6 H, CH3). FABMS: m/z (%) = 589 (100 [M + Na]+), 535 (70 [M + MeO]+). C31H27N2O9Cl: calculated for C31H27N2O9Cl: + 567.0840; found 567.0795.
Cyanodeoxy-β-glucosyl Derivatives as Substrates for Enzymatic Reactions

1 H, 2-H), 3.76 (ddd, J = 7.4 Hz, J = 9.7 Hz, 1 H, 5-H), 3.61 (dd, J = 8.7 Hz, J = 10.4 Hz, 1 H, 3-H), 3.33 (dd, J = 8.7 Hz, J = 10.4 Hz, 1 H, 4-H), 3.01 (dd, J = 3.3, J = 17.1 Hz, 1 H, 6-H), 2.80 (dd, J = 7.4 Hz, 1 H, 6-H), 1.97 (s, 3 H, Me-CO) ppm. 13C 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 (1-C), 74.3 (C=3), 74.5 (C=4), 73.3 (C=5), 57.1 (C-2), 22.9 (NHCOCH3), 21.4 (C-6) ppm. CHRM: calc. for C12H21N2O2 + H: 352.1145; found 352.1173.

2-Acetamido-2,6-dideoxy-β-L-gluco-heptopyranosylurononitride Azide (21): Compound 18b (762 mg, 2.12 mmol) was suspended in dry CH3Cl (40 mL) under argon. 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 (CH2Cl2:MeOH, 12:1). After 4 h, the reaction was quenched by adding sat. aq. NaHCO3 (40 mL), then CH3Cl (50 mL) was added and the organic phase was separated. The aqueous phase was extracted with CH2Cl2, 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 Na2SO4 and the solvents evaporated under vacuum. The crude 2-acetamido-2-deoxy-3,4-di-O-tosyl glucosylpyranosyl azide (21) was immediately used in the next step without further purification.

The crude compound 21 was dissolved in dry CH2Cl2 (10 mL) under argon and a solution of Bu4NF (1.7 g, 6.33 mmol) in dry CH2Cl2 (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 CHCl3 (80 mL) and the organic phase was washed with NaClO4 (2×40 mL) and water, dried (Na2SO4) and the solvent evaporated under vacuum to dryness. The corresponding residue was purified by column chromatography (diethyl ether/acetone, 20:1) to give 25 (145 mg, 58%) as a white solid. [α]D = +25 (c = 0.8, CH3Cl). IR: 3289, 2949, 2191, 1665, 1459, 1410, 1145 cm−1. 1H NMR (300 MHz, CDCl3): δ = 7.78 (2, 2-H), 7.36 (m, 2 H, Ph), 5.59 (br, J = 352.1173; found 352.1173. CH3Cl) IR: [α]D = +25 (c = 0.8, CH3Cl). IR: 3289, 2949, 2191, 1665, 1459, 1410, 1145 cm−1. 1H NMR (300 MHz, CDCl3): δ = 7.07 (4, 5, 6-H), 3.47 (J = 10.9 Hz, 1 H, 3-H), 4.27 (J = 3.1 Hz, 1 H, 6-H), 3.76 (J = 10.0 Hz, 1 H, 5-H), 3.62 (J = 1.8 Hz, 3 H, Me-CO), 1.29 (2s, 3 H each, Me) ppm. 13C NMR (75.4 MHz, CDCl3): 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, BDA), 87.4 (C-1), 73.4 (C-3), 67.2, 67.1 (C-3, C-6), 65.9 (C-2), 45.2 (C-3), 48.3, 48.0 (2 OMe), 23.5 (NHCOCH3), 21.7 (CH3 of tosyl), 17.6, 17.5 (2 CH2) ppm.

(1R,2R,AR,SR,7R,9R,9a)-N-(11-Azido-4,5-dimethoxy-4,5-dimethyl-3,6,1,3-trioxa-10-azacycloc[7,3.0]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. [α]D = +25 (c = 0.8, CH3Cl). IR: 3289, 2949, 2191, 1665, 1459, 1410, 1145 cm−1. 1H NMR (300 MHz, CDCl3): δ = 7.77 (2, 2-H), 7.36 (m, 2 H, Ph), 5.59 (br, J = 352.1173; found 352.1173. CH3Cl) IR: [α]D = +25 (c = 0.8, CH3Cl). IR: 3289, 2949, 2191, 1665, 1459, 1410, 1145 cm−1. 1H NMR (300 MHz, CDCl3): δ = 7.07 (4, 5, 6-H), 3.47 (J = 10.9 Hz, 1 H, 3-H), 4.27 (J = 3.1 Hz, 1 H, 6-H), 3.76 (J = 10.0 Hz, 1 H, 5-H), 3.62 (J = 1.8 Hz, 3 H, Me-CO), 1.29 (2s, 3 H each, Me) ppm. 13C NMR (75.4 MHz, CDCl3): 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, BDA), 87.4 (C-1), 73.4 (C-3), 67.2, 67.1 (C-3, C-6), 65.9 (C-2), 45.2 (C-3), 48.3, 48.0 (2 OMe), 23.5 (NHCOCH3), 21.7 (CH3 of tosyl), 17.6, 17.5 (2 CH2) ppm.

(1R,2R,AR,SR,7R,9R,9a)-N-(11-Azido-4,5-dimethoxy-4,5-dimethyl-3,6,1,3-trioxa-10-azacycloc[7,3.0]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. [α]D = +25 (c = 0.8, CH3Cl). IR: 3289, 2949, 2191, 1665, 1459, 1410, 1145 cm−1. 1H NMR (300 MHz, CDCl3): δ = 7.07 (4, 5, 6-H), 3.47 (J = 10.9 Hz, 1 H, 3-H), 4.27 (J = 3.1 Hz, 1 H, 6-H), 3.76 (J = 10.0 Hz, 1 H, 5-H), 3.62 (J = 1.8 Hz, 3 H, Me-CO), 1.29 (2s, 3 H each, Me) ppm. 13C NMR (75.4 MHz, CDCl3): 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, BDA), 87.4 (C-1), 73.4 (C-3), 67.2, 67.1 (C-3, C-6), 65.9 (C-2), 45.2 (C-3), 48.3, 48.0 (2 OMe), 23.5 (NHCOCH3), 21.7 (CH3 of tosyl), 17.6, 17.5 (2 CH2) ppm.
(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 (CHCl₃/MeOH, 50:1) to give 27 (35 mg, 71%). [α]D = +18.4 (c = 1, CH₂Cl₂). Rₜ = 328, 3082, 1667, 1593, 1346, 1111, 1067, 887, 745 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ = 6.36–6.47 (m, 2 H, Ph), 7.17–7.45 (m, 2 H, Ph), 7.76 (d, J = 8.7 Hz, 1 H, Ph), 7.98 (d, J = 1.5 Hz, 1 H, Ph), 8.12 (d, J = 1.5 Hz, 1 H, Ph), 8.78 (d, J = 8.7 Hz, 1 H, Ph), 8.82 (d, J = 1.5 Hz, 1 H, Ph), 9.16 (d, J = 1.5 Hz, 1 H, Ph). C NMR (150 MHz, CDCl₃): δ = 61.7 (CH₂), 127.0 (CH), 128.2 (CH), 128.6 (CH), 128.8 (CH), 129.1 (CH), 137.4 (CH), 138.4 (CH), 139.2 (CH), 143.2 (CH), 146.7 (CH), 151.0 (CH), 152.1 (CH), 168.8 (CO). Mass spectrum (EI): m/z = 364 (M⁺, 100). ¹³C NMR spectra for compounds 8–25 and 27. Supporting Information (for details see the footnote on the first page of this article): ¹³C NMR spectra for compounds 8–10, 19, 20, 23 and 27.

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Cyanodeoxy-Glycosyl Derivatives as Substrates for Enzymatic Reactions


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