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N-Alkylated Derivatives of 1,5-Dideoxy-1,5-iminoxylitol as β-Xylosidase and β-Glucosidase Inhibitors[a]

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Summary. A range of lipophilic derivatives of the D-xylosidase inhibitor 1,5-dideoxy-1,5-iminoxylitol was synthesized by N-alkylation of the parent compound with different alkyl halides. Inhibitory activities of the products obtained were measured with β-glucosidase from *Agrobacterium sp.*, a family 1 enzyme, as well as with β-xylosidase from *Thermoanaerobacterium saccharolyticum* belonging to family 39 of the glycohydrolases. Whereas the former enzyme, which has low β-xylosidase activity, was only moderately inhibited, with K_i values in the millimolar range, good inhibition was observed with the latter one. Inhibitors with terminally functionalized N-alkyl chains open up opportunities for novel applications as affinity ligands as well as for various types of tagging for diagnostic purposes.

Keywords. 1,5-Dideoxy-1,5-iminoxylitol; β-Glucosidase; β-Xylosidase; Glycosidase inhibitor.

Introduction

D-Xylans are polysaccharides consisting of β-1,4-linked D-xylopyranosyl moieties and can be found in the cell walls of all land plants and most plant parts [1]. Their biodegradation relies on β-1,4-xylanases (EC 3.2.1.8) and β-xylosidases (EC 3.2.1.37). Xylanases are endo-enzymes attacking internal glycosidic linkages, whereas xylosidases release xylose by attack of the terminal glycosidic bond at the non-reducing end of the oligomer. These enzymes are produced by biodegradative microorganisms such as fungi and bacteria that are important for carbon turnover in nature. For example, 35% of birch wood (dry weight) consists of xylans. In pulp and paper technology, these enzymes are employed in a prebleach treatment to remove residual xylans, thereby reducing the need for expensive and environmentally questionable chemicals in the process. The food

[a] Dedicated to Prof. *Günter Legler* on the occasion of his 75th birthday

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and feed industry has also been identified as a large potential area for application of xylanases.

Low molecular weight inhibitors of xylanases and xylosidases are not only useful tools for enzyme characterization and purification but might also serve other, more 'applied' purposes. For example, powerful and inexpensive inhibitors could be employed in crop protection against xylan degrading plant pathogens or as components in wood preservation coatings and paints. Iminosugars including iminoalditols have been found to be powerful inhibitors of many glycosidases with K_i values in the micromolar and, in quite a few cases, even nanomolar range [2]. Some of these compounds, due to their interference with biochemical pathways, exert interesting biological activities [2]. Many have become valuable diagnostic tools for studies of enzyme active sites and catalytic mechanisms. Despite the abundance of xylosidases and xylanases and the fact that they play important roles in the degradation of hemicelluloses, corresponding inhibitors such as 1,5-dideoxy-1,5-iminoxylitol (**1**) have not yet gained as much attention as the related hexopyranoside mimetics.

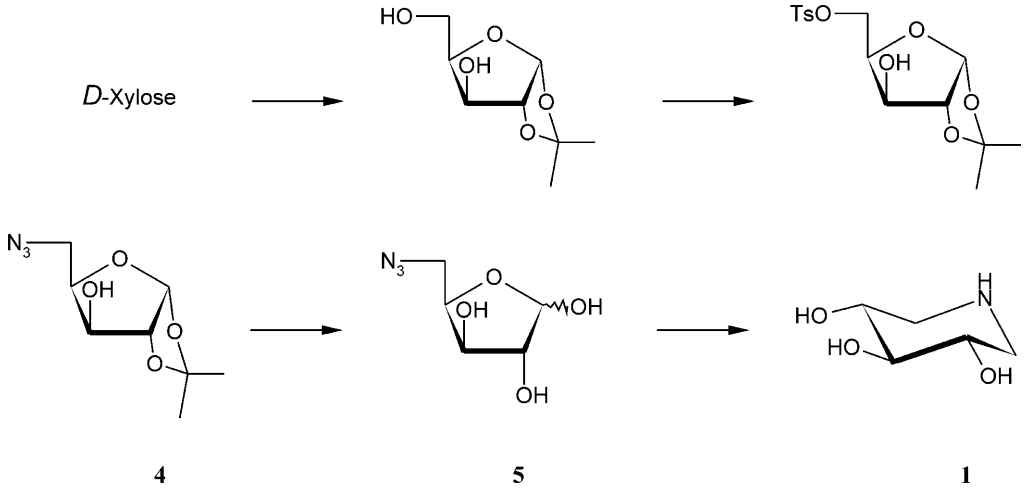
1,5-Dideoxy-1,5-iminoxylitol (**1**) is the paradigmatic example of a reversible, xyloside mimicking inhibitor. Its first synthesis has been reported by *Paulsen* in the mid-nineteen sixties (its precursor, 5-amino-5-deoxyxylose, was independently reported by both *Paulsen* and *Hanessian*) [3]. Subsequently, this compound was synthesized by several other groups [4]. Examples of its biochemical applications have remained rare and largely confined to studies of β-glucosidases, despite the fact that this compound could be a useful probe for xylanase/β-xylosidase research.

Based on recent findings with related glycosidase inhibitors, in particular derivatives of the powerful β-glucosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol, which indicated that lipophilic chain extensions can improve the inhibitory activity of such derivatives [5], we prepared a small exploratory set of simple N-alkylated derivatives of compound **1** featuring simple hydrocarbon substituents as well as terminally functionalized chains suitable for tagging or immobilization studies.

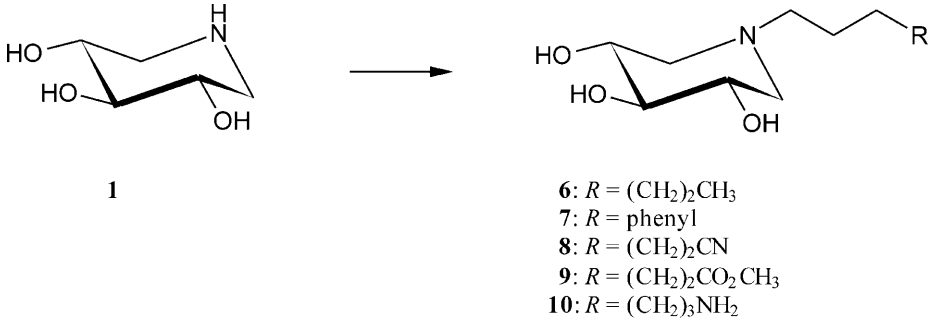
Results and Discussion

Parent compound **1** was readily accessible from D-xylose as mentioned previously according to Scheme 1. Controlled conversion of D-xylose gave the corresponding furanoid 1,2-O-isopropylidene protected derivative **2** which was regioselectively O-tosylated at the primary position in dichloromethane/pyridine employing the standard procedure. Conventional reaction of 1,2-O-isopropylidene-5-O-tosyl-α-D-xylofuranose (**3**) with sodium azide in N,N-dimethylformamide furnished the corresponding 5-azidodeoxysugar **4** [6]. Deprotection employing acidic ion exchange resin Amberlite IR 120 [H^+] gave the free sugar **5** which was cyclized by conventional catalytic hydrogenation and concomitant intramolecular reductive amination to yield the desired key intermediate **1**.

Its reaction with bromohexane, 3-phenyl-1-bromopropane, methyl 6-bromohexanoate, and 6-bromohexanoic nitrile led smoothly to the corresponding N-alkyl derivatives **6**–**9**. From **8**, the 6-aminohexyl substituted compound **10** was formed by catalytic hydrogenation over palladium hydroxide on charcoal. Compounds **8**–**10** can be modified at their terminal functional groups *e.g.* by



Scheme 1



Scheme 2

Table 1. Inhibition constants (K_i values, μM) for substituted iminoxylitols with β-glucosidase from *Agrobacterium sp*. at *pH* 7.0 as well as with β-xylosidase from *Thermoanaerobacterium saccharolyticum* at *pH* 5.5

	1	**6**	**7**	**8**	**9**	**10**
β-glucosidase	50	2600	1600	3600	2000	2500
β-xylosidase	206	88	135	25	35	118

attachment onto a polymer support for affinity chromatography or by other types of transformations.

Preliminary screening of inhibitory activities were conducted with β-glucosidase from *Agrobacterium sp*. as well as with β-xylosidase from *Thermoanaerobacterium saccharolyticum* (Table 1). All N-alkylated compounds were found to be moderate inhibitors of the β-glucosidase employed with activities lower than that of parent compound **1**.

Conversely, with the β-xylosidase employed in this study, N-alkylation improved activities by a factor of 2 to 3 (compounds **6**, **7**, and **10**) as compared

to the parent compound. Gratifyingly, in the case of nitrile **8**, an increase of inhibitory potency of nearly one order of magnitude was observed. These preliminary results suggest that β-xylosidase inhibitors of the iminoxylitol type with interesting properties should become available by fine-tuning the nature as well as the length of the N-alkyl substituent.

Experimental

Melting points were recorded on a Tottoli apparatus (Büchi) and are uncorrected. Optical rotations were measured on a JASCO Digital Polarimeter or with a Perkin Elmer 341 instrument with a path length of 10 cm. NMR spectra were recorded at 200 (^{1}H) and at 50.29 MHz (^{13}C) on a Varian Gemini spectrometer. Chemical shifts are given in δ relative to the solvent resonances. The signals of the protecting groups were found in the expected regions and are not listed explicitly. Thin-layer chromatography was performed on precoated aluminum sheets (Merck 5554). Plates were stained with a mixture of 10% ammonium molybdate (w/v) in 10% H_2SO_4 containing 0.8% $Ce(SO_4)_2$ (w/v). For column chromatography, Silica Gel 60 (E. Merck) was used employing $CHCl_3$:MeOH: concd. aq. ammonia = 100:100:1 as the solvent system. Elemental analyses agreed favourably with the calculated values.

General method for N-alkylation reactions employing bromoalkyl reagents

To a 5% solution of compound **1** in MeOH, 3 equivalents of the respective bromoalkane and 3 equivalents of pyridine were added, and the mixture was kept at ambient temperature for 48 h. Removal of solvents under reduced pressure followed by chromatography of the residue furnished compounds **6**–**8**.

N-Hexyl-1,5-dideoxy-1,5-iminoxylitol (**6**; $C_{11}H_{23}NO_3$)

Following the general procedure, 170 mg **1** (1.28 mmol) were reacted with 530 mm^3 1-bromohexane (3.78 mmol). Compound **6** (215 mg) was obtained in 78% yield.

M.p.: 79–82°C; ^{1}H NMR (CD_3OD, δ, 200 MHz): 3.78 (2H, m, H-2, H-4), 3.46 (1H, broad, H-3), 3.33 (2H, m, H-1a, H-5a), 2.98 (2H, m, H-1e, H-5e), 2.80 (2H, broad, H-1′, H-1″), 1.71 (2H, m, H-2′, H-2″), 1.37 (6H, m, H-3′, H-3″, H-4′, H-4″, H-5′, H-5″), 0.92 (3H, t, H-6′, H-6″, H-6‴) ppm; the poor resolution of signals is due to inherent conformational flexibility of the molecule; ^{13}C NMR (CD_3OD, δ, 50 MHz): 68.1 (C-2, C-3, C-4), 57.4 (C-1, C-5), 55.4 (C-1′), 31.3, 26.4, 24.5, 22.4, 13.1 (C-2′, C-3′, C-4′, C-5′, C-6′) ppm.

N-(3-Phenyl)-propyl-1,5-dideoxy-1,5-iminoxylitol (**7**; $C_{14}H_{21}NO_3$)

Employing the general procedure, 142 mg **1** (107 mmol) and 48 mm^3 3-phenyl-1-bromopropane (3.15 mmol) furnished 160 mg of **7** (59%) as a wax.

^{1}H NMR (CD_3OD, δ, 200 MHz): 3.67 (2H, ddd, $J_{1a,2} = J_{4,5a} = 7.5$ Hz, $J_{2,3} = J_{3,4} = 8.4$ Hz, $J_{1e,2} = J_{4,5e} = 4.4$ Hz, H-2, H-4), 3.15 (1H, H-3), 3.01 (2H, dd, $J_{1a,1e} = J_{5a,5e} = 11.4$ Hz, H-1a, H-5a), 2.70–2.12 (6H, m, H-1b, H-5b, H-1′, H-1″, H-3′, H-3″), 1.76 (2H, m, H-2′, H-2″) ppm; ^{13}C NMR (CD_3OD, δ, 50 MHz): 145.1, 132.3, 132.2, 129.9 (phenyl), 79.8 (C-3), 72.9 (C-2, C-4), 60.8 (C-1, C-5), 60.4 (C-1′), 36.7, 31.1 (C-2′, C-3′) ppm.

N-(5-Cyano)-pentyl-1,5-dideoxy-1,5-iminoxylitol (**8**; $C_{11}H_{20}N_2O_3$)

Iminoalditol **1** (170 mg, 1.28 mmol) was reacted with 250 mm^3 6-bromohexanoic nitrile (1.92 mmol) to give 134 mg (46%) **8**.

M.p.: 103–107°C; ^{1}H NMR (CD_3OD, δ, 200 MHz): 3.55 (2H, ddd, $J_{1a,2} = J_{4,5a} = J_{2,3} = J_{3,4} =$ 9.2 Hz, $J_{1e,2} = J_{4,5e} = 4.4$ Hz, H-2, H-4), 3.18 (1H, H-3), 3.06 (2H, dd, $J_{1a,1e} = J_{5a,5e} = 11.0$ Hz, H-1a, H-5a), 2.52 (4H, m, H-1′, H-1″, H-5′, H-5″), 2.14 (2H, m, H-1b, H-5b), 1.82–1.38 (6H, m, H-2′, H-2″, H-3′, H-3″, H-4′, H-4″) ppm; ^{13}C NMR (CD_3OD, δ, 50 MHz): 119.7 (CN), 79.0 (C-3), 69.7 (C-2, C-4), 57.6 (C-1, C-5), 57.2 (C-1′), 26.2, 25.3, 25.1, 16.1 (C-2′, C-3′, C-4′, C-5′) ppm.

N-Methoxycarbonylpentyl-1,5-dideoxy-1,5-iminoxylitol (**9**; $C_{12}H_{23}NO_5$)

To a 5% solution of 240 mg of the starting material **1** (1.80 mmol), 294 mm^3 adipic acid methyl ester hemialdehyde (2.7 equivalents) and 120 mg $Pd(OH)_2/C$ (20%) were added, and the mixture was stirred under H_2 for 5 h. After removal of the catalyst, the filtrate was concentrated under reduced pressure. Chromatography of the residue gave 394 mg **9** (89%).

M.p.: 118–119°C; ^{1}H NMR (CD_3OD, δ, 200 MHz): 3.66 (3H, s, OMe), 3.49 (2H, ddd, $J_{1a,2} = J_{4,5a} = 10.5$ Hz, $J_{1e,2} = J_{4,5e} = 4.8$ Hz, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-2, H-4), 3.09 (1H, H-3), 3.00 (2H, dd, $J_{1a,1b} = J_{5a,5b} = 11.0$ Hz, H-1a, H-5a), 2.37 (4H, m, H-1′, H-1″, H-5′, H-5″), 1.92 (2H, H-1b, H5b), 1.76–1.24 (6H, m, H-2′, H-2″, H-3′, H-3″, H-4′, H-4″) ppm; ^{13}C NMR (CD_3OD, δ, 50 MHz): 174.7 (C-6′), 79.2 (C-3), 70.2 (C-2, C-4), 58.3 (C-1, C-5), 57.6 (C-1′), 50.8 (OCH_3), 33.5 (C-5′), 26.8, 26.2, 24.7 (C-2′, C-3′, C-4′) ppm.

N-(6-Amino)-hexyl-1,5-dideoxy-1,5-iminoxylitol (**10**; $C_{11}H_{24}N_2O_3$)

To a 5% solution of 112 mg **8** (0.49 mmol), 105 mg $Pd(OH)_2/C$ (20%) were added, and the mixture was stirred under H_2 for 5 days. After removal of the catalyst, the filtrate was concentrated under reduced pressure. Chromatography of the residue gave 52 mg of syrupy **10** (46%).

^{1}H NMR (D_2O, δ, 200 MHz): 3.45 (2H, ddd, $J_{1e,2} = J_{4,5e} = 4.8$ Hz, $J_{2,3} = J_{3,4} = 10.1$ Hz, H-2, H-4), 3.15 (1H, H-3), 3.05 (2H, dd, $J_{1a,1b} = J_{5a,5b} = 11.0$ Hz, H-1a, H-5a), 2.81 (2H, t, $J = 7.3$ Hz, H-1′, H-1″), 2.49 (2H, m, H-6′, H-6″), 2.13 (2H, dd, H-1b, H-5b), 1.60–1.04 (8H, m, H-2′, H-2″, H-3′, H-3″, H-4′, H-4″, H-5′, H-5″) ppm; ^{13}C NMR (D_2O, δ, 50 MHz): 77.4 (C-3), 68.8 (C-2, C-4), 57.0 (C-1, C-5), 56.1 (C-1′), 39.5 (C-6′), 26.7, 26.0, 25.4, 24.8 (C-2′–C-5′) ppm.

Inhibition constants (K_i values, μM) for substituted iminoxylitols with *Agrobacterium sp.* β-glucosidase (8.5×10^{-5} mg · cm^{-3}) were determined at 37°C in *pH* 7.0 Na_3PO_4 buffer (45 *mM*) containing 0.1% bovine serum albumin using a fixed concentration of substrate, 4-nitrophenyl-β-*D*-glucopyranoside (0.11 $mM = 1.5 \times K_m$). Inhibition constants (K_i values, μM) for substituted iminoxylitols with *Thermoanaerobacterium saccharolyticum* β-xylosidase (0.10 mg · cm^{-3}) were determined at 37°C in *pH* 5.5 sodium citrate – phosphate buffer containing 0.01% bovine serum albumin using a fixed concentration of substrate, phenyl-β-*D*-xylopyranoside (1.7 m$M = 1.0 \times K_m$).

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