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Heteroaryl Thioglycosides, a New Class of Substrates for Glycosidases

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We have found that heteroaryl thioglycosides are useful substrates of β-glucosidase from almond and can act as a new class of donors in transglycosylation reaction.

Key words: glycosidase, thioglycosides, hydrolysis, transglycosidation

Glycoconjugates are one of the most functionally and structurally diverse molecules in nature; it is now well established that protein- and lipid-bounded saccharides play essential role in many molecular processes in living organisms [1–3]. The notion that carbohydrates are biologically important molecules and that many human diseases are associated with alterations of cellular carbohydrates resulted in a great interest in synthetic carbohydrate chemistry, especially in glycosidation reaction [4]. Chemical syntheses of such compounds are limited, at least for larger scalework, by many protection, activation and deprotection steps. The need for efficient methods for oligosaccharide synthesis has stimulated the development of enzymatic methods [5].

RESULTS AND DISCUSSION

Major advantages of glycosidase-catalyzed glycosyl transfer are usage unprotected derivatives of sugars; the defined configuration (only α or β) of the newly formed glycosyl bond can be controlled through the choice of the enzyme. Glycosylation catalyzed by glycosyl hydrolases was usually performed *via* kinetic control [6]. In this case activated glycosyl donor (often 4-nitro phenyl glycoside), which possesses an aglycon moiety with good leaving properties, is used.

Hydrolysis of donors is a competitive reaction and the sugar formed during the transglycosylation is also a substrate for the enzyme. To obtain a good yield of target glycoside, transglycosylation must be faster than hydrolysis of glycosyl donor and the formed product. Since the glycosylation under high concentration of donor diminishes the role of hydrolysis, glycosyl donor should be well soluble in water and in a water-organic solvent mixture.

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We now report that heteroaryl thioglycosides act as substrates of glycosidases and can be used as glycosyl donors in enzymatic reactions. They are stable, easily prepared and well soluble in water and water-organic solvent mixture.

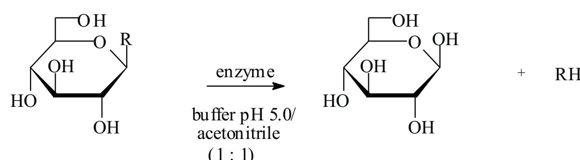
Looking for the inhibitors of β-glucosidase, we synthesized a series of heteroaryl thioglycosides derivatives of pyridine and imidazole. The conformation of 1-thiosugars is similar to the conformation of natural sugars and they can act as competitive enzyme inhibitors [7]. Analysis of the mechanism of glycosidase reactions shows that the low degree of protonation of the "soft" sulfur is probably the main cause of the thioglycosidic bond stability [8]. However, in preliminary experiments we have observed that compounds obtained by us are substrates of glycosyl hydrolases. This is an unexpected result, because it is well documented, that alkyl- and aryl thioglycosides are inert to the glycosidase hydrolysis [9].

In order to select the best substrates of glucosyl hydrolase, we have examined in a series of experiments the hydrolysis of heteroaryl thioglucosides, derivatives of pyridine and imidazole: 1-(5-nitropyrid-2-ylthio)-β-D-glucopyranoside (**1**), 1-(3-nitropyrid-2-ylthio)-β-D-glucopyranoside (**2**), 1-(1-methyl-4-nitro-5-imidazoylthio)-β-D-glucopyranoside (**3**), 1-(pyrid-2-ylthio)-β-D-glucopyranoside [10] (**4**) and (4-nitrophenyl)-β-D-glucopyranoside [11] (**5**) (Scheme 1).

Thioglycosides are very reactive substrates in nucleophilic reactions and are popular glycosyl donor [4]. Therefore, the enzyme-free hydrolysis was investigated as a control experiment. Substrates **1–5** were completely unreactive in buffer at pH 4–8. A slow degradation was observed at pH > 12. Kinetic analysis is an effective technique for characterizing enzyme-catalyzed mechanism and substrate specifity. Taking this into account, the kinetic parameters – the Michaelis constant K_m and the molar activity k_o of β-glucosidase-catalyzed reactions – were determined for glucosides **1**, **4** and **5** as substrates. The parameters K_m, k_o and V_{max} are listed in Table 1.

Enzymatic hydrolysis **2** and **3** carried out very slowly, thus we did not determine the kinetic parameters for these compounds.

Scheme 1



enzyme = β-glucosidase from almond

compound	**1**	**2**	**3**	**4**	**5**
R	S– N –NO_2	O_2N S– N	O_2N N S– N CH_3	S– N	O– –NO_2

Table 1. Michaelis parameters for β-glucosidase-catalyzed hydrolysis of compounds **1**, **4** and **5**.

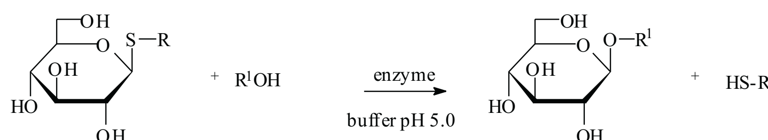
Substrate	K_m [mmol]	V_{max} [mmol min^{-1}]	k_o [min^{-1}]
(4-nitrophenyl)-β-D-glucopyranoside **(5)**	37	11	0.297
1-(5-nitropyrid-2-ylthio)-β-D-glucopyranoside **(1)**	109	21	0.193
1-(pyrid-2-ylthio)-β-D-glucopyranoside **(4)**	612	43	0.070

Kinetic parameters for β-glucosidase-catalyzed thioglucosides hydrolysis indicate that compounds with an electron withdrawing nitro group have larger values of k_o. On the other hand, we found that the 1-(4-nitrophenylthio)-β-D-glucoside is stable under the enzymatic hydrolysis conditions. This result shows that a "hard" basic center (nitrogen atom) in the aglycon is necessary in hydrolysis. Increasing the leaving ability of the aglycon by introduction of a nitro group in the 5-position of the pyridyl ring leads to the expected increase in hydrolysis rate. The activation of heteroaryl thioglucosides is only possible for compounds with appropriate conformational orientation of aglycone.

For compounds **2** and **3** steric demands in the surrounding active-side lead to the observed decreased activity. The possible application of thioglucosides in the glycosidation was tested in a reaction with methyl and ethyl alcohol (Scheme 2). A high yield and an excellent selectivity were found.

The present study is the first example of an enzymatic transglycosylation of thioglycosides. These studies on the application of heteroaryl thioglycosides in synthesis of glycosides and oligosaccharides are in progress.

Scheme 2



	R	Yields of glucosides [%]	
		$R^1 = CH_3$	$R^1 = C_2H_5$
(1)	S–(pyridyl, N)–NO_2	98	83
(4)	O–(phenyl)–NO_2	95	79

EXPERIMENTAL

General: Almond β-D-glucosidase was obtained from Sigma. The reactions was monitored by TLC. Optical rotations were measured with a Perkin Elmer 141 polarimeter, using sodium lamp (589 nm) at room temperature. ^{1}H NMR spectra were recorded with a Varian 300 MHz spectrometer in D_2O with TMS as an internal standard. Elemental analyses were performed with a Perkin Elmer 2400 analyzer.

1-(5-Nitropyrid-2-ylthio)-β-D-glucopyranoside (1). 1-(5-Nitropyrid-2-ylthio)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside was prepared as described in the literature [12] and deacetylated in usual way (NaOMe-MeOH); m.p. 59–62°C; $[\alpha]_D^{25}$–110° (c 0.5, MeOH); ^{1}H NMR δ: 3.30 (dd,1H, $J_{2,3}$ 8.3 Hz, $J_{2,1}$ 9.5 Hz, H-2), 3.35–3.51 (m, 3H, H-3, H-4, H-5), 3.66 (dd, 1H, $J_{6,5}$ 5.6 Hz, $J_{6,6a}$ 12.2 Hz, H-6), 3.85 (dd, 1H, $J_{6a,5}$ 2.0 Hz, H-6a), 5.45 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 7.56 (d, 1H, $J_{3Ar,4Ar}$ 8.7 Hz, H-3Ar), 8.40 (dd, 1H, $J_{4Ar,3Ar}$ 8.7 Hz, H-4Ar), 9.20 (d, 1H, H-6Ar); Anal. Calc. for $C_{11}H_{14}N_2O_7S$: C, 41.5; H, 4.4; N, 8.8; S, 10.1; Found: C, 41.3; H, 4.4; N, 8.5; S, 9.9.

1-(3-Nitropyrid-2-ylthio)-β-D-glucopyranoside (2). 1-(3-Nitropyrid-2-ylthio)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside was prepared like 1-(5-nitropyrid-2-ylthio)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside and deacetylated in usual way (NaOMe-MeOH); m.p. 120–122°C; $[\alpha]_D^{25}$ +59.2 (c 0.6, MeOH); ^{1}H NMR δ: 3.48–3.91 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b); 5.58 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1), 7.45 (dd, 1H, $J_{5Ar,4Ar}$ 4.6, $J_{5Ar,6Ar}$ 6.8 Hz, H-5Ar), 8.65 (d, 1H, $J_{6Ar,5Ar}$ 6.8 Hz, H-6Ar), 8.80 (d, 1-H, $J_{4Ar,5Ar}$ 4.6 Hz, H-4Ar); Anal. Calc. for $C_{11}H_{14}N_2O_7S$: C, 41.5; H, 4.4; N, 8.8; S, 10.10; Found: C, 41.3; H, 4.3; N, 8.9; S, 9.9.

1-(1-Methyl-4-nitro-5-imidazoylthio)-β-D-glucopyranoside (3). 1-(1-Methyl-4-nitro-5-imidazoylthio)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside was prepared as described in the literature [13] and deacetylated in usual way (NaOMe-MeOH); m.p. 168–170°C; $[\alpha]_D^{25}$–70° (c 0.3, MeOH; ^{1}H NMR δ: 3.2–3.5 (m, 4H, H-2, H-3, H-4, H-5), 3.3 (s, 3H, CH_3N), 3.66 (dd, 1H, $J_{6b,5}$ 5.1 Hz, H-6b), 3.8 (dd, 1H, $J_{6a,5}$ 2.2 Hz, H-6a), 4.78 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1); Anal. Calc. for $C_{10}H_{15}N_3O_7S$: C, 37.4; H, 4.7; N, 13.1; S, 10.0; Found: C, 37.4; H, 4.67; N, 12.93; S, 9.8.

Data of compounds **4** and **5** was reported previously [11,14,15].

Enzymatic hydrolysis with β-D-glucosidase: The enzyme (10 U) was added to a solution of appropriate glucoside **1–5** (10 mg) in a 0.1 M citrate-phosphate buffer pH = 5 (0.5 ml) and acetonitrile (0.5 ml) and left at 25°C for 5 min to 24 h.

Kinetic study: Kinetic constans were determined for compounds **1–5**. Affinity constans (K_m) and V_{max} were determined using linear regression plots of Lineweavera-Burk. In these cases enzymatic hydrolysis was carried on at 37°C. The course of reaction was determined spectrophotometrically (Cecil CE2501) by monitoring amount of glycosides [$\lambda_{max}(1) = 299$ nm, $\lambda_{max}(2) = 270$, $\lambda_{max}(3) = 380$ nm, $\lambda_{max}(4) = 240$ nm, $\lambda_{max}(5) = 300$ nm, solvent – 0.1 M NaOH and MeOH in the ratio of 1 to 1].

Transglucosylation reactions: To a solution of appropriate glycoside (**1** or **4**) (10 mg, 0.3 mmol) in a 0.1 M citrate-phosphate buffer pH = 5.0 (0.5 ml) was added β-D-glucosidase from almond (10 U) and methanol (0.5 ml, 16 mmol) or ethanol (0.5 ml, 11 mmol) and the mixture was allowed to stand at 25°C for 30–60 min. Then the mixture was diluted by water and a non-polar compound was extracted with CH_2Cl_2 and the aqueous solution concentrated *in vacuo*. The residue was dissolved in 10 ml of water. Glucose was determined spectrophotometrically (λ = 500 nm) using "Glukoza et new" test (from POCH S.A.). This test is base on series of enzymatic reactions – 1) oxidation of sugar in the presence of glucose oxidase led to glucoronic acid and H_2O_2; 2) the peroxidase catalyzed reaction H_2O_2-chromogene. The intensity of obtained tint is directly proportional to the concentration of glucose. The only products of reaction are D-glucose and alkyl glucoside. Thus, the yields of glucosides were calculated indirectly, based on the amount of glucose, formed by a hydrolysis of the donor.

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