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Nitropyridyl glycosides: new glycosyl donors for enzymatic transglycosylation

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Abstract

New glycosyl donors, 3-nitro-2-pyridyl and 5-nitro-2-pyridyl glycosides, proved to be effective for transglycosylation reaction catalyzed by glycosidases, such as β-galactosidase, β-glucosidase, and *N*-acetyl-β-hexosaminidase. The high solubility in water and the high reactivity of the nitropyridyl glycosides enabled the reactions under high concentrations of the donors and consequently rapid glycosyl transfer to glycosyl acceptors. The yields of the transglycosylated products with the nitropyridyl glycosides were much higher than those with conventional *p*-nitrophenyl glycosides.

Keywords: glycosidase; transglycosylation; glycosyl donor; 3-nitro-2-pyridyl glycoside.

Recently, glycosidase-catalyzed transglycosylation has attracted much attention for the synthesis of sugar chains, since stereoselective glycosylation can be achieved without multistep reactions of selective protection and deprotection procedures. Various glycosidases are readily available, and their rather low specificity for glycosyl acceptors allows the application of this method to a variety of compounds.[1,2] The yields of transglycosylation are, however, generally low because competitive hydrolysis of glycosyl donors and glycosylated products proceeds in parallel to the desired transglycosylation. In the present study, we describe a simple but effective transglycosylation system, where these undesired reactions are suppressed by using 3- and 5-nitro-2-pyridyl glycosides as new glycosyl donors.

p-Nitrophenyl (PNP) and *o*-nitrophenyl glycosides have been frequently used as donors for transglycosylation, since they are efficiently recognized by glycosidases and their high reactivity enables rapid glycosyl transfer to acceptors within short reaction times to suppress hydrolysis of the glycosylated products. Their solubilities in water are, however, not high enough because of the hydrophobicity of the nitrophenyl group. This is an obvious disadvantage of the nitrophenyl glycosides because the reaction under high concentrations of substrates is advantageous for glycosylation rather than hydrolysis of the donors. We therefore investigated the use of 3-nitro-2-pyridyl (3NPy) glycosides and 5-nitro-2-pyridyl

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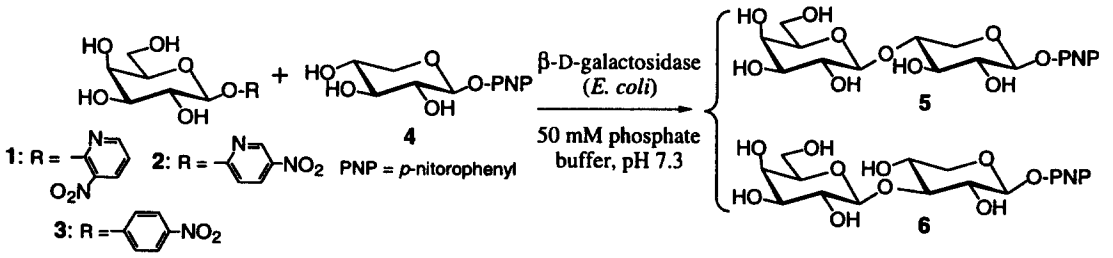
(5NPy) glycosides, which are expected to have higher reactivities and solubilities than those of the corresponding nitrophenyl glycosides.[3]

The transglycosylation reaction with β-galactosidase (*Escherichia coli*) was first examined by the use of 3-nitro-2-pyridyl β-D-galactoside (Gal-3NPy) (**1**) and 5-nitro-2-pyridyl β-D-galactoside (Gal-5NPy) (**2**) as donors and *p*-nitrophenyl β-D-xyloside (**4**) as an acceptor (Scheme 1).[4] The reactions were carried out by using 3 equiv. of a donor to the acceptor under saturated conditions of the donor.[5] Since both **1** and **2** are more soluble in water than the corresponding *p*-nitrophenyl galactoside (Gal-PNP (**3**)), the glycosylation reactions were carried out at a much higher concentration of either **1** or **2** (600 mM) than that of **3** (100 mM). As clearly shown in Table 1, the yields of the disaccharides **5** and **6** were remarkably improved by using nitropyridyl galactosides from the value with Gal-PNP (Table 1). Since Gal-3NPy (**1**) was more reactive than Gal-5NPy (**2**), the reaction with the former completed within shorter periods to give better yields of the disaccharides.



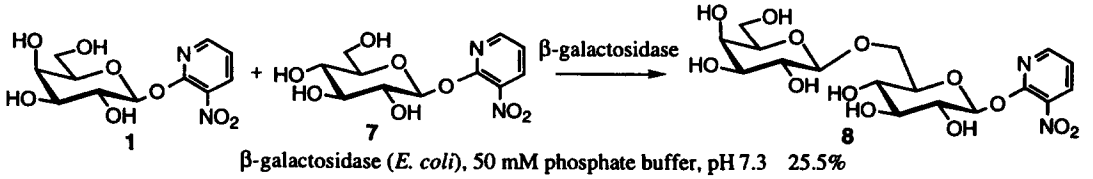
Scheme 1.

Table 1
The yields of transgalactosylation

Entry	Donor	Conc. / mM	Time / min	Yield / %[a]	
				5	6
1	1	600	35	32.1	3.8
2	2	600	70	26.2	3.1
3	3	100	90	16.9	2.3

a) Based on HPLC.

Gal-3NPy (**1**) was also effectively transferred to the 6-position of 3-nitro-2-pyridyl glucoside (Glc-3NPy) (**7**) at high concentrations of **1** (300 mM) and **7** (600 mM) (Scheme 2).

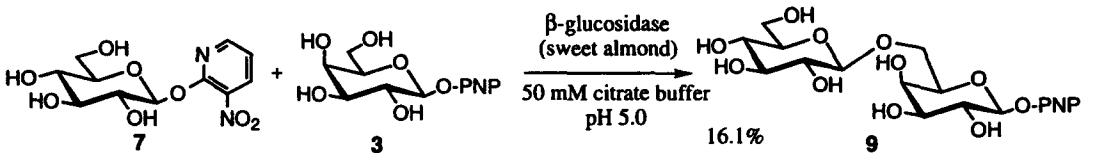


β-galactosidase (*E. coli*), 50 mM phosphate buffer, pH 7.3 25.5%
β-galactosidase (*A. oryzae*), 50 mM citrate buffer, pH 5.0 34.4%

Scheme 2.

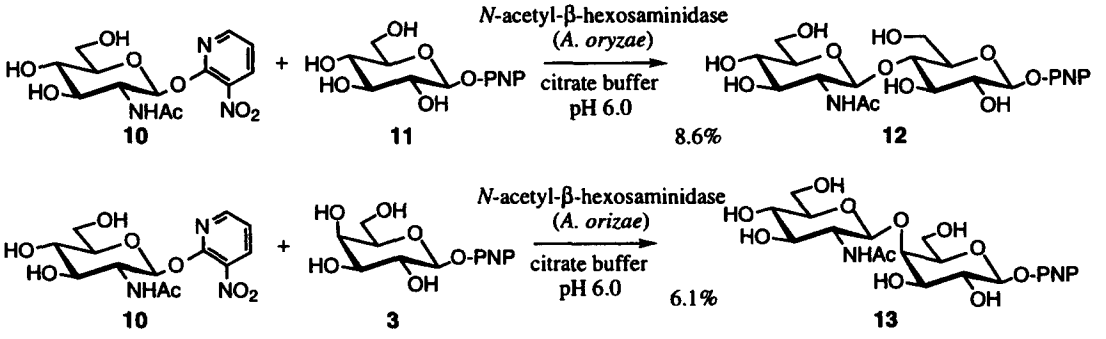
β-Glucosidase-catalyzed transglycosylation was then examined with 3-nitro-2-pyridyl β-D-glucoside (Glc-3NPy) (**7**) as a donor.[6] Glc-3NPy (**7**) also exhibits high solubility in water and high reactivity. The transglycosylation with β-glucosidase (sweet almond)[7] was effected by using 3 equiv. of **7** to Gal-PNP (**3**) under saturated conditions of **3** (100 mM) (the concentration of **7** was 300 mM) to afford the

β(1→6) disaccharide **9** selectively in 16.1% yield (Scheme 3). The yield of **9** was only 2.4% by using *p*-nitrophenyl β-D-glucoside (**11**) (Glc-PNP) as a donor under saturated conditions of **11** (100 mM) (the concentration of acceptor **3** was 33 mM).



Scheme 3.

3-Nitro-2-pyridyl *N*-acetyl-β-D-glucosaminide (GlcNAc-3NPy) (**10**) also showed much higher solubility (saturated concentration: 50 mM)[8] than GlcNAc-PNP (saturated concentration: 5 mM). The transglycosylation reactions with *N*-acetyl-β-hexosaminidase from *Aspergillus oryzae*[9] by using 2 equiv. of glycosyl acceptors Glc-PNP (**11**) or Gal-PNP (**3**) to saturated **10** gave the products **12** and **13** selectively in 8.6 and 6.1% yield, respectively (Scheme 4). The yields of the products were lower than those in the other examples but still higher than the yields obtained by using GlcNAc-PNP as a donor (**12**: 2.5% and **13**: 1.9%).



Scheme 4.

Transglycosylation reactions carried out by using highly reactive 3-nitro-2-pyridyl or 5-nitro-2-pyridyl glycosides at high concentrations, proved to be effective in improving the yields of transglycosylated products.

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3. Nitro-2-pyridyl glycosides are chemically more reactive than the corresponding nitrophenyl glycosides, since they have latent glycosyl imidate structures. 3-Methoxy-2-pyridyl glycosides, which were reported as donors for chemical glycosylation (see: Hanessian, S.; Ugolini, A.; Dubé, D.; Hodge, P. J.; André, C. *J. Am. Chem. Soc.* **1986**, *108*, 2776) also have glycosyl imidate structures and show higher solubilities than the corresponding *p*-nitrophenyl glycosides. The yields of transglycosylation of 3-methoxy-2-pyridyl galactoside were, however, lower than those of *p*-nitrophenyl galactoside.
4. Gal-3NPy (**1**) was prepared as follows. Silver 3-nitro-2-pyridoxide (**14**): To a solution of 2-hydroxy-3-nitropyridine (2.48 g, 16.1 mmol) and NaOH (0.701 g, 16.1 mmol) in water (500 ml) was added a solution of $AgNO_3$ (2.99 g, 16.1 mmol) in water (100 ml). The solution was stirred at rt for 10 min. The yellow solid was collected by filtration and washed with MeOH and ether: yield 2.15 g (94.0%). 3-Nitro-2-pyridyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**15**): To a solution of galactose pentaacetate (2.20 g, 5.79 mmol) in CH_2Cl_2 (20 ml) was added 20% HBr in acetic acid (5.8 ml) at 0°C. The solution was stirred at rt for 3 h and concentrated in vacuo. The mixture of the residue and **14** (1.75 g, 5.80 mmol) in CH_3CN (300 ml) and DMF (30 ml) was stirred at 80°C for 5 h. Insoluble materials were removed by filtration through Celite and the filtrate was concentrated in vacuo. The residue was dissolved in ether, washed with H_2O, dried over Na_2SO_4, concentrated, and purified with silica-gel column chromatography (toluene:acetone=3:1) to give a solid: yield 2.58 g (65.2%). 3-Nitro-2-pyridyl β-D-galactopyranoside (**1**): To a solution of **15** (2.15 g, 4.57 mmol) in anhydrous MeOH (180 ml) was added 1 M NaOMe in MeOH (0.20 ml). The solution was stirred at rt for 10 h and quenched by addition of dry ice. The solid obtained by concentration was crystallized from ethanol: yield 1.12 g (81.1%). Gal-5NPy (**2**) was prepared in a manner similar to the preparation of **1**.
5. A typical transglycosylation procedure is as follows. To a solution of 3-nitro-2-pyridyl galactoside (**1**) (60.0 mg, 0.199 mmol) and *p*-nitrophenyl xyloside (**4**) (18.1 mg, 0.0663 mmol) in a phosphate buffer (50 mM, pH 7.3, 33.3 μl) was added β-galactosidase (EC 3.2.1.23, *E. coli*, 25 U). After the mixture was allowed to stand at 25°C for 45 min, the reaction was stopped by addition of acetic acid (0.1 ml). The mixture was filtered and then concentrated. The residue was purified by HPLC [column: KC-PACK YM C18 20×250 mm; eluent: 18% MeCN-H_2O; flow rate: 8 ml/min; detection: 320 nm, retention time: 16.9 min (**5**) and 27.1 min (**6**)]. Lyophilization afforded Gal(β1-4)Xyl-PNP (**5**: 9.13 mg, 31.8%) and Gal(β1-3)Xyl-PNP (**6**: 1.09 mg, 3.81%) as a colorless powder.
6. Glc-3NPy (**7**) was prepared in a manner similar to the preparation of **1**. Crude **7** obtained after removal of acetyl group was purified by HP-20 column (H_2O→20% aq. MeOH→MeOH]. Glc-3NPy (**7**) was eluted at 20% aq. MeOH fractions, which were concentrated in vacuo after a few drops of Et_3N were added and lyophilized to give a powder.
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8. GlcNAc-3NPy (**10**) was prepared as follows. 3-Nitro-2-pyridyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**16**): To a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (1.51 g, 4.10 mmol) and 2-hydroxy-3-nitropyridine (0.574 g, 4.11 mmol) in CH_2Cl_2 (50 ml) were added 1 M aqueous NaOH solution (50 ml) and tetrabutylammonium bromide (0.862 g, 6.15 mmol). The solution was stirred vigorously for 50 min. The organic layer was separated, washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (toluene:acetone=4:1) to give a solid: yield 252 mg (28.1%). 3-Nitro-2-pyridyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**10**): To a solution of compound **16** (240 mg, 0.514 mmol) in anhydrous MeOH (60 ml) was added a solution of K_2CO_3 (10 mg, 0.072 mmol) in MeOH (1 ml) under Ar atmosphere. After 2 h, the reaction was

stopped by the addition of dry ice and the solution was concentrated in vacuo. The residue was purified by HPLC [column: KC-PACK YM C18 20×250 mm; eluent: 12% MeCN–H_2O; flow rate: 8 ml/min; detection: 320 nm, retention time 17.5 min) and lyophilized to give **10** as a powder: yield: 148 mg (84.1%).

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