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3-Nitro-2-pyridyl glycoside as donor for chemical glycosylation and its application to chemoenzymatic synthesis of oligosaccharide

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Abstract

3-Nitro-2-pyridyl (3NPy) glycosides, which act as versatile glycosyl donors in enzymatic transglycosylation, can also be chemically activated. Chemical glycosylation with protected 3NPy glycosides was readily effected at –20°C by using TMSOTf as a catalyst to afford the desired glycosides in good yields. A trisaccharide serine conjugate Gal(β1–3)Gal(β1–4)Xylβ-Ser was synthesized by combined use of enzymatic and chemical glycosylations. The trisaccharide 3NPy glycoside was prepared by stepwise transglycosylation of a Gal-3NPy donor to a Xyl-3NPy acceptor by using a β-galactosidase. After protection of the free hydroxy groups of the trisaccharide by acetylation, the 3NPy glycoside was then subjected to chemical glycosylation of a serine residue to afford the trisaccharide serine conjugate.

Keywords: glycosylation; glycosidase; glycosyl donor; 3-nitro-2-pyridyl glycoside; chemoenzymatic synthesis.

Enzymatic transglycosylation has often been successfully applied to synthesis of simple oligosaccharides generally in short reaction steps without or with minimal use of conventional protecting groups. Construction of complex structures is, however, not always easy by the enzymatic procedure alone. By contrast, a chemoenzymatic method, which utilizes the merits of both chemical and enzymatic procedures, is an attractive approach for synthesis of complex oligosaccharides and glycoconjugates.[1] We describe here a new chemical glycosylation by using 3-nitro-2-pyridyl (3NPy) glycosides as donors and its application to chemoenzymatic synthesis of Gal(β1–3)Gal(β1–4)Xylβ-Ser (**11**) which corresponds to the linkage region between glycosaminoglycan and protein in proteoglycan.[2]

In the preceding paper,[3] we reported that 3NPy or 5-nitro-2-pyridyl (5NPy) glycosides were useful donors for transglycosylation catalyzed by glycosidases. We expected that these glycosides in their fully protected form would serve as donors for chemical glycosylation catalyzed by Lewis acids, since these

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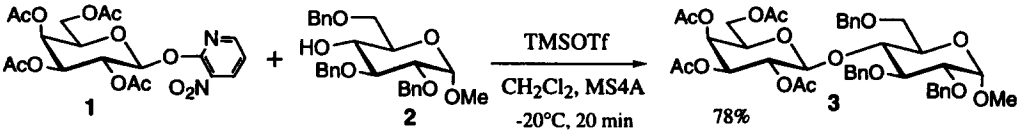
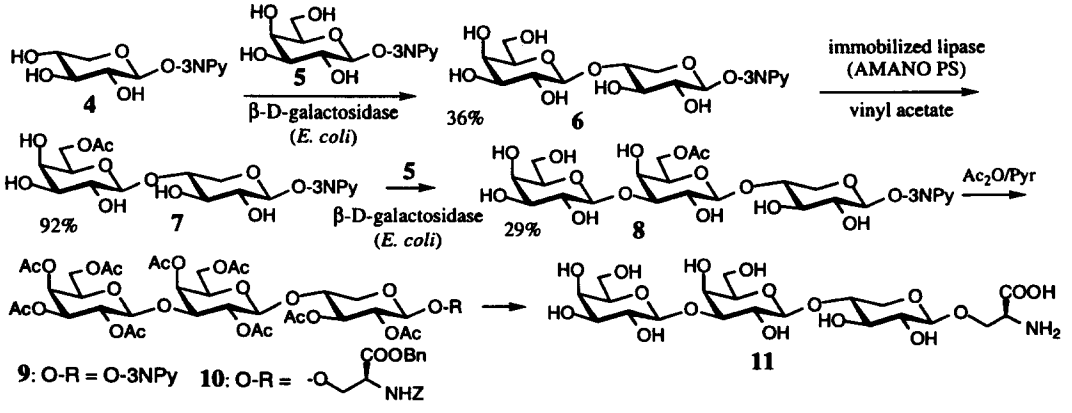
molecules have latent glycosyl imidate structures.[4] In fact, the glycosylation reaction of **2** with 3-nitro-2-pyridyl galactoside **1** smoothly proceeded at –20°C by the use of 0.1 equiv. of TMSOTf as a catalyst to give the desired disaccharide **3** in 78% yield. Interestingly, however, the corresponding 5-nitro-2-pyridyl galactoside was not activated even by the use of 3.0 equiv. of trimethylsilyl triflate (TMSOTf) (Scheme 1).



Scheme 1.

Since 3NPy glycosides had also been shown to be good acceptors in enzymatic glycosylations, the following new, simple but efficient approach should be realized: enzyme-aided preparation of an oligosaccharide 3NPy glycoside and its direct chemical activation to lead to a more complex glycoconjugate. This new strategy was applied to the synthesis of Gal(β1–3)Gal(β1–4)Xylβ-Ser (**11**) (Scheme 2).[2] The transglycosylation reaction with β-galactosidase (*Escherichia coli*) was carried out in a phosphate buffer (0.05 M, pH 7.3) by the use of 3-nitro-2-pyridyl galactoside (Gal-3NPy) (**5**) (600 mM) as a donor and 3-nitro-2-pyridyl xyloside (**4**) (200 mM) as an acceptor.[3,5] The desired disaccharide Gal(β1–4)Xylβ-3NPy (**6**) was obtained in 36% yield with perfect regioselectivity. The reactive 6′-hydroxy group of **6** was selectively protected with an acetyl group by using immobilized lipase as described previously to give 6-*O*-acetylated **7** in 92% yield.[3,6] Regioselective transglycosidation to the 3′-position was then effected by the use of Gal-3NPy (**5**) (5 equiv.) in a phosphate buffer (0.05 M, pH 7.3) at rt for 35 min under saturated conditions of **5** (600 mM). The desired trisaccharide **8** was successfully obtained in 29% yield with 54% recovery of **7**. After peracetylation of **8**, protected 3NPy glycoside **9** was directly coupled with Z-L-Ser-OBn by the use of TMSOTf (0.3 equiv.) in CH_2Cl_2 to give the protected serine conjugate **10** in 75% yield.[7] All the protecting groups of **10** were then removed by catalytic hydrogenation (H_2/Pd) and subsequent hydrazinolysis to give the desired Gal(β1–3)Gal(β1–4)Xyl(β)-L-Ser (**11**).[2] The present novel chemoenzymatic synthesis is much more straightforward than our previous work and thus opens an efficient way to complex oligosaccharides.



Scheme 2.

Acknowledgements

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2. We previously reported chemoenzymatic synthesis of Gal(β1–3)Gal(β1–4)Xylβ-Ser (**11**) via a trisaccharide *p*-nitrophenyl (PNP) glycoside which was converted, after selective cleavage of the PNP glycoside, to a glycosyl imidate for chemical coupling with serine: Fukase, K.; Yasukochi, T.; Suda, Y.; Yoshida, M.; Kusumoto, S. *Tetrahedron Lett.* **1996**, *37*, 6763.
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4. Glycosylation using imidates: (a) Pougny, J.-R.; Jacquinet, J.-C.; Nassr, M.; Duchet, D.; Milat, M.-L.; Sinaÿ, P. *J. Am. Chem. Soc.* **1977**, *99*, 6762. (b) Shoda, S.; Mukaiyama, T. *Chem Lett.* **1979**, *847*. (c) Schmidt, R. R.; Grundler, G.; *Synthesis* **1981**, 885. (d) Hanessian, S.; Ugolini, A.; Dubé, D.; Hodge, P. J.; André, C. *J. Am. Chem. Soc.* **1986**, *108*, 2776.
5. A typical transglycosylation procedure: To a solution of Gal-3NPy (**5**) (63.0 mg, 0.199 mmol) and Xyl-3NPy (**4**) (19.0 mg, 0.0699 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[3] to give **6** (10.4 mg, 36.1%) as colorless powder.
6. Yasukochi, T.; Fukase, K.; Takagaki, K.; Endo, M.; Kusumoto, S. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 2719. Acetylation using lipase: To a suspension of disaccharide **6** (30.0 mg, 0.0691 mmol) in THF (25 ml) and vinyl acetate (25 ml) was added immobilized lipase (Amano PS) (0.3 g). The mixture was stirred at rt for 48 h and then filtered. After the filtrate was concentrated in vacuo, the residue was purified by HPLC[3] to give **7** (30.1 mg, 31.6%) as colorless powder.
7. A typical chemical glycosylation procedure: To a solution of the trisaccharide **9** (9.01 mg, 9.24 μmol), Z-L-Ser-OBn (3.65 mg, 11.1 μmol), and molecular sieves 4A (50 mg) in CH_2Cl_2 (0.5 ml) was added TMSOTf (0.5 μl, 0.277 μmol) in CH_2Cl_2 (50 μl) at −10°C. The solution was stirred at −10°C. for 2.5 h and worked up as usual. The residue was purified by preparative silica gel TLC ($CHCl_3$:acetone=7:1) to give **10** (8.02 mg, 74.9%) as colorless powder.