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A Novel Method for Synthesis of Chitobiose via Enzymatic Glycosylation Using a Sugar Oxazoline as Glycosyl Donor

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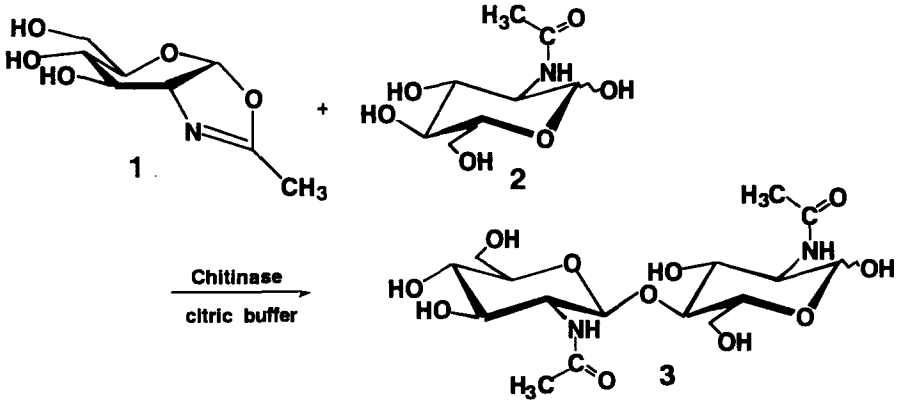
Abstract: A convenient method for synthesis of *N,N'*-diacetylchitobiose, an important building block for oligo and polysaccharide synthesis, has been developed by using a 1,2-oxazoline derivative of *N*-acetylglucosamine as new glycosyl donor for chitinase. © 1997 Elsevier Science Ltd.

N,N'-Diacetylchitobiose **3**, the smallest repeating unit of chitin, has been widely used as an important building block for synthesis of various complicated oligo and polysaccharides.[1] However, this compound is expensive and difficult to prepare. The preparation of **3** thus far has been through chemical,[2a,b] microbial,[2c,d] or enzymatic[2e] chitin degradation. Here, we report on a versatile synthetic method for preparing *N,N'*-diacetylchitobiose based on the coupling of two *N*-acetylglucosamine moieties. The coupling reaction occurred by combining a sugar oxazoline derivative **1** as a new glycosyl donor and *N*-acetylglucosamine **2** as a glycosyl acceptor for chitinase (from *Bacillus* sp.), a hydrolytic enzyme of chitin. The resulting *N,N'*-diacetylchitobiose was isolated as a peracetylated derivative after simple purification processes.



The oxazoline derivative **1** can be easily prepared starting from *N*-acetylglucosamine via three steps: conversion to peracetylated glycosyl chloride, formation of oxazoline ring by the action of a base in the presence of ammonium chloride, and removal of the acetyl group.[3] The substrate **1** was treated with an excess of *N*-acetylglucosamine in the presence of chitinase at pH 7.8. The resulting **3** was acetylated by acetic anhydride/pyridine. In this process, the excess **2** was also converted to the corresponding peracetate which

could be recovered for synthesis of **1** or **2**.

When only **2** was treated with chitinase under similar reaction conditions, no coupling product could be detected at all, clearly indicating that the usage of the distorted oxazoline moiety is essential for the promotion of the coupling reaction. The ^{1}H NMR spectrum of the reaction mixture of the acetylated products shows signals at 4.46 ppm (d, J = 8.5 Hz) and 3.74 ppm (dd, J = 9.4 Hz) assignable to the H-1' and H-4, respectively. Other signals derived from the isomeric β(1→3) or β(1→6) bond could not be observed. These results indicate that the control of regio and stereochemistry concerning the glycosidic bond formation is perfect, leading to the β(1→4) glycosidic linkage. The perfect regioselectivity shows that the recognization of chitinase towards **2** is very strong. The observed stereoselectivity can be explained by assuming the initial formation of an oxazolinium intermediate[4] followed by the attack of the 4-hydroxy group of **2** from β-face.

It is to be noted that the usage of the distorted monomer **1** whose conformation is close to that of the transition state of enzymatic hydrolysis enabled us to couple the two *N*-acetylglucosamine moieties effectively. The present method provides a sufficient amount of *N, N'*-diacetylchitobiose peracetate on a laboratory scale using experimental procedures that are very simple. All starting materials are inexpensive and readily available. By employing this method, it will be possible to prepare an *N,N'*-diacetylchitobiose derivative having a labeled atom in the *N*-acetylglucosamine unit, which cannot be realized by conventional methods including the degradation of natural chitin. This is the first example of enzymatic glycosylation using a sugar oxazoline derivative as glycosyl donor catalyzed by a glycosidase, which will further be applied to the regio and stereoselective synthesis of chitooligosaccharides with higher molecular weights.

Typical procedure for the preparation of *N,N'*-diacetylchitobiose hexaacetate: To a solution of **1** (135 mg, 0.67 mmol) in citric buffer (0.05 M, pH 7.8) (1.9 mL) was added a solution of *N*-acetylglucosamine (474 mg, 2.1 mmol) and chitinase (*Bacillus* sp.) (10 wt % to **1**) in citric buffer (0.05 M, pH 7.8) (2.0 mL), and the reaction mixture was stirred at 25 °C for 6 h . The resulting mixture was freeze dried and the residue was directly acetylated by using acetic anhydride (3 mL) in pyridine (6 mL) for 48 h. The reaction mixture was concentrated to dryness and the residue was washed with hot ethyl acetate (15 mL) and dried in vacuo to give 195 mg (43 %) of pure *N,N'*-diacetylchitobiose hexaacetate.

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References and Note

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3. NMR spectra of **1** are as follows. ^{1}H NMR (D_2O): δ 6.07 (d, $J_{1,2}$ = 7.25 Hz, 1H, H-1), 4.11 (1H, H-2), 3.96 (1H, H-3), 3.8-3.3 (4H, H-4,5,6), 2.03 (3H, methyl). ^{13}C NMR (D_2O): δ 168.9 (N=**C**), 101.3 (C-1), 73.8 (C-3), 72.5 (C-5), 69.5 (C-4), 66.7 (C-2), 62.5 (C-6), 13.8 (**C**H_3).
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