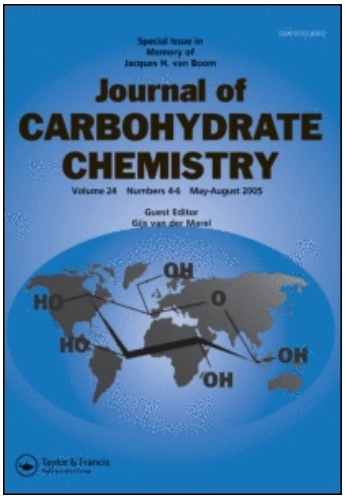

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LARGE-SCALE PREPARATION OF *N,N'*-DIACETYLCHITOBIOSE BY ENZYMIC DEGRADATION OF CHITIN AND ITS CHEMICAL MODIFICATIONS

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ABSTRACT

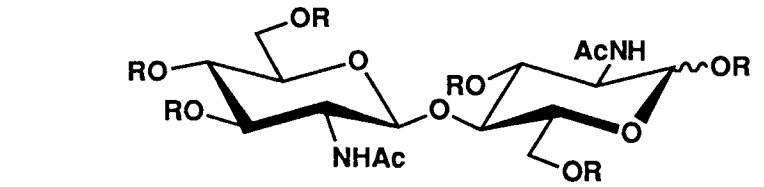
Hundred gram quantities of peracetylated chitobiose were prepared by degradation of colloidal chitin with commercially available chitinase (EC 3.2.1.14) from *Streptomyces griseus* and subsequent acetylation, the product being readily convertible into *N,N'*-diacetylchitobiose by conventional de-*O*-acetylation. These chitobiose derivatives were subjected to further chemical modifications to give novel disaccharide derivatives composed of a pair of 2-acetamido-2-deoxy-D-allopyranose moieties that are potential intermediates for the synthesis of an enzyme inhibitor.

INTRODUCTION

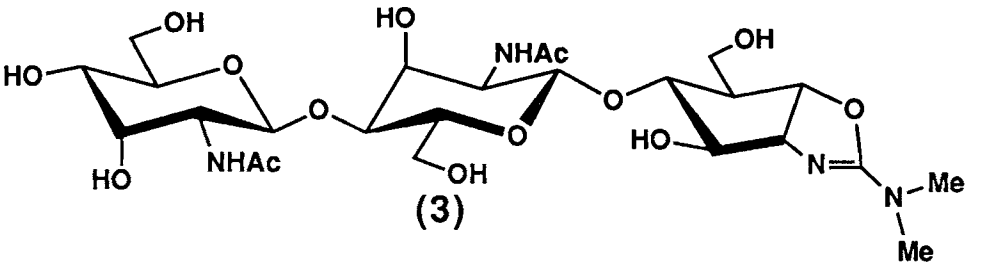
In the course of our studies on utilization of simple oligosaccharides as key starting materials for syntheses of biologically important compounds, we have reported successful conversions of several α- or β-(1→4)-linked and β-(1→3)-linked di- and trisaccharides, such as maltose, cellobiose, *N,N'*-diacetylchitobiose, laminaribiose, and maltotriose.[1-13]

These starting oligosaccharides have been produced by degradation of abundant natural or microbially produced polysaccharides such as starch, cellulose, chitin, curdlan, and pullulan. In the case of *N,N'*-diacetylchitobiose (**1**), however, its production has met difficulties because of the low efficiency or the poor reproducibility of chemical[14-16] or microbial[17] degradations of chitin. In particular, the large-scale preparation of **1** has been technically difficult.

This paper describes an efficient large-scale preparation of peracetylated **1** (**2**) through restricted degradation of chitin using a commercially available microbiologically produced chitinase followed by chemical peracetylation. De-*O*-acetylation of **2** by the Zemplen procedure gave **1** almost quantitatively. Compound **2** was further chemically modified to give derivatives of *O*-(2-acetamido-2-deoxy-β-D-allopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-allopyranose, a key component of an insect chitinase inhibitor, allosamidin **3**.[18]



(1) R=H
(2) R=Ac



(3)

RESULTS AND DISCUSSION

Hitherto, varieties of chitinase (EC 3.2.1.14) have been isolated from various sources such as plants, microorganisms, and insects etc.[19] Some such microbial enzymes are commercially available and have served in structural elucidation of fungous cell wall and for the preparation of protoplast.[19] To our knowledge, however, none of these enzymes have been

used for synthetic purposes such as the preparation of **1** from chitin. In order to attain such purpose, we employed chitinase derived from *Streptomyces griseus*, one of the most potent enzymes among various chitinases. As a result, we were able to overcome difficulties of handling a big jar-fermentor and monitoring the reaction process for preparation of **1**, and avoid uncertainty of process reproducibility, problems which we had encountered in a previous fermentation proccess with chitin.[17]

As the substrate for the enzymic degradation, "colloidal chitin" was prepared in the same way as described in the previous paper.[17] At this stage, partial de-*N*-acetylation might occur to some degree concomitantly with depolymerization of chitin. Before attempting a large-scale preparation of **1**, a preliminary small-scale test was conducted. The incubation was carried out at 40 °C with stirring in acetic acid-phosphate buffer adjusted to pH 6.3 according to the previous literature.[20] In order to make a graph showing the pattern of the hydrolyzate production, aliquots of incubation mixture were removed every 2 days for the subsequent work-up. Thus, each aliquot was heated at 100 °C and filtered to remove the unchanged colloidal chitin. The filtrate was concentrated, acetylated, and products separated by column chromatography. The weights of **2** and a mixture of by-products are plotted against time in Figure 1. For comparison, the incubation was also conducted at pH 4.75 and the results are plotted in the same way in Figure 1. Under both conditions, production of **1** was maximized after several days of incubation. As expected, higher yields of **1** were obtained at pH 6.3 than at pH 4.75, whereas almost the same amount of by-products was obtained at both pHs. HPLC analyses of the incubation mixture identified one of the by-products as 2-acetamido-2-deoxy-D-glucose, its weight being about 20% of **1**. The by-product mixture isolated after peracetylation included a couple of unidentified compounds in addition to expected 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-D-glucose. As all by-products moved on TLC similarly to each other but differently from **2** , they are most likely monosaccharide derivatives.

On the basis of these results from the preliminary experiments, the preparation of **2** was scaled up. After incubation of "colloidal chitin" with chitinase at 40 °C for 10 days with vigorous stirring, the unreacted substrate was filtered off and the filtrate was concentrated to dryness. The residue was heated at 80 °C with acetic anhydride and sodium acetate and

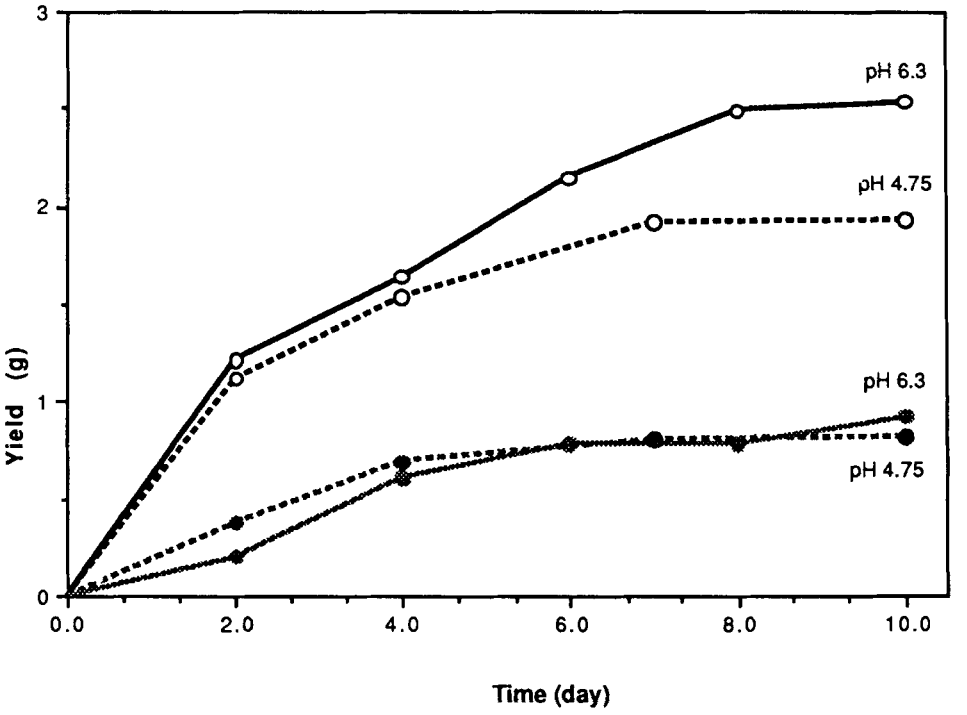
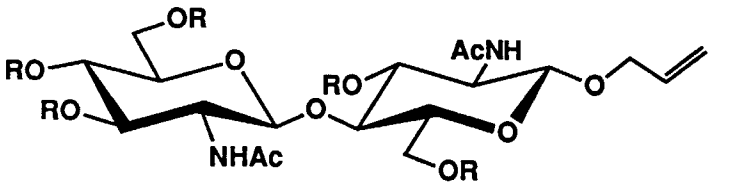


Fig. 1. Production Pattern of Peracetylated Chitin Hydrolyzates.

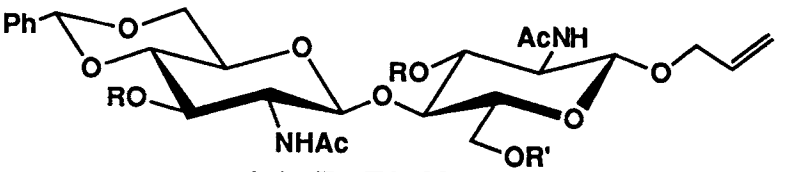
poured into ice-water to give a precipitate. When the resulting precipitate was dissolved in methanol, most of **2** crystallized as the α-anomer. After removal of the crystals by filtration, an α, β- mixture of **2** was further isolated from the mother liquor by column chromatography. Usually 200-250 g of **2** was obtained in one incubation run using 5000 units of enzyme. The yield of **2** varied in a moderate range depending on the batches of enzyme used but was not so much influenced by change of pHs employed in the range of 5.5-6.3.

Colloidal chitin (21.4 g with more than 85% of H_2O) was incubated with the enzyme (12.6 mg) at 40 °C in 200 mL of 0.1M acetic acid-phosphate buffer (pH 6.3 and 4.75). After filtration, the soluble degradation products were acetylated at 80 °C with Ac_2O-NaOAc and chromatographed on silica gel using 30 : 1($^v/_v$) $CHCl_3$-MeOH as the eluant. Solid and dotted lines with open circles show changes of the weight of peracetylchitobiose (**2**) isolated. The lines with filled circles correspond to the weight of combined monosaccharidic by-products involving peracetylated D-glucosamine.

Regarding chemical manipulations of **1**, we already reported several selective protections, configurational inversion of the 4'-hydroxyl group, and the use of derivatives of **1** for the preparation of a peripheral trisaccharide sequence of a hormonal glycoprotein.[10] Allyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside **4** was prepared in that process. Now, further chemical manipulations have been conducted starting from **4** in preparing the per-*O*-benzyl derivative (**5**) of **4** and its homolog (**10**), each with a D-*allo* configuration. Compound **10** was required for the synthesis of a potent insect-chitinase inhibitor **3** as one of the key intermediates and **5** was also necessary for syntheses directed towards analogs of **3**. Per-*O*-benzylation of **4** was performed at room temperature by treatment with benzyl bromide-barium oxide-barium hydroxide in *N,N*-dimethylformamide (DMF). More than 5 days of such treatment using a large excess of the reagents was needed to complete the reaction since some hydroxyl groups in **4** resisted benzylation. Usual work-up and chromatographic purification of the products gave **5** as crystals. On the other hand, **4** was benzylidenated by the Evans procedure[21] for the synthesis of **10**. The resulting allyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**6**) underwent selective tritylation of the primary hydroxyl group. Thus, **6** was heated at 60 °C for 6 days with trityl chloride in

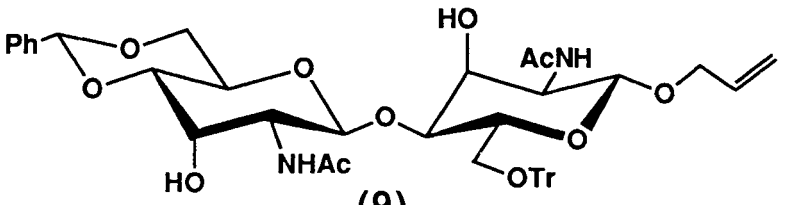


(4) R=H
(5) R=Bn

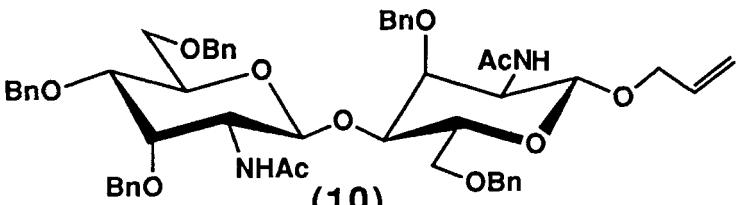


(6) R=R'=H
(7) R=H, R'=Tr
(8) R=Ms, R'=Tr

pyridine-DMF mixture in the presence of *p*-dimethylaminopyridine (DMAP), giving the 6-*O*-trityl derivative **7**. Mesylation of the two unprotected hydroxyl groups of **7** was carried out in the usual way with mesyl chloride-pyridine, giving the dimesylate (**8**) in very good yield. For configurational inversion from D-*gluco* to D-*allo*, **8** was subjected to solvolysis by treatment with aqueous methoxyethanol containing sodium acetate. The reaction proceeded smoothly, giving allyl *O*-(2-acetamido-4, 6-*O*-benzylidene-2-deoxy-β-D-allopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-allopyranoside (**9**) in good yield. The NMR spectrum of **9** revealed the C-3 and C-3' protons at δ 4.08 ($J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 2.9 Hz) and δ 3.99 ($J_{2',3'}$ = 3.0 Hz, $J_{3',4'}$ = 2.5 Hz), establishing formation of the *allo* configuration in each monosaccharide unit. After **9** was treated with aqueous acetic acid for removal of the benzylidene and trityl groups, the resulting pentaol was benzylated as described for the preparation of **5**. Again, some hydroxyl groups did not undergo benzylation readily with benzyl bromide-barium oxide-barium hydroxide-DMF. However, using potassium hydroxide as the base for the benzylation instead of barium oxide-barium hydroxide resulted in a good yield of **10**.



(9)



(10)

EXPERIMENTAL

General Methods and Materials. Melting points were determined with a Yamato micro melting point apparatus, and are uncorrected.

Optical rotations were determined with a JASCO Model DIP-370 polarimeter. ^1H NMR spectra were recorded at 500 MHz with a JEOL JNM-GX 500 spectrometer as solutions in $CDCl_3$, unless otherwise specified. HPLC was performed with a Kaseisorb LC NH_2-300-5 (4.6 × 300 mm) column in a Waters liquid chromatograph equipped with a UVIDEC 100 UV photometer. Elution was effected with acetonitrile-water (7:3, v/v) pumped at 1.0 mL/min. Column chromatography was performed on columns of silica gel (70-230 mesh; E. Merck, Darmstadt, Germany). Chitin (from *Chionoecetes japonicus* or *Chionoecetes opilio*) was purchased from Katakura Chikkarin Co. Ltd. and used without purification. Chitinase (EC 3.2.1.14, from *Strepomyces griseus* 4 units/mg solid) was purchased from SIGMA chemical company. Buffer solutions were prepared by mixing 0.2M acetic acid and 0.2M disodium hydrogen phosphate in appropriate ratios.

Preparation of "colloidal chitin". Colloidal chitin was prepared by treatment of chitin with 12M hydrochloric acid in the same way as described in the previous paper.[17] The product was collected on a Buchner funnel and after being washed the resulting salt-free, cake-like, colloidal chitin was used for the enzymic degradation or stored at 0 °C. This cake-like material was estimated to contain more than 85% water because it always lost 86-87% of its weight when heated at 80 °C for 4 h.

***O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose (2).** To a suspension of colloidal chitin (2.1 Kg) in a mixture of buffer solution of pH 6.3 (10 L) and water (10 L) was added chitinase (1.26 g). The mixture was vigorously stirred at 40 °C for 10 days and filtered. The filtrate was concentrated *in vacuo* to dryness. The residue was heated at 80 °C overnight with acetic anhydride (2.5 L) and anhydrous sodium acetate (180 g) with vigorous stirring. After most of acetic anhydride had been removed by evaporation, the residual syrup was poured into ice-water (1.5 L) and the mixture was vigorously stirred for 4 h. The resulting precipitate was filtered and dissolved in chloroform (3 L). The solution was washed with water, dried (Na_2SO_4), and concentrated to dryness to give a pale brown mass. When the mass was dissolved in hot methanol, α–**2** was obtained as crystals (ca. 150 g) on cooling. After filtration, the mother liquor was concentrated and chromatographed with chloroform-methanol (40:1, v/v) as eluant, giving

more α-**2** (48 g) and β-**2** (4 g). α-**2**: mp 303-304 °C (dec); $[\alpha]_D^{20}$ +56° (*c* 0.50, acetic acid), [ref. 17, mp 301-303 °C (dec), $[\alpha]_D^{30}$ +56° (*c* 0.52, acetic acid)]; ^{1}H NMR δ 6.10 (1H, d, $J_{1,2}$ = 3.7 Hz, H-1). β-**2**: mp 226-228 °C (dec); $[\alpha]_D^{19}$ -29° (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 1.94, 1.99, 2.02, 2.03, 2.09, 2.11, 2.13 (24H, each s, Ac), 3.63 (1H, brq, $J_{1',2'}$ = 8.6 Hz, $J_{2',NH'}$ = 8.0 Hz, $J_{2',3'}$ = 10 Hz, H-2'), 3.70 (1H, ddd, $J_{4',5'}$ = 8.0 Hz, $J_{5',6'a}$ = 2.1 Hz, $J_{5',6'b}$ = 4.6 Hz, H-5'), 3.76 (1H, ddd, $J_{4,5}$ = 7.9 Hz, $J_{5,6a}$ = 4.9 Hz, $J_{5,6b}$ = 2.4 Hz, , H-5), 3.83 (1H, dd, $J_{3,4}$ = 8.2 Hz, H-4), 4.05 (1H, dd, $J_{6'a,6'b}$ = 12 Hz, H-6'a), 4.19 (1H, dd, $J_{6a,6b}$ = 12 Hz, H-6a), 4.34 (1H, brq, $J_{1,2}$ = 7.9 Hz, $J_{2,NH}$ = 7.5 Hz, $J_{2,3}$ = 9.2 Hz, H-2), 4.39 (1H, dd, H-6b), 4.40 (1H, dd, H-6'b), 4.80 (1H, d, H-1'), 5.06 (1H, dd, $J_{3',4'}$ = 9.7 Hz, H-4'), 5.12 (1H, dd, H-3), 5.34 (1H, dd, H-3'), 5.51 (1H, H-1), 7.29~7.24 (2H, m, NH, NH').

Anal. Calcd for $C_{28}H_{40}N_2O_{17}$: C, 49.70; H, 5.96; N, 4.14. Found: C, 49.39; H, 5.97; N, 4.15.

***O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (*N,N'*-diacetylchitobiose, 1).** Sodium methoxide (0.1 g, 1.9 mmol) was added to a solution of **2** (1.0 g, 1.5 mmol) in dry methanol (70 mL) at 0 °C, and the mixture was stirred at room temperature for 3h, treated with Dowex-50W X-8 (H+) resin, and the suspension was filtered. The filtrate was concentrated to give **1** (0.60 g, 96%): mp 250 °C < (dec, aq. MeOH) [ref. 22a, mp 245~247 °C (dec, aq. MeOH), ref. 22b, mp 260~262 °C (dec, aq. MeOH)]; $[\alpha]_D^{27}$ +16° (*c* 1.0, H_2O, equilibrium) [ref. 22a, $[\alpha]_D^{25}$ +18.5° (*c* 1.0, H_2O, equilibrium), ref. 22b, $[\alpha]_D$ +17.2° (*c* 0.5, H_2O, equilibrium), ref. 22c, $[\alpha]_D^{30}$ +16.0° (*c* 1.0, H_2O, equilibrium)].

Allyl *O*-(2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (5). To a solution of **4** (tetrahydrate, 1.36 g, 2.5 mmol) in DMF (50 mL) were added barium oxide (7.97 g, 52 mmol), barium hydroxide octahydrate (7.95 g, 25.2 mmol), and benzyl bromide (6.0 mL, 50.4 mmol). After the mixture was stirred at room temperature for 3 days, additional barium oxide (8.0 g), barium hydroxide octaacetate (8.5 g), and benzyl bromide (6.0 mL) were added and stirring was continued at room temperature for 2 days. The mixture was diluted with ethyl acetate and filtered. The filtrate was washed with water, dried, concentrated, and chromatographed with chloroform-methanol (100:1, v/v) as eluant, giving crystalline **5** (2.07 g, 89%): mp 203 °C (EtOH); $[\alpha]_D^{18}$ -31° (*c* 0.5, $CHCl_3$); ^{1}H NMR δ 1.73 (3H, s,

NHAc), 1.96 (3H, s, NHAc), 3.33 (1H, brd, $J_{4,5}$ = 9.5 Hz, H-5), 3.48 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3), 3.62~3.80 (5H, m, H-5', H-6a, H-6b, H-6'a and H-6'b), 3.70 (1H, dd, H-4), 3.72~3.84 (2H, m, H-2 and H-4'), 3.95~4.00 (2H, m, H-3' and allyl), 4.09~4.14 (1H, m, H-2'), 4.26 (1H, dd, J = 13, 5.0 Hz, olefine), 4.29 (1H, d, $J_{1,2}$ = 8.6 Hz, H-1), 4.43~4.64 (8H, m, H-1' and ϕCH_2), 4.72~4.83 (4H, m, NH and ϕCH_2), 5.11 (1H, brd, J = 11 Hz, olefine), 5.22 (1H, dd, J = 17, 1.5 Hz, olefine), 5.81~5.87 (1H, m, olefine), 6.44 (1H, brd, $J_{2',NH'}$ = 8.9 Hz, NH'), 7.20~7.35 (25H, m, Ar).

Anal. Calcd for $C_{54}H_{62}N_2O_{11}$: C, 70.88; H, 6.83; N, 3.06. Found: C, 70.83; H, 6.77; N, 3.12.

Allyl *O*-(2-Acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-6-*O*-trityl-β-D-glucopyranoside (7). To a solution of **4** (4.64 g, 10 mmol) in DMF (30 mL) were added α,α-dimethoxytoluene (2.28 g, 15 mmol) and d-camphor sulfonic acid (116 mg, 0.5 mmol). After the mixture was stirred at 55-60 °C under reduced pressure (15-20 mmHg) for 5.5 h, additional α,α-dimethoxytoluene (1.11 g, 7.3 mmol) and d-camphor sulfonic acid (116 mg, 0.5 mmol) were added and stirring was continued for 10.5 h. The resulting mixture was then cooled, treated with Dowex-1 X-8 (OH-) resin and filtered. The filtrate was concentrated to give syrupy **6** (6.52 g). To a solution of **6** (6.52 g) in pyridine (70 mL) and DMF (6 mL) was added trityl chloride (6.58 g, 23.6 mmol) and p-*N,N*-dimethylaminopyridine (1.44 g, 11.2 mmol). The mixture was stirred at 60 °C for 6 days under Ar, cooled, diluted with MeOH and concentrated. The residue was dissolved in dichloromethane and the resulting solution was washed with aq. $NaHCO_3$, water, and brine and dried over $MgSO_4$. After concentration of the solution, the residue was chromatographed on silica gel using 50 : 3 : 1 (v/v) chloroform-methanol-triethylamine as the eluant, to give crystalline **7** (6.16 g, 78%): mp 273~274 °C (dec, EtOH); $[\alpha]_D^{28}$ -35° (*c* 1.0, $CHCl_3$); ^1H NMR δ 1.59 (3H, s, NHAc), 2.02 (3H, s, NHAc), 2.98 (1H, dd, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 10 Hz, H-6a), 3.20 (1H, d, $J_{3',OH}$ = 4.0 Hz, OH), 3.25 (1H, dt, $J_{5',6'b}$ = 4.9 Hz, $J_{4',5'}$ = $J_{5',6'a}$ = 9.5 Hz, H-5'), 3.38 (1H, dt, $J_{2',3'}$ = $J_{3',4'}$ = 9.5 Hz, H-3'), 3.42 (1H, dd, $J_{4,5}$ = 9.8 Hz, H-5), 3.47 (1H, brs, OH), 3.49 (1H, t, H-4'), 3.71 (1H, dd, $J_{6'a,6'b}$ =10 Hz, H-6'a), 3.74 (1H, d, H-6b), 3.79 (1H, t, $J_{2,3}$ = $J_{3,4}$ = 10 Hz, H-3), 3.89 (1H, q, $J_{1',2'}$ = 8.5 Hz, $J_{2',NH}$ = 9.6 Hz, H-2'), 3.91 (1H, q, $J_{1,2}$ = 7.9 Hz, $J_{2,NH}$ = 8.5 Hz, H-2), 4.07 (1H, dd, H-4), 4.14~4.18 (1H, m, allyl), 4.16 (1H, d, NH), 4.27 (1H, d, H-1'), 4.36~4.44 (1H, m, allyl),

4.40 (1H, dd, $J_{6'a,6'b}$ = 10 Hz, H-6'b), 4.59 (1H, d, H-1), 5.20 (1H, d, J = 11 Hz, olefine), 5.30 (1H, d, J = 7.4 Hz, olefine), 5.50 (1H, s, ϕCH), 5.9~6.0 (1H, m, olefine), 5.99 (1H, d, NH'), 7.29~7.37 (11H, m, Ar), 7.47~7.53 (9H, m, Ar).

Anal. Calcd for $C_{45}H_{50}N_2O_{11}\cdot H_2O$: C, 66.49; H, 6.45; N, 3.45. Found: C, 66.41; H, 6.19; N, 3.49.

Allyl *O*-(2-Acetamido-4,6-*O*-benzylidene-2- deoxy-β-D-allopyranosyl)-(1→4)-2-acetamido-2-deoxy-6-*O*-trityl-β-D-allopyranoside (9). To a stirred solution of **7** (3.0 g, 3.78 mmol) in pyridine (20 mL) was added methanesulfonyl chloride (1.12 g, 9.78 mmol) at 0 °C under Ar, and the mixture was stirred for 22 h. The resulting solution was poured into ice-water and extracted with chloroform. The extracts were washed with aq. $NaHCO_3$, water, and brine, dried over $MgSO_4$ and concentrated. The residual syrup was passed through a short silica gel column with chloroform-ethyl acetate-triethyl amine (100 : 200 : 1) as the eluant to give syrupy **8** (3.36 g, 94%), which was used for the next reaction without further purification. A mixture of dimesylate **8** (3.36 g, 3.53 mmol) and sodium acetate (4.52 g, 55.1 mmol) in methoxyethanol (143 mL) and water (7.5 mL) was stirred at 120 °C for 40 h under Ar and cooled. After removing the solvent, the residue was diluted with water, and extracted with dichloromethane. The extracts were washed with water and brine, dried over $MgSO_4$ and concentrated. The residual syrup was chromatographed on silica gel using 200 : 5 : 1 (v/v/v) chloroform-methanol-triethylamine to give **9** (2.33 g, 83%): mp 185~186 °C (EtOH); $[\alpha]_D^{28}$ -43° (*c* 1.0, $CHCl_3$); ^1H NMR δ 1.46 (3H, s, NHAc), 1.99 (3H, s, NHAc), 2.34 (1H, brs, OH), 2.79 (1H, brd, OH), 3.50 (1H, dd, $J_{3',4'}$ = 2.5 Hz, $J_{4',5'}$ = 8.7 Hz, H-4'), 3.55 (1H, dd, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 10 Hz, H-6a), 3.67~3.78 (4H, m, H-6b, H-6'a, H-5 and H-5'), 3.91 (1H, ddd, $J_{1',2'}$ = 8.2Hz, $J_{2',NH'}$ = 9.5 Hz, $J_{2',3'}$ = 3.0 Hz, H-2'), 3.99 (1H, dd, H-3'), 4.04 (1H, dd, J = 13, 6.0 Hz, allyl), 4.08 (1H, dd, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 2.9 Hz, H-3), 4.22 (1H, dd, $J_{4,5}$ = 9.0 Hz, H-4), 4.27 (1H, ddd, $J_{1,2}$ = 7.6 Hz, $J_{2,NH}$ = 9.5 Hz, H-2), 4.27~4.31 (1H, m, allyl), 4.40 (1H, brd, H-6'b), 4.60 (1H, d, H-1), 4.68 (1H, d, H-1'), 5.09 (1H, dd, J = 10, 1.5 Hz, olefine), 5.19 (1H, dd, J = 17, 1.5 Hz, olefine), 5.34 (1H, d, NH'), 5.50 (1H, s, ϕCH), 5.84 (1H, m, olefine), 6.16 (1H, d, NH), 7.20~7.49 (20H, m, Ar).

Anal. Calcd for $C_{45}H_{50}N_2O_{11}\cdot H_2O$: C, 66.49; H, 6.45; N, 3.45. Found: C, 66.61; H, 6.56; N, 3.39.

Allyl *O*-(2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-allopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-allopyranoside (10). A mixture of **9** (159 mg, 0.20 mmol) in AcOH (4 mL) and water (1 mL) was heated at 60 °C with stirring for 3 h and then cooled and concentrated. The residue was co-evaporated with ethanol-toluene to give crude pentaol (121 mg). Benzyl bromide (0.36 mL, 3.0 mmol) was added dropwise to a stirred mixture of crude pentaol (121 mg) and finely ground potassium hydroxide (337 mg, 6.0 mmol) in DMF (10 mL). After the mixture was stirred at room temperature for 18 h, the resulting suspension was diluted with chloroform and filtered, and the filtrate was concentrated. The residue was dissolved in chloroform and the resulting solution was washed with water and brine, dried over $MgSO_4$, concentrated, and chromatographed with chloroform-methanol (100 : 1, v/v) as eluant, giving crystalline **10** (170 mg, 93%): mp 181~182 °C (EtOH); $[\alpha]_D^{19}$ -71 (*c* 0.5, $CHCl_3$); ^1H NMR δ 1.65 (3H, s, NHAc), 1.69 (3H, s, NHAc), 3.60 (1H, brd, $J_{6a,6b}$ = 12 Hz, H-6a), 3.71~3.75 (1H, m, H-6b), 3.74 (1H, dd, $J_{3',4'}$ = 2.1 Hz, $J_{4',5'}$ = 9.8 Hz, H-4'), 3.79 (1H, dd, $J_{5',6'a}$ = 3.7 Hz, $J_{6'a,6'b}$ = 11 Hz, H-6'a), 3.83 (1H, dd, $J_{5',6'b}$ = 1.5 Hz, H-6'b), 3.84~3.88 (1H, m, H-4), 3.90~3.96 (2H, m, H-2', H-5), 3.97 (1H, dd, J = 13, 5 Hz, allyl), 4.11 (1H, m, H-5'), 4.09~4.20 (3H, m, H-2, H-3 and H-3'), 4.46~4.52 (5H, m, NH and ϕCH_2), 4.51 (1H, J1,2 = 7.9 Hz, H-1), 4.59 (4H, m, ϕCH_2), 4.73 (1H, d, J1',2' = 8.2 Hz, H-1'), 4.90 (2H, brd, J = 12 Hz, ϕCH_2), 5.11 (1H, dd, J = 11, 1.5 Hz, olefine), 5.20 (1H, dd, J = 17, 1.5 Hz, olefine), 5.78~5.90 (1H, m, olefine), 5.86 (1H, brs, NH'), 7.22~7.32 (25H, m, Ar).

Anal. Calcd for $C_{54}H_{62}N_2O_{11}$: C, 70.88; H, 6.83; N, 3.06. Found: C, 70.80; H, 6.88; N, 2.92.

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