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PERACETYLATED CHITOBIOSE: PREPARATION BY SPECIFIC DEGRADATIONS OF CHITIN, AND CHEMICAL MANIPULATIONS

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ABSTRACT

Chitobiose octaacetate (**3**) was preparable in moderate yield from chitin by microbial degradation followed by acetylation, or by modified chemical degradation. Compound **3** was chemically manipulated to give various compounds, including an oxazoline derivative, glycosides, and partially *O*-benzylated derivatives. The conformation of the oxazoline derivative is discussed.

INTRODUCTION

Such di- and tri-saccharides as maltose, cellobiose, and maltotriose are readily accessible by enzymic or chemical degradation, or both, of starch, cellulose, and pullulan, respectively. These α- and β-linked oligosaccharides have been employed as substrates for our studies on regioselective protection and modification of oligosaccharides[1–3], basic studies that have also been successfully applied to the synthesis of several bioactive compounds[4–7].

In order to extend the range of the utilizable oligosaccharides, our attention has been directed towards the amino sugar disaccharide, *N,N'*-diacetylchitobiose (**2**). The crucial role of **2** as a constituent of the "core structure" of the sugar chain of *N*-glycoproteins is well known. Furthermore, it has been reported that a chitohexaose regarded as a trimer of **2** is immunologically active, and suppressive against tumor cells[8].

We now describe a preparation of the peracetate (**3**) of **2** utilizing regiospecific degradation of chitin (**1**), subsequent chemical manipulations of **3**, including the processes of glycosidation and selective protection, and its conformational analysis.

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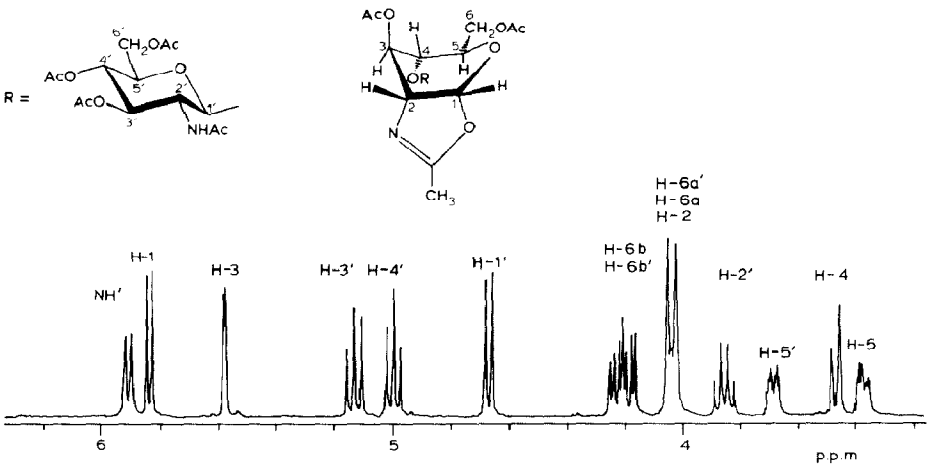


Fig. 1. 400-MHz, ^{1}H-n.m.r. spectrum of compound **4**.

RESULTS AND DISCUSSION

In contrast to the successful production of cellobiose octaacetate by acetolysis of cellulose[9], chitin (**1**) has given **3** under similar reaction conditions in <10% yield[10,11]. In order to obtain enough compound **3**, we first attempted to improve the acetolysis procedure. After chitin had been ultrasonicated in conc. hydrochloric acid, and the mixture diluted with an excess of water, the resulting "colloidal chitin" underwent acetolysis with acetic anhydride–conc. sulfuric acid, giving **3** in 16% yield. Recently, Defaye *et al.*[12] reported that hydrogen fluoride efficiently depolymerizes chitin, giving an oligosaccharide mixture (d.p. 2–10) from which *N,N'*-diacetylchitobiose (**2**) was isolated in 37% yield. Employment of anhydrous hydrogen fluoride, however, seems inappropriate for the large-scale preparation of *N,N'*-diacetylchitobiose. We now found that microbial degradation was applicable for the large-scale production of **3**. Thus, "colloidal chitin" was incubated for 30 h at 50° with[13] *Bacillus licheniformis* X-7u, and the culture broth was evaporated to dryness, the residue acetylated, and the product chromatographed, giving **3** in 31% overall yield.

As an example of chemical manipulation of **3**, oxazoline derivatives have been used as key intermediates for the preparation of various 2-acetamido-2-deoxy-β-D-glycosides. Therefore, a few procedures for the conversion of **3** into *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-methyl-(3,6-di-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**4**) were examined. Successive treatment of **3** with HBr in acetic acid and with pyridine[14] gave **4** in a yield of only 30–50% as a result of significant hydrolysis of the internal glycosidic linkage. The yield was remarkabley enhanced by treatment of **3** with titanium tetrabromide–triethylamine (83%) or trimethylsilyl trifluoromethanesulfonate–triethylamine[15] (99%). The chemical shifts and vicinal coupling-constants of the ring pro-

TABLE I

^{1}H-N.M.R. DATA FOR *N,N'*-DIACETYLCHITOBIOSE DERIVATIVES[a]

Hydrogen atom	*Chemical shift (δ) and multiplicity*				
	3[b]	**4**[c]	**5a**[d]	**5b**[e]	**5c**[f]
H-1	6.10d	5.84d	4.38d	4.38d	5.08d
H-2	4.36dd	4.04m	4.05m[g]	4.03dd	4.00dd
H-3	5.22dd	5.58d	5.10t	5.11t	5.11t
H-4	3.74t	3.46d	3.73t	3.73t	3.73t
H-5	3.62m	3.37m	3.64m	3.65m	3.57m
H-6a	4.02dd	4.04dd	4.05m[g]	4.05dd	4.02dd
H-6b	4.18dd	4.22dd	4.05m[g]	4.35m[g]	4.44dd
H-1'	4.47d	4.67d	4.49d	4.56d	4.82d
H-2'	3.95dd	3.86dd	3.89dd	3.89dd	4.21dd
H-3'	5.14t	5.13t	5.18t	5.19t	5.13t
H-4'	5.06t	5.00t	5.06t	5.05t	5.08t
H-5'	3.89dt	3.69m	3.64m	3.65m	3.61m
H-6'a	4.37dd	4.04dd	4.34m[g]	4.35m[g]	4.34m[g]
H-6'b	4.44dd	4.19dd	4.34m[g]	4.35m[g]	4.34m[g]
N-H	5.67d	—	5.68d	5.75d	6.02d
N'-H	5.95d	5.92d	5.86d	6.00d	6.43d

[a] ^{1}H-N.m.r. spectra were measured for solution in $CDCl_3$. [b]1.90–2.20 (24 H, 8 CH_3CO). [c]1.85 (3 H, CH_3-C) and 1.90–2.05 (18 H, 6 CH_3CO). [d]1.95–2.15 (21 H, 7 CH_3CO), and 3.47 (s, 3 H, OCH_3). [e]1.95–2.20 (21 H, 7 CH_3CO) and 4.37 (s, 2 H, $-CH_2CCl_3$). [f]Not analyzed, due to overlapping signals. [g]1.90–2.20 (21 H, 7 CH_3CO), 5.85 (m, 1 H, $-CH_2CH=CH_2$), 5.28 (m, 2 H, $-CH_2CH=CH_2$), and 4.34 (m, 2 H, $-CH_2CH=CH_2$).

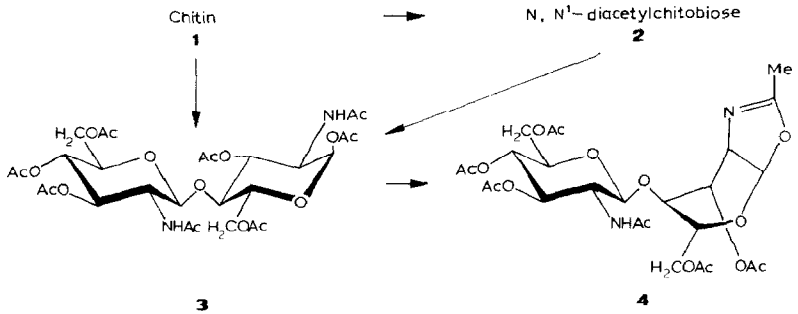
TABLE II

^{1}H–^{1}H COUPLING CONSTANTS OF *N,N'*-DIACETYLCHITOBIOSE DERIVATIVES

Coupled protons	J-*values (Hz)*				
	3	**4**	**5a**	**5b**	**5c**
1,2	3.4	7.3	7.6	7.8	6.8
2,3	9.2	2.4	9.5	9.8	9.3
3,4	9.2	0	8.5	8.5	8.5
4,5	9.2	9.5	8.5	8.5	8.5
5,6a	2.1	2.2	[a]	[a]	2.0
5,6b	3.7	4.6	4.7	[a]	3.9
6a,6b	12.5	12.2	[a]	12.2	12.5
1',2'	7.2	8.8	8.3	7.8	
2',3'	9.2	10.3	9.4	10.3	9.8
3',4'	9.2	9.6	9.5	9.5	9.5
4',5'	9.2	9.6	9.5	9.8	9.5
5',6'a	2.1	2.2	3.2	[a]	[a]
5',6'b	3.7	4.4	4.4	[a]	5.1
6'a,6'b	12.2	12.2	12.2	[a]	12.0
2,NH	8.9	—	9.3	9.3	9.3
2',N'H	9.5	8.8	9.0	8.8	9.5

[a]Not analyzed, due to overlapping signals.

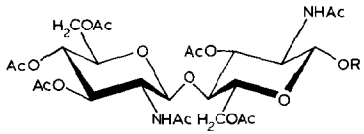
tons of **4** were readily determined from the 400-MHz ^{1}H-n.m.r. spectrum (see Fig. 1). The values are given in Tables I and II, together with the corresponding ones for compound **3** and glycosides **5a, 5b**, and **5c**. From the vicinal coupling-constants of **4**, the proton dihedral angle $\phi_{1,2}$ is in the range of 20–30°, and $\phi_{2,3}$ in the range of 50–60°. The angle $\phi_{3,4}$ was estimated to be nearly 90° ($J_{3,4}$ 0 Hz), and this value differed from the $\phi_{3,4}$ value of 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[16] ($\phi_{3,4}$ 110–120°, $J_{3,4}$ 2.2 Hz). Although a modified skew conformation had been proposed[16] for the corresponding oxazoline derivative of a monosaccharide, examination of a model of **4**, in conjunction with the values of all its dihedral angles, suggested that a slightly modified boat form ($^{3,O}B$; see inset, Fig. 1) is a plausible conformation. These results might suggest that the bulkiness of the substituents at C-4 greatly influence the stable conformation of the oxazoline-bearing sugar moiety.



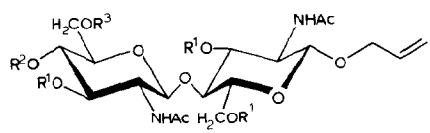
The glycosidation reactions of **4** with such simple alcohols as methanol, 2-propen-1-ol, and 2,2,2-trichloroethanol in the presence of trifluoromethanesulfonic acid as an acid catalyst gave the corresponding methyl, allyl, and trichloroethyl β-glycosides in 76, 53, and 40% yield, respectively. Only a faint trace of α anomer was observed in t.l.c. in the cases of the allyl and trichloroethyl glycosides. Employment of trifluoromethanesulfonic acid gave results superior to those with *p*-toluenesulfonic acid, as Lemieux *et al.*[17] had reported. The n.m.r. data for compounds **5a, 5b**, and **5c** are listed in Tables I and II. Differing from the simple primary alcohols just mentioned, methyl 3,4,6-tri-*O*-benzyl-α-D-mannoside or allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside failed to react with **4**.

To protect other hydroxyl groups, *O*-deacetylation of **5b** by the Zemplén procedure afforded compound **6** in quantitative yield. Treatment of **6** with α,α-dimethoxytoluene in *N,N*-dimethylformamide (DMF) in the presence of *p*-toluenesulfonic acid at 60° under diminished pressure and *O*-benzylation of the product with benzyl bromide–barium oxide–barium hydroxide octahydrate in DMF gave the 3,6,3′-tri-*O*-benzyl derivative (**7**) in 51% overall yield.

Selective cleavage of the benzylidene group of **7** by reduction with sodium cyanoborohydride–hydrochloric acid[18] in 10:1 (v/v) oxolane–DMF in the presence



5a R = CH_3
5b R = $CH_2CH{=}CH_2$
5c R = CH_2CCl_3



6 $R^1 = R^2 = R^3 = H$
7 R^1 = Bn, R^2, R^3 = PhCH
8 $R^1 = R^3$ = Bn, R^2 = H

of molecular sieves gave the 3,6,3′,6′-tetra-*O*-benzyl derivative[19] (**8**) in 62% yield. Compound **8** may be regarded as a useful disaccharidic glycosyl acceptor potentially having wide applicability.

EXPERIMENTAL

General methods. — Melting points were determined with a Yamato micro melting-point apparatus, and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter. I.r. spectra were recorded with a Shimazu IR-27 spectrophotometer, for potassium bromide disks or on KRS (thallium bromide–iodide) for thin films. ^{1}H-N.m.r. spectra were recorded at 400 MHz or 500 MHz with JEOL JNM-GX 400 or JEOL JNM-GX 500 spectrometers, using tetramethylsilane (Me_4Si) as the internal standard. Reactions were monitored by t.l.c. on a precoated plate of silica gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). Column chromatography was performed on columns of silica gel (70–230 mesh; E. Merck, Darmstadt, Germany). Preparative thin-layer chromatography was performed with a precoated plate of Silica Gel $60F_{254}$ (layer thickness 2 mm; E. Merck, Darmstadt, Germany). Solvent extracts were dried with anhydrous magnesium sulfate unless otherwise specified, and solutions were evaporated under diminished pressure below 40°. Chitin (EG) was purchased from Katokichi Co. Ltd., and used without purification.

Preparation of colloidal chitin. — Chitin (**1**; 100 g, 42-mesh) was added gradually to 12M hydrochloric acid (500 g) at room temperature with stirring. The mixture was then treated by an ultrasonic apparatus (27 kHz) for 30–40 min at room temperature. The viscous chitin solution was added in a thin stream to ice–water (5 L) with vigorous stirring; within a few minutes, a micro-powdered, fine precipitate was obtained. After being kept overnight at 5°, the product was filtered off on a sintered-glass funnel and repeatedly washed with water. The residue was neutralized with sodium hydroxide solution, and thoroughly washed with water. This salt-free, colloidal chitin was used for the microbial degradation procedure (Method *B*) as the substrate polysaccharide. Also, the colloidal chitin was successively washed with acetone and ether, and dried to a fine powder (80–85 g) for the acetolysis procedure (Method *A*).

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-α-D-glucopyranose (**3**). — *Method* A. The colloidal chitin (**1**; 80 g) was added in several portions, with stirring, to a mixture

of acetic anhydride (1 L) and 18M sulfuric acid (100 mL) at 55°. The mixture was stirred for 3 h at 55°, resulting in a thin, light-brown syrup. Stirring was continued for 36 h at 35°, with change of the color to a deep wine-red. The mixture was poured onto ice (2 L), made neutral with solid sodium acetate (500 g), kept for 12 h at room temperature, and extracted several times with chloroform; the extracts were successively washed with sodium hydrogencarbonate and water, dried (magnesium sulfate), and evaporated. The gummy product was triturated with methanol [some of the byproducts, such as 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose and *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-5,6-di-*O*-acetyl-2,3-dideoxy-*aldehydo*-D-*erythro*-hex-2-enose[20], were removed by this treatment], and the insoluble residue was chromatographed on silica gel (800 g) with 20:1 (v/v) chloroform–methanol. Fractions having R_F 0.4 (9:1 chloroform–methanol) in t.l.c. were collected and evaporated, to give crystalline **3** (15–20 g, 12–16%); m.p. 305–306° (dec.), $[\alpha]_D^{27}$ +55° (*c* 0.48, acetic acid) [lit.[10] m.p. 301–303° (dec.), $[\alpha]_D^{30}$ +56° (*c* 0.52, acetic acid); lit.[11] m.p. 307–309° (dec.) $[\alpha]_D^{20}$ +57° (*c* 1, acetic acid)]. Crude chitotriose dodeacetate was obtained from subsequent fractions in ~10% yield.

Method B. A suspension of colloidal chitin (**1**; 180 g/2 L) in basal medium solution (16 L: NH_4NO_3, 32 g; $Na_2HPO_4 \cdot 12\ H_2O$, 54.4 g; KH_2PO_4, 16 g; NaCl, 8 g; $MgSO_4 \cdot 7\ H_2O$, 8 g; $CaCl_2 \cdot 2\ H_2O$, 1.6 g; yeast extract, 16 g; pH 7.0) was inoculated with *Bacillus licheniformis* X-7u (initial cell concentration, 2×10^8 cells mL^{-1}), and the mixture was aerobically cultivated in a jar-fermentor (36 L) at 50°. The production of **2** in the culture medium was monitored by h.p.l.c. [column: Kaseisorb LC NH_2-60-5 (4.6 mm × 150 mm), Tokyo Kasei Co. Ltd., eluant: 7:3 (v/v) CH_3CN–H_2O, pressure: <100 $kg.cm^{-2}$], and the fermentation was stopped when the production of **2** reached the maximum (30–36 h). The culture broth was centrifuged (CEPA-Schnellzentrifuge No. 41, Carl Padberg G.m.b.H.) and the supernatant, liquor was evaporated *in vacuo*. The residual syrup was treated with acetic anhydride (500 mL) and pyridine (750 mL) for 18 h at room temperature. The mixture was evaporated, poured into ice–water (500 mL), and extracted several times with chloroform. The extracts were combined, successively washed with cold M sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried (magnesium sulfate), and evaporated. The residual syrup was chromatographed on silica gel (1 kg), with 20:1 (v/v) chloroform–methanol as the eluant, to give crystalline **3** (84 g, 31%); m.p. 305–306° (dec.), $[\alpha]_D^{25}$ +55° (*c* 0.5, acetic acid).

O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-methyl-(3,6-di-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-d]-2-oxazoline (**4**). — *Method* A. To a cooled solution of **3** (15.0 g, 22.2 mmol) in dry chloroform (500 mL) was gradually added, with stirring, titanium tetrabromide (15.0 g, 40.8 mmol) in dry ethyl acetate (180 mL) at 0°, and the mixture was stirred for 16 h at room temperature. Triethylamine (15.5 mL) was gradually added to the wine-red-colored solution at 0–5°, and the mixture was stirred for 1 h, poured into ice–water, washed successively with aqueous sodium hydrogencarbonate and water, dried, and

evaporated. The residue was chromatographed on silica gel (200 g), with 100:200:1 (v/v/v) toluene–ethyl acetate–triethylamine as the eluant, to give **4** (11.8 g, 83%) as an amorphous powder; $[\alpha]_D^{25}$ −8° (*c* 1.0, chloroform); [lit.[21] $[\alpha]_D^{20}$ −3° (*c* 1.1, chloroform) and lit.[22] $[\alpha]_D^{20}$ −8 ±2° (*c* 1, chloroform)].

Method B. A solution of **3** (7.7 g, 11.3 mmol) in 1,2-dichloroethane (40 mL) was treated with trimethylsilyl trifluoromethanesulfonate (2.3 mL, 11.9 mmol) under an argon atmosphere, and the mixture was stirred for 5 h at 50°, triethylamine (5 mL) was added, and the mixture was applied directly to a column of silica gel (250 g) and eluted with 100:200:1 (v/v/v) toluene–ethyl acetate–triethylamine, to give **4** (6.9 g, 99%); $[\alpha]_D^{25}$ −9° (*c* 1.0, chloroform).

*Methyl O-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**5a**). — A solution of the oxazoline derivative **4** (5.8 g, 9.4 mmol) in dry methanol (200 mL) containing trifluoromethanesulfonic acid (83 μL, 0.94 mmol) was stirred under an argon atmosphere for 15 min at 60°, cooled, made neutral with pyridine (5 mL), and co-evaporated with toluene. The syrupy residue was subjected to chromatography on silica gel with 20:1 (v/v) chloroform–methanol, to give pure **5a** (4.6 g, 76%); m.p. 284° (from methanol), $[\alpha]_D^{22}$ −44° (*c* 0.55, chloroform).

Anal. Calc. for $C_{27}H_{40}N_2O_{16} \cdot 0.3\ H_2O$: C, 49.59; H, 6.26; N, 4.28. Found: C, 49.48; H, 6.16; N, 4.17.

*Allyl O-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**5b**). — To a solution of **4** (11.8 g, 19.2 mmol) in dry chloroform (150 mL) were added 2-propen-1-ol (12.9 mL) and trifluoromethanesulfonic acid (160 μL, 1.8 mmol), and the mixture was stirred under an argon atmosphere for 5 h at 90°, cooled, poured into ice–water, washed successively with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 50:1 (v/v) chloroform-methanol as the eluant, to give **5b** (6.1 g, 53%); m.p. 254° (from ethanol), $[\alpha]_D^{22}$ −42° (*c* 0.43, chloroform).

Anal. Calc. for $C_{29}H_{42}N_2O_{16}$: C, 51.63; H, 6.27; N, 4.15. Found: C, 51.54; H, 6.23; N, 4.17.

*2,2,2-Trichloroethyl O-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**5c**). — A solution of the oxazoline **4** (320 mg, 0.52 mmol) in dry 2,2,2-trichloroethanol (5 mL) containing trifluoromethanesulfonic acid (20 μL) was stirred under an argon atmosphere for 2 h at 100°, cooled, diluted with chloroform (50 mL), and poured into ice–water, washed successively with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residual syrup was subjected to preparative thin-layer chromatography, developed with 1:1 (v/v) chloroform–ethyl acetate, to give **5c** (150 mg, 40%); m.p. 236–238° (from ethanol), $[\alpha]_D^{22}$ −35°, (*c* 0.43, chloroform).

Anal. Calc. for $C_{28}H_{39}Cl_3N_2O_{16}$: C, 43.91; H, 5.13; Cl, 13.89; N, 3.66. Found: C, 44.11; H, 5.18; Cl, 13.69; N, 3.54.

*Allyl O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→4)-2-acetamido-2-*

*deoxy-β-*D*-glucopyranoside* (**6**). — To a solution of **5b** (6.0 g, 8.9 mmol) in dry methanol (200 mL) was added sodium methoxide (600 mg, 11.2 mmol) at 0°, and the mixture was stirred for 3 h at room temperature, treated with Dowex-50W X-8 (H^+) resin, and the suspension filtered. The filtrate was evaporated to give **6** (4.0 g, 97%); m.p. 282–283° (from methanol), $[\alpha]_D^{22}$ −33° (*c* 0.31, H_2O); ν_{max}^{KBr} 3350, 1640, and 1550 cm^{-1}; δ_H (dimethyl sulfoxide-d_6): 1.80 (s, 3 H, NHCOCH_3), 1.82 (s, 3 H, NHCOCH_3), 4.70–4.72 (d, 2 H, H-1,1′), 4.98 (d, 1 H, *J* 5.4 Hz, N*H*COCH$_3$, D_2O-exchangeable), 5.07 (d, 1 H, *J* 5.4 Hz, N*H*COCH$_3$, D_2O-exchangeable), 5.10–5.25 (m, 2 H, $CH_2CH{=}CH_2$), and 5.79–5.88 (m, 1 H, $CH_2CH{=}CH_2$).

Anal. Calc. for $C_{19}H_{32}N_2O_{11}\cdot 4\ H_2O$: C, 46.60; H, 7.16; N, 5.72. Found: C, 46.81; H, 6.91; N, 5.57.

*Allyl O-(2-acetamido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-β-*D*-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-*D*-glucopyranoside* (**7**). — A solution of **6** (740 mg, 1.59 mmol), α,α-dimethoxytoluene (0.72 g, 4.7 mmol), and *p*-toluenesulfonic acid monohydrate (100 mg) in DMF (10 mL) was evacuated to ~1.33 kPa, rotated for 2 h at 60–80° in a rotary evaporator, diluted with DMF (20 mL), and cooled. To this mixture were added barium oxide (4.9 g), barium hydroxide octahydrate (5.0 g), and benzyl bromide (4.1 g), and the mixture was stirred for 16 h at room temperature, diluted with ethyl acetate (50 mL), filtered, and the filtrate washed with water, dried, and evaporated to a syrup that was chromatographed on silica gel with 100:1 (v/v) chloroform–methanol as the eluant, giving **7** (664 mg, 51%); $[\alpha]_D^{22}$ −38° (*c* 0.36, chloroform); δ_H: 1.80 (s, 3 H, NHCOCH_3), 1.95 (s, 3 H, NHCOCH_3), 3.23 (m, 1 H, H-5), 3.27 (t, 1 H, *J* 10.0 Hz, H-4′), 3.53 (t, 1 H, *J* 9.5 Hz, H-3), 3.60 (m, 1 H, H-3′), 3.62 (d, 1 H, *J* 10.5 Hz, H-6a), 3.69 (t, 1 H, *J* 9.4 Hz, H-4), 3.71–3.74 (m, 2 H, H-5′,6′a), 3.80 (m, 2 H, H-2,6′b), 3.93 (m, 1 H, H-2′), 3.99 (dd, 1 H, *J* 5.8, 13.2 Hz, OCH_2CH=CH$_2$), 4.24 (dd, 1 H, *J* 4.4, 10.5 Hz, H-6b), 4.28 (dd, 1 H, *J* 5.2, 12.9 Hz, OCH_2CH=CH$_2$), 4.36 (d, 1 H, *J* 8.3 Hz, H-1), 4.45–4.89 (m, 6 H, 3 OCH_2Ph), 4.67 (d, 1 H, *J* 8.8 Hz, H-1′), 4.77 (d, 1 H, *J* 8.8 Hz, N*H*), 5.12–5.25 (m, 2 H, OCH$_2$CH=CH_2), 5.54 (s, 1 H, PhC*H*), 5.81–5.87 (m, 1 H, OCH$_2$C*H*=CH$_2$), and 6.35 (d, 1 H, *J* 8.8 Hz, N*H*′).

Anal. Calc. for $C_{47}H_{54}N_2O_{11}\cdot 4\ H_2O$: C, 68.00; H, 6.65; N, 3.37. Found: C, 67.98; H, 6.60; N, 3.37.

*Allyl O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-*D*-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-*D*-glucopyranoside* (**8**). — Hydrogen chloride in diethyl ether was added at 0° to **7** (100 mg) and sodium cyanoborohydride (150 mg) in 10:1 (v/v) dry oxolane–DMF containing 3A molecular sieves until the evolution of gas ceased. The mixture was diluted with dichloromethane (20 mL) and filtered. The filtrate was poured into ice–water, washed with aqueous sodium hydrogencarbonate, dried, and evaporated. The resulting syrup was applied to a preparative thin-layer chromatograph and developed with 10:1 (v/v) chloroform–methanol, to give (62 mg, 62%); $[\alpha]_D^{22}$ −43° (*c* 0.18, chloroform); [lit.[19] m.p. 174.5–175.5°, $[\alpha]_D^{25}$ −38.2° (*c* 0.5, chloroform)]; δ_H: 1.78 (s, 3 H, NHCOCH_3), 1.95 (s, 3

H, NHCOCH_3), 3.08 (s, 1 H, O*H*-4′, D_2O-exchangeable), 3.30 (m, 1 H, H-5), 3.35 (dd, 1 H, *J* 8.5 Hz, H-4′), 3.60 (dd, 1 H, *J* 6.0, 10.0 Hz, H-6a), 3.64 (dd, 1 H, *J* 10.7, 10.9 Hz, H-3), 3.69 (m, 1 H, H-6b), 3.71–3.73 (m, 4 H, H-2,4,3′,5′), 3.73–3.75 (m, 1 H, H-6′a), 3.90–3.92 (m, 1 H, H-6′b), 3.98 (dd, 1 H, *J* 5.7, 12.9 Hz, OCH_2CH=CH_2), 4.01 (m, 1 H, H-2′), 4.26 (dd, 1 H, *J* 4.9, 13.2 Hz, OCH_2CH=CH_2), 4.29 (d, 1 H, *J* 8.3 Hz, H-1), 4.44–4.82 (m, 8 H, 4 OCH_2Ph), 4.52 (d, 1 H, *J* 9.3 Hz, H-1′), 4.83 (d, 1 H, *J* 8.8 Hz, N*H*), 5.11–5.25 (m, 2 H, OCH$_2$CH=CH_2), 5.81–5.87 (m, 1 H, OCH$_2$C*H*=CH$_2$), and 6.38 (d, 1 H, *J* 8.8 Hz, N*H*′).

Anal. Calc. for $C_{47}H_{56}N_2O_{11}\cdot 0.5\ H_2O$: C, 67.85; H, 6.92; N, 3.37. Found: C, 67.80; H, 6.94; N, 3.39.

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