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A New Synthesis of 2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3,6-tri-O-acetyl-2-deoxy-α-D-glucopyranose (Chitobiose Octaacetate)

Shigeyuki Oguri and Setsuzo Tejima

Faculty of Pharmaceutical Sciences, Nagoya City University[1]

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Condensation of 1,6: 2,3-dianhydro-β-D-mannopyranose with 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′: 4,5]-2-oxazoline (**2**) in the presence of a catalytic amount of anhydrous *p*-toluenesulfonic acid in boiling 1,2-dichloroethane afforded crystalline 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,6: 2,3-dianhydro-β-D-mannopyranose (**3**) in 42% yield. Azidolysis of the oxirane ring of **3**, reduction of the azido to an amino group, and N-acetylation afforded 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose (**6**) in 50% yield. Compound **6** was indistinguishable from the product prepared in 52.2% yield by direct condensation of 2-acetamido-3-O-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose with **2**. Acetolysis of **6** provided the title disaccharide.

Keywords——N-acetylglucosamine derivative; oxazoline method; di-N-acetylchitobiose; 1,6-anhydrochitobiose hexaacetate; azidolysis; oxirane ring; Fürst-Plattner rule

Recent work on glycoconjugates has clarified that di-N-acetylchitobiose exists in the internal region of many glycoproteins bearing N-acetylglucosaminyl-asparagine linkages. Although approaches to the synthesis of chitobiose derivatives have been reported by several investigators,[2] the title sugar is not readily available in laboratories. As a part of our program of syntheses of oligosaccharides bearing di-N-acetylchitobiose we now report a new synthesis of the title sugar.

The first step of this method is the condensation of 1,6: 2,3-dianhydro-β-mannopyranose (**1**) with acetylated 1,2-oxazoline of 2-acetamido-2-deoxy-α-D-glucopyranose to afford a β-D-(1→4)-linked disaccharide bearing 2-acetamido-2-deoxy-D-glucopyranose at the non-reducing terminus. The title disaccharide is then obtained by the following series of reactions: azidolysis of the oxirane ring, reduction of the azido to an amino group, N-acetylation, and finally, acetolysis of the 1,6-anhydro ring. Our synthetic method has the following three characteristics. 1) The oxazoline method was chosen because of its anomeric specificity and high yield of the condensation product.[2c,3] 2) As acceptors of the oxazoline, 1,6-anhydro derivatives of aldohexoses were chosen to overcome the poor nucleophilic properties of the C-4 hydroxyl group of aldohexoses having the $^4C_1$-D-conformation.[4] 3) The diaminodisaccharide derivative was prepared from the monoaminodisaccharide derivative containing 1,6: 2,3-dianhydro-β-D-mannopyranose *via* stereospecific cleavage of the oxirane ring with an azido group. An analogous synthetic route has been adopted in the synthesis of N-acetyllactosamine from 1,6-anhydro-β-lactose.[5]

1) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
2) *a*) K. Heyns, K. Propp, R. Harrison, and H. Paulsen, *Chem. Ber.*, **100**, 2655 (1967); *b*) F. Schmitt and P. Sinaÿ, *Carbohydr. Res.*, **29**, 99 (1973); *c*) C.D. Warren and R.W. Jeanloz, *ibid.*, **53**, 67 (1977); *d*) R.U. Lemieux, T. Takeda, and B.Y. Chung, "Synthetic Methods for Carbohydrates," ed. by H.S. El Khadem, ACS Symposium Series **39**, 90 (1977).
3) R. Kaifu, T. Osawa, and R.W. Jeanloz, *Carbohydr. Res.*, **40**, 111 (1975).
4) A.H. Haines, *Adv. Carbohydr. Chem. Biochem.*, **33**, 11 (1976).
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Compound **1** was easily prepared in 4 steps from 1,6-anhydro-β-D-glucopyranose according to the method of Černý *et al.*[6] Attempts to couple **1** with 3,4,6-tri-O-acetyl-2-deoxy-2-diphenoxyphosphorylamino-α-D-glucopyranosyl bromide[7] were unsuccessful. For example, when a mixture of **1**, the bromide, and mercuric cyanide in dry benzene was refluxed for 30 min, thin-layer chromatography (TLC) on silica gel plates revealed the formation of numerous by-products, while the desired disaccharide could not be identified. Cleavage of the oxirane ring in **1** and decomposition of the bromide probably occurred to give complex by-products before the coupling reaction proceeded. Thus, studies on this route were abandoned and, as a next step, the oxazoline method was investigated.

A mixture of **1** (1 mol) and 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,-1′: 4,5]-2-oxazoline (**2**)[8] (1.5 mol) in 1,2-dichloroethane containing a trace of anhydrous *p*-toluenesulfonic acid (TsOH) was boiled under reflux. Additional amounts of **2** (0.75 mol) and TsOH were added twice and heating was continued for a total of 5.5 hr. After neutralization, the reaction product was chromatographed on a column of silica gel to give the coupling disaccharide, 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,6: 2,3-dianhydro-β-D-mannopyranose (**3**) in 42% yield. Unchanged **1** was eluted with some by-products in earlier fractions. Thus, unchanged **1** could be recovered in 33.7% yield by re-chromatography. When recovered **1** was re-treated with **2**, the overall yield of **3** was improved.

Compound **3** crystallized as white needles. In the infrared (IR) spectrum of **3**, no signals due to hydroxyl groups could be detected. In the nuclear magnetic resonance (NMR) spectrum, the anomeric proton of the reducing terminus appeared as a doublet with $J_{1,2}=3$ Hz. Thus, the two anhydro rings in **1** were stable in the oxazoline method.

Azidolysis of the oxirane ring in **3** was performed by heating a mixture of **3** and sodium azide in aqueous hexamethylphosphoric triamide (HMPA) in the presence of ammonium chloride at 110° for 30 hr. The mixture was then diluted with ethyl acetate and water, and the organic layer was separated; from this, 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (**4**) was isolated in 15.8% yield. The product crystallized as white needles and, in the IR spectrum, showed signals due to a hydroxyl group and an azido group.

The aqueous layer in the azidolysis of **3** was concentrated to a syrup, which was acetylated to give white needles. On the other hand, acetylation of **4** gave a pentaacetate, 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (**5**), in 98% yield. Compound **5** was indistinguishable in terms of mp, $[\alpha]_D$, and IR spectrum from the white needles mentioned above. Therefore, the low yield (15.8%) of **4** separated from the azidolysis mixture of **3** is attributed to the formation of deacetylated products which are insoluble in ethyl acetate. When isolated **5** is added to **4**, the overall yield of the azidolysis is *ca.* 50%.

The oxirane ring attached to the rigid 1,6-anhydro system is known to undergo scission by nucleophiles, leading to predominantly *trans*-diaxial substitution (the Fürst–Plattner rule).[5,9] The validity of the structures of **4** and **5** tentatively assigned according to this rule was confirmed by the finding that 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose (**6**) could be derived from **5** *via* the unequivocal synthetic route described below.

Reduction of the azido group of **5** to an amino group was performed smoothly by catalytic hydrogenation in the presence of palladium black. After N-acetylation, **6** was isolated as white needles in 95.1% yield. In the NMR spectrum, the anomeric proton of the reducing

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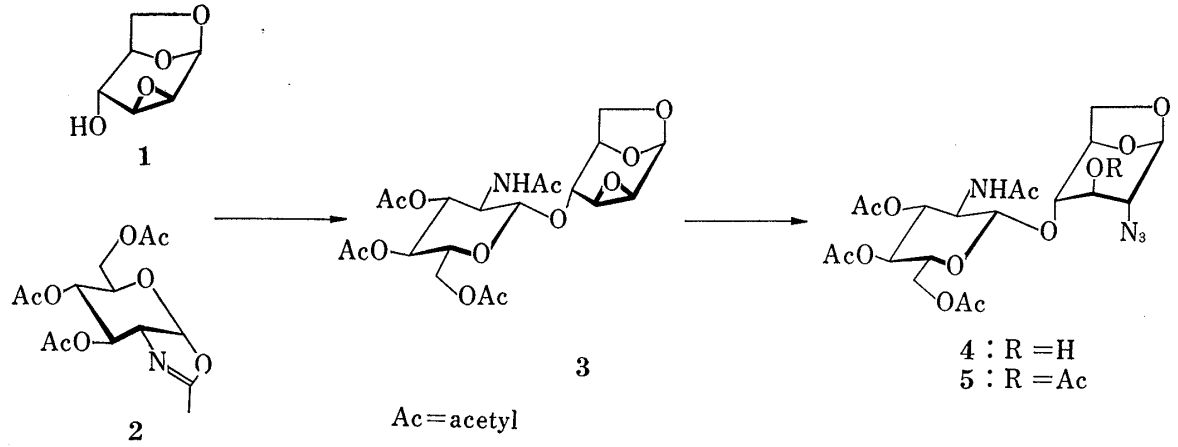

Chart 1

terminus appeared at 5.32 ppm as a singlet. During the synthetic studies on 1,6-anhydro-β-disaccharide derivatives so far reported from this laboratory,[10] we have often encountered NMR spectra in which the anomeric proton of the reducing terminus appears as a singlet. A reasonable interpretation of this has already been proposed.[11]

Compound **6** was also synthesized directly by the condensation of 2-acetamido-3-O-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose (**7**), prepared according to the method of Schmitt and Sinaÿ,[2b] with oxazoline (**2**) by a method analogous to that described for the preparation of **3**. However, in column chromatography of the reaction product, **6** was eluted along with unchanged **7** in the same fractions. Therefore, after removal of the solvent from the effluent of the preliminary chromatography, which was essential to remove the colored by-products from the reaction mixture, the residue was acetylated to transform unchanged **7** into 2-acetamido-3,4-di-O-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose (**8**),[12] and the acetylated product was re-chromatographed to isolate **6** from **8** in 52.2% yield.

Schmitt and Sinaÿ have also synthesized the title sugar *via* **6** by a different procedure,[2b] but they did not report detailed properties of **6**, except for limited IR data. Thus, our synthesis of **6** from **7** by the oxazoline method provides not only further proof of the validity of the assigned structures of **4** and **5**, but also useful intermediates for syntheses of oligosaccharides containing di-N-acetylchitobiose. The details will be reported in a subsequent paper.[13]

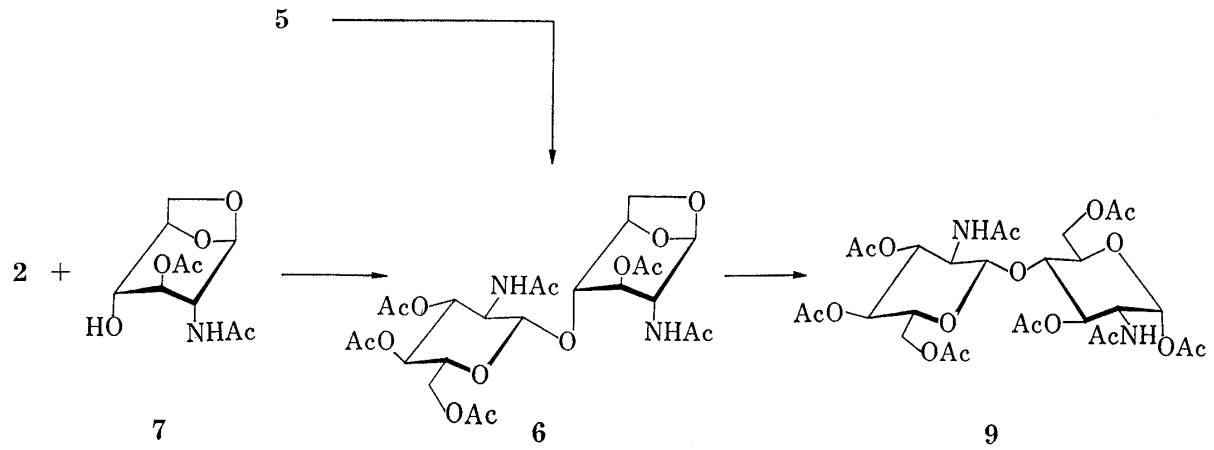

Chart 2

10) M. Černý and J. Staněk, Jr., *Adv. Carbohydr. Chem. Biochem.*, **34**, 24 (1977).
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The 1,6-anhydro ring of **6** was cleaved by mild acetolysis to afford the title disaccharide (**9**) as white needles in 51.3% yield. The product was indistinguishable from an authentic sample which was prepared from chitin according to the method of Osawa.[14]

Experimental

Solutions were concentrated in a rotary evaporator below 40° under a vacuum. Melting points were determined with a Yanagimoto MP-S2 micro melting point apparatus, and are uncorrected. Optical rotations were measured with a Union Giken PM-210 automatic digital polarimeter in a 0.5 dm tube. NMR spectra were recorded at 100 MHz with a JEOL JNM-MH-100 spectrometer. Tetramethylsilane (TMS) in $CDCl_3$ was used as an internal standard. Chemical shifts are given in ppm from TMS. TLC was performed on pre-coated silica gel plates 0.25 mm thick (Kiesel Gel $60F_{254}$, Merck) with the following solvent combinations (v/v): (A), $CHCl_3$–acetone (1: 1); (B), $CHCl_3$–MeOH (30: 1). Detection was effected with the spray reagent, anisaldehyde–H_2SO_4–EtOH (1: 1: 18) at 125°,[15] or by UV irradiation (short wavelength). Column chromatography was performed on Wako gel C-200 (Wako Pure Chemical Industries, Ltd., Osaka). Solvent combinations for elution are shown as v/v.

4-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,6: 2,3-dianhydro-β-D-mannopyranose (**3**)——A mixture of 1,6: 2,3-dianhydro-β-D-mannopyranose (**1**)[6] (720 mg, 5 mmol) and 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′: 4,5-]-2-oxazoline (**2**)[8] (2.4 g, 7.29 mmol) in 1,2-dichloroethane (30 ml) containing 0.01 N TsOH was boiled under reflux. After 1.5 and 3.5 hr, additional amounts of **2** (each 1.2 g, 3.64 mmol) in 1,2-dichloroethane (5 ml) containing 0.01 N TsOH were added, and heating was continued for a further 2 hr. The mixture was cooled, neutralized with pyridine (1 ml), and concentrated to give a residue which was chromatographed on a column (1.7×70 cm) of silica gel (80 g), eluting with $CHCl_3$–MeOH (50: 1); the fractions having *Rf* 0.43 (TLC, solvent A) were combined and concentrated to a syrup, which crystallized from MeOH to give pure **3** (1.08 g, 42%) as white needles, mp 243—245°, $[\alpha]_D^{22}$ −6.3° (c=0.63, $CHCl_3$). NMR ($CDCl_3$) δ: 1.96, 2.06, 2.15 (12H, each s, OAc×3, NAc), 5.70 (1H, d, $J_{1,2}$=3 Hz, H-1, β-Man). IR ν_{max}^{Nujol} cm^{-1}: 3280 (NH), 1745 (OAc), 1670 (amide I), 1560 (amide II). TLC: *Rf* 0.43 (solvent A), 0.09 (B). *Anal.* Calcd for $C_{20}H_{27}NO_{12}$: C, 50.74; H, 5.75; N, 2.96. Found: C, 50.82; H, 5.83; N, 2.84.

Unchanged **1** was eluted with some by-products in earlier fractions in the column chromatography of crude **3**. Thus, after removal of the solvent, the residue was re-chromatographed with $CHCl_3$–MeOH (100: 1), to recover **1** (243 mg, 33.7%).

4-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (**4**) **and 4-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose** (**5**)——A mixture of **3** (1 g, 1.94 mmol), NaN_3 (3 g, 4.62 mmol), and NH_4Cl (2 g, 37.4 mmol) in aqueous HMPA (50 ml) containing 20% (v/v) H_2O was stirred at 110° for 30 hr. After cooling at room temperature, the mixture was diluted with AcOEt (200 ml) and H_2O (200 ml) under stirring. The organic layer was separated, dried (Na_2SO_4), and evaporated to a syrup which crystallized on addition of MeOH. Recrystallization from MeOH gave pure **4** (172 mg, 15.8%) as white needles, mp 206—207°, $[\alpha]_D^{21}$ −5.3° (c=0.60, $CHCl_3$). NMR ($CDCl_3$) δ: 1.96, 2.06, 2.12 (12H, each s, OAc×3, NAc). IR ν_{max}^{Nujol} cm^{-1}: 3440, 3320 (OH, NH), 2110 (N_3), 1745 (OAc), 1675 (amide I), 1550 (amide II). TLC: *Rf* 0.42 (solvent A), 0.05 (B). *Anal.* Calcd for $C_{20}H_{28}N_4O_{12}$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.36; H, 5.37; N, 10.63.

The aqueous layer was evaporated to a mobile syrup which was treated with Ac_2O (10 ml) and pyridine (15 ml) at room temperature overnight, poured into ice-H_2O (200 ml), and extracted with AcOEt (100 ml×3). The organic layer was successively washed with aq. $NaHCO_3$ and H_2O. After desiccation over $CaCl_2$, the solvent was removed to yield a syrup which crystallized from EtOH. Recrystallization from EtOH gave pure **5** (440 mg, 37.3%) as white needles, mp 210—215° (dec.), $[\alpha]_D^{16}$ +38° (c=0.3, $CHCl_3$). NMR ($CDCl_3$) δ: 1.94, 2.02, 2.04, 2.08, 2.10 (15H, each s, OAc×4, NAc), 5.50 (1H, s, H-1 of reducing GlcNAc), 5.92 (1H, d, $J_{NH,2'}$=8 Hz, NH). IR ν_{max}^{Nujol} cm^{-1}: 3250 (NH), 2110 (N_3), 1740 (OAc), 1635 (amide I), 1565 (amide II). TLC: *Rf* 0.48 (solvent A), 0.17 (B). *Anal.* Calcd for $C_{22}H_{30}N_4O_{13}$: C, 47.31; H, 5.41; N, 10.03. Found: C, 47.08; H, 5.59; N, 9.88.

Acetylation of 4——Compound **4** (100 mg, 0.19 mmol) was acetylated with Ac_2O (1 ml) and pyridine (2 ml) at room temperature overnight. Excess Ac_2O was destroyed by dropwise addition of H_2O until no more heat was evolved. The mixture was concentrated to afford a crystalline residue which was recrystallized from EtOH. The product (106 mg, 98%) was indistinguishable from **5** in term of mp, $[\alpha]_D$, IR, NMR, and mobility on TLC.

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2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose (6)——1) From Compound **5**: A solution of **5** (100 mg, 0.19 mmol) in MeOH (10 ml) was hydrogenated with a Pd catalyst at room temperature under atmospheric pressure for 2 hr; the catalyst was freshly prepared from $PdCl_2$ (100 mg) according to the method of Schmidt and Staab.[16] After removal of the catalyst by filtration, the filtrate was evaporated to dryness and the residue was acetylated with Ac_2O (1 ml) and pyridine (2 ml) as described for the acetylation of **4** to afford **6**. The product crystallized from MeOH–ether as white needles (97.8 mg, 95.1%), mp 176—178°, $[\alpha]_D^{22}$ −75.5° (c=1.6, $CHCl_3$). NMR ($CDCl_3$) δ: 2.00, 2.08, 2.12, 2.15 (18H, each s, OAc×4, NAc×2), 5.32 (1H, s, H-1 of reducing GlcNAc), 6.98 (1H, d, $J_{NH,2}$=8 Hz, NH of reducing GlcNAc). TLC: *Rf* 0.32 (solvent A), 0.10 (B). *Anal.* Calcd for $C_{24}H_{34}N_2O_{14}$: C, 50.17; H, 5.96; N, 4.88. Found: C, 50.46; H, 6.02; N, 4.84.

2) From 2-Acetamido-3-O-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose (**7**) by the Oxazoline Method: A mixture of **7**[2b] (1 g, 3.93 mmol) and **2** (1.55 g, 4.72 mmol) in 1,2-dichloroethane (40 ml) containing 0.005 N TsOH was boiled under reflux. After 2, 3.5, and 5.5 hr, additional amounts of **2** (each 1.3 g, 3.95 mmol) in 1,2-dichloroethane (5 ml) containing 0.005 N TsOH were added, and heating was continued for a further 2 hr. The mixture was treated as described for the preparation of **3** to remove the colored by-products by column chromatography. However, **6** and unchanged **7** were eluted in the same fractions. Thus, after removal of the solvent from the effluent, the residue was acetylated with Ac_2O (5 ml) and pyridine (10 ml), and the acetylated product was re-chromatographed on a column (1.3×85 cm) of silica gel (40 g) with $CHCl_3$–MeOH (50: 1). From the earlier fractions, 2-acetamido-3,4-di-O-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose (**8**) (231 mg, 20.5%), mp 138—139°, $[\alpha]_D^{22}$ −91° (c=1, $CHCl_3$), was isolated after removal of the solvent [lit.[12] mp 137—138°, $[\alpha]_D^{20}$ −88.4° (c=1.1, MeOH)].

Compound **6** (1.18 g, 52.2%) was isolated from subsequent fractions having *Rf* 0.32 (TLC, solvent A). The product was indistinguishable from the product prepared by method 1).

2-Aetamido-4-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3,6-tri-O-acetyl-2-deoxy-α-D-glucopyranose (Chitobiose Octaacetate) (9)——Authentic chitobiose octaacetate was prepared from chitin (Wako) by acetolysis.[14]

Compound **6** (200 mg, 0.35 mmol) was dissolved in acetolysis mixture [4 ml, H_2SO_4–AcOH–Ac_2O (1: 30: 70, v/v)] at 0°, and the mixture was allowed to stand at 20° for 2 hr. The solution was then poured into ice-H_2O (100 ml), neutralized with $NaHCO_3$, and extracted with $CHCl_3$ (50 ml×4). The extract was dried (Na_2SO_4), then evaporated to dryness. After column chromatography, eluting with $CHCl_3$–MeOH (40: 1), white needles (120.8 mg, 51.3%), mp>300°, $[\alpha]_D^{20}$ +55.1° (c=0.62, AcOH), were isolated. The product was indistinguishable from authentic chitobiose octaacetate [lit.[14] m 301—303°, $[\alpha]_D^{30}$ +56° (c=0.52, AcOH)].

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