Synthesis of 2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-O-(α-L-fucopyranosyl)-N-glucopyranose (3-O-α-L-Fucopyranosyl-di-N-acetylchitobiose)

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A synthesis of the title trisaccharide (14) is reported. The first step of the synthetic route is stereospecific condensation of 2-acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-β-D-glucopyranosyl with 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyranono)-[2',1':4,5]-2-oxazoline to yield a fully protected β-D-(1→4)-linked disaccharide (8) bearing 2-acetamido-2-deoxy-β-D-glucopyranosyl (GlcNAC) at the non-reducing residue. After acetylation of the 1,6-anhydro ring of 8, the resulting fully-protected disaccharide is debenzylated to yield chitobiose heptaacetate (10) having a free hydroxy group at the C-3 position of the reducing GlcNAC. Compound 10 is glycosylated with 2,3,4,6-tetra-O-benzyl-α-L-fucopyranosyl bromide by a bromide ion-catalyzed reaction. After removing the protecting groups of the resulting trisaccharide by debenzylation and de-O-acetylation, an anomeric mixture of 14 is obtained as an amorphous solid. CMR spectral data for 14 are also presented.

Keywords——synthesis; 3-O-α-L-fucopyranosyl-di-N-acetylchitobiose; stem bromelain; serum-type glycoprotein; stereospecific condensation; GlcNAC derivative; oxazoline method; chitobiose heptaacetate; bromide ion-catalyzed condensation; CMR

The structure of the carbohydrate moiety of stem bromelain was recently proposed on the basis of results obtained in this laboratory. According to the proposed structure, the oligosaccharide has the characteristic feature that L-fucose is attached to the N-acetylgalcosamine (GlcNAC) involved in the protein-carbohydrate linkage by an α-L-(1→3) bond. Although attachment of L-fucose to the GlcNAC residue has been demonstrated in a number of cases, the mode of linkage is always α-L-(1→6). During the past few years, chemical syntheses of the terminal trisaccharide and tetrasaccharide units of human blood-group antigenic determinants have been achieved by several investigators. However, synthesis of the trisaccharide unit existing in the internal region of serum-type glycoproteins bearing N-acetylgalcosaminyl-asparagine linkages has not yet been undertaken. Synthesis of the title trisaccharide (14) was therefore investigated.

Our synthetic route is based on stepwise stereospecific condensation of monosaccharide units to yield the fully protected trisaccharide (13), from which 14 is prepared by removal of the protecting groups. A more detailed description of the synthesis of 13 is as follows. 1) The protected di-N-acetylchitobiose derivative (8) bearing a benzyl group at the C-3 position is prepared by condensation of the GlcNAC derivative having a free hydroxy group at the C-4 position with the acetylated oxazoline of GlcNAC. 2) Acetylation of 8 and debenzylation of the acetylosis product affords chitobiose heptaacetate (10) having a free hydroxy group at the

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1) Location: Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.
3) See the references cited in 2).
C-3 position of the reducing GlcNAc. 3) The disaccharide (10) is glycosylated with benzylated α-L-fucosyl bromide by a bromide ion-catalyzed reaction.

In the preceding paper from this laboratory,5) we have shown that 2-acetamido-3-O-acetyl-1,6-anhydro-2-deoxy-β-d-glucopyranose (1) is a useful aglycone for the synthesis of di-N-acetylgalactosamine derivatives by the oxazoline method.6) Therefore, 2-acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-β-d-glucopyranose (5) was chosen as a starting material for synthesis of the partially protected di-N-acetylgalactosamine derivative bearing a free hydroxyl group at the C-3 position.

Compound 1 was prepared from 1,6:2,3-dianhydro-4-O-benzyl-β-d-mannopyranose7) via 3 steps by a slight modification of the procedure of Schmitt and Šinaj8). instead of ammonolysis of the oxirane ring, azidolysis and successive reduction of the azido to an amino group were employed. Compound 1 was then treated with 3,4-dihydro-2H-pyran in order to protect temporarily the C-4 hydroxy group with tetrahydropryanyld ether (THP). The resulting 2-acetamido-3-O-acetyl-1,6-anhydro-2-deoxy-4-O-(tetrahydro-2-pyranyl)-β-d-glucopyranose (2) was isolated as a mixture of diastereoisomers. In the proton nuclear magnetic resonance (PMR) spectrum of 2, three methylene groups in the tetrahydropryanyl ring appeared as a multiplet; the methylene adjacent to the oxygen atom presumably does not appear in the field. Compound 2 was unstable and it gradually decomposed within several days.

De-O-acetylation of 2 afforded partially protected GlcNAc derivative having a free hydroxyl group at the C-3 position, 2-acetamido-1,6-anhydro-2-deoxy-4-O-(tetrahydro-2-pyranyl)-β-d-glucopyranose (3), which was unstable. Benzylation of 3 was performed at room temperature with benzyl bromide in N,N-dimethylformamide (DMF) in the presence of barium oxide and crystalline barium hydroxide. By column chromatography, 2-acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-4-O-(tetrahydro-2-pyranyl)-β-d-glucopyranose (4) was isolated in 99.2% yield. Direct benzylaion of 2 under alkaline conditions also gave 4 in 91.7% yield. Acid hydrolysis of 4 gave 2-acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-β-d-glucopyranose (5) in 82.7% yield. Acetylation of 5 gave the crystalline diacetate, 2-acetamido-4-O-acetyl-1,6-anhydro-3-O-benzyl-2-deoxy-β-d-glucopyranose (6), in 98.9% yield. The PMR spectra of 5 and 6 are in full agreement with their proposed structures. The overall yield from 1 to 5 was ca. 70%.

Condensation of the aglycone 5 with 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-d-glucopyranoyl)-[2',1':4,5]-2-oxazoline (7)9) in 1,2-dichloroethane in the presence of a trace of p-toluenesulfonic acid (TsOH) gave crude disaccharide, 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-d-glucopyranosyl)-1,6-anhydro-3-O-benzyl-2-deoxy-β-d-glucopyranose (8), which was contaminated with unchanged 5. After acetylation of 8, the unchanged 5 was separated as the diacetate (6) from 8 by column chromatography. Thus, 8 was isolated as white crystals in 41.4% yield and unchanged 5 could be recovered as the diacetate (6) in 55% yield; this material was recycled after de-O-acetylation. The assignment of β configuration

of the newly introduced glycosidic linkage in 8 is unequivocal because the oxazoline method is known to produce exclusively the β-anomer.

The C-3 hydroxyl group of 8 shows poor nucleophilicity because of the 4C-β-conformation of the reducing terminus.\textsuperscript{10} Therefore, before condensation with L-fucose, the conformation must be changed to 4C-β to increase the reactivity at the C-3 hydroxyl group.

The 1,6-anhydro ring in 8 was cleaved by mild acetylation with an acetylation mixture (see "Experimental") to give 1,3',4',6,6'-penta-O-acetyl-di-N-acetyl-3-O-benzyl-α-chitobiose (9) in 78.9% yield. The α configuration was confirmed as follows. 1) The specific rotation changed markedly from the levorotatory 8, [α]_D^{−113°}, to dextrorotatory 9, +41.8°. 2) In the PMR spectrum of 9 the anomic proton due to the reducing terminus appeared as a doublet with $J_{1,2}=4$ Hz.

Catalytic debenzylazation of 9 afforded 1,3',4',6,6'-penta-O-acetyl-α-di-N-acetylchitobiose (10) in 92.4% yield. Acetylation of 10 gave chitobiose octaacetate (11) in 96.7% yield; this was indistinguishable from an authentic sample.

![Chart 2](image)

The bromide ion-catalyzed reaction is known to be an excellent route to α-L-fucosides.\textsuperscript{4a,b,c}\textsuperscript{4a,b,c}\textsuperscript{4a,b,c}

A mixture of the glycone 10 with 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (12) in 1,2-dichloroethane–DMF containing tetraethylammonium bromide and powdered 4Å molecular sieves was stirred at 20° for 3 days under a nitrogen atmosphere. On chromatographic purification, the protected trisaccharide, 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxyβ-D-glucopyranosyl)-1,6-di-O-acetyl-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-α-D-glucopyranose (13), was isolated in 46.6% yield. Compound 13 crystallized from ethyl acetate–ether–hexane as white needles. The α configuration of the newly established glycosidic linkage was confirmed by carbon magnetic resonance (CMR) spectroscopy as mentioned later.

The protecting groups of 13 were removed by hydrogenolysis, followed by de-O-acetylation. On column chromatography, the title trisaccharide (14) was isolated in 55.1% yield. The low yield is presumably attributable to instability of 14 under alkaline conditions. Partial hydrolysis of 14 with hydrochloric acid resulted in the liberation of fucose and di-N-acetylchitobiose, while complete hydrolysis liberated fucose and glucosamine hydrochloride; they were identified by thin-layer chromatography (TLC).

The CMR spectrum of 10 or 13 was measured in deuterochloroform (CDCl$_3$), and that of 14 was measured in deuterium oxide (D$_2$O). 2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-glucopyranoses (15 and 16), and methyl 2,3,4-tri-O-benzyl-β-D-fucopyranosides (17 and 18) were used as reference compounds. The signals of 10, 13, and 14 were assigned by comparison with those of the reference compounds. Tetramethylsilane (TMS) was used as a standard and chemical shifts are given in ppm from TMS. The chemical-shift data on the anomic carbons are summarized in Table I. The results provided valuable information on the configurations of the anomic carbons and glycosidic linkages in 10, 13, and 14.

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. PMR spectra were recorded at 100 MHz with a JEOLENM-FX-100 spectrometer. TMS was used as a standard. Chemical shifts are given in ppm from TMS. TLC was performed on pre-coated silica gel plates 0.25 mm thick (Kiesel Gel 60 F\textsubscript{254}, Merck) with the following solvent combinations (v/v): (A) acetonitrile–CHCl\textsubscript{3} (1:1); (B) CHCl\textsubscript{3}–MeOH (30:1); (C) ether–hexane–MeOH (5:5:1); (D) CHCl\textsubscript{3}–MeOH–H\textsubscript{2}O (5:5:1); (E) PrOH–H\textsubscript{2}O–NH\textsubscript{4}OH (70:30:1). Detection was effected with the spray reagent, anisaldehyde–H\textsubscript{2}SO\textsubscript{4}–EtOH (1:1:18 at 125°) or by UV irradiation (short wavelength). Column chromatography was performed on Merck silica gel (70–230 mesh). Solvent combinations of eluates are given as v/v.

2-Acetamido-3-O-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose (1) — The product was prepared by a slight modification of the method reported by Schmitt and Sinay.\textsuperscript{9a}

Azidolysis of 1,6:2,3-dianhydro-4-O-benzyl-β-D-mannopyranose\textsuperscript{9} in aq. hexamethyldisiloxane triamide according to the method of Paulsen and Stenzel\textsuperscript{9b} gave 1,6-anhydro-2-azido-4-O-benzyl-2-deoxy-β-D-glucopyranose, mp 100–102°, [α]\textsubscript{D} –5.4° (c = 1, CHCl\textsubscript{3}) in 70% yield [lit.\textsuperscript{4b} mp 96°, [α]\textsubscript{D} –6° (c = 1.1, CHCl\textsubscript{3})]. Catalytic reduction of the azido group with Raney Ni catalyst, followed by acetylation yielded 2-acetamido-3-O-acetyl-1,6-anhydro-4-O-benzyl-2-deoxy-β-D-glucopyranose, [α]\textsubscript{D} –33.9° (c = 0.66, CHCl\textsubscript{3}). The benzyl group was removed by hydrolysis on Pd black to give 1, mp 139–142°, [α]\textsubscript{D} –79.6° (c = 1, MeOH). [lit.\textsuperscript{8} mp 143–144°, [α]\textsubscript{D} –82° (c = 1, MeOH)]. The total yield from 1,6:2,3-dianhydro-4-O-benzyl-β-D-mannopyranose was ca. 56%.

2-Acetamido-3-O-acetyl-1,6-anhydro-2-deoxy-4-O-(tetrahydro-2-pyryl)-β-D-glucopyranose (2) — A solution of 1 (2.54 g, 10 mmol), 3,4-dihydro-2H-pyran (1.6 ml, 17 mmol), and TsOH (20 mg) in dry dioxane (30 ml) was stirred at room temperature for 6 hr. The mixture was diluted with CHCl\textsubscript{3} (100 ml), washed successively with aq. NaHCO\textsubscript{3} and H\textsubscript{2}O, dried (CaCl\textsubscript{2}), and evaporated to dryness. The residue was chromatographed on a column (1.7 x 70 cm) of silica gel (80 g), eluting with CHCl\textsubscript{3}–MeOH (100:1). The fractions

having $R_f$ 0.66 (TLC, solvent A) were combined and evaporated to a syrup (3.03 g, 92.1%), $\delta$H (CDCl$_3$, 1H, s, H-1). TLC: $R_f$ 0.66 (solvent A), 0.39 (B), 0.20 (C).

2-Acetamido-1,6-anhydro-2-deoxy-4-O-(tetrahydro-2-pyanyl)-$\beta$-D-xylopyranose (3)—Methanolic NaOMe (0.5 m, 1 ml) was added to a solution of 2 (3.20 g, 9.72 mmol) in dry MeOH (30 ml), and the mixture was stirred at room temperature for 6 hr. After neutralization with dry Amberlite IR-120 (H$^+$) resin by stirring for 10 min, the solution was evaporated to a syrup, which was chromatographed on a column (1.7 x 70 cm) of silica gel (80 g), eluting with CHCl$_3$-MeOH (30:1). The fractions having $R_f$ 0.38 (TLC, solvent A) were combined and concentrated to give 3 (2.43 g, 87.1%), $\delta$H (CDCl$_3$, 1H, s, H-1), 1.96 (3H, s, NAc), 5.38 (1H, s, H-1). TLC: $R_f$ 0.38 (solvent A), 0.19 (B), 0.14 (C).

2-Acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-4-O-(tetrahydro-2-pyanyl)-$\beta$-D-dierythritopyranose (4)—From Compound 3: A mixture of 3 (500 mg, 1.74 mmol), BaO (2.3 g), Ba(OH)$_2$, 8H$_2$O (0.89 g), and benzyl bromide (0.5 ml, 4.2 mmol) in DMF (5 ml) was stirred at room temperature for 6 hr. After dilution with CHCl$_3$ (100 ml), salts were removed by filtration, and the filtrate was evaporated to dryness. The residue was chromatographed on a column (1.3 x 80 cm) of silica gel (40 g), eluting with CHCl$_3$. The fractions having $R_f$ 0.78 (TLC, solvent A) were combined and concentrated to give 4 (651 mg, 92.2%), $\delta$H (CDCl$_3$, 1H, s, H-1), 6.08 (1H, d, $J_{NH}$=8 Hz, NH), 7.30 (5H, s, aromatic protons). TLC: $R_f$ 0.78 (solvent A), 0.46 (B), 0.38 (C). Anal. Calcd for C$_{23}$H$_{26}$NO$_4$: C, 63.64; H, 7.21; N, 3.71. Found: C, 63.35; H, 7.08; N, 3.60.

2) From Compound 2: Benzylolation of 2 (2 g, 6.07 mmol) with BaO (4 g), Ba(OH)$_2$, 8H$_2$O (1.3 g), and benzyl bromide (1.3 ml, 11 mmol) in DMF (20 ml) as described in method 1 gave a syrup (2.1, 91.7%).

The product was indistinguishable from 4 in terms of $\delta$H, mobilities on TLC, and PMR spectrum.

2-Acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-$\alpha$-D-xylopyranose (5)—Amberlite IR-120 (H$^+$) resin (1.5 g), previously washed with EtOH, was added to a solution of 4 (1 g, 2.65 mmol) in 7/50% v/v aq. EtOH (30 ml), and the mixture was stirred at room temperature for 6 hr. After removal of the resin by filtration, the syrup was evaporated to dryness. The residue was chromatographed on a column (1.3 x 85 cm) of silica gel (40 g), eluting with CHCl$_3$-MeOH (20:1). The fractions having $R_f$ 0.43 (TLC, solvent A) were combined and concentrated to give 5 (643 mg, 82.7%), $\delta$H (CDCl$_3$, 1H, s, H-1) 8.86 (c=0.86, CHCl$_3$), as a syrup. PMR (CDCl$_3$) $\delta$: 1.92 (3H, s, NAc), 4.90 (1H, s, H-1), 5.90 (1H, d, $J_{NH}$=8 Hz, NH), 7.24 (5H, s, aromatic protons). TLC: $R_f$ 0.43 (solvent A), 0.13 (B), 0.19 (C). Anal. Calcd for C$_{24}$H$_{27}$NO$_5$: C, 59.59; H, 6.67; N, 4.63. Found: C, 59.36; H, 6.41; N, 4.53.

2-Acetamido-4-O-acetyl,1,6-anhydro-3-O-benzyl-2-deoxy-$\alpha$-D-xylopyranose (6)—Compound 5 (300 mg, 1 mmol) was acetylated with Ac$_2$O (3 mmol) and pyridine (5 ml) at room temperature. Excess Ac$_2$O was destroyed by azeotropic distillation of H$_2$O (1 ml), and the solution was evaporated to a syrup which crystallized from AcOEt-ether as crystals (329 mg, 98.9%), mp 115—116°C, $\delta$H (CDCl$_3$, 1H, s, H-1), 1.92 (3H, s, NAc), 5.35 (1H, s, H-1), 5.00 (1H, d, $J_{NH}$=8 Hz, NH), 7.31 (5H, s, aromatic protons). TLC: $R_f$ 0.43 (solvent A), 0.19 (B), 0.19 (C). Anal. Calcd for C$_{25}$H$_{30}$NO$_6$: C, 60.88; H, 6.31; N, 4.18. Found: C, 60.95; H, 6.34; N, 4.17.

2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-$\alpha$-D-xylopyranosyl)-1,6-anhydro-3-O-benzyl-2-deoxy-$\alpha$-D-xylopyranose (7)—A solution of 5 (1, 3.31 mmol) and 2-methyl-3,4,6-tri-O-acetyl-1,2-dideoxy-3-$\alpha$-D-glucopyranosyl-2-O-2',2'-amino-2-hydroxyethane (7) (15 g, 4.55 mmol) in 1,2-dichloroethane (20 ml) containing 0.005 N H$_2$SO$_4$ was heated under reflux. After 2 and 3.5 hr, additional amounts of 7 (each 1 g, 3 mmol) in 1,2-dichloroethane (5 ml) containing 0.005 N H$_2$SO$_4$ were added. Heating was continued for a further 3 hr. The mixture was cooled, neutralized with pyridine (0.5 ml), and evaporated to a syrup, which was chromatographed on a column (1.7 x 55 cm) of silica gel (60 g), eluting with CHCl$_3$-MeOH (100:1). The fractions having $R_f$ 0.48 (TLC, solvent A) were pooled and evaporated to dryness. The residue consisted of the desired disaccharide (8), unreacted 5, and by-products of 7.

The residue was then acetylated with Ac$_2$O (5 ml) and pyridine (10 ml) at room temperature for 12 hr, treated with H$_2$O (2 ml) to decompose excess Ac$_2$O, and evaporated to dryness. The residue was re-chromatographed on a column (1.3 x 85 cm) of silica gel (40 g), eluting with CHCl$_3$-MeOH (70:1). From the earlier fractions having $R_f$ 0.71 (TLC, solvent A), 6 (610 mg, 55%) was isolated. Compound 6 could be recycled after de-O-acetylation to 5.

The desired disaccharide (8) was isolated from the later fractions having $R_f$ 0.48 (TLC, solvent A). The product (485 mg, 41.4%) crystallized from MeOH-ether-hexane as needles, mp 205—207°C, $\delta$H (CDCl$_3$, 1H, s, H-1), 113°C (c=1, CHCl$_3$). PMR (CDCl$_3$) $\delta$: 2.00, 2.03, 2.06, 2.08, 2.12 (15H, each s, OAc $\times$ 3, NAc $\times$ 2), 5.34 (1H, s, H-1 of reducing GlcNAc), 6.02 (1H, d, $J_{NH}$=7 Hz, NH of reducing GlcNAc), 6.78 (1H, d, $J_{NH}$=8 Hz, NH of non-reducing GlcNAc), 7.37 (5H, s, aromatic protons). TLC: $R_f$ 0.48 (solvent A), 0.14 (B), 0.05 (C). Anal. Calcd for C$_{25}$H$_{32}$N$_2$O$_9$: C, 55.94; H, 6.15; H, 4.50. Found: C, 55.72; H, 6.39; N, 4.48.

2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-$\beta$-D-xylopyranosyl)-1,6-di-O-acetyl-3-O-benzyl-2-deoxy-*$\alpha$-D-xylopyranose (9)—A solution of 8 (1 g, 1.61 mmol) in acetoacetone (20 ml, Ac$_2$O-CH$_3$COOH (70:30:1, v/v/v)) was stirred at 20°C for 2 hr, poured into ice-H$_2$O (50 ml), and then neutralized with NaHCO$_3$. The resulting precipitate was filtered, dried in the air, and recrystallized from MeOH to give 9 as needles (915 mg, 78.9%), mp 277—280°C, $\delta$H (CD$_3$OD, 1H, s, H-1). TLC: $R_f$ 0.38 (solvent A), 0.19 (B), 0.14 (C).
3:1, v/v) δ: 1.84, 1.92, 2.00, 2.01 (21H, each s, OAc×5, NAc×2), 6.05 (1H, d, J1,4=4 Hz, H-1 of reducing GlcNAc), 7.35 (5H, s, aromatic protons). TLC: RF 0.46 (solvent A), 0.13 (B), 0.01 (C). Anal. Calcd for C33H47N2O24·C: 54.69; H: 6.12; N: 3.87. Found: C, 54.69; H, 6.05; N, 3.88.

2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,6-di-O-acetyl-2-deoxy-α-D-glucopyranose (10)—A solution of 9 (100 mg, 0.14 mmol) in MeOH (10 ml) was hydrogenated over a Pd catalyst at room temperature under atmospheric pressure until absorption of H2 ceased; the catalyst was then removed by filtration. The filtrate was evaporated to dryness. The residue was chromatographed on a column (0.9×50 cm) of silica gel (10 g), eluting with CHCl3-MeOH (40:1) to give 10 as a glass (83.2 mg, 92.4%), δ: 1.98, 2.00, 2.03, 2.09, 2.13, 2.16 (21H, each s, OAc×5, NAc×2), 5.61 (1H, d, J1,4=4 Hz, H-1 of reducing GlcNAc), 6.46 (1H, d, JNH,2=8 Hz, NH of non-reducing GlcNAc). TLC: RF 0.26 (solvent A), 0.08 (B), 0.01 (C). Anal. Calcd for C33H47N2O24·H2O: C, 47.85; H, 6.18; N, 4.29. Found: C, 48.08; H, 6.19; N, 4.45.

2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3,6-di-O-acetyl-2-deoxy-α-D-glucopyranose (Chibito Hegoctate) (11)—Acetylation of 10 (50 mg, 0.08 mmol) with Ac2O (0.5 ml) and pyridine (1 ml) as described in the preparation of 6 yielded 11 (49.6 mg, 95.7%). Recrystallization from MeOH gave a product as mp>300°C and δ: 5.54 (c=0.72, AcOH). The product was indistinguishable from authentic chibito octacetate prepared from chitin (Wako) by acetolysis.10

2,3,4-Tri-O-benzyl-α-L-fucopyranosyl Bromide (12)—The bromide was prepared by a slight modification of the method reported by Depj-Juszynski and Flowers.10

Dry HBr was introduced into dry CH2Cl2 to give a concentration of 0.50 n. To this solution (1.2 ml), a solution of 1,4-O-p-nitrobenzoyl-2,3,4-tri-O-benzyl-β-D-fucopyranose (322 mg, 0.56 mmol) in CH2Cl2 (5 ml) was added. The mixture was stirred for 12 min at room temperature, and the precipitated p-nitrobenzoic acid was removed by filtration. The filtrate was evaporated to a syrup (258 mg, 94.2%) which was immediately used for the coupling reaction.

2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,6-di-O-acetyl-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl) (13)—Compound 10 (100 mg, 0.16 mmol) was dissolved in a mixture of DMF (1.5 ml) and 1,2-dichloroethane (1.5 ml) which contained tetraethylammonium bromide (320 mg) and powdered 4 Å molecular sieves (360 mg). A solution of freshly prepared 12 (258 mg, 0.52 mmol) in dry 1,2-dichloroethane (3 ml) was added and the mixture was stirred under dry N2 gas at 20°C for 3 days. After addition of CHCl3 (50 ml), the solid was removed by filtration, and the filtrate was washed with H2O, dried (MgSO4) and evaporated to dryness. The residue was chromatographed on a column (0.9×50 cm) of silica gel (10 g), eluting with CHCl3-MeOH (100:1). Removal of the solvent from the effluent having RF 0.62 (TLG, solvent A) gave 13 (72.4 mg, 46.6%), which crystallized from AcOEt-ether-hexane, mp 165°C—166°C (c=0.72, MeOH). PMR (CDCl3): δ: 1.24 (3H, d, J= 7 Hz, CH3 in L-Fuc), 1.68, 1.74, 2.04, 2.12 (21H, each s, OAc×5, NAc×2), 5.92 (1H, d, JNH,2=7 Hz, NH of reducing GlcNAc), 6.28 (1H, d, J,1=3 Hz, H-1 of reducing GlcNAc), 7.12 (1H, d, JNH,2=7 Hz, NH of non-reducing GlcNAc), 7.20—7.40 (15H, aromatic protons). TLC: RF 0.62 (solvent A), 0.21 (B), 0.08 (C). Anal. Calcd for C33H47N2O24·H2O: C, 60.56; H, 6.33; N, 2.67. Found: C, 60.56; H, 6.32; N, 2.70.

2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-O-(α-L-fucopyranosyl)-D-glucopyranoses (3-O-α-L-Fucopyranosyl-di-N-acetylchitobiose) (14)—A solution of 13 (100 mg, 0.1 mmol) in MeOH (10 ml) was hydrogenated over a Pd catalyst; the catalyst was prepared15 from PdCl2 (100 mg). After removal of the catalyst by filtration, the filtrate was concentrated to give an amorbid powder (72.6 mg, 97.7%).

The resulting debenzylation product was, without purification, dissolved in freshly prepared 20% (w/v) NH4 in MeOH (5 ml) at 0°C. The mixture was left to stand at 4°C for 12 hr, then evaporated to a syrup, which was chromatographed on a column (0.6×35 cm) of silica gel (6 g), eluting with CHCl3-MeOH-H2O (5:5:1). The fractions having RF 0.27 (TLG, solvent D) were combined and evaporated to dryness. The residue was dissolved in MeOH (0.5 ml), from which crude 14 was precipitated by addition of acetone (10 ml). The procedure was repeated twice to yield pure 14 (32.5 mg, 55.1%), δ: 5.33 (3 min) → 4.75 (1 hr) (c=0.6, H2O), mp 169°C—172°C. PMR (D2O): δ: 1.37 (3H, d, J= 7 Hz, CH3 in L-Fuc), 2.14, 2.15 (6H, each s, NAc×2). TLC: RF 0.27 (solvent D), 0.20 (E). Anal. Calcd for C33H47N2O24·3H2O: C, 42.31; H, 7.10; N, 4.49. Found: C, 42.02; H, 7.21; N, 4.47.

TLC of Acid Hydrolysate of 14—1) Partial Hydrolysis: A mixture of 14 (1 mg) and 0.1 n HCl (2 ml) was heated at 90°C for 30 min, then evaporated to dryness. The residue was dissolved in a small amount of H2O and subjected to TLC; di-N-acetylchitobiose [RF 0.45 (solvent D), 0.31 (E)] and fucose [RF 0.63 (solvent D), 0.42 (E)] were identified.

2) Complete Hydrolysis: A mixture of 14 (1 mg) and 3 N HCl (2 ml) was heated at 90° for 3 hr. GlcN-HCl \( R_f \) 0.03 (solvent D), 0.28 (E) and fucose were identified in the hydrolysate.

Measurement of CMR Spectra—CMR spectra were measured at 25 MHz using a JEOL JNM-FX-100 spectrometer in the pulse Fourier transform mode. Spectra were taken in CDCl3 or D2O with TMS as an internal or external reference, respectively. The chemical shifts are given in ppm from TMS. The reference compounds, 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-\( \alpha \)-D-glucopyranose (15), \( \beta \)-D-glucopyranose (16), and methyl 2,3,4-tri-O-benzyl-\( \alpha \)-L-fucopyranoside (17) were prepared according to the cited references. Methyl 2,3,4-tri-O-benzyl-\( \beta \)-L-fucopyranoside (18), a syrup, \( [\alpha]_D^0 +6^\circ \) (c=0.7, CHCl3), was synthesized from methyl \( \beta \)-L-fucopyranoside17 by a procedure analogous to that reported for the benzylation of the corresponding \( \alpha \)-isomer.16)

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