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Synthesis of 2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-O-(α-L-fucopyranosyl)-D-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-glucopyranose with 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′: 4,5]-2-oxazoline to yield a fully protected β-D-(1→4)-linked disaccharide (**8**) bearing 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) at the non-reducing residue. After acetolysis of the 1,6-anhydro ring of **8**, the resulting fully-protected disaccharide is debenzylated to yield chitobiose heptaacetate (**10**) having a free hydroxyl group at the C-3 position of the reducing GlcNAc. Compound **10** is glycosylated with 2,3,4-tri-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.[2] According to the proposed structure, the oligosaccharide has the characteristic feature that L-fucose is attached to the N-acetylglucosamine (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).[3]

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.[4] However, synthesis of the trisaccharide unit existing in the internal region of serum-type glycoproteins bearing N-acetylglucosaminyl-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 hydroxyl group at the C-4 position with the acetylated oxazoline of GlcNAc. 2) Acetolysis of **8** and debenzylation of the acetolysis product afford chitobiose heptaacetate (**10**) having a free hydroxyl group at the

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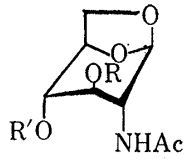
2) H. Ishihara, N. Takahashi, S. Oguri, and S. Tejima, *J. Biol. Chem.*, **254**, 10715 (1979).
3) See the references cited in 2).
4) *a*) R.U. Lemieux, K.B. Hendriks, R.V. Stick, and K. James, *J. Am. Chem. Soc.*, **97**, 4056 (1975); *b*) R.U. Lemieux and H. Driguez, *ibid.*, **97**, 4063, 4069 (1975); *c*) J.-C. Jacquinet and P. Sinaÿ, *J. Org. Chem.*, **42**, 720 (1977), *idem*, *Tetrahedron*, **35**, 365 (1979); *d*) H. Paulsen, W. Stenzel, and Č. Kolář, *Tetrahedron Lett.*, **1977**, 2785; H. Paulsen and Č. Kolář, *Angew. Chem. Int. Ed.*, **17**, 771 (1978); *Chem. Ber.*, **112**, 3190 (1979); H. Paulsen and W. Stenzel, *ibid.*, **111**, 2334, 2348 (1978); H. Paulsen, Č. Kolář, and W. Stenzel, *ibid.*, **111**, 2358, 2370 (1978).

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-acetylchitobiose 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-acetylchitobiose 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-mannopyranose[7] *via* 3 steps by a slight modification of the procedure of Schmitt and Sinaÿ[8]; 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 hydroxyl group with tetrahydropyranyl 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 tetrahydropyranyl 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 benzylation 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%.



1 : R=Ac, R′=H **4** : R=Bn, R′=THP
2 : R=Ac, R′=THP **5** : R=Bn, R′=H
3 : R=H, R′=THP **6** : R=Bn, R′=Ac

Ac=acetyl, Bn=benzyl,
THP=tetrahydropyranyl

Chart 1

Condensation of the aglycone **5** with 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[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

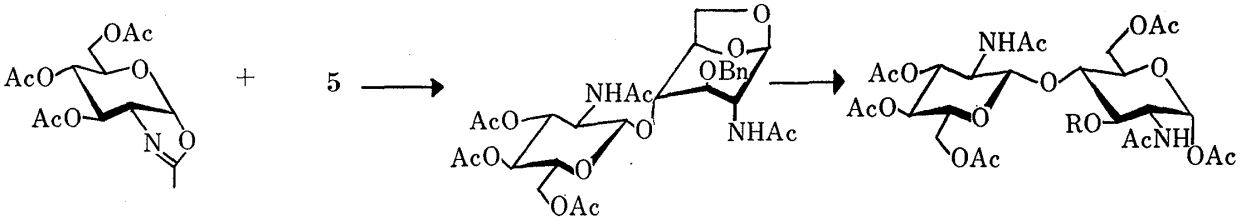
5) S. Oguri and S. Tejima, *Chem. Pharm. Bull.*, **28**, 3184 (1980).
6) C.D. Warren and R.W. Jeanloz, *Carbohydr. Res.*, **53**, 67 (1977).
7) M. Černý, J. Pacák, and J. Staněk, *Collect. Czech. Chem. Commun.*, **30**, 1155 (1956).
8) F. Schmitt and P. Sinaÿ, *Carbohydr. Res.*, **29**, 99 (1973).
9) K.L. Matta and O.P. Bahl, *Carbohydr. Res.*, **21**, 460 (1972).

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 $^1C_4$-D-conformation of the reducing terminus.[10] Therefore, before condensation with L-fucose, the conformation must be changed to $^4C_1$-D to increase the reactivity at the C-3 hydroxyl group.

The 1,6-anhydro ring in **8** was cleaved by mild acetolysis with an acetolysis 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**, $[\alpha]_D$ −113°, to dextrorotatory **9**, +41.8°. 2) In the PMR spectrum of **9** the anomeric proton due to the reducing terminus appeared as a doublet with $J_{1,2}$=4 Hz.

Catalytic debenzylation 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 95.7% yield; this was indistinguishable from an authentic sample.



7 **8** **9** : R=Bn, **10** : R=H, **11** : R=Ac

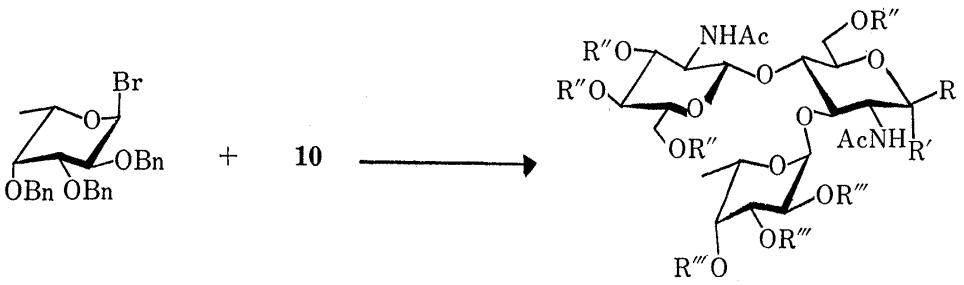
Chart 2

The bromide ion-catalyzed reaction is known to be an excellent route to α-L-fucosides.[4a,b,c] A mixture of the aglycone **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-deoxy-β-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_2O). 2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α- and β-D-glucopyranoses (**15** and **16**), and methyl 2,3,4-tri-O-benzyl-α- and β-L-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 anomeric carbons are summarized in Table I. The results provided valuable information on the configurations of the anomeric carbons and glycosidic linkages in **10**, **13**, and **14**.

10) M. Černý and J. Staněk, Jr., *Adv. Carbohydr. Chem. Biochem.*, **34**, 24 (1977).



12

13 : R=H, R′=OAc, R″=Ac, R‴=Bn
14 : R, R′=H, OH, R″=R‴=H

Chart 3

Table I. CMR Chemical Shifts; δ (ppm) from TMS

Solvent	$CDCl_3$						D_2O
Compound	**15**[a]	**16**[b]	**17**[c]	**18**[d]	**10**	**13**	**14**
C-1	90.6	92.5	98.7	104.8	90.4	89.2	91.95(α) 95.80(β)
C-1′	—	—	—	—	101.9	99.1	101.5
C-1″	—	—	—	—	—	99.1	99.7

a) 2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose.
b) 2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-glucopyranose.
c) Methyl 2,3,4-tri-O-benzyl-α-L-fucopyranoside.
d) Methyl 2,3,4-tri-O-benzyl-β-L-fucopyranoside.

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. PMR spectra were recorded at 100 MHz with a JEOL JNM-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_{254}, Merck) with the following solvent combinations (v/v): (A), acetone–$CHCl_3$ (1 : 1); (B), $CHCl_3$–MeOH (30 : 1); (C), ether–hexane–MeOH (5 : 5 : 1); (D), $CHCl_3$–MeOH–H_2O (5 : 5 : 1); (E), PrOH–H_2O–NH_4OH (70 : 30 : 1). Detection was effected with the spray reagent, anisaldehyde–H_2SO_4–EtOH (1 : 1 : 18) at 125°,[11] 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 Sinaÿ.[8]

Azidolysis of 1,6 : 2,3-dianhydro-4-O-benzyl-β-D-mannopyranose[7] in aq. hexamethylphosphoric triamide according to the method of Paulsen and Stenzel[4d] gave 1,6-anhydro-2-azido-4-O-benzyl-2-deoxy-β-D-glucopyranose, mp 100—102°, $[\alpha]_D^{24}$ −5.4° (c=1, $CHCl_3$) in 70% yield [lit.[4d] mp 96°, $[\alpha]_D^{20}$ −6° (c=1.1, $CHCl_3$)]. 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, $[\alpha]_D^{23}$ −33.6° (c=0.66, $CHCl_3$). The benzyl group was removed by hydrogenolysis on Pd black to give **1**, mp 139—142°, $[\alpha]_D^{22}$ −79.6° (c=1, MeOH). [lit.[8] mp 143—144°, $[\alpha]_D^{20}$ −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-pyranyl)-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 $CHCl_3$ (100 ml), washed successively with aq. $NaHCO_3$ and H_2O, dried ($CaCl_2$), and evaporated to dryness. The residue was chromatographed on a column (1.7×70 cm) of silica gel (80 g), eluting with $CHCl_3$–MeOH (100 : 1). The fractions

11) P.J. Dunphy, J.D. Kerr, J.F. Pennock, K.J. Whittle, and J. Feeney, *Biochem. Biophys. Acta*, **136**, 136 (1976).

having *Rf* 0.66 (TLC, solvent A) were combined and evaporated to a syrup (3.03 g, 92.1%), $[\alpha]_D^{20}$ −46.1° (c=1.1, $CHCl_3$). PMR ($CDCl_3$) δ: 1.40—1.90 (6H, m, $CH_2\times3$ in THP), 2.02, 2.12 (6H, each s, NAc, OAc), 5.38 (1H, s, H-1). TLC: *Rf* 0.66 (solvent A), 0.39 (B), 0.20 (C).

2-Acetamido-1,6-anhydro-2-deoxy-4-O-(tetrahydro-2-pyranyl)-β-D-glucopyranose (3)——Methanolic NaOMe (0.5 N, 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 (1.7×70 cm) of silica gel (80 g), eluting with $CHCl_3$–MeOH (30: 1). The fractions having *Rf* 0.38 (TLC, solvent A) were combined and concentrated to give **3** (2.43 g, 87.1%), $[\alpha]_D^{21}$ −43° (c=0.43, $CHCl_3$), as a syrup. PMR ($CDCl_3$) δ: 1.40—1.90 (6H, m, $CH_2\times3$ in THP), 2.00 (3H, s, NAc), 5.42 (1H, s, H-1), 6.24 (1H, d, $J_{NH,2}$=8 Hz, NH). TLC: *Rf* 0.38 (solvent A), 0.19 (B), 0.14 (C).

2-Acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-4-O-(tetrahydro-2-pyranyl)-β-D-glucopyranose (4)——1) From Compound **3**: A mixture of **3** (500 mg, 1.74 mmol), BaO (2.3 g), $Ba(OH)_2\cdot8H_2O$ (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 (1.3×80 cm) of silica gel (40 g), eluting with $CHCl_3$. The fractions having *Rf* 0.78 (TLC, solvent A) were combined and concentrated to give **4** (651 mg, 99.2%), $[\alpha]_D^{20}$ −82.6° (c=0.78, $CHCl_3$), as a syrup. PMR ($CDCl_3$) δ: 1.40—1.90 (6H, m, $CH_2\times3$ in THP), 1.96 (3H, s, NAc), 5.36 (1H, s, H-1), 6.08 (1H, d, $J_{NH,2}$=8 Hz, NH), 7.30 (5H, s, aromatic protons). TLC: *Rf* 0.78 (solvent A), 0.46 (B), 0.38 (C). *Anal.* Calcd for $C_{20}H_{27}NO_6$: C, 63.64; H, 7.21; N, 3.71. Found: C, 63.35; H, 7.08; N, 3.60.

2) From Compound **2**: Benzylation of **2** (2 g, 6.07 mmol) with BaO (4 g), $Ba(OH)_2\cdot8H_2O$ (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 g, 91.7%). The product was indistinguishable from **4** in terms of $[\alpha]_D$, mobilities on TLC, and PMR spectrum.

2-Acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-β-D-glucopyranose (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 70% (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 filtrate was evaporated to dryness. The residue was chromatographed on a column (1.3×85 cm) of silica gel (40 g), eluting with $CHCl_3$–MeOH (20: 1). The fractions having *Rf* 0.43 (TLC, solvent A) were combined and concentrated to give **5** (643 mg, 82.7%), $[\alpha]_D^{21}$ −88° (c=0.86, $CHCl_3$), as a syrup. PMR ($CDCl_3$) δ: 1.92 (3H, s, NAc), 5.30 (1H, s, H-1), 6.72 (1H, d, $J_{NH,2}$=8 Hz, NH), 7.24 (5H, s, aromatic protons). TLC: *Rf* 0.43 (solvent A), 0.13 (B), 0.19 (C). *Anal.* Calcd for $C_{15}H_{19}NO_5\cdot1/2H_2O$: 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-β-D-glucopyranose (6)——Compound **5** (300 mg, 1 mmol) was acetylated with Ac_2O (3 ml) and pyridine (5 ml) at room temperature. Excess Ac_2O was destroyed by dropwise addition of H_2O (1 ml), and the solution was evaporated to a syrup which crystallized from AcOEt–ether as prisms (329 mg, 98.9%), mp 115—116°, $[\alpha]_D^{19}$ −93.8° (c=1, $CHCl_3$). PMR ($CDCl_3$) δ: 2.00, 2.08, (6H, each s, OAc, NAc), 5.35 (1H, s, H-1), 5.90 (1H, d, $J_{NH,2}$=8 Hz, NH), 7.31 (5H, s, aromatic protons). *Anal.* Calcd for $C_{17}H_{21}NO_6$: 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-β-D-glucopyranosyl)-1,6-anhydro-3-O-benzyl-2-deoxy-β-D-glucopyranose (8)——A mixture of **5** (1 g, 3.31 mmol) and 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′: 4,5]-2-oxazoline (**7**)[9] (1.5 g, 4.55 mmol) in 1,2-dichloroethane (20 ml) containing 0.005 N TsOH was boiled 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 TsOH were added, and 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 (1.7×55 cm) of silica gel (60 g), eluting with $CHCl_3$–MeOH (100: 1). The fractions having *Rf* 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_2O (5 ml) and pyridine (10 ml) at room temperature for 12 hr, treated with H_2O (2 ml) to decompose excess Ac_2O, and evaporated to dryness. The residue was re-chromatographed on a column (1.3×85 cm) of silica gel (40 g), eluting with $CHCl_3$–MeOH (70: 1). From the earlier fractions having *Rf* 0.71 (TLC, solvent A), **6** (610 mg, 55%) was isolated after removal of the solvent. Compound **6** could be recycled after de-O-acetylation to **5**.

The desired disaccharide (**8**) was isolated from the later fractions having *Rf* 0.48 (TLC, solvent A). The product (857 mg, 41.4%) crystallized from MeOH–ether–hexane as needles, mp 205—207°, $[\alpha]_D^{22}$ −113° (c=1, $CHCl_3$). PMR ($CDCl_3$) δ: 2.00, 2.03, 2.06, 2.08, 2.12 (15H, each s, OAc×3, NAc×2), 5.34 (1H, s, H-1 of reducing GlcNAc), 6.02 (1H, d, $J_{NH,2}$=7 Hz, NH of reducing GlcNAc), 6.78 (1H, d, $J_{NH,2'}$=8 Hz, NH of non-reducing (GlcNAc), 7.37 (5H, s, aromatic protons). TLC: *Rf* 0.48 (solvent A), 0.14 (B), 0.05 (C). *Anal.* Calcd for $C_{29}H_{38}N_2O_{13}$: 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-β-D-glucopyranosyl)-1,6-di-O-acetyl-3-O-benzyl-2-deoxy-α-D-glucopyranose (9)——A solution of **8** (1 g, 1.61 mmol) in acetolysis mixture [20 ml, Ac_2O–AcOH–H_2SO_4 (70: 30: 1, v/v)] was stirred at 20° for 2 hr, poured into ice-H_2O (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°, $[\alpha]_D^{22}$ +41.8° (c=0.64, MeOH). PMR ($CDCl_3$–CD_3OD,

3:1, v/v) δ: 1.84, 1.92, 2.00, 2.01 (21H, each s, OAc×5, NAc×2), 6.05 (1H, d, $J_{1,2}$=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 $C_{33}H_{44}N_2O_{16}$: 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 H_2 ceased; the catalyst was freshly prepared from $PdCl_2$ (100 mg) according to the method of Schmidt and Staab.[12] After removal of the catalyst 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 $CHCl_3$–MeOH (40:1) to give **10** as a glass (83.2 mg, 92.4%), $[\alpha]_D^{22}$ +66.5° (c=0.4, MeOH). PMR ($CDCl_3$–CD_3OD, 3:1, v/v) δ: 1.98, 2.00, 2.03, 2.09, 2.13, 2.16 (21H, each s, OAc×5, NAc×2), 5.56 (1H, d, $J_{NH,2}$=8 Hz, NH of reducing GlcNAc), 6.19 (1H, d, $J_{1,2}$=4 Hz, H-1 of reducing GlcNAc), 6.46 (1H, d, $J_{NH,2'}$=8 Hz, NH of non-reducing GlcNAc). TLC: *Rf* 0.26 (solvent A), 0.08 (B), 0.01 (C). *Anal.* Calcd for $C_{26}H_{38}N_2O_{16}\cdot H_2O$: 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-tri-O-acetyl-2-deoxy-α-D-glucopyranose (Chitobiose Octaacetate) (11)——Acetylation of **10** (50 mg, 0.08 mmol) with Ac_2O (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 with mp>300° and $[\alpha]_D^{20}$ +55.4° (c=0.72, AcOH). The product was indistinguishable from authentic chitobiose octaacetate prepared from chitin (Wako) by acetolysis.[13]

2,3,4-Tri-O-benzyl-α-L-fucopyranosyl Bromide (12)——The bromide was prepared by a slight modification of the method reported by Dejter-Juszynski and Flowers.[14]

Dry HBr was introduced into dry CH_2Cl_2 to give a concentration of 0.50 N. To this solution (1.2 ml), a solution of 1-O-*p*-nitrobenzoyl-2,3,4-tri-O-benzyl-β-L-fucopyranose (322 mg, 0.55 mmol) in CH_2Cl_2 (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)-2-deoxy-α-D-glucopyranose (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 N_2 gas at 20° for 3 days. After addition of $CHCl_3$ (50 ml), the solid was removed by filtration, and the filtrate was washed with H_2O, dried ($MgSO_4$), and evaporated to dryness. The residue was chromatographed on a column (0.9×50 cm) of silica gel (10 g), eluting with $CHCl_3$–MeOH (100:1). Removal of the solvent from the effluent having *Rf* 0.62 (TLC, solvent A) gave **13** (72.4 mg, 46.6%), which crystallized from AcOEt–ether–hexane, mp 165—166°, $[\alpha]_D^{22}$ −39.7° (c=0.72, MeOH). PMR ($CDCl_3$) δ: 1.24 (3H, d, J=7 Hz, C<u>H</u>$_3$ in L-Fuc), 1.68, 1.74, 2.04, 2.12 (21H, each s, OAc×5, NAc×2), 5.92 (1H, d, $J_{NH,2}$=7 Hz, NH of reducing GlcNAc), 6.28 (1H, d, $J_{1,2}$=3 Hz, H-1 of reducing GlcNAc), 7.12 (1H, d, $J_{NH,2'}$=7 Hz, NH of non-reducing GlcNAc), 7.20—7.40 (15H, m, aromatic protons). TLC: *Rf* 0.62 (solvent A), 0.21 (B), 0.08 (C). *Anal.* Calcd for $C_{53}H_{66}N_2O_{20}$: 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-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 prepared[12] from $PdCl_2$ (100 mg). After removal of the catalyst by filtration, the filtrate was concentrated to give an amorphous powder (72.6 mg, 97.7%).

The resulting debenzylated product was, without purification, dissolved in freshly prepared 20% (w/v) NH_3 in MeOH (3 ml) at 0°. The mixture was left to stand at 4° 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 $CHCl_3$–MeOH–H_2O (5:5:1). The fractions having *Rf* 0.27 (TLC, 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%), $[\alpha]_D^{22}$ +8.3° (3 min)→+6.7° (1 hr) (c=0.6, H_2O), mp 169—172°. PMR (D_2O) δ: 1.37 (3H, d, J=7 Hz, C<u>H</u>$_3$ in L-Fuc), 2.14, 2.15 (6H, each s, NAc×2). TLC: *Rf* 0.27 (solvent D), 0.20 (E). *Anal.* Calcd for $C_{22}H_{38}N_2O_{15}\cdot 3H_2O$: 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° for 30 min, then evaporated to dryness. The residue was dissolved in a small amount of H_2O, 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.

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2) Complete Hydrolysis: A mixture of **14** (1 mg) and 3 N HCl (2 ml) was heated at 90° for 3 hr. GlcN-HCl [*Rf* 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 $CDCl_3$ or D_2O 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-α-D-glucopyranose (**15**),[15] -β-D-glucopyranose (**16**),[16] and methyl 2,3,4-tri-O-benzyl-α-L-fucopyranoside (**17**)[14] were prepared according to the cited references. Methyl 2,3,4-tri-O-benzyl-β-L-fucopyranoside (**18**), a syrup, $[\alpha]_D^{20}$ +6° (c=0.7, $CHCl_3$), was synthesized from methyl β-L-fucopyranoside[17] by a procedure analogous to that reported for the benzylation of the corresponding α-isomer.[14]

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