[Chem. Pharm. Bull.
28(11)3196-3202(1980)]

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)

SHIGEYUKI OGURI,¹ HIDEKO ISHIHARA, and SETSUZO TEJIMA

Faculty of Pharmaceutical Sciences, Nagoya City University¹⁾

(Received April 16, 1980)

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-glucopyranose)-[2',1':4,5]-2-oxazoline to yield a fully protected β -D-(1 $\rightarrow$ 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 $\rightarrow$ 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 $\rightarrow$ 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

1) Location: Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.

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. Sinay, *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-mannopyranose⁷⁾ *via* 3 steps by a slight modification of the procedure of Schmitt and Sinaý⁸⁾; 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%.

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

- 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

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. Sinaý, *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**, $[\alpha]_D -113^\circ$, to dextrorotatory **9**, $+41.8^\circ$. 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 debenzoylation 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.

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 deuteriochloroform ($CDCl_3$), 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).

Chart 3

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

Solvent	CDCl ₃						D ₂ O
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 F₂₅₄, Merck) with the following solvent combinations (v/v): (A), acetone-CHCl₃ (1:1); (B), CHCl₃-MeOH (30:1); (C), ether-hexane-MeOH (5:5:1); (D), CHCl₃-MeOH-H₂O (5:5:1); (E), PrOH-H₂O-NH₄OH (70:30:1). Detection was effected with the spray reagent, anisaldehyde-H₂SO₄-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 Sinay.⁸⁾

Azidolysis of 1,6:2,3-dianhydro-4-O-benzyl- β -D-mannopyranose⁷⁾ 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°, $[\alpha]_D^{25}$ -5.4° ($c=1$, CHCl₃) in 70% yield [lit.^{4d)} mp 96°, $[\alpha]_D^{20}$ -6° ($c=1.1$, CHCl₃)]. 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, $[\alpha]_D^{25}$ -33.6° ($c=0.66$, CHCl₃). The benzyl group was removed by hydrogenolysis on Pd black to give 1, mp 139–142°, $[\alpha]_D^{25}$ -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-pyran-2-yl)- 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 CHCl₃ (100 ml), washed successively with aq. NaHCO₃ and H₂O, dried (CaCl₂), and evaporated to dryness. The residue was chromatographed on a column (1.7 $\times$ 70 cm) of silica gel (80 g), eluting with CHCl₃-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 R_f 0.66 (TLC, solvent A) were combined and evaporated to a syrup (3.03 g, 92.1%), $[\alpha]_D^{20} -46.1^\circ$ ($c=1.1$, CHCl_3). PMR (CDCl_3) δ : 1.40–1.90 (6H, m, $\text{CH}_2 \times 3$ in THP), 2.02, 2.12 (6H, each s, NAc, OAc), 5.38 (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-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 $\times$ 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%), $[\alpha]_D^{20} -43^\circ$ ($c=0.43$, CHCl_3), as a syrup. PMR (CDCl_3) δ : 1.40–1.90 (6H, m, $\text{CH}_2 \times 3$ in THP), 2.00 (3H, s, NAc), 5.42 (1H, s, H-1), 6.24 (1H, d, $J_{\text{NH},2}=8\text{ Hz}$, NH). 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-pyranyl)- β -D-glucopyranose (4)—1) From Compound **3**: A mixture of **3** (500 mg, 1.74 mmol), BaO (2.3 g), $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (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 $\times$ 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, 99.2%), $[\alpha]_D^{20} -82.6^\circ$ ($c=0.78$, CHCl_3), as a syrup. PMR (CDCl_3) δ : 1.40–1.90 (6H, m, $\text{CH}_2 \times 3$ in THP), 1.96 (3H, s, NAc), 5.36 (1H, s, H-1), 6.08 (1H, d, $J_{\text{NH},2}=8\text{ Hz}$, NH), 7.30 (5H, s, aromatic protons). TLC: R_f 0.78 (solvent A), 0.46 (B), 0.38 (C). Anal. Calcd for $\text{C}_{20}\text{H}_{27}\text{NO}_6$: 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), $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (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 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 $\times$ 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%), $[\alpha]_D^{20} -88^\circ$ ($c=0.86$, CHCl_3), as a syrup. PMR (CDCl_3) δ : 1.92 (3H, s, NAc), 5.30 (1H, s, H-1), 6.72 (1H, d, $J_{\text{NH},2}=8\text{ Hz}$, NH), 7.24 (5H, s, aromatic protons). TLC: R_f 0.43 (solvent A), 0.13 (B), 0.19 (C). Anal. Calcd for $\text{C}_{15}\text{H}_{19}\text{NO}_5 \cdot 1/2\text{H}_2\text{O}$: 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°, $[\alpha]_D^{20} -93.8^\circ$ ($c=1$, 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, $J_{\text{NH},2}=8\text{ Hz}$, NH), 7.31 (5H, s, aromatic protons). Anal. Calcd for $\text{C}_{17}\text{H}_{21}\text{NO}_6$: 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-glucopyranose)-[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 $\times$ 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_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 $\times$ 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 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 R_f 0.48 (TLC, solvent A). The product (857 mg, 41.4%) crystallized from MeOH-ether-hexane as needles, mp 205–207°, $[\alpha]_D^{20} -113^\circ$ ($c=1$, CHCl_3). PMR (CDCl_3) δ : 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, $J_{\text{NH},2}=7\text{ Hz}$, NH of reducing GlcNAc), 6.78 (1H, d, $J_{\text{NH},2'}=8\text{ Hz}$, 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 $\text{C}_{29}\text{H}_{38}\text{N}_2\text{O}_{13}$: C, 55.94; H, 6.15; N, 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°, $[\alpha]_D^{20} +41.8^\circ$ ($c=0.64$, MeOH). PMR (CDCl_3 - CD_3OD ,

3: 1, v/v) δ : 1.84, 1.92, 2.00, 2.01 (21H, each s, OAc $\times$ 5, NAc $\times$ 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 $\times$ 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%), $[\alpha]_D^{25} + 66.5^\circ$ (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 $\times$ 5, NAc $\times$ 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 $>300^\circ$ and $[\alpha]_D^{20} + 55.4^\circ$ (c = 0.72, AcOH). The product was indistinguishable from authentic chitobiose octaacetate prepared from chitin (Wako) by acetylation.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 $\times$ 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° , $[\alpha]_D^{25} - 39.7^\circ$ (c = 0.72, MeOH). PMR ($CDCl_3$) δ : 1.24 (3H, d, J = 7 Hz, CH_3 in L-Fuc), 1.68, 1.74, 2.04, 2.12 (21H, each s, OAc $\times$ 5, NAc $\times$ 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 prepared¹² 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 $\times$ 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%), $[\alpha]_D^{25} + 8.3^\circ$ (3 min) $\rightarrow$ $+6.7^\circ$ (1 hr) (c = 0.6, H_2O), mp 169 – 172° . PMR (D_2O) δ : 1.37 (3H, d, J = 7 Hz, CH_3 in L-Fuc), 2.14, 2.15 (6H, each s, NAc $\times$ 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.

12) O. Th. Schmidt and W. Staab, *Chem. Ber.*, **87**, 388 (1954).

13) T. Osawa, *Carbohydr. Res.*, **1**, 435 (1966).

14) M. Dejter-Juszynski and H.M. Flowers, *Carbohydr. Res.*, **18**, 219 (1971).

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 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- 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, [α]_D²⁰ + 6° (*c*=0.7, CHCl₃), was synthesized from methyl β -L-fucopyranoside¹⁷⁾ by a procedure analogous to that reported for the benzylation of the corresponding α -isomer.¹⁴⁾

Acknowledgement We thank Mrs. T. Kumagai for the PMR and CMR spectral measurements, and Misses S. Iwauchi and T. Naito for the microanalyses.

15) Y. Inouye, K. Onodera, and S. Kitaoka, and H. Ochiai, *J. Am. Chem. Soc.*, **79**, 4218 (1957).

16) M. Bergmann and L. Zervas, *Chem. Ber.*, **64**, 975 (1931).

17) T. Chiba and S. Tejima, *Chem. Pharm. Bull.*, **27**, 2838 (1979).