[Chem. Pharm. Bull. 29(6)1629—1635(1981)]

Synthesis of 2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-6-O-(α-L-fucopyranosyl)-D-glucopyranose (6-O-α-L-Fucopyranosyl-di-N-acetylchitobiose)

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(Received December 22, 1980)

Benzyl 3,3′,4′,6′-tetra-O-benzoyl-β-di-N-acetylchitobioside (**8**) was prepared in 5 steps from 3,3′,4′,6′-tetra-O-acetyl-1,6-anhydro-β-di-N-acetylchitobiose [S. Oguri and S. Tejima, *Chem. Pharm. Bull.*, **28**, 3184 (1980)] by the following series of reactions; de-O-acetylation, benzoylation, acetolysis of the 1,6-anhydro-β-ring, benzyl glycosidation *via* oxazoline, and selective de-O-acetylation. Reaction of **8** with 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide by a bromide ion-catalyzed reaction afforded benzyl 3,3′,4′,6′-tetra-O-benzoyl-6-O-(2″,3″,4″-tri-O-benzyl-α-L-fucopyranosyl)-β-di-N-acetylchitobioside (**10**) in 83.4% yield. After removal of the protecting groups of **10**, 6-O-α-L-fucopyranosyl-di-N-acetyl chitobiose (**11**) was obtained as needles. ^{13}C-NMR spectra data for **11** are presented.

Keywords——synthesis; 6-O-α-L-fucopyranosyl-di-N-acetylchitobiose; 1,6-anhydro-di-N-acetylchitobiose derivative; oxazoline glycosidation method; benzyl di-N-acetylchitobioside derivative; bromide ion-catalyzed glycosidation; selective de-O-acetylation; ^{13}C-NMR

In recent years, studies in the fields of biology and immunology at the molecular level have revealed that carbohydrates participate in phenomena such as cell-cell interaction, differentiation, in phenomena such as cell-cell interaction, differentiation, and antigenic recognition. We are therefore interested in the chemical syntheses of carbohydrates, particularly those that are present in glycoconjugates possessing biological activities. In the preceding paper,[1] we reported a synthesis of 3-O-α-L-fucopyranosyl-di-N-acetylchitobiose, which was confirmed to exist in the internal region of the carbohydrate moiety of stem bromelain.[2] As an extension of the syntheses of oligosaccharides in the internal regions of asparagine-linked glycopeptides, we now report a synthesis of 6-O-α-L-fucopyranosyl-di-N-acetylchitobiose (**11**).

The occurrence of **11** in the reducing terminus of oligosaccharides in asparagine-linked glycopeptides has been reported in a number of cases.[3] In addition, the occurrence of **11** in glycopeptides isolated from glycoproteins of natural origin is now known to be quite widespread as a result of many biochemical studies on glycoconjugates.[4]

Our synthetic route is based on the condensation of an L-fucopyranosyl residue with benzyl 3,3′,4′,6′-tetra-O-benzoyl-β-di-N-acetylchitobioside (**8**) (having an unprotected hydroxyl group at the C-6 position) by the bromide ion-catalyzed reaction which is known to be an excellent route to α-L-fucosides.[5] Subsequent removal of the protecting groups from the resulting fully protected trisaccharide (**10**) provides **11**. The preparation of **8**, the key intermediate of this synthesis, is therefore described firstly.

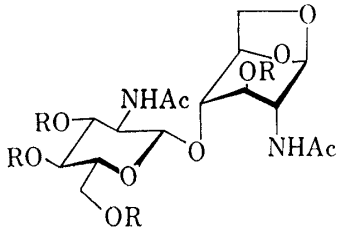
3,3′,4′,6′-Tetra-O-acetyl-1,6-anhydro-β-di-N-acetylchitobiose (**1**)[6] was selected as a starting material for this synthesis. Attempts to cleave the 1,6-anhydro-β-ring of **1** with titanium tetrachloride or tetrabromide to yield halides having an unprotected hydroxyl group at the C-6 position, which provides the shortest route to **8**, were unsuccessful. For example, when a mixture of **1** and titanium tetrahalides in chloroform was refluxed for 30 min, thin-layer chromatography (TLC) on a silica gel plate revealed several by-products as well as unreacted starting material (**1**), while the desired halides could not be identified; considerable decomposition of **1** occurred to give complex by-products. Thus, studies on this route were

abandoned and, as a next step, the following detour to **8** was investigated.

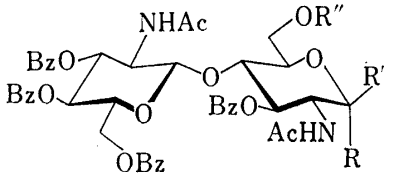
De-O-acetylation of **1** with sodium methoxide gave crystalline 1,6-anhydro-β-di-N-acetylchitobiose (**2**) in 94.2% yield. Subsequent benzoylation of **2** by the usual method provided crystalline 1,6-anhydro-3,3′,4′,6′-tetra-O-benzoyl-β-di-N-acetylchitobiose (**3**) in 85% yield. The 1,6-anhydro-β-ring of **3** was smoothly acetolyzed with acetic anhydride-boron trifluoride etherate at 20° for 2 hr. By column chromatographic purification, crystalline 1,6-di-O-acetyl-3,3′,4′,6′-tetra-O-benzoyl-α-di-N-acetylchitobiose (**4**) was isolated in 72.4% yield. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, a new doublet having a coupling constant of 3 Hz was recognized at 6.24 ppm, while the singlet at 5.36 ppm due to the anomeric proton (H-1) of the 1,6-anhydro-β-N-acetyl-D-glucosamine residue in **3** had disappeared. Thus, the doublet was assigned as H-1 of the α-acetate. Since benzoylated sugars are known to provide more stable glycosyl derivatives than acetylated sugars,[7] **3** must be able to resist decomposition during cleavage of the 1,6-anhydro-β-ring.

Benzyl glycosidation of **4** was carried out by the oxazoline glycosidation method. The α-acetate (**4**) was firstly treated with dry hydrogen chloride in acetic acid–acetic anhydride to give a crude chloride (**5**), which was contaminated with a trace of the oxazoline (**6**). Without further purification, **5** was converted to **6** by a method analogous to that described for the preparation of oxazoline of N-acetyl-D-glucosamine (GlcNAc).[8] The resulting oxazoline (**6**) was also contaminated with a trace of a by-product, and we could not separate **6** in a pure state. However, the ^{1}H-NMR spectrum of crude **6** showed satisfactory signals for the assigned structure. A mixture of **6** and benzyl alcohol in 1,2-dichloroethane containing 0.015 N anhydrous *p*-toluenesulfonic acid (TsOH) was subsequently stirred at 60° under nitrogen to carry out benzyl glycosidation. The crystalline benzyl 6-O-acetyl-3,3′,4′,6′-tetra-O-benzoyl-β-di-N-acetylchitobioside (**7**) was isolated in 35.7% yield from the reaction mixture after column chromatography. In the ^{1}H-NMR spectrum of **7**, the doublet at 6.04 ppm due to the anomeric proton of **6** had disappeared. The same product was also prepared in lower yield (20%) from the chloride (**5**) by the Koenigs–Knorr glycosidation method.

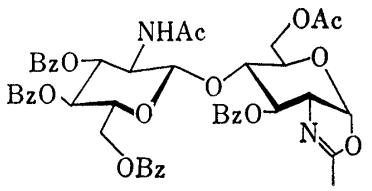
In order to convert the fully protected disaccharide (**7**) into the partially protected derivative (**8**), it was necessary to remove selectively the 6-O-acetate group without affecting the benzoate esters. Treatment of **7** with 10% (w/w) methanolic ammonia at 0° for 2 hr proceeded smoothly to achieve selective de-O-acetylation. The resulting benzyl 3,3′,4′,6′-tetra-O-benzoyl-β-di-N-acetylchitobioside (**8**) was isolated in 80.3% yield as an amorphous solid after column chromatography. Although selective de-O-acetylation provides a versatile procedure to prepare synthetic intermediates, only a few examples have been reported in carbohydrate chemistry.[9]



1: R=Ac
2: R=H
3: R=Bz



4: R=OAc, R′=H, R″=Ac
5: R=Cl, R′=H, R″=Ac
7: R=H, R′=OBn, R″=Ac
8: R=H, R′=OBn, R″=H



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Ac=acetyl, Bn=benzyl, Bz=benzoyl

Chart 1

The α-L-fucosidation of **8** was carried out by a method analogous to those described for the preparation of α-L-fucose-containing di- and trisaccharides by Lemieux and co-workers.[5] A mixture of **8** and 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (**9**) in 1,2-dichloroethane-

TABLE I. ^{13}C Chemical Shifts, δ (ppm) from TMS

Compound[a]		→6 →4 GlcNAc						GlcNAcβ1→						Fucα1→					
		C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″
GlcNAc	α	91.6	54.9	72.4	70.9	71.5	61.5												
	β	95.7	57.6	74.7	70.7	76.8	61.6												
GlcNAcβ1→Me								102.7	57.9	74.8	70.8	76.7	61.6						
GlcNAcβ1→4GlcNAc	α	91.6	54.8	71.1	81.0	70.5	61.2	102.6	57.3	74.7	70.9	77.4	61.7						
	β	96.0	56.8	73.7	80.6	75.7	61.2												
Fucα1→Me														100.7	73.0	69.1	70.8	67.6	16.5
Fucα1→6 4GlcNAc	α	91.7	54.9	71.0	80.6	69.4	68.0							100.8					
GlcNAcα1→ (11)								102.4	57.4	74.7	70.7	77.1	61.8		73.6	69.4	70.4	68.0	16.5
	β	96.1	56.9	73.0	80.3	74.7	68.4							100.5					

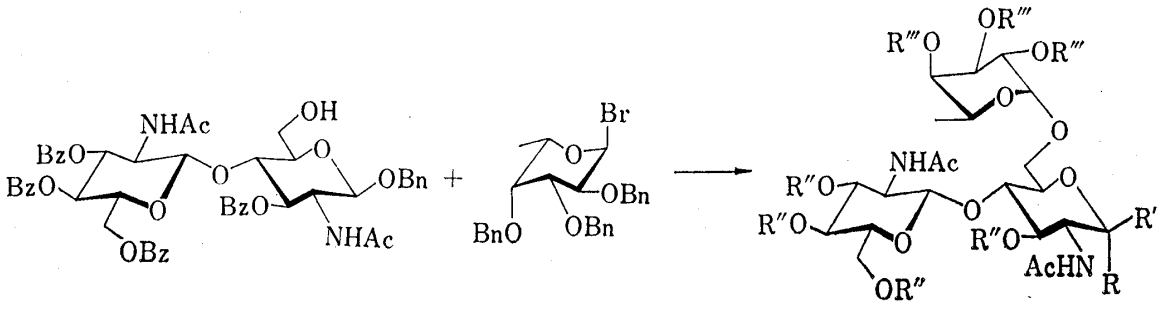
a) GlcNAc: 2-acetamido-2-deoxy-α,β-D-glucopyranose.
GlcNAcβ1→Me: methyl 2-acetamido-2-deoxy-β-D-glucopyranoside.
GlcNAcβ1→4GlcNAc: 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α,β-D-glucopyranose.
Fucα1→Me: methyl α-L-fucopyranoside.
GlcNAcβ1→4(Fucα1→6)GlcNAc: 2-Acetamido-4-O-(2-acetamido-2-deoxyl-β-D-glucopyranosyl)-2-deoxy-6-O-(α-L-fucopyranosyl)-α,β-D-glucopyranose (**11**).

N,N-dimethylformamide (DMF) was stirred at room temperature in the presence of tetraethylammonium bromide and molecular sieves under nitrogen. The fully protected trisaccharide (**10**), benzyl 3,3′,4′,6′-tetra-O-benzoyl-6-O-(2″,3″,4″-tri-O-benzyl-α-L-fucopyranosyl)-β-di-N-acetylchitobioside, was isolated from the reaction mixture as an amorphous solid in 83.4% yield after column chromatography.

The protecting groups of **10** were removed by debenzoylation with barium methoxide, followed by hydrogenolytic debenzylation, to yield the title trisaccharide (**11**) in 99.2% yield. The product was crystallized from aqueous ethanol as an anomeric mixture showing no mutarotation.

The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, in the Fourier transform mode with complete proton decoupling, of **11** was measured in deuterium oxide (D_2O) at room temperature. Tetramethylsilane (TMS) was used as an external standard. The signals anomeric carbons were assigned by selective proton decoupling of the corresponding anomeric protons, and those of other carbons were assigned as follows. Firstly, the signals for di-N-acetylchitobiose were assigned by comparison with literature values for 2-acetamido-2-deoxy-α,β-D-glucopyranoses[10] and methyl 2-acetamido-2-deoxy-β-D-glucopyranoside.[10] Secondly, the signals for **11** were assigned by comparison with the observed values for di-N-acetylchitobiose mentioned above and with reported values for methyl α-L-fucopyranoside.[11] The data are summarized in Table I.

According to values of carbon-13 chemical shifts hitherto reported for carbohydrates,[12] the signals due to the carbon of exocyclic hydroxymethyl groups (C-6, C-6′, *etc.*) appear at the 60—63 ppm region from TMS. In Table I, the signal of the C-6′ carbon of **11** is observed at 61.8 ppm, a reasonable value for the signal of a hydroxymethyl group. However, those due to the C-6 carbon are shifted from this region to lower fields (68.0 and 68.4 ppm for the α- and β-anomers, respectively). The results provide unequivocal proof of the position of the newly introduced L-fucosyl linkage in **11**, indicating that no migration of benzoyl groups had taken place during selective de-O-acetylation of **7**. The C-1″, carbon signal was observed as two singlets (100.5 and 100.8 ppm for the α- and β-anomers, respectively). The slight difference between these two values suggests that the angle between the planes of the di-N-acetylchitobiosyl and fucosyl residues may vary according to α- or β-configuration of the hydroxyl group at the anomeric center (C-1) of **11**.



8 **9** **10**: R=H, R′=OBn, R″=Bz, R‴=Bn
11: R,R′=H, OH, R″=R‴=H

Chart 2

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-201 automatic digital polarimeter in a 0.5 dm tube. Infrared (IR) spectra were recorded with a JASCO IRA-2 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded at 100 and 25 MHz, respectively, 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 plate 0.25 mm thick (Kieselgel 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°,[13] or by UV irradiation at 254 nm. Column chromatography was performed on Kieselgel (Merck, 70—230 mesh). Solvent combinations for elution are shown as v/v.

2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1,6-anhydro-2-deoxy-β-D-glucopyranose (1,6-Anhydro-β-di-N-acetylchitobiose) (2)——A 0.5 N methanolic solution of MeONa (1 ml) was added to a solution of 3,3′,4′,6′-tetra-O-acetyl-1,6-anhydro-β-di-N-acetylchitobiose (**1**)[6] (470 mg, 0.82 mmol) in MeOH (10 ml), and the mixture was stirred at room temperature for 3 hr. After neutralization with Amberlite IR-120B (H^+) resin, the mixture was filtered, and the filtrate was concentrated to a syrup, which was crystallized from MeOH–ether as white needles (313 mg, 94.2%), mp 171—174°, $[\alpha]_D^{30}$ −91.6° (c=1.4, MeOH). ^{1}H-NMR ($CDCl_3$): 2.02, 2.08 (6H, each s, $-NHCOCH_3\times2$), 4.48 (1H, d, $J_{1',2'}$=8 Hz, H-1′), 5.20 (1H, s, H-1), 7.18 (1H, d, $J_{NH,2\text{ or }2'}$=8 Hz, NH), 7.95 (1H, d, $J_{NH,2'\text{ or }2}$=8 Hz, NH). ^{13}C-NMR (CD_3OD): 101.3 (C-1′), 102.2 (C-1). IR ν_{max}^{Nujol} cm^{-1}: 3360, 3230 (OH, NH), 1650 (amide I), 1530 (amide II). TLC: *Rf* 0.59 (solvent D), 0.37 (E). *Anal.* Calcd for $C_{16}H_{26}N_2O_{10}\cdot H_2O$: C, 45.28; H, 6.65; N, 6.60. Found: C, 45.13; H, 6.61; N, 6.53.

2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-β-D-glucopyranosyl)-1,6-anhydro-3-O-benzoyl-2-deoxy-β-D-glucopyranose (1,6-Anhydro-3,3′,4′,6′-tetra-O-benzoyl-β-di-N-acetylchitobiose (3)——Compound **2** (313 mg, 0.74 mmol) was benzoylated with benzoyl chloride (1.1 ml, 9.48 mmol) and pyridine (5 ml) at 0° for 30 min. The mixture was poured into ice-H_2O (50 ml), and extracted with $CHCl_3$ (25 ml×2). The combined extracts were successively washed with satd. aqueous $NaHCO_3$ and H_2O. Desiccation ($MgSO_4$) and removal of the solvent provided a syrup, which was chromatographed on a column (1.3×85 cm) of Kieselgel (40 g) with $CHCl_3$–MeOH (50: 1). The major fraction eluted was crystallized from $CHCl_3$–ether as white needles. Recrystallization from the same solvents gave pure **3** (542 mg, 85%), which partially melts at 135° and completely melts at 148—150°, $[\alpha]_D^{22}$ −72.4° (c=0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.96, 2.20 (6H, each s, $-NHCOCH_3\times2$), 5.36 (1H, s, H-1), 6.95 (1H, d, $J_{NH,2\text{ or }2'}$=8 Hz, NH), 7.16—7.60 (12H, m, aromatic protons *meta* and *para* to C=O of benzoyl), 7.80—8.16 (8H, m, aromatic protons *ortho* to C=O of benzoyl). IR ν_{max}^{Nujol} cm^{-1}: 3260 (NH), 1710 (C=O), 1650 (amide I). TLC: *Rf* 0.67 (solvent A), 0.28 (B), 0.08 (C). *Anal.* Calcd for $C_{44}H_{42}N_2O_{14}$: C, 64.23; H, 5.14; N, 3.40. Found: C, 63.87; H, 4.93; N, 3.32.

2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-β-D-glucopyranosyl)-1,6-di-O-acetyl-3-O-benzoyl-2-deoxy-α-D-glucopyranose (1,6-Di-O-acetyl-3,3′,4′,6′-tetra-O-benzoyl-α-di-N-acetylchitobiose (4)——A solution of **3** (200 mg, 0.24 mmol) in an acetolysis mixture (5 ml, Ac_2O–$BF_3\cdot Et_2O$, 25: 1, v/v) was left to stand at 20° for 2 hr. The mixture was poured into ice-H_2O (50 ml), neutralized with $NaHCO_3$, and then the whole was extracted with $CHCl_3$ (15 ml×3). The combined extracts were successively washed with satd. aqueous $NaHCO_3$ and H_2O. Desiccation ($MgSO_4$) and removal of the solvent gave a syrup, which was chromatographed on a column (0.9×50 cm) of Kieselgel (10 g) with $CHCl_3$–MeOH (50: 1). The major fraction eluted was crystallized from CH_2Cl_2–ether as white needles (162 mg, 72.5%), mp 241—243°, $[\alpha]_D^{20}$ +1.1° (c=0.7, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.80, 1.85, 2.15, 2.20 (12H, all s, $-OCOCH_3\times2$, $-NHCOCH_3\times2$), 4.92 (1H, d, $J_{1',2'}$=8 Hz, H-1′), 5.96 (1H, d, $J_{NH,2\text{ or }2'}$=8 Hz, NH), 6.24 (1H, d, $J_{1,2}$=3 Hz, H-1), 6.32 (1H, d, $J_{NH,2'\text{ or }2}$=8 Hz, NH), 7.10—7.60 (12H, m, aromatic protons *meta* and *para* to C=O of benzoyl), 7.60—8.20 (8H, m, aromatic protons *ortho* to C=O of benzoyl). TLC: *Rf* 0.67 (solvent A), 0.22 (B), 0.06 (C). *Anal.* Calcd for $C_{48}H_{48}N_2O_{17}$: C, 62.33; H, 5.23; N, 3.03. Found: C, 62.19; H, 5.09; N, 3.19.

2-Methyl-[6-O-acetyl-3-O-benzoyl-1,2-dideoxy-4-O-(2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-β-D-glucopyranosyl)-α-D-glucopyrano][2,1-*d*]-2-oxazoline (6)——1) 2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-β-D-glucopyranosyl)-6-O-acetyl-3-O-benzoyl-2-deoxy-α-D-glucopyranosyl Chloride (**5**): A solution of **4** (190 mg, 0.21 mmol) in a mixture of AcOH (5 ml) and Ac_2O (2.5 ml) saturated with dry HCl at −10° was kept in a sealed tube for 24 hr at 0°. After dilution with $CHCl_3$ (30 ml), the solution was successively washed with H_2O, satd. aqueous $NaHCO_3$, and H_2O. Desiccation ($MgSO_4$) and removal of the solvent gave an amorphous powder (181 mg): TLC (solvent A) showed two spots of **5** (major) and **6** (minor) having *Rf* 0.71 (major) and 0.75 (minor), respectively. Since the minor product was assigned as the desired oxazoline (**6**), the crude chloride was used for further experiments without purification.

2) The Oxazoline Formation: The chloride **5** (181 mg) in dry acetonitrile (10 ml) was stirred with tetraethylammonium chloride (50 mg, 0.30 mmol) and $NaHCO_3$ (50 mg, 0.60 mmol) for 1 hr at room temperature. After dilution with $CHCl_3$ (40 ml), the mixture was filtered, and the filtrate was washed with H_2O. Desiccation ($MgSO_4$) and removal of the solvent gave **6** (140 mg) as an amorphous powder, $[\alpha]_D^{20}$ −37.6° (c=0.53, $CHCl_3$). TLC: *Rf* 0.75 (solvent A). IR ν_{max}^{Nujol} cm^{-1}: 1715 (C=O), 1661 (C=N), 1650 (amide I). ^{1}H-NMR ($CDCl_3$): 1.96 (9H, s, $-OCOCH_3$, $-NHCOCH_3$, and $-CH_3$ of oxazoline), 5.09 (1H, d, $J_{1',2'}$=8 Hz, H-1′), 6.04 (1H, d, $J_{1,2}$=5 Hz, H-1), 6.20 (1H, d, $J_{NH',2'}$=8 Hz, NH′), 7.20—8.00 (20H, m, aromatic protons). The product was contaminated with a trace of a by-product (TLC, *Rf* 0.55, solvent A).

Benzyl 2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-β-D-glucopyranosyl)-6-O-acetyl-3-O-benzoyl-2-deoxy-β-D-glucopyranoside (Benzyl 6-O-Acetyl-3,3′,4′,6′-tetra-O-benzoyl-di-N-acetylchitobioside (7)——1) By the Koenigs–Knorr Synthesis: A mixture of **5** (200 mg, *ca.* 0.22 mmol), benzyl alcohol (0.1 ml, 0.58 mmol), and mercuric cyanide (55 mg, 0.22 mmol) in dry nitromethane (3 ml) was stirred at 60° for 3 hr

under nitrogen. The mixture was diluted with $CHCl_3$ (40 ml), and the whole was successively washed with H_2O, satd. aqueous $NaHCO_3$, and H_2O. Desiccation ($MgSO_4$) and removal of the solvent gave a syrupy residue, which was treated with ether (40 ml) to remove excess benzyl alcohol. The mixture was filtered to provide a solid, which was dissolved in $CHCl_3$ (1 ml) and chromatographed on a column (0.9×50 cm) of Kieselgel (10 g) with $CHCl_3$–MeOH (100: 1). The major fraction eluted (TLC, *Rf* 0.75, solvent A) was crystallized from AcOEt–ether as white needles (43 mg, 20%), mp 263—264°, $[\alpha]_D^{21}$ −56.4° (c=0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.80, 1.84, 2.15 (9H, all s, $-OCOCH_3$, $-NHCOCH_3\times2$), 5.94 (1H, d, $J_{NH,\,2\text{ or }2'}$=8 Hz, NH), 6.24 (1H, d, $J_{NH,\,2'\text{ or }2}$=8 Hz, NH), 7.16—7.60 (17H, m, aromatic protons *meta* and *para* to C=O of benzoyl, and of benzyl), 7.64—8.08 (8H, m, aromatic protons *ortho* to C=O of benzoyl). *Anal.* Calcd for $C_{53}H_{52}N_2O_{16}$: C, 65.43; H, 5.39; N, 2.88. Found: C, 65.61; H, 5.51; N, 2.74.

2) By the Oxazoline Method: A mixture of **6** (200 mg, 0.23 mmol) and benzyl alcohol (0.1 ml, 0.58 mmol) in 1,2-dichloroethane (2 ml) containing 0.015 N anhyd. TsOH was stirred at 20° for 6 hr under nitrogen. The benzyl glycoside **7** (80 mg, 35.7%) was isolated from the mixture by treatment as described in method 1.

Benzyl 2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-β-D-glucopyranosyl)-3-O-benzoyl-2-deoxy-β-D-glucopyranoside (Benzyl 3,3′,4′,6′-Tetra-O-benzoyl-β-di-N-acetylchitobioside (8)——A solution of **7** (100 mg, 0.10 mmol) in 10% (w/w) methanolic ammonia (5 ml) was kept for 2 hr at 0°. The mixture was evaporated to dryness to give a syrupy residue, which was dissolved in $CHCl_3$ (1 ml) and chromatographed on a column (0.9×55 cm) of Kieselgel (10 g) with $CHCl_3$–MeOH (50: 1). The major fraction eluted (TLC, *Rf* 0.54, solvent A) gave **8** (78 mg, 80.3%). The product was solidified from $CHCl_3$–ether as an amorphous solid, $[\alpha]_D^{21}$ −50° (c=0.28, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3300, 3240 (OH, NH), 1710 (C=O), 1650 (amide I). TLC: *Rf* 0.54 (solvent A), 0.08 (B), 0.08 (C). ^{1}H-NMR ($CDCl_3$): 1.80, 1.84 (6H, each s, $-NHCOCH_3\times2$), 6.55 (1H, d, $J_{NH,\,2\text{ or }2'}$=8 Hz, NH), 6.86 (1H, d, $J_{NH,\,2'\text{ or }2}$=8 Hz, NH), 7.10—7.60 (17H, m, aromatic protons *meta* and *para* to C=O of benzoyl, and of benzyl), 7.64—8.20 (8H, m, aromatic protons *ortho* to C=O of benzoyl). *Anal.* Calcd for $C_{51}H_{50}N_2O_{15}\cdot H_2O$: C, 64.55; H, 5.52; N, 2.95. Found: C, 64.47; H, 5.25; N, 2.94.

Benzyl 2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-β-D-glucopyranosyl)-3-O-benzoyl-6-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (Benzyl 3,3′,4′,6′-Tetra-O-benzoyl-6-O-(2″,3″,4″-tri-O-benzyl-α-L-fucopyranosyl)-β-di-N-acetylchitobioside) (10)——A solution of 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide[14] (**9**, 154 mg, 0.31 mmol) in 1,2-dichloroethane (3 ml) was added to a mixture of **8** (100 mg, 0.11 mmol), tetraethylammonium bromide (225 mg, 1.07 mmol), and powdered molecular sieves 4 Å (500 mg) in 1,2-dichloroethane–DMF (1: 1, v/v, 3 ml). After being stirred for 48 hr at 20° under nitrogen, the mixture was diluted with $CHCl_3$ (40 ml), filtered, and the filtrate was washed with H_2O (40 ml×2). Desiccation ($MgSO_4$) and removal of the solvent gave a syrupy residue, which was dissolved in $CHCl_3$ (1 ml) and chromatographed on a column (0.9×50 cm) of Kieselgel (10 g) with $CHCl_3$–MeOH (100 : 1). Removal of the solvent from the major fraction and trituration of the residue with $CHCl_3$–ether gave **10** (118 mg, 83.4%), $[\alpha]_D^{21}$ −48.3° (c=0.41, $CHCl_3$), as an amorphous solid. TLC: *Rf* 0.79 (solvent A), 0.31 (B), 0.20 (C). ^{1}H-NMR ($CDCl_3$): 1.08 (3H, d, $J_{5'',6''}$=7 Hz, $-CH_3$), 1.69, 1.80 (6H, each s, $-NHCOCH_3\times2$), 5.88 (1H, d, $J_{NH,\,2\text{ or }2'}$=8 Hz, NH), 6.34 (1H, d, $J_{NH,\,2'\text{ or }2}$=8 Hz, NH), 7.00—7.60 (32H, m, aromatic protons *meta* and *para* to C=O of benzoyl, and of benzyl), 7.60—8.00 (8H, m, aromatic protons *ortho* to C=O of benzoyl). TLC: *Rf* 0.79 (solvent A), 0.31 (B), 0.20 (C). *Anal.* Calcd for $C_{78}H_{78}N_2O_{19}$: C, 69.53; H, 5.83; N, 2.08. Found: C, 69.10; H, 5.57; N, 2.06.

2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-6-O-(α-L-fucopyranosyl)-D-glucopyranose (6-O-α-L-Fucopyranosyl-di-N-acetylchitobiose) (11)——A 0.5 N methanolic solution of barium methoxide (0.43 ml) was added to a solution of **10** (50 mg, 0.04 mmol) in MeOH (10 ml), and the mixture was stirred at room temperature overnight. The mixture was neutralized with Amberlite IR-120B (H^+) resin. After removal of the resin by filtration, the filtrate was concentrated to an amorphous solid, which was washed with ether until the characteristic odor of methylbenzoate was no longer apparent. The resulting white powder was dissolved in MeOH (5 ml), and the solution was hydrogenated over a Pd catalyst at room temperature under atmospheric pressure until absorption of H_2 ceased; the catalyst was freshly prepared from $PdCl_2$ (50 mg) according to the method of Schmidt and Staab.[15] After removal of the catalyst by filtration, the filtrate was evaporated to dryness, and the residue was crystallized from EtOH–H_2O as white needles (21 mg, 99.2%), mp 261—265° (dec.), $[\alpha]_D^{21}$ −23.1° (no mutarotation, c=0.19, H_2O). ^{1}H-NMR (D_2O): 1.19 (3H, d, $J_{5'',6''}$=7 Hz, $-CH_3$), 2.01, 2.06 (6H, each s, $-NHCOCH_3\times2$), 4.62 (1H, d, $J_{1',2'}$=8 Hz, H-1′), 4.72 [<1H, d, $J_{1,2}$=8 Hz, H-1(β)], 5.16 [*ca.* 1H, d-like, H-1(α) and H-1″]. ^{13}C-NMR (D_2O): see Table I. TLC: *Rf* 0.38 (solvent D), 0.24 (E). *Anal.* Calcd for $C_{22}H_{38}N_2O_{15}\cdot1.5H_2O$: C, 44.22; H, 6.92; N, 4.69. Found: C, 44.05; H, 6.71; N, 4.58.

Acknowledgement We thank Mrs. T. Kumagai for the ^{1}H-NMR and ^{13}C-NMR spectral measurements, and Misses S. Iwauchi and T. Naito for the microanalyses.

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