Chem. Pharm. Bull. 28(1) 35—41 (1980)

A Preparation of the Branched Trisaccharide 2-Acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-3-O-(β-D-xylopyranosyl)-D-glucopyranose (3-O-β-D-Xylopyranosyl-N-acetyllactosamine)

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(Received May 8, 1979)

The title branched trisaccharide (**19**) was prepared from benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**1**) by stepwise Koenigs-Knorr condensation followed by removal of the protecting groups.

Benzyl 2-acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-2-deoxy-α-D-glucopyranoside (**4**), prepared by coupling **1** with 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide followed by debenzylidenation, is a key intermediate in this preparation. Removal of the benzyl and O-acetyl groups of **4** gave a crystalline 2-acetamido-2-deoxy-3-O-(β-D-xylopyranosyl)-α-D-glucopyranose. Preferential etherification and esterification of **4** were investigated. Selective benzylation of **4** afforded the 6-O-benzoate (**15**). Condensation of **15** with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide provided a crystalline, protected trisaccharide in 41% yield, from which **19** was obtained as a white powder after removal of the protecting groups.

Purified β-galactosidase from jack bean did not act on the β-galactosidic linkage in **19**.

Keywords——branched trisaccharide synthesis; benzyl α-N-acetylglucosaminide derivatives; N-acetyllactosamine; 3-O-xylosyl-β-N-acetylglucosamine; preferential etherification; preferential esterification; jack bean β-galactosidase

Many oligosaccharides and glycoconjugates of human origin have a structure with a trisaccharide such as 3-O-α-L-fucopyranosyl-N-acetyllactosamine located at the non-reducing end.[2] Other biologically active complex carbohydrates include trisaccharides having a structure with a monosaccharide attached to the 2-acetamido-2-deoxy-D-glucopyranose moiety of di-N-acetylchitobiose or N-acetyllactosamine.[3] Thus, amino sugar-containing branched trisaccharides are important in connection with studies of the action of glycosidases, the elucidation of the role of carbohydrates in complex saccharides, and the synthesis of more complex higher oligosaccharides.

We previously reported the synthesis of the branched trisaccharides, O-α- and O-β-D-galactopyranosyl-(1→6)-O-[α-D-glucopyranosyl-(1→4)]-D-glucopyranoses,[4] and recently reported a new synthesis of N-acetyllactosamine from lactose.[5] As an extension of the synthesis of N-acetyllactosamine-containing oligosaccharides, many of which have been isolated from human milk,[6] preparation of the title branched trisaccharide is described here. Our route starts from a partially protected benzyl α-N-acetylglucosaminide having only one unprotected hydroxyl group, and includes stepwise Koenigs-Knorr condensation and removal of the protecting groups.

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Stirring benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**1**)[7] with 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide (**2**)[8] in benzene-nitromethane in the presence of mercuric cyanide at 50°, followed by purification by column chromatography, afforded a protected benzyl 3-O-β-D-xylopyranosyl-α-D-glucosaminide (**3**) in 83% yield as white needles. Debenzylidenation of **3** with 50% aqueous acetic acid gave a partially acetylated benzyl 3-O-β-D-xylopyranosyl-α-D-glucosaminide (**4**) having the C-4 and the C-6 hydroxyl groups free. However, when debenzylidenation was performed by catalytic hydrogenation over 5% palladium on charcoal, debenzylation occurred simultaneously to give a partially acetylated 3-O-β-D-xylopyranosyl-D-glucosamine, from which 3-O-β-D-xylopyranosyl-α-N-acetylglucosamine (**5**) was isolated after de-O-acetylation. The product was easily crystallized from a mixture of methanol and ethanol as the α-form having $[\alpha]_D^{23}$ −44° (5 min)→−69.3° (24 hr, constant). The structure of the carbohydrate moiety of stem bromelain[9] was suggested to contain **5** as a partial structure, but more recent studies[10] do not support this.

To synthesize the title trisaccharide from **4**, it was necessary, in the first step, to protect the C-6 hydroxyl group of the glucosamine residue. Therefore, preferential etherification and esterification of **4** were investigated.

Tritylation of **4** in the usual way afforded a crystalline monotritylether (**6**) in 72% yield. The infrared (IR) spectrum showed an absorption due to the C-4 hydroxyl group. Compound **6** gave a crystalline acetate (**7**). The nuclear magnetic resonance (NMR) spectrum showed four separate singlets due to four O-acetyls, and one N-acetyl. Detritylation of **7** with 50% aqueous acetic acid gave the pentaacetate (**8**) having an unprotected hydroxyl group at the C-6 position in the D-glucosamine residue. No migration of acetyl groups took place during the detritylation step, as retritylation of **8** gave **7**. However, selective benzylation of the C-6 hydroxyl group of **4** in N,N-dimethylformamide with benzylbromide and silver oxide or barium hydroxide[11] resulted in the formation of many decomposition products of **4** having unidentified structures. Thus, no further studies of selective benzylation of **4** were carried out.

Preferential acetylation of the C-6 hydroxyl group of **4** using one equivalent of N-acetylimidazole[12] was unsuccessful: it gave a mixture of two products, the pentaacetate (**9**) and the hexaacetate (**10**). The reaction was monitored by integrating protons due to O- and N-acetyl moieties. Thus, it was necessary to synthesize **9** from **8** by acetyl migration as follows.

Heating **8** with glacial acetic acid at 95° for 3 hr, followed by purification by column chromatography, gave **9** as an amorphous powder in 81.6% yield. Acetylation of **9** gave a crystalline hexaacetate (**10**), which was also prepared from **4** by complete acetylation using excess acetylating reagent. Compounds **9** and **10** showed the same mobility on thin-layer chromatography (TLC). Thus, the products could not be isolated by column chromatography from the preferential acetylation product of **4** using N-acetylimidazole. Compound **9** gave a crystalline mesylate (**11**). The IR spectrum of **11** showed no hydroxyl absorption.

Tosylation of **4** with 2.9 molar equivalents of tosyl chloride in pyridine at 4° for 6 hr afforded an amorphous tosylate (**12**) in 82% yield after column chromatographic purification. From its NMR spectrum, **12** was confirmed to be a monotosylate. On heating **12** with sodium iodide in acetonitrile, substitution of the sulfonyloxy group by the iodo anion occurred readily to give the iodo compound (**13**). Therefore, the tosyl group of **12** is located at the

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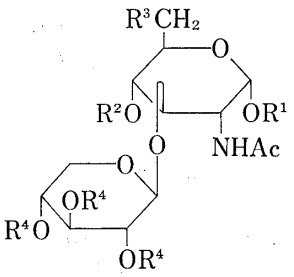
primary hydroxyl group (C-6). Mesylation of **12** gave a crystalline mesylate (**14**). The NMR spectrum of **14** indicated the presence of one mesyl and one tosyl groups. The IR spectrum of **14** showed no hydroxyl absorption.

Preferential benzoylation of **4** was carried out with 2 molar equivalents of benzoyl chloride in pyridine at 0° for 18 hr. After column chromatographic purification, the monobenzoate (**15**) was isolated as an amorphous powder in 72% yield. Mesylation of **15** gave a crystalline monomesylate (**16**).

On the basis of the low reactivity of the C-4 hydroxyl group in hexopyranose[13] and the differential reactivity of primary and secondary hydroxyl groups as indicated by the preferential tosylation of **4** mentioned above, it is reasonable to assume that preferential benzoylation occurred at the C-6 position in **4** under mild conditions. The strxcture of **15**, benzyl 2-acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-6-O-benzoyl-2-deoxy-α-D-glucopyranoside, was finally confirmed by the identification of N-acetyllactosamine in the partial hydrolysate of the title trisaccharide prepared from **15**.



3: R^1=Bn, R^2,R^3O=CHC_6H_5, R^4=Ac
4: R^1=Bn, R^2=H, R^3=OH, R^4=Ac
5: R^1=R^2=R^4=H, R^3=OH
6: R^1=Bn, R^2=H, R^3=OTr, R^4=Ac
7: R^1=Bn, R^2=R^4=Ac, R^3=OTr
8: R^1=Bn, R^2=R^4=Ac, R^3=OH
9: R^1=Bn, R^2=H, R^3=OAc, R^4=Ac
10: R^1=Bn, R^2=R^4=Ac, R^3=OAc
11: R^1=Bn, R^2=Ms, R^3=OAc, R^4=Ac
12: R^1=Bn, R^2=H, R^3=OTs, R^4=Ac
13: R^1=Bn, R^2=H, R^3=I, R^4=Ac
14: R^1=Bn, R^2=Ms, R^3=OTs, R^4=Ac
15: R^1=Bn, R^2=H, R^3=OBz, R^4=Ac
16: R^1=Bn, R^2=Ms, R^3=OBz, R^4=Ac

Ac=acetyl, Bn=benzyl, Bz=benzoyl, Ms=mesyl, Tr=trityl, Ts=tosyl

Chart 1

Among the four blocking groups tested, the benzoyl group appeared to be the most suitable for the preparation of the title trisaccharide from **4**. Therefore, we used **15** as a key intermediate for further trisaccharide synthesis.

Treatment of **15** and 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (**17**)[14] as described for the preparation of **3** provided the protected trisaccharide (**18**). Compound **18** was isolated by column chromatography as an amorphous powder, which was crystallized from methanol in 41% yield. Deacylation of **18** followed by catalytic debenzylation gave the title trisaccharide (**19**) as a white powder in 67% yield.

Complete hydrochloric acid hydrolysis of **19** resulted in the liberation of glucosamine hydrochloride, xylose, and galactose as indicated by paper partition chromatography (PPC). PPC of the products of partial hydrochloric acid hydrolysis showed the presence of N-acetyllactosamine and xylose. However, purified β-galactosidase from jack bean did not hydrolyze **19**.

According to Muramatsu *et al.*[15] the purified β-galactosidases from jack bean and almond emulsin did not act on the terminal β-galactosidic linkage in lacto-N-fucopentaitol, which

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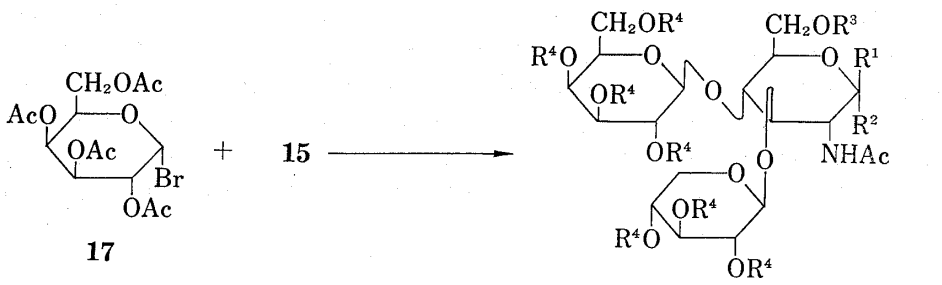
has an α-L-fucosidic linkage at the C-3 position in the N-acetyllactosamine moiety. They suggested that the action of the β-galactosidase is hindered by the presence of a neighboring α-L-fucosidic linkage. As shown by our experiments, a neighboring β-D-xylopyranosidic linkage also hinders the action of β-galactosidase. However, N-acetyllactosamine and the terminal β-galactosidic linkage in the synthetic linear tetrasaccharide[16] were easily hydrolyzed by this enzyme.



18: R^1=H, R^2=OBn, R^3=Bz, R^4=Ac
19: R^1, R^2=H, OH, R^3=R^4=H

Chart 2

Experimental

Solutions were evaporated down in a rotary evaporator below 40° under a vacuum. Melting points were determined with a Yanagimoto PM-52 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. IR spectra were measured with a Jasco IRA-2 spectrometer. NMR spectra were recorded at 100 MHz with a JEOL JNM-MH-100 spectrometer. Tetramethylsilane in $CDCl_3$ was used as an internal standard. Chemical shifts are given on the δ scale. TLC on pre-coated silica gel plates 0.25 mm thick (Kiesel Gel $60F_{254}$, E. Merck, Darmstadt, Germany) was performed with the following solvent combinations (v/v): (A), $CHCl_3$–acetone (1: 5); (B), $CHCl_3$–MeOH (30: 1); (C), hexane–ether–MeOH (7: 7: 1.5). Detection was effected with H_2SO_4 or by UV irradiation (short wavelength). Column chromatography was performed on Wakogel C-200 (Wako Pure Chemical Industries, Ltd., Osaka) with 1 g of a sample to be separated per 20 g of adsorbent. Solvent combinations are given as v/v. PPC was performed on Toyo Filter Paper No. 50 (Toyo Roshi Kaisha, Ltd., Tokyo) by the ascending method[17] with BuOH–pyridine–H_2O(6: 4: 3, v/v), and detection was effected with the alkaline silver nitrate reagent.[18]

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (3)——2,3,4-Tri-O-acetyl-α-D-xylopyranosyl bromide (2)[8] (672 mg, 2 mmol) was added to a solution of benzyl 2-acetamido-4,6-benzylidene-2-deoxy-α-D-glucopyranoside (1)[7] (800 mg, 2 mmol) in benzene–nitromethane (3: 5, v/v, 80 ml) containing $Hg(CN)_2$ (505 mg, 2 mmol), and the mixture was stirred at 50°. After 18 and 26 hr, further portions of 2 (each 336 mg) and $Hg(CN)_2$ (each 253 mg) were added, and stirring was continued for a further 15 hr. The mixture was diluted with benzene (100 mg), washed successively with H_2O, satd. $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. A solution of the residue in $CHCl_3$ (2 ml) was chromatographed on a column of silica gel, eluting with $CHCl_3$–MeOH (100: 1). Fractions having *Rf* 0.82 (solvent A) on TLC were pooled, and removal of the solvent gave a solid which was crystallized from EtOH. Recrystallization from EtOH gave 3 (1.1 g, 83%), mp 229—230°, $[\alpha]_D^{24}$ +4.1° (*c*=0.53, $CHCl_3$) as white needles. TLC: *Rf* 0.82 (solvent A), 0.60 (B), 0.01 (C). NMR $\delta_{ppm}^{CDCl_3}$: 1.92—2.20 (12H, m, OAc×3, NAc), 5.48 (1H, s, C_6H_5CH), 5.90 (1H, d, NH), 7.18—7.68 (10H, m, aromatic protons). *Anal.* Calcd for $C_{33}H_{39}NO_{13}$: C, 60.27; H, 5.98; N, 2.13. Found: C, 60.37; H, 5.92; N, 2.41.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-2-deoxy-α-D-glucopyranoside (4)—— A mixture of 3 (1.32 g, 2 mmol) in 50% (v/v) aq. AcOH (30 ml) was heated at 90° for 30 min with stirring. The solutions was evaporated to dryness by repeated co-distillation with toluene. The residue was dissolved in $CHCl_3$ (2 ml) and chromatographed on a column of silica gel, eluting with $CHCl_3$–MeOH (50: 1). After removal of the solvent from the fractions having *Rf* 0.71 (solvent A), the residue was solidified from CH_2Cl_2–ether. The product (1.16 g, 98%), $[\alpha]_D^{24}$ +57.5° (*c*=0.5, $CHCl_3$), was collected by filtration as a white powder.

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TLC: *Rf* 0.71 (solvent A), 0.21 (B), 0.06 (C). *Anal.* Calcd for $C_{26}H_{35}NO_{13}\cdot 1/2H_2O$: C, 53.98; H, 6.27; N, 2.42. Found: C, 53.72; H, 6.03; N, 2.45.

2-Acetamido-2-deoxy-3-O-(β-D-xylopyranosyl)-α-D-glucopyranose (5)——A solution of **3** (1 g 1.52 mmol) in glacial AcOH (50 ml) was hydrogenated in the presence of 5% Pd on charcoal (1.5 g). The absorption of H_2 ceased after 48 hr, and the catalyst was then removed by filtration, washed with AcOH, and the combined filtrate and washings were evaporated to dryness by repeated co-distillation with toluene. The residue was chromatographed on a column of silica gel, eluting with $CHCl_3$–MeOH (10: 1). Removal of the solvent from the eluate afforded an amorphous powder (570 mg), which was dissolved in MeOH (5 ml) and cooled to 0°. Methanolic NaOMe (0.5 N, 0.1 ml) was added to the solution, and the mixture was stirred for 2 hr at 4°. Dry Amberlite IR-120 (H^+) resin was added, and the mixture was stirred for 30 min, then filtered. Removal of the solvent gave an amorphous powder which was dissolved in MeOH. Addition of EtOH crystallized **5** (360 mg, 65%), mp 189—191°, $[\alpha]_D^{23}$ −44° (5 min)→−69.3° (24 hr, constant) (c=0.6, H_2O), as white needles. NMR $\delta_{ppm}^{CD_3OD}$: 2.01 (3H, s, NAc). PPC: *Rf* 0.44. *Anal.* Calcd for $C_{13}H_{23}NO_{10}\cdot 1/2H_2O$: C, 43.09; H, 6.68; N, 3.86. Found: C, 43.33; H, 6.72; N, 3.75.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-2-deoxy-6-O-trityl-α-D-glucopyranoside (6)——Trityl chloride (340 mg, 1.4 mmol) was added to a solution of **4** (500 mg, 0.85 mmol) in dry pyridine (5 ml). After stirring for 2.5 hr at 80°, the mixture was diluted with toluene and evaporated to dryness. A solution of the residue in $CHCl_3$ (2 ml) was applied to a column of silica gel, eluting with $CHCl_3$ until tritanol emerged, and then with $CHCl_3$–MeOH (100: 1) to remove the trityl ether. The fractions having *Rf* 0.85 (solvent A) were concentrated to dryness and the resulting amorphous powder was crystallized from AcOEt–ether as white needles (496 mg, 72%), mp 198—199°, $[\alpha]_D^{24}$ +38.9° (c=0.32, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3460, 3340 (OH, NH). NMR $\delta_{ppm}^{CDCl_3}$: 1.96, 2.02, 2.06 (12H, each s, OAc×3, NAc), 5.76 (1H, d, NH), 7.22—7.50 (20H, m, aromatic protons). TLC: *Rf* 0.85 (solvent A), 0.65 (B), 0.29 (C). *Anal.* Calcd for $C_{45}H_{49}NO_{13}$: C, 66.57; H, 6.08; N, 1.73. Found: C, 66.30; H, 6.05; N, 1.85.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-4-O-acetyl-2-deoxy-6-O-trityl-α-D-glucopyranoside (7)——Compound **6** (500 mg, 0.62 mmol) was acetylated with Ac_2O (2 ml) and pyridine (3 ml) at room temperature overnight, then poured into ice-H_2O (100 ml), and the mixture was extracted with $CHCl_3$. The organic layer was washed with aq. $NaHCO_3$ and H_2O, dried over $CaCl_2$, and evaporated down to give an amorphous powder which was crystallized from EtOH as white needles (478 mg, 91%), mp 239—240°, $[\alpha]_D^{24}$ +32.7° (c=1.08, $CHCl_3$). NMR $\delta_{ppm}^{DCCl_3}$: 1.79, 1.90, 1.99, 2.01 (15H, each s, OAc×4, NAc), 5.80 (1H, d, NH), 7.20—7.50 (20H, m, aromatic protons). TLC: *Rf* 0.85 (solvent A), 0.65 (B), 0.29 (C). *Anal.* Calcd for $C_{47}H_{51}NO_{14}$: C, 66.11; H, 6.02; N, 1.64. Found: C, 65.94; H, 5.97; N, 1.73.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-4-O-acetyl-2-deoxy-α-D-glucopyranoside (8)——Compound **7** (300 mg, 0.35 mmol) was detritylated with 50% aq. AcOH (5 ml) as described for the preparation of **4**: the heating time (15 min) and elution solvent for column chromatography [$CHCl_3$–MeOH (50: 1)] were slightly modified as indicated in parentheses. The product was crystallized from CH_2Cl_2–EtOH as white needles (198 mg, 92%), mp 198—202°, $[\alpha]_D^{23}$ +40° (c=0.87, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3420, 3300 (OH, NH). NMR $\delta_{ppm}^{CDCl_3}$: 1.96, 2.10 (15H, each s, OAc×4, NAc), 5.86 (1H, d, NH), 7.36 (5H, s, aromatic protons). TLC: *Rf* 0.74 (solvent A), 0.25 (B), 0.07 (C). *Anal.* Calcd for $C_{28}H_{37}NO_{14}$: C, 54.99; H, 6.10; N, 2.29. Found: C, 54.89; H, 6.06; N, 2.50.

Tritylation of **8** (50 mg 0.1 mmol) with trityl chloride (34 mg. 0.1 mmol) in dry pyridine (1 ml) at 100° for 1 hr and treatment of the mixture as described for the preparation of **6** afforded trityl ether (35 mg, 50%). The product was indistinguishable from **7** by mp, TLC, and NMR spectrum.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-6-O-acetyl-2-deoxy-α-D-glucopyranoside (9)——A solution of **8** (100 mg, 0.16 mmol) in glacial AcOH (3 ml) was heated at 95° for 3 hr, then AcOH was removed by repeated co-distillation with toluene. The residue was dissolved in $CHCl_3$ (2 ml) and chromatographed on a column of silica gel, eluting with $CHCl_3$–MeOH (100: 1). Removal of the solvent from the fractions having *Rf* 0.80 (solvent A) gave **9** (81.6 mg, 82%), $[\alpha]_D^{20}$ +32.4° (c=1.18, $CHCl_3$), as an amorphous powder. IR ν_{max}^{Nujol} cm^{-1}: 3420, 3330 (OH, NH). NMR $\delta_{ppm}^{CDCl_3}$: 1.92, 1.98, 2.00, 2.02, 2.03 (15H, each s, OAc×4, NAc), 5.86 (1H, d, NH), 7.36 (5H, s, aromatic protons). TLC: *Rf* 0.80 (solvent A), 0.53 (B), 0.11 (C). *Anal.* Calcd for $C_{28}H_{37}NO_{14}\cdot 3/2H_2O$: C, 52.66; H, 6.08; N, 2.19. Found: C, 52.94; H, 5.82; N, 2.42.

Further elution with the same solvent eluted the starting material (**8**) (14.6 mg) from the column.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-4,6-di-O-acetyl-2-deoxy-α-D-glucopranoside (10)——Acetylation of **9** (500 mg) with Ac_2O (2 ml) and pyridine (3 ml) as described for the preparation of **7** gave **10** (497 mg, 99%), mp 170.5—171°, $[\alpha]_D^{22}$ +37.5° (c=0.76, $CHCl_3$), as white needles from AcOEt–ether. NMR $\delta_{ppm}^{CDCl_3}$: 1.99, 2.01, 2.02, 2.08 (18H, each s, OAc×5, NAc), 6.08 (1H, d, NH), 7.36 (5H, s, aromatic protons). TLC: *Rf* 0.80 (solvent A), 0.53 (B), 0.11 (C). *Anal.* Calcd for $C_{30}H_{39}NO_{15}$: C, 55.13; H, 6.01; N, 2.41. Found: C, 55.04; H, 6.09; N, 2.17.

The product was also obtained from **4** (100 mg) by acetylation with pyridine (2 ml) and Ac_2O (2 ml) for 18 hr at room temperature.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-6-O-acetyl-2-deoxy-4-O-mesyl-α-D-glucopyranoside (11)——Mesyl chloride (0.35 ml, 4.5 mmol) was added dropwise with stirring to an ice-cold solution of **9** (550 mg, 0.86 mmol) in dry pyridine (5 ml), and the mixture was left to stand in a refrigerator for 18 hr.

The mixture was then poured into ice-H_2O (100 ml), extracted with $CHCl_3$, and the organic layer was washed several times with H_2O. After desiccation over $CaCl_2$, the solvent was removed. The residue was crystallized from EtOAc–ether as white needles (589 mg, 99%), mp 129—131°, $[\alpha]_D^{24}$ +26.5° (c=0.3, $CHCl_3$). NMR $\delta_{ppm}^{CDCl_3}$: 3.15 (3H, s, $SO_2C\underline{H}_3$). TLC: *Rf* 0.80 (solvent A), 0.53 (B), 0.11 (C). *Anal.* Calcd for $C_{29}H_{39}NO_{16}S$: C, 50.50; H, 5.70; N, 2.03. Found: C, 50.73; H, 5.86; N, 1.98.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-2-deoxy-6-O-tosyl-α-D-glucopyranoside (12)——Tosyl chloride (680 mg, 4.9 mmol) was added to an ice-cold solution of **4** (1 g, 1.7 mmol) in pyridine (10 ml) at 0°. The mixture was left to stand in a refrigerator for 6 hr, then poured into ice-H_2O (200 ml), and extracted with $CHCl_3$. The organic layer was washed several times with H_2O. After drying over Na_2SO_4, the solvent was evaporated off to afford an amorphous powder which was dissolved in $CHCl_3$ (2 ml) and chromatographed on a column of silica gel, eluting with $CHCl_3$–MeOH (100: 1). Removal of the solvent from the fractions having *Rf* 0.82 (solvent A) gave **12** as an amorphous powder (1.03 g, 82%), $[\alpha]_D^{21}$ +41.8° (c=1.03, $CHCl_3$). NMR $\delta_{ppm}^{CDCl_3}$: 1.90, 2.00, 2.01 (12H, each s, OAc×3, NAc), 2.42 (3H, s, $SO_2C_6H_4C\underline{H}_3$), 5.84 (1H, d, NH), 7.20—7.90 (9H, m, aromatic protons). TLC: *Rf* 0.82 (solvent A), 0.58 (B), 0.12 (C). *Anal.* Calcd for $C_{33}H_{41}NO_{15}S\cdot H_2O$: C, 53.44; H, 5.84; N, 1.89. Found: C, 53.27; H, 5.87 N, 1.89.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-2,6-dideoxy-6-iodo-α-D-glucopyranoside (13)——A mixture of **12** (100 mg, 0.13 mmol) and NaI (84 mg, 0.56 mmol) in CH_3CN (3 ml) was heated at 95° for 3 hr with stirring. It was then filtered, and the filtrate was evaporated to dryness. The residue was treated with $CHCl_3$ (5 ml), then the $CHCl_3$ layer was washed with H_2O, dried over Na_2SO_4, and chromatographed on a column of silica gel, eluting with $CHCl_3$–MeOH (100: 1) to yield **13** (82.2 mg, 88%), $[\alpha]_D^{23}$ +38.7° (c=0.6, $CHCl_3$). NMR $\delta_{ppm}^{CDCl_3}$: 1.96, 1.98, 1.99, 2.02 (12H, each s, OAc×3, NAc), 5.76 (1H, d, NH), 7.32 (5H, s, aromatic protons). TLC: *Rf* 0.82 (solvent A), 0.60 (B), 0.25 (C). *Anal.* Calcd for $C_{26}H_{34}INO_{12}\cdot 2/3H_2O$: C, 45.16; H, 5.15; N, 2.03. Found: C, 45.00; H, 4.89; N, 2.06.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-2-deoxy-4-O-mesyl-6-O-tosyl-α-D-glucopyranoside (14)——Compound **12** (100 mg, 0.13 mmol) in pyridine (2 ml) was mesylated with mesyl chloride (0.54 ml, 0.69 mmol) as described for the preparation of **11** to afford **14** (100 mg, 91%), which was crystallized from $CHCl_3$ as prisms, mp 218—224° (dec.), $[\alpha]_D^{21}$ +55.7° (c=0.35, $CHCl_3$). NMR $\delta_{ppm}^{CDCl_3}$: 2.47 (3H, s, $SO_2C_6H_4C\underline{H}_3$), 3.14 (3H, s, $SO_2C\underline{H}_3$). TLC: *Rf* 0.82 (solvent A), 0.58 (B), 0.12 (C). *Anal.* Calcd for $C_{34}H_{43}NO_{17}S_2$: C, 50.93; H, 5.41; N, 1.75. Found: C, 50.63; H, 5.31; N, 1.86.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-6-O-benzoyl-2-deoxy-α-D-glucopyranoside (15)——Benzoyl chloride (0.4 ml, 3.4 mmol) was added dropwise with stirring at 0° to a solution of **4** (1 g, 1.7 mmol) in dry pyridine (10 ml). The mixture was left in a refrigerator for 18 hr, then poured into ice-H_2O (200 ml) and extracted with $CHCl_3$. The extract was washed with H_2O, and after removal of the solvent from the dried extract, the residue was dissolved in $CHCl_3$ (2 ml) and chromatographed on a column of silica gel, eluting with $CHCl_3$–MeOH (50: 1). Removal of the solvent from the fractions having *Rf* 0.85 (solvent A) afforded **15** as an amorphous powder (988 mg, 72%), $[\alpha]_D^{22}$ +21.7° (c=0.88, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3450, 3350 (OH, NH). NMR $\delta_{ppm}^{CDCl_3}$: 1.96, 2.04, 2.06 (12H, each s, OAc×3, NAc), 5.60 (1H, d, NH), 7.00—8.20 (10H, m, aromatic protons). TLC: *Rf* 0.85 (solvent A), 0.62 (B), 0.16 (C). *Anal.* Calcd for $C_{33}H_{39}NO_{14}\cdot H_2O$: C, 57.30; H, 5.97; N, 2.02. Found: C, 57.59; H, 5.76; N, 2.13.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-6-O-benzoyl-2-deoxy-4-O-mesyl-α-D-glucopyranoside (16)——Mesylation of **15** (550 mg, 0.81 mmol) with mesyl chloride (0.32 ml, 4.1 mmol) in dry pyridine (15 ml) was performed as described for the preparation of **11** to afford the mesylate (529 mg, 86.2%). The product was crystallized from AcOEt–ether, yielding white needles, mp 192.5—193°, $[\alpha]_D^{24}$ +45.9° (c=0.23, $CHCl_3$). NMR $\delta_{ppm}^{CDCl_3}$: 3.12 (3H, s, $SO_2C\underline{H}_3$). TLC: *Rf* 0.85 (solvent A), 0.62 (B), 0.16 (C). *Anal.* Calcd for $C_{34}H_{41}NO_{16}S$: C, 54.32; H, 5.50; N, 1.86. Found: C, 54.38; H, 5.54; N, 1.71.

Benzyl 2-Acetamido-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-6-O-benzoyl-2-deoxy-α-D-glucopyranoside (18)——A mixture of **15** (300 mg, 0.44 mmol), 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (**17**)[14] (300 mg, 0.73 mmol), and $Hg(CN)_2$ (184 mg, 0.73 mmol) in dry benzene–nitromethane (3: 5, v/v, 4 ml) was stirred at 50°. After 18 hr, further portions of **17** (150 mg, 0.37 mmol) and $Hg(CN)_2$ (92 mg, 0.37 mmol) were added, and stirring was continued for a further 18 hr. The mixture was diluted with benzene (10 ml), washed successively with H_2O, satd. $NaHCO_3$ solution, and H_2O, dried over Na_2SO_4, and then evaporated to dryness. The residue was dissolved in $CHCl_3$ (2 ml) and chromatographed on a column of silica gel, eluting with $CHCl_3$–MeOH (100: 1). The fractions having *Rf* 0.85 (solvent A) were concentrated to dryness and the resulting amorphous powder was crystallized from MeOH as white needles (181 mg, 41%), mp 226—227.5°, $[\alpha]_D^{19}$ +14.1° (c=0.94, $CHCl_3$). NMR $\delta_{ppm}^{CDCl_3}$: 1.99—2.08 (24H, m, OAc×7, NAc), 5.76 (1H, d, NH), 7.20—8.20 (10H, m, aromatic protons). TLC: *Rf* 0.85 (solvent A), 0.61 (B), 0.16 (C). *Anal.* Calcd for $C_{47}H_{57}NO_{23}$: C, 56.23; H, 5.72; N, 1.40. Found: C, 56.03; H, 5.73; N, 1.37.

2-Acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-3-O-(β-D-xylopyranosyl)-D-glucopyranose (3-O-β-D-Xylopyranosyl-N-acetyllactosamine (19)——Methanolic NaOMe (0.5 N, 0.3 ml) was added dropwise to a solution of **18** (120 mg, 0.12 mmol) in dry MeOH (5 ml) at 0°, and the mixture was left to stand in a refrigerator for 6 hr. After neutralization with dry Amberlite IR-120 (H^+) resin, the solution was evaporated to dryness to afford an amorphous powder.

5% Pd on charcoal (100 mg) was added to a solution of the residue in glacial AcOH (2 ml), and the mixture was hydrogenated at room temperature under atmospheric pressure. After 48 hr, a further portion of the catalyst (100 mg) was added, and the hydrogenation was continued for a further 96 hr. The solvent was then removed by repeated co-distillation with toluene to afford an amorphous powder. The residue was dissolved in MeOH (1 ml), from which crude **19** was percipitated by the addition of acetone. The procedure was repeated twice to obtain pure **19** (42.5 mg, 67%), $[\alpha]_D^{22}$ +23.8° (c=0.2, H_2O), as a white powder. PPC: *Rf* 0.24. NMR $\delta_{ppm}^{CD_3OD}$: 1.96 (3H, s, NAc). *Anal.* Calcd for $C_{19}H_{33}NO_{15}\cdot H_2O$: C, 42.78; H, 6.61; N, 2.63. Found: C, 42.66; H, 6.52; N, 2.45.

PPC of Acid Hydrolysates of 19——1) Complete Hydrolysis: Compound **19** (10 mg) was heated with 3 N HCl (2 ml) at 90° for 3 hr, and then evaporated to dryness by repeated co-distillation with EtOH to give a syrupy residue, which was shown to contain glucosamine hydrochloride (*Rf* 0.31), galactose (*Rf* 0.34), and xylose (*Rf* 0.47) by PPC.

2) Partial Hydrolysis: Compound **19** (15 mg) was heated with 0.1 N HCl (1 ml) at 90° for 30 min. Treatment of the solution as described in 1) gave a syrupy residue, which was shown to contain N-acetyllactosamine[5] (*Rf* 0.36) and xylose (*Rf* 0.47) by PPC.

Action of β-Galactosidase from Jack Bean Meal on 19——β-Galactosidase was purified from jack bean meal (Sigma Chemical Co.) by the method of Muramatsu *et al.*[15] The enzyme solution (0.05 ml, 0.33 unit[19]) was added to a solution of **19** (0.5 mg) in 0.15 M sodium citrate buffer (0.1 ml), pH 3.5. Lactose, N-acetyllactosamine,[5] or methyl O-β-D-galactopyranosyl-(1→6)-O-α-D-galactopyranosyl-(1→6)-O-α-D-galactopyranosyl-(1→6)-O-β-D-glucopyranoside (**20**)[16] was used as a control substrate. Each mixture was incubated at 37° with a drop of toluene and the hydrolysis was monitored by TLC on plates (5×20 cm). TLC was performed by the ascending method with 2-PrOH–acetone–1 M lactic acid (4:4:2, v/v), and detection was effected with diphenylamine reagent.[20] After incubation for 2 hr, galactose (*Rf* 0.63) was identified in the incubation mixtures containing lactose, N-acetyllactosamine, and **20**, but **19** was not hydrolyzed, and it remained unaltered even after incubation for 22 hr.

Acknowledgement We thank Miss E. Masuda for technical assistance. Thanks are also due to Mrs. T. Kumagai for the NMR spectral measurements, and to Misses S. Iwaguchi and T. Naito for the microanalyses.

19) One enzyme unit is the amount of enzyme required to release 1 μmol of *p*-nitrophenol from *p*-nitrophenyl β-D-galactopyranoside per min.

20) S.A. Hansen, *J. Chromatogr.*, **107**, 224 (1975).