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Chemical Modification of Lactose. XIII.[1] Synthesis of Lacto-N-tetraose[2]

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The protected tetrasaccharide (**7**) was synthesized in 79% yield by condensation of 1,6-anhydro-2,2′,3,4′,6′-penta-O-benzyl-β-lactose (**4**) with the acetylated oxazoline derivative of lacto-N-biose I (**6**). The protecting groups of **7** were removed by the following series of reactions to provide lacto-N-tetraose (**13**): debenzylation, acetylation, acetolysis, and de-O-acetylation. The synthetic product (**13**) was crystallized from aqueous ethanol as hygroscopic fine needles, mp 225—228°, $[\alpha]_D^{21}$ +27° (4 min)→+21.3° (3 hr) (c=0.45, H_2O).

The homogeneity and the mobility of **13** were confirmed by gel permeation chromatography using a Bio-Gel P-4 column. The specific rotation and IR spectrum of **13** were in good agreement with those of the natural product reported by Kuhn, Gauhe, and Baer [*Chem. Ber.*, **86**, 827 (1953)]. ^{13}C-NMR spectral data for 1,6-anhydro-β-lacto-N-tetraose (**9**) are also presented.

Keywords——human milk oligosaccharide; lactosan pentabenzylether; oxazoline; lacto-N-biose I; 1,6-anhydro-β-lacto-N-tetraose; debenzylation; acetolysis; de-O-acetylation; Bio-Gel P-4 gel permeation chromatography; ^{13}C-NMR

Lacto-N-tetraose was the first aminodeoxy oligosaccharide shown to occur free in nature; it was isolated from human milk in a crystalline form.[4] The methods employed to establish the structure, O-β-D-galactopyranosyl-(1→3)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose (**13**), included partial hydrolysis and methylation.[5] Subsequent studies on the oligosaccharides in human milk have revealed that the sugar is the core structure of more complex oligosaccharides such as lacto-N-fucopentaose I and II, lacto-N-difucohexaose I and II, LS-tetrasaccharide a and b, and disialyllacto-N-tetraose.[6]

As part of our program of synthesis of oligosaccharides in human milk,[7] this paper reports a chemical synthesis of the title tetrasaccharide from lactose. The synthetic route is based on condensation of 1,6-anhydro-2,2′,3,4′,6′-penta-O-benzyl-β-lactose (**4**) (having an unprotected hydroxyl group at the C-3′ position) with the acetylated oxazoline derivative of O-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucopyranose (lacto-N-biose I) (**6**), followed by removal of the protecting groups.

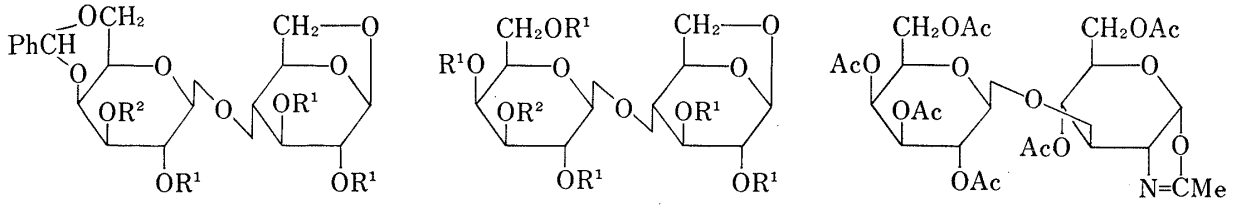
1,6-Anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**2**), isolated in 15% yield by partial tosylation of 1,6-anhydro-4′,6′-O-benzylidene-β-lactose (**1**),[8] was catalytically debenzylidenat-

1) Part XII: H. Matsuda, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull.*, **27**, 2564 (1979).
2) Preliminary communication: T. Takamura, T. Chiba, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull.*, **27**, 1497 (1979).
3) Location: *Tanabe-dori, Mizuho-ku, Nagoya 467, Japan.*
4) R. Kuhn, A. Gauhe, and H.H. Baer, *Chem. Ber.*, **86**, 827 (1953).
5) *a*) R. Kuhn, A. Gauhe, and H.H. Baer, *Chem. Ber.*, **87**, 289 (1954); *b*) R. Kuhn and H.H. Baer, *ibid.*, **89**, 504 (1956).
6) V. Ginsburg (ed.), "Methods in Enzymology," Academic Press, New York, San Francisco, and London, Vol. 28, 1972, p. 262; Vol. 50, 1978, p. 216.
7) T.G. Chung, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull.*, **26**, 2147 (1978).
8) T. Takamura and S. Tejima, *Chem. Pharm. Bull.*, **26**, 1117 (1978).

ed with a palladium catalyst to yield amorphous 1,6-anhydro-3′-O-tosyl-β-lactose (**3**) in 99.5% yield. The product crystallized from acetone with one mol of acetone.

Benzylation of **3** in N,N-dimethylformamide (DMF) with benzyl bromide, barium oxide, and crystalline barium hydroxide, followed by removal of the tosyl group[9] with 2% sodium amalgam in methanol, afforded 1,6-anhydro-2,2′,3,4′,6′-penta-O-benzyl-β-lactose (**4**) as a homogeneous syrup in 51% yield after column chromatography. Acetylation of **4** gave 3′-O-acetyl-1,6-anhydro-2,2′,3,4′,6′-penta-O-benzyl-β-lactose (**5**) as a syrup in 96.6% yield.

The acetylated oxazoline derivative of lacto-N-biose I (**6**), 2-methyl-[4,6-di-O-acetyl-1,2-dideoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano][2′,1′: 4,5]-2-oxazoline, was prepared according to the method of Augé and Veyrières.[10] The specific rotation and the infrared (IR) spectrum were in good agreement with the reported data.



1: $R^1=R^2=H$
2: $R^1=H$, $R^2=Ts$

3: $R^1=H$, $R^2=Ts$
4: $R^1=Bn$, $R^2=H$
5: $R^1=Bn$, $R^2=Ac$

6

Ac=acetyl, Bn=benzyl, Me=methyl, Ph=phenyl, Ts=tosyl

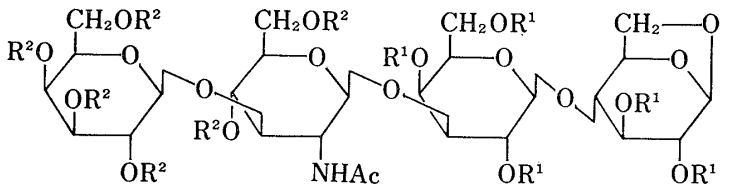
Chart 1

A mixture of **4** (1 mol eq.) and **6** (1.4 mol eq.) in toluene–nitromethane in the presence of a trace of *p*-toluenesulfonic acid was stirred at 60° for 24 hr under a nitrogen atmosphere. After 24 hr, further portions of **6** (1.4 mol eq.) were added, and stirring was continued for a further 24 hr. The mixture was neutralized with pyridine and evaporated to dryness. The residue was chromatographed on a column of silica gel, eluting first with benzene–ether and then with chloroform-ethanol. The protected tetrasaccharide (**7**), O-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-O-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-O-2,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose, was isolated as an amorphous powder in 79% yield. The synthetic route strongly suggests a β-configuration of the newly introduced 2-acetamido-2-deoxy-D-glucosidic linkage in **7**.[11] Further evidence of the β-configuration was obtained by carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy of 1,6-anhydro-β-lacto-N-tetraose (**9**), O-β-D-galactopyranosyl(1→3)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose, as mentioned later.

Catalytic debenzylation of **7** in methanol with palladium catalyst and successive acetylation of the debenzylated product provided the acetylated tetrasaccharide (**8**), O-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-O-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-O-2,4,6-tri-O-acetyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2,3-di-O-acetyl-β-D-glucopyranose, as an amorphous powder in 97.5% yield. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed 36 protons due to 11 O-acetyls and an N-acetyl as six singlets at the highest field. An aqueous methanolic solution of **8** was deacetylated with triethylamine at room temperature for 48 hr to afford 1,6-anhydro-β-lacto-N-tetraose (**9**) in 89% yield. The product crystallized from aqueous ethanol as hygroscopic fine needles, mp 278—

9) R.S. Tipson, *Advan. Carbohydr. Chem.*, **8**, 161 (1953).
10) C. Augé and A. Veyrières, *Carbohydr. Res.*, **46**, 293 (1976).
11) A.F. Bochkow and G.E. Zaikov, "Chemistry of the O-Glycosidic Bond: Formation and Cleavage," Pergamon Press, Oxford, New York, Toronto, Sydney, and Frankfurt, 1979, p. 48.

279°. The ^{13}C-NMR spectrum of **9** was measured in D_2O. The signals were assigned by comparison with those of 1,6-anhydro-β-lactose and lacto-N-biose I. The results provided substantial confirmation of the structure of **9**, and the details will be reported in a separate paper.[12)]



7: R^1=Bn, R^2=Ac
8: R^1=R^2=Ac
9: R^1=R^2=H

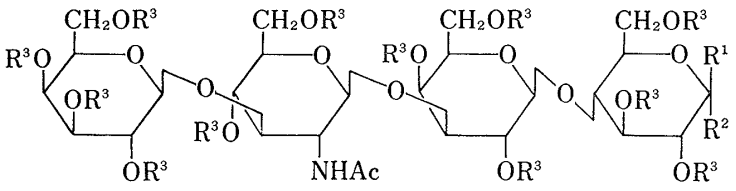
Chart 2

Acetolysis of **8** was performed with acetolysis mixture (see "Experimental") below 10° for 2 hr. The product was chromatographed on a column of silica gel, eluting with chloroform–acetone. Removal of the solvent from the effluent yielded an anomeric mixture of the acetylated lacto-N-tetraose (**10**), O-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-O-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-O-2,4,6-tri-O-acetyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-O-acetyl-D-glucopyranose, $[\alpha]_D^{19}$ +40°, as an amorphous powder in 88.2% yield. The ratio α: β in **10** was calculated as 6.5: 3.5 from the specific rotations of **10** and each anomer.

Each anomer was isolated from **10** in low yield by column chromatography, eluting with benzene–ether–methanol. The β-acetate (**11**) was eluted first and was isolated as an amorphous powder, $[\alpha]_D^{19}$ +22.9°, in 5.3% yield. After **11** emerged, the α-acetate (**12**) was eluted with the same solvent and isolated as an amorphous powder, $[\alpha]_D^{20}$ +49.3°, in 8.9% yield.

Compound **10** in a mixture of methanol, water, and triethylamine was left to stand at room temperature for 48 hr for de-O-acetylation. After removal of the solvent, treatment of the residue with aqueous ethanol induced crystallization of lacto-N-tetraose (**13**), mp 225—228°, $[\alpha]_D^{21}$ +27° (4 min)→+21.3° (3 hr) (c=0.45, H_2O), as hygroscopic fine needles in 81.9% yield.

The homogeneity and mobility of **13** were confirmed by gel permeation chromatography using a Bio-Gel P-4 column at 53°. Although the synthetic lacto-N-tetraose (**13**) had a slightly higher melting point that the natural product isolated from human milk,[5a,13)] the specific rotation and the IR spectrum of **13** were in good agreement with those of the natural product reported by Kuhn, Gauhe, and Baer.[4)]



11: R^1=OAc, R^2=H, R^3=Ac
12: R^1=H, R^2=OAc, R^3=Ac
13: R^1=R^3=H, R^2=OH

Chart 3

12) H. Matsuda and S. Tejima, *Yakugaku Zasshi*, **100**, 535 (1980).
13) F.H. Malpress and F.E. Hytten, *Biochem. J.*, **68**, 108 (1958).

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Solutions were concentrated in a rotary evaporator below 40° under a vacuum. Optical rotations were measured with a Union Giken PM-201 automatic digital polarimeter in a 0.5 dm tube. IR spectra were recorded with a Jasco IRA-2 spectrometer. ^{1}H-NMR spectra were recorded at 100 MHz with a JEOL JNM-MH-100 spectrometer or JNM-FX-100 spectrometer. ^{13}C-NMR spectra were recorded at 25 MHz with a JEOL JNM-FX-100 spectrometer. Tetramethylsilane was used as an internal standard. Chemical shifts are given on the δ scale. Thin-layer chromatography (TLC) was performed on pre-coated silica gel plates 0.25 mm thick (Kieselgel 60 F_{254}, Merck) using (A), $CHCl_3$–acetone (3: 1, v/v); (B), $CHCl_3$–acetone (1: 1); (C), benzene–ether (1: 1); and (D), benzene–ether–MeOH (7: 7: 1). Detection was effected with H_2SO_4 or by UV irradiation (short wavelength). Column chromatography was performed on Kieselgel 60 (Merck, 70—230 mesh).

1,6-Anhydro-3′-O-tosyl-β-lactose (3)——A suspension of **2**[8] (1 g, 1.76 mmol) in dry MeOH (30 ml) was hydrogenated overnight in the presence of Pd catalyst at room temperature under atmospheric pressure; the catalyst was freshly prepared[14] from $PdCl_2$ (500 mg). After removal of the catalyst by filtration, the filtrate was evaporated to dryness to afford **3** as a hygroscopic amorphous powder (856 mg, 99.5%), $[\alpha]_D^{21}$ −11.8° (c=1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 1596 (C=C in tosyl), 1340, 1175 (SO_2). ^{1}H-NMR $\delta_{ppm}^{CD_3OD}$: 2.46 (3H, s, $C_6H_4CH_3$), 5.28 (1H, s, H-1, β-Glc), 7.43 (2H, d, J=8 Hz, aromatic protons *meta* to SO_2), 7.88 (2H, d, J=8 Hz, aromatic protons *ortho* to SO_2). TLC: *Rf* 0.03 (solvent B). *Anal.* Calcd for $C_{19}H_{26}O_{12}S\cdot 1/2H_2O$: C, 46.81; H, 5.58. Found: C, 46.93; H, 5.58.

Crystallization of **3** from acetone gave colorless plates, $[\alpha]_D^{22}$ −11.5° (c=1.1, MeOH), which crystallized with 1 mol of acetone. The product started to melt at 136° and decomposed at 168°. IR ν_{max}^{KBr}: 3360 (OH), 1700 (CO in acetone), 1596 (C=C in tosyl), 1348, 1173 (SO_2). ^{1}H-NMR $\delta_{ppm}^{C_5D_5N}$: 2.02 [6H, s, $(CH_3)_2CO$], 2.16 (3H, s, $C_6H_4CH_3$), 5.82 (1H, s, H-1, β-Glc), 7.20 (2H, d, J=8 Hz, aromatic protons *meta* to SO_2), 8.12 (2H, d, J=8 Hz, aromatic protons *ortho* to SO_2). *Anal.* Calcd for $C_{19}H_{26}O_{12}S\cdot(CH_3)_2CO$: C, 49.25; H, 6.01. Found: C, 49.16; H, 6.08.

1,6-Anhydro-2,2′,3,4′,6′-penta-O-benzyl-β-lactose (4)——Benzyl bromide (2.5 ml, 21.05 mmol) was added dropwise with stirring at 0° to a suspension of **3** (350 mg, 0.72 mmol), BaO (1 g, 6.52 mmol), and $Ba(OH)_2\cdot 8H_2O$ (450 mg, 1.43 mmol) in dry DMF (10 ml). After stirring for 24 hr at room temperature, the mixture was poured into ice-H_2O (50 ml), stirred for 24 hr, and then extracted with CH_2Cl_2 (3×30 ml). The combined extracts were washed with H_2O, 10% H_2SO_4, H_2O, aq. $NaHCO_3$, and H_2O, dried ($MgSO_4$), and concentrated to give a syrup (1.84 g), which was dissolved in dry MeOH (20 ml). 2% sodium amalgam (7 g) was added to the solution and the mixture was stirred for 24 hr at room temperature. The mixture was filtered, neutralized with glacial AcOH, and evaporated to a syrup (1.34 g), which was chromatographed on a column of silica gel, eluting with benzene–ether (4: 1, v/v). Removal of the solvent from the eluate afforded pure **4** (283 mg, 51%) as a syrup, $[\alpha]_D^{21}$ −25.6° (c=0.65, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3450 (OH). ^{1}H-NMR $\delta_{ppm}^{CDCl_3}$: 2.44 (1H, br. s, OH), 5.46 (1H, s, H-1, β-Glc), 7.19—7.29 (25H, m, aromatic protons). TLC: *Rf* 0.55 (solvent A), 0.65 (B), 0.31 (C), 0.60 (D). *Anal.* Calcd for $C_{47}H_{50}O_{10}$: C, 72.85; H, 6.50. Found: C, 72.55; H, 6.37.

3′-O-Acetyl-1,6-anhydro-2,2′,3,4′,6′-penta-O-benzyl-β-lactose (5)——Acetic anhydride (3 ml) was added to an ice-cold solution of **4** (100 mg) in dry pyridine (3 ml). The mixture was stored overnight at 5°, and the solvent was removed by repeated co-distillation with toluene. A solution of the residue in CH_2Cl_2 (20 ml) was washed with H_2O, 10% H_2SO_4, H_2O, aq. $NaHCO_3$, and H_2O, then dried over $CaCl_2$, and concentrated to give **5** (102 mg, 96.6%) as a syrup, $[\alpha]_D^{19}$ −7.7° (c=0.8, $CHCl_3$). ^{1}H-NMR $\delta_{ppm}^{CDCl_3}$: 1.89 (3H, s, OAc), 5.46 (1H, s, H-1, β-Glc), 7.16—7.26 (25H, m, aromatic protons). TLC: *Rf* 0.62 (solvent A), 0.70 (B), 0.44 (C), 0.65 (D). *Anal.* Calcd for $C_{49}H_{52}O_{11}$: C, 72.04; H, 6.42. Found: C, 72.28; H, 6.37.

2-Methyl-[4,6-di-O-acetyl-1,2-dideoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano][2′,1′: 4,5]-2-oxazoline (6)——The product, $[\alpha]_D^{24}$ +5.1° (c=2.6, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 1740 (OAc), 1668 (C=N), 1225 (OAc) (NH and amide II bands were absent), was prepared by the method of Augé and Veyrières.[10] [lit.[10] $[\alpha]_D^{20}$ +5° (c=2.955, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 1740 (OAc), 1667 (C=N), 1225 (OAc)].

O-2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-O-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-O-2,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose (7)——A mixture of **4** (1 g, 1.29 mmol), oxazoline (**6**) (1.15 g, 1.86 mmol), and *p*-toluenesulfonic acid monohydrate (20 mg) in dry toluene–nitromethane (12 ml, 1: 1, v/v) was stirred at 60° for 24 hr under an N_2 atmosphere. After 24 hr, further portions of **6** (1.15 g) were added, and the stirring was continued for 24 hr. The mixture was neutralized with pyridine, and evaporated to dryness. The residue was chromatographed on a column of silica gel, eluting first with benzene–ether (3: 1, v/v) and then with $CHCl_3$–EtOH (60: 1, v/v). Removal of the solvent from the second effluent gave the protected tetrasaccharide (**7**) as an amorphous powder (1.42 g, 78.9%), $[\alpha]_D^{20}$ −33° (c=0.98, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3390 (NH), 1680 (amide I). ^{1}H-NMR $\delta_{ppm}^{CDCl_3}$: 1.59, 1.94, 1.99, 2.02, 2.04, 2.09, 2.13 (21H, all s, OAc×6, NAc), 5.42 (1H, s, H-1, β-Glc),

14) O. Th. Schmidt and W. Staab, *Chem. Ber.*, **87**, 393 (1954).

7.24—7.33 (25H, m, aromatic protons). TLC: *Rf* 0.29 (solvent A), 0.63 (B), 0.39 (D). *Anal.* Calcd for $C_{73}H_{85}NO_{26}$: C, 62.97; H, 6.15; N, 1.01. Found: C, 62.69; H, 6.22; N, 1.16.

O-2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-O-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-O-2,4,6-tri-O-acetyl-β-D-galactopyranosyl-(1→4)-1,6-anhydro-2,3-di-O-acetyl-β-D-glucopyranose (8)——A solution of **7** (500 mg, 0.36 mmol) in dry MeOH (15 ml) was hydrogenated overnight in the presence of Pd catalyst, prepared[14] from $PdCl_2$ (300 mg), at room temperature under atmospheric pressure. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was acetylated with Ac_2O (5 ml) and pyridine (5 ml). The mixture was processed as described for the preparation of **5** to yield an amorphous powder (432 mg). The product was purified on a column of silica gel, eluting with $CHCl_3$–acetone (5: 2, v/v). Removal of the solvent from the effluent gave pure **8** as an amorphous powder (408 mg, 97.5%), $[\alpha]_D^{21}$ +2.1° (c=1.2, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3360 (NH), 1675 (amide I), 1540 (amide II). ^{1}H-NMR $\delta_{ppm}^{CDCl_3}$: 1.98, 2.04, 2.06, 2.11, 2.12, 2.14 (36H, all s, OAc×11, NAc), 6.28 (1H, d, $J_{NH,2''}$=7 Hz, NH). TLC: *Rf* 0.04 (solvent A), 0.35 (B), 0.08 (D). *Anal.* Calcd for $C_{48}H_{65}NO_{31}$: C, 50.05; H, 5.69; N, 1.22. Found: C, 49.81; H, 5.77; N, 1.29.

O-β-D-Galactopyranosyl-(1→3)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (9)——A solution of **8** (120 mg, 0.1 mmol) in MeOH–H_2O–triethylamine (12 ml, 2: 3: 1, v/v) was stirred at room temperature for 48 hr to carry out de-O-acetylation. After removal of the solvent, the residue was crystallized from aq. EtOH to give hygroscopic fine needles (64 mg, 89%), mp 278—279°, $[\alpha]_D^{20}$ −29.1° (c=0.73, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3400 (br. OH, NH), 1630 (amide I), 1565 (amide II). ^{1}H-NMR $\delta_{ppm}^{D_2O}$: 2.46 (3H, s, NAc), 4.88 (1H, d, $J_{1''',2'''}$=8 Hz, H-1‴, β-Gal). 4.96 (1H, d, $J_{1',2'}$=8 Hz, H-1′, β-Gal), 5.89 (1H, s, H-1, β-Glc). ^{13}C-NMR $\delta_{ppm}^{D_2O}$: 23.5 ($NCO\underline{C}H_3$), 102.6 (C-1), 103.1 (C-1′), 103.8 (C-1″), 104.7 (C-1‴), 176.1 ($N\underline{C}OCH_3$). *Anal.* Calcd for $C_{26}H_{43}NO_{20}\cdot 1/2H_2O$: C, 44.70; H, 6.35; N, 2.00. Found: C, 44.57; H, 6.26; N, 1.99.

Anomeric Mixture of O-2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-O-2-acetamido-4,6-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-O-2,4,6-tri-O-acetyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-O-acetyl-D-glucopyranose (10)——A chilled acetolysis mixture (5 ml, H_2SO_4–Ac_2O–AcOH, 1: 70: 30, v/v) was added dropwise to **8** (300 mg, 0.26 mmol) with stirring at 0°, and stirring was continued for 2 hr below 10°. The solution was poured into a mixture of ice and aq. $NaHCO_3$ with stirring, and stirring was continued overnight. The mixture was extracted with CH_2Cl_2 (3×30 ml). The combined extracts were washed with aq. $NaHCO_3$ and H_2O, dried over $CaCl_2$, and evaporated to dryness. The residue was chromatographed on a column of silica gel, eluting with $CHCl_3$–acetone (3: 1, v/v). Removal of the solvent from the effluent gave **10** as an amorphous powder (288 mg, 88.2%), $[\alpha]_D^{19}$ +40° (c=1.4, $CHCl_3$). The ratio α: β in **10** was calculated as 6.5: 3.5 from the specific rotations of **10**, **11**, and **12**.

O-2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-O-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-O-2,4,6-tri-O-acetyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-O-acetyl-β-D-glucopyranose (11)——The anomeric mixture (**10**) (288 mg) was chromatographed on a column of silica gel, eluting with benzene–ether–MeOH (7: 7: 1, v/v). Compound **11** was isolated from the faster-moving effluent after removal of the solvent as an amorphous powder (15.3 mg, 5.3%), $[\alpha]_D^{19}$ +22.9° (c=0.53, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3380 (NH), 1670 (amide I), 1540 (amide II). ^{1}H-NMR $\delta_{ppm}^{CDCl_3}$: 1.97, 1.99, 2.04, 2.06, 2.11 (42H, all s, OAc×13, NAc), 5.71 (1H, d, $J_{1,2}$=8 Hz, H-1, β-Glc), 6.06 (1H, d, exchangeable with D_2O, $J_{NH,2''}$=7 Hz, NH). TLC: *Rf* 0.18 (solvent D). *Anal.* Calcd for $C_{52}H_{71}NO_{34}$: C, 49.80; H, 5.71; N, 1.12. Found: C, 49.54; H, 5.76; N, 1.04.

O-2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-O-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-O-2,4,6-tri-O-acetyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-O-acetyl-α-D-glucopyranose (12)——In column chromatography of **10** as mentioned above, **12** was eluted with the same solvent after **11** had emerged. Removal of the solvent gave **12** as an amorphous powder (25.6 mg, 8.9%), $[\alpha]_D^{20}$ +49.3° (c=0.5, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3400 (NH), 1670 (amide I), 1540 (amide II). ^{1}H-NMR $\delta_{ppm}^{CDCl_3}$: 1.95, 1.99, 2.05, 2.13, 2.17 (42H, all s, OAc×13, NAc), 5.81 (1H, d, exchangeable with D_2O, $J_{NH,2''}$=8 Hz, NH), 6.26 (1H, d, $J_{1,2}$=3.5 Hz, H-1, α-Glc). TLC: *Rf* 0.15 (solvent D). *Anal.* Calcd for $C_{52}H_{71}NO_{34}$: C, 49.80; H, 5.71; N, 1.12. Found: C, 49.66; H, 5.69; N, 1.42.

O-β-D-Galactopyranosyl-(1→3)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose (Lacto-N-tetraose) (13)——Compound **10** (500 mg, 0.4 mmol) in MeOH–H_2O–triethylamine (75 ml, 2: 3: 1, v/v) was stirred at room temperature for 48 hr to carry out de-O-acetylation. After removal of the solvent, treatment of the residue with aq. EtOH induced crystallization of **13** as hygroscopic fine needles (231 mg, 81.9%), mp 225—228°, $[\alpha]_D^{21}$ +27° (4 min)→+21.3° (3 hr) (c=0.45, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3250 (br. OH, NH), 1635 (amide I), 1558 (amide II). [lit. mp 204—205°,[13] 205±10° (dec.),[5a] $[\alpha]_D^{24}$ +25.2° (final value) (c=1.5, H_2O),[5b] $[\alpha]_D^{23}$ +38° (0 min)→+25.5° (final value) (H_2O)[5a]].

Paper Partition Chromatography (PPC) of 9 and 13——PPC was performed on Toyo No. 51 filter paper (Toyo Roshi Kaisha, Ltd., Tokyo) by the decending method with AcOEt–pyridine–H_2O (2: 1: 2, v/v, upper layer) for 20 hr at 18—19°. Detection was effected by spraying alkaline silver nitrate reagent[15] 30 min

15) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature* (London), **166**, 444 (1950).

after pre-spraying with 0.01 M KIO_4. Lactose: R_{Glc} 0.53 (lit.[5a] 0.51), lacto-N-biose I: R_{Glc} 0.78 (lit.[5a] 0.76), **9**: R_{Glc} 0.38, **13**: R_{Glc} 0.19 (lit.[5a] 0.17).

Gel Permeation Chromatography——Bio-Gel P-4 (under 400 mesh, BIO·RAD Laboratories) column chromatography was performed with a column (2 × 175 cm) equipped with a water jacket. During operation, the column was kept at 53° by circulation of warm water in the jacket. The column was eluted with water, and the eluate was collected in 3.3 ml fractions. The phenol–H_2SO_4 method[16] and the Morgan-Elson method[17] were used for monitoring neutral sugars and 2-acetamido-2-deoxy-D-glucose eluted from the column, respectively. D-Glucose, 2-acetamido-2-deoxy-D-glucose, and isomaltose oligomers were used as standard sugars. Isomaltose oligomers were prepared by partial hydrolysis[18] of dextran (MW 200000—300000, Wako Pure Chemical Industries, Ltd.) with 0.1 N HCl at 100° for 4 hr followed by isolation with a charcoal-Celite column.[19] One mg of each of the standard sugars and lacto-N-tetraose (**13**) was applied to the column separately.

Lacto-N-tetraose was eluted as a single peak at the same position as the peak of isomaltopentaose (at fraction number 94). On the other hand, 2-acetamido-2-deoxy-D-glucose was eluted at the same position as the peak of isomaltose (at fraction number 110). Thus, the lacto-N-tetraose and 2-acetamido-2-deoxy-D-glucose behaved as if they consisted of 5.0 and 2.0 glucose units, respectively.

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