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Chemical Modification of Lactose. XVI.[1] Synthesis of Lacto-N-neohexaose[2]

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Reaction of 1,6-anhydro-2,2′,3,4′-tetra-O-benzyl-β-lactose (**1**, 1 mol eq.) with the acetylated oxazoline of N-acetyllactosamine (**2**, 5 mol eq.) gave the derivatives of 6′-N-acetyllactosaminyllactose (**3**, 24.5%) and lacto-N-neohexaose (**8**, 53.5%). The protecting groups of **3** and **8** were removed by means of the following series of reactions to provide the corresponding tetrasaccharide (**7**) and hexasaccharide (**12**), respectively: debenzylation followed by acetylation, acetolysis, and de-O-acetylation. ^{13}C-nuclear magnetic resonance spectral data for the 1,6-anhydro-β-derivatives of **7** and **12** are presented.

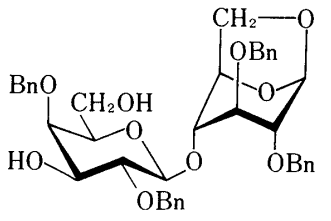
Keywords——synthesis; human milk oligosaccharide; lacto-N-neohexaose; oxazoline glycosidation method; 6′-N-acetyllactosaminyllactose; 1,6-anhydro-β-tetrasaccharide; 1,6-anhydro-β-hexasaccharide; ^{13}C-NMR

The occurrence and the structure of lacto-N-neohexaose (**12**) in human milk were reported by Kobata and Ginsburg,[3] and the existence of more complex Oligosaccharides having **12** as a partial structure has been described.[4] The occurrence of **12** in human milk suggests that the branched structure of this sugar may also exist as a distal part of some carbohydrate chains of blood group substances.[5] We now report a synthesis of **12** together with 6′-N-acetyllactosaminyllactose (**7**) as a by-product.

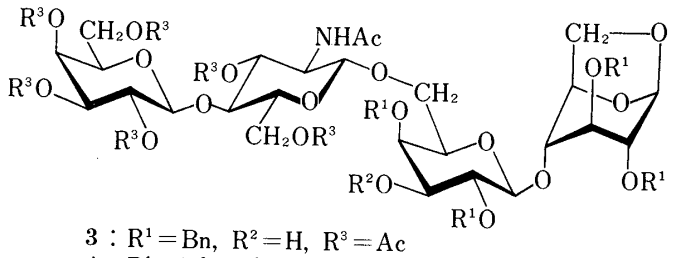
The synthetic route is based on the condensation of 1,6-anhydro-2,2′,3,4′-tetra-O-benzyl-β-lactose (**1**) (having two unprotected hydroxyl groups at the C-3′ and C-6′ positions) with five molar equivalents of the acetylated oxazoline derivative of N-acetyllactosamine (LacNAc) (**2**),[6] followed by removal of the protecting groups. The synthesis of **1** was reported in Part XIV[7] of this series. A mixture of **1** (1 mol eq.) and **2** (3 mol eq.) in dry 1,2-dichloroethane containing 0.01 M anhydrous *p*-toluenesulfonic acid (TsOH) was stirred at 60—65°C for 48 h under nitrogen. After 48 h, more **2** (2 mol eq.) was added and stirring was continued for a further 24 h. The mixture was neutralized and concentrated to dryness: thin-layer chromatography (TLC) showed two spots. The minor component was separated from these condensation products in the earlier fractions of silica gel column chromatography, in a yield of 24.5%. After purification by re-chromatography, the product was isolated as an amorphous powder, and it was designated as **3**. The infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectral data of **3** were consistent with those of the corresponding tetrasaccharide consisting of an acetylated LacNAc and **1**.

On debenzylation followed by acetylation, **3** gave the dodecaacetate (**4**) as an amorphous powder. De-O-acetylation of **4** provided the crystalline 1,6-anhydro-β-tetrasaccharide (**5**). The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of **5** was measured in deuterium oxide (D_2O) at room temperature. The results are shown in Table I. Each signal of the anomeric carbons in **5** was assigned by selective proton decoupling of the corresponding anomeric protons, and those of other carbons were assigned by comparison with the observed values for 1,6-anhydro-β-lactose (**13**)[1] and methyl β-N-acetyllactosaminide (**14**). The reference compound **14** was conveniently prepared from **2** and methanol by the oxazoline glycosidation method, whereas the reported diazomethane method provided **14** in only low yield (11%).[8] The chemical shifts of each carbon of **14** were assigned by comparison with the literature values for methyl 2-acetamido-2-deoxy-β-D-glucopyranoside[9] and methyl

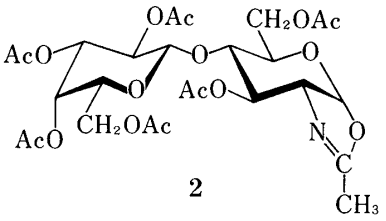
β-D-galactopyranoside.[10] The signals for the corresponding carbon atoms in **14** and the N-acetyllactosaminyl residue of **5** showed similar chemical shifts. On the other hand, the resonance for C-6′ of **5** appeared at 69.9 ppm, deshielded by 7.6 ppm as compared with the chemical shift for C-6′ of **13** (62.3 ppm). However, the chemical shift for C-5′ of **5** (74.8 ppm) was shifted upfield by 1.7 ppm as compared with that of C-5′ of **13** (76.5 ppm). These results provided unequivocal proof of the position (C-6′) of the newly introduced N-acetyllactosaminyl linkage in **5**. Therefore, compounds **3** and **4** were also assigned as tetrasaccharide derivatives having an acetylated LacNAc residue at the C-6′ position of a 1,6-anhydro-β-lactose residue.



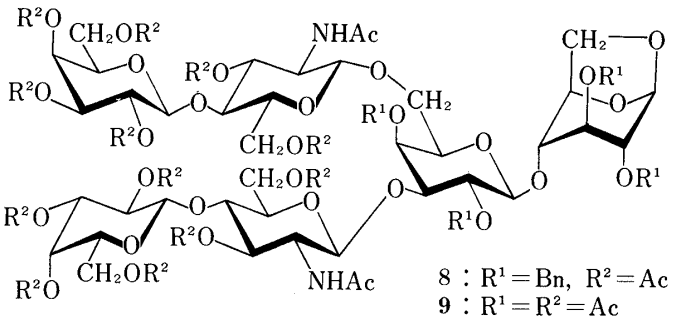
1



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



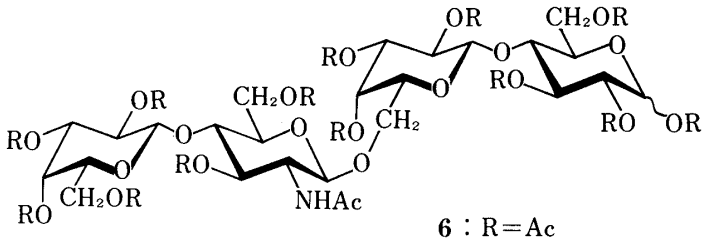
2



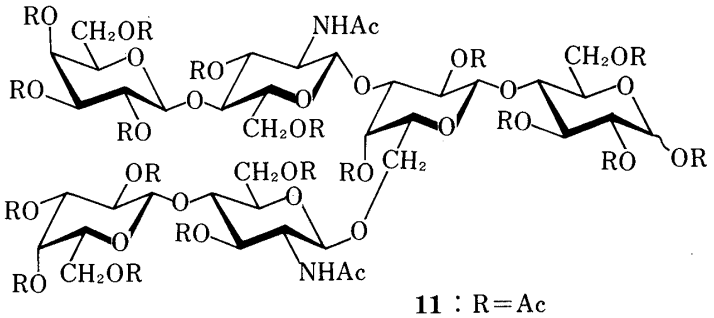
8 : R^1=Bn, R^2=Ac
9 : R^1=R^2=Ac
10 : R^1=R^2=H

Ac=acetyl, Bn=benzyl

Chart 1



6 : R=Ac
7 : R=H



11 : R=Ac
12 : R=H

Chart 2

The major component in the aforementioned condensation products of **1** and **2** was separated by column chromatography after **3** had been eluted and, on re-chromatography, it was isolated as an amorphous powder and designated as **8**. The structure of **8** was identified as the corresponding hexasaccharide consisting of two moles of acetylated LacNAc and **1**, based on the ^{1}H-NMR spectral data. The yield was 53.5%. Amorphous octadecaacetate (**9**) and powdered 1,6-anhydro-β-hexasaccharide (**10**) were prepared from **8** and **9**, respectively, by procedures similar to those described in the tetrasaccharide series.

In order to determine the positions of the newly introduced LacNAc residues, the ^{13}C-NMR spectrum of **10** was measured in D_2O. The signals were assigned by comparison with the chemical shifts of the aforementioned observed values for **5**, **13**, and **14**. The results are shown in Table I. The resonances for C-6′ and C-3′ of **10** appeared at 70.2 and 82.8 ppm, respectively. They were deshielded by 7.9 and 9.1 ppm as compared with the chemical shifts for C-6′ (62.3 ppm) and C-3′ (73.7 ppm) of **13**, respectively. However, the chemical shift of C-2′ of **10** (70.8 ppm) was shifted upfield by 1.1 ppm as compared with that of C-2′ of **13** (71.9 ppm). These results provided unequivocal proof that the newly introduced LacNAc residues in **10** are attached at the C-3′ and C-6′ positions of **13**.

The signals for the individual N-acetyl-D-glucosamines (GlcNAcs) branched at the C-3 and C-6 positions of D-galactose (Gal) were assigned as follows. The signals showing chemical shifts similar to those for the corresponding carbons of GlcNAc in **5** were assigned to the carbons of GlcNAc attached to the C-6 position of Gal. Therefore, the signals showing slightly

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

13: Galβ1→4 Glcsan
B A

14: Galβ1→4 Methyl β-GlcNAc
B A

5: Galβ1→4 GlcNAcβ1
D C
↘6 Galβ1→4 Glcsan
B A

10:
Galβ1→4 GlcNAcβ1
D C
↘6
Galβ1→4 Glcsan
B A
↗3
Galβ1→4 GlcNAcβ1
F E

Glcsan = 1,6-anhydro-β-D-glucopyranose

		C-1	C-2	C-3	C-4	C-5	C-6	NCOCH$_3$	NCOCH$_3$	OMe
13[a]	A	102.6	71.2	72.7	78.9	75.3	66.3			
	B	103.3	71.9	73.7	69.9	76.5	62.3			
14[b]	A	103.0	56.2	73.7	79.8	75.9	61.3	23.4	175.8	58.3
	B	104.1	72.2	73.7	69.8	76.5	62.2			
5[c]	A	102.5	70.9	72.5	78.7	75.1	66.2			
	B	103.1	71.7	73.7	69.7	74.8	69.9			
	C	102.3	56.2	73.5	79.6	75.9	61.2	23.4	175.8	
	D	104.0	72.1	73.5	69.7	76.5	62.2			
10[d]	A	102.5	70.8	72.5	78.5	75.0	66.1			
	B	103.0	70.8	82.8	69.7	74.7	70.2			
	C	102.5	56.2[f]	73.3[e]	79.6[f]	75.9[f]	61.2[f]	23.4	175.7	
	D	104.0	72.1	73.7[e]	69.7	76.5	62.2			
	E	104.0	56.4[f]	73.7[e]	79.5[f]	75.8[f]	61.2[f]	23.5	176.0	
	F	104.0	72.1	73.7[e]	69.7	76.5	62.2			

a) O-β-D-Galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose.
b) Methyl O-β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside.
c) O-β-D-Galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose.
d) O-β-D-Galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[O-β-D-galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1—6)]-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose.
e) Assignments may be interchangeable.
f) Assignments may be reversed.

different values from those mentioned above were assigned to the carbons of GlcNAc attached to the C-3 position of Gal.

According to the paper reported by Voelter and co-workers,[11] methylation of D-galactopyranose hydroxyls caused upfield shifts of about 4.5 ppm on β-carbons with axial hydroxyl groups. This observation has been generally recognized as useful for determining the position of substituents in the D-galactose series. However, according to a recent paper by Messer *et al.*,[12] only a very small upfield shift (0.1 ppm) was observed at the C-4′ position of 3′-β-D-galactopyranosyllacrose (69.3 ppm) as compared with the chemical shift for C-4′ of lactose (69.4 ppm). In compound **10**, the resonance for C-4′ (69.7 ppm) showed an upfield shift of only 0.2 ppm as compared with that for C-4′ of **13** (69.9 ppm). Thus, our result is fully consistent with that of Messer *et al.*

The 1,6-anhydro-β-rings of **4** and **9** were cleaved with an acetolysis mixture to give the tetrasaccharide tetradecaacetate (**6**) and hexasaccharide eicosaacetate (**11**) in 94.3 and 93.9% yields, respectively. The ^{1}H-NMR spectral data of **6** and **11** were in good agreement with their structures. De-O-acetylation of **6** and **11** gave 6′-N-acetyllactosaminyllactose (**7**) and lacto-N-neohexaose (**12**) in 73.5 and 80% yields, respectively. Compound **7** was a white powder having $[\alpha]_D^{19}$ +11.8° in water. Zurabyan *et al.*[13] reported that crystalline 6′-N-acetyllactosaminyllactose showed mp 185—187°C and $[\alpha]_D$ +8° in water. Compound **12** was crystallizable from aqueous ethanol as grains having mp 223—225°C and $[\alpha]_D^{21}$ +9.1° in water, which showed no mutarotation.

The mobilities *versus* lactose (R_{Lac}) of **5**, **7**, **10**, and **12**, on paper are also described.

Experimental

Instruments used and conditions for chromatography were the same as in Part XIII[14] unless otherwise indicated. TLC was performed with the following solvent combinations (v/v): (A), $CHCl_3$–acetone (3: 1); (B), $CHCl_3$–EtOH (93: 7); (C), $CHCl_3$–ether–MeOH (10: 10: 1). Solvent combinations for elution on column chromatography with Kieselgel 60 (Merck, 70—230 mesh) are shown as v/v.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-(2,4-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose (3) **and O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-O-(2,4-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose** (**8**)——A mixture of **1**[7] (280 mg, 0.41 mmol) and 2-methyl-[3,6-di-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline (**2**)[6] (760 mg, 1.23 mmol) in dry 1,2-dichloroethane containing 0.01 M anhydrous TsOH (10 ml) was stirred at 60—65°C for 48 h under nitrogen. After 48 h, more **2** (510 mg) was added and stirring was continued for a further 24 h. The mixture was neutralized with pyridine, and concentrated to afford a dark-brownish amorphous powder; TLC with solvent C showed two spots, *Rf* 0.18 (minor) and 0.08 (major). On column chromatography with $CHCl_3$–ether–MeOH (7: 7: 1), crude **3** (239 mg) was isolated as an amorphous powder from the earlier fractions, then crude **8** (565 mg) was separated from the later fractions. The former was purified by re-chromatography on a column of Kieselgel 60 with $CHCl_3$–acetone (3: 1). Removal of the solvent gave pure **3** (131 mg, 24.5%) as an amorphous powder, $[\alpha]_D^{21}$ −10.8° (*c*=0.65, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH, OH), 1743 (OAc), 1670 (amide I), 1535 (amide II). ^{1}H-NMR ($CDDl_3$): 1.84, 1.98, 2.01, 2.06, 2.16 (21H, all s, $-COC\underline{H}_3\times6$, $-NHCOC\underline{H}_3$), 5.51 (1H, s, H-1, β-Glc), 5.65 (1H, d, exchangeable with D_2O, $J_{NH,2''}$=8.5 Hz, NH), 7.20—7.44 (20H, m, aromatic protons). *Anal.* Calcd for $C_{66}H_{79}NO_{26}$: C, 60.87; H, 6.11; N, 1.08. Found: C, 60.54; H, 5.85; N, 1.37.

The aforementioned crude **8** was purified by re-chromatography through a column of Kieselgel 60 with $CHCl_3$–EtOH (19: 1). Removal of the solvent gave pure **8** (420 mg, 53.5%) as an amorphous powder, $[\alpha]_D^{21}$ −13.8° (*c*=0.26, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1745 (OAc), 1675 (amide I), 1525 (amide II). ^{1}H-NMR ($CDCl_3$): 1.53, 1.83, 1.99, 2.06, 2.09, 2.16 (42H, all s, $-COC\underline{H}_3\times12$, $-NHCOC\underline{H}_3\times2$), 5.88 (1H, d, exchangeable with D_2O, $J_{NH,2''\text{ or }2''''}$=8 Hz, NH), 7.24—7.44 (20H, m, aromatic protons). *Anal.* Calcd for $C_{92}H_{114}N_2O_{42}$: C, 57.56; H, 5.99; N, 1.46. Found: C, 57.23; H, 5.95; N, 1.43.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (**4**)——A solution of **3** (130 mg, 0.1 mmol) in dry MeOH (10 ml) was hydrogenated in the presence of a Pd catalyst, freshly prepared[15] from $PdCl_2$ (100 mg), at room temperature under atmospheric pressure to carry out debenzylation. After filtration, the filtrate was concentrated to provide an amorphous

powder (93 mg) that was acetylated with Ac_2O (2 ml) and pyridine (2 ml) at room temperature overnight. The mixture was poured into ice-H_2O (30 ml), and the whole was stirred for 3 h, then extracted with CH_2Cl_2 (3×10 ml). The extracts were successively washed with H_2O, 10% H_2SO_4, H_2O, aqueous $NaHCO_3$, and H_2O, dried ($MgSO_4$), and concentrated to yield an amorphous powder (117 mg) that was chromatographed on a column of Kieselgel 60 with $CHCl_3$–acetone (2: 1). The eluate provided **4** (106 mg, 92.4%) as an amorphous powder, $[\alpha]_D^{20}$ −27.8° ($c=1.5$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1745 (OAc), 1637 (amide I), 1532 (amide II). ^{1}H-NMR ($CDCl_3$): 1.95, 1.96, 2.05, 2.12, 2.13 (36H, all s, $-COCH_3 \times 11$, $-NHCOCH_3$), 5.46 (1H, s, H-1, β-Glc), 6.28 (1H, d, exchangeable with D_2O, $J_{NH,2''}=8.5$ Hz, NH). TLC: *Rf* 0.58 (solvent A), 0.21 (B), 0.19 (C). *Anal.* Calcd for $C_{48}H_{65}NO_{31}$: C, 50.05; H, 5.69; N, 1.22. Found: C, 49.84; H, 5.71; N, 1.16.

O-β-D-Galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (5)——Methanolic MeONa (0.5 N, 0.4 ml) was added dropwise under stirring to a chilled solution of **4** (70 mg, 0.06 mmol) in dry MeOH (4 ml), and stirring was continued at room temperature overnight. The mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to dryness to give **5** (31 mg, 73.5%) as an amorphous powder. The product was crystallized from a small amount of MeOH as fine needles, mp 197—199°C, $[\alpha]_D^{23}$ −34.8° ($c=0.37$, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3390 (br. OH, NH), 1635 (amide I), 1555 (amide II). ^{1}H-NMR (D_2O): 2.51 (3H, s, $-NHCOCH_3$), 4.55 (1H, d, $J_{1',2'}=8$ Hz, H-1′, β-Gal), 4.90 (1H, d, $J_{1''',2'''}=7$ Hz, H-1‴, β-Gal), 4.98 (1H, d, $J_{1'',2''}=6$ Hz, H-1″, β-GlcNAc), 5.90 (1H, s, H-1, β-Glc). ^{13}C-NMR: see Table I. *Anal.* Calcd for $C_{26}H_{43}NO_2$: C, 45.28; H, 6.28; N, 2.03. Found: C, 45.38; H, 6.09; N, 2.45.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-D-glucopyranose (6)——A chilled acetolysis mixture (2 ml, H_2SO_4–Ac_2O–AcOH, 1: 70: 30, v/v) was added to **4** (75 mg, 0.065 mmol) with stirring at 0°C. The solution was stirred for 2 h below 10°C, then poured into a mixture of ice and aqueous $NaHCO_3$ with stirring, and stirring was continued overnight. The mixture was extracted with CH_2Cl_2 (3×10 ml). The extracts were washed with aqueous $NaHCO_3$ and H_2O, dried ($MgSO_4$), and concentrated to dryness. The residue was chromatographed on a column of Kieselgel 60 with benzene–ether–MeOH (7: 7: 1) to obtain **6** (77 mg, 94.3%), as an amorphous powder, $[\alpha]_D^{22}$ +7.2° ($c=0.5$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3380 (NH), 1740 (OAc), 1674 (amide I), 1525 (amide II). ^{1}H-NMR ($CDCl_3$): 1.97, 1.99, 2.03, 2.08, 2.16, 2.19 (42H, all s, $-COCH_3 \times 13$, $-NHCOCH_3$), 5.81 (*ca.* 0.3H, d, $J_{1,2}=8$ Hz, H-1, β-Glc), 6.29 (1H, br. s, exchangeable with D_2O, NH), 6.37 (*ca.* 0.7H, d, $J_{1,2}=3.5$ Hz, H-1, α-Glc). TLC: *Rf* 0.56 (solvent A), 0.25 (B), 0.20 (C). *Anal.* Calcd for $C_{52}H_{71}NO_{34}$: C, 49.80; H, 5.71; N, 1.12. Found: C, 49.82; H, 5.84; N, 1.13.

O-β-D-Galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (6′-N-Acetyllactosaminyllactose, 7)——A solution of **6** (60 mg, 0.048 mmol) in dry MeOH (4 ml) was de-O-acetylated overnight with 0.5 N methanolic MeONa (0.4 ml) as described for the preparation of **5**. Removal of the solvent and treatment of the residue with MeOH–EtOH induced precipitation of **7** (30 mg, 73.5%) as a white powder, $[\alpha]_D^{19}$ +11.8° ($c=0.16$, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3370 (br. OH, NH), 1635 (amide I), 1555 (amide II). lit.[13] mp 185—187°C (crystallized from MeOH–EtOH), $[\alpha]_D$ +8° ($c=1$, H_2O).

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-O-(2,4-di-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (9)——A solution of **8** (410 mg, 0.21 mmol) in dry MeOH (15 ml) was hydrogenolytically debenzylated in the presence of a Pd catalyst, freshly prepared[15] from $PdCl_2$ (300 mg), then the resulting debenzylated product was acetylated with Ac_2O (3 ml) and pyridine (3 ml) as described for the preparation of **4** to afford an amorphous powder (366 mg). The crude product was purified by chromatography on a column of Kieselgel 60 with $CHCl_3$–acetone (1: 1). Removal of the solvent gave pure **9** (327 mg, 88.6%) as an amorphous powder, $[\alpha]_D^{25}$ −11.1° ($c=1.5$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1742 (OAc), 1670 (amide I), 1538 (amide II). ^{1}H-NMR ($CDCl_3$): 1.94, 1.98, 2.06, 2.12, 2.15 (54H, all s, $-COCH_3 \times 16$, $-NHCOCH_3 \times 2$), 5.48 (1H, s, H-1, β-Glc), 5.77 (1H, d, exchangeable with D_2O, $J_{NH,2''\text{ or }2''''}=8$ Hz, NH), 6.41 (1H, d, exchangeable with D_2O, $J_{NH,2''''\text{ or }2''}=8$ Hz, NH). TLC: *Rf* 0.19 (solvent A), 0.17 (B). *Anal.* Calcd for $C_{72}H_{98}N_2O_{46}$: C, 50.06; H, 5.72; N, 1.62. Found: C, 49.73; H, 5.72; N, 1.27.

O-β-D-Galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[O-β-D-galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (10)——A solution of **9** (89 mg, 0.05 mmol) in dry MeOH (4 ml) was de-O-acetylated overnight with methanolic MeONa (0.5 N, 0.4 ml) as described for the preparation of **5** to give **10** (36 mg, 72%), which was precipitated from aqueous EtOH as a white powder, $[\alpha]_D^{22}$ −26.3° ($c=0.4$, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3400 (br. OH, NH), 1634 (amide I), 1557 (amide II). ^{1}H-NMR (D_2O): 2.50, 2.53 (6H, each s, $-NHCOCH_3 \times 2$), 5.91 (1H, s, H-1, β-Glc). ^{13}C-NMR: see Table I. *Anal.* Calcd for $C_{40}H_{66}N_2O_{30} \cdot 3H_2O$: C, 43.32; H, 6.54; N, 2.53. Found: C, 43.57; H, 6.35; N, 2.42.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-O-(2,4-di-O-acetyl-β-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-D-

glucopyranose (11)——Compound **9** (200 mg, 0.115 mmol) was acetolyzed with an acetolysis mixture (5 ml, H_2SO_4–Ac_2O–AcOH, 1: 70: 30, v/v) as described for the preparation of **6** to give an amorphous powder (206 mg), which was purified by chromatography on a column of Kieselgel 60 with $CHCl_3$–acetone (1: 1). Removal of the solvent gave **11** (199 mg, 93.9%) as an amorphous powder, $[\alpha]_D^{22}$ +12.7° (c=1.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1744 (OAc), 1668 (amide I), 1537 (amide II). ^{1}H-NMR ($CDCl_3$): 1.93, 1.98, 2.07, 2.16 (60H, all s, $-COCH_3\times18$, $-NHCOCH_3\times2$), 5.62 (1H, d, exchangeable with D_2O, $J_{NH,2''\text{ or }2''''}$=8 Hz, NH), 6.30 (<1H, d, $J_{1,2}$=3.5 Hz, H-1, α-Glc), 6.40 (1H, d, exchangeable with D_2O, $J_{NH,2''''\text{ or }2''}$=8 Hz, NH). TLC: *Rf* 0.30 (solvent A), 0.20 (B), 0.04 (C). *Anal.* Calcd for $C_{76}H_{104}N_2O_{49}$: C, 49.89; H, 5.73; N, 1.53. Found: C, 49.70; H, 5.68; N, 1.65.

O-β-D-Galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[O-β-D-galactopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (Lacto-N-neohexaose, 12)——A solution of **11** (160 mg, 0.087 mmol) in dry MeOH (8 ml) was de-O-acetylated with methanolic MeONa (0.5 N, 0.8 ml) as described for the preparation of **5**. Removal of the solvent and treatment of the residue with aqueous EtOH induced crystallization of **12** (75 mg, 80%) as grains, mp 223—225°C, $[\alpha]_D^{21}$ +9.1° (no mutarotation, c=0.6, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3370 (br. OH, NH), 1635 (amide I), 1555 (amide II). *Anal.* Calcd for $C_{40}H_{68}N_2O_{31}\cdot2H_2O$: C, 43.32; H, 6.54; N, 2.53. Found: C, 43.28; H, 6.55; N, 2.71.

Methyl O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (Acetate of 14)——A solution of **2** (180 mg, 0.29 mmol) in dry MeOH containing 0.01 M anhydrous TsOH (1 ml) was stirred at 40°C overnight under nitrogen. The mixture was then neutralized with pyridine, and concentrated to dryness. The residue was purified by chromatography on a column of Kieselgel 60 with benzene–ether–MeOH (7: 7: 1). Removal of the solvent from the major fractions gave the acetate of **14** (102 mg, 54%) as an amorphous powder, $[\alpha]_D^{20}$ −9° (c=1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.97, 2.02, 2.04, 2.09, 2.11 (21H, all s, $-COCH_3\times6$, $-NHCOCH_3$), 3.43 (3H, s, $-OCH_3$), 6.25 (1H, d, exchangeable with D_2O, $J_{NH,2}$=9 Hz, NH).

Methyl O-β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (Methyl β-N-Acetyllactosaminide, 14)——A solution of the acetate of **14** (90 mg, 0.14 mmol) in dry MeOH (4 ml) was de-O-acetylated as described for the preparation of **5** to afford **14** (37.8 mg, 68.2%), which was crystallized from MeOH–EtOH as fine needles. The product began to turn brown at 270°C and decomposed at 283—285°C, $[\alpha]_D^{23}$ −16.7° (c=0.3, H_2O). ^{1}H-NMR (D_2O): 2.48 (3H, s, $-NHCOCH_3$), 3.95 (3H, s, $-OCH_3$), 4.89 (2H, d, $J_{1,2}$ and $J_{1',2'}$=8 Hz, H-1 and H-1′, β-GlcNAc and β-Gal). ^{13}C-NMR: see Table I. lit.[8] mp 243—245°C (dec.), $[\alpha]_D^{22}$ −23.1° (c=0.86, H_2O).

Paper Partition Chromatography (PPC) of Compounds 5, 7, 10, and 12——PPC was performed on Toyo No. 51 filter paper (Toyo Roshi Kaisha Ltd., Tokyo) by the descending method with AcOEt–pyridene–H_2O (2: 1: 2, v/v, upper layer) at 19—21°C for 20 h. Detection was effected by spraying alkaline silver nitrate reagent[16] 30 min after pre-spraying with 0.01 M KIO_4. **5**: R_{Lac} 0.60; **7**: R_{Lac} 0.43; **10**: R_{Lac} 0.31; **12**: R_{Lac} 0.21.

Measurement of ^{13}C-NMR Spectra——The ^{13}C-NMR spectra were measured at 25 MHz with a JEOL JNM-FX-100 spectrometer in the pulse Fourier transform mode. The spectra of 1,6-anhydro-β-lactose (**13**), methyl β-N-acetyllactosaminide (**14**), **5**, and **10** were measured in D_2O at room temperature. Tetramethylsilane (TMS) was used as external standard; chemical shifts are given in ppm from TMS. The results are shown in Table I.

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