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Chemical Modification of Lactose. XII.[1] Preparation of O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (6'-N-Acetylglucosaminyllactose)

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The title trisaccharide (**16**) is a constituent of the higher oligosaccharides in human milk. As part of our program of synthesis of oligosaccharides in human milk, the title trisaccharide was prepared.

Heating of a mixture of 1,6-anhydro-2,2',3,3',4'-penta-O-benzyl-β-lactose and 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)[2,1-*d*]-2-oxazoline in nitromethane–toluene in the presence of *p*-toluenesulfonic acid at 95° for 1 hr with stirring afforded the protected 1,6-anhydro-β-derivative of **16** (**6**). The protecting groups of **6** were removed by catalytic hydrogenation followed by deacetylation, or *vice versa*, to afford the 1,6-anhydro-β-derivative of **16** (**9**). Compound **9** gave a crystalline peracetate (**14**). The 1,6-anhydro-β-ring of **14** was cleaved by mild acetolysis to afford an anomeric mixture of the peracetate of **16**, from which the α-anomer (**15**) was isolated as needles. Deacetylation of **15** gave the title trisaccharide as a hygroscopic amorphous powder.

Keywords——trisaccharide synthesis; human milk; lactosan; oxazoline; Hakomori's methylation; methylated alditol acetates; GC–MS

The title trisaccharide is a constituent of the higher oligosaccharides in human milk, such as lacto-N-hexaose, lacto-N-neohexaose, fucosyl lacto-N-hexaose I, II, III and IV, fucosyl lacto-N-neohexaose I and II, sialyl lacto-N-hexaose, and sialyl fucosyl lacto-N-neohexaose I and II.[3] They are the antigenic determinants of ABH and Lewis blood group activity. Amino sugar-containing oligosaccharides are potent haptenic inhibitors of the agglutination of human blood group substances, and are useful substrates for studies of glycosidases acting on carbohydrate in glycoproteins. In Part IX of this series,[4] we reported the synthesis of 6'-galactosyllactose, which was isolated from the milk of "nonsecretor" women.[5] As part of our program of synthesis of oligosaccharides in human milk, this paper reports a preparation of the title trisaccharide. The synthetic route is based on condensation of a 1,6-anhydro-β-lactose derivative having an unprotected hydroxyl group at the C-6' position with the acetylated oxazoline of 2-acetamido-2-deoxy-D-glucopyranose, and subsequent removal of the protecting groups. The results are described in detail.

Only a limited number of studies have so far been reported on the tritylation of lactose or lactosides. In contrast to an unsuccessful attempt to tritylate benzyl β-lactoside,[6] tritylation of 1,6-anhydro-β-lactose (**1**) by a slight modification of the procedure used for the tritylation of 1,6-anhydro-β-maltose[7] gave a crystalline trityl ether (**2**) in 60.2% yield. Acetylation of **2** afforded a crystalline pentaacetate (**3**) in 93.3% yield. Benzylation of **2** by a slight modification of the procedure used for the preparation of 1,6-di-O-benzyl-2,4: 3,5-di-O-methylene-L-iditol[8] and subsequent removal of the trityl group afforded the pentabenzyl ether

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[1] Part XI: T. Takamura, T. Chiba, and S. Tejima, *Chem. Pharm. Bull.* (Tokyo), **27**, 721 (1979).
[2] Location: *Tanabe-dori, Mizuho-ku, Nagoya 467, Japan.*
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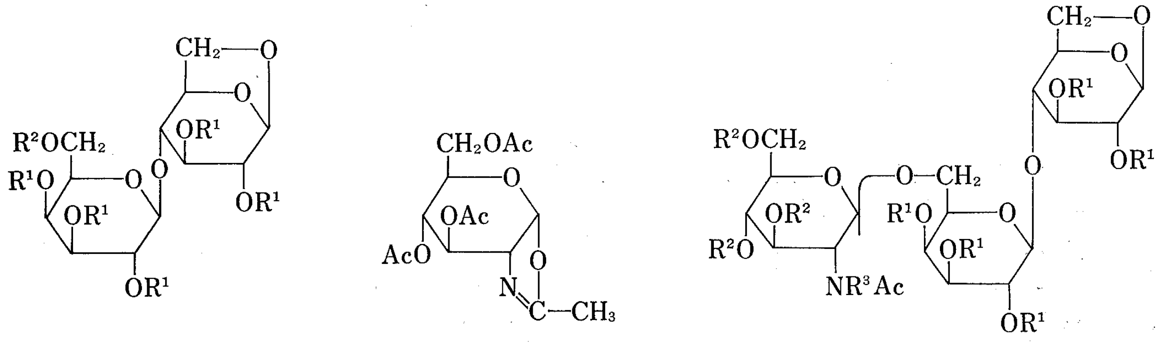
(**4**) having an unprotected hydroxyl group at the C-6′ position in **1**. Compound **4** was purified by chromatography on a column of silica gel and crystallized from ethanol as white needles in 69.8% yield. The infrared (IR) spectrum of **4** showed a band due to the hydroxyl group at 3520 cm^{-1} (Nujol).

Glycosylation with 2-methylglyco[2,1-*d*]-2-oxazoline is an efficient procedure for the synthesis of 1,2-*trans*-2-acetamido-2-deoxy-D-glycosides or oligosaccharides.[9] We used the oxazoline method for the synthesis of the title trisaccharide.

Heating of a mixture of 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)[2,1-*d*]-2-oxazoline (**5**)[10] and **4** in nitromethane-toluene in the presence of *p*-toluenesulfonic acid at 95° for 1 hr with stirring afforded the trisaccharide (**6**). Compound **6** was isolated after purification through a column of silica gel as an amorphous powder in 58.5% yield. The structures was confirmed by mass spectrometry of the permethylated trisaccahride (**10**), gas chromatographic (GC) analysis and gas chromatography–mass spectrometry (GC–MS) of the alditol acetates obtained from **10**.

Compound **10** was prepared from **6** as follows. The benzyl groups of **6** were removed by catalytic hydrogenation to afford a partially acetylated trisaccharide (**7**), which was deacetylated to give the 1,6-anhydro-β-trisaccharide (**9**). The order of removal of the protecting groups was reversible: deacetylation of **6** afforded a partially benzylated trisaccharide (**8**), which was debenzylated to give **9**. Compound **9** was dissolved in dimethylsulfoxide (DMSO) and methylated by Hakomori's procedure.[11] Compound **10** was isolated as a syrup in 31.9% yield after purification on a column of silica gel. In the nuclear magnetic resonance (NMR) spectrum, the singlet at δ 5.42 was assigned to the reducing terminus proton by reference to the NMR spectrum of 1,6-anhydro-2,2′,3,3′,4′,6′-hexa-O-methyl-β-lactose.[4] The doublets having a spacing of 6 Hz at δ 4.30 and 4.62 were assigned to 1″–H and 1′–H, respectively, by reference to data reported for various D-glycooligosaccharides.[9,12] Electron-impact mass spectrometry produced, by the pathways shown in Fig. 1 and 2, an ion with *m/e* 129 ($C_6H_{11}NO_2$), the formation of which, together with ions at *m/e* 142 ($C_7H_{12}NO_2$), *m/e* 158



2: $R^1=H$, $R^2=(C_6H_5)_3C$
3: $R^1=Ac$, $R^2=(C_6H_5)_3C$
4: $R^1=C_6H_5CH_2$, $R^2=H$

5

6: $R^1=C_6H_5CH_2$, $R^2=Ac$, $R^3=H$
7: $R^1=R^3=H$, $R^2=Ac$
8: $R^1=C_6H_5CH_2$, $R^2=R^3=H$
9: $R^1=R^2=R^3=H$
10: $R^1=R^2=R^3=CH_3$
14: $R^1=R^2=Ac$, $R^3=H$

Chart 1

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9) C. Augè and A. Veyrières, *Carbohyd. Res.*, **46**, 293 (1976), **54**, 45 (1977), and references cited therein.
10) R.U. Lemieux and H. Driguez, *J. Am. Chem. Soc.*, **97**, 4063 (1975).
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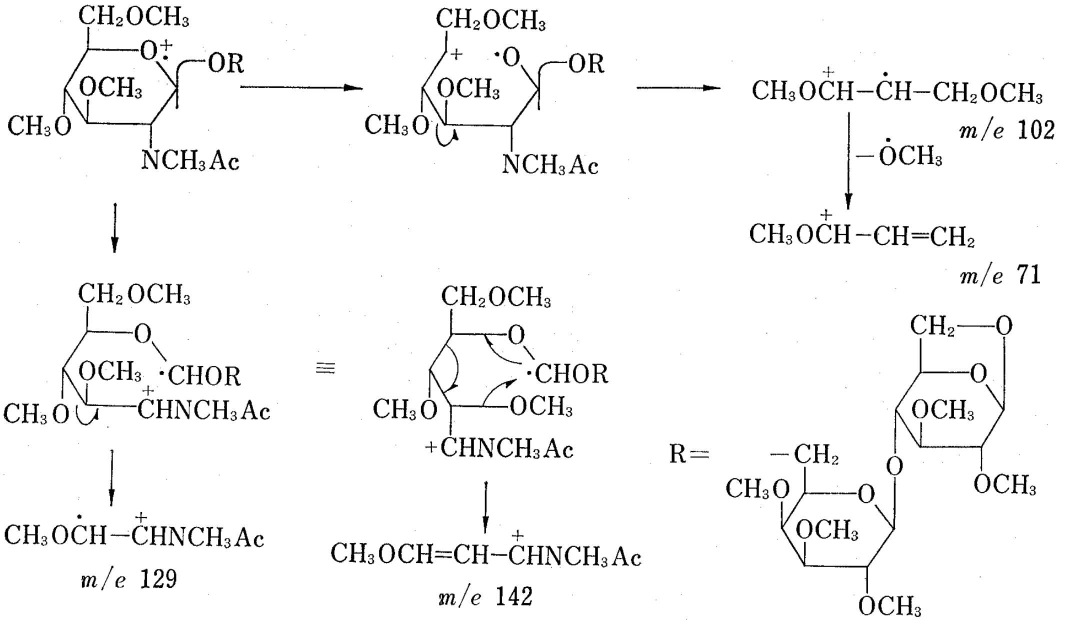

Fig. 1. Mass Fragmentation Scheme for **10**

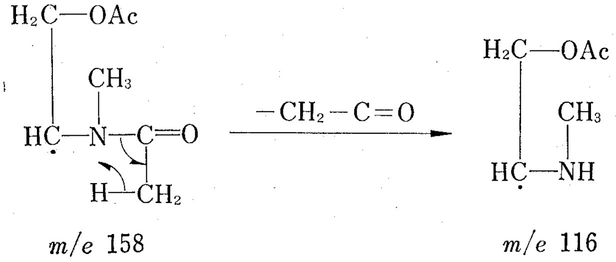

Fig. 2. Mass Fragmentation Scheme for the 1-O-Acetyl-2-deoxy-2-(N-methylacetamido) Group produced by Cleavage between C-2 and C-3 of 1,5-Di-O-acetyl-2-deoxy-2-(N-methylacetamido)-3,4,6-tri-O-methylglucitol

($C_7H_{12}NO_3$), and *m/e* 116 ($C_5H_{10}NO_2$), is characteristic of the 2-deoxy-2-(N-methylacetamido)-3,4,6-tri-O-methyl-D-glucopyranose ring system.[13]

A gas chromatogram of the methylated alditol acetates obtained from the hydrolysate of **10** showed three peaks, (a), (b), and (c). They were assigned to 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylgalactitol (**11**), 1,4,5,6-tetra-O-acetyl-2,3-di-O-methylglucitol (**12**), and 1,5-di-O-acetyl-2-deoxy-(N-methylacetamido)-3,4,6-tri-O-methylglucitol (**13**), respectively, by comparing their retention times and mass spectra with those of the products of 1,6-anhydro-2,2',-3,3',4',6'-hexa-O-methyl-β-lactose[4] and 2-deoxy-2-(N-methylacetamido)-3,4,6-tri-O-methyl-α-D-glucopyranose prepared from benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[14] by permethylation and subsequent debenzylation. The mass spectra of peaks (a), (b), and (c) were in good agreement with the reported spectra of partially methylated alditol acetates.[5,13,15]

Acetylation of compound **7** afforded a peracetylated 1,6-anhydro-β-trisaccharide (**14**) in 89.6% yield. The product was crystallized from ethanol. The 1,6-anhydro-ring of **14** was cleaved by mild acetolysis to yield an anomeric mixture of peracetylated trisaccharide as an amorphous powder in 46.7% yield. The low yield was probably a result of the instability of the (1→6) glycosidic linkage to acetolysis.[16] The α-acetate (**15**) was isolated from the anomeric mixture by crystallization from methanol-2-propanol as colorless needles in 27.8% yield. In the NMR spectrum, the anomeric proton of the reducing terminus appeared at δ 6.25 as a doublet having a spacing of 3 Hz.

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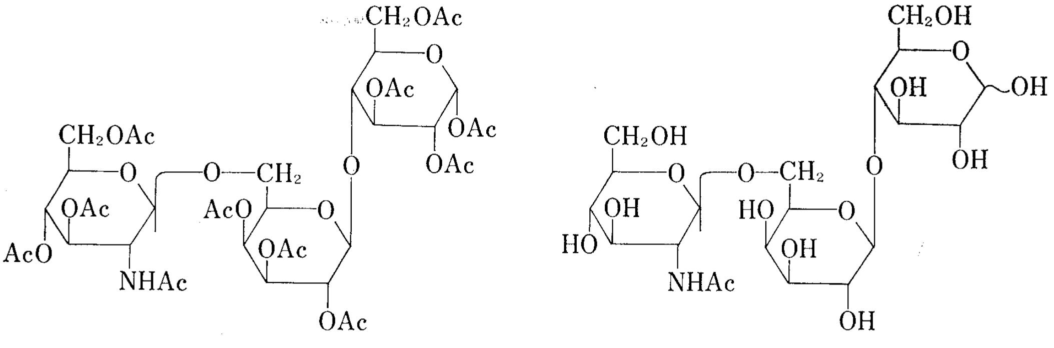
Deacetylation of **15** afforded the title trisaccharide (**16**) as a hygroscopic amorphous powder in 87% yield. Complete acid hydrolysis of **16** resulted in the liberation of glucose, galactose, and N-acetylglucosamine as determined by paper partition chromatography (PPC). PPC of the product of partial acid hydrolysis showed lactose and the title trisaccharide in addition to the above-mentioned monosaccharides.



15 **16**

Chart 2

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 unless otherwise indicated. 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 was used as an internal standard in $CDCl_3$. Chemical shifts are given in the δ scale. A Shimadzu GC-4BPTF gas chromatograph equipped with a hydrogen flame ionization detector was employed for GC analysis. Retention time (t_R) are given in minutes. Mass spectra were measured with a Hitachi M-52 GC-MS spectrometer.

TLC was performed on 0.25 mm precoated silica gel plates (Silica Gel $60F_{254}$, E. Merck, Darmstadt, Germany), using (A), $CHCl_3$–acetone (6: 1, v/v); (B), $CHCl_3$–acetone (2: 1); (C), $CHCl_3$–MeOH (6: 1); (D) 70% 2-PrOH–AcOEt (2: 1). Detection was effected with H_2SO_4 or by UV irradiation (short wavelength). Column chromatography was performed on a column of Wakogel C-200 (Wako Pure Chemical Industries, Ltd., Osaka) with 1 g of sample to be separated per 20 g of adsorbent. PPC was performed on Toyo Filter Paper No. 50 (Toyo Roshi, Ltd., Tokyo) by the ascending method[17] using BuOH–pyridine–H_2O (6: 4: 3, v/v) and detection was effected with alkaline silver nitrate.[18]

1,6-Anhydro-6′-O-trityl-β-lactose (6′-Trityllactosan) (2)——1,6-Anhydro-β-lactose[19] (1) (5.7 g, 17.6 mmol, powdered and dried over $CaCl_2$ *in vacuo*) was treated with freshly distilled dry pyridine (50 ml). Recrystallized trityl chloride (12 g, 43.1 mmol) was added and the mixture was stirred at room temperature (28°) for 2 hr. The solution was poured into ice-H_2O (1 l) and extracted with CH_2Cl_2 (3 × 150 ml). The combined extracts were washed with ice-H_2O (100 ml), cold 10% H_2SO_4 (3 × 100 ml), and ice-H_2O (3 × 100 ml), dried over $MgSO_4$, and then concentrated to give a hygroscopic amorphous powder which was crystallized from benzene–acetone. Recrystallization from EtOH gave fine colorless needles (6 g, 60.2%), mp 131—132°, $[\alpha]_D^{24}$ −48.7° (c=1.42, acetone); NMR $\delta_{\text{ppm}}^{\text{acetone-}d_6}$: 7.20—7.40 (15H, m, aromatic protons); TLC: *Rf* 0.20 (solvent C). *Anal.* Calcd. for $C_{31}H_{34}O_{10}\cdot H_2O$: C, 63.69; H, 6.21. Found: C, 63.95; H, 6.42.

2,2′,3,3′,4′-Penta-O-acetyl-1,6-anhydro-6′-O-trityl-β-lactose (3)——Compound 2 (1.2 g, 2.1 mmol) was acetylated with Ac_2O (7 ml) and pyridine (10 ml) for 14 hr at room temperature. The solution was poured into ice-H_2O (200 ml) and extracted with CH_2Cl_2 (3 × 30 ml). The combined extracts were washed with ice-H_2O (30 ml), satd. $NaHCO_3$ (3 × 30 ml), and ice-H_2O (3 × 30 ml), dried over $CaCl_2$, and evaporated to dryness by repeated co-distillation with toluene. Crystallization from EtOH gave fine needles (1.4 g, 93.3%), mp 191—192°, $[\alpha]_D^{24}$ −49° (c=1.45, $CHCl_3$): NMR $\delta_{\text{ppm}}^{CDCl_3}$: 1.87, 1.99, 2.03 (15H, each s, OAc × 5), 7.20—7.40 (15H, m, aromatic protons); TLC: *Rf* 0.36 (solvent A). *Anal.* Calcd. for $C_{41}H_{44}O_{11}$: C, 63.40; H, 5.71. Found: C, 63.43; H, 5.47.

1,6-Anhydro-2,2′,3,3′,4′-penta-O-benzyl-β-lactose (4)——Compound 2 (3 g, 5.3 mmol) was dissolved in distilled benzyl chloride (22 ml), and powdered KOH (13 g) was added. The mixture was heated in an oil bath with vigorous stirring at 120° for 4 hr. After cooling to room temperature, the mixture was poured

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into ice–H_2O (1 l), stirred overnight, and then extracted with CH_2Cl_2 (3×100 ml). The combined extracts were washed with ice-H_2O (100 ml), cold 10% H_2SO_4 (3×100 ml), ice-H_2O (100 ml), cold satd. $NaHCO_3$ (3×100 ml), and ice-H_2O (3×100 ml), dried over $MgSO_4$, and concentrated to afford a syrup. This was dissolved in 80% (v/v) AcOH (60 ml), and the solution was stirred at 100° for 2 hr. After cooling to room temperature, the solution was evaporated to dryness by repeated co-distillation with toluene. The residue was dissolved in CH_2Cl_2 (50 ml), and the solution was washed with ice-H_2O (3×40 ml), dried over $MgSO_4$, and evaporated to dryness. A solution of the residue in CH_2Cl_2 (10 ml) was purified on a column of silica gel, eluting with $CHCl_3$–acetone (10: 1, v/v). Removal of the solvent from the fraction having *Rf* 0.33 on TLC (solvent A) afforded an amorphous powder which was crystallized from EtOH. Recrystallization from EtOH gave white needles (2.86 g, 69.8%), mp 122—123°, $[\alpha]_D^{18}$ $-42.9°$ ($c=1.05$, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3520 (OH); NMR $\delta_{ppm}^{CDCl_3}$: 7.24—7.36 (25H, m, aromatic protons); TLC: *Rf* 0.33 (solvent A). *Anal.* Calcd. for $C_{47}H_{50}O_{10}$: C, 72.85; H, 6.50. Found: C, 72.61; H, 6.56.

O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose (6)——A mixture of **4** (2.35 g, 3 mmol), the oxazoline 5[10] (1.25 g, 3.8 mmol), and a catalytic amount of *p*-toluenesulfonic acid in a mixture of dry nitromethane (15 ml) and dry toluene (20 ml) was heated with stirring at 95° for 1 hr. After cooling to room temperature, the acid was neutralized with a few drops of pyridine. The solution was evaporated to a brown syrup, which was dissolved in CH_2Cl_2 (5 ml) and purified on a column of silica gel, eluting with $CHCl_3$–acetone (10: 1, v/v). Removal of the solvent from the fraction having *Rf* 0.22 on TLC (solvent A) gave an amorphous powder (1.95 g, 58.5%), $[\alpha]_D^{23}$ $-13.1°$ ($c=2.82$, $CHCl_3$); NMR $\delta_{ppm}^{CDCl_3}$: 1.88, 1.98 (12H, each s, OAc$\times$3, NAc), 5.91 (1H, d, $J=7.5$ Hz, NH), 7.22, 7.24, 7.27 (25H, m, aromatic protons); TLC: *Rf* 0.22 (solvent A). *Anal.* Calcd. for $C_{61}H_{69}NO_{18}$: C, 66.35; H, 6.30; N, 1.27. Found: C, 66.16; H, 6.29; N, 1.22.

O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (7)——A solution of **6** (1.52 g, 1.4 mmol) in MeOH (40 ml) was hydrogenated over a Pd catalyst at room temperature under atmospheric pressure until absorption of H_2 ceased; the catalyst was freshly prepared from $PdCl_2$ (0.7 g) according to the method of Schmidt and Staab.[20] After removal of the catalyst by filtration, the filtrate was evaporated to an amorphous powder (847 mg, 94.1%), $[\alpha]_D^{21}$ $-39.2°$ ($c=1.05$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (br, OH, NH); NMR $\delta_{ppm}^{pyridine\text{-}d_5}$: 2.00, 2.02, 2.16 (12H, each s, OAc$\times$3, NAc), 9.01 (1H, d, $J=8$ Hz, NH); TLC: *Rf* 0.26 (solvent C). *Anal.* Calcd. for $C_{26}H_{39}NO_{18}\cdot H_2O$: C, 46.50; H, 6.15; N, 2.09. Found: C, 46.47; H, 5.98; N, 2.49.

O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose (8)——A solution of **6** (973 mg, 0.88 mmol) in dry MeOH (10 ml) was treated with methanolic 0.5 N sodium methoxide (1 ml), and the mixture was stirred at room temperature for 2 hr; complete deacetylation was confirmed by TLC (solvent C). Dry Amberlite IR-120 (H^+) resin was added to neutralize the solution, which was then filtered. The filtrate was evaporated to dryness to afford an amorphous powder (786 mg, 91.2%), $[\alpha]_D^{22}$ $-10.9°$ ($c=0.38$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3390 (br, OH, NH); NMR $\delta_{ppm}^{pyridine\text{-}d_6}$: 2.05 (3H, s, NAc) 7.16—7.50 (25H, m, aromatic protons), 8.78 (1H, d, $J=8$ Hz, NH); TLC: *Rf* 0.48 (solvent C). *Anal.* Calcd. for $C_{55}H_{63}NO_{15}\cdot H_2O$: C, 66.32; H, 6.58; N, 1.41. Found: C, 66.62; H, 6.31; N, 1.38.

O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (9)——A solution of **8** (68 mg, 0.07 mmol) in MeOH (10 ml) was hydrogenated over a Pd catalyst and the mixture was treated as described for **7** to afford a hygroscopic amorphous powder (30.9 mg, 84.3%), $[\alpha]_D^{24}$ $-36.6°$ ($c=1.55$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (br, OH, NH); NMR $\delta_{ppm}^{pyridine\text{-}d_5}$: 2.17 (3H, s, NAc), 8.86 (1H, d, $J=6$ Hz, NH); TLC: *Rf* 0.66 (solvent D). *Anal.* Calcd. for $C_{20}H_{33}NO_{15}$: C, 45.54; H, 6.31; N, 2.66. Found: C, 45.77; H, 6.59; N, 2.86.

Deacetylation of 7 (54 mg, 0.05 mmol) in dry MeOH (5 ml) with methanolic 0.5 N sodium methoxide (0.5 ml) as described for **8** also gave **9** (38.5 mg, 88.5%).

O-[2-Deoxy-2-(N-methylacetamido)-3,4,6-tri-O-methyl-β-D-glucopyranosyl]-(1→6)-O-(2,3,4-tri-O-methyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-methyl-β-D-glucopyranose (10)——A suspension of NaH (133 mg, previously defatted with petr. ether) in dry DMSO (5 ml) was stirred at 80° for 2 hr under N_2 to furnish a solution of DMSO carbanion.[11] A solution of **9** (130 mg, 0.25 mmol) in dry DMSO (7 ml) was treated with the prepared carbanion. The mixture was stirred under N_2 at room temperature for 2 hr, treated with CH_3I (7 ml), and stirred for a further 14 hr in the dark. The solution was poured into ice-H_2O (100 ml) and extracted with AcOEt (3×15 ml). The combined extracts were washed with ice-H_2O (3×20 ml), cold $Na_2S_2O_3$ (3×20 ml), and ice-H_2O (3×20 ml), dried over Na_2SO_4, and then concentrated to a yellow syrup. A solution of the syrup in $CHCl_3$ (3 ml) was purified on a column of silica gel, eluting with $CHCl_3$–acetone (5: 1, v/v). Removal of the solvent from the fraction having *Rf* 0.17 on TLC (solvent B) afforded a syrup (52.7 mg, 31.9%), $[\alpha]_D^{21}$ $+14.7°$ ($c=0.34$, $CHCl_3$); NMR $\delta_{ppm}^{CDCl_3}$: 2.10 (3H, s, NAc), 3.06 (3H, s, NMe), 3.38—3.64 (24H, m, OMe$\times$8), 4.30 (1H, d, $J=6$ Hz, 1″-H, β-GlcNAc), 4.62 (1H, d, $J=6$ Hz, 1′-H, β-Gal), 5.42 (1H, s, 1-H, β-Glc); TLC: *Rf* 0.17 (solvent B).

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GC Analysis of the Acid Hydrolysate of 10——A glass column (2 m × 4 mm) packed with Gas-Chrom Q (80—100 mesh), coated with 2% OV-17 was used. The instrument was heated at 180° with a flow of 42 ml/min of N_2 passing through the column.

A mixture of **10** (2 mg) and 3 N HCl (0.6 ml) in a sealed tube was heated at 100° for 3 hr, then evaporated to dryness; traces of HCl were completely removed by repeated co-distillation with EtOH. The residue in H_2O (2 ml) was hydrogenated with $NaBH_4$ (20 mg) in H_2O (2 ml) with stirring at room temperature for 3 hr. Excess $NaBH_4$ was destroyed with AcOH, then the solution was evaporated to dryness. The residue was acetylated with Ac_2O (1 ml) and pyridine (1 ml), left overnight, and then concentrated by repeated co-distillation with EtOH and toluene to afford a syrup, which was dissolved in $CHCl_3$ and subjected to GC analysis. The product gave three peaks of methylated alditol acetates, (a), (b), and (c). They were assigned to 1,5,6-tri-O-acetyl-2,3,4-tri-O-methylgalactitol (**11**), 1,4,5,6-tetra-O-acetyl-2,3-di-O-methylglucitol (**12**), and 1,5-di-O-acetyl-2-deoxy-2-(N-methylacetamido)-3,4,6-tri-O-methylglucitol (**13**), respectively, by comparing their retention times with those of the products of 1,6-anhydro-2,2′,3,3′,4′,6′-hexa-O-methyl-β-lactose[4] and 2-deoxy-2-(N-methylacetamido)-3,4,6-tri-O-methyl-α-D-glucopyranose prepared from benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[14] by permethylation followed by debenzylation. t_R: (a) = 12.20; (b) = 16.80; (c) = 25.20; **11** = 12.20; **12** = 16.80; **13** = 25.20.

GC-MS of 10 and the Acid Hydrolysate of 10——A glass column (1.5 m × 3 mm) packed with Gas-Chrom Q (80—100 mesh), coated with 2% OV-17 was used. Conditions for recording mass spectra were as follows: injection temp., 215°; column temp., 150°.

Compound **10** was treated as described for GC analysis to prepare a sample for GC-MS. MS *m/e* (%) of **10** and methylated alditol acetates obtained by hydrolysis of **10** were as follows; **10**: 56 (11.5), 64 (10.7), 71 (29.5), 72 (19.7), 75 (23.8), 87 (82.3), 88 (49.2), 98 (24.6), 100 (18.9), 101 (27.1), 102 (34.4), 117 (53.3), 129 (69.7), 142 (100), 182 (9.8), 217 (6.6), 218 (6.6), 233 (7.4), 256 (6.6); (a): 43 (30.5), 57 (10.2), 87 (17.0), 99 (27.1), 101 (30.5), 117 (42.4), 129 (23.7), 149 (100), 159 (6.8), 161 (10.2), 173 (4.2), 189 (8.5), 205 (5.9), 223 (6.8), 233 (5.1); (b): 43 (87.0), 85 (30.4), 87 (17.4), 99 (36.2), 101 (49.3), 111 (10.1), 117 (100), 127 (44.9), 129 (13.0), 143 (10.9), 159 (13.0), 161 (14.5), 187 (8.7), 201 (14.5), 231 (4.4), 261 (33.3); (c): 43 (15.0), 45 (6.3), 74 (15.0), 87 (7.5), 99 (13.8), 116 (100), 129 (30.0), 142 (35.0), 145 (45.0), 158 (70.0), 161 (20.0), 202 (32.5), 205 (37.5).

O-(2-Acetamido-3,4,6-tri-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 (14)——Compound **7** (341 mg, 0.52 mmol) was acetylated with Ac_2O (3 ml) and pyridine (3 ml) at room temperature for 14 hr. The mixture was treated as described for **3** to afford an amorphous powder which was crystallized from EtOH. Recrystallization from EtOH gave fine needles (403 mg, 89.6%), mp 179—181°, $[\alpha]_D^{25}$ −30.3° (c = 1, $CHCl_3$); NMR $\delta_{ppm}^{CDCl_3}$: 1.91, 1.95, 1.99, 2.06, 2.12, 2.15 (27H, each s, OAc × 8, NAc), 6.19 (1H, d, J = 8 Hz, NH); TLC: *Rf* 0.33 (solvent A). *Anal.* Calcd. for $C_{36}H_{49}NO_{23}$: C, 50.06; H, 5.72; N, 1.62. Found: C, 50.09; H, 5.58; N, 1.93.

O-(2-Acetamido-3,4,6-tri-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-α-D-glucopyranose (15)——Compound **14** (144 mg, 0.17 mmol) was dissolved in acetolysis mixture (4 ml, 1: 70: 30, v/v, H_2SO_4–Ac_2O–AcOH) at 0° and the mixture was allowed to stand at 0° for 2 hr. The solution was poured into ice-H_2O (50 ml) and extracted with CH_2Cl_2 (3 × 10 ml). The combined extracts were washed with ice-H_2O (10 ml), satd. $NaHCO_3$ (3 × 10 ml), and ice-H_2O (3 × 10 ml), and dried over Na_2SO_4. The solvent was removed to give an amorphous powder which was dissolved in $CHCl_3$ (2 ml) and purified on a column of silica gel, eluting with $CHCl_3$–acetone (3: 1, v/v). Removal of the solvent from the fraction having *Rf* 0.30 on TLC (solvent B) gave an amorphous powder (70 mg, 46.7%). Crystallization from MeOH–2-PrOH gave colorless needles (42 mg, 27.8%), mp 175—176°, $[\alpha]_D^{21}$ +17.6° (c = 0.48, $CHCl_3$); NMR $\delta_{ppm}^{CDCl_3}$: 1.96, 2.04, 2.07, 2.08, 2.13, 2.16 (33H, each s, OAc × 10, NAc), 5.92 (1H, d, J = 8 Hz, NH), 6.25 (1H, d, J = 3 Hz, 1-H, α-Glc): TLC: *Rf* 0.30 (solvent B). *Anal.* Calcd. for $C_{40}H_{55}NO_{26}$: C, 49.74; H, 5.74; N, 1.45. Found: C, 49.46; H, 5.58; N, 1.47.

O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (16)——A solution of **15** (42 mg, 0.04 mmol) in dry MeOH (10 ml) was treated with methanolic 0.5 N sodium methoxide (1 ml), and the mixture was treated as described for **8** to afford a hygroscopic amorphous powder (20 mg, 87%), $[\alpha]_D^{20}$ −6.8° (c = 1.48, MeOH); TLC: *Rf* 0.57 (solvent D); PPC: *Rf* 0.16. *Anal.* Calcd. for $C_{20}H_{35}NO_{16}$: C, 44.04; H, 6.47; N, 2.57. Found: C, 44.02; H, 6.29; N, 2.76.

PPC of the Acid Hydrolysate of 16——1) Complete Hydrolysis: A mixture of **16** (5 mg) and 0.1 N HCl (2 ml) in a sealed tube was heated at 100° for 3 hr, then evaporated to dryness. Traces of HCl were completely removed by repeated co-distillation with EtOH. The residue was dissolved in a small amount of H_2O, and subjected to PPC; N-acetylglucosamine (*Rf* 0.49), glucose (*Rf* 0.39), and galactose (*Rf* 0.36) were identified.

2) Partial Hydrolysis: A mixture of **16** (7 mg) and 0.1 N HCl (2 ml) in a sealed tube was heated at 100° for 1 hr, and treated as described above to afford a hydrolysate, in which N-acetylglucosamine, glucose, galactose, lactose (*Rf* 0.23), and **16** (*Rf* 0.16) were identified by PPC.

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