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A chemical synthesis of UDP-LacNAc and its regioisomer for finding 'oligosaccharide transferases'

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Abstract A chemical synthesis of uridine 5'-diphospho-*N*-acetyllactosamine (Galβ(1→4)GlcNAc-UDP; UDP-LacNAc) and Galβ(1→3)GlcNAc-UDP is described. Coupling of the disaccharide imidate derivatives with dibenzylphosphate gave the corresponding 1-phosphates, which were condensed with UMP-imidazolate to give the target UDP-oligosaccharides after purification by anion exchange HPLC and gel filtration column chromatography. Using this methodology a variety of oligosaccharide nucleotide analogues can be synthesized. These UDP-oligosaccharides may be useful for finding so-called 'oligosaccharide transferases', the glycosyltransferases which transfer the oligosaccharide moiety onto glycosyl acceptors.

Keywords UDP-oligosaccharides · UDP-LacNAc · Glycosyltransferases

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Introduction

The biosynthesis of poly- or oligosaccharides is generally performed stepwise by glycosyltransferases using sugar nucleotides such as UDP-GlcNAc, GDP-Man, CMP-Neu5Ac, etc. as glycosyl donors (Figure 1). In 1960's, some UDP-oligosaccharides were isolated and characterized as elongated UDP-*N*-acetylglucosamine (UDP-GlcNAc) derivatives. For example, Galβ(1-4)GlcNAc-UDP (UDP-*N*-acetyllactosamine) and Neu5Acα(2-6)Galβ(1-4)GlcNAc-UDP were isolated from human milk [1] and goat colostrum [2], respectively (Figure 2). However, either their biosynthetic pathways or biological functions have not yet been elucidated, due to their limited availability. We guess that these oligosugar nucleotides may be the substrates of novel oligosaccharide transferases which can transfer the oligosaccharide unit to the suitable glycosyl acceptors in one step. In order to verify this conjecture, not only the naturally occurring UDP-oligosaccharides but also a wide variety of their analogues are required. The enzymatic synthesis of UDP-*N*-acetyllatosamine using galactosyltransferase [3] or galactosidase [4] has already been reported, however these methods are limited to natural substrates only. To address this issue, we directed to find out the synthetic methodology which allows the synthesis of a series of oligosugar nucleotides and its analogues. Toward this goal, we report here a chemical synthesis of type-2 sugar nucleotide UDP-*N*-acetyllactosamine (UDP-LacNAc) (**12**), and its type-1 regioisomer Galβ(1→3)GlcNAc-UDP (**22**) which may serve as the useful substrates for the novel glycosyltransferases.

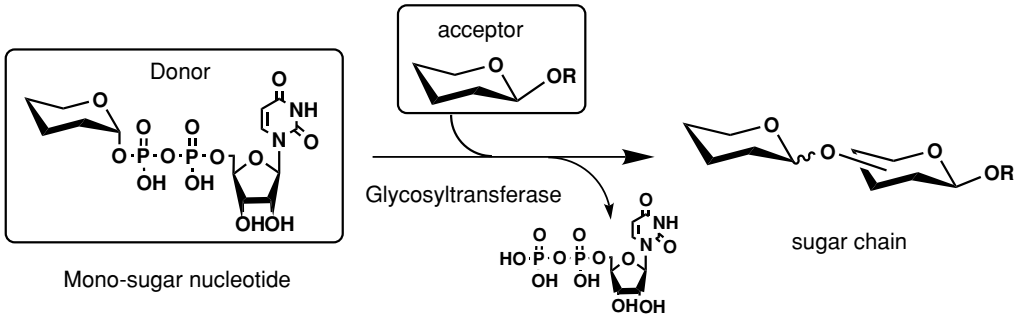


Fig. 1 Biosynthesis of sugar chain.

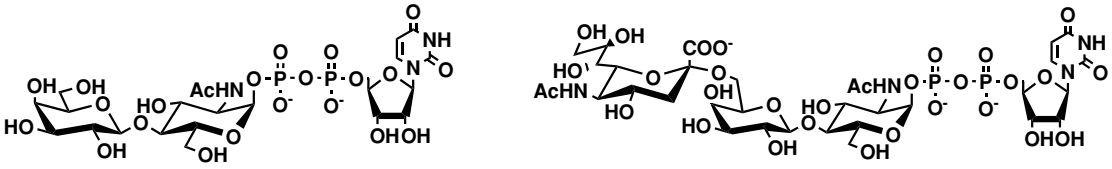


Fig. 2 UDP-oligosaccharides from human milk and goat colostrum.

Results and discussion

Synthesis of Galβ(1→4)GlcNAc-UDP (UDP-LacNAc)

For the synthesis of the UDP-LacNAc, the fully protected LacNAc-1-phosphate derivative (**9**) was prepared as a key compound.

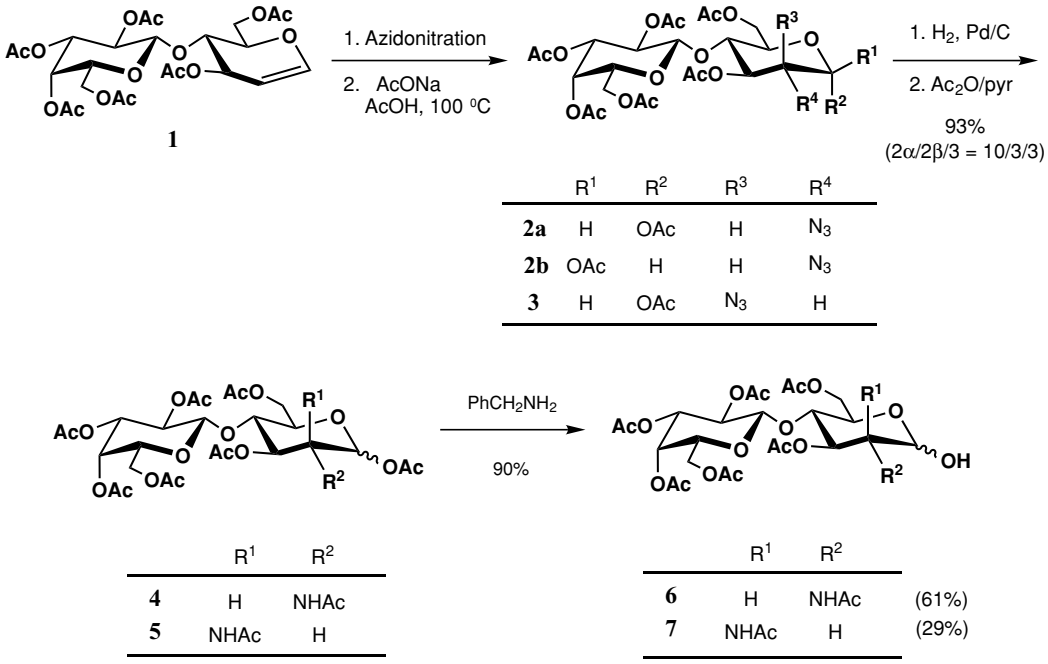
Azidonitration [5–7] of hexa-*O*-acetyllactal (**1**) followed by nitro group replacement with an acetoxy group by treatment with AcONa in AcOH at 100°C gave 2-azidolactose derivative (**2a**, **2b**) and its 2-epimer (**3**) in 61 % (**2a/2b/3** = 10/3/3 determined by ^{1}H-NMR). These compounds could not be separated by column chromatography on silica gel. Reduction of the azido group in **2a**, **2b** and **3** and subsequent *N*-acetylation with acetic anhydride in pyridine gave an inseparable mixture of **4** and **5** in 93 % yield. Selective removal of the *O*-acetyl group at the anomeric positions in **4** and **5** with benzylamine gave the 1-hydroxy compounds **6** and **7** in 61 % and 29 % yields, respectively. These diastereomers **6** and **7** were easily separated by column chromatography on silica gel (Scheme 1).

The treatment [8] of **6** with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2 at 0°C gave the imidate (**8**) in 88 % yield, which was coupled [9,10] with dibenzylphosphate in CH_2Cl_2 for 1 day at room temperature to afford the fully protected LacNAc-1-phosphate derivative (**9**) in 51 % yield. Significant signals in the ^{1}H-NMR spectrum of **9** were the one-proton doublet at δ4.49 ppm ($J_{1,2}$ = 7.9 Hz, H-1 of Gal) and one-proton doublet of doublets at δ 5.61 ppm ($J_{1,2}$ = 3.2 Hz, $^3J_{1,P}$ = 5.9 Hz, H-1 of GlcNAc), and the signal in the ^{31}P-NMR of **9** was a singlet at δ -1.89 ppm, indicating that the newly formed linkage between the LacNAc moiety and phosphoric acid moiety was α.

Reductive removal of the benzyl groups in **9** using 10 % palladium on activated carbon at room temperature gave **10** in 96 % yield. Condensation of **10** with UMP-imidazolate (**11**), which was prepared by Whitesides's procedure [11], in dry pyridine at room temperature and subsequent de-*O*-acetylation [12] in MeOH/H_2O/Et_3N (7/3/1) for 24 h at room temperature afforded 3-monoacetylated UDP-LacNAc as a main product. For removing the acetyl group at hindered position, de-*O*-acetylation was carried out in MeOH/H_2O/Et_3N (7/3/3) for 24 h at 35°C and followed by purification by anion-exchange column HPLC (Hamilton RCX-10, eluent: 0.3 M $HCOONH_4$) and column chromatography on Sephadex G-10 to give the UDP-LacNAc (**12**) as an ammonium salt in 77 % yield (Scheme 2). The significant signals in the ^{1}H-NMR spectrum of **12** were the one-proton doublet of doublets at δ 5.35 ppm ($J_{1,2}$ = 3.4 Hz, $^3J_{1,P}$ = 6.9 Hz, H-1 of GlcNAc), one-proton doublet at δ 5.79 ppm ($J_{1,2}$ = 4.8 Hz, H-1 of Rib), one-proton doublet at δ 5.80 ppm ($J_{5,6}$ = 8.2 Hz, H-5 of Ura) and one-proton doublet at δ 7.84 ppm ($J_{5,6}$ = 8.2 Hz, H-6 of Ura). The signals in the ^{31}P-NMR of **12** were two doublets at δ −10.8 and −12.6 ppm ($J_{P,P}$ = 19.7 Hz), indicating the assigned structure.

Synthesis of Galβ(1→3)GlcNAc-UDP

As previously described in the case of UDP-LacNAc, the fully protected Galβ(1→3)GlcNAc-1-phosphate derivative (**20**) was prepared as a key compound.

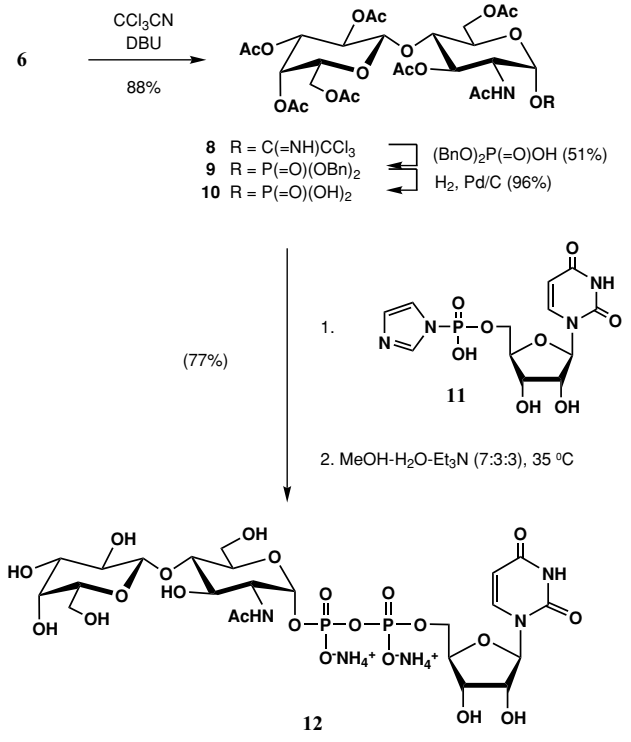


	R^1	R^2	R^3	R^4
2a	H	OAc	H	N_3
2b	OAc	H	H	N_3
3	H	OAc	N_3	H

	R^1	R^2
4	H	NHAc
5	NHAc	H

	R^1	R^2	
6	H	NHAc	(61%)
7	NHAc	H	(29%)

Scheme 1 Preparation of Galβ(1→4)GlcNAc derivative.

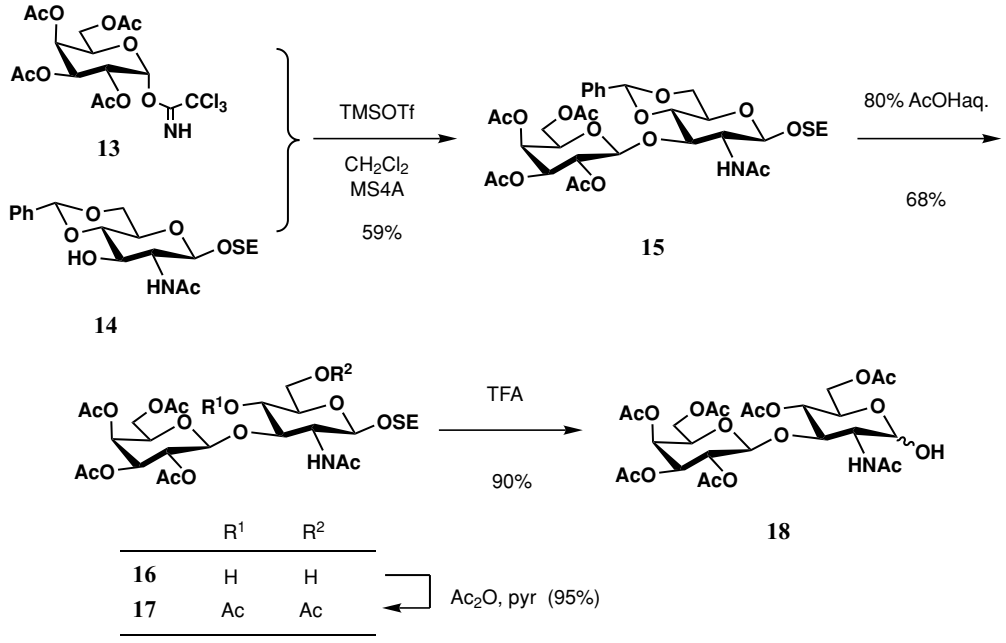


Scheme 2 Synthesis of Galβ(1→4)GlcNAc-UDP; UDP-LacNAc.

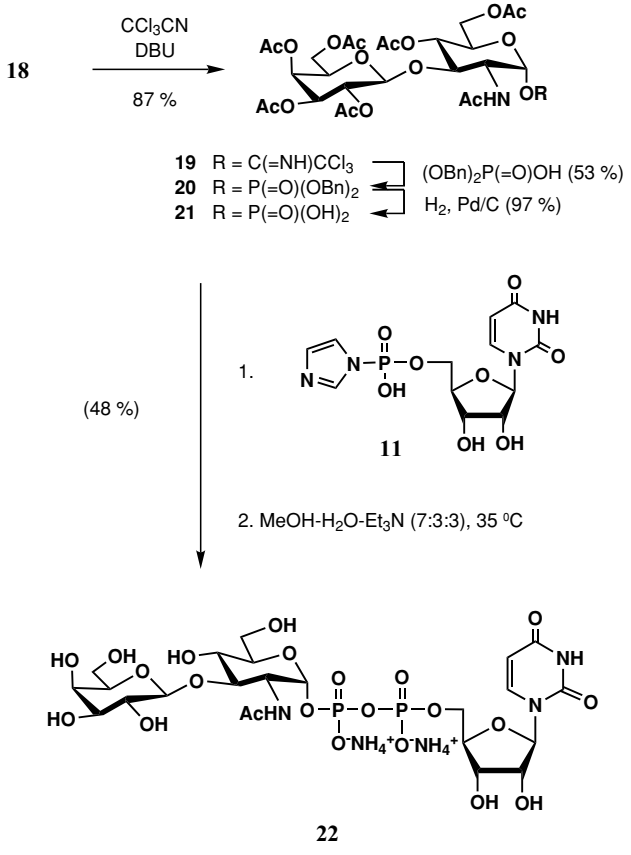
Coupling of the donor (**13**) [13] and the suitably protected acceptor (**14**) [14] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and powdered molecular sieves 4A gave the disaccharide (**15**) in 59 % yield. Deprotection of benzylidene group in **15** with 80 % aq. AcOH at 60°C gave **16** in 68 % yield, and the following acetylation of **16** afforded **17** in 95 % yield. Selective removal of the 2-(trimethylsilyl)ethyl (SE) group in **17** with trifluoroacetic acid (TFA) imparted **18** in 90 % yield (Scheme 3).

The synthesis of UDP-disaccharide (**22**) followed by phosphorylation was carried out in a manner similar to UDP-LacNAc (**12**). Compound **18** was transformed into the corresponding imidate (**19**) and successively coupled with dibenzylphosphate in 1,2-dichloroethane for 2.5 h at 45°C to give the entirely protected Galβ(1→3)GlcNAc-1-phosphate derivative (**20**) in 53 % yield. The newly formed α linkage between the disaccharide moiety and phosphoric acid moiety was confirmed by significant signals in the ^{1}H-NMR and ^{31}P-NMR spectrum. Reductive removal of the benzyl groups in **20** gave **21** in 97 % yield. Condensation of **21** with UMP-imidazolate (**11**) in dehydrated pyridine at room temperature and subsequent de-*O*-acetylation in MeOH/H_2O/Et_3N (7/3/3) for 24 h at 35°C produced the Galβ(1→3)GlcNAc-UDP (**22**) as an ammonium salt in 77 % yield after purification in the same way as described for **12** (Scheme 4). The significant signals in the ^{1}H-NMR spectrum of **22** were the one-proton doublet of doublets at δ 5.35 ppm ($J_{1,2} = 3.4$ Hz, $^3J_{1,P} = 7.6$ Hz, H-1 of GlcNAc), one-proton doublet at δ 5.82 ppm ($J_{1,2} = 5.5$ Hz, H-1 of Rib), one-proton doublet at δ 5.81 ppm ($J_{5,6} = 8.2$ Hz, H-5 of Ura) and one-proton doublet at δ 7.81 ppm ($J_{5,6} = 8.2$ Hz, H-6 of Ura). The signals in the ^{31}P-NMR of **22** were two doublets at δ -11.0 and -12.8 ppm ($J_{P,P} = 19.7$ Hz) indicating the assigned structure.

In conclusion, we have established the efficient procedure for the synthesis of two UDP-oligosaccharides. By extending the synthetic strategy, many kinds of UDP-oligosaccharides can be synthesized in order to search for the so-called 'oligosaccharide transferases' which transfer



Scheme 3 Preparation of Galβ(1→3)GlcNAc derivative.



Scheme 4 Synthesis of Galβ(1→3)GlcNAc-UDP.

oligosaccharide moieties onto an acceptor structure. We believe that complex glycoconjugates, such as gangliosides or glycoproteins could be synthesized using only a few enzymes if the oligosaccharide transferases are discovered.

Materials and methods

General procedures

Specific rotations were determined with a JASCO DIP-360 polarimeter at 25°C. ^{1}H NMR spectra were recorded with JEOL JNM-EX-400 (400 MHz) or JEOL JNM-ECA-600 (600 MHz) spectrometer. ^{13}C and ^{31}P NMR spectra were recorded with JEOL JNM-EX-400 at 100 MHz (^{13}C) or 162 MHz (^{31}P) respectively. ESI-TOF MS was recorded on Mariner^{TM}. HPLC was carried out on a HITACHI instrument: L-6200 intelligent pump and L-4250 UV-VIS detector on an anion exchange column HAMILTON RCX-10.7 μm (ϕ 4.1 mm×250 mm). TLC was performed on Silica gel 60 F_{254} (Merck) with detection by UV and/or by charring with sulfuric acid. Column chromatography on silica gel (Kanto Chemical, silica-gel 60N, spherical, neutral, 40–50 μm) was accomplished with the solvent systems (v/v) specified. All concentrations and evaporations were conducted *in vacuo*. The uridine 5'-monophosphate disodium salt was purchased from Wako Pure Chemical Industries, Ltd.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2- acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranose (**6**) and (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-mannopyranose (**7**)

A mixture of **2**α, **2**β, and **3** (508 mg, 749 μmol) in AcOEt (7 mL)-CH_2Cl_2 (1 mL)-AcOH (1 mL) was added to a suspension of 10 % Pd/C (0.45 g) in AcOEt (3 mL). Hydrogen gas was bubbled for 22 h at room temperature, and the

reaction mixture was filtered. The filtrate was concentrated to a residue, then treated with acetic anhydride (2 mL) in pyridine (4 mL) overnight at room temperature, and worked up. The products were extracted with CH_2Cl_2 and successively washed with 2 M aq. HCl, saturated aq. $NaHCO_3$, and brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on a column of silica gel (AcOEt) to give a mixture of **4** and **5**, which was then treated with benzylamine (0.23 mL, 2.1 mmol) in THF (5 mL) for 1 day at room temperature. The reaction mixture was concentrated to one third of volume, and extracted with CH_2Cl_2. The organic layer was washed with 2 M aq. HCl and water, dried over anhydrous Na_2SO_4, and concentrated. The products were chromatographed on a column of silica gel (AcOEt) to give compound **6** (270 mg, 57 %) and compound **7** (128 mg, 27 %, $\alpha/\beta = 0.79/0.21$) as colorless amorphous solids.

Compound **6**: $R_f = 0.24$ (AcOEt); $[\alpha]_D = +19.8^\circ$ (c 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.97 (s, 3H, NAc), 2.01, 2.05, 2.07, 2.08, 2.11, 2.15 (6s, 18H, OAc), 3.77 (t, 1H, $J_{3,4} = 9.5$ Hz, H-4), 3.88 (t, 1H, H-5′), 4.01-4.17 (m, 3H, H-5, H-6′a, H-6′b), 4.25 (td, 1H, $J_{1,2} = 3.9$ Hz, $J_{2,3} = 10.2$ Hz, $J_{2,\ NH} = 9.8$ Hz, H-2), 4.43 (d, 1H, $J_{1',2'} = 7.9$ Hz, H-1′), 4.53 (d, 1H, $J_{1,OH} = 4.1$ Hz, OH), 5.09 (dd, 1H, $J_{2',3'} = 10.5$ Hz, $J_{3',4'} = 3.0$ Hz, H-3′), 5.10 (dd, 1H, $J_{1',2'} = 7.9$ Hz, $J_{2',3'} = 10.5$ Hz, H-2'), 5.20 (t, 1H, $J_{1,OH} = 4.1$ Hz, $J_{1,2} = 3.9$ Hz, H-1), 5.37 (d, 1H, $J_{3',4'} = 3.0$ Hz, H-4′), 5.54 (t, 1H, $J_{2,3} = 10.2$ Hz, $J_{3,4} = 9.5$ Hz, H-3), 6.76 (d, 1H, $J_{2,NH} = 9.8$ Hz, NH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 20.5, 20.7, 20.8, 20.9, 21.0, 22.9, 43.8, 52.0, 60.6, 62.1, 66.5, 68.2, 69.6, 70.3, 70.6, 71.1, 75.8, 76.7, 77.0, 77.3, 91.4, 100.0, 127.4, 127.7, 128.6, 169.8, 169.9, 170.2, 170.3, 170.7, 170.8, 170.9; HRMS (ESI-TOF MS.): Calcd for $C_{23}H_{38}NO_{17}$ *m/z* $[M+H]^+$: 636.2134, found: 636.2174.

Compound **7**: $R_f = 0.15$ (AcOEt);

α **form**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.98 (s, 3H, NAc), 2.04, 2.051, 2.057, 2.063, 2.14, 2.17 (6s, 18H, OAc), 3.69 (m, 1H, H-5), 3.73–3.78 (m, 1H, H-4), 3.87–3.90 (m, 1H, H-5′), 4.01–4.05 (m, 1H, H-6a′), 4.17–4.23 (m, 4H, H-5, H-6a, H-6b′, OH), 4.35–4.38 (m, 1H, H-6b), 4.55–4.59 (m, 2H, H-2, H-1′), 4.97–5.00 (m, 1H, H-3'), 5.11–5.16 (m, 2H, H-1, H-2′), 5.34–5.35 (m, 1H, H-4), 5.39 (dd, 1H, H-3), 5.72 (d, 1H, NH),

β **form**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.98 (s, 3H, NAc), 2.051, 2.057, 2.063, 2.09, 2.13, 2.16 (6s, 18H, OAc), 3.69 (m, 1H, H-5), 3.73–3.78 (m, 1H, H-4), 3.87–3.90 (m, 1H, H-5′), 4.01–4.05 (m, 1H, H-6a′), 4.17–4.23 (m, 1H, H-6a), 4.35–4.38 (m, 1H, H-6b), 4.55–4.59 (m, 1H, H-1′), 4.63–4.64 (m, 1H, H-2), 4.82 (m, 1H, OH), 4.97–5.00 (m, 2H, H-1, H-3′), 5.07 (dd, 1H, H-3), 5.11–5.16 (m, 1H, H-2′), 5.34–5.35 (m, 1H, H-4), 5.91 (d, 1H, NH),

^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 20.56, 20.61, 20.64, 20.67, 20.91, 20.94, 21.0, 50.8 (α-C-2), 51.7 (β-C-2), 60.9 (α-C-6′, and β-C-6′), 62.8 (α-C-6, and β-C-6), 66.63 (β-C-4′), 66.68 (α-C-4′), 68.6 (α-C-5), 69.2 (α-C-2′, and β-C-2′), 70.0 (α-C-3), 70.5 (α-C-5′, andβ-C-5′), 70.86 (β-C-3′), 70.92 (α-C-3′), 72.3 (β-C-5), 73.1 (β-C-3), 73.9 (β-C-4), 74.6 (α-C-4), 93.3 (α-C-1), 93.4 (β-C-1), 100.9 (α-C-1′, and β-C-1′), 169.27, 169.30, 169.71, 169.80, 170.16, 170.23, 170.37, 170.47, 170.52, 170.6, 172.0, 177.0; HRMS (ESI-TOF MS.): Calcd for $C_{23}H_{38}NO_{17}$ *m/z* $[M+H]^+$: 636.2134, found: 636.2107.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl trichloroacetimidate (**8**)

To a solution of **6** (127 mg, 0.20 mmol) in CH_2Cl_2 (2 mL) were added trichloroacetonitrile (0.20 mL, 2.0 mmol) and DBU (9.0 μL, 60 mmol) at 0°C. The reaction mixture was stirred for 2 h at 0°C, and then concentrated. The product was purified by column chromatography on silica gel (AcOEt) to give compound **8** (137 mg, 88 %) as a colorless amorphous solid. $R_f = 0.52$ (AcOEt); $[\alpha]_D = +54.7^\circ$ (c 1.1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.93 (s, 3H, NAc), 1.97, 2.05, 2.07, 2.11, 2.16 (6s, 18H, OAc), 3.90–3.94 (m, 2H, H-4, H-5′), 4.00 (m, 1H, H-5), 4.10–4.17 (m, 3H, H-6a, H-6′a, H-6′b), 4.42–4.48 (m, 2H, H-2, H-6b), 4.55 (d, 1H, $J_{1',2'} = 7.8$ Hz, H-1'), 4.96 (dd, 1H, $J_{2',3'} = 10.5$ Hz, $J_{3',4'} = 3.4$ Hz, H-3′), 4.14 (dd, 1H, $J_{1',2'} = 7.8$ Hz, $J_{2',3'} = 10.5$ Hz, H-2′), 5.31 (dd, 1H, H-3), 5.36 (d, 1H, H-4′), 5.72 (d, 1H, $J_{2,NH} = 9.0$ Hz, NH), 6.29 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2, 20.5, 20.7, 20.8, 20.9, 23.1, 51.8, 60.4, 60.7, 61.5, 66.5, 69.0, 70.6, 70.8, 70.8, 75.6, 94.5, 101.2, 160.2, 169.0, 169.88, 169.91, 170.0, 170.1, 170.2, 171.0.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl dibenzyl phosphate (**9**)

Dibenzylphosphate (0.83 g, 3.0 mmol) was added to a solution of **8** (233 mg, 0.299 mmol) in CH_2Cl_2 (2 mL) at room temperature. The reaction mixture was stirred for 16 h at room temperature, and diluted with CH_2Cl_2 (10 mL). The organic layer was successively washed with saturated aq. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on a column of silica gel (AcOEt) to give compound **9** (137 mg, 51 %) as a colorless amorphous solid. $R_f = 0.49$ (AcOEt); $[\alpha]_D = +38.6^\circ$ (c 1.6, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.69 (s, 3H, NAc), 1.97, 2.02, 2.04, 2.05, 2.06, 2.16 (6s, 18H, OAc), 3.82 (t, 1H, $J_{3,4} = 9.1$ Hz, H-4), 3.86 (t, 1H, H-5′), 3.94 (ddd, 1H, $J_{4,5} = 10.1$ Hz, $J_{5,6} = 3.7$ Hz, H-5), 4.00 (dd, 1H, $J_{5,6a} = 3.7$ Hz, H-6a), 4.06–4.15 (m, 2H, H-6′a, H-6′b), 4.23–4.31 (m, 2H, H-2, H-6b), 4.49 (d, 1H, $J_{1',2'} = 7.9$ Hz, H-1′), 4.95 (dd, 1H, $J_{2',3'} = 10.3$ Hz, $J_{3',4'} = 3.4$ Hz,

H-3′), 5.11 (dd, 1H, $J_{1',2'} = 7.9$ Hz, $J_{2',3'} = 10.3$ Hz, H-2'), 5.34 (dd, 1H, $J_{3,4} = 9.1$ Hz, H-3), 5.34 (d, 1H, $J_{3',4'} = 3.4$ Hz, H-4′), 5.60–5.64 (m, 2H, H-1, NH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 20.6, 20.7, 20.8, 20.9, 22.8, 51.9, 60.7, 61.3, 66.5, 69.0, 69.8, 69.8, 69.9, 69.9, 70.2, 70.3, 70.6, 70.9, 75.4, 76.7, 77.0, 77.2, 77.3, 96.0, 96.0, 101.0, 128.0, 128.0, 128.7, 128.7, 128.8, 135.2, 169.0, 170.0, 170.0, 170.1, 170.2, 170.7; ^{31}P-NMR (162 MHz, $CDCl_3$): δ (ppm) –1.89 (s, 1P); HRMS (ESI-TOF MS.): Calcd for $C_{40}H_{50}NNaO_{20}P$ *m/z* $[M+Na]^+$: 918.2556, found: 918.2518.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl phosphate (**10**)

A solution of **9** (323 mg, 0.360 mmol) in THF (3 mL) was added to a suspension of 10 % Pd/C (350 mg) in THF (2 mL). After bubbling with hydrogen gas for 5 h at room temperature, triethylamine (0.2 mL) was added to the reaction mixture, and the catalyst was filtered off. The filtrate was concentrated and the residue was lyophilized with 1,4-dioxane to give compound **10** (268 mg, 96 %) as a colorless powder. $R_f =$ 0.21 ($CHCl_3$-MeOH-0.5M aq. $NH_4HCO_3 = 7:3:0.5$); $[\alpha]_D =$ +35.8° (c 0.91, MeOH); ^{1}H-NMR (400 MHz, CD_3OD): δ (ppm) 1.83 (s, 3H, NAc), 1.84, 1.93, 1.95, 1.96, 2.02, 2.03 (6s, 18H, OAc), 3.78 (t, 1H, $J_{3,4} = 9.5$ Hz, H-4), 3.99–4.25 (m, 6H, H-2, H-5, H-6a, H-5′, H-6′a, H-6′b), 4.00 (d, 1H, H-6b), 4.57 (d, 1H, $J_{1',2'} = 7.8$ Hz, H-1'), 4.91 (dd, 1H, $J_{1',2'} =$ 7.8 Hz, $J_{2',3'} = 10$ Hz, H-2′), 5.00 (dd, 1H, $J_{2',3'} = 10$ Hz, $J_{3',4'}$ = 3.2 Hz, H-3'), 5.15 (t, 1H, $J_{3,4} = 9.5$ Hz, H-3), 5.25 (d, 1H, $J_{3',4'} = 3.2$ Hz, H-4′), 5.33 (dd, 1H, $J_{1,P} = 6.3$ Hz, H-1); ^{13}C-NMR (100 MHz, CD_3OD): δ (ppm) 20.5, 20.6, 20.7, 20.8, 21.2, 22.6, 62.3, 63.2, 68.6, 70.5, 70.7, 71.7, 72.5, 72.8, 77.3, 102, 128.1, 128.5, 170.9, 171.3, 171.8, 171.9, 172.0, 172.4, 173; ^{31}P-NMR (162 MHz, $CDCl_3$): δ (ppm) –0.13 (s, 1P); HRMS (negative ion ESI-TOF MS.): Calcd for $C_{26}H_{37}NO_{20}P$ *m/z* $[M-H]^-$: 714.1652, found: 714.1689.

Uridine 5′-diphospho-[β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranose] diammonium salt (**12**)

Uridine 5′-monophosphoimidazolate (**11**) was prepared by Whitesides's procedure.[11]

To a solution of Uridine 5′-monophosphate disodium salt (21.2 mg, 57.6 μmol) in H_2O was added Amberlite IR-120 (H^+ form). After stirring for 30 min, the insoluble materials was filterd, and trioctylamine was added (25 μl, 58 μmol) to neutralize. The residue was co-evaporated with DMF (3 × 1.5 mL), then 1,1′-carbonylbisimidazole (39 mg, 0.24 mmol) and DMF (1.5 ml) was added. After stirring for 20 h, MeOH (7.6 μL, 0.19 mmol) was added to the reaction mixture and stirred for 30 min. The crude product **11** was used in the next step without further purification.

A mixture of compound **10** (16.9 mg, 20.7 μmol) and crude solution of Uridine 5′-monophosphoimidazolate (**11**) in DMF was evaporated and pyridine (1.5 ml) was added. After stirring for 42 h under argon atmosphere at room temperature, the reaction mixture was evaporated. The obtained residue was treated with MeOH-H_2O-Et_3N = 7:3:3 (12 ml) then stirred for 24 h at 35°C. The crude product was purified by anion-exchange HPLC column (Hamilton RCX-10), eluent: 0.3 M $HCOONH_4$) and column chromatography on Sephadex G-10 (eluent: H_2O). Lyophilization of the eluent gave compound **12** (15.9 μmol, 77 %) as a colorless powder. $R_f = 0.32$ ($CHCl_3$-MeOH-0.5M aq. $NH_4HCO_3 = 5:5:1$); $[\alpha]_D = +133°$ (c 0.45, H_2O); ^{1}H-NMR (600 MHz, D_2O): δ (ppm) 1.92 (s, 3H, NAc), 3.42 (dd, 1H, $J_{1',2'} = 7.6$ Hz, H-2'), 3.51 (dd, 1H, $J_{2',3'} = 9.6$ Hz, H-3'), 3.58 (dd, 1H, $J_{5,6a} = 4.1$ Hz, $J_{6a,6b} = 8.2$ Hz, H-6a), 3.61–3.66 (m, 3H, H-3, H-4, H-5′), 3.67 (dd, 1H, $J_{5,6b} = 2.7$ Hz, $J_{6a,6b} = 8.2$ Hz, H-6b), 3.73 (dd, 1H, $J_{5',6a'} = 3.4$ Hz, $J_{6'a,6'b} = 13.1$ Hz, H-6′a), 3.76 (dd, 1H, $J_{5',6'b} = 2.7$ Hz, $J_{6'a,6'b} = 13.1$ Hz, H-6'b), 3.78 (d, 1H, $J_{1',2'} = 3.4$ Hz, H-1'), 3.84 (td, 1H, $J_{5,6a} = 4.1$ Hz, $J_{5,6b} = 2.7$ Hz, H-5), 3.88 (td, 1H, $J_{1,2} = 3.4$ Hz, $J_{2,3} = 10.3$ Hz, $^3J_{2,P}$ = 2.7, H-2), 4.05 (ddd, 1H, H-5a of Rib), 4.13 (m, 1H, H-5b of Rib), 4.14 (m, 1H, H-4 of Rib), 4.20 (m, 2H, H-2b of Rib, H-3b of Rib), 4.30 (d, 1H, $J_{1',2'} = 7.6$ Hz, H-1'), 5.35 (dd, 1H, $J_{1,2} = 3.4$ Hz, $^3J_{1,P} = 6.9$ Hz, H-1), 5.79 (d, 1H, $J_{1,2} =$ 4.8 Hz, H-1 of Rib), 5.80 (d, 1H, $J_{Ura5,Ura6} = 8.2$ Hz, H-5 of Ura), 7.84 (d, 1H, $J_{Ura5,Ura6} = 8.2$ Hz, H-6 of Ura); ^{13}C-NMR (151 MHz, D_2O): δ (ppm) 57.7 (C of Me), 88.8 ($J_{C\text{-}2,P} = 8.7$ Hz, C-2), 95.3 (C-6′), 96.7 (C-6), 100.4 ($J_{C\text{-}5,P} = 4.3$ Hz, C-5 of Rib), 104.3 (C-4′), 104.8 (C-3 of Rib), 105.4 (C-3), 106.6 (C-2'), 107.4 (C-5), 108.3 (C-3′), 109.7 (C-2 of Rib), 111.0 (C-5′), 114.0 (C-4), 118.7 ($J_{C\text{-}4,P} = 10.1$ Hz, C-4 of Rib), 124.6 (C-1 of Rib), 130.0 ($J_{C\text{-}1,P} = 5.8$ Hz, C-1), 138.2 (C-5 of Ura), 138.8 (C-1′), 177.1 (C-6 of Ura), 187.4, 201.9, 210.4; ^{31}P-NMR (243 MHz, D_2O): δ (ppm) –10.8 (d, 1P, J = 19.7 Hz, P of Rib), −12.6 (d, 1P, P of LacNAc); HRMS (negative ion ESI-TOF MS.): Calcd for $C_{23}H_{36}N_3O_{22}P_2$ *m/z* $[M-H]^-$: 768.1271, found: 768.1304.

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**15**)

Molecular sieves 4Åpowder (5.0 g) was added to a solution of compound **13** (1.99 g, 5.22 mmol) and **14** (4.72 g, 9.58 mmol) in CH_2Cl_2 (100 ml) under an argon atmosphere. After stirring for 10 min at room temperature, TMSOTf (0.40 ml, 2.2 mmol) was added to the reaction mixture at 0°C. The reaction mixture was stirred for 12 h at room temperature, and filtered off. The filtrate was diluted with CH_2Cl_2 (50 ml) and the organic layer was successively washed with saturated

aq. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on a column of silica gel (CH_2Cl_2:MeOH = 80:1) to give compound **15** (2.20 g, 59 %) as a colorless amorphous solid. $R_f = 0.70$ (CH_2Cl_2:MeOH = 15:1); $[\alpha]_D = -23.1^\circ$ (c 0.32, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.83-1.00 (m, 2H, $CH_2CH_2SiMe_3$), 1.94 (s, 3H, NAc), 1.95, 1.98, 2.10 (4s, 12H, OAc), 2.96 (d, 1H, $J_{1,2} = 7.1$ Hz, $J_{2,3} = 8.1$ Hz, H-2), 3.55 (m, 3H, H-5, H-6a, $CH_2CH_2SiMe_3$), 3.65 (t, 1H, $J_{3,4} = 8.1$ Hz, $J_{4,5} = 8.3$ Hz, H-4), 3.76 (t, 1H, $J_{4',5'} = 10.3$ Hz, H-5′), 3.91 (m, 2H, H-6'b, $CH_2CH_2SiMe_3$), 4.03 (dd, 1H, $J_{5',6'a} = 7.8$ Hz, $J_{6'a,6'b} = 10.9$ Hz, H-6′a), 4.32 (t, 1H, $J_{6a,6b} = 4.9$ Hz, H-6b), 4.74 (m, 1H, H-3), 4.40 (dd, 1H, $J_{2',3'} = 3.4$ Hz, $J_{3',4'} = 10.5$ Hz, H-3′), 5.14 (dd, 1H, $J_{1',2'} = 7.8$ Hz, $J_{2',3'} = 10.5$ Hz, H-2′), 5.19 (d, 1H, $J_{1,2} = 8.1$ Hz, H-1), (dd, 1H, $J_{4',5'} = 1.0$ Hz, $J_{4',5'} = 3.4$ Hz, H-4′), 5.77(d, 1H, $J_{1,NH} = 6.8$ Hz, NH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) –1.38, 18.1, 20.6, 20.7, 20.8, 23.5, 59.1, 63.0, 77.0, 67.4, 68.7, 69.1, 74.1, 76.2, 77.1, 77.5, 79.7. 80.4, 82.4, 83.7, 99.3, 101.7, 105.7, 112.3, 126.0, 128.2, 129.0, 137.1, 169.8, 169.9, 170.0, 170.1, 170.6; HRMS (ESI-TOF MS.): Calcd for $C_{34}H_{50}NO_{15}Si$ *m/z* $[M+H]^+$: calcd. 740.2944, found: 740.2983.

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**16**)

80 % aq. AcOH (35 ml) was added to the compound **15** (1.62 g, 2.28 mmol) at room temperature. The reaction mixture was stirred for 4 h at 60°C, concentrated, and diluted with CH_2Cl_2 (70 ml). The organic layer was successively washed with saturated aq. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on a column of silica gel (AcOEt) to give compound **16** (1.01 g, 68 %) as a colorless amorphous solid. $R_f = 0.34$ (AcOEt); $[\alpha]_D = +9.66^\circ$ (c 0.58, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 0.83–1.00 (m, 2H, $CH_2CH_2SiMe_3$), 1.96 (s, 3H, NAc), 1.98, 2.05, 2.07, 2.14 (4, 12H, OAc), 2.95 (dd, 1H, $J_{1,2} = 8.3$ Hz, $J_{2,3} = 9.6$ Hz, H-2), 3.99 (ddd, 1H, $J_{4,5} = 4.1$ Hz, $J_{5,6a} = 4.1$ Hz, H-5), 3.47 (t, 1H, $J_{3,4} = 8.3$ Hz, $J_{4,5} = 9.6$ Hz, H-4), 3.54 (m, 1H, $CH_2CH_2SiMe_3$), 3.77 (m, 1H, H-6a), 3.91 (m, 2H, H-6b, $CH_2CH_2SiMe_3$), 4.01 (t, 1H, $J_{4',5'} = 6.9$ Hz, $J_{5',6'a} = 6.2$ Hz, H-5′), 4.11 (m, 2H, H-6′a, H-6′b), 4.43 (t, 1H, $J_{2,3} = 9.6$ Hz, $J_{3,4} = 8.3$ Hz, H-3), 4.56 (dd, 1H, $J_{1',2'} = 7.6$ Hz, H-1'), 4.95 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1), 5.00 (dd, 1H, $J_{2',3'} = 10.4$ Hz, $J_{3',4'} = 3.4$ Hz, H-3′), 5.20 (dd, 1H, $J_{1',2'} = 7.6$ Hz, $J_{2',3'} = 10.4$ Hz, H-2′), 5.36 (d, 1H, $J_{3',4'} = 3.4$ Hz, H-4′), 5.89 (s, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) –1.44, 18.2, 20.52, 20.54, 20.6, 20.83, 20.84, 57.9, 61.6, 62.9, 67.0, 67.4, 69.0, 70.4, 7-.8, 71.1, 75.1, 83.2, 98.5, 101.4, 169.1, 170.0, 170.1, 170.5, 170.7; HRMS (ESI-TOF MS.): Calcd for $C_{27}H_{45}NNaO_{15}Si$ *m/z* $[M+Na]^+$: calcd. 674.2451, found: 674.2455.

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**17**)

To a solution of **16** (1.01 g, 1.55 mmol) in pyridine (2 ml) was added acetic anhydride (15 ml) at room temperature. The reaction mixture was stirred for 24 h at room temperature. And the reaction mixture was diluted with CH_2Cl_2 (10 ml), then washed with 2 M aq. HCl, saturated aq. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on a column of silica gel (Hexane-AcOEt = 1:2) to give compound **17** (1.08 g, 95 %) as a colorless amorphous solid.; $R_f = 0.55$ (AcOEt); $[\alpha]_D = -7.58^\circ$ (c 0.33, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 0.83–1.00 (m, 2H, $CH_2CH_2SiMe_3$), 1.96 (s, 3H, NAc), 2.00, 2.03, 2.05, 2.06, 2.13 (6s, 18H, OAc), 3.12 (td, 1H, $J_{1,2} = 8.2$ Hz, $J_{2,3} = 9.6$ Hz, $J_{2,NH} = 6.9$ Hz, H-2), 3.55 (m, 1H, $CH_2CH_2SiMe_3$), 3.68 (ddd, 1H, $J_{4,5} = 9.6$ Hz, $J_{5,6b} = 4.8$ Hz, H-5), 3.86 (t, 1H, H-5′), 3.92 (m, 1H, $CH_2CH_2SiMe_3$), 4.06–4.12 (m, 3H, H-6a, H-6′a, H-6′b) 4.23 (dd, 1H, $J_{5,6b} = 4.8$ Hz, H-6b), 4.55 (d, 1H, $J_{1',2'} = 7.6$ Hz, H-1′), 4.53 (t, 1H, $J_{2,3} = 9.6$ Hz, $J_{3,4} = 9.6$ Hz, H-3), 4.92 (t, 1H, $J_{3,4} = 9.6$ Hz, $J_{4,5} = 9.6$ Hz, H-4), 4.96 (dd, 1H, $J_{2',3'} = 10.3$ Hz, $J_{3',4'} = 3.4$ Hz, H-3′), 4.97 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1), 5.05 (dd, 1H, $J_{1',2'} = 7.6$ Hz, $J_{2',3'} = 10.3$ Hz, H-2'), 5.33 (d, 1H, $J_{3',4'} = 3.4$ Hz, H-4′), 5.66 (d, 1H, $J_{2,NH} = 6.9$ Hz, NH); ^{13}C-NMR (151 MHz, $CDCl_3$): δ (ppm) –1.43, 18.2, 20.52, 20.54, 20.6, 20.83, 20.84, 23.8, 58.3, 61.0, 62.6, 66.9, 67.3, 69.1, 69.5, 70.6, 71.1, 71.7, 98.4, 100.6, 169.0, 169.4, 170.1, 170.2, 170.4, 170.8; (ESI-TOF MS.): Calcd for $C_{31}H_{50}NO_{17}Si$ *m/z* $[M+Na]^+$: calcd. 736.2843, found: 736.2887.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranose (**18**)

To a solution of **17** (1.01 g, 1.55 mmol) in CH_2Cl_2 (15 ml) was added TFA (10 ml) at room temperature. The reaction mixture was stirred for 1 h at room temperature, then co-evaporated three times with AcOEt (50 ml). The residue was chromatographed on a column of silica gel (AcOEt) to give compound **18** (886 mg, 95 %) as a colorless amorphous solid. $R_f = 0.18$ (AcOEt); $[\alpha]_D = 2.86^\circ$ (c 0.21, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 1.97 (s, 3H, NAc), 2.05, 2.06, 2.07, 2.08, 2.10, 2.14 (6s, 18H, OAc), 3.89 (t, 1H, $J_{5',6'a} = 7.6$ Hz, $J_{5',6'b} = 6.2$ Hz, H-5′), 4.00 (t, 1H, $J_{2,3} = 9.6$ Hz, $J_{3,4} = 9.6$ Hz, H-3), 4.04 (dd, 1H, $J_{5',6'a} = 7.6$ Hz, $J_{6'a,6'b} = 11.0$ Hz, H-6'a), 4.17–4.20 (m, 3H, H-5, H-6a, H-6b), 4.22 (dd, 1H, $J_{5',6'b} = 6.2$ Hz, $J_{6'a,6'b} = 11.0$ Hz, H-6′b), 4.32 (td, 1H, $J_{1,2} = 3.4$ Hz, $J_{2,3} = 9.6$ Hz, H-2), 4.59 (d, 1H, $J_{1',2'} = 7.6$ Hz, H-1′), 5.04 (dd, 1H, $J_{2',3'} = 10.3$ Hz, $J_{3',4'} = 3.4$ Hz, H-3′), 5.04 (t, 1H, $J_{3,4} = 9.6$ Hz, H-4), 5.04 (dd, 1H, $J_{1',2'} = 7.6$ Hz, $J_{2',3'} = 10.3$ Hz, H-2′), 5.17 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 5.35

(d, 1H, $J_{3',4'} = 3.4$ Hz, H-4′), 5.93 (m, 1H, NH); ^{13}C-NMR (151 MHz, $CDCl_3$): δ (ppm) 14.2, 20.6, 20.6, 20.7, 20.8, 20.8, 21.6, 23.4, 52.7, 60.4, 66.8, 67.9, 68.8, 69.0, 70.3, 71.0, 75.8, 76.8, 77.0, 92.0, 101.0, 169.7, 170.0, 170.2, 170.3, 170.4, 170.5, 170.6, 171.1; (ESI-TOF MS.): Calcd for $C_{26}H_{38}NO_{17}$ *m/z* $[M+H]^+$: calcd. 636.2134, found: 636.2172.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl trichloroacetimidate (**19**)

To a solution of **18** (17.0 mg, 26.7 μmol) in CH_2Cl_2 (2 ml) were added trichloroacetonitrile (27 μL, 0.27 mmol) and DBU (1.2 μL, 8.0 μmol) at 0°C. The reaction mixture was stirred for 4 h at 0°C, and concentrated. The residue was chromatographed on a column of silica gel (AcOEt) to give compound **19** (18.0 mg, 87 %) as a colorless amorphous solid. $R_f = 0.55$ (AcOEt); $[\alpha]_D = +34.4°$ (c 0.73, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 1.97 (s, 3H, NAc), 2.01, 2.07, 2.15 (6s, 18H, OAc), 3.95 (t, 1H, $J_{5',6'a} = 6.9$ Hz, $J_{5',6'b} = 6.9$ Hz, H-5′), 3.96 (dd, 1H, $J_{3,4} = 9.6$ Hz, H-4), 4.06 (dd, 1H, $J_{5',6'a} = 6.9$ Hz, $J_{6'a,6'b} = 11.0$ Hz, H-6′a), 4.13 (dd, 1H, $J_{6a,6b} = 13.1$ Hz, H-6a), 4.20 (dd, 1H, $J_{6a,6b} = 13.1$ Hz, H-6b), 4.21 (dd, 1H, $J_{5',6'b} = 6.9$ Hz, $J_{6'a,6'b} = 11.0$ Hz, H-6′b), 4.61–4.65 (m, 1H, H-2), 4.64 (d, 1H, $J_{1',2'} = 7.6$ Hz, H-1′), 5.00 (dd, 1H, $J_{2',3'} = 10.3$ Hz, $J_{3',4'} = 3.4$ Hz, H-3′), 5.05 (dd, 1H, $J_{1',2'} = 7.6$ Hz, $J_{2',3'} = 10.3$ Hz, H-2′), 5.37 (d, 1H, $J_{3',4'} = 3.4$ Hz, H-4′), 5.47 (d, 1H, NH of imidate), 6.24 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1); ^{13}C-NMR (151 MHz, $CDCl_3$): δ (ppm) 20.5, 20.6, 20.65, 20.7, 23.3, 51.8, 61.1, 61.7, 66.7, 68.0, 69.2, 70.3, 70.5, 70.7, 76.2, 90.9, 95.5, 100.9, 160.1, 169.0, 169.5, 169.6, 170.1, 170.2, 170.4, 170.7.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl dibenzyl phosphate (**20**)

Dibenzylphosphate (42 mg, 0.15 mmol) was added to a solution of **19** (10.1 mg, 12.8 μmol) in 1,2-dichloroethane (2 ml) under argon atmosphere at room temperature. The reaction mixture was stirred for 2.5 h under argon atmosphere at 45°C, then added Et_3N (21 μL, 0.15 mmol) at room temperature, and evaporated. The residue was chromatographed on a column of silica gel (AcOEt) to give compound **20** (6.1 mg, 53 %) as a colorless amorphous solid. $R_f = 0.49$ (AcOEt); $[\alpha]_D = +23.8°$ (c 0.8, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.80 (s, 3H, NAc), 1.96, 2.00, 2.02, 2.04, 2.08, 2.14 (6s, 18H, OAc), 3.84 (t, 1H, $J_{2,3} = 10.3$ Hz, $J_{3,4} = 9.6$ Hz, H-3), 3.09 (t, 1H, $J_{5',6'a} = 6.2$ Hz, $J_{5',6'b} = 6.9$ Hz, H-5′), 3.97 (dd, 1H, $J_{6a,6b} = 12.4$ Hz, H-6a), 4.00–4.02 (m, 1H, H-5), 4.08 (dd, 1H, $J_{5',6'a} = 6.2$ Hz, $J_{6'a,6'b} = 11.0$ Hz, H-6′a), 4.10 (dd, 1H, $J_{6a,6b} = 12.4$ Hz, H-6b), 4.18 (dd, 1H, $J_{5',6'a} = 6.2$ Hz, $J_{6'a,6'b} = 11.0$ Hz, H-6′b), 4.41(tt, 1H,, $J_{1,2} = 3.4$ Hz, $J_{2,3} = 10.3$ Hz, $J_{2,NH} = 9.6$ Hz, H-2), 4.48 (d, 1H, $J_{1',2'} = 8.2$ Hz, H-1'), 4.95 (dd, 1H, $J_{2',3'} = 11.0$ Hz, $J_{3',4'} = 3.4$ Hz, H-3′), 4.98 (t, 1H, $J_{3,4} = 9.6$ Hz, H-4), 5.03 (dd, 1H, $J_{1',2'} = 8.2$ Hz, $J_{2',3'} = 11.0$ Hz, H-2′), 5.03–5.12 (m, 4H, CH_2Ph), 5.36 (d, 1H, $J_{3',4'} = 3.4$ Hz, H-4′), 5.54 (dd, 1H, $J_{1,2} = 3.4$ Hz, $^3J_{1,P} = 5.5$ Hz, H-1), 5.65 (d, 1H, $J_{2,NH} = 9.6$ Hz, NH), 7.34–7.42 (m, 10H, Ph); ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 20.5, 20.6, 20.6, 20.7, 23.0, 30.0, 52.1, 61.0, 66.8, 69.9, 70.0, 70.1, 70.1, 70.5, 70.9, 75.2, 97.19, 97.24, 101.0, 128.2, 128.9, 128.9, 129.0, 135.0, 135.0, 135.3, 169.0, 169.5, 169.8, 170.1, 170.3, 170.4, 170.7; ^{31}P-NMR (242MHz, $CDCl_3$): δ (ppm) −2.14 (s, 1P). HRMS (ESI-TOF MS.): Calcd for $C_{40}H_{50}NNaO_{20}P$ *m/z* $[M+Na]^+$: 918.2556, found: 918.2573.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl phosphate (**21**)

A solution of **20** (50.9 mg, 56.9 μmol) in THF (3 ml) was added to a suspension of 10 % Pd/C (53.9 mg) in THF (2 ml). After bubbling with hydrogen gas for 18 h at room temperature, triethylamine (0.2 ml) was added to the reaction mixture, and the catalyst was filtered off. The filtrate was concentrated and the residue was lyophilized with 1,4-dioxane to give compound **21** (39.5 mg, 97 %) as a colorless powder. $R_f = 0.13$ ($CHCl_3$-MeOH-0.5M aq. NH_4HCO_3 = 7:3:0.5); $[\alpha]_D = +21.1°$ (c 0.93, H_2O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 1.82 (s, 3H, NAc), 1.93, 1.95, 1.97, 2.03 (4s, 18H, OAc), 3.95–4.01 (m, 2H, H-3, H-5′), 4.03–4.08 (m, 4H, H-2, H-6a, H-6′a, H-6'b), 4.13–4.17 (m, 2H, H-5, H-6b), 4.16 (d, 1H, $J_{1',2'} = 7.6$ Hz, H-1′), 4.86 (dd, 1H, $J_{1',2'} = 7.6$ Hz, $J_{2',3'} = 10.3$ Hz, H-2′), 4.88 (t, 1H, H-4), 4.97 (dd, 1H, $J_{2',3'} = 10.3$ Hz, $J_{3',4'} = 3.4$ Hz, H-3′), 5.26 (d, 1H, $J_{3',4'} = 3.4$ Hz, H-4′), 5.30 (dd, 1H, $J_{1,P} = 6.9$ Hz, H-1); ^{13}C-NMR (151 MHz, $CDCl_3$): δ (ppm) 20.5, 20.6, 20.7, 20.8, 21.1, 23.1, 22.6, 54.6, 54.6, 62.5, 63.2, 68.8, 69.8, 69.9, 70.5, 71.7, 72.6, 77.3, 95.3, 95.4, 101.9, 171.5, 171.6, 171.6, 171.9, 172.0, 172.6, 173.2; ^{31}P-NMR (243 MHz, $CDCl_3$): δ (ppm) −0.57 (s, 1P). HRMS (negative ion ESI-TOF MS.): Calcd for $C_{26}H_{37}NO_{20}P$ *m/z* $[M-H]^-$: 714.1652, found: 714.1639.

Uridine 5′-diphospho-[β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranose] diammonium salt (**22**)

A mixture of compound **21** (29.5 mg, 36.1 μmol) and crude solution of Uridine 5′-monophosphoimidazolate (**11**) (64.9 μmol) in DMF was evaporated and added pyridine (1.5 ml). After stirring for 24 h under argon atmosphere at room temperature, the reaction mixture was evaporated. The obtained residue was treated with MeOH-H_2O-Et_3N = 7:3:3 (16 ml) then stirred for 36 h at 35°C. The crude product was puri-

fied by anion-exchange HPLC column (Hamilton RCX-10), eluent: 0.3 M $HCOONH_4$) and column chromatography on Sephadex G-10 (eluent: H_2O). Lyophilization of the eluent gave compound **22** (17.4 μmol, 48 %) as a colorless powder. $R_f = 0.33$ ($CHCl_3$-MeOH-0.5M aq. NH_4HCO_3 = 5:5:1); $[\alpha]_D = +21.1^\circ$ (c 0.93, H_2O); ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 1.91 (s, 3H, NAc), 3.35 (dd, 1H, $J_{1',2'} = 7.6$ Hz, $J_{2',3'} = 9.6$ Hz, H-2′), 3.48 (t, 1H, $J_{3,4} = 9.6$ Hz, H-4), 3.50 (dd, 1H, $J_{2',3'} = 9.6$ Hz, $J_{3',4'} = 3.4$ Hz, H-3′), 3.55–3.63 (m, 3H, H-5′, H-6′a, H-6′b), 3.66 (dd, 1H, H-6a), 3.71–3.72 (m, 1H, H-6b), 3.76 (d, 1H, $J_{3',4'} = 3.4$ Hz, H-4′), 3.78–3.81 (m, 1H, H-5), 3.83 (t, 1H, $J_{3,4} = 9.6$ Hz, H-3), 4.00–4.05 (m, 2H, H-2, H-5a of Rib), 4.07–4.10 (m, 1H, H-4 of Rib), 4.12–4.13 (m,1H, H-4 of Rib), 4.20–4.22 (m, 2H, H-2 of Rib, H-3 of Rib), 4.32 (d, 1H, $J_{1',2'} = 7.6$ Hz, H-1′), 5.35 (dd, 1H, $^3J_{1,P} = 7.6$ Hz, H-1), 5.81 (d, 1H, $J_{Ura5,Ura6} = 8.2$ Hz, H-5 of Ura), 5.82 (d, 1H, H-1 of Rib), 7.81 (d, 1H, $J_{Ura5,Ura6} = 8.2$ Hz, H-6 of Ura); ^{13}C-NMR (151 MHz, D_2O): δ (ppm) 57.8 (C of Me), 88.2 ($J_{C\text{-}2,P} = 8.7$ Hz, C-2), 96.0 (C-6′), 96.6 (C-6), 100.6 ($J_{C\text{-}5,P} = 5.8$ Hz, C-5 of Rib), 103.9 (C-4), 104.2 (C-4′), 105.3 (C-3 of Rib), 106.4 (C-2′), 108.2 (C-3), 108.4 (C-5), 109.5 (C-2 of Rib), 110.8 (C-5′), 115.8 (C-3), 118.9 ($J_{C\text{-}4,P} = 8.7$ Hz, C-4 of Rib), 124.1 (C-1 of Rib), 130.3 ($J_{C\text{-}1,P} = 5.8$ Hz, C-1), 138.3 (C-5 of Ura), 139.2 (C-1′), 177.3 (C-6 of Ura), 187.5, 201.9, 210.5; ^{31}P-NMR (243 MHz, D_2O): δ (ppm) –11.0 (d, 1P, $J = 19.7$ Hz, P of Rib), −12.8 (d, 1P, P of GlcNAc); HRMS (negative ion ESI-TOF MS.): Calcd for $C_{23}H_{36}N_3O_{22}P_2$ m/z $[M\text{-}H]^-$: 768.1271, found: 768.1312.

References

1. Kobata, A.: The acid-soluble nucleotide of milk. II. Isolation and identification of two novel uridine nucleotide oligosaccharide conjugates from human milk and colostrums. J. Biochem. **53**, 167–75 (1963)
2. Jourdian, G.W., Shimizu, F., Roseman, S.: Isolation of nucleotide-oligosaccharide containing sialic acid. Fed. Proc. **20**, 161 (1961)
3. Elling, L., Zerrosen, A., Gallego, R.G., Nieder, V., Malissard, M., Benger, E.G., Vliegenhart, J.F.G., Karmerling, J.P.: UDP-*N*-Acetyl-α-glucosamine as acceptor substrate of β-1,4-galactosyltransferase. Enzymatic synthesis of UDP-*N*-acetyllactosamine, Glycoconjugate J. **16,** 327–36 (1999).
4. Zervosen, A., Nieder, V., Gallego, R.G., Karmerling, J.P., Vliegenthart, J.F.G., Elling, L.: Synthesis of nucleotide-activated oligosaccharides by beta-galactosidase from Bacillus circulans. Biol. Chem. **382**, 299–311 (2001)
5. Lemieux, R.U., Ratcliffe, R.M.: The azidonitration of tri-*O*-acetyl-D-galactal. Can. J. Chem. **57**, 1244–51 (1979)
6. Arnap, J., Lönngren, J.: Synthesis of a tri-, a penta-, and a hepta-saccharide containing terminal *N*-acetyl-β-D-lactosaminyl residues, part of the 'complex-type' carbohydrate moiety of glycoproteins. J C S Perkin I 2070–4 (1981)
7. Li, Q., Li, Z.-J., Li, H., Cai, M.-S.: Studies on carbohydrates XXXII – Chemoselective synthesis of mannosamine glycosides through non-regioselective azidonitration of lactal. Carbohydr Lett. **3,** 349–54 (1999)
8. Schmidt, R.R., Michel, J.: Facile synthesis of α- and -β-*O*-glycosyl imidates; preparation of glycosides and disaccharides, Angew Chem. Int. Ed. Engl. **19**, 731–2 (1980)
9. Schmidt, R.R., Stumpp, M.: Glycosylphosphate aus glycosyl(trichloracetimidaten). Liebigs Ann. Chem. 680–91 (1984)
10. Hoch, M., Heinz, E., Schmidt, R.R.: Synthesis of 6-deoxy-6-sulfo-α-D-glucopyranosyl phosphate. Carbohydr. Res. **191**, 21–28 (1989)
11. Simon, E.S., Grabowski, S., Whitesides, G.M.: Convenient synthesis of cytidine 5′-triphosphate, guanosine 5′-triphosphate, and uridine 5′-triphosphate and their use in the preparation of UDP-glucose, UDP-glucuronic acid, and GDP-mannose. J. Org. Chem. **55**, 1834–41 (1990)
12. Yuasa, H., Palcic, M.M., Hindsgaul, O.: Synthesis of the carbocyclic analog of uridine 5′-(α-D-galactopyranosyl diphosphate) (UDP-Gal) as an inhibitor of $\beta(1\rightarrow4)$-galactosyltransferase. Can. J. Chem. **73**, 2190–5 (1995)
13. Ren, T., Liu, D.: Synthesis of targetable cationic amphiphiles. Tetrahedron Lett. **40**, 7621–5 (1999)
14. Kiso, M., Ishida, H.: Preparation of sialic acid-containing oligosaccharide derivatives having antagonism against myelin-binding proteins, Kokai-Tokkyo-Koho, Heisei 10-251299 [in Japanese]