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Novel Aspects of Interaction between UDP-Gal and GlcNAc β-1,4-Galactosyltransferase: Transferability and Remarkable Inhibitory Activity of UDP-(mono-*O*-methylated Gal), UDP-Fuc and UDP-Man†

Tsuyoshi Endo,[a] Yasuhiro Kajihara,[b] Hisashi Kodama[b] and Hironobu Hashimoto[a,*]

[a]*Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama, 226 Japan*

[b]*Life Science Research Laboratory, Japan Tobacco Inc., 6-2 Umegaoka, Midori-ku Yokohama, 227 Japan*

Abstract—Four mono-*O*-methylated and one mono-*O*-acetylated UDP-D-Gal analogues and UDP-L-Fuc were synthesized. 2-*O*-Methyl-D-galactose residue was enzymatically transferred to give 2′-*O*-methyllactosaminide in high yield. UDP-Fuc and UDP-Man showed potent inhibitory activities against β-1,4-galactosyltransferase. Structural requirement and steric allowance for the ground and transition states of the enzyme reaction were discussed. Copyright © 1996 Elsevier Science Ltd

Introduction

Understanding of the mechanism of glycosyl transfer catalysed by glycosyltransferase may provide a way to obtain tailor-made oligosaccharide by controlling the biosynthesis. Although various glycosyltransferases participating in the biosynthesis of oligosaccharide chain have been already cloned,[1] none of their structures has yet been revealed by X-ray analysis. Very recently, the crystal structure of a recombinant β-glucosyltransferase characterized as a DNA modifying enzyme was reported.[2] Thus, even the active site of the most extensively studied enzyme, UDP-Gal: GlcNAc-OR β-1,4-galactosyltransferase (β-1,4-GalT) [EC 2.4.1.90] has scarcely been visualized.[3] Since the first structural features for both substrates, i.e. UDP-Gal and GlcNAc-OR, were reported by Berliner et al.,[4,5] the flexibility of this enzyme prompted many research groups to investigate substrate recognition for both the donor and acceptor sites.[6] For the Gal moiety of the former, more than 10 enzymatically transferable analogues or homologues such as deoxy analogues,[4,7–11] an amino analogue[12] at the 2-position, stereoisomers[4,12] at the 4-position, and the 5-thio analogue[13] have been reported.

In order to design bisubstrate analogues constructed from UDP-Gal and GlcNAc-OR analogues for elucidation of the binding arrangement of the two substrates based on the reported substrate specificities including our previous work,[10,14] we became interested in a series of mono-*O*-methylated analogues **1**–**4** and a mono-*O*-acetylated analogue **5**, which may provide the information about the steric allowance for the linker arm and the necessity of hydrogen donating or accepting property of each hydroxyl group of the Gal moiety. In addition, UDP-Fuc **6** and UDP-Man **7** have a structural resemblance to UDP-Gal as depicted in their structural formulae (see Fig. 1) and are interesting analogues for the inspection of the further functional allowance of the donor moiety. This paper describes the synthesis of UDP-Gal derivatives **1**–**5** and UDP-Fuc **6**. The transferability as well as inhibitory activity of these analogues together with UDP-Man **7** were examined.

Results and Discussion

Mono-*O*-methylated analogues **1**–**4** of UDP-Gal were synthesized by coupling uridine monophosphate (UMP) and the mono-*O*-methylated D-galactose-1-phosphates (Gal-1P), **13**, **19**, **25** and **30**, respectively. The diphosphate linkages between UMP and Gal-1P analogues were formed by activation of one of the coupling partners as the corresponding imidazolide with *N,N′*-carbonyldiimidazole. Protected Gal-1P derivatives **12**, **18**, **24** and **29** were derived from allyl 3,4-*O*-isopropylidene-6-*O*-trityl-α-D-galactopyranoside[15] (**8**) as shown in Scheme 1, allyl α-D-galactopyranoside[16] (**15**) in Scheme 2, allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside[17] in Scheme 3, and 1,2,3,4-tetra-*O*-acetyl-6-*O*-methyl-D-galactopyranose[18] (**27**) in Scheme 4, respectively. Phosphorylation of the anomeric hydroxyl group using butyl lithium and dibenzyl phosphorochloridate[19] gave the desired dibenzyl α-glycosyl phosphate in moderate yields (34–49%), where the β-anomers were formed up to 12%. The Gal-1P analogues **12**, **18** and **29** were converted by catalytic hydrogenolysis followed by coupling with *N,N′*-carbonyldiimidazole to the corre-

†Part of this work was presented at 17th International Carbohydrate Symposium, July 1994, Ottawa, Canada, Abstract p. 331.

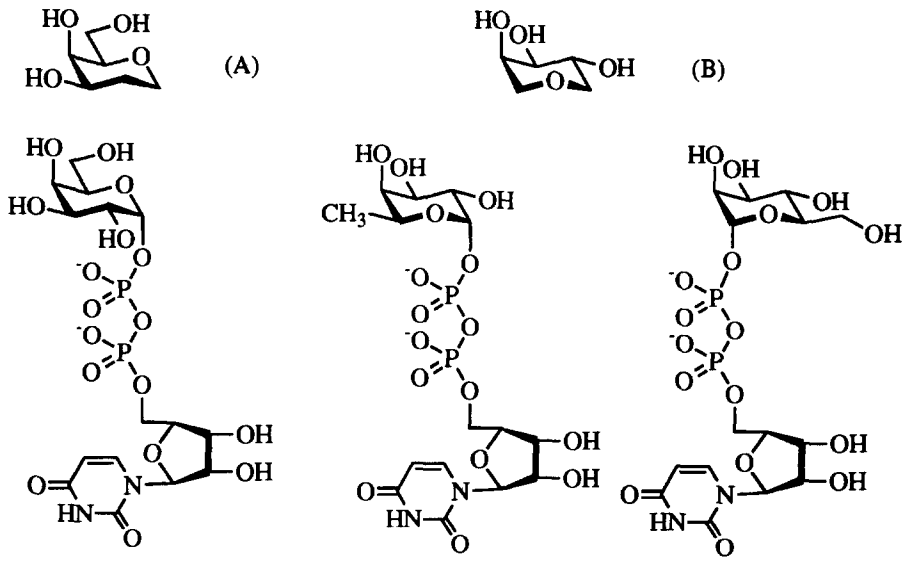
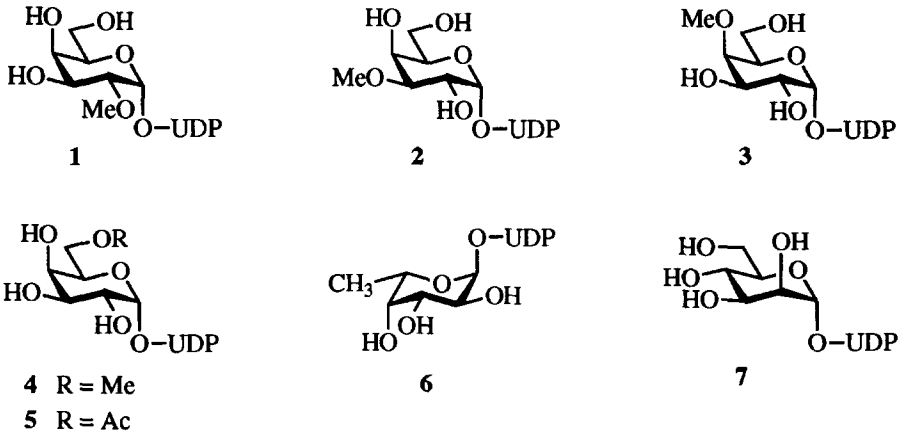
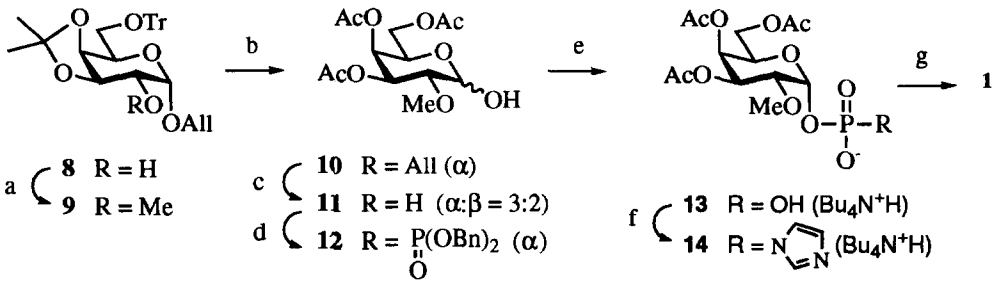


Figure 1. Alternative structure unit (B) of UDP-Fuc and UDP-Man effective for the binding as well as that (A) of UDP-Gal.

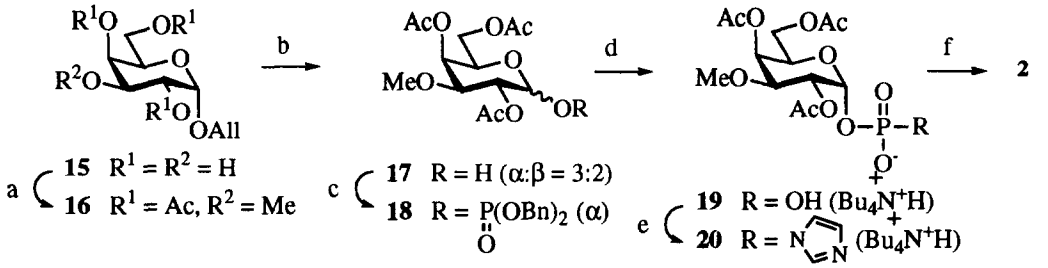
sponding imidazolides, **14**, **20** and **31**, respectively, which were coupled with UMP to give mono-*O*-methylated UDP-Gal analogues **1** and **2**, as disodium salts, and **4** as diammonium salt in 10–22% yields in three steps (Method A).

In the case of unprotected 4-*O*-Me Gal-1P (**25**), UMP was activated as the imidazolide **26**, which was coupled with **25** to give **3** as the disodium salt in 11% yield in two steps (Method B). According to the same coupling strategy, the 6-*O*-acetyl analogue **5** of UDP-Gal was also prepared from 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-galactose via 6-*O*-acetyl-Gal-1P (**34**).





Scheme 1. (a) MeI, NaH/DMF, 97%; (b) (1) 70% AcOH, 60 °C, (2) Ac_2O/pyridine, 93.5% (2 steps); (c) (1) Pd–C/MeOH, 60 °C, (2) $HgCl_2$, HgO/acetone–H_2O, 79% (2 steps); (d) BuLi, $(BnO)_2POCl$/THF, −78 °C α-anomer 34%, β-anomer 10%; (e) H_2, Pd–C, Bu_3N/MeOH; (f) *N*,*N*′-carbonyldimidazole/DMF; (g) (1) UMP·Bu_3N/DMF, (2) Et_3N–MeOH–H_2O, 14% from **12**.



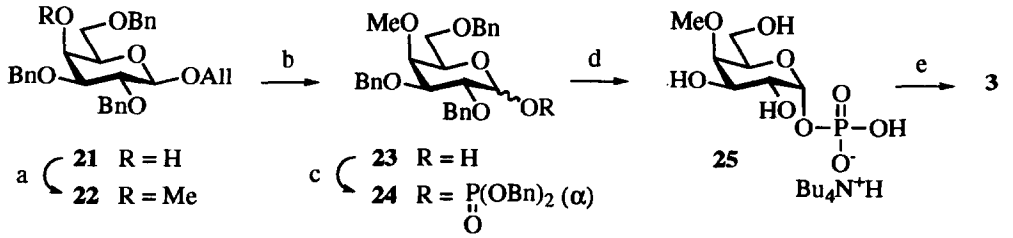
Scheme 2. (a) (1) Bu_2SnO/MeOH, reflux, (2) MeI, Bu_4NI/toluene, reflux, (3) Ac_2O/pyridine, 71% (3 steps); (b) (1) Pd–C/MeOH, 60 °C, 2) $HcCl_2$, HgO/acetone–H_2O, 64%; (c) BuLi, $(BnO)_2POCl$/THF, −78 °C, 41%; (d) H_2, Pd–C, Bu_3N/MeOH; (e) *N,N'*-carbonyldimidazole/DMF; (f) (1) UMP·Bu_3N/DMF, (2) Et_3N–MeOH–H_2O, 10% from **18**.

UDP-L-Fucose (**6**) was synthesized from 2,3,4-tri-*O*-acetyl-L-fucose via the known dibenzyl α-glycosyl phosphate[20] (**35**) by Method A.

Enzymatic glycosyl transfer of the synthesized donor analogues **1**–**6** and commercially available UDP-Man **7** using β-1,4-GalT was evaluated by measuring UDP,[21] which was liberated on glycosyl transfer using methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (GlcNAcβ-OMe) as an acceptor, after its conversion to uridine by treatment with alkaline phosphatase. Only the 2-*O*-methyl analogue **1** was transferred with a low relative rate (0.20% in comparison with UDP-Gal). A 10 μmol scale enzymatic glycosylation of GlcNAcβ-OMe with **1** (1.5 equiv), β-1,4-GalT (1.5 U), and alkaline phosphatase (100 U) at 37 °C for 38 h gave 2′-*O*-methyllactosaminide (**36**) in 91% yield. The newly formed glycosidic linkage was confirmed to be β→4 by ^{1}H NMR spectroscopy using the HMBC technique. Thus the enzymatic preparation described here is promising for the large scale preparation of **36**, which is useful for the assay of fucosyltransferase.[22]

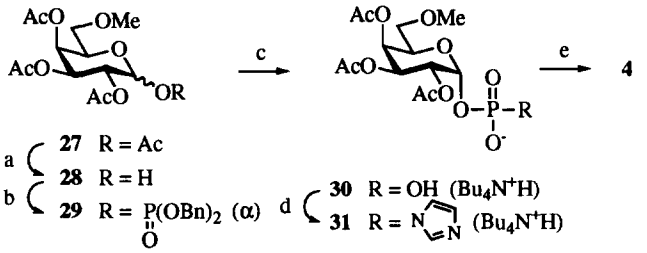
In order to assess the binding affinity of each donor analogue, an inhibition assay at 25 and 50 μM of the donor analogue was carried out using radioactive UDP-Gal and GlcNAcβ-OMe as previously reported.[14,23] The fractional inhibitions together with estimated apparent K_i values are summarized in Table 1. The strong inhibition by 2-*O*-methyl analogue **1** is rationalized by its high affinity to β-1,4-GalT and the extremely low transfer rate. This shows a distinct contrast to the activity of UDP-6-deoxy-Gal having a comparatively higher relative transfer rate (Table 1), whilst no inhibitory activity was observed at a concentration of 100 μM. Three other analogues, 3-OMe **2**, 6-OMe **4**, and 6-O-Ac **5**, seem to have almost the same moderate affinities to the enzyme, although the last two analogues showed no inhibition at a concentration of 25 μM. The discrepancy between the activities at 25 and 50 μM observed for **4** and **5**, remains to be answered and seems to be related to the stability of the diphosphate linkage. In the presence of 10 mM Mn^{2+} ion, 2-OMe analogue **1** and UDP-Man **7** are stable for 24 h, while 4-OMe **3** and 6-OAc **5** are reduced by half after 24 h and the others vanish completely during the same period. It is noteworthy that remarkable inhibitions, i.e. much more potent than the hitherto best inhibitor UDP,[24] were observed in the cases of UDP-Fuc **6** and UDP-Man **7**.

These results suggest the essential structural requirements of the glycosyl donor (UDP-Gal) for the ground state (recognition step) and the transition state with conformational change of the enzyme (glycosyl transfer step). For the ground state ES complex, the nucleotide moiety is essential for the donor binding as deduced from the low K_i value of UDP itself. The appended hexopyranose residue contributes only slightly and the following allowances are made for the functional groups and in the steric bulkiness. (1) The relatively strong extra-bindings due to the appended hexose were observed for UDP-Fuc and UDP-Man. This indicates that the upper half of the galactosyl residue including the hydroxyl groups at C-4, C-6 and ring oxygen as depicted in Figure 1(A) could be substituted with one axial and two equatorial hydroxyl groups as shown in Figure 1(B) for UDP-Fuc and UDP-Man. (2) The very weak inhibitory activity of the 4-OMe analogue **3** indicates a restricted steric allowance around the 4-hydroxyl group. (3) The relative positioning between



Scheme 3. (a) MeI, NaH/DMF, 73.5%; (b) (1) *t*-BuOK/DMSO, 60 °C, (2) $HgCl_2$, HgO/acetone–H_2O, quant. (2 steps); (c) BuLi, $(BnO)_2POCl$/THF, −78 °C 45%; (d) H_2, Pd–C, Bu_3N/MeOH; (e) UMP–imidazolide (**26**)/DMF, 11% from **24**.

the diphosphate group and the hexose moiety. Furthermore, as the transfer reaction proceeds, the binding of hexose moiety becomes crucial, as reflected in the relative rates of transfer. The following characteristics deserve comment. (4) For the glycosyl transfer, the hydroxyl group at the C-2 position is not prerequisite as indicated by the previous work.[6] This position has an allowance in the functional groups as well as in the steric bulkiness. (5) The C-3 hydroxyl group seems to be important as a hydrogen acceptor for the glycosyl transfer, which is supported by the fact that the relative transfer rate of UDP-3-deoxy-Gal is extremely low, while the 3-OMe analogue **2** can also bind to the active site, showing a little steric allowance for the binding. (6) Substitution of hydroxyl groups at C-3 and C-6 has a serious effect on the glycosyl transfer step, i.e. cleavage and formation of the C—O bond at the anomeric center, indicating less steric allowance for this step.



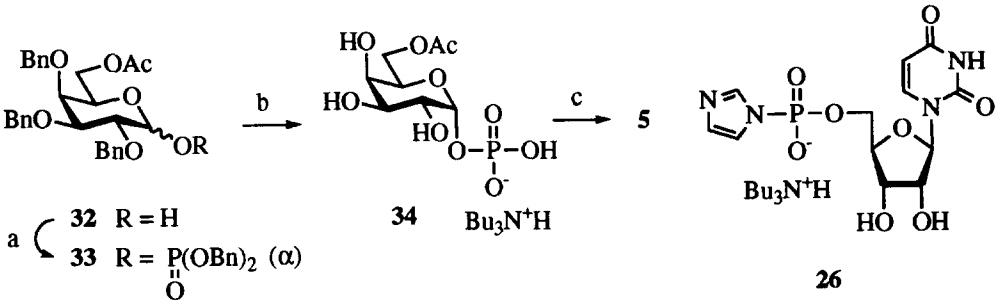
Scheme 4. (a) H_2NNH_2 AcOH, 60 °C, (b) BuLi, $(BnO)_2POCl$/THF, −78 °C 49%; (c) H_2, Pd–C, Bu_3N/Me; (d) *N,N'*-carbonyldimidazole/DMF; (e) 1) UMP·Bu_3N/DMF, 2) Et_3N–MeOH–H_2O, 22% from **29**.

In conclusion, among mono-*O*-methyl analogues of UDP-Gal only the 2-*O*-methyl one can be transferred enzymatically, while the 2-*O*-methyl and 3-*O*-methyl analogues inhibit the galactosyl transfer significantly. Very strong inhibition by UDP-Fuc as well as by UDP-Man was also found. The results suggest a new aspect for the structural requirement for the glycosyl transfer of β-1,4-GalT.

Experimental

General

Melting points were measured with a Yanagimoto MP apparatus and were uncorrected. All ^1H and ^{13}C NMR spectra except those for compound **36** were recorded with a JEOL EX-270 spectrometer at 270 and 67.8 MHz, respectively, for sols in $CDCl_3$ (internal Me_4Si) or in D_2O (^1H, HOD: δ 4.81; ^{13}C, external 1,4-dioxane: δ 67.4). ^1H and ^{13}C NMR spectra of **36** were recorded



Scheme 5. (a) *t*-BuLi, $(BnO)_2POCl$/THF, −78 °C 35%; (b) H_2, Pd–C, Bu_3N/MeOH; (c) UMP–imidazolide (**26**)/DMF, 14% from **24**.

Table 1. The fractional inhibition at 25 μM and estimated K_i values of UDP-Gal analogues and other UDP-sugars

Inhibitor	Inhibitory activity $[I] = 25$ μM	K_i[a] (μM)	Relative transfer rate of UDP-monodeoxy-Gal UDP-Gal = 100 (Ref.)	
UDP-2-OMe-Gal (**1**)	51%	20	90	(8)
UDP-3-OMe-Gal (**2**)	31%	46	0.16	(9)
UDP-4-OMe-Gal (**3**)	7%	270	5.5	(4)
UDP-6-OMe-Gal (**4**)	(48%)[b]	44	1.6	(10)
UDP-6-OAc-Gal (**5**)	(48%)[b]	44		
UDP-Fuc (**6**)	67%	10		
UDP-Man (**7**)	70%	8.8		
UDP	30%	48[c]		

UDP-Gal: 3 μM, GlcNAc; 500 μM.

[a] Apparent K_i values estimated assuming the inhibition is competitive and calculated from the equation $i = [I]/\{[I] + K_i(1 + [S]/K_m)\}$, where i is the fractional inhibition, $[I]$ the inhibitor concentration, and $[S]$ the substrate concentration. Among the reported K_m values[4,8] for UDP-Gal 13.7 μM[4] was used for the estimation considering the assay conditions.

[b] Inhibitory activity at $[I] = 50$ μM.

[c] A reported K_i value for *N*-acetyllactosamine formation reaction (competitive inhibition).

with Bruker AM-500 spectrometer at 500 and 125.8 MHz, respectively, for solution in D_2O. ^{31}P NMR were recorded at 109.25 MHz (JEOL EX-270) for solutions in D_2O (external 85% phosphoric acid). All chemical shifts and coupling constants are expressed in ppm and Hz, respectively. High-resolution mass spectra were recorded on a Shimadzu-Kratos concept-IIH instrument under FAB conditions. Optical rotations were measured with a Jasco DIP-4 instrument at 23 °C. All reactions were monitored by TLC (silica gel 60 F_{254}, E. Merck) by charring after spraying with 5% H_2SO_4 in MeOH. Wako-Gel C-300 and silica gel 60 (Art. 7734) were used for flash column chromatography. Bovine β-(1→4)-galactosyltransferase (EC 2.4.1.22) and calf intestine alkaline phosphatase (EC 3.1.3.1) were purchased from Sigma Chemical Co. and Boehringer-Mannheim, respectively. UDP-α-D-[U-^{14}C]galactose was purchased from NEN Research Products and UDP-α-D-galactose, UDP-α-D-mannose and uridine 5-monophosphate from Sigma Chemical Co.

Synthesis of UDP-sugar from protected glycosyl dibenzyl phosphate

Method A. Into a soln of a protected glycosyl dibenzyl phosphate (1 mmol), 10% Pd/C (350 mg) and tributylamine (1.3 mmol) in dry MeOH (25 mL) was bubbled hydrogen for 2–12 h at room temperature. The mixture was filtered through charcoal and the filtrate concd in vacuo and thrice coevaporated with pyridine to give crude protected glycosyl phosphate tributylammonium salt. The salt was dissolved in DMF (8 mL) and coupled with *N,N'*-carbonyldiimidazole (1.1 mmol) by stirring for 12 h at room temperature. After checking the disappearance of the starting compound on TLC, the reaction mixture was quenched with a small amount of MeOH for 30 min. The mixture was concd in vacuo and coevaporated twice with pyridine and twice further with DMF to give a residue, which was dissolved in DMF (8 mL) together with tributylammonium uridine 5'-monophosphate (1.1–1.3 mmol) coevaporated thrice with pyridine. The coupling reaction was carried out under an Ar atmosphere for 12 h at room temperature. The mixture was concd in vacuo and the residue deacetylated with Et_3N–MeOH–H_2O (2:13:6 v/v, 19 mL) for 24 h. The reaction mixture was concd to give a residue, which was loaded on a column of anion exchange resin (Dowex 1 × 2, formate form) and fractionated by a gradient elution with aq NH_4HCO_3 (0.05–1 M). The frs eluted with 0.3–0.6 M NH_4HCO_3 were lyophilized to give UDP-sugar diammonium salt, which was further converted to the corresponding disodium salt by exchange on a column of cation exchange resin (Dowex 50W × 8, pyridinium form and then Na). The final purification by gel filtration on a Sephadex G-15 column was performed 2–4 times, until the purity of the UDP-sugars was confirmed by ^{1}H as well as ^{31}P NMR spectra.

Method B. Into a mixture of protected glycosyl dibenzyl phosphate, 10% Pd/C (350 mg) and tributylamine (1.3 mmol), was bubbled hydrogen for 12–24 h at room temperature. The mixture was filtered with charcoal and the filtrate concd to give protected glycosyl phosphate tributylammonium salt. To a soln of tributylammonium salt uridine 5'-monophosphate (1.2 mmol) coevaporated thrice with pyridine in DMF (8 mL) was added *N,N'*-carbonyldiimidazole (1.44 mmol) and the mixture stirred under an atmosphere of Ar for 2 h at room temperature. The mixture quenched with a small amount of MeOH for 30 min was concd to give crude UMP-imidazolide (**26**). The glycosyl phosphate and imidazolide thus obtained were coevaporated twice with pyridine and twice with DMF, respectively, dissolved in DMF (8 mL) and the mixture was kept under an atmosphere of Ar at room temperature for 12 h. Further work-up was carried out in the same manner as described in method A.

Allyl 3,4-*O*-isopropylidene-2-*O*-methyl-6-*O*-triphenylmethyl-α-D-galactopyranoside (9). To a soln of allyl 3,4-*O*-isopropylidene-6-*O*-triphenylmethyl-α-D-galactopyranoside[15] (**8**, 1.74 g, 3.45 mmol) in DMF (5 mL) was added NaH (452 mg, 10.4 mmol, 55%) and then after stirring at room temperature for 1 h, methyl iodide (0.64 mL, 10.4 mmol). After a further 20 min stirring the reaction mixture was diluted with EtOAc, washed once with water and twice with aq NaCl, dried with $MgSO_4$, and concd. The residue obtained was purified by flash column chromatography (5:1 hexane–EtOAc) to afford **9** (1.72 g, 97.2%).

$[\alpha]_D^{20}$ +65.7° (*c* 1.00; $CHCl_3$); mp 94–97 °C; ^{1}H NMR: δ 7.49–7.20 (m, 15H, Ar), 6.01–5.89 (m, 1H, CH), 5.36–5.20 (m, 2H, CH_2), 4.98 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.27–4.03 (m, 5H, CH_2, H-3, H-4, H-5), 3.50 (s, 3H, CH_3), 3.43 (dd, 1H, $J_{6a,5}$ = 6.9, $J_{6a,6b}$ = 9.6 Hz, H-6a), 3.39 (dd, 1H, $J_{6b,5}$ = 5.9 Hz, H-6b), 3.35 (dd, 1H, $J_{2,3}$ = 7.3 Hz, H-2), 1.48, 1.32 (each s, each 3H, CH_3); Anal calcd for $C_{32}H_{36}O_6$: C, 74.40; H, 7.62. Found: C, 74.44; H, 7.20.

Allyl 3,4,6-tri-*O*-acetyl-2-*O*-methyl-α-D-galactopyranoside (10). The 2-*O*-methylated galactoside **9** (55.0 mg, 0.106 mmol) was mixed with 70% aq acetic acid (2 mL), and the mixture stirred at 60 °C for 2 h and concd. The residue obtained was acetylated with Ac_2O–pyridine (1:1, 2 mL) for 2 h. The mixture was concd and the syrup obtained was purified by flash column chromatography with 2:1 hexane–EtOAc to give **10** (35.7 mg, 93.5%).

$[\alpha]_D^{20}$ +130.6° (*c* 3.11; $CHCl_3$); ^{1}H NMR: δ 6.00–5.88 (m, 1H, CH), 5.44 (bd, 1H, $J_{4,3}$ = 3.3 Hz, H-4), 5.38–5.23 (m, 3H, CH_2, H-3), 5.12 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.26–4.05 (m, 5H, CH_2, H-5, H-6), 3.67 (dd, 1H, $J_{2,3}$ = 10.6 Hz, H-2), 3.46 (s, 3H, CH_3), 2.14, 2.05, 2.02 (each s, each 3 H, Ac); Anal calcd for $C_{16}H_{24}O_9$: C, 53.33; H, 6.71. Found: C, 52.99; H, 7.02.

3,4,6-Tri-*O*-acetyl-2-*O*-methyl-α,β-D-galactopyranose(11). To a solution of the peracetylated 2-*O*-methyl-D-galactoside **10** (1.09 g, 3.03 mmol) in dry MeOH (15 mL) was added 10% Pd/C (520 mg), the mixture was stirred

at 60 °C for 4 h and, after filtration, concd to give a residue, which was dissolved in Me_2CO–H_2O (4:1, 25 mL) and stirred with $HgCl_2$ (1.64 g, 6.06 mmol), HgO (1.31 g, 6.06 mmol) at room temperature for 10 min. The mixture was filtered with charcoal, the filtrate diluted with EtOAc, washed thrice with satd aq NaCl, dried with $MgSO_4$ and concd. The residue obtained was purified on by flash chromatography (2:1 hexane–EtOAc) to give **11** (769 mg, 79.4%) as a 3:2 mixture of α- and β-anomers.

^{1}H NMR: δ 5.47 (bt, 0.6H, α H-1), 5.43 (bd, 0.6H, α H-4), 5.36 (bd, 0.4H, α H-4), 5.26 (dd, 0.6H, $J_{3,2}=10.6$, $J_{3,4}=3.3$ Hz, α H-3), 4.91 (dd, 0.4H, $J_{3,2}=9.9$, $J_{3,4}=3.3$ Hz, β H-3), 4.73 (dd, 0.4H, $J_{1,2}=7.6$, $J_{1,OH}=5.3$ Hz, β H-1), 4.44 (bt, 0.6H, α H-5), 3.91 (bt, 0.4H, β H-5), 3.64 (dd, 0.6H, $J_{2,1}=3.3$ Hz, α H-2), 3.57 (s, 1.2 H, β CH_3), 3.50 (s, 1.8 H, α CH_3), 3.35 (dd, 0.4H, β H-2), 2.15, 2.05, 2.04 (each s, each 3H, Ac). Anal. calcd for $C_{13}H_{20}O_9$: C, 48.75; H, 6.29. Found: C, 48.63; H, 6.67.

3,4,6-Tri-*O*-acetyl-2-*O*-methyl-α-D-galactopyranosyl 1-phosphate, dibenzyl ester (12). To a soln of the α-*O*-methyl-D-galactose triacetate **11** (318 mg, 0.994 mmol, α:β=3:2) in dry THF (24 mL) was added dropwise a solution of BuLi in THF (0.63 mL, 1.01 mmol) under an atmosphere of Ar at −78 °C, and then after 5 min dibenzyl phosphorochloridate (3 equiv.) in THF (6 mL) carefully. After 15 min the mixture was dild with Et_2O, washed with satd aq $NaHCO_3$ and twice with satd aq NaCl, dried with $MgSO_4$ and concd. The residue was purified by flash column chromatography (1:1 hexane–EtOAc containing 1% Et_3N) to give α-phosphate (**12**α 194 mg, 33.6%) and its β-anomer **12** β (58.9 g, 10.2%).

$[\alpha]_D^{22}+92.1°$ (c 0.59, $CHCl_3$); ^{1}H NMR: δ 7.38–7.28 (m, 10H, Ar), 6.04 (dd, 1H, $J_{1,2}=3.3$, $J_{1,P}=6.9$ Hz, H-1), 5.42 (bd, 1H, H-4), 5.22 (dd, 1H, $J_{3,2}=10.2$, $J_{3,4}=3.0$ Hz, H-3), 5.13–5.08 (m, 4H, CH_2 ×2), 4.25 (bt, 1H, H-5), 4.04 (dd, 1H, $J_{6a,5}=6.3$, $J_{6a,6b}=11.2$ Hz, H-6a), 3.93 (dd, 1H, $J_{6b,5}=6.9$ Hz, H-6b), 3.69 (dt, $J_{2,P}=3.0$ Hz, H-2), 3.47 (s, 3H, CH_3), 2.14, 2.04, 1.91 (each s, each 3H, Ac);^{13}C NMR: δ 170.15, 169.90, 135.53, 135.42, 128.50, 128.46, 127.94, 127.66, 94.95, 94.88, 74.90, 74.79, 69.54, 69.45, 69.24, 69.17, 68.84, 68.16, 67.58, 61.21, 58.69, 20.69, 20.51, 20.40; ^{31}P NMR: δ −1.81. Anal. calcd for $C_{27}H_{33}O_{12}P$: C, 55.86; H, 5.73. Found: C, 55.57; H, 5.84.

Uridine 5′(2-*O*-methyl-α-D-galactopyranosyl) diphosphate, disodium salt (1). According to method A the glycosyl dibenzyl phosphate **12**α (79 mg, 0.136 mmol) was first converted to 3,4,6-tri-*O*-acetyl-2-*O*-methyl-α-D-galactopyranosyl 1-phosphate tributylammonium salt (**13**): ^{1}H NMR (D_2O, HOD=4.81): δ 5.84 (dd, 1H, $J_{1,2}=3.3$, $J_{1,P}=6.3$ Hz, H-1), 5.51 (bd, 1H, H-4), 5.22 (dd, 1H, $J_{3,2}=10.6$ Hz, $J_{3,4}=3.3$ Hz, H-3), 4.55 (bt, 1H, H-5), 3.90 (dt, 1H, $J_{2,P}=3.3$ Hz, H-2), 3.36 (s, 3H, Me), 2.21, 2.10, 2.09 (each s, each 3H, Ac); ^{31}P NMR: δ −0.58. This salt was activated as the imidazolide and coupled with uridine 5′-monophosphate tributylammonium salt to give **1** (12.0 mg, 14%).

^{1}H NMR (D_2O, HOD=4.81): δ 7.99 (d, 1H, $J_{6,5}=8.3$ Hz, H-6), 6.01–5.98 (m, 2H, H-5, H-1′), 5.85 (dd, 1H, $J_{1'',2''}=3.3$, $J_{1'',P}=7.3$ Hz, H-1″), 4.42–4.21 (m, 5H, H-2′, H-3′, H-4′, H-5′), 4.18 (bt, 1H, H-5″), 4.04 (bd, 1H, H-4″), 3.96 (dd, 1H, $J_{3'',2''}=10.2$, $J_{3'',4''}=3.6$ Hz, H-3″), 3.77 (dd, 1H, $J_{6''a,5''}=6.0$, $J_{6''a,6''b}=11.6$ Hz, H-6″a), 3.71 (dd, 1H, $J_{6''b,5''}=5.6$ Hz, H-6″b), 3.57 (dt, 1H, $J_{2'',P}=3.3$ Hz, H-2″), 3.52 (s, 3H, CH_3); ^{31}P NMR: δ −10.76, −12.18 ($J_{P,O,P}=19.8$ Hz); HRFABMS (positive-ion): calcd for $C_{16}H_{25}O_{17}N_2P_2Na_2$ (M+H): 625.0425, Found: 625.0450.

Allyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-galactopyranoside (16). To a solution of allyl α-D-galactopyranoside[16] (**15**, 1.00 g, 4.55 mmol) in dry MeOH (17.5 mL) was added dibutyltin oxide (1.13 g, 4.55 mmol), the mixture was heated at reflux for 2 h, concd and coevaporated thrice with toluene. To a solution of the residue in dry toluene (35 mL) was added Bu_4NI (2.01 g, 5.45 mmol) and MeI (1.41 mL, 22.7 mmol), the mixture was heated at reflux for 4 h, quenched with excess MeONa, neutralized with acetic acid and concd. The residue dried in vacuo was acetylated with 1:1 acetic anhydride–pyridine (40 mL) at room temperature for 2 h. The mixture was diluted with EtOAc and stirred with aq KI for 5 h, and extracted with CH_2Cl_2. The organic layer was washed with satd aq $NaHCO_3$ and NaCl, dried with $MgSO_4$ and concd. The residue was purified by flash column chromatography (2:1 hexane–EtOAc) to give **16** (1.17 g, 71.3%).

$[\alpha]_D^{20}+145.8°$ (*c* 1.80; $CHCl_3$); ^{1}H NMR: δ 5.97–5.82 (m, 1H, CH), 5.53 (bd, 1H, H-4), 5.34–5.21 (m, 2H, CH_2), 5.11 (d, 1H, $J_{1,2}=3.9$ Hz, H-1), 5.03 (dd, 1H, $J_{2,3}=10.6$ Hz, H-2), 4.21–3.99 (m, 5H, CH_2, H-5, H-6), 3.73 (dd, 1H, $J_{3,4}=3.6$ Hz, H-3), 3.39 (s, 3H, CH_3), 2.14, 2.12, 2.05 (each s, each 3H, Ac). Anal. calcd for $C_{16}H_{24}O_9$: C, 53.33; H, 6.71. Found: C, 53.09; H, 6.90.

2,4,6-Tri-*O*-acetyl-3-*O*-methyl α,β-D-galactopyranose (17). De-*O*-allylation of **16** (412 mg, 2.53 mmol) was performed in the same manner as described for the preparation of **11** to give **17** (518 mg, 63.8%, α:β=6:1).

^{1}H NMR: δ 5.55 (bd, 0.87H, α H-4), 5.47 (t, 0.87H, $J_{1,2}=J_{1,OH}=3.3$ Hz, αH-1), 5.02 (dd, 0.87H, $J_{2,3}=10.6$ Hz, α H-2), 4.92 (dd, 0.13H, $J_{2,1}=8.3$, $J_{2,3}=10.2$ Hz, β H-2), 4.61 (bt, 0.13H, β H-1), 4.38 (bt, 0.87H, α H-5), 3.86 (bt, 0.13H, β H-5), 3.76 (dd, 0.87H, $J_{3,4}=3.3$ Hz, α H-3), 3.40 (s, 2.61H, α CH_3), 3.39 (s, 0.39H, β CH_3), 2.16, 2.14, 2.05 (each s, each 0.39H, β Ac), 2.15, 2.13, 2.08 (each s, each 2.61H, α Ac). Anal. calcd for $C_{13}H_{20}O_9$: C, 48.75; H, 6.29. Found : C, 49.20; H, 6.67.

2,4,6-Tri-*O*-acetyl-3-*O*-methyl-α-D-galactopyranosyl 1-phosphate, dibenzyl ester (18). Dibenzyl phosphorylation of **17** (50.0 mg, 0.156 mmol, α:β=6:1) was performed in the same manner as described for the preparation of **12** with BuLi (0.10 mL, 0.159 mmol) and dibenzyl phosphorochloridate (3 equiv) in THF (5 mL) to give **18** (36.9 mg, 40.7%).

$[\alpha]_D^{17}$ +70.9° (*c* 0.93; $CHCl_3$); ^1H NMR: δ 7.37–7.27 (m, 10H, Ar), 5.90 (dd, 1H, $J_{1,2}=3.3$, $J_{1,P}=6.6$ Hz, H-1), 5.52 (bd, 1H, H-4), 5.12–5.03 (m, 5H, CH_2 ×2, H-2), 5.28 (bt, 1H, H-5), 4.11 (dd, 1H, $J_{6a,5}=5.6$, $J_{6a,6b}=11.2$ Hz, H-6a), 3.97 (dd, 1H, $J_{6b,5}=6.9$ Hz, H-6b), 3.63 (dd, 1H, $J_{3,2}=10.6$, $J_{3,4}=3.3$ Hz, H-3), 3.37 (s, 3H, CH_3), 2.13, 1.93, 1.92 (each s, each 3H, Ac); ^{13}C NMR: δ 170.31, 170.22, 170.08, 128.73, 128.63, 127.96, 94.84, 94.75, 74.63, 69.58, 69.51, 68.79, 68.72, 66.02, 61.85, 57.90, 29.65, 20.63, 20.52; ^{31}P NMR: δ −1.91. Anal. calcd for $C_{27}H_{33}O_{12}P$: C, 55.86; H, 5.73. Found: C, 56.13; H, 5.80.

Uridine 5′-(3-*O*-methyl-α-D-galactopyranosyl) diphosphate, disodium salt (2). According to method A the glycosyl dibenzyl phosphate **18** (158 mg, 0.272 mmol) was first converted to 3,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-galactopyranosyl 1-phosphate tributylammonium salt (**19**): ^1H NMR (D_2O, HOD=4.81): δ 5.73–5.70 (m, 2H, H-1, H-4), 5.04 (bd, 1H, H-2), 4.53 (bt, 1H, H-5), 4.28 (dd, 1H, $J_{6a,5}=5.6$, $J_{6a,6b}=10.9$ Hz, H-6a), 4.18 (dd, 1H, $J_{6b,5}=6.3$ Hz, H-6b) 4.07 (dd, 1H, $J_{3,2}=10.6$ Hz, $J_{3,4}=3.3$ Hz, H-3), 3.45 (s, 3H, Me), 2.22, 2.19, 2.13 (each s, each 3H, Ac).

This salt was activated as the imidazolide and coupled with uridine 5′-monophosphate tributylammonium salt to give **2** (17.3 mg, 10%).

^1H NMR (D_2O, HOD=4.81): δ 7.99 (d, 1H, $J_{6,5}=8.3$ Hz, H-6), 6.02–5.98 (m, 2H, H-5, H-1′), 5.65 (dd, 1H, $J_{1'',2''}=3.6$, $J_{1'',P}=7.3$ Hz, H-1″), 4.40–4.23 (m, 6H, H-2′, H-3′, H-4′, H-5′, H-4″), 4.17 (bt, ^1H, H-5″), 3.86 (ddd, $J_{2'',3''}=10.6$, $J_{2'',P}=3.0$ Hz, H-2″), 3.79–3.73 (m, 2H, H-6″), 3.64 (dd, 1H, $J_{3'',4''}=3.3$ Hz, H-3′), 3.47 (s, 3H, CH_3); ^{31}P NMR: δ −10.40, −12.02 ($J_{P,O,P}=20.6$ Hz); HRFABMS: calcd for $C_{16}H_{25}O_{17}N_2P_2Na_2$ (M+H): 625.0425, Found: 625.0438.

Allyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (21). To solution of allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside[16] (218 mg, 0.456 mmol) in dry THF (7 mL) was added $NaBH_3CN$ (258 mg, 4.10 mmol) and molecular sieves 3 Å (100 mg), the mixture was stirred under an atmosphere of Ar at room temperature for 30 min, acidified with HCl in Et_2O until evolution of gas ceased, poured into ice-water and extracted with $CHCl_3$. The organic layer was washed twice with H_2O and once with satd $NaHCO_3$, dried with $MgSO_4$ and concd. The residue was purified by flash column chromatography (3:1 hexane–EtOAc) to give **21** (157 mg, 71.9%).

$[\alpha]_D^{23}$ −5.6° (*c* 3.67; $CHCl_3$); ^1H NMR: δ 7.38–7.25 (m, 15H, Ar), 6.02–5.88 (m, 1H, CH), 5.36–5.16 (m, 2H, CH_2), 4.92, 4.73 (each d, each 1H, *J*=10.9 Hz, CH_2), 4.71, 4.58 (each d, each 1H, *J*=10.9 Hz, CH_2), 4.46–4.39, 4.17–4.10 (each m, each 1H, CH_2), 4.41 (d, 1H, $J_{1,2}=7.6$ Hz, H-1), 4.01 (bs, 1H, H-4), 3.80 (dd, 1H, $J_{6a,5}=6.3$, $J_{6a,6b}=9.9$ Hz, H-6a), 3.72 (dd, 1H, $J_{6b,5}=5.9$ Hz, H-6b), 3.68 (dd, 1H, $J_{2,3}=9.6$ Hz, H-2), 3.55 (bt, 1H, H-5), 3.49 (dd, 1H, $J_{3,4}=3.6$ Hz, H-3), 2.50 (bs, 1H, OH). Anal. calcd for $C_{30}H_{34}O_6$: C, 73.45; H, 6.99. Found: C, 73.19; H, 6.95.

Allyl 2,3,6-tri-*O*-benzyl-4-*O*-methyl-β-D-galactopyranoside (22). *O*-Methylation of **21** (133 mg, 0.27 mmol) was performed in DMF (0.5 mL) in the same manner as described for the preparation of **10** by treatment with NaH (35 mg, 10.36 mmol, content 55%) for 2 h and then with MeI (50 μL 0.81 mmol) for 10 min to give **22** (101 mg, 73.5%).

$[\alpha]_D^{20}$ −13.4° (*c* 1.07, $CHCl_3$); ^1H NMR: δ 7.38–7.24 (m, 15H, Ar), 6.00–5.86 (m, 1H, CH), 5.35–5.14 (m, 2H, CH_2), 4.91, 4.74 (each d, each 1H, *J*=10.9 Hz, CH_2), 4.74 (s, 1H, CH_2), 4.58, 4.53 (each d, each 1H, *J*=10.9 Hz, CH_2), 4.43–4.35, 4.13–4.06 (each m, each 1H, CH_2), 4.39 (d, 1H, $J_{1,2}=7.6$ Hz, H-1), 3.74 (dd, 1H, $J_{2,3}=9.9$ Hz, H-2), 3.73 (dd, 1H, $J_{6a,5}=6.6$, $J_{6a,6b}=9.2$ Hz, H-6a), 3.64 (bd, 1H, H-4), 3.63 (dd, 1H, $J_{6b,5}=5.6$ Hz, H-6b), 3.56 (s, 3H, CH_3), 3.47 (dd, 1H, $J_{3,4}=3.0$ Hz, H-3). Anal. calcd for $C_{31}H_{36}O_6$: C, 73.79; H, 7.19. Found: C, 73.53; H, 7.56.

2,3,6-Tri-*O*-benzyl-4-*O*-methyl-α,β-D-galactopyranose (23). To a soln of **22** (85 mg, 0.17 mmol) in DMSO (2 mL) was added t-BuOK (66 mg, 0.59 mmol), the mixture was stirred at 60 °C for 30 min and, after cooling, poured into H_2O and extracted with EtOAc. The organic layer was washed twice with H_2O, dried with $MgSO_4$ and concd. A mixture of the residue, $HgCl_2$ (91 mg, 0.34 mmol) and HgO (73 mg, 0.34 mmol) in 4:1 Me_2CO–H_2O (2.5 mL) was stirred for 10 min and filtered with charcoal. The filtrate diluted with EtOAc was washed with H_2O and twice with satd NaCl, dried and concd. The residue was purified by flash column chromatography (2:1 hexane–EtOAc) to give **23** (78 mg, quantitative, α:β=3:1).

^1H NMR: δ 7.40–7.24 (m, 15H, Ar), 5.25 (d, 0.75H, $J_{1,2}=3.6$ Hz, α H-1), 4.92–4.48 (m, 6H, CH_2 ×3), 3.93 (dd, 0.75H, $J_{2,3}=9.9$ Hz, α H-2), 3.85 (dd, 0.75H, $J_{3,4}=2.6$ Hz, α H-3), 3.71 (bd, 0.75H, α H-4), 3.68 (bd, 0.25H, β H-4), 3.56 (s, 0.75H, β CH_3), 3.55 (s, 2.25H, α CH_3). Anal. calcd for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94. Found: C, 72.24; H, 6.79.

2,3,6-tri-*O*-Benzyl-4-*O*-methyl-α-D-galactopyranosyl 1-phosphate, dibenzyl ester (24). Dibenzyl phosphorylation of **23** (250 mg, 0.54 mmol, α:β=3:1) was performed in the same manner as described for the preparation of **12** by treatment with BuLi (1.0 mL, 1.6 mmol) for 10 min and then with dibenzyl phosphorochloridate (3 equiv.) for 15 min in dry THF (8 mL) to give **24** (176 mg, 45%) after purification by flash column chromatography (3:1 hexane–EtOAc).

$[\alpha]_D^{20}$ +45.3° (*c* 1.32; $CHCl_3$); ^1H NMR: δ 7.37–7.17 (m, 25H, Ar), 5.95 (dd, 1H, $J_{1,2}=3.3$, $J_{1,P}=6.6$ Hz, H-1), 5.07–4.92 (m, 4H, CH_2 ×2), 4.76, 4.70 (each d, each 1H, *J*=10.9 Hz, CH_2), 4.74 (s, 2H, CH_2), 4.48, 4.42 (each d, each 1H, *J*=10.9 Hz, CH_2), 4.09 (bt, 1H, H-5), 4.02 (dt, 1H, $J_{2,3}=10.2$, $J_{2,P}=3.3$ Hz, H-2), 3.81 (dd, 1H, $J_{3,4}=2.6$ Hz, H-3), 3.72 (bd, 1H, H-4), 3.62 (dd, 1H,

$J_{6a,5}$=7.3, $J_{6a,6b}$=9.2 Hz, H-6a), 3.55 (s, 3H, CH_3), 3.46 (dd, 1H, $J_{6b,5}$=5.9 Hz, H-6b); ^{13}C NMR: δ 138.29, 137.97, 137.70, 135.90, 135.76, 128.46, 128.37, 128.30, 128.21, 128.14, 127.94, 127.85, 127.73, 127.69, 127.62, 127.58, 127.46, 96.50, 96.39, 77.72, 76.59, 75.68, 73.37, 72.72, 71.20, 69.15, 69.08, 68.95, 68.88, 68.03, 61.40; ^{31}P NMR: δ-1.47. Anal. calcd for $C_{42}H_{45}O_9P$: C, 69.60; H, 6.26. Found: C, 69.30; H, 6.18.

Uridine 5′-(4-*O*-methyl-α-D-galactopyranosyl) diphosphate, disodium salt (3). According to method B, the glycosyl phosphate **25** derived from **24** (176 mg, 0.24 mmol) was first converted to 3,4,6-tri-*O*-acetyl-4-*O*-methyl-α-D-galactopyranosyl 1-phosphate tributylammonium salt (**25**): ^{31}P NMR: δ −0.45.

This salt was coupled with the imidazolide **26** derived from UMP to give **3** (17.4 mg, 11%).

^{1}H NMR (D_2O, HOD=4.81): δ 7.99 (d, 1H, $J_{6,5}$=8.3 Hz, H-6), 6.03–5.98 (m, 2H, H-5, H-1′), 5.64 (dd, 1H, $J_{1'',2''}$=3.6, $J_{1'',P}$=7.3 Hz, H-1″), 4.43–4.19 (m, 6H, H-2′, H-3′, H-4′, H-5′, H-5″), 4.01 (dd, 1H, $J_{3'',2''}$=10.2, $J_{3'',4''}$=3.3 Hz, H-3″), 3.82–3.75 (m, 4H, H-2″, H-4″, H-6″), 3.57 (s, 3H, CH_3); ^{31}P NMR: δ −10.40, −11.98 ($J_{P,O,P}$=20.6 Hz); HRFABMS: calcd for $C_{16}H_{25}O_{17}N_2P_2Na$ (M+H): 625.0425, Found: 625.0461.

2,3,4-Tri-*O*-acetyl-6-*O*-methyl-α,β-D-galactopyranose (28). To a soln of 6-*O*-methyl-D-galactopyranose tetraacetate[18] **27** (1.61 g, 4.44 mmol) in DMF (25 mL) was added H_2NNH_2·AcOH (490 mg, 5.33 mmol), the mixture was stirred at 60 °C for 4 h, after cooling diluted with EtOAc, washed thrice with satd NaCl, dried and concd. The residue was purified by flash column chromatography (2:1 hexane–EtOAc) to give **28** (1.05 g, 73.9%, α:β=4:1).

^{1}H NMR: δ 5.53 (d, 0.8H, $J_{1,2}$=3.6 Hz, α H-1), 5.46 (dd, 0.8H, $J_{4,3}$=3.3, $J_{4,5}$=1.0 Hz, α H-4), 5.42 (bd, 0.2H, β H-4), 5.41 (dd, 0.8H, $J_{3,2}$=10.6 Hz, α H-3), 5.18 (dd, 0.8H, α H-2), 5.09 (d, 0.2H, $J_{1,2}$=6.9 Hz, β H-1), 4.44 (ddd, 0.8H, $J_{5,6a}$=6.6, $J_{5,6b}$=4.6 Hz, α H-5), 3.88 (bt, 0.2H, β H-5), 3.61–3.35 (m, 2H, H-6), 3.35 (s, 3H, CH_3), 2.16 (s, 0.6H, β Ac), 2.15, 2.10 (each s, each 3H, α,β Ac), 2.05 (s, 2.4H, α Ac). Anal. calcd for $C_{13}H_{20}O_9$: C, 48.75; H, 6.29. Found: C, 48.83; H, 6.63.

2,3,4-Tri-*O*-acetyl-6-*O*-methyl-α-D-galactopyranosyl 1-phosphate, dibenzyl ester (29α). Dibenzyl phosphorylation of **28** (336 mg, 1.05 mmol, α:β=4:1) was performed in the same manner as described for the preparation of **12** with BuLi (0.67 mL, 1.07 mmol) and dibenzyl phosphorochloridate (3 equiv.) in THF (35 mL) to give **29α** (298 mg, 49.0%) and **29β** (74.1 mg, 12.2%).

$[\alpha]_D^{17}$ +75.6° (*c* 2.42; $CHCl_3$); ^{1}H NMR: δ 7.36–7.31 (m, 10H, Ar), 5.98 (dd, 1H, $J_{1,2}$=3.3, $J_{1,P}$=6.6 Hz, H-1), 5.48 (bd, 1H, H-4), 5.34 (dd, 1H, $J_{3,2}$=10.9, $J_{3,4}$=3.3 Hz, H-3), 5.24 (dt, 1H, $J_{2,P}$=3.3 Hz, H-2), 5.11–5.06 (m, 4H, CH_2 ×2), 4.26 (bt, 1H, H-5), 3.34 (d, 2H, $J_{6,5}$=5.9 Hz, H-6), 3.23 (s, 3H, CH_3), 2.14, 2.00, 1.91 (each s, each 3H, Ac); ^{13}C NMR: δ 170.03, 169.81, 135.51, 135.45, 135.35, 128.59, 128.55, 127.96, 127.91, 94.68, 94.61, 70.28, 69.60, 69.53, 69.42, 68.11, 67.17, 67.10, 59.16, 20.58, 20.43; ^{31}P NMR: δ −1.90. Anal. calcd for $C_{27}H_{33}O_{12}P$: C, 55.86; H, 5.73. Found: C, 56.07; H, 6.06.

Uridine 5′-(6-*O*-methyl-α-D-galactopyranosyl) diphosphate, diammonium salt (4). According to method A, the glycosyl dibenzyl phosphate **29α** (298 mg, 0.514 mmol) was first converted to 3,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-galactopyranosyl 1-phosphate tributylammonium salt (**30**): ^{1}H NMR (D_2O, HOD=4.81): δ 5.76 (dd, 1H, $J_{1,2}$=3.3, $J_{1,P}$=7.9 Hz, H-1), 5.57 (bd, 1H, H-4), 5.45 (dd, 1H, $J_{3,2}$=10.6, $J_{3,4}$=3.3 Hz, H-3), 5.26 (ddd, 1H, $J_{2,P}$=2.0 Hz, H-2), 4.57 (bt, 1H, H-5), 3.67–3.55 (m, 2H, H-6), 3.38 (s, 3H, Me), 2.24, 2.17, 2.08 (each s, each 3H, Ac); ^{31}P NMR: δ −0.32.

This salt was activated as the imidazolide and coupled with uridine 5′-monophosphate tributylammonium salt to give **4** (71.6 mg, 22%).

^{1}H NMR (D_2O, HOD=4.81): δ 7.99 (d, 1H, $J_{6,5}$=8.3 Hz, H-6), 6.03–6.00 (m, 2H, H-5, H-1′), 5.67 (dd, 1H, $J_{1'',2''}$=3.6, $J_{1'',P}$=7.6 Hz, H-1″), 4.43–4.23 (m, 6H, H-2′, H-3′, H-4′, H-5′, H-5″), 4.03 (bd, 1H, H-4″), 3.95 (dd, $J_{3'',2''}$=10.2, $J_{3'',4''}$=3.0 Hz, H-3″), 3.82 (dt, 1H, $J_{2'',P}$=3.0 Hz, H-2″), 3.67 (d, 2H, $J_{6'',5''}$=5.6 Hz, H-6″), 3.43 (s, 3H, CH_3); ^{31}P NMR: δ −10.32, −11.94 ($J_{P,O,P}$=20.6 Hz): HRFABMS: $C_{16}H_{30}O_{17}N_3P_2$ (monoammonium salt): 598.1050 (M+H), Found: 598.1060.

6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl 1-phosphate, dibenzyl ester (33). Dibenzyl phosphorylation of 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α,β-D-galactopyranose (**32**, 300 mg, 0.607 mmol, α:β=3:2) was performed in the same manner as described for the preparation of **12** by treatment with BuLi (0.36 mL, 0.62 mmol) for 2 min and the with dibenzyl phosphorochloridate (3 equiv.) for 20 min in dry THF (2.5 mL) to give **33** (161 mg, 35%) after purification by flash column chromatography (2:1 hexane–EtOAc).

$[\alpha]_D^{24}$ +46.4° (*c* 2.25; $CHCl_3$); ^{1}H NMR: δ 7.36–7.24 (m, 25H, Ar), 5.95 (dd, 1H, $J_{1,2}$=3.3, $J_{1,P}$=6.6 Hz, H-1), 5.08–4.96 (m, 4H, CH_2 ×2), 4.86–4.58 (m, 6H, CH_2 ×3), 4.10–4.03 (m, 4H, H-2, H-5, H-6), 3.90–3.87 (m, 2H, H-3, H-4), 1.82 (s, 3H, CH_3); ^{13}C NMR: δ 170.37, 138.28, 137.88, 135.85, 135.81, 135.74, 128.45, 128.39, 128.34, 128.27, 128.01, 127.69, 127.51, 127.42, 96.41, 96.32, 77.95, 75.67, 75.58, 74.64, 74.25, 73.32, 73.24, 70.64, 69.20, 69.13, 69.00, 68.93, 63.11, 20.54; ^{31}P NMR: δ −1.57. Anal. calcd for $C_{43}H_{45}O_{10}P$: C, 68.61; H, 6.03. Found: C, 68.31; H, 6.11.

Uridine 5′-(6-*O*-acetyl-α-D-galactopyranosyl) diphosphate, disodium salt (5). According to method B, the dibenzyl glycosyl phosphate **33** (77 mg, 0.10 mmol) was first converted to 6-*O*-acetyl-α-D-galactopyranosyl 1-phosphate tributylammonium salt (**34**):^{1}H NMR (D_2O, HOD=4.81): δ 5.58 (dd, 1H, $J_{1,2}$=3.3, $J_{1,P}$=6.9 Hz, H-1), 4.36–4.28 (m, 3H, H-5, H-6), 4.11 (bd, 1H,

$J_{4,3}$=3.0 Hz, H-4), 3.97 (dd, 1H, $J_{3,2}$=10.6 Hz, H-3), 3.62 (dt, 1H, $J_{2,P}$=3.3 Hz, H-2), 2.17 (s, 3H, Ac); ^{31}P NMR: δ -0.20.

This salt was coupled with the imidazolide derived from to give **5** (9.8 mg, 14%).

^{1}H NMR (D_2O, HOD=4.81): δ 7.99 (d, 1H, $J_{6,5}$=6.9 Hz, H-6), 6.00–5.95 (m, 2H, H-5, H-1′), 5.66 (dd, 1H, $J_{1'',2''}$=3.3, $J_{1'',P}$=5.9 Hz, H-1″), 4.39–4.18 (m, 8H, H-2′, H-3′, H-4′, H-5′, H-5″, H-6″), 4.08 (bd, 1H, H-4″), 3.96 (dd, $J_{3'',2''}$=9.6, $J_{3'',4''}$=2.6 Hz, H-3″), 3.83 (dt, 1H, $J_{2'',P}$=3.3 Hz, H-2″), 2.14 (s, 3H, CH_3); ^{31}P NMR: δ −10.40, −12.12 ($J_{P,O,P}$=20.6 Hz); HRFABMS: calcd for $C_{17}H_{24}O_{18}P_2Na_2$ (M+H): 653.0373, Found 653.0395.

Uridine 5-(α-L-fucopyranosyl) diphosphate, diammonium salt (6). According to method A, 2,3,4-tri-acetyl-α-L-fucopyranosyl dibenzyl phosphate[20] (**35**, 152 mg, 0.28 mmol) was converted to **6** (23.9 mg, 15%).

^{1}H NMR (D_2O, HOD=4.81): δ 7.99 (d, 1H, $J_{6,5}$=8.3 Hz, H-6), 6.01 (d, 1H, $J_{1',2'}$=5.0 Hz, H-1′), 6.00 (d, 1H, H-5), 5.59 (dd, 1H, $J_{1'',2''}$=3.3, $J_{1'',P}$=6.3 Hz, H-1″), 4.41–4.22 (m, 6H, H-2′, H-3′, H-4′, H-5′, H-5″), 3.95 (dd, $J_{3'',2''}$=10.6, $J_{3'',4''}$=3.3 Hz, H-3″), 3.85 (bd, 1H, H-4″), 3.78 (dt, 1H, $J_{2'',P}$=3.3 Hz, H-2″), 1.25 (d, 3H, $J_{6'',5''}$=6.6 Hz, H-6″); ^{31}P NMR: δ −10.52, −12.04 ($J_{P,O,P}$=20.6 Hz).

Glycosyl transfer assay of UDP-sugars

A soln containing a UDP-sugar (150 μM) methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (10 mM), β-1,4-galactosyltransferase (7 munit; in the case of UDP-galactose 0.07 munit), $MnCl_2$ (10 mM or 1 mM), alkaline phosphatase (25 unit) and bovine serum albumin (30 μg) in HEPES buffer (pH 7.0, 60 mM, total vol. 101 μL) was incubated at 37 °C for 30 min. A 50 μL aliquot was subjected to HPLC (Asahipak GS320 column, 200 mM phosphate buffer, pH 3.0) for determination of uridine formed by UV absorption at 254 nm. The solution without the glycosyl acceptor was used as a control.

Methyl *O*-(2-*O*-methyl-β-D-galactopyranosyl)-(1→4))-2-acetamido-2-deoxy-β-D-glucopyranoside (36). A glycosyl transfer reaction was performed in the same manner as described for transfer assay using UDP-2-OMe-Gal **1** (9.0 mg, 1.5 equiv), GlcNAcβ-OMe (2.1 mg) and β-1,4-galactosyltransferase (1.5 unit) for 38 h to give (**36**, 91%), which was purified, after deionized through anion exchange resin (Dowex 1×2, Cl^- form), on a column of reversed phase silica gel.

^{1}H NMR (500 MHz, D_2O, HOD=4.81): δ 4.54 (d, 1H, $J_{1',2'}$=7.7 Hz, H-1′), 4.50 (d, 1H, $J_{1,2}$=7.8 Hz, H-1), 3.95 (bd, 1H, $J_{4',3'}$=3.3 Hz, H-4′), 3.63 (s, 3H, Gal 2-OMe), 3.55 (s, 3H, GlcNAc 1-*O*-Me), 3.30 (dd, 1H, $J_{2',3'}$=10.0 Hz, H-2′), 2.07 (s, 3H, Ac); ^{13}C NMR δ′ 175.38, 103.32, 102.56, 81.63, 79.23, 75.94, 75.67, 73.20, 72.95, 69.31, 61.66, 61.31, 60.78, 57.80, 55.71, 22.87. HRFABMS: calcd for $C_{16}H_{29}O_{11}NNa$: (M+Na) 434.1639. Found: 434.1641.

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