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ORIGINAL ARTICLE

Stereoselective synthesis of UDP-2-(2-ketopropyl)galactose aided by di-*tert*-butylsilylene protecting group

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Abstract UDP-2-(2-ketopropyl)galactose (**1**) has been utilized as a valuable probe for profiling proteins modified by *O*-GlcNAc. In this work, we developed a protocol for efficient synthesis of **1**. Thus, 2-methallylgalactose derivative **11**, a synthetic intermediate for the compound **1**, was prepared by stereoselective iodination and methallylation at *C*-2 position, through exploitation of 4,6-*O*-di-*tert*-butylsilylene protecting group.

Keywords Stereoselective synthesis · Galactose derivative · Di-*tert*-butylsilylene · Iodination · Radical methallylation

Abbreviations

AIBN	α,α′-azobisisobutyronitrile
DMF	*N*,*N*-dimethylformamide
DTBS	di-*tert*-butylsilylene
ESI	electrospray ionization
GalT	β-1,4-galactosyltransferase
NIS	*N*-iodosuccinimide
OGA	*O*-linked β-*N*-acetylglucosamine hydrolase
O-GlcNAc	*O*-linked β-*N*-acetylglucosamine
OGT	*O*-linked β-*N*-acetylglucosamine transferase
PEG	polyethylene glycol
Py	pyridine
THF	tetrahydrofuran
UDP	uridine diphosphate
UMP	uridine monophosphate

Introduction

Among various types of protein glycosylations, *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modification (*O*-GlcNAcylation) is unique as it occurs on serine or threonine residues of nuclear and cytoplasmic proteins [1–4]. Introduction of *O*-GlcNAc is dynamically regulated by a pair of counteracting enzymes, *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA) [5–12]. *O*-GlcNAcylation, which has been found on more than 1,000 sites of various proteins, controls transcription, translation, signaling, and cytoskeleton organization in competition or cooperation with protein phosphorylation [3]. Furthermore, it has been attracting growing attention as a histone code [13–16].

UDP-2-(2-ketopropyl)galactose (**1**) is utilized as a valuable tool for facile profiling of proteins modified by *O*-GlcNAc [17–19] (Scheme 1). Namely, *O*-GlcNAc-modified proteins are chemoenzymatically labeled with UDP-ketogalactose **1** by a β-1,4-galactosyltransferase mutant (Y289L GalT) and introduced ketogalactose serves as a handle for chemoselective ligation with aminooxy-functionalized biotin- [17, 18] or PEG-tag [19].

Previously reported syntheses of **1** [17, 20, 21] employed iodination of tri-*O*-acetylgalactal (**2**) and following radical methallylation of the resulting iodide **3** as key reactions. The iodination, however, was reported to afford a mixture of

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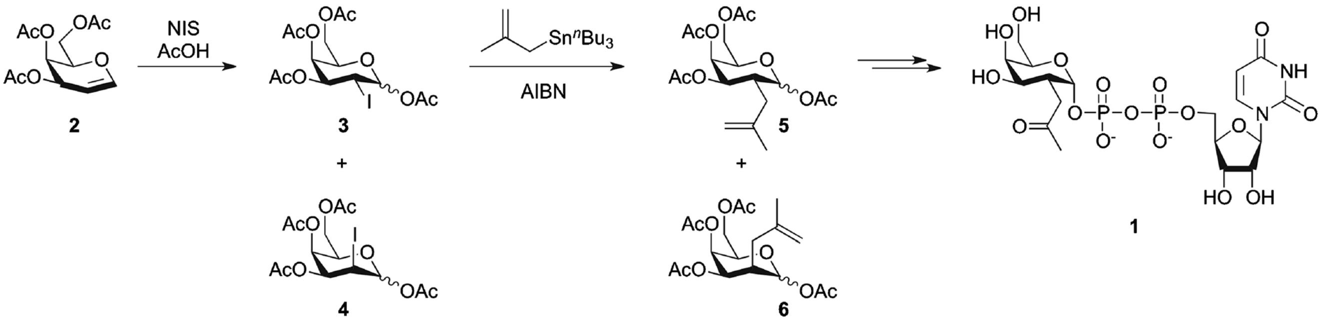


Scheme 1 Reported synthesis of UDP-ketogalactose **1**

equatorial (**3**) and axial (**4**) iodides. For subsequent transformation, the minor isomer **3** isolated in 35 % yield was utilized [21]. In addition, methallylation of **3** was not entirely stereoselective (**5**:**6**=ca. 7:1) [20].

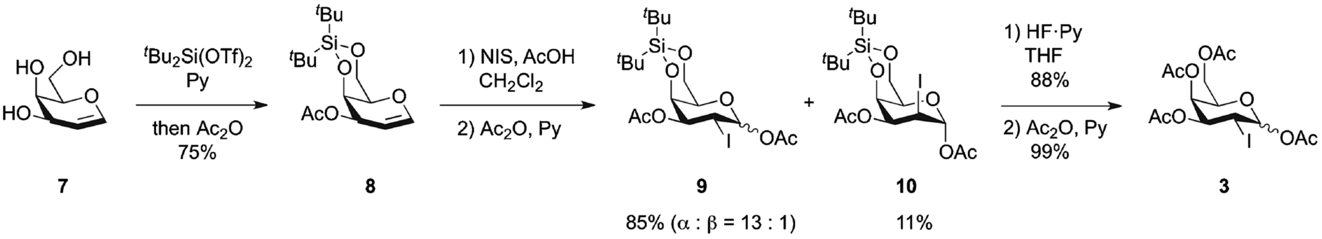
Recently, α-selective glycosylation of galactose and galactosamine derivatives protected with 4,6-*O*-di-*tert*-butylsilylene (DTBS) group was reported [22–24]. In these cases, bulky DTBS group forces nucleophiles to attack from the convex side, giving α-glycosides with high selectivity. By analogy, we anticipated that the iodination and methallylation would proceed with favorable stereoselectivity when a DTBS group is installed to galactal, leading to an efficient access to intermediates for 2-(2-ketopropyl)galactose derivatives such as **1**.

Results and discussion

To begin with, the galactal (**7**) was converted to 4,6-*O*-DTBS derivative **8** by sequential treatment with ^{t}Bu$_2$Si(OTf)$_2$ and Ac$_2$O in pyridine (Scheme 2). Subsequent iodination was carried out by treatment with NIS in the presence of AcOH at room temperature, which, after acetylation, gave the equatorial iodide **9** as a 13:1 mixture of α- and β-anomers in 85 %, together with the axial isomer **10** which was isolated in 11 % as an α-anomer. Therefore, the introduction of 4,6-*O*-DTBS group into the galactal was shown to exhibit a favorable effect in controlling the stereochemistry of the *C*-2 position in an equatorial selective manner (**9**:**10**=7.7:1). Deprotection of the DTBS group of **9** by using HF•Py in THF and successive acetylation afforded the known iodide **3** [20, 21], *albeit* with anomeric composition (α:β=13:1) different from previous reports.

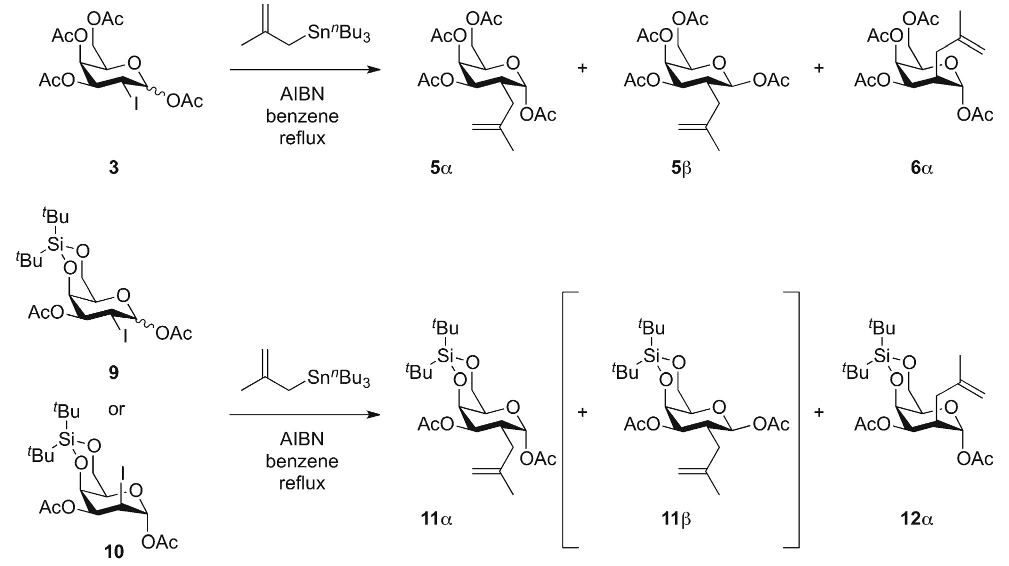
Methallylation of the iodides was conducted under Keck conditions [25] based on the reported procedure [11] (Table 1). As an initial attempt, reaction of the tetraacetate **3** (α:β=13:1) with methallyltributyltin in the presence of AIBN was conducted in refluxing benzene, which gave a mixture of isomers composed of the equatorially methallylated product **5** (α:β=1:0.23) and its *C*-2 epimer **6** (α only) in 62 % yield (**5**:**6**=0.65:1) (Table 1, entry 1). This result was somewhat unexpected in light of the previous report that the tetraacetate **3** (mainly β) gave the desired **5** as the major product [20]. On the other hand, methallylation of **9** (α:β=13:1) having the 4,6-*O*-DTBS group preferentially gave the equatorial product **11** together with the axial product **12** (**11**:**12**=4.6:1) in 89 % yield (entry 2). In this case, a bulky silylene group exhibited a positive effect indeed in enhancing a proportion of the desired isomer. We also observed that the β-anomer of **9** exclusively gave **11**, while the α-anomer afforded an isomeric mixture of **11** and **12** (4.2:1). These results indicated that the configuration of the anomeric acetate affects the stereochemistry of the methallylation.

With the above knowledge in hand, acetylation of the hemiacetal **13** was examined to seek for conditions that give higher proportion of the β-acetate. To prepare **13**, iodination of the galactal **8** was conducted under aqueous conditions, which gave the equatorial iodide **13** along with **14**, which were used for the following acetylation without separation. As shown in Table 2, the use of Ac$_2$O and pyridine in CH$_2$Cl$_2$ provided **9** in which the β-anomer was dominant (α:β=1:2.1) (entry 2), while more reactive AcCl provided larger proportion



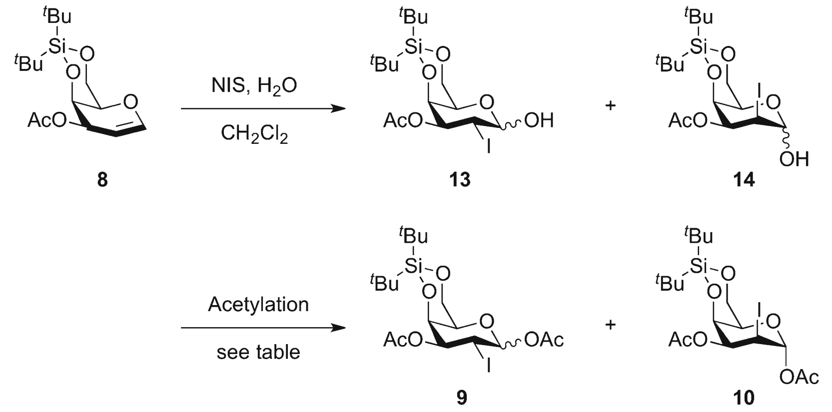
Scheme 2 Iodination of 4,6-*O*-DTBS-protected galactal **8**

Table 1 Radical methallylation of 2-iodogalactose derivatives



Entry	Iodide (α:β)	Products (proportion)	Yield	**11**:**12** (**5**:**6**)
1	**3** (13:1)	**5**α/**5**β/**6**α (0.53/0.12/1)	62%	(0.65:1)
2	**9** (13:1)	**11**α/**11**β/**12**α (4.2/0.41/1)	89%	4.6:1
3	**9** (1:2.1)	**11**α/**11**β/**12**α (3.8/11.1/1)	97%	14.9:1
4	**10** (α)	**11**α/**12**α (4.2/1)	89%	4.2:1

Table 2 Stereoselectivity of acetylation for 1-hydroxy-2-iodogalactose derivative



Entry	Solvent[a]	Reagents (eq)	Temp.	Time (h)	**9**		**10**
					Yield[b]	α:β	Yield[b]
1	CH_2Cl_2	AcCl (6), Py (12)	0 ˚C to r.t.	2.5	56%	1.8:1	1%
2	CH_2Cl_2	Ac_2O (3), Py (6)	r.t.	20	82%	1:2.1	13%
3	CH_3CN	Ac_2O (3), Py (6)	r.t.	21	85%	1:2.0	4%
4	EtOAc	Ac_2O (3), Py (6)	r.t.	24	84%	1:1.3	5%

[a] Concentration of substrate was 0.2 M.

[b] Isolated yield in 2 steps.

of the α-anomer (entry 1). Other solvents were shown to be less suitable (entry 3 and 4). Interestingly, acetylation of the axial iodide **14** afforded only α-anomer **10** under any reaction conditions.

As expected, methallylation of the iodide **9** now provided the desired equatorial product **11** as a major (**11**:**12**=14.9:1) (Table 1, entry 3). In addition, the axial iodide **10** was also led predominantly to **11** (**11**:**12**=4.2:1) (entry 4). Noticeably, the product ratio in the latter case was nearly identical to that observed for the α-anomer of equatorial iodide **9** (entry 2–4). These results indicate that no significant anomerization occurs and anomeric configuration of the acetate is a decisive factor for stereoselective methallylation.

Having established a stereoselective access to the methallylated derivative **11**, our experimentation was continued to lead the latter to UDP-2-(2-ketopropyl)galactose (**1**) (Scheme 3). Namely, a mixture of the methallylated compounds **11** and **12** (**11**:**12**=14.9:1) produced from the iodide **9** was subjected to deprotection of the DTBS group by using HF•Py in THF, which was followed by acetylation to afford a mixture of tetraacetates **5** and **6** in 89 % yield. Subsequent conversion to **1** was conducted based on the reported procedure [21]. It was commenced by partial deacetylation with hydrazine acetate in DMF, giving the hemiacetal **15**, which was readily isolated by simple chromatographic purification. Subsequent coupling with dibenzyl *N*,*N*-diisopropylphosphoramidite and ozonolysis to provide the phosphate **17**, which was isolated as a single isomer in high yield. Finally, debenzylation, coupling with the UMP component, and deprotection of acetyl groups afforded the aimed compound **1**.

In conclusion, we developed a renewed procedure to prepare UDP-2-(2-ketopropyl)galactose (**1**), through exploitation of 4,6-*O*-DTBS protecting group as an α-directing element. The present procedure gave UDP-2-(2-ketopropyl)galactose (**1**) in 22 % overall yield. Furthermore, it was revealed that configuration of anomeric acetate affected stereoselectivity of the radical methallylation at *C*-2 position.

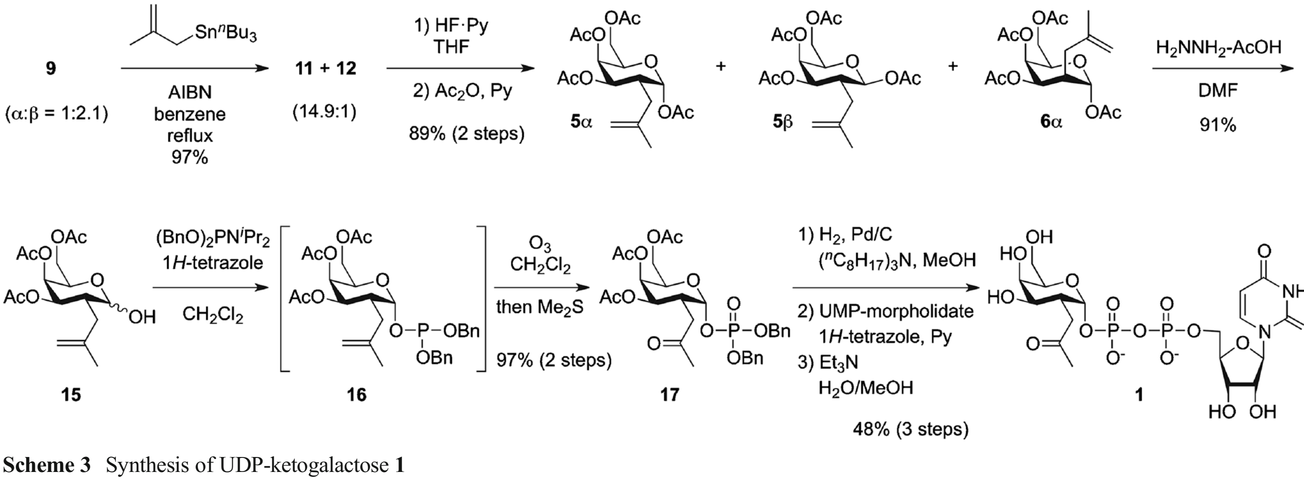
Experimental

General methods

All moisture-sensitive reactions were carried out in dehydrated solvents (Kanto Chemical Co., Inc.) under nitrogen atmosphere. Column chromatography was performed on silica gel 60 N (spherical, neutral, 40–100 μm, Kanto Chemical Co., Inc.). Optical rotations were measured by a JASCO DIP 370 polarimeter. NMR spectra were recorded on a JEOL ECX 400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C, 162 MHz for ^{31}P) in the indicated solvent. Chemical shifts are reported in ppm related to internal $CHCl_3$ (7.26 ppm) in $CDCl_3$ or DHO (4.79 ppm) in D_2O for ^{1}H, $CDCl_3$ (77.0 ppm) in $CDCl_3$ or native scale in D_2O for ^{13}C, and external H_3PO_4 (0.00 ppm) in D_2O for ^{31}P. ESI mass spectra were recorded on a Waters SYNAPT G2 mass spectrometer.

3-*O*-Acetyl-1,5-anhydro-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-D-*lyxo*-hex-1-enitol (8)

To a solution of D-galactal (**7**) (1.36 g, 9.31 mmol) in dry pyridine (279 mL) was added di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (3.4 mL, 10.4 mmol) at room temperature. After being stirred for 3 h at the same temperature, acetic anhydride (2.7 mL, 28.6 mmol) was added to the reaction mixture at room temperature. After being stirred for 1 h at the same temperature, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc, washed with ice-cooled 1 N HCl solution, saturated $NaHCO_3$ solution, brine, and dried over $MgSO_4$. After filtration and removal of the solvent *in vacuo*, the residue was purified by column chromatography on silica gel (hexane/EtOAc=30/1) to give **8** (2.28 g, 6.94 mmol, 75 %). $[\alpha]^{23}_D$ +132 (*c* 1.00, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.42 (dd, *J*=6.3, 1.8 Hz, 1H, H-1), 5.25 (m, 1H, H-3), 4.81 (brd, *J*=4.5 Hz, 1H, H-4), 4.65 (dt, *J*=6.3, 1.8 Hz, 1H, H-2), 4.27 (dd, *J*=12.6, 1.8 Hz,



Scheme 3 Synthesis of UDP-ketogalactose **1**

1H, H-6), 4.23 (dd, J=12.6, 1.8 Hz, 1H, H-6), 3.87 (brs, 1H, H-5), 2.10 (s, 3H, Ac), 1.02 (s, 9H, ^{t}Bu), 1.01 (s, 9H, ^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 145.7, 98.4, 73.1, 67.6, 67.3, 65.0, 27.6, 26.9, 23.3, 20.9, 20.8; HR-ESI-MS: $[M+Na]^+$ m/z calcd for $C_{16}H_{28}O_5SiNa$ 351.1604, found 351.1595.

1,3-Di-*O*-acetyl-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-2-iodo-D-galactopyranose (9, 10)

Method A using acetic acid as a nucleophile

To a solution of **8** (1.44 g, 4.38 mmol) and acetic acid (1.3 mL, 22.7 mmol) in dry CH_2Cl_2 (88 mL) was added *N*-iodosuccinimide (1.49 g, 6.62 mmol) at room temperature. After being stirred for 2 h at the same temperature, a mixture of 1 M $Na_2S_2O_3$ solution and saturated $NaHCO_3$ solution was added to the reaction mixture at room temperature. After being stirred for 5 min at the same temperature, the organic layer was separated and washed with a mixture of 1 M $Na_2S_2O_3$ solution and saturated $NaHCO_3$ solution, brine, and dried over $MgSO_4$. After filtration and removal of the solvent *in vacuo*, the residue was dissolved in pyridine (26 mL). Acetic anhydride (26 mL) was added to this solution at room temperature. After being stirred for 63 h at the same temperature, the reaction mixture was concentrated *in vacuo* azeotropically with toluene. The residue was purified by column chromatography on silica gel (hexane/EtOAc=10/1 for **9** then 2/1 for **10**) to give a mixture of **9** (1.92 g, 3.73 mmol, 85 %, α:β=13:1) and **10** (0.260 g, 0.505 mmol, 11 %).

Method B using water as a nucleophile

To a solution of **8** (1.09 g, 3.32 mmol) and H_2O (3.3 mL) in CH_2Cl_2 (66 mL) was added *N*-iodosuccinimide (2.24 g, 9.96 mmol) at room temperature. After being stirred for 3 h at the same temperature, a mixture of 1 M $Na_2S_2O_3$ solution and saturated $NaHCO_3$ solution was added to the reaction mixture at room temperature. After being stirred for 5 min at the same temperature, the organic layer was separated and washed with a mixture of 1 M $Na_2S_2O_3$ solution and saturated $NaHCO_3$ solution, brine, and dried over $MgSO_4$. After filtration and removal of the solvent *in vacuo*, the residue was dissolved in CH_2Cl_2 (16 mL). To this solution was added pyridine (1.62 mL, 20.0 mmol) and acetic anhydride (0.95 mL, 10.1 mmol) at room temperature. After being stirred for 20 h at the same temperature, the reaction mixture was concentrated *in vacuo* azeotropically with toluene. The residue was purified by column chromatography on silica gel (hexane/EtOAc=10/1 for **9** then 1/1 for **10**) to give **9** (1.40 g, 2.72 mmol, 82 %, α:β=1:2.1) and **10** (0.217 g, 0.422 mmol, 13 %). **9**: HR-ESI-MS: $[M+Na]^+$ m/z calcd for $C_{18}H_{31}O_7ISiNa$ 537.0781, found 537.0779; α-anomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.41 (d, J=3.2 Hz, 1H, H-1), 5.31 (dd, J=11.7, 2.7 Hz, 1H, H-3), 4.60 (m, 1H, H-4), 4.59 (dd, J=11.7, 3.2 Hz, 1H, H-2), 4.19 (dd, J=12.8, 2.3 Hz, 1H, H-6), 4.14 (dd, J=12.8, 1.6 Hz, 1H, H-6), 3.92 (brs, 1H, H-5), 2.17 (s, 3H, Ac), 2.14 (s, 3H, Ac), 1.03 (s, 9H, ^{t}Bu), 1.00 (s, 9H, ^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 168.9, 92.8, 73.0, 70.7, 69.2, 66.7, 27.5, 27.2, 23.2, 23.1, 20.9, 20.8, 20.7; β-anomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.86 (d, J=9.4 Hz, 1H, H-1), 4.87 (dd, J=11.6, 3.1 Hz, 1H, H-3), 4.48 (m, 1H, H-4), 4.32 (dd, J=11.6, 9.4 Hz, 1H, H-2), 4.17–4.26 (m, 2H, H-6), 3.64 (m, 1H, H-5), 2.18 (s, 3H, Ac), 2.15 (s, 3H, Ac), 1.04 (s, 9H, ^{t}Bu), 1.00 (s, 9H, ^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 169.0, 94.5, 76.7, 72.3, 70.1, 66.5, 27.5, 27.3, 25.9, 23.2, 20.8 (two carbons), 20.7. **10**: $[\alpha]^{27}_D$ +92.6 (*c* 1.03, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.59 (brs, 1H, H-1), 4.73 (m, 1H, H-4), 4.62 (dd, J=5.4, 3.6 Hz, 1H, H-3), 4.30 (dd, J=13.0, 3.6 Hz, 1H, H-6), 4.29 (m, 1H, H-2), 4.21 (dd, J=13.0, 1.8 Hz, 1H, H-6), 3.97 (m, 1H, H-5), 2.20 (s, 3H, Ac), 2.09 (s, 3H, Ac), 1.13 (s, 9H, ^{t}Bu), 1.03 (s, 9H, ^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 168.4, 97.3, 69.4, 68.0, 67.1, 66.9, 27.9, 27.1, 23.2, 21.1, 20.93, 20.86, 17.5; HR-ESI-MS: $[M+Na]^+$ m/z calcd for $C_{18}H_{31}O_7ISiNa$ 537.0781, found 537.0781.

*General procedure for methallylation of 2-iodo-sugars (**9** and **10**)*

A solution of **9** or **10** and methallyltri-*n*-butyltin (12 eq) in benzene (10 mL/mmol) bubbled with nitrogen gas for 30 min. To the resulting solution was added AIBN (0.2 eq) at room temperature. After being stirred for 80 min under reflux, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane/EtOAc=15/1) to give 2-methallylated products (**11α**, **11β**, **12α**). HR-ESI-MS: $[M+Na]^+$ m/z calcd for $C_{22}H_{38}O_7SiNa$ 465.2284, found 465.2275; **11α**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.06 (d, J=3.6 Hz, 1H, H-1), 4.92 (dd, J=11.7, 2.7 Hz, 1H, H-3), 4.77 (brs, 1H, vinyl), 4.61 (brs, 1H, vinyl), 4.56 (brd, J=2.7 Hz, 1H, H-4), 4.20 (dd, J=12.6, 2.2 Hz, 1H, H-6), 4.14 (dd, J=12.6, 1.8 Hz, 1H, H-6), 3.75 (brs, 1H, H-5), 2.80 (dddd, J=11.7, 10.8, 4.9, 3.6 Hz, 1H, H-2), 2.21 (dd, J=13.9, 4.9 Hz, 1H, allyl), 2.11 (s, 3H, Ac), 2.09 (s, 3H, Ac), 1.92 (dd, J=13.9, 10.8 Hz, 1H, allyl), 1.73 (brs, 3H, Me), 1.04 (s, 9H, ^{t}Bu), 1.01 (s, 9H, ^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 169.4, 141.7, 112.9, 92.8, 72.6, 69.2, 69.1, 67.0, 35.3, 33.7, 27.5, 27.2, 23.2, 21.7, 20.88, 20.86, 20.7. **11β**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.50 (d, J=9.0 Hz, 1H, H-1), 4.67 (brs, 1H, vinyl), 4.61 (brs, 1H, vinyl), 4.58 (dd, J=11.2, 2.7 Hz, 1H, H-3), 4.47 (brd, J=2.7 Hz, 1H, H-4), 4.16–4.23 (m, 2H, H-6), 3.50 (m, 1H, H-5), 2.60 (m, 1H, H-2), 2.01–2.21 (m, 2H, allyl), 2.08 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.76 (brs, 3H, Me), 1.06 (s, 9H, ^{t}Bu), 1.00 (s, 9H, ^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 169.4, 143.4, 111.6, 94.9, 75.1, 71.5, 68.5, 66.9, 36.4, 35.7, 27.4, 27.3, 23.2, 21.9, 20.9, 20.72, 20.66.

12α: ^{1}H NMR (400 MHz, CDCl$_3$): δ 6.18 (brs, 1H, H-1), 5.12 (dd, *J*=5.8, 3.1 Hz, 1H, H-3), 4.83 (brs, 1H, vinyl), 4.76 (brs, 1H, vinyl), 4.63 (brd, *J*=3.1 Hz, 1H, H-4), 4.29 (dd, *J*=13.0, 3.6 Hz, 1H, H-6), 4.19 (dd, *J*=13.0, 1.3 Hz, 1H, H-6), 3.88 (m, 1H, H-5), 2.69 (dd, *J*=15.2, 11.2 Hz, 1H, allyl), 2.50 (brd, *J*=15.2 Hz, 1H, allyl), 2.14 (s, 3H, Ac), 2.10–2.18 (m, 1H, H-2), 2.07 (s, 3H, Ac), 1.71 (brs, 3H, Me), 1.07 (s, 9H, ^{t}Bu), 1.02 (s, 9H, ^{t}Bu); ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.5, 169.0, 143.3, 112.1, 94.8, 69.6, 69.1, 68.7, 67.3, 37.2, 34.4, 27.7, 27.2, 23.1, 22.2, 21.1, 21.0, 20.6.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-methallyl-D-galactopyranose (5, 6)

A mixture of **11α**, **11β**, and **12α** (3.8:11.1:1; 0.835 g, 1.89 mmol) in dry THF (20 mL) was added hydrogen fluoride pyridine (0.9 mL) at room temperature. After being stirred for 70 min at the same temperature, EtOAc (50 mL) and saturated NaHCO$_3$ solution (50 mL) was added to the reaction mixture at room temperature. After being stirred for 5 min at the same temperature, the reaction mixture was extracted with EtOAc (150 mL). The aqueous layer was further extracted with EtOAc (50 mL) three times. The combined organic layer was dried over MgSO$_4$. After filtration and removal of the solvent *in vacuo*, the residue was dissolved in pyridine (12 mL). Acetic anhydride (12 mL) was added to this solution at room temperature. After being stirred for 16 h at the same temperature, the reaction mixture was concentrated *in vacuo* azeotropically with toluene. The residue was purified by column chromatography on silica gel (hexane/EtOAc=9/2) to give 0.651 g (89 %) of corresponding tetraacetates (**5α**:**5β**:**6α**=3.4:10.7:1). HR-ESI-MS: [M+Na]$^+$ *m/z* calcd for $C_{18}H_{26}O_9Na$ 409.1475, found 409.1469; **5α**: ^{1}H NMR (400 MHz, CDCl$_3$): δ 6.07 (d, *J*=3.1 Hz, 1H, H-1), 5.34 (m, 1H, H-4), 5.12 (dd, *J*=11.7, 3.1 Hz, 1H, H-3), 4.77 (brs, 1H, vinyl), 4.61 (brs, 1H, vinyl), 4.21 (ddd, *J*=7.2, 6.7, 0.9 Hz, 1H, H-5), 4.09 (dd, *J*=11.7, 7.2 Hz, 1H, H-6), 4.04 (dd, *J*=11.7, 6.7 Hz, 1H, H-6), 2.53 (m, 1H, H-2), 2.15 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.10–2.20 (m, 1H, allyl), 2.01 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.91 (dd, *J*=13.9, 10.3 Hz, 1H, allyl), 1.72 (brs, 3H, Me); ^{13}C NMR (100 MHz, CDCl$_3$): δ 170.44, 170.35, 170.27, 169.0, 141.1, 113.3, 92.0, 69.6, 68.4, 66.2, 61.5, 35.3, 35.2, 21.9, 20.8, 20.70, 20.68, 20.65; **5β**: ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.55 (d, *J*=9.4 Hz, 1H, H-1), 5.27 (dd, *J*=3.1, 0.9 Hz, 1H, H-4), 4.84 (dd, *J*=11.7, 3.1 Hz, 1H, H-3), 4.67 (brs, 1H, vinyl), 4.61 (brs, 1H, vinyl), 4.14 (dd, *J*=11.2, 7.2 Hz, 1H, H-6), 4.09 (dd, *J*=11.2, 6.7 Hz, 1H, H-6), 3.95 (ddd, *J*=7.2, 6.7, 0.9 Hz, 1H, H-5), 2.40 (m, 1H, H-2), 2.14 (s, 3H, Ac), 2.10–2.19 (m, 1H, allyl), 2.08 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.00–2.09 (m, 1H, allyl), 1.97 (s, 3H, Ac), 1.73 (brs, 3H, Me); ^{13}C NMR (100 MHz, CDCl$_3$): δ 170.4, 170.3, 170.0, 168.9, 142.7, 112.2, 94.7, 72.0, 71.4, 65.6, 61.4, 37.3, 36.4, 21.9, 20.8, 20.70, 20.65, 20.5; **6α**: ^{1}H NMR (400 MHz, CDCl$_3$): δ 6.13 (brs, 1H, H-1), 5.36 (dd, *J*=5.8, 3.6 Hz, 1H, H-3), 5.34 (m, 1H, H-4), 4.85 (brs, 1H, vinyl), 4.79 (brs, 1H, vinyl), 4.29 (dt, *J*=1.8, 6.7 Hz, 1H, H-5), 4.15 (dd, *J*=11.2, 6.7 Hz, 1H, H-6), 4.08 (dd, *J*=11.2, 6.7 Hz, 1H, H-6), 2.43 (dd, *J*=14.8, 11.2 Hz, 1H, allyl), 2.32 (brd, *J*=14.8 Hz, 1H, allyl), 2.11–2.20 (m, 1H, H-2), 2.11 (s, 6H, two Acs), 2.033 (s, 3H, Ac), 2.026 (s, 3H, Ac), 1.70 (brs, 3H, Me); ^{13}C NMR (100 MHz, CDCl$_3$): δ 170.5, 170.0, 169.9, 168.9, 142.5, 113.2, 93.7, 68.5, 66.6, 66.3, 61.7, 37.7, 34.2, 22.0, 21.1, 20.79, 20.72, 20.67.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-methallyl-D-galactose (15)

To a solution of the mixture of **5α**, **5β**, and **6α** (3.4:10.7:1; 0.201 g, 0.520 mmol) in dry DMF (1.8 mL) was added a powdered hydrazine acetate (95.8 mg, 1.04 mmol) at room temperature. During being stirred for 4 h at the same temperature, hydrazine acetate (50.2 mg, 0.545 mmol) in DMF (2 mL) was hourly added to the reaction mixture three times. The reaction mixture was diluted with EtOAc (60 mL) and washed with brine (40 mL). The aqueous layer was extracted with EtOAc three times and the combined organic layer was washed with brine and dried over MgSO$_4$. After filtration and removal of the solvent *in vacuo*, the residue was purified by column chromatography on silica gel (hexane/EtOAc=3/1) to give **15** (0.163 g, 0.473 mmol, 91 %, α:β=9:1 in CDCl$_3$). $[\alpha]^{22}_{D}$ +85.2 (*c* 1.01, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) α-anomer: δ 5.34 (dd, *J*=3.1, 1.3 Hz, 1H, H-4), 5.26 (d, *J*=3.1 Hz, 1H, H-1), 5.16 (dd, *J*=11.7, 3.1 Hz, 1H, H-3), 4.80 (brs, 1H, vinyl), 4.78 (brs, 1H, vinyl), 4.43 (ddd, *J*=6.7, 6.3, 1.3 Hz, 1H, H-5), 4.11 (dd, *J*=11.2, 6.3 Hz, 1H, H-6), 4.07 (dd, *J*=11.2, 6.7 Hz, 1H, H-6), 2.39 (dddd, *J*=11.7, 9.4, 5.4, 3.1 Hz, 1H, H-2), 2.15 (s, 3H, Ac), 2.09–2.17 (m, 2H, allyl), 2.05 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.75 (brs, 3H, Me); β-anomer: δ 5.25 (m, 1H, H-4), 4.84 (dd, *J*=11.2, 3.2 Hz, 1H, H-3), 4.75 (brs, 1H, vinyl), 4.71 (brs, 1H, vinyl), 4.64 (d, *J*=8.5 Hz, 1H, H-1), 4.15 (dd, *J*=11.7, 6.3 Hz, 1H, H-6), 4.12 (dd, *J*=11.7, 6.7 Hz, 1H, H-6), 3.88 (ddd, *J*=6.7, 6.3, 0.9 Hz, 1H, H-5), 2.20 (m, 1H, H-2), 2.11 (s, 3H, Ac), 2.09–2.17 (m, 2H, allyl), 2.05 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.77 (brs, 3H, Me); ^{13}C NMR (100 MHz, CDCl$_3$) α-anomer: δ 170.6, 170.39, 170.38, 142.2, 112.5, 93.2, 69.5, 67.1, 66.4, 62.4, 36.4, 35.3, 22.3, 20.73, 20.71, 20.70; β-anomer: δ 170.6, 170.4, 170.2, 143.5, 112.2, 98.0, 72.1, 70.7, 66.1, 62.0, 40.3, 36.3, 22.6, 20.69, 20.67, 20.5; HR-ESI-MS: [M+Na]$^+$ *m/z* calcd for $C_{16}H_{24}O_8Na$ 367.1369, found 367.1360.

Dibenzyl (2-acetonyl-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl) phosphate (17)

To a solution of **15** (77.4 mg, 0.225 mmol) and 1*H*-tetrazole (63.0 mg, 0.899 mmol) in dry CH$_2$Cl$_2$ (1.6 mL) was added dibenzyl *N*,*N*-diisopropylphosphoramidite (190 μL, 0.571 mmol) at −30 °C. After the reaction mixture was slowly warmed up to room temperature for 3 h, dibenzyl *N*,*N*-diisopropylphosphoramidite (38 μL, 0.11 mmol) was added to the reaction mixture at room temperature. After being stirred for 1 h at the same temperature, the reaction mixture was diluted with Et_2O (30 mL), washed with cold brine twice, and dried over $MgSO_4$. After filtration and removal of the solvent *in vacuo*, the residue was dissolved in dry CH_2Cl_2 (1.8 mL). Ozone was bubbled into this solution at −78 °C until a blue color became obvious (2 min). Oxygen was then bubbled into the reaction mixture. After the blue color disappeared, dimethylsulfide (1.3 mL) was added to the reaction mixture at −78 °C. After being stirred overnight at room temperature, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane/EtOAc=1/1) to give **17** (0.132 g, 0.218 mmol, 97 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30–7.40 (m, 10H, Ph), 5.87 (dd, *J*=6.3, 2.9 Hz, 1H, H-1), 5.31 (m, 1H, H-4), 4.98–5.12 (m, 4H, benzyl), 4.95 (dd, *J*=12.1, 3.1 Hz, 1H, H-3), 4.27 (brdd, *J*=6.7, 6.3 Hz, 1H, H-5), 4.06 (dd, *J*=11.2, 6.3 Hz, 1H, H-6), 3.95 (dd, *J*=11.2, 6.7 Hz, 1H, H-6), 2.87 (m, 1H, H-2), 2.38 (dd, *J*=18.4, 8.1 Hz, 1H, acetonyl), 2.36 (dd, *J*=18.4, 5.4 Hz, 1H, acetonyl), 2.12 (s, 3H), 1.97 (s, 3H), 1.93 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 205.9, 170.24, 170.17, 170.0, 135.4 (d, J_{CP}=6.6 Hz), 135.3 (d, J_{CP}=6.6 Hz), 128.7, 128.63, 128.60, 128.0, 97.8 (d, J_{CP}=6.6 Hz), 69.6 (d, J_{CP}=5.6 Hz), 69.5 (d, J_{CP}=5.6 Hz), 68.3, 68.0, 65.9, 61.7, 39.6, 34.3 (d, J_{CP}=7.5 Hz), 29.9, 20.6 (two carbons), 20.5.

Uridine diphosphate 2-deoxy-2-(2-oxopropyl)-α-D-galactopyranose (1)

To a solution of **17** (0.148 g, 0.244 mmol) and tri-*n*-octylamine (65 μL, 0.147 mmol) in dry MeOH (3.7 mL) was added palladium on carbon (10 %, 24.2 mg) at room temperature. After being stirred for 24 h at the same temperature under H_2, the reaction mixture was filtered through Celite® and concentrated *in vacuo*. The residue was dissolved in dry pyridine (2 mL). To this solution was added uridine 5′-monophosphomorpholidate 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt (0.252 g, 0.367 mmol), activated 4 A molecular sieves (ca. 300 mg), and 1*H*-tetrazole (68.6 mg, 0.545 mmol) in dry pyridine (1 mL) at room temperature. After being stirred for 3 days at the same temperature, the reaction mixture was filtered through Celite® and concentrated *in vacuo*. The residue was dissolved in MeOH (3 mL). To this solution was added H_2O (1.5 mL) and triethylamine (0.75 mL) at room temperature. After being stirred for 23 h at the same temperature, the reaction mixture was diluted with H_2O (20 mL), washed with CH_2Cl_2 (30 mL) twice. The aqueous layer was lyophilized and the residue was purified by short-path column chromatography using Sep-Pak® C18 cartridge with 0.1 M NH_4HCO_3 solution, gel permeation column chromatography on Sephadex G-15 with 0.1 M NH_4HCO_3 solution and then lyophilization to give **1** (69.9 mg, 0.116 mmol, 48 %). After extensive purification by HPLC using ODS column (Mightysil RP-18, 20×250 mm, 5 μm) with 0.1 M NH_4HCO_3 solution, 40.5 mg of **1** was provided. ^{1}H NMR (400 MHz, D_2O): δ 7.94 (d, *J*=8.1 Hz, 1H, H-6″), 5.95 (d, *J*=2.7 Hz, 1H, H-1′), 5.94 (d, *J*=8.1 Hz, 1H, H-5″), 5.55 (dd, *J*=7.2, 3.1 Hz, 1H, H-1), 4.31–4.37 (m, 2H, H-2′,3′), 4.25 (m, 1H, H-4′), 4.21 (ddd, *J*=11.7, 4.5, 2.2 Hz, 1H, H-5′), 4.15 (ddd, *J*=11.7, 5.4, 3.1 Hz, 1H, H-5′), 4.12 (brdd, *J*=6.7, 5.2 Hz, 1H, H-5), 3.86 (brd, *J*=3.1 Hz, 1H, H-4), 3.75 (dd, *J*=11.2, 3.1 Hz, 1H, H-3), 3.73 (dd, *J*=11.7, 6.7 Hz, 1H, H-6), 3.68 (dd, *J*=11.7, 5.2 Hz, 1H, H-6), 2.79 (dd, *J*=17.9, 5.8 Hz, 1H, acetonyl), 2.73 (dd, *J*=17.9, 7.2 Hz, 1H, acetonyl), 2.50 (m, 1H, H-2), 2.23 (s, 3H, acetonyl); ^{13}C NMR (100 MHz, D_2O): δ 214.3, 166.3, 151.8, 141.7, 102.6, 96.2 (d, J_{CP}=6.6 Hz), 88.5, 83.2 (d, J_{CP}=9.4 Hz), 73.8, 71.9, 69.6, 68.2, 68.0, 64.9 (d, J_{CP}=5.6 Hz), 61.4, 41.2, 36.8 (d, J_{CP}=8.5 Hz), 29.9; ^{31}P NMR (162 MHz, D_2O) δ –11.42 (brd, *J*=20.1 Hz), −12.73 (brdd, *J*=20.1, 5.3 Hz); HR-ESI-MS: $[M+Na]^+$ *m*/*z* calcd for $C_{18}H_{28}N_2O_{17}P_2Na$ 629.0761, found 629.0750.

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