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First total synthesis of 6-tuliposide B

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ABSTRACT

Labile (+)-6-tuliposide B, an antimicrobial compound produced by tulip, was synthesized in nine steps from D-glucose via the Baylis–Hillman reaction of 2-(*tert*-butyldimethylsilyloxy)-acetaldehyde with 6-*O*-acryloyl-1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside, followed by a mild deprotection procedure using TFA in CH_2Cl_2.

1. Introduction

Collectively, plants produce a diverse array of low molecular weight natural products numbering over 100,000. This rich diversity is the result of an evolutionary process driven by selection for improved defense against microbial, insect, or animal attack.[1] The simplest definition of such biologically active substances recognizes phytoalexins, which are synthesized de novo, and phytoanticipins as pre-formed infection inhibitors.[2]

Tulip cultivars accumulate phytoanticipins, named 6-tuliposide B **1** and 6-tuliposide A **3**, in flowers, stems, leaves, and bulbs. The non-sugar groups released by enzymatic or spontaneous hydrolysis of tuliposides are easily converted to the corresponding α-methylene-γ-butyrolactones, tulipalin B **2** and tulipalin A **4**, under non-alkaline conditions[3] (Fig. 1). Although **3** and **4** are known to cause allergic contact dermatitis, **1** is non-allergenic.[4]

Recently, we found compound **1** to have potent antimicrobial activity against Gram-positive, Gram-negative, and certain fungicide-tolerant strains of bacteria, but it was not active against yeast. Both compounds **1** and **3** were detected in tulip tissues such as petal, pistil, leaf, stem, bulb scale, and root. In the anther, **1** was detected, however **3**, which is a precursor of the insecticidal compound **4**,[5] was not detected under the cultivation conditions used in the agricultural field. These observations suggest that the anther-specific production of **1** is a novel defense mechanism evolved in tulips to protect pollens from bacterial infection that could be introduced during the reproductive process.[6] To verify this hypothesis and survey the potential of **1** as an antimicrobial agent against multidrug-resistant microorganisms, we undertook the total synthesis of **1**.

Several synthetic methods to produce **2** have already appeared,[7] however, the total synthesis of **1** or **3** has not been reported, although there have been three unsuccessful attempts,[8] including our effort.[9] These failures probably stem from the extremely labile nature of these tuliposides. Herein, we report the first total synthesis of labile tuliposide B **1**.

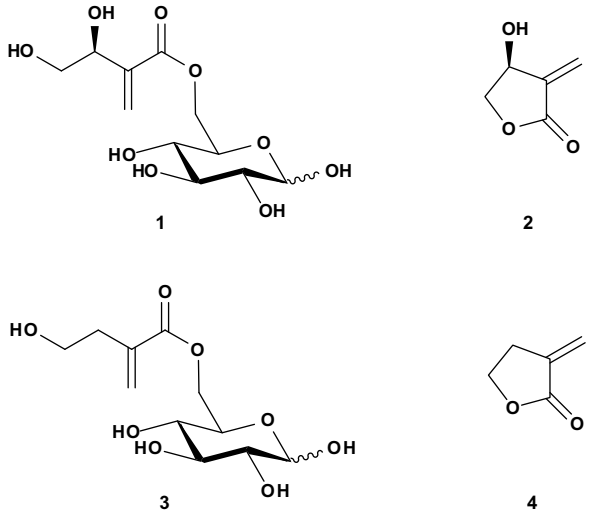

Figure 1. Structures of tuliposides B **1** and A **3**, and tulipalins B **2** and A **4**.

2. Results and discussion

2.1. Preparation of 6-*O*-acryloyl glucosides 8a–8f

Sugar acrylates **8a** and **8b** depicted in Table 1 were prepared as previously described.[9] The synthesis of 2-trimethylsilylethyl (TMSET) glucoside derivative **8c** began with 2,3,4,6-tetra-*O*-acetyl-1-*O*-(2-triethylsilylethyl)-β-D-glucopyranoside **5**, which was prepared from D-glucose in three steps according to the reported procedure,[10] shown in Scheme 1. Methanolysis of the acetate gave

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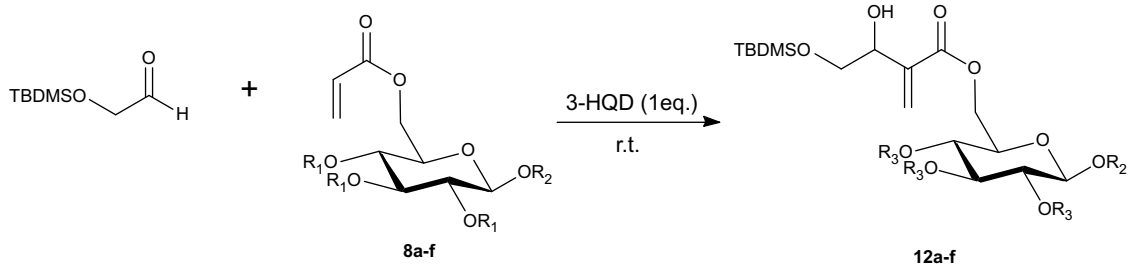
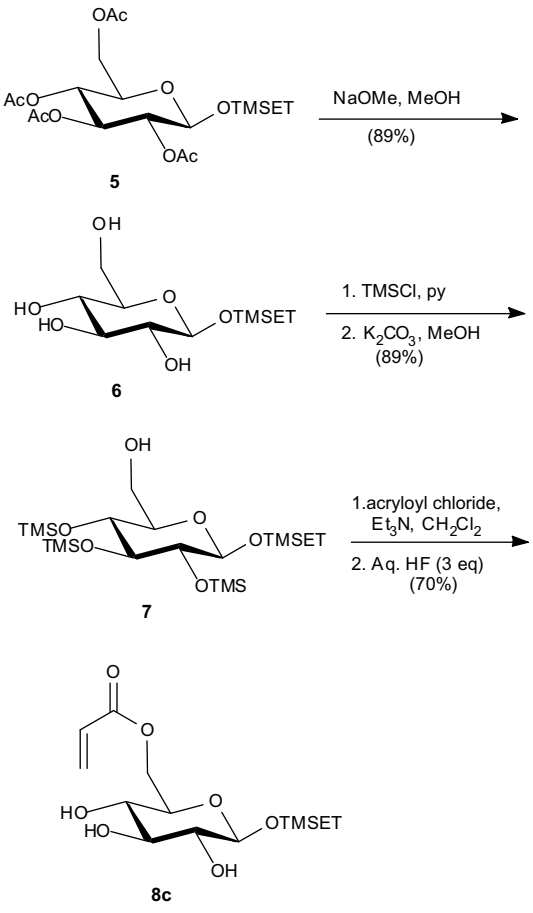
Table 1
Baylis–Hillman reaction of *tert*-butyldimethylsilyloxy-acetaldehyde and sugar acrylates[a]



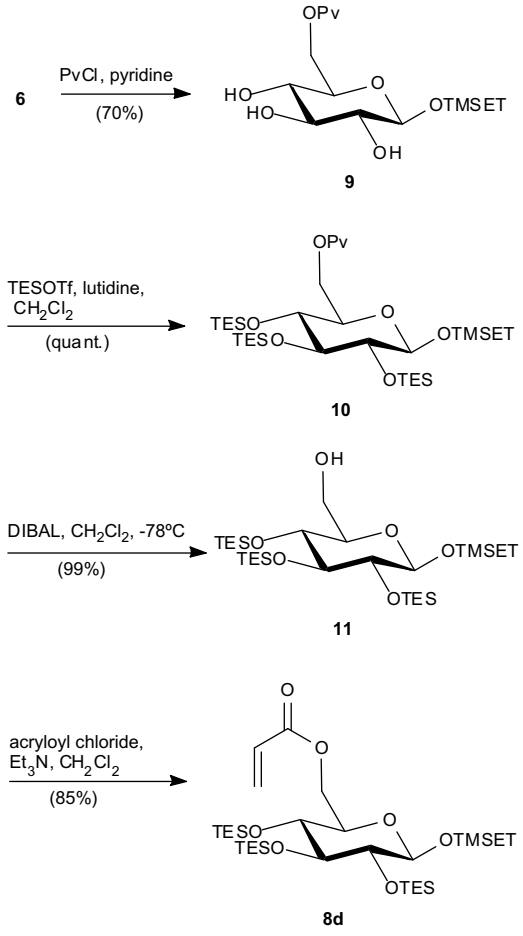
Entry	Acrylate	R_1	R_2	Product	Solvent	Time	Yield (%)
1	**8a**	TMS	Me	**12a** (R_3 = H)	DMSO	8 days	29
2	**8b**	TES	Me	**12b** (R_3 = TES)	DMSO	15 days	47
3	**8c**	H	TMSET	**12c** (R_3 = H)	MeCN	44 h	27
4	**8c**	H	TMSET	**12c** (R_3 = H)	DMSO	40 h	40
5	**8d**	TES	TMSET	**12d** (R_3 = TES)	DMSO	23 days	0[b]
6	**8e**	TES	TCE	**12e** (R_3 = TES)	DMSO	15 days	50
7	**8f**[c]	TES	TES	**12f**[c] (R_3 = TES)	DMSO	30 days	6

[a] Reaction was run with 3 equiv of acetaldehyde.
[b] Starting material **8c** was recovered in 51% yield.
[c] Diastereomeric mixture (1:1) at the anomeric center of the sugar.

tetraol **6** and trimethylsilylation of **6** was followed by selective deprotection[11] of the TMS group at C-6 to produce free alcohol **7**. Acylation of **7** with acryloyl chloride followed by HF treatment led to the formation of 6-*O*-acryloyl-1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside **8c**.



Scheme 1. Synthesis of TMSET glucoside **8c**.



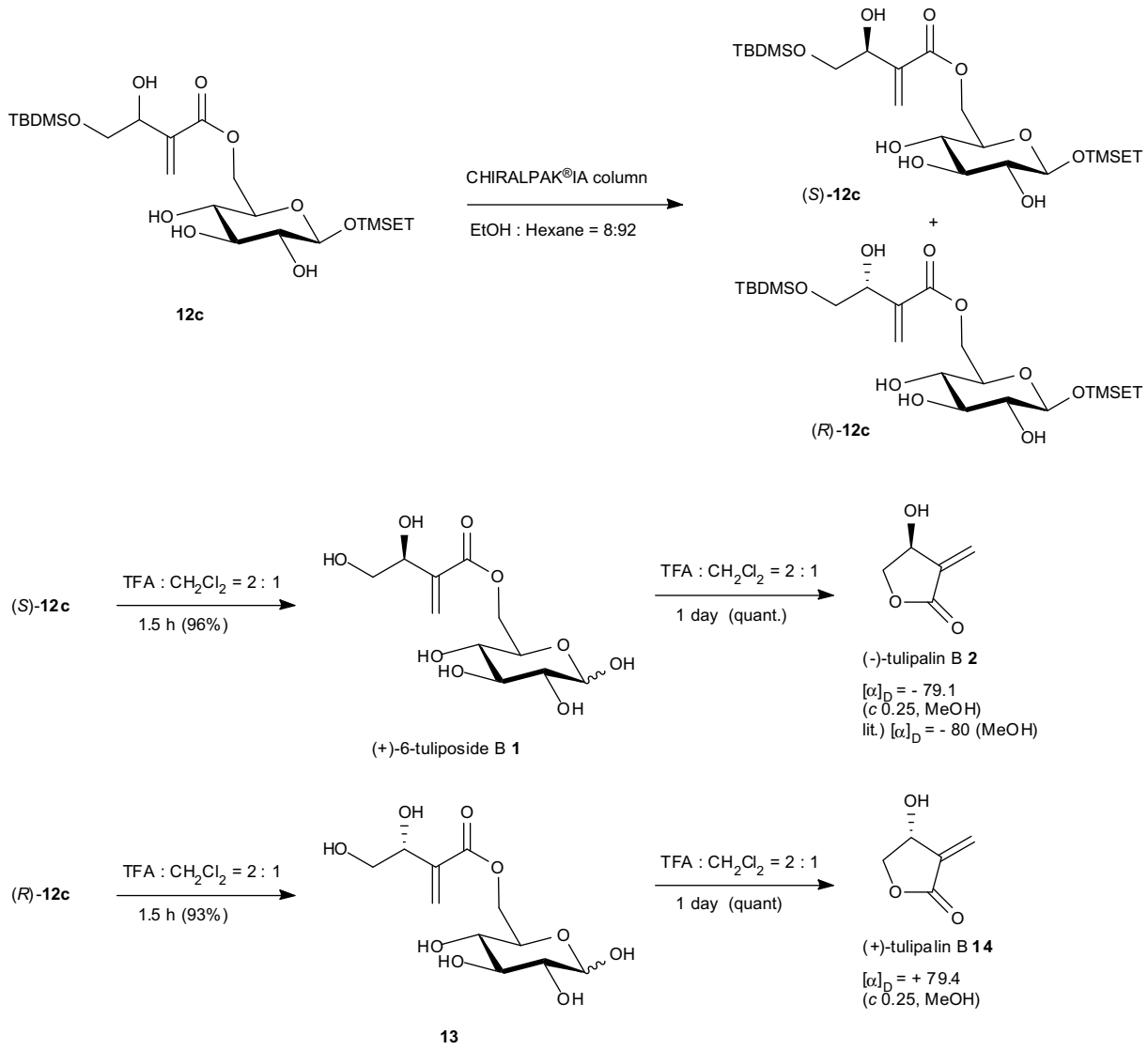
Scheme 2. Synthesis of TMSET glucoside **8d**.

Scheme 2 shows the synthesis of 6-*O*-acryloyl-2,3,4-tri-*O*-triethylsilyl-1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside **8d**. Protection of **6** with pivaloyl chloride provided monopivaloyl derivative **9** in 70% yield which was converted in quantitative yield to the TES derivative **10** using TESOTf. Removal of the pivaloyl group was achieved by DIBAL reduction to give the free alcohol in **11** in 99% yield and acylation of **11** with acryloyl chloride produced compound **8d** in 85% yield.

2,2,2-Trichloroethyl (TCE) glucoside **8e** and triethylsilyl glucoside **8f** as depicted in Table 1 were synthesized by procedures similar to those used for the synthesis of TMSET glucoside **8d**, shown in Scheme 2.

2.2. Baylis–Hillman reaction of sugar acrylates 8a–f with 2-(*tert*-butyldimethylsilyloxy)-acetaldehyde

The results of the Baylis–Hillman reaction of sugar acrylates **8a**–**f** with 2-(*tert*-butyldimethylsilyloxy)-acetaldehyde, easily prepared from 1,4-di-(*tert*-butyldimethylsilyloxy)-2-butene,[12] are summarized in Table 1. During the Baylis–Hillman reaction using 3-HQD (3-hydroxyquinuclidine),[13] the loss of the TMS groups from the methyl glucoside **8a** was observed to give **12a** in 29% yield, whereas the TES derivative **8b** produced the desired Baylis–Hillman product **12b** in moderate yield without loss of the protective groups. The yield of the Baylis–Hillman reaction for the TES-protected methyl glucoside **8b** was 47% and better than the yield in the case of TMS-protected methyl glucoside **8a** as shown in entries 1 and 2. However, the Baylis–Hillman reaction of **8d** did not give the desired products even after 23 days and only 51% of starting material **8d** was recovered (entry 5). The Baylis–Hillman reaction of 2,2,2-trichloroethyl glucoside acrylate **8e**, derived from 2,2,2-trichloroethyl β-D-glucoside,[14] gave **12e** in 50% yield after 15 days (entry 6), whereas another acrylate **8f** produced **12f** in 6% yield after 30 days (entry 7). The reaction of the polar and less bulky sugar acrylate **8c** with 2-(*tert*-butyldimethylsilyloxy)-acetaldehyde in the presence of 3-HQD formed the desired product **12c** in 40% yield after 40 h (entry 4). Although an aprotic polar solvent, such as DMSO or DMF, accelerated the Baylis–Hillman reaction between methyl acrylate with 2-(*tert*-butyldimethylsilyloxy)-acetaldehyde, the use of MeCN instead of DMSO as the reaction solvent resulted in a convenient separation step for the products. However, the yield of **12c** when MeCN was used was lower than when DMSO was used (entry 3).



Scheme 3. Synthesis of natural 6-tuliposide B (**1**) and its 3′*R*-epimer (**13**).

2.3. Synthesis of 6-tuliposide B 1

One of the most critical points for the total synthesis of **1** was the avoidance of the lactonization of **1** and its derivatives. Deprotection of the TES glucoside derivative **12f** was successful in giving a 1:1 mixture of **1** and **13** without lactonization. However, because of the poor yield (6%) for **12f**, suitable deprotection conditions for methyl glucoside **12b**, TMSET glucoside **12c**, and TCE glucoside **12e** were examined. Demethylation of **12b** was unsuccessful under the various conditions described previously.[9] TBDMS and TES groups of **12e** were removed by treating with 46% aqueous HF in MeCN for 2 h to give 6-*O*-(3,4-dihydroxy-2-methylene-butanoyl)-1-*O*-(2,2,2-trichloroethyl)-β-D-glucopyranoside in quantitative yield. However, deprotection of the TCE group with Zn in AcOH only produced (±)-tulipalin. We succeeded in the removal of the TMSET group from **12c** by treating with TFA in CH_2Cl_2 for 1.5 h. Scheme 3 shows the synthetic route for 6-tuliposide B **1** and its (3′*R*) epimer **13**. The diastereomeric mixture of the Baylis–Hillman adduct **12c** was separated using a chiral HPLC column (CHIRALPAK® IA. DAICEL Co. Ltd, Japan) to give (*S*)-**12c** and (*R*)-**12c**. Deprotection of (*S*)-**12c** produced **1** whose ^{1}H NMR data and ^{13}C NMR data were comparable to the reported NMR data of natural 6-tuliposide B. In contrast, the NMR data of (3′*R*)-epimer **13** derived from (*R*)-**12c** were distinguishable from those of natural **1**. In order to confirm the structure of synthetic 6-tuliposide B, we converted both diastereomers **1** and **13** into **2** and **14**, respectively. The synthetic compound **2** showed a negative sign for the specific rotation {$[\alpha]_D = -79.1$ (*c* 0.25, MeOH); $[\alpha]_D = -80$ (MeOH) in the literature[12]} that unambiguously indicates the synthetic compounds **1** and **2** to be 6-tuliposide B and tulipalin B, respectively. In contrast, compound **14** showed a positive specific rotation {$[\alpha]_D = +79.4$ (*c* 0.25, MeOH)}, which indicates **14** to be the antipode of tulipalin B and compound **13** to be (3′*R*)-epimer of 6-tuliposide B.

3. Conclusion

We have succeeded in the total synthesis of 6-tuliposide B **1**. The key point in the synthesis of **1** was to find deprotection conditions for a suitable Baylis–Hillman adduct. The Baylis–Hillman reaction of **8d** or **8f** with 2-(*tert*-butyldimethylsilyloxy)-acetaldehyde failed, probably because of the poor accessibility of 3-HQD to the bulky and less polar compound **8d** or **8f**. The reaction of the more polar and less bulky acrylates, **8b**, **8c**, and **8e**, proceeded to give the corresponding Baylis–Hillman adducts **12b**, **12c**, and **12e**, respectively. After the separation of the diastereomers, (*S*)-**12c** yielded 6-tuliposide B **1**. Thus, both stereoisomers of 6-tuliposide B **1** and **13** and the enantiomers of tulipalin B **2** and **14** are in hand. These compounds may contribute to the clarification of the defense mechanism of the tulip and the systematic structure–activity relationship of tuliposide and tulipalin.

4. Experimental

4.1. General

Unless otherwise stated, chemicals of the highest commercial purity were used without further purification. Thin-layer and silica gel column chromatographies were performed using Merck Silica Gel 60 F_{254} and Kanto Chemicals Co. Silica Gel 60 N (spherical, neutral), respectively. Chiral HPLC was performed using a DAICEL CHIRALPAK® IA column (∅ 20 mm × 20 cm) and a HITACHI L-7455 photodiode array detector at 30 °C. IR spectra were recorded on a Digilab FTS-50A. ^{1}H, ^{13}C, HH-COSY, HMBC, and HMQC NMR spectra were measured in $CDCl_3$ or methanol-d_4, with a Bruker AMX-500. Chemical shifts are reported in δ ppm using tetramethylsilane as the internal standard. Coupling constants (*J*) are given in Hertz. Mass spectra were acquired using FD and FAB techniques using a JMS-SX102A. All of the NMR and mass spectra were measured at the GC–MS and NMR Laboratory, Faculty of Agriculture, Hokkaido University. Optical rotations were determined on a JASCO DIP-370 polarimeter in ∅ 3.4 mm × 5.0 cm cells at 25 °C. Dichloromethane and acetonitrile were distilled from phosphorous oxide, and pyridine was distilled from calcium hydride.

4.1.1. 1-*O*-(2-Trimethylsilylethyl)-β-D-glucopyranoside 6

Sodium methoxide (504 μl, 28% in MeOH) was added to a solution of **5** (5.65 g, 12.6 mmol) in 90 ml of MeOH at room temperature. Stirring was continued for 1 h, whereupon the reaction mixture was neutralized by adding Amberlyst R-150 and filtered. After evaporation of the filtrate, the crude product was purified by short silica gel column chromatography (MeOH/$CHCl_3$, 1:4) to give 3.13 g of **6** (89%) as a white solid. IR (ν_{max}, cm^{-1}) 3539, 3351, 1441, 1405, 1250, 1188, 1078, 1031, 868, and 836. ^{1}H NMR (500 MHz, CD_3OD), 0.02 (9H, s, CH_2CH_2*TMS*), 1.00 (2H, m, CH_2CH_2TMS), 3.15 (1H, dd, *J* = 9.1, 7.9 Hz, H-2), 3.26–3.34 (3H, m, H-3, 4, 5), 3.59–3.67 (2H, m, H-6a and CH_2CH_2TMS), 3.86 (1H, dd, J = 11.9, 1.9 Hz, H-6b), 4.01 (1H, ddd, *J* = 11.7, 9.6, 5.6 Hz, CH_2CH_2TMS), 4.26, (1H, d, *J* = 7.9 Hz, H-1), ^{13}C NMR (125 MHz, CD_3OD) −1.4 (CH_2CH_2*TMS*), 19.1 (CH_2CH_2TMS), 62.8 (C-6), 68.1 (CH_2CH_2TMS), 71.7 (C-4), 75.1 (C-2), 77.9 (C-5), 78.2 (C-3), 103.9 (C-1), HR-FAB-MS *m*/*z* [M−H]$^-$, calcd for $C_{11}H_{23}O_6Si$, 279.1264; found, 279.1250.

4.1.2. 6-*O*-Pivaloyl-1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside 9

Pivaloyl chloride solution (1.35 ml, 1.10 mmol/30 ml of pyridine) was added dropwise to a solution of **6** (3.01 g, 10.7 mmol) in 20 ml of pyridine for 15 min. The reaction was allowed to stir at room temperature for 20 h and then at 40 °C for 4 h. Pyridine was azeotropically removed with the repeated addition of toluene, followed by evaporation which was repeated several times. The residual mixture was purified by silica gel column chromatography with 10% MeOH in $CHCl_3$. After evaporation, 2.74 g of **9** (70%) was obtained as a white solid. IR (ν_{max}, cm^{-1}) 3400, 1734, 1457, 1420, 1364, 1287, 1250, 1166, 1077, 862, 836. ^{1}H NMR (500 MHz, $CDCl_3$), 0.01 (9H, s, CH_2CH_2*TMS*), 1.01 (2H, m, CH_2CH_2TMS), 1.21 (9H, s, *t*-Bu), 3.36 (1H, d, *J* = 7.8 Hz, H-2), 3.50 (1H, ddd, *J* = 9.3, 6.8, 2.3 Hz, H-5), 3.54–3.62 (3H, m, H-3, H-4 and CH_2CH_2TMS), 3.93–3.98 (1H, m, CH_2CH_2TMS), 4.28 (1H, d, *J* = 7.8 Hz, H-1), 4.27–4.31 (1H, m, H-6a), 4.39, (1H, dd, *J* = 11.8, 2.1 Hz, H-6b), ^{13}C NMR (125 MHz, $CDCl_3$) −1.5 (CH_2CH_2*TMS*), 18.2 (CH_2CH_2TMS), 27.1 (CMe_3), 38.9 (CMe_3), 63.7 (C-6), 67.4 (CH_2CH_2TMS), 70.5 (C-4), 73.6 (C-2), 73.9 (C-5), 76.2 (C-3), 101.9 (C-1), 179.0 (carbonyl). HR-FD-MS *m*/*z* [M+H]$^+$ calcd for $C_{16}H_{33}O_7Si$, 365.1996: found, 365.1987.

4.1.3. 6-*O*-Pivaloyl-2,3,4-tri-*O*-triethylsilyl-1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside 10

Compound **9** (2.02 g, 5.55 mmol) was dissolved in 25 ml of dry CH_2Cl_2 and cooled to 0 °C. 2,6-Lutidine (3.0 ml, 25.9 mmol) and triethylsilyl trifluoromethanesulfonate (4.3 ml, 19.2 mmol) were added and the mixture was stirred for 5.5 h. The reaction mixture was diluted with 100 ml of diethyl ether and washed with 1 M HCl aqueous solution (100 ml). After washing with satd $NaHCO_3$ (100 ml) and brine (100 ml), the organic layer was dried over anhydrous Na_2SO_4. Evaporation and silica gel column chromatography (EtOAc/hexane, 1:4) produced 4.05 g of **10** (quant.) as a colorless syrup. IR (ν_{max}, cm^{-1}) 1737, 1460, 1415, 1284, 1249, 1149, 1117, 1090, 1006, 860, 838, and 741. ^{1}H NMR (500 MHz, $CDCl_3$), 0.01 (9H, s, CH_2CH_2*TMS*), 0.62–0.69 (18H, m, $SiCH_2CH_3$), 0.93–1.04 (29H, m, CH_2CH_2TMS and $SiCH_2CH_3$), 1.21 (9H, s, *t*-Bu), 3.42–3.47

(2H, m, H-2 and C*H*$_2$CH$_2$TMS), 3.53 (1H, t, *J* = 6.3 Hz, H-4), 3.58–3.64 (2H, m, H-3 and H-5), 3.94 (1H, ddd, *J* = 11.7, 9.4, 6.2 Hz, C*H*$_2$CH$_2$TMS), 4.05 (1H, dd, *J* = 11.3, 8.0, H-6a), 4.36 (1H, d, *J* = 7.0 Hz, H-1), 4.36–4.40, (1H, m, H-6b), ^{13}C NMR (125 MHz, $CDCl_3$) −1.6 (CH$_2$CH$_2$*TMS*), 5.1, 5.1, 5.2 (Si*C*H$_2$CH$_3$), 6.9, 6.9, 7.0 (SiCH$_2$*C*H$_3$), 18.1 (CH$_2$*C*H$_2$TMS), 27.2 (*C*Me$_3$), 38.7 (*C*Me$_3$), 64.8 (C-6), 66.3 (*C*H$_2$CH$_2$TMS), 72.2 (C-4), 76.1 (C-5), 77.0 (C-2, overlapping with solvent peak), 76.2 (C-3), 102.1 (C-1), 178.2 (carbonyl). HR-FD-MS *m*/*z* [M+H]$^+$, calcd for $C_{34}H_{75}O_7Si_4$, 707.4590; found, 707.4600.

4.1.4. 2,3,4-Tri-*O*-triethylsilyl-1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside 11

Pivaloyl ester **10** (4.45 g, 5.73 mmol) was dissolved in 80 ml of CH_2Cl_2 and cooled to −78 °C. DIBAL (12 mmol) was added dropwise and left for 3 h under an argon atmosphere at −78 °C. The reaction was monitored by TLC, and when it was completed, the solution was quenched by MeOH and then diluted with diethyl ether (100 ml). After warming to room temperature, the mixture was washed with 1 M HCl (100 ml), satd $NaHCO_3$ (100 ml), and brine (100 ml). The organic layer was dried over Na_2SO_4 and evaporated to give a crude product, which was purified by silica gel column chromatography (EtOAc/hexane, 1:9). Evaporation yielded 3.51 g of **11** (99%) as a syrup. IR (ν_{max}, cm^{-1}) 3503, 1459, 1416, 1250, 1142, 1086, 1006, 860, 837, 802, 740. ^{1}H NMR (500 MHz, $CDCl_3$), 0.02 (9H, s, CH$_2$CH$_2$*TMS*), 0.64–0.70 (18H, m, Si*C*H$_2$CH$_3$), 0.94–1.06 (29H, m, CH$_2$*C*H$_2$TMS and SiCH$_2$*C*H$_3$), 3.42–3.52 (3H, m, H-2, H-5 and CH$_2$*C*H$_2$TMS), 3.57–3.60 (2H, m, H-3 and H-4), 3.70 (1H, m, H-6a), 3.78 (1H, ddd, *J* = 8.6, 6.2, 2.9 Hz, H-6b), 3.94, (1H, ddd, *J* = 11.7, 9.4, 6.2 Hz, C*H*$_2$CH$_2$TMS), 4.38 (1H, d, *J* = 7.0 Hz, H-1), ^{13}C NMR (125 MHz, $CDCl_3$) −1.5 (CH$_2$CH$_2$*TMS*), 5.0, 5.1, 5.2 (Si*C*H$_2$CH$_3$), 6.9, 6.9, 7.0 (SiCH$_2$*C*H$_3$), 18.2 (CH$_2$*C*H$_2$TMS), 63.1 (C-6), 66.6 (*C*H$_2$CH$_2$TMS), 72.0 (C-4), 76.9 (C-2), 78.6 (C-5), 78.9 (C-3), 102.6 (C-1), HR-FD-MS *m*/*z* [M+H]$^+$ calcd for $C_{29}H_{67}O_6Si_4$, 623.4015: found, 623.3997.

4.1.5. 6-*O*-Acryloyl-2,3,4-tri-*O*-triethylsilyl-1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside 8d

Silylated sugar **11** (3.52 g, 5.64 mmol) was dissolved in 100 ml of dry CH_2Cl_2. To the 0 °C-cooled solution, 2.0 ml of triethylamine (14.3 mmol) and 650 μl of acryloyl chloride (8.0 mmol) were added sequentially. After 3 h of stirring at room temperature, the solvent was evaporated. The mixture was dissolved into petroleum ether and the insoluble part was filtered off. The filtrate was evaporated and the residue was purified by silica gel column chromatography (CH_2Cl_2/hexane, 1:1). Evaporation of the eluant gave 3.26 g of **8d** (85%) as a syrup. IR (ν_{max}, cm^{-1}) 1734, 1636, 1458, 1407, 1249, 1190, 1118, 1090, 1006, 860, 838, 808, 740. ^{1}H NMR (500 MHz, $CDCl_3$) 0.01 (9H, s, CH$_2$CH$_2$*TMS*), 0.64–0.69 (18H, m, Si*C*H$_2$CH$_3$), 0.93–1.04 (29H, m, SiCH$_2$*C*H$_3$ and CH$_2$*C*H$_2$TMS), 3.43–3.49 (2H, m, H-2 and C*H*$_2$CH$_2$TMS), 3.60–3.68 (3H, m, H-3, H-4 and H-5), 3.92 (1H, ddd, *J* = 11.7, 9.4, 6.2 Hz, C*H*$_2$CH$_2$TMS), 4.25 (1H, dd, *J* = 11.4, 6.8 Hz, H-6a), 4.39 (1H, d, *J* = 6.7 Hz, H-1), 4.41 (1H, dd, *J* = 11.2, 4.4 Hz, H-6b), 5.83 (1H, dd, *J* = 10.5, 1.4 Hz, H-b), 6.14 (1H, dd, *J* = 17.3, 10.5 Hz, H-a), 6.42 (1H, dd, *J* = 17.3, 1.5 Hz, H-b), ^{13}C NMR (125 MHz, $CDCl_3$) −1.5 (CH$_2$CH$_2$*TMS*), 5.0, 5.1, 5.2 (Si*C*H$_2$CH$_3$), 6.9, 6.9, 7.0 (SiCH$_2$*C*H$_3$), 18.1 (CH$_2$*C*H$_2$TMS), 64.7 (C-6), 66.4 (*C*H$_2$CH$_2$TMS), 71.9 (C-4), 76.0 (C-5), 77.0 (C-2, overlapping with the solvent peak), 79.0 (C-3), 102.1 (C-1), 128.3 (C-β), 130.8 (C-α), 165.9 (carbonyl), HR-FD-MS *m*/*z* [M+H]$^+$ calcd for $C_{32}H_{69}O_7Si_4$, 677.4120: found, 677.4118.

4.1.6. 2,3,4-Tri-*O*-trimethylsilyl-1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside 7

Trimethylsilyl chloride (6.5 ml, 51.3 mmol) was added dropwise to a solution of **6** (3.53 g, 12.0 mmol) in 25 ml of pyridine at 0 °C. After 2 h, toluene was added to the reaction mixture and evaporated. The addition and evaporation of toluene were repeated twice. The resulting mixture was dissolved into petroleum ether and the insoluble part was filtered off. The filtrate was evaporated and the resulting crude product was dissolved in dry MeOH and then methanolic K_2CO_3 (2.7 ml, 4.48 mg/ml) was added to the solution at 0 °C. After 2 h, the solution was neutralized by adding AcOH (4.0 ml, 2.78 mg/ml) and evaporated yielding a white solid. The white solid obtained was purified by silica gel column chromatography (EtOAc/$CHCl_3$, 1:29). Evaporation of the eluant produced 4.33 g of **7** (73%) as a syrup. IR (ν_{max}, cm^{-1}) 3452, 1446, 1250, 1143, 1124, 1080, 869, 841. ^{1}H NMR (500 MHz, $CDCl_3$) 0.02 (9H, s, CH$_2$CH$_2$*TMS*), 0.16, 0.16, 0.18 (9H, s, OSi*Me*$_3$), 1.01 (2H, dd, *J* = 9.7, 8.0 Hz, CH$_2$*C*H$_2$TMS), 3.28 (2H, m, H-2 and H-5), 3.44 (2H, overlapped t, H-3 and H-4), 3.54 (1H, dd, *J* = 9.5, 7.8 Hz, C*H*$_2$CH$_2$TMS), 3.66 (1H, ddd, *J* = 11.9, 6.5, 5.6 Hz, H-6a), 3.81 (1H, ddd, *J* = 11.7, 6.5, 2.8 Hz, H-6b), 3.92 (1H, dd, *J* = 9.7, 8.2 Hz, C*H*$_2$CH$_2$TMS), 4.24 (1H, d, *J* = 7.6 Hz, H-1), ^{13}C NMR (125 MHz, $CDCl_3$) −1.5 (CH$_2$CH$_2$*TMS*), 0.9, 1.2, 1.3 (OSi*Me*$_3$), 18.3 (CH$_2$*C*H$_2$TMS), 62.3 (C-6), 67.2 (*C*H$_2$CH$_2$TMS), 71.7 (C-4), 75.9, 76.0 (C-2 and C-5), 78.2 (C-3), 103.0 (C-1), HR-FD-MS *m*/*z* [M]$^+$ calcd for $C_{20}H_{48}O_6Si_4$, 496.2528; found, 496.2538.

4.1.7. 6-*O*-Acryloyl-1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside 8c

The same procedure as in the case of the synthesis of **8d** was applied to **7** (3.42 g, 6.89 mmol). Acryloyl chloride (800 ml, 9.8 mmol), 2.0 ml of triethylamine (14.3 mmol), and 55 ml of dry CH_2Cl_2 gave 2.66 g of 6-*O*-acryloyl-2,3,4-tri-*O*-trimethylsilyl-1-*O*-(2-trimethylsilylethyl)-β-D-gluco-pyranoside (4.83 mmol, 70%) as a syrup after purification by silica gel column chromatography (EtOAc/hexane, 1:19). IR (ν_{max}, cm^{-1}) 1733, 1637, 1408, 1250, 1179, 1159, 1092, 1079, 870, and 841. ^{1}H NMR (500 MHz, $CDCl_3$) 0.01 (9H, s, CH$_2$CH$_2$*TMS*), 0.16, 0.16, 0.17 (9H, s, OSi*Me*$_3$), 0.99 (2H, m, CH$_2$*C*H$_2$TMS), 3.33 (1H, t, *J* = 8.0, H-2), 3.41–3.54 (4H, m, H-3, H-4, H-5 and C*H*$_2$CH$_2$TMS), 3.89 (1H, m, C*H*$_2$CH$_2$TMS), 4.15 (1H, dd, *J* = 11.8, 6.2 Hz, H-6a), 4.18 (1H, d, *J* = 7.5 Hz, H-1), 4.47 (1H, dd, *J* = 11.7, 2.3 Hz, H-6b), 5.84 (1H, dd, *J* = 10.4, 1.4 Hz, H-β), 6.15 (1H, dd, *J* = 17.4, 10.4 Hz, H-α), 6.42 (1H, dd, *J* = 17.4, 1.4 Hz, H-β), ^{13}C NMR (125 MHz, $CDCl_3$) −1.5 (CH$_2$CH$_2$*TMS*), 0.9, 1.2, 1.3 (OSi*Me*$_3$), 18.3 (CH$_2$*C*H$_2$TMS), 64.0 (C-6), 66.8 (*C*H$_2$CH$_2$TMS), 72.2 (C-4), 73.7 (C-2), 76.0 (C-5), 78.7 (C-3), 102.7 (C-1), 128.3 (C-β), 130.9 (C-α), 166.0 (carbonyl), HR-FD-MS *m*/*z* [M]$^+$ calcd for $C_{23}H_{50}O_7Si_4$, 550.2634; found, 550.2654.

The above trimethylsilyl derivative (2.36 g, 4.29 mmol) was dissolved in MeCN (60 ml) and cooled to 0 °C. To the reaction mixture, a stoichiometric amount of aqueous HF aqueous (800 μl, 13.3 mmol) was slowly added and stirring was continued for 70 min. Evaporation of solvent and subsequent short silica gel column chromatography (MeOH/$CHCl_3$, 1:9) produced 1.44 g of **8c** (quant.) as a colorless syrup. IR (ν_{max}, cm^{-1}) 3391, 1730, 1636, 1618, 1409, 1250, 1195, 1083, 861, and 836. ^{1}H NMR (500 MHz, $CDCl_3$) 0.02 (9H, s, CH$_2$CH$_2$*TMS*), 1.01 (2H, m, CH$_2$*C*H$_2$TMS), 3.38 (1H, t, *J* = 8.6 Hz, H-2), 3.41 (1H, t, *J* = 9.3 Hz, H-4), 3.51 (1H, ddd, *J* = 9.7, 5.0, 2.2 Hz, H-5), 3.58 (1H, t, *J* = 8.9 Hz, H-3), 3.58–3.63 (1H, m, C*H*$_2$CH$_2$TMS), 3.98 (1H, ddd, *J* = 11.2, 9.9, 6.1 Hz, C*H*$_2$CH$_2$TMS), 4.30 (1H, d, *J* = 7.8 Hz, H-1), 4.40 (1H, dd, *J* = 12.1, 2.2 Hz, H-6a), 4.54 (1H, dd, *J* = 12.1, 5.0 Hz, H-6b), 5.88 (1H, dd, *J* = 10.5, 1.3 Hz, H-β), 6.17 (1H, dd, *J* = 17.3, 10.4 Hz, H-α), 6.46 (1H, dd, *J* = 17.3, 1.3 Hz, H-β), ^{13}C NMR (125 MHz, $CDCl_3$) −1.5 (CH$_2$CH$_2$*TMS*), 18.2 (CH$_2$*C*H$_2$TMS), 63.4 (C-6), 67.6 (*C*H$_2$CH$_2$TMS), 70.0 (C-4), 73.6 (C-2), 73.9 (C-5), 76.0 (C-3), 102.1 (C-1), 127.9 (C-β), 131.8 (C-α), 166.8 (carbonyl), HR-FD-MS *m*/*z* [M+H]$^+$ calcd for $C_{14}H_{27}O_7Si$, 335.1526; found, 335.1553.

4.1.8. 1-*O*-(2-Trimethylsilylethyl)-6-*O*-[4′-(*tert*-butyldimethylsilyloxy)-3′-hydroxy-2′-methylenebutanoyl]-β-D-glucopyranoside 12c

To a solution of **8c** (54.3 mg, 162 μmol) in dry DMSO (1.62 ml), 2-(*tert*-butyldimethylsilyloxy)-acetaldehyde (84.9 mg, 487 μmol) and 3-hydroxy quinuclidine (20.6 mg, 162 μmol) were added at room temperature. After 40 h, the reaction mixture was diluted with EtOAc (2.0 ml) and extracted with 2.0 ml of brine. The water layer was then washed with EtOAc (2.0 ml × 2). The combined organic layer was dried over Na_2SO_4 and evaporated. Silica gel column chromatography (5% then 10% MeOH in $CHCl_3$) yielded 32.4 mg of **12c** (64 μmol, 40%, 3% de) as a colorless syrup. IR (ν_{max}, cm^{-1}) 3417, 1717, 1630, 1412, 1362, 1251, 1167, 1080, 860, and 837. HR-FD-MS *m*/*z* $[M+H]^+$ calcd for $C_{22}H_{45}O_9Si_2$, 509.2602; found, 509.2597.

The obtained mixture of diastereomers was further separated by chiral HPLC (EtOH/hexane, 8:92; CHIRALPAK® IA column (∅ 20 mm × 20 cm)).

4.1.8.1. First eluted diastereomer. (***R***)-**12c** (rt = 87.1 min), $[\alpha]_D = -35.1$ (*c* 1.0, $CHCl_3$), ^1H NMR (500 MHz, $CDCl_3$) 0.02 (9H, s, CH_2CH_2*TMS*), 0.06 and 0.07 (3H, s, *Me*Si), 0.90 (9H, s, *t*-Bu), 1.01 (2H, m, CH_2*C*H_2TMS), 3.34 (1H, t, *J* = 8.5 Hz, H-2), 3.41 (1H, t, *J* = 9.3 Hz, H-4), 3.48–3.56 (3H, m, H-3,5 and H-4′a), 3.59 (1H, ddd, *J* = 11.2, 9.9, 6.0 Hz, C*H*$_2$CH$_2$TMS), 3.85 (1H, dd, J = 10.1, 4.2 Hz, H-4′b), 3.95 (1H, ddd, *J* = 11.2, 9.9, 6.0 Hz, C*H*$_2$CH$_2$TMS), 4.28 (1H, d, *J* = 7.8 Hz, H-1), 4.44–4.45 (2H, m, H-6a and H-6b), 4.56 (1H, br q, H-3′), 6.00 (1H, s, =CH$_2$, *Ha*), 6.40 (1H, s, =CH$_2$, *Hb*), ^{13}C NMR (125 MHz, $CDCl_3$) −5.4 and −5.3 (*Me*$_2$Si), −1.4 (CH_2CH_2*TMS*), 18.2 (CH_2*C*H2TMS), 18.2 (Me$_3$*C*), 25.9 (*Me*$_3$C), 63.8 (C-6), 66.3 (C-4′), 67.4 (*C*H$_2$CH$_2$TMS), 70.2 (C-4), 70.7 (C-3′), 73.5 (C-5), 73.7 (C-2), 76.1 (C-3), 102.1 (C-1), 127.4 (=*C*H$_2$), 139.0 (C-2′), and 166.2 (C-1′).

4.1.8.2. Second eluted diastereomer. (***S***)-**12c** (rt = 94.5 min), $[\alpha]_D = -24.0$ (*c* 1.0, $CHCl_3$), ^1H NMR (500 MHz, $CDCl_3$), 0.02 (9H, s, CH_2CH_2*TMS*), 0.06 and 0.07 (3H, s, *Me*Si), 0.90 (9H, s, *t*-Bu), 1.01 (2H, m, CH_2*C*H_2TMS), 3.35 (1H, t, *J* = 8.6 Hz, H-2), 3.42 (1H, t, *J* = 9.3 Hz, H-4), 3.49–3.53 (3H, m, H-3,5 and H-4′a), 3.60 (1H, ddd, J = 11.3, 9.8, 6.0 Hz, C*H*$_2$CH$_2$TMS), 3.86 (1H, dd, *J* = 10.0 Hz, 4.1 Hz, H-4′b), 3.96 (1H, ddd, *J* = 11.3, 9.8, 6.0 Hz, C*H*$_2$CH$_2$TMS), 4.28 (1H, d, *J* = 7.6 Hz, H-1), 4.40 (2H, dd, *J* = 12.0, 5.5 Hz, H-6a), 4.47 (2H, dd, *J* = 12.0, 2.2 Hz, H-6b), 4.56 (1H, br q, H-3′), 5.99 (1H, s, =CH$_2$, *Ha*), 6.39 (1H, s, =CH$_2$, *Hb*), ^{13}C NMR (125 MHz, $CDCl_3$) −5.4 and −5.3 (*Me*$_2$Si), −1.4 (CH_2CH_2*TMS*), 18.2 (CH_2*C*H$_2$TMS), 18.3 (Me$_3$*C*), 25.9 (*Me*$_3$C), 63.9 (C-6), 66.3 (C-4′), 67.5 (*C*H$_2$CH$_2$TMS), 70.3 (C-4), 70.8 (C-3′), 73.5 (C-5), 73.6 (C-2), 76.2 (C-3), 102.0 (C-1), 127.4 (=CH$_2$), 139.0 (C-2′), and 166.2 (C-1′).

4.1.9. Deprotection of Baylis–Hillman adducts

BH adduct (***S***)-**12c** (17.2 mg, 33.9 μmol) was dissolved in 172 μl of deprotection reagent (trifluoroacetic acid/CH_2Cl_2, 2:1, v/v) and left for 1.5 h at room temperature. Dilution of the reaction mixture with toluene followed by evaporation was repeated until the TFA was completely removed. The resulting mixture was then purified via silica gel column chromatography (H_2O/MeCN, 1:5). Subsequent evaporation of the solvent provided 9.6 mg of **1** (96%) as a colorless syrup. (3′*R*)-Diastereomer **13** was obtained from (*R*)-**12c** by the same procedure as for **1** and provided a 93% yield. IR (ν_{max}, cm^{-1}) 3326, 1718, 1653, 1277, and 1020. HR-FD-MS *m*/*z* $[M+Na]^+$ calcd for $C_{11}H_{18}O_9Na$, 317.0849; found, 317.0840.

4.1.9.1. *epi*-6-Tuliposide B. Compound **13**: $[\alpha]_D = +30.9$ (*c* 1.0, MeOH), ^1H NMR (500 MHz, CD_3OD), 3.15 (d, *J* = 8.4 Hz, H-2(β)), 3.30-3.39 (m, H-2(α), H-3(β), H-4(α) and H-4(β)), 3.45 (1H, overlapped dd, *J* = 11.3, 6.7 Hz, H-4′a(α) and H-4′a(β)), 3.53 (ddd, *J* = 9.4, 5.6, 1.9 Hz, H-5(β)), 3.68 (t, *J* = 9.3 Hz, H-3(α)), 3.73 (1H, overlapped dd, *J* = 11.3, 3.1 Hz, H-4′b(α) and H-4′b(β)), 4.01 (ddd, *J* = 10.0, 5.1, 2.1 Hz, H-5(α)), 4.26 (dd, *J* = 12.0, 5.6 Hz, H-6a(β)), 4.28 (dd, *J* = 12.3, 4.8 Hz, H-6a(α)), 4.47 (dd, *J* = 11.9, 2.3 Hz, H-6b(α)), 4.50 (d, *J* = 7.8 Hz, H-1 (β)), 4.51 (dd, *J* = 11.9, 2.2 Hz, H-6b(β)), 4.59 (1H, br q, H-3′), 5.10 (d, *J* = 3.8 Hz, H-1 (α)), 6.00 (1H, s, =CH$_2$, Ha), 6.36 (1H, s, =CH$_2$, Hb), ^{13}C NMR (125 MHz, CD_3OD) 65.1 (C-6(α)), 65.2 (C-6(β)), 66.7 and 66.8 (C-4′), 70.7 (C-5(α)), 71.8 (C-4(β)), 72.1 (C-4(α)), 72.1 (C-3′), 73.8 (C-2(α)), 74.8 (C-3(α)), 75.3 (C-5(β)), 76.2 (C-2(β)), 77.9 (C-3(β)), 94.0 (C-1(α)), 98.3 (C-1(β)), 126.7 and 126.8 (=*C*H$_2$), 142.2 and 142.3 (C-2′), 167.4 and 167.5 (C-1′).

4.1.9.2. 6-Tuliposide B. Compound **1**: $[\alpha]_D = +37.7$ (*c* 1.0, MeOH), ^1H NMR (500 MHz, CD_3OD), 3.14 (dd, *J* = 8.9, 7.9 Hz, H-2(β)), 3.30–3.38 (m, H-2(α), H-3(β), H-4(α), and H-4(β)), 3.44 (1H, overlapped dd, *J* = 11.3, 6.8 Hz, H-4′a(α) and H-4′a(β)), 3.53 (ddd, *J* = 9.4, 5.8, 2.2 Hz, H-5(β)), 3.68 (t, *J* = 9.4 Hz, H-3(α)), 3.72 and 3.73 (1H, overlapped dd, *J* = 11.3, 3.7 Hz, H-4′b(α), and H-4′b(β)), 4.01 (ddd, *J* = 10.1, 5.4, 2.2 Hz, H-5(α)), 4.28 (dd, *J* = 12.0, 5.8 Hz, H-6a(β)), 4.30 (dd, *J* = 11.8, 5.4 Hz, H-6a(α)), 4.42 (dd, *J* = 11.8, 2.3 Hz, H-6b(α)), 4.48 (dd, *J* = 11.9, 2.0 Hz, H-6b(β)), 4.50 (d, *J* = 7.8 Hz, H-1 (β)), 4.57 (1H, br q, *J* = 6.8, 3.5 Hz, H-3′), 5.09 (d, *J* = 3.7 Hz, H-1 (α)), 5.99 (1H, s, =CH$_2$, Ha), 6.35 (1H, s, =CH$_2$, Hb), ^{13}C NMR (125 MHz, CD_3OD) 65.0 (C-6(α)), 65.1 (C-6(β)), 66.6 (C-4′), 70.7 (C-5(α)), 71.7 (C-4(β)), 72.0 (C-4(α)), 72.1 (C-3′), 73.8 (C-2(α)), 74.7 (C-3(α)), 75.3 (C-5(β)), 76.2 (C-2(β)), 77.9 (C-3(β)), 94.0 (C-1(α)), 98.2 (C-1(β)), 126.8 and 126.9 (=*C*H$_2$), 142.1 (C-2′), 167.3 and 167.4 (C-1′).

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References

1. Dixon, R. A. *Nature* **2001**, *411*, 843–847.
2. VanEtten, H.; Mansfield, J. W.; Bailey, J. A.; Farmer, E. E. *Plant Cell* **1994**, *6*, 1191–1192.
3. (a) Tschesche, R.; Kämmerer, F. J.; Wulff, G.; Schönbeck, F. *Tetrahedron Lett.* **1968**, *6*, 701–706; (b) Tschesche, R.; Kämmerer, F. J.; Wulff, G. *Chem. Ber.* **1969**, *102*, 2057–2071; (c) Christensen, L. P. *Phytochemistry* **1999**, *51*, 969–974.
4. (a) Solb, A. *Phytochemistry* **1973**, *12*, 811–815; (b) Solb, A. *Phytochemistry* **1975**, *14*, 1997–2005; (c) Hausen, B. M.; Prater, E.; Schubert, H. *Contact Dermatitis* **1983**, *9*, 46–54; (d) Santucci, B.; Picardo, M.; Iavarone, C.; Trogolo, C. *Contact Dermatitis* **1985**, *12*, 215–219.
5. (a) Kim, C.-S.; Hara, T.; Probal, K. D.; Itoh, E.; Horiike, M. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1546–1594; (b) Probal, K. D.; Kim, C.-S.; Hara, T.; Itoh, E. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 760–761.
6. Shoji, K.; Ubukata, M.; Momonoi, K.; Tsuji, T.; Morimatsu, T. *J. Jpn. Soc. Hort. Sci.* **2005**, *74*, 469–475.
7. (a) Tanaka, A. *Agric. Biol. Chem.* **1980**, *44*, 199–203; (b) Papagerorgiou, C.; Benezra, C. *J. Org. Chem.* **1985**, *50*, 1144–1145; (c) Ohgiya, T.; Nishiyama, S. *Heterocycles* **2004**, *63*, 2349–2354.
8. (a) Hutchinson, C. R. *J. Org. Chem.* **1974**, *39*, 1854–1858; (b) Corbet, J. P.; Benezra, C. *J. Org. Chem.* **1981**, *46*, 1141–1147.
9. Preliminary accounts for the synthesis of 1-*O*-methyl 6-tuliposide B: Shigetomi, K.; Kishimoto, T.; Shoji, K.; Ubukata, M. *Heterocycles* **2006**, *69*, 63–67.
10. Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G. *J. Org. Chem.* **1988**, *53*, 5629–5647.
11. (a) Richard, F. H.; John, R.; Ronald, D. H. *Carbohydr. Res.* **1992**, *229*, 183–194; Preparation of methyl 2,3,4-tris-*O*-trimethylsilyl-α-D-glucoside: (b) Hurst, D. T.; McInnes, A. G. *Can. J. Chem.* **1965**, *43*, 2004–2011.
12. (a) Enders, D.; Schübeler, T. *Synthesis* **2002**, *15*, 2280–2288; (b) Lafontaine, J. A.; Provencal, D. P.; Gardelli, C.; Leahy, J. W. *J. Org. Chem.* **2003**, *68*, 4215–4234.
13. (a) Drewes, S. E.; Freese, S. D.; Emslie, N. D.; Roos, G. H. P. *Synth. Commun.* **1988**, *18*, 1565–1572; (b) Ameer, F.; Drewes, S. E.; Freese, S.; Kaye, P. T. *Synth. Commun.* **1988**, *18*, 495–500.
14. Lemiux, R. U.; Driguez, H. *J. Am. Chem. Soc.* **1975**, *97*, 4069–4075.