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The antibacterial properties of 6-tuliposide B. Synthesis of 6-tuliposide B analogues and structure–activity relationship

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ABSTRACT

6-Tuliposide B is a secondary metabolite occurring specifically in tulip anthers. Recently, a potent antibacterial activity of 6-tuliposide B has been reported. However, its molecular target has not yet been established, nor its action mechanism. To shed light on such issues, 6-tuliposide B and tulipalin B analogues were synthesized and a structure–activity relationship (SAR) was examined using a broad panel of bacterial strains. As the results of SAR among a total of 25 compounds, only tulipalin B and the compounds having 3′,4′-dihydroxy-2′-methylenebutanoate (DHMB) moieties showed any significant antibacterial activity. Moreover, the 3′*R* analogues of these compounds displayed essentially the same activities as 6-tuliposide B and the structure of the 3′*R*-DMBA moiety was the same as that of the proposed active moiety of cnicin. These results suggest that 6-tuliposide B has the same action mechanism as proposed for cnicin and bacterial MurA is one of the major molecular targets of 6-tuliposide B.

1. Introduction

Tuliposides are secondary metabolites occurring mainly in the Liliaceae and Alstroemeriaceae (Slob, 1973; Slob et al., 1975; Christensen, 1995b). At present, tuliposides A, B, D, E and F have been reported together with their chemical structures (Tschesche et al., 1969; Christensen and Kristiansen, 1995a; Christensen, 1995c, 1999), with all tuliposides composed of D-glucose and 4′-hydroxy-2′-methylenebutanoyl and/or (3′*S*)-3′,4′-dihydroxy-2′-methylenebutanoyl side-chains. Both types of side-chains can be released by either pH-dependent or enzymatic lactonization to form tulipalin A (**3**) and tulipalin B (**2**) (Fig. 1), respectively (Tschesche et al., 1968; Kato et al., 2009). Principally, these tulipalins are known to be allergic and causative agents of "tulip fingers" (Barbier and Benezra, 1982, 1986; Lahti, 1986; Marks, 1988; Gette and Marks, 1990).

Recently, we found 6-tuliposide B (6-*O*-[(3′*S*)-3′,4′-dihydroxy-2′-methylenebutanoyl]-D-glucose) (**1**) to have potent antimicrobial activity (Shoji et al., 2005), with its simple and low-molecular weight structure displaying a broad antibacterial effect against Gram-positive, Gram-negative, and certain fungicide-tolerant strains of bacteria. However, neither its antibacterial molecular target has been established, nor its action mechanism.

In this study, we describe the synthesis of 6-tuliposide B (**1**) and tulipalin B (**2**) analogues based on our previous total synthesis of 6-tuliposide B and its 3′*R*-epimer (***epi*-1**) (Shigetomi et al., 2008). Furthermore, we report a broad structure–activity relationship (SAR) study to identify the structures responsible for antibacterial activity, and thereafter discuss the target molecule of 6-tuliposide B and its action mechanism.

2. Results and discussion

2.1. Synthesis of 6-tuliposide B and tulipalin B analogues

2.1.1. Synthesis of 4′-deoxy and hexanoyl-type 6-tuliposide B analogues (*5*, *epi-5*, *7* and *epi-7*)

The syntheses of 4′-deoxy and hexanoyl-type 6-tuliposide Bs were conducted on the basis of the total synthesis of 6-tuliposide B (**1**). By employing acetaldehyde rather than (*tert*-butyldimethylsilyloxy)-acetaldehyde in the Baylis–Hillman reaction, both the 4′-deoxy 6-tuliposide B precursor and its 3′-epimer were obtained via chiral HPLC separation. Both diastereomers were deprotected by TFA treatment to yield 4′-deoxy 6-tuliposide B (**5**) and its 3′-epimer (***epi*-5**), respectively (Scheme 1). Hexanoyl-type analogues (**7** and ***epi*-7**) were synthesized from 4-(*tert*-butyldimethylsilyloxy)-butanal (**6**) (Taillier et al., 2005) and sugar acrylate (**4**) in a similar way.

2.1.2. Synthesis of hydrogenated 6-tuliposide Bs and hydrogenated tulipalin Bs (*8*, *epi-8*, *9* and *ent-9*)

To survey the significance of the α,β-unsaturated moiety, hydrogenated 6-tuliposide B (**8**) and hydrogenated tulipalin B (**9**) were synthesized by Pd/C catalytic hydrogenation of 6-tuliposide

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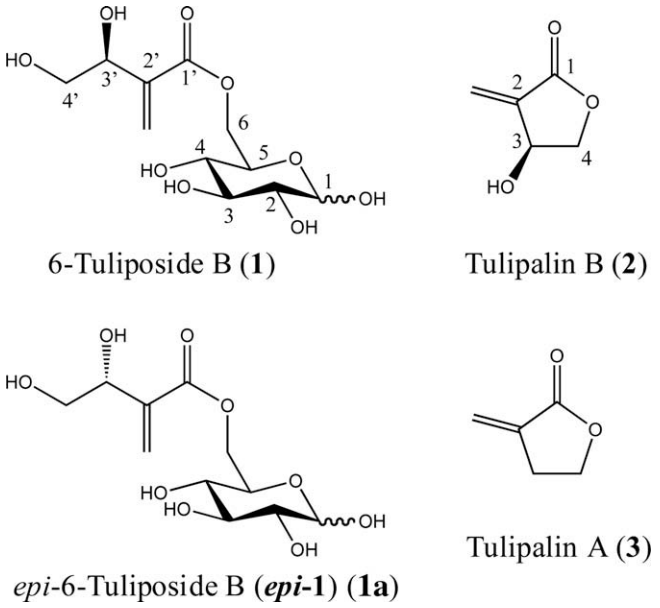


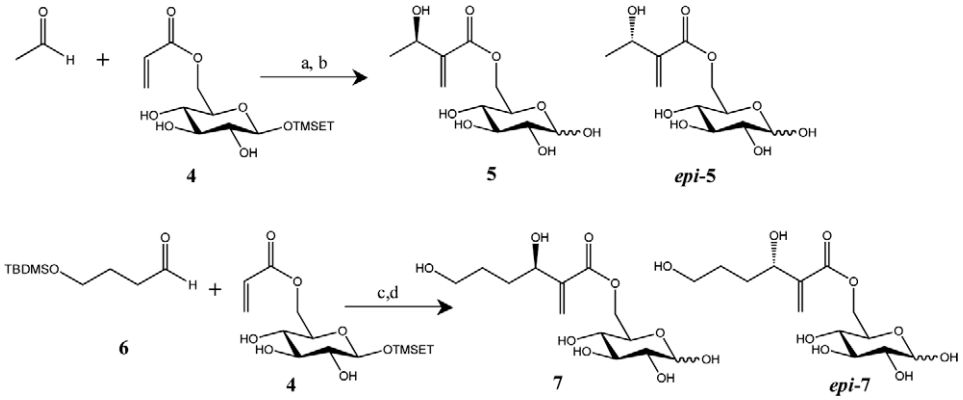
Fig. 1. Structures of 6-tuliposide B (**1**), epi-6-tuliposide (**1a**) (−)-tulipalin B (**2**) and tulipalin A (**3**).

B (**1**) and (−)-tulipalin B (**2**), respectively. In general, α,β-unsaturated carbonyl compounds are known to be reactive and to have various biological activities (Shibaoka et al., 1967; Iino et al., 1972; Kim et al., 1998; Rastelli et al., 2008) because of their Michael acceptor properties. Based on the signal intensities of the α-H in the ^{1}H NMR spectra, the diastereomeric *syn*/*anti* ratio of **8** and **9** were estimated to be 3:2 and 4:1, respectively. Diastereomeric mixtures were subjected to the antibacterial assays without further separation. Similarly, hydrogenated products of non-natural type (***epi*-8** and ***ent*-9**) were prepared from *epi*-6-tuliposide B (***epi*-1**) and (+)-tulipalin B (***ent*-2**) (see Scheme 2).

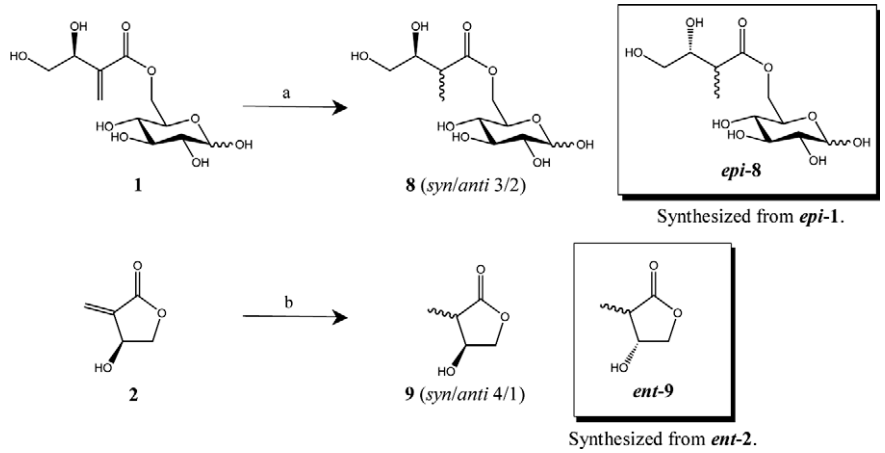
2.1.3. Synthesis of amide-type 6-tuliposide B and 1,2-dideoxy 6-tuliposide Bs (*19* and *epi-19*)

As mentioned above, 6-tuliposide B (**1**) can be converted into tulipalin B (**2**) even at neutral conditions and is relatively stable in mild acidic solutions below pH 5.0. To avoid this pH-dependent lactonization in antibacterial assays, amide-linked 6-tuliposide B analogues were synthesized. Amide-type analogues were constructed by a condensation of butanoic acid and amino sugar because aliphatic acrylamide has a low Baylis–Hillman reactivity due to its low Michael acceptor properties (Guo et al., 2005).

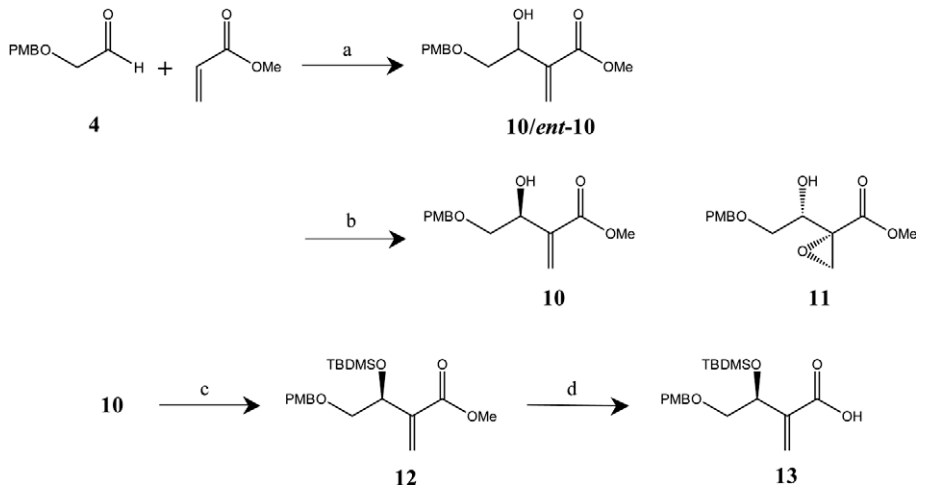
The synthetic route of the butanoyl side chain is depicted in Scheme 3. Baylis–Hillman reaction of (*p*-methoxybenzyloxy)-acetaldehyde (Smith and Fox, 2004) and methyl acrylate provided the racemic adduct in good yield. The so-obtained racemate was then separated by Katsuki-Sharpless kinetic resolution to afford 3*S*-enantiomer (**10**, >99%ee) in 43% yield and the 3*S*-epoxide (**11**, 96%ee) in 40% yield, respectively. Similarly, the 3*R*-enantiomer (***epi*-10**) and 3*R*-epoxide (***epi*-11**) were obtained by using (−)-diisopropyl tartrate in kinetic resolution. The pure 3*S*-enatiomer (**10**)



Scheme 1. Reaction and reagents: (a) 3-hydroxy quinuclidine (1.0 eq), CH_3CN, 40 h, 31%, (b) TFA:CH_2Cl_2 = 2:1, **5**:96%, ***epi*-5**:98%, (c) 3-hydroxy quinuclidine (1.0 eq), DMSO, 40 h, 40%, and (d) TFA:CH_2Cl_2 = 2:1, **7**:95%, ***epi*-7**:95%.

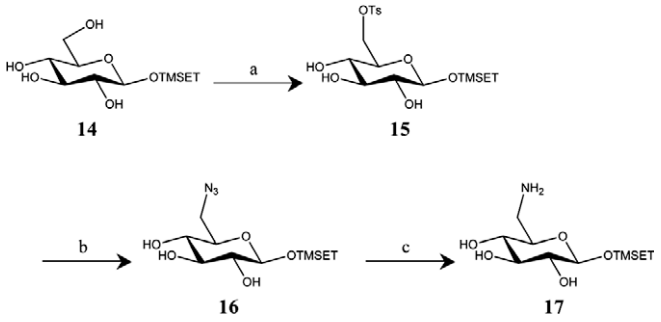


Scheme 2. Reaction and reagents: (a) Pd/C, H_2, MeOH, 72% and (b) Pd/C, H_2, EtOAc, 65%.

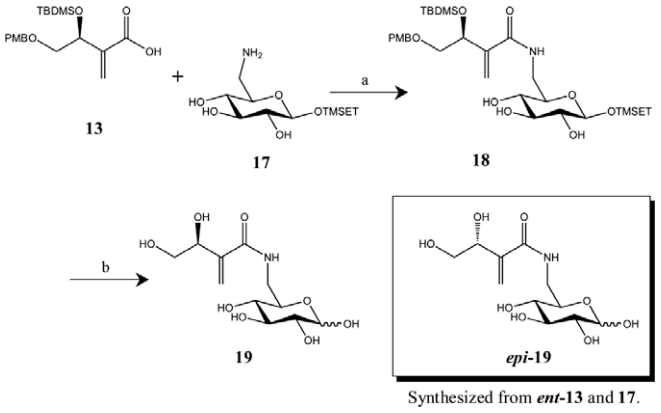


Scheme 3. Reaction and reagents: (a) 3-hydroxy quinuclidine (0.5 eq), DMSO, 15 h, 84%, (b) (+)-diisopropyl tartrate, Ti(O*i*Pr)$_4$, cumenehydroperoxide, MS 4A, CH_2Cl_2, **10**; 43% (>99%ee), **11**; 40% (96%ee), (c) *tert*-butyldimethylsilyl trifuluoromethanesulfonate, Et_3N, CH_2Cl_2, quant., and (d) LiOH (1.1 eq), MeCN:H_2O = 1:1, 60 °C, 92%.

was protected by a TBDMS group and hydrolyzed to give butanoic acid (**13**). The amino sugar (**17**) was synthesized from 1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside (**14**) in three steps (Scheme 4). The 6-hydroxyl group of (2-trimethylsilylethyl)- glucoside **14** was selectively tosylated and then substituted by azide, which was reduced to the amine to give the amino sugar (**17**). Condensation of butanoic acid (**13**) with the amino sugar (**17**) afforded an amide-type 6-tuliposide B analogue (**19**) after TFA treatment as shown in Scheme 5. Likewise, the 3′*R*-epimer (***epi*-19**) was prepared from the 3*R* Baylis–Hillman adduct (***ent*-13**).

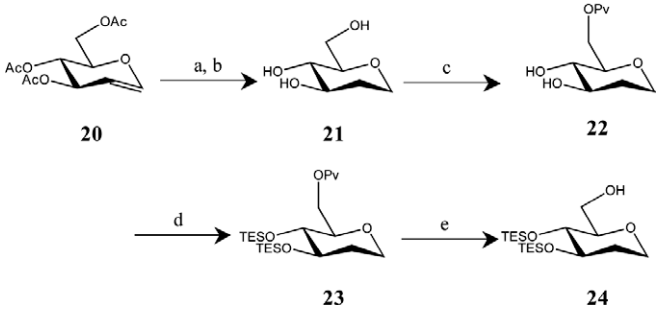


Scheme 4. Reaction and reagents: (a) TsCl, pyridine, 0 °C, 86%, (b) NaN_3, DMF, 90 °C, 92%, and (c) Pd/C, H_2, EtOAc, quant.

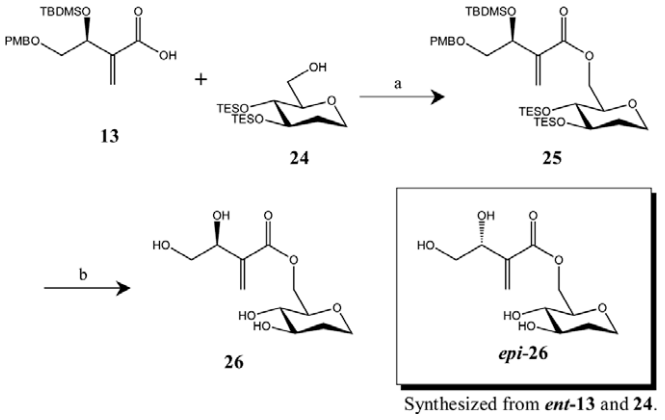


Scheme 5. Reaction and reagents: (a) diisopropyl carbodiimide, 4-dimethylaminopyridine, CH_2Cl_2, 0 °C, 81% and (b) TFA:CH_2Cl_2 = 2:1, 90%.

To study the role of the sugar moiety in 6-tuliposide B (**1**), 1,2-dideoxy-6-tuliposide B (**26**) was synthesized (Scheme 6). The synthesis was achieved by a condensation of butanoic acid with 1,2-dideoxy-D-glucopyranose derivatives (**24**), which was prepared from 3,4,6-tri-*O*-acetyl-D-glucal (**20**) in five steps. Hydrogenation followed by methanolysis provided 1,2-dideoxy-D-glucopyranose (**21**). Subsequently, the primary alcohol and 3,4-hydroxyl groups were protected by pivaloylation and triethylsilylation, respectively.



Scheme 6. Reaction and reagents: (a) Pd/C, H_2, EtOAc, (b) NaOMe, MeOH, 69% (two steps), (c) PvCl, pyridine, 83%, and (d) triethylsilyl trifuluoromethanesulfonate2,6-lutidine, CH_2Cl_2, 99%, (d) DIBAL, CH_2Cl_2, 79%.



Scheme 7. Reaction and reagents: (a) diisopropyl carbodiimide, 4-dimethylaminopryidine, CH_2Cl_2, 62% and (b) TFA:CH_2Cl_2 = 2:1, 70%.

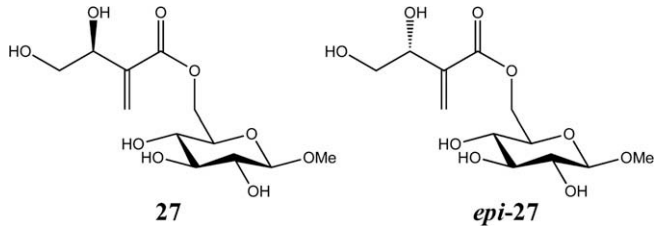
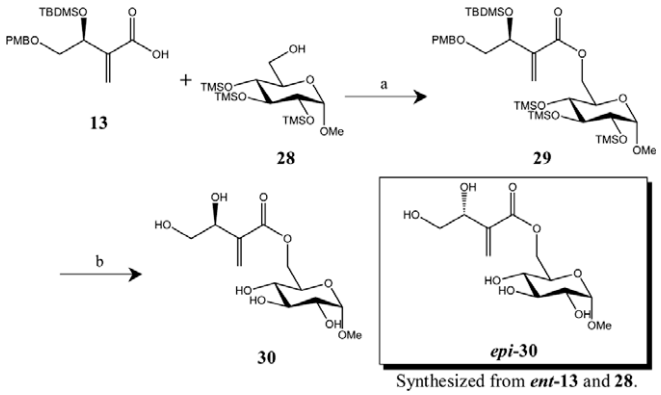


Fig. 2. Structures of 1-*O*-methyl-β-6-tuliposide Bs (**27** and ***epi*-27**).



Scheme 8. Reaction and reagents: (a) diisopropyl carbodiimide, 4-dimethylaminopyridine, CH_2Cl_2, 60% and (b) TFA:CH_2Cl_2 = 2:1, 80%.

The pivaloyl group was then removed by DIBAL reduction to give **24**. Finally, 1,2-dideoxy-6-tuliposide B (**26**) was synthesized by coupling of the sugar moiety (**24**) with butanoic acid (**13**) followed by TFA treatment as shown in Scheme 7. The 3′*R*-epimer of **26** (***epi*-26**) was also prepared in the same way.

*2.1.4. Synthesis of methyl glucosides of 6-tuliposide B (**27**, ***epi*-27**, **30** and ***epi*-30**)*

As sugar-modified analogues, the 1-*O*-methyl-β-D-glucoside derivatives (**27** and ***epi*-27**) and 1-*O*-methyl-α-D-glucoside derivatives (**30** and ***epi*-30**) were also synthesized. The preparation of the diastereomeric mixture of 1-*O*-methyl-β-D-glucoside derivatives was previously described (Shigetomi et al., 2006). To validate the SAR analysis, the diastereomeric mixture was separated into its natural-type 1-*O*-methyl-β-D-glucoside derivative (**27**) and its epimer (***epi*-27**) by chiral HPLC. The 1-*O*-methyl-α-D-glucoside derivative (**30**) and its 3′*R*-epimer (***epi*-30**) were individually synthesized via condensation of acid (**13**, ***ent*-13**) with sugar (**28**) as shown in Scheme 8 (see also Fig. 2).

*2.1.5. Synthesis of methyl esters of tuliposide B side chain (**31** and ***ent*-31**) and their epoxides (**32** and e**nt*-32**)*

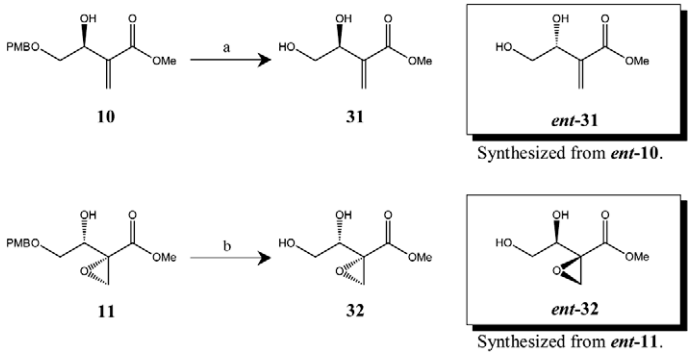
The Baylis–Hillman adduct (**10**) and the epoxide (**11**) were deprotected by TFA treatment to give the methyl ester of 6-tuliposide B side chain (**31**) and its epoxide (**32**), respectively. Moreover, the 3*R*-enantiomer of each product was prepared from the corresponding starting materials. These products were also used in antibacterial tests to probe the importance of the sugar moiety and the double bond of the α,β-unsaturated carbonyl moiety (see Scheme 9).

2.2. Antibacterial activity evaluation

Antibacterial assays were performed against reported seven strains (Shoji et al., 2005) that are *Escherichia coli* (NBRC 3972, ATCC 8739), *Salmonella enteritidis* (NBRC 3313), *Pseudomonas aeruginosa* (NBRC 13275, ATCC 9027), *Burkholderia glumae* (NBRC T 12119, ATCC 49703), *Acidovorax avenae* (NBRC T 9020), *Staphylococcus aureus* (NBRC 13276, ATCC 6538) and *Bacillus subtilis* (NBRC 3007, ATCC 70385). *A. avenae* is a resistant strain against oxolinic acid, and *B. glumae* is resistant to both oxolinic acid and kasugamycin. To test the antibacterial generality of 6-tuliposide B (**1**) against drug-resistant strains, methicillin-resistant *S. aureus* (ATCC 700699) was also subjected to assays.

2.2.1. Antibacterial activities of 6-tuliposide B and its analogues

At first, 6-tuliposide B (**1**) and its analogues were evaluated for their antibacterial activities. Tested compounds were namely



Scheme 9. Reaction and reagents: (a) TFA:CH_2Cl_2 = 2:1, 80% and (b) TFA:CH_2Cl_2 = 2:1, 80%.

Table 1
MIC values of 6-tuliposide B (**1**) and its analogues against bacteria (mM).

Compounds	*E. coli*	*S. enteritidis*	*P. aeruginosa*	*B. glumae*	*A. avenae*	*S. aureus*	MRSA	*B. subtilis*
1 (***epi*-1**)	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)	0.3 (0.2)	0.1 (0.1)	0.2 (0.2)	0.3 (0.3)	0.5 (0.5)
26 (***epi*-26**)	0.3 (0.3)	0.2 (0.3)	0.2 (0.3)	0.3 (0.3)	0.1 (0.1)	0.2 (0.2)	0.2 (0.2)	0.5 (1.0)
27 (***epi*-27**)	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)	0.3 (0.2)	0.1 (0.1)	0.2 (0.2)	0.3 (0.3)	0.5 (0.5)
30 (***epi*-30**)	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)	0.1 (0.1)	0.3 (0.3)	0.3 (0.4)	0.5 (0.6)

Compounds **5**, **7**, **8** and **19** and their epimers (***epi*-5**, ***epi*-7**, ***epi*-8** and ***epi*-19**) did not show any activity at 1.0 mM.

6-tuliposide B (**1**), *epi*-6-tuliposide B (***epi*-1**) and 14 synthetic analogues (**5**, **7**, **8**, **19**, **26**, **27**, **30** and their epimers).

The results are shown in Table 1. The antibacterial activities were presented as minimal inhibitory concentration (MIC). All of the analogues having 3′,4′-dihydroxy-2-methylenebutanoate structures (**26**, **27**, **30** and their epimers) retained antibacterial activities irrespective of their sugar modification. Moreover, their MIC values were essentially the same as 6-tuliposide B (**1**), which suggests that the sugar moiety of 6-tuliposide B (**1**) is not indispensable for inhibitory activities. Likewise, the absolute configurations at the 3′-position seemed to not affect activities. Active compounds exhibited growth inhibition against MRSA as well as the reported seven strains.

On the other hand, hydrogenated 6-tuliposide B (**8** and ***epi*-8**) did not show any activities at 1 mM. This indicates that the α,β-unsaturated enone is essential for antibacterial activities. The 4′-deoxy (**5** and ***epi*-5**), hexanoyl-type (**7** and ***epi*-7**) and amide-type analogues (**19** and ***epi*-19**) were also inactive. These results might stem from their inabilities to form tulipalin Bs (**2** and ***ent*-2**), because **5**, ***epi*-5**, **7** and ***epi*-7** were structurally unable to form tulipalin Bs. Likewise, the amide-type analogues (**19** and ***epi*-19**) would be difficult to lactonize due to their strong amide bonds. In contrast, all of the antibacterial compounds tested (**1**, **26**, **27**, **30** and their epimers) possess a 3′,4′-dihydroxy-2′-methylenebutanoate structure, which can be converted into tulipalin Bs. Although the assay was carried out under weak acidic conditions, a part of these compounds would be converted into tulipalin Bs at pH 6.0. Therefore, SAR of tulipalin B (**2**) and its analogues should be studied to establish the role of tulipalin B (**2**) in these activities.

2.2.2. SAR study of (−)-tulipalin B (2) and its analogues

Secondly, SAR of (−)-tulipalin B (**2**) and its analogues were studied. (−)-Tulipalin B (**2**), synthetic (+)-tulipalin B (***ent*-2**) and other six analogues described above (**9**, **31**, **32** and their enantiomers) were assayed. As a 3-deoxy analogue of tulipalin B (**2**), commercially available tulipalin A (**3**) was also assayed.

As shown in Table 2, tulipalins (**2**, **3** and ***ent*-2**) and the methyl esters of tuliposide B side chain (**31** and ***ent*-31**) showed antibacterial activities. The MIC values of the 3*S*- and 3*R*-enantiomers were almost the same, this being analogous to the results of 6-tuliposide B analogues. The methyl esters of the 6-tuliposide B side chain (**31** and ***ent*-31**) showed equal activities to sugar esters, thus it was confirmed that the sugar moiety was not necessary for growth inhibition of bacteria. Tulipalin Bs (**2** and ***ent*-2**) showed a little higher activity than linear (not lactonized) esters such as **1, 26, 27, 30, 31** and their epimers. These results reflect that tulipalin B formation plays a significant role for the antibacterial activity of 6-tuliposide B (**1**). Tulipalin A (**3**) showed a lower activity, which is approximately a tenth to a twentieth part of tulipalin Bs (**2** and ***ent*-2**). This indicated that presence of a hydroxyl group at β-position is important, but its configuration is not significant for antibacterial activities. Hydrogenated tulipalin Bs (**9** and ***ent*-9**) and epoxidized derivatives (**32** and ***ent*-32**) exhibited no growth inhibition at 1.0 mM and this is consistent with the results of **8** and ***epi*-8**.

2.2.3. Discussion of the molecular target of 6-tuliposide B (1) and its action mechanism

Recently, cnicin occurring in *Cnicus benedictus* has been shown to inhibit bacterial MurA by an unusual 1,2-addition (Steinbach et al., 2008). MurA is a bacterial cytoplasmic enzyme, which catalyzes the first committed step of peptidoglycan biosynthesis that is the coupling reaction of UDP-*N*-acetylglucosamine (UNAG) with phosphoenolpyruvate (PEP) to yield a muramic acid precursor. Cnicin consists of a large sesquiterpenoid moiety and the same butanoate side chain as the synthetic 3′*R*-epimer of 6-tuliposide B (***epi*-1**) (Fig. 3). Steinbach's X-ray crystallographic analysis has established that 3,4-dihydroxy-2-methylenebutanoate (DHMB) moiety of cnicin reacts with UNAG to form an unusual adduct in the active site of bacterial enzyme MurA by mimicking PEP (Fig. 3).

Results of the present SAR study proved that the 3′,4′-dihydroxy-2′-methylenebutanoate structure is responsible for the activities and both the 3′*S*- and 3′*R*-epimers show the same activities, suggesting that 6-tuliposide B (**1**) has the same action mechanism as cnicin. In general, MurA prefers high polar and low-molecular weight substrates or inhibitors (e.g. PEP or fosfomycin); therefore, it would be difficult especially for cnicin to interact with MurA in its original form. 6-Tuliposide B (**1**) and cnicin could express their antibacterial activities by formation of (−)- and (+)-tulipalin B (**2** and ***ent*-2**), respectively, and also released tulipalins could be hydrolyzed into 3,4-dihydroxy-2-methylenebutanoic acids to form UNAG-tulipalin B adducts as substrate-mimics. Hydrolysis of tulipalin Bs (**2** and ***ent*-2**) would proceed effectively in the active site of MurA. The lower activity of tulipalin A (**3**) can be attributed to its low polarity, because it has been considered that the 3,4-dihydroxy moiety of the butanoic acid mimics phosphate group of PEP. The fact that the α,β-unsaturated carbonyl compounds, **5**, ***epi*-5**, **7**, and ***epi*-7** do not show any antibacterial

Table 2
MIC values of (−)-tulipalin B (**2**) and its analogues against bacteria (mM).

Compounds	*E. coli*	*S. enteritidis*	*P. aeruginosa*	*B. glumae*	*A. avenae*	*S. aureus*	MRSA	*B. subtilis*
2 (***ent*-2**)	0.2 (0.2)	0.1 (0.1)	0.1 (0.1)	0.2 (0.2)	0.1 (0.1)	0.2 (0.2)	0.2 (0.2)	0.3 (0.3)
3	3.4	1.8	2.0	2.8	1.2	3.2	4.8	>5.0
31 (***ent*-31**)	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)	0.1 (0.1)	0.3 (0.3)	0.4 (0.5)	0.8 (1.0)

Compounds **9** and **32** and their enantiomers (***ent*-9** and ***ent*-32**) did not show any activity at 1.0 mM.

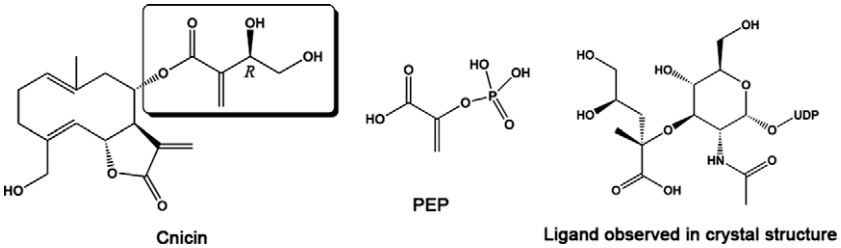


Fig. 3. Structures of cnicin, PEP and the UNAG-DHMB adduct observed in the crystal structure (Steinbach et al., 2008).

activity at 1.0 mM indicates that a non-specific Michael addition of biomolecule is not the major cause of antibacterial activity, whereas a non-specific Michael addition might weaken the antibacterial activity of 6-tuliposide B. The α,β-unsaturated structure would be required for the unusual 1,2-addition of UNAG to the double bond, although inhibition of the other PEP-related enzymes could not be excluded completely.

3. Conclusion

6-Tuliposide B (**1**) and (−)- tulipalin B (**2**) analogues were synthesized and their structure–activity relationships studied against a broad panel of bacteria. The results of the SAR study proved that the 3′,4′-dihydroxy-2′-methylenebutanoate structure is responsible for antibacterial activities and the glucose moiety of 6-tuliposide B did not directly relate to antibacterial activities. 6-Tuliposide B (**1**) showed antibacterial activity against MRSA; however, no MIC differences were observed between 3′*S*- and 3′*R*-epimers, and tulipalin B (**2**) showed a little higher activity than linear esters. Therefore, formation of tulipalin B (**2**) is considered to be important for bacterial growth inhibition of 6-tuliposide B (**1**). These results suggest that the molecular target of 6-tuliposide B (**1**) is bacterial MurA on which the (*S*)-3′,4′-dihydroxy-2′-methylenebutanoic acid acts in a similar fashion to cnicin. Although the sugar moiety is not important for antibacterial activity in vitro, the existence of tuliposide-converting enzymes in tulip tissues (Kato et al., 2009) might explain the improved defense mechanism against both microbial and insect attack. Given the present SAR study of tuliposide B (**1**) and its analogues, the inhibitory activities against MurA and further studies on action mechanism of these particular compounds will be reported elsewhere.

4. Experimental

Unless otherwise stated, chemicals of the highest commercial purity were used without further purification. Thin-layer and silica gel column chromatographic steps were performed using Merck SilicaGel 60 F_{254} and Kanto Chemicals Co. Silica Gel 60 N (spherical, neutral), respectively. Chiral HPLC used a DAICEL CHIRALPAK® IA column (φ 20 mm × 25 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 (500 MHz) or a JEOL JNM-EX270 (270 MHz). Chemical shifts are reported in δ ppm using tetramethylsilane as internal standard. Coupling constants (*J*) are given in Hertz. Mass spectra were acquired using FD and FAB techniques using a JMS-SX102A. A part of the NMR spectra and all of the 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 or a P-2200 polarimeter in φ 3.4 mm × 5.0 cm cells at 25 °C. CH_2Cl_2 and CH_3CN were distilled from phosphorous oxide, and pyridine was distilled from calcium hydride.

4.1. 6-*O*-(3′-hydroxy-2′-methylenebutanoyl)-β-D-glucopyranoside (*5*, *epi-5*)

To a solution of **4** (234.7 mg, 0.70 mmol) in dry CH_3CN (7.0 ml), acetaldehyde (88.3 mg, 2.10 mmol) and 3-hydroxy quinuclidine (89.1 mg, 0.70 mmol) were added at room temperature. After 40 h, the reaction mixture was diluted with EtOAc (3.0 ml) and extracted with 3.0 ml of brine. The water layer was then washed with EtOAc (3.0 ml × 2). The combined organic layer was dried over anhydr. Na_2SO_4 and evaporated. Silica gel CC ($MeOH–CHCl_3$, 1:9, v/v) yielded 83.6 mg of 6-*O*-(3′-hydroxy-2′-methylenebutanoyl)-1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside (0.22 mmol, 31%) as a colorless syrup: IR (cm^{-1}) 3400, 1683, 1297, 1207, 1080 and 801, HR-FAB-MS *m/z* $[M-H]^+$ calcd for $C_{16}H_{29}O_8Si$, 377.1632; found, 377.1608. The obtained mixture of diastereomers was further separated by chiral HPLC (EtOH/hexane, 20:80; CHIRALPAK® IA column (φ 20 mm × 25 cm)).

4.1.1. First eluted (3′*R*)- diastereomer

rt = 25.6 min, $[\alpha]_D^{25}$ = −9.1 (*c* 1.77, MeOH), ^{1}H NMR (500 MHz, $CDCl_3$) 0.03 (9H, s, $CH_2CH_2Si\underline{Me}_3$), 0.94–1.06 (2H, m, $CH_2C\underline{H}_2SiMe_3$), 1.37 (1H, d, *J* = 6.5 Hz, CH_3), 3.18 (1H, dd, *J* = 4.7, 4.4 Hz, H-2), 3.30–3.40 (2H, m, H-3 and H-4), 3.52 (1H, ddd, *J* = 9.3, 6.6, 2.4 Hz, H-5), 3.63 (1H, m, $C\underline{H}_2CH_2SiMe_3$), 3.92 (1H, ddd, *J* = 13.3, 7.1, 3.8 Hz, $C\underline{H}_2CH_2SiMe_3$), 4.27 (1H, m, H-6a), 4.33 (1H, d, *J* = 7.8 Hz, H-1), 4.52 (1H, dd, *J* = 6.4, 1.2 Hz, H-6b), 4.68 (1H, br q, *J* = 6.4 Hz, H-3′), 5.93 (1H, dd, *J* = 1.4 Hz, =CH_2, Ha), 6.24 (1H, s, =CH_2, Hb), ^{13}C NMR (125 MHz, $CDCl_3$) −1.4 ($CH_2CH_2Si\underline{Me}_3$), 19.1 ($CH_2\underline{C}H_2SiMe_3$), 23.5 ($CH_3$), 65.0 (C-6), 66.8 ($\underline{C}H_2CH_2SiMe_3$), 68.1 (C-3′), 71.9 (C-4), 75.1 (C-5), 75.2 (C-2), 78.0 (C-3), 103.9 (C-1), 123.9 (=CH_2), 146.7 (C-2′), and 167.5 (C-1′).

4.1.2. Second eluted (3′*S*)- diastereomer

rt = 28.5 min, $[\alpha]_D^{25}$ = −23.6 (*c* 2.22, MeOH), ^{1}H NMR (500 MHz, $CDCl_3$) 0.04 (9H, s, $CH_2CH_2Si\underline{Me}_3$), 0.95–1.04 (2H, m, $CH_2C\underline{H}_2SiMe_3$), 1.34 (1H, d, *J* = 6.4 Hz, CH_3), 3.19 (1H, dd, *J* = 4.5 Hz, H-2), 3.36–3.40 (2H, m, H-3 and H-4), 3.52 (1H, ddd, *J* = 12.9, 9.2, 2.4 Hz, H-5), 3.62 (1H, m, $C\underline{H}_2CH_2SiMe_3$), 3.91 (1H, ddd, *J* = 13.3, 6.9, 3.7 Hz, $C\underline{H}_2CH_2SiMe_3$), 4.27 (1H, dd, *J* = 6.4, 3.3 Hz, H-6a), 4.33 (1H, d, *J* = 7.9 Hz, H-1), 4.57 (1H, dd, *J* = 6.4, 1.2 Hz, H-6b), 4.66 (1H, br q, *J* = 6.4 Hz, H-3′), 5.93 (1H, dd, *J* = 1.4 Hz, =CH_2, Ha), 6.25 (1H, s, =CH_2, Hb), ^{13}C NMR (125 MHz, $CDCl_3$) −1.4 ($CH_2CH_2Si\underline{Me}_3$), 19.1 ($CH_2\underline{C}H_2SiMe_3$), 23.4 ($CH_3$), 64.9 (C-6), 66.9 ($\underline{C}H_2CH_2SiMe_3$), 68.1 (C-3′), 71.8 (C-4), 75.1 (C-5), 75.2 (C-2), 78.0 (C-3), 103.8 (C-1), 123.9 (=CH_2), 146.7 (C-2′), and 167.5 (C-1′).

The above (3′*S*)-epimer (10.2 mg, 27.0 μmol) was dissolved in 500 μl of deprotection solution (CF_3CO_2H/CH_2Cl_2, 2:1, v/v) and stirred for 2 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 by silica gel CC (H_2O/CH_3CN, 1:5). Subsequent evaporation of the solvent provided **5** (7.20 mg, 96%) as a colorless syrup: IR (cm^{-1}) 3412, 1684, 1289, 1205 and 1080, HR-FAB-MS *m/z* $[M + Na]^+$ calcd for $C_{11}H_{18}O_8Na$, 301.0911; found, 301.0905. (3′*R*)-Diastereomer ***epi*-5** was obtained from (*R*)-Baylis–Hillman adduct in 98% yield by the same procedure.

4.1.3. 4′-Deoxy-6-tuliposide B (*5*)

$[\alpha]_D^{25}$ = +55.6 (*c* 1.30, MeOH), ^{1}H NMR (270 MHz, CD_3OD) 1.32 (3H, d, *J* = 6.3 Hz, CH_3), 3.14 (dd, *J* = 7.6 Hz, H-2(β)), 3.31–3.56 (m, H-2(α), H-3(β), H-4(α) and H-4(β)), 3.53 (m, H-5(β)), 3.69 (t, *J* = 9.1 Hz, H-3(α)), 4.03 (ddd, *J* = 9.1, 4.1, 1.9 Hz, H-5(α)), 4.23–4.32 (m, H-6a(α) and H-6a(β)), 4.45–4.55 (m, H-1(β), H-6b(α) and H-6b(β)), 4.64 (br q, *J* = 6.3 Hz, H-3′), 5.09 (d, *J* = 3.6 Hz, H-1(α)), 5.91 (1H, s, =CH_2, Ha), 6.23 (1H, s, =CH_2, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 23.3 (CH_3) 65.0 (C-6), 66.9 (C-3′), 70.7 (C-5(α)), 71.8 (C-4(β)), 72.0 (C-4(α)), 73.8 (C-2(α)), 74.8 (C-3(α)), 75.4 (C-5(β)), 76.3 (C-2(β)), 78.0 (C-3(β)), 94.0 (C-1(α)), 98.3 (C-1(β)), 123.8 and 126.9 (=CH_2), 146.7 and 146.8 (C-2′), 167.7 (C-1′).

4.1.4. 4′-Deoxy-epi-6-tuliposide B (***epi*-5**)

$[\alpha]_D^{25}$ = +34.7 (*c* 1.72, MeOH), ^{1}H NMR (270 MHz, CD_3OD) 1.29 (3H, d, *J* = 6.4 Hz, CH_3), 3.12 (dd, *J* = 8.2 Hz, H-2(β)), 3.29–3.33 (m, H-2(α), H-3(β), H-4(α) and H-4(β)), 3.51 (m, H-5(β)), 3.66 (t, *J* = 9.2 Hz, H-3(α)), 4.03 (m, H-5(α)), 4.23–4.31 (m, H-6a(α) and H-6a(β)), 4.39–4.49 (m, H-1(β), H-6b(α) and H-6b(β)), 4.56–4.65

(m, H-3′), 5.07 (d, J = 3.5 Hz, H-1(α)), 5.90 (1H, s, =CH_2, Ha), 6.21 (1H, s, =CH_2, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 23.3 (CH_3) 65.0 and 65.1 (C-6(α) and C-6(β)), 66.8 and 66.9 (C-3′ (α) and C-3′(β)), 70.7 (C-5(α)), 71.8 (C-4(β)), 72.0 (C-4(α)), 73.8 (C-2(α)), 74.7 (C-3(α)), 75.3 (C-5(β)), 76.2 (C-2(β)), 78.0 (C-3(β)), 94.0 (C-1(α)), 98.3 (C-1(β)), 123.9 (=CH_2), 146.6 and 146.7 (C-2′), 167.6 and 167.7 (C-1′).

4.2. 6-O-(3′,6′-dihydroxy-2′-methylenehexanoyl)-D-glucose (**7, epi-7**)

Following the same procedure as **4.1**, hexanoyl-type precursors were synthesized from **4** (234.7 mg, 0.51 mmol), 4-(*tert*-butyldimethylsilyloxy)-butanal (**6**, 310.0 mg, 1.53 mmol) and 3-hydroxy quinuclidine (65.0 mg, 0.51 mmol) in dry DMSO (5.1 ml). Silica gel CC was conducted using MeOH/$CHCl_3$ (5:95) then MeOH/$CHCl_3$ (1:9) as developing solvent to yielded 114.9 mg of 6-*O*-[6′-(*tert*-butyldimethylsilyloxy)-3′-hydroxy-2′-methylenehexanoyl]-1-*O*-(2-trimethylsilylethyl)-β-D-glucopyranoside (0.21 mmol, 42%) as a colorless syrup: IR (cm^{-1}) 3410, 1715, 1250, 1161, 1079, 860 and 834, HR-FD-MS m/z $[M+H]^+$ calcd for $C_{24}H_{49}O_9Si_2$, 537.2916; found, 537.2921. The obtained mixture of diastereomers was further separated by chiral HPLC (EtOH/hexane, 8:92; CHIRALPAK® IA column (φ 20 mm × 25 cm)).

4.2.1. First eluted (3′*R*)- diastereomer

rt = 97.8 min, $[\alpha]_D^{25}$ = −14.7 (c 1.68, MeOH), ^{1}H NMR (500 MHz, $CDCl_3$) 0.01–0.06 (15H, s, Me_2Si and $CH_2CH_2SiMe_3$), 0.89 (9H, a, *t*-Bu), 0.94–1.03 (2H, m, $CH_2CH_2SiMe_3$), 1.54–1.77 (4H, m, H-5′ and H-6′), 3.16 (1H, t, J = 8.4 Hz, H-2), 3.29–3.37 (2H, m, H-4 and H-4′a), 3.49 (1H, ddd, J = 5.0, 3.5, 1.3 Hz, H-5), 3.60–3.66 (3H, m, H-3, H-4′b and $CH_2CH_2SiMe_3$), 3.92 (1H, m, CH_2CH_2TMS), 4.24–4.28 (1H, m, H-6a), 4.27 (1H, d, J = 7.8 Hz, H-1), 4.48–4.51 (2H, m, H-3′ and H-6b), 5.89 (1H, s, =CH_2, Ha), 6.25 (1H, s, =CH_2, Hb), ^{13}C NMR (125 MHz, $CDCl_3$) −5.1 (Me_2Si), −1.3 ($CH_2CH_2SiMe_3$), 19.1 ($CH_2CH_2SiMe_3$), 19.2 (Me_3C), 26.5 (Me_3C), 30.1 and 34.1 (C-5′ and C-6′), 64.3 (C-6), 65.0 (C-4′), 68.1 ($CH_2CH_2SiMe_3$), 70.6 (C-4), 71.9 (C-3′), 75.1 (C-5), 75.2 (C-2), 78.0 (C-3), 103.8 (C-1), 124.6 (=CH_2), 145.6 (C-2′), and 167.5 (C-1′).

4.2.2. Second eluted (3′*S*)- diastereomer

rt = 104.9 min, $[\alpha]_D^{25}$ = −39.5 (c 2.01, MeOH), ^{1}H NMR (500 MHz, $CDCl_3$) 0.01–0.06 (15H, s, Me_2Si and $CH_2CH_2SiMe_3$), 0.89 (9H, a, *t*-Bu), 0.93–1.05 (2H, m, $CH_2CH_2SiMe_3$), 1.53–1.78 (4H, m, H-5′ and H-6′), 3.16 (1H, t, J = 8.3 Hz, H-2), 3.28–3.37 (2H, m, H-4 and H-4′a), 3.49 (1H, ddd, J = 5.0, 3.5, 1.3 Hz, H-5), 3.61–3.66 (3H, m, H-3, H-4′b and $CH_2CH_2SiMe_3$), 3.92 (1H, ddd, J = 7.2, 3.8, 2.0 Hz, $CH_2CH_2SiMe_3$), 4.23 (1H, dd, J = 6.4, 3.3 Hz, H-6a), 4.27 (1H, d, J = 7.8 Hz, H-1), 4.49 (1H, m, H-3′), 4.52 (1H, dd, J = 6.4, 1.2 Hz, H-6b), 5.89 (1H, s, =CH_2, Ha), 6.25 (1H, s, =CH_2, Hb), ^{13}C NMR (125 MHz, $CDCl_3$) −5.1 (Me_2Si), −1.3 ($CH_2CH_2SiMe_3$), 19.1 ($CH_2CH_2SiMe_3$), 19.2 (Me_3C), 26.5 (Me_3C), 30.2 and 34.2 (C-5′ and C-6′), 64.3 (C-6), 65.0 (C-4′), 68.1 ($CH_2CH_2SiMe_3$), 70.7 (C-4), 71.8 (C-3′), 75.1 (C-5), 75.2 (C-2), 78.0 (C-3), 103.8 (C-1), 127.7 (=CH_2), 145.6 (C-2′), and 167.5 (C-1′).

Hexanoyl-type 6-tuliposide B (**7**) and its epimer (***epi*-7**) were obtained from the above 3′*R*-diastereomer and 3′*S*-diastereomer in 95% yields, respectively, by using essentially the same deprotection procedures as mentioned in **4.1.2** except for reaction time (1.5 h): IR (cm^{-1}) 3375, 1709, 1679, 1205, 1143 and 1057, HR-FD-MS m/z $[M+H]^+$ calcd for $C_{13}H_{23}O_9$, 323.1342; found, 323.1370.

4.2.3. Hexanoyl-type 6-tuliposide B (**7**)

$[\alpha]_D^{25}$ = +37.6 (c 1.39, MeOH), ^{1}H NMR (270 MHz, CD_3OD) 1.48–1.83 (4H, m, H-5′ and H-6′), 3.13 (t, J = 8.4 Hz, H-2(β)), 3.30–3.38 (m, H-4′a, H-2(α), H-3(β), H-4(α) and H-4(β)), 3.54–3.58 (H-4′b and H-5(β)), 3.67 (1H, t, J = 9.3 Hz, H-5(α)), 4.00 (1H, ddd, J = 9.7, 4.9, 2.0 Hz, H-5(α)), 4.21–4.29 (1H, m, H-6a), 4.45–4.53 (m, H-6b, H-3′ and H-1(β)), 5.08 (d, J = 3.6 Hz, H-1(α)), 5.89 (1H, s, =CH_2, Ha), 6.25 (1H, s, =CH_2, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 29.9 and 34.3 (C-5′ and C-6′) 63.0 (C-6), 65.0 (C-3′), 70.7 (C-5(α)), 71.8 (C-4(β)), 72.0 (C-4(α)), 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)), 124.7 (=CH_2), 146.7 (C-2′), 167.7 (C-1′).

4.2.4. Hexanoyl-type epi-6-tuliposide B (***epi*-7**)

$[\alpha]_D^{25}$ = +25.7 (c 1.52, MeOH), ^{1}H NMR (270 MHz, CD_3OD) 1.45–1.76 (4H, m, H-5′ and H-6′), 3.09 (t, J = 8.2 Hz, H-2(β)), 3.26–3.33 (m, H-4′a, H-2(α), H-3(β), H-4(α) and H-4(β)), 3.50–3.55 (H-4′b and H-5(β)), 3.63 (1H, t, J = 9.0 Hz, H-5(α)), 3.94–3.98 (1H, m, H-5(α)), 4.23–4.30 (1H, m, H-6a), 4.34–4.56 (m, H-6b, H-3′ and H-1(β)), 5.04 (d, J = 3.3 Hz, H-1(α)), 5.85 (1H, s, =CH_2, Ha), 6.21 (1H, s, =CH_2, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 29.9 and 34.3 (C-5′ and C-6′) 63.0 (C-6), 65.0 and 65.1 (C-3′), 70.7 (C-5(α)), 71.8 (C-4(β)), 72.0 (C-4(α)), 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)), 124.7 (=CH_2), 145.6 and 145.7 (C-2′), 167.7 (C-1′).

4.3. Hydrogenation of 6-tuliposide Bs

To a solution of 6-tuliposide B (**1**) (8.70 mg, 29.5 μmol) in MeOH (200 μl) was added Pd/C (2.1 mg). The mixture was allowed to stir under H_2 atmosphere at room temperature overnight. Filtration with a Celite pad and evaporation of solvent were followed by purification via silica gel CC (H_2O/MeCN, 1:5). Subsequent evaporation provided **8** (6.35 mg, 72%) as a colorless syrup. The (3′*R*)-diastereomer ***epi*-8** was obtained from ***epi*-1** by the same procedure in 68% yield.

IR (cm^{-1}) 3401, 1769, 1182 and 1041, HR-FAB-MS m/z $[M+Na]^+$ calcd for $C_{11}H_{20}O_9Na$, 319.1006; found, 319.1003.

4.3.1. Hydrogenated 6-Tuliposide B (***8***)

^{1}H NMR (270 MHz, CD_3OD) 1.14–1.28 (3H, m, CH_3), 2.42–2.77 (1H, m, H-2′), 3.08–3.16 (m, H-2(β)), 3.25–3.90 (m, H-2(α), H-3, H-4, H-5(β), H-3′ and H-4′), 3.93–4.00 (m, H-5(α)), 4.12–4.25 (m, H-6a), 4.31–4.48 (m, H-6b and H-1(β)), 5.07–5.10 (m, H-1 (α)). ^{13}C NMR (67.5 MHz, CD_3OD) 11.9, 12.0, 13.7 and 13.8 (CH_3),43.7, 44.3 and 44.3 (C-2′), 64.7, 64.9, 65.0 and 65.1 (C-6), 70.7 (C-5(α)), 71.5, 71.8, 71.9 and 72.0 (C-4), 73.8 and 73.9 (C-2(α) and C-3′), 74.7 (C-3(α)), 75.0 (C-4′), 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)), 176.4 and 176.5 (C-1′).

4.3.2. Hydrogenated epi-6-tuliposide B (***epi*-8**)

^{1}H NMR (270 MHz, CD_3OD) 1.13–1.19 (3H, m, CH_3), 2.58–2.74 (1H, m, H-2′), 3.09–3.15(m, H-2(β)), 3.25–3.69 (m, H-2(α), H-3, H-4 and H-4′), 3.73–3.81 (m, H-5(β) and H-3′), 3.89 (t, J = 6.0 Hz, H-3′), 3.91–4.00 (m, H-5(α)), 4.16–4.27 (m, H-6a), 4.32–4.47 (m, H-6b), 4.46 (d, J = 7.7 Hz, H-1(β)), 5.07 (d, J = 3.7 Hz, H-1 (α)), ^{13}C NMR (67.5 MHz, CD_3OD) 11.8, 12.0, 13.6 and 13.7 (CH_3),43.7, 43.7, 44.3 and 44.3 (C-2′), 64.6, 64.8, 64.9 and 65.1 (C-6), 70.7 (C-5(α)), 71.6, 71.7, 71.9 and 72.0 (C-4), 73.8 and 73.9 (C-2(α) and C-3′), 74.7 (C-3(α)), 75.0 (C-4′), 75.3 and 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)), 176.4 and 176.6 (C-1′).

4.4. Methyl 3-hydroxy-4-(p-methoxybenzyloxy)-2-methylenebutanoate (***10*/*ent*-10**)

3-hydroxy quinuclidine (2.10 g, 16.5 mmol) was dissolved in DMSO (10 ml) and methylacrylate (100 ml) was added. To the solution, 6.13 g of (*p*-methoxybenzyloxy)-acetaldehyde (34.1 mmol) was added and stirred for 15 h at room temperature. The reaction mixture was diluted with Et_2O, washed with H_2O

(50 ml), and subsequently with brine (50 ml). The resulting organic layer was dried (anhydr. Na_2SO_4) and evaporated. Following purification by silica gel CC (EtOAc/hexane, 1:4–1:2) afforded the Baylis–Hillman adduct (**10**/***ent*-10**, 7.61 g, 84%).

IR (cm^{-1}) 3432, 1709, 1514, 1248, 1180, 1105, 1085 and 1033, ^1H NMR (270 MHz, $CDCl_3$), 3.39 (1H, dd, J = 9.6, 7.3 Hz, H-4a), 3.71 (1H, dd, J = 9.8, 3.6 Hz, H-4b), 3.76 (3H, s, OMe), 3.81 (3H, s, PhOMe), 4.51 (2H, m, benzyl), 4.73 (1H, dd, J = 7.2, 3.3 Hz, H-3), 6.01 (1H, dd, J = 1.3 Hz, =CH_2, H-a), 6.36, (1H, dd, J = 1.1 Hz, =CH_2, H-b), 6.89, (2H, dd, J = 8.8, 2.5 Hz, aromatic), 7.26, (2H, m, aromatic), ^{13}C NMR (67.5 MHz, $CDCl_3$), 51.8 (OMe), 55.2 (PhOMe), 69.5 (C-3), 72.9 (C-4), 73.2 (benzyl), 113.8 (aromatic), 126.6 (=CH_2), 129.4 (aromatic), 129.9 (aromatic), 139.1 (C-2), 159.3 (aromatic), 166.4 (C-1), HR-FD-MS m/z $[M]^+$, calcd for $C_{14}H_{18}O_5$, 266.1154; found, 266.1168.

4.4.1. Katsuki-Shrapless kinetic resolution of BH adduct (*10*/*ent-10*, *11*/*ent-11*)

To a stirred suspension of MS 4A (1.50 g, activated by use of a microwave) in CH_2Cl_2 (25 ml) was added $Ti(O^iPr)_4$ (0.50 g, 1.76 mmol) at room temperature. The mixture was then cooled to −25 °C to which was added (+)-diisopropyl tartrate (0.70 g, 2.99 mmol) in CH_2Cl_2 (5.0 ml) dropwise. After stirring for 1 h, the racemate of **10**/***ent*-10** (1.00 g, 3.50 mmol) in CH_2Cl_2 (20 ml) was added dropwise and left for an additional 1 h. Cumenehydroperoxide was added dropwise and the reaction mixture was allowed to stir at −20 °C for 24 h. The reaction was quenched by addition of H_2O/acetone (15 ml: 4 ml) and stirred at room temperature. The resultant emulsion was filtered through a Celite pad. The filtrate was then diluted with Et_2O and washed with 1 M HCl (80 ml), $NaHCO_3$ (80 ml) and brine (80 ml), respectively. The organic layer was dried (anhydr.Na_2SO_4), evaporated, and then the resulting residue was purified by silica gel CC (EtOAc/hexane, 1:2) to give the allyl alcohol and the epoxide. Evaporation yielded **10** (430 mg) (43%, >99%ee) and **11** (403 mg) (40%, 96%ee) as syrups. Kinetic resolution with (−)-diisopropyl tartrate gave (3*R*)-allyl alcohol (***ent*-10**) and (3*R*)-epoxide (***ent*-11**), respectively, with enantiomeric excesses measured by chiral HPLC using a CHIRALPAK IA column. Allyl alcohols were eluted with EtOH:hexane = 25:75, and epoxides were eluted with EtOH:hexane = 60:40, *S*-allyl alcohol (**10**, rt = 26.9 min.) $[\alpha]_D^{25} = +5.3$ (c 0.60, $CHCl_3$), *R*-allyl alcohol (***ent*-10**, rt = 21.1 min.) $[\alpha]_D^{25} = -5.4$ (c 0.52, $CHCl_3$), *S*-epoxide (**11**, rt = 18.1 min.) $[\alpha]_D^{25} = -10.7$ (c 1.10, $CHCl_3$), *R*-epoxide (***ent*-11**, rt = 21.3 min.) $[\alpha]_D^{25} = +11.3$ (c 1.73, $CHCl_3$), ^1H NMR (270 MHz, $CDCl_3$) 3.05 (1H, d, J = 5.8 Hz, H_2C-β), 3.12 (1H, d, J = 5.8 Hz, H_2C-β), 3.66–3.80 (2H, m, H_2C-γ, 3.71 (3H, s, OMe), 3.80 (3H, s, PhOMe), 4.14 (1H, br t, J = 4.5 Hz, H-β), 4.48 (2H, s, benzyl), 6.87, (2H, d, J = 8.8 Hz, aromatic), 7.23, (2H, d, J = 8.8 Hz, aromatic). ^{13}C NMR (67.5 MHz, $CDCl_3$) 50.0 (oxyranyl-C-β), 52.6 (OMe), 55.2 (PhOMe), 56.3 (C-2), 69.2 (C-3), 70.4 (C-4), 73.1 (benzyl), 113.8 (aromatic), 129.4 (aromatic), 129.9 (aromatic), 159.3 (aromatic), 169.7 (C-1), HR-FD-MS m/z $[M]^+$ calcd for $C_{14}H_{18}O_6$, 282.1103; found, 282.1106.

4.5. Methyl 3-(tert-butyldimethylsilyloxy)-4-(p-methoxybenzyloxy)-2-methylenebutanoate (*12, ent-12*)

To a solution of **10** (1.00 g, 3.50 mmol) in CH_2Cl_2 (20 ml), triethylamine (976 μl, 7.00 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (1.02 g, 3.85 mmol) was added in series. The reaction mixture was allowed to stir at room temperature for 1 h. After that, the reaction was quenched by satd. NH_4Cl (20 ml) and washed with Et_2O (30 ml). The organic phase was dried (anhydr. Na_2SO_4) and the solvent was removed by evaporation. The resulting residue was subjected to short silica gel CC (EtOAc:hexane, 1:4) to afford 1.33 g (**12**, quant.) as a colorless syrup. ***ent*-12** was yielded from ***ent*-10** by the same procedure as above. **12** $[\alpha]_D^{25} = +17.3$ (c 1.12 $CHCl_3$), ***ent*-12** $[\alpha]_D^{25} = -18.4$ (c 1.08, $CHCl_3$), IR (cm^{-1}) 1721, 1514, 1250, 1100, 1038 and 834, ^1H NMR (270 MHz, $CDCl_3$) 0.03 and 0.09 (3H, s, $SiMe_2$), 0.90 (9H, s, *t*-Bu), 3.37 (1H, dd, J = 10.1, 6.5 Hz, H-4a), 3.51 (1H, dd, J = 10.1, 3.3 Hz, H-4b), 3.73 (3H, s, OMe), 3.80 (3H, s, PhO<u>Me</u>), 4.48 (2H, m, benzyl), 4.79–4.83 (1H, m, H-3), 6.01 (1H, dd, J = 1.3 Hz, =CH_2, H-a), 6.31, (1H, dd, J = 1.9, 0.9 Hz, =CH_2, H-b), 6.86, (2H, dd, J = 8.8, 2.5 Hz, aromatic), 7.26, (2H, m, aromatic), ^{13}C NMR (67.5 MHz, $CDCl_3$) −5.0 and −4.8 ($SiMe_2$), 18.2 ($Me_3\underline{C}$), 25.8 ($\underline{Me}_3C$), 51.8 (OMe), 55.2 (PhO<u>Me</u>), 70.4 (C-3), 72.8 (C-4), 74.7 (benzyl), 113.6 (aromatic), 126.1 (=CH_2), 129.1 (aromatic), 130.6 (aromatic), 141.2 (C-2), 159.0 (aromatic), 166.5 (C-1), HR-FD-MS m/z $[M]^+$ calcd for $C_{20}H_{32}O_5Si$, 380.2019; found, 380.2001.

4.6. 3-(tert-Butyldimethylsilyloxy)-4-(p-methoxybenzyloxy)-2-methylenebutanoic acid (*13*)

To a solution of **12** (1.28 g, 3.37 mmol) in 64 ml of H_2O/CH_3CN (1:1, v/v), lithium hydroxide monohydrate (320 mg, 7.62 mmol) was added and stirred at 55 °C. After 1 h, the reaction mixture was acidified to pH 3 with dilute HCl and washed with EtOAc (50 ml × 2). The combined organic layer was then washed with brine (50 ml) and dried (anhydr. Na_2SO_4) followed by evaporation. The crude product so obtained was further purified using silica gel CC (EtOAc/hexane, 1:2) to give 1.13 g of **13** (3.10 mmol, 92%) as a white solid. **13** $[\alpha]_D^{25} = +26.0$ (c 1.20, $CHCl_3$), ***ent*-13** $[\alpha]_D^{25} = -28.8$ (c 1.03, $CHCl_3$). IR (cm^{-1}) 3008, 1696, 1514, 1251 1104 and 833, ^1H NMR (270 MHz, $CDCl_3$) 0.05 and 0.09 (3H, s, $SiMe_2$), 0.90 (9H, s, *t*-Bu), 3.41 (1H, dd, J = 10.0, 6.4 Hz, H-4a), 3.53 (1H, dd, J = 10.1, 3.6 Hz, H-4b), 3.79 (3H, s, PhO<u>Me</u>), 4.48 (2H, m, benzyl), 4.76 (1H, br dd, J = 6.2 3.5 Hz, H-3), 6.03 (1H, s, =CH_2, H-a), 6.43, (1H, s, =CH_2, H-b), 6.86, (2H, dd, J = 8.7, 2.4 Hz, aromatic), 7.23, (2H, m, aromatic), ^{13}C NMR (67.5 MHz, $CDCl_3$) −5.0 and −4.8 ($SiMe_2$), 18.2 ($Me_3\underline{C}$), 25.7 ($\underline{Me}_3C$), 55.2 (PhO<u>Me</u>), 70.8 (C-3), 72.9 (C-4), 74.4 (benzyl), 113.7 (aromatic), 128.5 (=CH_2), 129.2 (aromatic), 130.2 (aromatic), 140.2 (C-2′), 159.1 (aromatic), 169.9 (C-1′), HR-FAB-MS m/z $[M-H]^-$ calcd for $C_{19}H_{29}O_5Si$, 365.1822; found, 365.1803.

4.7. 6-O-(p-Toluenesulfonyl)-1-O-(2-trimethylsilylethyl)-β-D-glucopyranoside (*15*)

To a stirred solution of (2-trimethylsilylethyl)- glucoside (**14**, 1.22 g, 4.35 mmol) in pyridine (18 ml), *p*-toluenesulfonyl chloride (860 mg, 4.51 mmol) solution in pyridine (2.0 ml) was added dropwise at 0 °C. After 15 h, the reaction was quenched by addition of few drops of MeOH and diluted with toluene. The solvent was then azeotropically removed by evaporation, with the addition and evaporation of toluene repeated twice. The obtained crude product was purified by silica gel CC (MeOH/$CHCl_3$, 1:9) to give 1.58 g of **15** (3.63 mmol, 83%) as a white solid.

IR (cm^{-1}) 3392, 1364, 1248, 1190, 1178, 1096, 1040 and 836, ^1H NMR (270 MHz, $CDCl_3$) 0.00 (9H, s, $CH_2CH_2Si\underline{Me}_3$), 0.88–1.08 (2H, m, $CH_2\underline{CH}_2SiMe_3$), 2.43 (3H, s, $PhC\underline{H}_3$), 3.32 (1H, t, J = 8.4 Hz, H-2), 3.39–3.61 (4H, m, H-3, 4, 5 and $C\underline{H}_2CH_2SiMe_3$), 3.82–3.92 (1H, m, $C\underline{H}_2CH_2SiMe_3$), 4.22–4.33 (2H, m, H-1, H-6a and H-6b), 7.33 (2H, d, J = 8.2 Hz, aromatic), 7.80, (2H, d, J = 8.2 Hz, aromatic), ^{13}C NMR (67.5 MHz, $CDCl_3$) −1.5 ($CH_2CH_2Si\underline{Me}_3$), 18.2 ($CH_2\underline{C}H_2SiMe_3$), 21.6 ($Ph\underline{C}H_3$), 67.4 (C-6), 69.1($CH_2CH_2SiMe_3$), 69.6 (C-4), 73.2 (C-2), 73.3 (C-5), 76.1 (C-3), 101.8 (C-1), 128.0 (aromatic), 129.8(aromatic), 132.7 (aromatic), 144.8 (aromatic), HR-FAB-MS m/z $[M + Na]^+$ calcd for $C_{18}H_{30}O_8SiSNa$, 457.1328; found, 457.1348.

*4.8. 6-Azido-6-deoxy-1-O-(2-trimethylsilylethyl)-β-D-glucopyranoside (**16**)*

Tosylate **15** (1.58 g, 3.63 mmol) and NaN_3 (484 mg, 7.44 mmol) were dissolved in DMF (33 ml). The reaction mixture was allowed to stir at 60 °C for 24 h. After that, the mixture was added satd. $NaHCO_3$ (30 ml) and washed with EtOAc (50 ml × 2). The combined organic phase was then dried (anhydr. Na_2SO_4) and the solvent was removed under reduced pressure. The resulting crude product was next subjected to further purification by silica gel CC (MeOH/$CHCl_3$, 1:5–1:9) to give 1.02 g of **16** (3.34 mmol, 92%) as a white solid.

IR (cm^{-1}) 3417, 2172, 2128, 2103, 1078, 1051 and 838, ^1H NMR (270 MHz, $CDCl_3$) 0.00 (9H, s, $CH_2CH_2Si\underline{Me}_3$), 0.89–1.08 (2H, m, $CH_2C\underline{H}_2SiMe_3$), 3.12–3.42 (6H, m, H-2, H-3, H-4, H-5, H-6a and H-6b), 3.57–3.67 (1H, m, $C\underline{H}_2CH_2SiMe_3$), 3.92–4.02 (1H, m, $C\underline{H}_2CH_2SiMe_3$), 4.28 (1H, d, J = 7.7 Hz, H-1), ^{13}C NMR (67.5 MHz, $CDCl_3$) −1.5 ($CH_2CH_2Si\underline{Me}_3$), 19.4 ($CH_2\underline{C}H_2SiMe_3$), 52.8 (C-6), 68.0($\underline{C}H_2CH_2SiMe_3$), 72.6 (C-4), 75.1 (C-2), 77.2 (C-5), 77.9 (C-3), 103.7 (C-1), HR-FAB-MS *m/z* $[M + Na]^+$, calcd for $C_{11}H_{23}O_5N_3SiNa$, 328.1305; found, 328.1297.

*4.9. 6-Amino-6-deoxy-1-O-(2-trimethylsilylethyl)-β-D-glucopyranoside (**17**)*

The azido sugar **16** (309 mg, 1.01 mmol) was dissolved in EtOAc (3.0 ml) and Pd activated C (15 mg) was subsequently added, with the suspension allowed to stir under H_2 atmosphere overnight. After that, the Pd/C was removed by filtration through a Celite pad and the filtrate was evaporated to give the amino sugar **17** (280 mg quant., slightly impure).

IR (cm^{-1}) 3370, 1364, 1250, 1075, 1050, 862 and 837, ^1H NMR (270 MHz, CD_3OD) 0.03 (9H, s, $CH_2CH_2Si\underline{Me}_3$), 1.00 (2H, m, $CH_2C\underline{H}_2SiMe_3$), 2.72 (1H, dd, J = 13.4, 6.8 Hz, H-6a), 3.02 (1H, dd, J = 13.3, 2.5 Hz, H-6b), 3.11–3.34 (4H, m, H-2, H-3, H-4 and H-5), 3.66 (1H, m, $C\underline{H}_2CH_2SiMe_3$), 3.97 (1H, m, $C\underline{H}_2CH_2SiMe_3$), 4.27, (1H, d, J = 7.7 Hz, H-1), ^{13}C NMR (67.5 MHz, CD_3OD) −1.4 ($CH_2CH_2Si\underline{Me}_3$), 19.1 ($CH_2\underline{C}H_2SiMe_3$), 43.9 (C-6), 68.1 ($\underline{C}H_2CH_2SiMe_3$), 73.3 (C-4), 75.2 (C-2), 77.6 (C-5), 78.0 (C-3), 103.9 (C-1), HR-FAB-MS *m/z* $[M-H]^-$, calcd for $C_{11}H_{24}NO_5Si$, 278.1424; found, 278.1428.

*4.10. 6-[3′-(tert-Butyldimethylsilyloxy)-4′-(p-methoxybenzyloxy)-2′-methylenebutanoyl]amino-6-deoxy-1-O-(2-trimethylsilylethyl)-β-D-glucopyranoside (**18, epi-18**)*

To a solution of the amino sugar **17** (279 mg, 1.00 mmol) in CH_2Cl_2 (3.0 ml), dimethylaminopyridine (61.0 mg, 0.5 mmol) and carboxylic acid **13** (402 mg, 1.10 mmol) were added at −20 °C. After that, diisopropylcabodiimide (126 mg, 1.00 mmol) was added slowly dropwise and stirred at 0 °C overnight. The reaction mixture was then diluted with EtOAc, whereupon it was washed with satd. NH_4Cl, brine and dried (anhydr. Na_2SO_4). Subsequent removal of solvent by evaporation and purification by silica gel CC (MeOH/$CHCl_3$, 1:9) gave acrylamide **18** (508 mg, 0.81 mmol, 81%) as a syrup. The 3′-epimer ***epi*-18** was synthesized in a similar way in 70% yield.

IR (cm^{-1}) 3556, 3460, 3340, 1610, 1513, 1465, 1361, 1250, 1056, 861, 836 and 780, HR-FD-MS *m/z* $[M]^+$ calcd for $C_{30}H_{53}NO_9Si_2$, 627.3259; found, 627.3249.

*4.10.1. Acrylamide **18** (3′S)*

$[\alpha]_D^{25} = -68.4$ (*c* 2.22, $CHCl_3$), ^1H NMR (270 MHz, CD_3OD) 0.01 (9H, s, $CH_2CH_2Si\underline{Me}_3$), 0.06 and 0.09 (6H, s, $SiMe_2$), 0.90 (9H, s, *t*-Bu), 0.90–0.97 (2H, m, $CH_2C\underline{H}_2SiMe_3$), 3.15 (1H, t, J = 8.8 Hz, H-2), 3.30–3.63 (8H, m, H-3, H-4, H-5, H-6a, H-6b and $C\underline{H}_2CH_2SiMe_3$), 3.77 (3H, s, OMe), 3.91–4.01 (1H, m, $C\underline{H}_2CH_2SiMe_3$), 4.24 (1H, d, J = 7.7 Hz, H-1), 4.46, (2H, s, benzyl), 4.78 (1H, m, H-3′), 5.64 (1H, s, $=CH_2$, Ha), 5.87 (1H, s, $=CH_2$, Hb), 6.87 (2H, d, J = 8.5 Hz, aromatic), 7.21 (2H, d, J = 8.5 Hz, aromatic), ^{13}C NMR (125 MHz, CD_3OD) −4.8 and −4.6 ($SiMe_2$), −1.3 ($CH_2CH_2Si\underline{Me}_3$), 19.1 ($CH_2\underline{C}H_2SiMe_3$ and $Me_3\underline{C}$), 26.4 ($\underline{Me}_3C$), 41.5 (C-6), 55.7 (PhO$\underline{Me}$), 68.2 ($\underline{C}H_2CH_2SiMe_3$), 73.0 (C-4′), 73.5 (C-4), 73.8 (benzyl), 75.2 (C-3′), 75.8 (C-5), 75.6 (C-2), 77.6 (C-3), 103.9 (C-1), 114.8 (aromatic), 121.7 ($=CH_2$), 130.4 (aromatic), 131.4 (aromatic), 146.0 (), 160.8 (aromatic), 169.9 (C-1′).

*4.10.2. Acrylamide **epi-18** (3′R)*

$[\alpha]_D^{25} = -94.8$ (*c* 1.88, $CHCl_3$), ^1H NMR (270 MHz, CD_3OD) 0.01 (9H, s, $CH_2CH_2Si\underline{Me}_3$), 0.07 and 0.11 (6H, s, $SiMe_2$), 0.90 (9H, s, *t*-Bu), 0.90–1.02 (2H, m, $CH_2C\underline{H}_2SiMe_3$), 3.13 (1H, t, J = 7.8 Hz, H-2), 3.19–3.26 (1H, m, H-5), 3.30–3.57 (2H, m, H-3, H-4, H-6a and $C\underline{H}_2CH_2SiMe_3$), 3.66 (1H, dd, J = 14.0, 2.7 Hz, H-6b), 3.78 (3H, s, OMe), 3.91 (1H, ddd, J = 13.3, 7.2, 3.8 Hz, $C\underline{H}_2CH_2SiMe_3$), 4.12 (1H, d, J = 7.8 Hz, H-1), 4.44, (2H, s, benzyl), 4.79 (1H, br dd, J = 5.4, H-3′), 5.64 (1H, s, $=CH_2$, Ha), 5.89 (1H, s, $=CH_2$, Hb), 6.87 (2H, d, J = 8.5 Hz, aromatic), 7.21 (2H, d, J = 8.5 Hz, aromatic), ^{13}C NMR (125 MHz, CD_3OD) −4.8 and −4.6 ($SiMe_2$), −1.3 ($CH_2CH_2Si\underline{Me}_3$), 19.1 ($CH_2\underline{C}H_2SiMe_3$ and $Me_3\underline{C}$), 26.4 ($\underline{Me}_3C$), 41.5 (C-6), 55.7 (PhO$\underline{Me}$), 68.2 ($\underline{C}H_2CH_2SiMe_3$), 72.9 (C-4′), 73.4 (C-4), 74.0 (benzyl), 75.2 (C-3′), 75.4 (C-5), 75.6 (C-2), 77.6 (C-3), 104.0 (C-1), 114.7 (aromatic), 121.4 ($=CH_2$), 130.5 (aromatic), 131.5 (aromatic), 146.2 (C-2′), 160.8 (aromatic), 170.0 (C-1′).

*4.11. 6-Deoxy-6-(3′,4′-dihydroxy-2′-methylenebutanoamido)-β-D-glucopyranoside (**19, epi-19**)*

Following the procedure described in **4.2**, amide product **19** and ***epi*-19** were obtained in 90% and 92% yield, respectively.

IR (cm^{-1}) 3402, 1687, 1206, 1141 and 1055, HR-FAB-MS *m/z* $[M-H]^-$ calcd for $C_{11}H_{18}NO_8$, 292.1033; found, 292.1050.

*4.11.1. Amide-type 6-tuliposide B (**19**)*

$[\alpha]_D^{25} = +24.7$ (*c* 0.84, MeOH), ^1H NMR (270 MHz, CD_3OD) 3.08–3.19 (m, H-6a and H-2(β)), 3.30–3.70 (m, H-2(α), H-3(α), H-3(β), H-4(α), H-6b, H-4′a, H-5(β), H-4′b and H-4(β)), 3.81–3.88 (m, H-5(α)), 4.47 (d, J = 7.8 Hz, H-1(β)), 4.49–4.51 (m, H-3′), 5.10 (d, J = 3.6 Hz, H-1(α)), 5.64 (1H, d, J = 3.5 Hz, $=CH_2$, Ha), 5.87 (1H, s, $=CH_2$, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 41.6 and 41.7 (C-6(α) and C-6(β)), 66.4 and 66.5 (C-4′), 71.2 (C-5(α)), 73.0 (C-4(β)), 73.2 (C-4(α)), 73.7 (C-3′), 73.8 (C-2(α)), 74.4 (C-3(α)), 75.9 (C-5(β)), 76.3 (C-2(β)), 77.5 (C-3(β)), 94.0 (C-1(α)), 98.2 (C-1(β)), 121.5 and 121.6 ($=CH_2$), 145.4 (C-2′), 170.7 and 170.8 (C-1′).

*4.11.2. Amide-type epi-6-tuliposide B (**epi-19**)*

$[\alpha]_D^{25} = +11.9$ (*c* 1.03, MeOH), ^1H NMR (270 MHz, CD_3OD) 3.08–3.18 (m, H-6a and H-2(β)), 3.29–3.69 (m, H-2(α), H-3(α), H-3(β), H-4(α), H-6b, H-4′a, H-5(β), H-4′b and H-4(β)), 3.80–3.87 (m, H-5(α)), 4.46 (d, J = 7.7 Hz, H-1(β)), 4.48–4.52 (m, H-3′), 5.08 (d, J = 3.7 Hz, H-1(α)), 5.62 (1H, d, J = 4.0 Hz, $=CH_2$, Ha), 5.86 (1H, s, $=CH_2$, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 41.6 and 41.7 (C-6(α) and C-6(β)), 66.5 and 66.6 (C-4′), 71.2 (C-5(α)), 73.0 (C-4(β)), 73.3 (C-4(α)), 73.6 and 73,7 (C-3′), 73.9 (C-2(α)), 74.4 (C-3(α)), 75.9 (C-5(β)), 76.4 (C-2(β)), 77.5 (C-3(β)), 94.0 (C-1(α)), 98.3 (C-1(β)), 121.0 and 121.2 ($=CH_2$), 145.7 and 145.8 (C-2′), 170.6 and 170.6 (C-1′).

*4.12. 1,2-Dideoxy-D-glucopyranose (**21**)*

To a solution of 3,4,6-tri-*O*-acetylglucal (5.11 g, 18.8 mmol) in MeOH (50 ml) was added Pd/C (500 mg). The mixture was allowed to stir under H_2 atmosphere for 22 h at room temperature. After that, the suspension was filtered through a Celite pad to remove

Pd/C and the filtrate was evaporated. The crude mixture was purified by silica gel CC (EtOAc:hexane = 1:2) to give 3,4,6-tri-*O*-acetyl-1,2-dideoxy-glucopyranose (4.12 g, 15.0 mmol, 80%) as a viscous syrup. IR (cm^{-1}) 1735, 1368, 1240 and 1049, ^1H NMR (270 MHz, $CDCl_3$) 1.74–1.90 (1H, m, H-2a), 2.03–2.12 (1H, m, H-2b), 2.03, 2.04 and 2.09 (9H, s, Ac), 3.47–3.57 (2H, m, H-1a and H-5), 4.01–4.26 (1H, m, H-1b), 4.06 (1H, dd, J = 5.0, 1.6 Hz, H-6a), 4.06 (1H, dd, J = 5.0, 1.6 Hz, H-6a), 4.24 (1H, dd, J = 12.3, 5.0 Hz, H-6b), 4.92–5.03 (2H, m, H-3 and H-4), ^{13}C NMR (67.5 MHz, $CDCl_3$) 20.6, 20.6 and 20.8 (Me), 30.9 (C-2), 62.6 (C-1), 65.3 (C-6), 69.2 (C-4), 72.2 (C-3), 76.4 (C-5), 169.7, 170.3 and 170.6 (carbonyl), HR-FD-MS *m/z* $[M]^+$, calcd for $C_{12}H_{18}O_7$, 274.1052; found, 274.1040.

NaOMe (400 μl, 28% in MeOH) was added to a solution of above 3,4,6-tri-*O*-acetyl-1,2-dideoxy-glucopyranose (4.12 g, 15.0 mmol) in MeOH (70 ml) at room temperature. Stirring was continued for 1 h, whereupon the reaction mixture was neutralized by adding Amberlyst R-150 and filtered to give a solution. After evaporation of the filtrate, the crude product was purified by short silica gel CC (MeOH/$CHCl_3$, 15:85) to give **21** (1.95 g, 87%) as a white solid. IR (cm^{-1}) 1735, 1368, 1240 and 1049, ^1H NMR (270 MHz, $CDCl_3$) 1.74–1.90 (1H, m, H-2a), 2.03–2.12 (1H, m, H-2b), 2.03, 2.04 and 2.09 (9H, s, Ac), 3.47–3.57 (2H, m, H-1a and H-5), 4.01–4.26 (1H, m, H-1b), 4.06 (1H, dd, J = 5.0, 1.6 Hz, H-6a), 4.06 (1H, dd, J = 5.0, 1.6 Hz, H-6a), 4.24 (1H, dd, J = 12.3, 5.0 Hz, H-6b), 4.92–5.03 (2H, m, H-3 and H-4), ^{13}C NMR (67.5 MHz, $CDCl_3$) 20.6, 20.6 and 20.8 (Me), 30.9 (C-2), 62.6 (C-1), 65.3 (C-6), 69.2 (C-4), 72.2 (C-3), 76.4 (C-5), 169.7, 170.3 and 170.6 (carbonyl), HR-FD-MS *m/z* $[M]^+$, calcd for $C_{12}H_{18}O_7$, 274.1052; found, 274.1040.

4.13. 1,2-Dideoxy-6-O-pivaloyl-D-glucopyranose (**22**)

Pivaroyl chloride solution (1.34 ml, 10.9 mmol)/30 ml of pyridine was added dropwise to a solution of **21** (1.47 g, 9.94 mmol) in pyridine (30 ml). The reaction was allowed to stir at room temperature for 24 h. Pyridine was azeotropically removed with repeated addition of toluene, followed by evaporation which was repeated twice. The residual mixture was purified by silica gel CC (MeOH/$CHCl_3$, 1:9) to give **22** (1.91 g, 83%) as a white solid.

IR (cm^{-1}) 3413, 1727 and 901, ^1H NMR (270 MHz, $CDCl_3$) 1.21 (9H, s, *t*-Bu), 1.66 (1H, dddd, J = 12.8, 11.5, 5.0 Hz, H-2a), 1.95 (1H, br dd, J = 13.0, 4.7 Hz, H-2b), 3.15 (1H, t, J = 9.0 Hz, H-4), 3.27 (1H, ddd, J = 9.6, 4.4, 2.1 Hz, H-5), 3.45 (1H, dd, J = 12.2, 1.9 Hz, H-3), 3.97 (1H, dd, J = 11.2, 4.9 Hz, H-1b), 4.28 (1H, dd, J = 12.1, 2.1 Hz, H-6a), 4.42–4.46 (1H, m, H-6b), ^{13}C NMR (67.5 MHz, $CDCl_3$) 27.1 ($\underline{Me}_3C$), 33.1 (C-2), 38.9 ($Me_3\underline{C}$), 63.7 (C-6), 65.8 (C-1), 72.3 (C-4), 72.6 (C-3), 78.5 (C-5), 179.8 (carbonyl), HR-FD-MS *m/z* $[M]^+$ calcd for $C_{11}H_{20}O_5$, 232.1311; found, 232.1307.

4.14. 1,2-Dideoxy-6-O-pivaloyl-3,4-di-O-triethylsilyl-D-glucopyranose (**23**)

1,2-Dideoxy-D-glucopyranose **22** (1.85 g, 7.96 mmol) was dissolved in CH_2Cl_2 (40 ml) to which was added 2,6-lutidine (3.7 ml, 31.9 mmol) and triethylsilyl trifluoromethanesulfonate (4.0 ml, 17.9 mmol) in series. The reaction mixture was allowed to stir at room temperature for 1 h. After that, the reaction was quenched by 1 M HCl (40 ml) and washed with Et_2O (50 ml). The organic phase was washed with satd. $NaHCO_3$ (40 ml), brine (40 ml) and dried (anhyd. Na_2SO_4). After evaporation, the resulting residue was subjected to short silica gel CC (EtOAc:hexane, 1:4) to afford **23** (3.63 g, 99%) as a colorless syrup.

IR (cm^{-1}) 1733, 1481, 1461, 1284, 1240, 1152, 1131, 1108, 809 and 740, ^1H NMR (270 MHz, $CDCl_3$) 0.47–0.68 (12H, m, $SiC\underline{H}_2CH_3$), 0.90–0.68 (18H, m, $SiCH_2C\underline{H}_3$), 1.22 (9H, s, *t*-Bu), 1.52–1.67 (1H, m, H-2a), 1.88–1.97 (1H, m, H-2b), 3.27–3.44 (3H, m, H-3, H-4 and H-5), 3.63 (1H, ddd, J = 11.2, 9.7, 3.3 Hz, H-1a), 3.89 (1H, ddd, J = 11.7, 6.0, 4.4 Hz, H-1b), 4.10 (1H, dd, J = 11.7, 5.5 Hz, H-6a), 4.46 (1H, dd, J = 11.7, 2.5 Hz, H-6b), ^{13}C NMR (67.5 MHz, $CDCl_3$) 5.2–6.9 ($SiCH_2CH_3$), 27.2 ($\underline{Me}_3C$), 34.4 (C-2), 38.8 ($Me_3\underline{C}$), 63.5 (C-6), 64.4 (C-1), 73.1 (C-4), 74.3 (C-3), 79.0 (C-5), 178.3 (carbonyl), HR-FD-MS *m/z* $[M-Et]^+$ calcd for $C_{21}H_{43}O_5Si_2$, 431.2649; found, 431.2661.

4.15. 1,2-Dideoxy-3,4-di-O-triethylsilyl-D-glucopyranose (**24**)

The pivaroyl ester **23** (3.60 g, 7.87 mmol) was dissolved in dry CH_2Cl_2 (40 ml), and cooled to −78 °C. DIBAL (16 mmol) was added dropwise and left for 2 h at −78 °C. The reaction was monitored by TLC, and quenched by MeOH and then diluted with Et_2O (100 ml) when it was completed. 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 purified by silica gel CC (EtOAc:hexane, 1:4). Evaporation yielded **24** (2.33 g, 6.18 mmol, 78%).

IR (cm^{-1}) 3478, 1460, 1415, 1239, 1128, 1104, 810 and 740, ^1H NMR (270 MHz, $CDCl_3$) 0.59–0.70 (12H, m, $SiC\underline{H}_2CH_3$), 0.94–1.01 (18H, m, $SiCH_2CH_3$), 1.52–1.67 (1H, m, H-2a), 1.88–1.97 (1H, m, H-2b), 3.16 (1H, ddd, J = 8.8, 5.9, 2.9 Hz, H-5), 3.37 (1H, t, J = 7.8 Hz, H-4), 3.37–3.47 (1H, m, H-1a), 3.58–3.77 (2H, m, H-3 and H-6a), 4.46 (1H, dd, J = 11.7, 2.5 Hz, H-1b), ^{13}C NMR (67.5 MHz, $CDCl_3$) 5.2 and 5.4 ($SiC\underline{H}_2CH_3$), 6.9 ($SiCH_2\underline{C}H_3$), 34.9 (C-2), 62.7 (C-6), 65.1 (C-1), 73.6 (C-4), 74.5 (C-3), 80.9 (C-5), HR-FD-MS *m/z* $[M-Et]^+$ calcd for $C_{16}H_{35}O_4Si_2$, 347.2073; found, 347.2065.

4.16. 6-O-[3′-(tert-Butyldimethylsilyloxy)-4′-(p-methoxybenzyloxy)-2′-methylenebutanoyl]-1,2-dideoxy-3,4-di-O-triethylsilyl-D-glucopyranose (***25, epi-25***)

Following the procedure described in **4.10**, **25** and ***epi*-25** were obtained from **24** and corresponding carboxylic acids (**13** and ***ent*-13**) in 62% and 71% yield, respectively. The reactions were conducted at 0 °C. IR (cm^{-1}) 1717, 1514, 1463, 1415, 1380, 1303, 1249, 1129, 1109, 835, 809, 740 and 729, HR-FD-MS *m/z* $[M]^+$ calcd for $C_{37}H_{68}O_8Si_3$, 724.4222; found, 724.4200.

4.16.1. 1,2-Dideoxy-6-tuliposide B precursor (**25**)

$[\alpha]_D^{25} = +28.8$ (*c* 1.55, $CHCl_3$), ^1H NMR (270 MHz, $CDCl_3$) 0.03 and 0.08 (6H, s, SiMe), 0.58–0.68 (12H, m, $SiC\underline{H}_2CH_3$), 0.90–1.00 (27H, m, *t*-Bu and $SiCH_2C\underline{H}_3$), 1.54–1.69 (1H, m, H-2a), 1.89–1.95 (1H, m, H-2b), 3.28–3.47 (4H, m, H-3, H-4, H-5 and H-4′a), 3.55 (1H, dd, J = 10.2, 2.6 Hz, H-4′b), 3.64 (1H, ddd, J = 14.0, 6.4, 4.3 Hz, H-1a), 3.79 (3H, s, OMe), 3.88 (1H, ddd, J = 12.5, 5.0, 2.6 Hz, H-1b), 4.22 (1H, dd, J = 11.8, 4.3 Hz, H-6a), 4.41–4.48 (3H, m, H-6b and benzyl), 4.84 (1H, br dd, H-3′), 6.05 (1H, t, J = 1.6 Hz, $=CH_2$, Ha), 6.36 (1H, s, $=CH_2$, Hb), 6.85 (2H, d, J = 8.8 Hz, aromatic), 7.24 (2H, d, J = 8.8 Hz, aromatic), ^{13}C NMR (125 MHz, $CDCl_3$) −5.0 and −4.8 (Me_2Si), 5.2–6.9 (Et), 18.2 ($\underline{Me}_3C$), 25.8 ($Me_3\underline{C}$), 34.6 (C-2), 55.2 (OMe), 64.0 (C-6), 64.7 (C-1), 70.5 (C-3′), 72.7 (benzyl), 73.2 (C-4′), 74.4 (C-4), 74.9 (C-3), 78.8 (C-5), 113.6 (C-1), 126.5 (aromatic), 129.0 ($=CH_2$), 130.7 (aromatic), 140.9 (C-2′), 158.9 (aromatic), 165.8 (C-1′).

4.16.2. 1,2-Dideoxy-epi-6-tuliposide B precursor (***epi*-25**)

$[\alpha]_D^{25} = -10.5$ (*c* 1.68, $CHCl_3$), ^1H NMR (270 MHz, $CDCl_3$) 0.04 and 0.08 (6H, s, SiMe), 0.60–0.68 (12H, m, $SiC\underline{H}_2CH_3$), 0.89–1.01 (27H, m, *t*-Bu and $SiCH_2C\underline{H}_3$), 1.55–1.71 (1H, m, H-2a), 1.89–2.00 (1H, m, H-2b), 3.31–3.47 (4H, m, H-3, H-4, H-5 and H-4′a), 3.54 (1H, dd, J = 10.2, 2.9 Hz, H-4′b), 3.63 (1H, ddd, J = 10.9, 5.6, 3.9 Hz,, H-1a), 3.79 (3H, s, OMe), 3.88 (1H, ddd, J = 10.9, 5.6, 3.9 Hz,, H-1b), 4.25 (1H, dd, J = 11.7, 5.5 Hz, H-6a), 4.41–4.48 (3H, m, H-6b and benzyl), 4.84 (1H, br dd, J = 6.9, 2.7 Hz, H-3′), 6.05 (1H, s, $=CH_2$, Ha), 6.36 (1H, s, $=CH_2$, Hb), 6.85 (2H, d, J = 8.7 Hz,

aromatic), 7.24 (2H, d, J = 8.7 Hz, aromatic), ^{13}C NMR (125 MHz, $CDCl_3$) −5.0 and −4.8 (Me_2Si), 5.2–6.9 (Et), 18.2 ($Me_3\underline{C}$), 25.8 ($\underline{Me}_3C$), 34.5 (C-2), 55.2 (OMe), 64.1 (C-6), 64.6 (C-1), 70.4 (C-3′), 72.7 (benzyl), 73.2 (C-4′), 74.4 (C-4), 74.8 (C-3), 78.8 (C-5), 113.6 (aromatic), 126.4 (aromatic), 129.0 (=CH_2), 130.7 (aromatic), 140.9 (C-2′), 159.0 (aromatic), 165.8 (C-1′).

4.17. 1,2-Dideoxy-6-O-(3′,4′-dihydroxy-2′-methylenebutanoyl)-D-glucopyranose (**26, epi-26**)

The same deprotection procedures as mentioned in **4.1.2** was applied for **25** and ***epi*-25** to yield **26** (70%) and ***epi*-26** (68%), respectively. IR (cm^{-1}) 3407, 1709, 1277, 1204 and 1087, HR-FAB-MS m/z $[M-H]^-$ calcd for $C_{11}H_{17}O_9$, 261.0975; found, 261.0982.

4.17.1. 1,2-Dideoxy-6-tuliposide B (**26**)

$[\alpha]_D^{25}$ = +24.7 (c 0.84, MeOH), ^{1}H NMR (270 MHz, CD_3OD) 1.52–1.67 (1H, m, H-2a), 1.87–1.94 (1H, m, H-2a), 3.20 (1H, t, J = 9.1 Hz, H-4), 3.29–3.37 (1H, m, H-5), 3.41–3.57 (3H, m, H-4′a, H-1a and H-4), 3.71 (1H, dd, J = 11.3, 3.4 Hz, H-4′b), 3.89 (1H, br dd, J = 11.6, 4.7 Hz, H-5), 4.24 (1H, dd, J = 11.9, 5.5 Hz, H-6a), 4.49 (1H, dd, J = 11.9, 1.9 Hz, H-6b), 4.57 (1H, br m, H-3′), 6.00 (1H, s, =CH_2, Ha), 6.35 (1H, s, =CH_2, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 35.0 (C-2), 65.5 (C-6), 66.7 (C-1), 66.8 (C-4′), 72.1 (C-3′), 73.5 (C-4), 73.9 (C-3), 79.8 (C-5), 126.7 (=CH_2), 142.2 (C-2′), 167.5 (C-1′).

4.17.2. 2. 1,2-Dideoxy-epi-6-tuliposide B (***epi*-26**)

$[\alpha]_D^{25}$ = +11.9 (c 1.03, MeOH), ^{1}H NMR (270 MHz, CD_3OD) 1.52–1.67 (1H, m, H-2a), 1.87–1.94 (1H, m, H-2a), 3.19 (1H, t, J = 9.1 Hz, H-4), 3.29–3.37 (1H, m, H-5), 3.41–3.57 (3H, m, H-4′a, H-1a and H-4), 3.71 (1H, dd, J = 11.3, 3.6 Hz, H-4′b), 3.90 (1H, ddd, J = 10.4, 4.6, 1.6 Hz, H-1b), 4.27 (1H, dd, J = 11.9, 5.7 Hz, H-6a), 4.45 (1H, dd, J = 11.9, 2.1 Hz, H-6b), 4.57 (1H, br dd, J = 6.6, 3.3 Hz, H-3′), 6.00 (1H, t, J = 1.6 Hz, =CH_2, Ha), 6.35 (1H, dd, J = 1.4, 0.9 Hz, =CH_2, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 35.0 (C-2), 65.5 (C-6), 66.7 (C-1), 66.8 (C-4′), 72.1 (C-3′), 73.5 (C-4), 73.9 (C-3), 79.8 (C-5), 126.7 (=CH_2), 142.2 (C-2′), 167.5 (C-1′).

4.18. 6-O-(3′,4′-Dihydroxy-2′-methylenebutanoyl)-1-O-methyl-β-D-glucopyranoside (**27, epi-27**)

To a solution of **4** (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 brine (2.0 ml). The water layer was then washed with EtOAc (2.0 ml × 2). The combined organic layer was dried (anhydr.Na_2SO_4) and evaporated. Silica gel CC (5% then 10% MeOH in $CHCl_3$) yielded 6-*O*-[4′-(*tert*-butyldimethylsilyloxy)-3′-hydroxy-2′-methylenebutanoyl]-1-*O*-methyl-β-D-glucopyranoside (32.4 mg, 64 μmol, 40%, 3% d.e.) as a colorless syrup:

IR (cm^{-1}) 3421, 1718, 1257, 1053 and 838, HR-FD-MS m/z $[M+H]^+$, calcd for $C_{18}H_{35}O_9Si$, 423.2050; found, 423.2023. The obtained mixture of diastereomers was further separated by chiral HPLC (EtOH/hexane, 8:92; CHIRALPAK® IA column (φ 20 mm × 25 cm)).

4.18.1. First eluted (3′*R*)-diastereomer

$[\alpha]_D^{25}$ = −8.6 (c 1.13, CH_3OH), ^{1}H NMR (270 MHz, CD_3OD) 0.05 and 0.06 (3H, s, MeSi), 0.88 (9H, s, *t*-Bu), 3.16 (1H, dd, J = 9.0, 7.9 Hz, H-2), 3.28–3.37 (2H, m, H-3 and H-4), 3.48 (3H, s, OMe) 3.48–3.51 (1H, m, H-5), 3.60 (1H, dd, J = 10.4, 6.1 Hz, H-4′a), 3.77 (1H, dd, J = 10.4, 4.1 Hz, H-4′b), 4.17 (1H, d, J = 7.8 Hz, H-1), 4.27 (1H, dd, J = 11.8, 6.2 Hz, H-6a), 4.50 (1H, dd, J = 11.8, 2.2 Hz, H-6b), 4.56 (1H, br dd, J = 5.1 Hz, H-4′), 5.97 (1H, dd, J = 1.5 Hz, =CH_2, Ha), 6.33 (1H, s, =CH_2, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) −5.2 and −5.1 ($SiMe_2$), 19.2 ($Me_3\underline{C}$), 26.4 ($\underline{Me}_3C$), 57.2 (OMe), 65.0 (C-6), 68.0 (C-4′), 71.8 (C-4), 72.0 (C-3′), 75.0 (C-5), 75.3 (C-2), 77.9 (C-3), 105.4 (C-1), 126.7 (C-β), 142.4 (C-α), 167.4 (carbonyl).

4.18.2. Second eluted (3′*S*)-diastereomer

$[\alpha]_D^{25}$ = −11.1 (c 1.31, CH_3OH), ^{1}H NMR (270 MHz, CD_3OD) 0.05 and 0.06 (3H, s, MeSi), 0.89 (9H, s, *t*-Bu), 3.16 (1H, dd, J = 8.4, 8.4 Hz, H-2), 3.30–3.37 (2H, m, H-3 and H-4), 3.48 (3H, s, OMe) 3.48–3.51 (1H, m, H-5), 3.59 (1H, dd, J = 10.5, 6.2 Hz, H-4′a), 3.79 (1H, dd, J = 10.4, 4.0 Hz, H-4′b), 4.17 (1H, d, J = 7.8 Hz, H-1), 4.25 (1H, dd, J = 11.9, 6.0 Hz, H-6a), 4.50 (1H, dd, J = 11.9, 2.0 Hz, H-6b), 4.56 (1H, br dd, J = 4.8 Hz, H-4′), 5.97 (1H, s, =CH_2, Ha), 6.35 (1H, s, =CH_2, Hb), ^{13}C NMR (67.5 MHz, CD_3OD), −5.2 and −5.1 ($SiMe_2$), 19.2 ($Me_3\underline{C}$), 26.4 ($\underline{Me}_3C$), 57.2 (OMe), 65.0 (C-6), 68.0 (C-4′), 71.8 (C-4), 72.0 (C-3′), 75.0 (C-5), 75.3 (C-2), 77.9 (C-3), 105.4 (C-1), 126.9 (C-β), 142.4 (C-α), 167.4 (carbonyl).

The (3′*S*)-Baylis–Hillman adduct (39.3 g, 93.0 μmol) was dissolved in CH_3CN (2.0 ml) and cooled to 0 °C. To the reaction mixture, a stoichiometric amount of aqueous HF aqueous (10.0 μl) was slowly added and stirring was continued for 1 h. Evaporation of solvent and subsequent short silica gel CC (MeOH/$CHCl_3$, 1:9) produced **27** (25.8 g, 90%) as a colorless syrup. The 3′*R*-epimer (***epi*-27**) was prepared following the same procedure in 93% yield.

IR (cm^{-1}) 3392, 1720, 1269, 1164 and 1083, HR-FAB-MS m/z $[M-H]^-$, calcd for $C_{12}H_{19}O_9$, 307.1029; found, 307.1018.

4.18.3. 1-O-Methyl-β-epi-6-tuliposide B (epi-27)

$[\alpha]_D^{25}$ = −20.6 (c 1.67, CH_3OH), ^{1}H NMR (500 MHz, CD_3OD) 3.17 (1H, dd, J = 7.8, H-2), 3.30–3.38 (2H, m, H-3, H-4), 3.44 (1H, dd, J = 11.4, 6.7 Hz, H-4′a), 3.49 (3H, s, OMe), 3.49–3.53 (1H, m, H-5), 3.71 (1H, dd, J = 11.3, 3.5 Hz, H-4′b), 4.18 (1H, d, J = 7.8 Hz, H-1), 4.30 (1H, dd, J = 11.8, 6.1 Hz, H-6a), 4.48 (1H, dd, J = 11.9, 2.2 Hz, H-6b), (1H, br dd, J = 6.8, 3.5 Hz, H-3′), 6.00 (1H, t, J = 1.5 Hz, =CH_2, Ha), 6.36, (1H, s, =CH_2, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 57.3 (OMe), 65.0 (C-6), 66.7 (C-4′), 71.8 (C-4), 72.1 (C-3′), 75.0 (C-5), 75.2 (C-2), 77.9 (C-3), 105.4 (C-1), 126.8 (=CH_2), 142.2 (C-2′), 167.3 (C-1′).

4.18.4. 1-O-Methyl-β-6-tuliposide B (**27**)

$[\alpha]_D^{25}$ = −4.2 (c 1.14, CH_3OH), ^{1}H NMR (500 MHz, CD_3OD) 3.15 (1H, dd, J = 7.8, H-2), 3.31–3.36 (2H, m, H-3, H-4), 3.43 (1H, dd, J = 11.3, 6.8 Hz, H-4′a), 3.47 (3H, s, OMe), 3.47–3.50 (1H, m, H-5), 3.71 (1H, dd, J = 11.3, 3.5 Hz, H-4′b), 4.16 (1H, d, J = 7.8 Hz, H-1), 4.25 (1H, dd, J = 11.9, 5.9 Hz, H-6a), 4.52 (1H, dd, J = 11.9, 2.2 Hz, H-6b), (1H, br dd, J = 6.6, 3.4 Hz, H-3′), 6.00 (1H, t, J = 1.5 Hz, =CH_2, Ha), 6.34, (1H, s, =CH_2, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 57.3 (OMe), 64.9 (C-6), 66.7 (C-4′), 71.7 (C-4), 72.1 (C-3′), 75.0 (C-5), 75.2 (C-2), 77.8 (C-3), 105.4 (C-1), 126.8 (=CH_2), 142.2 (C-2′), 167.3 (C-1′).

4.19. 1-O-Methyl-6-O-(3′,4′-dihydroxy-2′-methylenebutanoyl)-α-D-glucopyranoside (**30, epi-30**)

1-*O*-Methyl-6-*O*-[3′-(*tert*-butyldimethylsilyloxy)-4′-(*p*-methoxybenzyloxy)-2′-methylenebutanoyl]-2,3,4-tri-*O*-trimethylsilyl-α-D-glucopyranoside (**29**, ***epi*-29**) were prepared by the same procedure as described in **4.10**. IR (cm^{-1}) 1718, 1515, 1473, 1362, 1303, 1251, 1163, 1099, 1080, 1043, 897, 871 and 841, HR-FD-MS m/z $[M]^+$, calcd for $C_{35}H_{66}O_{10}Si_4$, 758.3734; found, 758.3734.

4.19.1. 3′*S* product (**29**)

$[\alpha]_D^{25}$ = +49.8 (c 0.79, $CHCl_3$), ^{1}H NMR (270 MHz, $CDCl_3$) 0.02 and 0.07 (3H, s, MeSi), 0.15, 0.15 and 0.16 (27H, s, TMS), 0.89

(9H, s, *t*-Bu), 3.29–3.57 (4H, m, H-2, H-4, H-5 and H-4′a), 3.34 (3H, s, OMe), 3.73–3.85 (2H, m, H-3 and H-4′b), 3.80 (3H, s, PhOMe), 4.12 (1H, dd, J = 11.8, 5.9 Hz, H-6a), 4.47 (1H, d, J = 6.8 Hz, benzyl), 4.49 (1H, dd, J = 11.7, 2.2 Hz, H-6b), 4.61 (1H, d, J = 3.6 Hz, H-1), 4.81–4.85 (1H, m, H-3′), 6.07 (1H, dd, J = 1.7 Hz, $=CH_2$, Ha), 6.37 (1H, dd, J = 1.3 Hz, $=CH_2$, Hb), 6.85 (2H, d, J = 8.7 Hz, aromatic), 7.23 (2H, d, J = 8.7 Hz, aromatic), ^{13}C NMR (67.5 MHz, $CDCl_3$) −5.0 and −4.9 ($SiMe_2$), 0.5, 0.9 and 1.3 (TMS), 18.2 ($Me_3\underline{C}$), 25.8 ($\underline{Me}_3C$), 54.8 (OMe), 55.2 (PhO$\underline{Me}$), 63.9 (C-6), 69.5 (C-5), 70.5 (C-3′), 72.6 (C-2), 72.8 (C-4′), 73.8 (C-4), 74.8 (benzyl), 75.1 (C-3), 99.7 (C-1), 113.6 (aromatic), 126.6 ($=CH_2$), 129.0 (aromatic), 130.6 (aromatic), 140.9 (C-2′), 159.0 (aromatic), 165.7 (carbonyl).

*4.19.2. 3′R product (**epi-29**)*

$[\alpha]_D^{25} = +33.1$ (*c* 2.66, $CHCl_3$), ^{1}H NMR (270 MHz, $CDCl_3$) 0.02 and 0.07 (3H, s, MeSi), 0.15, 0.16 and 0.16 (27H, s, TMS), 0.89 (9H, s, *t*-Bu), 3.30–3.56 (4H, m, H-2, H-4, H-5 and H-4′a), 3.33 (3H, s, OMe), 3.74–3.81 (2H, m, H-3 and H-4′b), 3.80 (3H, s, PhO$\underline{Me}$), 4.14 (1H, dd, J = 11.8, 5.9 Hz, H-6a), 4.42–4.53 (1H, m, H-6b and benzyl), 4.61 (1H, d, J = 3.6 Hz, H-1), 4.84 (1H, br dd, J = 6.7, 2.7 Hz, H-3′), 6.07 (1H, dd, J = 1.6 Hz, $=CH_2$, Ha), 6.37 (1H, s, $=CH_2$, Hb), 6.85 (2H, d, J = 8.7 Hz, aromatic), 7.23 (2H, d, J = 8.6 Hz, aromatic), ^{13}C NMR (67.5 MHz, $CDCl_3$) −5.0 and −4.9 ($SiMe_2$), 0.5, 0.9 and 1.3 (TMS), 18.2 ($Me_3\underline{C}$), 25.8 ($\underline{Me}_3C$), 54.8 (OMe), 55.2 (PhO$\underline{Me}$), 63.9 (C-6), 69.4 (C-5), 70.3 (C-3′), 72.6 (C-2), 72.8 (C-4′), 73.7 (C-4), 74.7 (benzyl), 75.1 (C-3), 99.7 (C-1), 113.6 (aromatic), 126.6 ($=CH_2$), 129.0 (aromatic), 130.6 (aromatic), 141.0 (C-2′), 159.0 (aromatic), 165.7 (carbonyl).

IR (cm^{-1}) 3392, 1712, 1271, 1148 and 1056, HR-FAB-MS *m/z* $[M-H]^-$, calcd for $C_{12}H_{19}O_9$, 307.1029; found, 307.1018.

*4.19.3. 1-O-Methyl-α-6-tuliposide B (**30**)*

Deprotection of **29** and ***epi*-29** were carried out by procedure described in **4.1.2.**

$[\alpha]_D^{25} = +111.8$ (*c* 0.72, CH_3OH), ^{1}H NMR (500 MHz, CD_3OD) 3.28–3.30 (1H, m, H-4), 3.38–3.41 (4H, m, OMe and H-2), 3.43 (1H, dd, J = 11.3, 6.8 Hz, H-4′a), 3.60 (1H, t, J = 9.3 Hz, H-3), 3.71 (1H, dd, J = 11.3, 3.5 Hz, H-4′b), 3.72–3.76 (1H, m, H-5), 4.25 (1H, dd, J = 11.8, 6.0, H-6a), 4.49 (1H, dd, J = 11.8, 2.1, H-6b), 4.57 (1H, br dd, J = 6. 7, 3.4 Hz, H-3′), 4.65 (1H, d, J = 3.8 Hz, H-1), 5.99 (1H, t, J = 1.5 Hz, $=CH_2$, Ha), 6.34, (1H, s, $=CH_2$, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 55.6 (OMe), 65.1 (C-6), 66.7 (C-4′), 71.0 (C-5), 72.0 (C-3′), 72.1 (C-2), 73.5 (C-4), 75.0 (C-3), 101.3 (C-1), 126.7 ($=CH_2$), 142.3 (C-2′), 167.3 (C-1′).

*4.19.4. 1-O-Methyl-α-epi-6-tuliposide B (**epi-30**)*

$[\alpha]_D^{25} = +144.0$ (*c* 0.95, CH_3OH), ^{1}H NMR (500 MHz, CD_3OD) 3.27–3.31 (1H, m, H-4), 3.37–3.40 (4H, m, OMe and H-2), 3.43 (1H, dd, J = 11.3, 6.8 Hz, H-4′a), 3.61 (1H, t, J = 9.3 Hz, H-3), 3.70 (1H, dd, J = 11.3, 3.5 Hz, H-4′b), 3.73–3.77 (1H, m, H-5), 4.27 (1H, dd, J = 11.8, 6.2, H-6a), 4.45 (1H, dd, J = 11.8, 2.2, H-6b), 4.57 (1H, br dd, J = 6.8, 3.5 Hz, H-3′), 4.65 (1H, d, J = 3.8 Hz, H-1), 5.99 (1H, t, J = 1.5 Hz, $=CH_2$, Ha), 6.34, (1H, s, $=CH_2$, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 55.6 (OMe), 65.2 (C-6), 66.7 (C-4′), 71.0 (C-5), 72.0 (C-3′), 72.0 (C-2), 73.5 (C-4), 75.0 (C-3), 101.3 (C-1), 126.7 ($=CH_2$), 142.3 (C-2′), 167.4 (C-1′).

*4.20. Methyl 3,4-dihydroxy-2-methylenebutanoate (**31, ent-31**)*

To a stirred solution of PMB ether (**10**, 280 mg, 1.00 mmol) in CH_2Cl_2 (1.0 ml) was added CF_3CO_2H (250 μl). After the reaction mixture turned deep magenta, it was immediately diluted with toluene and the solvent was azeotropically removed. The residual mixture was subjected to silica gel CC (MeOH/$CHCl_3$, 1:9) to give **31** (116.8 mg, 80%).

S-diol (**31**) $[\alpha]_D^{25} = +12.7$ (*c* 1.80, MeOH), *R*-diol (***ent*-31**) $[\alpha]_D^{25} = -12.1$ (*c* 1.60, MeOH), IR (cm^{-1}) 3393, 1718, 1273, 1197 and 1085, ^{1}H NMR (270 MHz, CD_3OD) 3.42 (1H, dd, J = 11.3, 6.7 Hz, H-4a), 3.68 (1H, dd, J = 11.2, 3.5 Hz, H-4b), 3.74 (3H, s, OMe), 4.55 (1H, br dd, J = 6.4, 3.4 Hz, H-3), 5.97 (1H, J = 1.5 Hz, $=CH_2$, Ha), 6.31 (1H, J = 1.4 Hz, 0.9 Hz, $=CH_2$, Hb), ^{13}C NMR (67.5 MHz, CD_3OD) 52.3 (OMe), 66.7 (C-4), 72.0 (C-3), 126. 5 ($=CH_2$), 142.1 (C-2), 168.0 (C-1), HR-FAB-MS *m/z* $[M+H]^+$ calcd for $C_6H_{11}O_4$, 147.0657; found, 147.0672.

*4.21. Methyl 3,4-dihydroxy-2-oxyranyl-butanoate (**32 ent-32**)*

The deprotection procedure shown in **4.1.2**. was applied for **11** and ***ent*-11** to yield **32** (80%) and ***ent*-32** (80%), respectively.

S-Oxide (**32**) $[\alpha]_D^{25} = +0.5$ (*c* 1.01, MeOH), *R*-Oxide (***ent*-32**) $[\alpha]_D^{25} = -2.2$ (*c* 1.34, MeOH), IR (cm^{-1}) 3392, 1788, 1252, 1207, 1159, 1119 and 1001, ^{1}H NMR (270 MHz, CD_3OD) 3.03 (2H, m, H_2C-β), 3.62 (2H, m, H_2C-γ), 3.75 (3H, s, OMe), 4.17 (1H, m, H-β), ^{13}C NMR (67.5 MHz, CD_3OD) 50.0 (C-β*O*C-α), 53.0 (OMe), 58.3 (C-β*O*C-α), 63.5 (C-4), 70.6 (C-3), 171.5 (C-1), HR-FD-MS *m/z* $[M+H]^+$ calcd for $C_6H_{11}O_5$, 163.0606; found, 163.0615.

4.22. Assay for Antibacterial activity

Antibacterial activities were evaluated by a broth microdilution method based on the National Committee for Clinical Laboratory Standards (NCCLS) guideline. Bacteria were maintained with NA medium (5.0 g/l meat extract, 10.0 g/l Bacto™ Pepton, 5.0 g/l NaCl, and 15.0 g/l agar) plates at 37 °C. Assay for antibacterial activity was tested using Mueler-Hinton (MH) liquid medium containing 3.0 g/l meat extract, 17.5 g/l Bacto™ Pepton, and 1.5 g/l soluble starch. MH liquid medium was acidified to pH 6.0 with aqueous HCl, because 6-tuliposide B (**1**) is rapidly converted into tulipalin B (**2**) within 1–2 h even at neutral conditions. The tested compounds were diluted with Milli Q-water except tulipalin A (**3**), which was diluted with 5% DMSO. Tulipalin A (**3**) was tested at final concentrations of 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8 and 5.0 mM and other compounds were 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mM. Minimum inhibitory concentration (MIC) was defined as the lowest concentrations at which no growth was observed. Each bacterium was pre-cultured for 6–10 h with MH liquid medium at 170 rpm, 35 °C and then used for preparation of bacterial inoculum. The cell density of obtained suspensions was adjusted to 1.0×10^7 colony-forming unit (CFU)/ml and further diluted 19-fold with MH medium. After that, 5 μl of test compound solutions and 95 μl of bacterial inoculums were mixed to 5.0×10^5 CFU/ml in 96-well microtiter plates. The plates were covered with sterile sealer and incubated at 600 rpm, 35 °C for 16 h. Bacterial growth was evaluated by measuring OD_{595} value (microplate spectrophotometer, Sunrise Remote, TECAN). Assays were carried out in triplicate.

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