

Synthesis of new sugar derivatives from *Stachys sieboldi* Miq and antibacterial evaluation against *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Staphylococcus aureus*

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Received 3 September 2006; revised 22 January 2007; accepted 9 February 2007

Available online 13 February 2007

Abstract—A series of sugar derivatives (**7–14**) were synthesized from stachyose, a sugar compound of *Stachys sieboldi* Miq, and evaluated for antibacterial activity against *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Staphylococcus aureus*, and their structure–activity relationships were studied. The results showed that the compound OCT359 (allyl *O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranosyl)-(1 \rightarrow 6)-*O*-(2,3,4-tri-*O*-acetyl- α -D-galactopyranosyl)-(1 \rightarrow 6)-*O*-2,3,4-tri-*O*-acetyl- β -D-glucopyranoside) (**12**) exhibited in vitro antibacterial activity. The allyl group at C-1 and the acetoxy groups of the manninotriose were requisite for the antibacterial activity.

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Manninotriose is obtained by hydrolysis of stachyose, a sugar compound abundantly present in the root of *Stachys sieboldi* Miq. Chung et al. have previously reported the synthesis of methyl *O*- β -D-galactopyranosyl-(1 \rightarrow 6)-*O*- α -D-galactopyranosyl-(1 \rightarrow 6)-*O*- α -D-galactopyranosyl-(1 \rightarrow 6)-*O*- β -D-glucopyranoside,¹ from manninotriose (**1**). Biological activities of manninotriose are largely unknown. Kubo et al. studied the biological effect of a stachyose-containing extract from the root of *Rehmannia glutinosa* on hemorheology. A 50% ethanolic extract from the steamed root of *R. glutinosa* decreased erythrocyte deformability, inhibited polybrene-induced erythrocyte aggregation, and promoted the activity of the fibrinolytic system.² In the present study, we showed that a new sugar compound derived from manninotriose exhibited antibacterial activity against *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Staphylococcus aureus*.

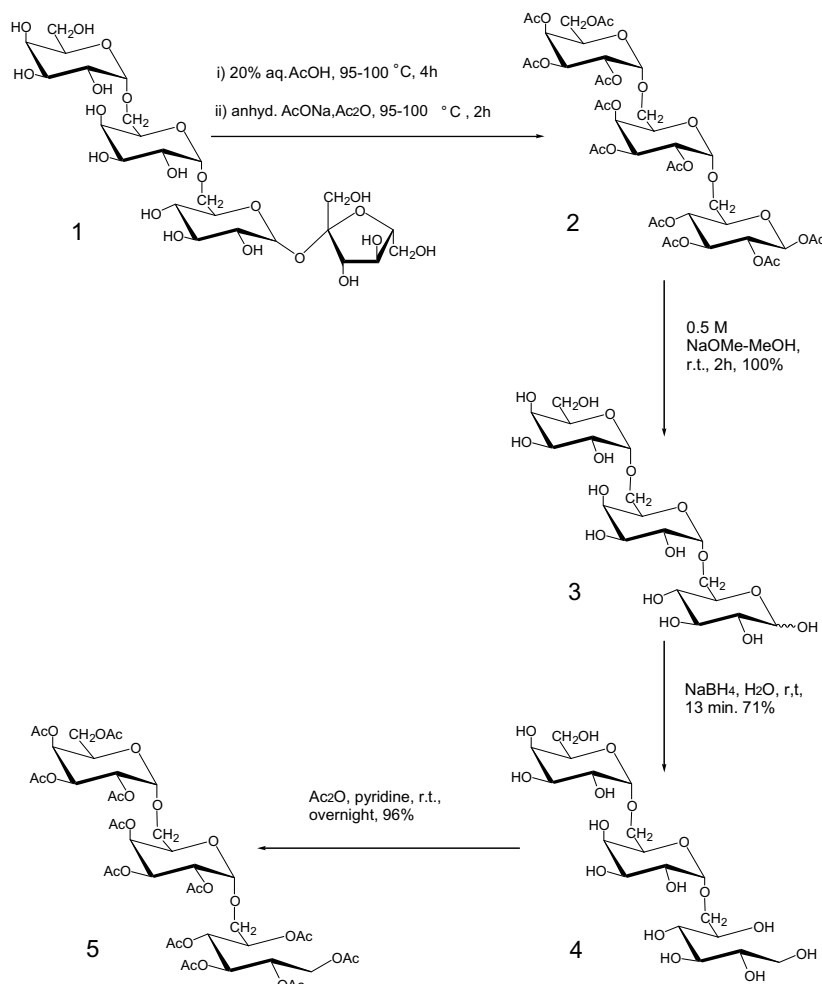
The trisaccharide OCT359 (allyl *O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranosyl)-(1 \rightarrow 6)-*O*-(2,3,4-tri-*O*-acetyl- α -D-galactopyranosyl)-(1 \rightarrow 6)-*O*-2,3,4-tri-*O*-acetyl- β -D-

glucopyranoside) (**12**) consists of one glucose and two galactose residues. This compound was prepared from manninotriose obtained by partial hydrolysis of stachyose (**1**), followed by acetylation, bromo-substitution, and allylation (Schemes 1 and 2). An aqueous solution of *O*- α -D-galactopyranosyl-(1 \rightarrow 6)-*O*- α -D-galactopyranosyl-(1 \rightarrow 6)-*O*-D-glucopyranose (manninotriose), prepared from *O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranosyl)-(1 \rightarrow 6)-*O*-(2,3,4-tri-*O*-acetyl- α -D-galactopyranosyl)-(1,2,2',2'', 3,3',3'',4,4',4'',6''-undeca-*O*-acetyl- β -manninotriose) (**2**) known in the literature and positive for Fehling's reaction, was treated with sodium borohydride to afford a sugar alcohol as an amorphous powder (94.6%) which was then negative for Fehling's reaction. After acetylation with acetic anhydride and pyridine, the acetate of a sugar alcohol was obtained as an amorphous powder (100%) and its ¹H NMR spectrum showed signals of acetyl groups at δ 1.96 ($\times 2$), 2.04, 2.05, 2.07, 2.08, 2.09, 2.13 ($\times 2$), 2.14 ($\times 2$), and 2.15 (Scheme 1).

The allyl group of OCT359 (**12**) was replaced with seven other types of functional groups; namely, methyl (**7**), ethyl (**8**), *n*-propyl (**9**), *i*-propyl (**10**), *n*-butyl (**11**), crotyl (**14**), and propargyl (**13**) (Scheme 2). The general method for modification is as follows. The mixture of *O*-(2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranosyl)-(1 \rightarrow 6)-

Keywords: Stachyose; β -Allyl manninotriose; Antibacterial activity.

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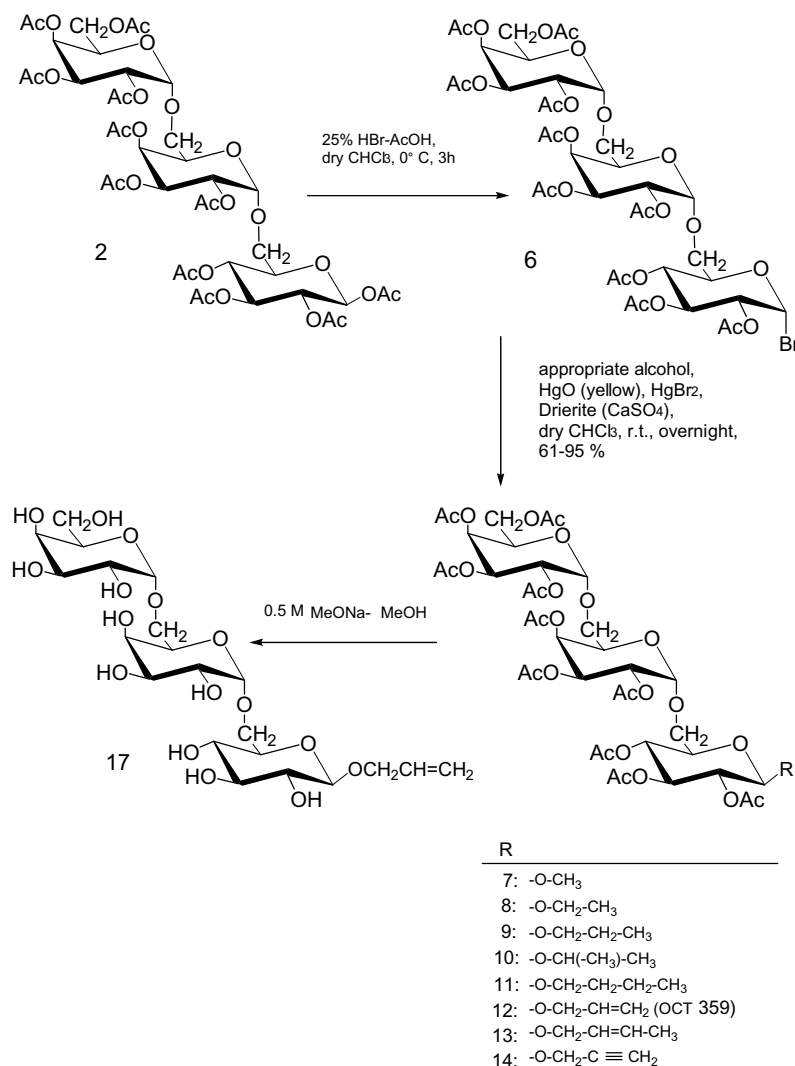


Scheme 1. Synthesis of sugar alcohol of manninotriose.

O-(2,3,4-tri-*O*-acetyl- α -D-galactopyranosyl)-(1 \rightarrow 6)-*O*-2,3,4-tri-*O*-acetyl- α -D-glucopyranosyl bromide (2,2',2'',3,3',3'',4,4',4'',6''-deca-*O*-acetyl- α -manninotriosyl bromide) (**6**), an appropriate alcohol, mercury (II) oxide (yellow), mercury (II) bromide, and Drierite (calcium sulfate) in dry chloroform was stirred overnight at room temperature. After workup, the resulting syrup or amorphous powder was purified by column chromatography. The yield of each compound was from ca. 61% to 94% (Scheme 2). The antibacterial activity of compounds (**7–14**) was investigated. The target bacteria were *M. tuberculosis* H₃₇Rv, *M. avium* 724S, and *S. aureus* including multi drug-resistant *M. tuberculosis* (MDR-MTB) and methicillin-resistant *S. aureus* MRSA (Tables 1 and 2). The MICs of OCT359 (R=allyl) (**12**) with these bacteria were between 3.13 and 25 μ g/ml. However, no other derivatives exhibit antibacterial activity (>400 μ g/ml) (Table 1). The isomer (**16**) of α -configuration did not exhibit antibacterial activity, either (>400 μ g/ml) (Table 1). The deacetylated form (**17**) of (**12**) did not exhibit antibacterial activity (Table 1). It suggests that hydrophobic nature of the compound could play an important role in an integration of the compound to the thick lipidic cell wall of bacilli.

In conclusion, the allyl group at C-1 of manninotriose is critical for antibacterial activity. The acetyla-

tion of all the hydroxyl groups of β -manninotriose is also critical for the antibacterial effect. One of the compounds with an allyl group, allyl isothiocyanate (AITC), is found in plants and AITC was reported to be mutagenic^{3,4} and clastogenic,⁵ while other reports indicated no genotoxic activity in this compound.^{6–8} Wei and his co-workers also reported that not only allyl but also methyl isothiocyanate exhibit bactericidal activity against a rifampicin-resistant strain of *Salmonella* Montevideo, streptomycin-resistant strains of *Escherichia coli* O157:H7 and *Listeria monocytogenes* Scott A.⁹ Other groups of researchers reported that modification of penam sulfones with an allyl group rendered the compounds to be inhibitors of β -lactamase.¹⁰ However, neither allyl alcohol (>400 μ g/ml) nor AITC (>800 μ g/ml) exhibited antibacterial activity against *M. tuberculosis* H₃₇Rv and *S. aureus* (Table 1). These findings strongly suggest that allyl β -manninotriose (OCT359) (**12**) exerts antibacterial activity by a mechanism distinct from that of AITC. The cell wall architecture of mycobacteria is only partly known. Daffe et al. reported that oligosaccharide fragments, 5-Gal-(1 \rightarrow 6)-Gal-(1 \rightarrow 5)-Gal or 5-Gal-(1 \rightarrow 6)-Gal, were components in the cell wall of *M. tuberculosis*.¹¹ But manninotriose structure



Scheme 2. Chemical modification of C-1 site of β-manninotrioside.¹²

Table 1. Antibacterial effects of manninotriose derivatives (MIC, μg/ml)^a

Compound	Organisms						
	<i>M. tuberculosis</i> H ₃₇ Rv	<i>M. avium</i> 724S	<i>S. aureus</i>	MRSA 873	MRSA 906	MRSA 908	MRSA 910
4	>800	ne	>800	ne	ne	ne	ne
5	>800	ne	>800	ne	ne	ne	ne
7	>800	ne	>800	ne	ne	ne	ne
8	>800	ne	>800	ne	ne	ne	ne
9	>800	ne	>800	ne	ne	ne	ne
10	>800	ne	>800	ne	ne	ne	ne
11	>800	ne	>800	ne	ne	ne	ne
12	3.13	3.13	3.13	3.77	15.1	7.54	15.1
13	>400	ne	>400	ne	ne	ne	ne
14	>800	ne	>800	ne	ne	ne	ne
16	>800	ne	>800	ne	ne	ne	ne
17	>100	>100	>100	100	>100	>100	>100
Allyl alcohol	>400	ne	>400	ne	ne	ne	ne
AITC	>800	ne	>800	ne	ne	ne	ne
<i>Anti-MTB Antibiotics</i>							
INH	0.075	12.5	>100	>100	>100	>100	>100
RIP	0.008	0.004	0.004	0.004	0.004	0.004	0.004
STM	0.20	0.40	100	>100	100	100	50
EMB	1.25	3.00	>100	>100	>100	>100	>100

^a Broth dilution methods¹⁰ using MiddleBrook 7H9 broth containing albumin, dextrose, and catalase for derivatives (ne, not examined). For *Staphylococcus aureus*, we used the heart-infusion broth. INH, isoniazide; RIF, rifampicin; STM, streptomycin; EMB, ethambutol.

Table 2. Antimycobacterial effects of OCT359 on drug-sensitive and resistant clinical isolates of *M. tuberculosis*

Clinical isolates	Resistance to	MIC for OCT359 (μg/ml)
<i>Drug-susceptible strains</i>		
A-1-1		6.25
A-1-2		6.25
A-1-3		6.25
A-2-5		12.5
A-3-1		25
A-3-2		25
A-3-5		6.25
A-3-6		6.25
A-3-9		3.13
A-3-11		3.13
A-3-12		6.25
A-3-15		3.13
A-3-16		6.25
A-3-17		3.13
A-3-19		3.13
A-3-20		6.25
A-3-21		6.25
A-3-22		3.13
<i>Drug-resistant strains</i>		
A-2-1	INH	3.13
A-2-3	INH, RIF	3.13
A-2-4	RIF	12.5
A-2-6	INH, RIF, STR, EMB	6.25
A-3-47	STR	3.13
A-4-8	STR	6.25
A-4-25	INH, STR	12.5
A-4-30	INH, RIF, EMB	6.25
C-1-29	INH, RIF, STR, EMB	12.5
E-1-40	INH, RIF, STR, EMB	6.25
J-1-19	INH, RIF, EMB	3.13
K-3-6	INH	3.13
M-1-32	INH, RIF, STR, EMB	3.13
N-4-11	INH, RIF, EMB	3.13
N-5-2	INH, RIF, STR, EMB	3.13
P-1-50	INH, RIF, STR, EMB	3.13
P-4-11	INH, RIF, STR, EMB	3.13
Q-4-1	INH, RIF, STR, EMB	6.25
R-1-38	INH, RIF, STR, EMB	6.25
S-1-14	INH, RIF, STR, EMB	6.25
U-2-15	INH, RIF, STR, EMB	6.25
U-4-6	INH, RIF, STR, EMB	1.56
V-1-16	INH, RIF, STR, EMB	6.25
Z-1-2	INH, RIF, STR, EMB	3.13
Z-1-4	INH, RIF, STR, EMB	6.25
<i>Reference strain</i>		
<i>M. tuberculosis</i> H ₃₇ Rv		3.13

Proportion methods using Middlebrook 7H11 agar plates for INH (isoniazide), RIF (rifampicin), STR(streptomycin), EMB (ethambutol) and 7H9 broth for OCT359. Cut off concentrations of each antibiotic were 10, 10, 100, and 100 μg/ml, respectively.

is not known. But, OCT359 may show anti-bacterial activity by inhibiting the glycosyltransferases of bacilli. It should be noted that 25 clinical isolates of drug-resistant MTB and 19 drug-sensitive MTB were sensitive to OCT359. The MICs of OCT359 with these clinical isolates were from 3.13 to 25 μg/ml. The results strongly indicate that OCT359 could be a useful anti-bacterial compound against *M. tuberculosis*, *M. avium*, and *S. aureus*.

Acknowledgments

The authors thank Ms. Maki Kondo and Mr. Toshiyuki Ishiguro (Nagoya City University) for their technical assistance. The authors also thank Drs. Masao Imai (Nagoya City Public Health Research Institute) and Katsumi Kishida (Nagoya City University Hospital), for providing strains of *S. aureus* and MRSA, respectively. This work was supported in part by grants from the Grant-in-Aid for Scientific Research (C) from Japan Society for the Promotion of Sciences, Ohyama Health Foundation, Takeda Science Foundation, Ueda Yasushi Memorial Foundation of Japanese Society of Chemotherapy, and the U.S.-Japan Cooperative Medical Sciences Program.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bmcl.2007.02.024](https://doi.org/10.1016/j.bmcl.2007.02.024).

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the elute provided alkyl β -glycoside as an amorphous powder in 61–94% yields. The appropriate alcohols used were: methanol, ethanol, *n*-propyl alcohol, *i*-propyl alcohol, *n*-butyl alcohol, allyl alcohol, propargyl alcohol, and crotyl alcohol. Methyl β -glycoside (**7**): 74%, an amorphous powder, $[\alpha]_D^{22} + 118.3^\circ$ (*c* 1.09, CHCl₃) lit. (2) an amorphous powder, $[\alpha]_D^{19} + 128.2^\circ$ (*c* 1.70, CHCl₃), IR (Nujol) cm⁻¹: 1750 (C=O). ¹H NMR (CDCl₃) δ : 1.98, 2.01, 2.05 ($\times 2$), 2.07 ($\times 3$), 2.08, 2.11, 2.14 (s, 30H, OAcx10), 3.53 (s, 3H, OMe), 4.45 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1), 4.97 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 4.0$ Hz, H-1' or H-1'') and 5.10 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 4.3$ Hz, H-1' or H-1''). Ethyl β -glycoside (**8**): 95%, an amorphous powder, $[\alpha]_D^{22} + 120.5^\circ$ (*c* 1.15, CHCl₃), IR (Nujol) cm⁻¹: 1750 (C=O). ¹H NMR (CDCl₃) δ : 1.21 (t, 3H, $J = 7.0$ Hz, OCH₂CH₃), 1.98, 2.01, 2.05 ($\times 2$), 2.07 ($\times 2$), 2.08, 2.11, 2.13, 2.14 (s, 30H, OAcx10), 4.53 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1), 4.97 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 3.4$ Hz, H-1' or H-1'') and 5.10 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 3.4$ Hz, H-1' or H-1''). Anal. Calcd for C₄₀H₅₆O₂₆: C, 50.42; H, 5.92. Found: C, 50.50; H, 6.00. *n*-Propyl β -glycoside (**9**): 68%, an amorphous powder, $[\alpha]_D^{23} + 116.8^\circ$ (*c* 1.01, CHCl₃), IR (Nujol) cm⁻¹: 1750 (C=O). ¹H NMR (CDCl₃) δ : 0.91 (t, 3H, $J = 7.5$ Hz, OCH₂CH₂CH₃), 1.60 (m, 2H, OCH₂CH₂CH₃), 1.98 ($\times 2$), 2.01, 2.04, 2.06, 2.08 ($\times 2$), 2.12, 2.14, 2.15 (s, 30H, OAcx10), 3.47, 3.80 (m, 2H, OCH₂CH₂CH₃), 4.05 (dd, 1H, $J_{5,6a} = 7.3$ Hz, $J_{6a,6b} = 11.3$ Hz, H-6a), 4.17 (dd, 1H, $J_{5,6b} = 5.8$ Hz, H-6b), 4.50 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1), 4.95 (d and dd, 2H, $J_{2,3} = 9.1$ Hz, $J_{1',2' \text{ or } 1'',2''} = 4.3$ Hz, H-2, H-1' or H-1''), and 5.16 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 3.7$ Hz, H-1' or H-1''). Anal. Calcd for C₄₁H₅₈O₂₆: C, 50.93; H, 6.05. Found: C, 50.88; H, 6.09. *i*-Propyl β -glycoside (**10**): 77%, an amorphous powder, $[\alpha]_D^{22} + 111.8^\circ$ (*c* 1.17, CHCl₃), IR (Nujol) cm⁻¹: 1750 (C=O). ¹H NMR (CDCl₃) δ : 1.15, 1.24 (each d, 6H, CH(CH₃)₂), 1.98 ($\times 2$), 2.01, 2.04, 2.06, 2.09 ($\times 2$), 2.12, 2.14, 2.15 (s, 30H, OAcx10), 3.93 (m, 1H, CH(CH₃)₂), 4.05 (dd, 1H, $J_{5,6a} = 7.3$ Hz, $J_{6a,6b} = 11.3$ Hz, H-6a), 4.17 (dd, 1H, $J_{5,6b} = 5.8$ Hz, H-6b), 4.56 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1), 4.90 (dd, 1H, $J_{2,3} = 9.4$ Hz, H-2), 4.95 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 3.4$ Hz, H-1' or H-1''), and 5.15 (d, 2H, $J_{1',2' \text{ or } 1'',2''} = 3.4$ Hz, H-1' or H-1''). Anal. Calcd for C₄₁H₅₈O₂₆: C, 50.93; H, 6.05. Found: C, 50.76; H, 6.02. *n*-Butyl β -glycoside (**11**): 78%, an amorphous

powder, $[\alpha]_D^{22} + 127.0^\circ$ (*c* 0.74, CHCl₃), IR (Nujol) cm⁻¹: 1750 (C=O). ¹H NMR (CDCl₃) δ : 0.91 (t, 3H, $J = 7.3$ Hz, OCH₂CH₂CH₂CH₃), 1.35 (m, 2H, OCH₂CH₂CH₂CH₃), 1.56 (m, 2H, OCH₂CH₂CH₂CH₃), 1.98 ($\times 2$), 2.01, 2.04, 2.06, 2.08 ($\times 2$), 2.12, 2.14 ($\times 2$) (s, 30H, OAcx10), 3.50, 3.85 (m, 2H, OCH₂CH₂CH₂CH₃), 4.04 (dd, 1H, $J_{5,6a} = 7.3$ Hz, $J_{6a,6b} = 11.3$ Hz, H-6a), 4.17 (dd, 1H, $J_{5,6b} = 5.5$ Hz, H-6b), 4.50 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1), 4.94 (dd, 1H, $J_{2,3} = 8.9$ Hz, H-2), 4.95 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 3.7$ Hz, H-1' or H-1'') and 5.16 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 3.4$ Hz, H-1' or H-1''). Anal. Calcd for C₄₂H₆₀O₂₆: C, 51.43; H, 6.17. Found: C, 51.22; H, 6.18. Allyl β -glycoside (**12**): OCT359: 84%, an amorphous powder, $[\alpha]_D^{23} + 123.6^\circ$ (*c* 1.01, CHCl₃), IR (Nujol) cm⁻¹: 1750 (C=O), 1648 (C=C). ¹H NMR (CDCl₃) δ : 1.98 ($\times 2$), 2.01, 2.05, 2.06, 2.08, 2.09, 2.12, 2.14 ($\times 2$) (s, 30H, OAcx10), 4.05 (dd, 1H, $J_{5,6a} = 7.4$ Hz, $J_{6a,6b} = 11.3$ Hz, H-6a), 4.13, 4.33 (m, 2H, OCH₂CH=CH₂), 4.18 (dd, 1H, $J_{5,6b} = 5.5$ Hz, H-6b), 4.57 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1), 4.96 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 3.7$ Hz, H-1' or H-1''), 5.17 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 4.3$ Hz, H-1' or H-1''), and 5.87 (m, 1H, OCH₂CH=CH₂). Anal. Calcd for C₄₁H₅₆O₂₆·H₂O: C, 50.10; H, 5.95. Found: C, 50.19; H, 5.95. Propargyl β -glycoside (**13**): 81%, an amorphous powder, $[\alpha]_D^{22} + 117.8^\circ$ (*c* 1.07, CHCl₃), IR (Nujol) cm⁻¹: 1750 (C=O), 2150 (C \equiv C). ¹H NMR (CDCl₃) δ : 1.98 ($\times 2$), 2.01, 2.05, 2.06, 2.07, 2.09, 2.11, 2.13 ($\times 2$) (s, 30H, OAcx10), 2.59 (t, 1H, $J = 2.4$ Hz, OCH₂C \equiv CH), 4.39, 4.44 (dd, 2H, $J = 15.9$ Hz, OCH₂C \equiv CH), 4.80 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1), 4.96 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 3.1$ Hz, H-1' or H-1''), and 5.16 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 3.4$ Hz, H-1' or H-1''). Anal. Calcd for C₄₁H₅₄O₂₆: C, 51.14; H, 5.65. Found: C, 50.85; H, 5.60. Crotyl β -glycoside (**14**): 61%, an amorphous powder, $[\alpha]_D^{22} + 120.0^\circ$ (*c* 1.10, CHCl₃), IR (Nujol) cm⁻¹: 1750 (C=O), 1648 (C=C). ¹H NMR (CDCl₃) δ : 1.73 (dd, 3H, $J = 6.7$ Hz, $J = 0.9$ Hz, OCH₂CH=CHCH₃), 1.98 ($\times 2$), 2.01, 2.04, 2.05, 2.08 ($\times 2$), 2.12, 2.13, 2.14 (s, 30H, OAcx10), 4.04, 4.25 (m, 2H, OCH₂CH=CHCH₃), 4.56 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1), 4.96 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 3.7$ Hz, H-1' or H-1''), 5.17 (d, 1H, $J_{1',2' \text{ or } 1'',2''} = 3.7$ Hz, H-1' or H-1''), 5.50 (m, 1H, OCH₂CH=CHCH₃), and 5.74 (m, 1H, OCH₂CH=CHCH₃). Anal. Calcd for C₄₂H₅₈O₂₆: C, 51.53; H, 5.97. Found: C, 51.27; H, 5.98.