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Synthesis and anti-herpes simplex viral activity of monoglycosyl diglycerides

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Abstract

Based on the discovery of antiviral β-galactosyl diglycerides from *Clinacanthus nutans* leaves, 19 monoglycosyl diglycerides were synthesized and examined for inhibitory effect on herpes simplex virus types 1 and 2 (HSV-1, HSV-2). A study of the structure–activity relationships of the synthetic monoglycosyl diglycerides indicated that the fatty acyl moieties were critical for inhibitory action with higher activity displayed as the acyl groups became more olefinic in character. The sugar moiety was also important for anti-HSV action; however, the type of sugar (glucose or galactose) did not affect activity. The stereochemistry at C-2 of the glycerol backbone displayed no significant effect on anti-HSV activity. Among the compounds synthesized, 1,2-*O*-dilinolenoyl-3-*O*-β-D-glucopyranosyl-*sn*-glycerol showed the highest inhibitory activity against HSV-1 and HSV-2 with IC_{50} values of 12.5±0.5 and 18.5±1.5 μg/ml, respectively.

Keywords: *Clinacanthus nutans*; Acanthaceae; Synthesis; Anti-herpes simplex activity; Monoglycosyl diglycerides; 1,2-*O*-diacyl-3-*O*-β-D-glycopyranosyl-glycerols

1. Introduction

Galactosyl diglycerides are major constituents of the stacked thylakoid membrane of chloroplasts and also widely distributed in some bacterial membranes (Douce and Joyard, 1980; Yagi and Maruyama, 1999). Although various galactosyl diglycerides have been isolated and characterized, the biological functions of these compounds have not been fully elucidated (Kopitz, 1997). Studies on glycoglycerolipids reported them as having antitumor-promoting (Shirahashi et al., 1996; Colombo et al., 1998, 1999), oxygen scavenging (Nakata, 2000), and virus neutralizing (Nakata et al., 2000) activities. Recently, we reported that trigalactosyl and digalactosyl diglycerides isolated from *Clinacanthus nutans* leaves exhibited anti-herpes simplex virus (anti-HSV) activity (Satakhun, 2001), with the latter plant being a well-known medicinal plant in Thailand promoted in primary and public health care units for the treatment of herpes simplex, herpes zoster, skin pruritis and insect bites. Previous pharmacological and clinical studies of extracts from this plant showed both anti-HSV activity and anti-inflammatory action (Kittisiripornkul, 1984; Jayawasu et al., 1992). These findings prompted us to investigate structure–activity relationships of this class of compounds against HSV. Using monoglycosyl diglycerides as a starting point. In order to examine these analogs for anti-HSV activity, a series of mono-acid and mixed-acid 1,2-*O*-diacyl-3-*O*-β-glycopyranosyl-*rac*-glycerols containing either a glucose or a galactose moiety were synthesized, as well as studying the effects of the stereochemistry at C-2 of the glycerol backbone of some selected compounds on anti-HSV activity.

2. Results and discussion

2.1. Synthesis

Glucosyl diglycerides and galactosyl diglycerides bearing either saturated or unsaturated fatty acids were

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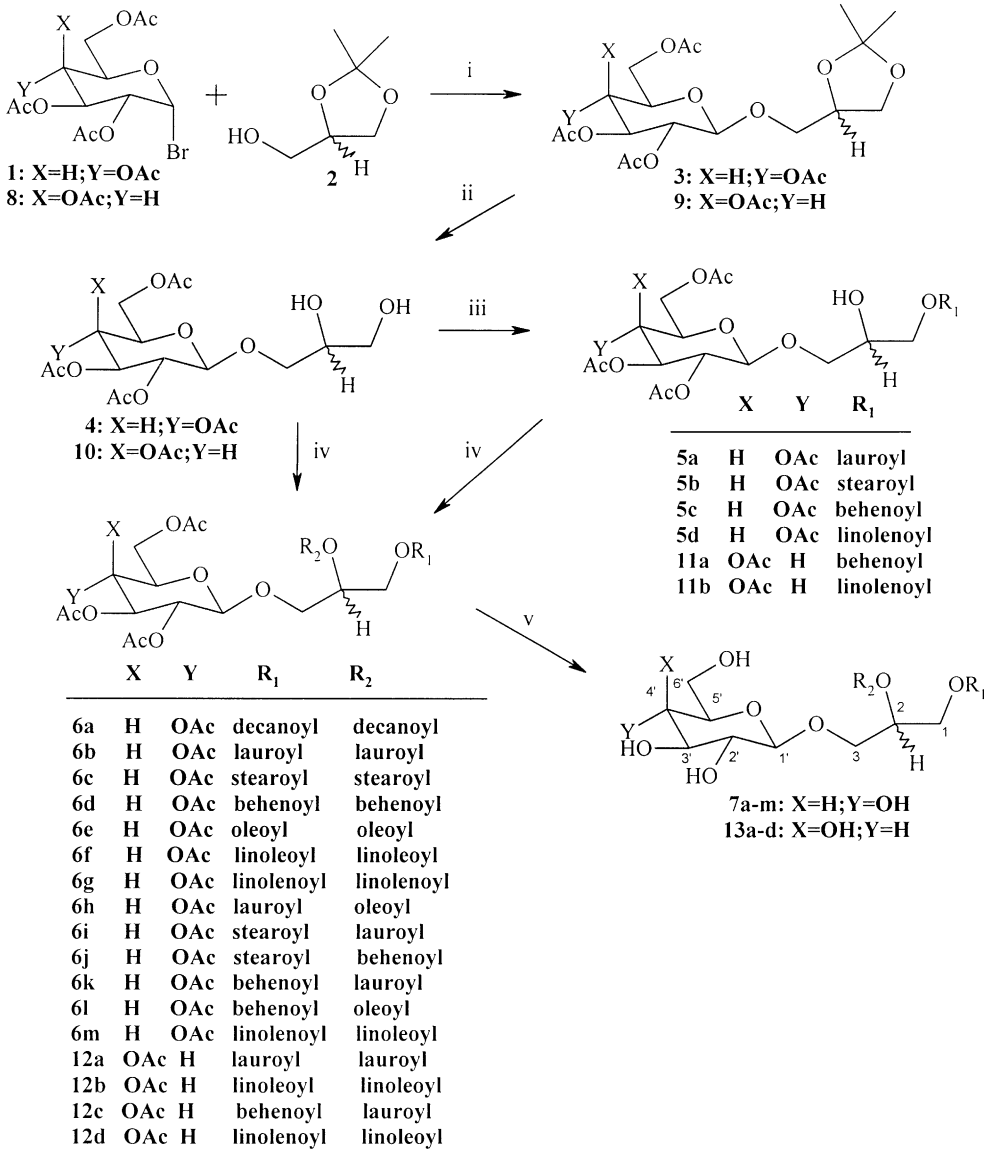
synthesized as illustrated in Scheme 1. For the preparation of glucosyl diglycerides (**7a–m**), condensation of commercially available acetobromo-α-D-glucose (**1**) with D,L-α,β-isopropylideneglycerol (**2**) in the presence of Ag_2CO_3 using Koenigs–Knorr conditions afforded the peracetylated glucoside (**3**). The ^{1}H-NMR signals of anomeric protons at δ 4.61, 4.63 (two overlapped doublets, $J = 7.6$ Hz) in **3** indicated a 1:1 ratio of diastereomeric products, with the stereochemistry of the new glycosidic bond being in the β-orientation. Then **3** was allowed to react with 60% acetic acid in aqueous solution at 60 °C to remove the isopropylidene protecting group, the diol intermediate (**4**) was obtained in quantitative yield. The mono-acid 1,2-*O*-diacyl-3-D-(β-D-2′,3′,4′,6′-tetra-*O*-acetyl-glucopyranosyl)-*rac*-glycerols (**6a–g**) were prepared by one step diacylation of **4** with 2 equivalents of the desired fatty acids in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) at room temperature, whereas the desired mixed-acid 1,2-*O*-diacyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetyl-glucopyranosyl)-*rac*-glycerols (**6h–m**) could be furnished by stepwise acylation of **4** with the required fatty acid at 0 °C to provide 1-*O*-acyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetyl-glucopyranosyl)-*rac*-glycerols (**5a–d**). The ^{1}H-NMR spectra of **5a–d** showed H_2-1 and H-2 signals in the range of 4.05–4.30 and 3.85–4.00 ppm, respectively, indicating that the fatty acyl groups have attached to C-1 hydroxyl of the glycerol backbone.



Scheme 1. Reagent and conditions: (i) Ag_2CO_3, Drierite, CH_2Cl_2, rt; (ii) 60% CH_3COOH, 60 °C; (iii) fatty acid, DCC, DMAP, CH_2Cl_2, 0 °C; (iv) fatty acid, DCC, DMAP, CH_2Cl_2, rt; (v) NH_2NH_2, 85% EtOH, 40–60 °C.

Subsequent acylation of the products obtained with the second fatty acid at room temperature provided compounds **6h–m**. The difficult step in this synthesis was the selective removal of the acetate moieties of **6a–m** without affecting the fatty acyl ester groups on the glycerol backbone. Successful deacetylation of **6a–m** was carried out by hydrazine hydrate (3 moles per acetate group) in 85% aq. ethanol to give the corresponding 1,2-*O*-diacyl-3-*O*-β-D-glucopyranosyl-*rac*-glycerols (**7a–m**).

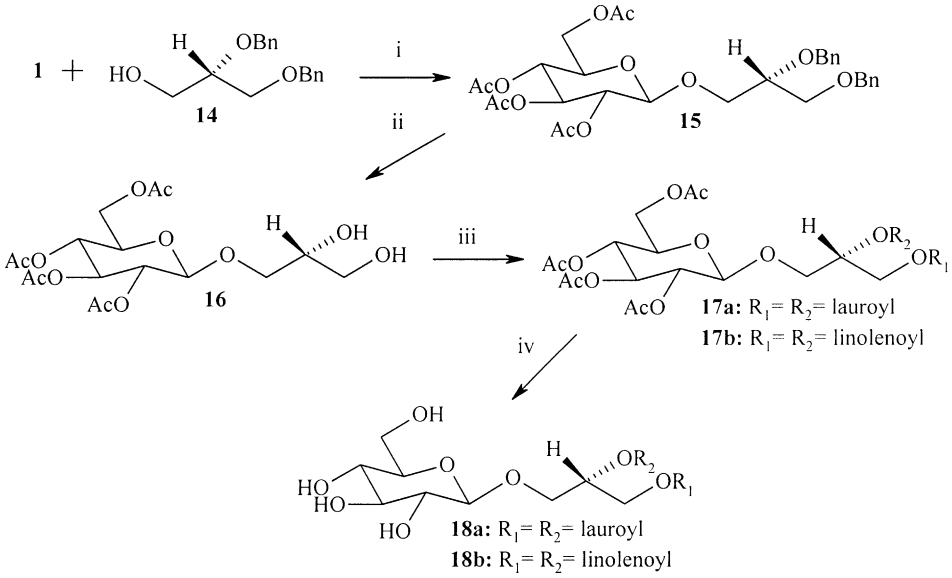
Galactosyl diglycerides (**13a–d**) were prepared from acetobromo-α-D-galactose (**8**) following the procedure for the synthesis of 1,2-*O*-diacyl-3-*O*-β-D-glucopyranosyl-*rac*-glycerols as described above. The ^{1}H-NMR spectral data of these compounds (Table 1) showed signals attributable to glycosyl diglycerides. The proton signals could be assigned by the correlations in the 2D H–H COSY spectra. Interestingly, the anomeric protons of all peracetylated products were observed as 1:1 ratio of two overlapped doublets ($J = 7.5$–7.8 Hz) indicating 1:1 mixtures of the diastereomers, while the same protons of the corresponding deacetylated final products appeared as a doublet. However, the ^{13}C-NMR spectra of these final products (Table 2), showing a 1:1 ratio of anomeric carbon signals separated by about 0.1–0.2 ppm, confirmed the 1:1 mixtures of the final diastereomeric glycosyl diglycerides. In addition, the observed carbons are less than the actual carbons due to the overlapped methylene carbon signals of the fatty acyl chains in the region of 29–30 ppm.

For the synthesis of 1,2-*O*-diacyl-3-*O*-β-D-glucopyranosyl-*sn*-glycerols (**18a–b**) (Scheme 2), **1** was allowed to react with 1,2-*O*-dibenzyl-*sn*-glycerol (**14**) (prepared from D-mannitol according to the method of Mannock et al., 1987) in the presence of Ag_2CO_3 to give 1,2-*O*-dibenzyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetyl-glucopyranosyl)-*sn*-glycerol (**15**), which was then debenzylated by catalytic hydrogenolysis with 10% Pd/C in ethyl acetate. The 3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetyl-glucopyranosyl)-*sn*-glycerol (**16**) obtained was subsequently acylated with the desired fatty acid to give 1,2-*O*-diacyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetyl-glucopyranosyl)-*sn*-glycerols (**17a–b**). Finally, **17a–b** were reacted with hydrazine to give the corresponding 1,2-*O*-diacyl-3-*O*-β-D-glucopyranosyl-*sn*-glycerols (**18a–b**). The ^{1}H- and ^{13}C-NMR spectral data of **18a–b** are listed in Table 3.

2.2. Biological evaluation

To study the effect of fatty acids on anti-HSV activity, a series of 1,2-*O*-diacyl-3-*O*-β-D-glycopyranosyl-*rac*-glycerols bearing various fatty acyl moieties were determined. The data (Table 4) showed that glucosyl diglycerides bearing the same saturated fatty acyls (**7a–d**) and different fatty acyls (**7i–k**) at C-1 and C-2 displayed little or no inhibitory activity at 50 μg/ml, except for **7b** which contains a dilauroyl (C12:0) moiety. On the other hand, compounds bearing the same unsaturated fatty acyls (**7e–g**) and different fatty acyls (**7h**, **7l–m**) exhibited higher inhibitory activity than those bearing saturated fatty acyls. By comparing anti-HSV activity of four compounds containing 18 carbon atom fatty acyls (**7e–g**, **7m**), the data showed that inhibitory activity of these compounds followed the order **7g** > **7m** > **7f** > **7e**. These results suggested that more olefinic derivatives displayed higher anti-HSV activity.

To study the effect of the sugar moiety on anti-HSV activity, the selected galactosyl diglycerides bearing the same fatty acyls (**13a–b**) and the different fatty acyls



Scheme 2. Reagent and conditions: (i) Ag_2CO_3, Drierite, CH_2Cl_2, rt; (ii) H_2, 10% Pd/C, EtOAc; (iii) fatty acid, DCC, DMAP, CH_2Cl_2, rt; (iv) NH_2NH_2, 85% EtOH, 40–60 °C.

Table 1
^{1}H-NMR spectral data for compounds **7a**–**m** and **13a**–**d** (in $CDCl_3$)

H	7a	7b	7c	7d	7e	7f	7g	7h	7i
Glycerol moiety									
1-Ha	4.08, *dd*, 4.13, *dd*, 12.0, 6.7	4.08, *dd*, 4.12, *dd*, 12.0, 6.7	4.06, *dd*, 4.12, *dd*, 12.0, 6.7	4.07, *dd*, 4.14, *dd*, 12.0, 6.7	4.07, *dd*, 4.14, *dd*, 12.0, 6.7	4.06, *dd*, 4.12, *dd*, 12.0, 6.7	4.07, *dd*, 4.13, *dd*, 12.0, 6.7	4.07, *dd*, 4.14, *dd*, 12.0, 6.7	4.09, *dd*, 4.15, *dd*, 12.0, 6.6
1-Hb	4.35, *br d*, 12.1	4.38, *br d*, 10.4	4.35, *br d*, 11.0	4.35, *br d*, 11.0	4.34, *br d*, 11.8	4.35, *br d*, 10.4	4.35, *br d*, 10.5	4.35, *br d*, 10.5	4.35, *br d*, 11.0
2	5.24, *br*	5.22, *br*	5.24, *br*	5.24, *br*	5.22, *br*	5.23, *br*	5.23, *br*	5.23, *br*	5.24, *br*
3-Ha	3.62, *dd*, 3.65, *dd*, 10.7, 6.4	3.62, *dd*, 3.65, *dd*, 10.7, 6.4	3.62, *dd*, 3.65, *dd*, 10.7, 6.4	3.66, *m*	3.62, *dd*, 3.65, *dd*, 10.7, 6.4	3.62, *dd*, 3.65, *dd*, 10.7, 6.4	3.62, *dd*, 3.65, *dd*, 10.7, 6.4	3.64, *dd*, 3.67, *dd*, 10.7, 6.4	3.62, *dd*, 3.65, *dd*, 10.7, 6.4
3-Hb	3.87, *br d*, 10.7	3.85, *br d*, 10.5	3.85, *br d*, 10.5	3.88, *m*	3.86, *br d*, 10.6	3.86, *br d*, 10.6	3.85, *br d*, 10.5	3.87, *br d*, 10.6	3.89, *br d*, 10.6
Sugar moiety									
1′	4.28, *d*, 7.8	4.25, *d*, 7.7	4.28, *d*, 7.8	4.28, *d*, 7.8	4.29, *d*, 7.5	4.27, *d*, 7.6	4.27, *d*, 7.5	4.28, *d*, 7.8	4.28, *d*, 7.8
2′	3.35, *m*	3.36, *m*	3.39, *m*	3.31, *m*	3.33, *m*	3.33, *m*	3.32, *m*	3.38, *m*	3.38, *m*
3′	3.57, *m*	3.58, *m*	3.60, *m*	3.48, *m*	3.54, *m*	3.56, *m*	3.56, *m*	3.57, *m*	3.55, *m*
4′	3.53, *m*	3.55, *m*	3.57, *m*	3.44, *m*	3.50, *m*	3.52, *m*	3.52, *m*	3.55, *m*	3.53, *m*
5′	3.28, *m*	3.29, *m*	3.32, *m*	3.24, *m*	3.26, *m*	3.26, *m*	3.25, *m*	3.30, *m*	3.30, *m*
6′-Methylene	3.80, *br s*	3.79, *br s*	3.79, *br s*	3.80, *br s*	3.79, *br s*	3.79, *br s*	3.80, *br* s	3.79, *br s*	3.79, *br* s
Fatty acyl moiety									
α-Methylene	2.29, *br t*, 7.0	2.26, *br t*, 7.0	2.29, *br t*, 6.9	2.20, *br t*, 6.9	2.25, *br t*, 7.0	2.24, *br t*, 7.0	2.25, *br t*, 6.9	2.29, *br t*, 6.9	2.29, *br t*, 6.9
β-Methylene	1.57, *br*	1.55, *br*	1.57, *br*	1.49, *br*	1.57, *br*	1.54, *br*	1.54, *br*	1.57, *br*	1.57, *br*
Bulk methylene	1.24, *br*	1.22, *br*	1.23, *br*	1.16, *br*	1.25, *br*	1.24, *br*	1.24, *br*	1.23, *br*	1.23, *br*
Allylic methylene	–	–	–	–	1.99, *br*	1.99, *br*	2.10, *br*	1.98, *br*	–
Methylene[a]	–	–	–	–	–	2.78, *br t*, 5.5	2.76, *br t*, 5.3	–	–
Olefinic	–	–	–	–	5.27–5.36, *br*	5.24–5.40, *br*	5.21–5.42, *br*	5.28–5.32, *br*	–
Methyl	0.85, *br t*, 6.7	0.81, *br t*, 6.7	0.84, *br t*, 6.7	0.76, *br t*, 6.5	0.85, *br t*, 6.7	0.85, *br t*, 6.6	0.93, *t*, 7.5	0.85, *br t*, 6.5	0.85, *br t*, 6.8

H	7j	7k	7l	7m	13a	13b	13c	13d
Glycerol moiety								
1-Ha	4.07, *dd*, 4.13, *dd*, 12.0, 6.7	4.08, *dd*, 4.15, *dd*, 12.0, 6.7	4.07, *dd*, 4.14, *dd*, 12.0, 6.6	4.07, *dd*, 4.13, *dd*, 12.0, 6.6	4.11, *dd*, 4.15, *dd*, 12.0, 3.3	4.08, *dd*, 4.12, *dd*, 12.0, 3.3	4.09, *dd*, 4.14, *dd*, 12.0, 3.3	4.08, *dd*, 4.13, *dd*, 12.0, 4.4
1-Hb	4.35, *br d*, 11.0	4.35, *br d*, 12.1	4.34, *br d*, 12.0	4.35, *br d*, 12.0	4.39, *br d*, 11.4	4.35, *br d*, 10.4	4.35, *br d*, 12.1	4.38, *br d*, 10.4
2	5.27, *br*	5.23, *br*	5.23, *br*	5.23, *br*	5.23, *br*	5.21, *br*	5.25, *br*	5.28, *br*
3-Ha	3.62, *dd*, 3.65, *dd*, 10.7, 6.4	3.62, *dd*, 3.65, *dd*, 10.7, 6.4	3.62, *dd*, 3.65, *dd*, 10.7, 6.4	3.62, *dd*, 3.65, *dd*, 10.7, 6.4	3.63, *dd*, 3.70, *dd*, 10.9, 5.4	3.63, *dd*, 3.70, *dd*, 10.9, 5.4	3.68, *dd*, 3.71, *dd*, 11.0, 5.6	3.65, *dd*, 3.70, *dd*, 10.9, 5.3
3-Hb	3.87, *br d*, 10.6	3.88, *br d*, 10.6	3.89, *br d*, 10.6	3.87, *br d*, 10.6	3.86, *dd*, 3.92, *dd*, 10.9, 4.9	3.84, *dd*, 3.89, *dd*, 10.9, 4.9	3.86, *dd*, 3.91, *dd*, 10.9, 4.9	3.87, *dd*, 3.91, *dd*, 10.9, 4.9
Sugar moiety								
1′	4.29, *d*, 7.8	4.28, *d*, 7.8	4.28, *d*, 7.8	4.27, *d*, 7.5	4.22, *d*, 7.8	4.21, *d*, 7.5	4.21, *d*, 7.8	4.21, *d*, 7.5
2′	3.39, *m*	3.39, *m*	3.39, *m*	3.32, *m*	3.60, *m*	3.61, *m*	3.60, *m*	3.61, *m*
3′	3.59, *m*	3.59, *m*	3.58, *m*	3.56, *m*	3.58, *m*	3.58, *m*	3.57, *m*	3.58, *m*
4′	3.56, *m*	3.56, *m*	3.55, *m*	3.52, *m*	3.96, *br s*	3.96, *br s*	3.97, *br s*	3.98, *br s*
5′	3.31, *m*	3.31, *m*	3.31, *m*	3.25, *m*	3.52, *m*	3.52, *m*	3.53, *m*	3.52, *m*
6′-Methylene	3.79, *br* s	3.79, *br* s	3.80, *br* s	3.80, *br s*	3.80, *br s*	3.80, *br s*	3.81, *br s*	3.81, *br s*
Fatty acyl moiety								
α-Methylene	2.29, *br t*, 7.0	2.29, *br t*, 7.0	2.29, *br t*, 6.9	2.25, *br t*, 7.0	2.24, *br t*, 7.0	2.28, *br t*, 7.0	2.29, *br t*, 7.0	2.31, *br t*, 7.0
β-Methylene	1.57, *br*	1.57, *br*	1.57, *br*	1.54, *br*	1.52, *br*	1.58, *br*	1.57, *br*	1.60, *br*
Bulk methylene	1.23, *br*	1.23, *br*	1.23, *br*	1.24, *br*	1.24, *br*	1.30, *br*	1.23, *br*	1.28, *br*
Allylic methylene	–	–	1.98, *br*	2.10, *br*	–	2.02, *br*	–	2.02, *br*
Methylene[a]	–	–	–	2.76, *br t*, 5.5	–	2.76, *br t*, 5.5	–	2.78, *br t*, 5.5
Olefinic	–	–	5.28–5.32, *br*	5.21–5.42, *br*	–	5.21–5.30, *br*	–	5.22–5.40, *br*
Methyl	0.84, *br t*, 6.8	0.85, *br t*, 6.9	0.85, *br t*, 6.8	0.85, *br t*, 6.7, 0.93, *t*, 7.5	0.85, *br t*, 6.7	0.85, *br t*, 6.7	0.85, *br t*, 6.9	0.85, *br t*, 7.0 0.95, *t*, 7.5

[a] Methylene lying between double bonds.

Table 2
^{13}C-NMR spectral data for compounds **7a**–**m** and **13a**–**d** (in $CDCl_3$)

C	7a	7b	7c	7d	7e	7f	7g	7h	
Glycerol moiety									
1	63.2, 63.3	63.2, 63.3	63.1, 63.2	63.1, 63.2	63.2, 63.3	63.0, 63.1	63.2, 63.3	63.1, 63.2	
2	70.5	70.4	70.4, 70.5	70.4, 70.5	70.4, 70.5	70.4, 70.5	70.5, 70.6	70.5	
3	68.6	68.4	68.5	68.5	68.5	68.6	68.5	68.6	
Sugar moiety									
1′	103.7, 103.8	103.7, 103.8	103.7, 103.9	103.7, 103.9	103.7, 103.8	103.7, 103.8	103.7, 103.8	103.7, 103.9	
2′	73.7, 73.8	73.6, 73.7	73.7, 73.8	73.7, 73.8	73.6, 73.7	73.7, 73.8	73.7	73.8	
3′	69.8	69.6	69.8	69.8	69.7	69.7	69.7	69.8	
4′	76.7	76.6	76.6	76.6	76.6, 76.7	76.6	76.9	76.6	
5′	76.2, 76.3	76.2	76.2	76.2	76.2, 76.3	76.1	76.2, 76.3	76.2	
6′	61.9	61.7	61.9	61.9	61.7	62.1	61.8	61.8	
Fatty acyl moiety									
Carbonyl	173.6, 173.7, 173.9, 174.0	173.7, 173.8, 174.1, 174.2	173.8, 173.9, 174.1, 174.2	173.8, 173.9, 174.1, 174.2	173.7, 173.8, 174.1, 174.2	173.8, 173.9, 174.1	173.5, 173.6, 173.9, 174.0	173.8, 173.9, 174.1, 174.2	
α-Methylene	34.6, 34.8	34.5, 34.6	34.6, 34.7	34.6, 34.7	34.6	34.5, 34.6	34.6, 34.7	34.5, 34.7	
β-Methylene	25.4	25.2, 25.3	25.3	25.3	25.2, 25.3	25.1, 25.2	25.4	25.3	
Bulk methylene[a]	23.2–32.4	23.0–32.3	23.0–32.3	23.0–32.3	23.0–32.3	22.9–32.3	21.1–30.2	23.1–32.3	
Allylic methylene	–	–	–	–	27.5, 27.7	27.5	27.5, 27.6, 27.7	27.5, 27.6	
Methylene[b]	–	–	–	–	–	26.0	26.0, 26.1	–	
Olefinic	–	–	–	–	130.1, 130.4	128.3, 128.5, 130.4, 130.5	127.4, 128.0, 128.5, 130.1, 130.4, 132.2	130.1, 130.4	
Methyl	14.6	14.5	14.5	14.5	14.5	14.4	14.8	14.5	

	7i	7j	7k	7l	7m	13a	13b	13c	13d
Glycerol moiety									
1	63.1, 63.2	62.9, 63.0	63.2, 63.3	62.9, 63.0	63.2, 63.3	63.4	63.1, 63.2	63.2, 63.3	63.2, 63.3
2	70.5, 70.6	70.5, 70.6	70.4, 70.5	70.4, 70.5	70.5, 70.6	70.6	70.6	70.5, 70.6	70.6
3	68.6	68.4, 68.5	68.5	68.7	68.5	68.5	68.5, 68.6	68.5	68.5, 68.6
Sugar moiety									
1′	103.7, 103.8	103.8, 103.9	103.7, 103.8	103.7, 103.9	103.7, 103.8	104.2, 104.3	104.2, 104.4	104.2, 104.4	104.1, 104.3
2′	73.7	73.9, 74.0	73.5, 73.6	73.7, 73.8	73.7	71.5	71.5, 71.6	71.4, 71.5	71.6
3′	70.0	70.0	69.6	70.0	69.7	73.6	73.6	73.6	73.7
4′	76.5	76.9	76.6	76.8	76.9	69.2	69.4	69.2	69.4
5′	76.2, 76.3	76.3, 76.4	76.2	76.4	76.5, 76.6	74.9, 75.0	74.8, 74.9	74.8, 74.9	74.8, 74.9
6′	62.0	62.5	61.6	62.1	61.8	61.8	62.3, 62.4	61.9, 62.0	62.3
Fatty acyl moiety									
Carbonyl	173.6, 173.7, 173.9	173.5, 173.6, 173.7	173.8, 173.9, 174.2, 174.3	173.9, 174.0, 174.2	173.5, 173.6, 173.9, 174.0	173.7, 173.8, 174.1	173.8, 173.9, 174.1	173.9, 174.0, 174.2, 174.3	173.7, 173.8, 174.1, 174.2
α-Methylene	34.7, 34.8	34.6, 34.8	34.5, 34.7	34.5, 34.7	34.6, 34.7	34.7, 34.8	34.5, 34.6	34.6, 34.7	34.6, 34.8
β-Methylene	25.4, 25.5	25.4	25.3	25.3	25.4	25.5	25.3	25.3	25.4
Bulk methylene	23.2–32.4	23.2–32.4	23.1–32.3	23.1–32.3	21.1–30.2	23.2–32.4	22.2–31.2	23.0–32.3	21.1–32.0
Allylic methylene	–	–	–	27.5, 27.6	27.5, 27.6, 27.7	–	27.3, 27.5	–	27.7
Methylene[b]	–	–	–	–	26.0, 26.1, 26.2	–	26.0	–	26.0, 26.2
Olefinic	–	–	–	130.1, 130.4	127.4, 128.0, 128.5, 130.1, 130.4, 132.2	–	128.3, 128.5, 130.4, 130.6	–	127.4, 128.0, 128.2, 128.4, 130.3, 132.2
Methyl	14.6	14.6	14.5	14.5	14.8	14.7	14.7	14.5	14.6, 14.7

[a] Overlapped signals.
[b] Methylene lying between double bonds.

Table 3
^{1}H and ^{13}C-NMR spectral data for compounds **18a** and **18b**

Position	**18a**		**18b**	
	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$
Glycerol moiety				
1-Ha	63.2	4.15, *dd*, 11.9, 6.6	63.3	4.15, *dd*, 11.9, 6.6
1-Hb		4.38, *br d*, 10.4		4.38, *br d*, 10.4
2	70.5	5.22, *br*	70.6	5.22, *br*
3-Ha	68.5	3.66, *dd*, 10.8, 6.6	68.5	3.66, *dd*, 10.8, 6.6
3-Hb		3.87, *dd*, 11.0, 5.2		3.85, *dd*, 11.0, 5.2
Sugar moiety				
1′	103.9	4.25, *d*, 7.7	103.8	4.28, *d*, 7.7
2′	73.8	3.35, *m*	73.8	3.36, *m*
3′	69.8	3.57, *m*	69.8	3.58, *m*
4′	76.6	3.53, *m*	76.6	3.54, *m*
5′	76.2	3.28, *m*	76.2	3.29, *m*
6′-Methylene	61.9	3.72, *br s*	61.9	3.72, *br s*
Fatty acyl moiety				
Carbonyl	173.8, 174.2		173.5, 174.0	
α-Methylene	34.5, 34.6	2.26, *br t*, 7.0	34.6, 34.7	2.25, *br t*, 7.0
β-Methylene	25.2, 25.3	1.55, *br*	25.4	1.55, *br*
Bulk methylene[a]	29.5–32.3	1.22, *br*	29.6–32.0	1.25, *br*
Allylic methylene	–	–	27.5, 27.6, 27.7	1.95–2.12, *br*
Methylene[b]	–	–	26.0, 26.2	2.75, *br t*, 5.5
Olefinic	–	–	127.4, 128.1, 128.5, 128.6, 130.4, 132.2	5.18–5.45, *br*
Methyl	14.5	0.81, *br t*, 6.7	14.8	0.93, *t*, 7.5

[a] Overlapped signals.
[b] Methylene lying between double bonds.

(**13c–d**) based on the above-mentioned evidence were synthesized and evaluated. Similar results were observed in the series of galactosyl diglycerides. However, the *O*-acetylated forms of glycosyl diglycerides displayed no activity, indicating a requirement for free hydroxyl groups in these glycosyl diglycerides for anti-HSV action. In addition, neither glycoside derivatives lacking fatty acyl moieties nor 1,2-diacylglycerol exhibited anti-HSV activity (data not shown). To evaluate the effect of the stereochemistry at C-2 of the glycerol backbone, we also synthesized and determined the anti-HSV activity of 1,2-*O*-diacyl-3-*O*-β-D-glucopyranosyl-*sn*-glycerols (**18a–b**). The IC_{50} and toxicity of **18a** and **18b** (Table 5) showed that **18b** displayed about two times the potency of **18a** and both compounds were not toxic to the Vero cell lines at concentrations up to 100 μg/ml. The anti-HSV activity of diastereomeric mixtures **7b** compared with its diastereomeric pure isomer **18a** was not significantly different. A similar result was observed in the case of **7g** and its diastereomerically pure isomer **18b**. This observation suggests that the stereochemistry at C-2 of the glycerol backbone of these compounds does not influence anti-HSV activity.

Even though the anti-HSV mechanism of these compounds is still unknown, there was evidence that some monoglycerides exhibited antiviral activities against enveloped viruses by destruction of viral envelopes (Thormar et al., 1987). We might postulate that the mechanism of action of these monoglycosyl diglycerides conforms to that of monoglycerides to some extent.

In conclusion, the synthesis of 1,2-*O*-diacyl-3-*O*-β-D-glycopyranosyl glycerols have been obtained. We have examined the inhibitory action of all compounds against HSV-1 and HSV-2, and some structure-activity relationships were explored. The results showed that compounds containing fatty acyl moieties bearing more olefinic character displayed higher activity, except for **7b** which despite having no olefinic character still showed activity. The sugar moiety, whether it be glucose or galactose was essential for activity and the stereochemistry at C-2 of the glycerol backbone did not affect anti-HSV activity. Compound **18b** displayed moderate anti-HSV-1 and HSV-2 action with the IC_{50} 12.5±0.5 and 18.5±1.5 μg/ml, respectively. These results reveal the potential of these compounds as a new type of anti-HSV agents, although the mechanism of action must be further investigated.

3. Experimental

3.1. General

^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker AVANCE DPX-300 FT-NMR spectrometer in

Table 4
Anti-HSV activity of the synthetic monoglycosyl diglycerides

Compd[a]	R_1	R_2	Inhibition (%)[b]	
			HSV-1	HSV-2
7a	Decanoyl (C10:0)	Decanoyl	n.a.[c]	n.a.
7b	Lauroyl (C12:0)	Lauroyl	80±6	50±5
7c	Stearoyl (C18:0)	Stearoyl	n.a.	n.a.
7d	Behenoyl (C22:0)	Behenoyl	n.a.	n.a.
7e	Oleoyl (C18:1)	Oleoyl	n.a.	25±3
7f	Linoleoyl (C18:2)	Linoleoyl	50±2	50±4
7g	Linolenoyl (C18:3)	Linolenoyl	94±4	97±4
7h	Lauroyl	Oleoyl	13±2	17±1
7i	Stearoyl	Lauroyl	n.a.	n.a.
7j	Stearoyl	Behenoyl	n.a.	n.a.
7k	Behenoyl	Lauroyl	n.a.	20±1
7l	Behenoyl	Oleoyl	n.a.	10±2
7m	Linolenoyl	Linoleoyl	90±5	70±2
13a	Lauroyl	Lauroyl	80±4	50±2
13b	Linoleoyl	Linoleoyl	55±2	50±2
13c	Behenoyl	Lauroyl	n.a.	25±1
13d	Linolenoyl	Linoleoyl	100±0	90±2
18a	Lauroyl	Lauroyl	80±8	50±4
18b	Linolenoyl	Linolenoyl	100±0	100±0

[a] Performed at concentration 50 μg/ml.
[b] Values are expressed as means±S.D. of three replicates.
[c] n.a. = No activity.

Table 5
IC_{50} for anti-HSV activity and toxicity of compounds **18a** and **18b**

Compd	IC_{50} (μg/ml)[a]		Toxicity (μg/ml)[b]
	HSV-1	HSV-2	
18a	23.0±2.2	40.0±4.0	>100
18b	12.5±0.5	18.5±1.5	>100

[a] Values are expressed as means±S.D. of three replicates.
[b] Concentration of the compound required to reduce the number of viable Vero cells by 50%.

$CDCl_3$ with tetramethylsilane as the internal standard. FAB-MS were determined on a Jeol JMS-700 spectrometer. Reactions were monitored by TLC on silica gel plates; spots were visualized by spraying with 2% K_2CrO_4 in 30% aq. H_2SO_4 followed by heating.

3.2. Synthesis of 1,2-O-diacyl-3-O-β-D-glycopyranosyl-rac-glycerols

3.2.1. General procedure for glycosylation

A mixture of D,L-α,β-isopropylideneglycerol (**2**) (2.0 mmol), Ag_2CO_3 (2.0 mmol) and fine Drierite (2.5 g) in dichloromethane (40 ml) was stirred in the dark under N_2. After being stirred for half an hour, iodine (0.2 mmol) was added and acetobromo-α-D-glucose (**1**) [or acetobromo-α-D-galactose (**8**)] (2.0 mmol) in dry dichloromethane (10 ml) was then added dropwise over a period of 1 h. The reaction mixture was stirred overnight at room temperature and then filtered. The residue was washed with dry dichloromethane, and the filtrate was evaporated in vacuo to give a colorless syrupy mixture. The mixture was purified by CC on silica gel, eluted with EtOAc–hexane (1:8) to give the 1:1 mixtures of the two diastereomers of **3** (or **9**).

*3.2.1.1. 1,2-Isopropylidene-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (**3**).* (470 mg, 50.8%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.34, 1.42 (each 2×3H, *s*), 2.01, 2.03, 2.05, 2.11(each 2×3H, *s*), 3.60–4.33 (2×8H), 4.61 and 4.63 (each 1H, *d*, *J* = 7.6 Hz), 5.01 and 5.03 (each 1H, *dd* (apparent *t*), *J* = 9.8 and 7.5 Hz), 5.09 (2×1H, *dd* (apparent *t*), *J* = 9.8 and 9.5 Hz), 5.22 and 5.23 (each 1H, *dd* (apparent *t*), *J* = 9.4 and 9.4 Hz).

*3.2.1.2. 1,2-Isopropylidene-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-galactopyranosyl)-rac-glycerol (**9**).* (458 mg, 49.7%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.35, 1.43 (each 2×3H, *s*), 1.98, 2.01, 2.02, 2.13 (each 2×3H, *s*), 3.60–4.20 (2×8H), 4.49 and 4.51 (each 1H, *d*, *J* = 7.6 Hz), 5.01 (2×1H, *br d*, *J* = 10.4 Hz), 5.18 (2×1H, *br t*, *J* = 8.3 Hz), 5.38 (2×1H, *br s*).

3.2.2. General procedure for deacetonization

A solution of **3** (or **9**) (400 mg) in 60% aq. acetic acid (20 ml) was stirred at 60 °C for 1.5 h. The mixture was concentrated and co-evaporated with toluene three times. The residue was then purified by silica gel CC, eluted with EtOAc–hexane (5:1) to give a semisolid substance **4** (or **10**).

*3.2.2.1. 3-O-(β-D-2′,3′,4′,6′-Tetra-O-acetyl-glucopyranosyl)-rac-glycerol (**4**).* (363 mg, 100%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.01, 2.04, 2.06, 2.10 (each 2×3H, *s*), 3.52–3.95 (2×6H), 4.22 (2×2H, *br s*), 4.56 (2×1H, *d*, *J* = 7.8 Hz), 5.02 (2×1H, *dd* (apparent *t*), *J* = 9.4 and 7.5 Hz), 5.09 (2×1H, *dd* (apparent *t*), *J* = 9.4 and 9.5 Hz), 5.23 (2×1H, *dd* (apparent *t*), *J* = 9.4 and 9.3 Hz).

*3.2.2.2. 3-O-(β-D-2′,3′,4′,6′-Tetra-O-acetyl-galactopyranosyl)-rac-glycerol (**10**).* (360 mg, 99%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.98, 2.01, 2.02, 2.13 (each 2×3H, *s*), 3.53–3.96 (2×6H), 4.14 (2×2H, *br* s), 4.48 (2×1H, *d*, *J* = 7.8 Hz), 5.01 (2×1H, *dd*, *J* = 10.4 and 3.3 Hz), 5.18 (2×1H, *dd*, *J* = 9.9 and 8.4 Hz), 5.38 (2×1H, *br d*, *J* = 3.0 Hz).

3.2.3. General procedure for preparation of mono-acid 1,2-O-diacyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glycopyranosyl)-rac-glycerols

DCC (0.4 mmol) was added to a solution of **4** (or **10**) (0.3 mmol), fatty acid (0.7 mmol), and DMAP (0.03

mmol) in dry dichloromethane (8 ml), and stirred for 24 h at room temp. The solid was removed by filtration and the solvent evaporated in vacuo. The residue was purified by CC on silica gel, eluted with EtOAc–hexane (1:3) to give the products **6a–g** (or **12a–b**). All compounds were obtained as the 1:1 mixtures of two diastereomers.

*3.2.3.1. 1,2-O-Didecanoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (**6a**).* (198.2 mg, 90.5%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.80 (2×6H, *br t*, *J* = 6.9 Hz), 1.19 (2×24H, *br s*), 1.50 (2×4H, *br*), 1.90, 1.92, 1.94, 1.98 (each 2×3H, *s*), 2.21 (2×4H, *br t*, *J* = 6.9 Hz), 3.61–3.72 (2×2H, *m*), 3.89 (2×1H, *dd*, *J* = 10.9 and 4.9 Hz), 3.95–4.10 (2×2H, *m*), 4.12–4.28 (2×2H, *m*), 4.42 and 4.44 (each 1H, *d*, *J* = 7.8 Hz), 4.88 and 4.89 (each 1H, *dd* (apparent *t*), *J* = 9.5 and 7.9 Hz), 4.98 (2×1H, *dd* (apparent *t*), *J* = 9.5 Hz), 5.12 (2×1H, overlapped), 5.11 and 5.13 (each 1H, *dd* (apparent *t*), *J* = 9.4 and 7.2 Hz).

*3.2.3.2. 1,2-O-Dilauroyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (**6b**).* (212.8 mg, 90.2%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.79 (2×6H, *br t*, *J* = 6.9 Hz), 1.18 (2×32H, *br s*), 1.51 (2×4H, *br*), 1.90, 1.92, 1.94, 2.01 (each 2×3H, *s*), 2.20 (2×4H, *br t*, *J* = 7.0 Hz), 3.53–3.68 (2×2H, *m*), 3.83 (2×1H, *dd*, *J* = 10.9, 4.9 Hz), 3.97–4.10 (2×2H, *m*), 4.11–4.26 (2×2H, *m*), 4.43 and 4.45 (each 1H, *d*, *J* = 7.8 Hz), 4.89 and 4.90 (each 1H, *dd* (apparent *t*), *J* = 9.4 and 7.6 Hz), 4.99 (2×1H, *dd* (apparent *t*), *J* = 9.6 Hz), 5.09 (2×1H, overlapped), 5.10 and 5.11 (each 1H, *dd* (apparent *t*), *J* = 9.6 and 7.4 Hz).

*3.2.3.3. 1,2-O-Distearoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (**6c**).* (223.5 mg, 78.1%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 (2×6H, *br t*, *J* = 6.9 Hz), 1.22 (2×56H, *br s*), 1.56 (2×4H, *br*), 1.91, 1.92, 1.94, 1.98 (each 2×3H, *s*), 2.23 (2×4H, *br t*, *J* = 6.9 Hz), 3.58–3.72 (2×2H, *m*), 3.92 (2×1H, *dd*, *J* = 11.0 and 4.9 Hz), 4.03–4.17 (2×2H, *m*), 4.19–4.32 (2×2H, *m*), 4.50 and 4.51 (each 1H, *d*, *J* = 7.7 Hz), 4.96 and 4.97 (each 1H, *dd* (apparent *t*), *J* = 9.5 and 7.9 Hz), 5.05 (2×1H, *dd* (apparent *t*), *J* = 9.5 Hz), 5.10 (2×1H, overlapped), 5.11 and 5.12 (each 1H, *dd* (apparent *t*), *J* = 9.4 and 7.2 Hz).

*3.2.3.4. 1,2-O-Dibehenoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (**6d**).* (176.1 mg, 55.1%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 (2×6H, *br t*, *J* = 6.9 Hz), 1.25 (2×72H, *br s*), 1.51 (2×4H, *br*), 2.01, 20.2, 2.04, 2.08 (each 2×3H, *s*), 2.29 (2×4H, *br t*, *J* = 6.9 Hz), 3.61–3.72 (2×2H, *m*), 3.90 (2×1H, *dd*, *J* = 10.8 and 4.8 Hz), 4.05–4.18 (2×2H, *m*), 4.19–4.32 (2×2H, *m*), 4.50 and 4.51 (each 1H, *d*, *J* = 7.8 Hz), 4.96 and 4.97 (each 1H, *dd* (apparent *t*), *J* = 9.5 and 7.9 Hz), 5.05 (2×1H, *dd* (apparent *t*), *J* = 9.7 Hz), 5.12 (2×1H, overlapped), 5.13 and 5.14 (each 1H, *dd* (apparent *t*), *J* = 9.6 and 7.3 Hz).

*3.2.3.5. 1,2-O-Dioleoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (**6e**).* (207.1 mg, 72.6%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 (2×6H, *br t*, *J* = 6.7 Hz), 1.30 (2×40H, *br s*), 1.51 (2×4H, *br*), 2.02 (2×8H, overlapped), 2.01, 2.03, 2.05, 2.08 (each 2×3H, *s*), 2.29 (2×4H, *br t*, *J* = 7.0 Hz), 3.60–3.72 (2×2H, *m*), 3.92 (2×1H, *dd*, *J* = 10.9 and 4.9 Hz), 4.03–4.18 (2×2H, *m*), 4.19–4.33 (2×2H, *m*), 4.49 and 4.50 (each 1H, *d*, *J* = 7.8 Hz), 4.96 and 4.97 (each 1H, *dd* (apparent *t*), *J* = 9.5 and 7.5 Hz), 5.05 (2×1H, *dd* (apparent *t*), *J* = 9.6 Hz), 5.12 (2×1H, overlapped), 5.13 and 5.14 (each 1H, *dd* (apparent *t*), *J* = 9.8 and 7.4 Hz), 5.28–5.38 (2×4H, *br*).

*3.2.3.6. 1,2-O-Dilinoleoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (**6f**).* (198.3 mg, 69.9%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.87 (2×6H, *br t*, *J* = 6.8 Hz), 1.26 (2×28H, *br s*), 1.59 (2×4H, *br*), 2.02 (2×8H, overlapped), 2.02, 2.04, 2.06, 2.12 (each 2×3H, *s*), 2.28 (2×4H, *br t*, *J* = 7.0 Hz), 2.78 (2×4H, *br t*, *J* = 5.5 Hz), 3.60–3.74 (2×2H, *m*), 3.93 (2×1H, *dd*, *J* = 10.9 and 4.9 Hz), 4.02–4.17 (2×2H, *m*), 4.18–4.34 (2×2H, *m*), 4.50 and 4.51 (each 1H, *d*, *J* = 7.8 Hz), 4.96 and 4.97 (each 1H, *dd* (apparent *t*), *J* = 9.3 and 8.1 Hz), 5.06 (2×1H, *dd* (apparent *t*), *J* = 9.6 Hz), 5.12 (2×1H, overlapped), 5.13 and 5.14 (each 1H, *dd* (apparent *t*), *J* = 9.4 and 7.3 Hz), 5.22–5.38 (2×8H, *br*).

*3.2.3.7. 1,2-O-Dilinolenoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (**6g**).* (158.5 mg, 56.1%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.92 (2×6H, *t*, *J* = 7.5 Hz), 1.29 (2×16H, *br s*), 1.56 (2×4H, *br*), 2.02 (2×8H, overlapped), 1.93, 2.01, 2.03, 2.08 (each 2×3H, *s*), 2.25 (2×4H, *br t*, *J* = 7.0 Hz), 2.75 (2×8H, *br t*, *J* = 5.5 Hz), 3.58–3.68 (2×2H, *m*), 3.90 (2×1H, *dd*, *J* = 10.7 and 4.6 Hz), 4.01–4.15 (2×2H, *m*), 4.16–4.30 (2×2H, *m*), 4.50 and 4.51 (each 1H, *d*, *J* = 7.6 Hz), 4.95 and 4.96 (each 1H, *dd* (apparent *t*), *J* = 9.3 and 7.8 Hz), 5.05 (2×1H, *dd* (apparent *t*), *J* = 9.6 Hz), 5.12 (2×1H, overlapped), 5.13 and 5.14 (each 1H, *dd* (apparent *t*), *J* = 9.4 and 7.3 Hz), 5.20–5.40 (2×12H, *br*).

*3.2.3.8. 1,2-O-Dilauroyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-galactopyranosyl)-rac-glycerol (**12a**).* (210.2 mg, 89.1%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 (2×6H, *br t*, *J* = 6.5 Hz), 1.29 (2×32H, *br s*), 1.51 (2×4H, *br*), 1.98, 2.01, 2.02, 2.14 (each 2×3H, *s*), 2.25 (2×4H, *br t*, *J* = 7.0 Hz), 3.65 (2×1H, *dd*, *J* = 10.8 and 5.5 Hz), 3.82–3.95 (2×2H, *m*), 4.01–4.15 (2×3H, *m*), 4.27 (2×1H, *br d*, *J* = 11.0 Hz), 4.44 and 4.46 (each 1H, *d*, *J* = 7.6 Hz), 4.96 (2×1H, *br d*, *J* = 10.3 Hz), 5.12–5.22 (2×2H, *br*), 5.35 (2×1H, *br s*).

*3.2.3.9. 1,2-O-Dilinoleoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-galactopyranosyl)-rac-glycerol (**12b**).* (149.1 mg,

52.7%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 (2×6H, *br t*, J=6.8 Hz), 1.23 (2×28H, *br s*), 1.58 (2×4H, *br*), 1.92, 1.99, 2.00, 2.17 (each 2×3H, *s*), 2.02 (2×8H, overlapped), 2.23 (2×4H, *br t*, J=7.0 Hz), 2.76 (2×4H, *br*), 3.61 (2×1H, *dd*, J=10.8 and 5.5 Hz), 3.82–3.95 (2×2H, *m*), 4.01–4.15 (2×3H, *m*), 4.27 (2×1H, *br d*, J=11.0 Hz), 4.42 and 4.44 (each 1H, *d*, J=7.6 Hz), 4.96 (2×1H, *br d*, J=10.2 Hz), 5.10–5.18 (2×2H, *br*), 5.35 (2×1H, overlapped), 5.20–5.40 (2×8H, *br*).

3.2.4. General procedure for preparation of mixed-acid 1,2-O-diacyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glycopyranosyl)-rac-glycerols

To a solution of **4** (or **10**) (0.3 mmol), the first fatty acid (0.4 mmol) and DMAP (0.03 mmol) in dry dichloromethane (8 ml), DCC (0.4 mmol) was added and stirred for 6 h at 0 °C. The solid was filtered off and the solvent was evaporated in vacuo. The residue was purified by CC on silica gel column, eluted with EtOAc–hexane (1:3) to give the glycosyl monoglycerides (**5a–d** or **11a–b**) which were subsequently reacted with equimolar amounts of the second fatty acid, DCC and a little amount of DMAP at room temperature overnight. The solid was filtered off and the solvent was evaporated in vacuo. The residue was purified by CC on silica gel, eluted with EtOAc–hexane (1:3) to give the 1:1 mixtures of two diastereomers **6h–m** (or **12c–d**).

3.2.4.1. 1-O-Lauroyl-2-O-oleoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (6h).

(187.5 mg, 72.0%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.84 (2×6H, *br t*, J=6.7 Hz), 1.25 (2×36H, *br s*), 1.54 (2×4H, *br*), 2.02 (2×4H, overlapped) 1.95, 2.00, 2.02, 2.05 (each 2×3H, *s*), 2.25 (2×4H, *br t*, J=7.0 Hz), 3.60–3.70 (2×2H, *m*), 3.90 (2×1H, *dd*, J=10.9 and 4.9 Hz), 4.01–4.14 (2×2H, *m*), 4.15–4.30 (2×2H, *m*), 4.49 and 4.50 (each 1H, *d*, J=7.8 Hz), 4.95 and 4.96 (each 1H, *dd* (apparent *t*), J=9.5 and 7.5 Hz), 5.06 (2×1H, *dd* (apparent *t*), J=9.6 Hz), 5.14 (2×1H, overlapped), 5.15 and 5.16 (each 1H, *dd* (apparent *t*), J=9.4 and 7.2 Hz), 5.28–5.32 (2×2H, *br*).

3.2.4.2. 1-O-Stearoyl-2-O-lauroyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (6i).

(221.5 mg, 84.9%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 (2×6H, *br t*, J=6.7 Hz), 1.27 (2×44H, *br s*), 1.51 (2×4H, *br*), 1.98, 2.01, 2.03, 2.06 (each 2×3H, *s*), 2.29 (2×4H, *br t*, J=7.0 Hz), 3.60–3.72 (2×2H, *m*), 3.92 (2×1H, *dd*, J=10.9 and 4.9 Hz), 4.01–4.18 (2×2H, *m*), 4.19–4.33 (2×2H, *m*), 4.49 and 4.50 (each 1H, *d*, J=7.8 Hz), 4.96 and 4.97 (each 1H, *dd* (apparent *t*), J=9.5 and 7.5 Hz), 5.05 (2×1H, *dd* (apparent *t*), J=9.6 Hz), 5.13 (2×1H, overlapped), 5.14 and 5.15 (each 1H, *dd* (apparent *t*), J=9.8 and 7.4 Hz).

3.2.4.3. 1-O-Stearoyl-2-O-behenoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (6j).

(196.3 mg, 64.8%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.87 (2×6H, *br t*, J=6.7 Hz), 1.38 (2×64H, *br s*), 1.56 (2×4H, *br*), 2.01, 2.03, 2.05, 2.08 (each 2×3H, *s*), 2.30 (2×4H, *br t*, J=7.0 Hz), 3.61–3.75 (2×2H, *m*), 3.92 (2×1H, *dd*, J=10.9 and 4.9 Hz), 4.02–4.19 (2×2H, *m*), 4.20–4.33 (2×2H, *m*), 4.50 and 4.51 (each 1H, *d*, J=7.8 Hz), 4.96 and 4.97 (each 1H, *dd* (apparent *t*), J=9.5 and 7.5 Hz), 5.07 (2×1H, *dd* (apparent *t*), J=9.6 Hz), 5.15 (2×1H, overlapped), 5.16 and 5.17 (each 1H, *dd* (apparent *t*), J=9.8 and 7.4 Hz).

3.2.4.4. 1-O-Behenoyl-2-O-lauroyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (6k).

(194.2 mg, 69.9%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.88 (2×6H, *br t*, J=6.7 Hz), 1.25 (2×52H, *br s*), 1.54 (2×4H, *br*), 1.98, 2.01, 2.03, 2.07 (each 2×3H, *s*), 2.28 (2×4H, *br t*, J=7.0 Hz), 3.60–3.72 (2×2H, *m*), 3.92 (2×1H, *dd*, J=10.9 and 4.9 Hz), 4.01–4.17 (2×2H, *m*), 4.18–4.32 (2×2H, *m*), 4.50 and 4.51 (each 1H, *d*, J=7.8 Hz), 4.96 and 4.97 (each 1H, *dd* (apparent *t*), J=9.5 and 7.5 Hz), 5.05 (2×1H, *dd* (apparent *t*), J=9.6 Hz), 5.14 (2×1H, overlapped), 5.15 and 5.16 (each 1H, *dd* (apparent *t*), J=9.8 and 7.4 Hz).

3.2.4.5. 1-O-Behenoyl-2-O-oleoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (6l).

(205.8 mg, 68.1%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.87 (2×6H, *br t*, J=6.7 Hz), 1.25 (2×56H, *br s*), 1.58 (2×4H, *br*), 2.02 (2×4H, overlapped), 2.01, 2.03, 2.05, 2.10 (each 2×3H, *s*), 2.25 (2×4H, *br t*, J=7.0 Hz), 3.60–3.73 (2×2H, *m*), 3.92 (2×1H, *dd*, J=10.9 and 4.9 Hz), 4.04–4.17 (2×2H, *m*), 4.19–4.33 (2×2H, *m*), 4.50 and 4.51 (each 1H, *d*, J=7.8 Hz), 4.96 and 4.97 (each 1H, *dd* (apparent *t*), J=9.5 and 7.5 Hz), 5.05 (2×1H, *dd* (apparent *t*), J=9.6 Hz), 5.14 (2×1H, overlapped), 5.15 and 5.16 (each 1H, *dd* (apparent *t*), J=9.8 and 7.4 Hz), 5.27–5.35 (2×2H, *br*).

3.2.4.6. 1-O-Linolenoyl-2-O-linoleoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-rac-glycerol (6m).

(169.5 mg, 59.9%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 (2×3H, *br t*, J=6.7 Hz), 0.92 (2×3H, *t*, J=7.6 Hz), 1.29 (2×22H, *br s*), 1.56 (2×4H, *br*), 2.02 (2×8H, overlapped), 1.93, 2.01, 2.03, 2.08 (each 2×3H, *s*), 2.25 (2×4H, *br t*, J=6.9 Hz), 2.75 (2×6H, *br t*, J=5.5 Hz), 3.58–3.68 (2×2H, *m*), 3.90 (2×1H, *dd*, J=10.9 and 4.9 Hz), 4.01–4.15 (2×2H, *m*), 4.16–4.30 (2×2H, *m*), 4.50 (2×1H, *br d*, J=7.6 Hz), 4.95 (2×1H, *dd* (apparent *t*), J=9.3 and 8.1 Hz), 5.04 (2×1H, *dd* (apparent *t*), J=9.6 Hz), 5.12 (2×1H, overlapped), 5.15 (2×1H, *dd* (apparent *t*), J=9.4 and 7.3 Hz), 5.20–5.38 (2×10H, *br*).

3.2.4.7. 1-O-Behenoyl-2-O-lauroyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-galactopyranosyl)-rac-glycerol (12c).

(190.0 mg, 68.4%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 (2×6H, *br t*, J=6.7 Hz), 1.29 (2×52H, *br s*), 1.51

(2×4H, *br*), 1.98, 2.01, 2.02, 2.14 (each 2×3H, *s*), 2.25 (2×4H, *br t*, $J=7.0$ Hz), 3.64 (2×1H, *dd*, $J=10.8$ and 5.5 Hz), 3.82–3.96 (2×2H, *m*), 4.01–4.17 (2×3H, *m*), 4.26 (2×1H, *br d*, $J=11.0$ Hz), 4.44 and 4.46 (each 1H, *d*, $J=7.6$ Hz), 4.95 (2×1H, *br d*, $J=10.4$ Hz), 5.12–5.21 (2×2H), 5.35 (2×1H, *br s*).

*3.2.4.8. 1-O-Linolenoyl-2-O-linoleoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-galactopyranosyl)-rac-glycerol (**12d**).* (151.5 mg, 53.5%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 (2×3H, *br t*, $J=6.7$ Hz), 0.95 (2×3H, *t*, $J=7.6$ Hz), 1.29 (2×22H, *br s*), 1.51 (2×4H, *br*), 1.98, 2.01, 2.02, 2.14 (each 2×3H, *s*), 2.00 (2×8H, overlapped), 2.30 (2×4H, *br t*, $J=6.9$ Hz), 2.78 (2×6H, *br t*, $J=5.5$ Hz), 3.64 (2×1H, *dd*, $J=10.8$ and 5.5 Hz), 3.82–3.95 (2×2H, *m*), 4.01–4.15 (2×3H, *m*), 4.27 (2×1H, *br d*, $J=11.0$ Hz), 4.44 and 4.46 (each 1H, *d*, $J=7.6$ Hz), 4.96 (2×1H, *br d*, $J=10.4$ Hz), 5.12–5.22 (2×2H), 5.35 (2×1H, overlapped), 5.30–5.40 (2×10H, *br*).

3.2.5. General procedure for selective removal of acetyl protecting groups from 1,2-O-diacyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glycopyranosyl)glycerols

To a solution of **6a–m** (or **12a–d**) (0.1 mmol) in 85% EtOH, $NH_2NH_2{\cdot}H_2O$ (1.2 mmol) was added. Each mixture was stirred for 4–6 h at 40–60 °C, the mixture poured into ice-cold water and extracted with $CHCl_3$ (3×50 ml). The combined $CHCl_3$ layers were dried (anhydr Na_2SO_4) and individually evaporated to give a syrupy mixture. Each mixture was purified by CC on silica gel column, eluted with $CHCl_3$–MeOH (8:1) to give 1:1 mixtures of two diastereomers **7a–m** (or **13a–d**).

*3.2.5.1. 1,2-O-Didecanoyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7a**).* (31.9 mg, 56.7%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 585.3600 (calc. 585.3614) for $C_{29}H_{54}O_{10}Na$.

*3.2.5.2. 1,2-O-Dilauroyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7b**).* (31.6 mg, 51.1%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+K]^+$ *m/z*: 657.3901 (calc. 657.3979) for $C_{33}H_{62}O_{10}K$.

*3.2.5.3. 1,2-O-Distearoyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7c**).* (27.8 mg, 35.4%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 809.6144 (calc. 809.6118) for $C_{45}H_{86}O_{10}Na$.

*3.2.5.4. 1,2-O-Dibehenoyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7d**).* (25.2 mg, 28.1%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 921.7365 (calc. 921.7344) for $C_{53}H_{102}O_{10}Na$.

*3.2.5.5. 1,2-O-Dioleoyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7e**).* (28.0 mg, 35.9%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 805.5820 (calc. 805.5805) for $C_{45}H_{82}O_{10}Na$.

*3.2.5.6. 1,2-O-Dilinoleoyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7f**).* 25.7 mg, 33.0%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 801.5455 (calc. 801.5492) for $C_{45}H_{78}O_{10}Na$.

*3.2.5.7. 1,2-O-Dilinolenoyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7g**).* (23.5 mg, 30.4%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 797.5190 (calc. 797.5179) for $C_{45}H_{74}O_{10}Na$.

*3.2.5.8. 1-O-Lauroyl-2-O-oleoyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7h**).* (30.7 mg, 43.9%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 723.5023 (calc. 723.5023) for $C_{39}H_{72}O_{10}Na$.

*3.2.5.9. 1-O-Stearoyl-2-O-lauroyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7i**).* (28.2 mg, 40.2%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z* : 725.5159 (calc. 725.5179) for $C_{39}H_{74}O_{10}Na$.

*3.2.5.10. 1-O-Stearoyl-2-O-behenoyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7j**).* (26.1 mg, 31.0%); for ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 865.6743 (calc. 865.6745) for $C_{49}H_{94}O_{10}Na$.

*3.2.5.11. 1-O-Behenoyl-2-O-lauroyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7k**).* (28.6 mg, 37.7%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 781.5831 (calc. 781.5805) for $C_{43}H_{82}O_{10}Na$.

*3.2.5.12. 1-O-Behenoyl-2-O-oleoyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7l**).* (26.8 mg, 31.9%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 863.6566 (calc. 863.6588) for $C_{49}H_{92}O_{10}Na$.

*3.2.5.13. 1-O-Linolenoyl-2-O-linoleoyl-3-O-β-D-glucopyranosyl-rac-glycerol (**7m**).* (24.0 mg, 30.9%)); for ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz,

$CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 799.5366 (calc. 799.5336) for $C_{45}H_{76}O_{10}Na$.

*3.2.5.14. 1,2-O-Dilauroyl-3-O-β-D-galactopyranosyl-rac-glycerol (**13a**).* (30.8 mg, 49.8%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 641.4249 (calc. 641.4240) for $C_{33}H_{62}O_{10}Na$.

*3.2.5.15. 1,2-O-Dilinoleoyl-3-O-β-D-galactopyranosyl-rac-glycerol (**13b**).* (24.0 mg, 31.3%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 801.5496 (calc. 801.5492) for $C_{45}H_{78}O_{10}Na$.

*3.2.5.16. 1-O-Behenoyl-2-O-lauroyl-3-O-β-D-galactopyranosyl-rac-glycerol (**13c**).* (30.2 mg, 39.8%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 781.5866 (calc. 781.5805) for $C_{43}H_{82}O_{10}Na$.

*3.2.5.17. 1-O-Linolenoyl-2-O-linoleoyl-3-O-β-D-galactopyranosyl-rac-glycerol (**13d**).* (23.2 mg, 29.9%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Tables 1 and 2; HR-FABMS $[M+Na]^+$ *m/z*: 779.5303 (calc. 779.5336) for $C_{45}H_{76}O_{10}Na$.

3.3. Synthesis of 1,2-O-diacyl-3-O-β-D-glycopyranosyl-sn-glycerols

*3.3.1. 1,2-O-Dibenzyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-sn-glycerol (**15**)*

15 was prepared from **14** according to the general procedure for glycosylation as described in Section 3.2.1; (618.2 mg, 51.6%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.90, 1.96, 1.97, 2.01 (each 3H, *s*), 3.50–3.78 (5H, *m*), 3.86–3.97 (1H, *m*), 4.09 (1H, *br d*, *J* = 12.1 Hz), 4.28 (1H, *dd*, *J* = 12.2 and 4.3 Hz), 4.51 (1H, overlapped), 4.51 and 4.63 (each 2H, *s*), 4.97 (1H, *dd* (apparent *t*), *J* = 8.7 Hz), 5.01 (1H, *dd* (apparent *t*), *J* = 9.6 Hz), 5.16 (1H, *dd* (apparent *t*), *J* = 9.2 Hz), 7.30 (10H, *br*).

*3.3.2. 3-O-(β-D-2′,3′,4′,6′-Tetra-O-acetyl-glucopyranosyl)-sn-glycerol (**16**)*

A mixture of **15** (100 mg) and 10% Pd/C (45 mg) in EtOAc (10 ml) containing 0.25 ml each of EtOH and HOAc was vigorously shaken under H_2 gas using a Parr apparatus for 5 h. The catalyst was filtered off, the filtrate was evaporated and purified by CC, eluted with EtOAc–hexane (5:1) to give semisolid substance **16**; (56 mg, 80.4%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.01, 2.04, 2.06, 2.10 (each 3H, *s*), 3.52–3.95 (6H, *m*), 4.22 (2H, *br s*), 4.56 (1H, *d*, *J* = 7.8 Hz), 5.02 (1H, *dd* (apparent *t*), *J* = 9.4 and 7.5 Hz), 5.09 (1H, *dd* (apparent *t*), *J* = 9.4 Hz), 5.23 (1H, *dd* (apparent *t*), *J* = 9.4 Hz).

*3.3.3. 1,2-O-Dilauroyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-sn-glycerol (**17a**)*

17a was prepared from **16** according to the general procedure for preparation of mono-acid 1,2-*O*-diacyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetyl-glycopyranosyl)-*rac*-glycerols as described in Section 3.2.3 (210.2 mg, 89.1%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.83 (6H, *br t*, *J* = 6.9 Hz), 1.22 (32H, *br s*), 1.54 (4H, *br*), 1.94, 1.96, 1.98, 2.02 (each 3H, *s*), 2.23 (4H, *br t*, *J* = 7.9 Hz), 3.57–3.72 (2H, *m*), 3.89 (1H, *dd*, *J* = 10.4 and 4.9 Hz), 4.05 (1H, *dd*, (apparent *t*), *J* = 6.2 Hz) 4.09 (1H, *br s*), 4.15 (1H, *br d*, 4.6 Hz) 4.24 (1H, *dd*, *J* = 12.1 and 3.3 Hz), 4.48 (1H, *d*, *J* = 7.8 Hz), 4.93 (1H, *dd* (apparent *t*), *J* = 9.4 and 8.1 Hz), 5.01 (1H, *dd* (apparent *t*), *J* = 9.6 Hz), 5.12 (1H, overlapped), 5.14 (1H, *dd* (apparent *t*), *J* = 9.3 Hz).

*3.3.4. 1,2-O-Dilinolenoyl-3-O-(β-D-2′,3′,4′,6′-tetra-O-acetyl-glucopyranosyl)-sn-glycerol (**17b**)*

17b was prepared from **16** according to the general procedure for preparation of mono-acid 1,2-*O*-diacyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetyl-glycopyranosyl)-*rac*-glycerols as described in Section 3.2.3; (160.2 mg, 56.7%); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.92 (6H, *t*, *J* = 7.5 Hz), 1.25 (16H, *br s*), 1.53 (4H, *br*), 2.02 (8H, overlapped), 1.93, 2.01, 2.03, 2.08 (each 3H, *s*), 2.25 (4H, *br t*, *J* = 7.0 Hz), 2.76 (8H, *br t*, *J* = 6.3 Hz), 3.58–3.68 (2H, *m*), 3.90 (1H, *dd*, *J* = 10.9 and 4.9 Hz), 4.05 (1H, *dd* (apparent *t*), *J* = 6.3 Hz) 4.11 (1H, *br* s), 4.19 (1H, *br d*, *J* = 4.6 Hz) 4.27 (1H, *dd*, *J* = 12.0 and 3.6 Hz), 4.50 (1H, *d*, *J* = 7.8 Hz), 4.93 (1H, *dd* (apparent *t*), *J* = 9.3 and 8.0 Hz), 5.03 (1H, *dd* (apparent *t*), *J* = 9.7 Hz), 5.14 (1H, overlapped), 5.16 (1H, *dd* (apparent *t*), *J* = 9.3 Hz), 5.20-5.41 (12H, *br*).

*3.3.5. 1,2-O-Dilauroyl-3-O-β-D-glucopyranosyl-sn-glycerol (**18a**)*

18a was prepared from **17a** according to the general procedure for selective removal of acetyl protecting groups as described in Section 3.2.5; (29.8 mg, 48.2%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Table 3; HR-FABMS $[M+Na]^+$ *m/z*: 641.4247 (calc. 641.4240) for $C_{33}H_{62}O_{10}Na$.

*3.3.6. 1,2-O-Dilinolenoyl-3-O-β-D-glucopyranosyl-sn-glycerol (**18b**)*

18b was prepared from **17b** according to the general procedure for selective removal of acetyl protecting groups as described in Section 3.2.5; (21.5 mg, 27.7%); for ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$) assignments, see Table 3; HR-FABMS $[M+Na]^+$ *m/z*: 797.5192 (calc. 797.5179) for $C_{45}H_{74}O_{10}Na$.

3.4. Biological assay

The anti-HSV activity was determined via the plaque reduction assay (Abou-Karam and Shier, 1990). The cells used for the assay were from the Vero cell line. They were cultured as a monolayer in minimal essential medium (MEM) supplemented with 10% fetal calf serum. One hour prior to viral infection, HSV-1 (KOS strain) or HSV-2 (186 strain) and the test compounds were added to a 96-well microtiter plate, the Vero cells were added to each well, and then incubated at 37 °C, 5% CO_2 for 3 days. The viral plaques formed in the Vero cell cultures were counted under an inverted microscope. Antiviral activity was expressed as a score of% inhibition and 50% inhibitory concentration (IC_{50}). Toxicity was evaluated by the ability of the compounds to reduce the number of viable Vero cells by 50% at different concentrations.

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