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Chemoenzymatic Synthesis and Antitumor Promoting Activity of 6′- and 3-Esters of 2-*O*-β-D-Glucosylglycerol

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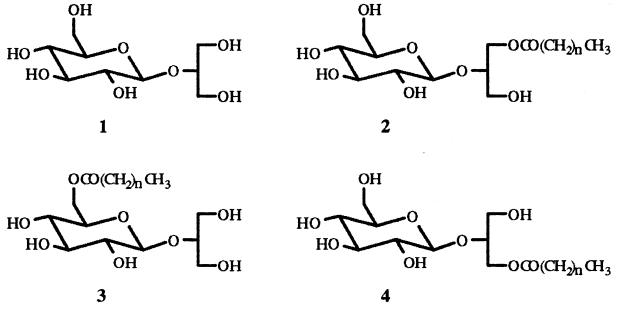
Abstract—Through a simple chemoenzymatic approach 6′- and 3-esters of 2-*O*-β-D-glucosylglycerol **1**, with short-medium length fatty acid acyl chains, were prepared. The study of their in vitro antitumor promoting effect on Epstein–Barr virus early antigen (EBV-EA) activation, in comparison with that of the 1-esters previously described, confirms the significant activity of such monoacylated glycoglycerolipid analogues and establishes for the glucose series that the 1-substitution and the hexanoyl chain are the proper structural features for the maximum activity.

Introduction

Recently tumor inhibitory activity of glycosylglycerols and glycoglycerolipids has been demonstrated on the basis of their in vitro and in vivo antitumor promoting effect on Epstein–Barr virus early antigen (EBV-EA) activation induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[1–3] Our efforts in this field have been directed toward the synthesis of different gluco-[4,5] and galactosylglycerols[6,7] in order to study the relationship between the molecular structure of glycoglycerolipids and their activity. In particular, we could easily prepare[5] highly enriched 1-*O*-acyl derivatives of 2-*O*-β-D-glucosylglycerol (**1**), such as **2**, containing small amounts of their 6′-*O*- and 3-*O*-acyl isomers, through a direct lipase catalyzed monoacylation of the substrate mediated by *Pseudomonas cepacia* lipase (LPS). These compounds were then assayed as cancer chemopreventive agents and resulted highly active especially for short-medium length fatty acid acyl chains.[3]

However, the biological activity might be related not to the 1-*O*-acyl derivatives **2**, but to the small amounts of the isomers **3** and **4**. Therefore, we report here on the synthesis of short-medium length (from C_4 to C_{10}) 6′- and 3-*O*-acyl-2-*O*-β-D-glucosylglycerols **3a–d** and **4a–d** and on the dependence of their antitumor promoting potential on the acylation site and on the chain length.



a: $n=2$; **b**: $n=4$; **c**: $n=6$; **d**: $n=8$.

Results and Discussion

Chemoenzymatic synthesis of esters 3 and 4

1,3-Di-*O*-benzyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**5**) was chosen as the key compound for the preparation of both the 6′- and 3-*O*-acylglucosylglycerols **3** and **4**.

To get the 6′-esters **3** (Scheme 1), compound **5**, obtained from the known[8,9] 1,3-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*sn*-glycerol, was directly

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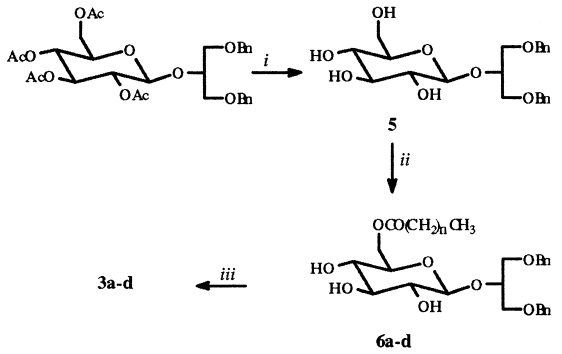
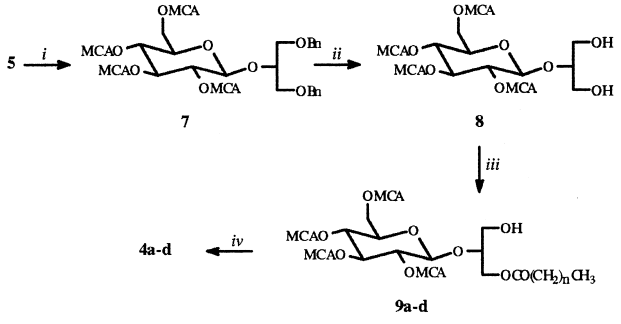


Scheme 1. Reagents: (i) MeOH, MeONa; (ii) LPS, $CF_3CH_2OCO(CH_2)_nCH_3$, THF; (iii) H_2, Pd/C.

submitted to transesterification catalyzed by LPS in the presence of the appropriate trifluoroethylester in tetrahydrofuran as the solvent. The reactions were completely regioselective and afforded, through the expected acylation of the primary hydroxyl group,[10] the 1,3-di-*O*-benzyl-2-*O*-(6-*O*-acyl-β-D-glucopyranosyl)-*sn*-glycerols **6** in good yields. Catalytic hydrogenolysis of **6** with palladium on activated carbon in methanol finally yielded almost quantitatively pure 2-*O*-(6-*O*-acyl-β-D-glucopyranosyl)-*sn*-glycerols **3**.

In order to obtain the 3-esters **4**, the hydroxyl groups of the glucosyl moiety of **5** were temporary protected as chloroacetates (MCA); thus, after removal of the benzyl groups, the glycerol hydroxymethyls should be available for the selective acylation catalyzed by lipase from *Candida antarctica* (LCA) which is known to occur on such substrates with opposite diastereoselectivity with respect to LPS.[4]

So **5** was converted (Scheme 2) into 1,3-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-chloroacetyl-β-D-glucopyranosyl)-*sn*-glycerol (**7**) by chloroacetic anhydride treatment in a dichloromethane–pyridine mixture at 0°C.[11] Catalytic hydrogenolysis of **7** yielded 2-*O*-(2,3,4,6-tetra-*O*-chloroacetyl-β-D-glucopyranosyl)-*sn*-glycerol (**8**), which was submitted to LCA transesterification with the appropriate trifluoroethyl esters in tetrahydrofuran. The



Scheme 2. Reagents: (i) $(ClCH_2CO)_2O$, CH_2Cl_2/Py, 0°C; (ii) H_2, Pd/C; (iii) LCA, $CF_3CH_2OCO(CH_2)_nCH_3$, THF; (iv) NH_2NH_2.AcOH, AcOEt/MeOH; MCA = $COCH_2Cl$.

enzymatic reaction afforded the 3-*O*-acyl-2-*O*-(2,3,4,6-tetra-*O*-chloroacetyl-β-D-glucopyranosyl)-*sn*-glycerols (**9**) in good diastereoselectivity and yields; the desired 3-*O*-acyl-2-*O*-β-D-glucosylglycerols (**4**) were finally obtained pure by mild removing of the MCA groups of **9** by hydrazine–acetate treatment[12] followed by crystallization.

The configuration at C-2 of compounds **4** was determined by chemical correlation to commercial 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerol. Compounds **4** were hydrolyzed with β-glucosidase from almonds; the monoacyglycerols present in the reaction mixtures were transformed into the isopropylidene derivatives and analyzed by chiral GLC in comparison with authentic samples as described in ref 7 for the corresponding 3-galactosylesters. In this way the configuration of 3-*O*-acyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerols was assigned to the monoesters **4**.

Inhibitory effects of 3 and 4 on EBV-EA

Compounds **3a–d** and **4a–d** were assayed for their antitumor promoting activities using a short-term in vitro assay for Epstein–Barr virus early antigen activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[2] Their inhibitory effects on activation of the early antigen (% to control) and the viabilities of Raji cells (% viability) are shown in Table 1, in comparison with those of 1-esters **2a–d**, previously described by us.[3]

As reported in Table 1 all the tested compounds showed significant and comparable activity even if the already studied 1-esters **2**[3] resulted the most active. In particular the antitumor promoting activity followed the order: 1-esters > 6′-esters > 3-esters for all the concentrations and all the acyl chain length employed, and for each isomeric series the hexanoyl derivatives **2b**, **3b** and **4b** resulted the most potent compounds, strengthening previous findings.[3] These results establish that the activity previously ascertained for compounds **2** is actually due to them and not to the small amounts of compounds **3** and **4** they contain as byproducts.

In conclusion, this study has allowed to prepare other series of interestingly active glucosylglycerols, once more synthetic analogues more potent than the natural compounds,[3] and establishes the hexanoyl chain as the proper length for the best activity in the series. The role of the acyl chain, which appear not strictly related to a positional requirement, needs further elucidation.

Experimental

General procedures

^1H NMR spectra were recorded with a Bruker AM-500 spectrometer on $CDCl_3$ solutions at 303 K, unless otherwise stated; chemical shifts are reported as δ (ppm) relative to tetramethylsilane as internal standard. Mass experiments were performed through chemical ionisation mass spectrometry (CI–MS) as described in ref 13.

Table 1. Percentages of EBV-EA induction in the presence of **2**, **3** and **4** with respect to positive control (100%)

Compound	Relative concentration (mol ration/TPA)[a]			
	1000	500	100	10
	% to positive control ± SE ($n = 3$)			
2a[3]	0.0[b] ± 0.1 (60)[c]	15.7 ± 0.4	37.2 ± 1.5	79.4 ± 2.0
2b[3]	0.0 ± 0 (70)	10.6 ± 0.6	30.9 ± 1.8	68.2 ± 2.5
2c[3]	0.0 ± 0 (70)	16.9 ± 0.7	55.0 ± 1.9	89.2 ± 2.2
2d[3]	0.0 ± 0 (60)	18.4 ± 1.0	56.5 ± 2.5	88.2 ± 1.9
3a	0.0 ± 0 (70)	27.9 ± 0.8	51.3 ± 1.3	88.4 ± 1.0
3b	0.0 ± 0 (70)	19.3 ± 0.3	37.6 ± 1.5	78.1 ± 1.8
3c	0.0 ± 0 (70)	21.1 ± 0.4	57.2 ± 1.4	90.6 ± 1.1
3d	0.0 ± 0 (70)	27.3 ± 0.6	62.8 ± 2.3	92.1 ± 1.2
4a	0.0 ± 0.5 (70)	31.6 ± 0.5	56.2 ± 1.4	93.6 ± 0.2
4b	0.0 ± 0 (70)	25.1 ± 0.6	42.3 ± 1.1	82.7 ± 1.1
4c	0.0 ± 0 (70)	27.7 ± 0.4	69.5 ± 1.3	92.1 ± 0.3
4d	0.0 ± 0.7 (70)	36.2 ± 0.6	76.3 ± 1.3	95.8 ± 0.3

[a] TPA (32 pmol/mL).
[b] Values represent relative percentages to the positive control value (at least 500 cells were counted).
[c] Values in parentheses are viability percentages of Raji cells.

Melting points were recorded on a Büchi 510 capillary melting point apparatus and were uncorrected. Optical rotations were determined on a Perkin–Elmer 241 polarimeter in methanol solutions ($c = 1.0$) in a 1 dm cell at 20°C unless otherwise stated. Analytical thin layer chromatography (TLC) was carried out on Merck 60 F_{254} silica gel plates (0.25 mm thickness) and the spots were detected by spraying with 50% aqueous H_2SO_4 or with anisaldehyde based reagent and heating at 110°C. Flash chromatography was performed with Merck 60 silica gel (230–400 mesh). *P. cepacia* lipase (lipase PS, LPS, specific activity 30.5 triacetin units/mg solid), a generous gift from Amano Pharmaceutical Co (Mitsubishi Italia), was supported on Celite;[4] *C. antarctica* lipase SP 435 L, immobilized on a macroporous acrylic resin, (Novozym® 435, LCA, specific activity 9.5 PL units/mg solid), was a generous gift from Novo Nordisk A/S; β-glucosidase from almonds (specific activity about 6 salicin units/mg solid) was purchased from Fluka. LPS and LCA were kept under vacuum overnight prior to use. The trifluoroethyl esters were synthesized according to ref 5. Evaporation under reduced pressure was always effected with the bath temperature kept below 40°C. Tetrahydrofuran was distilled over sodium-benzophenone, and pyridine over calcium hydride prior to use. The elemental analyses of the new compounds were consistent with the theoretical ones.

1,3-Di-*O*-benzyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (5). 50 mL of 0.7 M sodium metoxide in methanol were added to a solution of the known[8,9] 1,3-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*sn*-glycerol (12 g, 20 mmol) in methanol (18 mL). After 2 h at room temperature the reaction mixture was neutralized with Dowex 50×8 H^+ and filtered. The solvent was removed under vacuum and flash chromatography (ethyl acetate:methanol, 90:10, v/v) of the crude product afforded pure **5** (87% yield): oil; $[\alpha]_D$ −5.0 (chloroform); ^{1}H NMR: δ 3.21 (ddd, 1H, $J_{5',4'} = 9.0$ Hz, $J_{5',6'a} = 3.0$ Hz, $J_{5',6'b} = 4.0$ Hz, H-5′), 3.40 (dd, 1H, $J_{2',1'} = 8.0$ Hz, $J_{2',3'} = 9.0$ Hz, H-2′), 3.49 (dd, 1H, $J_{3',4'} = 9.0$ Hz, H-3′), 3.51–3.62 (m, 5H, H_2-1, H_2-3 and H-4′), 3.71 (dd, 1H, $J_{6'a,6'b} = 12.0$ Hz, H-6′a), 3.74 (dd, 1H, H-6′b), 4.0 (m, 1H, H-2), 4.43 (d, 1H, H-1′), 4.44–4.50 (m, 4H, 2 CH_2Ph), 7.20–7.35 (m, 10H, Ph).

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1,3-Di-*O*-benzyl-2-*O*-(6-*O*-acyl-β-D-glucopyranosyl)-*sn*-glycerols (6). 1,3-Di-*O*-benzyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**5**) (0.50 g, 1.15 mmol) was dissolved in tetrahydrofuran (6 mL); the appropriate trifluoroethyl ester (3.45 mmol) and LPS (2.50 g) were added in the order and the suspension was stirred at 45°C. The reaction was monitored by TLC and stopped after 24 h by filtering off the enzyme which was washed with tetrahydrofuran. The solvent was removed under reduced pressure and the crude residue was submitted to silica gel flash chromatography (ethyl acetate:petroleum ether, 80:20, v/v). In this way 6′-*O*-acylderivatives **6** were obtained. For the ^{1}H NMR signals see Table 2.

1,3-Di-*O*-benzyl-2-*O*-(6-*O*-butanoyl-β-D-glucopyranosyl)-*sn*-glycerol (6a). (83% yield): oil, $[\alpha]_D$ −14.3 (chloroform).

1,3-Di-*O*-benzyl-2-*O*-(6-*O*-hexanoyl-β-D-glucopyranosyl)-*sn*-glycerol (6b). (80% yield): oil, $[\alpha]_D$ −14.8 (chloroform).

1,3-Di-*O*-benzyl-2-*O*-(6-*O*-octanoyl-β-D-glucopyranosyl)-*sn*-glycerol (6c). (81% yield): oil, $[\alpha]_D$ −14.4 (chloroform).

1,3-Di-*O*-benzyl-2-*O*-(6-*O*-decanoyl-β-D-glucopyranosyl)-*sn*-glycerol (6d). (80% yield): oil, $[\alpha]_D$ −13.8 (chloroform).

2-*O*-(6-*O*-Acyl-β-D-glucopyranosyl)-*sn*-glycerols (3). Compound **6** (0.40 mmol) was dissolved in methanol (18 mL) and 10% palladium on activated carbon (0.12 g) was added. This mixture was shaken under hydrogen

Table 2. Significant ^{1}H NMR signals of compounds **3**[a], **4**[a], **6** and **9**

	Chemical shifts, δ[b]								
	H-1′	H-6′a	H-6′b	H-2	H-1a	H-1b		H-3a	H-3b
3a	5.11	4.70	4.96	4.39	4.11		—	4.26	
3b	5.12	4.71	4.98	4.38	4.15		—	4.24	
3c	5.12	4.73	4.98	4.38	4.15		—	4.25	
3d	5.13	4.73	4.99	4.38	4.14		—	4.25	
4a	5.13	4.36	4.50	4.46	4.07–4.15			4.60	4.66
4b[c]	5.15	4.36	4.51	4.48	4.08–4.16			4.62	4.69
4c[d]	5.15	4.36	4.52	4.50	4.09–4.17			4.63	4.70
4d	5.15	4.37	4.51	4.50	4.09–4.17			4.64	4.70
6a	4.44	4.25	4.42	4.05	3.50		—	3.64	
6b	4.44	4.24	4.42	4.05	3.50		—	3.66	
6c	4.44	4.24	4.42	4.05	3.50		—	3.65	
6d	4.45	4.24	4.43	4.05	3.49		—	3.65	
9a[e]	4.78	4.29	4.35	3.90	3.62	3.68		4.09	4.27
9b[e]	4.78	4.29	4.35	3.90	3.62	3.67		4.08	4.28
9c[e]	4.78	4.29	4.34	3.90	3.61	3.67		4.08	4.27
9d[e]	4.78	4.29	4.34	3.89	3.61	3.67		4.08	4.27

[a] Pyridine-d_5 solutions.
[b] When measurable, the coupling constants in Hz results: $J_{1',2'} = 8.0$, $J_{6'a,6'b} = 12.0$; **3**: $J_{6'b,5'} = 0.5$, $J_{6'a,5'} = 5.5$; **4**: $J_{3a,3b} = 12.0$, $J_{6'b,5'} = 2.0$, $J_{6'a,5'} = 5.0$, $J_{3b,2} = 5.5$, $J_{3a,2} = 5.5$; **6**: $J_{6'b,5'} = 4.0$, $J_{6'a,5'} = 2.0$; **9**: $J_{1a,1b} = 12.0$, $J_{3a,3b} = 12.0$, $J_{6'b,5'} = 5.0$, $J_{6'a,5'} = 2.0$, $J_{1b,2} = 3.5$, $J_{1a,2} = 6.0$, $J_{3b,2} = 5.0$, $J_{3a,2} = 6.0$.
[c] Significant ^{1}H NMR signals for the 1-*O*-isomer **2b**: 4.66 (dd, 1H, $J_{1a,1b} = 12.0$ Hz, $J_{1a,2} = 4.5$ Hz, H-1a), 4.69 (dd, 1H, $J_{1b,2} = 5.5$ Hz, H-1b), 5.10 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1′).
[d] Significant ^{1}H NMR signals for the 1-*O*-isomer **2c**: 4.67 (dd, 1H, $J_{1a,1b} = 12.0$ Hz, $J_{1a,2} = 4.5$ Hz, H-1a), 4.70 (dd, 1H, $J_{1b,2} = 5.5$ Hz, H-1b), 5.10 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1′).
[e] Significant ^{1}H NMR signals for the 1-*O*-isomers **9**; **a**: 4.71 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), **b**: 4.71 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), **c**: 4.71 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), **d**: 4.71 (d, 1H $J_{1',2'} = 8.0$ Hz, H-1′).

atmosphere for 1 h and then filtered through a Celite bed affording quantitatively the debenzylated compounds **3**. For the ^{1}H NMR signals see Table 2.

2-*O*-(6-*O*-Butanoyl-β-D-glucopyranosyl)-*sn*-glycerol (3a). (98% yield), mp 76–78°C (ethanol), $[\alpha]_D$ −4.0, MS *m*/*z* 342 $[M + NH_4]^+$.

2-*O*-(6-*O*-Hexanoyl-β-D-glucopyranosyl)-*sn*-glycerol (3b). (97% yield), mp 103–105°C (ethanol), $[\alpha]_D$ −8.0, MS *m*/*z* 370 $[M + NH_4]^+$.

2-*O*-(6-*O*-Octanoyl-β-D-glucopyranosyl)-*sn*-glycerol (3c). (98% yield), mp 109–111°C (ethanol), $[\alpha]_D$ −6.0, MS *m*/*z* 398 $[M + NH_4]^+$.

2-*O*-(6-*O*-Decanoyl-β-D-glucopyranosyl)-*sn*-glycerol (3d). (97% yield), mp 113–114°C (ethanol), $[\alpha]_D$ −4.0, MS *m*/*z* 426 $[M + NH_4]^+$.

General procedure for the chemoenzymatic synthesis of monoesters 4

1,3-Di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-chloroacetyl-β-D-glucopyranosyl)-*sn*-glycerol (7). Chloroacetic anhydride (3.92 g, 22.92 mmol) was added at 0°C to a solution of **5** (2.00 g, 4.61 mmol) in dichloromethane (40 mL) and pyridine (5.4 mL). After 15 min the reaction mixture was diluted with dichloromethane (160 mL) and washed with 1 M HCl (80 mL), water (80 mL), 10% $NaHCO_3$ (80 mL), and two volumes of water (80 mL). Organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. Flash chromatography (ethyl acetate:petroleum ether, 30:70, v/v) of the crude product yielded pure 1,3-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-chloroacetyl-β-D-glucopyranosyl)-*sn*-glycerol (**7**), (90% yield): oil; $[\alpha]_D$ +0.6 (chloroform); ^{1}H NMR: δ 3.46–3.65 (m, 4H, H_2-1 and H_2-3), 3.70 (ddd, 1H, $J_{5',4'} = 10.0$, $J_{5',6'a} = 3.0$, $J_{5',6'b} = 4.0$ Hz, H-5′), 3.82, 3.96 and 4.04 (3s, 6H, 3 CH_2Cl), 3.97–4.00 (m, 2H, CH_2Cl), 4.02 (m, 1H, H-2), 4.23 (dd, 1H, $J_{6'a,6'b} = 12.0$ Hz, H-6′a), 4.27 (dd, 1H, H-6′b), 4.46–4.52 (m, 4H, 2 CH_2Ph), 4.84 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), 5.03 (dd, 1H, $J_{2',3'} = 10.0$ Hz, H-2′), 5.13 (dd, 1H, $J_{4',3'} = 10.0$ Hz, H-4′), 5.29 (dd, 1H, H-3′), 7.20–7.35 (m, 10H, Ph); MS *m*/*z* 758 $[M + NH_4]^+$.

2-*O*-(2,3,4,6-Tetra-*O*-chloroacetyl-β-D-glucopyranosyl)-*sn*-glycerol (8). Compound **7** (3.00 g, 4.05 mmol) was submitted to hydrogenolysis as described above affording 2-*O*-(2,3,4,6-tetra-*O*-chloroacetyl-β-D-glucopyranosyl)-*sn*-glycerol (**8**), (88% yield): oil; $[\alpha]_D$ +2.4 (chloroform); ^{1}H NMR: δ 3.58–3.68 (m, 4H, H_2-1 and H_2-3), 3.78 (m, 1H, H-2), 3.88 (ddd, 1H, $J_{5',4'} = 10.0$, $J_{5',6'a} = 6.0$, $J_{5',6'b} = 3.0$ Hz, H-5′), 3.97 and 4.12 (2s, 4H, 2 CH_2Cl), 3.99–4.05 (m, 4H, 2 CH_2Cl), 4.30 (dd, 1H, $J_{6'a,6'b} = 12.0$ Hz, H-6′a), 4.37 (dd, 1H, $J_{6'b}$), 4.77 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), 5.10 (dd, 1H, $J_{2',3'} = 10.0$ Hz, H-2′), 5.13 (dd, 1H, $J_{4',3'} = 10.0$ Hz, H-4′), 5.36 (dd, 1H, H-3′).

3-*O*-Acyl-2-*O*-(2,3,4,6-tetra-*O*-chloroacetyl-β-D-glucopyranosyl)-*sn*-glycerols (9). 2-*O*-(2,3,4,6-Tetra-*O*-chloroacetyl-β-D-glucopyranosyl)-*sn*-glycerol (**8**) (0.50 g, 0.89 mmol) was dissolved in 10 mL of tetrahydrofuran; the appropriate trifluoroethyl ester (2.67 mmol) and LCA (1.50 g) were added in the order and the suspension was stirred at 45°C for 8 h. The reaction was treated as

reported for the LPS transesterification procedure and after flash chromatography (ethyl acetate:petroleum ether from 50:50 to 70:30, v/v) 3-*O*-acyl-2-*O*-(2,3,4,6-tetra-*O*-chloroacetyl-β-D-glucopyranosyl)-*sn*-glycerols **9**, contaminated by about 10% of the corresponding 1-*O*-diastereoisomers detected by ^{1}H NMR, were obtained in 60–65% yield. For the ^{1}H NMR signals see Table 2.

3-*O*-Acyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerols. (4). To a solution of **9** (0.50 mmol) in a 1:1 ethyl acetate: methanol mixture (9.5 mL) hydrazine acetate (0.69 g, 7.50 mmol) was added. After 6 h at room temperature the solvent was removed under reduced pressure and the obtained residue submitted to flash chromatography (dichloromethane:methanol, 80:20, v/v). Final crystallization allowed to remove the 1-isomer yielding pure **4**. For the ^{1}H NMR signals see Table 2.

3-*O*-Butanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (4a). (60% yield), mp 138–140°C (ethanol-diisopropylether), $[\alpha]_D$ −8.6, MS *m/z* 342 $[M+NH_4]^+$.

3-*O*-Hexanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (4b). (67% yield), mp 135–137°C (ethanol), $[\alpha]_D$ −8.4, MS *m/z* 370 $[M+NH_4]^+$.

3-*O*-Octanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (4c). (65% yield), mp 136–137°C (ethanol), $[\alpha]_D$ −7.1; MS *m/z* 398 $[M+NH_4]^+$.

3-*O*-Decanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (4d). (68% yield), mp 140–141°C (ethanol), $[\alpha]_D$ −7.0; MS *m/z* 426 $[M+NH_4]^+$.

Assignment of configuration of 4a–d

Standard samples. Standard 3-*O*-acyl-1,2-*O*-isopropylidene-*sn*-glycerols were prepared from commercial 1,2-*O*-isopropylidene-*sn*-glycerol according to ref 7.

Analytical samples. 0.014 mmol of **4** were dissolved in water (0.5 mL) and 5 mg of β-glucosidase from almonds were added. After 1.5 h at 30°C the mixture was extracted and processed according to ref 7.

Chromatographic conditions. Standard and analytical samples were analyzed on a chiral GLC-capillary column (dimethylpentyl-β-cyclodextrin, 25 m, ID 0.25 mm, from MEGA-Italy) in the conditions described in ref 7.

Short term in vitro bioassay for antitumor promoters

The inhibitions were assayed using a short term in vitro assay for Epstein–Barr virus activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as a primary screening test for antitumor promoters.[2] For each compound assays were performed in triplicate. No sample exhibited significant toxicity against Raji cells. The results are reported in Table 1.

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