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Short Regioselective Chemoenzymatic Synthesis and Biological Evaluation of 1-*O*-Acyl-2-*O*-(β-D-sulfoquinovopyranosyl)-*sn*-glycerols

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A convenient chemoenzymatic synthesis of a new class of non-natural sulfo-glycolipids – 2-*O*-(β-D-sulfoquinovosyl)-monoacylglycerols (2-*O*-β-D-SQMG) – derived from 2-*O*-(β-D-glucopyranosyl)glycerol and carrying acyl chains of various lengths at the 1-position of the *sn*-glycerol moiety, was performed with the aid of a key step involving regioselective lipase-catalyzed acylation of 2-*O*-(6-deoxy-6-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (**4**) at its 1-position, reported here for the first time. Elaboration of the sugar moiety through thioacetate substitution of the selectively inserted tosyl group with subsequent Oxone® oxidation in the presence of unprotected primary and secondary hydroxy groups efficiently afforded the target compounds, the hexanoyl, dodecanoyl, and octadecanoyl derivatives **1a–c**, which were active when tested in the EBV-EA in vitro assay for antitumor promoters.

Introduction

Sulfoquinovosylacylglycerols are a family of sulfoglycolipids in which 6-deoxy-6-sulfoglucose (sulfoquinovose) is α-linked to the *sn*-3 position of an acylglycerol.[1,2] Among them, sulfoquinovosyldiacylglycerols (SQDGs, Figure 1), generally extracted from green plants or photosynthetic microorganisms together with glycoglycerolipids (lacking the characteristic SQDG sulfonate), are characterized by the presence of pairs of fatty acid acyl chains. Conversely, the presence of only single chains is distinctive of synthetic sulfoquinovosylmonoacylglycerols (SQMGs). Recently, both SQDGs and SQMGs have come to be considered promising compounds for cancer therapy and prevention, due to their noteworthy biological activities.[3–6] With regard to cancer prevention, 2-*O*-(β-D-glycopyranosyl)-*sn*-glycerol-related lipids (synthetic analogues of natural glycoglycerolipids) have recently shown high in vitro and in vivo antitumor-promoting activities[7] and, in particular, the in vitro activities of fatty acid monoesters of 2-*O*-(β-D-glucopyranosyl)-*sn*-glycerol[8] [namely 1-*O*-acyl-2-*O*-(β-D-glucopyranosyl)-*sn*-glycerols] proved to be strictly related to the length of the acyl chain.[9]

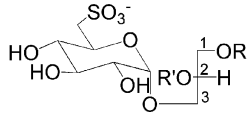

Figure 1. Structures of SQDGs (R and R′ = acyl chain) and SQMGs (R = acyl chain, R′ = H).

In view of these results we set out to synthesize a new class of SQMGs derived from 2-*O*-β-D-glucopyranosyl-*sn*-glycerol in which acyl chains of different length would be linked to one of the two free hydroxy groups in the glycerol part and to test their in vitro antitumor-promoting activities. Here we report a rapid chemoenzymatic synthesis of the sulfoquinovosides **1a–c** (Scheme 1, below) – 1-*O*-octadecanoyl, -dodecanoyl, and -hexanoyl-2-*O*-(β-D-sulfoquinovopyranosyl)-*sn*-glycerols – in which the key steps were selective 6′-*O*-tosylation of the glucose moiety of a suitable starting compound, selective lipase-catalyzed 1-*O*-monoacylation of the glycerol moiety, and a final oxidation reaction without any protection of the sugar unit. The in vitro antitumor activities of the obtained compounds **1a–c** are also reported.

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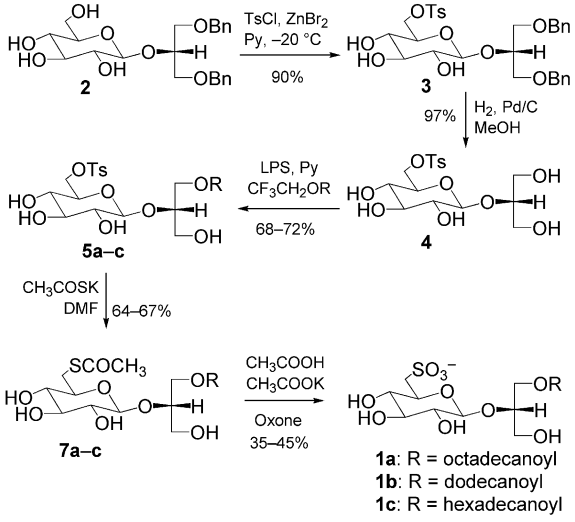
Results and Discussion

Synthesis

To perform the synthesis of the target monoesters **1a**–**c** we decided to exploit the convenient known regioselectivity of lipase from *Pseudomonas cepacia* (LPS) towards 2-*O*-(β-D-glucopyranosyl)-*sn*-glycerol.[8] In particular, in transesterification reactions carried out in pyridine with 2,2,2-trifluoroethyl alkanoates as acyl carriers, LPS had selectively acylated this polyhydroxylated compound at the 1-*O* position in its *sn*-glycerol moiety.[8] The selectivity was especially high (94%) with short-chain acyl carriers (2,2,2-trifluoroethyl butanoate) and decreased (80%) with the lengthening of the chain (2,2,2-trifluoroethyl octadecanoate), becoming accompanied by increased acylation (15%) of the glucose primary hydroxy group.[8] In view of these previous results, our strategy was to use a tosyl group to protect the glucose 6′-OH of 2-*O*-(β-D-glucopyranosyl)-*sn*-glycerol before the enzymatic regioselective acylation of the 1-OH group in the *sn*-glycerol moiety and, in the meantime, also to use it as a leaving group for insertion of the desired sulfonate group into the molecule by means of thioacetate substitution and final oxidation (Scheme 1).[10]



Scheme 1. Synthesis of SQMG analogues **1a**–**c**.

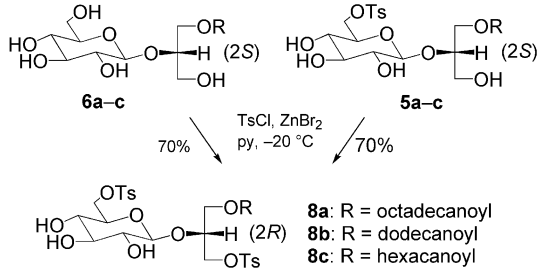
The starting 1,3-di-*O*-benzyl-2-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (**2**) was prepared from glucose pentaacetate and 1,3-dibenzylglycerol as previously reported.[11] $ZnBr_2$-catalyzed selective tosylation[12] of the primary 6′-hydroxy group of **2** in pyridine at –20 °C then yielded 1,3-di-*O*-benzyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (**3**) in high yields. Pd/C-catalyzed benzyl hydrogenolysis removed the benzyl groups, quantitatively affording 2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (**4**), which was the substrate for the LPS-catalyzed transesterifications.[8]

The enzymatic reactions were run in pyridine with the appropriate 2,2,2-trifluoroethyl ester as acyl carrier until almost complete disappearance of the starting material. In this way, the pure 1-*O*-octadecanoyl, -dodecanoyl, and -hexanoyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerols **5a**–**c** were obtained in yields of about 70% (see Exp. Section). The 2*S* configurations were assigned to **5a**–**c** on the basis of chemical correlation with the known 1-*O*-octadecanoyl, -dodecanoyl, and -hexanoyl-2-*O*-(β-D-glucopyranosyl)-*sn*-glycerols **6a**–**c**, as discussed in the next section.[8,11]

Compounds **5a**–**c** were then converted into the corresponding 1-*O*-acyl-2-*O*-(6-deoxy-6-thioacetyl-β-D-glucopyranosyl)-*sn*-glycerols **7a**–**c** by potassium thioacetate treatment in DMF in 64–67% yields. The final oxidation step was carried out with Oxone® (potassium peroxymonosulfate) in acetic acid[10] directly on the unprotected compounds **7a**–**c**. Although 4′-*O*-acetylated sulfoquinovosides were formed in a side reaction (diagnostic ^{1}H NMR signals for the dodecanoyl derivative in $CDCl_3/CD_3OD/D_2O$: H-4′, dd, at δ = 4.66 ppm and CH_3CO, s, at δ = 2.08 ppm), this procedure allowed us to obtain the target 1-*O*-acyl-2-*O*-(β-D-sulfoquinovopyranosyl)-*sn*-glycerols **1a**–**c** directly in 35–45% yields, avoiding the need for protecting group manipulation.

Configuration Assignment

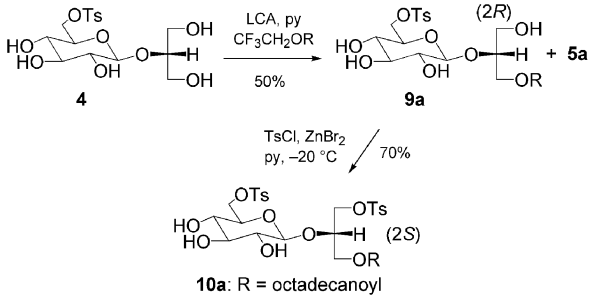
The 6′-tosyl derivatives **5a**–**c** obtained from enzymatic transesterification were transformed into the corresponding ditosyl derivatives **8a**–**c** (Scheme 2) by selective $ZnBr_2$-catalyzed tosylation under the conditions reported above (see Exp. Section). When the known 1-*O*-octadecanoyl, -dodecanoyl, and -hexanoyl-2-*O*-(β-D-glucopyranosyl)-*sn*-glycerols **6a**–**c** (2*S* configurations)[8,11] were subjected to selective 3,6′-ditosylation, 1-*O*-octadecanoyl, -dodecanoyl, and -hexanoyl-3-*O*-tosyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (**8a**–**c**) were also obtained (Scheme 2). The complete superimposability of the ^{1}H and ^{13}C NMR spectra of the two batches in [D_5]pyridine (see Exp. Section) allowed us to assign 2*R* configurations to **8a**–**c** and, consequently, 2*S* configurations to **5a**–**c**.



Scheme 2. Configuration assignment of compounds **8a**–**c**.

To validate this assignment we considered performing an enzymatic transesterification (Scheme 3) on the 6′-tosylate **4** with the aid of lipase from *Candida antarctica* (LCA) as the catalyst, because it is reported that this enzyme shows an opposite, although lower, regioselectivity to that of LPS in the acylation of the glycerol moiety of 2-*O*-(β-D-glucopyranosyl)-*sn*-glycerol, affording both the 2*S* and the 2*R* stereoisomers (in a 25:75 ratio in the presence of trifluoromethyl decanoate as acyl carrier).[8] Here we decided to pre-

pare only the C-18 derivatives (Scheme 3), by treatment of **4** with trifluoroethyl octadecanoate in pyridine in the presence of LCA. A mixture of the two monotosyl octadecanoates **5a** and **9a** in a 47:53 ratio (by ^{1}H NMR) was obtained, with **5a** proving to be the less polar compound by TLC analysis and **9a** (easily purified by column chromatography) being established to be its 2-diastereomer, because its acyl chain was unambiguously shown by ^{1}H NMR analysis to be linked to one of the two primary positions in the *sn*-glycerol component (see Exp. Section). Finally, 3-*O*-octadecanoyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (**9a**) was converted into the 1,6′-ditosyl derivative 3-*O*-octadecanoyl-1-*O*-tosyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (**10a**, 2*S* configuration). This showed different ^{1}H and ^{13}C NMR spectra from those of the 1-*O*-octadecanoyl derivative **8a** (2*R* configuration; see Exp. Section), further confirming the previously performed configuration assignment.



Scheme 3. LCA-catalyzed synthesis of **10a** (diastereomer of **8a**).

EBV-EA Assay for Antitumor Promoters

Epstein–Barr Virus (EBV) is known to be activated by tumor promoters to produce viral early antigens (EAs), and an evaluation of its inhibition is often used as a primary screening for in vitro antitumor-promoting activities.[13] The inhibitory effects of the 2-*O*-β-D-SQMGs **1a**–**c** were assayed in a short-term in vitro assay for EBV-EA activation induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells, as described in ref.[6,14] (see Exp. Section). Table 1 shows the in vitro tumor-promoting inhibitory activities of compounds **1a**–**c**; the in vitro inhibitory effects of the already studied 1-*O*-acyl-2-*O*-(β-D-glucopyranosyl)-*sn*-glycerols **6a**–**c**[9] are also reported for comparison.

Only weak cytotoxicities against Raji cells were observed for compounds **1a**–**c** (50% viability at 1000 mol ratio/TPA, Table 1) and, in general, all compounds were active as indicated by their percentages to control values (53.6–61.3 at 500 mol ratio/TPA, Table 1). As already reported for compounds **6a**–**c**,[9] shortening of the acyl chain, from C18 (**1a**) to C6 (**1c**), resulted in increasing inhibitory activity (IC_{50} 543 for **1a** vs. 417 for **1c**; see Table 1). In general, the sulfonate group seems to have little influence on the antitumor-promoting activities of glycoglycerolipids bearing medium or long chains (IC_{50} 543 and 463 for **1a** and **1b** vs. IC_{50} 550 and 504 for **6a** and **6b**; Table 1) in this in vitro experimental model. However, when a short acyl chain is present, the sulfonate exerts a strong negative effect on the activity (IC_{50} 417 for **1c** vs. 29.3 for **6c**; Table 1).

Conclusions

A new class of sulfoquinovosylmonoacylglycerols based on 2-*O*-(β-D-glucopyranosyl)-*sn*-glycerol – 1-*O*-acyl-2-*O*-(β-D-sulfoquinovopyranosyl)-*sn*-glycerols **1a**–**c** (2-*O*-β-D-SQMG) – were prepared by means of a short chemoenzymatic procedure using the tosyl group as a convenient temporary protecting group and LPS as an efficient and selective biocatalyst for polyhydroxylated substrates. It is noteworthy that, due to the practicability of chromatographic separation of the two 2-diastereomers **5a** and **9a**, the same procedure could in principle also be exploited to synthesize 3-*O*-acyl-2-*O*-(β-D-sulfoquinovopyranosyl)-*sn*-glycerols simply by changing the employed biocatalyst, as in the case of LCA vs. LPS. A preliminary bioassay was carried on the obtained compounds, showing their antitumor-promoting activities, and some new structure–activity relationship conclusions on the influence of the sulfonate group could also be drawn. In particular, a highly negative effect of this group was observed for the short-chain compound **1c** in relation to the corresponding non-sulfonated **6c**. In contrast, when a long acyl chain is present on the glycerol moiety (see compound **1a** vs. **6a**), the simultaneous presence of a sulfonate moiety does not significantly induce variation

Table 1. Inhibitory effects of **1a**–**c** and **6a**–**c** on TPA-induced EBV-EA activation.

	Concentration (mol ratio/TPA)				
	1000	500	100	10	
	% to control ± SE (n = 3)[a]				IC_{50}
1a	22.5 ± 1.6 (50)[b]	61.3 ± 2.2 (70)	83.5 ± 1.7 (80)	100 ± 0.4 (80)	543
1b	17.0 ± 1.5 (50)	57.2 ± 2.1 (70)	81.5 ± 1.9 (80)	100 ± 0.4 (80)	463
1c	14.3 ± 1.2 (50)	53.6 ± 2.0 (70)	80.8 ± 1.9 (80)	100 ± 0.5 (80)	417
6a[c]	19.6 ± 0.7 (60)	57.4 ± 2.2 (70)	90.6 ± 0.5 (80)	100 ± 0.0 (80)	550
6b[c]	0.0 ± 0.4 (60)	52.5 ± 2.3 (70)	82.5 ± 1.6 (80)	100 ± 0.2 (80)	504
6c[c]	0.0 ± 0.0 (70)	10.6 ± 0.6 (70)	30.9 ± 1.8 (80)	68.2 ± 2.5 (80)	29.3

[a] Values are EBV-EA activation (%) in the presence of the test compound relative to the control (100%). Activation was achieved by treatment with TPA (32 pmol). IC_{50} represents the mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol TPA. [b] Values in parentheses are viability percentages of Raji cells. [c] See ref.[9]

in activity, showing that the effects of the "negative" groups (sulfonate and long chain) are not additive. This conclusion is strengthened by the recent observation that 1,3-di-*O*-octadecanoyl-2-*O*-(β-D-sulfoquinovopyranosyl)-*sn*-glycerol, which contains three different "negative" factors (i.e., sulfonate, long chain, and double acylation of glycerol) has an activity similar to those of compounds **1a** and **6a** (IC_{50} 543 and 550, Table 1, vs. 632, ref.[15]). Work to further characterize the biological potential of these new synthetically available sulfoquinovosyl-monoacylglycerols is in progress.

Experimental Section

General: *Pseudomonas cepacia* lipase (LPS, lipase PS, specific activity 30.5 triacetin units/mg solid), from Amano Pharmaceutical Co (Mitsubishi Italia), was supported on celite;[8] *Candida antarctica* lipase SP 435 L, immobilized on a macroporous acrylic resin (Novozym® 435, LCA, specific activity 9.5 PL units/mg solid) was purchased from Novo Nordisk. LPS and LCA were kept under vacuum overnight prior to use. Pyridine was distilled from calcium hydride prior to use. The acyl carriers – 2,2,2-trifluoroethyl esters – were synthesized as described.[8] 1,3-Di-*O*-benzyl-2-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (**2**) was synthesized by a literature procedure.[11] Optical rotations were determined with a Perkin–Elmer 241 polarimeter in 1% $CHCl_3$, CH_3OH or $CHCl_3/CH_3OH/H_2O$ (65:25:4) solutions at 20 °C, in a 1 dm cell. Melting points (m.p.) were recorded on a Büchi 510 capillary melting point apparatus and are uncorrected. All reagents and solvents used were reagent grade and were purified before use by standard methods. Dry solvents and liquid reagents were distilled prior to use or were dried with molecular sieves (4 Å). Column chromatography was carried out on flash silica gel (Merck 230–400 mesh). TLC analysis was carried out on silica gel plates (Merck $60F_{254}$) with development with 50% sulfuric acid or anisaldehyde-based reagent. Evaporation under reduced pressure was always performed with a bath temperature below 40 °C. The structures of all the new synthesized compounds were confirmed through full ^1H and ^{13}C NMR characterization and mass spectroscopy. ^1H NMR analysis were performed at 500 MHz with a Bruker FT-NMR AVANCE™ DRX 500 spectrometer with a 5 mm z-PFG (pulsed field gradient) broadband reverse probe at 298 K unless otherwise stated, and ^{13}C NMR spectra of all the new compounds were measured at 125.76 MHz. The signals were unambiguously assigned by 2D COSY and HSQC experiments (standard Bruker pulse program). Chemical shifts are reported as δ (ppm) relative to residual $CHCl_3$, pyridine, or CH_3OH (when $CDCl_3/CD_3OD/D_2O$ solvent mixture was used) fixed at 7.24, 7.19 (higher field signal), and 3.30 ppm, respectively, for ^1H NMR spectra and relative to $CDCl_3$ fixed at δ = 77.0 ppm (central line), [D_5]pyridine 123.0 (higher field signal, central line), or CD_3OD at δ = 49.00 ppm (central line) for ^{13}C NMR spectra; scalar coupling constants are reported in Hz. Mass spectra were recorded in negative- or positive-ion electrospray (ESI) mode with a Thermo Quest Finnigan LCQ™ DECA ion-trap mass spectrometer equipped with a Finningan ESI interface; sample solutions were injected with a ionization spray voltage of 4.5 or 5.0 kV (positive- and negative-ion mode, respectively), a capillary voltage of 32 V or –15 V (positive- and negative-ion mode, respectively), and capillary temperature of 250 °C. Data were processed with the aid of the Finnigan Xcalibur software system.

Synthesis of 1,3-Di-*O*-benzyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (3): Zinc bromide (6.21 g, 27.6 mmol) was added at –20 °C to a solution of compound **2** (3.00 g, 6.90 mmol) in dry pyridine (240 mL). After the addition of tosyl chloride (6.58 g, 34.5 mmol) the reaction mixture was stirred at –20 °C for 0.5 h and monitored by TLC ($EtOAc/CH_3OH$, 95:5 v/v). After quenching with CH_3OH, the solvent was evaporated under vacuum, the residue was dissolved in EtOAc (450 mL), the organic solution was washed with HCl (1 M, 2 × 120 mL), H_2O (120 mL), saturated $NaHCO_3$ solution (120 mL), and H_2O (2 × 120 mL), and the aqueous phases were extracted again with EtOAc (2 × 360 mL). The collected organic layers were dried with Na_2SO_4, concentrated under vacuum, and purified by flash column chromatography with EtOAc to provide the tosyl derivative **3** (3.65 g, 6.20 mmol, 90% yield) as a gel-like compound. $[\alpha]_D^{20}$ = –6.3 ($CHCl_3$). ^1H NMR ($CDCl_3$): δ = 2.39 (s, 3 H, CH_3), 3.33 (dd, $J_{1',2'}$ = 7.8, $J_{2',3'}$ = 8.4 Hz, 1 H, 2′-H), 3.39–3.49 (m, 3 H, 3′-H, 4′-H, 5′-H), 3.50–3.64 (m, 4 H, 1a-H, 1b-H, 3a-H, 3b-H), 4.02 (m, 1 H, 2-H), 4.21 (dd, $J_{6'a,5'}$ = 4.6, $J_{6'a,6'b}$ = 10.9 Hz, 1 H, 6′a-H), 4.26 (dd, $J_{6'b,5'} < 1.0$ Hz, 1 H, 6′b-H), 4.40 (d, 1 H, 1′-H), 4.46–4.54 (m, 4 H, 2 × CH_2), 7.23–7.34 (m, 12 H, Ph), 7.76 (d, J = 8.2 Hz, 2 H, Ph) ppm. ^{13}C NMR ($CDCl_3$): δ = 21.64 ($PhCH_3$), 68.65 (C6′), 69.44 (C4′ or C5′), 70.25 and 70.61 (C1 or C3), 73.09 (C2′), 73.49 and 73.51 (2 × Ph*C*H_2), 73.70 (C4′ or C5′), 75.98 (C3′), 77.60 (C2), 103.04 (C1′), 127.5–128.6 (10 C, 2 × Ph), 127.95 and 129.85 (4 C, Ts), 132.83 (Ts), 137.60 and 137.74 (2 C, Ph), 144.91 (Ts) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): m/z (%) = 611.1 (100) $[M + Na]^+$. $C_{30}H_{36}O_{10}S$ (588.67): calcd. C 61.21, H 6.16, S 5.45; found C 61.30, H 6.18, S 5.44.

Synthesis of 2-*O*-(6-*O*-Tosyl-β-D-glucopyranosyl)-*sn*-glycerol (4): Compound **3** (3.00 g, 5.10 mmol) was dissolved in methanol (160 mL), and Pd on activated carbon (10%, 1.0 g) was added. The mixture was shaken under hydrogen for 2 h with monitoring by TLC ($EtOAc/CH_3OH$ 100:1 v/v) and was then filtered through a celite bed to afford the debenzylated **4** (2.02 g, 4.95 mmol, 97% yield); m.p. 168 °C (amorphous solid). $[\alpha]_D^{20}$ = +4.1 (CH_3OH). ^1H NMR (CD_3OD): δ = 2.45 (s, 3 H, CH_3), 3.18 (dd, $J_{1',2'}$ = 7.8, $J_{2',3'}$ = 9.2 Hz, 1 H, 2′-H), 3.21 (dd, $J_{3',4'}$ = 9.5, $J_{4',5'}$ = 9.5 Hz, 1 H, 4′-H), 3.32 (dd, 1 H, 3′-H), 3.45 (ddd, $J_{5',6'a}$ = 6.0, $J_{5',6'b}$ = 1.7 Hz, 1 H, 5′-H), 3.55–3.72 (m, 5 H, 1a-H, 1b-H, 2-H, 3a-H, and 3b-H), 4.12 (dd, $J_{6'a,6'b}$ = 10.7 Hz, 1 H, 6′a-H), 4.31 (dd, 1 H, 6′b-H), 4.38 (d, 1 H, 1′-H), 7.45 (d, J = 8.2 Hz, 2 H, Ph), 7.80 (d, 2 H, Ph) ppm. ^{13}C NMR (CD_3OD): δ = 21.58 (Ph*C*H_3), 62.67 and 62.98 (C1 or C3), 70.67 (C6′), 71.05 (C4), 74.96 (C2′), 75.05 (C5′), 77.56 (C3′), 82.95 (C2), 104.37 (C1′), 129.08 and 131.11 (4 C, Ph), 134.26 and 146.57 (2 C, Ph) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): m/z (%) = 431.1 (100) $[M + Na]^+$. $C_{16}H_{24}O_{10}S$ (408.42): calcd. C 47.05, H 5.92, S 7.85; found C 47.19, H 5.94, S 7.88.

General Procedure for the Enzymatic Synthesis of Monoesters 5a–c: Tosyl derivative **4** (0.600 g, 1.47 mmol) was dissolved in pyridine (10 mL) and the appropriate trifluoroethyl ester (8.82 mmol) and LPS (3.6 g) were added in that order. The suspension was stirred at 45 °C overnight and monitored by TLC (CH_2Cl_2/CH_3OH, 90:10 v/v). The reaction was stopped by filtering off the enzyme, which was washed with pyridine. After removal of the solvent under vacuum, the resulting crude material was subjected to flash column chromatography (CH_2Cl_2/CH_3OH, 95:5 v/v) to yield the pure 1-*O*-monoesters **5a**–**c**.

1-*O*-Octadecanoyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (5a): Reaction time 21 h, yield 72%; m.p. 96 °C (amorphous solid). $[\alpha]_D^{20}$ = –11.3 ($CHCl_3$). ^1H NMR ([D_5]pyridine, 312 K): δ = 0.87 (t,

J = 7.0 Hz, 3 H, CH_3), 1.17–1.35 (m, 28 H, 14 × CH_2), 1.66 (m, 2 H, CH_2), 2.18 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 3.86 (dd, $J_{1',2'}$ = 7.8, $J_{2',3'}$ = 8.8 Hz, 1 H, 2′-H), 3.94 (dd, $J_{3',4'}$ = 8.8, $J_{4',5'}$ = 9.7 Hz, 1 H, 4′-H), 4.00 (ddd, $J_{5',6'a}$ = 6.0, $J_{5',6'b}$ = 1.6 Hz, 1 H, 5′-H), 4.10 (dd, 1 H, 3′-H), 4.11–4.18 (m, 2 H, 3a-H, 3b-H), 4.38 (m, 1 H, 2-H), 4.62–4.73 (m, 3 H, 1a-H, 1b-H, and 6′a-H), 4.91 (dd, $J_{6'a,6'b}$ = 10.4 Hz, 1 H, 6′b-H), 4.98 (d, 1 H, 1′-H), 7.24 (d, J = 8.1 Hz, 2 H, Ph), 8.00 (d, 2 H, Ph) ppm. ^{13}C NMR ([D$_5$]pyridine, 312 K): δ = 13.76 (CH_3), 20.79 (PhCH_3), 22.44 (CH_2), 24.77 (CH_2), 28.90–29.60 (12 × CH_2), 31.64 (CH_2), 33.92 (*C*H$_2$CO), 62.23 (C3), 63.87 (C1), 70.41 (C6′), 70.46 (C4′), 74.23 (C2′), 74.52 (C5′), 77.53 (C3′), 78.71 (C2), 104.20 (C1′), 127.84 and 129.82 (4 C, Ph), 133.42 and 145.55 (2 C, Ph), 173.14 (CO) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): *m*/*z* (%) = 697.3 (100) [M + Na]$^+$. $C_{34}H_{58}O_{11}S$ (674.88): calcd. C 60.51, H 8.66, S 4.75; found C 60.68, H 8.60, S 4.81.

1-*O*-Dodecanoyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (5b): Reaction time 21 h, yield 68%; gel-like compound. $[\alpha]_D^{20}$ = –13.0 ($CHCl_3$). ^{1}H NMR ([D$_5$]pyridine, 312 K): δ = 0.86 (t, J = 7.0 Hz, 3 H, CH_3), 1.17–1.30 (m, 16 H, 8 × CH_2), 1.65 (m, 2 H, CH_2), 2.18 (s, 3 H, CH_3), 2.37 (m, 2 H, CH_2), 3.86 (dd, $J_{1',2'}$ = 7.8, $J_{2',3'}$ = 8.8 Hz, 1 H, 2′-H), 3.94 (dd, $J_{3',4'}$ = 8.8, $J_{4',5'}$ = 9.7 Hz, 1 H, 4′-H), 4.00 (ddd, $J_{5',6'a}$ = 5.9, $J_{5',6'b}$ = 1.7 Hz, 1 H, 5′-H), 4.10 (dd, 1 H, 3′-H), 4.11–4.18 (m, 2 H, 3a-H, 3b-H), 4.38 (m, 1 H, 2-H), 4.65–4.72 (m, 3 H, 1a-H, 1b-H, 6′a-H), 4.90 (dd, $J_{6'a,6'b}$ = 10.4 Hz, 1 H, 6′b-H), 4.98 (d, 1 H, 1′-H), 7.24 (d, J = 8.3 Hz, 2 H, Ph), 8.00 (d, 2 H, Ph) ppm. ^{13}C NMR ([D$_5$]pyridine, 312 K): δ = 13.76 (CH_3), 20.79 (PhCH_3), 22.43 (CH_2), 24.76 (CH_2), 28.94 (CH_2), 29.09 (2CH_2), 29.26 (CH_2), 29.37 (2 × CH_2), 31.62 (CH_2), 33.92 (*C*H$_2$CO), 62.23 (C3), 63.87 (C1), 70.41 (C6′), 70.46 (C4′), 74.22 (C2′), 74.51 (C5′), 77.53 (C3′), 78.71 (C2), 104.19 (C1′), 127.83 and 129.82 (4 C, Ph), 133.41 and 145.55 (2 C, Ph), 173.14 (CO) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): *m*/*z* (%) = 613.2 [M + Na]$^+$ (100). $C_{28}H_{46}O_{11}S$ (590.72): calcd. C 56.93, H 7.85, S 5.43; found C 57.10, H 7.93, S 5.37.

1-*O*-Hexanoyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (5c): Reaction time 21 h, yield 70%; oil. $[\alpha]_D^{20}$ = –16.4 ($CHCl_3$). ^{1}H NMR ([D$_5$]pyridine, 312 K): δ = 0.78 (t, J = 7.0 Hz, 3 H, CH_3), 1.19 (m, 4 H, 2 × CH_2), 1.60 (m, 2 H, CH_2), 2.18 (s, 3 H, CH_3), 2.33 (m, 2 H, CH_2), 3.85 (dd, $J_{1',2'}$ = 7.7, $J_{2',3'}$ = 8.8 Hz, 1 H, 2′-H), 3.93 (dd, $J_{3',4'}$ = 8.8, $J_{4',5'}$ = 9.7 Hz, 1 H, 4′-H), 4.00 (ddd, $J_{5',6'a}$ = 5.9, $J_{5',6'b}$ = 1.7 Hz, 1 H, 5′-H), 4.09 (dd, 1 H, 3′-H), 4.10–4.17 (m, 2 H, 3a-H, 3b-H), 4.37 (m, 1 H, 2-H), 4.59–4.71 (m, 3 H, 1a-H, 1b-H, 6′a-H), 4.91 (dd, $J_{6'a,6'b}$ = 10.4 Hz, 1 H, 6′b-H), 4.96 (d, 1 H, 1′-H), 7.24 (d, J = 8.3 Hz, 2 H, Ph), 8.00 (d, 2 H, Ph) ppm. ^{13}C NMR ([D$_5$]pyridine, 312 K): δ = 13.49 (CH_3), 20.78 (PhCH_3), 22.02 (CH_2), 24.36 (CH_2), 30.98 (CH_2), 33.83 (*C*H$_2$CO), 62.22 (C3), 63.89 (C1), 70.42 (C6′), 70.46 (C4′), 74.22 (C2′), 74.51 (C5′), 77.53 (C3′), 78.69 (C2), 104.19 (C1′), 127.83 and 129.82 (4 C, Ph), 133.40 and 145.56 (2 C, Ph), 173.09 (CO) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): *m*/*z* (%) = 529.1 [M + Na]$^+$ (100). $C_{22}H_{34}O_{11}S$ (506.56): calcd. C 52.16, H 6.77, S 6.33; found C 52.29, H 6.82, S 6.27.

General Procedure for the Synthesis of Thioacetates 7a–c: A compound **5** (0.770 mmol) was dissolved in dry DMF (30 mL), and potassium thioacetate (0.390 g, 3.41 mmol) was added. The mixture was stirred under Ar at room temperature and the changing of its color from blue to brown indicated progress of the reaction that was monitored by TLC (CH_2Cl_2/CH_3OH, 97:3 v/v). Owing to co-elution of the starting and target compound, the reaction was stopped when the TLC spot color had turned completely brown from green (anisaldehyde-based reagent). DMF was then co-evaporated with cyclohexane at reduced pressure at 45 °C and subsequent flash column chromatography (CH_2Cl_2/CH_3OH, 98:2–95:5 v/v) of the crude residue yielded the desired derivative **7**.

2-*O*-(6-Deoxy-6-thioacetyl-β-D-glucopyranosyl)-1-*O*-octadecanoyl-*sn*-glycerol (7a): Reaction time 17 h, yield 64%; m.p. 90 °C (brown amorphous solid). $[\alpha]_D^{20}$ = –20.8 ($CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.85 (t, J = 7.1 Hz, 3 H, CH_3), 1.17–1.31 (m, 28 H, 14 × CH_2), 1.58 (m, 2 H, CH_2), 2.30 (m, 2 H, CH_2), 2.36 (s, 3 H, –SCOCH_3), 3.20 (dd, $J_{6'a,5'}$ = 6.2, $J_{6'a,6'b}$ = 14.4 Hz, 1 H, 6′a-H), 3.29 (dd, $J_{3',4'}$ = 9.3, $J_{4',5'}$ = 9.3 Hz, 1 H, 4′-H), 3.33 (dd, $J_{6'b,5'}$ = 2.8 Hz, 1 H, 6′b-H), 3.37 (dd, $J_{1',2'}$ = 7.7, $J_{2',3'}$ = 8.2 Hz, 1 H, 2′-H), 3.46 (ddd, 1 H, 5′-H), 3.54 (dd, 1 H, 3′-H), 3.57–3.70 (m, 2 H, 3a-H, 3b-H), 3.88 (m, 1 H, 2-H), 4.13 (dd, $J_{1a,2}$ = 5.7, $J_{1a,1b}$ = 11.7 Hz, 1 H, 1a-H), 4.24 (dd, $J_{1b,2}$ = 5.0 Hz, 1 H, 1b-H), 4.36 (d, 1 H, 1′-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.15 (CH_3), 22.72 (CH_2), 24.90 (CH_2), 29.16–29.78 (12 × CH_2), 30.57 (SCO*C*H$_3$), 30.67 (C6′), 31.96 (CH_2), 34.22 (*C*H$_2$CO), 62.61 (C3), 63.03 (C1), 72.02 (C4′), 73.31 (C2′), 74.49 (C5′), 75.45 (C3′), 80.34 (C2), 103.01 (C1′), 174.11 (CO), 197.40 (SCO) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): *m*/*z* (%) = 601.3 [M + Na]$^+$ (100). $C_{29}H_{54}O_9S$ (578.8): calcd. C 60.18, H 9.40, S 5.54; found C 60.07, H 9.36, S 5.59.

2-*O*-(6-Deoxy-6-thioacetyl-β-D-glucopyranosyl)-1-*O*-dodecanoyl-*sn*-glycerol (7b): Reaction time 17 h, yield 65%; brown, gel-like compound. $[\alpha]_D^{20}$ = –30.8 ($CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.84 (t, J = 7.0 Hz, 3 H, CH_3), 1.16–1.30 (m, 16 H, 8 × CH_2), 1.56 (m, 2 H, CH_2), 2.28 (m, 2 H, CH_2), 2.33 (s, 3 H, –SCOCH_3), 3.07 (dd, $J_{6'a,5'}$ = 7.5, $J_{6'a,6'b}$ = 14.2 Hz, 1 H, 6′a-H), 3.29 (dd, $J_{3',4'}$ = 9.0, $J_{4',5'}$ = 9.0 Hz, 1 H, 4′-H), 3.35–3.45 (m, 3 H, 2′-H, H5′-H, 6′b-H), 3.51 (dd, $J_{2',3'}$ = 8.5 Hz, 1 H, 3′-H), 3.56–3.73 (m, 2 H, 3a-H, 3b-H), 3.89 (m, 1 H, 2-H), 4.12–4.21 (m, 2 H, 1a-H, 1b-H), 4.38 (d, $J_{1',2'}$ = 7.7 Hz, 1 H, 1′-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.05 (CH_3), 22.62 (CH_2), 24.82 (CH_2), 29.13 (CH_2), 29.28 (2 × CH_2), 29.46 (CH_2), 29.58 (2 × CH_2), 30.50 (SCO*C*H$_3$), 30.73 (C6′), 31.85 (CH_2), 34.12 (*C*H$_2$CO), 62.48 (C3), 62.77 (C1), 72.52 (C4′), 73.08 (C2′), 74.58 (C5′), 75.57 (C3′), 79.05 (C2), 102.48 (C1′), 173.94 (CO), 196.71 (SCO) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): *m*/*z* (%) = 517.1 (100) [M + Na]$^+$. $C_{23}H_{42}O_9S$ (494.64): calcd. C 55.85, H 8.56, S 6.48; found C 56.03, H 8.61, S 6.39.

2-*O*-(6-Deoxy-6-thioacetyl-β-D-glucopyranosyl)-1-*O*-hexanoyl-*sn*-glycerol (7c): Reaction time 17 h, yield 67%; pink, gel-like compound. $[\alpha]_D^{20}$ = –37.0 ($CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, J = 7.0 Hz, 3 H, CH_3), 1.21–1.33 (m, 4 H, 2 × CH_2), 1.57 (m, 2 H, CH_2), 2.29 (m, 2 H, CH_2), 2.34 (s, 3 H, –SCOCH_3), 3.08 (dd, $J_{6'a,5'}$ = 8.0, $J_{6'a,6'b}$ = 14.8 Hz, 1 H, 6′a-H), 3.30 (dd, $J_{3',4'}$ = 9.1, $J_{4',5'}$ = 9.1 Hz, 1 H, 4′-H), 3.34–3.44 (m, 3 H, 2′-H, H5′-H, 6′b-H), 3.51 (dd, $J_{2',3'}$ = 9.0 Hz, 1 H, 3′-H), 3.56–3.72 (m, 2 H, 3a-H, 3b-H), 3.90 (m, 1 H, 2-H), 4.15–4.20 (m, 2 H, 1a-H, 1b-H), 4.38 (d, $J_{1',2'}$ = 7.8 Hz, 1 H, 1′-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.83 (CH_3), 22.24 (CH_2), 24.51 (CH_2), 30.48 (SCO*C*H$_3$), 30.77 (C6′), 31.25 (CH_2), 34.13 (*C*H$_2$CO), 62.58 (C3), 63.01 (C1), 72.46 (C4′), 73.30 (C2′), 74.64 (C5′), 75.70 (C3′), 79.59 (C2), 102.75 (C1′), 173.96 (CO), 196.78 (SCO) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): *m*/*z* (%) = 433.1 (100) [M + Na]$^+$. $C_{17}H_{30}O_9S$ (410.48): C 49.74, H 7.37, S 7.81; found C 49.83, H 7.45, S 7.67.

General Procedure for the Synthesis of Sulfonates 1a–c: Potassium monopersulfate triple salt (Oxone®, 0.830 g, 1.35 mmol) and potassium acetate (1.30 g, 13.2 mmol) were added in that order to a solution of a compound **7** (0.450 mmol) in glacial acetic acid (15 mL). The suspension was stirred at room temperature and the reaction was monitored by TLC ($CHCl_3$/CH_3OH/H_2O, 65:25:4). After disappearing of the starting material, the solvent was evaporated un-

der vacuum. The obtained crude residue was purified directly by flash column chromatography (from $CHCl_3/CH_3OH$, 80:20 v/v to $CHCl_3/CH_3OH/H_2O$, 65:25:4 v/v) to yield the pure sulfonate **1**.

1-*O*-Octadecanoyl-2-*O*-(β-D-sulfoquinovopyranosyl)-*sn*-glycerol Potassium Salt (1a): Reaction time 7 h; yield 37%; m.p. 220 °C (amorphous solid). $[\alpha]_D^{20} = -6.5$ ($CHCl_3/CH_3OH/H_2O$, 65:25:4). ^1H NMR ($CDCl_3/CD_3OD/D_2O$, 65:25:4, 320 K): $\delta = 0.84$ (t, $J = 7.1$ Hz, 3 H, CH_3), 1.17–1.34 (m, 28 H, $14 \times CH_2$), 1.56 (m, 2 H, CH_2), 2.29 (m, 2 H, CH_2), 3.00 (dd, $J_{6'a,5'} = 8.6$, $J_{6'a,6'b} = 14.6$ Hz, 1 H, 6′a-H), 3.22 (dd, $J_{3',4'} = 9.2$, $J_{4',5'} = 9.2$ Hz, 1 H, 4′-H), 3.24 (dd, $J_{1',2'} = 7.9$, $J_{2',3'} = 9.2$ Hz, 1 H, 2′-H), 3.32 (dd, $J_{6'b,5'} = 2.4$ Hz, 1 H, 6′b-H), 3.40 (dd, 1 H, 3′-H), 3.62 (dd, $J_{2,3a} = 6.0$, $J_{3a,3b} = 12.0$ Hz, 1 H, 3a-H), 3.68 (dd, $J_{2,3b} = 3.9$ Hz, 1 H, 3b-H), 3.72 (ddd, 1 H, 5′-H), 3.94 (m, 1 H, 2-H), 4.15 (dd, $J_{2,1a} = 5.2$, $J_{1a,1b} = 11.8$ Hz, 1 H, 1a-H), 4.18 (dd, $J_{2,1b} = 5.4$ Hz, 1 H, 1b-H), 4.40 (d, 1 H, 1′-H) ppm. ^{13}C NMR ($CDCl_3/CD_3OD/D_2O$, 65:25:4, 320 K): $\delta = 14.19$ (CH_3), 22.96 (CH_2), 25.19 (CH_2), 29.41–30.24 ($12 \times CH_2$), 32.25 (CH_2), 34.46 (*C*H_2CO), 53.05 (C6′), 62.27 (C3), 63.84 (C1), 72.97 (C5′), 73.25 (C4′), 73.88 (C2′), 76.40 (C3′), 80.10 (C2), 103.45 (C1′), 175.08 (CO) ppm. ESI-MS (CH_3OH, negative-ion mode, relative intensity): *m*/*z* (%) = 583.3 $[M]^-$ (100). Calcd for $C_{27}H_{51}O_{11}S^-$, *m*/*z* 583.3 $[M]^-$. $C_{27}H_{51}KO_{11}S$ (622.85): calcd. C 52.07, H 8.25, S 5.15; found C 52.24, H 8.19, S 5.21.

1-*O*-Dodecanoyl-2-*O*-(β-D-sulfoquinvopyranosyl)-*sn*-glycerol Potassium Salt (1b): Reaction time 7 h; yield 35%; m.p. 180 °C (amorphous solid). $[\alpha]_D^{20} = -7.0$ ($CHCl_3/CH_3OH/H_2O$, 65:25:4). ^1H NMR ($CDCl_3/CD_3OD/D_2O$, 65:25:4, 320 K): $\delta = 0.84$ (t, $J = 7.1$ Hz, 3 H, CH_3), 1.18–1.31 (m, 16 H, $8 \times CH_2$), 1.56 (m, 2 H, CH_2), 2.29 (m, 2 H, CH_2), 3.00 (dd, $J_{6'a,5'} = 8.6$, $J_{6'a,6'b} = 14.6$ Hz, 1 H, 6′a-H), 3.21 (dd, $J_{3',4'} = 8.9$, $J_{4',5'} = 8.9$ Hz, 1 H, 4′-H), 3.23 (dd, $J_{1',2'} = 7.9$, $J_{2',3'} = 9.1$ Hz, 1 H, 2′-H), 3.32 (dd, $J_{6'b,5'} = 2.3$ Hz, 1 H, 6′b-H), 3.39 (dd, 1 H, 3′-H), 3.62 (dd, $J_{2,3a} = 6.0$, $J_{3a,3b} = 12.1$ Hz, 1 H, 3a-H), 3.68 (dd, $J_{2,3b} = 3.9$ Hz, 1 H, 3b-H), 3.72 (ddd, 1 H, 5′-H), 3.94 (m, 1 H, 2-H), 4.15 (dd, $J_{2,1a} = 5.4$, $J_{1a,1b} = 11.8$ Hz, 1 H, 1a-H), 4.18 (dd, $J_{2,1b} = 5.1$ Hz, 1 H, 1b-H), 4.41 (d, 1 H, 1′-H) ppm. ^{13}C NMR ($CDCl_3/CD_3OD/D_2O$ 65:25:4, 320 K): $\delta = 14.19$ (CH_3), 22.96 (CH_2), 25.20 (CH_2), 29.52 (CH_2), 29.63 ($2CH_2$), 29.83 (CH_2), 29.94 ($2 \times CH_2$), 32.24 (CH_2), 34.47 (*C*H_2CO), 53.11 (C6′), 62.28 (C3), 63.89 (C1), 72.99 (C5′), 73.32 (C4′), 73.92 (C2′), 76.45 (C3′), 80.02 (C2), 103.43 (C1′), 175.14 (CO) ppm. ESI-MS (CH_3OH, negative-ion mode, relative intensity): *m*/*z* (%) = 499.2 $[M]^-$ (100). Calcd for $C_{21}H_{39}O_{11}S^-$, *m*/*z* 499.2 [M]. $C_{21}H_{39}KO_{11}S$ (538.69): calcd. C 46.82, H 7.30, S 5.95; found C 46.98, H 7.41, S 5.84.

1-*O*-Hexanoyl-2-*O*-(β-D-sulfoquinvopyranosyl)-*sn*-glycerol Potassium Salt (1c): Reaction time 7 h; yield 45%; m.p. 120 °C (amorphous solid). $[\alpha]_D^{20} = -6.9$ ($CHCl_3/CH_3OH/H_2O$ 65:25:4). ^1H NMR ($CDCl_3/CD_3OD/D_2O$ 65:25:4, 320 K): $\delta = 0.85$ (t, $J = 7.0$ Hz, 3 H, CH_3), 1.21–1.33 (m, 4 H, $2 \times CH_2$), 1.57 (m, 2 H, CH_2), 2.30 (m, 2 H, CH_2), 3.00 (dd, $J_{6'a,5'} = 8.7$, $J_{6'a,6'b} = 14.6$ Hz, 1 H, 6′a-H), 3.22 (dd, $J_{3',4'} = 9.0$, $J_{4',5'} = 9.0$ Hz, 1 H, 4′-H), 3.23 (dd, $J_{1',2'} = 7.8$, $J_{2',3'} = 9.1$ Hz, 1 H, 2′-H), 3.32 (dd, $J_{6'b,5'} = 2.3$ Hz, 1 H, 6′b-H), 3.40 (dd, 1 H, 3′-H), 3.63 (dd, $J_{2,3a} = 6.0$, $J_{3a,3b} = 12.1$ Hz, 1 H, 3a-H), 3.68 (dd, $J_{2,3b} = 3.7$ Hz, 1 H, 3b-H), 3.72 (ddd, 1 H, 5′-H), 3.94 (m, 1 H, 2-H), 4.15 (dd, $J_{2,1a} = 5.4$, $J_{1a,1b} = 11.7$ Hz, 1 H, 1a-H), 4.19 (dd, $J_{2,1b} = 5.1$ Hz, 1 H, 1b-H), 4.41 (d, 1 H, 1′-H) ppm. ^{13}C NMR ($CDCl_3/CD_3OD/D_2O$ 65:25:4, 320 K): $\delta = 13.92$ (CH_3), 22.54 (CH_2), 24.81 (CH_2), 31.59 (CH_2), 34.41 (*C*H_2CO), 52.99 (C6′), 62.20 (C3), 63.87 (C1), 73.04 (C5′), 73.25 (C4′), 73.87 (C2′), 76.42 (C3′), 80.05 (C2), 103.45 (C1′), 175.14 (CO) ppm. ESI-MS (CH_3OH, negative-ion mode, relative intensity): *m*/*z* (%) = 415.1 $[M]^-$ (100). Calcd for $C_{15}H_{27}O_{11}S^-$, *m*/*z* 415.1 [M]. $C_{15}H_{27}KO_{11}S$ (454.53): calcd. C 39.64, H 5.99, S 7.05; found C 39.72, H 6.10, S 6.98.

Configuration Assignment of Tosylates 5a–c: The converging synthesis of the 2*R* ditosyl derivatives **8a**–**c** was achieved by starting from **5a**–**c** and **6a**–**c** as reported here.

General Procedure for the Synthesis of (2*R*)-Ditosylates 8a–c from 5a–c: Zinc bromide (0.135 g, 0.600 mmol) was added at –20 °C to a solution of a compound **5** (0.150 mmol) in dry pyridine (5.0 mL). After addition of tosyl chloride (0.143 g, 0.750 mmol) the reaction mixture was stirred at –20 °C for 1 h and monitored by TLC (CH_2Cl_2/CH_3OH, 95:5 v/v). After quenching with CH_3OH, the solvent was evaporated under vacuum by repeated addition of toluene and the obtained crude material was subjected to flash column chromatography (CH_2Cl_2/CH_3OH, 95:5 v/v) to yield the ditosyl derivative **8** (about 70%).

1-*O*-Octadecanoyl-3-*O*-tosyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (8a): M.p. 208 °C (from Et_2O). $[\alpha]_D^{20} = -2.2$ ($CHCl_3$). ^1H NMR ([D_5]pyridine, 312 K): $\delta = 0.86$ (t, $J = 7.1$ Hz, 3 H, CH_3), 1.20–1.32 (m, 28 H, $14 \times CH_2$), 1.61 (m, 2 H, CH_2), 2.23 (s, 3 H, CH_3), 2.24 (s, 3 H, CH_3), 2.30 (m, 2 H, CH_2), 3.71 (dd, $J_{1',2'} = 7.8$, $J_{2',3'} = 8.9$ Hz, 1 H, 2′-H), 3.85 (dd, $J_{3',4'} = 9.0$, $J_{4',5'} = 9.9$ Hz, 1 H, 4′-H), 3.95 (ddd, $J_{5',6'a} = 6.1$, $J_{5',6'b} = 1.7$ Hz, 1 H, 5′-H), 4.03 (dd, 1 H, 3′-H), 4.40–4.46 (m, 2 H, 2-H, 1a-H), 4.51–4.61 (m, 3 H, a-H, 3b-H, 1b-H), 4.62 (dd, $J_{6'a,6'b} = 10.5$, $J_{6'a,5'} = 6.1$ Hz, 1 H, 6′a-H), 4.86 (d, 1 H, 1′-H), 4.88 (dd, 1 H, 6′b-H), 7.29 (m, 4 H, Ph), 8.01 (d, $J = 8.1$ Hz, 2 H, Ph), 8.02 (d, $J = 8.1$ Hz, 2 H, Ph) ppm. ^{13}C NMR ([D_5]pyridine, 312 K): $\delta = 13.75$ (CH_3), 20.82 ($2 \times PhCH_3$), 22.42 (CH_2), 24.63 (CH_2), 28.80–29.80 ($12 \times CH_2$), 31.62 (CH_2), 33.67 (*C*H_2CO), 62.16 (C1), 69.75 (C3), 70.30 (C4′, C6′), 73.99 (C2′), 74.33 (C2), 74.54 (C5′), 77.37 (C3′), 104.26 (C1′), 127.83 (2 C, Ph), 128.03 (2 C, Ph), 129.87 (4 C, Ph), 133.03 and 133.42 (2 C, Ph), 144.66 and 144.76 (2 C, Ph), 172.69 (CO) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): *m*/*z* (%) = 851.2 $[M + Na]^+$ (100). $C_{41}H_{64}O_{13}S_2$ (829.07): calcd. C 59.40, H 7.78, S 7.74; found C 59.51, H 7.85, S 7.68.

1-*O*-Dodecanoyl-3-*O*-tosyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (8b): M.p. 196 °C (from Et_2O). $[\alpha]_D^{20} = -1.8$ ($CHCl_3$). ^1H NMR ([D_5]pyridine, 312 K): $\delta = 0.86$ (t, $J = 7.1$ Hz, 3 H, CH_3), 1.18–1.30 (m, 16 H, $8 \times CH_2$), 1.61 (m, 2 H, CH_2), 2.22 (s, 3 H, CH_3), 2.23 (s, 3 H, CH_3), 2.30 (m, 2 H, CH_2), 3.71 (dd, $J_{1',2'} = 7.8$, $J_{2',3'} = 8.9$ Hz, 1 H, 2′-H), 3.86 (dd, $J_{3',4'} = 9.0$, $J_{4',5'} = 9.9$ Hz, 1 H, 4′-H), 3.95 (ddd, $J_{5',6'a} = 6.1$, $J_{5',6'b} = 1.8$ Hz, 1 H, 5′-H), 4.04 (dd, 1 H, 3′-H), 4.41–4.46 (m, 2 H, 2-H, 1a-H), 4.52–4.62 (m, 3 H, 4a-H, 3b-H, 1b-H), 4.62 (dd, $J_{6'a,6'b} = 10.5$, $J_{6'a,5'} = 6.1$ Hz, 1 H, 6′a-H), 4.86 (d, 1 H, 1′-H), 4.89 (dd, 1 H, 6′b-H), 7.29 (m, 4 H, Ph), 8.01 (d, $J = 8.1$ Hz, 2 H, Ph), 8.02 (d, $J = 8.1$ Hz, 2 H, Ph) ppm. ^{13}C NMR ([D_5]pyridine, 312 K): $\delta = 13.75$ (CH_3), 20.81 ($2 \times PhCH_3$), 22.42 (CH_2), 24.63 (CH_2), 28.90 (CH_2), 29.07 ($2 \times CH_2$), 29.25 (CH_2), 29.36 ($2 \times CH_2$), 31.61 (CH_2), 33.67 (*C*H_2CO), 62.16 (C1), 69.76 (C3), 70.30 (C4′, C6′), 73.99 (C2′), 74.33 (C2), 74.54 (C5′), 77.37 (C3′), 104.26 (C1′), 127.83 (2 C, Ph), 128.04 (2 C, Ph), 129.88 (4 C, Ph), 133.04 and 133.39 (2 C, Ph), 144.66 and 144.77 (2 C, Ph), 172.69 (CO) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): *m*/*z* (%) = 767.2 (100) $[M + Na]^+$. $C_{35}H_{52}O_{13}S_2$ (744.91): calcd. C 56.43, H 7.04, S 8.61; found C 56.27, H 7.12, S 8.50.

1-*O*-Hexanoyl-3-*O*-tosyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (8c): M.p. 180 °C (from Et_2O). $[\alpha]_D^{20} = -1.2$ ($CHCl_3$). ^1H NMR ([D_5]pyridine, 312 K): $\delta = 0.78$ (t, $J = 7.1$ Hz, 3 H, CH_3), 1.14–1.21 (m, 4 H, $2 \times CH_2$), 1.55 (m, 2 H, CH_2), 2.22 (s, 6 H, $2 \times CH_3$), 2.25 (m, 2 H, CH_2), 3.71 (dd, $J_{1',2'} = 7.8$, $J_{2',3'} = 8.9$ Hz, 1 H, 2′-H), 3.85 (dd, $J_{3',4'} = 8.9$, $J_{4',5'} = 9.9$ Hz, 1 H, 4′-H), 3.95

(ddd, $J_{5',6'a}$ = 6.0, $J_{5',6'b}$ = 1.6 Hz, 1 H, 5′-H), 4.04 (dd, 1 H, 3′-H), 4.39–4.45 (m, 2 H, 2-H, 1a-H), 4.50–4.61 (m, 3 H, 3a-H, 3b-H, 1b-H), 4.62 (dd, $J_{6'a,6'b}$ = 10.5, $J_{6'a,5'}$ = 6.2 Hz, 1 H, 6′a-H), 4.86 (d, 1 H, 1′-H), 4.89 (dd, 1 H, 6′b-H), 7.28 (d, *J* = 8.1 Hz, 4 H, Ph), 8.00 (d, *J* = 8.1 Hz, 2 H, Ph), 8.02 (d, *J* = 8.1 Hz, 2 H, Ph) ppm. ^{13}C NMR ([D$_5$]pyridine, 312 K): δ = 13.46 (CH_3), 20.80 (2 × $PhCH_3$), 21.97 (CH_2), 24.20 (CH_2), 30.90 (CH_2), 33.56 (CH_2CO), 62.14 (C1), 69.73 (C3), 70.24 (C4′ or C6′), 70.28 (C4′ or C6′), 73.95 (C2′), 74.29 (C2), 74.50 (C5′), 77.33 (C3′), 104.22 (C1′), 127.80 (2 C, Ph), 128.00 (2 C, Ph), 129.86 (4 C, Ph), 132.98 and 133.36 (2 C, Ph), 144.66 and 144.76 (2 C, Ph), 172.63 (CO) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): *m*/*z* (%) = 683.1 (100) [M + Na]$^+$. $C_{29}H_{40}O_{13}S_2$ (660.2): calcd. C 52.71, H 6.10, S 9.71; found C 52.77, H 6.19, S 9.63.

General Procedure for the Synthesis of 2*R* Ditosylates 8a–c from 6a–c: Zinc bromide (0.126 g, 0.560 mmol) was added at –20 °C to a solution of a compound **6** (0.140 mmol) in dry pyridine (3.0 mL). After addition of tosyl chloride (0.133 g, 0.700 mmol) the reaction mixture was stirred at –20 °C for 1 h with monitoring by TLC (CH_2Cl_2/CH_3OH, 95:5 v/v). After conventional workup, compounds identical (NMR, MS, m.p.) to the previously obtained **8a–c** were obtained in about 70% yields.

Chemoenzymatic Synthesis of the (2*S*)-Ditosylate 10a: The tosyl derivative **4** (0.080 g, 0.196 mmol) was dissolved in pyridine (1.0 mL), and trifluoroethyl octadecanoate (0.360 g, 0.982 mmol) and LCA (0.250 g) were added in that order. The suspension was stirred at 45 °C and monitored by TLC (CH_2Cl_2/CH_3OH, 90:10 v/v). The reaction was stopped after 28 h by filtering off of the enzyme, which was washed with pyridine. After removal of the solvent under vacuum, the crude product was subjected to flash column chromatography (CH_2Cl_2/CH_3OH, 95:5 v/v), yielding the 1-*O*-monoester **5a** (0.059 g, 0.087 mmol) and 3-*O*-octadecanoyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (**9a**, 0.066 g, 0.098 mmol) in that order; m.p. 98 °C (amorphous solid). $[\alpha]_D^{20}$ = –10.9 ($CHCl_3$). ^{1}H NMR ([D$_5$]pyridine, 312 K): δ = 0.86 (t, *J* = 7.0 Hz, 3 H, CH_3), 1.19–1.38 (m, 28 H, 14 × CH_2), 1.68 (m, 2 H, CH_2), 2.22 (s, 3 H, CH_3), 2.42 (m, 2 H, CH_2), 3.91 (dd, $J_{1',2'}$ = 7.8, $J_{2',3'}$ = 9.0 Hz, 1 H, 2′-H), 3.93 (dd, $J_{3',4'}$ = 9.0, $J_{4',5'}$ = 9.8 Hz, 1 H, 4′-H), 3.99 (ddd, $J_{5',6'a}$ = 5.9, $J_{5',6'b}$ = 1.5 Hz, 1 H, 5′-H), 4.05–4.13 (m, 3 H, 1a-H, 1b-H, 3′-H), 4.41 (m, 1 H, 2-H), 4.60 (dd, $J_{3a,2}$ = 4.9, $J_{3a,3b}$ = 11.4 Hz, 1 H, 3a-H), 4.68 (dd, $J_{3b,2}$ = 5.4 Hz, 1 H, 3b-H), 4.70 (dd, $J_{6'a,6'b}$ = 10.4 Hz, 1 H, 6′a-H), 4.93 (dd, 1 H, 6′b-H), 5.04 (d, 1 H, 1′-H), 7.27 (d, *J* = 8.1 Hz, 2 H, Ph), 8.02 (d, 2 H, Ph) ppm. ^{13}C NMR ([D$_5$]pyridine, 312 K): δ = 13.76 (CH_3), 20.82 ($PhCH_3$), 22.44 (CH_2), 24.82 (CH_2), 28.90–29.69 (12 × CH_2), 31.64 (CH_2), 33.91 (CH_2CO), 61.56 (C1), 64.06 (C3), 70.46 (C4′ and C6′), 74.62 (C2′ and C5′), 77.64 (C3′), 78.95 (C2), 104.23 (C1′), 127.86 and 129.82 (4 C, Ph), 133.50 and 145.54 (2 C, Ph), 173.09 (CO) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): *m*/*z* (%) = 697.3 (100) [M + Na]$^+$. $C_{34}H_{58}O_{11}S$ (674.88): calcd. C 60.51, H 8.66, S 4.75; found C 60.73, H 8.59, S 4.81.

Zinc bromide (0.067 g, 0.297 mmol) was added at –20 °C to a solution of compound **9a** (0.050 g, 0.074 mmol) in dry pyridine (3.0 mL). After addition of tosyl chloride (0.070 g, 0.367 mmol) the reaction mixture was stirred at –20 °C for 3 h and monitored by TLC (CH_2Cl_2/CH_3OH, 95:5 v/v). The reaction was stopped and worked up as described previously, yielding the ditosyl derivative 3-*O*-octadecanoyl-1-*O*-tosyl-2-*O*-(6-*O*-tosyl-β-D-glucopyranosyl)-*sn*-glycerol (**10a**, 0.050 g, 0.060 mmol) after flash column chromatography (CH_2Cl_2/CH_3OH, 95:5 v/v); m.p. 190 °C (amorphous solid). $[\alpha]_D^{20}$ = –3.2 ($CHCl_3$). ^{1}H NMR ([D$_5$]pyridine, 312 K): δ = 0.86 (t, *J* = 6.9 Hz, 3 H, CH_3), 1.19–1.35 (m, 28 H, 14 × CH_2), 1.63 (m, 2 H, CH_2), 2.21 (s, 3 H, CH_3), 2.23 (s, 3 H, CH_3), 2.32 (m, 2 H, CH_2), 3.84 (dd, $J_{1',2'}$ = 7.8, $J_{2',3'}$ = 8.6 Hz, 1 H, 2′-H), 3.90 (dd, $J_{3',4'}$ = 9.0, $J_{4',5'}$ = 9.6 Hz, 1 H, 4′-H), 3.93 (ddd, $J_{5',6'a}$ = 5.6, $J_{5',6'b}$ < 1.0 Hz, 1 H, 5′-H), 4.04 (dd, 1 H, 3′-H), 4.46 (m, 1 H, 3a-H), 4.50 (m, 1 H, 2-H), 4.52 (m, 1 H, 1a-H), 4.57 (m, 1 H, 1b-H), 4.59 (m, 1 H, 3b-H), 4.65 (dd, $J_{6'a,6'b}$ = 10.5 Hz, 1 H, 6′a-H), 4.87 (d, 1 H, 1′-H), 4.89 (dd, 1 H, 6′b-H), 7.25 (d, *J* = 8.0 Hz, 2 H, Ph), 7.28 (d, *J* = 8.0 Hz, 2 H, Ph), 8.01 (m, 4 H, Ph) ppm. ^{13}C NMR ([D$_5$]pyridine, 312 K): δ = 13.76 (CH_3), 20.82 (2 × $PhCH_3$), 22.43 (CH_2), 24.69 (CH_2), 28.86–29.70 (12 × CH_2), 31.63 (CH_2), 33.68 (CH_2CO), 63.08 (C3), 68.75 (C1), 70.33 (C4′ and C6′), 74.10 (C2′), 74.33 (C2), 74.58 (C5′), 77.49 (C3′), 103.97 (C1′), 127.83 (2 C, Ph), 127.93 (2 C, Ph), 129.86 (4 C, Ph), 133.01 (2 C, Ph), 133.40 (2 C, Ph), 144.60 and 144.76 (2 C, Ph), 172.65 (CO) ppm. ESI-MS (CH_3OH, positive-ion mode, relative intensity): *m*/*z* (%) = 851.4 (100) [M + Na]$^+$. $C_{41}H_{64}O_{13}S_2$ (829.07): calcd. C 59.40, H 7.78, S 7.74; found C 59.28, H 7.89, S 7.61.

Short-Term In-Vitro Bioassay for Antitumor Promoters: Inhibition was tested by a short-term in-vitro assay for EBV activation in Raji cells (originally obtained from Prof. G. Klein, Karolinska Institute, Stockholm, Sweden) cultivated in RPMI 1640 medium containing fetal calf serum (10%) and induced with TPA as described previously.[6,14] Raji cells (1×10^6 mL^{-1}) were incubated at 37 °C for 48 h in a medium (1 mL) containing *n*-butyric acid (4 mM), TPA in DMSO (32 pmol), and a known amount of the test compound in DMSO. The cells were stained with high-titer EBV-positive sera from nasopharyngeal carcinoma patients and fluorescein-isothiocyanate-labeled antihuman IgG. After staining, they were detected by a conventional indirect immunofluorescence technique. The assays were performed in triplicate for each compound, with at least 500 cells being counted. The average EBV-EA inhibitory activities of the test compounds were determined by comparison with control experiments (100%) with butyric acid (4 mM) and TPA (32 pM), in which EBV-EA induction was typically around 30%. The viability of the cells was assayed against treated cells by the Trypan Blue staining method. For an accurate determination of cytotoxicity, the cell viability was required to be more than 60% 2 d after treatment with the compounds.

Supporting Information (see also the footnote on the first page of this article): ^{13}C NMR spectra of target compounds **1a–c**.

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