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Chemo-enzymatic preparation of α-6-sulfoquinovosyl-1,2-*O*-diacylglycerols

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ABSTRACT

Synthesis of α-6-sulfoquinovosyl-1,2-*O*-diacylglycerols is achieved by a versatile chemo-enzymatic stereoselective procedure that involves the use of α-D-glucosidase activity from the Mediterranean mollusc *Aplysia fasciata*. The synthetic procedure is designed to obtain a wide diversity of regio- and stereo-isomers of these compounds that have gained great interest as antineoplastic agents and potent inhibitors of DNA polymerases.

1. Introduction

Sulfoquinovosyl-diacylglycerols (SQDGs), namely 1,2-di-*O*-acyl-3-*O*-(6′-deoxy-6′-sulfo-α-D-glucopyranosyl)-*sn*-glycerols, first described by Benson et al. in 1959,[1] are major constituents of chloroplast membranes of photosynthetic organisms,[2,3] but have been also reported in bacteria.[4] SQDGs play a direct role in the photosynthetic processes and, in the last years, have attracted bio-medical attention because of their important activities as antitumors,[4,5] antivirals,[6,7] immunomodulators[8] and inhibitors of DNA polymerase.[2,9] Despite their biological potential, SQDGs have been scarcely investigated because of the difficulties related to their isolation from natural sources and chemical synthesis,[9b,c,e,10] mainly challenged by partial loss of stereoselectivity in formation of the α-glycosidic bond[11] hampering the access to these products in laboratory.

Use of enzymes for the stereospecific construction of glycosidic linkages is a new technological tool that involves the ability of glycosyl hydrolases (*endo*- and *exo*-glycosidases)[12] or glycosyltransferases[13] to transfer a sugar unit from different donors to specific acceptors. In particular, glycosyl hydrolases (EC 3.2.1.) are functionally committed to the hydrolysis of glycosidic bonds but are also able to catalyze the stereospecific formation of such linkages (transglycosylation processes), thus finding application in the enzymatic synthesis of oligosaccharides and glycoconjugates.[12]

Recently, a study of the catalytic activity from the marine mollusk *Aplysia fasciata* yielded a library of glycoside hydrolases able to form glycosidic bonds.[14] In a preliminary assessment, the prominent activity found in the visceral mass of *A. fasciata* resulted in an α-glucosidase enzyme showing a promising potential to glycosylate free glycerol.[15] Here we report the use of *A. fasciata* α-glucosidase activity as the key step of a novel and simple synthetic approach for the preparation of SQDGs.

2. Results and discussion

As shown in Scheme 1 for the synthesis of natural sulfoquinovosyl-diacylglycerols,[16] transglycosylation catalyzed by the α-glucosidase activity of *A. fasciata* (step 1) controlled the transfer of glucose from maltose (4-*O*-α-D-glucopyranosyl-D-glucose) to (*rac*)-1,2-*O*-isopropylidene glycerol. In a 10:1 molar ratio of donor/acceptor the reaction gave a 1,2 glycerol acetonide conversion of 59% in 29 h. Reaction products were exclusively 3-α-glycosyl derivatives of 1,2-*O*-isopropylidene glycerol. In particular 3-α-glucosyl-1,2-*O*-isopropylidene glycerol (**1**) was produced as the major component (30% yield) together with a mixture of di- and tri-saccharide analogues (23 and 6% yields, respectively).

NMR spectroscopic data for H-1′ (δ=4.82 ppm, J=3.29 Hz) and C-1′ (99.31 ppm) confirmed the α configuration of the anomeric centre of compound **1**, whereas further glucosylation in the oligosaccharides occurred with the preferential formation of α-1→4 and α-1→6 linkages (in molar ratio 1:1), in agreement with the capability of *A. fasciata* α-glucosidase to perform polyglycosylation reactions.[17]

To date few examples of enzymatic *O*-α-D-glucosylation of glycerol have been reported in the literature.[18–20] Interestingly, *A.*

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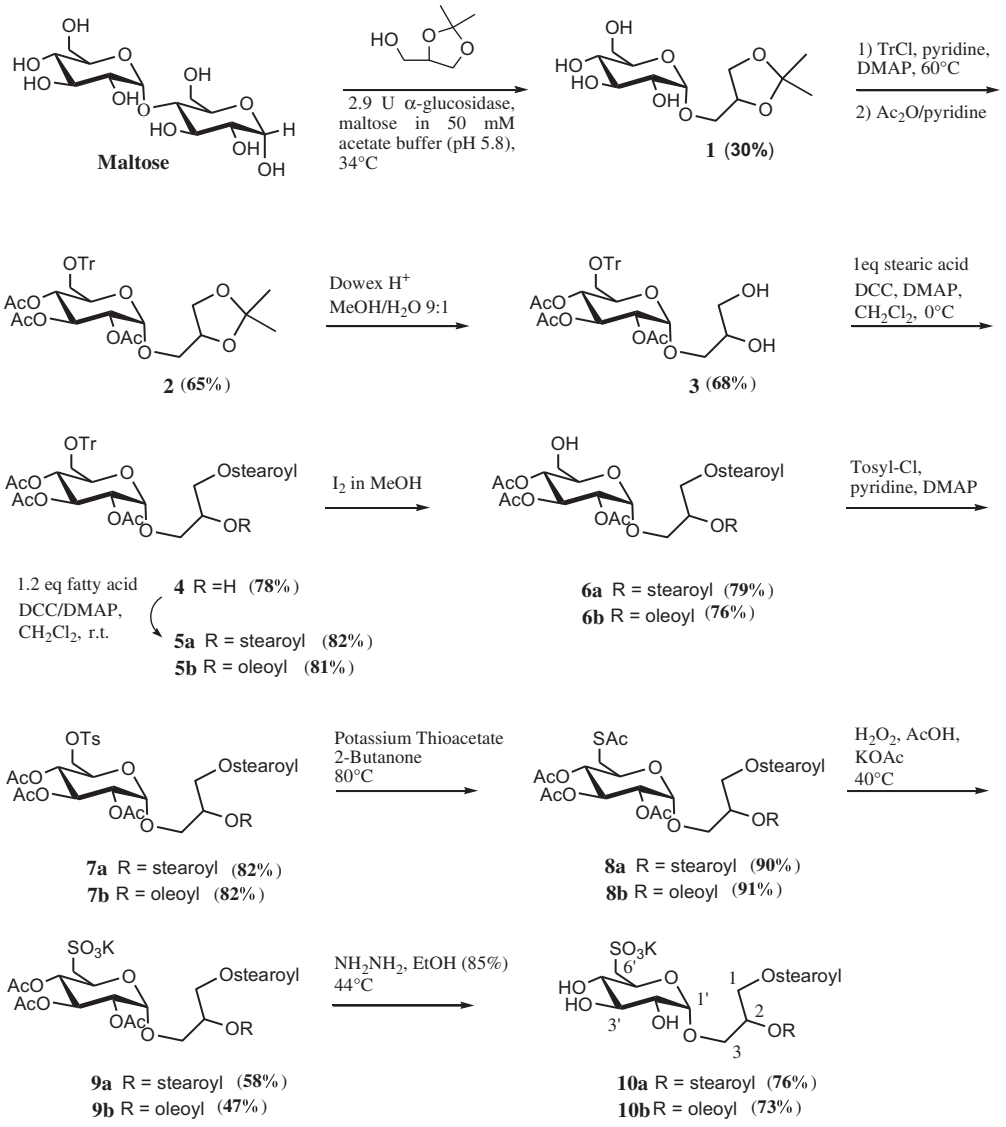

Scheme 1. Synthesis of natural sulfoquinovosyl-diacylglycerols.

fasciata activity was higher or at least comparable with these known processes. In support of the synthetic potential of the mollusk enzyme, we also tested the reactivity of commercial glycosidases with glycerol. Under conditions similar to those used for *A. fasciata* fraction (maltose/glycerol 10:1; 24 h at 37 °C), 3.75 U/mmol of maltose of the transglycosidase of *Aspergillus niger* and 3.43 U/mmol of maltose of α-glucosidase of *Bacillus sterearothermophilus* gave compound **1** with yields below 2%. These results are largely lower than that obtained with the α-glucosidase activity of *A. fasciata*, suggesting that this latter enzyme may offer a viable option for the synthesis of different types of glycolipids.

Tritylation of the primary alcohol of compound **1** followed by peracetylation gave compound **2** that was converted to **3** by selective hydrolysis of the isopropylidene residue with Dowex H^+ in methanol/water 9:1.[21] In this last step, the reaction with common catalysts such as HCl, HBr or TFA was hampered by the undesired removal of the trityl group. In the ^{1}H NMR spectrum of compound **2**, anomeric signals of two diastereoisomers at δ 5.18 and 5.16 ppm were distinguishable in a ratio of 1:1, showing the absence of *A. fasciata* α-glucosidase diastereospecificity. Subsequent DCC-mediated condensation with 1 or 2 equiv of free fatty acids (i.e., stearic or oleic) yielded diacyl derivatives **5a** and **5b**. In agreement with previous observations,[22] acylation of the primary hydroxyl group was largely favoured in the coupling reaction with equimolar amounts of reagents (compound **4**). Thus 2-acylglycerol derivatives were not observed when 1 equiv of acid was used, although esterification of the secondary hydroxyl group of glycerol has been sometimes reported.[23]

Selective removal of the trityl group with iodine in methanol[24] (**6a**/**6b**) followed by tosylation of the primary alcohol gave compounds **7a**/**7b** that, after replacement of the tosyl group with thioacetate[25] (**8a**/**8b**) and oxidation by hydrogen peroxide to the sulfonic function,[25] was easily converted to compounds **9a**/**9b**. Finally, removal of the acetate groups with hydrazine hydrate completed the reaction sequence and yielded the target products 1,2-di-*O*-stearoyl- and 1-*O*-stearoyl-2-*O*-oleoyl-3-*O*-(6′-α-sulfoquinovosyl)glycerols (**10a** and **10b**, respectively).[22,26]

The above procedure allowed both the step-wise introduction of different acyl residues, and the control of fatty acid regiospecificity of the resulting SQDGs. Use of palmitic, decanoic and 5-dodecenoic acids gave a number of regioisomers, including a few natural products (Table 1). Overall yields were good (from 14 to 23%) independent of the acyl substituents. As expected, saturated compounds (e.g., 10:0/16:0) led to the best results because of the minor susceptibility to be oxidized by hydrogen peroxide in the conversion to sulfonate.

Table 1
Sulfoquinovosyl-diacylglycerol derivatives prepared according to the synthetic strategy of Scheme 1

	Acyl residue C-1	Acyl residue C-2	Yield[a] (%)
Compound **10a**	C18:0	C18:0	~21
Compound **10b**	C18:0	C18:1	~16
Compound **11**	C18:1	C18:1	~14
Compound **12**	C16:0	C18:1	~18
Compound **13**	C18:0	C16:0	~22
Compound **14**	C10:0	C12:1	~17
Compound **15**	C10:0	C10:0	~23
Compound **16**	C12:1	C12:1	~14

[a] Overall yields are calculated from the esterification reaction of compound **3.**

3. Conclusion

Synthesis of 6-α-sulfoquinovosyl-diacylglycerols was achieved by a versatile chemo-enzymatic process. This is the first time that an enzymatic approach has been used successfully for the preparation of this class of molecules. Avoiding procedures involving the activation of chemical donors and using maltose as a cheap donor, the key-intermediate 3-α-glucosyl-1,2-*O*-isopropylidene (**1**) was obtained by a stereoselective glycosylation of the protected glycerol by α-glucosidase activity from the marine mollusc *A. fasciata*. The enzymatic coupling allowed a strict control of the stereoselectivity of the α-glycosidic linkage, whereas the use of acetate as protecting group favoured the synthesis of saturated, unsaturated, or mixed derivatives in high yields.[22] Although both enantiopure commercially available 1,2-*O*-isopropylidene glycerols were not tested in this work, their use is compatible with the mild enzymatic coupling conditions (pH 5.8).[27] The proposed procedure is of general application and allows the preparation of regio- and stereo-pure derivatives of α-6-sulfoquinovosyl-1,2-*O*-diacylglycerols, thus giving a realistic access to the biological use of these compounds.

4. Experimental section

4.1. General experimental procedures

All products and synthetic intermediates structure were assigned using ^{1}H, ^{13}C, COSY, TOCSY (mixing time=68 ms), HSQC, HMBC and NOESY (mixing time=250 ms) NMR spectra.

^{1}H, ^{13}C and 2D NMR spectra were acquired by the NMR Service of the Institute of Biomolecular Chemistry of the National Council of Research (ICB-CNR) and recorded on a Bruker DRX-600 spectrometer, equipped with a TCI CryoProbe™, fitted with a gradient along the *Z*-axis and on Bruker instruments at 400 and/or 300 MHz.

Samples for NMR spectroscopic analysis were dissolved in the appropriate solvent; spectra in D_2O were referenced to internal sodium 3-(trimethyl-silyl)-(2,2,3,3-2H4) propionate (Aldrich, Milwaukee, WI); for other solvents the downfield shift of the signal of the solvent was used as internal standard: $CDCl_3$ (^{1}H, ^{13}C): 7.26, 77.0 ppm; CD_3OD (^{1}H, ^{13}C): 3.34, 49.0 ppm.

High resolution ESIMS were performed on a Micromass Q-TOF Micro™ coupled with an HPLC Waters Alliance 2695. TLC plates (Kieselgel 60 F_{254}) and silica gel powder (Kieselgel 60, 0.063–0.200 mm) were from Merck.

All the reagents were purchased from Sigma–Aldrich and used without any further purification.

4.2. Enzyme sources

A clear enzymatic homogenate from *A. fasciata* visceral mass was prepared by homogenization in K-acetate buffer (50 mM, pH 5.5), subsequent centrifugation and dialysis procedures, and finally concentration by ultrafiltration, as previously described.[15] Since the most abundant hydrolytic enzyme in the *A. fasciata* visceral mass extract was an α-D-glucosidase, this enzymatic solution, with a total protein content of 8.1 mg/mL and a specific activity corresponding to 1.2 U/mg (using *p*-nitrophenyl α-D-glucopyranoside as substrate), was considered useful for this work without further purification. One unit of α-D-glucosidase activity was defined as that amount of enzyme required to catalyze the release of 1.0 μmol of *p*-nitrophenol per minute. Transglucosidases from *A. niger* and α-glucosidase from *B. sterearothermophilus* were purchased from Megazyme. Enzymatic reaction with *A. niger* was performed in K-acetate buffer (50 mM, pH 5.5).[28]

Enzymatic reactions with *A. niger* and *B. sterearothermophilus* were performed in K-phosphate buffer (0.1 M, pH 6.5).[29]

4.3. Synthetic procedures

4.3.1. 1,2-O-Isopropylidene glycerol. Glycerol (2.0 g, 0.022 mol) was dissolved in *N,N*-dimethylformamide (4 mL); 2,2-dimethoxypropane (4 mL) and *p*-toluenesulfonic acid (300 mg) were added; after stirring overnight at room temperature, the mixture was portioned between water and dichloromethane; the organic phase was purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether to give 1,2-*O*-isopropylidene glycerol (2.0 g, 0.015 mol, 68%) as colourless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.24 (1H, m, H-2), 4.09 (1H, dd, J=6.7, 8.5 Hz, H-3a), 3.82 (1H, dd, J=6.4, 8.5 Hz, H-3b), 3.65 (2H, m, H_2-1), 1.46 (3H, s, CH_3), 1.40 (3H, s, CH_3); HRESIMS m/z calcd for $C_6H_{12}O_3Na$: 155.0684; found:155.0689.

*4.3.2. Compound **1**.* 1,2-*O*-Isopropylidene glycerol (1.9 mmol), previously prepared and dissolved in DMSO (1 mL), was added to a solution of maltose 0.5 M (19 mmol, 38 mL) in K-acetate buffer (50 mM, pH 5.5) containing 600 μL of *A. fasciata* visceral mass enzymatic homogenate (5.83 U); the reaction system was put under magnetic stirring at 34 °C for 29 h up to almost total maltose consumption and regularly monitored by TLC analysis (system solvent: EtOAc/MeOH/H_2O 70:20:10 vol). After 5 h 1,2-*O*-isopropylidene glycerol (acceptor) was totally consumed, but being maltose still present, a second aliquot of it (1.9 mmol) was added into the reaction medium; a third similar aliquot of acceptor (1.9 mmol) was added at 8 h of reaction time.

Reaction was stopped after 29 h by cooling to −20 °C and the reaction mixture was fractionated by reverse phase RP-18 column (eluting firstly with two column volumes of water and then with methanol). The product mixture, consisting of the α-glucosyl derivative of 1,2-*O*-isopropylidene glycerol (**1**) and its corresponding di- and tri-saccharides were recovered in the methanol fraction and isolated by silica gel column chromatography (eluting with gradient of methanol in ethyl acetate); 1.71 mmol (502 mg) of pure compound **1** (pale yellow oil) was isolated; IR (liquid film) ν_{max} 3400, 2941, 2851, 1252, 1240 cm^{-1}; ^{1}H NMR (400 MHz, D_2O): δ 4.82 (1H, d, J=3.29 Hz, H-1′), 4.40–4.37 (1H, m, H-2), 4.07–3.73 (2H, m, H_2-1), 3.75–3.74 (1H, m, H-6′a), 3.65–3.52 (2H, m, H_2-3), 3.63–3.61 (1H, m, H-6′b), 3.62–3.60 (1H, m, H-3′), 3.57–3.55 (1H, m, H-4′), 3.43 (1H, dd, H-2′), 3.28 (1H, dd, H-5′), 1.38 (3H, s, CH_3), 1.29 (3H, s, CH_3); ^{13}C NMR (100 MHz, D_2O): δ 99.3 (CH, C1′), 75.3 (CH, C2), 74.0 (CH, C3′), 72.7 (CH, C4′), 72.3 (CH, C2′), 70.4 (CH, C5′), 69.1 (CH_2, C3), 66.4 (CH_2, C1), 61.4 (CH_2, C6′), 26.5, 25.1 (acetonide methyls); HRESIMS m/z calcd for $C_{12}H_{22}NaO_8$: 317.1212; found: 317.1218.

*4.3.3. Compound **2**.* Compound **1** (0.500 g, 0.0017 mol) was dissolved in pyridine (4 mL); 1.6 equiv of trityl chloride (0.756 g, 2.7 mmol) and 0.3 equiv of DMAP (57 mg, 0.51 mmol) were added; the reaction mixture was stirred for 3 h at 60 °C; subsequently, acetic anhydride (1 mL) was added and after stirring overnight the mixture was evaporated under a stream of nitrogen and purified by

silica gel chromatography using a chloroform/methanol gradient to give compound **2** (0.732 g, 1.1 mmol, 65%) as pale yellow oil; R_f (light petroleum ether/diethyl ether 3:7)=0.65; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.25 (15H, m, trityl portion), 5.41 (1H, dd, J=9.4, 9.4 Hz, H-3′), 5.18 and 5.16 (each 1H, d, J=3.5 Hz, H-1′), 5.07 (1H, dd, J=9.8, 9.8 Hz, H-4′), 4.90 (1H, m, H-2′), 4.30–4.28 (1H, m, H-2), 4.05–4.03 (2H, m, H_2-1), 3.97–3.95 (1H, m, H-5′), 3.79–3.77 (1H, m, H-3a), 3.56–3.54 (1H, m, H-3b), 3.17 (1H, br d, J=10.5 Hz, H-6′a), 3.07 (1H, dd, J=5.1, 10.5 Hz, H-6′b), 2.04 (6H, s, OAc), 1.96 (3H, s, OAc), 1.40 (3H, s, CH_3), 1.32 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 128.5, 127.6, 126.9 (CH, aromatic methynes), 109.4 (C, acetonide carbon), 95.7 (CH, C1′), 70.7 (CH, C2′), 74.1 (CH, C2),70.2 (CH, C3′), 68.8 (CH, C4′), 68.6 (CH_2, C1), 68.4 (CH, C5′), 66.4 (CH_2, C3),61.9 (CH_2, C6′), 26.5, 25.5 (CH_3, acetonide methyls), 20.6, 20.5, 20.4 (CH_3, acetate methyls); HRESIMS m/z calcd for $C_{37}H_{42}NaO_{11}$: 685.2625; found: 685.2630.

*4.3.4. Compound **3**.* Compound **2** (0.732 g, 1.1 mmol) was dissolved in methanol/water solution (25 mL, 9:1) and Dowex 50WX8-H^+ resin (18 g) was added; after 40 min under stirring, the reaction mixture was filtered, evaporated and purified by silica gel chromatography using a gradient of light petroleum ether/diethyl ether to give compound **3** (0.465 g, 0.748 mmol, 68%) as pale yellow oil; R_f (light petroleum ether/diethyl ether 3:7)=0.10; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.21 (15H, m, trityl portion), 5.41 (1H, dd, J=9.4, 9.4 Hz, H-3′), 5.16 and 5.13 (each 1H, d, J=3.5 Hz, H-1′), 5.06 (1H, dd, J=9.8, 9.8 Hz, H-4′), 4.94 and 4.92 (each 1H, dd, J=9.39, 3.5 Hz, H-2′), 4.01–3.99 (1H, m, H-5′), 3.94–3.92 (1H, m, H-2), 3.79–3.77 (1H, m, H-3a), 3.72–3.70 (1H, m, H-1a), 3.65–3.63 (1H, m, H-3b), 3.61 (1H, m, H-1b), 3.19 (1H, br d, J=10.5 Hz, H-6′a), 3.10 (1H, br d, J=10.5 Hz, H-6′b), 2.06 (3H, s, OAc), 1.97 (3H, s, OAc), 1.82 (3H, s, OAc); ^{13}C NMR (100 MHz, $CDCl_3$): 128.5, 127.5, 126.7 (CH, aromatic methynes), 96.3 (CH, C1′), 71.0 (CH, C2′), 70.4 (CH, C3′), 70.3 (CH, C2), 70.2 (CH_2, C3), 69.0 (CH, C4′), 68.8 (CH, C5′), 63.2 (CH_2, C1),62.0 (CH_2, C6′), 20.7, 20.6, 20.5 (CH_3, acetate methyls); HRESIMS m/z calcd for $C_{34}H_{38}NaO_{11}$: 645.2312; found: 645.2306.

*4.3.5. Compound **4**.* Compound **3** (0.20 g, 0.32 mmol) was dissolved in anhydrous dichloromethane (6 mL); stearic acid (0.90 g, 0.32 mmol), dicyclohexylcarbodiimide (0.0326 g, 0.32 mmol) and DMAP (0.0038 g, 0.032 mmol) were added under argon at 0 °C; the reaction mixture was stirred overnight at 0 °C; after evaporation under reduced pressure, the mixture was purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether to give compound **4** (0.220 g, 0.250 mmol, 78%) as a colourless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40–7.22 (15H, m, trityl portion), 5.39–5.37 (1H, m, H-3′), 5.15 and 5.11 (each 1H, br d, J=2.7 Hz, H-1′), 5.08 (1H, dd, J=10.1, 10.1 Hz, H-4′), 4.87 (1H, dd, J=10.5, 2.7 Hz, H-2′), 4.31–4.29 (1H, m, H-1a), 4.15–4.13 (1H, m, H-1b), 4.00–3.98 (1H, m, H-5′), 3.94–3.92 (1H, m, H-3a), 3.88–3.86 (1H, m, H-2), 3.65–3.63 (1H, m, H-3b), 3.17 (1H, br d, J=9.8 Hz, H-6′a), 3.07 (1H, dd, J=9.8, 4.7 Hz, H-6′b), 2.31–2.28 (2H, m, α-methylene of acyl portion), 2.04 (3H, s, OAc), 2.02 (3H, s, OAc), 1.98 (3H, s, OAc), 1.64–1.60 (2H, m, β-methylene of acyl portion), 1.38–1.23 (28H, m, aliphatic protons), 0.90 (3H, t, J=6.9 Hz, CH_3); HRESIMS m/z calcd for $C_{52}H_{72}NaO_{12:}$ 912.4921; found: 912.4925.

*4.3.6. Compound **5a**.* Compound **4** (0.115 g, 0.130 mmol) was dissolved in anhydrous dichloromethane (5 mL); stearic acid (0.044 g, 0.156 mmol), dicyclohexylcarbodiimide (0.0303 g, 0.156 mmol) and DMAP (0.00190 g, 0.0156 mmol) were added under argon; the reaction mixture was stirred overnight at room temperature; after evaporation under reduced pressure, the mixture was purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether to give compound **5a** (yellow oil) (0.122 g, 0.106 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.22 (15H, m, trityl portion), 5.39–5.37 (1H, m, H-3′), 5.26–5.24 (1H, m, H-2), 5.15 and 5.14 (each 1H, br d, J=2.7 Hz, H-1′), 5.08 (1H, dd, J=10.1, 10.1 Hz, H-4′), 4.89 (1H, dd, J=10.5, 2.7 Hz, H-2′), 4.33–4.31 (1H, m, H-1a), 4.16–4.14 (1H, m, H-1b), 3.97–3.95 (1H, m, H-5′), 3.91–3.89 (1H, m, H-3a), 3.66–3.64 (1H, m, H-3b), 3.18 (1H, br d, J=9.8 Hz, H-6′a), 3.09 (1H, dd, J=9.8, 4.7 Hz, H-6′b), 2.31–2.27 (4H, m, α-methylenes of acyl portions), 2.05 (3H, s, OAc), 2.02 (3H, s, OAc), 1.98 (3H, s, OAc), 1.64–1.60 (4H, m, β-methylenes of acyl portions), 1.38–1.23 (56H, m, aliphatic protons), 0.92–0.90 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.8, 172.6 (C, acyl ester carbons), 128.6, 127.7, 127.1 (CH, aromatic methynes), 96.1 (CH, C1′), 71.1 (CH, C2′), 70.4 (CH, C3′), 70.0 (CH, C2), 69.3 (CH, C5′), 68.8 (CH, C4′), 66.0 (CH_2, C3), 62.4 (CH_2, C1), 62.2 (CH_2, C6′), 34.2, 34.1 (CH_2, α-methylenes of acyl portions), 29.8–29.0 (CH_2, aliphatic methylenes), 25.2, 24.9 (CH_2, β-methylenes of acyl portions), 20.7, 20.5, 20.4 (CH_3, acetate methyls), 14.2, 14.0 (CH_3, acyl terminal methyls); HRESIMS m/z calcd for $C_{70}H_{106}NaO_{13}$: 1177.7531; found: 1177.7535.

*4.3.7. Compound **5b**.* Compound **4** (0.115 g, 0.130 mmol) was dissolved in anhydrous dichloromethane (5 mL); oleic acid (0.044 g, 0.156 mmol), dicyclohexylcarbodiimide (0.0303 g, 0.156 mmol) and DMAP (0.00190 g, 0.0156 mmol) were added under argon; the reaction mixture was stirred overnight at room temperature; after evaporation under reduced pressure, the mixture was purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether to give compound **5b** (yellow oil) (0.120 g, 0.105 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.22 (15H, m, trityl portion), 5.40–5.38 (1H, m, H-3′), 5.38–5.36 (2H, m, olefinic protons), 5.26–5.24 (1H, m, H-2), 5.16 and 5.13 (each 1H, br d, J=2.7 Hz, H-1′), 5.08 (1H, dd, J=10.1, 10.1 Hz, H-4′), 4.89 (1H, dd, J=10.5, 2.7 Hz, H-2′), 4.33–4.31 (1H, m, H-1a), 4.16–4.14 (1H, m, H-1b), 3.97–3.95 (1H, m, H-5′), 3.91–3.89 (1H, m, H-3a), 3.66–3.64 (1H, m, H-3b), 3.18 (1H, br d, J=9.8 Hz, H-6′a), 3.09 (1H, dd, J=9.8, 4.7 Hz, H-6′b), 2.31–2.27 (4H, m, α-methylenes of acyl portions), 2.04–2.02 (4H, m, allylic protons), 2.05 (3H, s, OAc), 2.02 (3H, s, OAc), 1.98 (3H, s, OAc), 1.63–1.61 (4H, m, β-methylenes of acyl portions), 1.38–1.23 (48H, m, aliphatic protons), 0.92–0.89 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.8, 172.7 (C, acyl ester carbons), 131.0, 128.6, 127.7, 127.1 (CH, olefinic and aromatic methynes), 96.1 (CH, C1′), 71.1 (CH, C2′), 70.4 (CH, C3′), 70.0 (CH, C2), 69.3 (CH, C5′), 68.8 (CH, C4′), 66.0 (CH_2, C3), 62.5 (CH_2, C1), 62.2 (CH_2, C6′), 34.2, 34.1 (CH_2, α-methylenes of acyl portions), 29.8–29.0 (CH_2, aliphatic methylenes), 27.8 (2 CH_2, allylic methylenes), 25.2, 24.9 (CH_2, β-methylenes of acyl portions), 20.7, 20.5, 20.4 (CH_3, acetate methyls), 14.2, 14.0 (CH_3, acyl terminal methyls); HRESIMS m/z calcd for $C_{70}H_{104}NaO_{13}$: 1175.7375; found: 1175.7381.

*4.3.8. Compound **6a**.* Compound **5a** (0.124 g, 0.107 mmol) was dissolved in iodine–methanol solution (5 mL, 1%); after stirring for 30 min at 60 °C, the mixture was concentrated and purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether to give compound **6a** (0.077 g, 0.084 mmol, 79%) as a pale yellow oil; R_f (light petroleum ether/diethyl ether 3:7)=0.22; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.52–5.50 (1H, m, H-3′), 5.22–5.20 (1H, m, H-2), 5.11 (1H, br d, 2.9 Hz, H-1′), 5.02–5.00 (1H, m, H-4′), 4.84 (1H, dd, J=9.9, 2.9 Hz, H-2′), 4.33–4.31 (1H, m, H-1a), 4.18–4.16 (1H, m, H-1b), 3.83–3.81 (1H, m, H-3a), 3.82–3.80 (1H, m, H-5′), 3.69 (1H, br d, J=9.6 Hz, H-6′a), 3.64–3.62 (1H, m, H-3b), 3.59 (1H, dd, J=9.6, 4.4 Hz, H-6′b), 2.31–2.27 (4H, m, α-methylenes of acyl portions), 2.05 (3H, s, OAc), 2.01 (6H, s, OAc), 1.63–1.60 (4H, m, β-methylenes of acyl portions), 1.38–1.23 (56H, m, aliphatic protons), 0.91–0.89 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.0, 172.8 (C, acyl ester carbons), 96.4 (CH, C1′), 70.9 (CH, C2′), 70.0 (CH, C2), 69.8 (CH, C3′), 69.8 (CH, C5′), 68.9 (CH, C4′), 66.4 (CH_2, C3), 62.3 (CH_2, C1), 61.1 (CH_2, C6′), 34.0, 33.9 (CH_2, α-methylenes of acyl portions), 29.9–29.0 (CH_2, aliphatic methylenes), 25.2, 25.1

(CH_2, β-methylenes of acyl portions), 20.8, 20.7, 20.4 (CH_3, acetate methyls), 14.6, 14.1 (CH_3, acyl terminal methyls); HRESIMS *m/z* calcd for $C_{51}H_{92}NaO_{13}$: 935.6436; found: 935.6441.

*4.3.9. Compound **6b**.* Compound **5b** (0.124 g, 0.107 mmol) was dissolved in iodine–methanol solution (5 mL, 1%). After stirring for 30 min at 60 °C, the mixture was concentrated and purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether to give compound **6b** (0.074 g, 0.081 mmol, 76%) as a pale yellow oil; R_f (light petroleum ether/diethyl ether 3:7)=0.22; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.52–5.50 (1H, m, H-3′), 5.38–5.36 (2H, m, olefinic protons), 5.22–5.20 (1H, m, H-2), 5.11 (1H, br d, 2.9 Hz, H-1′), 5.02–5.00 (1H, m, H-4′), 4.84 (1H, dd, *J*=9.9, 2.9 Hz, H-2′), 4.33–4.31 (1H, m, H-1a), 4.18–4.16 (1H, m, H-1b), 3.83–3.81 (1H, m, H-3a), 3.82–3.80 (1H, m, H-5′), 3.69 (1H, br d, *J*=9.6 Hz, H-6′a), 3.64–3.62 (1H, m, H-3b), 3.59 (1H, dd, *J*=9.6, 4.4 Hz, H-6′b), 2.31–2.27 (4H, m, α-methylenes of acyl portions), 2.05 (3H, s, OAc), 2.04–2.01 (4H, m, allylic protons), 2.01 (6H, s, OAc), 1.64–1.60 (4H, m, β-methylenes of acyl portions), 1.38–1.23 (48H, m, aliphatic protons), 0.92–0.89 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9, 172.8 (C, acyl ester carbons), 131.0 (2CH, olefinic methynes), 96.3 (CH, C1′), 70.9 (CH, C2′), 70.0 (CH, C2), 69.8 (CH, C3′), 69.8 (CH, C5′), 68.9 (CH, C4′), 66.4 (CH_2, C3), 62.3 (CH_2, C1), 61.1 (CH_2, C6′), 34.0, 33.9 (CH_2, α-methylenes of acyl portions), 29.8–29.0 (CH_2, aliphatic methylenes), 27.9 ($2CH_2$, allylic methylenes), 25.2, 25.1 (CH_2, β-methylenes of acyl portions), 20.8, 20.7, 20.4 (CH_3, acetate methyls), 14.7, 14.1 (CH_3, acyl terminal methyls); HRESIMS *m/z* calcd for $C_{51}H_{90}NaO_{13}$: 933.6279; found: 933.6275.

*4.3.10. Compound **7a**.* Compound **6a** (0.081 g, 0.089 mmol) was dissolved in anhydrous pyridine (3 mL). *p*-Tosylchloride (0.169 g, 0.888 mmol) and DMAP (0.010 g, 0.089 mmol) were added at 0 °C under argon and the reaction mixture was stirred overnight at room temperature. After evaporation under a stream of nitrogen, the mixture was purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether to give compound **7a** (0.076 g, 0.073 mmol, 82%) as a pale yellow oil; R_f (light petroleum ether/diethyl ether 1:1)=0.28; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (2H, d, *J*=8.3 Hz, Ar-H), 7.34 (2H, d, *J*=8.3 Hz, Ar-H), 5.40–5.38 (1H, m, H-3′), 5.18–5.16 (1H, m, H-2), 5.02–5.00 (1H, m, H-4′), 4.92–4.90 (1H, m, H-2′), 4.73 and 4.75 (each 1H, d, *J*=3.4 Hz, H-1′), 4.31–4.29 (1H, m, H-1a), 4.26 (1H, br d, *J*=9.8 Hz, H-6′a), 4.17–4.15 (1H, m, H-1b), 4.16–4.14 (1H, m, H-5′), 4.09 (1H, dd, *J*=9.8, 4.1 Hz, H-6′b), 3.75–3.73 (1H, m, H-3a), 3.56–3.54 (1H, m, H-3b), 2.44 (3H, s, aromatic methyl), 2.31–2.29 (4H, m, α-methylenes of acyl portions), 2.04 (6H, s, OAc), 1.98 (3H, s, OAc), 1.60–1.58 (4H, m, β-methylenes of acyl portions), 1.38–1.23 (56H, m, aliphatic protons), 0.91–0.88 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.7, 172.5 (C, acyl ester carbons), 129.6, 127.9 (CH, aromatic methynes), 95.8 (CH, C1′), 70.4 (CH, C2′), 69.8 (CH, C3′), 69.5 (CH, C2), 68.4 (CH, C4′), 66.5 (CH_2, C3), 62.0 (CH_2, C1), 62.0 (CH_2, C6′), 34.0, 33.9 (CH_2, α-methylenes of acyl portions), 29.9–29.4 (CH_2, aliphatic methylenes), 24.8, 24.6 (CH_2, β-methylenes of acyl portions), 20.6, 20.5, 20.4 (CH_3, acetate methyls), 14.0, 13.9 (CH_3, acyl terminal methyls); HRESIMS *m/z* calcd for $C_{58}H_{98}NaO_{15}S$: 1089.6524; found: 1089.6521.

*4.3.11. Compound **7b**.* Compound **6b** (0.081 g, 0.089 mmol) was dissolved in anhydrous pyridine (3 mL). *p*-Tosylchloride (0.169 g, 0.888 mmol) and DMAP (0.010 g, 0.089 mmol) were added at 0 °C under argon and the reaction mixture was stirred overnight at room temperature. After evaporation under a stream of nitrogen, the mixture was purified by silica gel chromatography using a gradient of petroleum ether/diethyl ether to give compound **7b** (0.076 g, 0.073 mmol, 82%) as a colourless oil; R_f (light petroleum ether/diethyl ether 1:1)=0.28; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (2H, d, *J*=8.3 Hz, Ar–H), 7.34 (2H, d, *J*=8.3 Hz, Ar–H), 5.40–5.38 (1H, m, H-3′), 5.38–5.36 (2H, m, olefinic protons), 5.18–5.16 (1H, m, H-2), 5.01–4.99 (1H, m, H-4′), 4.92–4.90 (1H, m, H-2′), 4.73 and 4.75 (each 1H, d, *J*=3.4 Hz, H-1′), 4.31–4.29 (1H, m, H-1a), 4.26 (1H, br d, *J*=9.8 Hz, H-6′a), 4.17–4.15 (1H, m, H-1b), 4.16–4.14 (1H, m, H-5′), 4.09 (1H, dd, *J*=9.8, 4.1 Hz, H-6′b), 3.75–3.73 (1H, m, H-3a), 3.56–3.54 (1H, m, H-3b), 2.44 (3H, s, aromatic methyl), 2.31–2.29 (4H, m, α-methylenes of acyl portions), 2.06–2.03 (4H, m, allylic protons), 2.04 (6H, s, OAc), 1.98 (3H, s, OAc), 1.60–1.57 (4H, m, β-methylenes of acyl portions), 1.38–1.23 (48H, m, aliphatic protons), 0.90–0.88 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.6, 172.5 (C, acyl ester carbons), 130.2, 129.6, 127.9 (CH, olefinic and aromatic methynes), 95.9 (CH, C1′), 70.4 (CH, C2′), 69.8 (CH, C3′), 69.5 (CH, C2), 68.4 (CH, C4′), 66.5 (CH_2, C3), 62.0 (CH_2, C1), 62.0 (CH_2, C6′), 34.0, 33.9 (CH_2, α-methylenes of acyl portions), 29.9–29.4 (CH_2, aliphatic methylenes), 27.9 ($2CH_2$, allylic methylenes), 24.8, 24.6 (CH_2, β-methylenes of acyl portions), 20.6, 20.5, 20.4 (CH_3, acetate methyls), 14.1, 13.9 (CH_3, acyl terminal methyls); HRESIMS *m/z* calcd for $C_{58}H_{96}NaO_{15}S$: 1099.6368; found: 1099.6373.

*4.3.12. Compound **8a**.* Compound **7a** (0.076 g, 0.073 mmol) was dissolved in 2-butanone (7 mL) and potassium thioacetate (0.021 g, 0.183 mmol) was added. The reaction mixture was stirred at 80 °C 2.5 h, and after evaporation under reduced pressure, the mixture was purified by silica gel chromatography using a light petroleum ether/diethyl ether gradient to give compound **8a** (0.064 g, 0.065 mmol, 90%) as a colourless oil; R_f (light petroleum ether/diethyl ether 1:1)=0.60; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.41–5.39 (1H, m, H-3′), 5.20–5.18 (1H, m, H-2), 5.04–5.02 (1H, m, H-4′), 4.94–4.92 (1H, m, H-2′), 4.82 and 4.79 (each 1H, br d, *J*=3.9 Hz, H-1′), 4.33–4.31 (1H, m, H-1a), 4.19 (1H, br d, m, H-1b), 3.83–3.81 (1H, m, H-5′), 3.80–3.78 (1H, m, H-3a), 3.60–3.58 (1H, m, H-3b), 3.19–3.18 (1H, m, H-6′a), 3.12–3.10 (1H, m, H-6′b), 2.34 (3H, s, SCH_3), 2.32–2.30 (4H, m, α-methylenes of acyl portions), 2.07 (3H, s, OAc), 2.05 (3H, s, OAc), 1.99 (3H, s, OAc), 1.60–1.58 (4H, m, β-methylenes of acyl portions), 1.37–1.23 (56H, m, aliphatic protons), 0.90–0.87 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.8, 172.6 (C, acyl ester carbons), 95.8 (CH, C1′), 70.6 (CH, C4′), 70.7 (CH, C2′), 69.7 (CH, C3′), 69.4 (CH, C2), 66.1 (CH_2, C3), 62.0 (CH_2, C1), 34.1, 34.0 (CH_2, α-methylenes of acyl portions), 30.5 (CH_3, thioacetate methyl), 29.1–29.7 (CH_2, aliphatic methylenes), 29.7 (CH_2, C6′), 24.9, 24.7 (CH_2, β-methylenes of acyl portions), 20.6, 20.5, 20.5 (CH_3, acetate methyls), 14.0, 13.9 (CH_3, acyl terminal methyls); HRESIMS *m/z* calcd for $C_{53}H_{94}NaO_{13}S$: 993.6313 found: 993.6316.

*4.3.13. Compound **8b**.* Compound **7b** (0.076 g, 0.073 mmol) was dissolved in 2-butanone (7 mL) and potassium thioacetate (0.021 g, 0.183 mmol) was added. The reaction mixture was stirred at 80 °C for 2.5 h, and after evaporation under reduced pressure, the mixture was purified by silica gel chromatography using a light petroleum ether/diethyl ether gradient to give compound **8b** (0.065 g, 0.066 mmol, 91%) as a pale yellow oil; R_f (light petroleum ether/diethyl ether 1:1)=0.60; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.41–5.39 (1H, m, H-3′), 5.38–5.36 (2H, m, olefinic protons), 5.20–5.18 (1H, m, H-2), 5.04–5.02 (1H, m, H-4′), 4.94–4.92 (1H, m, H-2′), 4.82 and 4.80 (each 1H, br d, *J*=3.9 Hz, H-1′), 4.33–4.31 (1H, m, H-1a), 4.19 (1H, br d, m, H-1b), 3.83–3.80 (1H, m, H-5′), 3.80–3.78 (1H, m, H-3a), 3.60–3.58 (1H, m, H-3b), 3.19–3.17 (1H, m, H-6′a), 3.12–3.11 (1H, m, H-6′b), 2.34 (3H, s, SCH_3), 2.32–2.30 (4H, m, α-methylenes of acyl portions), 2.07 (3H, s, OAc), 2.02–2.00 (4H, m, allylic protons), 2.05 (3H, s, OAc), 1.99 (3H, s, OAc), 1.61–1.59 (4H, m, β-methylenes of acyl portions), 1.37–1.23 (48H, m, aliphatic protons), 0.91–0.88 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.7, 172.6 (C, acyl ester carbons), 130.1 (2CH, olefinic methynes), 95.8 (CH, C1′), 70.6 (CH, C4′), 70.7 (CH, C2′), 69.7 (CH, C3′), 69.4 (CH, C2), 66.1 (CH_2, C3), 62.0 (CH_2, C1), 34.1, 34.0 (CH_2, α-methylenes of

acyl portions), 30.5 (CH_3, thioacetate methyl), 29.1–29.7 (CH_2, aliphatic methylenes), 29.7 (CH_2, C6′), 27.8 ($2CH_2$, allylic methylenes), 24.9, 24.7 (CH_2, β-methylenes of acyl portions), 20.6, 20.5, 20.5 (CH_3, acetate methyls), 14.1, 13.9 (CH_3, acyl terminal methyls); HRESIMS *m*/*z* calcd for $C_{53}H_{92}NaO_{13}S$: 991.6156 found: 991.6159.

*4.3.14. Compound **9a**.* Compound **8a** (0.065 g, 0.0.066 mmol) was dissolved in a mixture of potassium acetate (0.032 g, 0.323 mmol), aq H_2O_2 (0.161 mL) (34% w/v) and acetic acid (1.9 mL); the reaction mixture was stirred overnight at 40 °C; after evaporation under a stream of nitrogen, the mixture was purified by silica gel chromatography using a gradient of chloroform/methanol to give compound **9a** (0.038 g, 0.038 mmol, 58%) as a colourless oil; R_f (chloroform/methanol 9:1)=0.32; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.41–5.39 (1H, m, H-3′), 5.23–5.21 (1H, m, H-2), 5.15–5.13 (1H, overlapped, H-1′), 5.01–4.99 (1H, m, H-4′), 4.93–4.91 (1H, m, H-2′), 4.33–4.31 (1H, m, H-1a), 4.27–4.25 (1H, m, H-1b), 4.26–4.24 (1H, m, H-5′), 3.72–3.70 (1H, m, H-3a), 3.64–3.62 (1H, m, H-3b), 3.23–3.21 (1H, m, H-6′a), 3.12–3.10 (1H, m, H-6′b), 2.31–2.29 (4H, m, α-methylenes of acyl portions), 2.04 (6H, s, OAc), 1.99 (3H, s, OAc), 1.60–1.57 (4H, m, β-methylenes of acyl portions), 1.37–1.23 (56H, m, aliphatic protons), 0.90–0.87 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.7, 172.5 (C, acyl ester carbons), 95.5 (CH, C1′), 70.9 (CH, C4′), 70.4 (CH, C3′), 70.3 (CH, C2), 70.2 (CH, C2′), 66.4 (CH_2, C3), 62.9 (CH_2, C1), 51.0 (CH_2, C6′), 34.1, 34.0 (CH_2, α-methylenes of acyl portions), 29.4–29.9 (CH_2, aliphatic methylenes), 24.9, 24.7 (CH_2, β-methylenes of acyl portions), 20.6, 20.5 (CH_3, acetate methyls), 14.0, 13.8 (CH_3, acyl terminal methyls); HRESIMS *m*/*z* calcd for $C_{51}H_{91}NaO_{15}KS$: 1037.5613; found: 1037.5616.

*4.3.15. Compound **9b**.* Compound **8b** (0.065 g, 0.0.066 mmol) was dissolved in a mixture of potassium acetate (0.032 g, 0.323 mmol), aq H_2O_2 (0.161 mL) (34% w/v) and acetic acid (1.9 mL); the reaction mixture was stirred overnight at 40 °C; after evaporation under a stream of nitrogen, the mixture was purified by silica gel chromatography using a gradient of chloroform/methanol to give compound **9b** (0.031 g, 0.031 mmol, 47%) as a colourless oil; R_f (chloroform/methanol 9:1)=0.32; IR (liquid film) ν_{max} 3400, 2940, 2829, 1748, 1200, 1081 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.41–5.39 (1H, m, H-3′), 5.38–5.36 (2H, m, olefinic protons), 5.23–5.21 (1H, m, H-2), 5.15–5.13 (1H, m, H-1′), 5.01–5.00 (1H, m, H-4′), 4.93–4.91 (1H, m, H-2′), 4.33–4.31 (1H, m, H-1a), 4.27–4.25 (1H, m, H-1b), 4.26–4.24 (1H, m, H-5′), 3.72–3.70 (1H, m, H-3a), 3.63–3.62 (1H, m, H-3b), 3.23–3.21 (1H, m, H-6′a), 3.11–3.10 (1H, m, H-6′b), 2.31–2.29 (4H, m, α-methylenes of acyl portions), 2.04 (6H, s, OAc), 2.03–2.00 (4H, m, allylic protons), 1.99 (3H, s, OAc), 1.59–1.57 (4H, m, β-methylenes of acyl portions), 1.37–1.23 (48H, m, aliphatic protons), 0.89–0.87 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.7, 172.6 (C, acyl ester carbons), 130.2 (2CH, olefinic methynes), 95.5 (CH, C1′), 70.9 (CH, C4′), 70.4 (CH, C3′), 70.3 (CH, C2), 70.2 (CH, C2′), 66.4 (CH_2, C3), 62.9 (CH_2, C1), 51.0 (CH_2, C6′), 34.1, 34.0 (CH_2, α-methylenes of acyl portions), 29.4–29.9 (CH_2, aliphatic methylenes), 27.7 ($2CH_2$, allylic methylenes), 24.9, 24.7 (CH_2, β-methylenes of acyl portions),20.6, 20.5 (CH_3, acetate methyls), 14.1, 13.8 (CH_3, acyl terminal methyls); HRESIMS *m*/*z* calcd for $C_{51}H_{89}NaO_{15}KS$: 1035.5457; found: 1035.5461.

*4.3.16. Compound **10a**.* Compound **9a** (0.042 g, 0.042 mmol) was dissolved in aq ethanol (85%) (4.7 mL), hydrazine monohydrate (0.064 g, 1.28 mmol) was added, and the reaction mixture was stirred for 3 h at 44 °C. After evaporation under a stream of nitrogen, the mixture was purified by silica gel chromatography using a gradient of chloroform/methanol to give compound **10a** (0.027 g, 0.031 mmol, 76%) as a white solid, mp 87–92 °C; R_f (chloroform/methanol 7:3)=0.15; IR (liquid film) ν_{max} 3400, 2940, 2862, 1750, 1351, 1343 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ 5.35–5.33 (1H, m, H-2), 4.81 and 4.79 (each 1H, br d, *J*=3.3 Hz, H-1′), 4.35 (1H, dd, *J*=12.2, 7.3 Hz, H-1a), 4.22 (1H, dd, *J*=12.2, 6.6 Hz, H-1b), 4.15–4.13 (1H, m, H-4′), 4.11–4.08 (1H, m, H-5′), 4.08–4.06 (1H, m, H-3a), 3.69–3.67 (1H, m, H-3′), 3.67–3.65 (1H, m, H-3b), 3.46–3.44 (1H, m, H-2′), 3.12 (1H, dd, *J*=9.3, 9.3 Hz, H-6′a), 2.95 (1H, dd, *J*=14.4, 9.3 Hz, H-6′b), 2.38–2.34 (4H, m, α-methylenes of acyl portions), 1.64–1.62 (4H, m, β-methylenes of acyl portions), 1.39–1.27 (56H, m, aliphatic protons), 0.91–0.89 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, CD_3OD): δ 175.2, 174.9 (C, acyl ester carbons), 99.1 (CH, C1′), 74.8 (CH, C4′), 74.1 (CH, C3′), 72.9 (CH, C2′), 70.8 (CH, C2), 66.4 (CH_2, C3), 63.1 (CH_2, C1), 53.8 (CH_2, C6′), 34.1, 34.0 (CH_2, α-methylenes of acyl portions), 29.4–29.9 (CH_2, aliphatic methylenes), 24.9, 24.7 (CH_2, β-methylenes of acyl portions), 14.0, 13.8 (CH_3, acyl terminal methyls); HRESIMS *m*/*z* calcd for $C_{45}H_{85}NaO_{12}KS$: 911.5297; found: 911.5300.

*4.3.17. Compound **10b**.* Compound **9b** (0.042 g, 0.042 mmol) was dissolved in aq ethanol (85%) (4.7 mL), hydrazine monohydrate (0.064 g, 1.28 mmol) was added, and the reaction mixture was stirred for 3 h at 44 °C. After evaporation under a stream of nitrogen, the mixture was purified by silica gel chromatography using a gradient of chloroform/methanol to give compound **10b** (0.026 g, 0.030 mmol, 73%) as a white solid, mp 92–98 °C; R_f (chloroform/methanol 7:3)=0.15; IR (liquid film) ν_{max} 3400, 2940, 2829, 1750, 1352, 1340 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ 5.39–5.36 (2H, m, olefinic protons), 5.34–5.32 (1H, m, H-2), 4.81 and 4.79 (each 1H, br d, *J*=3.3 Hz, H-1′), 4.36 (1H, dd, *J*=12.2, 7.3 Hz, H-1a), 4.22 (1H, dd, *J*=12.2, 6.6 Hz, H-1b), 4.15–4.13 (1H, m, H-4′), 4.11–4.09 (1H, m, H-5′), 4.08–4.06 (1H, m, H-3a), 3.69–3.67 (1H, m, H-3′), 3.67–3.65 (1H, m, H-3b), 3.46–3.44 (1H, m, H-2′), 3.12 (1H, dd, *J*=9.3, 9.3 Hz, H-6′a), 2.95 (1H, dd, *J*=14.4, 9.3 Hz, H-6′a), 2.36–2.32 (4H, m, α-methylenes of acyl portions), 2.03–2.01 (4H, m, allylic protons), 1.65–1.62 (4H, m, β-methylenes of acyl portions), 1.39–1.27 (48H, m, aliphatic protons), 0.90–0.88 (6H, overlapped, $2CH_3$); ^{13}C NMR (100 MHz, CD_3OD): δ 175.1, 174.9 (C, acyl ester carbons), 130.5 (2CH, olefinic methynes), 99.1 (CH, C1′), 74.8 (CH, C4′), 74.1 (CH, C3′), 72.9 (CH, C2′), 70.8 (CH, C2), 66.4 (CH_2, C3), 63.1 (CH_2, C1), 53.7 (CH_2, C6′), 34.1, 34.0 (CH_2, α-methylenes of acyl portions), 29.4–29.9 (CH_2, aliphatic methylenes), 27.6 (CH_2, allylic methylene), 24.9, 24.7 (CH_2, β-methylenes of acyl portions), 14.1, 13.8 (CH_3, acyl terminal methyls); HRESIMS *m*/*z* calcd for $C_{45}H_{83}NaO_{12}KS$: 909.5140; found: 909.5144.

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References and notes

1. Benson, A. A.; Daniel, H.; Wiser, R. *Proc. Natl. Acad. Sci. U.S.A.* **1959**, *45*, 1582–1587.
2. Mizushina, Y.; Watanabe, I.; Ohta, K.; Takemura, M.; Sahara, H.; Takahashi, N.; Gasa, S.; Sugawara, F.; Matsukage, A.; Yoshida, S.; Sakaguchi, K. *Biochem. Pharmacol.* **1998**, *55*, 537–541.
3. (a) Tulloch, A. P.; Heinz, F.; Fisher, W. *Hoppe-Seyler's Z. Physiol. Chem.* **1973**, *354*, 879–889; (b) Kates, M. *Adv. Lipid Res.* **1970**, *8*, 225–265; (c) Davies, W. H.; Mercer, F. I.; Goodwin, T. W. *Phytochemistry* **1965**, *4*, 741–749; (d) Weier, T. E.; Benson, A. A. *Am. J. Bot.* **1967**, *54*, 389–402; (e) Ohta, K.; Mizushina, Y.; Hirata, N.; Takemura, M.; Sugawara, F.; Matsukage, A.; Yoshida, S.; Sakaguchi, K. *Chem. Pharm. Bull.* **1998**, *46*, 684–686.
4. Sahara, H.; Ishikawa, M.; Takahashi, N.; Ohtani, S.; Sato, N.; Gasa, S.; Akino, T.; Kikuchi, K. *Br. J. Cancer* **1997**, *75*, 324–332.
5. Ohta, K.; Mizushina, Y.; Yamazaki, T.; Hanashina, S.; Sugawara, F.; Sakaguchi, K. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 893–900.

6. Gustafson, R.; Cardelina, J. H.; Fuller, R. W.; Weislow, O. S.; Kiser, R. F.; Snader, K. M.; Patterson, G. M. L.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1254–1258.
7. (a) Loya, S.; Reshef, V.; Mizrachi, E.; Silberstein, C.; Rachamim, Y.; Carmeli, S.; Hizi, A. *J. Nat. Prod.* **1998**, *61*, 891–895; (b) Reshef, V.; Mizrachi, E.; Maretzki, T.; Silberstein, C.; Loya, S.; Hizi, A.; Carmeli, S. *J..Nat. Prod.* **1997**, *60*, 1251–1260.
8. Matsumoto, Y.; Sahara, H.; Fujita, T.; Shimozawa, K.; Takenouchi, M.; Torigoe, T.; Hanashima, S.; Yamazaki, T.; Takahashi, S.; Sugawara, F.; Mizushina, Y.; Ohta, K.; Takahashi, N.; Gasa, S.; Jimbow, K.; Sakaguchi, K.; Sato, N. *Transplantation* **2002**, *74*, 261–267.
9. (a) Murakami, C.; Yamazaki, T.; Hanashima, S.; Takahashi, S.; Ohta, K.; Yoshida, H.; Sugawara, F.; Sakaguchi, K.; Mizushina, Y. *Arch. Biochem. Biophys.* **2002**, *403*, 229–236; (b) Hanashima, S.; Mizushina, Y.; Yamazaki, T.; Ohta, K.; Takahashi, S.; Sahara, H.; Sagakuchi, K.; Sugawara, F. *Bioorg. Med. Chem.* **2001**, *9*, 367–376; (c) Hanashima, S.; Mizushina, Y.; Yamazaki, T.; Ohta, K.; Takahashi, S.; Koshino, H.; Sahara, H.; Sagakuchi, K.; Sugawara, F. *Tetrahedron Lett.* **2000**, *41*, 4403–4407; (d) Mizushina, Y.; Maeda, N.; Kawasaki, M.; Ichikawa, H.; Murakami, C.; Takemura, M.; Xu, X.; Sugawara, F.; Fukumori, Y.; Yoshida, H.; Sakaguchi, K. *Lipids* **2003**, *38*, 1065–1074; (e) Hanashima, S.; Mizushina, Y.; Ohta, K.; Yamazaki, T.; Sugawara, F.; Sakaguchi, K. *Jpn. J. Cancer Res.* **2000**, *91*, 1073–1083; (f) Ohta, K.; Hanashima, S.; Mizushina, Y.; Yamazaki, T.; Saneyoshi, M.; Sugawara, F.; Sakaguchi, K. *Mutat. Res.* **2000**, *467*, 139–152; (g) Mizushina, Y.; Xu, X.; Asahara, H.; Takemura, M.; Yamaguchi, T.; Kuroda, K.; Linn, S.; Yoshida, H.; Koiwai, O.; Saneyoshi, M.; Sugawara, F.; Sakaguchi, K. *Biochem. J.* **2003**, *370*, 299–305.
10. (a) Gordon, D. M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1992**, *114*, 659–663; (b) Gigg, R.; Penglis, A. A. E.; Conant, R. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2490–2493.
11. (a) Demchenko, A. V. *Synlett* **2003**, 1225–1240; (b) Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3215–3237.
12. Trincone, A.; Giordano, A. *Curr. Org. Chem.* **2006**, *10*, 1163–1193.
13. Koeller, K. M.; Wong, C. H. *Chem. Rev.* **2000**, *100*, 4465–4493.
14. Trincone, A.; Tramice, A.; Giordano, A.; Andreotti, G. *Biotechnol. Genet. Eng. Rev.* **2008**, *25*, 129–148.
15. Andreotti, G.; Giordano, A.; Tramice, A.; Mollo, E.; Trincone, A. *J. Biotechnol.* **2006**, *122*, 274–284.
16. (a) Sassaki, G. L.; Gorin, P. A. J.; Tisher, C. A.; Iacomini, M. *Glycobiology* **2001**, *11*, 345–351; (b) Cedergren, R. A.; Hollingsworth, R. I. *J. Lipid Res.* **1994**, *35*, 1452–1461.
17. (a) Trincone, A.; Pagnotta, E.; Tramice, A. *Bioresource Technol.* **2012**, *115*, 79–83; (b) Tramice, A.; Andreotti, G.; Trincone, A. *Mar. Biotechnol.* **2011**, *13*, 773–781; (c) Tramice, A.; Andreotti, G.; Trincone, A. *Biotechnol. J.* **2008**, *3*, 545–554.
18. Sawai, T.; Hehere, E. J. *J. Biol. Chem.* **1962**, 2047–2052.
19. Takenaka, F.; Uchiyama, H. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 1821–1826.
20. Nakano, H.; Kiso, T.; Okamoto, K.; Tomita, T.; Bin Abdul Manan, M.; Kitahata, S. *J. Biosci. Bioeng.* **2003**, *95*, 583–588.
21. Park, K. H.; Yoon, Y. J.; Lee, S. G. *Tetrahedron Lett.* **1994**, *35*, 9737–9740.
22. Manzo, E.; Ciavatta, M. L.; Pagano, D.; Fontana, A. *Tetrahedron Lett.* **2012**, *53*, 879–881.
23. Chen, J.; Profit, A. A.; Prestwitch, G. D. *J. Org. Chem.* **1996**, *61*, 6305–6312.
24. Wahlstrom, J. L.; Ronald, R. C. *J. Org. Chem.* **1998**, *63*, 6021–6022.
25. Fernandez-Bolanos, J. G.; Morales, J.; Garcia, S.; Dianez, M. J.; Estrada, M. D.; Lopez-Castro, A.; Perez, S. *Carbohydr. Res.* **1993**, *248*, 1–14.
26. (a) Janwitayanuchit, W.; Sunwanborirux, K.; Patarapanich, C.; Pummangura, S.; Lipipun, V.; Vilaivan, T. *Phytochemistry* **2003**, *64*, 1253–1264; (b) Bashkatova, A. I.; Smirnova, V. N.; Shvets, V. I.; Evstigneeva, R. P. *Zh. Org. Khim.* **1971**, *7*, 1707–1714; (c) Bashkatova, A. I.; Volynskayaya, V. N.; Smirnova, V. N.; Shvets, V. I.; Evstigneeva, R. P. *Zh. Org. Khim.* **1972**, *8*, 548–552; (d) Bashkatova, A. I.; Senyushkina, N. N.; Shvets, V. I.; Evstigneeva, R. P. *Zh. Org. Khim.* **1972**, *8*, 2317–2322; (e) Bashkatova, A. I.; Shvets, V. I.; Evstigneeva, R. P. *Zh. Org. Khim.* **1972**, *8*, 2323–2325; (f) Bashkatova, A. I.; Smirnova, V. N.; Volynskaya, V. N.; Shvets, V. I.; Evstigneeva, R. P. *Zh. Org. Khim.* **1973**, *9*, 1422–1429; (g) Kaplum, A. P.; Shvets, V. I.; Evstigneeva, R. P. *Bioorg. Khim.* **1977**, *3*, 165–172; (h) Kaplum, A. P.; Shvets, V. I.; Evstigneeva, R. P. *Zh. Org. Khim.* **1977**, *13*, 1483–1488; (i) Shvets, V. I.; Bashkatova, A. I.; Evstigneeva, R. P. *Chem. Phys. Lipids* **1973**, *3*, 267–285; (l) Batrakov, S. G.; Il'ina, E. F.; Panoysan, A. G. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1976**, *3*, 626–632; (m) Nagatsu, A.; Watanabe, M.; Ikemoto, K.; Hashimoto, M.; Murakami, N.; Sakakibara, J.; Tokuda, H.; Nishino, H.; Iwashima, A.; Yazawa, K. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1619–1622; (n) Mannock, D.; Lewis, R. N. A. H.; McElhaney, R. N. *Chem. Phys. Lipids* **1987**, *43*, 113–127.
27. *Protective Groups in Organic Synthesis*; Green, T. W., Wuts, P. G. M., Eds.; Wiley-Intersciences: 1999, pp 207–215, 716–719.
28. Ota, M.; Okamoto, T.; Wakabayashi, H. *Carbohydr. Res.* **2009**, *344*, 460–465.
29. Sárka, M.; Hana, D.; Richard, H.; Blanka, K. *Carbohydr. Res.* **1999**, *322*, 209–218.