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ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 815–826
Bioorganic & Medicinal Chemistry

Chemoenzymatic synthesis and antimicrobial activity evaluation of monoglucosyl diglycerides

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Received 19 June 2006; revised 17 October 2006; accepted 23 October 2006
Available online 25 October 2006

Abstract—Monoglucosyl diglycerides with medium-long length fatty acid acyl chains were prepared and examined for antimicrobial activity against Gram-positive, Gram-negative bacteria and fungi. The study of their in vitro antimicrobial activity confirms the significant activity of some monoglucosyl diacylglycerol analogues and establishes for the glucose series that the 1,2-disubstitution and the octanoyl chain are the proper structural features for the maximum activity.

1. Introduction

Glycoglycerolipids are common constituents of plant cell membranes and bacterial cell walls.[1–4]

Various glycolipids have been isolated from plants, such as spinach,[5,6] from algae,[7] and bacteria.[4,8] The recent discovery of a multigenic family of monogalactosyl diacylglycerol synthases raised the possibility that multiple isoenzymes might carry out monogalactosyl diacylglycerol synthesis in various tissues and developmental stages.[9,10]

Recently, we investigated the active principles from various species of Euphorbiaceae and isolated monogalactosyl diacylglycerols (MGDGs), monogalactosyl monoacylglycerols (MGMGs), and digalactosyl diacylglycerols (DGDGs) as the active components, after repeated chromatography.[11] MGDGs, MGMGs, and DGDGs, a class of glycolipids, have attracted much attention in recent years because of their biological activities, such as anti-tumor-promoting,[5,6,8,12,13] oxygen scavenging,[14] anti-viral,[15,16] anti-inflammatory,[17] and anti-hyperlipemic activities.[18] The activity of such compounds seems strictly related to the acyl chain length.[12,13,18]

Previous pharmacological studies of methanol extracts from Euphorbiaceae showed both antimicrobial and anti-inflammatory activities.[19,20] Antimicrobial monogalactosyl diacylglycerols (MGDGs) have been isolated and characterized from the brown algae *Sargassum stolonifolium* Phang et Yoshida.[7] However, glycoglycerolipids are usually available from natural sources in only limited quantities and as hardly separable mixtures in terms of their fatty acid compositions so that their versatile synthesis seems to be of importance, especially for their biological investigation. Currently, different synthetic studies of various analogues of natural glycoglycerolipids and their modified forms with unnatural structures are intensively being developed.[21–25] Besides, a method for identifying monoglycosyl diglycerides in lipid extracts by using combined gas chromatography-mass spectrometry was reported.[26]

On the basis of these evidences, which indicate the nature of the lipophilic chain as a crucial structural feature for the activity of many compounds, we decided to prepare glycoglycerolipid analogues in order to study the effect against Gram-positive, Gram-negative bacteria and fungi. In this paper, we report the synthesis of a series of mono-acid and mixed-acid 1,2-*O*-diacyl-3-*O*-β-glucopyranosyl-*rac*-glycerols (**9a**–**h**, **13a**–**c**, and **14a**–**c**)

Keywords: Synthesis; Monoglucosyl diglycerides; 1,2-*O*-Diacyl-3-*O*-β-D-glucopyranosyl-glycerols; Antimicrobial activity; Lipases.
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doi:10.1016/j.bmc.2006.10.045

with medium-long length (from C_8 to C_{18}) acyl chains. In particular, as concerning the mixed-acid 1,2-*O*-diacyl-3-*O*-β-glucopyranosyl-*rac*-glycerols (**13a**–**c**, **14a**–**c**), the effects on antimicrobial activity of long-chain polyunsaturated fatty acids (PUFA) located at the mid-position with medium-chain fatty acids (MCFA) at one of the end-position of the glycerol back-bone have been studied.

Besides, the compounds obtained in this study, in analogy with the structured lipids, whose biological activity seems to be directly influenced by the presence of determined fatty acids in predetermined positions of the glycerol moiety, should have an interesting application as nutraceutic compounds.[27–29]

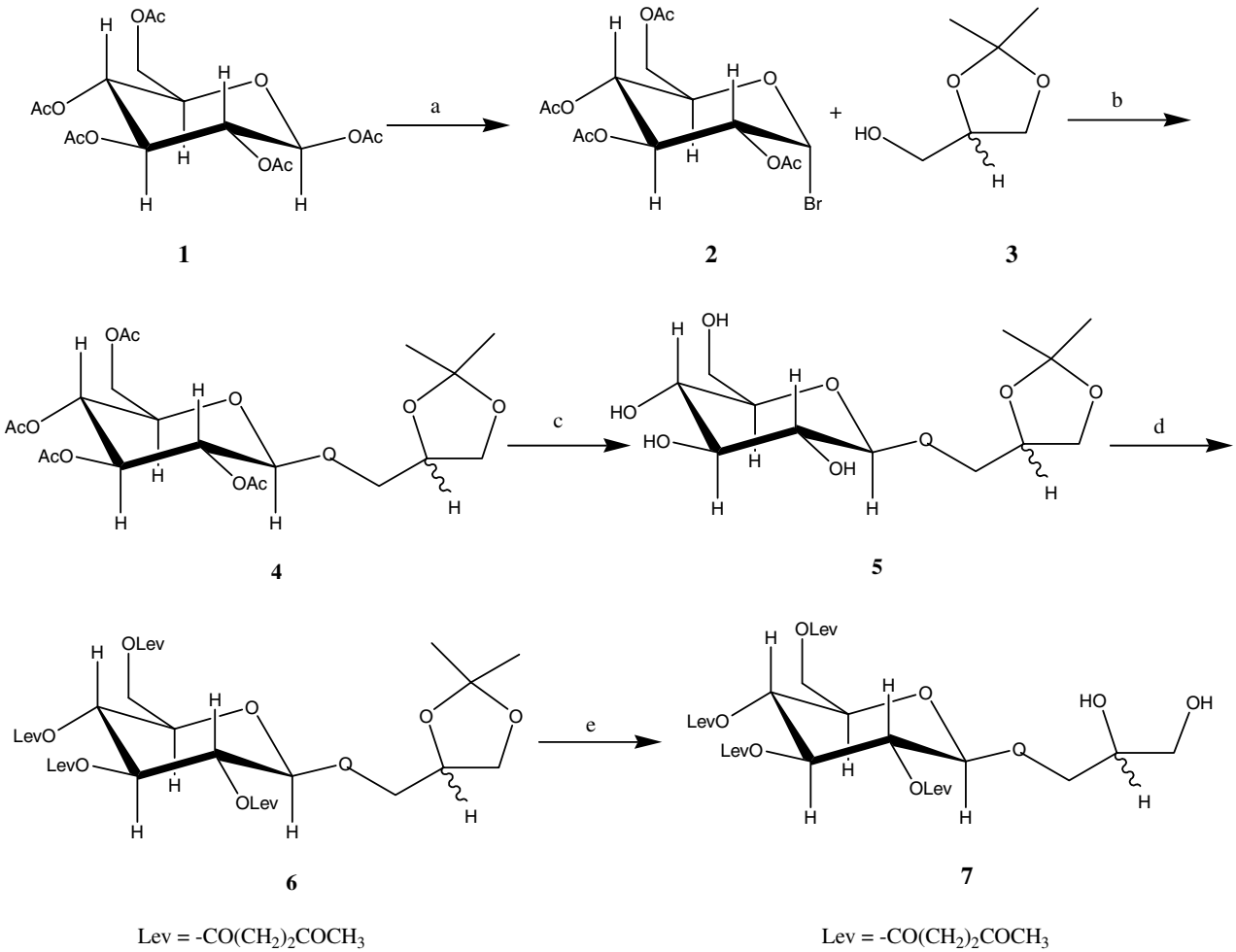
2. Results and discussion

2.1. Chemistry

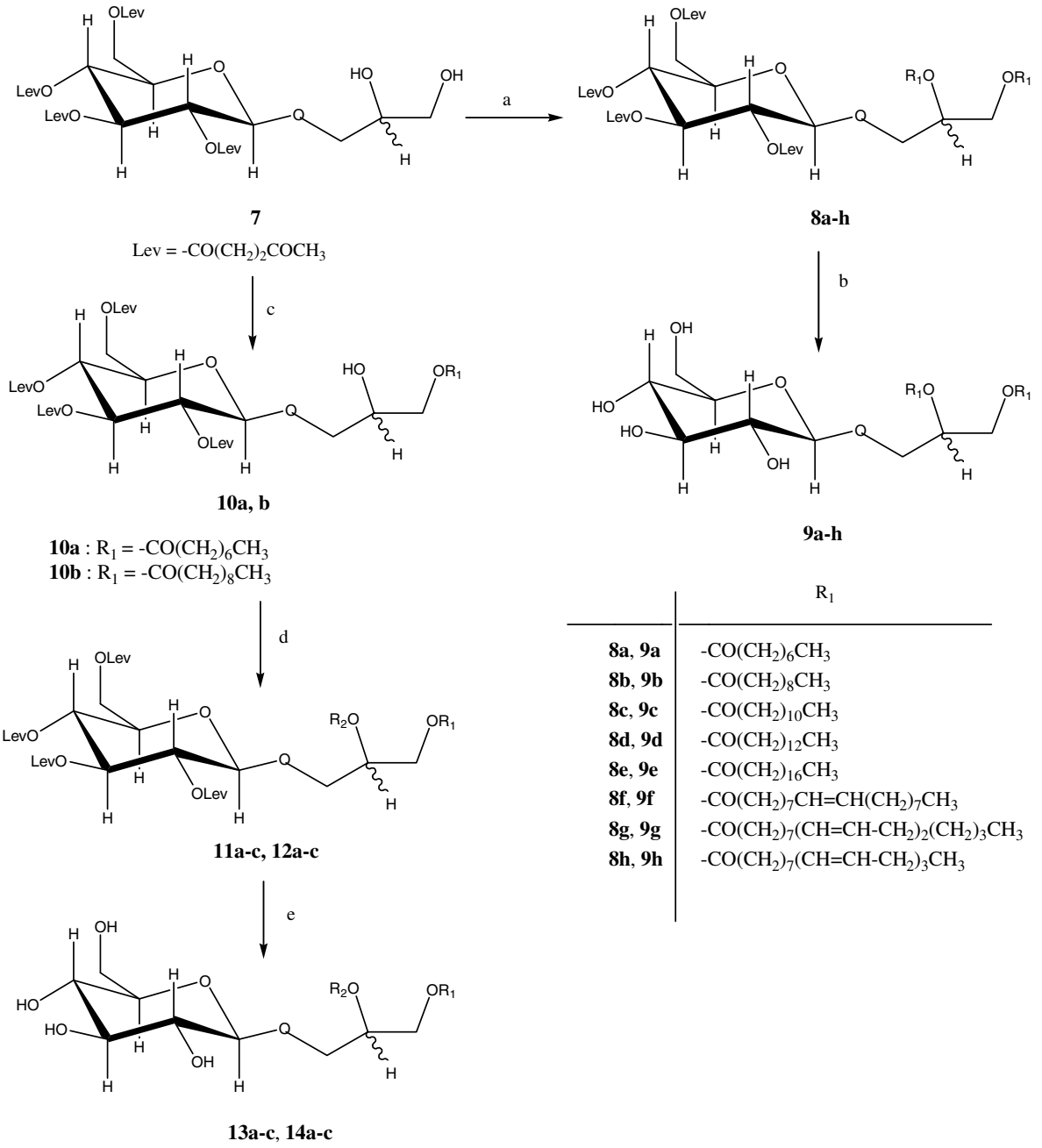
Glucosyl diglycerides (**9a**–**h**, **13a**–**c**, and **14a**–**c**) bearing either saturated or unsaturated fatty acids were synthesized as depicted in Schemes 1 and 2. Glucosidation of D,L-α,β-isopropylideneglycerol (**3**) under Köenigs-Knorr conditions[16] by using tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**2**) in dichloromethane in the presence of silver carbonate gave the desired peracetylated β-glucoside (**4**) [^{1}H NMR signals of anomeric protons: δ 4.61, 4.63 (dd, $J = 8$ Hz) indicated a 1:1 ratio of diastereomeric products] in 70% yield.

Removal of all the acyl protective groups of **4** by alkaline hydrolysis afforded **5** which was subjected to protection of the resulting free hydroxyl groups as levulinate esters (**6**). Thus, successive treatment of **6** with 70% acetic acid in aqueous solution at 60 °C in order to remove the isopropylidene protecting group provided the diol intermediate (**7**) in quantitative yield (Scheme 1). The intermediate compound **7** represents the key synthon for the synthesis of the mono-acid and mixed-acid 1,2-*O*-diacyl-3-*O*-glucosylglycerols (**9a**–**b**, **13a**–**c**, and **14a**–**c**) (Scheme 2).

The mono-acid 1,2-*O*-diacyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerols (**8a**–**h**) were prepared by one-step diacylation of **7** with 2 equivalents of the desired fatty acids in the presence of dicyclohexylcarbodiimide (DCCD) and 4-dimethylaminopyridine (DMAP) in dry dichloromethane at room temperature.



Scheme 1. Reagents and conditions: (a) HBr, CH_3CO_2H, rt; (b) Ag_2CO_3, $CaSO_4$, I_2, CH_2Cl_2, rt; (c) $NaOCH_3$, CH_3OH; (d) $[CH_3CO(CH_2)_2CO]_2O$, Py; (e) 70% CH_3CO_2H, 60 °C.



	R_1
8a, **9a**	$-CO(CH_2)_6CH_3$
8b, **9b**	$-CO(CH_2)_8CH_3$
8c, **9c**	$-CO(CH_2)_{10}CH_3$
8d, **9d**	$-CO(CH_2)_{12}CH_3$
8e, **9e**	$-CO(CH_2)_{16}CH_3$
8f, **9f**	$-CO(CH_2)_7CH{=}CH(CH_2)_7CH_3$
8g, **9g**	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_2(CH_2)_3CH_3$
8h, **9h**	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_3CH_3$

	R_1	R_2
11a, **13a**	$-CO(CH_2)_6CH_3$	$-CO(CH_2)_7CH{=}CH(CH_2)_7CH_3$
11b, **13b**	$-CO(CH_2)_6CH_3$	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_2(CH_2)_3CH_3$
11c, **13c**	$-CO(CH_2)_6CH_3$	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_3CH_3$
12a, **14a**	$-CO(CH_2)_8CH_3$	$-CO(CH_2)_7CH{=}CH(CH_2)_7CH_3$
12b, **14b**	$-CO(CH_2)_8CH_3$	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_2(CH_2)_3CH_3$
12c, **14c**	$-CO(CH_2)_8CH_3$	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_3CH_3$

Scheme 2. Reagents and conditions: (a) fatty acid, DCCD, DMAP, CH_2Cl_2, rt; (b) NH_2NH_2 (1 M, Py/CH_3CO_2H/3:2); (c) *Mucor miehi*, vinyl caprylate (vinyl caprate), 37 °C; (d) fatty acid, DCCD, DMAP, CH_2Cl_2, rt; (e) NH_2NH_2 (1 M, Py/CH_3CO_2H/3:2).

We selected octanoate, caprate, laurate, myristate, and stearate as introduction acyl groups, because they are components of bioactive analogues of natural glycoglycerolipids,[21–25] and oleate, linolate, and linolenate that are the major acyl components of MGDGs, MGMGs, and DGDGs isolated from Euphorbiaceae.[11] Finally, the levulinyl protective groups of **8a–h** were removed by treatment with hydrazine hydrate and mono-acid 1,2-*O*-diacyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol derivatives with different acyl groups (**9a–h**) were obtained (Scheme 2).

The synthesis of mixed-acid 1,2-*O*-diacyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerols (**13a–c**, **14a–c**) requires full regioselectivity control and can hardly be undertaken by synthetic organic chemistry methods without multi-step protection-deprotection processes.[18] Based on their high regioselectivity, lipases are ideally suited as biocatalysts for the synthesis of structured lipids, by acting preferably or exclusively at the primary positions of the glycerol moiety.[27–29] Another important feature offered by lipases is the mild conditions under which they act, which may become crucial in hampering intramolecular acyl-migration side-reactions. A chemoenzymatic approach was developed for the synthesis of 2-*O*-β-D-glucosylglycerol derivatives through a direct lipase-catalyzed monoacylation of the substrate mediated by *Pseudomonas cepacia* lipase (LPS).[22]

To get the intermediate adducts **10a** and **10b** (Scheme 2), compound **7**, obtained by standard procedures as depicted in Scheme 1, was directly submitted to transesterification catalyzed by *Mucor miehei* lipase in the presence of the appropriate octanoate (**10a**) and caprate (**10b**) vinyl esters in dry dichloromethane as solvent. The reactions were completely regioselective and afforded, through the expected acylation of the primary hydroxyl group,[27–29] the 1-*O*-acyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerols **10a** and **10b** in good yields (95–98%) (Scheme 2). There were no signs of any acyl-migration taking place and subsequently, in the second step, pure oleic, linoleic, and linolenic fatty acids were chemically introduced to the remaining mid-position by DCCD coupling agent in excellent yields (58–87%) to give the intermediate products **11a–c**, **12a–c** with the hydroxyl groups of the glucosyl moiety temporarily protected as levulinates. Finally, the levulinyl protective groups of **11a–c** and **12a–c** were removed by treatment with hydrazine in acetic acid/pyridine mixture at room temperature and 1,2-*O*-diacyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerols (**13a–c**, **14a–c**) with medium-chain fatty acids (MCFAs) at the end-position and long-chain polyunsaturated fatty acids (PUFA) located at the mid-position of the glycerol back-bone were obtained.

2.2. Biological evaluation

All the synthesized 1,2-*O*-diacyl-3-*O*-β-D-glucopyranosyl-*rac*-glycerols (**9a–h**, **13a–c**, and **14a–c**) were submitted for preliminary evaluation of their in vitro antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*,

Table 1. Antimicrobial activity of the compounds **9a–h**, **13a–c**, and **14a–c** against Gram-positive, Gram-negative, tubercular bacteria and *Candida* spp.

	MIC (μg/mL)																
	9a	**9b**	**9c**	**9d**	**9e**	**9f**	**9g**	**9h**	**13a**	**13b**	**13c**	**14a**	**14b**	**14c**	Ciprofloxacin	Miconazol
Staphylococcus aureus ATCC 25923	16	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	8	>128	>128	0.5	—
Staphylococcus aureus ATCC 105487	32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32	>128	0.25	—
Enterococcus faecalis ATCC 29212	32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	1	—
Bacillus subtilis ATCC 6633	16	>128	>128	>128	>128	>128	128	>128	128	128	128	32	128	>128	0.25	—
Escherichia coli ATCC 25922	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	0.25	—
Pseudomonas aeruginosa ATCC 27753	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1	—
Staphylococcus aureus 52	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>4	—
Staphylococcus aureus 85	16	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	32	64	0.5	—
Staphylococcus aureus 87	16	64	>128	>128	>128	>128	64	>128	64	64	64	>128	32	32	0.5	—
Mycobacterium tuberculosis H37Rv	64	128	>128	>128	>128	>128	64	>128	64	64	64	>128	>128	>128	0.5	—
Mycobacterium tuberculosis H160	64	64	>128	>128	>128	>128	64	>128	64	128	128	>128	>128	>128	0.5	—
Mycobacterium tuberculosis H190	64	64	>128	>128	>128	>128	128	>128	64	64	64	>128	>128	>128	0.5	—
Candida albicans	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	—	8
Candida pseudotropicalis	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	—	0.5

Pseudomonas aeruginosa, and *Mycobacterium tuberculosis*, and for antimycotic activity against *Candida albicans* and *Candida pseudotropicalis*. Antimicrobial activity was always evaluated by reference methods. Ciprofloxacin was chosen as a standard in antibacterial activity measurements, as it has excellent activity against most Gram-negative and Gram-positive bacteria, and is known as an antibacterial drug in the treatment of a wide range of infections.[30] Table 1 shows MIC values of the synthesized compounds. No activity was exhibited against Candida spp.

The data (Table 1) showed that glucosyl diglycerides bearing the same saturated fatty acyls (**9a**–**e**) at C-1 and C-2 displayed no inhibitory activity, except for **9a** and **9b** which contain a dicapryl (C8:0) (**9a**) and a dicaprinyl (C10:0) (**9b**) moiety, respectively.

On the other hand, compounds bearing the same unsaturated fatty acyls (**9f**–**h**) and mixed fatty acyls (**13a**–**c**, **14a**–**c**) exhibited higher inhibitory activity than those bearing saturated fatty acyls. A total of 6 compounds were active against a *M. tuberculosis* reference strain (H37Rv) and two different human clinical isolates (H160, H190) with MICs ranging from 64 to 128 μg/mL.

The antibacterial activity of the compounds was lost increasing the length of the saturated acyl moiety (**9c**–**e**). The presence of a single double bond within the acyl chain (18:1) (**9f**) did not modify the biological activity. The compound **9g**, characterized by the presence of two linolate acyl groups in the positions 1 and 2 of the glycerol moiety, displayed inhibitory activity against *M. tuberculosis* reference and clinical strains, *B. subtilis*, and one clinical strain of *S. aureus* with MIC ranging from 64 to 128 μg/mL. The compound **9h** bearing three unsaturations within the acyl moiety did not show any antibacterial activity.

3. Conclusions

In conclusion, this study has allowed us to prepare and characterize a series of 1,2-*O*-diacyl-3-*O*-β-D-glucopyranosyl glycerols and some of them proved to be antibacterial agents. We have examined the antimicrobial and antifungal activities of all compounds and some structure–activity relationships were explored. It was observed that the antimicrobial activity in this class of compounds is dependent on the nature of fatty acids; the octanoyl chain is the proper structural feature for the maximum activity (**9a**). This observation is confirmed by the results showing that compounds containing the octanoyl fatty acyl moiety at C-1 and unsaturated fatty acids at C-2 position of glycerol (**13a**–**c**) displayed significant activity against *B. subtilis*, *S. aureus* 87, and *M. tuberculosis* reference and clinical strains. The compounds **14a**–**c**, which differ from the derivatives **13a**–**c** for the presence of a decanoyl chain at the C-1 position of glycerol, displayed an interesting activity against *S. aureus* (**14a**–**c**), *E. faecalis* (**14b**), *B. subtilis* (**14a**–**b**), but they were devoid of activity against *M. tuberculosis*.

These results reveal the potential of these compounds as a new type of antimicrobial agents, although the mechanism of action must be further investigated.

4. Experimental

4.1. General procedures

^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Gemini 200 MHz spectrometer and a Varian Unity 400 MHz spectrometer. ^{13}C NMR: 90.5 MHz, Gemini 200 spectrometer. NMR spectra were obtained by using $CDCl_3$ as solvent; chemical shifts are expressed as δ units (ppm) relative to tetramethylsilane (TMS) as internal standard. The abbreviations s, d, dd, t, q, m, and sb refer to singlet, doublet, doublet of doublet, triplet, quartet, multiplet, and singlet broad signal, respectively. The EI-MS spectra were measured with a VG-ZAB 2F spectrometer. The ionizing energy was 70 eV in all cases and compounds were introduced by direct insertion. Electrospray analysis: API Perkin Elmer (voltage + 5600 with orifice 90 and/or 120).

The column chromatography was performed by using silica gel (Kieselgel 60, 230–400 Mesh, 60 Å Merck). 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 and heating at 110 °C. *M. miehei* (Lipozyme®, specific activity 42 U/g) was purchased from Fluka, Italy. Infrared spectra were recorded as film on a Jasco FT-IR-200 model spectrophotometer. Reagents (Fluka, Italy) used were of AR grade and all solvents for synthesis, extraction, and column chromatography were distilled and dried before use. Evaporation under reduced pressure was always effected with the bath temperature kept below 40 °C. The elemental analyses of all the compounds were consistent with the theoretical.

4.1.1. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (2). HBr in glacial acetic acid (33%) was added dropwise to a solution of pentaacetylglucose (**1**) (2 mmol) in acetic acid (3 mL). The reaction mixture was stirred in the dark overnight at room temperature, then ice-cold water (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3× 20 mL). The combined CH_2Cl_2 layers were washed with a saturated $NaHCO_3$ solution, H_2O, and brine, and the filtrate was evaporated in vacuo to give a colorless syrupy mixture recrystallized from Et_2O. Yield (**2**): 70%. R_f = 0.85 (EtOAc/hexane/1:1, v:v). ^{1}H NMR ($CDCl_3$): δ 2.0–2.16 (s, 12H, 4× CH_3CO), 4.05 (m, 1H, H-5), 4.10–4.40 (m, 2H, H_2-6), 5.06 (dd, J_1 = 10.4 Hz, J_2 = 3.4 Hz, 1H, H-3), 5.33 (dd, J_1 = 8.4 Hz, J_2 = 10.4 Hz, 1H, H-2), 5.39 (dd, J_1 = 3.4 Hz, J_2 = 1.2 Hz, 1H, H-4), 6.12 (d, J_1 = 3.8 Hz, 1H, H-1). ESI-MS: m/z = 434 (M+Na, 15%)$^+$.

4.2. General procedure for glycosylation

A mixture of 2,2-dimethyl-4-hydroxymethyl-1,3-dioxolan (**3**) (1.2 mmol), Ag_2CO_3 (1 mmol), and fine Drierite

(2.0 g) in dry dichloromethane (30 mL) was stirred in the dark under N_2. After being stirred for half an hour, iodine (0.1 mmol) was added and acetobromo-α-D-glucose (**2**) (1 mmol) in dry dichloromethane (10 mL) was then added dropwise over a period of 1 h. The reaction mixture was stirred overnight at room temperature, filtered on Celite, and the filtrate was evaporated in vacuo to give a colorless syrupy mixture.

The mixture was purified by 'flash chromatography' on silica gel, eluted with EtOAc/hexane (1:1, v:v) to give the 1:1 mixtures of the two diastereoisomers of **4**.

4.2.1. 1,2-Isopropylidene-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetyl-glucopyranosyl)-*rac*-glycerol (4). Yield: 70%. R_f = 0.60 (EtOAc/hexane/1:1, v:v). ^{1}H NMR ($CDCl_3$): δ 1.38, 1.43 (s, 6H, 2× CH_3), 2.01, 2.03, 2.05, 2.10 (s, 12H, 4× COCH_3), 3.60–4.10 (m, 6H, 5H-glycerol, H-5′), 4.0–4.35 (m, 2H, H_2-6′), 4.61 (dd, J = 8.0 Hz, 1H, H-1′), 4.90 and 5.01 (dd, J = 9.9 and 7.8 Hz, 1H, H-3′), 5.10 (dd, J = 9.9 and 9.6 Hz, 1H, H-2′), 5.22 (dd, J = 9.4 Hz, 1H, H-4′). ESI-MS: m/z = 485 (M+Na, 18%)$^+$.

4.3. General procedure for removal of acetyl protecting groups from 1,2-isopropylidene-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-acetyl-glucopyranosyl)-*rac*-glycerol (4)

CH_3ONa (10 mmol) was added to a solution of **4** (1 mmol) in dry methanol (10 mL). The reaction mixture was stirred at room temperature for 2 h, then the solution was neutralized with Amberlist-15, filtered through Celite, and concentrated. The mixture was purified by 'flash chromatography' on silica gel, eluted with EtOAc/MeOH (1:1, v:v) to give the 1:1 mixtures of the two diastereoisomers of **5**.

4.3.1. 1,2-Isopropylidene-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (5). Yield: 95%. R_f = 0.40 (EtOAc/MeOH/5:1, v:v). ^{1}H NMR ($CDCl_3$): δ 1.38, 1.43 (s, 6H, 2× CH_3), 3.23 (dd, 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′), 3.53–4.10 (m, 6H, 5H-glycerol and H-4′), 3.71 (dd, 1H, $J_{6'a,6'b}$ = 12.0 Hz, H-6′a), 3.75 (dd, 1H, H-6′b), 4.95 (d, 1H, H-1′). EI-MS (70 eV): m/z = 293 (M, 18%)$^+$, 179 (M−$C_6H_{11}O_2$, 28%)$^+$, 113 (M−$C_6H_{11}O_6$, 100%)$^+$.

4.4. General procedure for preparation of levulinate esters

To a solution of 1,2-isopropylidene-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (**5**) (2.1 mmol) in dry pyridine (10 mL) at room temperature, under an inert atmosphere, levulinic anhydride (16 mmol) dissolved in dry pyridine (6 mL) was added dropwise. The reaction mixture was stirred for 24 h and then ice water was added. The mixture was extracted with chloroform (3× 30 mL) and then the combined organic layers were extracted with a 10% solution of sodium bicarbonate (2× 50 mL) first and then with water, dried over Na_2SO_4, and concentrated in vacuo.

The compound **6** was employed in the next step without further purification.

4.4.1. 1,2-Isopropylidene-3-*O*-(2′,3′,4′,6′-tetra-*O*-levulinyl-β-D-glucopyranosyl)-*rac*-glycerol (6). Yield: 80%. R_f = 0.60 (CH_2Cl_2/MeOH/1:1, v:v). ^{1}H NMR ($CDCl_3$): δ 1.22, 1.27 (2s, 6H, 2× CH_3), 2.10 (4s, 12H, 4× CH_3CO), 2.60 (br t, 8H, 4× CH_2OCO), 2.84 (br t, 8H, 4× CH_2COCH$_3$), 3.60–3.82 (m, 6H, 5H-glycerol, H-5′), 4.0–4.35 (dd, 2H, J = 12.0 Hz, H_2-6′), 4.60 (dd, $J_{1',2'}$ = 8.0 Hz, 1H, H-1′), 5.01 (dd, $J_{2',3'}$ = 9.8 Hz, 1H, H-2′), 5.13 (dd, $J_{4',3'}$ = 10.0 Hz, 1H, H-4′), 5.36 (dd, 1H, H-3′). ESI-MS: m/z = 773 (M+Na, 12%)$^+$.

4.5. General procedure for deacetonization

A solution of **6** (1.90 mmol) in 70% aq acetic acid (10 mL) was stirred at 60 °C for 4 h. The mixture was concentrated and co-evaporated with toluene four times. The residue was then purified by silica gel 'flash chromatography,' eluted with $CHCl_3$/MeOH (10:1, v:v) to give **7** as a semisolid.

4.5.1. 3-*O*-(β-D-2′,3′,4′,6′-Tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (7). Yield: 92%. R_f = 0.36 (EtOAc/MeOH/10:1, v:v). ^{1}H NMR ($CDCl_3$): δ 2.10 (4s, 12H, 4× CH_3CO), 2.60 (br t, 8H, 4× CH_2OCO), 2.84 (br t, 8H, 4× CH_2COCH$_3$), 3.58–3.69 (m, 4H, H_2-1, H_2-3), 3.79 (m, 1H, H-2), 3.88 (dd, 1H, J = 6.0, 3.0 Hz, H-5′), 4.0–4.35 (dd, 2H, J = 12.0 Hz, H_2-6′), 4.60 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.01 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, H-2′), 5.13 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4′), 5.37 (dd, 1H, H-3′). ESI-MS: m/z = 669 (M+Na, 50%)$^+$.

4.6. General procedure for preparation of mono-acid 1,2-*O*-diacyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerols

DCCD (0.4 mmol) was added to a solution of **7** (0.3 mmol), fatty acid (0.7 mmol), and DMAP (0.03 mmol) in dry dichloromethane (10 mL), and stirred for 24 h at room temperature. The solid was removed by filtration and the solvent evaporated in vacuo. The residue was purified by 'flash chromatography' on silica gel, eluted with EtOAc/hexane (8:1, v:v) to give the products **8a**–**h**. All compounds were obtained as the 1:1 mixtures of two diastereoisomers.

4.6.1. 1,2-*O*-Dicaprilyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (8a). Yield: 75%. R_f = 0.55 (EtOAc/hexane/8:1, v:v). ^{1}H NMR ($CDCl_3$): δ 0.90 (br t, 6H, J = 6.7 Hz), 1.22 (br s, 16H, CH_2 aliph.), 1.55 (br t, 4H, COCH$_2$CH_2), 2.10 (4s, 12H, 4× CH_3CO), 2.30 (br t, 4H, COCH_2), 2.60 (br t, 8H, 4× CH_2OCO), 2.84 (br t, 8H, 4× CH_2COCH$_3$), 3.66–3.85 (dd, 2H, J = 10.7, 6.4 Hz, H_2-3), 3.87 (dd, 1H, $J_{5',4'}$ = 10.1, $J_{5',6'a}$ = 6.0, $J_{5',6'b}$ = 3.0 Hz, H-5′), 4.0–4.13 (dd, 2H, J = 11.9, 6.6 Hz, H_2-1), 4.30–4.35 (dd, 2H, $J_{6'a,6'b}$ = 12.0 Hz, H_2-6′), 4.61 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.01 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, H-2′), 5.13 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4′), 5.24 (br s, 1H, H-2), 5.36 (dd, 1H, H-3′). ESI-MS: m/z = 921 (M+Na, 30%)$^+$.

4.6.2. 1,2-*O*-Didecanoyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (8b). Yield: 68%. R_f = 0.58 (EtOAc/hexane/8:1, v:v). ^{1}H NMR ($CDCl_3$):

δ 0.90 (br t, 6H, J = 6.7 Hz), 1.23 (br s, 24H, CH_2 aliph.), 1.55 (br t, 4H, COCH$_2$CH_2), 2.10 (4s, 12H, 4× CH_3CO), 2.31 (br t, 4H, COCH_2), 2.60 (br t, 8H, 4× CH_2OCO), 2.84 (br t, 8H, 4× CH_2COCH$_3$), 3.66–3.85 (dd, 2H, J = 10.7, 6.4 Hz, H$_2$-3), 3.87 (dd, 1H, $J_{5',4'}$ = 10.1, $J_{5',6'a}$ = 6.0, $J_{5',6'b}$ = 3.0 Hz, H-5′), 4.03–4.13 (dd, 2H, J = 11.9, 6.6 Hz, H$_2$-1), 4.30–4.37 (dd, 2H, $J_{6'a,6'b}$ = 12.0 Hz, H$_2$-6′), 4.65 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.03 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, H-2′), 5.13 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4′), 5.24 (br s, 1H, H-2), 5.36 (dd, 1H, H-3′). ESI-MS: m/z = 977 (M+Na, 26%)$^+$.

4.6.3. 1,2-*O*-Dilauroyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (8c). Yield: 66%. R_f = 0.58 (EtOAc/hexane/8:1, v:v). ^{1}H NMR (CDCl$_3$): δ 0.89 (br t, 6H, J = 6.7 Hz), 1.24 (br s, 32H, CH_2 aliph.), 1.54 (br t, 4H, COCH$_2$CH_2), 2.11 (4s, 12H, 4× CH_3CO), 2.31 (br t, 4H, COCH_2), 2.60 (br t, 8H, 4× CH_2OCO), 2.83 (br t, 8H, 4× CH_2COCH$_3$), 3.66–3.85 (dd, 2H, J= 10.6, 6.4 Hz, H$_2$-3), 3.87 (dd, 1H, $J_{5',4'}$ = 10.1, $J_{5',6'a}$ = 6.0, $J_{5',6'b}$ = 3.0 Hz, H-5′), 4.02–4.15 (dd, 2H, J = 11.9, 6.6 Hz, H$_2$-1), 4.30–4.37 (dd, 2H, $J_{6'a,6'b}$ = 12.0 Hz, H$_2$-6′), 4.64 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.03 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, H-2′), 5.13 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4′), 5.24 (br s, 1H, H-2), 5.36 (dd, 1H, H-3′). ESI-MS: m/z = 1033 (M+Na, 24%)$^+$.

4.6.4. 1,2-*O*-Dimyristoyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (8d). Yield: 86%. R_f = 0.65 (EtOAc/hexane/8:1, v:v). ^{1}H NMR (CDCl$_3$): δ 0.90 (br t, 6H, J = 6.7 Hz), 1.24 (br s, 40H, CH_2 aliph.), 1.55 (br t, 4H, COCH$_2$CH_2), 2.10 (4s, 12H, 4× CH_3CO), 2.31 (br t, 4H, COCH_2), 2.60 (br t, 8H, 4× CH_2OCO), 2.84 (br t, 8H, 4× CH_2COCH$_3$), 3.66–3.85 (dd, 2H, J = 10.7, 6.4 Hz, H$_2$-3), 3.87 (dd, 1H, $J_{5',4'}$ = 10.1, $J_{5',6'a}$ = 6.0, $J_{5',6'b}$ = 3.0 Hz, H-5′), 4.0–4.15 (dd, 2H, J = 11.9, 6.6 Hz, H$_2$-1), 4.30–4.37 (dd, 2H, $J_{6'a,6'b}$ = 12.0 Hz, H$_2$-6′), 4.65 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.03 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, H-2′), 5.13 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4′), 5.24 (br s, 1H, H-2), 5.36 (dd, 1H, H-3′). ESI-MS: m/z = 1089 (M+Na, 48%)$^+$.

4.6.5. 1,2-*O*-Distearoyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (8e). Yield: 83%. R_f = 0.65 (EtOAc/hexane/8:1, v:v). ^{1}H NMR (CDCl$_3$): δ 0.89 (br t, 6H, J = 6.6 Hz), 1.24 (br s, 56H, CH_2 aliph.), 1.59 (br t, 4H, COCH$_2$CH_2), 2.12 (4s, 12H, 4× CH_3CO), 2.40 (br t, 4H, COCH_2), 2.60 (br t, 8H, 4× CH_2OCO), 2.83 (br t, 8H, 4× CH_2COCH$_3$), 3.66–3.85 (dd, 2H, J = 10.7, 6.4 Hz, H$_2$-3), 3.87 (dd, 1H, $J_{5',4'}$ = 10.1, $J_{5',6'a}$ = 6.0, $J_{5',6'b}$ = 3.0 Hz, H-5′), 4.0–4.15 (dd, 2H, J = 11.9, 6.6 Hz, H$_2$-1), 4.30–4.37 (dd, 2H, $J_{6'a,6'b}$ = 12.0 Hz, H$_2$-6′), 4.65 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.03 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, H-2′), 5.13 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4′), 5.24 (br s, 1H, H-2), 5.37 (dd, 1H, H-3′). ESI-MS: m/z = 1202 (M+Na, 41%)$^+$.

4.6.6. 1,2-*O*-Dioleoyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (8f). Yield: 72%. R_f = 0.70 (EtOAc/hexane/8:1, v:v). ^{1}H NMR (CDCl$_3$): δ 0.91 (br t, 6H, 2× CH_3 term.), 1.24 (br s, 40H, CH_2 aliph.), 1.62 (t, 4H, COCH$_2$CH_2), 2.05 (m, 8H, 4× CH_2CH=CH), 2.10 (4s, 12H, 4× CH_3CO), 2.40 (br t, 4H, COCH_2), 2.61 (br t, 8H, 4× CH_2OCO), 2.83 (br t, 8H, 4× CH_2COCH$_3$), 3.66–3.85 (dd, 2H, J = 10.6, 6.3 Hz, H$_2$-3), 3.88 (dd, 1H, $J_{5',4'}$ = 10.1, $J_{5',6'a}$ = 6.0, $J_{5',6'b}$ = 3.0 Hz, H-5′), 4.0–4.14 (dd, 2H, J = 11.9, 6.6 Hz, H$_2$-1), 4.30–4.36 (dd, 2H, $J_{6'a,6'b}$ = 12.0 Hz, H$_2$-6′), 4.65 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.03 (dd, 1H, $J_{2',3'}$ = 9.4 Hz, H-2′), 5.13 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4′), 5.24 (br s, 1H, H-2), 5.35 (dd, 1H, H-3′), 5.40 (m, 4H, 2× CH=CH). ESI-MS: m/z = 1198 (M+Na, 83%)$^+$.

4.6.7. 1,2-*O*-Dilinoleoyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (8g). Yield: 54%. R_f = 0.71 (EtOAc/hexane/8:1, v:v). ^{1}H NMR (CDCl$_3$): δ 0.91 (br t, 6H, 2× CH_3 term.), 1.32 (br s, 28H, CH_2 aliph.), 1.63 (br t, 4H, COCH$_2$CH_2), 2.02 (m, 8H, 4× CH_2CH=CH), 2.12 (4s, 12H, 4× CH_3CO), 2.35 (br t, 4H, COCH_2), 2.60 (br t, 8H, 4× CH_2OCO), 2.83 (br t, 8H, 4× CH_2COCH$_3$), 2.89 (m, 4H, 2× =CHCH_2CH=), 3.65–3.84 (dd, 2H, J = 10.6, 6.3 Hz, H$_2$-3), 3.88 (dd, 1H, $J_{5',4'}$ = 10.1, $J_{5',6'a}$ = 6.0, $J_{5',6'b}$ = 3.0 Hz, H-5′), 4.0–4.14 (dd, 2H, J = 11.8, 6.5 Hz, H$_2$-1), 4.30–4.35 (dd, 2H, $J_{6'as,6'b}$ = 12.0 Hz, H$_2$-6′), 4.65 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.03 (dd, 1H, $J_{2',3'}$ = 9.4 Hz, H-2′), 5.13 (dd, 1H, $J_{4',3'}$ = 9.9 Hz, H-4′), 5.23 (br s, 1H, H-2), 5.34 (dd, 1H, H-3′), 5.41 (m, 8H, 4× CH=CH). ESI-MS: m/z = 1194 (M+Na, 70%)$^+$.

4.6.8. 1,2-*O*-Dilinolenoyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (8h). Yield: 45%. R_f = 0.72 (EtOAc/hexane/8:1, v:v). ^{1}H NMR (CDCl$_3$): δ 0.97 (br t, 6H, 2× CH_3 term.), 1.32 (br s, 16H, CH_2 aliph.), 1.63 (br t, 4H, COCH$_2$CH_2), 2.07 (m, 8H, 4× CH_2CH=CH), 2.11 (4s, 12H, 4× CH_3CO), 2.41 (br t, 4H, COCH_2), 2.62 (br t, 8H, 4× CH_2OCO), 2.83 (br t, 8H, 4× CH_2COCH$_3$), 2.90 (m, 8H, 2× =CHCH_2CH=), 3.67–3.85 (dd, 2H, J = 10.5, 6.2 Hz, H$_2$-3), 3.87 (dd, 1H, $J_{5',4'}$ = 10.1, $J_{5',6'a}$ = 6.0, $J_{5',6'b}$ = 2.9 Hz, H-5′), 4.0–4.14 (dd, 2H, J = 11.9, 6.6 Hz, H$_2$-1), 4.30–4.36 (dd, 2H, $J_{6'a,6'b}$ = 12.0 Hz, H$_2$-6′), 4.65 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.02 (dd, 1H, $J_{2',3'}$ = 9.3 Hz, H-2′), 5.12 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, H-4′), 5.24 (br s, 1H, H-2), 5.34 (dd, 1H, H-3′), 5.40 (m, 12H, 6× CH=CH). ESI-MS: m/z = 1190 (M+Na, 72%)$^+$.

4.7. General procedure for preparation of mixed-acid 1,2-*O*-diacyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerols

To a mixture of **7** (1 mmol) and vinyl caprylate (or vinyl capriate) (1.25 mmol) in dry dichloromethane (3.0 mL) was added immobilized *M. miehei* lipase (0.06 g). The resulting mixture was stirred at 37 °C for 8 h. Then, additional lipase (0.01 g) was added to the reaction mixture which was stirred for additional 40 h at the same temperature. The lipase was separated off by filtration and the solvent removed in vacuo on a rotary evaporator. The residue was purified by 'flash chromatography' on silica gel column, eluted with EtOAc/hexane (8:1/v:v) to give the glucosyl monoglyce-

rides (**10a** and **b**) which were subsequently reacted with equimolar amounts of the second fatty acid, DCC, and a little amount of DMAP at room temperature overnight.

The solid was filtered off and the solvent was evaporated in vacuo. The residue was purified by 'flash chromatography' on silica gel column, eluted with EtOAc/hexane (8:1, v:v) to give the 1:1 mixture of two diastereoisomers **11a–c** and **12a–c**.

4.7.1. 1-*O*-Caprylyl-2-*O*-oleoyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (11a). Yield: 39%. $R_f = 0.52$ (EtOAc/hexane/8:1, v:v). ^{1}H NMR ($CDCl_3$): δ 0.95 (br t, 6H, 2× CH_3 term.), 1.28 (br s, 28H, CH_2 aliph.), 1.62 (br t, 4H, $COCH_2CH_2$), 2.00 (m, 4H, 2× CH_2CH=CH), 2.10 (4s, 12H, 4× CH_3CO), 2.40 (br t, 4H, $COCH_2$), 2.62 (br t, 8H, 4× CH_2OCO), 2.82 (br t, 8H, 4× CH_2COCH_3), 3.67–3.84 (dd, 2H, $J = 10.5$, 6.2 Hz, H_2-3), 3.87 (dd, 1H, $J_{5',4'} = 10.1$, $J_{5',6'a} = 6.0$, $J_{5',6'b} = 3.0$ Hz, H-5′), 4.10–4.14 (dd, 2H, $J = 11.9$, 6.6 Hz, H_2-1), 4.30–4.36 (dd, 2H, $J_{6'a,6'b} = 12.0$ Hz, H_2-6′), 4.64 (dd, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), 5.02 (dd, 1H, $J_{2',3'} = 9.3$ Hz, H-2′), 5.12 (dd, 1H, $J_{4',3'} = 10.0$ Hz, H-4′), 5.23 (br s, 1H, H-2), 5.34 (dd, 1H, H-3′), 5.40 (m, 2H, CH=CH). ESI-MS: m/z = 1060 (M+Na, 80%)$^+$.

4.7.2. 1-*O*-Caprylyl-2-*O*-linoleoyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glyce-rol (11b). Yield: 38%. $R_f = 0.52$ (EtOAc/hexane/8:1, v:v). ^{1}H NMR ($CDCl_3$): δ 0.93 (br t, 6H, 2× CH_3 term.), 1.28 (br s, 24H, CH_2 aliph.), 1.61 (br t, 4H, $COCH_2CH_2$), 2.01 (m, 4H, 2× CH_2CH=CH), 2.10 (4s, 12H, 4× CH_3CO), 2.40 (br t, 4H, $COCH_2$), 2.63 (br t, 8H, 4× CH_2OCO), 2.82 (br t, 8H, 4× CH_2COCH_3), 2.90 (m, 2H, =CHCH_2CH=), 3.67–3.84 (dd, 2H, $J = 10.5$, 6.2 Hz, H_2-3), 3.87 (dd, 1H, $J_{5',4'} = 10.1$, $J_{5',6'a} = 6.0$, $J_{5',6'b} = 3.0$ Hz, H-5′), 4.10–4.14 (dd, 2H, $J = 11.9$, 6.6 Hz, H_2-1), 4.30–4.36 (dd, 2H, $J_{6'a,6'b} = 12.0$ Hz, H_2-6′), 4.64 (dd, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), 5.02 (dd, 1H, $J_{2',3'} = 9.3$ Hz, H-2′), 5.12 (dd, 1H, $J_{4',3'} = 10.0$ Hz, H-4′), 5.23 (br s, 1H, H-2), 5.34 (dd, 1H, H-3′), 5.40 (m, 2H, CH=CH). ESI-MS: m/z = 1058 (M+Na, 80%)$^+$.

4.7.3. 1-*O*-Caprylyl-2-*O*-linolenoyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glyce-rol (11c). Yield: 40%. $R_f = 0.51$ (EtOAc/hexane/8:1, v:v). ^{1}H NMR ($CDCl_3$): δ 0.96 (br t, 6H, 2× CH_3 term.), 1.28 (br s, 16H, CH_2 aliph.), 1.60 (br t, 4H, $COCH_2CH_2$), 1.98 (m, 4H, 2× CH_2CH=CH), 2.11 (4s, 12H, 4× CH_3CO), 2.40 (br t, 4H, $COCH_2$), 2.61 (br t, 8H, 4× CH_2OCO), 2.82 (br t, 8H, 4× CH_2COCH_3), 2.90 (m, 4H, 2× =CHCH_2CH=), 3.66–3.85 (dd, 2H, $J = 10.5$, 6.2 Hz, H_2-3), 3.87 (dd, 1H, $J_{5',4'} = 10.0$, $J_{5',6'a} = 6.0$, $J_{5',6'b} = 3.0$ Hz, H-5′), 4.10–4.14 (dd, 2H, $J = 11.9$, 6.6 Hz, H_2-1), 4.30–4.36 (dd, 2H, $J_{6'a,6'b} = 12.0$ Hz, H_2-6′), 4.65 (dd, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), 5.02 (dd, 1H, $J_{2',3'} = 9.3$ Hz, H-2′), 5.11 (dd, 1H, $J_{4',3'} = 9.9$ Hz, H-4′), 5.23 (br s, 1H, H-2), 5.33 (dd, 1H, H-3′), 5.39 (m, 6H, 3× CH=CH). ESI-MS: m/z = 1054 (M+Na, 90%)$^+$.

4.7.4. 1-*O*-Caprinyl-2-*O*-oleoyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (12a). Yield: 36%. $R_f = 0.51$ (EtOAc/hexane/8:1, v:v). ^{1}H NMR ($CDCl_3$): δ 0.96 (br t, 6H, 2× CH_3 term.), 1.28 (br s, 32H, CH_2 aliph.), 1.62 (br t, 4H, $COCH_2CH_2$), 1.99 (m, 4H, 2× CH_2CH=CH), 2.10 (4s, 12H, 4× CH_3CO), 2.40 (br t, 4H, $COCH_2$), 2.63 (br t, 8H, 4× CH_2OCO), 2.83 (br t, 8H, 4× CH_2COCH_3), 3.67–3.85 (dd, 2H, $J = 10.5$, 6.2 Hz, H_2-3), 3.87 (dd, 1H, $J_{5',4'} = 10.1$, $J_{5',6'a} = 6.0$, $J_{5',6'b} = 3.0$ Hz, H-5′), 4.10–4.14 (dd, 2H, $J = 11.9$, 6.7 Hz, H_2-1), 4.30–4.36 (dd, 2H, $J_{6'a,6'b} = 12.0$ Hz, H_2-6′), 4.64 (dd, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), 5.02 (dd, 1H, $J_{2',3'} = 9.3$ Hz, H-2′), 5.12 (dd, 1H, $J_{4',3'} = 10.0$ Hz, H-4′), 5.23 (br s, 1H, H-2), 5.34 (dd, 1H, H-3′), 5.39 (m, 2H, CH=CH). ESI-MS: m/z = 1062 (M+Na, 100%)$^+$.

4.7.5. 1-*O*-Caprinyl-2-*O*-linoleoyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (12b). Yield: 39%. $R_f = 0.50$ (EtOAc/hexane/8:1, v:v). ^{1}H NMR ($CDCl_3$): δ 0.95 (br t, 6H, 2× CH_3 term.), 1.28 (br s, 28H, CH_2 aliph.), 1.60 (br t, 4H, $COCH_2CH_2$), 2.00 (m, 4H, 2× CH_2CH=CH), 2.10 (4s, 12H, 4× CH_3CO), 2.40 (br t, 4H, $COCH_2$), 2.63 (br t, 8H, 4× CH_2OCO), 2.82 (br t, 8H, 4× CH_2COCH_3), 2.90 (m, 2H, =CHCH_2CH=), 3.66–3.84 (dd, 2H, $J = 10.5$, 6.2 Hz, H_2-3), 3.88 (dd, 1H, $J_{5',4'} = 10.0$, $J_{5',6'a} = 6.0$, $J_{5',6'b} = 3.0$ Hz, H-5′), 4.10–4.14 (dd, 2H, $J = 11.8$, 6.5 Hz, H_2-1), 4.30–4.36 (dd, 2H, $J_{6'a,6'b} = 12.0$ Hz, H_2-6′), 4.64 (dd, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), 5.02 (dd, 1H, $J_{2',3'} = 9.3$ Hz, H-2′), 5.11 (dd, 1H, $J_{4',3'} = 10.0$ Hz, H-4′), 5.24 (br s, 1H, H-2), 5.34 (dd, 1H, H-3′), 5.40 (m, 4H, 2× CH=CH). ESI-MS: m/z = 1086 (M+Na, 75%)$^+$.

4.7.6. 1-*O*-Caprynyl-2-*O*-linolenoyl-3-*O*-(β-D-2′, 3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerol (12c). Yield: 42%. $R_f = 0.51$ (EtOAc/hexane/8:1, v:v). ^{1}H NMR ($CDCl_3$): δ 0.93 (br t, 6H, 2× CH_3 term.), 1.29 (br s, 20H, CH_2 aliph.), 1.61 (br t, 4H, $COCH_2CH_2$), 2.08 (m, 4H, 2× CH_2CH=CH), 2.10 (4s, 12H, 4× CH_3CO), 2.36 (br t, 4H, $COCH_2$), 2.64 (br t, 8H, 4× CH_2OCO), 2.83 (br t, 8H, 4× CH_2COCH_3), 2.90 (m, 4H, 2× =CHCH_2CH=), 3.66–3.84 (dd, 2H, $J = 10.5$, 6.2 Hz, H_2-3), 3.88 (dd, 1H, $J_{5',4'} = 10.0$, $J_{5',6'a} = 6.0$, $J_{5',6'b} = 3.0$ Hz, H-5′), 4.10–4.14 (dd, 2H, $J = 11.7$, 6.4 Hz, H_2-1), 4.30–4.36 (dd, 2H, $J_{6'a,6'b} = 12.0$ Hz, H_2-6′), 4.64 (dd, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), 5.02 (dd, 1H, $J_{2',3'} = 9.3$ Hz, H-2′), 5.10 (dd, 1H, $J_{4',3'} = 10.0$ Hz, H-4′), 5.24 (br s, 1H, H-2), 5.34 (dd, 1H, H-3′), 5.37 (m, 6H, 3× CH=CH). ESI-MS: m/z = 1084 (M+Na, 87%)$^+$.

4.8. General procedure for selective removal of levulinyl protecting groups from 1,2-*O*-diacyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerols

To a solution of **8a–h**, **11a–c**, and **12a–c** (0.1 mmol) in dry pyridine, 1 M NH_2NH_2 H_2O in pyridine/glacial acetic acid (3:2, v:v) was added. Each mixture was stirred for 1 h at room temperature, under inert atmosphere, the mixture poured into ice-cold water, extracted with CH_2Cl_2 (3× 50 mL), and washed with 10% $NaHCO_3$

and brine. The combined CH_2Cl_2 layers were dried (anhydrous Na_2SO_4) and individually evaporated to give a syrupy mixture. Each mixture was purified by 'flash chromatography' on silica gel column, eluted with CH_2Cl_2/MeOH (10:1) to give 1:1 mixtures of two diastereoisomers **9a**–**h**, **13a**–**c**, and **14a**–**c**.

4.8.1. 1,2-*O*-Dicaprylyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (9a). Yield: 85%. R_f = 0.48 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (br t, 6H, J = 6.7 Hz, CH_3 term.), 1.25 (br s, 16H, CH_2 aliph.), 1.55 (br t, 4H, COCH$_2$CH_2), 2.30 (br t, 4H, J = 7.0 Hz, COCH_2), 3.29 (m, 1H, H-5′), 3.35 (m, 1H, H-2′), 3.53 (m, 1H, H-4′), 3.57 (m, 1H, H-3′), 3.66, 3.63 (dd, 1H, J = 10.6, 6.3 Hz, H_a-3), 3.79 (br s, 2H, H_2-6′), 3.87 (br d, 1H, J = 10.6 Hz, H_b-3), 4.09, 4.13 (dd, 1H, J = 11.9, 6.6 Hz, H_a-1), 4.28 (d, 1H, J = 7.8 Hz, H-1′), 4.38 (br d, 1H, J = 12.0 Hz, H_b-1), 5.22 (br s, 1H, H-2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 173.2 (*C*OO), 102.9 (C-1′), 76.4 (C-4′), 76.1 (C-5′), 73.6 (C-2′), 70.5 (C-2), 70.0 (C-3′), 68.4 (C-3), 63.0, 62.9 (C-1), 61.8 (C-6′), 33.8, 33.7 (CO*C*H$_2$), 32.3–23.2 (*C*H$_2$ aliph.), 25.5, 25.4 (COCH$_2$*C*H$_2$), 14.5 (*C*H$_3$). ESI-MS: m/z = 529 (M+Na, 25%)$^+$.

4.8.2. 1,2-*O*-Dicaprinyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (9b). Yield: 83%. R_f = 0.48 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (br t, 6H, J = 6.7 Hz, CH_3 term.), 1.24 (br s, 24H, CH_2 aliph.), 1.54 (br t, 4H, COCH$_2$CH_2), 2.34 (br t, 4H, J = 7.0 Hz, COCH_2), 3.28 (m, 1H, H-5′), 3.36 (m, 1H, H-2′), 3.55 (m, 1H, H-4′), 3.58 (m, 1H, H-3′), 3.65, 3.62 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.80 (br s, 2H, H_2-6′), 3.85 (br d, 1H, J = 10.5 Hz, H_b-3), 4.08, 4.12 (dd, 1H, J = 12.0, 6.7 Hz, H_a-1), 4.27 (d, 1H, J = 7.7 Hz, H-1′), 4.38 (br d, 1H, J = 10.4 Hz, H_b-1), 5.23 (br s, 1H, H-2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 173.1 (*C*OO), 102.8 (C-1′), 76.5 (C-4′), 76.3 (C-5′), 73.7 (C-2′), 70.4 (C-2), 69.8 (C-3′), 68.4 (C-3), 63.2, 63.1 (C-1), 61.9 (C-6′), 33.8, 33.7 (CO*C*H$_2$), 32.4–23.0 (*C*H$_2$ aliph.), 25.3, 25.2 (COCH$_2$*C*H$_2$), 14.6 (*C*H$_3$). ESI-MS: m/z = 585 (M+Na, 26%)$^+$.

4.8.3. 1,2-*O*-Dilauroyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (9c). Yield: 81%. R_f = 0.49 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (br t, 6H, J = 6.7 Hz, CH_3 term.), 1.23 (br s, 32H, CH_2 aliph.), 1.55 (br t, 4H, COCH$_2$CH_2), 2.36 (br t, 4H, J = 6.9 Hz, COCH_2), 3.27 (m, 1H, H-5′), 3.35 (m, 1H, H-2′), 3.55 (m, 1H, H-4′), 3.59 (m, 1H, H-3′), 3.65, 3.62 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.79 (br s, 2H, H_2-6′), 3.86 (br d, 1H, J = 10.6 Hz, H_b-3), 4.06, 4.12 (dd, 1H, J = 12.0, 6.7 Hz, H_a-1), 4.27 (d, 1H, J = 7.5 Hz, H-1′), 4.36 (br d, 1H, J = 10.4 Hz, H_b-1), 5.24 (br s, 1H, H-2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 173.1 (*C*OO), 102.8 (C-1′), 76.5 (C-4′), 76.3 (C-5′), 73.5 (C-2′), 70.6, 70.5 (C-2), 69.7 (C-3′), 68.4 (C-3), 63.1, 62.9 (C-1), 61.9 (C-6′), 33.8, 33.7 (CO*C*H$_2$), 32.3–23.0 (*C*H$_2$ aliph.), 25.3, 25.2 (COCH$_2$*C*H$_2$), 14.5 (*C*H$_3$). ESI-MS: m/z = 641 (M+Na, 24%)$^+$.

4.8.4. 1,2-*O*-Dimyristoyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (9d). Yield: 80%. R_f = 0.49 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (br t, 6H, J = 6.6 Hz, CH_3 term.), 1.23 (br s, 40H, CH_2 aliph.), 1.55 (br t, 4H, COCH$_2$CH_2), 2.33 (br t, 4H, J = 7.0 Hz, COCH_2), 3.30 (m, 1H, H-5′), 3.36 (m, 1H, H-2′), 3.42 (m, 1H, H-4′), 3.56 (m, 1H, H-3′), 3.62, 3.65 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.78 (br s, 2H, H_2-6′), 3.85 (br d, 1H, J = 10.5 Hz, H_b-3), 4.07, 4.13 (dd, 1H, J = 12.0, 6.7 Hz, H_a-1), 4.28 (d, 1H, J = 7.8 Hz, H-1′), 4.35 (br d, 1H, J = 10.5 Hz, H_b-1), 5.22 (br s, 1H, H-2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.4, 173.3 (*C*OO), 106.0 (C-1′), 76.6 (C-4′), 76.2 (C-5′), 73.6 (C-2′), 70.6, 70.5 (C-2), 69.9 (C-3′), 68.6 (C-3), 63.3, 63.2 (C-1), 62.0 (C-6′), 34.2, 33.8 (CO*C*H$_2$), 32.4–23.1 (*C*H$_2$ aliph.), 25.4, 25.3 (COCH$_2$*C*H$_2$), 14.5 (*C*H$_3$). ESI-MS: m/z = 697 (M+Na, 48%)$^+$.

4.8.5. 1,2-*O*-Distearoyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (9e). Yield: 81%. R_f = 0.51 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (br t, 6H, J = 6.7 Hz, CH_3 term.), 1.25 (br s, 56H, CH_2 aliph.), 1.54 (br t, 4H, COCH$_2$CH_2), 2.39 (br t, 4H, J = 7.0 Hz, COCH_2), 3.29 (m, 1H, H-5′), 3.37 (m, 1H, H-2′), 3.57 (m, 1H, H-3′), 3.58 (m, 1H, H-4′), 3.62, 3.65 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.79 (br s, 2H, H_2-6′), 3.87 (br d, 1H, J = 10.6 Hz, H_b-3), 4.07, 4.13 (dd, 1H, J = 11.9, 6.6 Hz, H_a-1), 4.28 (d, 1H, J = 7.8 Hz, H-1′), 4.35 (br d, 1H, J = 11.2 Hz, H_b-1), 5.25 (br s, 1H, H-2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.7, 173.8 (*C*OO), 106.0 (C-1′), 76.6 (C-4′), 76.2 (C-5′), 73.7 (C-2′), 70.6, 70.5 (C-2), 70.0 (C-3′), 68.5 (C-3), 63.2, 63.1 (C-1), 62.0 (C-6′), 34.2, 34.1 (CO*C*H$_2$), 32.3–23.0 (*C*H$_2$ aliph.), 25.4, 25.3 (COCH$_2$*C*H$_2$), 14.6 (*C*H$_3$). ESI-MS: m/z = 810 (M+Na, 41%)$^+$.

4.8.6. 1,2-*O*-Dioleoyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (9f). Yield: 75%. R_f = 0.50 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (br t, 6H, J = 6.6 Hz, CH_3 term.), 1.24 (br s, 40H, CH_2 aliph.), 1.56 (br t, 4H, COCH$_2$CH_2), 2.0 (br, 8H, 4× CH_2CH=CH), 2.35 (br t, 4H, J = 6.9 Hz, COCH_2), 3.30 (m, 1H, H-5′), 3.38 (m, 1H, H-2′), 3.46 (m, 1H, H-4′), 3.58 (m, 1H, H-3′), 3.62, 3.65 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.79 (br s, 2H, H_2-6′), 3.88 (br d, 1H, J = 10.6 Hz, H_b-3), 4.08, 4.15 (dd, 1H, J = 12.0, 6.7 Hz, H_a-1), 4.29 (d, 1H, J = 7.8 Hz, H-1′), 4.35 (br d, 1H, J = 12.1 Hz, H_b-1), 5.24 (br s, 1H, H-2), 5.25–5.40 (m, 4H, 2× C*H*=C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.9, 173.4 (*C*OO), 130.3, 130.1 (*C*H=*C*H), 103.2 (C-1′), 76.5 (C-4′), 76.3 (C-5′), 73.6 (C-2′), 70.5, 70.4 (C-2), 69.8 (C-3′), 68.6 (C-3), 63.0, 62.9 (C-1), 61.9 (C-6′), 33.8, 33.7 (CO*C*H$_2$), 32.3–22.9 (*C*H$_2$ aliph.), 27.7, 27.5 (*C*H$_2$CH=CH), 25.5, 25.4 (COCH$_2$*C*H$_2$), 14.5 (*C*H$_3$). ESI-MS: m/z = 806 (M+Na, 100%)$^+$.

4.8.7. 1,2-*O*-Dilinoleoyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (9g). Yield: 70%. R_f = 0.50 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (br t, 6H, J = 6.7 Hz, CH_3 term.), 1.22 (br s, 28H, CH_2 aliph.), 1.56 (br t, 4H, COCH$_2$CH_2), 1.99 (br, 8H, 4× CH_2CH=CH), 2.33 (br t, 4H, J = 6.9 Hz, COCH_2), 2.82 (br t, 4H, 2× =CHCH_2CH=), 3.30 (m, 1H, H-5′), 3.37 (m, 1H, H-2′), 3.55 (m, 1H, H-4′), 3.58 (m, 1H, H-3′), 3.63, 3.66 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.80

(br s, 2H, H_2-6′), 3.89 (br d, 1H, J = 10.6 Hz, H_b-3), 4.07, 4.14 (dd, 1H, J = 12.0, 6.6 Hz, H_a-1), 4.29 (d, 1H, J = 7.8 Hz, H-1′), 4.34 (br d, 1H, J = 12.0 Hz, H_b-1), 5.23 (br s, 1H, H-2), 5.30–5.38 (m, 8H, 4× C*H*═C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 173.4 (*C*OO), 130.5, 130.4, 128.4, 128.3 (*C*H═*C*H), 103.5 (C-1′), 76.7 (C-4′), 76.1 (C-5′), 73.5 (C-2′), 70.5 (C-2), 69.7 (C-3′), 68.6 (C-3), 63.3, 63.2 (C-1), 61.7 (C-6′), 33.8, 33.7 (CO*C*H_2), 30.3–21.1 (*C*H_2 aliph.), 27.5 (*C*H_2CH═CH), 26.0 (═CH*C*H_2CH═), 25.5, 25.4 (COCH$_2$*C*H_2), 14.4 (*C*H_3). ESI-MS: m/z = 802 (M+Na, 100%)$^+$.

4.8.8. 1,2-*O*-Dilinolenoyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (9h). Yield: 70%. R_f = 0.50 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97 (br t, 6H, J = 6.8 Hz, C*H*$_3$ term.), 1.23 (br s, 16H, C*H*$_2$ aliph.), 1.54 (br t, 4H, COCH$_2$C*H*$_2$), 2.10 (br, 8H, 4× C*H*$_2$CH═CH), 2.35 (br t, 4H, J = 6.9 Hz, COC*H*$_2$), 2.90 (br t, 8H, J = 5.6 Hz, 4× ═CHC*H*$_2$CH═), 3.29 (m, 1H, H-5′), 3.33 (m, 1H, H-2′), 3.57 (m, 1H, H-4′), 3.60 (m, 1H, H-3′), 3.63, 3.66 (dd, 1H, J = 10.6, 6.3 Hz, H_a-3), 3.79 (br s, 2H, H_2-6′), 3.87 (br d, 1H, J = 10.6 Hz, H_b-3), 4.09, 4.13 (dd, 1H, J = 11.9, 6.7 Hz, H_a-1), 4.28 (d, 1H, J = 7.7 Hz, H-1′), 4.38 (br d, 1H, J = 12.0 Hz, H_b-1), 5.22 (br s, 1H, H-2), 5.30–5.41 (m, 12H, 6× C*H*═C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 173.4 (*C*OO), 132.2, 130.4, 130.1, 128.6, 128.1, 127.5 (*C*H═*C*H), 103.5 (C-1′), 76.7 (C-4′), 76.1 (C-5′), 73.6 (C-2′), 70.5 (C-2), 70.0 (C-3′), 68.7 (C-3), 63.2, 63.1 (C-1), 61.8 (C-6′), 34.3, 34.1 (CO*C*H_2), 30.3–21.1 (*C*H_2 aliph.), 27.7, 27.6, 27.5 (*C*H_2CH═CH), 26.1, 26.0 (═CH*C*H_2CH═), 25.5, 25.4 (COCH$_2$*C*H_2), 14.7 (*C*H_3). ESI-MS: m/z = 798 (M+Na, 100%)$^+$.

4.8.9. 1-*O*-Caprylyl-2-*O*-oleoyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (13a). Yield: 71%. R_f = 0.49 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (br t, 6H, J = 6.8 Hz, C*H*$_3$ term.), 1.23 (br s, 28H, C*H*$_2$ aliph.), 1.56 (br t, 4H, COCH$_2$C*H*$_2$), 1.99 (br, 4H, 2× C*H*$_2$CH═CH), 2.39 (br t, 4H, J = 7.0 Hz, COC*H*$_2$), 3.28 (m, 1H, H-5′), 3.35 (m, 1H, H-2′), 3.54 (m, 1H, H-4′), 3.59 (m, 1H, H-3′), 3.62, 3.65 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.80 (br s, 2H, H_2-6′), 3.88 (br d, 1H, J = 10.6 Hz, H_b-3), 4.08, 4.15 (dd, 1H, J = 12.0, 6.7 Hz, H_a-1), 4.27 (d, 1H, J = 7.5 Hz, H-1′), 4.35 (br d, 1H, J = 12.1 Hz, H_b-1), 5.24 (br s, 1H, H-2), 5.20–5.40 (m, 2H, C*H*═C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.2, 174.1 (*C*OO), 130.4, 130.1 (*C*H═*C*H), 103.7 (C-1′), 76.5 (C-4′), 76.3 (C-5′), 73.7 (C-2′), 70.6, 70.5 (C-2), 70.0 (C-3′), 68.5 (C-3), 63.0, 62.9 (C-1), 61.8 (C-6′), 34.6, 34.5 (CO*C*H_2), 32.3–23.2 (*C*H_2 aliph.), 27.6, 27.5 (*C*H_2CH═CH), 25.3, 25.2 (COCH$_2$*C*H_2), 14.5 (*C*H_3). ESI-MS: m/z = 668 (M+Na, 100%)$^+$.

4.8.10. 1-*O*-Caprylyl-2-*O*-linoleoyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (13b). Yield: 72%. R_f = 0.50 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (br t, 6H, J = 6.8 Hz, C*H*$_3$ term.), 1.25 (br s, 24H, C*H*$_2$ aliph.), 1.55 (br t, 4H, COCH$_2$C*H*$_2$), 2.0 (br, 4H, 2× C*H*$_2$CH═CH), 2.30 (br t, 4H, J = 7.0 Hz, COC*H*$_2$), 2.87 (br t, 2H, J = 5.6 Hz, ═CHC*H*$_2$CH═), 3.28 (m, 1H, H-5′), 3.36 (m, 1H, H-2′), 3.56 (m, 1H, H-4′), 3.58 (m, 1H, H-3′), 3.63, 3.66 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.80 (br s, 2H, H_2-6′), 3.89 (br d, 1H, J = 10.5 Hz, H_b-3), 4.07, 4.14 (dd, 1H, J = 12.0, 6.6 Hz, H_a-1), 4.29 (d, 1H, J = 7.7 Hz, H-1′), 4.34 (br d, 1H, J = 12.0 Hz, H_b-1), 5.23 (br s, 1H, H-2), 5.20–5.40 (m, 4H, 2× C*H*═C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 173.2 (*C*OO), 130.6, 130.4, 128.4, 128.3 (*C*H═*C*H), 103.3 (C-1′), 76.5 (C-4′), 76.3 (C-5′), 73.8 (C-2′), 70.6, 70.5 (C-2), 69.9 (C-3′), 68.5 (C-3), 63.3, 63.2 (C-1), 61.8 (C-6′), 34.3, 34.1 (CO*C*H_2), 32.4–23.2 (*C*H_2 aliph.), 27.6 (*C*H_2CH═CH), 26.0 (═CH*C*H_2CH═), 25.3, 25.2 (COCH$_2$*C*H_2), 14.7 (*C*H_3). ESI-MS: m/z = 666 (M+Na, 100%)$^+$.

4.8.11. 1-*O*-Caprylyl-2-*O*-linolenoyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (13c). Yield: 69%. R_f = 0.50 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (br t, 6H, J = 6.8 Hz, C*H*$_3$ term.), 1.25 (br s, 16H, C*H*$_2$ aliph.), 1.57 (br t, 4H, COCH$_2$C*H*$_2$), 2.10 (br, 4H, 2× C*H*$_2$CH═CH), 2.31 (br t, 4H, J = 7.0 Hz, COC*H*$_2$), 2.90 (br t, 4H, J = 5.5 Hz, 2× ═CHC*H*$_2$CH═), 3.29 (m, 1H, H-5′), 3.34 (m, 1H, H-2′), 3.56 (m, 1H, H-4′), 3.57 (m, 1H, H-3′), 3.62, 3.65 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.80 (br s, 2H, H_2-6′), 3.85 (br d, 1H, J = 10.5 Hz, H_b-3), 4.07, 4.13 (dd, 1H, J = 11.9, 6.6 Hz, H_a-1), 4.28 (d, 1H, J = 7.7 Hz, H-1′), 4.35 (br d, 1H, J = 10.5 Hz, H_b-1), 5.22 (br s, 1H, H-2), 5.22–5.39 (m, 6H, 3× C*H*═C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 173.5 (*C*OO), 132.3, 130.4, 130.2, 128.5, 128.0, 127.6 (*C*H═*C*H), 103.3 (C-1′), 76.5 (C-4′), 76.3 (C-5′), 73.7 (C-2′), 70.6 (C-2), 69.8 (C-3′), 68.6 (C-3), 63.1, 62.9 (C-1), 62.0 (C-6′), 34.3, 34.2 (CO*C*H_2), 32.3–23.1 (*C*H_2 aliph.), 27.5, 27.6, 27.7 (*C*H_2CH═CH), 26.2, 26.0 (═CH*C*H_2CH═), 25.5, 25.4 (COCH$_2$*C*H_2), 14.8 (*C*H_3). ESI-MS: m/z = 662 (M+Na, 100%)$^+$.

4.8.12. 1-*O*-Caprinyl-2-*O*-oleoyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (14a). Yield: 75%. R_f = 0.50 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.94 (br t, 6H, J = 6.8 Hz, C*H*$_3$ term.), 1.24 (br s, 32H, C*H*$_2$ aliph.), 1.55 (br t, 4H, COCH$_2$C*H*$_2$), 1.99 (br, 4H, 2× C*H*$_2$CH═CH), 2.40 (br t, 4H, J = 6.9 Hz, COC*H*$_2$), 3.29 (m, 1H, H-5′), 3.32 (m, 1H, H-2′), 3.54 (m, 1H, H-4′), 3.60 (m, 1H, H-3′), 3.62, 3.65 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.79 (br s, 2H, H_2-6′), 3.85 (br d, 1H, J = 10.5 Hz, H_b-3), 4.08, 4.12 (dd, 1H, J = 12.0, 6.7 Hz, H_a-1), 4.27 (d, 1H, J = 7.8 Hz, H-1′), 4.38 (br d, 1H, J = 10.4 Hz, H_b-1), 5.23 (br s, 1H, H-2), 5.20–5.40 (m, 2H, C*H*═C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5, 173.4 (*C*OO), 130.3, 130.2 (*C*H═*C*H), 103.2 (C-1′), 76.6 (C-4′), 76.2 (C-5′), 73.8 (C-2′), 70.5, 70.4 (C-2), 69.7 (C-3′), 68.4 (C-3), 63.3, 63.2 (C-1), 61.9 (C-6′), 34.1, 33.9 (CO*C*H_2), 32.3–23.0 (*C*H_2 aliph.), 27.6, 27.5 (*C*H_2CH═CH), 25.5, 25.4 (COCH$_2$*C*H_2), 14.5 (*C*H_3). ESI-MS: m/z = 696 (M+Na, 100%)$^+$.

4.8.13. 1-*O*-Caprinyl-2-*O*-linoleoyl-3-*O*-(β-D-glucopyrano- syl)-*rac*-glycerol (14b). Yield: 69%. R_f = 0.50 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (br t, 6H, J = 6.7 Hz, C*H*$_3$ term.), 1.24 (br s, 28H, C*H*$_2$ aliph.), 1.54 (br t, 4H, COCH$_2$C*H*$_2$), 2.0 (br, 4H, 2× C*H*$_2$CH═CH), 2.33 (br t, 4H, J = 7.0 Hz, COC*H*$_2$), 2.88 (br t, 2H, J = 5.6 Hz, ═CHC*H*$_2$CH═), 3.31 (m, 1H, H-5′), 3.35 (m, 1H, H-2′), 3.56 (m, 1H, H-4′), 3.58

(m, 1H, H-3′), 3.62, 3.65 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.80 (br s, 2H, H_2-6′), 3.89 (br d, 1H, J = 10.6 Hz, H_b-3), 4.07, 4.13 (dd, 1H, J = 12.0, 6.7 Hz, H_a-1), 4.29 (d, 1H, J = 7.8 Hz, H-1′), 4.35 (br d, 1H, J = 10.5 Hz, H_b-1), 5.22 (br s, 1H, H-2), 5.22–5.40 (m, 4H, 2× C*H*=C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 173.4 (*C*OO), 130.6, 130.4, 128.4, 128.3 (*C*H=*C*H), 103.2 (C-1′), 76.6 (C-4′), 76.3 (C-5′), 73.7 (C-2′), 70.6 (C-2), 69.7 (C-3′), 68.5 (C-3), 63.2, 63.1 (C-1), 62.3 (C-6′), 34.3, 33.6 (CO*C*H_2), 32.4–23.2 (*C*H_2 aliph.), 27.6 (*C*H_2CH=CH), 26.0 (=CH*C*H_2CH=), 25.3, 25.2 (COCH$_2$*C*H_2), 14.7 (*C*H_3). ESI-MS: m/z = 694 (M+Na, 100%)$^+$.

4.8.14. 1-*O*-Caprinyl-2-*O*-linolenoyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerol (14c). Yield: 66%. R_f = 0.50 (CH_2Cl_2/MeOH/10:1, v:v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (br t, 6H, J = 6.8 Hz, C*H*$_3$ term.), 1.24 (br s, 20H, C*H*$_2$ aliph.), 1.56 (br t, 4H, COCH$_2$C*H*$_2$), 2.10 (br, 4H, 2× C*H*$_2$CH=CH), 2.35 (br t, 4H, J = 6.9 Hz, COC*H*$_2$), 2.91 (br t, 4H, J = 5.5 Hz, 2× =CHC*H*$_2$CH=), 3.30 (m, 1H, H-5′), 3.38 (m, 1H, H-2′), 3.55 (m, 1H, H-4′), 3.57 (m, 1H, H-3′), 3.62, 3.65 (dd, 1H, J = 10.7, 6.4 Hz, H_a-3), 3.80 (br s, 2H, H_2-6′), 3.88 (br d, 1H, J = 10.6 Hz, H_b-3), 4.08, 4.15 (dd, 1H, J = 12.0, 6.7 Hz, H_a-1), 4.27 (d, 1H, J = 7.7 Hz, H-1′), 4.35 (br d, 1H, J = 12.1 Hz, H_b-1), 5.24 (br s, 1H, H-2), 5.20–5.40 (m, 6H, 3× C*H*=C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 173.5 (*C*OO), 132.3, 130.4, 130.2, 128.5, 128.0, 127.6 (*C*H=*C*H), 103.6 (C-1′), 76.8 (C-4′), 76.3 (C-5′), 73.7 (C-2′), 70.6, 70.5 (C-2), 70.0 (C-3′), 68.5 (C-3), 63.3, 63.2 (C-1), 62.4 (C-6′), 34.4, 34.2 (CO*C*H_2), 32.3–23.0 (*C*H_2 aliph.), 27.5, 27.6, 27.7 (*C*H_2CH=CH), 26.2, 26.0 (=CH*C*H_2CH=), 25.3, 25.4 (COCH$_2$*C*H_2), 14.8 (*C*H_3). ESI-MS: m/z = 692 (M+Na, 100%)$^+$.

4.9. Biological assays

All the synthesized 1,2-*O*-diacyl-3-*O*-β-D-glucopyranosyl-*rac*-glycerols (**9a–h**, **13a–c**, **14a–c**) have been evaluated for their in vitro activity against a number of Gram-positive and Gram-negative reference strains: *S. aureus* ATCC 25923, *S. aureus* ATCC 105487, *E. faecalis* ATCC 29212, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27753, *M. tuberculosis* H37Rv; and against four different *S. aureus*, clinical strains SA52, SA85, SA87, and two *M. tuberculosis* clinical isolates, H160, H190.

The antifungal activity of all compounds was evaluated against two strains, clinical isolates, of *C. albicans* and *C. pseudotropicalis*. Antibacterial activity was evaluated by a reference agar dilution method.[31] MICs were determined twice in duplicate experiments. Antitubercular activity was evaluated by a standard agar dilution method and by a recently developed color assay,[32] MIC was defined as the lowest drug concentration that prevented Resazurin color change and was determined twice in duplicate experiments. Ciprofloxacin was chosen as a standard in antibacterial activity measurements. Antifungal activity has been tested against *Candida spp.* clinical isolates measuring MICs by a microdilution RPMI reference method, in comparison with miconazole.[33]

Acknowledgment

This study was supported by MURST (Rome, Italy).

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