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Bioorganic & Medicinal Chemistry Letters 17 (2007) 1971–1978
Bioorganic & Medicinal Chemistry Letters

Chemoenzymatic synthesis and in vitro studies on the hydrolysis of antimicrobial monoglycosyl diglycerides by pancreatic lipase

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Received 22 November 2006; revised 8 January 2007; accepted 9 January 2007
Available online 19 January 2007

Abstract—Monoglucosyl and monogalactosyl diglycerides (MGDGs) with medium-long length acyl chains, identified as active components in Euphorbiaceae, were synthesized. They were examined for antimicrobial activity against Gram-positive, Gram-negative bacteria and fungi. MGDGs with two octanoyl groups at both 1- and 2-positions showed the most potent activity. The stereoselectivity of pancreatic lipase was investigated in vitro where the preference for the 1 position in MGDGs is strictly related to the length of the acyl chains.

Glycoglycerolipids are common constituents of plant cell membranes and bacterial cell walls.[1–4] Various glycolipids have been isolated from plants,[5,6] algae,[7] and bacteria.[4,8]

Recently, we investigated the active principles from various species of Euphorbiaceae and isolated monogalactosyl diacylglycerols (MGDGs), monogalactosyl monoacylglycerols (MGMGs) and digalactosyl diacylglycerols (DGDGs) as active components, after repeated chromatography.[9] MGDGs, MGMGs, and DGDGs have attracted much attention in recent years because of their biological activities, such as anti-tumor-promoting,[5,6,8,10,11] oxygen scavenging,[12] anti-viral,[13,14] anti-inflammatory[15] and anti-hyperlipidemic[16] activities. The activity of such compounds seems to be strictly related to the acyl chain length.[10,11,16]

Previous pharmacological studies of methanol extracts from Euphorbiaceae showed both antimicrobial and anti-inflammatory actions.[17,18] 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 particular, it is difficult to isolate MGDGs, MGMGs, and DGDGs with different acyl groups from Euphorbiaceae, because they have almost the same polarity and size. Their versatile synthesis seems to be of importance, especially for their biological investigation. Different synthetic studies of various analogs of natural glycoglycerolipids and their modified forms with unnatural structures are currently under development.[19–23]

On the basis of these evidences, which indicate that the nature of the lipophilic chain is a crucial structural feature for the activity of many compounds, we decided to investigate structure–activity relationships of this class of compounds against Gram-positive, Gram-negative bacteria and fungi. In addition, the hydrolysis of the synthesized MGDGs as release of free fatty acids by pancreatic lipase was studied. In order to examine these analogs for antimicrobial activity and to investigate the stereoselectivity of pancreatic lipase, a series of mono-acyl and mixed-acyl 1,2-di-*O*-acyl-3-*O*-β-glycopyranosyl-*rac*-glycerols con-

Keywords: Synthesis; Monoglycosyl diglycerides; 1,2-Di-*O*-acyl-3-*O*-β-D-glucopyranosyl-glycerols; 1,2-Di-*O*-acyl-3-*O*-β-D-galactopyranosyl-glycerols; Antimicrobial activity; Pancreatic lipase.
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doi:10.1016/j.bmcl.2007.01.019

taining glucose and galactose moieties were synthesized with medium-long length (from C8 to C18) acyl chains. Mixed-acyl 1,2-di-*O*-acyl-3-*O*-β-glycopyranosyl-*rac*-glycerols possess long-chain polyunsaturated fatty acids (PUFA) at the mid-position and medium-chain fatty acids (MCFA) at the C-1 position of the glycerol backbone. For this class of compounds, the hydrolysis by pancreatic lipase and the effects on antimicrobial activity were studied. 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 acyls in predetermined positions of the glycerol moiety, could have an interesting application as nutraceutic compounds.[24–26]

Pancreatic lipase hydrolyzes ester bonds at the 1- and 3-positions in triglycerides, and shows higher activity toward long-chain fatty acids (especially PUFA) than toward medium-chain ones (8:0, 10:0).[27,28] Medium-chain glycerides (MCGs) are mainly utilized as a nutritional supplement for patients suffering from malabsorption caused by intestinal resection. They are also useful to treat a number of medicinal disorders that involve impaired or damaged lipid metabolism, which include obstructive jaundice, biliary cirrhosis, pancreatitis, cystic fibrosis, and celiac disease.[29] Besides, in humans, pancreatic lipase plays an important role in nutrition processes and is one of the main enzymes in the digestive tract involved in the hydrolysis of dietary triacylglycerols (TAGs). Long-chain triglycerides, most of the natural oils and fats, are hydrolyzed to 2-monoglycerides and fatty acids by the lipase, and the hydrolysis products are absorbed into the intestinal mucosa. These products are converted to triglycerides again in the mucosal cells. Upon reconstructing triglycerides, about 75% of the fatty acids located at the 2-position in the triglycerides are conserved in the original position, while fatty acids released from the 1- and 3-positions bind to glycerol at random.[30] Triglycerides with medium-chain fatty acids at the 1- and 3-positions and functional fatty acids at the 2-position are absorbed efficiently into mucosal cells.[31,32]

In this paper, we have examined the hydrolysis of the synthesized MGDGs as release of free fatty acids.

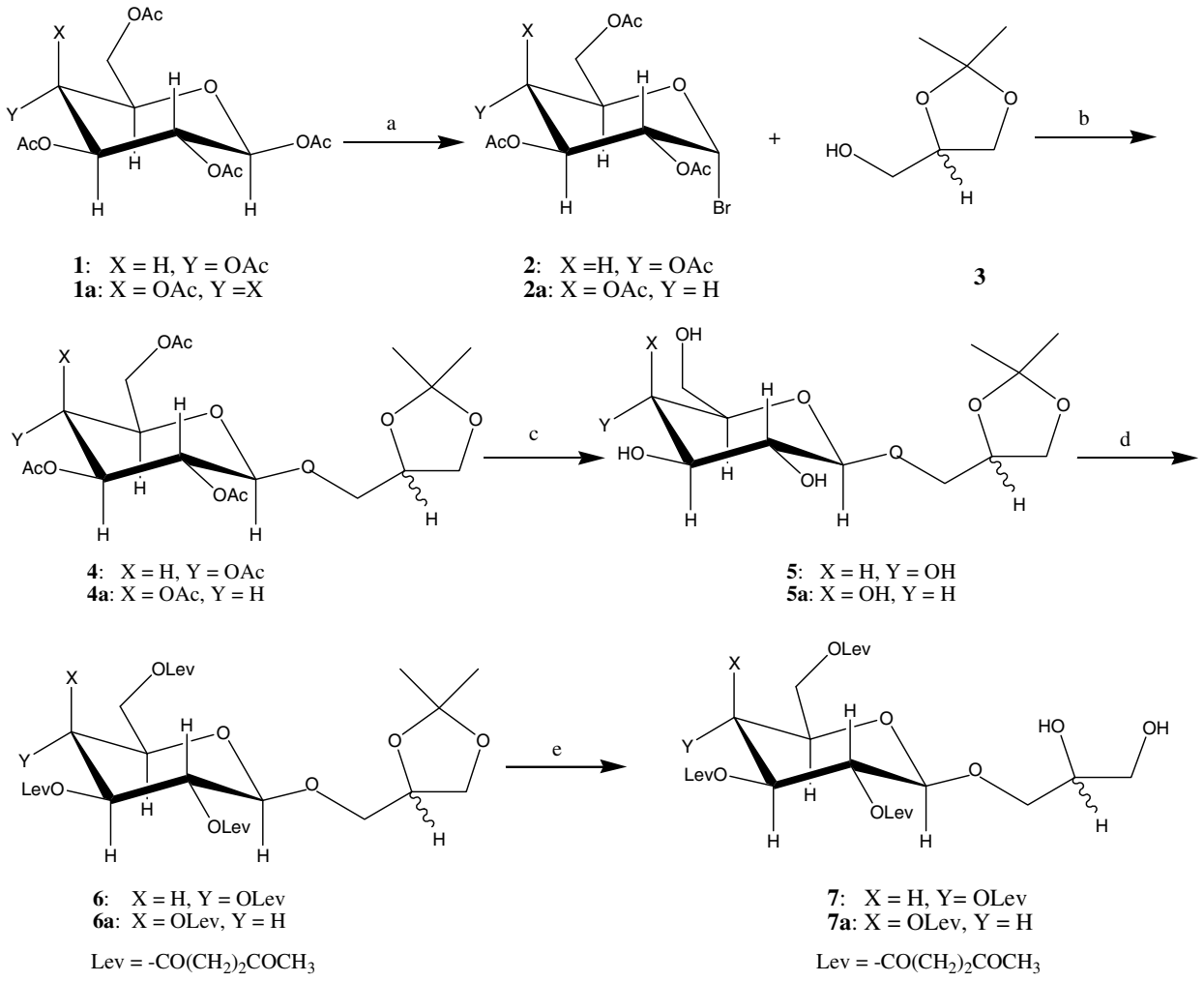
Glycosyl diglycerides bearing either saturated or unsaturated acyls were synthesized as depicted in Schemes 1 and 2. Glycosylation of D,L-α,β-isopropylideneglycerol (**3**) under Köenigs–Knorr conditions,[14] by using tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**2**) and tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**2a**) in dichloromethane in the presence of silver carbonate, gave the desired peracetylated β-glycosides (**4**, **4a**) [^{1}H NMR signals of anomeric protons at δ 4.61, 4.63 ($J_{1,2}$ = 8.0 Hz) in **4**, **4a** indicated a 1:1 ratio of diastereomeric products] in 70% yields.

Removal of all the acyl protective groups of **4**, **4a** by alkaline hydrolysis afforded **5**, **5a** which were subjected to protection of the resulting free hydroxyl groups as levulinate esters (**6**, **6a**). Thus, successive treatment of **6** and **6a** with 70% acetic acid in aqueous solution at 60 °C in order to remove the isopropylidene protecting group provided the diol intermediates (**7**, **7a**) in 92% (**7**) and 80% (**7a**) yields, respectively (Scheme 1). The intermediate compounds **7**, **7a** represent the key synthons for the synthesis of mono-acyl and mixed-acyl 1,2-di-*O*-acyl-3-*O*-β-glycosylglycerols (Scheme 2).

The mono-acyl 1,2-di-*O*-acyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glucopyranosyl)-*rac*-glycerols (**8a**–**h**) and 1,2-di-*O*-acyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-galactopyranosyl)-*rac*-glycerols (**8i**–**r**), obtained in satisfactory yields (54–80%), were prepared by one-step diacylation of **7** and **7a** with 2 equiv of the desired fatty acids in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in dry CH_2Cl_2 at room temperature. We selected octanoate, caprate, laurate, myristate, and stearate as introduction acyl groups, because they are components of bioactive analogs of natural glycoglycerolipids,[19–23] and oleate, linolate, and linolenate that are the major acyl components of MGDGs, MGMGs and DGDGs isolated from Euphorbiaceae.[9] Finally, the levulinyl protective groups of **8a**–**r** were removed by treatment with hydrazine hydrate and mono-acyl 1,2-di-*O*-acyl-3-*O*-(β-D-glycopyranosyl)-*rac*-glycerol derivatives with different acyl groups (**9a**–**r**) were obtained in quantitative yields (80%)[33] (Scheme 2).

On the other hand, the synthesis of mixed-acyl 1,2-di-*O*-acyl-3-*O*-(β-D-glycopyranosyl)-*rac*-glycerols (**13a**–**f**, **14a**–**f**) requires full regioselectivity control and can hardly be undertaken by synthetic organic chemistry methods without multi-step protection–deprotection processes.[16] 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.[24–26] 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).[20]

To get the intermediate adducts **10a**–**d** (Scheme 2), compounds **7**, **7a**, obtained by standard procedures as depicted in Scheme 1, were directly submitted to transesterification catalyzed by *Mucor miehei* lipase in the presence of the appropriate octanoate (**10a**, **10c**) and caprate (**10b**, **10d**) vinyl esters in dry dichloromethane as solvent. The reactions were completely regioselective and afforded, through the expected acylation of the primary hydroxyl group,[24–26] the 1-*O*-acyl-3-*O*-(β-D-2′,3′,4′,6′-tetra-*O*-levulinyl-glycopyranosyl)-*rac*-glycerols **10a**–**d** 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 DCC coupling agent in excellent yields (58–87%) to give the intermediate products **11a**–**f**, **12a**–**f** with the hydroxyl groups of the glycosyl moiety temporarily protected as levulinates. Finally,



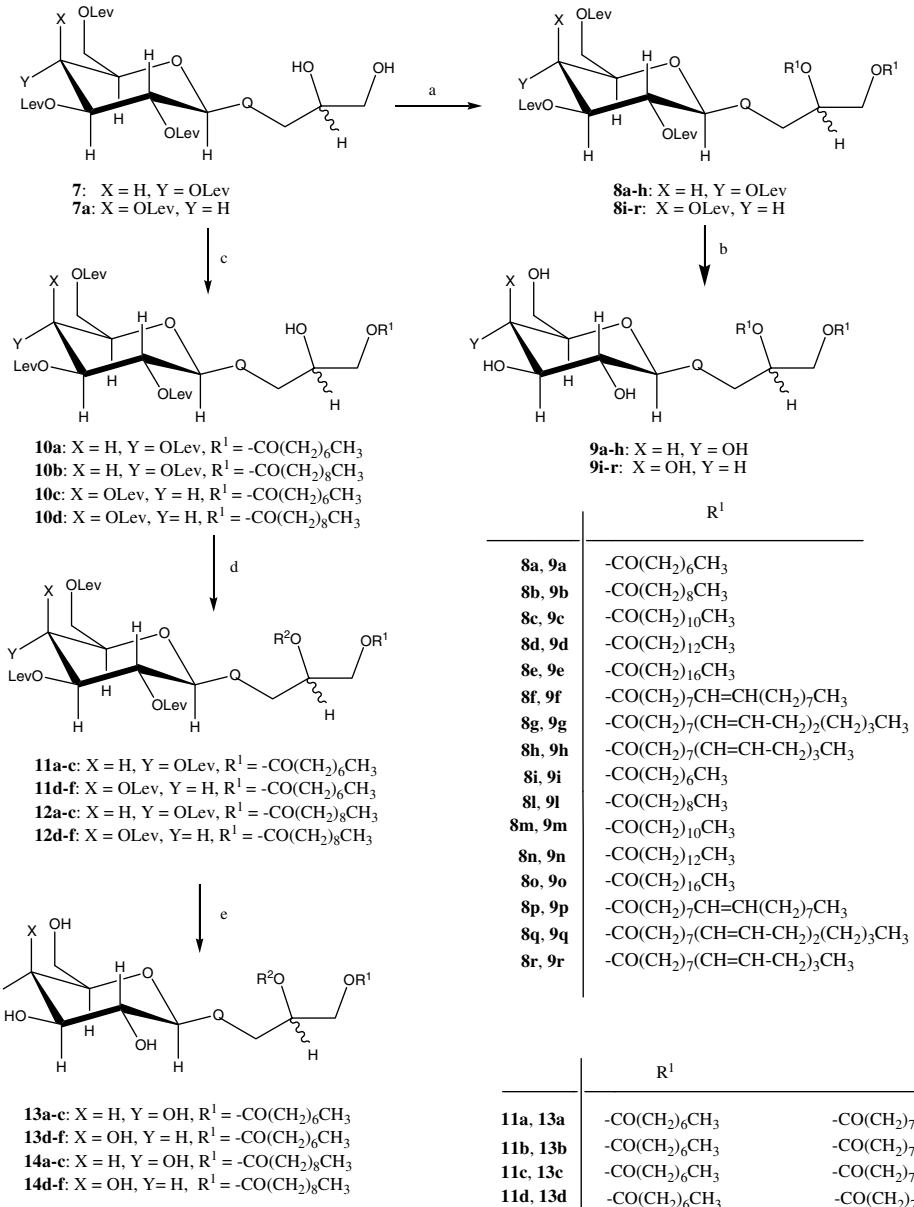
Scheme 1. Reagents and conditions: (a) HBr, CH_3CO_2H, rt; (b) Ag_2CO_3, $CaSO_4$, I_2, CH_2Cl_2, rt; (c) $NaOCH_3$, CH_3OH; (d) $[CH_3CO(CH_2)_2CO]_2O$, Py; (e) 70% CH_3CO_2H, 60 °C.

the levulinyl protective groups of **11a**–**f** and **12a**–**f** were removed by treatment with hydrazine and 1,2-di-*O*-acyl-3-*O*-(β-D-glycopyranosyl)-*rac*-glycerols (**13a**–**f**, **14a**–**f**) 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 backbone were obtained.[34]

Hydrolysis with porcine pancreatic lipase (PPL) was carried out for each synthesized compound and the data are reported in Table 1. Ten milligrams of each synthesized MGDG was weighted into Teflon-stoppered glass tubes. The reaction buffer [1 ml of 50 mM Tris–HCl (pH 7.6), 2.2% $CaCl_2$] was added and substrate emulsion was prepared by vigorously vortexing the mixture for 1 min. After adding 10 mg of lipase, the substrate emulsion was incubated in a shaking water bath at 37 °C. The enzymatic reaction was stopped after 40 min adding 0.2 ml of HCl (6 N). The aqueous phase was extracted three times with ethyl ether; the organic phase was dried over anhydrous sodium sulfate and the solvent was removed under a stream of nitrogen. The course of lipolysis was monitored qualitatively by thin-layer chromatography (TLC) on silica gel. Free fatty acids and fractions corresponding to each glyceride type were scraped from the plates; the lipolysis products were separated using the solvent system dichloromethane/methanol (5:1, v/v) and visualized with iodine vapor.

The mono-acyl compounds **9a**–**h** (glucosyl derivatives) and **9i**–**r** (galactosyl derivatives) contain acyl chains characterized by the presence of caprylic (**9a**, **9i**), caprynic (**9b**, **9l**), lauric (**9c**, **9m**), myristic (**9d**, **9n**), stearic (**9e**, **9o**), oleic (**9f**, **9p**), linoleic (**9g**, **9q**), and linolenic (**9h**, **9r**) acids, respectively. The mixed-acyl compounds **13a**–**c**, **14a**–**c** (glucosyl derivatives) and **13d**–**f**, **14d**–**f** (galactosyl derivatives) bear at the C-1 position of the glycerol backbone caprylic acid (**13a**–**f**) or caprynic acid (**14a**–**f**), while at the C-2 position oleic (**13a**, **13d**, **14a**, **14d**), linoleic (**13b**, **13e**, **14b**, **14e**), and linolenic (**13c**, **13f**, **14c**, **14f**) acids, respectively.

The compounds bearing, at the primary position of the glycerol backbone, acyl chains with a number of carbons $\leqslant 10$ (**9a**, **9b**, **9i**, **9l**, **13a**–**f**, **14a**–**f**) do not show important hydrolysis effects so that from the TLC analysis, the starting compounds (MGDGs) are evident. Instead, the compounds which present acyl chains with at



	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**	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_3CH_3$
8i, **9i**	$-CO(CH_2)_6CH_3$
8l, **9l**	$-CO(CH_2)_8CH_3$
8m, **9m**	$-CO(CH_2)_{10}CH_3$
8n, **9n**	$-CO(CH_2)_{12}CH_3$
8o, **9o**	$-CO(CH_2)_{16}CH_3$
8p, **9p**	$-CO(CH_2)_7CH{=}CH(CH_2)_7CH_3$
8q, **9q**	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_2(CH_2)_3CH_3$
8r, **9r**	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_3CH_3$

	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**	$-CO(CH_2)_6CH_3$	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_3CH_3$
11d, **13d**	$-CO(CH_2)_6CH_3$	$-CO(CH_2)_7CH{=}CH(CH_2)_7CH_3$
11e, **13e**	$-CO(CH_2)_6CH_3$	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_2(CH_2)_3CH_3$
11f, **13f**	$-CO(CH_2)_6CH_3$	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_3CH_3$
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**	$-CO(CH_2)_8CH_3$	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_3CH_3$
12d, **14d**	$-CO(CH_2)_8CH_3$	$-CO(CH_2)_7CH{=}CH(CH_2)_7CH_3$
12e, **14e**	$-CO(CH_2)_8CH_3$	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_2(CH_2)_3CH_3$
12f, **14f**	$-CO(CH_2)_8CH_3$	$-CO(CH_2)_7(CH{=}CH\text{-}CH_2)_3CH_3$

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

least 12 carbons (**9c**–**h**, **9m**–**r**) are hydrolyzed: the free acyl residues and the monoacyl derivatives are evident, while the starting compounds disappeared.

In order to follow quantitatively the course of reaction, fatty acid composition was determined by a gas–liquid chromatographic (HRGC) method after converting the fatty acids into methyl esters.[35] The methyl esters were analyzed with a Shimadzu GC 14A, connected with a Shimadzu CR4A data integrator. The GLC was fitted with a capillary column SP 2330 (30 m × 0.32 mm ID, 0.20 μm film thickness, Supelco). The oven temperature

Table 1. Free fatty acid composition of the compounds **9a–r**, **13a–f**, **14a–f** hydrolyzed by PPL

Compound	% free fatty acid composition							
	C8:0	C10:0	C12:0	C14:0	C18:0	C18:1	C18:2	C18:3
9a	0.74	—	—	—	—	—	—	—
9b	—	1.05	—	—	—	—	—	—
9c	—	—	96.1	—	—	—	—	—
9d	—	—	—	97.5	—	—	—	—
9e	—	—	—	—	98.8	—	—	—
9f	—	—	—	—	—	99.1	—	—
9g	—	—	—	—	—	—	98.9	—
9h	—	—	—	—	—	—	—	99.2
9i	0.88	—	—	—	—	—	—	—
9l	—	1.12	—	—	—	—	—	—
9m	—	—	95.4	—	—	—	—	—
9n	—	—	—	97.3	—	—	—	—
9o	—	—	—	—	99.1	—	—	—
9p	—	—	—	—	—	98.9	—	—
9q	—	—	—	—	—	—	99.1	—
9r	—	—	—	—	—	—	—	99.6
13a	0.86	—	—	—	—	—	—	—
13b	0.78	—	—	—	—	—	—	—
13c	0.84	—	—	—	—	—	—	—
13d	1.01	—	—	—	—	—	—	—
13e	0.74	—	—	—	—	—	—	—
13f	0.81	—	—	—	—	—	—	—
14a	1.27	—	—	—	—	—	—	—
14b	1.45	—	—	—	—	—	—	—
14c	1.32	—	—	—	—	—	—	—
14d	1.15	—	—	—	—	—	—	—
14e	1.08	—	—	—	—	—	—	—
14f	1.26	—	—	—	—	—	—	—

was programmed from 100 °C to 250 °C at 10 °C/min. The split/splitless injector (1:20) and detector (FID) block temperatures were maintained at 260 and 275 °C, respectively. Helium (flow rate 2 ml/min) was used as the carrier gas. The fatty acid esters peaks were identified and calibrated with standard methyl esters: data are averages of three determinations.

All the synthesized 1,2-di-*O*-acyl-3-*O*-β-D-glycopyranosyl-*rac*-glycerols (**9a–r**, **13a–f**, **14a–f**) were submitted for preliminary evaluation of their in vitro antibacterial activity against *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 105487, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27753, *Mycobacterium 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 *Candida albicans* and *Candida pseudotropicalis*. Antimicrobial activity was always evaluated by reference methods.[36–38] The data are shown in Table 2. 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.[39] Miconazole was chosen as a standard in antifungal activity measurements. MIC was defined as the lowest concentration of compound that inhibited bacterial/fungal growth.

Table 2 shows MIC values of the synthesized compounds. No inhibiting activity was exhibited by any compound against *Candida* spp. As can be seen from the presented data, generally, glucosyl diglycerides (**9a–h**, **13a–c**, **14a–c**) present more potent effect as their activity was high, compared to galactosyl diglycerides (**9i–r**, **13d–f**, **14d–f**). Glycosyl diglycerides bearing the same saturated fatty acyls (**9a–e**, **9i–o**) at C-1 and C-2 displayed no inhibitory activity, except for **9a**, **9i** and **9b**, **9l** which contain a dicapryl (C8:0) (**9a**, **9i**) and a dicaprinyl (C10:0) (**9b**, **9l**) moiety, respectively.

On the other hand, compounds bearing the same unsaturated fatty acyls (**9f–h**, **9p–r**) and mixed fatty acyls (**13a–c**, **14a–c**, **13d–f**, **14d–f**) exhibited higher inhibitory activity than those bearing saturated fatty acyls. A total of 12 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 by increasing the length of the saturated acyl moiety (**9c–e**, **9m–o**). The presence of a single double bond within the acyl chain (18:1) (**9f**, **9p**) did not improve biological activity. The compounds **9g** and **9q**, characterized by the presence of two linoleic acids 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,

Table 2. Antimicrobial activity of the compounds **9a–r**, **13a–f**, **14a–f** 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
S. aureus ATCC 25923	16	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	8	>128	>128	0.5	—
S. aureus ATCC 105487	32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32	>128	0.25	—
E. faecalis ATCC 29212	32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	1	—
B. subtilis ATCC 6633	16	>128	>128	>128	>128	>128	128	>128	128	128	128	32	128	>128	0.25	—
E. coli ATCC 25922	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	0.25	—
P. aeruginosa ATCC 27753	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1	—
S. aureus 52	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>4	—
S. aureus 85	16	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	32	64	0.5	—
S. aureus 87	16	64	>128	>128	>128	>128	64	>128	64	64	64	>128	32	32	0.5	—
M. tuberculosis H37Rv	64	128	>128	>128	>128	>128	64	>128	64	64	64	>128	>128	>128	0.5	—
M. tuberculosis H160	64	64	>128	>128	>128	>128	64	>128	64	128	128	>128	>128	>128	0.5	—
M. tuberculosis H190	64	64	>128	>128	>128	>128	128	>128	64	64	64	>128	>128	>128	0.5	—
C. albicans	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	—	8
C. pseudotropicalis	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	—	0.5
	9i	**9l**	**9m**	**9n**	**9o**	**9p**	**9q**	**9r**	**13d**	**13e**	**13f**	**14d**	**14e**	**14f**	Ciprofloxacin	Miconazol
S. aureus ATCC 25923	32	32	>128	>128	>128	>128	>128	128	>128	>128	>128	128	>128	>128	0.5	—
S. aureus ATCC 105487	32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	0.25	—
E. faecalis ATCC 29212	32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	1	—
B. subtilis ATCC 6633	32	>128	>128	>128	>128	>128	128	>128	128	128	128	32	128	>128	0.25	—
E. coli ATCC 25922	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	0.25	—
P. aeruginosa ATCC 27753	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1	—
S. aureus 52	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>4	—
S. aureus 85	32	32	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	64	64	0.5	—
S. aureus 87	32	128	>128	>128	>128	>128	128	>128	64	128	64	>128	64	32	0.5	—
M. tuberculosis H37Rv	128	128	>128	>128	>128	>128	128	>128	128	64	128	>128	>128	>128	0.5	—
M. tuberculosis H160	128	64	>128	>128	>128	>128	128	>128	64	64	128	>128	>128	>128	0.5	—
M. tuberculosis H190	128	64	>128	>128	>128	>128	128	>128	64	128	128	>128	>128	>128	0.5	—
C. albicans	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	—	8
C. pseudotropicalis	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	—	0.5

while the analog **9r**, characterized by the presence of a galactosyl residue at the C-3 position of the glycerol instead of a glucosyl (**9h**), exhibited activity against *S. aureus* with a MIC of 128 μg/ml.

In conclusion, a series of 1,2-di-*O*-acyl-3-*O*-β-D-glycopyranosyl glycerols was synthesized and characterized, and some of them proved to be antibacterial agents. The stereoselectivity of pancreatic lipase was investigated in vitro where the preference for the 1 position in MGDGs is strictly related to the length of the acyl chains. From the presented data, pancreatic lipase, under our experimental conditions, is not able to hydrolyze the glycosidic bond, while the hydrolysis of the ester bond at the C-1 position of the glycerol backbone takes place exactly, as for triglycerides (TGs) and structured lipids.

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**, **9i**). This observation is confirmed by the results showing that compounds containing the octanoyl acyl moiety at C-1 and unsaturated fatty acids at C-2 position of glycerol (**13a**–**c**, **13d**–**f**) displayed significant activity against *B. subtilis*, *S. aureus 87*, and *M. tuberculosis* reference and clinical strains. The compounds **14a**–**c** and **14d**–**f**, which differ from the derivatives **13a**–**c** and **13d**–**f** for the presence of a decanoyl chain at the C-1 position of glycerol, displayed an interesting activity against *S. aureus* (**14a**–**c**, **14d**–**f**), *E. faecalis* (**14b**, **14c**), *B. subtilis* (**14a**–**b**, **14d**–**e**), but they lack 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.

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33. Data for **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 (COO), 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 (COCH_2), 32.3–23.2 (CH_2 aliph.), 25.5, 25.4

($COCH_2CH_2$), 14.5 (CH_3); ESI-MS *m/z*: 529 (M+Na, 25%)$^+$.

34. Data for **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, CH_3 term.), 1.25 (br s, 16H, CH_2 aliph.), 1.57 (br t, 4H, $COCH_2CH_2$), 2.10 (br, 4H, $2\times CH_2CH{=}CH$), 2.31 (br t, 4H, J = 7.0 Hz, $COCH_2$), 2.90 (br t, 4H, J = 5.5 Hz, $2\times{=}CHCH_2CH{=}$), 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, $3\times CH{=}CH$); ^{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 ($COCH_2$), 32.3–23.1 (CH_2 aliph.), 27.5, 27.6, 27.7 ($CH_2CH{=}CH$), 26.2, 26.0 ($=CHCH_2CH=$), 25.5, 25.4 ($COCH_2CH_2$), 14.8 (CH_3); ESI-MS *m/z*: 662 (M+Na, 100%)$^+$.
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