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Design and synthesis of benzyl 4-*O*-lauroyl-α-L-rhamnopyranoside derivatives as antimicrobial agents

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C H R O N I C L E

Article history:
Received August 21, 2016
Received in revised form
October 14, 2016
Accepted 15 October 2016
Available online
15 October 2016

Keywords:
Benzyl α-L-rhamnopyranoside
Lauroylation
Antimicrobial agents
Structure activity relationship (SAR)

A B S T R A C T

Benzyl α-L-rhamnopyranoside, prepared by both conventional and microwave assisted glycosidation techniques, was converted into benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside which after lauroylation followed by removal of isopropylidene group gave the benzyl 4-*O*-lauroyl-α-L-rhamnopyranoside in good yield. Several derivatives of benzyl 4-*O*-lauroyl-α-L-rhamnopyranoside were prepared and assessed *in vitro* for their antimicrobial activity against ten human pathogenic bacteria and seven fungi. The structure activity relationship (SAR) study revealed that incorporation of 4-*O*-lauroyl group in rhamnopyranoside frame work along with 2,3-di-*O*-acyl group increased the antifungal potentiality of the rhamnopyranoside**s**.

1. Introduction

L-Rhamnose, an important member of the monosaccharide series,[1] is widely distributed in nature, it was found in plant gums, plant glycosides and in bacterial polysaccharides.[1,2] Some disaccharides having L-rhamnose as the aglycone has been synthesized and are important for the determination of the immunodominant site in antigenic lipopolysaccharides.[3] The aldobiouronic acid 4-*O*-(8-D-glucopyranosyluronic acid)-L-rhamnose has been isolated from hydrolysates of *Acrosiphonia centralis*, *Ulva lactuca* and *Klebsiella* K9 capsular polysaccharide.[3] Also, 5-*O*-α-L-rhamnopyranosyl-β-L-arabinofuranose (**1**, Fig. 1) has been found as the sugar component of sitosterol glycoside and showed rhamnosidase specificity in *Aspergillus niger*.[4] The diacetyl derivative of the natural product kaempferol-3-*O*-(3',4'-di-*O*-acetyl-α-L-rhamnopyranoside), also called SL0101 (**2**), is a highly specific protein kinase (RSK) inhibitor.[5] Compound **2** was isolated from *Forsteronia refracta*, a variety of dogbane found in the South American rainforest. This diacetyl compound **2** was found 12 times more inhibitor of RSK *in vitro* than that of its non-acetyl analogue **3**. Thus, diacyl compound **2** inhibits the growth of cancer cell lines.[5] Acylation of the rhamnose moiety in these natural products is necessary

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doi: 10.5267/j.ccl.2016.10.001

for high affinity binding and selectivity. These results should facilitate the development of RSK inhibitors derived from SL0101 as anticancer agents.[5]

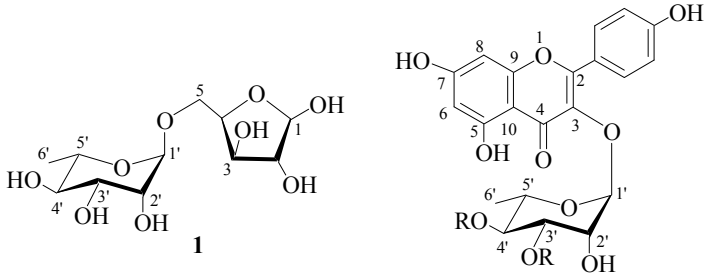


Fig. 1. Naturally occurring rhamnopyranosides **1-3**

The branched L-rhamnopyranosides are found abundant in nature.[6] Protected carbohydrate derivatives are also used as intermediate in syntheses of many biologically active natural products.[7-9] Although, regioselectivity is a major challenge as carbohydrates contain several hydroxyl groups of similar reactivity. Small differences in reactivity cannot be utilized for selective protection and modification of hydroxyl group. However, desired protection pattern can be achieved in one or few steps making use of complex reaction sequences.[10] For example, organotin reagents, such as tributyltin oxide or dibutyltin oxide[11-12] are often used to accomplish regioselective protection, including acylation[13-14] of hydroxyl group of carbohydrate derivatives. Typically, the regioselectivity is difficult to control due to the similarity of the secondary 2, 3 and 4-trihydroxyls of rhamnose.[12,15-17] In this context, our main aim was to establish a method for the synthesis of 4-*O*-lauroylrhamnopyranoside *via* protection-deprotection technique.

In recent years, search for new antibacterial agents with novel mode of action represents a major target in chemotherapy[18] as the emergence of multiple antibiotic resistant pathogenic bacteria causing threat to human health worldwide. Sugar esters have been widely used as cosmetic and pharmaceutical industries for many years because they are considered to be biocompatible, biodegradable, and nontoxic.[19-20] The sugar moieties present in these esters can increase drug water solubility, decrease toxicity, and contribute to the bioactivity of the natural products. Hence, sugar esters are used as anticancer agents,[21] insecticides,[22] antibacterial, and antifungal agents.[23-26] Attachment of aryl and acyl group(s) to the sugar molecules enhances the biological activities many times than that of the parent sugar[27,28]. Considering these important observations, we are interested to the introduction of lauroyl group at position C-4 of benzyl α-L-rhamnopyranoside (**4**) instead of acyl group at C-3 position. This may provide important information about positional effects of the acyl group in its role as antimicrobial functionality.

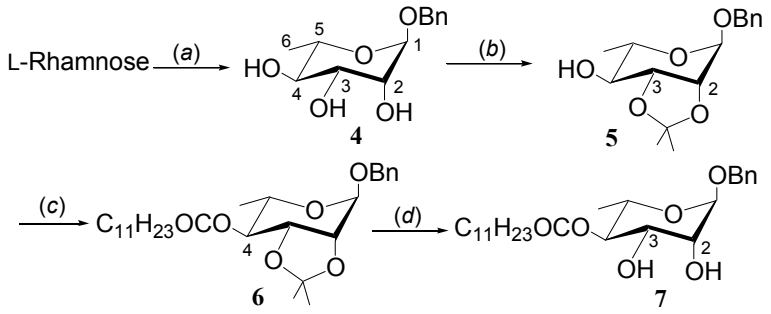
2. Results and Discussion

Our present research work mainly describes the synthesis of benzyl 4-*O*-lauroyl-α-L-rhamnopyranoside (**7**) with its 2,3-di-*O*-acyl derivatives (**8**-**10**) and antimicrobial evaluation/studies of all the synthesized products.

2.1 Synthesis of benzyl 4-O-lauroyl-α-L-rhamnopyranoside (7)

For the selective 4-*O*-lauroylation of benzyl α-L-rhamnopyranoside (**4**) dibutyltin oxide method was found to be unsuccessful and furnished the 3-*O*-acyl derivatives only.[15-17] Thus, protection-deprotection method was employed successfully for the 4-*O*-lauroylation of rhamnopyranoside **4**. Initially, benzyl α-L-rhamnopyranoside (**4**) was prepared from L-(+)-rhamnose according to the

literature procedure[12,29] (Scheme 1) in 82% yield.



Reagents and conditions: (*a*) BnOH, Amberlite IR 120 (H^+) resin, 120 °C, 30 h[12,29], 82%, or BnOH (excess), IR 120 H^+ resin, Microwave irradiation, 90 sec, 96%; (*b*) 2,2-dimethoxypropane, *p*-TsOH (cat), rt, 2 h, 93%; (*c*) $C_{11}H_{23}COCl$, pyridine, dimethylaminopyridine (cat), 0 ºC - rt, 12 h, 95%; (*d*) AcOH, 40 ºC, 18 h, 82%.

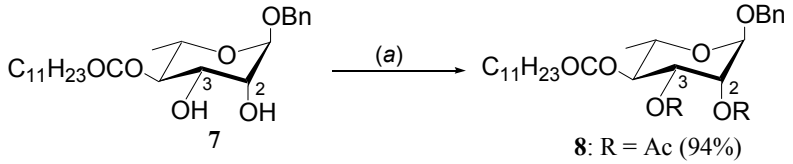
Scheme 1. Synthesis of benzyl 4-*O*-lauroyl-α-L-rhamnopyranoside (**7**)

To improve the yield of **4**, we have applied microwave irradiation to conventional Fischer glycosidation. Thus, microwave irradiation of finely powdered L-rhamnose with little excess dry benzyl alcohol (in a porcelain dish) and Amberlite IR 120 (H^+) ion exchange resin at 160 watts for 90 sec in a domestic microwave oven followed by short silica gel column provided pure benzyl rhamnopyranoside **4** almost in quantitative yield (96%), as a brownish thick liquid. Notable, the achieved in this method yield was very high and the reaction time was shorter (only 90 sec) compare to the conventionaly heated reaction (20 h). Having benzyl α-L-rhamnopyranoside (**4**) in hand, we have protected its *cis*-vicinal glycol group at position C-2 and C-3 by isopropylidene protecting group. Treatment of **4** with excess 2,2-DMP in the presence of catalytic amount of *p*-TSA afforded **5**, as an oil, in 79% yield. In its IR spectrum, stretching bands at 3450-3300 (br) and 1381 cm^{-1} belong to hydroxyl group and isopropylidene group, respectively. In the ^1H NMR spectrum, two three-proton singlets at δ 1.33 and 1.32 ppm confirm the presence of one acetonide group in the molecule. Based on the spectral analysis, the structure of **5** was established as benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside. The acetonide protection was formed between *cis*-vicinal 2,3-diol positions of **4** and Liptak *et al*. reported the similar type of acetonide formation.[30] The monoacetonide **5**, having free hydroxyl group at C-4 position, was used in mono-lauroylation in reaction with lauroyl chloride in dry pyridine to afford **6** as a viscouscous oil (Scheme 1). IR spectrum of the compound **6** possessed the carbonyl-stretching band at 1708 cm^{-1} instead of the C-4 hydroxyl group band at 3450-3300 cm^{-1}. The proton spectrum was consistent with the structure of compound **6**. The presence of lauroyl group was confirmed by the integrating the regions of ^1H NMR spectrum at about 0.87 (3H), 1.21-1.34 (16H, overlapping multiple signals) 1.59-1.65 (2H, m), and 2.36 (2H, t) ppm, totaling to 23 proton equivalents. In addition, the downfield shift of H-4 (4.90 ppm) as compared to the precursor compound **5** (4.42-4.48 ppm) confirmed the attachment of the lauroyloxy group at C-4 position of the molecule. Thus, the structure of benzyl 2,3-*O*-isopropylidene-4-*O*-lauroyl-α-L-rhamnopyranoside (**6**) was confirmed. In the subsequent step, removal of the acetonide functionality was achieved by stirring 4-*O*-lauroate **6** with glacial acetic acid at 40 ºC for 18 h to give a semi-solid **7** (82%). In the IR spectrum of **7**, the presence of a new broad band at 3510-3280 cm^{-1} corresponding to hydroxyl groups witnessed the removal of isopropylidene moiety. This fact was also confirmed by observation of the absence of isopropylidene protons in the ^1H NMR spectrum, while a broad two-proton singlet (exchanged with D_2O) at 1.87-2.16 ppm in that spectrum corresponds to two hydroxyl groups. Thus, the structure benzyl 4-*O*-lauroyl-α-L-rhamnopyranoside (**7**) was unambiguously assigned.

*2.2 Synthesis of 2,3-di-O-acyl derivatives **8-10** of 4-O-lauroate 7*

To get new biologicaly active derivatives of L-rhamnose three 2,3-di-*O*-acyl derivatives (**8-10**) containing various groups (e.g. acetyl, mesyl and benzoyl) (Scheme 2), were prepared. Initially,

treatment of diol **7** with acetic anhydride in pyridine gave a compound **8** in 94% yield. Its IR spectrum gave signals at 1751, 1740 and 1716 cm^{-1} (CO) and showed no signals for hydroxyl stretching indicating acetylation of the molecule. In the ^1H NMR spectrum, two three-proton singlets at 2.11 and 1.96 ppm, corresponding to two acetyl-methyl groups, clearly indicated the attachment of two acetyloxy groups in the molecule. Also, H-2 (5.19 ppm) and H-3 (5.34 ppm) protons were shifted considerably downfield as compared to its precursor 2,3-diol compound **7** (4.04-4.07) which indicated the attachment of acetyloxy groups at C-2 and C-3 positions. This confirm the assignment of the structure of benzyl 2,3-di-*O*-acetyl-4-*O*-lauroyl-α-L-rhamnopyranoside (**8**).



7

8: R = Ac (94%)
9: R = Ms (81%)
10: R = Bz (87%)

Reagents and conditions: (*a*) Ac_2O/MsCl/BzCl, pyridine, dimethylaminopyridine, 0 ºC-rt, 12 h.

Scheme 2. Synthesis of compounds **8-10**

Similarly, mesylation of 4-*O*-lauroate **7** gave a compound **9** in 81% yield. Its IR spectrum showed no signal for hydroxyl group and thus indicated the mesylation of the compound. In its ^1H NMR spectrum, two three-proton singlets at 3.15 and 3.12 ppm clearly indicated the attachment of two mesyloxy groups in the molecule. The reasonable downfield shift of H-2 (4.98 ppm) and H-3 (5.05 ppm) protons as compared to that of compound **7** (4.04-4.07 ppm) confirmed the attachment of two mesyloxy groups at position C-2 and C-3. The rest of the ^1H NMR spectrum was in complete agreement with the structure assigned as benzyl 2,3-di-*O*-methanesulfonyl-4-*O*-lauroyl-α-L-rhamnopyranoside (**9**). Finally, dimolar benzoylation of laureate **7** gave a solid benzyl 2,3-di-*O*-benzoyl-4-*O*-lauroyl-α-L-rhamnopyranoside (**10**) in 87% yield as confirmed a complete analysis of its IR and ^1H NMR spectra.

2.3 Conformational study of the L*-rhamnopyranosides (**4-10**): Distortion of **5** and **6***

Table 1. Coupling constants of rhamnopyranosides **5-10.**

Rhamnopyranoside	coupling constants (Hz)		
	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
5	5.8	6.9	--
6	3.0	10.0	6.3
7	3.4	9.6	10.0
8	3.2	10.1	9.9
9	2.7	--	--
10	--	9.8	9.8

Methyl α-L-rhamnopyranoside (**11**) is well known to exist in $^1C_4$ conformation.[31-32] Similarly, benzyl α-L-rhamnopyranoside (**4**) was found to exist in regular $^1C_4$ conformation.[29] However, in case of derivatives **5-6**, the presence of isopropylidene functionality at C-2 and C-3 positions and/or acyl group(s) increases the steric hindrance in these molecules. Therefore, the conformations of **5-10** were proposed based on the analyses of ^1H NMR spectral data. The coupling constants determined from the 400 MHz ^1H NMR spectra in $CDCl_3$ of **5-10** are shown in Table 1. In case of **7**, appearance of a distinct triplet for H-4 at 5.02 ($J_{4,3} = J_{4,5} = 10.0$ Hz) and a doublet of doublet for H-3 at 4.04 ($J_{3,4} = 9.6$ and $J_{3,2} = 3.4$ Hz) ppm were informative. The large coupling constants ($J_{4,3} = J_{4,5} = \sim 10.0$ Hz) for the H-4 axial proton requires *trans*-diaxial relationship with H-3 and H-5 protons. This clearly requires H-3 and H-5 protons to be axial. Again, the small coupling constant between H-3 and H-2 protons requires *cis* axial-equatorial relationship. As H-3 is axially oriented, H-2 must be present in equatorial position. These observation confirmed that 4-*O*-lauroate **7** exists in regular $^1C_4$ conformation with C-5 substituent (–

CH_3) equatorially oriented [(5*S*)]. Compound **7** was obtained from monoacetonide **6**. Hence, in compound **6**, the relative stereochemistry of the substituents at C-2, C-3 is *cis* and C-3, C-4 is *trans* (as the same stereochemistry is retained in the product **7** formation). But the ^{1}H NMR spectrum of rhamnopyranoside **6** contains a doublet of doublet for H-4 at 4.90 ppm ($J_{4,3}$ = 10.0 and $J_{4,5}$ = 6.3 Hz). The smaller value of coupling constant between H-4 and H-5 (6.3 Hz) than the expected one (~10.0 Hz) could be explained by the presence of a five-membered isopropylidene ring fused to the six-membered rhamnopyranoside ring. This clearly indicated the slight distortion of the pyranose ring from regular $^1C_4$ conformation. Similar distortion of the pyranose ring from regular $^1C_4$ conformation was also observed for monoacetonide **5**. It could be anticipated from the Table 1 that coupling constants of compounds **8**-**10** were in good agreement with regular $^1C_4$ conformation with C-5 substituent (–CH_3) equatorially oriented [(5*S*) configuration].

2.4 Antimicrobial studies

In vitro zone of inhibitions of four Gram-positive and six Gram-negative bacteria due to the effect of the rhamnopyranoside derivatives **4**-**10** are shown in Table 2. The Table 2 indicates that the tested rhamnopyranosides **4**-**10** were less effective against these Gram-positive and Gram-negative organisms than that of the standard antibiotic kanamycin. Only 2,3-di-*O*-benzoate **10** exhibited considerable inhibition against these bacterial pathogens.

Table 2. Inhibition against bacterial organism by the rhamnopyranosides (**4**-**10**)

	Diameter of zone of inhibition in mm, 50 μg.dw./disc							
Name of bacteria	**4**	**5**	**6**	**7**	**8**	**9**	**10**	****Kanamycin**
Bacillus cereus	NI	NI	NI	NI	NI	NI	08	*20
Bacillus megaterium	NI	NI	05	06	08	11	15	*20
Bacillus subtilis	NI	07	NI	NI	NI	09	12	*21
Staphylococcus aureus	NI	NI	NI	NI	NI	NI	*20	*22
Escherichia coli	NI	NI	NI	06	09	06	19	*22
Pastunella maltosida	NI	NI	NI	NI	08	NI	NI	*23
Salmonella gallinarium	NI	NI	08	07	12	15	NI	*24
Salmonella typhi	05	NI	06	06	11	12	17	*23
Shigella dysenteriae	NI	10	10	NI	10	NI	18	*24
Vibrio cholerae	NI	NI	NI	NI	06	NI	14	18

"*" shows good inhibition, "NI" indicates no inhibition,
"**" indicates standard antibiotic, "dw" means dry weight

Table 3. Antifungal activities of the rhamnopyranoside derivatives (**4**-**10**)

	% inhibition of fungal mycelial growth, sample 100 μg.dw./mL PDA							
Name of fungus	**4**	**5**	**6**	**7**	**8**	**9**	**10**	****Fluconazole**
Aspergillus acheraccus	NI	35	40	45	25	NI	43	58
Aspergillus flavus	NI	18	22	33	*66	42	*62	*62
Aspergillus fumigatus	NI	26	24	41	46	44	NI	*70
Aspergillus niger	NI	28	35	48	51	49	41	58
Aspergillus nodusus	NI	NI	NI	31	NI	33	46	*64
Candida albicans	18	32	33	NI	32	28	37	*60
Fuserium equiseti	10	NI	38	49	44	45	51	*65

"*" shows good inhibition, "NI" indicates no inhibition,
"**" indicates standard antibiotic, "dw" means dry weight

In vitro percentage inhibition results of mycelial growth of seven plant pathogenic fungi due to the effect of rhamnopyranoside derivatives (**4**-**10**) are presented in Table 3. All the acylated rhamnopyranosides were found comparatively more active against the tested fungal pathogens than that of bacterial organisms. In case of *Aspergillus flavus*, diacetate **8** (*66%) and dibenzoate **10** (*62%) showed excellent inhibition, which were comparable to that of standard antifungal antibiotic

fluconazole (*62%).

2.5 Structure activity relationship (SAR)

It was evident from Table 2 and Table 3 that incorporation of lauroyl group increased the antimicrobial potentiality of rhamnopyranoside **4**. Again, the rhamnopyranoside derivatives **4-10** were more active against fungal pathogens than against the bacterial organisms. An important observation was that, compounds **7-10** were found to be more active than compounds **5-6** against the tested pathogens. Compounds **4-7** contain more hydroxyl groups (more hydrophilic) than that of compound **8-10**. Compounds **8-10** having fewer or no hydroxyl groups (more hydrophobic) showed much better antimicrobial potentiality than compounds **4-7**. The hydrophobicity of compounds is an important parameter for bioactivity such as toxicity or alteration of membrane integrity, and is directly related to membrane permeation.[33] Hunt[34] proposed that the antimicrobial activities of alcoholic compounds is directly related to their lipid solubility through the hydrophobic interaction between alkyl chains of alcohols and lipid regions in the membrane. A similar hydrophobic interaction might occur between the acyl chains of glucofuranoses accumulated in the lipid like nature of the bacteria membranes. As a consequence of their hydrophobic interaction, bacteria lose their membrane permeability, ultimately causing death of the organism.[33-35]

It was observed from Table 2 and Table 3 that 4-*O*-lauroyl-2,3-di-*O*-acetate/mesylate/benzoate (**8**/**9**/**10**) exhibited excellent activity against both bacterial and fungal pathogens which were, in some cases, comparable to that of the standard antibiotic. This led us to conclude that incorporation of 4-*O*-lauroyl group in rhamnopyranoside frame work along with 2,3-di-*O*-acetyl/mesyl/benzoyl group increased the antimicrobial potentiality of the rhamnopyranoside **4**.

3. Conclusions

Thus, benzyl 4-*O*-lauroyl-α-L-rhamnopyranoside (**7**) was successfully synthesized in reasonably good yield (improved by application of microwave irradiation) from benzyl α-L-rhamnopyranoside (**4**). Three 2,3-di-*O*-acyl substituted derivatives (**8-10**) of **7** were also prepared for biological study. Rhamnopyranosides **5** and **6** may have a slightly distorted, due to the presence of isopropylidene, pyranose ring. *In vitro* antimicrobial functionality tests and structure activity relationship (SAR) study revealed that incorporation of 4-*O*-lauroyl and 2,3-di-*O*-acetyl/mesyl/benzoyl groups in rhamnopyranoside frame increased the antimicrobial potentiality of rhamnopyranoside **4**.

Acknowledgements

The authors would like to thank the Ministry of Science and Technology, Dhaka, Bangladesh for financial support (BS-10/2013-14). Mr. A.K.M.S. Azad is grateful to Bangladesh University Grants Commission for Ph.D. fellowship (2009).

4. Experimental

4.1. Materials and methods

All reagents were commercially available from Merck and Aldrich and used as received unless otherwise specified. Melting points (mp) were determined on an electrothermal melting point apparatus and are uncorrected. Thin layer chromatography was performed on Kieselgel GF_{254} and visualization was accomplished by spraying the plates with 1% H_2SO_4 followed by heating the plates at 150-200 ºC until coloration took place. Evaporations were performed under diminished pressure on a Büchi rotary evaporator. Column chromatography was carried out with silica gel (100-200 mesh). IR spectra were recorded on a FT-IR spectrophotometer (Shimadzu, IR Prestige-21) in $CHCl_3$ solution. ^1H (400 MHz, AVANCE III, ASCEND,™ Bruker, Switzerland) NMR spectra were recorded in $CDCl_3$ solution using tuneable multinuclear probe. The microwave heating was provided by a domestic microwave oven (LG

microwave oven, MB-3947C, 800 W, 2450 MHz). Chemical shifts were reported in δ unit (ppm) with reference to TMS as an internal standard and *J* values are given in Hz.

4.2. General procedure: Synthesis

Benzyl α-L-rhamnopyranoside (4):

(a) Literature method: The compound **4** was prepared from L-rhamnose (Merck) and anhydrous benzyl alcohol with Amberlite IR 120 (H^+) resin (stirring at 120 °C for 30 h) in 82% yield as a thick syrup by a literature procedure.[12,29] R_f = 0.52 ($CHCl_3$/MeOH = 10/1); IR ($CHCl_3$): 3480-3310 cm^{-1} (br, OH); ^1H NMR (400 MHz, CD_3OD): δ = 7.12-7.28 (5H, m, Ar-*H*), 4.75 (1H, s, H-1), 4.68 (1H, d, *J* = 12.0 Hz, PhCH_AH_B), 4.59 (1H, m, H-2), 4.50 (1H, d, *J* = 12.0 Hz, PhCH_AH_B), 3.76-3.84 (1H, m, H-3), 3.57-3.69 (1H, m, H-5), 3.38 (1H, t, *J* = 10.6 Hz, H-4), 3.27-3.32 (3H, br s, exchange with D_2O, 3×O*H*), and 1.26 (3H, d, *J* = 6.4 Hz, 6-CH_3) ppm.

(b) Microwave assisted method: Finely powdered L-rhamnose (0.8 g, 4.873 mmol) was taken in a porcelain dish followed by addition of dry benzyl alcohol (1.0 mL) and Amberlite IR 120 (H^+) ion exchange resin (0.8 g). The reaction mixture was mixed with a spatula and covered with a glass plate. The mixture was then placed in a domestic microwave oven (LG microwave oven, MB-3947C, 800 W, 2450 MHz) and irradiated at 160 watts for 1.5 minutes (30 sec×3). Progress of the reaction was monitored every 30 sec intervals by TLC ($CHCl_3$/MeOH = 10/1). The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to leave a thick syrup. The syrup was then passed through a short silica gel column to give pure benzyl rhamnopyranoside (1.19 g, 96%) as brownish thick liquid. The IR and ^1H NMR spectra of this compound were indistinguishable to that of earlier prepared (**4**) by conventional glycosidation method (literature method).

Benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (5): A solution of benzyl α-L-rhamnopyranoside (**4**) (2.0 g, 7.865 mmol), excess 2,2-dimethoxypropane (DMP, 40 mL) and catalytic amount of *p*-toluenesulfonic acid (*p*-TSA, 0.02 mg) was refluxed for 30 min. Here DMP acts both as a solvent and as a reagent. The mixture was cooled, added 10% $NaHCO_3$ solution (2 mL) and extracted with ethyl acetate (3×5 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuum to leave a thick syrup which on column chromatography (*n*-hexane/ethyl acetate = 10/1) afforded compound **5** as an oil (1.829 g, 79%). R_f = 0.45 (*n*-hexane/ethyl acetate = 4/1); IR ($CHCl_3$): 3450-3300 (br, OH), 1381 cm^{-1} [$C(CH_3)_2$]; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.09-7.36 (5H, m, Ar-*H*), 4.92 (1H, s, H-1), 4.72 (1H, d, *J* = 11.8 Hz, PhCH_AH_B), 4.70 (1H, d, *J* = 5.0 Hz, H-2), 4.66 (1H, dd [apparent t], *J* = 6.9 and 5.8 Hz, H-3), 4.58 (1H, d, *J* = 11.8 Hz, PhCH_AH_B), 4.51-4.57 (1H, m, H-5), 4.42-4.48 (1H, m, H-4), 1.90-2.20 (1H, br s, exchange with D_2O, O*H*), 1.33 [3H, s, $C(CH_3)_2$], 1.32 [3H, s, $C(CH_3)_2$], and 1.28 (3H, d, *J* = 6.1 Hz, 6-CH_3) ppm.

General procedure for acylation: To a solution of the benzyl rhamnopyranoside having hydroxyl groups in anhydrous pyridine (1 mL) was added acyl halide at 0 ºC followed by addition of catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was allowed to attain room temperature and stirring was continued for 10-16 h. A few pieces of ice was added to the reaction mixture to decompose unreacted (excess) acyl halide and extracted with dichloromethane (DCM, 3×5 mL). The DCM layer was washed successively with 5% hydrochloric acid, saturated aqueous sodium hydrogen carbonate solution and brine. The DCM layer was dried and concentrated under reduced pressure. The residue thus obtained on column chromatography (*n*-hexane/ethyl acetate) gave the corresponding acylated product.

Benzyl 2,3-*O*-isopropylidene-4-*O*-laurouyl-α-L-rhamnopyranoside (6): Thick syrup; yield 85%; R_f= 0.57 (*n*-hexane/ethyl acetate = 6/1); IR ($CHCl_3$): 1708 (CO), 1375 cm^{-1} [$C(CH_3)_2$]; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.51-7.60 (5H, m, Ar-*H*), 5.11 (1H, s, H-1), 4.90 (1H, dd, *J* = 10.0 and 6.3 Hz,

H-4), 4.71 (1H, d, J = 11.8 Hz, PhCH_AH$_B$), 4.55 (1H, d, J = 11.8 Hz, PhCH$_A$$H_B$), 4.26 (1H, dd, J = 10.0 and 3.0 Hz, H-3), 4.15 (1H, d, J = 3.0 Hz, H-2), 3.76-3.80 (1H, m, H-5), 2.36 [2H, t, J = 7.5 Hz, CH$_3$(CH$_2$)$_9$CH_2CO], 1.59-1.65 [2H, m, CH$_3$(CH$_2$)$_8$CH_2CH$_2$CO], 1.52 [3H, s, C(CH_3)$_2$], 1.34 [3H, s, C(CH_3)$_2$], 1.21-1.34 [16H, br m, CH$_3$(CH_2)$_8$CH$_2$CH$_2$CO], 1.17 (3H, d, J = 6.2 Hz, 6-CH_3), and 0.87 [3H, t, J = 6.5 Hz, CH_3(CH$_2$)$_{10}$CO] ppm.

Benzyl 4-*O*-lauroyl-α-L-rhamnopyranoside (7): 4-*O*-Lauroate **6** (1.8 g, 3.776 mmol) was gently dissolved in acetic acid (96%, 25 mL) at room temperature. The solution was slowly warmed to 40 ºC and stirred at this temperature for 18 h. After completion of the reaction, acetic acid was evaporated in *vacuum* and co-evaporated with toluene (3×3 mL) to remove traces of acetic acid. The residue thus obtained on chromatography with *n*-hexane/ethyl acetate (4/1) afforded 2,3-diol **7** (1.352 g, 82%) as semi-solid. R_f = 0.46 (*n*-hexane/ethyl acetate = 2/1); IR ($CHCl_3$): 3510-3280 (br, OH), 1705 cm^{-1} (CO); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38-7.46 (5H, m, Ar-*H*), 5.02 (1H, t, J = 10.0 Hz, H-4), 4.95 (1H, s, H-1), 4.72 (1H, d, J = 11.8 Hz, PhCH_AH$_B$), 4.53 (1H, d, J = 11.8 Hz, PhCH$_A$$H_B$), 4.07 (1H, d, J = 3.4 Hz, H-2), 4.04 (1H, dd, J = 9.6 and 3.4 Hz, H-3), 3.94-4.02 (1H, m, H-5), 2.36 [2H, t, J = 7.2 Hz, CH$_3$(CH$_2$)$_9$CH_2CO], 1.87-2.16 (2H, br s, exchange with D_2O, 2×O*H*), 1.55-1.64 [2H, m, CH$_3$(CH$_2$)$_8$CH_2CH$_2$CO], 1.18-1.30 [16H, m, CH$_3$(CH_2)$_8$CH$_2$CH$_2$CO], 1.16 (3H, d, J = 6.0 Hz, 6-CH_3), and 0.87 [3H, t, J = 6.5 Hz, CH_3(CH$_2$)$_{10}$CO] ppm.

Benzyl 2,3-di-*O*-acetyl-4-*O*-lauroyl-α-L-rhamnopyranoside (8): Semi-solid; yield 94%; R_f = 0.56 (*n*-hexane/ethyl acetate = 5/1); IR ($CHCl_3$): 1751, 1740, 1716 cm^{-1} (CO); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41-7.48 (5H, m, Ar-*H*), 5.34 (1H, dd, J = 10.1 and 3.2 Hz, H-3), 5.27 (1H, t, J = 9.9 Hz, H-4), 5.19 (1H, d, J = 3.2 Hz, H-2), 4.85 (1H, s, H-1), 4.75 (1H, d, J = 12.0 Hz, PhCH_AH$_B$), 4.59 (1H, d, J = 12.0 Hz, PhCH$_A$$H_B$), 4.01-4.06 (1H, m, H-5), 2.25 [2H, t, J = 7.4 Hz, CH$_3$(CH$_2$)$_9$CH_2CO], 2.11 (3H, s, COCH_3), 1.96 (3H, s, COCH_3), 1.51-1.60 [2H, m, CH$_3$(CH$_2$)$_8$CH_2CH$_2$CO], 1.20-1.29 [16H, br s, CH$_3$(CH_2)$_8$CH$_2$CH$_2$CO], 1.20 (3H, d, J = 6.5 Hz, 6-CH_3), and 0.86 [3H, t, J = 6.6 Hz, CH_3(CH$_2$)$_{10}$CO] ppm.

Benzyl 2,3-di-*O*-mesyl-4-*O*-lauroyl-α-L-rhamnopyranoside (9): Semi-solid; yield 81%; R_f = 0.50 (*n*-hexane/ethyl acetate = 6/1); IR ($CHCl_3$): 1746 (CO), 1318 cm^{-1} (SO_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37-7.46 (5H, m, Ar-*H*), 5.04-5.13 (2H, m, H-3 and H-4), 4.98 (1H, d, J = 2.7 Hz, H-2), 4.84 (1H, s, H-1), 4.78 (1H, d, J = 12.1 Hz, PhCH_AH$_B$), 4.58 (1H, d, J = 12.1 Hz, PhCH$_A$$H_B$), 3.89-3.98 (1H, m, H-5), 3.15 (3H, s, SO_2CH_3), 3.12 (3H, s, SO_2CH_3), 2.34 [2H, t, J = 7.4 Hz, CH$_3$(CH$_2$)$_9$CH_2CO], 1.56-1.64 [2H, m, CH$_3$(CH$_2$)$_8$CH_2CH$_2$CO], 1.22-1.30 [16H, m, CH$_3$(CH_2)$_8$CH$_2$CH$_2$CO], 1.20 (3H, d, J = 6.4 Hz, 6-CH_3), and 0.85 [3H, t, J = 6.8 Hz, CH_3(CH$_2$)$_{10}$CO] ppm.

Benzyl 2,3-di-*O*-benzoyl-4-*O*-lauroyl-α-L-rhamnopyranoside (10): Solid, mp 55-56 ºC; yield 87%; R_f = 0.54 (*n*-hexane/ethyl acetate = 7/1); IR ($CHCl_3$): 1744, 1728, 1708 cm^{-1} (CO); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05 (2H, d, J = 8.2 Hz, Ar-*H*), 7.96 (2H, d, J = 8.2 Hz, Ar-*H*), 7.41-7.53 (11H, m, Ar-*H*), 5.54- 5.62 (2H, m, H-2 and H-3), 5.34 (1H, t, J = 9.8 Hz, H-4), 4.85 (1H, d, J = 12.0 Hz, PhCH_AH$_B$), 4.80 (1H, s, H-1), 4.69 (1H, d, J = 12.0 Hz, PhCH$_A$$H_B$), 3.97-4.02 (1H, m, H-5), 2.15-2.18 [2H, m, CH$_3$(CH$_2$)$_9$CH_2CO], 1.40-1.48 [2H, m, CH$_3$(CH$_2$)$_8$CH_2CH$_2$CO], 1.32 (3H, d, J = 6.2 Hz, 6-CH_3), 1.04-1.20 [16H, br m, CH$_3$(CH_2)$_8$CH$_2$CH$_2$CO], and 0.81 [3H, t, J = 7.0 Hz, CH_3(CH$_2$)$_{10}$CO] ppm.

4.3 Test human and phytopathogens

The rhamnopyranoside derivatives (**4-10**) were tested against ten human pathogenic bacteria. Of these four were Gram-positive viz. *Bacillus cereus* BTCC 19, *Bacillus megaterium* BTCC 18, *Bacillus subtilis* BTCC 17 and *Staphylococcus aureus* ATCC 6538 and six were Gram-negative bacteria viz. *Escherichia coli* ATCC 25922, *Pastunella maltosida, Salmonella gallinarium, Salmonella typhi* AE 14612, *Shigella dysenteriae* AE 14369 and *Vibrio cholerae*. Seven plant pathogenic fungi viz. *Aspergillus acheraccus*, *Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Aspergillus*

nodusus, Candida albicans and *Fuserium equiseti* (Corda) Sacc. were selected for *in vitro* mycelial growth test for these rhamnopyranoside derivatives (**4-10**).

4.4 Antimicrobial screening procedure

Screening of antibacterial activity: For the detection of antibacterial activities, the disc diffusion method[23] was followed. Dimethylformamide (DMF) was used as a solvent for test chemicals and a 2% solution of the compound was used in the investigation. The plates were incubated at 37 °C for 48 h. Proper control was maintained with DMF without chemicals. Mueller-Hinton (agar and broth) medium was used for culture of bacteria. Each experiment was carried out three times. All the results were compared with the standard antibacterial antibiotic kanamycin (50 μg/disc, Taj Pharmaceuticals Ltd., India).

Screening of mycelial growth: The antifungal activities of the newly synthesized rhamnopyranosides (**4-10**) were investigated based on food poisoning technique.[25,26] Sabouraud (agar and broth, PDA) medium was used for culture of fungi. Linear mycelial growth of fungus was measured after 3~5 days of incubation. The percentage inhibition of radial mycelial growth of the test fungus was calculated as $I = \left\{ \frac{C-T}{C} \right\} \times 100$ where, I = percentage of inhibition, C = diameter of the fungal colony in control (DMF), T = diameter of the fungal colony in treatment. The results were compared with standard antifungal antibiotic fluconazole (100 μg/mL medium, brand name Omastin, Beximco Pharmaceuticals Ltd., Bangladesh).

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