

Infrared, ¹H-NMR Spectral Studies of some Methyl 6-O-Myristoyl- α -D-Glucopyranoside Derivatives: Assessment of Antimicrobial Effects

Sarkar M. A. Kawsar^{1,3*}, Khaleda Mymona¹, Refat Asma¹,
Mohammad A. Manchur², Yasuhiro Koide³ and Yasuhiro Ozeki³

¹Laboratory of Carbohydrate and Protein Chemistry, Department of Chemistry, Faculty of Science, University of Chittagong, Chittagong-4331, Bangladesh

²Department of Microbiology, Faculty of Biological Sciences, University of Chittagong, Chittagong-4331, Bangladesh

³Laboratory of Glycobiology and Marine Biochemistry, Department of Life and Environmental System Science, Graduate School of NanoBio Sciences, Yokohama City University, 22-2 Seto, Kanazawa-ku, Yokohama 236-0027, Japan

*E-mail address: akawsarabe@yahoo.com

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ABSTRACT. This study was carried out to regioselective myristoylation of methyl α -D-glucopyranoside (**1**) using the direct acylation method gave the corresponding methyl 6-O-myristoyl- α -D-glucopyranoside (**2**) in fair yield. A number of 2,3,4-tri-O-acyl derivatives (**3-15**) of this 6-O-substitution product using a wide variety of acylating agents were also prepared in order to obtain newer derivatives of synthetic and biological importance. The reaction conditions are reasonably simple and yields were very good. The structures of the title compounds (**2-15**) were established by using analytical, physicochemical techniques and spectroscopic data (IR and ¹H-NMR). All the synthesized compounds were employed as test chemicals for *in vitro* antimicrobial functionality test against Gram-positive *Bacillus subtilis*, *Staphylococcus aureus*, Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* bacteria and plant pathogenic fungi *Aspergillus niger* and *Candida albicans*. For comparative studies, antimicrobial activity of standard antibiotics, Ampicillin and Nystatin were also carried out against these microorganisms. The study revealed that the tested samples exhibited moderate to good antibacterial and antifungal activities. It was also observed that the test substances were more effective against fungal phytopathogens than those of the human bacterial strains. Encouragingly, a number of tested chemicals showed nearest antibacterial and antifungal activities with the standard antibiotics employed.

1. INTRODUCTION

Carbohydrates are key molecules in nature with multiple roles in biological processes. For a long time, carbohydrates have been very attractive field for scientists due to their immense importance in biological systems [1]. They are the source of the metabolic energy supply, but also for the fine-tuning of cell-cell interactions and other crucial processes [2,3]. As a consequence, the chemistry and biochemistry of carbohydrate derivatives is an essential part of biochemical and medicinal research. Owing to the many functional groups and the configurational variety, the number of possible carbohydrate derivatives is huge. Therefore, the synthesis of carbohydrate derivatives is complicated, generally requiring many steps, and a range of selectivity problems has to be solved. So, selective acylation is very important in the field of carbohydrate chemistry because of its usefulness for the synthesis of biologically active products. Protection of a particular functional group of an organic compound is not only necessary for the modification of properties of the remaining functional groups but also for the synthesis of newer derivatives of great importance. Various methods for acylation of carbohydrates and nucleosides have so far been developed and

employed successfully [4-8]. Of these, the direct acylation method is considered as one of the most effective [8,9] for selective acylation of carbohydrates.

Microbial food contamination problems have been the cause of much public concern over the last few decades because of an increase in the number of infections and diseases originating from the consumption of spoiled food [10]. Antibacterial and antifungal agents are necessary for food preservation, especially for food processors, because bacterial and fungal growth are important causes of food spoilage. For this reason, many investigators have focused their research efforts on finding new efficient, low toxicity and environmentally friendly antibacterial and antifungal agents.

It was found from the literature survey that a large number of biologically active compounds also possess aromatic, heteroaromatic and acyl substituents [11]. The benzene, substituted benzene and also nitrogen, sulphur and halogen containing substituents are known to enhance the biological activity of the parent compound [12]. It also known that if an active nucleus is linked to another active nucleus, the resulting molecule may possess greater potential for biological activity [11]. Results of an ongoing our research project on selective acylation of carbohydrates [13-17] and also evaluation of microbial activities [18-21] reveal that in many cases the combination of two or more heteroaromatic nuclei and acyl groups enhances the biological activity manifold than its parent nucleus [22,23].

Guided by these observations, we deliberately synthesized some acylated products of methyl 6-*O*-myristoyl- α -D-glucopyranoside (**2**) (**Fig. 1**) containing various acyl groups (e.g. acetyl, pentanoyl, hexanoyl, octanoyl, decanoyl, lauroyl, myristoyl, palmitoyl, pivaloyl, methanesulfonyl, benzenesulfonyl, 4-chlorobenzoyl, 4-*t*-butylbenzoyl and cinnamoyl) in a single molecular framework. Fourteen synthesized derivatives of glucopyranoside were screened for *in vitro* antibacterial and antifungal activities against some number of human and phyto- pathogenic microorganisms as first time.

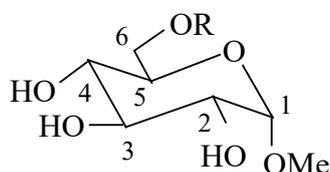


Fig. 1. Methyl 6-*O*-myristoyl- α -D-glucopyranoside (**2**)

2. EXPERIMENTAL

2.1. Materials and methods

In this present work, all chemicals used were purchased from Sigma-Aldrich and Merck Company unless reported otherwise and used without further purifications. Infrared spectra were recorded on a FTIR spectrophotometer (SHIMADZU) using the CHCl_3 technique at the Department of Chemistry, University of Chittagong, Bangladesh. $^1\text{H-NMR}$ (400 MHz) spectra were recorded using CDCl_3 as a solvent with TMS as the internal standard at the Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Dhaka, Bangladesh. Evaporations were carried out under reduced pressure using vacuum rotary evaporator (Germany). Melting points were determined using an electro-thermal melting point apparatus (England) and are uncorrected. Thin layer chromatography was performed on GF_{254} and spots were detected by spraying the plates with 1% H_2SO_4 . Column chromatography was carried out with silica gel G_{60} (100-200 mesh).

2.2. Reaction of methyl- α -D-glucopyranoside (1) with myristoyl chloride

A suspension of methyl α -D-glucopyranoside (1) (200 mg, 1.04 mmol) in dry pyridine (3 ml) was cooled to 0°C (maintained by ice and common salt) whereupon myristoyl chloride (0.3 ml, 1.1 molar eq.) was added to it. The mixture was stirred at this temperature for 5-6 hrs and then allowed to stand overnight. The progress of the reaction was checked by t.l.c (methanol-chloroform, 1:12) which indicated full conversion of the starting material into a single product ($R_f = 0.50$). A few pieces of ice were added to the flask and then extracted the product mixture with chloroform (3×10 ml).

The combined CHCl_3 layer was washed successively with dil. HCl acid (10%), saturated aqueous NaHCO_3 solution and distilled H_2O . The CHCl_3 layer was dried with anhydrous MgSO_4 , filtered and the filtrate was concentrated. The syrup was passed through a silica gel column chromatography and eluted with methanol-chloroform (1:12) provided the myristoyl chloride derivative (2) as crystalline solid. Recrystallization from CHCl_3 - $n\text{C}_6\text{H}_6$ gave the methyl 6-*O*-myristoyl- α -D-glucopyranoside (2).

Methyl 6-*O*-myristoyl- α -D-glucopyranoside (2): FTIR (CHCl_3) ν_{max} (cm^{-1}): 1702 (-CO), 3335-3510 cm^{-1} (br -OH). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ_{H} 4.89 (1H, d, $J = 3.6$ Hz, H-1), 4.66 (1H, dd, $J = 5.0$ and 12.1 Hz, H-6a), 4.45 (1H, dd, $J = 2.0$ and 12.1 Hz, H-6b), 4.10 (1H, t, $J = 9.3$ Hz, H-3), 3.94 (1H, t, $J = 9.7$ Hz, H-4), 3.72 (1H, dd, $J = 3.6$ and 10.0 Hz, H-2), 3.65 (1H, ddd, $J = 2.9$, 9.9 and 12.8 Hz, H-5), 3.36 (3H, s, 1-OCH₃), 2.34 {2H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 1.24 {22H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 0.86 {3H, m, $\text{CH}_3(\text{CH}_2)_{12}\text{CO-}$ }. Anal. Calcd for $\text{C}_{21}\text{H}_{40}\text{O}_7$: C, 62.38; H, 9.90%; found C, 62.41; H, 9.95%.

2.3. General procedure for the synthesis of methyl 6-*O*-myristoyl- α -D-glucopyranoside derivatives (3-15)

To a rapidly stirred and cooled (-5°C) solution of the triol (2) (78 mg, 0.19 mmol), in dry $\text{C}_3\text{H}_5\text{N}$ (3 ml) was added acetic anhydride (0.1 ml, 5 molar eq.). The reaction mixture was stirred at -5°C temperature for 8 hrs and then stirred overnight at room temperature. The progress of the reaction was monitored by t.l.c (ethyl acetate-*n*-Hexane, 1:2) which indicated the complete conversion of the starting material into faster moving product ($R_f=0.52$). Work-up as described earlier and purification by silica gel column chromatography (with ethyl acetate-*n*-hexane, 1:2 as eluant) afforded the acetyl derivative (3).

Similar reaction and purification procedure was applied to prepare compound 4 (pentanoyl derivative, (CHCl_3 - $n\text{C}_6\text{H}_6$); compound 5 (hexanoyl derivative); compound 6 (octanoyl derivative); compound 7 (decanoyl derivative); compound 8 (lauroyl derivative) ($\text{C}_2\text{H}_5\text{CO}_2\text{CH}_3$ - $n\text{C}_6\text{H}_6$); compound 9 (palmitoyl derivative); compound 10 (pivaloyl derivative) ($\text{C}_2\text{H}_5\text{CO}_2\text{CH}_3$ - $n\text{C}_6\text{H}_6$); compound 11 (methanesulfonyl derivative); compound 12 (benzenesulfonyl derivative) (CHCl_3 - $n\text{C}_6\text{H}_6$); compound 13 (4-chlorobenzoyl derivative) ($\text{C}_2\text{H}_5\text{CO}_2\text{CH}_3$ - $n\text{C}_6\text{H}_6$); compound 14 (4-*t*-butylbenzoyl derivative) ($\text{C}_2\text{H}_5\text{CO}_2\text{CH}_3$ - $n\text{C}_6\text{H}_6$) and compound 15 (cinnamoyl derivative) ($\text{C}_2\text{H}_5\text{CO}_2\text{CH}_3$ - $n\text{C}_6\text{H}_6$).

Methyl 2,3,4-tri-*O*-acetyl-6-*O*-myristoyl- α -D-glucopyranoside (3): FTIR (CHCl_3) ν_{max} (cm^{-1}): 1742, 1705, 1680 cm^{-1} (-CO). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ_{H} 5.45 (1H, d, $J = 3.5$ Hz, H-1), 5.04 (1H, dd, $J = 3.6$ and 10.0 Hz, H-2), 4.92 (1H, t, $J = 9.6$ Hz, H-3), 4.86 (1H, t, $J = 9.7$ Hz, H-4), 4.20 (1H, dd, $J = 5.0$ and 12.1 Hz, H-6a), 4.12 (1H, dd, $J = 2.0$ and 12.1 Hz, H-6b), 3.95 (1H, m, H-5), 3.38 (3H, s, 1-OCH₃), 2.32 {2H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 2.07, 2.00, 1.98 (3×3H, 3×s, 3×CH₃CO-), 1.23 {22H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 0.85 {3H, m, $\text{CH}_3(\text{CH}_2)_{12}\text{CO-}$ }. Anal. Calcd for $\text{C}_{27}\text{H}_{46}\text{O}_{10}$: C, 61.13; H, 8.68%; found C, 61.19; H, 8.70%.

Methyl 6-*O*-myristoyl-2,3,4-tri-*O*-pentanoyl- α -D-glucopyranoside (4): FTIR (CHCl₃) ν_{\max} (cm⁻¹): 1740 cm⁻¹ (-CO). ¹H-NMR (CDCl₃, 400 MHz): δ_{H} 5.48 (1H, d, J = 3.6 Hz, H-1), 5.06 (1H, dd, J = 3.7 and 10.1 Hz, H-2), 4.89 (1H, t, J = 9.5 Hz, H-3), 4.84 (1H, t, J = 9.6 Hz, H-4), 4.40 (1H, dd, J = 5.0 and 12.0 Hz, H-6a), 4.08 (1H, dd, J = 2.0 and 12.0 Hz, H-6b), 3.97 (1H, m, H-5), 3.38 (3H, s, 1-OCH₃), 2.33 {6H, m, 3×CH₃(CH₂)₂CH₂CO-}, 2.30 {2H, m, CH₃(CH₂)₁₁CH₂CO-}, 1.60 {6H, m, 3×CH₃CH₂CH₂CH₂CO-}, 1.31 {6H, m, 3×CH₃CH₂(CH₂)₂CO-}, 1.23 {22H, m, CH₃(CH₂)₁₁CH₂CO-}, 0.90 {9H, m, 3×CH₃(CH₂)₃CO-}. 0.86 {3H, m, CH₃(CH₂)₁₂CO-}. Anal. Calcd for C₃₆H₆₄O₁₀: C, 65.85; H, 9.76%; found C, 65.92; H, 9.77%.

Methyl 2,3,4-tri-*O*-hexanoyl-6-*O*-myristoyl- α -D-glucopyranoside (5): FTIR (CHCl₃) ν_{\max} (cm⁻¹): 1728, 1690 cm⁻¹ (-CO). ¹H-NMR (CDCl₃, 400 MHz): δ_{H} 5.42 (1H, d, J = 3.6 Hz, H-1), 5.02 (1H, dd, J = 3.6 and 10.0 Hz, H-2), 4.87 (1H, t, J = 9.6 Hz, H-3), 4.81 (1H, t, J = 9.6 Hz, H-4), 4.48 (1H, m, H-6a), 4.06 (1H, t, J = 10.2 Hz, H-6b), 3.95 (1H, m, H-5), 3.34 (3H, s, 1-OCH₃), 2.35 {2H, m, CH₃(CH₂)₁₁CH₂CO-}, 2.26 {6H, m, 3×CH₃(CH₂)₃CH₂CO-}, 1.51 {6H, m, 3×CH₃(CH₂)₂CH₂CH₂CO-}, 1.24 {12H, m, 3×CH₃(CH₂)₂CH₂CH₂CO-}, 1.22 {22H, m, CH₃(CH₂)₁₁CH₂CO-}, 0.87 {3H, m, CH₃(CH₂)₁₂CO-}, 0.84 {9H, m, 3×CH₃(CH₂)₄CO-}. Anal. Calcd for C₃₉H₇₀O₁₀: C, 67.05; H, 10.03%; found C, 67.11; H, 10.07%.

Methyl 6-*O*-myristoyl-2,3,4-tri-*O*-octanoyl- α -D-glucopyranoside (6): FTIR (CHCl₃) ν_{\max} (cm⁻¹): 1712 cm⁻¹ (-CO). ¹H-NMR (CDCl₃, 400 MHz): δ_{H} 5.47 (1H, d, J = 3.5 Hz, H-1), 5.27 (1H, m, H-2), 5.04 (1H, t, J = 9.4 Hz, H-3), 4.86 (1H, m, H-4), 4.19 (1H, dd, J = 5.0 and 12.0 Hz, H-6a), 4.10 (1H, m, H-6b), 3.94 (1H, m, H-5), 3.35 (3H, s, 1-OCH₃), 2.36 {6H, m, 3×CH₃(CH₂)₅CH₂CO-}, 2.20 {2H, m, CH₃(CH₂)₁₁CH₂CO-}, 1.58 {6H, m, 3×CH₃(CH₂)₄CH₂CH₂CO-}, 1.29 {24H, m, 3×CH₃(CH₂)₄(CH₂)₂CO-}, 1.22 {22H, m, CH₃(CH₂)₁₁CH₂CO-}, 0.89 {9H, m, 3×CH₃(CH₂)₆CO-}, 0.83 {3H, m, CH₃(CH₂)₁₂CO-}. Anal. Calcd for C₄₅H₈₂O₁₀: C, 69.05; H, 10.49%; found C, 69.15; H, 10.51%.

Methyl 2,3,4-tri-*O*-decanoyl-6-*O*-myristoyl- α -D-glucopyranoside (7): FTIR (CHCl₃) ν_{\max} (cm⁻¹): 1685 cm⁻¹ (-CO). ¹H-NMR (CDCl₃, 400 MHz): δ_{H} 5.29 (1H, d, J = 3.6 Hz, H-1), 4.87 (1H, dd, J = 3.6 and 10.0 Hz, H-2), 4.35 (1H, t, J = 9.6 Hz, H-3), 4.25 (1H, t, J = 9.6 Hz, H-4), 4.01 (1H, dd, J = 4.8 and 10.0 Hz, H-6a), 3.93 (1H, t, J = 10.0 Hz, H-6b), 3.75 (1H, m, H-5), 3.31 (3H, s, 1-OCH₃), 2.33 {6H, m, 3×CH₃(CH₂)₇CH₂CO-}, 2.31 {2H, m, CH₃(CH₂)₁₁CH₂CO-}, 1.62 {6H, m, 3×CH₃(CH₂)₆CH₂CH₂CO-}, 1.23 {36H, m, 3×CH₃(CH₂)₆CH₂CH₂CO-}, 1.22 {22H, m, CH₃(CH₂)₁₁CH₂CO-}, 0.85 {9H, m, 3×CH₃(CH₂)₈CO-}, 0.83 {3H, m, CH₃(CH₂)₁₂CO-}. Anal. Calcd for C₅₁H₉₄O₁₀: C, 70.67; H, 10.85%; found C, 70.70; H, 10.87%.

Methyl 2,3,4-tri-*O*-lauroyl-6-*O*-myristoyl- α -D-glucopyranoside (8): FTIR (CHCl₃) ν_{\max} (cm⁻¹): 1718 cm⁻¹ (-CO). ¹H-NMR (CDCl₃, 400 MHz): δ_{H} 5.46 (1H, d, J = 3.5 Hz, H-1), 5.04 (1H, dd, J = 3.5 and 10.0 Hz, H-2), 4.88 (1H, t, J = 9.6 Hz, H-3), 4.82 (1H, t, J = 9.7 Hz, H-4), 4.15 (1H, dd, J = 2.0 and 12.1 Hz, H-6b), 4.06 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 3.97 (1H, m, H-5), 3.35 (3H, s, 1-OCH₃), 2.34 {2H, m, CH₃(CH₂)₁₁CH₂CO-}, 2.31 {6H, m, 3×CH₃(CH₂)₉CH₂CO-}, 1.58 {6H, m, 3×CH₃(CH₂)₈CH₂CH₂CO-}, 1.21 {48H, m, 3×CH₃(CH₂)₈CH₂CH₂CO-}, 1.20 {22H, m, CH₃(CH₂)₁₁CH₂CO-}, 0.85 {9H, m, 3×CH₃(CH₂)₁₀CO-}, 0.83 {3H, m, CH₃(CH₂)₁₂CO-}. Anal. Calcd for C₅₇H₁₀₆O₁₀: C, 72.0; H, 11.15%; found C, 72.05; H, 11.19%.

Methyl 6-*O*-myristoyl-2,3,4-tri-*O*-palmitoyl- α -D-glucopyranoside (9): FTIR (CHCl₃) ν_{\max} (cm⁻¹): 1730 cm⁻¹ (-CO). ¹H-NMR (CDCl₃, 400 MHz): δ_{H} 5.43 (1H, d, J = 3.7 Hz, H-1), 5.01 (1H, dd, J = 3.6 and 10.2 Hz, H-2), 4.88 (1H, t, J = 9.6 Hz, H-4), 4.54 (1H, m, H-3), 4.22 (1H, m, H-6a), 4.0 (1H, t, J = 10.2 Hz, H-6b), 3.89 (1H, m, H-5), 3.31 (3H, s, 1-OCH₃), 2.34 {6H, m, 3×CH₃(CH₂)₁₃CH₂CO-}, 2.33 {2H, m, CH₃(CH₂)₁₁CH₂CO-}, 1.26 {78H, m, 3×CH₃(CH₂)₁₃CH₂CO-}, 1.24 {22H, m, CH₃(CH₂)₁₁CH₂CO-}, 0.87 {9H, m, 3×CH₃(CH₂)₁₄CO-},

0.84 {3H, m, $\text{CH}_3(\text{CH}_2)_{12}\text{CO-}$ }. Anal. Calcd for $\text{C}_{69}\text{H}_{130}\text{O}_{10}$: C, 74.06; H, 15.70%; found C, 74.11; H, 15.73%.

Methyl 6-*O*-myristoyl-2,3,4-tri-*O*-pivaloyl- α -D-glucopyranoside (10): FTIR (CHCl_3) ν_{max} (cm^{-1}): 1752 (-CO). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ_{H} 5.50 (1H, d, $J = 3.6$ Hz, H-1), 5.05 (1H, dd, $J = 3.7$ and 10.2 Hz, H-2), 4.92 (1H, t, $J = 9.7$ Hz, H-3), 4.85 (1H, t, $J = 9.6$ Hz, H-4), 4.30 (1H, m, H-5), 4.14 (2H, m, H-6a and H-6b), 3.37 (3H, s, 1- OCH_3), 2.34 {2H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 1.23 {27H, s, $3 \times (\text{CH}_3)_3\text{CCO-}$ }, 1.17 {22H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 0.85 {3H, m, $\text{CH}_3(\text{CH}_2)_{12}\text{CO-}$ }. Anal. Calcd for $\text{C}_{36}\text{H}_{64}\text{O}_{10}$: C, 65.85; H, 9.76%; found C, 65.91; H, 9.77%.

Methyl 2,3,4-tri-*O*-methanesulfonyl-6-*O*-myristoyl- α -D-glucopyranoside (11): FTIR (CHCl_3) ν_{max} (cm^{-1}): 1735 (-CO), 1365 (-SO_2). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ_{H} 5.37 (1H, d, $J = 3.6$ Hz, H-1), 5.0 (1H, dd, $J = 3.6$ and 10.1 Hz, H-2), 4.82 (1H, t, $J = 9.6$ Hz, H-3), 4.54 (1H, t, $J = 9.6$ Hz, H-4), 4.22 (1H, m, H-6a), 3.82 (1H, m, H-6b), 3.76 (1H, ddd, $J = 2.8$, 9.7 and 12.6 Hz, H-5), 3.38 (3H, s, 1- OCH_3), 3.18, 3.10, 3.06 ($3 \times 3\text{H}$, $3 \times \text{s}$, $3 \times \text{CH}_3\text{SO}_2-$), 2.31 {2H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 1.24 {22H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 0.86 {3H, m, $\text{CH}_3(\text{CH}_2)_{12}\text{CO-}$ }. Anal. Calcd for $\text{C}_{24}\text{H}_{46}\text{S}_3\text{O}_{13}$: C, 45.14; H, 7.21%; found C, 45.20; H, 7.26%.

Methyl 2,3,4-tri-*O*-benzenesulfonyl-6-*O*-myristoyl- α -D-glucopyranoside (12): FTIR (CHCl_3) ν_{max} (cm^{-1}): 1762 (-CO), 1365 (-SO_2). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ_{H} 7.85 (6H, m, Ar-H), 7.63 (3H, m, Ar-H), 7.52 (6H, m, Ar-H), 5.58 (1H, d, $J = 3.7$ Hz, H-1), 5.48 (1H, dd, $J = 3.6$ and 10.0 Hz, H-2), 5.05 (1H, t, $J = 9.6$ Hz, H-3), 4.86 (1H, m, H-4), 4.20 (1H, m, H-6a), 4.11 (1H, dd, $J = 2.0$ and 12.0 Hz, H-6b), 3.95 (1H, m, H-5), 3.35 (3H, s, 1- OCH_3), 2.31 {2H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 1.24 {22H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 0.86 {3H, m, $\text{CH}_3(\text{CH}_2)_{12}\text{CO-}$ }. Anal. Calcd for $\text{C}_{39}\text{H}_{52}\text{S}_3\text{O}_{13}$: C, 56.80; H, 6.31%; found C, 56.88; H, 6.39%.

Methyl 2,3,4-tri-*O*-(4-chlorobenzoyl)-6-*O*-myristoyl- α -D-glucopyranoside (13): FTIR (CHCl_3) ν_{max} (cm^{-1}): 1746 (-CO). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ_{H} 7.92 (6H, m, Ar-H), 7.67 (6H, m, Ar-H), 5.54 (1H, d, $J = 3.6$ Hz, H-1), 5.05 (1H, dd, $J = 3.6$ and 10.0 Hz, H-2), 4.88 (1H, m, H-3), 4.67 (1H, t, $J = 9.6$ Hz, H-4), 4.16 (1H, m, H-6a), 3.95 (1H, t, $J = 10.2$ Hz, H-6b), 3.59 (1H, m, H-5), 3.36 (3H, s, 1- OCH_3), 2.36 {2H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 1.23 {22H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 0.88 {3H, m, $\text{CH}_3(\text{CH}_2)_{12}\text{CO-}$ }. Anal. Calcd for $\text{C}_{42}\text{H}_{49}\text{O}_{10}\text{Cl}_3$: C, 61.50; H, 5.98%; found C, 61.55; H, 6.03%.

Methyl 2,3,4-tri-*O*-(4-*t*-butylbenzoyl)-6-*O*-myristoyl- α -D-glucopyranoside (14): FTIR (CHCl_3) ν_{max} (cm^{-1}): 1662 (-CO). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ_{H} 8.02 (6H, m, Ar-H), 7.49 (6H, m, Ar-H), 5.40 (1H, d, $J = 3.7$ Hz, H-1), 5.08 (1H, dd, $J = 3.6$ and 10.6 Hz, H-2), 4.83 (1H, t, $J = 9.6$ Hz, H-4), 4.78 (1H, t, $J = 9.2$ Hz, H-3), 4.66 (1H, m, H-6a), 4.52 (1H, m, H-6b), 3.95 (1H, m, H-5), 3.32 (3H, s, 1- OCH_3), 2.36 {2H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 1.33 {27H, s, $3 \times (\text{CH}_3)_3\text{C-}$ }, 1.25 {22H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 0.87 {3H, m, $\text{CH}_3(\text{CH}_2)_{12}\text{CO-}$ }. Anal. Calcd for $\text{C}_{54}\text{H}_{76}\text{O}_{10}$: C, 73.30; H, 8.60%; found C, 73.37; H, 8.66%.

Methyl 2,3,4-tri-*O*-cinnamoyl-6-*O*-myristoyl- α -D-glucopyranoside (15): FTIR (CHCl_3) ν_{max} (cm^{-1}): 1705 (-CO), 1631 (-CH=CH-). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): δ_{H} 7.67 7.50, 7.36 ($3 \times 1\text{H}$, $3 \times \text{d}$, $J = 16.0$ Hz, $3 \times \text{PhCH=CHCO-}$), 7.60 (6H, m, Ar-H), 7.25 (9H, m, Ar-H), 6.46, 6.36, 6.30 ($3 \times 1\text{H}$, $3 \times \text{d}$, $J = 16.1$ Hz, $3 \times \text{PhCH=CHCO-}$), 5.62 (1H, d, $J = 3.6$ Hz, H-1), 5.35 (1H, dd, $J = 3.6$ and 10.2 Hz, H-2), 5.01 (1H, t, $J = 9.6$ Hz, H-3), 4.88 (1H, t, $J = 9.6$ Hz, H-4), 4.28 (1H, m, H-6a), 4.09 (1H, m, H-6b), 3.82 (1H, m, H-5), 3.37 (3H, s, 1- OCH_3), 2.32 {2H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 1.23 {22H, m, $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO-}$ }, 0.87 {3H, m, $\text{CH}_3(\text{CH}_2)_{12}\text{CO-}$ }. Anal. Calcd for $\text{C}_{48}\text{H}_{58}\text{O}_{10}$: C, 72.54; H, 7.30%; found C, 72.59; H, 7.35%.

2.4. Antibacterial screening studies

2.4.1. Test microorganisms

Test tube cultures of bacterial and fungal pathogens were obtained from the Microbiology Laboratory, Department of Microbiology, University of Chittagong, Bangladesh. The synthesized test compounds (**Schemes 1 & 2**) were subjected to antibacterial screening against two Gram-positive and two Gram-negative bacterial strains viz., *Bacillus subtilis* BTCC 17, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* CRL (ICDDR'B). The name of phytopathogenic fungi viz., *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231. In all cases, a 2% solution (in CHCl₃) of the chemicals was used. Standard NA (Nutrient Agar) medium was used throughout the work (**Table 1**).

Table 1. List of constituents of NA and PDA media.

Name of Media	Beef extract (g)	Agar (g)	Peptone (g)	Potato (g)	Dextrose (g)	NaCl (g)	H ₂ O (ml)
NA	3	20	5	0	NA	0.5	1000
PDA	NA	15	0	200	20	0	1000

Note: NA = Nutrient Agar and PDA = Potato Dextrose Agar.

2.4.2. Antibacterial activity assay

The *in vitro* antibacterial spectrum of the synthesized chemicals were done by disc diffusion method [24] with little modification [25]. Sterilized paper discs of 4 mm in diameter and Petri dishes of 150 mm in diameter were used throughout the experiment. The autoclaved Mueller-Hinton agar medium, cooled to 45°C, was poured into sterilized Petri dishes to a depth of 3 to 4 mm and after solidification of the agar medium the plates were transferred to an incubator at 37°C for 15 to 20 minutes to dry off the moisture that developed on the agar surface. The plates were inoculated with the standard bacterial suspensions (as McFarland 0.5 standard) followed by spread plate method and allowed to dry for three to five minutes. Dried and sterilized filter paper discs were treated separately with 50 µg dry weight/disc from 2% solution (in CHCl₃) of each test chemical using a micropipette, dried in air under aseptic condition and were placed at equidistance in a circle on the seeded plate. A control plate was also maintained in each case without any test chemical. These plates were kept for 4-6 hours at low temperature (4-6°C) and the test chemicals diffused from disc to the surrounding medium. The plates were then incubated at 35±2°C for 24 hours to allow maximum growth of the microorganisms. The antibacterial activity of the test agent was determined by measuring the mean diameter of zone of inhibitions (in millimeter). Each experiment was repeated thrice. All the results were compared with the standard antibacterial antibiotic Ampicillin (20µg/disc, BEXIMCO Pharm. Bangladesh Ltd).

2.5. Antifungal activity assay

The antifungal activities of the D-glucopyranoside derivatives (scheme-1) were investigated by food poisoned technique [26]. Two percent solution of the test chemical (in CHCl₃) was mixed with sterilized melted Sabouraud agar medium to obtain the desired concentration (2%) and this was poured in sterilized Petri dishes. At the center of each plate, 5 days old fungal mycelial block (4 mm in diameter) was inoculated and incubated at 27°C. A control set was also maintained in each experiment. 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 follows:

$$I = \left\{ \frac{C - T}{C} \right\} \times 100$$

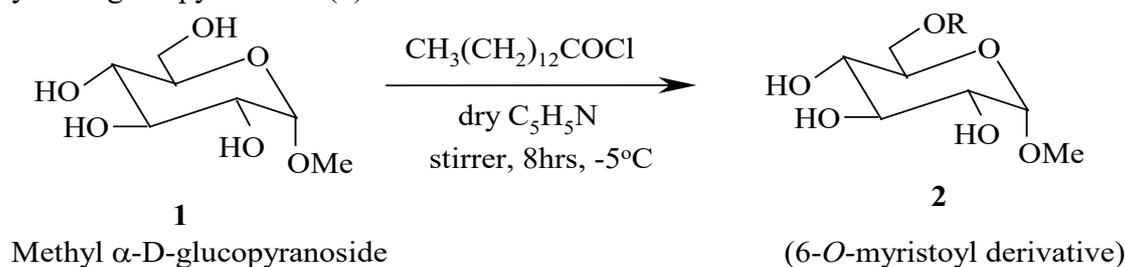
Where, I = Percentage of inhibition, C = Diameter of the fungal colony in control (CHCl₃), T = Diameter of the fungal colony in treatment. All the results were compared with the standard antifungal antibiotic nystatin (100 µg/ml medium, BEXIMCO Pharm. Bangladesh Ltd.).

3. RESULTS AND DISCUSSION

3.1. Characterization and chemistry

The main objective of the piece of work reported in this dissertation was to perform regioselective myristoylation of methyl α -D-glucopyranoside (**1**) using the direct acylation method (**scheme 1**). The resulting myristoylation product was converted to a number of derivatives using a series of acylating agents (**scheme 2**). The structure of the main acylation product and their derivatives were ascertained by analyzing their IR and $^1\text{H-NMR}$ spectra. In continuation of a project going on in our laboratory of Carbohydrate and Protein Chemistry, we intended to prepare a series of D-glucose derivatives for use as test chemicals for biological evaluation. Keeping this objective in mind, we thus prepared a set of derivatives containing a wide variety of substituents in a single molecular framework (**Table 2** and **3**). All the acylation products thus prepared were employed as test chemicals for antibacterial activity studies against a number of Gram-positive and Gram-negative human pathogenic bacteria. The antifungal activities of these derivatives were also performed against a number of phytopathogenic fungi. The selection of a wide variety of acylating agents was deliberate with the aim of finding biologically active agents.

Our initial effort was to prepare the methyl 6-*O*-myristoyl- α -D-glucopyranoside (**2**). Thus, treatment of methyl α -D-glucopyranoside (**1**) with myristoyl chloride as acylating agent in dry pyridine at -5°C and after usual work-up, compound **2** was obtained in high yields. This compound was sufficiently pure for use in the next stages. However, an analytical sample was prepared by recrystallisation from chloroform-hexane. Its IR spectrum showed absorption bands at 1702 cm^{-1} (for $-\text{CO}$ stretching) and $3335\text{--}3510\text{ cm}^{-1}$ (br) (for $-\text{OH}$ stretching). In its $^1\text{H-NMR}$ spectrum, a two-proton multiplet at δ 2.34 $\{\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO}-\}$, a twenty-two proton multiplet at δ 1.24 $\{\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO}-\}$ and a three-proton multiplet at δ 0.86 $\{\text{CH}_3(\text{CH}_2)_{12}\text{CO}-\}$ indicated the introduction of one myristoyl group in the molecule. The downfield shift of the C-6 protons to δ 4.66 (as dd, $J = 5.0$ and 12.1 Hz, H-6a) and δ 4.45 (as dd, $J = 2.0$ and 12.1 Hz, H-6b) from their precursor (**1**) values and the resonances of other protons in their anticipated positions showed the attachment of myristoyl group at position 6. The rest of the IR and $^1\text{H-NMR}$ spectra was in complete agreement with the structure accorded to the myristoyl derivative as methyl 6-*O*-myristoyl- α -D-glucopyranoside (**2**).

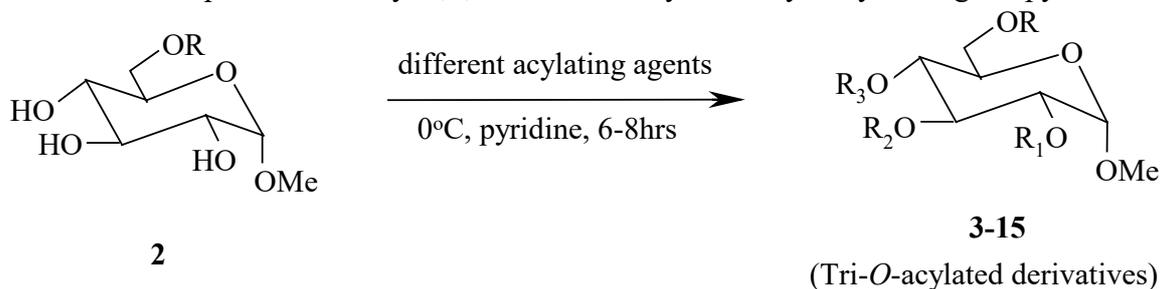


Scheme 1. Synthesis of methyl 6-*O*-myristoyl- α -D-glucopyranoside.

The structure of the myristoyl derivative (**2**) was further ascertained by its conversion to and identification of its acetyl derivative (**3**). Thus, reaction of compound **2** with an excess of acetic anhydride in pyridine, followed by usual work-up procedure and silica gel column chromatographic purification, provided the acetyl derivative (**3**). The IR spectrum of this compound showed the absorption peaks at 1742 , 1705 and 1680 cm^{-1} due to carbonyl ($-\text{CO}$) stretching. The introduction of three acetyl groups in the molecule was demonstrated by the appearance of three three-proton singlets at δ 2.07, δ 2.00 and 1.98 in its $^1\text{H-NMR}$ spectrum. The C-2 proton resonated at δ 5.04 (as dd, $J = 3.6$ and 10.0 Hz) and shifted downfield from the precursor triol (**2**) (δ 3.72); C-3 proton resonated at δ 4.92 (as t, $J = 9.6$ Hz) and shifted downfield from the precursor triol (**2**) (δ 4.10); also, C-4 proton resonated downfield to δ 4.86 (as t, $J = 9.7$ Hz) as compared to the precursor compound **2** (δ 3.94), thereby suggesting the attachment of the acetyl groups at positions 2, 3 and 4. By complete analysis of the IR and $^1\text{H-NMR}$ spectra, the structure of the triacetate was ascertained as methyl 2,3,4-tri-*O*-acetyl-6-*O*-myristoyl- α -D-glucopyranoside (**3**).

When the myristoyl derivative (2) was allowed to react with equimolecular amount of pentanoyl chloride at freezing temperature, followed by conventional work-up procedure and column chromatography, it afforded the tri-*O*-pentanoyl derivative (4). In its IR spectrum, the absorption bands at 1740 cm^{-1} corresponded to carbonyl stretching. In its $^1\text{H-NMR}$ spectrum, three six-proton multiplet at $\delta 2.33$ $\{3\times\text{CH}_3(\text{CH}_2)_2\text{CH}_2\text{CO}-\}$, $\delta 1.60$ $\{3\times\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}-\}$ and $\delta 1.31$ $\{3\times\text{CH}_3\text{CH}_2(\text{CH}_2)_2\text{CO}-\}$ and one nine-proton multiplet at $\delta 0.90$ $\{3\times\text{CH}_3(\text{CH}_2)_3\text{CO}-\}$ were indicative of the presence of three pentanoyl groups in the compound. The deshielding of C-2, C-3 and C-4 protons to $\delta 5.06$ (as dd, $J=3.7$ and 10.1 Hz), $\delta 4.89$ (as t, $J=9.5$ Hz) and $\delta 4.84$ (as t, $J=9.6$ Hz) from their usual values, confirmed the attachment of three pentanoyl groups at the 2, 3 and 4 positions. The rest of the IR and $^1\text{H-NMR}$ spectra was in conformity with the structure accorded as methyl 6-*O*-myristoyl-2,3,4-tri-*O*-pentanoyl- α -D-glucopyranoside (4).

Additional support for the structure accorded to compound (2) was obtained by its conversion to its hexanoyl derivative (5). Thus, reaction of compound 2 with hexanoyl chloride at freezing temperature followed by conventional aqueous work-up and chromatography, furnished the compound (5) as semi solid mass which resisted crystallization. The IR spectrum of this compound showed the absorption peaks at 1728, 1690 due to $-\text{CO}$ stretching. The $^1\text{H-NMR}$ spectrum of compound 5 displayed two six-proton multiplet at $\delta 2.26$ $\{3\times\text{CH}_3(\text{CH}_2)_3\text{CH}_2\text{CO}-\}$, and $\delta 1.51$ $\{3\times(\text{CH}_3)_2\text{CH}_2\text{CH}_2\text{CO}-\}$, a twelve-proton multiplet at $\delta 1.24$ $\{3\times\text{CH}_3(\text{CH}_2)_2\text{CH}_2\text{CH}_2\text{CO}-\}$ and one nine-proton multiplet at $\delta 0.84$ $\{3\times\text{CH}_3(\text{CH}_2)_4\text{CO}-\}$ showing the attachment of three hexanoyl groups in the molecule. The resonance for C-2, C-3 and C-4 appeared at $\delta 5.02$, $\delta 4.87$ and $\delta 4.81$ which shifted downfield from their usual values indicating the presence of the hexanoyl groups at positions 2, 3 and 4. Complete analysis of the IR and $^1\text{H-NMR}$ spectrum enabled us to propose the structure of this compound as methyl 2,3,4-tri-*O*-hexanoyl-6-*O*-myristoyl- α -D-glucopyranoside (5).



Scheme 2. Synthesis of methyl 6-*O*-myristoyl- α -D-glucopyranoside derivatives.

Table 2. Substituted acyl residues for the synthesis of methyl α -D-glucopyranoside derivatives.

Compound	R	$\text{R}_1=\text{R}_2=\text{R}_3$	Compound	R	$\text{R}_1=\text{R}_2=\text{R}_3$
2	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	H	9	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$\text{CH}_3(\text{CH}_2)_{14}\text{CO}-$
3	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$\text{CH}_3\text{CO}-$	10	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$(\text{CH}_3)_3\text{CCO}-$
4	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$\text{CH}_3(\text{CH}_2)_3\text{CO}-$	11	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	CH_3SO_2-
5	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$\text{CH}_3(\text{CH}_2)_4\text{CO}-$	12	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$\text{C}_6\text{H}_5\text{SO}_2-$
6	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$\text{CH}_3(\text{CH}_2)_6\text{CO}-$	13	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$4.\text{Cl}.\text{C}_6\text{H}_4\text{CO}-$
7	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$\text{CH}_3(\text{CH}_2)_8\text{CO}-$	14	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$(\text{CH}_3)_3\text{CC}_6\text{H}_4\text{CO}-$
8	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$\text{CH}_3(\text{CH}_2)_{10}\text{CO}-$	15	$\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$	$\text{C}_6\text{H}_5\text{CH}=\text{CHCO}-$

The same myristoyl derivative **2** was then converted to the octanoyl derivative (**6**). Its IR spectrum displayed the absorption band at 1712 cm^{-1} (for $-\text{CO}$). In its $^1\text{H-NMR}$ spectrum, two six-proton multiplet at $\delta 2.36$ $\{3\times\text{CH}_3(\text{CH}_2)_5\text{CH}_2\text{CO}-\}$ and 1.58 $\{3\times\text{CH}_3(\text{CH}_2)_4\text{CH}_2\text{CH}_2\text{CO}-\}$, a twenty four-proton multiplet at $\delta 1.29$ $\{3\times\text{CH}_3(\text{CH}_2)_4(\text{CH}_2)_2\text{CO}-\}$ and a nine-proton multiplet at $\delta 0.89$ $\{3\times\text{CH}_3(\text{CH}_2)_6\text{CO}-\}$ due to the presence of three octanoyl groups to the molecule. The downfield shift of C-2, C-3 and C-4 protons to $\delta 5.27$, $\delta 5.04$ and $\delta 4.86$ from their values, indicative the attachment of the three octanoyl groups at positions 2, 3 and 4. The rest of the IR and $^1\text{H-NMR}$ signals were in their anticipated positions to enable the structure of this compound as methyl 6-*O*-myristoyl-2,3,4-tri-*O*-octanoyl- α -D-glucopyranoside (**6**).

Being encouraged by the results so far, we then used decanoyl chloride as the acylating agent. Thus, the triol (**2**) was treated with 5.0 molar amount of decanoyl chloride in dry $\text{C}_5\text{H}_5\text{N}$, followed by usual work-up and silica gel column chromatography, provided the decanoyl derivative (**7**). The IR spectrum of compound **7** showed the 1685 cm^{-1} absorption bands for $-\text{CO}$ stretching. The $^1\text{H-NMR}$ spectrum of this compound (**7**) provided the following characteristic peaks : two six-proton multiplet at $\delta 2.33$ $\{3\times\text{CH}_3(\text{CH}_2)_7\text{CH}_2\text{CO}-\}$ and 1.62 $\{3\times\text{CH}_3(\text{CH}_2)_6\text{CH}_2\text{CH}_2\text{CO}-\}$, a thirty six-proton multiplet at $\delta 1.23$ $\{3\times\text{CH}_3(\text{CH}_2)_6\text{CH}_2\text{CH}_2\text{CO}-\}$ and a nine-proton multiplet at $\delta 0.85$ $\{3\times\text{CH}_3(\text{CH}_2)_8\text{CO}-\}$, thereby suggesting the presence of three decanoyl groups to the triol molecule. Also C-2, C-3 and C-4 resonated to $\delta 4.87$ (as dd, $J=3.6$ and 10.0 Hz), $\delta 4.35$ (as t, $J=9.6$ Hz) and $\delta 4.25$ (as t, $J=9.6$ Hz) downfield to that of triol (**2**) values thus suggesting the introduction of the three decanoyl groups at positions 2, 3 and 4. On the basis of complete analysis of the IR and $^1\text{H-NMR}$ spectra of this compound was accorded as methyl 2,3,4-tri-*O*-decanoyl-6-*O*-myristoyl- α -D-glucopyranoside (**7**).

The myristoyl derivative (**2**) was then allowed to react with lauroyl chloride in pyridine at room temperature overnight to afford the lauroylate (**8**). The IR spectrum of this compound (**8**) displayed absorption band at 1718 cm^{-1} due to carbonyl stretching. Its $^1\text{H-NMR}$ spectrum displayed two six-proton multiplet at 2.31 $\{3\times\text{CH}_3(\text{CH}_2)_9\text{CH}_2\text{CO}-\}$, $\delta 1.58$ $\{3\times\text{CH}_3(\text{CH}_2)_8\text{CH}_2\text{CH}_2\text{CO}-\}$ a forty eight-proton multiplet at $\delta 1.21$ $\{3\times\text{CH}_3(\text{CH}_2)_8\text{CH}_2\text{CH}_2\text{CO}-\}$ and a nine-proton multiplet at $\delta 0.85$ $\{3\times\text{CH}_3(\text{CH}_2)_{10}\text{CO}-\}$, thereby suggesting the presence of three lauroyl groups in the compound. Also the C-2, C-3 and C-4 protons were deshielded considerably to $\delta 5.04$ (as dd, $J=3.5$ and 10.0 Hz), $\delta 4.88$ (as t, $J=9.6$ Hz) and $\delta 4.82$ (as t, $J=9.7$ Hz) from their usual values ($\delta 3.72$, $\delta 4.10$ and $\delta 3.94$), thus showing that the three lauroyl groups were introduced at positions 2, 3 and 4. By analysis of the IR and $^1\text{H-NMR}$ spectra, the structure of the lauroyl derivative was assigned as methyl 2,3,4-tri-*O*-lauroyl-6-*O*-myristoyl- α -D-glucopyranoside (**8**).

Further support for the structure accorded to compound (**2**) was obtained by preparation of its palmitoyl derivative (**9**). Thus, treatment of compound (**2**) with palmitoyl chloride in pyridine, followed by conventional work-up and chromatographic purification, afforded the palmitoyl derivative (**9**). The IR spectrum showed carbonyl stretching band at 1730 cm^{-1} and absence of hydroxyl stretching bands. The $^1\text{H-NMR}$ spectrum, the presence of three palmitoyl groups in the molecule was ascertained by observing the following resonance peaks: $\delta 2.34$ {6H, m, $3\times\text{CH}_3(\text{CH}_2)_{13}\text{CH}_2\text{CO}-$ }, $\delta 1.26$ {78H, m, $3\times\text{CH}_3(\text{CH}_2)_{13}\text{CH}_2\text{CO}-$ } and $\delta 0.87$ {9H, m, $3\times\text{CH}_3(\text{CH}_2)_{14}\text{CO}-$ }. The introduction of the palmitoyl groups at position 2, 3 and 4 were indicated by appearance of C-2, C-3 and C-4 resonance peaks at $\delta 5.01$ (as dd), $\delta 4.54$ (as m) and $\delta 4.88$ (as t), deshielded considerably from its precursor triol (**2**). The IR and $^1\text{H-NMR}$ spectrum of this compound was in complete agreement with the structural assigned as methyl 6-*O*-myristoyl-2,3,4-tri-*O*-palmitoyl- α -D-glucopyranoside (**9**).

Pivaloylation of compound **2** by direct method using pivaloyl chloride in dry pyridine, furnished the pivaloyl derivative (**10**). The IR spectrum of compound **10** showed peaks at 1752 cm^{-1} due to carbonyl stretching. In its $^1\text{H-NMR}$ spectrum, a twenty seven-proton singlet at $\delta 1.23$ $\{3\times(\text{CH}_3)_3\text{CCO}-\}$ corresponded to the presence of three pivaloyl groups in the molecule. The introduction of the pivaloyl groups at positions 2, 3 and 4 were demonstrated by downfield shift of C-2 to $\delta 5.05$, C-3 to $\delta 4.92$ and C-4 to $\delta 4.85$ from their precursor triol δ values (**2**). Complete

analysis of the IR and $^1\text{H-NMR}$ spectra was consistent with the structure of the compound assigned as methyl 6-*O*-myristoyl-2,3,4-tri-*O*-pivaloyl- α -D-glucopyranoside (**10**).

The myristoyl derivative (**2**) was then derivatised by using methanesulphonyl chloride in pyridine followed by usual work-up and purification procedures. The methanesulphonyl derivative (**11**) was isolated as semi-solid. Its IR spectrum exhibited absorption bands at 1735 cm^{-1} and 1365 cm^{-1} due to $-\text{CO}$ and $-\text{SO}_2$ stretchings, respectively. The $^1\text{H-NMR}$ spectrum, of this compound showed the following characteristic peaks: three three-proton singlets at δ 3.18, δ 3.10 and δ 3.06 ($3\times\text{CH}_3\text{SO}_2-$) ascertaining the presence of three methanesulphonyl groups. However, the C-2, C-3 and C-4 protons shifted downfield to δ 5.0 (as dd, $J=3.6$ and 10.1 Hz), δ 4.82 (as t, $J = 9.6$ Hz) and δ 4.54 (as t, $J = 9.6$ Hz) from its precursor compound **2**, thereby suggesting the attachment of the methanesulphonyl groups at positions 2, 3 and 4. Complete analysis of the IR and $^1\text{H-NMR}$ spectra led us to establish its structure as methyl 2,3,4-tri-*O*-methanesulfonyl-6-*O*-myristoyl- α -D-glucopyranoside (**11**).

For derivatization, we then used benzenesulfonyl chloride as acylating agent for this purpose. Thus treatment of compound (**2**) with 5 molar equivalent of benzenesulfonyl chloride in pyridine, followed by usual work-up and column chromatography, we obtained compound **12** as needless. IR spectrum of this compound displayed absorption bands at 1762 cm^{-1} ($-\text{CO}$ stretching) and 1365 cm^{-1} ($-\text{SO}_2$ stretching). In its $^1\text{H-NMR}$ spectrum, the peaks at δ 7.85 (6H, m), δ 7.63 (3H, m) and δ 7.52 (6H, m) corresponded the protons of three phenyl groups. The downfield shift of C-2 to δ 5.48, C-3 to δ 5.05 and C-4 to δ 4.86 from their precursor compound (**2**) ascertained the attachment of benzenesulfonyl groups at positions 2, 3 and 4. Complete analysis of the IR and $^1\text{H-NMR}$ spectra, led us to assign its structure as methyl 2,3,4-tri-*O*-benzenesulfonyl-6-*O*-myristoyl- α -D-glucopyranoside (**12**).

Table 3. Physicochemical properties of synthesized methyl α -D-glucopyranoside derivatives.

Compound no	Mol. formula	Time (h)	R _f	Yield (mg)	%	State/mp (°C)
2	C ₂₁ H ₄₀ O ₇	7.5	0.5	141	70.5	needles, 48-50
3	C ₂₇ H ₄₆ O ₁₀	6	0.52	64	82.5	thick syrup
4	C ₃₆ H ₆₄ O ₁₀	6.5	0.54	68	82	needles, 44-46
5	C ₃₉ H ₇₀ O ₁₀	6.5	0.51	85	80.95	semi-solid
6	C ₄₅ H ₈₂ O ₁₀	6	0.5	90	95.57	thick syrup
7	C ₅₁ H ₉₄ O ₁₀	6.5	0.54	54	91.52	homogen. syrup
8	C ₅₇ H ₁₀₆ O ₁₀	6	0.55	70	89.97	needles, 54-55
9	C ₆₉ H ₁₃₀ O ₁₀	6	0.51	63	78.75	solid mass
10	C ₃₆ H ₆₄ O ₁₀	6.5	0.52	89	80.18	needles, 58-60
11	C ₂₄ H ₄₆ S ₃ O ₁₃	6	0.51	90	90	semi-solid
12	C ₃₉ H ₅₂ S ₃ O ₁₃	6	0.53	55	84.61	needles, 55-56
13	C ₄₂ H ₄₉ O ₁₀ Cl ₃	6.5	0.51	62	77.5	needles, 198-200
14	C ₅₄ H ₇₆ O ₁₀	6	0.52	91	91	needles, 158-160
15	C ₄₈ H ₅₈ O ₁₀	6	0.52	70	88.6	needles, 45-46

4-Chlorobenzoylation of compound (**2**) by direct method using 4-chlorobenzoyl chloride in dry C_6H_5N and after similar work-up and purification techniques, the product (**13**) was isolated in 77.5% yield. The IR spectrum of this compound showed absorption band at 1746 cm^{-1} for carbonyl stretching. In its 1H -NMR spectrum, the two six-aromatic proton multiplet at δ 7.92 (as m), δ 7.67 (as m) are characteristic of *p*-substituted benzoyl groups. The deshielding of C-2, C-3 and C-4 protons from their usual values (compound **2**) and the resonance of other protons in their anticipated positions confirmed the structure of this compound as methyl 2,3,4-tri-*O*-(4-chlorobenzoyl)-6-*O*-myristoyl- α -D-glucopyranoside (**13**).

In a similar way, the myristoyl derivative (**2**) was converted to compound **14** by reaction with with 4-*t*-butylbenzoyl chloride in anhydrous pyridine and after usual work-up and chromatographic purification, it yielded 4-*t*-butylbenzoate (**14**). The IR spectrum of this compound indicated absorption bands at 1662 cm^{-1} corresponding to carbonyl (-CO) stretching. Its 1H -NMR spectrum displayed the following characteristic aromatic two six-proton multiplet peaks at δ 8.02 (as m, Ar-H), δ 7.49 (as m, Ar-H) and a twenty seven-proton singlet at δ 1.33 {as s, $3\times(CH_3)_3C$ -} which corresponded to the presence of three 4-*t*-butylbenzoyl groups in the compound. The deshielding of C-2, C-3 and C-4 protons to δ 5.40, δ 5.08 and δ 4.83 from their usual values confirmed the attachment of three 4-*t*-butylbenzoyl groups at these positions. By complete analysis of the IR and 1H -NMR spectrum led us to establish its structure as methyl 2,3,4-tri-*O*-(4-*t*-butylbenzoyl)-6-*O*-myristoyl- α -D-glucopyranoside (**14**).

Finally, we have carried out cinnamoylation of **2** with an excess of cinnamoyl chloride in pyridine as same work-up and purification techniques, we isolated compound (**15**) as needles. IR spectrum showed absorption bands at 1705 cm^{-1} (for -CO stretching) and 1631 cm^{-1} (for -CH=CH-stretching). In the 1H -NMR spectrum three one-proton doublets at δ 7.67, 7.50, 7.36 ($3\times 1H$, $3\times d$, $J = 16.0\text{ Hz}$, $3\times PhCH=CHCO$ -) and also three one-proton doublets at δ 6.46, 6.36, 6.30 ($3\times 1H$, $3\times d$, $J = 16.1\text{ Hz}$, $3\times PhCH=CHCO$ -) due to the presence of three cinnamoyl groups in the molecule. In addition a six-proton multiplet at δ 7.60 (as m, Ar-H) and a nine-proton multiplet at δ 7.25 (as m, Ar-H) due to the three aromatic ring protons. The downfield shift of C-2, C-3 and C-4 to δ 5.35 (as dd), δ 5.01 (as t) and 4.88 (as t) from their usual values in the precursor compound **2** and the resonances of other protons in their anticipated positions, showed the presence of the cinnamoyl group at positions 2, 3 and 4. The rest of the IR and 1H -NMR spectrum was in accord with the structure of this compound assigned as methyl 2,3,4-tri-*O*-cinnamoyl-6-*O*-myristoyl- α -D-glucopyranoside (**15**).

Thus, a series of acylated methyl α -D-glucopyranoside derivatives (**scheme 1 & 2**) were prepared using a wide variety of acylating agents. These acyl chlorides were chosen so as to contain probable biologically prone atoms or groups in order to find biologically active carbohydrate derivatives. All the acylation products thus prepared were employed as test chemicals for determining their antibacterial and antifungal activities against a number of human and plant pathogens.

3.2. Assessment of antimicrobial effects

The antibacterial results of the test chemicals and the standard antibiotic, Ampicillin against Gram-positive bacteria and Gram-negative bacteria are presented in **Fig. 2**.

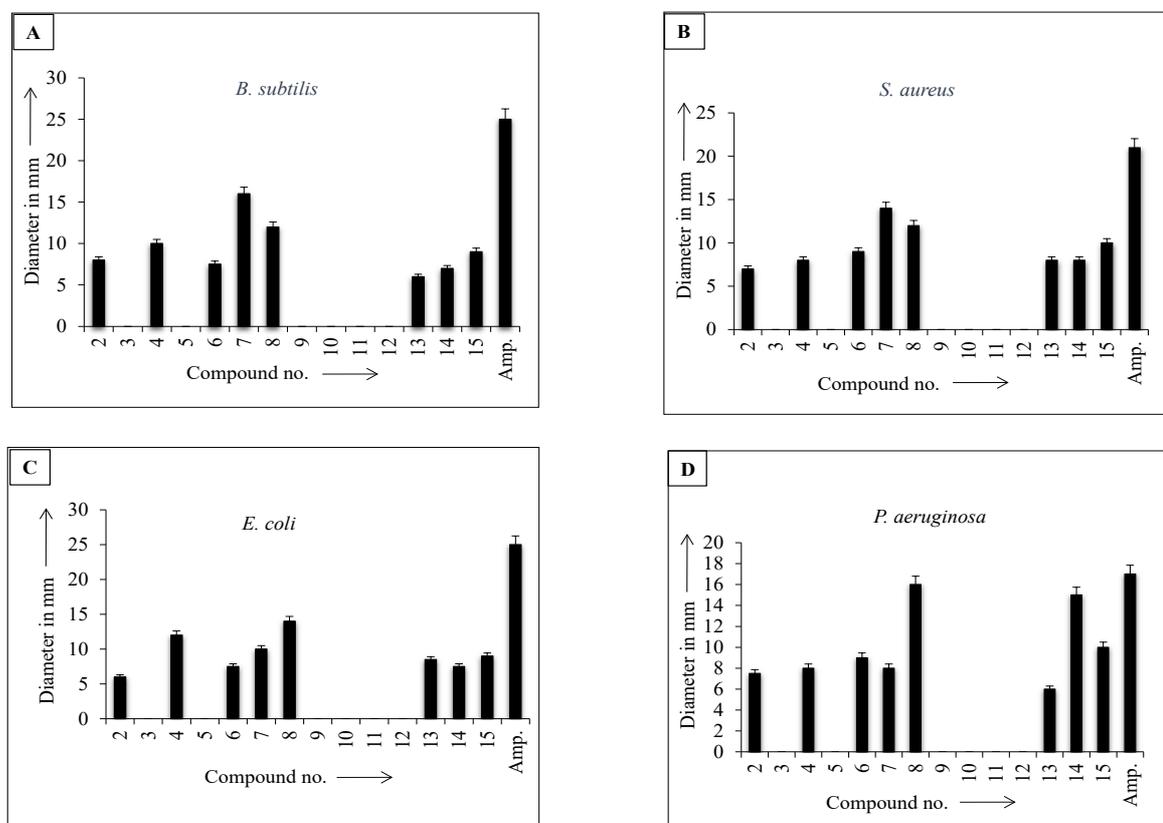


Fig. 2. Antibacterial assessment activity against Gram +Ve bacteria (A) & (B) and Gram -Ve bacteria (C) & (D) by the synthesized compounds (2-15) and standard antibiotic, Ampicillin.

From the result we observed that compound 7 and 8 were very sensitive towards all of both Gram-positive and Gram-negative bacterial organisms. In case of 7 *B. subtilis* (16 mm), *S. aureus* (14 mm), *E. coli* (10 mm), *P. aeruginosa* (08 mm) and in case of 8 *B. subtilis* (12 mm), *S. aureus* (12 mm), *E. coli* (14 mm), *P. aeruginosa* (16 mm) were found very sensitive (**Fig. 3**). The inhibition (15 mm) of the growth of *P. aeruginosa* by 14 was remarkable. Compounds 3, 5, 9, 10, 11 and 12 were quite insensitive towards any of the Gram-positive or Gram-negative bacteria. So the test compounds 7 and 8 were exhibited highest potential antibacterial power against all tested microorganisms.

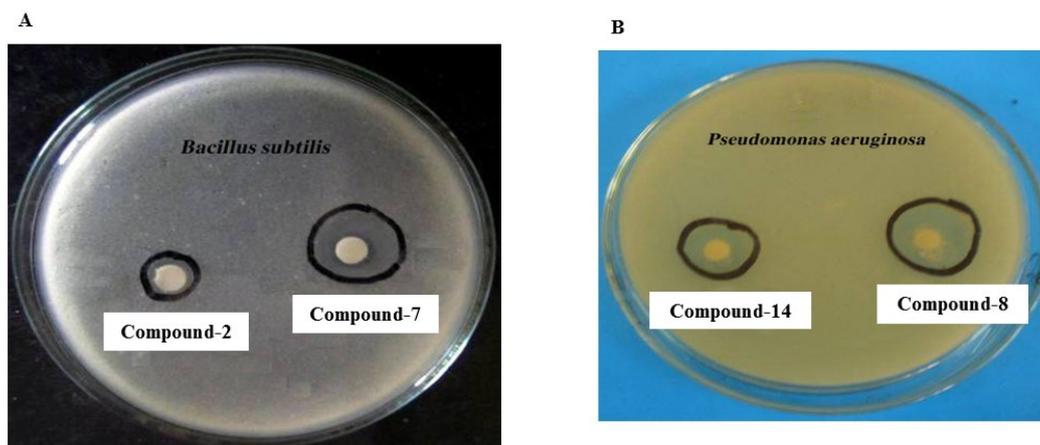


Fig. 3. Inhibition of zone against *B. subtilis* by the compounds **2** and **7** (A) and *P. aeruginosa* by the compounds **14** and **8** (B).

Table 4. Antifungal activity of the tested compounds with standard antibiotic, Nystatin.

Compound no.	% of fungal mycelial growth inhibition ^a		Compound no.	% of fungal mycelial growth inhibition ^a	
	<i>Aspergillus niger</i>	<i>Candida albicans</i>		<i>Aspergillus niger</i>	<i>Candida albicans</i>
2	*52.00	20.15	10	6	9.1
3	21	18	11	14	6.67
4	5	3.45	12	*55.50	*53.50
5	12.45	6	13	6	8
6	*51.25	*51.25	14	31.25	25
7	*62.00	*55.00	15	4	9
8	*58.00	*60.00	**Nystatin	**66.41	**63.10
9	3.51	16.67			

N.B: '*=' marked inhibition, '**=' standard antibiotic, ^agrowth measured-radial growth in cm & conc. (100 µg (dw)/ml).

The results of the *in vitro* antifungal studies of the test chemicals and the standard antibiotic, Nystatin is furnished in **Table 4**. From the results we found that compound **7** and **8** were very sensitive towards the mycelial growth of all the fungal test organisms. It was observed that the decanoyl derivative (**7**) showed the highest inhibition (62.00%) against *Aspergillus niger* which was comparable to the standard antibiotic, Nystatin (66.41%) (**Fig. 4**). Also the lauroyl derivative (**8**) showed maximum inhibition (60.00%) against *Candida albicans* which is also very close to that of standard antibiotic, Nystatin (63.10%). Again *Aspergillus niger* in case of **2**, **6**, **12** and *Candida albicans* in case of **6**, **12** were found very sensitive. Rest of the test chemicals showed their antifungal activities by different degrees.

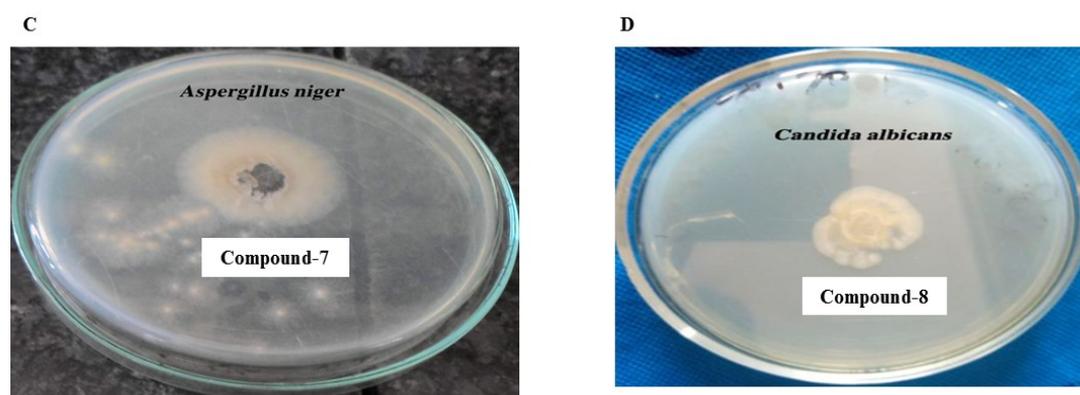


Fig. 4. Mycelial growth inhibition against *A. niger* by the compound 7 (C) and *C. albicans* by the compound 8 (D).

From the results discussed above it is clear that the presence of some particular groups in the test compounds enhanced their sensitivities towards the growth of bacteria and fungi. The presence of decanoyl and lauroyl group made the test chemicals very effective towards the growth of both Gram-positive and Gram-negative bacteria. The test chemicals containing octanoyl, decanoyl, lauroyl and benzenesulphonyl group were found to show very high antifungal activity which was in accordance with our previous work [27-30]. So these compounds may be targeted for future studies for their usage as broad spectrum antibiotics.

4. CONCLUSION

In the present study, we have described the regioselective synthesis of fourteen methyl α -D-glucopyranoside derivatives with various acyl groups containing different carbon chain length. This direct acylation method demonstrates a very simple and efficient method for the total synthesis and methyl 6-*O*-myristoyl-2,3,4-tri-*O*-octanoyl- α -D-glucopyranoside (**6**) was found to be encouraging in terms of high selectivity and excellent yield as 95.57%. Thus, a good number of test compounds reported herein exhibited promising antimicrobial effects. This is the first report regarding the effectiveness of the selected compounds against the selected pathogens.

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