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Synthesis, spectral characterization and biological studies of some newly acylated carbohydrate derivatives

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ABSTRACT

Selective 4-methoxybenzoylation of methyl α-**D**-glucopyranoside (1) using direct acylation method afforded the methyl 2,6-di-*O*-(4-methoxybenzoyl) α-**D**-glucopyranoside (2) in an excellent yield. In order to obtain newer products for antimicrobial evaluation studies, the 2,6-di-*O*-(4-methoxybenzoyl) derivative was further transformed to a series of 3,4-di-*O*-acyl derivatives (2-12) containing a wide variety of functionalities in a single molecular framework. The structure of the synthesized compounds was confirmed by spectral data and elemental analysis. All synthesized compounds were screened for their *in vitro* antimicrobial activity against some human and phytopathogens. Some of the obtained compounds exhibited good antimicrobial activity comparable to standard antibiotics like ampicillin and nystatin.

Keywords: *Carbohydrate, synthesis, antimicrobial, inhibition zone, pathogens.*

1. INTRODUCTION

The increasing interest in the chemistry of carbohydrates has led to a continuing search for new and efficient methods for selective acylation of a of a particular sugar hydroxyl group. 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 [1-5]. Of these, the direct method is considered as one of the most effective [5] for selective acylation of carbohydrates. In the last few decades considerable works have been done in the field biological activities by chemical compounds [6]. It must, however, be admitted that a lot of the reports on the benefits of one or the other chemicals were based on empirical knowledge. Different classes of compounds have been screened for antimicrobial activities all over the world. Acylated carbohydrates, glycosides and glycoses are very important due to their effective biological activity [7]. It was found from the literature survey that a large number of biologically active compounds also possess aromatic, heteroaromatic and acyl substituents [8]. The benzene, substituted benzene and also nitrogen, sulphur and halogen containing substituents are known to enhance the biological activity of the parent compound [9]. It also known that if an active nucleus is linked to another active nucleus, the resulting molecule may possess greater potential for biological activity [8]. Results of an ongoing research project on selective acylation of carbohydrates

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[10-12] and also evaluation of microbial activities [13-15] 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 [16,17]. Guided by these observations, we deliberately synthesized some acylated products of methyl α-**D**-glucopyranoside (1).

2. EXPERIMENTAL SECTION

2.1. Materials, method and instruments. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Thin layer chromatography (t.l.c) was performed on Kieselgel GF_{254} and spots were detected with 1% sulphuric acid spray reagent. Evaporations were carried out under reduced pressure using VV-1 type vacuum rotary evaporator (Germany) with a bath temperature below 40°C. ^{1}H-NMR spectra (300 MHz) were recorded for solutions in deuteriochloroform ($CDCl_3$) (internal Me_4Si) with a Bruker DPX-40C spectrometer. Column chromatography was performed with silica gel G_{60}.

2.2. Synthesis of Methyl 2,6-di-*O*-(4-methoxybenzoyl)-α-D-glucopyranoside (2). A solution of methyl α-D-glucopyranoside (1) (0.5g, 2.57 mmol) in dry pyridine (8 mL) was cooled to -5°C whereupon 4-methoxybenzoyl chloride (0.48 mL, 1.1 molar eq.) was added to it. The mixture was stirred at this temperature for 4 hours and then stirred overnight at room temperature. The progress of the reaction was monitored by t.l.c. (methanol-chloroform 1:21), which indicated formation of two products, the faster-moving component being the major one. A few pieces of ice was added to the flask and then extracted the product mixture with chloroform (3 × 10 mL). The combined chloroform layer was washed successively with dilute hydrochloric acid (10%), saturated aqueous sodium hydrogen carbonate ($NaHCO_3$) solution and distilled water. The chloroform layer was dried ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure to leave a syrup. The syrup was passed through a silica gel column and eluted with methanol-chloroform (1:21), provided the faster-moving component as methyl 2,6-di-*O*-(4-methoxybenzoyl)-α-D-glucopyranoside (2) (0.54 g, 45%) as white syrup. R_f 0.51 (MeOH : $CHCl_3$, 1:21); ^{1}H-NMR δ_H ($CDCl_3$) ppm: 7.40 (2H, d, J = 8.4 Hz, Ar-H), 7.37 (2H, d, J = 8.4 Hz, Ar-H), 6.86 (2H, d, J = 8.4 Hz, Ar-H), 6.82 (2H, d, J = 8.5 Hz, Ar-H), 4.88 (1H, dd, J = 3.6 and 9.6 Hz, H-2), 4.82 (1H, d, J = 3.6 Hz, H-1), 4.76 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 4.68 (1H, t, J = 10.2 Hz, H-6b), 3.78, 3.76 (2×3H, 2×s, 2×4 – OC*H*$_3$.C_6H_4CO-), 3.70 (1H, m, H-5), 3.65 (1H, t, J = 9.6 Hz, H-3), 3.62 (1H, t, J = 9.6 Hz, H-4), 3.42 (3H, s, 1-OC*H*$_3$).

2.3. General procedure for the synthesis of 3,4-di-*O*-acyl derivatives (3-12). A solution of compound 2 (80 mg, 0.29 mmol) in dry pyridine (3 mL) was cooled to -5^{0}C when acetyl chloride (0.2 mL, 1.66 mmol) was added. The reaction mixture was stirred at 0^{0}C for two hours and then kept standing overnight in the refrigerator. The progress of the reaction was monitored by t.l.c. which showed complete conversion of reactant into a single product. A few pieces of ice was added to the flask and then extracted the product mixture with chloroform (3×10 mL). The combined chloroform layer was washed successively with dilute hydrochloric acid (10%), saturated aqueous sodium hydrogen carbonate ($NaHCO_3$) solution and distilled water. The chloroform layer was then dried Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to leave a syrup. The syrup was passed through a silica gel column and eluted with ethyl acetate-hexane (1:8), to afford the acetyl derivative (**3**). Similar reaction and purification procedure was applied to prepare compounds **4-12**.

2.3.1. Methyl 3,4-di-*O*-acetyl-2,6-di-*O*-(4-methoxybenzoyl)-α-D-glucopyranoside (3). Yield (85 mg, 89%) as a syrup. R_f 0.52 (EtOAc : n-C_6H_{14}, 1:8); ^{1}H-NMR δ_H ($CDCl_3$) ppm: 7.52 (2H, d, J = 8.5

Hz, Ar-H), 7.41 (2H, d, *J* = 8.4 Hz, Ar-H), 6.95, 6.88 (2×2H, 2×d, *J* = 8.6 Hz, Ar-H), 4.93 (1H, t, *J* = 9.6 Hz, H-3), 4.89 (1H, dd, *J* = 3.6 Hz and 4.6 Hz, H-2), 4.82 (1H, dd, *J* = 4.8 and 10.2 Hz, H-6a), 4.78 (1H, t, *J* = 9.6 Hz, H-4), 4.75 (1H, t, *J* = 10.2 Hz, H-6b), 4.71 (1H, d, *J* = 3.6 Hz, H-1), 3.80, 3.78 (2×3H, 2×s, 2× 4-OC*H*$_3$.C$_6$H$_4$CO-), 3.73 (1H, m, H-5), 3.40 (3H, s, 1-OC*H*$_3$), 2.04, 2.01 (2×3H, 2×s, 2× C*H*$_3$CO-).

2.3.2. Methyl 3,4-di-*O*-hexanoyl-2,6-di-*O*-(4-methoxybenzoyl)-α-D-glucopyranoside (4). Yield (97mg, 85%) as a pasty mass. R$_f$ 0.48 (EtOAc : n-C$_6$H$_{14}$, 1:10); ^{1}H-NMR δ$_H$ (CDCl$_3$) ppm: 7.40 (2H, d, *J* = 8.3 Hz, Ar-H), 7.36 (2H d, *J* = 8.4 Hz, Ar-H), 6.85, (2H, d, *J* = 8.5 Hz, Ar-H), 6.82 (2H, d, *J* = 8.4 Hz, Ar-H), 4.90 (1H, t, *J* = 9.6 Hz, H-3), 4.86 (1H, dd, *J* = 3.6 and 9.6 Hz, H-2), 4.81 (1H, dd, *J* = 4.8 and 10.0 Hz, H-6a), 4.77 (1H, t, *J* = 9.6 Hz, H-4), 4.72 (1H, t, *J* = 10.1 Hz, H-6b), 4.70 (1H, d, *J* = 3.6 Hz, H-1), 3.79, 3.77 (2 ×3H, 2 × s, 2 × 4-OC*H*$_3$. C$_6$H$_4$ CO-), 3.42 (3H, s, 1-OC*H*$_3$), 2.36 {4H, m, 2 × CH$_3$ (CH$_2$)$_3$ C*H*$_2$ CO-}, 1.65 {4H, m, 2× CH$_3$ (CH$_2$)$_2$ C*H*$_2$ CH$_2$ CO-}, 1.33 {8H, m, 2 × CH$_3$ (C*H*$_2$)$_2$ (CH$_2$)$_2$ CO-}, 0.90 {3H, t, *J* = 7.4 Hz, C*H*$_3$ (CH$_2$)$_4$ CO-}, 0.86 {3H, t, *J* = 7.4 Hz, C*H*$_3$ (CH$_2$)$_4$ CO-}.

2.3.3. Methyl 3,4-di-*O*-decanoyl -2,6-di-*O*-(4 -methoxybenzoyl)-α-D-glucopyranoside (5). Yield (120 mg, 80 %) as a semi-solid mass which could not be crystallized. R$_f$ 0.55 (EtOAc : n-C$_6$H$_{14}$, 1:13); ^{1}H-NMR δ$_H$ (CDCl$_3$) ppm: 7.48 (2H, d, *J* = 8.3 Hz, Ar-H), 7.40 (2H, d, *J* = 8.3 Hz, Ar-H), 6.92 (2H, d, *J* = 8.3 Hz, Ar-H), 6.88 (2H, d, *J* = 8.3 Hz, Ar-H), 4.91 (1H, t, *J* = 9.6 Hz, H-3), 4.88 (1H, dd, *J* = 3.6 and 9.6 Hz, H-2), 4.83 (1H, dd, *J* = 4.8 and 10.2 Hz, H-6a), 4.80 (1H, t, *J* = 10.1 Hz, H-6b), 4.77 (1H, t, *J* = 9.6 Hz, H-4), 4.73 (1H, d, *J* = 3.6 Hz, H-1), 3.80, 3.76 (2 × 3H, 2 × s, 2 × 4 – OC*H*$_3$ .C$_6$H$_4$ CO-), 3.44 (3H, s, 1-OC*H*$_3$), 2.33 {4H, m, 2 × CH$_3$ (CH$_2$)$_7$ C*H*$_2$ CO-}, 1.63 {4H, m, 2 × CH$_3$ (CH$_2$)$_6$ C*H*$_2$ CH$_2$ CO-}, 1.30 {24H, m, 2 × CH$_3$ (C*H*$_2$)$_6$ (CH$_2$)$_2$ CO-}, 0.91 {3H, t, *J* = 7.3 Hz, C*H*$_3$ (CH$_2$)$_8$ CO-}, 0.87 {3H, t, *J* = 7.3 Hz, C*H*$_3$ (CH$_2$)$_8$ CO-}.

2.3.4. Methyl 3,4-di-*O*-(3-chlorobenzoyl)-2,6-di-*O*-(4-methoxybenzoyl)-α-D-glucopyranoside (6). Yield (107 mg, 93%) as a syrup. R$_f$ 0.55 (EtOAc : n-C$_6$H$_{14}$, 1:13); ^{1}H-NMR δ$_H$ (CDCl$_3$) ppm: 7.98 (1H, s, Ar-H), 7.93, 7.90 (2 × 2H, 2 × d, *J* = 8.4 Hz, Ar-H), 7.54 (2H, d, *J* = 8.0 Hz, Ar-H), 7.37 (1H, t, *J* = 7.9 Hz, Ar-H), 6.95, 6.91 (2 × 2H, 2 × d, *J* = 8.4 Hz, Ar-H), 4.93 (1H, t, *J* = 9.6 Hz, H-3), 4.89 (1H, dd, *J* = 3.6 and 9.6 Hz, H-2), 4.86 (1H, dd, *J* = 4.8 and 10.3 Hz, H-6a), 4.82 (1H, t, *J* = 9.6 Hz, H-4), 4.78 (1H, t, *J* = 10.2 Hz, H-6b), 4.73 (1H, d, *J* = 3.6 Hz, H-1), 3.79, 3.76 (2 × 3H, 2 × s, 2 × 4-OC*H*$_3$ .C$_6$H$_4$ CO-), 3.72 (1H, m, H-5), 3.41 (3H, s, 1-OC*H*$_3$).

2.3.5. Methyl 3,4-di-*O*-(4-chlorobenzoyl)-2,6-di-*O*-(4-methoxybenzoyl)-α-D-glucopyranoside (7). Yield (0.55g, 80%) as a syrup. R$_f$ 0.55 (EtOAc : n-C$_6$H$_{14}$, 1:10); ^{1}H-NMR δ$_H$ (CDCl$_3$) ppm: 7.90 (2H, d, *J* = 8.3 Hz, Ar-H), 7.86 (2H, d, *J* = 8.4 Hz, Ar-H), 7.42 (2H, d, *J* = 8.4 Hz, Ar-H), 6.88 (2H, d, *J* = 8.4 Hz, Ar-H), 4.93 (1H, t, *J* = 9.6 Hz, H-3), 4.88 (1H, dd, *J* = 3.6 and 9.6 Hz, H-2), 4.82 (1H, t, *J* = 9.6 Hz, H-4), 4.78 (1H, d, *J* = 3.6 Hz, H-1), 4.75 (1H, dd, *J* = 4.8 and 10.2 Hz, H-6a), 4.70 (1H, t, *J* = 10.2 Hz, H-6b), 3.80, 3.77 (2 × 3H, 2 × s, 2 × 4–OC*H*$_3$.C$_6$H$_4$ CO-), 3.70 (1H, m, H-5), 3.44 (3H, s, 1-OC*H*$_3$).

2.3.6. Methyl 3,4-di-*O*-(2,6-dichlorobenzoyl)-2,6-di-*O*-(4-methoxybenzoyl)-α-D-glucopyranoside (8). Yield (117 mg, 90%) as a thick syrup. R$_f$ 0.51 (EtOAc : n-C$_6$H$_{14}$, 1:8); ^{1}H-NMR δ$_H$ (CDCl$_3$) ppm: 7.48, 7.44 (2 ×2H, 2 × d, *J* = 8.4 Hz, Ar-H), 7.35 (4H, m, Ar-H), 7.28 (2H, m, Ar-H), 6.98, 6.93 (2 ×2H, 2 × d, *J* = 8.4 Hz, Ar-H), 4.95 (1H, t, *J* = 9.6 Hz, H-3), 4.91 (1H, dd, *J* = 3.6 and 9.6 Hz, H-2), 4.87 (1H, dd, *J* = 4.8 and 10.1 Hz, H-6a), 4.83 (1H, t, *J* = 10.2 Hz, H-6b), 4.78 (1H, t, *J* = 9.6 Hz, H-4), 4.74 (1H, d, *J* = 3.6 Hz, H-1), 3.78, 3.76 (2 × 3H, 2 × s, 2 × 4-OC*H*$_3$ .C$_6$H$_4$ CO-), 3.73 (1H, m, H-5), 3.42 (3H, s, 1-OC*H*$_3$).

2.3.7. Methyl 2,6-di-*O*-(4-methoxybenzoyl)-3,4-di-*O*-(4-nitrobenzoyl)-α-D-glucopyranoside (9). Yield (133 mg, 92 %) as a syrup. R_f 0.51 (EtOAc : n-C_6H_{14}, 1:6); ^{1}H-NMR δ_H ($CDCl_3$) ppm: 8.30, 8.23, 7.85, 7.81 (4 ×2H, 4 × d, *J* = 8.7 Hz, Ar-H), 7.45, 7.41, 6.98, 6.93 (4 × 2H, 4 × d, *J* = 8.3 Hz, Ar-H), 5.50 (1H, t, *J* = 9.6 Hz, H-3), 5.10 (1H, t, *J* = 9.6 Hz, H-4), 4.96 (1H, dd, *J* = 3.6 and 9.6 Hz, H-2), 4.92 (1H, dd, *J* = 4.8 and 10.2 Hz, H-6a), 4.83 (1H, t, *J* = 10.2 Hz, H-6b), 4.77 (1H, d, *J* = 3.6 Hz, H-1), 3.78, 3.75 (2 × 3H, 2 × s, 2 × 4-OC*H*$_3$.C_6H_4CO-), 3.68 (1H, m, H-5), 3.44 (3H, s, 1-OC*H*$_3$).

2.3.8. Methyl 3,4-di-*O*-(3,5-dinitrobenzoyl)-2,6-di-*O*-(4-methoxybenzoyl)-α-D-glucopyranoside (10). Yield (123 mg, 86%) as a thick syrup. R_f 0.51 (EtOAc : n-C_6H_{14}, 1:8); ^{1}H-NMR δ_H ($CDCl_3$) ppm: 9.12, 9.10, (2 ×1H, 2 ×s, Ar-H), 9.06, 9.04 (2 × 2H, 2 × s, Ar-H), 7.44, 7.40 (2 × 2H, 2 ×d, *J* = 8.3 Hz, Ar-H), 6.92, 6.90 (2 ×2H, 2 ×d, *J* = 8.3 Hz, Ar-H), 4.92 (1H, t, *J* = 9.6 Hz, H-3), 4.88 (1H, dd, *J* = 3.6 and 9.6 Hz, H-2), 4.83 (1H, dd, *J* = 4.8 and 10.2 Hz, H-6a), 4.78 (1H, t, *J* = 9.6 Hz, H-4), 4.74 (1H, t, *J* = 10.2 Hz, H-6b), 4.71 (1H, d, *J* = 3.6 Hz, H-1), 3.78, 3.76 (2 ×3H, 2 ×s, 2 ×4-OC*H*$_3$.C_6H_4CO-), 3.42 (3H, s, 1-OC*H*$_3$).

2.3.9. Methyl 3,4-di-*O*-(4-t-butylbenzoyl)-2,6-di-*O*-(4-methoxybenzoyl)-α-D-glucopyranoside (11). Yield (167 mg, 88%) as a syrup. R_f 0.58 (EtOAc : n-C_6H_{14}, 1:2); ^{1}H-NMR δ_H ($CDCl_3$) ppm: 7.96, 7.92, (2 ×2H, 2 × d, *J*= 8.4 Hz, Ar-H), 7.46, 7.41 (2 ×2H, 2 ×d, *J*=8.3 Hz, Ar-H), 7.36, 7.33 (2 ×2H, 2 ×d, *J* = 8.3 Hz, Ar-H), 6.92, 6.88 (2 ×2H, 2 ×d, *J* = 8.5 Hz, Ar-H), 4.90 (1H, t, *J* = 9.7 Hz, H-3), 4.87 (1H, dd, *J* = 3.6 and 9.6 Hz, H-2), 4.83 (1H, dd, *J* = 4.8 and 10.2 Hz, H-6a), 4.80 (1H, t, *J* = 10.2 Hz, H-6b), 4.76 (1H, t, *J* = 9.6, H-4), 3.77, 3.75 (2 ×3H, 2 ×s, 2 × 4-OC*H*$_3$.C_6H_4 CO-), 3.42 (3H, s, 1- OC*H*$_3$), 1.30, 1.27 {2 ×9H, 2 × s, 2 × (C*H*$_3$)$_3$C-}.

2.3.10. Methyl 2,6-di-*O*-(4-methoxybenzoyl)-3,4-di-*O*-pivaloyl-α-D-glucopyranoside (12). Yield (103 mg, 87 %) as a pasty mass. R_f 0.56 (EtOAc : n-C_6H_{14}, 1:10); ^{1}H-NMR δ_H ($CDCl_3$) ppm: 7.45, 7.40 (2 ×2H, 2 × d, *J*= 8.4 Hz, Ar-H), 6.92, 6.88 (2 ×2H, 2 ×d, *J*=8.4 Hz, Ar-H), 4.95 (1H, t, *J* = 9.6 Hz, H-3), 4.90 (1H, dd, *J* = 3.6 and 9.6 Hz, H-2), 4.86 (1H, dd, *J* = 4.8 and 10.1 Hz, H-6a), 4.82 (1H, t, *J* = 9.6 Hz, H-4), 4.78 (1H, t, *J* = 10.2 Hz, H-6b), 4.73 (1H, d, *J* = 3.6 Hz, H-1), 3.80, 3.77 (2 ×3H, 2 ×s, 2 × 4-OC*H*$_3$.C_6H_4 CO-), 3.72 (1H, m, H-5), 3.44 (3H, s, 1-OC*H*$_3$), 1.09, 1.06 {2 ×9H, 2 ×s, 2 ×(C*H*$_3$)$_3$CCO-}.

2.4. Microorganisms. Some partially protected derivatives of **D**-glucopyranoside (2-12) were tested for their antibacterial activity against ten human pathogenic bacteria, i.e., *Bacillus subtilis* BTCC 17, *Bacillus megaterium* BTCC 18, *Bacillus cereus* BTCC 19, *Staphylococcus aureus* ATCC 6538, *Shigella dysenteriae* AE 14396, *Escherichia coli* ATCC 25922, *Salmonella typhi* AE 14612, *Salmonella paratyphi* AE 14613, *Pseudomonas* sp. CRL (ICDDR, B) and *Vibrio cholerae* AE 14748 and three pathogenic fungi i.e., *Fusarim equiseti* (*corda*) *Sacc*., *Macrophomina phaseolina* (Tassi) Goid and *Alternaria alternata* (Fr.) Kedissler.

2.5. Antibacterial activity. The *in vitro* susceptibility of the bacterial strains to the synthesized methyl 4,6-*O*-benzylidene-α-**D**-glucopyranoside derivatives (2-10) was done by disc diffusion method [18] with little modification [19]. 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 (adjusted to 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 $CHCl_3$) of each test chemical using a micropipette, dried in air under aseptic condition and were placed at equidistance in a circle

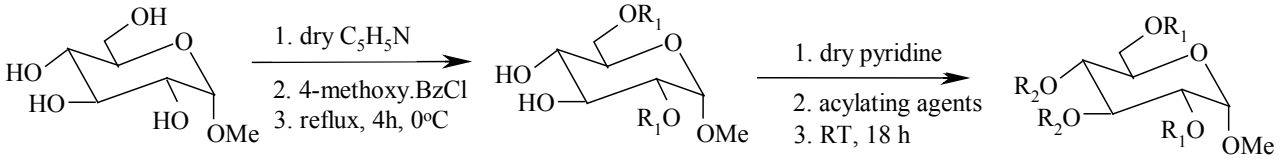
on the seeded plate. A control plate was also maintained in each case without any test chemical. These plates were kept for 4-6 hours at low temperature (4-6˚C) and the test chemicals diffused from disc to the surrounding medium by this time. The plates were then incubated at 35±2C for 24 hours to allow maximum growth of the organisms. 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 (20 µg/disc, BEXIMCO Pharm Bangladesh Ltd).

2.6. Antifungal activity. The *in vitro* antifungal activities of the synthesized **D**-glucopyranoside derivatives (2-10) were determined by poisoned food technique [20]. Two percent solution of the test chemical (in $CHCl_3$) was mixed with sterilized melted Saburaud 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 27C. 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, T = Diameter of the fungal colony in treatment. All the results were compared with the standard antifungal antibiotic nystatin (100 µg/mL medium, MEXIMCO Pharm Bangladesh Ltd.).

3. RESULTS SECTION

3.1. Synthesis and spectral characterization. The structure of the 4-methoxybenzoyl derivative (2) was established by analyzing its ^{1}H-NMR spectrum. Four two-proton doublets at δ 7.40 (J = 8.4 Hz), δ 7.37 (J = 8.4 Hz), δ 6.86 (J = 8.4 Hz) and δ 6.82 (J = 8.5 Hz) and two-three proton singlets at δ 3.78 and δ 3.76 indicated the attachment of two 4-methoxybenzoyl groups in the molecule. The downfield shift of C-2 proton to δ 4.88 (as dd, J = 3.6 and 9.6 Hz) and C-6 proton to δ 4.76 (as dd, J = 4.8 and 10.2 Hz, H-6a) and δ 4.68 (as t, J = 10.2 Hz, H-6b) from their usual values in the precursor compound 1 and the resonances of other protons in their anticipated positions, showed the presence of the 4-methoxybenzoyl groups at positions 2 and 6.



1
Methyl α-**D**-glucopyranoside

2: R_1 = 4-Met.Bz
(2,6-di-*O*-acyl derivative)

3: R_1 = 4-Met.Bz ; R_2 = Ac
4: R_1 = 4-Met.Bz ; R_2 = Hexanoyl
5: R = 4-Met.Bz ; R_2 = Decanoyl
6: R = 4-Met.Bz ; R_2 = 3-Cl.BzO
7: R = 4-Met.Bz ; R_2 = 4-Cl.BzO
8: R = 4-Met.Bz ; R_2 = 2,6-di.Cl.Bz
9: R = 4-Met.Bz ; R_2 = 4NO_2.Bz
10: R = 4-Met.Bz ; R_2 = 3,5-di.NO_2.Bz
11: R = 4-Met.Bz ; R_2 = 4-t-Bu.Bz
12: R = 4-Met.Bz ; R_2 = Pivaloyl

Scheme 1: Synthesis of compounds 2-12.

The structure of this compound was confidently assigned as methyl 2,6-di-*O*-(4-methoxybenzoyl)-α-**D**-glucopyranoside (2) by complete analysis of its ^{1}H-NMR spectrum. Further support for the structure of the 4-methoxybenzoyl derivative (2) was obtained by its conversion to and identification

of the acetyl derivative (3). Thus, treatment of compound 2 with acetic anhydride in pyridine, followed by usual aqueous work-up provided the diacetate (3) as a syrup. In its ^{1}H-NMR spectrum, two three-proton singlets at δ 2.04 and 2.01 was attributed to the presence of two acetoxy groups in the molecule. The C-3 proton appeared at δ 4.93 (as t, J = 9.6 Hz) from their values in the precursor diol (2) (δ 3.65, t, J = 9.6 Hz, H-4) ascertaining the introduction of the acetyl groups at position 3 and 4. The resonances of other protons were observed in their anticipated positions .The structure of the acetate was thus assigned as methyl 3,4-di-*O*-acetyl-2,6-*O*-(4-methxybenzoyl)-α-**D**-glucopyranoside (3) by complete analysis of its ^{1}H-NMR spectrum. The structure of the 4-methoxybenzoyl derivative (2) was then derivatised using two fatty acid chlorides such as hexanoyl chloride and decanoyl chloride. Thus, treatment of compound 2 with hexanoyl chloride in pyridine, followed by usual work-up gave the hexanoyl derivative (4). In its ^{1}H-NMR spectrum, two four-proton multiplets at δ 2.36 and δ 1.65, one eight-proton multiplet at δ 1.33 and two three-proton triplets at δ 0.90 (J = 7.4 Hz) and δ 0.86 (J = 7.4 Hz), indicated the introduction of two hexanoyl groups in the molecule. Complete analysis of its ^{1}H-NMR spectrum enabled us to assign its structure as methyl 3,4-di-*O*-hexanoyl-2,6-di-*O*-(4-methoxybenzoyl)-α-**D**-glucopyranoside (4). The ^{1}H-NMR spectrum of compound 5 displayed two four-proton multiplets at δ 2.33 and δ 1.63, a twenty-four proton multiplet at δ 1.30 and two three-proton triplets at δ 0.91 (J = 7.3 Hz) and δ 0.87 (J = 7.3 Hz) showing the attachment of two decanoyl groups in the molecule. The resonances of all the ring protons are in their anticipated positions thus enabling us to ascertain its structure as methyl 3,4-di-*O*-decanoyl-2,6-di-*O*-(4-methoxybenzoyl)-α-**D**-glucopyranoside (5). Reactions of compound 2 with 3-chlorobenzoyl chloride and 4-chlorobenzoyl chloride in pyridine medium, afforded the corresponding 3,4-di-*O*-(3-chlorobenzoyl) and 3,4-di-*O*-(4-chlorobenzoyl) derivatives (6 and 7) in high yields, both as chromatographically homogeneous syrup . The ^{1}H-NMR spectra of compounds 6 and 7 were consistent with the structure assigned to them. Complete analysis of its ^{1}H-NMR spectrum and by analogy with similar derivatives, the structure of this compound was assigned as methyl 3,4-di-*O*-(2,6-dichlorobenzoyl)-2,6-di-*O*-(4-methoxybenzoyl)-α-**D**-glucopyranoside (8). The ^{1}H-NMR spectrum of compound 9, four low field two-proton doublets at δ 8.30, δ 8.23, δ 7.85 and δ 7.81 corresponded to two 4-nitrobenzoyl groups. The rest of the spectrum was consistent with the structure of the compound assigned as methyl 2,6-di-*O*-(4-methoxybenzoyl)-3,4-di-*O*-(4-nitrobenzoyl)-α-**D**-glucopyranoside (9). Similarly, the 3,5-dinitrobenzoyl derivative (10) of compound 2 was prepared and its ^{1}H-NMR spectrum was compatible with the structure assigned as methyl 3,4-di-*O*-(3,5-dinitrobenzoyl)-2,6-di-*O*-(4-methoxybenzoyl)-α-**D**-glucopyranoside (10). Treatment of compound 2 with 4-t-butylbenzoyl chloride using direct acylation method provided the 4-t-butylbenzoyl derivative (11) in 88% yield as a syrup. In its ^{1}H-NMR spectrum, eight two-proton doublets at δ 7.96, δ 7.92, δ 7.46, δ 7.41, δ 7.36, δ 7.33, δ 6.92 and δ 6.88 corresponded to two 4-t-butylbenzoyl and two 4-methoxybenzoyl groups in the molecule. The C-3 proton deshielded to δ 4.90 (as t, J = 9.7 Hz) and the C-4 proton to δ 4.76 (as t, J = 9.6 Hz), as compared to the precursor compound (2) (δ 3.65, t, J = 9.6 Hz, H-3; δ 3.62, t, J = 9.6 Hz, H-4), indicating the introduction of the 4-t-butylbenzoyl groups at positions 3 and 4. Complete analysis of the ^{1}H-NMR spectrum enabled us to ascertain its structure as methyl 3,4-di-*O*-(4-t-butylbenzoyl)-2,6-di-*O*-(4-methoxybenzoyl)-α-**D**-glucopyranoside (11). The pivaloyl derivative (12) was obtained in 87% yield as syrup. In its ^{1}H-NMR spectrum, two nine-proton singlets at δ 1.09 and δ 1.06 corresponded to two pivaloyl groups. The deshielding of C-3 and C-4 protons to δ 4.95 (as t, J = 9.6 Hz, H-3) and δ 4.82 (as t, J = 9.6 Hz, H-4) from its usual values and the resonances of other protons in their anticipated positions led us to assigned its structure as methyl 2,6-di-*O*-(4-methoxybenzoyl)-3,4-di-*O*-pivaloyl-α-**D**-glucopyranoside (12). Thus, selective acylation of methyl α-**D**-glucopyranoside (1) with a

number of rather non-traditional acylating agents such as acetic anhydride, hexanoyl chloride, decanoyl chloride, 3-chlorobenzoyl chloride, 4-chlorobenzoyl chloride, 2,6-dichlorobenzoyl chloride, 4-nitrobenzoyl chloride, 3,5-dinitrobenzoyl chloride, 4-t-benzoyl chloride and pivaloyl chloride, was successfully carried out using the direct method. These acylations were found to be very promising since in all the reactions a single substitution product was isolated in reasonably high yields.

3.2. Antimicrobial activity. The *in vitro* antibacterial screening against four Gram-positive and six Gram-negative human pathogens are shown in Table 1 and Table 2.

Table 1: Antibacterial screening studies against Gram-positive bacteria.

Compound	Diameter of growth inhibition zone in mm 200 µg dw/disc			
	B. subtilis	***B. cereus***	***B. megaterium***	***S. aureus***
2	9	8	10	11
3	2	5	8	9
4	NF	NF	NF	NF
5	*20	15	13	*15
6	14	14	10	12
7	NF	NF	NF	NF
8	NF	NF	NF	NF
9	NF	NF	NF	NF
10	NF	NF	NF	NF
11	12	13	5	7
12	NF	NF	NF	NF
**Ampicillin	*19	*18	*16	*22

N.B: '*' = marked inhibition, '**' = standard antibiotic, 'NF' = not found, 'dw' = dry weight.

Table 2: Antibacterial screening studies against Gram-negative bacteria.

Compound	Diameter of growth inhibition zone in mm 200 µg dw/disc					
	E. coli	***S. typhi***	***S. paratyphi***	***S. dysenteriae***	***Pseudomonas sp.***	***V. cholerae***
2	NF	NF	NF	NF	NF	NF
3	6	10	12	8	8	8
4	NF	10	7.5	NF	6.5	NF
5	15	*14	12	8	*15	12
6	NF	NF	NF	NF	NF	NF
7	8.5	12	8	9	7.5	7.5
8	NF	12	11	NF	9	NF
9	NF	NF	NF	NF	*14	NF
10	NF	NF	NF	NF	NF	NF
11	12	*16	11.5	8	*19	10
12	NF	8	6	NF	7	NF
**Ampicillin	*20	*20	*18	*22	*18	*15

N.B: '*' = marked inhibition, '**' = standard antibiotic, 'NF' = not found, 'dw' = dry weight.

The synthesized compounds showed a different degree of inhibitory activity against both Gram-positive and Gram-negative test bacteria. Test compounds 2, 3, 5, 6 and 11 were found to be active against all the Gram-positive bacteria, while 3, 5, 7 and 11 were very active against Gram-negative bacteria tested herein, although the degrees of inhibition were different. Whereas, the test compounds 3, 5 and 11 were recorded active against all the ten Gram-positive and Gram-negative test bacteria. So, these chemicals may be targeted for future studies for their usage as broad spectrum antibiotics. Highest inhibition by acylated derivative **5** was observed against *B. subtilis* (20 mm). No

inhibition was observed with the compounds 4, 7-10 and 12 against all the Gram-positive bacterial strains. Both Gram-positive and Gram-negative bacteria were found resistant towards the compound 10. Antibacterial activity better than standard ampicillin was recorded with the chemical 2 against *B. subtilis* and with the compound 11 against *P. species*. Among the acylated products, the chemicals 5 and 11 were found more prone and wide spectrum towards antibacterial functionality against both Gram-positive and Gram-negative bacteria. These results are in concurrence with the findings of our previous results [15-17].

The *in vitro* antifungal results of our synthesized test compounds and the standard nystatin is shown in Table 3. The results indicated that most of the compounds were more or less sensitive towards the three fungal phytopathogens. It was found that the compounds 11 (40%) showed highest inhibition against *F. equiseti*. However, the inhibition of mycelial growth of the compound 7 (40%) against *M. phaseolina*, compound 5 (35%) against *F. equiseti* and *A. alternata*, and 4 (34%) against *M. phaseolina* were reasonably high, though not as high as the standard antibiotic, nystatin. Antifungal activity of our test chemicals are in accordance with the results we observed before [13, 16].

Table 3: Antifungal activities of the test chemicals & nystatin.

Compound	% Inhibition of fungal mycelial growtha (100 µg (dw)/mL medium)		
	F. equiseti	***A. alternata***	***M. phaseolina***
2	*31	30	20
3	28	30	*32
4	25	15	*34
5	*35	*35	18
6	NF	NF	23
7	25	22	*40
8	NF	17	22
9	16	25	NF
10	NF	15	NF
11	*40	19	NF
12	*32	21	25
**Nystatin	*44	*51	*71

N.B: '*' = marked inhibition, '**' = standard antibiotic, 'NF' = not found, 'dw' = dry weight,
[a]growth measured-radial growth in cm.

Our synthesized and reported compounds (2-12) have not been tested before against the selected bacterial and fungal phytopathogens. This is the first report regarding the effectiveness of the selected chemicals against the selected pathogens. The results of the present investigation showed that some of the newly developed compounds may be tested against a wide range of phytopathogenic fungi and bacterial microorganisms.

4. CONCLUSIONS

All acylation products were found to have a long shelf life and may further be utilized as probable starting materials for the synthesis of newer derivatives. Methyl 3,4-di-*O*-(3-chlorobenzoyl)-2,6-di-*O*-(4-methoxybenzoyl)-α-D-glucopyranoside (6) and Methyl 2,6-di-*O*-(4-methoxybenzoyl)-3,4-di-*O*-(4-nitrobenzoyl)-α-D-glucopyranoside (9) were found to be encouraging in terms of high selectivity and excellent yields as 93% and 92%, respectively. The antimicrobial screening data indicated the tested compounds to possess promising biological activities and can be used as good source of antimicrobial agents at least in the field of agriculture.

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