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A SYNTHETIC APPROACH OF D-GLUCOSE DERIVATIVES: SPECTRAL CHARACTERIZATION AND ANTIMICROBIAL STUDIES

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Abstract. A new series of methyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside derivatives was synthesized using the direct acylation method. Methyl-α-D-glucopyranoside was selectively converted to methyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside by the reaction with 4-methoxybenzaldehyde dimethylacetal in a reasonable yield. Using a wide variety of acylating agents, a series of 2,3-di-*O*-acyl derivatives of this product was also prepared in order to gather additional information for structure elucidation. The structures of the newly synthesized compounds were elucidated by their spectral and elemental analysis. All synthesized compounds were screened for *in vitro* antimicrobial activities against ten human pathogenic bacteria and four plant pathogenic fungi. Encouragingly, a number of test compounds showed better antimicrobial activity than the standard antibiotics employed. It observed that the test compounds were more effective against fungal phytopathogens than those of the bacterial organisms.

Keywords: synthesis, D-glucose, antibacterial, antifungal, inhibition, spectroscopy.

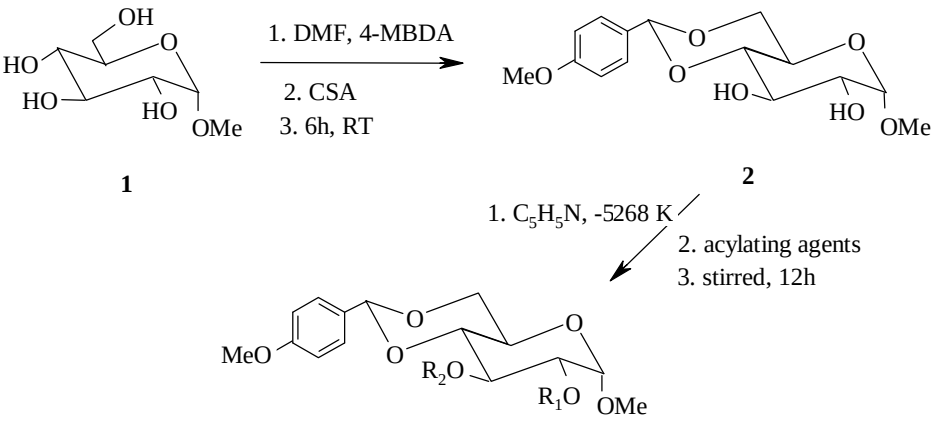
1. Introduction

The study of carbohydrates is exhilarating in organic chemistry which attracts much attention in various newer fields of application by their chemical modification. So far considerable works were performed in the field of screening studies of chemical compounds [1]. Carbohydrates, especially acylated glycoses and glycosides, are important due to their effective biological activity [2-5].

A considerable number of heterocyclic compounds is known to be bioactive. They display antibacterial [6], antitumor [7] and antimicrobial [8] herbicidal [9] activities. Compounds having amino acid and sulfonamide moieties are also known to possess a wide range of antibacterial and antifungal activities [10].

Literature survey revealed that a large number of biologically active compounds possess aromatic and heteroaromatic nuclei [6, 11]. It was found that nitrogen, sulphur and halogens-containing heterocyclic compounds showed the marked antimicrobial activities [12, 13]. When the heterocyclic part becomes attached to carbohydrates, their efficiency to inhibit bacteria or fungus sharply increased. It is also known that if an active nucleus is linked to another active nucleus, the resulting molecule may possess a greater potential for biological activity [6]. Detailed studies on selective acylation of monosaccharide derivatives [14, 15] and evaluation of their antimicrobial activities [16, 17] revealed that in many cases, the combination of two or more acyl groups enhanced the biological activity much over their parent nuclei.

So, the selective acylation is one of the most important and fundamental methods for protection of hydroxyl groups in carbohydrate and nucleoside chemistry. Protection of a particular functional group of carbohydrates, especially monosaccharides, is not only necessary for modification of the remaining functional groups but also for synthesis of newer derivatives of great importance [18]. Various methods for acylation of carbohydrates and nucleosides have so far been developed and employed successfully [19-22]. Of these, the direct method is considered as one of the most effective for selective acylation of carbohydrates [23].

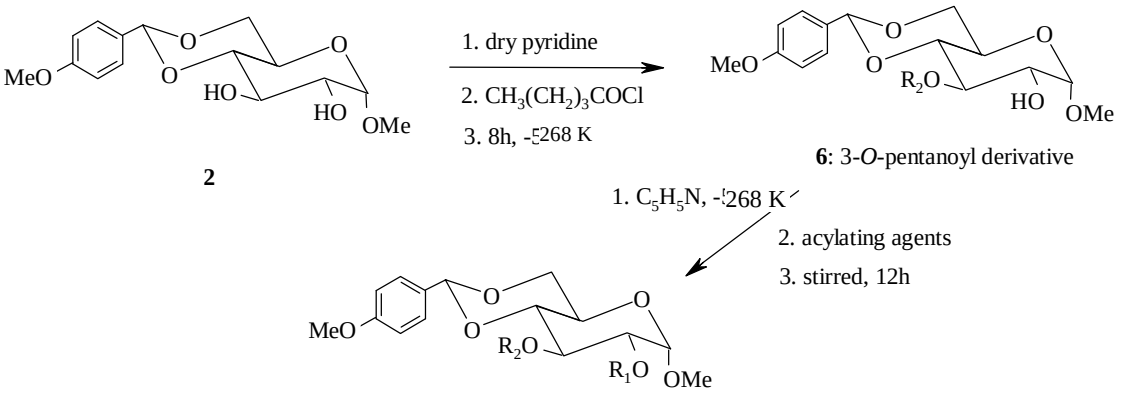


1 **2**

Acylated products

Compounds	R_1	R_2
3	$(CH_3CO)O$-	$(CH_3CO)O$-
4	CH_3OSO-	CH_3OSO-
5	C_6H_5CO-	C_6H_5CO-

Scheme 1. Conversion of the compound **2** into diacyclic derivatives **3–5**: dimethylformamide (DMF); 4-methoxybenzaldehyde dimethyl acetal (4-MBDA) and camphor-10-sulphonic acid (CSA)



2

6: 3-*O*-pentanoyl derivative

Acylated products

Compounds	R_1	R_2	Compounds	R_1	R_2
7	$(CH_3CO)O$-	$CH_3(CH_2)_3CO$-	**11**	-OC–Cl	$CH_3(CH_2)_3CO$-
8	CH_3OSO-	$CH_3(CH_2)_3CO$-	**12**	-OC–NO_2	$CH_3(CH_2)_3CO$-
9	Cl_3CCO-	$CH_3(CH_2)_3CO$-			
10	-OC–(Cl)	$CH_3(CH_2)_3CO$-	**13**	-OC–(NO_2, NO_2)	$CH_3(CH_2)_3CO$-

Scheme 2. Conversion of the compound **2** into the monocyclic derivative **6** and the following synthesis of disubstituted compounds **7–13**

As a continuation of a research project on selective acylation and antimicrobial activities of carbohydrates guided by some positive results observed in this field [24], we deliberately synthesized a number of D-glucose derivatives (Schemes 1 and 2) containing various groups such as acetyl, benzoyl, *etc*. and tested their antimicrobial activities for the first time.

2. Experimental

2.1. Reagents and Instruments

Column chromatography was performed with a silica gel G_{60} and thin layer chromatography (t.l.c.) was performed on Kieselgel GF_{254} and spots were detected by spraying the plates with 1 % H_2SO_4. ^{1}H NMR spectra (300 MHz) recorded the solution in deuteriochloroform (internal Me_4Si) with a Bruker DPX-40C spectrometer. The reagents used were commercially available (Aldrich) and were used as received, unless otherwise specified. Melting points were determined on an electro-thermal melting point apparatus and are uncorrected. Evaporations were conducted under reduced pressure using VV-1 type vacuum rotary evaporator (Germany) with a water bath at the appropriate temperature.

2.2. Synthesis

2.2.1. Procedure for synthesis of methyl 4,6-***O***-(4-methoxybenzylidene)-a-D-glucopyranoside 2

A solution of methyl α-D-glucopyranoside **1** (5.0 g, 27.78 mmol) in dry dimethylformamide (DMF; 30 ml) was treated with 4-methoxybenzaldehyde dimethylacetal (4-MBDA; 5 ml) and camphor-10-sulphonic acid (CSA; 100 mg) and the mixture was heated and stirred at room temperature for 6 h. After cooling to room temperature, the mixture was neutralized with triethyl amine (Et_3N), diluted with ethyl acetate, washed with saturated $NaHCO_3$ and brine and dried over Na_2SO_4. The progress of the reaction was monitored by t.l.c. (ethyl acetate-hexane, 1:3) and the solvent was then removed. The residue was purified by a passage through a silica gel column with ethy1 acetate-hexane (1:3) as an eluant to afford the compound **2**.

Methyl 4,6-O-(4-methoxybenzylidene)-a-D-glucopyranoside **2.** Yield 78 %. Mp 383 K (decomp.). R_f = 0.51 (EtOAc:C_6H_{14}, 1:3). Found: C 57.63; H 6.45. $C_{15}H_{20}O_7$. Calc.: C 57.69; H 6.44. ^{1}H NMR ($CDCl_3$, 300 MHz) *d* (ppm): 7.40 (2H, d, J = 8.4 Hz, Ar-H), 6.88 (2H, d, J = 8.8 Hz, Ar-H), 5.50 (1H, s, 4-OCH_3.C_6H_4CH-), 4.71 (1H, d, J = 4.0 Hz, H-1), 4.18 (1H, m, H-5), 3.78 (1H, t, J = 9.6 Hz, H-3), 3.77 (3H, s, 4-$OCH_3C_6H_4$CH-), 3.71 (2H, m, H-6a and H-4), 3.50 (1H, dd, J = 4.0 and 9.6 Hz, H-2), 3.42 (3H, s, 1-OCH_3), 3.30 (1H, t, J = 10.2 Hz, H-6b).

2.2.2. General procedure for synthesis of 2,3-di-***O***-substituted compounds 3–5

A solution of 2, 3-diol **2** (300 mg, 0.96 mmol) in anhydrous pyridine (5 ml) was cooled to 268 K when acetic anhydride (1.0 ml) was added. The solution was stirred at this temperature for 3 h and at room temperature overnight. T.l.c. (ethyl acetate-hexane, 1:2) showed the completion of the reaction with the formation of one faster-moving product. The solution was poured into the ice water under constant stirring. It was then extracted with chloroform (3×10 ml). The combined chloroform layer was washed successively with a dilute hydrochloric acid, saturated aqueous sodium hydrogen carbonate solution and distilled water. The organic layer was dried (Na_2SO_4), filtered and concentrated. The organic layer was dried ($MgSO_4$), filtered and the filtrate was evaporated off. The resulting syrup was purified by a column chromatography (with ethyl acetate-hexane 1:2, as eluant) to afford diacetate **3**. Similar reaction and purification procedure were applied to prepare dimesylate compound **4** (using methanesulphonyl chloride, 0.5 ml) and dibenzoate compound **5** (using benzoyl chloride, 1.0 ml).

Methyl 2,3-di-O-acetyl-4,6-O-(4-methoxybenzylidene)-a-D-glucopyranoside **3.** Yield 95 %. Mp 359 K (decomp.). R_f = 0.52 (EtOAc:C_6H_{14}, 1:2). Found: C 57.94; H 6.18. $C_{19}H_{24}O_9$. Calc.: C 57.61; H 6.09. ^{1}H NMR ($CDCl_3$, 300 MHz): *d* (ppm): 7.34 (2H, d, J = 8.4Hz, Ar-H), 6.85 (2H, d, J = 8.8 Hz, Ar-H), 5.56 (1H, t, J = 10.0 Hz, H-3), 5.49 (1H, s, 4-OCH_3.C_6H_4CH-), 4.91 (1H, d, J = 3.6 Hz, H-1), 4.89 (1H, dd, J = 3.6 and 10.0 Hz, H-2), 4.26 (1H, m, H-5), 3.93, (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 3.77 (3H, s, 4-$OCH_3C_6H_4$CH-), 3.74 (1H, t, J = 10.2 Hz, H-6b), 3.60 (1H, t, J = 9.9 Hz, H-4), 3.39 (3H, s, 1-OCH_3), 2.07, 2.02 (2·3H, 2s, $2CH_3$CO-).

Methyl 2,3-di-O-methanesulphonyl-4,6-O-(4-methoxybenzylidene)-a-D-glucopyranoside **4**. Yield 90 %) as a syrup which resisted crystallization. R_f = 0.50 (EtOAc:C_6H_{14}, 1:1). Found: C 69.88; H 8.43. $C_{17}H_{24}O_{11}S_2$. Calc.: C 69.83; H 8.26. ^{1}H NMR ($CDCl_3$, 300 MHz): *d* (ppm): 7.33 (2H, d, J = 8.8 Hz, Ar-H), 6.85 (2H, d, J = 8.8Hz, Ar-H), 5.47 (1H, s, 4-$OCH_3C_6H_4$C*H*-), 5.03 (1H, t, J = 9.6 Hz, H-3), 4.99 (1H, d, J = 3.6 Hz, H-1), 4.59 (1H, dd, J = 3.6 and 9.6Hz, H-2), 4.28 (1H, dd, J = 4.8 and 10.4 Hz, H-6a), 3.88 (1H, m, H-5), 3.77 (3H, s, 4-$OCH_3C_6H_4$CH-), 3.71 (2H, m, H-4 and H-6b), 3.45 (3H, s, 1-OCH_3), 3.13, 2.93 (2·3H, 2s, $2CH_3SO_2$-).

Methyl 2,3-di-O-benzoyl-4,6-O-(4-methoxybenzylidene)-a-D-glucopyranoside **5.** Yield 92 %. Mp 369 K.

R_f = 0.51 (EtOAc:C_6H_{14}, 1:2). Found: C 61.86; H 5.48. $C_{27}H_{28}O_{11}$. Calc.: C 61.36; H 5.33. ^{1}H NMR ($CDCl_3$, 300 MHz) *d* (ppm): 7.97 (3H, m, Ar-H), 7.49 (2H, m, Ar-H), 7.41 (3H, m, Ar-H), 7.34 (3H, m, Ar-H), 6.83 (3H, m, Ar-H), 6.04 (1H, t, J = 10.0 Hz, H-3), 5.52 (1H, s, 4-$OCH_3C_6H_4CH$-), 5.23 (1H, dd, J = 3.6 and 9.8 Hz, H-2), 5.17 (1H, d, J = 3.6 Hz, H-1), 4.35 (1H, dd, J = 4.8 and 10.4 Hz, H-6a), 4.06 (1H, m, H-5a), 3.88 (1H, t, J = 10.4 Hz, H-6b), 3.83 (1H, t, J = 9.8 Hz, H-4), 3.78 (3H, s, 4-$OCH_3C_6H_4CH$-), 3.45 (3H, s, 1-OCH_3).

2.2.3. Synthesis of methyl 4,6-*O*-(4-methoxybenzylidene)-3-*O*-pentanoyl-α-D-glucopyranoside 6

A solution of methyl 4,6-(4-methoxybenzylidene)-α-D-glucopyranoside **2** (1000 mg, 3.20 mmol) in dry pyridine (5 ml) was cooled to 268 K and treated with pentanoyl chloride (0.45 ml, 0.90 mmol). It was stirred at low temperature for 8 h and then allowed to stand overnight at room temperature. The progress of the reaction was monitored by t.l.c. A few pieces of ice were then added to the reaction flask with constant shaking and were extracted with $CHCl_3$ (3×15 ml). The combined $CHCl_3$ layer was washed successively with dil. HCl (10 %), saturated aq. $NaHCO_3$ and finally with distilled H_2O. The $CHCl_3$ layer was dried by $MgSO_4$, filtered and the filtrate was evaporated under reduced pressure to leave a syrupy mass, which was passed through a silica gel column and eluted with EtOAc-C_6H_{14} furnished compound **6**.

Methyl 4,6-O-(4-methoxybenzylidene)-3-O-pentanoyl-a-D-glucopyranoside **6.** Yield 42 % as yellow syrup which resisted crystallization. R_f = 0.50 (EtOAc:C_6H_{14}, 1:1). Found: C 60.72; H 7.48. $C_{20}H_{28}O_8$. Calc.: C 60.61; H, 7.11. ^{1}H NMR ($CDCl_3$, 300 MHz) *d* (ppm): 7.84 (2H, d, J = 8.7 Hz, Ar-H), 6.95 (2H, d, J = 8.6 Hz, Ar-H), 5.49 (1H, t, J = 9.7 Hz, H-3), 5.44 (1H, s, 4-$OCH_3.C_6H_4.CH$-), 4.96 (1H, d, J = 3.6 Hz, H-1), 4.20 (1H, dd, J = 4.7 and 10.1 Hz, H-6a), 4.12 (1H, dd, J = 3.6 and 9.6 Hz, H-2), 4.07 (1H, t, J = 10.1 Hz, H-6b), 4.02 (1H, t, J = 9.7 Hz, H-4), 3.97 (1H, m, H-5), 3.81 (3H, s, 4-$OCH_3C_6H_4CH$-), 3.42 (3H, s, 1-OCH_3), 2.28 {2H, m, $CH_3(CH_2)_2CH_2CO$-}, 1.62 (2H, m, $CH_3CH_2CH_2CH_2CO$-), 1.33 {2H, m, $CH_3CH_2(CH_2)_2CO$-}, 0.91 {3H, t, J = 7.5 Hz, $CH_3(CH_2)_3CO$-}.

2.2.4. General procedure for synthesis of 3-*O*-pentanoyl derivatives 7−13

A solution of the compound **6** (50 mg, 0.13 mmol) in dry pyridine (4 ml) was cooled to 268 K when acetic anhydride (0.04 ml, 0.4 mmol) was added. The solution was stirred at this temperature for 6 h and then allowed to stand at room temperature for 12 h. The progress of the reaction was checked by t.l.c. (ethyl acetate-cyclohexane), which indicated full conversion of the starting material into a single product. Excess reagent was destroyed by the addition of a few pieces of ice and the reaction mixture was extracted with chloroform (3×8 ml). The combined chloroform layer was washed successively with dilute hydrochloric acid, saturated aqueous sodium hydrogen carbonate solution and water. The organic layer was dried ($MgSO_4$), filtered and the filtrate was evaporated off. The resulting syrup was purified by the column chromatography (with ethyl acetate-cyclohexane as eluant) to afford the monoacetate **7**. Similar reaction and purification procedure were used to isolate compounds **8**–**13**.

Methyl 2-O-acetyl-4,6-O-(4-methoxybenzylidene)-3-O-pentanoyl-a-D-glucopyranoside **7.** Yield 82 % as a pasty mass, which resisted crystallization. R_f = 0.51 (EtOAc:C_6H_{12}, 1:4). Found: C 60.41; H 7.01. $C_{22}H_{30}O_9$. Calc.: C 60.26; H 6.91. ^{1}H NMR ($CDCl_3$, 300 MHz) *d* (ppm): 7.82 (2H, d, J = 8.2 Hz, Ar-H), 6.99 (2H, d, J = 8.6 Hz, Ar-H), 5.47 (1H, t, J = 9.6 Hz, H-3), 5.42 (1H, s, 4-$OCH_3C_6H_4CH$-), 5.03 (1H, dd, J = 3.6 and 9.6 Hz, H-2), 4.98 (1H, d, J = 3.6 Hz, H-1), 4.23 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 4.19 (1H, t, J = 10.2 Hz, H-6b), 4.09 (1H, t, J = 9.6 Hz, H-4), 3.86 (3H, s, 4-$OCH_3C_6H_4CH$-), 3.38 (3H, s, 1-OCH_3), 2.30 {2H, m, $CH_3(CH_2)_2CH_2CO$-}, 2.08 (3H, s, CH_3CO-), 1.60 (2H, m, $CH_3CH_2CH_2CH_2CO$-), 1.32 {2H, m, $CH_3CH_2(CH_2)_2CO$-}, 0.90 {3H, t, J = 7.3 Hz, $CH_3(CH_2)_3CO$-}.

Methyl 2-O-methanesulphonyl-4,6-O-(4-methoxybenzylidene)-3-O-pentanoyl-a-D-glucopyranoside **8.** Yield 72 % as a homogeneous syrup, which resisted crystallization. R_f = 0.50 (EtOAc:C_6H_{12}, 1:3). Found: C 53.35; H 6.41. $C_{21}H_{30}SO_{10}$. Calc.: C 53.16; H 6.36. ^{1}H NMR ($CDCl_3$, 300 MHz) *d* (ppm): 7.78 (2H, d, J = 8.6 Hz, Ar-H), 6.96 (2H, d, J = 8.6 Hz, Ar-H), 5.28 (1H, s, 4-$OCH_3C_6H_4CH$-), 5.10 (1H, t, J = 9.2 Hz, H-3), 4.93 (1H, d, J = 3.6 Hz, H-1), 4.88 (1H, dd, J = 3.6 and 9.3 Hz, H-2), 4.80 (1H, t, J = 10.1 Hz, H-6b), 4.35 (1H, dd, J = 4.8 and 10.1 Hz, H-6a), 4.31 (1H, t, J = 9.3 Hz, H-4), 3.97 (1H, m, H-5), 3.78 (3H, s, 4-$OCH_3C_6H_4CH$-), 3.37 (3H, s, 1-OCH_3), 3.14 (3H, s, CH_3SO_2-), 2.37 {2H, m, $CH_3(CH_2)_2CH_2CO$-}, 1.61 (2H, m, $CH_3CH_2CH_2CH_2CO$-), 1.35 {2H, m, $CH_3CH_2(CH_2)_2CO$-}, 0.90 {3H, t, J = 7.3 Hz, $CH_3(CH_2)_2CO$-}.

Methyl 4,6-O-(4-methoxybenzylidene)-3-O-pentanoyl-2-O-trichloroacetyl-a-D-glucopyranoside **9.** Yield 96 % as a yellow color syrup, which resisted crystallization. R_f = 0.51 (EtOAc:C_6H_{12}, 1:6). Found: C 48.74; H 5.29. $C_{22}H_{28}O_9Cl_3$. Calc.: C 48.66; H 5.18. ^{1}H NMR ($CDCl_3$, 300 MHz) *d* (ppm): 7.85 (2H, d, J = 8.7 Hz, Ar-H), 6.99 (2H, d, J = 8.6 Hz, Ar-H), 5.72 (1H, t, J = 9.6 Hz, H-3), 5.45 (1H, s, 4-$OCH_3C_6H_4CH$-),

5.28 (1H, dd, J = 3.6 and 9.6 Hz, H-2), 5.10 (1H, d, J = 3.6 Hz, H-1), 4.45 (1H, dd, J = 4.7 and 10.2 Hz, H-6a), 4.33 (1H, t, J = 10.2 Hz, H-6b), 4.10 (1H, t, J = 9.7 Hz, H-4), 3.99 (1H, m, H-5), 3.77 (3H, s, 4-$OCH_3C_6H_4CH$-), 3.44 (3H, s, 1-OCH_3), 2.34 {2H, m, $CH_3(CH_2)_2CH_2CO$-}, 1.59 (2H, m, $CH_3CH_2CH_2CH_2CO$-), 1.33 {2H, m, $CH_3CH_2(CH_2)_2CO$-}, 0.91 {3H, t, J = 7.3 Hz, $CH_3(CH_2)_3CO$-}.

Methyl 2-O-(3-chlorobenzoyl)-4,6-O-(4-methoxybenzylidene)-3-O-pentanoyl-α-D-glucopyranoside **10.** Yield 60 % as the light yellow color pasty mass, which resisted crystallization. R_f = 0.50 (EtOAc:C_6H_{12}, 1:5). Found: C 60.65; H 5.92. $C_{27}H_{31}O_9Cl$. Calc.: C 60.61; H 5.83. ^{1}H NMR ($CDCl_3$, 300 MHz) *d* (ppm): 7.87 (2H, m, Ar-H), 7.82 (2H, d, J = 8.6 Hz, Ar-H), 7.76 (1H, s, Ar-H), 7.42 (1H, d, J = 7.8 Hz, Ar-H), 6.98 (2H, d, J = 8.6 Hz, Ar-H), 5.90 (1H, t, J = 9.7 Hz, H-3), 5.45 (1H, s, 4-$OCH_3C_6H_4CH$-), 5.13 (1H, dd, J = 3.6 and 9.6 Hz, H-2), 5.02 (1H, d, J = 3.6 Hz, H-1), 4.37 (2H, m, H-6a and H-6b), 4.22 (1H, t, J = 9.6 Hz, H-4), 3.98 (1H, m, H-5), 3.79 (3H, s, 4-OCH_3.C_6H_4CH-), 3.47 (3H, s, 1-OCH_3), 2.35 {2H, m, $CH_3(CH_2)_2CH_2CO$-}, 1.60 (2H, m, $CH_3CH_2CH_2CH_2CO$-), 1.33 {2H, m, $CH_3CH_2(CH_2)_2CO$-}, 0.89 {3H, t, J = 7.4 Hz, $CH_3(CH_2)_3CO$-}.

Methyl 2-O-(4-chlorobenzoyl)-4,6-O-(4-methoxybenzylidene)-3-O-pentanoyl-α-D-glucopyranoside **11.** Yield 71 % as the pasty mass, which resisted crystallization. R_f = 0.51 (EtOAc:C_6H_{12}, 1:6). Found: C 60.72; H 5.89. $C_{27}H_{31}O_9Cl$. Calc.: C 60.61; H 5.83. ^{1}H NMR ($CDCl_3$, 300 MHz) *d* (ppm): 7.91 (2H, d, J = 8.6 Hz, Ar-H), 7.43 (2H, d, J = 8.2 Hz, Ar-H), 7.30 (2H, d, J = 8.1 Hz, Ar-H), 6.96 (2H, d, J = 8.6 Hz, Ar-H), 5.88 (1H, t, J = 9.6 Hz, H-3), 5.52 (1H, dd, J = 3.6 and 9.6 Hz, H-2), 5.42 (1H, s, 4-$OCH_3C_6H_4CH$-), 5.10 (1H, d, J = 3.6 Hz, H-1), 4.42 (1H, dd, J = 4.9 and 10.3 Hz, H-6a), 4.31 (1H, t, J = 10.2 Hz, H-6b), 4.18 (1H, t, J = 9.6 Hz, H-4), 4.01 (1H, m, H-5), 3.81 (3H, s, 4-$OCH_3C_6H_4CH$-), 3.45 (3H, s, 1-OCH_3), 2.34 {2H, m, $CH_3(CH_2)_2CH_2CO$-}, 1.62 (2H, m, $CH_3CH_2CH_2CH_2CO$-), 1.30 {2H, m, $CH_3CH_2(CH_2)_2CO$-}, 0.90 {3H, t, J = 7.3 Hz, $CH_3(CH_2)_3CO$-}.

Methyl 4,6-O-(4-methoxybenzylidene)-2-O-(4-nitrobenzoyl)-3-O-pentanoyl-α-D-glucopyranoside **12.** Yield 64 % as the semi-solid mass, which resisted crystallization. R_f = 0.52 (EtOAc:C_6H_{12}, 1:4). Found: C 59.55; H 5.81. $C_{27}H_{31}O_{11}N$. Calc.: C 59.44; H 5.72. ^{1}H NMR ($CDCl_3$, 300 MHz) *d* (ppm): 8.25 (2H, d, J = 8.7 Hz, Ar-H), 8.22 (2H, d, J = 8.8 Hz, Ar-H), 7.90 (2H, d, J = 8.6 Hz, Ar-H), 6.97 (2H, d, J = 8.6 Hz, Ar-H), 5.65 (1H, t, J = 9.8 Hz, H-3), 5.52 (1H, s, 4-$OCH_3C_6H_4CH$-), 5.32 (1H, dd, J = 3.6 and 9.7 Hz, H-2), 5.02 (1H, d, J = 3.6 Hz, H-1), 4.38 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 4.25 (1H, t, J = 10.2 Hz, H-6b), 4.13 (1H, t, J = 9.6 Hz, H-4), 3.99 (1H, m, H-5), 3.75 (3H, s, 4-$OCH_3C_6H_4CH$-), 3.47 (3H, s, 1-OCH_3), 2.28 {2H, m, $CH_3(CH_2)_2CH_2CO$-}, 1.59 (2H, m, $CH_3CH_2CH_2CH_2CO$-), 1.19 {2H, m, $CH_3CH_2(CH_2)_2CO$-}, 0.92 {3H, t, J = 7.4 Hz, $CH_3(CH_2)_3CO$-}.

Methyl 2-O-(3,5-dinitrobenzoyl)-4,6-O-(4-methoxybenzylidene)-3-O-pentanoyl-α-D-glucopyranoside **13.** Yield 60 % as the yellow pasty mass, which resisted crystallization. R_f = 0.51 (EtOAc:C_6H_{12}, 1:4). Found: C 55.15; H 5.21. $C_{27}H_{30}O_{13}N_2$. Calc.: C 55.01; H 5.11. ^{1}H NMR ($CDCl_3$, 300 MHz) *d* (ppm): 9.78, 9.18, 9.10 (3·1H, 3s, Ar-H), 7.91 (2H, d, J = 8.7 Hz, Ar-H), 6.92 (2H, d, J = 8.6 Hz, Ar-H), 5.72 (1H, t, J = 9.7 Hz, H-3), 5.44 (1H, s, 4-$OCH_3C_6H_4CH$-), 5.18 (1H, dd, J = 3.6 and 9.7 Hz, H-2), 5.03 (1H, d, J = 3.6 Hz, H-1), 4.45 (1H, dd, J = 4.9 and 10.1 Hz, H-6a), 4.31 (1H, t, J= 10.2 Hz, H-6b), 4.10 (1H, t, J = 9.6 Hz, H-4), 4.05 (1H, m, H-5), 3.73 (3H, s, 4-$OCH_3C_6H_4CH$-), 3.43 (3H, s, 1-OCH_3), 2.32 {2H, m, $CH_3(CH_2)_2CH_2CO$-}, 1.64 (2H, m, $CH_3CH_2CH_2CH_2CO$-), 1.35 {2H, m, $CH_3CH_2(CH_2)_2CO$-}, 0.91 {3H, t, J = 7.3 Hz, $CH_3(CH_2)_3CO$-}.

2.3. Antimicrobial Studies

2.3.1. Bacterial and fungal cultures

The antibacterial activities of synthesized compounds, as shown in the scheme, were determined *in vitro* against ten pathogenic microorganisms *viz Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* BTCC 17, *Bacillus megaterium* BTCC 18, *Bacillus cereus* BTCC 19, *Shigella dysenteriae* AE 14396, *Escherichia coli* ATCC 25922, *Salmonella typhi* AE 14612, *Salmonella paratyphi* AE 146313, *Pseudomonas* species CRL (ICDDRB), *Vibrio choleare* INABAET. Antifungal activities of the same compounds were also studied against four fungi *viz Fusarim equiseti* (*corda*) *Sacc.*, *Colletotrichum corchori* (Ikata Yoshida), *Curvularia lunata* (Wakker Becdijin) and *Alternaria alternata* (Fr.) Kedissler.

2.3.2. Evaluation of antibacterial activity

In vitro antibacterial activities of the synthesized compounds were detected by the disc diffusion method [25, 26]. 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 318 K, was poured into sterilized Petri dishes to the depth of 3–4 mm and after solidification of the agar medium; the plates were transferred to an incubator at 310 K for 15–20 min 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 a spread plate method and allowed to dry for 3–5 min. 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 the 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 chemical test. These plates were kept for 4–6 h at low temperature (277–279 K) and the test chemicals diffused from disc to the surrounding medium by this time. The plates were then incubated at 308 ± 2 K for 24 h to allow maximum growth of the organisms. The antibacterial activity of the test agent was determined by measuring the mean diameter of inhibitions zone in millimeters. Each experiment was repeated thrice. All the results were compared with the standard antibacterial antibiotic ampicillin (20 µg/disc, BEXIMCO Pharm. Bangladesh Ltd).

2.3.3. Evaluation of antifungal growth inhibition

In vitro antifungal functionality tests of the synthesized compounds were performed by the mycelial growth tests which were based on "food poisoned" technique [27]. 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. In the center of each plate, a 5-day-old mycelium colonies (4 mm in diameter) were inoculated and incubated at 300 K. 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:

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

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, BEXIMCO Pharm. Bangladesh Ltd).

3. Results and Discussion

3.1. Synthesis and Characterization

The main aim of the present work involves regioselective acylation of methyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside **2** and synthesis of some newer derivatives (**3**–**13**). A number of acylating agents such as acetic anhydride, methanesulphonyl chloride, trichloroacetyl chloride, 3-chlorobenzoyl chloride, 4-chlorobenzoyl chloride, 4-nitrobenzoyl chloride and 3,5-dinitrobenzoyl chloride was employed for this purpose. Methyl α-D-glucopyranoside **1** was treated with 4-methoxybenzaldehyde dimethylacetal and camphor-10-sulphonic acid as a catalyst in dry DMF at 323 K and after usual work-up, compound **2** was obtained in high yields as a crystalline solid. The structure of compound **2** was confirmed by interpretation of ^{1}H NMR spectrum and further ascertained by its conversion to the acetyl derivative **3**. Thus, conventional actylation of compound **2** with acetic anhydride in pyridine furnished the acetyl derivative **3**. In its ^{1}H NMR spectrum, two three-proton singlets at d = 2.07 and d = 2.02 corresponded to two acetyl groups in the molecule. Also, C-2 proton deshielded to d = 4.89 (as dd, J = 3.6 and 10.0 Hz) and C-3 proton also deshielded to d = 5.56 (as t, J = 10.0 Hz), as compared with the starting diol **1** (d = 3.50, dd, J = 4.0 and 9.6 Hz, H-2 ; d = 3.78, t, J = 9.6 Hz, H-3), thus conforming that the acetyl groups were introduced at positions 2 and 3. Complete analysis of ^{1}H NMR spectrum led us to deduce its structure as methyl 2,3-di-*O*-acetyl-4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside **3**. The benzylidene derivative **2**, when treating with methanesulphonyl chloride in pyridine, yielded the mesylate **4**. In its ^{1}H NMR, the two three-proton singlets at d = 3.13 and d = 2.93 were due to the methyl protons of two mesyloxy groups. The deshielding of C-2 and C-3 protons to d = 4.59 (as dd, J = 3.6 and 9.6 Hz, C-2) and d = 5.03 (as t, J = 9.6 Hz, H-3) from their values in the precursor diol **2**, indicates the introduction of the mesyloxy groups at positions 2 and 3. The diol **2** was then converted to the benzoate **5** by the direct method using benzoyl chloride in pyridine. ^{1}H NMR spectrum of this compound was compatible with the structure assigned as methyl 2,3-di-*O*-benzoyl-4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside **5**.

4-Methoxybenzylidene derivative **2** was then allowed to react with a unimolecular amount of pentanoyl chloride in pyridine and provided compound **6**. ^{1}H NMR of compound **6** displayed three two-proton multiplets at d = 2.28, 1.62 and 1.33 and a three-proton triplet at d = 0.91 (J = 7.5 Hz), which were due to the presence of one pentanoyl group in the molecule. The attachment of the pentanoyl group at position 3 was supported by observation of C-3 proton at d = 5.49 (as t, J = 9.7 Hz), deshielded from its value in the precursor diol **2** (d = 3.78, t, J = 9.6 Hz). By complete analysis of its ^{1}H NMR spectrum, the structure of this compound was assigned as methyl 4,6-*O*-(4-methoxybenzylidene)-3-*O*-pentanoyl-α-D-glucopyranoside **6**. We then employed 3-*O*-pentanoyl derivative **6** for synthesizing a series of derivatives containing a wide variety of probable biologically prone atoms/groups for conducting antibacterial and antifungal evaluation studies.

Thus, acylation of compound **6** with acetic anhydride in pyridine, afforded the acetate **7**, and in its ^{1}H NMR spectrum, the three-proton singlet at *d* = 2.08 corresponded to the methyl protons of one acetyloxy group. The deshielding of C-2 proton to *d* = 5.03 (as dd, J = 3.6 and 9.6 Hz) from its value in the precursor compound **6**, ascertained introduction of the acetyl group at position 2. Thus, the structure of the acetate was assigned as methyl 2-*O*-acetyl-4,6-*O*-(4-methoxybenzylidene)-3-*O*-pentanoyl-α-D-glucopyranoside **7**. Confirmation of the structure of compound **6** was also achieved by its conversion to and identification of the mesylate **8**. By complete analysis of ^{1}H NMR spectrum, we assigned the structure of this compound as methyl 2-*O*-methanesulphonyl-4,6-*O*-(4-methoxybenzylidene)-3-*O*-pentanoyl-α-D-glucopyranoside **8**. We then treated compound **6** with trichloroacetyl chloride in pyridine under similar reaction conditions; isolated the desired compound **9**. By complete analysis of its ^{1}H NMR spectrum and by analogy, the structure was assigned as methyl 4,6-*O*-(4-methoxybenzylidene)-3-*O*-pentanoyl-2-*O*-trichloroacetyl-α-D-glucopyranoside **9**.

Reaction of compound **6** with 3-chlorobenzoyl chloride and 4-chlorobenzoyl chloride in pyridine, followed by the usual work-up and chromatographic purification, provided 3-chlorobenzoyl derivative **10** and 4-chlorobenzoyl derivative **11**. ^{1}H NMR spectrum of this compound was in complete agreement with the structure assigned as methyl 2-*O*-(4-chlorobenzoyl)-4,6-*O*-(4-methoxybenzylidene)-3-*O*-pentanoyl-α-D-glucopyranoside **11**.

Our next effort was to react compound **6** with 4-nitrobenzoyl chloride in pyridine at freezing temperature and the targeted compound **12** was obtained. In its ^{1}H NMR, two downfield two-proton doublets at *d* = 8.25 (J = 8.7 Hz) and *d* = 8.22 (J = 8.8 Hz) corresponded to the aromatic protons of one 4-nitrobenzoyl group. The downfield shift H-2 to *d* = 5.32 (as dd, J = 3.6 and 9.7 Hz) indicated the attachment of this group at position 2. Finally we employed 3,5-dinitrobenzoyl chloride for derivatizing compound **6** using the similar reaction, and the expected 3,5-dinitrobenzoyl derivative **13** was obtained. In its ^{1}H NMR spectrum, the lowfield three one-proton singlets at *d* = 9.78, 9.18 and 9.10 corresponded to the aromatic protons of one 3,5-dinitrobenzoyl group. The resonance of C-2 proton shifted downfield as expected to *d* = 5.18 (as dd, J = 3.6 and 9.7 Hz) confirming the attachment of 3,5-dinitrobenzoyl group at position 2. The rest of the protons resonated at their anticipated positions ascertaining the structure of this compound as methyl 2-*O*-(3,5-dinitrobenzoyl)-4,6-*O*-(4-methoxybenzylidene)-3-*O*-pentanoyl-α-D-glucopyranoside **13**. The aim of preparing all the derivatives was, in some cases, to obtain supportive evidences for structure elucidation and also to obtain newer products of synthetic and biological importance.

3.2. Biological Evaluation

Antibacterial activities of the tested compounds **3–13** are presented in Tables 1 and 2. In the case of Gram-positive organisms, the trichloroacetyl derivative, **9** exhibited the highest activity (30 mm) against *Bacillus subtilis* which is higher than that of the standard antibiotic, ampicillin (19 mm). The acylated compounds **7** and **11** also showed maximum inhibition against Gram-negative bacteria *viz Pseudomonas* species (26 mm) and *Vibrio choleare* (26 mm) which are also higher than the standard antibiotic. It was evident that these compounds **3**–**13** were more active against Gram-positive organisms than that of Gram-negative organisms. *In vitro* result of % inhibition of mycelial growth in mm is listed in Table 3. It was observed from Table 3 that the methanesulphonyl derivative **4** (Fig. 1) showed the highest inhibition

Table 1

Zone of inhibition observed against Gram-positive bacteria by the test compounds

Compounds	Diameter of the inhibition zone in mm 200 µg dw/disc			
	B. subtilis	*B. cereus*	*B. megaterium*	*S. aureus*
3	8	9	10	*22
4	NF	NF	NF	NF
5	NF	NF	NF	13
6	12	*19	NF	NF
7	10	8	7	NF
8	7	13	NF	7
9	*30	11	NF	11
10	NF	NF	NF	NF
11	8	11	NF	8
12	12	8	NF	8
13	7	*22	NF	6
**Ampicillin	*19	*18	*16	*22

Table 2

Zone of inhibition observed against Gram-negative bacteria by the test compounds

Compounds	Diameter of the inhibition zone in mm 200 µg dw/disc					
	E. coli	*S. typhi*	*S. paratyphi*	*S. dysenteriae*	*P. species*	*V. cholerae*
3	NF	10	10	12	12	10
4	NF	6	NF	6	NF	NF
5	NF	8	NF	10	NF	NF
6	NF	10	8	9	NF	6
7	NF	10	NF	8	*26	6
8	NF	9	NF	NF	NF	7
9	NF	NF	NF	13	12	10
10	NF	NF	NF	NF	NF	6
11	NF	6	6	NF	NF	*26
12	NF	13	8	NF	NF	9
13	NF	10	8	NF	14	7
**Ampicillin	*10	*20	*18	*22	*20	*15

Table 3

Antifungal activities of the synthesized test compounds by the selected phytopathogens

Compound	% Inhibition of the fungal mycelial growth (100 µg (dw)/ml medium)			
	F. equiseti	*A. alternata*	*C. corchori*	*C. lunata*
3	29.54	15.38	35.70	35.00
4	*65.70	47.38	12.69	20.68
5	37.14	39.84	21.58	13.80
6	25.21	44.76	27.67	62.86
7	40.11	21.42	34.92	23.91
8	40.02	19.04	12.90	48.69
9	37.15	16.66	35.48	13.04
10	14.29	35.25	19.70	12.22
11	48.57	19.04	*48.38	30.43
12	31.43	21.05	29.03	57.78
13	31.42	47.61	*50.16	56.52
**Nystatin	*44.7	*51.55	*40.51	*75.05

Notes for Tables 1-3: * – marked inhibition; ** – standard antibiotic; NF – not found; dw – dry weight.

(65.7 %) against *Fusarim equiseti* that was higher than that of standard antibiotic – nystatin (44.7 %). Among all acylated compounds **4** (65.7 %), **11** (48.38 %) and **13** (50.16 %) showed relatively higher inhibition than the standard antibiotic. An important observation was that, these acylated derivatives (Schemes 1 and 2) were more active against fungal pathogens than that of bacterial organisms. These results are in concurrence with the results of our previous investigations [28, 29]. Our newly synthesized and reported compounds (Schemes 1 and 2) have not been tested before against the selected fungal pathogens. 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 synthesized acylated methyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside derivatives may be tested against a wide range of phytopathogenic fungi and bacteria, before sending them to pesticide producing companies for further tests.

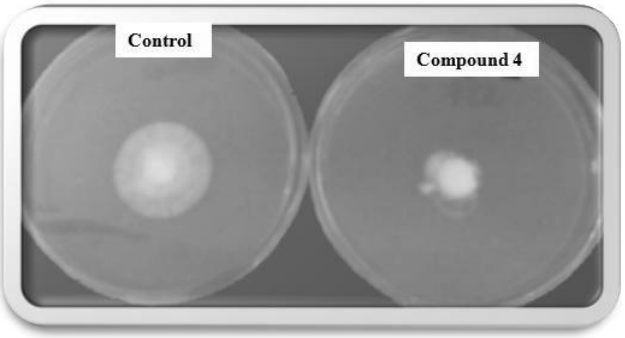


Fig. 1. Inhibition of mycelial growth of the compound **4** against *Fusirum equiseti* phytopathogen

4. Conclusions

This paper describes the synthesis of a new series of methyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside containing a wide variety of acyl substituent groups and thus, obtained acylated products had high yields under easy experimental conditions. All the compounds were subjected to biological screening. Most of compounds exhibited good to moderate activities whereas methyl 4,6-*O*-(4-methoxybenzylidene)-3-*O*-pentanoyl-2-*O*-trichloroacetyl-α-D-glucopyranoside exhibited the highest inhibition activity (30 mm) against *Bacillus subtilis*. The methanesulphonyl derivative **4** showed the highest growth inhibition (65.7 %) against *Fusarim equiseti* that was higher than that of standard antibiotic. This proves the high therapeutic value of these compounds and encourages further study to explore their biological potential.

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СИНТЕТИЧНИЙ ПІДХІД ДО ОДЕРЖАННЯ ПОХІДНИХ D-ГЛЮКОЗИ: СПЕКТРАЛЬНИЙ АНАЛІЗ ТА АНТИМІКРОБНІ ДОСЛІДЖЕННЯ

Анотація. *З використанням методу ацілування синтезовано нові похідні метил 4,6-О-(4-метоксібензіліден)-а-D-глюкопіранозиду. Показано, що метил-а-D-глюкопіранозид селективно перетворюється на метил-4,6-О-(4-метоксібензіліден)-а-D- глюкопіранозид за реакцією з 4-метоксибензальдегід диметилформамідом з достатньо непоганим виходом. Використовуючи широкий спектр ацілюючих агентів, одержано ряд 2,3-ді-О-ацильних похідних цього продукту для визначення їх структури. Структуру синтезованих сполук підтверджено методами спектрального і елементного аналізу. Визначено антимікробну діяльність* in vitro *сполук під дією десяти хвороботворних бактерій і чотирьох патогенних рослинних грибів. Показано, що синтезовані сполуки мають кращу антимікробну активність, у порівнянні з стандартними антибіотиками; тестовані сполуки є більш ефективними проти грибкових фітопатогенів, ніж бактеріальних організмів.*

Ключові слова: *синтез, D-глюкоза, антибактеріальний, протигрибковий, інгібування, спектроскопія.*