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SYNTHESIS, CHARACTERIZATION AND MICROBIAL SCREENING OF SOME NEW METHYL 4, 6-*O*-(4-METHOXYBENZYLIDENE)-α-D-GLUCOPYRANOSIDE DERIVATIVES

SARKAR M. A. KAWSAR[*], ABUL K. M. S. KABIR[†], MOHAMMAD M. R. BHUIYAN[1], JANNATUL FERDOUS AND MOHAMMAD S. RAHMAN[2]

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

ABSTRACT

Regioselective pentanoylation of methyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside by the direct acylation method provided the methyl 4,6-*O*-(4-methoxybenzylidene)-2-*O*-pentanoyl-α-D-glucopyranoside in good yield. A number of 3-*O*-acyl derivatives of this 2-*O*-pentanoylation product were also prepared in order to obtain new compounds and also gather additional information for structure elucidation. The chemical structure of the newly synthesized compounds was characterized by analytical and spectral methods. Synthesized acylated derivatives of D-glucopyranoside were screened for *in vitro* antimicrobial activities against ten human pathogenic bacteria and four plant pathogenic fungi. The study revealed that the acylated products exhibited moderate to good antimicrobial activities. It was interesting to observe that the selected compounds were more sensitive against fungal phytopathogens than those of the bacterial strains.

Key words: Synthesis, Glucopyranoside, Spectroscopic studies, Antimicrobial

INTRODUCTION

The study of carbohydrates is one of the most exciting fields in organic chemistry. Carbohydrate chemistry has witnessed a rapid evolution during the last decades of this century not only due to the increasing evidence for the importance of carbohydrates in biological systems (Dwek 1996, Varki 1993) but also due to the recognition both in public and in the scientific community, that the complex/or derivatives of carbohydrates have many beneficial effects in food, such as health. Carbohydrates isolated from plants or other sources can be acylated directly or by applying the blocking-deblocking methods (Andary *et al*. 1982, Williams *et al*. 1967). Selective acylation is very important in the field of carbohydrate and nucleoside chemistry because of its usefulness for the synthesis of biologically active products. Various methods for acylation of carbohydrates and

[*] Corresponding author: <akawsarabe@yahoo.com>.
[†] Recently deceased.
[1] Department of Chemistry, School of Science and Technology, University of New England, Armidale NSW 2351, Australia.
[2] Department of Microbiology, Faculty of Biological Science, University of Chittagong, Chittagong-4331, Bangladesh.

nucleosides have so far been developed and employed successfully (Kim *et al*. 1985, Wagner *et al*. 1974, Zhou *et al*. 2012). Of these, direct method has been found to be the most encouraging for acylation of carbohydrates and nucleosdies (Kabir *et al*. 2005).

Literature survey revealed that a large number of biologically active compounds possess aromatic and heteroaromatic nuclei (Ichinari *et al*. 1988, Gawande *et al*. 1987). It is also known that, if an active nucleus is linked to another active nucleus, the resulting molecule may possess greater potential for biological activity (Gupta *et al*. 1997). The benzene and substituted benzene nuclei play important role as common denominator of various biological activities (Singh *et al*. 1990). Results of an ongoing research work on selective acylation of carbohydrates (Kawsar *et al*. 2012), nucleosides (Kabir *et al*. 1998) and also evaluation of antimicrobial activities revealed that in many cases the combination of two or more heteroaromatic nuclei (Gupta *et al*. 1997) and acyl groups enhances the biological activity many-folds than its parent nucleus. It is also found that nitrogen (N), sulfur (S) and halogen (X) containing substituents show marked antimicrobial activities (Kawsar *et al*. 2012, Kabir *et al*. 2009, Kabir *et al*. 2008, Kabir *et al*. 2004). Encouraged by literature reports their own findings, the authors synthesized some selectively acylated derivatives of methyl α-D-glucopyranoside (**1**) containing various substituents in a single molecular framework and evaluated their antibacterial and antifungal activities using a variety of bacterial and fungal pathogens.

MATERIALS AND METHODS

Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Evaporations were performed under diminished pressure on a Buchi rotary evaporator (Germany). ^{1}H-NMR spectra (300 MHz) were recorded for solutions in deuteriochloroform ($CDCl_3$) using tetramethylsilane (TMS) as internal standard with a Bruker DPX-40C spectrometer (300 MHz) and elemental analyses were done at the Department of Chemistry, University of New England, Armidale, Australia. Thin layer chromatography (t.l.c.) was accomplished by spraying the plates with 1% H_2SO_4, followed by heating the plates at 150 - 200^{o}C until coloration took place. Column chromatography was performed with Merck silica gel G_{60}. All reagents were commercially available and were used as received unless otherwise specified.

Synthesis of methyl 4,6-O-(4-methoxybenzylidene)-α-D-glucopyranoside 2: A solution of methyl α-D-glucopyranoside (**1**) (1.0 g, 3.64 mmol in dry DMF (30 ml) was treated with 4-methoxybenzaldehyde dimethylacetal (5 ml) and camphor-10-sulphonic acid (100 mg) and the mixture was heated and stirred at. 50^{0}C for six hours. After cooling to room temperature, the mixture was neutralized with triethylamine (Et_3N), diluted with ethyl acetate, washed with saturated $NaHCO_3$ solution and brine and dried over Na_2SO_4. The progress of the reaction was monitored by t. l. c. and the solvent was then removed.

The residue was purified by passage through a silica gel column with ethy1acetate-hexane (1 : 3) as an eluant to afford compound 2.

Yield (1.02 g, 70%) as a crystalline solid, m.p. 110 - 111°C from (ethyl acetate-hexane), R_f 0.51 (EtOAc:C_6H_{14}, 1:3). Anal. calcd. for $C_{15}H_{20}O_7$: C, 57.69; H, 6.41. Found: C, 57.48; H, 7.83. ^{1}H-NMR ($CDCl_3$): δ_H 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_4.C*H*-), 4.71 (1H, d, J = 4.0 Hz, H-1), 4.18 (1H, m, H-5), 3.78, 3.76 (2×3H, 2×s, 2×4-OC*H*$_3$.C_6H_4CO-), 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-OC*H*$_3$), 3.30 (1H, t, J = 10.2 Hz, H-6b).

Synthesis of methyl 4,6-O-(4-methoxybenzylidene)-2-O-pentanoyl-α-D-glucopyranoside 3: A solution of the methyl 4,6-(4-methoxybenzylidene)-α-D-glucopyranoside (2) (1 g, 3.20 mmol) in dry pyridine (12 ml) was cooled to 0^0C and treated with pentanoyl chloride (0.45ml, 0.90 mmol). It was stirred at 0^0C temperature for five hours and then allowed to stand overnight at room temperature. The progress of the reaction was monitored by t. l. c., which indicated the formation of a major product. A few pieces of ice were added to the flask with constant shaking and the reaction mixture was extracted three times with chloroform. The combined chloroform layer was washed successively with dilute hydrochloric acid (10%), saturated aqueous sodium hydrogen carbonate ($NaHCO_3$) solution and distilled water. The organic layer was dried ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure to leave a syrupy mass. Purification of the mass by silica gel column chromatography (with ethyl acetate-hexane, 1 : 3 as eluant) furnished the 2-*O*-pentanoyl derivative (3).

Yield (45 mg, 36%) as a white crystalline solid, as needles, m.p.101 - 102°C from (ethyl acetate-hexane), R_f 0.51 (EtOAc:C_6H_{14}, 1:3). Anal. calcd. for $C_{20}H_{28}O_8$: C, 60.61; H, 7.07. Found: C, 60.98; H, 7.45. ^{1}H-NMR ($CDCl_3$): δ_H 7.40 (2H, d, J = 8.7 Hz, Ar-H), 6.88 (2H, d, J = 8.6 Hz, Ar-H), 5.49 (1H, s, 4-OCH_3.C_6H_4.C*H*-), 4.94 (1H, d, J = 3.7 Hz, H-1), 4.78 (1H, dd, J = 3.7 and 9.7 Hz, H-2), 4.26 (1H, dd, J = 4.2 and 9.6 Hz, H-6a), 4.15 (1H, t, J = 9.5 Hz, H-3), 3.79 (3H, s, 4-OC*H*$_3$.C_6H_4.CH-), 3.73 (1H, t, J = 9.8 Hz, H-4), 3.52 (1H, t, J = 9.5 Hz, H-6b), 3.38 (3H, s, 1-OC*H*$_3$), 2.39 {2H, m, $CH_3(CH_2)_2CH_2$CO-}, 1.63 (2H, m, $CH_3CH_2CH_2CH_2$CO-), 1.34 {2H, m, $CH_3CH_2(CH_2)_2$CO-}, 1.34 {2H, m, $CH_3CH_2(CH_2)_2$CO-}, 0.91 {3H, t, J = 7.3 Hz, $CH_3(CH_2)_3$CO-}.

General procedure for synthesis of 2-O-pentanoyl derivatives 4-12: A cooled (0^oC) and stirred solution of the pentanoyl derivative (3) (50 mg, 0.13 mmol) in dry pyridine (3 ml) was treated with acetic anhydride (0.04 ml, 0.40 mmol) and stirring was continued at room temperature for 18 hours when t. l. c examination showed complete conversion of the starting material into one product. Work-up, as described earlier and chromatographic purification (with ethyl acetate-cyclohexane, 1:4) yielded the acetyl derivative (4) as pasty mass. Using the similar reaction procedure, compound 3 was converted to compound 5, 6, 7, 8, 9, 10, 11 and compound 12.

Methyl 3-O-acetyl-4,6-O-(4-methoxybenzelidene)-2-O-pentanoyl-α-D-glucopyra-noside 4: Yield (40 mg, 72%) as a pasty mass, which resisted crystallization, R_f 0.51 (EtOAc:C_6H_{12}, 1 : 4). Anal. calcd. for $C_{22}H_{30}O_9$: C, 60.27; H, 6.88. Found: C, 60.47; H, 6.83.

Methyl3-O-methanesulphonyl-4,6-O-(4-methoxybenzylidene)-2-O-pentanoyl-α-D-glucopyranoside 5: Yield (50 mg, 83%) as a pasty mass, which resisted crystallization, R_f 0.52 (EtOAc:C_6H_{12}, 1 : 4). Anal. calcd. for $C_{21}H_{30}SO_{10}$: C, 53.76; H, 6.36. Found: C, 53.87; H, 6.83. *Methyl 3-O-hexanoyl-4,6-O-(4-methoxybenzylidene)-2-O-pentanoyl-α-D-glucopyranoside 6*: yield (55 mg, 88%) as a colorless semi-solid mass, which resisted crystallization, R_f 0.50 (EtOAc:C_6H_{12}, 1 : 5). Anal. calcd. for $C_{26}H_{38}O_9$: C, 63.15; H, 7.69. Found: C, 63.69; H, 7.73. *Methyl 3-O-lauroyl-4,6-O-(4-methoxybenzylidene)-2-O-pentanoyl-α-D-glucopyranoside 7*: Yield (39 mg, 47%) as a colorless syrup, R_f 0.51 (EtOAc:C_6H_{12}, 1 : 6). Anal. calcd. for $C_{32}H_{49}O_9$: C, 66.53; H, 8.53. Found: C, 66.77; H, 8.83.

Methyl 4,6-O-(4-methoxybenzylidene)-3-O-palmitoyl-2-O-pentanoyl-α-D-glucopyranoside 8: Yield (72 mg, 90%) as a needless, m. p. 62-63^{0}C (from ethyl acetate-cyclohexane), R_f 0.50 (EtOAc:C_6H_{12}, 1 : 6). Anal. calcd. for $C_{36}H_{58}O_9$: C, 68.12; H, 9.21. Found: C, 68.44; H, 9.68. *Methyl3-O-(3-chlorobenzoyl)-4,6-O-(4-methoxybenzylidene)-2-O-pentanoyl-α-D-glucopyranoside 9*: Yield (80 mg, 85%) as a homogeneous syrup, which resisted crystallization. R_f 0.52 (EtOAc:C_6H_{12}, 1 : 5). Anal. calcd. for $C_{27}H_{31}O_9Cl$: C, 60.61; H, 5.83. Found: C, 60.77; H, 6.03.

Methyl3-O-(4-chlorobenzoyl)-4,6-O-(4-methoxybenzylidene)-2-O-pentanoyl-α-D-glucopyranoside 10: Yield (90 mg, 67%) as a pasty mass, which resisted crystallization. R_f 0.51 (EtOAc:C_6H_{12}, 1 : 5). Anal. calcd. for $C_{27}H_{31}O_9Cl$: C, 60.61; H, 5.83. Found: C, 61.05; H, 5.92.

Methyl4,6-O-(4-methoxybenzylidene)-3-O-(4-nitrobenzoyl)-2-O-pentanoyl-α-D-glucopyranoside 11: Yield (75 mg, 90%) as a colorless semi-solid mass, which resisted crystallization. R_f 0.50 (EtOAc:C_6H_{12}, 1 : 5). Anal. calcd. for $C_{27}H_{31}O_{11}N$: C, 59.44; H, 5.72. Found: C, 60.04; H, 6.03. *Methyl 3-O-(3,5-dinitrobenzoyl)-4,6-O-(4-methoxybenzylidene)-2-O-pentanoyl-α-D-glucopyranoside 12*: Yield (82 mg, 55%) as a yellow pasty mass, which resisted crystallization. R_f 0.50 (EtOAc:C_6H_{12}, 1:4). Anal. calcd. for $C_{27}H_{30}O_{13}N_2$: C, 54.91; H, 5.11. Found: C, 55.07; H, 5.22.

Microbial screening studies for test bacteria and fungi: The synthesized test chemicals (3 - 12) (Fig. 1) were subjected to antibacterial screening against four Gram-positive and six Gram-negative bacterial strains used are: *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 (ICDDR,B),

Vibrio choleare INABA ET. The phytopathogenic fungi used are: *Fusarim equiseti* (*corda*) *Sacc.*, *Colletotrichum corchori* (Ikata Yoshida), *Curvularia lunata* (Wakker Becdijin) and *Alternaria alternata* (Fr.) Kedissler. In all cases, a 2% solution (in $CHCl_3$) of the chemicals was used.

Antibacterial activity assay: The *in vitro* antibacterial activities of the synthesized chemicals were detected by disc diffusion method (Bauer *et al*. 1966, Miah *et al*. 1990). Paper discs of 4 mm in diameter and glass petri plate of 90 mm in diameter were used throughout the experiment. Paper discs were sterilized in an autoclave and dried at 100^oC in an oven. Then the discs were soaked with test chemicals at the rate of 50μg (dry weight) per disc for antibacterial analysis. For pour plate technique, one drop of bacterial suspension was taken in a sterile Petri dish and approximately 20 ml of melted sterile nutrient agar (NA) (~45^oC) was poured into the plate, and then mixed thoroughly with the direction of clockwise and anticlockwise. After solidification of the seeded NA medium, paper disc after soaking with test chemicals (2% in $CHCl_3$) were placed at the centre of the inoculated Petri dish. A control plate was also maintained in each case with chloroform. Firstly, the plates were kept for 4 hrs at low temperature (4^oC) and the test chemicals diffused from disc to the surrounding medium by this time. The plates were then incubated at (35± 2^oC) for growth of test organisms and were observed at 24 hrs. intervals for two days. The activity was expressed in terms of inhibition zone diameter in mm. Each experiment was repeated thrice. The standard antibiotic ampicillin from FISONS Ltd. (Bangladesh) was used as a positive control and compared with tested chemicals under identical conditions.

Antifungal activity assay: The *in vitro* antifungal functionality tests of the synthesized chemicals were tested by mycelial growth test (Grover *et al*. 1962). Required amount of medium was taken in a conical flask separately and was sterilized in autoclave. After autoclaving, weighted amount of test chemicals (2%) was added to the sterilized medium in conical flask at the point of pouring to obtain the desired concentration. The flask was shaken thoroughly to mix the chemical with the medium homogeneously before pouring. The medium with definite concentration (2%) of chemical was poured at the rate of 10μl in sterilized glass Petri dishes individually. Proper control was maintained separately with sterilized PDA (potato dextrose agar) medium without chemicals and three replications were prepared for each treatment. After solidification of medium, the fungal inoculums (5 mm approximately) were placed at the centre of each Petri dish in an inverted position. All the plates were inoculated at room temperature on the laboratory desk for five days. The linear growth of fungal colony was measured in two directions at right angle to each other after five days of incubation and average of three replicates was taken as the diameter of a colony in mm. The percentage inhibition of mycelial growth of test fungi was calculated as follow:

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

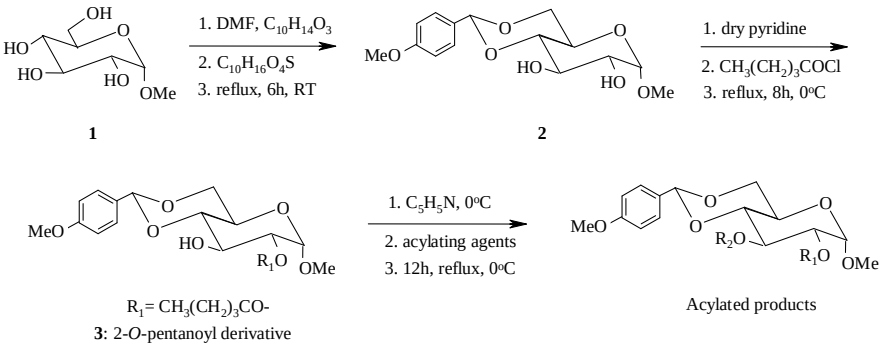
Where, I = Percentage of inhibition, C = Diameter of the fungal colony in control, T = Diameter of the fungal colony in treatment. The antifungal results were compared with that of the standard antibiotic, nystatin (100 μg dw./disc, BEXIMCO Pharm. Bangladesh Ltd.).

RESULTS AND DISCUSSION

Synthesis and characterization: In the present investigation, the researchers carried out selective acylation of methyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside (2) (Fig. 1). A series of derivatives of the resulting acylation products were prepared in order to gather supportive evidences for structure elucidation and also to obtain newer derivatives of synthetic and biological importance.

The author initial effort was to prepare the starting methyl 4, 6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside (2). Thus, treatment of methyl α-D-glucopyranoside (1) with 4-methoxybenzaldehyde dimethylacetal and camphor-10-sulphonic acid as catalyst in dry DMF at 50^0C 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 recrystallization from ethyl acetate-hexane. In its ^{1}H- NMR spectrum, the characteristic peaks at δ 7.40 (2H, d, J = 8.4 Hz), δ 6.88 (2H, d, J = 8.8 Hz), δ 5.50 (1H, s, 4-OCH$_3$.C$_6$H$_4$.C*H*-) and δ 3.77 (3H, s, 4-OC*H*$_3$.C$_6$H$_4$.CH-) indicated the introduction of one 4-methoxybenzylidene group in the molecule. The structure of compound 2 was further ascertained by its conversion to and identification of the pentanoyl derivative (3). Thus, conventional pentanoylation of compound 2 with pentanoyl chloride in pyridine, followed by usual work-up and silica gel chromatographic purification, furnished the pentanoyl derivative (326) in 36% yield as a crystalline solid, m. p. 101-102^{o}C (ethyl acetate-hexane). In the ^{1}H-NMR of compound 3, the resonance peaks at δ 2.39 (2H, m) and δ 1.63 (2H, m), δ 1.34 (2H, m) and δ 0.91 (3H, t, J = 7.3 Hz) corresponded to the presence of one pentanoyl group in the molecule. The C-2 proton shifted downfield to δ 4.78 (as dd, J = 3.7 and 9.7 Hz) from its value in the precursor diol 2 (δ 3.50, dd, J = 4.0 and 9.6 Hz) indicating the attachment of the pentanoyl group at position 2. The resonances of other protons were observed in their anticipated positions supporting the structure of the compound assigned as methyl 4,6-*O*-(4-methoxybenzylidene)-2-*O*-pentanoyl-α-D-glucopyranoside (3). We then prepared a series of derivatives of the 2-*O*-pentanoyl derivative (3) using a wide variety of acylating agents containing various probable biological prone atoms/groups. Thus, reaction of compound 3 with acetic anhydride in pyridine by direct acylation method, followed by

usual work-up and purification procedure, the acetyl derivative (4) was obtained in 72% yield as a pasty mass. In its ^{1}H-NMR spectrum (Table 1), a three-proton singlet at δ 2.01 corresponded to the methyl protons of one acetyloxy group. The C-3 proton resonated at δ 5.56 (as t, J = 9.7 Hz), shifted downfield as compared to the precursor compound 2 (δ 4.15, t, J = 9.5 Hz), thus suggesting the incorporation of the acetyl group at position 3. Complete analysis of the ^{1}H-NMR spectrum was in conformity with the structure assigned as methyl 3-*O*-acetyl-4,6-*O*-(4-methoxybenzylidene)-2-*O*-pentanoyl-α-D-glucopyranoside (4).



Compounds	R_1	R_2
4	$CH_3(CH_2)_3CO$-	$(CH_3CO)_2O$-
5	$CH_3(CH_2)_3CO$-	CH_3OSO-
6	$CH_3(CH_2)_3CO$-	$CH_3(CH_2)_4CO$-
7	$CH_3(CH_2)_3CO$-	$CH_3(CH_2)_{10}CO$-
8	$CH_3(CH_2)_3CO$-	$CH_3(CH_2)_{14}CO$-

Compounds	R_1	R_2
9	$CH_3(CH_2)_3CO$-	-OC–C$_6$H$_4$–Cl
10	$CH_3(CH_2)_3CO$-	-OC–C$_6$H$_4$–Cl
11	$CH_3(CH_2)_3CO$-	-OC–C$_6$H$_4$–NO_2
12	$CH_3(CH_2)_3CO$-	-OC–C$_6$H$_3$–(NO_2)$_2$

Fig. 1. Synthesis of the compounds 2-12.

The introduction of the pentanoyl group at position 2 in compound 3 was further confirmed by its conversion to and identification of the methanesulphonyl derivative (5) using direct acylation method. The ^{1}H-NMR spectrum (Table-1) of the resulting mesylate contained a three-proton singlet at δ 3.05 corresponding to the methyl protons of one mesyloxy group. The attachment of the mesyl group at C-3 was ascertained by observing the deshielding of the C-3 proton to δ 5.58 (as t, J = 9.6 Hz) from its value of δ 4.15 (as t, J = 9.5 Hz) in the precursor compound 3. Compound 2 was also similarly derivatised to the hexanoate (6) using hexanoyl chloride in pyridine. The data obtained by complete analysis of the ^{1}H-NMR spectrum of this compound was compatible with the structure assigned as methyl 3-*O*-hexanoyl-4,6-*O*-(4-methoxybenzylidene)-2-*O*-pentanoyl-α-D-glucopyranoside (6).

Table 1. ^{1}H-NMR spectra of the compounds 4-12.

	Compounds (δ-values)								
Protons	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Ar-H	7.34(2H, d,J 8.6) 6.92(2H, d,J 8.8)	7.39(2H, d,J 8.6) 6.88(2H, d,J 8.5)	7.34(2H, d,J 8.6) 6.85(2H, d,J 8.6)	7.34(2H, d,J 8.7) 6.89(2H, d,J 8.6)	7.34(2H, d,J 8.6) 6.85(2H, d,J 8.7)	7.97 (1H,s) 7.82 (2H,d,J8.6) 7.54 (2H,d,J8.0) 7.37 (1H,t,J7.9) 6.99 (2H,d,J8.6)	7.93 (2H,d,J8.6) 7.38 (2H,d,J8.6) 7.31 (2H,d,J7.2) 6.82 (2H,d,J8.6)	8.27(2H,d,J 8.8) 8.17(2H,d,J 8.8) 7.81(2H,d,J 8.8 6.99(2H,d,J 8.7)	9.86, 9.10, 9.09 (3× 1H, 3×s) 7.82 (2H, d, J 8.7) 6.80 (2H, d, J 8.6)
H-3	5.56 (1H, t,J9.7)	5.58 (1H,t,J 9.6)	5.58 (1H,t,J 9.7)	5.58 (1H,t,J 9.7)	5.55 (1H, t,J 9.7)	5.53 (1H,t,J 9.5)	5.81 (1H,t,J 9.7)	5.59 (1H,t,J 9.4)	5.85 (1H,t,J 9.7)
4-OCH_3.C$_6$H$_4$.C*H*	5.44 (1H, s)	5.52 (1H, s)	5.44 (1H, s)	5.44 (1H, s)	5.55 (1H, s)	5.28 (1H, s)	5.46 (1H, s)	5.55 (1H, s)	5.49 (1H, s)
H-2	5.30 (1H, dd,J3.8, 9.6)	4.90 (1H,dd, J 3.6, 9.7)	4.88 (1H,dd, J 3.6 and 9.7)	4.88 (1H,dd, J3.7 and 9.7)	4.90 (1H, dd, J 3.7 and 9.7)	5.03 (1H, dd, J 3.6 and 10.2)	5.08 (1H,dd,J 3.6 and 9.7)	5.05 (1H,dd,J 3.6 and 9.6)	5.12 (1H,dd,J 3.7 and 9.8)
H-1	5.28 (1H, d,J3.8)	4.86 (1H,d,J 3.6)	4.91 (1H,d,J 3.5)	4.92 (1H,d,J 3.7)	4.92 (1H, d,J 3.7)	4.93 (1H, d,J 3.5)	4.93 (1H,d,J 3.6)	4.92 (1H,d,J 3.5)	5.00 (1H,d,J 3.7)
H-6a	4.90 (1H, dd,J4.8 and 10.2)	4.33 (1H,dd, J 4.8 and 10.1)	4.27 (1H,dd, J 4.7 and 10.1)	4.26 (1H,dd, J 4.7 and 10.1)	4.28 (1H, dd, J 4.8 and 10.2)	3.75 (1H,dd,J 4.8 and 10.2)	4.31 (1H,dd,J 4.7 and 10.1)	3.90 (2H,m)	4.32 (1H,dd,J 4.7 and 10.2)
H-5	4.26 (1H, m)	3.95 (1H,m)	3.90 (1H,m)	3.90 (1H,ddd,J 4.7, 9.9, 14.5)	3.88 (1H,m)	3.89 (1H,m)	3.97 (1H,m)	3.90 (2H,m)	4.00 (1H,m)
4-OC*H*$_3$.C$_6$H$_4$.CH	3.77 (3H,s)	3.75 (3H,s)	3.78 (3H,s)	3.77 (3H,s)	3.78 (3H,s)	3.80 (3H,s)	3.75 (3H,s)	3.87 (3H,s)	3.74 (3H,s)
H-6b	3.71 (1H, t, J10.2)	3.78 (1H,t,J 10.2)	3.74 (1H, t,J 10.3)	3.74 (1H, t,J 10.2)	3.75 (1H, t,J 10.2)	3.78 (1H, t,J 10.2)	3.84 (2H,m)	3.78 (2H,m)	3.92 (1H,t,J 10.2)
H-4	3.61 (1H,t)	3.68 (1H,t,J 9.7)	3.61 (1H,t,J 9.6	3.61 (1H, t, J 9.6)	3.63 (1H, t,J 9.6)	3.82 (1H,t,J 9.7)	3.84 (2H,m)	3.78 (2H,m)	3.80 (1H,t,J 9.8)
1-OC*H*$_3$	3.38 (3H,s)	3.47 (3H,s)	3.39 (3H,s)	3.79 (3H,s)	3.39 (3H,s)	3.42 (3H,s)	3.43 (3H,s)	3.42 (3H,s)	3.45 (3H,s)
C*H*$_3$SO$_2$		3.05 (3H,s)							
CH$_3$(CH$_2$)$_2$C*H*$_2$CO	2.32 (2H,m)	2.27 (2H,m)	2.33 (2H,m)	2.29 (2H,m)	2.33 (2H,m)	2.24 (2H,m)	2.23 (2H,m)	2.23 (2H,m)	2.23 (2H,m)
CH$_3$(CH$_2$)$_{13}$C*H*$_2$CO					2.26 (2H,m)				
CH$_3$(CH$_2$)$_9$C*H*$_2$CO				2.29 (2H,m)					

(Contd.)

$CH_3(CH_2)_3CH_2CO$			2.26 (2H,m)						
CH_3CO	2.01 (3H,s)								
$CH_3(CH_2)_2CO$ and $CH_3(CH_2)_{11}(CH_2)_2CO$					1.60 (8H,m)				
$CH_3(CH_2)_2CH_2CO$ and $CH_3(CH_2)_7(CH_2)_2CH_2CO$				1.58 (8H,m)					
$CH_3CH_2CH_2CH_2CO$	1.57 (2H,m)	1.56 (2H,m)	1.60 (2H,m)			1.44 (2H,m)	1.41 (2H,m)	1.42 (2H,m)	1.45 (2H,m)
$CH_3(CH_2)_2CH_2CH_2CO$			1.53 (2H,m)						
$CH_3CH_2(CH_2)_2CO$	1.31 (2H,m)	1.28 (2H,m)	1.35 (2H,m)			1.18 (2H,m)	1.15 (2H,m)	1.14 (2H,m)	1.19 (2H,m)
$CH_3(CH_2)_7(CH_2)_3CO$				1.29 (14H,m)					
$CH_3(CH_2)_2(CH_2)_2CO$			1.25 (4H,m)						
$CH_3(CH_2)_{11}(CH_2)_3CO$					1.25 (22H,m)				
$CH_3(CH_2)_3CO$	0.89 (3H, t,J7.3)	0.88 (3H,t,J 7.3)	0.89 (3H,t,J 7.3)	0.92 (3H,m)	0.88 (3H,m)	0.71 (3H,t,J 7.3)	0.70 (3H,t,J 6.0)	0.69 (3H,t,J 7.3)	0.73 (3H,t,J 7.3)
$CH_3(CH_2)_{10}CO$				0.92 (3H,m)					
$CH_3(CH_2)_{16}CO$					0.88 (3H,m)				
$CH_3(CH_2)_4CO$			0.82 (3H,t,J 7.3)						

Lauroylation of compound 3 with lauroyl chloride in pyridine, followed by usual work-up and purification procedure, provided the 3-*O*-laurate (7) in 47% yield as syrup. The introduction of one lauroyl group in the molecule and its attachment at position 3 was ascertained by complete analysis of its ^{1}H-NMR spectrum. We then employed another fatty acid chloride (palmitoyl chloride) for derivatising compound 3 using direct acylation method. The resulting palmitate (8) was isolated in 90% yield as needles, m. p. 62-63^{0}C. By detailed analysis of the ^{1}H-NMR spectrum (Table 1), its structure was confidently assigned as methyl 4,6-*O*-(4-methoxybenzylidene)-3-*O*-palmitoyl-2-*O*-pentanoyl-α-D-glucopyranoside (8).

Reaction of the 2-*O*-pentanoyl derivative (3) with 3-chlorobenzoyl chloride in pyridine, followed by aqueous work-up and purification procedure, furnished the 3-chlorobenzoyl derivative (9) in 85% yield as a syrup. In its ^{1}H-NMR spectrum (Table 1), the presence of a one- proton singlet at δ 7.97, a two-proton doublet at δ 7.82 (J = 8.6 Hz), a one-proton singlet at δ 7.37 indicated attachment of one 3-chlorobenzoyl group in the molecule. Its point of attachment was also established by ^{1}H-NMR spectroscopy in which the C-3 proton shifted downfield to δ 5.53 (as t, J = 9.6 Hz) from its values in the precursor compound 3. We then allowed compound 3 to react with 4-chlorobenzoyl chloride under direct acylation conditions and using similar isolation procedures. The 4-chlorobenzoyl derivative (10) was isolated in 67% yield as a pasty mass. Complete analysis of its ^{1}H-NMR spectrum (Table-1) and by analogy with other derivatives as already prepared, the structure of this compound was assigned as methyl 3-*O*-(4-chlorobenzoyl)-4, 6-*O*-(4-methoxybenzylidene) -2-*O*-pentanoyl -α-D-glucopyranoside (10). Reaction of compound 3 with 4-nitrobenzoyl chloride in pyridine, followed by similar work-up and purification procedures, furnished compound (11) in 55% yield as a semi-solid mass. In its ^{1}H-NMR spectrum (Table 1), two lowfield two-proton doublets at δ 8.27 (J = 8.8 Hz) and δ 8.17 (J = 8.8 Hz) corresponded to the aromatic protons of one 4-nitrobenzoyl group. The much deshielding of H-3 to δ 5.59 (as t, J = 9.4 Hz) as compared to its precursor supported the attachment of the 4-nitrobenzoyl group at C-3.

Finally we used 3, 5-dinitrobenzoyl chloride for derivatising compound 3 using similar procedures. The derivative 12 was obtained in 55% yield as a pasty mass. In its ^{1}H-NMR spectrum (Table 1), three very lowfield singlets at δ 9.86, δ 9.10 and δ 9.09 corresponded to the presence of one 3, 5-dinitrobenzoyl group in the molecule. Here also, the C-3 proton deshielded downfield to δ 5.85 (as t, J = 9.7 Hz) as compared to its precursor compound 3, thereby suggesting the introduction of this group at C-3. The resonances of other protons appeared at their anticipated positions enabled us to assign the structure of this compound as methyl 3-*O*-(3,5-dinitrobenzoyl)-4,6-*O*-(4-methoxybenzylidene)-2-*O*-pentanoyl-α-D-glucopyranoside (12). All these products have been employed as test chemicals for antimicrobial screening experiments.

Screening of antimicrobial activities: From the experimental results obtained by using a number of selected human pathogenic bacteria (Tables 2 and 3) were found that selectively acylated derivatives 3, 5, 6, 9, 11 and 12 showed moderate to marked inhibition against Gram-positive bacteria while compounds 5, 6, 9, 11 and 12 are very active against Gram-negative bacteria.

Table 2. Zone of inhibition observed against Gram-positive bacteria by the test chemicals.

Compound	Diameter of inhibition zone in mm 200 μg dw/disc			
	B. subtilis	*B. cereus*	*B. megaterium*	*S. aureus*
3	18	8	*19	9
4	10	10	NF	6
5	NF	*22	NF	9
6	NF	*20	NF	7
7	8	11	NF	11
8	NF	NF	NF	NF
9	12	*20	NF	14
10	12	10	NF	NF
11	*19	11	NF	NF
12	18	11	NF	*23
**Ampicillin	*19	*18	*16	*22

'*' = Marked inhibition. '**' = Standard antibiotic. 'NF' = Not found. 'dw' = Dry weight.

The authors also observed that some compounds such as 5, 6, 9, 11 and 12 are active against both the Gram-positive and Gram-negative organisms. So these compounds may be targeted for future studies for their usage as broad-spectrum antibiotics.

Table 3. Zone of inhibition observed against Gram-negative bacteria by the test chemicals.

Compound	Diameter of inhibition zone in mm 200 μg dw/disc					
	E. coli	*S. typhi*	*S. paratyphi*	*S. dysenteriae*	*P.* species	*V. cholerae*
3	NF	NF	12	NF	9	9
4	NF	NF	12	NF	NF	NF
5	NF	*26	14	NF	NF	*22
6	NF	NF	*23	11	11	14
7	NF	11	NF	NF	NF	9
8	NF	NF	NF	NF	NF	NF
9	NF	14	*22	NF	NF	14
10	NF	NF	8	10	NF	8
11	NF	19	8	16	9	*16
12	NF	*28	12	12	6	14
**Ampicillin	*10	*20	*18	*22	*20	*15

'*' = Marked inhibition. '**' = Standard antibiotic. 'NF' = Not found. 'dw' = Dry weight.

In general, it has been observed that antibacterial results of the selectively acylated D-glucopyranoside derivatives obtained by using various acylating agents follow the order for Gram-positive organisms: 12 > 5 > 6 = 9 > 3 = 11 > 10 > 7 > 4 > 8 and Gram-negative bacteria follow the order 12 > 5 > 6 > 9 > 11 > 3 = 4 > 7 > 10 > 8. From this study it was found that among the acylated products, compound 5 showed effective activity and compounds 6 and 9 showed high activity against the selected bacteria. Some of the tested chemicals showed moderate to marked inhibition against the bacterial pathogens employed. It was also found that some tested chemicals were unable to show any inhibition at all against the bacterial pathogens employed. It was also observed that the carbohydrate derivatives were found comparatively more effective against Gram-positive micro-organisms than that of Gram-negative micro-organisms.

The results obtained from the present investigation of antifungal studies mentioned in Table 4 clearly demonstrate that compounds 4, 5 and 9 showed the highest inhibition (65.70%, 60.00%, 60.00% and 100%) against the *Fusarium equiseti*. Excellent inhibition was observed in case of compound 9 (100%) in which the percent inhibition is more than nystatin (44.70%) against *Fusariuum equiseti*.

Table 4. Antifungal activities of the synthesized test chemicals.

Compound	% inhibition of fungal mycelial growtha (100 µg (dw)/ml medium)			
	F. equiseti	*A. alternata*	*C. corchori*	*C. lunata*
3	47.14	*63.84	12.41	26.89
4	*60	37.69	34.92	34.79
5	*60	18.57	33.33	22.27
6	34.29	44.73	12.69	19.09
7	14.29	33.84	11.11	33.44
8	11.42	19.23	12.69	13.79
9	*100	50.29	12.69	*85.63
10	42.46	15.38	39.52	65.57
11	18.57	10.5	17.93	37.14
12	31.43	31.43	38.7	17.39
**Nystatin	*44.7	*51.55	*40.51	*75.05

'*' = Marked inhibition. '**' = Standard antibiotic. 'NF' = Not found. 'dw' = Dry weight.

Again compound 9 (85.63%) showed the highest inhibition against the *Curvularia lunata* which is also higher than that of the standard antibiotic (75.05%). Compounds 3 (63.84%) and 9 (50.29%) showed the highest inhibition against the *Alternaria alternata*. However, most of the chemicals showed to be less active or toxic to the *Colletotrichum corchori* and *Alternaria alternata* as compared to the standard antibiotic (Nystatin). So, it was found that the newly synthesized and reported chemicals were not very much

effective against *Colletotrichum corchori* and *Alternaria alternata.* Antifungal and antibacterial activities of our test chemicals are in accordance with the results we observed before (Kawsar *et al*. 2012, Kabir *et al*. 2005). It is expected that this piece of work employing D-glucose derivatives as test chemicals will open the scope for further work on the development of pesticides and medicines sectors. This is the first report regarding the effectiveness of the selected chemicals against the selected pathogens.

CONCLUSIONS

The present study reports the synthesis of a new series of methyl α-D-glucopyranoside (1) derivatives 2-12. Antibacterial and antifungal activity of the new synthesized compounds bearing benzene and various acyl moiety, revealed that some tested compounds showed good activities against selected human and phytopathogenic strains. Therefore, it is expected that the newly acylated derivatives might show potential antiviral, antitumor, anticancer and anti-inflammatory activities.

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