RESEARCH ARTICLE

Chemical Sciences Journal, Vol. 2012: CSJ-73

Selective Synthesis of Some New Carbohydrate Derivatives: Antimicrobial Screening Studies against Human and Phytopathogens

Selective Synthesis of Some New Carbohydrate Derivatives: Antimicrobial Screening Studies against Human and Phytopathogens

SMA Kawsar[1]*, AKMS Kabir[1]†, MM Manik[1], MN Anwar[2]
[1]Laboratory of Carbohydrate and Protein Chemistry, Department of Chemistry, Faculty of Physical Science, University of Chittagong, Chittagong, Bangladesh.
[2]Department of Microbiology, Faculty of Biological Science, University of Chittagong, Chittagong, Bangladesh.

***Correspondence to:** Sarkar MA Kawsar, akawsarabe@yahoo.com
†Recently deceased

Accepted: Sep 05, 2012; Published: Sep 21, 2012

Abstract
Methyl 4,6-*O*-benzylidene-α-**D**-glucopyranoside (**1**) was selectively converted to methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-α-**D**-glucopyranoside (**2**) by reaction with 2,6-dichlorobenzoyl chloride using direct method in good yield. Using a wide variety of acylating agents, a series of 3-*O*-acyl derivatives (**2-10**) of this 2-substitution product were also prepared. These synthesized derivatives were screened for *in vitro* antimicrobial activity against ten human pathogenic bacteria and three fungal phytopathogens. The study revealed that the acylated derivatives exhibit promising antibacterial and antifungal activities. The acylated derivatives were found to be more effective against the fungal strains than those of the bacterial pathogens. However, a good number of methyl 4,6-*O*-benzylidene-α-**D**-glucopyranoside derivatives exhibited better antimicrobial activity than the standard antibiotics.

Keywords: Carbohydrates; synthesis; antimicrobial activity; inhibition; pathogens.

1. Introduction

The study of carbohydrates is one of the exciting fields of organic chemistry. Selective acylation of monosaccharide derivatives is of growing importance in the field of carbohydrate chemistry because of its usefulness for the synthesis of biologically active products [1, 2]. Carbohydrate, especially acylated monosaccharides, is very important due to their effective biological activity [3]. It is also known that if an active nucleus or molecule is linked to another nucleus, the resulting molecule may possess great potential for biological activity [4]. A number of fruitful and efficient methods for selective acylation have so far been developed and employed successfully [5-7]. Most of these methods are based on the blocking-deblocking technique [8, 9]. Carbohydrate isolated from natural sources, acyl glycoses and acyl glycosides have immense importance and some of them have effective biological activity [7]. The acyl derivatives of carbohydrates are essential for the synthesis of various natural products and also have great synthetic importance because the products thus obtained may further be utilized as versatile intermediates for the synthesis of higher carbon sugars and other carbohydrate derivatives. From literature survey, it was revealed [10] that a large number of biological compounds possess aromatic, heteroaromatic acyl substituents, nitrogen, sulphur and halogen containing substituents are also known to enhance the biological activity of the parent compound [10].

Over the last few years, active researchers in our laboratory carried out selective acylation of monosaccharide derivatives [11-13] and also biological evaluation of the synthesized compounds [14-16]. It was observed that the combination of two or more acyl substituents in a single molecular framework enhances the biological activity many fold than their parent nuclei, for example, some acylated derivatives of **D**-glucopyranose were found more than those of the standard antibiotics [17]. Encouraged by these results, we synthesized some acyl derivatives of methyl 4,6-*O*-benzylidene-α-**D**-glucopyranoside (**1**) containing a benzene moiety and various acyl groups e.g., 2,6-dichlorobenzoyl, pentanoyl, hexanoyl, lauroyl, myristoyl, pivaloyl, 4-t-butylbenzoyl and 3-chlorobenzoyl (Figure 1) in a single molecular framework and antimicrobial activities of these compounds were carried out using a variety of bacterial and fungal strains and the results are reported here for the first time.

2. Methods

2.1. Physical measurements

All reagents used were commercially available (Aldrich) and were used as received, unless otherwise specified. Solvents were distilled from appropriate drying agents immediately prior to use. Melting points were determined on an electrothermal melting point apparatus (England) and are uncorrected. Evaporations were carried out under reduced pressure using VV-1 type vacuum rotary evaporator (Germany) with a bath temperature below 40^{o}C. ^{1}H-NMR spectra (300 MHz) and were recorded for solutions in deuteriochloroform ($CDCl_3$) (internal Me_4Si) with a Bruker DPX-40C spectrometer. Thin layer chromatography (TLC) was performed on Kieselgel GF_{254} and spots were detected by spraying the plates with 1% H_2SO_4 and heating at 150-200^{o}C until coloration took place. Column chromatography was performed with silica gel G_{60}. Solvent system employed for TLC analyses was ethyl acetate-cyclohexane.

2.2. Synthesis of Methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-α-D-glucopyranoside (2)

A solution of methyl 4, 6-*O*-benzylidene-α-**D**-glucopyranoside, (**1**) (500 mg, 1.77 mmol) in dry pyridine (3 mL) was cooled to 0^{0}C (maintained by ice and common salt) whereupon 2,6-dichlorobenzoyl chloride (0.28 mL, 1.95 mmol) was added to it. The mixture was stirred at this temperature for 5 hours and then allowed to stand overnight in the refrigerator. The progress of the reaction was monitored by TLC, which indicated the formation of a faster-moving product. A few pieces of ice were 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 syrupy residue. The syrup was passed through a silica gel column and eluted with ethyl acetate-cyclohexane (1:3), to obtain compound, **2**.

It was obtained as pasty white mass in 76%, R_f = 0.54 (ethyl acetate/cyclohexane = 1/3): ^{1}H-NMR ($CDCl_3$): δ 7.33 (2H, m, Ar-H), 7.29 (6H, m, Ar-H), 5.54 (1H, s, PhC*H*-), 5.17 (1H, d, J = 3.7 Hz, H-1), 5.14 (1H, dd, J = 3.7 and 9.8 Hz, H-2), 4.36 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 4.33 (1H, t, J = 9.8 Hz, H-3), 3.87 (1H, m, H-5), 3.78 (1H, t, J = 10.2 Hz, H-6b), 3.65 (1H, t, J = 9.8 Hz, H-4), 3.48 (3H, s, 1-OC*H*$_{3)}$. Anal. Calcd. for $C_{21}H_{20}O_7Cl_2$ (455.03): C, 55.38; H, 4.41. Found: C, 55.79; H, 4.64%.

Synthesis of Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside derivatives (3-10) - General procedure

A cooled (0 ^{0}C) and stirred solution of the 2,6-dichlorobenzoyl derivative (**2**) (59 mg, 0.13 mmol) in dry pyridine (3 mL) was treated with acetic anhydride (0.1 mL). The mixture was stirred at this temperature for 5 hours and then it was allowed to stand at room temperature overnight. The completion of the reaction was confirmed by TLC, which indicated the formation of a faster-moving product. A few pieces of ice were 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 were concentrated under reduced pressure to leave syrup. The syrup was passed through a silica gel column and eluted with ethyl acetate-cyclohexane (1:5), to afford the 3-*O*-acetyl derivative (**3**). Similar reaction and purification procedure was applied to prepare compounds **4-10**.

Methyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-α-D-glucopyranoside **(3)**. It was obtained as colorless syrup in 85%, R_f = 0.51 (ethyl acetate/cyclohexane = 1/5): ^{1}H-NMR ($CDCl_3$): δ 7.48 (3H, m, Ar-H), 7.34 (5H, m, Ar-H), 5.72 (1H, t, J = 9.8 Hz, H-3), 5.54 (1H, s, PhC*H*-), 5.23 (1H, dd, J = 3.7 and 9.9 Hz, H-2), 5.15 (1H, d, J = 3.7 Hz, H-1), 4.35 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 3.98 (1H, m, H-5), 3.83 (1H, t, J = 10.2 Hz, H-6b), 3.72 (1H, t, J = 9.8 Hz, H-4), 3.48 (3H, s, 1-OC*H*$_{3)}$, 2.01 {3H, s, C*H*$_3$CO-). Anal. Calcd. for $C_{23}H_{22}O_8Cl_2$ (497.03): C, 55.52; H, 4.45. Found C, 56.02; H, 5.01%.

Methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-3-*O*-pentanoyl-α-D-glucopyranoside **(4).** It was obtained as syrup in 92%, R_f = 0.55 (ethyl acetate/cyclohexane = 1/6): ^{1}H-NMR ($CDCl_3$): δ 7.41 (3H, m, Ar-H), 7.32 (5H, m, Ar-H), 5.71 (1H, t, J = 9.8 Hz, H-3), 5.52 (1H, s, PhC*H*-), 5.21 (1H, dd, J = 3.7 and 10.0 Hz, H-2), 5.13 (1H, d, J = 3.7 Hz, H-1), 4.33 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 3.99 (1H, ddd, J = 4.9, 9.9 and 14.6 Hz, H-5), 3.80 (1H, t, J = 10.2 Hz, H-6b), 3.69 (1H, t, J = 9.6 Hz, H-4), 3.45 (1H, s, 1-OC*H*$_{3)}$, 2.31 {2H, m, $CH_3(CH_2)_2CH_2CO$-}, 1.57 {2H, m, $CH_3CH_2CH_2CH_2CO$-}, 1.30 {2H, m, $CH_3CH_2(CH_2)_2CO$-}, 0.81 {3H, t, J = 7.3 Hz, C*H*$_3(CH_2)_3CO$-}. Anal. Calcd. for $C_{26}H_{28}O_8Cl_2$ (539.06): C, 57.88; H, 5.22. Found C, 57.77; H, 5.15%.

Methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-3-*O*-hexanoyl-α-D-glucopyranoside **(5).** It was obtained as thick syrup in 55%, R_f = 0.52 (ethyl acetate/cyclohexane = 1/5): ^{1}H-NMR ($CDCl_3$,): δ 7.42 (2H, m, Ar-H), 7.31 (5H, m, Ar-H), 2H, m, Ar-H), 5.71 (1H, t, J = 9.8 Hz, H-3), 5.52 (1H, s, PhC*H*-), 5.21 (1H, dd, J = 3.7 and 9.9 Hz, H-2), 5.13 (1H, d, J = 3.7 Hz, H-1), 4.33 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 3.97 (1H, m, H-5), 3.80 (1H, t, J = 10.2 Hz, H-6b), 3.69 (1H, t, J = 9.6 Hz, H-4), 3.45 (3H, s, 1-OCH_3), 2.31 {2H, m, $CH_3(CH_2)_3CH_2CO$-}, 1.58 {2H, m, $CH_3(CH_2)_2CH_2CH_2CO$-}, 1.23 {4H, m, $CH_3(CH_2)_2(CH_2)_2CO$-}, 0.78 {3H, t, J = 6.9 Hz, $CH_3(CH_2)_4CO$-}. Anal. Calcd. for $C_{27}H_{30}O_8Cl_2$ (553.07): C, 58.59; H, 5.45, Found C, 58.42; H, 5.36%.

Methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-3-*O*-lauroyl-α-D-glucopyranoside **(6).** It was obtained as colorless thick syrup in 94%, R_f = 0.53 (ethyl acetate/cyclohexane = 1/6): ^{1}H-NMR ($CDCl_3$): δ 7.41 (2H, m, Ar-H), 7.31 (6H, m, Ar-H), 5.72 (1H, t, J = 9.8 Hz, H-3), 5.51 (1H, s, PhC*H*-), 5.20 (1H, dd, J = 3.7 and 9.9 Hz, H-2), 5.12 (1H, d, J = 3.6 Hz, H-1), 4.32 (1H, dd, J = 4.7 and 10.2 Hz, H-6a), 3.98 (1H, m, H-5), 3.80 (1H, t, J = 10.2 Hz, H-6b), 3.69 (1H, t, J = 9.8 Hz, H-4), 3.44 (3H, s,1-OCH_3), 2.32 {2H, m, $CH_3(CH_2)_9CH_2CO$-}, 1.61 {2H, m, $CH_3(CH_2)_8CH_2CH_2CO$-}, 1.25 {16H, m, $CH_3(CH_2)_8CH_2CH_2CO$-}, 0.86 {3H, t, J = 6.2 Hz, $CH_3(CH_2)_{10}CO$-}. Anal. Calcd. for $C_{33}H_{42}O_8Cl_2$ (637.13): C, 62.15; H, 6.63, Found C, 62.66; H, 6.83%.

Methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-3-*O*-myristoyl-α-D-glucopyranoside **(7).** It was obtained as pasty mass in 72%, R_f = 0.50 (ethyl acetate/cyclohexane = 1/6): ^{1}H-NMR ($CDCl_3$): δ 7.42 (2H, m, Ar-H), 7.32 (6H, m, Ar-H), 5.71 (1H, t, J = 9.8 Hz, H-3), 5.52 (1H, s, PhC*H*-), 5.21 (1H, dd, J = 3.7 and 9.9 Hz, H-2), 5.13 (1H, d, J = 3.7 Hz, H-1), 4.33 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 3.98 (1H, m, H-5), 3.80 (1H, t, J = 10.3 Hz, H-6b), 3.69 (1H, t, J = 9.6 Hz, H-4), 3.45 (3H, s, 1-OCH_3), 2.31 {2H, m, $CH_3(CH_2)_{11}CH_2CO$-}, 1.58 {2H, m, $CH_3(CH_2)_{10}CH_2CH_2CO$-}, 1.25 {20H, m, $CH_3(CH_2)_{10}(CH_2)_2CO$-}, 0.87 {3H, t, J = 6.4 Hz, $CH_3(CH_2)_{12}CO$-}. Anal. Calcd. for $C_{35}H_{46}O_8Cl_2$ (665.15): C, 63.14; H, 6.95, Found C, 63.74; H, 6.85%.

Methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-3-*O*-pivaloyl-α-D-glucopyranoside **(8).** It was obtained as syrup in 56%, R_f = 0.51 (ethyl acetate/cyclohexane = 1/6): ^{1}H-NMR ($CDCl_3$): δ 7.42 (2H, m, Ar-H), 7.36 (6H, m, Ar-H), 5.70 (1H, t, J = 9.8 Hz, H-3), 5.53 (1H, s, PhC*H*-), 5.23 (1H, dd, J = 3.7 and 9.9 Hz, H-2), 5.16 (1H, d, J = 3.7 Hz, H-1), 4.33 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 3.98 (1H, m, H-5), 3.79 (1H, t, J = 10.2 Hz, H-6b), 3.69 (1H, t, J = 9.6 Hz, H-4), 3.44 (3H, s, 1-OCH_3), 1.02 {9H, s, $(CH_3)_3CCO$-}. Anal. Calcd. for $C_{26}H_{28}O_8Cl_2$ (539.06): C, 57.88; H, 5.22, Found C, 57.45; H, 5.16%.

Methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-3-*O*-(4-t-butylbenzoyl)-α-D-glucopyranoside **(9).** It was obtained as thick syrup in 93%, R_f = 0.52 (ethyl acetate/cyclohexane = 1/5): ^{1}H-NMR ($CDCl_3$): δ 8.07 (2H, d, J = 8.07 Hz, Ar-H), 7.97 (2H, d, J = 8.00 Hz, Ar-H), 7.52 (4H, m, Ar-H), 7.42 (2H, m, Ar-H), 7.29 (2H, m, Ar-H), 5.96 (1H, t, J = 9.8 Hz, H-3), 5.52 (1H, s, PhC*H*-), 5.44 (1H, dd, J = 3.5 and 10.0 Hz, H-2), 5.15 (1H, d, J = 3.5 Hz, H-1), 4.36 (1H, dd, J = 4.7 and 10.2 Hz, H-6a), 4.08 (1H, m, H-5), 3.84 (1H, t, J = 10.2 Hz, H-6b), 3.81 (1H, t, J = 10.0 Hz, H-4), 3.48 (3H, s, 1-OCH_3), 1.35 {9H, s, $(CH_3)_3C$-}. Anal. Calcd. for $C_{32}H_{32}O_8Cl_2$ (615.08): C, 62.43; H, 5.29, Found C, 62.49; H, 5.79%.

Methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-3-*O*-(3-chlorobenzoyl)-α-D-glucopyranoside **(10).** It was obtained as syrup in 61%, R_f = 0.55 (ethyl acetate/cyclohexane = 1/6): ^{1}H-NMR ($CDCl_3$): δ 7.50 (1H, s, Ar-H), 7.49 (2H, m, Ar-H), 7.41 (2H, m, Ar-H), 7.37 (3H, m, Ar-H), 7.31 (4H, m, Ar-H), 5.94 (1H, t, J = 9.8 Hz, H-3), 5.54 (1H, s, PhC*H*-), 5.42 (1H, dd, J = 3.7 and 10.0 Hz, H-2), 5.16 (1H, d, J = 3.7 Hz, H-1), 4.36 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 4.07 (1H, m, H-5), 3.87 (1H, t, J = 10.2 Hz, H-6b), 3.82 (1H, t, J = 9.8 Hz, H-4), 3.48 (3H, s, 1-OCH_3). Anal. Calcd. for $C_{28}H_{23}O_8Cl_3$ (593.54): C, 56.61; H, 4.25, Found C, 56.67; H, 4.76%.

2.3. Screening of antimicrobial activities

2.3.1. Test organisms

The synthesized methyl 4,6-*O*-benzylidene-α-**D**-glucopyranoside derivatives (**2-10**) were tested for their antibacterial activity against ten human pathogenic bacteria, viz., *Bacillus subtilis* BTCC 17, *Bacillus megaterium* BTCC 18, *Bacillus cereus* BTCC 19, *Staphylococcus aureus* ATCC 6538, *Shigella dysenteriae* AE 14396, *Escherichia coil* ATCC 25922, *Salmonella typhi* AE 14612, *Salmonella paratyphi* AE 14613, *Pseudomonas* species CRL (ICDDR,B) and *Vibrio cholerae* AE 14748 and three pathogenic fungi viz., *Fusarim equiseti* (*corda*) Sacc., *Curvularia lunata* (Wakker Becdijin) and *Alternaria alternata* (Fr.) Kedissler.

2.3.2. Determination of antibacterial activity

The *in vitro* sensitivity of the bacteria 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 (as McFarland 0.5 standard) followed by spread plate method and allowed to dry for three to five minutes. Dried and sterilized filter paper discs were treated separately with 50 µg dry weight/disc from 2% solution (in $CHCl_3$) 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±2˚C 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).

2.3.3. Determination of antifungal activity

The *in vitro* antifungal activities of the synthesized **D**-glucopyranoside derivatives (**2-10**) were determined by poisoned food technique [20] with some modification [19]. 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 27˚C. A control set was also maintained in each experiment. Linear mycelial growth of fungus was measured after 3-5 days of incubation. The percentage inhibition of radial mycelial growth of the test fungus was calculated as follows:

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

where, I = Percentage of inhibition, C = Diameter of the fungal colony in control ($CHCl_3$), 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

The main objective of the research work reported in this paper was to study regioselective acylation of methyl 4,6-*O*-benzylidene-α-**D**-glucopyranoside (**1**) with 2,6-dichlorobenzoylation using the direct acylation method. The resulting acylation products were then converted to a series of derivatives using various acylating agents containing a wide variety of biologically prone atoms/groups. The structure of the main acylation products and their derivatives were established by analyzing their ^{1}H-NMR spectra. All the acylation products thus prepared were used as test chemicals for antibacterial and antifungal evaluation studies against a number of pathogenic strains.

Initially, we carried out 2,6-dichlorobenzoylation of compound **1** with unimolecular amount of 2,6-dichlorobenzoyl chloride using direct acylation method. By employing conventional reaction, work-up and purification procedures, we obtained compound **2** in 76% yields as a pasty mass. In its ^{1}H-NMR spectrum, the aromatic protons resonated at their anticipated positions. Also, observed the downfield shift of C-2 proton to δ 5.14 (as dd, J = 3.7 and 9.8 Hz) from its value in the precursor diol **1**, thereby confirming the attachment of the 2,6-dichlorobenzoyl group at position C-2. Complete analysis of the ^{1}H-NMR spectrum enabled us to assign its structure as methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-α-**D**-glucopyranoside (**2**). The structure of compound **2** was further ascertained by its conversion to and identification of its acetyl derivative (**3**). Thus, reaction of compound **2** with an excess of acetic anhydride in pyridine followed by usual work-up procedure and silica gel column chromatographic purification, furnished the acetyl derivative (**3**) in 85% yield as syrup. The introduction of one acetyl group in the molecule was demonstrated by the appearance of one three-proton singlet at δ 2.01 in its ^{1}H-NMR spectrum. The C-3 proton resonated at δ 5.72 (as t, J = 9.8 Hz) and shifted downfield from the precursor compound **2** (δ 4.33, t, J = 9.8 Hz), thereby suggesting the attachment of the acetyl group at position 3. By complete analysis of the ^{1}H-NMR spectrum, the structure of the acetate was ascertained as methyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-α-**D**-glucopyranoside (**3**).

The structure of compound **2** was further supported by its transformation to and identification of a number of fatty acid derivatives using pentanoyl chloride, hexanoyl chloride, lauroyl chloride and myristoyl chloride by direct acylation method. Thus, treatment of compound **2** with pentanoyl chloride in pyridine provided the pentanoyl derivative (**4**) in 92% yield as syrup. In its ^{1}H-NMR spectrum, the presence of three two-proton multiplets at δ 2.31, δ 1.57, δ 1.30 and a three-proton triplet at δ 0.81 (J= 7.3 Hz) were indicative of the introduction of one pentanoyl group. The de-shielding of the C-3 proton to δ 5.71 (as t, J = 9.8 Hz) from its value in the precursor compound **2** (δ 4.33, t, J = 9.8 Hz), suggesting the attachment of the pentanoyl group at C-3.

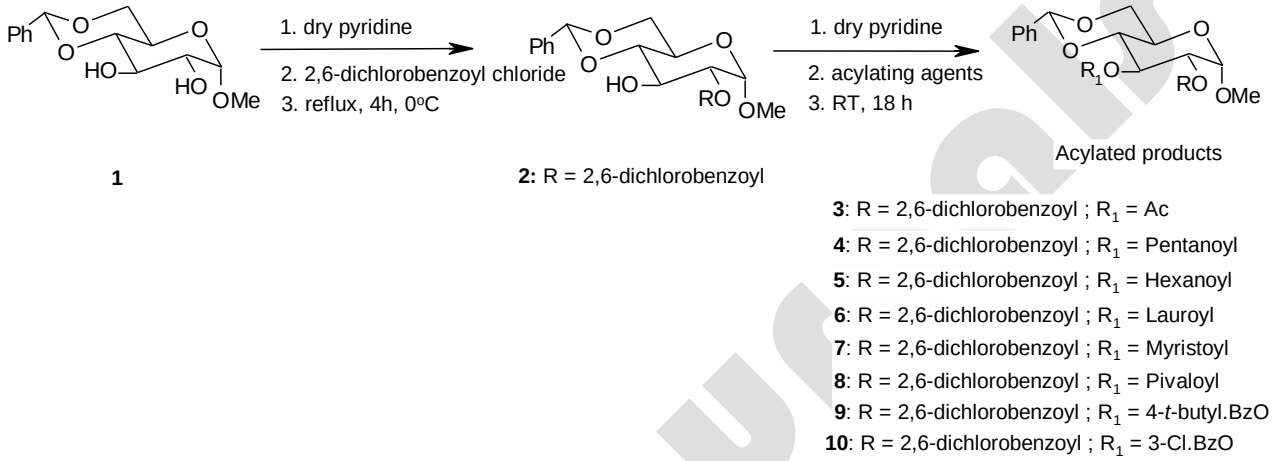

Fig. 1: Reaction scheme

We then performed hexanoylation of compound **2** using similar procedures and isolated the hexanoyl derivative (**5**) in 55% yield as syrup. In its ^{1}H-NMR spectrum, two two-proton multiplets at δ 2.31, δ 1.58, one four-proton multiplet at δ 1.23 and a three-proton triplet at δ 0.78 (J = 6.9 Hz) indicated the presence of one hexanoyl group in the molecule. The attachment of the hexanoyl group at C-3 was confirmed by observing considerable downfield shift of H-3 to δ 5.71 (as t, J = 9.8 Hz) from its value in its precursor compound **2**. Complete analysis of the ^{1}H-NMR spectrum of this compound established its structure as methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-3-*O*-hexanoyl-α-**D**-glucopyranoside (**5**). The 2,6-dichlorobenzoyl derivative (**2**) was then converted to the 3-*O*-lauroyl and 3-*O*-myristoyl derivatives (**6** and **7**) by using similar procedures. The structures of these derivatives were confidently assigned by completely analyzing their ^{1}H-NMR spectra. In both the cases, the introduction of the substituents at C-3 was ascertained. We then employed pivaloyl chloride for derivatizing compound **2**. By applying conventional reaction, work-up and purification procedures, we isolated the pivaloyl derivative (**8**) in 56% yield as syrup. The presence of one pivaloyl group in the molecule was shown by the appearance of a nine-proton singlet at δ 1.02 and C-3 proton shifted downfield to δ 5.70 (as t, J = 9.8 Hz), as observed in its ^{1}H-NMR spectrum. The rest of the protons resonated in their anticipated positions and this led us to propose a structure of this compound as methyl 4,6-*O*-benzylidene-2-*O*-(2,6-dichlorobenzoyl)-3-*O*-pivaloyl-α-**D**-glucopyranoside (**8**). Compound **2** was then allowed to react with 4-t-butylbenzoyl chloride in dry pyridine; we obtained compound **9** in high yield as thick syrup. In its ^{1}H-NMR spectrum, the characteristic peaks at δ 8.07 (2H, d, J = 8.7 Hz), δ 7.97 (2H, d, J = 8.0 Hz) and δ 1.35 (9H, s) were due to the presence of one 4-t-butylbenzoyl group in the molecule. The incorporation of the 4-t-butylbenzoyl group at C-3 was ascertained by considerable downfield shift H-3 to δ 5.96 (as t, J = 9.8 Hz) as compared to its precursor **2** (δ 4.33, t, J = 9.8 Hz). Finally, we used 3-chlorobenzoyl chloride for derivatizing compound **2** by direct acylation method and obtained the 3-chlorobenzoyl derivative (**10**) in 57% yield. By complete analysis of its ^{1}H-NMR spectrum and by analogy with similar derivatives described earlier, the structure of this compound was assigned as methyl 4,6-*O*-benzylidene-3-*O*-(3-chlorobenzoyl)-2-*O*-(2,6-dichlorobenzoyl)-α-**D**-glucopyranoside (**10**).

Table 1: Zone of inhibition observed against Gram-positive microorganisms by the test chemicals.

Compound	Diameter of inhibition zone in mm 200 µg dw/disc			
	B. subtilis	***B. cereus***	***B. megaterium***	***S. aureus***
2	8	NF	8	NF
3	6.5	6	NF	7
4	7	7.5	NF	NF
5	7	7	8	6.5
6	*20	8	*18	*18
7	7	*12	*10.5	6.5
8	8	6.5	9.5	7
9	*11	NF	*12.5	7.5
10	7	6.5	6	7
****Ampicillin**	*19	*18	*16	*22
NB: '*' = marked inhibition, '**' = standard antibiotic, 'NF' = not found, 'dw' = dry weight.				

Table 2: Zone of inhibition observed against Gram-negative test organisms 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***
2	7	11	NF	6.5	8	NF
3	NF	8	7.5	7	8	8.5
4	8	8	7	6.5	8	6.5
5	9	7.5	7.5	7	NF	7
6	*12.5	*13	*13	*15	*14	*13
7	7.5	7	*12.5	7	6	9
8	8.5	9	8	7	NF	8
9	*9.5	8.5	*12	10.5	*14	*12
10	7	7.5	7	8	NF	7
****Ampicillin**	*10	*20	*18	*22	*18	*15
NB: '*' = marked inhibition, '**' = standard antibiotic, 'NF' = not found, 'dw' = dry weight.						

The results of *in vitro* antibacterial activity of the test chemicals (**2**-**10**) against four Gram-positive and six Gram-negative human pathogens are presented in Table 1 and 2. For comparative study, the antimicrobial activity of two standard antibiotics, ampicillin and nystatin, were also evaluated against these microorganisms. Maximum synthesized compounds showed different degree of sensitivity against both Gram-positive and Gram-negative bacteria. Test chemicals **5**, **6**, **7**, **8** and **10** were found to be active against all the Gram-positive bacteria while **4**, **6**, **7** and **9** were very active against Gram-negative bacteria tested herein, although the degrees of inhibition were different. Whereas, the test chemicals **6** and **7** were active against both 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 **6** was observed against *B. subtilis* (20 mm), *B. megaterium* (18 mm) and *S. aureus* (18 mm). Antibacterial activity better than standard ampicillin was recorded with the chemical **6** against *B. subtilis*, *B. megaterium*, *S. aureus* and *E. coli*. Among the acylated products, the chemicals **6** and **7** 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 results of our previous investigations [16, 17, 21].

The results obtained from the present investigation of antifungal studies mentioned in Table 3 clearly demonstrated that chemical **9** showed excellent inhibition in which the percent inhibition (90.76%) is more than standard antibiotic, nystatin (44.70%) against *F. equiseti*. However, the inhibition of mycelial growth of the chemical **6** (58.09%) against *C. lunata*, chemicals **7** (35.33%) and **9** (36.47%) against *A. alternata* 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 [15, 16].

Table 3: Antifungal activities of the test chemicals.

Compound	% Inhibition of fungal mycelial growth[a] (100 µg (dw)/mL medium)		
	F. equiseti	*A. alternata*	*C. lunata*
2	12.69	17.39	5.87
3	NF	13.04	21.07
4	20	*25.16	13.24
5	21.54	NF	8.87
6	26.15	8.69	*58.09
7	*30	*35.33	9.54
8	19.23	19.56	*25.07
9	*90.76	*36.47	20.31
10	21.54	13.04	*24.81
****Nystatin**	*44.7	*51.55	*75

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

Our synthesized and reported chemicals (**2**-**10**) have not been tested before against the selected bacterial and fungal pathogens. This is the first report regarding the effectiveness of the selected chemicals against the selected pathogens.

4. Conclusion

The results of the present investigation showed that some of the newly synthesized acylated methyl 4,6-*O*-benzylidene-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. It is also expected that this work employing carbohydrate derivatives as test chemicals will help further the development of pesticides and medicines for plant/human disease control. So it is hoped that the acylated derivatives of methyl 4,6-*O*-benzylidene-α-**D**-glucopyranoside (**2**-**10**) might show potential antiviral, anti-tubercular and anti-inflammatory activities.

Competing Interests

Authors do not have any competing interests.

Authors' Contributions

SMAK study designed and drafted the manuscript. AKMSK was responsible for execution of the work, interpretation of spectral and analytical data. MMM was involved in research work and literature survey. MNA performed the biological work.

Acknowledgement

The authors are indebted to the Chairman, Department of Chemistry, University of New England, Australia for providing the facility for recording the 1H-NMR spectra.

References

[1] Andry C, Wylde R, Laffite C, *et al*., 1982. Structures of verbascoside and orobanchoside caffeic acid sugar esters from *Orobanche rapumgenistae*. Phytochemistry, 21: 1123-1127.

[2] Tsuda Y, Haque E, 1983. Regioselective introduction of p-coumaroyl group to α-L-arabinopyranosides. Total synthesis of inundoside-G and inundoside-D1. Chemistry & Pharmaceutical Bulletin, 31: 1437-1439.

[3] Ishii H, Nakamura M, Seo S, *et al*., 1980. Isolation, characterization and nuclear magnetic spectra of new saponins from the roots of *Bupleurum falcatum* L. Chemistry & Pharmaceutical Bulletin, 28: 2367-2373.

[4] Gupta R, Paul S, Gupta AK, *et al*., 1997. Synthesis and biological activities of some 2-substituted phenyl-3-(3-alkyl/aryl-5,6-dihydro-s-triazolo[3,4-b][1,3,4]thiazolo-6-yl)-indoles. Indian Journal of Chemistry, 36: 707-710.

[5] Williams JM, Richardson AC, 1967. Selective acylation of pyranosides-I. Benzoylation of methyl α-D-glycopyranosides of mannose, glucose and galactose. Tetrahedron, 23: 1369-1378.

[6] Kondo Y, 1982. Selective benzoylation of methyl α- and β-D-xylopyranoside. Carbohydrate Research, 107: 303-311.

[7] Tsuda Y, Haque ME, Yoshimoto K, 1983. Biological activity of inundosides-D, F and G. Chemistry & Pharmaceutical Bulletin, 31: 1-12.
[8] Wagner D, Verheyden JPH, Moffatt JG, 1974. Preparation and synthetic utility of some organotin derivatives of nucleosides. Journal of Organic Chemistry, 39: 24-30.
[9] Sing R, Gupta BB, Mallik OM, *et al*., 1987. Studies on pesticides based on coumarin 1. Antifungal activity of 6-alkyl-3-n-butyl-7-hydroxy-4-methyl coumarins. Pesticide Science, 20: 125-130.
[10] Ghorab MM, Ismail ZH, Gaward SMA, *et al*., 2004. Antimicrobial activity of amino acid, imidazole and sulfonamide derivatives of pyrazole[3,4-d] pyrimidine. Heteroatom Chemistry, 15: 57-62.
[11] Kabir AKMS, Kawsar SA, Bhuiyan MMR, *et al*., 2003. Synthesis and characterization of some D-glucopyranose derivatives. Journal of the Bangladesh Academy of Sciences, 27: 149-159.
[12] Kabir AKMS, Dutta P, Kawsar SMA, *et al*., 2006. Regioselective 2-chlorobenzoylation of methyl α-D-mannopyranoside and related studies. Ceylon Journal of Sciences (Physical Sciences), 11: 57-62.
[13] Kabir AKMS, Kawsar SMA, Bhuiyan MMR, *et al*., 2008. Synthesis of some new derivatives of methyl 4,6-*O*-cyclohexylidene-α-D-glucopyranoside. Journal of the Bangladesh Chemical Society, 21: 72-80.
[14] Kabir AKMS, Kawsar SMA, Bhuiyan MMR, *et al*., 2003. Biological evaluation of some uridine derivatives. The Chittagong University Journal of Sciences, 27: 105-111.
[15] Kabir AKMS, Kawsar SMA, Bhuiyan MMR, *et al*., 2008. Biological evaluation of some octanoyl derivatives of methyl 4,6-*O*-cyclohexylidene-α-D-glucopyranoside. The Chittagong University Journal of Biological Sciences, 3: 53-64.
[16] Kabir AKMS, Kawsar SMA, Bhuiyan MMR, *et al*., 2009. Antimicrobial screening of some derivatives of methyl α-D-glucopyranosid. Pakistan Journal of Scientific and Industrial Research, 52: 138-142.
[17] Kabir AKMS, Rahman MS, Matin MM, *et al*., 2001. Antimicrobial activities of some D-glucose derivatives. The Chittagong University Journal of Sciences, 25: 123-128.
[18] Bauer AW, Kirby WMM, Sherris JC, *et al*., 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45: 439-476.
[19] Miah MAT, Ahmed HU, Sharma NR, *et al*., 1990. Antifungal activity of some plant extracts. Bangladesh Journal of Botany, 19: 05-10.
[20] Grover RK, Moore JD, 1962. Toximetric studies of fungicides against the brown rot organisms *Sclerotinia flucticola* and *S. laxa*. Phytopathology, 52: 876-880.
[21] Kabir AKMS, Dutta P, Kawsar SMA, *et al*., 2004. Biological evaluation of some derivatives of methyl-α-D-mannopyranoside. Bulletin of Pure and Applied Sciences, 23: 39-45.