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Synthesis, Characterization and Antibacterial Susceptibility of some Benzenesulfonyl and N-Acetylsulfanilyl Derivatives of Methyl α-D-Glucopyranoside

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ABSTRACT

A novel regioselective benzenesulphonylation and N-acetylsulfanilylation series of methyl α-D-glucopyranoside derivative (1) has been carried out by direct method and afforded the 6-O-benzenesulfonyl derivative (2) and 6-O-N-acetylsulfanilyl derivative (7) in an excellent yields. In order to obtain newer products, the 6-O-glucopyranoside derivative was further transformed to a series of 2,3,4-tri-O-acyl derivatives (3-6 and 8-11) containing a wide variety of functionalities in a single molecular framework. The structures of the newly synthesized compounds were elucidated with the aid of FTIR, ^{1}H-NMR spectroscopy and elemental analysis. All the synthesized compounds were also tested for their antibacterial activity against some human pathogenic bacterial microorganisms. For comparative studies, antibacterial activity of standard antibiotics, ampicillin was also carried out against these microorganisms. The study revealed that the acylated products exhibit moderate to good antimicrobial activities. It was interesting to observe that the selected compounds were more sensitive against gram-negative bacteria than that of the gram-positive bacterial strains. We carried out the antibacterial activities of the tested chemicals *in vitro*. *In vivo* screening studies of the tested compounds showing promising results will be the subject of our future investigation.

Key words: Synthesis, glucopyranoside, spectroscopy, antibacterial, susceptibility

INTRODUCTION

Carbohydrates have attracted an increasing amount of attention up to today on account of their diverse biological function. For example, specific protein-carbohydrate interactions are involved in cell differentiation (Wang *et al*., 2014), cell adhesion (Iniguez-Palomares *et al*., 2011), immune response (De Schutter and Van Damme, 2015) and tumor cell metastasis (Simone *et al*., 2013). These important processes occur between carbohydrates (glycoproteins, glycolipids and polysaccharide entities at cell surfaces) and lectins, proteins with carbohydrate-binding domains.

Carbohydrate derivatives have been widely used as cosmetic and pharmaceutical industries for many years because they are considered biocompatible, biodegradable and nontoxic and can be synthesized from renewable resources (Farran *et al*., 2015; Kadajji and Betageri, 2011). It is also used in medicine, for example as anticoagulants, antibiotics and vaccines (Liu and Linhardt, 2014; Song *et al*., 2015). Furthermore, carbohydrate derivatives have attracted considerable research interest in recent years because they have exhibited a variety of biological activities, including antioxidant, cytotoxic (Deng *et al*., 2015), antitumor (Il'ina and Varlamov, 2015; Sudha *et al*., 2014), antimicrobial and antifungal properties (Shen *et al*., 2012).

Regioselective chemical monoacylation of carbohydrates is not easy due to their multifunctionality (Ren *et al*., 2014) and frequently, protection/deprotection sequences are needed (Guo and Ye, 2010; Filice *et al*., 2012). Acetylation and acylation of alcohols is a basic and widely used transformation in organic chemistry (Sartori *et al*., 2004), primarily to synthetically protect hydroxyl groups and as an aid to structurally elucidate poly-hydroxyl containing natural products such as oligosaccharides. In the context of carbohydrate chemistry, fully acetylated monosaccharides are widely used as starting materials to synthesize oligosaccharides and glycoconjugates.

Of the carbohydrates isolated from natural sources, acyl glycoses and acyl glycosides have immense importance and some of them have effective biological activity (Gupta *et al*., 1997). It was found from the literature survey that nitrogen, sulphur and halogen containing heterocyclic compounds showed marked antimicrobial activities (Tsuda and Haque, 1983). When heterocyclic parts become attached to carbohydrates (Singh *et al*., 1987), their efficiency to inhibit bacteria or fungus sharply increased. A large number of biologically active compounds also possess aromatic, heteroaromatic and acyl substituents (Singh *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 (Singh *et al*., 1987).

From our previous works (Kawsar *et al*., 2013a, 2015; Kabir *et al*., 2008, 2009), it also observed that in many cases the combination of two or more acyl substituents in a single molecular framework enhances the biological profile manifold than their parent nuclei (Kabir *et al*., 2004, 2005a; Kawsar *et al*., 2012a). Encouraged by our own findings and also literature reports, it synthesized a series of methyl α-D-glucopyranoside (1) derivatives (Fig. 1 and 2) using direct method and deliberately incorporating a wide variety of probable biologically active components to the D-glucose moiety. Antibacterial screening of these newly synthesized compounds were also evaluated using a number of human pathogenic bacterial microorganism and the results are reported here as first time.

MATERIALS AND METHODS

General procedures: Melting temperatures were determined on an electrothermal melting temperature apparatus (England) and are uncorrected. Thin layer chromatography was performed on Kieselgel GF_{254} and visualization was effected by spraying the plates with 1% H_2SO_4 followed by heating at 150-200°C until colouration took place. Column chromatography was performed with silica gel G_{60}. ^{1}H-(400 MHz) NMR (Germany) spectra were recorded in $CDCl_3$ (TMS as an internal standard) by a Bruker spectrospin DPX-400 spectrophotometer. FTIR (SHIMADZU, Japan) spectra were recorded at the Department of Chemistry, University of Chittagong, Bangladesh. Evaporation was performed under reduced pressure on a Buchi rotary evaporator (Switzerland). All reagents used were commercially available (Aldrich) and were used as received, unless otherwise specified. The reaction pathways have been summarized in Fig. 1 and 2.

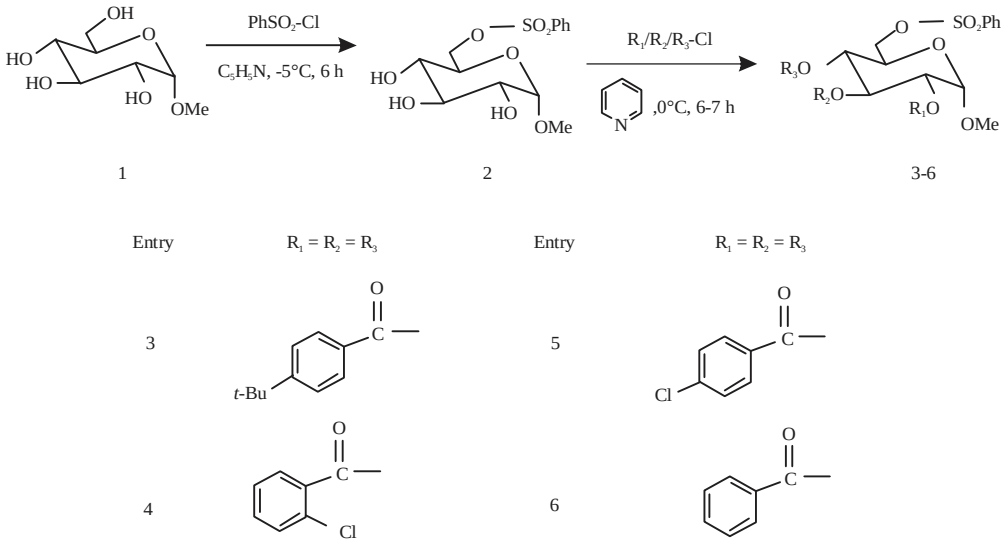

Fig. 1: Synthesis of methyl 6-O-benzenesulphonyl-α-D-glucopyranoside derivatives (3-6)

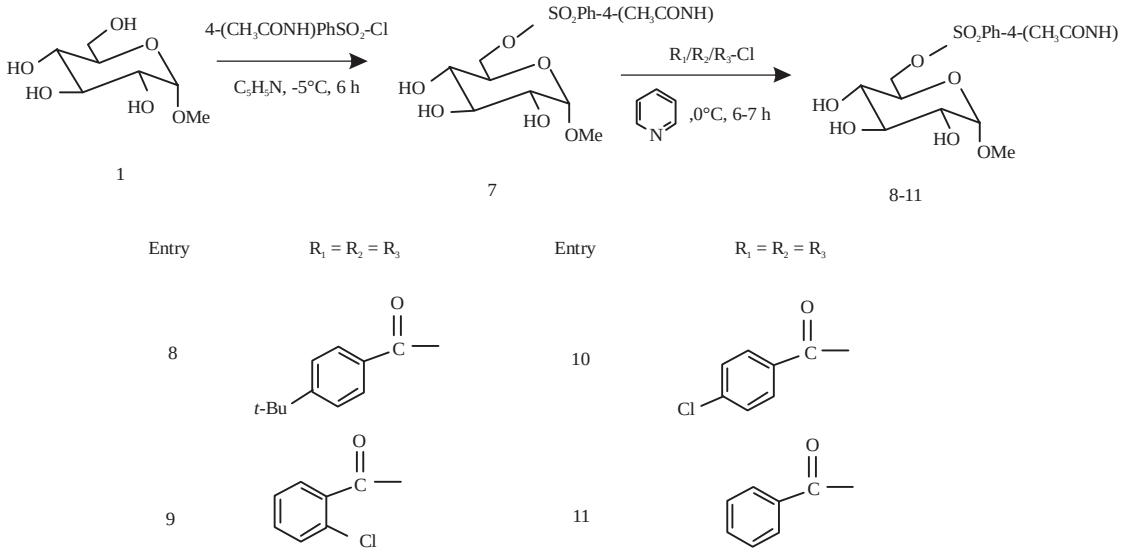

Fig. 2: Synthesis of methyl 6-O-N-acetylsulfanilyl-α-D-glucopyranoside derivatives (8-11)

Synthesis of methyl 6-O-benzenesulfonyl-α-D-glucopyranoside (2): A suspension of methyl-α-D-glucopyranoside (1 mg, 0.41 mmol), was made with dry pyridine (3 mL) in a round-bottom flask. It was then cooled to -5°C in an ice bath whereupon benzenesulfonyl chloride (0.10 mL, 1.1 M equivalent) was added. The reaction mixture was continuously stirred for 6 h at 0°C temperature and then the reaction mixture was stand for overnight at room temperature with continuous stirring. The progress of the reaction was monitored by TLC (methanol-chloroform, 1:16) which indicated full conversion of the starting material into a single product ($R_f = 0.53$). A few pieces of ice was added to the flask and then extracted the product mixture with chloroform (30 mL).

The combined chloroform layer was washed successively with dilute hydrochloric acid (10%), saturated aqueous $NaHCO_3$ solution and distilled water. The chloroform layer was dried with anhydrous $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure to leave syrup. The syrup was passed through a silica gel column and eluted with methanol-chloroform (1:16) to afford the 6-O-benzenesulfonyl derivative (2).

FTIR (KBr, cm^{-1}): ν = 1701 (C=O), 3510 (-OH), 1360 (-SO_2). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.90 (2H, m, Ar-H), 7.70 (1H, m, Ar-H), 7.50 (2H, m, Ar-H), 5.30 (1H, d, H-1), 4.51 (1H, dd, H-6a), 4.30 (1H, dd, H-6b), 4.21 (1H, t, H-4), 3.87 (1H, t, H-3), 3.70 (1H, dd, H-2), 3.49 (1H, ddd, H-5), 3.21 (3H, s, 1-OCH_3).

Anal calcd for $C_{13}H_{18}SO_8$: C 46.70; H 5.39; found C 46.73; H 5.43.

General procedure for the synthesis of benzenesulfonyl-α-D-glucopyranoside derivatives (3-6): A suspension of compound 2 (253 mg, 0.76 mmol), was made with dry pyridine (3 mL) in a flask. It was then cooled to -5°C in an ice bath whereupon 4-t-butylbenzoyl chloride (0.68 mL, 5 M equivalent) was added. The reaction mixture was then stirred for 6 h at 0°C temperature and then the reaction mixture was standing for overnight at room temperature with continuous stirring. The progress of the reaction was monitored by TLC (methanol-chloroform, 1:25) which indicated the complete conversion of the starting material into faster moving product (R_f = 0.53). Work-up as usual and purification by passage through a silica gel column chromatography with methanol-chloroform (1:25 as elutant) yielded the 4-tert-butylbenzoyl derivative (3) as a crystalline solid. Recrystallization from chloroform-hexane gave the 2,3,4-tri-O-(4-t-butylbenzoyl) derivative (3) as needless, m.p.148-152°C.

Similar reaction and purification procedure was applied to prepare compound 4 (MeOH-$CHCl_3$, 1:25), compound 5 (MeOH-$CHCl_3$, 1:22), compound 6 (MeOH-$CHCl_3$, 1:23).

Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-(4-t-butylbenzoyl)-α-D-glucopyranoside (3): FTIR (KBr, cm^{-1}): ν = 1687 (C = O), 1362 (-SO_2). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): *δ* 8.05 (2H, m, Ar-H), 7.97 (6H, m, Ar-H), 7.49 (2H, m, Ar-H), 7.45 (6H, m, Ar-H), 7.38 (1H, m, Ar-H), 5.38 (1H, d, H-1), 4.80 (1H, dd, H-2), 4.73 (1H, t, H-4), 4.69 (1H, t, H-3), 4.36 (1H, m, H-6a), 4.12 (1H, m, H-6b), 3.80 (1H, m, H-5), 3.33 (3H, s, 1-OCH_3), 1.33 {27H, s, 3×(CH3)3C-}.

Anal calcd for $C_{43}H_{54}SO_{11}$: C 66.32; H 6.94; found C 66.33; H 6.98.

Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-(2-chlorobenzoyl)-α-D-glucopyranoside (4): FTIR (KBr, cm^{-1}): ν = 1768, 1708 (-CO), 1362 cm^{-1} (-SO_2). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.88 (2H, m, Ar-H), 7.78 (3H, m, Ar-H), 7.68 (1H, m, Ar-H), 7.50 (2H, m, Ar-H), 7.41 (6H, m, Ar-H), 7.29 (3H, m, Ar-H), 5.21 (1H, d, H-1), 4.75 (1H, dd, H-2), 4.61 (1H, t, H-3), 4.30 (1H, t, H-4), 4.08 (1H, m, H-6a), 3.90 (1H, m, H-6b), 3.52 (1H, m, H-5), 3.31 (3H, s, 1-OCH_3).

Anal calcd for $C_{34}H_{27}SO_{11}$: C 63.45; H 4.20; found C 63.48; H 4.25.

Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-(4-chlorobenzoyl)-α-D-glucopyranoside (5): FTIR (KBr, cm^{-1}): ν = 1740, 1680 (C = O), 1360 (-SO_2). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.95 (6H, m, Ar-H), 7.58 (2H, m, Ar-H), 7.41 (6H, m, Ar-H), 7.38 (1H, m, Ar-H), 7.29 (2H, m, Ar-H), 5.41 (1H, d, H-1), 5.22 (1H, dd, H-2), 5.16 (1H, m, H-3), 4.26 (1H, t, H-4), 4.16 (1H, m, H-6a), 3.94 (1H, t, H-6b), 3.49 (1H, m, H-5), 3.36 (3H, s, 1-OCH_3).

Anal calcd for $C_{34}H_{27}SO_{11}$: C 63.45; H 4.20; found C 63.49; H 4.27.

Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (6): FTIR (KBr, cm^{-1}): ν = 1756, 1682 (C = O), 1365 (-SO_2). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): *δ* 8.12 (6H, m, Ar-H), 7.70 (3H, m, Ar-H), 7.51 (6H, m, Ar-H), 5.28 (1H, d, H-1), 5.11 (1H, m, H-2), 4.85 (1H, t, H-3), 4.69 (1H, t, H-4), 4.22 (1H, dd, H-6a), 4.12 (1H, dd, H-6b), 3.78 (1H, m, H-5), 3.31 (3H, s, 1-OCH_3).

Anal calcd for $C_{34}H_{30}SO_{11}$: C 63.16; H 4.64; found C 63.19; H 4.67.

Synthesis of methyl 6-O-N-acetylsulfanilyl-α-D-glucopyranoside (7): A stirred solution of methyl α-D-glucopyranoside (1) (200 mg, 1.03 mmol) in dry pyridine (3 mL) was cooled to -5°C where upon N-acetylsulfanilyl chloride (0.35 g, 1.5 M equivalent) was added to it. The reaction mixture was stirred at -5°C temperature for 8 h and then stirred overnight at room temperature. The progress of the reaction was monitored by TLC (methanol-chloroform, 1:8) which indicated the formation of one product, (R_f = 0.50). A few pieces of ice were added to the flask and then extracted the product mixture with chloroform (30 mL).

The combined chloroform layer was washed successively with dilute hydrochloric acid (10%), saturated aqueous sodium hydrogen carbonate ($NaHCO_3$) solution and distilled water. The chloroform layer was dried with anhydrous magnesium sulphate ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure to leave a syrup. The syrup was passed through a silica gel column chromatography and eluted with methanol-chloroform (1.8) provided the N-acetylsulfanilyl derivative (7).

FTIR (KBr, cm^{-1}): ν = 1738 (C=O), 3510 (-OH), 3320 (-NH), 1365 (-SO_2). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.70 (2H, d, Ar-H), 7.68 (2H, d, Ar-H), 7.53 (1H, s, -NH), 5.31 (1H, d, H-1), 4.50 (1H, dd, H-6a), 4.32 (1H, dd, H-6b), 4.09 (1H, t, H-4), 3.88 (1H, t, H-3), 3.76 (1H, dd, H-2), 3.52 1 (1H, ddd, H-5), 3.26 (3H, s, 1-OCH_3), 2.22 (3H, s, CH_3CON-).

Anal calcd for $C_{15}H_{21}SO_9$: C 47.75; H 5.57; found C 47.81; H 5.63.

General procedure for the synthesis of N-acetylsulfanilyl-α-D-glucopyranoside derivatives (8-11): To a rapidly stirred and cooled (-5°C) solution of the triol (7) (75 mg, 0.18 mmol) in dry pyridine (3 mL) was added acetyl chloride (0.18 mL, 5 molar eq.) was added. The reaction mixture was stirred at -5°C temperature for 8 h and then stirred overnight at room temperature. The progress of the reaction was monitored by TLC (methanol-chloroform, 1:16) which indicated the complete conversion of the starting material into faster moving product (R_f = 0.51). Work-up as described earlier and purification by silica gel column chromatography (with methanol-chloroform, 1:16, as eluant) afforded the acetyl derivative (8) (59 mg, 78.51%) as a semi solid which resisted crystallization.

Similar reaction, isolation and purification procedure was applied to prepare compound 9 (MeOH-$CHCl_3$, 1:16), compound 10 (MeOH-$CHCl_3$, 1:17) and compound 11 (MeOH-$CHCl_3$, 1:18).

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-(4-t-butylbenzoyl)-α-D-glucopyranoside (8): FTIR (KBr, cm^{-1}): ν = 1701 (C = O), 3320 (-NH), 1362 (-SO_2). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.98 (6H, m, Ar-H), 7.83 (2H, d, Ar-H), 7.71 (2H, d, Ar-H), 7.58 (1H, s, -NH), 7.47 (6H, m, Ar-H), 5.38 (1H, d, H-1), 4.78 (1H, m, H-2), 4.62 (1H, m, H-3), 4.55 (1H, m, H-4), 4.31 (1H, dd, H-6a), 4.27 (1H, dd, H-6b), 3.78 (1H, ddd, H-5), 3.35 (3H, s, 1-OCH_3), 2.25 (3H, s, CH_3CON-), 1.34 {27H, s, 3×$(CH_3)_3C$-}.

Anal calcd for $C_{48}H_{31}SO_{12}$: C 69.31; H 3.73; found C 69.35; H 3.77.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-(2-chlorobenzoyl)-α-D-glucopyranoside (9): FTIR (KBr, cm^{-1}): ν = 1698 (C = O), 3360 (-NH), 1361 ($-SO_2$). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.78 (3H, m, Ar-H), 7.70 (2H, d, Ar-H), 7.67 (2H, d, Ar-H), 7.59 (6H, m, Ar-H), 7.54 (1H, s, -NH), 7.48 (3H, m, Ar-H), 5.20 (1H, d, H-1), 4.73 (1H, m, H-2), 4.60 (1H, t, H-3), 4.45 (1H, t, H-4), 4.03 (1H, m, H-6a), 3.94 (1H, m, H-6b), 3.72 (1H, m, H-5), 3.33 (3H, s, 1-OCH_3), 2.22 (3H, s, CH_3CON-).

Anal calcd for $C_{36}H_{30}SO_{12}$: C 62.97; H 4.37; found C 62.99; H 4.41.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-(4-chlorobenzoyl)-α-D-glucopyranoside (10): FTIR (KBr, cm^{-1}): ν = 1700 (C = O), 3358 (-NH), 1359 ($-SO_2$). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 8.01 (6H, m, Ar-H), 7.96 (2H, d, Ar-H), 7.62 (6H, m, Ar-H), 7.40 (2H, d, Ar-H), 7.23 (1H, s, -NH), 5.70 (1H, d, H-1), 5.25 (1H, dd, H-2), 4.80 (1H, t, H-3), 4.41 (1H, t, H-4), 4.21 (1H, m, H-6a), 4.10 (1H, m, H-6b), 3.89 (1H, m, H-5), 3.38 (3H, s, 1-OCH_3), 2.21 (3H, s, CH_3CON-).

Anal calcd for $C_{36}H_{30}SO_{12}$: C 62.97; H 4.37; found C 63.1; H 4.43.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (11): FTIR (KBr, cm^{-1}): ν = 1765 (-CO), 3323 (-NH), 1362 cm^{-1} ($-SO_2$). ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 8.10 (6H, m, Ar-H), 7.71 (2H, d, Ar-H), 7.69 (3H, m, Ar-H), 7.65 (2H, d, Ar-H), 7.53 (1H, s, -NH), 7.49 (6H, m, Ar-H), 5.28 (1H, d, H-1), 5.11 (1H, m, H-2), 4.85 (1H, t, H-3), 4.69 (1H, t, H-4), 4.22 (1H, dd, H-6a), 4.12 (1H, dd, H-6b), 3.78 (1H, m, H-5), 3.31 (3H, s, 1-OCH_3), 2.21 (3H, s, CH_3CON-).

Anal calcd for $C_{33}H_{33}SO_{12}$: C 60.64; H 5.05; found C 60.67; H 5.09.

Biological studies: Antibacterial susceptibilities of the synthesized compounds (Fig. 1 and 2) were determined against four pathogenic bacterial strains e.g., *Bacillus cereus* BTCC 19, *Bacillus megaterium* BTCC 18, *Salmonella typhi* AE 14612 and *Salmonella paratyphi* AE 146313. The test tube cultures of the bacterial pathogens were collected from the Department of Microbiology, University of Chittagong, Bangladesh. In all cases, a 1% solution (in $CHCl_3$) of the chemicals and standard Nutrient Agar (NA) medium was used throughout the antibacterial study.

Antibacterial susceptibility: The *in vitro* antibacterial spectrums of the synthesized chemicals were done by disc diffusion method (Bauer *et al*., 1966) with little modification (Miah *et al*., 1990). 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 3-4 mm and after solidification of the agar medium the plates were transferred to an incubator at 37°C 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 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 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 h at low temperature (4-6°C) and the test chemicals diffused from disc to the surrounding medium. The plates were then incubated at 35±2°C for 24 h to allow maximum growth of the microorganisms. The antibacterial activity of the test agent was determined by measuring the mean diameter of zone of inhibitions (in millimeter). Each experiment was repeated thrice. All the results were compared with the standard antibacterial antibiotic Ampicillin (20 µg/disc, BEXIMCO Pharm. Bangladesh Ltd).

RESULTS AND DISCUSSION

Synthesis: Carbohydrates are key molecules in nature with multiple roles in biological processes. For a long time, carbohydrates have been very attractive field for scientists due to their immense importance in biological systems (Appelt *et al*., 2013; Taylor and Drickamer, 2011; Varki *et al*., 2009). They are the source of the metabolic energy supply but also for the fine-tuning of cell-cell interactions and other crucial processes. As a consequence, the chemistry and biochemistry of carbohydrate derivatives is an essential part of biochemical and medicinal research. Owing to the many functional groups and the configurational variety, the number of possible carbohydrate derivatives is huge. Therefore, the synthesis of carbohydrate derivatives is complicated, generally requiring many steps and a range of selectivity problems has to be solved. So, selective acylation is very important in the field of carbohydrate chemistry because of its usefulness for the synthesis of biologically active products. Protection of a particular functional group of an organic compound is not only necessary for the modification of properties of the remaining functional groups but also for the synthesis of newer derivatives of great importance.

The main aim of the present work reported here was to study selective benzenesulphonylation (Fig. 1) and N-acetylsulfanilylation (Fig. 2) of methyl α-D-glucopyranoside (1) using some non-traditional acylating agents namely, 4-t-butylbenzoyl chloride, 2-chlorobenzoyl chloride, 4-chlorobenzoyl chloride and benzoyl chloride. According to the well-known method i.e., direct acylation method (Kawsar *et al*., 2015; Kabir *et al*., 2005b), two series (Fig. 1 and 2) were synthesized using methyl α-D-glucopyranoside as the starting material. The acylated mono-substituted 2,3,4-tri-O-acyl derivatives (3-6 and 8-11) were synthesized from the corresponding substituted 6-O-benzenesulfonyl (2) and 6-O-N-acetylsulfanilyl (7) derivatives as previously described (Kawsar *et al*., 2014a). The structure of the various suitably substituted monosaccharide derivatives were ascertained by analyzing their FTIR (Taleb-Mokhtari *et al*., 2016; Brauer *et al*., 2011), ^{1}H-NMR (Loss and Lutteke, 2015; Ojha *et al*., 2013) spectra, elemental analysis and physicochemical properties (Table 1). All the acylation products were employed as test chemicals for antibacterial susceptibility studies against a number of human pathogenic bacteria.

Spectral characterization of benzenesulfonyl-α-D-glucopyranoside derivatives

Chemistry: Our initial effort was to carry out regioselective benzenesulphonylation of methyl α-D-glucopyranoside (1) (Fig. 1). A number of derivatives of the resulting benzenesulphonylation product were also prepared in order to achieve supportive evidences for structure elucidation and also to obtain newer derivatives of synthetic and biological importance. The treatment of methyl α-D-glucopyranoside (1) with 1.1 M equivalent of benzenesulfonyl chloride in pyridine under freezing conditions, followed by usual work up and separation by silica gel column chromatography, afforded compound 2. The structure of the benzenesulfonyl derivative (2) was established by

Table 1: Physicochemical data of synthesized D-glucopyranoside derivatives (2-11)

Compound No.	RT (h)	R_f	Yield (mg)	Percentage	State/mp (°C)
2	6.0	0.50	70	48.0	Needles, 130-132
3	6.5	0.51	61	84.3	Needles, 149-151
4	6.5	0.52	91	67.6	Needles, 146-148
5	6.0	0.50	89	90.0	Needles, 138-140
6	6.5	0.53	87	78.0	Semi-solid
7	6.0	0.53	98	71.5	Semi-solid
8	6.5	0.54	99	91.0	Needles, 139-140
9	6.5	0.52	63	78.5	Thick syrup
10	6.0	0.55	53	84.4	Needles, 142-144
11	6.0	0.52	62	87.6	Needles, 135-137

RT: Retention time

analyzing its IR and ^{1}H-NMR spectroscopy. The FTIR spectrum showed absorption bands at 1701, 3510 and 1360 cm^{-1}, thereby suggesting the presence of carbonyl, hydroxyl and sulfonyl groups in the molecule. The IR spectra of the synthesized compounds are similar to the IR values of which were stated in the study of (Taleb-Mokhtari *et al*. (2016) and Brauer *et al*. (2011). In its ^{1}H-NMR spectrum, the peaks at δ 7.90 (2H, m), δ 7.70 (1H, m) and δ 7.50 (2H, m) corresponded the protons of one phenyl group. The downfield shift of H-6 to *δ* 4.51 (as dd, J = 4.8 and 10.2 Hz, 6a) and 4.30 (as dd, J = 2.0 and 12.0 Hz, 6b) from its usual value (~4.00 ppm) indicated the introduction of the benzenesulfonyl group at position 6. The ^{1}H-NMR spectrum were found to show very similar which was in accordance with our previous work (Kawsar *et al*., 2012c, 2014b). Complete analysis of the FTIR and ^{1}H-NMR spectrum suggested that the structure of this compound may be assigned as methyl 6-*O*-benzenesulfonyl-α-D-glucopyranoside (2).

It then allowed the triol (2) to react with 4-t-butylbenzoyl chloride as usual work-up and chromatographic purification, yielded 4-t-butylbenzoate (3). The FTIR spectrum indicated absorption bands at 1687 cm^{-1} corresponding to carbonyl (-CO) and 1362 cm^{-1} to sulfonyl (-SO_2) stretchings. Its ^{1}H-NMR spectrum displayed two six-proton multiplet peaks at δ 7.97 (as m, Ar-H), δ 7.45 (as m, Ar-H) and a 27 proton singlet at δ 1.33 {as s, 3×$(CH_3)_3$C-} which corresponded to the presence of three 4-t-butylbenzoyl groups in the compound. The deshielding of C-2, C-3 and C-4 protons to δ 4.80 (as dd, J=3.6 and 10.6 Hz), δ 4.69 (as t, J = 9.2 Hz) and δ 4.73 (as t, J = 9.6 Hz) from their usual values. So, the above information led us to establish its structure as methyl 6-O-benzenesulfonyl-2,3,4-tri-O-(4-t-butylbenzoyl)-α-D-glucopyranoside (3).

Encouraged by the results obtained by above acylation of the triol (2), It then used 2-chlorobenzoyl chloride as the next acylating agent and isolated compound 4. The FTIR spectrum of this compound showed absorption bands at 1768 and 1708 cm^{-1} for carbonyl and 1362 cm^{-1} due to -SO_2 stretchings. In the ^{1}H-NMR spectrum, the peaks at δ 7.78 (3H, m), δ 7.41 (6H, m) and δ 7.29 (3H, m) were due to the aromatic protons of three 2-chlorobenzoyl group. The deshielding of C-2, C-3 and C-4 protons to *δ* 4.75 (as dd, J = 3.4 and 10.2 Hz), δ 4.61 (as t, J = 9.4 Hz) and δ 4.30 (as t, J = 9.6, Hz) from their precursor triol (2) values. By the analysis of the rest of the IR and ^{1}H-NMR spectrum of this compound assigned as methyl 6-O-benzenesulfonyl-2,3,4-tri-O-(2-chlorobenzoyl)-α-D-glucopyranoside (4).

4-Chlorobenzoylation of triol (2) using 4-chlorobenzoyl chloride similar purification techniques, the product (5) was isolated as crystalline solid. In its ^{1}H-NMR spectrum, the two six-aromatic proton multiplet at δ 7.95 (as m), δ 7.41 (as m) are characteristic of *p*-substituted benzoyl groups. The deshielding of C-2, C-3 and C-4 protons from their usual values (compound 2) and the resonance of other protons in their anticipated positions confirmed the structure of this compound as methyl 6-O-benzenesulfonyl-2,3,4-tri-O-(4-chlorobenzoyl)-α-D-glucopyranoside (5). Final confirmation of the structure of compound 2 was provided by preparation of its benzoyl derivative (6). Reaction of compound 2 with benzoyl chloride in pyridine, gave the benzoyl derivative (6). In its ^{1}H-NMR spectrum the aromatic proton peaks at δ 8.12 (as 6H, m), δ 7.70 (as 3H, m), δ 7.51 (as 6H, m) corresponded to three benzoyl groups present in the compound. The H-2, H-3 and H-4 protons resonated shifted downfield from their precursor compound (2), which were indicative of the attachment of the benzoyl group at C-2, C-3 and C-3 positions. The FTIR and ^{1}H-NMR spectrum of this compound was in complete agreement with the structural assigned as methyl 6-O-benzenesulfonyl-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (6).

Spectral characterization of N-acetylsulfanilyl-α-D-glucopyranoside derivatives

Chemistry: Next, it applied the direct method for selective N-acetylsulfanilylation of methyl α-D-glucopyranoside (1) using a rarely used acylating agent N-acetylsulfanilyl chloride (Fig. 2). Reaction of methyl α-D-glucopyranoside (1) with an equimolecular amount of N-acetylsulfanilyl chloride in dry pyridine at -5°C, followed by removal of solvent and silica gel column chromatographic purification, afforded the N-acetylsulfanilyl derivative (7).

When triol (7) was allowed to react with 4-t-butylbenzoyl chloride in dry pyridine, followed by conventional work-up procedure, it yielded 4-t-butylbenzoate (8). The FTIR spectrum bands at 1701 cm^{-1} corresponding to -CO, 3320 cm^{-1} corresponding to -NH and 1362 cm^{-1} to -SO_2 stretchings. Its ^{1}H-NMR spectrum exhibited aromatic two six-proton multiplet peaks at δ 7.98 (as m), δ 7.47 (as m) and a 27 proton singlet at δ 1.34 {as s, 3×$(CH_3)_3$C-} which corresponded to the presence of three 4-t-butylbenzoyl groups in the molecule. The deshielding of H-2, H-3 and H-4 protons to δ 4.78 (as m), δ 4.62 (as m) and δ 4.55 (as m) from their precursor compound 7 established the attachment of the three 4-t-butylbenzoyl groups at these positions. Therefore, by analysis of the rest of FTIR and ^{1}H-NMR spectrum supported the structure assigned as methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-(4-t-butylbenzoyl)-α-D-glucopyranoside (8).

Derivatization of compound 7 with 2-chlorobenzoyl chloride and the FTIR spectrum of this compound showed absorption bands at 1698 cm^{-1} for carbonyl, 3360 cm^{-1} for -NH and 1361 cm^{-1} for -SO_2 stretchings. In the ^{1}H-NMR spectrum the peaks at δ 7.78 (3H, m), δ 7.59 (6H, m) and δ 7.48 (3H, m) were due to the aromatic protons of three 2-chlorobenzoyl group. The deshielding of H-2, H-3 and H-4 protons to δ 4.73 (as m), δ 4.60 (as t, J = 9.4 Hz) and δ 4.45 (as t, J = 9.6 Hz) from their values in the precursor compound 7 confirmed the attachment of the three 2-chlorobenzoyl groups. The structure of this compound assigned as methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-(2-chlorobenzoyl)-α-D-glucopyranoside (9). 4-Chlorobenzoylation of N-acetylsulfanilyl derivative (7) and after similar work-up techniques, the 4-chlorobenzoyl derivative (10). Its FTIR spectrum showed absorption peaks at 1700, 3358 and 1359 cm^{-1} corresponding to -CO, -NH and -SO_2 stretchings, respectively. In its ^{1}H-NMR spectrum the two six-aromatic proton multiplet at δ 8.01 (as m), δ 7.62 (as m) are characteristic of p-substituted benzoyl groups. The deshielding of H-2, H-3 and H-4 protons from their precursor compound (7) and the resonance of other protons in their anticipated positions confirmed the structure of this compound as methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-(4-chlorobenzoyl)-α-D-glucopyranoside (10).

Final confirmation of the structure of compound 7 was provided by preparation of its benzoyl derivative (11). Reaction of compound 7 with benzoyl chloride in pyridine, followed by conventional work-up procedure, gave the benzoyl derivative (11) in good yields,. In its ^{1}H-NMR spectrum the aromatic proton peaks at δ 8.10 (as 6H, m), δ 7.69 (as 3H, m), δ 7.49 (as 6H, m) corresponded to three benzoyl groups present in the compound. The H-2, H-3 and H-4 protons resonated at d 5.11 (as m), d 4.85 (as t, J = 9.7 Hz) and d 4.69 (as t, J = 9.7 Hz) shifted downfield from their precursor compound (7), which were indicative of the attachment of the benzoyl group at C-2, C-3 and C-3 positions. On the basis of complete analysis of the FTIR and ^{1}H-NMR spectra of this compound was accorded as methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (11).

All the compounds thus prepared were employed as test chemicals for evaluating their antibacterial activities against a number of human pathogenic bacteria.

Evaluation of antibacterial susceptibility: The antibacterial susceptibility results of the test compounds and the standard antibiotic, ampicillin against gram-positive and gram-negative bacteria are listed in Table 2. As shown in Table 2 the test chemicals 2, 3, 4, 6, 8, 9 and 11 were

Table 2: Zone inhibition observed against gram-positive and gram-negative test bacteria by the test compounds

	Zone of inhibition (mm) at 200 µg dw/disc			
	Gram-positive bacteria		Gram-negative bacteria	
Compound No.	*B. cereus*	*B. megaterium*	*S. typhi*	*S. paratyphi*
2	6.5	6.5	NA	NA
3	6.5	6.5	6	6
4	6	7	*10	7.5
5	8	NA	6	6
6	7	6	NA	NA
7	NA	NA	6.5	6.5
8	7	6.5	6.5	6.5
9	7.5	8	6	7
10	NA	NA	6	7
11	7	6.5	*10	8.5
**Ampicillin	*19	*16	*20	*18

*Marked inhibition, **Standard antibiotic, NA: Not active, dw: Dry weight

found to inhibit the growth of all the Gram-positive bacteria. The inhibition zone of *B. cereus* by 5 (8 mm), 9 (7.5 mm) and of *B. megaterium* by 9 (8 mm), 4 (7 mm) were exhibited prominent susceptibility. All the gram-positive bacteria were insensitive towards the test chemicals 7 and 10. From the Table 1 it is clear that except two test chemicals e.g., 2, 6 all other test chemicals were prone to inhibit the growth of gram-negative bacteria. The inhibitions of growth of bacteria were very remarkable in many cases. Of the test chemicals 3, 4, 5, 7, 8, 9, 10 and 11 were very effective towards the inhibition of growth of maximum gram-negative bacterial strains used. The zone of inhibition of the growth of *S. typhi* by 4 (10 mm), 11 (10 mm) and of *S. paratyphi* by 4 (7.5 mm), 11 (8.5 mm) were very high.

Thus we observed that the high antimicrobial efficiency of the test chemicals were due to the presence of different acyl groups like 2-chlorobenzoyl, 4-chlorobenzoyl etc., groups. As seen in our previous investigations (Kawsar *et al*., 2012b, 2013b, c) the presence of same acyl groups in the test chemicals increased the antimicrobial capacity, here in this investigation we also found that the 2-chlorobenzoyl, 4-chlorobenzoyl acyl groups improved the antimicrobial power of the test chemicals. These highly efficient antimicrobiotic agents can be chosen for the further work on the development of medicines for human disease control. So, it is hoped that the acylated methyl α-D-glucopyranoside derivatives (Fig. 1 and 2) might show potential antiviral, antituberculatic and anti-inflammatory activities.

CONCLUSION

In conclusion, it have explored the synthesis, characterization and antibacterial screening studies of some acylated methyl α-D-glucopyranoside (1) derivatives obtained from the direct acylation method. This method demonstrates a very simple reaction procedure, shorter reaction time, good to excellent yields of products and efficient for the synthesis. Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-(4-chlorobenzoyl)-α-D-glucopyranoside (5) and methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-(4-t-butylbenzoyl)-α-D-glucopyranoside (8) were found to be encouraging in terms of high selectivity and excellent yields as 90 and 91%, respectively. A good number of test compounds reported herein exhibited promising antibacterial activity. This piece of work, in our opinion has created an opportunity for further work with these test compounds, ultimately leading to develop new pesticides/medicines for human disease.

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