

## Benzenesulfonylation of Methyl $\alpha$ -D-Glucopyranoside: Synthesis, Characterization and Antibacterial Screening

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**ABSTRACT.** A novel series of benzenesulfonyl derivatives of methyl  $\alpha$ -D-glucopyranoside (**1**) were synthesized by reacting benzenesulfonyl chloride in pyridine followed by direct acylation method to yield 6-*O*-benzenesulfonyl derivative (**2**). In order to obtain newer products for antibacterial evaluation studies, the 6-*O*-benzenesulfonyl derivative was further transformed to a series of 2,3,4-tri-*O*-acyl derivatives (**3-11**) containing a wide variety of functionalities in a single molecular framework. All the synthesized compounds have been confirmed by IR, <sup>1</sup>H NMR and elemental analysis. These newly synthesized compounds were screened for *in vitro* antibacterial activity against some human pathogenic bacterial strains. The study revealed that the acylated products exhibit moderate to good antibacterial activities. It was interesting to observe that the selected compounds were more sensitive against Gram-ve bacteria than that of the Gram-+ve bacterial strains.

### 1. INTRODUCTION

Carbohydrates are known to be involved in a wide range of biological and pathological processes [1, 2], including cancer metastasis, cell adhesion, cell signaling, embryo development, egg fertilization, protein function regulation, cellular communications and so on [3-6]. Four major classes of macromolecules in biology are DNA, proteins, carbohydrates, and lipids. Among these, carbohydrates allow almost unlimited structural variations. Although carbohydrates can be present without being attached to other molecules, the majority of carbohydrates present in cells are attached to proteins or lipids and the terminology glycoprotein and glycolipid is used to reflect this. Glycoproteins and glycolipids are major components of the outer surface of mammalian cells. They are fundamental to many important functions including immune defense, viral replication, parasitic infection, degradation of blood clots and inflammation [7, 8].

Carbohydrates, specially monosaccharides, lend themselves to us to study on the relative reactivity of various hydroxyl groups at different positions. Using the idea of relative reactivity and reaction sequence that clearly display the dexterity of the modern carbohydrate chemist, a broad range of biologically active natural products can be synthesized. Various methods for acylation of carbohydrates and nucleosides have so far been developed and employed successfully [9-13]. Of these, the direct method is considered as one of the most effective for selective acylation of carbohydrates. A considerable number of heterocyclic compounds are known to be bioactive. They display antibacterial [14], anti-inflammatory [15] and antimicrobial [16] activities. Compounds having amino acid and sulfonamide moieties are also known to possess a wide range of antibacterial and antifungal activities [17]. In the field of carbohydrate chemistry, acylated glycosides were

considered as very important test chemicals due to their biological activity [10]. Literature survey revealed that a wide variety of biologically active substances contain aromatic, heteroaromatic and acyl substituents [18].

It is also known that the combination of two or more potent acyl substituents in a single molecular framework enhances the biological profile many-fold than its parent nuclei [19-25]. The benzene and substituted benzene nuclei play important role as common denominator for various biological activities. In the context of our studies, we observed that some acylated derivatives of L-lyxose [26], D-glucose [27], D-mannose [28] and L-rhamnose [29] also exhibited effective antibacterial and antifungal activities.

Inspired by our own findings and also literature reports, we synthesized some derivatives of methyl  $\alpha$ -D-glucopyranoside (**Scheme 1**) containing a benzene moiety and various acyl groups (e.g. acetyl, pentanoyl, hexanoyl, pivaloyl, methanesulfonyl, decanoyl, lauroyl, myristoyl and palmitoyl) in a single molecular framework. Antibacterial screening of these compounds were also carried out using a variety of bacterial strains.

## 2. MATERIALS AND METHODS

All reagents used were commercially available (Sigma-Aldrich) and were used as received, unless otherwise specified. IR spectra were recorded on a FTIR spectrophotometer (SHIMADZU) using KBr and  $\text{CHCl}_3$  technique.  $^1\text{H-NMR}$  (400 MHz) spectra were recorded for solutions in  $\text{CDCl}_3$  using TMS as internal standard with a Bruker DPX-400 spectrometer. Evaporations were carried out under reduced pressure using VV-1 type vacuum rotary evaporator (GERMANY). Melting points were determined on an electro-thermal melting point apparatus (ENGLAND) and are uncorrected. Column chromatography was performed with silica gel G<sub>60</sub>. Thin layer chromatography (t.l.c) was performed on Kieselgel GF<sub>254</sub>. The reaction pathways have been summarized in **Scheme 1**.

### 2.1. Procedure for the synthesis of methyl 6-*O*-benzenesulfonyl- $\alpha$ -D-glucopyranoside (**2**)

A suspension of methyl- $\alpha$ -D-glucopyranoside (**1** mg, 0.41 mmol), was made with dry pyridine (3 ml) in a round bottle flask. It was then cooled to  $-5^\circ\text{C}$  in an ice bath whereupon benzenesulfonyl chloride (0.10 ml, 1.1 molar eq.) was added. The reaction mixture was continuously stirred for 6 hours at  $0^\circ\text{C}$  temperature and then the reaction mixture was stand for overnight at room temperature with continuous stirring. The progress of the reaction was monitored by t.l.c which indicated full conversion of the starting material into a single product. 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  $\text{NaHCO}_3$  solution and distilled water. The chloroform layer was dried with anhydrous  $\text{MgSO}_4$ , 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**.

**Methyl 6-*O*-benzenesulfonyl- $\alpha$ -D-glucopyranoside (**2**):** Yield 43% as a solid mass which resisted crystallization.  $R_f = 0.53$  (MeOH: $\text{CHCl}_3$ , 1:16).  $^1\text{H NMR}$  data ( $\text{CDCl}_3$ , 400 MHz):  $\delta_{\text{H}}$  7.90 (2H, m, Ar-H), 7.70 (1H, m, Ar-H), 7.50 (2H, m, Ar-H), 5.30 (1H, d,  $J = 3.7$  Hz, H-1), 4.51 (1H, dd,  $J = 4.8$  and 10.2 Hz, H-6a), 4.30 (1H, dd,  $J = 2.0$  and 12.0 Hz, H-6b), 4.21 (1H, t,  $J = 9.7$  Hz, H-4), 3.87 (1H, t,  $J = 9.6$  Hz, H-3), 3.70 (1H, dd,  $J = 3.6$  and 10.2 Hz, H-2), 3.49 (1H, ddd,  $J = 2.9$ , 9.8 and 12.8 Hz, H-5), 3.21 (3H, s, 1- $\text{OCH}_3$ ). Anal. Calcd for  $\text{C}_{13}\text{H}_{18}\text{SO}_8$ : C, 46.72; H, 5.40%; found C, 46.70; H, 5.39.

### 2.2. General procedure for the synthesis of benzenesulfonyl derivatives (**3-11**)

A stirred solution of compound **2** (87 mg, 0.26 mmol), was made with dry pyridine (3 ml) in a R.B. It was then cooled to  $-5^\circ\text{C}$  in ice bath whereupon acetic anhydride (0.12 ml, 5 molar eq.) was

added. The reaction mixture was continuously stirred for 5 hrs at 0<sup>0</sup>C temperature and then the reaction mixture was standing for overnight at room temperature with continuous stirring. Work-up as usual and purification by passage through a silica gel column chromatography with methanol-chloroform yielded the triacetate compound **3**. Similar reaction and purification procedure were applied to prepare tripentanoate compound **4**, trihexanoate compound **5**, tripivaloate **6**, trimesylate **7**, tridecanoate **8**, trilauroate **9**, trimyristoate **10** and tripalmitoate **11**.

**Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-acetyl- $\alpha$ -D-glucopyranoside (3):** Yield 71% as a pasty mass which resisted crystallization.  $R_f = 0.50$  (MeOH:CHCl<sub>3</sub>, 1:25). <sup>1</sup>H NMR data (CDCl<sub>3</sub>, 400 MHz):  $\delta_H$  7.87 (2H, m, Ar-H), 7.63 (1H, m, Ar-H), 7.57 (2H, m, Ar-H), 5.38 (1H, d, J = 3.6 Hz, H-1), 4.91 (1H, dd, J = 3.6 and 10.0 Hz, H-2), 4.80 (1H, t, J = 9.6 Hz, H-3), 4.75 (1H, t, J = 9.7 Hz, H-4), 4.38 (1H, dd, J = 5.0 and 12.1 Hz, H-6a), 4.10 (1H, dd, J = 2.0 and 12.1 Hz, H-6b), 3.91 (1H, m, H-5), 3.32 (3H, s, 1-OCH<sub>3</sub>), 2.06, 2.00, 1.98 (3 $\times$ 3H, 3 $\times$ s, 3 $\times$ CH<sub>3</sub>CO-). Anal. Calcd for C<sub>19</sub>H<sub>24</sub>SO<sub>11</sub>: C, 55.35; H, 5.88; found C, 55.33; H, 5.83.

**Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-pentanoyl- $\alpha$ -D-glucopyranoside (4):** Yield 87% as a semi-solid which resisted crystallization.  $R_f = 0.55$  (MeOH:CHCl<sub>3</sub>, 1:25). <sup>1</sup>H NMR data (CDCl<sub>3</sub>, 400 MHz):  $\delta_H$  7.89 (1H, m, Ar-H), 7.62 (1H, m, Ar-H), 7.53 (2H, m, Ar-H), 7.25 (1H, m, Ar-H), 5.41 (1H, d, J = 3.7 Hz, H-1), 4.90 (1H, dd, J = 3.7 and 10.1 Hz, H-2), 4.81 (1H, t, J = 9.5 Hz, H-3), 4.73 (1H, t, J = 9.6 Hz, H-4), 4.40 (1H, m, H-6a), 4.02 (1H, dd, J = 2.1 and 12.0 Hz, H-6b), 3.95 (1H, m, H-5), 3.33 (3H, s, 1-OCH<sub>3</sub>), 2.34 {6H, m, 3 $\times$ CH<sub>3</sub>(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CO-}, 1.61 {6H, m, 3 $\times$ CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO-}, 1.37 {6H, m, 3 $\times$ CH<sub>3</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>CO-}, 0.91 {9H, m, 3 $\times$ CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>CO-}. Anal. Calcd for C<sub>28</sub>H<sub>42</sub>SO<sub>11</sub>: C, 62.48; H, 7.84; found C, 62.45; H, 7.81.

**Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-hexanoyl- $\alpha$ -D-glucopyranoside (5):** Yield 72% as a solid mass which resisted crystallization.  $R_f = 0.54$  (MeOH:CHCl<sub>3</sub>, 1:25). <sup>1</sup>H NMR data (CDCl<sub>3</sub>, 400 MHz):  $\delta_H$  7.93 (2H, m, Ar-H), 7.63 (1H, m, Ar-H), 7.52 (2H, m, Ar-H), 5.40 (1H, d, J = 3.6 Hz, H-1), 4.92 (1H, dd, J = 3.5 and 10.2 Hz, H-2), 4.79 (1H, t, J = 9.5 Hz, H-3), 4.75 (1H, t, J = 9.6 Hz, H-4), 4.38 (1H, dd, J = 4.8 and 10.2 Hz, H-6a), 4.08 (1H, dd, J = 2.0 and 12.0 Hz, H-6b), 3.95 (1H, m, H-5), 3.30 (3H, s, 1-OCH<sub>3</sub>), 2.21 {6H, m, 3 $\times$ CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>CO-}, 1.51 {6H, m, 3 $\times$ (CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO-}, 1.25 {12H, m, 3 $\times$ CH<sub>3</sub>(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO-}, 0.83 {9H, m, 3 $\times$ CH<sub>3</sub>(CH<sub>2</sub>)<sub>4</sub>CO-}. Anal. Calcd for C<sub>31</sub>H<sub>48</sub>SO<sub>11</sub>: C, 64.18; H, 8.30; found C, 64.14; H, 8.28.

**Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-pivaloyl- $\alpha$ -D-glucopyranoside (6):** Yield 77% as a pasty mass which resisted crystallization.  $R_f = 0.54$  (MeOH:CHCl<sub>3</sub>, 1:25). <sup>1</sup>H NMR data (CDCl<sub>3</sub>, 400 MHz):  $\delta_H$  7.91 (2H, m, Ar-H), 7.70 (1H, m, Ar-H), 7.52 (2H, m, Ar-H), 5.50 (1H, d, J = 3.4 Hz, H-1), 4.91 (1H, dd, J = 3.6 and 10.0 Hz, H-2), 4.80 (1H, t, J = 9.6 Hz, H-3), 4.61 (1H, t, J = 9.6 Hz, H-4), 4.52 (1H, m, H-5), 4.0 (2H, m, H-6a and H-6b), 3.30 (3H, s, 1-OCH<sub>3</sub>), 1.20 {27H, s, 3 $\times$ (CH<sub>3</sub>)<sub>3</sub>CCO-}. Anal. Calcd for C<sub>28</sub>H<sub>24</sub>SO<sub>11</sub>: C, 64.68; H, 4.63; C, 64.62; H, 4.61.

**Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-methanesulfonyl- $\alpha$ -D-glucopyranoside (7):** Yield 65% as a semi-solid mass.  $R_f = 0.56$  (MeOH:CHCl<sub>3</sub>, 1:24). <sup>1</sup>H NMR data (CDCl<sub>3</sub>, 400 MHz):  $\delta_H$  7.95 (1H, m, Ar-H), 7.88 (1H, m, Ar-H), 7.72 (1H, m, Ar-H), 7.62 (2H, m, Ar-H), 5.30 (1H, d, J = 3.5 Hz, H-1), 5.10 (1H, dd, J = 3.5 and 10.2 Hz, H-2), 4.62 (1H, t, J = 9.5 Hz, H-3), 4.48 (1H, t, J = 9.6 Hz, H-4), 4.21 (1H, dd, J = 5.0 and 12.1 Hz, H-6a), 3.92 (1H, dd, J = 2.0 and 12.0 Hz, H-6b), 3.63 (1H, ddd, J = 2.9, 9.8 and 12.7 Hz, H-5), 3.40 (3H, s, 1-OCH<sub>3</sub>), 3.19, 3.11, 3.08 (3 $\times$ 3H, 3 $\times$ s, 3 $\times$ CH<sub>3</sub>SO<sub>2</sub>-). Anal. Calcd for C<sub>16</sub>H<sub>24</sub>S<sub>4</sub>O<sub>14</sub>: C, 33.83; H, 4.26; C, 33.80; H, 4.23.

**Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-decanoyl- $\alpha$ -D-glucopyranoside (8):** Yield 86% as a thick mass which resisted crystallization.  $R_f = 0.56$  (MeOH:CHCl<sub>3</sub>, 1:25). <sup>1</sup>H NMR data (CDCl<sub>3</sub>, 400 MHz):  $\delta_H$  7.87 (2H, m, Ar-H), 7.65 (1H, m, Ar-H), 7.52 (2H, m, Ar-H), 5.39 (1H, d, J = 3.6 Hz, H-1), 4.88 (1H, dd, J = 3.6 and 10.0 Hz, H-2), 4.81 (1H, t, J = 9.6 Hz, H-3), 4.40 (1H, t, J = 9.6 Hz, H-4), 4.08 (1H, dd, J = 4.8 and 10.0 Hz, H-6a), 3.98 (1H, t, J = 10.0 Hz, H-6b), 3.63 (1H, m, H-5), 3.33 (3H, s, 1-OCH<sub>3</sub>), 2.31 {6H, m, 3 $\times$ CH<sub>3</sub>(CH<sub>2</sub>)<sub>7</sub>CH<sub>2</sub>CO-}, 1.61 {6H, m,

$3 \times \text{CH}_3(\text{CH}_2)_6\text{CH}_2\text{CH}_2\text{CO}-$ }, 1.23 {36H, m,  $3 \times \text{CH}_3(\text{CH}_2)_6\text{CH}_2\text{CH}_2\text{CO}-$ }, 0.86 {9H, m,  $3 \times \text{CH}_3(\text{CH}_2)_8\text{CO}-$ }. Anal. Calcd for  $\text{C}_{43}\text{H}_{54}\text{S}_4\text{O}_{14}$ : C, 70.71; H, 7.44; C, 70.68; H, 7.40.

**Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-lauroyl- $\alpha$ -D-glucopyranoside (9):** Yield 77%. mp 150-152°C (decomp.).  $R_f = 0.58$  (MeOH:CHCl<sub>3</sub>, 1:25). <sup>1</sup>H NMR data (CDCl<sub>3</sub>, 400 MHz):  $\delta_{\text{H}}$  7.92 (1H, m, Ar-H), 7.66 (1H, m, Ar-H), 7.56 (2H, m, Ar-H), 7.25 (1H, m, Ar-H), 5.44 (1H, d, J = 3.4 Hz, H-1), 4.90 (1H, dd, J = 3.4 and 9.8 Hz, H-2), 4.79 (1H, t, J = 9.5 Hz, H-3), 4.73 (1H, t, J = 9.6 Hz, H-4), 4.10 (1H, dd, J = 2.2 and 12.2 Hz, H-6b), 4.0 (1H, m, H-6a), 3.64 (1H, m, H-5), 3.31 (3H, s, 1-OCH<sub>3</sub>), 2.35 {6H, m,  $3 \times \text{CH}_3(\text{CH}_2)_9\text{CH}_2\text{CO}-$ }, 1.63 {6H, m,  $3 \times \text{CH}_3(\text{CH}_2)_8\text{CH}_2\text{CH}_2\text{CO}-$ }, 1.24 {48H, m,  $3 \times \text{CH}_3(\text{CH}_2)_8\text{CH}_2\text{CH}_2\text{CO}-$ }, 0.87 {9H, m,  $3 \times \text{CH}_3(\text{CH}_2)_{10}\text{CO}-$ }. Anal. Calcd for  $\text{C}_{49}\text{H}_{66}\text{SO}_{11}$ : C, 72.27; H, 8.14; C, 72.24; H, 8.11.

**Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-myristoyl- $\alpha$ -D-glucopyranoside (10):** Yield 66%. mp 146-147°C (decomp.).  $R_f = 0.59$  (MeOH:CHCl<sub>3</sub>, 1:25). <sup>1</sup>H NMR data (CDCl<sub>3</sub>, 400 MHz):  $\delta_{\text{H}}$  7.91 (1H, m, Ar-H), 7.69 (1H, m, Ar-H), 7.54 (2H, m, Ar-H), 7.30 (1H, m, Ar-H), 5.41 (1H, d, J = 3.6 Hz, H-1), 4.93 (1H, dd, J = 3.5 and 9.8 Hz, H-2), 4.82 (1H, t, J = 9.6 Hz, H-3), 4.75 (1H, m, H-4), 4.07 (1H, m, H-6a), 3.96 (1H, m, H-6b), 3.64 (1H, m, H-5), 3.31 (3H, s, 1-OCH<sub>3</sub>), 2.28 {6H, m,  $3 \times \text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO}-$ }, 1.24 {66H, m,  $3 \times \text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{CO}-$ }, 0.86 {9H, m,  $3 \times \text{CH}_3(\text{CH}_2)_{12}\text{CO}-$ }. Anal. Calcd for  $\text{C}_{55}\text{H}_{78}\text{SO}_{11}$ : C, 73.57; H, 8.71; C, 73.50; H, 8.69.

**Methyl 6-O-benzenesulfonyl-2,3,4-tri-O-palmitoyl- $\alpha$ -D-glucopyranoside (11):** Yield 75%. mp 154-155°C (decomp.).  $R_f = 0.55$  (MeOH:CHCl<sub>3</sub>, 1:25). <sup>1</sup>H NMR data (CDCl<sub>3</sub>, 400 MHz):  $\delta_{\text{H}}$  8.0 (2H, m, Ar-H), 7.81 (1H, m, Ar-H), 7.55 (2H, m, Ar-H), 5.40 (1H, d, J = 3.7 Hz, H-1), 4.90 (1H, dd, J = 3.6 and 10.2 Hz, H-2), 4.81 (1H, t, J = 9.5 Hz, H-4), 4.69 (1H, m, H-3), 4.11 (1H, m, H-6a), 4.02 (1H, t, J = 10.2 Hz, H-6b), 3.71 (1H, m, H-5), 3.30 (3H, s, 1-OCH<sub>3</sub>), 2.31 {6H, m,  $3 \times \text{CH}_3(\text{CH}_2)_{13}\text{CH}_2\text{CO}-$ }, 1.25 {78H, m,  $3 \times \text{CH}_3(\text{CH}_2)_{13}\text{CH}_2\text{CO}-$ }, 0.88 {9H, m,  $3 \times \text{CH}_3(\text{CH}_2)_{14}\text{CO}-$ }. Anal. Calcd for  $\text{C}_{61}\text{H}_{108}\text{SO}_{11}$ : C, 73.24; H, 10.81; C, 73.20; H, 10.80.

All synthesized compounds IR spectra were presented in the **Table 3**.

## 2.3. Antibacterial screening

### 2.3.1. Bacterial cultures

In vitro antibacterial activities of the synthesized compounds (**Scheme 1**) were determined against four pathogenic microorganism as shown in the **Table 1**. 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<sub>3</sub>) of the chemicals and standard NA medium (**Table 2**) was used throughout the microbial study.

**Table 1.** List of tested organisms used.

Type	Name of tested organisms	Strain no.
Gram +ve	<i>Bacillus cereus</i>	BTCC 19
	<i>Bacillus megaterium</i>	BTCC 18
Gram -ve	<i>Salmonella typhi</i>	AE 14612
	<i>Salmonella paratyphi</i>	AE 146313

**Table 2.** Composition of the Nutrient Agar (NA) media.

Name of composition	Amounts (g/ml)
Beef extract	3.0 g
Peptone	5.0 g
Agar	20.0 g
NaCl	0.5 g
Distilled water	1000 ml
Final pH 7.0 adjusted with 5N NaOH	

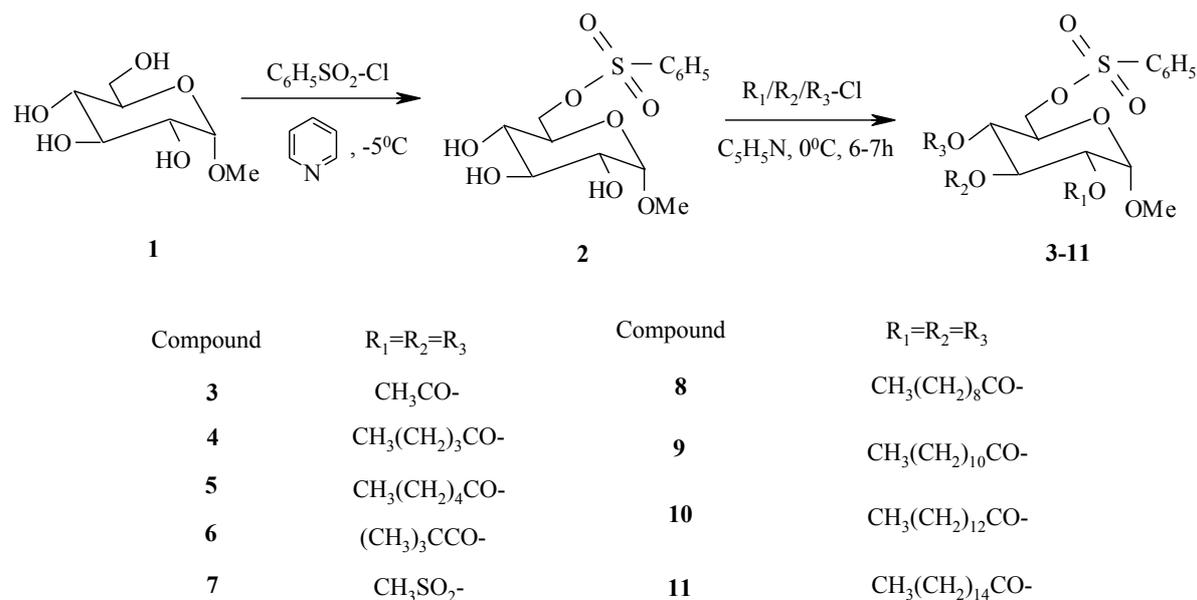
### 2.3.2. Evaluation of antibacterial activity

*In vitro* antibacterial screening of the synthesized chemicals were done by disc diffusion method [30] with little modification [31]. 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<sub>3</sub>) 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. The plates were then incubated at 35±2°C for 24 hours 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. Each experiment was repeated thrice. All the results were compared with the standard antibacterial antibiotic Ampicillin (20µg/disc, BEXIMCO Pharm. Bangladesh Ltd).

## 3. RESULTS AND DISCUSSION

### 3.1. Chemistry and spectral characterization

The main aim of the piece of work presented in this manuscript was to carry out selective benzenesulfonylation of methyl  $\alpha$ -D-glucopyranoside (**1**) with benzenesulfonyl chloride using the direct acylation method (**Scheme 1**). A series of derivatives of the resulting benzenesulfonylation products were also prepared using a wide variety of acylating agents in order to achieve supportive evidences for structure elucidation and also to obtain newer derivatives of synthetic and biological importance. All these acylation products and their derivatives were employed as test chemicals for antibacterial screening studies against a number of human pathogenic bacteria.



**Scheme 1.** Synthetic pathway of benzenesulfonyl derivatives of methyl  $\alpha$ -D-glucopyranoside **3-11**.

Our initial effort was to treatment of methyl  $\alpha$ -D-glucopyranoside (**1**) with 1.1 molar 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 IR spectrum (**Table 3**) of **2** showed absorption bands at  $1701\text{ cm}^{-1}$ ,  $3510\text{ cm}^{-1}$  and  $1360\text{ cm}^{-1}$ , suggested the presence of -CO, -OH and -SO<sub>2</sub> groups in the molecule. In its <sup>1</sup>H-NMR spectrum, the peaks at  $\delta$  7.90 (2H, m),  $\delta$  7.70 (1H, m) and  $\delta$  7.50 (2H, m) corresponded the protons of one phenyl group. The downfield shift of H-6 to  $\delta$  4.51 and 4.30 from its usual value ( $\sim$ 4.00 ppm) indicated the introduction of the benzenesulfonyl group at position 6. Complete analysis of the IR and <sup>1</sup>H-NMR spectrum suggested that the structure of this compound may be assigned as methyl 6-O-benzenesulfonyl- $\alpha$ -D-glucopyranoside (**2**).

**Table 3.** Infra-red (IR) values of the synthesized compounds.

Compound	IR ( $\nu\text{ cm}^{-1}$ )		
	Hydroxyl (-OH)	Carbonyl (-CO)	Sulphonyl (-SO <sub>2</sub> )
<b>2</b>	3510	1701	1360
<b>3</b>		1770, 1760, 1740	1362
<b>4</b>		1760	1368
<b>5</b>		1781, 1772	1366
<b>6</b>		1760	1360
<b>7</b>		1710	1358
<b>8</b>		1760, 1710	1369
<b>9</b>		1768	1365
<b>10</b>		1762, 1705	1361
<b>11</b>		1700	1358

The structure of the benzenesulfonyl derivative (**2**) was further ascertained by its conversion to and identification of its acetyl derivative (**3**). The IR spectrum of this compound showed the following characteristic peaks:  $1770$ ,  $1760$ ,  $1740\text{ cm}^{-1}$  (for -CO) and  $1362\text{ cm}^{-1}$  (for -SO<sub>2</sub>) stretchings. The introduction of three acetyl groups in the molecule was demonstrated by the

appearance of three three-proton singlets at  $\delta$  2.06,  $\delta$  2.00 and 1.98 in its  $^1\text{H-NMR}$  spectrum. By complete analysis of the IR and  $^1\text{H-NMR}$  spectra, the structure of the triacetate was ascertained as methyl 2,3,4-tri-*O*-acetyl-6-*O*-benzenesulfonyl- $\alpha$ -D-glucopyranoside (**3**). Acylation of compound **2** was done using excess pentanoyl chloride in pyridine and the pentanoyl derivative (**4**) was obtained. In its IR spectrum, the absorption bands at  $1760\text{ cm}^{-1}$  and  $1368\text{ cm}^{-1}$  corresponded to carbonyl and sulfonyl stretchings, respectively. In its  $^1\text{H-NMR}$  spectrum, three six-proton multiplet at  $\delta$  2.34 and  $\delta$  1.61 and  $\delta$  1.37 and one nine-proton multiplet at  $\delta$  0.91 were indicative of the presence of three pentanoyl groups in the molecule. Analysis of the rest of the spectrum led us to assign its structure as methyl 6-*O*-benzenesulfonyl-2,3,4-tri-*O*-pentanoyl- $\alpha$ -D-glucopyranoside (**4**).

The IR spectrum of the compound **5** showed the peaks at 1781, 1772 (-CO stretching) and  $1366\text{ cm}^{-1}$  (-SO<sub>2</sub>) stretching. The  $^1\text{H-NMR}$  spectrum of compound **5** displayed two six-proton multiplet at  $\delta$  2.21, and  $\delta$  1.51, a twelve-proton multiplet at  $\delta$  1.25 and one nine-proton multiplet at  $\delta$  0.83 showing the attachment of three hexanoyl groups in the molecule. The resonance for H-2, H-3 and H-4 appeared at downfield from their usual values indicating the attachment of the hexanoyl groups at positions 2, 3 and 4. Complete analysis of the IR and  $^1\text{H-NMR}$  spectrum enabled us to assign the structure of this derivative as methyl 6-*O*-benzenesulfonyl-2,3,4-tri-*O*-hexanoyl- $\alpha$ -D-glucopyranoside (**5**). The IR spectrum of compound **6** showed peaks at  $1760\text{ cm}^{-1}$  and  $1360\text{ cm}^{-1}$  due to carbonyl and sulfonyl stretchings, respectively. In the  $^1\text{H-NMR}$  spectrum of **6** a twenty seven-proton singlet at  $\delta$  1.20 was due to the methyl protons of pivaloyl groups which indicated the introduction of three pivaloyl groups. Complete analysis of the spectra was consistent with the structure of the compound assigned as methyl 6-*O*-benzenesulfonyl-2,3,4-tri-*O*-pivaloyl- $\alpha$ -D-glucopyranoside (**6**).

The structure of compound **2** was also supported by its transformation to and identification of the methanesulphonyl derivative (**7**). Its IR spectrum exhibited absorption bands at  $1710$  and  $1358\text{ cm}^{-1}$  due to -CO stretching and -SO<sub>2</sub> stretching respectively. The presence of three methanesulphonyl groups was demonstrated by its  $^1\text{H-NMR}$  spectrum which displayed three three-proton singlets at  $\delta$  3.19,  $\delta$  3.11 and  $\delta$  3.08 due to the methyl protons of three methanesulphonyloxy groups. Complete analysis of the IR and  $^1\text{H-NMR}$  spectra led us to establish its structure as methyl 6-*O*-benzenesulfonyl-2,3,4-tri-*O*-methanesulfonyl- $\alpha$ -D-glucopyranoside (**7**). We then triol (**2**) was treated with 5.0 molar amount of decanoyl chloride in dry C<sub>5</sub>H<sub>5</sub>N, provided the decanoyl derivative (**8**). The  $^1\text{H-NMR}$  spectrum of this compound (**8**) provided the following characteristic peaks: two six-proton multiplet at  $\delta$  2.31 and 1.61, a thirty six-proton multiplet at  $\delta$  1.23 and a nine-proton multiplet at  $\delta$  0.86 indicating the introduction of three decanoyl groups to the triol molecule. On the basis of complete analysis of the IR and  $^1\text{H-NMR}$  spectra of this compound was accorded as methyl 6-*O*-benzenesulfonyl-2,3,4-tri-*O*-decanoyl- $\alpha$ -D-glucopyranoside (**8**).

As a continuation our next effort was to lauroylation, and the IR spectrum of this compound (**9**) displayed absorption bands at  $1768$ ,  $1365\text{ cm}^{-1}$  due to carbonyl and sulfonyl stretchings. Its  $^1\text{H-NMR}$  spectrum displayed two six-proton multiplet at 2.35 and  $\delta$  1.63 a forty eight-proton multiplet at  $\delta$  1.24 and a nine-proton multiplet at  $\delta$  0.87, thereby suggesting the presence of three lauroyl groups in the compound. By analysis of the IR and  $^1\text{H-NMR}$  spectra, the structure of the lauroyl derivative was assigned as methyl 6-*O*-benzenesulfonyl-2,3,4-tri-*O*-lauryl- $\alpha$ -D-glucopyranoside (**9**). As same as, the structure of the myristoyl derivative (**10**) was established by analyzing its IR and  $^1\text{H-NMR}$  spectra. By analysis of the IR and  $^1\text{H-NMR}$  spectra, the structure of the tri-*O*-myristoate was assigned as methyl 6-*O*-benzenesulfonyl-2,3,4-tri-*O*-myristoyl- $\alpha$ -D-glucopyranoside (**10**). Finally, we were able to isolate palmitoyl derivative **11** in 74.76% as needless. The IR and  $^1\text{H-NMR}$  spectrum of this compound was in complete agreement with the structural assigned as methyl 6-*O*-benzenesulfonyl-2,3,4-tri-*O*-palmitoyl- $\alpha$ -D-glucopyranoside (**11**).

Thus selective benzenesulfonylation of methyl  $\alpha$ -D-glucopyranoside (**1**) was successfully carried out using the direct acylation method. A series of acylated derivatives of the 6-*O*-benzenesulfonylate (**2**) were prepared employing a wide variety of acylating agents containing some probable biologically prone atoms/groups. The aim of preparing all the derivatives was, in some

cases, to obtain supportive evidences for structure elucidation and also to obtain newer products of synthetic and biological importance. All the compounds thus prepared were employed as test chemicals for evaluating their antibacterial activities against a number of human pathogenic bacteria.

### 3.2. Antibacterial screening studies

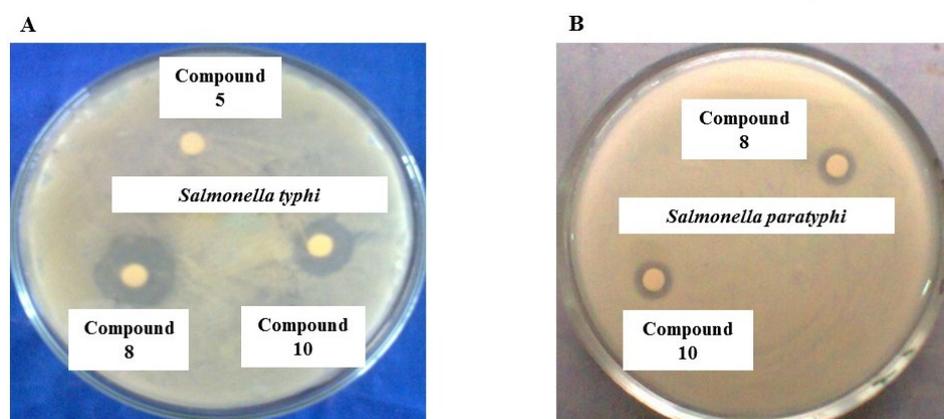
The results of antibacterial activity of the test chemicals (2-11) were measured in terms of zone of inhibition and are presented in **Table 4** and **Fig. 1**.

The number of compounds showed a promising inhibitory activity against a number of both Gram-positive and Gram-negative bacteria. The inhibition data (**Table 4**) indicated that compounds **8** (12 mm) and **10** (10 mm) were exhibited highest inhibition against *S. typhi* AE 14612; compounds **6**, **8** and **10** were more active on *B. cereus* and showed 7, 7.5 and 7.5 mm diameter of inhibition zone. On the other hand, compounds **4** and **8** showed more activity on *B. megaterium*. However, compound **8** showed the highest inhibition zone (8 mm) against *S. paratyphi*.

**Table 4.** Zone of inhibition against tested organisms by the synthesized derivatives.

Compound no.	Zone of inhibition (mm) at 200 µg dw/disc			
	Gram +Ve organisms		Gram -Ve organisms	
	<i>B. cereus</i>	<i>B. megaterium</i>	<i>S. typhi</i>	<i>S. paratyphi</i>
<b>2</b>	6.5	6.5	6.5	NF
<b>3</b>	6	NF	7	7
<b>4</b>	7	7	6	7
<b>5</b>	NF	NF	NF	NF
<b>6</b>	7	6	7	NF
<b>7</b>	NF	NF	NF	NF
<b>8</b>	7.5	7.5	*12	8
<b>9</b>	NF	6	6.5	6
<b>10</b>	7.5	7.5	*10	6.5
<b>11</b>	NF	NF	NF	NF
**Ampicillin	*19	*16	*20	*18

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



**Fig. 1.** Zone of inhibition observed against (A) *Salmonella typhi* by three test chemicals **5**, **8** and **10**. (B): *Salmonella paratyphi* by two test chemicals **8** and **10**.

From the above experimental results obtained by using a number of selected human pathogenic bacteria, we found that selectively acylated derivatives **2**, **4**, **6**, and **8** showed moderate to marked inhibition against Gram-positive bacteria while chemicals **3**, **4**, **8**, **9** and **10** are very active against Gram-negative bacteria. We also observed that some compounds such as **4**, **8** and **10** 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.

#### 4. CONCLUSION

In the present paper, we report the synthesis, spectral characterization and *in vitro* antibacterial activity of new series of methyl 6-*O*-benzenesulfonyl- $\alpha$ -D-glucopyranoside (**3–11**) followed by direct method in presence of pyridine. The results of the antibacterial investigation showed that some of the newly synthesized acylated derivatives may possess a wide range of susceptibilities activities. So the acylated derivatives of methyl 6-*O*-benzenesulfonyl- $\alpha$ -D-glucopyranoside (Scheme 1) may be considered as a potential source for developing new and better antibacterial agents against a number of pathogenic organism. Further, it is also expected that this piece of work employing carbohydrate derivatives as test chemicals will help further work to the development of pesticides and medicine for plant/ human disease control.

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