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Acylation of D-Glucose Derivatives over C_5H_5N: Spectral Characterization and *in vitro* Antibacterial Activities

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ABSTRACT

Methyl α-D-glucopyranoside was easily prepared by the treatment of D-glucose with anhydrous methyl alcohol in presence of hydrogen chloride at freezing temperature in good yield. Then N-acetylsulfanilylation of methyl α-D-glucopyranoside has been carried out by the direct method and afforded the 6-O-N-acetylsulfanilyl derivative in an excellent yield. In order to obtain newer products, the 6-O-N-acetylsulfanilyl derivative was further transformed to a series of 2,3,4-tri-O-acyl derivatives containing a wide variety of functionalities in a single molecular framework. The chemical structures of the newly synthesized compounds were elucidated by Fourier Transform Infrared spectroscopy (FTIR), ^{1}H-NMR (Proton nuclear magnetic resonance) spectroscopy elemental and physicochemical properties analysis. All the newly synthesized D-glucose derivatives were tested for their *in vitro* antibacterial activity against some human pathogenic bacterial strains. The study revealed that a good number of acylated products exhibited promising antibacterial activities. It is expected that the acylated derivatives of D-glucose may be considered as a potential source for developing new and better antibacterial agents against a number of pathogenic organism.

Key words: Carbohydrate, N-acetylsulfanilyl, structure, spectroscopy, inhibition, H-NMR, 6-O-N-acetylsulfanilyl

INTRODUCTION

In recent years, it has been widely recognized that carbohydrates play an important roles in diverse biological processes, including viral and bacterial infections, cell growth and proliferation, cell-cell communication as well as immune response (Rudd *et al*., 2001; Bertozzi and Kiessling, 2001; Murrey and Hsieh-Wilson, 2008; Walker-Nasir *et al*., 2008; Chen and Fukuda, 2006). Studies on carbohydrates draw unprecedented attention but still fall behind that on proteins and nucleic acids. A major obstacle is related to the fact that it is difficult to get enough and structurally

well-defined carbohydrates, which often exist in nature at low-concentrations and in micro-heterogeneous forms. Chemical synthesis of carbohydrates is one of the approaches to solve this problem (Miljkovic, 2009; Lindhorst, 2007).

Carbohydrates are polyhydroxy compounds and can be acylated directly by applying the blocking-deblocking techniques (Andary *et al*., 1982; Williams and Richardson, 1967). Carbohydrates can also be acylated selectively to give important derivatives which may be biologically active. Of the methods used for selective acylation, the direct method was found to be more encouraging (Kabir *et al*., 2005a). Carbohydrates isolated from natural sources, acyl glycoses and acyl glycosides have immense importance and some of them have effective biological activity (Tsuda *et al*., 1983). 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 (Tiwari and Mishra, 2011; Miljkovic, 2009).

Methyl α-D-glucopyranoside is a component of emulsifier applied for personal care products, skin creams, lotions and other cosmetics, particularly for leave on skin care systems to reduce tacky feel and synergistic humectancy performance. Different concentrations of methyl α-D-glucopyranoside were used to vary echo decay times in a study that assessed the effects of cryoprotection on the structure and activity of p21ras (Halkides *et al*., 1998). Methyl α-D-glucopyranoside has also been used in a study to investigate saccharide-mediated protection of chaotropic-induced deactivation of concanavalin A (Figlas *et al*., 1997).

Antimicrobial agents inhibit or kill the growth of microorganisms such as bacteria or fungus. By means of antimicrobial drug discovery, it is believed that microbial infections will end up. However, rapid increases of microorganism originated diseases make it difficult to happen. Furthermore, senseless usage of antimicrobials exposed another big problem, drug resistance (Byarugaba, 2004; Projan and Shlaes, 2004). As a result of this, the need for the synthesis and development of new antimicrobial agents has emerged (Boggs and Miller, 2004; Barker, 2006; Bakht *et al*., 2010; Jha *et al*., 2010; Kumar *et al*., 2011).

From literature survey it was revealed that a large number of biologically active compounds contain aromatic, heteroaromatic and acyl substituents (Gupta *et al*., 1997). Nitrogen, sulphur and halogen containing substituents are also known to enhance the biological activity of the parent compound (Kabir *et al*., 2009, 2004, 2003; Kawsar *et al*., 2014a, 2013a, 2012a). It is also known that if an active nucleus is linked to another active nucleus, the resulting molecule may show greater potential for biological activity (Gupta *et al*., 1997). From our previous works we also observed that in many cases the combination of two or more acyl substituents in a single molecular framework enhances the biological activity by many fold than their parent nuclei (Kabir *et al*., 2008, 2005b; Kawsar *et al*., 2014b, 2013b, 2012b). Encouraged by the own findings and also literature reports, synthesized a series of D-glucose (Fig. 1) derivatives deliberately incorporating a wide variety of probable biologically active components to the D-glucose moiety and also evaluated their antibacterial activities against some human pathogenic microorganism as a first time.

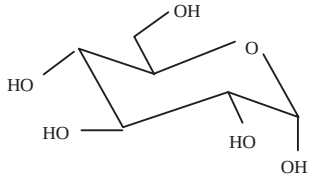

Fig. 1: D-Glucose

MATERIALS AND METHODS

Thin Layer Chromatography (TLC) was performed on Kieselgel GF_{254} with 1% H_2SO_4 and heating at 150-200°C until coloration took place. Column chromatography was performed with silica gel G_{60}. The ^{1}H-NMR (400 MHz) spectra were recorded for solutions in $CDCl_3$ 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) with a bath temperature below 40°C. Melting points were determined on an electro-thermal melting point apparatus (England) and are uncorrected. All reagents were purchased from commercial sources and used as received.

Synthesis

Methyl α-D-glucopyranoside (2): From (a) D-glucose (2) methyl α-D-glucopyranoside was prepared as described in the literature (Helferich and Schafer, 1926).

Methyl 6-O-N-acetylsulfanilyl-α-D-glucopyranoside (3): A stirred solution of methyl α-D-glucopyranoside (2) (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 molar eq.) was added to it. The reaction mixture was stirred at -5°C temperature for 7 h and then stirred overnight at room temperature. The progress of the reaction was monitored by TLC which indicated the formation of one product. 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 (3).

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 47.79, H 5.61.

General procedure for the synthesis of 6-O-N-acetylsulfanilyl derivatives of D-glucopyranoside (4-13): To a rapidly stirred and cooled (-5°C) solution of the triol (2) (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 cold temperature for 6-7 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. Work-up as described earlier and purification by silica gel column chromatography (with methanol-chloroform, 1:16) afforded the acetyl derivative (4).

Similar reaction and purification procedure was applied to prepare compound (5) (CH_3OH-$CHCl_3$, 1:15), (6) (CH_3OH-$CHCl_3$, 1:16), (7) (CH_3OH-$CHCl_3$, 1:16), (8) (CH_3OH-$CHCl_3$, 1:17), (9) (CH_3OH-$CHCl_3$, 1:15), (10) (CH_3OH-$CHCl_3$, 1:16), (11) (CH_3OH-$CHCl_3$, 1:16), (12) (CH_3OH-$CHCl_3$, 1:16) and (13) (CH_3OH-$CHCl_3$, 1:16).

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-acetyl-α-D-glucopyranoside (4): FTIR (KBr, cm^{-1}): ν = 1690 (C = O), 3325 (-NH), 1362 (-SO_2), ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.72 (2H, d, Ar-H),

7.69 (2H, d, Ar-H), 7.54 (1H, s, -NH), 5.40 (1H, d, H-1), 5.11 (1H, dd, H-2), 4.93 (1H, t, H-3), 4.79 (1H, H-4), 4.33 (1H, dd, H-6a), 4.21 (1H, dd, H-6b), 3.72 (1H, ddd, H-5), 3.33 (3H, s, 1-OCH_3), 2.23 (3H, s, CH_3CON-), 2.08, 2.00, 1.98 (3×3H, 3×s, 3×CH_3CO-).

Anal calcd for $C_{21}H_{27}SO_{12}$: C 50.10, H 5.37, found C 50.15, H 5.42.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-pentanoyl-α-D-glucopyranoside (5): FTIR (KBr, cm^{-1}): ν = 1715, 1710 (C=O), 3350 (-NH), 1358 (-SO_2); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.75 (2H, d, Ar-H), 7.66 (2H, d, Ar-H), 7.54 (1H, s, -NH), 5.33 (1H, d, H-1), 4.92 (1H, m, H-2), 4.78 (1H, t, H-3), 4.70 (1H, t, H-4), 4.38 (1H, m, H-6a), 4.01 (1H, dd, H-6b), 3.79 (1H, m, H-5), 3.31 (3H, s, 1-OCH_3), 2.34 {6H, m, 3×$CH_3(CH_2)_2CH_2CO$-}, 2.23 (3H, s, CH_3CON-), 1.61 {6H, m, 3×$CH_3CH_2CH_2CH_2CO$-}, 1.38 { 6H, m, 3×$CH_3CH_2(CH_2)_2CO$-}, 0.88 {9H, m, 3×$CH_3(CH_2)_3CO$-}.

Anal calcd for $C_{30}H_{45}SO_{12}$: C 57.23, H 7.15, found C 57.27, H 7.20.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-hexanoyl-α-D-glucopyranoside (6): FTIR (KBr, cm^{-1}): ν = 1760 (C=O), 3310 (-NH), 1363 (-SO_2); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.85 (2H, d, Ar-H), 7.48 (2H, d, Ar-H), 7.27 (1H, s, -NH), 5.39 (1H, d, H-1), 4.90 (1H, m, H-2), 4.78 (1H, t, H-3), 4.73 (1H, m, H-4), 4.37 (1H, dd, H-6a), 4.10 (1H, dd, H-6b), 3.83 (1H, m, H-5), 3.32 (3H, s, 1-OCH_3), 2.31(3H, s, CH_3CON-), 2.21 {6H, m, 3×$CH_3(CH_2)_3CH_2CO$-}, 1.63 {6H, m, 3×$CH_3(CH_2)_2CH_2CH_2CO$-}, 1.29 {12H, m, 3×$CH_3(CH_2)_2CH_2CH_2CO$-}, 0.92 {9H, m, 3×$CH_3(CH_2)_4CO$-}.

Anal calcd for $C_{33}H_{51}SO_{12}$: C 59.02, H 7.60, found C 59.07, H 7.62.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-decanoyl-α-D-glucopyranoside (7): FTIR (KBr, cm^{-1}): ν = 1701 (C=O), 3320 (-NH), 1361 (-SO_2); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.80 (2H, d, Ar-H), 7.72 (2H, d, Ar-H), 7.65 (1H, s, -NH), 5.39 (1H, d, H-1), 5.01 (1H, m, H-2), 4.89 (1H, t, H-3), 4.62 (1H, t, H-4), 4.11 (1H, dd, H-6a), 3.99 (1H, dd, H-6b), 3.73 (1H, ddd, H-5), 3.30 (3H, s, 1-OCH_3), 2.33 {6H, m, 3×$CH_3(CH_2)_7CH_2CO$-}, 2.24 (3H, s, CH_3CON-), 1.63 {6H, m, 3×$CH_3(CH_2)_6CH_2CH_2CO$-}, 1.24 {36H, m, 3×$CH_3(CH_2)_6CH_2CH_2CO$-}, 0.88 {9H, m, 3×$CH_3(CH_2)_8CO$-}.

Anal calcd for $C_{45}H_{75}SO_{12}$: C 64.36, H 8.94, found C 64.37, H 8.99.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-lauroyl-α-D-glucopyranoside (8): FTIR (KBr, cm^{-1}): ν = 1718 (C=O), 3319 (-NH), 1364 (-SO_2); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.91 (2H, d, Ar-H), 7.77 (2H, d, Ar-H), 7.31 (1H, s, -NH), 5.42 (1H, d, H-1), 4.92 (1H, dd, H-2), 4.78 (1H, t, H-3), 4.70 (1H, t, H-4), 4.23 (1H, dd, H-6a), 4.19 (1H, dd, H-6b), 3.64 (1H, m, H-5), 3.58 (3H, s, 1-OCH_3), 2.34 {6H, m, 3×$CH_3(CH_2)_9CH_2CO$-} 2.29 (3H, s, CH_3CON-), 1.64 {6H, m, 3×$CH_3(CH_2)_8CH_2CH_2CO$-}, 1.26 {48H, m, 3×$CH_3(CH_2)_8CH_2CH_2CO$-}, 0.88 {9H, m, 3×$CH_3(CH_2)_{10}CO$-}.

Anal calcd for $C_{51}H_{87}SO_{12}$: C 66.31, H 9.43, found C 66.34, H 9.49.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-myristoyl-α-D-glucopyranoside (9): FTIR (KBr, cm^{-1}): ν = 1725 (C=O), 3326 (-NH), 1366 (-SO_2); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.78 (2H, d, Ar-H), 7.67 (2H, d, Ar-H), 7.54 (1H, s, -NH), 5.40 (1H, d, H-1), 4.93 (1H, dd, H-2), 4.80 (1H, t, H-3), 4.73 (1H, m, H-4), 4.06 (1H, m, H-6a), 3.97 (1H, m, H-6b), 3.70 (1H, m, H-5), 3.30 (3H, s, 1-OCH_3), 2.28 {6H, m, 3×$CH_3(CH_2)_{11}CH_2CO$-}, 2.23 (3H, s, CH_3CON-), 1.23 {66H, m, 3×$CH_3(CH_2)_{11}CH_2CO$-}, 0.87 {9H, m, 3×$CH_3(CH_2)_{12}CO$-}.

Anal calcd for $C_{57}H_{99}SO_{12}$: C 67.92, H 9.83, found C 67.94, H 9.89.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-palmitoyl-α-D-glucopyranoside (10): FTIR (KBr, cm^{-1}): ν = 1728 (C=O), 3331 (-NH), 1367 ($-SO_2$); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.79 (2H, d, Ar-H), 7.69 (2H, d, Ar-H), 7.58 (1H, s, -NH), 5.39 (1H, d, H-1), 5.02 (1H, dd, H-2), 4.89 (1H, t, H-3), 4.75 (1H, m, H-4), 4.55 (1H, m, H-6a), 4.34 (1H, t, H-6b), 3.79 (1H, m, H-5), 3.30 (3H, s, 1-OCH_3), 2.32 {6H, m, 3×$CH_3(CH_2)_{13}CH_2CO$-}, 2.22 (3H, s, CH_3CON-), 1.24 {78 H, m, 3×$CH_3(CH_2)_{13}CH_2CO$-}, 0.86 {9H, m, 3×$CH_3(CH_2)_{14}CO$-}.

Anal calcd for $C_{63}H_{111}SO_{12}$: C 69.30, H 10.17, found C 69.33, H 10.19.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-methanesulfonyl-α-D-glucopyranoside (11): FTIR (KBr, cm^{-1}): ν = 1705 (C=O), 3322 cm^{-1} (-NH), 1364 ($-SO_2$); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.82 (2H, d, Ar-H), 7.70 (2H, d, Ar-H), 7.35 (1H, s, -NH), 5.30 (1H, d, H-1), 5.10 (1H, m, H-2), 4.56 (1H, t, H-3), 4.35 (1H, m, H-4), 4.08 (1H, dd, H-6a), 3.90 (1H, dd, H-6b), 3.65 (1H, m, H-5), 3.40 (3H, s, 1-OCH_3), 3.18, 3.10, 3.08 (3×3H, 3×s, 3×CH_3SO_2-), 2.21 (3H, s, CH_3CON-).

Anal calcd for $C_{18}H_{27}S_4O_{15}$: C 35.35, H 4.42, found C 35.39, H 4.44.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-benzenesulfonyl- α-D-glucopyranoside (12): FTIR (KBr, cm^{-1}): ν = 1765 (C=O), 3319 (-NH), 1374 ($-SO_2$); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 8.00 (6H, m, Ar-H), 7.75 (3H, m, Ar-H), 7.70 (2H, d, Ar-H), 7.69 (2H, d, Ar-H), 7.65 (6H, m, Ar-H), 7.54 (1H, s, -NH), 5.30 (1H, d, H-1), 5.02 (1H, dd, H-2), 4.84 (1H, m, H-3), 4.63 (1H, m, H-4), 4.32 (1H, m, H-6a), 4.27 (1H, t, H-6b), 3.79 (1H, ddd, H-5), 3.28 (3H, s, 1-OCH_3), 2.22 (3H, s, CH_3CON-).

Anal calcd for $C_{33}H_{33}S_4O_{15}$: C 49.68; H 4.14, found C 49.71; H 4.18.

Methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-pivaloyl-α-D-glucopyranoside (13): FTIR (KBr, cm^{-1}): ν = 1722, 1684 (C=O), 3327 (-NH), 1365 ($-SO_2$); ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 7.84 (2H, d, Ar-H), 7.49 (2H, d, Ar-H), 7.40 (1H, s, -NH), 5.41 (1H, d, H-1), 4.87 (1H, dd, H-2), 4.77 (1H, t, H-3), 4.69 (1H, t, H-4), 4.31 (1H, m, H-6a), 4.08 (1H, m, H-6b), 3.79 (1H, m, H-5), 3.33 (3H, s, 1-OCH_3), 2.24 (3H, s, CH_3CON-), 1.20 {27H, s, 3×$(CH_3)_3CCO$-}.

Anal calcd for $C_{30}H_{45}SO_{12}$: C 57.23, H 7.15, found C 57.25, H 7.18.

Biological evaluation: *In vitro* antibacterial activities of the synthesized compounds (Fig. 2 and Table 1) were determined against four pathogenic bacterial strains. 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.

Evaluation of *in vitro* antibacterial activity: The *in vitro* antibacterial spectrum 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 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

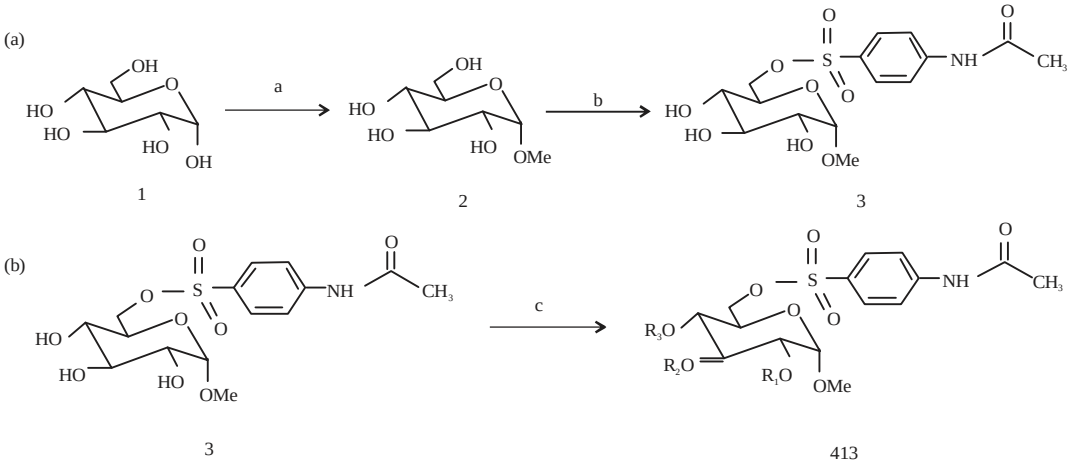

Fig. 2(a-b): Reaction pathway. Reagents and conditions, A: a = ref.[31], b = C_5H_5N/4-($CH_3CONH)C_6H_4SO_2Cl$, -5°C, 8 h., B: c = various acylating agents, 0°C, 8-7 h., C_5H_5N. 1: D-glucose, 2: methyl α-D-glucopyranoside, 3: methyl 6-O-N-acetylsulfanilyl-α-D-glucopyranoside and other compounds 4-13

Table 1: Synthesized D-glucose derivatives

Compounds	$R_1 = R_2 = R_3$
4	CH_3CO-
5	$CH_3(CH_2)_3CO$-
6	$CH_3(CH_2)_4CO$-
7	$CH_3(CH_2)_8CO$-
8	$CH_3(CH_2)_{10}CO$-
9	$CH_3(CH_2)_{12}CO$-
10	$CH_3(CH_2)_{14}CO$-
11	CH_3SO_2-
12	$C_6H_5SO_2$-
13	$(CH_3)_3CCO$-

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

Carbohydrates are the most abundant class of biomolecules, making up 75% of the biomass on Earth (Ferreira *et al*., 2009). Carbohydrates are used to store energy but also perform other important functions to life (Stick and Williams, 2008). Recently, carbohydrates and their derivatives have emerged as an important tool for selective synthesis and as a chiral pool for the design of chiral ligands. They are used as chiral building blocks, precursors for drug synthesis and chiral catalysts in asymmetric catalysis (Dieguez *et al*., 2004a, 2004b, 2007; Woodward *et al*., 2010; Boysen, 2007; Appelt *et al*., 2008). Despite the importance of carbohydrates in biological events, the pace of development of carbohydrate based therapeutics has been relatively slow. This is mainly

due to practical synthetic and analytical difficulty. Recent advances in the field, however, have demonstrated that many of these problems can be circumvented and evidence the importance of carbohydrates as bioactive substances with regard to antibacterial, antiviral, antineoplastic, antiprotozoal and antifungal activity among others, related recently in literature (Nogueira *et al*., 2009; Wong, 2003). So, the study of carbohydrates is one of the most exciting fields in organic chemistry. 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.

Chemistry and spectral characterization: The main objective of the work reported here was to study regioselective acylation of methyl α-D-glucopyranoside (2) with N-acetylsulfanilyl chloride in presence of anhydrous C_5H_5N. A well-known direct acylation method (Kawsar *et al*., 2015; Kabir *et al*., 2005a) were used for the synthesis of D-glucose derivatives (Fig. 2 and Table 1). The resulting N-acetylsulfanilylation products (3) were converted to a number of derivatives using a series of acylating agents and their physicochemical properties are presented in the Table 2. The structure of the main acylation product and their derivatives were ascertained by analyzing their IR (Taleb-Mokhtari *et al*., 2016; Brauer *et al*., 2011) and ^{1}H-NMR (Loss and Lutteke, 2015; Ojha *et al*., 2013) spectra. All the acylation products thus prepared were employed as test chemicals for antibacterial activity studies against a number of Gram-positive and Gram-negative human pathogenic bacteria.

Initial effort was to prepare the starting material, (2) methyl α-D-glucopyranoside. Thus, (1) reaction of D-glucose with anhydrous methyl alcohol and hydrogen chloride in ice cool system provided the (2) methyl α-D-glucopyranoside. Then the (2) methyl α-D-glucopyranoside were treated with N-acetylsulfanilyl chloride in dry pyridine at -5°C, followed by removal of solvent and silica gel column chromatographic purification, afforded the (3) N-acetylsulfanilyl derivative. The IR spectrum of this compound showed the following characteristic peaks: 1738 cm^{-1} (-CO stretching), 3510 cm^{-1} (-OH stretching), 3320 cm^{-1} (-NH stretching) and 1365 cm^{-1} (-SO_2 stretching). The IR spectra of the synthesized compounds were accordance to the IR values of which were stated in the literature (Taleb-Mokhtari *et al*., 2016; Brauer *et al*., 2011). In its ^{1}H-NMR spectrum displayed two two-proton doublets at d 7.70 (J = 8.8 Hz) and d 7.68 (J = 8.8 Hz) corresponding to the aromatic ring protons, one one-proton singlet at d 7.53 (-NH), one three-proton singlet at d 2.22 (-$NCOCH_3$) thereby suggesting the introduction of one N-acetylsulfanilyl (4-acetamidobenzenesulfonyl) group in the molecule. Also, the C-6 proton was deshielded considerably to d 4.50 (as dd, J = 4.8 and 10.2

Table 2: Physicochemical data of synthesized D-glucose derivatives

Compounds No.	RT (h)	R_f	Yield (mg)	Percentage	State/mp (°C)
3	8.0	0.51	102	51.0	Needles, 134-136
4	7.5	0.50	59	78.5	Needles, 140-142
5	7.5	0.51	73	95.5	Thick syrup
6	7.0	0.50	53	74.7	Semi-solid
7	7.5	0.53	60	88.1	Needles, 138-140
8	7.0	0.55	53	81.1	Thick syrup
9	7.0	0.52	93	81.0	Needles, 134-136
10	7.5	0.52	59	78.5	Needles, 139-140
11	7.0	0.54	61	84.3	Semi-solid
12	7.0	0.55	63	79.9	Semi-solid
13	7.0	0.53	52	70.6	Pasty mass

Hz, 6a) and 4.32 (as dd, J = 2.2 and 12.2 Hz, 6b) from its usual value (~4.00 ppm), thus showing that the *N*-acetylsulfanilyl group was introduced at this position. The ^{1}H-NMR spectrum were found to show very similar which was in accordance with our previous work (Kawsar *et al*., 2014b, 2012b). By complete analysis of the IR and ^{1}H-NMR spectra, the structure of this compound was assigned as methyl 6-O-N-acetylsulfanilyl-a-D-glucopyranoside (3).

The structure of the (3) N-acetylsulfanilyl derivative was further supported by its conversion to and identification of its (4) acetyl derivatives. Thus, reaction of compound 3 with an excess of acetic anhydride in pyridine, followed by conventional work-up procedure and purification by silica gel column chromatography, furnished the (4) tri-O-acetyl derivative. The IR spectrum of the triacetate showed the following characteristics absorption bands at 1690, 3325 and 1362 cm^{-1} stretching. The introduction of three acetyl group in the molecule was demonstrated by the appearance of three three-proton singlet at d 2.08, d 2.00 and d 1.98 in its ^{1}H-NMR spectrum. The H-2 proton resonated at δ 5.11 (as dd, J = 3.6 and 9.8 Hz) and shifted downfield from the precursor triol (2) (δ 3.76); H-3 proton resonated at δ 4.93 (as t, J = 9.7 Hz) and shifted downfield from the precursor triol (2) (δ 3.88); also, H-4 proton resonated downfield to δ 4.79 (as t, J = 9.7 Hz) as compared to the precursor compound 2 (δ 4.09), thereby suggesting the attachment of the acetyl groups at positions 2, 3 and 4. Analysis of the rest of the IR and ^{1}H-NMR spectra supported the structure ascertained as (4) methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-acetyl-α-D-glucopyranoside.

The (3) N-acetylsulfanilyl derivative was then converted to a number of fatty acid derivatives using pentanoyl chloride, hexanoyl chloride, decanoyl chloride, lauroyl chloride, myristoyl chloride and palmitoyl chloride in order to get further support to its structure and also prepare newer products. Thus, pentanoylation of compound 3 in pyridine provided the (5) pentanoyl derivative. In its IR spectrum, the absorption bands at 1715 and 1710 cm^{-1} (carbonyl), 3350 cm^{-1} (-NH) and 1358 cm^{-1} (-SO_2). In its ^{1}H-NMR spectrum, the resonance peaks three six-proton multiplet at δ 2.34 {3×$CH_3(CH_2)_2CH_2CO$-}, δ 1.61 {3×$CH_3CH_2CH_2CH_2CO$-}, δ 1.38 {3×$CH_3CH_2(CH_2)_2$ CO-} and one nine-proton multiplet at δ 0.88 {3×$CH_3(CH_2)_3CO$-} showed the presence of three pentanoyl groups in the compound. The deshielding of H-2, H-3 and H-4 protons to *δ* 4.92 (as m), δ 4.78 (as t, J= 9.6 Hz) and δ 4.70 (as t, J= 9.6 Hz) from their precursor compound 3 values (δ 3.76), (δ 3.88) and (δ 4.09), respectively indicated the introduction of the three pentanoyl groups at positions 2, 3 and 4. So, we were able to propose a structure of this compound as (5) methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-pentanol-*α*-D-glucopyranoside. The (6) hexanoyl derivative obtained and its IR spectrum absorption bands showed at 1760, 3310 and 1363 cm^{-1} corresponded to -CO, -NH and -SO_2 stretchings, respectively. The ^{1}H-NMR spectrum of compound 6 displayed two six-proton multiplet at δ 2.21 {3×$CH_3(CH_2)_3CH_2CO$-} and δ 1.63 {3×$(CH_3)_2CH_2CH_2CO$-}, a twelve-proton multiplet at δ 1.29 {3×$CH_3(CH_2)_2CH_2CH_2CO$-} and one nine-proton multiplet at δ 0.92 {3×$CH_3(CH_2)_4CO$-} showing the attachment of three hexanoyl groups. The resonance for H-2, H-3 and H-4 appeared at δ 4.90 (as m), δ 4.78 (as t, J=9.4 Hz) and δ 4.73 (as m) which shifted downfield from their values (compound 3). The analysis of the IR and ^{1}H-NMR spectra it was assigned as (6) methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-hexanoyl-α-D-glucopyranoside.

Compound 3 was then converted to the (7) decanoyl derivative. The IR spectrum showed the absorption bands: 1701 cm^{-1} (-CO stretching), 3320 cm^{-1} (-NH stretching) and 1361 cm^{-1} (-SO_2 stretching). The ^{1}H-NMR spectrum of this compound exhibited the following characteristic peaks: two six-proton multiplet at *δ* 2.33 {3×$CH_3(CH_2)_7CH_2CO$-} and 1.63 {3×$CH_3(CH_2)_6CH_2CH_2CO$-}, a thirty six-proton multiplet at δ 1.24 {3×$CH_3(CH_2)_6CH_2CH_2CO$-} and a nine-proton multiplet at δ 0.88 {3×$CH_3(CH_2)_8CO$-} suggesting the introduction of three decanoyl groups to the molecule. The

downfield shift of H-2, H-3 and H-4 resonances to δ 5.01 (as m), δ 4.89 (as t, J = 9.6 Hz) and δ 4.62 (as t, J = 9.6 Hz) as compared to the triol (3) Values was indicative of the attachment of the three decanoyl groups at positions 2, 3 and 4. This compound was accorded as methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-decanoyl-α-D-glucopyranoside (7).

Further support for the structure accorded to compound 3 was then subjected to lauroylation by reaction with lauroyl chloride and isolated the lauroyl derivative (8). The structure of this compound was confidently assigned as (8) methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-lauroyl-α-D-glucopyranoside by complete analyzing its IR and ^{1}H-NMR spectrum. The myristoryl derivative 9 was furnished. Its IR spectrum showed absorption bands at 1725 cm^{-1} (-CO stretching), 3326 cm^{-1} (-NH stretching) and 1366 cm^{-1} (-SO_2 stretching). In its ^{1}H-NMR spectrum, a six-proton multiplet at δ 2.28 {3×$CH_3(CH_2)_{11}CH_2CO$-}, a sixty-six proton multiplet at δ 1.23 {3×$CH_3(CH_2)_{11}CH_2CO$-} and a nine-proton multiplet at δ 0.87 {3×$CH_3(CH_2)_{12}CO$-} indicated the attachment of three myristoyl groups in the molecule. The downfield shift of the H-2, H-3 and H-4 protons to δ 4.93 (as dd, J=3.5 and 9.8 Hz), δ 4.80 (as t, J = 9.6 Hz) and δ 4.73 (as m) from their values and the resonances of other protons in their anticipated positions showed the attachment of myristoyl groups at positions 2, 3 and 4. The IR and ^{1}H-NMR spectra of this compound was in complete agreement with the structure accorded to it as the structure of the tri-O-myristoate was assigned as (9) methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-myristoyl-α-D-glucopyranoside. Now, we used palmitoyl chloride for derivatizing compound 3 by direct acylation method. After usual work-up and purification procedure, we obtained the palmitoyl derivative (10). By complete analysis of its IR and ^{1}H-NMR spectrum (please see experimental section for details) and by analogy with similar derivatives described earlier, the structure of this compound was confidently assigned as (10) methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-palmitoyl-α-D-glucopyranoside.

Additional support for the structure accorded to compound (3) was obtained by its conversion to its methanesulfonyl derivative (11). Its IR spectrum displayed absorption bands at 1705, 3322 and 1364 cm^{-1} due to -CO, -NH and -SO_2 stretchings. The presence of three methanesulphonyl groups was demonstrated by its ^{1}H-NMR spectrum which displayed three three-proton singlets at δ 3.18, δ 3.10 and δ 3.08 due to the methyl protons of three methanesulphonyloxy (CH_3SO_2-) groups. Also, the H-2, H-3 and H-4 protons shifted downfield to δ 5.10 (as m), δ 4.56 (as t, J = 9.4 Hz) and δ 4.35 (as m) from its precursor compound 3 (δ 3.76, *δ* 3.88 and δ 4.09), thereby led us to establish its structure as (11) methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-methanesulfonyl-α-D-glucopyranoside. The IR spectrum of benzenesulfonyl derivative 12 showed absorption bands at 1765 cm^{-1} (-CO), 3319 cm^{-1} (-NH) and 1374 cm^{-1} (-SO_2). In its ^{1}H-NMR spectrum, the peaks at δ 8.00 (6H, m), δ 7.75 (3H, m) and δ 7.65 (6H, m) corresponded the protons of three phenyl groups. The downfield shift of the H-2, H-3 and H-4 protons to δ 5.02 (as dd, J = 3.7 and 10.2 Hz), δ 4.84 (as m) and δ 4.63 (as m) from their values and the resonances of other protons in their anticipated positions showed the attachment of benzenesulfonyl groups and the structure of the compound was assigned (12) as methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-benzenesulfonyl-*α*-D-glucopyranoside.

Finally, treatment of compound 3 with pivaloyl chloride followed by same procedure, afforded the (13) pivaloyl derivative. The IR spectrum showed carbonyl stretching bands at 1722 and 1684 cm^{-1}, -NH stretching at 3327 cm^{-1} and sulfonyl stretching at 1365 cm^{-1}. In the ^{1}H-NMR spectrum displayed a twenty seven-proton singlet at δ 1.20 {3×$(CH_3)_3CCO$-} was due to the methyl protons of pivaloyl groups which indicated the introduction of three pivaloyl groups. The downfield shift of H-2 proton to δ 4.87 (as dd, J = 3.6 and 10.0 Hz), H-3 proton to δ 4.77 (t, J = 9.5 Hz) and H-4 proton to δ 4.69 (as t, J = 9.6 Hz) from their precursor triol (3) δ values showed the attachment of

the pivaloyl groups at positions 2, 3 and 4. The rest of the IR and ^{1}H-NMR spectra was consistent with the structure accorded as (13) methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-pivaloyl-α-D-glucopyranoside.

Thus selective N-acetylsulfanilylation of methyl α-D-glucopyranoside (2) with a number of rather non-traditional acylating agents (using the direct acylation method) was found to be very promising since in all the reactions, a single monosubstitution product was isolated reasonably high yields. Furthermore, this acylation product and its derivatives were found to have long shelf life and these products may further be utilized as probable starting materials for the synthesis of newer derivatives.

Evaluation of antibacterial activity: In the *in vitro* antibacterial investigation eleven acylated derivatives (3-13) of D-glucose (1) (Fig. 1) were considered as the test chemicals. In this purpose, four human pathogenic bacteria were used (Table 3).

The test chemicals contained different type of acyl groups in the molecular framework. The results of antibacterial screening studies of the test chemicals and the standard antibiotic, ampicillin against Gram-positive and Gram-negative bacteria are delivered in Table 4.

The results in Table 4 revealed that the test chemicals 5 and 10 were highly active towards the growth of all the tested bacteria. Chemical 3, 7 and 9 were completely insensitive towards any of the Gram-positive bacteria. Again, the results in Table 4 showed that except the test chemicals 3, 7, 9 and 12 all other test chemicals were found to be effective towards the different Gram-negative bacteria in different degrees. The test chemical 5 very significantly inhibited the growth of all Gram-negative bacterial strains used. We also observed that the chemicals 5, 8 and 10 are highly active against both the Gram-positive and Gram-negative organisms. So, these chemicals may be targeted for future studies for their usage as broad spectrum antibiotics. As seen in our previous investigations (Kawsar *et al*., 2015, 2013c) the presence of some acyl groups in the test chemicals increased the antibacterial capacity, here in this investigation we found that the

Table 3: Tested bacteria

Type of bacteria	Name of tested bacteria	Strain no.
Gram +Ve	*Bacillus cereus*	BTCC 19
	Bacillus megaterium	BTCC 18
Gram -Ve	*Salmonella typhi*	AE 14612
	Salmonella paratyphi	AE 146313

Table 4: Antibacterial activity against both Gram+Ve and Gram-Ve bacteria by the D-glucose derivatives

	Zone of inhibition (mm) at 200 µg dw/disc			
	Gram +Ve bacteria		Gram -Ve bacteria	
Compounds No.	*B. cereus*	*B. megaterium*	*S. typhi*	*S. paratyphi*
3	N/A	N/A	N/A	6.5
4	6	6	6	6
5	*10	*10	*10	7.5
6	N/A	6.5	6.5	6.5
7	N/A	N/A	N/A	N/A
8	7	7	7	7
9	N/A	N/A	N/A	N/A
10	0	*10	6	N/A
11	6.5	N/A	6.5	6.5
12	6.5	7	6	N/A
13	7	7	6.5	6.5
**Ampicillin	*19	*16	*20	*18

dw: Dry weight, *: Marked inhibition, **: Standard antibiotic, N/A: Not action

presence of pentanoyl, lauroyl, palmitoyl etc. acyl groups improved the antimicrobial power of the test chemicals. This is the first report regarding the effectiveness of the selected chemicals (3-13) against the selected bacterial strains.

CONCLUSION

In conclusion, here report the synthesis of some new derivatives of D-glucose (3-13) obtained by the direct acylation method. This method demonstrates an efficient and convenient one, providing good yields comparatively with the other methods. Compound (5) methyl 6-O-N-acetylsulfanilyl-2,3,4-tri-O-pentanoyl-α-D-glucopyranoside showed the highest antibacterial activities against all tested microorganisms. It was also observed that the selected compounds were more sensitive against Gram-positive bacteria than that of the Gram-negative bacterial strains. Based on the above studies, the promising compounds can be submitted to *in vivo* antibacterial studies as a future perspective.

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