Chemistry & Biology Interface, 2014, 4, 1, 37-47

RESEARCH PAPER ISSN: 2249 –4820

CHEMISTRY & BIOLOGY INTERFACE

An official Journal of ISCB, Journal homepage; www.cbijournal.com

Synthesis and Spectroscopic Characterization of Some Novel Acylated Carbohydrate Derivatives and Evaluation of their Antimicrobial Activities

Sarkar M. A. Kawsar,[1*] Md O. Faruk,[1] Golam Mostafa[2] and Mohammad S. Rahman[3]

[1]Laboratory of Carbohydrate and Protein Chemistry, Department of Chemistry, Faculty of Science, University of Chittagong, Chittagong-4331, Bangladesh; E-mail: akawsarabe@yahoo.com; Tel +88 01762717081; Fax +88 031 2606014

[2]Department of Chemistry, Faculty of Science, Comilla Govt. Women's University College, Comilla-3000, Bangladesh

[3]Department of Microbiology, Faculty of Biological Science, University of Chittagong, Chittagong-4331, Bangladesh

Received 27 November 2013; Accepted 13 January 2014

Abstract: Selective acylation of methyl 4,6-*O*-cyclohexylidene-α-D-glucopyranoside, methyl 3-*O*-pivaloyl-α-L-rhamnopyranoside, 1,2-*O*-isopropylidine-6-*O*-pivaloyl-α-D-glucofuranose and 3,6-di-*O*-benzoyl-α-D-mannopyranoside using the direct method, afforded the corresponding acylated derivatives in an excellent yield. The structures of the acylated products were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR spectroscopy and elemental analysis. All the acylation products thus prepared were used as test chemicals for *in vitro* antibacterial and antifungal evaluation against a number of human and phytopathogenic strains. For comparison, biological activity of standard antibiotics Ampicillin and Nystatin, was also determined. The study revealed that the acylated derivatives exhibited moderate to good antibacterial and antifungal activities.

Keywords: Synthesis, Monosaccharide, Acylating agents, Antimicrobial, Inhibition

Introduction

The study of carbohydrates is one of the most exciting fields in organic chemistry. Carbohydrates, especially monosaccharides, are different concerning 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.

With the development of modern and sophisticated isolation, purification and identification techniques, various natural

products from plants and other sources become readily available. Of the carbohydrates isolated from natural sources, acyl glycoses and acyl glycosides have immense importance and some of them have effective biological activity [1-6]. For instance, the sulfated polysaccharide, heparin, plays an essential role in blood coagulation [7]. Carbohydrates also serve as attachment sites for infectious bacteria, viruses, toxins and hormones that result in pathogenesis [8]. One of the important applications of monomers containing carbohydrates is their utilization in glycopolymers [9]. Some of these natural products can also be prepared by selective modification of the hydroxyl groups of carbohydrates which include acylation, alkylation, etc. A number of methods for selective acylation of carbohydrates have been developed and successfully employed [10-14]. Of these, the direct method is considered as one of the most effective [5] for selective acylation of carbohydrates. From literature survey it was revealed that a large number of biologically active compounds contain aromatic, heteroaromatic and acyl substituents [15]. Nitrogen, sulphur and halogen containing substituents are also known to enhance the biological activity of the parent compound [16-18]. 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 [15]. In 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 manifold compared to their parent nuclei [19-21]. Encouraged by the above-mentioned results, we deliberately synthesized a number of monosaccharide compounds (Schemes 1, 2, 3 and 4) containing various groups and evaluated their antibacterial and antifungal activities.

Materials and Methods

Experimental

Melting points were determined on an electro-thermal melting point apparatus (England) and are uncorrected. The IR spectra were recorded on KBr pellets with an IRAffinity-1 FTIR spectrophotometer, SHIMADZU Corporation, Japan. ^{1}H-NMR spectra (400 MHz) and ^{13}C-NMR spectra (100 MHz) were recorded for solutions in deuteriochloroform ($CDCl_3$), with a Bruker spectrospin spectrometer. Evaporations were carried out under reduced pressure using VV-1 type vacuum rotary evaporator with a bath temperature below 40°C. Thin layer chromatography (t.l.c) was performed on Kieselgel GF_{254} and spots were detected by spraying the plates with 1% H_2SO_4. Column chromatography was performed with silica gel G_{60}. All reagents and chemicals used were commercially available (Aldrich) and were used as received, unless otherwise specified. The reaction pathways have been summarized in Schemes 1-4.

General Procedure of the Synthesis of Methyl 4,6-*O*-cyclohexylidene-α-D-glucopyranoside (1) with Pentanoyl Chloride and Compounds (2-3)

A cooled (-5°C) and stirred solution of methyl 4,6-*O*-cyclohexylidene-α-D-glucopyranoside (**1**) [22] (200 mg, 0.729 mmol) in dry pyridine (3 ml) was treated with pentanoyl chloride (0.1 ml, 0.83 mmol) and stirring was continued at (-5°C) for 6 hrs. The solution was then allowed to stand overnight at room temperature. T.l.c. (ethyl-acetate-hexane, 1:4) examination showed formation of two close-moving products, the faster-moving one being the major component. Excess reagent was decomposed by the addition of ice to the flask and the product was extracted with chloroform. The chloroform layer was then processed as usual. The residual syrup was then packed in a silica gel column. Initial elution with ethyl acetate-hexane (1:8) gave the faster-moving 2-*O*-pentanoyl derivative i.e., methyl 4,6-*O*-cyclohexylidene-2-*O*-pentanoyl-α-D-glucopyranoside (**2**) as chromatographically

homogeneous syrup. Further elution with ethyl acetate-hexane (1:6) provided the 3-*O*-pentanoyl derivative **3**.

Methyl 4,6-*O*-cyclohexylidene-2-*O*-pentanoyl-α-D-glucopyranoside (2)

Yield: 62 %; m.p. 110-112 °C; Anal Calcd. for $C_{18}H_{30}O_7$: C, 60.32; H, 8.41 %. Found: C, 60.56; H, 8.78 %; R_f = 0.52 (ethyl acetate/hexane = 1/8); IR (KBr, cm^{-1}): 1722 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$ δ / ppm): 4.87 (1H, *d*, *J* = 3.70 Hz, H-1), 4.72 (1H, *dd*, *J* = 3.7 and 9.7 Hz, H-2), 3.98 (1H, *t*, *J* = 9.2 Hz, H-3), 3.83 (1H, *dd*, *J* = 4.7 and 10.2 Hz, H-6a), 3.73 (1H, *t*, *J* = 9.9 Hz, H-6b), 3.63 (1H, *ddd*, *J* = 5.1, 9.7 and 10.2 Hz, H-5), 3.57 (1H, *t*, *J* = 9.5 Hz, H-4), 3.33 (3H, *s*, 1- OCH_3), 2.37 {2H, *t*, *J* = 7.4 Hz, $CH_3(CH_2)_2CH_2CO$-}, 1.61 {2H, *t*, *J* = 7.4 Hz, $CH_3CH_2CH_2CH_2CO$-}, 1.57-1.38 (10H, *m*, C_6H_{10}), 1.32 {2H, *m*, $CH_3CH_2(CH_2)_2CO$-}, 0.81 {3H, *t*, *J* = 7.3 Hz, $CH_3(CH_2)_3CO$-}; ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 173.52 {$CH_3(CH_2)_3CO$-}, 99.97 {$C_5H_{10}C(O)_2$}, 97.63 (C_1), 73.45 (C_2), 73.22 (C_3), 69.09 (C_4), 63.06 (C_5), 61.55 (C_6), 55.22 (1-OCH_3), 37.89, 27.75, 25.53, 22.75, 22.48 {$C_5H_{10}C(O)_2$}, 33.84, 26.97, 22.08 {$CH_3(CH_2)_3CO$-}, 13.63 {$CH_3(CH_2)_3CO$-}.

Methyl 4,6-*O*-cyclohexylidene-3-*O*-pentanoyl-α-D-glucopyranoside (3)

Yield: 10 %; m.p. 105-107 °C; Anal Calcd. for $C_{18}H_{30}O_7$: C, 60.32; H, 8.41 %. Found: C, 60.66; H, 8.93 %; R_f = 0.51 (ethyl acetate/hexane = 1/6); IR (KBr, cm^{-1}): 1718 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$ δ / ppm): 5.14 (1H, *t*, *J* = 9.5 Hz, H-3), 4.74 (1H, *d*, *J* = 3.7 Hz, H-1), 3.86 (1H, *dd*, *J* = 5.0 and 10.2 Hz, H-6a), 3.76 (1H, *t*, *J* = 10.1 Hz, H-6b), 3.67 (1H, *ddd*, *J* = 5.0, 9.4 and 10.1 Hz, H-5), 3.58 (2H, *m*, H-2 and H-4), 3.42 (3H, *s*, 1-OCH_3), 2.36 {2H, *t*, *J* = 7.2 Hz, $CH_3(CH_2)_2CH_2CO$-}, 1.63 {2H, *t*, *J* = 8.0 Hz, $CH_3CH_2CH_2CH_2CO$-}, 1.59-1.39 (10H, *m*, C_6H_{10}), 1.37 {2H, *m*, $CH_3CH_2(CH_2)_2CO$-}, 0.90 {3H, *t*, *J* = 7.3 Hz, $CH_3(CH_2)_3CO$-}; ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 173.92 {$CH_3(CH_2)_3CO$-}, 100.20 {$C_5H_{10}C(O)_2$}, 99.67 (C_1), 72.66(C_2), 71.90 (C_3), 70.69 (C_4), 63.84 (C_5), 61.80 (C_6), 55.45 (1-OCH_3), 37.87, 27.67, 25.60, 22.69, 22.47, {$C_5H_{10}C(O)_2$}, 34.28, 27.30, 22.17 {$CH_3(CH_2)_3CO$-}, 13.72 {$CH_3(CH_2)_3CO$-}.

General Procedure for the Synthesis of Compounds (5-6)

A solution of methyl 3-*O*-pivaloyl-α-L-rhamnopyranoside (**4**) [23] (100 mg, 0.38 mmol) in anhydrous pyridine (4 ml) was cooled to 0°C when pentanoyl chloride (0.2ml, 1.66 mmol) was added to it. The mixture was stirred at this temperature for 4 hours and then left standing overnight at room temperature. The progress of the reaction was monitored by T.l.c. (ethyl acetate-hexane, 1:4), which indicated completion of reaction with the formation of a faster-moving product. A few pieces of ice was added to the flask and then the reaction mixture was processed as usual. The residual syrup was passed through a silica gel column and eluted with ethyl acetate-hexane (1:4) to furnish the methyl 2-*O*-pentanoyl-3-*O*-pivaloyl-α-L-rhamnopyranoside (**5**). Similarly, the hexanoyl derivative, methyl 2,4-di-*O*-hexanoyl-3-*O*-pivaloyl-α-L-rhamnopyranoside **6** was prepared in good yield.

Methyl 2-*O*-pentanoyl-3-*O*-pivaloyl-α-L-rhamnopyranoside (5)

Yield: 90 %; m.p. 115-117 °C; Anal Calcd. for $C_{17}H_{30}O_8$: C, 56.34; H, 8.32 %. Found: C, 56.84; H, 8.56 %; R_f = 0.51 (ethyl acetate/hexane = 1/4); IR (KBr, cm^{-1}): 1726 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$ δ / ppm): 5.21 (1H, *d*, *J* = 3.3 Hz, H-2), 5.09 (1H, *dd*, *J* = 3.3 and 9.8 Hz, H-3), 4.57 (1H, *s*, H-1), 3.71 (1H, *dd*, *J* = 6.2 and 9.4 Hz, H-5), 3.59 (1H, *t*, *J* = 9.7 Hz, H-4), 3.36 (3H, *s*, 1-OCH_3), 2.35 {2H, *t*, *J* = 7.6 Hz, $CH_3(CH_2)_2CH_2CO$-}, 1.60 {2H, *t*, *J* = 7.5 Hz, $CH_3CH_2CH_2CH_2CO$-}, 1.36 {2H, underlying *m*, $CH_3CH_2(CH_2)_2CO$-}, 1.34 {3H, *d*, *J* = 6.0 Hz, 6-CH_3), 1.16 {9H, *s*, $(CH_3)_3C$-}, 0.90 {3H, *t*, *J* = 7.3 Hz, $CH_3(CH_2)_3CO$-}; ^{13}C-NMR (100

MHz, $CDCl_3$, δ / ppm): 178.97{$(CH_3)_3CCO$-}, 172.67 {$CH_3(CH_2)_3CO$-}, 98.58 (C_1), 72.23 (C_2), 71.84 (C_3), 69.80 (C_4), 68.55 (C_5), 54.98 (1-OCH_3), 38.93 {$(CH_3)_3CCO$-}, 33.90, 22.22, 17.53 {$CH_3(CH_2)_3CO$-}, 27.00 (×3) {$(CH_3)_3CCO$-}, 13.67 {$CH_3(CH_2)_3CO$-}.

Methyl 2,4-di-*O*-hexanoyl-3-*O*-pivaloyl-α-L-rhamnopyranoside (6)

Yield: syrupy mass, 88 %; Anal Calcd. for $C_{24}H_{42}O_8$: C, 62.86; H, 9.21 %. Found: C, 62.88; H, 9.34 %; R_f = 0.51 (ethyl acetate/hexane = 1/12); IR (KBr, cm^{-1}): 1710 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$ δ / ppm): 5.36 (1H, *m*, H-3), 5.23 (1H, *d*, *J* = 3.4 Hz, H-2), 5.12 (1H, *t*, *J* = 9.7 Hz, H-4), 4.60 (1H, *s*, H-1), 3.85 (1H, *ddd*, *J* = 6.2, 9.4 and 12.5 Hz, H-5), 3.37 (3H, *s*, 1-OCH_3), 2.37 {2H, *t*, *J* = 7.4 Hz, $CH_3(CH_2)_3CH_2CO$-}, 2.28 {2H, *t*, *J* = 7.5 Hz, $CH_3(CH_2)_3CH_2CO$-}, 1.60 {4H, *m*, 2×$CH_3(CH_2)_2CH_2CH_2CO$-}, 1.29 {8H, *m*, 2×$CH_3(CH_2)_2(CH_2)_2CO$-}, 1.21 {3H, *d*, *J* = 6.3 Hz, 6-CH_3), 1.09 {9H, *s*, $(CH_3)_3CCO$-}, 0.89 {6H, *m*, 2×$CH_3(CH_2)_4CO$-}; ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 177.17 {$(CH_3)_3CCO$-}, 172.78, 172.66 {2× $CH_3(CH_2)_4CO$-}, 98.63 (C_1), 70.81 (C_2), 69.49 (C_3), 69.07 (C_4), 66.27 (C_5), 55.14 (1-OCH_3), 38.74 {$(CH_3)_3CCO$-}, 34.17, 34.08, 31.31, 31.25, 24.53 (×2), 22.29, 17.45 {2× $CH_3(CH_2)_4CO$-}, 26.93 (×3) {$(CH_3)_3CCO$-}, 13.86 {2× $CH_3(CH_2)_4CO$-}.

General Procedure of the Synthesis of Compounds (8-10)

A solution of 1,2-*O*-isopropylidine-6-*O*-pivaloyl-α-D-glucofuranose (**7**) [24] (100 mg, 0.33 mmol) in dry pyridine (4 ml) was cooled to 0°C when pentanoyl chloride (0.16 ml, 1.33 mmol) was added. The solution was stirred at this temperature for 6 hr. and then at room temperature overnight. T.l.c. (ethyl acetate-hexane, 1:15), which indicated full conversion of the starting material into a single product, Usual work-up and chromatographic purification afforded the 1,2-*O*-isopropylidene-3,5-di-*O*-pentanoyl-6-*O*-pivaloyl-α-D-glucofuranose (**8**). Similar reaction and purification method was employed to synthesize compound 3,5-di-*O*-hexanoyl-1,2-*O*-isopropylidene-6-*O*-pivaloyl-α-D-glucofuranose (**9**) and 1,2-*O*-isopropylidene-3,5-di-*O*-(4-methoxybenzoyl)-6-*O*-pivaloyl-α-D-glucofuranose (**10**).

1,2-*O*-Isopropylidene-3,5-di-*O*-pentanoyl-6-*O*-pivaloyl-α-D-glucofuranose (8)

Yield: Crystalline solid, 92 %; m.p. 57-58 °C; Anal Calcd. for $C_{24}H_{40}O_9$: C, 61; H, 8.51 %. Found: C, 61.95; H, 8.76 %; R_f = 0.50 (ethyl acetate/hexane = 1/15); IR (KBr, cm^{-1}): 1728 (C=O), 1362 (-CMe_2); ^{1}H-NMR (400 MHz, $CDCl_3$ δ / ppm): 5.89 (1H, *d*, *J* = 3.3 Hz, H-1), 5.30 (1H, *d*, *J* = 2.7 Hz, H-3), 5.22 (1H, *m*, H-5), 4.54 (1H, *m*, H-6a), 4.44 (1H, *d*, *J* = 3.0 Hz, H-2), 4.42 (1H, *m*, H-4), 4.10 (1H, *dd*, *J* = 4.9 and 12.2 Hz, H-6b), 2.29 {2H, *t*, *J* = 7.2 Hz, $CH_3(CH_2)_2CH_2CO$-}, 2.21 {2H, *t*, *J* = 7.5 Hz, $CH_3(CH_2)_2CH_2CO$-}, 1.55 {4H, *m*, $CH_3(CH_2)_2CH_2CO$-}, 1.50, 1.26 {2×3H, 2×*s*, $(CH_3)_2C$-}, 1.32 {4H, *m*, $CH_3(CH_2)_2CH_2CO$-}, 1.17 {9H, *s*, $(CH_3)_3CCO$-}, 0.88 {6H, *m*, 2× $CH_3(CH_2)_3CO$-}; ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 177.93 {$(CH_3)_3CCO$-}, 172.37, 172.19 {2×$CH_3(CH_2)_3CO$-}, 112.44 {$(CH_3)_2C$-}, 105.11 (C_1), 83.29 (C_2), 76.60 (C_3), 74.57 (C_4), 67.42 (C_5), 63.17 (C_6), 38.85 {$(CH_3)_3CCO$-}, 33.76, 33.62, 26.74, 26.27, 22.18 (×2) {2×$CH_3(CH_2)_3CO$-}, 26.74, 26.64 {$(CH_3)_2C$-}, 13.67 {2×$CH_3(CH_2)_3CO$}.

3,5-Di-*O*-hexanoyl-1,2-*O*-isopropylidene-6-*O*-pivaloyl-α-D-glucofuranose (9)

Yield: 85 %; m.p. 66-67 °C; Anal Calcd. for $C_{26}H_{44}O_9$: C, 62.38; H, 8.84 %. Found: C, 62.78; H, 8.98 %; R_f = 0.52 (ethyl acetate/hexane = 1/8); IR (KBr, cm^{-1}): 1710 (C=O), 1364 (-CMe_2); ^{1}H-NMR (400 MHz, $CDCl_3$ δ / ppm): 5.88 (1H, *d*, *J* = 3.5 Hz, H-1), 5.29 (1H, *d*, *J* = 2.7 Hz, H-3), 5.21 (1H, *ddd*, *J* = 2.9, 5.1 and 9.5 Hz, H-5), 4.52 (1H, *dd*, *J* = 1.9 and 12.2 Hz, H-6a), 4.43 (1H, *d*, *J* = 3.3 Hz, H-2), 4.42 (1H, *dd*, *J* = 2.9 and 10.0 Hz, H-4), 4.09 (1H, *dd*, *J* = 5.1 and 12.3 Hz, H-6b), 2.28

{4H, *m*, 2×$CH_3(CH_2)_3CH_2CO$-}, 2.19 {4H, *t*, *J*= 7.5 Hz, 2× $CH_3(CH_2)_2CH_2CH_2CO$-}, 1.55 {4H, *m*, 2×$CH_3CH_2CH_2(CH_2)_2CO$-},1.49,1.29{2×3H,2×*s*, $(CH_3)_2C$-}, 1.25 {4H, *m*, 2×$CH_3CH_2(CH_2)_3CO$-}, 1.16 {9H, *s*, $(CH_3)_3CCO$-}, 0.86 {6H, *m*, 2×$CH_3(CH_2)_4CO$-}; ^{13}C-NMR (100 MHz, $CDCl_3$, *δ* / ppm): 177.90 {$(CH_3)_3CCO$-}, 172.34, 172.16 {2×$CH_3(CH_2)_4CO$-}, 112.42 {$(CH_3)_2C$-}, 105.01 (C_1), 83.27 (C_2), 76.60 (C_3), 74.53 (C_4), 67.39 (C_6), 63.18 (C_5), 38.83 {$(CH_3)_3CCO$-}, 33.98, 33.83, 31.18 (×2), 24.34, 24.23, 22.26 (×2) {2×$CH_3(CH_2)_4CO$-}, 27.10 (×3) {$(CH_3)_3CCO$-}, 26.72, 26.25 {$(CH_3)_2C$}, 13.85, 13.82 {2×$CH_3(CH_2)_4CO$-}.

1,2-*O*-Isopropylidene-3,5-di-*O*-(4-methoxybenzoyl)-6-*O*-pivaloyl-α-D-glucofuranose (10) Yield: 78 %; m.p. 69-70 °C; Anal Calcd. for $C_{30}H_{36}O_9$: C, 66.65; H, 6.7 %. Found: C, 66.68; H, 6.91 %; R_f = 0.51 (ethyl acetate/hexane = 1/2); IR (KBr, cm^{-1}): 1726 (C=O), 1365 (-CMe_2); ^{1}H-NMR (400 MHz, $CDCl_3$ *δ* / ppm): 8.08 (4H, *d*, *J* = 8.8 Hz, Ar-H), 6.97 (4H, *d*, *J* = 8.8 Hz, Ar-H), 5.97 (1H, *d*, *J* = 3.6 Hz, H-1), 5.93 (1H, *d*, *J* = 3.4 Hz, H-3), 5.52 (1H, *d*, *J* = 2.2 Hz, H-2), 5.32 (1H, *ddd*, *J* = 4.1, 8.9 and 9.5 Hz, H-5), 4.56 (1H, *dd*, *J* = 3.4 and 8.6 Hz, H-4), 4.33 (1H, *dd*, *J* = 2.4 and 9.2 Hz, H-6a), 4.21 (1H, *dd*, *J* = 5.1 and 11.2 Hz, H-6b), 3.88, 3.80 (2×3H, 2×*s*, 2×Ar-OCH_3),1.48, 1.30 {2×3H, 2×*s*, $(CH_3)_2C$}, 1.15 {9H, *s*, $(CH_3)_3CCO$-}.^{13}C-NMR (100 MHz, $CDCl_3$, *δ* / ppm): 178.88 {$(CH_3)_3CCO$-}, 164.61, 162.30 {2×4-OCH_3.C_6H_4CO-), 132.83 (×4), 132.27, 132.09, 121.33 (×2), 114.16 (×4) (2×4-OCH_3.C_6H_4CO-), 113.91 {$(CH_3)_2C$}, 105.07 (C_1), 84.42 (C_2), 83.32 (C_3), 78.91 (C_4), 67.10 (C-6), 63.20 (C-5), 55.59, 55.53 (2 ×4-OCH_3. C_6H_4CO-), 38.80 {$(CH_3)_3CCO$-}, 27.21, 27.12 {$(CH_3)_2C$}.

Synthesis of Methyl 3,6-di-*O*-benzoyl-2,4-di-*O*-pentanoyl-α-D-mannopyranoside (12)

A cooled (0°C) and stirred solution of methyl 3,6-di-*O*-benzoyl-α-D-mannopyranoside (**11**) [25] (100 mg, 0.25 mmol) in anhydrous pyridine (3 ml) was treated with pentanoyl chloride (0.14 ml, 1.04 mmol). The low temperature (0°C) was maintained by ice and common salt for four hours with continuous stirring. The progress of the reaction was monitored by T.l.c. (ethyl acetate-hexane, 1:8), which indicated completion of the reaction and formation of a faster-moving product. Work-up as described earlier and purification by silica gel column chromatography (with ethyl acetate-hexane, 1:10, as eluant) afforded the title compound (**12**).

Methyl 3,6-di-*O*-benzoyl-2,4-di-*O*-pentanoyl-α-D-mannopyranoside (12)
Yield: 90 %; m.p. 101-102 °C; Anal Calcd. for $C_{31}H_{38}O_{10}$: C, 65.25; H, 6.7 %. Found: C, 65.55; H, 6.88 %; R_f = 0.51 (ethyl acetate/hexane = 1/8); IR (KBr, cm^{-1}): 1717 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$ *δ* / ppm): 8.09 (2H, *d*, *J* = 7.4 Hz, Ar-H), 7.94 (2H, *d*, *J* = 7.3 Hz, Ar-H), 7.55 (2H, *m*, Ar-H), 7.40 (4H, *m*, Ar-H), 5.67 (1H, *t*, *J* = 10.1 Hz, H-4), 5.59 (1H, *dd*, *J* = 3.2 and 10.1 Hz, H-3), 5.41 (1H, *dd*, *J* = 1.7 and 3.2 Hz, H-2), 4.78 (1H, s, H-1), 4.56 (1H, *dd*, *J* =2.2 and 12.1 Hz, H-6a), 4.40 (1H, *dd*, *J* = 5.2 and 12.1 Hz, H-6b), 4.19 (1H, *m*, H-5), 3.45 (3H, *s*, 1-OCH_3), 2.34 {2H, *dd*, *J* = 7.5 and 12.7 Hz, $CH_3(CH_2)_2CH_2CO$-}, 2.23 {2H, *dd*, *J* = 7.9 and 12.7 Hz, $CH_3(CH_2)_2CH_2CO$-}, 1.55 {2H, *m*, $CH_3CH_2CH_2CH_2CO$-}, 1.41 {2H, *m*, $CH_3CH_2CH_2CH_2CO$-}, 1.27 {2H, *m*, $CH_3CH_2(CH_2)_2CO$-}, 1.10 {2H, *m*, $CH_3CH_2(CH_2)_2CO$-}, 0.83 {3H, *t*, *J* = 7.3 Hz, $CH_3(CH_2)_3CO$-}, 0.66 {3H, *t*, J=7.3 Hz, $CH_3(CH_2)_3CO$-}; ^{13}C-NMR (100 MHz, $CDCl_3$, *δ* / ppm): 172.63 (×2) {2×$CH_3(CH_2)_3CO$-}, 166.18, 165.32 (2×C_6H_5CO-), 133.33, 133.16, 129.84, 129.77 (×2), 129.69 (×3), 129.18, 128.39 (×3) (2× C_6H_5CO-), 98.59 (C_1), 70.18 (C_3), 69.53 (C_2), 68.64 (C_4), 65.85(C_5), 62.98 (C_6), 55.38 (1-OCH_3), 33.88 (×2), 26.96, 26.87, 22.12, 22.03 {2×$CH_3(CH_2)_3CO$-}, 13.58, 13.42 {2×$CH_3(CH_2)_3CO$-}.

Antimicrobial Screening Studies

Test Microorganisms

Test tube cultures of bacterial and fungal pathogens were obtained from the Microbiology Laboratory, Department of Microbiology, University of Chittagong, Bangladesh. The synthesized test compounds (Schemes 1-4) were subjected to antibacterial screening against four Gram-positive and six Gram-negative bacterial strains viz., *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* BTCC 17, *Bacillus megaterium* BTCC 18, *Bacillus cereus* BTCC 19, *Shigella dysenteriae* AE 14396, *Shigella sonnei* CRL (ICDDR,B), *Salmonella typhi* AE 14612, *Salmonella paratyphi* AE 146313, *Pseudomonas* Species CRL (ICDDR,B), INABA ET (*Vibrio*) AE 14748. The name of phytopathogenic fungi viz., *Fusarim equiseti* (*corda*) *Sacc*., *Macrophomina phaseolina* (Tassi) Goid, *Colletotrichum corchori* (Ikata Yoshida), *Curvularia lunata* (Wakker Becdijin), *Alternaria alternata* (Fr.) Kedissler. In all cases, a 2% solution (in $CHCl_3$) of the chemicals was used.

Antibacterial Activity Assay

The *in vitro* antibacterial spectrum of the synthesized chemicals were done by disc diffusion method [26] with little modification [27]. Sterilized paper discs of 4 mm in diameter and Petri dishes of 150 mm in diameter were used throughout the experiment. The autoclaved Mueller-Hinton agar medium, cooled to 45°C, was poured into sterilized Petri dishes to a depth of 3 to 4 mm and after solidification of the agar medium the plates were transferred to an incubator at 37°C for 15 to 20 minutes to dry off the moisture that developed on the agar surface. The plates were inoculated with the standard bacterial suspensions (as McFarland 0.5 standard) followed by spread plate method and allowed to dry for three to five minutes. Dried and sterilized filter paper discs were treated separately with 50 µg dry weight/disc from 2% solution (in $CHCl_3$) of each test chemical using a micropipette, dried in air under aseptic condition and were placed at equidistance in a circle on the seeded plate. A control plate was also maintained in each case without any test chemical. These plates were kept for 4-6 hours at low temperature (4-6°C) and the test chemicals diffused from disc to the surrounding medium. 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 (in millimeter). Each experiment was repeated thrice. All the results were compared with the standard antibacterial antibiotic Ampicillin (20µg/disc, BEXIMCO Pharm. Bangladesh Ltd).

Antifungal Activity Assay

The *in vitro* antifungal activity of the acylated chemicals were done by Poisons Food technique [28] with modification [27]. Two percent solution of the test chemical (in $CHCl_3$) was mixed with sterilized melted Saburaud agar medium to obtain the desired concentration (2%) and this was poured in sterilized Petri dishes. At the center of each plate, 5 days old fungal mycelial block (4 mm in diameter) was inoculated and incubated at 27°C. A control set was also maintained in each experiment. Linear mycelial growth of fungus was measured after 3-5 days of incubation. The percentage inhibition of radial mycelial growth of the test fungus was calculated as follows:

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

Where, I = Percentage of inhibition, C = Diameter of the fungal colony in control ($CHCl_3$), T = Diameter of the fungal colony in treatment. All the results were compared with the standard antifungal antibiotic Nystatin (100 µg/ml medium, BEXIMCO Pharmaceutical

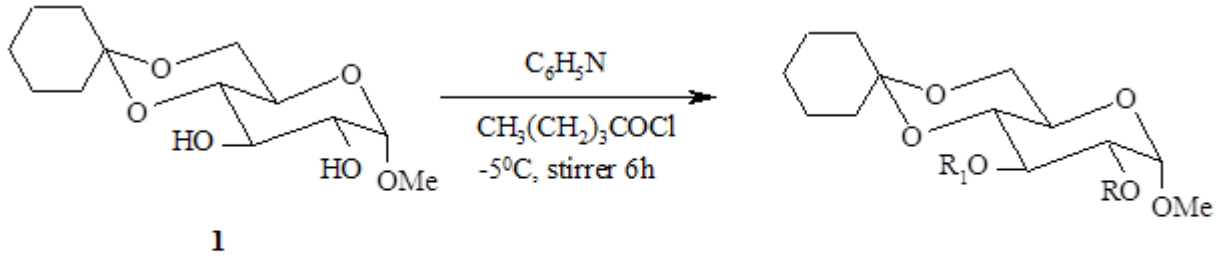
Bangladesh Ltd).

Results and Discussion

Chemistry

The main objective of the research work reported in this paper was to study the selective acylation of monosaccharide derivatives (Schemes 1-4) using a direct method. A number of rarely used acylating agents such as pentanoyl chloride, hexanoyl chloride and 4-methoxy benzoyl chloride were successfully employed for this purpose. All the acylation products thus prepared were used as test chemicals for antibacterial and antifungal evaluation studies against ten human pathogenic bacterial and five phytopathogenic fungal strains.

Initially we derivatized the compound methyl 4,6-*O*-cyclohexylidene-α-D-glucopyranoside (**1**) (Scheme 1) with 1.1 molar equivalent of pentanoyl chloride in pyridine, followed by usual work-up and chromatography, we obtained compound **2** (62%) and compound **3** (10%), both as crystalline solid and m.p. 110-112°C and 105-107 °C, respectively. The IR spectrum of this compound (**2**) displayed absorption bands at 1722 cm^{-1} due to carbonyl stretching. The ^{1}H-NMR spectrum of compound **2** displayed peaks at δ 2.37 (2H, *t*, J=7.4 Hz), *δ* 1.61 (2H, *t*, J=7.4 Hz), *δ* 1.32 (2H, *m*) and *δ* 0.81 (3H, *t*, J=7.3 Hz) and indicated the presence of one pentanoyl group. The deshielding of C-2 proton to *δ* 4.72 (as *dd*, J=3.7 and 9.7 Hz) supports the introduction of the pentanoyl group at position 2. The presence of one pentanoyl group in the molecule was also shown by its ^{13}C-NMR spectrum by displaying the following characteristic peaks: *δ* 173.52 {$CH_3(CH_2)_3CO$-}, *δ* 33.84, 26.97, 22.08 {$CH_3(CH_2)_3CO$-} and *δ* 13.63 {$CH_3(CH_2)_3CO$-}. The slower moving minor component **3** was established as the 3-*O*-pentanoyl derivative by analyzing its IR, ^{1}H-NMR and ^{13}C-NMR spectra. Its IR spectrum showed absorption bands at 1718 cm^{-1} due to –CO stretching. In its ^{1}H-NMR spectrum, the resonance peaks at *δ* 2.36 (2H, *t*, J=7.2 Hz), *δ* 1.63 (2H, *t*, J=8.0 Hz), *δ* 1.37 (2H, *m*) and *δ* 0.90 (3H, *t*, J=7.3 Hz) corresponded to one pentanoyl group. The downfield shift of H-3 to *δ* 5.14 (as *t*, J = 9.5 Hz) was indicative of the attachment of the pentanoyl group at position 3. The presence of one pentanoyl group in the molecule was also ascertained from its ^{13}C-NMR spectrum which gave the following resonance peaks: *δ* 173.92 {$CH_3(CH_2)_3CO$-}, *δ* 34.28, 27.30, 22.17 {$CH_3(CH_2)_3CO$-}and *δ* 13.72 {$CH_3(CH_2)_3CO$-}.



1

2: R = $CH_3(CH_2)_3CO$-; R_1 = H

3: R = H; R_1 = $CH_3(CH_2)_3CO$-

Scheme 1. Synthetic route for the synthesis of compounds 2-3

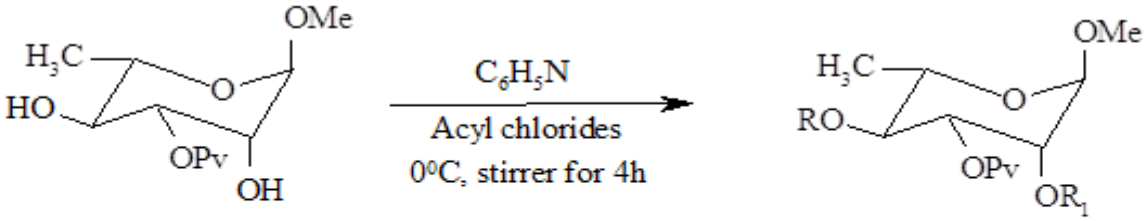
Next target molecule was methyl 3-*O*-pivaloyl-α-L-rhamnopyranoside (**4**) (Scheme 2) and we acylated this compound with pentanoyl chloride and hexanoyl chloride. Thus, pentanoylation of compound **4** with an excess of pentanoyl choloride gave principally compound **5** (90%)

as needless m.p. 115-117 °C. The IR spectrum of this compound indicated absorption bands at 1726 cm^{-1} corresponding to carbonyl stretchings (-CO). The introduction of one pentanoyl group was confirmed by analyzing its ^{1}H-NMR spectrum which showed the following characteristic peaks: δ 2.35 (2H, *t*, J=7.6 Hz), δ 1.60 (2H, *t*, J=7.5 Hz), 1.36 (2H, *m*) and δ 0.90 (3H, *t*, J=7.3 Hz). Its ^{1}H-NMR spectrum also ascertained the introduction of the pentanoyl group at position 2, since H-2 resonated downfield to δ 5.21 (as *d*, J = 3.3 Hz) as compared to its usual value (~4.00 ppm). The introduction of one pentanoyl group was also supported by ^{13}C-NMR spectrum in which the following peaks were observed: δ 178.97{$(CH_3)_3C\underline{C}O$-}, δ 33.90, 22.22, 17.53 {$CH_3(\underline{C}H_2)_3CO$-}and δ 13.67 {$\underline{C}H_3(CH_2)_3CO$-.

The rhamnopyranoside (**4**) was then transformed to the hexanoyl derivative (**6**) in 88% yield using an excess of hexanoyl chloride in pyridine. Its IR spectrum exhibited absorption bands at 1710 cm^{-1} due to –CO stretching. In its ^{1}H-NMR spectrum, the presence of the following peaks showed the presence of two hexanoyl groups: δ 2.37 (2H, *t*, J=7.4 Hz), δ 2.28 (2H, *t*, J=7.5 Hz), δ 1.60 (4H, m), δ 1.29 (8H, m) and δ 0.89 (6H, *m*). The presence of two hexanoyl group in the molecule was also confirmed by analyzing its ^{13}C-NMR spectrum which displayed the following characteristic peaks: δ 172.78, δ 172.66 {2× $CH_3(CH_2)_4\underline{C}O$-}, δ 34.17, 34.08, 31.31, 31.25, 24.53 (×2), 22.29, 17.45 {2× $CH_3(\underline{C}H_2)_4CO$-} and δ 13.86 (×2){2× $\underline{C}H_3(CH_2)_4CO$-}. By complete analysis of the ^{1}H-NMR and ^{13}C-NMR spectra, the structure of the hexanoyl derivative was elucidated as methyl 2,4-di-*O*-hexanoyl-3-*O*-pivaloyl-α-L-rhamnopyranoside (**6**).

Next effort was to derivatize the monosaccharide derivative 1,2-*O*-isopropylidene-6-*O*-pivaloyl-α-D-glucofuranose (**7**) (Scheme 3) and for this purpose we used pentanoyl chloride, hexanoyl chloride and 4-methoxybenzoyl chloride as the acylating agents. Thus, treatment of compound **7** with pentanoyl chloride in pyridine, followed by usual work-up and purification procedure, afforded compound 8 as needles, m.p. 57-58 °C. The IR spectrum of compound **8** showed absorption bands at 1728 cm^{-1} (-CO stretching) and 1362 cm^{-1} (carbon-hydrogen stretching of $>CMe_2$), thereby suggesting the presence of carbonyl and $>CMe_2$ groups in the molecule. In its ^{1}H-NMR spectrum, the resonance peaks at δ 2.29 (2H, *t*, J=7.2 Hz), δ 2.21 (2H, *t*, J=7.5 Hz), δ 1.55 (4H, *m*), δ 1.32 (4H, *m*), δ 0.88 (6H, *m*), corresponded to two pentanoyl groups. The ^{13}C-NMR spectrum also supported the attachment of two pentanoyl groups by displaying *inter alia* the following characteristic peaks: δ 172.37, 172.19 {2× $CH_3(CH_2)_3\underline{C}O$-}, δ 33.76, 33.62, 26.74, 26.27, 22.18 (×2) {2×$CH_3(\underline{C}H_2)_3CO$-}and δ 13.67 (×2) {2× $\underline{C}H_3(CH_2)_3CO$}.

The isopropylidene derivative (**7**) was then treated with hexanoyl chloride and after usual

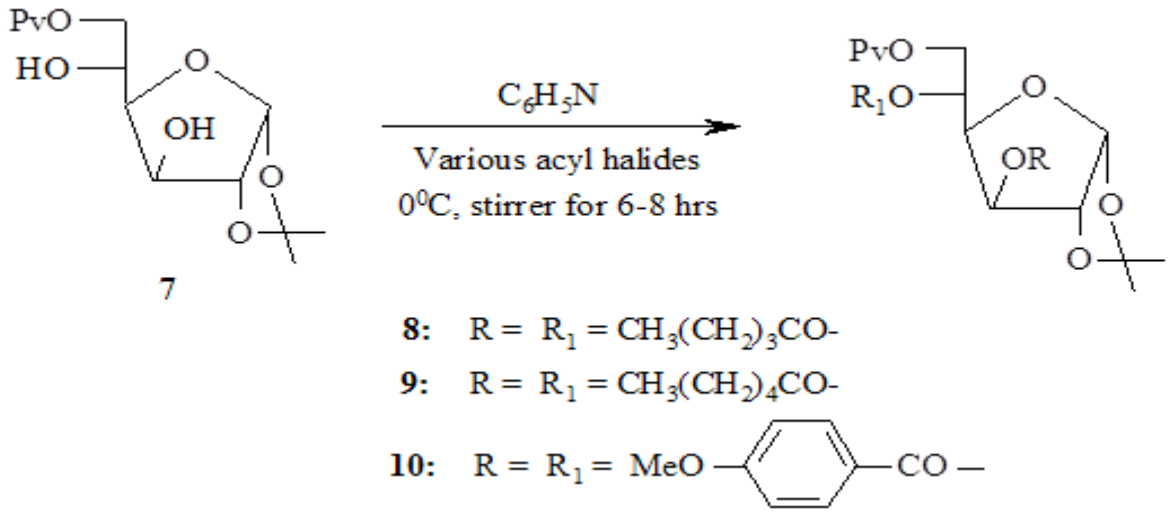


4

5: R = H; $R_1 = CH_3(CH_2)_3CO$-

6: R = $R_1 = CH_3(CH_2)_4CO$-

Scheme 2. Synthetic route for the synthesis of compounds **5-6**



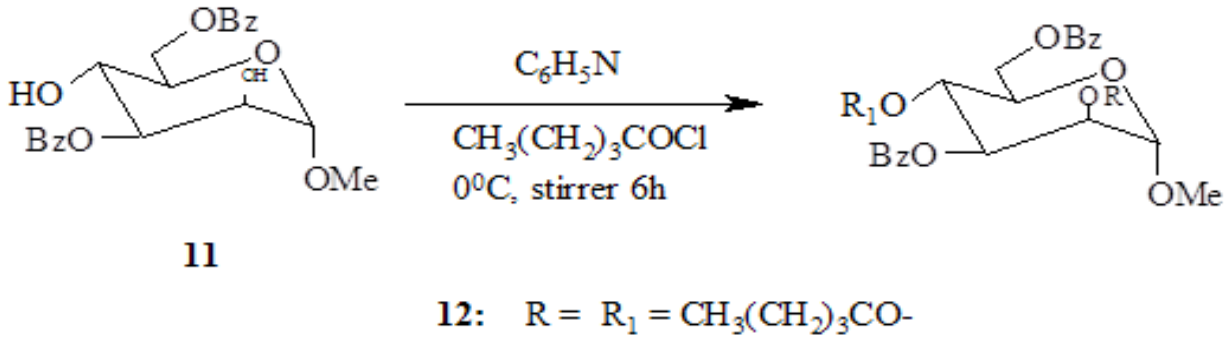
Scheme 3. Synthetic route for the synthesis of compounds **8-10**

work-up and purification procedures, compound **9** was obtained in 85% yield as crystalline solid, m.p. 66-67 °C. The IR spectrum of this compound showed the following characteristic peaks: 1710 (-CO stretching) and 1364 cm^{-1} (>CMe_2) stretching. Compound 9 was found to be a di-*O*-hexanoyl derivative by completely analyzing its ^{1}H-NMR and ^{13}C-NMR spectra and its structure was established as 3,5-di-*O*-hexanoyl-1,2-*O*-isopropylidene-6-*O*-pivaloyl-α-D-glucofuranose. In a similar way, the isopropylidene derivative (**7**) was converted to compound **10** by reaction with 4-methoxybenzoyl chloride in pyridine. The ^{1}H-NMR and ^{13}C-NMR spectra of this compound was compatible with the structure assigned as 1,2-*O*-isopropylidene-3,5-di-*O*-(4-methoxybenzoyl)-6-*O*-pivaloyl-α-D-glucofuranose (**10**).

Finally, we carried out pentanoylation of methyl 3,6-di-*O*-benzoyl-α-D-mannopyranoside (**11**) (Scheme 4) with an excess of pentanoyl chloride in pyridine. After usual work-up and chromatographic purification, compound **12** was obtained in 90% yield as a solid m.p. 101-102 °C. By complete analysis of its IR, NMR spectra, the structure of this compound was ascertained as methyl 3,6-di-*O*-benzoyl-2,4-di-*O*-pentanoyl-α-D-mannopyranoside (**12**).

Antimicrobial Activities

The test chemicals (Schemes 1-4) in the present investigation contain a variety of functional groups. Since we have previously observed [29-30] that various acylated monosaccharide derivatives showed effective biological activity,



Scheme 4. Synthesis of the compound **12**

the test chemicals under investigation are expected to show such activity. For comparative study, antimicrobial activities of the standard antibiotics (Ampicillin and Nystatin) were also determined.

Table 1. Zone of inhibition observed against Gram-positive bacteria by the test compounds 2-12

Compound	Diameter of inhibition zone (mm) 200 µg dw/disc			
	B. subtilis	*B. cereus*	*B. megaterium*	*S. aureus*
2	NF	NF	NF	NF
3	6.21	NF	8.13	NF
5	NF	NF	NF	NF
6	NF	NF	NF	NF
8	NF	NF	NF	NF
9	NF	NF	NF	NF
10	8.11	6.15	*17.3	7.12
12	NF	NF	NF	NF
**Ampicillin	*19.0	*18.0	*16.0	*22.0

* = marked inhibition, ** = standard antibiotic, dw = dry weight; NF = not found

The *in vitro* antibacterial screening against four Gram-positive and six Gram-negative human pathogens are shown in Table 1 and Table 2. Test chemical **10** was found to be active against all the Gram-positive and Gram-negative bacteria tested herein. The chemicals **2, 5, 6, 8**, **9** and **12** were recorded inactive against all the Gram-positive and Gram-negative bacteria. Compound **10** (17.3 mm) showed higher activity than standard antibiotic, Ampicillin (16.0 mm) against *B. megaterium* and showed comparable activity (14.5 mm) against *S. typhi*. The inhibitions of compound **3** showed somewhat lesser activity against *B. subtilis* (6.21 mm), *B. megaterium* (8.13 mm), *S. sonnei* (7.5 mm) and INABAET (*vibrio*) (7.5 mm).

The *in vitro* antifungal results of our synthesized chemicals and the standard antibiotic is shown in Table 3. The results indicated that most of the chemicals were moderate or less sensitive towards the five test fungal phytopathogens. Compound **10** exhibited highest sensitivity against *Fusarim equiseti* (40.0%), *Macrophomina phaseolina* (40.0%) and *Colletotricum corchori* (35.0%).

Table 2. Zone of inhibition observed against Gram-negative bacteria by the test compounds 2-12

Compound	Diameter of inhibition zone in mm 200 µg dw/disc					
	Pseudomona sp	*S. typhi*	*S. paratyphi*	*S. dysenteriae*	*S. sonnei*	*INABAET (vibrio)*
2	NF	NF	NF	NF	NF	NF
3	NF	NF	NF	NF	7.5	7.5
5	NF	NF	NF	NF	NF	NF
6	NF	NF	NF	NF	NF	NF
8	NF	NF	NF	NF	NF	NF
9	NF	NF	NF	NF	NF	NF
10	6.5	*14.5	7.5	11	8	10
12	NF	NF	NF	NF	NF	NF
****Ampicillin**	*18	*20	*18	*22	*20	*15

* = marked inhibition, ** = standard antibiotic, dw = dry weight; NF = not found

Table 3. Antifungal activities of the test compounds 2-12 and nystatin

Compound	Inhibition % of fungal mycelial growth[a] (100 µg dw/mL medium)				
	F. equiseti	*M. phaseolina*	*C. corchori*	*C. lunata*	*A. alternata*
2	NF	9.11	4.35	12.11	6.21
3	8.09	NF	10.08	15.15	NF
5	14.13	NF	NF	NF	NF
6	8.09	18.12	10.23	NF	10.22
8	NF	15.05	8.33	NF	8.33
9	NF	12.15	4.25	10.31	8.21
10	*40.0	*40.0	*35.0	25.14	*30.0
12	4.11	6.21	NF	8.11	NF
****Nystatin**	*44.7	*71.78	*40.51	*75.0	*51.55

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

Our newly synthesized monosaccharide derivatives (Schemes 1-4) have not been studied against these microorganisms, as mentioned before. These acylated derivatives were used for a comparative study of antibacterial and antifungal activity in our research laboratory.

Conclusion

The results of the present investigation showed that some of the newly synthesized acylated derivatives of 1,2-*O*-isopropylidene-6-*O*-pivaloyl-α-D-glucofuranose may possess a wide range of antimicrobial activities. From the results we observed that the introduction of some

specific functionalities in the test chemicals improved their antimicrobial activities. In this series the presence of 4-methoxybenzoyl and pentanoyl groups might be responsible for the improvement of the antimicrobial capacity of the test chemicals. 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.

Acknowledgements

The authors are thankful to the Ministry of Science and Technology for special allocation project (sl. no. 8 (*physical sci.*), Gr. sl. no. 13; ref. 39.009.006.01.00.042.2012-2013/Phys-8/599/1(5)), Bangladesh for financial assistance in carrying out the research. We are also grateful to the Chairman, BCSIR laboratory, Dhaka, for recording the ^{1}H-NMR and ^{13}C-NMR spectra.

References

1. C. Andry, R. Wyld, C. Laffite, G. Privat, K. Winternitz, *Phytochemistry,* 1982, 21, 1123-1127.
2. S. Elliott, J. Eqrie, J. Browne, T. Lorenzini, L. Busse, N. Roqers, I. Ponting *Exp. Hematol.,* 2004, 32, 1146-1155.
3. H. Ishji, M. Nakamura, S. Seo, K. Tori, T. Tyzoyo, Y. Yoshimura, *Chem. Pharm. Bull.,* 1980, 28, 2367-2373.
4. B.G. Moyer, P.E. Pfeffer, J.L. Moniot, M. Shamina, D.L. Gustine, *Phytochemistry,* 1977, 16, 375-377.
5. R. Tacheche, F.J. Kannerer, G. Wulff, *Chem. Ber.,* 1969, 104, 2057-2063.
6. Y. Tsuda, E. Haque, *Chem. Pharm. Bull.,* 1983, 31, 1437-1439.
7. B.D. Martin, S.A. Ampofo, J.S. Dordick, *Macromolecules,* 1992, 25, 7081-7086.
8. Y. Tokiwa, H. Fan, Y. Hiraguri, R. Kurane, *Macromolecules,* 2000, 33, 1636-1639.
9. H.M.I. Osborn, V.A. Brome, *Carbohydr. Res.,* 2001, 332, 157-166.
10. H. Namazi, R. Sharifzadeh, *Molecules,* 2005, 10, 772-782.
11. D. Wagner, J.P.H.Verheyden, J.G. Moffatt, *J. Org. Chem.,* 1974, 39, 24-30.
12. T. Ogawa, M. Matsui, *Tetrahedron,* 1981, 37, 2363-2369.
13. R.M. Munavu, H.H. Szmant, *J. Org. Chem.,* 1976, 41, 1832-1835.
14. J.J. Willard, J.S. Brimacombe, R.P. Brueton, *Can. J. Chem.,* 1964, 42, 2560-2567.
15. R. Gupta, S. Paul, A.K. Gupta, P.K. Kachroo, S. Bani, *Ind. J. Chem.,* **1997**, 36B, 707-710.
16. A.K.M.S. Kabir, S.M.A. Kawsar, M.M.R. Bhuiyan, M.R. Islam, M.S. Rahman, *Bull. Pure Appl. Sci.,* **2004**, 23C, 83-91.
17. A.K.M.S. Kabir, P. Dutta, M.N. Anwar, *Int. J. Agric. Biol.,* **2005**, **7**, 760-763.
18. S.M.A. Kawsar, A.K.M.S. Kabir, M.M.R. Bhuiyan, J. Ferdous, M.S. Rahman, *J. Bang. Acad. Sci*., **2013**, 37, 145-158.
19. S.M.A. Kawsar, M.M. Islam, S.A. Chowdhury, T. Hasan, M.K. Hossain, M.A. Manchur, Y. Ozeki, *Hace. J. Biol. Chem*., **2013**, 41, 195-206.
20. S.M.A. Kawsar, A.K.M.S. Kabir, M.M. Manik, M.K. Hossain, M.N. Anwar, *Int. J. Biosci*., **2012**a, 2, 66-73.
21. S.M.A. Kawsar, T. Hasan, S.A. Chowdhury, M.M. Islam, M.K. Hossain, M.A. Manchur, *Int. J. Pure Appl. Chem*., **2013**, 8, 125-135.
22. A.K.M.S. Kabir, S.M.A. Kawsar, M.M.R. Bhuiyan, B. Banu, *J. Bang. Chem. Soc*., **2008**, 21, 72-80.
23. A.K.M.S. Kabir, M.P.I. Bhuiyan, *J. Bang. Chem. Soc*., **1999**, 12, 1-7.
24. A.K.M.S. Kabir, *J. Bang. Chem. Soc*., **1996**, 9, 01-11.
25. Y. Tsuda, M.E. Haque, K. Yoshimoto, *Chem. Pharm. Bull*., **1983**, 31, 1662-1668.
26. A.W. Bauer, W.M.M. Kirby, J.C. Sherris, M. Turck, *Am. J. Clinic. Pathol.,* **1966**, 45, 439-476.
27. M.A.T. Miah, H.U. Ahmed, N.R. Sharma, A. Ali, S.A. Miah, *Bang. J. Bot*. 1990, 19, 05-10.
28. R.K. Grover, J.D. Moore, *Phytopathology,* **1962**, 52, 876-880.
29. S.M.A. Kawsar, A.K.M.S. Kabir, M.M.R. Bhuiyan, A. Siddiqa, M.N. Anwar, *RGUHS J. Pharm. Sci*., **2012**b, 2, 107-115.
30. A.K.M.S. Kabir, P. Dutta, S.M.A. Kawsar, M.M.R. Bhuiyan, M.N. Anwar, *Bull. Pure Appl. Sci*,. (India), 2004, 23C, 39-45.