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Full Paper

Comparative Antibacterial Activities of Some Monosaccharide and Disaccharide Benzoates

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Abstract: For comparative antibacterial studies a number of furanose (**3**, **5**), pyranose (**7**, **9**, **11**, **13**), and disaccharide benzoates (**14**, **15**) were prepared by direct benzoylation method. Synthesized benzoates (**3**, **5**, **7**, **9**, **11**, **13**-**15**) along with some starting materials were screened for *in vitro* antibacterial activity against ten human pathogenic bacteria viz. *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Staphylococcus aureus*, *Escherichia coli*, INABA ET (Vibrio), *Pseudomonas* species, *Salmonella paratyphi*, *Salmonella typhi*, and *Shigella dysenteriae*. The study revealed that the pyranose benzoate derivatives (**7**, **9**, **11**, **13**) were more prone towards antibacterial functionality than that of the furanose benzoate (**3**, **5**) and disaccharide benzoates (**14, 15**).

Keywords: monosaccharides; rhamnopyranoside; benzoylation; antibacterial activity; structure activity relationship (SAR)

1. INTRODUCTION

Monosaccharides are wide spread in nature, being a component of some plant glycosides and bacterial polysaccharides of immunological importance [1, 2]. In monosaccharides, protection of a particular hydroxyl group is not only necessary for the modification of the remaining functional groups but also for the synthesis of newer derivatives of great importance [3, 4]. The most common hydroxyl-protecting acyl groups are acetyl, benzoyl *etc*. Both the acetyl and benzoyl groups are very cheap protecting groups that can be easily removed and the parent alcoholic components can be recovered under basic or acidic conditions [5]. Per-*O*-acetyl and per-*O*-benzoyl derivatives of sugars are important intermediates in carbohydrate transformation and synthesis [6]. Acetylation and benzoylation of carbohydrates will convert unprotected and polar sugars into substances soluble in many organic solvents and the resulting sugar peracetates and perbenzoates have been utilized as glycosyl donors in monosaccharide transformations and oligosaccharide syntheses [7]. In comparison with sugar peracetates, sugar perbenzoates even possess some additional advantages. For example, benzoylated sugar derivatives are significantly less reactive than their acetylated analogues and tend to be more bench-stable [6, 8].

Monosaccharides and nucleosides in combination with acyl nuclei (e.g. acetyl, mesyl, benzoyl, *etc*) play an important role as common denominator for various biological activities, which is also revealed by a number of our previous works [9-11]. Catelani *et al.* [12] reported the synthesis of various 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives (**1a-c**) from triol **4** (Figure 1) and tested their effects in augmenting the proportion of benzidine-positive (hemoglobin-containing) cells in treated K562cell populations. The results obtained demonstrated that two of these newly synthesized compounds (**1b** and **1c**) were potent inducers of erythroid differentiation of K562cells. Very recently, we reported the synthesis and comparative antimicrobial studies of a number of furanose, pyranose and disaccharide acetates [13], and acylates [14]. The structure activity relationship

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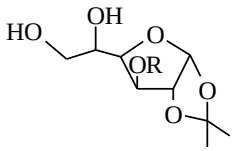
(SAR) study revealed that the pyranose acetate derivatives were more prone towards antimicrobial functionality than those of the furanose and disaccharide acetates. Considering the synthetic and biological importance, we were interested to extend our research work for the synthesis of some benzoyl derivatives of various monosaccharides and disaccharides for antibacterial studies.



1a: R = $COCH_2CH_2CH_3$;
1b: R = $COCH_2CH(CH_3)CH_3$;
1c: R = $COC(CH_3)_3$;
4: R = H.

Figure 1

2. MATERIAL AND METHODS

General experimental procedures

Evaporations were performed under diminished pressure on a Büchi rotary evaporator. Melting points (mp) were determined on an Electrothermal melting point apparatus and are uncorrected. FT-IR spectra were recorded on a FT IR spectrophotometer (Shimadzu, IR Prestige-21) using KBr and $CHCl_3$ technique. Thin layer chromatography was performed on Kieselgel GF_{254} and visualization was accomplished by spraying the plates with 1% H_2SO_4 followed by heating the plates at 150-200 ºC until coloration took place. Column chromatography was carried out with silica gel (100-200 mesh). ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded using $CDCl_3$ as a solvent. Chemical shifts were reported in δ unit (ppm) with reference to TMS as an internal standard and *J* values are given in Hz. All reagents used were commercially available (Aldrich) and were used as received unless otherwise specified.

Synthesis

1,2:5,6-Di-O-isopropylidene-α-D-gluco-1,4-furanose (2): The title compound **2** was prepared from D-glucose and anhydrous acetone according to the literature procedure [15]. The product was obtained in 46% yield as a white amorphous solid, mp. 108-110 °C (lit. [15] mp. 108-109 °C).

General procedure for direct benzoylation: To a solution of the hydroxyl compound in anhydrous pyridine (1 mL) was added benzoyl chloride at 0 ºC followed by addition of catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was allowed to attain room temperature and stirring was continued for 10-16 h. A few pieces of ice was added to the reaction mixture to decompose unreacted (excess) benzoyl chloride and the reaction mixture was extracted with dichloromethane (DCM, 3×5 mL). The organic (DCM) layer was washed successively with 5% hydrochloric acid, saturated aqueous sodium hydrogen carbonate solution, and brine. The DCM layer was dried and concentrated under reduced pressure. The residue thus obtained on column chromatography (*n*-hexane/ethyl acetate) gave the corresponding benzoyl product.

3-O-Benzoyl-1,2:5,6-di-O-isopropylidene-α-D-gluco-1,4-furanose (3): Benzoylation of the bisacetone D-glucose **2** (0.5 g, 1.92 mmol) with benzoyl chloride (0.297 g, 2.113 mmol) gave 3-*O*-benzoate **3** (0.644 g, 92%) as a colorless solid, mp 86-88 °C.

R_f = 0.58 (*n*-hexane/ethyl acetate = 5/1). IR ($CHCl_3$): 1730 (CO), 1375 cm^{-1} [$C(CH_3)_2$]. ^1H NMR (400 MHz, $CDCl_3$): δ 7.25-7.53 (3H, m, Ar-*H*), 7.82-8.22 (2H, m, Ar-*H*), 6.88 (1H, d, *J* = 3.6 Hz, H-1), 6.32 (1H, d, *J* = 2.8 Hz, H-3), 4.68 (1H, d, *J* = 3.6 Hz, H-2), 4.61-4.64 (1H, m, H-5), 4.61 (1H, dd, *J* = 8.9 and 3.0 Hz, H-6a), 4.50 (1H, dd, *J* = 8.6 and 7.0 Hz, H-4), 4.42 (1H, dd, *J* = 8.9 and 5.9 Hz, H-6b), 1.59 [3H, s, $C(CH_3)_2$], 1.52 [3H, s, $C(CH_3)_2$], 1.35 [6H, s, $2\times C(CH_3)_2$].

1,2-O-Isopropylidene-α-D-gluco-1,4-furanose (4): Selective deprotection of 5,6-*O*-isopropylidene functionality of bisacetone D-glucose **2** with methanolic H_2SO_4 using reported procedure [16] gave the title compound **4** as a white solid (76%), mp. 158-160 ºC (reported [16] mp. 159-160 ºC).

3,5,6-Tri-O-benzoyl-1,2-O-isopropylidene-α-D-gluco-1,4-furanose (5): Benzoylation of triol **4** (0.4 g, 1.816 mmol) with 3.3 molar equivalent of benzoyl chloride (1.149 g, 8.174 mmol) yielded 3,5,6-tri-*O*-benzoate **5** (0.803 g, 83%) as a clear oil, which turned pale-yellow after a couple of weeks.

R_f = 0.51 (*n*-hexane/ethyl acetate = 5/1). IR (neat): 1745 (CO), 1366 cm^{-1} [$C(CH_3)_2$]. ^1H NMR (400 MHz, $CDCl_3$): δ 7.95-8.09 (5H, m, Ar-H), 7.24-7.58 (10H, m, Ar-H), 6.00 (1H, d, *J* = 3.7 Hz, H-1), 5.59 (1H, d, *J* = 2.8 Hz, H-3), 5.48 (1H, m, H-5), 4.71 (1H, dd, *J* = 12.0 and 3.1 Hz, H-6a), 4.46 (1H, d, *J* = 3.7 Hz, H-2), 4.35 (1H, dd, *J* = 12.0 and 5.2 Hz, H-6b), 4.08-4.13 (1H, m, H-4), 1.53 [3H, s, $C(CH_3)_2$], 1.33 [3H, s, $C(CH_3)_2$].

Methyl 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranoside (7): Methyl α-D-glucopyranoside (**6**) (0.4 g, 2.06 mmol) on benzoylation with benzoyl chloride (1.274 g, 9.063 mmol) afforded 2,3,4,6-tetra-*O*-benzoate **7** (1.069 g, 85%) as a white solid, mp 90-92 °C.

R_f = 0.55 (*n*-hexane/ethyl acetate = 4/1). IR (KBr): 1746, 1732, 1714 cm^{-1} (CO). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (1H, d, *J* = 8.0 Hz, Ar-*H*), 8.06 (1H, d, *J* = 7.4 Hz, Ar-*H*), 7.99 (1H, d, *J* = 7.6 Hz, Ar-*H*), 7.95 (1H, d, *J* = 8.0 Hz, Ar-*H*), 7.88 (1H, d, *J* = 7.6 Hz, Ar-*H*), 7.32-7.56 (13H, m, Ar-*H*), 7.27 (2H, t, *J* = 7.8 Hz, Ar-*H*), 6.22 (1H, app t, *J* = 10.0 Hz, H-3), 5.72 (1H, app t, *J* = 9.8 Hz, H-4), 5.33 (1H, dd, *J* = 10.0 and 3.6 Hz, H-2), 5.27 (1H, d, *J* = 3.6 Hz, H-1), 4.62 (1H, dd, *J* = 12.8 and 2.8 Hz, H-6a), 4.52 (1H, dd, *J* = 12.8 and 6.5 Hz, H-6b), 4.42-4.48 (1H, m, H-5), 3.50 (3H, s, OCH_3).

Methyl 6-O-triphenylmethyl-α-D-glucopyranoside (8): The title compound **8** was prepared from methyl α-D-glucopyranoside (**6**) and triphenylmethyl (trityl) chloride (3.73 g, 13.38 mmol) in 72% yield as a crystalline solid, mp. 150-151 ºC (lit. [17] mp. 151-152 ºC employing reported procedure [17].

Methyl 2,3,4-tri-O-benzoyl-6-O-triphenylmethyl-α-D-glucopyranoside (9): Benzoylation **8** (0.4 g, 0.916 mmol) with benzoyl chloride (0.425 g, 3.023 mmol) furnished the tribenzoate **9** (0.611 g, 89%), as a white solid, mp 96-98 ºC.

R_f = 0.56 (*n*-hexane/ethyl acetate = 4/1). IR ($CHCl_3$): 1730, 1714 cm^{-1} (CO). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22-8.40 (8H, m, Ar-*H*), 7.95-8.17 (3H, m, Ar-*H*), 7.73-7.91 (3H, m, Ar-*H*), 7.48-7.88 (12H, m, Ar-*H*), 7.18-7.45 (4H, m, Ar-*H*), 6.25 (1H, t, *J* = 9.5 Hz, H-3), 5.72 (1H, t, *J* = 9.6 Hz, H-4), 5.49 (1H, d, *J* = 3.6 Hz, H-1), 5.44 (1H, dd, *J* = 9.5 and 3.6 Hz, H-2), 4.36 (1H, m, H-5), 3.67 (3H, s, OCH_3), 3.45 (2H, m, H-6a and H-6b).

Methyl 2,3,4,6-tetra-O-benzoyl-α-D-mannopyranoside (11): Benzoylation of methyl α-D-mannopyranoside (**10**) (0.3 g, 1.545 mmol) with benzoyl chloride (0.956 g, 6.80 mmol) afforded the title compound **11** (0.887 g, 94%) as a solid, mp 131-132 °C.

R_f = 0.52 (*n*-hexane/ethyl acetate = 4/1). IR ($CHCl_3$): 1723 cm^{-1} (CO). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00-8.25 (5H, m, Ar-*H*), 7.95 (2H, d, *J* = 7.6 Hz, Ar-*H*),7.83 (2H, d, *J* = 7.8 Hz, Ar-*H*), 7.32-7.65 (10H, m, Ar-*H*), 7.24 (1H, d, *J* = 8.1 Hz, Ar-*H*), 6.10 (1H, dd, *J* = 10.0 and 3.0 Hz, H-3), 5.91 (1H, app t, *J* = 9.9 Hz, H-4), 5.69 (1H, d, *J* = 3.0 Hz, H-2), 5.00 (1H, s, H-1), 4.69 (1H, dd, *J* = 12.2 and 5.3 Hz, H-6a), 4.50 (1H, dd, *J* = 12.2 and 2.0 Hz, H-6b), 4.40-4.48 (1H, m, H-5), 3.54 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.6, 167.1, 167.0, 168.7 (*C*OPh), 126-131 (Ar-C), 97.6 (C-1), 70.5 (C-2), 69.9 (C-3), 68.8 (C-4), 66.7 (C-5), 63.8 (C-6), 55.7 (O*C*H_3).

Methyl 2,3,4-tri-O-benzoyl-α-L-rhamnopyranoside (13): Benzoylation of methyl α-L-rhamnopyranoside (**12**) (0.4 g, 2.245 mmol) with benzoyl chloride (1.04 g, 7.40 mmol) gave 2,3,4-tri-*O*-benzoate **13** (1.024 g, 93%) as a semi solid, which resisted crystallization.

R_f = 0.52 (*n*-hexane/ethyl acetate = 4/1). IR ($CHCl_3$): 1730 cm^{-1} (CO). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10-8.15 (3H, m, Ar-*H*), 7.98 (1H, d, *J* = 7.6 Hz, Ar-*H*), 7.83 (1H, d, *J* = 7.5 Hz, Ar-*H*), 7.60 (2H, t, *J* = 7.6 Hz, Ar-*H*), 7.34-7.53 (6H, m, Ar-*H*), 7.24 (2H, t, *J* = 8.0 Hz, Ar-*H*), 5.85 (1H, dd, *J* = 10.3 and 3.4 Hz, H-3), 5.69 (1H, t, *J* = 10.0 Hz, H-4), 5.67 (1H, dd, *J* = 3.4 and 1.2 Hz, H-2), 4.93 (1H, d, *J* = 1.2 Hz, H-1), 4.19-4.25 (1H, m, H-5), 3.51 (3H, s, *O*-CH_3), 1.39 (3H, d, *J* = 6.4 Hz, 6-CH_3).

Octa-O-benzoylsucrose (14): Finely powdered and dried sucrose (0.40 g, 1.168 mmol) on benzoylation for 16 h gave a clear solution. The mixture was poured into an ice water with the formation of solid crystals of sucrose octabenzoate. This on column chromatography with *n*-hexane-ethyl acetate (6/1) afforded octa-*O*-benzoylsucrose (**14**) (0.906 g, 66%) as a hygroscopic semi-solid (reported [18] mp 89 ºC).

R_f = 0.45 (*n*-hexane/ethyl acetate = 3/1). IR ($CHCl_3$): 1728 cm^{-1} (CO). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78-8.20 (14H, m, Ar-*H*), 7.09-7.62 (26H, m, Ar-*H*), 6.20 (1H, t, *J* = 10.1 Hz, H-3), 6.18 (1H, d, *J* = 3.6 Hz, H-1), 5.98 (1H, d, *J* = 5.6 Hz, H-3′), 5.95 (1H, t, *J* = 5.7 Hz, H-4′), 5.75 (1H, t, *J* = 10.0 Hz, H-4), 5.39 (1H, dd, *J* = 10.4 and 3.6 Hz, H-2), 4.57-4.62, 4.64-4.74 (6H, 2×m, H-5, H-1′a, H-1′b, H-5′, H-6′a and H-6′b,), 4.40 (1H, dd, *J* = 12.8 and 3.3 Hz, H-6b), 4.32 (1H, dd, *J* = 12.8 and 2.9 Hz, H-6a). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 166.1, 166.0(×2), 165.8, 165.7, 165.6, 165.4 (8×*C*OPh), 128.1-130.2 (Ar-*C*), 105.7 (C-2′), 91.8 (C-1), 80.2 (C-5′), 77.5 (C-4′), 73.8 (C-3′), 72.0 (C-2), 71.2 (C-3), 69.9 (C-4), 69.8 (C-5), 65.3, 64.8 (C-1′,C-6′), 63.0 (C-6).

Octa-O-benzoyl-α-lactose (15): To a solution of powdered and dried lactose (0.5 g, 1.46 mmol) in anhydrous pyridine (2 mL) was added benzoyl chloride (1.65 g, 11.738 mmol) and stirred for

overnight. Usual work-up and chromatography afforded octa-*O*-benzoyl-α-lactose (**15**) (1.184 g, 69%) as a hygroscopic semi-solid.

R_f = 0.58 (*n*-hexane/ethyl acetate = 2/1). IR ($CHCl_3$): 1733, 1717 cm^{-1} (CO). ^1H NMR (400 MHz, $CDCl_3$): δ 7.88-8.19 (14H, m, Ar-*H*), 7.15-7.76 (26H, m, Ar-*H*), 6.75 (1H, d, *J* = 3.6 Hz, H-1), 6.20 (1H, t, *J* = 10.0 Hz, H-3), 5.78 (1H, d, *J* = 3.6 Hz, H-4′), 5.75 (1H, dd, *J* = 10.4 and 8.0 Hz, H-2′), 5.62 (1H, dd, *J* = 10.4 and 3.6 Hz, H-2), 5.38 (1H, dd, *J* = 3.6 and 10.4 Hz, H-3′), 4.95 (1H, d, *J* = 8.0 Hz, H-1′), 4.56 (2H, br s, H-6a and H-6b), 4.38 (1H, dd, *J* = 10.0 and 9.2 Hz, H-4), 4.28-4.32 (1H, m, H-5), 3.87-3.92 (1H, m, H-5′), 3.77 (1H, dd, *J* = 12.6 and 6.0 Hz, H-6a′), 3.74 (1H, dd, *J* = 12.6 and 4.0 Hz, H-6b′).

Antibacterial screening tests

In vitro antibacterial functionality was evaluated against ten bacterial pathogens. Of these four were Gram-positive viz. *Bacillus cereus* BTCC 19, *Bacillus megaterium* BTCC 18, *Bacillus subtilis* BTCC 17 and *Staphylococcus aureus* ATCC 6538 and six were Gram-negative viz. *Escherichia coli* ATCC 25922, *INABAET* (*vibrio*) AE 14748, *Pseudomonas aeruginosa* CRL (ICDDR,B*)*, *Salmonella paratyphi* AE 14613, *Salmonella typhi* AE 14612 and *Shigella dysenteriae* AE 14369. For the detection of antibacterial activities, the disc diffusion method described by Bauer *et al.* [19] was followed. Dimethylformamide (DMF) was used as a solvent to initially prepare desired solution (1%) of the compounds. The plates were incubated at 37 °C for 48 h. Proper control was maintained with DMF without chemicals. Mueller-Hinton (agar and broth) medium was used for culture of bacteria. All the results were compared with the standard antibacterial antibiotic ampicillin (50 µg/disc, Beximco Pharmaceuticals Ltd., Bangladesh). Each experiment was carried out in triplicate.

3. RESULTS AND DISCUSSION

We observed that some acylated derivatives of monosaccharides exhibited effective antibacterial and antifungal activities [9-11, 13]. Encouraged by these results and to compare biological activities of monosaccharide (furanose and pyranose form) benzoates with that of disaccharide (e.g. sucrose and lactose) benzoates, our main aim was to synthesize some benzoyl derivatives of D-*gluco*-1,4-furanose, D-glucopyranose, D-mannopyranoside, L-rhamnopyranoside, sucrose, and lactose (Figure 2).

Synthesis of furanose benzoates 3 and 5

Our first effort was to synthesize 3-*O*-benzoyl derivative of 1,2:5,6-di-*O*-isopropylidene-α-D-*gluco*-1,4-furanose (**2**). For this reason, initially, 1,2:5,6-di-*O*-isopropylidene-α-D-*gluco*-1,4-furanose (**2**) was prepared from D-glucose [15]. In the 1,2:5,6-*O*-protected glucofuranose (**2**), the C-3 position OH remain free and can be easily acylated. Thus, treatment of **2** with benzoyl chloride in pyridine gave a compound in quantitative yield (92%). The FT-IR spectrum of the compound showed a band at 1730 cm^{-1} corresponding to carbonyl frequency and the absence of hydroxyl stretching band indicated the attachment of benzoyl group. In the ^1H NMR spectrum, the presence of a three-proton multiplet at δ 7.25-7.53 and a two-proton multiplet at δ 7.82-8.22 were due to the benzoyl group. Also, C-3 proton shifted considerably to downfield at δ 6.32 (d, *J* = 2.8 Hz) as compared to its usual value (~4.30 ppm), thus confirming the attachment of the benzoyl group at position C-3 of the molecule.

In the next step, we prepared 1,2-*O*-isopropylidene-α-D-*gluco*-1,4-furanose (**4**) from bisacetone D-glucose (**2**) by selective de-protection of 5,6-acetonide functionality [16]. Having 3,5,6-triol **4** in hand, we carried out tri-*O*-benzoylation. Reaction of triol **4** with benzoyl chloride in pyridine afforded the product as oil. FT-IR spectrum of this compound showed signals at 1745 (CO) and 1366 cm^{-1} [$C(CH_3)_2$]. It showed no peak corresponding to hydroxyl stretching and hence indicated the complete benzoylation. In its ^1H NMR spectrum, one five-proton multiplet at δ 7.95-8.09 and one ten-proton multiplet at δ 7.24-7.58 indicated the incorporation of three benzoyloxy groups in the molecule. Thus, the structure of the compound was assigned as 3,5,6-tri-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-*gluco*-1,4-furanose (**5**).

Synthesis of pyranose benzoates

For pyranose sugar benzoates, our first effort was to prepare methyl tetra-*O*-benzoyl-D-glucopyranoside. Thus, benzoylation of methyl α-D-glucopyranoside (**6**) afforded a white solid, mp 90-92 °C in 85% yield. The FTIR spectrum of this solid exhibited no band for hydroxyl stretching. It also

showed bands at 1746, 1732 and 1714 cm^{-1} corresponding to carbonyl frequency and thus indicated the attachment of benzoyloxy groups in the molecule. In the ^1H NMR spectrum, a three-proton singlet at δ 3.50 was due to glycosidic (C-1) methoxy group. Also, H-1 appeared at δ 5.27 as doublet with small coupling constant (J = 3.6 Hz) indicating the α-glucosidic nature. In addition, the appearance of twenty protons in the aromatic region were assigned for the four benzoyloxy groups. Thus the structure was accorded as methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (**7**).

To increase biological activities we were interested to synthesize benzoylated glucopyranoside with trityl moiety. So, we prepared methyl 6-*O*-trityl-α-D-glucopyranoside (**8**) according to the literature procedure [17]. Trimolar benzoylation of triol **8** furnished a white solid (89%), mp 96-98 ºC. The FT-IR spectrum of this solid showed bands at 1730 and 1714 cm^{-1} corresponding to carbonyl frequency and exhibited no band for hydroxyl stretching. In the ^1H NMR spectrum, the compound exhibited the following signals in the aromatic region: δ 8.22-8.40 (8H, m), 7.95-8.17 (3H, m), 7.73-7.91 (3H, m), 7.48-7.88 (12H, m) and 7.18-7.45 (4H, m). Of these thirty aromatic protons, fifteen were for trityl group and the rest fifteen protons were assigned for the three benzyloxy protons. The rest of the FT-IR and ^1H NMR spectra were in complete agreement with the structure accorded as methyl 2,3,4-tri-*O*-benzoyl-6-*O*-trityl-α-D-glucopyranoside (**9**).

In the next step, benzoylation of methyl α-D-mannopyranoside (**10**) gave a solid in 94% yield, mp 131-132 °C. In the FT-IR spectrum of this compound, a band at 1723 cm^{-1} was observed for carbonyl frequency and it showed no band for hydroxyl stretching. In the ^1H NMR spectrum, a five-proton multiplet at δ 8.00-8.25, a two-proton doublet at δ 7.95, a two-proton doublet at δ 7.82, a ten-proton multiplet at δ 7.32-7.65 and a one-proton doublet at δ 7.24 were assigned for the four benzoyloxy groups in the molecule. The ^{13}C NMR spectrum showed four carbonyl carbon peaks at δ 168.6, 167.1, 167.0 and 168.7. Complete analyses of the FT-IR, ^1H and ^{13}C NMR spectra confirmed the structure as methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (**11**).

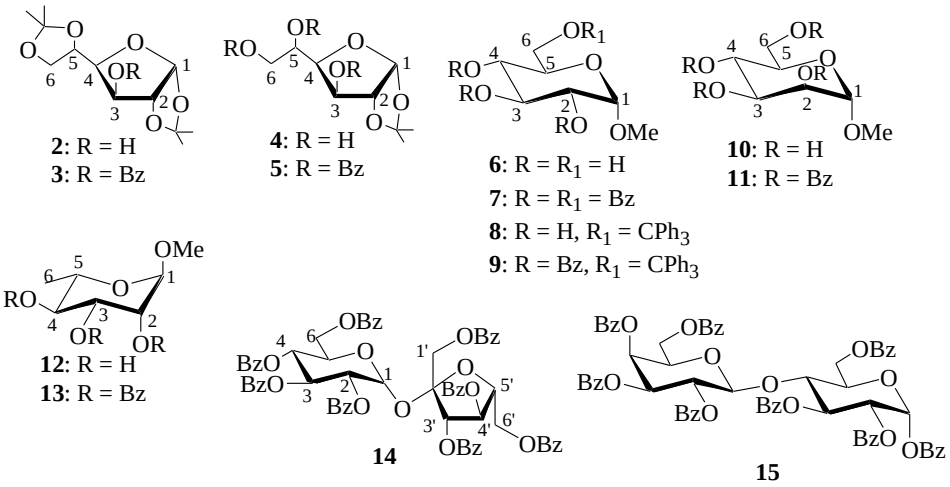


Figure 2

Finally, direct benzoylation methyl α-L-rhamnopyranoside (**12**) afforded a semi-solid (93%). The FT-IR spectrum of the compound showed a band at 1730 cm^{-1} corresponding to carbonyl frequency. In the ^1H NMR spectrum, fifteen protons resonated in the aromatic region at δ 8.10-8.15 (3H, m), 7.98 (1H, d, J = 7.6 Hz), 7.83 (1H, d, J = 7.5 Hz), 7.60 (2H, t, J = 7.6 Hz), 7.34-7.53 (6H, m) and 7.24 (2H, t, J = 8.0 Hz), hence indicated the attachment of three benzoyloxy groups in the compound. The H-2, H-3 and H-4 protons appeared considerably downfield at δ 5.67, 5.85 and 5.69, respectively as compared to its precursor methyl α-L-rhamnopyranoside (**12**). These downfield shifts clearly indicated the attachment of benzoyloxy groups at C-2, C-3 and C-4 positions. Based on the FT-IR and ^1H NMR spectra, the structure was accorded as methyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranoside (**13**).

Synthesis of per-*O*-benzoylated disaccharides 14 and 15

To compare the antibacterial activities of furanose and pyranose sugar benzoates with that of disaccharide benzoates we deliberately prepared octa-*O*-benzoylsucrose (**14**) and octa-*O*-benzoyl-α-lactose (**15**). Thus, sucrose on reaction with excess benzoyl chloride in anhydrous pyridine afforded a semi-solid in 66% yield. In its FT-IR spectrum, the carbonyl stretching peaks were observed at 1728 cm^{-1}. However, absence of frequency corresponding to hydroxyl group indicated the per-*O*-benzoylation of the molecule. In the ^{1}H NMR spectrum, forty aromatic protons appeared as two multiplets at δ 7.78-8.20 (14H) and δ 7.09-7.62 (26H) corresponding to eight benzoyloxy groups. This was further confirmed by its ^{13}C NMR spectrum where eight benzoyl carbonyl peaks appeared at δ 166.2, 166.1, 166.0(×2), 165.8, 165.7, 165.6, and 165.4. Therefore, the compound was unambiguously assigned the structure as 1′,2,3,3′,4,4′,6,6′-octa-*O*-benzoylsucrose (**14**).

Perbenzoylation of lactose, having eight hydroxyl groups, was conducted with the treatment with excess benzoyl chloride in anhydrous pyridine and afforded a semi-solid in 69% yield. FT-IR spectrum of the compound showed signals at 1733 and 1717 cm^{-1} corresponding to the carbonyl stretching peaks. Also, absence of frequency corresponding to hydroxyl group indicated the per-*O*-benzoylation of the molecule. In the ^{1}H NMR spectrum, two broad multiplets at δ 7.88-8.19 (14H, Ar-*H*) and 7.15-7.76 (26H, Ar-*H*) totaling forty aromatic protons indicated the attachment of eight benzoyloxy groups. The appearance of H-1 at δ 6.75 as doublet with small coupling constant (J = 3.6 Hz) indicated that it is equatorially oriented. So, the glycosidic (C-1) benzoyl group must be α. Therefore, the structure was assigned as 1′,2′,3′,6′,2,3,4,6-octa-*O*-benzoyl-α-lactose (**15**).

Antibacterial potentiality of the synthesized compounds

The results of the *in vitro* inhibition zone against the selected Gram-positive bacteria due to the effect of the chemicals (**2-15**) are mentioned in Table 1. The tested chemicals showed less inhibitory properties against these Gram-positive organisms. Only methyl 6-*O*-trityl-α-D-glucopyranoside (**8**) exhibited considerable inhibition (18 mm) against *Bacillus subtilis*.

Table 1. Inhibition against Gram-positive organisms by the chemicals (**2-15**).

Compound no.	Diameter of zone of inhibition in mm (50 µg. dw./ disc)			
	Bacillus cereus	***Bacillus megaterium***	***Bacillus subtilis***	***Staphylococcus. aureus***
2	NF	NF	NF	NF
3	07	NF	NF	08
4	NF	NF	NF	NF
5	NF	NF	12	NF
6	NF	NF	NF	NF
7	09	NF	07	NF
8	NF	NF	18	07
9	07	06	NF	NF
10	NF	NF	NF	NF
11	07	NF	11	NF
12	NF	NF	NF	NF
13	12	NF	NF	NF
14	08	NF	NF	NF
15	18	NF	NF	NF
****Ampicillin**	*22	*19	*25	*21

NB. NF indicates no inhibition, dw. = dry weight,
"**" indicates standard antibiotic, "*" shows good inhibition.

Inhibition zone against the selected Gram-negative bacteria due to the effect of the chemicals (**2-15**) are mentioned in Table 2. The study revealed that the tested chemicals were more effective against these

Gram-negative organisms. These compounds were more prone against *Salmonella paratyphi* and *Salmonella typhi*. In addition monosaccharides in the six-membered pyranose form viz. methyl 6-*O*-trityl-α-D-glucopyranoside (**8**), methyl 2,3,4,6-tetra-*O*-benzoyl-6-*O*-trityl-α-D-glucopyranoside (**9**), methyl α-D-mannopyranoside (**10**), methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (**11**) and methyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranoside (**13**) were the most effective against these pathogens. These pyranose monosaccharides were more effective than that of five-membered furanose (**2**-**5**) and disaccharide octabenzoates (**14**,**15**).

Table 2. Inhibition against Gram-negative organisms by the chemicals (**2**-**15**).

Compound no.	Diameter of zone of inhibition in mm (50 µg.dw./disc)					
	Escherichia coli	***INABAET (vibrio)***	***Pseudomonas aeruginosa***	***Salmonella paratyphi***	***Salmonella typhi***	***Shigella dysenteriae***
2	NF	NF	NF	16	NF	NF
3	06	NF	NF	12	NF	NF
4	NF	NF	NF	16	NF	NF
5	NF	NF	11	10	10	17
6	NF	NF	NF	08	NF	NF
7	06	NF	NF	10	08	NF
8	NF	08	10	10	18	17
9	15	NF	09	16	*20	NF
10	NF	NF	NF	*21	*20	NF
11	06	NF	08	19	18	NF
12	NF	NF	NF	NF	NF	NF
13	15	NF	NF	19	18	NF
14	08	NF	NF	11	12	NF
15	11	NF	NF	08	09	NF
****Ampicillin**	*25	*24	17	*35	13	*35

NB. NF indicates no zone of inhibition, dw. = dry weight,
"**" indicates standard antibiotic, "*" shows good inhibition.

Structure activity relationship (SAR)

Incorporation of benzoyl group increased the antimicrobial potentiality of different manosaccharide and disaccharides (Table 1 and Table 2). These synthesized benzoates were more active against Gram-negative pathogens than that of Gram-positive bacterial organisms. Benzoylated sugars with five-membered furanose form are less effective against both Gram-negative and Gram-positive than that of the corresponding six-membered pyranose form. This is because of the slight distortion of furanose ring in the presence of 1,2-*O*-isopropylidene ring. But monosaccharides (**6**-**13**) in pyranose form with regular $^4C_1$ or $^1C_4$ conformation exhibited better antibacterial potentiality. An important observation was that, compounds **2**, **4**, **6,** and **12** showed poor toxicity (except mannopyranoside **10**) than that of partially or fully benzoylated compounds **5**, **9**, **11,** and **13** against the tested pathogens. This is probably due to the presence of more hydroxyl groups in **2**, **4**, **6,** and **12**. While compounds **5**, **9**, **11,** and **13** having fewer or no hydroxyl groups showed much better antimicrobial potentiality. The hydrophobicity of the molecules increased gradually from compound **2**, **4**, and **6** and **12** to **5**, **9**, **11,** and **13**, which is an important parameter with respect to such bioactivity and toxicity or alteration of membrane integrity, because it is directly related to membrane permeation [20]. A similar hydrophobic interaction might occur between the benzoyl groups of glycopyranoses accumulated in the lipid like nature of the bacterial membranes. As a consequence of their hydrophobic interaction, bacteria lose their membrane permeability, ultimately causing death of the organisms [20-22].

In vitro antimicrobial activities of similar type of monosaccharide and disaccharide acetates [13] and structure activity relationship study also exhibited the almost similar inhibitory properties. Although, we expected better antibacterial potentiality for the benzoates because a large number of biologically active compounds contain aromatic and heteroaromatic nuclei [23-25]. An important observation between these two series was that both acetates and benzoates are more prone against Gram-

negative *Salmonella paratyphi* and *Salmonella typhi* organisms.

4. CONCLUSION

Thus, we have successfully synthesized benzoylated furanose (**3**, **5**), pyranose (**7**, **9**, **11**, **13**) and disaccharide (**14**, **15**) derivatives. A comparative study of *in vitro* antibacterial activities of monosaccharide (furanose and pyranose forms) benzoates with disaccharide benzoates was also carried out. The structure activity relationship (SAR) study revealed that the benzoylated sugar derivatives were more prone towards Gram-negative organisms than that of Gram-positive organisms. Benzoylated monosaccharides were strong inhibitors towards antibacterial functionality than that of the corresponding disaccharides.

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