Original Article

Synthesis and Antimicrobial Evaluation of Some *n*-Butyl α- and β-D-Glucopyranoside Derivatives

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ABSTRACT

Glycosidation of D-*glucose* with *n*-butanol yielded *n*-butyl α-D-glucopyranoside (**3a**) and *n*-butyl β-D-glucopyranoside (**3b**) which were converted to their corresponding acetates (**4a** and **4b**). Glucopyranosides (**3a** and **3b**) on treatment with benzaldehyde provided 4,6-*O*-benzylidene derivatives (**5a** and **5b**). Finally, reaction of compounds **5a** and **5b** with acetic anhydride in dry pyridine gave diacetates **6a** and **6b**, respectively. All the synthesized compounds were employed as test chemicals for *in vitro* antibacterial and antifungal functionality test against ten human pathogenic bacteria and seven fungi. The study revealed that the tested *n*-butyl glucopyranoside derivatives showed moderate antimicrobial functionalities as compared to the standard antibiotic.

Keywords: D-*Glucose*, *n*-butyl D-glucopyranoside, antimicrobial activities, inhibition, structure activity relationship.

INTRODUCTION

Acylation is found to be one of the most important and fundamental methods for protection of the hydroxyl groups in carbohydrate chemistry.[1] Considerable works have been done in the field of antimicrobial activities[2] by chemical compounds. Different classes of chemicals have been screened all over the world. Carbohydrates, especially acylated glycoses and glycosides are very important due to their effective biological activities.[3] Literature survey reveal that a large number of biologically active compounds possess aromatic, heteroaromatic nucleus and acyl groups.[4] In this context, we have reported the synthesis and antimicrobial activities of some selectively acylated derivatives of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**1**).[5]

Alkyl glucosides are a group of carbohydrate derived non-ionic surfactants of particular interest in the detergent, food, and pharmaceutical industries.[6] For example, *n*-octyl β-D-glucopyranoside and other similar compounds have been used as detergents to solubilize membrane proteins and to study the hydrophilic requirements of several enzymes.[7] Another alkyl glucopyranoside viz. *n*-propyl α-D-glucopyranoside (**2a**) was found to be a good polymer stabilizer.[8] Alkyl glucosides are also present in natural sources viz. ethyl α-D-glucopyranoside (**2b**) and *n*-butyl α-D-glucopyranoside (**3a**) were isolated from the mollusk *Cryptochiton stelleri*.[9] Guided by some positive observations in this area, we extend the research work for *n*-butyl α/β-D-glucopyranoside (**3a,b**) derivatives to get newer derivatives of biological importance.

MATERIALS AND METHODS

Evaporations were performed under diminished pressure on a Buchi rotary

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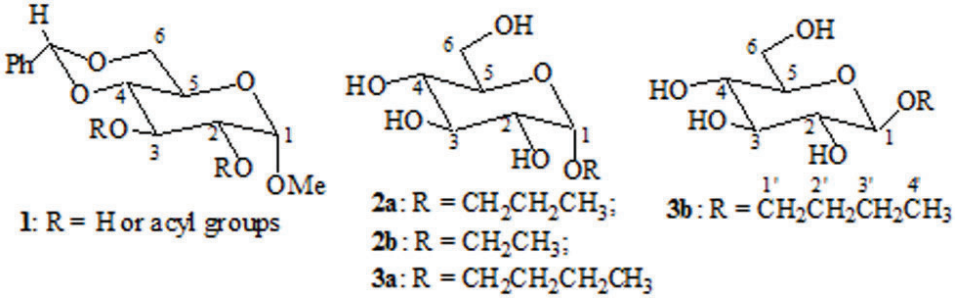


evaporator. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on a FT IR spectrophotometer (Shimadzu, IR Prestige-21) using KBr and $CHCl_3$ technique. Thin layer chromatography was performed on Kieselgel GF_{254} and visualization was accomplished by spraying the plates with 1% H_2SO_4 followed by heating the plates at 150–200°C 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 $CDCl_3$ or CD_3OD 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. The assignments of the signals were confirmed by decoupling and DEPT experiments. All reagents used were commercially available (Aldrich) and were used as received unless otherwise specified.

Procedure for synthesis of *n*-butyl α-D-glucopyranoside (3a) and *n*-butyl β-D-glucopyranoside (3b)

D-*Glucose* (3.0 g) was dissolved in *n*-butanol (8 ml) with stirring. Catalytic amount of conc. sulfuric acid (0.1 ml) was added to the mixture and the mixture was refluxed at 100–105°C for 5 h. The reaction mixture was cooled to room temperature and neutralized with aqueous ammonium hydroxide. The solution was concentrated to leave a syrupy residue which was passed through a packed silica gel column. Initial elution with dichloromethane/methanol (10/1) provided **3a** (1.61 g, 41%) as a yellow solid (dec. at 200°C).

R_f = 0.52 (dichloromethane/methanol = 4/1). IR (KBr): 3445 cm^{-1} (OH). ^1H NMR (400 MHz, CD_3OD): δ 5.01 (1H, d, *J* = 3.8 Hz, H-1), 3.95 (1H, dd, *J* = 12.3 and 1.9 Hz, H-6a), 3.86 (1H, dd, *J* = 12.3 and 4.8 Hz, H-6b), 3.81 (1H, ddd, *J* = 9.8, 4.8 and 1.9 Hz, H-5), 3.79 (1H, t, *J* = 9.6 Hz, H-3), 3.65 (1H, dd, *J* = 9.6 and 3.8 Hz, H-2), 3.63 (1H, dt, *J* = 9.6 and 7.1 Hz, H-1′a), 3.50 (1H, dd, *J* = 9.8 and 9.6 Hz, H-4), 3.45 (1H, dt, *J* = 9.6 and 7.1 Hz, H-1′b), 1.70 (2H, m, H-2′), 1.50 (2H, m, H-3′), 1.01 (3H, t, *J* = 7.2 Hz, H-4′). ^{13}C NMR (100 MHz, CD_3OD): δ 98.5 (C-1), 73.6 (C-3), 72.3 (C-5), 71.8 (C-2), 70.2 (C-4), 68.6 (C-1′), 61.1 (C-6), 31.2 (C-2′), 19.1 (C-3′), 13.5 (C-4′). Anal Calcd. for $C_{10}H_{20}O_6$ (236.13): C, 50.84; H,8.53%: found C, 50.93; H, 8.56%.

Further elution with dichloromethane/methanol (7/1) provided **3b** as a colourless solid (1.14 g, 29%), mp. 53–54°C (lit[10] mp. 52–54°C).

R_f = 0.49 (dichloromethane/methanol = 4/1). IR (KBr): 3448 cm^{-1} (OH). ^1H NMR (400 MHz, CD_3OD): δ 4.43 (1H, d, *J* = 7.9 Hz, H-1), 3.90 (1H, dd, *J* = 12.2 and 5.6 Hz, H-6a), 3.89 (1H, dt, *J* = 9.5 and 7.3 Hz, H-1′a), 3.70 (1H, dd, *J* = 12.2 and 2.0 Hz, H-6b), 3.62 (1H, dt, *J* = 9.5 and 7.4 Hz, H-1′b), 3.47 (1H, t, *J* = 9.6 Hz, H-3), 3.41 (1H, ddd, *J* = 9.8, 4.8 and 2.0 Hz, H-5), 3.36 (1H, dd, *J* = 9.8 and 9.6 Hz, H-4), 3.24 (1H, dd, *J* = 9.6 and 7.9 Hz, H-2), 1.57 (2H, m, H-2′), 1.34 (2H, m, H-3′), 0.89 (3H, t, *J* = 7.3 Hz, H-4′). ^{13}C NMR (100 MHz, CD_3OD): δ 98.5 (C-1), 73.3 (C-3), 72.8 (C-5), 71.9 (C-2), 70.4 (C-4), 66.5 (C-1′), 61.00 (C-6), 31.3 (C-2′), 19.0 (C-3′), 13.9 (C-4′). Anal Calcd. for $C_{10}H_{20}O_6$ (236.13): C, 50.84; H,8.53%: found C, 50.95; H, 8.55%.

n-Butyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (4a)

To a cooled (0°C) and stirred solution of glucoside **3a** (0.3 g, 1.27 mmol) in dry pyridine (1 ml) was added acetic anhydride (0.61 g, 5.98 mmol) slowly followed by addition of catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was allowed to attain the room temperature and stirred at this temperature for 12 h. The reaction mixture was treated with cold water (2 ml) and extracted with dichloromethane (3 × 3 ml). Work-up followed by silica gel column chromatography with *n*-hexane/ethyl acetate (8/1) afforded the tetraacetate **4a** (0.457 g, 89%) as a colourless oil.

R_f = 0.44 (*n*-hexane/ethyl acetate = 5/1). IR ($CHCl_3$): 1760, 1745 cm^{-1} (CO). ^1H NMR (400 MHz, $CDCl_3$): δ 5.47 (1H, t, *J* = 9.6 Hz, H-3), 5.07 (1H, d, *J* = 3.9 Hz, H-1), 5.05 (1H, dd, *J* = 10.2 and 9.6 Hz, H-4), 4.85 (1H, dd, *J* = 9.6 and 3.9 Hz, H-2), 4.27 (1H, dd, *J* = 12.2 and 4.8 Hz, H-6a), 4.07 (1H, dd, *J* = 12.2 and 2.2 Hz, H-6b), 4.01 (1H, ddd, *J* = 10.2, 4.8 and 2.2 Hz, H-5), 3.66 (1H, dt, *J* = 9.8 and 7.0 Hz, H-1′a), 3.43 (1H, dt, *J* = 9.8 and 7.0 Hz, H-1′b), 2.10 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 2.03 (3H, s, $COCH_3$), 2.02 (3H, s, $COCH_3$),

1.58 (2H, m, H-2′), 1.29 (2H, m, H-3′), 0.88 (3H, t, J = 7.0 Hz, H-4′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3 (*C*OCH$_3$), 169.9 (*C*OCH$_3$), 169.6 (*C*OCH$_3$), 169.0 (*C*OCH$_3$), 95.5 (C-1), 70.7 (C-3), 70.2 (C-5), 68.5 (C-2), 68.2 (C-1′), 67.0 (C-4), 61.8 (C-6), 31.1 (C-2′), 20.4 (CO*C*H$_3$), 20.3 (2 × CO*C*H$_3$), 20.0 (CO*C*H$_3$), 19.0 (C-3′), 13.5 (C-4′). Anal Calcd. for $C_{18}H_{28}O_{10}$ (404.17): C, 53.46; H, 6.98%: found C, 53.52; H, 6.96%.

n-Butyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (4b)

To a solution of glucoside **3b** (0.3 g, 1.27 mmol) in anhydrous pyridine (1 ml) was added acetic anhydride (0.60 g, 5.88 mmol) slowly at 0°C followed by addition of catalytic amount of DMAP. The reaction mixture was stirred overnight at room temperature. The mixture was treated with ice water (2 ml) and extracted with dichloromethane (3 × 3 ml). Usual work-up followed by chromatography with *n*-hexane/ethyl acetate (8/1) afforded the tetraacetate **4b** (0.472 g, 92%) as a white solid which on recrystallization (*n*-hexane/ethyl acetate) gave pure **4b**, mp 63–65°C (Lit[11] mp. 65–66°C).

R_f = 0.46 (*n*-hexane/ethyl acetate = 5/1). IR (KBr): 1758, 1746 cm^{-1} (CO). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.20 (1H, t, J = 9.5 Hz, H-3), 5.08 (1H, dd, J = 9.8 and 9.5 Hz, H-4), 4.98 (1H, dd, J = 9.5 and 8.0 Hz, H-2), 4.49 (1H, d, J = 8.0 Hz, H-1), 4.26 (1H, dd, J = 12.3 and 4.8 Hz, H-6a), 4.13 (1H, dd, J = 12.3 and 2.3 Hz, H-6b), 3.87 (1H, dt, J = 9.6 and 7.1 Hz, H-1′a), 3.68 (1H, ddd, J = 9.8, 4.8 and 2.3 Hz, H-5), 3.47 (1H, dt, J = 9.6 and 7.1 Hz, H-1′b), 2.08 (3H, s, COC*H*$_3$), 2.04 (3H, s, COC*H*$_3$), 2.02 (3H, s, COC*H*$_3$), 2.00 (3H, s, COC*H*$_3$), 1.52 (2H, m, H-2′), 1.35 (2H, m, H-3′), 0.91 (3H, t, J = 7.2 Hz, H-4′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6 (*C*OCH$_3$), 170.2 (*C*OCH$_3$), 169.2 (*C*OCH$_3$), 169.1 (*C*OCH$_3$), 100.7 (C-1), 72.9 (C-3), 71.8 (C-5), 71.2 (C-2), 69.9 (C-1′), 68.4 (C-4), 62.1 (C-6), 31.4 (C-2′), 20.8 (CO*C*H$_3$), 20.7 (2 × CO*C*H$_3$), 20.6 (CO*C*H$_3$), 19.0 (C-3′), 13.8 (C-4′). Anal Calcd. for $C_{18}H_{28}O_{10}$ (404.17): C, 53.46; H, 6.98%: found C, 53.51; H, 6.97%.

n-Butyl 4,6-*O*-benzylidene-α-D-glucopyranoside (5a)

Benzaldehyde (3.0 g, 28.27 mmol) was added to *n*-butyl α-D-glucopyranoside (**3a**) (0.6 g, 2.54 mmol) followed by addition of anhydrous copper sulfate (1.0 g, 6.27 mmol) at room temperature. The reaction mixture was stirred for 12 h and filtered through celite. The resulting solution was concentrated under reduced pressure to yield a residue which upon chromatographic purification with *n*-hexane/ethyl acetate (2/1) yielded a **5a** (0.577 g, 70%) as a thick syrup.

R_f = 0.66 (*n*-hexane/ethyl acetate = 5/3). IR ($CHCl_3$): 3444 cm^{-1} (OH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49, 7.33 (5H, 2×m, Ar-H), 5.54 (1H, s, C*H*Ph), 4.94 (1H, d, J = 3.7 Hz, H-1), 4.22 (1H, dd, J = 9.8 and 9.6 Hz, H-4), 4.07 (1H, t, J = 9.6 Hz, H-3), 3.89 (1H, ddd, J = 9.8, 4.8 and 1.9 Hz, H-5), 3.78 (1H, dd, J = 12.3 and 1.9 Hz, H-6a), 3.66 (1H, dd, J = 9.6 and 3.7 Hz, H-2), 3.62 (1H, dd, J = 12.3 and 4.8 Hz, H-6b), 3.59 (1H, dt, J = 9.6 and 7.1 Hz, H-1′a), 3.44 (1H, dt, J = 9.6 and 7.1 Hz, H-1′b), 1.68 (2H, m, H-2′), 1.49 (2H, m, H-3′), 0.98 (3H, t, J = 7.2 Hz, H-4′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.1, 130.2, 129.2, 128.5, 128.3, 126.3 (Ar-*C*), 103.2 (Ph*C*H-), 101.1 (C-1), 80.9, 72.9, 72.1, 70.3 (C-2/C-3/C-4/C-5), 68.6 (C-1′), 61.1 (C-6), 31.2 (C-2′), 19.1 (C-3′), 13.5 (C-4′). Anal Calcd. for $C_{17}H_{24}O_6$ (324.16): C, 62.95; H, 7.46%: found C, 63.01; H, 7.49%.

n-Butyl 4,6-*O*-benzylidene-β-D-glucopyranoside (5b)

To a mixture of *n*-butyl β-D-glucopyranoside (**3b**) (0.5 g, 2.12 mmol) was added benzaldehyde (3.0 g, 28.27 mmol) and anhydrous copper sulfate (1.2 g, 7.52 mmol) at room temperature. The reaction mixture was stirred for 12 h at room temperature and filtered through celite. The resulting solution was concentrated and chromatography with *n*-hexane/ethyl acetate (2/1) yielded a **5b** (0.501 g, 73%) as an oil.

R_f = 0.66 (*n*-hexane/ethyl acetate = 5/3). IR ($CHCl_3$): 3446 cm^{-1} (OH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50, 7.33 (5H, 2×m, Ar-H), 5.51 (1H, s, C*H*Ph), 4.38 (1H, d, J = 7.8 Hz, H-1), 4.02 (1H, dd, J = 9.8 and 9.6 Hz, H-4), 3.99 (1H, t, J = 9.6 Hz, H-3), 3.87 (1H, ddd, J = 9.8, 4.8 and 2.0 Hz, H-5), 3.82 (1H, dd, J = 12.2 and 5.6 Hz, H-6a), 3.69 (1H, dt, J = 9.5 and 7.3 Hz, H-1′a), 3.67 (1H, dd, J = 12.2 and 2.0 Hz, H-6b), 3.53 (1H, dt, J = 9.5 and 7.4 Hz, H-1′b), 3.26 (1H, dd, J = 9.6 and 7.8 Hz, H-2), 1.55 (2H, m, H-2′), 1.34 (2H, m, H-3′), 0.92 (3H, t, J = 7.3 Hz, H-4′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.0, 133.7, 129.4, 128.4, 128.3, 126.3 (Ar-*C*), 103.1 (Ph*C*H-), 98.8 (C-1), 80.6, 74.7, 73.2, 72.1 (C-2/C-3/C-4/C-5), 66.5 (C-1′), 61.00 (C-6), 31.3 (C-2′), 19.0 (C-3′), 12.9 (C-4′). Anal Calcd. for $C_{17}H_{24}O_6$ (324.16): C, 62.95; H, 7.46%: found C, 62.99; H, 7.50%.

n-Butyl 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-α-D-glucopyranoside (6a)

Diol **5a** (0.3 g, 0.924 mmol) was dissolved in anhydrous pyridine (1 ml) and the solution was cooled to 0°C. Acetic anhydride (0.24 g, 2.35 mmol) followed by catalytic amount of DMAP was added to the solution. The reaction mixture was allowed to attain room temperature and stirring was continued for 10 h. A few pieces of ice was added to the reaction mixture to decompose unreacted acetic anhydride and extracted with dichloromethane (DCM) (3 × 3 ml). Usual work-up followed by chromatography with *n*-hexane/ethyl acetate (12/1) afforded 2,3-di-*O*-acetates, **6a** (0.355 g, 94%) as a white solid, mp.108–109°C.

R_f = 0.50 (*n*-hexane/ethyl acetate = 9/1). IR (KBr): 1755, 1746 cm^{-1} (CO). ^1H NMR (400 MHz, $CDCl_3$): δ 7.409, 7.31 (5H, 2×m, Ar-H), 5.52 (1H, t, *J* = 9.6 Hz, H-3), 5.44 (1H, s, C*H*Ph), 4.90 (1H, d, *J* = 3.7 Hz, H-1), 4.76 (1H, dd, *J* = 9.6 and 3.7 Hz, H-2), 4.20 (1H, dd, *J* = 9.8 and 9.6 Hz, H-4), 3.87 (1H, ddd, *J* = 9.8, 4.8 and 1.9 Hz, H-5), 3.77 (1H, dd, *J* = 12.3 and 1.9 Hz, H-6a), 3.60 (1H, dd, *J* = 12.3 and 4.8 Hz, H-6b), 3.58 (1H, dt, *J* = 9.6 and 7.1 Hz, H-1′a), 3.42 (1H, dt, *J* = 9.6 and 7.1 Hz, H-1′b), 2.04 (3H, s, COCH_3), 2.00 (3H, s, COCH_3), 1.68 (2H, m, H-2′), 1.48 (2H, m, H-3′), 0.95 (3H, t, *J* = 7.2 Hz, H-4′). Anal Calcd. for $C_{21}H_{28}O_8$ (408.18): C, 61.75; H, 6.91%: found C, 61.82; H, 6.93%.

n-Butyl 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-β-D-glucopyranoside (6b)

Acetic anhydride (0.24 g, 2.35 mmol) was added to a stirred solution of the diol **5b** (0.3 g, 0.924 mmol) in anhydrous pyridine (1 ml) at 0°C followed by addition of catalytic amount of DMAP. The reaction mixture was allowed to attain room temperature and stirring was continued for 10 h. A few pieces of ice was added to the reaction mixture and extracted with dichloromethane (DCM) (3 × 5 ml). Usual work-up followed by chromatography with *n*-hexane/ethyl acetate (12/1) gave 2,3-di-*O*-acetates, **6b** (0.34 g, 90%) as a clear syrup.

R_f = 0.49 (*n*-hexane/ethyl acetate = 10/1). IR ($CHCl_3$): 1756, 1743 cm^{-1} (CO). ^1H NMR (400 MHz, $CDCl_3$): δ 7.50, 7.33 (5H, 2×m, Ar-H), 5.53 (1H, t, *J* = 9.6 Hz, H-3), 5.45 (1H, s, C*H*Ph), 4.86 (1H, d, *J* = 7.9 Hz, H-1), 4.78 (1H, dd, *J* = 9.5 and 7.9 Hz, H-2), 4.22 (1H, dd, *J* = 9.8 and 9.6 Hz, H-4), 3.92 (1H, ddd, *J* = 9.8, 4.8 and 2.0 Hz, H-5), 3.80 (1H, dd, *J* = 12.2 and 5.6 Hz, H-6a), 3.70 (1H, dt, *J* = 9.5 and 7.3 Hz, H-1′a), 3.66 (1H, dd, *J* = 12.2 and 2.0 Hz, H-6b), 3.51 (1H, dt, *J* = 9.5 and 7.4 Hz, H-1′b), 2.00 (3H, s, COCH_3), 1.98 (3H, s, COCH_3), 1.57 (2H, m, H-2′), 1.34 (2H, m, H-3′), 0.92 (3H, t, *J* = 7.4 Hz, H-4′). Anal Calcd. for $C_{21}H_{28}O_8$ (408.18): C, 61.75; H, 6.91%: found C, 61.77; H, 6.89%.

ANTIMICROBIAL SCREENING STUDIES

Test human and phytopathogens

All the *n*-butyl D-glucopyranoside derivatives (**3a, b-6a,b**) were tested against ten human pathogenic bacteria. Of these four were Gram-positive viz. *Bacillus cereus*, *Bacillus megaterium* and *Bacillus subtilis* and six were Gram-negative viz. *Staphylococcus aureus*, *Escherichia coli*, *Pastunella maltosida*, *Salmonella gallinarium*, *Salmonella typhi*, *Shigella dysenteriae* and *Vibro cholera*. Seven plant pathogenic fungi viz. *Aspergillus acheraccus*, *Aspergillus flavus*, *Aspergillus fumigates*, *Aspergillus niger*, *Aspergillus nodusus*, *Fuserium equiseti* and *Candida albicans* were selected for mycelial growth test for these glucopyranosides (**3a,b-6a,b**).

Screening of antibacterial activity

For the detection of *in vitro* antibacterial activities, the disc diffusion method[12a] slightly modified by Mia *et al*.,[12b] was followed. Nutrient agar (NA) was used as basal medium for test bacteria. Dimethyl formamide (DMF) was used as a solvent to prepare desired solution (0.5% and 1%) of the compounds. The plates were incubated at 37°C for 48 h. Proper control was maintained with DMF. Each experiment was carried out three times.

Screening of mycelial growth

The *in vitro* antifungal activities of the glucopyranosides (**3a,b-6a,b**) were assessed by poisoned food technique.[13] Potato dextrose agar (PDA) was used as basal medium for test fungi. 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 (DMF), *T* = Diameter of the fungal colony in treatment. All the results were compared with the standard antifungal antibiotic fluconazole (trade name Flugal-50) (100 μg/ml).

RESULTS AND DISCUSSION

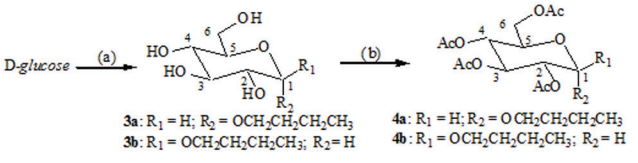
Synthesis of *n*-butylglucopyranosides

The main aim of the present work involves preparation of *n*-butyl glucopranoside and synthesis of its 4,6-*O*-benzylidene-2,3-di-*O*-acetyl derivatives. Many chemical and enzymatic methods were developed for the synthesis of alkyl glucopyranosides. Chemical methods generally used to synthesize alkyl glucosides involves several steps, complexity arises in the separation of anomers, require expensive reagents and are time-consuming.[14a] Enzyme catalyzed synthesis produce very poor yield.[14b]

We attempted to synthesize *n*-butyl D-glucopyranosides by heating D-*glucose* with *n*-butanol. Thus, D-*glucose* on treatment with *n*-butanol at 100–105°C in the presence of catalytic sulfuric acid provided a syrupy residue (Scheme 1). The residue on column chromatography elution with dichloromethane/methanol (10/1) gave a yellow solid having R_f 0.52. In its ^1H NMR spectrum, a one-proton doublet at δ 5.01 was assigned for H-1 and its small coupling constant (*J* = 2.4 Hz) indicated the formation of α-anomer. In its ^1H NMR spectrum, peaks at δ 3.63 (1H, dt, *J* = 9.6 and 7.1 Hz, H-1′a), 3.45

(1H, dt, J = 9.6 and 7.1 Hz, H-1′b), 1.70 (2H, m, H-2′), 1.50 (2H, m, H-3′) and 1.01 (3H, t, J = 7.2 Hz, H-4′) indicated the presence of *n*-butyl group in the molecule. In its ^{13}C NMR spectrum, signals corresponding to *n*-butyl group appeared at δ 68.6 (C-1′), 61.1 (C-6), 31.2 (C-2′), 19.1 (C-3′) and 13.5 (C-4′). Complete analysis of the ^{1}H and ^{13}C NMR spectra helped us to assign the structure of this compound as *n*-butyl α-D-glucopyranoside (**3a**).

Further elution with dichloromethane/methanol (7/1) provided a colourless solid, mp. 53–54°C with lower R_f value (0.49) (Scheme 1). In its ^{1}H NMR spectrum, a one-proton (H-1) doublet at δ 4.43 with high coupling constant (J = 7.9 Hz) along with the singnals at δ 3.89 (1H, dt, J = 9.5 and 7.3 Hz, H-1′a), 3.62 (1H, dt, J = 9.5 and 7.4 Hz, H-1′b), 1.57 (2H, m, H-2′), 1.34 (2H, m, H-3′) and 0.89 (3H, t, J = 7.3 Hz, H-4′) corresponding to *n*-butyl group indicated the formation of butyl glucopyranoside. On the basis of its IR, ^{1}H and ^{13}C NMR spectra, the structure of the compound was accorded as *n*-butyl β-D-glucopyranoside (**3b**).

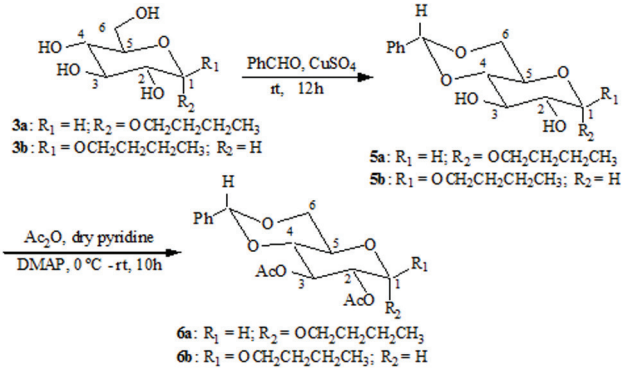


Scheme 1. *Reagents and conditions:* (a) *n*-butanol, H_2SO_4 100–105°C, 5h; (b) Ac_2O, pyridine, DMAP, 0°C – rt, 12h.

The structures of **3a** and **3b** were also confirmed by their conversion into corresponding tetraacetates (**4a** and **4b**, Scheme 1). Treatment of **3a** with acetic anhydride in dry pyridine followed by chromatography provided a syrupy residue. Its IR spectrum showed bands at 1760 and 1745 cm^{-1} due to carbonyl stretchings. In ^{13}C NMR spectrum, signals at δ 170.3 (*C*OCH$_3$), 169.9 (*C*OCH$_3$), 169.6 (*C*OCH$_3$), 169.0 (*C*OCH$_3$), 20.4 (CO*C*H$_3$), 20.3 (2×CO*C*H$_3$) and 20.0 (CO*C*H$_3$) confirmed the attachment of four acetyl groups in the molecule. Its ^{1}H NMR spectrum showed four three-proton singlets at δ 2.10, 2.07, 2.03 and 2.02 indicating the presence of four acetyl methyl groups. The downfield shift of H-2, H-3, H-4 and H-6 to δ 4.85, 5.47, 5.05 and 4.27, respectively as compared to the precursor glucopyranoside **3a**, indicated the introduction of acetyl groups at position 2, 3, 4 and 5. The rest of the ^{1}H and ^{13}C NMR spectra were in complete agreement with the structure accorted as *n*-butyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**4a**).

Similarly, reaction of β-glucopyranoside **3b** with acetic anhydride afforded the tetraacetate **4b** as a white solid, mp 63–65°C. In its IR spectrum, absorption bands at 1758 and 1746 cm^{-1} were due to carbonyl stretching and the spectrum showed absence of hydroxyl stretching bands. In the ^{1}H NMR spectrum, four three-proton signals at δ 2.08, 2.04, 2.02 and 2.00 were due to the methyl protons of four acetyloxy groups. Complete analysis of its IR, ^{1}H and ^{13}C NMR spectra enabled us to establish its structure as *n*-butyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**4b**).

Having butyl glucosides (**3a,b**) in hand, our next effort was to introduce 4,6-*O*-benzylidene functionality in the molecule (Scheme 2). At first, reaction of **3a** with excess benzaldehyde in the presence of anhydrous copper sulfate followed by chromatographic purification furnished a syrup in 70% yield. Its IR spectrum showed absorption band at 3444 cm^{-1} due to hydroxyl group. In the ^{1}H NMR spectrum, two multiplets at δ 7.49 and 7.33 integrated for five aromatic protons and a singlet at δ 5.54 corresponding to C*H*Ph indicated the formation of benzylidene acetal. The compound also showed signals at δ 137.1, 130.2, 129.2, 128.5, 128.3, 126.3 (Ar-*C*) and 103.2 (Ph*C*H-) in its ^{13}C NMR spectrum due to benzylidene group. Thus, the structure of the compound was accorded as *n*-butyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**5a**) by complete analysis of its all spectra.



Scheme 2. Synthesis of *n*-butyl 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-D-glucopyranosides.

Again, reaction of **3b** with excess benzaldehyde in the presence of anhydrous copper sulfate followed by chromatographic purification gave an oil in 73% yield (Scheme 2). In its IR spectrum, absorption band at 3446 cm^{-1} was due to hydroxyl stretching. In its ^{1}H NMR spectrum, two multiplets at δ 7.50 and 7.33 integrated for five aromatic protons and a singlet at δ 5.51 corresponding to C*H*Ph indicated the formation of benzylidene acetal. The compound also showed signals at δ 137.0, 133.7, 129.4, 128.4, 128.3, 126.3 (Ar-*C*) and 103.1 (Ph*C*H-) in its ^{13}C NMR spectrum due to benzylidene group. By comparing its all spectra with that of **5a**, the structure of the compound was assigned as *n*-butyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**5b**).

Finally, benzylidene glucopyranosides **5a** and **5b** were converted to their 2,3-di-*O*-acetates. Thus, reaction of **5a** with acetic anhydride in dry pyridine followed by chromatography afforded a white solid, mp. 108–109°C in 94% yield. In its IR spectrum, two strong absorption bands

at 1755 and 1746 cm^{-1} were due to carbonyl stretching and the spectrum showed absence of hydroxyl stretching bands. In the ^1H NMR spectrum, two three-proton signals at δ 2.04 and 2.00 were due to the methyl protons of two acetyloxy groups. The downfield shift of H-2 and H-3 to δ 4.76 and 5.52, respectively as compared to the precursor glucopyranoside **5a**, indicated the introduction of acetyl groups at position 2 and 3. Complete analysis of its IR and ^1H NMR spectra enabled us to establish its structure as *n*-butyl 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-α-D-glucopyranoside (**6a**).

Similarly, **5b** was dissolved in dry pyridine and reacted with acetic anhydride to afford a compound in 90% yield (Scheme 2). Its IR didn't show any absorption band for hydroxyl stretching and showed two carbonyl stretching bands at 1756 and 1743 cm^{-1}. In the ^1H NMR spectrum, two three-proton signals at δ 2.00 and 1.98 were due to the methyl protons of two acetyloxy groups. The downfield shift of H-2 and H-3 to δ 4.78 and 5.53, respectively as compared to the precursor glucopyranoside **5b**, indicated the introduction of acetyl groups at position 2 and 3. Complete analysis of its IR and ^1H NMR spectra were in agreement with its structure assigned as *n*-butyl 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-β-D-glucopyranoside (**6b**).

Antimicrobial activities

All the *n*-butyl glucopyranoside derivatives **(3a,b-6a,b)** were used as test chemicals for antimicrobial activities. The results of the inhibition zone against the selected bacteria due to the effect of acylated glucopyranosides **(3a,b-6a,b)** are mentioned in Table 1. The study revealed that the tested *n*-butyl glucopyranoside derivatives **(3a,b-6a,b)** showed moderate to good antibacterial functionalities.

The results of the percentage inhibition of mycelial growth of seven fungi due to the effect of chemicals (**3a,b**-**6a,b**) are presented in Table 2. The study revealed that the tested *n*-butyl glucopyranoside derivatives (**3a,b**-**6a,b**) showed moderate antifungal functionalities.

Structure activity relationship (SAR)

In vitro antimicrobial study revealed that *n*-butyl glucopyranosides (**3a,b**-**6a,b**) are moderate to good antimicrobial agents. From Table 1 and Table 2, we noticed that β-glucosides are more potential than that of α-glucosides against the tested microorganisms. Glucopyranoside **3a,b** and **4a,b** showed more antibacterial potentiality than that of *n*-butyl glucopyranoside **5a,b** and **6a,b**. The same result was observed for antifungal activities also. It has been observed that monosaccharides and their derivatives in

Table 1: Inhibition Against Bacterial Organism by the Glucopyranosides (3a,b-6a,b).

Name of Bacteria	Diameter of Zone of Inhibition in mm, 50 µg.dw./disc								
	3a	3b	4a	4b	5a	5b	6a	6b	**Kenamycin
Bacillus cereus	12	14	13	16	11	12	10	12	20
Bacillus megaterium	10	11	11	14	11	13	NF	NF	20
Bacillus subtilis	15	17	12	14	10	12	09	11	*21
Staphylococcus aureus	15	16	14	16	13	14	12	10	*22
Escherichia coli	12	11	14	14	10	12	13	12	*22
Pastunella maltosida	12	13	11	13	08	10	10	12	*23
Salmonella gallinarium	14	15	12	10	NF	NF	NF	NF	*24
Salmonella typhi	13	14	15	14	12	13	11	12	*23
Shigella dysenteriae	13	15	17	15	13	12	11	13	*24
Vibro cholera	12	13	12	16	10	14	NF	NF	18

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

Table 2: Antifungal Activities of the Glucopyranosides (3a,b-6a,b).

Name of Fungas	% Inhibition of Fungal Mycelial Growth, Sample 100 µg.dw./ml PDA								
	3a	3b	4a	4b	5a	5b	6a	6b	**Fluconazole
Aspergillus acheraccus	44	51	36	47	NF	32	NF	46	58
Aspergillus flavus	38	47	NF	50	30	34	32	39	*62
Aspergillus fumigatus	24	44	40	42	NF	27	42	44	*70
Aspergillus niger	43	44	29	38	20	36	NF	42	58
Aspergillus nodusus	40	45	42	53	33	38	38	35	*64
Fuserium equiseti	38	51	NF	54	26	37	NF	35	*65
Candida albicans	35	43	48	53	NF	29	NF	39	*60

"*" shows good inhibition, "NF" indicates no inhibition,
"**" indicates standard antibiotic, "dw" means dry weight

furanose form or distorted pyranose form are very weak inhibitor towards antimicrobial functionality.[15] But in pyranose form with regular $^4C_1$ or $^1C_4$ conformation these compounds exhibited excellent antimicrobial potentiality.[16] In this context, our present *n*-butyl glucopyranosides (**3a,b-4a,b**) posses regular $^4C_1$ conformation and exhibited better antimicrobial functionality. In addition, attachment of *n*-butyl group at glucosidic position instead of methyl or benzyl group didn't change the antimicrobibial potentiality significantly as compared to the standard antibiotics.

CONCLUSION

In conclusion, we have prepared *n*-butyl D-glucopyranosides (**3a** and **3b**) and converted them to *n*-butyl 4,6-*O*-benzylidene-2,3-di-*O*-acetyl-α- and β-D-glucopyranosides (**6a** and **6b**). We have also discussed their structure activity relationship (SAR) against some microbial strains. Incorporation of *n*-butyl group at glucosidic position didn't increase the antimicrobibial potentiality very much.

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