Asian Journal of Chemistry Vol. 22, No. 4 (2010), 3135-3146

Regioselective Synthesis of Fatty Acid Esters of Methyl α-D-glucopyranoside with Dibutyltin Dimethoxide Method and Biological Test against *Staphylococcus aureus* and *Salmonella agona*

XIN LOU*, JULIE O'BRIEN†, GARY HENEHAN† and SEAMAS CASSIDY†
Chemical and Biological Science Department, Chuzhou Uniersity, Chuzhou 239000, P.R. China
E-mail: franklinee@hotmail.com

Fatty acid esters of methyl α-D-glucopyranoside were regioselectively synthesized using dibutyltin dimethoxide (DBDM) as stannylating agent, general factors affecting regioselectivity has been examined. Comparison work indicated that DBDM has some advantage as stannylating agent over dibutyltin oxide for regioselective acylation at the 2-position of unprotected methyl α-D-glucopyranoside. Microbiological tests show that methyl 2-lauryl-α-D-glucopyranoside is effective inhibitor against gram-negative bacterial *Staphylococcus aures*.

Key Words: Dibutyltin dimethoxide, Fatty acid esters of carbohydrates, Regioselectivity, Antibacterial.

INTRODUCTION

Sugar esters of fatty acids, which have glycerol as the hydrophilic group and fatty acids as the lipophilic group are widely used in processed foods as stabilizing agents or emulsifiers[1]. These compounds are recently discovered to exhibit antibacterial activity, particularly against gram-positive bacteria[2], for instance sucrose esters of fatty acids[3], which are used to reduce flat-sour spoilage caused by *Bacillus* and *Clostridium* in canned coffee milk drinks in Japan[4]. The antimicrobial features of other carbohydrate esters have been reported[5]. It has been demonstrated that commercial sucrose fatty acid esters can decrease acid production from sugar by oral bacteria[6] and also reduce the development of dental caries in rats when added to sucrose-rich diets[7]. Use of fatty acids and their sugar esters potentially represent a nontoxic and nonallergenic means of controlling the acidogenic organisms associated with dental. Typically these fatty acid esters of sugars as inhibitors of bacterial were regioselectively synthesized with emzyme method and the 6-OH of the glucose moiety was selectively acylated.

Tin-containing intermediates have found many applications in carbohydrate chemistry, including protection of pair of hydroxyl groups[8], the formation of carbo-

†The School of Food Science and Environmental Health, Dublin Institute of Technology, Dublin 1, Ireland.

hydrate cluster[9], regioselective deoxygen[10], *etc.* Dibutyltin oxide (DBO) is common selective agent to activate specific hydroxyl groups of mono and disaccharide derivatives[9-12]. Dibutyltin dimethoxide (DBDM) also has been used as stannylating agent with the advantage of better yields and ease of use[13-15]. This reagent allows the rapid formation of dibutylstannylene acetals at room temperature[13]. In this study, the regioselective substitution of carbohydrates with long chain fatty acid has been probed with dibutyltin dimethoxide (DBDM) method. Herein, a regioselective synthesis of a series of fatty acid esters of methyl α-D-glucopyranoside using DBDM as stannylating agent is reported. The antibacterial activity of these compounds against a gram-positive organism *Staphylococcus aureus* and a gram-negative organism *Salmonella agona* was assessed.

EXPERIMENTAL

^{1}H and ^{13}C spectra were recorded with Varian 300 and 500 spectrometers, respectively. ^{1}H chemical shifts in CD_3OD were referenced to CHD_2OD (3.30 ppm); ^{13}C chemical shifts in CD_3OD were referenced to CHD_2OD (49.0 ppm). Coupling constants are reported in hertz. FTIR spectra were recorded with a Nicolet FTIR 3000 using KBr discs, as specified. Melting points were measured on a STUART melting point apparatus. Elemental analysis was performed on an Exeter Analytical CE 440 elemental analyzer. Low and high-resolution mass spectra were measured on electrospray mass spectrometry in ES negative mode unless otherwise indicated. TLC was performed on aluminum sheets precoated with silica gel 60 (HF_{254}, E. Merck) and spots visualized by UV and charring with H_2SO_4-EtOH (1:20). Flash column chromatography was carried out with silica gel 60 (0.040-0.630 mm, E. Merck) and using a solvent mixture system correlated with TLC mobility. Chromatography solvents used were $CHCl_3$ (Riedel-deHaen), MeOH (Riedel-deHaen). The Waters Assoc. HPLC system used consisted of a M 6000Pump, a U6K injector and a R401 refractive index detector. The column system consisted of a 15 cm × 4.6 mm luna C_{18} (analytic column), columns were equilibrated and developed isocratically with methanol:acetonitrile:water = 70:20:10 at a flow rate of 1 mL/min. HPLC-grade methanol. Acetonitrile, water were filtered through a 0.45 μm nylon filter (millipore) and degassed prior to chromatography.

Acetonitrile, toluene, benzene and methanol reaction solvents were of HPLC grade. Anhydrous dioxane, DME, DMF were used as received from Sigma-Aldrich. DBDM, DBO, methyl α-D-glucopyranosides (99 %), octanoyl chloride, decanoyl chloride, lauroyl chloride, myristoyl chloride, palmitoyl chloride, stearoyl chloride, TEA were purchased from Sigma-Aldrich. 4-Dimethylaminopyridine (DMAP) was recrystallized from benzene and dried in vacuum.

General procedure for the tin mediated acylation of methyl α-D-glucopyranoside

Method A (Bu_2SnO method): Methyl α-D-glucopyranoside (1.00 g, 5.15 mmol) and dibutyltin oxide (1.28 g, 5.15 mmol, 1 mol equiv.) in toluene (300 mL) were refluxed for 12 h with azeotropic removal of water. The clear solution was then

concentrated to 100 mL *in vacuo* and cooled to room temperature. The solution (50 mL) was evaporated again to dryness under reduced pressure and the residue dissolved in dioxane (35 mL) and acylated with palmitoyl chloride (0.76 g, 2.75 mmol, 1.1 mol equiv.) for two days at room temperature with periodically monitoring of the reaction progress by TLC. 4-Dimethylaminopyridine (0.76 g, 6.76 mmol) was added as a catalyst after the first 2 h. The progress of the reaction was monitored by TLC. Thereafter, the solvent was evaporated off *in vacuo* to leave dry solids *in vacuo*. The dry residue from flask (a) was purified by chromatography on a silica gel column using a solvent mixture of chloroform and methanol (v/v=10:1). An isomeric mixture was given (0.83 g, 1.98 mmol, overall yield 77 %).

Method B (DBDM method): Methyl α-D-glucopyranoside (0.97 g, 5 mmol) was dissolved in solvent (40 mL) and DBDM (1.47 mL, 5.5 mmol) was added in the solution, some effervescence was noticed. The reaction mixture was kept at 5 ºC and a solution of fatty acid chloride in 10 mL solvent was added dropwise over 1 h. The reaction was monitored using thin layer chromatography TLC and HPLC, terminated when all the starting material converted. The mixture was evaporated under vacuum to give syrup. This was fractionated by flash chromatography using a solvent mixture of chloroform:methanol = 15:1.

HPLC Development method for analysis and separation of fatty acid ester isomers of methyl α-D-glucopyranoside: Thin-layer (TLC), gas-liquid (GLC) and high-performance liquid chromatographic (HPLC) methods have ever been tried to separate and identify isomers of sugar fatty acid ester. Torres *et al.*[17] separated sucrose monostearate isomers by TLC methods. However separation of the positional isomers of methyl α-D-glucopyranoside has not been reported. To develop a suitable analytical method is essential in order to examine the regioselectivity of this reaction and to separate products. After a series of solvent composition tests eventually we develop a three solvent system method methanol:acetonitrile:water (70:20:10 method H) and found that the three element solvent mixture gave the desired separation, all isomers of O-3, O-2, O-6 isomer were well separated with retention times of 14.2, 19.2, 22.4 min, respectively (Fig. 1).

Data for fatty acid esters of methyl α-D-glucopyranoside: Methyl 2-O-stearoyl-α-D-glucopyranoside: m.p. 117-119 ºC, $[\alpha]_D$ +79.42 (C 0.42, $CHCl_3$); ^{1}H NMR (500 Hz, CD_3OD): 4.84 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.58 (dd, 1H, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.83 (dd, 1H, $J_{5,6a}$ = 2.3 Hz, $J_{6a,6b}$ = 12 Hz, H-6a), 3.80 (apt t, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3), 3.69 (dd, 1H, $J_{5,6b}$ = 5.6 Hz, $J_{6a,6b}$ = 12 Hz, H-6b), 3.55 (ddd, 1H, $J_{5,6a}$ = 2.3 Hz, $J_{5,6b}$ = 5.6 Hz, $J_{4,5}$ = 10.0 Hz, H-5), 3.37 (apt t, 1H, $J_{3,4} = J_{4,5}$ = 10.0 Hz, H-4), 3.36 (s, 3H, OCH_3), 2.37 (t, 2H, J = 7.5 Hz, $COCH_2$), 1.63 (m, 2H, $COCH_2CH_2$), 1.29 (m, 28H, -CH_2CH_2-), 0.90 (t, 3H, J = 7.0 Hz, CH_3); ^{13}C NMR (500 Hz, CD_3OD) δ: 175.8 (CO), 98.8 (C-1), 75.3 (C-2), 73.9 (C-5), 72.8 (C-3), 72.2 (C-4), 62.9 (C-6), 55.9 (OCH_3), 35.4 ($COCH_2$), 33.4 ($COCH_2CH_2$), 31.1, 31.1, 31.1, 31.1, 31.1, 31.1, 31.1, 30.9, 30.9, 30.9, 30.7, 30.4 (-CH_2CH_2-), 26.3 ($CH_2CH_2CH_3$), 24.1 ($CH_2CH_2CH_3$), 14.8 ($CH_2CH_2CH_3$); FTIR (KBr, cm^{-1}): 3512,

3363 (OH), 2916, 2844, 1725(CO), 1463, 1380, 1196, 1041, 723; Anal. calcd. (%) for $C_{25}H_{48}O_7$: C, 65.22; H, 10.43 Found (%): C, 64.92; H,10.63; ES-LRMS: Found 477.4 required 477.3 [M.H_2O-H].

Methyl 2-O-palmitoyl-D-glucopyranoside: m.p. 130-132 ºC; $[\alpha]_D$ +85.64 (C 0.15, $CHCl_3$); ^{1}H NMR (500 Hz, CD_3OD): 4.80 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.55 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.80 (dd, 1H, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a), 3.76 (apt t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3), 3.66 (dd, 1H, $J_{5,6b}$ = 5.6 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6b), 3.52 (ddd, 1H, $J_{5,6a}$ = 2.4 Hz, $J_{5,6b}$ = 5.6 Hz, $J_{4,5}$ = 10.0 Hz, H-5), 3.34 (apt t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4), 3.33 (s, 3H, OCH_3), 2.27 (t, 2H, J = 7.4 Hz, $COCH_2$), 1.60 (m, 2H, $COCH_2CH_2$), 1.25 (m, 24H, -CH_2CH_2-), 0.86 (t, 3H, J = 7.0, CH_3); ^{13}C NMR (500 Hz, CD_3OD) δ: 175.2 (CO), 98.4 (C-1), 74.9 (C-2), 73.5 (C-5), 72.4 (C-3), 71.9 (C-4), 62.6 (C-6), 55.5 (OCH_3); 35.2 ($COCH_2$), 33.2 ($COCH_2CH_2$), 31.1, 31.1, 31.1, 31.1, 31.1, 30.9, 30.8, 30.7, 30.6, 30.5 (-CH_2CH_2-), 26.1 ($CH_2CH_2CH_3$), 23.8 ($CH_2CH_2CH_3$), 14.5 ($CH_2CH_2CH_3$); FTIR (KBr, cm^{-1}): 3522, 3296 (OH), 2911, 2844, 1719(CO), 1468, 1386, 1201, 1047, 934, 723. Anal. calcd. (%) for $C_{23}H_{44}O_7$: C, 63.89; H, 10.19 Found (%): C, 63.69; H, 10.39; CI-LRMS: Found 433.2 required 433.3 $[M+H]^+$.

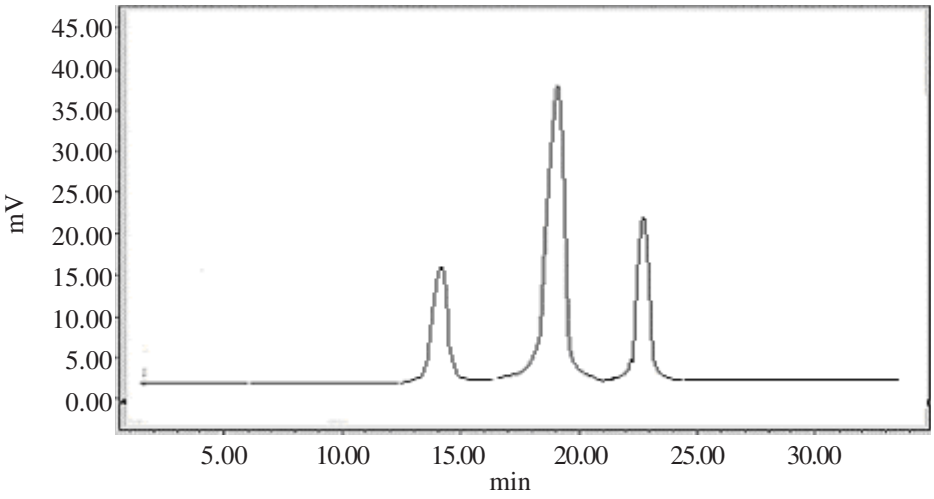


Fig. 1. HPLC-RI Chromatograph of methyl 2-palmitoyl-α-D-glucopyranoside using the mobile phase of methanol:acetonitrile:water = 70:20:10 at flow rate = 1.0 mL/min

Methyl 2-O-myristoyl-α-D-glucopyranoside: m.p. 132-134 ºC, $[\alpha]_D$ +87.96 (C 0.14, $CHCl_3$); ^{1}H NMR (300 Hz, CD_3OD): 4.84 (overlap with OH, H-1), 4.52 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.76 (dd, 1H, $J_{5,6a}$ = 2.2 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.77 (apt t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 3.69 (dd, 1H, $J_{5,6b}$ = 5.6 Hz, $J_{6a,6b}$ = 11.9 Hz, H-6b), 3.48 (m, H-5), 3.24 (apt t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.30 (s, 3H, OCH_3), 2.31 (t, 2H, J = 7.3 Hz, $COCH_2$), 1.56 (m, 2H, $COCH_2CH_2$), 1.24 (m,

20H, -CH_2CH_2-), 0.84 (t, 3H, J = 6.7, CH_3); ^{13}C NMR (500 Hz, CD_3OD) δ: 175.5 (CO), 98.6 (C-1), 75.1 (C-2), 73.7 (C-5), 72.6 (C-3), 72.1 (C-4), 62.8 (C-6), 55.7 (OCH_3), 35.2 ($COCH_2$), 33.3 ($COCH_2CH_2$), 31.1, 31.0, 30.9, 30.8, 30.7,30.6, 30.4, 30.3 (-CH_2CH_2-), 26.26 (-$CH_2CH_2CH_3$), 23.98 ($CH_2CH_2CH_3$), 14.69 ($CH_2CH_2CH_3$); FTIR (KBr, cm^{-1}): 3522, 3296 (OH), 2921, 2844, 1719 (CO), 1463, 1375, 1185, 1041, 939, 723, 687, 507; Anal. calcd. (%) for $C_{21}H_{40}O_7$: C, 62.38; H, 9.90 Found (%): C, 61.98; H, 10.03; ES-LRMS: Found 427.3 required 427.3 [M+Na]$^+$.

Methyl 2-O-lauoryl-α-D-glucopyranoside: m.p. 82-84 ºC; $[\alpha]_D$ +44.82 (C 0.12, $CHCl_3$); ^{1}H NMR (500 Hz, CD_3OD): 4.84 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.58 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.82 (dd, 1H, $J_{5,6a}$ = 2.5 Hz, $J_{6a,6b}$ = 12 Hz, H-6a), 3.80 (apt t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3), 3.68 (dd, 1H, $J_{5,6b}$ = 6.0 Hz, $J_{6a,6b}$ = 12 Hz, H-6b), 3.54 (ddd, 1H, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 6.0 Hz, $J_{4,5}$ = 10.0 Hz, H-5), 3.37 (apt t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4), 3.36 (s, 3H, OCH_3), 2.37 (t, 2H, J = 7.3 Hz, $COCH_2$), 1.63 (m, 2H, $COCH_2CH_2$), 1.29 (m, 16H, -CH_2CH_2-), 0.89 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (500 Hz, CD_3OD) 175.5 (CO), 98.8 (C-1), 75.3 (C-2), 73.9 (C-5), 72.8 (C-3), 72.2 (C-4), 62.9 (C-6), 55.9 (OCH_3), 35.3 ($COCH_2$), 33.4 ($COCH_2CH_2$), 31.0, 30.9, 30.8, 30.7, 30.7, 30.4, 30.4 (-CH_2CH_2-), 26.38 ($CH_2CH_2CH_3$), 24.1 ($CH_2CH_2CH_3$), 14.8 ($CH_2CH_2CH_2$); FTIR (KBr, cm^{-1}): 3512, 3363 (OH), 2916, 2844, 1725 (CO), 1463, 1380, 1196, 1041, 723; Anal. calcd. (%) for $C_{19}H_{36}O_7$: C, 65.22; H, 10.43 Found (%): C, 64.92; H, 10.63; ES- LRMS: Found 399.3 required 399.2 [M+Na]$^+$.

Methyl 2-O-decanoyl-α-D-glucopyranoside: m.p. 75-77 ºC, $[\alpha]_D$ +84.17 (C 0.20, $CHCl_3$); ^{1}H NMR (300 Hz, CD_3OD): δ 4.83 (overlap with OH, H-1), 4.58 (dd, 1H, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.83 (dd, 1H, $J_{5,6a}$ = 2.3 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a), 3.78 (apt t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3), 3.68 (dd, 1H, $J_{5,6b}$ = 5.6 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6b), 3.54 (m, 1H, H-5), 3.36 (apt t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4), 3.35 (s, 3H, OCH_3), 2.37 (t, 2H, J = 7.3 Hz, $COCH_2$), 1.63 (m, 2H, $COCH_2CH_2$), 1.30 (m, 12H, -CH_2CH_2-), 0.90 (t, 3H, J = 6.8, CH_3); ^{13}C NMR (300 Hz, CD_3OD) δ: 175.0 (CO), 98.5 (C-1), 75.0 (C-2), 73.6 (C-5), 72.5 (C-3), 71.9 (C-4), 62.7 (C-6), 55.6 (OCH_3), 35.1 ($COCH_2$), 33.1 ($COCH_2CH_2$), 30.8, 30.7, 30.7, 30.4 (-CH_2CH_2-), 26.2 ($CH_2CH_2CH_3$), 24.0 ($CH_2CH_2CH_3$), 14.6 ($CH_2CH_2CH_3$); FTIR (KBr, cm^{-1}): 3532, 3311 (OH), 2921, 2844, 1714 (CO), 1463, 1380, 1185, 1041, 928, 846, 764, 682, 507; Anal. calcd. (%) for $C_{17}H_{32}O_7$: C, 58.62; H, 9.20 Found (%): C, 58.33; H, 9.30; ES-LRMS: Found 371.3; required 371.2 [M+Na]$^+$.

Methyl 2-O-octoryl-α-D-glucopyranoside: m.p. 99-101 ºC; $[\alpha]_D$ +22.81 (C 0.31, $CHCl_3$); ^{1}H NMR (300 Hz, CD_3 OD): δ 4.77 (overlapping with OH, H-1), 4.52 (dd, 1H, $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.77 (m, 1H, H-6a), 3.74 (apt t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 3.62 (dd, 1H, $J_{5,6b}$ = 5.5 Hz, $J_{6a,6b}$ = 12 Hz, H-6b), 3.55 (m,1H, H-5), 3.32 (apt t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4), 3.30 (s, 3H, OCH_3), 2.31 (t, 2H, J = 7.3 Hz, $COCH_2$), 1.56 (br t, 2H, $COCH_2CH_2$), 1.25 (m, 8H, -CH_2CH_2-), 0.86 (apt t, 3H, J = 6.25, CH_3); ^{13}C NMR (300 Hz, CD_3OD): δ 175.4 (CO), 98.6 (C-1), 75.1

(C-2), 73.8 (C-5), 72.7 (C-3), 72.1 (C-4), 62.8 (C-6), 55.8 (OCH_3), 35.2 ($COCH_2$-), 33.2 ($COCH_2CH_2$), 30.4, 30.3 (-CH_2CH_2-), 26.4 (-$CH_2CH_2CH_3$), 24.1 (-$CH_2CH_2CH_3$), 14.8 (-$CH_2CH_2CH_3$); FTIR (KBr, cm^{-1}): 3524, 3316 (OH), 2926, 2844, 1723 (CO), 1473, 1388, 1182, 1042, 847, 762, 683, 512; Anal. calcd. (%) for $C_{15}H_{28}O_7$: C, 56.25; H, 8.75. Found (%): C, 56.76; H, 8.76; ES-LRMS: Found 343.3 required 343.2 $[M+Na]^+$.

Methyl 3-O-palmitoyl-α-D-glucopyranoside was identified as isomer **1** formed from method A after purification of flash chromatography. m.p. 90-92 ºC, ^{1}H NMR (500 Hz, CD_3OD): 5.23 (apt t, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 4.78 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1), 3.89 (dd, 1H, $J_{5,6} = 2.2$ Hz, $J_{6,6} = 11.5$ Hz, H-6), 3.77 (dd, 1H, $J_{5,6} = 5.4$ Hz, $J_{6,6} = 11.5$ Hz, H-6); 3.71 (m, H-5), 3.59 (dd, 1H, $J_{1,2} = 3.5$ Hz, $J_{2,3} = 9.5$ Hz, H-2), 3.52 (apt t, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), ^{13}C NMR (500 Hz, CD_3OD) δ: 101.3 (C-1), 76.8, 73.6, 71.9, 69.8 (C-4), 62.4 (C-6), 55.7 (OCH_3); FTIR (KBr, cm^{-1}): 3393 (OH), 2918, 2848, 1700 (C=O), 1466, 1389, 1268, 1202, 1042, 906, 805, 719, 595.

Methyl 6-O-palmityol-α-D-glucopyranoside was identified as isomer **2** formed by method A as a monoester isomer within the isomeric mixture after purification. ^{1}H NMR (500 Hz, CD_3OD): δ 5.2-5.3 (overlapping signals, 4H, H-2, 3, 4, 5) 4.71 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 4.44 (dd, 1H, $J_{5,6} = 2.2$ Hz, $J_{6,6} = 12$ Hz, H-6), 4.25 (dd, 1H, $J_{5,6} = 6.0$ Hz, $J_{6,6} = 12$ Hz, H-6); ^{13}C NMR (500 Hz, CD_3OD): 101.32 (C-1), 75.08 (C-3), 73.52 (C-2), 71.95 (C-4), 71.12 (C-5), 64.78 (C-6), 55.40 (OCH_3); FTIR (KBr, cm^{-1}): 3398 (OH), 2916, 2848, 1720 (C=O), 1460, 1381, 1197, 1156, 1050, 718.

Microbiological experiment

Incubate the bacterial: Make up 3l BHI solution (37 g/L), sterilize the solution in the autoclave at a temperature of 110 ºC for 15 min. Incubate the *Salmonella agona* and *Staphylococcus aureus*, respectively into 30 mL BHI broth solution (in duplicate), leave them at 37 ºC overnight.

Dilution of bacterial solution (1:100): Transfer 1mL of 30mL BHI solution into 100 mL BHI, keep it at 37 ºC for 14 h.

Centrifugation: Centrifuge 50 mL of superior BHI solution at 4,800 rpm for 10 min, descant the superior solution and wash. resuspend in 9 mL MRD, centrifuge once again for 5 min to get a 9 mL solution, dilute to get 1:100 solution.

Addition of sugar ester solution: Transfer 1 mL aliquot into 10 mL of 100 ppm sugar ester solution(in duplicate), incubate for 6 h at 37 ºC.

Dilution: Serially dilute solution to such concentration: 10^{-1} N, 10^{-2} N, 10^{-3} N, 10^{-4} N on PCA plate in duplicate, incubate at 37 ºC one day.

Count bacterial number: Select the PCA plate in which bacterial number between 25 and 500, count the number of bacterial and work out the log N.

RESULTS AND DISCUSSION

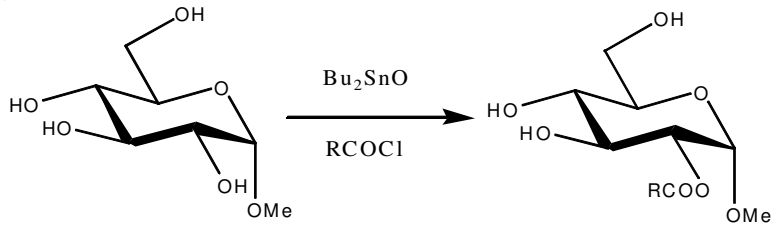
Regioselective synthesis of fatty acid ester of methyl α-D-glucopyranoside using DBO and DBDM as stannylating reagent: The fatty acid esters of methyl α-D-glucopyranoside using DBO (method A) and DBDM (method B) as stannylating reagent was carried out (Table-1). The DBO method involved the change of solvents from the reaction to form stannlyene complex to acylation reaction while DBDM method keep the solvent unchanged.

TABLE-1
YIELD AND COMPOSITION DATA OF METHYL MONO-O-PALMITOYL-α-D-GLUCOPYRANOSIDE OBTAINED FROM METHOD A AND B

	Monoester yield (%)	Ratio of composition			
		2-O	3-O	4-O	6-O
Method A (dioxane as solvent)	77	87	6	0	2
Method B (dioxane as solvent)	75	100	0	0	0

Method A: Bu_2SnO method 1: methyl-α-D-glucopyranoside, DBO, toluene, reflux;
2: dioxane, palmitoyl chloride, room temperature.
Method B: $Bu_2Sn(OMe)_2$ method: methyl-α-D-glucopyranoside, dioxane, palmitoyl chloride, room temperature.
Note: The ratio of isomers was determined with the intensity of H-1 of isomers.

It can be seen from Table-1 when DBO (method A) was used, the reaction gave 2-O-palmitoyl ester as major product and 3-O, 6-O-palmitoyl esters as minor products. The ratio of 2-, 3-, 6-mono-O-palmitoyl esters is 17:6:2. When DBDM (method B) was used, it was more selective than former method, giving only the 2-O-palmitoyl ester though the yield of mono-O-palmitoyl ester was a bit lower. In both methods appreciable formation of di-O-palmitoyl ester was observed, which could be avoidable on the condition of lower temperature (0-5 ºC) and use of a limited amount of acylating reagent. It indicated that DBDM was a superior reagent for regioselective acylation at the 2-position of unprotected methyl α-D-glucopyranoside. Dibutyltin dimethoxide (DBDM) was chosen as stannylating agent to regioselectively synthesize a series of fatty acid esters of methyl α-D-glucopyranoside (**Scheme-I**).



1 R=$C_{17}H_{35}$, **2** R=$C_{15}H_{31}$, **3** R=$C_{13}H_{27}$,
4 R=$C_{11}H_{23}$, **5** R= C_9H_{19}, **6** R=C_7H_{15}

Scheme-I: Regioslective synthesis of methyl 2-acyl-α-D- glucopyranoside using DBDM as stannylating agent

The structure elucidation of a series of fatty acid esters of methyl α-D-glucopyranoside is determined by use of the acylation shift rule and shift parameters[16]. The yield of synthesized compounds **1-6** varied between 70-90 and the regioselective position is confirmed with NMR (that C-1 and C-3 of all these esters have a shift of 2-3 ppm upfield and the C-2 have a 1-2 ppm shift downfield; H-2 has a shift +1.1-1.2 ppm) all esters are 2-O isomers.

Factors that influence reaction regioselectivity: The regioselectivity is complicated for polyol system such as methyl α-D-glucopyranoside. Several factors can affect the regioselectivity of this reaction. General factors affecting the regioselectivity have been examined.

Effect of solvent on regioselectivity: In order to probe the solvent effect on regioselectivity, different solvents were investigated (Table-2).

TABLE-2
SOLVENT EFFECT ON REGIOSELECTIVE ACYLATION OF METHYL α-D-GLUCOPYRANOSIDE

Solvent	No of monoester isomers (visible peaks)	Yield of monoester (%)
DMF	4	85
Acetonitrile	2	65
Dioxane	1	75
DME	2	58
Toluene	3	56

Note: The number of visible peaks were detected by HPLC analytical method: mobile phase: methanol:acetonitrile:water = 70:20:10; Flow rate =1.0 mL/min; detector: RI (method H).

Table-2 shows that choice of solvent can significantly affect the regioselectivity. It is worth noting that in different solvents a major product was formed, which was proved by NMR to be the O-2 isomer (R_t 19.2 min). The different regioselectivity for acylation of methyl α-D-glucopyranoside in different solvents indicate solvents more or less interact with stannylene complexes to form a equilibrium mixture.

Effect of added nucelophiles on regioselectivity: Generally an added nucleophile can improve the yield of the acylation reaction, however it can also diversify regioselectivity. For instance the addition of DMAP can increase the yield of fatty acid ester of methyl α-D-glucopyranoside, however other isomers are also given along with a major isomer. Table-3 shows the effect of added nucleophile on the ratio of isomers formed by the acylation of methyl α-D-glucopyranoside with palmitoyl chloride.

TABLE-3
ADDED NUCLEOPHILE EFFECT ON REGIOSELECTIVE ACYLATION OF METHYL α-D-GLUCOPYRANOSIDE (COMPOUND **2**)

Solvent	Added nucleophile	Ratio of isomers (peak area)	Overall yield of products (%)
Dioxane	-	100	75
Dioxane	DMAP	39:9	87
Dioxane	TEA	2:4:37:7	81

Effect of the reaction temperature on regioselectivtiy: At higher temperature 1 to 2 diester products are formed along with monoester. The diester was identified by its longer migration on TLC plates and longer retention time on HPLC analysis (method H). This prompted an investigation of the effect of temperature on the production of diester. Fig. 2 is a study effect of temperature on the production of distearate and dioctorate derivatives of methyl α-D-glucopyranoside.

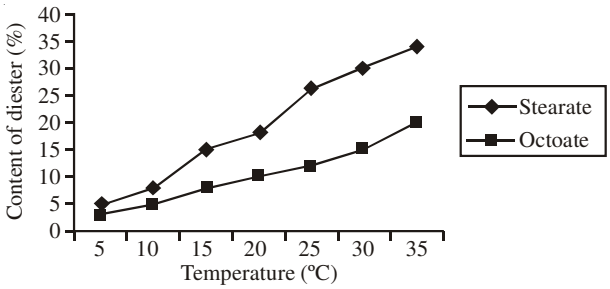


Fig. 2. Plot of the content of diester against reaction temperatures. Acylation was run without the presence of added nucleophile at the designed temperature. The content ratio was determined with HPLC (method H); Reaction conditiosn: methyl α-D-glucopyranoside. DBDM. Acylating agent (1:1:1) in dioxane

From Fig. 2 it can be seen that diester is formed along with the monoester. When the reaction is carried out at lower temperatures (5 ºC) the amount of diester formed is very little. The chain length of the fatty acid chloride used for the acylation reaction was found to influence the formation of the diester. Longer fatty acid chains yield greater amounts of diester product.

Microbiology evaluation of fatty acid esters of methyl α-D-glucopyranoside

Preliminary assessment of antibacterial activity of fatty acid esters of methyl α-D-glucopyranoside: A series of fatty esters of methyl α-D glucopyranoside, which were regioselectively synthesized using DBDM as stannylating agent, were screened for antibacterial activity against a gram-positive organism *Staphylococcus aureus* and a gram-negative organism *Salmonella agona*. The bacteria were grown as described in the experimental section and the sugar ester added to a final concentration of 100 ppm. Fig. 3 shows the results of screening of α-D-glucopyranoside fatty acid esters and water control (Log_{10} CFU/mL), varying in chain length from 8 to 18 carbons, as inhibitors of the growth of *S. aureus*. Fig. 4 shows a similar screen for *S. agona* (Log_{10} CFU/mL).

Preliminary results indicate that only the laurate derivative of methyl α-D-glucopyranoside had a significant effect on the growth of *Staphylococcus aureus* and the population of *S. aureus* was reduced from 5.445 (water control) to 0.814 Log_{10} CFU/mL (Fig. 3), while other esters had little effect on the growth of *S. aureus*. None of these esters had a significant effect on the growth of *Salmonella agona* (Fig. 4).

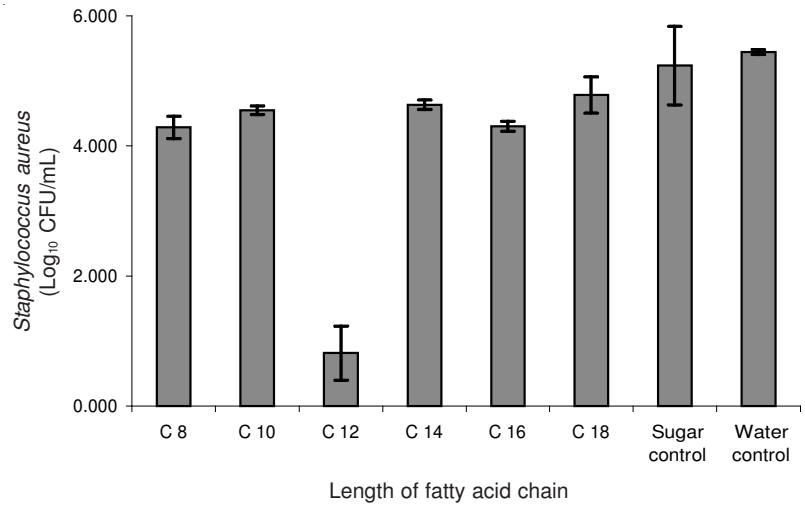


Fig. 3. Influence of chain length of fatty acid esters of methyl α-D-glucopyranoside on the growth of *Staphylococcus aureus* in culture. The esters were added to a final concentration of 100 ppm

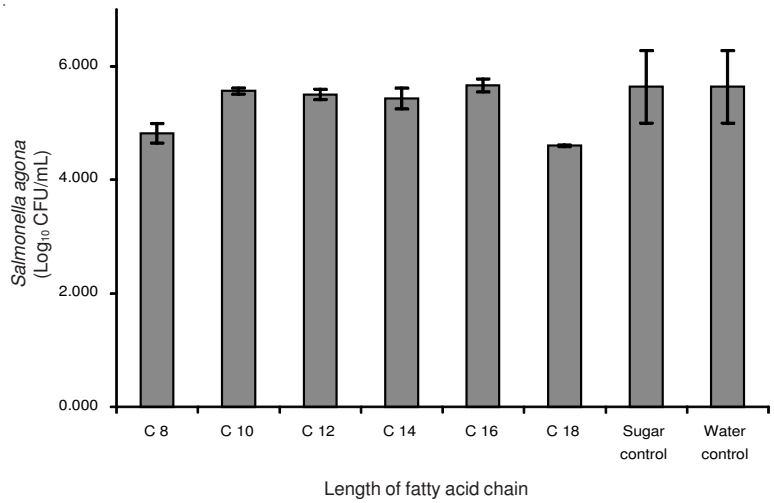


Fig. 4. Influence of chain length of fatty acid esters of methyl α-D-glucopyranoside on the growth of *Salmonella agona* in culture. The esters were added to a final concentration of 100 ppm

Effect of concentration on the antibacterial activity of fatty acid esters of methyl α-D-glucopyranoside: To confirm the antibacterial results, three fatty acid esters of methyl α-D-glucopyranoside **3** (myristate C14), 4 (laurate C12), 5 (decanoate C10) were screened for antibacterial activity against *Staphylococcus aureus* using varying concentrations (Fig. 5).

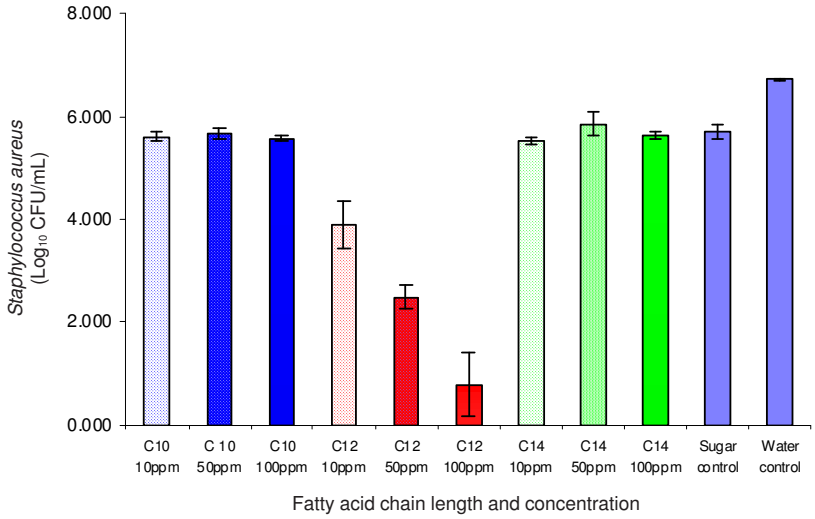


Fig. 5. Influence of concentration on the antibacterial activity of esters 3, 4 and 5 on *Staphylococcus aureus* in culture

The results showed that increasing the concentration of methyl 2-lauroyl-α-D-glucopyranoside (**4**) from 10 to 100 ppm increased the antibacterial effect against *Staphylococcus aureus*. The populations of *S. aureus* were reduced by 2.83, 4.23 and 5.93 Log_{10} CFU/mL compared to water control by 10, 50 and 100 ppm. However, increasing the concentration of two other esters: methyl 2-myristoyl-α-D-glucopyranoside (**3**), methyl 2-decanoyl-α-D-gluco-pyranoside (**5**), had no influence on their antibacterial activity against *S. aureus*.

These results confirmed preliminary screening where the antibacterial effect of esters of methyl α-D-glucopyranoside were confined to the laurate ester activity against *Staphylococcus aureus*.

The comparison experiments show DBDM is a superior reagent for regioselective acylation at the 2-position of unprotected methyl α-D-glucopyranoside to DBO. The regioselectivity consistence with benzoylation reaction[13], which indicate long chain fatty acids don't cause steric problem for substitution of stannylene complex of methyl α-D-glucopyranoside. The antibacterial screening for fatty acid esters of methyl α-D-glucopyranoside indicated the length of fatty acid is critical for antimicrobiological effect.

ACKNOWLEDGEMENTS

The authors thank Ms. Geraldine Fitzpatrick for her valuable help on NMR analysis. Thanks also to Dr. Declan Bolton and Ms. Jean Kennedy, (Teagasc, The National Food Centre) for the antibacterial testing. This work was funded by the Applied Chemistry key subject of Anhui Province (No. 2008021870) and Dublin Institute of Technology.

REFERENCES

1. T. Kato, *Shokuhin Kako Gijutsu*, **19**, 81 (1999) (in Japanese).
2. A.J. Conlay and J.J. Kabara, *Antimicrob. Agents Chemother.*, **4**, 501 (1973).
3. L.R. Beuchat, *Appl. Environ. Microbiol.*, **39**, 1178 (1980)
4. N. Suwa, N. Shirota and H. Michida, *J. Japan Soc. Food Sci. Technol.*, **33**, 44 (1988).
5. C.C. Akoh and B.G. Swanson, Carbohydrate Polyesters as Fat Substitutes, Marcel Dekker, New York, pp. 149-167 (1994).
6. Y. Iwami, C.F. Schachtele and T. Yamada, *J. Dent. Res.*, **74**, 1613 (1995).
7. K.A. Williams, B.R. Schemehorn, J.L. MacDonald Jr, G.K. Stookey and S. Katz, *Arch. Oral. Biol.*, **27**, 1027 (1982).
8. C.W. Holzapfel, J.J. Huyser, T.L. van der Merwe and F.R. van Heerden, *Heterocycles*, **32**, 1445 (1991).
9. M. Basson, M.W. Bredenkamp and C.W. Holzapfel, *Tetrahedron Lett.*, **30**, 591 (1989).
10. M.E. Haque, T. Kikuchi, K. Kanemitsu and Y. Tsuda, *Chem. Pharm. Bull.*, **35**, 1016 (1987).
11. D.D. Manning, C.R. Bertozzi, P. Beck and L.L. Kiessing, *J. Am. Chem. Soc.*, **119**, 3161 (1997).
12. T.B. Grindley and H. Namazi, *Tetrahedron Lett.*, **37**, 991 (1996).
13. G.J. Boons, G.H. Castle, J.A. Clase, P. Grice, S.V. Ley and C. Pinel, *Synlett*, 913 (1993).
14. A.V. Kornilov, E.V. Sukhova and N.E. Nifantiev, *Carbohydr. Res.*, **336**, 309 (2001).
15. T. Nakano, Y. Ito and T. Ogawa, *Carbohydr. Res.*, **243**, 43 (1993).
16. Y. Tsuda, M.E. Haque and K. Yoshimoto, *Chem. Pharm. Bull.*, **31**, 1612 (1983).
17. M.C. Torres, M.A. Dean and F.W. Wagner, *J. Chromatogr.*, **522**, 245 (1990).

(*Received*: 7 August 2009; *Accepted*: 2 January 2010) AJC-8250