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Regioselectivity of Esterification of Lactose with Fatty Acid *via* the Stannylene Acetal Method

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Regioselectivity of lactose esterification with fatty acid using dibutyltin dimethoxide as stannylating agent were probed. The general factors affecting regioselectivity have been examined. The result shows the C-3' position of galactose and the β anomer of C-1 position of the glucose residue have some advantage of being acylated at the condition of N-methyl-1-pyrrolidione as solvent and lower temperature (-15 ºC), the corresponding isomers of lactose fatty acid esters were separated by preparative HPLC method. Explanations for the regioselectivities observed during this tin-mediated reaction were also proposed based on the stannylene acetal in solution.

Key Words: Lactose, Esterification, Stannylene acetal, Regioselectivity.

INTRODUCTION

The diary industry produces large amounts of lactose as a by-product of cheese production. Attempts to utilize excess lactose are mainly limited to production of some fermentation products such as galactose- oligosaccharides and organic acids. Demand is insufficient to utilize all available lactose so that almost half the lactose produced each year remains unused and is a significant waste problem[1].

Fatty acid esters of lactose are attracting attention from a number of sectors ranging from agrochemicals to personal care products and foods[2-4]. The degree of substitution, position and type of fatty acid ester determines the functional properties of these derivatives. For example the polyesters of lactosides (oleates and palmitates) have been found to be suitable as low-calorie fat substitutes for frying and as components of foods, *e.g.*, salad dressings. Sugar fatty acid esters were also reported as effective inhibitors of some typical anaerobic spore forming bacteria including Clostridia[5]. These esters had the interesting characteristic that the higher the heat resistance of the bacterium, the smaller the amount of sugar ester required to have a growth inhibitory effect. However most of the products are used as mixtures of regioisomers as well as mono-, di- and trimesters. In order to get some precise structure-activity relationships in this series of compounds it is necessary to prepare pure lactose fatty acid esters of defined structure. Regioselective modification of lactose is still a challenging task contrast to sucrose due to little solubility in most of the organic solvents. The enzymatic method, which works on most of unprotected sugars, can't be used to regioselectively synthesize fatty acid esters of lactose[6]. Hence selective acylation of lactose necessitate a number of tedious protection and deprotection steps. For instance, Sarney and Vlusion[7] prepared tri-O-isopropylidene-lactose dimethyl acetal with only free two hydroxyl groups (2- and 6'-OH) as the precursor for the regioselective esterification with fatty acids.

Carbohydrate stannyl ethers and stannylene acetals have proven to be useful intermediates in the regioselective synthesis of carbohydrate derivatives[8-10], 1,6-dibutylstannylene acetals are reactive intermediates that have been treated with acylating and alkylating agents to provide mono-substituented derivatives of diols or polyols with high regioselectivity[11,12]. In this study we describe the investigation on the regioselective acylation of lactose *via* stannylene acetal method. Dibutyltin dimethoxide (DBDM), in place of dibutyltin oxide, is used as stannylating agent to activate certain hydroxyl groups on lactose. This reagent allows the rapid formation of dibutylstannylene acetals at room temperature[13].

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were recorded with Varian Inova 300, 500 NMR spectrometers. ^{1}H NMR chemical shifts in $CDCl_3$ or CD_3OD were referenced to DMSO-d_6 (2.50 ppm), CHD_2OD (3.30 ppm); ^{13}C NMR chemical shifts in DMSO-d_6 or CD_3OD were referenced to DMSO-d_6 or (39.5 ppm), CHD_2OD (49.0 ppm). Coupling constants are reported in hertz. FTIR spectra were recorded with a Nicolet FTIR 3000 using

either thin film or 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 aluminium 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) using a solvent mixture system correlated with TLC mobility. Chromatography solvents used were $CHCl_3$ (Riedel-deHaen), MeOH (Riedel-deHaen).

α-Lactose monohydrate was a 97 % ACS reagent. N-Methyl-1-pyrrolidione, fatty acid chloride were purchased from Sigma-Aldrich.

General method for syntheses of fatty acid ester of lactose: α-Lactose monohydrate (1.08 g, 3 mmol) was heated in an oven at 150 ºC for 1 h and transferred to a flask into which solvent (40 mL) was added. Dibutyltin dimethoxide (0.76 mL, 3.3 mmol, 1.1 equiv.) was added as stannylating agent into the solution, some effervescence was noticed, followed by the addition of fatty acid chloride (3.3 mmol, 1.1 equiv.). The reaction proceeded for 24 h and was monitored by TLC. The reaction solution was washed three times with petroleum ether (30 mL) to remove the organotin compounds. The solvent was evaporated at 90 ºC under vacuum and the product partitioned between 1-butanol and 10 % aqueous NaCl. The aqueous phase was washed with 1-butanol three times and combined organics were extracted three times with 10 % aqueous NaCl and evaporated. The residue was fractionated by flash chromatography using chloroform:methanol = 100:30 as eluent to give a mixture of fatty acid esters of lactose.

Chromatographic separations of structural isomers of lactose monoester: The reaction progress detection of esters mixture firstly referred to that of sucrose monopalmitate (using chloroform-methanol-acetic acid (85:5:10) as mobile phase on silica gel G plates)[14]. This method, however, was found unable to give an adequate separation of our lactose palmitate monoesters. HPLC method was probed to separate the lactose palmitate isomers , the isomers were well resolved using methanol: water = 83:17, flow rate = 1.0 mL/min (method A) as mobile phase (Fig. 1).

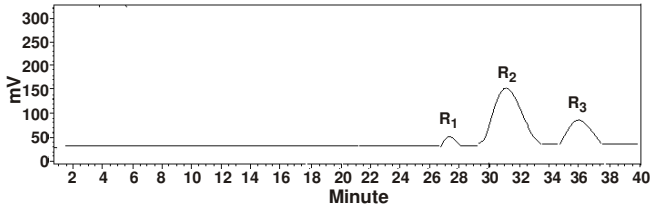


Fig. 1. HPLC-RI chromatograph of lactose palmitate isomers using methanol:water = 83:17 as mobile phase at flow rate of 1.0 mL/min (R_1 = 28.72 min, R_2 = 31.06 min, R_3 = 36.15 min)

RESULTS AND DISCUSSION

Lactose was acylated with varying acylating agents using DBDM as stannylating reagent in DMF, gave lactose esters. Our preliminary studies showed that acyl chloride was the most effective acylating agent, albeit giving only 30 % yield under our DBDM system. General factors affecting regioselectivity has been examined.

It can be seen from Table-1 that solvent affects both the yield and the regioselectivity. Generally polar solvents facilitate reactions, but can interact with tin-complexes and decrease regioselectivity. N-Methyl-1-pyrrolidione is proved to be the most suitable solvent for esterification of lactose. The effect of temperature was also investigated to probe the regioselectivity of lactose acylation. Table-2 shows the experimental results.

TABLE-1
EFFECT OF SOLVENT ON THE ESTERIFICATION REACTION OF LACTOSE USING PALMITOYL CHLORIDE AS ACYLATING AGENT AT ROOM TEMP.

Solvent	Number of derivatives*	Overall yield of monoester (%)
Toluene	0	0
DMF	4	30
Pyridine	7	65
DMF/pyridine	6	52
N-Methyl-1-pyrrolidione	4	81

*Number of isomers was determined by HPLC method A, analysing the crude reaction mixture, reaction conditions: lactose: DBDM: palmitoyl chloride = 1:1.1:1.1, r.t., 24 h.

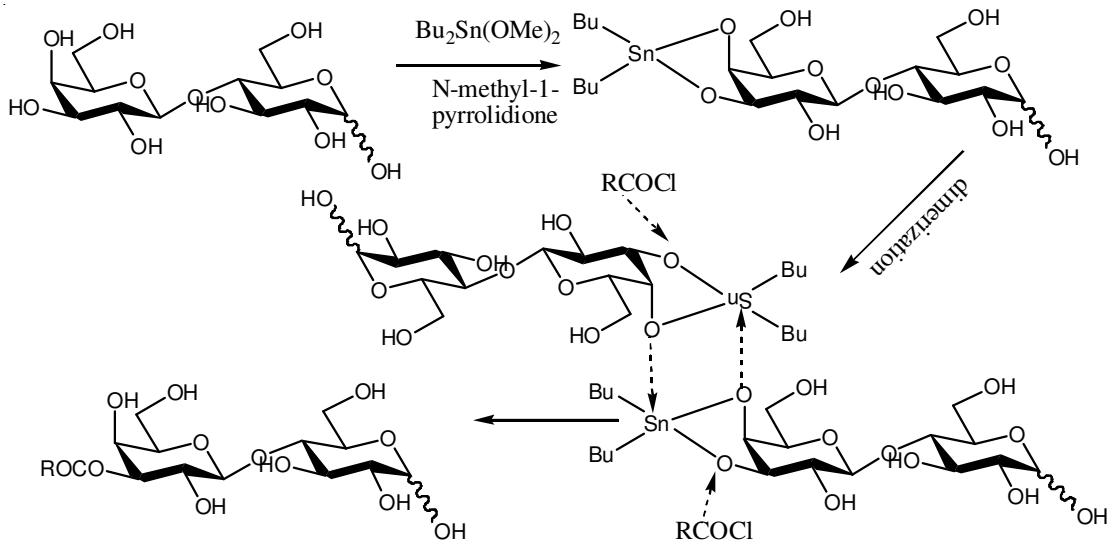
TABLE-2
EFFECT OF TEMPERATURE ON ESTERIFICATION OF LACTOSE USING PALMITOYL CHLORIDE AS ACYLATING AGENT

Temperature (ºC)	Number of isomers	Ratio of isomers*	Yield of overall monoester (%)
60	6	5:10:12:15:65a:8b	84
25	4	12:8:60a:5b	79
0	4	9:5:62a:12b	81
-10	3	6:68:26	78
-15	2	79a:32b	76

*The number of isomers and ratio of isomers were determined by HPLC method A; Reaction conditions: lactose: DBDM: palmitoyl chloride = 1:1.1:1.1, solvent: N-methyl-1-pyrrolidione a: compound **1a**, b: compound **1b**.

The results show that temperature affects regioselectivity of ester formation dramatically. At lower temperatures, regioselectivity was enhanced. When it reaches -15 ºC satisfactory regioselectivity was obtained with reasonable conversion, the ester mixtures were separated with flash chromatography and the ester isomers were fully separated by preparative HPLC and their structures determined using NMR. The two isomers are identified as 3'-O-palmitoyl-D-lactose **1a** and 1-O-palmitoyl-β-D-lactose **1b**, respectively, the β configuration of **1b** was conducted from similar sugar esters in literatures[15,16].

Data for compound isomers of lactose palmitoyl: 3'-O-Palmitoyl-D-lactose Yield: 42 % m.p. 176 ºC; $[\alpha]_D$ +55.11º (C 0.24, EtOH); ^{1}H NMR (500 MHz, CD_3OD): δ 6.63 (d, 1H, *J* = 6.5 Hz, OH), 6.30 (d, 1H, *J* = 4.7 Hz, OH), 4.67 (d, 1H, *J* = 3.7 Hz, H-1α), 4.62 (d, 1H, *J* = 6.8 Hz, H-1β), 4.33 (d, 1H, *J* = 7.8 Hz, H-1'), 4.43 (apt t, 1H, *J* = 6.0 Hz, H-3'), 3.97 (1H, dd, *J* = 9.1 Hz, 13.2, H-2'), 3.80-3.88 (4H, m, H-6a, H-6b, H-6a', H-6b'), 3.67-3.72 (m, 2H, H-3, H-4), 3.58 (m, 1H, H-5'), 3.43-3.45 (m, 2H, H-2, H-5), 2.31 (apt t, *J* = 7.5 Hz, 2H, $COCH_2$), 1.53 (m, 2H, $COCH_2CH_2$), 1.24 (m, 24H, $-CH_2CH_2-$), 0.85 (t, 3H, *J* = 7.0 Hz, CH_3); ^{13}C NMR (500 Hz,



Scheme-I: Proposed mechanism for regioseletively acylation of lactose with fatty acid on 3'-OH position

DMSO): δ 172.9 (CO), 103.7 (C-1'), 96.8 (C-1β), 92.2 (C-1α), 81.3 (C-4α), 80.9 (C-4β), 75.9 (C-5'), 75.2 (C-3'), 75.1 (C-5β), 74.8 (C-3β), 74.7 (C-2β), 72.3 (C-3α), 71.5 (C-5α), 69.9 (C-2α), 67.9 (C-2'), 65.5 (C-4'), 60.7 (C-6'), 60.0 (C-6), 33.8 ($COCH_2$), 31.4 ($COCH_2CH_2$), 31.4, 29.2, 29.2, 29.2, 29.1, 29.1, 29.0, 28.9, 28.8, 28.6, (-CH_2CH_2-), 24.7 ($CH_2CH_2CH_3$), 22.2 ($CH_2CH_2CH_3$), 14.1 ($CH_2CH_2CH_3$); anal. calcd. (%) for $C_{17}H_{32}O_7$: C, 57.91; H, 9.03; found (%): C, 58.62; H, 9.32 FTIR (KBr, ν_{max}, cm^{-1}): 3378 (OH), 2916, 2844, 1725 (CO), 1463, 1377, 1176, 1088, 782, 717; HRMS-ES: Found (%) 579.3394, required 579.3381 [M-H]-.

1-O-Palmitoyl-β-D-lactose yield: 18 % m.p. 158 ºC; $[\alpha]_D$ +22.40º (C 0.257, EtOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.40 (d, 1H, *J* = 8.2 Hz, H-1), 4.22 (d, 1H, *J* = 7.6 Hz, H-1'), 3.80-3.88 (4H, m, H-6a, H-6b, H-6a', H-6b'), 3.70 (dd, 1H, *J* = 3.5 Hz, 10.0 Hz, H-2'), 3.67-3.72 (m, 2H, H-3, H-4), 3.58 (m, 1H, H-5'), 3.43-3.45 (m, 2H, H-2, H-5), 2.31 (apt t, *J* = 7.5 Hz, 2H, $COCH_2$), 1.53 (m, 2H, $COCH_2CH_2$), 1.24 (m, 24H, -CH_2CH_2-), 0.85 (t, 3H, *J* = 7.0 Hz, CH_3); ^{13}C NMR (500 MHz, DMSO): δ 172.0 (CO), 103.9 (d, C-1'), 93.9 (C-1), 80.1 (C-4), 75.9 (C-5'), 75.7 (C-5), 74.8 (C-3), 73.4 (C-3'), 72.3 (C-2), 70.7 (C-2'), 68.3 (C-4'), 60.3 (C-6'), 60.1 (C-6), 33.5 ($COCH_2$), 31.4 ($COCH_2CH_2$), 29.2, 29.1, 29.1, 29.1, 29.1, 29.0, 28.8, 28.8, 28.6, 28.5 (-CH_2CH_2-), 24.5 ($CH_2CH_2CH_3$), 22.2 ($CH_2CH_2CH_3$), 14.1 ($CH_2CH_2CH_3$); FTIR (KBr, ν_{max}, cm^{-1}): 3391 (OH), 2916, 2847, 1760 (CO), 1470, 1377, 1090, 1015, 892, 782, 717; HRMS-ES: Found (%) 579.3354, required 579.3381 [M-H]-.

The generally accepted explanation for the regioselectivities observed during tin-mediated reaction invokes dimmers and higher oligomer of the five-coordinated tin as the reaction species. For lactose there exist a *cis*-diol system on the glucose moiety and a trans-diol system of which adjacent group in apical position, both type of structure of substrate can be anticipated to regioselectively acylated, for the instance of methyl α-D-gluco pyanoside fatty acids were regioselectively introduced on the 2-OH position[17], whereas for the galactoside the acylation of fatty acid happen on the 3-OH position[18], the result showed the *cis*-diol system has some advantage being sannylated with DBDM when both system co-exist and the following acylation happen on the 3-OH position (**Scheme-I**). The formation of 1-O-palmitoyl-β-D-lactose was possibly directly acylated with lactose, generally it need some base to facilitate, for instance glucose and galactose were acylated with fatty acid to give corresponding 1-O-acyl-β-D-glucose-(galctose)[16].

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