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# **B-Galactosidase Catalysed Transglycosylation in Aqueous Organic Media** Using Glycosylasparagine Mimics As Novel Acceptors

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Abstract:  $\beta$ -1-N-acetamido-D-glucopyranose (2), a model of N-glycoprotein linkage region, and its benzamido analogue were explored as novel acceptors in transglycosylation catalysed by  $\beta$ -galactosidase from *Bacillus circulans*. Acceptor ability of 2 was shown to be as good as or better than several O-glycosides employed earlier. Systematic variation of reaction conditions led to improvement of yield from 5 % to 41 %. Interestingly, use of aqueous organic media has led to increased yield of transglycosylation and the 1,3-linked disaccharide was formed in considerable amounts under all the conditions examined. @ 1999 Elsevier Science Ltd. All rights reserved.

## INTRODUCTION

Synthesis of oligosaccharides is currently receiving world wide attention owing to their important roles in many biological processes including cell-cell recognition, communication and viral infection.<sup>1</sup> Glycosidases are proving to be attractive catalysts for oligosaccharide synthesis as these hydrolases are readily available, inexpensive, highly stable, easy to handle and require no cofactors. Glycosylation catalysed by a variety of glycosidases has been achieved both under equilibrium controlled condition (also known as reverse hydrolysis) and kinetically controlled condition (also called transglycosylation). Due to the use of activated donors in transglycosylation, the oligosaccharide synthesis occurs rapidly and often in greater yields than under reverse hydrolysis.<sup>2</sup> The major drawbacks of glycosidases, as compared to glycosyltransferases, are the moderate to low yields and lack of regiospecificity in glycosylation.

Several efforts have been directed towards evaluating the influence of various factors on the yield<sup>3,4</sup> of transglycosylation. These factors include concentration and nature of the aglycon group of acceptor sugar,

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temperature and reaction time. Since water (>50 M) and the acceptor sugar nucleophile ( $\equiv$ 100 mM) typically compete with each other in trapping the enzyme-held oxocarbenium ion intermediate leading to hydrolysis and synthesis respectively, use of organic co-solvents and effective acceptors should significantly contribute to improving the disaccharide yield. Contrary to the expected increase due to lowering of water activity, earlier studies<sup>5, 6</sup> on the use of glycosidases in aqueous organic media have observed a decrease in transglycosylation yield. Clearly, a better understanding of the structural aspects of glycosidase-acceptor sugar interactions in aqueous and aqueous organic media is vital for transforming the glycosidase-based synthetic methodology into an efficient technology.

In the present paper, we report for the first time 1) transglycosylation using a N-glycosidic acceptor that mimics glycosylasparagine and 2) improved yield of glycosylation conducted in aqueous organic media catatylsed by ß-galactosidase form Bacillus circulans. The newer acceptor sugar, ß-1-N-acetamido-Dglucopyranose (2) represents a simple model of the linkage region (glycosylasparagine) in N-glycoproteins. Successful demonstration of the acceptor ability of 2, it was reasoned, would pave way for the enzymic synthesis of larger glycopeptide models. Elucidation of the conformation of such models of the linkage region is fundamental to the better understanding of the structural basis of the functions of protein-linked glycans.<sup>7,8</sup> β-D-Glucopyranosylamine that serves as a synthon for compound 2 can be readily prepared on large scale with high yield and stereoselectivity<sup>9</sup> and is also versatile enough for the introduction of a variety of aglycon groups to modulate yield and regioselectivity.<sup>4</sup> Following enzymic synthesis, removal of the acetamido aglycon moiety of the product disaccharides can also be readily effected using hydrazine in a manner similar to that followed for the preparation of free oligosaccharides from glycoproteins.<sup>10</sup> Keeping these advantages in mind, the biomimetic approach was explored systematically in the present study. The stability of B-galactosidase from B. circulans in various aqueous organic media was first evaluated. A comparative evaluation of 2 with an analogue,  $\beta$ -1-Nbenzamido-D-glucopyranose, and several O-glycosides demonstrated its good acceptor ability. Transgalactosylations to 2 under varying donor / acceptor ratio, organic co-solvent, pH and temperature were carried out to understand the influence of these parameters on both yield and regioselectivity.

#### **RESULTS AND DISCUSSION**

 $\beta$ -Galactosidase from *B. circulans* is commercially available and inexpensive. This crude enzyme preparation has been shown to be an economical substitute for  $\beta$  1,4-galactosyltransferase for synthesising  $\beta$ -(1,4)-galactosyl disaccharides bearing a N-acetyl-D-glucosamine or N-acetyl-D-galactosamine residue at the reducing end.<sup>11</sup> Though transglycosylations catalysed by this enzyme have been carried out in aqueous acetonitrile,<sup>11,12</sup> no detailed study of its stability in various aqueous organic media is reported to the best of our knowledge.

The effect of five common organic solvents on the stability of  $\beta$ -galactosidase from *B. circulans* was examined in the present work. The percentage of retained activity after one hour of incubation in 30% (v/v)



Figure 1. Effect of organic co-solvents [30% (v/v) concentration] on the activity of  $\beta$ -galactosidase from *B. circulans* 



Figure 2. Effect of acetone concentration on the activity of β-galactosidase from *B. circulans* 

concentration of organic solvents in aqueous phosphate buffer turned out to be: 85 (dimethyl sulphoxide); 70 (acetone); 67 (dimethyl formamide); 59 (dioxane); 12 (acetonitrile) [Figure 1]. Considerable lowering of enzyme activity observed in acetonitrile is consistent with the unusually long transglycosylation time (>100 h) reported earlier.<sup>11</sup> The effect of varying concentrations (10 to 40%) of less toxic and easily removable solvents viz., acetone and dioxane was also studied [Figure 2] to choose the optimum concentration for transglycosylation. The decrease in enzyme activity upto 30% concentration of these solvents was gradual and reasonable. Considerable loss in stability, however, was noticed at 40%. At 50% concentration, precipitation of the protein was noticed.



Figure 3. Effect of various acceptors on the activity of β-galactosidase from B. circulans; ◆ glucose (400 mM, 52%); ■, compound 2 (400 mM, 83%); ▲, β-1-N-benzamido-D-glucopyranose (75 mM, 48%); ×, methyl α-D-glucopyranoside (400 mM, 85%); □, methyl α-D-galactopyranoside (75 mM, 85%). The percentages refer to maximum enzyme activity at the specified concentration.

All glycosides employed earlier as acceptors in transglycosylation contained the aglycon groups either O- or S-linked to the sugar. The linkage region model was prepared according to literature procedure<sup>13</sup> and its acceptor ability was evaluated by assaying the enzymic activity in phosphate buffered solution containing varying concentrations (0-400 mM) of **2**. For comparison, D-glucose, methyl  $\alpha$ -D-glucopyranoside and *p*-nitrophenyl  $\beta$ -D-glucopyranoside were included in this study. Methyl  $\beta$ -D-galactopyranoside was chosen as a negative control. The observed enzyme activities at various concentrations of the acceptor are expressed relative to that of zero acceptor concentration which is normalised to 100%. A plot of acceptor sugar concentration versus relative activity is shown in Figure 3. Compound **2** turns out to be as good an acceptor as methyl  $\alpha$ -D-glucopyranoside and better than other acceptors examined, contributing to 83% increase in relative activity and showing no signs of enzyme inhibition even at 400 mM concentration.  $\beta$ -1-N-Benzamido-D-glucopyranoside, used as a negative control, resulted in decrease in activity of 75% at 400 mM concentration. Incidentally, the activity data presented in Figures 1, 2 and 3 represent the average of activities obtained from two sets of experiments and there was very good agreement between them.



#### Scheme 1

Transgalactosylation from o-nitrophenyl  $\beta$ -D-galactopyranoside (1), the donor, to the newer acceptor sugar 2 (Scheme 1) was performed under different conditions varying the donor to acceptor ratio, organic cosolvent, pH and temperature. After completion of the reaction, the solution was lyophilised and the solid obtained was acetylated using pyridine and acetic anhydride. The per-O-acetylated derivative of the excess unreacted acceptor sugar was separated from the product mixture by simple crystallisation. Separation of the regioisomeric disaccharide derivatives was performed on silica gel by flash column chromatography. The 1,4isomer was obtained in pure form, while the 1, 3 and 1,6-isomers were obtained as a mixture that was difficult to separate. Unambiguous structural elucidation of the regioisomeric per-O-acetylated disaccharide products was accomplished by the combined use of NMR experiments including two-dimensional homonuclear proton COSY and NOESY and heteronuclear (<sup>1</sup>H - <sup>13</sup>C) HMQC and HMBC. These powerful NMR methods enabled the unambiguous assignment as well as quantitation of column fractions containing even a mixture of 1,6 and 1,3-linked regioisomers. It is clear from Table 1 that increased donor to acceptor ratio (1:10), use of aqueous acetone (30%), lower pH (5.0) and temperature (15°C) cumulatively contributed to the transglycosylation yield improving it from 5 % to 41 %. During long reaction time (11 h) observed at 15°C, the secondary hydrolysis of product disaccharides presumably takes place to some extent. Use of more amount of enzyme reduced the reaction time and increased the yield as well. It is interesting to note the absence of 1,4-regioisomeric disaccharide, when the transgalactosylation was carried out in phosphate buffer with a donor to acceptor ratio of 1:2. In all other cases, the 1,3 and 1,4-isomers predominated while the 1,6-isomer is formed in minor amounts.

Table 1:	Yield and regioselectivity ratios obtained for the transgalactosylation of	f 2
	under various conditions	

No.	Donor/ Acceptor Ratio	Reaction Medium	Time (h)	Yield (%)	Regioselectivity Ratio (3 : 4 : 5)
1.	1:2	Phosphate buffer pH 7.0	1.5	5	71 : - : 29
2.	1:5	11	1.0	16	41 : 40 : 19
3.	1:5	30% acetone in phosphate buffer	2.3	25	50 : 43 : 07
4.	1:5	Acetate buffer pH 5.0	2.0	-23	33 : 59 : 08
5.	1 : 5	30% acetone in acetate buffer	4.9	28	42 : 44 : 14
6.	1 : 10	tt.	4.3	36	44 : 38 : 17
7.	1 : 10ª	11	11.0	37	35 : 54 : 07
8.	1 : 10 <sup>b</sup>	н	2.7	41	35:47:16

a : 2 U of  $\beta$ -galactosidase and at 15°C ; b : 6 U of  $\beta$ -galactosidase and at 15°C while in all other cases 2U of  $\beta$ -galactosidase was employed at 30°C.

Similar distribution of regioisomeric disaccharides was recently observed in the transglycosylation catalysed by the same enzyme using methyl  $\beta$ -D-glucopyranoside as the acceptor.<sup>14</sup> Use of ethylthio  $\beta$ -D-glucopyranoside as an acceptor under similar conditions, however, did not afford any 1,3-linked disaccharide.<sup>15</sup> The extent of hydrogen bonding interactions between the glycosidic atom (O and N vs S) and an electrophilic centre in the acceptor binding site of the enzyme<sup>16</sup> appears to be important for the formation of 1, 3 - linkage. The galactosyl  $\beta$ -1,3-linkage represents an interesting structural motif of such biologically important oligosaccharides as sialyl Lewis a and the T-antigen.<sup>17</sup> Considering the non-availability of  $\beta$  1,3-galactosyltransferase, the  $\beta$ -galactosidase based methodology, reported here, using a glycosylasparagine mimic (2) as acceptor sugar offers a potentially practical and economical route for the synthesis of such targets.

In conclusion,  $\beta$ -1-N-acetamido-D-glucopyranose, a simple model of the linkage region in Nglycoproteins, has been shown to be a good acceptor for transgalactosylation. The biomimetic approach to acceptor design reported here, exploits glycosylamines as versatile synthons and thus offers a ready and efficient access to a variety of structurally diverse acceptors. A detailed study of transglycosylation involving such acceptors would be very useful in obtaining a better understanding of factors influencing yield and regioselectivity. The present observation of increased yield of transglycosylation in aqueous organic media catalysed by  $\beta$ -galactosidase from *B. circulans* is noteworthy and further illustrates the synthetic utility of this enzyme. Experiments are currently under way to explore the modulation in regioselectivity by employing structurally diverse glycosylasparagine mimics derived from glycosylamines and glycosyl isothiocyanates.

#### EXPERIMENTAL SECTION

#### Materials

Commercially available  $\beta$ -D-galactosidase (E.C.3.2.1.23) from *B. circulans* ("Biolacta N5") was a gift from Daiwa Kasei Co. Ltd., Osaka, Japan. Methyl  $\alpha$ -D-galactopyranoside and methyl  $\beta$ -D-galactopyranoside was purchased from Sigma. Methyl  $\alpha$ -D-glucopyranoside was a gift from Chimique Laboratories, Chennai. *o*-Nitrophenyl  $\beta$ -D-galactopyranoside and *p*-nitrophenyl  $\beta$ -D-glucopyranoside were synthesised according to the literature procedure.<sup>18</sup>  $\beta$ -1-N-Acetamido-D-glucopyranose was prepared from  $\beta$ -D-glucopyranosylamine according to the literature procedure.<sup>13</sup>  $\beta$ -1-N-Benzamido-D-glucopyranose was prepared by the de-Obenzoylation of  $\beta$ -1-N-benzamido-2,3,4,6-tetra-O-benzoyl-D-glucopyranose<sup>19</sup> using sodium methoxide in methanol. All other chemicals purchased were of reagent grade and purified according to standard procedures.

### Methods

FT-IR spectra were recorded on a BRUKER IFS 66V FT-IR spectrometer. NMR spectra were recorded on Varian UXR300, JEOL JNM-GS-400 and Bruker AMX-600 MHz spectrometers using tetramethylsilane as standard. The structure of the enzymatically synthesised disaccharides were assigned by COSY, HMQC, HMBC and DEPT experiments. Quantitation of regioisomers was done by integrating the anomeric proton of  $\beta$ galactose residues appearing at 4.59 ppm, 4.45 ppm and 4.44 ppm corresponding to the 1,3, 1,4 and 1,6-linked disaccharides. In the listing of NMR spectral data, the atoms of Gal residue are indicated with (') and those of Glc have no superscripts. High resolution FAB-MS analyses were made with a *m*-nitrobenzyl alcohol / cesium iodide matrix on a VG ZAB-VSE double focusing high resolution mass spectrometer in the positive-ion mode. Optical rotation was measured on a JASCO-DIP 200 digital polarimeter. Melting points were determined on a Toshniwal melting point apparatus and are uncorrected. TLC was performed on 25 mm E.Merck silica gel plates (60F-254). Detection was done by spraying the plates with 10% H<sub>2</sub>SO<sub>4</sub> in ethanol and heating. Flash column chromatography was performed on silica gel (230-400 mesh).

#### Enzyme assay<sup>9</sup>

A mixture containing 2 mM *o*-nitrophenyl  $\beta$ -D-galactopyranoside (1, 0.9 ml) in 50 mM sodium phosphate buffer (pH 7.0) and an appropriate amount of enzyme (0.1 ml) in the same buffer was incubated for 10 min at 30°C. The reaction was stopped by adding 0.1 M sodium carbonate (2 ml). Absorbance of the liberated *o*-nitrophenol was read spectrophotometrically at 420 nm. One unit of activity is defined as the amount of enzyme releasing one µmole of *o*-nitrophenol per minute.

## Effect of organic solvent on enzyme activity

A solution (1 ml) of enzyme (0.5 mg) in 50 mM phosphate buffer (pH 7.0) containing 30% v/v organic co-solvent was incubated at 30°C for 60 min. Aliquots (0.1 ml) were drawn at various intervals of time (5, 10, 20, 30 and 60 min) and the enzymic activity present in each case was assayed using 1 as the substrate as described above. Enzymic activity in standard 50 mM phosphate buffer was expressed as 100%.

#### Evaluation of acceptor sugar

ß-Galactosidase was assayed in 50 mM phosphate buffer (pH 7.0) containing 1.8 mM of 1 and varying concentrations of each acceptor sugar (20, 50, 75, 125, 200, 300 and 400 mM) for 10 min. In the case of *p*-nitrophenyl ß-D-glucopyranoside, concentrations beyond 80 mM could not be achieved in phosphate buffer. The reaction was quenched using 0.1 M sodium carbonate (2 ml) and absorbance measured spectrophotometrically at 420 nm. Glucose was used as the standard. Enzymic activity at zero concentration of acceptor was taken as 100%.

### Typical procedure for enzymatic galactosylation

To a solution of 2 (0.367g, 1.66 mmol) in 50 mM sodium phosphate buffer (4 ml) containing  $\beta$ -galactosidase (2 U) from *B. circulans* was added a solution of 1 (0.1g, 0.33 mmol) in the same buffer (9 ml) over a period of 30 min. The reaction was followed by TLC (CHCl<sub>3</sub> : MeOH : aq.NH<sub>3</sub> = 7 : 3 : 0.1). After the disappearance of donor sugar, the reaction was quenched by heating in a boiling water bath for 10 min followed by cooling in ice. The released *o*-nitrophenol was removed from the reaction mixture by extraction with ether. The aqueous solution was freeze-dried and the residue (0.63 g) peracetylated using acetic anhydride and pyridine (2 ml each). Pyridine was removed by successive evaporation using toluene and the resulting solid obtained was dissolved in ethyl acetate-hexane for fractional crystallisation of the per-O-acetate of the excess acceptor sugar (2). The mother liquor enriched in the product disaccharides was impregnated onto celite and purified by flash column chromatography on silica gel. Initial elution with CHCl<sub>3</sub> : acetone : EtOH = 92 : 8 : 0.5 eluted out the per-O-acetate of the hydrolysis product (D-galactose) and that of excess acceptor sugar (2). The product disaccharides were eluted out using CHCl<sub>3</sub> : acetone: EtOH = 89 :11 : 0.5 to obtain 15 mg (6.7%) of 3 together with 7 mg (3%) of 5 and 14.5 mg of 4 (6.5%), in that order. The disaccharides 3 and 5 obtained as an inseparable mixture were characterised as such unambiguously by various 2D NMR spectral methods.

**B-1-N-Acetamido-2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-B-D-galactopyranosyl)-D-glucopyranose** (3): granular solid;  $R_f = 0.50$  (silica, CHCl<sub>3</sub> : acetone : EtOH = 9.2: 0.8 : 0.1, 3 runs); IR (KBr)  $v_{max}$ : 3371, 2958, 1753, 1701, 1538, 1434, 1373, 1225, 1171, 1045, 899 and 600 cm<sup>-1</sup>; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>):  $\delta 6.21$  (d, 1H, J = 9.5 Hz, N<u>H</u>), 5.34 (dd, 1H, J = 1, 3.5 Hz, H-4'), 5.15 (t, 1H, J = 9.5 Hz, H-1), 5.07 (dd, 1H, J = 8.0, 10.5 Hz, H-2'), 4.97 (t, 1H, J = 9.5 Hz, H-4), 4.93 (dd, 1H, J = 3.5, 10.5 Hz, H-3'), 4.85 (t, 1H, J = 9.5 Hz, H-2), 4.59 (d, 1H, J = 8.0 Hz, H-1'), 4.21 (dd, 1H, J = 4.7, 12.5 Hz, H-6b), 4.14 (dd, 1H, J = 6.5, 11.2 Hz, H-6b'), 4.09 (m, 1H, H-6a), 4.06 (m, 1H, H-6a'), 3.96 (t, 1H, 9.4 Hz, H-3), 3.90 (m, 1H, H-5'), 3.75 (m, 1H, H-5), 2.14, 2.12, 2.07, 2.06, 2.03, 1.97 1.95 (7s, 24 H, acetyl-C<u>H<sub>3</sub></u>); <sup>13</sup>C NMR (90 MHz,CDCl<sub>3</sub>):  $\delta$  170.84, 170.66, 170.38, 170.33, 170.16, 170.11, 169.18 & 169.00 (s each, -<u>C</u>OCH<sub>3</sub>), 101.16 (C-1'), 78.88 (C-3), 77.93 (C-1), 73.61 (C-5), 72.82 (C-2), 70.97 (C-3'), 70.62 (C-5'), 68.84 (C-2'), 67.86 (C-4), 66.81 (C-4'), 61.90 (C-6), 60.98 (C-6'), 23.34, 20.88, 20.73, 20.62, 20.57, 20.48 & 20.35 (s each, -COCH<sub>3</sub>); FAB-MS: *m* / *z* 810.1250 (M+Cs)<sup>+</sup>, 100%; calcd. = 810.1221.

(1-6) linked isomer (5):  $R_f = 0.54$  (silica, CHCl<sub>3</sub> : acetone: EtOH = 9.2 : 0.8 : 0.1, 3 runs); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>):  $\delta$  6.31 (d, 1H, J = 9.6 Hz, N<u>H</u>), 5.34 (dd, 1H, H4<sup>2</sup>), 5.28-5.15 (m,3H), 5.02-4.95 (m,2H), 4.85 (t, 1H, J = 9.6 Hz) 4.44 (d, 1H, J = 7.8 Hz, H-1<sup>2</sup>), 4.19 - 4.04 (m,2H), 3.97 (dd, 1H, J = 2.1, 11.4 Hz), 3.84 (m, 1H), 3.72 (m, 1H) 3.51 (dd, 1H, J = 4.2, 11.4 Hz, H-6a) 2.12, 2.08, 2.04, 2.03, 2.01, 1.98, 1.97, 1.96 (8s, 24H, acetyl-C<u>H</u><sub>3</sub>); <sup>13</sup>C NMR (90 MHz,CDCl<sub>3</sub>):  $\delta$  170.91, 170.49, 170.20, 170.11, 169.91, 169.82 & 169.40 (s each, -<u>C</u>OCH<sub>3</sub>), 101.23, (C-1<sup>2</sup>), 77.99, 74.34, 72.90, 70.81, 70.58, 70.47, 68.48, 67.27, 66.91, 61.17 (C-6<sup>2</sup>), 23.30, 20.65, 20.61, 20.55 (s each, -COC<u>H</u><sub>3</sub>); IR and FAB-MS data as given above.

**β-1-N-Acetamido-2,3,6-tri-O-a 4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-D-glucopyranose** (4): colourless crystal;  $R_r = 0.45$  (silica, CHCl<sub>3</sub> : acetone : EtOH = 9.2 : 0.8 : 0.1, 3 runs) ; m.p. = 131 - 132°C ;  $[\alpha]_D^{27} = + 11.7$  (c=1, CH<sub>2</sub>Cl<sub>2</sub>) ; IR (KBr)  $v_{max}$ : 3337, 2937, 1755, 1709, 1683, 1536, 1434, 1373, 1232, 1173, 1131, 1049, 949, 930, 907, 646 and 587 cm<sup>-1</sup>; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) : δ 6.18 (d, 1H, J = 9.3 Hz, N<u>H</u>) ; 5.34 (dd, 1H, J = 1.1, 3.5 Hz, H-4'), 5.29 (dd, 1H, J = 8.6, 9.6 Hz H-3), 5.18 (t, 1H, J = 9.4 Hz, H-1), 5.09 (dd, 1H, J = 7.9, 10.4 Hz, H-2'), 4.93 (dd, 1H, J = 3.5, 10.4 Hz, H-3'), 4.81 (t, 1H, J = 9.5 Hz, H-2), 4.45 (d, 1H, J = 7.9 Hz, H-1'), 4.42 (dd, 1H, J = 1.8, 12.1 Hz, H-6b), 4.14 (dd, 1H, J = 6.3, 11.1 Hz, H-6b'), 4.13 (dd, 1H, J = 4.6, 12.1 Hz, H-6a), 4.06 (dd, 1H, J = 7.4, 11.1 Hz, H-6a'), 3.86 (m, 1H, H-5'), 3.76 (dd, 1H, J = 8.6, 9.9 Hz, H-4), 3.72 (ddd, 1H, J = 1.8, 4.6, 9.9Hz, H-5), 2.15, 2.11, 2.06, 2.04, 2.03, 1.97, 1.95 (7s, 24 H, acetyl-C<u>H<sub>3</sub></u>). <sup>13</sup>C NMR (90 MHz, CDCl<sub>3</sub>): δ 171.30, 170.31, 170.30, 170.17, 170.11, 170.04, 169.27 & 168.93 (s each, -<u>C</u>OCH<sub>3</sub>), 100.87 (C-1'), 78.05 (C-1), 75.94 (C-4), 74.43 (C-5), 72.30 (C-3), 70.97 & 70.95 (C-2 & C-3', interchangeable), 70.71 (C-5'), 68.96 (C-2'), 66.59 (C-4'), 61.96 (C-6), 60.82 (C-6'), 23.36, 20.84, 20.73, 20.67, 20.61, 20.57 & 20.47 (s each, -CO<u>C</u>H<sub>3</sub>); FAB-MS: *m / z* 810.1250 (M+Cs)<sup>+</sup>, 100%; calcd. = 810.1221.

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