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On the Regioselectivity of the Protease Subtilisin Towards the Acylation of Enantiomeric Pairs of Benzyl and Naphthyl Glycopyranosides. Part 2.

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Abstract: Subtilisin-catalyzed esterification of several enantiomeric benzyl and naphthyl glycopyranosides has been investigated. The D-sugar derivatives were all good substrates and subtilisin regioselectivity was similar with all the compounds tested, the 3-OH being acylated predominantly. On the other hand, most of the L-glycopyranosides were transformed during longer reaction times with a lower regioselectivity, the 2-OH being preferentially but not exclusively acylated.

1. Introduction

Regioselectivity is one of the properties of hydrolytic enzymes that has been recently exploited for synthetic applications.[1] Specifically, lipases and proteases have been used to catalyze protection steps in the chemo-enzymatic synthesis of sugar derivatives,[2] to prepare sugar-based surfactants[3] and sugar acrylates,[4] and to synthesize specific esters of natural glycosides as, for instance, cinnamoyl esters of flavanoid glycosides.[5] In most cases these applications are based on a "try and see" approach and, up to now, little effort has been made in order to rationalize the results obtained.

Being particularly interested in the regioselective enzymatic modification of natural compounds,[6] we have previously found that the protease subtilisin was able to acylate the D-glucose of the flavanol isoquercitrine (**1**) in the presence of the activated ester trifluoroethyl butanoate (TFEB) affording the 6"-*O*-butanoyl derivative together with a small amount of the 3",6"-di-*O*-butanoyl analogue. In contrast, the L-rhamnose moiety of the congener flavanol quercetrine (**2**) remained completely unaffected.[7] As these two molecules differ only in their sugar moiety, it was clear that the reaction outcomes had been dictated by the differences of the two

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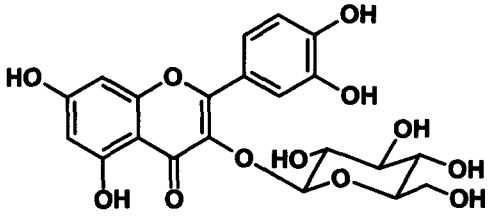
carbohydrates. The two sugars have several structural differences but, more importantly, they belong to opposite steric series. Therefore, it might be possible that L-rhamnose has the "wrong" configuration to fit correctly into the chiral environment of the enzyme active site. This possibility stimulated us to study the behaviour of enantiomeric pairs of sugars towards their subtilisin-mediated regioselective acylation.



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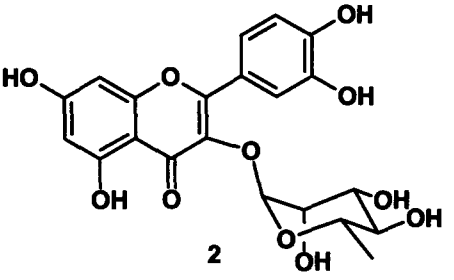

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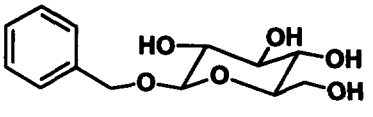
Acylation of enantiomeric methyl glycopyranosides by different lipases has already been described.[8] These investigations have been focused on the characterization of the reaction outcomes (percentage of the formed regioisomers after the complete disappearance of starting materials or after a fixed reaction time) and the results obtained were interpreted on the basis of the relative orientation of hydroxyls at C-2, C-3 and C-4, independently from the sugar steric series. The different possible "triplets" (i.e. Axial, Equatorial, Equatorial) were compared, and a relative scale was suggested to predict the degree of regioselectivity (EEE > AEE >> EEA) and the hydroxyl that could be acylated.[8]

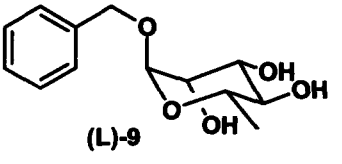
In this paper we report on the results obtained in the subtilisin-catalyzed esterification of several enantiomeric benzyl and naphthyl glycopyranosides. The D-sugar derivatives were all good substrates and subtilisin regioselectivity was similar with all the compounds tested; on the other hand, most of the L-glycopyranosides were transformed during longer reaction times with a lower regioselectivity. To get additional information about the observed enzymatic performances, the kinetic constants of the reactions were determined *via* a measure of the initial rates with different concentrations of sugars and ester. A possible rationale based on the different sugar structures is suggested.

2. Results and Discussion

In starting our investigation, we decided to study the behavior of benzyl β-D-glucopyranoside **(D)-3** and benzyl α-L-rhamnopyranoside **(L)-9**, two derivatives that mimic **1** and **2**.



(D)-3



(L)-9

These compounds have several structural differences in their carbohydrate moieties: besides belonging to the opposite steric series of **(D)-3**, the compound **(L)-9** is a 6-deoxy sugar and its C-2 OH and anomeric substituent have an axial conformation instead of an equatorial one. Therefore we decided to "move" step by step from the glucopyranoside **(D)-3** to the rhamnopyranoside **(D)-9** by preparing new derivatives with a single structural variation compared to their immediate "precursors". The same was carried out in order to synthesize the corresponding derivatives belonging to the L-series, and the performances of the enantiomers of each compound were finally compared. As a preliminary experiment clearly indicated that, as expected, both enantiomers **(D)-3** and **(L)-3** were rapidly monoacylated by subtilisin at their primary OH with similar efficiency, we started from the corresponding benzyl 6-*O*-acetyl β-D-glucopyranoside **(D)-4** and benzyl 6-*O*-acetyl β-L-glucopyranoside **(L)-4** for our subsequent investigation, with the aim of observing a discrimination between the two enantiomeric sugars during the acylation of the secondary hydroxyls. ***Figure 1*** shows the structural modifications performed along two pathways on **(D)-4** to reach **(D)-9**, that is either C-6 deoxygenation followed by C-1 anomerization and final C-2 epimerization, or C-1 anomerization followed by C-2 epimerization and final C-6 deoxygenation.

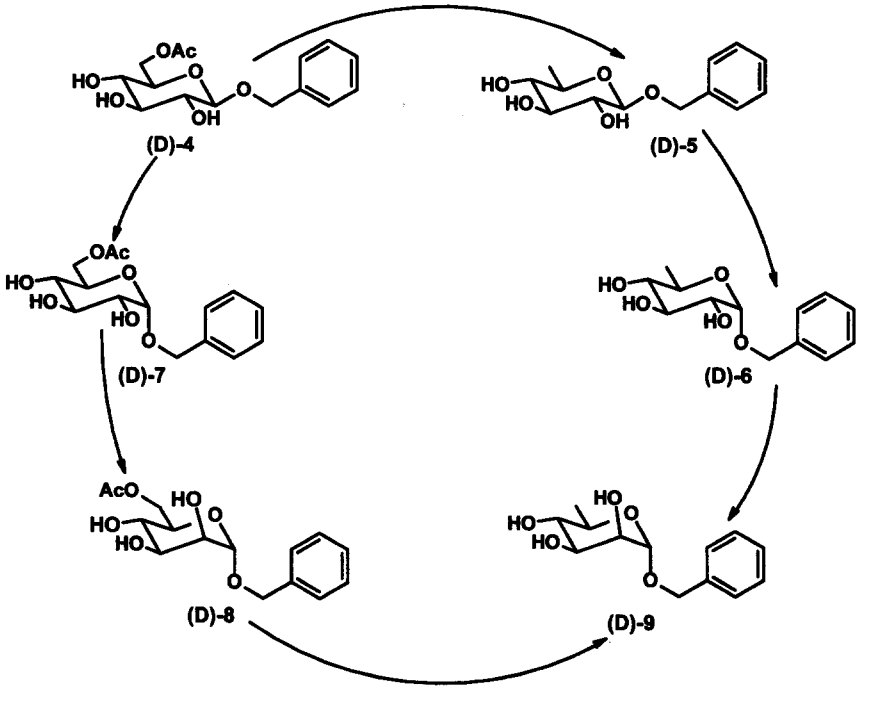
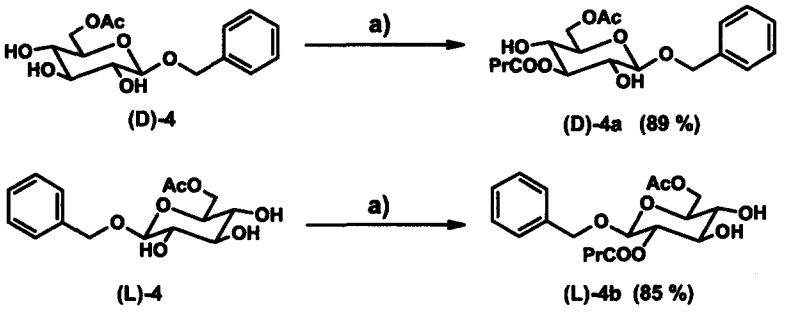


Figure 1

In a preliminary communication (Part 1 of this series),[9] we have described the peculiar results obtained in the acylation of the enantiomers **(D)-** and **(L)-4**. When **(D)-4** was dissolved in acetone and treated with 5 eq of

trifluoroethyl butanoate (TFEB) in the presence of subtilisin, the benzyl 6-*O*-acetyl 3-*O*-butanoyl β-D-glucopyranoside **(D)-4a** was formed in 89 % yield after 24 hours; on the other hand, **(L)-4** gave the 2-*O*-butanoyl ester **(L)-4b** in 85 % yield (***Scheme 1***). Thus the two enantiomers gave rise to different acylated regioisomers in comparable yields.

Scheme 1



a) TFEB , acetone , subtilisin

Analogously, the D-sugar derivatives were all good substrates and subtilisin regioselectivity was similar with all the compounds tested. Deoxygenation of the C-6 OH and/or epimerization of the anomeric substituent or of the C-2 OH did not affect enzyme selectivity, the 3-OH always being overwhelmingly acylated. On the other hand, structural variations deeply influenced subtilisin performances with the L-sugars. Specifically, while a single structural variation such as 6-OH deoxygenation (as in **(L)-5**) or anomeric epimerization (as in **(L)-7**) did not change the enzymatic preference for the acylation of the C-2 OH, a combination of structural variations from the "parent" **(L)-4** decreased reactivity and produced the formation of derivatives acylated at the C-3 OH in addition to the expected C-2 OH esterified products. Significantly, the benzyl α-L-rhamnopyranoside **(L)-9** (differing from **(L)-5** in three structural variations) was very poorly acylated (less than 20 % conversion after 3 days at 45 °C) and, additionally, the regioselectivity was completely lost, a mixture of the three possible monoesters being formed (***Figure 2***. For details concerning reaction times, % conversion, isolated yields and product characterization, see the Experimental Part).

To gain a deeper insight into the observed enzymatic behavior towards the enantiomeric substrates, we determined the kinetic constants of the reactions *via* a measure of the initial rates with different concentrations of sugars and ester. A comparison of the kinetic constants for different substrates is essential for an evaluation of the enzyme preference. However, to our knowledge this study has never been accomplished in any of the enzymatic regioselective acylation of sugars reported so far, in spite of the importance of the information that can be obtained.

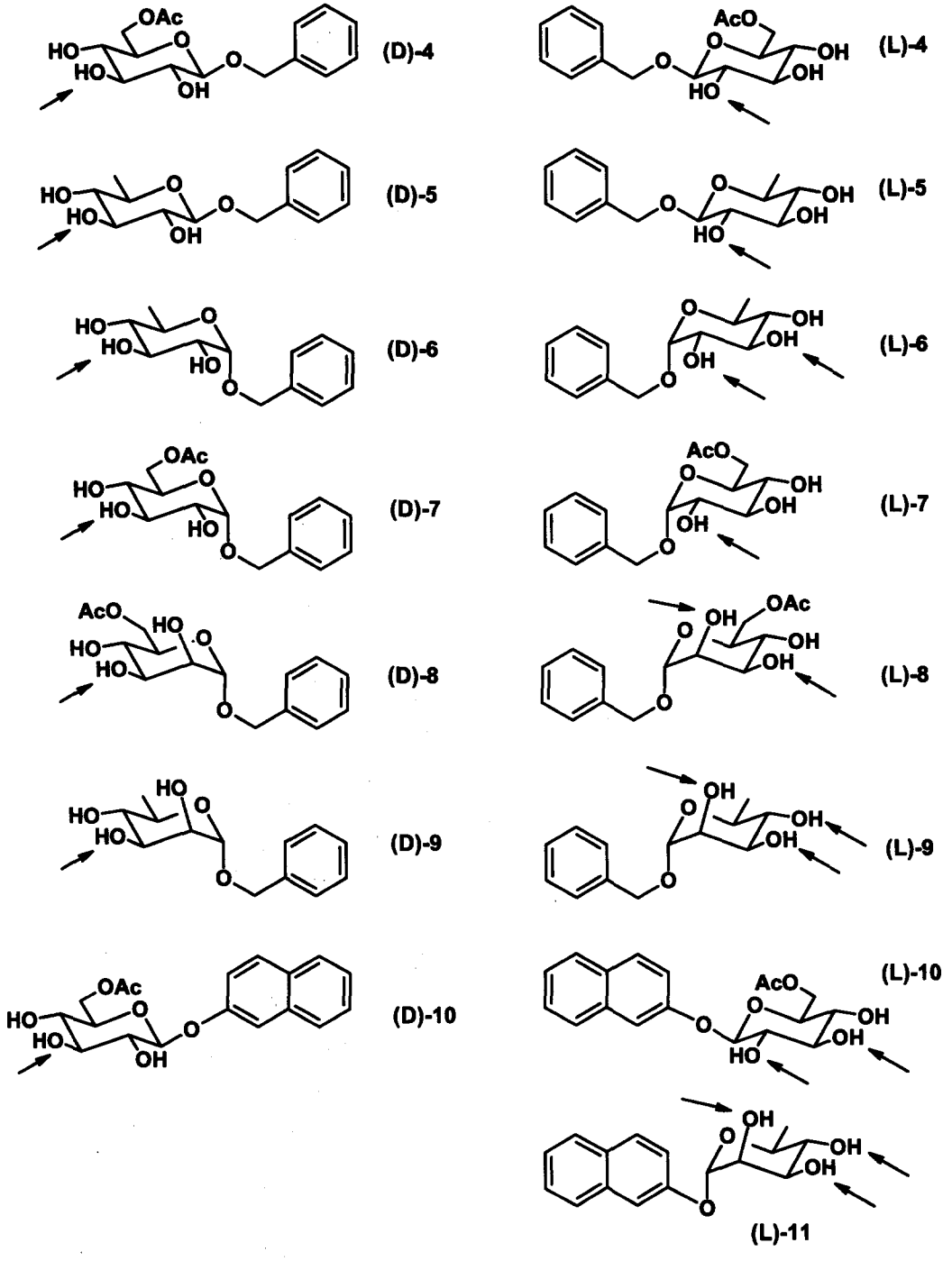


Figure 2. **(D)- and (L)-glycopyranosides substrates. Arrows indicate the positions acylated by subtilisin.**

The catalytic mechanism of subtilisin is of the "PING-PONG-Bi-Bi" type (according to the symbolism introduced by Cleland [10]), and the kinetic equation describing this mechanism is:

$$\frac{1}{v} = \frac{K_{m\,Sugar}}{V_{max}} * \frac{1}{[S]} + \frac{1}{V_{max}} * \left(1 + \frac{K_{m\,Ester}}{[E]}\right)$$

where [S] is $[sugar]_o$ and [E] is $[ester]_o$. Accordingly, the double reciprocal plot of the initial rates of acylation *versus* sugar concentration, obtained at fixed ester concentrations, gives a set of parallel lines (primary plot). From this a secondary plot is obtained, from which it is possible to determine $K_{m\,Sugar}$, $K_{m\,Ester}$ and V_{max} (for details see ref. 11).

The initial rates of acylation were measured by GC analysis of the corresponding TMS-derivatives, and the obtained values of $K_{m\,Sugar}$, $K_{m\,Ester}$ and V_{max} for the compounds **(D)-4** and **(L)-4** are reported in *Table 1*. As $V_{max} = k_{cat} \times [enzyme]$, the ratio $(V_{max}/K_m)_D/(V_{max}/K_m)_L$ is equal to $(k_{cat}/K_m)_D/(k_{cat}/K_m)_L$, which is the ratio of the specificity constants (also called enantiomeric ratio, E) for the two enantiomeric sugars.[12] As it can be observed (sixth column of *Table 1*), the E value for these enantiomers is equal to 1. This means that, in spite of the fact that the two enantiomers gave rise to a different regiochemical outcome, they were kinetically equivalent for the enzyme.

The same kinetic investigation was performed on the compounds **5-9** and the results are reported in *Table 1*, together with the derived E values for the pairs of enantiomeric sugars.

These data clearly indicated that, from a kinetic point of view, whereas **(L)-5** was a better substrate than **(D)-5**, the other α-benzyl derivatives of the (L)-sugars were worse substrates than the corresponding D-enantiomers.

The data obtained so far could be explained by comparing the molecular models of the enantiomeric pairs of sugars. We first considered the substrates **(D)-4** and **(L)-4**, compounds that, despite the different regioselective outcome, were similarly recognized by subtilisin. If the six-member rings with all equatorially oriented substituents were overlaid in such a way that the 3-OH of **(D)-4** and the 2-OH of **(L)-4** (the hydroxyls that were acylated) were superimposed, we could observe that in this way the 4-OH of **(D)-4** and 3-OH of **(L)-4** also overlapped and the acetoxymethyl and benzyloxy appendages were on adjacent positions on the overlapped cyclohexanic structures (***Figure 3***). Therefore it is likely that these molecules accommodate in a very similar way into subtilisin active site environment, when the 3-OH of **(D)-4** and the 2-OH of **(L)-4** face the acylated serine of the catalytic triad, thus accounting for the kinetic equivalence of these two compounds.

Of course any structural modification of the sugar substituents could be responsible of a less favorable interaction into subtilisin active site. This was particularly true with the manno- and rhamno- derivatives belonging to the (L)–series, because both the 2-OH and the anomeric oxygen were in a different orientation (axial instead of equatorial) and, therefore, a good molecular overlapping with the **(D)-4** structure was not possible anymore, as reflected by the kinetic data and by the reaction outcomes (lower yields and selectivities).

We reasoned that this trend might be even strengthened by a more rigid and bulkier anomeric substituent, more similar to the flavanol moiety of **1** and **2**. To verify this hypothesis, we prepared the two enantiomeric naphthyl

β-glucopyranosides **(D)-10** and **(L)-10** as well as the naphthyl α-L-rhamonpyranoside **(L)-11**. As expected, the two enantiomers **(D)-10** and **(L)-10** were not kinetically equivalent anymore (*Table 1*) and the reaction outcomes were also different, as subtilisin regioselectivity was partially lost with **(L)-10** (the acylation ratio between 2-OH and 3-OH was approximately 4 : 1). Additionally, the rhamnopyranoside **(L)-11** proved to be the worst substrate among the sugar derivatives tested in this work, its specifity constant being one order of magnitude lower than that of the corresponding benzyl derivative **(L)-9**.

Table 1. Kinetic data of the acylation of the sugars 4-11 by the protease subtilisin.

Sugar	$K_{M\ ester}$ (M)	$K_{M\ sugar}$ (M)	V_{max} (mmol/min)	$V_{max}/K_{M\ sugar}$	E [a]	Enantiomeric Preference
(D)-4	1.3	4.0×10^{-2}	1.50×10^{-3}	37.0×10^{-3}	1.0	D ≈ L
(L)-4	1.7	5.5×10^{-2}	1.97×10^{-3}	35.8×10^{-3}		
(D)-5	2.3	24.0×10^{-2}	2.50×10^{-3}	10.4×10^{-3}	0.3	D < L
(L)-5	2.0	13.0×10^{-2}	4.30×10^{-3}	33.1×10^{-3}		
(D)-6	4.5	43.0×10^{-2}	3.70×10^{-3}	8.6×10^{-3}	3.2	D > L
(L)-6	0.8	4.2×10^{-2}	0.16×10^{-3}	3.8×10^{-3}		
(D)-7	0.8	6.1×10^{-2}	0.87×10^{-3}	14.3×10^{-3}	5.2	D > L
(L)-7	2.4	20.7×10^{-2}	0.56×10^{-3}	2.7×10^{-3}		
(D)-8	1.7	2.6×10^{-2}	1.01×10^{-3}	38.8×10^{-3}	34.5	D > L
(L)-8	3.2	22.0×10^{-2}	0.24×10^{-3}	1.1×10^{-3}		
(D)-9	1.6	6.8×10^{-2}	1.62×10^{-3}	23.8×10^{-3}	6.2	D > L
(L)-9	1.4	8.0×10^{-2}	0.30×10^{-3}	3.7×10^{-3}		
(D)-10	0.4	3.3×10^{-2}	0.41×10^{-3}	12.4×10^{-3}	2.8	D > L
(L)-10	0.5	3.9×10^{-2}	0.17×10^{-3}	4.4×10^{-3}		
(L)-11	18.5	65.9×10^{-2}	0.16×10^{-3}	2.5×10^{-4}		

[a] $E = (V_{max}/K_m)_D/(V_{max}/K_m)_L$

By comparing the molecular models of **(D)-10** and **(L)-10** it was evident that the possible structural overlapping of the two molecules was lower than that observed with **(D)-4** and **(L)-4**, as the rigid naphthyl aglycones pointed to adjacent but different space regions when the six-member rings with all equatorially oriented substituents were superimposed as previously described (***Figure 3***).

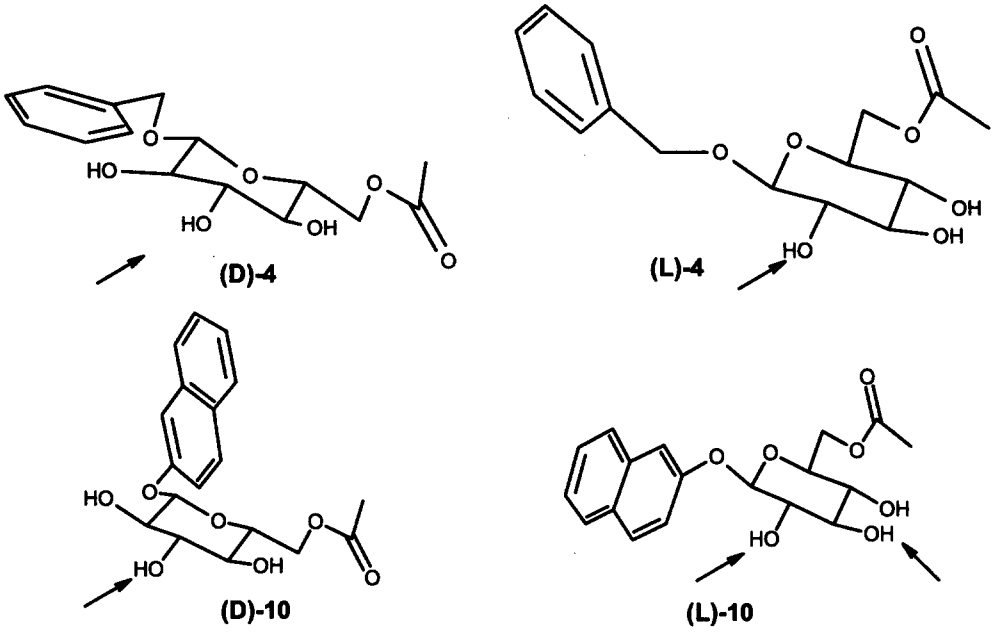


Figure 3. **Structures of glycopyranosides (D)-4, (L)-4, (D)-10 and L-(10). Arrows indicate the acylation positions.**

As it might have been expected, these results indicate that enzymatic regioselectivity in the acylation of sugars is determined by the whole substrate molecule and not only by the relative orientation of the secondary hydroxyls, as it had been suggested in previous work.[8]

If we consider the bulkiness of the flavanol moiety of **1** and **2**, the above data give also a plausible explanation to the selectivity observed in the acylation of **1** and **2** by subtilisin. However, no explanations can be given to clear up why the preferred acylation site is the C-3 OH of β-D-glycopyranosides (or the C-2 OH of the enantiomeric β-L-glycopyranosides). This information could be obtained by molecular modeling, docking the sugar derivatives into subtilisin active site, though this is not an easy task to be accomplished.[13] We are currently performing such an investigation and the results will be presented in due course.

3. Experimental Part

Materials and Methods. Subtilisin was purchased from Sigma (Protease Type VIII). Lipase from *Chromobacterium viscosum* was from Finnsugar Biochem Inc. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-300 at 300 MHz and 75.4 MHz, respectively, in $CDCl_3$ milieu, unless otherwise stated. FT-IR spectra were recorded on a Jasco 610 instrument equipped with a DTGS detector. The system was managed by a JASCO software and the spectra were recorded in $CHCl_3$ solution using a 18 μm spacer in a demountable specac cell with CaF_2 windows. Optical rotations were measured using a Perkin-Elmer 141 polarimeter. TLC was performed on precoated silica gel 60 F_{254} plates (Merck), and visualized spraying with a molybdic reagent

(prepared by dissolving 1 g of $CeSO_4$ and 21 g of $(NH_4)_6Mo_7O_{24}\cdot 4\,H_2O$ in 500 mL of water and adding 31 mL of concentrated H_2SO_4) followed by heating. Preparative chromatography was performed by flash chromatography using Silica Gel 60A (230-400 mesh) with the appropriate eluents. Enzymatic esterifications were followed by gas chromatography with a 25 m HP1 capillary silica gel column coated with methylsilicon gum (Hewlett Packard).

Benzyl 6-O-Acetyl-β-D-Glucopyranoside **((D)-4)** and *Benzyl 6-O-Acetyl-β-L-Glucopyranoside* **((L)-4)**. The preparation of these compounds has been described elsewhere.[9]

Benzyl 6-O-Acetyl-α-D-Glucopyranoside **((D)-7)**. D-Glucose (5 g, 28 mmol) was dissolved in 150 mL of benzyl alcohol presaturated with gaseous HCl. The solution was heated at 60 °C for 6 h, monitoring the degree of conversion by TLC (AcOEt, MeOH, H_2O 95:3:2). The alcohol was evaporated and the crude residue dissolved in 200 mL of 50 mM acetate buffer pH 5. β-Glucosidase (150 mg) was added and the solution left overnight at 37 °C. The pH was adjusted to 7, water was evaporated and the residue purified by flash chromatography (AcOEt, MeOH, H_2O 95:3:2) to give 3.45 g (46 % yield) of benzyl α-D-glucopyranoside. This intermediate was dissolved in 200 mL of acetone and 4 mL of vinyl acetate. *Chromobacterium viscosum* lipase supported on celite (5 g) was added and the suspension was shaken at 45 °C for 3 h. The enzyme was filtered, the solvent evaporated and the crude residue purified by flash chromatography (AcOEt, MeOH, H_2O 95 : 5 : 3) to give 2.78 g (72 % yield) of benzyl 6-*O*-acetyl-α-D-glucopyranoside **((D)-7)**. Oil. $[\alpha]_D$ - 95.8 (c 0.5, MeOH). ^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.68 and 4.51 (d, each 1H, J = 12 Hz, CH_2Ar); 4.90 (d, 1H, J = 3.5 Hz, H-1); 4.36 (dd, 1H, J_1 = 12.8 Hz, J_2 = 1.7 Hz, H-6a); 4.16 (dd, 1H, J_1 = 12.8 Hz, J_2 = 5.0 Hz, H-6b); 3.73 (m, 2H, H-3 and H-5); 3.47 (dd, 1H, J_1 = 9.5 Hz, J_2 = 3.5 Hz, H-2); 3.32 (t, 1H, J = 9.5 Hz, H-4); 2.10 (s, 3H, CH_3CO). ^{13}C-NMR, δ = 137.6; 129.1; 128.9;128.7; 98.3; 74.9; 72.7; 70.6; 70.5; 63.9; 21.5. IR ν_{max} 3407, 3017, 2883, 1737, 1366, 1250, 1089. Anal. Calcd. for $C_{15}H_{20}O_7$; C, 57.69; H, 6.41. Found C, 57.44; H, 6.57.

Benzyl 6-O-Acetyl-α-L-Glucopyranoside **((L)-7)**. α-L-Glucose pentaacetate (4.2 g, 10.7 mmol), obtained by acetylation of 2 g of L-glucose with Ac_2O/Py was brominated with 3.5 mL of a 40 % HBr solution in AcOH. The crude 2,3,4,6-tetra-*O*-acetyl-α-L-glucopyranosyl bromide was benzylated under standard conditions (benzyl alcohol, HgO, $HgBr_2$) to give, after purification (flash chromatography, eluent hexane-AcOEt 7 : 3), 3.062 g (57 % yield) of benzyl 2,3,4,6-tetra-*O*-β-L-glucopyranoside. This compound was dissolved in 125 mL of anhydrous CH_2Cl_2. $TiCl_4$ (6.4 mL of a 1 M solution in CH_2Cl_2) was added dropwise under nitrogen and the reaction was heated at 60 °C for 3 h, following the anomeric epimerization by TLC (eluent hexane-AcOEt 7 : 3, 2 runs). The solution was then poured on ice and the organic layer washed with a 5 % $NaHCO_3$ solution and H_2O. Purification by flash chromatography (eluent hexane-AcOEt 7 : 3) gave 1.318 g (43 % yield) of benzyl 2,3,4,6-tetra-*O*-acetyl-α-L-glucopyranoside. This compound was deacetylated by treatment with MeONa / MeOH to give benzyl α-L-glucopyranoside quantitatively which, in the last step, was regioselectively acetylated by catalysis of *Chromobacterium viscosum* lipase (1.8 g of lipase supported on celite, 1 mL of vinyl

acetate, 50 mL of acetone, 45 °C, 2 days) to give, after purification (eluent $CHCl_3$, MeOH 9.5 : 0.5) 892 mg (95 % yield) of oily benzyl 6-*O*-acetyl-α-L-glucopyranoside (**(L)-7**). $[\alpha]_D$ + 96.4 (c 0.5, MeOH). Anal. Calcd. for $C_{15}H_{20}O_7$; C, 57.69; H, 6.41. Found C, 57.50; H, 6.55.

Benzyl 6-O-Acetyl-α-D-Mannopyranoside (**(D)-8**). D-Mannose (1g, 5.6 mmol) was dissolved in 10 mL of benzyl alcohol presaturated with gaseous HCl. The solution was heated at 60 °C for 4 h (TLC : AcOEt, MeOH, H_2O 95:3:2). Usual work up and purification by flash chromatography gave 1 g (67 % yield) of benzyl α-D-mannopyranoside **6**. This compound was dissolved in 80 mL of anhydrous acetone; vinyl acetate (1 mL) and *Chromobacterium viscosum* lipase on celite (2 g) were added and the suspension was shaken for 2 h at 45 °C. Purification (eluent AcOEt, MeOH, H_2O 9:0.5:0.5) gave 905 mg (78 % yield) of benzyl 6-*O*-acetyl-α-D-mannopyranoside (**(D)-8**). Oil. $[\alpha]_D$ + 65.3 (c 0.5, MeOH); ^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.69 and 4.51 (d, each 1H, J = 12 Hz, CH_2Ar); 4.91 (br s, 1H, H-1); 4.51 (dd, 1H, J_1 = 12.5 Hz, J_2 = 5.5 Hz, H-6a); 4.18 (dd, 1H, J_1 = 12.5 Hz, J_2 = 2 Hz, H-6b); 3.97 (m, 1H, H-2); 3.84 (dd, 1H, J_1 = 10 Hz, J_2 = 3 Hz, H-3); 3.73 (1H, ddd, J_1= 10 Hz, J_2= 5.5 Hz, J_3= 2 Hz, H-5); 3.60 (t, 1H, J = 10 Hz, H-4); 2.15 (s, 3H, CH_3CO). ^{13}C-NMR, δ = 137.6; 129.1; 128.9; 128.6; 99.7; 72.0; 71.1; 69.9; 68.1; 64.1; 21.5. IR ν_{max} 3019, 2881, 1736, 1363, 1248, 1090. Anal. Calcd. for $C_{15}H_{20}O_7$; C, 57.69; H, 6.41. Found C, 57.45; H, 6.58.

Benzyl 6-O-Acetyl-α-L-Mannopyranoside (**(L)-8**). This compound was obtained in comparable yields following the same procedure used for its enantiomer. **(L)-8**: Oil. $[\alpha]_D$ - 66.4 (c 0.5, MeOH). Anal. Calcd. for $C_{15}H_{20}O_7$; C, 57.69; H, 6.41. Found C, 57.52; H, 6.53.

Benzyl α-D-Rhamnopyranoside (**(D)-9**). Benzyl α-D-mannopyranoside (2g, 7.4 mmol) was dissolved in 6 mL of pyridine. The flask was cooled with an ice bath and a solution of *p*-toluensulphonyl chloride (2 g, 10.5 mmol) in pyridine (6 mL) was slowly dropped. The solution was left at room temperature for 6 h, the solvent evaporated and the product purified by flash chromatography (eluent AcOEt) to give 1.85 g (59 % yield) of benzyl 6-*O*-tosyl-α-D-mannopyranoside. This intermediate was dissolved in 10 mL of anhydrous THF and was dropped in a flask containing 40 mL of a 1 M solution of $LiAlH_4$ in THF. The reaction was then refluxed under nitrogen for 1 h (TLC, AcOEt, MeOH, H_2O 9:0.5:0.5). The solution was then poured on 200 mL of ice, acidified with 2 M HCl and extracted with AcOEt. The residue was cristallized from diisopropyl ether to give 450 mg (40 % yield) of pure benzyl α-D-rhamnopyranoside (**(D)-9**) as white crystals. M.p. (iPr$_2$O): 74-75 °C. [$\alpha]_D$ + 85.2 (c 0.5, MeOH). ^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.66 and 4.43 (d, each 1H, J = 12 Hz, CH_2Ar); 4.80 (br s, 1H, H-1); 3.91 (br s, 1H, H-2); 3.75 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.7 Hz, H-3); 3.66 (1H, m, H-5); 3.42 (t, 1H, J = 8.8 Hz, H-4); 1.25 (d, 3H, J = 6.5 Hz, CH_3-6). ^{13}C-NMR, δ = 137.2; 128.5; 128.4; 128.0; 127.9; 99.1; 73.0; 71.8; 71.1; 69.2; 68.3; 17.5. IR ν_{max} 3021, 2886, 1211, 1063. Anal. Calcd. for $C_{13}H_{18}O_5$; C, 61.42; H, 7.09. Found C, 61.49; H, 7.27.

Benzyl α-L-Rhamnopyranoside (**(L)-9**). L-Rhamnose (3 g) was heated at 60 °C for 7 h in 30 mL of benzyl alcohol presaturated with gaseous HCl. The solvent was evaporated and the residue purified by flash

chromatography (eluent AcOEt, MeOH, H_2O 95:3:1) and crystallized from iPr_2O to give 2.74 g (59 % yield) of benzyl α-L-rhamnopyranoside (**(L)-9**) as white crystals. M.p. (iPr_2O): 75-76 °C. $[\alpha]_D$ - 87.0 (c 0.5, MeOH). Anal. Calcd. for $C_{13}H_{18}O_5$; C, 61.42; H, 7.09. Found C, 61.53; H, 7.25.

Benzyl 6-Deoxy-β-D-Glucopyranoside (**(D)-5**) *and Benzyl 6-Deoxy-β-L-Glucopyranoside* (**(L)-5**). Compound **(D)-5** was prepared from benzyl β-D-glucopyranoside (**(D)-3**, 1.3 g) following the two-steps protocol used for obtaining **(D)-9** and isolated in 47 % yield (580 mg). White crystals, m.p. (iPr_2O): 104-106 °C. $[\alpha]_D$ - 69.0 (c 0.5, MeOH). ^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.92 and 4.58 (d, each 1H, J = 12.3 Hz, CH_2Ar); 4.35 (d, 1H, J = 7 Hz, H-1); 3.50-3.33 (m, 3H, H-2, H-3 and H-5); 3.25 (t, 1H, J = 8.8 Hz, H-4); 1.38 (d, 3H, J = 6.5 Hz, CH_3-6). ^{13}C-NMR, δ = 137.6; 129.1; 128.8; 128.5; 102.0; 76.9; 75.9; 74.4; 72.4; 71.6; 18.6. IR ν_{max} 3401, 3021, 2886, 1214, 1065. Anal. Calcd. for $C_{13}H_{18}O_5$; C, 61.42; H, 7.09. Found C, 61.50; H, 7.26.

Compound **(L)-5** was similarly prepared from benzyl β-L-glucopyranoside (**(L)-3**) in comparable yields. White crystals, m.p. (iPr_2O): 105-107 °C. $[\alpha]_D$ + 69.8 (c 0.5, MeOH). Anal. Calcd. for $C_{13}H_{18}O_5$; C, 61.42; H, 7.09. Found C, 61.55; H, 7.22.

Benzyl 6-Deoxy-α-D-Glucopyranoside (**(D)-6**) *and Benzyl 6-Deoxy-α-L-Glucopyranoside* (**(L)-6**). The same two-steps protocol (tosylation and reduction with $LiAlH_4$) was used to prepare these two compounds from the corresponding benzyl α-glucopyranosides. Benzyl 6-deoxy-α-D-glucopyranoside (**(D)-6**) was isolated in 42 % yield. Oil. $[\alpha]_D$ + 141.0 (c 0.5, MeOH); ^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.63 and 4.44 (d, each 1H, J = 12.5 Hz, CH_2Ar); 4.78 (d, 1H, J = 4 Hz, H-1); 3.67 (t, 1H, J = 9 Hz, H-3); 3.59 (dt, 1 H, J_1 = 9 Hz, J_2 = 6 Hz, H-5); 3.41 (dd, 1H, J_1 = 9 Hz, J_2 = 4 Hz, H-2); 3.04 (t, 1H, J = 9 Hz, H-4); 1.18 (d, 3H, J = 6 Hz, CH_3-6). ^{13}C-NMR, δ = 137.6; 129.1; 128.7; 128.5; 98.1; 76.1; 75.0; 72.9; 70.2; 68.2; 18.1. IR ν_{max} 3021, 2880, 1211, 1070. Anal. Calcd. for $C_{13}H_{18}O_5$; C, 61.42; H, 7.09. Found C, 61.55; H, 7.24.

Benzyl 6-deoxy-α-L-glucopyranoside (**(L)-6**) was isolated in 48 % yield. Oil. $[\alpha]_D$ - 140.6 (c 0.5, MeOH). Anal. Calcd. for $C_{13}H_{18}O_5$; C, 61.42; H, 7.09. Found C, 61.53; H, 7.26.

Naphthyl 6-O-Acetyl-β-D-Glucopyranoside (**(D)-10**) *and Naphthyl 6-O-Acetyl-β-L-Glucopyranoside* (**(L)-10**). These compounds were prepared according to the procedure suggested by Mottadelli *et al.*[16] a) *Tributyltin 2-naphthoxide.* Under nitrogen, Bu_3SnOMe (2 mL, 7 mmol) was added dropwise to a solution of β-naphthol (925 mg, 6.4 mmol) in 1,2-dichloroethane (20 mL). The mixture was stirred at room temperature for 2 h, untill the starting materials were no longer detectable (GLC analysis). The solvent was then evaporated under reduced pressure. b) Under nitrogen, $SnCl_4$ (1 mL, 8.55 mmol) was added to a solution of D-glucose pentaacetate (2.5 g, 6.4 mmol) in 1,2-dichloroethane (20 mL) containing 3 Å molecular sieves. The solution was stirred for 15 min, then the tributyltin 2-naphthoxide was added and the reaction was stirred at r.t. overnight. Satured aq. $NaHCO_3$ was then added. The insoluble materials were filtered off and washed twice with CH_2Cl_2. Organic materials were combined and washed with 10 % NaF, dried (Na_2SO_4) and concentrated. The residue was chromatographed (eluent hexane, AcOEt 7 :3) to give 2-naphthyl 2,3,4,6-*O*-tetraacetyl-β-D-glucopyranoside

(1.89 g, 60 % yield). This compound was then deacetylated with MeONa/MeOH to give 2-naphthyl β-D-glucopyranoside and regioselectively acetylated (2-naphthyl β-D-glucopyranoside, 740 mg; vinyl acetate, 0.9 mL; acetone, 45 mL; *Chromobacterium viscosum* lipase on celite, 1.6 g; 45 °C, 4 h) to give, after chromatographic purification (eluent $CHCl_3$, MeOH 9.5:0.5), 376 mg (56 % yield) of 2-naphthyl 6-*O*-acetyl-β-D-glucopyranoside (**(D)-10**). White crystals, m.p. 173-174 °C (iPr_2O), $[\alpha]_D$ -105.8 (c 0.5, MeOH). ^{1}H-NMR (DMSO-d_6, 300 MHz), δ = naphthyl protons : 9.10 (d, 1H, J = 8.7 Hz), 9.02 (dd, 2H, J_1= 14 Hz, J_2= 8.7 Hz), 8.60 (dt, 2H, J_1= 14 Hz, J_2= 8.7 Hz), 8.55 (d, 1H, J = 3.5 Hz), 8.40 (dd, 2H, J_1= 8.7 Hz, J_2= 3.5 Hz); 5.58 (d, 1H, J = 7.5 Hz, H-1), 4.83 (br d, 1H, J = 12 Hz, H-6a); 4.52 (dd, 1H, , J_1= 12 Hz, J_2= 6 Hz, H-6b); 3.92 (t, 1H, J = 7.5 Hz); 3.65-3.45 (m, 3H); 1.90 (s, 3H, CH_3CO). ^{13}C-NMR, δ = 172.0; 155.4; 134.8; 130.6; 130.1; 128.3; 127.7; 127.1; 125.1; 119.5; 112.0; 101.9; 76.7; 74.2; 73.9; 71.5; 70.7; 64.1; 21.4. IR ν_{max} 3388, 3015, 2931, 1738, 1597, 1256, 1110, 1061. Anal. Calcd. for $C_{18}H_{20}O_7$: C, 68.57; H, 4.76. Found C, 68.71; H, 4.89.

Naphthyl 6-*O*-acetyl-β-L-glucopyranoside (**(L)-10**) was prepared similarly in 20% yield. M.p. 174-175°C, $[\alpha]_D$ + 106.2 (c 0.5, MeOH). Anal. Calcd. for $C_{18}H_{20}O_7$: C, 68.57; H, 4.76. Found C, 68.69; H, 4.90.

Naphthyl α-L-Rhamnopyranoside (**(L)-11**). Naphthyl α-L-rhamnopyranoside was prepared following the previously described procedure.[16] *a) Tributyltin-2-naphthoxide.* Under nitrogen Bu_3SnOMe (4.2 mL, 14.75 mmol) was added dropwise to a solution of β-naphthol (1.95 g, 13.5 mmol) in 1,2-dichloroethane (40 mL). The mixture was stirred at room temperature for 2 h, until the starting materials were no longer detectable (GLC analysis). The solvent was then evaporated under reduced pressure. *b)* Under nitrogen $SnCl_4$ (2 mL, 17.1 mmol) was added to a solution of L-rhamnose tetraacetate (4.482g, 13.5 mmol), obtained by acetylation of 2g of L-rhamnose with Ac_2O/py, in 1,2-dichloroethane (40 mL) containing 3 Å molecular sieves. The solution was stirred for 15 min, then the tributyltin-2-naphthoxide was added and the reaction was stirred at r.t. overnight. Satured aq. $NaHCO_3$ was then added. The insoluble materials were filtered off and washed twice with CH_2Cl_2. Organic materials were combined and washed with 10% NaF, dried (Na_2SO_4) and concentrated. The residue was chromatographed (eluent hexane, AcOEt 7:3) to give 2-naphthyl 2,3,4-triacetyl-α-L-rhamnopyranoside (388 mg, 7% yield). This compound was then deacetylated with MeONa/MeOH to give 2-naphthyl α-L-rhamnopyranoside (**(L)-11**) Oil. $[\alpha]_D$ - 82.6 (c 0.5, MeOH). ^{1}H-NMR, δ = naphtyl protons : 7.80 (d, 1H, J = 8.7 Hz), 7.75 (dd, 2H, J_1= 14 Hz, J_2= 8.7 Hz), 7.47 (d, 1H, J = 3.5 Hz), 7.30 (dt, 2H, J_1= 14 Hz, J_2= 8.7 Hz), 7.13 (dd, 2H, J_1= 8.7 Hz, J_2= 3.5 Hz); 5.56 (br s, 1H, J = 7.5 Hz, H-1), 4.09 (br s, 1H, H-2); 3.92 (dd, 1H, , J_1= 9 Hz, J_2= 3 Hz, H-3); 3.70 (dq, 1H, J_1= 9 Hz, J_2= 6.5 Hz, H-5); 3.47 (t, 1H, J = 9 Hz, H-4); 1.21 (d, 3H, CH_3-6). ^{13}C-NMR, δ = 153.9; 134.2; 129.5; 129.2; 127.3; 126.9; 126.1; 123.9; 118.4; 110.4; 98.1; 72.6; 71.1; 70.4; 68.9; 17.1. IR ν_{max} 3388, 3015, 2931, 1597, 1256, 1108, 1063. Anal. Calcd. for $C_{16}H_{18}O_5$: C, 66.21, H, 6.21. Found C, 66.30; H, 6.35.

GLC analysis. In details, the experimental procedures were as follows. a) *Derivatization.* 20 μL of the reaction mixture were evaporated with N_2 in a mini tube. The residue was redissolved with 50 μL of pyridine and

derivatized by adding 50 μL of hexamethyldisylazane and 5 μL of trifluoroacetic acid. After 1 minute the solution was diluted 5 folds with DMF, and 5 μL were injected into the GC. During the gas chromatographic run, oven temperature was between 230 and 250 °C (H_2 was used as a carrier gas). b) *Kinetics*. In all the experiments 30 mg of lyophilized enzyme were added to a vial containing the sugar dissolved in an appropriate mixture of acetone and trifluoroethyl butanoate (TFEB), total volume, 1 mL). The suspension was shaken at 45°C and, periodically, a sample was taken and analyzed as previously described.

Regioselective Acylation of D- and L-Glycopyranosides Catalyzed by Subtilisin. General procedure: subtilisin (300 mg) was added to a mixture of acetone (8 mL) and TFEB (5 mL, 33.5 mmol) containing 0.6 mmol of glycopyranoside. The suspension was shaken at 45 °C for 24-96 h. The enzyme was filtered and the solvent evaporated. The crude residue was purified by flash chromatography (using a mixture of $CHCl_3$, MeOH in an appropriate ratio) to give the pure monobutanoyl derivative. The conditions used for any specific substrate (reaction time, degree of conversion determined by GLC analysis, $CHCl_3$, MeOH ratio) are reported in the following, together with the isolated yields and the physico-chemical and spectroscopic data of the products.

Benzyl 3-O-Butanoyl-6-O-Acetyl-β-D-Glucopyranoside (**(D)-4a**) and *Benzyl 2-O-Butanoyl-6-O-Acetyl-β-L-Glucopyranoside* (**(L)-4b**). The preparation of these compounds has been described elsewhere.[9]

Benzyl 3-O-Butanoyl-6-O-Acetyl-α-D-Glucopyranoside (**(D)-7a**). React. time, 48 h; conversion, 90 %; eluent, $CHCl_3$, MeOH 30:1; isol. yield, 144 mg (63 %). Oil. ^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.68 and 4.51 (d, each 1H, J = 12 Hz, CH_2Ar); 5.10 (t, 1H, J = 9.5 Hz, H-3); 4.90 (d, 1H, J = 3.5 Hz, H-1); 4.42 (dd, 1H, J_1 = 12.8 Hz, J_2 = 1.7 Hz, H-6a); 4.17 (dd, 1H, J_1 = 12.8 Hz, J_2 = 5.0 Hz, H-6b); 3.82 (m, 1H, H-5); 3.59 (dd, 1H, J_1 = 9.5 Hz, J_2 = 3.5 Hz, H-2); 3.48 (t, 1H, J = 9.5 Hz, H-4); 2.47 (t, 2H, J = 7 Hz, CH_2CO); 1.68 (m, 2H, CH_2); 0.92 (s, 3H, CH_3); 2.10 (s, 3H, CH_3CO). ^{13}C-NMR, δ = 137.6; 129.1; 128.9; 128.7; 98.3; 74.9; 72.7; 70.6; 70.5; 63.9; 21.5. IR ν_{max} 3021, 2879, 1733, 1366, 1243, 1211, 1082. Anal. Calcd. for $C_{19}H_{26}O_8$: C, 59.69; H, 6.81. Found C, 59.81; H, 6.95.

Benzyl 2-O-Butanoyl-6-O-Acetyl-α-L-Glucopyranoside (**(L)-7b**). React. time, 96 h; conversion, 76 %; eluent, $CHCl_3$, MeOH 50:1; isol. yield, 151 mg (66 %). Oil. $[\alpha]_D$ -96.4 (c 0.5, MeOH).^{1}H-NMR ($CDCl_3$, 300 MHz), δ = 7.5-7.3 (s, 5H, Ar); 4.68 and 4.50 (d, each 1H, J = 12 Hz, CH_2Ar); 5.08 (d, 1H, J = 3.5 Hz, H-1); 4.68 (dd, 1H, J_1 = 12.8 Hz, J_2 = 1.7 Hz, H-6a); 4.42 (m, 2H, H-2 and H-6b); 4.01 (t, 1H, J = 9.0 Hz, H-3); 3.80 (m, 1H, H-5); 3.42 (t, 1H, J = 9.0 Hz, H-4); 2.32(t, 2H, J = 7 Hz, CH_2CO);1.61 (m, 2H, CH_2);0.96 (s, 3H, CH_3);2.10 (s, 3H, CH_3CO). ^{13}C-NMR ($CDCl_3$, 62.5 MHz), 137.5; 129.0; 128.9; 128.5; 96.1; 73.6; 71.9; 71.1; 70.3; 63.7; 36.5; 21.4; 18.9; 14.1. IR ν_{max} 3021, 2879, 1733, 1366, 1243, 1211, 1082. Anal. Calcd. for $C_{19}H_{26}O_8$: C, 59.69; H, 6.81. Found C, 59.85; H, 6.98.

Benzyl 3-O-Butanoyl-6-O-Acetyl-α-D-Mannopyranoside (**(D)-8a**). React. time, 48 h; conversion, 80 %; eluent, $CHCl_3$-MeOH 30:1; isol. yield, 117 mg (51 %). Oil. $[\alpha]_D$ + 19.3 (c 0.5, MeOH).^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.76 and 4.55 (d, each 1H, J = 12 Hz, CH_2Ar); 5.06 (dd, 1H, J_1 = 10 Hz, J_2 = 3 Hz, H-3); 4.85 (br s, 1H,

H-1); 4.41 (dd, 1H, J_1 = 12.5 Hz, J_2 = 2 Hz, H-6a); 4.26 (dd, 1H, J_1 = 12.5 Hz, J_2 = 5.5 Hz, H-6b); 4.05 (m, 1H, H-2); 3.90 (2H, m, H-4 and H-5); 2.32 (t, 2H, J = 7.5 Hz, CH_2CO), 1.62 (m, 2H, CH_2), 0.93 (t, 3H, J = 7.5 Hz); 2.05 (s, 3H, CH_3CO). ^{13}C-NMR, δ = 137.7; 129.3; 128.9; 128.6; 100.3; 75.3; 70.0; 69.7; 68.4; 64.5; 36.6; 20.9; 18.9; 13.9. IR ν_{max} 3018, 2880, 1736, 1368, 1224, 1080. Anal. Calcd. for $C_{19}H_{26}O_8$: C, 59.69; H, 6.81. Found C, 59.78; H, 6.70.

Benzyl 2-O-Butanoyl-6-O-Acetyl-α-L-Mannopyranoside **((L)-8b)** and *Benzyl 3-O-Butanoyl-6-O-Acetyl-α-L-Mannopyranoside* **((L)-8a)**. React. time, 96 h; conversion, 60 %; eluent, $CHCl_3$-MeOH 30:1.

(L)-8a, 41 mg (18 %). Oil. $[\alpha]_D$ -19.8 (c 0.5, MeOH). Anal. Calcd. for $C_{19}H_{26}O_8$: C, 59.69; H, 6.81. Found C, 59.83; H, 6.75.

Isol. yield, **(L)-8b**, 55 mg (34 %). Oil. $[\alpha]_D$ -36.6 (c 0.5, MeOH).^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.69 and 4.51 (d, each 1H, J = 12 Hz, CH_2Ar); 5.14 (1H, br d, J = 3 Hz, H-2); 4.91 (br s, 1H, H-1); 4.49 (dd, 1H, J_1 = 12.5 Hz, J_2 = 5.5 Hz, H-6a); 4.21 (dd, 1H, J_1 = 12.5 Hz, J_2 = 2 Hz, H-6b); 4.06 (dd, 1H, J_1 = 10 Hz, J_2 = 3 Hz, H-3); 3.78 (1H, ddd, J_1= 10 Hz, J_2= 5.5 Hz, J_3= 2 Hz, H-5); 3.63 (t, 1H, J = 10 Hz, H-4); 2.34 (t, 2H, J = 7 Hz, CH_2CO), 1.65 (m, 2H, J = 7 Hz, CH_2), 0.95 (t, 3H, J = 7 Hz, CH_3); 2.15 (s, 3H, CH_3CO). ^{13}C-NMR, δ = 137.5; 129.1; 128.9; 128.6; 99.7; 72.0; 71.1; 69.9; 68.1; 64.1; 21.5. IR ν_{max} 3018, 2880, 1736, 1368, 1224, 1080. Anal. Calcd. for $C_{19}H_{26}O_8$: C, 59.69; H, 6.81. Found C, 59.80; H, 6.69.

Benzyl 3-O-Butanoyl-α-D-Rhamnopyranoside **((D)-9a)** React. time, 48 h; conversion, 80 %; eluent, $CHCl_3$, MeOH 50:1; isol. yield, 101 mg (44 %). Oil. ^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.72 and 4.52 (d, each 1H, J = 12 Hz, CH_2Ar); 5.08 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.7 Hz, H-3); 4.84 (br s, 1H, H-1); 4.02 (d, 1H, J = 1.7 Hz, H-2); 3.77 (1H, m, H-5); 3.61 (t, 1H, J = 8.8 Hz, H-4); 1.25 (d, 3H, J = 6.8 Hz, CH_3-6); 2.35 (t, 2H, J = 7 Hz, CH_2CO), 1.51 (m, 2H, J = 7 Hz, CH_2), 0.90 (t, 3H, J = 7 Hz, CH_3). IR ν_{max} 3024, 2878, 1739, 1219, 1076. Anal. Calcd. for $C_{17}H_{24}O_6$: C, 62.96; H, 7.41. Found C, 63.10; H, 7.23.

Benzyl 3-O-Butanoyl-6-Deoxy-β-D-Glucopyranoside **((D)-5a)**. React. time, 20 h; conversion, 98 %; eluent, $CHCl_3$, MeOH 50:1; isol. yield, 135 mg (73 %). Oil. $[\alpha]_D$ -38.4 (c 0.5, MeOH). ^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.90 and 4.62 (d, each 1H, J = 12.3 Hz, CH_2Ar); 4.82 (t, 1H, J = 8.5 Hz, H-3); 4.38 (d, 1H, J = 7.5 Hz, H-1); 3.53 (dd, 1H, J_1= 8.5 Hz, J_2= 7.5 Hz, H-2); 3.45 (dq, 1H, J_1= 8.5 Hz, J_2= 7 Hz, H-5); 3.28 (t, 1H, J = 8.5 Hz, H-4); 1.38 (d, 3H, J = 7.5 Hz, CH_3-6); 2.40 (t, 2H, J = 7.5 Hz, CH_2CO), 1.70 (m, 2H, J = 7.5 Hz, CH_2), 0.96 (t, 3H, J = 7.5 Hz, CH_3). ^{13}C-NMR, δ = 137.5; 129.0; 128.8; 128.6; 102.0; 78.8; 75.2; 72.9; 71.6; 36.6; 18.9; 18.0; 14.0. IR ν_{max} 3382, 3021, 2880, 1740, 1221, 1076. Anal. Calcd. for $C_{17}H_{24}O_6$: C, 62.96; H, 7.41. Found C, 63.12; H, 7.48.

Benzyl 2-O-Butanoyl-6-Deoxy-β-L-Glucopyranoside **((L)-5b)**. React. time, 20 h; conversion, 89 %; eluent, $CHCl_3$, MeOH 50:1; isol. yield, 153 mg (83 %). Oil. $[\alpha]_D$ + 55.4 (c 0.5, MeOH). ^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.85 and 4.57 (d, each 1H, J = 12.3 Hz, CH_2Ar); 4.80 (dd, 1H, J_1= 8.5 Hz, J_2= 7.5 Hz, H-2); 4.45 (d, 1H, J = 7.5 Hz, H-1); 3.48 (t, 1H, J = 8.5 Hz, H-3); 3.45 (dq, 1H, J_1= 8.5 Hz, J_2= 7 Hz, H-5); 3.25 (t, 1H, J = 8.5 Hz,

H-4); 1.45 (d, 3H, J = 7 Hz, CH_3-6); 2.30 (t, 2H, J = 7.5 Hz, CH_2CO), 1.61 (m, 2H, J = 7.5 Hz, CH_2), 0.95 (t, 3H, J = 7.5 Hz, CH_3). ^{13}C-NMR, δ = 137.4; 128.9; 128.3; 100.0; 76.6; 76.2; 74.8; 72.4; 71.1; 36.8; 18.9; 18.1; 14.2. IR ν_{max} 3382, 3021, 2880, 1740, 1221, 1076. Anal. Calcd. for $C_{17}H_{24}O_6$: C, 62.96; H, 7.41. Found C, 63.06; H, 7.53.

Benzyl 3-O-Butanoyl-6-Deoxy-α-D-Glucopyranoside (**(D)-6a**). React. time, 48 h; conversion, 87 %; eluent, $CHCl_3$-MeOH 50:1; isol. yield, 148 mg (80 %). Oil. $[\alpha]_D$ + 113.6 (c 0.5, MeOH). ^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.74 and 4.54 (d, each 1H, J = 12.5 Hz, CH_2Ar); 5.02 (t, 1H, J = 9 Hz, H-3); 4.92 (d, 1H, J = 4 Hz, H-1); 3.74 (dq, 1 H, J_1 = 9 Hz, J_2 = 7 Hz, H-5); 3.60 (dd, 1H, J_1 = 9 Hz, J_2 = 4 Hz, H-2); 3.22 (t, 1H, J = 9 Hz, H-4); 1.30 (d, 3H, J = 7 Hz, CH_3-6); 2.39 (t, 2H, J = 7 Hz, CH_2CO), 1.68 (m, 2H, J = 7Hz, CH_2), 0.95 (t, 3H, J = 7 Hz, CH_3). ^{13}C-NMR, δ = 137.6;129.1; 128.9; 128.6; 98.1; 77.4; 75.1; 71.6; 70.3; 68.6; 36.9; 19.0; 18.0; 14.1. IR ν_{max} 3021, 2880, 1738, 1221, 1076. Anal. Calcd. for $C_{17}H_{24}O_6$: C, 62.96; H, 7.41. Found C, 63.03; H, 7.49.

Benzyl 2-O-Butanoyl-6-Deoxy-α-L-Glucopyranoside (**(L)-6b**) *and Benzyl 3-O-Butanoyl-6-Deoxy-α-L-Glucopyranoside* (**(L)-6a**). React. time, 48 h; conversion, 80 %; eluent, $CHCl_3$, MeOH 50:1.

Isol. yield, **(L)-6a**, 78 mg (42 %). Oil. $[\alpha]_D$ -111.2 (c 0.5, MeOH). Anal. Calcd. for $C_{17}H_{24}O_6$: C, 62.96; H, 7.41. Found C, 63.11; H, 7.52.

(L)-6b, 28 mg (15 %). Oil. $[\alpha]_D$ -113.6 (c = 0.5, MeOH). ^{1}H-NMR, δ = 7.5-7.3 (m, 5H, Ar); 4.74 and 4.54 (d, each 1H, J = 12.5 Hz, CH_2Ar); 5.02 (t, 1H, J = 9 Hz, H-3); 4.92 (d, 1H, J = 4 Hz, H-1); 3.74 (dq, 1 H, J_1 = 9 Hz, J_2 = 7 Hz, H-5); 3.60 (dd, 1H, J_1 = 9 Hz, J_2 = 4 Hz, H-2); 3.22 (t, 1H, J = 9 Hz, H-4); 1.30 (d, 3H, J = 7 Hz, CH_3-6); 2.39 (t, 2H, J = 7 Hz, CH_2CO), 1.68 (m, 2H, J = 7 Hz, CH_2), 0.95 (t, 3H, J = 7 Hz, CH_3). ^{13}C-NMR, δ = 137.6; 129.1; 128.8; 128.6; 98.1; 77.4; 75.1; 71.6; 70.3; 68.6; 36.9; 19.0; 18.0; 14.1. IR ν_{max} 3019, 2883, 1735, 1217, 1067. Anal. Calcd. for $C_{17}H_{24}O_6$: C, 62.96; H, 7.41. Found C, 63.15; H, 7.42.

Naphthyl 3-O-Butanoyl-6-O-Acetyl-β-D-Glucopyranoside (**(D)-10a**). React. time, 36 h; conversion, 90 %; eluent, $CHCl_3$, MeOH 50:1; isol. yield, 203 mg (67 %). Oil. $[\alpha]_D$ -48.2 (c 0.5, MeOH). ^{1}H-NMR, δ = naphthyl protons: 7.70 (m, 3H), 7.42 (t, 1H, J= 10 Hz), 7.34 (m, 2H), 7.20 (dd, 2H, J_1= 9.0 Hz, J_2= 3 Hz); sugar protons: 5.05 (t, 1H, J = 8.5 Hz, H-3), 5.03 (d, 1H, J = 8.5 Hz, H-1), 4.40 (d, 1H, J_1 = 12 Hz, J_2= 3 Hz, H-6a), 4.32 (dd, 1H, J_1= 12 Hz, J_2= 6 Hz, H-6b), 3.79 (t, 1H, J = 8.4 Hz, H-2), 3.68 (m, 1H, H-5), 3.58 (t, 1H, J = 9 Hz, H-4); acyl moieties : 2.37 (t, 2H, J = 7 Hz, CH_2CO), 2.02 (s, 3H, CH_3CO), 1.65 (m, 2H, J = 7 Hz, CH_2), 0.92 (t, 3H, J = 7 Hz, CH_3). ^{13}C-NMR, δ = 175.7; 172.0; 155.4; 134.8; 130.6; 130.1; 128.3; 127.7; 127.1; 125.1; 119.5; 112.0; 101.6; 78.0; 74.8; 72.6; 69.8; 64.0; 36.8; 21.4; 19.0; 14.1. IR ν_{max} 3015, 2931, 1738, 1597, 1256, 1110, 1061. Anal. Calcd. for $C_{22}H_{26}O_8$: C, 63.16; H, 6.22. Found C, 63.27; H, 6.16.

Naphthyl 2-O-Butanoyl-6-O-Acetyl-β-L-Glucopyranoside (**(L)-10b**) and *Naphthyl 3-O-Butanoyl-6-O-Acetyl-β-L-Glucopyranoside* (**(L)-10a**). React. time, 48 h; conversion, 78 %; eluent, $CHCl_3$-MeOH 50:1.

Isol. yield, **(L)-10a**, 36 mg (12 %). Oil. $[\alpha]_D$ + 59.2 (c 0.5, MeOH). Anal. Calcd. for $C_{22}H_{26}O_8$: C, 63.16; H, 6.22. Found C, 63.30; H, 6.10.

(L)-10b, 140 mg (46 %). Oil. $[\alpha]_D$ + 65.2 (c 0.5, MeOH). ^{1}H-NMR, δ = naphthyl protons: 7.70 (m, 3H), 7.42 (t, 1H, J= 10 Hz), 7.34 (m, 2H), 7.20 (dd, 2H, J_1= 9.0 Hz, J_2= 3 Hz); sugar protons: 5.05 (t, 1H, J = 8.5 Hz, H-3), 5.03 (d, 1H, J = 8.5 Hz, H-1), 4.40 (d, 1H, J_1 = 12 Hz, J_2= 3 Hz, H-6a), 4.32 (dd, 1H, J_1= 12 Hz, J_2= 6 Hz, H-6b), 3.79 (t, 1H, J = 8.4 Hz, H-2), 3.68 (m, 1H, H-5), 3.58 (t, 1H, J = 9 Hz, H-4); acyl moieties : 2.37 (t, 2H, J = 7 Hz, CH_2CO), 2.02 (s, 3H, CH_3CO), 1.65 (m, 2H, J = 7 Hz, CH_2), 0.92 (t, 3H, J = 7 Hz, CH_3). ^{13}C-NMR, δ = 175.7; 172.0; 155.4; 134.8; 130.6; 130.1; 128.3; 127.7; 127.1; 125.1; 119.5; 112.0; 101.6; 78.0; 74.8; 72.6; 69.8; 64.0; 36.8; 21.4; 19.0; 14.1. IR ν_{max} 3015, 2931, 1738, 1597, 1256, 1110, 1061. Anal. Calcd. for $C_{22}H_{26}O_8$: C, 63.16; H, 6.22. Found C, 63.06; H, 6.29.

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