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Note

Regioselective acylation of disaccharides by enzymatic transesterification

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Regioselective acylation of hydroxyl groups in carbohydrates is a basic challenge for organic chemists. In fact, even discrimination between primary and secondary hydroxyls often involves multistep procedures, while there is no general basis for the regiospecific acylation of secondary hydroxyls [1].

In recent years, the use of hydrolases (lipases and proteases) in aqueous or organic media has been growing continuously [2]. When applied to carbohydrates, these enzymes showed remarkable regioselectivity of action, both in hydrolysis and transesterification, often allowing the isolation of monoacyl and diacyl derivatives in good yields [3–7]. Studies have been mainly conducted on monosaccharides, while only few examples have been reported on disaccharides. In a pioneering investigation [8], Klibanov et al. showed that subtilisin is able to catalyze the acylation of the disaccharides sucrose, maltose, cellobiose, and lactose dissolved in *N*,*N*-dimethylformamide. Esterification was regioselective and, in the case of the last three sugars, acylation of the primary hydroxyl group of the disaccharide non-reducing moiety occurred in good yield. More recently, some examples of acetylation and deacetylation of simple derivatives of these sugars have been reported in the literature [4,5,7].

Because of the importance of disaccharides as building blocks for the preparation of more complex oligosaccharides, we decided to study the enzymatic acylation of glycosides of common disaccharides (Scheme 1) more systematically.

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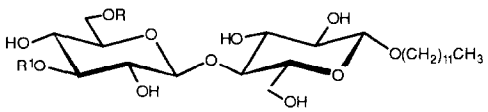
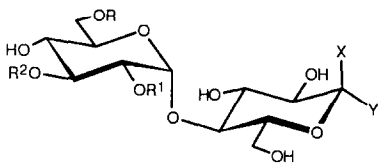


1 R = R^1 = H **1a** R = Ac; R^1 = H

1b R = R^1 = Ac



2 X = O(CH$_2$)$_{11}$CH$_3$; Y = R = R^1 = R^2 = H

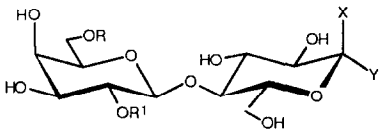
2a R = Ac; X = O(CH$_2$)$_{11}$CH$_3$; Y = R^1 = R^2 = H

2b R = R^1 = R^2 = Ac; X = O(CH$_2$)$_{11}$CH$_3$; Y = H

3 X = R = R^1 = R^2 = H; Y = O(CH$_2$)$_{11}$CH$_3$

3a R = Ac; X = R^1 = R^2 = H; Y = O(CH$_2$)$_{11}$CH$_3$

3b R = R^2 = Ac; X = R^1 = H; Y = O(CH$_2$)$_{11}$CH$_3$



4 X = O(CH$_2$)$_{11}$CH$_3$; Y = R = R^1 = H

4a R = Ac; X = O(CH$_2$)$_{11}$CH$_3$; Y = R^1 = H

5 X = R = R^1 = H; Y = O(CH$_2$)$_{11}$CH$_3$

5a R = Ac; X = R^1 = H; Y = O(CH$_2$)$_{11}$CH$_3$

5b R = R^1 = Ac, X = H; Y = O(CH$_2$)$_{11}$CH$_3$

6 X = R = R^1 = H; Y = OBn

6a R = Ac; X = R^1 = H; Y = OBn

6b R = ClCH$_2$CO; X = R^1 = H; Y = OBn

6c R = Pent; X = R^1 = H; Y = OBn

6d R = Lev; X = R^1 = H; Y = OBn

6e R = R^1 = Lev; X = H; Y = OBn

6f R = Pent; R^1 = Lev; X = H; Y = OBn

6g R = R^1 = Ac, X = H; Y = OBn

Pent = -COOCH$_2$CH$_2$CH$_2$CH=CH$_2$

Scheme 1.

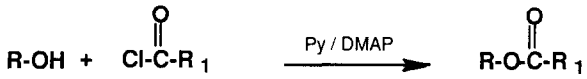
In a preliminary screening, the dodecyl glycosides of cellobiose, maltose, and lactose (**1–5**) were used as substrates due to their enhanced solubility in organic solvents as compared to the parent free sugars [9]. It is known that only the protease subtilisin has residual activity in such polar solvents as *N*,*N*-dimethylformamide, while all the lipases tested so far prefer less polar, water immiscible organic solvents. Therefore, as a compromise between solubility of the substrates and good residual activity of the enzymes, 2-methyl-2-butanol (*tert*-amyl alcohol) was the solvent of choice, while vinyl acetate was the acylating agent. Fifteen commercially available crude lipase preparations were tested and, from this preliminary screening, the lipases from *Pseudomonas cepacia* (lipase P) and *Candida antarctica* were selected as they gave better results. Subtilisin was also chosen because of the previous positive results obtained with this enzyme in the acylation of free disaccharides in *N*,*N*-dimethylformamide [8]. The results obtained are summarized in Table 1. *Candida antarctica* showed high selectivity, giving exclusively the 6′-*O*-acetyl derivatives **1a–5a** in good yields. Lipase P showed a similar behavior, but with an interesting exception. The acylation of the dodecyl β-lactoside gave a mixture of mono- and di-acetyl derivatives **5a** and **5b**, showing that lipase P was not only selective for the primary hydroxyl group of the disaccharide non-reducing end but also for the secondary 2′-OH position. On the other hand, quite surprisingly, subtilisin was a catalyst with poor selectivity and, with the exception of **3b**, complex mixtures of mono-, di-, and tri-acetates were always obtained (Table 1 shows the yield of the most abundant product).

Having in hand these preliminary results, we subsequently focused our attention on lactose. This sugar is a very cheap widespread compound that is present in many biologically important complex oligosaccharides. Therefore, our finding that lipase P

Table 1
Acetylation of some disaccharides using vinyl acetate as acyl donor

Substrate	Enzymes					
	Candida antarctica		*Pseudomonas cepacia*		Subtilisin	
	Reaction time	Product (amount, yield)	Reaction time	Product (amount, yield)	Reaction time	Product [a] (amount, yield)
Dodecyl β-cellobioside **1**	2 days	**1a** (81 mg, 75%)	2 days	**1a** (78 mg, 72%)	7 days	**1b** (44 mg, 41%)
Dodecyl α-maltoside **2**	1 day	**2a** (103 mg, 95%)	2 days	**2a** (96 mg, 89%)	7 days	**2b** (38 mg, 35%)
Dodecyl β-maltoside **3**	1 day	**3a** (104 mg, 96%)	2 days	**3a** (95 mg, 88%)	7 days	**3b** (77 mg, 71%)
Dodecyl α-lactoside **4**	3 days	**4a** (89 mg, 82%)	2 days	**4a** (96 mg, 89%)	7 days	**4a** (40 mg, 37%)
Dodecyl β-lactoside **5**	5 days	**5a** (80 mg, 74%)	2 days	**5a** (58 mg, 53%) **5b** (33 mg, 30%)	2 days	**5a** (39 mg, 36%)

[a] Isolated yield of the most abundant product.



7 R = CH_2CF_3; $R_1 = OCH_2Ph$ **11** R = CH_2CCl_3; $R_1 = OCH_2CH_2CH_2CH{=}CH_2$

8 R = CH_2CF_3; $R_1 = OCH_2CH{=}CH_2$ **12** R = CH_2CF_3; $R_1 = CH_2Cl$

9 R = CH_2CCl_3; $R_1 = OCH_2CH_2Si(CH_3)_3$ **13** R = CH_2CF_3; $R_1 = CH_2CH_2COCH_3$

10 R = CH_2CH_2Cl; $R_1 = OCH_2CH_2Cl$

Scheme 2.

selects one of its five secondary hydroxyls can be exploited for the preparation of lactose-containing oligosaccharides. In order to have a starting material that was both soluble in organic solvents and synthetically useful, the dodecyl aglycon was replaced with a benzyl group. A second screening was performed using the previously selected lipases and considering other more useful acylating agents in order to broaden the scope of this method by introducing different esters which can be selectively removed. As shown in Scheme 2, these activated esters and carbonates [10], apart from the commercially available vinyl acetate,were prepared from the corresponding acyl chlorides and the appropriate alcohol in the presence of 4-(dimethylamino)-pyridine [11], according to a general methodology. As an exception, trifluoroethyl levulinate was synthesized by esterification of levulinic anhydride (prepared in situ by reacting levulinic acid with *N,N'*-dicyclohexylcarbodiimide) with trifluoroethanol.

Our new activated carbonates (**7–11**) and esters (**12**, **13**), as well as vinyl acetate, were used following the previously described conditions. Besides vinyl acetate, compounds **11** and **12** proved to be efficient acyl donors in the *Candida antarctica* lipase-catalyzed esterification of the benzyl lactoside **6**, and the corresponding products **6a–c** were obtained in fair to good yields. Carbonates **7–10** showed only sluggish conversion or no reaction at all. Surprisingly, when the levulinate **13** was used, a mixture of mono- and di-substituted derivatives **6d** and **6e** was obtained. A similar result, with respect to the disubstituted derivative **6e** was obtained using lipase P. The results are summarized in Table 2.

The above information was exploited to selectively acylate the positions 6'-OH and 2'-OH of **6** with two different protecting groups. Accordingly, the previously obtained monoester **6c** was converted to the mixed diester **6f** in 65% yield by treatment with **12** and *Candida antarctica* lipase. It is noteworthy that, in this way, an easy selective protection of the 2'-OH was achieved, otherwise difficult by chemical methods. Compound **6f** is potentially interesting because, in principle, all the remaining hydroxyl groups can be selectively protected in a different way with chemical methods, making this sugar derivative a useful synthetic intermediate for the preparation of more complex oligosaccharides containing lactose.

1. Experimental

1.1. General methods.—Lipase P was obtained from Amano Pharmaceutical Corporation and adsorbed on Celite [12]. *Candida antartica* lipase, immobilized on macrop-

Table 2
Acylation of benzyl β-lactoside

Lipase	Substrate	Acylating Agent	Reaction Time	Product (amount, yield)
Candida antarctica	**6**	vinyl acetate	5 days	**6a** (83 mg, 75%)
Candida antarctica	**6**	**12**	1 day	**6b** (95 mg, 81%)
Candida antarctica	**6**	**11**	5 days	**6c** (92 mg, 73%)
Candida antarctica	**6**	**13**	2 days	**6d** (90 mg, 73%)
				6e (27 mg, 19%)
Candida antarctica	**6**	**7–10**	7 days	No reaction
Pseudomonas cepacia	**6**	vinyl acetate	4 days	**6g** (80 mg, 67%)
Pseudomonas cepacia	**6**	**12**	4 days	**6b** (36 mg, 30%)
Pseudomonas cepacia	**6**	**13**	2 days	**6d** (87 mg, 66%)
				6e (33 mg, 23%)
Pseudomonas cepacia	**6**	**11**	7 days	**6c** (80 mg, 64%)
Pseudomonas cepacia	**6**	**7–10**	7 days	no reaction
Candida antarctica	**6c**	**13**	3 days	**6f** (75 mg, 65%)

orus acrylic resin, was obtained from Novo-Nordisk A/S, Denmark. NMR spectra were recorded with a Bruker WP 80 or AC 300 spectrometer for solutions in $CDCl_3$ or CD_3OD. NMR spectroscopy data of all compounds were in agreement with the proposed structures. Assignment of the signals of proton resonances was obtained from COSY experiments. Thin-layer chromatography (TLC) was performed on Merck Silica Gel 60 F_{254} plates, and visualized spraying with a 1:1 mixture of 20% sulfuric acid and 10 g I_2–100 g KI in 500 mL of H_2O followed by heating. Preparative chromatography was performed by flash chromatography using Merck Silica Gel 60 (230–400 mesh) with the appropriate eluent. Dodecyl α- and β-maltosides were obtained from Sigma. Dodecyl α- and β-lactosides [13], dodecyl β-cellobioside [14] and benzyl β-lactoside [15] were prepared according to published procedures.

1.2. Trifluoroethyl levulinate (**13**).—To a solution of levulinic acid (40 g, 0.344 mol) in CH_2Cl_2 (200 mL), *N,N'*-dicyclohexylcarbodiimide (35.5 g, 0.172 mol) was added at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The mixture was filtered and the filtrate was added dropwise to a solution of trifluoroethanol (12 mL, 0.168 mol), triethylamine (24 mL, 0.173 mol), and 4-(*N,N*-dimethylamino)pyridine (100 mg, 0.82 mmol) in CH_2Cl_2 (70 mL) at −78°C. The reaction mixture was stirred for 1 h at this temperature, then it was allowed to warm to room temperature and stirred for further 2 h. The mixture was washed with water, 5% aq $NaHCO_3$, water, dried (Na_2SO_4), and the solvent evaporated under reduced pressure. The residue was distilled to give **13** (20.3 g, 61%); bp 96–97°C at 20 mm Hg; ^1H NMR (80 MHz, $CDCl_3$), δ 4.41 (q, 2 H, $J_{H,F}$ 9 Hz, CH_2CF_3), 2.9–2.5 (m, 4 H, 2 CH_2CO), 2.16 (s, 3 H, CH_3CO). Anal. Calcd for $C_7H_9F_3O_3$: C, 42.43; H, 4.58. Found: C, 42.12; H, 4.83.

1.3. General procedure for the acylation of disaccharide alkyl glycosides.—In a typical procedure, 100 mg of the starting compound (**1–6**) and 2 mL of activated ester in 10 mL of *tert*-amyl alcohol were shaken at 45 °C in the presence of 300 mg of

Table 3
Physical and ^{1}H NMR data for acylated disaccharides

Compound	Eluent	$[\alpha]_D$ (MeOH, deg)	Selected ^{1}H NMR data (δ, 300 MHz, CD_3OD unless stated otherwise)	Elemental analysis	
				Calcd for:	Found
1a	EtOAc–MeOH–H_2O 8:1:0.3	−9.6 (*c* 1.0)	4.46 (dd, *J* = 11.9, 1.9 Hz, H-6′a); 4.41 (d, *J* = 7.9 Hz, H-1′); 4.27 (d, *J* = 8.0 Hz, H-1); 4.10 (dd, *J* = 11.9, 6.8 Hz, H-6′b).	$C_{26}H_{48}O_{12}$ C, 56.51; H, 8.75	C, 56.32; H, 8.92
1b	EtOAc–MeOH 9:0.3	−8.7 (*c* 1.0)	4.95 (t, *J* = 9.6 Hz, H-3′; 4.52 (d, *J* = 7.9 Hz, H-1′); 4.45 (dd, *J* = 11.7, 1.7 Hz, H-6′a); 4.27 (d, *J* = 8.0 Hz, H-1); 4.17 (dd, *J* = 11.7, 6.7 Hz, H-6′b).	$C_{28}H_{50}O_{13}$ C, 56.55; H, 8.47	C, 56.21; H, 8.78
2a	EtOAc–MeOH–H_2O 8:1:0.3	+99 (*c* 1.0)	5.10 (d, *J* = 3.8 Hz, H-1′); 4.70 (d, *J* = 3.8 Hz, H-1); 4.37 (dd, *J* = 11.8, 1.8 Hz, H-6′a); 4.17 (dd, *J* = 11.8, 6.7 Hz, H-6′b).	$C_{26}H_{48}O_{12}$ C, 56.51; H, 8.75	C, 56.66; H, 8.61
2b	EtOAc–MeOH 9:0.3	+127 (*c* 1.0)	5.10 (d, *J* = 3.8 Hz, H-1′); 5.30 (t, *J* = 9.7 Hz, H-3′); 4.76 (d, *J* = 3.8 Hz, H-1); 4.74 (dd, *J* = 9.7, 3.8 Hz, H-2′); 4.40 (dd, *J* = 11.7, 1.5 Hz, H-6′a); 4.17 (dd, *J* = 11.7, 5.7 Hz, H-6′b).	$C_{30}H_{52}O_{14}$ C, 56.59; H, 8.23	C, 56.24; H, 8.51
3a	EtOAc–MeOH–H_2O 8:1:0.3	+36 (*c* 1.0)	5.10 (d, *J* = 3.8 Hz, H-1′); 4.38 (dd, *J* = 11.8, 1.8 Hz, H-6′a); 4.27 (d, *J* = 7.7 Hz, H-1); 4.15 (dd, *J* = 11.8, 6.7 Hz, H-6′b).	$C_{26}H_{48}O_{12}$ C, 56.51; H, 8.75	C, 56.78; H, 8.98
3b	EtOAc–MeOH 9:0.3	+39 (*c* 1.0)	5.19 (d, *J* = 3.7 Hz, H-1′); 5.13 (t, *J* = 9.5 Hz, H-3′); 4.38 (dd, *J* = 11.7, 1.8 Hz, H-6′a); 4.28 (d, *J* = 7.8 Hz, H-1); 4.18 (dd, *J* = 11.8, 6.3 Hz, H-6′b).	$C_{28}H_{50}O_{13}$ C, 56.55; H, 8.47	C, 56.27; H, 8.69
4a	EtOAc–MeOH–H_2O 8:1:0.3	+44 (*c* 1.0)	4.75 (d, *J* = 3.7 Hz, H-1); 4.34 (d, *J* = 7.1 Hz, H-1′); 4.31 (dd, *J* = 11.4, 8.0 Hz, H-6′a); 4.20 (dd, *J* = 11.4, 4.1 Hz, H-6′b).	$C_{26}H_{48}O_{12}$ C, 56.51; H, 8.75	C, 56.31; H, 9.03
5a	EtOAc–MeOH–H_2O 8:1:0.3	−4.6 (*c* 1.4)	4.35 (d, *J* = 7.6 Hz, H-1′); 4.33–4.19 (m, H-1, 2 H-6′).	$C_{26}H_{48}O_{12}$ C, 56.51; H, 8.75	C, 56.74; H, 8.87
5b	EtOAc–MeOH–H_2O 10:1:0.2	+7.5 (*c* 1.0)	5.05 (dd, *J* = 9.7, 8.3 Hz, H-2′); 4.56 (d, *J* = 8.3 Hz, H-1′); 4.31–4.22 (m, H-1 and 2 H-6′).	$C_{28}H_{50}O_{13}$ C, 56.55; H, 8.47	C, 56.19; H, 8.81

6a	EtOAc–MeOH 10:1.7	−13 (*c* 1.0)	4.41 (d, J = 7.9 Hz, H-1′); 4.37 (d, J = 7.2 Hz, H-1); 4.31 (dd, J = 11.4, 8.6 Hz, H-6′a); 4.20 (dd, J = 11.4, 4.1 Hz, H-6′b).	$C_{21}H_{30}O_{12}$ C, 53.16; H, 6.37	C, 53.44; H, 6.49
6b	EtOAc–MeOH 10:1.2	−8.4 (*c* 1.4)	4.35–4.24 (m, H-1, H-1′, 2 H-6′, $ClCH_2CO$).	$C_{21}H_{29}ClO_{12}$ C, 49.56; H, 5.74	C, 49.37; H, 5.93
6c	EtOAc–MeOH 10:0.8	−14 (*c* 1.4)	5.85–4.90 (m, $CH{=}CH_2$); 4.35–4.24 (m, H-1, H-1′, 2 H-6′).	$C_{25}H_{36}O_{13}$ C, 55.14; H, 6.66	C, 54.94; H, 6.89
6d	EtOAc–MeOH 10:0.9	−7.9 (*c* 1.0)	4.40 (d, J = 7.9 Hz, H-1′); 4.37 (d, J = 7.1 Hz, H-1); 4.29–4.26 (m, 2 H-6); 2.16 (s, CH_3CO).	$C_{24}H_{34}O_{13}$ C, 54.34; H, 6.46	C, 54.61; H, 6.21
6e	EtOAc MeOH 10:0.9	+7.4 (*c* 1.7)	5.03 (dd, J = 7.9, 10.1 Hz, H-2′); 4.58 (d, J = 7.9 Hz, H-1′); 4.39 (d, J = 7.8 Hz, H-1); 4.31–4.28 (m, 2 H-6); 2.17 (s, CH_3CO); 2.16 (s, CH_3CO).	$C_{29}H_{40}O_{15}$ C, 55.41; H, 6.41	C, 55.73; H, 6.69
6f	*n*-Hexane–EtOAc 0.5:10	+3.2 (*c* 0.7)	($CDCl_3$): 5.80–5.60 (m, CH=); 5.10–4.90 (m, $=CH_2$, H-2′); 4.52 (d, J = 7.9 Hz, H-1′); 4.50–4.30 (m, H-1, 2 H-6′); 2.18 (s, CH_3CO).	$C_{30}H_{42}O_{15}$ C, 56.07; H, 6.59	C, 56.26; H, 6.71
6g	EtOAc–MeOH 10:0.8	+2.7 (*c* 1.0)	5.06 (dd, J = 9.7, 8.5 Hz, H-2′); 4.57 (d, J = 7.9 Hz, H-1′); 4.38 (d, J = 7.6 Hz, H-1); 4.31 (dd, J = 10.2, 7.9 Hz, H-6′a); 4.25 (dd, J = 10.2, 4.4 Hz, H-6′b).	$C_{23}H_{32}O_{13}$ C, 53.49; H, 6.24	C, 53.25; H, 6.52

immobilized lipase [12] or subtilisin. The reactions were carried out until the starting material was almost completely transformed; otherwise they were stopped after 7 days. Filtration of the enzyme and evaporation of the solvent gave a crude mixture. Pure compounds were isolated by flash chromatography using the eluents reported in Table 3.

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