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Invited Review

Lipases as Useful Tools for the Stereo- and Regioselective Protection and Deprotection of Carbohydrates

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Summary. Stereo- and regioselective acylation and deacylation of carbohydrates can be achieved using lipases as biocatalysts. These enzymes are extremely versatile, quite stable in aqueous and organic solvents, easily available, and easy to handle. Recently, they have become the object of site directed mutagenesis which suggests that they soon could be 'built *ad hoc*' for specific necessities.

Keywords. Biocatalyst; Lipases; Protective groups; Carbohydrates.

Introduction

Among all synthetic transformations, the proper introduction and removal of protecting groups is one of the most widely adopted in synthetic organic chemistry. Chemo-, regio-, and stereoselection are daily beneath our eyes; all chemical reactions nature has realized have these amazing characteristics. It is therefore not surprising that many chemists make efforts to somehow try to lean towards nature's perfection, even if this target still represents a 'beautiful dream'.

Many classical chemical methods have been developed for the manipulation of protecting groups under mild conditions [1, 2]. Such methods are necessary, in particular, in the construction of complex, polyfunctional molecules, among which are oligosaccharides and their conjugates. Nevertheless, the synthesis of these and other complex polyfunctional molecules still presents severe problems which can be solved only with great difficulties and long multistep strategies. Attempts to exploit the same or similar tools as nature to perform comparable reactions have led to an extended use of biocatalysts which have substantially enriched the arsenal of available protecting group techniques, offering viable alternatives to classical methods [3, 4].

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More specifically, the protection/deprotection of carbohydrates, necessary for the synthesis of more complex saccharidic structures or other glycoconjugates, represent challenging goals. In fact, the numerous hydroxyl groups with 'similar' reactivity that characterize these compounds need to be manipulated selectively during the synthesis. Although numerous chemical techniques are available to mask and liberate hydroxyl groups [1, 2, 5, 6], the development and exploitation of enzymatic methods has been progressing and becoming a common tool for workers in this field.

Lipases as Biocatalysts

Many biocatalysts are used in the carbohydrate field, among them hydrolitic enzymes such as lipases, which are particularly suitable for the regioselective acylation and deacylation of hydroxyl groups.

Lipases hydrolyze fat in the digestive tract but are extremely flexible for the acylation or deacylation of a wide range of unnatural substrates. Unlike most other enzymes, they can accommodate a wide range of substrates, are quite stable in non-aqueous organic solvents, and can be used to either hydrolyze or esterify depending on the solvent system used.

A recent review [7] extensively treats these enzymes with respect to their occurrence, preparation, analysis, structure, and mechanism. Moreover, it deals with the application of these enzymes in oleochemistry as well as in detergents, paper, and food industries.

Ester hydrolysis

A freely dissolved lipase in absence of an aqueous/lipid interface resides in its inactive state, and part of the enzyme molecule covers the active site. When the enzyme contacts the interface of a biphasic water–oil system, a short α-helix, the lid, is folded back. Thus, opening its active site the lipase is rearranged into its active state. As a consequence, lipase-catalyzed hydrolyses should be performed in a biphasic medium. It is sufficient to employ a substrate alone at elevated concentrations, such that it constitutes the second organic phase, or, alternatively, it may be dissolved in a water-immiscible organic solvent such as hexane, a dialkyl ether, or an aromatic liquid. Since most substrates are only sparingly soluble in water, the reaction mixture will be an emulsion or a suspension. Especially with solid substrates (suspension) the addition of cosolvents (5–20%), such as low aliphatic alcohols (methanol, ethanol, *tert*-butanol), water-soluble ethers (*THF*, dioxane), low aliphatic ketones (acetone), or *DMSO* and *DMF*, will be advantageous. Some cosolvents must be used in very low percentage as they may reduce the activity of the enzyme (Table 1).

Another important feature which must be taken into consideration during ester hydrolysis is the liberation of the carboxylic acid lowering the *pH* of the reaction mixture (which is neutral at the beginning of the reaction). It is essential to keep the *pH* value constant during the reaction because of lower *pH* could stop the reaction and may damage the enzyme. Thus, a phosphate buffer is usually adopted to keep the *pH* constant.

Table 1. Solvent conditions used in biocatalyzed ester hydrolysis

Solvent system		Cosolvent (5–20%)
Water	hexane	aliphatic alcohol (MeOH,
buffter	dialkyl ether	EtOH, *t*BuOH)
–	aromatic	water-soluble ether (*THF*,
	liquid	dioxane)
		aliphatic ketone (acetone)
		DMSO, *DMF*

Table 2. Solvents in transesterification

Organic solvents
tert-butanol
methyl-*tert*-butyl ether
hexane
THF
methylene chloride
dioxane
acetone
acetonitrile
vinyl acetate
benzene/pyridine
DMF
pyridine

Transesterification (acylation)

Several studies have indicated that many enzymes, among them lipases such as porcine pancreatic lipase (PPL), are more thermostable in organic solvents than in water. For example, PPL remains active for many hours (half-life longer than 12 h) when incubated at 100°C in an almost anhydrous mixture of heptanol and tributyrin (Fig. 1) [8]. Lower stability was observed in the presence of 0.8% (w/v) water, whereas inactivation occurred almost instantaneous in aqueous buffer or water.

The high thermal stability of enzymes in organic solvents, especially in hydrophobic ones and at low water contents, was attributed to increased conformational rigidity and to the absence of nearly all covalent reactions which cause irreversible thermo-inactivation in water [9]. It should be mentioned that, as demonstrated by FTIR spectroscopic studies on lysozyme and subtilisin, enzyme structure is much more similar to the native form in pure organic solvents such as acetonitrile, *THF*, or 1-propanol than in aqueous solvent mixture [10]. This behaviour was found to be kinetically controlled, that is, due to the inherent restriction on protein

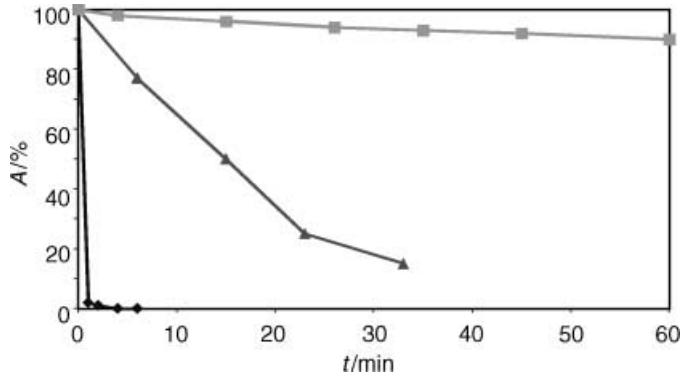


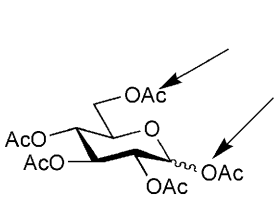
Fig. 1. Activity decay of dried powder of porcine pancreatic lipase; –◆–: H_2O or 0.1 *M* phosphate buffer; –▲–: 2 *M* solution of heptanol + 0.8% H_2O; –■–: 2 *M* solution of heptanol + 0.015% H_2O

conformational mobility in anhydrous media in contrast to aqueous-organic solvent mixtures.

The solvent also affects the selectivity of enzyme. Indeed, a great number of papers have reported that the enantio-, prochiral, and regioselectivity of enzymes can be affected, sometimes very remarkably, by the nature of the organic solvent used as the reaction medium [11]. *Klibanov* and coworkers first coined the term 'medium engineering' referring to the possibility of influencing enzyme properties by changing the nature of the solvent in which the reaction is carried out. An example of different enzymatic reactivity in different solvents is that observed for lipase from *Candida antarctica* on the 2-azidodeoxy derivative of β-D-*Gal*$(1 \rightarrow 3)$-D-*Glc*NAc [60]. Various hypotheses have been formulated to rationalize this phenomenon. For instance, the solvent, depending on its polarity, could modify the enzyme's conformation and, thus, influence the selectivity by altering the molecular recognition process between substrate and enzyme [12]. According to another theory, selectivity depends on the energetics of substrate solvation [13], whereas a third model envisages that solvent molecules could bind within the active site and, depending on their structure, interfere with the association or transformation of one enantiomer more than with that of the other [14, 15]. However, both the hypotheses based on the physico-chemical properties of the solvents and that based on solvent structure are unsatisfactory from the point of view of predictive value. At present, no link among the various hypotheses appears to exist, even though it is likely that the solvent influences enzymatic selectivity through more than a single mechanism. Table 2 reports a list of the organic solvents used in enzymatic acylation of carbohydrates.

Lipases as hydrolytic enzymes on acylated carbohydrates

First studies on the application of lipases in hydrolysis date back to 1969 when *Fink* and *Hay* [16] and *Sachder* and *Starkovsky* [17] used lipases from wheat germ (WGL) on penta-O-acetyl-D-glucose (**1**). The results were poor (only a mixture of partially deacetylated glucoses was obtained), but the authors made the interesting observation that C-1 and C-6 acyl groups were preferentially removed. The hydrolytic application of lipases was abandoned for about seventeen years. Then, in 1986 *Klibanov*, *Shaw* and, independently, *Wong* published their results [18–20].



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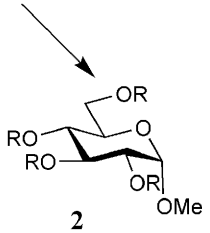

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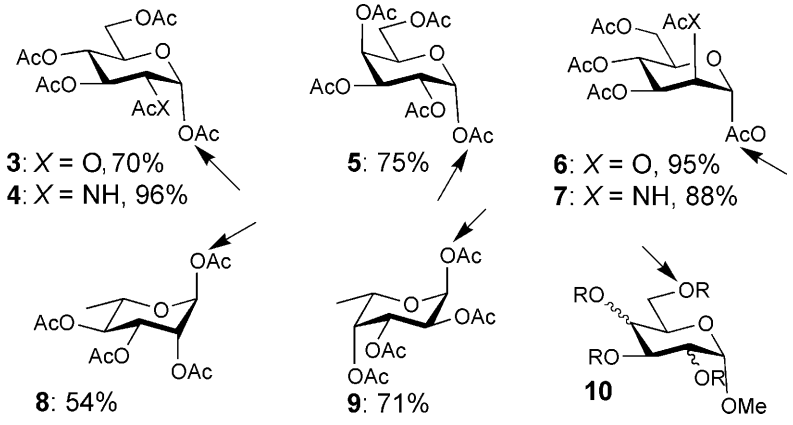
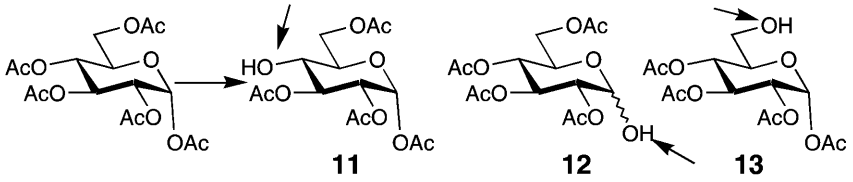
Lipase from *Candida cilindracea* (CCL) was the most adequate for methyl α- and β-2,3,4,6-tetra-O-acylpyranosides (**2**) [20], resulting in a 80–90% deacylation of O-6 (Fig. 2). Pentanoyl esters were preferred for technical reasons despite the fact the lipase best reacted with octanoyl derivatives. Moreover, the reaction was much cleaner for glucose with respect to other pyranosides tested and proceeded

five times faster for α- than for β-glycosides. The hydrolysis on β-D-penta-O-acetylglucose [18, 19] was studied using *Aspergillus niger* lipase (ANL). In particular, it was analyzed how reaction conditions (time, temperature, and solvent system) influenced the product composition, *i.e.* the ratio of tetra-, tri-, and diacetylated glucose derivatives obtained. Particularly interesting was the observation that the same lipase was able to catalyze also the alcoholysis of penta-O-acetyl-β-D-glucose affording quantitatively the 2,3,4,6-tetra-O-acetylglucose.

In further studies carried out on different pyranosides [21] bearing an acetate at the anomeric position, porcine pancreas lipase (PPL) was found to hydrolyze the anomeric acetate selectively in 54–96% yield (**3**–**9**). On pyranosides lacking the acyl group at C-1, the primary hydroxy group was removed in good yield using lipase *Candida cylindracea* (CCL) (**10**).



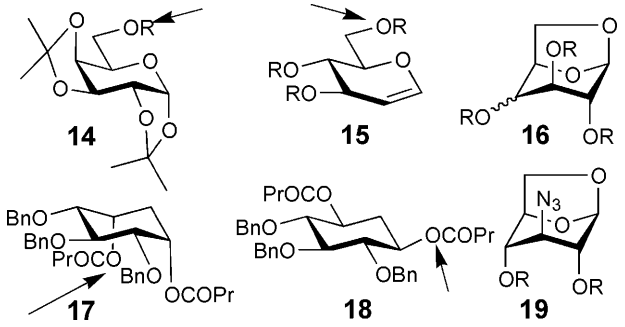
More recently, progress has been achieved in the regioselective deacetylation of penta-O-acetyl-α-D-glucose at positions other than C-1 and C-6 [22]. Testing different lipases at different *pH* values revealed a very interesting behaviour of CCL: this enzyme was not selective for the anomeric position and showed a dramatic change in regioselectivity at different *pH* (Scheme 1). Particularly interesting is the product selectively deprotected at O-4, a useful intermediate for the synthesis of many 1–4 linked oligosaccharides.



Lipase	*pH*	Yield / %	**11**	**12** α/β	**13**	Others
PFL	7	98	1	91	–	8
PFL	5	98	1	94	–	4
CCL	7	78	71	–	24	5
CCL	5	80	4	–	96	–

Scheme 1

The results so far observed are obviously of great interest, but none of them seem to be of fundamental importance for a synthetic chemist. Things become more attractive when differently protected substrates carrying various and liable functionalities showed to be well tolerated and region- and stereoselectively de-O-acylated by these enzymes. Among them are the 1,6-anhydro-2,3,4-tri-O-acyl sugars **16**, substrates bearing different functionalities such as acetals (*e.g.* **14**), glycitols such as **17** and **18**, and glycals (*e.g.* **15**).



Substrates bearing acetals as protecting groups such as **14** [23] are hydrolyzed by CCL quantitatively. Studies on 2,6-di-O-acetyl-3,4-O-isopropylidenegalactosides with different anomeric substitutents showed the possibility to hydrolyze selectively the O-6 acetate in good yields (75–90%) adopting Porcine pancreas (PPL) or *Mucor miehei* (MML) lipases. Once again, the α-anomers reacted much faster than β-configured compounds and C-1 deoxygenated derivatives [24].

Lipase from *Pseudomonas fluorescens* (PFL) turned out to be extremely efficient and regioselective for the hydrolysis of tri-O-acetyl-D-glucal (**15**, Fig. 4) at C-6 (90% yield). Less selective was the reaction with tri-O-acetyl galactal, which afforded a complex mixture of di- and monoacetylated products [25, 26].

1,6-Anhydropyranoses, being useful starting materials in carbohydrate chemistry, have been the objects of studies by many groups [27–30]. Interesting results obtained on differently protected substrates **20** and adopting different lipases are summarized in Table 3.

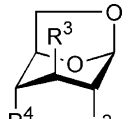
Of great importance seems to be the stereochemistry at C-4. When the hydroxyl group is equatorially oriented, only CCL and PPL are effective in hydrolyzing the C-2 butanoate in high yields (90%) and, on prolonged reaction time, PPL can also hydrolyze the C-4 butanoate in 65% yield [30].

The following is just one of the many examples of the applications of lipases in a complex synthesis. In order to prepare D-galacturonic acid glycosyl acceptors from the protected 1,6-lactone **21** (Scheme 2), it has been tried to adopt many classical techniques for the selective hydrolysis of the C-2 acetate, but no attempt gave satisfactory results. Only mild conditions offered by lipase from wheat germ afforded the desired compound **22** in 60% yield [31].

Lipase from *Candida cilindracea* (CCL) was also adopted for the stereoselective hydrolysis of (*R*)-configured propionates of cyclohexitols **17** and **18** [32]. Lipases being able to hydrolyze esters on pyranosides are also efficient on protected five-membered rings and exhibit a similar behaviour. The primary acetyl groups of furanosides **23**–**26** are cleaved with good yield using CCL [21], whereas the

Table 3. Hydrolysis of 1,6-anhydropyranoses; WGL = wheat germ; *RJL* = *Rhizopus javanicus*; CVL = *Chromobacterium viscosum*; PSL = *Pseudomonas Sp*; MML = *Mucor mihei*

Substrate			Lipase	Products			Yield/%
R^1	R^2	R^3		R^1	R^2	R^3	
OAc	OAc	OAc	PPL	OAc	OAc	OH	63–69
OAc	OAc	OAc	WGL	OAc	OH	OAc	42–67
OAc	OAc	OAc	RJL	OH	OAc	OAc	47
OBu	OBu	OBu	CVL/PSL/MML	OBu	OBu	OH	91
OBu	OBu	OBu	CCL	OH	OBu	OH	47
OAc	N_3	OAc	CCL	OAc	N_3	OH	85–90



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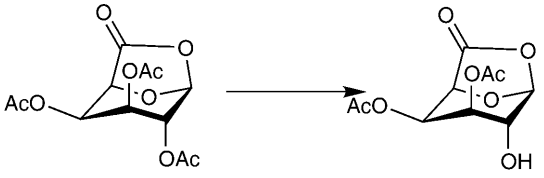
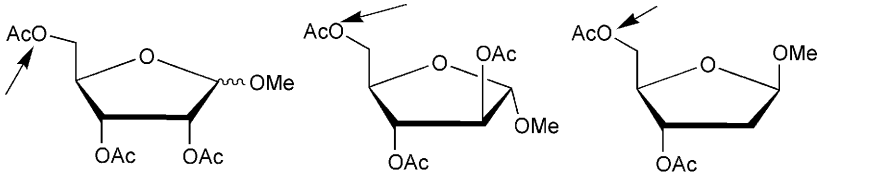
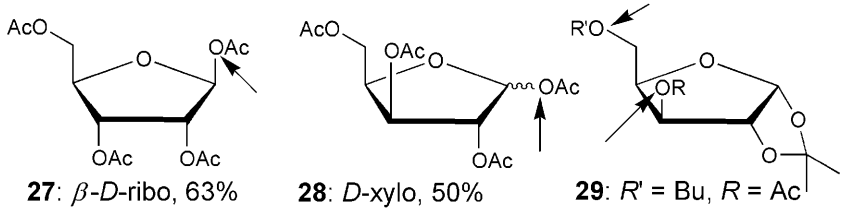
peracetylated furanoses **27** and **28** are deprotected at the anomeric position by lipase from *Aspergillus niger*. It is also interesting the observation that the regioselectivity of lipases is partially influenced by the presence of different acyl groups.



Scheme 2

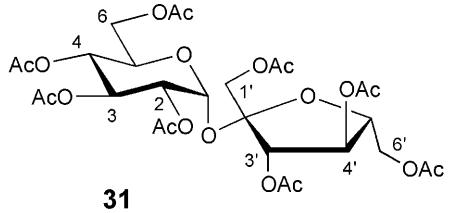




For example, CCL selectively removes the primary butyryl ester in presence of the secondary acetate on compound **29** in a 90% yield; if the bulky pivaloate ester is present on the primary group (**30**), the lipase prefers to hydrolyze the secondary butyryl chain [23].

Disaccharides have also been the object of hydrolytic reactions with lipases. First of all, sucrose **31** has been used as a substrate. Most enzymes evidenced a preference for hydrolysis at the fructosidic moiety. Using lipase from wheat germ (WGL) [33], the octa-O-acetyl sucrose was deacetylated at positions O-1′,4′,6′ affording the pentaacetyl derivative in 40% yield. Lipase from CCL preferentially hydrolyzes the O-4′ ester [34, 35], whereas lipase from *Candida antartica* deacetylates the O-6′ position [36]. Quite surprisingly, lipase from *Aspergillus niger* acts on the glucose moiety, affording a 1:1-mixture of heptaacetyl sucroses with the free hydroxyl groups at C-4 and C-6.

Other common per-O-acetylated disaccharides (maltose (**32**), cellobiose (**33**), lactose (**34**), and melibiose (**35**)) have been submitted to this methodology [37–39]. Lipase from *Aspergillus niger* gave the highest reaction rate and afforded selectively the corresponding heptaacetates with a free hydroxyl group at C-1 after 30 min. Prolonged reaction time on cellobiose and lactose per-O-acetates gave the hexaacetates with free hydroxyl groups at C-1 and C-2, whereas maltose and melibiose gave a complex mixture of products [39].



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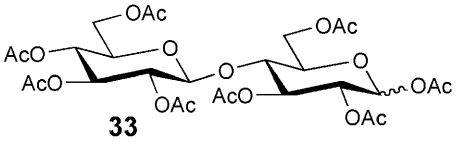

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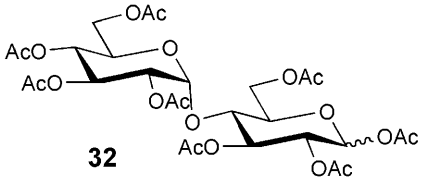

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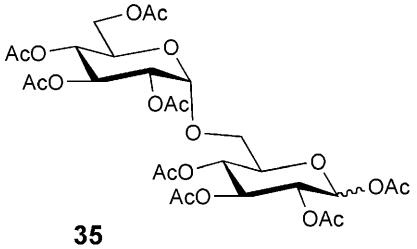

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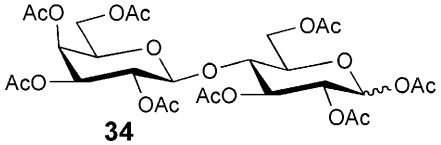

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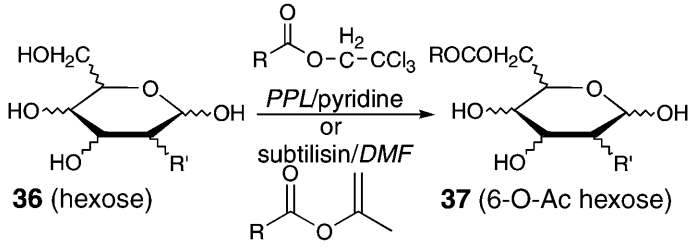
Lipases as acylating biocatalysts

Studies on the use of lipases as biocatalysts for the transesterification between sugars and activated esters date back to 1986. In his pioneering studies, *Klibanov* [40, 41] has demonstrated that lipases can also work in dry organic solvents. Under these conditions the enzymes reverse their hydrolytic activity and are able to catalyze acyl transfer reactions from activated esters to suitable acceptors. First studies employing pyridine as solvent and activated esters showed that lipase from Porcine



36 (hexose) **37** (6-O-Ac hexose)

Hexose	Enzyme	*R*	Yield/%	Ref.
Glucose	*PPL*	CH_3	76	[73]
Glucose	*PPL*	C_3H_7	50	[73]
Galactose	*PPL*	CH_3	60	[73]
Mannose	*PPL*	CH_3	85	[73]
Sialic acid [a]	*PPL*	CH_3	64	[74]
Glucose	Subtilisin	C_3H_7	61–64	[76]
Mannose	Subtilisin	CH_3	40	[77]
Sialic acid	Subtilisin	CH_3	73	[77]

[a] 9-O-acetyl-N-neuraminic acid was formed

Scheme 3

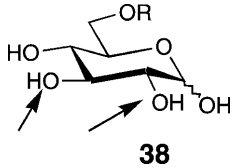
pancreas (PPL) could regioselectively transfer acyl groups to the primary hydroxyl groups (Scheme 3). The protease subtilisin was tested as well.

Acyl donors that proved to be suitable for the process and which are commonly adopted are 2,2,2-trichloroethyl, 2,2,2-trifluoroethyl [42], enol, [43, 44] and oxime [45] esters. In particular, enol esters have the advantage that the liberated enol tautomerizes to a ketone or a aldehyde, thereby shifting the equilibrium. Glucoses already protected in the primary hydroxyl group have been selectively acylated at C-2 or C-3 according to different lipases employed (Table 4) [46].

Many research groups have concentrated their studies on anomerically protected pyranosides which are easier to handle and better soluble in organic solvents

Table 4. Acylation of C-6 protected glucoses; CVL = *Chromobacterium viscosum*; ANL = *Aspergillus niger*

R	Lipase	Acylating agent	C-2	C-3	Yield/%
Bu	CVL/ANL	trichloroethylBu	OH	OBu	80
Bu	PPL	”	OBu	OH	51
Trt	CVL	”	OH	OBu	88
TBDPS	CCL	”	OBu	OH	75



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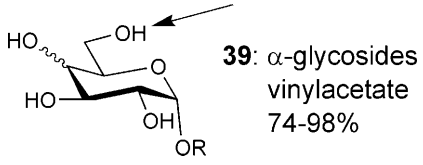
than the free sugars [46, 47]. Lipase from *Candida antarctica* (CAL) was used on methyl, phenyl, and octyl pyranosides [47] (Table 5).



This enzyme is able to acylate regioselectively not only primary but also secondary hydroxyl groups, and the study seems to outline that lipase activity is dramatically influenced by the stereochemistry of the anomeric position. As a matter of fact, α-glycosides (**39**) are acylated only at C-6, whereas β-glycosides (**40**) are partially acylated also at positions C-2 and/or C-3 as summarized in Table 5. D- and L-fucopyranosides, rhamnopyranosides [47, 48], and mannopyranosides under lipase acylation conditions all afforded selectively protected products (Table 6).

Lipase from *Pseudomonas fluorescens* (PFL) converts both D-6-deoxysugars (Table 6, entries 1,2) to the 2-monobutyrates with high regioselectivity, whereas

Table 5. Acylation of anomerically protected pyranosides

X	Galactosides		Glucosides	
	Yield/%	Acetylated	Yield/%	Acetylated
	24	C-6		
Me	44	C-6, C-3	97	C-6, C-3
	31	C-6, C-2		
Ph	92	C-6, C-3	77	C-6, C-3
	6	C-6, C-2	23	C-6, C-2
Octyl	–	–	99	C-6

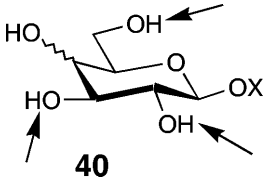


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Table 6. Acylation of fuco-, rhamno-, and mannopyranosides; a: *THF*:*Py* = 4:1; b: *THF*; c: acetone: Py = 10:1; d: acetone; *TFEB* = trifluoroethylbutyrate

Entry	Substrate	Lipase	Solvent	Product	Yield/%	Acylating agent
1	Me-*D*-fuco	PFL	a	C-2	88	*TFEB*
2	Me-*D*-rhamno	PFL	b	C-2	42	*TFEB*
3	Me-*L*-fuco	PFL	a	C-4	45	*TFEB*
4	Me-*L*-rhamno	PFL	b	C-4	63	*TFEB*
5	*Bn*-*D*-rhamno	CAB	d	C-4	20	Vinyl acetate
6	*Bn*-*D*-manno	CAB	c	C-6	97	Vinyl acetate
7	*Bn*-*L*-rhamno	CAB	d	C-4	90	Vinyl acetate
8	*Bn*-*L*-manno	CAB	c	C-6 (C-4,6)	64 (29)	Vinyl acetate

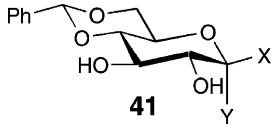
the L-enantiomers (Table 6, entries 3,4) are esterified at C-4. The same result is obtained using lipase from *Candida antarctica* (CAB) on benzyl L-rhamnopyranosides (Table 6, entry 7), but unlike with PFL, also the D-enantiomer is acylated at C-4 (Table 6, entry 5). As expected, mannosides are esterified at the primary position, the L-enantiomer affording also 29% of diacetylated compound (Table 6, entry 8).

As already observed for the hydrolitic methodology, lipases are able to accept also sugars with functional groups such as acetals and azides, glycals, disaccharides, glycoconjugates, and other complex unnatural moieties as substrates.

Among the monosaccharides bearing acetals as protecting groups, the 4,6-O-benzylidene pyranosides are the most studied so far [47, 49]. Different lipases have been tested on protected glucose and galactopyranosides with different anomeric substituents (Table 7).

Table 7. Lipase selectivity on 4,6-O-benzylidene pyranosides

Entry	Lipase	*X*	*Y*	C-2	C-3
1	PFL	SEt	H	–	100
2	PPL	SEt	H	–	100
3	CCL	SEt	H	64	36
4	PFL	SPh	H	14	86
5	PPL	SPh	H	18	82
6	PFL	SePh	H	47	53
7	PPL	SePh	H	35	65
8	CAB	OMe	H	21	79
9	CAB	H	OMe	58	–



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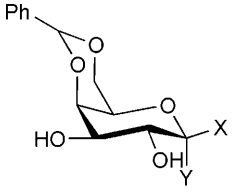
The thioethyl β-D-glucoside **41** (Table 7, entries 1,2) was well accepted by PPL; PFL afforded mainly the C-3 acetylated compound, whereas CCL showed the opposite regioselectivity (Table 7, entry 3). If the anomeric substituent is more hindered like SPh and SePh, the regioselectivity is sensibly decreased (Table 7, entries 4–7). CAB reveals a preference for the acetylation of C-3 in the methyl β-D-glucopyranoside (Table 7, entry 8), whereas the regiochemistry is completely reversed for the α-anomer (Table 7, entry 9). *Pseudomonas Sp* lipase (PSL) is able to selectively acylate the primary hydroxyl group of 3,4-O-isopropylidene-D-galactopyranosides **43** [24]; the 1-deoxy derivative showed the highest acylating rate along with the α-glycosides (Table 8).

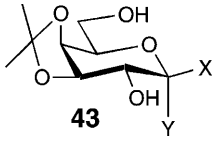


42: very poor substrate preference for C-2

Glycals are very useful precursors in the synthesis of complex oligosaccharides as well as for the formation of C-2 functionalized compounds. *Holla* [25], as cited

Table 8. Acylation of 3,4-O-isopropylidene galactopyranosides; solvent: vinyl acetate: *THF* = 7:3

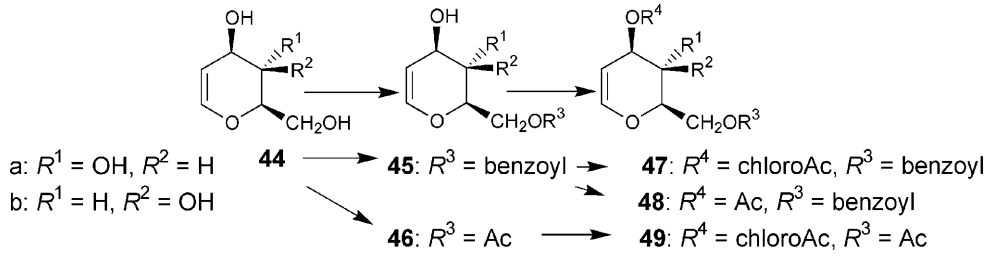
Substrate	*t*/h	Lipase	Yield/%
1a; *X* = OMe, *Y* = H	48	PSL	90
1b; *X* = H, *Y* = OMe	24	PSL	93
1c; *X* = OBn, *Y* = H	48	PSL	87
1d; *X* = H, *Y* = H	24	PSL	94



43

in the hydrolysis paragraph, dedicated part of his work to the study of enzymatic protection and deprotection of these compounds. *Candida* lipases OF and S-VII were found to be suitable for the selective acetylation of the primary hydroxyl group, whereas PPL afforded the 3,6-diacetylated compounds. For the complete differentiation of the three hydroxyl groups, also benzoylation and chloroacetylation were investigated (Scheme 4). Combining the use of the cited enzymes and lipase AY-20 with different acylating agents it was possible to obtain the completely differentiated glycals (Table 9).

Furanosides and furanoses, although more flexible in solution than pyranoses, can also be regioselectively protected by lipases. As expected, the primary hydroxyl

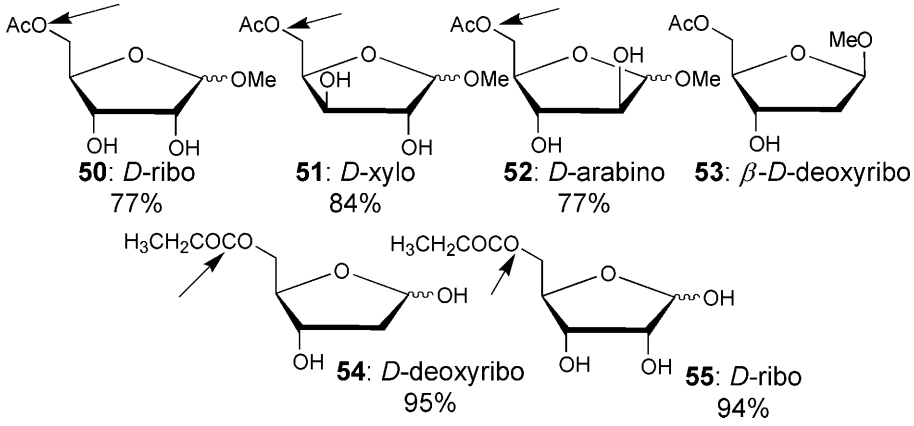


Scheme 4

Table 9. Conditions for the generation of completely differentiated glycals; *VA* = vinyl acetate; *VB* = vinyl benzoate; *VCA* = vinyl chloroacetate

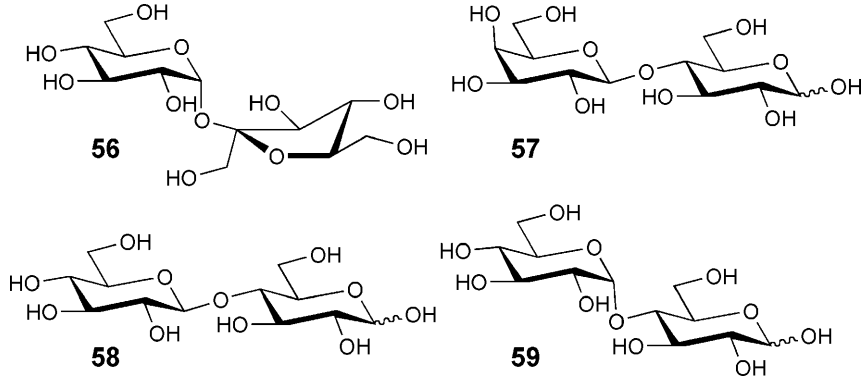
Entry	Compound	Lipase	Acylating agent, conditions	Product	Yield/%
1	**44a**	OF	*VA*, 20% EtOAC	**46a**	90
2	**44a**	AY-20	*VB*, 30–40% *THF*	**45a**	70
3	**44b**	S-VII	*VA*, 1–4% H_2O	**46b**	93
4	**44b**	AY-20	*VB*, 5–10% H_2O	**45b**	67
5	**46a**	PPL	*VCA*, 20–30% *DME*	**49a**	83
6	**46b**	PPL	*VCA*, 20–30% *DME*	**49b**	80
7	**45a**	PPL	*VCA*, 20–30% *DME*	**47a**	82
8	**45b**	PPL	*VCA*, 20–30% *DME*	**47b**	80
9	**45a**	PPL	*VA*	**48a**	92
10	**45b**	PPL	*VA*, 20–30% *DME*	**48b**	80

group is always the first to be acylated. D-Ribo-, D-xylo-, and D-arabinofuranosides were acylated using PPL [21] affording **50**, **51**, and **52** in good yields, whereas 2-deoxyribofuranoside **53** afforded a mixture of regioisomers. The problem was overcome using the protease subtilisin instead of a lipase [50, 51]. The free sugars D-2-deoxyribose and D-ribose were propionylated at C-5-OH using CAL, propionic anhydride in *THF* affording **54** and **55**, respectively [52].



Lipases acylate the free primary hydroxyl groups of furanosides partially protected with acetals [53]. If the primary hydroxyl group is already protected, some lipases are able to discriminate between the secondary hydroxyls [54]. Of particular interest is the possibility of using lipases on more complex oligosaccharides and glycoconjugates both in hydrolysis (as previously reported) and in esterification. It must be pointed out that most, if not all, results obtained on lipase selective protection/deprotection of complex and unnatural compounds are empirical, obtained often after a careful screening of many lipases under different exper-imental conditions. This work is generally tedious and time consuming, but once the right conditions are found these reactions are mild and regioselective, and it is difficult (sometimes impossible) to achieve the same result by means of chemical methods.

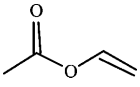
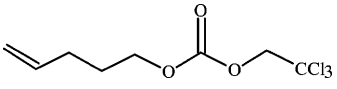
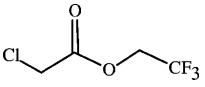
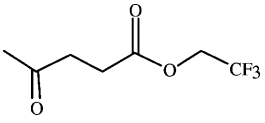
Very few disaccharides have been subjected to a systematic study applying lipases as acylating catalysts; in his pioneering investigation [55], *Klibanov* has shown that not a lipase but a protease (subtilisin) was able to catalyze the acylation of disaccharides such as sucrose **56**, lactose **57**, cellobiose **58**, and maltose **59** dissolved in *DMF*.

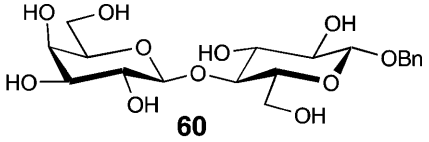


The acylation is regioselective; the non reducing sucrose is esterified at the 1′-OH, whereas on the other disaccharides the acyl group is always transferred to the primary hydroxyl group of the nonreducing end. This means that the enzyme is able to discriminate between the two primary positions, which is not easily achieved with chemical methods. Some examples of acylation of simple derivatives of these sugars have been reported in the literature [36, 56, 57]. A systematic study on the acylation of disaccharides adopting lipases has been carried out recently [58]. Selective esterification of dodecyl glycosides of cellobiose, maltose, and lactose was achieved using *Candida antarctica* (CAL) and *Pseudomonas cepacia* (PCL) employing different acylating agents. All substrates were acylated at the primary C-6′-OH; only β-lactoside afforded a mixture of mono- and diacetylated derivatives with PCL where the second acetate is transferred on the 2′-OH.

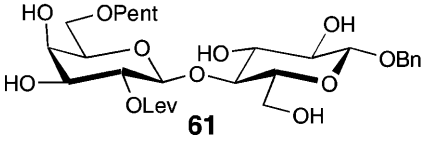
A further study was carried out on benzyl β-lactoside **60** (Table 10) using the same two lipases and different acylating agents, affording different mono- and diacylated derivatives; particularly interesting is the product in which two different protecting groups were introduced at C-6′ and C-2′ (**61**). Such protected derivatives have been used for the synthesis of human milk oligosaccharides [59].

Table 10. Regioselectivity of benzyl β-lactoside

Acylating agent	Product	CAL	PCL
	6′ OAc	75%	67%
	6′ O*Pent*	73%	64%
	6′ OAcCl	81%	30%
	6′ O*Lev* 2′,6′ di-O*Lev*	73% 19%	66% 23%



60

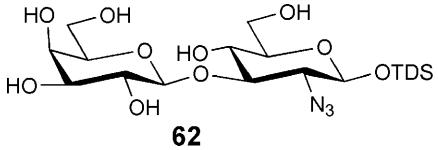


61

Table 11. Enzymatic selective acylation of disaccharides **52** and **53**; for abbreviations, see Table 9

Entry	Compound	Conditions	/°C, *t*/h	Products	Acylation position	Yield/%
1	**62**	*THF*, *VA*	40°, 24 h	**64**	6′-OAc	90
2	**62**	CH_3CN, *VA*	40°, 24 h	**64**	6′-OAc	8
				65	6,6′-OAc	70
				66	6,2′,6′-OAc	16
3	**63**	*TMF*, *VA*	45°, 40 h	**70**	6′-OAc	90
4	**63**	CH_3CN, *VA*	45°, 40 h	**70**	6′-OAc	90
5	**64**	CH_3CN, *VCA*	28°C, 4 h	**67**	6′-OAc, 6-OChloroAc	90
6	**64**	CH_3CN, *VCA*	35°C, 1 h	**67**	6′-OAc, 6-OChloroAc	67
				68	6′-OAc, 6,2′-OChloroAc	29
7	**65**	CH_3CN, *VCA*	40°C, 12 h	**69**	6′,6-OAc, 2′-OChloroAc	91

CAB was also employed for a systematic study of the regioselective protection of the 2-azidodeoxy derivatives **62** and **63**, synthetic equivalents of *β*-D-*Gal*(1-3)-D-*Glc*NAc and *β*-D-*Gal*(1-4)-D-*Glc*NAc [60]. The latter compounds are among the most important components of glycoproteins and glycolipids; therefore, it is extremely important to protect them selectively for the synthesis of their complex derivatives. The 2-azidodeoxy compounds were submitted to selective acylation using CAB under different conditions (Table 11).



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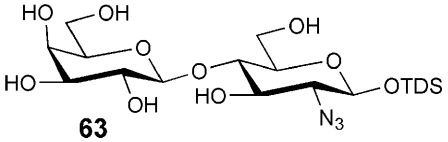
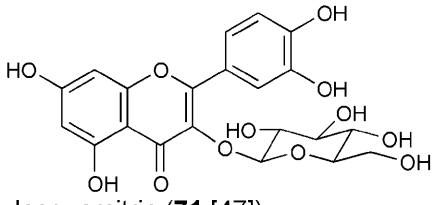


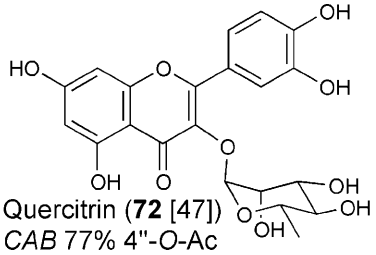
63

The regioselectivity depended on the solvent as well as on the nature of substrate and acylating agent. The azido derivative **62** in CH_3CN afforded as main product the 6,6′-diacetylated compound (Table 11, entry 2); as lipases usually acylate only the non-reducing moiety of a disaccharide, this behaviour appeared quite surprising. The possibility of modulating the regioselectivity in different solvents was then exploited for the preparation of compounds with two diverse acyl groups on the two primary hydroxyl groups (Table 11, entries 5,6), a result that can be achieved chemically only in many steps.

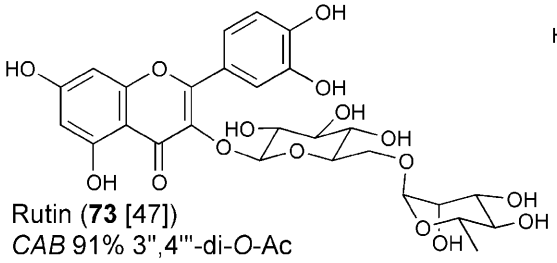
Finally, some selected examples of the use of regioselective lipase acylations of complex glycoconjugated compounds are reported to stress once more the versatility of these enzymes.



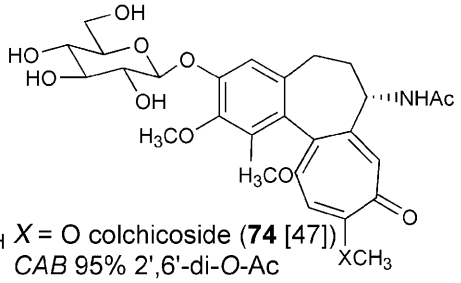
Isoquercitrin (**71** [47])
CAB 79% 3'',6''-di-*O*-Ac



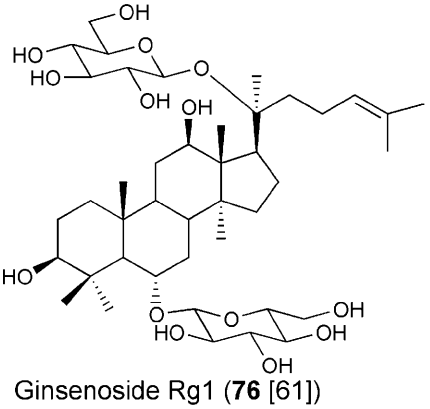
Quercitrin (**72** [47])
CAB 77% 4''-*O*-Ac



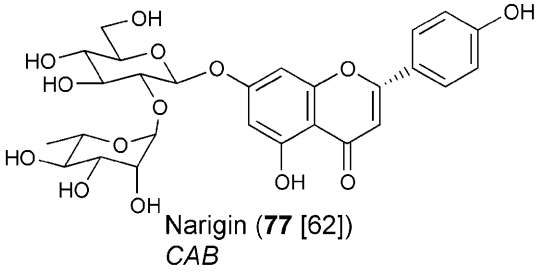
Rutin (**73** [47])
CAB 91% 3'',4'''-di-*O*-Ac



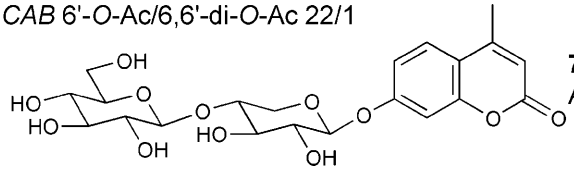
X = O colchicoside (**74** [47])
CAB 95% 2',6'-di-*O*-Ac
X = S thiocolchicoside (**65**)
CAB 93% 2',6'-di-*O*-Ac



Ginsenoside Rg1 (**76** [61])
CAB 6'-*O*-Ac/6,6'-di-*O*-Ac 22/1



Narigin (**77** [62])
CAB



78 [63]
Amano *PS* 89% 6''-*O*-Ac

Lipases have also been used for the resolution of racemic β-*D*,*L*-amicetosides **79** (Scheme 5); the pure free *D*-enantiomer, amicetose (a deoxy sugar), is a constituent of the antibiotic substances amicetin and axemomycin. Among the many lipases tested, PPL, *Candida rugosa*, and *Pseudomonas PS*, the latter gave the best result (Scheme 5) [64].

D,*L*-amicetoside **79** → 79%, *ee* = 99% **80** + 77%, *ee* = 30% **81**

Scheme 5

Conclusions

Quite a lot of work has been done in the study and application of lipases in carbohydrate chemistry; lipases have already found important applications, especially in the industrial synthesis of fatty acid esters of carbohydrates, compounds that have important applications in detergents, food, cosmetics, and pharmaceuticals because of their properties as non-ionic surfactants. In organic synthesis these enzymes are the most extensively used as biocatalysts; moreover, the increasing knowledge of their structure and function has already led to site directed mutagenesis of *Rhizopus delemar* lipase to shift the preference of the mutants for the hydrolysis of medium-chain triglycerides [65]. This suggests that very soon these already versatile chemical tools could be designed and built as required for specific substrates and acylating agents.

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