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An update of biocatalytic selective acylation and deacylation of monosaccharides

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Abstract: Partially acylated monosaccharides (PAMs) exhibit diverse and interesting applications but due to their polyhydroxylated nature, their synthesis requires regio- and stereoselective reactions. These features are provided by biocatalytic processes and in particular by hydrolases, which offer mild conditions and selective routes for the preparation of PAMs. Since this strategy has been extensively explored, the aim of the present review is to update research on enzymatic selective acylation and deacylation of monosaccharides, focusing on enzymatic preparation of synthetic useful PAMs and drug-monosaccharide conjugates involving PAMs.

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1. Introduction

Carbohydrates exert important biochemical functions (energy supply, molecular recognition processes, structural roles, cell-surface functions) and are starting materials for the synthesis of varied molecules, in particular, bioactive compounds such as oligosaccharides, glycoconjugates and nucleosides. Synthesis involving carbohydrates must address an inherent and troublesome issue: due to the presence of different hydroxyl groups of similar reactivity, the availability of suitable selective reactions is crucial. The traditional approach dealing with the application of protective groups involves several steps that affect yield, cost and time of the syntheses. In particular, esters are among the most useful protective groups in sugar chemistry and therefore, the synthesis of partially acylated monosaccharides (PAMs) by means of selective acylation and deacylation reactions is of key importance. Despite the extensive work carried out to differentiate reactivity of similar hydroxyl groups, this problem is still challenging and it remains a central matter in efficient carbohydrate synthesis.[1]

PAMs are not only useful synthetic intermediates in the synthesis of bioactive compounds but also possess other applications. On the one hand, conjugation of a drug with a monosaccharide moiety can afford a prodrug with higher hydrophilicity and improved bioavailability than the parent drug. On the other hand, due to the low cost, biocompatibility and biodegradability of sugars, PAMs bearing fatty acyl moieties have found applications as biosurfactants and emulsifiers in pharmacy, cosmetic and food.[2] All these applications need efficient synthesis of PAMs, facing the mentioned problem of hydroxyl groups selective recognition.

Enzymes are mild, regio-, chemo- and stereoselective green catalysts. They are active in organic solvents and non conventional media and their activity can be improved today through protein engineering techniques. Owing to these features, enzymes offer

nowadays useful alternatives in organic synthesis. Among biocatalysts, lipases display wide substrate structure acceptance and besides their hydrolytic activity *in vivo*, they are active in non aqueous media, catalysing reactions such as esterification, transesterification and aminolysis. Hydrolytic enzymes, mainly lipases and proteases, provide an efficient access to PAMs, by catalysing selective acylation and deacylation of carbohydrates. The pioneering works of Klibanov[3,4] and Hong[5] groups in the 80´s opened the gate to research in the field of carbohydrates, which has been comprehensively reviewed.[6-9] Enzymatic selective acylation involves the reaction of a nucleophilic hydroxyl group with a carboxylic acid (esterification) or an ester (transesterification); generally, the latter is preferred, since the use of activated esters, such as vinyl esters, shifts the equilibrium towards the target PAM. In enzymatic selective deacylation, water (hydrolysis) or an alcohol (transesterification) are employed as nucleophiles, affording PAMs bearing selectively free hydroxyl groups. Mechanistically, most of the hydrolytic enzymes applied in carbohydrate acylation and deacylation are serine hydrolases, whose accepted catalytical mechanisms are widely documented in literature.[10]

As a very general trend, regioselective acylation and deacylation of the least hindered functional group (primary alcohol or ester of primary alcohol) is observed, while enzymatic recognition of secondary hydroxyl groups is often difficult to predict. Moreover, monosaccharide stereochemistry and conformation affect enzyme recognition. Since prediction of the selectivity may be limited, a screening of enzymes is usually necessary when previous assays are not available.

Taking into account the previous discussion, the aim of the present work is to review research on enzymatic selective acylation and deacylation of monosaccharides, in the period dating from 2003 until the middle of 2015, focusing on enzymatic preparation of

synthetic useful PAMs (Section 2) and on the preparation of drug-monosaccharide conjugates involving PAMs (Section 3). Enzymatic sugar fatty acid esters synthesis has been the target of reviews of the last decade[11-15] and will be then not dealt in the present work. **Table 1** lists the enzymes most frequently mentioned through this review.

2. Enzymatic selective acylation and deacylation in the preparation of synthetic useful partially acylated monosaccharides

The following examples in this section show the tendency of many hydrolases towards regioselective recognition of carbohydrate least hindered position, both in acylation and deacylation reactions. However, it will be appreciated that yields and results are dependent on many variables such as enzyme, sugar stereochemistry, anomeric substituent structure, reaction solvent, acyl moiety structure and nucleophile.

2.1. Pyranoside rings

2.1.1. Deacylations

Peracylated pyranoses, which bear the chemically more reactive anomeric ester, illustrate the potential of enzymes for regio- and chemoselective transformations of monosaccharides. Exploiting lipase regioselectivity towards deacylation at C6, Ferrero, Gotor and coworkers[16] developed a chemoenzymatic synthesis of D-glucose-6-phosphate (**4**, **Scheme 1**) in 77% overall yield, in which the key precursor **2α** was obtained through a lipase-catalysed hydrolysis. Six lipases were tested and lipase from *Candida rugosa* (CRL) gave the best results; the effect of temperature, pH and cosolvent was also studied. In this way, an almost quantitative conversion of

pentaacetylated α-D glucose (**1α**) into product **2α** was reached, with excellent regioselectivity and total stereoselectivity towards the α-anomer. Starting from pure **1α**, **2α** could be obtained in 95% yield on a 4 g-scale; the authors also showed that formation of the minoritary product **3α** was due to acetyl migration and not to lipase catalysis. Molecular modelling was employed to rationalise the stereoselective outcome of the reaction, supporting that an additional H bond between the carbonylic oxygen at C1 in the α-anomer and the OH of the Ser 450 residue in the surroundings of CRL active site fixes the substrate favourably for regioselective deacetylation at C6; this interaction is not found in the β-anomer. This shows the influence of a site remote from the reaction centre on enzyme recognition and indicates the importance of the anomeric substituent structure, in relation with selective enzyme recognition. Furthermore, this research group applied the precursor **2α** to synthetise chemically nucleoside-carbohydrate phosphodiester prodrugs[17], which are more soluble in water than the non conjugated nucleosides.

Guisán, Fernández Lafuente, Terreni, Palomo and coworkers applied the so called conformational engineering of lipases and studied extensively different immobilisation techniques. These involve distinct areas and microenvironments of the lipase, conferring different rigidity to the enzyme structure; the choice of the immobilisation procedure allowed a modulation of the catalytic properties of the enzyme. Four very different immobilisation protocols were applied: (i) Immobilisation on hydrophobic supports (octyl agarose: agarose coated with a dense layer of octyl groups), by interfacial activation of the lipase on support hydrophobic surface; ii) Immobilisation on cyanogen bromide (CNBr)-activated agarose, through covalent attachment through amino terminal groups on the enzyme surface; (iii) Immobilisation on glyoxyl-agarose, by multipoint covalent attachment involving areas on the enzyme surface rich in lysine;

(iv) Immobilisation on agarose beads coated with polyethylenimine (PEI), by means of anionic exchange concerning areas with the highest negative net charge of the enzyme.

The immobilisation procedure results crucial in order to find the most satisfactory biocatalyst for each assayed monosaccharide and it can provide, for a given lipase, biocatalysts with different selectivity. For instance (**Scheme 2**), the octyl agarose-*Thermomyces lanuginosus* lipase (octyl-TLL) preparation removed chemoselectively the 6-*O*-acetate of pentaacetylated β-D-galactose (**5β**) in excellent yield (**10β**, 95%), while the CNBr-TLL biocatalyst exhibited preference for the anomeric acetate (**11**).[18] This research group applied this strategy to many substrates and lipases;[19-24] examples given in **Scheme 2** show that modulation of enzyme selectivity and activity afforded an access to C6- and C1-deprotected products. Moreover, synthetic useful glycals (**20** and **21, Scheme 3**) were also studied as substrates and yielded C6- (**22** and **24**) or C3- (**23** and **25**) monodeacetylated products, depending on the applied immobilised biocatalyst.[21,23]

In **Schemes 2** and **3** differences in obtained yields and in the optimal biocatalyst reflect the influence of ring stereochemistry on enzyme recognition. On the other hand, by assaying a set of peracetylated 1-β-substituted D-gluco-[23] and galactopyranosides,[24] and by comparing with substrates carrying an anomeric acetate, it was concluded that the structure of the anomeric substituent affected the catalytic properties of the biocatalysts: an example of this behaviour can be appreciated through results of octyl-CRL catalysed deacetylation of β-galacto substrates **5β** and **8β** (**Scheme 2**). Regarding to the influence of the anomeric centre stereochemistry, in a study[25] assaying a set of peracetylated α- and β-D-pyranoses and hydrolases immobilised on octyl agarose (CRL, lipase from *Aspergillus niger* (ANL) and phospholipase Lecitase (LECI)), the biocatalysts showed the highest activity and selectivity towards the α-anomers, as exemplified by octyl-CRL

catalysed deacetylation of substrates **5β** and **5α** (**Scheme 2**). It is also worth mentioning that the xylopyranoside **9β**, bearing only secondary acetate groups, was deacetylated at C4 with complete regioselectivity and in nearly quantitative yield, using immobilised-ANL preparations.

Further, the C6 monodeacetylated monosaccharides obtained by the above discussed immobilisation strategies were chemically transformed into the corresponding C4 and C3 monodeacetylated regioisomers, through a mild acyl group migration at pH 8-9.[25,26] In this way, these researchers developed an enzymatic or chemoenzymatic access to monosaccharides regioselectively monodeacetylated at C1, C3, C4, C5 and C6, useful as glycosyl acceptors in the synthesis of oligosaccharides.[26]

In addition to previous examples involving deacetylation by hydrolysis, Hietanen and Kanerva[27] reported the regioselective methanolysis of peracetylated methyl α-D-glycopyranosides **26-28α** (**Table 2**)**:** results were again very dependent on monosaccharide stereochemistry.

On the other hand, microbial carbohydrate esterases (CEs) deacylating plant polysaccharides have gained attention due to their potential for the bioconversion of plant biomass residues into fermentable sugars.[28] A few papers have reported the application of microbial CEs for the deacetylation of peracetylated D-pyranosides, observing regioselective formation of monoacetates of secondary hydroxyl groups. Moriyoshi *et al*.[29] assayed the cellulose acetate esterase from *Neisseria sicca* in the hydrolysis at pH 7 of peracetylated methyl β-D-gluco- and galactopyranoside and found the former to react more regioselectively, affording the monodeacetylated product at C3 in about 70% conversion. Moen and Anthonsen[30] tested the carbohydrate esterase from *Bacillus pumilus* PS 213, belonging to the CE family 7, in the hydrolysis of peracetylated methyl- (**27α**), benzyl α-D-galactopyranoside (**29α**) and methyl α-L-

glucopyranoside (**30α**). At the monoacetylation stage, products 2-*O*- (from **27α** and **29α**) and 4-*O*-monoacetylated (from **30α**) were mainly formed but results were very dependent on the stereochemistry of both pyranose ring and anomeric centre: the α-D-gluco-, α-D-manno- and β-counterparts of **27α** reacted with low selectivity.

As protective groups, benzoates are more stable and less prone to migration than acetates. Esmurziev *et al*.[31] reported CRL-catalysed hydrolysis of a set of perbenzoylated methyl D-pyranosides (**Scheme 4**). This enzyme afforded most satisfactory results, while other tested lipases, such as lipases B and A from *Candida antarctica* (CAL-B and CAL-A, respectively), TLL and *Rhizomucor miehei* (RML) did not exhibit useful activity; the choice of the solvent affected reaction rates. Results in **Scheme 4** indicate, as already pointed out, that the stereochemistry at both the anomeric centre and at the rest of the pyranoside ring influenced drastically the attained yields. By extending enzymatic catalysis to benzoylated PAMs, this work provides a methodology complementing the enzymatic procedures towards methyl 2,3,4-tri-*O*-acetyl-D-pyranosides.[27,32]

Beyond the works above presented, an interesting example of the explotation of the chemoselectivity and mild selective reaction conditions of biocatalysts is given in **Scheme 5.** Some acylglucosides containing pyrrole-2-carboxylic acid moieties, named buprestins, act as ant deterrents and studies on their biological activity required their synthesis. Attemps to obtain **32**, the key precursor for all the family members (buprestins A, B, D-H), involved the difficult selective deacetylation of pentaester **31**: unsatisfactory results and mixtures of products were obtained by employing conventional basic reagents. In the approach developed by Unverzagt and coworkers,[33,34] a set of twelve lipases was tested for **31** deacetylation and only CAL-B resulted successful, affording the desired completely deacetylated intermediate **32** in

86% isolated yield, whose further Mitsunobu esterification afforded two of the target buprestins (A and H). Direct Mitsunobu esterification failed when hydroxybenzoic acids were used, and this was circumvented by using acetoxybenzoic acids, which were subsequently deacetylated applying again a CAL-B-catalysed hydrolysis, affording Buprestins B and G. A similar strategy led to Buprestins D and E, but it was unsuccessful for Buprestin F since the involved di-*O*-acetylcaffeylated substrate gave unsatisfactory results in CAL-B-catalysed deacetylation.

Another interesting example of the selective recognition of different aliphatic acyl moieties deals with mannosylerythrithol lipids (MELs). MELs are biosurfactants produced *in vivo* from sugars and vegetable oils by yeasts of the genus *Pseudozyma*; in particular, *Pseudozyma tsukubaensis* produces large amounts of MEL-B (**33**, **Scheme 6**) as the sole MEL, rendering its isolation and purification easy. Taking MEL-B as starting material, Kitamoto and coworkers[35] obtained quantitatively the new MEL homolog MEL-D (**34**), not found as microbial product, through a highly chemoselective CAL-B-catalysed alcoholysis; removal of the 6-*O*-acetyl group did not proceed with other tested enzymes (RML and TLL). Bearing two free hydroxyl groups, MEL-D displayed a higher hydrophicity and critical aggregation concentration compared to known MELs.

1-β-*O*-Acylglucuronides (**37a-c**, **Scheme 7**) are metabolites of pharmaceuticals, such as non-steroidal anti-inflammatory drugs (NSAID), and due to their lability at physiological pH, these compounds can be involved in drugs adverse effects. The chemical synthesis of 1-β-*O*-acylglucuronides for toxicological studies requires the chemoselective removal of all ester protecting groups, without reaction of the most reactive ester at C1; the use of nucleophilic reagents afforded unsatisfactory results.[36] Baba and Yoshioka[37] applied enzymatic chemoselective hydrolysis to accede to NSAID glucuronides **37a-c**. In their approach, acetates of chemically prepared substrates **35a-c**

were chemoselectively removed by ANL and the resulting diesters **36a-c** selectively hydrolysed by pig liver esterase (PLE), to afford target glucuronides **37a-c**. In a latter study[38] these authors showed that the nature of the acyl group at C1 influenced LAS chemoselectivity, as exemplified with substrates **38** and **39** (**Scheme 8**): by screening seventeen enzymes for the removal of the acetates of **39**, the carboxylesterase from *Streptomyces rochei* (SRC) resulted the most chemoselective biocatalyst. Moreover (**Scheme 9**), in the step of C6-methyl ester deprotection, CAL-B exhibited more chemoselectivity and efficience than PLE, affording the corresponding 1-β-*O*-acyl glucuronides. Based on these results, a one-pot procedure to 1-β-*O*-acyl glucuronides **43a-c** was developed,[39] in which two enzymes were consecutively applied. The position of the phenyl substituent in the benzoyl moiety affected the chemoselectivity of *O*-deacetylation: ANL was highly chemoselective towards the *o*- isomer (**40a**), while SRC exhibited higher chemoselectivity towards the *m*- (**40b**); the satisfactory *O*-deacetylation of the *p*-isomer (**40c**) required the concurrent use of both enzymes. Additionally, in an early stage of the deacetylation step, it was found that ANL exhibited regioselectivity towards 3-*O*-deacetylation, allowing the isolation of the corresponding 2,4-di-*O*-acetylated products **41a-c** in 52-83% yields.

Moreover, (*S*)-naproxen-1-β-*O*-acyl glucopyranoside was obtained from its tetra-*O*-acetylated derivative by a SRC-catalysed hydrolysis.[38] In the screening of enzymes carried out, ANL and PLE resulted in low chemoselectivity while CAL-B was chemoselective but exhibited low activity. This contrasts to the behaviour of CAL-B towards (*S*)-naproxen derivative **35c** (**Scheme 7**), showing the influence of monosaccharide structure on enzyme recognition.

In relation to the use of non conventional reaction media for the enzymatic deacylation of pyranosic monosaccharides, a few examples deal with ionic liquids (ILs). In the

hydrolysis of the β-D-thioglucopyranoside **44** (**Scheme 10**)**,** the regioselectivity of CRL was modulated through the 1-butyl-3-methylimidazolium hexafluorophosphate [BMIM] [PF_6] content in the biphasic buffer-IL reaction mixture;[40] additional experiments showed that the IL does not prevent acetyl migration but influences CRL selectivity. The same IL was tested in *Pseudomonas cepacia* lipase (PCL)-catalysed hydrolysis of the glucal **20** (**Scheme 3**), giving 84% conversion towards the 3-hydroxy free product **23** and higher selectivity (98%) than hydrophilic [BMIM] [BF_4] and THF[41], although buffer as the only reaction medium gave comparable results.

On the other hand, Hernáiz and coworkers[42] have recently reported the application of *Pseudomonas stutzeri* lipase (PStL) to deacetylate chemoselectively the anomeric acetate of peracetylated β-D-pyranoses (**Scheme 11**). Wet solvents (10% v/v) derived from biomass, such as glycerol derivatives and dimethyl amide derivatives, were assayed as the reaction medium. Under these conditions, CAL-B afforded much worse results than PStL. No reaction took place when peracetylated α-D-glycopyranoses were tested; in this way, this enzyme exhibits potential in the resolution of anomeric mixtures. Moreover, results in **Scheme 11** show that structural variation at C2 strongly influences the activity of the enzyme. On the basis of molecular modelling and docking studies, it was explained that the arrangement of **5β** anomeric acetate in the neighbourhood of the catalytic Ser-109 site is favoured by a hydrogen bond between the Try-54 residue and the acetate carbonyl at C2; thus, the C2 substituent and its stereochemistry play a crucial role.

In addition to the deacetylation through hydrolysis involved in **Scheme 11**, PStL-catalysed alcoholysis of substrates **1β** and **5β** was also studied, exploiting the nucleophilic hydroxyl group of one of the assayed solvents. In this case, dry solvent was employed and almost quantitative conversions towards the corresponding 1-OH free

product were reached. Besides, conversions in the range 82-87% were obtained by testing simple alcohols such as 1-butanol and isopropanol.

2.1.2. Acylations

Many examples in the previous Section show that the configuration at the anomeric centre influences radically monosaccharide enzyme recognition and the regioselectivity of the biotransformation. Roberts and coworkers[43] profited lipase different recognition of α- and β-anomers to separate anomeric mixtures (**48-50 α,β**, **Scheme 12**), based on diasteroselective acylations. Depending on the anomer, a different acylation pattern was obtained, giving products of different degree of acylation and chromatographical mobility. Consequently, a separative procedure alternative to unsatisfactory chromatography of anomers or to unavailable glycosidases was developed. The examples in **Scheme 12** further depict both the influence of anomeric substituent structure (for ethyl α,β-D-glucopyranoside the β-anomer (**48β**) reacted with lower regioselectivy than the α- (**48α**), while this was inverse for benzyl α,β-D-glucopyranoside **49α,β**)) and of pyranose stereochemistry (CAL-B-catalysed butyrylation of benzyl β-D-glucopyranoside **49β** stopped at the monoacylation step (**54α** + **55β**), while it proceeded further to diacylation (**57β**) for benzyl β-D-galactopyranoside **50β**).

In a related strategy, the unsatisfactory separation of **58** epimers (**Scheme 13**) by silicagel column chromatography could be overcome by Sakakibara *et al.*[44] through an efficient diasteroselective enzymatic acylation. PCL acetylated quantitatively only the epimer bearing an equatorial hydroxyl, allowing the separation.

Monoacetylated derivatives of *N*-acetylhexosamines have interest as sugar acceptors, in transglycosylation reactions oriented to the synthesis of chitooligomers derivatives for application in inmunology. To obtain these precursors, Křen, Riva and coworkers[45] carried out the regioselective enzymatic 6-*O*-acylation of a set of *N*-acetylhexosamine pyranoses displaying low solubility in organic solvents (**60-62**, **Scheme 14**), by choice of the reaction conditions (solvent, enzyme, acylating agent). Taking gluco compound **60** as a reference substrate for the screening, proteases from various *Bacillus* species (subtilisins, EC 3.4.21.62) gave better results than lipases and 87% conversion into the 6-*O*-acetylated derivative (**63**) could be reached using an acetonitrile/DMSO 8:2 mixture as the reaction solvent. Under these reaction conditions, galacto and manno compounds **61** and **62** afforded respectively monoacetylated derivatives at C6 **64** and **65** in good yields. Further CAL-B-catalysed acetylation of the latter to give **66** showed the effect of the sugar stereochemistry on enzyme recognition, since **64** was unreactive.

In order to obtain potential inhibitors of a fungal β-*N*-acetylhexosaminidase, these researchers[46] have more recently reported the regioselective preparation of a set of novel 6-*O*-acyl derivatives (**68a-e**, **Scheme 15**) of the thiazoline **67** derived from glucosamine, as well as the diesters **69a,b**, although in lower yields.

Furthermore, Sundell and Kanerva[47] studied the regioselective preparation of sugar esters bearing a glyceric acid moiety at C6 (**74-77, Scheme 16**). Methyl α-D-glycopyranosides (**70-72**) and the same set of lipases (except CRL) applied to the methanolysis of peracetylated **26-28α** (Section 2.1.1**, Table 2**) was assayed. CAL-B (**Scheme 16**) was one of the enzymes affording most satisfactory results, contrasting with its performance in the deacetylation of **26-28α** (**Table 2**), and showing that the same biocatalyst can exhibit very different activities in pyranoside acylation and in peracylated pyranoside deacylation. Again, pyranoside ring stereochemistry influenced

the results, since only mannopyranoside **72** did not afford any diacetylated product, while some amounts (< 20%) of 2,6-di-*O*-acylated derivatives were formed from **70** and **71**. Further experiments on selective acylation at C6 were carried out using methyl α-D-galactopyranoside derivatives having higher solubility in organic solvent than **71**; in one case (**73**), a conversion of 79% into the glycerylated product (**77**) could be reached.

Enzymatic regioselective monosaccharide acylation has also been applied to prepare vinyl sugar esters as monomers, whose subsequent polymerization gives water soluble polymers and biocompatible hydrogels, suitable for biomedical and membrane applications (**Table 3**).[48-50] CAL-B and protease from *Bacillus subtilis* (BSP) were the biocatalysts and the screening of different experimental parameters (solvent, CAL-B content, excess of the acetylating agent, temperature, initial concentration of glucoside) allowed the quantitative formation of the 6-*O*-acryloyl and 6-*O*-methacryloyl derivatives of methyl β-D-glucopyranoside. Besides, vinyl sugar diesters have been also obtained (**Scheme 17**). Using BSP in anhydrous pyridine -other assayed enzymes and solvents gave less satisfactory results- and divinyl dicarboxylates as acylating agents, Lin and coworkers[51] acylated pyranoses at C-6, obtaining a set of polymerizable vinyl sugar diesters derived from D-glucose (**78-80α,β**), D-galactose (**81-83α,β**) and D-mannose (**84-86α,β**) in 35-85% yields. Additionally, the enzyme displayed stereoselectivity towards the α-anomer, which in some cases (**79-83**) was total (100% de). The authors also studied the effect of ultrasound on the previously commented reaction of D-glucose and found that higher power (120 W) of ultrasound irradiation at a continual operative procedure accelerated the biotransformations, allowing higher yields than by shaking.[52] Additionally, enzymatically obtained **83α** was chemically converted into a glycopolymer and incorporated into polyacrilonitrile using

electrospinning; the resulting material was used as support for covalent immobilisation of catalase.[53]

Debuigne and coworkers[54] applied CAL-B-catalysed regioselective esterifications of unprotected D-mannose to obtain regioselectively a 3-mercaptopropanoylated derivative at C6 in 48% yield, which after a subsequent thiol-Michael addition with α,β-insaturated carboxylic acids gave surface-active carbohydrate esters. By using hemifluorinated carboxylic acids a set of mannose derivatives fluorinated at C6 was prepared, which reduced the surface tension of water more efficiently than their non-fluorinated counterparts.[55]

A strategy based on enzymatic regioselective monosaccharide acylation has also been applied to functionalize polyhydroxyalkanoates with a glycosyl moiety, in order to obtain biomaterials with better biodegradability.[56] The biocatalyst LECI was employed and a 1:1 mixture of DMSO-chloroform was the most effective medium for the assayed biotransformation. Under these conditions, a 38% conversion towards 6-*O*-glucosyl-poly(3-hydroxyalkanoate) was reached.

In addition to the commercial lipases previously mentioned for pyranose acylation, a thermostable lipase from a bacterium of the genus *Streptomyces* (*Streptomyces thermocarboxydus* ME168) has been purified, characterized and immobilised on Celite. Its application to the regioselective acetylation of D-glucose in a mixture of tert-butanol-pyridine gave 6-*O*-acetyl-D-glucose in 93% conversion.[57]

As already discussed, selective recognition of secondary hydroxyl groups is particularly difficult. Raku and Tokiwa[58] screened proteases in the reaction of some 6-deoxypyranoses (**87-89**, **Table 4**), bearing only secondary hydroxyls, with divinyl adipate. Using proteases BSP and from *Streptomyces griseus* in DMF, conversions in

the range 61-97% towards acylated products at C2 (**90** and **91**) were reached. Addition of water accelerated reaction rate but, as predictable, had a detrimental effect on the yield. Although D- (**87**) and L-fucose (**88**) exhibited the same regioselectivity, sugar stereochemistry had effect on substrate recognition, since L-rhamnose (**89**) afforded by-products. For this substrate, a 59% conversion into the derivative at C4 (**92**) could be obtained with PSL in pyridine. The resulting vinyl sugars **90-92** were chemically polymerized to yield macromolecules having a number average molecular weight in the range 5000-14000.

Xylopyranoses are other examples of monosaccharides bearing only secondary hydroxyl groups. In the context of microbial carbohydrate esterases (CEs) with biotechnological interest outlined in Section 2.1.1, acetylxylan esterases (AcXEs, EC 3.1.1.72) are a group of CEs which deacetylate the plant cell wall polysaccharide xylan and they are involved in the degradation of cellulose and hemicelluloses of plant cell walls.[28] Partially acetylated xylopyranosides are useful as substrates to investigate the catalytic properties of AcXEs. In a screening of solvents of different polarity for the acetylation of 4-nitrophenyl β-D-xylopyranoside reported by Mastibuhová and Biely, PCL gave mixtures of the 2,4- and 3,4-di-*O*-acetates; using toluene as the biotransformation medium, these products were respectively obtained in 62% and 25% yield.[59] Low regioselectivity was obtained in the monoacetylation stage; it is worth mentioning that the isomeric 2,3-di-*O*-acetate **18β** could be obtained in almost quantitative conversion by a ANL-catalysed deacetylation (**Scheme 2,** Section 2.1.1). The group of Biely also assayed the ability of the AcXE from the wood-rotting fungus *Schizophyllum commune* (CE family 1) to catalyse acylations, testing methyl β-D-xylopyranoside in a microemulsion system consisting of hexane, vinylacetate, sodium

dioctylsulfosuccinate and water, obtaining above 60% conversion of the substrate but with low regioselectivity.[60]

CEs have also been tested for the acylation of monosaccharides other than xylopyranosides. In the case of the AcXE from the wood-rot fungus *Trichoderma reesei* (CE5 family), this enzyme was able to catalyse the acetylation of methyl- and *p*-nitrophenyl β-D-galactopyranoside in water at room temperature, yielding respectively 70% and 56% of the corresponding 3-*O*-monoacetylated products;[61] unprotected hexoses and pentoses reacted unselectively.

Besides, Topakas, Biely and coworkers[62] have assayed acetyl esterases from the saprophytic bacteria *Cellvibrio japonicus* (CJE) and *Clostridium thermocellum* (CTE) (CE family 2). As *Trichoderma reesei* AcXE, CJE and CTE also catalysed the acetylation of monosaccharides in aqueous medium, but displaying a regioselectivity different from that of typical CEs families:[28] D-glucose, D-galactose and D-mannose were acetylated at C6 in 71-89% conversions by CTE catalysis. In contrast, D-xylose remained unreacted.

Due to the poor solubility of underivatised monosaccharides in low polar solvents compatible with enzymes, alternative media for biocatalysed reactions have been searched. Among them, ionic liquids (ILs) have been studied over the last decade[63] and their application in the field of sugars has been reviewed, focusing especially on sugar fatty acid esters.[64] In the context of the present review and its covered period, Kim *et al*.[65] addressed regioselective secondary hydroxyl group differentation by studying in different reaction media CRL-catalysed acetylation of D-gluco- (**93α,β**; **Scheme 18**) and galactopyranoside derivatives (**94α,β**) blocked at C-6. In some cases, the reactions were much more regioselective towards 2-*O*-acetylation (**95**) in ILs than in organic solvents, as well as faster. In the acetylation of **93β** with the same enzyme in a set of

organic solvents of different hydrophobicity, lower regioselectivities than those attained by using the ILs in **Scheme 18** were again obtained.[66] On the other hand, highly regioselective 2-*O*-acetylation of **93,94α,β** has been reported by assaying CRL in neat vinyl acetate.[67] A reverse in C2-regioselectivity using CRL in supercritical CO_2 was reported by Palocci and coworkers: by appling a pressure of 20 Mpa, the 3-*O*-acetylated derivative **96β** was obtained from **93β** as the sole isomer and isolated in 90% yield.[68]

On the whole, results seem to depend strongly on the carbohydrate structure and on the IL: for instance, in lipase-catalysed acetylation of levoglucosan, acetonitrile afforded better results than the assayed ILs.[69]

2.2. Furanoside rings

In comparison to pyranosic rings, enzymatic deacylation and acylation of conformationally more flexible furanoses and furanosides had been studied to a lesser extent.[8,9]

2.2.1. Deacylations

Regioselectively acetylated furanosic rings bearing one free hydroxyl are building blocks for the synthesis of modified nucleosides and oligosaccharides. Interest on enzymatic access to these synthons can be appreciated in **Schemes 19-21** and **Table 5**.

Our research group has studied the enzymatic preparation of acetylated furanoses carrying free 5-hydroxyl and applied them as precursors for nucleoside enzymatic synthesis, by combining a phosphopentomutase and nucleoside phosphorylases.[70,71] A first study on CAL-B-catalysed deacetylation of peracetylated methyl α- (**97α**) and β-D-ribofuranoside (**97β**, **Scheme 19**) in ethanol showed a different recognition of each

anomer.[72] While **97α** was regioselectively deacetylated at C5, **97β** reacted unselectively, affording quantitatively fully deacetylated methyl β-D-ribofuranoside at long reaction time. Besides, the choice of the reaction medium resulted crucial: in contrast to alcoholysis, enzymatic hydrolysis of both anomers proceeded with low selectivity. A stereopreference towards the α-anomer was also observed in the alcoholysis of peracetylated alkyl α,β-D-ribofuranosides **97,98α,β** (**Scheme 19**), although substrate anomeric mixtures were enriched in the β-anomer. The length of the substituent at the anomeric centre also affected CAL-B regioselectivity, since propyl (**100α,β**) and butyl (**101α,β**) α,β-D-ribofuranosides yielded mixtures of partially acetylated compounds.[73]

By assaying the alcoholysis of chromogenic peracetylated aryl α,β-D-ribofuranosides **102-109α,β** (**Scheme 20**), Prasad, Parang and coworkers[74] have reported a highly stereo- and regiopreference of TLL towards α-anomer 5-deacetylation. CAL-B afforded less selective results, while CAL-B immobilised on Accurel and CRL did not provide selective results. The stereoselective TLL-catalysed deacylation has been exploited by the authors to obtain pure aryl α- (**110-117α**) and β-D-ribofuranosides (**102-109β**), which are difficult to be separated by conventional chromatographic procedures. Recently, this research group found the same stereo- and regioselective trend in TLL-catalysed alcoholysis of some peracylated aryl α,β-D-ribofuranosides and observed that reaction time decreased by increasing the acyl moiety lenght from acetyl to pentanoyl.[75]

Beyond substrates with ribo configuration, the stereochemistry of the furanose moiety had a marked effect on the selectivity of CAL-B alcoholysis and in some cases (**118-120α,β**, **Table 5**), products bearing free 5-hydroxyls (**121-123α**) were formed exclusively as the corresponding α-anomer (diasteromeric excess: 100%) through highly stereoselective deacetylations reported by our group.[76] In contrast, peracetylated

methyl α,β-D-xylofuranoside reacted with low stereoselectivity but gave regioselectively the C6 deacetylated product in 72% yield.[77]

In addition to previously discussed deacetylations involving alcoholysis, CRL-catalysed hydrolyses exhibited the regioselectivity of this enzyme towards the primary acetate of **124β**[32,78] (**Scheme 21**) and **125α**.[79] Complementary regioselectivities were obtained by a preparation of PFL immobilised on glutaraldehyde-agarose, which hydrolysed the C3 acetate of **124β**,[32] and by action on **125α** of *Rhizopus oryzae* esterase in buffer-solvent mixtures.[79] In this case, the regioselective removal of the C2 acetate was observed and using solvents such as acetone, n-hexane and dimethyl sulfoxide, conversions up to 91-100 % could be reached.

The effect of ionic liquids (ILs) on the selectivity of CAL-B- and CRL-catalysed hydrolysis of some peracetylated methyl α,β-D-furanosides was also studied; it depended on the furanose structure, the IL and its content in the hydrolysis mixture.[80] CRL in a 1:1 mixture of phosphate buffer (pH 7) and [BMIM] [PF_6] allowed the preparation of methyl 2,3-di-*O*-acetyl-α,β-D-arabinofuranoside from **125α,β** (**Scheme 21**) in 77% yield. On the other hand, *Musa sapientum* was assayed by our group as a cheap source of hydrolases for the deacetylation of furanoside rings and the selective outcome of these biotransformations showed a strong dependence on substrate structure.[81] With this biocatalyst, the 5-hydroxylated product from **97α** (**Scheme 21**) could be obtained in nearly quantitative yield.

2.2.2. Acylations and combination of acylations and deacylations

An example of regioselective enzymatic acylation of a D-ribofuranosic ring was reported by Nascimento and coworkers[82] and involves D-ribonolactone. It was

acetylated exclusively at C5 in 94% yield using CAL-B in dry acetonitrile, displaying again the preference of this lipase towards the less hindered hydroxyl group. TLL displayed the same regioselectivity but resulted less efficient and PCL afforded mixtures of products.

α-L-Arabinofuranosyl residues are widely distributed in plant cell wall polisaccharides, such as arabinan and arabinoxylans, and they are partially esterified by a feruloyl (3-methoxy-4-hydroxycinnamoyl) moiety. Prompted by the need of chromogenic substrates to investigate the catalytic properties of microbial glycosidases and previously mentioned CEs involved in biodegradation of plant cell walls (Section 2.1.2),[28] Biely and coworkers prepared all mono- and diacetates of p-nitrophenyl α-L-arabinofuranoside (**126**) using lipases[83] (**Scheme 22**). Regioselective 5-*O*-acetylation of **126** by porcine pancreas lipase (PPL) afforded **131** and CRL deacetylation of **127** primary acetate group gave diacetylated **130**. To prepare diacetylated regioisomers **128** and **129**, **126** was treated with an excess of vinyl acetate and a screening of enzymes and solvents was carried out. The polarity of the solvent affected PCL activity and reaction conversion, but it had little effect on the regioselectivity, contrasting with the results obtained by these researchers with p-nitrophenyl β-xylopyranoside (Section 2.1.2)[59]. Moreover, CRL-catalysed hydrolysis of **128** and **129** allowed a regioselective access to monoacetylated **132** and **133**, not available by direct enzymatic acetylation. Diacetylated isomers **128-130** were chemically feruloylated and subsequently chemically deacetylated to yield carbohydrate ferulates,[84] which were applied to the study of plant cell wall degradating enzymes.

In another example concerning biocatalytic acylation of L-arabinose and aiming at the preparation of a biodegradable polymer from it, the enzymatic synthesis of the involved monomer has been recently reported,[85] based on the BSP-catalysed reaction of L-

arabinose with divinyl adipate in DMF at 50 ºC. A mixture of 5-*O*-vinyladipoyl-α,β-L-arabinofuranose and 4-*O*-vinyladipoyl-α,β-L-arabinopyranose was isolated in 67% yield and subsequently submitted to radical polymerization to obtain a water soluble biodegradable copolymer.

Furthermore, Ferrero, Gotor and coworkers[86] have reported the enzymatic regioselective manipulation of 1,2-dideoxy-D-ribose (**134**, **Scheme 23**). Both PCL and CAL-B exhibited the same regiopreference in acylation reactions, giving the C3-hydroxylated products **136a,b** in excellent yields. Free 5-hydroxyl derivative **137** was obtained in excellent conversion and yield through hydrolysis catalysed by PCL immobilised on ceramic beads, which exhibited an unusual regioselectivity towards the C5 ester. Similar conversions were observed using *Chromobacterium viscosum* lipase and CAL-B, but the latter proceeded much slower; immobilised PCL was then chosen for scale-up experiments. It is worth remarking the same regioselectivity displayed by PCL and CAL-B, in contrast to PCL-catalysed hydrolysis of 3′,5′-di-*O*-acyl-β–D-2′-deoxynucleosides,[87] showing that the base moiety is crucial in substrate recognition by the enzyme. By further chemical transformation of the isomeric products **136b** and **137**, dimethoxytritylated monomers **138** and **139** were obtained, which are building blocks in the synthesis of therapeutical abasic site oligonucleotides.

Beyond previous examples, regioselective manipulation of the hexose D-fructose (**140**, **Scheme 24**) involves the additional problem posed by two primary hydroxy groups; most of previous enzymatic attempts to discriminate between them had been unsuccessfull. Nicolosi and coworkers[88] applied a combination of different lipases to obtain regioselectively a set of D-fructose acylated derivatives, based on acylation and deacylation reactions. In a screening of lipases in THF for acetylation, CAL-B acetylated regioselectively the primary hydroxyl groups, allowing the formation of **141**

in 95% yield without further conversion; RML, PCL and CRL gave no conversion or poor reactivity. **141** was converted into different products of further regioselective acylation, depending on the employed lipase: PCL and CRL recognised C-4 hydroxyl group with total regioselectivity, the latter affording products **142** and **143** in 90% yield, while product of interesterification **144** was obtained by RML or CAL-B catalysis (80% yield). In order to get fructose protected derivatives bearing a free hydroxyl group, chemically peracetylated **145**, obtained as a 1:1 mixture of α,β-anomers was submitted to enzymatic alcoholysis. Interestingly, the configuration at **145** anomeric centre influenced strongly the regioselectivity of the tested biocatalysts and the reactions were also highly stereoselective: depending of the anomer of **145**, CAL-B displayed a different regiopreference towards the primary acetate, yielding **146β** and **147α**. In contrast, CRL gave a different pattern, affording **146β** and unreacted **145α**, showing thus a regioselectivity (C-1 acetate) different to the observed in the assayed acylations (C-4 hydroxyl). Moreover, these authors subsequently converted chemically **142** into the hexoketose D-psicose.

Further examples concerning the troublesome discrimination of two primary hydroxyl groups focuse on 4-*C*-branched pentofuranose derivatives (**Scheme 25**), which are key intermediates in the synthesis of modified nucleosides, antisense oligonucleotides and locked nucleic acids (LNA).[89] Parmar, Prasad and coworkers aimed at diasteroselective transformations based on lipase catalysis: depending on the employed enzyme, **148** was converted into either the epimer **149** or **150**.[90] Furthermore, CAL-B showed inverse selectivity, affording **151**, when a bulkier acylating was employed instead of vinyl acetate. This reversal was not observed in CAL-B catalysed acylation of **152**[91] and the authors suggested an explanation for this behaviour in terms of the different configuration and substituent polarity at C3 in **148** and **152**: this would result in

different interactions with the active site of CAL-B for **148** when a bulkier acylating agent is used. Recently, in studies dealing with the chemoenzymatic synthesis of LNA monomers, the group of Prasad reported that CAL-B could be utilised efficiently for ten cycles in the preparation of **150**[92] and **153a**[93]; the enzymatic synthesis of the former was successfully scaled up to 5 g scale. In the case of **154**, the reaction depicted in **Scheme 25** conducting to **155** was the key step in the enzymatic synthesis of a polymer contaning a carbohydrate moiety, useful as carrier for a drug delivery system, and as part of the interest of Gross group in enzyme-catalysed preparation of monomers and polymers.[94] Besides, enzymatic acylation of **157a,b** was also studied.[95]

Additionally,[96,97] diasteroselective deacylation of peracylated furanosides **160a-c** (**Scheme 26**) was accomplished through a butanolysis catalysed by TLL. PPL, CRL, and CAL-B (immobilised on Lewatit and on Accurel) did not accept any of the assayed compounds as substrates. In order to confirm that isolated products **161a-c** did not arise from a deacylation of other groups followed by acyl migration, mixed esters **162b-e** were tested as substrates for TLL: only one product (**161a**) was formed in all cases, except for benzoylated **162e**, which did not result a substrate for the enzyme. In contrast to the lack of reactivity of CAL-B towards **160a-c**, substrates **163a-c** were diasteroselectively deacylated in excellent yields,[98] reflecting the dramatic effect of furanose substituents on CAL-B recognition. The authors discarded again the formation of **164a-c** through acyl migration; furthermore, **164a** was chemically converted into *C*-4′-spiro-oxetanoribonucleosides **165a,b**.

Peracetylated L-ribofuranose **174** (**Scheme 27**), a synthetic precursor for L-nucleosides, has been synthesised from D-lyxose by Sugai and coworkers,[99] by employing biocatalysts in two key steps of a strategy involving furanoses and pyranoses. To obtain the key intermediate of the synthesis (**167**), which bears the C-4 free hydroxyl of a

lyxose moiety, the peracetylated pyranosic form of D-lyxose was treated with *p*-methoxyphenol to give *p*-methoxyphenyl lyxopyranoside **166**, exclusively as the α-anomer. The researchers remarked structural similarity between **166** and *p*-nitrophenyl β-D-xylopyranoside and aimed at the desired regioselective deacetylation at C-4, inspired on the previously commented C-4 regioselective PCL-acetylation of *p*-nitrophenyl β-D-xylopyranoside reported by Mastihubová and Biely (Section 2.1.2).[59] Applying a butanolysis catalysed by PCL in toluene, low yield of **167** was obtained; change of 1-butanol by cyclopentanol raised the yield to 50%. With the latter nucleophile, use of CAL-B instead of PCL afforded the desired intermediate **167** in 97% yield; interestingly, *p*-methoxyphenyl group at C-1 showed to play an important role, since CAL-B-catalysed deacetylation of peracetylated D-lyxopyranose was less regioselective.

The needed inversion of the configuration at C-4 in **167** was subsequently accomplished chemically, affording the needed compound **168** along with a small amount of epimer **167**, which formed an inseparable mixture. This mixture was then chemically treated to remove the *p*-methoxyphenyl group, conducting to a complex mixture of furanosic and pyranosic forms (**169-172**, **Scheme 27**). The key furanose **170** could be obtained free from contaminating pyranoses **169** and **171** and undesired C-4 epimeric ribose (**172**) by a selective CAL-B-catalysed acetylation, which yielded **173**. The application of CAL-B to distinguish between furanosic and pyranosic forms of a pentose has been reported by Prasad *et al.*[100] for the regioselective 5-*O*-acylation of 2-deoxy-D-ribose in high yield and further applied by our research group[76] to obtain 5-*O*-acetylated-D-pentoses from D-ribose and D-arabinose, without formation of acylated pyranosic counterparts. Finally, the last step of the synthesis consisted of a conventional chemical acetylation, providing target peracetylated L-ribose **174**.

3. Conjugation of monosaccharides with bioactive molecules through enzymatic selective acylation and deacylation

Following examples present the potential of enzymatic regioselective acylation of monosaccharides as a simple access to prodrugs, modifying the water solubility and biodisponibility of the parent drug.

3.1. Aminoacid conjugates

Many carbohydrate-peptide conjugates possess therapeutic value and some α-aminoacid esters of carbohydrates are found among glycopeptides having biological activity. Few studies have been reported dealing with enzymatic sugar aminoacid ester synthesis,[8] which presents the drawback of the polar medium required to solubilise sugars and aminoacids, what tends to enzyme damage. Due to the limited available examples, these will cover the period from 2001.

Applying the protease from *Bacillus lentus*, Davis and coworkers[101] reported the regioselective 6-*O*-acylation of free D-hexoses (D-manno-, D-gluco-, D-galacto and *N*-acetyl-D-gluco-) and different glycosides (methyl, thiophenyl and selenophenyl) of the mentioned hexoses. Results depended crucially on carbohydrate structure. *N*-Protected L-α-aminoacid vinyl esters were employed as acylating agents in anhydrous pyridine at 45 ºC and on the whole, 23-76% yields of the 6-acylated derivatives were obtained. Among the assayed aminoacid (phenylalanine, aspartic acid and glutamic acid) vinyl esters, only *N*-protected phenylalanine derivative was reactive; a similar trend was found when cross-linked enzyme crystals of the protease thermolysin were tested.

Among the assayed *N*-protected phenylalanine vinyl esters, the *N*-acetylated ones afforded the best results.

Also based on a subtilisin, Goto and coworkers[102] prepared and assayed surfactant-enzyme complexes for the acylation of a set of carbohydrates. In a screening of different lipase- and protease-surfactant complexes for the regioselective 6-*O*-acylation of D-glucose with *N*-acetylphenylalanine trifluoroethyl ester in pyridine, the *Bacillus licheniformis* protease -surfactant complex showed the highest activity; this preparation was also active in all the polar solvents tested (pyridine, tert-butanol, DMSO). Employing the cyanomethyl ester of several *N*-aceylated L-α-aminoacids (phenylalanine, leucine, methionine, tryrosine and tryptophan) in tert-butanol containing 10% DMSO at 40 ºC, D-glucose was regioselectively 6-*O*-acylated in 65-71% conversions. Other tested monosaccharides (D-galactose, D-mannose and D-fructose) gave conversions in the range 59-68%, while lower reactivity was obtained from methyl α- and β-glucopyranoside (7% and 28% respectively).

On the other hand, Lohith *et al.*[103,104] assayed the reaction of a wide set of L-α-aminoacids with monosaccharides (D-ribose, D-fructose, D-galactose and D-mannose) catalysed by CRL and RML in 9:1 mixtures of buffer-organic solvents, obtaining mixtures of partially acylated carbohydrate esters in low yields, probably due to the reaction medium favouring hydrolysis. More recently,[105] the use of CAL-B in tert-butanol-DMSO 9:1 to catalyse the esterification of D-glucose with L-cysteine has been reported by Valepyn and coworkers. The effect of the initial water content on the conversion was studied: although at a 0.2% v/v content a 64% conversion was reached, a mixture of partially acylated derivatives was formed.

Beyond α-aminoacids, other aminoacids were also assayed. The delivery of the neurotransmisor γ-aminobutyric acid (GABA) into the nervous central system is

facilitated by its linkage to a glucose moiety; De Torres and Mayoralas[106] described a chemoenzymatic polymer-supported liquid phase synthesis of glucose 6-*O*-(4-aminobutyryl) ester (**178, Scheme 28**). Prior to the synthesis, benzyl β-D-glucopyranoside (**49β**) was taken as a model of glucosylated linker and its enzymatic acylation by lipases was studied, since chemical esterification of **49β** with *N*-Cbz-protected GABA gave a mixture of esters. The trichloroethyl ester of *N*-Cbz-protected GABA was employed as the acylating agent and after a screening of biocatalysts (CAL-B, PPL and PCL), best results were found with CAL-B in acetonitrile with 4 A molecular sieves, affording exclusively the 6-*O*-acyl derivative **175** in 82% yield. Extension of the methodology to the glucosyl polymer **176** was conducted in toluene and gave the acetylation product **177** with the desired regioselectivity at C6 in 84%; subsequent hydrogenolysis accomplished the cleavage of the polymeric support employed for the synthesis and the Cbz protecting group, giving the target glycoconjugate **178**.

More recently, Kanerva and coworkers[107] have recently studied the regioselective enzymatic synthesis of sugar-β-aminoacid conjugates (**180a,b, Scheme 29**). In a first screening of experimental parameters (lipase, solvent) using methyl α-D-galactopyranoside (**71**) and β-lactams **179a-c** as acyldonors, PCL and tert-amyl alcohol gave most satisfactory results and were chosen for further experiments. Extension of the methodology to methyl α-D-glucopyranoside conducted to lower product formation, while methyl α-D-mannopyranoside gave no appreciable conversion; in all cases, no product was formed using the β-lactam **179c**.

3.2. Other conjugates

Besides aminoacids, diverse bioactive molecules have been conjugated to monosaccharides through enzymatic acylation. An example is arbutin (**181**, **Scheme 30**), an inhibitor of tyrosinase, an enzyme involved in the formation of melanin and hyperpigmentation in the epidermis. Its 6-*O*-undecenoyl ester (**182a**)[108-110] showed a higher antimelanogenesis activity than arbutin in murine melanome cells.[108] Additionally, a set of arbutin aromatic esters at C6 were regioselectively obtained in a wide range of yields;[111] in particular, the 6-*O*-ferulate **182b** exhibited higher antioxidant activity than its precursors.[112] Low yields obtained with aromatic acylating agents bearing a phenolic moiety reflect again (**Scheme 5,** Section 2.1.1) the low affinity of many lipases towards phenolic aromatic carboxylic acids.

Regarding to interest on modification of biomass-derived antioxidanting agents, such as ferulic acid, hydrolases have also been assayed to obtain monosaccharide ferulates; a recent review deals with the application of hydrolytic enzymes in the acylation of carbohydrates with hydroxycinnamic acids.[113] **Table 6** shows the regioselective feruloylation of a set of assayed furanoses and pyranoses; a feruloyl esterase (EC 3.1.1.73; Entries 1 and 4), commercial multienzyme preparations containing feruloyl esterases (Entries 3, 5 and 8) and the lipase TLL (Entries 2, 6 and 7) were employed as biocatalysts. Moreover, Mastihubová and Mastihuba[119] extended more recently TLL-catalysed regioselective 6-*O*-acylation of methyl α-D-glucopyranoside to a wide set of phenolic and non phenolic acylating agents. For the former (vinyl hydroxybenzoates, hydroxycinnamates and hydroxyphenylalkanoates), yields in the range 5-77% were attained, while the latter (vinyl benzoates, cinnamates and two heterocycles analogues) conducted to 52-79% yields along with 2-13% of 2,6-di-*O*-acylated products.

On the other hand, recent applications of the chemoselective behaviour of lipases involve the synthesis of D-glucosylated and D-xylosylated aromatic acid derivatives

with potential interest as antioxidanting agents through a CAL-B-catalysed methanolysis (**Scheme 31**). Shimotori and coworkers[120,121] have reported the selective formation of glucosylated products dideacetylated at C4 and C6 (**184a-d**) and xylosylated monodeacetylated products at C4 (**186a-e**); overall, yields in the range 65-98% were attained.

Hydrolases have also been applied to conjugate non-steroidal anti-inflammatory (NSAI) drugs with monosaccharides. Unlike examples presented in **Schemes 7-9** (Section 2.1.1), where the target was the synthesis of NSAI-1-β-*O*-acylglucuronides, in the following examples a simple access to NSAI water-soluble prodrugs was pursued. Water-soluble (*S*)-ketoprofen derivatives (**190**,**191**; **Scheme 32**)[122] were obtained in high conversions through a protease-catalysed regioselective reaction of (*S*)-ketoprofen vinyl ester (**189**) with a hexose moiety -among other tested carbohydrates-. Preparation of a glucopyranosyl derivative of ibuprofen was reported through a CAL-B-catalysed esterification of methyl α-D-glucopyranoside with the racemic drug in acetonitrile: the ester at C6 was obtained in about 50% yield and 49% optical purity.[123,124]

Villo and coworkers[125] developed a chemoenzymatic approach for the stereoselective preparation of pyranosic 3,4-dideoxy-D-ribose esters (**192** and **193**, **Scheme 33**), based on the combination of CAL-B-catalysed acetylation and deacetylation reactions. This strategy allowed obtaining the target stereoisomeric esters **192** and **193** –both as an equilibrium mixture of anomers- in high enantiomeric excess. The methodology depicted in **Scheme 33** was also applied to prepare 3,4-dideoxyribose conjugates with carboxylic acids, such as deoxycholic acid, prostaglandin $F_{2\alpha}$ and (*S*)-mandelic acid; it was further extended to furanosic rings, obtaining esters of 3-deoxy-D-erythrose in high stereochemical purity,[126] although in lower yields than with pyranose deoxy sugars.

Enzymatic regioselective acylation of monosaccharides with diesters has been exploited to get a facile access to polyhydroxylated hybrid compounds, in which a bioactive molecule and a sugar moiety are linked by a dicarboxylate moiety, resulting in a hybrid with enhanced properties or efficacy. Following a pioneering work from the group of Dordick,[127] in which paclitaxel was linked to glucose in two steps by thermolysin-catalysed reaction with vinyl adipate, Lin and coworkers[128] obtained *N*-substituted imidazole derivatives containing a glucose branch (**194a-c**, **Scheme 34**). The strategy consisted of a one-pot synthesis, in which the two steps were catalysed by BSP; in addition to acylation catalysis, the enzyme showed a promiscuous Michael addition activity. In a variant of the above mentioned approach, the same research group[129] prepared saccharide derivatives (**197, 198**) of the antimicrobial agent metronidazole (**195, Scheme 35**): in this case, the best results were found through two steps catalyzed by different hydrolases. The general strategy of linking a drug with a monosaccharide through a dicarboxylate moiety was applied to water insoluble drugs, obtaining amphipatic prodrugs of muscle relaxants mephenesin and chlorphenesin,[130] β-blockers such as chlorprenalin[131] and metoprolol[132]: some representative examples (**207-213**) are depicted in **Scheme 36**. Moreover, exploiting the regioselectivity displayed by hydrolases in the acylation of nucleosides,[133-135] Lin and coworkers also prepared nucleoside-sugar conjugates (**216, Scheme 37**) from antitumour nucleosides 5-fluorouridine (**214**)[136] and floxuridine.[137]

Furthermore, Riva and coworkers[138] linked two monosaccharide moieties through a dicarboxylate moiety, in a two-step procedure catalysed by CAL-B; some representative examples (**221, 222**) are shown in **Scheme 38**. This strategy was also employed to obtain a set of novel polyhydroxylated hybrid compounds, such as conjugates of bioactive thiocolchicoside and cortisone.

4. Conclusion

This review intends providing an update of research on biocatalytic acylation and deacylation of monosaccharides, exploiting the selective behaviour of hydrolases and their advantages as green catalysts. Examples presented and discussed in Sections 2 and 3 witness current interest on application of lipases, proteases and esterases in the field of monosaccharide chemistry, and allow to appreciate the opportunities provided by biocatalysts in the synthesis of PAMs.

On the whole, examples through the review further confirm that enzyme recognition and selectivity may be dramatically affected not only but the biocatalyst itself but also by parameters such as monosaccharide stereochemistry, anomeric substituent structure and stereochemistry, and engineering of the reaction medium. Since prediction remains quite limited in this field, update of accomplished advances is useful to compilate the battery of tools available in this area of research, integrating the present work with valuable previous reviews in this subject.

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Table 1. Enzymes most frequently mentioned through this review

Abbreviation	Enzyme
ANL	*Aspergillus niger* lipase
BSP	Protease from *Bacillus subtilis*
CAL-B	*Candida antarctica* lipase B[1]
CRL	*Candida rugosa* lipase[2]
LECI	Phospholipase Lecitase[3]
PLE	Pig liver esterase
PPL	Porcine pancreatic lipase
PCL	*Pseudomonas cepacia* lipase[4]
SRC	Carboxylesterase from *Streptomyces rochei*
TLL	*Thermomyces lanuginosus* lipase[5]
RML	*Rhizomucor miehei* lipase[6]

[1]Frequently designed by commercial names (Novozym 435, SP 435). [2]Formerly *Candida cylindracea* lipase. [3]This enzyme preparation is obtained from the fusion of the genes of *Thermomyces lanuginose* lipase and *Fusarium oxysporum* phospholipase and possesses phospholipase A_1 activity. [4] Owing to the successive classifications of the microorganism, the denominations lipase from *Pseudomonas fluorescens*, *Pseudomonas cepacia* and *Burkholderia cepacia* currently coexist in the literature. The enzyme is frequently designed by commercial names (lipase PS, lipase PS-C (immobilised on ceramic), lipase PS-D (immobilised on Celite), lipase PS-30, etc). [5]Formerly *Humicola lanuginosus* lipase. [6]Formerly *Mucor miehei* lipase.

Table 2- Lipase-catalysed regioselective methanolysis of peracetylated methyl α-D-glycopyranosides **26-28α** (Reference 27)

Substrate	Conversion to the C6-free hydroxyl derivative (%)					
	Lipase[1]					
	PS-D	PS-C II	TLL	CAL-B	CAL-A	CRL
Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**26α**)	6	6	69	0	17	64
Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**27α**)	80	72	95	14	10	15
Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**28α**)	0	0	92	36	0	28

[1]PS-D: *Burkholderia cepacia* adsorbed on Celite. PS-C II: *Burkholderia cepacia* lipase immobilised on ceramic. TLL: *Thermomyces lanuginosus* lipase. CAL-B: lipase B from *Candida antarctica*. CAL-A: lipase A from *Candida antarctica*. CRL: lipase from *Candida rugosa*.

Table 3- Sugar vinyl esters obtained by enzyme-catalysed regioselective acylation

Substrate	Product (sugar vinyl ester)	Conv.[1] (%)	Enzyme	Acylating agent; solvent, temperature	Reference
D-Glucose	6-*O*-(10-Undecenoyl)-D-glucose	64	BSP[2]	10-Undecenoic acid vinyl ester; MeCN, 27 °C	48
Methyl β-D-glucopyranoside	Methyl 6-*O*-acryloyl-β-D-glucopyranoside	100	CAL-B[3]	Vinyl acrylate; tBuOH, 50 °C	49
Methyl β-D-glucopyranoside	Methyl 6-*O*-methacryloyl-β-D-glucopyranoside	100	CAL-B	Vinyl methacrylate; tBuOH, 50 °C	49
Methyl β-D-glucopyranoside	Methyl 6-*O*-acryloyl-β-D-glucopyranoside	59	CAL-B	Acrylic acid; tBuOH, 50 °C	50
Methyl β-D-glucopyranoside	Methyl 6-*O*-methacryloyl-β-D-glucopyranoside	71	CAL-B	Methacrylic acid; tBuOH, 45 °C	50

[1]Conversion. [2]*Bacillus subtilis* protease. [3]*Candida antarctica* lipase B.

Table 4- Enzymatic regioselective acylation of 6-deoxypyranoses **87-89** with divinyl adipate at 30 °C (Reference 58)

Substrate	Product	Conv.[1] (%)	Enzyme	Solvent
D-Fucose (**87**)	2-*O*-Vinyladipoyl-D-fucose (**90**)	90 97	SGP[2] BSP[3]	DMF DMF
L-Fucose (**88**)	2-*O*-Vinyladipoyl-L-fucose (**91**)	61	BSP	DMF
L-Rhamnose (**89**)	4-*O*-Vinyladipoyl-L-rhamnose (**92**)	59	PSL[4]	Pyridine

[1]Conversion. [2]*Streptomyces griseus* protease. [3]*Bacillus subtilis* protease. [4]*Pseudomonas sp* lipase.

Table 5- *Candida antarctica* lipase B (CAL-B)-catalysed regio- and stereoselective ethanolysis of peracetylated D-furanosides and furanoses **118-120α,β** (Reference 76)

Substrate	Product	de[1] (%)	α-Anomer recovery (%)	Solvent
1,2,3,5-Tetra-*O*-acetyl-α,β-D-arabinofuranose (**118α,β**)	1,2,3-Tri-*O*-acetyl-α-D-arabinofuranose (**121α**)	100	69	EtOH-DMF
Methyl 3,5-di-*O*-acetyl-2-deoxy-α,β-D-ribofuranoside (**119α,β**)	Methyl 3-*O*-acetyl-2-deoxy-α-D-ribofuranoside (**122α**)	100	67	EtOH
1,3,5-Tri-*O*-acetyl-2-deoxy-α,β-D-ribofuranose (**120α,β**)	1,3-Di-*O*-acetyl-2-deoxy-α-D-ribofuranose (**123α**)	100	58	EtOH-CH_2Cl_2

[1]Diasteromeric excess

Table 6- Enzymatic regioselective feruloylation of furanoses and pyranoses

Substrate	Product (%)	Biocatalyst	Acylating agent, solvent, T	Reference
L-Arabinose	5-*O*-Feruloyl-L-arabinose (40[1])	STFE[2]	Methyl ferulate; hexane-tBuOH-buffer pH 6; 35 ºC	114,115
Methyl α-L-arabinofuranoside	Methyl 5-*O*-feruloyl-α-L-arabinofuranoside (90[3])	TLL[4]	Vinyl ferulate; methyl isobutyl ketone; 27 ºC	116
D-Arabinose	5-*O*-Feruloyl-D-arabinose (37[1])	MBA[5]	Ferulic acid; hexane-nBuOH-buffer pH 6; 35 ºC	117
D-Arabinose	5-*O*-Feruloyl-D-arabinose (45[1])	STFE	Methyl ferulate; hexane-tBuOH-buffer pH 6; 35 ºC	118
D-Xylose	5-*O*-Feruloyl-D-xylose (31[1])	MBA	Ferulic acid; hexane-butanone-buffer pH 6; 35 ºC	117
Methyl α-D-glucopyranoside	Methyl 6-*O*-feruloyl-α-D-glucopyranoside (64[3])	TLL	Vinyl ferulate; acetonitrile, 27 ºC	116
n-Pentyl β-D-galactopyranoside	n-Pentyl 6-*O*-feruloyl-β-D-galactopyranoside (88[3])	TLL	Vinyl ferulate; methyl isobutyl ketone; 27 ºC	116
D-Galactose	6-*O*-Feruloyl-D-galactose (61[1])	DTR[6]	Ferulic acid; hexane-nBuOH-buffer pH 6; 35 ºC	117

[1]Conversion. [2]*Sporotrichum thermophile* type C feruloyl esterase. [3]Yield. [4]*Thermomyces lanuginosus* lipase. [5]Multifect P3000 from *Bacillus amyloliquefaciens*. [6]Depol 670 from *Trichoderma reesei*.

Captions of Schemes

Scheme 1 – *Candida rugosa* lipase (CRL)-catalysed preparation of a precursor (**2α**) of the synthesis of D-glucose-6-phosphate (**4**)

Scheme 2 – Lipase selectivity modulation through different immobilisation techniques applied to the deacetylation of peracetylated pyranoses and pyranosides

Scheme 3 – Application of biocatalysts obtained through different immobilisation techniques to C6- and C3- monodeacetylation of glycals **20** and **21**

Scheme 4 – CRL-catalysed hydrolysis of perbenzoylated methyl α-D-glycopyranosides (buffer pH 4.8, 20% dioxane, 30-40 ºC)

Scheme 5 – Chemoenzymatic synthesis of Buprestins by applying *Candida antarctica* lipase B (CAL-B)-catalysed selective hydrolyses

Scheme 6 – Preparation of the mannosylerythritol lipid analogue **34** through a CAL-B-catalysed selective alcoholysis

Scheme 7 – Enzymatic synthesis of 1-β-*O*-acylglucuronides **37a-c**

Scheme 8 – Enzymatic selective hydrolysis of 1-β-*O*-acylglucuronide precursors **38** and **39**

Scheme 9 – Enzymatic synthesis of 1-β-*O*-acylglucuronides **43a-c** and selective 3-*O*-monodeacetylation (**41a-c**) of their precursors

Scheme 10 – Modulation of CRL regioselectivity in the hydrolysis of the β-D-thioglucopyranoside **44** through the 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIM.PF_6$) content

Scheme 11 – *Pseudomonas stutzeri* lipase-catalysed 1-*O*-deacetylation of peracetylated β-D-glycopyranoses in solvent-water 9:1 mixtures at 30 ºC

Scheme 12 – Lipase-catalysed regio- and stereoselective acylation of anomeric mixtures of D-glycopyranosides **48-50α,β**

Scheme 13 – Separation of the epimers of **58** by a selective PCL (*Burkholderia cepacia* lipase)-catalysed acylation

Scheme 14 – Enzymatic regioselective acetylation of *N*-acetylhexosamines **60-62**

Scheme 15 – CAL-B-catalysed regioselective acylation of the thiazoline **67** derived from D-glucosamine

Scheme 16 – Lipase-catalysed regioselective obtention of esters of methyl α-D-glycopyranosides bearing a gliceric acid moiety (**74-77**)

Scheme 17 – Polymerizable vinyl fatty sugar esters (**78-86α,β**) obtained by *Bacillus subtilis* protease-catalysed reaction of free D-pyranoses with divinyl carboxylates in pyridine at 50 ºC

Scheme 18 – Effect of the reaction medium on CRL-catalysed acetylation of methyl 6-*O*-trityl-D-glycopyranosides **93-94α,β**

Scheme 19 – CAL-B-catalysed ethanolysis of peracetylated alkyl D-ribofuranosides **97-101**

Scheme 20 – *Thermomyces lanuginosus* lipase (TLL)-catalysed regio- and stereoselective butanolysis of peracetylated aryl α,β-D-ribofuranosides **102-109α,β**

Scheme 21 – Lipase-catalysed hydrolysis of peracetylated D-furanoses and furanosides

Scheme 22 – Lipase-catalysed preparation of the mono- and diacetylated derivatives of p-nitrophenyl α-L-arabinofuranoside (**126**)

Scheme 23 – Regioselective preparation of monoacylated derivatives (**136, 137**) of 1,2-dideoxy-D-ribose (**134**) by *Pseudomonas cepacia* lipase (PCL)-catalysed reactions

Scheme 24 – Regio- and stereoselective differentiation of D-fructose (**140**) hydroxyls by lipase-catalysed reactions

Scheme 25 – Lipase-catalysed stereoselective acylation of 4-*C*-branched pentofuranose derivatives

Scheme 26 – Lipase-catalysed stereoselective alcoholysis of 4-*C*-branched pentofuranose peracylated derivatives (**160**, **162**, **163**)

Scheme 27 – Chemoenzymatic synthesis of peracetylated L-ribofuranose (**174**)

Scheme 28 – Regioselective synthesis of the GABA (γ-aminobutyric acid) derivative of D-glucose (**178**)

Scheme 29 – PS-D (*Burkholderia cepacia* lipase immobilised on Celite)-catalysed regioselective enzymatic synthesis of sugar-β-aminoacid conjugates (**180**)

Scheme 30 – Enzymatic acylation of arbutin (**181**). BSP: *Bacillus subtilis* protease. PEL: *Penicillum expansum* lipase

Scheme 31 – CAL-B-catalysed regioselective alcoholysis of glycosylated aromatic acid derivatives **183** and **185**

Scheme 32 – Enzymatic conjugation of (*S*)-ketoprofen with monosaccharides

Scheme 33 – CAL-B-catalysed synthesis of 3,4-dideoxy-D-ribose esters **192** and **193**

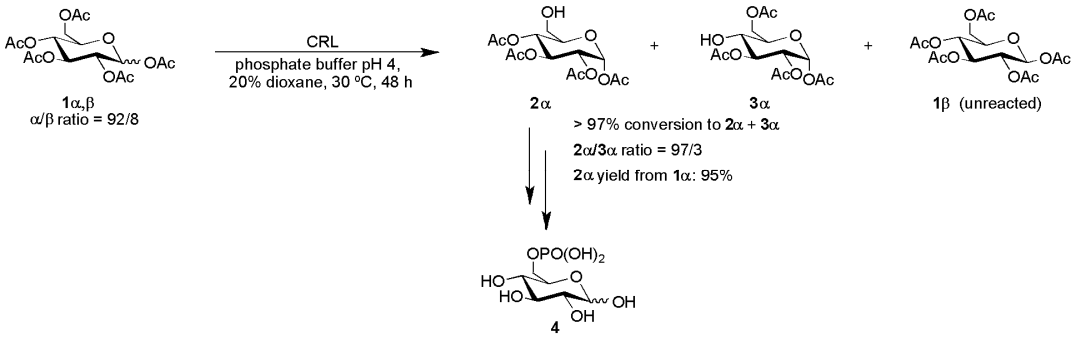
Scheme 34 – BSP-catalysed conjugation of imidazole and imidazole derivatives with D-glucose

Scheme 35 – BSP-catalysed conjugation of metronidazole (**195**) with D-hexoses

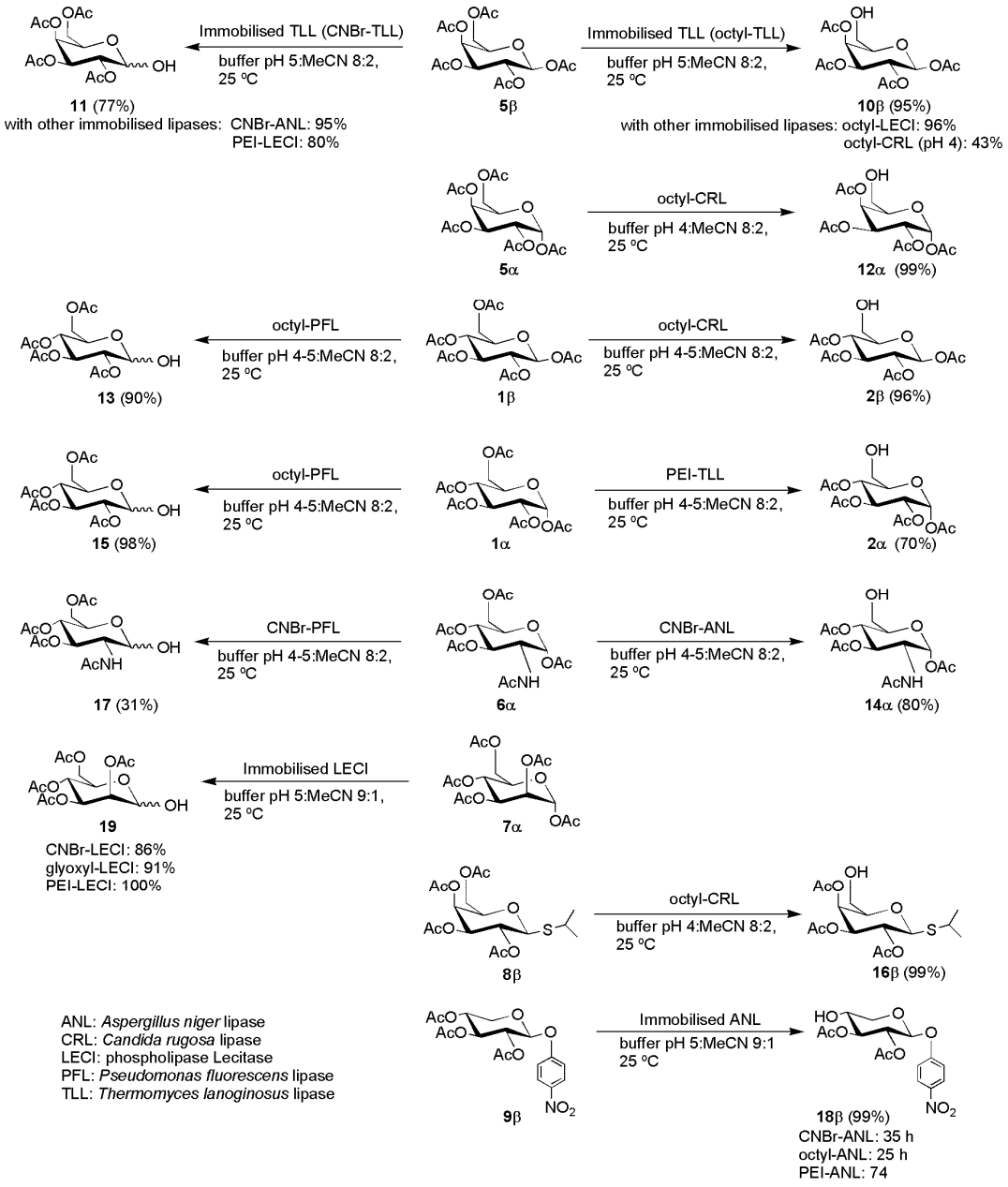
Scheme 36 – Enzymatic conjugation of drugs **199-201** with D-hexoses

Scheme 37 – Preparation of a nucleoside-sugar conjugate (**216**) through hydrolase-catalysed acylations

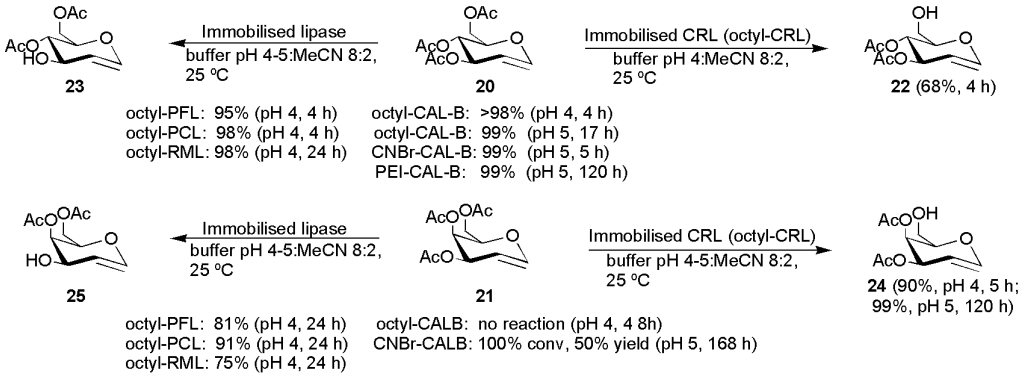
Scheme 38 – Linking of monosaccharides through CAL-B-catalysed acylation with divinyl esters



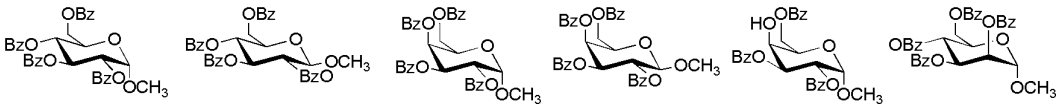
Scheme 1



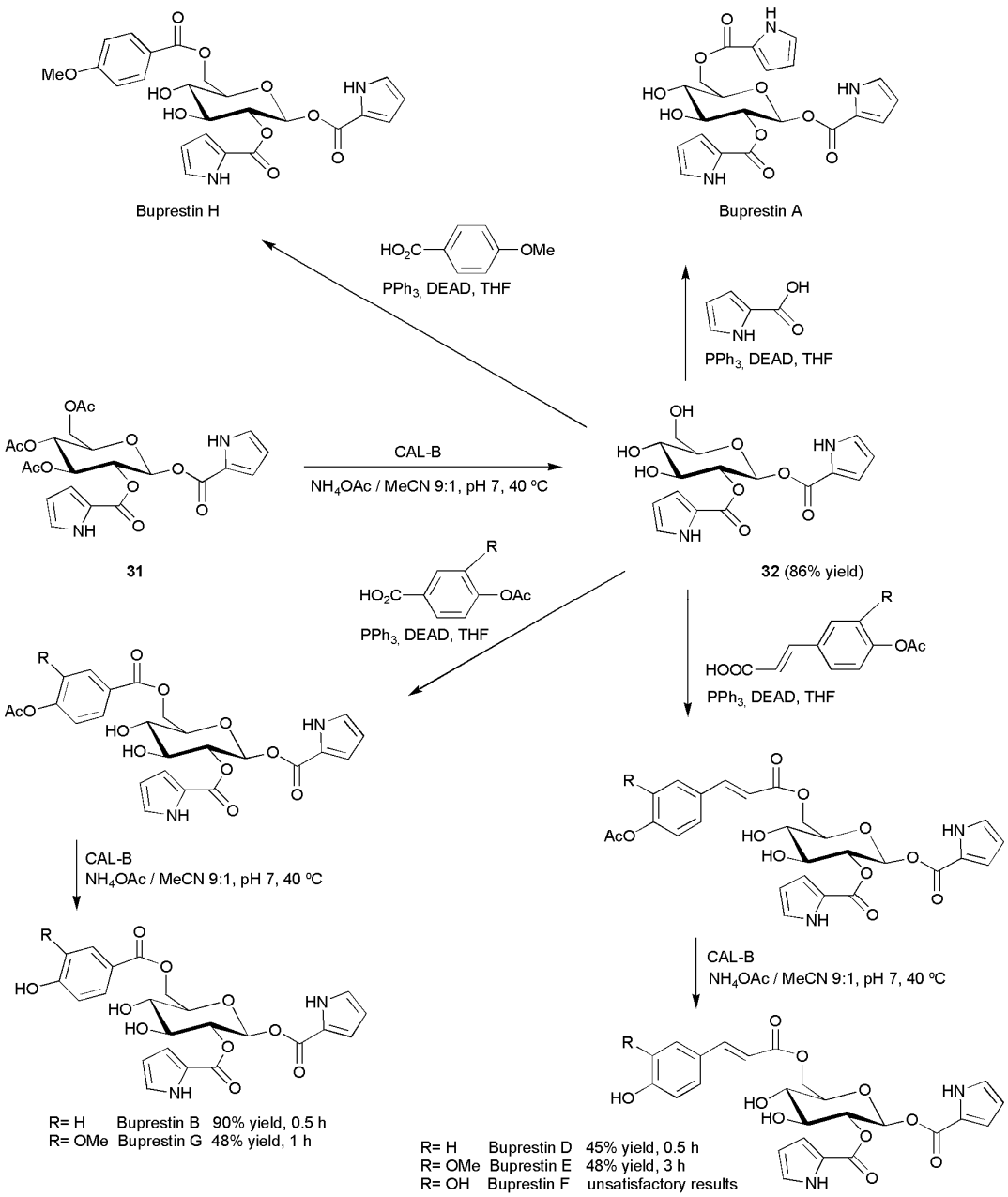
Scheme 2



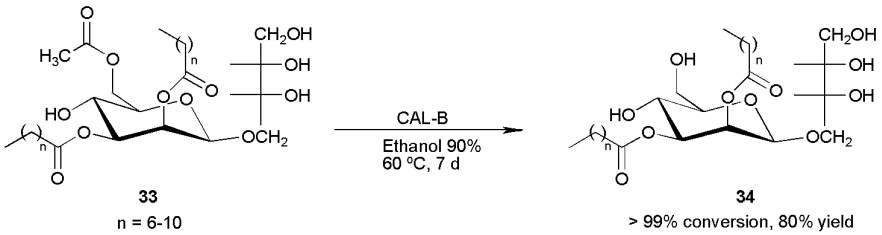
Scheme 3



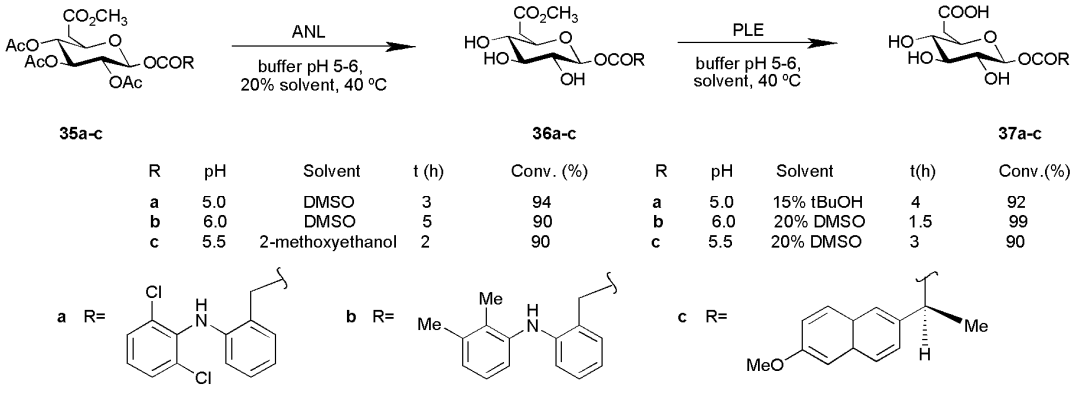
Scheme 4



Scheme 5

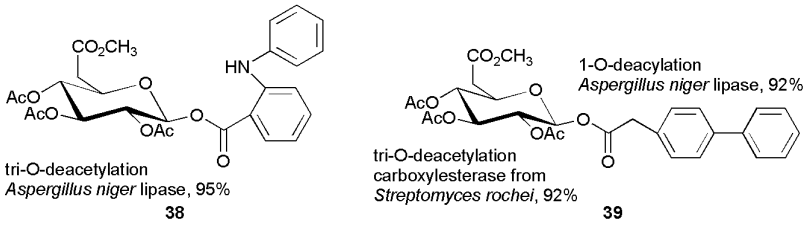


Scheme 6

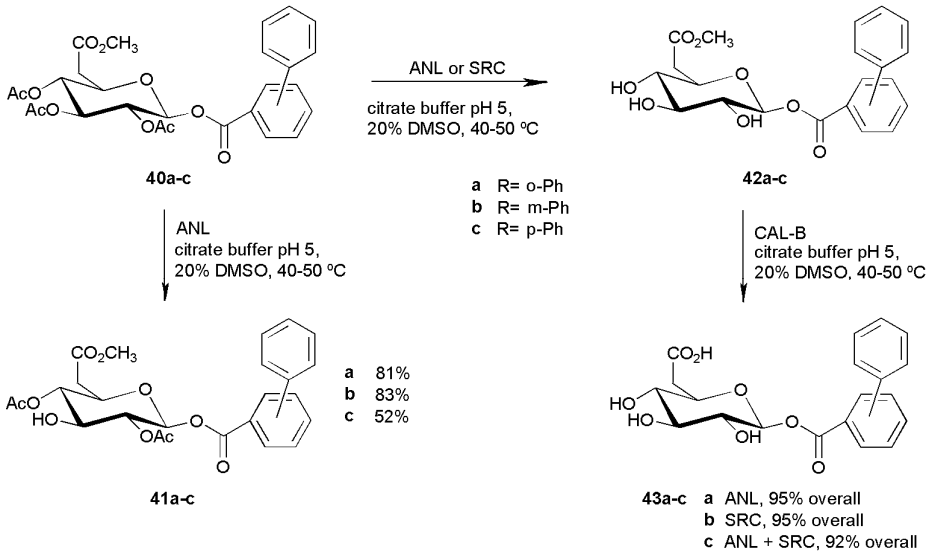


	R	pH	Solvent	t (h)	Conv. (%)	R	pH	Solvent	t(h)	Conv.(%)
	a	5.0	DMSO	3	94	**a**	5.0	15% tBuOH	4	92
	b	6.0	DMSO	5	90	**b**	6.0	20% DMSO	1.5	99
	c	5.5	2-methoxyethanol	2	90	**c**	5.5	20% DMSO	3	90

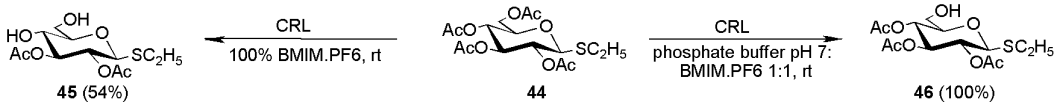
Scheme 7



Scheme 8

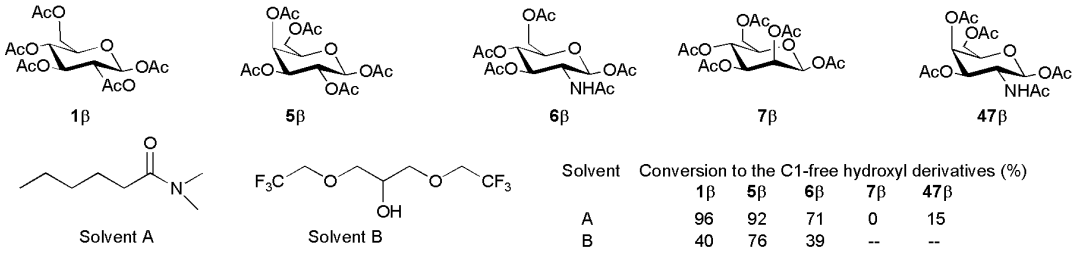


Scheme 9



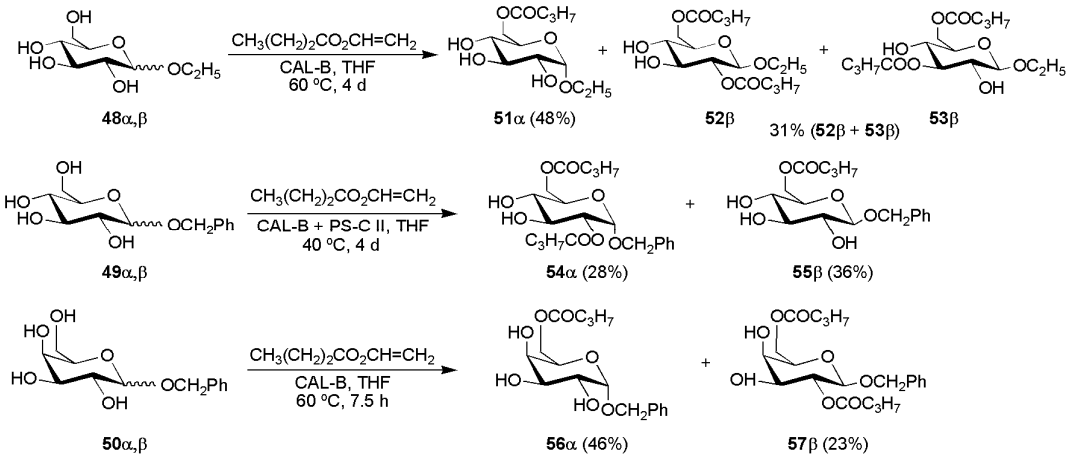
45 (54%) **44** **46** (100%)

Scheme 10

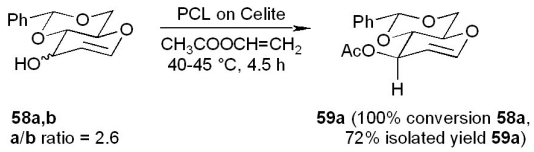


Solvent	Conversion to the C1-free hydroxyl derivatives (%)				
	1β	**5**β	**6**β	**7**β	**47**β
A	96	92	71	0	15
B	40	76	39	--	--

Scheme 11



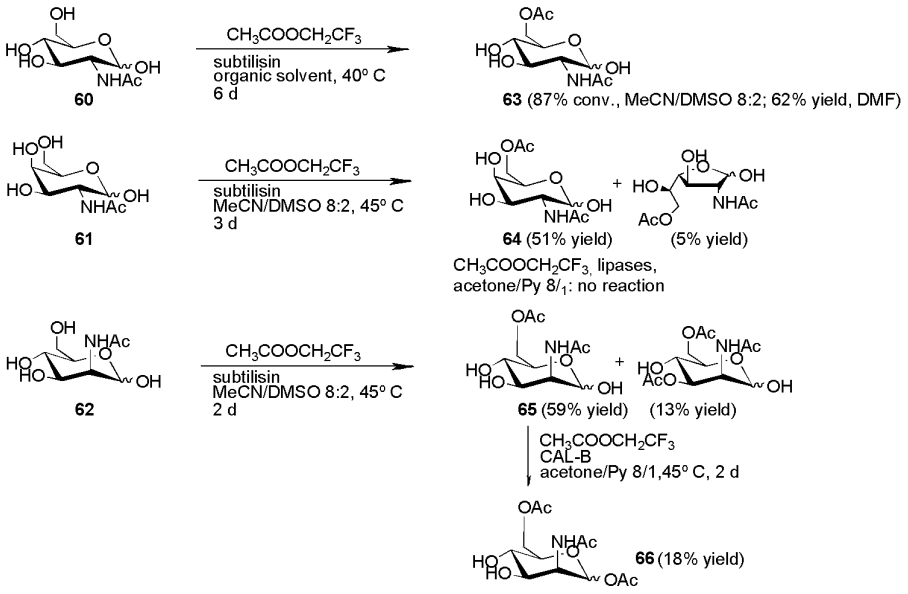
Scheme 12



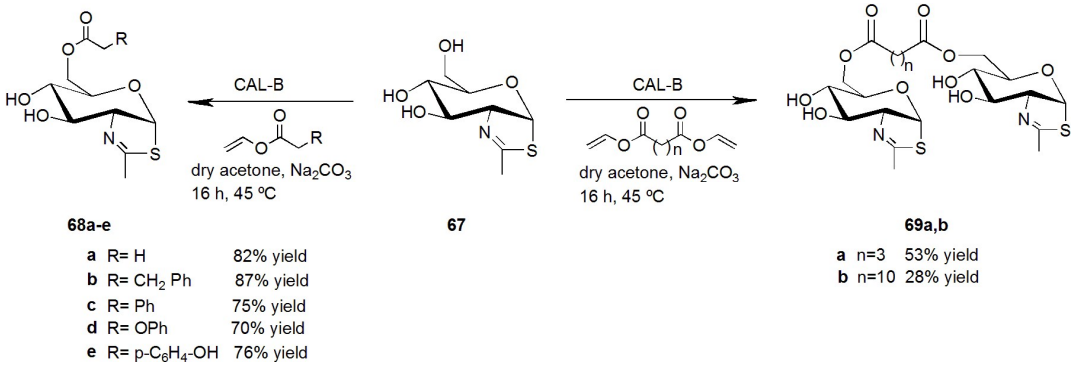
58a,b
a/**b** ratio = 2.6

59a (100% conversion **58a**,
72% isolated yield **59a**)

Scheme 13



Scheme 14



68a-e

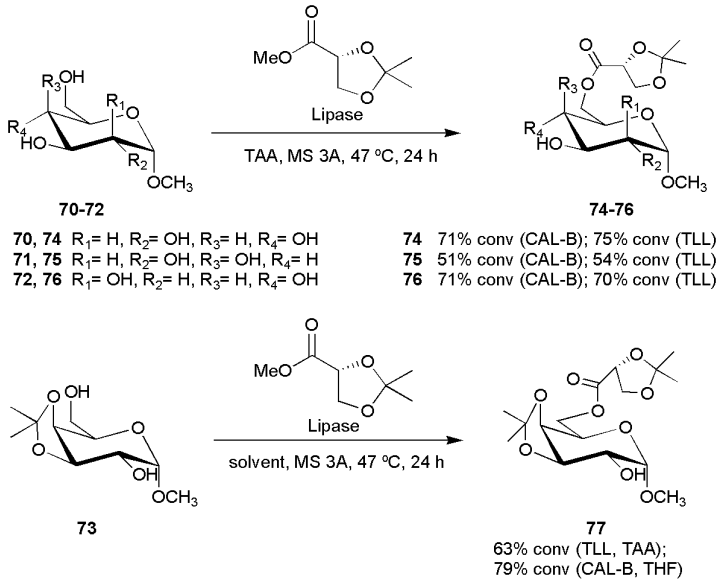
a R= H 82% yield
b R= CH_2 Ph 87% yield
c R= Ph 75% yield
d R= OPh 70% yield
e R= p-C_6H_4-OH 76% yield

67

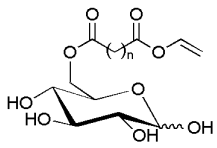
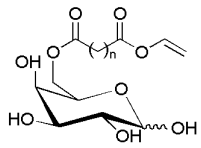
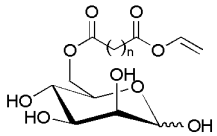
69a,b

a n=3 53% yield
b n=10 28% yield

Scheme 15



Scheme 16

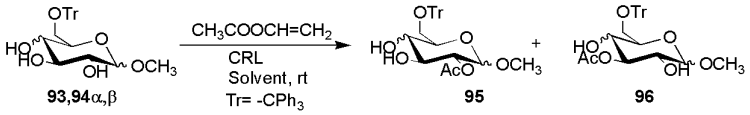
78-80α,β | **81-83**α,β | **84-86**α,β

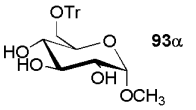
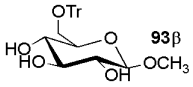
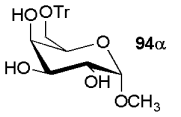
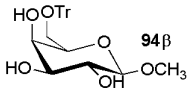
78α,β n=2 80% yield, α/β ratio = 1/1
79α,β n=4 53% yield, α/β ratio = 100/0
80α,β n=8 35% yield, α/β ratio = 100/0

81α,β n=2 70% yield, α/β ratio = 100/0
82α,β n=4 47% yield, α/β ratio = 100/0
83α,β n=8 35% yield, α/β ratio = 100/0

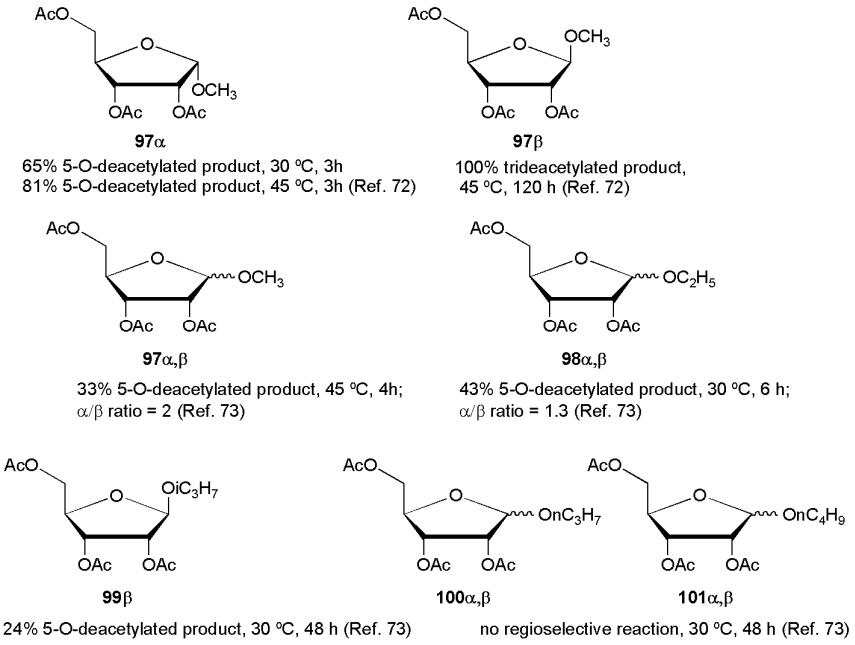
84α,β n=2 85% yield, α/β ratio = 81/18
85α,β n=4 69% yield, α/β ratio = 90/10
86α,β n=8 43% yield, α/β ratio = 91/9

Scheme 17

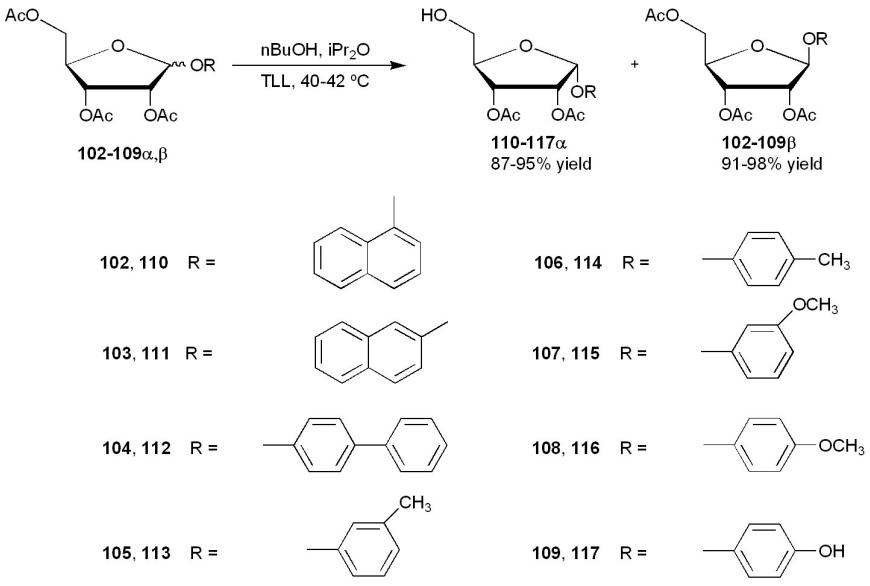


Substrate	Solvent	**95/96** ratio	Yield (**95**+**96**, %)
 **93**α	THF	4.0	40
	$CHCl_3$	15.7	88
	$BMIM.PF_6$	> 98	90
	$MOEMIM.PF_6$	> 98	84
 **93**β	THF	1.6	20
	$CHCl_3$	3.3	25
	$BMIM.PF_6$	9.0	82
	$MOEMIM.PF_6$	9.0	76
 **94**α	THF	15.7	82
	$CHCl_3$	19.0	94
	$BMIM.PF_6$	> 98	93
	$MOEMIM.PF_6$	> 98	94
 **94**β	THF	2.1	30
	$CHCl_3$	6.7	82
	$BMIM.PF_6$	24.0	93
	$MOEMIM.PF_6$	24.0	95

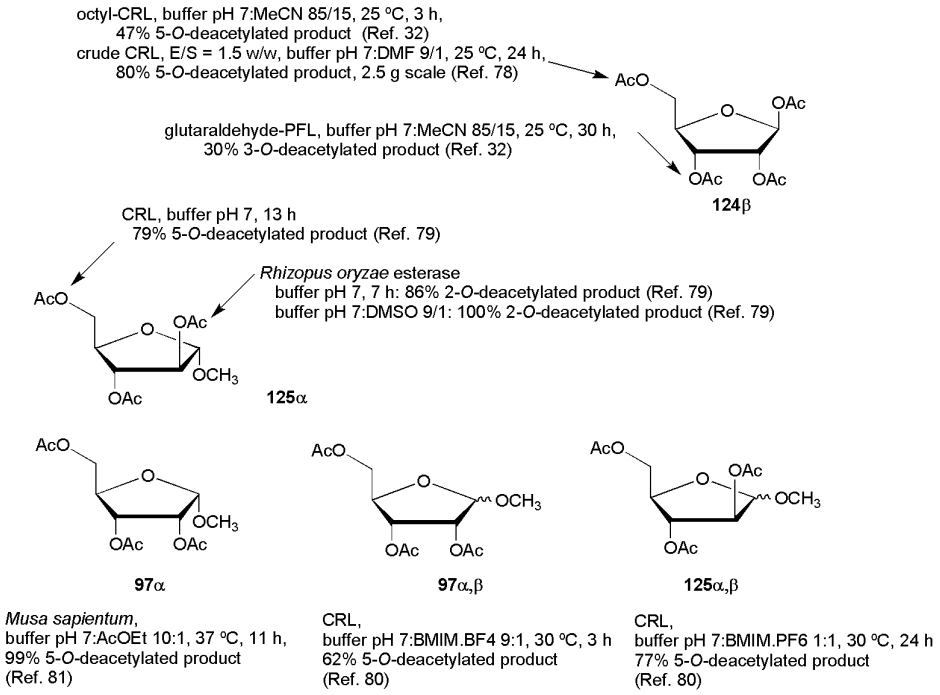
Scheme 18



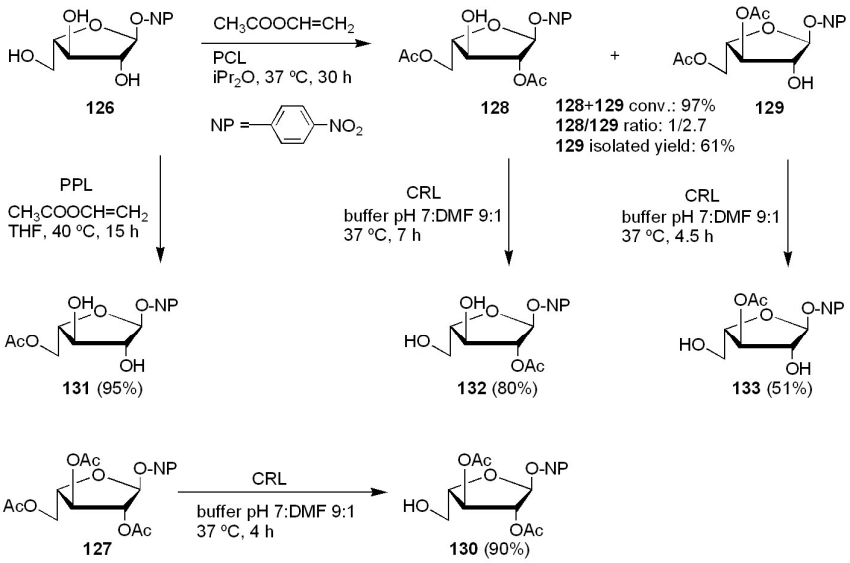
Scheme 19



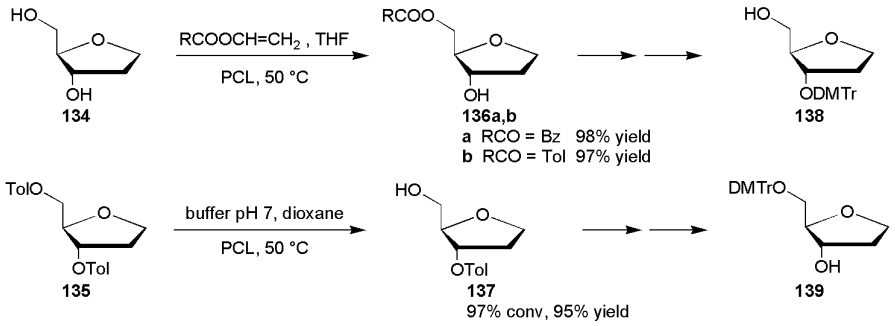
Scheme 20



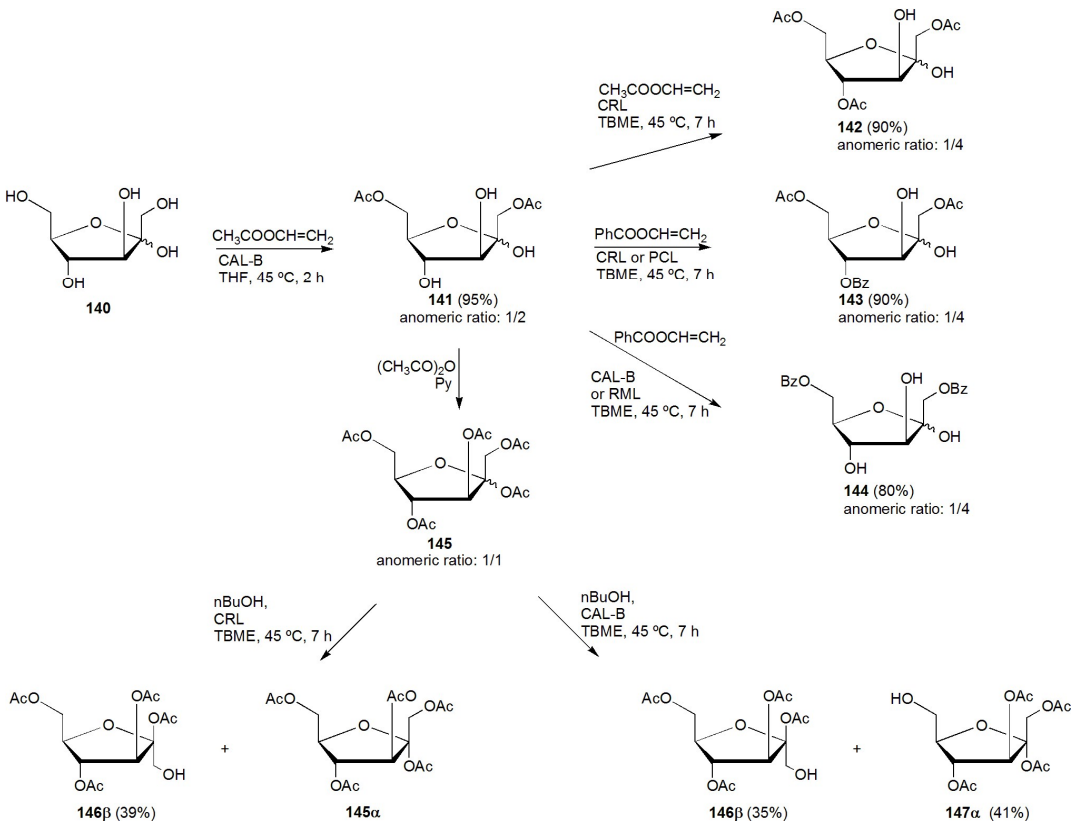
Scheme 21



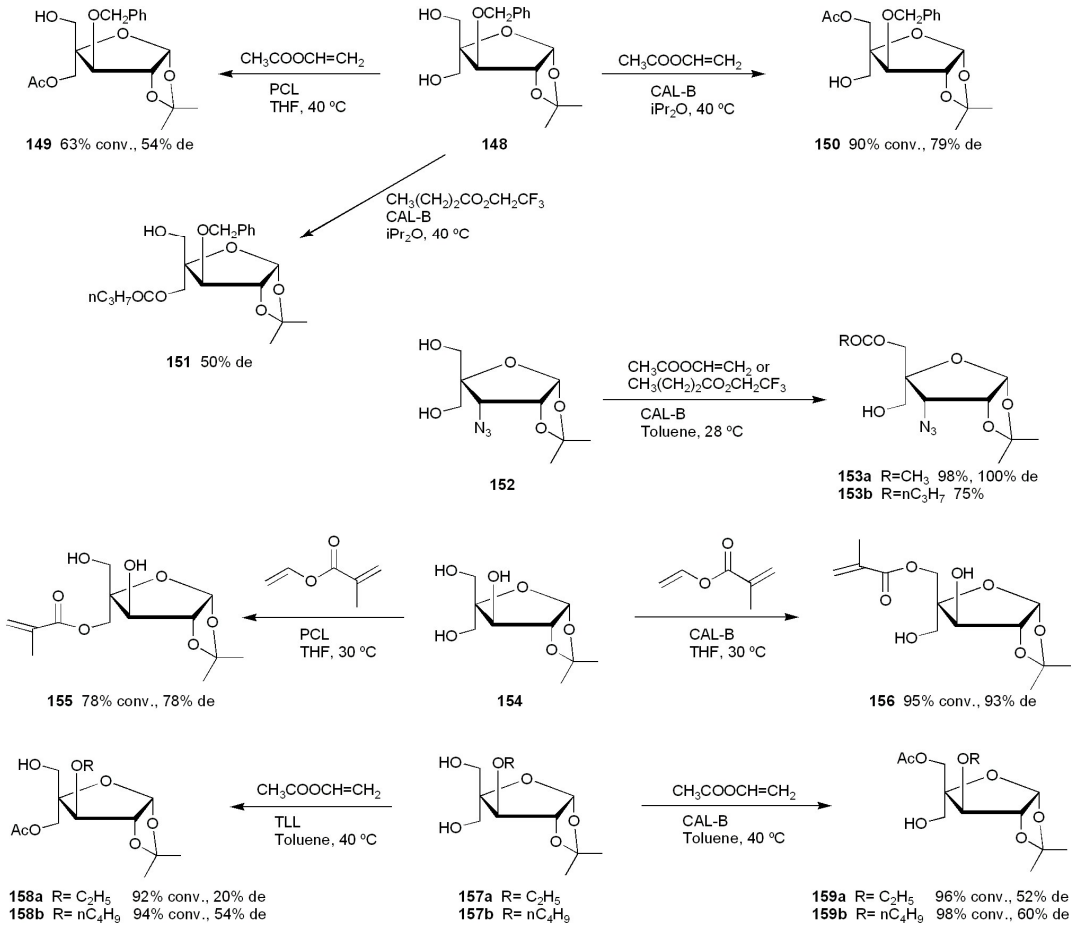
Scheme 22



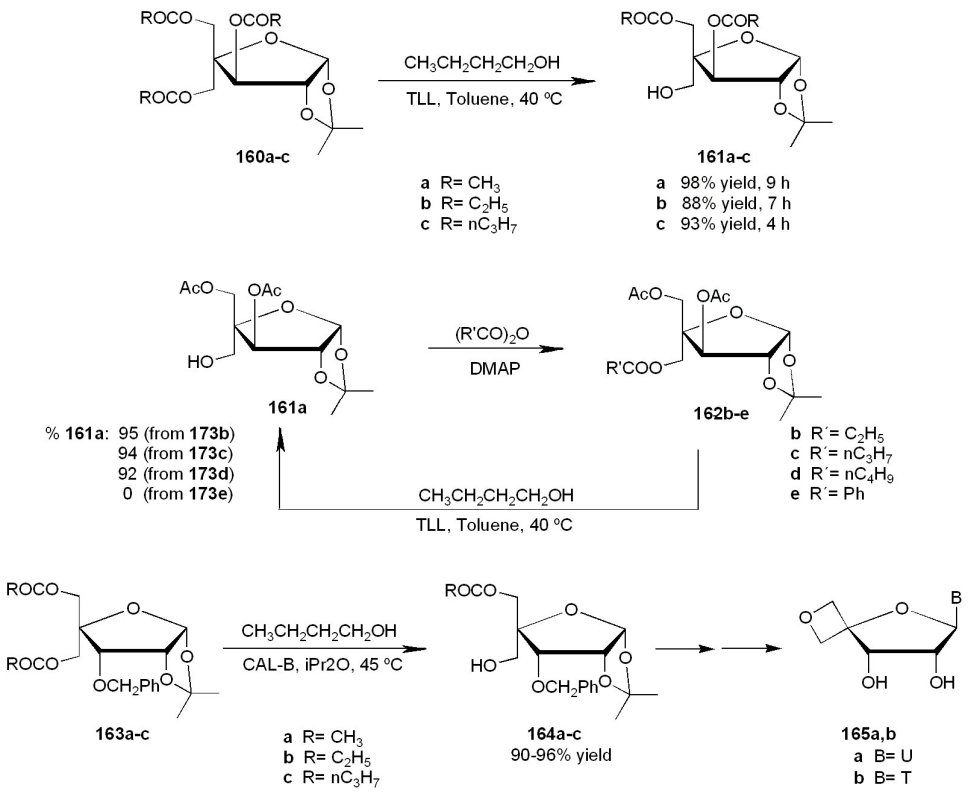
Scheme 23



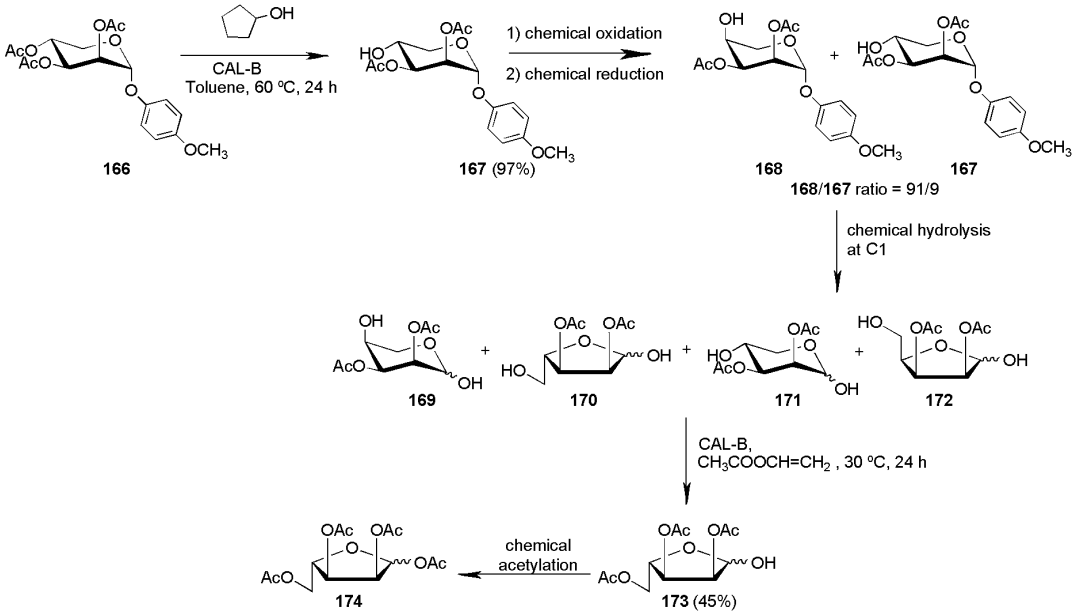
Scheme 24



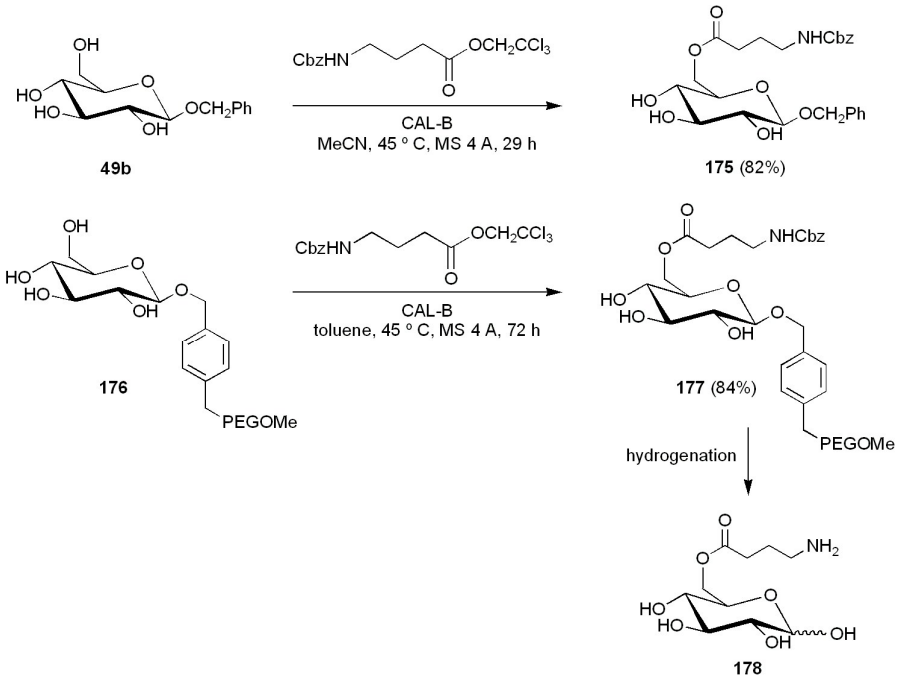
Scheme 25



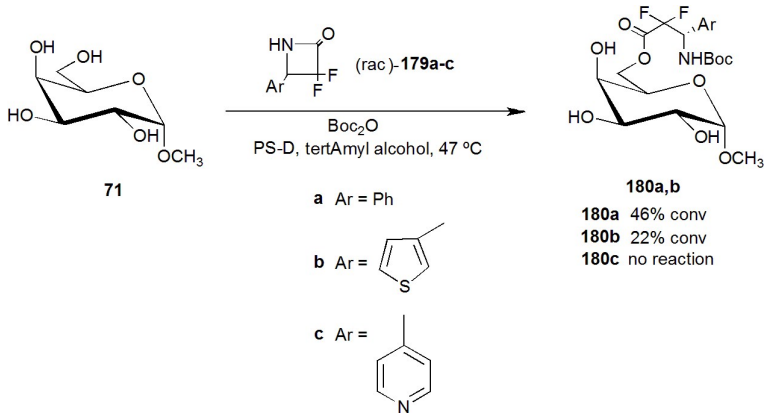
Scheme 26



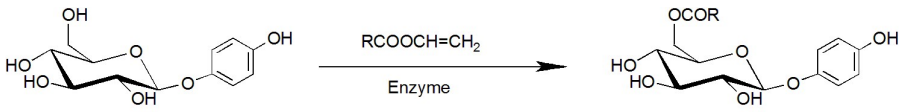
Scheme 27



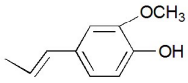
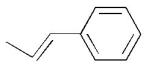
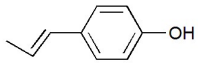
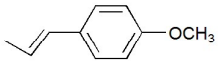
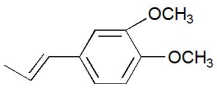
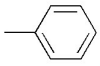
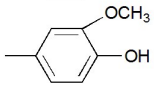
Scheme 28



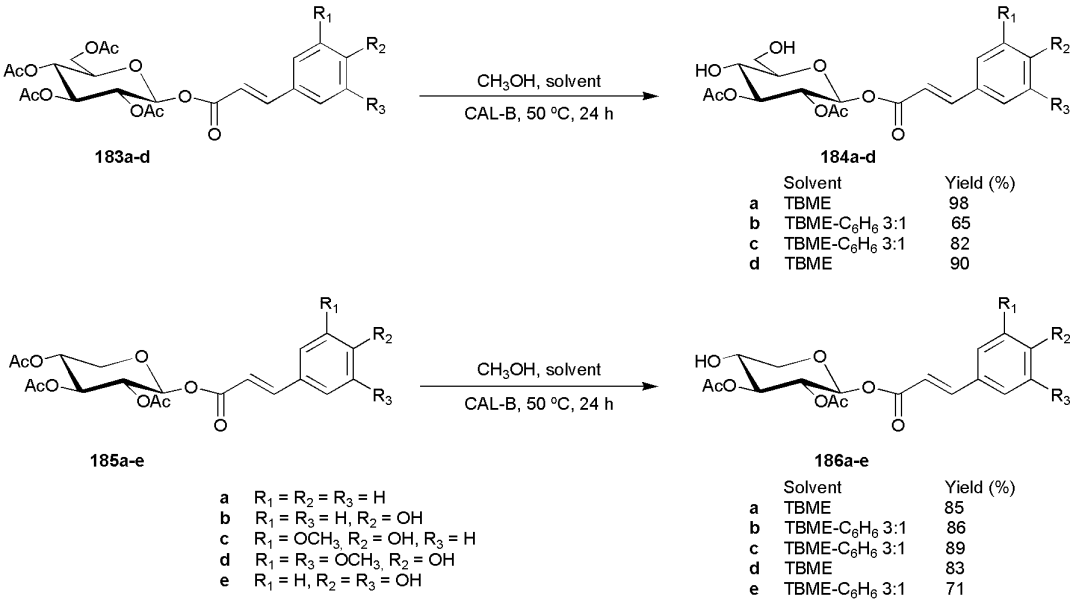
Scheme 29



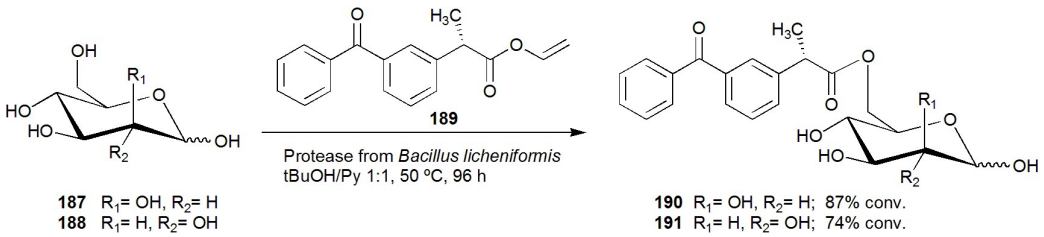
181 **182a-h**

Product **182a-h**	R	Yield (%)	Enzyme	Solvent, T (ºC)
a	$-(CH_2)_8CH=CH_2$	90	BSP	DMF/$H_2$0 95:5, 40
		93	PEL	anh. THF, 35
b		50	CAL-B	MeCN, 45
c		88	PEL	anh. THF, 50
d		34		
e		78		
f		68		
g		86		
h		28		

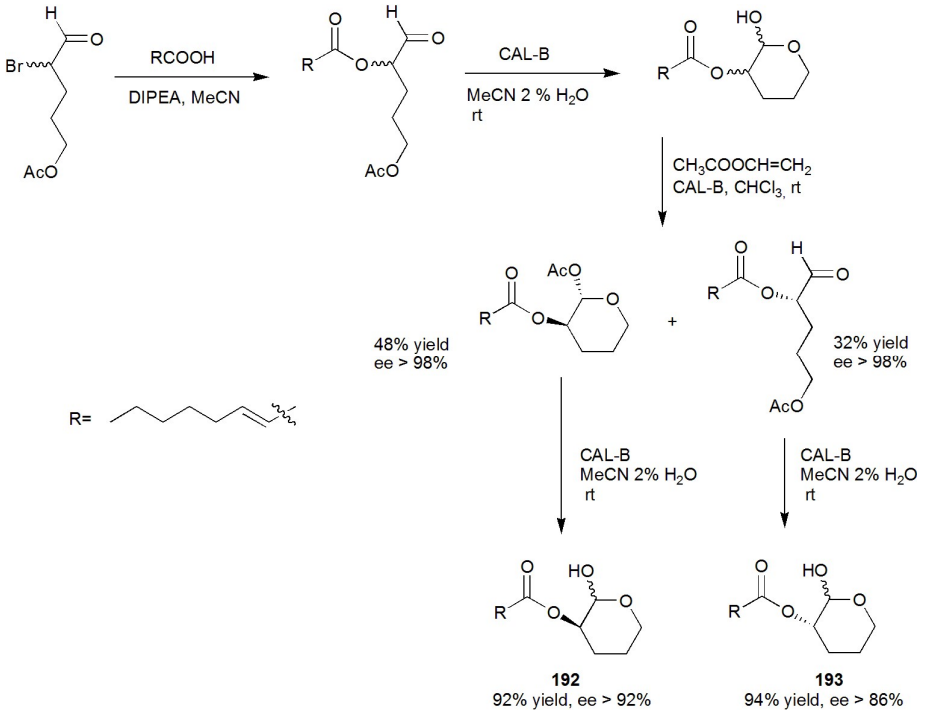
Scheme 30



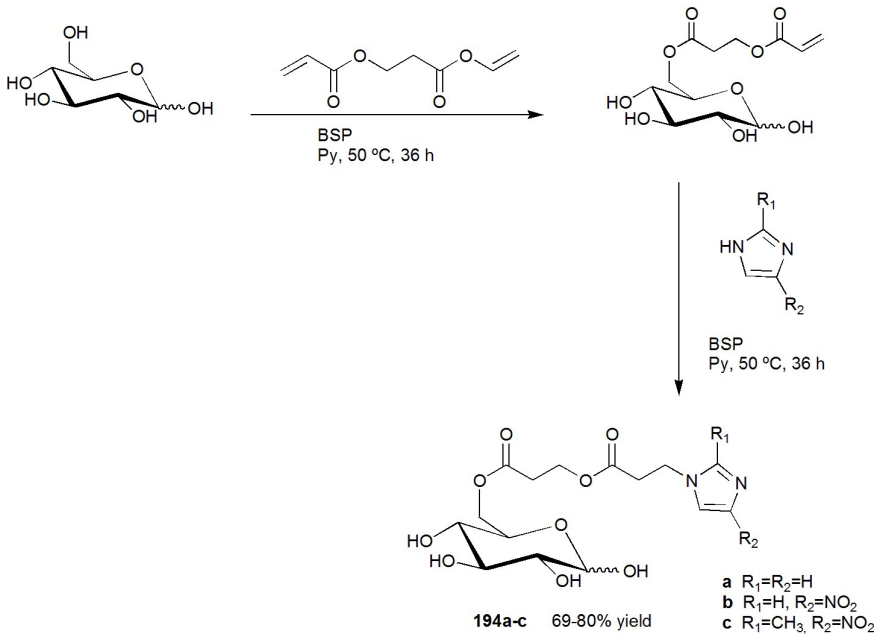
Scheme 31



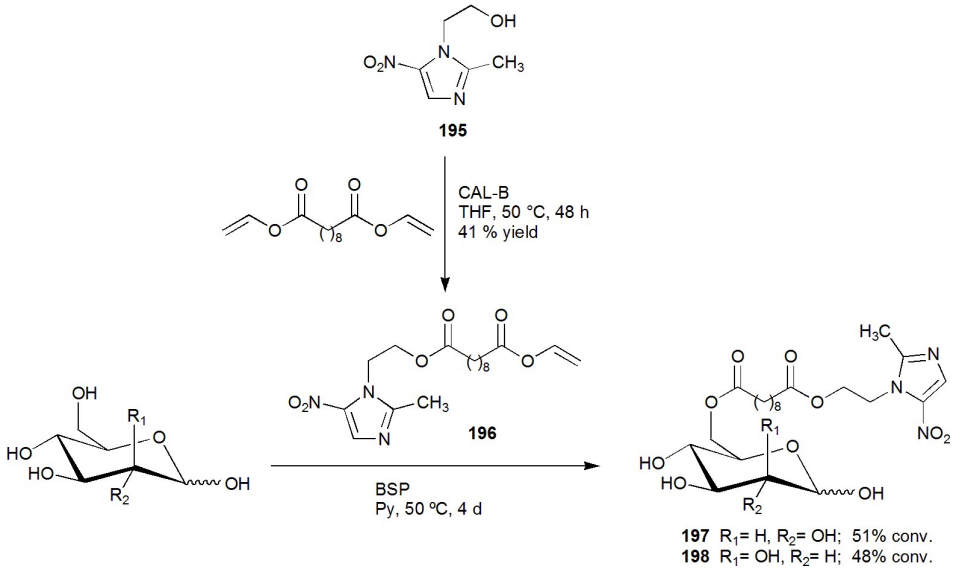
Scheme 32



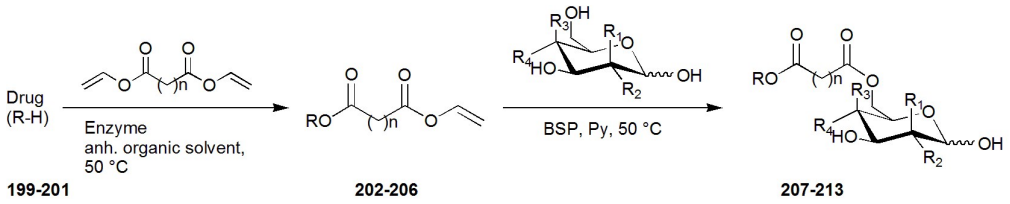
Scheme 33



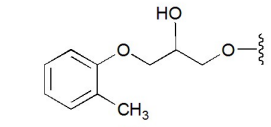
Scheme 34

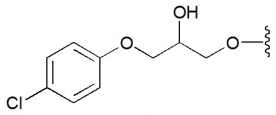


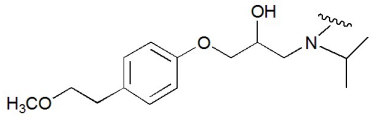
Scheme 35



R

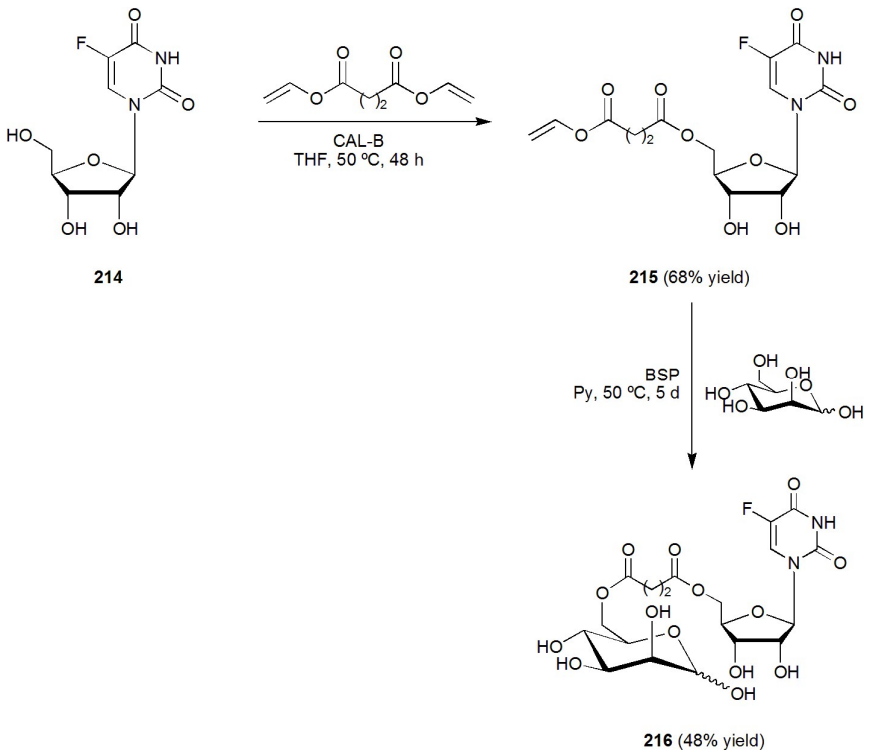

199


200


201

Drug	Enzyme	Solvent	**202-206** (n, conv. %)	**207-213** (yield, %)
Mephenesin (**199**)	CAL-B, RML	acetone	**202** (2, 86)	**207** (78) $R_1=R_3=H$, $R_2=R_4=OH$
	CAL-B	acetone	**203** (4, 79)	**208** (74) $R_1=R_3=H$, $R_2=R_4=OH$
Chlorphenesin (**200**)	CAL-B, RML	acetone	**204** (2, 85)	**209** (78) $R_1=R_3=H$, $R_2=R_4=OH$
	CAL-B	acetone	**205** (4, 73)	**210** (70) $R_1=R_3=H$, $R_2=R_4=OH$
Metoprolol (**201**)	PPL	CCl_4	**206** (4, 74)	**211**(69) $R_1=R_3=H$, $R_2=R_4=OH$ **212** (41) $R_1=R_4=H$, $R_2=R_3=OH$ **213** (57) $R_2=R_3=H$, $R_1=R_4=OH$

Scheme 36

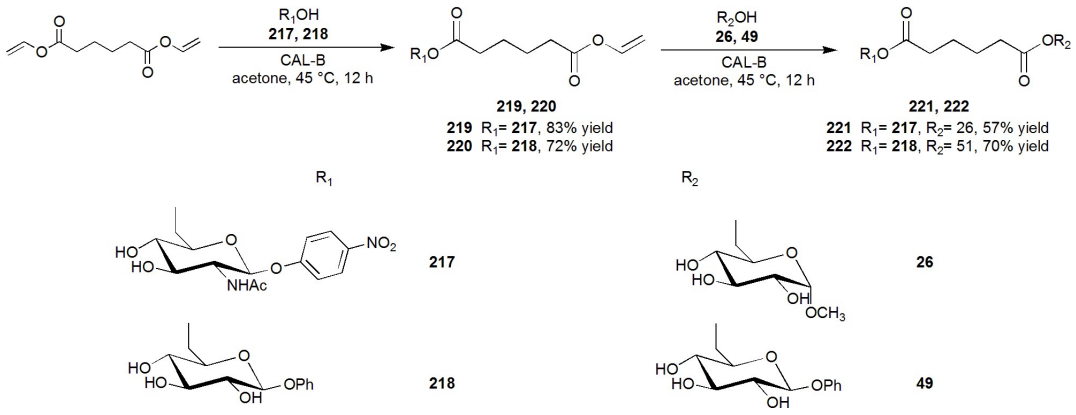


214

215 (68% yield)

216 (48% yield)

Scheme 37



Scheme 38

An update of biocatalytic selective acylation and deacylation of monosaccharides

Adolfo M. Iribarren and Luis E. Iglesias*

Partially acylated monosaccharides (PAMs) exhibit diverse and interesting applications but due to their polyhydroxylated nature, their synthesis requires regio- and stereoselective reactions. These features are provided by biocatalytic processes and in particular by hydrolases, which offer mild conditions and selective routes for the preparation of PAMs. Since this strategy has been extensively explored, the aim of the present review is to update research on enzymatic selective acylation and deacylation of monosaccharides, focusing on enzymatic preparation of synthetic useful PAMs and drug-monosaccharide conjugates involving PAMs.