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Porcine Pancreatic Lipase (PPL) – a Versatile Biocatalyst in Organic Synthesis

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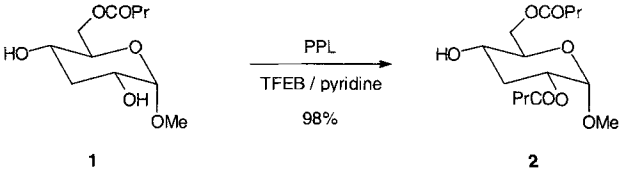
In the past two decades whole cell systems and (crude) enzyme preparations have been increasingly appreciated as valuable tools for the large scale preparation of isomerically pure compounds [1]. Out of the various enzyme classes, lipases and esterases represent the most valuable biocatalysts, since their catalytic competence only depends on their unique arrangement of functional groups (amino acid sequence) at the active site. Cofactors are not required. Among the many lipases which have been previously employed in Organic Synthesis [2], a crude enzyme preparation from porcine pancreas, **PPL** (E.C. 3.1.1.3.), possesses a particularly advantageous combination of selectivity, versatility and low cost. Although PPL is a complex mixture of isoenzymes, the biocatalyst can conveniently be used "straight from the jar". Like all lipolytic and esterolytic enzymes, PPL catalyses transacylations *via* an acyl-enzyme intermediate, and, depending on the type and relative concentration of a nucleophile (H–OH, R–OH, R–SH and R–NH_2) in the medium, either saponification or (thio)ester and amide formation can be achieved. The transformations can be carried out in buffered solutions as well as in anhydrous organic solvents. The exceptionally mild conditions (around pH 7, 3 °C to r.t.) facilitate introduction and cleavage of acyl groups in the presence of acid and/or base labile functional groups [3].

If acylations in organic media are slow, simple azeotropic distillation with removal of water serves to increase the reaction rate up to 4–70 fold [4]. Bath-ultrasonification is another simple and sometimes effective (83 fold) method [5]. Support of PPL on e.g. celite not only allows simple work-up of the reaction mixture and recycling of the catalyst, but may also result in an enhanced reaction rate. With vinyl acetate as solvent and as an irreversal acyl-donor [6] such a Celite-supported PPL can catalyse acylations 7–20 fold faster than the crude enzyme [7]. The effect of mole sieves appears to be limited. Furthermore, the use of activated esters (e.g. trifluoroethylbutyrate, TFEB) facilitates a rapid formation of the acyl–enzyme intermediate [8].

Regioselective Group Manipulations

Selective transformations of individual groups within polyfunctional substrates generally require cumbersome protecting group strategies resulting in low overall yields. However, PPL permits the regioselective manipulation of functional groups in polyhydroxy compounds like carbohydrates and polyphenols without protection [3]. For example, the lipase catalysed acetylation of the primary hydroxy group (6-OH) of glucose, galactose and mannose is readily achieved with good regioselectivity (85 to 100%) and high yield (60 to 85%) [9]. Many further monoacylations have been described, including butyrylation of desoxy sugar **1** which proceeds with complete regioselectivity [10] (Scheme 1). If the enzyme is used in the hydrolytic mode, the anomeric hydroxy group (1-OH) of pentaacylated pyranoses is preferentially liberated [11].

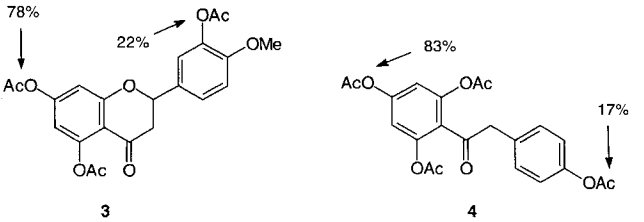


1 2

Pr = propyl; TFEB = trifluoroethylbutyrate

Scheme 1

Certain derivatives of naturally occurring flavones are interesting target molecules because of their potential antiviral and anticancer activity. The regioselective deacylation (78 % at C-7, 22 % at C-3) of peracetylated flavanone **3** is possible upon treatment with PPL, whereas the acetoxy group next to the carbonyl function remains completely unaffected. With polyphenolic peracetate **4** the same high regioselectivity is observed [12] (Scheme 2).

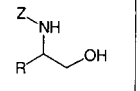
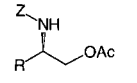
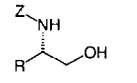
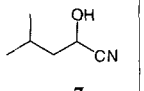
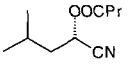
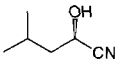
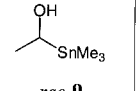
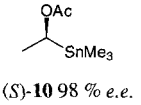
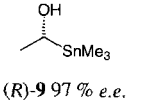
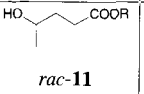
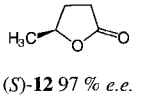
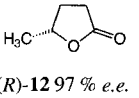
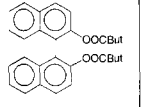
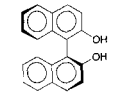
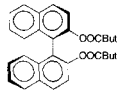


3 4

Scheme 2

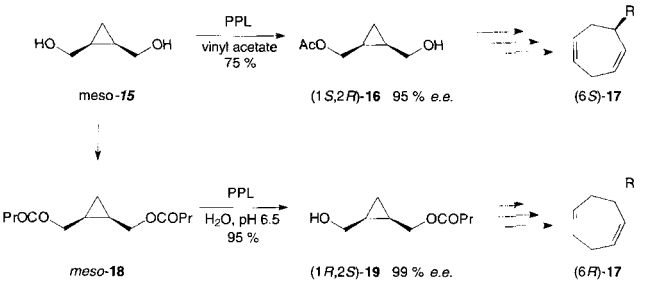
Synthesis of Optically Active Compounds

A large number of optically active compounds has been prepared using PPL (especially when required in substantial quantities), either through kinetic resolution of racemic substrates or by asymmetrisation of prochiral *meso*-compounds. Examples of racemate separation, involving porcine pancreatic lipase are given in table 1.

educt	conversion	product	recovered	ref.
 *rac*-**5**	50 %	 (*R*)-**6** 99 % *e.e.*	 (*S*)-**5** 99 % *e.e.*	[13]
 *rac*-**7**	48 %	 (*S*)-**8** 92 % *e.e.*	 (*R*)-**7** 83 % *e.e.*	[14]
 *rac*-**9**	40 %	 (*S*)-**10** 98 % *e.e.*	 (*R*)-**9** 97 % *e.e.*	[15]
 *rac*-**11**	36 %	 (*S*)-**12** 97 % *e.e.*	 (*R*)-**12** 97 % *e.e.*	[16]
 *rac*-**13**	46 %	 (*S*)-**14** 95 % *e.e.*	 (*R*)-**13** 84 % *e.e.*	[17]

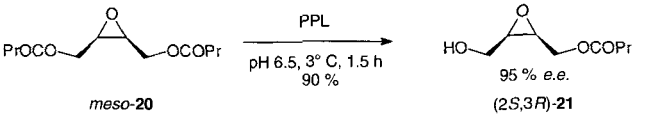
Z = benzyloxycarbonyl; Me = methyl; But = butyl; Pr = propyl

Besides conventional organic compounds such as amino alcohol *rac*-**5** [13] and cyanohydrin *rac*-**7** [14], the enzyme also accepts organometallic substrates like *rac*-**9**, representing valuable building units in Stille-couplings [15]. Chiral lactones (*R/S*)-**12** are obtained, if the nucleophile, which attacks the intermediary acyl-enzyme, is located within the same molecule [16]. As a chiral host, PPL is able to discriminate between axially dissymmetric compounds. Thus, the biaryl (*S*)-**13** is hydrolysed faster than (*R*)-**13**, and enantiomerically enriched (*S*)-1,1'-binaphthyl-2,2'-diol (*S*)-**14** is obtained [17] (see Table 1). If only one enantiomer is required, a kinetic resolution of racemates is inefficient because the maximum yield cannot exceed 50%. This limitation is easily overcome by using C_s-symmetric, prochiral *meso*-compounds as substrates which, at least in theory, adopt only a single, well defined orientation at the active site of the enzyme. This highly effective dissymmetrisation of prochiral compounds, known as the "*meso*-trick", has been applied to numerous cyclic and acyclic substrates. For example, stereocontrolled esterification of the diol **15** with PPL allows the large scale preparation the *cis*-cyclopropane (2*S*,3*R*)-**16**, an important building block for the synthesis of gamete-attracting and -releasing pheromones of brown algae **17** [18]. The enantiomer of the signal compound(s) is available by either selective group transformations on the monoester **16**, or using PPL for hydrolysis of the corresponding *meso*-diacyl derivative **18** [19] (Vertauschungsprinzip) (Scheme 3).

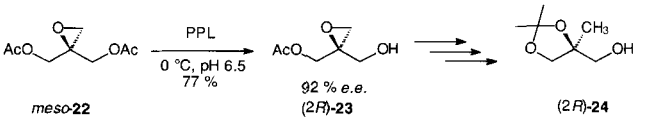


Scheme 3

The lipase mediated hydrolysis of *meso-cis*-2,3-epoxybutane-1,4-diol diester *meso*-**20** provides a useful alternative to the *Sharpless*-epoxidation which often displays a low degree of enantioselectivity (ca. 80-85% *e.e.*) when applied to *Z*-allylic alcohols [20]. Using PPL under pH-controlled conditions, monoester (2*S*,3*R*)-**21** is available in high chemical (90%) and optical (95% *e.e.*) yield (Scheme 4). Again, the saponification can be scaled up to molar quantities. Recrystallisation of the product affords virtually pure epoxyalcohol (2*S*,3*R*)-**21** [21], which represents a versatile building block in the synthesis of insect pheromones [22]. A similar approach allows the preparation of chiral glycidol (2*R*)-**24** (Scheme 5) [23].



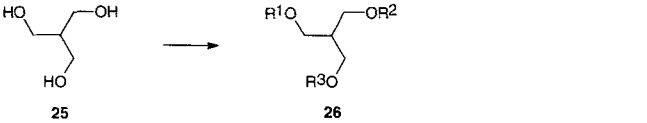
Scheme 4



Scheme 5

The kinetically controlled enzymatic resolution of non-*meso*-2,3-epoxyalkohols or their esters is a generally applicable alternative to the *Sharpless*-methodology, and esters of different chain length can be used to optimise the *e.e.* of the products [24].

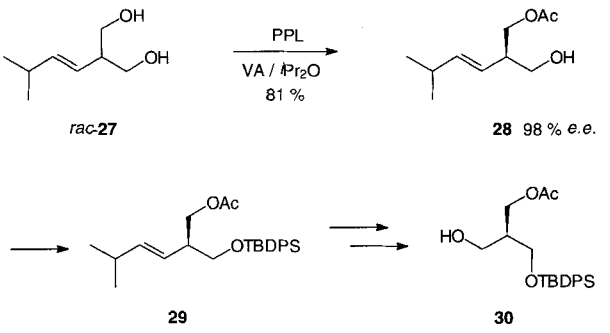
"Asymmetrised tris(hydroxymethyl)methane" (THYM) **26** is a highly interesting chiral starting material for the synthesis of optically active tripod ligands and chiral starburst dendrimers [25].



Scheme 6

This goal to distinguish between the three equivalent hydroxymethyl groups of the C_{3v}-symmetric triol **25** has been successfully approached by PPL catalysed monoacylation of the diol **27** [5]. Silylation of the free hydroxy group of monoacetate **28** and subsequent cleavage of the olefinic moiety generates the chiral tripod ligand **30** (Scheme 7). Owing to the orthogonal stability of the protecting groups, the substituents can be modified individually. For the success of the PPL catalysed acylation (98% *e.e.*), the presence of the π-

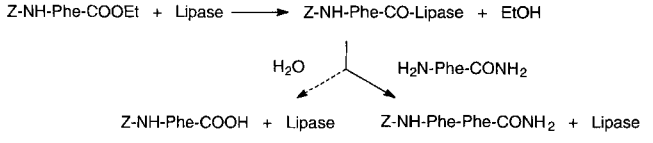
system next to the prochiral centre has a beneficial effect [26].



Scheme 7 VA = vinyl acetate; TBDPS = *tert*-butyldiphenylsilyl

Peptide Synthesis

If amines act as nucleophiles on the intermediary acyl–enzyme, the synthesis of amides becomes feasible. A very useful application of this kind of aminoacylation is the enzymatic peptide synthesis. Owing to the mild reaction conditions, racemisation does not occur. The involved functional groups require only minimal activation; side chain protection is generally not required. Unlike proteases, PPL does virtually not attack peptide bonds in the presence of water.



Scheme 8 Z = benzyloxycarbonyl; Phe = phenylalanine

Thus, peptide synthesis can be carried out with PPL in a medium of water and water-miscible organic solvent using Z-protected amino acid esters and amino acid amides as *N*-nucleophiles (Scheme 8) [27]. Surprisingly, and in contrast to proteases, PPL displays no L-specificity and, hence, peptides of the D-series are accessible with equal ease.

Since PPL represents only one enzyme out of a broad spectrum of commercially available lipases and esterases of sometimes complementary selectivity, a large variety of highly selective transformations may be performed that are difficult to emulate using more traditional methods.

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