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Biocatalytic routes toward pharmaceutically important precursors and novel polymeric systems*

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Abstract: The synthetic potential of enzymes related to organic synthesis has been applied profusely, especially since the introduction of their use in organic solvents. Enzymes offer the opportunity to carry out highly chemo-, regio-, and enantioselective transformations. The use of enzymes in the synthetic sequence provides unique advantages of efficiency and environmental friendliness. Owing to their low cost and applicability to a broad range of substrates, lipases have become the most versatile class of biocatalysts in organic synthesis. We have screened a battery of lipases to carry out highly selective reactions for the synthesis of a wide range of organic compounds and polymeric materials.

INTRODUCTION

The proper introduction and removal of protecting groups is one of the most important and widely carried out synthetic transformations in preparative organic chemistry. In particular, in the highly selective construction of complex, polyfunctional molecules (e.g., oligonucleotides, oligosaccharides, peptides, and conjugates thereof) and in the synthesis of alkaloids, macrolides, polyether antibiotics, prostaglandins, and further natural products, regularly the problem arises that a given functional group has to be protected or deprotected selectively under the mildest conditions and in the presence of functionalities of similar reactivity as well as in the presence of structures being sensitive to acids, bases, oxidation, and reduction. Organic chemists have responded to these challenges in a befitting manner, and one of the recent advances in this direction is the application of catalysis as a tool to achieve selectivity in organic synthesis. Catalytic organic reactions can be achieved either by chemocatalysis or biocatalysis. Among the important challenges facing organic chemists today is the synthesis of compounds in a highly selective and cost-effective manner, utilizing renewable raw materials through environmentally benign processes. In this endeavor, the synthetic potential of enzymes related to organic synthesis has been applied profusely, especially since the introduction of organic solvent methodology

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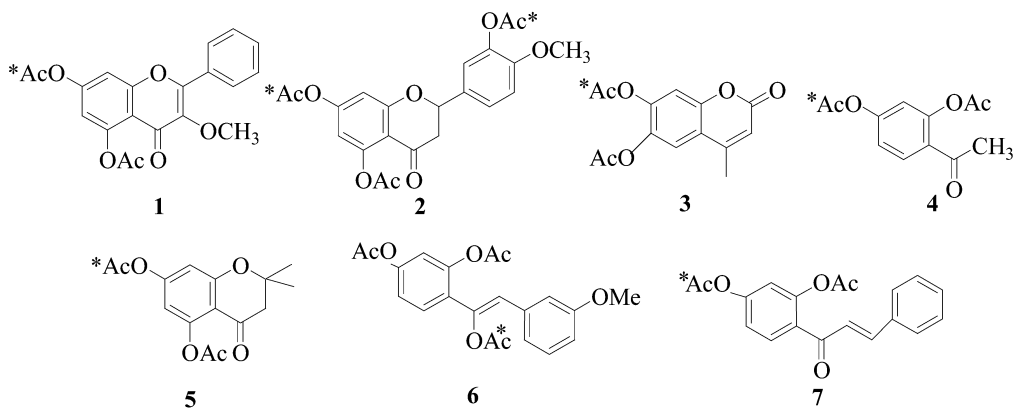
[1]. In addition to their stereoisomeric discriminating properties, enzymes offer the opportunity to carry out highly chemo- and regioselective transformations. They often operate at neutral, weakly acidic, or weakly basic pH values and in many cases combine the high selectivity for the reactions they catalyze and the structures they recognize with a broad substrate tolerance. Therefore, the applications of these biocatalysts offer viable alternatives to classical chemical methods [2–8].

In biocatalysis, enzymes (in particular, hydrolases) dominate. Yeast reductions have also found wide uses, whereas catalytic antibodies have not yet found the broad practical uses that enzymes have [9]. Triacylglycerol hydrolases (EC 3.1.1.3) are termed lipases and have become one of the most versatile classes of biocatalysts in organic synthesis [10,11]. This is because lipases can accept a wide range of organic substrates and they work very well in organic solvents. Lipases can be used as catalysts in either hydrolysis reactions or ester synthesis (acylation reactions). In both types of reactions, lipases usually react with a high degree of chemo-, regio-, enantio-, and diastereoselectivity. Owing to the fact that lipases have found applications in biotechnology and as additives in detergents, some lipases are produced in amounts of hundreds of tons per year. Commercial manufacturing of such lipases is done by genetic engineering techniques. By gene expression in an appropriate microorganism such as a fungus, yeast, or bacteria (e.g., *Escherichia coli*), large-scale production of lipases has been realized [9]. We have screened a battery of lipases to achieve selectivity in the synthesis of a wide range of organic compounds of different classes as discussed below.

POLYPHENOLICS

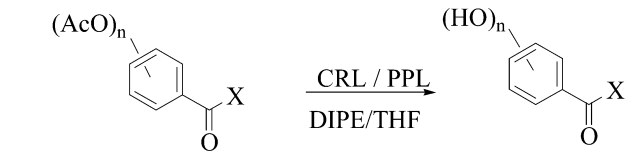
Polyphenolics occur widely in nature, and being the secondary metabolites of plants, many of their analogs possess a variety of biological activities. Suitably substituted polyhydric phenols are used as starting materials for the synthesis of different classes of natural polyphenolics, viz. chalcones, flavones, flavanones, isoflavonoids, coumarins, xanthones, catechins, etc. Selective protection and deprotection steps are often required to achieve the total synthesis of these compounds, which are rather difficult; moreover, they increase the number of steps and decrease the net yields, thus making the overall synthesis quite cumbersome. In order to ease the problems of protection/deprotection in the synthesis of biopolyphenolics, we systematically investigated the deacetylation of a large number of different types of peracetylated compounds **1**–**7** representing various classes of natural products over the years (flavones, flavanones, coumarins, ketones, chromanones, diphenyl ethenes, chalcones, catechins, etc.) by lipases from porcine pancreas, *Candida*, *Aspergillus*, *Fusarium*, and *Pseudomonas* species (Scheme 1) [12–21].



Scheme 1 Regioselective deacetylation of flavone, flavanone, coumarin, ketone, and chromanone using lipases. *Deacetylation occurs at selected positions.

Interestingly, we observed a selective deacetylation from a position other than *ortho* or *peri* (in heterocyclic compounds) to the nuclear carbonyl group while using porcine pancreatic lipase (PPL) as the biocatalyst. These results are not only interesting but important as deacetylation by chemical means would preferentially have occurred at the *ortho* or *peri* position as the resulting *ortho*/*peri* hydroxy group would undergo chelation with the nuclear carbonyl group.

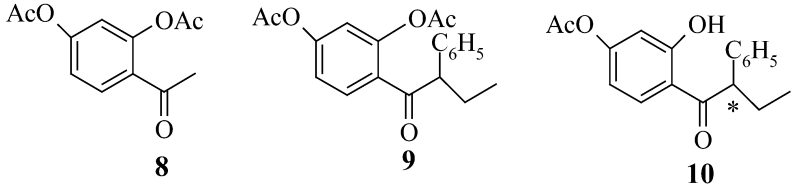
Based on the above, it was postulated that the nuclear carbonyl group present in the substrate forms a dynamic Schiff's base-type complex with PPL, which we believe is responsible for regioselective deacetylation [17]. However, no concrete proof for this mechanism could be given as the active site of PPL is not known and also Schiff's base formation is a transient (dynamic) process. To confirm our hypothesis, we studied the lipase-catalyzed deacetylation of differently substituted amides [22] and esters [23] (Scheme 2).



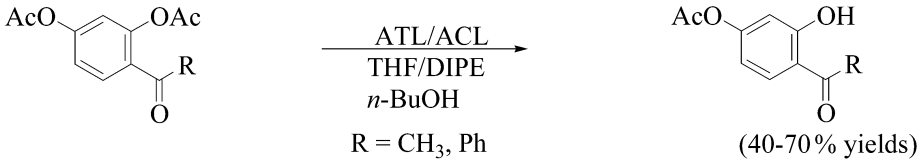
Scheme 2 Nonselective deacetylation of polyacetoxy amides and esters.

Since amides and esters cannot form Schiff's bases easily, it is expected that deacetylation reactions on the esters and amides of polyacetoxy aromatic acids catalyzed by lipases would not involve formation of Schiff's base complexes and as a result random orientation of different acetoxy groups in the active site of PPL would result in deacetylation of all the acetoxy groups, including the one at the *ortho* position. So no selectivity should be observed in such systems, our results were in accordance with expectations as no clear-cut regioselectivity was observed with PPL in THF [22,23] in the case of polyacetoxy aromatic esters and amides (Scheme 2). A useful feature of these reactions is the exclusive chemoselectivity in de-esterification of esters of polyacetoxy aromatic acids, i.e., exclusive de-esterification of the ester groups derived from the phenolic hydroxy and aliphatic acid over the ester group of the aromatic carboxylic acid moiety and aliphatic alcohol has been achieved, affording the corresponding esters of polyphenolic acids (Scheme 2).

To prove our hypothesis of dynamic Schiff's base formation, we have modified the enzyme by pre-shaking PPL with acetophenones; this should deprive the ability of the enzyme to form Schiff's base with the substrate. This modified PPL was used to catalyze the deacetylation of **8**, leading to the formation of completely deacetylated product, i.e., 2,4-dihydroxyacetophenone [24]. The formation of dihydroxyketone with modified PPL, which in the natural form exclusively mediates the deacetylation of *para*-acetoxy function of **8** over the *ortho*-acetoxy group with respect to nuclear carbonyl function, indicates that it does not show any preference for the *ortho*- or *para*-acetoxy function. This observation lends support to our proposed mechanism of action of PPL in THF involving a dynamic Schiff's base complex formation [16,17]. Similarly, racemic ketone **9** was incubated with modified PPL, leading to the formation of a completely deacetylated compound and a partially deacetylated optically enriched compound **10** (unpublished results). This random selectivity of modified PPL is further in line with the hypothesis on the mechanism of action of PPL in THF [17].

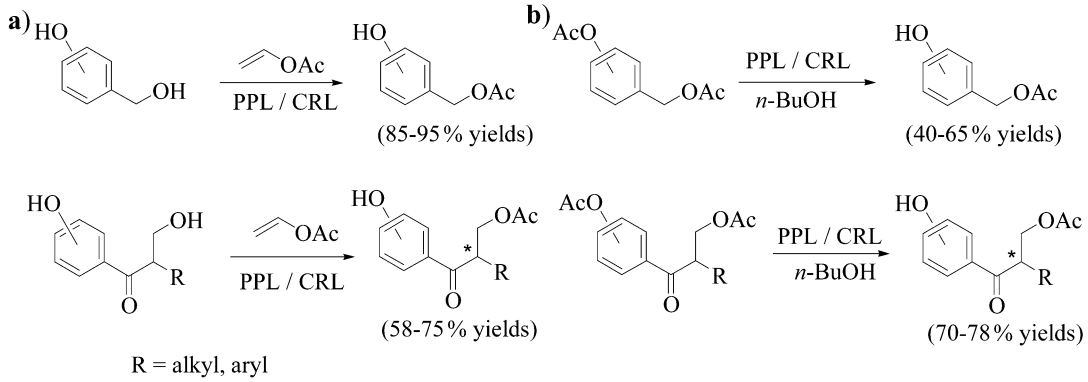


However, the use of *Aspergillus terreus* (ATL) and *Aspergillus carneus* (ACL) lipases for the deacetylation of the polyacetoxy aromatic ketones in organic solvents leads to a reversal of regioselectivity to that obtained during deacetylation with PPL (Scheme 3) [25,26]. These complementary results may prove to be useful for the synthesis of complex natural products.



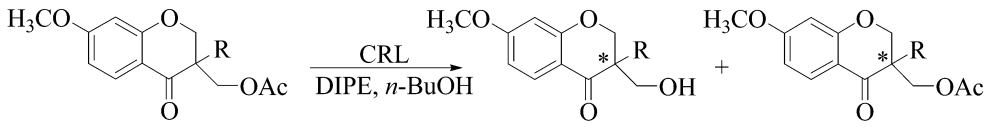
Scheme 3 Regioselective deacetylation of aromatic ketones with *Aspergillus* lipases.

The chemoselective capabilities of PPL (in THF) and *Candida rugosa* lipase (CRL) (in diisopropyl ether, DIPE) have been investigated for the selective acetylation and deacetylation of hydroxymethylated phenols and hydroxyaryl alkyl ketones and their peracetylated derivatives. Both PPL and CRL exhibited exclusive selectivity for the acetylation of alcoholic hydroxyl group over the phenolic hydroxyl group(s) (Scheme 4), and for the deacetylation of ester group involving the phenolic hydroxyl group over the alcoholic hydroxyl group (Scheme 4).



Scheme 4 Chemo- and regioselective acetylation/deacetylation of hydroxymethylated phenols and hydroxyaryl alkyl ketones and their peracetylated derivatives.

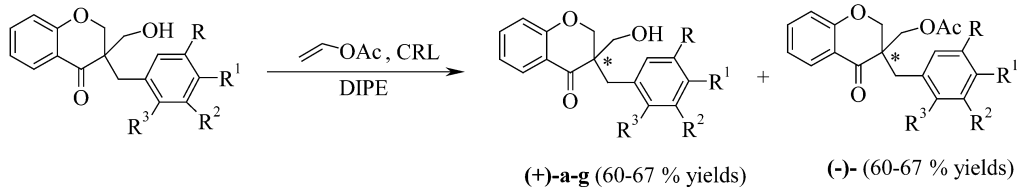
These results suggested that this strategy of acetylation can also be used in the enantiomeric resolution of racemic ketones, i.e., the enantioselective acetylation of racemic 2-alkyl/aryl-3-hydroxypropiophenones has been observed leading to the formation of enantiomerically enriched monoacetates (Scheme 4) [27], which are important precursors in the synthesis of biologically active chromanones and isoflavanones. These results enabled us to synthesize six racemic 3-acetoxymethyl-3-alkylchromanones and study their enantioselective deacetylation using CRL (Scheme 5) [28].



R	% ee
a CH_3	38
b CH_2CH_3	14
c $(CH_2)_2CH_3$	56
d $CH(CH_3)_2$	18
e $(CH_2)_3CH_3$	29
f $(CH_2)_4CH_3$	20

Scheme 5 Enantioselective deacetylation of 3-acetoxymethyl-3-alkyl-7-methoxy-chroman-4-ones.

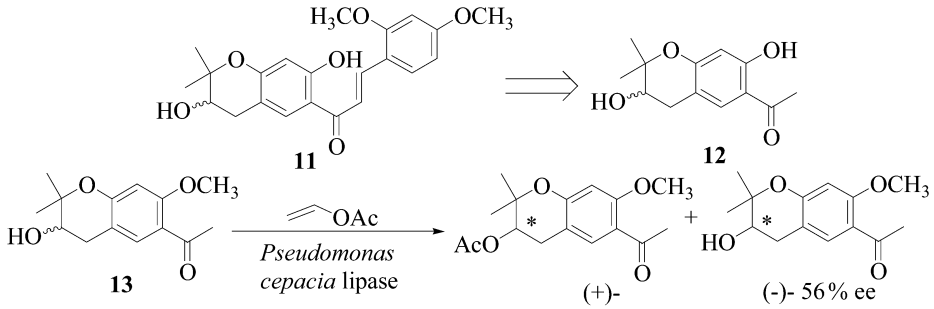
We have expanded this study by synthesizing (±)-3-benzyl-3-hydroxymethyl-2,3-dihydrobenzopyran-4-ones and subjecting them for enantioselective resolution. The 3-hydroxymethylated chromanones were synthesized in three steps starting with the coupling of 2-hydroxyacetophenone with corresponding benzaldehydes leading to the formation of 2-hydroxychalcones, which upon hydrogenation and hydroxymethylation afforded racemic 3-hydroxymethyl chromanones in 70–82 % yields. These 3-hydroxymethylated chromanones were subjected to lipase-catalyzed acylation reaction (Scheme 6) (unpublished results).



R	R^1	R^2	R^3	% ee
a H	H	H	H	4
b H	OCH_3	H	H	33
c H	OCH_3	OCH_3	H	79
d H	Cl	H	H	10
e H	O-CH_2-O		H	65
f OCH_3	H	H	OCH_3	8
g OCH_3	OCH_3	OCH_3	H	59

Scheme 6 Enantioselective acylation of 3-benzyl-3-hydroxymethyl-2,3-dihydro-benzopyran-4-ones.

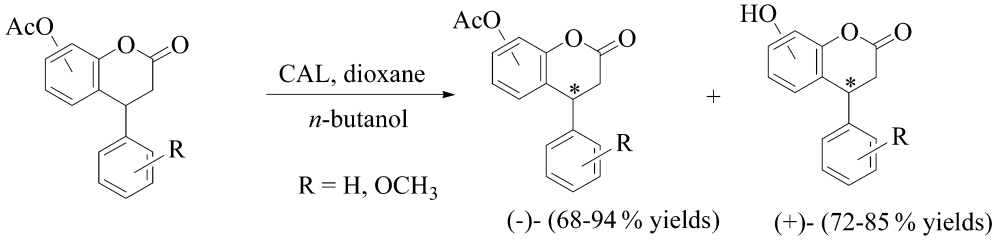
As a part of our ongoing research program toward the synthesis of bioactive compounds, we came across a chalcone derivative **11** [29], which possesses strong anti-invasive activity against human breast carcinoma cells [30]. This compound has some cytotoxic effects too, it is quite reasonable to assume that one of the enantiomers may be bioactive and the other may have some cytotoxic affects. Therefore, we envisaged the synthesis of its analogs in optically pure/enriched form. The retroanalysis suggested that the resolution of its precursor **12** may be helpful in synthesizing the pure enantiomer (Scheme 7). We used the methoxy analog **13** of **12** for biocatalytic resolution, *Pseudomonas cepacia* lipase (Amano PS) was found to be selective for the enantioselective acetylation of the racemic chromanol **13** (Scheme 7) [31].



Scheme 7 Lipase-catalyzed enantioselective acetylation of 6-acetyl-3,4-dihydro-2,2-dimethyl-3-hydroxy-7-methoxy-2*H*-1-benzopyran.

4-Phenyldihydrocoumarins are naturally occurring compounds, and this skeleton is also common in various classes of other natural products, for example, flavonoids, tannins, etc. [32–35]. The compounds of this class are indicated to be involved in the defense mechanism of plants by interfering with signal transduction in fungal pathogens and herbivores [36].

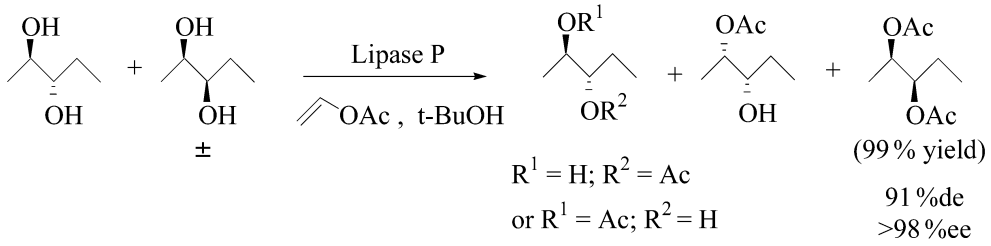
We synthesized eleven (±)-5/6/7-acetoxy-4-aryl-3,4-dihydrocoumarins in two steps starting from the coupling of cinnamic acid/substituted cinnamic acids with appropriate phenols, followed by acetylation in 50–83 % overall yields. All the acetoxycoumarins were subjected to *C. antarctica* lipase (CAL)-catalyzed deacetylation in dioxane, moderate enantioselectivity was observed (Scheme 8). This is one of the rare examples of resolution using phenolic ester moiety as a remote handle for chiral recognition by a lipase [37]. We attempted to determine the enantiomeric excess (ee) values of the enzymatically deacetylated hydroxy dihydrocoumarins and those of the recovered acetoxy dihydrocoumarins and racemic acetoxy dihydrocoumarins by HPLC using Chiracel OJ and Chiracel OD columns. However, separation of enatiomers was not observed in either racemic hydroxy- or in racemic acetoxy dihyrocoumarins. Further, we synthesized racemic *O*-acetylmandelates and studied the ^{1}H NMR spectra of the diastereomeric mandelates. No separation of the signals in the ^{1}H NMR spectra of diastereomeric mandelates was observed. Therefore, the enantiomeric excess (ee) of the compounds (Scheme 8) could not be determined.



Scheme 8 Enantioselective deacetylation of acetoxy dihydrocoumarins.

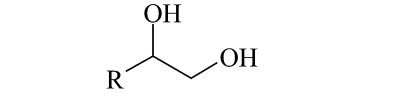
POLYOLS AND NUCLEOSIDES

We have exploited the extraordinary selectivity of the lipase Amano PS to convert butane-2,3-diol into the (2*R*,3*R*)-diacetate with 91 % de and >98 % ee on esterification with vinyl acetate (Scheme 9) [38].



Scheme 9 Diastereo- and enantioselective esterification of butane-2,3-diol.

On the other hand, PPL and CRL allow discrimination of the primary and secondary hydroxyl groups. We have studied the regioselective transesterification of 1,2-diols **14** with the acylating agent 2,2,2-trifluroethyl butyrate (TFEB) in defferent organic solvents, viz. pyridine, dimethylformamide, acetonitrile, acetone, tetrahydrofuran, and iso-octane by PPL and CRL. The reaction proceeds with high regioselectivity, and the acylation takes place at the primary hydroxy groups over the secondary in 65–75 % yields [39]. In the case of compound **15**, which has two primary and one secondary hydroxyl groups, only the C-3' primary hydroxyl group at the far end position to the asymmetric carbon gets acylated in 90 % yields [39]. These results are in conformity with the active-site model of the lipase proposed by Bhalerao et al. [40].



R= C_6H_5, CH_3, CH_2CH_3, $(CH_2)_2CH_3$, $(CH_2)_3CH_3$, $(CH_2)_5CH_3$

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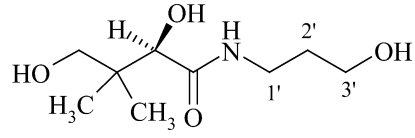
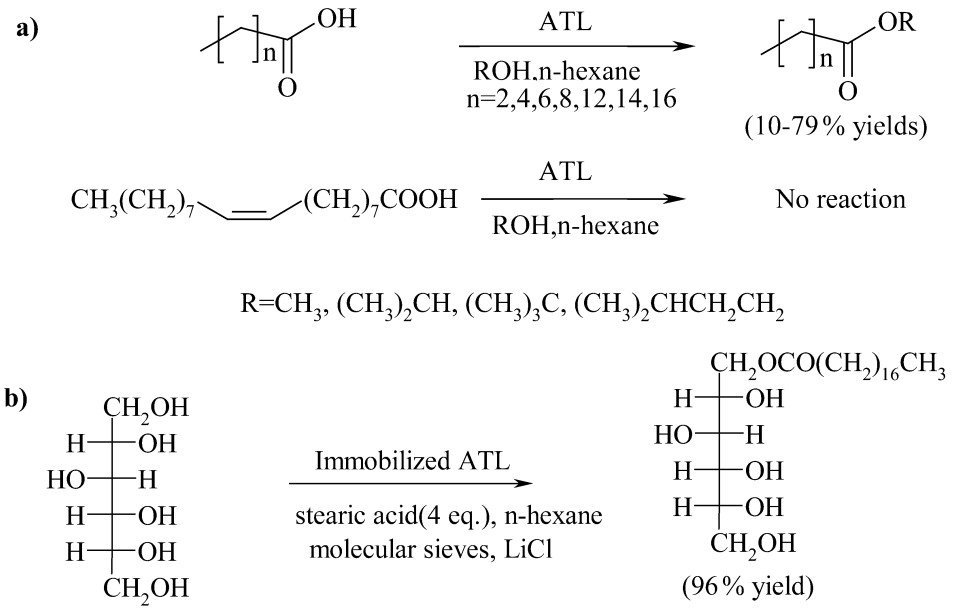


15

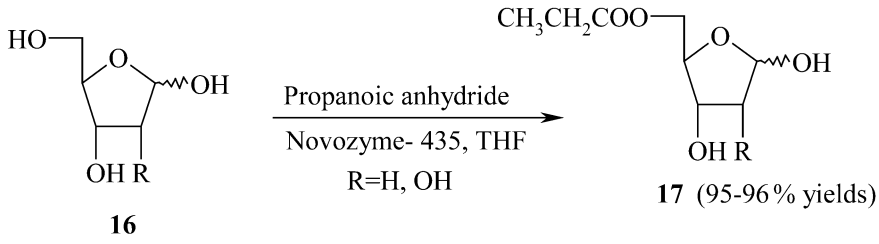
In another study, we observed that ATL discriminates between saturated and unsaturated fatty acids toward their esterification reaction with primary, secondary, and tertiary alcohols, i.e., no esterification reaction was observed with unsaturated fatty acids (Scheme 10a) [41]. Expanding these reactions, we have obtained sorbitol 1(6)-monostearate in very high yields by ATL-catalyzed esterification of sorbitol with stearic acid, i.e., esterification occurs at one of the two primary hydroxyl groups without any reaction at any of the four secondary hydroxyl groups (Scheme 10b) [41].

Nucleosides are fundamental building blocks of biological systems that show a wide range of biological activities [42]. Since the latter part of the 1980s, nucleoside analogs have been investigated with renewed vigor and urgency in the search for agents effective against the human immunodeficiency virus (HIV), the causative agent of the AIDS epidemic, in addition to finding more effective treatment for other viral infections which can prove lethal to AIDS patients and other immuno-compromised individuals. This has resulted in an explosion of synthetic activity in the field of nucleosides, and in the discovery of a number of derivatives with potent antitumor and antiviral activities. Consequently, extensive modifications have been made to both the heterocyclic base and the sugar moieties in order to avoid the drawbacks shown by nucleosides or analogs in certain applications mainly due to enzymatic degradations. The intense search for clinically useful nucleoside derivatives has resulted in a wealth of new approaches for their synthesis, especially enzyme-catalyzed reactions, are becoming standard procedures in their synthesis due to their feasibility and efficiency [43].



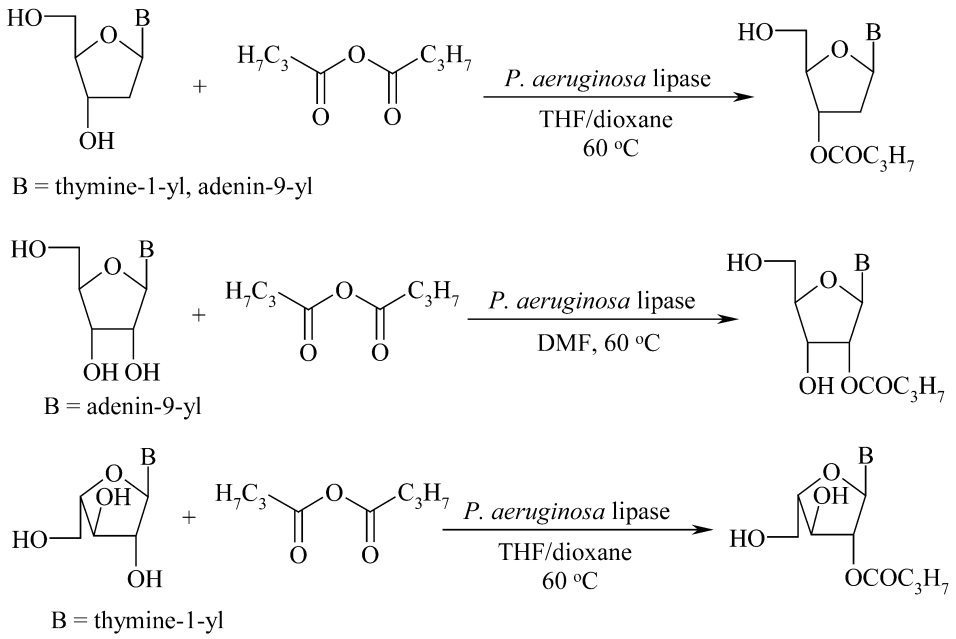
Scheme 10 Regioselective esterification reactions on (a) fatty acids and (b) synthesis of sorbitol 1 (6) monostearate.

The CAL (Novozyme-435) was used in regioselective acylation of the primary hydroxyl group of pentoses **16** with propanoic anhydride (Scheme 11) in dry THF, affording exclusively monoacylated derivatives **17** in 95–96 % yields [44]. Therefore, this method should be useful for monoprotection of the primary hydroxyl group of a large variety of carbohydrate derivatives.



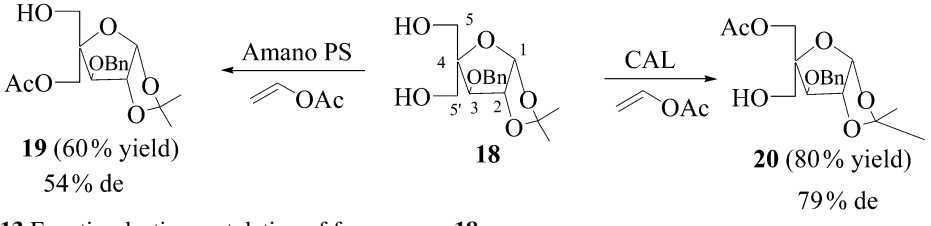
Scheme 11 Regioselective acylation of pentose sugar **16**.

There are few reports in the literature for the regioselective acylation of secondary hydroxyl groups of deoxyribo- and ribonucleosides using lipases, e.g., *Pseudomonas cepacea* lipase (PSL) and lipase KWI-56 (a lipase from *Pseudomonas sp*.) [45–47]. We explored the use of lipases for the regioselective acylation of secondry hydroxyl groups of deoxyribo-, arabino-, and ribonucleosides by a novel bacterial lipase, i.e., *P. aeruginosa* lipase using butanoic anhydride as acylating agent, affording exclusively monoacylated derivatives in 30–50 % yields (Scheme 12) (unpublished results).



Scheme 12 Regioselective acylation of deoxyribo- and ribonucleosides.

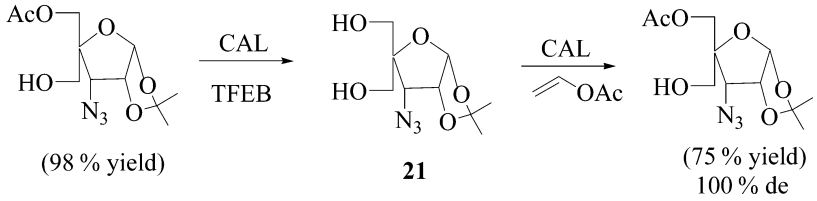
Also, we employed the versatile synthone 4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-3-*O*-(phenylmethyl)-α-D-pentofuranose (**18**) [48] as a key intermediate toward the synthesis of modified nucleosides, as one of the hydroxymethyl groups at the C-4 position can be exploited for conversion into various functionalities as well as for the synthesis of bicyclo sugar derivatives which have recently been reported to be of immense importance in the antisense/antigene technology. Our primary interest was in the selective protection of one of the two primary hydroxyl groups of **18**; our initial attempts for selective protection by chemical means, however, were not successful. Based on our earlier experience of carrying out regioselective reactions using lipases, a screening program involving PPL, CRL, Amano PS, and CAL for the preferential asymmetrization of the prochiral center C-4 in **18** was undertaken. It was observed that using vinyl acetate as the acyl donor, PPL did not catalyze the reaction, while with CRL no clear-cut selectivity was observed, as both the monoacetylated compounds **19** and **20** along with the diacetate were obtained. High diastereoselectivity was observed with both Amano PS and CAL, and interestingly different monoacetylated epimers **19** and **20** were obtained, with Amano PS the acetylation occurs preferentially at C-5' hydroxyl affording **19** as major product (54 % de, 63 % conversion), while the reaction with CAL yielded **20** as the main product with de as high as 79 % (optimized, 90 % conversion, (Scheme 13) [49].



Scheme 13 Enantioselective acetylation of furanosugar **18**.

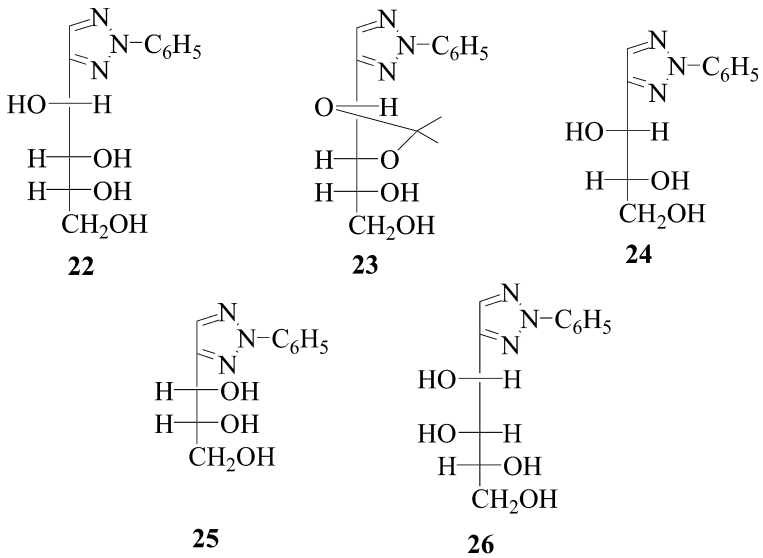
Further, on using 2,2,2-trifluoroethyl butyrate as an alternative acyl donor in the presence of CAL, a reversal of diastereoselectivity with the preferential acylation of the C-5' hydroxyl group was observed with 50 % de [49].

CAL has also been used in the asymmetrization of a diastereotopic furanose diol **21** (Scheme 14) [50], which could serve as an important intermediate for the synthesis of bicyclic analogs of AZT.



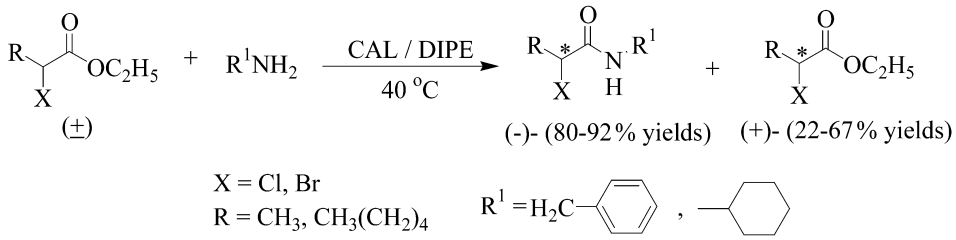
Scheme 14 Regioselective acetylation of an azido sugar.

Capabilities of lipases from *C. antarctica*, *C. rugosa*, and porcine pancreas have been evaluated for regioselective acetylation of 2-phenyl-4-(D-*arabino*-tetrahydroxybutyl)-2*H*-1,2,3-triazole (**22**), 2-phenyl-4-(D-*arabino*-*O*-1',2'-*iso*propylidene-3',4'-dihydroxybutyl)-2*H*-1,2,3-triazole (**23**), and 2-phenyl-4-(D-*threo*-trihydroxypropyl)-2*H*-1,2,3-triazole (**24**). CAL and PPL exhibited exclusive selectivity for the acetylation of primary hydroxyl group over secondary hydroxyl group(s) in all three cases [51]. We have expanded this study to CAL-catalyzed regioselective acylation of hydroxyl groups in **22**, **24**, 2-phenyl-4-(D-*erythro*-1',2',3'-trihydroxypropyl)-*2H*-1,2,3-triazole (**25**), and 2-phenyl-4-(D-*lyxo*-1',2',3',4'-tetrahydroxybutyl)-*2H*-1,2,3-triazole (**26**), using anhydrides of acetic, propanoic, butanoic, pentanoic, hexanoic, heptanoic, and benzoic acids, and 2,2,2-trifluoroethyl butyrate and vinyl acetate as acylating agents. This study clearly demonstrated the specificity of CAL for acylation of primary hydroxyl groups over the secondary hydroxyl groups for all the triazolyl sugars. Amongst the different acid anhydrides used as acylating agents, butanoic anhydride was found to be the most suitable using DIPE as solvent; it gave the corresponding monobutanoyl derivatives in 95–98 % yields [52].



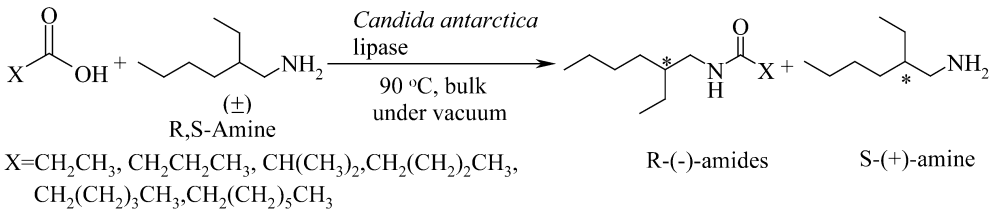
AMIDES

The synthesis of optically active amides is an area of growing interest in synthetic organic chemistry [53]. We are interested particularly in α-haloamides as they constitute an important class of compounds and they are used as starting materials for a wide variety of compounds, such as α-lactams, dioxopiperazines, oxazolidines, and depsipeptides [54–56]. Halogenated amides in optically pure form are also being used as herbicides. These observations encouraged us to synthesize α-haloamides in optically enriched forms using lipases as catalysts. Gotor et al. [57] have earlier reported the CRL-catalyzed enantioselective aminolysis of the esters of (±)-2-chloropropanoic acid with aliphatic as well as aromatic amines, but the reactions were carried out at 2 and 60 °C with aliphatic and aromatic amines, respectively. In order to overcome this problem of the wide temperature range, we have carried out aminolysis at a relatively more standard temperature (40 °C) using CAL in DIPE (Scheme 15) [58].



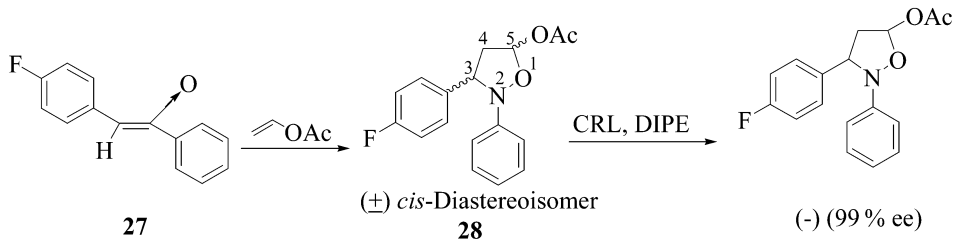
Scheme 15 Lipase-catalyzed reactions on α-haloesters.

We have performed lipase-catalyzed enantioselective amidation reactions by reacting the *racemic* amines with aliphatic acids under solvent-less conditions (bulk system). The reaction equilibrium was shifted toward amide synthesis by the removal of water under reduced pressure (Scheme 16) (unpublished results). This method may find general utility toward the efficient "green" synthesis of analogs in optically enriched form as it is difficult to synthesize amides directly from carboxylic acids and amines by purely chemicals means in enantiomerically pure form. The optically enriched amides were exclusively obtained in high yields (80–91 %).



Scheme 16 Lipase-catalyzed enantioselective amidation of aliphatic acids.

Isoxazolidines are masked amino acids and under appropriate conditions give rise to hydroxyamino acids and β-aminoalcohols, which are important components in the synthesis of a wide range of biologically active compounds, such as neopolyoxins [59], theonellamide F [60], and WS 47083 [61]. Isoxazolidines themselves possess a variety of biological activities, for example, antiviral [62], antifungal [63], anti-inflammatory [64], etc. In view of the importance of isoxazolidines in medicinal chemistry, we envisaged to synthesize 5-oxygenated 2,3-diarylisoxazolidines by [3+2] cycloaddition reaction of α,*N*-diarylnitrones with vinyl and allyl acetates to provide handles for resolution. The racemic isoxazolidine **28** was synthesized by such a reaction between α-(4-fluorophenyl)-*N*-phenylnitrone **27** and vinyl acetate in 75 % yield (Scheme 17); it was found to be diastereomerically pure as only one diastereomer with the C-3 aryl and the C-5 acetoxy groups *cis* to each other was detected. Based on our



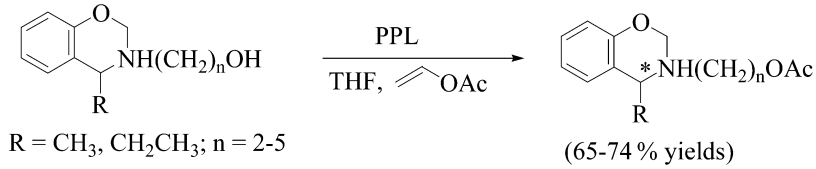
27 28

Scheme 17 Enantioselective deacetylation of 5-acetoxy-3-(4-fluorophenyl)-2-phenylisoxazolidine.

earlier studies on the use of lipases in regio- and stereocontrol of transacylation reactions on different types of compounds, we attempted enzymatic resolution of (±)-**28** with CRL and PPL in different organic solvents, viz. toluene, DIPE, tetrahydrofuran, dioxane, and acetonitrile. While no deacetylation of (±)-**28** was observed with PPL in any solvent, CRL in DIPE led to a highly enantioselective biotransformation involving the deacetylation of (±)-isoxazolidine **28** (Scheme 17) [65].

Encouraged by these results, we synthesized two series of isoxazolidines, viz. 5-acetoxy- and 5-acetoxymethyl-3-aryl-2-phenylisoxazolidines and studied their enantioselective acylation using CRL. Varying degree of enantioselectivity have been observed for different substrates, these compounds showed interesting antitubercular and anticancer activities [66].

Five (±)-4-alkyl-3,4-dihydro-3-ω-hydroxyalkyl-2*H*-1,3-benzoxazines (**29**) have been synthesized by Mannich-type condensation of *N*-ω-hydroxyalkyl-*N*-[1-(2-hydroxyphenyl)alkyl]amines and formaldehyde in 55–60 % yields. PPL in THF catalyzed the acetylation of **29** in an enantioselective fashion (Scheme 18) [67]. This perhaps is the first report of the resolution of benzoxazines involving the primary alcohol group situated far away from the asymmetric center as a remote handle for chiral recognition by PPL.



29

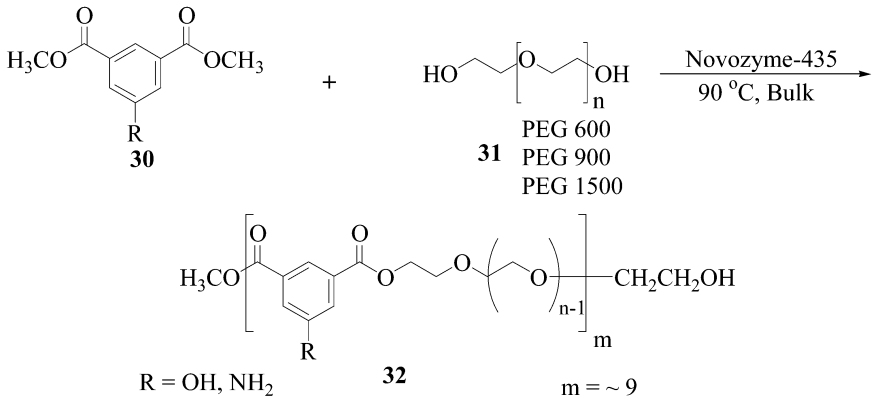
Scheme 18 Enantioselective acetylation of benzoxazines.

POLYMER SYNTHESIS

Because of their diversity and renewability, microbial polymers such as polysaccharides, bacterial polyhydroxyalkanoates, and polyanions such as poly(L-glutamic acid) have received increasing attention as candidates for industrial applications. In many cases, microorganisms carry out polymer syntheses that are impractical or impossible to accomplish with conventional chemistry. Thus, microbial catalysts enable the production of materials that might otherwise be unavailable. In addition, microbial polymers provide products that, when disposed, can degrade to nontoxic products.

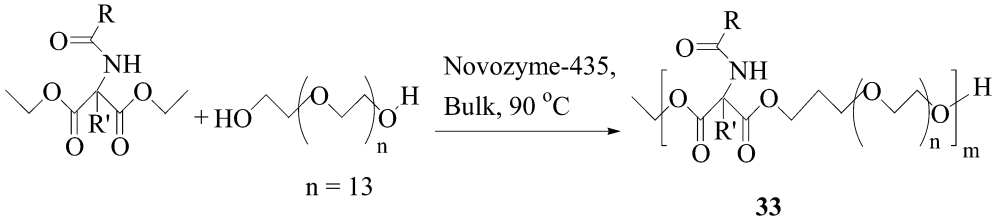
A separate activity that has taken an increased importance in recent years is the use of isolated enzymes as catalysts for in vitro polymer synthesis. Polymers with well-defined structures can be prepared by enzyme-catalyzed processes. In contrast, attempts to attain similar levels of polymer structural control by conventional methods may require harsh experimental conditions and undesirable protection-deprotection steps.

We have successfully employed Novozyme-435 (CAL-B) in the copolymerization of dimethyl 5-hydroxy/amino isophthalate **30** with poly(ethylene glycol) (PEG) **31** to give the copolymers **32** (Scheme 19) [68].



Scheme 19 Biocatalytic synthesis of amphiphilic polymers.

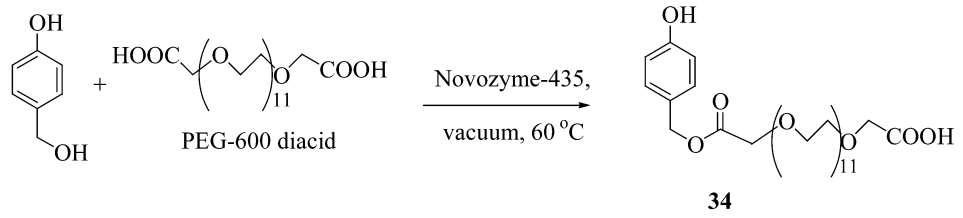
The amphiphilic polymers (obtained by alkylation of **32**) aggregate in aqueous medium, forming nanospheres of size 20–35 nm and act as potential drug delivery agents [69] as they can encapsulate small hydrophobic drugs. We have studied the influence of EDA-π interactions in drug encapsulation using the nanospheres generated from these polymers [70]. We have extended the above method to synthesize polymers with a number of linker molecules with hydroxyl, amino, carboxylic acid, alkoxy, amido, and alkoxycarbonyl moieties [71]. Besides, we have used CAL-B to synthesize multicomponent polyesters and mixed polymers having polyester and polyamide linkages under solvent-less conditions. The effect of a third component, i.e., a series of 1,ω-alkanediols on the copolymerization reaction of dimethyl 5-hydroxyisophthalate with PEG has been studied [72]. In another approach, we screened five different lipases (PPL, CRL, CAL, *Pseudomonas AY*, and *Pseudomonas cepacia*) for the condensation copolymerization of nonproteinogenic amino acid derivatives with PEG, we found that CAL (Novozyme-435) produced the polymer **33** with fine characteristics (Scheme 20) [73,74].



R	R'	m
CH_3	H	4
CH_3	3-CF_3-C_6H_4	25
CH_3	3-Cl-C_6H_4	18

Scheme 20 Enzymatic synthesis of copolymers of diethylmalonate derivatives with PEG.

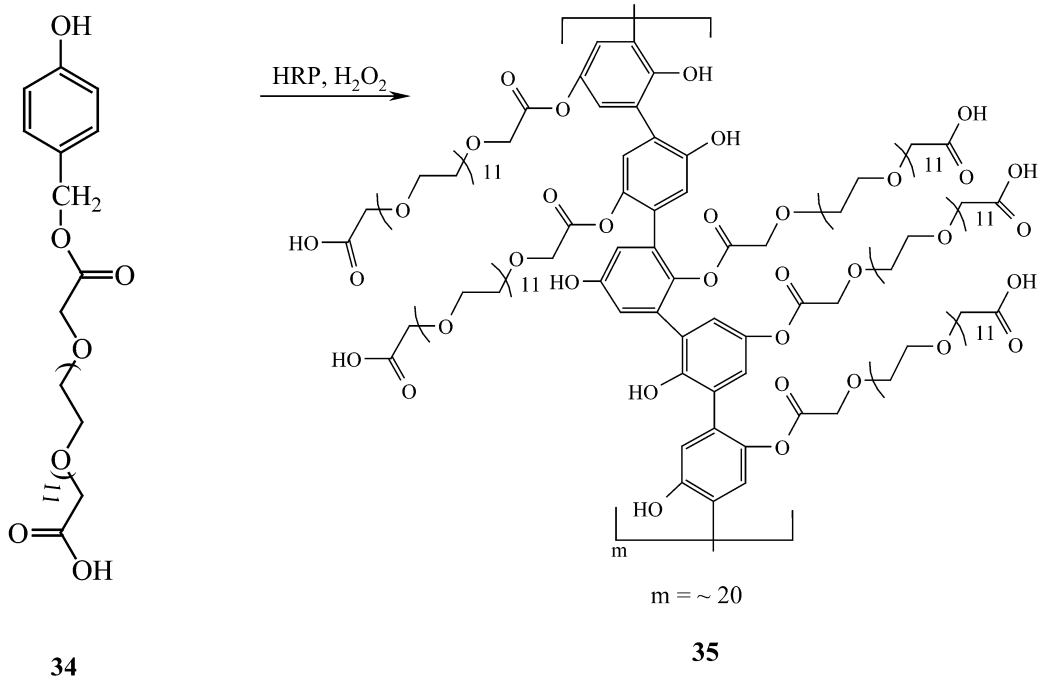
In an ongoing research program to synthesize conducting polymers, we combined the extraordinary selectivities of a lipase and oxidase to develop polymeric electrolytes for applications in dye-sensitized solar cells [75]. Initially, we synthesized a pegylated macromer by Novozyme-435 mediated biocatalytic reaction of PEG-600 diacid with 4-hydroxybenzyl alcohol (Scheme 21). The reaction proceeds in a highly chemo- and regioselective fashion, i.e., reaction occurs at alcoholic group over phenolic and



34

Scheme 21 Biocatalytic synthesis of pegylated macromer.

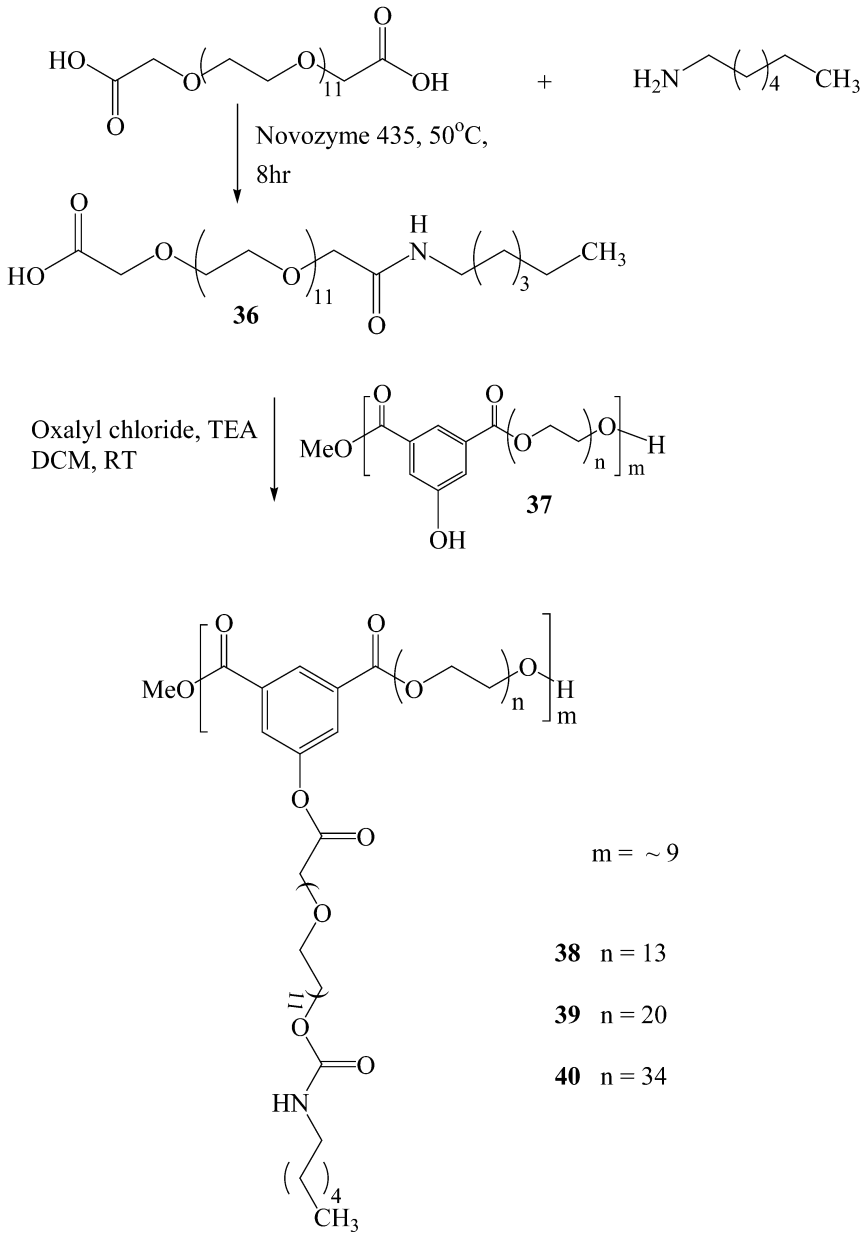
also only one end of the PEG diacid undergoes esterification. The macromer **34** has better solubility in common organic/aqueous solvents and was in turn subjected to (horse radish peroxidase) HRP mediated polymerization to yield the polymer **35** (Scheme 22). The beauty of this method is that the PEG units confer better solubility to the polymer and PEG units could be easily removed hydrolytically at later stages.



34 **35**

Scheme 22 HRP-mediated synthesis of pegylated polymeric polyphenolics.

Also, we have developed a novel biocatalytic route to synthesize quasi-solid electrolytes by coupling pegylated-amide **36** with the copolymer **37** (unpublished results). The Novozyme-catalyzed amidation of PEG diacid/(M_n 600) was found to be highly selective as the amidation reaction occurred at only one acid end (Scheme 23). The photovoltaic characteristics of these polymeric materials **38**–**40** were studied and found to have reasonably good PV efficiency (Table 1) (unpublished results).



Scheme 23 Chemo-enzymatic synthesis of quasi-solid electrolytes.

Table 1 Photovoltaic (PV) characteristics of biocatalytically derived quasi-solid electrolytes.

Biocatalytically derived quasi-solid electrolyte	Open circuit voltage (V)	Short circuit current density (mA/cm^2)	Efficiency (%)	Fill factor
38	0.648	9.664	4.04	0.645
39	0.681	9.485	4.32	0.668
40	0.671	9.712	4.27	0.656

We are extending our studies to the development of polymeric materials that can find applications in diverse areas such as drug delivery, gene delivery, flame-retardants, and photoelectrolytes.

Thus, lipases have allowed new options for the control of polymer molecular weight as well as the morphology and architecture of polymeric products.

CONCLUSION

In general, lipases are inexpensive and ecologically beneficial natural catalysts. Owing to these advantages, it is to be expected that lipase-catalyzed reactions will play an increasing role, primarily in the development of economic, efficient, and "greener" methodologies for the preparation of nonracemic chiral biologically active compounds and polymeric systems in the laboratory as well as in industrial sectors.

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