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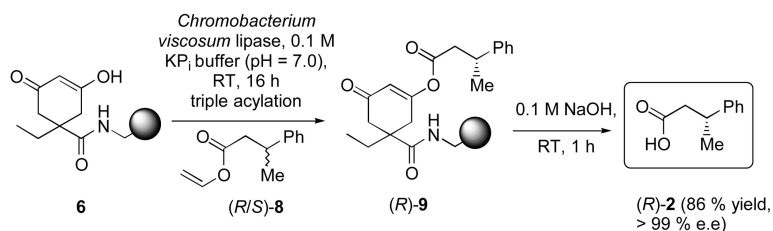
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Lipase-Catalyzed Kinetic Resolution on Solid-Phase via a "Capture and Release" Strategy

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Lipases are industrially important enzymes that have found widespread use in the resolution of chiral alcohols, amines and carboxylic acids.[1] In view of their diverse substrate specificity they have also been used as reagents for the solution-phase parallel synthesis of libraries of compounds based upon pharmacophoric scaffolds.[2] However, in comparison with proteases, little is known about the activity of lipases toward resin-bound substrates,[3] an area of contemporary interest with applications in solid-phase synthesis[4] including enzyme-cleavable linkers.[5] Herein we report the first example of a lipase-catalyzed acylation reaction on a resin-bound substrate, the results of which may have applications in parallel solid-phase synthesis and screening methodologies.

We recently reported the synthesis of resin-bound cyclohexane-1,3-dione (CHD) and demonstrated its use as a capture and release reagent for the synthesis of amides.[6] Furthermore, we have shown that esters of cyclic-1,3-diketones are good substrates for lipases and esterases[7] and thus sought to combine these two ideas to investigate lipase resolutions on resin-bound substrates. Screening of a range of lipases and esterases against enol ester (*R/S*)-**1** (Scheme 1) identified *Chromobacterium viscosum* lipase (CVL) as possessing good activity and high enantioselectivity [(*E*) = 88],[8] and hence this enzyme was selected for initial studies. Previous work has shown that enzyme-catalyzed reactions on solid-phase are greatly enhanced by the use of PEGA$_{1900}$ resin (commercially available from Polymer Laboratories, Ltd.) which swells in aqueous solvents.[9] The resin also has an open-pore structure allowing large enzyme molecules access to the resin active sites.[10]

PEGA$_{1900}$ supported CHD resin **6** was prepared as shown in Scheme 2. Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid **3**, obtained by the Birch reduction and alkylation of 3,5-dimethoxy-benzoic acid,[11] was coupled (× 2) directly to PEGA$_{1900}$ resin **4** using DIC in DCM/DMF (1:1) to give resin bound bis-enol ether **5**. Acidic hydrolysis of **5**, using a TFA/H$_2$O/DMF (90:5:5) mixture, yielded CHD resin **6**. The loading of CHD resin **6** was determined as 19 μmol/g (swollen) and 130 μmol/g (dry), by coupling with (*R/S*)-3-phenylbutyryl chloride followed by standard sodium hydroxide release of the resin bound racemate.

PEGA$_{1900}$ supported CHD **6** was subjected to CVL-catalyzed acylation using (*R/S*)-methyl 3-phenylbutanoate **7** and (*R/S*)-vinyl 3-phenylbutanoate **8** (Scheme 2) to yield resin-bound 1,3-enol ester **9**. As is typical for solid-phase reactions, a large excess (approximately 10 equiv) of acylating agent was used to ensure high conversions. The reaction was performed in aqueous potassium phosphate buffer at neutral pH in the absence of organic solvent. Under these conditions, the concentration of hydrophobic acylating agent at the reaction site, within the PEGA$_{1900}$ microenvironment, is high.[12] After overnight reaction, the resin was extensively washed,

Scheme 1. CVL-Catalyzed Hydrolysis of Dimedone 1,3-Enol Ester **1**[a]
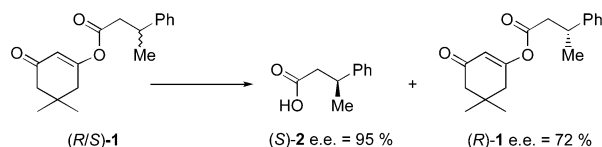
^{a} Reagents and conditions: CVL, 10% MeCN/0.1 M KPi buffer (pH = 7.0), rt, 1.5 h (49%).

Scheme 2. Synthesis of CHD-PEGA$_{1900}$ Resin **6** and Lipase-Catalyzed Resolution Involving a "Capture and Release" Strategy[a]
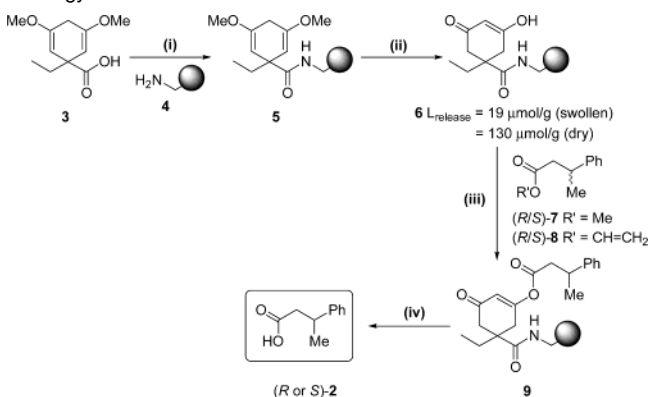
^{a} Reagents and conditions: **(i)** DIC, DCM/DMF (1:1), rt, 2.5 h × 2; **(ii)** TFA/H$_2$O/DMF (90:5:5), rt, 2.5 h; **(iii)** lipase, 0.1 M KP$_i$ buffer (pH = 7.0), rt, 16 h; **(iv)** 0.1 M NaOH, rt 1 h.

Table 1. Lipase-Catalyzed Resolution by "Capture and Release"

entry	ester	lipase	product	yield[a]	ee
1	(*R/S*)-**7**	CVL	(*R*)-**2**	74	65
2	(*R/S*)-**8**	CVL	(*R*)-**2**	38	>99
3	(*R*)-**8**[b]	CVL	(*R*)-**2**	92	>99
4	(*S*)-**8**[c]	CVL	(*R*)-**2**	7	>99
5	(*R/S*)-**8**	PCL	(*R*)-**2**	77	59
6	(*R/S*)-**8**	PPL	(*R*)-**2**	83	62
7	(*R/S*)-**8**	CVL[d]	(*R*)-**2**	78	>99
8	(*R/S*)-**8**	CVL[e]	(*R*)-**2**	86	>99

^{a} Calculated by comparison of HPLC peak areas with a control [NaOH hydrolysis of chemically synthesized racemic enol ester]. ^{b} ee = 96%. ^{c} ee = 92%. ^{d} Double acylation. ^{e} Triple acylation.

followed by acyl group release from the resin (aqueous NaOH) to give 3-phenylbutyric acid **2** which was analyzed by HPLC (Chiracel-ODH column) to determine the yield and enantiomeric excess (ee) of the reaction (Table 1). In each case the predominant enantiomer observed was the (*R*)-acid **2** rather than the expected (*S*)-acid despite the known preference for CVL to catalyze hydrolysis of the (*S*)-enantiomer in solution (Scheme 1). Similar results were obtained with *Pseudomonas cepacia* lipase (PCL) and

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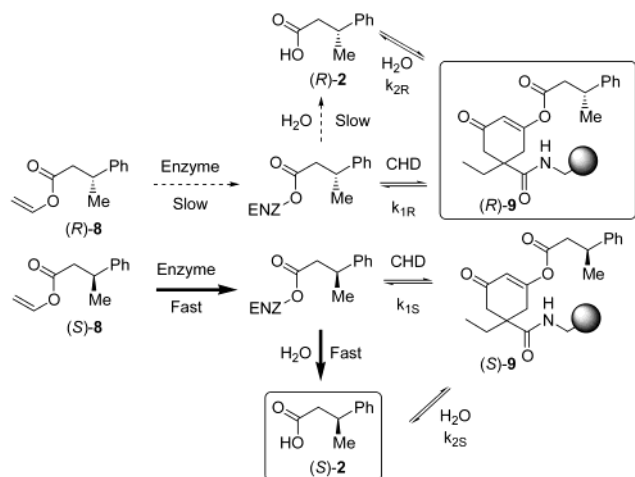
Figure 1. Explanation of enantiospecificity of reaction.

porcine pancreatic lipase (PPL), both of which are also known to be (*S*)-selective. Use of the methyl ester **7** (Table 1, entry 1) resulted in a reasonable yield of (*R*)-acid **2** with modest ee, whereas the more reactive vinyl ester **8** (Table 1, entry 2) gave a lower yield but excellent ee. Subjecting the resin to a second acylation reaction, after washing, resulted in an increased yield (78%) (Table 1, entry 7) which could be further enhanced (86%) with a triple acylation (Table 1, entry 8).

To probe this unexpected enantiospecificity, the acylation reactions were repeated using enantiomerically enriched acyl donors. When (*R*)-vinyl ester **8** (ee = 96%) was used, the (*R*)-acid **2** was obtained in high yield and ee (Table 1, entry 3). However, switching to the enantiomerically enriched (*S*)-vinyl ester **8** (ee = 92%) also gave the (*R*)-acid **2** in low yield but high ee (Table 1, entry 4). In this case the lipase selectively catalyses acylation of the minor contaminant (*R*)-enantiomer, in the presence of excess (*S*)-enantiomer.

The reaction in the absence of lipase showed negligible levels of formation of 3-phenylbutyric acid **2** by HPLC, eliminating the possibility of background reaction between CHD resin **6** and vinyl ester **8**. Similarly, the corresponding solution-phase reactions, in the absence of lipase, between dimedone and either vinyl ester **8** or 3-phenylbutyric acid **2** showed no evidence of conversion.

The enantiospecificity of the reaction can be rationalized by considering the competing enzymatic hydrolysis of the (*R*)- and (*S*)-enantiomers of **8** in a lipase-catalyzed parallel kinetic resolution analogous to that reported by Rakels et al.[13] (Figure 1).

(*S*)-Vinyl ester **8** undergoes rapid formation of an acyl-enzyme intermediate followed by hydrolysis to form (*S*)-acid **2**. Solution-phase hydrolysis of vinyl ester **8** revealed that the (*S*)-enantiomer is completely hydrolyzed in less than 15 min under these conditions. The corresponding hydrolysis of the (*R*)-enantiomer is much slower than that of the (*S*)-enantiomer allowing the enzyme intermediate to undergo transesterification to form the resin bound (*R*)-enol ester **9**. An alternative scenario, which we cannot exclude at present, involves initial acylation by both (*R*)- and (*S*)-esters ($k_{1S} > k_{1R}$) followed by rapid hydrolysis of the (*S*)-enantiomer ($k_{2S} > k_{2R}$), thus releasing (*S*)-acid into solution with accumulation of the (*R*)-enantiomer on the resin.[14]

The difference in ee for transesterification with methyl versus vinyl ester (Table 1, entries 1 and 2) is consistent with the faster rate of hydrolysis of vinyl versus methyl ester. Previous unpublished studies in our laboratories have shown that diffusion of enzymes into PEGA$_{1900}$ resin occurs over a period of ca. 1 h. When CVL was allowed to preequilibrate with the resin for 1 h before addition of vinyl ester **8**, the ee of the liberated (*R*)-acid **2** decreased to 90%. Under these conditions the (*S*)-vinyl ester **8** presumably undergoes a small degree of transesterification onto the CHD resin, prior to hydrolysis. Longer preequilibration times did not lead to further reductions in ee.

In conclusion, we have demonstrated for the first time that the lipase-catalyzed kinetic resolution of racemic esters can be carried on solid-phase using an acylation/deacylation capture and release strategy. The reactions exhibit enantiospecificity which is consistent with the operation of a parallel kinetic resolution process. Such resin-mediated reactions should be amenable to automation, particularly using parallel synthetic approaches, and in view of the broad substrate specificity of available lipases they can be exploited for the preparation of combinatorial libraries suitable for screening against biological targets of interest.

Supporting Information Available: Experimental preparations for ester (*R/S*)-**1**, resins **5**, **6**, esters **7**, **8**, and standard procedures for lipase-catalyzed hydrolysis and transesterifications (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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