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# Lipase Catalysis in Organic Solvents. In Search of Practical Derivatizing Agents for the Kinetic Resolution of Alcohols.

**Hanamanthsa S. Bevinakatti* and Ravindra V. Newadkar**

Alchemie Research Centre, ICI India Ltd Site, Thane-Belapur Road, Thane-400 601, India

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***Abstract***: 2-Phenyloxazolin-5-one ( **2** ) acts as a 'derivatizing agent' in the lipase catalyzed kinetic resolution of N-ethoxycarbonyl-2-amino-1-butanol ( **1** ) in organic solvents. Unique structural alteration possible due to condensation with **2** gives enough scope for the separation of product and substrate isomers without using column chromatography.

Enzymatic synthesis has now become a common tool in organic chemistry to prepare optically pure compounds[1]. Lipases are presently the most widely used enzymes for the kinetic resolution of esters and alcohols. While the conventional approach to achieve the kinetic resolution of alcohols is through the aqueous hydrolysis of their simple esters like acetates, now this can also be achieved in non-aqueous systems using n-butanol as a nucleophile[2]. Complimentary to these 'deacylation approaches', kinetic resolution of alcohols can also be achieved using 'acylation approaches' by using acylating agents such as ethyl acetate[3], trihaloalkyl butyrates[4], vinyl acetate[5] and acetic anhydride[6].

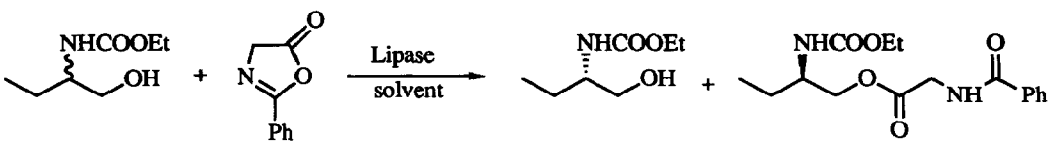
Despite best enantioselectivities, commercial exploitation of this reaction at the expected rate has not been possible due to some of the drawbacks with the above approaches such as - a) careful monitoring of the reaction so as to stop at the required conversion and, b) column separation of product and substrate isomers. These are impractical on an industrial scale. These drawbacks can, however, be overcome if it is possible to use an 'ideal acylating/derivatizing agent' which can be - a) highly reactive so as to limit its usage only to required amounts. This not only would avoid the need to remove excess of it but would also save the problem of careful monitoring of the reaction. The reaction would automatically stop after complete consumption of the derivatizing agent ; b) capable of altering the structure of the product to such an extent that it should be possible to separate the product from substrate without column chromatography. From an industrial viewpoint, it is important to find such an 'ideal derivatizing agent' and we report herein our efforts towards finding one.

While working on the enzymatic synthesis of optically active amino acids via lipase catalyzed ring-opening of oxazolin-5-ones[7], we found that 2-phenyloxazolin-5-one[8] (**2**) readily undergoes ring-opening with

n-butanol in diisopropyl ether (DIPE) to afford butyl hippurate in quantitative yields[7a]. The ease with which **2** reacted prompted us to further test its ability to act as an enantioselective derivatizing agent in the kinetic resolution of alcohols. Due to the industrial importance of 2-amino-1-butanol, a precursor to antitubercular drug Ethambutol which has already been the subject of several lipase catalysis studies[9], we chose its N-protected derivative, N-ethoxycarbonyl-2-amino-1-butanol[10] (**1**) as a substrate for our present study.

Kinetic resolution of **1** in presence of 0.4 (or 0.6) equivalents of the azlactone **2** and catalytic amounts of lipase from *Mucor miehei* ( Lipozyme[11] ) was carried out in three different solvents - DIPE, dichloromethane and benzene in which both the substrates were completely soluble. Reactions stopped automatically after the complete consumption of **2**, thus avoiding the need to closely monitor the conversion of the reactions. In all the solvents, the (*R*)-isomer of **1** preferentially cleaved the azlactone to afford (*R*)-2-[(N-ethoxycarbonyl)amino]butyl hippurate (**3**) ( see Table 1 ). However, DIPE turned out to be the solvent of choice for this reaction. The solid product (*R*)-**3**, being insoluble in DIPE, dropped out of the reaction mixture as and when formed thus avoiding the need for a tedious separation of it from the unreacted isomer. It was separated from the soluble (*S*)-**1** by a simple filtration. Reaction products from dichloromethane and benzene were also separated without column chromatography by suspending them in DIPE[12].

***Scheme 1***




(*R,S*)-**1** **2** (*S*)-(-)-**1** (*R*)-(+)-**3**

***Table 1*** : Lipase catalyzed kinetic resolution of **1**.

| solvent | ratio **1 : 2** | (*S*)-(-)-**1** | | (*R*)-(+)-**3** | |
|---|---|---|---|---|---|
| | | $[\alpha]_D$[a] | ee[b] % | $[\alpha]_D$[a] | ee[c] % |
| DIPE | 1:0.4 | -22.1 | 63 | +19.9 | 93 |
| | 1:0.6 | -26.2 | 75 | +12.3 | 57 |
| $CH_2Cl_2$ | 1:0.4 | -22.8 | 65 | +17.5 | 81 |
| $C_6H_6$ | 1:0.4 | -22.0 | 63 | +15.8 | 73 |

[a] Measured in 1-5% EtOH at 25°C. [b] Based on Lit.[9c] value for ( S )- 1, $[\alpha]^{25}_D$ = -32.2 (2, EtOH) ( 92% ee ). [c] Based on authentic sample's $[\alpha]^{25}_D$ = +21.5 (6, EtOH) (see ref. 13).

In summary, this study[14] shows that some of the disadvantages associated with the present approaches for the commercial exploitation of enzyme catalyzed kinetic resolution of alcohols can be overcome by properly designing the derivatizing agents in combination with the right solvent system.

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10. Prepared from 2-amino-1-butanol following the known method : see ref. 9c.
11. Lipozyme$^{TM}$ (31 BIU/g), a commercially available lipase from fungus *Mucor miehei,* immobilized on a macroporous anion exchange resin was kindly provided by Novo-Nordisk.
12. A typical experimental procedure consisted of stirring a mixture of **1** (1.61 g, 10 mmol), **2** (0.64 g, 4 mmol or 0.97 g, 6 mmol) and Lipozyme (0.3 g) in DIPE (50 ml) (or 15 ml $CH_2Cl_2$ or 30 ml benzene, see Table ) at room temperature until GC analysis showed complete disappearance of **2** ( 6-12 h ). Filtration followed by removal of DIPE from filtrate gave (*S*)-**1**. (*R*)-**3** was separated from enzyme

residue by suspending in $CH_2Cl_2$, filtration and then removal of solvent.

$CH_2Cl_2/C_6H_6$ reactions : Filtrate containing (*S*)-**1** and (*R*)-**3** was evaporated to dryness, suspended in DIPE ( 50 ml ) and filtered to give (*R*)-**3** as the insoluble solid residue. (*S*)-**1** was recovered from filtrate.

Yields of (*S*)-**1** and (*R*)-**3** varied between 85-90% of the theoretically expected. Analytically pure samples of (*S*)-**1** and (*R*)-**3** for ee determination were further purified by column chromatography.

13. An authentic sample of (*R*)-**3** ( $[\alpha]^{25}_D$ = +21.5 (6, EtOH) ) was prepared by condensing equimolar quantities of (*R*)-**1** ( $[\alpha]^{25}_D$ = +34.65 (5, EtOH), prepared from (*R*)-(-)-2-amino-1-butanol ) and **2** in presence of Lipozyme in DIPE as mentioned above.
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