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Enzymatic Synthesis of Optically Active Amino Acids. Effect of Solvent on the Enantioselectivity of Lipase-catalysed Ring-opening of Oxazolin-5-ones.

Hanamanthsa S. Bevinakatti,* Ankur A. Banerji, Ravindra V. Newadkar, and Asmita A. Mokashi

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

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Abstract : *Reaction medium plays an important role in governing the enantioselectivity of the lipase-catalysed ring opening of 4-substituted-2-phenyl-oxazolin-5-ones.*

Synthesis of optically active α-amino acids continues to be a field of growing interest in organic chemistry.[1] We have recently reported a new method of preparing optically active α-amino acids via lipase-catalysed enantioselective ring opening of 2-phenyl-oxazolin-5-ones using n-butanol as the nucleophile in diisopropyl ether.[2] The prediction that this method has the potential to be an alternative high yielding route to prepare optically active α-amino acids[3] is further strengthened by a recent study by Sih et al.,[4] where marked improvements in optical purities (up to >99% ee) were attained after screening nearly a dozen lipases in aqueous medium. Moreover, the reaction could be maneuvered to give the desired isomer, either (*R*) or (*S*), by simply choosing the right enzyme.

While screening of enzymes is one approach to optimise the enantioselectivity of an enzyme catalysed reaction, another

alternative and simpler approach to achieve this is through 'solvent engineering'-by simply changing the reaction medium.[5] We report herein the application of the latter approach in enhancing the enantioselectivity of the *Mucor miehei* lipase (Lipozyme[6]) catalysed ring opening of 4-substituted-2-phenyl-oxazolin-5-ones using n-butanol as the nucleophile to give optically active α-amino acid esters.

2-Phenyl-4-benzyl-oxazolin-5-one (**1a**), derived from (*R S*) - phenylalanine,[7] was selected as a substrate for our 'solvent engineering' studies. **1a** was subjected to Lipozyme-catalysed ring opening using n-butanol[8] in nine different solvents to give, after quantitative conversion, optically active butyl-N-benzoylphenylalanine (Scheme 1). In all the solvents used, Lipozyme showed selectivity towards the (*S*)-isomer, however, with different degree of selectivity. While dichloromethane showed the best selectivity (69% ee), diisopropyl ether (DIPE), the solvent reported in our previous study, interestingly showed the least selectivity (33% ee). Three other azlactones derived from alanine, leucine and norvaline (R = Me (**1b**); *i*-butyl (**1c**); *n*-propyl (**1d**) respectively) showed a similar trend when tried in three solvents - DIPE, 1-butanol and CH_2Cl_2 (Table 1).

In summary, this study shows that it is possible to increase the optical purity of the α-amino acid derivatives obtained from the lipase catalysed ring opening of oxazolin-5-ones by simply changing the reaction medium. By carefully selecting the solvent and lipase it should now be possible to prepare any α-amino acid by this route not only in excellent optical purity but also in desired stereochemical form.

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Table 1. Effect of solvent on enantioselective ring opening of **1a-d.**

Solvent	ee% of (*S*)-(2)			
	2a	**2b**	**2c**	**2d**
DIPE	33	34	39	26
CCl_4	43			
C_6H_6	51			
t-BuOH	59			
n-BuOH	59	42	66	39
$CHCl_3$	60			
t-AmylOH	61			
THF	62			
CH_2Cl_2	69	47	61	43

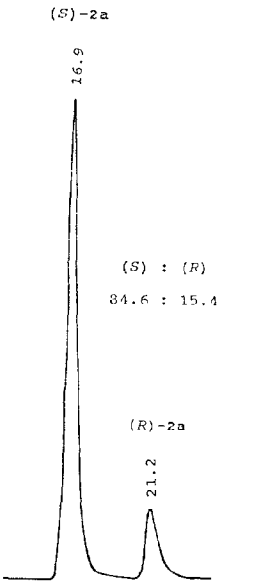
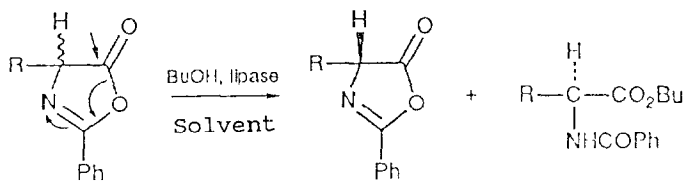


Fig. 1. Chiral HPLC Seperation of (*R*)- and (*S*)-**2a**

Scheme 1



(*R,S*)-**1a-d** (*R*)-**1a-d** (*S*)-**2a-d**

Partial *in situ* racemisation

R = $PhCH_2$ (**a**); CH_3 (**b**); $(CH_3)_2CHCH_2$ (**c**); $CH_3CH_2CH_2$ (**d**)

References and Notes

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6 Lipozyme IM20(31 BIU g^{-1}), a commercially available lipase from the fungus *Mucor miehei*, immobilised on a macroporous anion exchange resin, was a gift from Novo-Nordisk.

7 4-Substituted-2-phenyl-oxazolin-5-ones were readily prepared treating respective amino acid with PhCOCl in presence of aq.NaOH followed by Ac_2O dehydration.

8 *General Procedure*: A mixture of oxazolin-5-one **1a-d** (20 mmol), butanol (40 mmol), and 1 g Lipozyme in 40 ml solvent was stirred at ambient temperature until GC/TLC showed complete disappearance of **1**. Filtration followed by removal of solvent gave product **2**, the absolute configuration and ee of which were determined by direct chiral HPLC analysis using Pirkle DNBPG-column (hexane-IPA 90:10). Retention time, t_R, (in minutes) for (*S*)- and (*R*)-isomers, respectively, are : **2a** 16.9 and 21.2; **2b** 18.2 and 22.2; **2c** 12.3 and 15.4; **2d** 13.6 and 16.3. (Fig. 1). Pure **2** was isolated by purification on a silica-gel column (dichloromethane) (>90% yield).