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Dynamic kinetic resolution: synthesis of optically active α-amino acid derivatives

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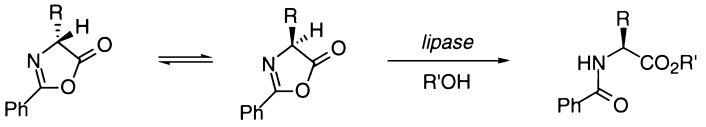
Abstract

Candida antarctica lipase B (Novozyme) is an effective catalyst for the dynamic kinetic resolution of racemic 2-benzyl-4-substituted-5(4*H*)-oxazolones, in the presence of an alcohol, yielding optically active *N*-benzoyl amino acid esters. The reaction has been optimised with respect to the solvent and the effect of a catalytic amount of organic base is investigated.

Despite the success of enzyme-catalysed kinetic resolutions for the generation of a wide range of chiral building blocks,[1] there is an increasing desire to develop transformations that are not limited by a maximum yield of only 50% of the desired product. Increasing attention has been given in recent years to the discovery of dynamic kinetic resolution (DKR) processes in which the unreactive enantiomer equilibrates, in situ under the reaction conditions, with the reactive antipode.[2] DKR reactions can thus result in quantitative yields with enantiomeric excesses (e.e.s) approaching 100%. Previously, we[3] and others[4] have reported the lipase-catalysed DKR of 5(4*H*)-oxazolones **1** as an entry into enantiomerically pure amino acids. Oxazolones are excellent substrates for DKR reactions on account of (i) the low p*K*a of the C-4 proton;[5] and (ii) their inherent reactivity towards lipase-catalysed alcoholysis.



(R)-**1** (S)-**1**

In our initial studies we described an efficient method for the synthesis of L-(*S*)-*tert*-leucine using the oxazolone approach based upon *Rhizomucor miehei* lipase (Lipozyme) as the catalyst.[5]

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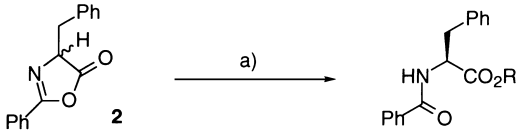
Although this lipase gives excellent results for *tert*-leucine (94% yield, 99.5% e.e.), less sterically demanding substrates result in a dramatic drop in the e.e. and additionally the reaction displays a pronounced dependence on the chain length of the alcohol.[6] Herein we report that these limitations can be overcome by employing *Candida antarctica* lipase B (Novozyme) as the catalyst, enabling a wider range of substrates to be transformed with high yield and e.e.

Initially Lipozyme and Novozyme were compared as catalysts using 2-phenyl-4-benzyl-5(4*H*)-oxazolone **2** as the substrate in toluene with *n*-butanol as the nucleophile (Table 1).[7] The results in Table 1 illustrate clearly that the use of Novozyme results in a higher yield and e.e. compared to Lipozyme. The data also confirm our previous observation that addition of Et_3N (0.25 equiv.) results in a significant increase in the e.e. of the product as deduced by comparing entries 1–2 and 3–4, respectively. The initial moderate yield obtained for the Novozyme reaction (entry 4) was due to the competing hydrolysis reaction which could be substantially reduced by pre-drying the crushed lipase over phosphorus pentoxide to remove excess water. An increase in the yield from 51% to 81% (entry 5) was observed with no detrimental effect on the e.e.

Table 1
Dynamic kinetic resolution of oxazolone **1** using Lipozyme and Novozyme



Entry	Lipase	RO'H	Et_3N	Yield/ %	e.e./ %[b]
1	Lipozyme	$CH_3CH_2CH_2CH_2$	no	59	55
2	Lipozyme	$CH_3CH_2CH_2CH_2$	yes	64	69
3	Novozyme	$CH_3CH_2CH_2CH_2$	no	40	64
4	Novozyme	$CH_3CH_2CH_2CH_2$	yes	51	95
5	**Novozyme[c]**	**$CH_3CH_2CH_2CH_2$**	**yes**	**81**	**95**

a) toluene, lipase, Et_3N (0.25 equiv.), R'OH (2 eq.), 37°C, b) e.e's. measured by chiral HPLC using a Chiracel OD column, c) crushed and dried over P_2O_5.

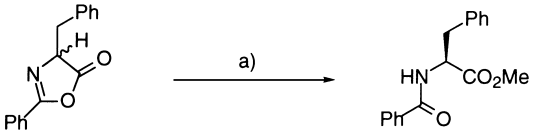
The alkyl chain length of the alcohol nucleophile was also varied and found to have little effect on the e.e. of the product (with the exception of *n*-pentanol) in the Novozyme mediated biotransformation, which is in direct contrast to the results found with Lipozyme (Table 2).

Table 2
Effect of nucleophile alkyl chain length

	Novozyme		Lipozyme	
R-OH	Yield/ %	e.e./ %	Yield/%	e.e./ %
CH_3	79	**94**	55	40
CH_3CH_2	82	**97**	53	83
$CH_3CH_2CH_2$	83	**97**	n.d.	n.d.
$CH_3CH_2CH_2CH_2$	81	**95**	69	73
$CH_3CH_2CH_2CH_2CH_2$	32	**88**	n.d.	n.d.

In an attempt to increase the e.e. of the products in the Novozyme reaction the solvent used was varied in the presence and absence of Et_3N (Table 3). Chlorinated solvents gave poorer yields and e.e.s. while ethers gave comparable results to those obtained in toluene. In general the effect of the Et_3N was to enhance the e.e. of the product, in some cases in a dramatic fashion (i.e. ^{t}BuOMe, DIPE). However, the use of tetrahydrofuran and acetonitrile as solvent produced the reverse effect. In both cases the e.e. remained high and the yield in fact increased in the absence of Et_3N. Although the initial reason for adding Et_3N to these DKR reactions was to accelerate the rate of substrate racemisation it appears that it has a more complex role. Kinetic experiments[8] have shown that, in non-polar solvents such as hexane and toluene, small amounts of carboxylic acid by-product (e.g. *N*-benzoyl phenylalanine), resulting from hydrolysis of the ozaxolone substrate, cause a marked lowering of the e.e. of the product. Addition of triethylamine reverses this effect, presumably due to the formation of a soluble ion-pair.[9] In the absence of the base, the carboxylic acid generated may remain bound to the lipase surface thus altering the ionogenic state of the enzyme and leading to a change in conformation and hence enantioselectivity. In polar solvents, such as acetonitrile and tetrahydrofuran, the acid by-product is more soluble and therefore less likely to interact with the lipase, resulting in no reduction of the observed e.e. In terms of overall simplicity the use of acetonitrile as solvent is to be preferred.

Table 3
Dynamic kinetic resolution of oxazolone **2** using different solvents in the presence/absence of Et_3N



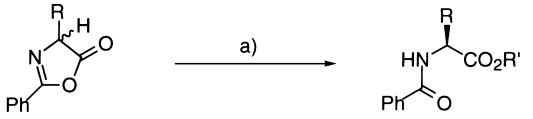
Solvent	Et_3N Present		Et_3N Absent	
	Yield/ %	e.e./ %	Yield/ %	e.e./ %
DCM	78	89	65	75
$CHCl_3$	66	75	63	83
THF	**64**	**95**	**71**	**97**
Et_2O	87	97	90	58
^{t}BuOMe	90	96	91	34
DIPE	86	96	90	33
Toluene	82	94	82	71
CH_3CN	**44**	**97**	**88**	**98**

a) Solvent, Novozyme, (Et_3N, 0.25 equiv.), CH_3OH, 37°C.

Finally we investigated the substrate specificity of Novozyme. A series of C-4 substituted oxazolones were examined under conditions of either CH_3CN or toluene/Et_3N as solvent. The results are shown in Table 4. It can be seen that for a range of different side chains (e.g. R = CH_2Ph, $CH_2CH(CH_3)_2$, $CH(CH_3)_2$) the yields and e.e.s of the products are generally good to excellent and that the reaction conditions have little effect. For entries 4 and 5, acetonitrile was found to be inferior to toluene/Et_3N. Perhaps expectedly, when the R group is either sterically demanding, e.g. *tert*-butyl or the small methyl group, the enantioselectivity is observed to be poor.

In summary we have demonstrated that Novozyme is a versatile catalyst for the synthesis of optically active α-amino acid derivatives based upon DKR of 5(4*H*)-oxazolone substrates.

Table 4
Dynamic kinetic resolution of 4-substituted oxazolones



Entry	R	R'	Solvent	Yield/ %	e.e./ %
1	CH_2Ph	CH_3	CH_3CN	88	98
			Toluene, Et_3N	81	95
2	$CH_2CH(CH_3)_2$	CH_3	CH_3CN	96	97
			Toluene, Et_3N	96	97
3	$CH(CH_3)_2$	CH_3	CH_3CN	83	97
			Toluene, Et_3N	82	95
4	Indolemethylene	CH_3	CH_3CN	0	-
			Toluene, Et_3N	90	90
5	$CH_2CH_2SCH_3$	CH_3	CH_3CN	79	73
			Toluene, Et_3N	69	80
6	CH_3	i. CH_3	CH_3CN	94	10
		ii. CH_3CH_2 CH_2	Toluene, Et_3N	60	14
7	$C(CH_3)_3$	CH_3	CH_3CN	0	-
			Toluene, Et_3N	40[b]	35

a) solvent, Novozyme, R'OH, 37°C, b) based on recovered starting material.

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7. To a solution of oxazolone (100 mg) dissolved in organic solvent (8 mL) were added alcohol (2.0 equiv.), Et_3N (0.25 equiv., optional) and lipase (100 mg). The flask was stoppered and placed in an orbital incubator at 37°C and the reaction monitored by TLC. Upon consumption of the starting material the lipase was filtered off and washed with solvent (2×10 mL). The combined organic fractions were evaporated under reduced pressure and the crude product purified by column chromatography to give the desired product as a colourless solid. In all cases the (*S*)-isomer of the product predominated.
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