Enzymatic Synthesis of Amides with Two Chiral Centres

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Candida cylindracea lipase and subtilisin protease were utilised in the enantioselective aminolysis of ethyl (±)-2-chloropropionate with racemic amines.

In recent years, interest in the use of enzymes for the preparation of optically active compounds has been growing continuously. This application has attracted the attention of organic chemists because of their usefulness as chiral catalysts.[1] Moreover, the fact that some enzymes can work in organic solvents[2] offers new possibilities in synthetic organic chemistry.

The use of lipases and proteases to catalyse amide bond formation is an interesting alternative to conventional methods. Lipases have been used in anhydrous organic solvents in the synthesis of peptides.[3] Recently, we have studied the formation of chiral amides[4] by reaction of a racemic ester and amines with *Candida cylindracea* lipase (CCL); and Klibanov *et al.*[5] have described the resolution of racemic amines *via* a subtilisin-catalysed aminolysis reaction. However, the formation of amides with two chiral centres using racemic substrates has never been reported.

We report here an easy, enantioselective preparation of amides with two chiral centres through the enzymatic reaction of ethyl (±)-2-chloropropionate (**1**) and racemic amines (**2**). The enzymes chosen to catalyse this reaction were CCL and subtilisin, because of their high enantioselectivity for the ester[4] and amines[5] respectively. The results are summarised in Table 1.

$$\underset{(\pm)\ (\mathbf{1})}{MeCHClCO_2Et} + \underset{(\pm)\ (\mathbf{2})}{R\text{-}NH_2} \xrightarrow{\text{Enzyme}} \underset{\text{(e.e. 45—95\%)}\ (\mathbf{3})}{MeCHClCONHR}$$

Table 1. Asymmetric synthesis of amides (**3**) from ethyl (±)-2-chloropropionate and amines (**2**).[a]

Entry	R-NH_2	Yield (%)	Isomer proportion (%)[b] (t_R min)	$[\alpha]_D^{25}$/°	Isomer	E.e.(%)[c]
(**3a**)	Isobutylamine[d]	30	63 (24.5)	−0.42(*c* 0.47, $CHCl_3$)	(2*S*,2′*S*)	95
			37 (27)	−32.0(*c* 0.42, $CHCl_3$)	(2*S*,2′*R*)	77
(**3a**)	Isobutylamine[e]	45	45 (24.5)	−0.39(*c* 0.25, $CHCl_3$)	(2*S*,2′*S*)	90
			55 (27)	+34.0(*c* 0.31, $CHCl_3$)	(2*R*,2′*S*)	82
(**3b**)	2-Aminoheptane[d]	40	60 (18)	−8.3(*c* 0.36, $CHCl_3$)	(2*S*,2′*S*)	48
			40 (21)	−19.1(*c* 1.24, $CHCl_3$)	(2*S*,2′*R*)	91
(**3b**)	2-Aminoheptane[e]	29	20 (18)	−7.7(*c* 0.15, $CHCl_3$)	(2*S*,2′*S*)	45
			80 (21)	+17.2(*c* 0.35, $CHCl_3$)	(2*R*,2′*S*)	82
(**3c**)	α-Methylbenzylamine[d]	15	25	−60.9(*c* 0.20, EtOH)	(2*S*,1′*S*)	46
			75	+125.6(*c* 0.34, EtOH)	(2*S*,1′*R*)	95
(**3c**)	α-Methylbenzylamine[e]	30	13	+87.4(*c* 0.22, EtOH)	(2*R*,1′*R*)	66
			87	−115.5(*c* 0.52, EtOH)	(2*R*,1′*S*)	88

[a] Reaction conditions 8 mM ethyl (±)-2-chloropropionate (**1**), 8 mM racemic amine (**2**); 25 °C; 250 rev. min^{-1}; 0.1 g CCL per ml in hexane (40 ml) or 2 mg subtilisin per ml in 3-methylpentan-3-ol (10 ml). [b] Determined by ^1H NMR, confirmed and isolated by HPLC with a semi-preparative μ-porasil column (7.8 × 300 mm); eluant: hexane–aceone, 100:2.5; flow 2 ml min^{-1} for (**3a**) and (**3b**). The mixture of diastereoisomers (**3c**) separated by crystallization from hexane–di-isopropyl ether. [c] Enantiomeric excess (e.e.) calculated from ^1H NMR device (300 MHz) in the presence of tris-3-(2,2,2,-trifluoro-1-hydroxyethylidene)-(+)-camphoratoeuropium, Eu(tfc)$_3$. [d] Enzyme CCL; reaction time 120 h. [e] Enzyme subtilisin; reaction time 24 h.

As one can see from Table 1, high enantiomeric excesses (e.e.) were obtained in most instances; in some cases for example (**3c**), the ratio of diastereoisomers† was satisfactory, but in none of them did the enzyme show an absolute selectivity towards ester and amine simultaneously. However, CCL preferentially utilizes the (*S*) enantiomer of the ester, and subtilisin exhibits a high selectivity towards the (*S*) isomer of the amine. On the other hand, although the ratio of diastereoisomers is not high, these mixtures are easily resolved by HPLC.

The strategy described here provides a facile entry to amides with two chiral centres and with high enantiomeric excess. Furthermore, the simplicity of the procedure and the availability of the materials used are noteworthy.

† The configurations were determinated as follows: For (**3c**), the isomer (2*S*,1′*R*) by analogy with the optically active amide obtained from ethyl (*S*)-(−)-2-chloropropionate and (*R*)-(+)-α-methylbenzylamine; the isomer (2*S*,1′*S*) by comparison with the corresponding diastereoisomer isolated from the mixture obtained with ethyl (*S*)-(−)-2-chloropropionate and the racemic amine. For (**3a**) and (**3b**), by analogy with (**3c**) and published data (ref. 5), one can assume that when the reaction is catalysed by subtilisin in 3-methylpentan-3-ol the more reactive amine isomer has the (*S*) configuration; the C(2) configuration was established by comparison with the corresponding diastereoisomer separated from the mixture obtained with ethyl (*S*)-(−)-2-chloropropionate and the corresponding racemic amine.

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