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Optical Resolution of Unusual Amino–Acids by Lipase-catalysed Hydrolysis

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The 2-chloroethyl esters of the *N*-benzyloxycarbonyl (Z) derivatives of several unusual amino-acids are converted by *Aspergillus niger* lipase into enantiomerically enriched Z-amino-acids with fairly high optical purities, the L-enantiomers being preferentially hydrolysed.

Lipases are a group of enzymes often used as practical catalysts for organic synthesis, because they are easily available, inexpensive, and stable, and they require no added cofactors.[1] Recently a number of reports have appeared on the lipase-catalysed resolution of racemic alcohols[2] *via* enantioselective hydrolyses of their corresponding esters. Several examples have also been reported on the similar resolution of racemic carboxylic acids.[3] The usefulness of lipases from microbial sources has been recognized in these studies. Lipases, however, have not previously been employed for the resolution of racemic amino-acids. We report here the first attempt to resolve unusual amino-acids by microbial lipase-catalysed hydrolysis of the 2-chloroethyl esters (**1**) of their *N*-benzyloxycarbonyl (Z) derivatives. Enantiomerically enriched unusual amino-acids are useful as building blocks for the synthesis of analogues of biologically active peptides and as chiral starting materials or chiral catalysts in other organic syntheses.

First, enzymatic hydrolyses of the heptyline (2-aminoheptanoic acid) derivative (**1e**) were surveyed in 0.2 M phosphate buffer (pH 7.0) with several commercially available lipases. Of the enzymes tested, lipases from *Aspergillus niger*, *Pseudomonas fluorescens*, and *Candida cylindracea* showed rather high values of enantiomeric excess (e.e.). Accordingly

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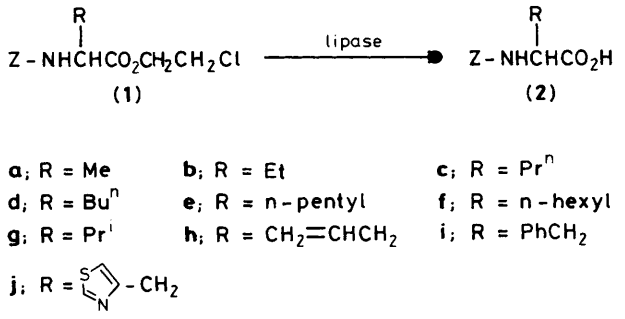
Table 1. Lipase-catalysed hydrolysis of (**1**).[a]

Sub-strate	Lipase *A. niger*[b] % convn.	% e.e.[e]	*P. fluorescens*[c] % convn.	% e.e.[e]	*C. cylindracea*[d] % convn.	% e.e.[e]
(**1a**)	36	89	46	16	38	7
(**1b**)	40	96	49	52	36	7
(**1c**)	27	86	43	58		
(**1d**)	32	85	52	60	42	30
(**1e**)[f]	44	95	25	80	27	66
(**1f**)	31	94	47	87		
(**1g**)	14	92	8	53		
(**1h**)	34	86	40	52	31	27
(**1i**)	32	94	12	57	35	63
(**1j**)	37	94	40	70	35	43

[a] The reactions were run as illustrated in the text. The course of reaction was followed by the amount of NaOH necessary to neutralize the liberated Z-amino-acid. The reaction conditions were not optimized. [b] Amano A-6, 49 h for (**1b**), 23 h for (**1h**), 12 h for (**1j**). [c] Amano P, 22 h for (**1b**), 20 h for (**1h**), 115 h for (**1j**). [d] Meito MY, 22 h for (**1b**), 16 h for (**1h**), 12 h for (**1j**). [e] Enantiomeric excess of the acid. [f] Other results: *Mucor javanicus* (Amano M), 27% e.e. at 35% convn.; *Rhizopus javanicus* (Amano F-AP15), 45% e.e. at 29% convn.; *Rhizopus japonicus* (Saiken 50), 13% e.e. at 41% convn.

the hydrolyses of other substrates were performed using these three lipases. The results are summarized in Table 1, which also includes the results with some usual protein amino-acids. The optical purity and the absolute configuration of a liberated Z-amino-acid [Z-AA, (**2**)] were determined by reversed-phase high performance liquid chromatography (h.p.l.c.) of Z-AA–Gly–L-Phe–OMe [for (**2a**)—(**2h**)] or Z-AA–Sar–L-Phe–OMe† [for (**2i**) and (**2j**)] obtained by coupling the Z–AA with Gly–L-Phe–OMe or Sar–L-Phe–OMe by the EDC method.‡

For any amino-acid derivative listed in Table 1, the enantioselectivities vary markedly with the enzymes used and increase in the general order, *Candida cylindracea* lipase < *Pseudomonas fluorescens* lipase < *Aspergillus niger* lipase. The three types of unusual amino-acids, (i) with a straight carbon chain [(**1b**)—(**1f**)], (ii) with an unsaturated bond [(**1h**)], and (iii) with a heteroaromatic ring [(**1j**)], in the side-chain R of (**1**), were resolved with fairly high optical purities (85—95% e.e.) by lipase from *Aspergillus niger*, although the reaction conditions have not yet been optimized. The good result obtained in the lipase-catalysed hydrolysis of (**1j**) is especially significant, because the other enzymatic procedure using acylase I was not applicable to this heteroaromatic amino-acid. In the case of (**1a**)—(**1i**), the L-enantiomer was preferentially hydrolysed by all the enzymes studied here, which was confirmed by comparison with the authentic samples of Z–L-AA–Gly–L-Phe–OMe or Z–L-AA–Sar–L-Phe–OMe on h.p.l.c. Furthermore, the same enantiomeric preference was suggested in the hydrolysis of (**1j**), from the regularity of the elution order of diastereomers of Z-L/D-AA′–Sar–L-Phe–OMe (AA′ denotes various amino-acid residues).§



In a typical procedure, to 1 mmol of the Z-amino-acid 2-chloroethyl ester (**1b**)¶ and 10 mg of poly(vinyl alcohol) suspended in 2 ml of 0.2 M phosphate buffer (pH 7.0) was added 50 mg of *Aspergillus niger* lipase (Amano A-6) dissolved in 0.3 ml of the buffer. The resulting suspension was stirred at 25 °C with a controlled addition of 0.5 M NaOH to maintain the pH at 7.0. After 49 h of stirring, the liberated Z-amino-acid (**2b**) was extracted from the reaction mixture as usual, and a part of the sample was coupled with Gly–L-Phe–OMe by the EDC method in dichloromethane overnight. The resulting tripeptide was analysed by reversed-phase h.p.l.c. under the following conditions to afford the e.e. value shown in Table 1: column, Cosmosil $5C_{18}$ (4.6 mm I.D. × 150 mm); mobile phase, 60% MeOH aq; flow rate, 1.0 ml/min; column temperature, 30 °C; detection, 254 nm.

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† Abbreviations: Sar, sarcosine (*N*-methylglycine); EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

‡ The kinetic resolution during the coupling was negligible, probably because achiral Gly or Sar occupies the coupling site in the amino-component (cf. refs. 4 and 5).

§ In general, the L–L-isomer of Z–L/D–AA′–Sar–L–Phe–OMe (and Z–L/D–AA′–Gly–L-Phe–OMe as well) is eluted first through an ODS column with aqueous MeOH as eluent.

¶ This ester was prepared by reaction of the Z-amino-acid with 2-chloroethanol in the presence of 4-dimethylaminopyridine and EDC·HCl in dichloromethane.