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# AN ENZYMATIC METHOD FOR THE PREPARATION OF CHIRAL DIAMIDES

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Summary The enzymatic aminolysis from ethyl (±)-2-chloropropionate with different diamines has been studied In these processes the isomer and the enantiomeric excess of the diamide obtained depend dramatically on the enzyme and solvent used

Lipases and proteases have found widespread application in the synthesis of chiral organic compounds [1] However, their utility for the preparation of simple chiral amides has not been fully developed Recently , it has been shown that Candida cylindracea lipase (CCL)[2] and subtilisin[3] are good biocatalysts for the preparation of chiral amides Moreover, CCL catalyses the reaction between ethylpropiolate and aromatic amines to afford propargylamides [4]

In previous works,[2,4,5] we have reported the usefulness of enzymes as biocatalysts in the synthesis of nitrogen compounds We herein report the synthesis of diamide (3a) by the double aminolysis process between a racemic ester (1) and ethylenediamine (2a), employing CCL, lipase Amano P, and subtilisin protease in different organic solvents (Table 1) Other lipases, porcine pancreatic, Rhizzopus Arrhizus, and Aspergillus Niger, showed a very low catalytic activity in this reaction

As shown in Tables, CCL catalysed the formation of the (S,S) isomer with high enantiomeric excess, whereas subtilisin and lipases Amano P exhibit an opposite and lower selectivity, yielding a mixture of the isomers (R,R) and (R,S) In order to form the second amide bond the acyl-enzyme complex must already be formed, but, interactions, may occur between the new nucleophile and the active site of the enzyme too This, may mean that the enzyme should be more selective for the formation of the diamide

We have extended this reaction to other diamides (2b and 2c), the processes being carried out under the best conditions found for the formation of diamide (3a)[6] It can be seen in Table 2 that the yield and enantioselectivity of these reactions depend on the starting diamine (2)

In conclusion, CCL and subtilisin are excellent catalysts for obtaining chiral diamides, the configuration of these diamides depending on the enzyme used

$$\underset{(1)}{2\ CH_3\text{-}CHCl\text{-}CO_2C_2H_5} + \underset{(2)}{H_2N\text{-}(CH_2)_n\text{-}NH_2} \longrightarrow \underset{(3)}{CH_3\text{-}CHCl\text{-}CONH\text{-}(CH_2)_n\text{-}HNOC\text{-}CHCl\text{-}CH_3}$$

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Table 1 Diamide 3a formation with different enzymes[a] and solvents

| Enzyme | Solvent | Reaction time, h | Yield,% | Isomer (ratio)[b] (R,R+S,S R,S) | d e , (%) | $\|\alpha\|_D^{25}$ (mixture) |
|---|---|---|---|---|---|---|
| CCL | tetrachloromethane | 48 | 50 | (100 0) | 100 | -17 5 (c 0 24) |
| Subtilisin | 3-methyl-3-pentanol | 24 | 85 | (89 11) | 78 | +10 4 (c 0 75) |
| Subtilisin | tetrahydrofuran | 24 | 40 | (86 14) | 72 | + 6 8 (c 0 85) |
| Amano P | tetrachloromethane | 24 | 45 | (85 15) | 70 | + 5 0 (c 0 40) |
| Amano P | diisopropyl ether | 24 | 70 | (97 3 ) | 94 | + 3 3 (c 0 69) |

a) The yeast Candida cylindracea lipase ( E C 3 1 1 3 ), Type VII crude and subtilisin Carlsberg protease, Type VIII are purchased from Sigma, and the Amano P lipase from Amano Pharmaceutical Co , Ltd b) The ratio of isomers was determined by HPLC using a Spherisorb S5W column (0 46cm x 30cm) and as eluent, hexane-dichloromethane-methanol 180 15 15, Flow, 1ml/min, $t_R$ (min), isomer R,S (7 12), isomer R,R (9 17) c) In chloroform

Table 2 Optically active diamides (3) from (1) and diamines (2)

| Entry | n | Configuration | $\|\alpha\|_D^{25}$ ($HCCl_3$) | e e ,%[a] |
|---|---|---|---|---|
| (3a)[b] | 2 | S,S | -17 5 (c 0 24) | 88 |
| (3a)[c] | 2 | R,R | +11 8 (c 0 56) | 60 |
| (3b)[b] | 3 | S,S | -16 4 (c 0 68) | 69 |
| (3b)[c] | 3 | R,R | +7 1 (c 0 20) | 30 |
| (3c)[b] | 8 | S,S | -5 2 (c 0 95) | 32 |
| (3c)[c] | 8 | R,R | +3 3 (c 0 31) | 21 |

a) The e e s and the configuration were determined by analogy with the optically active diamide obtained from the S-(-)-ester and the corresponding diamine b) With CCL c)With subtilisin protease in 3-methyl-3-pentanol

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6 - The reaction was carried out as follows To a solution of 16mM of (1) and 4mM of (2a) in the apropiate solvent, 5g of CCL (0 12 g/ml), 20 mg of subtilisin (2mg/ml), or 0 5 g of Amano P (30 mg/ml) was added