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Enzymatic Aminolysis and Transamidation Reactions

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Abstract *Chiral amides can be obtained from (±)-2-chloropropionate esters and a wide range of amines when the reaction is catalyzed by Candida cylindracea lipase The enantioselection of the enzyme in this aminolysis reaction depends on the substrate and nucleophile structure and reaction conditions This lipase can catalyze a transamidation reaction if N-trifluoroethyl-2-chloropropionamide is used as substrate In this way, amides are obtained in high-moderate enantiomeric excesses The aminolysis of ethyl (±)-2-methylbutyrate with aliphatic amines is achieved using CC and PS lipases as catalysts*

INTRODUCTION

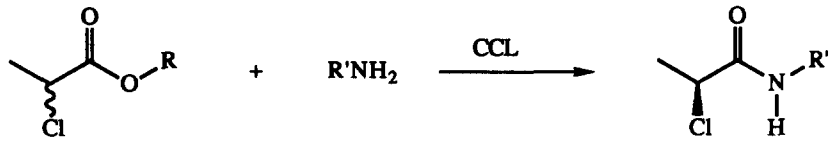
Nowadays, there is an increasing demand for efficient processes for the synthesis of optically active compounds For this purpose, biocatalysts have widely been utilized[1] Despite most of the biotransformations occurring in aqueous medium, hydrolytic enzymes such as lipases are being successfully used to catalyze stereoselective esterifications and transesterifications in organic solvents[2,3] Thus, a great number of primary and secondary alcohols, acids or ester derivatives are being resolved On the other hand, there is a great interest on the development of new methods for the preparation of amides, which may also be utilized in peptide and lactam synthesis With respect to the amidation reaction catalyzed by lipases, it has previously been applied in peptide synthesis from conveniently protected D or L aminoacids[4], but because the lipases have the ability to accept a broad range of substrates, we though that it will be interesting to study if these enzymes could catalyze the simple amide formation in an enantioselective way from racemic substrates

In a preliminary communication[5], we described the synthesis of optically active amides from a racemic ester and different amines using yeast *Candida cylindracea* lipase as catalyst We have introduced some modifications into the substrate and reaction conditions in order to improve the optical purity of the amides and the results are reported here Besides, we have studied the potentiality of this enzyme to catalyze a transamidation reaction The aminolysis of an unactivated ester, ethyl (±)-2-methylbutyrate, has been investigated too

RESULTS AND DISCUSSION

Yeast *Candida cylindracea* lipase catalyzes the reaction between ethyl (±)-2-chloropropionate (**1a**) and aliphatic or aromatic amines giving in all the cases the corresponding (*S*)-amide The enantioselectivity of this reaction depends on the amine In general, aliphatic amides are obtained with higher optical purity than aromatic amides Bulky substituent on the amine (*tert*-butyl) has a strong negative influence on the enantioselective activity of the enzyme

As one can see in Table I, the yeast *Candida cylindracea* lipase acts in a broad range of temperature With aromatic amines, the reaction was carried out to 60°C to accelerate the reaction, but with some aliphatic amines which spontaneously react with ethyl (±)-2-chloropropionate, it was necessary to carry the reaction at temperatures lower than room temperature, and despite this, the yeast lipase showed a high catalytic activity The solvent effect is important too, with aliphatic amines, hexane was the best solvent (hexane, log P= 3 5, CCl_4, log P= 3)[6], but CCl_4 was the most suitable for aromatic amines because these amines have low solubility in hexane In addition, we have checked little modifications on the ester substrate Although the difference between an ethyl or methyl ester is not very significative (the reaction time is similar), when methyl (±)-2-chloropropionate (**1b**) was used in this reaction, the optical purity of some amides (Table II) was appreciably improved In the remaining aminolysis reactions, small differences were observed With aromatic amines, both with methyl and ethyl ester, the reaction is carried out to 60°C Even though in the most cases a rise in temperature does not lead to a decrease of the catalytic activity of the lipase in organic solvents[7], in some cases the enantioselectivity can be improved when milder temperatures are used For this reason, we tried the aminolysis reaction with aromatic amines at room temperature using activated esters such as 2,2,2-trichloroethyl (±)-2-chloropropionate (**1c**) and for a similar percentage of conversion, the optical yields were poorer (30%, 52% and 91% for 3f, 3g and 3h respectively)



1a, R= CH_2-CH_3 **2** **3**

1b, R= CH_3

1c, R= CH_2-CCl_3

Table I (*S*)-Amides (**3**) from (±)-(**1a**) and amines (**2**)

Entry	R'	Time, h (T, °C)	Yield, %	ee, %
3a	*n*-butyl	3 (2)	62	95
3b	allyl	7 (2)	60	92
3c	*t*-butyl	3 (2)	50	<5
3d	cyclohexyl	3 (2)	44	63
3e	benzyl	21 (25)	56	74
3f	phenyl	48 (60)	52	80
3g	*p*-tolyl	31 (60)	81	65
3h	*p*-methoxyphenyl	35 (60)	83	>95

Table II. (*S*)-Amides (**3**) from (±)-(**1b**)

Entry	Yield, %	ee, %
3a	60	>95
3e	50	95
3g	80	75

Analyzing the results showed, we can deduce the following

- In all the cases CCL prefers the *S* enantiomer of the (±)-2-chloropropionate ester in contrast to what it is found in the esterification reaction[8], for which this lipase showed a total preference towards the *R* enantiomer of the acid

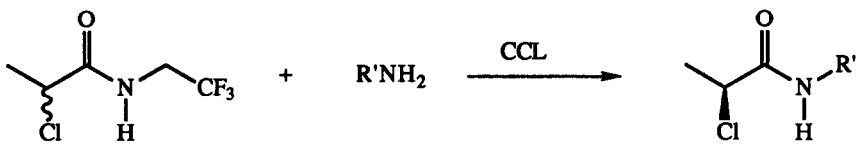
- The enantioselective activity of the CCL is dramatically influenced by the nucleophile structure With the same racemic substrate, the optical yield of the amides ranges from <5 to >95%

- In the case of aliphatic amines, the e e of the amide decreases in line with the ramification level of the amine

- Even though we cannot give an explanation about variations observed with respect to the size of the R group of the ester, this can be used in each particular case to improve the enantiomeric excess of the product

The transamidation is known to be a difficult reaction. Generally, high temperatures or critical pH are required[9] In this respect, we though that as lipases have proved to be efficient catalysts in the acyl transfer reactions, it would be possible that these enzymes could catalyze transamidation reactions First, we choose N-

methyl-N-phenyl-2-chloropropionamide as substrate because the leaving group is a secondary amine (we have noticed that CCL does not catalyze the aminolysis of 2-chloropropionate esters when secondary amines such as N methylaniline or diphenylamine are used) so it could not compete with the primary amine used as nucleophile, however CCL does not show any catalytic activity in this reaction, probably due to steric hindrance. To alleviate this steric congestion and to avoid the reversibility of the process, we have used N-(2,2,2-trifluoroethyl)-2-chloropropionamide (**4**) as substrate and in this case CCL catalyzed the reaction. The results are summarized in Table III.



4 **2** **3**

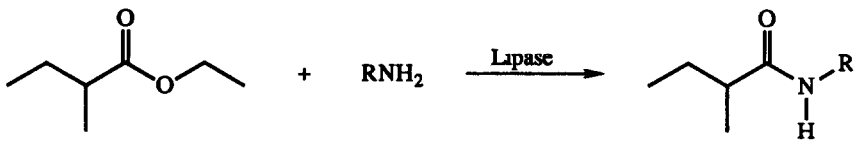
Table III. Transamidation reaction of (**4**) with amines (**2**)

Entry	R'	Solvent	Yield, %	ee, %
3a	*n*-butyl	hexane	48	78
3b	allyl	hexane	40	60
3f	phenyl	tetrachloromethane	3	52
3h	*p*-methoxyphenyl	tetrachloromethane	20	74

Although the chemical and optical yields of the amides by this last method are lower than by aminolysis of the ester, it is the first example of lipase catalyzed transamidation and may be an alternative route to the resolution of chiral amines and amides

Finally, we have investigated the aminolysis of ethyl (±)-2-methylbutyrate Despite the fact that the best ester substrates in lipase catalyzed reactions are those having an electronwithdrawing group in C_2-position[8], recently the resolution of 2-methylalkanoic acids by CCL catalyzed esterification yielding the (*S*)-ester has been published[10]. We started the study of this aminolysis reaction by checking the catalytic power of some lipases in different organic solvents The best results (Table IV) were obtained when lipases from *Pseudomonas cepacia* (PS) and *Candida cylindracea* were used as catalysts As it can be seen in Table IV, both lipases exhibit opposite

selectivity With CCL the (*R*)-amide (**6a**) is obtained, again this enzyme showed an opposite enantioselectivity with respect to the esterification reaction of the (±)-2-methylbutanoic acid[10]



(±)-(**5**) (*R*) or (*S*)-(**6**)

Table IV Amides (**6**) from (±)-(**5**) and amines

Entry	R	Lipase	Solvent	Yield, %	ee, % (Conf)
6a	*n*-butyl	CC	hexane	18	84 (*R*)
6a	*n*-butyl	PS	1,4-dioxane	20	40 (*S*)
6b	allyl	PS	diisopropyl ether	15	62 (*S*)

CONCLUSION

Candida cylindracea lipase is a very useful catalyst in aminolysis and transamidation reactions It is of great importance the influence of the amine on the enantioselective activity of this enzyme As a result, aminolysis reaction is an alternative and complementary method to the corresponding esterification

EXPERIMENTAL

Candida cylindracea lipase, Type VII crude, was purchased from Sigma Chemical Co *Pseudomonas cepacia* lipase from Amano Pharmaceutical Co All reagents were of commercially quality and were purchased from Aldrich Chemie N-(2,2,2-Trifluoroethyl)-2-chloropropionamide was obtained by reaction of (±)-2-chloropropionic chloride and 2,2,2-trifluoroethylamine Solvents were distilled over an adequate desiccant and stored under argon For column chromatography, Merck silica gel 60/230-400 mesh was used Melting points

were taken using a Gallenkamp apparatus and are uncorrected. Optical rotations were measured using a Perkin-Elmer 241 polarimeter IR spectra were recorded on a Perkin-Elmer 170-X Infrared fourier transform spectrophotometer ^{1}H- and ^{13}C-NMR were obtained with TMS (tetramethylsilane) as internal standard, using a Bruker AC-300 (^{1}H- 300 MHz and ^{13}C- 75.5 MHz) spectrometer Mass spectra were recorded on a Hewlett-Packard 5897 A spectrometer All the new compounds gave satisfactory elemental analysis and were performed by Microanalytisches Perkin-Elmer 240

Determination of enantiomeric excess and configuration was as follows For amides (**3**) the ee was calculated by ^{1}H-NMR spectroscopy using the chiral shift reagent tris[3-(trifluoromethylhydroxymethylen)-(+)-camphorato]europium (III), the configuration was determined by analogy with the optically active amide obtained from ethyl (*S*)-(-)-2-chloropropionate and the corresponding amine For amides (**6**), both ee and configuration were calculated by comparison with the optically active amide prepared from (*S*)-(+)-2-methylbutyric anhydride and the appropiate amine

***Candida cylindracea* lipase catalyzed aminolysis of (±)-2-chloropropionate esters (1).**

To a solution of 10 mmol of ester (**1**) and 5 mmol of the corresponding amine (**2**) in 30 mL of hexane (aliphatic amine) or tetrachloromethane (aromatic amine) was added CCL (4 g) The suspension was stirred at 2°C, 25°C or 60°C Reaction was terminated by removal of the enzyme by filtration Organic solvent and ester were evaporated under reduced pressure to give amide (**3**) after recrystallization in hexane/CCl_4 (aliphatic amide) or CCl_4 (aromatic amide).

***Candida cylindracea* lipase catalyzed transamidation of (±)-N-(2,2,2-trifluoroethyl)-2-chloropropionamide (4).**

To a solution of 1 6 mmol of amide (**4**) and 0.8 mmol of the corresponding amine (**2**) in 15 mL of solvent, CCL (2 g) was added The mixture was stirred at 50°C for 11 days and then the enzyme was removed by filtration and the solvent evaporated The residue was subjected to flash chromatography on silica using hexane-ethyl acetate-diethylamine (20 10·0 1) as eluent.

(*S*)-(-)-N-*n*-butyl-2-chloropropionamide (3a) mp 32-34°C, $[\alpha]_D^{25}$ -16 6° (c 1 47, ethanol), ee 95%, IR (nujol) $\nu_{C=O}$ 1630 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ (ppm) 0 87 (t, 3H, CH_3), 1 32 (m, 2H, CH_2), 1 48 (m, 2H, CH_2), 1 67 (d, 3H, CH_3), 3 20 (m, 2H, CH_2), 4 35 (q, 1H, CH), 6.60 (bs, 1H, NH), ^{13}C-NMR ($CDCl_3$) δ (ppm) 13 57 (CH_3), 19 84 (CH_2), 22 56 (CH_3), 31 24 (CH_2), 39 51 (CH_2), 55 95 (CH), 169 30 (C=O), MS m/z 163 (M+)

(*S*)-(-)-N-allyl-2-chloropropionamide (3b) oil, $[\alpha]_D^{25}$ -5 2° (c 1 08, ethanol), ee 92%, IR (neat) $\nu_{C=O}$ 1630 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1 68 (d, 3H, CH_3), 3 87 (m, 2H, CH_2), 4 39 (q, 1H, CH), 5 15 (m, 2H, CH_2), 5 80 (m, 1H, CH), 6 62 (bs, 1H, NH), ^{13}C-NMR ($CDCl_3$) δ (ppm) 22 73 (CH_3), 42 08 (CH_2), 55 92 (CH), 116 69 (CH_2), 133 29 (CH), 169.31 (C=O), MS m/z 147 (M+)

N-*t*-butyl-2-chloropropionamide (3c) mp 103-105°C; IR (nujol): $\nu_{C=O}$ 1635 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.25 (s, 9H, CH_3), 1.57 (d, 3H, CH_3), 4.18 (q, 1H, CH), 6.30 (bs, 1H, NH), ^{13}C-NMR ($CDCl_3$) δ (ppm): 22 54 (CH_3), 28.32 (CH_3), 51 36 (C), 56.15 (CH), 168.50 (C=O), MS m/z: 163 (M+)

(*S*)-(-)-N-cyclohexyl-2-chloropropionamide (3d) mp 102-104°C, $[\alpha]_D^{25}$ -4.6° (c 1 09, ethanol), ee 30%, IR (nujol)· $\nu_{C=O}$ 1634 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ (ppm): 1 13-1 90 (m, 10H, CH_2), 1.72 (d, 3H, CH_3), 3 73 (m, 1H, CH), 4.38 (q, 1H, CH), 6 51 (bs, 1H, NH), ^{13}C-NMR ($CDCl_3$) δ (ppm) 22 67 (CH_3), 24 61 (CH_2), 25 33 (CH_2), 32 64 (CH_2), 48 48 (CH), 55 95 (CH), 168 34 (C=O), MS m/z 189 (M+)

(*S*)-(-)-N-benzyl-2-chloropropionamide (3e)· mp 64-66°C, $[\alpha]_D^{25}$ -4 3° (c 0 35, ethanol), ee 74%, IR (nujol) $\nu_{C=O}$ 1640 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1 75 (d, 3H, CH_3), 4 50 (m, 3H, CH_2 and CH), 6 90 (bs, 1H, NH), 7 22-7 42 (m, 5H, aromatic), ^{13}C-NMR ($CDCl_3$) δ (ppm) 22 57 (CH_3), 43 73 (CH_2), 55 72 (CH), 127 51 (CH), 127.56 (CH), 137.38 (C), 163 37 (C=O), MS m/z. 197 (M+)

(*S*)-(-)-N-phenyl-2-chloropropionamide (3f). mp 81-83°C, $[\alpha]_D^{25}$ -48 7° (c 0 95, ethanol), ee 80%, IR (nujol) $\nu_{C=O}$ 1665 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1 82 (d, 3H, CH_3), 4 58 (q, 1H, CH), 7 1-7 6 (m, 5H,aromatic), 8 32 (bs, 1H, NH), ^{13}C-NMR ($CDCl_3$) δ (ppm) 22 53 (CH_3), 56 08 (CH), 119 99 (CH), 124.98 (CH), 129 01 (CH), 136 84 (C), 167 40 (C=O), MS m/z 183 (M+)

(*S*)-(-)-N-*p*-tolyl-2-chloropropionamide (3g) mp 114-116°C, $[\alpha]_D^{25}$ -41 7° (c 0 48, ethanol), ee 65%, IR (nujol) $\nu_{C=O}$ 1660 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1 80 (d, 3H, CH_3), 2 31 (s, 3H, CH_3), 4 50 (q, 1H, CH), 7 1-7 4 (m, 4H, aromatic), 8 22 (bs, 1H, NH), ^{13}C-NMR ($CDCl_3$) δ (ppm) 20.62 (CH_3), 22 01 (CH_3), 55 46 (CH), 120 25 (CH), 129 22 (CH), 134 23 (C), 134 43 (C), 167 63 (C=O), MS m/z 197 (M+).

(*S*)-(-)-N-*p*-methoxyphenyl-2-chloropropionamide (3h)· mp 103-105°C, $[\alpha]_D^{25}$ -58.2° (c 0 48, ethanol), ee >95%; IR (nujol): $\nu_{C=O}$ 1677 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ (ppm) 1 91 (d, 3H, CH_3), 3 89 (s, 3H, CH_3), 4 10 (q, 1H, CH), 6 9-7.5 (m, 4H, aromatic), 8 10 (bs, 1H, NH), ^{13}C-NMR ($CDCl_3$) δ (ppm) 21 99 (CH_3), 55 13 (CH_3), 55.36 (CH), 113 82 (CH), 122 12 (CH), 129 84 (C), 156 62 (C), 167 71 (C=O), MS m/z 213 (M+)

Lipase catalyzed aminolysis of ethyl (±)-2-methylbutyrate (5).

Ethyl (±)-2-methylbutyrate (10 mmol) and the corresponding amine (7 mmol) were dissolved in the appropiate solvent (Table IV) and then 50 mg of lipase per mL of solvent was added (10 g of CCL or 1 g of PSL). After being stirred at 50°C (with CCL) or 30°C (with PSL) for 5 days, the enzyme was filtered and the solvent evaporated under vacuum The chromatographic separation on silica of the resulting residue yield the amide (**6**) (eluent hexane-ethyl acetate 1 1)

(*S*)-(+)-N-*n*-butyl-2-methylbutyramide (6a) oil, $[\alpha]_D^{25}$ +7 3° (c 0.22, chloroform), ee 40%, IR (neat) $\nu_{C=O}$ 1646 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ (ppm) 0 90 (t, 3H, CH_3), 0.93 (t, 3H, CH_3), 1 22 (d, 3H, CH_3), 1 36 (m, 2H, CH_2), 1 47-1.55 (m, 2H, CH_2 and 1H, CH_2), 1 65 (m, 1H, CH_2), 2 12 (m, 1H, CH), 3 25 (m, 2H,

CH_2), 6.02 (bs, 1H, NH), ^{13}C-NMR ($CDCl_3$) δ (ppm): 11 82 (CH_3), 13 66 (CH_3), 17.45 (CH_3), 19 98 (CH_2), 27 25 (CH_2), 31 69 (CH_2), 39 02 (CH_2), 43 18 (CH), 176.61 (C=O); MS m/z: 157 (M^+).

(*S*)-(+)-N-allyl-2-methylbutyramide (6b): oil, $[\alpha]_D^{25}$ +12 6° (c 0 36, chloroform), ee 62%; IR (neat) $\nu_{C=O}$ 1646 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.85 (t, 3H, CH_3), 1 09 (d, 3H, CH_3), 1 36 (m, 1H, CH_2), 1 51 (m, 1H, CH_2), 2 05 (m, 1H, CH), 3 83 (m, 2H, CH_2), 5.08 (m, 2H, CH_2), 5.61 (bs, 1H, NH), 5 80 (m, 1H, CH), ^{13}C-NMR ($CDCl_3$) δ (ppm). 11.90 (CH_3), 17 51 (CH_3), 27.29 (CH_2), 41.67 (CH_2), 43.24 (CH), 116 16 (CH_2), 134 46 (CH), 176 24 (C=O); MS m/z 141 (M^+)

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