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Review Article

Non-Conventional Hydrolase Chemistry: Amide and Carbamate Bond Formation Catalyzed By Lipases

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Abstract—Biocatalysis in nonaqueous media is becoming increasingly important in organic synthesis. Lipases are the most used enzymes, especially in transesterification reactions. However, in the last years the amidation reaction catalyzed by lipases has also been shown to be a useful tool for the organic chemists. In this review, we discuss the possibilities of the enzymatic aminolysis and ammonolysis reactions for the preparation of different amides and for the resolution of esters, amines and aminoalcohols. The enzymatic alkoxycarbonylation of amines opens a new way for the synthesis of chiral carbamates.

Introduction

The use of enzymes in organic solvents has been increasingly developed in the last few years.[1–4] Nowadays, biocatalysis in non-conventional media is a well-established methodology, and many organic chemists are using this tool for the preparation of a great variety of organic compounds. The most frequently used biocatalysts are lipases because of their acceptance of a broad range of substrates.[5,6]

Lipases have been widely used for the preparation and resolution of chiral alcohols, esters, carboxylic acids and lactones through the corresponding hydrolysis and transesterification reactions.[7–10] However; the amidation reaction has been much less studied. The enzymatic acylation is the most common reaction in transesterification and amidation processes, but the enzymatic alkoxycarbonylation of alcohols and amines using lipases in organic solvents has scarcely been studied, and only a few examples have been reported in the last years.[11]

The synthetic potential of lipases in organic solvents has been quickly recognized. The present article reviews the utility of the enzymatic aminolysis and ammonolysis reaction using lipases in organic solvents, especially for the preparation of amide-bond formation and the synthesis of enantiomerically pure compounds (EPC).

Enzymatic Acylation of Amines and Ammonia

Enzymatic preparation of amides

Although in an enzymatic transesterification reaction the solvent plays an important role,[12] the election of the acyl donor is also essential and it is necessary to know the reactivity of the starting materials well to choose the acylating agent. In order to avoid the reversibility of these processes, several acylating agents can be used such as activated esters,[13] enol esters,[14] anhydrides,[15] oxime esters,[16] thioesters,[17] or 1-ethoxyvinyl esters.[18] However, the above mentioned acylating agents can not be employed in the enzymatic acylation of amines, due to the higher nucleophilicity of amines with respect to the corresponding alcohols, and frequently these reagents react with amines in absence of an enzyme.

The use of lipases to generate amide bonds in organic solvents was predicted several years ago,[19] and the enzymatic aminolysis reaction was applied to the preparative synthesis of peptides by Klibanov[20] and Wong,[21] but the utility of lipases for the preparation of chiral amides was first demonstrated in the reaction of racemic ethyl chloropropionate with several aliphatic and aromatic amines.[22] *Candida cylindracea* lipase (CCL) proves to be an efficient biocatalyst in this process and also catalyses the reaction with diamines for

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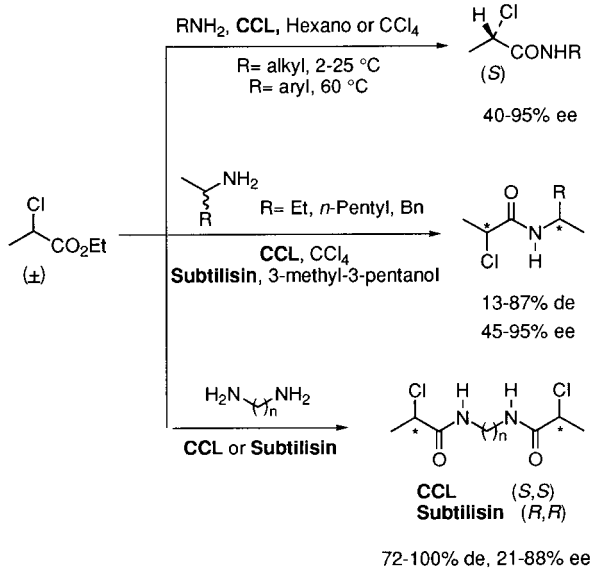
the formation of chiral diamides.[23] In addition, if racemic amines are used, amides with two stereogenic centers can be prepared (Scheme 1).[24]

Lipases also catalyse the formation of amides from non-activated esters. The preparation of *N*-octyl alkylamides, for instance has been carried out in anhydrous hexane,[25] and *Candida antarctica* lipase (CAL) exhibits a very high activity and specificity in the acylation of primary amines with ethyl butyrate.[26] Fatty acid amides have been prepared with moderate yields using *Rhizmucor miehei* lipase.[27] Kobata et al. have investigated the enzymatic synthesis of capsaicin analogues. Several fatty acids derivatives react with vanillylamine in the presence of various lipases, achieving in moderate yields capsaicin analogues, the reaction takes place in a two-phase system. Among the lipases used, PSL, gave the highest yield.[28,29]

Ethyl (±)-2-methylbutyrate yields chiral amides using CCL and *Pseudomonas cepacia* lipase (PSL). Interestingly both lipases show opposite enantioselectivity.[30] The same enantioselectivity as that of CCL is found with CAL, a lipase which presents a high versatility in this kind of reaction.[31] The aminolysis reaction of racemic ethyl 2-methyloctanoate with enantiomerically pure (*S*) or (*R*) 1-phenylethylamine[32] yields the corresponding chiral amides with good enantioselectivity.

PSL also catalyses the amidation of benzyl esters producing high yields of amides with a variety of different primary amines.[33] Reactions with α-hydroxyesters result in rapid conversion to good yields of α-hydroxyamides, while with methyl or ethyl esters the reaction takes place, although with longer reaction times.[34] Recently, an efficient enzymatic procedure has been described for the one-pot conversion of carboxylic acids to the corresponding amides via in situ formation of the ester and subsequent aminolysis.[35]



Scheme 1.

Chemoselective aminolysis from bifunctional esters

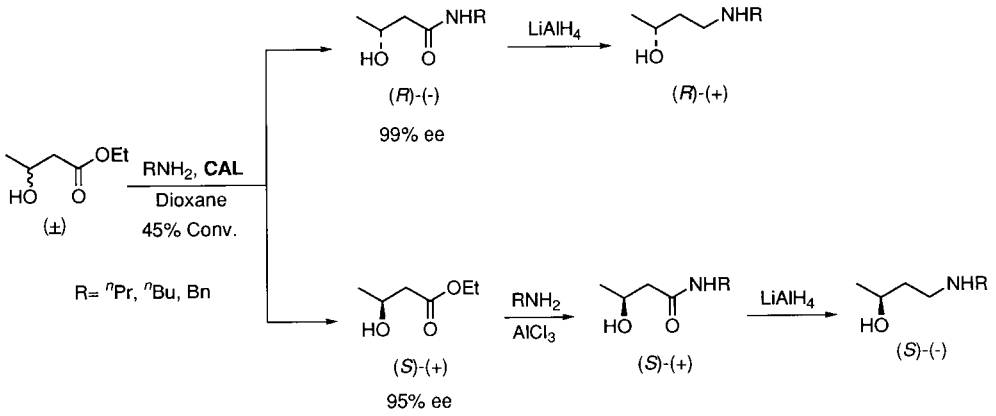
Lipases are now widely recognized as efficient catalysts in the preparation of amides from bifunctional compounds. Moreover, some lipases accept other nucleophiles as hydrazines.[36] A representative example about the utility of lipases in chemoselective processes, is the enzymatic aminolysis of acrylic and propyolic esters, since in absence of enzyme the formation of Michael adducts takes place. CCL catalyzes the reaction between ethyl propyolate and aromatic amines to afford the corresponding propyolic amides. This reaction has a limitation; it is not possible to use aliphatic amines because the chemical reaction is faster than the enzymatic process, and Michael adducts are always obtained.[37] CAL is the most active biocatalyst for the preparation of acrylic amides. This lipase catalyzes the aminolysis of different acrylic esters and aliphatic amines, in this case the reaction with aromatic amines takes place with very low yields. If racemic amines are used, the corresponding optically active acrylamides are obtained in moderately-high enantiomeric excesses.[38] The reaction of α,β-ethylenic esters produced from benzaldehyde and furfural, with amines is also catalysed by CAL, and the unsaturation in the ester has a significant effect in the course of the reaction.[39] When aromatic amines are used a longer reaction time is required.

Of particular interest is the enantiopure preparation of β-hydroxyesters and β-hydroxyamides. Both types of compounds are highly versatile intermediates in organic synthesis. Optically active 3-hydroxyamides and the remain β-hydroxyester are obtained from racemic ethyl 3-hydroxybutyrate and aliphatic amines. When the reaction is catalysed by CAL the corresponding (*S*)-hydroxyamide is isolated with high optical yield, but if PSL is used a lower and opposite enantioselectivity is observed (Scheme 2).[40] CAL also catalyzes the aminolysis of racemic ethyl 3,4-epoxybutyrate, and the chiral epoxyamide is formed with high enantiomeric excess.[41] From these results, it is easy to conclude that aminolysis processes can be an alternative to the enzymatic hydrolysis and transesterification for the resolution of esters.

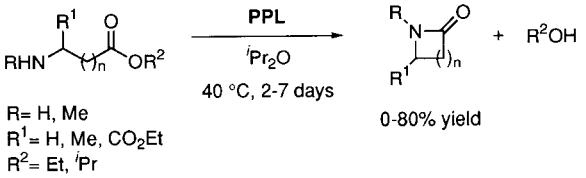
The intramolecular aminolysis of different aminoesters catalysed by porcine pancreatic lipase (PPL), give the corresponding lactams of ring size 5–7, but the preparation of a 4-membered ring has not been described. For this reaction of intramolecular cyclisation of aminoesters, it is necessary to suppress the uncatalyzed cyclisation. The enantioselectivity of this process is very low and the intramolecular cyclisation is faster with primary amines (Scheme 3).[42]

Enzymatic ammonolysis reaction

The lipase-catalyzed ammonolysis of esters has been reported by us[43] and others.[44] Proteases have also been employed to catalyze the synthesis of *N*-protected aminoacids or peptide amides using ammonia as the nucleophile.[45] CAL proves to be a very efficient biocatalyst in the synthesis of great variety of fatty acid



Scheme 2.



Scheme 3.

amides by enzymatic ammonolysis.[46] Recently, we described a new and simple procedure for the preparation of *N*-unsubstituted acrylic and propyolic unsubstituted amides through an enzymatic ammonolysis reaction (Scheme 4).[47]

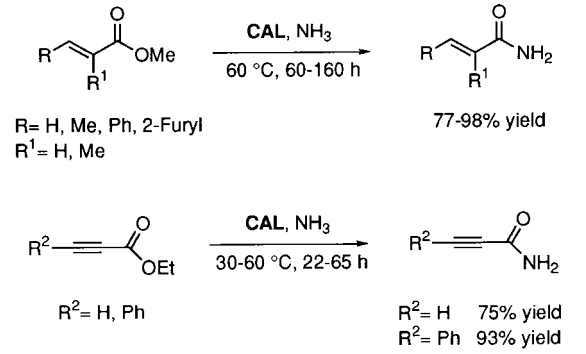
The enzymatic aminolysis and ammonolysis reactions of diethyl fumarate and maleate have been studied. Surprisingly, the influence of the π system geometry has a notable influence in this process. When ethyl fumarate reacts with amines or ammonia in the presence of CAL, the corresponding *trans*-amidoester is isolated in good yields, however, the reaction with diethyl maleate gives the same compounds as in the case of fumarate.[48] The formation of these compounds can be rationalized via a previous Michael/retro-Michael type isomerization of diethyl maleate to fumarate, before the enzymatic reaction takes place. In consequence, diethylmaleate is not an adequate substrate for the enzyme.

3-Oxoamides are difficult to obtain by ammonolysis or aminolysis reactions, because ketoesters react with amines or ammonia in the absence of a catalyst to yield the corresponding enaminoesters. However, if the reaction is carried out in the presence of CAL, only the chemoselective formation of β-ketoamides takes place (Scheme 5).[43] If racemic amines are allowed to react, optically active 3-oxoamides are obtained with moderate to high enantiomeric excesses.[49]

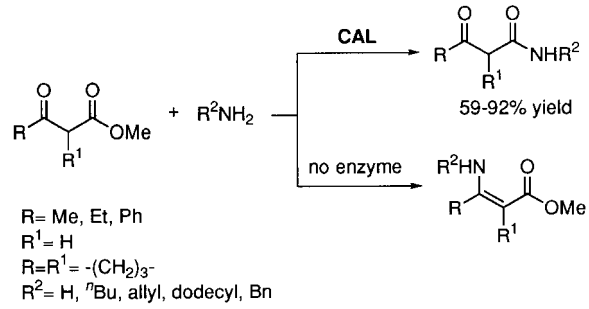
The enzymatic ammonolysis of amino acid esters has been performed with lipases, yielding the corresponding amino acid amides with moderate enantioselectivity. Of interest is the formation of the D-(−)-amide of the phenylglycine ester because of their importance in the enzymatic coupling in the synthesis of antibiotics.[50] Recently, the ammonolysis of D,L-phenylglycine methyl ester has been carried out in *t*-butyl alcohol and CAL as biocatalyst. The combination of this reaction with racemisation of the unconverted ester in a single step is also investigated (Scheme 6).[51]

Resolution and asymmetrization of esters

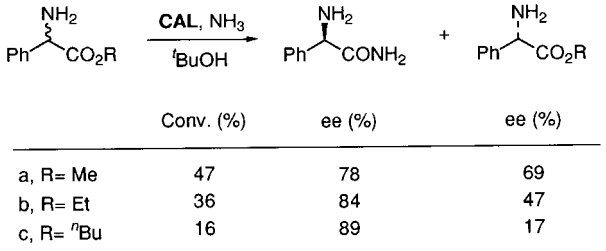
As we have mentioned previously, aminolysis and ammonolysis reactions can be of utility for the resolution of esters.[40] The ammonolysis of ibuprofen 2-chloroethyl ester is more enantioselective that the enzymatic hydrolysis (Scheme 7).[44] Ethyl esters of β-aminocarboxylic acids can be resolved by lipases through the acylation of the amino group at the *R*-stereogenic centre.[52] Recently, racemic ethyl-3-aminobutyrate has been successfully resolved by CAL catalysed *N*-acetylation,[53] and Zwanenburg et al.[54] have described the resolution of racemic



Scheme 4.



Scheme 5.



	Conv. (%)	ee (%)	ee (%)
a, R= Me	47	78	69
b, R= Et	36	84	47
c, R= ^{n}Bu	16	89	17

Scheme 6.

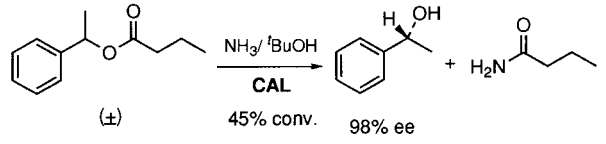
N-alkylated azetidines by CAL in an ammonolysis reaction (Scheme 8).

We have reported the first example of the asymmetrization of a prochiral diester through an ammonolysis and amminolysis processes. The reaction of dimethyl 3-hydroxyglutarate with ammonia or amines and CAL as biocatalyst gives the corresponding enantiopure amidoester. In all solvent tested the nucleophile reacts with the *pro-R* ester grouping, and the *S* amidoster is always obtained. The monoamide prepared from ammonia is an excellent starting compound for the preparation of biologically active (*R*)-GABOB (Scheme 9).[55]

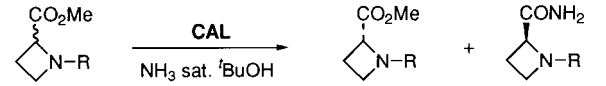
Resolution of amines

Chiral amines constitute an important class of organic compounds because of their utility as starting material for the preparation of important compounds of pharmaceutical and industrial interest.[56,57] Proteases have been used for the kinetic resolution of some amines.[58,59] However, lipases present several advantages, mainly the very low amidase activity and the possibility of carrying out the reaction in low hydrated organic solvents.[19]

Reetz et al.[60] have reported the enantioselective acetylation of chiral primary amines with CAL, and have also showed that Pd-catalyzed racemization of a chiral amine is compatible with lipase-catalyzed enantioselective *N*-acetylation. The racemization takes place only with the amine, and thus it is possible to obtain good

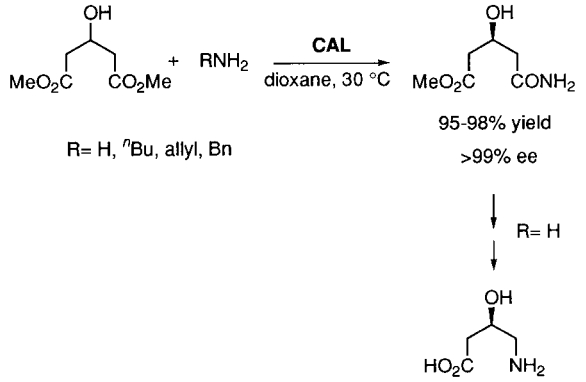


Scheme 7.



R	conv. (%)	ee (%)	ee (%)
Bn	56	99	80
*p*MeOBn	54	99	84
allyl	50	99	97

Scheme 8.

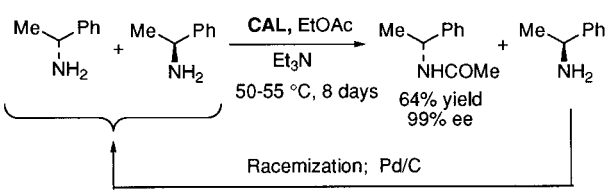


Scheme 9.

yields of one enantiomer, achieving an interesting dynamic kinetic resolution of racemic phenyethylamine (Scheme 10).[61] In addition, this group has also described the kinetic resolution of 2-pentylamine with *Pseudomonas aeruginosa* lipase[62] via a *N*-acetylation reaction, and this process has been extended to other amines. In this reaction, those amines possessing bulky cyclohexyl or phenyl group are efficiently acylated giving very high enantiomeric excesses.[63]

Racemic amines can be resolved using ethyl methoxyacetate as acylating agent in a lipase-catalyzed reaction.[64] PSL catalyses the *N*-acetylation of azidoamines for example. This process is of interest because the resolution of these amines, which are starting compounds for the synthesis of azasugars, is possible.[65] Recently, racemic 1-(heteroaryl)ethylamines have been resolved by an enzymatic acetylation using ethyl acetate as solvent and acyl donor. Again CAL catalyses this reaction very efficiently.[66] The resolution of alcohols and their corresponding primary amines and thiols has been studied by Hult et al.[67] In this report it is possible to observe the different behavior of these three classes of compounds in the enzymatic acylation reaction.

Cyclohexan-1,2-diamines are an important class of compounds, especially because of their usefulness as chiral auxiliaries in asymmetric synthesis.[68] The enzymatic aminolysis with ethyl octanoate of a mixture of *cis*/*trans* compounds has been carried out with CAL, and the reaction is found to take place with acceptable stereoselectivity.[69] A more interesting process is the double resolution of racemic *trans*-cyclohexane-1,2-diamine,[70] the reaction being carried out with dimethyl malonate and using CAL as a biocatalyst. The formation of the (*R,R*)-bisamidoester involves two biocatalytic



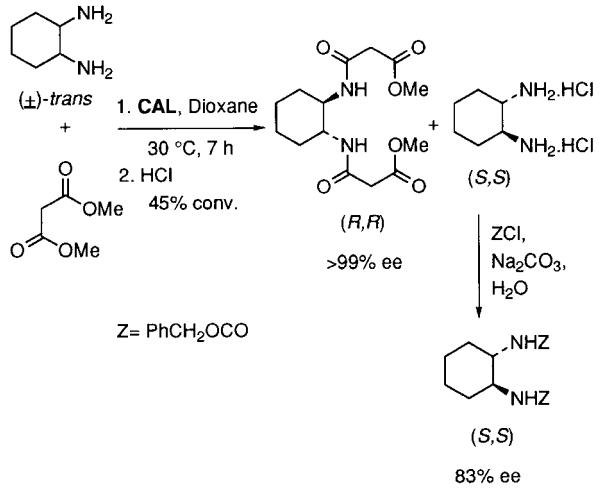
Scheme 10.

steps; the enzyme shows the same stereochemical preference towards the (*R,R*) enantiomer of the substrate in both steps. Moreover, choosing carefully the reaction conditions, it is possible to obtain substrate and product in enantiopure form (Scheme 11).

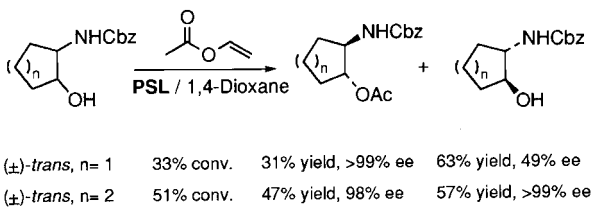
In most examples studied in the aminolysis reaction with racemic amines, lipases and especially CAL, always exhibit preference towards the (*R*) isomer of the amines. Kazlauskas[71] has described a structure-based rationalization of the enantiopreference of subtilisin and lipases with primary amines. While lipases favor the (*R*) enantiomer of amines, subtilisin has preference towards the opposite enantiomer. Recently, Sinisterra[72] has studied the influence of several variables for the aminolysis of esters catalysed by several lipases from different origin.

Resolution of aminoalcohols

The chemoenzymatic monoacylation of aminoalcohols has been carried out by Klibanov[73] using 6-amino-l-hexanol as a model of a bifunctional compound. The enzymatic resolution of aminoalcohols via *N*-acetylation reaction is not easy because acyl migration takes place in some cases in these processes.[74] The resolution of chiral 2-amino-1-alcohols has been carried out with PPL and ethyl acetate[75] through an enzymatic aminolysis reaction, however the enantioselectivity depends dramatically on the nature of the substrate. Better results are obtained by a transesterification reaction following earlier *N*-protection of the amino group.[76] The kinetic resolution of *N,O*-diacetyl-2-amino-1-butanol is carried out by an enzymatic transesterification reaction in diisopropyl ether with butanol,[77] and several aminoalcohols can be resolved by transesterification using the corresponding carbamates.[78] The resolution of different aminoalcohols derivatives is almost always achieved by *O*-acylation; 2-amino-1-phenylethanols for instance can be resolved by *O*-acylation with PSL,[79] and recently, by a similar mechanism, racemic *trans*-2-aminocyclohexanol and 2-aminocyclopentanol have also been efficiently resolved (Scheme 12).[80]



Scheme 11.



Scheme 12.

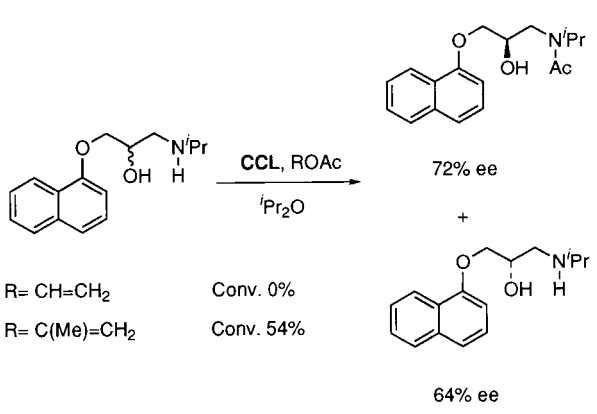
Enzymatic acylation of secondary amines

The structure of the amine has a great influence in the lipase-catalyzed aminolysis reaction. Although primary amines can be efficiently resolved, few examples have been reported of the acylation or resolution of secondary amines. The *N*-acylation of piperidine derivatives has been carried out with ethyl acetate using PPL and PSL,[81] although it is difficult to explain the results obtained in this reaction.

Djeghaba and De Jeso[82] have studied the acylation of various cyclic and acyclic secondary amines with esterases and lipases. Although some lipases, are active in this reaction, the best results are found with one particular esterase (horse liver acetonic powder). The most efficient reaction is when pyrrolidine react with ethyl butyrate in hexane as solvent. It is of note the different behavior of piperidine and its corresponding substituted 2- or 2,5 derivatives. In the last case no reaction is observed. On the other hand, acyclic secondary amines are not good substrates for this enzymatic reaction and their acylation has not been described yet.

Surprisingly, the kinetic resolution of propranolol has recently been described by a lipase-catalyzed *N*-acylation of the secondary amino group. Several acyl agents have been tested, but only isopropenyl acetate is efficient in this reaction. Among various lipases checked, CCL is found to be the most reactive and enantioselective for catalysing the *N*-acetylation of propanolol. The influence of the solvent has also been studied, and isopropyl ether is shown to be the most suitable (Scheme 13).[83]

The enzymatic *N*-acylation of *N*-methyl-glucamine in hexane using lipase from *Rhizomucor miehei* has



Scheme 13.

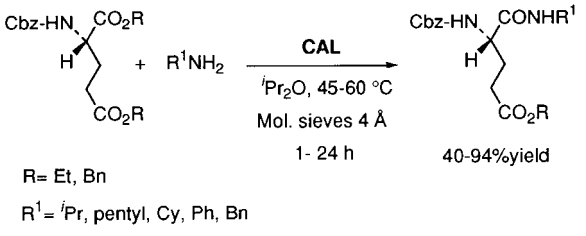
recently been described. *N*-Methyl-glucamine is solubilized by oleic acid addition and the formation of an ion-pair between acid and amine function takes place. This ion-pair formation looks essential for amide or ester synthesis.[84] In addition, the synthesis of glucamide surfactants has been carried out by acylation of *N*-methyl-glucamine with fatty acids in the presence of CAL and 2-methyl-2-butanol as solvent.[85]

Enzymatic monoamidation of diesters

We have discussed here two processes for the amidation of diesters, the enzymatic aminolysis and ammonolysis of diethyl maleate and fumarate,[48] and the resolution of *trans*-cyclohexane-1,2-diamine with dimethyl malonate.[70] These kinds of processes are of interest, due to the importance of amidoesters as starting materials for the synthesis of compounds of biological relevance, such as polyamines and azamacrocycles.

CAL is again the most efficient biocatalyst in all processes described about the aminolysis and ammonolysis of diesters with amines and ammonia. This lipase catalyses the selective monoammonolysis and aminolysis of dimethyl succinate; the solvent has an important influence in this reaction, and the best results are obtained when dioxane is employed. If racemic amines are used, the corresponding amidoesters are obtained, and CAL is selective towards the (*R*) isomer of the amine. In addition, the enzymatic amidation of racemic α-methyl-succinate has also been studied, and a mixture of regioisomers amido esters are formed, but the aminolysis takes place preferably by the less hindered ester group.[86]

Conde et al.[87] have studied the amidation of Cbz-glutamic acid diesters with different primary amines and CAL in anhydrous diisopropylether as solvent. The reaction takes place in a regio- and enantioselective manner to give the corresponding monoamide derivatives. When the starting amine is racemic α-methyl-benzylamine only reacts with the (*R*) isomer. It is of note, the influence of the *N*-blocking group in the regioselectivity of this reaction, and the corresponding α-monoamides are obtained in all cases. However, there is a broad range of reaction rates depending on the size and electronic features of the blocking group (Scheme 14).[88] The enzymatic amidation of (*R*) and (*S*) diethyl glutamate and ethyl pyroglutamate has also been studied; the amidation rate of the isomer (*S*) is higher than that of the (*R*) enantiomer.[89]



Scheme 14.

The enzymatic aminolysis of non-activated diesters with diamines has been investigated.[90] Among the different lipases tested in this reaction, CCL and PSL showed a very low catalytic activity, while CAL is very efficient in this process. When propane 1,2-diamine is used, the enzyme catalyses the aminolysis of diesters with very good enantioselectivity. It is of note that depending of the starting esters, *N,N′*-polymethylene succinimides and glutarymides are obtained. Activated monochloroethyl diesters react with diamines catalysed by PPL giving macrocyclic bislactams. The reaction is carried out in dichloromethane or chloroform at reflux.[43] Aminoalcohols react with diesters in the presence of CAL to yield, in some cases, the corresponding macrocycles by an enzymatic aminolysis and intramolecular transesterification processes.[91] The asymmetrization of the prochiral diester 3-hydroxyglutarate is also an example of monoamidation of diesters.[55]

Enzymatic Alkoxycarbonylation of Amines

Synthesis of carbamates

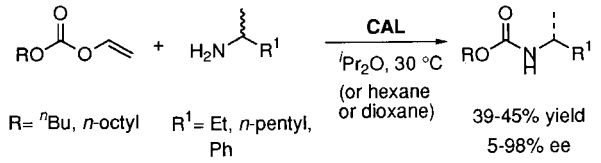
The enzymatic alkoxycarbonylation reaction, has scarcely been investigated,[92] despite the fact that this process is of a great utility since it permits the possibility of the introduction of protected or activated groups in alcohols or amines. For instance, the application of this reaction has been demonstrated by our group in the regioselective alkoxycarbonylation of carbohydrates[93] and nucleosides.[94–96]

Carbamate derivatives are products of considerable interest in some areas of medicinal chemistry,[97] and although some chemical methods have been described for the synthesis of several kinds of carbamates,[98] the enzymatic alkoxycarbonylation of amines is a methodology worth considering for the preparation of this class of important compounds.

To carry out the carbonation of amines, several alkoxycarbonylation agents have been investigated. Alkyl carbonates are much less reactive than the corresponding esters in enzymatic processes, although vinyl alkyl carbonates prove very useful alkoxycarbonylation agents for alcohols and amines. CAL catalyses the carbonation of butylamine with benzyl and octyl vinyl carbonates, giving with moderate yields the corresponding carbamates. This reaction is the first example of a lipase-catalyzed carbonation of amines.[99]

Preparation of chiral carbamates

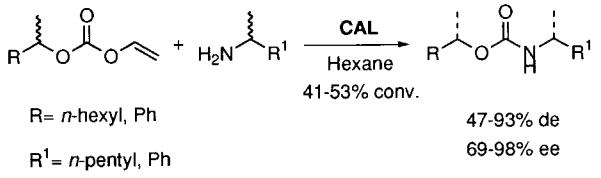
Chiral carbamates can be prepared by the enzymatic alkoxycarbonylation of vinyl carbonates and racemic amines using CAL as biocatalyst. The enantioselectivity achieved depends on the nature of the substrate and the solvent. In all cases the (*R*) isomer of the amine reacts faster (Scheme 15).[100] This procedure also allows the resolution of amines, and with a combination of long-medium length amine and carbonate alkyl chains high enantiomeric excesses can be achieved. When this



Scheme 15.

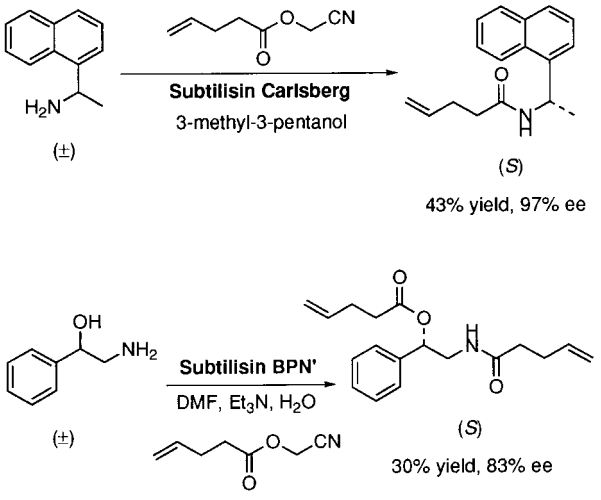
process is allowed to react with racemic vinyl carbonates and primary amines, the corresponding (*S*) carbamates are prepared with higher enantioselectivity in shorter reaction times.[101]

The double enantioselective lipase-catalyzed alkoxycarbonylation of racemic amines with racemic vinyl carbonates has been carried out using CAL, to yield carbamates with two stereogenic centers. This process shows that the use of enzymes in combination with an adequate matching between vinyl carbonate and amine, yields high enantiomeric excesses in the preparation of urethanes with two stereogenic centers in one step (Scheme 16).[102]



Scheme 16.

The enzymatic alkoxycarbonylation of (±)-*trans*-2-aminocyclohexanol catalysed by CAL has been studied. When this aminoalcohol is allowed to react with dibenzyl carbonate, only the product of aminolysis is obtained. However, the yield is low and this reaction is not an adequate methodology for this resolution. The *O*-alkoxycarbonylation compound is not detected.[80]



Scheme 17.

Recently Wong et al.[103] have described a novel enzymatic method for protecting amines as carbamates with high enantioselectivity, using homocarbonates as substrates for lipases and proteases. The best results are obtained when diallyl carbonate is allowed to react with an aziridine. This process opens an opportunity to resolve secondary amines, difficult to achieve using ester aminolysis.

Racemic amines and aminoalcohols can be enzymatically resolved via an enzymatic alkoxycarbonylation reaction using diallyl carbonate. However, proteases are more active enzymes than lipases in these processes. On the other hand, of several carbonates tested, cyanomethyl pent-4-enoate results in being the most efficient acylating agent to achieve a good resolution in this reaction (Scheme 17).[104]

Concluding Remarks

Although lipase-catalyzed transesterification reaction is a tool of great utility for organic chemists, the amidation reaction has also emerged in the last few years as an alternative of high value for the synthesis and resolution of amines. The preparation of chiral carbamates with potential biological activity is also possible to achieve. There are reasons to be optimistic due to the constant presence of new lipases that, they will extend this methodology to the synthesis of new compounds of biological and industrial interest. On the other hand, the fine chemical industry is showing their interest for several of the procedures described here in the preparation of optically pure amines in large scale preparations.

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Biography

Vicente Gotor was born in 1947 in Calatayud, Spain. He recieved his Ph.D from the University of Zaragoza in 1974. After leaving Zaragoza, Dr. Gotor carried out two years of postdoctoral studies at Max Planck Institut für Kohlenforschung (Mülhein/Ruhr, Germany) in the area of organometallic chemistry. He joined the Chemistry Faculty at the University of Oviedo as assistant Professor in 1977; he assumed his current position as Professor of Organic Chemistry at the same institution in 1982. His research fields include the areas of Heterocyclic and Bioorganic Chemistry. He worked in heterocyclic chemistry until 1988. In this year, he started his work in the field of biotransformations. Specific areas of his research interest are enzymatic amidation reactions with hydrolases, enzymatic transformations on natural products and biotransformations with oxynitrilases and oxidoreductases. At present, he is Vice-chancellor of Research of Oviedo University.