TUTORIAL REVIEW

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Biocatalytic and biomimetic aminolysis reactions: useful tools for selective transformations on polyfunctional substrates

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Aminolysis is a deeply studied reaction, but the development of new catalysts for this process is still an emerging area of organic and bioorganic chemistry. Two different approaches are reviewed in this article: the biomimetic *de novo* designed synthetic catalysts and the use of natural enzymes. Brief mechanistic considerations are discussed. Some important aspects like chemo-, regio- and stereoselectivity towards the substrates are highlighted on selected examples with synthetic applications.

Introduction

The amide group is present in many chemicals, proteins, peptides and other biomolecules. Usually, the introduction of the amide functional group is carried out by aminolysis of a carboxylic acid itself or different derivatives such as oxo- and thiolesters. In spite of that, the use of efficient and selective catalysts for aminolysis reaction is yet a very important task in many areas of chemistry. As the mechanism for that reaction has been deeply studied, natural and non-natural catalysts have been applied to increase the reaction rate or substrate selectivity. The development of new catalysts can be done by pure synthetic or by biomimetic approaches. In all the cases, the basis for the catalysis are very similar. In this paper, we will review on natural and biomimetic non-natural catalysts of the process, focusing especially on the most recent applications for selective organic and bio-organic transformations.

General concepts in catalysis for aminolysis reaction

The aminolysis reaction is a basic example for the interaction of a carbonyl group with nucleophiles as well as a model for the formation of peptide bonds. There are numerous kinetic and theoretical[1] studies on this reaction. From these studies three main schemes have been mechanistically considered: (a) a stepwise mechanism through zwitterionic intermediates; (b) a stepwise path through neutral intermediates; and (c) a concerted pathway (Scheme 1).

Related to the design and use of catalysts for the process, many factors have to be taken into account. The catalytic influences of solvent and general acid-base catalysis are crucial in the reaction. Theoretical calculations showed that the most favoured pathway is the general base catalysed neutral stepwise mechanism. In this

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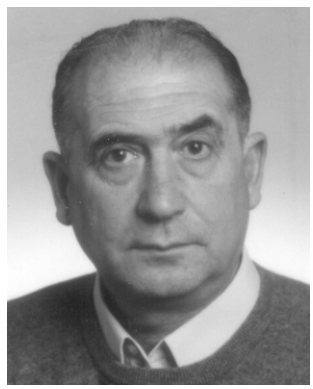
Vicente Gotor received his PhD from the University of Zaragoza in 1974. After leaving Zaragoza, Dr. Gotor carried out two years of postdoctoral studies at Max Planck Institut für Kohlenforschung (Mülhein/Rhur, Germany) in the area of organometallic chemistry. He joined the Chemistry Faculty at the University 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 chemoselective transformations on natural products, biotransformations with oxynitrilases and oxidoreductases, and chiral recognition with azamacrocycles. He was Vice-chancellor of Research of Oviedo University for four years until June 2000. At present, he is the leader of the Bioorganic group in the Chemistry Faculty of Oviedo University and Head of Department of Organic and Inorganic Chemistry. He has published more of 200 papers, is director of 35 Doctoral Theses and coauthor of 8 patents.



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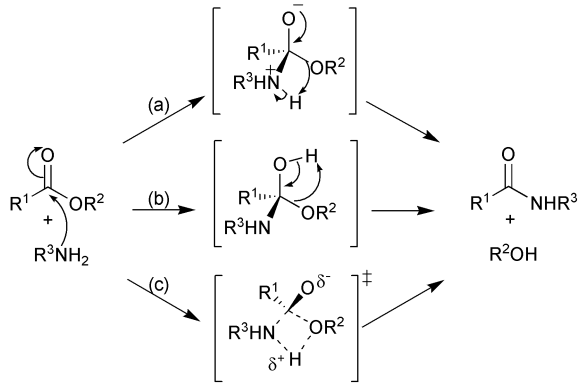
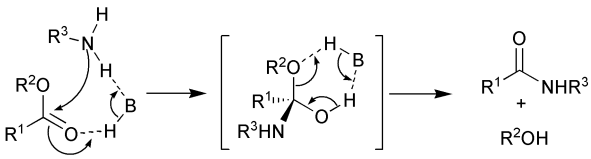


Scheme 1

mechanism (Scheme 2) a general base interacts with both the partially negatively charged carbonyl of the ester and the entering nucleophile, stabilising the transition state for the first step of the process and leading to the tetrahedral non-charged intermediate shown in Scheme 2. This intermediate evolves to the final amide



Scheme 2

through a similar process, in which the base now interacts with the hydroxy group and the oxygen of the alcohol leaving group. The overall balance is the breaking of a C–O single bond, the formation of a new C–N single bond and a proton transference from the incoming amine nucleophile to the leaving group, in which the base would act as a proton shuttle.

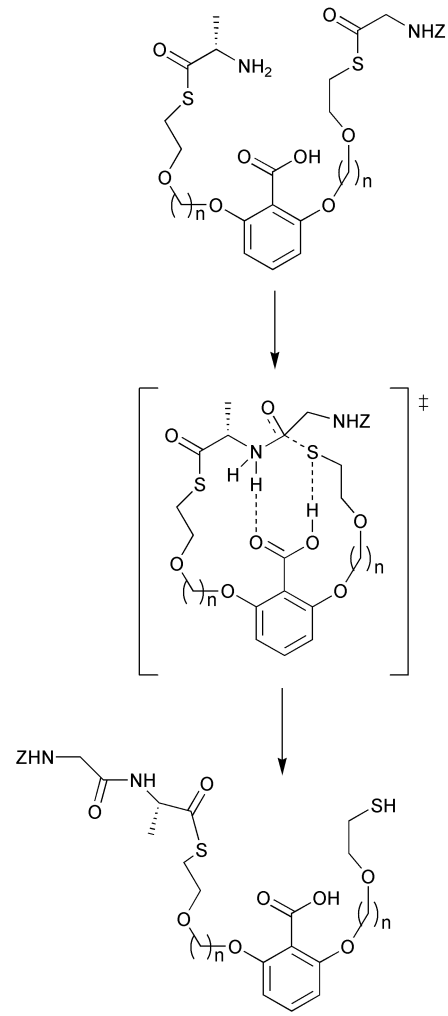
Another important factor for the catalysis is, as usual, the stabilization of the transition state, which is very similar to the tetrahedral intermediate. The general acid-base scheme serves as stabilizing interaction itself. Besides, the use of polar environment also facilitates the process.

Considering all these factors, we can define a good candidate for catalysing an aminolysis reaction. The system has to be able to approximate reactants in the proper orientation in order to overcome the entropic contribution to catalysis. It must also stabilise the tetrahedral intermediate and thus, decrease the energy of the transition states. Moreover, the catalyst should ideally have acid-base groups to act as proton shuttle in the catalytic process. Finally, the undesired hydrolysis side reaction should be avoided by water exclusion from the catalytic centre.

De novo designed approaches to a catalyst

One of the earliest approximations to a synthetic catalyst for amide bond formation was inspired by natural enzymes such us the ones responsible for non-ribosomal peptides syntheses. The fundamentals of this biosynthetic path are through the formation of an activated thiolester and the subsequent aminolysis to yield the final amide bond. Factors like the spatial approximation of the reacting centres and general acid-base catalysis are also important in this example. Thus, different C_2 symmetrical catalysts were prepared bearing two pendant thiol groups and a bifunctional acid-base catalyst to facilitate the necessary proton transfer on the transition state (Scheme 3). Although the rate enhancement of these biomimetic catalysts is far away from natural enzyme efficiency, they are a good example of a *de novo* designed catalyst, based on biocatalytic principles.[2]

Very recently, Tee and co-workers reported the use of different cyclodextrins for the catalysis of amide bond formation.[3] Cyclodextrins (CD) have been widely used in molecular recognition, with



Scheme 3

examples in many fields of basic and applied chemistry.[4] In this paper, the authors used a model reaction (aminolysis of esters) to perform a deep kinetic study of the effect of different CDs on the reaction rate. Conclusions of this work show that the formation of inclusion complexes (see Fig. 1) stabilise the transition state due to

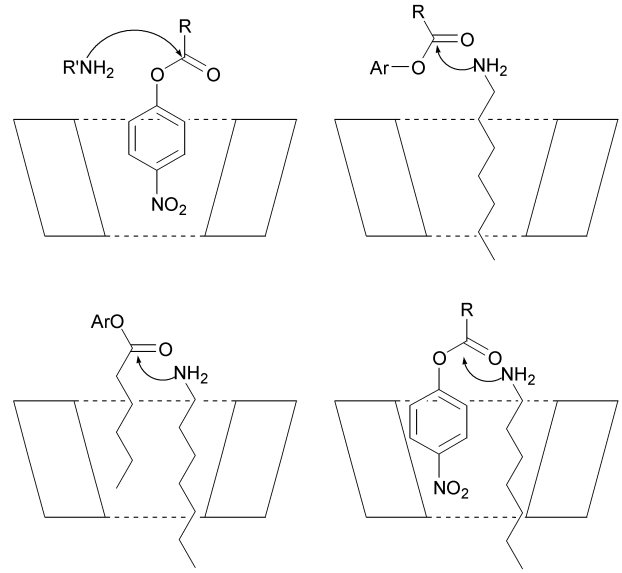


Fig. 1 Schematic representation of inclusion complexes proposed for CD-catalysed aminolysis reaction.

de-solvation of either the carbonyl group or amine side chain. Besides, proposed ternary complexes could approximate amine to

carbonyl and increase in that way the effective concentration of the reactants.

Although *de novo* construction of efficient catalysts based on natural biopolymers is still elusive, Ghadiri and co-workers reported the first fully synthetic peptide ligase with impressing rate enhancement in the catalysis of amide bond formation.[5] The system is formed by a 33-residue synthetic peptide, based on the coiled-coil structural motif, which efficiently catalyses the condensation of two shorter peptide fragments with high selectivity. Coiled-coils are assemblies of two or more α-helical peptides which wrap around each other in a left-handed superhelical twist. Their sequences usually show a seven-residue repeat $(abcdefg)_n$ in which the *a* and *d* positions are typically occupied by hydrophobic amino acids and the *e* and *g* ones by charged side chains (Fig. 2). The

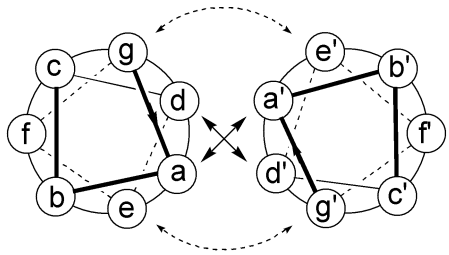


Fig. 2 Helical wheel representation of a general structure of a dimeric coiled-coil superhelical assembly. Hydrophobic interactions are represented by solid double-headed arrows, while electrostatic secondary interactions are shown by dashed double headed arrows.

identity of these amino acids determines the stability of the coiled-coil supramolecular assembly based on knot-to-holes van der Waals interactions (*a* and *d*) and self-complementary salt bridges (*e* and *g*).[6]

In Ghadiri's design, the catalyst (E) in the helical conformation has a hydrophobic surface able to provide the thermodynamic stability to bind two shorter complementary fragments (S_E and S_N) (Fig. 3). Given the appropriate activation of the fragments, the

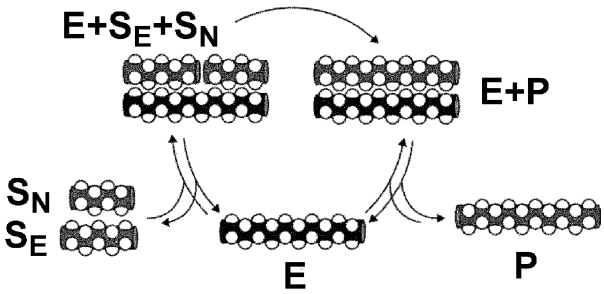
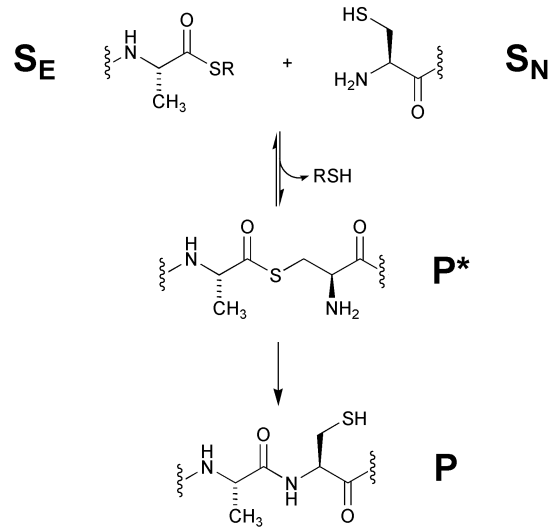


Fig. 3 Schematic representation of the catalytic process. Electrophilic ($\mathbf{S_E}$) and Nucleophilic ($\mathbf{S_N}$) peptide fragments are bound to the catalyst (**E**), forming a ternary complex which facilitates ligation reaction. Peptide backbones are shown as cylinders and side chains as spheres.

effect of the catalyst is to increase the effective concentration within the ternary complex. This non-covalent complex evolves to a covalent one by a reversible transthiolesterification reaction, leading to the thiolester intermediate P* (Scheme 4). The S to N acyl transfer produces the intramolecular rearrangement to the final product P.[7] Thus, the effect of the catalyst is, in this case, the approximation of the substrates with the proper orientation for the reaction to take place. Some features, similar to natural biocatalysts, are also observed in this non-natural system. The synthetic peptide ligase shows product inhibition as the product of the reaction also forms a very stable complex with the catalyst. It also presents substrate selectivity as the stability of the ternary catalytically active complex depends on the sequence of the two shorter substrate peptides. Mutations on *e* and *g* positions reduce the catalytic activity as they reduce the attractive electrostatic secondary interactions in the coiled-coil motif and thus the stability of the ternary complex.[5] Moreover, a deeper mechanistic study



Scheme 4

showed a dependence on the identity of the amino acids surrounding the ligation site. The initial rate of electrophilic substrates (S_E) with different thiol leaving groups correlates with their pK_a values. On the other hand, the system needs to bear a free thiol residue on the N-terminus of the nucleophilic peptide substrate (S_N). Mutation of L-cystein to glycine kills the process as it avoids the first step of transthiolesterification. Substrate specificity is also demonstrated through mutation to less active homocystein-nucleophile, or the lower reactivity of D-cystein diastereomer.[5] The stereoselectivity of the reaction has been further demonstrated by the development of a chiroselective self-replicating peptide, with a very similar design.[8]

The use of natural enzymes

Another different approach to efficient catalysts for aminolysis reaction is the use of natural enzymes.[9] Some advantages of using biocatalysts are easy manipulation, recovery and re-utilisation of the catalyst, smooth reaction conditions, high substrate specificity, low environmental impact and, after the advances of molecular biology, easy non-expensive availability. Since the discovery of the possibility of using hydrolases in organic solvents, the number of papers published describing the application of these enzymes for amide synthesis has increased tremendously. As the hydrolysis of amide bond is a process occurring *in vivo*, the early use of natural proteases to catalyse amide-bond formation is not surprising. However, the hydrolysis reaction is favoured by these biocatalysts and, consequently, special care is necessary to avoid low final yields of the product. For that reason, other hydrolases, like lipases have been applied for these purposes. Some lipases are able to catalyse aminolysis of differently activated esters, but the cleavage of the amide bond is not produced in the presence of the biocatalyst. Besides, the discovery of the stability of some lipases in organic solvents broadens their versatility due to substrate solubility and stability, avoiding aqueous medium. The accepted mechanism for the whole family of hydrolases is that of serine hydrolase (Fig. 4).[10] The catalytic site of the enzyme is formed by a catalytic triad (serine, aspartic and histidine residues). The serine residue accepts the acyl group of the ester, leading to a covalent acyl-enzyme activated intermediate. This acyl-enzyme intermediate reacts with the nucleophile (an amine) to yield the final amide product and leaves the free biocatalyst, which can enter again into the catalytic cycle. A histidine residue, activated by an aspartate side chain, is responsible for the proton transference necessary for the catalysis. Another important factor is the oxyanion hole, formed by different residues able to stabilize the negatively charged oxygen present in both the transition state and tetrahedral intermediate. The complex structure of the enzyme can show a very large substrate-enzyme

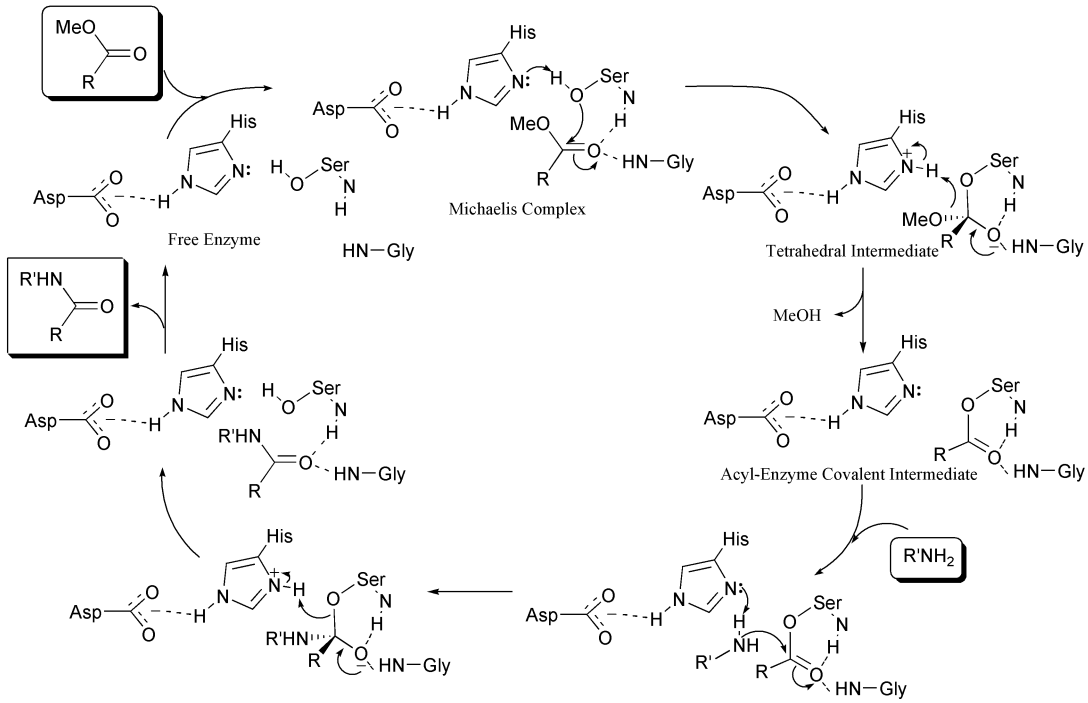


Fig. 4 Schematic representation of the mechanism of serine hydrolase catalysed aminolysis reaction.

interaction specificity, which can be traduced in high degree of chemo-, regio-, or stereoselectivity. These properties have made these biotransformations a very useful technique in organic and bioorganic chemistry.[9] A selection of examples to highlight the latest developments in this field is next reviewed in this paper.

Chemoselectivity

The selective transformation of a unique functional group in a molecule presenting more complex functionality and avoiding side-reactions is one of the most important tasks in organic synthesis. This goal is not easily reached by using conventional reactants or catalysts, especially when the target molecule is not stable under very drastic physico-chemical conditions (temperature, pressure, or presence of acidic or basic compounds). The conventional solution to this problem usually goes through tedious protection/de-protection steps, which lower the yields and make the total synthesis of some compounds very difficult. Considering these problems, the use of biocatalysts is an attractive alternative for carrying out chemoselective transformations in polyfunctionalised substrates.

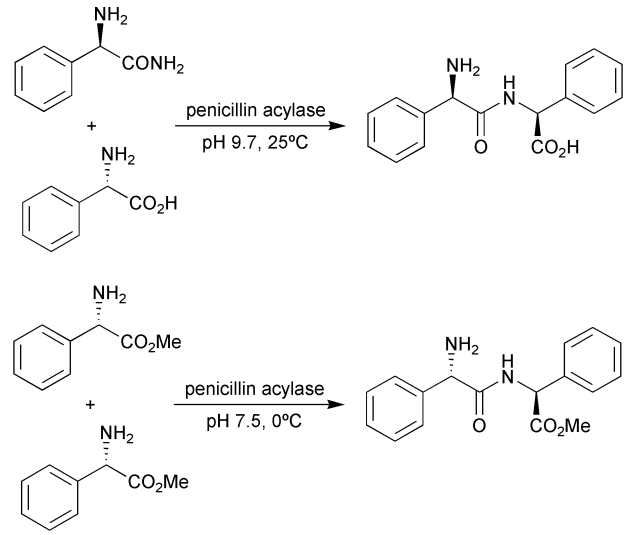
A very important example of these substrates are polypeptides, which are present in many interesting chemicals, including proteins and enzymes themselves. Related to that, Čeřovský *et al*. described the *C*-terminal peptide amidation catalysed by orange flavedo peptide amidase (Scheme 5).[11] The presence of a *C*-terminal amido

$$R^1CONHCH(R^2)COOH + NH_3 \overset{\text{amidase}}{\rightleftharpoons} R^1CONHCH(R^2)CONH_2 + H_2O$$

Scheme 5

group on the peptide chain is usually essential for its bioactivity. The developments in peptide solid phase chemical synthesis and recombinant DNA technology allow the production of many different peptidic compounds. The selective *C*-terminal transformation by enzymatic amidation emerges as an interesting complementary reaction to these techniques. Although some factors have to be considered, and the final yield of the amidated peptide depends on the sequence, the process seems to be a simple and reliable way for selective transformation of these substrates.

Another example of enzymatic selective peptide synthesis is the one described by Sheldon *et al*.[12] Penicillin acylase from *E. coli* catalyses the formation of a di-peptide from the monomeric phenylglycine, avoiding the use of protection-deprotection sequences (Scheme 6). As, for this enzyme, the acyl donor subsite is

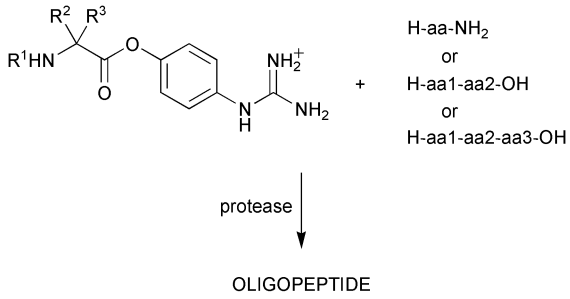


Scheme 6

moderately stereoselective but the nucleophile pocket shows an extremely high preference towards the L enantiomer, the authors used different reaction conditions for the efficient preparation of two diastereomeric dipeptides.

Despite of the problems of using proteases in peptide synthesis, these enzymes have been used for catalysing this reaction both in bulky solution and on solid phase. As we already commented, proteases catalyse both formation and hydrolysis of the amide bond, and the use of these biocatalysts could lead to low product yields. However, there have been some clever approaches to overcome this limitation.

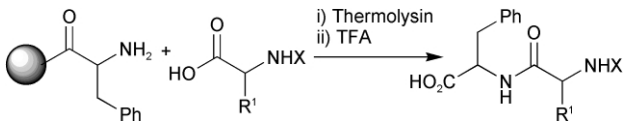
Koksch and co-workers described a method of preparing different di-, tri-, and tetra-peptides in aqueous solution using trypsin and α-chymotrypsin (Scheme 7).[13] They take advantage of the specificity of the enzymes towards the substrate structure and



Scheme 7

prepare 4-guanidinophenyl esters as substrate mimetics and frozen-state reaction conditions to carry out the process. Some interesting $C^{\alpha},^{\alpha}$-disubstituted and C^{α}-fluoroalkyl amino acids can be efficiently introduced in a peptidic sequence with this methodology.

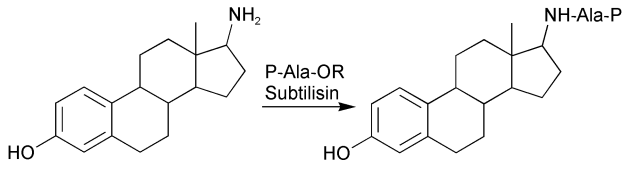
The most versatile approach to peptide syntheses is, by far, using solid supported substrates. Very recently, a very interesting solid-phase enzymatic approach has been reported.[14] Thermolysin is able to catalyse the amide bond formation between a free amino-supported substrate and a *N*-blocked amino acid in moderate to excellent yields (Scheme 8). The reasons for the shift of the



Scheme 8

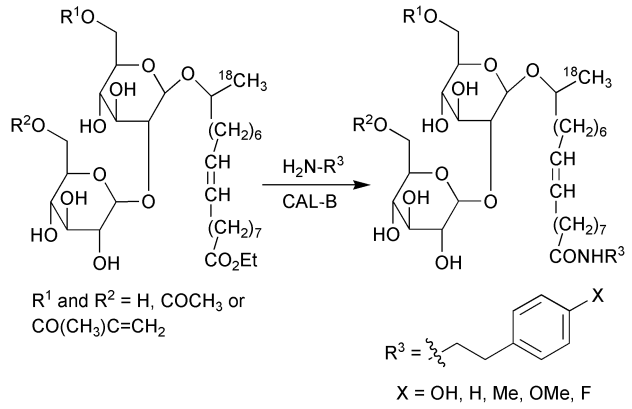
equilibrium to the peptide synthesis when the amine is immobilized are three. One advantage is that a large excess of acyl donor can be used to drive the reaction to completion. A second contribution is the suppression of ionisation of the free amino group on the solid phase due to the positive charge repulsion on the resin. Finally, there is an improved solvation of the hydrophobic acyl donor substrate in the PEGA resin when compared to bulky aqueous solution. The process allows the use of side chain deprotected amino acids as acyl donors and shows a very high L-enantioselectivity.

Another emerging application of chemoselective transformations is the conjugation of biologically interesting molecules. Very frequently, the conjugation of different molecules to biologically active compounds can change their properties, like transport conditions, lipophilicity, bio-availability, stability or even the biological effect. The usually very selective bio-transformations under smooth reaction conditions is a useful alternative to prevent undesired side reactions on the substrates. Related to that, aminoacid-estradiol derivatives have been prepared *via* protease-catalysed condensation.[15] The reaction can be carried out in organic solvent, under smooth reaction conditions and without racemisation of the starting materials (Scheme 9).



Scheme 9

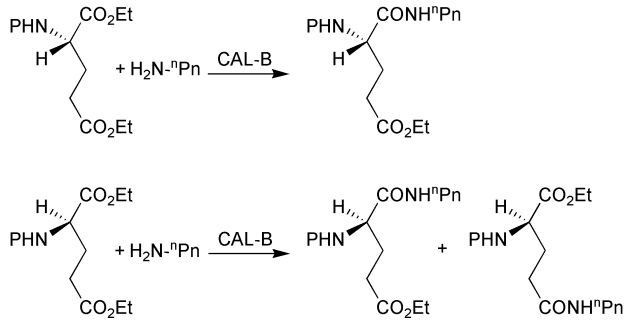
A final example of chemoselective enzymatic amidation on biologically interesting compounds has been recently reported.[16] A polymer supported preparation of lipase B from *Candida antarctica* (Novozyme 435) is able to catalyse the mild amidation reactions between primary amines and different derivatives of sophorolipid ethyl ester (Scheme 10). Fatty acid moiety or glycosidic bonds and acyl groups on the alcohols of the disaccharide remain unaffected during the process and the obtained yields are excellent.



Scheme 10

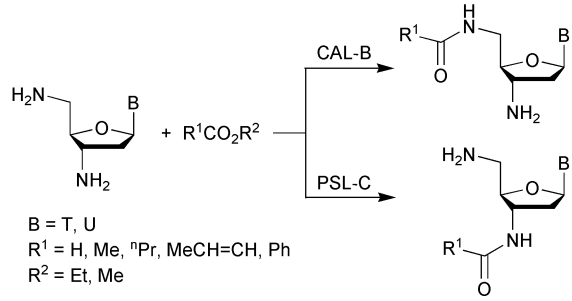
Regioselectivity

Very commonly in a synthetic pathway, the same functional group is present at different positions of a polyfunctionalised molecule. Sometimes, the selective transformation of only one of these groups is not an easy process. Here again, the enzymatic amidation is a powerful solution to this problem. We will show two examples in which either a diester or a diamine are regioselectively transformed using an enzyme. Conde *et al*. have recently described the regioselective amidation of *N*-blocked glutamic acid diesters catalysed by CAL-B.[17] Depending on both the absolute configuration of the sterocentre and the *N*-protecting group of the aminodiester, different molecular ratio of γ:α monoamides are obtained. As a conclusion, L-glutamic derivatives produced α-regioisomers but D-glutamic mainly led to γ-derivatives, being the regioselectivity dependent on the nature of the *N*-protecting group of the diester (Scheme 11).



Scheme 11

The regioselectivity of the lipases in aminolysis reaction is also demonstrated in the case of the nucleophile. Thus, the regioselective acylation of pyrimidine 3′,5′-diamino-2′,3′,5′-trideoxynucleosides can be carried out by choosing different biocatalysts (Scheme 12).[18] Immobilised lipase from *Pseudomonas cepacia*



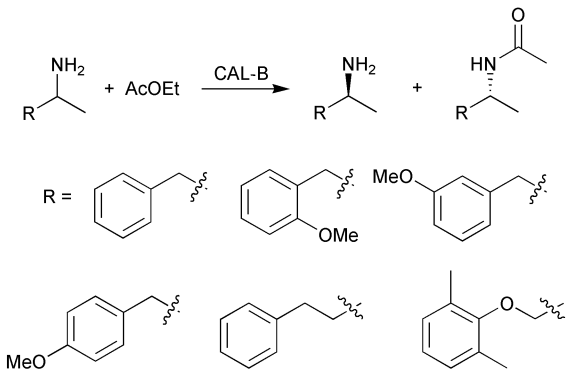
Scheme 12

(PSL-C) preferentially catalyses the acylation at the 3′-position while CAL-B shows an excellent regioselectivity towards the 5′-amino substituent. Some 3′,5′-diacylated derivatives were isolated

in some cases and the regioselectivity also depends on the acyl donor structure.

Stereoselectivity

Probably the most interesting application of enzymatic amidation is the preparation of enantio-enriched compounds.[9] Enzymes, as chiral entities, usually show high degree of preference for one enantiomer from a racemic pool or an enantiotopic group in a *meso* substrate. Applications of this technology in the pharmaceutical industry or synthesis of chiral ligands and synthons make the biocatalytic aminolysis a very attractive process for organic and bioorganic chemists. The enzyme can show enantiopreference for both substrates of the reaction, leading to the resolution of either acyl donor, nucleophile or even both of them. The source of this enantioselection is the faster reaction of one enantiomer compared with the other, being consequently a kinetic resolution. The ratio of the reaction rates between both enantiomers is expressed by the parameter called enantioselectivity (*E*). The most used enzymes for this transformation are lipases from *Candida antarctica*. Related to the nucleophile, amine acylation in the presence of lipases usually takes place on the *R* enantiomer of the substrate, following the Kazlasukas' rule, an empirical rule useful for the resolution of secondary alcohols.[19] This rule is based on the steric difference between the groups attached to the stereocentre and predicts that the larger is the steric difference, the higher is the enantioselectivity of the reaction. Thus, many 1-substituted ethyl amines[20] (Scheme 13)



Scheme 13

and 1-heteroaryl amines[21] (Fig. 5) have been successfully resolved by using CAL-B as biocatalyst. Also some efforts to give an explanation for the fitting of the substrates to the empirical rule have been carried out.[20]

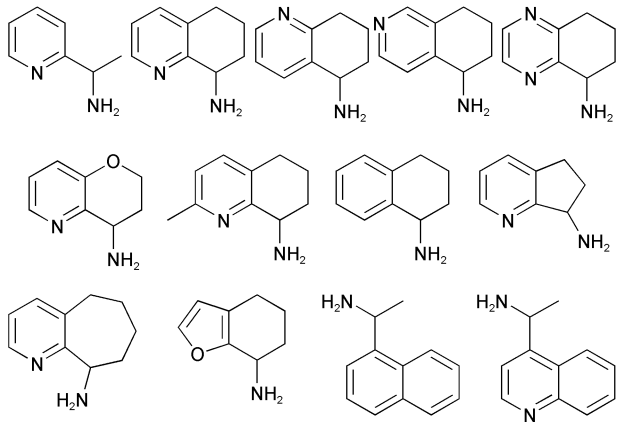
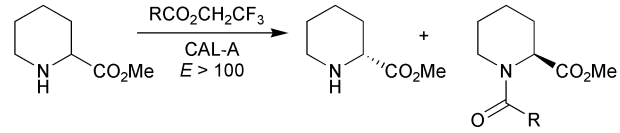


Fig. 5 Heteroaryl amines resolved by CAL-B catalysed acetylation.

Until now, all the described reactions have been performed with primary amines as substrates. To date, there are not many examples of enzymatic acylation of secondary amines. However, Kanerva *et al*. reported very recently the acylation of pipecolic acid derivatives catalysed by lipase A from *Candida antarctica* (CAL-A) (Scheme 14).[22] This enzyme seems to have a larger pocket in the active site and accepts this bulkier substrate. Besides, the amino group in a cyclic structure makes the nitrogen more reactive than a general secondary amine. A combination of these two factors makes possible the efficient resolution of the substrate.



Scheme 14

The enantiodiscrimination of the biocatalysts is not only restricted to asymmetric centres, and other chirality elements (such us chirality axe[23] or atropisomerism[24]) have been efficiently resolved through lipase catalysed acylation of the amino group (Fig. 6).

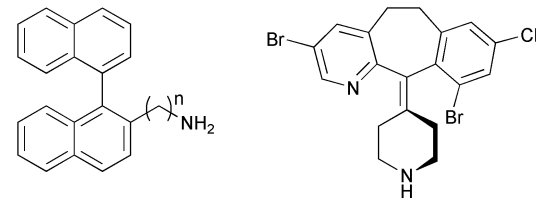
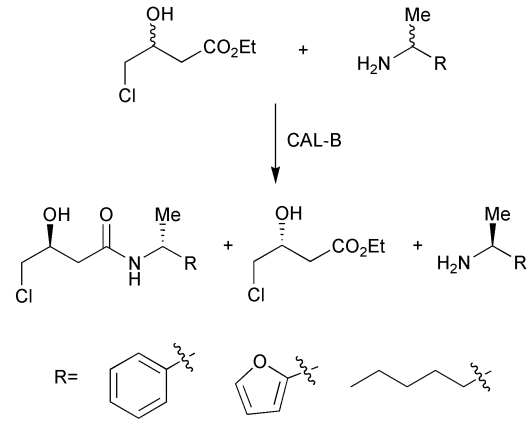


Fig. 6 Different chirality elements are also biocatalytically resolved.

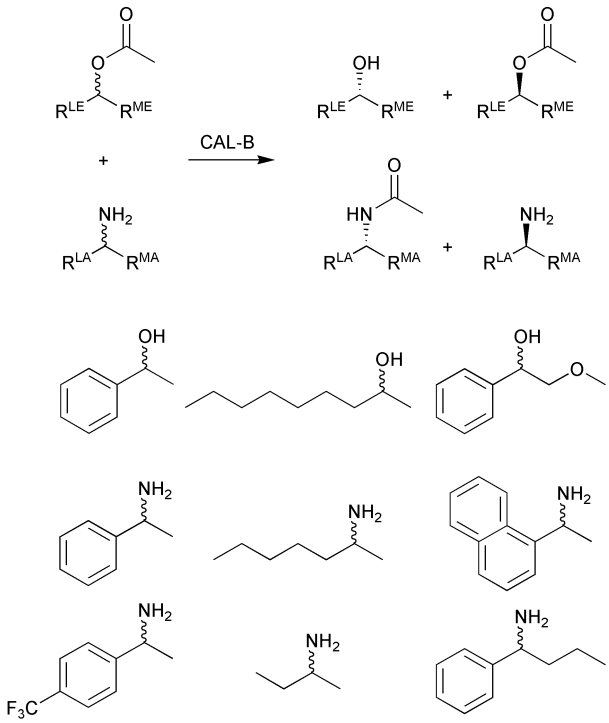
The power of biocatalysts for the production of chiral compounds can be elevated to a second stage when the proper use of the process leads to a larger number of different enantio-enriched products from the same reaction. Some efforts have been made in this direction. As we previously stated, the enzyme can be selective to either nucleophile or acyl donor. The elegance of the reaction is increased when the process is carried out for the resolution of both. Scheme 15 depicts this possibility.[25]



Scheme 15

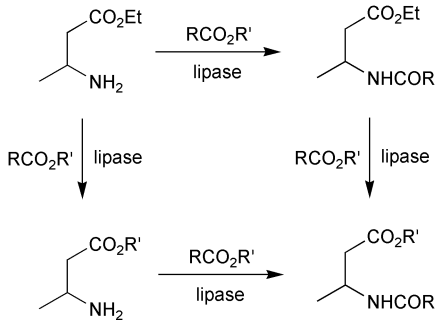
For this reaction, CAL-B catalyses the amidation between a racemic β-hydroxyester and racemic amines, leading to the corresponding amide with very high enantiomeric and diastereomeric excesses. Also, the remaining ester and amine are recovered from the reaction media, showing good ee's as well. In this way, three enantio-enriched interesting compounds are obtained from an easy one-step reaction.

Another philosophy would be the one-pot resolution of two different nucleophiles, alcohol and amine.[26] An acylated racemic alcohol reacts with a racemic amine in the presence of CAL-B to yield four separable enantio-enriched compounds. The *R*-alcohol acts as the leaving group in the acylation of the *R*-amine. The remaining *S*-ester and *S*-amine are also recovered from the reaction media (Scheme 16). Apart from the synthetic utility of the reaction, the authors use it for studying the effect of the leaving group in the resolution of the nucleophile.



Scheme 16

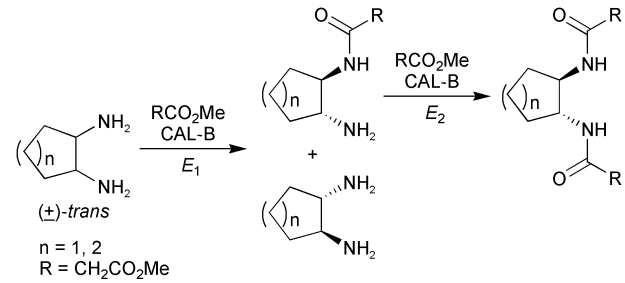
In some cases there is no suitable enzyme or reaction conditions for the efficient resolution of a given substrate. In these situations some substrate engineering can be undertaken. Related to that, maybe one of the most useful ways of increasing the ee of the final product is performing a sequential biocatalytic process. This consists of two or more consecutive enzymatic reactions. The already enantio-enriched product of the first step undergoes another process which increases the ee of the final product even more. Kanerva *et al*. applied this methodology for the resolution of ethyl-3-aminobutyrate (Scheme 17).[27] A one-pot transesterification–



Scheme 17

aminolysis sequence yielded the final product in very high ee. Several enzymes and acyl donors were tested, a preparation of lipase B from *Candida antarctica* (Chirazyme L2) being once again the most efficient.

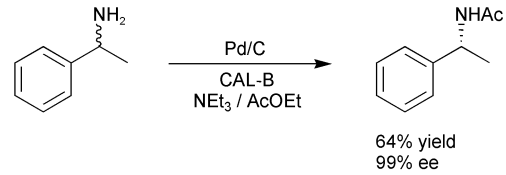
Considering the aminolysis reaction itself, there are only two examples of sequential biocatalytic resolution by one-pot double aminolysis. In our research group, we have used this approach for the CAL-B catalysed resolution of cyclic 1,2-diamines, obtaining the final diacylated compounds of *trans*-cyclohexane-1,2-diamine and *trans*-cyclopentane-1,2-diamine in enantiopure forms (Scheme 18).[28] Careful study of the enantiomeric ratio for each step, allowed us to extract an interesting structural effect. The enantioselectivity of the second step is always higher than the first one in a good agreement with Kazlauskas' rule, because the monoacylated compound has a bigger steric difference in the substituents of the stereocentre than the free diamine. Moreover the difference in the



Scheme 18

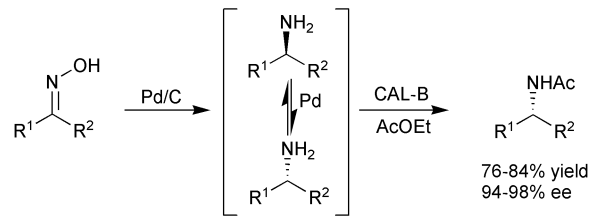
enantioselectivity of both steps is larger for the five-membered ring (E_1 = 21, E_2 > 200) than for the cyclohexane moiety (E_1 = 45, E_2 = 68).

We have shown that the enzymatic resolution through the aminolysis reaction is a powerful tool for obtaining both enantiomers of a substrate in high enantiomeric excess. Sometimes, only one enantiomer is interesting for synthetic purposes. In those cases, the mayor disadvantage of this methodology is the inherent 50% theoretical conversion as the upper limit for the chemical yield. Two elegant approaches have been developed to overcome this problem: the dynamic kinetic resolution and the desymmetrisation of *meso* and prochiral compounds. Dynamic kinetic resolution consists of coupling an enantioselective aminolysis process with a racemisation of the remaining substrate. With a proper control of the kinetics of both competing reactions, the enzyme always faces a racemate of the substrate, ideally yielding the product with high ee and 100% conversion. Here, the key reaction is the racemisation process, because this has to be faster than the enzymatic resolution and also faster for the substrate than for the product. Thus, Reetz *et al*. reported the first example of a Pd(0) catalysed racemisation of 1-phenylethylamine to carry out a dynamic kinetic resolution of the substrate by coupling with CAL-B catalysed acylation (Scheme 19).[29] The racemisation process proceeds *via* palladium(0) pro-



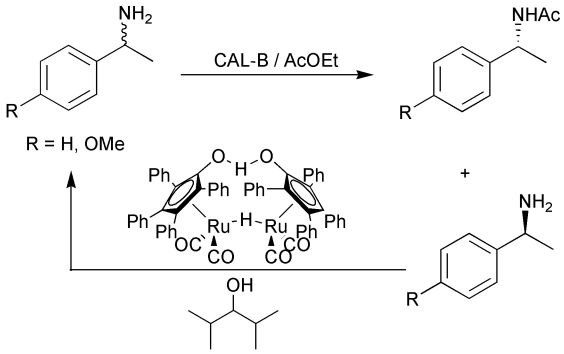
Scheme 19

moted amine–imine equilibrium. This reaction is very slow and by-products from reductive amination and elimination of ammonia are observed. Kim and co-workers have recently improved the process using ketoximes as starting materials under hydrogen atmosphere (Scheme 20).[30] In this way, the concentration of amine is low and the formation of by-products *via* reductive amination less favoured.



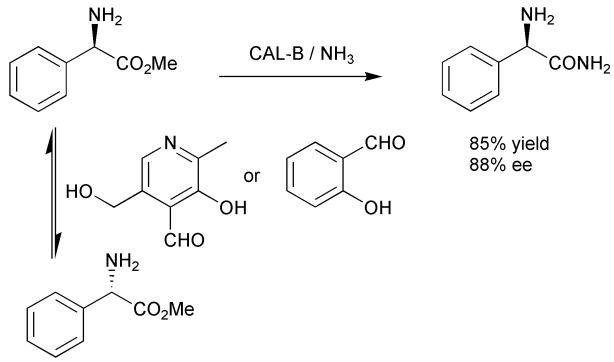
Scheme 20

Finally, another metal promoted racemisation for a dynamic kinetic resolution *via* enzymatic aminolysis has been reported by Bäckwald *et al*. (Scheme 21).[31] The use of a ruthenium hydrogen transfer catalyst for the racemisation process in combination with CAL-B acetylation allows the isolation of the corresponding acetamides in good combined yields and ee′s after two kinetic resolutions and one racemisation (in a two step manner).



Scheme 21

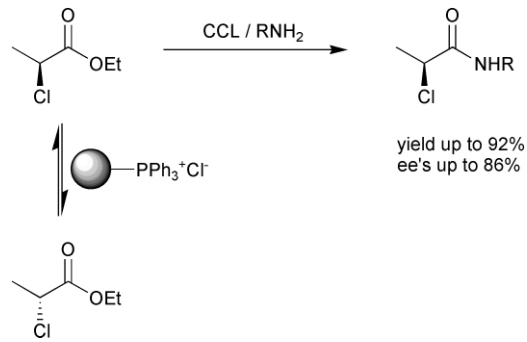
Sheldon and co-workers described one of the first examples of DKR of acyl donor, using ammonolysis of phenylglycine methyl ester, by the addition of different aldehydes (Scheme 22). The



Scheme 22

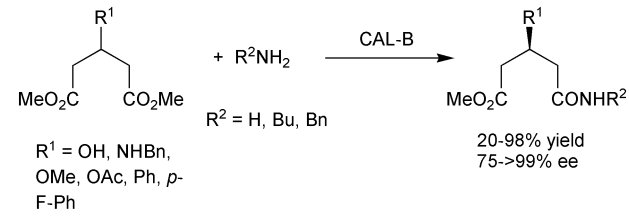
equilibrium in the formation of the corresponding imine and the imine–enamine tautomerism produce the racemisation of the methyl ester, while the final amide remains configurationally stable.[32]

A different possibility for racemization is a nucleophilic displacement on the stereocentre, as reported by Kostic and co-workers.[33] They performed the dynamic kinetic resolution of ethyl 2-chloropropionate *via* aminolysis catalysed by encapsulated *Candida cylindracea* lipase (CCL) in the presence of triphenylphosphonium chloride immobilized on Merrifield resin (Scheme 23). Again, a high degree of percent conversion and good ee's are obtained.



Scheme 23

A final approach to enantio-enriched synthons with >50% conversion is the desymmetrisation of *meso* and prochiral compounds. The selective transformation of an enantiotopic group in a prochiral substrate has been efficiently carried out in our research group.[34] The aminolysis of different 3-substituted glutarate diesters in the presence of CAL-B exclusively led to the corresponding monoamide of *S* configuration (Scheme 24). The conversion and the enantiomeric excess of the final product depend on the substrate structure. In general, when R^1 has a heteroatom (O, N) the process



Scheme 24

is very efficient, leading almost quantitatively (98% yield) to enantiopure monoamide. However both ee and yield drop when R^1 is aliphatic or aromatic. Some of these enantiopure monoamides have been used to prepare biologically interesting β-amino acids.[35]

Summary and outlook

Although the aminolysis reaction is a well known process, the development of new catalysts is still an emerging area in chemistry. Both synthetic and natural biomimetic catalysts have been described in this review. The *de novo* designed catalysts are still less efficient than the natural enzymes, but their study provides a tool for a better understanding of the factors affecting the catalysis, as they serve as a simplified model of the more complicated natural biocatalysts. On the other hand, the use of natural biocatalysts is already becoming a conventional process for organic and bio-organic chemists. Their utility in carrying out very chemo-, regio- and stereoselective transformations under mild reaction conditions make them a very attractive catalyst to perform some transformations in a synthetic route. Besides, the advances of molecular biology allow the production of new enzymes on a gram scale. Thus, expression, site directed mutagenesis, molecular evolution, purification and immobilisation of new enzymes provide a new battery of biocatalysts, easy to handle and ready for synthetic organic chemists to carry out new reactions. All these topics make the enzymatic aminolysis a very useful and exciting area to work in, with still unlimited possibilities.

Acknowledgements

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