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Lipases: Useful biocatalysts for the preparation of pharmaceuticals

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Abstract

Biocatalysis offers a clean and ecological way to perform chemical processes, in mild reaction conditions and with high degree of selectivity. The use of enzymes, specially lipases, in organic solvents proves an excellent methodology for the preparation of single-isomer chiral drugs. This review covers some general aspects and representative examples of the use of lipases for the enantioselective or regioselective preparation of alcohol and amine intermediates in the synthesis of pharmaceuticals.

Keywords: Biocatalysis; Lipases; Enzymatic resolution; Desymmetrization; Pharmaceuticals

1. Introduction

The use of enzymes, specially lipases, for the synthesis of chiral drugs is well established since years ago [1], and biotransformations are now accepted as a common methodology for the preparation of chiral pharmaceuticals [2]. Over the last few years, interesting processes have appeared, using hydrolases, oxidoreductases or lyases as biocatalysts for the synthesis of chiral drugs [3]. However, the use of lipases in organic solvents proves an excellent methodology for the preparation of single-isomer chiral drugs by enzymatic hydrolysis, transesterification or aminolysis reactions.

Applications of lipases in asymmetric synthesis include kinetic resolution of racemic alcohols, acids, esters or amines [4], as well as the desymmetrization of prochiral compounds [5]. During the last few years, there has been an ever-increasing trend for chiral drug substances to focus on single stereoisomers, that is, enantiomers instead of racemic mixtures, for this reason kinetic resolution (KR) processes or enantioslective enzymatic desymmetrization (EED) of prochiral compounds have special relevance for many pharmaceutical companies [6].

As above commented, the stereoselective biotransformations with lipases can be classified in two main groups: KR of mixtures, and EED of proquiral or *meso* compounds. Since, in EED a maximum yield of 100% in one enantiomer can be attained, in a KR only half of the starting material is used. When only one enantiomer of a substrate is required this fact constitutes a disadvantage of KRs and different approaches have been developed to overcome this limitation. The one on which more attention has been recently paid is the dynamic KR (DKR) and consists in carrying out an in situ continuous racemization of the substrate. In this manner, theoretically all of the racemic starting material can be used for the transformation into one enantiomer [7]. This new strategy has appeared in asymmetric catalysis during the last decade and the most common approach is the use of lipases and a metal–organic catalyst to produce the racemization of the substrate [8].

This review covers some general aspects of the use of lipases in organic solvents, through the above commented reactions and representative examples of these enzymatic processes for the enantioselective preparation of intermediates in the synthesis of chiral drugs. Also, the utility of lipases in regioselective processes in compounds of physiological interest will be briefly commented.

2. Lipases in organic solvents

One of the most serious drawbacks of the use of enzymes for organic synthesis is the poor solubility of the majority of organic compounds with more than four carbon atoms when the process is carried out in water. Water is a poor solvent for nearly all applications in industrial chemistry since many organic compounds are sometimes unstable in aqueous solution. Furthermore, the removal of water is more tedious and expensive than when organic solvents are used due to the lower boiling point of them. The use of organic solvents presents several advantages, such as: (a) easy recovery of the substrate and

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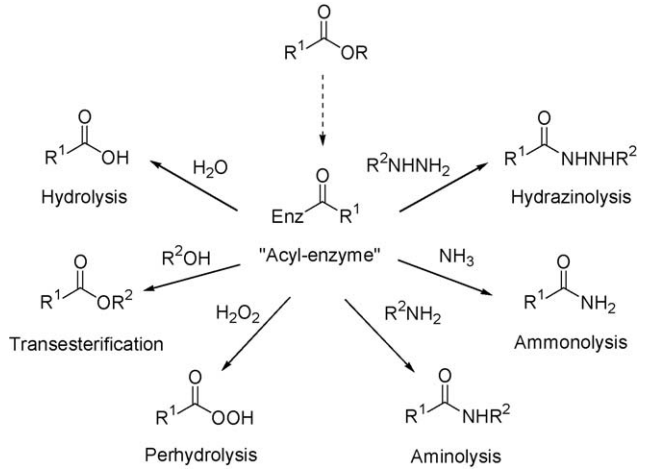
product with high yield; (b) it is possible to use non-polar substrates; (c) avoids side reactions; (d) in many cases the lipase is thermodynamically more active; (e) shifting thermodynamic equilibrium to favour synthesis over hydrolysis [9].

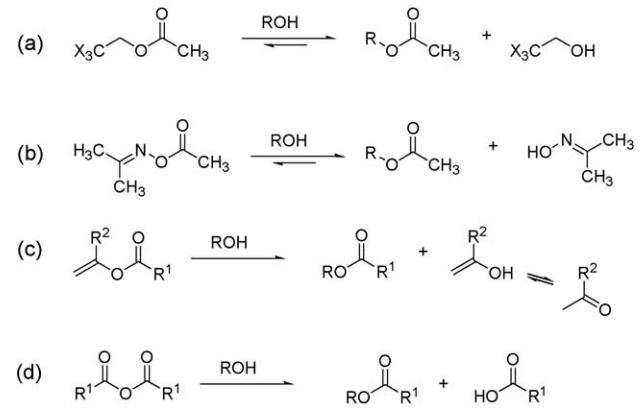
Biocatalysis in non-aqueous media has been widely used for the resolution of alcohols, acids or lactones through enzymatic transesterificacion reactions using different lipases [10]. Moreover, other processes such as the enzymatic acylation of amines or ammonia, have shown themselves to be of great utility for the resolution of amines and the preparation of chiral amides [11,12]. The mechanism for these processes is known as the serine-hydrolase mechanism, which is common for the hydrolysis of esters, and also for enzymatic processes of transesterification, aminolysis, ammonolysis, hydrazinolysis and also perhydrolysis of esters, in which the natural nucleophile water is replaced by an alcohol, amine, ammonia, hydrazine or hydroperoxide (Scheme 1).

The acyl donor more appropriate for each process depends on the nucleophile used; to carry out the resolution of alcohols, activated esters are the most adequate reagents to avoid the reversibility of the process, however, for the acylation of amines, which are more nucleophilic than alcohols, these acyl donors cannot be employed as they react in absence of the lipase, and non-activated esters must be used. The most common reagents for the resolution of alcohols [13] are, halogen-ethyl or methyl esters, oxime ester, anhydrides and especially vinyl esters (Scheme 2). In some cases, thioesters [14] and 1-ethoxyvinyl esters [15] have also been successfully used. Meanwhile vinyl acetate (VA) is the most common acyl reagent for the acylation of secondary alcohols, ethyl acetate is the most used for KR of amines. Both reagents can act in many examples as acyl donors and solvents.

Although enzymatic acylation reaction has been the process most exhaustively used in organic synthesis, enzymatic alkoxycarbonylation reactions in organic solvents have been scarcely reported, however, they have a great value for the activation and protection of hydroxyl or amino groups. Benzyl or allyl carbonates together with oxime or vinyl carbonates are the best alkoxycarbonylation reagents [16].



Scheme 1. Biotransformations of an ester in organic solvents.



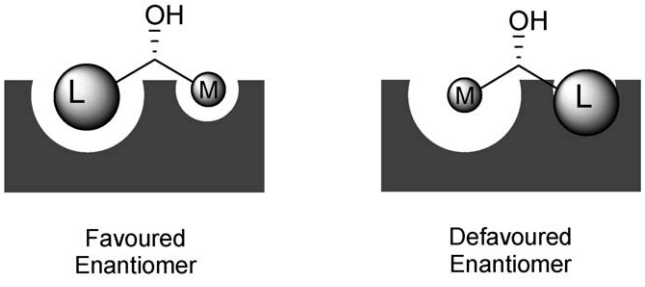
Scheme 2. Common reagents for the enzymatic resolution of alcohols.

3. Resolution of alcohols

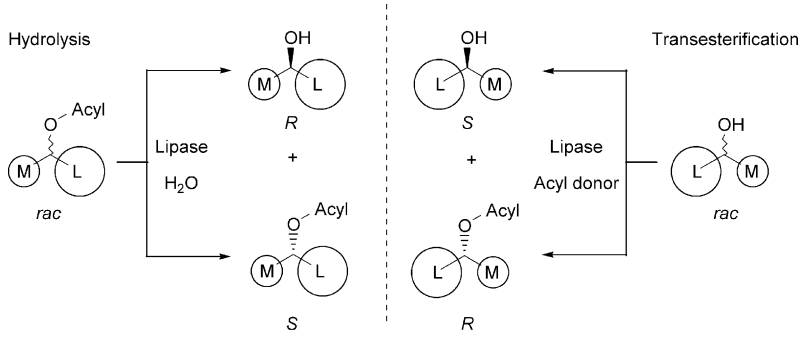
Lipases usually show much higher enantioselectivity in KR with secondary alcohols than primary or tertiary alcohols. Kazlauskas' rule predicts which enatiomer reacts faster in the acylation of secondary racemic alcohols [17]. The model is represented in Scheme 3, if the group of bigger size (L) has priority again the medium (M) according to Prelog's rule the (*R*)-alcohol reacts to yield the corresponding ester. The same stereopreference is observed for the hydrolysis of esters, in this case the (*R*)-alcohol (product) is always obtained.

There are many examples to prepare enantiomerically pure secondary alcohols, structure that contains many chiral drugs or intermediates for the synthesis of pharmaceuticals, their resolution can be carried out by acylation of alcohols or hydrolysis of esters using lipases in organic solvents. Enzymatic hydrolysis and transesterification reactions can be complementary processes for the resolution of secondary alcohols. In Scheme 4 is shown the symmetry of both processes according to Kazlauskas' rule.

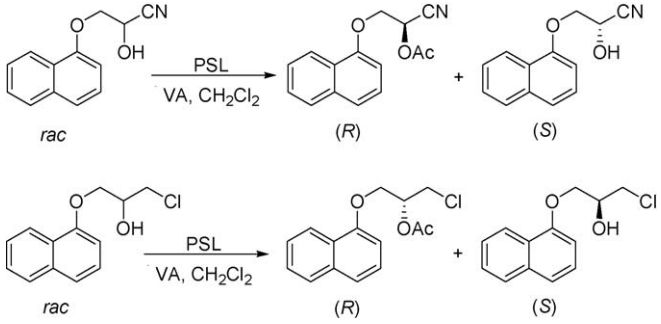
Due to the vastness of the bibliographic material related to the resolution of secondary alcohols by enzymatic hydrolysis from the acylated derivatives or by transesterification processes with different acyl donors, we have summarized some representative examples, choosing for instance, the resolution of some bifunctional compounds, which have been synthesized for the preparation of β-adrenergic blocking agents with structure of 1,2-aminoalcohols. Thus, the resolution through enzymatic acylation of halogenalcohols [18] or cianoalcohols [19]



Scheme 3. Kazlauskas' rule for resolution of secondary alcohols.



Scheme 4. Symmetry of enzymatic hydrolysis and transesterification reactions according to Kazlauskas' rule.

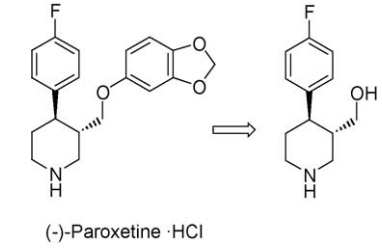


Scheme 5. Resolution of alcohol intermediates in the preparation of (*S*)-propranolol.

using *Pseudomonas cepacia* lipase (PSL), and more recently a dynamic kinetic resolution of azidoalcohols [20] have been reported towards the synthesis of (*S*)-propranolol. The general strategy for the resolution of these alcohols by enzymatic acylation is shown in Scheme 5.

Recently, an elegant strategy to carry out an enantioselective synthesis of chiral β-azidoalcohols via the reduction of the corresponding ketoazides with $NaBH_4$ in the presence of moist aluminium oxide followed by an in situ lipase-mediated resolution has been described [21] (Scheme 6).

Enantiomerically pure 2-methoxy-2-phenylethanol (Scheme 7) has been reported as a useful chiral auxiliary

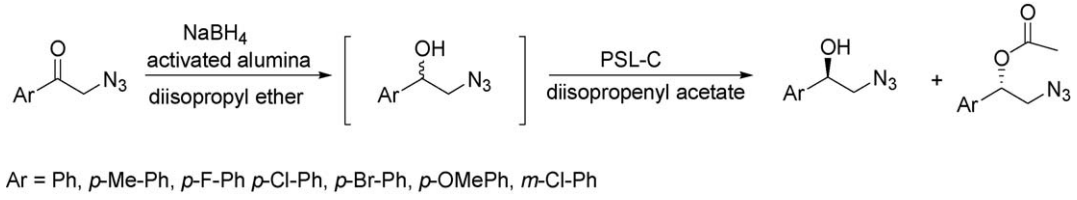


Scheme 8. Retrosynthetic pathway for the synthesis of optically pure (−)-Paroxetine.

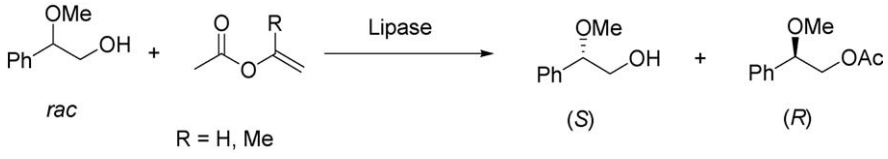
in the synthesis of optically pure 1,4-dihydropyridine derivatives, compounds of great interest as calcium antagonists and which preparation and resolution are one of the most important targets in the pharmaceutical industry. The resolution of this primary alcohol can be carried out by enzymatic acylation with different lipases. *Candida antarctica* lipase B (CAL-B) was the most efficient biocatalyst, achieving a *E* of 47 [22].

(−)-Paroxetine hydrochloride is a selective serotonin (5-HT) reuptake inhibitor that is used as an antidepressant. The chemoenzymatic synthesis can be carried out through the piperidine intermediate shown in Scheme 8.

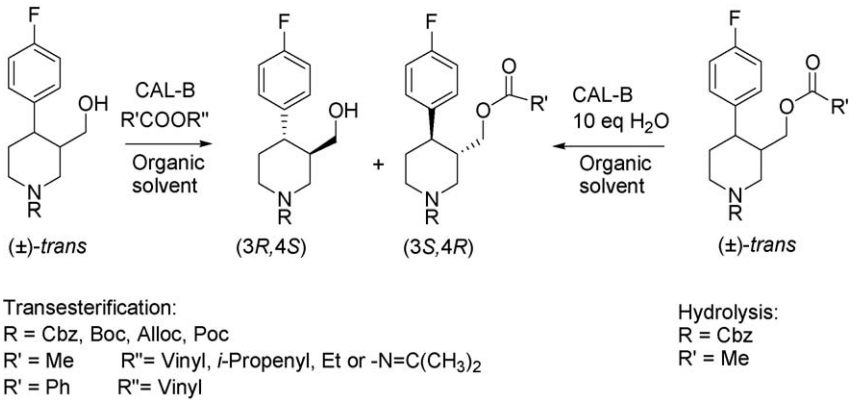
In principle, there are two possible approaches for the preparation of the (−)-Paroxetine alcohol intermediate, the enzymatic hydrolysis of the corresponding ester derivative or the enzymatic acylation of the primary alcohol (Scheme 9). We have



Scheme 6. Chemoenzymatic synthesis of chiral β-azidoalcohols.



Scheme 7. Lipase-catalyzed resolution of 2-methoxy-2-phenylethanol.



Scheme 9. Lipase-catalyzed preparation of the enantiopure (−)-Paroxetine precursor.

investigated both processes with several hydrolases and different reaction conditions [23]. The best results are obtained in the acylation catalyzed by the two *Candida antarctica* lipases CAL-A and CAL-B. In both cases good yields and high enantioselectivities ($E > 100$) can be achieved by an appropriate selection of the reaction parameters. It is remarkable the opposite stereochemical preference of the two *Candida antarctica* lipases in these processes. Since CAL-A catalyzes the acylation of the (3*S*,4*R*) alcohol, CAL-B prefers the (3*R*,4*S*) enantiomer. In the last case, the remaining alcohol posses the correct absolute configuration (3*S*,4*R*) for the synthesis of (−)-Paroxetine.

We have also studied the enzymatic hydrolysis of acetylated derivative. In most cases low enantioselectivities were observed except in the reaction catalyzed by CAL-A in hexane ($E = 68$).

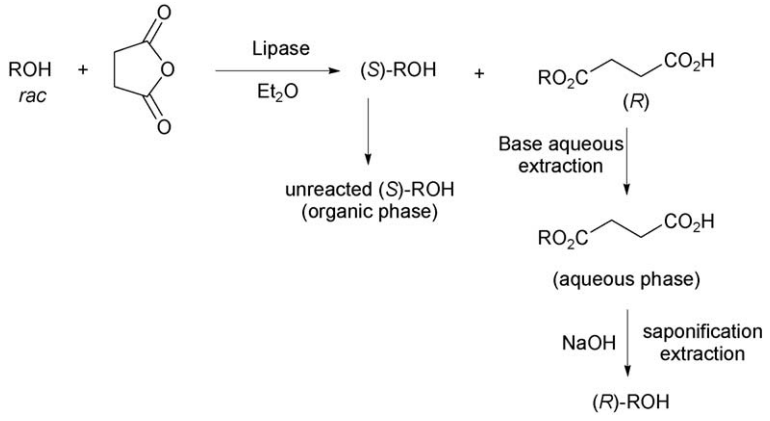
In some cases the use of vinyl or alkyl esters, as acyl donors, has the drawback of the separation of the ester (product) and the alcohol (substrate). A practical strategy to avoid this problem is the use of cyclic anhydrides [24]. In this case an acid is obtained as product, which can be readily separated of the unreacted alcohol by a simple aqueous base–organic solvent liquid–liquid extraction (Scheme 10).

This strategy has also been applied for the synthesis of (−)-Paroxetine [25]. The best results are obtained with CAL-B and glutaric anhydride in toluene (Scheme 11). In addition, the reuse of the immobilized lipase afforded the same enantioselectivity in the second and third cycles, a moderate loss of enzyme activity is observed in the fourth and fifth cycles.

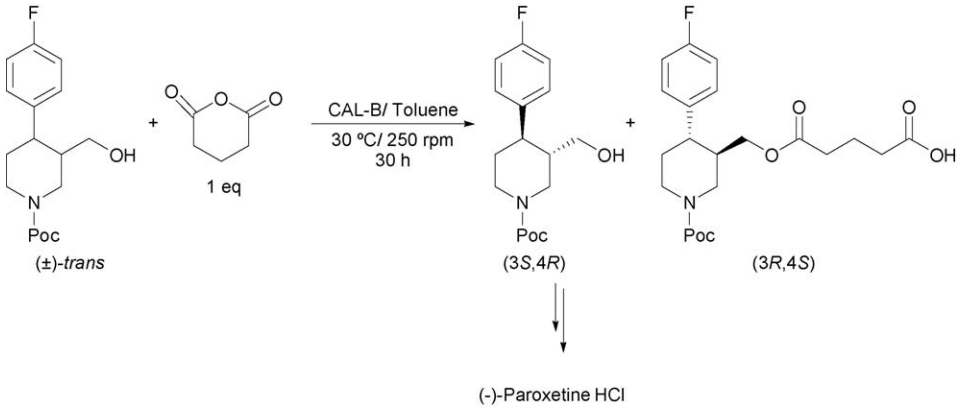
Citalopram, as Paroxetine, is a very selective inhibitor of serotonin and also is an efficient human antidepressant. It has been demonstrated that almost the entire inhibition activity resides in the (*S*)-(+)-enantiomer [26]. To obtain enantiomerically pure Citalopram a suitable strategy is the resolution of the corresponding cyanodiol, which presents a primary and a tertiary hydroxyl groups (Scheme 12). Although, the resolution of primary alcohols is easy by enzymatic acylation, few examples have been described about resolution of tertiary alcohols [27].

The enzymatic resolution of 4-[(4-dimethylamino)-1-(4′-fluorophenyl)-1-hydroxy-1-butyl]-3-(hydroxymethyl)benzonitrile, useful intermediate for the synthesis of optically pure Citalopram, has been investigated using several lipases and different acyl donors in different reaction conditions [28]. Best results are observed with CAL-B, vinyl acetate and acetonitrile ($E = 70$). It is remarkable that CAL-B catalyzes the enzymatic acetylation of the primary benzylic alcohol with high enantioselectivity at the quaternary quiral center (Scheme 13).

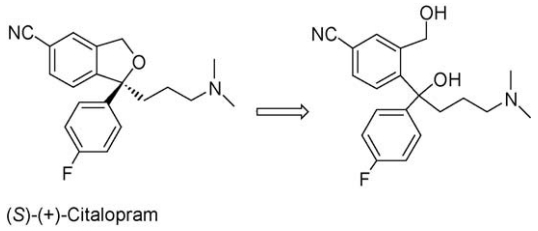
(±)-Zopiclone is a chiral cyclopyrrolone with hypnotic properties, possessing a pharmaceutical profile of high efficacy



Scheme 10. General work up procedure in enzymatic acylations with cyclic anhydrides.



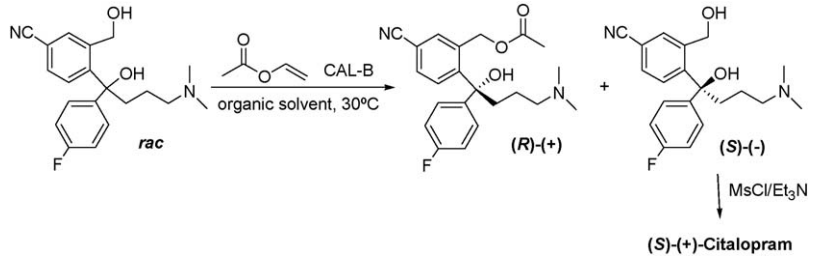
Scheme 11. Cyclic anhydrides in the chemoenzymatic synthesis of (−)-Paroxetine.



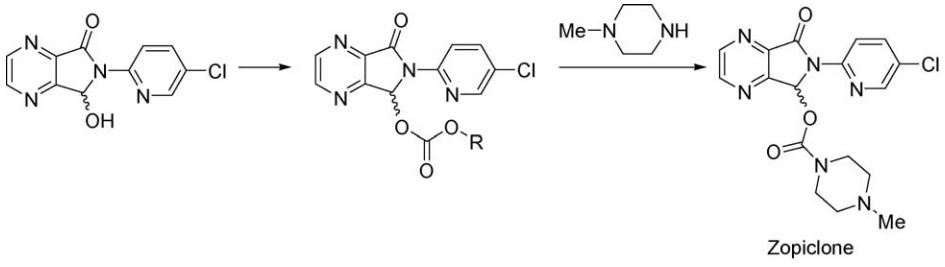
Scheme 12. Retrosynthetic pathway of the synthesis of (*S*)-(+)-Citalopram.

and low toxicity, similar to that of benzodiazepines. Zopiclone has been commercialized as a racemic mixture, however, the (*S*)-enantiomer is more active and less toxic than the (*R*)-enantiomer [29]. Racemic Zopiclone is currently produced as outlined in Scheme 14, where the final step is the reaction of *N*-methylpiperazine with the phenyl carbonate.

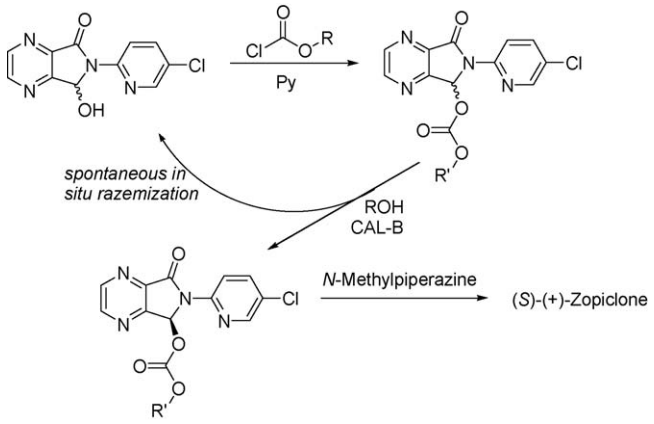
Although, as we have commented, enzymatic hydrolysis or transesterification processes have been widely applied for enzymatic resolution or desymmetrization processes, the enzymatic hydrolysis of carbonates or alkoxycarbonylation of alcohols has been scarcely reported. However, to carry out the synthesis of enantiomerically Zopiclone is more practical to obtain the corresponding carbonate intermediate for the synthesis of the drug. We have studied both processes and better results are achieved by hydrolysis or transcarbonatation of the carbonate than when the direct enzymatic alkoxycarbonylation is carried out (Scheme 15). We have resolved the vinyl carbonate intermediate by lipase-catalyzed hydrolysis or transcarbonatation [30]. The (*R*)-alcohol, product of the enzymatic reaction suffers a spontaneous racemization in the reaction medium. So, it can be directly recycled just after work-up of the enzymatic reaction. Thus, the overall formal yield of the enzymatic process is 100%, even though this enzymatic step is a kinetic resolution. However, with the aim to obtain better yields in the last step of the synthesis of (*S*)-(+)-Zopiclone (only a 30% yield was obtained in the treatment of the vinyl carbonate with *N*-methylpiperazine), the enzymatic hydrolysis in organic solvents of several carbonates has been studied [31]. The optimal process was reached when



Scheme 13. Chemoenzymatic synthesis of (*S*)-(+)-Citalopram.



Scheme 14. Last steps in the industrial preparation of Zopiclone.



Scheme 15. Chemoenzymatic preparation of (*S*)-(+)-Zopiclone.

the less expensive chloromethylchloroformate is used as starting material to prepare the corresponding carbonate (R=CH_2Cl). In this case the last step takes place with an isolated yield higher than 90%.

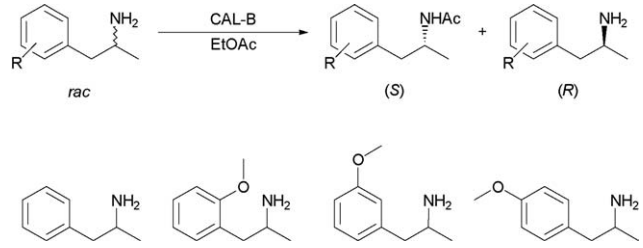
From the data reported on the literature, it seems that better enantioselectivities are achieved by resolution of secondary alcohols, but we have demonstrated that intermediates from pharmaceuticals with structure of primary alcohol are also of utility because high or moderated enantioselectivities can also be achieved.

4. Resolution of amines

Amines and their derived amides are important compounds in organic synthesis, because of the presence of these functions in many pharmacologically active compounds. Due to the increasing demand of optically active compounds in the pharmaceutical industry, the design of efficient methods for the preparation of optically active amines is of special interest. Biocatalysis offers a clean and ecological way to perform chemical processes, in mild reaction conditions and with high degree of selectivity. CAL-B has proven to be the most effective catalyst for the enzymatic aminolysis reaction in organic solvents, allowing the preparation of a variety of optically active primary amines and amides [11,12].

Normally ethyl acetate is used for the acylation of amines, in many cases as acyl donor and solvent. Other acylating agents such alkyl methoxy acetates are also useful, however, vinyl esters, the best reagents for the resolution of alcohols, are not adequate reagents for the resolution of primary amines due to their high reactivity.

The enzymatic acylation of amines has been used for the preparation of pharmacologically interesting β-substituted isopropylamines (Scheme 16). The presence of methyl groups on the nitrogen atom or methoxy groups on the aromatic ring is known to increase the effects of the drug. In addition, the stereochemistry of these amines has a great influence on their pharmacological properties. Both enantiomers of amphetamine and the isomeric *o*-, *m*-, and *p*-methoxyamphetamines have been prepared with very high ee by CAL-B catalyzed resolution of the corresponding racemic amines using ethyl acetate as acyl



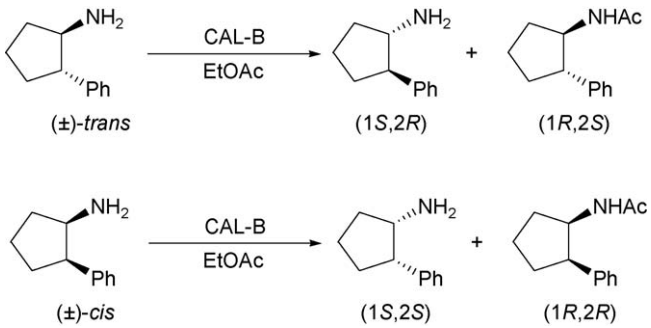
Scheme 16. Enzymatic kinetic resolution of β-substituted isopropylamines.

donor and solvent [32]. The unreacted amines and the converted acetamides are easily separated by selective extraction.

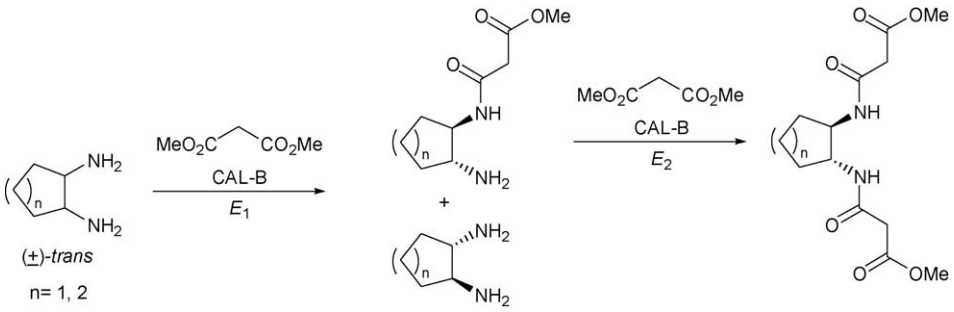
Recently, we have reported the resolution of (±)-*trans*- and (±)-*cis*-2-phenylcyclopentanamine by CAL-B catalyzed aminolysis of a variety of esters [33], the *trans* isomer, cypenamine, is an antidepressant, and both isomers are precursors of semicyclic amidines (lactamimides) with potent hypoglycemic activity. Whereas reaction between (±)-*trans*-2-phenylcyclopentanamine and ethyl acetate proceeds with very high *E* (>200) and conversion (50%), the corresponding acetylation of (±)-*cis*-2-phenylcyclopentanamine occurs with low *E* (16) and conversion (28%). Nevertheless, this problem is overcome using other acyl donors such as (±)-1-phenylethyl and (±)-*cis*-2-phenylcyclopentyl methoxyacetates (Scheme 17).

Cyclic 1,2-diamines are an important class of compounds, especially because of their usefulness as chiral auxiliaries in asymmetric synthesis and because they are intermediates of compounds with pharmaceutical interest. We have studied a sequential biocatalytic resolution by *one-pot* double aminolysis, and we have achieved the resolution of cyclic 1,2-diamines obtaining the final diacylated products in enantiopure forms from *trans*-cyclohexane-1,2-diamine [34] and *trans*-cyclopentane-1,2-diamine [35]. Additionally a C_2-symmetry fluorosensor has been developed and employed to analyze in real-time the enzymatic kinetic resolution of *trans*-cyclohexane-1,2-diamine, eliminating cumbersome purification and derivatization steps required by traditional methods [36].

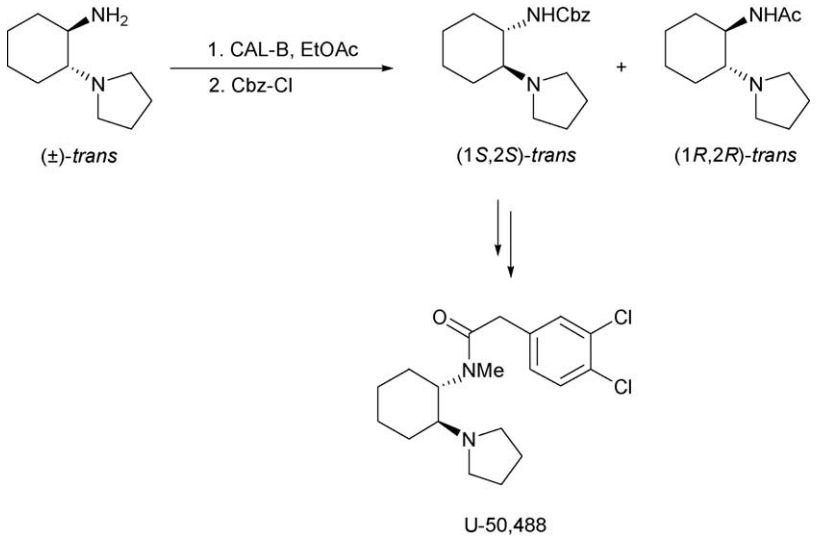
The sequential resolution is carried out with dimethyl malonate and using CAL-B as biocatalyst (Scheme 18). The formation of the (*R*,*R*)-bisamidoester involves two biocatalytic steps; the enzyme shows the same stereochemical preference towards the (*R*,*R*)-enantiomer of the substrate in both steps. Careful study of the enantiomeric ratio for each step, allowed us to suggest an interesting structural effect. The enantioselectivity of the sec-



Scheme 17. Enzymatic kinetic resolution of 2-phenylcyclopentanamine.



Scheme 18. Kinetic resolution of *trans*-cycloalcane-1,2-diamine.

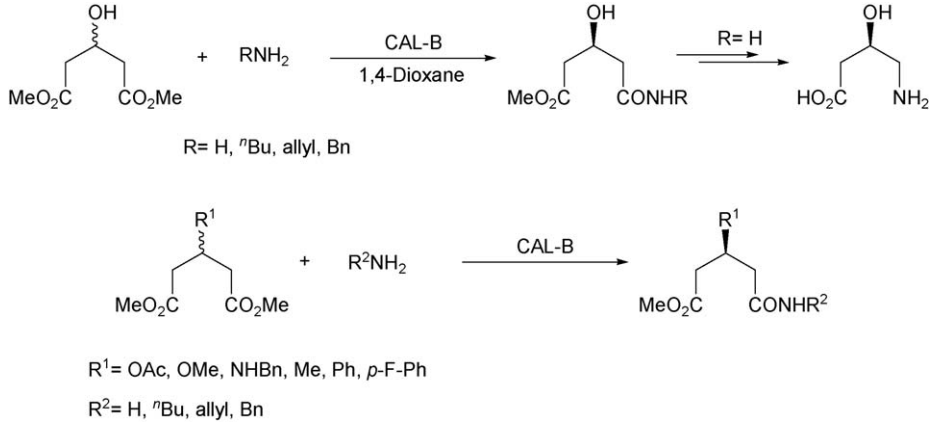


Scheme 19. Chemoenzymatic approach for the synthesis of the analgesic U-50,488.

ond step is always higher than the first one in agreement with Kazlauskas' rule, because the monoacylated compound has a bigger steric difference in the substituents of the sterocenter than the free diamine.

Compound U-50,488 (Scheme 19) and other structural analogues have been reported to be highly selective κ-opioid agonists, free from the adverse side effects of μ-agonists like morphine. The preparation of this drug is easily obtained starting from cyclohexene oxide [37], where the key step is the resolution of the intermediate (±)-*trans*-2-(pyrrolidin-1-yl)cyclohexanamine using CAL-B as biocatalyst (Scheme 19). In the enzymatic process CAL-B shows very high enantioselectivity preferentially catalyzing the acetylation of the (1*R*,2*R*) isomer according to Kazlauskas' rule.

There are many examples of enzymatic desymmetrization of prochiral esters, by enzymatic hydrolysis, however, the desymmetrization by enzymatic aminolysis has been scarcely explored. Of interest is the aminolysis of dimethyl 3-substituted



Scheme 20. Enzymatic desymmetrization of dimethyl 3-substituted glutarates.

glutarates with amines and ammonia in the presence of CAL-B (Scheme 20). In these processes the yields and the enantioslectivity obtained depends of the substituent in the three position, however, the (*S*)-isomer is always obtained [38]. The amidoesters prepared are valuable intermediates for the synthesis of compounds of pharmaceutical interest, for instance, the enantiopure monoamide from ammonia and 3-hydroxyglutarate has been used to prepare a biologically interesting β-amino acid such as (*R*)-3-hydroxy-4-aminobutanoic acid [(*R*)-GABOB]. Others of these enantiopure monoamides have been used to prepare many biologically interesting β-amino acids [39].

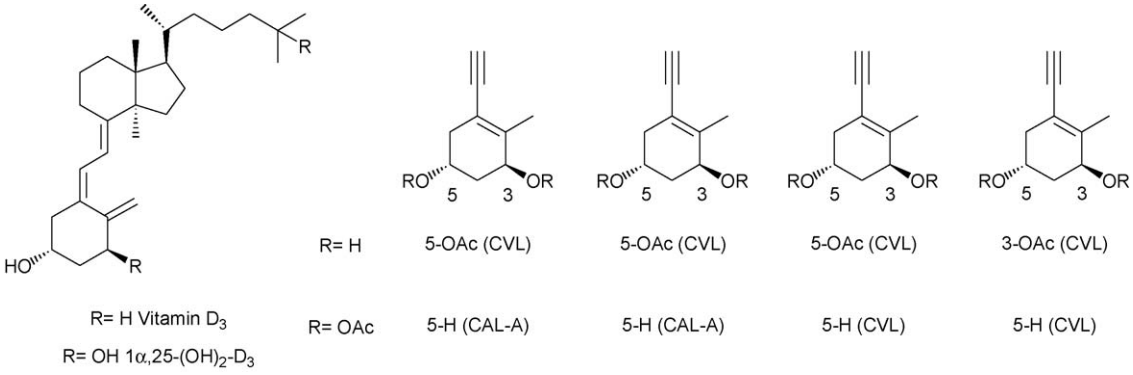
5. Regioselective transformations

Selective biotransformations of polyhydroxylated compounds have been used for the activation or protection of some of their hydroxyl groups. For instance, regioselective enzymatic transesterification or alkoxycarbonylation in the chemoenzymatic transformation of natural products, allow to avoid tedious protection and deprotection steps. We show here some applications of these enzymatic processes that we have carried out in our laboratory with the aim of prepare new derivatives of Vitamin D and nucleosides of pharmaceutical interest.

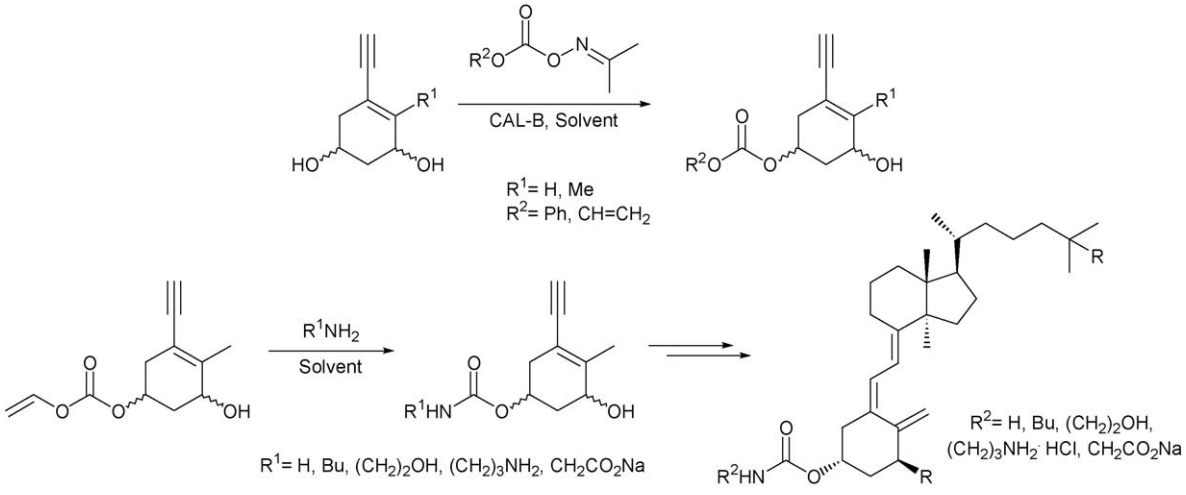
We have studied the enzymatic regioselective transformation of the four different isomers of the A-ring synthon of 1α,25-dihydrovitamin D_3 (Scheme 21), the active metabolite of Vitamin D_3, which can promote cell differentiation, inhibit cell proliferation, and show immunosuppressive activity in addition to its classical calciotropic effects [40,41].

In Scheme 21 are shown the results obtained in the enzymatic acetylation and its complementary hydrolysis to reach the formation of the eight possible monoacetylated stereoisomers of an A-ring synthon of the 1α,25-$(OH)_2$-D_3 [42,43].

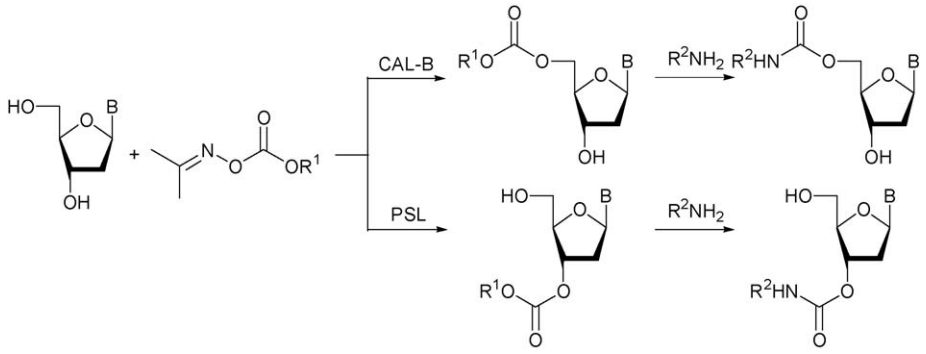
However, the introduction of an alkoxycarbonyl group through an enzymatic alkoxycarbonylation has advantages due to the selective protection or activation of the hydroxyl groups is achieved so later it is possible to introduce different functional groups in the molecule (Scheme 22). It has been investigated the enzymatic alkoxycarbonylation with all four A-ring diol isomers using different alkoxycarbonylation agents, being the most effective oxime carbonates. We have also studied the enzymatic alkoxycarbonylation with the four stereoisomers of the A-ring of the corresponding previtamins [44,45]. With oxime vinyl carbonate the regioselectivities are not very good, however, better results can be achieved when the alkoxycarbonylation agent is phenyloxime carbonate. As occurs with the diacetylated compounds, the enzymatic hydrolysis of the correspond-



Scheme 21. Enzymatic regioselective acylation or hydrolysis of the stereoisomers of an A-ring synthon of 1α,25-dihydrovitamin D_3.



Scheme 22. Chemoenzymatic synthesis of carbamate derivatives of (1α,25)-$(OH)_2$-D_3.



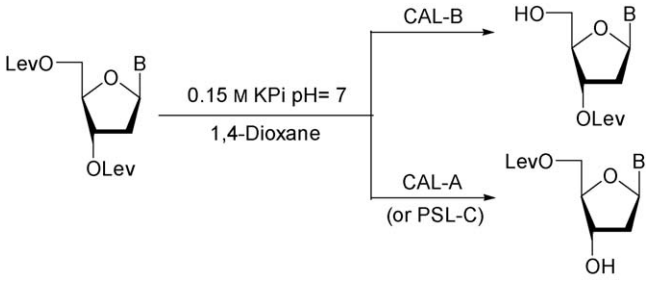
Scheme 23. Regioselective enzymatic alcoxycarbonylation of 2′-deoxynucleosides.

ing dicarbonate yields the opposite regioisomer respect to the alkoxycarbonylation reaction [46].

The introduction of a vinylalkoxycarbonyl group allows us the preparation of new derivatives, such as carbamates, by reaction of the corresponding carbonate obtained by enzymatic alkoxycarbonylation reaction with amines or ammonia in absence of biocatalyst [47]. In Scheme 22 it is showed that the combination between chemical catalysis and biocatalysis can be an excellent strategy to achieve new analogues of Vitamin D_3.

Deoxynucleosides are compounds of special interest in medicinal chemistry as they have important applications because of their antiviral and antitumoral properties. Application of biocatalysis over these compounds has acquired great importance for the preparation of new derivatives with interesting pharmacological activity. Two lipases, CAL-B and PSL, are selective towards one of the two hydroxyl groups of different 2′-deoxynucleosides (Scheme 23). Thus, it is possible to prepare the acylated or the carbonate compounds in 5′-position with CAL-B, meanwhile PSL is selective towards the secondary hydroxyl group. The introduction of an alkoxycarbonyl group, by enzymatic carbonation allows the preparation of new carbamate derivatives, compounds of physiological interest [48].

According with the behaviour of these two lipases, a short and convenient synthesis of 3′- and 5′-*O*-levulinyl-2′-deoxynucleosides has been developed from the corresponding 3′,5′-di-*O*-levulinyl derivatives by regioselective hydrolysis [49], avoiding several tedious chemical protection/deprotection steps. As a result, these key building blocks needed for solution-phase for the synthesis of oligonucleotides *antisense*, can be prepared with high yields (Scheme 24). These transformations



Scheme 24. Enzymatic hydrolysis of 2′-deoxynucleosides carbonate derivatives.

can be also carry out by enzymatic acylation showing both processes the symmetry commented [50].

6. Concluding remarks

Nowadays, lipases are used in the pharmaceutical industry as a routine tool for the preparation of chiral drugs. The possibility of carry out these enzymatic processes under mild conditions and their high selectivity, make lipases very attractive catalysts to perform some transformations which are difficult to achieve by chemical procedures. We have shown here the versatility of these biocatalysts when are used in organic solvents for the preparation of a great variety of pharmaceutical intermediates. In the last time, directed evolution of lipases allows to obtain new biocatalysts, which will broad new possibilities in the industry, specially in the pharmaceutical sector.

References

[1] A.L. Margolin, Enzyme Microb. Technol. 15 (1993) 266–280.
[2] R.N. Patel, Curr. Opin. Drug Disc. Dev. 6 (2003) 902–920.
[3] V. Gotor, Org. Proc. Res. Dev. 6 (2002) 420–426.
[4] A. Ghanem, H.Y. Aboul-Enein, Tetrahedron: Asymmetry 15 (2004) 3331–3351.
[5] E. García-Urdiales, I. Alfonso, V. Gotor, Chem. Rev. 105 (2005) 313–354.
[6] A.J.J. Straathof, S. Panke, A. Schmid, Curr. Opin. Biotechnol. 13 (2002) 548–556.
[7] H. Pellissier, Tetrahedron 59 (2003) 8291–8327.
[8] O. Pàmies, J.-E. Bäckvall, Chem. Rev. 103 (2003) 3247–3262.
[9] G. Carrea, S. Riva, Angew. Chem. Int. Ed. 39 (2000) 226–2254.
[10] A.M. Klibanov, Nature 409 (2001) 241–246.
[11] I. Alfonso, V. Gotor, Chem. Soc. Rev. 33 (2004) 201–209.
[12] F. van Rantwijk, R.A. Sheldon, Tetrahedron 60 (2004) 501–519.
[13] K. Faber, Biotransformations in Organic Chemistry, Springer-Verlag, Heidelberg, 2004.
[14] H. Frykman, N. Öhrner, T. Norin, K. Hult, Tetrahedron Lett. 34 (1993) 1367–1370.
[15] Y. Kita, Y. Takebe, K. Murata, T. Naka, S. Akai, Tetrahedron Lett. 37 (1996) 7369–7372.
[16] S. Takayama, S.T. Lee, S.-C. Hung, C.-H. Wong, Chem. Commun. (1999) 127–128.
[17] R.J. Kazlauskas, A.N.E. Weissfloch, A.T. Rappaport, L.A. Cuccia, J. Org. Chem. 56 (1991) 2656–2665.
[18] Y.-F. Wang, S.T. Chen, K.-C. Liu, Tetrahedron Lett. 30 (1989) 1917–1920.
[19] H.S. Bevinakatti, A.A. Banerji, J. Org. Chem. 56 (1991) 5372–5375.
[20] O. Pàmies, J.-E. Bäckvall, J. Org. Chem. 66 (2001) 4022–4025.

[21] A. Kamal, A.A. Shaik, M. Sandbhor, M.S. Malik, Tetrahedron: Asymmetry 15 (2004) 935–939.
[22] M. Monterde, R. Brieva, V. Gotor, Tetrahedron: Asymmetry 13 (2002) 1091–1096.
[23] G. de Gonzalo, R. Brieva, V.M. Sánchez, M. Bayod, V. Gotor, J. Org. Chem. 66 (2001) 8947–8953.
[24] N. Bouzemi, H. Debbeche, L. Aribi-Zouiou, J.-C. Fiaud, Tetrahedron Lett. 45 (2004) 627–630.
[25] G. de Gonzalo, R. Brieva, V.M. Sánchez, M. Bayod, V. Gotor, J. Org. Chem. 68 (2003) 3333–3336.
[26] K.P. Boegeso, EP Patent 0347066 (1989);
K.P. Boegeso, Chem. Abstr. 113 (1990) 78150.
[27] E. Henke, J. Pleiss, U.T. Bornscheuer, Angew. Chem. Int. Ed. 41 (2002) 3211–3213.
[28] L. Fernández-Solares, R. Brieva, M. Quirós, I. Llorente, M. Bayod, V. Gotor, Tetrahedron: Asymmetry 15 (2004) 341–345.
[29] G. Blaschke, G. Hempel, W. Muller, Chirality 5 (1993) 419–421.
[30] V. Gotor, F. Limeres, R. García, M. Bayod, R. Brieva, Tetrahedron: Asymmetry 8 (1997) 995–997.
[31] L. Fernández-Solares, M. Díaz, R. Brieva, V.M. Sánchez, M. Bayod, V. Gotor, Tetrahedron: Asymmetry 13 (2002) 2577–2582.
[32] J. González-Sabín, V. Gotor, F. Rebolledo, Tetrahedron: Asymmetry 12 (2002) 1315–1320.
[33] J. González-Sabín, V. Gotor, F. Rebolledo, Tetrahedron: Asymmetry 14 (2004) 481–488.
[34] I. Alfonso, C. Astorga, F. Rebolledo, V. Gotor, Chem. Commun. (1996) 2471–2472.
[35] A. Luna, I. Alfonso, V. Gotor, Org. Lett. 4 (2002) 3627–3629.
[36] G.E. Tumambac, C. Wolf, Org. Lett. 7 (2005) 4045–4048.
[37] J. González-Sabín, V. Gotor, F. Rebolledo, Chem. Eur. J. 10 (2004) 5788–5794.
[38] S. Puertas, F. Rebolledo, V. Gotor, J. Org. Chem. 61 (1996) 6024–6027.
[39] M. López-García, I. Alfonso, V. Gotor, J. Org. Chem. 68 (2003) 648–651.
[40] R. Bouillon, W.H. Okamura, A.W. Norman, Endocrine Rev. 16 (1995) 200–257.
[41] S. Fernández, V. Gotor-Fernández, M. Ferrero, V. Gotor, Curr. Org. Chem. 6 (2002) 453–469.
[42] S. Fernández, M. Ferrero, V. Gotor, W.H. Okamura, J. Org. Chem. 60 (1995) 6057–6061.
[43] V. Gotor-Fernández, M. Ferrero, S. Fernández, V. Gotor, J. Org. Chem. 67 (2002) 1266–1270.
[44] V. Gotor-Fernández, M. Ferrero, S. Fernández, V. Gotor, J. Org. Chem. 64 (1999) 7504–7510.
[45] M. Díaz, V. Gotor-Fernández, M. Ferrero, S. Fernández, V. Gotor, J. Org. Chem. 62 (2001) 4227–4232.
[46] D. Oves, V. Gotor-Fernández, S. Fernández, M. Ferrero, V. Gotor, Tetrahedron: Asymmetry 15 (2004) 2881–2887.
[47] V. Gotor-Fernández, S. Fernández, M. Ferrero, V. Gotor, R. Bouillon, A. Verstuyf, Biorg. Med. Chem. 12 (2004) 5443–5451.
[48] L.F. García-Alles, V. Gotor, Tetrahedron 51 (1995) 307–316.
[49] J. García, S. Fernández, M. Ferrero, Y.S. Sanghvi, V. Gotor, J. Org. Chem. 67 (2002) 4513–4519.
[50] J. García, S. Fernández, M. Ferrero, Y.S. Sanghvi, V. Gotor, Tetrahedron: Asymmetry 14 (2003) 3533–3540.