ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 19–20 (2002) 21–30

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

# Biocatalysis applied to chemoselective transformations on Vitamin D and nucleoside derivatives

Vicente Gotor*

*Departamento de Química Orgánica e Inorgánica, Facultad de Química, Universidad de Oviedo, 33006 Oviedo, Spain*

Received 19 October 2001; received in revised form 6 May 2002; accepted 20 May 2002

**Abstract**

Vitamin $D_3$ and 2′-deoxynucleosides are compounds of special relevance in some areas of medicinal chemistry. On the other hand, some derivatives of these compounds have shown new and important physiological properties. Biotransformations are a tool of great utility to carry out selective modifications on these compounds in very mild conditions. In this account, we report the preparation of new analogues of Vitamin D and deoxynucleosides derivatives through a combination between biocatalysis and chemical catalysis. The key step is the regioselective enzymatic acylation, and specially alkoxycarbonylation using lipases in organic solvents.
© 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Lipases; Acylation; Alkoxycarbonylation; Nucleosides; Vitamin D

## 1. Introduction

In the last decade enzyme-catalyzed reactions have been well recognized as an excellent strategy for the preparation of fine chemicals [1]. Hydrolytic enzymes are widely used in organic synthesis as environmentally friendly catalysts that possess broad substrate specificities, display high stereoselectivity, are commercially available, and do not require the use of cofactors [2]. Among these hydrolytic enzymes, lipases are of the greatest utility in organic synthesis. These biocatalysts have been exploited for asymmetric synthesis transformations, fuelled by the growing demand for enantiopure pharmaceuticals. Furthermore, lipase-catalyzed reactions are normally carried out under mild conditions, such as room temperature and can be used in organic solvents.

Over the last few years, biocatalysis in non-aqueous media has been widely used for the resolution of alcohols, acids or lactones through enzymatic transesterificacion reactions using hydrolytic enzymes, especially lipases [3]. Recently, other processes, such as the enzymatic acylation of amines or alkoxycarbonylation of alcohols and amines, have shown themselves to be of great utility for the resolution of amines and the preparation of chiral amides, carbonates or carbamates [4].

Nowadays, for many organic chemists, biotransformation is an area of great interest for the preparation of products, which are difficult to obtain by conventional chemical methods [5]. It is recognized that lipases are capable of accepting a wide array of substrates, and catalyze enantio-, chemo- and regioselective reactions.

Selective biotransformations of polyhydroxylated compounds, such as carbohydrates, have been used for

* Tel.: +34-98-510-3448; fax: +34-98-510-3448.
*E-mail address:* vgs@sauron.quimica.uniovi.es (V. Gotor).

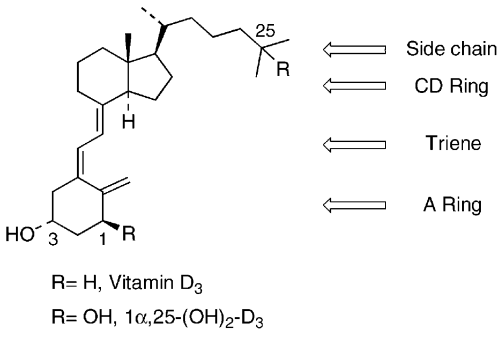
1381-1177/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S1381-1177(02)00148-0

the activation or protection of some of their hydroxyl groups [6] allowing different chemical transformations to be carried out without the need for tedious protection and deprotection steps. Monoesters of some monosaccharides and disaccharides have been obtained using lipases or proteases in polar organic solvents. An interesting transformation in the carbohydrate field is regioselective alkoxycarbonylation, and in particular enzymatic alkoxycarbonylation using oxime carbonates as alkoxycarbonylation agents [7]. This process allows the regioselective acylation of secondary hydroxyl groups through the corresponding enzymatic alkoxycarbonylation reaction [8].

More recently, biotransformations have also shown their utility for other kinds of natural products, such as steroids [9] and nucleosides [10], whilst here we shown some applications of enzymatic acylations and alkoxycarbonylations that we have carried out in our laboratory with the aim of prepare new derivatives of Vitamin D and nucleosides.

## 2. Chemoenzymatic transformations on the A-ring synthon of Vitamin $D_3$ derivatives

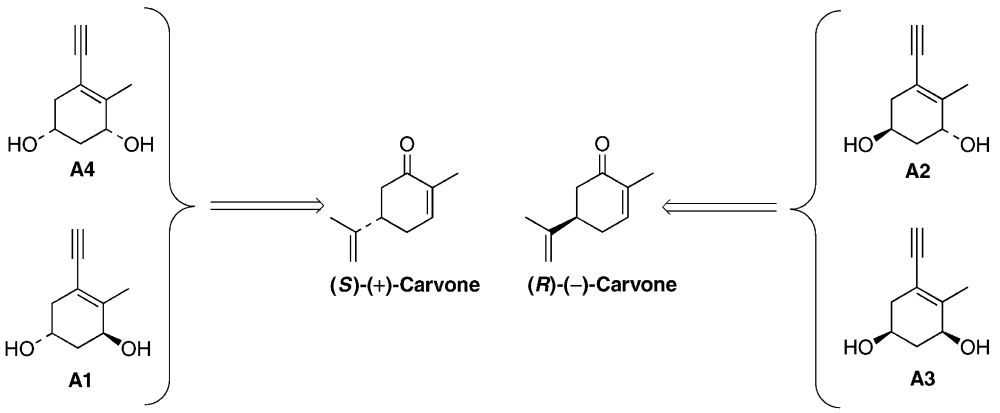
Vitamin $D_3$ through its hormonally active form 1α, 25-dihydrovitamin $D_3$ plays an important role in the endocrine system, and this metabolite exhibits a much broader biological spectrum than the expected [11]. The structure of Vitamin $D_3$ can be divided in four parts (Scheme 1): (1) the side chain; (2) the CD ring; (3) the trienic part; and (4) the A ring. Different transformations have mainly been carried out on the side chain. However, chemical and, of course, biotransformations have been investigated much less in the A-ring synthon than in the other parts of the molecule. For this reason, we have worked in chemoenzymatic transformations on the A-ring synthon with the aim of regioselectively preparing different kinds of derivatives for the synthesis of new analogues of Vitamin D, as well as carrying out a biological evaluation of the new compounds obtained.



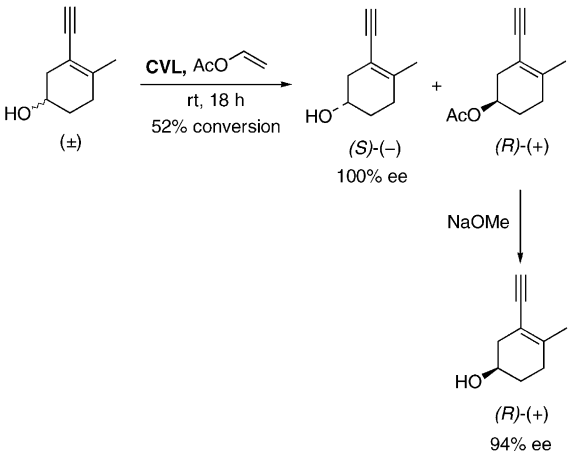

Scheme 1.

Starting from (*S*) carvone, it is possible to obtain the natural stereoisomer A1 (Scheme 2) and the corresponding diasteroisomer A4 through chemical transformations. On the other hand, if the starting material is the (*R*) carvone, the other two non-natural diastereoisomers A2 and A3 can be also prepared [12].

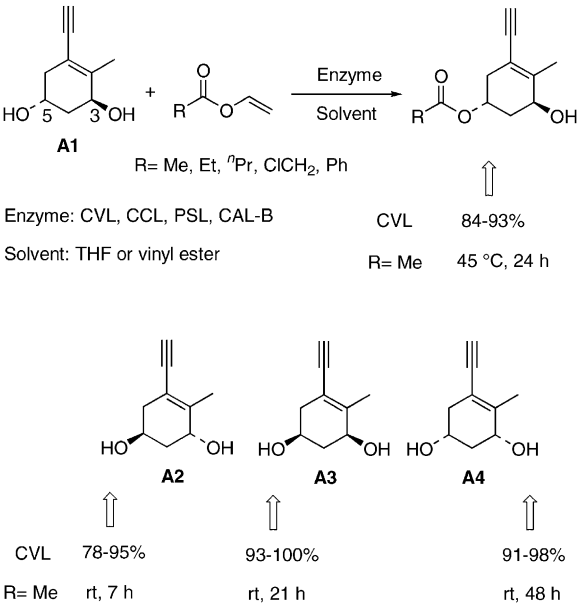
Before studying the enzymatic regioselective transformation on the different isomers of the A-ring synthon of 1α, 25-dihydrovitavin $D_3$, we considered it of




Scheme 2.




Scheme 3.

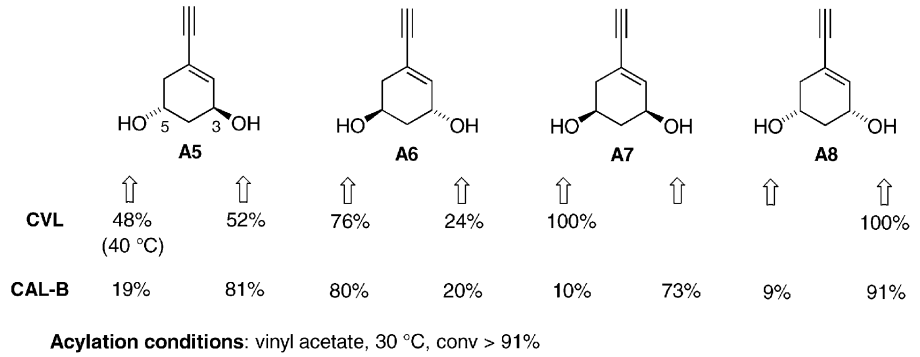



Scheme 4.

interest to carry out the enzymatic resolution of the A ring of Vitamin $D_3$, ring with only one hydroxyl group in the 5 position. The resolution was carried out using *Chromobacterium viscosum* lipase (CVL) with vinyl acetate acting as both acylating agent and solvent. As is shown in Scheme 3, the enzyme is selective towards the *R* isomer of the alcohol, obtaining the enantiopure substrate that is the natural isomer of Vitamin $D_3$ [13], with a conversion of 52%.

We started with regioselective enzymatic acylation, studying first the acylation of the natural stereoisomer with stereochemistry (3*S*, 5*R*) A1 using different enzymes with several vinyl esters (Scheme 4). The best biocatalyst was CVL, and this lipase showed a great selectivity towards the hydroxyl group at the 5 position. This process takes place faster with the enantiomer of A1, and also with total regioselectivity towards the same hydroxyl group. A good regioslectivity was also observed with the *cis* diastereoisomers, and it is of note that in the case of the *cis* isomer with 3*R*, 5*R* configuration (A4), the enzyme showed opposite regioselectivity [13].

Normally, enzymatic hydrolysis is a complementary reaction of the acylation reaction, and for this reason we considered of interest the study of the enzymatic hydrolysis of the corresponding diacylated stereoisomers, with the aim of obtaining the acylated compound with opposite regioselectivity than that achieved in the

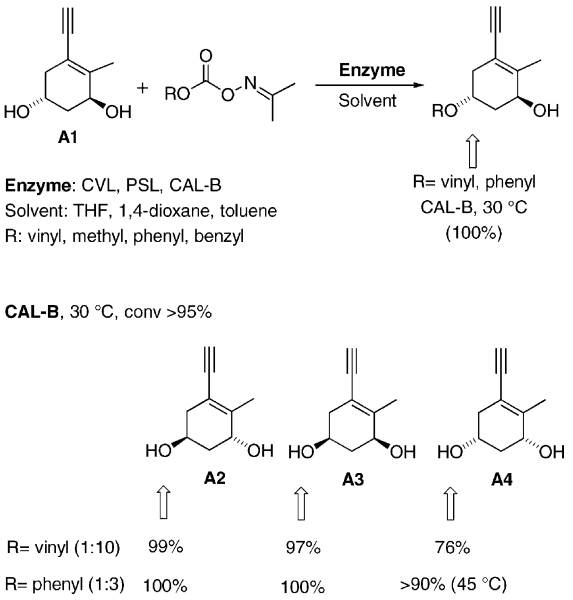



Scheme 5.

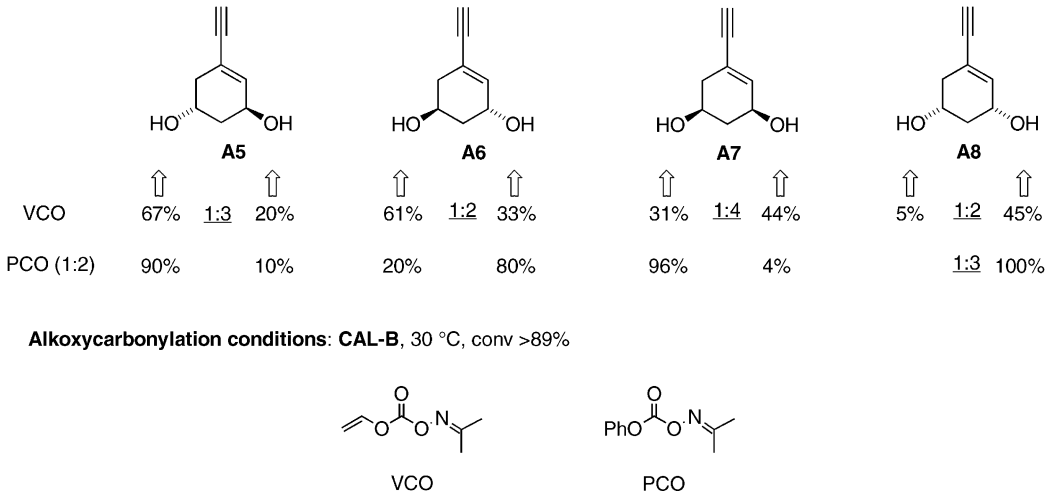
enzymatic acylation. At the moment, we are testing this enzymatic hydrolysis with different lipases.

Recently, we prepared the four stereoisomers of the A-ring synthon of the 1α, 25-dihydroxy-3-epi-19-nor-previtamin $D_3$, where the difference with respect to vitamin is the absence of the methyl group in the ring [14]. We observed that the methyl group has a great influence in the regioselectivity of the enzymatic acylation. We tested different lipases and proteases and the best results are shown in Scheme 5. For example, very good regioselectivity was achieved with the CVL for the two *cis* isomers, where the regioselectivity is similar to that obtained for the corresponding isomers with the methyl group [15]. However, for the stereoisomers with *trans* configuration the regioselectivity was only moderate and the best results were achieved with the lipase of *Candida antarctica* B (CAL-B).

Although, the enzymatic acylation has been exhaustively studied, the corresponding alkoxycarbonylation reaction has been much less investigated. However, the introduction of an alkoxycarbonyl group has advantages for the protection or activation of the hydroxyl groups in the chemistry of natural products. For instance, by this methodology it is possible the introduction of a benzyl oxycarbonyl group as protecting agent, and also the activation through the formation of vinyl carbonate. First, we investigated the enzymatic alkoxycarbonylation with the isomer of natural configuration (A1) using different alkoxycarbonylation agents, the most effective being oxime carbonates. The reaction takes place with an excess of oxime carbonate, although in some cases, depending of the amount of the alkoxycarbonyl reagent used, we have observed inhibition. Scheme 6 shows this regioselective alkoxycarbonylation reaction, which takes place with excellent regioselectivity [16]. The




Scheme 6.

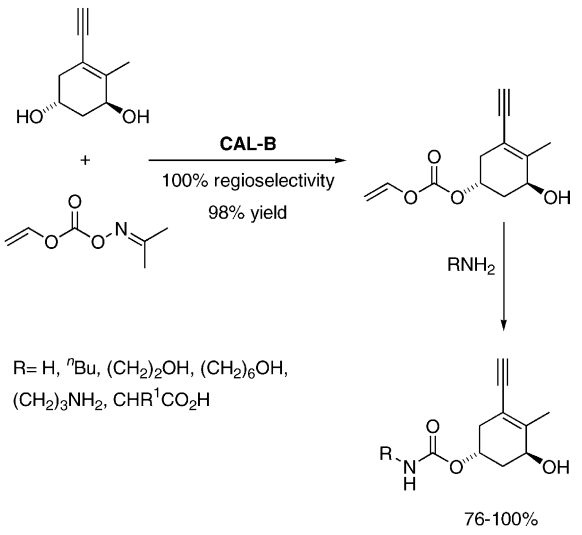



Scheme 7.

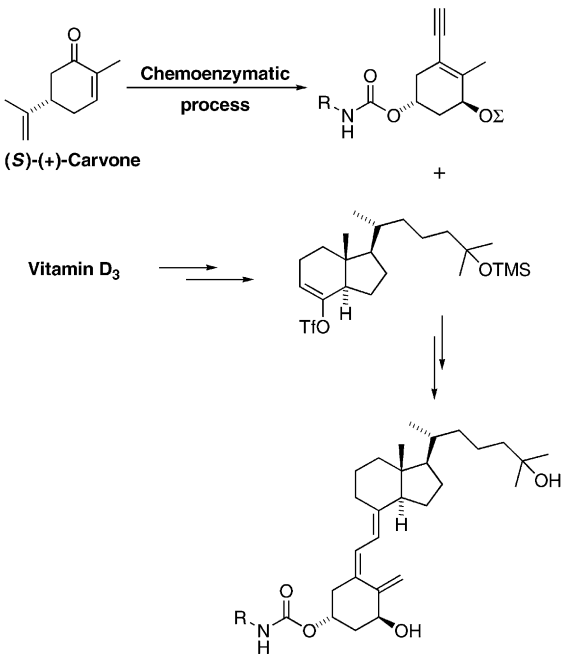
alkoxycarbonylation process with the other three diastereoisomers takes place also with good regioselectivity and always in the same position even with the *cis* isomer A4 [17]. The best results were obtained with CAL-B. We observed that the alkoxycarbonylation agent has a notable influence in this process. Thus with the A4-ring synthon, better regioselectivity was achieved using phenyloxime carbonate than with other reagents [18].

We have also studied the enzymatic alkoxycarbonylation with the four stereoisomers of the A ring of the corresponding previtamins [18]. With oxime vinyl carbonate the regioselectivity is not very good, although better results can be achieved when the alkoxycarbonylation agent is phenyloxime carbonate (Scheme 7). Again the selectivity is lower than in the case of the A-ring synthons of the previtamin than in the case of vitamin.

The introduction of a vinylalkoxycarbonyl group allows the preparation of new derivatives, such as carbamates, by reaction of the corresponding carbonate obtained by an enzymatic alkoxycarbonylation reaction with amines or ammonia in the absence of biocatalyst. Scheme 8 shows the preparation through this chemoenzymatic procedure of new A-ring synthons introducing ammonia, butylamine, aminoalcohols, diamine and even aminoacids, compounds obviously that are difficult to prepare by chemical methods. In




Scheme 8.



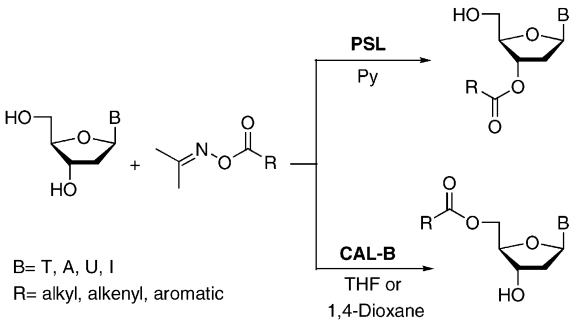

Scheme 9.

all cases the yields are practically quantitative [16]. We have also prepared carbamates with the other three stereoisomers in a similar manner to that with the natural isomer, introducing also hydrazine and spermine [17] with the aim of preparing new analogues of Vitamin $D_3$.

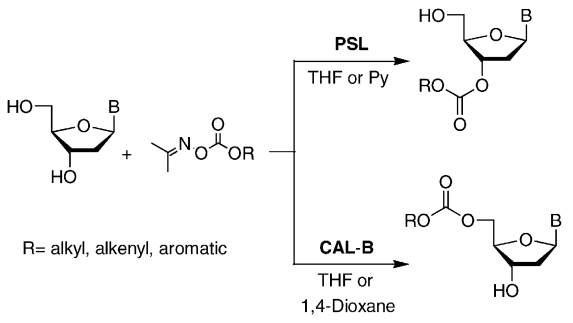
In Scheme 9 we show that the combination between chemical catalysis and biocatalysis can be an excellent strategy to achieve new analogues of Vitamin $D_3$. Thus, starting from (*S*) or (*R*) carvone, it is possible as we have already commented to obtain carbamates from the four ring synthons of Vitamin $D_3$. These intermediates can be used as starting material for the synthesis of new analogues of Vitamin $D_3$. At the moment, we have prepared some derivatives with the A1 isomer, with the aim of examining certain physiological properties.

## 3. Chemoenzymatic transformations of 2′-deoxynucleosides

Several years ago we started a systematic study of regioselective acylation reactions of 2′-deoxinucleosides. We found two lipases that were selective towards




Scheme 10.

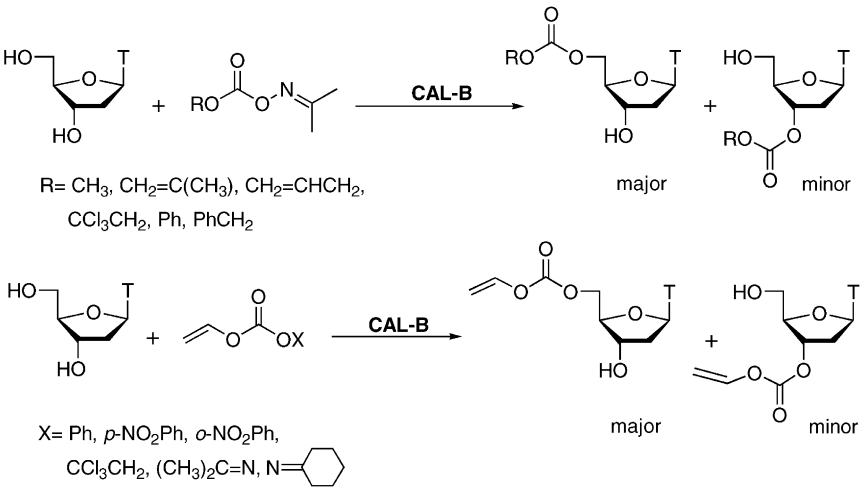



Scheme 11.

one of the two hydroxyl groups of different 2′-deoxynucleosides. Thus, we could prepare the acylated compounds in 5′ position with CAL-B [19], *Pseudomonas cepacia* lipase (PSL) being selective towards the secondary hydroxyl group [20]. We believe that this reaction is an interesting process because selective modifications of natural products which contain several functional groups with similar chemical reactivity, represents an interesting challenge for organic chemists, and it is of note that the secondary hydroxyl group can be modified without the need to protect the most reactive primary alcohol. We studied this reaction with different acyl reagents and we found oxime ester the most efficient reagents for this kind of process (Scheme 10).

Recently, we studied this reaction exhaustively with different oxime ester. The nature of the R group has a great influence on the regioselectivity. Poor regioselectivity is obtained, for instance, when R is a methyl group, although in the other cases very high regioselectivity was observed. We carried out this study with the assistance of professor Kazlauskas [21], who has rationalized this reaction by studies of molecular modelization. In base to these studies, it is possible explain the unusual stereo preference of the PSL, because this enzyme has a hydrophobic alternative pocket where the tymine base is sited, whilst the large hydrophobic pocket (common in all lipases) accommodates the acyl chain.

Several years ago we reported the utility of the enzymatic alkoxycarbonylation reaction in 2′-deoxynucleosides as is summarized in Scheme 11. Using oximecarbonates as alkoxycarbonylation agents, the protection of 5′-OH group is possible when CAL-B

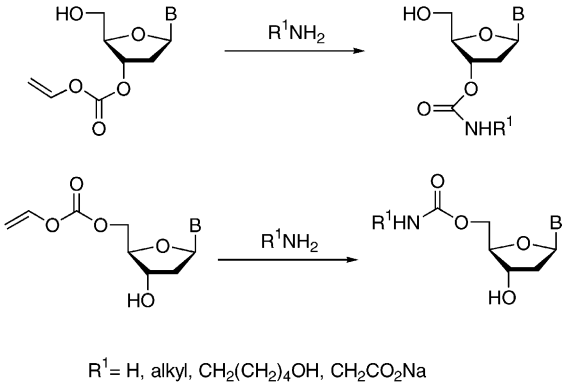



Scheme 12.

catalyses this reaction [22], although PSL shows opposite regioselectivity and the 3′-carbonates are achieved [23]. The introduction of a vinyl oxycarbonyl group is of interest because this allows the preparation of new derivatives of nucleosides by reaction with different nucleophiles [24].

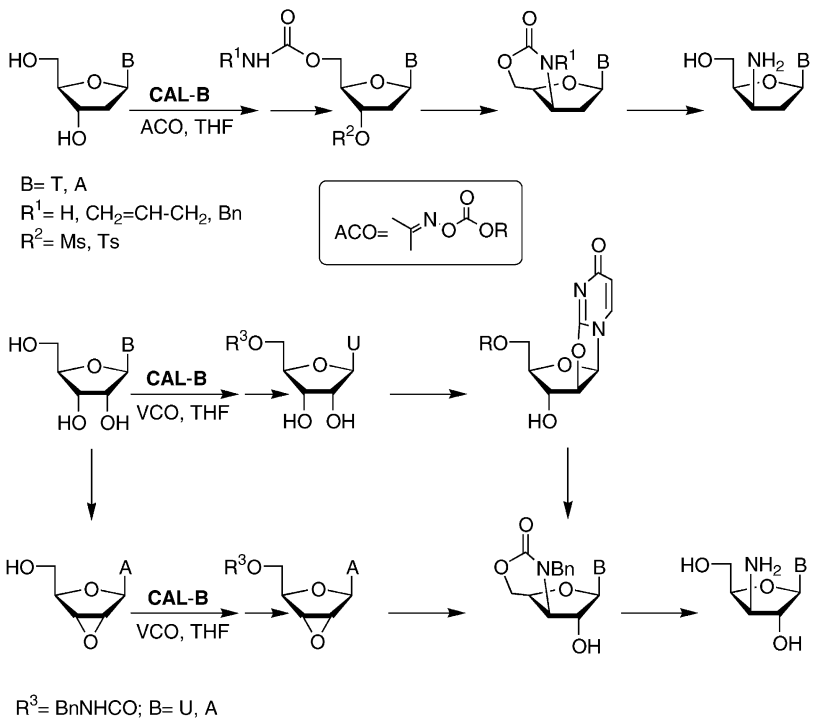
We have studied the influence of the R group and the leaving group of the alkoxycarbonylation agent in the enzymatic alkoxycarbonylation reaction of thymidine using CAL-B as biocatalyst. Scheme 12 shows the different reagents used in this study, the regioselectivity depending of the structure of the R group, but not of the leaving group, which only influences the reaction rate [25].

The introduction of an alkoxyvinylcarbonyl group, as we have already seen for Vitamin D, allows the preparation of other deoxynucleoside derivatives. Thus, we have prepared different new 2′-deoxynucleoside derivatives with the introduction of a carbamate group [26]. The value of nucleoside and also carbamate groups in some areas of medicinal chemistry is of note (Scheme 13).




Scheme 13.

This chemoenzymatic reaction yields new amino nucleosides that are very difficult to prepare only by chemical procedures [27]. Scheme 14 shows a novel and general chemoenzymatic procedure, developed in our group, to obtain 3′-amino-*xylo*-nucleosides. This scheme is based on the 5′-directed intramolecular nucleophilic substitution at the 3′-activated position of

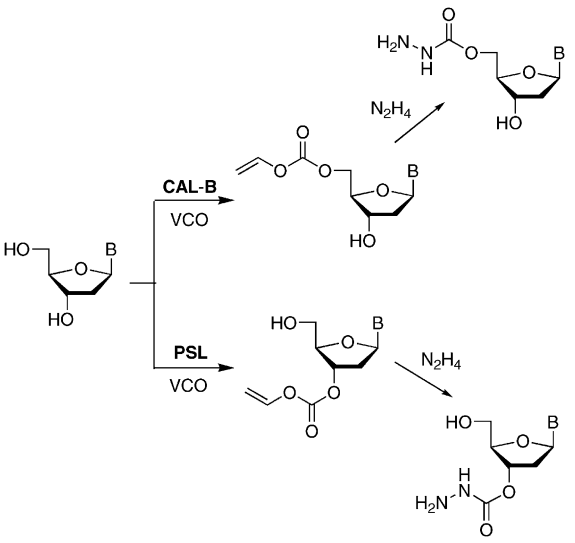



Scheme 14.

the nucleoside. This methodology is applicable to ribonucleosides and 2′-deoxyribonucleosides.

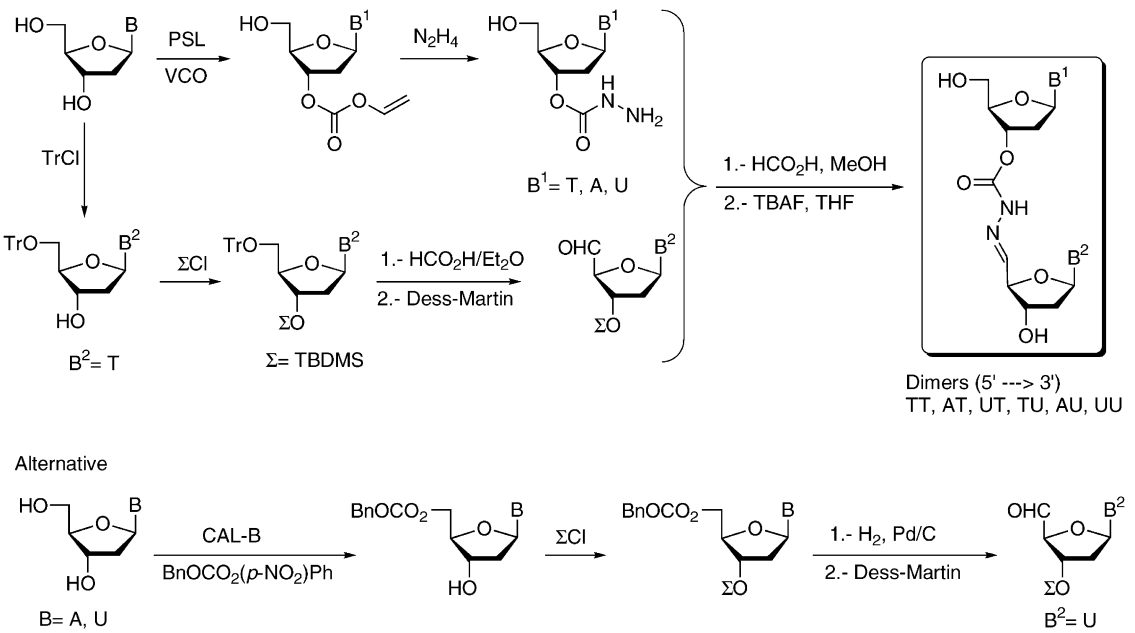
An interesting combination of biocatalysis and chemical catalysis has been carried out for the preparation of new oligonucleotides with a carbazoyl bridge. We have reported a chemoenzymatic procedure for the first synthesis of 3′- and 5′-carbazoyl nucleoside derivatives [28]. This process involves the regioselective enzymatic alkoxycarbonylation of nucleosides and the subsequent transformation with hydrazine into novel carbazoyl nucleoside derivatives (Scheme 15). Before achieving the preparation of oligonucleotides, we studied a standard chemical reaction between the carbazoyl nucleosides prepared and carbonyl compounds, with the aim of finding the best conditions for the coupling with carbonyl nucleosides. Through this process new alkylidencarbazoyl-2′-deoxynucleosides were prepared.

The synthesis of backbone modified dinucleotide analogues is shown in Scheme 16, where natural phosphodiester linkage is replaced by a 3′-, 5′-carbazoyl linkage [29]. The bridge was formed via a coupling reaction between an appropriate 3′-carbazoyl nucleoside analogue and an aldehyde nucleoside derivative obtained by chemical or chemoenzymatic procedure. At the moment, we are using these new dinucleotides as starting material for the synthesis of *antisense* oligonucleotides.

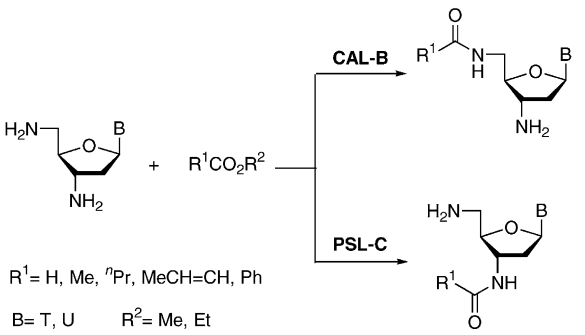



Scheme 15.

Recently, we optimized the synthesis of aminodeoxinucleosides in order to apply this methodology to




Scheme 16.




Scheme 17.

these derivatives because they can be of interest in medicinal chemistry. In these cases, oxime esters cannot be used as acyl reagents because the amino group is more nucleophilic than the hydroxyl group, and reacts with oxime esters in absence of catalyst. For this reason, to achieve good regioselectivity non-activated esters must be used [30]. Again, PSL is selective towards the 3′-amino group and CAL-B was selective for the amino group on the five carbon atom (Scheme 17). The great utility of CAL-B in aminolysis reactions [4] is also corroborated in this process, because better results are obtained with CAL-B than with PSL. However, to improve the yields in the 3′ position we have used immobilized PSL (PSL-C).

## 4. Conclusions

At present, many research groups employ biotransformations for the preparation of different kinds of organic compounds, and it is well recognized that biocatalysis is of special relevance in the fine chemicals industry for the manufacture of enantiopure compounds. Among the enzymes tested for organic synthesis, lipases have demonstrated a great versatility and around 50% of biotransformations take place using hydrolytic enzymes, especially lipases. In this account, we have explored the utility of some lipases for regioselective enzymatic transformations in two important classes of natural products. This enzymatic methodology allows the synthesis of products of a great added value, and we have shown here that biocatalysis and chemical catalysis is an excellent combination in synthetic organic chemistry.

## Acknowledgements

The success of this work is due to the many contributions from a number of co-workers, whose names are mentioned in the references. I thank specially to Drs. S. Fernández and M. Ferrero. This work is supported by Spanish Ministry of Science and Technology (projects BIO95-0677 and BIO98-0770). The author thanks to Novo Nordisk and Genzyme for the generous donation of CAL-B and CVL.

## References

[1] S.C. Stinson, Chem. Eng. News 9 (2001) 65.
[2] K. Faber, Biotransformations in Organic Chemistry, 4th ed., Springer, Berlin, 2000.
[3] G. Carrea, S. Riva, Angew. Chem. Int. Ed. 39 (2000) 2226.
[4] V. Gotor, Bioorg. Med. Chem. 7 (1999) 2189.
[5] K.M. Koeller, C.-H. Wong, Nature 409 (2001) 232.
[6] C.-H. Wong, Pure Appl. Chem. 67 (1995) 1609.
[7] R. Pulido, V. Gotor, J. Chem. Soc., Perkin Trans. I (1993) 589.
[8] R. Pulido, V. Gotor, Carbohydr. Res. 252 (1994) 55.
[9] M. Ferrero, V. Gotor, Stereoselective biocatalysis, in: R.N. Patel (Ed.), Marcel Dekker, New York, Chapter 11, p. 289.
[10] (a) M. Ferrero, V. Gotor, Monatsh. Chem. 131 (2000) 585; (b) M. Ferrero, V. Gotor, Chem. Rev. 100 (2000) 4319.
[11] R. Bouillon, W.H. Okamura, A.W. Norman, Endocrine Rev. 16 (1995) 200.
[12] (a) W.H. Okamura, J.M. Aurrecoechea, J.M. Gibs, A.W. Norman, J. Org. Chem. 54 (1989) 4072;
(b) K.R. Muralidharan, A.R. de Lera, S.D. Issaeff, A.W. Norman, W.H. Okamura, J. Org. Chem. 58 (1993) 1895.
[13] S. Fernández, M. Ferrero, V. Gotor, W. Okamura, J. Org. Chem. 60 (1995) 6057.
[14] (a) M. Diaz, M. Ferrero, S. Fernández, V. Gotor, Tetrahedron Lett. 41 (2000) 775;
(b) M. Díaz, M. Ferrero, S. Fernández, V. Gotor, J. Org. Chem. 65 (2000) 5647.
[15] M. Diaz, M. Ferrero, S. Fernández, V. Gotor, Tetrahedron: asymmetry 13 (2002) 539.
[16] M. Ferrero, S. Fernández, V. Gotor, J. Org. Chem. 62 (1997) 4358.
[17] V. Gotor-Fernández, M. Ferrero, S. Fernández, V. Gotor, J. Org. Chem. 64 (1999) 7504.
[18] M. Díaz, V. Gotor-Fernández, M. Ferrero, S. Fernández, V. Gotor, J. Org. Chem. 62 (2001) 4227.
[19] F. Morís, V. Gotor, J. Org. Chem. 58 (1993) 653.
[20] F. Morís, V. Gotor, Synthesis (1992) 626.
[21] Kazlauskas, not published.
[22] F. Morís, V. Gotor, Tetrahedron 48 (1992) 9869.
[23] F. Morís, V. Gotor, J. Org. Chem. 57 (1992) 2490.

[24] L. García-Allés, F. Morís, V. Gotor, Tetrahedron Lett. 34 (1993) 6337.
[25] L. García-Allés, V. Gotor, J. Mol. Catal. B: Enzymatic 6 (1999) 407.
[26] L. García-Allés, V. Gotor, Tetrahedron 51 (1995) 307.
[27] L. García-Allés, J. Magdalena, V. Gotor, J. Org. Chem. 61 (1996) 6980.
[28] J. Magdalena, S. Fernández, M. Ferrero, V. Gotor, J. Org. Chem. 63 (1998) 8873.
[29] J. Magdalena, S. Fernández, M. Ferrero, V. Gotor, Tetrahedron Lett. 40 (1999) 1787.
[30] I. Lavandera, S. Fernández, M. Ferrero, V. Gotor, J. Org. Chem. 66 (2001) 4079.