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Regioselective enzymatic syntheses of C-3 and C-5 carbonate A-ring stereoisomeric precursors of vitamin D

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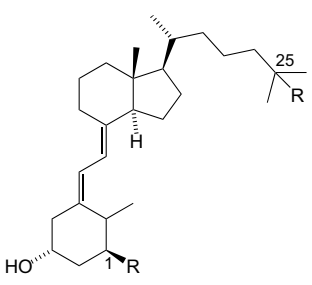
Abstract—The synthesis of selectively modified A-ring precursors for the preparation of 1α,25-dihydroxyvitamin D_3 analogues by enzymatic hydrolysis reaction of corresponding dicarbonates has been accomplished. Thus, *Candida rugosa* lipase (CRL) was found to hydrolyze with high selectivity the C-3 carbonate of stereoisomers **4a**,**b**, and **4d**, furnishing C-5 vinyloxycarbonates **5a**,**b**, and **5d**. On the other hand, *Chromobacterium viscosum* lipase exhibit opposite regioselectivity with *cis* enantiomers **4c** and **4d**, catalyzing hydrolysis at the C-5 carbonate for **4c** and at C-3 position for **4d**. In addition, CRL catalyzes the alkoxycarbonylation reaction at C-3 of diol **3d** affording the monocarbonate complementary to the one obtained by the enzymatic hydrolysis process.

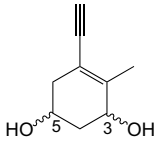
1. Introduction

To perform regio- and stereoselective transformations, enzymes have become standard catalysts in organic synthesis.[1] Lipases in particular have received most attention because they are one of the most versatile classes of biocatalysts acting with high efficiency in acylation or hydrolysis as well as alkoxycarbonylation processes.[2] In general, these catalysts are inexpensive and in many cases able to adapt to a wide range of substrate structures. Moreover, lipases are ecologically beneficial natural catalysts. Over the last few years, a biotransformation has sometimes proven to be the key step in the synthesis of biologically active natural products and their analogues.[2,3] Just as with enantioselectivity, the ability of the enzymes to catalyze the regioselective modification of several functional groups is also of great interest for organic chemists. Esterification and hydrolysis reactions have been commonly used whereas the alkoxycarbonylation process has scarcely been investigated.

1α,25-Dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3, **1**, Chart 1], the hormonally active form of vitamin D_3 **2**, is a key calcium-regulating hormone but also displays potent prodifferentiating and antiproliferative activities



1, R = OH 1α,25-$(OH)_2$-D_3
2, R = H Vitamin D_3



3a-d

Compound	Positions	
	3	5
a	*S*	*R*
b	*R*	*S*
c	*S*	*S*
d	*R*	*R*

Chart 1.

on normal and malignant cell types.[4] Since 1α,25-$(OH)_2$-D_3 itself is of limited therapeutic value due to its hypercalcemic effects, interest has been focused on the development of analogues having strong cell differentiating and weak calcemic effects. The therapeutical usefulness of 1α,25-$(OH)_2$-D_3 has led to the synthesis of new vitamin D analogues.[5] Most of the analogues are altered in the side chain, although modifications in the A-ring, less accessible synthetically, provide vitamin D derivatives with unique biological profiles.[6]

In our ongoing research related to the synthesis of selectively modified vitamin D_3 analogues,[7] appropriate

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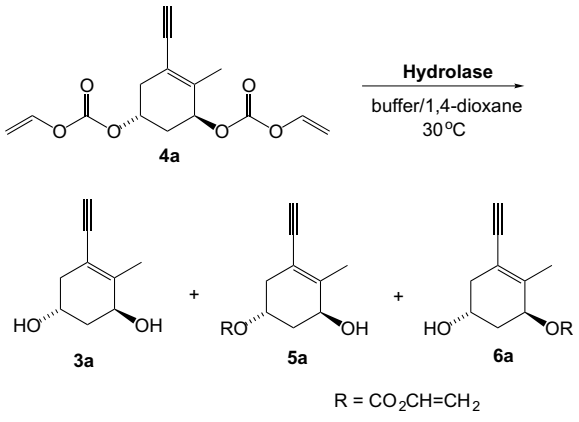
routes for the preparation of precursors are required. Thus, we have described the selective enzymatic acylation of diols[8] and hydrolysis[9] of corresponding diacylated derivatives of 1α,25-$(OH)_2$-D_3 and 1α,25-$(OH)_2$-19-nor-pre-D_3 A-ring stereoisomeric synthons. We have demonstrated the complementary regioselective behavior showed by enzymes in acylation and hydrolysis processes to prepare the monoacylated derivatives of the A-ring. Moreover, we have reported the enzyme-catalyzed regioselective alkoxycarbonylation processes in organic solvents of the A-ring precursors to obtain carbonate derivatives,[10] very interesting intermediates, which provide us the access to new A-ring synthons of vitamin D analogues. Thus, *Candida antarctica* lipase B (CAL-B) mainly allows the hydroxyl group at C-5 of **3a**–**d** to react giving place to C-5 carbonates.

Herein we report the enzyme mediated regioselective hydrolysis of A-ring carbonate synthons as useful precursors for the synthesis of vitamin D analogues. This study will allow us to have in hand all possible C-3 and C-5 monocarbonate A-ring stereosiomeric precursors from diols **3a**–**d**. Recently, the enzymatic resolution of a carbonate intermediate has been disclosed as a useful procedure for the preparation of (*S*)-(+)-Zoplicone.[11] Also, Matsumoto has described the enantioselective hydrolysis of various cyclic carbonates to obtain optically active 1,2-[12] and 1,3-diol[13] derivatives.

2. Results and discussion

In order to prepare carbonates as intermediates for the introduction of other functionalities, it is desirable that the substrate has a good leaving group. For this reason, we chose vinyl carbonates **4a**–**d** as starting materials. For that, diols **3a**–**d** were treated with vinyl chloroformate in the presence of pyridine and methylene chloride as solvent.

We first studied the enzymatic hydrolysis of dicarbonate **4a** with several commercial lipases and esterases (Scheme 1). Initially, the reactions were performed at 30 °C in 0.1 M phosphate buffer (pH 7) using 1,4-dioxane as co-solvent.



Scheme 1.

As shown in entry 1 of Table 1, *Chromobacterium viscosum* lipase (CVL) give rise to an approximately 1:1 mixture of C-3 and C-5 alkoxycarbonylated products, indicating poor recognition. Similar behavior was observed with *Pseudomonas cepacia* lipase (PSL-C) (Table 1, entry 4). In the case of CAL-B, no formation of the C-5 carbonate derivative **5a** was observed. However, significant amounts of the diol **3a** were isolated in addition to the C-3 carbonate **6a** (Table 1, entries 2 and 3). *C. antarctica* lipase A (CAL-A), Chirazyme L2 (a lipase from *C. antarctica* B immobilized in a different support), and porcine pancreas lipase (PPL) did not show good selectivities (Table 1, entries 5–8). Nevertheless, just Chirazyme L2 was the biocatalyst that showed moderate selectivity toward the C-5 position, obtaining carbonate **6a** with 47% isolated yield (Table 1, entry 6). Interestingly, excellent selectivity was found with *Candida rugosa* lipase (CRL) as catalyst. This enzyme catalyzes the hydrolysis at the C-3 carbonate group affording exclusively the C-5 vinyloxycarbonyl derivative **5a** (Table 1, entries 9 and 10). However, low conversions were achieved using a 58% buffer/dioxane even after longer reaction times and using more amount of the biocatalyst. The conversion was further improved by increasing buffer concentration up to 67%. Thus, after 90 h, 96% conversion was raised although selectivity decrease slightly, 78% of **5a** (62% isolated yield) being obtained in addition to the C-3 carbonate derivative **6a** and diol **3a** (Table 1, entry 11). Higher concentrations of buffer are not appropriate for this process due to the lack of selectivity (Table 1, entry 12). We also tested an esterase as catalyst (pig liver esterase, PLE), but not selectivity was observed giving place to diol **3a** as unique reaction product (Table 1, entry 13).

The results of the enzymatic hydrolysis of dicarbonate **4b** are summarized in Table 2. Among the enzymes screened only CRL displayed high selectivity toward the hydrolysis of the carbonate at the C-3 position (Scheme 2). Under the conditions of 30 °C and 58% buffer/dioxane during 24 h, the reaction proceeded to afford carbonate **5b** with total regioselectivity and 95% of conversion (Table 2, entry 9). Longer reaction times increased conversion up to 98%. In both cases, compound **5b** was isolated with >90% yield by flash chromatography (Table 2, entries 9 and 10). All other enzymes furnished exclusively diol **3b**.

When the hydrolysis was performed on the *cis*-dicarbonate **4c** (Scheme 3 and Table 3), the preliminary screening with the eight enzymes was unsatisfactory. Changing the reaction conditions did not have any significant influence on the regioselectivity of the enzyme except when CVL was the biocatalyst. The processes when carried out with 83% buffer/dioxane, both at 30 or 10 °C, gave place to the total hydrolysis of the dicarbonate **4c** (Table 3, entries 1 and 2). However, a drastic reduction of the amount of buffer (5%) led to a completely selective process. This lipase catalyzes the carbonate hydrolysis on the C-5 position in compound **4c** with excellent regioselectivity (Table 3, entry 3), yielding derivative **6c** (93% isolated yield). It is noteworthy that through a hydrolysis reaction, CVL allows the synthesis of the

Table 1. Enzymatic hydrolysis of A-ring synthon (3*S*,5*R*)–**4a**[a]

Entry	Enzyme	Buffer (%)[b]	*t* (h)	Conv. (%)[c]	**3a** (%)[c]	**5a** (%)[c]	**6a** (%)[c]
1	CVL[d]	18	112	42		20	22
2	CAL-B[e]	41	114	45	29		16
3	CAL-B[e]	83	93	64	30		34
4	PSL-C[f]	58	14	63		28	35
5	CAL-A[g]	83	48	54	13	27	14
6	Chirazyme L2[h]	34	216	77	8	10	59
7	Chirazyme L2[h]	58	37	98	31	15	52
8	PPL[i]	41	114	15	11		4
9	CRL[j]	58	48	15		15	
10	CRL[k]	58	216	46		46	
11	CRL[k]	67	90	96	12	78	6
12	CRL[k]	76	24	69	7	46	16
13	PLE[l]	83	25	78	78		

[a] The reactions were carried out at 30 °C, 250 rpm, in 0.04 M concentration with 17 mg of **4a**.
[b] Percentage of buffer solution (0.1 M KH_2PO_4/KOH, pH 7.0) in 1,4-dioxane; in all cases, total reaction volume was 1.5 mL.
[c] Based on ^{1}H NMR signal integration (±3% error).
[d] Ratio 5.7:1 of **4a**:CVL (w/w).
[e] Ratio 1:5.3 of **4a**:CAL-B (w/w).
[f] Ratio 1:5.3 of **4a**:PSL-C (w/w).
[g] Ratio 1:2.6 of **4a**:CAL-A (w/w).
[h] Ratio 1:5.3 of **4a**:Chirazyme L2 (w/w).
[i] Ratio 1:3.5 of **4a**:PPL (w/w).
[j] Ratio 1:3.5 of **4a**:CRL (w/w).
[k] Ratio 1:4.4 of **4a**:CRL (w/w).
[l] Ratio 1:1.2 of **4a**:PLE (w/w).

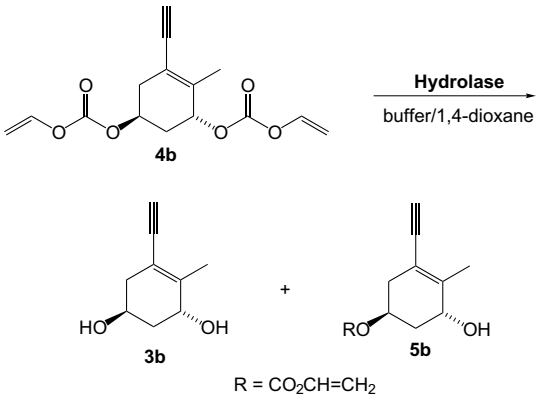
Table 2. Enzymatic hydrolysis of A-ring synthon (3*R*,5*S*)–**4b**[a]

Entry	Enzyme	Buffer (%)[b]	*T* (°C)	*t* (h)	Conv. (%)[c]	**3b** (%)[c]	**5b** (%)[c]
1	CVL[d]	58	30	96	20	20	
2	CVL[d]	83	30	24	98	98	
3	CAL-B[e]	83	10	24	6	6	
4	CAL-B[e]	83	30	48	77	77	
5	PSL-C[f]	83	30	48	22	22	
6	CAL-A[g]	83	30	48	46	46	
7	Chirazyme L2[h]	83	30	24	28	28	
8	PPL[i]	83	30	42	53	53	
9	CRL[j]	58	30	24	95		95
10	CRL[j]	58	30	48	98		98
11	CRL[j]	83	30	48	100	100	
12	PLE[k]	83	20	3	35	35	

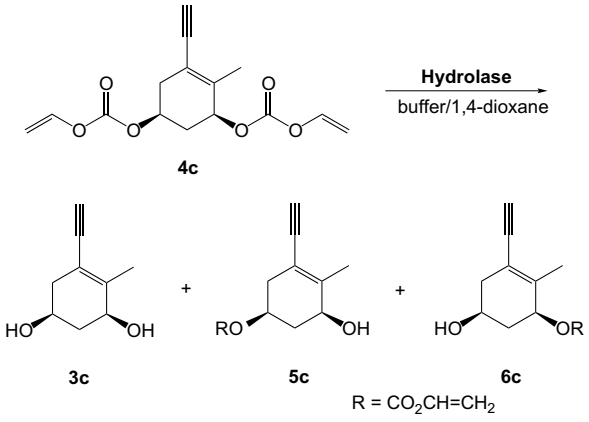
[a] The reactions were carried out at 250 rpm in 0.04 M concentration with 17 mg of **4b**.
[b] Percentage of buffer solution (0.1 M KH_2PO_4/KOH, pH 7.0) in 1,4-dioxane; in all cases, total reaction volume was 1.5 mL.
[c] Based on ^{1}H NMR signal integration (±3% error).
[d] Ratio 5.7:1 of **4b**:CVL (w/w).
[e] Ratio 1:5.3 of **4b**:CAL-B (w/w).
[f] Ratio 1:5.3 of **4b**:PSL-C (w/w).
[g] Ratio 1:2.6 of **4b**:CAL-A (w/w).
[h] Ratio 1:5.3 of **4b**:Chirazyme L2 (w/w).
[i] Ratio 1:3.5 of **4b**:PPL (w/w).
[j] Ratio 1:3.5 of **4b**:CRL (w/w).
[k] Ratio 1:1.2 of **4b**:PLE (w/w).

A-ring carbonate precursor of 1α,25-$(OH)_2$-D_3, which is complementary to that obtained with CAL-B by direct alkoxycarbonylation reaction.

As in the previous *cis*-dicarbonate **4c**, CVL exhibits an excellent selectivity in the hydrolysis reaction of its enantiomer **4d** (Scheme 4). Best results were obtained at 20 °C and 14% buffer/dioxane, with the monocarbonate **5d** being obtained as the major product after 15 h (81% isolated yield) (Table 4, entry 2). It is worth noting the opposite selectivity observed with CVL for both enantiomers: that is, (3*S*,5*S*)-**4c** showed preference for hydrolysis of the C-5 carbonate group meanwhile (3*R*,5*R*)-**4d** was hydrolyzed at C-3. Similarly, CRL catalyzes the synthesis of the C-5 monocarbonate derivative **5d** (71% isolated yield) from dicarbonate **4d** with high regioselectivity using 67% buffer/dioxane at 30 °C (Table 4, entry 10). Other enzymes such as CAL-B, PSL-C, CAL-A, Chirazyme L2, PPL, and PLE exhibited lower selectivities than CVL and CRL. This process

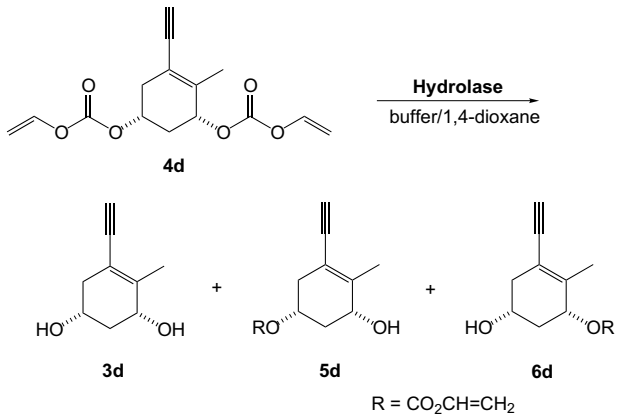


Scheme 2.



Scheme 3.

improves the synthesis of **5d**, previously described for ourselves,[10b] from diol **3d** through alkoxycarbonylation with CAL-B using acetone *O*-[(vinyloxy)carbonyl]oxime as carbonylating agent.



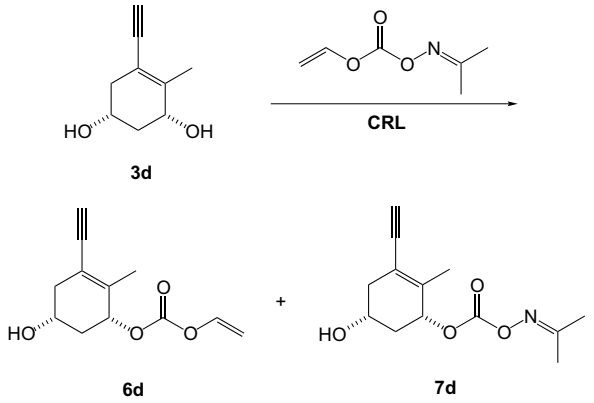
Scheme 4.

In view of the selectivity displayed by CRL with isomers **4a**,**b**, and **4d** and taking into account one of our aims to prepare the C-3 monocarbonate A-ring precursors, we tested this lipase in the alkoxycarbonylation reaction of diols **3a**,**b**, and **3d** with acetone *O*-[(vinyloxy)carbonyl]oxime in similar conditions as previously described for CAL-B (Scheme 5).[10b] The CRL-catalyzed enzymatic alkoxycarbonylation of *trans*-enantiomers **3a** and **3b** took place without selectivity and a complex mixture of monocarbonates and dicarbonates were obtained. In contrast, CRL was a very efficient catalyst for the regioselective transformation of substrate **3d**. This lipase catalyzed exclusively the alkoxycarbonylation in the C-3 position although a mixture of both vinyloxy and oxime carbonates were obtained **6d** and **7d**, respectively. The conversions were lower in 1,4-dioxane or THF than in toluene (Table 5, entries 1–3). Compounds **6d** and **7d** were formed even if the reaction was carried out at lower temperature or with lesser amounts of carbonylation agent (Table 5, entries 4–6). The fact to obtain the mixture **6d/7d** is not an inconvenience from

Table 3. Enzymatic hydrolysis of A-ring synthon (3*S*,5*S*)-**4c**[a]

Entry	Enzyme	Buffer (%)[b]	*T* (°C)	*t* (h)	Conv. (%)[c]	**3c** (%)[c]	**5c** (%)[c]	**6c** (%)[c]
1	CVL[d]	83	30	25	98	98		
2	CVL[d]	83	10	24	40	40		
3	CVL[d]	5	30	21	100			100
4	CAL-B[e]	41	30	72	26	18	8	
5	PSL-C[f]	41	30	22	64	26	10	28
6	CAL-A[g]	83	30	24	46	46		
7	Chirazyme L2[h]	41	30	70	35	22	13	
8	Chirazyme L2[h]	83	30	24	66	66		
9	PPL[i]	76	30	24	35	35		
10	CRL[j]	67	30	72	32	18	7	7
11	PLE[k]	67	30	30	64	35	21	8

[a] The reactions were carried out at 250 rpm in 0.04 M concentration with 17 mg of **4c**.
[b] Percentage of buffer solution (0.1 M KH_2PO_4/KOH, pH 7.0) in 1,4-dioxane; in all cases, total reaction volume was 1.5 mL.
[c] Based on ^1H NMR signal integration (±3% error).
[d] Ratio 5.7:1 of **4c**:CVL (w/w).
[e] Ratio 1:5.3 of **4c**:CAL-B (w/w).
[f] Ratio 1:5.3 of **4c**:PSL-C (w/w).
[g] Ratio 1:2.6 of **4c**:CAL-A (w/w).
[h] Ratio 1:5.3 of **4c**:Chirazyme L2 (w/w).
[i] Ratio 1:3.5 of **4c**:PPL (w/w).
[j] Ratio 1:3.5 of **4c**:CRL (w/w).
[k] Ratio 1:1.2 of **4c**:PLE (w/w).



Scheme 5.

the synthetic point of view because both carbonates are appropriate synthons to introduce additional functionalities.

3. Summary

Several chiral monocarbonylated precursors for the preparation of 1α,25-dihydroxyvitamin D_3 analogues have been synthesized. *C. rugosa* lipase has catalyzed with high selectivity the hydrolysis of the carbonate at the C-3 position of A-ring dicarbonate precursors **4a**,**b**, and **4d** to afford the corresponding C-5 monocarbonate derivatives **5a**,**b**, and **5d**. However, this lipase was not efficient in the hydrolysis of substrate **4c**. On the other hand, *Chromobacterium viscosum* lipase has shown opposite selectivity with *cis* enantiomers **4c** and **4d**: the hydrolysis occurs at the C-5 carbonate for **4c** and at the C-3 position in case of **4d**. Additionally, CRL allowed the selective synthesis of C-3 derivatives **6d** and **7d** through direct alkoxycarbonylation reaction. Although preparation of C-3 monocarbonates **6** was not achieved in all cases with high selectivities, the results obtained in this study could be useful in the selective deprotection of similar precursors of 1α,25-$(OH)_2$-D_3 analogues.

4. Experimental

Chromobacterium viscosum lipase, (CVL, 4100 U/mg) was a gift from Genzyme Co. *C. antarctica* lipase B (CAL-B, Novozym 435, 10,000 PLU/g), Chirazyme L-2 (lipase from *C. antarctica* B, 400 U/g), and immobilized *Pseudomonas cepacia* lipase (PSL-C, 904 U/g) were purchased from Novo Nordisk Co., Roche Diagnostics,

Table 4. Enzymatic hydrolysis of A-ring synthon (3*R*,5*R*)-**4d**[a]

Entry	Enzyme	Buffer (%)[b]	*T* (°C)	*t* (h)	Conv. (%)[c]	**3d** (%)[c]	**5d** (%)[c]	**6d** (%)[c]
1	CVL[d]	7	30	73	87	11	76	
2	CVL[d]	14	20	15	98	8	90	
3	CVL[d]	18	20	37	100	34	66	
4	CAL-B[e]	67	30	19	25	18		7
5	PSL-C[f]	29	30	17	91	56	12	23
6	CAL-A[g]	67	30	69	35	25	10	
7	Chirazyme L2[h]	58	30	17	49	24		25
8	PPL[i]	67	30	70	8	8		
9	CRL[j]	67	30	25	90	7	83	
10	CRL[j]	67	30	46	100	12	88	
11	PLE[k]	67	30	25	23	14	5	4

[a] The reactions were carried out at 250 rpm in 0.04 M concentration with 17 mg of **4d**.
[b] Percentage of buffer solution (0.1 M KH_2PO_4/KOH, pH 7.0) in 1,4-dioxane; in all cases, total reaction volume was 1.5 mL.
[c] Based on ^{1}H NMR signal integration (±3% error).
[d] Ratio 5.7:1 of **4d**:CVL (w/w).
[e] Ratio 1:5.3 of **4d**:CAL-B (w/w).
[f] Ratio 1:5.3 of **4d**:PSL-C (w/w).
[g] Ratio 1:2.6 of **4d**:CAL-A (w/w).
[h] Ratio 1:5.3 of **4d**:Chirazyme L2 (w/w).
[i] Ratio 1:3.5 of **4d**:PPL (w/w).
[j] Ratio 1:3.5 of **4d:**CRL (w/w).
[k] Ratio 1:1.2 of **4d**:PLE (w/w).

Table 5. Enzymatic alkoxycarbonylation catalyzed by CRL of A-ring diol (3*R*,5*R*)-**3d**[a]

Entry	Solvent	VCO[b] (equiv)	*T* (°C)	*t* (h)	Conv. (%)[c]	**6d** (%)[c]	**7d** (%)[c]
1	THF	10	40	96	46	20	26
2	1,4-Dioxane	10	40	96	47	19	28
3	Toluene	10	40	96	100	26	74
4	Toluene	10	30	1	100	35	65
5	Toluene	10	20	1.5	100	40	60
6	Toluene	5	20	1.5	100	39	61

[a] The reactions were carried out at 250 rpm in 0.03 M concentration with 15 mg of **3d**, dissolved in 3.5 mL of solvent; Ratio 1:6.7 of **3d**:CRL (w/w).
[b] VCO, acetone *O*-[(vinyloxy)carbonyl]oxime.
[c] Based on ^{1}H NMR signal integration (3% error).

and Amano Pharmaceuticals, respectively. *C. rugosa* lipase (CRL, 724 U/mg), pig liver esterase (PLE, 3500 U/mg), and porcine pancreas lipase (PPL, 46 U/mg) were purchased from Sigma. *C. antarctica* lipase A (CAL-A, 50 kU/19.24 g) was purchased from Roche.

Solvents were distilled over an adequate desiccant under nitrogen. A-ring synthons **3a** and **3b** were obtained according to the method of Okamura et al.[14] A-ring synthons **3c** and **3d** were obtained through Mitsunobu inversion.[10a] Acetone *O*-[(vinyloxy)carbonyl]oxime was synthesized as previously reported.[15]

4.1. General procedure for the syntheses of dicarbonates 4a–d

To a solution of diol **3** (197 mg, 1.30 mmol) in CH_2Cl_2 (15 mL) and pyridine (0.6 mL, 6.48 mmol) at 0 °C was added dropwise vinyl chloroformate (0.62 mL, 6.48 mmol). The progress of the reaction was followed by TLC (40% EtOAc/hexane). After consumption of the starting material (1 h) the solvent was removed under reduced pressure, and the crude was extracted with CH_2Cl_2. Then, the residue was subjected to *flash* chromatography (20% EtOAc/hexane) to afford a colorless oil.

4.1.1. (3*S*,5*R*)-1-Ethynyl-2-methyl-3,5-bis[(vinyloxy)carbonyloxy]-1-cyclohexene 4a. Yield 87%. This compound has been previously described.[10c] $[\alpha]_D^{20} = -112$ (*c* 0.65, $CHCl_3$).

4.1.2. (3*R*,5*S*)-1-Ethynyl-2-methyl-3,5-bis[(vinyloxy)carbonyloxy]-1-cyclohexene 4b. Yield 70%. Same data as for its enantiomer, **4a**. $[\alpha]_D^{20} = +108$ (*c* 0.85, $CHCl_3$).

4.1.3. (3*S*,5*S*)-1-Ethynyl-2-methyl-3,5-bis[(vinyloxy)carbonyloxy]-1-cyclohexene 4c. Yield 92%. This compound has been previously described.[10b] $[\alpha]_D^{20} = -41$ (*c* 0.57, $CHCl_3$).

4.1.4. (3*R*,5*R*)-1-Ethynyl-2-methyl-3,5-bis[(vinyloxy)carbonyloxy]-1-cyclohexene 4d. Yield 81%. This compound has been previously described.[10b] $[\alpha]_D^{20} = +37$ (*c* 0.6, $CHCl_3$).

4.2. General procedure for the enzymatic hydrolysis of dicarbonates 4a–d

In a typical procedure, to a solution of dicarbonate **4** (17 mg, 0.058 mmol) in 1.5 mL of solvent (mixture of 1,4-dioxane and buffer) was added one of the following enzymes: 90 mg of CAL-B, PSL-C, or Chirazyme L2, 60 mg of CRL or PPL, 45 mg of CAL-A, 20 mg of PLE, or 3 mg of CVL. The suspension was shaken (250 rpm) at 10, 20, or 30 °C and the progress of the reaction was followed by TLC (40% EtOAc/hexane). After removal of the enzyme by filtration, the crude was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, evaporated, and the residue was analyzed by ^1H NMR. All these data are summarized in Tables 1–4.

4.2.1. (3*S*,5*R*)-1-Ethynyl-3-hydroxy-2-methyl-5-[(vinyloxy)carbonyloxy]-1-cyclohexene 5a. This compound has been previously described.[10c] $[\alpha]_D^{20} = -45$ (*c* 0.35, $CHCl_3$).

4.2.2. (3*R*,5*S*)-1-Ethynyl-3-hydroxy-2-methyl-5-[(vinyloxy)carbonyloxy]-1-cyclohexene 5b. Same data as for its enantiomer, **5a**. $[\alpha]_D^{20} = +50$ (*c* 0.58, $CHCl_3$).

4.2.3. (3*S*,5*S*)-1-Ethynyl-3-hydroxy-2-methyl-5-[(vinyloxy)carbonyloxy]-1-cyclohexene 5c. This compound has been previously described.[10b] $[\alpha]_D^{20} = -48$ (*c* 0.72, $CHCl_3$).

4.2.4. (3*R*,5*R*)-1-Ethynyl-3-hydroxy-2-methyl-5-[(vinyloxy)carbonyloxy]-1-cyclohexene 5d. Same data as for its enantiomer, **5c**. $[\alpha]_D^{20} = +51$ (*c* 0.8, $CHCl_3$).

4.2.5. (3*S*,5*R*)-1-Ethynyl-5-hydroxy-2-methyl-3-[(vinyloxy)carbonyloxy]-1-cyclohexene 6a. $[\alpha]_D^{20} = -104$ (*c* 0.31, $CHCl_3$); IR (NaCl): *v* 3441, 3288, 2934, 2094, 1756, and 1649 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz): δ 1.88 (ddd, 1H, H_4, $^2J_{HH}$ 14.0, $^3J_{HH}$ 10.6, $^3J_{HH}$ 4.7 Hz), 1.95 (s, 3H, $3H_9$), 2.17 (m, 2H, H_4+H_6), 2.60 (dd, 1H, H_6, $^2J_{HH}$ 16.9, $^3J_{HH}$ 4.2 Hz), 3.17 (s, 1H, H_8), 4.12 (m, 1H, H_5), 4.60 (dd, 1H, $H_{12\text{-}cis}$, $^3J_{HH}$ 6.4, $^2J_{HH}$ 2.1 Hz), 4.93 (dd, 1H, $H_{12\text{-}trans}$, $^3J_{HH}$ 13.6, $^2J_{HH}$ 2.1 Hz), 5.34 (m, 1H, H_3), and 7.07 (dd, 1H, H_{11}, $^3J_{HH}$ 13.6, $^3J_{HH}$ 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 18.4 (C_9), 36.6 (C_4), 38.4 (C_6), 63.1 (C_5), 76.1 (C_3), 81.8 (C_8), 82.2 (C_7), 98.0 (C_{12}), 118.1 (C_1), 137.6 (C_2), 142.4 (C_{11}), and 152.4 (C_{10}); MS (FAB^+, *m*/*z*): 222 (M^+, 4%), 204 (11), 134 (49), 116 (60), 115 (100), 105 (23), and 91 (61); Anal. Calcd (%) for $C_{12}H_{14}O_4$: C, 64.84; H, 6.35. Found: C, 64.8; H, 6.3.

4.2.6. (3*S*,5*S*)-1-Ethynyl-5-hydroxy-2-methyl-3-[(vinyloxy)carbonyloxy]-1-cyclohexene 6c. $[\alpha]_D^{20} = -39$ (*c* 0.61, $CHCl_3$); IR (NaCl): *v* 3385, 3298, 2921, 2855, 2095, 1756, and 1650 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz): δ 1.96 (s, 3H, $3H_9$), 2.01 (ddd, 1H, H_4, $^2J_{HH}$ 13.6, $^3J_{HH}$ 7.9, $^3J_{HH}$ 6.1 Hz), 2.25 (dddd, 1H, H_4, $^2J_{HH}$ 13.6, $^3J_{HH}$ 5.9, $^3J_{HH}$ 3.1, $^4J_{HH}$ 1.0 Hz), 2.42 (m, 2H, $2H_6$), 3.18 (s, 1H, H_8), 4.05 (m, 1H, H_5), 4.61 (dd, 1H, $H_{12\text{-}cis}$, $^3J_{HH}$ 6.2, $^2J_{HH}$ 2.1 Hz), 4.93 (dd, 1H, $H_{12\text{-}trans}$, $^3J_{HH}$ 13.9, $^2J_{HH}$ 2.1 Hz), 5.32 (m, 1H, H_3), and 7.09 (dd, 1H, H_{11}, $^3J_{HH}$ 13.9, $^3J_{HH}$ 6.2 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 17.9 (C_9), 35.7 (C_4), 38.3 (C_6), 63.8 (C_5), 75.1 (C_3), 81.9 (C_8), 82.3 (C_7), 98.1 (C_{12}), 116.8 (C_1), 138.1 (C_2), 142.4 (C_{11}), and 152.4 (C_{10}); MS (FAB^+, *m*/*z*): 222 (M^+, 3%), 204 (6), 178 (5), 134 (50), 116 (78), 115 (100), 105 (29), and 91 (89); Anal. Calcd (%) for $C_{12}H_{14}O_4$: C, 64.84; H, 6.35. Found: C, 64.9; H, 6.2.

4.2.7. (3*R*,5*R*)-1-Ethynyl-5-hydroxy-2-methyl-3-[(vinyloxy)carbonyloxy]-1-cyclohexene 6d. Same data as for its enantiomer, **6c**. $[\alpha]_D^{20} = +42$ (*c* 0.63, $CHCl_3$).

4.3. General procedure for the enzymatic alkoxycarbonylation of diols 3

To a solution of **3a**,**b**, or **3d** (15 mg, 0.098 mmol) in 3.5 mL of solvent (THF, 1,4-dioxane, or toluene) was added 100 mg of CRL and acetone *O*-[(vinyloxy)carbon-

yl]oxime (141 mg, 0.986 mmol or 70.5 mg, 0.493 mmol). The suspension was shaken (250 rpm) at 20, 30, or 40 °C, and the progress of the reaction was followed by TLC (50% EtOAc/hexane) until no further reaction was apparent. After removal of the enzyme by filtration and evaporation, the crude was analyzed by ^{1}H NMR. All these data are summarized in Table 5.

4.3.1. (3*R*,5*R*)-3-[(Acetonoxime)carbonyloxy]-1-ethynyl-5-hydroxy-2-methyl-1-cyclohexene 7d. $[\alpha]_D^{20} = +57$ (*c* 0.5, $CHCl_3$); IR (NaCl): *v* 3476, 3289, 2926, 2096, 1768, 1654, and 1378 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.98 (s, 3H, $3H_{12}$), 2.02 (s, 3H, $3H_{12}$), 2.04 (s, 3H, $3H_9$), 2.07 (m, 1H, $1H_4$), 2.27 (ddd, $^2J_{HH}$ 16.5, $^3J_{HH}$ 5.7, $^3J_{HH}$ 3.1 Hz), 2.33–2.55 (m, 2H, $2H_6$), 3.18 (s, 1H, H_8), 4.08 (m, 1H, H_5), and 5.42 (m,1H, H_3); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 16.8 (C_{12}), 17.9 (C_{12}), 21.7 (C_9), 35.7, 38.2 ($C_4 + C_6$), 63.8 (C_5), 74.7 (C_3), 81.7 (C_8), 82.4 (C_7), 116.7 (C_1), 138.4 (C_2), 153.6 (C_{10}), and 163.7 (C_{11}). MS (EI, *m*/*z*): 251 (M^+, 2%), 135 (M-$OCO_2NC_3H_6$, 50), 133 (26), 105 (29), 91 (89) and 56 (100); Anal. Calcd (%) for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.0; H, 6.9; N, 5.6.

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