CAL-B-Catalyzed Alkoxycarbonylation of A-Ring Stereoisomeric Synthons of 1α,25-Dihydroxyvitamin D_3 and 1α,25-Dihydroxy-19-*nor*-previtamin D_3: A Comparative Study. First Regioselective Chemoenzymatic Synthesis of 19-*nor*-A-Ring Carbonates

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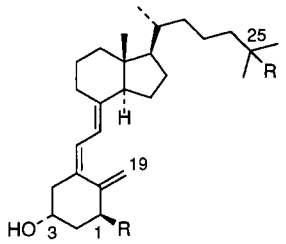
A comparative study of alkoxycarbonylation processes of both 19-*nor*-A-ring and A-ring stereoisomers of 1α,25-dihydroxyvitamin D_3 analogues catalyzed by *Candida antarctica* lipase B (CAL-B) has been described. The presence of the methyl group in the A-ring at C-2, as in **3**−**6**, has a determining role in the regioselectivity of the biocatalysis, mainly allowing the hydroxyl group at C-5 position to react. For the 19-*nor*-A-ring stereoisomers **7**−**10**, which lack the C-2 methyl group, the configurations at C-3 and C-5 have a high influence in the selectivity exhibited by CAL-B. Thus, each couple of enantiomers showed opposing regioselectivities depending on the C-3 configuration. When C-3 possesses an (*S*)-configuration, enzymatic alkoxycarbonylations took place at the C-5-(*R*) or C-5-(*S*) hydroxyl groups. However, if the chiral centers at C-3 are (*R*), CAL-B alkoxycarbonylated the C-3-(*R*) hydroxyl group independently of the configuration at C-5. The corresponding carbonates are useful A-ring precursors of 1α,25-dihydroxyvitamin D_3 analogues, selectively modified at the C-1 or C-3 positions. In addition, an improved synthesis of *cis* A-ring synthons **5** and **6** is described using a Mitsunobu methodology.

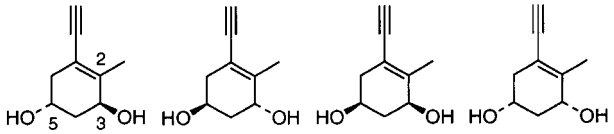
Introduction

To perform regio- and stereoselective transformations, and because of their simple feasibility and high efficiency, enzyme-catalyzed reactions have become standard procedures in organic synthesis.[1] In general, these catalysts are inexpensive and in many cases able to adapt to a wide range of substrate structures. Moreover, biocatalysts are ecologically beneficial natural catalysts. Previously, we reported the regioselective enzymatic acylation[2] and alkoxycarbonylation[3] reactions of 1α,25-dihydroxyvitamin D_3 A-ring precursors **3**−**6** (Chart 1). The hormonal active form (**2**, Chart 1) of vitamin D_3 (**1**) has, apart from its normal role as a calcium regulator, a wide range of activities such as modulation of cell proliferation and differentiation.[4] Most of the analogues[5] are altered in the side chain, although modifications in the A-ring, less accessible synthetically, provide vitamin D derivatives with an unique biological profile.[6] The A-ring synthon possesses two hydroxyl groups of similar reactivity, and as a result it is very difficult to discern between these

Chart 1



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



3 **4** **5** **6**

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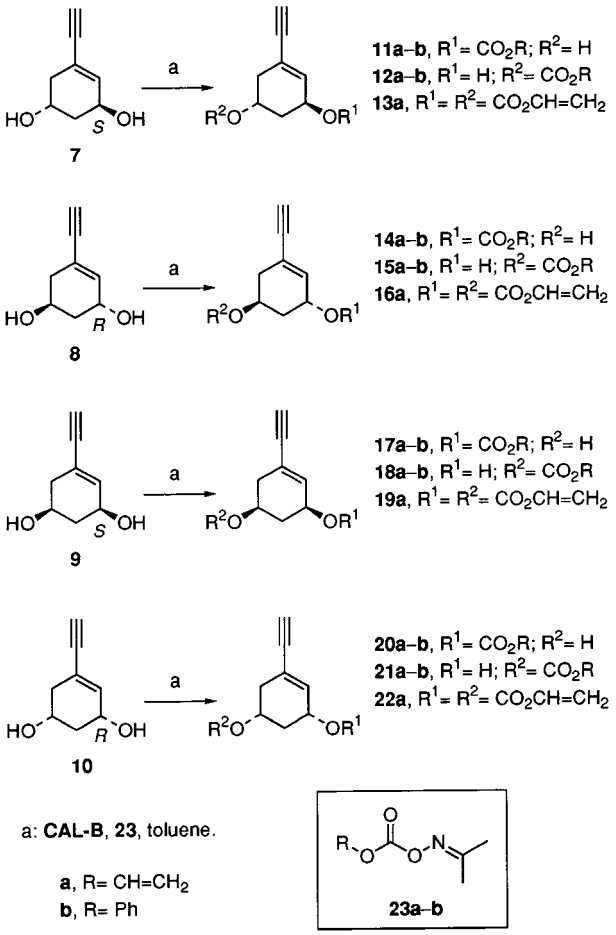
Scheme 1



two groups from a chemical point of view. An important and synthetically relevant transformation in lipase-mediated reactions in organic solvents is the selective modification of polyfunctionalized compounds, such as carbohydrates,[7] steroids,[8] and nucleosides.[9]

Candida antarctica lipase B (CAL-B) catalyzes selectively the alkoxycarbonylation of the C-5 hydroxyl group of the four stereoisomeric vitamin D A-ring precursors **3**, **4**, **5**, and **6**.[3] It is possible to obtain the monocarbonate derivatives at C-5 with excellent regioselectivity using acetone *O*-[(vinyloxy)carbonyl]oxime (**23a**) (Scheme 1) as the alkoxycarbonylation reagent in toluene. Here we report a systematic study of the influence of the methyl group at C-2 in the selectivity of the process. The study aims to go into the selectivity of CAL-B with these substrates in depth, and to provide easy access to selectively modified 19-*nor*-A-ring synthons. Different alkoxycarbonylation agents are investigated. In addition, we describe an alternative approach to synthesize the *cis*-diol isomers **5** and **6**, this being more efficient on the basis of its fewer steps an high overall yields.

Results and Discussion

Alkoxycarbonylation of 19-*nor*-A-Ring Synthons 7−10. The synthesis of these A-ring precursors as synthons to prepare 6-*s*-*cis*-locked analogues of 1α,25-dihydroxyvitamin D_3 has been previously described.[10] To compare both enzymatic alkoxycarbonylation processes, similar reaction conditions were used to those previously described for **3**−**6**. All reactions were carefully monitored by GC to determine the conversion. Ratio of C-3, C-5, or C-3,5 derivatives were calculated by ^{1}H NMR since these alkoxycarbonylation products were too close in GC chromatograms. Thus, reaction of A-ring synthon **7** (Scheme 1) with 10 equiv of acetone *O*-[(vinyloxy)carbonyl]oxime (**23a**) in toluene at 30 °C in the presence of CAL-B gave, after 3 h and 98% conversion, moderate selectivity toward C-5 (compound **12a**) in addition to C-3 (**11a**) and a small amount of C-3,5 (**13a**) derivatives (entry 1, Table 1). This result contrasts with that reported for compound **3**, in which total regioselectivity toward C-5 position was achieved.[3b] This indicates that the presence of the C-2 methyl group has an important influence in the enzymatic catalysis. To increase the selectivity, and taking into account the high reaction rate, fewer equivalents of **23a** were added. This change did not drastically increase the ratio of **12a** (entry 2, Table 1). The shorter reaction time, compared with entry 1, is probably due to the inhibition of the enzyme when large amount of alkoxycarbonylation agent were added to the reaction mixture. A decrease in temperature did not lead to an increase of selectivity (entry 3, Table 1). When the substrate was 19-*nor*-A-ring **8**, C-5 carbonate **15a** was obtained as the major product, both at 15 or 30 °C (entries 4 and 5, Table 1), although the regioselectivity was similar to that shown by its enantiomer **7**. The selectivity of the process did not experience an improvement when the biocatalysis was carried out with stereoisomers **9** and **10** (entries 6−9, Table 1). CAL-B mainly catalyzed the formation of the corresponding C-3,5 carbonates **19a** and **22a**.

In view of these unsuccessful results and taking into account our aim to prepare regioselective modified 19-*nor*-A-ring synthons, we decided to study the enzymatic alkoxycarbonylation process with acetone *O*-(phenoxycarbonyl)oxime (**23b**). The latter carbonate has shown better regioselectivities than acetone *O*-[(vinyloxy)carbonyl]oxime (**23a**) in CAL-B-catalyzed alkoxycarbonylation in nucleosides.[11] Besides their intrinsic value, the A-ring phenoxycarbonates generated are excellent intermediates for consecutive modifications since phenol is, like acetaldehyde, a good leaving group. Table 2 summarizes the results obtained for the alkoxycarbonylation of isomers **7**−**10** with CAL-B and carbonate **23b** at 30 °C in toluene. This lipase catalyzes carbonate formation on the C-5 hydroxyl group in compound **7** with excellent regioselectivity (entry 1, Table 2), isolating derivative **12b**. A parallel experiment was carried out with its stereoisomer, **8**. The high selectivity of the enzyme toward the C-3 position (isolating **14b**) is noteworthy and is the very opposite of enantiomer **7** (entry 2, Table 2). When the reaction was carried out on *cis*-diol **9**, excellent selectivity toward the C-5 hydroxyl was achieved. Since this compound is less reactive than that previously mentioned, the reaction took place with 4 equiv of **23b**, yielding 97% conversion in 24 h (entry 3, Table 2). To get total selectivity, 2 equiv of alkoxycarbonylating agent

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Table 1. Enzymatic Alkoxycarbonylation Catalyzed by CAL-B of 19-*nor*-A-Ring Synthons 7−10 with Carbonate 23a

entry	substrate	*T* (°C)	**23a** (equiv)[a]	*t* (h)	conv (%)[b]	C-3 (%)[c,d]	C-5 (%)[c,d]	C-3,5 (%)[c,d]
1	**7**	30	10	3	98	30	60	8
2	**7**	30	3	1	99	20	66	13
3	**7**	15	4[e]	20	98	30	41	27
4	**8**	30	2	1.5	98	32	60	6
5	**8**	15	2	3	96	32	64	
6	**9**	30	4	26	97	43	30	24
7	**9**	30	5	15	92	25	31	36
8	**10**	15	5	72	90	24	4	62
9	**10**	30	2	36	95	43	5	47

[a] Equivalents of **23a** were chosen according to their relative reactivity compared to A-ring synthons **3**−**6** which has already been reported. [b] Calculated by GC, using Method A. [c] Ratio of regioselectivity at position C-3, C-5, or C-3,5, calculated by ^{1}H NMR. [d] Some of these percentages contains, in addition to the vinyl derivative, the corresponding oxime. [e] 3 + 1.

Table 2. Enzymatic Alkoxycarbonylation Catalyzed by CAL-B at 30 °C of 19-*nor*-A-Ring Synthons 7−10 with Carbonate 23b

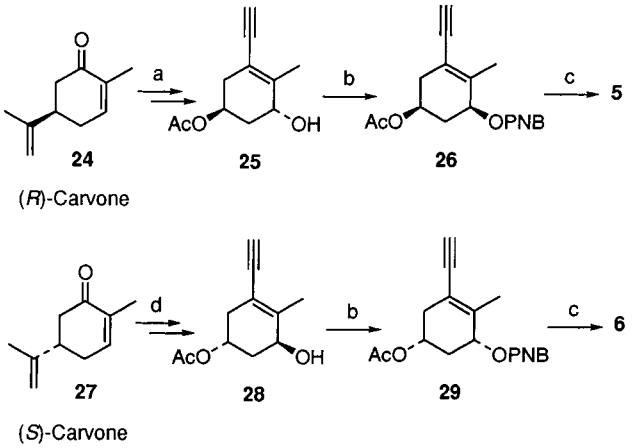
entry	substrate	**23b** (equiv)	*t* (h)	conv (%)[a]	C-3 (%)[b]	C-5 (%)[b]
1	**7**	2	14	100	10	90
2	**8**	2	18	100	80	20
3	**9**	4	24	97	5	92
4	**9**	2	26	100	4	96
5	**10**	3	20	98	98	

[a] Calculated by GC, using Method A. [b] Ratio of regioselectivity at position C-3 or C-5, calculated by ^{1}H NMR.

23b was used. Thus after 26 h, 100% conversion was achieved with a selectivity of 96% for **18b** (entry 4, Table 2). Opposite regioselectivity was obtained with isomer **10**, forming compound **20b** exclusively (entry 5, Table 2). From the experimental data reported in Table 2, we can conclude that if the configuration of C-3 is (*S*), as in **7** and **9**, CAL-B-catalyzed alkoxycarbonylation in the C-5 position, independent of its configuration. On the other hand, when C-3 possesses an (*R*)-configuration, as in **8** and **10**, the enzyme prefers this center to perform the catalysis. It is worth noting that regioselectivity is opposite for each couple of enantiomers, that is, **7** vs **8** and **9** vs **10**. Results in Table 1 and Table 2 reveal that the nature of the carbonate employed have a key influence on the regioselectivity of the biocatalytic alkoxycarbonylation reaction. While the acetone *O*-[(vinyloxy)carbonyl]oxime gave poor selectivities (Table 1), acetone *O*-(phenoxycarbonyl)oxime showed from high to excellent regioselectivities for substrates **7**−**10**.

Improved Synthesis of A-Ring *Cis* Stereoisomers 5 and 6. The synthesis of the stereoisomeric diols **5** and **6** has been previously described[2] by ourselves from diols **3** and **4**[12] through the oxidation of the C-3 allylic alcohol using Dess−Martin reagent, and subsequent reduction of the resulting ketones in a 3:1 *cis* to *trans* selectivity. Recently, an alternative procedure to protected diol **5** has also been reported[13] but with the problem of the very poor yield with which the ozonolysis step takes place on the terminal alkene in a (*R*)-carvone derivative. Because of these drawbacks, here we propose an alternative preparation to obtain single isomers **5** and **6** (Scheme 2). Thus, starting from (*R*)-carvone (**24**), alcohol **25** was obtained

Scheme 2



a: ref. 12a, 5 steps, 46% yield; b: PNBOH, PPh_3, DEAD, THF; c: NaOMe, MeOH; d: ref. 12b,c, 5 steps, 46% yield.

PNB= *p*-nitrobenzoyl

in five steps and 46% overall yield. Inversion of the later alcohol under Mitsunobu conditions using *p*-nitrobenzoic acid afforded *p*-nitrobenzoate ester **26** with total inversion of the configuration and high yield.[12a] Acetic acid instead of *p*-nitrobenzoic acid gave lower yields, since substantial amounts of starting alcohol **25** were recovered, even when longer reaction times, higher temperatures, and more equivalents of reactants were used. Simultaneous deprotection of both ester groups in derivative **26** was carried out with NaOMe in MeOH to obtain *cis*-diol **5** with excellent yield. Similarly, *cis*-diol **6** was obtained from (*S*)-carvone.

Alkoxycarbonylation of A-Ring Synthons 3−6. As previously mentioned, the CAL-B-catalyzed enzymatic alkoxycarbonylation of stereoisomeric diols **3**−**6**, which possess the C-2 methyl group in their structure, using acetone *O*-[(vinyloxy)carbonyl]oxime (**23a**), takes place with excellent regioselectivity (>97%) toward the C-5 position for compounds **3**−**5**, whereas *cis*-isomer **6** shows moderate regioselectivity (76%) to the same position, in addition to a considerable percentage (22%) of C-3,5 divinyl derivative.[3] Taking into account the excellent regioselectivity obtained when carbonate **23b** is used as the alkoxycarbonylating agent in 19-*nor*-A-ring substrates **7**−**10**, as well as the different behavior between enantiomers, we set out to study the effect of carbonate **23b** on diols **3**−**6** with two goals in mind. The first was to increase the regioselectivity of the process for isomer **6**. The second goal was to check if the enzyme keeps its structural preferences by changing the alkoxycarbony-

(12) Compounds **3** and **4** have been synthesized by Okamura from (*S*)- and (*R*)-carvone, respectively: (a) Muralidharan, K. R.; de Lera, A. R.; Issaeff, S. D.; Norman, A. W.; Okamura. W. H. *J. Org. Chem.* **1993**, *58*, 1895−1899. (b) Okamura, W. H.; Aurrecoechea, J. M.; Gibbs, R. A.; Norman, A. W. *J. Org. Chem.* **1989**, *54*, 4072−4083. (c) Aurrecoechea, J. M.; Okamura, W. H. *Tetrahedron Lett.* **1987**, *28*, 4947−4950.

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Scheme 3

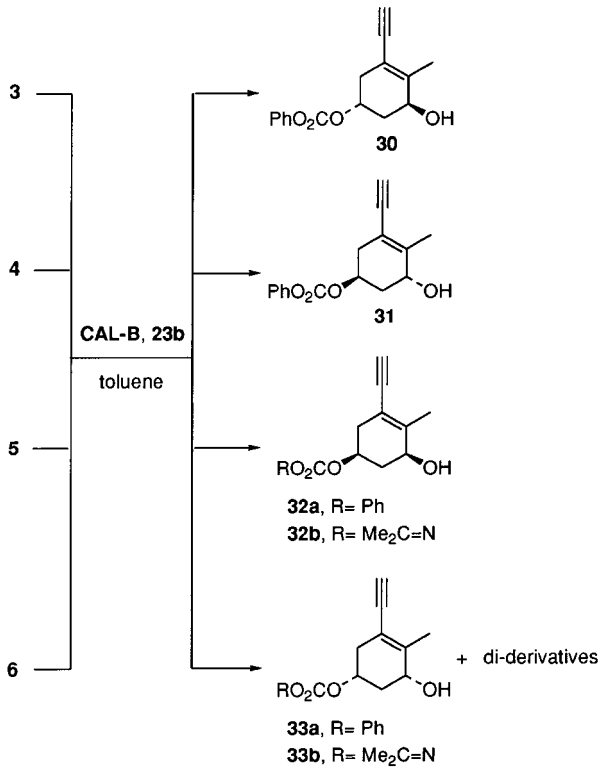


Table 3. Enzymatic Alkoxycarbonylation Catalyzed by CAL-B of A-Ring Synthons 3−6 with Carbonate 23b

entry	substrate	T (°C)	**23b** (equiv)	*t* (h)	conv (%)[a]	yield C-5 (%)[b] Ph	yield C-5 (%)[b] $Me_2C{=}N$
1	**3**	30	3	24	100	90	
2	**4**	30	3	2	100	87	
3	**5**	30	3	96	89	56	26
4	**5**	45	3	32	88	56	20
5	**5**	45	6	24	87	55	28
6	**6**	30	3	96	41[c]	6	16
7	**6**	45	3	80	92[c]	17	61
9	**6**	45	6	24	64[c]	10	38

[a] Calculated by GC, using Method B. [b] Isolated yields after flash chromatography. [c] Di-derivatives such as C-3,5 dioxime and C-3,5 phenyl-oxime carbonates were formed as minor compounds.

lating agent. We found that the use of acetone *O*-(phenoxycarbonyl)oxime for the alkoxycarbonylation of *trans*-diols **3** and **4** did not affect the regioselectivity of the reaction, and we isolated exclusively C-5 carbonates **30** and **31** (Scheme 3), respectively, with high yields (entries 1 and 2, Table 3). When the same process was carried out with isomer **5**, total regioselectivity toward the C-5 position was obtained although two products are formed, C-5 phenyl carbonate **32a**, and C-5 oxime carbonate **32b** (entry 3, Table 3). Considering the hypothesis that longer reaction times favor the formation of **32b**, and in order to prepare just **32a**, higher temperature and more equivalents of carbonate **23b** were used. No differences were observed with these changes (entries 4 and 5, Table 3). A similar pattern of results was observed with isomer **6**. In this case, C-5 oxime carbonate **33b** was the major product and, in addition to the C-5 phenyl carbonate **33a**, di-derivatives such as C-3,5 dioxime and C-3,5 phenyl-oxime were formed as minor compounds. Although two different C-5 derivatives are obtained, this fact is not an inconvenience from the synthetic point of view because both compounds, **32a**,**b** or **33a**,**b**, are appropriate synthons for use as precursors to introduce additional functionalities, taking into account that the combined isolated yields are high.

From the results in Table 2 and Table 3, it seems that the methyl group in the C-2 position is crucial to accommodate substrates **3**−**6**, independent of their stereochemistry, inside the enzyme active site in a way that mainly allows the available hydroxyl group to react at C-5 position.

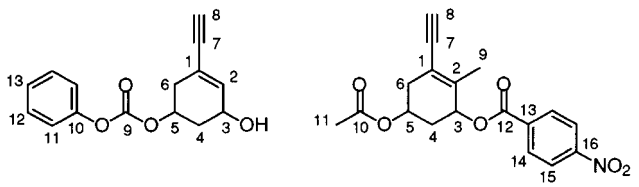
Summary

Candida antarctica lipase B (CAL-B) has catalyzed with excellent selectivity the alkoxycarbonylation process of 19-*nor*-A-ring stereoisomers **7**−**10** of 1α,25-dihydroxy-19-*nor*-previtamin D_3 with acetone *O*-(phenoxycarbonyl)-oxime (**23b**). The opposite regioselectivity shown by each couple of enantiomers (**7** vs **8** and **9** vs **10**) is noteworthy. Thus, when C-3 possesses an (*S*)-configuration (as **7** or **9**), CAL-B-catalyzed alkoxycarbonylation in the C-5 position, independent of its own configuration. Meanwhile, if C-3 has an (*R*)-configuration (as **8** or **10**), the enzyme performed the reaction at this center. For the A-ring stereoisomers of 1α,25-dihydroxyvitamin D_3 **3**−**6**, which possess the C-2 methyl group, the enzymatic process took place toward the C-5 position independent of the stereochemistry at the chiral centers. The methyl group in the C-2 position is crucial in the accommodation of these substrates inside the enzyme active site. Besides the intrinsic importance of these A-ring carbonates, this group facilitates the introduction of different functionalities. Selective modification at the C-1 or C-3 positions of 6-*s*-*cis* locked analogues (previtamin forms) or 6-*s*-*trans* derivatives of 1α,25-dihydroxyvitamin D_3, could be obtained. In addition, an improved synthesis of *cis*-diols **5** and **6** was described using a Mitsunobu procedure.

Experimental Section[14]

General. *Candida antarctica* lipase B (CAL-B, 7300 PLU/g) was a gift from Novo Nordisk Co. Reagents were purchased from Aldrich or Fluka. Solvents were distilled over an appropriate desiccant under nitrogen. Syntheses of A-ring **3**−**6**[2,12] and 19-*nor*-A-ring **7**−**10**[10] precursors were previously reported. Carbonates **30**, **32b**, and **33b** were described.[3] Oxime carbonates **23a**,**b** were synthesized as has been described.[15] Dowex 50WX4-400 ion-exchange resin (200−400 mesh) was washed with H_2O prior to use. Gas chromatography was carried out with flame ionization detection (FID) and a HP-1 capillary column (25 m × 0.2 mm × 0.33 μm) coated with methylsilicone gum, with nitrogen as carrier gas and following two different methods. **Method A**: injector and detector temperatures set at 300 °C, column initial temperature 130 °C (3 min), rate 7 °C/min until 200 °C (15 min) and then 15 °C/min, column final temperature 270 °C; acetanilide (as internal standard) appeared at 6.8 min; **7** and **8** at 5.9 min; **9** and **10** at 5.8 min; **11a** and **14a** at 10.4 min; **12a** and **15a** at 10.3 min; **13a** and

(14) Structures of the products are numbered as follows:



(15) Fernández, S.; Menéndez, E.; Gotor, V. *Synthesis* **1991**, 713−716.

16a at 14.0 min; **17a** and **20a** at 10.2 min; **18a** and **21a** at 10.3 min; **19a** and **22a** at 14.3 min. Conversion of the reaction for phenoxycarbonyl derivatives was calculated by disappearance of the starting material with respect to internal standard. **Method B**: injector and detector temperatures set at 250 °C and 275 °C, respectively, column initial temperature 150 °C (3 min), rate 18 °C/min, column final temperature 260 °C (20 min); naphthalene (as internal standard) appeared at 3.3 min; **3** and **4** at 4.7 min; **5** and **6** at 4.6 min; **30** and **31** at 11.1 min; **32a** and **33a** at 7.5 min; **32b** and **33b** at 7.6 min.

(3*S*,5*S*)-1-Ethynyl-3,5-dihydroxy-2-methylcyclohex-1-ene (5). A solution of MeONa in MeOH, prepared in situ by addition of Na (67 mg, 2.93 mmol) to MeOH (2.5 mL), was added dropwise to a solution of **26** (430 mg, 1.25 mmol) in MeOH (14 mL) at 0 °C. The reaction was stirred at this temperature for 2.5 h and then acidified with Dowex 50WX4-400 ion-exchange resin (200−400 mesh). After removal of the resin by filtration, the solution was evaporated, and the residue was purified by flash chromatography (50% EtOAc/hexane) to afford 183 mg (96%) of diol **5** as a white solid: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.55 (br s, 1H, OH), 1.86 (ddd, 1H, H4, $^2J_{HH}$ = 14.2, $^3J_{HH}$ = 5.0, $^3J_{HH}$ = 2.1 Hz), 2.06 (s, 3H, H9), 2.13−2.24 (m, 1H, H4), 2.25−2.52 (m, 2H, H6), 3.02 (br s, 1H, OH), 3.10 (s, 1H, H8), 4.04 (m, 1H, H5), and 4.28 (m, 1H, H3); HRMS (EI, *m*/*z*): Calcd for $C_9H_{12}O_2$ (M^+): 152.0837. Found: 152.0846.

(3*R*,5*R*)-1-Ethynyl-3,5-dihydroxy-2-methylcyclohex-1-ene (6). The same procedure as that described for **5** yielded **6** (89%). Spectral data are identical to that of **5** given above.

Enzymatic Alkoxycarbonylation of 7−10. Synthesis of Carbonates 11−22. In a typical procedure, CAL-B (45 mg) was added to a solution of diol **7**−**10** (9 mg, 0.066 mmol) and carbonate **23a**,**b** in toluene (2.5 mL, acetanilide is present as internal standard in 0.013 M) under nitrogen. Equivalents of alkoxycarbonylation agent, temperature, and reaction time are given in Table 1 and Table 2. The suspension was shaken, and the progress of the reaction was followed by GC analysis. Close to 100% conversion the mixture was filtered, and the solvent was removed under reduced pressure. After ^{1}H NMR analysis, the crude material was subjected to flash chromatography (15% EtOAc/hexane) to give compounds **11**−**22**.

(3*S*,5*R*)- and (3*R*,5*S*)-1-Ethynyl-5-hydroxy-3-[(vinyloxy)-carbonyloxy]cyclohex-1-ene (11a and 14a). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90−2.80 (several m, 4H, H4+H6), 2.80 (s, 1H, H8), 4.15 (m, 1H, H5), 4.55 (dd, 1H, H11-*cis*, $^3J_{HH}$ = 6.4, $^2J_{HH}$ = 2.1 Hz), 4.92 (dd, 1H, H11-*trans*, $^3J_{HH}$ = 13.6, $^2J_{HH}$ = 2.1 Hz), 5.50 (m, 1H, H3), 6.22 (m, 1H, H2), and 7.05 (dd, 1H, H10, $^3J_{HH}$ = 13.6, $^2J_{HH}$ = 6.4 Hz); MS (ESI^+, *m*/*z*): 209 [M + H]$^+$.

(3*S*,5*R*)- and (3*R*,5*S*)-1-Ethynyl-5-hydroxy-3-(phenoxy-carbonyloxy)cyclohex-1-ene (11b and 14b). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90−2.26 (m, 3H, 2H4+H6), 2.63 (dd, 1H, H6, $^2J_{HH}$ = 17.4, J_{HH} = 5.2 Hz), 2.99 (s, 1H, H8), 4.25 (m, 1H, H5), 5.44 (m, 1H, H3), 6.30 (m, 1H, H2), and 7.21−7.41 (m, 5H, ArH); MS (ESI^+, *m*/*z*): 281 [M + Na]$^+$.

(3*S*,5*R*)- and (3*R*,5*S*)-1-Ethynyl-3-hydroxy-5-[(vinyloxy)-carbonyloxy]cyclohex-1-ene (12a and 15a). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90−2.80 (several m, 4H, H4+H6), 2.80 (s, 1H, H8), 4.45 (m, 1H, H3), 4.55 (dd, 1H, H11-*cis*, $^3J_{HH}$ = 6.3, $^2J_{HH}$ = 1.9 Hz), 4.92 (dd, 1H, H11-*trans*, $^3J_{HH}$ = 13.6, $^2J_{HH}$ = 1.9 Hz), 5.21 (m, 1H, H5), 6.24 (m, 1H, H2), and 7.05 (dd, 1H, H10, $^3J_{HH}$ = 13.6, $^3J_{HH}$ = 6.3 Hz); MS (ESI^+, *m*/*z*): 209 [M + H]$^+$.

(3*S*,5*R*)- and (3*R*,5*S*)-1-Ethynyl-3-hydroxy-5-(phenoxy-carbonyloxy)cyclohex-1-ene (12b and 15b). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.02 (m, 1H, H4), 2.22 (m, 1H, H4), 2.42 (ddd, 1H, H6, $^2J_{HH}$ = 17.9, J_{HH} = 5.9, J_{HH} = 1.8 Hz), 2.71 (ddd, 1H, H6, $^2J_{HH}$ = 17.9, J_{HH} = 5.1, J_{HH} = 2.3 Hz), 2.94 (s, 1H, H8), 4.54 (m, 1H, H3), 5.19 (m, 1H, H5), 6.25 (m, 1H, H2), and 7.21−7.41 (m, 5H, ArH); MS (ESI^+, *m*/*z*): 281 [M + Na]$^+$.

(3*S*,5*R*)- and (3*R*,5*S*)-1-Ethynyl-3,5-di[(vinyloxy)carbo-nyloxy]cyclohex-1-ene (13a and 16a). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90−2.80 (several m, 4H, H4+H6), 2.81 (s, 1H, H8), 4.55 (m, 2H, H11+H14), 4.92 (m, 2H, H11+H14), 5.25 (m, 1H, H3), 5.55 (m, 1H, H5), 6.22 (m, 1H, H2), and 7.05 (m, 2H, H10+H13); MS (ESI^+, *m*/*z*): 279 [M + H]$^+$ and 301 [M + Na]$^+$.

(3*S*,5*S*)- and (3*R*,5*R*)-1-Ethynyl-5-hydroxy-3-[(vinyloxy)-carbonyloxy]cyclohex-1-ene (17a and 20a). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90−2.80 (several m, 4H, H4+H6), 2.92 (s, 1H, H8), 4.15 (m, 1H, H5), 4.53 (dd, 1H, H11-*cis*, $^3J_{HH}$ = 6.2, $^2J_{HH}$ = 2.1 Hz), 4.82 (dd, 1H, H11-*trans*, $^3J_{HH}$ = 13.9, $^2J_{HH}$ = 2.1 Hz), 5.33 (m, 1H, H3), 6.24 (m, 1H, H2), and 6.98 (dd, 1H, H10, $^3J_{HH}$ = 13.9, $^3J_{HH}$ = 6.2 Hz); MS (ESI^+, *m*/*z*): 231 [M + Na]$^+$.

(3*S*,5*S*)- and (3*R*,5*R*)-1-Ethynyl-5-hydroxy-3-(phenoxy-carbonyloxy)cyclohex-1-ene (17b and 20b). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.93−2.10 (m, 2H, H4), 2.28−2.57 (m, 2H, H6), 3.00 (s, 1H, H8), 4.09 (m, 1H, H5), 5.41 (m, 1H, H3), 6.27 (m, 1H, H2), and 7.17−7.45 (m, 5H, ArH); MS (ESI^+, *m*/*z*): 281 [M + Na]$^+$.

(3*S*,5*S*)- and (3*R*,5*R*)-1-Ethynyl-3-hydroxy-5-[(vinyloxy)-carbonyloxy]cyclohex-1-ene (18a and 21a). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90−2.80 (several m, 4H, H4+H6), 2.82 (s, 1H, H8), 4.55 (m, 1H, H3), 4.53 (dd, 1H, H11-*cis*, $^3J_{HH}$ = 6.1, $^2J_{HH}$ = 2.0 Hz), 4.82 (dd, 1H, H11-*trans*, $^3J_{HH}$ = 13.8, $^2J_{HH}$ = 2.0 Hz), 5.13 (m, 1H, H5), 6.24 (m, 1H, H2), and 6.98 (dd, 1H, H10, $^3J_{HH}$ = 13.8, $^3J_{HH}$ = 6.1 Hz); MS (ESI^+, *m*/*z*): 231 [M + Na]$^+$.

(3*S*,5*S*)- and (3*R*,5*R*)-1-Ethynyl-3-hydroxy-5-(phenoxy-carbonyloxy)cyclohex-1-ene (18b and 21b). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.95−2.21 (m, 2H, H4), 2.57 (m, 2H, H6), 2.96 (s, 1H, H8), 4.37 (m, 1H, H3), 5.15 (m, 1H, H5), 6.32 (m, 1H, H2), and 7.16−7.42 (m, 5H, ArH); MS (ESI^+, *m*/*z*): 281 [M + Na]$^+$.

(3*S*,5*S*)- and (3*R*,5*R*)-1-Ethynyl-3,5-di[(vinyloxy)carbo-nyloxy]cyclohex-1-ene (19a and 22a). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90−2.80 (several m, 4H, H4+H6), 2.82 (s, 1H, H8), 4.53 (m, 2H, H11+H14), 4.82 (m, 2H, H11+H14), 4.95 (m, 1H, H3), 5.43 (m, 1H, H5), 6.25 (m, 1H, H2), and 6.99 (m, 2H, H10+H13); MS (ESI^+, *m*/*z*): 279 [M + H]$^+$ and 301 [M + Na]$^+$.

(3*S*,5*S*)-5-Acetoxy-1-ethynyl-2-methyl-3-[(4-nitrophenyl)-carbonyloxy]cyclohex-1-ene (26). To a stirred solution of **25** (300 mg, 1.54 mmol) in THF (21 mL) under nitrogen were added 4-nitrobenzoic acid (516 mg, 3.09 mmol), PPh_3 (810 mg, 3.09 mmol), and diethyl azodicarboxylate (0.48 mL, 3.09 mmol). The mixture was stirred for 1 h at room temperature and then evaporated under reduced pressure to leave a residue which was purified by flash chromatography (10% EtOAc/hexane); oil, yield 472 mg (89%): ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.95 (s, 3H, H9), 1.98 (s, 3H, H11), 2.12 (ddd, 1H, H4, $^2J_{HH}$ = 13.9, $^3J_{HH}$ = 8.3, $^3J_{HH}$ = 6.1 Hz), 2.31 (ddd, 1H, H4, $^2J_{HH}$ = 13.5, $^3J_{HH}$ = 5.7, $^3J_{HH}$ = 3.1 Hz), 2.43 (m, 1H, H6), 2.58 (d, 1H, H6, $^2J_{HH}$ = 17.0 Hz), 3.21 (s, 1H, H8), 5.08 (m, 1H, H5), 5.70 (apparent t, 1H, H3, $^3J_{HH}$ = ∼5.6 Hz), 8.19 (d, 2H, H15, $^3J_{HH}$ = 2.2 Hz), and 8.29 (d, 2H, H14, $^3J_{HH}$ = 2.2 Hz); MS (ESI^+, *m*/*z*): 366 [M + Na]$^+$.

(3*R*,5*R*)-5-Acetoxy-1-ethynyl-2-methyl-3-[(4-nitrophen-yl)carbonyloxy]cyclohex-1-ene (29). The same procedure as that described for **26** yielded **29** (87%). Spectral data are identical to that of **26** given above.

Enzymatic Alkoxycarbonylation of 3−6. Synthesis of Carbonates 30−33. In a typical procedure, CAL-B (89 mg) was added to a solution of diol **3**−**6** (20 mg, 0.13 mmol), and carbonate **23b** in toluene (5 mL, naphthalene is present as internal standard in 0.066 M) under nitrogen. Equivalents of **23b**, temperature, and reaction time are given in Table 3. The suspension was shaken and the progress of the reaction was followed by GC analysis. After removal of the enzyme by filtration, evaporation of the solvent, and ^{1}H NMR analysis, the residual mixture was purified by flash chromatography (gradient eluent 10−50% EtOAc/hexane) to give compounds **30**−**33**.

(3*S*,5*R*)- and (3*R*,5*S*)-1-Ethynyl-3-hydroxy-2-methyl-5-(phenoxycarbonyl)cyclohex-1-ene (30 and 31). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90 (br s, 1H, OH), 2.05 (s, 3H, H9), 2.11 (m, 2H, H4), 2.41 (dd, 1H, H6, $^2J_{HH}$ = 17.2, $^3J_{HH}$ = 7.0 Hz), 2.76 (dd, 1H, H6, $^2J_{HH}$ = 17.2, $^3J_{HH}$ = 5.0 Hz), 3.14 (s, 1H, H8), 4.34 (br s, 1H, H3), 5.14 (m, 1H, H5), and 7.15−745

(m, 5H, ArH); HRMS (EI, *m*/*z*) Calcd. for $C_{16}H_{16}O_4$: 272.1049. Found: 272.1050.

(3*S*,5*S*)- and (3*R*,5*R*)-1-Ethynyl-3-hydroxy-2-methyl-5-(phenoxycarbonyloxy)cyclohex-1-ene (32a and 33a). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.70 (br s, 1H, OH), 2.09 (s, 3H, H9), 2.13 (m, 1H, H4), 2.30 (ddd, 1H, H4, $^2J_{HH} = 14.6$, $^3J_{HH} = 6.4$, $^3J_{HH} = 4.1$ Hz), 2.59 (m, 2H, H6), 3.16 (s, 1H, H8), 4.13 (m, 1H, H3), 5.17 (m, 1H, H5), 7.15−7.45 (m, 5H, ArH); MS (ESI$^+$, *m*/*z*): 272 $[M]^+$, 295 $[M + Na]^+$, and 311 $[M + K]^+$.

(3*S*,5*S*)- and (3*R*,5*R*)-1-Ethynyl-3-hydroxy-2-methyl-5-[(acetonoxime)carbonyloxy]cyclohex-1-ene (32b and 33b): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.98 (s, 3H, H12), 1.99 (s, 3H, H12), 1.97−2.10 (m, 1H, H4), 2.05 (br s, 3H, H9), 2.24 (dddd, 1H, H4, $^2J_{HH} = 14.4$, $^3J_{HH} = 5.9$, $^3J_{HH} = 3.6$, $^4J_{HH} = 1.0$ Hz), 2.41−2.67 (m, 2H, H6), 2.70 (br s, 1H, OH), 3.11 (s, 1H, H8), 4.06 (m, 1H, H3), and 5.20 (m, 1H, H5).); MS (70 eV, *m*/*z*): 251 (M + , <1%), 134 (19), 119 (23), 91 (60), and 156 (100).

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Supporting Information Available: Complete ^{1}H and ^{13}C NMR spectral data in addition to mp, IR, microanalysis, and MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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