Selective Alkoxycarbonylation of A-Ring Precursors of Vitamin D Using Enzymes in Organic Solvents. Chemoenzymatic Synthesis of 1α,25-Dihydroxyvitamin D_3 C-5 A-Ring Carbamate Derivatives[1]

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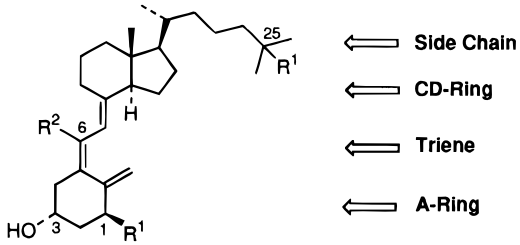
A-Ring modification of 1α,25-dihydroxyvitamin D_3 [**2**, 1α,25-$(OH)_2$-D_3] is an important area of analog studies to investigate biological activity of vitamin D-related structures. An efficient synthesis of 1α,25-$(OH)_2$-D_3 C-5 A-ring carbamate derivatives **19** and amino acid derivatives **21** was developed by applying a two-step chemoenzymatic strategy, involving the enzymatic synthesis of carbonates followed by reaction with amino derivatives. Accordingly, we began the studies of enzymatic alkoxycarbonylation of 1α,25-$(OH)_2$-D_3 A-ring precursor **7**. *Candida antarctica* lipase (CAL) was found to be the best catalyst in toluene. Regioselective alkoxycarbonylation occurred only at the C-5-(*R*) hydroxyl group. Good to excellent yields were achieved by chemical reaction of these carbonates with amino derivatives. The procedure provided convenient synthesis of carbamates **19** and **21** under mild reaction conditions.

Introduction

Vitamin D_3 (**1**), through its hormonally active form 1α,25-dihydroxyvitamin D_3 [**2**, 1α,25-$(OH)_2$-D_3], plays an important role in the endocrine system (Chart 1). Because of the biological profile of **2** and the promising clinical application of some analogs, which have been investigated for treatment of diverse diseases such as psoriasis, renal osteodystrophy, rickets, osteoporosis, several cancer types, AIDS, and Alzheimer's disease,[2] the synthesis of potential chemotherapeutic vitamin D drugs has been one of the most interesting aims of vitamin D research.[3]

The classical biological responses associated with vitamin D actions are calcitropic, which induces intestinal calcium absorption (ICA) and bone calcium mobilization (BCM). However, it has been shown that 1α,25-$(OH)_2$-D_3 induces differentiation and affects cellular proliferation, opening its use in the treatment of certain cancers and skin disorders.[4] The clinical utility of 1α,25-$(OH)_2$-D_3 is limited because therapeutically effective doses induce hypercalcemia through its normal action in stimulating ICA and BCM.[5] This has prompted discovery of more selective analogs, specifically directed toward derivatives with high cell differentiating ability and low calcitropic action (ICA and BCM).

Chart 1



1, $R^1 = R^2 = H$ Vitamin D_3

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

3, $R^1 = H$; $R^2 = F$ 6-F-D_3

One of the strategies entails studies of analogs in terms of their ability to bind to various receptors and other proteins. For example, the binding to the plasma protein (DBP) is a crucial factor determining the half-life and uptake of the analogs in different tissues by regulating their free or available concentration.[6] In the vitamin D field, the indirect affinity-labeling approach might still be the only practical method to determine the topography of the binding sites of the hormone-specific proteins (DBP or VDR) as long as a reasonable quantity of ligand bound complex is difficult to obtain.[7]

A-ring modification of 1α,25-$(OH)_2$-D_3 is the second most extensive area of analog studies next to side chain modifications. The two hydroxyl groups at C-1 and C-25 in 1α,25-$(OH)_2$-D_3 are known to be essential for optimum

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(1) (a) Note that α (down) and *β* (up) have their usual definitions for the structures of vitamin D_3 and its analogs. Because vitamin D is typically drawn in its more stable nonsteroidal, 6-s-trans conformation, the α's (up) and *β*'s (down) are reversed in orientation for its A-ring only. Analogously, the numbering of the vitamin D and derivatives are the same as steroidal structures, but note that A-ring alone has different numbering, compare Chart 1 with Scheme 2. Thus, carbons 1 and 3 in vitamin D structures correspond with carbons 3 and 5 in the A-ring, respectively. (b) For a preliminary account of part of this study, see: Ferrero, M.; Fernández, S.; Gotor, V. *New Frontiers in Screening for Microbial Biocatalysts*; Ede: The Netherlands Dec 15−18, 1996, commun. 18 (to be published).

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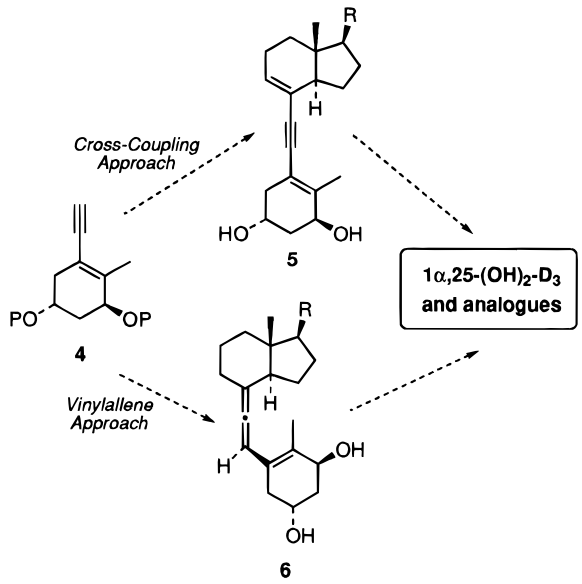
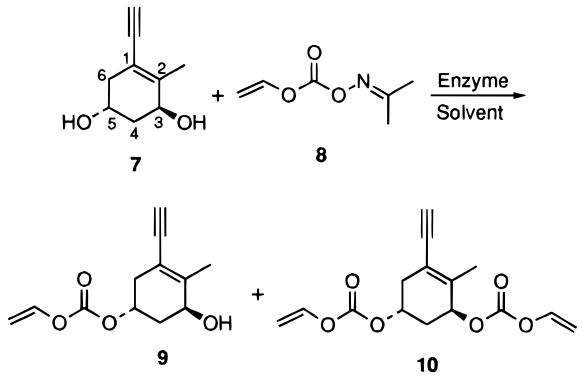
Scheme 1



Scheme 2



binding to the VDR, but 6-fluorovitamin D_3 (**3**, Chart 1) is the first analog lacking these hydroxyls to be observed to bind significantly to the n-VDR in vitro.[8] It is the purpose of this article to provide several A-ring precursors which could be converted by known chemical strategies to analogs of steroid hormone 1α,25-$(OH)_2$-D_3. Two of the major synthetic routes utilized in recent years to synthesize the hormone 1α,25-$(OH)_2$-D_3 and its various analogs are depicted in Scheme 1. The cross-coupling approach, wherein type **5** dienynes are semihydrogenated to a previtamin structure which undergoes rearrangement to the corresponding vitamin D analogs, was developed by Lythgoe.[9] A main contribution to this method was the synthesis Okamura developed for **4**, starting with (*S*)-carvone.[10] The vinylallene approach[11] involves the production of **6** from **4** and the subsequent rearrangement of the former using either heat or a combination of metal-catalyzed isomerization followed by sensitized photoisomerization.[12]

The synthetic potential of enzymes in organic solvents has been well documented in the last few years.[13] Esterification reactions have been commonly used whereas the alkoxycarbonylation reaction has scarcely been investigated.[14] Previously we reported regioselective enzymatic transformations of several A-ring precursors using lipase-mediated reactions in organic solvents.[15] Here we report a systematic study of the enzyme-catalyzed alkoxycarbonylation in organic solvents of the 1α,25-$(OH)_2$-D_3 A-ring precursors to obtain very interesting intermediates that provide us with access to new and very promising A-ring synthons of vitamin D analogs, including **19** and **21**.

Results and Discussion

The studies of enzymatic alkoxycarbonylation were focused on the A-ring fragment **7** (Scheme 2),[10,11] which possesses the natural enyne stereochemistry (3*S*,5*R*) used to prepare certain 1α,25-$(OH)_2$-D_3 analogs. Initial experiments concerned screening *Chromobacterium viscosum* lipase (CVL), *Pseudomonas cepacia* lipase (PSL), and *Candida antarctica* lipase (CAL) as catalysts to determine which enzyme gives the best regioselectivity for alkoxycarbonylation. For the initial studies (vinyloxy)carbonyl oxime **8** was used. In principle, alkoxycarbonylation could occur in two ways, since the leaving group could be the acetone oxime moiety[16] or the vinyloxy group.[17] We first focused our attention on CVL in THF using a ratio of carbonate **8** to diol **7** of 10:1 because this gave the best results in previous studies of acylation.[15]

At 30 °C the starting material was recovered, while at 60 °C the reaction proceeded with poor conversion of the hydroxyl group at the C-5 position, the acetone oxime moiety acting as leaving group (see entries 1 and 2; Table 1). Recognizing that significant changes in enzymatic properties[18] can be affected simply by changing the solvent utilized in the reaction, we investigated the effect of other solvents on this lipase-catalyzed alkoxycarbonylation process. Different behavior was observed depending on solvent. When 1,4-dioxane or toluene was used, the conversion was higher. In both cases, traces of **10**, which is the bis-vinyloxycarbonylation product, were observed by gas chromatography (GC) analysis.

In order to find the best enzyme-solvent system for alkoxycarbonylation of the C-5-(*R*) hydroxyl group, with total conversion and good yield, other enzymes were tested. Negative results were observed when PSL was used as catalyst and THF as solvent at 30 °C or 60 °C (entries 5 and 6; Table 1), when no reaction was produced. The change from THF to 1,4-dioxane or toluene showed an improvement in the process. The combination

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Table 1. Reaction of Diol 7 with (Vinyloxy)carbonyl Oxime 8 Catalyzed by Enzymes[a]

entry	enzyme	solvent	*T* (°C)	*t* (h)	conv (%)[b]	**7** (%)[b]	**9** (%)[b]	**10** (%)[b]
1	CVL	THF	30	94.5	0	100	–	–
2	CVL	THF	60	87	38	62	38	–
3	CVL	1,4-dioxane	30	94	52.4	47.7	51.5	0.9
4	CVL	toluene	30	80	80.7	19.3	78.9	1.8
5	PSL	THF	30	94.5	0	100	–	–
6	PSL	THF	60	87	0	100	–	–
7	PSL	1,4-dioxane	30	94	49.0	51.1	43.5	5.5
8	PSL	toluene	30	23	100	–	80	20
9	CAL[c]	THF	30	94.5	0	100	–	–
10	CAL[c]	THF	60	87	82	18	82	–
11	CAL[c]	1,4-dioxane	30	94	83.4	16.6	79.4	4.0
12	CAL[c]	toluene	30	4	100	–	100[d]	–

[a] These processes were carried out using a ratio of 1:10 (**7**:**8**). [b] Calculated by GC analysis. [c] SP 435L. [d] 98% isolated yield.

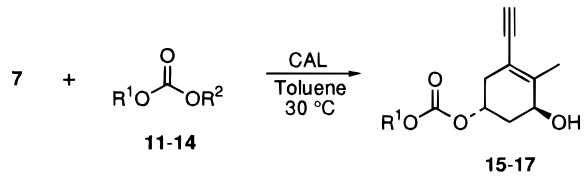
of PSL and toluene gave a higher conversion of **9** (80%), although an important amount of diprotected product **10** was also obtained (20%) (entry 8; Table 1).

The best results were obtained when CAL was used as catalyst. In the initial screening no reaction was observed in THF at 30 °C. But when the temperature was raised, the process took place with good conversion and generated a unique C-5-(*R*) regioisomer. When 1,4-dioxane was used, the percentage of conversion was also high, but in this case only a small amount of product **10** was formed (entry 11; Table 1). Of the enzyme-solvent systems studied, CAL-toluene gave the best result. It catalyzed vinyloxycarbonylation with total conversion in 4 h at 30 °C. Only the C-5-(*R*) hydroxyl group was protected and with excellent yield (98% of isolated product) (entry 12; Table 1). It is noteworthy that no C-3 alkoxycarbonylation products were observed in any of these processes, and alkoxycarbonylation did not take place in the absence of enzyme even if stronger conditions were used. However, a great difference in reaction time was observed when the solvent was changed from 1,4-dioxane to toluene in all three cases. Especially significant was when CAL was used as catalyst. Thus, after 94 h in 1,4-dioxane the reaction did not evolve further (83.4% conversion) because the enzyme became inactive, whereas in toluene CAL catalyzed the alkoxycarbonylation process with 100% of conversion in 4 h.

The above-mentioned structures were determined by means of their spectral data. With regard to the ^{1}H-NMR, the more significant change occurs on the multiplet corresponding to H_5 (Scheme 2), which changes from 4.11 ppm in **7** to 5.07 ppm in **9**. In addition, ^{13}C-NMR spectrum presents a shift of ca. 8 ppm on the C-5 of carbonate **9** (from 62.9 ppm in **7** to 71.3 ppm in **9**). However, dicarbonate **10** displays a downfield shift of both protons corresponding to the triplet of H_3 (from 4.26 ppm to 5.37 ppm) and the multiplet of H_5 (from 4.11 ppm to 5.06 ppm), and in ^{13}C-NMR spectrum a shift of 6 ppm on the C-3 of **10** (from 68.8 ppm in **7** to 74.8 ppm in **10**) plus ca. 8 ppm on the C-5 of **10** (from 62.9 ppm in **7** to 70.5 ppm in **10**). Complete ^{1}H- and ^{13}C-NMR spectral data are given in the Experimental Section, as well as microanalyses and high resolution mass spectra (HRMS).

We subsequently examined a variety of alkoxycarbonylating agents to study the utility of CAL in toluene and take advantage of the fact that CAL shows excellent vinyloxycarbonylation selectivity and yield (Scheme 3). Reactions were run at 30 °C using a ratio of carbonates **11**−**14** to diol **7** of 10:1. As Scheme 3 indicates, good to excellent yields of carbonates **15**−**17** were achieved in 6 to 72 h. Alkoxycarbonylation occurred selectively at the C-5-(*R*) hydroxyl in all cases. Thus, the methoxycarbonyl

Scheme 3



Carbonate	R^1	R^2	Ratio	t (h)	Product	Yield (%)
11	Me	$Me_2C{=}N$	1:10	6	**15**	80
11	Me	$Me_2C{=}N$	1:5	12.5	**15**	70
12	$PhCH_2$	$Me_2C{=}N$	1:10	72	**16**	85
13	Ph	$Me_2C{=}N$	1:10	36	**17**	90
14	Ph	$CH_2{=}CH$	1:10	24	**17** + **9**	[a]

[a] As a mixture 1.4:1 of carbonates **17** (54% isolated yield) and **9** (33.5% isolated yield).

moiety was incorporated into **7** when methoxycarbonyl oxime **11** was used, obtaining **15** exclusively. When we used smaller ratio of carbonates (1:5), longer reaction times were required and a slight decrease in yield was observed. In both cases, after purification of compound **15** by flash chromatography, carbonate **11** was recovered. When acetone *O*-[(benzyloxy)carbonyl]oxime (**12**) was used this process proved to be of great utility in the one-step regioselective protection (through introduction of Cbz) of the enynediol **7** in mild conditions and with high yield (85%). With the aim of testing the possible different reactivity between oxime carbonates and vinyl carbonates, reactions with acetone *O*-(phenoxycarbonyl)oxime (**13**) and phenyl vinyl carbonate (**14**) were run. Carbonate **17** was obtained with excellent yield (90%) when the process was carried out with carbonate **13**. On the other hand, if carbonate **14** was used, the starting material reacted completely after 24 h, but phenyl carbonate **17** and vinyl carbonate **9** were obtained as a mixture, 1.4:1, respectively (by GC).

In order to demonstrate the synthetic utility of these A-ring fragment derivatives, the carbamates **19** were synthesized from carbonates **9**. Four kinds of compounds have been tested, as a proof of the versatility of the procedure: (a) ammonia, (b) amines (primary and secondary aliphatic and primary aromatic), (c) amino alcohols, and (d) diamines. In the first place, ammonolysis of **9** was carried out in THF, bubbling NH_3 through at 0 °C for 30 min, and then leaving to react for 18 h at room temperature, until the disappearance of the starting

Scheme 4

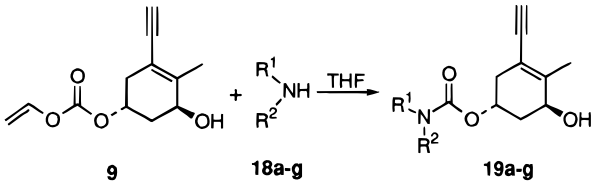


Table 2. Reaction of Carbonate 9 with Amino Derivatives 18[a]

	compound 18					
entry	R^1	R^2	ratio **9**:**18**	*T* (°C)	*t* (h)	**19** (%)[b]
1	H	H	c	25	18	100
2	Bu	H	1:2.5	30	7	–
3	Bu	H	1:2.5	60	39	–
4	Bu	H	1:10	60	24	78
5	Ph	H	1:3	60	96	–
6	Ph	H	excess **18c**	reflux	72	–
7	Pr	Pr	excess **18d**	reflux	90	–
8	$HO(CH_2)_2$	H	1:2.5	60	48	80
9	$HO(CH_2)_6$	H	1:2.5	60	48	82
10	$H_2N(CH_2)_3$	H	1:2.5	60	48	76

[a] These processes were carried out in THF. [b] Isolated yield. [c] Bubbling at 0 °C on the reaction solution.

carbonate (TLC monitoring) (Scheme 4). Carbamate **19a** was formed in quantitative yield, as the sole product (entry 1; Table 2), which was shown to be essentially pure by TLC and spectroscopic analysis.

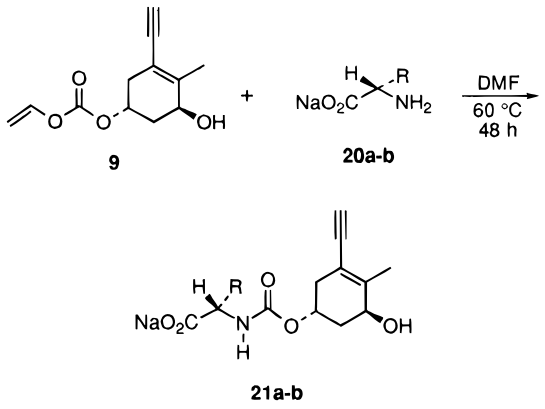
The next step was the reaction of primary aliphatic amines with vinyl carbonate **9**. Aminolysis reaction between butylamine and carbonate **9** required heating at 60 °C in THF with excess amine **18b** (10:1) for 24 h to provide carbamate **19b** (entries 2−4; Table 2). In the case of aromatic amines, no reaction was observed even when a big excess of aniline was used at reflux in THF (entries 5 and 6; Table 2). Secondary amine, dipropylamine in excess in THF at reflux over 90 h gave no reaction (entry 7; Table 2).

Interesting products to synthesize are A-ring carbamate derivatives reached from linear amino alcohols or diamines. Carbonate **9** was allowed to react with 2-amino-1-ethanol (**18e**), 6-amino-1-hexanol (**18f**), and 1,3-propanediamine (**18g**) at 60 °C in THF for approximately 2 days to provide carbamates **19e**−**g** in good yields (entries 8−10; Table 2).

We extended the aforementioned methodology to synthesize some amino acid A-ring precursors. The best results were obtained submitting the corresponding vinyloxycarbonylated C-5-(*R*) 1α,25-$(OH)_2$-D_3 A-ring precursor **9** to reaction with the amino acid sodium salts **20a**,**b** in DMF at 60 °C to provide **21a**,**b** (Scheme 5). Generally, sonication of the amino acid salts was performed to improve dissolution.

The structural assignment of the compounds described in this paper is based on the analysis of their ^{1}H- and ^{13}C-NMR spectra. Additional DEPT experiments and the correct assignment were confirmed by ^{1}H−^{13}C heteronuclear correlation experiments. Thus for example, heteronuclear correlation of compound **19g** showed, for the methyl protons at 2.16 ppm, the cross peaks with the carbon atoms at 68.95, 115.21, and 144.54 ppm corresponding to the C_3 ($^3J_{CH}$), C_1 ($^3J_{CH}$), and C_2 ($^2J_{CH}$), respectively.[19] The signal at 115.21 ppm further correlates with the double doublets at 2.33 and 2.74 ppm arising from the methylene diasterotopic protons H_6 ($^2J_{CH}$), with the triplet at 4.38 ppm corresponding to the

Scheme 5



R	Amino Acid	A-Ring Derivative	Yield (%)
H	**20a**, Glycine	**21a**	87
CH_2SMe	**20b**, *S*-Methyl-L-Cysteine	**21b**	91

H_3 ($^3J_{CH}$), and with the multiplet at 5.17 ppm which belongs to the H_5 ($^3J_{CH}$). The full assignment of the ^{1}H- and ^{13}C-NMR spectra is given in the Experimental Section.

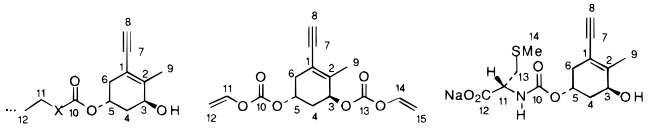
Summary

A chemoenzymatic procedure has been shown, involving the enzymatic transesterification of carbonates and the subsequent transformation with ammonia, amines, amino alcohols, diamines, and amino acids to obtain the corresponding 1α,25-$(OH)_2$-D_3 A-ring C-5 carbamates synthons. Evaluation of the enzymatic alkoxycarbonylation of the vitamin D A-ring enyne **7** revealed that CAL is the best enzyme for effecting practical levels of regioselectivity. The leaving group was always the acetone oxime moiety (except for carbonate **14**), giving rise to regioselective formation of the corresponding C-5-(*R*) carbonate derivatives. Direct application of these carbonate derivatives provided convenient formation of carbamates **19** and amino acid derivatives **21**, useful precursors of analogs of 1α,25-$(OH)_2$-D_3 for pharmaceutical research.

Experimental Section[19]

General. *Chromobacterium viscosum* lipase, CVL, was a gift from Genzyme Co.; *Pseudomonas cepacia* lipase, PSL, was obtained from Amano Pharmaceutical Co. and *Candida antarctica* lipase, CAL SP 435L, was donated by Novo Nordisk Co. All other reagents were purchased from Aldrich. Solvents were distilled over an adequate desiccant under nitrogen. A-ring synthon **7** was obtained according to the method of Okamura.[10,11] Vinyl carbonate **14**, oxime carbonates **8** and **11**-**13** were synthesized as has been previously reported.[16b] Amino acid sodium salts were prepared dissolving the corresponding L-amino acids in water adding NaOH (1 N) and evaporating under vacuum.

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



Enzymatic Alkoxycarbonylation of Diol 7 with Acetone *O*-[(Vinyloxy)carbonyl]oxime (8). Synthesis of (3*S*,5*R*)-1-Ethynyl-3-hydroxy-2-methyl-5-[(vinyloxy)carbonyl]-1-cyclohexene (9). To a solution of **7** (10 mg, 0.066 mmol) in 2.5 mL of solvent (THF, 1,4-dioxane, or toluene) was added one of the following enzymes: 200 mg of PSL, 10 mg of CVL, or 45 mg of CAL and carbonate **8** (94.5 mg, 0.66 mmol). The suspension was shaken at 30 °C or 60 °C, and the progress of the reaction was followed by TLC and GC analysis until no further reaction was apparent. After removal of the enzyme by filtration and evaporation of the solvent and ^{1}H-NMR analysis, the residual mixture was purified by HPLC (Spherisorb W, 1 × 25 cm, 5 μm silica gel 60 column, 15% ethyl acetate/hexanes, 4 mL/min) to give monovinyloxycarbonylation product **9** (or **9** and bis(vinyl carbonate) **10** depending on conditions). All these data are summarized in Table 1. **9**: ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.01 (s, 3H, H_9), 2.05 (m, 2H, H_4), 2.16 (br s, 1H, OH), 2.31 (dd, 1H, H_6, $^2J_{HH}$ 17.2, $^3J_{HH}$ 7.5 Hz), 2.67 (dd, 1H, H_6, $^2J_{HH}$ 17.2, $^3J_{HH}$ 3.7 Hz), 3.10 (s, 1H, H_8), 4.29 (br s, 1H, H_3), 4.58 (dd, 1H, H_{12}-cis, $^3J_{HH}$ 6.4, $^2J_{HH}$ 2.1 Hz), 4.91 (dd, 1H, H_{12}-trans, $^3J_{HH}$ 13.8, $^2J_{HH}$ 2.1 Hz), 5.07 (m, 1H, H_5), and 7.06 (dd, 1H, H_{11}, $^3J_{HH}$ 13.8, $^3J_{HH}$ 6.4 Hz); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 18.32 (C_9), 34.66 (C_4), 35.99 (C_6), 68.03 (C_3), 71.29 (C_5), 80.93 (C_8), 82.37 (C_7), 97.88 (C_{12}), 113.56 (C_1), 142.37 (C_{11}), 142.81 (C_2), and 152.00 (C_{10}). Anal. Calcd (%) for $C_{12}H_{14}O_4$: C, 64.84; H, 6.35. Found: C, 64.7; H, 6.4. HRMS (*m*/*z*) Calcd for $C_{12}H_{14}O_4$: 222.0892. Found: 222.0895.

(3*S*,5*R*)-1-Ethynyl-2-methyl-3,5-bis[(vinyloxy)carbonyl]-1-cyclohexene (10): ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.97 (s, 3H, H_9), 2.13 (m, 1H, H_4), 2.25 (m, 1H, H_4), 2.35 (dd, 1H, H_6, $^2J_{HH}$ 16.6, $^3J_{HH}$ 6.4 Hz), 2.75 (dd, 1H, H_6, $^2J_{HH}$ 16.6, $^3J_{HH}$ 3.4 Hz), 3.18 (s, 1H, H_8), 4.60 (dd, 1H, H_{12}-cis, $^3J_{HH}$ 6.0, $^2J_{HH}$ 2.1 Hz), 4.62 (dd, 1H, H_{15}-cis, $^3J_{HH}$ 6.2, $^2J_{HH}$ 1.9 Hz), 4.93 (dd, 1H, H_{12}-trans, $^3J_{HH}$ 13.8, $^2J_{HH}$ 2.1 Hz), 4.94 (dd, 1H, H_{15}-trans, $^3J_{HH}$ 14.0, $^2J_{HH}$ 1.9 Hz), 5.06 (m, 1H, H_5), 5.37 (t, 1H, H_3, $^3J_{HH}$ 4.7 Hz), 7.07 (dd, 1H, H_{11}, $^3J_{HH}$ 12.5, $^3J_{HH}$ 6.9 Hz), and 7.09 (dd, 1H, H_{14}, $^3J_{HH}$ 12.5, $^3J_{HH}$ 6.9 Hz); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 18.23 (C_9), 32.87 (C_4), 34.52 (C_6), 70.51 (C_5), 74.77 (C_3), 81.64 (C_7), 82.23 (C_8), 98.06 (C_{12}), 98.17 (C_{15}), 116.88 (C_1), 137.90 (C_2), 142.33 (C_{11}), 142.43 (C_{14}), 151.84 (C_{10}), and 152.33 (C_{13}). Anal. Calcd (%) for $C_{15}H_{16}O_6$: C, 61.62; H, 5.52. Found: C, 61.7; H, 5.3. HRMS (*m*/*z*) Calcd for $C_{15}H_{16}O_6$: 292.0947. Found: 292.0951.

Enzymatic Alkoxycarbonylation of 7 with other Carbonates. In a typical procedure, *C. antarctica* lipase (90 mg) was added to a solution of **7** (20 mg, 0.131 mmol) and carbonates **11**−**14** (0.657 mmol or 1.314 mmol) in 5 mL of toluene as summarized in Scheme 3. The suspension was shaken at 30 °C, and the progress of the reaction was followed by TLC and GC analysis. At 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 HPLC (Kromasil 60, 2 × 25 cm, 7 μm silica gel column, 20% ethyl acetate/hexanes for **15** and **16** and 15% EtOAc/hexanes for **17**, 8 mL/min) to give compounds **15**−**17**.

(3*S*,5*R*)-1-Ethynyl-3-hydroxy-5-(methoxycarbonyl)-2-methyl-1-cyclohexene (15). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.01 (s, 3H, H_9), 2.02 (m, 2H, H_4), 2.11 (br s, 1H, OH), 2.28 (dd, 1H, H_6, $^2J_{HH}$ 17.0, $^3J_{HH}$ 7.1 Hz), 2.65 (dd, 1H, H_6, $^2J_{HH}$ 17.0, $^3J_{HH}$ 3.7 Hz), 3.09 (s, 1H, H_8), 3.77 (s, 3H, H_{11}), 4.27 (br s, 1H, H_3), and 5.01 (m, 1H, H_5); ^{13}C-NMR ($CDCl_3$, 50.3 MHz): δ 18.31 (C_9), 34.83 (C_4), 36.16 (C_6), 54.68 (C_{11}), 68.17 (C_3), 70.44 (C_5), 80.79 (C_8), 82.49 (C_7), 113.76 (C_1), 142.76 (C_2), and 155.03 (C_{10}). Anal. Calcd (%) for $C_{11}H_{14}O_4$: C, 62.83; H, 6.72. Found: C, 63.0; H, 6.7. HRMS (*m*/*z*) Calcd for $C_{11}H_{14}O_4$: 210.0892. Found: 210.0900.

(3*S*,5*R*)-5-[(Benzyloxy)carbonyl]-1-ethynyl-3-hydroxy-2-methyl-1-cyclohexene (16). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.78 (br s, 1H, OH), 2.01 (s, 3H, H_9), 2.03 (m, 2H, H_4), 2.30 (dd, 1H, H_6, $^2J_{HH}$ 17.1, $^3J_{HH}$ 7.3 Hz), 2.67 (dd, 1H, H_6, $^2J_{HH}$ 17.1, $^3J_{HH}$ 4.5 Hz), 3.10 (s, 1H, H_8), 4.28 (t, 1H, H_3, $^3J_{HH}$ 4.7 Hz), 5.04 (m, 1H, H_5), 5.15 (s, 2H, H_{11}), and 7.37 (m, 5H, ArH); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 18.31 (C_9), 34.80 (C_4), 36.12 (C_6), 68.14 (C_3), 69.58 (C_{11}), 70.54 (C_5), 80.78 (C_8), 82.49 (C_7), 113.74 (C_1), 128.36 (C_{13}), 128.55 (C_{12}+C_{14}), 134.96 (C_{15}), 142.77 (C_2), and 154.39 (C_{10}). Anal. Calcd (%) for $C_{17}H_{18}O_4$: C, 71.30; H, 6.34. Found: C, 71.5; H, 6.6. HRMS (*m*/*z*) Calcd for $C_{17}H_{18}O_4$: 286.1205. Found: 286.1211.

(3*S*,5*R*)-1-Ethynyl-3-hydroxy-2-methyl-5-(phenoxycarbonyl)-1-cyclohexene (17). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.90 (br s, 1H, OH), 2.05 (s, 3H, H_9), 2.11 (m, 2H, H_4), 2.41 (dd, 1H, H_6, $^2J_{HH}$ 17.2, $^3J_{HH}$ 7.0 Hz), 2.76 (dd, 1H, H_6, $^2J_{HH}$ 17.2, $^3J_{HH}$ 5.0 Hz), 3.14 (s, 1H, H_8), 4.34 (br s, 1H, H_3), 5.14 (m, 1H, H_5), 7.25 (m, 3H, $2H_m$+H_p), and 7.40 (m, 2H, H_o); ^{13}C-NMR ($CDCl_3$, 50.3 MHz): δ 18.32 (C_9), 34.78 (C_4), 36.12 (C_6), 68.13 (C_3), 71.39 (C_5), 80.92 (C_8), 82.44 (C_7), 113.68 (C_1), 120.93 (C_{12}), 126.00 (C_{14}), 129.42 (C_{13}), 142.82 (C_2), 150.92 (C_{11}), and 152.97 (C_{10}). Anal. Calcd (%) for $C_{16}H_{16}O_4$: C, 70.56; H, 5.93. Found: C, 70.6; H, 6.1. HRMS (*m*/*z*) Calcd for $C_{16}H_{16}O_4$: 272.1049. Found: 272.1050.

Synthesis of Carbamates 19: General Procedure. Carbonate **9** (15 mg, 0.068 mmol) and ammonia (bubbled during 30 min at 0 °C), amines (0.680 mmol), amino alcohols (0.170 mmol), or diamines (0.170 mmol) were dissolved in 5 mL of THF. The solution was stirred under nitrogen atmosphere at 60 °C (30 °C for ammonolysis reaction) until no starting material **9** remained (18−48 h). Then, solvent was evaporated under reduced pressure, and the residue subjected to flash chromatography.

(3*S*,5*R*)-5-(Carbamoyloxy)-1-ethynyl-3-hydroxy-2-methyl-1-cyclohexene (19a). ^{1}H-NMR (MeOH-d_4, 200 MHz): δ 2.13 (m, 2H, H_4), 2.17 (s, 3H, H_9), 2.35 (dd, 1H, H_6, $^2J_{HH}$ 17.1, $^3J_{HH}$ 7.0 Hz), 2.75 (dd, 1H, H_6, $^2J_{HH}$ 17.1, $^3J_{HH}$ 3.4 Hz), 3.66 (s, 1H, H_8), 4.38 (t, 1H, H_3, $^3J_{HH}$ 4.9 Hz), and 5.16 (m, 1H, H_5); ^{13}C-NMR (MeOH-d_4, 50.3 MHz): δ 18.86 (C_9), 36.85 (C_4), 37.94 (C_6), 68.56 (C_3), 68.98 (C_5), 82.17 (C_8), 84.17 (C_7), 115.26 (C_1), 144.51 (C_2), and 159.66 (C_{10}). Anal. Calcd (%) for $C_{10}H_{13}NO_3$: C, 61.51; H, 6.72; N, 7.18. Found: C, 61.3; H, 6.8; N, 7.0. HRMS (*m*/*z*) Calcd for $C_{10}H_{13}NO_3$: 195.0895. Found: 195.0898.

(3*S*,5*R*)-5-[(*N*-Butylcarbamoyl)oxy]-1-ethynyl-3-hydroxy-2-methyl-1-cyclohexene (19b). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.91 (t, 3H, H_{14}, $^3J_{HH}$ 7.3 Hz), 1.33 (m, 2H, H_{13}), 1.45 (m, 2H, H_{12}), 1.97 (m, 2H, H_4), 2.01 (s, 3H, H_9), 2.17 (br s, 1H, OH), 2.19 (m, 1H, H_6), 2.60 (dd, 1H, H_6, $^2J_{HH}$ 17.0, $^3J_{HH}$ 2.2 Hz), 3.08 (s, 1H, H_8), 3.15 (q, 2H, H_{11}, $^3J_{HH}$ 6.6 Hz), 4.23 (br s, 1H, H_3), 4.65 (br s, 1H, NH), and 5.04 (m, 1H, H_5); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 13.65 (C_{14}), 18.27 (C_9), 19.81 (C_{13}), 31.91 (C_{12}), 35.34 (C_4), 36.57 (C_6), 40.60 (C_{11}), 66.74 (C_5), 68.28 (C_3), 80.47 (C_8), 82.84 (C_7), 113.90 (C_1), 143.11 (C_2), and 155.87 (C_{10}). Anal. Calcd (%) for $C_{14}H_{21}NO_3$: C, 66.89; H, 8.43; N, 5.58. Found: C, 66.7; H, 8.5; N, 5.4. HRMS (*m*/*z*) Calcd for $C_{14}H_{21}NO_3$: 251.1521. Found: 251.1522.

(3*S*,5*R*)-1-Ethynyl-3-hydroxy-5-[[*N*-(2-hydroxyethyl)-carbamoyl]oxy]-2-methyl-1-cyclohexene (19e). ^{1}H-NMR (MeOH-d_4, 300 MHz): δ 2.13 (m, 2H, H_4), 2.17 (s, 3H, H_9), 2.35 (dd, 1H, H_6, $^2J_{HH}$ 17.2, $^3J_{HH}$ 6.4 Hz), 2.76 (d, 1H, H_6, $^2J_{HH}$ 17.2 Hz), 3.39 (t, 2H, H_{11}, $^3J_{HH}$ 5.7 Hz), 3.67 (s, 1H, H_8), 3.76 (t, 2H, H_{12}, $^3J_{HH}$ 5.7 Hz), 4.39 (t, 1H, H_3, $^3J_{HH}$ 4.8 Hz), and 5.19 (m, 1H, H_5); ^{13}C-NMR (MeOH-d_4, 75.5 MHz): δ 18.86 (C_9), 36.90 (C_4), 37.96 (C_6), 44.40 (C_{11}), 62.18 (C_{12}), 68.74 (C_3), 68.97 (C_5), 82.24 (C_8), 84.17 (C_7), 115.23 (C_1), 144.53 (C_2), and 158.89 (C_{10}). Anal. Calcd (%) for $C_{12}H_{17}NO_4$: C, 60.22; H, 7.17; N, 5.86. Found: C, 60.3; H, 7.0; N, 6.0. HRMS (*m*/*z*) Calcd for $C_{12}H_{17}NO_4$: 239.1158. Found: 239.1147.

(3*S*,5*R*)-1-Ethynyl-3-hydroxy-5-[[*N*-(6-hydroxyhexyl)-carbamoyl]oxy]-2-methyl-1-cyclohexene (19f). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.36 (m, 4H, H_{12} + H_{13}), 1.53 (m, 4H, H_{14} + H_{15}), 1.83 (br s, 1H, OH), 1.98 (m, 2H, H_4), 2.02 (s, 3H, H_9), 2.19 (dd, 1H, H_6, $^2J_{HH}$ 17.3, $^3J_{HH}$ 4.9 Hz), 2.39 (br s, 1H, OH), 2.60 (d, 1H, H_6, $^2J_{HH}$ 17.3 Hz), 3.09 (s, 1H, H_8), 3.15 (m, 2H, H_{11}), 3.63 (t, 2H, H_{16}, $^3J_{HH}$ 6.5 Hz), 4.22 (m, 1H, H_3), 4.73 (t, 1H, N-H, $^3J_{HH}$ 5.8 Hz), and 5.03 (m, 1H, H_5); ^{13}C-NMR ($CDCl_3$, 50.3 MHz): δ 18.29 (C_9), 25.15 (C_{13}), 26.22 (C_{14}), 29.81 (C_{15}), 32.40 (C_{12}), 35.34 (C_6), 36.56 (C_4), 40.61 (C_{11}), 62.54 (C_{16}), 66.85 (C_5), 68.21 (C_3), 80.47 (C_8), 82.86 (C_7), 113.80 (C_1), 143.21 (C_2), and 156.00 (C_{10}). Anal. Calcd (%) for $C_{16}H_{25}NO_4$: C, 65.05; H, 8.54; N, 4.74. Found: C, 65.2; H, 8.6; N, 5.0. HRMS (*m*/*z*) Calcd for $C_{16}H_{25}NO_4$: 295.1784. Found: 295.1787.

(3*S*,5*R*)-5-[[*N*-(3-Aminopropyl)carbamoyl]oxy]-1-ethynyl-3-hydroxy-2-methyl-1-cyclohexene (19g). ^{1}H-NMR (MeOH-d_4, 400.1 MHz): δ 1.82 (p, 2H, H_{12}, $^3J_{HH}$ 7.0 Hz),

2.12 (m, 2H, H_4), 2.16 (s, 3H, H_9), 2.33 (dd, 1H, H_6, $^2J_{HH}$ 16.9, $^3J_{HH}$ 6.4 Hz), 2.74 (dd, 1H, H_6, $^2J_{HH}$ 16.9, $^3J_{HH}$ 3.5 Hz), 2.85 (t, 2H, H_{13}, $^3J_{HH}$ 6.1 Hz), 3.34 (t, 2H, H_{11}, $^3J_{HH}$ 6.8 Hz), 3.66 (s, 1H, H_8), 4.38 (t, 1H, H_3, $^3J_{HH}$ 4.8 Hz), and 5.17 (m, 1H, H_5); ^{13}C-NMR (MeOH-d_4, 100.6 MHz): δ 18.87 (C_9), 33.98 (C_{12}), 36.93 (C_6), 37.97 (C_4), 39.20 (C_{11}), 39.87 (C_{13}), 68.65 (C_5), 68.95 (C_3), 82.25 (C_8), 84.17 (C_7), 115.21 (C_1), 144.54 (C_2), and 158.92 (C_{10}). Anal. Calcd (%) for $C_{13}H_{20}N_2O_3$: C, 61.87; H, 7.99; N, 11.11. Found: C, 62.0; H, 7.9; N, 11.3. HRMS (*m*/*z*) Calcd for $C_{13}H_{20}N_2O_3$: 252.1474. Found: 252.1475.

Synthesis of Amino Acid Derivatives 21a,b. Amino acid sodium salts **20** (0.170 mmol) were suspended in 4 mL of DMF and sonicated in order to dissolve the amino acid faster. Carbonate **9** (15 mg, 0,068 mmol) was added, and the mixture was stirred at 60 °C until there was no more starting carbonate remaining in solution. The solvent was then evaporated under vacuum and the residue subjected to flash chromatography (30% MeOH/EtOAc).

(3*S*,5*R*)-*N*-[[(1-Ethynyl-3-hydroxy-2-methyl-1-cyclohexen-5-yl)oxy]carbonyl]glycine (21a). ^{1}H-NMR (MeOH-d_4, 300 MHz): δ 2.12 (m, 2H, H_4), 2.17 (s, 3H, H_9), 2.37 (dd, 1H, H_6, $^2J_{HH}$ 17.1, $^3J_{HH}$ 6.6 Hz), 2.76 (d, 1H, H_6, $^2J_{HH}$ 17.1 Hz), 3.65 (s, 1H, H_8), 3.85 (s, 2H, H_{11}), 4.40 (t, 1H, H_3, $^3J_{HH}$ 4.6 Hz), and 5.19 (m, 1H, H_5); ^{13}C-NMR (MeOH-d_4, 75.5 MHz): δ 18.88 (C_9), 31.09 (C_{11}), 36.91 (C_4), 37.98 (C_6), 68.76 (C_3), 68.97 (C_5), 82.19 (C_8), 84.21 (C_7), 115.31 (C_1), 144.53 (C_2), 158.61 (C_{10}), and 177.67 (C_{12}). Anal. Calcd (%) for $C_{12}H_{14}NNaO_5$: C, 52.35; H, 5.13; N, 5.09. Found: C, 52.4; H, 5.0; N, 5.1; MS (FAB$^-$, glycerol): *m*/*z* 252 (M^- − Na, 12%), 183 (100), and 92 (42).

(3*S*,5*R*,11*S*)-*N*-[[(1-Ethynyl-3-hydroxy-2-methyl-1-cyclohexen-5-yl)oxy]carbonyl]-*S*-methyl-L-cysteine (21b). ^{1}H-NMR (MeOH-d_4, 300 MHz): δ 2.13 (m, 2H, H_4), 2.17 (s, 3H, H_9), 2.31 (s, 3H, H_{14}), 2.37 (d, 1H, H_6, $^2J_{HH}$ 16.7 Hz), 2.76 (d, 1H, H_6, $^2J_{HH}$ 16.7 Hz), 2.99 (dd, 1H, H_{13}, $^3J_{HH}$ 13.7, $^3J_{HH}$ 7.5 Hz), 3.19 (dd, 1H, H_{13}, $^3J_{HH}$ 13.7, $^3J_{HH}$ 4.2 Hz), 3.67 (s, 1H, H_8), 4.41 (m, 2H, H_3 + H_{11}), and 5.19 (m, 1H, H_5); ^{13}C-NMR (MeOH-d_4, 50.3 MHz): δ 16.35 (C_{14}), 18.89 (C_9), 36.88 (C_4), 37.94 (C_6), 38.82 (C_{13}), 57.11 (C_{11}), 68.79 (C_3), 68.94 (C_5), 82.19 (C_8), 84.20 (C_7), 115.23 (C_1), 144.52 (C_2), 158.02 (C_{10}), and 178.38 (C_{12}). Anal. Calcd (%) for $C_{14}H_{18}NNaO_5S$: C, 50.14; H, 5.41; N, 4.18. Found: C, 50.2; H, 5.5; N, 4.1; MS (FAB$^-$, glycerol): *m*/*z* 312 (M^- − Na, 21%), 275 (36), 183 (100), and 92 (41).

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Supporting Information Available: Spectral and analytical data (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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