Pergamon

Tetrahedron: *Asymmetry* 13 (2002) 539–546

TETRAHEDRON: *ASYMMETRY*

Novel chiral precursors of 6-*s*-*cis* locked 1α,25-dihydroxyvitamin D_3 analogues through selective enzymatic acylation

Mónica Díaz, Miguel Ferrero, Susana Fernández and Vicente Gotor*

Departamento de Química Orgánica e Inorgánica, Facultad de Química, Universidad de Oviedo, 33071 Oviedo, Spain

Received 26 February 2002; accepted 21 March 2002

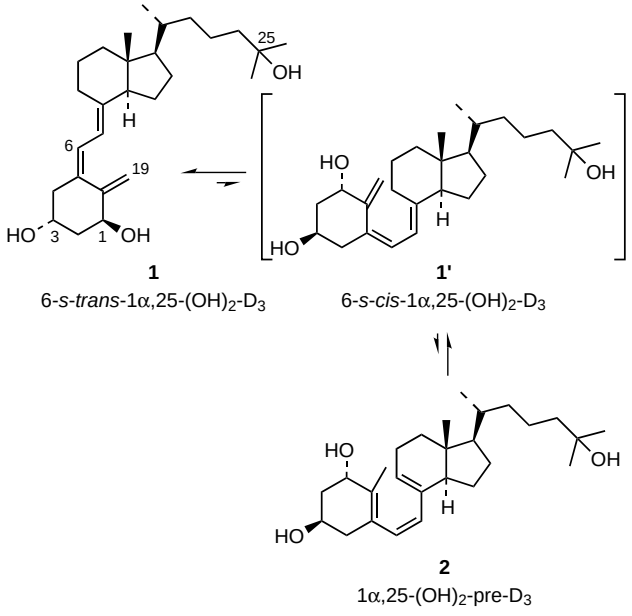
Abstract—The syntheses of selectively modified chiral A-ring precursors for the preparation of 1α,25-dihydroxyvitamin D_3 analogues by regioselective enzymatic acylation are described. *Candida antarctica* lipase B (CAL-B) catalyzes the acylation of 1α,25-dihydroxy-19-*nor*-previtamin D_3 *trans* A-ring precursors **4** and **5** with high selectivity. The opposing regioselectivities observed for each pair of enantiomers is noteworthy: whereas CAL-B acylates the C-3 hydroxyl groups for derivatives of (3*S*,5*R*)-configuration, it catalyzes acylation at the C-5 hydroxyl group for substrates which possess (3*R*,5*S*)-stereochemistry. In relation to stereoisomer **4b**, *Chromobacterium viscosum* lipase (CVL) showed opposite behavior to CAL-B, catalyzing acylation at the C-5 hydroxyl group with acceptable selectivity. In the enzymatic acylation of *cis* A-ring synthons **6** and **7**, CVL gave total selectivity for acylation of the C-5 hydroxyl group of (3*S*,5*S*)-**6** and the C-3 hydroxyl group of (3*R*,5*R*)-**7**. CAL-B also exhibits high selectivity towards the acylation of the C-3 hydroxyl in 19-*nor*-A-ring precursors with (3*R*,5*R*)-configuration.

1. Introduction

The efficacy of 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3] **1** (Scheme 1) analogues as chemotherapeutic agents in a variety of human diseases is highly promising.[1] This secosteroid, the bioactive metabolite of vitamin D_3, can generate biological responses via both genomic[2] and non-genomic[3] pathways by interaction with vitamin D receptors placed in the nucleus (n-VDR) and/or in the cellular membrane (m-VDR).

1α,25-$(OH)_2$-D_3 exists as two conformationally interequilibrating forms (6-*s*-*trans* **1** and a minor form 6-*s*-*cis* **1′**), which are present in slow chemical equilibrium (5–10%) with 1α,25-dihydroxyprevitamin D_3 [1α,25-$(OH)_2$-pre-D_3, **2**]. Due to the spontaneous isomerization of previtamin **2** back to the thermally more stable vitamin **1**, biological evaluation of **2** is very difficult. 6-*s*-*cis*-1α,25-$(OH)_2$-D_3 was proposed as the active conformer in eliciting non-genomic effects, with 1α,25-$(OH)_2$-pre-D_3 **2** simply behaving as an excellent analogue of this conformer.[4]

In our laboratory we have synthesized[5] the A-ring diastereoisomers of 1α,25-$(OH)_2$-19-*nor*-pre-D_3 (**3**, Fig. 1) which are unable to undergo rearrangement to the respective vitamin form because of the absence of the C(19) methyl group, and are therefore potential tools for exploring the biological significance of the previtamin form. The synthesis of A-ring analogues of these



Scheme 1.

* Corresponding author. Tel.: +34 98 510 3448; fax: +34 98 510 3448; e-mail: vgs@sauron.quimica.uniovi.es

0957-4166/02/$ - see front matter
PII: S0957-4166(02)00149-0

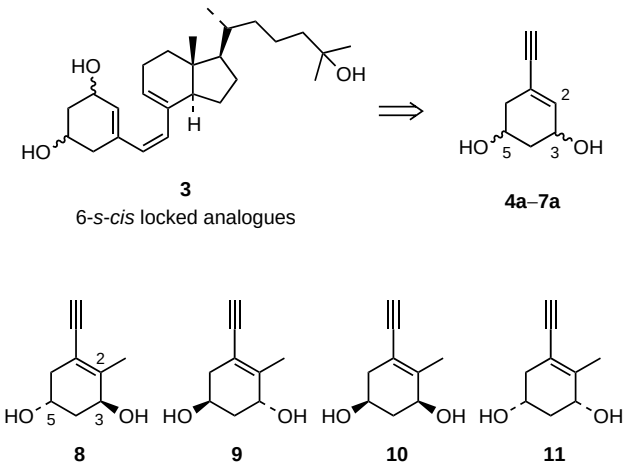


Figure 1.

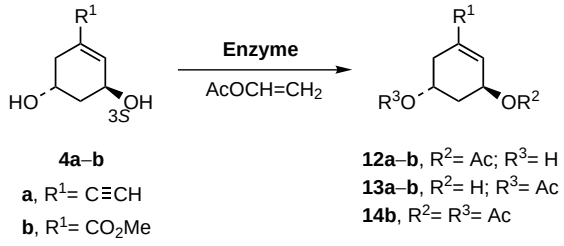
derivatives is a difficult task since the A-ring synthon possesses two stereogenic centers and two hydroxyl groups of similar reactivity, as a result of which it is very difficult to discern between these two groups from a chemical point of view. An important and synthetically relevant transformation mediated by lipases[6] is the selective modification of polyfunctionalized compounds, such as carbohydrates,[7] steroids,[8] and nucleosides.[9] In this way, we described recently the enzymatic alkoxycarbonylation processes of 1α,25-$(OH)_2$-D_3 and 1α,25-$(OH)_2$-19-*nor*-pre-D_3 A-ring synthons.[10] Previously, we reported the regioselective enzymatic acylation of 1α,25-$(OH)_2$-D_3 A-ring precursors **8**–**11** (Fig. 1) catalyzed by *Chromobacterium viscosum* lipase (CVL).[11] Whereas CVL selectively catalyzes the acylation of the C-5 hydroxyl of the three stereoisomeric vitamin D A-ring precursors **8**–**10**, only the C-3 hydroxyl group of the fourth stereoisomer **11** is acylated under these conditions in organic solvent. Here we report the synthesis of novel chiral 19-*nor*-A-ring precursors selectively modified at C-3 or C-5 through selective enzymatic acylation of ethynyl synthons **4a**–**7a** or methyl ester derivatives **4b**–**7b**. Different lipases such as *Candida antarctica* B (CAL-B), *C. viscosum* (CVL) and *Pseudomonas cepacia* (PSL) are used to obtain the monoacylated derivatives in a highly regioselective way.

2. Results and discussion

Diols **4**–**7** were obtained through deprotection of the corresponding silylether derivatives,[5] previously described, with hydrochloric acid in methanol.

2.1. Acylation of A-ring precursors 4a–b

CVL catalyzes the acylation of A-ring **8** at the C-5 hydroxyl group with high selectivity when vinyl acetate is used as both the solvent and acylation reagent.[11] When similar conditions were applied to 19-*nor*-A-ring **4a** (Scheme 2), which has the same stereochemistry but lacks the methyl group at the C(2) position, no selectivity was observed with this lipase at 30 or 40°C (entries 1 and 2, Table 1). However, when CAL-B was used as catalyst, reasonably high regioselectivity towards the C-3 position was achieved, with the monoacetate **12a** being obtained as the major product after 16 h at 30°C (entry 3, Table 1). If THF is used as solvent, with a 10:1 ratio of vinyl acetate to diol, longer reaction times were required and no improvement in the selectivity



Scheme 2.

Table 1. Enzymatic acylation of 19-*nor*-A-ring synthons **4a**–**b**

Entry	Substrate	Enzyme	*T* (°C)	*t* (h)	Conv. (%)[a]	C-3 (%)[b]	C-5 (%)[b]	C-3,5 (%)[b]
1	**4a**	CVL	30	240	91	42	49	
2	**4a**	CVL	40	96	93	48	45	
3	**4a**	CAL-B	30	16	100	81	19	
4	**4a**	CAL-B[c]	30	41	100	80	20	
5	**4a**	PSL-C	30	40	100	64	36	
6	**4a**	CRL	30	24	100	65	35	
7	**4b**	CVL	30	240	92	16	76	
8	**4b**	CVL	40	192	93	9	80	4
9	**4b**	CAL-B	30	50	94	74	20	
10	**4b**	CAL-B[d]	30	96	100	80	20	
11	**4b**	PSL	30	43	98	48	32	18
12	**4b**	CRL	30	60	100	50	50	

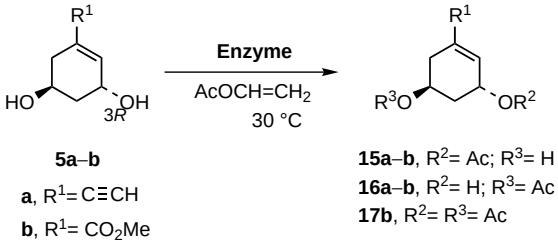
[a] Calculated by GC.
[b] Ratio of regioselectivity at position C-3, C-5 or C-3,5 calculated by ^{1}H NMR.
[c] THF as solvent and 10 equiv. of vinyl acetate.
[d] Isopropenyl acetate as both the solvent and acylating reagent.

was observed (entry 4, Table 1). Other lipases such as PSL-C (immobilized *P. cepacia* lipase) and CRL (*Candida rugosa* lipase) exhibited lower selectivities than CAL-B (entries 5 and 6, Table 1).

With the aim of determining the influence of the substitution at C(1), the methyl ester derivative **4b** was also studied. Thus, the acylation of diol **4b** with CVL at 30°C is very slow but shows moderate regioselectivity toward the C-5 hydroxyl group (entry 7, Table 1). Shorter reaction times and a slight increase in the selectivity for the formation of **13b** is observed if the process is carried out at 40°C (entry 8, Table 1). CVL displayed higher regioselectivity for ester **4b** than for the ethynyl analogue **4a**. The enzymatic acylation using CAL-B is much faster and still maintains high selectivity toward the C-3 position, just the opposite of CVL, when vinyl acetate is the solvent and the acylating agent (entry 9, Table 1). If isopropenyl acetate is used, instead of vinyl acetate, the process is slower but slightly more regioselective (entry 10, Table 1). When other lipases, such as PSL or CRL, are used the enzymatic reaction takes place without selectivity (entries 11 and 12, Table 1).

2.2. Acylation of A-ring precursors 5a–b

Table 2 summarizes the results obtained for the acylation of (3*R*,5*S*)-**5a** at 30°C, shown in Scheme 3.



5a–b

a, R^1= C≡CH

b, R^1= CO_2Me

15a–b, R^2= Ac; R^3= H

16a–b, R^2= H; R^3= Ac

17b, R^2= R^3= Ac

Scheme 3.

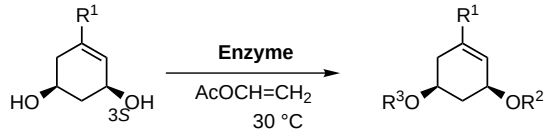
In this case, CVL exhibited moderate preference for the C-5 position when vinyl acetate was used as solvent and acylating agent (entry 1, Table 2). The best results are obtained when the process is catalyzed by CAL-B, giving rise after 2 h to the monoacetate **16a** with 80% regioselectivity (entry 2, Table 2). This selectivity decreased when THF was used as the solvent (entry 3, Table 2).

It is worth noting the opposing selectivity observed with CAL-B for both enantiomers: that is, (3*S*,5*R*)-**4a** showed preference for acylation of the C-3 hydroxyl group meanwhile (3*R*,5*S*)-**5a** was acylated at C-5. PSL showed low selectivity in this process (entry 4, Table 2).

Replacement of the ethynyl group by the methyl ester functionality at C-1 gives rise to a decrease in the selectivity of the acylation with CVL although this lipase still maintains preference towards the same position (entry 5, Table 2). However, CAL-B shows higher selectivity with the methyl ester **5b** than the ethynyl analogue **5a** catalyzing the formation of **16b** in 87% yield after 1 h. When THF was used as the solvent, no improvement in the selectivity was achieved (entry 7, Table 2). PSL did not show preference for any of the hydroxyl groups in **5b** (entry 8, Table 2). For example, in the enyne enantiomers, CAL-B catalyzed the acylation of **4b** and **5b** with opposite selectivity, whereas CVL preferentially acylated the C-5 position of both stereoisomers.

2.3. Acylation of A-ring precursors 6a–b

The enzymatic acylation of **6a** with CVL and vinyl acetate takes place with total selectivity towards the C-5 OH (Scheme 4), isolating the monoacetate **19a** exclusively after 50 h (entry 1, Table 3). CAL-B displayed a slight preference for acylation at C-3 instead of C-5 for this isomer (entry 2, Table 3).



6a–b

a, R^1= C≡CH

b, R^1= CO_2Me

18a–b, R^2= Ac; R^3= H

19a–b, R^2= H; R^3= Ac

20a–b, R^2= R^3= Ac

Scheme 4.

Table 2. Enzymatic acylation of 19-*nor*-A-ring synthons **5a–b**

Entry	Substrate	Enzyme	*t* (h)	Conv. (%)[a]	C-3 (%)[b]	C-5 (%)[b]	C-3,5 (%)[b]
1	**5a**	CVL	18	100	30	70	
2	**5a**	CAL-B	2	100	20	80	
3	**5a**	CAL-B[c]	36	100	31	69	
4	**5a**	PSL	2	100	40	60	
5	**5b**	CVL	26	97	32	65	
6	**5b**	CAL-B	1	100	7	87	6
7	**5b**	CAL-B[c]	6	100	10	82	8
8	**5b**	PSL	4	100	53	47	

[a] Calculated by GC.

[b] Ratio of regioselectivity at position C-3, C-5 or C-3,5 calculated by ^{1}H NMR.

[c] THF as solvent and 10 equiv. of vinyl acetate.

Table 3. Enzymatic acylation of 19-*nor*-A-ring synthons **6a–b**

Entry	Substrate	Enzyme	*t* (h)	Conv. (%)[a]	C-3 (%)[b]	C-5 (%)[b]	C-3,5 (%)[b]
1	**6a**	CVL	50	97		97	
2	**6a**	CAL-B	18	91	66	9	16
3	**6a**	PSL	8	100		90	10
4	**6a**	PSL[c]	18	100		100	
5	**6b**	CVL	40	100		100	
6	**6b**	CAL-B	13	93	40	48	5
7	**6b**	PSL	8	98		3	95
8	**6b**	PSL[c]	20	99		61	38

[a] Calculated by GC.
[b] Ratio of regioselectivity at position C-3, C-5 or C-3,5 calculated by ^{1}H NMR.
[c] Isopropenyl acetate as both the solvent and acylating reagent.

Surprisingly, PSL also catalyzed the acylation process at C-5 with excellent regioselectivity (entry 3, Table 3). This selectivity is complete if the reaction is carried out with isopropenyl acetate as both the solvent and acylating reagent (entry 4, Table 3).

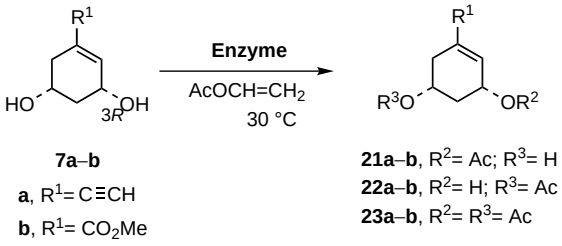
When methyl ester **6b** was used as substrate, CVL maintained the total selectivity at the C-5 hydroxyl group (entry 5, Table 3). However, CAL-B and PSL were not suitable biocatalysts for this reaction (entries 6, 7 and 8, Table 3).

2.4. Acylation of A-ring precursors 7a–b

Among all A-ring stereoisomers of 1α,25-$(OH)_2$-D_3, A-ring synthon **11** was the only one acylated at the C-3 allylic hydroxyl group with CVL as biocatalyst. Similarly, CVL catalyzed the synthesis of the C-3 monoacetylated derivate **21a** from diol **7a** (Scheme 5) with total regioselectivity using vinyl acetate at 30°C (entry 1, Table 4). The opposite selectivity achieved for the *cis* enantiomers **6a** and **7a** is noteworthy.

The total selectivity seen with CVL could not be obtained with CAL-B, although this enzyme gives rise to a mixture of the regioisomers **21a** and **22a** in a ratio of approximately 10:1 (entry 2, Table 4). PSL was less selective than CVL or CAL-B and provided diacetylated **23a** as the major product (entry 3, Table 4).

Methyl ester **7b** was acylated at the same position with excellent regioselectivities when CVL, CAL-B, or PSL were used (entries 4, 5 and 6, Table 4). The process catalyzed by CAL-B is of note, forming monoacetate



Scheme 5.

21b exclusively after 8 h with 100% conversion (entry 5, Table 4). Also, the opposing regioselectivity was obtained for methyl ester enantiomers **6b** and **7b** if CVL was used.

3. Summary

Several chiral monoacylated precursors for the preparation of 6-*s*-*cis* locked analogues of 1α,25-dihydroxyvitamin D_3 have been synthesized. *trans* A-ring precursors **4** and **5** have been acylated toward *S*-configuration positions (at C-3 in **4** and at C-5 in **5**) with high selectivity using *C. antarctica* lipase B (CAL-B), independently of the functionality at C-1 (ethynyl or methyl ester). The opposing regioselectivities shown by each couple of enantiomers is noteworthy. In relation to stereoisomer **4b**, *C. viscosum* lipase (CVL) showed opposite behavior to CAL-B, catalyzing the acylation at C-5 position with acceptable selectivity.

For *cis* enyne and methyl ester 19-*nor*-A-ring precursors **6** and **7**, CVL gave the excellent regioselectivity

Table 4. Enzymatic acylation of 19-*nor*-A-ring synthons **7a–b**

Entry	Substrate	Enzyme	*t* (h)	Conv. (%)[a]	C-3 (%)[b]	C-5 (%)[b]	C-3,5 (%)[b]
1	**7a**	CVL	22	99	99		
2	**7a**	CAL-B	26	98	89	9	
3	**7a**	PSL	10	100	32		68
4	**7b**	CVL	52	99	95	4	
5	**7b**	CAL-B	8	100	100		
6	**7b**	PSL	1.5	100	90		10

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

shown with A-ring isomers **10** and **11**. Thus, the regioselectivity shown by each couple of enantiomers was opposite and independent of the functionality at the C-1 or C-2 positions. From the results of the acylation it is clear that for *cis* compounds with stereochemistry (3*S*,5*S*), C-5-(*S*) position are selectively acylated. However, if the substrates possess (3*R*,5*R*) configuration, the enzyme chooses the hydroxyl group at C-3-(*R*). CAL-B also shows high selectivities toward the C-3 position in 19-*nor*-A-ring precursors with configuration (3*R*,5*R*). Moreover, PSL was an adequate biocatalyst to prepare monoacetate derivatives **19a** and **21b**. In general, the enzymatic processes with *cis* synthons take place with higher selectivities than for *trans* stereoisomers.

4. Experimental[12]

Candida antarctica lipase B (CAL-B, 7300 PLU/g) and *C. viscosum* lipase (CVL, 4918 U/mg protein) were a gift from Novo Nordisk Co. and Genzyme, respectively. *Pseudomonas cepacia* lipase (PSL, 30000 U/g) and immobilized *P. cepacia* lipase (PSL-C, 783 U/g) were purchased from Amano Pharmaceutical Co. *Candida rugosa* lipase (CRL, 4570 U/mg protein) was purchased from Sigma. Gas chromatography (GC) 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 a method with injector and detector temperatures set at 300°C, press head column 15 psi and split 40:1, column initial temperature 150°C (3 min), rate 10°C/min until 228°C and then at 15°C/min until 270°C (3 min). Acetanilide (as internal standard) appeared at 4.91 min; **4a**–**5a** at 4.27 min; **6a**–**7a** at 4.29 min; **4b**–**5b** at 6.89 min; **6b**–**7b** at 6.78 min. Conversion of the reaction was calculated by disappearance of the starting material with respect to the internal standard.

4.1. Syntheses of 1-ethynyl-3,5-dihydroxycyclohex-1-ene 4a–7a

To a solution of the corresponding 3,5-di[(*tert*-butyldimethylsilyl)oxy]-1-ethynylcyclohex-1-ene previously described[5] (500 mg, 1.36 mmol) in MeOH (10 mL) was added three drops of HCl_{conc}. The reaction mixture was stirred at rt during 3 h and then solid $NaHCO_3$ was added. Solvent was evaporated and the residue was dissolved in EtOAc. After separation of the solid by filtration, the filtrate was evaporated to afford diols **4a**–**7a** (yields >90%).

4.1.1. (3*S*,5*R*)- and (3*R*,5*S*)-1-Ethynyl-3,5-dihydroxycyclohex-1-ene 4a and 5a. R_f (80% EtOAc/hex): 0.2; IR (Nujol): ν 3320 and 1648 cm^{-1}; ^1H NMR (MeOH-d_4, 200 MHz): δ 1.99 (dd, 2H, 2H4, J_{HH} 6.5, 6.5 Hz), 2.23 (dddd, 1H, H6a′, $|^2J_{HH}|$ 17.3, $^3J_{HH}$ 6.8, J_{HH} 1.8, 1.8 Hz), 2.63 (m, 1H, H6e′, $|^2J_{HH}|$ 17.3, $^3J_{HH}$ 4.8 Hz), 3.46 (s, 1H, H8), 4.27 (m, 1H, H5), 4.55 (m, 1H, H3) and 6.29 (m, 1H, H2); ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 39.1 and 39.8 (C4, C6), 64.5 and 65.5 (C3, C5), 78.1 (C8), 85.1 (C7), 121.5 (C1) and 137.4 (C2); MS (EI, m/z): 366 (M^+, 1%), 351 (2), 309 (19), 285 (4), 234 (88), 178 (28), 151 (73) and 147 (49); HRMS calcd for $C_8H_{10}O_2$ (M^+): 138.0681. Found: 138.0684. **4a**: $[\alpha]_D^{20}=-111.1$ (c 0.80, MeOH). Anal. calcd (%) for $C_8H_{10}O_2$: C, 69.55; H, 7.3. Found: C, 69.6; H, 7.4. **5a**: $[\alpha]_D^{20}=+113.9$ (c 0.44, MeOH). Anal. calcd (%) for $C_8H_{10}O_2$: C, 69.55; H, 7.3. Found: C, 69.4; H, 7.4.

4.1.2. (3*S*,5*S*)- and (3*R*,5*R*)-1-Ethynyl-3,5-dihydroxycyclohex-1-ene 6a and 7a. R_f (80% EtOAc/hex): 0.2; IR (Nujol): ν 3320 and 1648 cm^{-1}; ^1H NMR (MeOH-d_4, 200 MHz): δ 1.65 (ddd, 1H, H4a, $|^2J_{HH}|$ 11.6, J_{HH} 11.6, 9.5 Hz), 2.26 (dddd, 1H, H6a′, $|^2J_{HH}|$ 16.7, $^3J_{HH}$ 9.2, J_{HH} 3.1, 3.1 Hz), 2.41 (m, 1H, H4e), 2.56 (m, 1H, H6e′, $|^2J_{HH}|$ 16.7, $^3J_{HH}$ 5.4 Hz), 3.45 (s, 1H, H8), 4.01 (m, 1H, H5), 4.50 (m, 1H, H3) and 6.20 (m, 1H, H2); ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 39.5 and 41.5 (C4, C6), 66.3 and 67.7 (C3, C5), 78.2 (C8), 84.8 (C7), 120.5 (C1) and 139.3 (C2); MS (EI, m/z): 366 (M^+, 1%), 351 (2), 309 (19), 285 (4), 234 (88), 178 (28), 151 (73) and 147 (49); HRMS calcd for $C_8H_{10}O_2$ (M^+): 138.0681. Found: 138.0685. **6a**: $[\alpha]_D^{20}=+49.3$ (c 0.50, MeOH). Anal. calcd (%) for $C_8H_{10}O_2$: C, 69.55; H, 7.3. Found: C, 69.3; H, 7.4. **7a**: $[\alpha]_D^{20}=-50.0$ (c 0.30, MeOH). Anal. calcd (%) for $C_8H_{10}O_2$: C, 69.55; H, 7.3. Found: C, 69.6; H, 7.2.

4.2. Syntheses of methyl 3,5-dihydroxycyclohex-1-enecarboxylate 4b–7b

Using the same procedure as that described for **4a**–**7a** yielded **4b**–**7b** from the corresponding silyl ether derivatives previously described.[5]

4.2.1. Methyl (3*S*,5*R*)- and (3*R*,5*S*)-3,5-dihydroxycyclohex-1-enecarboxylate 4b and 5b. R_f (90% EtOAc/hex): 0.1; IR (NaCl): ν 3383, 1718 and 1648 $cm^{-1;}$ ^1H NMR (MeOH-d_4, 300 MHz): δ 1.95 (ddd, 1H, H4a, $|^2J_{HH}|$ 13.2, $^3J_{HH}$ 6.4, 2.8 Hz), 2.09 (ddd, 1H, H4e, $|^2J_{HH}|$ 13.2, $^3J_{HH}$ 8.1, 5.3 Hz), 2.35 (m, 1H, H6a′, $|^2J_{HH}|$ 17.8, $^3J_{HH}$ 6.1 Hz), 2.79 (m, 1H, H6e′, $|^2J_{HH}|$ 17.8 Hz), 3.94 (s, 3H, H8), 4.32 (m, 1H, H5), 4.66 (m, 1H, H3) and 7.06 (m, 1H, H2); ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 34.2 and 39.5 (C4, C6), 52.7 (C8), 65.1 and 65.3 (C3, C5), 130.4 (C1), 140.9 (C2) and 169.2 (C7); MS (EI, m/z): 172 (M^+, 1%), 155 (3), 154 (29), 140 (36), 126 (45) and 95 (100); HRMS calcd for $C_8H_8O_2$ (M^+-2H_2O): 136.0524. Found: 136.0495. **4b**: $[\alpha]_D^{20}=-106.0$ (c 0.38, MeOH). Anal. calcd (%) for $C_8H_{12}O_4$: C, 55.81; H, 7.02. Found: C, 55.9; H, 7.1. **5b**: $[\alpha]_D^{20}=+103.0$ (c 0.45, MeOH). Anal. calcd (%) for $C_8H_{12}O_4$: C, 55.81; H, 7.02. Found: C, 55.7; H, 7.1.

4.2.2. Methyl (3*S*,5*S*)- and (3*R*,5*R*)-3,5-dihydroxycyclohex-1-enecarboxylate 6b and 7b. R_f (90% EtOAc/hex): 0.1; IR (NaCl): ν 3391, 2953, 2252, 1712 and 1652 cm^{-1}; ^1H NMR (MeOH-d_4, 200 MHz): δ 1.68 (ddd, 1H, H4a, $|^2J_{HH}|$ 11.8, $^3J_{HH}$ 11.8, 9.8 Hz), 2.25 (dddd, 1H, H6a′, $|^2J_{HH}|$ 17.0, $^3J_{HH}$ 9.2, J_{HH} 3.6, 2.7 Hz), 2.45 (m, 1H, H6e′, $|^2J_{HH}|$ 17.0, $^3J_{HH}$ 5.6, J_{HH} 3.2, 1.7 Hz), 2.85 (m, 1H, H4e, $|^2J_{HH}|$ 11.8, $^3J_{HH}$ 4.0 Hz), 3.93 (s, 3H, H8), 4.03 (dddd, 1H, H5, $^3J_{HH}$ 9.8, 5.9, 3.3, 2.1 Hz), 4.58 (m, 1H, H3) and 7.06 (m, 1H, H2); ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 34.8 and 41.5 (C4, C6), 52.7 (C8), 66.7

and 67.7 (C3, C5), 129.9 (C1), 142.6 (C2) and 168.9 (C7); MS (EI, m/z): 172 (M^+, <1%), 154 (1) and 43 (100); HRMS calcd for $C_8H_{12}O_4$ (M^+): 172.0735. Found: 172.0739. **6b**: $[\alpha]_D^{20}=+29.0$ (c 0.81, MeOH). Anal. calcd (%) for $C_8H_{12}O_4$: C, 55.81; H, 7.02. Found: C, 55.7; H, 6.9. **7b**: $[\alpha]_D^{20}=-27.0$ (c 1.05, MeOH). Anal. calcd (%) for $C_8H_{12}O_4$: C, 55.81; H, 7.02. Found: C, 55.8; H, 7.1.

4.3. Enzymatic acylation of 4–7. Syntheses of acetates 12–23

In a typical procedure, a lipase (15 mg of CAL-B, CRL, or PSL; or 9 mg of CVL) was added to a solution 0.055 M of diol **4**–**7** (**4a**–**7a**: 15 mg, 0.11 mmol; **4b**–**7b**: 19 mg, 0.11 mmol) in vinyl acetate (2 mL, acetanilide is present as internal standard in 0.013 M) under nitrogen. Temperature, other solvents or acylating agent and reaction time are given in Tables 1–4. The suspension was shaken at 250 rpm 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 (gradient eluent 5–30% EtOAc/hexane) to give compounds **12**–**23**.

4.3.1. (3*S*,5*R*)- and (3*R*,5*S*)-3-Acetoxy-1-ethynyl-5-hydroxycyclohex-1-ene 12a and 15a. R_f (5% ^{i}PrOH/hex): 0.4; IR (NaCl): ν 3391, 1712 and 1652 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.55–1.95 (m, 2H, 2H4), 2.04 (s, 3H, H10), 2.18 (m, 1H, H6), 2.56 (m, 1H, H6, $|^2J_{HH}|$ 17.4, $^3J_{HH}$ 4.8 Hz), 2.94 (s, 1H, H8), 4.15 (m, 1H, H5), 5.46 (m, 1H, H3) and 6.15 (m, 1H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.2 (C10), 35.6 and 37.8 (C4, C6), 63.5 and 67.4 (C3, C5), 77.6 (C8), 83.2 (C7), 122.5 (C1), 131.7 (C2) and 170.3 (C9); MS (EI, m/z): 180 (M^+, <1%), 165 (3), 162 (22), 151 (8), 137 (12), 120 (100) and 91 (97); HRMS calcd for $C_{10}H_{12}O_3$ (M^+): 180.0786. Found: 180.0779.

4.3.2. Methyl (3*S*,5*R*)- and (3*R*,5*S*)-3-acetoxy-5-hydroxycyclohex-1-enecarboxylate 12b and 15b. R_f (90% ^{i}PrOH/hex): 0.3; IR (NaCl): ν 3391, 1712 and 1652 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.97 (m, 1H, H4), 2.06 (s, 3H, H10), 2.07–2.40 (m, 2H, H4′+H6), 2.75 (m, 1H, H6, $|^2J_{HH}|$ 18.2 Hz), 3.74 (s, 3H, H8), 4.20 (m, 1H, H5), 5.58 (m, 1H, H3) and 6.84 (m, 1H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.0 (C10), 32.9 and 35.1 (C4, C6), 51.9 (C8), 64.3 and 67.2 (C3, C5), 131.4 (C1), 134.6 (C2), 166.8 (C7) and 170.3 (C9); MS (EI, m/z): 214 (M^+, <1%), 182 (7), 172 (7), 154 (90), 139 (68), 126 (67), 122 (70), 111 (42) and 95 (100); HRMS calcd for $C_9H_{10}O_4$ (M^+–MeOH): 182.0579. Found: 182.0576. **12b**: Anal. calcd (%) for $C_{10}H_{14}O_5$: C, 56.07; H, 6.54. Found: C, 56.1; H, 6.6.

4.3.3. (3*S*,5*R*)- and (3*R*,5*S*)-5-Acetoxy-1-ethynyl-3-hydroxycyclohex-1-ene 13a and 16a. R_f (5% ^{i}PrOH/hex): 0.4; IR (NaCl): ν 3391, 1712 and 1652 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.83 (m, 1H, H4, $|^2J_{HH}|$ 13.8, J_{HH} 6.4, 3.6 Hz), 1.93–2.32 (m, 2H, H4′+H6), 2.04 (s, 3H, H10), 2.57 (m, 1H, H6, $|^2J_{HH}|$ 17.9 Hz), 2.90 (s, 1H, H8), 4.44 (m, 1H, H3), 5.20 (m, 1H, H5) and 6.21 (m, 1H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.1 (C10), 34.2 and 35.2 (C4, C6), 64.2 and 66.8 (C3, C5), 77.1 (C8), 83.2 (C7), 119.5 (C1), 136.4 (C2) and 170.5 (C9); MS (EI, m/z): 180 (M^+, <1%), 165 (3), 162 (34), 151 (17), 137 (34), 120 (93), 102 (19), 92 (38) and 91 (100); HRMS calcd for $C_{10}H_{12}O_3$ (M^+): 180.0786. Found: 180.0794. **13a**: Anal. calcd (%) for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.4; H, 6.8. **16a**: Anal. calcd (%) for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.5; H, 6.6.

4.3.4. Methyl (3*S*,5R)- and (3*R*,5*S*)-5-acetoxy-3-hydroxycyclohex-1-enecarboxylate 13b and 16b. R_f (90% ^{i}PrOH/hex): 0.3; IR (NaCl): ν 3391, 1712 and 1652 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.80 (ddd, 1H, H4a, $|^2J_{HH}|$ 13.0, $^3J_{HH}$ 7.3, 2.8 Hz), 2.04 (s, 3H, H10), 2.07–2.77 (m, 2H, H4e+H6), 2.68 (m, 1H, H6, $|^2J_{HH}|$ 18.2 Hz), 3.76 (s, 3H, H8), 4.55 (m, 1H, H3), 5.26 (m, 1H, H5) and 6.95 (m, 1H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.2 (C10), 29.7 and 35.0 (C4, C6), 52.0 (C8), 64.1 and 67.3 (C3, C5), 128.7 (C1), 139.2 (C2), 166.9 (C7) and 170.4 (C9); MS (EI, m/z): 214 (M^+, <1%), 182 (7), 172 (14), 154 (88), 139 (32), 126 (32), 111 (20) and 95 (100); HRMS calcd for $C_8H_{12}O_4$ (M^+): 214.0841. Found: 214.0843. **13b**: Anal. calcd (%) for $C_{10}H_{14}O_5$: C, 56.07; H, 6.54. Found: C, 56.0; H, 6.6. **16b**: Anal. calcd (%) for $C_{10}H_{14}O_5$: C, 56.07; H, 6.54. Found: C, 56.1; H, 6.4.

4.3.5. Methyl (3*S*,5R)- and (3*R*,5*S*)-di(acetoxy)cyclohex-1-enecarboxylate 14b and 17b. R_f (5% ^{i}PrOH/hex): 0.8; IR (NaCl): ν 1722 and 1657 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90 (ddd, 1H, H4a, $|^2J_{HH}|$ 13.6, $^3J_{HH}$ 6.7, 3.1 Hz), 2.03–2.20 (m, 1H, H4e), 2.06 (s, 3H, H10), 2.09 (s, 3H, H10′), 2.35 (m, 1H, H6, $|^2J_{HH}|$ 18.5, $^3J_{HH}$ 4.9 Hz), 2.74 (m, 1H, H6, $|^2J_{HH}|$ 18.5, $^3J_{HH}$ 5.3 Hz), 3.78 (s, 3H, H8), 5.25 (m, 1H, H5), 5.60 (m, 1H, H3) and 6.85 (m, 1H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.0 and 21.1 (C10, C10′), 29.7 and 31.9 (C4, C6), 52.0 (C8), 66.5 and 66.8 (C3, C5), 130.8 (C1), 134.9 (C2), 166.4 (C7) and 170.3 (C9, C9′); MS (ESI$^+$, m/z): 279 [M+Na]$^+$.

4.3.6. (3*S*,5*S*)- and (3*R*,5*R*)-3-acetoxy-1-ethynyl-5-hydroxycyclohex-1-ene 18a and 21a. R_f (5% ^{i}PrOH/hex): 0.4; IR (NaCl): ν 3391, 1712 and 1652 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.82 (ddd, 1H, H4a, $^2J_{HH}$ 13.1, $^3J_{HH}$ 8.7, 6.9 Hz), 2.07 (s, 3H, H10), 2.22 (m, 1H, H4e), 2.28 (m, 1H, H6a′, $|^2J_{HH}|$ 17.3, J_{HH} 6.8 Hz), 2.49 (m, 1H, H6e′, $|^2J_{HH}|$ 17.3, J_{HH} 5.0 Hz), 2.95 (s, 1H, H8), 4.06 (m, 1H, H5), 5.45 (m, 1H, H3) and 6.12 (m, 1H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.1 (C10), 35.3 and 37.6 (C4, C6), 64.3 and 67.6 (C3, C5), 77.7 (C8), 83.1 (C7), 121.5 (C1), 136.5 (C2) and 170.2 (C9); MS (EI, m/z): 180 (M^+, <1%), 165 (1), 162 (10), 151 (3), 137 (4), 120 (100), 102 (21), 92 (22) and 91 (81); HRMS calcd for $C_8H_{12}O_4$ (M^+): 180.0786. Found: 180.0781. **21a**: Anal. calcd (%) for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.8; H, 6.6.

4.3.7. Methyl (3*S*,5*S*)- and (3*R*,5*R*)-3-acetoxy-5-hydroxycyclohex-1-enecarboxylate 18b and 21b. R_f (90% ^{i}PrOH/hex): 0.3; IR (NaCl): ν 3391, 1712 and 1652 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.78 (ddd, 1H,

H4a, $|^2J_{HH}|$ 12.6, $^3J_{HH}$ 9.8, 7.8 Hz), 2.09 (s, 3H, H10), 2.70 (m, 1H, H4e, $|^2J_{HH}|$ 12.6, J_{HH} 6.3 Hz), 2.33 (m, 1H, H6a′, $|^2J_{HH}|$ 17.6, J_{HH} 7.6 Hz), 2.69 (m, 1H, H6e′, $|^2J_{HH}|$ 17.6, J_{HH} 5.2 Hz), 3.76 (s, 3H, H8), 4.06 (m, 1H, H5), 5.53 (m, 1H, H3) and 6.80 (m, 1H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.0 (C10), 33.2 and 35.8 (C4, C6), 52.0 (C8), 64.8 and 67.8 (C3, C5), 130.9 (C1), 135.4 (C2), 166.6 (C7) and 170.2 (C9); MS (EI, m/z): 214 (M^+, <1%), 182 (13), 172 (33), 154 (100) and 93 (85); HRMS calcd for $C_{10}H_{14}O_5$ (M^+): 214.0841. Found: 214.0843. **21b**: Anal. calcd (%) for $C_{10}H_{14}O_5$: C, 56.07; H, 6.54. Found: C, 56.0; H, 6.6.

4.3.8. (3*S*,5*S*)- and (3*R*,5*R*)-5-Acetoxy-1-ethynyl-3-hydroxycyclohex-1-ene 19a and 22a. R_f (5% ^{i}PrOH/hex): 0.4; IR (NaCl): ν 3391, 1712 and 1652 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90 (ddd, 1H, H4a, $|^2J_{HH}|$ 13.8, $^3J_{HH}$ 8.4, 6.1 Hz), 2.06 (s, 3H, H10), 2.13 (m, 1H, H4e, $|^2J_{HH}|$ 13.8 Hz), 2.32 (m, 1H, H6, $|^2J_{HH}|$ 17.8 Hz), 2.48 (m, 1H, H6, $|^2J_{HH}|$ 17.8 Hz), 2.92 (s, 1H, H8), 4.34 (m, 1H, H3), 5.12 (m, 1H, H5) and 6.24 (m, 1H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.2 (C10), 34.0 and 35.1 (C4, C6), 64.5 and 67.2 (C3, C5), 77.3 (C8), 83.2 (C7), 118.5 (C1), 136.8 (C2) and 170.1 (C9); MS (EI, m/z): 180 (M^+, 1%), 162 (2), 149 (2), 137 (3), 121 (30), 120 (98), 102 (28), 92 (89) and 91 (100); HRMS calcd for $C_8H_{12}O_4$ (M^+): 180.0786. Found: 180.0782. **19a**: Anal. calcd (%) for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.5; H, 6.8.

4.3.9. Methyl (3*S*,5*S*)- and (3*R*,5*R*)-3-acetoxy-5-hydroxycyclohex-1-enecarboxylate 19b and 22b. R_f (90% ^{i}PrOH/hex): 0.3; IR (NaCl): ν 3391, 1712 and 1652 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90 (ddd, 1H, H4a, $|^2J_{HH}|$ 13.0, $^3J_{HH}$ 8.9, 6.7 Hz), 2.05 (s, 3H, H10), 2.17 (m, 1H, H4e, $|^2J_{HH}|$ 13.0 Hz), 2.45 (m, 1H, H6, $|^2J_{HH}|$ 18.0, J_{HH} 6.5 Hz), 2.60 (m, 1H, H6, $|^2J_{HH}|$ 18.0, J_{HH} 5.2 Hz), 3.76 (s, 3H, H8), 4.45 (m, 1H, H3), 5.15 (m, 1H, H5) and 6.95 (m, 1H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.2 (C10), 29.7 and 35.3 (C4, C6), 52.0 (C8), 64.8 and 67.5 (C3, C5), 128.2 (C1), 139.2 (C2), 166.8 (C7) and 170. 1 (C9); MS (ESI^+, m/z): 237 $[M+Na]^+$. **19b**: Anal. calcd (%) for $C_{10}H_{14}O_5$: C, 56.07; H, 6.54. Found: C, 56.0; H, 6.5.

4.3.10. (3*S*,5*S*)- and (3*R*,5*R*)-di(acetoxy)-1-ethynylcyclohex-1-ene 20a and 23a. R_f (5% ^{i}PrOH/hex): 0.8; IR (NaCl): ν 1718 and 1662 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.93 (ddd, 1H, H4a, $|^2J_{HH}|$ 13.1, $^3J_{HH}$ 9.5, 7.2 Hz), 2.07 (s, 6H, H10+H10′), 2.15–2.40 (m, 2H, H4e+H6), 2.56 (m, 1H, H6, $|^2J_{HH}|$ 17.2 Hz), 2.91 (s, 1H, H8), 5.14 (m, 1H, H5), 5.45 (m, 1H, H3) and 6.09 (m, 1H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.2 (C10, C10′), 32.0 and 34.2 (C4, C6), 66.3 and 66.7 (C3, C5), 77.3 (C8), 83.2 (C7), 118.7 (C1), 132.1 (C2) and 170.3 (C9, C9′); MS (ESI^+, m/z): 245 $[M+Na]^+$.

4.3.11. Methyl (3*S*,5*S*)- and (3*R*,5*R*)-5-di(acetoxy) cyclohex-1-enecarboxylate 20b and 23b. R_f (5% ^{i}PrOH/hex): 0.4; IR (NaCl): ν 1715 and 1657 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90 (ddd, 1H, H4a, $|^2J_{HH}|$ 12.6, $^3J_{HH}$ 10.0, 7.7 Hz), 2.08 (s, 3H, H10), 2.11 (s, 3H, H10′), 2.20–2.80 (m, 2H, H4e+H6), 2.74 (m, 1H, H6, $|^2J_{HH}|$ 17.7, J_{HH} 3.6 Hz), 3.75 (s, 3H, H8), 5.15 (m, 1H, H5), 5.57 (m, 1H, H3) and 6.80 (m, 1H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 20.9 and 21.0 (C10, C10′), 29.8 and 32.3 (C4, C6), 52.0 (C8), 66.6 and 67.0 (C3, C5), 130.3 (C1), 135.6 (C2), 166.2 (C7) and 170.1 and 170.2 (C9, C9′); MS (ESI^+, m/z): 279 $[M+Na]^+$.

Acknowledgements

We express our appreciation to Novo Nordisk Co. and Genzyme for the generous gift of the lipase CAL-B and CVL, respectively. Financial support from Principado de Asturias (Spain; Project GE-EXP01-03) and MCYT (Spain; Project PPQ-2001-2683) is gratefully acknowledged. S.F. also thanks MCYT (Spain) for a personal grant (Ramón y Cajal Program).

References

1. (a) *Vitamin D*; Feldman, D.; Glorieux, F. H.; Pike, J. W., Eds.; Academic Press: New York, 1997; (b) Ettinger, R. A.; DeLuca, H. F. *Adv. Drug Res.* **1996**, *28*, 269–312; (c) Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocrine Rev.* **1995**, *16*, 200–257.
2. (a) Lowe, K. E.; Maiyar, A. C.; Norman, A. W. *Crit. Rev. Eukar. Gene Exp.* **1992**, *2*, 65–109; (b) Pike, J. W. *Annu. Rev. Nutr.* **1991**, *11*, 189–216; (c) *Vitamin D: Gene Regulation, Structure–Function Analysis and Clinical Application*; Norman, A. W.; Bouillon, R.; Thomasset, M., Eds.; Walter de Gruyter: Berlin, 1991; (d) Norman, A. W. *Vitamin D*: *The calcium Homeostatic Steroid Hormone*; Academic Press: New York, 1979.
3. (a) Norman, A. W.; Nemere, I.; Zhou, L. X.; Bishop, J. E.; Lowe, K. E.; Maiyar, A. C.; Collins, E. D.; Taoka, T.; Sergeev, I.; Farach-Carson, M. C. *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 231–240; (b) Deboland, A. R.; Nemere, I.; Norman, A. W. *Biochem. Biophys. Res. Commun.* **1990**, *166*, 217–222.
4. Okamura, W. H.; Midland, M. M.; Norman, A. W.; Hammond, M. W.; Dormanen, M. C.; Nemere, I. *Ann. N.Y. Acad. Sci.* **1995**, *761*, 344–348.
5. (a) Díaz, M.; Ferrero, M.; Fernández, S.; Gotor, V. *J. Org. Chem.* **2000**, *65*, 5647–5652; (b) Díaz, M.; Ferrero, M.; Fernández, S.; Gotor, V. *Tetrahedron Lett.* **1999**, *41*, 775–779.
6. (a) Carrea, G.; Riva, S. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 2226–2254; (b) Faber, K. *Biotransformations in Organic Chemistry*, 4th ed.; Springer-Verlag: Berlin, 2000; (c) Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*: *Regio- and Stereoselective Biotransformations*; Wiley-VCH: Weinheim, 1999.
7. (a) Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443–473; (b) Drueckhammer, D. G.; Hennen, W. J.; Pederson, R. L.; Barbas, C. F., III; Gautheron, C. M.; Krach, T.; Wong, C.-H. *Synthesis* **1991**, 499–525.

8. Ferrero, M.; Gotor, V. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker: New York, 2000; Chapter 20, pp. 579–631.
9. Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319–4347.
10. (a) Díaz, M.; Gotor-Fernández, V.; Ferrero, M.; Fernández, S.; Gotor, V. *J. Org. Chem.* **2001**, *66*, 4227–4232; (b) Gotor-Fernández, V.; Ferrero, M.; Fernández, S.; Gotor, V. *J. Org. Chem.* **1999**, *64*, 7504–7510; (c) Ferrero, M.; Fernández, S.; Gotor, V. *J. Org. Chem.* **1997**, *62*, 4358–4363.
11. Fernández, S.; Ferrero, M.; Gotor, V.; Okamura, W. H. *J. Org. Chem.* **1995**, *60*, 6057–6061.
12. Structures of the products are numbered as follows: