Synthesis of Monoacyl A-Ring Precursors of 1α,25-Dihydroxyvitamin D_3 through Selective Enzymatic Hydrolysis

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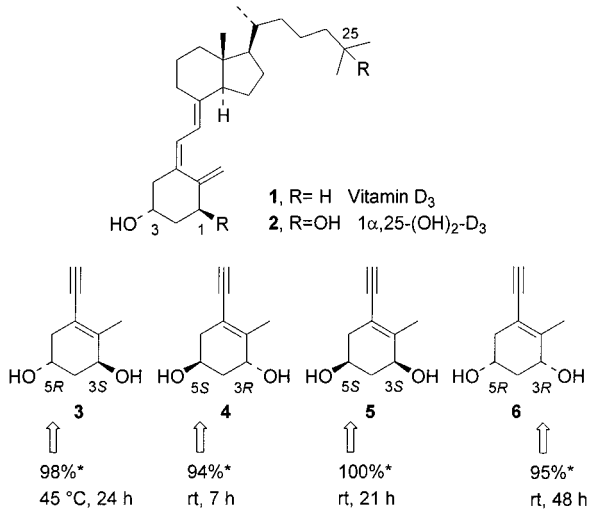
An efficient synthesis of monoacylated 1α,25-dihydroxyvitamin D_3 A-ring precursors **15**, **16**, **18**, and **19** has been described through an enzymatic hydrolysis process. *Candida antarctica* A lipase (CAL-A) hydrolyzes the C-5 acetate ester in trans stereoisomers **9** and **13**, with complete and high selectivity, respectively. In the case of cis isomers **11** and **14**, *Chromobacterium viscosum* lipase (CVL) is the enzyme of choice, exhibiting opposite selectivity for these two enantiomers. This lipase selectively catalyzes the hydrolysis at the C-3 acetate in diester **11** and at C-5 position in diester **14**. It is noteworthy that through a hydrolysis reaction CAL-A and CVL allow the synthesis of the four A-ring monoacetylated precursors of 1α,25-dihydroxyvitamin D_3, precursors which are complementary to those obtained by the enzymatic acylation process. In addition, with excellent yield CVL selectively hydrolyzes the C-3 chloroacetate ester instead of the C-5 acetate in diester **22**, a key intermediate in the synthesis of new A-ring modified 1α,25-dihydroxyvitamin D_3 analogues.

Introduction

The potential of enzymes in organic chemistry is well recognized.[1] The chiral nature of enzymes results in the stereo- and regiospecific formation of products with remarkable rate acceleration. Substrates with several functional groups of similar reactivity can be selectively manipulated by enzymatic acylation and/or deacylation processes. Direct chemical methods possess low regioselectivities, and the procedures often necessarily involve low temperatures and long reaction times.

In addition to its classical calciotropic effects,[2] 1α,25-dihydroxyvitamin D_3 [**2**, 1α,25-$(OH)_2$-D_3] (Chart 1), the major active metabolite of vitamin D_3 (**1**), has also been shown to possess immunosuppressive activity,[3] to inhibit cellular proliferation, and induce cellular differentiation.[4] As a result, considerable effort has been made toward the synthesis of structurally related congeners that show interesting pharmacological applications.[5] Most of the analogues prepared have modifications on the upper side chain, whereas A-ring manipulation is rare because of difficulties in synthesis. These metabolites possess several hydroxyl groups of similar reactivity, and as result it is very difficult to discern between them from a chemical point of view.

Chart 1



In our ongoing research related to the synthesis of selectively modified vitamin D_3 analogues, appropriate routes for the preparation of the corresponding precursors are required. In this way, we have described the selective enzymatic acylation[6] and alkoxycarbonylation[7] of A-ring

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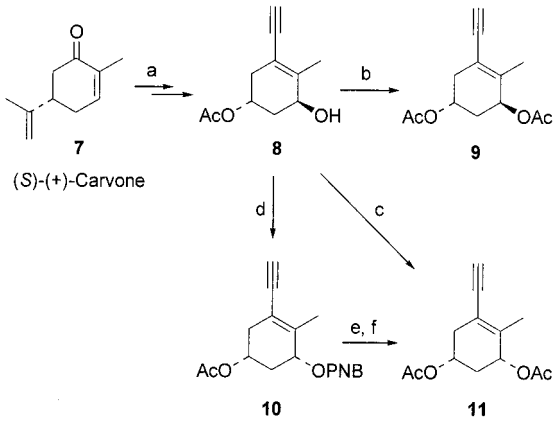
Scheme 1[a]

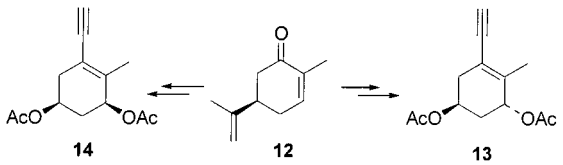


[a] (a) Reference 8; (b) Ac_2O, DMAP, Py, CH_2Cl_2, rt, 12 h (92%); (c) AcOH, PPh_3, DEAD, toluene, rt, 2 h (36% conv); (d) PNBA, PPh_3 DEAD, THF, rt, 1 h (87%); (e) NaOMe, MeOH, 2.5 h, 0 °C (89%); (f) Ac_2O, DMAP, Py, CH_2Cl_2, rt, 12 h (96%).

stereoisomeric synthons of 1α,25-$(OH)_2$-D_3 and 1α,25-$(OH)_2$-19-*nor*-pre-D_3. With respect to the acylation reaction[6] of A-ring precursors **3**−**6** (Chart 1), *Chromobacterium viscosum* lipase (CVL) has selectively catalyzed the acylation with vinyl esters of the C-5 hydroxyl of the three stereoisomeric vitamin D A-ring synthons **3**−**5**. However, the stereoisomer **6** was acylated at the C-3 hydroxyl under the same conditions. Taking into account the complementary behavior shown by enzymes in acylation and hydrolysis processes, here we report the lipase-catalyzed hydrolysis of the corresponding diacyl substrates **9**, **11**, **13**, and **14** to prepare the monoacylated derivatives of the A-ring, which are complementary to those mentioned above.

Results and Discussion

First, diacetates **9**, **11**, **13**, and **14** were synthesized. Derivative **9** was obtained by acetylation of monoacetate **8**, an intermediate en route to 1α,25-$(OH)_2$-D_3 A-ring precursor **3** from (*S*)-(+)-carvone described by Okamura et al.[8] (Scheme 1). It was possible to prepare cis diester **11** directly, through the inversion of the allylic alcohol **8** under Mitsunobu[9] conditions using acetic acid. Several solvents, phosphines, and equivalents of reagents were tried. However, although the process gave the desired inversion, the yield of **11** was very poor, the highest conversion achieved only reaching 36%. An alternative approach to isomer **11** implies inversion of configuration at C-3 in **8** using *p*-nitrobenzoic acid (PNBA) affording *p*-nitrobenzoate ester **10** with total inversion of the configuration and high yield. Deprotection of both ester groups with NaOMe in MeOH, and subsequent acetylation with acetic anhydride, gave diacetate **11**. Similarly, diacyl derivatives **13** and **14** were obtained starting from (*R*)-(−)-carvone.



Enzymatic Hydrolysis of Diacetate 9. Since CVL catalyzes with excellent selectivity the formation of **8** from the diol **3**, our aim was to find an enzyme that hydrolyzes exclusively the ester group at the C-5 position of **9** in order to obtain **15**. The enzymatic hydrolysis reactions were carried out at 30 °C using 0.1 M KH_2PO_4 (pH 7) in 1,4-dioxane as cosolvent (Scheme 2). In these conditions, CVL showed low selectivity, isolating diol **3** as the major compound (entry 1, Table 1). When *Candida antarctica* B lipase (CAL-B) was used, high regioselectivity toward the hydrolysis of C-3 acetate was achieved, with **8** being obtained (entry 2, Table 1). The long reaction time required to reach moderate conversion in the enzymatic hydrolysis made the enzymatic acylation process more suitable from a synthetic point of view. Other lipases such as immobilized *Pseudomonas cepacia* (PSL-C), porcine pancreas (PPL), or *Candida rugosa* (CRL) did not show any improvement in the hydrolysis process (entries 3, 4, and 5, Table 1). Better results were obtained when the reaction was carried out with porcine liver esterase (PLE). This enzyme gave rise to a mixture of regioisomers **15** and **8** in a ratio of approximately 4.3:1, and traces of diol (entry 6, Table 1). However, total selectivity toward the hydrolysis of the C-5 ester was exhibited by *Candida antarctica* A lipase (CAL-A). This lipase catalyzed exclusively the formation of monoacetate **15** after 31 h and 91% conversion (entry 7, Table 1).

Scheme 2

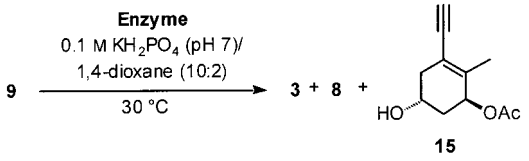
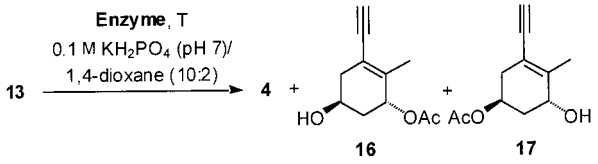


Table 1. Enzymatic Hydrolysis of A-Ring Synthon (3*S*,5*R*)-9

entry	enzyme	*t* (h)	conv (%)[a]	**3** (%)[a]	**8** (%)[a]	**15** (%)[a]
1	CVL	262	91	69	1	21
2	CAL-B	335	74	8	65	1
3	PSL-C	70	99	9	51	39
4	PPL	336	24	3	7	14
5	CRL	155	100	38	8	54
6	PLE	270	89	4	16	69
7	CAL-A	31	91			91

[a] Calculated by ^{1}H NMR and GC.

Scheme 3



Enzymatic Hydrolysis of Diacetate 13. Reaction of **13** with phosphate buffer/1,4-dioxane in the presence of CVL gave, after 130 h, diol **4** (Scheme 3) as the unique reaction product (entry 1, Table 2). CAL-B, PSL-C, and PPL showed poor reactivity, and no selectivity was observed (entries 2, 3, and 4, Table 2). CRL and PLE

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Table 2. Enzymatic Hydrolysis of A-Ring Synthon (3*R*,5*S*)-13

entry	enzyme	*T* (° C)	*t* (h)	conv (%)[a]	**4** (%)[a]	**16** (%)[a]	**17** (%)[a]
1	CVL	30	130	97	97		
2	CAL-B	60	264	30	10	9	11
3	PSL-C	30	222	5	5		
4	PPL	30	336	45	22	23	
5	CRL	30	4	100	77	10	13
6	PLE	30	9	90	50	11	29
7	CAL-A	30	48	85	5	74	6
8	CAL-A[b]	30	63	91	12	75	4
9	CAL-A	40	23	92	8	79	5

[a] Calculated by ^{1}H NMR and GC. [b] Enzyme was added in two portions: 55 mg at the beginning and 35 mg after 18 h of reaction.

Scheme 4

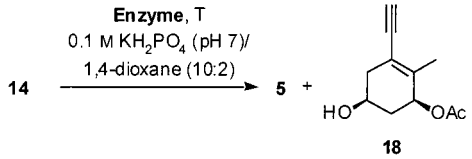


Table 3. Enzymatic Hydrolysis of A-Ring Synthon (3*S*,5*S*)-14

entry	enzyme	*T* (°C)	*t* (h)	conv (%)[a]	**5** (%)[a]	**18** (%)[a]
1	CVL	30	25	97	3	94
2	CAL-A	30	52	31		31
3	CAL-A[b]	30→40	130	96	8	88
4	CAL-A	40	81	93	7	86

[a] Calculated by ^{1}H NMR and GC. [b] An extra fraction of 45 mg was added at 52 h; after 74 h, temperature was increased up to 40 °C.

exhibited moderate regioselectivity toward the hydrolysis of C-3 ester at low conversions; however, as the reaction evolves, diol **4** was the major compound (entries 5 and 6, Table 2). The best results were observed with CAL-A. Thus, after 48 h, 85% conversion was achieved with a selectivity of 74% for **16** (entry 7, Table 2). To enhance that selectivity, the influence of temperature and the amount of lipase were studied. Since the enzyme displays Michaelis−Menten kinetics, it possesses its highest activity at the beginning of the reaction. For this reason, CAL-A was added in two portions (entry 8, Table 2) during the process. Although a slight rise conversion was obtained, the percentage of derivative **16** was the same. When the process took place at 40 °C, a shorter reaction time was observed for high conversion, in addition to a slight increase in the regioselectivity exhibited by CAL-A, the ratio of C-3 monoacetate **16** being 79% (entry 9, Table 2). CAL-A showed selectivity toward the C-5 position for both enantiomers **9** and **13**, although **9** displayed total selectivity and higher reactivity than **13**.

Enzymatic Hydrolysis of Diacetate 14. As in the acylation process, CVL still maintained excellent selectivity toward the C-5 position in the hydrolysis reaction (Scheme 4). As shown in entry 1 of Table 3, when the reaction was run at 30 °C, hydrolysis of the C-5 ester occurred with almost complete regioselectivity, and only trace amounts of diol **5** was observed. CAL-A also revealed total selectivity toward the acetate at the C-5 position (entry 2, Table 3), but to get a higher conversion, a temperature of 40 °C and more enzyme were used (entries 3 and 4, Table 3). Under these conditions, approximately 95% conversions were achieved with high selectivities, although CVL still offered the best result.

Scheme 5

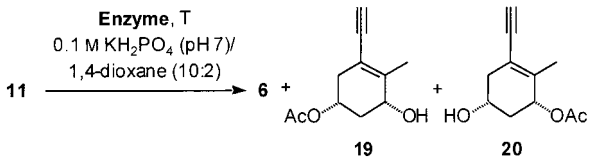
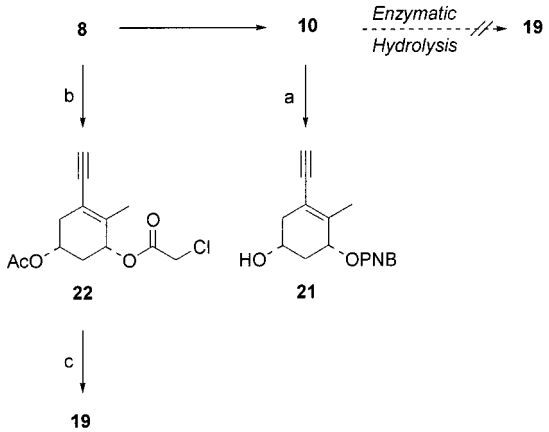


Table 4. Enzymatic Hydrolysis of A-Ring Synthon (3*R*,5*R*)-11

entry	enzyme	*T* (°C)	*t* (h)	conv (%)[a]	**6** (%)[a]	**19** (%)[a]	**20** (%)[a]
1	CVL	30	29	95	15	77	3
2	CVL	40	10	94	48	40	6
3	CVL	20	43	94	17	74	3
4	CAL-A	30	74	78	23	3	52
5	CAL-A	40	32	87	21	3	63
6	CAL-A	20	144	69	21	1	47

[a] Calculated by ^{1}H NMR and GC.

Scheme 6[a]



[a] (a) 0.1 M KH_2PO_4 (pH 7)/1,4-dioxane (10:2), 30 °C, CVL or PSL-C; (b) $ClCH_2CO_2H$, PPh_3, DEAD, THF, rt, 1 h (93%); (c) 0.1 M KH_2PO_4 (pH 7)/1,4-dioxane (10:2), 30 °C, CVL, 5 h (94%).

Enzymatic Hydrolysis of Diacetate 11. Table 4 and Scheme 5 summarize similar hydrolysis studies of the cis stereoisomer **11**. CVL catalyzed the hydrolysis of **11** at 30 °C with high selectivity toward the acyl group at the C-3 position (entry 1, Table 4), in complete contrast to its enantiomer **14**, and in the same way as the acylation reaction of the corresponding diol **6**. When the process was performed at 40 °C or 20 °C, the enzyme did not exhibit any improvement in the selectivity (entries 2 and 3, Table 4). However, the opposite regioselectivity, somewhat favoring the hydrolysis of the C-5 ester group, was achieved when CAL-A was used as catalyst, although conditions for selective hydrolysis at C-5 could not be defined (entries 4, 5, and 6, Table 4).

Improved Synthesis of Monoacetate 19. In the synthesis of new 1α,25-$(OH)_2$-D_3 analogues carried out in our laboratory, cleavage of *p*-nitrobenzoate ester **10** in the presence of acetate ester was necessary. As indicated above, CVL catalyzed the hydrolysis of the C-3 ester in the corresponding diacetate derivative **11**, with high regioselectivity. When the same process was carried out with diacyl **10**, CVL hydrolyzed exclusively the acetate group, isolating **21** as the unique reaction product (Scheme 6). The enzyme probably cannot accommodate a large group as *p*-nitrobenzoyl in the active site. The same result was obtained with PSL-C, and because of this drawback, we designed a new route to A-ring synthon **19** through the inversion of **8** under Mitsunobu

conditions using chloroacetic acid. The process took place with excellent yield in 1 h to obtain **22**. Subsequent enzymatic hydrolysis with CVL of diacyl derivative **22** gave exclusively monoacetate **19** with 94% yield.

Summary

Candida antarctica A lipase has catalyzed with high selectivity the hydrolysis of the ester at the C-5 position of 1α,25-$(OH)_2$-D_3 A-ring diacetate precursors **9**, **13**, and **14**. However, this enzyme has exhibited low regioselectivity with stereoisomer **11**. Better results were obtained for cis synthons **11** and **14** with *Chromobacterium viscosum* lipase. This enzyme maintains the opposite selectivity shown with respective diol enantiomers **6** and **5** in the enzymatic acylation process: the hydrolysis is performed at the C-5 ester for **14** and at the C-3 position in case of **11**. It is noteworthy that through a hydrolysis reaction, both enzymes (CAL-A for trans isomers **9** and **13**, and CVL for cis isomers **11** and **14**) allowed the synthesis of the four A-ring monoacetylated precursors of 1α,25-$(OH)_2$-D_3, which are complementary to those obtained by the acylation reaction. From this exhaustive study, the selective deprotection of diacyl derivative **22**, a key intermediate in the synthesis of new A-ring modified 1α,25-$(OH)_2$-D_3 analogues, has been carried out. CVL hydrolyzed the C-3 chloroacetate ester instead of the C-5 acetate selectively and with excellent yield.

Experimental Section[10]

General. *Chromobacterium vicosum* lipase (CVL, 3800 U/mg of solid) and *Candida antarctica* B lipase (CAL-B, 7300 PLU/g) were a gift from Genzyme and Novo Nordisk Co., respectively. Immobilized *Pseudomonas cepacia* lipase (PSL-C, 783 U/g) was obtained from Amano Pharmaceutical Co. Porcine pancreas lipase (PPL, type II, 46 U/mg of protein using triacetin), *Candida rugosa* lipase (CRL, typeVII, 950 U/mg of solid), and porcine liver esterase [PLE, 260 U/mg of protein, 3.2 M $(NH_4)_2SO_4$ solution pH 8] were purchased from Sigma. *Candida antarctica* A lipase (CAL-A, chirazyme L-5, c−f, lyo., 1000 U/g using tributyrin) was obtained from Roche. Reagents were purchased from Aldrich, Fluka, Merck, or Sigma. Solvents were distilled over an appropriate desiccant under nitrogen. Gas chromatography (GC) was carried out with flame ionization detection (FID) and a HP-1 capillary column (25 m × 0.2 mm × 0.2 μm) coated with methylsilicone gum, with nitrogen as carrier gas. In this method the injector and detector temperatures were set at 250 °C and 275 °C, respectively, column initial temperature was 80 °C (3 min), rate was 10 °C/min until 170 °C (5 min) and then 18 °C/min, column final temperature was 260 °C; **3** and **4** appeared at 11.65 min; **5** and **6** at 11.55 min; **8** and **17** at 13.59 min; **9** and **13** at 15.08 min; **11** and **14** at 15.72 min; **15** and **16** at 13.41 min; **18** and **20** at 13.12 min; and **19** at 13.01 min.

Synthesis of (3*S*,5*R*)-3,5-Diacetoxy-1-ethynyl-2-methylcyclohex-1-ene (9). To a solution of **8** (150 mg, 0.77 mmol) in CH_2Cl_2 (12.5 mL) under nitrogen were added pyridine (187 μL, 2.32 mmol), DMAP (14 mg, 0.23 mmol), and Ac_2O (73 μL, 1.54 mmol). The mixture was stirred at room temperature for 12 h, after which it was poured into water (10 mL), and the organic phase was washed with 1 N HCl (3 × 5 mL). Solvent was evaporated, and the residue was purified by flash chromatography (10% EtOAc/hexane) to afford 167 mg (92%) of diacetate **9** as an oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.88 (s, 3H, H_9), 1.86−1.99 (m, 2H, H_4), 2.03 (s, 3H, H_{11}), 2.07 (s, 3H, H_{13}), 2.20 (dd, 1H, H_6, $^2J_{HH}$ 17.3, $^3J_{HH}$ 7.4 Hz), 2.63 (dd, 1H, H_6, $^2J_{HH}$ 16.8, $^3J_{HH}$ 4.2 Hz), 3.14 (s, 1H, H_8), 5.07 (m, 1H, H_5), and 5.45 (t, 1H, H_3, $^3J_{HH}$ 4.7 Hz); MS (ESI$^+$, m/z): 259 [(M + Na)$^+$, 100%].

Enzymatic Hydrolysis of 9. In a standard procedure, CVL (10 mg), CAL-B (90 mg), CRL (180 mg), PSL-C (90 mg), PLE (20 μL), PPL (90 mg), or CAL-A (90 mg) was added to a solution of diacetate **9** (20 mg, 0.085 mmol) in 2 mL of 0.1 M KH_2PO_4 (pH 7)/1,4-dioxane (10:2). The suspension was shaken at 250 rpm, and the progress of the reaction was followed by GC analysis (conversions and percentages of compounds are summarized in Table 1). The mixture was filtered, and the solution was extracted with EtOAc (3 × 5 mL). The crude was purified by flash chromatography (10−60% EtOAc/hexane) to give compounds **3**,[6] **8**,[6] and **15**.

(3*S*,5*R*)-3-Acetoxy-1-ethynyl-5-hydroxy-2-methylcyclohex-1-ene (15).[11] ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.76-1.86 (m, 2H, H_4), 1.88 (s, 3H, H_9), 1.97−2.19 (m, 2H, H_4+H_6), 2.07 (s, 3H, H_{11}), 2.54−2.61 (dd, 1H, H_6, $^2J_{HH}$ 16.9, $^3J_{HH}$ 4.0 Hz), 3.14 (s, 1H, H_8), 4.06 (m, 1H, H_5), and 5.45 (m, 1H, H_3).

Synthesis of (3*R*,5*R*)-5-Acetoxy-1-ethynyl-2-methyl-3-[(4-nitrophenyl)carbonyloxy]cyclohex-1-ene (10). This compound was prepared as previously reported.[7c]

Synthesis of (3*R*,5*R*)-3,5-Diacetoxy-1-ethynyl-2-methylcyclohex-1-ene (11). 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 **10** (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 170 mg (89%) of diol **6** as a white solid. To a solution of **6** (145 mg, 0.95 mmol) in CH_2Cl_2 (12 mL) were added pyridine (308 mL, 3.81 mmol), DMAP (23 mg, 0.38 mmol), and Ac_2O (180 μL, 3.81 mmol). The mixture was stirred at room temperature for 12 h, after which it was poured into water (10 mL), and the organic phase was washed with 1 N HCl (3 × 5 mL). Solvent was evaporated, and the residue was purified by flash chromatography (10% EtOAc/hexane) to afford 216 mg (96%) of diacetate **11** as a white solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.86 (s, 3H, H_9), 1.86−2.00 (m, 1H, H_4), 2.01 (s, 3H, H_{11}), 2.05 (s, 3H, H_{13}), 2.14 (ddd, 1H, H_4, $^2J_{HH}$ 14.4, $^3J_{HH}$ 7.4, $^3J_{HH}$ 2.3 Hz), 2.19−2.56 (m, 2H, H_6), 3.14 (s, 1H, H_8), 4.96 (m, 1H, H_5), and 5.43 (t, 1H, H_3, $^3J_{HH}$ 5.9 Hz); MS (ESI$^+$, m/z): 259 [(M + Na)$^+$, 100%].

Enzymatic Hydrolysis of 11. The same procedure as that described for the enzymatic hydrolysis of **9** yielded compounds **6**,[6] **19**, and **20**[6] (conversions, percentages of compounds, and temperatures are summarized in Table 4).

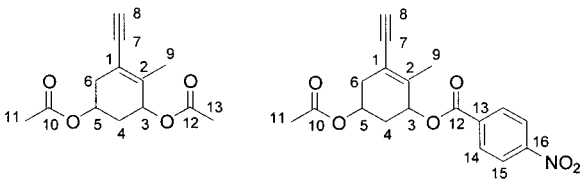
(3*R*,5*R*)-5-Acetoxy-1-ethynyl-3-hydroxy-2-methylcyclohex-1-ene (19).[11] ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.98−2.17 (m, 8H, H_9+H_{11}+H_4), 2.33−2.54 (m, 2H, H_6), 3.11 (s, 1H, H_8), 4.09 (m, 1H, H_3), and 5.19 (m, 1H, H_5).

Synthesis of (3*R*,5*S*)-3,5-Diacetoxy-1-ethynyl-2-methylcyclohex-1-ene (13). The same procedure as that described for **9** yielded **13** (91%). Spectral data are identical to that of **9** given above.

Enzymatic Hydrolysis of 13. The same procedure as that described for enzymatic hydrolysis of **9** yielded compounds **4**,[6] **16**, and **17**[6] (conversions, percentages of compounds, and temperatures are summarized in Table 2). The spectral data of compound **16** were identical to that of **15**.

Synthesis of (3*S*,5*S*)-3,5-Diacetoxy-1-ethynyl-2-methylcyclohex-1-ene (14). The same procedure as that described

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



(11) This A-ring synthon was previously described in the Supporting Information of ref 6 as a minor compound.

for **11** yielded **14** (91%). Spectral data are identical to that of **11** given above.

Enzymatic Hydrolysis of 14. The same procedure as that described for enzymatic hydrolysis of **9** yielded compounds **5**[6] and **18** (conversions, percentages of compounds, and temperatures are summarized in Table 3).

(3*S*,5*S*)-3-Acetoxy-1-ethynyl-5-hydroxy-2-methylcyclohex-1-ene (18).[11] ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.87 (m, 1H, H_4), 1.90 (s, 3H, H_9), 2.09 (s, 3H, H_{11}), 2.13 (ddd, 1H, H_4, $^2J_{HH}$ 14.4, $^3J_{HH}$ 5.8, $^3J_{HH}$ 3.0 Hz), 2.17−2.53 (m, 2H, H_6), 3.15 (s, 1H, H_8), 4.03 (m, 1H, H_5), and 5.46 (t, 1H, H_3, $^3J_{HH}$ 5.7 Hz).

Synthesis of (3*R*,5*R*)-5-Acetoxy-1-ethynyl-3-hydroxy-2-methylcyclohex-1-ene (19) from 22. The same procedure as that described for enzymatic hydrolysis of **9** yielded compound **19** (94%). Spectral data are given above.

Synthesis of (3*R*,5*R*)-1-Ethynyl-5-hydroxy-2-methyl-3-[(4-nitrophenyl)carbonyloxy]cyclohex-1-ene (21). The same procedure as that described for enzymatic hydrolysis of **9** yielded compound **21**. The crude was purified by flash chromatography (10−25% EtOAc/hexane) as an oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.61 (br s, 1H, OH), 1.88−2.05 (m, 1H, H_4), 1.96 (s, 3H, H_9), 2.32−2.60 (m, 3H, H_4+H_6), 3.21 (s, 1H, H_8), 4.11 (t, 1H, H_5, $^3J_{HH}$ 7.0 Hz), 5.74 (t, 1H, H_3, $^3J_{HH}$ 6.1 Hz), 8.20 (d, 2H, H_{13}, $^3J_{HH}$ 2.0 Hz), and 8.29 (d, 2H, H_{12}, $^3J_{HH}$ 2.0 Hz); MS (ESI$^+$, *m*/*z*): 340 [(M + K)$^+$, 7%] and 324 [(M + Na)$^+$, 69].

Synthesis of (3*R*,5*R*)-5-Acetoxy-3-(chloroacetoxy)-1-ethynyl-2-methyl-cyclohex-1-ene (22). To a solution of **8** (50 mg, 0.26 mmol) in THF (3.5 mL) under nitrogen atmosphere were added chloroacetic acid (49 mg, 0.52 mmol), PPh_3 (135 mg, 0.52 mmol), and diethyl azodicarboxylate (80 μL, 0.52 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) to obtain 68 mg (97%) of **22** as a white solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.89 (s, 3H, H_9), 1.89−2.10 (m, 1H, H_4), 2.02 (s, 3H, H_{11}), 2.14−2.25 (ddd, 1H, H_4, $^2J_{HH}$ 13.7, $^3J_{HH}$ 6.1, $^3J_{HH}$ 3.1 Hz), 2.28−2.57 (m, 2H, H_6), 3.18 (s, 1H, H_8), 4.04 (s, 2H, H_{13}), 5.00 (m, 1H, H_5), and 5.50 (t, 1H, H_3, $^3J_{HH}$ 5.8 Hz); MS (ESI$^+$, *m*/*z*): 309 [(M + K)$^+$, 15%], 295 [(M(^{37}Cl) + Na)$^+$, 15], and 293 [(M + Na)$^+$, 40].

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Supporting Information Available: Complete ^{1}H and ^{13}C NMR spectral data in addition to mp, IR, microanalysis, optical rotation, and MS data for the new compounds. The level of purity is indicated by the inclusion of copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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