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TETRAHEDRON: *ASYMMETRY*

Chemoenzymatic synthesis of 1α,24(*R*)-dihydroxycholesterol

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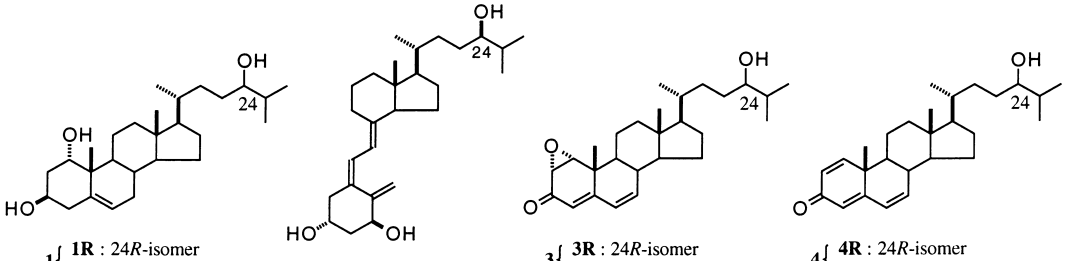
Abstract

1α,24(*R*)-Dihydroxycholesterol, which is the key intermediate for the synthesis of 1α,24(*R*)-dihydroxyvitamin D_3, was effectively synthesized via stereoselective esterification of the 24(*R*)-hydroxy group using a lipase in combination with inversion of configuration of the 24(*S*)-hydroxy group using the Mitsunobu reaction (*R*:*S*=99:1).

1. Introduction

1α,24(*R*)-Dihydroxycholesterol[1] **1R** is known to be the key intermediate of 1α,24(*R*)-dihydroxyvitamin D_3[2] **2**, which is a potent analogue of active vitamin D_3 and used as a therapeutic agent for psoriasis. The triol **1R** is obtained through the chromatographic separation of a diastereomeric mixture[3a] and the remaining diastereoisomer **1S** can be converted into the desired synthon[3b] **1R**. Previously, we reported a new methodology for the synthesis of **1R** using the diastereoselective isopropylation of steroidal 24-aldehyde precursors with diisopropylzinc in the presence of certain chiral β-amino alcohols.[4] We wish to report here the novel synthesis of **1R** employing the lipase catalyzed stereoselective esterification and the Mitsunobu reaction.



1 { **1R** : 24*R*-isomer, **1S** : 24*S*-isomer } **2** **3** { **3R** : 24*R*-isomer, **3S** : 24*S*-isomer } **4** { **4R** : 24*R*-isomer, **4S** : 24*S*-isomer }

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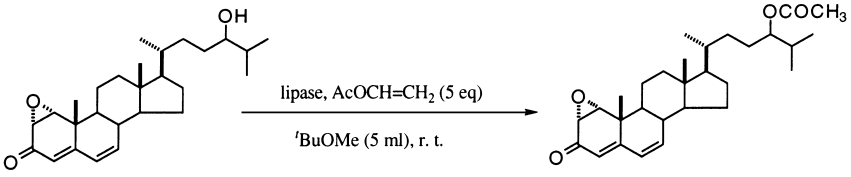
The lipase-catalyzed esterification of an enantiomeric mixture of hydroxy compounds in organic solvents is well established for obtaining enantiomerically pure compounds.[5] Since the direct application of the lipase-catalyzed esterification to the triol **1** is considered difficult with respect to regioselectivity, we focused on the esterification of the epoxy dienone **3** and the trienone **4**, which are known to be the precursors of the triol[6] **1**.

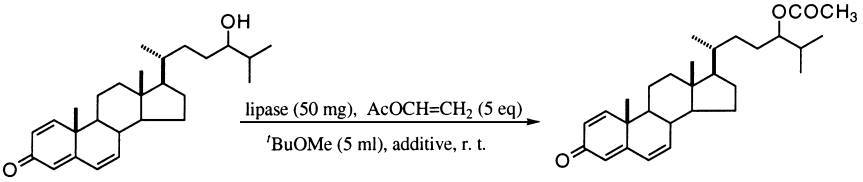
2. Results and discussion

We first examined the stereoselective esterification of the epoxydienone **3** using a lipase. The alcohol **3** was reacted with vinyl acetate in ^{t}BuOMe at room temperature for 48 h in the presence of several kinds of lipases (Table 1, entries 1–12). Among the 12 tested lipases, LIP-300 and Chirazyme L-6 were promising to preferentially acetylate **3R**, giving unreacted alcohol **3S** in high stereoselectivity (Table 1, entries 6 and 12). The reaction only occurred slightly in the presence of Chirazyme L-1, Chirazyme L-2, Chirazyme L-3, Chirazyme L-5, Chirazyme L-7, Chirazyme L-8, Amano PS-D, Amano AD, and Amano PS, while Chirazyme L-4 gave a moderate result (Table 1). By shortening the reaction time, both LIP-300 and Chirazyme L6 gave the desired ester **5** in higher stereoselectivity (Table 1, entries 13–16). LIP-300 was found to be more useful than Chirazyme L-6 because LIP-300 gave both **3** and **5** in a highly stereoselective manner. Thus, the LIP-300 catalyzed esterification furnished the ester **5R** in 56% yield (*R*:*S*=98:2) and alcohol **3S** in 44% yield (*R*:*S*=1:99) starting from the diastereomeric mixture **3** (*R*:*S*=56:44).



3 (200 mg, R : S = 56 : 44) **5**

We then studied the reaction of the trienone **4** with vinyl acetate in the presence of LIP-300 or Chirazyme L-6 to expand the scope of this diastereoselective acylation reaction of the steroidal hydroxy group at position 24. However, esterification of alcohol **4** using LIP-300 or Chirazyme L-6 resulted in a lower reactivity and lower selectivities than that of epoxydienone **3** (Table 2, entries 1–3). The results showed that the 1,2-epoxide moiety of **3** plays a crucial role in this reaction. Although adding MS 4A to the reaction mixture[8] increased the reaction rate, it did not improve the diastereoselectivity of the product **6** (Table 2, entries 4–5).



4 (200 mg, R : S = 56 : 44) **6**

While we have established the method to obtain both the ester **5R** and alcohol **3S** in high yield, we attempted the transformation of **3S** into **5R**. Among methods for the inversion of configuration of the hydroxy group,[9,10] the Mitsunobu reaction[10] is one of the mildest and most suitable for the reaction of **3**, which has an unstable epoxydienone moiety. Although the ester **5R** and alcohol **3S** can be easily separated, the reaction mixture of **5R** and **3S** was directly subjected to the Mitsunobu reaction conditions to simplify the process of obtaining **5R**. The resulting reaction mixture of **5R** and **3S** through

Table 1
Lipase-catalyzed acetylation of diastereomeric mixture of epoxydienone **3**

entry	lipase		time	**5**		**3**		*E* value[a]
	name	amount		yield	R : S	yield	R : S	
1	Chirazyme L-1[b]	50 mg	48 h	7%	12 : 88	91%	59 : 41	3
2	Chirazyme L-2[c]	10 mg	48 h	no reaction				
3	Chirazyme L-3[d]	200 mg	48 h	7%	95 : 5	92%	54 : 46	21
4	Chirazyme L-4[e]	50 mg	48 h	52%	92 : 8	44%	18 : 82	22
5	Chirazyme L-5[f]	100 mg	48 h	2%	24 : 76	97%	55 : 45	4
6	Chirazyme L-6[g]	50 mg	48 h	62%	90 : 10	35%	1 : 99	—
7	Chirazyme L-7[h]	200 mg	48 h	no reaction				
8	Chirazyme L-8[i]	50 mg	48 h	no reaction				
9	Lipase PS[j]	200 mg	48 h	no reaction				
10	Lipase PS-D[k]	200 mg	48 h	no reaction				
11	Lipase AK[l]	200 mg	48 h	no reaction				
12	LIP-300[m]	50 mg	48 h	65%	87 : 13	35%	0 : 100	—
13	Chirazyme L-6	50 mg	6 h	48%	99 : 1	35%	16 : 84	142
14	Chirazyme L-6	50 mg	24 h	59%	95 : 5	40%	2 : 98	
15	LIP-300	50 mg	2 h	52%	99 : 1	47%	7 : 93	256
16	LIP-300	50 mg	4 h	56%	98 : 2	44%	1 : 99	

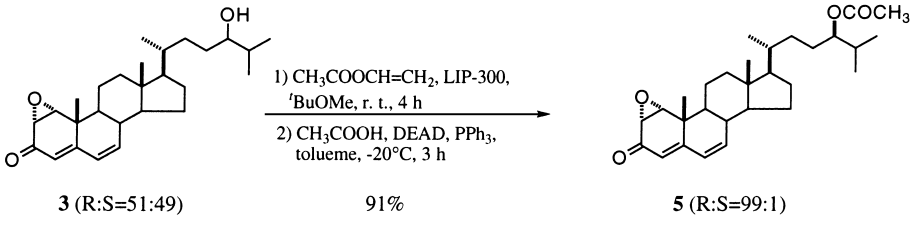
a. Calculated according to ref 7. b. Burkholderia sp. (Boehringer Mannheim), c. Candida antractica (Boehringer Mannheim), d. Candida rugosa (Boehringer Mannheim), e. Pseudomonas sp. (Boehringer Mannheim), f. Candida antractica (Boehringer Mannheim), g. Pseudomonas sp.(Boehringer Mannheim), h. porcine pancreas (Boehringer Mannheim), i. Humicola sp. (Boehringer Mannheim), j. Pseudomonas cepacia (Amano),k. Pseudomonas cepacia (Amano), l. Pseudomonas cepacia (Amano), m. Pseudomonas sp.(Toyobo)

Table 2
Lipase-catalyzed acetylation of diastereomeric mixture of trienone **4**

entry	lipase	time	additive	**6**		**4**		*E* value
				yield	R : S	yield	R : S	
1	LIP-300	23 h	———	45%	96 : 4	55%	24 : 76	36
2	LIP-300	47 h	———	54%	94 : 6	46%	10 : 90	
3	Chirazyme L-6	23 h	———	45%	93 : 7	55%	20 : 80	24
4	LIP-300	23 h	MS4A (50 mg)	65%	86 : 14	35%	1 : 99	33
5	LIP-300	16 h	MS4A (50 mg)	59%	93 : 7	41%	6 : 94	

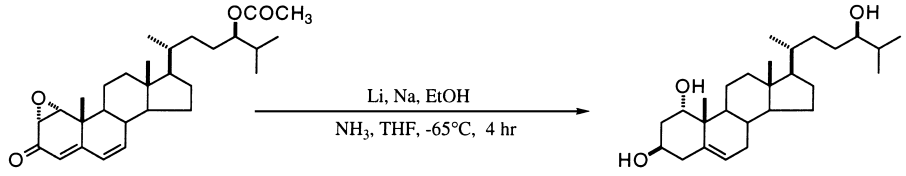
esterification of **3** (*R*:*S*=51:49) using LIP-300 was reacted with acetic acid in the presence of an excess amount of diethylazodicarboxylate (DEAD) and triphenylphosphine at −20°C to give **5R** in 91% yield with high stereoselectivity (*R*:*S*=99:1, Scheme 1).

To complete the synthetic route to the vitamin D_3 synthon **1R**, the thus obtained **5R** was subjected to Birch reduction[1,3b] using a mixture of Li and Na as a reducing metal to give the desired **1R** with the same diastereomeric purity (*R*:*S*=99:1, Scheme 2).



3 (R:S=51:49) 91% **5** (R:S=99:1)

Scheme 1.



5 (R:S=99:1) 80% **1** (R:S=99:1)

Scheme 2.

3. Conclusion

The lipase-catalyzed esterification of the diastereomeric mixture of the epoxyalcohol **3** was found to proceed in a highly stereoselective manner. The combination of this esterification with the Mitsunobu reaction gave desired ester **5R** in high yield with excellent stereoselectivity. The following Birch reduction of **5R** completed the synthesis of the vitamin D_3 synthon **1R**. This method provides an efficient route for the synthesis of **1R**, which is the key intermediate of 1α,24(*R*)-dihydroxyvitamin D_3 **2**.

4. Experimental

IR spectra were recorded on a Shimadzu 8100M spectrometer. NMR spectra were obtained using a Varian Gemini 200 (200 MHz) spectrometer with $CDCl_3$. Chemical shifts and coupling constants (*J*) are given in ppm relative to internal tetramethylsilane and hertz, respectively. The following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and b (broad). Mass spectra (MS) were taken at 70 eV using an HP 5971 mass spectrometer. For the high-performance liquid chromatography (HPLC) analysis, a Shimadzu Model LC-6A equipped with a Shimadzu SPD-6A UV detector (210 or 254 nm) and a Shimadzu C-R3A chromatopac were employed. Melting points were taken with a Mettler FP 81 and are uncorrected. All the lipases used are commercially available.

*4.1. Stereoselective esterification of **3***

To a solution of 1α,2α-epoxy-24-hydroxycholesta-4,6-dien-3-one **3** (200 mg, 0.49 mmol, **3R**:**3S**=56:44) in ^{t}BuOMe (5 ml) was added at rt vinyl acetate (0.23 ml, 2.50 mmol) and LIP-300 (50 mg), and the resulting mixture was stirred for 4 h. After filtration, the filtrate was evaporated to give a crude product, which was subjected to silica gel chromatography (20 g) with hexane and EtOAc (20:1 up to 3:1) providing 24-acetoxy-1α,2α-epoxycholesta-4,6-dien-3-one **5** (124 mg, 0.27 mmol, **5R**:**5S**=98:2, 56%) and 1α,2α-epoxy-24-hydroxycholesta-4,6-dien-3-one **3** (88 mg, 0.21 mmol, **3R**:**3S**=2:98, 44%). The obtained **5** was subjected to HPLC analysis (Chiralpak OJ, 25 cm×4.6 mm I.D.) using hexane:ethanol:methanol (100:1:0.3) as the mobile phase at 1.0 ml/min to estimate the ratio of **5R** and **5S** (**5R**: 20.2 min, **5S**: 18.2 min). The obtained **3** was subjected to HPLC analysis

(YMC AM-303, 25 cm×4.6 mm I.D.) using acetonitrile:H_2O (7:3) as the mobile phase at 1.0 ml/min to estimate the ratio of **3R** and **3S** (**3R**: 21.8 min, **3S**: 20.9 min). Other data are summarized in Table 1. **5R**: Mp 99–101°C (hexane–ethyl acetate); $[\alpha]_D^{20}$=+151 (*c* 0.20, EtOH); IR (KBr): 2861, 1732, 1650, 1620, 1590, 1456, 1364, 1280 cm^{-1}; ^1H NMR: δ 0.79 (s, 3H), 0.90–1.00 (m, 9H), 1.20 (s, 3H), 1.00–2.10 (m, 18H), 2.06 (s, 3H), 3.40–3.60 (m, 2H), 4.60–4.80 (m, 1H), 5.60–5.65 (m, 1H), 6.00–6.15 (m, 2H); MS (m/z): 454 (M^+); high-resolution MS for $C_{29}H_{42}O_4$ (M^+): calcd m/z: 454.3083; found: 454.3023. **3S**: Mp 145–147°C (hexane–ethyl acetate); $[\alpha]_D^{20}$=+169 (*c* 0.20, EtOH); IR (KBr): 3200, 2880, 1667, 1617, 1586, 1450, 1380, 1297 cm^{-1}; ^1H NMR: δ 0.78 (s, 3H), 0.90–1.00 (m, 9H), 1.18 (s, 3H), 1.00–2.10 (m, 18H), 3.25–3.35 (b, 1H), 3.40–3.60 (m, 2H), 4.60–4.80 (m, 1H), 5.60–5.65 (m, 1H), 6.00–6.15 (m, 2H); MS (m/z): 412 (M^+); high-resolution MS for $C_{27}H_{40}O_3$ (M^+): calcd m/z: 412.2977; found: 412.2927.

*4.2. Stereoselective esterification of **4***

To a solution of 24-hydroxycholesta-1,4,6-trien-3-one **4** (200 mg, 0.51 mmol, **4R**:**4S**=56:44) in ^{t}BuOMe (5 ml) were added at rt vinyl acetate (0.23 ml, 2.50 mmol) and LIP-300 (50 mg), and the resulting mixture was stirred for 23 h. After filtration, the filtrate was evaporated to give a crude product, which was subjected to silica gel chromatography (20 g) with hexane and EtOAc (20:1 up to 3:1) providing 24-acetoxycholesta-1,4,6-trien-3-one **6** (100 mg, 0.23 mmol, **6R**:**6S**=96:4, 45%) and 24-hydroxycholesta-1,4,6-trien-3-one **4** (110 mg, 0.28 mmol, **4R**:**4S**=20:80, 55%). The obtained **4** was subjected to HPLC analysis (YMC AM-303, 25 cm×4.6 mm I.D.) using acetonitrile:H_2O (7:3) as the mobile phase at 1.0 ml/min to estimate the ratio of **4R** and **4S** (**4R**: 38.8 min, **4S**: 34.7 min). The diastereomeric ratio of **6** was determined using HPLC analysis after converting to **4** by deacetylation. Other data are summarized in Table 2. **6**: IR (KBr): 2950, 2880, 1718, 1652, 1602, 1455, 1375, 1245 cm^{-1}; ^1H NMR: δ 0.77 (s, 3H), 0.85–1.00 (m, 9H), 1.20 (s, 3H), 1.00–2.20 (m, 18H), 2.04 (s, 3H), 4.60–4.75 (b, 1H), 6.00–6.10 (m, 2H), 6.20–6.30 (m, 2H), 7.08 (d, 2H, *J*=12 Hz); MS (m/z): 438 (M^+); high-resolution MS for $C_{29}H_{42}O_3$ (M^+): calcd m/z: 438.3135; found: 438.3134. **4**: Mp 97–98°C (hexane–ethyl acetate); IR (KBr): 3440, 1765, 1603, 1460, 1380, 1287 cm^{-1}; ^1H NMR: δ 0.76 (s, 3H), 0.90–1.00 (m, 9H), 1.19 (s, 3H), 1.00–2.10 (m, 18H), 3.25–3.35 (b, 1H), 6.00–6.10 (m, 2H), 6.20–6.30 (m, 2H), 7.08 (d, 2H, *J*=12 Hz); MS (m/z): 396 (M^+); high-resolution MS for $C_{27}H_{40}O_2$ (M^+): calcd m/z: 396.3029; found: 396.3028.

*4.3. Consecutive stereoselective esterification and Mitsunobu reaction of **3***

To a solution of 1α,2α-epoxy-24-hydroxycholesta-4,6-dien-3-one **3** (200 mg, 0.49 mmol, **3R**:**3S**=51:49) in ^{t}BuOMe (5 ml) was added at rt vinyl acetate (0.23 ml, 2.50 mmol) and LIP-300 (50 mg), and the resulting mixture was stirred for 4 h. After filtration, the filtrate was evaporated to give a crude product, which was dissolved in toluene (5 ml) followed by the addition of Ph_3P (286 mg, 1.10 mmol) and acetic acid (61 μl, 1.10 mmol). To the mixture was added 2.3 mol/l of a toluene solution of diethylazodicarboxylate (0.42 ml, 0.97 mmol) at −20°C. After stirring for 3 h, aq. $NaHCO_3$ solution (30 ml) was added to the mixture and extracted with EtOAc (30 ml). The organic layer was washed with brine (30 ml) and dried over $MgSO_4$. After filtration, evaporation of the solvent gave a crude product, which was purified by silica gel chromatography (20 g) with hexane and EtOAc (20:1 up to 3:1) providing 24-acetoxy-1α,2α-epoxycholesta-4,6-dien-3-one **5** (200 mg, 0.44 mmol, **5R**:**5S**=99:1, 91%). The obtained **5** was subjected to HPLC analysis (Chiralpak OJ, 25 cm×4.6 mm I.D.) using hexane:ethanol:methanol (100:1:0.3) as the mobile phase at 1.0 ml/min to estimate the ratio of **5R** and **5S** (**5R**: 20.2 min, **5S**: 18.2 min).

4.4. Preparation of 1α,24(R)-dihydroxycholesterol **1R**

To a solution of Li (1.8 g, 300 mmol) and Na (0.8 g, 34.7 mmol) in liq. NH_3, a solution of 24-acetoxy-1α,2α-epoxycholesta-4,6-dien-3-one **5** (3.0 mg, 6.61 mmol, **5R**:**5S**=99:1) in THF (150 ml) was added dropwise at −60°C. After stirring for 1 h, EtOH (51 ml, 870 mmol) was added and the resulting mixture was stirred for 3 h. The mixture was warmed to rt to vaporize the NH_3 followed by the addition of 6 mol/l HCl solution (100 ml) and EtOAc (100 ml). The organic layer was separated, washed with aq. $NaHCO_3$ solution (100 ml) and brine (30 ml), and dried over $MgSO_4$. After filtration, evaporation of the solvent gave a crude product, which was purified by silica gel chromatography (100 g) with toluene and acetone (4:1 up to 2:1) providing 1α,24(*R*)-dihydroxycholesterol **1** (2.21 g, 5.31 mmol, 80%, **1R**:**1S**=99:1). The obtained **1** was subjected to HPLC analysis (YMC A-303, 25 cm×4.6 mm I.D.) using acetonitrile:water (6:4) at 1.0 ml/min. The diastereomeric ratio of **1** was estimated by comparison of the peak area of **1R** and **1S** (**1R**: 34 min, **1S**: 28 min); **1R**: IR (KBr): 3400, 1460, 1370, 1050 cm^{-1}; ^1H NMR: δ 0.65 (s, 3H), 0.85–1.00 (m, 9H), 1.07 (s, 3H), 1.00–2.20 (m, 25H), 2.20–2.40 (m, 2H), 3.28 (m, 1H), 3.83 (m, 1H), 3.90–4.10 (m, 1H), 5.60 (m, 1H); MS (m/z): 418 (M^+).

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