Enantioselective Reaction of (±)-1-Hydroxy-5-methyl-3-vinylcyclohex-2-ene and Its Acyl Derivatives Using Lipid-Lipase Aggregates in Organic Solvent

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The enzymatic esterification or hydrolysis of 1-hydroxy-((±)-1), 1-acetoxy-((±)-2) and 1-butyryloxy-5-methyl-3-vinylcyclohex-2-ene ((±)-3) was carried out using lipid-lipase aggregates. The esterification of (±)-1 provided (1*S*,5*S*)-1 with >99% ee using lipid-lipase "Amano P" with vinyl butyrate as the acylating reagent.

Key words lipid-lipase aggregate; enantioselective esterification; 1-butyryloxy-5-methyl-3-vinylcyclohex-2-ene; mevinolin derivative

Enantioselective synthesis using biocatalysts has been widely used and is one of the most effective methods for transformation of racemic compounds into chiral ones. In particular, enantioselective esterification and hydrolysis with lipase have proved to be broadly useful. The use of organic solvents is convenient for the transformation of racemic lipophilic substrates into chiral ones,[1] but inactivation and denaturation of lipase occur. However, immobilized enzymes have remarkable activity in organic solvents and there are numerous methods of immobilization.[2] For example, the use of reverse micelles is effective. They lack the outer layer of liposomes and are spheroidal aggregates formed by certain amphiphiles such as phospholipids and water containing lipase under mild conditions in nonpolar solvents. Such micelles show affinity for external nonpolar solvents and may retain the enzyme activity for a long time. There are many examples of synthesis using these micelles,[3] but only a few reports of enantioselective esterification and hydrolysis using reverse micelles such as lipid-coated lipase.[4] (1*S*)-Hydroxy-(5*S*)-methyl-3-vinylcyclohex-2-ene (+)-**1** is expected to be useful as a synthon for optically active natural products such as compactin and mevinolin, which are potent competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase, EC 1.1.1.34), the rate-limiting enzyme in cholesterol biosynthesis.[5] A few examples of enantioselective reaction with lipase of monocyclic enol derivatives are known,[6] but there has been no report on that of conjugated dienol derivatives so far. We now report the enantioselective reactions of the dienol (±)-**1** and its derivatives using lipid-lipase aggregates in organic solvents.

Results and Discussion

In preliminary experiments, it was found that 1:1 mixtures of two racemates, [(±)-**1** and (±)-**2**] and [(±)-**1** and (±)-**3**],[7] were well separated by high-performance liquid-chromatographic (HPLC) analysis with a chiral column [Chiralcell OD-H (4.6 × 250 mm)]. Thus, chemical and optical yields of the reaction products can be determined by using this method. Next, we examined the asymmetric hydrolysis of (±)-**2** with twenty-seven kinds of commercially available lipases in phosphate buffer. We then selected four lipases, "Amano P" (P) from *Pseudomonas* sp., OF-360 (OF) from *Candida cylindracea*, MY-30 (MY) from *Candida cylindracea* and "Amano AY" (AY) from *Candida cylindracea* and prepared aggregates with these lipases and a phospholipid analogue

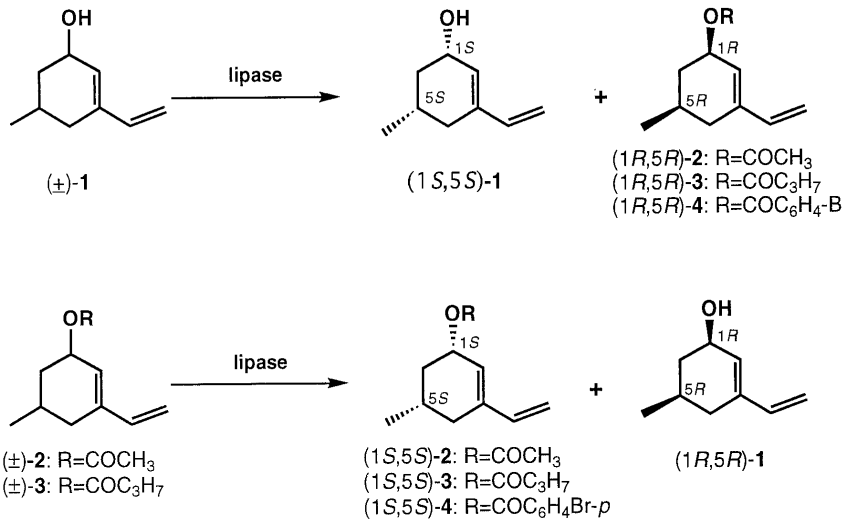

Chart 1

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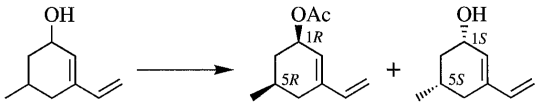
(1,2-di-*O*-hexadecyl-*rac*-3-phosphonoxy ethyl morpholium) by using the reported procedure.[4d] The absolute structures of enzymatic reaction products were determined by using the CD exciton chirality method.[8] The CD spectrum of the (+)-benzoate (**4**) prepared from (−)-**1** (90% ee) exhibited the first exciton CD Cotton effect with positive sign (ε 45316 at 243 nm), while that of the (−)-benzoate (**4**) derived from (−)-**2** (64% ee) showed the first exciton CD Cotton effect with negative sign (ε 45359 at 243 nm). Therefore the absolute structures of (−)-**1** and (−)-**2** were established to be 1*R*,5*R* and 1*S*,5*S*, respectively.

Enantioselective Esterification Using Lipid-Lipase Aggregates The lipid-lipase aggregates showed higher catalytic activity than that of the native lipases when esterification of the dienol (±)-**1** was carried out using isopropenyl acetate (IPA) as the acetylating agent in absolute isopropyl ether (IPE), as shown in entries 1—8 of Table 1. Lack of reaction in entries 6—8 may be due to inactivation of the enzymes. In a more lipophilic solvent (hexane), the catalytic activities of aggregates and native lipases using IPA in the acetylation of (±)-**1** did not differ, as shown in entries 9—16. The lipid-lipase aggregates also showed similar activity to that of native enzymes when acetylation of the dienol was tried using vinyl acetate (VA) as the acylating agent in IPE (entries 17–24). In hexane, the reaction (entry 25) with lipid-lipase "Amano P" aggregate (aggregate P) using VA showed a higher reaction rate and gave (1*S*,5*S*)-**1** with high optical purity in comparison with entries 26—32. Butyrylation of (±)-**1** was conducted with vinyl butyrate (VB) as the acylating agent in IPE. The lipid-lipase aggregates, particularly, aggregate P showed higher enzymatic activity compared with that of native enzymes (entries 5—8 of Table 2). The reason why no reaction was observed in entry 5 may be the same as mentioned above (entries 6—8 of Table 1). The butyrylation of (±)-**1** with aggregate P using the same agent in hexane proceeded with the highest activity [(1*S*,5*S*)-**1**, 23% yield, 99% ee, 2.3 h)], as shown in entry 9, and lipid-lipase MY-30 (aggregate MY) also showed higher enantioselective activity [(1*S*,5*S*)-**1**, 35% yield, 86% ee, 2 h] compared with that of the native enzyme (entry 15). It was found that both aggregate P and native P were well suited for esterification and showed excellent activities.

Enantioselective Hydrolysis of (±)-2 and (±)-3 Using Lipid-Lipase Aggregates We carried out the enzymatic hydrolysis of the acetate (±)-**2** with lipid-lipase aggregates in phosphate buffer and water-saturated isopropenyl ether (H_2O/IPE) in order to compare the results with those using native lipase (Table 3). As shown in entries 1—8, the rates of hydrolysis of (±)-2 in phosphate buffer were much faster than those in the other examples mentioned below. The hydrolysis using aggregate P in phosphate buffer produced unchanged (1*S*,5*S*)-**2** with the high optical purity of 94% ee (entry 1), while the hydrolysis of (±)-**2** using native P yielded the same acetate (1*S*,5*S*)-**2** with 57% ee (entry 5). The rate of hydrolysis of (±)-**2** using the lipid-lipase "OF-360" (aggregate OF) was much faster than that using native OF (entry 2), while the optical purity of (1*S*,5*S*)-2 using native OF was high at 97% ee (entry 6). In organic solvent (H_2O/IPE), hydrolysis of the acetate (±)-**2** using the aggregate OF and native OF produced the alcohol (−)-**1** with the same high optical purity of 90% ee in similar yields (entries 10 and 14). In organic solvent, no difference between the activities of the aggregate and the native lipase was observed. Hydrolysis of the more lipophilic substrate (±)-**3** in buffer solution

Table 1. Enantioselective Acetylation of the Dienol (±)-**1**

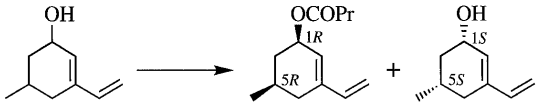


(±)-**1** (+)-**2** (+)-**1**

Aggregate							Native						
Entry	Enzyme	Agent	Solvent	Time (d)	Yield (%)/ee (%)		Entry	Enzyme	Agent	Solvent	Time (d)	Yield (%)/ee (%)	
					(1*R*,5*R*)-**2**	(1*S*,5*S*)-**1**						(1*R*,5*R*)-**2**	(1*S*,5*S*)-**1**
1	AP	IPA	IPE	1	83/14	14/91	5	P	IPA	IPE	5	15/69	44/28
2	AOF	IPA	IPE	10	16/71	73/17	6	OF	IPA	IPE	10	No reaction	
3	AMY	IPA	IPE	10	21/81	61/35	7	MY	IPA	IPE	21	No reaction	
4	AAY	IPA	IPE	10	9/63	36/25	8	AY	IPA	IPE	21	No reaction	
9	AP	IPA	Hexane	2.7	17/36	59/26	13	P	IPA	Hexane	5.5	17/30	60/ 9
10	AOF	IPA	Hexane	27.3	19/68	54/25	14	OF	IPA	Hexane	27.3	5/60	60/ 6
11	AMY	IPA	Hexane	27.3	15/76	62/16	15	MY	IPA	Hexane	27.3	7/81	70/ 9
12	AAY	IPA	Hexane	22.9	36/74	39/ 6	16	AY	IPA	Hexane	27.3	8/62	60/ 7
17	AP	VA	IPE	0.8	58/44	40/68	21	P	VA	IPE	2.8	42/48	28/77
18	AOF	VA	IPE	9.6	6/ 4	87/ 3	22	OF	VA	IPE	9.6	12/31	82/ 0
19	AMY	VA	IPE	9.6	14/62	84/11	23	MY	VA	IPE	8.2	10/71	54/14
20	AAY	VA	IPE	5.8	29/41	63/18	24	AY	VA	IPE	8.2	11/37	65/ 7
25	AP	VA	Hexane	0.1	87/ 3	2/>99	29	P	VA	Hexane	2.2	19/64	52/19
26	AOF	VA	Hexane	3.9	15/25	74/ 6	30	OF	VA	Hexane	5.8	10/ 7	74/12
27	AMY	VA	Hexane	2	11/45	85/ 8	31	MY	VA	Hexane	11	3/44	91/ 1
28	AAY	VA	Hexane	2	9/57	89/ 7	32	AY	VA	Hexane	11	2/22	83/ 0

AP; lipid-lipase "Amano P" aggregate. AOF; lipid-lipase "OF-360" aggregate. AMY; lipid-lipase "MY-30" aggregate. AAT; lipid-lipase "Amano AY" aggregate.

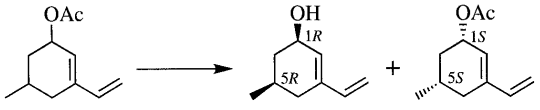
Table 2. Enantioselective Butyrylation of the Dienol (±)-**1**



(±)-**1** (+)-**3** (+)-**1**

Aggregate							Native						
Entry	Enzyme	Agent	Solvent	Time (d)	Yield (%)/ee (%)		Entry	Enzyme	Agent	Solvent	Time (d)	Yield (%)/ee (%)	
					(1*R*,5*R*)-**3**	(1*S*,5*S*)-**1**						(1*R*,5*R*)-**3**	(1*S*,5*S*)-**1**
1	AP	VB	IPE	3	11/78	50/18	5	P	VB	IPE	9	No reaction	
2	AOF	VB	IPE	3	28/72	24/75	6	OF	VB	IPE	7	36/52	55/37
3	AMY	VB	IPE	3	30/66	35/60	7	MY	VB	IPE	7	30/69	60/37
4	AAY	VB	IPE	3	38/45	24/68	8	AY	VB	IPE	7	12/ 5	80/11
9	AP	VB	Hexane	2.3	69/30	23/99	13	P	VB	Hexane	0.1	32/66	54/40
10	AOF	VB	Hexane	2	20/54	72/17	14	OF	VB	Hexane	5	22/21	61/ 7
11	AMY	VB	Hexane	2	52/33	35/86	15	MY	VB	Hexane	5	4/46	76/ 2
12	AAY	VB	Hexane	2	32/67	56/39	16	AY	VB	Hexane	5	9/74	69/ 5

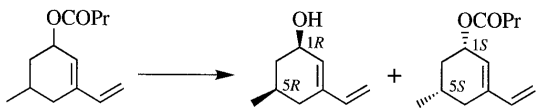
Table 3. Enantioselective Hydrolysis of the Acetate (±)-**2**



(±)-**2** (−)-**1** (−)-**2**

Aggregate						Native					
Entry	Enzyme	Solvent	Time (d)	Yield (%)/ee (%)		Entry	Enzyme	Solvent	Time (h)	Yield (%)/ee (%)	
				(1*S*,5*S*)-**2**	(1*R*,5*R*)-**1**					(1*S*,5*S*)-**2**	(1*R*,5*R*)-**1**
1	AP	Buffer	3	15/94	61/38	5	P	Buffer	2	13/57	35/45
2	AOF	Buffer	2	44/62	43/82	6	OF	Buffer	16.5	11/97	63/49
3	AMY	Buffer	2	42/18	28/62	7	MY	Buffer	2	25/32	25/55
4	AAY	Buffer	2	52/ 2	28/16	8	AY	Buffer	2	46/15	29/39
9	AP	IPE	3	60/45	34/69	13	P	IPE	5	81/ 7	8/71
10	AOF	IPE	2	58/52	27/90	14	OF	IPE	5	73/27	23/90
11	AMY	IPE	3	43/17	8/86	15	MY	IPE	5	89/ 3	4/78
12	AAY	IPE	3	73/23	24/76	16	AY	IPE	5	80/ 4	7/66

Table 4. Enantioselective Hydrolysis of the Butyrate (±)-**3**



(±)-**3** (−)-**1** (−)-**3**

Aggregate						Native					
Entry	Enzyme	Solvent	Time (d)	Yield (%)/ee (%)		Entry	Enzyme	Solvent	Time (h)	Yield (%)/ee (%)	
				(1*S*,5*S*)-**3**	(1*R*,5*R*)-**1**					(1*S*,5*S*)-**3**	(1*R*,5*R*)-**1**
1	AP	Buffer	3	77/11	22/43	5	P	Buffer	3	53/10	2/55
2	AOF	Buffer	3	16/24	51/ 9	6	OF	Buffer	3	31/36	22/27
3	AMY	Buffer	3	40/ 9	27/11	7	MY	Buffer	3	50/17	34/26
4	AAY	Buffer	3	28/13	22/10	8	AY	Buffer	3	44/18	46/21
9	AP	IPE	7	76/14	11/85	13	P	IPE	21	No reaction	
10	AOF	IPE	1	43/40	22/81	14	OF	IPE	1	48/33	20/82
11	AMY	IPE	1.1	75/29	19/85	15	MY	IPE	6	64/18	15/78
12	AAY	IPE	1.1	72/28	25/79	16	AY	IPE	6	53/39	29/69

gave lower optical purities and chemical yields, as shown in entries 1—8 of Table 4. On the other hand, in organic solvent (H_2O/IPE), no marked difference between the enzymatic activities of aggregates and native lipases for (±)-**3** was obseved, as shown in entries 10—16, except for 13, of Table 4. The reason why no reaction occurred in entry 13 would be the same as that mentioned above (entries 6—8 of Table 1). Hydrolysis by use of aggregate P in organic solvent gave (−)-**1** in the high optical purity of 85% ee and chemical yield of 11%, while (±)-**3** remained intact when native P was used as the catalyst (entry 13). In the asymmetric hydrolysis, both aggregate OF and native OF were found to be well suited for hydrolyses in buffer and organic solvents. In conclusion, the lipid-lipase aggregates are better catalysts than the native lipases for both enantioselective esterification and hydrolysis. In particular, the aggregates were effective for both the asymmetric hydrolysis of the lipophilic substrate (±)-**3** and the enantioselective esterification of (±)-**1** using lipophilic acylating agents such as VB.

Experimental

Melting points were measured with a Kofler micro melting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured on a JEOL GX-270 spectrometer and spectra were taken as 5—10% (w/v) solutions in $CDCl_3$ with Me_4Si as an internal reference. Infrared (IR) spectra (KBr) were measured on a JASCO A-3 spectrophotometer. High-resolution mass spectra (HR-MS) were taken on a JEOL JMS D-300 (JMA-200 data analysis system) mass spectrometer. Optical rotations were measured with a JASCO DIP-360 polarimeter in $CHCl_3$ solution. CD spectra were measured in a 0.05 cm cell with a JASCO J-500A spectropolarimeter. The HPLC system was composed of a Shimadzu LC 10AD flow system and a Soma S-310A UV detector.

General Procedure for Enantioselective Acylation of *cis*-5-Methyl-3-vinylcyclohex-2-en-1-ol [(±)-1] A mixture of substrate (5 mg), acylating reagent (5 mg) and lipid-lipase aggregate (5 mg) or native lipase (5 mg) in a solvent (5 ml) was shaken at 33 °C for a suitable time. The reaction mixture was applied to a silica gel cartridge (Sep-pack) using hexane–ethyl acetate (EtOAc) (1 : 1) as the eluent, and each fraction was evaporated to afford a product, which was analyzed by HPLC. The results are shown in Tables 1 and 2.

General Procedure for Enantioselective Hydrolysis of *cis*-1-Acetoxy-[(±)-2] and *cis*-1-Butyryloxy-5-methyl-3-vinylcyclohex-2-ene [(±)-3] A mixture of substrate (5 mg) and lipid-lipase aggregate (5 mg) or native lipase (5 mg) in phosphate buffer (5 ml, pH 7.25) or solvent (5 ml, H_2O/IPE) was shaken at 33 °C for a suitable time. The reaction mixture was extracted with EtOAc, and the organic solution was dried over anhydrous $MgSO_4$ and evaporated to afford a crude product (in the case of phosphate buffer). The product or reaction mixture (in the case of organic solvent) was applied to a silica gel cartridge (Sep-pack) using hexane–EtOAc (1 : 1) as the eluent, and each fraction was evaporated to afford a product, which was analyzed by HPLC. The results are shown in Tables 3 and 4.

HPLC Analysis of Two Pairs of Two Racemates, [(±)-1 and (±)-2)] and [(±)-1 and (±)-3], Using a Chiral Column Two 1 : 1 mixtures of two racemates [(±)-**1** and (±)-**2**] and [(±)-**1** and (±)-**3**] gave well separated peaks [(±)-**1**; 71.8 min, 74.0 min, (±)-**2**; 35.6 min, 37.8 min and (±)-**3**; 34.4 min, 36.2 min] corresponding to each enantiomer under the following analytical conditions (eluent, 0—4.8% hexane–isopropanol; detection, UV at 230 nm: flow rate, 0.2–0.8 ml/min. The assignment of these peaks was achieved by comparing them with those of authentic samples (1*R*,5*R*)-**1** (t_R = 74.0 min), (1*S*,5*S*)-**2** (t_R = 35.6 min) and (1*S*,5*S*)-**3** (t_R = 34.4 min).

Preparation of (1*R*,5*R*)-1 and (1*S*,5*S*)-2 from *cis*-1-Acetoxy-5-methyl-3-vinylcyclohex-2-ene [(±)-2] A mixture of substrate (200 mg) and aggregate OF (50 mg) in H_2O/IPE (20 ml) was shaken at 33 °C for 19 h. The reaction mixture was filtered and the filtrate was evaporated to afford a crude product, which was subjected to preparative TLC [silica gel, 20 × 20 cm; developing solvent, hexane–EtOAc (2 : 1)] to afford (−)-**1** (47 mg, 31% yield, 94% ee) and (−)-**2** (100 mg, 50% yield, 48% ee). (−)-**1**: $[\alpha]_D^{26}$ −56° (*c* = 0.949, $CHCl_3$). IR (KBr) cm^{-1}: 3330 (OH), 1642 and 1607 (C=C–C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.06 (d, 3H, *J* = 6 Hz, >CHC<u>H</u>$_3$), 4.39 (br s, 1H, >C<u>H</u>OH), 5.04 (d, 1H, *J* = 11 Hz, –CH=C<u>H</u>$_2$), 5.19 (d, 1H, *J* = 17 Hz, –CH=C<u>H</u>$_2$), 5.69 (s, 1H, –C<u>H</u>=C<), 6.37 (dd, 1H, *J* = 11, 17 Hz, –C<u>H</u>=CH$_2$). HR-MS *m/z*: 138.1034 (Calcd for $C_9H_{14}O$: 138.1043). EI-MS *m/z* (rel. int. %): 138 (M^+, 51), 123 (22), 109 (18), 113 (17). (−)-**2**: $[\alpha]_D^{26}$ −17° (*c* = 0.701, $CHCl_3$). IR (KBr) cm^{-1}: 1736 (–OCOCH$_3$), 1646 and 1609 (C=C–C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.06 (d, 3H, *J* = 7 Hz, >CHC<u>H</u>$_3$), 2.06 (s, 3H, –COC<u>H</u>$_3$), 5.05 (d, 1H, *J* = 11 Hz, –CH=C<u>H</u>$_2$), 5.21 (d, 1H, *J* = 18 Hz, –CH=C<u>H</u>$_2$), 5.46 (br s, 1H, >C<u>H</u>OCOCH$_3$), 5.60 (s, 1H, –C<u>H</u>=C<), 6.35 (dd, 1H, *J* = 11, 18 Hz, –C<u>H</u>=CH$_2$). HR-MS *m/z*: 180.1138 (Calcd for $C_{11}H_{16}O_2$: 180.1148). EI-MS *m/z* (rel. int. %): 180 (M^+, 62), 166 (13), 155 (30), 138 (100), 120 (41).

Preparation of (1*R*,5*R*)-1 and (1*S*,5*S*)-3 from 1-Butyryloxy-5-methyl-3-vinylcyclohex-2-ene [(±)-3] A mixture of substrate (118 mg) and aggregate OF (128 mg) in H_2O/IPE (130 ml) was shaken at 33 °C for 6 d. The reaction mixture was filtered and evaporated to afford a crude product, which was subjected to preparative TLC [silica gel, 20 × 20 cm; developing solvent, hexane–EtOAc (2 : 1)] to afford (−)-**1** (31 mg, 39% yield, 69% ee) and (−)-**3** (54 mg, 48% yield, 72% ee). (−)-**1**: $[\alpha]_D^{22}$ −38° (*c* = 0.714, $CHCl_3$). (−)-**3**: $[\alpha]_D^{22}$ −20° (*c* = 0.805, $CHCl_3$). IR (KBr) cm^{-1}: 1737 (–OCO–), 1639 and 1608 (C=C–C=C). ^{1}H-NMR ($CDCl_3$) δ: 0.95 (t, 3H, *J* = 7 Hz, –CH$_2$C<u>H</u>$_3$), 1.06 (d, 3H, *J* = 6 Hz, >CH$_2$C<u>H</u>$_3$), 1.66 (tq, 2H, *J* = 7, 7 Hz, –C<u>H</u>$_2$CH$_3$), 2.29 (t, 2H, *J* = 7 Hz, –COC<u>H</u>$_2$CH$_2$–), 5.05 (d, 1H, *J* = 11 Hz, –CH=C<u>H</u>$_2$), 5.20 (d, 1H, *J* = 17 Hz, –CH=C<u>H</u>$_2$), 5.48 (br s, 1H, >C<u>H</u>OCOCH$_2$-), 5.60 (s, 1H, –C<u>H</u>=C<), 6.35 (dd, 1H, *J* = 11, 17 Hz, –C<u>H</u>=CH$_2$). HR-MS *m/z*: 208.1477 (Calcd for $C_{13}H_{20}O_2$: 208.1464). EI-MS *m/z* (rel. int. %): 208 (M^+, 23), 196 (6), 137 (35), 122 (24), 105 (43).

Preparation of (1*R*,5*R*)-Benzoate (4) from (1*R*)-Hydroxy-(5*R*)-methyl-3-vinylcyclohex-2-ene (1) Pyridine (0.5 ml) was added to a mixture of the alcohol (1*R*,5*R*-**1**) (39 mg, 90% ee), *p*-bromobenzoyl chloride (145 mg) and 4-dimethylaminopyridine (DMAP) (10 mg) and the reaction mixture was stirred at room temperature for 1 h. After addition of H_2O, the reaction mixture was extracted with EtOAc. The extract was washed with saturated aqueous NaCl, dried over anhydrous $MgSO_4$ and concentrated. The crude product was subjected to preparative TLC [silica gel, 20 × 20 cm, developing solvent, hexane–EtOAc (1 : 1)] to provide the *p*-bromobenzoate (1*R*,5*R*-**4**) (47 mg, 52% yield), which was recrystallized from hexane–EtOAc to give colorless prisms, mp 63—64 °C. $[\alpha]_D^{26}$ +96° (*c* = 1.07, $CHCl_3$). UV λ_{max}^{EtOH} nm (ε): 205 (451000), 230 (650000), 240 (699000). IR (KBr) cm^{-1}: 1719 (–OCOAr), 1646 and 1604 (sh, C=C–C=C), 1596 (Ar), 750. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (d, 3H, *J* = 6 Hz, >CHC<u>H</u>$_3$), 5.09 (d, 1H, *J* = 11 Hz, –CH=C<u>H</u>$_2$), 5.25 (d, 1H, *J* = 17 Hz, –CH=C<u>H</u>$_2$), 5.72 (br s, 1H × 2, –OC<u>H</u><, –C<u>H</u>=C<), 6.40 (dd, 1H, *J* = 11, 17 Hz, –C<u>H</u>=CH$_2$), 7.57 (d, 2H, *J* = 8 Hz, ArH), 7.91 (d, 2H, *J* = 8 Hz, ArH). HR-MS *m/z*: 322.0381 (Calcd for $C_{16}H_{17}BrO_2$: 322.0391). EI-MS *m/z* (rel. int. %): 322 (M^+, 21), 320 (66), 186 (32), 184 (35), 155 (23), 137 (33), 120 (45), 105 (100). CD (*c* = 1.96 × 10^{-3} MeOH) $[\theta]^{26}$ (nm): +45316 (243) (positive maximum).

Preparation of (1*S*,5*S*)-Benzoate (4) from (1*S*)-Acetoxy-(5*S*)-methyl-3-vinylcyclohex-2-ene (2) Methanol (0.3 ml) was added to a mixture of the acetate (1*S*,5*S*-**2**) (57 mg, 64% ee) and potassium carbonate (10 mg), then the mixture was stirred at room temperature for 2 h. After addition of H_2O, the reaction mixture was extracted with EtOAc. The extract was washed with saturated aqueous NaCl, dried over anhydrous $MgSO_4$ and concentrated. Pyridine (0.5 ml) was added to a mixture of the above crude alcohol (43 mg), *p*-bromobenzoyl chloride (144 mg) and DMAP (10 mg), then the mixture was stirred at room temperature for 1 h. After addition of H_2O, whole was extracted with EtOAc. The extract was washed with saturated aqueous NaCl, dried over anhydrous $MgSO_4$ and concentrated. The crude product was subjected to preparative TLC [silica gel, 20 × 20 cm, developing solvent, hexane–EtOAc (1 : 1)] to provide the *p*-bromobenzoate (1*S*,5*S*)-**4** (45 mg, 50% yield), which was recrystallized from hexane–EtOAc to give colorless prisms, mp 63—65 °C. $[\alpha]_D^{27}$ −81° (*c* = 0.92, $CHCl_3$). CD (*c* = 1.43 × 10^{-3} MeOH) $[\theta]^{26}$ (nm): −45359 (243) (negative maximum).

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