Synthesis of Four Chiral Isomers of β-Lactone DU-6622 and Inhibition of HMG-CoA Synthase by the Specific (2*R*,3*R*)-Isomer

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Four chiral forms of the β-lactone DU-6622 (3-hydroxy-2-(hydroxymethyl)-5-[7-(methoxycarbonyl)-naphthalen-1-yl]pentanoic acid 1,3-lactone) were prepared to investigate their inhibitory activity against 3-hydroxy-3-methylglutarly-CoA (HMG-CoA) synthase. The (2*R*,3*R*)-β-lactone isomer (+)-**8a**, having the same stereochemistry as that of the fungal β-lactone 1233A, showed the most potent HMG-CoA synthase inhibitory activity (IC_{50}: 0.098 μM). The other three β-lactone isomers, (2*S*,3*R*)-((−)-**8b**), (2*S*,3*S*)- ((−)-**8a**), and (2*R*,3*S*)-isomers ((+)-**8b**), were weaker inhibitors with larger IC_{50} values of 9.4, 31, and 360 μM, respectively. Thus, it was concluded that the (2*R*,3*R*) stereochemistry of the β-lactone ring is responsible for HMG-CoA synthase inhibition.

Introduction

A fungal β-lactone 1233A (also known as F-244 or L-659,699) (Figure 1) originally isolated as an antibiotic by Aldridge et al.[1] was rediscovered as a potent and specific inhibitor of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase.[2] The compound inhibited the enzyme *in vitro* using a rat liver cytosolic fraction and in intact cell systems using Vero and HepG2 cells.[2] Importantly, it proved orally active in mice by inhibiting hepatic sterol biosynthesis in a dose-dependent fashion.[3] Our early study on 1233A modification revealed that the 2-(hydroxymethyl)-β-lactone moiety of 1233A is essential for the inhibition.[2b] The stereochemistry of the β-lactone was determined to be 2*R*,3*R* by Chiang et al.[4] Interestingly, we found that ebelactones A and B,[5] reported as esterase inhibitors having a very similar planar structure to 1233A except for the opposite (2*S*,3*S*)-β-lactone ring (Figure 1), showed no inhibitory activity against HMG-CoA synthase even at 500 μM.[2b] Therefore, the importance of the β-lactone stereochemistry was suggested for inhibition of HMG-CoA synthase, but the exact correlation between the stereochemistry and the inhibition still remains to be defined.

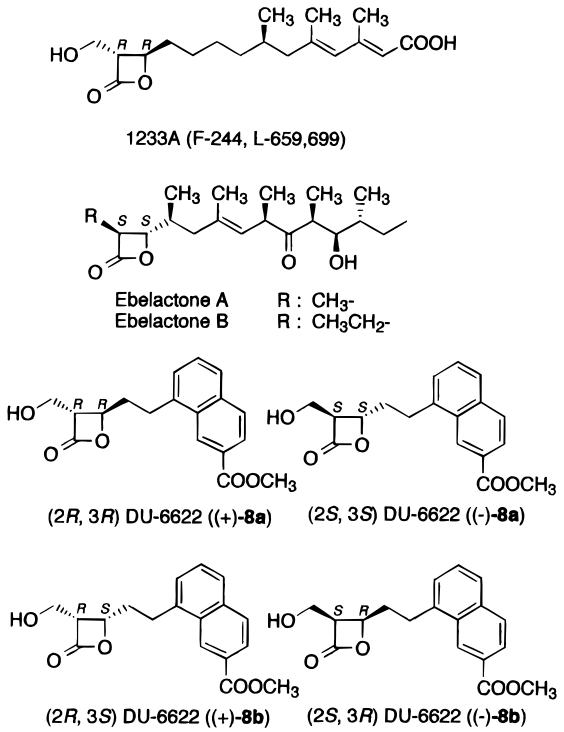

Figure 1. Structure of β-lactone stereochemistry of 1233A, ebelactones, and four chiral DU-6622.

During our synthetic study of 1233A analogs,[6] a derivative named *trans*-DU-6622 (*erythro*-3-hydroxy-2-(hydroxymethyl)-5-[7-(methoxycarbonyl)naphthalen-1-yl]-

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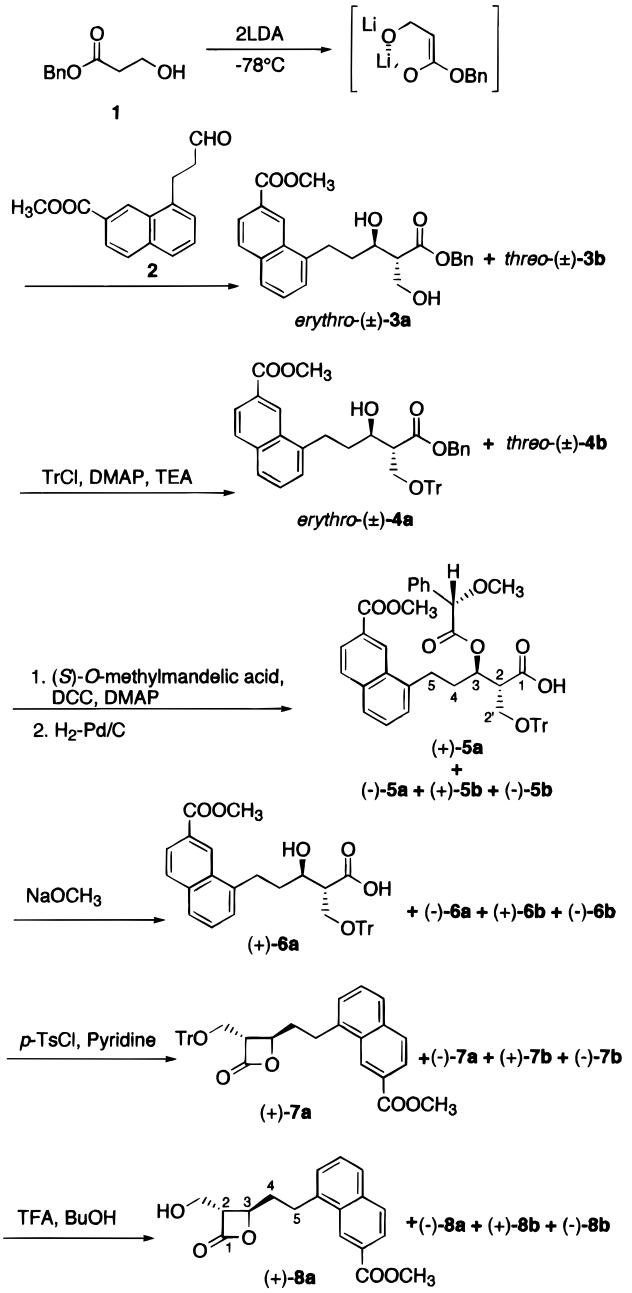
Scheme 1. Synthetic Route for the Four Stereoisomers of DU-6622[a]



[a] Abbreviations: LDA, lithium diisopropylamide; TrCl, triphenylmethyl chloride; DMAP, (dimethylamino)pyridine; TEA, triethylamine; DCC, dicyclohexylcarbodiimide; *p*-TsCl, *p*-toluenesulfonyl chloride; TFA, trifluoroacetic acid; BuOH, *n*-butanol.

pentanoic acid 1,3-lactone) was selected as a potent HMG-CoA synthase inhibitor. The IC_{50} value (0.15 μM) is analogous to that of 1233A (0.20 μM). We chose this compound as a model inhibitor of HMG-CoA synthase to answer the above question. In this paper, we describe the preparation of the four chiral isomers of DU-6622 to compare their inhibitory activity against HMG-CoA synthase. Thus, we demonstrate that the (2*R*,3*R*)-β-lactone structure is primarily responsible for HMG-CoA synthase inhibition.

Results and Discussion

Synthesis of Four Chiral Isomers of DU-6622. *trans*-DU-6622 (a mixture of (2*R*,3*R*)- and (2*S*,3*S*)-β-lactone isomers) was synthesized as reported previously.[6b] Four chiral ((2*R*,3*R*)- ((+)-**8a**), (2*S*,3*S*)- ((−)-**8a**), (2*R*,3*S*)- ((+)-**8b**), and (2*S*,3*R*)- ((−)-**8b**)) isomers of DU-6622 (Figure 1) were synthesized by the synthetic sequence shown in Scheme 1.

The essential carbon skeleton of DU-6622 was formed by an aldol condensation with 3-hydroxypropionic acid benzyl ester (**1**) and the corresponding aldehyde (**2**) to afford *erythro*-(±)-**3a** and *threo*-(±)-**3b** according to the method reported previously.[6a] The selective protection of the primary alcohol in (±)-**3a** and (±)-**3b** was performed with a triphenylmethyl group. Then, each enantiomer could be isolated by esterification of the secondary alcohols of (±)-**4a** (or (±)-**4b**) with (*S*)-*O*-methylmandelic acid to form the corresponding diastereomers (+)-**5a** and (−)-**5a** (or (+)-**5b** and (−)-**5b**). On the basis of an NMR-configurational correlation model,[4,7] the nonequivalence of the chemical shifts of the proton resonances from the groups attached to the carbinyl carbon can be used to assign the absolute configuration of that stereogenic center. The assumed low-energy conformation upon which this model depends is illustrated by the structures (+)-**5a** and (−)-**5a** in Figure 2. The protons of the substituent that is eclipsed by the phenyl ring always show an upfield shift possibly due to the shielding effect of the phenyl ring. In the structures (+)-**5a** and (−)-**5a**, the substituents are L2 and L3, respectively.

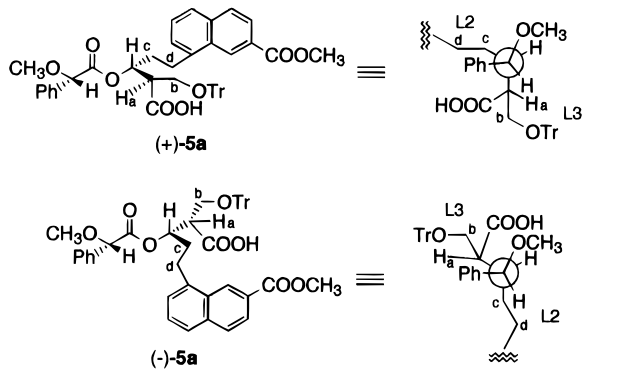


Figure 2. Configurational correlation model for (*S*)-*O*-methylmandalate derivatives **(+)-5a** and **(−)-5a**.

Table 1. Nonequivalent ^{1}H NMR Chemical Shifts for Selected Protons of (*S*)-*O*-Methylmandelates (+)-5a, (−)-5a, (+)-5b, and (−)-5b

proton		(+)-**5a**	(−)-**5a**	(+)-**5b**	(−)-**5b**
L3	Ha	2.95	2.73	3.17	3.13
	Hb	3.40, 3.44	3.08, 3.25	3.28, 3.27	3.28, 3.49
L2	Hc	1.75, 1.90	1.92, 2.05	1.94, 2.05	1.71, 1.87
	Hd	2.55 2.73	2.88, 3.08	2.88 3.09	2.44, 2.90

Table 1 summarizes the nonequivalent chemical shifts for (+)-**5a**, (−)-**5a**, (+)-**5b**, and (−)-**5b**. The data show that the protons Ha and Hb in (−)-**5a** resonate at higher field than the corresponding protons in (+)-**5a**. On the other hand, the Hc and Hd in (+)-**5a** resonate at higher field than those in (−)-**5a**. As illustrated by the correlation model in Figure 2, the shift data are consistent with the assignment of *R*- and *S*-configurations at the secondary alcohol in (+)-**5a** and (−)-**5a**, respectively. Since these compounds have *erythro*-structures, the absolute

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Table 2. Effect of Four Chiral and *trans*-DU-6622 on HMG-CoA Synthase

compd	config (C-2, C-3)	IC_{50} (μM) HMG-CoA synthase
(+)-**8a**	(*R*,*R*)	0.098
(−)-**8a**	(*S*,*S*)	31
(+)-**8b**	(*R*,*S*)	360
(−)-**8b**	(*S*,*R*)	9.4
(±)-**8a**	(*R*,*R*) + (*S*,*S*)	0.15

configurations of (+)-**5a** and (−)-**5a** were assigned as 2*R*,3*R* and 2*S*,3*S*, respectively. Similarly, the absolute configurations of (+)-**5b** and (−)-**5b** were determined to be 2*R*,3*S* and 2*S*,3*R*, respectively (Table 1).

Further synthesis was performed according to our established procedures.[6] After removal of the methylmandelic acid ester from each optically pure isomer ((+)-**5a**, (−)-**5a**, (+)-**5b**, and (−)-**5b**), they were cyclized by treatment with *p*-toluenesulfonyl chloride to form the β-lactone ((+)-**7a**, (−)-**7a**, (+)-**7b**, and (−)-**7b**). Finally, the triphenylmethyl protecting group was removed by TFA treatment to yield the four DU-6622 isomers ((+)-**8a**, (−)-**8a**, (+)-**8b**, and (−)-**8b**, Figure 1).

Inhibition of HMG-CoA Synthase by the Specific Isomer (2*R*,3*R*)-DU-6622. The importance of the stereochemistry of a β-lactone ring was suggested due to the following evidence using natural β-lactone compounds: (1) the fungal 1233A with a (2*R*,3*R*)-β-lactone ring showed potent HMG-CoA synthase inhibitory activity, but (2) microbial ebelactones with an opposite (2*R*,3*R*)-β-lactone ring did not express the inhibitory activity.[2b] To make this point clear, the effect of the four optically active isomers of DU-6622 on HMG-CoA synthase was tested, and the results are summarized in Table 2. Among the four stereoisomers, the (2*R*,3*R*)-isomer (+)-**8a** exhibited the most potent inhibition of HMG-CoA synthase. The IC_{50} value was calculated to be 0.098 μM, indicating that this isomer is more potent than *trans*-DU-6622 and 1233A (IC_{50}: 0.15 and 0.20 μM, respectively). However, the other three isomers were substantially weaker inhibitors. Especially, the (2*S*,3*S*)-isomer (−)-**8a** showed very weak inhibitory activity with an over 300-fold higher IC_{50} value than (+)-**8a**. Therefore, we concluded that the 2*R*,3*R* stereochemistry of the β-lactone ring is responsible for elicitng HMG-CoA synthase inhibition. Furthermore, the (2*S*,3*R*)-isomer (−)-**8b** is more potent than the (2*S*,3*S*)-isomer (−)-**8a**, suggesting that the C-3 geometry of the β-lactone is more important for the inhibitory activity than the C-2 geometry. Miziorko et al.[8] reported that the final product HMG-CoA possesses inhibitory activity against HMG-CoA synthase by competing with the substrate acetyl-CoA at the acetyl-CoA binding site of the enzyme. Therefore, it might be plausible that DU-6622 is structurally similar to HMG-CoA as an inhibitor. In fact, if the respective β-lactone carbons (C-1 to -3, see (+)-**8a** in Scheme 1) of DU-6622 are fixed to the $HOO^1C^2CH_2{}^3C(OH)(CH_3)-$ carbons of HMG-CoA, the C-3 carbon corresponds to the stereogenic ^3C carbon and the bulky ethyl naphthalene part of the inhibitor orients to the same direction as the $-CH_2$-CoA moiety of HMG-CoA. This might be the reason for the importance of the C-3 geometry of the β-lactone for HMG-CoA synthase inhibition.

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Experimental Section

Materials. Isolation of 1233A from the culture broth of *Scopulariopsis* sp. F-244 was performed as reported.[9] Lovastatin was purchased from Merck, Sharp and Dohme (Rahway, NJ). Acetyl-CoA, acetoacetyl-CoA, and cholestyramine were obtained from Sigma (St. Louis, MO). [1-^{14}C]Acetyl-CoA (53 μCi/μmol) was purchased from Dupont/NEN Research Products. All other chemicals used in this work were of the highest purity available commercially and were used without further purification.

Syntheses and Characterization of Analogs. Four optically active isomers of DU-6622 were prepared in the synthetic sequence shown in Scheme 1. All NMR spectra were measured in $CDCl_3$ at 400 MHz for ^{1}H and at 100 MHz for ^{13}C. Chemical shifts are reported in parts per million relative to tetramethylsilane used as internal standard.

***erythro*-3-Hydroxy-5-[7-(methoxycarbonyl)naphthalen-1-yl]-2-[(trityloxy)methyl]pentanoic Acid Benzyl Ester ((±)-4a) and *threo*-3-Hydroxy-5-[7-(methoxycarbonyl)-naphthalen-1-yl]-2-[(trityloxy)methyl]pentanoic Acid Benzyl Ester ((±)-4b).** To a cold solution (−40 °C) of diisopropylamine (15.8 mL) in tetrahydrofuran (88.3 mL) was added a solution of *n*-butyllithium (15% in hexane) (86.1 mL) dropwise over 25 min under an argon atmosphere. After the addition was complete, the solution was warmed to 0 °C for 15 min and then cooled to −78 °C. A solution of 3-hydroxypropionic acid benzyl ester (**1**) (10.6 g) in tetrahydrofuran (32 mL) was added dropwise over 20 min to the lithium diisopropylamide (LDA) solution at −78 °C. After 10 min, the reaction mixture was kept at −25 °C for 5 min and then cooled to −78 °C. After 10 min, a solution of **2** (15.67 g) in tetrahydrofuran (65 mL) was added dropwise over 30 min to the reaction mixture at −78 °C, and the resulting mixture was stirred at −78 °C for 50 min. The reaction was quenched by addition of saturated NH_4Cl solution (450 mL), and the mixture was extracted with ether (3 × 450 mL). The combined extracts were dried over $MgSO_4$, and the solvent was removed *in vacuo* to obtain a yellow oil (24.24 g). This oil was purified by column chromatography on silica gel ($CHCl_3$−acetone = 20:1) to afford *erythro*-3-hydroxy-2-(hydroxymethyl)-5-[7-(methoxycarbonyl)-naphthalen-1-yl]pentanoic acid benzyl ester ((±)-**3a**) (2.85 g, 10%) and *threo*-3-hydroxy-2-(hydroxymethyl)-5-[7-(methoxycarbonyl)naphthalen-1-yl]pentanoic acid benzyl ester ((±)-**3b**) (3.11 g, 11%).

A solution of (±)-**3a** (2.84 g) in CH_2Cl_2 was cooled to 0 °C under argon, 4-(dimethylamino)pyridine (163 mg), triethylamine (1.09 g), and triphenylmethyl chloride (2.81 g) were added, and then the mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (150 mL), washed with saturated $NaHCO_3$ solution (150 mL), and dried over $MgSO_4$, and the solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$−diisopropyl ether = 100:1) to afford (±)-**4a** (4.0 g, 90%): ^{1}H NMR ($CDCl_3$) δ 1.62−1.86 (m, 2H), 2.68−3.35 (m, 4H), 3.47 (d, 2H), 3.85−4.10 (m, 1H), 3.92 (s, 3H), 5.18 (s, 2H), 7.00−8.11 (m, 25H), 8.81 (t, 1H). Anal. Calcd for $C_{44}H_{40}O_6$: C, 79.49; H, 6.06. Found: C, 79.75; H, 6.32.

Compound (±)-**3b** (3.10 g) was treated in a manner similar to that described for the preparation of (±)-**4a** to give (±)-**4b** (3.83 g, 79%): ^{1}H NMR δ 1.62−1.86 (m, 2H), 2.45−3.45 (m, 4H), 3.56 (d, J = 5.5 Hz, 2H), 3.80−4.20 (m, 1H), 3.91 (s, 3H), 5.14 (s, 2H), 7.00−8.10 (m, 25H), 8.77 (t, J = 0.7 Hz, 1H). Anal. Calcd for $C_{44}H_{40}O_6$: C, 79.49; H, 6.06. Found: C, 79.50; H, 6.22.

(+)-*erythro*-3-Hydroxy-5-[7-(methoxycarbonyl)naphthalen-1-yl]-2-[(trityloxy)methyl]pentanoic Acid (*S*)-(+)-α-Methoxyphenylacetic Acid Ester ((+)-5a) and (−)-*erythro*-3-Hydroxy-5-[7-(methoxycarbonyl)naphthalen-1-yl]-2-[(trityloxy)methyl]pentanoic Acid (*S*)-(+)-α-Methoxyphenylacetic Acid Ester ((−)-5a). A mixture of (±)-**4a** (2.0 g), dicyclohexylcarbodiimide (1.24 g), (*S*)-(+)-α-methoxy-

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phenylacetic acid (750 mg), and 4-(dimethylamino)pyridine (375 mg) in CH_2Cl_2 (3 mL) was stirred at room temperature for 30 min. The reaction mixture was diluted with ether and filtered, and then the solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel (hexane−ethyl acetate = 10:1−2:1) to afford an oily material. The oily material was dissolved in EtOH (30 mL), and 5% Pd/C (2.0 g) was added to the solution. The mixture was stirred for 1 h at room temperature under a hydrogen atmosphere. The reaction mixture was filtered through a Celite pad, concentrated under reduced pressure, and purified by column chromatography on silica gel (CH_2Cl_2−MeOH = 95:5) to afford (+)-**5a** (1.05 g, 49%) and (−)-**5a** (1.05 g, 49%) as colorless solids.

(+)-5a: $[\alpha]^{23}_D$ + 7.2 (*c* 1.0, EtOH); ^{1}H NMR δ 1.60−1.95 (m, 2H), 2.50−3.50 (m, 5H), 3.33 (s, 3H), 3.90 (s, 3H), 4.69 (s, 1H), 5.24−5.55 (m, 1H), 7.10−8.10 (m, 25H), 8.55 (s, 1H). Anal. Calcd for $C_{46}H_{42}O_8$: C, 76.43; H, 5.85. Found: C, 76.58; H, 5.95. **(−)-5a:** $[\alpha]^{23}_D$ +3.6 (*c* 1.0, EtOH); ^{1}H NMR ($CDCl_3$, δ ppm) 1.70−2.20 (m, 2H), 2.50−3.60 (m, 5H), 3.33 (s, 3H), 3.95 (s, 3H), 4.73 (s, 1H), 5.24−5.54 (m, 1H), 7.10−8.12 (m, 25H), 8.75 (s, 1H). Anal. Calcd for $C_{46}H_{42}O_8$: C, 76.43; H, 5.85. Found: C, 76.60; H, 5.92.

(+)-*erythro*-3-Hydroxy-5-[7-(methoxycarbonyl)naphthalan-1-yl]-2-[(trityloxy)methyl]pentanoic Acid ((+)-6a). To a solution of **(+)-5a** (700 mg) in MeOH (7 mL) was added 4 M CH_3ONa (1 mL), and the mixture was stirred at room temperature for 3 h. The pH of the reaction mixture was adjusted to 2.0 by addition of 1 M HCl, and the mixture was extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried over $MgSO_4$, and the solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2−MeOH = 9.1) to afford **(+)-6a** (500 mg, 90%) as a colorless solid: $[\alpha]^{23}_D$ +13.0 (*c* 1.0, EtOH); ^{1}H NMR δ 1.68−2.00 (m, 2H), 2.60−2.87 (m, 1H), 3.05−3.33 (m, 2H), 3.50 (d, *J* = 5.9 Hz, 2H), 3.70−4.20 (m, 1H), 3.88 (s, 3H), 6.95−8.10 (m, 20H), 8.80 (s, 1H). Anal. Calcd for $C_{37}H_{34}O_6$: C, 77.33; H, 5.96. Found: C, 77.51; H, 5.65.

(−)-*erythro*-3-Hydroxy-5-[7-(methoxycarbonyl)naphthalen-1yl]-2-[(trityloxy)methyl]pentanoic Acid ((−)-6a). Compound **(−)-5a** (722 mg) was treated in a manner similar to that described for the preparation of **(+)-6a** to give **(−)-6a** (517 mg, 90%) as a colorless solid: $[\alpha]^{20}_D$ −13.9 (*c* 1.0, EtOH); ^{1}H NMR δ 1.68−2.00 (m, 2H), 2.60−2.87 (m, 1H), 3.05−3.33 (m, 2H), 3.50 (d, *J* = 5.9 Hz, 2H), 3.70−4.20 (m, 1H), 3.88 (s, 3H), 6.95−8.10 (m, 20H), 8.80 (s, 1H). Anal. Calcd for $C_{37}H_{34}O_6$: C, 77.33; H, 5.96. Found: C, 77.52; H, 5.73.

(+)-*erythro*-3-Hydroxy-5-[7-(methoxycarbonyl)naphthalen-1-yl]-2-[(trityloxy)methyl]pentanoic Acid 1,3-Lactone ((+)-7a). To a solution of **(+)-6a** (500 mg) in pyridine (1 mL) was added *p*-toluenesulfonyl chloride (700 mg) at 0 °C, and the mixture was stirred at 3 °C overnight. MeOH (5 mL) was added to the mixture, and the mixture was stirred at room temperature for an additional 30 min and diluted with CH_2Cl_2 (50 mL). The solution was washed with saturated NaCl solution (50 mL) and dried over $MgSO_4$, and the solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$−MeOH = 10:1) to afford **(+)-7a** (445 mg, 92%) as colorless solid: $[\alpha]^{20}_D$ +33.2 (*c* 1.0, EtOH); MS *m*/*z* 556 (M^+); HRMS calcd for $C_{37}H_{32}O_5$ 556.2250, found 556.2237; ^{1}H NMR δ 2.10−2.41 (m, 2H), 3.10−3.38 (m, 4H), 3.42−3.67 (m, 1H), 3.95 (s, 3H), 4.58 (dt, *J* = 4.1, 6.6 Hz, 1H), 7.00−8.21 (m, 20H), 8.75 (s, 1H). Anal. Calcd for $C_{37}H_{32}O_5$: C, 79.83; H, 5.79. Found: C, 79.77; H, 5.70.

(−)-*erythro*-3-Hydroxy-5-[7-(methoxycarbonyl)naphthalen-1-yl]-2-[(trityloxy)methyl]pentanoic Acid 1,3-Lactone ((−)-7a). Compound **(−)-6a** (500 mg) was treated in a manner similar to that described for the preparation of **(+)-7a** to give **(−)-7a** (480 mg, 96%) as a colorless solid: $[\alpha]^{20}_D$ −17.4 (*c* 1.0, EtOH); MS *m*/*z* 556 (M^+); HRMS calcd for $C_{37}H_{32}O_5$ 556.2250, found 556.2234; ^{1}H NMR δ 2.10−2.41 (m, 2H), 3.10−3.38 (m, 4H), 3.42−3.67 (m, 1H), 3.95 (s, 3H), 4.58 (dt, *J* = 4.1, 6.6 Hz, 1H), 7.00−8.21 (m, 20H), 8.75 (s, 1H). Anal. Calcd for $C_{37}H_{32}O_5$: C, 79.83; H, 5.79. Found: C, 79.85; H, 5.66.

(+)-*erythro*-3-Hydroxy-2-(hydroxymethyl)-5-[7-(methoxycarbonyl)naphthalen-1-yl]pentanoic Acid 1,3-Lactone ((+)-8a). To a solution of **(+)-7a** (445 mg) in *n*-butanol (10 mL) was added trifluoroacetic acid (4.4 mL) dropwise over 10 min at 0 °C, and the mixture was stirred at room temperature. After 2 h, the mixture was diluted with EtOAc (150 mL), washed with saturated $NaHCO_3$, and dried over $MgSO_4$, and the solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$−acetone = 50:1) to afford **(+)-8a** (195 mg, 78%) as a colorless solid: $[\alpha]^{20}_D$ +69.0 (*c* 1.0, EtOH); MS *m*/*z* 314 (M^+); HRMS calcd for $C_{18}H_{18}O_5$ 314.1154, found 314.1177; ^{1}H NMR δ 2.16−2.46 (m, 2H), 3.16−3.51 (m, 3H), 3.81−4.18 (m, 2H), 3.96 (s, 3H), 4.67 (dt, *J* = 3.6, 6.8 Hz, 1H), 7.30−8.24 (m, 20H), 8.75 (s, 1H). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.73; H, 5.90.

(−)-*erythro*-3-Hydroxy-2-(hydroxymethyl)-5-[7-(methoxycarbonyl)naphthalen-1-yl]pentanoic Acid 1,3-Lactone ((−)-8a). Compound **(−)-7a** (480 mg) was treated in a manner similar to that described for the preparation of **(+)-8a** to give **(−)-8a** (210 mg, 78%) as a colorless solid: $[\alpha]^{20}_D$ −68.9 (*c* 1.0, EtOH); MS *m*/*z* 314 (M^+); HRMS calcd for $C_{18}H_{18}O_5$ 314.1154, found 314.1172; ^{1}H NMR δ 2.16−2.46 (m, 2H), 3.16−3.51 (m, 3H), 3.81−4.18 (m, 2H), 3.96 (s, 3H), 4.67 (dt, *J* = 3.6, 6.8 Hz, 1H), 7.30−8.24 (m, 20H), 8.75 (s, 1H). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.50; H, 5.90.

(+)-*threo*-3-Hydroxy-5-[7-(methoxycarbonyl)naphthalen-1-yl]-2-[(trityloxy)methyl]pentanoic Acid (*S*)-(+)-α-Methoxyphenylacetic Acid Ester ((+)-5b) and (−)-*threo*-3-Hydroxy-5-[7-(methoxycarbonyl)naphthalen-1-yl]-2-[(trityloxy)methyl]pentanoic Acid (*S*)-(+)-αMethoxyphenylacetic Acid Ester ((−)-5b). Compound **(±)-4b** (1.5 g) was treated in a manner similar to that described for the preparation of **(+)-5a** and **(−)-5b** to give **(+)-5b** (680 mg, 42%) and **(−)-5b** (680 mg, 42%).

(+)-5b: $[\alpha]^{20}_D$ +31.0 (*c* 1.0, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.70−2.10 (m, 2H), 2.20−3.70 (m, 5H), 3.42 (s, 3H), 3.98 (s, 3H), 4.58 (s, 1H), 5.12−5.42 (m, 1H), 7.05−8.10 (m, 25H), 8.63 (s, 1H). Anal. Calcd for $C_{46}H_{42}O_8$: C, 76.43; H, 5.85. Found: C, 76.45; H, 5.80. **(−)-5b:** $[\alpha]^{20}_D$ +8.2 (*c* 1.0, EtOH); ^{1}H NMR δ 1.86−2.16 (m, 2H), 2.60−3.40 (m, 5H), 3.32 (s, 3H), 4.00 (s, 3H), 4.72 (s, 1H), 5.10−5.40 (m, 1H), 7.10−8.15 (m, 25H), 8.75 (s, 1H). Anal. Calcd for $C_{46}H_{42}O_8$: C, 76.43; H, 5.85. Found: C, 76.40; H, 5.85.

(+)-*threo*-3-Hydroxy-5-[7-(methoxycarbonyl)naphthalen-1-yl]-2-[(trityloxy)methyl]pentanoic Acid 1,3-Lactone ((+)-7b). Compound **(+)-5b** (55 mg) was treated in a manner similar to that described for the preparation of **(+)-6a** to give **(+)-6b** (36 mg, 82%) and then treated in a manner similar to that described for the preparation of **(+)-7a** to give **(+)-7b** (30 mg, 86%): $[\alpha]^{20}_D$ +13.3 (*c* 1.0 EtOH); ^{1}H NMR δ 1.92−2.40 (m, 2H), 2.87−3.76 (m, 4H), 3.82−4.07 (m, 1H), 3.95 (s, 3H), 4.61 (dt, *J* = 7.9, 6.9 Hz, 1H), 7.00−8.15 (m, 20H), 8.64 (s, 1H). Anal. Calcd for $C_{37}H_{32}O_5$: C, 79.83; H, 5.79. Found: C, 79.88; H, 5.58.

(−)-*threo*-3-Hydroxy-5-[7-(methoxycarbonyl)naphthalen-1-yl]-2-[(trityloxy)methyl]pentanoic Acid 1,3-Lactone ((−)-7b). Compound **(−)-5b** (68 mg) was treated in a manner similar to that described for the preparation of **(+)-6a** to give **(−)-6b** (45 mg, 86%) and then treated in a manner similar to that described for the preparation of **(+)-7a** to give **(−)-7b** (30 mg, 67%): $[\alpha]^{20}_D$ −5.0 (*c* 1.0, EtOH); ^{1}H NMR δ 1.92−2.40 (m, 2H), 2.87−3.76 (m, 4H), 3.82−4.07 (m, 1H), 3.95 (s, 3H), 4.61 (dt, *J* = 7.9, 6.9 Hz, 1H), 7.00−8.15 (m, 20H), 8.64 (s, 1H). Anal. Calcd for $C_{37}H_{32}O_5$: C, 79.83; H, 5.79. Found: C, 79.70; H, 5.71.

(+)-*threo*-3-Hydroxy-2-(hydroxymethyl)5-[7-(methoxycarbonyl)naphthalen-1-yl]pentanoic Acid 1,3-Lactone ((+)-8b). Compound **(+)-7b** (30 mg) was treated in a manner similar to that described for the preparation of **(+)-8a** to give **(+)-8b** (10 mg, 59%): $[\alpha]^{20}_D$ +62.9 (*c* 1.0, EtOH); MS *m*/*z* 314 (M^+); HRMS calcd for $C_{18}H_{18}O_5$ 314.1154, found 314.1179; ^{1}H NMR δ 2.24−2.53 (m, 2H), 2.65 (brs, 1H), 3.20−3.42 (m, 2H), 3.72−4.35 (m, 3H), 3.98 (s, 3H), 4.71 (dt, *J* = 5.9, 6.8 Hz, 1H),

7.31−8.14 (m, 5H), 8.88 (s, 1H). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.72; H, 5.82.

(−)-*threo*-3-Hydroxy-2-(hydroxymethyl)-5-[7-(methoxycarbonyl)naphthalen-1-yl]pentanoic Acid 1,3-Lactone ((−)-8b). Compound **(−)-7b** (30 mg) was treated in a manner similar to that described for the preparation of **(+)-8a** to give **(−)-8b** (8 mg, 47%): $[\alpha]^{20}_D$ −49.3 (*c* 1.0, EtOH); MS *m*/*z* 314 (M^+); HRMS calcd for $C_{18}H_{18}O_5$ 314.1154, found 314.1162; ^{1}H NMR δ 2.24−2.53 (m, 2H), 2.65 (brs, 1H), 3.20−3.42 (m, 2H), 3.72−4.35 (m, 3H), 3.98 (s, 3H), 4.71 (dt, J = 5.9, 6.8 Hz, 1H), 7.31−8.14 (m, 5H), 8.88 (s, 1H). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.81; H, 5.85.

Assay for HMG-CoA Synthase. HMG-CoA synthase was prepared from livers of rats fed lovastatin (0.1%) and cholestyramine (5%) for 7 days. The enzyme was purified through the DEAE-cellulose step according to the method of Mehrabian et al.[10] with some modification. The 30−55% ammonium sulfate precipitation and a dialysis were carried out prior to the DEAE-cellulose step. The 60 and 100 mM potassium phosphate elutes contained β-ketothiolase and HMG-CoA synthase, respectively. The enzyme was concentrated by ammonium sulfate precipitation, dissolved in the buffer used for elution, and stored at −70 °C.

HMG-CoA synthase was assayed as described previously.[2b] The reaction mixture contained 100 mM Tris−HCl (pH 8.2), 100 μM EDTA, 100 μM acetoacetyl-CoA, 200 μM [1-^{14}C]acetyl-CoA (0.06 μCi), β-lactone compounds (dissolved in 5 μL of EtOH), and enzyme protein in a total volume of 200 μL. The reaction was initiated by addition of the enzyme. The reaction mixture was incubated at 37 °C for 30 min and then stopped by the removal of 40 μL of the reaction mixture to 100 μL of 6 M HCl in a glass counting vial. The vials were heated to dryness at 90 °C for 90 min. The residues were determined for radioactivity with a liquid scintillation spectrometer (Aloka).

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