SYNTHESIS OF 1233A ANALOGS AND THEIR INHIBITORY ACTIVITY AGAINST HYDROXYMETHYLGLUTARYL COENZYME A SYNTHASE

TOSHIAKI SUNAZUKA, KAZUO TSUZUKI, HIDETOSHI KUMAGAI, HIROSHI TOMODA, HARUO TANAKA, HAJIME NAGASHIMA†, HIROKAZU HASHIZUME†† and SATOSHI ŌMURA*

Research Center for Biological Function, The Kitasato Institute, and School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108, Japan
†Daiichi Pharmaceutical Co., Ltd., Edogawa-ku, Tokyo 134, Japan
††Fuji Chemical Industries Ltd., Takaoka, Toyama 933, Japan

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Simple and efficient syntheses of 1233A analogs were developed and the inhibitory activity of the analogs against hydroxymethylglutaryl coenzyme A (HMG-CoA) synthase was determined. Study of the structure-activity relationships revealed that not only the geometry in β-lactone moiety but also the length of the carbon side chain is important for inhibitory activity against HMG-CoA synthase.

1233A, a natural occurring, β-lactone isolated independently from *Scopulariopsis* sp.[1] and *Fusarium* sp.[2] is a potent and specific inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A synthase (HMG-CoA synthase) and, hence, cholesterol biosynthesis in cell culture[1,2].

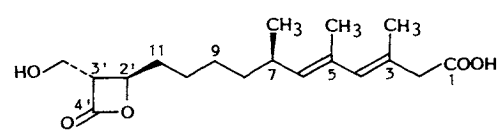
1233A first isolated as an antibiotic from *Cephalosporin* sp. in 1971 by ALDRIDGE *et al.* was identified as (*E*,*E*)-11-[3′-(hydroxymethyl)-4′-oxo-2′-oxetanyl]-3,5,7-trimethyl-2,4-undecadienoic acid[3]. The absolute configuration has been recently determined as 2′*R*, 3′*R*, and 7*R* (Fig. 1)[4].

We prepared several derivatives of 1233A and their inhibition of HMG-CoA synthase was compared to 1233A[5]. The saturated derivative was about 50% as active as the native polyunsaturated compound. The methyl ester derivative exhibited inhibitory activity comparable to 1233A, whereas acylation of the hydroxyl residue reduced the inhibition considerably. Opening of the β-lactone ring resulted in complete loss of inhibitory activity.

Through these findings, it is obvious that not only the lactone ring but also the hydroxymethyl moiety of 1233A is essential for potent inhibitory activity against HMG-CoA synthase. The carboxyl group can be tampered with without much loss of activity.

So, a synthetic study directed toward the development of structurally simplified 1233A was initiated in our laboratory. In this paper we describe the synthesis and the inhibitory activity of 1233A analogs.

Fig. 1. Structure of 1233A.



Chemistry

The synthesis of 1233A analogs was accomplished *via* an aldol condensation with β-hydroxypropionate and oxocarboxylate, and then β-

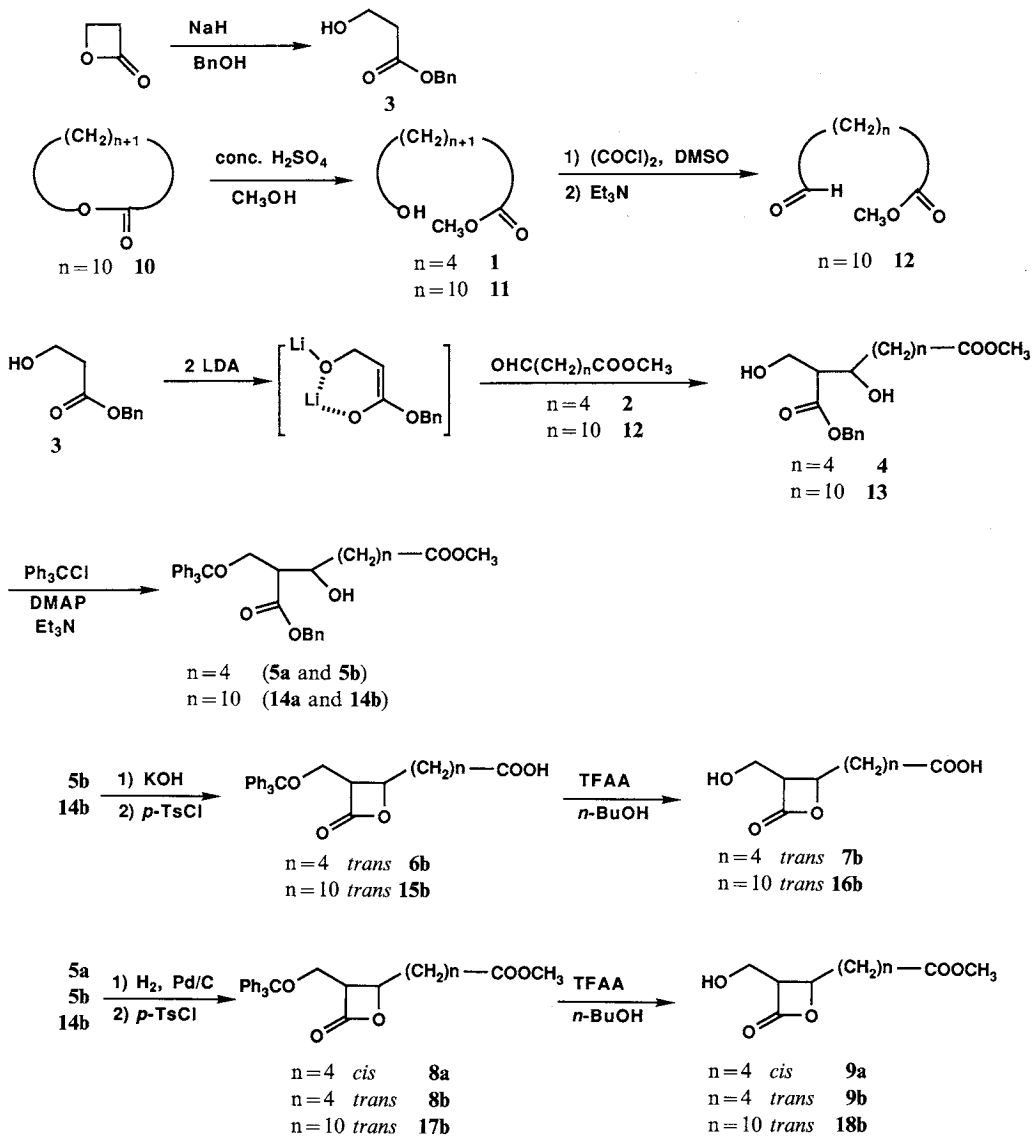
lactonization as outlined in Scheme 1.

β-Hydroxypropionic acid benzyl ester (**3**) was obtained from β-propiolactone as described by GRESHAM *et al.*[6].

Oxocarboxylates (**2** and **12**) were each prepared by acid-methanolysis of the corresponding macrolactone followed by Swern oxidation.

The aldol condensation was carried out berween β-hydroxypropionic acid benzyl ester (**3**) and the corresponding aldehyde (**2** or **12**) using the following conditions according to the procedure of FRÁTER *et al.*[7]. For the formation of the dianion, **3** was added in solution (THF) to two equivalents of lithium diisopropylamide (LDA) at −78°C, normally at such a rate that the temperature rose to −20°C or even to 0°C. In this manner the formation of the dianion is complete in minutes. The resulting dianion was

Scheme 1.



quenched by the corresponding aldehydes at $-78°C$ to give the mixture of *erythro* and *threo* aldol products (**4** and **13**) in moderate yield.

When the hydroxy group of the ester (**3**) was protected using groups, such as THP, TBDMS or Bn, the enolate was not formed, but β-elimination occurred rapidly at $-78°C$.

The selective protection of the primary alcohol in **4** and **13** was performed with triphenylmethyl chloride, 4-dimethylaminopyridine, and triethylamine at room temperature prior to separating the 1:1 mixture of the *threo* compound **5a** and **14a**, from their *erythro* isomers **5b** and **14b**, respectively.

The *erythro* compound (**5b**) was hydrolyzed quantitatively to the dicarboxylic acid with KOH in aqueous ethanol at room temperature without any epimerization at C(2). The crystalline product was further converted to the β-lactone (**6b**) by treating it with *p*-toluenesulfonyl chloride in pyridine at 3°C overnight[8]. The *erythro* compound (**14b**) was treated in the same way to obtain the desired β-lactone. On the other hand, when the *threo* compound (**5a**) was treated in the same procedure, the desired β-lactone was not obtained because of formation of the 7-membered lactone. Similarly the *threo* compound (**14a**) was not converted to the desired β-lactone.

Removal of the triphenylmethyl protecting group from **6b** and **15b** was performed with trifluoroacetic acid (TFAA) in *n*-butanol[9] to obtain the α-hydroxymethyl β-lactones **7b** and **16b**, respectively.

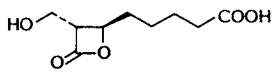
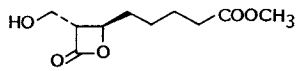
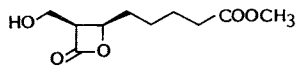
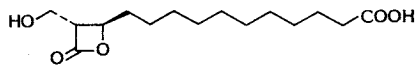
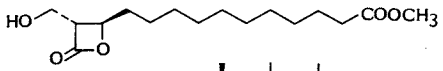
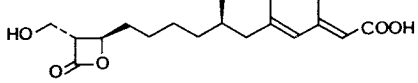
The *erythro* compound (**5b**) and the *threo* compound (**5a**) were each hydrogenated, followed by lactonization to obtain the desired β-lactones **8a** and **8b**, respectively. The *erythro* compound (**14b**) with the same procedure yielded the desired β-lactone (**17b**). Finally, **8a**, **8b** and **17b** were deprotected by TFAA in *n*-butanol to obtain the α-hydroxymethyl β-lactone **9a**, **9b** and **18b**, respectively.

The coupling constant, $J(3,4)$ in the β-lactone (**9b**) was 4.0 Hz, which is indicative for the *trans* geometry[10]. In contrast, $J(3,4)$ in **9a** was 6.5 Hz, indicative of *cis* geometry. By the same analysis, **7b** and **18b** were assigned *cis* geometry about the 2,3 bond.

Biological Activity

The inhibitory activities of five analogs of 1233A against HMG-CoA synthase and the growth of

Table 1. Effects of 1233A analogs on HMG-CoA synthase and the growth of Vero cells without or with mevalonolactone (1 mM).

Compound	HMG-CoA synthase IC_{50} (μg/ml)	Vero cells, MIC (μg/ml)	
		None	+MVA (1 mM)
7b 	20	25	100
9b 	45	12.5	>100
9a 	>50	50	50
16b 	1.2	12.5	>100
18b 	1.4	3.13	100
1233A 	0.11	0.031	>100

Vero cells are summarized in Table 1.

1233A has *trans* geometry in the β-lactone moiety. Considering the short side chain methyl esters of structure **9**, only the *trans* β-lactone (**9b**) was active against HMG-CoA synthase, whereas the *cis* β-lactone (**9a**) showed no inhibitory activity even at 50 μg/ml. Therefore, the geometry of the β-lactone moiety is important for inhibitory activity against HMG-CoA synthase.

The β-lactone with a short side chain (**9b**) exhibited a very weak inhibitory activity with IC_{50} value of 45 μg/ml, which was about 1/400 as active as that of 1233A. However, the β-lactone with a long side chain (**18b**) was about one tenth as active as that of 1233A. So the length of carbon side chain appears to be another factor affecting the inhibitory activity.

The derivatives possessing a free carboxylic acid (**7b** and **16b**) showed more potent inhibitory activity against HMG-CoA synthase than the corresponding methyl esters (**9b** and **18b**). In contrast, the methyl esters (**9b** and **18b**) showed stronger growth inhibition of Vero cells than the corresponding carboxylic acids (**7b** and **16b**). The difference between the potency of the two activities might be derived from the membrane permeability of drugs.

As previously reported[11], when 1 mM mevalonate was added to the culture medium, both morphological change and growth inhibition of Vero cells by 1233A were overcome and the cells grew normally. All 1233A analogs tested except **9a**, showed the same reversal of activity against Vero cells upon the mevalonate addition as did 1233A, indicating that the synthetic analogs of 1233A also inhibited mevalonate biosynthesis specifically in cultured cells.

Experimental

NMR spectra were measured on either a Jeol FX-90 Q or a Varian XL-400 spectrometer in $CDCl_3$ solution. Chemical shifts are reported in parts per million relative to Me_4Si as the internal standard.

IR spectra were measured on a Jeol A-102 spectrometer. Column chromatography were performed on silica gel (60 Merck, 230~400 mesh). Preparative TLC were performed on silica gel (Merck 60 PF 254) of 0.5 mm thickness.

Mass spectra were obtained on a Jeol D-100 and a DX-300 spectrometer at 20 eV.

β-Hydroxypropionic Acid Benzyl Ester (3)

A mixture of 60% sodium hydride (224 mg) in benzyl alcohol (71.4 ml) was stirred at 0°C under argon for 10 minutes.

β-Propiolactone (7 ml) was added dropwise to the mixture at 0°C and the reaction mixture was stirred at 0°C for 30 minutes.

The reaction mixture was quenched with conc HCl, diluted with $CHCl_3$ (100 ml) and washed with H_2O (150 ml). The $CHCl_3$ solution was dried over Na_2SO_4, filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatogaphy on silica gel eluted with hexane-ethyl acetate (3:1) to afford β-hydroxypropionic acid benzyl ester (**3**) (12.7 g, 83%). Mass *m/z* 180 (M^+). HR-MS calcd for $C_{10}H_{12}O_3$: 180.0785, found: 180.0805. IR ($CHCl_3$) cm^{-1} 3500, 1720, 1495 and 1490. ^1H NMR ($CDCl_3$) δ 2.61 (2H, t, J=5.6 Hz), 3.88 (2H, q, J=5.8 Hz), 5.15 (2H, s), 7.35 (5H, s).

6-Hydroxyhexanoic Acid Methyl Ester (1)

To a mixture of ε-caprolactone (15 g) in methanol (300 ml), conc H_2SO_4 (1.5 ml) was added dropwise and the mixture was refluxed for 2 hours. After cooling, the mixture was evaporated. The residue was dissolved in $CHCl_3$ (450 ml) and washed with saturated $NaHCO_3$ (450 ml) and H_2O (450 ml). The $CHCl_3$ layer was dried over Na_2SO_4, filtered and the filtrate was concentrated *in vacuo* to afford 6-hydroxyhexanoic acid methyl ester (**1**) (18.4 g, 96%). Mass *m/z* 146 (M^+). HR-MS calcd for $C_7H_{14}O_3$: 146.0942, found

146.0938. IR ($CHCl_3$) cm^{-1} 3450 and 1720. ^1H NMR ($CDCl_3$) δ 1.30~1.70 (6H, m), 2.33 (2H, t, J=5.4 Hz), 3.62 (2H, t, J=5.0 Hz), 3.67 (3H, s).

5-Formylvaleric Acid Methyl Ester (**2**)

A solution of DMSO (29.2 ml) in dichloromethane (100 ml) was added dropwise over 10 minutes to a solution of oxalyl chloride (18 ml) in dichloromethane (180 ml) at −35°C under argon. The mixture was stirred for 2 minutes and a solution of alcohol (**1**) (20.0 g) in dichloromethane (100 ml) was then added dropwise over 10 minutes.

The resultant mixture was stirred for 15 minutes at −35°C, treated with triethylamine (95 ml), stirred for 5 minutes further, and warmed to room temperature. After addition of H_2O (200 ml) and extraction with dichloromethane (200 ml × 2). The organic layers were dried over Na_2SO_4, filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluted with hexane-ethyl acetate (5:1) to afford 5-formylvaleric acid methyl ester (**2**) (18.4 g, 93%). Mass *m/z* 143 (M^+-H). HR-MS calcd for $C_7H_{12}O_3-H$: 143.0706, found: 143.0700. IR ($CHCl_3$) cm^{-1} 1725 and 1710. ^1H NMR ($CDCl_3$) δ 1.30~1.70 (6H, m), 2.33 (2H, t, J=5.4 Hz), 2.41 (2H, dt, J=6.9 and 1.8 Hz), 9.77 (1H, t, J=1.8 Hz).

Dodecanolactone (**10**)

A mixture of cyclododecanone (9.1 g) and *m*-chloroperbenzoic acid (17.3 g) and boron trifluoride etherate (6.2 ml) in chloroform (50 ml) was heated at 60°C for 16 hours. After cooling, the mixture was filtered, diluted with chloroform (500 ml) and washed by saturated $NaHCO_3$ (500 ml), then with H_2O (500 ml). The chloroform solution was dried over Na_2SO_4, filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluted with hexane-ethyl acetate (20:1) to afford dodecanolactone (**10**) (8.71 g, 88%). Mass *m/z* 198 (M^+). HR-MS calcd for $C_{12}H_{22}O_2$: 198.1618, found: 198.1628. IR ($CHCl_3$) cm^{-1} 1715. ^1H NMR ($CDCl_3$) δ 1.30~1.70 (18H, m), 2.37 (2H, t, J=6.1 Hz), 4.16 (2H, t, J=5.0 Hz).

12-Hydroxydodecanoic Acid Methyl Ester (**11**)

10 (8.7 g) was treated in a similar manner to that described for the preparation of **1** to give 12-hydroxydodecanoic acid methyl ester (**11**) (9.62 g, 95%). Mass *m/z* 231 (M^++1). HR-MS calcd for $C_{13}H_{26}O_3+H$: 231.1958, found: 231.1951. IR ($CHCl_3$) cm^{-1} 3450 and 1725. ^1H NMR ($CDCl_3$) δ 1.30~1.70 (18H, m), 2.31 (2H, t, J=7.4 Hz), 3.62 (2H, t, J=6.3 Hz), 3.66 (3H, s).

11-Formylundecanoic Acid Methyl Ester (**12**)

A solution of DMSO (14.8 ml) in dichloromethane (100 ml) was added dropwise over 10 minutes to a solution of oxalyl chloride (8 ml) in dichloromethane (100 ml) at −35°C under argon. The mixture was stirred for 2 minutes and a solution of alcohol (**11**) (9.2 g) in dichloromethane (40 ml) was then added dropwise over 10 minutes.

The resultant mixture was stirred for 20 minutes at −35°C, treated with triethylamine (60 ml), stirred for 5 minutes further, and warmed to room temperature. After addition of H_2O (200 ml) and extraction with dichloromethane (200 ml × 2), the organic layers were dried over Na_2SO_4, filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluted with hexane-ethyl acetate (20:1) to afford 11-formylundecanoic acid methyl ester (**12**) (9.3 g, 98%). Mass *m/z* 227 (M^+-1). HR-MS calcd for $C_{13}H_{24}O_3-H$: 227.1645, found: 227.1650. IR ($CHCl_3$) cm^{-1} 1725 and 1710. ^1H NMR ($CDCl_3$) δ 1.30~1.70 (18H, m), 2.30 (2H, t, J=7.6 Hz), 2.41 (2H, dt, J=6.9 and 1.8 Hz), 3.66 (3H, s), 9.76 (1H, t, J=1.8 Hz).

threo-3-Hydroxy-7-methoxycarbonyl-2-trityloxymethylheptanoic Acid Benzyl Ester (**5a**) and *erythro*-3-Hydroxy-7-methoxycarbonyl-2-trityloxymethylheptanoic Acid Benzyl Ester (**5b**)

A solution of diisopropylamine (4.95 ml) in tetrahydrofuran (20 ml) was cooled to −20°C under argon, and a solution of 1.53 M *n*-butyllithium in hexane (19.5 ml) added dropwise over 10 minutes. After the addition was complete, the solution was stirred at −20°C for 15 minutes and then cooled to −78°C for 15 minutes.

A solution of 3-hydroxypropionic acid benzyl ester (**3**) (2.4 g) in tetrahydrofuran (7.5 ml) was added dropwise over 5 minutes to the LDA solution at −78°C. After 10 minutes, the reaction mixture was warmed to −20°C for 5 minutes, and then cooled to −78°C again. After 10 minutes, a solution of **2** (2.1 g) in tetrahydrofuran (13 ml) was added dropwise over 15 minutes to the reaction mixture at −78°C and the resultant mixture was stirred at −78°C for 50 minutes.

The reaction mixture was quenched with saturated NH_4Cl solution (100 ml) and extracted three times with ether (100 ml × 3). Combined extracts were dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo* to obtain a yellow oil.

This was purified by column chromatography on silica gel, eluted with hexane-ethyl acetate (3 : 1) to obtain a mixture of *erythro*- and *threo*-3-hydroxy-2-hydroxymethyl-7-methoxycarbonylheptanoic acid benzyl ester (**4a**+**4b**) (3.5 g, 74%).

A mixture of **4a** and **4b** (3.5 g) in CH_2Cl_2 was cooled to 0°C under argon, and 4-dimethylaminopyridine (65 mg), triethylamine (2.3 ml) and triphenylmethyl chloride (3.3 g) were added and then the mixture was stirred at room temperature for 23 hours. The reaction mixture was diluted with CH_2Cl_2 (300 ml), washed with saturated $NaHCO_3$ (300 ml) and then with H_2O (300 ml), dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo*.

The resultant residue was purified by column chromatography on silica gel, eluted with hexane-ethyl acetate (5 : 1) to afford *threo*-3-hydroxy-7-methoxycarbonyl-2-trityloxymethylheptanoic acid benzyl ester (**5a**) (1.42 g, 23%) and *erythro*-3-hydroxy-7-methoxycarbonyl-2-trityloxymethylheptanoic acid benzyl ester (**5b**) (2.66 g, 44%). **5a**: Mass *m/z* 566 (M^+). HR-MS calcd for $C_{36}H_{38}O_6$: 566.2666, found: 566.2694. IR ($CHCl_3$) cm^{-1} 3500, 1720, 1490 and 1450. ^1H NMR ($CDCl_3$) δ 1.20~1.60 (6H, m), 2.23 (2H, t, J=6.1 Hz), 2.67 (1H, q, J=5.5 Hz), 3.52 (2H, d, J=6.1 Hz), 3.64 (3H, s), 3.90 (1H, m), 5.18 (2H, s), 7.20~7.50 (20H, m). **5b**: Mass *m/z* 566 (M^+). HR-MS calcd for $C_{36}H_{38}O_6$: 566.2666, found: 566.2688. IR ($CHCl_3$) cm^{-1} 3500, 1725, 1490 and 1450. ^1H NMR ($CDCl_3$) δ 1.20~1.60 (6H, m), 2.25 (2H, t, J=6.0 Hz), 2.72 (1H, q, J=3.0 Hz), 3.45 (2H, d, J=6.0 Hz), 3.64 (3H, s), 3.90 (1H, m), 5.18 (2H, s), 7.20~7.50 (20H, m).

threo-3-Hydroxy-13-methoxycarbonyl-2-trityloxymethyltridecanoic Acid Benzyl Ester (**14a**) and *erythro*-3-Hydroxy-13-methoxycarbonyl-2-trityloxymethyltridecanoic Acid Benzyl Ester (**14b**)

A solution of diisopropylamine (4.86 ml) in tetrahydrofuran (20 ml) was cooled to −20°C under argon, and a solution of 1.53 M *n*-butyllithium in hexane (17.9 ml) was added dropwise over 10 minutes. After the addition was complete, the solution was stirred at −20°C for 15 minutes and then cooled to −78°C for 15 minutes.

A solution of 3-hydroxypropionic acid benzyl ester (**3**) (2.4 g) in tetrahydrofuran (7.5 ml) was added dropwise over 10 minutes to the LDA solution at −78°C. After 10 minutes, the reaction mixture was warmed to −20°C for 5 minutes, and then cooled to −78°C again. After 10 minutes, a solution of **12** (3.05 g) in tetrahydrofuran (12 ml) was added dropwise over 15 minutes to the reaction mixture at −78°C and the resultant mixture was stirred at −78°C for 40 minutes.

The reaction mixture was quenched with saturated NH_4Cl solution (100 ml) and extracted three times with ether (100 ml × 3). Combined extracts were dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo*.

The residue was purified by column chromatography on silica gel, eluted with hexane-ethyl acetate (4 : 1) to obtain a mixture of *erythro*- and *threo*-3-hydroxy-2-hydroxymethyl-13-methoxycarbonyltridecanoic acid benzyl ester (**13a**+**13b**) (3.78 g, 88%).

A mixture of **15a** and **15b** (2.80 g) in CH_2Cl_2 was cooled to 0°C under argon, and 4-dimethylaminopyridine (42 mg), triethylamine (1.43 ml) and triphenylmethyl chloride (2.1 g) were added and then the mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (200 ml), washed with saturated $NaHCO_3$ (200 ml) and then with H_2O (200 ml). The extract was dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo*.

The resultant residue was purified by column chromatography on silica gel, eluted with hexane-ethyl acetate (7 : 1) to afford *threo*-3-hydroxy-13-methoxycarbonyl-2-trityloxymethyltridecanoic acid benzyl ester (**14a**) (1.05 g, 23%) and *erythro*-3-hydroxy-13-methoxycarbonyl-2-trityloxymethyltridecanoic acid benzyl ester (**14b**) (2.22 g, 50%). **14a**: Mass *m/z* 650 (M^+). HR-MS calcd for $C_{42}H_{50}O_6$: 650.3604, found: 650.3612. IR ($CHCl_3$) cm^{-1} 3550, 1725, 1490 and 1450. ^1H NMR ($CDCl_3$) δ 1.20~1.70 (18H, m), 2.30 (2H, t,

J=7.4 Hz), 2.73 (1H, q, J=5.9 Hz), 3.45 (2H, d, J=6.4 Hz), 3.65 (3H, s), 3.89 (1H, m), 5.17 (2H, s), 7.20~7.50 (20H, m). **14b**: Mass m/z 650 (M^+). HR-MS calcd for $C_{42}H_{50}O_6$: 650.3604, found: 650.3592. IR ($CHCl_3$) cm^{-1} 3550, 1725, 1490 and 1450. ^1H NMR ($CDCl_3$) δ 1.20~1.70 (18H, m), 2.30 (2H, t, J=7.4 Hz), 2.73 (1H, q, J=5.9 Hz), 3.45 (2H, d, J=6.4 Hz), 3.65 (3H, s), 3.90 (1H, m), 5.20 (2H, s), 7.20~7.50 (20H, m).

erythro-6-Hydroxy-7-trityloxymethyloctanedioic Acid 6,8-Lactone (**6b**)

Potassium hydroxide (280 mg) was added to a solution of **5b** (566 mg) in 70% aqueous ethanol (16.6 ml) and the mixture was stirred at room temperature for 3 hours. The mixture was evaporated. The resultant residue was dissolved in saturated aqueous $NaHCO_3$ (30 ml) and the solution was washed with ether (30 ml). The aqueous layer was adjusted to pH 2 by addition of 9 N HCl (5 ml) and extracted with ether (50 ml × 3). The ether solution was dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo* to a residue.

To a solution of the residue in pyridine (8 ml) at 0°C, *p*-toluenesulfonyl chloride (524 mg) was added and the mixture was stirred at 3°C for 13 hours. The reaction was quenched by addition of methanol (5 ml) and was then diluted with CH_2Cl_2 (40 ml). The CH_2Cl_2 solution was washed with saturated NaCl solution (40 ml) and dried over $MgSO_4$. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluted with $CHCl_3$-MeOH (20:1) to afford *erythro*-6-hydroxy-7-trityloxymethyloctanedioic acid 6,8-lactone (**6b**) (181 mg, 59%). Mass m/z 444 (M^+). HR-MS calcd for $C_{28}H_{28}O_5$: 444.1935, found: 444.1937. IR ($CHCl_3$) cm^{-1} 3600, 1820, 1720, 1490 and 1450. ^1H NMR ($CDCl_3$) δ 1.20~1.60 (6H, m), 2.23 (2H, t, J=6.1 Hz), 3.41 (1H, m), 3.62 (2H, m), 4.61 (1H, m).

threo-3-Hydroxy-7-methoxycarbonyl-2-trityloxymethylheptanoic Acid 1,3-Lactone (**8a**)

Compound **5a** (200 mg) was dissolved in methanol (4 ml) and palladium black (50 mg) added. After flashing the flask three times with hydrogen, the mixture was stirred for 45 minutes at room temperature under an atmosphere of hydrogen. The reaction was filtered through a Celite pad and concentrated *in vacuo* to obtain an oily material.

This was dissolved in pyridine (2.9 ml), cooled to 0°C, and *p*-toluenesulfonyl chloride (116 mg) was added. The mixture was stirred at 3°C for 18 hours. The reaction was quenched with methanol (1 ml) and diluted with CH_2Cl_2. The CH_2Cl_2 solution was washed with saturated NaCl solution (20 ml), dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluted with hexane-ethyl acetate (5:1) to afford *threo*-3-hydroxy-7-methoxycarbonyl-2-trityloxymethylheptanoic acid 1,3-lactone (**8a**) (39 mg, 28%). Mass m/z 458 (M^+). HR-MS calcd for $C_{29}H_{30}O_5$: 458.2091, found: 458.2091. IR (CH_3Cl) cm^{-1} 1820, 1730, 1490 and 1450. ^1H NMR ($CDCl_3$) δ 1.20~1.60 (6H, m), 2.23 (2H, t, J=6.8 Hz), 3.41 (2H, m), 3.62 (3H, s), 3.89 (1H, m), 4.50 (1H, m), 7.20~7.60 (15H, m).

erythro-3-Hydroxy-7-methoxycarbonyl-2-trityloxymethylheptanoic Acid 1,3-Lactone (**8b**)

Compound **5b** (550 mg) was treated in a similar manner to that described for the preparation of **8a** to give *erythro*-3-hydroxy-7-methoxycarbonyl-2-trityloxymethylheptanoic acid 1,3-lactone (**8b**) (350 mg, 81%). Mass m/z 458 (M^+). HR-MS calcd for $C_{29}H_{30}O_5$: 458.2091, found: 458.2108. IR ($CHCl_3$) cm^{-1} 1820, 1730, 1490 and 1450. ^1H NMR ($CDCl_3$) δ 1.20~1.60 (6H, m), 2.23 (2H, t, J=6.8 Hz), 3.35 (2H, m), 3.51 (1H, m), 3.59 (3H, s), 4.52 (1H, m), 7.20~7.60 (15H, m).

erythro-12-Hydroxy-13-trityloxymethyltetradecanedioic Acid 12,14-Lactone (**15b**)

Compound **14b** (500 mg) was treated in a similar manner to that described for the preparation of **6b** to give *erythro*-12-hydroxy-13-trityloxymethyltetradecanedioic acid 12,14-lactone (**15b**) (75 mg, 26%). Mass m/z 528 (M^+). IR ($CHCl_3$) cm^{-1} 3550, 1820, 1720, 1490 and 1450. ^1H NMR ($CDCl_3$) δ 1.20~1.60 (18H, m), 2.24 (2H, t, J=8.6 Hz), 3.32 (1H, m), 3.65 (2H, m), 4.49 (1H, m), 7.20~7.60 (15H, m).

erythro-3-Hydroxy-13-methoxycarbonyl-2-trityloxymethyltridecanoic Acid 1,3-Lactone (**17b**)

Compound **14b** (200 mg) was treated in similar manner to that described for the preparation of **8b**

to give *erythro*-3-hydroxy-13-methoxycarbonyl-2-trityloxymethyltridecanoic acid 1,3-lactone (**17b**) (69 mg, 48%). Mass *m/z* 542 (M^+). IR ($CHCl_3$) cm^{-1} 1820, 1730, 1490 and 1450. ^1H NMR ($CDCl_3$) δ 1.20~1.70 (18H, m), 2.30 (2H, t, J=6.8 Hz), 3.31 (2H, m), 3.49 (1H, m), 3.60 (3H, s), 4.49 (1H, m), 7.20~7.60 (15H, m).

erythro-6-Hydroxy-7-hydroxymethyloctanedioic Acid 6,8-Lactone (**7b**)

Trifluoroacetic acid (0.52 ml) was added to a solution of **6b** (67 mg) in *n*-butanol (1.2 ml) at 0°C, dropwise over 5 minutes and the mixture was stirred for 4 hours at room temperature. The mixture was diluted with EtOAc (20 ml), and washed with saturated $NaHCO_3$ (20 ml), then with saturated NaCl solution (20 ml), and dried over $MgSO_4$. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluted with $CHCl_3$ - MeOH (10 : 1) to afford *erythro*-6-hydroxy-7-hydroxymethyloctanedioic acid 6,8-lactone (**7b**) (24 mg, 79%). MS *m/z* 202 (M^+). IR ($CHCl_3$) cm^{-1} 3490, 1820 and 1725. ^1H NMR ($CDCl_3$) δ 1.30~1.80 (6H, m), 2.34 (2H, t, J=7.0 Hz), 3.41 (1H, m), 3.95 (2H, m), 4.60 (1H, m).

threo-3-Hydroxy-2-hydroxymethyl-7-methoxycarbonylheptanoic Acid 1,3-Lactone (**9a**)

Trifluoroacetic acid (0.18 ml) was added to a solution of **8a** (30 mg) in *n*-butanol (0.54 ml) at 0°C, dropwise over 5 minutes and the mixture was stirred for 3 hours at room temperature.

The mixture was diluted with EtOAc (10 ml), and washed with saturated $NaHCO_3$ (10 ml), then saturated NaCl solution (10 ml), and then dried over $MgSO_4$. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluted with hexane - ethyl acetate (2 : 1) to afford *threo*-3-hydroxy-2-hydroxymethyl-7-methoxycarbonylheptanoic acid 1,3-lactone (**9a**) (11 mg, 78%). FAB-MS *m/z* 217 (M^++H). IR ($CHCl_3$) cm^{-1} 3490, 1820 and 1730. ^1H NMR ($CDCl_3$) δ 1.50~2.00 (6H, m), 2.21 (2H, t, J=6.8 Hz), 3.58 (3H, s), 3.89 (3H, m), 4.48 (1H, m).

erythro-3-Hydroxy-2-hydroxymethyl-7-methoxycarbonylheptanoic Acid 1,3-Lactone (**9b**)

Compound **8b** (50 mg) was treated in a similar manner to that described for the preparation of **8a** to give *erythro*-3-hydroxy-2-hydroxymethyl-7-methoxycarbonylheptanoic acid 1,3-lactone (**9b**) (16 mg, 68%). FAB-MS *m/z* 217 (M^++H). IR ($CHCl_3$) cm^{-1} 3500, 1820 and 1730. ^1H NMR ($CDCl_3$) δ 1.50~2.05 (6H, m), 2.34 (2H, t, J=6.8 Hz), 3.38 (1H, m), 3.72 (3H, s), 3.90 (2H, m), 4.61 (1H, m).

erythro-3-Hydroxy-2-hydroxymethyltetradecanedioic Acid 1,3-Lactone (**16b**)

Compound **15b** (30 mg) was treated in a similar manner to that described for the preparation of **7b** to give *erythro*-3-hydroxy-2-hydroxymethyltetradecanedioic acid 1,3-lactone (**16b**) (8 mg, 49%). FAB-MS *m/z* 287 (M^++H). IR ($CHCl_3$) cm^{-1} 3490 and 1820. ^1H NMR ($CDCl_3$) δ 1.20~1.60 (18H, m), 2.28 (2H, m), 3.41 (1H, m), 3.90 (2H, m), 4.52 (1H, m).

erythro-3-Hydroxy-2-hydroxymethyl-13-methoxycarbonyltridecanoic Acid 1,3-Lactone (**18b**)

Compound **17b** (54 mg) was treated in a similar manner to that described for the preparation of **9a** to give *erythro*-3-hydroxy-2-hydroxymethyl-13-methoxycarbonyltridecanoic acid 1,3-lactone (**18b**) (18 mg, 60%). FAB-MS *m/z* 301 (M^++H). IR ($CHCl_3$) cm^{-1} 3600, 1820 and 1730. ^1H NMR ($CDCl_3$) δ 1.20~1.70 (18H, m), 2.31 (2H, t, J=7.4 Hz), 3.39 (3H, s), 3.98 (2H, m), 4.58 (1H, m).

Inhibitory Activities against HMG-CoA Synthase and Growth of Vero Cells

Inhibitory activities against HMG-CoA synthase and growth of Vero cells were assayed as described previously[5,11].

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