Synthesis and Biological Activity of New 3-Hydroxy-3-methylglutaryl-CoA Synthase Inhibitors: 2-Oxetanones with a *meta*-Substituent on the Benzene Ring in the Side Chain

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Received October 12, 1993; accepted December 24, 1993

Isosteric side chain analogs of 3a were synthesized and tested for inhibitory activities towards 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase and upon cholesterol production in Hep G2 cells and in mouse liver. It became clear that the lipophilic substituent on the aromatic ring and the terminal hydrophilic group in the side chain were important in the enhancement of activity. 4-[2-(3-*n*-Hexyloxyphenyl)ethyl]-3-hydroxy-methyl-2-oxetanone (5a) showed equivalent inhibitory activity *in vivo* to that of 1233A.

Keywords 3-hydroxy-3-methylglutaryl-CoA synthase; inhibitor; cholesterol biosynthesis; 2-oxetanone; 1233A analog; structure–activity relationship

Following the discovery of the biological activity of 1233A, a potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase,[1] a series of studies directed toward the development of 1233A analogs was initiated in these laboratories. Compounds were designed to fix the folded structure of side chain in 1233A by introduction of an aromatic ring, and synthesized.[2] Their intrinsic inhibitory potencies were ascribed to the 2-oxetanone moiety itself, but were greatly modulated by a) the distance between the 2-oxetanone and the aromatic ring, b) the lipophilicity and size of the aromatic ring and its substituent(s). Among the synthesized compounds, compound **2** had the highest activity *in vitro* and strongly inhibited the cholesterol biosynthesis in mouse liver. It also induced a considerable increment in serum triglyceride level, and so we stopped the investigation of naphthalene analogs. We then directed our attention to compound **3a**, which was half as active as 1233A *in vitro*, but showed neither cholesterol synthesis inhibition nor triglyceride increment *in vivo*. The inactivity *in vivo* could be explained in terms of the fast esterase-catalyzed hydrolysis of the ester on the side chain of **3a** to give the less active carboxylic acid **3c**. In practice, **3c** was prepared from the corresponding methyl ester **3b** by hydrolysis with porcine liver esterase (PLE).[2]

To avoid this hypothetical process, it would be effective to replace the *n*-hexyl carboxylic ester moiety with some other functional group by isosteric transformation. This hypothesis promoted us to investigate the modification of **3a** to obtain more potent *in vivo* inhibitors without triglyceride-enhancing activity.

Figure 2 showed the process of design based upon this

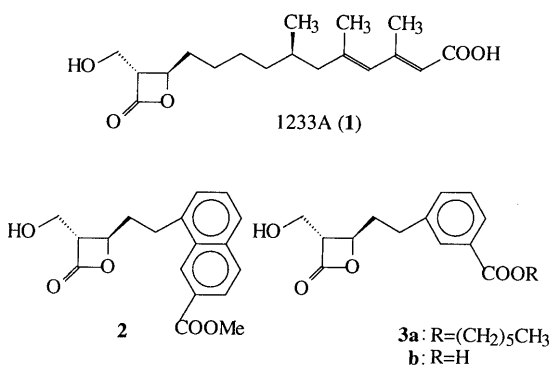


Fig. 1

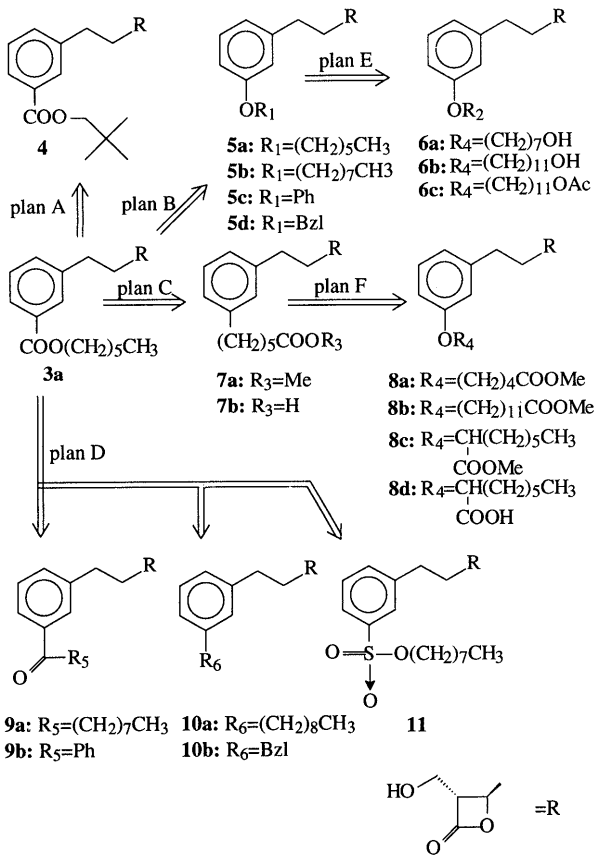
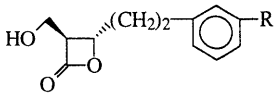


Fig. 2. Drug Design by Isosteric Transformation of **3a**

TABLE I. Physical Data for 4-Sustituted-3-hydroxymethyl-2-oxetanones



Compd. No.	R	mp (°C)	Formula	Elementary analysis Calcd (Found) C	H	MS M^+	High MS Calcd (Found)	^{1}H-NMR ($CDCl_3$) δ
4a	$COOCH_2C(CH_3)_3$	Oil	$C_{18}H_{24}O_5$			320 (FD)		1.06 (9H, s), 1.80—2.45 (3H, s), 2.70—3.00 (2H, m), 3.44—3.60 (1H, m), 3.71—4.12 (4H, m), 4.66 (1H, dt, J=4.0, 7.2 Hz), 7.33—7.60 (2H, m), 7.85—8.10 (2H, m)
5a	$O(CH_2)_5CH_3$	43—44.0 (*n*-hexane)	$C_{18}H_{26}O_4$			306 (EI)	306.1831 (306.1855)	0.80—1.97 (12H, m), 1.97—2.53 (2H, m), 2.60—3.01 (2H, m), 3.25—3.50 (2H, m), 3.60—4.24 (2H, m), 3.94 (1H, t, J=7.0 Hz), 4.59 (1H, dt, J=4.0, 7.0 Hz), 6.64—7.46 (4H, m)
5b	$O(CH_2)_7CH_3$	51—52.0 (*n*-hexane)	$C_{20}H_{30}O_4$	71.82 (71.77	9.04 9.09)	334 (EI)		0.76—1.96 (15H, m), 1.98—2.31 (3H, m), 2.61—2.89 (2H, m), 3.25—3.47 (1H, m), 3.63—4.12 (4H, m), 4.58 (1H, dt, J=4.3, 7.2 Hz), 6.63—6.86 (3H, m), 7.08—7.31 (1H, m)
5c	OPh	Oil	$C_{18}H_{18}O_4$			298 (EI)	298.1205 (298.1223)	1.96—2.40 (2H, m), 2.50—3.05 (3H, m), 3.28—3.42 (1H, m), 3.50—4.20 (2H, m), 4.58 (1H, dt, J=4.0, 6.8 Hz), 6.75—7.42 (9H, m)
5d	OBzl	78.5—79.5 (IPE)	$C_{19}H_{20}O_4$	73.06 (73.01	6.45 6.43)	312 (EI)		1.87 (1H, br t), 2.00—2.30 (2H, m), 2.63—2.88 (2H, m), 3.25—3.40 (1H, m), 3.61—4.10 (2H, m), 4.59 (1H, dt, J=3.6, 7.2 Hz), 5.08 (2H, s), 6.77—7.62 (9H, m)
6a	$O(CH_2)_7OH$	64—65.0 (AcOEt–IPE)	$C_{19}H_{28}O_5$	67.83 (67.91	8.39 8.39)	336 (EI)		1.20—3.10 (16H, m), 3.28—3.50 (1H, m), 3.50—4.20 (6H, m), 4.61 (1H, dt, J=3.6, 7.2 Hz), 6.70—6.96 (3H, m), 7.13—7.40 (1H, m)
6b	$-O(CH_2)_{11}OH$	79.5—81.0	$C_{23}H_{36}O_5$	70.37 (70.25	9.24 9.43)	392 (EI)		1.10—2.00 (20H, m), 2.00—2.45 (2H, m), 2.55—3.00 (2H, m), 3.27—3.55 (1H, m), 3.55—4.25 (6H, m), 4.63 (1H, dt, J=3.6, 7.2 Hz), 6.60—7.05 (3H, m), 7.10—7.32 (1H, m)
6c	$O(CH_2)_{11}OAc$	52.0—53.0 (*n*-hexane–IPE)	$C_{25}H_{38}O_6$	69.09 (68.85	8.81 9.10)	434 (EI)		1.05—2.00 (19H, m), 2.05 (3H, s), 2.05—2.33 (2H, m), 2.66—2.91 (2H, m) 3.31—3.50 (1H, m), 3.70—4.25 (6H, m), 4.64 (1H, dt, J=4.2, 7.2 Hz), 6.73—6.95 (3H, m), 7.18—7.40 (1H, m)
7a	$(CH_2)_5COOMe$	Oil	$C_{19}H_{26}O_5$			334 (EI)		1.20—1.88 (7H, m), 2.07—2.45 (4H, m), 2.42—2.90 (4H, m), 3.30—3.50 (1H, m), 3.65 (3H, s), 3.70—4.17 (2H, m), 4.61 (1H, dt, J=3.6, 6.8 Hz), 6.97—7.39 (4H, m)
7b	$(CH_2)_5COOH$	70.0—72.5 (IPE)	$C_{18}H_{24}O_5$	67.48 (67.40	7.75 7.59)	320 (EI)		1.05—1.88 (6H, m), 1.90—2.42 (4H, m), 2.42—2.90 (4H, m), 3.20—3.50 (1H, m), 3.57—4.15 (2H, m), 4.61 (1H, dt, J=3.6, 7.2 Hz), 4.72 (2H, br s), 6.90—7.40 (4H, m)
8a	$O(CH_2)_4COOMe$	Oil	$C_{18}H_{24}O_6$			336 (EI)	336.1573 (336.1605)	1.65—2.55 (8H, m), 2.61—3.02 (3H, m), 3.28—3.50 (1H, m), 3.68 (3H, s), 3.62—4.20 (4H, m), 4.62 (1H, dt, J=3.6, 7.2 Hz), 6.69—6.92 (3H, m), 7.10—7.40 (1H, m)
8b	$O(CH_2)_{10}COOMe$	57.5—59.5 (*n*-hexane–IPE)	$C_{24}H_{36}O_6$	68.54 (68.44	8.63 8.50)	420 (EI)		0.84—2.54 (21H, m), 2.60—2.97 (2H, m), 3.27—3.60 (1H, m), 3.70 (3H, s), 3.80—4.24 (4H, m), 4.63 (1H, dt, J=4.0, 6.8 Hz), 6.70—7.10 (3H, m), 7.13—7.50 (1H, m)
8c	$OCH(COOMe)(CH_2)_5CH_3$	Oil	$C_{21}H_{30}O_6$			378 (FD)		0.70—1.80 (16H, m), 2.60—2.96 (2H, m), 3.25—3.45 (1H, m), 3.78 (3H, s), 3.60—4.12 (2H, m), 4.42—4.77 (2H, m), 6.63—7.05 (3H, m), 7.10—7.42 (1H, m)
8d	$OCH(COOH)(CH_2)_5CH_3$	Oil	$C_{20}H_{28}O_6$			364 (EI)	364.1886 (364.1849)	0.70—1.80 (13H, m), 1.80—2.40 (2H, m), 2.61—2.90 (2H, m), 3.18—3.42 (1H, m), 3.50—4.00 (2H, m), 4.50 (1H, dt, J=4.0, 6.8 Hz), 4.69 (1H, br t), 5.59 (2H, br s), 6.60—7.00 (3H, m), 7.20—7.40 (1H, m)
9a	$CO(CH_2)_7CH_3$	52—53.0 (*n*-hexane–IPE)	$C_{21}H_{30}O_4$			346 (EI)	346.2144 (346.2149)	0.73–2.50 (18H, m), 2.71—3.14 (4H, m), 3.35—3.54 (1H, m), 3.70—4.30 (2H, m), 4.64 (1H, dt, J=3.6, 7.2 Hz), 7.40—7.60 (2H, m), 7.75—7.97 (2H, m)
9b	COPh	Oil	$C_{19}H_{18}O_4$			310 (EI)	310.1205 (310.1221)	1.88—2.40 (2H, m), 2.52—3.24 (3H, m), 3.32—3.50 (1H, m), 3.60—4.15 (2H, m), 4.61 (1H, dt, J=3.9, 6.8 Hz), 7.20—8.04 (9H, m)
10a	$(CH_2)_8CH_3$	54—55.0 (*n*-hexane–IPE)	$C_{21}H_{32}O_3$			332 (EI)	332.2351 (332.2353)	0.75—1.80 (17H, m), 1.82 (1H, br t), 2.02—2.32 (2H, m), 2.45—2.80 (4H, m), 3.29—3.50 (1H, m), 3.73—4.18 (2H, m), 4.62 (1H, dt, J=3.6, 7.2 Hz, 1H), 6.98—7.40 (4H, m)
10b	CH_2Ph	Oil	$C_{19}H_{20}O_3$			296 (EI)	296.1412 (296.1408)	1.90 (1H, br t), 2.05—2.30 (2H, m), 2.60—2.87 (2H, m), 3.24—3.40 (1H, m), 3.56—4.07 (2H, m), 4.00 (2H, s), 4.59 (1H, dt, J=4.0, 6.8 Hz), 7.00—7.50 (9H, m)
11	$SO_2(CH_2)_7CH_3$	Oil	$C_{20}H_{30}O_5S$			382 (FD)		0.65—1.90 (15H, m), 2.00—2.38 (2H, m), 2.50 (1H, br t), 2.76—3.24 (4H, m), 3.38—3.58 (1H, m), 3.66—4.22 (2H, m), 4.63 (1H, dt, J=4.2, 7.2 Hz), 7.48—7.70 (2H, m), 7.70—7.90 (2H, m)

IPE = isopropyl ether.

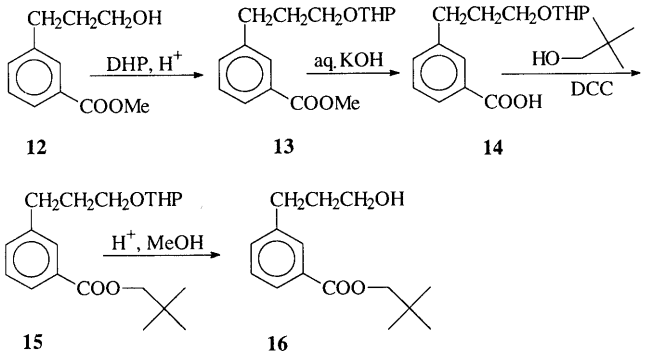


Chart 1. Synthesis of the Propanol **16**

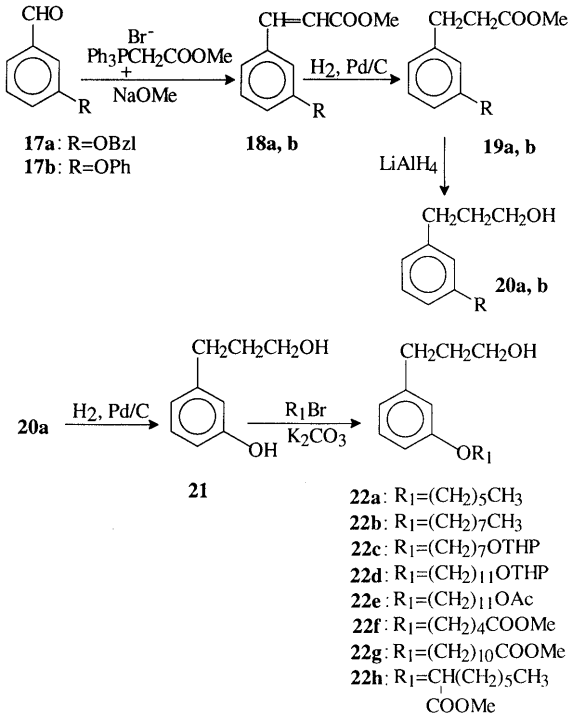


Chart 2. Synthesis of Propanols **22**

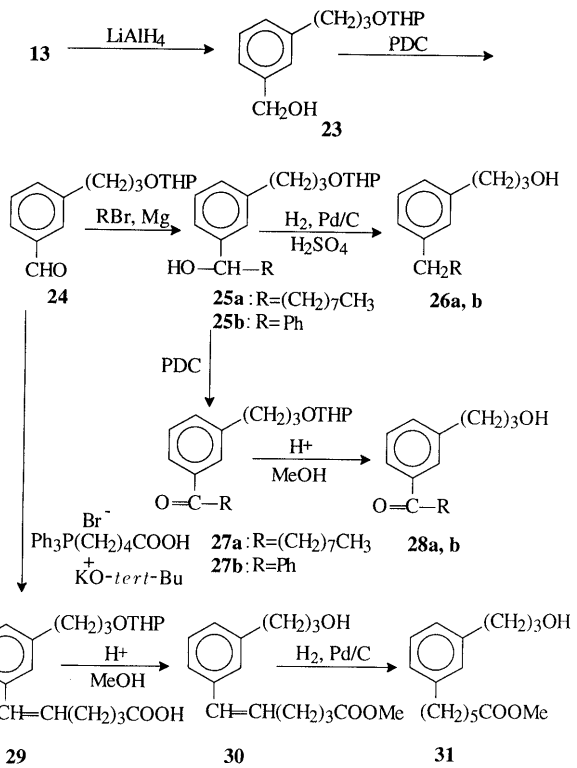


Chart 3. Synthesis of Propanols **26**, **28** and **31**

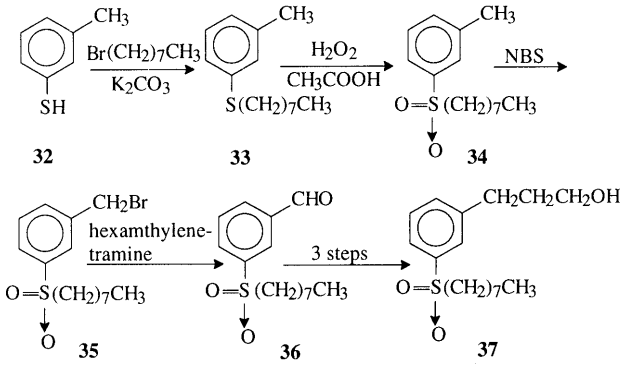


Chart 4. Synthesis of the Propanol **37**

hypothesis. Plan A was transformation to a neopentyl ester. Plans B, C and D were isosteric transformations to other functional groups, which are roughly equal in size to the *n*-hexyl carboxylic ester moiety. In plans E and F, a hydrophilic group was introduced to investigate its effect on inhibitory activity and triglyceride increment.

Chemistry 2-Oxetanones explored in this study are listed in Table I and were prepared from the key propanols shown in Table II. The key propanols were prepared as shown in Charts 1—4.

As shown in Chart 1, the propanol **16** with a neopentyl ester group was prepared by the esterification of **14**. Chart 2 shows the preparation of propanols **20**, based on the reported method.[2] The alkylation of **21** provided the propanols **22**. As illustrated in Chart 3, Grignard reaction products **25** of the benzaldehyde derivative **24** were subjected to hydrogenolysis and oxidation to give alkyl and alkylcarbonyl analogs (**26** and **28**), respectively. Wittig reaction of **24** followed by esterification and hydrogenation afforded the methoxycarbonylpentyl analog **31**. 3-Thiocresol (**32**) was alkylated, oxidized and treated in a manner similar to that reported[2] to give the propanol **37**.

A typical procedure for the preparation of 2-oxetanones as 1233A analogs is outlined in Chart 5, and is similar to the reported method.[2] In this procedure, although the yield of aldol condensation with lithium diisopropylamide (LDA) as a base was low[2] (20—30%), the use of lithium bistrimethylsilylamide gave a moderate yield (40—70%). Analogs **7b** and **8d** were prepared from the corresponding methyl ester **7a** and **8c** by hydrolysis with PLE.[2] All of these 2-oxetanones were racemic *trans*-form.

Inhibition of HMG-CoA Synthase and Cholesterol Biosynthesis in the Mouse Liver by the Synthesized 2-Oxetanone Derivatives Synthesized 1233A analogs were evaluated for inhibitory activities against HMG-CoA synthase in a cell-free system and cholesterol synthesis in Hep G2 cells and in mouse liver. The results are summarized in Table III.

TABLE II. Physical Data for Sustituted Propanols

R–Ph–$CH_2CH_2CH_2OH$

Compd. No.	R	mp (°C)	Formula	EI-MS M^+	High MS Calcd (Found)	^{1}H-NMR ($CDCl_3$) δ
16	3-$COOCH_2C(CH_3)_3$	Oil	$C_{15}H_{22}O_3$	250	250.1570 (250.1552)	1.07 (9H, m), 1.75—2.11 (2H, m), 2.68—2.96 (2H, m), 3.59—3.76 (2H, m), 4.06 (2H, s), 7.33—7.50 (2H, m), 7.83—8.02 (2H, m)
20a	3-OBzl	Oil	$C_{16}H_{18}O_2$	242	242.1307 (242.1326)	1.59 (1H, br s), 1.60—2.10 (2H, m), 2.61—2.89 (2H, m), 3.40—3.75 (2H, m), 5.10 (2H, s), 6.83—7.60 (9H, m)
20b	3-OPh	Oil	$C_{15}H_{16}O_2$	228	228.1150 (228.1162)	1.50—2.08 (3H, m), 2.58—2.76 (2H, m), 3.64 (2H, t, J=6.8 Hz), 6.75—7.43 (9H, m)
22a	3-OC_6H_{13}	Oil	$C_{15}H_{24}O_2$	236	236.1776 (236.1785)	0.74—2.10 (14H, m), 2.56—2.83 (2H, m), 3.70 (2H, t, J=7.0 Hz), 3.98 (2H, t, J=7.0 Hz), 6.70—7.00 (3H, m), 7.10—7.43 (1H, m)
22b	3-OC_8H_{17}	Oil	$C_{17}H_{28}O_2$	264	264.2089 (264.2115)	0.75—2.10 (18H, m), 2.55—2.84 (2H, m), 3.70 (2H, t, J=6.8 Hz), 3.97 (2H, t, J=7.0 Hz), 6.67—6.91 (3H, m), 7.12—7.38 (1H, m)
22c	3-$O(CH_2)_7OTHP$	Oil	$C_{21}H_{34}O_4$	350	350.2457 (350.2484)	1.15—2.10 (19H, m), 2.59—2.84 (2H, m), 3.20—4.10 (8H, m), 4.57 (1H, br s), 6.60—6.95 (3H, m), 7.05—7.37 (1H, m)
22d	3-$O(CH_2)_{11}OTHP$	Oil	$C_{25}H_{42}O_4$	406	406.3083 (406.3072)	1.20—2.10 (27H, m), 2.60—2.83 (2H, m), 3.26—4.15 (8H, m), 4.60 (1H, br s), 6.65—6.95 (3H, m), 7.15—7.40 (1H, m)
22e	3-$O(CH_2)_{11}OAc$	33.5—35.5 (*n*-hexane–IPE)	$C_{22}H_{36}O_4$[a]	364	364.2614 (364.2602)	1.20—2.05 (21H, m), 2.06 (3H, s), 2.60—2.83 (2H, m), 3.72 (2H, t, J=6.8 Hz), 3.98 (2H, t, J=7.0 Hz), 4.18 (2H, t, J=6.8 Hz), 6.70—6.95 (3H, m), 7.16—7.40 (1H, m)
22f	3-$O(CH_2)_4COOMe$	Oil	$C_{15}H_{22}O_4$	266	266.1518 (266.1536)	1.40—2.10 (7H, m), 2.22—2.52 (2H, m), 2.57—2.80 (2H, m), 3.50—3.75 (5H, m), 3.87—4.10 (2H, m), 6.62—6.92 (3H, m), 7.08—7.41 (1H, m)
22g	3-$O(CH_2)_{10}COOMe$	Oil	$C_{21}H_{34}O_4$	350	350.2459 (350.2447)	1.20—2.08 (19H, m), 2.32 (2H, t, J=7.2 Hz), 2.60—2.81 (2H, m), 3.70 (3H, s), 3.70 (2H, t, J=7.0 Hz), 3.97 (2H, t, J=6.8 Hz), 6.70—6.92 (3H, m), 7.12—7.43 (1H, m)
22h	3-$CH(COOMe)C_6H_{13}$	Oil	$C_{18}H_{28}O_4$	308	308.1989 (308.1977)	0.70—1.70 (12H, m), 1.74—2.10 (4H, m), 2.53—2.80 (2H, m), 3.57—3.87 (5H, m), 4.67 (1H, t, J=6.5 Hz), 6.70—6.94 (3H, m), 7.09—7.37 (1H, m)
26a	3-$(CH_2)_8CH_3$	Oil	$C_{18}H_{30}O$	262	262.2299 (262.2310)	0.70—1.70 (18H, m), 1.70—2.08 (2H, m), 2.43—2.83 (4H, m), 3.57—3.82 (2H, m), 6.94—7.37 (4H, m)
26b	3-CH_2Ph	Oil	$C_{16}H_{18}O$	226	226.1359 (226.1369)	1.42 (1H, br s), 1.60—2.05 (2H, m), 2.54—2.80 (2H, m), 3.69 (2H, t, J=6.4 Hz), 4.00 (2H, s), 7.05—7.96 (9H, m)
28a	3-$CO(CH_2)_7CH_3$	Oil	$C_{18}H_{28}O_2$	276	276.2091 (276.2068)	0.65—2.10 (18H, m), 2.63—3.10 (4H, m), 3.45—3.84 (2H, m), 7.25—7.54 (2H, m), 7.68—7.94 (2H, m)
28b	3-COPh	Oil	$C_{16}H_{16}O_2$	240	240.1151 (240.1127)	1.76—2.22 (2H, m), 2.06 (1H, s), 2.60—3.00 (2H, m), 3.67 (2H, t, J=6.4 Hz), 7.05—7.96 (9H, m)
31	3-$(CH_2)_5COOMe$	Oil	$C_{16}H_{24}O_3$	264	264.1727 (264.1711)	1.14—2.10 (9H, m), 2.31 (2H, t, J=7.0 Hz), 2.47—2.84 (4H, m), 3.69 (3H, s), 3.69 (2H, t, J=7.0 Hz), 6.94—7.38 (4H, m)
37	3-$SO_2(CH_2)_7CH_3$	Oil	$C_{17}H_{28}O_3S$	312		0.70—2.10 (18H, m), 2.70—3.23 (4H, m), 3.69 (2H, t, J=6.8 Hz), 7.40—7.64 (2H, m), 7.64—7.88 (2H, m)

a) Elementary analysis: Calcd: C, 72.49; H, 9.96. Found: C, 72.12; H, 9.69. IPE = isopropyl ether.

Analog **4** with a neopentyl ester group was designed to resist hydrolysis in serum, but it was considerably less active than **3a**. Among analogs **5a—d**, analogs except **5d** were as active as **3a** *in vitro* and analogs **5a, b** showed stronger inhibition of cholesterol biosynthesis in mouse liver than that expected from the *in vitro* results. In analogs **6a, b**, analog **6b** with the bulky substituent was more active than 1233A (**1**). However, **6b** was inactive *in vivo*. Although the reason is unknown, a more lipophilic analog **6c** was synthesized to modify the absorbability of **6b**, but it showed low activity in vitro and it was inactive *in vivo*.

Among analogs **7** and **8**, **7a** showed similar activity to that of **3a**, and **8c** was as active as 1233A (**1**). However, analogs **8a, b** were less active. As reported,[2] analogs with carboxy group were less active *in vitro* than the corresponding methyl esters (**7a** *vs.* **7b** and **8c** *vs.* **8d**). In analogs **9** with a carbonyl group and **10** with an alkyl group, many analogs were about as active as **3a** *in vitro*, as we had expected (**9a, b** and **10a, b**) and analog **10b** was active *in vivo*. Analog **11** with an *n*-octylsulfonyl group was less active than **3a**. The *n*-octylsulfonyl group can not be regarded as an isoster for the *n*-hexyl carboxylic ester group.

These results indicate that a lipophilic *meta*-substituent on the benzene ring contributes to the inhibitory activity of these analogs regardless of its nature. It is interesting that a terminal carboxy group favored the inhibitory activity *in vivo* (**7b**).

TABLE III. Results of *in Vitro* and *in Vivo* Assays (Mice, $n=6$)

Compound No.	*In vitro* test		*In vivo* test		
	Inhibition of HMG-CoA synthase IC_{50} (μM)	Inhibition of sterol synthesis in Hep G2 Cells IC_{50} (μM)	Dose (mg/kg) body w.t. *p.o.*	Inhibition of sterol synthesis (%) in liver	Increase of serum triglyceride level ×fold[a]
4	1.91	1.2	150	4.7 (−)[b]	0.95
5a	0.74	25	500	80.1 (++)	1.53[c]
5b	0.60	7.10	500	76.3 (++)	1.80[d]
5c	0.50	n.d.	n.d.	n.d.	n.d.
5d	1.40	n.d.	500	16.0 (−)	1.68
6a	1.25	4.70	200	33.8 (−)	0.94
6b	0.09	1.30	500	−4.5 (−)	1.05
6c	2.70	1.45	500	21.6 (−)	1.13
7a	0.65	2.55	500	59.6 (−)	1.20[c]
7b	2.29	0.95	500	65.2 (+)	1.37[c]
8a	3.12	n.d.	450	22.4 (−)	0.89
8b	1.80	1.4	500	−38.7 (−)	1.45[c]
8c	0.12	2.00	500	39.2 (−)	1.51
8d	0.38	11.50	500	42.1 (+)	1.23
9a	0.61	6.8	n.d.	n.d.	n.d.
9b	0.97	3.3	n.d.	n.d.	n.d.
10a	0.55	>25	500	59.0 (−)	2.08[c]
10b	0.45	>25	500	59.7 (+)	1.49[d]
11	1.91	>25	500	69.0 (+)	1.30
3c	140	n.d.	n.d.	n.d.	n.d.
3a	0.47	3.7	500	−17.0 (−)	1.29
2	0.09	2.1	500	80.6 (++)	11.9
1	0.20	0.42	500	83.0 (++)	1.03

a) The triglyceride level of control groups was assigned a value of 1.00. *b*) +, significant inhibition (<70%); ++, significant inhibition (>70%); −, not significant. *c*) $p<0.01$ *vs.* control. *d*) $p<0.05$. n.d., not determined.

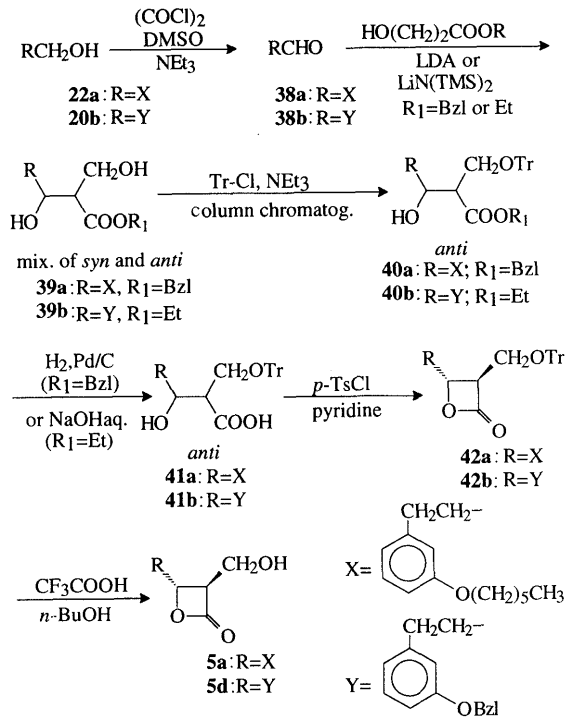


Chart 5. Typical Procedure for the Synthesis of 2-Oxetanones

Inhibition of Cholesterol Biosynthesis in Hep G2 Cells by Synthesized 2-Oxetanones Derivatives Synthesized analogs except **10a, b** and **11** inhibited cholesterol biosynthesis. However, the results did not show a good correlation to the inhibitory activities against HMG-CoA synthase and cholesterol biosynthesis in mouse liver (*e.g.*, **4** *vs.* **5a**). The inconsistency might be attributed to the different abilities of analogs to pass through the membrane of Hep G2 cells.

Discussion of Triglyderide Level Increment in Serum In comparison with compound **2**, 1233A analogs prepared in this study did not greatly increase the triglyceride level in serum. However, there were a tendency for compounds having a substituent with high lipophilicity and high bulkiness on the benzene ring enhance the triglyceride level (**5a** *vs.* **5b**, **5b** *vs.* **6a**, **8a** *vs.* **8b** and **5b** *vs.* **10a**). The analogs with a hydroxy group at terminal did not increase the level (**6a** and **6b**) and analogs **7b** and **8d** with a carboxy group slightly increased it. Hence, an analog with a hydrophilic group might not affect the triglyceride level.

Conclusion

By the isosteric transformation of the *n*-hexyl ester group in the side chain of **3a**, 1233A analogs active *in vivo* (*e.g.* **5a**) were obtained. A lipophilic *meta*-substituent on the benzene ring contributed to the inhibitory activity of these analogs. It is noteworthy that a terminal carboxy group in the substituent on the benzene ring enhanced the activity *in vivo* with a slight serum triglyceride increment (**7b**). The results suggest a direction for further investigation to obtain an active 1233A analog without a triglyceride-increasing effect.

Experimental

Melting points were measured on a Yanagimoto hot stage apparatus and are uncorrected. In work-up, extracted solutions were dried over anhydrous $MgSO_4$, and concentrated under reduced pressure (rotary

evaporator). ^{1}H-NMR spectra were measured on a JEOL FX-90 unless otherwise noted and chemical shift values are reported in parts per million relative to tetramethylsilane as an internal standard. IR spectra were measured on a Hitachi 270-30 infrared spectrometer. Mass spectra were measured on a JEOL HX-110, JEOL JMS AX-505W, or JEOL JMS D300 instrument. The results of elementary analyses for carbon and hydrogen were within $\pm 0.4\%$ of the theoretical values. Physical data of 2-oxetanones and propanols were summarized in Tables I and II, respectively. All starting materials are commercially available unless otherwise indicated.

3-[3-(2-Tetrahydropyranyloxy)propyl]benzoic Acid (14) Compound **12**[2] was treated with 2,3-dihydropyran in the presence of *p*-TsOH to give **13**. To a solution of **13** (10.67 g, 38.3 mmol) in MeOH (59.5 ml), 1 N KOH (59.5 ml) was added dropwise at room temperature. The mixture was stirred for 3 h, concentrated, and extracted with Et_2O. The aqueous layer was acidified with 5% HCl, and extracted with Et_2O. The extract was dried and concentrated to give **14** (8.83 g, 87.2%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.40—2.18 (2H, m), 2.64—2.97 (2H, m), 3.28—4.10 (4H, m), 4.63 (1H, br s), 7.38—7.62 (2H, m), 7.90—8.10 (2H, m), 9.45 (1H, br s).

Neopentyl 3-(3-Hydroxypropyl)benzoate (16) Neopentyl alcohol (2.00 g, 22.6 mmol), **14** (3.99 g, 15.1 mmol) and dicyclohexylcarbodimide (DCC) (6.23 g, 30.2 mmol) were dissolved in CH_2Cl_2 (12 ml). 4-Dimethylaminopyridine (DMAP) (0.37 g, 30.2 mmol) was added, and the mixture was stirred for 30 min at room temperature. After addition of Et_2O (30 ml), the mixture was filtered. The filtrate was concentrated and the residue was subjected to silica gel column chromatography with $CHCl_3$ to give **15** (2.99 g, 59.2%) as an oil, which was dissolved in tetrahydrofuran (THF)–AcOH–H_2O (2 : 3 : 3 v/v/v, 30 ml). The solution was stirred for 2 h at 50 °C, poured into water (60 ml), and extracted with AcOEt. The extract was washed with water and saturated aqueous $NaHCO_3$, dried, and concentrated. The residue was subjected to silica gel column chromatography with $CHCl_3$ to give **16** (1.72 g, 78.1% based on **15**).

Methyl 3-(3-Benzyloxyphenyl)propenoate (18a) 3-Benzyloxybenzaldehyde (**17a**) (11.65 g, 54.9 mmol) and methoxycarbonylmethyltriphenylphosphonium bromide (34.19 g, 82.3 mmol) were dissolved in CH_2Cl_2 (233 ml), and NaOMe (4.74 g, 87.8 mmol) was added. The mixture was stirred overnight and washed with water. The organic layer was dried and concentrated, and the residue was subjected to silica gel column chromatography with *n*-hexane–AcOEt (3 : 1 v/v) to give **18a** (12.8 g, 86.9%), which was a mixture of *trans* and *cis* isomers. Recrystallization of the mixture from *n*-hexane–AcOEt gave the *trans*-isomer. *trans*-Isomer: mp 95—95.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.82 (3H, s), 5.13 (2H, s), 6.44 (1H, d, J = 15.1 Hz), 6.91—7.54 (9H, m), 7.70 (1H, d, J = 15.1 Hz).

3-(3-Benzyloxyphenyl)propanol (20a) A solution of **18a** (11.7 g, 43.6 mmol) in MeOH (200 ml) was stirred with 5% Pd/C (3 g, 50% wet) under H_2 at room temperature for 1 h, then filtered. The filtrate was concentrated to give an oily product. A solution of this product in THF (84 ml) was added dropwise to a suspension of $LiAlH_4$ (1.84 g, 48.4 mmol) in Et_2O (84 ml). The mixture was refluxed for 1 h. Unreacted $LiAlH_4$ was decomposed with MeOH, then the mixture was poured into water (1000 ml) containing concentrated HCl (20 ml). The organic layer was separated, dried, and concentrated. The residue was subjected to silica gel column chromatography with $CHCl_3$–MeOH (100 : 1 v/v) to give two oily products, **20a** (4.89 g, 46.3%) and **21** (2.30 g, 34.6%), each as an oil. **21**: ^{1}H-NMR ($CDCl_3$) δ: 1.68—2.08 (2H, m), 2.35 (1H, br s), 2.50—2.80 (2H, m), 3.70 (2H, t, J = 7.2 Hz), 6.03 (1H, br s), 6.50—7.30 (4H, m).

Compound **20b** was prepared in a similar manner.

3-(3-Hydroxyphenyl)propanol (21) A solution of **20a** (33.7 g, 139 mmol) in MeOH (337 ml) was stirred with 5% Pd/C (12 g, 50% wet) for 1 h under H_2, and then filtered. The filtrate was concentrated to give **21** (20.3 g, 96.0%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: described above.

3-(3-*n*-Hexyloxyphenyl)propanol (22a) K_2CO_3 (5.01 g, 36.3 mmol) and *n*-hexyl bromide (5.56 g, 33.7 mmol) were added to a solution of **21** (3.94 g, 25.9 mmol) in *N*,*N*-dimethylformamide (DMF) (88 ml). The mixture was stirred overnight at 70—80 °C, poured into water (300 ml) and extracted with AcOEt. The extract was washed with water, dried and concentrated. The residue was subjected to silica gel column chromatography with *n*-hexane–AcOEt (3 : 1 v/v) to give **22a** (4.33 g, 70.7%) as an oil.

Compounds **22b** and **22f—g** were prepared in a similar manner.

3-[3-[11-(2-Tetrahydropyranyloxy)undecanyloxy]phenyl]-propanol (22d) Bromoundecanol(9.88 g, 39.3 mmol) prepared by the method of Kang *et al.*[3] and 3,4-dihydropyran (4.66 ml, 51.1 mmol) were dissolved in CH_2Cl_2 (69 ml), and *p*-TsOH–H_2O (0.24 g, 1.23 mmol) was added. The mixture was stirred for 30 min at room temperature, washed with saturated aqueous $NaHCO_3$, dried, and concentrated. The residue was subjected to silica gel column chromatography with *n*-hexane–AcOEt (5 : 1 v/v) to give 11-bromoundecanyl 2-tetrahydropyranylether (11.46 g, 87.0%) as an oil: ^{1}H-NMR ($CDCl_3$) δ: 1.10—2.10 (24H, m), 3.22—4.10 (6H, m), 4.60 (1H, br s). Compound **21** was treated with this compound in a similar manner as described for the preparation of **22a** to give **22d** (70.9%).

Compounds **22c** and **22e** were prepared in a similar manner.

3-[3-(2-Tetrahydropyranyloxy)propyl]benzyl Alcohol (23) Compound **13** (23.2 g, 83.4 mmol) was reduced with $LiAlH_4$ as described for the preparation of **20a** to give **23** (19.2 g, 91.9%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.37—2.30 (9H, m), 2.60—2.88 (2H, m), 3.20—4.10 (4H, m), 4.54—4.82 (3H, m), 7.10—7.40 (4H, m).

3-[3-(2-Tetrahydropyranyloxy)propyl]benzaldehyde (24) Compound **23** (27.2 g, 109 mmol) was dissolved in CH_2Cl_2 (27 ml) and pyridinium dichromate (PDC) (82.0 g, 218 mmol) was added at ambient temperature. The mixture was stirred for 2 d and filtered. The filtrate was concentrated and the residue was subjected to silica gel column chromatography with *n*-hexane–AcOEt (20 : 1 v/v) to give **24** (19.8 g, 73.1%) as an oil. 1H NMR (CDCl3) d: 1.37—2.16 (8H, m), 2.83 (2H, t, J = 6.8Hz), 3.28—4.04 (4H, m), 4.59 (1H, br s), 7.41—7.60 (2H, m), 7.60—7.87 (2H, m), 10.0 (1H, s).

1-[3-[3-(2-Tetrahydropyranyloxy)propyl]phenyl]nonanol (25a) A solution of *n*-octyl bromide (3.72 ml, 21.4 mmol) in dry Et_2O (37 ml) was added dropwise to Mg turnings (0.32 g, 13 mmol). The mixture was refluxed for 30 min, and cooled to room temperature. A solution of **24** (2.48 g, 9.99 mmol) in dry Et_2O (10 ml) was added dropwise. The mixture was refluxed for 1 h, poured into saturated aqueous NH_4Cl (100 ml) and extracted with Et_2O. The extract was dried, and concentrated. The residue was subjected to silica gel column chromatography with *n*-hexane–AcOEt (3 : 1 v/v) to give **25a** (3.23 g, 89.1%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 0.69—2.20 (25H, m), 2.60—2.86 (2H, m), 3.20—4.05 (5H, m), 4.40—4.70 (2H, m), 7.00—7.50 (4H, m).

3-(3-*n*-Nonanylphenyl)propanol (26a) Concentrated sulfuric acid (0.22 g), **25a** (2.70 g, 7.45 mmol) and 5% Pd/C (1.5 g, 50% wet) were dissolved in MeOH (27 ml). The mixture was stirred for 2 h under H_2 at room temperature, and filtered. The filtrate was poured into water (200 ml), and extracted with $CHCl_3$. The extract was washed with water, dried, and concentrated. The residue was subjected to silica gel column chromatography with *n*-hexane–AcOEt (3 : 1 v/v) to give **26a** (1.54 g, 78.7%).

Compound **26b** was prepared in a similar manner.

3′-[3-(2-Tetrahydropyranyloxy)propyl]-*n*-nonanophenone (27a) Compound **25a** (3.11 g, 8.58 mmol) was treated with PDC in a similar manner as described for the preparation of **24** from **23** to give **27a** (2.49 g, 80.5%) as an oil. ^{1}H-NMR (CDCl3) δ: 0.70—1.70 (23H, m), 2.60—3.10 (4H, m), 3.20—4.05 (4H, m), 4.61 (1H, br s), 7.30—7.54 (2H, m), 7.70—7.94 (2H, m).

3′-(3-Hydroxypropyl)-*n*-nonanophenone (28a) *p*-TsOH–H_2O (0.26 g, 1.40 mmol) was added to a solution of **27a** (2.48 g, 6.88 mmol) in MeOH (25 ml). The mixture was stirred for 1 h at room temperature, poured into water, and extracted with $CHCl_3$. The extract was washed with saturated aqueous $NaHCO_3$, dried, and concentrated. The residue was subjected to silica gel column chromatography with *n*-hexane–AcOEt (3 : 1 v/v) to give **28a** (1.90 g, 100%) as an oil.

Compound **28b** was prepared in a similar manner.

Methyl 6-[3-(3-Hydroxypropyl)phenyl]-5-hexenoate (30) A solution of 4-carboxybutyltriphenylphosphonium bromide[4] (33.7 g, 76.1 mmol) in dimethyl sulfoxide (DMSO) (145 ml) was treated with KO-*tert*-Bu (17.1 g, 152 mmol). The mixture was stirred for 15 min at room temperature, then a solution of **24** (14.53 g, 58.5 mmol) in DMSO (15 ml) was added. The whole was stirred for 1 h, poured into water (1500 ml), acidified with concentrated HCl, and extracted with $CHCl_3$. The extract was washed with water, dried, and concentrated. The residue was dissolved in MeOH (300 ml). After addition of acetyl chloride (8.3 ml, 117 mmol), the mixture was refluxed for 2 h, concentrated and dissolved in $CHCl_3$. The resultant mixture was washed with saturated aqueous $NaHCO_3$, dried, and concentrated. The residue was subjected to silica gel column chromatography with *n*-hexane–AcOEt (2 : 1 v/v) to give **30** (12.45 g, 81.1%) as an oil. ^{1}H-NMR ($CDCl_3$) δ : 1.56 (1H, br s), 1.67—2.08 (4H, m), 2.10—2.54 (4H, m), 2.56—2.87 (2H, m), 3.60—3.85

(5H, m), 5.50—6.61 (2H, m), 7.00—7.40 (4H, m).

Methyl 6-[3-(3-Hydroxypropyl)phenyl]hexanoate (31) A solution of **30** (12.5 g, 47.5 mmol) in MeOH (124 ml) was stirred with 5% Pd/C (3.7 g, 50% wet) under H_2 for 3 h and then filtered. The filtrate was concentrated to give **31** (11.9 g, 94.8%) as an oil.

***n*-Octyl 3-Tolyl Sulfone (34)** *n*-Octyl 3-tolyl sulfide (**33**) (31.1 g, 132 mol) prepared from 3-thiocresol (**32**) in a similar manner described for the preparation of **22a**, was dissolved in AcOH (93 ml). To this solution, 30% H_2O_2 (74 ml, 740 mmol) was added dropwise at 30—40 °C. The mixture was stirred for 3 h at ambient temperature and then poured into water. The resultant mixture was extracted with AcOEt. The extract was washed with water and 2% aqueous Na_2SO_3, dried, and concentrated. The residue was subjected to silica gel column chromatography with *n*-hexane–AcOEt (3 : 1 v/v) to give **34** (31.6 g, 89.2%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 0.70—1.90 (15H, m), 2.47 (3H, s), 2.96—3.22 (2H, m), 7.48—7.90 (4H, m).

3-(3-*n*-Octylsulfonylphenyl)propanol (37) Compound **34** was treated as reported previously[2] to give **37** as an oil.

3-(3-*n*-Hexyloxyphenyl)propanal (38a) Compound **22a** was oxidized by Swern's method to give **38a** as an oil. ^{1}H-NMR ($CDCl_3$) δ: 0.85—2.00 (11H, m), 2.62—3.10 (4H, m), 3.97 (2H, t, J=6.8 Hz), 6.67—7.00 (2H, m), 7.08—7.44 (2H, m), 9.84 (1H, t, J=1.2 Hz).

The other propanols were similarly prepared.

Benzyl 5-(3-*n*-Hexyloxyphenyl)-3-hydroxy-2-hydroxymethylpentanoate (39a) Benzyl 3-hydroxypropanoate[5] (24.8 g, 138 mmol) was dissolved in THF (100 ml) and cooled to −70 °C under an N_2 atmosphere. A solution (1 mol/l, 302.5 ml) of lithium bistrimethylsilylamide in THF was added dropwise. The mixture was stirred for 20 min, stirred for 10 min at −40 °C and cooled to −70 °C again. A solution of **38a** (35.4 g, 151 mmol) in THF (140 ml) was added dropwise. The mixture was stirred for 50 min, poured into saturated aqueous NH_4Cl (500 ml), and extracted with Et_2O. The extract was washed with water, dried, and concentrated. The residue was subjected to silica gel column chromatography with *n*-hexane–AcOEt (2 : 1 v/v) to give **39a** (39.2 g, 68.7%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 0.80—2.00 (13H, m), 2.37—3.16 (5H, m), 3.68—4.30 (3H, m), 3.92 (2H, t, J=7.0 Hz), 5.18 (2H, s), 6.62—7.42 (9H, m). EI-MS *m/z*: 414 (M^+). High MS: Calcd for $C_{25}H_{34}O_5$, 414.2406. Found: 414.2360.

Ethyl 5-(3-Benzyloxyphenyl)-3-hydroxy-2-hydroxymethylpentanoate (39b) Compound **38b** was treated with ethyl 3-hydroxypropanoate[5] in a similar manner to the preparation of **39a** to give **39b** (66.6%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, t, J=7.2 Hz), 1.73—2.10 (2H, m), 2.44—3.10 (5H, m), 3.75—4.10 (3H, m), 4.23 (2H, q, J=7.2 Hz), 5.06 (2H, s), 6.72—6.90 (2H, m), 7.20—7.52 (7H, m).

Benzyl *anti*-5-(3-*n*-Hexyloxyphenyl)-3-hydroxy-2-triphenylmethoxymethylpentanoate (40a) Compound **39a** (39.2 g, 94.6 mmol) was treated with triphenylmethyl chloride (39.5 g, 142 mmol), 4-dimethylaminopyridine (0.58 g, 4.70 mmol) and NEt_3 (15.3 g, 151 mmol) in the reported manner[2] to give 40a (19.9 g, 32.1%) and the *syn* compound (19.3 g, 31.2%). ^{1}H-NMR ($CDCl_3$) δ: 0.85—2.00 (13H, m), 2.43—2.95 (4H, m), 3.46 (2H, d, J=6.5 Hz), 3.75—4.03 (1H, m), 3.89 (2H, t, J=6.9 Hz), 5.19 (2H, s), 6.56—7.62 (24H, m). FD-MS *m/z*: 656 (M^+).

Compound **40b** was prepared in a similar manner.

***trans*-4-[2-(3-*n*-Hexyloxyphenyl)ethyl]-3-triphenylmethoxymethyl-2-oxetanone (42a)** Compound **40a** (4.00 g, 6.09 mmol) was subjected to hydrogenolysis and lactonization with *p*-TsCl and pyridine in the reported manner[2] to give **42a** (2.98 g, 89.2%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.92 (11H, m), 1.92—2.36 (2H, m), 2.36—2.97 (2H, m), 3.05—3.73 (3H, m), 3.89 (2H, t, J=7.0 Hz), 4.53 (1H, dt, J=4.9, 7.2 Hz), 6.53—7.82 (19H, m). FD-MS *m/z*: 548(M^+).

***trans*-4-[2-(3-*n*-Hexyloxyphenyl)ethyl]-3-hydroxymethyl-2-oxetanone (5a)** Compound **42a** (2.90 g, 5.29 mmol) was treated with CF_3COOH (29 ml) by the reported manner[2] to give **5a** (1.32 g, 81.4%) as crystals, mp 43—44 °C.

Compounds **4**, **5b—c**, **6a—c**, **7a**, **8a—c**, **9a—b**, **10a** and **11** were prepared in a similar manner.

***trans*-4-[2-(3-Benzyloxyphenyl)ethyl]-3-hydroxymethyl-2-oxetanone (5d)** Compound **40b** was hydrolyzed with aqueous alkali, followed by lactonization (42.5%) with *p*-TsCl and pyridine to give **42b**. Deprotection was carried out as reported[2] to give **5d** (59.4%) as an oil.

Compound **10b** was prepared in a similar manner.

5-[3-[2-(3-Hydroxymethyl-4-*trans*-oxooxetan-2-yl)ethyl]phenyl]-pentanoic Acid (7b) Compound **7a** was treated with PLE in the reported manner[2] to give **7b** (75%). mp: 70.5—72.5 °C.

Compound **8d** was prepared in a similar manner.

Inhibition of HMG-CoA Synthase (*in Vitro* Assay) and Cholesterol Biosynthesis (*in Vivo* Assay) Inhibitory activities against HMG-CoA synthase[6] and cholesterol biosynthesis in standard chow-fed male ddY mice[2] were assayed as reported.

Inhibition of Sterol Synthesis in Hep G2 Cells Hep G2 cells were obtained from ATCC and maintained in Dulbecco's modified Eagle's Medium (DMEM, Gibco430-1600EF) supplemented with 10% fetal bovine serum (FBS), penicillin (100 unit/ml), and kanamycin (50 μg/ml) in an atmosphere of 95% air/5% CO_2 at 37 °C. Lipoprotein-deficient serum (LPDS) was obtained by ultracentrifugation of FBS at d= 1.215 g/ml. The lipoprotein-free fraction (the bottom fraction) was then dialyzed extensively against 0.15 M NaCl, and filter-sterilized. The protein concentration was adjusted to 50 mg/ml. Sterol synthesis was determined by measuring the incorporation of ^{14}C-acetate radioactivity into the cell-associated digitonin precipitable sterol fraction. For this assay, Hep G2 cells (4 × 105 cells/well) were seeded in 35 mm 6-well plastic culture dishes and grown for 2 d in 2 ml of 10% FBS-DMEM medium. Then the cells were incubated with fresh DMEM medium containing 10% LPDS for 24 h. These cells were then incubated with fresh 10% LPDS–DMEM medium containing varying concentrations of test compounds. After incubation for 1 h, [^{14}C]acetate (0.5 μCi/ml) was added to the medium and incubation was continued for an additional 1 h. Since the test compounds examined in this study were rather hydrophobic, they were first dissolved in DMSO and then added to the cell culture medium. The final DMSO concentration in the medium was 0.5%. At the end of the incubation, the cells were washed with phosphate-buffered saline 3 times and dissolved in 1 ml of 0.1 N NaOH. A small aliquot of the cell lysate was kept for 1 h at 75 °C and extracted with petroleum ether. The extracts were dried, precipitated as digitonide and counted as described by Endo *et al.*[7]

References

1) S. Ōmura, H. Tomoda, H. Kumagai, M. D. Greenspan, J. B. Yodkovitz, J. S. Chen, A. W. Alberts, I. Martin, S. Mochales, R. L. Monaghan, J. C. Chabala, R. E. Schwarts, A. A. Patchett, *J. Antibiot.*, **40**, 1356 (1987).
2) H. Hashizume, H. Ito, K. Yamada, H. Nagashima, M. Kanao, H. Tomoda, T. Toshiaki, H. Kumagai, S. Ōmura, *Chem. Pharm. Bull.*, **42**, 512 (1994).
3) S. Kang, W. Kim, B. Moon, *Synthesis*, **1985**, 161
4) E. J. Corey, N. M. Weinshenkel, T. K. Schaaf, W. Huber, *J. Am. Chem. Soc.*, **91**, 5675 (1961).
5) T. Sunazuka, K. Tsuzuki, H. Kumagai, H. Tomoda, H. Nagashima, H. Hashizume, S. Ōmura, *J. Antibiot.*, **45**, 1139 (1992).
6) *a*) H. Tomoda, H. Kumagai, H. Tanaka, S. Ōmura, *Biochim. Biophys. Acta*, **922**, 351 (1987); *b*) H. Kumagai, H. Tomoda, S. Ōmura, *J. Antibiot.*, **43**, 397 (1990).
7) A. Endo, Y. Tsujita, M. Kuroda, K. Tanzawa, *Eur. J. Biochem.*, **77**, 31 (1977).