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-Substituted-3-hydroxymethyl-2-oxetanones

Compd. No.	R	mp (°C)	Formula	Elementary analysis Calcd (Found)		MS M ⁺	High MS Calcd (Found)	¹ H-NMR (CDCl ₃) δ
				C	H			
4a	COOCH ₂ C(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, <i>J</i> =4.0, 7.2 Hz), 7.33—7.60 (2H, m), 7.85—8.10 (2H, m)
5a	O(CH ₂) ₅ CH ₃	43—44.0 (<i>n</i> -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, <i>J</i> =7.0 Hz), 4.59 (1H, dt, <i>J</i> =4.0, 7.0 Hz), 6.64—7.46 (4H, m)
5b	O(CH ₂) ₇ CH ₃	51—52.0 (<i>n</i> -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, <i>J</i> =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, <i>J</i> =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, <i>J</i> =3.6, 7.2 Hz), 5.08 (2H, s), 6.77—7.62 (9H, m)
6a	O(CH ₂) ₇ OH	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, <i>J</i> =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, <i>J</i> =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 (<i>n</i> -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, <i>J</i> =4.2, 7.2 Hz), 6.73—6.95 (3H, m), 7.18—7.40 (1H, m)
7a	(CH ₂) ₅ COOMe	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, <i>J</i> =3.6, 6.8 Hz), 6.97—7.39 (4H, m)
7b	(CH ₂) ₅ COOH	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, <i>J</i> =3.6, 7.2 Hz), 4.72 (2H, br s), 6.90—7.40 (4H, m)
8a	O(CH ₂) ₄ COOMe	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, <i>J</i> =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 (<i>n</i> -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, <i>J</i> =4.0, 6.8 Hz), 6.70—7.10 (3H, m), 7.13—7.50 (1H, m)
8c	OCH(COOMe)(CH ₂) ₅ CH 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) ₅ CH 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, <i>J</i> =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) ₇ CH ₃	52—53.0 (<i>n</i> -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, <i>J</i> =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, <i>J</i> =3.9, 6.8 Hz), 7.20—8.04 (9H, m)
10a	(CH ₂) ₈ CH ₃	54—55.0 (<i>n</i> -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, <i>J</i> =3.6, 7.2 Hz), 6.98—7.40 (4H, m)
10b	CH ₂ Ph	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, <i>J</i> =4.0, 6.8 Hz), 7.00—7.50 (9H, m)
11	SO ₂ (CH ₂) ₇ CH ₃	Oil	C ₂₀ H ₃₀ O ₃ S			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, <i>J</i> =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

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.

Chart 3. Synthesis of Propanols 26, 28 and 31

Chart 4. Synthesis of the Propanol 37

3-Thiocresol (32) was alkylated, oxidized and treated in a manner similar to that reported²⁾ 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 low²⁾ (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 Substituted Propanols

R-Ph-CH ₂ CH ₂ CH ₂ OH						
Compd. No.	R	mp (°C)	Formula	EI-MS M ⁺	High MS Calcd (Found)	¹ H-NMR (CDCl ₃) δ
16	3-COOCH ₂ C(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, <i>J</i> = 6.8 Hz), 6.75—7.43 (9H, m)
22a	3-OC ₆ H 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, <i>J</i> = 7.0 Hz), 3.98 (2H, t, <i>J</i> = 7.0 Hz), 6.70—7.00 (3H, m), 7.10—7.43 (1H, m)
22b	3-OC ₈ H 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, <i>J</i> = 6.8 Hz), 3.97 (2H, t, <i>J</i> = 7.0 Hz), 6.67—6.91 (3H, m), 7.12—7.38 (1H, m)
22c	3-O(CH ₂) ₇ OTHP	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 (<i>n</i> -hexane-IPE)	33.5—35.5	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, <i>J</i> = 6.8 Hz), 3.98 (2H, t, <i>J</i> = 7.0 Hz), 4.18 (2H, t, <i>J</i> = 6.8 Hz), 6.70—6.95 (3H, m), 7.16—7.40 (1H, m)
22f	3-O(CH ₂) ₄ COOMe	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, <i>J</i> = 7.2 Hz), 2.60—2.81 (2H, m), 3.70 (3H, s), 3.70 (2H, t, <i>J</i> = 7.0 Hz), 3.97 (2H, t, <i>J</i> = 6.8 Hz), 6.70—6.92 (3H, m), 7.12—7.43 (1H, m)
22h	3-CH(COOMe)C ₆ H 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, <i>J</i> = 6.5 Hz), 6.70—6.94 (3H, m), 7.09—7.37 (1H, m)
26a	3-(CH ₂) ₈ CH 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 ₂ Ph	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, <i>J</i> = 6.4 Hz), 4.00 (2H, s), 7.05—7.96 (9H, m)
28a	3-CO(CH ₂) ₇ CH 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, <i>J</i> = 6.4 Hz), 7.05—7.96 (9H, m)
31	3-(CH ₂) ₅ COOMe	Oil	C ₁₆ H ₂₄ O ₃	264	264.1727 (264.1711)	1.14—2.10 (9H, m), 2.31 (2H, t, <i>J</i> = 7.0 Hz), 2.47—2.84 (4H, m), 3.69 (3H, s), 3.69 (2H, t, <i>J</i> = 7.0 Hz), 6.94—7.38 (4H, m)
37	3-SO ₂ (CH ₂) ₇ CH ₃	Oil	C ₁₇ H ₂₈ O ₃ S	312		0.70—2.10 (18H, m), 2.70—3.23 (4H, m), 3.69 (2H, t, <i>J</i> = 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**).

evaporator). $^1\text{H-NMR}$ 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 Et₂O. The aqueous layer was acidified with 5% HCl, and extracted with Et₂O. The extract was dried and concentrated to give **14** (8.83 g, 87.2%) as an oil. $^1\text{H-NMR}$ (CDCl₃) 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 dicyclohexylcarbodiimide (DCC) (6.23 g, 30.2 mmol) were dissolved in CH₂Cl₂ (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 Et₂O (30 ml), the mixture was filtered. The filtrate was concentrated and the residue was subjected to silica gel column chromatography with CHCl₃ to give **15** (2.99 g, 59.2%) as an oil, which was dissolved in tetrahydrofuran (THF)–AcOH–H₂O (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 NaHCO₃, dried, and concentrated. The residue was subjected to silica gel column chromatography with CHCl₃ 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 CH₂Cl₂ (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. $^1\text{H-NMR}$ (CDCl₃) 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 H₂ 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 LiAlH₄ (1.84 g, 48.4 mmol) in Et₂O (84 ml). The mixture was refluxed for 1 h. Unreacted LiAlH₄ 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 CHCl₃–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**: $^1\text{H-NMR}$ (CDCl₃) 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 H₂, and then filtered. The filtrate was concentrated to give **21** (20.3 g, 96.0%) as an oil. $^1\text{H-NMR}$ (CDCl₃) δ : described above.

3-(3-*n*-Hexyloxyphenyl)propanol (22a) K₂CO₃ (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 CH₂Cl₂ (69 ml), and *p*-TsOH–H₂O (0.24 g, 1.23 mmol) was added. The mixture was stirred for 30 min at room temperature, washed with saturated aqueous NaHCO₃, 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-tetrahydropyranyloxy (11.46 g, 87.0%) as an oil. $^1\text{H-NMR}$ (CDCl₃) 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 LiAlH₄ as described for the preparation of **20a** to give **23** (19.2 g, 91.9%) as an oil. $^1\text{H-NMR}$ (CDCl₃) 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 CH₂Cl₂ (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. $^1\text{H-NMR}$ (CDCl₃) δ : 1.37–2.16 (8H, m), 2.83 (2H, t, *J* = 6.8 Hz), 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 Et₂O (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 Et₂O (10 ml) was added dropwise. The mixture was refluxed for 1 h, poured into saturated aqueous NH₄Cl (100 ml) and extracted with Et₂O. 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. $^1\text{H-NMR}$ (CDCl₃) 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*-Nonanilyphenyl)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 H₂ at room temperature, and filtered. The filtrate was poured into water (200 ml), and extracted with CHCl₃. 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. $^1\text{H-NMR}$ (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–H₂O (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 CHCl₃. The extract was washed with saturated aqueous NaHCO₃, 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 bromide⁴⁾ (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 CHCl₃. 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 CHCl₃. The resultant mixture was washed with saturated aqueous NaHCO₃, 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. $^1\text{H-NMR}$ (CDCl₃) 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 H₂ 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% H₂O₂ (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 Na₂SO₃, 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. ¹H-NMR (CDCl₃) 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 previously²¹ 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. ¹H-NMR (CDCl₃) 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-(3-Hexyloxyphenyl)-3-hydroxy-2-hydroxymethylpentanoate (39a) Benzyl 3-hydroxypropanoate⁵¹ (24.8 g, 138 mmol) was dissolved in THF (100 ml) and cooled to –70 °C under an N₂ 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 NH₄Cl (500 ml), and extracted with Et₂O. 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. ¹H-NMR (CDCl₃) 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 C₂₅H₃₄O₅, 414.2406. Found: 414.2360.

Ethyl 5-(3-Benzoyloxyphenyl)-3-hydroxy-2-hydroxymethylpentanoate (39b) Compound **38b** was treated with ethyl 3-hydroxypropanoate⁵¹ in a similar manner to the preparation of **39a** to give **39b** (66.6%) as an oil. ¹H-NMR (CDCl₃) 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 NEt₃ (15.3 g, 151 mmol) in the reported manner²¹ to give **40a** (19.9 g, 32.1%) and the *syn* compound (19.3 g, 31.2%). ¹H-NMR (CDCl₃) 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 manner²¹ to give **42a** (2.98 g, 89.2%) as an oil. ¹H-NMR (CDCl₃) 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 CF₃COOH

(29 ml) by the reported manner²¹ 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-Benzoyloxyphenyl)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 reported²¹ to give **5d** (59.4%) as an oil.

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

5-[3-[2-(3-Hydroxymethyl-4-*trans*-oxoxetan-2-yl)ethyl]phenyl]-pentanoic Acid (7b) Compound **7a** was treated with PLE in the reported manner²¹ 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 synthase⁶¹ and cholesterol biosynthesis in standard chow-fed male ddY mice²¹ 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% CO₂ 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 ¹⁴C-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, [¹⁴C]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.*⁷¹

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. Schwartz, 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).