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Syntheses and Pharmacological Activities of Novel Optically Active Inhibitors of Acyl-CoA : Cholesterol *O*-Acyltransferase: EAB-309 ((*R*)-*N*-2-(1,3-Benzodioxol-4-yl)heptyl-*N'*-2,6-diisopropylphenylurea) and Its Enantiomer

Tetsuo SEKIYA,* Shinya INOUE, Tadashi SHIRASAKA, Chiaki MIYAJIMA, Hiromi OKUSHIMA, Kazuo SUZUKI, Mizue KAWAI, Masayuki MITSUKA, and Kouhei UMEZU

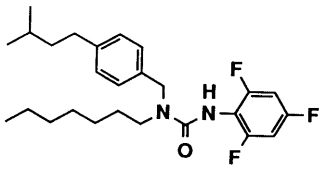
Pharmaceuticals Laboratory, Research Center, Mitsubishi Kasei Corporation, 1000 Kamoshida-cho, Midori-ku, Yokohama, Kanagawa 227, Japan. Received September 3, 1993; accepted November 2, 1993

Novel and potent ACAT (acyl-CoA : cholesterol *O*-acyltransferase) inhibitors, (*R*)-*N*-2-(1,3-benzodioxol-4-yl)heptyl-*N'*-2,6-diisopropylphenylurea (2a, EAB-309), and its enantiomer 2b (EAB-310), were prepared from 4-(1,3-benzodioxole)carbaldehyde (7) *via* optically active (*R* or *S*)-2-(1,3-benzodioxol-4-yl)heptanoic acid (12a or 12b). Compound 2a showed potent inhibitory effects on ACATs *in vitro*, and lowered plasma cholesterol *in vivo*. The IC_{50} value for inhibition of rat hepatic microsomal ACAT was 5 nM. The ED_{30} values of hypolipidemic activities in hamster and rat models were 0.25 and 0.75 mg/kg *p.o.*, respectively. The results indicate that 2a has potential to be a novel hypocholesterolemic and antiatherosclerotic agent. The activities of 2a *in vitro* and *in vivo* were only several times more potent than those of the enantiomer 2b. Modeling studies suggested that the three-dimensional structures of the two enantiomers are similar to each other.

Keywords acyl-CoA : cholesterol *O*-acyltransferase; hypocholesterolemic urea; phenylurea; antiatherosclerotic urea; optical resolution; (*R*)-2-(1,3-benzodioxol-4-yl)heptanoic acid

The enzyme acyl-CoA : cholesterol *O*-acyltransferase (ACAT, EC 2.3.1.26) is responsible for catalyzing the intracellular esterification of cholesterol. Studies both in cultured cells and in arterial tissue have suggested that ACAT activity is increased when cells are exposed to cholesterol-rich lipoproteins.[1] Since the intracellular accumulation of esterified cholesterol is one of the distinctive features of the atherosclerotic plaque, the regulation of ACAT is likely to be of great importance in the treatment of atherosclerosis.[2] Furthermore, ACAT facilitates the absorption of dietary cholesterol by esterifying free cholesterol taken up by the villous cells from the lumen.[3] The liver receives cholesterol from a number of sources, such as endogenous biosynthesis, removal of the lipolytic remnants of chylomicrons *via* the chylomicron/remnant receptor, and catabolism of low-density lipoprotein (LDL) *via* the LDL receptor pathway.[4] The cholesterol is esterified and stored within hepatocytes, and a portion of these cholesteryl ester stores is utilized in the assembly of very-low-density lipoprotein (VLDL), which is then secreted into the plasma to initiate the VLDL-IDL (intermediate density lipoprotein)-LDL endogenous fat transport pathway. Thus, inhibition of this pivotal enzyme in lipoprotein metabolism may yield a new therapy for the treatment of hypercholesterolemia and atherosclerosis.

A number of compounds have been reported to inhibit ACAT-catalyzed cholesterol esterification.[5] These early inhibitors are exemplified by Sah 57-118,[6] CL-277082,[7] CL-283546 (**1**, Fig. 1),[8] CI-976,[9] NAT-04152,[10] and YM-17E.[11] In the course of the investigation of novel ACAT inhibitors, we found that (±)-*N*-2-(1,3-benzodioxol-4-yl)hepty-*N'*-2,6-diisopropylphenylurea (**2**, Fig. 1) had not only potent ACAT-inhibitory activity but also a strong lowering effect on plasma cholesterol level in cholesterol-fed hamsters. Compound **2** has one asymmetric carbon, so both enantiomers were prepared and evaluated for *in vitro* ACAT-inhibitory activity and *in vivo* hypocholesterolemic effect.



CL-283546 (1)

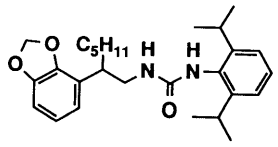


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Fig. 1. Structures of CL-283546 (**1**) and **2**

Chemistry

The racemic urea **2** was prepared from 1,3-benzodioxole-4-acetonitrile (**3**) in 3 steps, as shown in Chart 1. The nitrile **3** was alkylated with 1-bromopentane in the presence of 50% aqueous sodium hydroxide in dimethyl sulfoxide (DMSO) to give the alkylated derivative **4**, which was subsequently reduced with lithium aluminum hydride to yield 2-(1,3-benzodioxol-4-yl)heptylamine (**5**). Condensation of **5** with 2,6-diisopropylphenyl isocyanate (**6**) gave **2** in 66% overall yield.

For the preparation of the optically active isomer of **2**, the resolution of the racemic amine **5** was first attempted by recrystallizing the salts of some optically active acids such as tartaric acid. However, the resolution efficiencies were very poor, probably because **5** has the asymmetric center at the β-position with respect to the amino group.

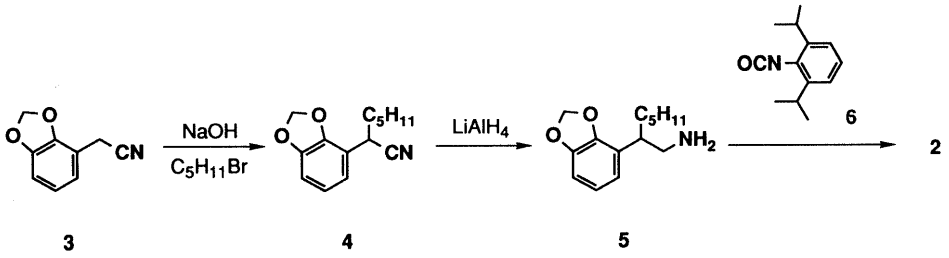

Chart 1

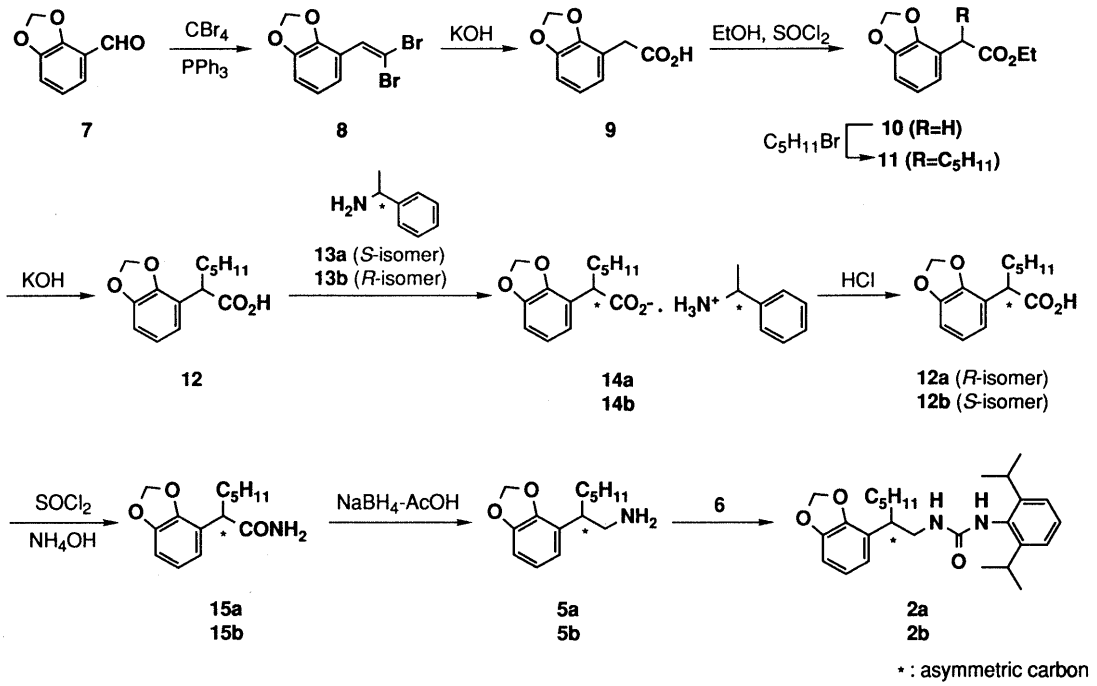

Chart 2

Then the optically active amines **5a** and **5b** were prepared from the resolved 2-(1,3-benzodioxol-4-yl)heptanoic acids (**12a** and **12b**), which were synthesized from 4-(1,3-benzodioxole)carbaldehyde (**7**) in six steps including the resolution process, as shown in Chart 2.

The aldehyde **7** was treated with triphenylphosphine and carbon tetrabromide to give the vinyl derivative **8**, which was hydrolyzed to yield 1,3-benzodioxol-4-ylacetic acid (**9**). Compound **9** was esterified to afford ethyl 1,3-benzodioxol-4-ylacetate (**10**), which was alkylated with 1-bromopentane in the presence of sodium hydride, and the resulting ester **11** was hydrolyzed to give racemic 2-(1,3-benzodioxol-4-yl)heptanoic acid (**12**) in 56% yield based on **7**. The racemic acid **12** was converted to the salt of (*S*)-1-phenylethylamine (**13a**). Recrystallization of the salt from 10% 2-propanol–heptane was repeated three times to provide the optically resolved salt **14a** in 38% yield (76% yield based on the isomer **12a**). The optical purity was found to be 98.5% ee by HPLC analysis using a chiral column. The configuration of the acid **12a** derived from **14a** was determined to be *R* by X-ray crystallographic analysis of **14a**.[12] The optically active acid **12a** obtained by acidification of **14a** was treated with thionyl chloride to convert it to the acid chloride, which was reacted with 28% aqueous ammonia solution to give the amide **15a**. Reduction of **15a** with $NaBH_4$ and acetic acid[13] yielded the *R*-configured amine **5a**. Condensation of **5a** with 2,6-diisopropylphenyl isocyanate (**6**) gave the optically active urea **2a** (EAB-309) of 98.2% ee in 71% yield based on **14a**. Its enantiomer **2b** (EAB-310, 98.0% ee) was prepared *via* the same route as **2a**, except for using (*R*)-1-phenylethylamine (**13b**) instead of **13a** for the resolution of the racemic acid **12**.

Results and Discussion

The inhibitory activities of **2**, **2a**, **2b**, and CL-283546 (**1**)[8] on rat hepatic and dog intestinal microsomal ACATs and cellular ACATs derived from mouse foamed macrophages and human hepatoma cells HepG2 were measured, and the results are shown in Table I.

The hypocholesterolemic activities were evaluated with cholesterol-fed rats, hamsters, and rabbits. The results are given in Table II and Fig. 2.

The data in Table I indicate that the inhibitory effects of **2**, **2a**, and **2b** are more potent than those of CL-283546 on rat hepatic microsomal ACAT and cellular ACAT derived from foamed macrophages and those of **2**, **2a**, **2b**, and CL-283546 are at a similar level on ACAT derived from HepG2. Furthermore, **2a** effectively inhibited dog intestinal ACAT. The data in Table II show that **2a** has

TABLE I. Inhibitory Effects of Urea Derivatives on ACAT

Enzyme or cell system	IC_{50} (nM)[a]			
	2	**2a**	**2b**	CL-283546 (**1**)
Rat liver ACAT[b]	8.6	5.0	11.8	98.0
Dog intestinal ACAT[b]	NT	25.0	NT	NT
Foamed macrophages[c]	37.3	37.7	42.8	309.0
HepG2[d]	18.6	17.4	53.7	37.7

a) The inhibitory effect on microsomal ACAT activity was determined by measuring the ratio of cholesteryl [^{14}C]oleate formation from cholesterol and [^{14}C]oleoyl-CoA with and without test compound. In the case of the cell system, [^{14}C]oleate-BSA was used as the substrate. *b*) Microsomes. *c*) Mouse peritoneal macrophages. *d*) Human hepatoma cells. NT, not tested.

TABLE II. Effects of **2a** and **2b** on Hamster and Rat Serum Cholesterol Levels

In vivo model[a]	ED_{30} (mg/kg)[b]	
	2a	**2b**
Cholesterol-fed hamster	0.25	2.69
Cholesterol-fed rat	0.76	6.33

a) Cholesterol-fed hamsters and rats were dosed with the compounds for 8 d and final values of serum total cholesterol levels were obtained. *b*) The ED_{30} value is the dose at which the decrease of the serum cholesterol level with the test compound amounted to 30% of the serum cholesterol level without test compound.

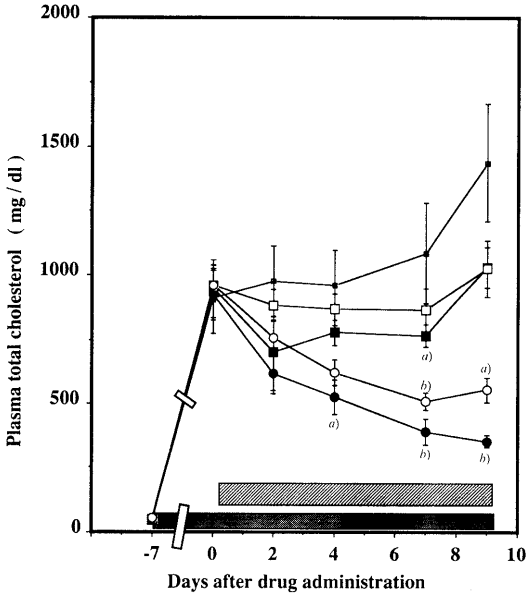

Fig. 2. Effect of **2a** and **2b** on Total Plasma Cholesterol Level in Rabbits with Hypercholesterolemia Induced by Cholesterol Diet

—⊞—, control; —■—, **2a**, 0.3 mg/kg; —●—, **2a**, 3 mg/kg; —□—, **2b**, 0.3 mg/kg; —○—, **2b**, 3 mg/kg. ▨, diet containing 0.5% cholesterol; ▨, drug administration (once a day); *a*) $p<0.05$. *b*) $p<0.01$.

potent hypolipidemic activities in hamster and rat models. Although the structure of ACAT is unknown at present, it has been reported that there are two subtypes of ACAT, liver and aorta ones.[14] The results mentioned above indicate that **2a** is a potent inhibitor of both types of ACAT, and may be available to treat both hypercholesterolemia and atherosclerosis.

The data in Table I also show that the *R*-isomer **2a** is a little more active than the *S*-isomer **2b** *in vitro*; the inhibitory effects of **2a** were slightly greater for foamed macrophages and two or three times greater for rat liver ACAT and HepG2 cell ACAT than those of **2b**. The *R*-isomer **2a** is about 10 times more active than the *S*-isomer **2b** *in vivo* (Table II). The ratio of the activity of **2a** to **2b** *in vivo* was slightly larger than that *in vitro*. Since the activities *in vivo* are influenced by absorption, metabolism, distribution, and excretion of the compound in addition to its inhibitory activity at the receptor (or enzyme), the difference between the ratio of the activities of **2a** to **2b** *in vitro* and that *in vivo* presumably reflects differences of these factors.

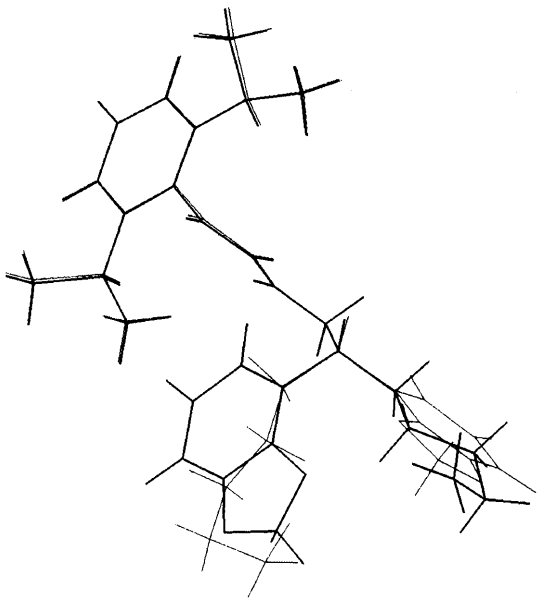

Fig. 3. Superposition of Wire Models of **2a** and **2b**
The model of **2a** is expressed by bold lines.

It is interesting to consider the finding of the small ratio of activities of **2a** to **2b** *in vitro*. Preliminary molecular modeling studies revealed that the two isomers can be well superposed in a certain conformation, when the 2,6-diisopropylphenyl groups of the isomers are overlapped (Fig. 3).[15]

In this superposition, the benzodioxole moiety of one enantiomer is roughly equivalent to the pentyl group of the other enantiomer. This finding may explain why both compounds show similar activities *in vitro*, and suggests that the requirements of the binding sites of ACAT corresponding to these moieties are not strict. As ACAT catalyzes the esterification of cholesterol by fatty acyl-CoA, it is assumed that there are three binding sites, *i.e.*, for cholesterol, fatty acid, and CoA. Sufficient information is not yet available to estimate the relationship between the binding sites of ACAT and the three parts of **2a** and **2b**.

Experimental

Melting points (mp) were measured with a Yanaco melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO IR-102 or FT/IR-5300 spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Bruker AC-250 instrument, using tetramethylsilane as an internal standard. Specific rotations were measured with a JASCO DIP-4 polarimeter. Optical purities were determined by high-performance liquid chromatographic

(HPLC) analysis using an ULTRON ES-OVM column (Shinwa Kako Co., Ltd., 4.6 mm × 15 cm). The 1-phenylethylamine enantiomers were purchased from Tokyo Kasei Kogyo Co., Ltd.

(±)-2-(1,3-Benzodioxol-4-yl)heptanenitrile (4) 1-Bromopentane (0.87 g, 5.8 mmol) and 50% NaOH (1.9 g) were added simultaneously to a solution of the nitrile **3**[16] (0.93 g, 5.8 mmol) in DMSO (10 ml) at 20 °C, and the mixture was stirred at room temperature for 30 min, diluted with H_2O (30 ml), and extracted with Et_2O. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (Et_2O : hexane = 1 : 20 as eluent) to give **4** (1.12 g, 84%) as a clear oil. IR (neat): 2957, 2930, 2863, 2243, 1460, 1252, 1061, 932 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.82—0.98 (3H, m), 1.28—1.38 (4H, m), 1.41—1.53 (2H, m), 1.82—1.97 (2H, m), 3.90 (1H, dd, J = 6.8, 8.2 Hz), 5.98—6.08 (2H, m), 6.77—6.88 (3H, m). High-resolution MS *m/z*: Calcd for $C_{14}H_{17}NO_2$: 231.1259. Found: 231.1214 (M^+).

(±)-2-(1,3-Benzodioxol-4-yl)heptylamine (5) A solution of **4** (1.01 g, 4.37 mmol) in dry Et_2O (5 ml) was added dropwise to a suspension of $LiAlH_4$ (0.25 g, 6.6 mmol) in dry Et_2O (10 ml), and the mixture was refluxed for 2 h. After the mixture had cooled, H_2O (0.25 ml), 15% NaOH (0.25 ml), and H_2O (0.75 ml) were added successively with stirring, and the precipitated solid was filtered off. The filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel (MeOH : CH_2Cl_2 = 1 : 19 as eluent) to give **5** (0.895 g, 87%) as a clear oil. IR (neat): 2940, 2870, 1450, 1250, 1055 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.80—0.92 (3H, m), 1.17—1.33 (8H, m), 1.57—1.66 (2H, m), 2.71—2.83 (1H, m), 2.90 (2H, d, J = 7.2 Hz), 5.92 (2H, s), 6.64—6.83 (3H, m). The oxalate of **5** was recrystallized from EtOH to give a sample of mp 147—148 °C. IR(KBr): 2957, 2930, 1636, 1458, 1252, 1059 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.70—0.88 (3H, m), 1.00—1.25 (6H, m), 1.44—1.75 (2H, m), 2.90—3.10 (3H, m), 5,97 (2H, s), 6.69—6.82 (3H, m). *Anal.* Calcd for $C_{16}H_{23}NO_6$: C, 59.07; H, 7.13; N, 4.30. Found. C, 58.72; H, 7.13; N, 4.15.

(±)-*N*-2-(1,3-Benzodioxol-4-yl)heptyl-*N'*-2,6-diisopropylphenylurea (2) A solution of **5** (634 mg, 2.69 mmol) in heptane–EtOAc (50, 5 ml) was treated with pure 2,6-diisopropylphenyl isocyanate (6)[17] (574 mg, 2.82 mmol), and the mixture was stirred overnight at room temperature. The precipitated solid was collected by filtration and recrystallized from heptane–EtOAc to give **2** (1.06 g, 90%) as colorless needles. mp 165—166 °C. IR (KBr): 3400, 3300, 2960, 2930, 1650, 1555, 1450, 1245 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, t, J = 6.5 Hz), 1.10 (12H, d, J = 6.9 Hz), 1.01—1.20 (6H, m), 1.53—1.61 (2H, m), 2.73—2.85 (1H, m), 3.16—3.32 (3H, m), 3.48—3.58 (1H, m), 4.21 (1H, br s), 5.60—5.68 (3H, m), 6.47 (1H, dd, J = 7.3, 1.6 Hz), 6.58—6.69 (2H, m), 7.13 (2H, d, J = 7.7 Hz), 7.30 (1H, t, J = 7.7 Hz). *Anal.* Calcd for $C_{27}H_{38}N_2O_3$: C, 73.94; H, 8.73; N, 6.39. Found: C, 74.01; H, 8.98; N, 6.30.

4-(2,2-Dibromovinyl)-1,3-benzodioxole (8) A solution of triphenylphosphine (747 g, 2.85 mol) in CH_2Cl_2 (2000 ml) was treated with CBr_4 (470 g, 1.42 mol) in portions at 5—15 °C, and then the aldehyde **7**[18] (203 g, 1.35 mol) in CH_2Cl_2 (440 ml) was added dropwise at 10—17 °C. The whole was stirred at 13—19 °C for 45 min, then triphenylphosphine (36.4 g, 139 mmol) and CBr_4 (23.2 g, 70 mmol) were added successively, and the reaction mixture was stirred for 1 h at room temperature. H_2O (1500 ml) was added dropwise to the cooled mixture, and the organic layer was separated, concentrated *in vacuo*, and diluted with EtOAc (2000 ml). It was allowed to stand overnight, then the deposited solid was removed by filtration, and the filtrate was concentrated *in vacuo*. EtOAc (880 ml) and hexane (880 ml) were added to the residue, and the deposited solid was removed by filtration. The filtrate was concentrated *in vacuo*, and hexane (1100 ml) was added to the residue. The insoluble solid was removed by filtration, and the filtrate was concentrated *in vacuo*. The residual oil was passed through a short silica gel (800 g) column (EtOAc : hexane = 1 : 10 as eluent) to give crude **8** (389 g) as a brown oil. ^{1}H-NMR ($CDCl_3$) δ: 5.96 (2H, s), 6.76—6.83 (2H, m), 7.25—7.49 (1H, m), 7.48 (1H, s). Compound **8** was used in the next step without further purification.

1,3-Benzodioxol-4-ylacetic Acid (9) A mixture of crude **8** (389 g) and 30% aqueous KOH (1240 g) in diethylene glycol (1590 ml) was refluxed for 13 h. The cooled reaction mixture was diluted with H_2O (2100 ml) and washed with toluene (2 × 800 ml). The aqueous layer was acidified with concentrated HCl and extracted with EtOAc (2 × 800 ml, 500 ml). The combined extracts were washed with H_2O (2 × 300 ml) and brine (300 ml), dried over anhydrous $MgSO_4$, treated with active carbon (10 g), and concentrated *in vacuo* to give crude **9** (192 g) as a yellow solid. A small amount of crude **9** was purified by column chromatography on silica gel (EtOAc : hexane = 1 : 1 as the eluent) followed by recrystallization from EtOAc–hexane to give pure **9**. mp 107—108 °C (lit.,[19] 103—104 °C).

Ethyl 1,3-Benzodixol-4-ylacetate (10) A solution of crude **9** (192 g) in EtOH (1200 ml) was treated dropwise with $SOCl_2$ (156 ml, 2.14 mol) with cooling, and the mixture was stirred at 50—60 °C for 1 h. After concentration *in vacuo*, the residual oil was mixed with H_2O (1000 ml), stirred for 20 min at 40 °C, cooled, and extracted with toluene (2 × 500 ml). The combined extracts were washed with saturated $NaHCO_3$ (400 ml) and brine (400 ml), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was distilled under reduced pressure to give **10** (192 g, 68% based on **7**) as a clear oil. bp 112—115 °C (0.4 mmHg). IR (neat): 2980, 2900, 1735, 1460, 1250, 1060 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7.1 Hz), 3.60 (2H, s), 4.17 (2H, q, J = 7.1 Hz), 5.95 (2H, s), 6.71—6.83 (3H, m). High-resolution MS *m/z*: Calcd for $C_{11}H_{12}O_4$: 208.0736. Found: 208.0724 (M^+).

(±)-Ethyl 2-(1,3-Benzodioxol-4-yl)heptanoate (11) A solution of **10** (192.3 g, 924 mmol) in dry *N*,*N*-dimethylformamide (DMF) (280 ml) was added dropwise to a suspension of NaH (60% in oil, 37.0 g, 925 mmol) in dry DMF (470 ml) over a period of 1 h at 2—8 °C, and the mixture was stirred for an additional 1 h. To the resulting clear solution was added 1-bromopentane (139.6 g, 924 mmol) over a period of 25 min at 15—25 °C, and the reaction mixture was stirred for 1 h at room temperature and poured into ice-water (2200 ml) containing concentrated HCl (5 ml). The mixture was extracted with toluene (1250, 400 ml), and the combined extracts were washed with saturated $NaHCO_3$ (560 ml), H_2O (2 × 750 ml), and concentrated *in vacuo* to give crude **11** (261 g) as a brown oil. An analytical sample was obtained by column chromatography on silica gel (Et_2O : hexane = 1 : 25 as the eluent) as a clear oil. IR (neat): 2975, 2950, 1735, 1460, 1250, 1185, 1060 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.83—0.88 (3H, m), 1.22 (3H, t, J = 7.1 Hz), 1.22—1.33 (6H, m), 1.74—1.83 (1H, m), 1.98—2.08 (1H, m), 3.72 (1H, t, J = 7.6 Hz), 4.03—4.21 (2H, m), 5.93—5.95 (2H, m), 6.70—6.83 (3H, m). High-resolution MS *m/z*: Calcd for $C_{16}H_{22}O_4$: 278.1518. Found 278.1504 (M^+).

(±)-2-(1,3-Benzodioxol-4-yl)heptanoic Acid (12) A mixture of crude **11** (261 g) and KOH (85% purity, 175 g, 2.65 mol) in EtOH (1100 ml) and H_2O (1550 ml) was stirred for 3 h at 55 °C, and then EtOH was removed by evaporation *in vacuo*. The aqueous mixture was washed with toluene (2 × 500 ml), acidified with HCl, and extracted with toluene (1300, 500 ml). The combined extracts were washed with H_2O (2 × 800 ml), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was dissolved in hot hexane (250 ml) and cooled to 0 °C. The deposited solid was collected by filtration, washed with cold hexane (125 ml), and dried to give **12** (191.0 g, 83% based on **10**). mp 58.5—60.5 °C. IR (KBr): 2970, 2940, 2875, 1705, 1460, 1260, 1065, 925 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.83—0.88 (3H, m) 1.20—1.35 (6H, m), 1.77—1.86 (1H, m), 1.99—2.10 (1H, m) 3.75 (1H, t, J = 7.7 Hz), 5.94—5.96 (2H, m), 6.70—6.83 (3H, m). *Anal.* Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 66.89; H, 7.29.

(*S*)-1-Phenylethylammonium (*R*)-2-(1,3-Benzodioxol-4-yl)heptanoate (14a) A mixture of **12** (130 g, 0.52 mol) and (*S*)-1-phenylethylamine (**13a**) (63.0 g, 0.52 mol) in 2-propanol (130 ml) and heptane (1170 ml) was heated to reflux, and gradually cooled to 15 °C with stirring. The deposited salt was collected by filtration, washed with heptane (130 ml), and dried. This salt was recrystallized three times from 2-propanol–heptane (1 : 9 (v/v), 1230, 1320, 1170 ml) to give **14a** (74.0 g, 38%) as colorless needles. mp 128—129 °C. $[\alpha]_D^{22}$ −33.9° (c = 1.19, MeOH). IR (KBr): 2940, 1565, 1455, 1375, 1365, 1250 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, t, J = 6.5 Hz), 1.13—1.28 (6H, m), 1.32 (3H, d, J = 6.8 Hz), 1.59—1.70 (1H, m), 1.79—1.89 (1H, m), 3.44 (1H, dd, J = 8.5, 6.9 Hz), 3.96 (1H, q, J = 6.8 Hz), 5.79—5.81 (2H, m), 6.06 (3H, br s), 6.63—6.72 (3H, m), 7.26 (5H, s). *Anal.* Calcd for $C_{22}H_{29}NO_4$: C, 71.13; H, 7.87; N, 3.77. Found: C, 70.88; H, 7.96; N, 3.76.

(*R*)-2-(1,3-Benzodioxol-4-yl)heptanoic Acid (12a) A suspension of **14a** (55.0 g, 148 mmol) in CH_2Cl_2 (150 ml) and H_2O (50 ml) was treated with 6 M HCl (40 ml), and the mixture was stirred and separated. The aqueous layer was extracted with CH_2Cl_2 (50 ml). The combined organic layers were washed with 1 M HCl (30 ml), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give **12a** (37.0 g, quantitatively) as a colorless solid. The optical purity was determined by chiral HPLC analysis to be 98.5% ee (20 mM KH_2PO_4 : MeOH = 65 : 35 as the eluent, 0.8 ml/min). Recrystallization from hexane gave an analytical sample as

colorless needles. mp 47—48 °C. $[\alpha]_D^{25}$ −64.5° (c=1.01, MeOH). IR (KBr): 3240, 2960, 2880, 1730, 1460, 1245, 1185, 1060 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.83—0.88 (3H, m), 1.20—1.35 (6H, m) 1.74—1.88 (1H, m), 1.99—2.10 (1H, m), 3.75 (1H, t, J=7.7 Hz), 5.94—5.96 (2H, m), 6.72—6.83 (3H, m). *Anal.* Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25; Found: C, 67.21; H, 7.36.

(*R*)-2-(1,3-Benzodioxol-4-yl)heptanamide (15a) Thionyl chloride (7.3 ml, 100 mmol) and DMF (0.12 ml) were added to a solution of **12a** (12.51 g, 50 mmol) in CH_2Cl_2 (50 ml), and the mixture was stirred for 2.5 h at room temperature and then concentrated *in vacuo* to give (*R*)-2-(1,3-benzodioxol-4-yl)heptanoyl chloride as an oil. This crude acid chloride (13.6 g) in CH_2Cl_2 (50 ml) was added dropwise to a stirred mixture of 28% NH_4OH (15 ml) and CH_2Cl_2 (30 ml) over a period of 20 min at 9—15 °C. The mixture was stirred for 1.5 h at room temperature and after addition of H_2O (25 ml), the organic layer was separated, washed with H_2O (2×25 ml), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give crude **15a** (12.45 g, quantitatively) as a white solid, which was pure enough to be used in the next step. Recrystallization from toluene gave an analytical sample as colorless flakes. mp 106—107 °C. $[\alpha]_D^{25}$ −53.1° (c=1.02, MeOH). IR (KBr): 3470, 3440, 3340, 3230, 2950, 2870, 1660, 1635, 1460, 1255, 1065 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.80—0.95 (3H, m), 1.10—1.35 (6H, m), 1.67—1.88 (1H, m), 2.06—2.20 (1H, m), 3.56 (1H, t, J=7.7 Hz), 5.66 (2H, br s), 5.95—5.98 (2H, m), 6.73—6.87 (3H, m). *Anal.* Calcd for $C_{14}H_{19}NO_3$: C, 67.45; H, 7.65; N, 5.62. Found: C, 67.55; H, 7.82; N, 5.57.

(*R*)-2-(1,3-Benzodioxol-4-yl)heptylamine (5a) A solution of **15a** (24.93 g, 100 mmol) and AcOH (28.6 ml, 500 mmol) in dioxane (125 ml) was added dropwise to a suspension of $NaBH_4$ (18.92 g, 500 mmol) in dioxane (250 ml) over a period of 1 h at 69—75 °C (caution! H_2 evolution), and the mixture was refluxed for 3 h. The cooled suspension was added dropwise to well-stirred cold 2.8 M HCl (325 ml) (caution! H_2 evolution). The clear solution was stirred at 60 °C for 1 h, cooled, washed with hexane (2×225 ml), made basic with 25% NaOH, and extracted with heptane (2×250 ml). The combined extracts were washed with brine (2×75 ml), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give crude **5a** (21.59 g) as a slightly brown oil. Purification by column chromatography on silica gel (MeOH : CH_2Cl_2 = 1 : 19 as the eluent) gave a clear oil. $[\alpha]_D^{25}$ −8.1° (c=1.13, MeOH). IR (neat): 2950, 2875, 1455, 1255, 1060, 775, 735 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.77—0.92 (3H, m), 1.17—1.26 (8H, m), 1.57—1.66 (2H, m), 2.71—2.85 (1H, m), 2.90 (2H, d, J=7.0 Hz), 5.92 (2H, s), 6.64—6.83 (3H, m). The oxalate of **5a** was recrystallized from EtOH to give colorless crystals. mp 140-142 °C. IR (KBr): 3067, 2955, 2930, 1616, 1458, 1254, 1059 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.73—0.88 (3H, m), 1.00—1.25 (6H, m), 1.47—1.75 (2H, m), 2.90—3.10 (3H, m), 5.98 (2H, s), 6.68—6.84 (3H, m). *Anal.* Calcd for $C_{16}H_{23}NO_6$: C, 59.07; H, 7.13; N, 4.30. Found: C, 58.96; H, 7.15, N, 4.21.

(*R*)-*N*-2-(1,3-Benzodioxol-4-yl)heptyl-*N'*-2,6-diisopropylphenylurea (2a) Pure **6** (17.3 g, 85.1 mmol) in heptane (70 ml) was added to a solution of crude **5a** (20.0 g) in heptane–EtOAc (1630, 170 ml), and the mixture was stirred at room temperature for 2 h. The deposited solid was collected by filtration, washed with heptane (125 ml), and recrystallized from heptane–EtOAc (1450, 145 ml) to give **2a** (28.75 g, 71% based on **15a**) as colorless needles. mp 157—158 °C. $[\alpha]_D^{25}$ +17.8° (c=1.03, MeOH). The optical purity was determined by chiral HPLC analysis to be 98.2% ee (MeOH : H_2O = 43.5 : 56.5 as eluent, 0.8 ml/min). IR (KBr): 3340, 2960, 2930, 2875, 1630, 1590, 1565, 1455, 1250, 1055 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, t, J=6.5 Hz), 1.10 (12H, d, J=6.9 Hz), 1.01—1.20 (6H, m), 1.53—1.66 (2H, m), 2.73—2.85 (1H, m), 3.16—3.32 (3H, m), 3.48—3.58 (1H, m), 4.21 (1H, br s), 5.61—5.64 (3H, m), 6.47 (1H, dd, J=7.3, 1.6 Hz), 6.58—6.69 (2H, m), 7.13 (2H, d, J=7.7 Hz), 7.30 (1H, t, J=7.7 Hz). *Anal.* Calcd for $C_{27}H_{38}N_2O_3$: C, 73.94; H, 8.73; N, 6.39. Found: C, 74.20; H, 9.03; N, 6.41.

(*S*)-*N*-2-(1,3-Benzodioxol-4-yl)heptyl-*N'*-2,6-diisopropylphenylurea (2b) The urea derivative **2b** was prepared by a procedure similar to that used for the preparation of **2a**, from (*S*)-2-(1,3-benzodioxol-4-yl) heptanoic acid (**12b**), which was obtained by the resolution of **12** using (*R*)-1-phenylethylamine (**13b**). mp 156—157 °C. $[\alpha]_D^{25}$ −17.9° (c=1.03, MeOH). The optical purity was 98.0% ee. IR and ^{1}H-NMR spectra were in consonance with those of **2a**. *Anal.* Calcd for $C_{27}H_{38}N_2O_3$: C, 73.94; H, 8.73; N, 6.39. Found: C, 74.22; H, 8.90; N, 6.29.

Preparation of Foamed Macrophages Murine peritoneal macrophages were obtained from unstimulated ddY mice (female) by peritoneal lavage with phosphate-buffered saline (PBS). The peritoneal fluid from 30—50 mice was pooled and the cells were collected by centrifugation (400 *g*, 10 min, 4 °C), washed once with Dulbecco's modified Eagle's medium (DMEM) and resuspended in DMEM containing 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 μg/ml). Aliquots (2×10^6 cells) of the cell suspension were dispensed in 12-well dishes (Costar Corp., Cambridge, MA) and incubated in a humidified incubator at 37 °C (5% CO_2). On the second day, each dish was extensively washed with DMEM without serum until there were no non-adherent cells visible under the microscope. Foamed macrophages were induced by the addition of DMEM containing 20 μg protein/ml of acetylated low-density lipoprotein (Ac-LDL) for 18—24 h.

Assay of ACAT Activity The activity of ACAT was determined by the formation of cholesteryl [^{14}C]oleate from [^{14}C]oleoyl-CoA and endogenous cholesterol. Liver and intestinal microsomes were prepared as described previously.[20] The standard ACAT assay started with a 5-min preincubation at 37 °C of a mixture containing 100 μg of microsomal protein, 1 mg/ml bovine serum albumin (BSA), and a graded concentration of a test compound (dissolved in DMSO). The reaction was initiated by the addition of 10 nmol of [^{14}C]oleoyl-CoA (1.85 kBq/ml, 1.96 kBq/μmol) in a final volume of 300 μl. After a 5-min incubation period, the reaction was stopped by the addition of chloroform–methanol (2 : 1 (v/v) 4 ml). The phases were separated by the addition of water (1 ml) and the lower phase was removed. Cholesteryl esters were then isolated by TLC for determination of total ^{14}C radioactivity by use of a Bioimage analyzer BAS 2000 (Fuji Film, Tokyo). Cell-derived ACAT activity was determined in terms of the [^{14}C]oleate (165 kBq/nmol) incorporation into cellular cholesteryl esters. Murine foamed cells were incubated for 1 h with a test compound dissolved in DMSO (final concentration 0.4%). Albumin-bound [^{14}C]oleate was then added and the cells were further incubated for 2 h. The cellular lipids were extracted with hexane–2-propanol (3 : 2 (v/v) 3 ml) after the culture medium was removed.

Efficacy in Cholesterol-Fed Animals Hypolipidemic activities of the compounds were tested in rats, hamsters, and rabbits. Male Wistar rats (Japan Laboratory Animals, Tokyo) weighing 140—160 g and male golden Syrian hamsters (Japan Laboratory Animals, Tokyo) weighing 80—100 g were fed with a 1% cholesterol–0.5% cholic acid diet for 8 d. The test compounds were administrated by daily oral gavage using aqueous Tween 80 (0.1%) suspensions. Control animals received vehicle alone by the appropriate route. On the 8th day, plasma cholesterol concentrations were determined by the emzymatic method using a clinical assay kit (Kyowa Medex, Tokyo, Determiner TC5). Efficacy was assessed in terms of the ability (dose) of the test compounds to reduce the plasma cholesterol level by 30% compared with the control (vehicle) level (ED_{30} value). In the case of rabbits, male New Zealand White rabbits (Japan SLC, Inc., Hamamatsu) weighing 2.0—3.0 kg were fed with a 0.5% cholesterol diet. After one week, the rabbits were bled and grouped on the basis of the concentration of plasma cholesterol. The test compounds were administrated to the grouped animals by daily oral gavage using aqueous Tween 80 (0.1%) suspensions for 9 d. On days 2, 4, 7, and 9, plasma cholesterol concentrations were determined by enzymatic procedures.

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