Structure-Activity Relationship of *N*-[2-(Dimethylamino)-6-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propoxy]phenyl]-*N'*-pentylurea and Analogues. Novel Potent Inhibitors of Acyl-CoA:Cholesterol *O*-Acyltransferase with Antiatherosclerotic Activity

Teiji Kimura,* Yasutaka Takase, Kenji Hayashi, Hiroshi Tanaka, Issei Ohtsuka, Takao Saeki, Motoji Kogushi, Toshie Yamada, Tohru Fujimori, Isao Saitou, and Kozo Akasaka

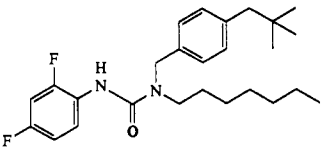
Tsukuba Research Laboratories, Eisai Company, Ltd., 5-1-3 Tokodai, Tsukuba-shi, Ibaraki 300-26, Japan

Received April 30, 1992

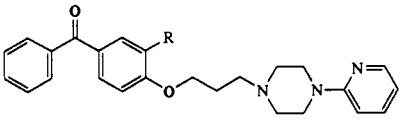
We have discovered *N*-butyl-*N'*-[2-(dimethylamino)-6-[3-(4-phenyl-1*H*-imidazol-1-yl)propoxy]-phenyl]urea (**4**), a novel, potent, and systemically bioavailable inhibitor of ACAT (acylCoA:cholesterol *O*-acyltransferase). The structure-activity relationships (SARs) of this lead compound **4** were investigated by systematic modification of four regions in the molecule. The compounds prepared in this study were tested for in vitro inhibitory activity toward both aortic and intestinal ACATs, and selected compounds were further tested for in vivo hypocholesterolemic activity. The studies not only resulted in the discovery of *N*-[2-(dimethylamino)-6-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propoxy]phenyl]-*N'*-pentylurea (**24**), with potent activity and moderate plasma level after oral administration, but also revealed the SAR in each modified region. Four compounds (**4**, **13**, **14**, **24**) were further selected for testing of in vivo antiatherosclerotic activity; **4**, **13**, and **24** reduced atherosclerotic plaque development to 38-45% of the control value in terms of area, while **14** did not have a significant antiatherosclerotic effect.

The entry of cholesterol into cells by receptor-mediated endocytosis of plasma lipoprotein and the subsequent intracellular fate of free and esterified cholesterol have received much attention from researchers interested in cholesterol metabolism and its role in pathological conditions such as coronary heart disease.[1] AcylCoA:cholesterol *O*-acyltransferase (ACAT, EC 2.3.1.26) is one of the enzymes responsible for catalyzing the formation of cholesteryl ester.[2-4] This enzyme plays an important role in the intestinal absorption of dietary cholesterol, because ACAT activity is greatest in the jejunum, where the majority of cholesterol absorption occurs,[3,5] and in vitro and ex vivo inhibition of ACAT activity is correlated with the inhibition of cholesterol absorption, at least in rats[6] and rabbits.[7] In addition, studies both in cultured cells[8-12] and in arterial tissue[13-15] have suggested that ACAT activity is regulated and increased when cells are exposed to cholesterol-rich media. Since the intracellular accumulation of esterified cholesterol is one of the characteristic features of the atherosclerotic plaque, the regulation of ACAT is likely to be of great importance for the treatment of atherosclerosis.[16]

Several ACAT inhibitors have been shown to be effective in treating hypercholesterolemia[7,17-24] and atherosclerotic plaque formation. The latter was assessed by measuring aortic cholesterol content and lesion severity[18,21,25,26] in animal models during cholesterol feeding. Recently, it has been reported that a systemically bioavailable potent ACAT inhibitor prevented the accumulation of monocytes-macrophages, reduced the foam cell area, and blunted the development of thoracic aortic fatty streak-like lesions even in the absence of a plasma cholesterol lowering effect.[27] These results suggest that inhibition of ACAT in the arterial wall may well be considered a true antiatherosclerotic therapy. A nonabsorbable ACAT inhibitor (one that is not detected in plasma after oral administration) may retard the progression of atherosclerosis only by reducing serum cholesterol levels. However, a systemically bioavailable ACAT inhibitor may also delay or impede atherosclerosis by preventing the accumulation of esterified cholesterol in arterial tissue as well as by reducing serum cholesterol levels. We therefore searched for novel, potent, and systemically bioavailable ACAT inhibitors. Our screening program identified the benzophenone derivatives **2** and **3** as weak inhibitors of



1. CL-277082

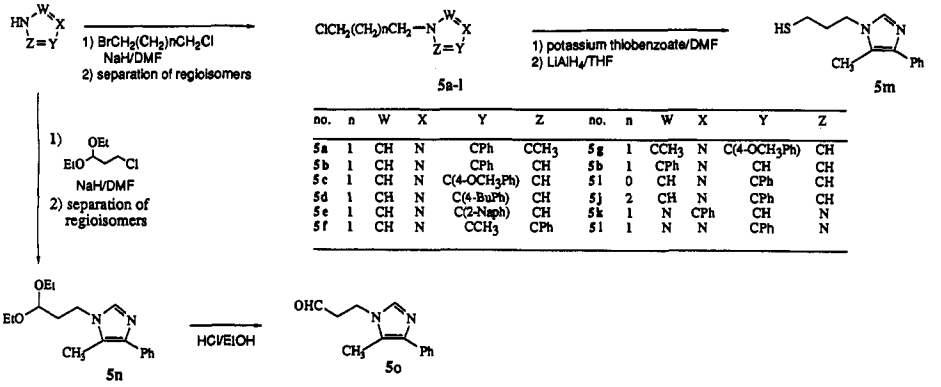


2. R= NO_2
3. R= $NHCOC_4H_9$

ACAT activity.[28] We then prepared a wide variety of derivatives of compounds **2** and **3** and evaluated their in vitro activity.[29] This led to the selection of *N*-butyl-*N'*-[2-(dimethylamino)-6-[3-(4-phenyl-1*H*-imidazol-1-yl)propoxy]phenyl]urea (**4**) as an example of a novel, potent, and systemically bioavailable inhibitor of ACAT.[30] The aim of the present studies was to search for analogues more potent than **4** in terms of in vitro ACAT inhibitory activity, in vivo hypocholesterolemic effect, and antiatherosclerotic effect, as well as to establish the SAR of **4** for in vitro ACAT inhibitory activity and in vivo hypocholesterolemic effect in four regions of the molecule. The results obtained, which led to the discovery of a candidate drug with potent hypocholesterolemic and antiatherosclerotic activities, are presented herein.

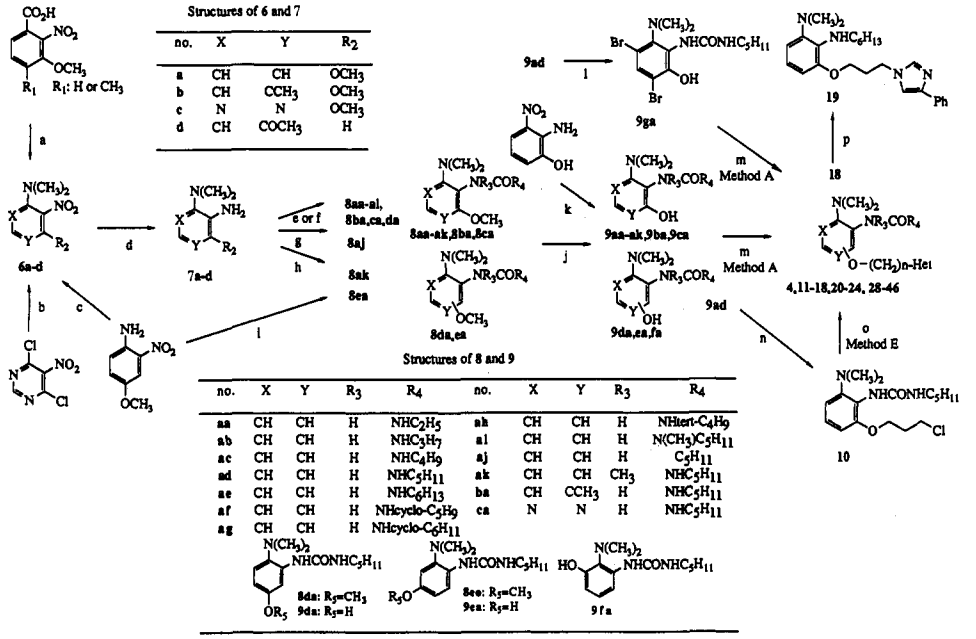
0022-2623/93/1836-1630$04.00/0

Scheme I. Preparation of Heterocycle Intermediates



no.	n	W	X	Y	Z	no.	n	W	X	Y	Z
5a	1	CH	N	CPh	CCH_3	5g	1	CCH_3	N	C(4-OCH_3Ph)	CH
5b	1	CH	N	CPh	CH	5h	1	CPh	N	CH	CH
5c	1	CH	N	C(4-OCH_3Ph)	CH	5i	0	CH	N	CPh	CH
5d	1	CH	N	C(4-BuPh)	CH	5j	2	CH	N	CPh	CH
5e	1	CH	N	C(2-Naph)	CH	5k	1	N	CPh	CH	N
5f	1	CH	N	CCH_3	CPh	5l	1	N	N	CPh	N

Scheme II. Preparation of Target Molecules by Method A and Method E[a]



no.	X	Y	R_2
a	CH	CH	OCH_3
b	CH	CCH_3	OCH_3
c	N	N	OCH_3
d	CH	$COCH_3$	H

no.	X	Y	R_3	R_4	no.	X	Y	R_3	R_4
aa	CH	CH	H	NHC_2H_5	ah	CH	CH	H	NHtert-C_4H_9
ab	CH	CH	H	NHC_3H_7	al	CH	CH	H	$N(CH_3)C_5H_{11}$
ac	CH	CH	H	NHC_4H_9	aj	CH	CH	H	C_5H_{11}
ad	CH	CH	H	NHC_5H_{11}	ak	CH	CH	CH_3	NHC_5H_{11}
ae	CH	CH	H	NHC_6H_{13}	ba	CH	CCH_3	H	NHC_5H_{11}
af	CH	CH	H	NHcyclo-C_5H_9	ca	N	N	H	NHC_5H_{11}
ag	CH	CH	H	NHcyclo-C_6H_{11}					

[a] Reagents: (a) (1) $SOCl_2$, (2) NaN_3/acetone–H_2O, (3) Δ/benzene, (4) NaOH, (5) HCl, (6) MeI, K_2CO_3/DMF; (b) (1) NaOMe/DMF, (2) $HNMe_2$/DMF; (c) MeI, K_2CO_3/DMF; (d) H_2, Pd–C/EtOAc; (e) alkyl isocyanate/$CHCl_3$; (f) (1) phenyl chloroformate/pyridine, (2) alkylamine/toluene; (g) caproyl chloride, Et_3N/$CHCl_3$; (h) (1) HCO_2H, (2) MeI, K_2CO_3/DMF, (3) HCl/1,4-dioxane, (4) pentyl isocyanate/$CHCl_3$; (i) (1) HCO_2H, (2) BzlBr, NaH/DMF, (3) H_2, Pd–C/EtOAc, (4) MeI, K_2CO_3/DMF, (5) HCl/1,4-dioxane, (6) H_2, $Pd(OH)_2$–C/EtOH, (7) pentyl isocyanate/$CHCl_3$; (j) BBr_3/CH_2Cl_2; (k) (1) MOMCl, K_2CO_3/DMF, (2) MeI, K_2CO_3/DMF, (3) H_2, Pd–C/EtOAc, (4) pentyl isocyanate/$CHCl_3$, (5) HCl/THF; (l) Br_2/$CHCl_3$; (m) **5a–l**, K_2CO_3, NaI/DMF; (n) 1-bromo-3-chloropropane, K_2CO_3/DMF; (o) Het-H, K_2CO_3, NaI/DMF; (p) $LiAlH_4$/THF.

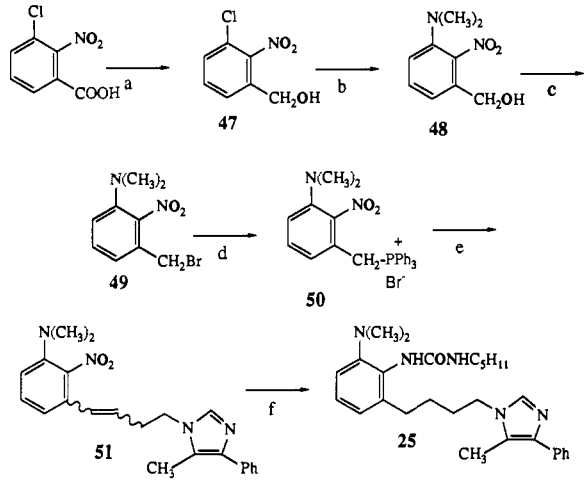
Chemistry

The chloroalkylated heterocycles required for these syntheses were prepared as illustrated in Scheme I. Alkylation of arylated imidazoles,[31] 4-phenyl-1,2,3-triazole,[32] and 5-phenyltetrazole[33] with 1-bromo-ω-chloroalkanes in the presence of NaH provided a mixture of regioisomers, which were separated by flash column chromatography. The regioisomers of imidazole derivatives were identified from ^{1}H-NMR spectral data. In $CDCl_3$, the $ClCH_2CH_2CH_2$ protons of the 5-phenyl isomer **5f** were shifted upfield, relative to the corresponding protons of the 4-phenyl isomer **5a**, by the shielding effect of the benzene ring.[34,35] 1,2,3-Triazole (**5k**) and tetrazole (**5l**) derivatives were structurally identified by comparison of the spectral data with literature values for analogous compounds.[36,37] Treatment of **5a** with sodium thiobenzoate followed by reductive deprotection with $LiAlH_4$ afforded 5-methyl-4-phenyl-1-(3-propylthio)imidazole (**5m**). Alkylation of 5-methyl-4-phenylimidazole with 3-chloropropionaldehyde diethyl acetal and subsequent separation of the regioisomers afforded the intermediate **5n**. Deprotection of **5n** with hydrochloric acid gave 3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propionaldehyde (**5o**).[38]

Scheme II shows the preparation of the target molecules by methods A and E. Synthesis of nitroanisole derivatives **6a–d** was accomplished by the usual methods. In brief, 3-(dimethylamino)-2-nitroanisole (**6a**) was prepared from 3-methoxy-2-nitrobenzoic acid by Curtius rearrangement, hydrolysis of isocyanate to amine, and methylation. By the same method, 3-(dimethylamino)-6-methyl-2-nitroanisole (**6b**) was prepared from 3-methoxy-4-methyl-2-nitrobenzoic acid. The pyrimidine derivative **6c** was obtained from 4,6-dichloro-5-nitropyrimidine by sequential treatment with sodium methoxide and dimethylamine. 4-(Dimethylamino)-3-nitroanisole (**6d**) was prepared from 4-methoxy-2-nitroaniline by methylation. Aniline derivatives **7a–d** were obtained from the corresponding nitroanisoles **6a–d** by catalytic hydrogenation.

Treatment of **7a–d** with either isocyanate or phenyl chloroformate followed by reaction with alkylamines gave urea derivatives **8aa–ai**, **8ba**, **8ca**, and **8da**. Acylation of **7a** with caproyl chloride gave the amide derivative **8aj**.

Scheme III. Preparation of **25** by Method B[a]



[a] Reagents: (a) B_2H_6/THF; (b) $HNMe_2$/DMF; (c) PBr_3/DMF; (d) PPh_3/toluene; (e) **5o**, EtONa/EtOH; (f) (1) H_2, Pd–C/EtOAc, (2) pentyl isocyanate/$CHCl_3$.

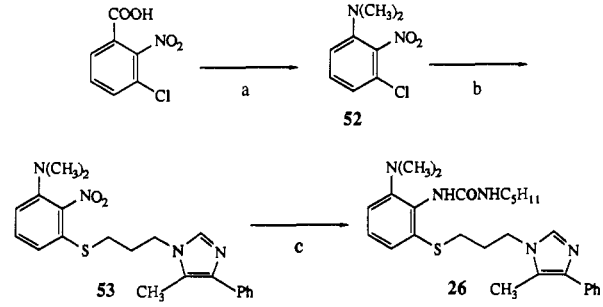
The *N*-methyl-*N*-phenyl-*N'*-pentylurea derivative **8ak** was afforded from **7a** by formylation, methylation, deprotection of the formyl group, and treatment with pentyl isocyanate. The other (methoxyphenyl)urea (**8ea**) was prepared from 4-methoxy-2-nitroaniline by formylation, benzylation, catalytic hydrogenation of the nitro group, methylation, deprotection, and reaction with pentyl isocyanate. Demethylation of the anisole derivatives **8aa–ak**, **8ba**, **8ca**, **8da**, and **8ea** was accomplished with BBr_3 to give the corresponding phenol derivatives **9aa–ak**, **9ba**, **9ca**, **9da**, and **9ea**. The other phenol derivative (**9fa**) was prepared from 2-amino-3-nitrophenol by *O*-methoxymethylation, *N*-dimethylation, catalytic hydrogenation, reaction with pentyl isocyanate, and deprotection. The 4,6-dibromo derivative **9ga** was afforded by treatment of **9ad** with bromine.

The preparation of compounds by method A was accomplished by reaction of the above phenol derivatives **9aa–ak**, **9ba**, **9ca**, **9da**, **9ea**, **9fa**, and **9ga** with chloroalkylated heterocycles **5a–l** in the presence of potassium carbonate. The amine analogue **19** was prepared from compound **18** by reduction with $LiAlH_4$.

Preparation by method E was accomplished by treatment of **10**, which was afforded by chloropropylation of 3-(dimethylamino)-2-(*N'*-pentylureido)phenol (**9ad**), with various heterocycles in the presence of potassium carbonate. In the case of the benzotriazole analogue **33**, the position of the substituent was assigned on the basis of the ^{1}H-NMR spectral data, which showed a symmetrical pattern of the protons of the benzotriazole moiety.

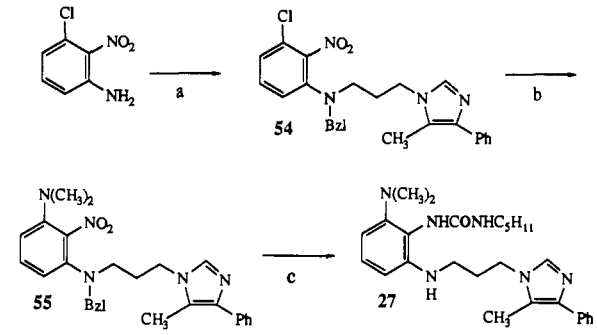
In order to examine the effect of oxygen in the bridging portion of **24**, compounds **25–27**, listed in Table III, were prepared as illustrated in Schemes III–V. Scheme III shows the preparation of the carbon analogue **25** by method B. Reduction of 3-chloro-2-nitrobenzoic acid with diborane gave the benzyl alcohol derivative **47**, which was heated with dimethylamine in an autoclave to provide 3-(dimethylamino)-2-nitrobenzyl alcohol (**48**). Bromination of **48** with phosphorus tribromide gave the benzyl bromide derivative **49**. Formation of the phosphonium salt **50** was accomplished by reaction of **49** with triphenylphosphine. The Wittig reaction of **50** with 3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propionaldehyde (**5o**) in the presence of sodium ethoxide in EtOH afforded the olefin derivative **51** as a cis–trans mixture. Catalytic hydrogenation of **51** and subsequent treatment with pentyl isocyanate led to the carbon analogue **25**.

Scheme IV. Preparation of **26** by Method C[a]



[a] Reagents: (a) (1) $SOCl_2$, (2) NaN_3/acetone–H_2O, (3) NaOH, (4) HCl, (5) MeI, K_2CO_3/DMF; (b) *t*-BuOK, **5m**/DMF; (c) (1) H_2, Pd–C/EtOAc, (2) pentyl isocyanate/$CHCl_3$.

Scheme V. Preparation of **27** by Method D[a]



[a] Reagents: (a) (1) HCO_2H, (2) K_2CO_3, NaI, **5a**/DMF, (3) HCl/EtOH, (4) BzlBr, NaH/DMF; (b) $HNMe_2$/DMF; (c) (1) $SnCl_2$/HCl, (2) pentyl isocyanate/$CHCl_3$, (3) H_2, $Pd(OH)_2$–C/EtOH.

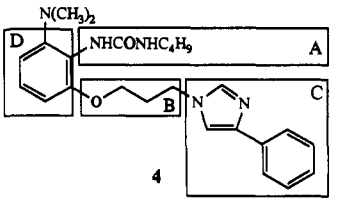
Scheme IV shows the preparation of the sulfur analogue **26** by method C. The Curtius rearrangement of 3-chloro-2-nitrobenzoic acid, hydrolysis of the isocyanate to amine, and methylation afforded the dimethylamine derivative **52**. Treatment of **52** with 5-methyl-4-phenyl-1-(3-propylthio)imidazole (**5m**) in the presence of potassium butoxide afforded the intermediate **53**. The desired analogue **26** was obtained by catalytic hydrogenation of **53** and subsequent treatment with pentyl isocyanate.

Scheme V shows the preparation of the nitrogen analogue **27** by method D. Formylation of 3-chloro-2-nitroaniline with formic acid, alkylation with 1-(3-chloropropyl)-5-methyl-4-phenylimidazole (**5a**) in the presence of NaH, deprotection with 36% hydrochloric acid in THF, and benzylation with benzyl bromide gave the intermediate **54**. The intermediate **54** was heated with dimethylamine in an autoclave to provide the dimethylamine derivative **55**. The nitrogen analogue **27** was obtained by reduction of **55** with $SnCl_2$, treatment with pentyl isocyanate, and hydrogenolysis of the benzyl group.

Biological Results and Structure–Activity Relationship

We wished to find a compound able to act on both intestinal and aortic ACATs to prevent cholesterol absorption in the intestine and the accumulation of cholesteryl esters in arterial wall. Since Lange et al.[39] had suggested the possibility of the existence of isozymes of ACAT, the synthesized compounds were evaluated for in vitro ACAT inhibitory activity according to the method of Heider et al.[7] using two enzyme sources, rabbit aorta homogenate and rabbit small intestine microsomes. The potency of activity was expressed as the micromolar

Chart I. Four Structural Regions of Lead Compound 4 Examined in SAR Studies



4

concentration of a compound required to inhibit enzyme activity by 50% (IC_{50}).

Selected analogues were then tested for in vivo hypocholesterolemic activity. In vivo serum cholesterol lowering activity was assessed in male Sprague–Dawley rats (5-weeks-old) fed a hypercholesterolemic diet along with the compound administered orally once a day for 2 or 5 days. Effect was expressed as a percentage of the control value. For the compounds listed in Table VII, ED_{30} values, the mg/kg dose of a compound required to decrease serum cholesterol level to 30% of the control value, were obtained.

To study structure–activity relationships, we divided the parent compound 4 into four regions, as illustrated in Chart I: (A) urea moiety, (B) bridging portion, (C) heterocycle moiety, (D) phenylurea nucleus. In region A, we examined the optimal size of the alkyl group. The results are shown in Table I. When the linear alkyl groups (4 and 11–14) were compared, lengthening the alkyl chain increased in vitro activities. The hexylurea derivative 14 showed the most potent in vitro aortic activity, while the pentylurea compound 13 showed the most potent in vitro intestinal activity. Cyclic and branched analogues 15–17 were also evaluated. The in vitro activity was decreased compared with linear analogues. The in vivo hypocholesterolemic activity was increased in the order *tert*-butyl (17) < cyclopentyl (16) < *n*-butyl (4) < cyclohexyl (15) < *n*-pentyl (13) < *n*-hexyl (14). The discrepancy between in vitro intestinal inhibitory activity and in vivo hypocholesterolemic effect (13 > 14 for in vitro intestinal inhibition and 13 < 14 for in vivo hypocholesterolemic activity) might be due not only to the difference in species employed (rabbit enzyme in vitro and rat in vivo) but also to many other factors, including absorption and metabolism. The order of in vivo activity correlated with the *L* lengths of STERIMOL parameters[40] for alkyl groups of 4 and 13–17. Although this correlation indicates that longer alkyl analogues may have more potent in vivo hypocholesterolemic activity, we also found in a preliminary experiment[30] that lengthening the alkyl chain of 4, 13, and 14 reduced the concentration of the compounds in plasma after oral administration; in particular, the plasma level of 14 was much lower than those of 4 and 13. Since our aim was to explore compounds which directly inhibited arterial ACAT after oral administration, we discontinued studies on compounds with alkyl chains longer than hexyl (14), which gave poor plasma levels. Replacement of the urea group (13) with an amide group (18) resulted in almost unchanged in vitro aortic activity, while the in vitro intestinal activity of 18 was about one-seventh as potent as that of 13, and 18 lacked in vivo hypocholesterolemic activity. When an amine group (19) was used, the activity nearly disappeared. Introduction of a methyl group on the nitrogen atom of the urea group (20 and 21) reduced the activity. The in vitro activity of the *N*-methyl isomer 20 was lower than that of the *N'*-methyl isomer 21.

In order to examine the effect of length and heteroatom in the bridging portion between the phenylimidazole and phenylurea moieties, the compounds listed in Tables II and III were prepared. When the length of the bridging portion (13, 22, and 23) was varied, a propoxy group (13) was found to give the greatest activity. The in vitro intestinal activity of the ethoxy compound 22 was equal to that of the butoxy compound 23, while the in vitro aortic activity of 22 was half that of 23. When oxygen (24) was replaced with carbon (25), sulfur (26), and nitrogen (27), aortic activity was decreased in the order sulfur (26) > oxygen (24) > carbon (25) > nitrogen (27), and intestinal activity was decreased in the order oxygen (24) > sulfur (26) > carbon (25) > nitrogen (27). The in vivo hypocholesterolemic effect of the oxygen analogue 24 was more potent than that of the carbon analogue 25, correlating with the in vitro intestinal activities. Although the activity of the sulfur analogue was equal to that of the oxygen analogue, on the basis of the ease of synthesis, we selected the oxygen analogue.

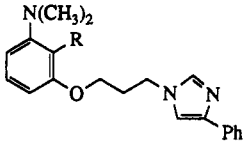
Next, the heterocyclic ring moiety was examined, since variation of the heterocycle may lead to a change in molecular properties such as basicity or hydrophilicity, and such a change of molecular properties may affect absorption or distribution, which is expected to influence in vivo activity. The results are shown in Table IV.

Although replacement of the imidazole (13) with 1,2,3-triazole (28), tetrazole (29), or piperazine (30) left the in vitro activity almost unchanged, it strongly increased the in vivo hypocholesterolemic activity. Piperidine (31) reduced the in vitro activity, while the in vivo activity was maintained. Phenethylamine (32), which is a ring-opened analogue of a heterocycle, reduced both in vitro and in vivo activities. Replacement of phenylated heterocycles (13 and 28) with benzo analogues (33 and 34) reduced the potency. Although compounds 28–31 were all potent in vivo, they showed lower plasma concentrations after oral administration compared with 13 in a preliminary pharmacokinetic study.[30] For this reason, the imidazole analogue was selected for further deviation.

Introduction of a substituent on the phenyl group of the phenylimidazole moiety (36 and 37) or replacement of the phenyl group with a naphthyl group (38) decreased in vitro aortic activity, but increased in vivo hypocholesterolemic activity. In particular, compound 37, which is a more lipophilic compound, possessed more potent in vivo hypocholesterolemic activity than 13. However, 37 was not detectable in plasma after oral administration in the preliminary experiment.[30] As regards substitution position, the 2-phenylimidazole analogue 35 was less potent than the 4-phenylimidazole analogue (13). Introduction of a methyl group at the 2-position (39) of the imidazole ring of 36 reduced in vivo hypocholesterolemic activity. Introduction at the 5-position (24) of the imidazole ring of 13 strongly increased in vivo hypocholesterolemic activity. When 24 was compared with the regioisomer 40, 24 had slightly more potent activity than 40.

Effects of substituents in the phenylurea nucleus (D region) are shown in Table V. Introduction of substituents (41 and 42) into the phenylurea nucleus and replacement of phenyl with pyrimidine (43) decreased the activity. To examine the effect of the location of the propoxy phenylimidazole moiety on the phenylurea nucleus, 44–46 were synthesized (Table VI). The 3-position derivative (24) was the most potent and the activity decreased in the

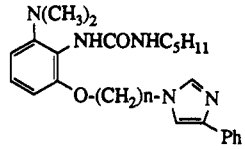
Table I. Physical Data and Biological Activities of *N*-Alkyl-*N'*-[2-(dimethylamino)-6-[3-(4-phenyl-1*H*-imidazol-1-yl)propoxy]phenyl]ureas, Amide, and Amine (Variable A Region)



no.	R	yield[a] (method)	mp (°C)	formula[b]	ACAT inhibn aorta[c]	ACAT inhibn intestine[d]	cholesterol lowering[e]
1	CL-277082				0.90	0.18	
11	$NHCONHC_2H_5$	53 (A)	115–116	$C_{23}H_{29}N_5O_2$	>10	0.87	+2
12	$NHCONHC_3H_7$	51 (A)	121–123	$C_{24}H_{31}N_5O_2$	7.23	0.66	NT[f]
4	$NHCONHC_4H_9$	36 (A)	98–100	$C_{25}H_{33}N_5O_2$	0.81	0.14	−37
13	$NHCONHC_5H_{11}$	51 (A)	110–111	$C_{26}H_{35}N_5O_2$	0.16	0.031	−51
14	$NHCONHC_6H_{13}$	48 (A)	124–126	$C_{27}H_{37}N_5O_2$	0.050	0.060	−57
15	NHCONHcyclo-C_6H_{11}	40 (A)	129–131	$C_{27}H_{35}N_5O_2$	1.07	0.073	−42
16	NHCONHcyclo-C_5H_9	55 (A)	112–113	$C_{26}H_{33}N_5O_2$	3.53	0.24	−24
17	NHCONHtert-C_4H_9	51 (A)	127–128	$C_{25}H_{33}N_5O_2$	0.99	0.13	−12
18	$NHCOC_5H_{11}$	34 (A)	*g*	$C_{26}H_{34}N_4O_2$[h]	0.10	0.20	+3
19	NHC_6H_{13}	91[i]	*g*	$C_{27}H_{36}N_4O$[h]	3.3	11	NT[f]
20	$N(CH_3)CONHC_5H_{11}$	33 (A)	108–109	$C_{27}H_{37}N_5O_2$	0.40	0.93	NT[f]
21	$NHCON(CH_3)C_5H_{11}$	19 (A)	90–92	$C_{27}H_{37}N_5O_2$	0.28	0.55	NT[f]

[a] Yield (%) of final step. [b] Satisfactory elemental analyses were obtained for C, H, N unless otherwise indicated. [c] IC_{50} (μM) for the enzyme derived from rabbit aorta homogenate. [d] IC_{50} (μM) for the enzyme derived from rabbit intestine microsomes. [e] Compounds were administered to rats at 50 mg/kg once a day for 2 days. Cholesterol lowering activity expressed as percentage of control value ($n \geq 4$). [f] Not tested. [g] Compound obtained as an oil. [h] Elemental analysis was not performed. Spectral data were consistent with the indicated structure. See Experimental Section. [i] Compound 19 was prepared from 18 by reduction with $LiAlH_4$ as illustrated in Scheme II.

Table II. Physical Data and Biological Activities of *N*-[2-(Dimethylamino)-6-(4-phenyl-1*H*-imidazol-1-yl)-alkoxy]phenyl]-*N'*-pentylureas (Variable B Region)



no.	*n*	yield[a] (method)	mp (°C)	formula[b]	ACAT inhibn aorta[c]	ACAT inhibn intestine[d]
22	2	48 (A)	138–140	$C_{25}H_{33}N_5O_2$	1.4	0.17
13	3	51 (A)	110–111	$C_{26}H_{35}N_5O_2$	0.16	0.031
23	4	51 (A)	142–144	$C_{27}H_{37}N_5O_2$	0.78	0.16

[a–d] See corresponding footnotes of Table I.

order 3-position (**24**) > 4-position (**44**) > 5-position (**45**) > 6-position (**46**).

On the basis of in vitro ACAT inhibitory activity, in vivo hypocholesterolemic activity, and the results of the preliminary pharmacokinetic study, the lead compound **4** and the more potent derivatives **13**, **14**, and **24** were selected for in vivo antiatherosclerotic testing. The in vitro ACAT inhibitory activity and in vivo hypocholesterolemic activity of these compounds are summarized in Table VII. In vivo antiatherosclerotic activity (Table VIII) was evaluated in New Zealand white rabbits (NZW) fed an atherogenic diet for 12 weeks by mixing each compound with the diet for the last 4 weeks. The antiatherosclerotic effect was assessed from the development of fatty streaks (surface involvement) and the cholesterol content in the aortic wall. The plasma cholesterol level after administration of **4**, **13**, **14**, and **24** was decreased by 51%, 66%, 62%, and 67%, respectively. Compounds **4**, **13**, **14**, and **24** diminished the surface involvement (in terms of area) by 38%, 40%, 20%, and 45%, respectively, and decreased cholesterol content by 59%, 56%, 23%, and 68%, respectively. Compounds **4**, **13**, and **24** exhibited significant antiatherosclerotic effect, while **14** did not. Although the reason for the poor antiatherosclerotic effect of **14** is not clear, we observed a reduction in food consumption and body weight gain in **14**-treated rabbits. These changes may reflect some toxic effect of **14** and could have contributed to its poor antiatherosclerotic effect.

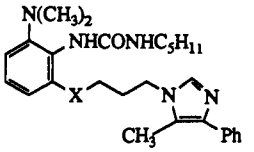
Conclusion

To develop a novel, potent, and systemically bioavailable ACAT inhibitor, many analogues of compound **4** were synthesized and evaluated as ACAT inhibitors. Structure–activity relationship studies (see Chart I) revealed the following features: (i) In the A region of the molecule, a urea binding pattern was desirable, and introduction of substituents on the nitrogen atom of the urea group reduced the activity. Alkyl groups longer than butyl were necessary for potent activity. It was also found that in vivo hypocholesterolemic activity correlated with the *L* length of STERIMOL parameters of alkyl groups. (ii) In the B region, the *n*-propoxy moiety was optimal in length and the oxygen atom could be replaced with carbon or sulfur. (iii) In the C region, 4-phenylimidazole, 4-phenyl-1,2,3-triazole, 5-phenyltetrazole, and 4-phenylpiperaine had potent activity. Among the imidazole derivatives, introduction of a substituent at the para position on the phenyl ring or substitution with a methyl group at the 5-position of the imidazole ring of the 4-phenylimidazole moiety resulted in enhanced in vivo hypocholesterolemic activity. (iv) In the D region, 2,6-disubstituted phenylurea was the optimal nucleus. Introduction of substituents into the phenyl nucleus and replacement of phenyl with pyrimidine decreased the activity. On the basis of these SARs and the biological test data, compound **24** was identified as a potent and orally bioavailable inhibitor of ACAT with hypocholesterolemic and antiatherosclerotic activity and was selected as a candidate for further preclinical studies.

Experimental Section

The purity of each product was checked by thin-layer chromatography (TLC) on silica gel plates (Kieselgel 60 F_{254}, thickness 0.25 mm). Column chromatography was performed

Table III. Physical Data and Biological Activities of *N*-[2-(Dimethylamino)-6-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)heteropropyl]phenyl]-*N'*-pentylureas (Variable B Region)



no.	X	yield[a] (method)	mp (°C)	formula[b]	ACAT inhibn: aorta[c]	ACAT inhibn: intestine[d]	cholesterol lowering[e]
24	O	69 (A)	128–130	$C_{27}H_{37}N_5O_2$	0.11	0.043	−49
25	CH_2	57 (B)	117–119	$C_{28}H_{39}N_5O$	0.14	0.17	−37
26	S	44 (C)	*g*	$C_{27}H_{37}N_5OS$[h]	0.058	0.056	NT[f]
27	NH	28 (D)	*g*	$C_{27}H_{38}N_6O$[h]	2.2	0.47	NT[f]

[a–d–f–h] See corresponding footnotes of Table I. [e] Compounds were administered to rats at 10 mg/kg once a day for 5 days. Cholesterol lowering activity expressed as percentage of control value ($n \geq 4$).

on silica gel (Merck; particle size 0.063–0.200 mm for normal chromatography and 15 μm for flash chromatography). All melting points (mp) were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured on a JEOL JNM-FX90Q (90 MHz) instrument. Chemical shifts are reported in δ units from tetramethylsilane as an internal standard; coupling constants (*J*) are reported in hertz. Mass spectra were obtained on a JEOL HX100 mass spectrometer and data are tabulated as *m/e*. Elemental analyses were performed at the Analytical Chemistry Section of Eisai Tsukuba Research Laboratories.

1-(3-Chloropropyl)-5-methyl-4-phenylimidazole (5a). To a suspension of NaH (55% oil suspension, 50.0 g, 1.14 mol) in DMF was added a solution of 5-methyl-4-phenylimidazole (150 g, 0.95 mol) in DMF at 40–50 °C. The mixture was stirred at 50 °C for 1 h and then added dropwise to a solution of 1-bromo-3-chloropropane in DMF at 50–60 °C over 1 h. The reaction mixture was stirred at 60 °C for 3 h, poured into water, and extracted with EtOAc. The organic layer was dried over $MgSO_4$, and evaporated to remove the solvent. The residue was chromatographed with benzene–acetone (3:1) to give 121 g (54%) of **5a** as an oil: ^{1}H NMR ($CDCl_3$) δ 2.16 (2H, quintet, J = 5.6), 2.20 (3H, s), 3.50 (2H, t, J = 5.6), 4.08 (2H, t, J = 5.6), 7.08–7.69 (6H, m).

1-(3-Chloropropyl)-4-methyl-5-phenylimidazole (5f): ^{1}H NMR ($CDCl_3$) δ 1.88 (2H, quintet, J = 5.6), 2.20 (3H, s), 3.35 (2H, t, J = 5.6), 4.07 (2H, t, J = 5.6), 7.23–7.52 (6H, m).

5-Methyl-4-phenyl-1-(3-propylthio)imidazole (5m). A solution of 1-(3-chloropropyl)-5-methyl-4-phenylimidazole (**5a**, 5.00 g, 21.3 mmol) and potassium thiobenzoate (3.75 g, 21.3 mmol) in DMF was stirred at 60 °C for 1 h. The mixture was poured into water and extracted with EtOAc. The organic layer was washed with 1 N NaOH, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography (benzene–acetone 2:1) to give 4.9 g (68%) of 5-methyl-4-phenyl-1-(3-thiobenzoylpropyl)imidazole as an oil.

A mixture of the oil (1.26 g, 3.75 mmol) and $LiAlH_4$ (142 mg, 3.74 mmol) in THF was stirred for 1 h, and then water (0.14 mL), 15% NaOH (0.14 mL), and water (0.42 mL) were added consecutively at 0 °C. The resulting precipitate was filtered off and the filtrate was evaporated in vacuo to give **5m** as an oil. The product was used without further purification.

3-(5-Methyl-4-phenyl-1*H*-imidazol-1-yl)propionaldehyde (5o). 3-(5-Methyl-4-phenyl-1*H*-imidazol-1-yl)propionaldehyde diethyl acetal (**5n**) was prepared by the same procedure as reported for **5a**. A solution of **5n** (5.00 g, 17.4 mmol) and 7 N HCl (10 mL) in THF was stirred at room temperature for 1 h. The mixture was poured into water, neutralized with saturated sodium hydrogen carbonate, and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated to give 890 mg (24%) of **5o** as an oil, which was used without further purification.

2-(Dimethylamino)-6-(pentylureido)phenol (9ad). A mixture of 3-methoxy-2-nitrobenzoic acid (98.6 g, 0.50 mol) and thionyl chloride (500 mL) was heated under reflux for 3 h. The excess thionyl chloride was removed under reduced pressure and the residue was washed with hexane to give 3-methoxy-2-nitrobenzoyl chloride.

A solution of the acid chloride in acetone (500 mL) was added dropwise to a solution of sodium azide (40.0 g, 0.60 mol) in water (290 mL) at below 10 °C over 1 h. The mixture was stirred at room temperature for 30 min and poured into water (1 L). The resulting precipitate was collected and washed with water (500 mL × 2) to give 3-methoxy-2-nitrobenzoyl azide.

The benzoyl azide was refluxed in benzene to give 3-methoxy-2-nitrophenyl isocyanate until nitrogen gas evolution ceased. The reaction mixture was cooled, 5 N NaOH (200 mL) was added at 0 °C, and the whole was stirred at room temperature for 1 h. Then 10 N HCl (200 mL) was added to the solution at 0 °C, and the reaction mixture was heated at 80 °C for 2 h. The benzene layer was separated, and the water layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The resulting solid was recrystallized from EtOAc–hexane to give 63.8 g (76%) of 3-methoxy-2-nitroaniline.

To a suspension of the aniline derivative (110 g, 0.65 mmol) and potassium carbonate (455 g, 3.2 mol) in DMF was added methyl iodide (410 mL, 6.5 mol) while stirring. The mixture was stirred for 24 h at 50 °C. Water was then added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give 88.3 g (69%) of 3-methoxy-2-nitrodimethylaniline (**6a**).

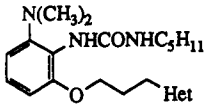
A solution of **6a** (88.3 g, 0.45 mol) in EtOAc was hydrogenated over 10% palladium on carbon (1.5 g) at 1 atm for 1 h. The catalyst was filtered off and the filtrate was evaporated in vacuo to give 74.6 g (99%) of 2-(dimethylamino)-6-methoxyaniline (**7a**).

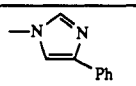
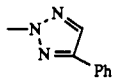
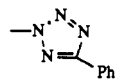
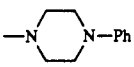
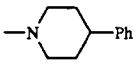
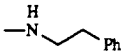
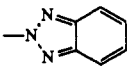
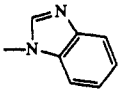
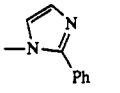
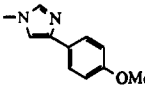
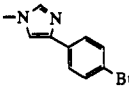
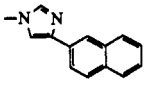
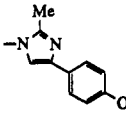
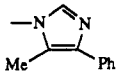
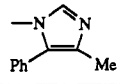
A solution of **7a** (74.6 g, 0.45 mol) and pentyl isocyanate (51 g, 0.45 mol) in $CHCl_3$ was heated under reflux for 24 h. The solvent was removed in vacuo. The solid residue was recrystallized from EtOAc to give 124 g (99%) of *N*-[2-(dimethylamino)-6-methoxyphenyl]-*N'*-pentylurea (**8ad**).

To a solution of **8ad** (81.3 g, 0.29 mol) in CH_2Cl_2 was added BBr_3 (2 M CH_2Cl_2 solution, 370 mL, 0.74 mol) at 0 °C. The mixture was heated under reflux for 2 h and then poured into water, neutralized with sodium hydrogen carbonate, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give 69.8 g (91%) of **9ad** as an amorphous solid. The compound was used without further purification: ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, J = 7.5), 1.15–1.60 (6H, m), 2.62 (6H, s), 3.30 (2H, q, J = 7.5), 5.00 (1H, brs), 6.62 (1H, dd, J = 8.0, 1.5), 6.71 (1H, dd, J = 8.0, 1.5), 6.98 (1H, t, J = 8.0), 7.62 (1H, brs), 10.0 (1H, s).

Method A. *N*-[2-(Dimethylamino)-6-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propoxy]phenyl]-*N'*-pentylurea (24). A solution of 1-(3-chloropropyl)-5-methyl-4-phenylimidazole (**5a**, 8.84 g, 37.7 mmol) in DMF was added dropwise to a suspension of 2-(dimethylamino)-6-(pentylureido)phenol (**9ad**, 10.0 g, 37.7 mmol), sodium iodide (100 mg), and potassium carbonate (10.4 g, 76.5 mmol) in DMF at 60 °C over 2 h. The mixture was stirred at 60 °C for 2 h. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (benzene–acetone

Table IV. Physical Data and Biological Activities of *N*-[2-(Dimethylamino)-6-[3-(heterocycle)propoxy]phenyl]-*N'*-pentylureas (Variable C Region)



no.	Het	yield[a] (method)	mp (°C)	formula[b]	ACAT inhibn: aorta[c]	ACAT inhibn: intestine[d]	cholesterol lowering[e]
13		51 (A)	110–111	$C_{26}H_{35}N_5O_2$	0.16	0.031	−21
28		49 (A)	129–131	$C_{25}H_{34}N_6O_2$	0.18	0.032	−32
29		55 (A)	146–147	$C_{24}H_{33}N_7O_2$	0.23	0.023	−48
30		37 (E)	148–150	$C_{27}H_{41}N_5O_2$	0.18	0.017	−44
31		66 (E)	129–131	$C_{28}H_{42}N_4O_2$	0.47	0.34	−27
32		30 (E)	171–173	$C_{25}H_{38}N_4O_2$	3.44	0.96	−18
33		41 (E)	149–151	$C_{23}H_{32}N_6O_2$	0.29	0.77	−3.4
34		35 (E)	137–139	$C_{24}H_{33}N_5O_2$	0.62	0.18	−12
35		31 (A)	125–126	$C_{26}H_{35}N_5O_2$	0.48	0.38	−12[f]
36		41 (A)	98–101	$C_{27}H_{37}N_5O_3$	1.33	0.059	−36
37		22 (A)	111–114	$C_{30}H_{43}N_5O_2$	0.43	0.054	−46
38		33 (A)	71–75	$C_{30}H_{37}N_5O_2$	1.55	0.065	−30
39		53 (A)	140–143	$C_{28}H_{39}N_5O_3$	0.73	0.16	−15
24		69 (A)	128–130	$C_{27}H_{37}N_5O_2$	0.11	0.043	−49
40		45 (A)	119–121	$C_{27}H_{37}N_5O_2$	0.21	0.085	−46

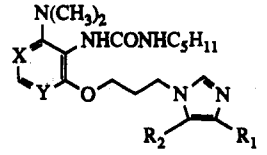
[a–d] See corresponding footnotes of Table I. [e] Compounds were administered to rats at 10 mg/kg once a day for 5 days, unless otherwise indicated. Cholesterol lowering activity expressed as percentage of control value ($n \geq 4$). [f] The compound was administered to rats at 30 mg/kg once a day for 5 days.

2:1), and recrystallization from EtOAc–hexane gave 12.0 g (69%) of **24**: mp 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, J = 6.0), 1.16–1.71 (6H, m), 2.21 (2H, quintet, J = 5.0), 2.28 (3H, s), 2.78 (6H, s), 3.19 (2H, q, J = 5.0), 3.95 (2H, t, J = 6.0), 4.13 (2H, t, J = 6.0), 6.19 (1H, brs), 6.70 (1H, brt), 6.40–7.60 (9H, m). Anal. ($C_{27}H_{37}N_5O_2$) C, H, N.

1-[*N*-[2-(Dimethylamino)-6-[3-(4-phenyl-1*H*-imidazol-1-yl)propoxy]phenyl]carbamoyl]pentane (18): MS *m/e* (FAB) 435 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, J = 6.0), 1.08–1.90 (6H, m), 2.19 (2H, quintet, J = 7.0), 2.63 (6H, s), 3.36 (2H, t, J = 6.5), 3.89 (2H, t, J = 7.0), 4.11 (2H, t, J = 7.0), 6.41–7.80 (11H, m).

2-(Hexylamino)-3-[3-(4-phenyl-1*H*-imidazol-1-yl)propoxy]dimethylaniline (19). To a solution of 18 (330 mg, 0.76 mmol) in THF was slowly added $LiAlH_4$ (40 mg). The mixture was stirred for 1 h at room temperature, and 1 N NaOH solution was added to it. The resulting precipitate was filtered off and the filtrate was evaporated in vacuo. The residue was purified by column chromatography (benzene–acetone 4:1) to give

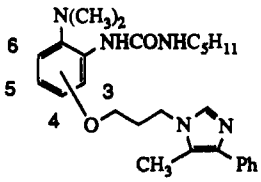
Table V. Physical Data and Biological Activities of *N*-[2-(Dimethylamino)-6-[3-(4-phenyl-1*H*-imidazol-1-yl)-propoxy]aryl]-*N'*-pentylureas (Variable D Region)



no.	X	Y	R_1	R_2	yield[a] (method)	mp (°C)	formula[b]	ACAT inhibn aorta[c]	ACAT inhibn intestine[d]
13	CH	CH	Ph	H	51 (A)	110–111	$C_{26}H_{35}N_5O_2$	0.16	0.031
41	CH	CCH_3	Ph	H	50 (A)	119–121	$C_{27}H_{37}N_5O_2$	0.25	0.41
42	CBr	CBr	4-OCH_3Ph	H	65 (A)	124–126	$C_{27}H_{35}N_5O_3Br_2$	1.3	2.4
43	N	N	Ph	CH_3	17 (A)	*e*	$C_{25}H_{35}N_7O_2$[f]	11	5.5

[a–d] See corresponding footnotes of Table I. [e] See footnote *g* of Table I. [f] See footnote *h* of Table I.

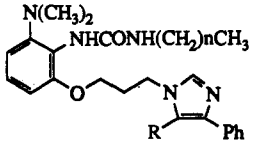
Table VI. Physical Data and Biological Activities of **24** and the Regioisomers (Variable D Region)



no.	position	yield[a] (method)	mp (°C)	formula[b]	ACAT inhibn aorta[c]	ACAT inhibn intestine[d]
24	3	69 (A)	128–130	$C_{27}H_{37}N_5O_2$	0.11	0.043
44	4	6 (A)	142–144	$C_{27}H_{37}N_5O_2$	0.41	0.41
45	5	22 (A)	125–126	$C_{27}H_{37}N_5O_2$	1.5	2.4
46	6	25 (A)	178–180	$C_{27}H_{37}N_5O_2$	3.2	5.8

[a–d] See corresponding footnotes of Table I.

Table VII. Biological Activities of Selected Compounds



no.	R	*n*	ACAT inhibn aorta[a]	ACAT inhibn intestine[b]	hypocholesterolemic activity (ED_{30}, mg/kg)[c]
4	H	3	0.81	0.14	75
13	H	4	0.16	0.031	14
14	H	5	0.050	0.060	1.3
24	CH_3	4	0.11	0.043	4.5

[a] See footnote c of Table I. [b] See footnote *d* of Table I. [c] The values are the effective dose required to decrease serum total cholesterol to 30% of the control value, calculated from the dose–response curve derived from experiments with at least 3 doses ($n = 4$ or 5). Each compound was administered orally as a suspension in 2% Sefuzol for 5 days.

300 mg (91%) of **19**: MS *m/e* (FAB) 421 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 0.90 (3H, t, $J = 6.5$), 1.25–1.47 (8H, m), 2.19 (2H, quintet, $J = 7.0$), 2.63 (6H, s), 3.30 (2H, q, $J = 6.5$), 3.89 (2H, t, $J = 7.0$), 4.11 (2H, t, $J = 7.0$), 6.41–7.80 (11H, m).

4-(Dimethylamino)-6-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propoxy]-5-(*N'*-pentylureido)pyrimidine (43): MS *m/e* (FAB) 466 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 0.87 (3H, t, $J = 6.2$), 1.12–1.78 (6H, m), 2.19 (2H, quintet, $J = 6.0$), 2.33 (3H, s), 3.10 (6H, s), 3.17 (2H, q, $J = 6.8$), 3.83 (2H, t, $J = 6.0$), 3.95 (2H, t, $J = 6.0$), 5.58 (1H, brt), 6.65 (1H, brs), 7.21–7.71 (7H, m).

Method B. *N*-[2-(Dimethylamino)-6-[4-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)butyl]phenyl]-*N'*-pentylurea (25). To a solution of 3-chloro-2-nitrobenzoic acid (25.0 g, 124 mmol) in THF was added a solution of B_2H_6 (1 M THF solution, 300 mL) at 0 °C. The mixture was stirred at room temperature for 2 h, then MeOH was added until gas evolution ceased. After 10 min, the mixture was poured into water and extracted with EtOAc. The organic layer was washed with aqueous sodium hydrogen carbonate and brine, dried over $MgSO_4$, and evaporated to give 20.5 g (88%) of 3-chloro-2-nitrobenzyl alcohol (**47**).

Table VIII. Effect of Selected Compounds on Plasma Cholesterol Level, Atheromatous Lesion Development, and Esterified Cholesterol Content in New Zealand White Rabbits Fed Cholesterol-Containing Atherogenic Diet

		plasma total cholesterol[c] (mg/dL)	aortic atheromatous lesion index[c] % surface involvement	aortic atheromatous lesion index[c] cholesterol content
control	($n = 12$)	2428 ± 113	38.2 ± 5.0	32.9 ± 3.5
4[a]	($n = 9$)	1192 ± 127[d]	23.5 ± 3.2[g]	13.6 ± 2.7[d]
13[a]	($n = 8$)	830 ± 76[d]	22.8 ± 4.0[g]	14.4 ± 3.3[e]
14[a]	($n = 7$)	924 ± 151[d]	30.6 ± 5.8	25.2 ± 8.7
24[b]	($n = 9$)	800 ± 84[d]	21.0 ± 1.9[f]	10.4 ± 2.1[d]

[a] Compounds **4**, **13**, and **14** were administered at 0.2% (w/w) for the first 4 days of drug treatment, and then the dosage was changed to 0.1% (w/w). [b] Compound was dosed at 0.1% (w/w). [c] Values are mean ± SEM. Statistical comparisons against control values were performed by means of Student's *t* test. [d] $p < 0.001$. [e] $p < 0.01$. [f] $p < 0.02$. [g] $p < 0.05$.

A mixture of **47** (20.0 g, 107 mmol) and dimethylamine (50% water solution, 50 mL) in DMF was heated at 120 °C in an autoclave for 4 h. The reaction mixture was evaporated to give a brown oil. This crude oil was purified by silica gel column chromatography (hexane–EtOAc 4:1) to give 15.9 g (76%) of 3-(dimethylamino)-2-nitrobenzyl alcohol (**48**).

To a solution of **48** (15.9 g, 81.3 mmol) in DMF (20 mL) was slowly added phosphorus tribromide (2.82 mL) at 0 °C for 30 min. The mixture was stirred at room temperature for 1 h and then poured into water. The mixture was neutralized with 1 N NaOH and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel with hexane–EtOAc (8:1) to give 10.3 g (49%) of 3-(dimethylamino)-2-nitrobenzyl bromide (**49**).

A mixture of **49** (10.3 g, 39.8 mmol) and triphenylphosphine (11.4 g, 43.7 mmol) in toluene (200 mL) was heated under reflux for 1 h. The mixture was cooled to room temperature and allowed to stand for 5 h. The resulting precipitate was collected by suction filtration, washed with cold toluene, and dried in vacuo to give 20.0 g (96%) of [3-(dimethylamino)-2-nitrobenzyl]triphenylphosphonium bromide (**50**).

To a solution of **50** (2.17 g, 4.17 mmol) and 3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propionaldehyde (**5o**, 890 mg, 4.17 mmol) in EtOH was added a solution of sodium ethoxide (21 wt% ethanol solution, 1.3 mL). The mixture was stirred under reflux for 2 h, and then the dark mixture was cooled and concentrated in vacuo. The residue was diluted with Et_2O (100 mL) and extracted with 2 N HCl (50 mL × 2). The combined aqueous layer was basified with 2 N NaOH and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography (benzene–acetone 3:1) to give 980 mg (63%) of 3-[4-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)-1-butenyl]-2-nitro-*N*,*N*-dimethylaniline (**51**).

A solution of **51** (980 mg, 2.61 mmol) in EtOAc was hydrogenated over 10% palladium on carbon at 5 kg/cm^2 for 4 h. The

catalyst was filtered off and the filtrate was evaporated to give 880 mg (97%) of 2-(dimethylamino)-6-[4-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)butyl]aniline, which was used without further purification.

A mixture of the aniline derivative (880 mg, 2.53 mmol) and pentyl isocyanate (430 mg, 3.81 mmol) in $CHCl_3$ was refluxed for 10 h. The excess pentyl isocyanate and solvent were removed under reduced pressure. The residue was purified by column chromatography (benzene–acetone 3:1), and the resulting solid was recrystallized from EtOAc–hexane to give 670 mg (57%) of **25**: mp 117–119 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, t, J = 6.8), 1.26 (4H, m), 1.43 (2H, quintet, J = 7.2), 1.63 (2H, quintet, J = 7.2), 1.79 (2H, quintet, J = 7.2), 2.37 (3H, s), 2.66 (2H, t, J = 7.2), 2.68 (6H, s), 3.18 (2H, q, J = 6.8), 3.86 (2H, t, J = 7.2), 5.24 (1H, brt), 6.00 (1H, brs), 6.86–7.65 (9H, m). Anal. ($C_{28}H_{39}N_5O$) C, H, N.

Method C. *N*-[2-(Dimethylamino)-6-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propylthio]phenyl]-*N'*-pentylurea (26). 3-Chloro-2-nitro-*N*,*N*-dimethylaniline (**52**) was prepared by the same procedure as reported for 3-methoxy-2-nitro-*N*,*N*-dimethylaniline (**6a**).

A mixture of **52** (1.97 g, 9.82 mmol), 5-methyl-4-phenyl-1-(3-propylthio)imidazole (**5m**, 2.28 g, 9.83 mmol), and potassium *tert*-butoxide (1.21 g, 10.8 mmol) was stirred at 50 °C for 1 h. The mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to remove the solvent. The residue was purified by column chromatography (benzene–acetone 2:1) to give 690 mg (18%) of 3-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propylthio]-2-nitro-*N*,*N*-dimethylaniline (**53**).

A solution of **53** (690 mg, 1.74 mmol) in EtOAc was hydrogenated over 10% palladium on carbon (1.5 g) at 1 atm for 5 h. The catalyst was filtered off and the filtrate was evaporated to give 190 mg (30%) of 2-(dimethylamino)-6-[[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propyl]thio]aniline.

A solution of the above aniline derivative (190 mg, 0.52 mmol) and pentyl isocyanate (88 mg, 0.78 mmol) in $CHCl_3$ was heated under reflux for 10 h. The mixture was evaporated under reducing conditions to remove the excess reagent and solvent. The residue was chromatographed on silica gel (benzene–acetone 1:1) to give 110 mg (44%) of **26** as an oil: MS m/e (FAB) 480 $(M + H)^+$; ^{1}H NMR δ 0.89 (3H, t, J = 6.4), 1.29 (4H, m), 1.48 (2H, m), 2.20 (2H, quintet, J = 6.8), 2.36 (3H, s), 2.77 (6H, s), 2.86 (2H, t, J = 6.8), 3.21 (2H, q, J = 6.8), 4.02 (2H, t, J = 6.8), 6.34 (1H, brs), 6.47 (1H, brs), 6.97–7.64 (9H, m).

Method D. *N*-[2-(Dimethylamino)-6-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propylamino]phenyl]-*N'*-pentylurea (27). A mixture of 3-chloro-2-nitroaniline (6.73 g, 39.0 mmol) and formic acid (50 mL) was heated under reflux for 1 h. The excess formic acid was removed in vacuo, and the precipitate was recrystallized from Et_2O–hexane to give 6.33 g (81%) of 3-chloro-2-nitro-*N*-formylaniline.

A mixture of the formylaniline derivative (6.33 g, 31.6 mmol), potassium carbonate (8.71 g), sodium iodide (100 mg), and **5a** (6.96 g, 31.6 mmol) in DMF was stirred at 60 °C for 3 h. The reaction mixture was cooled and diluted with water until all solids were dissolved. The mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by flash chromatography (benzene–acetone 1:1 and 1:2) to give 6.13 g (50%) of 3-chloro-2-nitro-*N*-formyl-*N*-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propyl]aniline.

A solution of the above compound (4.78 g, 12.43 mmol) and 2 N HCl (20 mL) in 1,4-dioxane (10 mL) was heated under reflux for 30 min. The mixture was poured into ice water, basified with 1 N NaOH, and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated to give 4.43 g (100%) of 3-chloro-2-nitro-*N*-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propyl]aniline as an oil.

A solution of the above oil (4.43 g, 12.4 mmol) and benzyl bromide (2.4 g, 14.0 mmol) in DMF was added dropwise to a suspension of NaH (55% oil suspension, 650 mg, 14.9 mmol) in DMF at 0 °C over 1 h. The mixture was stirred at room temperature for 1 h, poured into water, and extracted with EtOAc. The organic layer was dried over $MgSO_4$, and evaporated to give 5.55 g (100%) of 3-chloro-2-nitro-*N*-benzyl-*N*-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propyl]aniline (**54**).

A solution of **54** (5.55 g, 12.4 mmol) and dimethylamine (50% water solution, 22.4 mL) in DMF was heated at 200 °C for 3 h in an autoclave. The reaction mixture was cooled to room temperature, poured into water, and extracted with EtOAc. The organic layer was dried over $MgSO_4$, and evaporated to remove the solvent. The residue was purified by column chromatography (benzene–acetone 3:1) to give 2.20 g (39%) of 3-(dimethylamino)-2-nitro-*N*-benzyl-*N*-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propyl]aniline (**55**).

To a solution of **55** (2.20 g, 5.60 mmol) in 36% HCl (10 mL) was added $SnCl_2 \cdot H_2O$ (3.79 g) at 0 °C. The reaction mixture was stirred at room temperature for 1 h, poured into ice water, basified with 5 N NaOH, and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, and evaportaed to give 880 mg (37%) of 2-[*N*-benzyl-*N*-[[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propyl]-amino]]-6-(dimethylamino)aniline.

A solution of the above aniline derivative (800 mg, 2.20 mmol) and pentyl isocyanate (250 mg) in $CHCl_3$ was refluxed for 5 h and then evaporated to remove excess reagent and solvent. The residue was chromatographed on silica gel with benzene–acetone (1:1) to give 470 mg (45%) of *N*-[2-[*N*-benzyl-*N*-[3-(5-methyl-4-phenyl-1*H*-imidazol-1-yl)propyl]amino]-6-(dimethylamino)-phenyl]-*N'*-pentylurea.

A solution of the above compound (470 mg, 0.87 mmol) in EtOH was hydrogenated over 20% palladium hydroxide on carbon at 5 kg/cm^2 for 2 h. The catalyst was filtered off and the filtrate was evaporated. The residue was purified by column chromatography (CH_2Cl_2–MeOH 10:1) to give 110 mg (28%) of **27** as an oil. MS m/e (FAB) 463 $(M + H)^+$; ^{1}H NMR δ 0.87 (3H, t, J = 6.5), 1.20–1.54 (6H, m), 2.10 (2H, quintet, J = 7.2), 2.39 (3H, s), 2.68 (6H, s), 3.18 (2H, q, J = 7.3), 3.23 (2H, t, J = 7.2), 4.05 (2H, t, J = 7.2), 4.78 (1H, brt), 5.00 (1H, brs), 6.16 (1H, brt), 6.39 (1H, dd, J = 7.6, 1.0), 6.49 (1H, dd, J = 7.6, 1.0), 7.08 (1H, t, J = 7.6), 7.26 (1H, t, J = 4.8), 7.40 (2H, t, J = 8.4), 7.64 (2H, dd, J = 8.4, 1.2).

Method E. *N*-[2-(Dimethylamino)-6-[3-(4-phenylpiperazine)propoxy]phenyl]-*N'*-pentylurea (30). A mixture of 3-(dimethylamino)-2-(*N'*-pentylureido)phenol (**9ad**, 5.00 g, 18.9 mmol), 1-bromo-3-chloropropane (4.46 g), and potassium carbonate (6.5 g) in DMF was stirred at 50 °C for 3 h. The mixture was poured into water, and the resulting precipitate was collected by suction filtration. This product was recrystallized from EtOAc to give 5.73 g (89%) of *N*-[2-(3-chloropropoxy)-6-(dimethylamino)phenyl]-*N'*-pentylurea (**10**).

To a suspension of **10** (1.00 g, 3.03 mmol), potassium carbonate (810 mg, 5.79 mmol), and sodium iodide (100 mg, 0.667 mmol) in DMF was added a solution of 1-phenylpiperazine (490 mg, 3.03 mmol) in DMF at 60 °C. The mixture was stirred at 60 °C for 2 h, poured into water, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was crystallized from benzene–hexane and the product was recrystallized from EtOAc to give 500 mg (37%) of **30**: mp 148–150 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (3H, t, J = 6.0), 1.15–1.74 (6H, m), 2.01 (2H, quintet, J = 6.5), 2.58 (2H, t, J = 6.5), 2.64 (4H, m), 3.20 (6H, m), 4.09 (2H, t, J = 6.5), 6.34 (1H, brs), 6.51–7.38 (9H, m). Anal. ($C_{27}H_{41}N_5O_2$) C, H, N.

Assay of ACAT Activity. The intestinal microsomes were prepared according to the method of Field et al.[4] Briefly, the intestinal mucosa of an NZW rabbit was homogenized in a buffered sucrose solution (0.1 N sucrose, 0.05 M KCl, 0.04 M KH_2PO_4, 0.03 M EDTA, pH 7.4) in a glass Dounce homogenizer. The whole homogenate was spun at 10000g for 20 min at 4 °C. The resulting supernatant was centrifuged at 105000g for 1 h at 4 °C. The microsomal pellet thus obtained was rehomogenized in cold buffered sucrose solution and used as intestinal ACAT.

Arterial ACAT was prepared as follows. The aortae of cholesterol-fed NZW rabbits were homogenized in 154 mM potassium phosphate buffer (pH 7.4) containing 2.4 mg/mL BSA. The whole homogenate was spun at 300g for 5 min to remove cellular debris. The resulting supernatant was used as arterial ACAT.

ACAT activity was determined essentially by the method described by Heider et al.[7] Endogenous cholesterol (of microsomal fraction or homogenate) and exogenous [1-^{14}C]oleoyl-CoA were used as the substrates. The reaction mixture for intestinal ACAT consisted of 250 μL of K_2HPO_4 buffer (pH 7.4) containing

2.4 mg/mL BSA and 40 μM [1-^{14}C]oleoyl-CoA, and the reaction mixture for arterial ACAT consisted of 250 μL of the same buffer containing 2.4 mg/mL BSA and 5 μM [1-^{14}C]oleoyl-CoA. Then, 30 μL of test compound solution was added, and the mixture was preincubated at 37 °C for 3 min before the addition of 20 μL of the enzyme (8 mg protein/mL). The reaction mixture was incubated for a certain time (5 min for aorta and 2 min for intestine), and then the reaction was terminated by the addition of 3 mL of chloroform/methanol (2:1; v/v) and 500 μL of 0.04 N HCl. After shaking, the chloroform phase was taken to dryness and the residue was separated by thin-layer chromatography on plastic sheets (TLC plastic sheets, silica gel 60, Merck Co.,) using heptane–diethyl ether–acetic acid (90:30:1; v/v/v). Cholesteryl oleate was visualized with iodine vapor, and the cholesterol ester zone was cut out and placed directly in a scintillation vial for counting. The values are expressed as percent inhibition or IC_{50} values. Calculation of IC_{50} values was performed with data from triplicate assay tubes at each drug concentration.

Serum Cholesterol Level. Male Sprague–Dawley rats (5-weeks-old) were fed a standard rat chow, MF (Oriental Yeast Co., Ltd., Tokyo, Japan), containing 1% cholesterol and 0.5% cholic acid. At the same time, the drug suspended in 2% Sefuzol (Nikko Chemical, Tokyo, Japan) was administered orally once a day. Rats were starved for 5 h after the last administration and bled via the abdominal aorta while under ether anesthesia. Serum concentration of total cholesterol was determined enzymatically using the Iatrolipo TC kit (Iatron Lab., Tokyo, Japan). After the last day of the test, the control rat serum cholesterol levels were in the range of 180–230 mg/dL, while the normal rat serum cholesterol levels were in the range of 50–60 mg/dL. The values shown in Tables I–IV are expressed as percentage of control value, while the values shown in Table VII are ED_{30} (mg/kg). The values of ED_{30} were calculated from the dose–response curve derived from experiments with at least three doses (n = 4 or 5).

Cholesterol-Fed Rabbits. Male New Zealand white rabbits weighing 2.5–3.0 kg were fed standard chow (Oriental, ORC-400) supplemented with 0.5% cholesterol and 0.5% olive oil. All rabbits received 100 g of food/day and water ad libitum during the course of the study. After 8 weeks, the rabbits were randomized to standardize plasma cholesterol levels. The grouped animals were placed on the following diets: control = cholesterol-containing diet with 0.5% olive oil; drug treatment = control diet with a test compound. The dosage in the drug-treated rabbits during the subsequent 4 weeks of the study was 30 mg/kg per day. Blood samples were obtained from the ear vein in the presence of EDTA, and plasma total cholesterol level was measured enzymatically using Iatrolipo TC kit (Iatron Lab., Tokyo, Japan). At the termination of the experiment, the rabbits were anesthetized with pentobarbital and exsanguinated from the aortic arch, and the thoracic aorta was separated at 5 mm proximal to the outlet of the first intercostal artery. These tissues were then longitudinally cut into two halves of about equal size. One half was fixed in buffered formalin and stained with Sudan IV. The positively stained areas were measured with an image analyzer (Nachet). Values are mean ± SEM percent of 7–12 animals per group. The other half was used for the determination of cholesterol content by use of a TLC-FID analyzer (Iatron). Values are mean ± SEM mg/g of wet tissue.

Acknowledgment. We thank the members of the Analytical Chemistry Section of Eisai Tsukuba Research Laboratories for interpretation of the mass spectra and elemental analysis data. We are grateful to Dr. Masaji Ohno and Dr. Toshiharu Ohgou for their invaluable advice. We also wish to thank Dr. Wendy Gray for her help in preparing the manuscript.

References

(1) Brown, M. S.; Goldsteine, J. L. How LDL Receptors Influence Cholesterol and Atherosclerosis. *Sci. Am.* **1984**, *251*, 52–60.
(2) Goodman, D. S. Cholesterol Ester Metabolism. *Physiol. Rev.* **1965**, *45*, 747–839.
(3) Suckling, K. E.; Stange, E. F. Role of Acyl-CoA:Cholesterol Acyltransferase in Cellular Cholesterol Metabolism. *J. Lipid Res.* **1985**, *26*, 647–671.
(4) Field, F. J.; Mathur, S. N. β-Sitosterol: Esterification by Intestinal Acylcoenzyme A: Cholesterol Acyltransferase (ACAT) and Its Effect on Cholesterol Esterification. *J. Lipid Res.* **1983**, *24*, 409–417.
(5) Helgerud, P.; Saarem, K.; Norum, K. R. Acyl-CoA:Cholesterol Acyltransferase in Human Small Intestine: Its Activity and Some Properties of the Enzyme Reaction. *J. Lipid Res.* **1981**, *22*, 271–277.
(6) Largis, E. E.; Wang, C. H.; De Vries, V. G.; Schaffer, S. A. CL 277,082: a Novel Inhibitor of ACAT-Catalyzed Cholesterol Esterification and Cholesterol Absorption. *J. Lipid Res.* **1989**, *30*, 681–690.
(7) Heider, J. G.; Pickens, C. E.; Kelly, L. A. Role of Acyl-CoA: Cholesterol Acyltransferase in Cholesterol Absorption and Its Inhibition by 57-118 in the Rabbit. *J. Lipid Res.* **1983**, *24*, 1127–1134.
(8) Smith, B. P.; St. Clair, R. W.; Lewis, J. C. Cholesterol Esterification and Cholesterol Ester Accumulation in Cultured Pigeon and Monkey Arterial Smooth Muscle Cells. *Exp. Mol. Pathol.* **1979**, *30*, 190–208.
(9) Rothblat, G. H.; Naftulin, M.; Arbogast, L. Y. Stimulation of Acyl-CoA:Cholesterol Acyltransferase Activity by Hyperlipemic Serum Lipoproteins (39838). *Proc. Soc. Exp. Biol. Med.* **1977**, *155*, 501–506.
(10) Brown, M. S.; Ho, Y. K.; Goldsteine, J. L. The Cholesterol Ester Cycle in Macrophage Foam Cells. *J. Biol. Chem.* **1980**, *255*, 9344–9352.
(11) Mathur, S. N.; Field, F. J.; Megan, M. B.; Armstrong, M. L. A Defect in Mobilization of Cholesterol Esters in Rabbit Macrophages. *Biochim. Biophys. Acta* **1985**, *834*, 48–57.
(12) Rothblat, G. H.; Arbogast, L. Y.; Ray, E. K. Stimulation of Esterified Cholesterol Accumulation in Tissue Culture Cells Exposed to High Density Lipoproteins Enriched in Free Cholesterol. *J. Lipid Res.* **1978**, *19*, 350–358.
(13) Hashimoto, S.; Dayton, S.; Alfine-slater, R. B. Esterification of Cholesterol by Homogenates of Atherosclerotic and Normal Aortas. *Life Sci.* **1973**, *12*, 1–11.
(14) St. Clair, R. W.; Lofland, H. B.; Clarkson, T. B. Influence of Duration of Cholesterol Feeding on Esterification of Fatty Acids by Cell-Free Preparation of Pigeon Aorta. *Circ. Res.* **1970**, *27*, 213–225.
(15) Brecher, P.; Chan, C. T. Properties of Acyl-CoA:Cholesterol *O*-Acyltransferase in Aortic Microsomes from Atherosclerotic Rabbits. *Biochim. Biophys. Acta* **1980**, *617*, 458–471.
(16) Sliskovic, D. R.; White, A. D. Therapeutic Potential of ACAT Inhibitors as Lipid Lowering and Anti-atherosclerotic Agents. *Trends Pharmacol. Sci.* **1991**, *12*, 194–199.
(17) Larsen, S. D.; Spilman, C. H.; Bell, F. P.; Dinh, D. M.; Martineborough, E.; Wilson, G. J. Synthesis and Hypocholesterolemic Activity of 6,7-Dihydro-4H-pyrazolo[1,5-a]pyrrolo[3,4-d]pyrimidine-5,8-diones, Novel Inhibitors of Acyl-CoA:Cholesterol *O*-Acyltransferase. *J. Med. Chem.* **1991**, *34*, 1721–1727.
(18) Gammill, R. B.; Bell, F. P.; Bell, L. T.; Bisaha, S. N.; Wilson, G. J. Antiatherosclerotic Agents. A Structurally Novel Bivalent Inhibitor of Acyl-CoA:Cholesterol *O*-Acyltransferase with Systemic Activity. *J. Med. Chem.* **1990**, *33*, 2685–2687.
(19) De Vries, V. G.; Bloom, J. D.; Dutia, M. D.; Katocs, A. S.; Largies, E. E. Potential Antiatherosclerotic Agents. 6. Hypocholesterolemic Trisubstituted Urea Analogues. *J. Med. Chem.* **1989**, *32*, 2318–2325.
(20) Ishida, F.; Sato, A.; Iizuka, Y.; Kitani, K.; Sawasaki, Y.; Kamei, T. Effects of MK-733 (Simvastatine), an Inhibitor of 3-Hydroxy-3-methylglutaryl CoenzymeA Reductase, on Intestinal Acylcoenzyme A:Cholesterol Acyltrasferase Activity in Rabbits. *Biochim. Biophys. Acta* **1989**, *1004*, 117–123.
(21) Rucker, W.; Prop, G.; Huther, A. M. Antiatherosclerotic and Antihyperlipidemic Effects of Octimibate Sodium in Rabbits. *Atherosclerosis* **1988**, *69*, 155–160.
(22) Matsubara, K.; Matsuzawa, Y.; Jiao, S.; Kihara, S.; Takama, T.; Nakamura, T.; Tokunaga, K.; Kubo, M.; Tarui, S. Cholesterol-Lowering Effect of N-(α-Methylbenzyl)linoleamide (Melinamide) in Cholesterol-Fed Diabetic Rats. *Atherosclerosis* **1988**, *72*, 199–204.
(23) Natori, K.; Okazaki, Y.; Nakajima, J.; Hirohoshi, T.; Aono, S. Mechanism of the Inhibition of Cholesterol Absorption by DL-Melinamide: Inhibition of Cholesterol Esterification. *Jpn. J. Pharmacol.* **1986**, *42*, 517–523.
(24) De Vries, V. G.; Schaffer, S. A.; Largis, E. E.; Dutia, M. D.; Wang, C.; Bloom, J. D.; Katocs, A. S. Potential Antiatherosclerotic Agents. 5. An Acyl-CoA:Cholesterol O-Acyltransferase Inhibitor with Hypocholesterolemic Activity. *J. Med. Chem.* **1986**, *29*, 1131–1133.
(25) Bell, F. P.; Schaub, R. G. Chloropromazine Inhibits Arterial ACAT and Reduces Arterial Cholesterol and Cholesteryl Ester Accumulation in Cholesterol-fed Rabbits. *Arteriosclerosis* **1986**, *6*, 42–49.
(26) Schaffer, S. A.; Bloom, J. D.; De Vries, V. G.; Dutia, M.; Katocs, A. S., Jr.; Largis, E. E. CL277,082, a Novel Inhibitor of Cholesterol Esterification and Cholesterol Absorption. In *Atherosclerosis VII*; Fidge, N. H., Nestel, P. J., Eds.; Elsevier Science Publishers BV: Amsterdam, 1986, pp 633–636.

(27) Bocan, T. M. A.; Mueller, S. B.; Uhlendorf, P. D.; Newton, R. S.; Krause, B. R. Comparison of CI-976, an ACAT Inhibitor, and Selected Lipid-Lowering Agents for Antiatherosclerotic Activity in Iliac-Femoral and Thoracic Aortic Lesions. *Arteriosclerosis Thrombosis* **1991**, *11*, 1830–1843.

(28) As our strategy to discover a novel ACAT inhibitor with non fatty acid structure, we prepared many possible folding conformation models of oleoyl-CoA, which is the optimal substrate for ACAT, and our available compounds that seemed to fit the models were evaluated for in vitro ACAT inhibitory activity using rat liver microsomes. Compound **2** was reported by Sugimoto et al. in JP-88297067. Compound **3** was afforded by catalytic hydrogenation of **2** followed by reaction with valeryl chloride. The inhibitory activities of **2** and **3** were 58% at 5×10^{-5} M and 61% at 1×10^{-5} M, respectively.

(29) Kimura, T.; et al. Unpublished results.

(30) After oral administration of test compounds (30 mg/kg) to NZW rabbits ($n = 2$), C_{max} values were 3173 and 2069 ng/mL (**4**), 1671 and 1276 ng/mL (**13**), 202 and 161 ng/mL (**14**), 1920 and 508 ng/mL (**24**), <30 ng/mL (**28**, **29**, **31**, and **37**), and 355 and 202 ng/mL (**30**). Nose, K.; et al. Unpublished results.

(31) Bredereck, H.; Theilig, G. *Ber. Dtsch. Chem. Ges.* **1953**, *86*, 88–96.

(32) Birkofer, L.; Wegner, P.; Richter, P. *Ber. Dtsch. Chem. Ges.* **1966**, *99*, 2515–2517.

(33) Finnegan, W. G.; Henry, R. A.; Lofquist, R. An Improved Synthesis of 5-Substituted Tetrazoles. *J. Am. Chem. Soc.* **1958**, *80*, 3908–3911.

(34) Olofson, R. A.; Kendall, R. V. Protection by Acylation in the Selective Alkylation of Heterocycles. *J. Org. Chem.* **1970**, *35*, 2246–2248.

(35) Identification of regioisomers by alternative synthesis will be described in *J. Med. Chem.* (following paper in this issue).

(36) Avendano, C.; Gomenz-Molinero, E.; Pascual, C.; Elgueo, J. The Tautomerism of Diphenyl[5(4)-phenyl-1H-1,2,3-triazol-4(5)-yl]-methanol. A Carbon-13 Nuclear Magnetic Study. *J. Chem. Res. Synop.* **1986**, *2*, 78–79.

(37) Henry, R. A. Methylation of 5-Phenyltetrazole. *J. Am. Chem. Soc.* **1951**, *73*, 4470.

(38) Gante, J.; Kurmeier, H. A. Pharmacologically Active Basically Substituted Phenyl Ether(s). DE-2842759, **1979**.

(39) Kinnunen, P. M.; DeMichele, A.; Lange, L. G. Chemical Modification of Acyl-CoA:Cholesterol O-Acyltransferase. 1. Identification of Acyl-CoA:Cholesterol O-Acyltransferase Subtypes by Differential Diethyl Pyrocarbonate Sensitivity. *Biochemistry* **1988**, *27*, 7344–7350.

(40) Verloop, A.; Hoogenstraaten, M.; Tipker, J. *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1976, Vol. VII, pp 180–185.