Novel Tetrahydroisoquinoline Derivatives with Inhibitory Activities against Acyl-CoA: Cholesterol Acyltransferase and Lipid Peroxidation

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To find a novel acyl-CoA: cholesterol acyltransferase (ACAT) inhibitor with anti-lipid peroxidative activity, a series of tetrahydroisoquinoline derivatives were synthesized and evaluated. A compound with a *N*-(4-hydroxy-2,3,5-trimethylphenyl)carbamoyl moiety at the 3-position and an octanoyl moiety at the 2-position (7) was demonstrated to show anti-foam cell formation activity stronger than and anti-lipid peroxidative activity comparable to those of Pactimibe, while it was hardly absorbed orally. To increase its bioavailability, the acyl chain at the 2-position was shortened and various polar or basic moieties were introduced at the 7-position of 7. Among the synthesized derivatives, (*S*)-7-dimethylamino-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-2-isobutyryl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide hydrochloride (21) showed about 16-fold stronger anti-foam cell formation activity, 3-fold stronger hepatic ACAT inhibitory activity, similar anti-low density lipoprotein (LDL) oxidative activity and 2-fold more potent protective activity against macrophage cell death by oxidative stress in comparison with Pactimibe. Compound 21 was efficiently absorbed after oral administration at 10 mg/kg in rats and dogs and its C_{max} values were higher than its IC_{50} values for *in vitro* activities. In conclusion, a tetrahydroisoquinoline structure is a useful scaffold for designing a phenolic anti-oxidative ACAT inhibitor, and compound 21 is expected to effectively prevent atherosclerosis.

Key words acyl-CoA: cholesterol acyltransferase; tetrahydroisoquinoline; foam cell formation; lipid peroxidation; oxidative stress-induced cell death; Pactimibe

A great number of acyl-CoA: cholesterol acyltransferase (ACAT) inhibitors have been reported as potential hypolipidemic drugs and anti-atherosclerotic drugs, since ACAT plays an important role in intestinal absorption and hepatic secretion of cholesterol and accumulation of cholesterol in macrophages in atherosclerotic plaque[1—4]; however, none of the reported inhibitors have been successfully developed. Most of them were highly lipophilic, since ACAT inhibitory activity is dependent on the inhibitor's lipophilicity,[5,6] and were thus demonstrated to have low bioavailability. We previously synthesized a new indoline-based ACAT inhibitor, Pactimibe, with a carboxymethyl group at the 5-position, which showed moderate ACAT inhibitory activities; however, it was highly water soluble, and showed good oral absorption.[7] Pactimibe also has potent anti-oxidative activities, which were expected to exert synergetic anti-atherosclerotic effects with ACAT inhibitory activities, since oxidized low density lipoprotein (LDL) is taken up by macrophages and then cholesterol is esterified by ACAT to be accumulated in foam cells in atherosclerosis. Pactimibe decreased atherosclerotic areas in apo-E knockout mice and stabilized aortic plaques in Watanabe heritable hyperlipidemic rabbits (WHHL rabbits)[8,9]; however, a clinical study using an intravascular-ultrasonography catheter did not show the retardation of plaques at a dose of 100 mg in patients with coronary artery disease (CAD).[10] Pactimibe may have not fully inhibited plaque ACAT activity, and/or its ACAT-inhibitory and anti-oxidative activities may have not exerted synergetic anti-atherosclerotic effects in humans. The anti-oxidative activity of Pactimibe is due to its indoline structure, of which the tertiary amine at the 1-position chemically resembles that of aromatic dialkylamine. Indeed, ACAT inhibitors containing dimethylamino moiety, such as NTE-122, also show potent anti-oxidative activities[11]; however, the pharmacological effects of the aromatic amine-derived anti-oxidative activities have not been elucidated in atherosclerosis. On the other hand, a number of phenolic compounds have been reported to have anti-oxidative activities. α-Tocopherol and Probucol have been reported to show anti-atherosclerotic effects, probably due to their preventive activities against LDL oxidation.[12,13]

During the course of studies to find a new ACAT inhibitor with structural and biological properties different from Pactimibe, a tetrahydroisoquinoline derivative with a *N*-(4-hydroxy-2,3,5-trimethylphenyl)carbamoyl moiety at the 3-position and an octanoyl moiety at the 2-position (**7**) was found to have ACAT inhibitory and anti-oxidative activities comparable to those of Pactimibe, but to show poor bioavailability. To increase the bioavailability of **7**, a series of tetrahydroisoquinoline derivatives with polar or basic moieties at the 7-position were synthesized and evaluated.

Chemistry The synthetic routes of various 2-acyl-*N*-aryl-1,2,3,4-tetrahydroisoquinoline-3-carboxamides are shown in Chart 1—3. In Chart 1, the synthetic routes of *N*-aryl-2-octanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamides with no substitution at the 7-position (**7**, **8**, **10**) are outlined. The starting material **1**, which was easily prepared from L-phenylalanine,[14] was acylated with octanoyl chloride to give **2**. The methyl ester of **2** was hydrolyzed with NaOH to provide carboxylic acid (**3**), which was condensed with three anilines, 4-amino-2,3,6-trimethylphenyl acetate (**4**),[15] 2,6-diisopropylaniline, and 2,6-diisopropyl-4-nitroaniline (**5**), to give **6**, **8**, **9**, respectively. Compound **6** was hydrolyzed with LiOH to give **7**. The nitro moiety of compound **9** was

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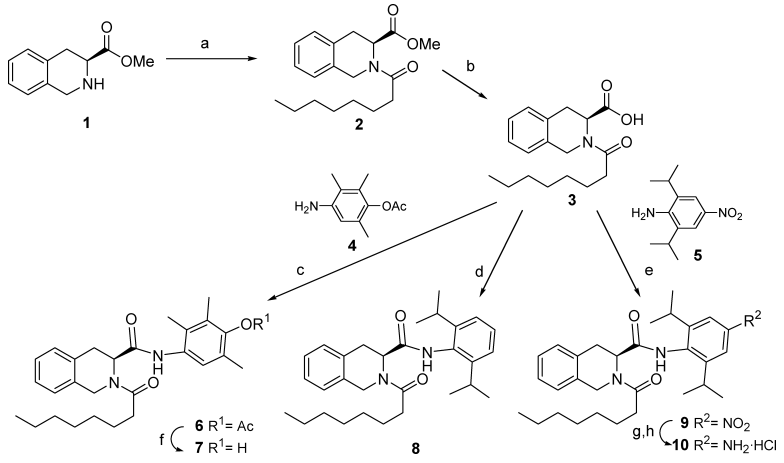
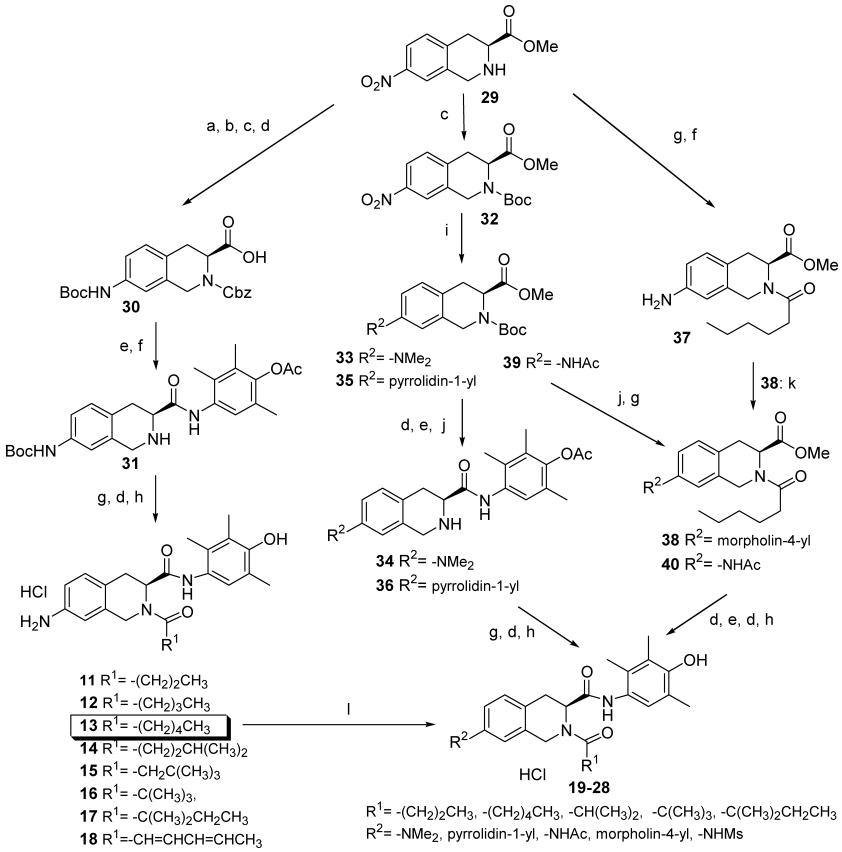


Reagents: (a) *n*-octanoyl chloride, Et_3N, CH_2Cl_2; (b) NaOH aq., MeOH; (c) **4**, $POCl_3$, pyridine, CH_2Cl_2; (d) 2,6-diisopropylaniline, EDC·HCl, CH_2Cl_2; (e) **5**, $POCl_3$, pyridine, CH_2Cl_2; (f) LiOH aq., MeOH; (g) H_2, Pd–C, MeOH; (h) HCl in *i*-PrOH, MeOH.

Chart 1. Synthesis of *N*-Aryl-2-octanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Derivatives with No Substitution at the 7-Position



Reagents: (a) CbzCl, MgO, acetone; (b) Fe, HCl aq., MeOH; (c) Boc_2O, $CHCl_3$ or THF; (d) LiOH aq., MeOH–THF; (e) **4**, EDC·HCl, CH_2Cl_2; (f) H_2, Pd–C, MeOH; (g) acyl chloride, Et_3N, CH_2Cl_2; (h) HCl in *i*-PrOH, MeOH; (i) **33**: H_2, Pd–C, formalin, HCl aq., MeOH; **35**: 1,4-dibromobutane, K_2CO_3, DMF; **39**: Ac_2O, Et_3N, CH_2Cl_2; (j) HCl in *i*-PrOH, HCO_2H; (k) **38**: $(ClCH_2CH_2)_2O$, K_2CO_3, KI, NMP; (l) **28**: MsCl, pyridine, $CHCl_3$.

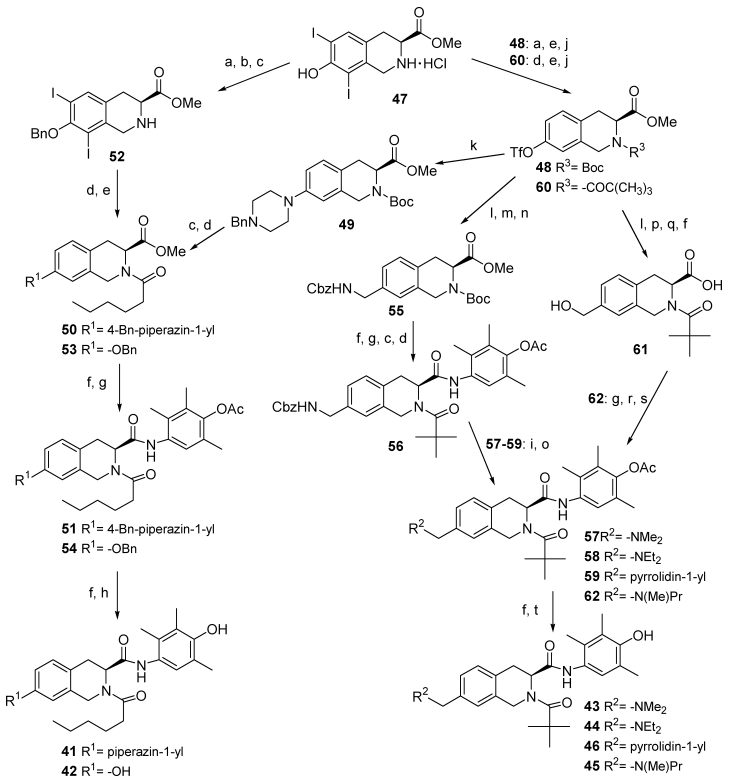
Chart 2. Synthesis of 2-Acyl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Derivatives with an Amino, Dimethylamino, Acetylamino, Methanesulfonylamino, Pyrrolidinyl, and Morpholinyl Moiety at the 7-Position

reduced by hydrogenation, and the product was converted to a HCl salt (**10**).

In Chart 2, the synthetic routes of 2-acyl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide derivatives with an amino, dimethylamino, acetylamino, methanesulfonylamino, pyrrolidinyl, and morpholinyl moiety at the 7-position (**11**—**28**) are outlined. Starting material **29**, which was obtained from nitration of **1**,[16] was protected with Cbz group, and the nitro moiety was reduced selectively with iron, protected with Boc_2O, and then hydrolyzed to give **30**. After condensation with the aniline **4**, the Cbz group was removed to give **31**. Compound **31** was acylated with eight acyl chlorides, and deprotected of the acetyl moiety and the Boc group to afford **11**—**18**. Sepa-

rately, compound **29** was protected with Boc_2O to give **32**, and the nitro moiety of **32** was converted to dimethylamino moiety by reductive alkylation to afford compound **33**, which was converted to **34** by hydrolyzation, condensation with the aniline **4** using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)·HCl, and removal of the Boc group. Compound **34** was acylated with five acyl chlorides, and the products were hydrolyzed and treated with hydrochloride to give **19**—**23**. Likewise, the nitro moiety of **32** was reduced, and the generated amino moiety was cyclized to 1-pyrrolidinyl moiety to give **35**, and then compound **35** was converted to **24** and **25** *via* **36** as described in the synthesis of **19**—**23**. To synthesize of **26**, the Boc group of **32** was changed to hexanoyl moiety, and the nitro moiety was reduced to give **37**, and then the amino moiety of **37** was cyclized with bis(2-chloroethyl) ether to give **38** having a morpholinyl moiety. Compound **38** was converted to **26** in a similar manner to the synthesis of **19**—**23**. Separately, compound **32** was reduced, and acetylated to give **39**. Compound **39** was treated with hydrochloride, and then acylated with hexanoyl chloride to afford **40**. Compound **40** was converted to **27** as described in the synthesis of **19**—**23**. Compound **28** was obtained directly from **13** by mesylation with pyridine as a base.

In Chart 3, the synthetic routes of 2-acyl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide derivatives with a hydroxyl, piperazinyl, and dialkylaminomethyl moiety at the 7-position (**41**—**46**) are outlined. To synthesize **41**, starting compound **47**, which was prepared from L-tyrosine in accordance with a reported method,[17] was converted to **48** with the triflate moiety at the 7-position by the usual methods. Compound **48** was reacted with 1-benzylpiperazine by palladium-catalyzed amination to give **49**,[18] and the Boc group at the 2-position was changed to the hexanoyl moiety to afford **50**. Compound **50** was hydrolyzed, and condensed with the aniline **4** to give **51**. The acetyl moiety of **51** was removed, and then the benzyl protection group of piperazinyl moiety was removed by hydrogenolyzation with $Pd(OH)_2$–C to afford **41**. To synthesize **42**, compound **47** was protected with benzyl bromide at the 7-position by temporary protection with Boc group at the 2-position, to give **52**. Compound **52** was acylated with hexanoyl chloride, and deiodination was achieved by hydrogenolyzation with Pd–C to give **53** without debenzylation. Compound **53** was converted to **42** *via* **54** in a similar way to the conversion of **50** to **41**. Separately, to synthesize **57**—**59**, the triflate moiety of **48** was replaced with a nitrile moiety by application of the palladium-catalyzed coupling reaction,[19] and the nitrile was hydrogenated in the presence of ammonia and Pd–C, and then the generated aminomethyl moiety was protected with CbzCl to give **55**. Conversion of **55** to **56** was accomplished as mentioned in the synthesis of **19**—**23**. The Cbz group of **56** was removed by hydrogenolyzation, and the aminomethyl moiety of the product was alkylated by reductive alkylation to give **57** and **58**, and cyclized with 1,4-dibromobutane to give **59**. The synthesis of **62** with an asymmetric tertiary amino moiety was difficult by this method;



Reagents: (a) Boc_2O, Et_3N, $CHCl_3$; (b) BnBr, K_2CO_3, acetone; (c) HCl in *i*-PrOH, HCO_2H; (d) acyl chloride, Et_3N, CH_2Cl_2; (e) H_2, Et_3N, Pd–C, MeOH; (f) LiOH aq, MeOH–THF; (g) **4**, EDC·HCl, CH_2Cl_2; (h) H_2, $Pd(OH)_2$–C, MeOH; (i) H_2, Pd–C, MeOH; (j) Tf_2O, 2,6-lutidine, CH_2Cl_2; (k) 1-benzylpiperazine, $Pd(OAc)_2$, BINAP, Cs_2CO_3, 1,4-dioxane; (l) KCN, $Pd(OAc)_2$, BINAP, NMP; (m) H_2, $Pd(OH)_2$–C, NH_4OH, MeOH; (n) CbzCl, MgO, AcOEt; (o) **57**: formalin, $NaBH_3CN$, MeOH; **58**: acetaldehyde, $NaBH_3CN$, MeOH; **59**: 1,4-dibromobutane, K_2CO_3, DMF; (p) HCO_2H, Raney nickel; (q) *tert*-butylamine borane, AcOEt; (r) MsCl, Et_3N, CH_2Cl_2; (s) PrNH(Me), THF; (t) **43**, **44**, **46**: HCl in *i*-PrOH, MeOH.

Chart 3. Synthesis of 2-Acyl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Derivatives with a Hydroxyl, Piperazinyl, and Dialkylaminomethyl Moiety at the 7-Position

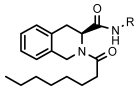
therefore, an alternative route was utilized. Compound **47** was converted to **60**, and the triflate moiety of **60** was replaced with a nitrile moiety, and the nitrile was reduced to aldehyde by Raney nickel.[20] Selective reduction of the aldehyde was accomplished with *tert*-butylamine borane complex,[21] and the product was hydrolyzed to give **61**. After condensation of **61** with the aniline **4**, the hydroxyl moiety was mesylated, and to the mesylate was attached *N*-methylpropylamine to give **62**. Compounds **57**—**59**, **62** were hydrolyzed, and the products were converted to HCl salts (**43**, **44**, **46**) or obtained as a free form (**45**), respectively.

Results and Discussion

Pactimibe exerted inhibitory effects on macrophage ACAT activities and LDL oxidation, and was efficiently absorbed orally, being expected to show potent anti-atherosclerotic effects by the synergism of both activities; however, Pactimibe failed to show a plaque-reducing effect in CAD patients.[10] Pactimibe may have not fully inhibited plaque ACAT activity and LDL oxidation, or both activities may not be effective in human atherosclerotic plaque. To clarify these possibilities, it is important to find new bioavailable ACAT inhibitors with structural and biological properties different from Pactimibe and to experimentally and clinically investigate its effects on atherosclerosis.

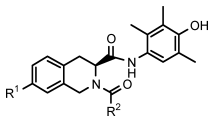
In the present study, a tetrahydroisoquinoline structure instead of indoline structure was employed to design new ACAT inhibitors. To find the lead compound, 3 derivatives (**7**, **8**, **10**) were synthesized and their log $D_{7.0}$, inhibitory activities against rabbit hepatic ACAT, foam cell formation induced by acetyl LDL, and LDL peroxidation were determined (Table 1). Among them, compound **7** showed the most potent inhibitory activities against ACAT, foam cell formation, and LDL peroxidation; however, all these compounds were highly lipophilic and were not detected in plasma after oral administration at 10 mg/kg in rats. Therefore, to decrease lipophilicity and increase bioavailability, the acyl chain at the 2-position was shortened and a polar or basic

Table 1. Chemical Structures, Molecular Weights, log $D_{7.0}$, and Inhibitory Activities against Liver ACAT, Foam Cell Formation, and LDL Oxidation of Tetrahydroisoquinoline Derivatives

	Compound	R	M.W.[a]	log $D_{7.0}$	Liver ACAT[b] (*vs.* Pac)	AFCF[c] (*vs.* Pac)	Anti-Ox[d] (*vs.* Pac)
	Pactimibe		416.6	3.26	1.0	1.0	1.0
	**7**		436.6	3.35	10	6.3	0.74
	8		462.7	4.84	2.2	1.9	<0.05
	10		477.7	4.13	0.96	2.4	<0.05

a) Molecular weight of free form. *b*) Ratio of inhibitory activities against normocholesterolemic rabbit liver ACAT activity (IC_{50} of Pactimibe/IC_{50} of test compound), duplicate assay. *c*) Ratio of inhibitory activities against foam cell formation in THP-1 cells (IC_{50} of Pactimibe/IC_{50} of test compound), duplicate assay. *d*) Ratio of inhibitory activities against LDL peroxidation (IC_{50} of Pactimibe/IC_{50} of test compound), duplicate assay.

Table 2. Chemical Structures, Molecular Weights, log $D_{7.0}$, and Inhibitory Activities against Foam Cell Formation of Tetrahydroisoquinoline Derivatives

	Compound	R^1	R^2	M.W.[a]	log $D_{7.0}$	AFCF[b] (*vs.* Pac)
	Pactimibe			416.6	3.26	1.0
	**11**			395.5	0.79	1.3
	12			409.5	1.19	5.0
	13			423.6	1.59	7.0
	14			423.6	1.51	7.0
	15			423.6	1.46	2.9
	16			409.5	1.10	1.5
	17			423.6	1.38	6.5
	18			419.5	1.18	5.2
	19			423.6	1.99	9.2
	29			451.6	2.80	40
	21			423.6	1.93	16
	22			437.6	2.35	113
	23			451.6	2.67	597

a) Molecular weight of free form. *b*) Ratio of inhibitory activities against foam cell formation in THP-1 cells (IC_{50} of Pactimibe/IC_{50} of test compound), duplicate assay.

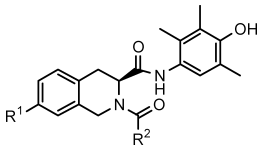
moiety was introduced at the 7-position in compound **7**.

In Table 2, compounds with a primary amino group at the 7-position and various acyl groups (**11**—**18**) had lower $\log D_{7.0}$ than 1.6, and still had 1.3 to 7.0-fold more potent anti-foam cell formation activities than Pactimibe. The activities increased in a $\log D_{7.0}$-dependent manner, and tended to be higher with straight alkyl chains than branched alkyl and straight alkenyl chains. They showed poor oral absorption; a primary amino moiety may have been easily metabolized. The most potent compound **13** with activity 7-fold higher than Pactimibe and comparable to **7** showed low plasma concentration after oral administration at 10 mg/kg in rats (C_{max}: 0.33 μg/ml). Compounds with a dimethylamino group at the 7-position (**19**—**23**) had lower $\log D_{7.0}$ (1.93—2.80) and markedly stronger anti-foam cell formation activity (9.2—597-fold) than Pactimibe. Interestingly, unlike derivatives with a primary amino group, activity was higher at branched alkyl chains than straight alkyl chains (**19** *vs.* **21** and **20** *vs.* **23**), and was extremely higher than the corresponding derivatives with a primary amino group. Further study is needed to clarify the interaction of derivatives with amino and dimethylamino groups, and ACAT protein. The C_{max} of **19**, **21**, **22**, **23** at 10 mg/kg (*per os* (*p.o.*)) in rats was 0.15, 0.91, 0.55 and 0.23 μg/ml, respectively. The C_{max} of **19** with butyryl was lower than that of **21** with isobutyryl, suggesting the lower metabolic stability of a straight alkyl chain than a branched chain. In **21**, **22** and **23**, C_{max} decreased dependently on $\log D_{7.0}$.

Finally, various basic and amide moieties were introduced at the 7-position (Table 3). Compounds with pyrrolidinyl and morpholinyl groups (**24**—**26**) showed relatively high $\log D_{7.0}$ (2.31—3.94) and markedly more potent activity (16—37-fold) than Pactimibe. Compounds with piperazinyl, acetylamino, methanesulfonylamino and hydroxyl groups (**41**, **27**, **28**, **42**) showed lower $\log D_{7.0}$ (1.36—1.79) and higher activity (2.7—9.8-fold). Compounds with a dialkylaminomethyl group (**43**—**46**) showed low $\log D_{7.0}$ and activity. Compound **43** with a dimethylaminomethyl group was markedly reduced compared to compound **22** with a dimethylamino group. Compound **45** with *N*-methylpropylaminomethyl group showed relatively high activities, but was hardly absorbed orally in rats.

In atherosclerosis, anti-oxidants are expected to inhibit LDL oxidation, resulting in reduction of the uptake of LDL

Table 3. Chemical Structures, Molecular Weights, $\log D_{7.0}$, and Inhibitory Activities against Foam Cell Formation of Tetrahydroisoquinoline Derivatives



Compound	R^1	R^2	M.W.[a]	$\log D_{7.0}$	AFCF[b] (*vs.* Pac)
Pactimibe			416.6	3.26	1.0
24			463.6	3.22	29
25			477.6	3.94	16
26			493.6	2.31	37
41			492.7	1.36	6.9
27			465.6	1.74	2.7
28			501.6	1.61	5.7
42			424.5	1.79	9.8
43			451.6	0.42	0.56
44			479.7	1.23	1.1
45			479.7	1.21	5.2
46			477.6	1.00	0.45

a) Molecular weight of free form. *b*) Ratio of inhibitory activities against foam cell formation in THP-1 cells (IC_{50} of Pactimibe/IC_{50} of test compound), duplicate assay.

Table 4. $\log D_{7.0}$, Inhibitory Activities against Liver ACAT, Foam Cell Formation, LDL Peroxidation, and Oxidative Stress-Induced Cell Death, and Plasma Concentrations of Compound **21** and Pactimibe

Compound	$\log D_{7.0}$	Liver ACAT[a]	AFCF[b]	Anti-Ox[c]	THP-1 death[d]	C_{max}[e]	
						Rat	Beagle
21	1.93	0.113±0.004	0.15±0.02	4.8	1.5±0.1	0.91±0.13	1.63±0.19
Pactimibe	3.26	0.312±0.027	2.45±0.25	3.8	3.1±0.4	0.15±0.02	6.98±0.35

a) IC_{50} (μM) against normocholesterolemic rabbit liver ACAT activity, mean±S.E. (n=3). *b*) IC_{50} (μM) against foam cell formation in THP-1 cells, mean±S.E. (n=3). *c*) IC_{50} (μM) against LDL peroxidation. *d*) IC_{50} (μM) against oxidative stress-induced cell death, mean±S.E. (n=6). *e*) Maximal plasma concentration (μg/ml) after oral administration at 10 mg/kg, mean±S.E. (n=3).

by macrophages and foam cell formation, and preventing oxidative stress-induced foam cell death, resulting in the reduction of cell debris, extracellular cholesterol deposition and inflammatory response. Probucol and KY-455, an indoline-based ACAT inhibitor, have been reported to attenuate the oxidative stress-induced apoptosis of THP-1 derived macrophages.[22,23] Compound **21** and Pactimibe concentration-dependently decreased macrophage cell death induced by co-incubation with LDL and Cu^{2+} (Table 4). The effect of compound **21** was 2-fold stronger than Pactimibe, whereas both compounds showed similar anti-LDL oxidative activities. The C_{max} of compound **21** was higher in rats and lower in dogs than Pactimibe, and exceeded the IC_{50} values for foam cell formation, LDL oxidation and cell death, anticipating that **21** would exert these effects *in vivo*. Phenolic antioxidants such as Probucol are also known to have anti-inflammatory effects *via* inhibition of adhesion molecule expression.[24] Future studies will reveal whether phenolic ACAT inhibitors have such anti-inflammatory effects beneficial for atherosclerosis therapy.

In conclusion, the present study demonstrated that a tetrahydroisoquinoline structure with a phenol moiety is useful for the design of bioavailable anti-oxidative ACAT inhibitors, and that **21** is expected to show anti-atherosclerotic effects by ACAT inhibitory and phenolic anti-oxidative activities.

Experimental

General Procedures Chemicals were obtained from commercial sources and used without purification. Reactions were monitored by TLC on Merck precoated silica gel 60 F_{254} (0.25 mm) plates. Column chromatography was performed on silica gel (Daisogel No. 1001W; Daiso, Osaka, Japan). Melting points were measured on melting point apparatus (MP-500P; Yanaco, Kyoto, Japan) and are uncorrected. IR spectra were obtained with an infrared spectrometer (FT-IR 8200PC; Shimadzu, Kyoto, Japan). ^{1}H-NMR spectra were recorded on a nuclear magnetic resonance spectrometer at 90 MHz (R-1900; Hitachi, Tokyo, Japan) or 400 MHz (JNM-AL400; JEOL, Tokyo, Japan) using tetramethylsilane as an internal standard. MS spectra were obtained on a QTRAP LC/MS/MS system (API2000; Applied Biosystems, Foster, CA, U.S.A.).

Procedure for the Synthesis of 7 4-Amino-2,3,6-trimethylphenyl Acetate (**4**): 2,3,6-Trimethylphenol was nitrated as described in a previous article.[15] The nitrated product was acetylated with AcCl and Et_3N, and hydrogenated in the presence of Pd–C in a standard manner to give **4** as a crystalline solid (78% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.04 (3H, s), 2.18 (6H, s), 2.30 (3H, s), 3.20—3.60 (2H, br), 6.41 (1H, s).

Methyl (*S*)-2-Octanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**2**): To a solution of **1** (1.00 g, 5.23 mmol) in CH_2Cl_2 (10 ml) were added Et_3N (1.09 ml, 7.82 mmol) and *n*-octanoyl chloride (0.98 ml, 5.7 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was washed with 10% citric acid solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography to give **2** as an oil (1.32 g, 80% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, br-t), 1.10—1.90 (10H, m), 2.21—2.60 (2H, m), 3.08—3.40 (2H, m), 3.61 (3H, s), 4.50—4.98 (2H, m), 5.48 (1H, t, J=5.4 Hz), 7.02—7.35 (4H, m).

(*S*)-2-Octanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (**3**): To a solution of **2** (1.00 g, 3.15 mmol) in MeOH (10 ml) was added 5.0 M NaOH aqueous solution (1.26 ml, 6.3 mmol) at room temperature, and the mixture was stirred at 50 °C for 1 h. After evaporation under reduced pressure, water and 6 M hydrochloric acid (1.1 ml) were added, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give **3** as an oil (947 mg, 99% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, br-t), 1.10—1.85 (10H, m), 2.20—2.60 (2H, m), 2.90—3.50 (2H, m), 4.30—5.05 (2H, m), 5.40 (1H, t, J=5.2 Hz), 6.98—7.42 (4H, m), 8.80 (1H, br-s).

(*S*)-*N*-(4-Hydroxy-2,3,5-trimethylphenyl)-2-octanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**7**): To a solution of 4-amino-2,3,6-trimethylphenyl acetate (**4**) (665 mg, 2.19 mmol) and compound **3** (364 mg, 1.88 mmol) in CH_2Cl_2 (7 ml) in an ice bath were added pyridine (0.46 ml, 5.7 mmol) and $POCl_3$ (0.28 ml, 3.0 mmol), and the mixture was stirred for 15 min. AcOEt was added, and the mixture was washed with 10% citric acid solution, saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography to give 2,3,6-trimethyl-4-{[(*S*)-2-octanoyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl]amino}phenyl acetate (**6**) as a powder (580 mg). To a solution of **6** (550 mg, 1.15 mmol) in MeOH (11 ml) was added 1.0 M LiOH aqueous solution (3.5 ml, 3.5 mmol), and the mixture was stirred at room temperature for 1 h. After addition of 6 M hydrochloric acid (0.6 ml), the mixture was concentrated under reduced pressure. The residue was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was rinsed with *i*-Pr_2O, and the precipitate was collected by filtration to give **7** as a solid (360 mg, 47% yield). mp 157—160 °C. IR (Nujol) cm^{-1}: 1647. MS *m/z*: 437 ($M+H^+$). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, br-t), 0.9—2.2 (13H, m), 2.04 (6H, s), 2.51 (2H, br-t), 2.8—3.4 (1H, m), 3.51 (1H, dd, J=15.4, 4.1 Hz), 4.3—5.4 (3H, m), 6.46 (1H, br-s), 6.95 (1H, s), 7.0—7.4 (5H, m), 7.86 (1H, br-s). *Anal.* Calcd for $C_{27}H_{36}N_2O_3$: C, 74.28; H, 8.31; N, 6.42. Found: C, 74.11; H, 8.39; N, 6.39.

Procedure for the Synthesis of 8 and 10 (*S*)-*N*-(2,6-Diisopropylphenyl)-2-octanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**8**): To a solution of **3** (913 mg, 3.01 mmol) in CH_2Cl_2 (9 ml) were added 2,6-diisopropylphenylamine (0.57 ml, 3.0 mmol) and EDC·HCl (692 mg, 3.61 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was washed with 10% citric acid solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography. The residue was recrystallized from benzene to **8** as a crystalline solid (335 mg, 24% yield). mp 146—148 °C. IR (Nujol) cm^{-1}: 1655. MS *m/z*: 463 ($M+H^+$). ^{1}H-NMR ($CDCl_3$) δ: 0.70—1.18 (15H, m), 1.18—1.92 (10H, m), 2.30—2.80 (4H, m), 2.88—3.78 (2H, m), 4.49—5.08 (2H, m), 5.40 (1H, br-t), 6.92—7.60 (8H, m). *Anal.* Calcd for $C_{30}H_{42}N_2O_2$: C, 77.88; H, 9.15; N, 6.05. Found: C, 77.78; H, 9.28; N, 5.95.

(*S*)-*N*-(4-Amino-2,6-diisopropylphenyl)-2-octanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**10**): To a solution of **3** (600 mg, 1.98 mmol) and compound **5** (396 mg, 1.80 mmol) in CH_2Cl_2 (6 ml) were added pyridine (0.48 ml, 5.9 mmol) and $POCl_3$ (0.24 ml, 2.6 mmol) in an ice bath, and the mixture was stirred for 3 h. The mixture was washed with 10% citric acid solution, saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography to give (*S*)-*N*-(2,6-diisopropyl-4-nitrophenyl)-2-octanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**9**) (812 mg). A solution of **9** (795 mg, 1.57 mmol) in MeOH (10 ml) was hydrogenated at 0.3 MPa in the presence of 10% Pd–C (160 mg) at 40 °C for 2.5 h. After filtration, the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography. To a solution of the residue in MeOH (2.6 ml) was added 10 M HCl in *i*-PrOH (0.13 ml, 1.3 mmol) in an ice bath. After evaporation under reduced pressure, Et_2O was added, and the precipitate formed was collected by filtration to give **10** as a solid (453 mg, 56% yield). mp 187—197 °C. IR (Nujol) cm^{-1}: 1636. MS *m/z*: 478 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.35—1.80 (25H, m), 2.10—4.00 (6H, m), 4.38—5.32 (3H, m), 7.03 (2H, s), 7.10—7.50 (4H, m), 8.91 (1H, s), 9.41 (1H, s), 8.60—11.50 (2H, br). *Anal.* Calcd for $C_{30}H_{43}N_3O_2 \cdot HCl \cdot 1.2H_2O$: C, 67.25; H, 8.73; N, 7.84. Found: C, 67.24; H, 8.67; N, 7.91.

Procedure for the Synthesis of 19—23 Compound **21** was synthesized from **29** *via* **32**, **33**, **34** as follows. Compounds **19**, **20**, **22**, and **23** were synthesized in a similar manner to the synthesis of **21**.

Methyl (*S*)-2-*tert*-Butoxycarbonyl-7-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**32**): To a solution of **29** (1.64 g, 6.94 mmol) in $CHCl_3$ (30 ml) was added Boc_2O (1.89 g, 8.66 mmol), and the mixture was stirred at room temperature for 14 h. After evaporation under reduced pressure, the residue was purified by column chromatography to give **32** as a crystalline solid (2.43 g, 100% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.51 (9H, s), 3.2—3.4 (2H, m), 3.64 (3H, s), 4.55 (1H, d, J=17.1 Hz), 4.85 (1H, d, J=17.1 Hz), 5.0—5.4 (1H, m), 7.2—7.4 (1H, m), 7.9—8.2 (2H, m).

Methyl (*S*)-2-*tert*-Butoxycarbonyl-7-dimethylamino-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**33**): A solution of **32** (3.57 g, 10.6 mmol) in MeOH (50 ml) was hydrogenated at 0.3 MPa in the presence of 10% Pd–C (0.25 g) at room temperature for 1.5 h. To the mixture were added formalin 2.4 ml (32 mmol) and 2.0 M hydrochloric acid (1.05 ml, 2.1 mmol), and the mixture was hydrogenated at 0.3 MPa for 3 h. After filtration, the filtrate was evaporated under reduced pressure. A solution of the residue in AcOEt was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was recrystallized from

AcOEt–hexane to give **33** as a crystalline solid (2.33 g, 66% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.45, 1.52 (total 9H, s, s), 2.8—3.3 (2H, m), 2.91 (6H, s), 3.63 (3H, s), 4.2—5.2 (3H, m), 6.4—6.7 (2H, m), 7.00 (1H, d, J=8.4 Hz).

4-{[(*S*)-7-Dimethylamino-1,2,3,4-tetrahydroisoquinoline-3-carbonyl]amino}-2,3,6-trimethylphenyl Acetate (**34**): To a solution of **33** (1.79 g, 5.35 mmol) in tetrahydrofuran (THF)–MeOH (3 : 1, 30 ml) in an ice bath was added 1.0 M LiOH aqueous solution (11 ml, 11 mmol), and the mixture was stirred at room temperature for 2 h. After neutralization with 5% citric acid solution, the mixture was concentrated under reduced pressure, and the residue was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the residue in CH_2Cl_2 (50 ml) in an ice bath were added **4** (1.03 g, 5.33 mmol) and EDC·HCl (1.23 g, 6.42 mmol), and the mixture was stirred for 30 min. After evaporation under reduced pressure, 5% citric acid solution was added to the residue, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography to give 4-{[(*S*)-2-*tert*-butoxycarbonyl-7-dimethylamino-1,2,3,4-tetrahydroisoquinoline-3-carbonyl]amino}-2,3,6-trimethylphenyl acetate as a crystalline solid (2.42 g, 91% yield). To a solution of the product (2.38 g, 4.80 mmol) in HCO_2H (7 ml) in an ice bath was added 10 M HCl in *i*-PrOH (1.5 ml, 15 mmol), and the mixture was stirred for 30 min. After neutralization with $NaHCO_3$, the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. Et_2O (50 ml) was added, and the precipitate formed was collected by filtration to give **34** as a crystalline solid (1.66 g, 87% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.71 (3H, s), 1.9—2.1 (1H, br), 2.12 (6H, s), 2.32 (3H, s), 2.6—3.5 (2H, m), 2.91 (6H, s), 3.5—4.0 (1H, m), 4.01 (2H, s), 6.47 (1H, d, J=1.6 Hz), 6.62 (1H, dd, J=8.5, 1.6 Hz), 7.08 (1H, d, J=8.5 Hz), 7.67 (1H, s), 9.23 (1H, s).

(*S*)-7-Dimethylamino-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-2-isobutyryl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**21**): Compound **34** was acylated with isobutyryl chloride as described in the preparation of **2**. A crystalline solid. Yield 89%. To a solution of the product (304 mg, 0.65 mmol) in THF–MeOH (3 : 1, 6 ml) in an ice bath was added 1.0 M LiOH aqueous solution (2.0 ml, 2.0 mmol), and the mixture was stirred for 1 h at room temperature. After neutralization with 10% citric acid solution, the mixture was concentrated under reduced pressure, and the residue was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a suspension of the residue (0.34 g) in MeOH (0.6 ml) in an ice bath was added 10 M HCl in *i*-PrOH (0.10 ml, 1.0 mmol), and the mixture was stirred for 10 min. After addition of Et_2O (20 ml), the mixture was stirred for 30 min, and the precipitate formed was collected by filtration to give **21** as a solid (302 mg, 100% yield). mp 161—164 °C. IR (Nujol) cm^{-1}: 1622. MS *m/z*: 424 (M+H$^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.9—1.4 (6H, m), 1.71 (3H, s), 2.04 (6H, s), 2.65 (2H, br-t), 2.8—3.5 (3H, m), 3.05 (6H, s), 4.0—6.0 (3H, m), 6.53 (1H, s), 7.2—7.7 (3H, m), 9.01, 9.32 (total 1H, br-s, br-s). *Anal.* Calcd for $C_{25}H_{33}N_3O_3\cdot1.5HCl\cdot2H_2O$: C, 58.39; H, 7.55; N, 8.17. Found: C, 58.77; H, 7.25; N, 8.19.

(*S*)-2-Butyryl-7-dimethylamino-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**19**): Compound **19** was synthesized from **34** using butyryl chloride instead of isobutyryl chloride. A solid. mp 144—148 °C. IR (Nujol) cm^{-1}: 1622. MS *m/z*: 424 (M+H$^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, br-t), 1.6—2.0 (5H, m), 2.03 (3H, s), 2.05 (3H, s), 2.0—2.6 (2H, m), 2.9—3.6 (2H, m), 3.05 (6H, s), 4.4—6.4 (5H, m), 6.50 (1H, s), 7.2—7.7 (3H, m), 8.96, 9.30 (total 1H, br-s, br-s). *Anal.* Calcd for $C_{25}H_{33}N_3O_3\cdot HCl\cdot2.5H_2O$: C, 59.45; H, 7.78; N, 8.32. Found: C, 59.71; H, 7.66; N, 8.45.

(*S*)-7-Dimethylamino-2-hexanoyl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**20**): Compound **20** was synthesized from **34** using hexanoyl chloride instead of isobutyryl chloride. A solid. mp 133—139 °C. IR (Nujol) cm^{-1}: 1651. MS *m/z*: 452 (M+H$^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.88 (3H, br-t), 1.0—1.8 (6H, m), 1.66 (3H, s), 2.03 (6H, s), 2.2—2.7 (2H, m), 3.0—3.6 (2H, m), 3.05 (6H, s), 4.0—6.0 (5H, m), 6.49 (1H, s), 7.2—7.7 (3H, m), 8.96, 9.33 (total 1H, br-s, br-s). *Anal.* Calcd for $C_{27}H_{37}N_3O_3\cdot HCl\cdot2.5H_2O$: C, 60.83; H, 8.13; N, 7.88. Found: C, 60.56; H, 7.98; N, 7.87.

(*S*)-7-Dimethylamino-2-(2,2-dimethylpropionyl)-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**22**): Compound **22** was synthesized from **34** using 2,2-dimethylpropionyl chloride instead of isobutyryl chloride. A solid. mp 162—166 °C. IR (Nujol) cm^{-1}: 1614. MS *m/z*: 438 (M+H$^+$). ^{1}H-NMR (DMSO-d_6) δ: 1.29 (9H, s), 1.79 (3H, s), 2.05 (6H, s), 2.9—3.4 (2H, m), 3.05 (6H, s), 4.0—6.0 (2H, br), 4.5—5.3 (3H, m), 6.60 (1H, s), 7.2—7.7 (3H, m), 9.18 (1H, br-s). *Anal.* Calcd for $C_{26}H_{35}N_3O_3\cdot HCl\cdot3H_2O$: C, 59.13; H, 8.02; N, 7.96. Found: C, 59.47; H, 7.83; N, 8.07.

(*S*)-7-Dimethylamino-2-(2,2-dimethylbutyryl)-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**23**): Compound **23** was synthesized from **34** using 2,2-dimethylbutyryl chloride instead of isobutyryl chloride. A solid. mp 150—156 °C. IR (Nujol) cm^{-1}: 1614. MS *m/z*: 452 (M+H$^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.80 (3H, br-t), 1.25 (6H, s), 1.6—2.0 (2H, m), 1.81 (3H, s), 2.06 (6H, s), 2.9—3.6 (2H, m), 3.05 (6H, s), 4.4—6.4 (2H, br), 4.62 (1H, d, J=16.3 Hz), 4.99 (1H, d, J=16.3 Hz), 5.11 (1H, br-t), 6.61 (1H, s), 7.2—7.7 (3H, m), 9.19 (1H, br-s). *Anal.* Calcd for $C_{27}H_{37}N_3O_3\cdot HCl\cdot2.8H_2O$: C, 60.22; H, 8.16; N, 7.80. Found: C, 60.22; H, 8.00; N, 7.94.

Procedure for the Synthesis of 11—18 Compound **11** was synthesized *via* **30**, **31** as follows. Compounds **12**—**18** were synthesized in a similar manner to the synthesis of **11**.

Methyl (*S*)-2-Benzyloxycarbonyl-7-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**65**): To a suspension of **29** (8.00 g, 33.9 mmol) in acetone (80 ml) in an ice bath were added MgO (2.05 g, 50.9 mmol) and CbzCl (5.3 ml, 37 mmol), and the mixture was stirred at room temperature for 16 h. After filtration, the filtrate was evaporated under reduced pressure. Hexane (20 ml) was added to the oily residue, and the mixture was stirred for 30 min. The supernatant was removed by decantation, and the residue was dried under reduced pressure to give **65** as an oil (9.30 g, 74% yield). ^{1}H-NMR ($CDCl_3$) δ: 3.28 (2H, br-d), 3.57, 3.63 (total 3H, s, s), 4.5—5.4 (3H, m), 5.24 (2H, s), 7.2—7.6 (6H, m), 7.9—8.2 (2H, m).

(*S*)-2-Benzyloxycarbonyl-7-*tert*-butoxycarbonylamino-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (**30**): To a suspension of **65** (9.30 g, 25.1 mmol) in 65% MeOH (260 ml) was added concentrated hydrochloric acid (2.6 ml), and the mixture was heated to be refluxed. Under refluxing, iron powder (5.61 g, 100 mmol) was added in portions over 10 min, and then the mixture was stirred for 1.5 h. Concentrated hydrochloric acid (2.6 ml) and iron powder (2.80 g, 50 mmol) were added, and the mixture was stirred for 1 h. After allowing to cool, the mixture was neutralized with $NaHCO_3$. The mixture was filtrated, and the filtrate was concentrated under reduced pressure. The residue was extracted with AcOEt, and the organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the residue in THF (40 ml) was added Boc_2O (5.48 g, 25.1 mmol), and the mixture was stirred at 45 °C for 16 h. After evaporation under reduced pressure, the residue was purified by column chromatography to give a Boc protected derivative. The product was hydrolyzed as described in the preparation of **3** to give **30** as a crystalline solid (6.91 g, 65% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.50 (9H, s), 3.0—3.4 (2H, m), 4.3—5.3 (3H, m), 5.19 (2H, s), 5.4—6.6 (1H, br), 6.57 (1H, br-s), 6.8—7.6 (8H, m).

4-{[(*S*)-7-*tert*-Butoxycarbonylamino-1,2,3,4-tetrahydroisoquinoline-3-carbonyl]amino}-2,3,6-trimethylphenyl Acetate (**31**): Compound **30** (6.91 g, 16.2 mmol) was condensed with compound **4** as described in the synthesis of **34**, and then the Cbz group of the product was removed by hydrogenolyzation in the presence of 10% Pd–C in MeOH to give **31**. A crystalline solid. Yield 58%. ^{1}H-NMR ($CDCl_3$) δ: 1.51 (9H, s), 1.86 (1H, br-s), 2.07 (3H, s), 2.12 (6H, s), 2.29 (3H, s), 2.85 (1H, dd, J=16.0, 9.5 Hz), 3.27 (1H, dd, J=16.0, 5.5 Hz), 3.70 (1H, dd, J=9.5, 5.5 Hz), 4.01 (2H, s), 6.47 (1H, br-s), 6.9—7.2 (2H, m), 7.2—7.4 (1H, m), 7.64 (1H, s), 9.21 (1H, br-s).

(*S*)-7-Amino-2-butyryl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**11**): Compound **31** (400 mg, 0.856 mmol) was acylated with butyryl chloride, hydrolyzed, and treated with HCl to give **11** as a solid (253 mg, 77% yield) in a manner similar to that described in the synthesis of **21**. mp 166—175 °C. IR (Nujol) cm^{-1}: 1622. MS *m/z*: 396 (M+H$^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.94 (3H, br-t), 1.4—2.0 (2H, m), 1.67 (3H, br-s), 2.05 (6H, s), 2.1—2.7 (2H, m), 2.8—4.4 (4H, br), 2.9—3.6 (2H, m), 4.4—5.3 (3H, m), 6.50 (1H, s), 7.0—7.5 (3H, m), 9.00 (1H, br-s). *Anal.* Calcd for $C_{23}H_{29}N_3O_3\cdot HCl\cdot2.5H_2O$: C, 57.91; H, 7.40; N, 8.81. Found: C, 57.79; H, 7.16; N, 8.71.

(*S*)-7-Amino-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-2-pentanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**12**): Compound **12** was synthesized from **31** using pentanoyl chloride instead of butyryl chloride. A solid. mp 167—171 °C. IR (Nujol) cm^{-1}: 1622. MS *m/z*: 410 (M+H$^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.91 (3H, br-t), 1.2—1.8 (4H, m), 1.66, 1.71 (total 3H, s, s), 2.03 (3H, s), 2.05 (3H, s), 2.3—2.7 (2H, m), 2.9—3.6 (2H, m), 4.4—5.3 (3H, m), 7.0—7.5 (4H, m), 8.99 (1H, br-s). *Anal.* Calcd for $C_{24}H_{31}N_3O_3\cdot HCl\cdot2H_2O$: C, 59.80; H, 7.53; N, 8.72. Found: C, 59.69; H, 7.47; N, 8.66.

(*S*)-7-Amino-2-hexanoyl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**13**): Compound **13**

was synthesized from **31** using hexanoyl chloride instead of butyryl chloride. A solid. mp 198—204 °C. IR (Nujol) cm^{-1}: 1653. MS *m/z*: 424 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.88 (3H, br-t), 1.0—1.8 (6H, m), 1.70 (3H, s), 2.03 (3H, s), 2.05 (3H, s), 2.2—2.7 (2H, m), 3.0—3.6 (2H, m), 4.4—5.3 (3H, m), 6.50 (1H, s), 7.0—7.5 (3H, m), 8.99, 9.34 (total 1H, br-s, br-s).

(*S*)-7-Amino-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-2-(4-methylpentanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**14**): Compound **14** was synthesized from **31** using 4-methylpentanoyl chloride instead of butyryl chloride. A solid. mp 165—175 °C. IR (Nujol) cm^{-1}: 1628. MS *m/z*: 424 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.91 (6H, d, J=5.0 Hz), 1.4—1.9 (3H, m), 1.66 (3H, s), 2.05 (6H, s), 2.1—2.6 (2H, m), 3.0—3.6 (2H, m), 4.4—5.3 (3H, m), 6.49 (1H, s), 7.1—7.6 (3H, m), 8.99, 9.36 (total 1H, br-s, br-s). *Anal*. Calcd for $C_{25}H_{33}N_3O_3S\cdot HCl\cdot 2.5H_2O$: C, 59.45; H, 7.78; N, 8.32. Found: C, 59.66; H, 7.60; N, 8.32.

(*S*)-7-Amino-2-(3,3-dimethylbutyryl)-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**15**): Compound **15** was synthesized from **31** using 3,3-dimethylbutyryl chloride instead of butyryl chloride. A solid. mp 165—170 °C. IR (Nujol) cm^{-1}: 1622. MS *m/z*: 424 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 1.04 (9H, s), 1.74 (3H, s), 2.05 (6H, s), 2.4—2.6 (2H, m), 3.0—3.4 (2H, m), 4.4—5.3 (3H, m), 6.53 (1H, s), 7.0—7.4 (3H, m), 9.04 (1H, br-s). *Anal*. Calcd for $C_{25}H_{33}N_3O_3\cdot 1.4HCl\cdot 1.8H_2O$: C, 59.22; H, 7.55; N, 8.29. Found: C, 59.29; H, 7.20; N, 8.42.

(*S*)-7-Amino-2-(2,2-dimethylpropionyl)-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**16**): Compound **16** was synthesized from **31** using 2,2-dimethylpropionyl chloride instead of butyryl chloride. A solid. mp 171—185 °C. IR (Nujol) cm^{-1}: 1651. MS *m/z*: 410 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 1.28 (9H, s), 1.80 (3H, s), 2.05 (6H, s), 2.8—4.0 (3H, m), 4.57 (1H, d, J=17.3 Hz), 4.95 (1H, d, J=17.3 Hz), 5.06 (1H, br-t), 6.59 (1H, s), 7.0—7.4 (3H, m), 9.18 (1H, br-s). *Anal.* Calcd for $C_{24}H_{31}N_3O_3\cdot HCl\cdot 2.5H_2O$: C, 58.71; H, 7.60; N, 8.56. Found: C, 58.49; H, 7.54; N, 8.50.

(*S*)-7-Amino-2-(2,2-dimethylbutyryl)-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**17**): Compound **17** was synthesized from **31** using 2,2-dimethylbutyryl chloride instead of butyryl chloride. A solid. IR (Nujol) cm^{-1}:1655. MS *m/z*: 424 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.79 (3H, br-t), 1.24 (6H, s), 1.5—1.9 (2H, m), 1.83 (3H, s), 2.06 (6H, s), 3.0—3.6 (2H, m), 4.3—5.2 (3H, m), 6.60 (1H, s), 7.0—7.5 (3H, m), 9.17 (1H, br-s).

(*S*)-7-Amino-2-(2*E*,4*E*)-hexa-2,4-dienoyl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**18**): Compound **18** was synthesized from **31** using *trans*-2,4-hexadienoyl chloride instead of butyryl chloride. A solid. mp 224—230 °C (dec.). IR (Nujol) cm^{-1}: 1651. MS *m/z*: 420 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 1.70 (3H, s), 1.83 (3H, d, J=4.6 Hz), 2.03 (3H, s), 2.14 (3H, s), 3.0—3.6 (2H, m), 4.5—5.4 (4H, m), 6.0—6.8 (3H, m), 6.52 (1H, s), 7.0—7.5 (4H, m), 9.0—9.5 (1H, m). *Anal.* Calcd for $C_{25}H_{29}N_3O_3\cdot HCl\cdot 0.8H_2O$: C, 63.83; H, 6.77; N, 8.93. Found: C, 63.70; H, 6.76; N, 8.91.

Procedure for the Synthesis of Compounds 24 and 25 Methyl (*S*)-2-*tert*-Butoxycarbonyl-7-pyrrolidin-1-yl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**35**): Compound **32** (2.92 g, 8.68 mmol) was hydrogenated as described in the preparation of **33**. A mixture of the residue (2.57 g), K_2CO_3 (2.90 g, 21.0 mmol) and 1,4-dibromobutane (1.2 ml, 10 mmol) in *N*,*N*-dimethylformamide (DMF) (26 ml) was stirred at 60 °C for 18 h. After addition of water, the mixture was extracted with AcOEt, and the organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography to give **35** as a crystalline solid (2.25 g, 74% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.44, 1.51 (total 9H, s, s), 1.7—2.2 (4H, m), 2.9—3.5 (6H, m), 3.62 (3H, s), 4.2—5.2 (3H, m), 6.2—6.5 (2H, m), 6.97 (1H, d, J=8.4 Hz).

2,3,6-Trimethyl-4-{[(*S*)-7-pyrrolidin-1-yl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl]amino}phenyl Acetate (**36**): Compound **35** was hydrolyzed, and condensed with compound **4**, and then the Boc group was removed with HCl as described in the preparation of **34** to afford **36**. A crystalline solid. Yield 73%. ^{1}H-NMR ($CDCl_3$) δ: 1.6—2.3 (4H, m), 2.07 (3H, s), 2.13 (6H, s), 2.32 (3H, s), 3.12 (1H, dd, J=18.0, 10.0 Hz), 3.0—3.5 (6H, m), 3.68 (1H, dd, J=10.0, 5.5 Hz), 3.99 (2H, s), 6.29 (1H, br-s), 6.45 (1H, dd, J=8.3, 2.3 Hz), 7.05 (1H, d, J=8.3 Hz), 7.67 (1H, s), 9.26 (1H, s).

(*S*)-*N*-(4-Hydroxy-2,3,5-trimethylphenyl)-2-pentanoyl-7-pyrrolidin-1-yl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**24**): Compound **36** was acylated with pentanoyl chloride, hydrolyzed, and converted to a HCl salt in a similar manner to that described in the synthesis of **21**. A solid. Yield 73%. mp 141—145 °C. IR (Nujol) cm^{-1}: 1647. MS *m/z*: 464 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 1.04 (9H, s), 1.60—1.9.0 (4H, m), 2.04 (9H, s), 2.50 (2H, s), 2.90—3.60 (6H, m), 4.40—5.30 (3H, m), 6.54 (1H, s), 6.80—7.30 (4H, m), 8.95, 9.25 (total 1H, s, s). *Anal*. Calcd for $C_{28}H_{37}N_3O_3\cdot HCl\cdot 2.5H_2O$: C, 61.69; H, 7.95; N, 7.71. Found: C, 61.73; H, 7.70; N, 7.76.

(*S*)-2-(3,3-Dimethylbutyryl)-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-7-pyrrolidin-1-yl-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**25**): Compound **25** was obtained from **36** using 3,3-dimethylbutyryl chloride, as described in the synthesis of **24**. A solid. Yield 54%. mp 144—148 °C. IR (Nujol) cm^{-1}: 1622. MS *m/z*: 478 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.9—1.4 (6H, m), 1.71 (3H, s), 2.04 (6H, s), 2.65 (2H, br-t), 2.8—3.5 (3H, m), 3.05 (6H, s), 4.0—6.0 (2H, br), 4.6—5.3 (3H, m), 6.53 (1H, s), 7.2—7.7 (3H, m), 9.01, 9.32 (total 1H, br-s, br-s). *Anal*. Calcd for $C_{29}H_{39}N_3O_3\cdot HCl\cdot 2.5H_2O$: C, 62.29; H, 8.11; N, 7.52. Found: C, 62.08; H, 7.86; N, 7.45.

Procedure for the Synthesis of 26 Methyl (*S*)-7-Amino-2-hexanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**37**): Compound **29** (2.00 g, 8.47 mmol) was acylated with hexanoyl chloride as described in the preparation of **2**. A solution of the obtained residue in MeOH (30 ml) was hydrogenated at 0.3 MPa in the presence of 10% Pd–C (290 mg) at room temperature for 3.5 h. After filtration, the filtrate was evaporated under reduced pressure, and the residue was purified by column chromatography to give **37** as an oil (2.29 g, 89% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, br-t), 1.0—2.0 (6H, m), 2.2—2.6 (2H, m), 2.9—3.4 (2H, m), 3.4—4.0 (2H, br), 3.61 (3H, s), 4.4—5.0 (2H, m), 5.43 (1H, br-t), 6.4—6.7 (2H, m), 6.93 (1H, d, J=7.9 Hz).

Methyl (*S*)-2-Hexanoyl-7-(morpholin-4-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**38**): A mixture of **37** (700 mg, 2.30 mmol), bis(2-chloroethyl) ether (329 mg, 2.30 mmol), K_2CO_3 (636 mg, 4.60 mmol), KI (191 mg, 1.15 mmol), and *N*-methyl-2-pyrrolidinone (3.5 ml) was heated at 100 °C for 5 h. After addition of water, the mixture was extracted with AcOEt, and the organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography to give **38** as a viscous oil (626 mg, 73% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, br-t), 1.1—1.8 (6H, m), 2.35 (2H, br-t), 2.8—3.5 (2H, m), 3.11 (4H, br-t), 3.61 (3H, s), 3.85 (4H, br-t), 4.4—5.6 (3H, m), 6.6—6.9 (2H, m), 7.07 (1H, d, J=8.3 Hz).

(*S*)-2-Hexanoyl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-7-(morpholin-4-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**26**): Compound **38** was hydrolyzed, condensed with compound **4**, and then hydrolyzed to give **26**, as described in the synthesis of **21**. A solid. Yield 87%. mp 133—137 °C. IR (Nujol) cm^{-1}: 1634. MS *m/z*: 494 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.88 (3H, br-t), 1.0—2.0 (6H, m), 1.65 (3H, s), 2.04 (6H, s), 2.2—2.6 (2H, m), 2.9—3.5 (6H, m), 3.7—4.2 (4H, m), 4.2—5.4 (5H, m), 6.51 (1H, s), 7.2—7.6 (3H, m), 8.94 (1H, br-s). *Anal.* Calcd for $C_{29}H_{39}N_3O_4\cdot HCl\cdot 2H_2O$: C, 61.52; H, 7.83; N, 7.42. Found: C, 61.42; H, 7.84; N, 7.51.

Procedure for the Synthesis of 27 Methyl (*S*)-7-Acetylamino-2-*tert*-butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**39**): A solution of **32** (1.00 g, 2.97 mmol) in MeOH (10 ml) was hydrogenated at 0.3 MPa in the presence of 10% Pd–C (100 mg) at room temperature for 2 h. After filtration, the filtrate was evaporated under reduced pressure. To a solution of the residue in CH_2Cl_2 (10 ml) in an ice bath were added Et_3N (0.62 ml, 4.5 mmol) and Ac_2O (0.25 ml, 3.5 mmol), and the mixture was stirred for 15 min. The mixture was washed with 5% citric acid solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The solid residue was washed with Et_2O to give **39** as a crystalline solid (740 mg, 71% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.14 (3H, s), 2.9—3.4 (2H, m), 3.61 (3H, s), 4.3—5.2 (3H, m), 7.0—7.6 (4H, m).

Methyl (*S*)-7-Acetylamino-2-hexanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**40**): Compound **39** was treated with HCl, and acylated with hexanoyl chloride to give **40** in a manner similar to the preparation of **21**. An oil. Yield 68%. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, br-t), 1.1—1.8 (6H, m), 2.15 (3H, s), 2.47 (2H, br-t), 2.8—3.5 (2H, m), 3.60 (3H, s), 4.3—5.0 (2H, m), 5.0—5.2 (0.2H, m), 5.50 (0.8H, dd, J=5.6, 4.1 Hz), 6.8—7.2 (2H, m), 7.3—7.8 (2H, m).

(*S*)-7-Acetylamino-2-hexanoyl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**27**): Compound **40** was converted to **27** in a similar manner to the synthesis of **26**. A crystalline solid. Yield 65%. mp 147—151 °C. IR (Nujol) cm^{-1}: 1651. MS *m/z*: 466 ($M+H^+$). ^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ: 0.90 (3H, br-t), 1.1—1.8 (6H, m), 1.85 (3H, s), 2.10 (9H, s), 2.51 (2H, br-t), 2.8—3.6 (3H, m), 4.3—5.5 (3H, m), 6.86 (1H, br-s), 6.5—8.1 (4H, m), 8.94 (1H, br-s). *Anal.* Calcd for $C_{27}H_{35}N_3O_4\cdot H_2O$: C, 67.06; H, 7.71; N, 8.69. Found: C, 67.24; H, 7.61; N,

8.64.

Procedure for the Synthesis of 28 (*S*)-2-Hexanoyl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-7-methanesulfonylamino-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**28**): To a solution of **13** (350 mg, 0.826 mmol) in $CHCl_3$ (3.5 ml) in an ice bath were added pyridine (0.12 ml, 1.2 mmol) and MsCl (0.06 ml, 0.78 mmol), and the mixture was stirred at room temperature for 1 h. After addition of 10% citric acid solution, the precipitate formed was collected by filtration to give **28** as a crystalline solid (297 mg, 72% yield). mp 168—173 °C. IR (Nujol) cm^{-1}: 1649, 1634. MS *m/z*: 502 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.70—1.10 (3H, br-t), 1.10—1.80 (6H, m), 2.03 (9H, s), 2.10—2.70 (2H, m), 2.70—3.50 (2H, m), 2.94 (3H, s), 4.40—5.20 (3H, m), 6.45 (1H, s), 6.80—7.30 (3H, m), 7.60—8.20 (1H, br), 8.88, 9.15 (total 1H, s, s), 9.62 (1H, s). *Anal.* Calcd for $C_{26}H_{35}N_3O_5S\cdot 0.5H_2O$: C, 61.15; H, 7.11; N, 8.23. Found: C, 61.21; H, 6.91; N, 8.07.

Procedure for the Synthesis of 41 Methyl (*S*)-2-*tert*-Butoxycarbonyl-7-trifluoromethanesulfonyloxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**48**): Compound **47** was protected with Boc_2O at the 2-position, and hydrogenolyzed to give methyl (*S*)-2-*tert*-butoxycarbonyl-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**63**) as described in the synthesis of **32** and **53**. To a solution of **63** (5.00 g, 16.3 mmol) in CH_2Cl_2 (50 ml) in an ice bath were added 2,6-lutidine (2.7 ml, 23 mmol) and Tf_2O (5.53 g, 19.6 mmol) slowly, and then the mixture was stirred for 30 min. After evaporation under reduced pressure, 1.0 M hydrochloride acid (25 ml) was added, and the mixture was extracted with Et_2O. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and then evaporated under reduced pressure. The residue was purified by column chromatography to give **48** as an oil (6.92 g, 97% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.51 (9H, s), 3.0—3.3 (2H, m), 3.64 (3H, s), 4.3—5.3 (3H, m), 6.9—7.3 (3H, m).

Methyl (*S*)-7-(4-Benzylpiperazin-1-yl)-2-hexanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**50**): Under a nitrogen atmosphere, a suspension of $Pd(OAc)_2$ (85 mg, 0.32 mmol) and *rac*-BINAP (262 mg, 0.42 mmol) in dehydrated 1,4-dioxane (1 ml) was stirred for 30 min, and a solution of **48** (1.42 g, 3.23 mmol) and 1-benzylpiperazine (684 mg, 3.88 mmol) in 1,4-dioxane (4 ml) and Cs_2CO_3 (1.47 g, 4.51 mmol) were added. The mixture was aerated with nitrogen for 10 min, and stirred at 80 °C for 5 h. After allowing to cool, AcOEt was added to the mixture. After filtration, the filtrate was evaporated under reduced pressure, and the residue was purified by column chromatography to give **49** as a viscous oil (745 mg, 50% yield). The Boc group of **49** was changed to hexanoyl moiety, as described in the synthesis of **38** to give **50**. An oil. Yield 83%. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, br-t), 1.1—2.0 (6H, m), 2.2—2.8 (6H, m), 3.0—3.4 (6H, m), 3.56 (2H, s), 3.60 (3H, s), 4.3—5.6 (3H, m), 6.6—6.9 (2H, m), 7.03 (1H, d, *J*=8.1 Hz), 7.2—7.5 (5H, m).

4-{[(*S*)-7-(4-Benzylpiperazin-1-yl)-2-hexanoyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl]amino}-2,3,6-trimethylphenyl Acetate (**51**): Compound **50** was hydrolyzed, and condensed with compound **4** in a manner similar to the synthesis of **21** to give **51**. A crystalline solid. Yield 74%. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, br-t), 1.0—2.0 (6H, m), 1.98 (3H, s), 2.04 (6H, s), 2.2—2.8 (6H, m), 2.30 (3H, s), 2.9—3.5 (6H, m), 3.57 (2H, s), 4.3—5.4 (3H, m), 6.6—7.0 (2H, m), 7.0—7.6 (7H, m), 8.09 (1H, br-s).

(*S*)-2-Hexanoyl-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-7-(piperazin-1-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**41**): Compound **51** was deacetylated by usual method. Yield 76%. The obtained deacetylated compound (570 mg, 0.98 mmol) in MeOH (20 ml) was hydrogenolyzed at 0.4 MPa in the presence of 20% $Pd(OH)_2$–C (110 mg) at 40 °C for 9 h. After filtration, the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography to give **41** as a crystalline solid (283 mg, 59% yield). mp 155—174 °C. IR (Nujol) cm^{-1}: 1622. MS *m/z*: 493 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 0.87 (3H, br-t), 1.0—1.8 (6H, m), 1.69 (3H, s), 2.04 (6H, s), 2.1—4.0 (14H, m), 4.4—5.2 (3H, m), 6.51 (1H, s), 6.6—7.2 (3H, m), 9.13 (1H, br-s). *Anal.* Calcd for $C_{29}H_{40}N_4O_3\cdot 2H_2O$: C, 65.88; H, 8.39; N, 10.60. Found: C, 66.13; H, 8.13; N, 10.48.

Procedure for the Synthesis of 42 Methyl (*S*)-7-Benzyloxy-6,8-diiodo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate Hydrochloride (**52**): To a suspension of compound **47** (20.0 g, 40.4 mmol) in $CHCl_3$ (150 ml) in an ice bath were added Et_3N (12.4 ml, 89.0 mmol) and a solution of Boc_2O (9.69 g, 44.4 mmol) in $CHCl_3$ (50 ml), and the mixture was stirred at room temperature for 16 h. The mixture was washed with 1.0 M hydrochloric acid and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography. To a solution of the obtained residue in acetone (200 ml) were added benzyl bromide (5.4 ml, 45 mmol) and K_2CO_3 (7.90 g, 57.2 mmol), and the mixture was stirred at 60 °C for 2.5 h. After addition of AcOEt (100 ml), the mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography. To a solution of the obtained residue in Et_2O (30 ml) in an ice bath were added HCO_2H (90 ml) and 8.8 M HCl in *i*-PrOH (12.7 ml, 0.11 mol), and the mixture was stirred for 1.5 h. After addition of Et_2O (360 ml), the precipitate formed was collected by filtration to give **52** as a crystalline solid (18.8 g, 79% yield). ^{1}H-NMR (DMSO-d_6) δ: 3.26 (2H, d, *J*=6.5 Hz), 3.82 (3H, s), 4.13 (2H, s), 4.49 (1H, br-t), 4.93 (2H, s), 7.3—8.0 (6H, m), 10.2—11.0 (2H, br).

Methyl (*S*)-7-Benzyloxy-2-hexanoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**53**): Compound **52** (4.00 g, 6.83 mmol) was acylated with hexanoyl chloride, as described in the preparation of **2**. A solution of the residue in MeOH (40 ml) was hydrogenated at 0.3 MPa in the presence of 10% Pd–C (200 mg) at 25 °C for 2 h. After filtration, the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography to give **53** as an oil (1.93 g, 71% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, br-t), 1.1—1.9 (6H, m), 2.2—2.7 (2H, m), 2.8—3.4 (2H, m), 3.66 (3H, s), 4.3—4.9 (2H, m), 4.8—5.0 (0.2H, m), 5.03 (2H, s), 5.39 (0.8H, dd, *J*=5.7, 4.4 Hz), 6.4—7.0 (2H, m), 7.11 (1H, d, *J*=7.8 Hz), 7.2—7.6 (5H, m).

4-{[(*S*)-7-Benzyloxy-2-hexanoyl-1,2,3,4-tetrahydroisoquinoline-3-carbonyl]amino}-2,3,6-trimethylphenyl Acetate (**54**): Compound **53** was hydrolyzed, and condensed with compound **4** in a manner similar to the synthesis of **21** to give **54**. A crystalline solid. Yield 61%. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, br-t), 1.1—2.0 (6H, m), 1.58 (3H, s), 2.04 (6H, s), 2.2—2.6 (2H, m), 2.30 (3H, s), 2.7—3.6 (2H, m), 4.4—5.4 (3H, m), 5.04 (2H, s), 6.7—7.0 (2H, m), 7.0—7.6 (7H, m), 8.13 (1H, br-s).

(*S*)-2-Hexanoyl-7-hydroxy-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**42**): Compound **42** was obtained from **54** in a manner similar to the synthesis of **41**. A crystalline solid. Yield 63%. mp 213—217 °C. IR (Nujol) cm^{-1}: 3600—3100, 1636. MS *m/z*: 425 ($M+H^+$). ^{1}H NMR (DMSO-d_6) δ: 0.88 (3H, br-t), 1.1—2.0 (6H, m), 1.68 (3H, s), 2.03 (6H, s), 2.2—2.6 (2H, m), 2.7—3.6 (2H, m), 4.3—5.0 (2H, m), 5.01 (1H, br-t), 6.4—6.8 (3H, m), 6.97 (1H, d, *J*=7.9 Hz), 7.92, 8.84, 9.11, 9.19 (total 3H, br-s, br-s, br-s, br-s). *Anal.* Calcd for $C_{25}H_{32}N_2O_4\cdot 0.3H_2O$: C, 69.84; H, 7.64; N, 6.52. Found: C, 69.75; H, 7.55; N, 6.49.

Procedure for the Synthesis of 43, 44, and 46 Compound **43** was synthesized from **48** *via* **55**, **56** as follows. Compounds **44** and **46** were synthesized from **56** in a similar manner to the synthesis of **43**.

Methyl (*S*)-2-*tert*-Butoxycarbonyl-7-cyano-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**64**): After a suspension of $Pd(OAc)_2$ (153 mg, 0.579 mmol) and *rac*-BINAP (467 mg, 0.750 mmol) in dehydrated *N*-methyl-2-pyrrolidinone (NMP) (3 ml) was stirred at room temperature for 1 h under a nitrogen atmosphere, KCN (892 mg, 13.7 mmol) and a solution of **48** (3.00 g, 6.83 mmol) in NMP (6 ml) were added, and the mixture was stirred at 80 °C for 10 h. After allowing to cool, water (60 ml) and Et_2O (20 ml) were added, and the mixture was stirred for 10 min. After filtration, the filtrate was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. After the residue had been purified by column chromatography, the residue was washed with hexane, and the precipitate was collected by filtration to give **64** as a crystalline solid (1.65 g, 76% yield). IR (neat) cm^{-1}: 2230. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (9H, s), 3.0—3.4 (2H, m), 3.63 (3H, s), 4.3—5.3 (3H, m), 7.25 (1H, d, *J*=8.1 Hz), 7.3—7.5 (2H, m).

7-Benzyloxycarbonylaminomethyl-2-*tert*-butoxycarbnoyl-2-(2,2-dimethylpropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**55**): A mixture of **64** (2.19 g, 6.92 mmol), 28% aqueous ammonia (8.4 g, 0.14 mol), and MeOH (22 ml) was hydrogenated at 0.4 MPa in the presence of 20% $Pd(OH)_2$–C (0.22 g) at 35 °C for 15 h. After filtration, the filtrate was evaporated under reduced pressure. A solution of the residue in $CHCl_3$ was dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography to give an aminomethyl derivative as an oil (712 mg, 32% yield). To a solution of the product (900 mg, 2.81 mmol) in AcOEt (9 ml) were added MgO (340 mg, 8.43 mmol) and CbzCl (0.60 ml, 4.2 mmol), and the mixture was stirred at room temperature for 24 h. After filtration, the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography to give **55** as an oil (882 mg, 69% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.47, 1.51 (total 9H, s, s), 3.0—3.3 (2H, m), 3.61 (3H, s), 4.33 (2H, d, *J*=5.8 Hz), 4.4—5.4 (3H, m), 5.14 (2H, s), 7.0—7.2 (4H, m), 7.2—7.5 (5H, m).

4-{[(*S*)-7-Benzyloxycarbonylaminomethyl-2-(2,2-dimethylpropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carbonyl]amino}-2,3,6-trimethylphenyl Acetate (**56**): Compound **55** was hydrolyzed, condensed with compound **4**, treated with acid to remove the Boc group, and acylated with 2,2-dimethylpropionyl chloride in a similar manner to the preparation of **21**. A crystalline solid. Yield 63%. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (9H, s), 1.98 (3H, br-s), 2.03

(3H, s), 2.06 (3H, s), 2.31 (3H, s), 3.05 (1H, dd, J=16.2, 6.4 Hz), 3.49 (1H, dd, J=16.2, 4.6 Hz), 4.35 (2H, br-t), 4.50 (1H, d, J=16.1 Hz), 4.96 (1H, d, J=16.1 Hz), 5.00—5.10 (1H, br), 5.13 (2H, s), 5.32 (1H, dd, J=6.4, 4.6 Hz), 7.07 (1H, s), 7.16 (1H, d, J=7.8 Hz), 7.21 (1H, d, J=7.8 Hz), 7.30—7.40 (5H, m), 7.44 (1H, s), 7.96 (1H, br-s).

(*S*)-7-Dimethylaminomethyl-2-(2,2-dimethylpropionyl)-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**43**): A solution of **56** (315 mg, 0.525 mmol) in MeOH (6 ml) was hydrogenolyzed at 0.3 MPa in the presence of 10% Pd–C (30 mg) at 25 °C for 4 h. After filtration, the filtrate was evaporated under reduced pressure. To a solution of the residue in MeOH (2 ml) were added formalin (172 mg, 2.1 mmol) and $NaBH_3CN$ (74 mg, 1.1 mmol), and the mixture was stirred at room temperature for 1 h. After evaporation under reduced pressure, saturated $NaHCO_3$ solution was added, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give **57**. To a solution of the residue in THF–MeOH (3 : 1, 5 ml) in an ice bath was added 1.0 M LiOH aqueous solution (1.6 ml, 1.6 mmol), and the mixture was stirred for 1.5 h. After addition of 2.0 M hydrochloric acid (0.8 ml), the mixture was concentrated under reduced pressure. Saturated $NaHCO_3$ solution was added, and the mixture was extracted with AcOEt. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography. To a solution of the residue in MeOH (0.5 ml) in an ice bath was added 8.7 M HCl in *i*-PrOH (0.05 ml, 0.4 mmol), and the mixture was stirred for 5 min. Et_2O (60 ml) was added, and stirred for 30 min. The precipitate formed was collected by filtration to give **43** as a solid (128 mg, 50% yield). IR (Nujol) cm^{-1}: 1668. MS *m*/*z*: 452 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 1.29 (9H, s), 1.77 (3H, br-s), 2.04 (3H, s), 2.06 (3H, s), 2.66 (3H, s), 2.67 (3H, s), 3.15—3.40 (2H, m), 4.21 (2H, br-d), 4.60 (1H, d, J=16.7 Hz), 4.91 (1H, d, J=16.7 Hz), 4.95—5.20 (1H, m), 6.56 (1H, br-s), 7.31 (1H, d, J=7.6 Hz), 7.39 (1H, d, J=7.6 Hz), 7.44 (1H, s), 8.00 (1H, br-s), 9.21 (1H, br-s), 10.53 (1H, br-s).

7-Diethylaminomethyl-2-(2,2-dimethylpropionyl)-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**44**): Compound **44** was obtained from **56** *via* **58** in a similar manner to the synthesis of **43** using acetaldehyde instead of formalin. A solid. Yield 49%. mp 157—165 °C. IR (Nujol) cm^{-1}:1674. MS *m*/*z*: 480 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 1.24 (6H, t, J=16.2 Hz), 1.29 (9H, s), 1.75 (3H, br-s), 2.04 (3H, s), 2.06 (3H, s), 2.90—3.20 (4H, m), 3.20—3.50 (2H, m), 4.23 (2H, br-s), 4.60 (1H, d, J=16.1 Hz), 4.92 (1H, d, J=16.1 Hz), 4.95—5.20 (1H, m), 6.57 (1H, br-s), 7.25—7.55 (3H, m), 8.00 (1H, br-s), 9.20 (1H, br-s), 10.40—10.70 (1H, br). *Anal.* Calcd for $C_{29}H_{41}N_3O_3\cdot HCl\cdot 1.7H_2O$: C, 63.71; H, 8.37; N, 7.69. Found: C, 63.73; H, 8.30; N, 7.63.

4-{[(*S*)-2-(2,2-Dimethylpropionyl)-7-(pyrrolidin-1-ylmethyl)-1,2,3,4-tetrahydroisoquinoline-3-carbonyl]amino}-2,3,6-trimethylphenyl Acetate (**59**): Compound **56** (4.02 g, 8.71 mmol) was hydrogenolyzed. A mixture of the residue, DMF (40 ml), 1,4-dibromobutane (1.14 ml, 9.55 mmol), and K_2CO_3 (3.01 g, 21.8 mmol) was heated at 55 °C for 3 h. After allowing to cool, 0.26 M hydrochloric acid (200 ml) was added, and $NaHCO_3$ was added until pH>8. The mixture was extracted with AcOEt, and the organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography to give **59** as a crystalline solid (1.50 g, 33% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.37 (9H, s), 1.70—1.85 (4H, m), 1.98 (3H, br-s), 2.03 (3H, s), 2.07 (3H, s), 2.31 (3H, s), 2.40—2.60 (4H, m), 3.06 (1H, dd, J=16.0, 6.7 Hz), 3.50 (1H, dd, J=16.0, 5.0 Hz), 3.58 (1H, d, J=12.9 Hz), 3.62 (1H, d, J=12.9 Hz), 4.53 (1H, d, J=15.6 Hz), 4.96 (1H, d, J=15.6 Hz), 5.29 (1H, dd, J=6.7, 5.0 Hz), 7.10—7.25 (3H, m), 7.41 (1H, s), 7.93 (1H, br-s).

(*S*)-2-(2,2-Dimethylpropionyl)-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-7-(pyrrolidin-1-ylmethyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Hydrochloride (**46**): Compound **59** was converted to **46** as described in the synthesis of **43**. A solid. Yield 89%. mp 169—173 °C. IR (Nujol) cm^{-1}: 1674. MS *m*/*z*: 478 ($M+H^+$). ^{1}H-NMR (DMSO-d_6) δ: 1.29 (9H, s), 1.70—1.95 (4H, m), 2.00 (3H, br-s), 2.04 (3H, s), 2.06 (3H, s), 2.95—3.10 (2H, m), 3.15—3.45 (4H, m), 4.28 (2H, br-s), 4.60 (1H, d, J=16.7 Hz), 4.91 (1H, d, J=16.7 Hz), 4.95—5.20 (1H, m), 6.57 (1H, br-s), 7.30 (1H, d, J=7.1 Hz), 7.35—7.55 (2H, m), 7.99 (1H, br-s), 9.18 (1H, br-s), 10.20—11.00 (1H, br). *Anal*. Calcd for $C_{29}H_{39}N_3O_3\cdot HCl\cdot 1.6H_2O$: C, 64.15; H, 8.02; N, 7.74. Found: C, 64.01; H, 7.72; N, 7.69.

Procedure for the Synthesis of 45 Compound **45** was obtained from **47** *via* **60**, **61**, and **62**.

Methyl (*S*)-2-(2,2-Dimethylpropionyl)-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**66**): Compound **47** was acylated at the 2-position with 2,2-dimethylpropionyl chloride, and hydrogenolyzed as described in the preparation of **48** to give **66**. The obtained crude crystal was recrystallized from AcOEt–hexane. A crystalline solid. Yield 78%. ^{1}H-NMR ($CDCl_3$) δ: 1.34 (9H, s), 3.00—3.25 (2H, m), 3.65 (3H, s), 4.56 (1H, br-d), 4.93 (1H, d, J=16.4 Hz), 5.05—5.25 (1H, m), 5.50—6.50 (1H, br), 6.60—6.80 (2H, m), 7.00 (1H, d, J=8.6 Hz).

Methyl (*S*)-2-(2,2-Dimethylpropionyl)-7-trifluoromethanesulfonyloxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**60**): Compound **66** was triflated as described in the preparation of **48** to give **60**. A crystalline solid. Yield 90%. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (9H, s), 3.17 (1H, dd, J=16.2, 6.1 Hz), 3.28 (1H, br-dd), 3.67 (3H, s), 4.61 (1H, br-d), 5.04 (1H, d, J=16.8 Hz), 5.31 (1H, dd, J=6.1, 4.4 Hz), 7.06 (1H, d, J=2.4 Hz), 7.12 (1H, dd, J=8.4, 2.4 Hz), 7.25 (1H, d, J=8.4 Hz).

Methyl (*S*)-7-Cyano-2-(2,2-dimethylpropionyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**67**): Compound **60** was reacted with KCN as described in the preparation of **64** to give **67**. A crystalline solid. Yield 80%. IR (Nujol) cm^{-1}: 2235. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (9H, s), 3.21 (1H, dd, J=16.6, 6.2 Hz), 3.31 (1H, br-dd), 3.67 (3H, s), 4.61 (1H, br-d), 5.04 (1H, d, J=16.8 Hz), 5.33 (1H, dd, J=6.2, 4.4 Hz), 7.29 (1H, d, J=7.9 Hz), 7.44 (1H, s), 7.49 (1H, d, J=7.9 Hz).

Methyl (*S*)-2-(2,2-Dimethylpropionyl)-7-formyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**68**): To a solution of **67** (34.1 g, 114 mmol) in 80% formic acid (340 ml) heated at 90 °C was added Raney nickel (34 g) in portions, and the mixture was refluxed for 20 min. The mixture was poured into ice-water (3.4 l), and stirred for 1 h at room temperature. After collecting the precipitate by filtration, a solution of the residue in AcOEt was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was recrystallized from AcOEt–hexane to give **68** as a crystalline solid (18.1 g, 53% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (9H, s), 3.23 (1H, dd, J=16.4, 6.2 Hz), 3.64 (1H, br-dd), 3.66 (3H, s), 4.68 (1H, br-d), 5.09 (1H, d, J=16.4 Hz), 5.31 (1H, dd, J=6.2, 4.9 Hz), 7.35 (1H, d, J=7.7 Hz), 7.67 (1H, s), 7.72 (1H, d, J=7.7 Hz), 9.97 (1H, s).

(*S*)-2-(2,2-Dimethylpropionyl)-7-hydroxymethyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (**61**): To a solution of **68** (23.0 g, 75.8 mmol) in AcOEt (230 ml) in an ice bath was added *tert*-butylamine borane (7.25 g, 83.4 mmol), and the mixture was stirred at room temperature for 1.5 h. 2.0 M hydrochloric acid was added, and the mixture was stirred for 1 h. The AcOEt layer was washed with 1.0 M hydrochloric acid and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was hydrolyzed as described in the preparation of **34** to give **61** as a crystalline solid (18.2 g, 82% yield). ^{1}H-NMR (DMSO-d_6) δ: 1.25 (9H, s), 3.00—3.20 (2H, m), 4.35—4.60 (3H, m), 4.80—5.20 (2H, m), 4.90 (1H, br-d), 7.10—7.30 (3H, m), 12.30—13.10 (1H, br).

(*S*)-3-(4-Acetoxy-2,3,5-trimethylphenylcarbamoyl)-2-(2,2-dimethylpropionyl)-1,2,3,4-tetrahydroisoquinolin-7-ylmethyl Methanesulfonate (**69**): Compound **61** was condensed with compound **4** as described in the preparation of **21**. Yield 98%. To a solution of the amide derivative (1.50 g, 3.21 mmol) and Et_3N (0.58 ml, 4.2 mmol) in CH_2Cl_2 (15 ml) in an ice bath was added MsCl (0.30 ml, 3.9 mmol), and the mixture was stirred for 3 h. The mixture was washed with saturated $NaHCO_3$ solution, 5% citric acid solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure to give **69** as a crystalline solid (1.77 g, 100%). ^{1}H-NMR ($CDCl_3$) δ: 1.38 (9H, br-s), 2.02 (3H, br-s), 2.04 (3H, s), 2.07 (3H, s), 2.32 (3H, s), 2.92 (3H, s), 3.08 (1H, dd, J=16.4, 6.8 Hz), 3.54 (1H, dd, J=16.4, 4.5 Hz), 4.52 (1H, d, J=16.0 Hz), 5.02 (1H, d, J=16.0 Hz), 5.29 (2H, s), 5.37 (1H, dd, J=6.8, 4.5 Hz), 7.18 (1H, s), 7.20—7.30 (2H, m), 7.45 (1H, br-s), 8.03 (1H, br-s).

4-{[(*S*)-2-(2,2-Dimethylpropionyl)-7-[(methylpropylamino)methyl]-1,2,3,4-tetrahydroisoquinoline-3-carbonyl]amino}-2,3,6-trimethylphenyl Acetate (**62**): To a solution of **69** (909 mg, 1.87 mmol) in THF (4.5 ml) was added *N*-methylpropylamine (0.51 ml, 5.1 mmol), and the mixture was stirred at room temperature for 19 h. After evaporation under reduced pressure, 1.0 M hydrochloric acid was added, and washed with Et_2O. After neutralization with $NaHCO_3$, the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give **62** as a crystalline solid (683 mg, 70% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t, J=7.4 Hz), 1.37 (9H, br-s), 1.52 (2H, sextet, J=7.4 Hz), 1.98 (3H, br-s), 2.03 (3H, s), 2.07 (3H, s), 2.18 (3H, s), 2.25—2.35 (2H, m), 2.31 (3H, s), 3.06 (1H, dd, J=16.0, 6.8 Hz), 3.45 (2H, s), 3.51 (1H, dd, J=16.0, 4.9 Hz), 4.52 (1H, d, J=15.9 Hz), 4.96 (1H, d, J=15.9 Hz), 5.56 (1H, dd, J=6.8, 4.9 Hz), 7.13 (1H, s), 7.15—7.25 (2H, m), 7.41 (1H, br-s), 7.94 (1H, br-s).

(*S*)-2-(2,2-Dimethylpropionyl)-*N*-(4-hydroxy-2,3,5-trimethylphenyl)-7-[(methylpropylamino)methyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**45**): Compound **62** was hydrolyzed as described in the synthesis of **43** to

give **45**. A crystalline solid. Yield 32%. mp 165—170 °C. IR (Nujol) cm^{-1}; 1680. MS *m/z*: 480 ($M+H^+$). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.5 Hz), 1.38 (9H, br-s), 1.52 (2H, sextet, J=7.5 Hz), 1.90 (3H, br-s), 2.09, 2.10, 2.12, 2.13 (total 6H, s, s, s, s), 2.18 (3H, s), 2.31 (2H, t, J=7.5 Hz), 3.06 (1H, dd, J=15.8, 6.8 Hz), 3.45 (2H, s), 3.51 (1H, dd, J=15.8, 5.1 Hz), 4.56 (1H, d, J=15.9 Hz), 4.95 (1H, d, J=15.9 Hz), 4.70—5.00 (1H, br), 5.35 (1H, dd, J=6.8, 5.1 Hz), 6.95—7.05 (1H, br), 7.13 (1H, s), 7.15—7.25 (2H, m), 7.69 (1H, br-s). *Anal.* Calcd for $C_{29}H_{41}N_3O_3$: C, 72.62; H, 8.62; N, 8.70. Found: C, 72.27; H, 8.63; N, 8.70.

Partition Coefficient at pH 7.0 Log $D_{7.0}$ values (logarithm of octanol–water partition coefficients at pH 7.0) were determined by HPLC methods.[25] Acetanilide, benzonitrile, benzene, bromobenzene, biphenyl and hexachlorobenzene, the log $D_{7.0}$ values of which are known, were used as reference substances. Test compounds and reference substances were dissolved in acetonitrile containing 1% dimethylsulfoxide (DMSO) at 10 μg/ml, and then 10 μl of the solution was injected into the HPLC system. The HPLC equipment consisted of a pump (PU-980; JASCO, Tokyo, Japan), a UV detector (UV-970; JASCO), an autoinjector (AS-950; JASCO), and a Cosmosil 5C18-AR-II column (5 μm, 4.6 mm×150 mm; Nacalai Tesque, Kyoto, Japan). Phosphate buffer (pH 7.0)–MeOH (8 : 2) was used as the eluent. The capacity factors of test substances and reference substances were calculated from their retention time. The log $D_{7.0}$ values of test compounds were calculated using these capacity factors and the reported log $D_{7.0}$ values of reference substances.

Biological Evaluations of Compounds All experiments were conducted according to the guidelines for animal experiments of our institute and the Guidelines for Animal Experimentation approved by the Japanese Association of Laboratory Animal Science and the Japanese Pharmacological Society.

Effect on *in Vitro* LDL Peroxidation: The effects of test compounds on LDL oxidation were determined according to the method reported previously.[6] Briefly, the LDL fraction was isolated from the plasma of male KHC rabbits and incubated with $CuSO_4$ (5 μM) at 37 °C for 1 h in the presence of vehicle or test compounds. Oxidized LDL was determined as MDA. IC_{50} values were calculated using data from duplicate assay tubes at each concentration of test compounds.

In Vitro ACAT Activity: Male Japanese white rabbits (2.5 kg, Japan SLC) were anesthetized with sodium pentobarbital (30 mg/kg, intravenously (i.v.)), exsanguinated from the common carotid artery, and then the liver was isolated. Microsomes were prepared according to the method of Field and Mathur.[26] Briefly, each sample was homogenized in a buffered sucrose solution (250 mM sucrose, 5 mM K_2HPO_4/KH_2PO_4, 1 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM dithioerythritol, pH 7.4) using a Teflon-glass homogenizer. The homogenate was centrifuged at 12000×***g*** for 15 min at 4 °C. The resulting supernatant was centrifuged at 105000×***g*** for 30 min at 4 °C. The microsomal fraction was used as the ACAT preparation. ACAT activity was determined according to the method described by Heider *et al.*[27] The microsomes were incubated in 154 mM phosphate buffer (pH 7.4) containing bovine serum albumin. Test compounds were applied and preincubated at 37 °C for 5 min, then 30 nmol of [1-^{14}C]oleoyl-CoA (PerkinElmer, Waltham, MA, U.S.A.) was added. The reaction mixture was incubated at 37 °C for 20 min and EC was extracted with $CHCl_3$/MeOH (2 : 1) and separated by thin-layer chromatography. The EC produced in microsomes treated with vehicle and test compounds was determined. IC_{50} values were calculated using data from duplicate assay tubes at the concentrations of test compounds in each experiment.

Esterified Cholesterol (EC) Accumulation in THP-1 Cell-Derived Macrophages: The effects of the test compounds on EC accumulation in THP-1 cells were determined during differentiation and foam cell formation. In order to cause them to differentiate into macrophages and to form foam cells, THP-1 cells were suspended in RPMI-1640 medium containing fetal bovine serum (FBS, 10%) and phorbol 12-myristate 13-acetate (PMA, 200 nM) with acetyl LDL (400 μg protein/ml), and then they were incubated at 4×10^5 cells/well in a humidified atmosphere of 95% air, 5% CO_2 at 37 °C for 3 d in the presence or absence of the test compounds. Acetyl LDL was prepared from the serum of male KHC rabbits (6—8 months old; Japan Laboratory Animals, Inc., Tokyo, Japan). Cellular cholesterol was extracted by hexane/*i*-PrOH (3 : 2) and determined by enzymatic methods. EC content was calculated by subtracting the amount of free cholesterol from the total amount of cholesterol. Cellular protein was measured by Lowry's method.

Oxidative Stress-Induced Cell Death: THP-1 cells were suspended in RPMI-1640 medium containing FBS (10%) and PMA (200 nM), and then incubated at 4×10^4 cells/well in a humidified atmosphere of 95% air, 5% CO_2 at 37 °C for 24 h. Differentiated cells were incubated with LDL (400 μg/ml), $CuSO_4$ (5 μM) and test compounds in serum-free RPMI-1640 medium for 24 h, and cell death was observed using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay (DOJINDO LABORATORIES, Kumamoto, Japan). LDL was prepared from the serum of male KHC rabbits (12 months old; Japan Laboratory Animals, Inc.).

Plasma Levels after Oral Administration: Test compounds were suspended in 5% arabic gum and administered orally at 10 mg/kg to male Sprague-Dawley (SD) rats (6—7 weeks old; Japan SLC), and beagles (9—14 months; NARC Co., Yamatake, Japan). Blood samples were drawn from the jugular vein using a heparinized syringe at 0.25, 0.5, 1, 2, 3, 5, 8, and 24 h after administration. Blood was centrifuged at 3000 rpm for 10 min at room temperature. The concentrations of test compounds in the plasma were determined using HPLC.

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