Inhibitors of Acyl-CoA:Cholesterol Acyltransferase (ACAT). 2. Modification of Fatty Acid Anilide ACAT Inhibitors: Bioisosteric Replacement of the Amide Bond[1]

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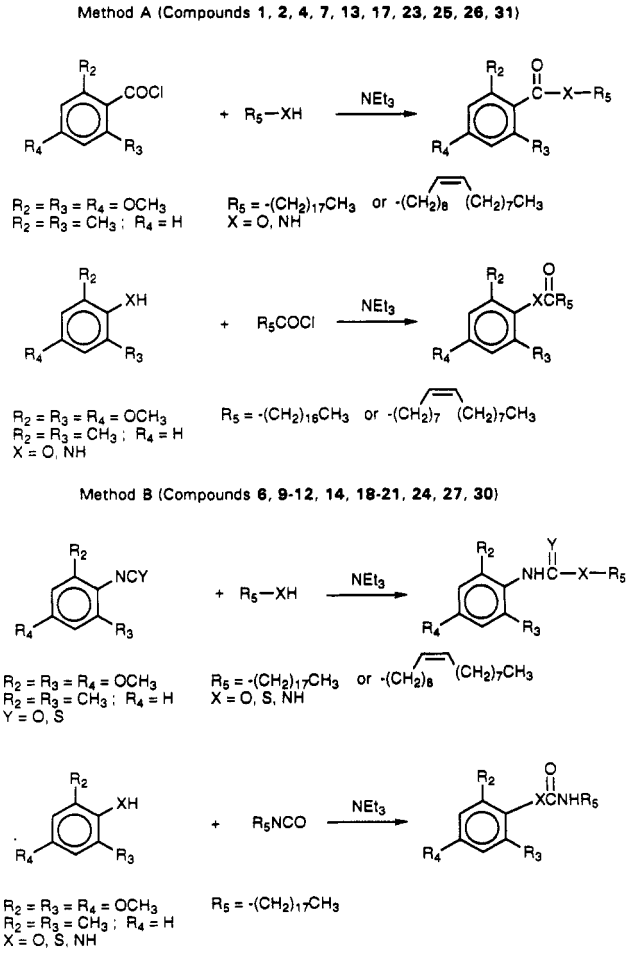
Received March 4, 1993

In order to further define the structural features necessary for potent inhibition of acyl-coenzyme A:cholesterol acyltransferase (ACAT) in vitro and cholesterol lowering in vivo, a systematic study of bioisosteric replacements for the amide bond in our previously identified series of fatty acid anilide ACAT inhibitors was undertaken. Only replacement of amide bonds with isosteres having both hydrogen bond donor and acceptor functionalities yielded compounds retaining ACAT inhibitory activity. Replacement of the amide bond with the urea bioisostere yielded compounds that were potent ACAT inhibitors in vitro and efficacious hypocholesterolemic agents in vivo. Examination of the structure activity relationships in the phenyl ring and alkyl portion of the *N*-phenyl-*N'*-alkylureas revealed that 2,6-diisopropyl substitution was optimal in the phenyl ring. When the 2,6-diisopropyl moiety was kept constant, potency in vitro and in vivo was maintained with straight and branched alkyl groups from 6 to 18 carbons in length.

A prominent feature of atherosclerosis is the accumulation of cholesterol and its esters in coronary arteries. The enzyme acyl-coenzyme A:cholesterol acyltransferase (ACAT) catalyzes the formation of cholesteryl esters from cholesterol and fatty acyl-CoA. In addition to its role in arterial cholesteryl ester accumulation,[2] evidence suggests that ACAT is necessary for hepatic very low density lipoprotein (VLDL) secretion[3] and intestinal absorption of cholesterol.[3,4] Thus, inhibition of ACAT may be of therapeutic value in the treatment of atherosclerosis by reducing the amount of cholesterol absorbed from the intestine, reducing hepatic VLDL secretion, and retarding the accumulation of cholesteryl esters in the artery wall.[4]

Previously, oleic[5] and linoleic acid[6] amides were shown to inhibit ACAT in vitro and to lower plasma total cholesterol in cholesterol-fed animal models. We recently described a series of oleic and related fatty acid anilides that are potent inhibitors of ACAT in vitro and lower plasma total cholesterol in cholesterol-fed rats.[7] Some of these compounds also unexpectedly produced an elevation in high-density lipoprotein cholesterol (HDL-C) in this model. Fatty acid amides derived from 2,4,6-trimethoxy- and 2,6-dimethylanilines yielded compounds that were among the most potent in vitro and most efficacious in vivo. To further define the structural requirements for potent ACAT inhibition in vitro and cholesterol lowering in vivo, we employed a strategy prevalent in the design of peptidomimetics, wherein peptide amide bonds are replaced with bioisosteres.[8,9] Applying this strategy to our previous series of ACAT inhibitors, we replaced the amide moieties in *N*-(2,4,6-trimethoxyphenyl)- and *N*-(2,6-dimethylphenyl)oleamides and stearamides with various bioisosteres and examined the effect on biological activity. Upon identification of urea as the only satisfactory amide replacement, we examined the structure–activity relationships in a series of *N*-phenyl-*N'*-alkylureas.

Scheme I



Chemistry

The majority of compounds were prepared by two general methods (Scheme I). Method A involved either acylation of a fatty alcohol or amine with 2,6-dimethyl- or 2,4,6-trimethoxybenzoyl chloride or acylation of 2,6-dimethylphenol, 2,6-dimethylaniline, or 2,4,6-trimethoxy-

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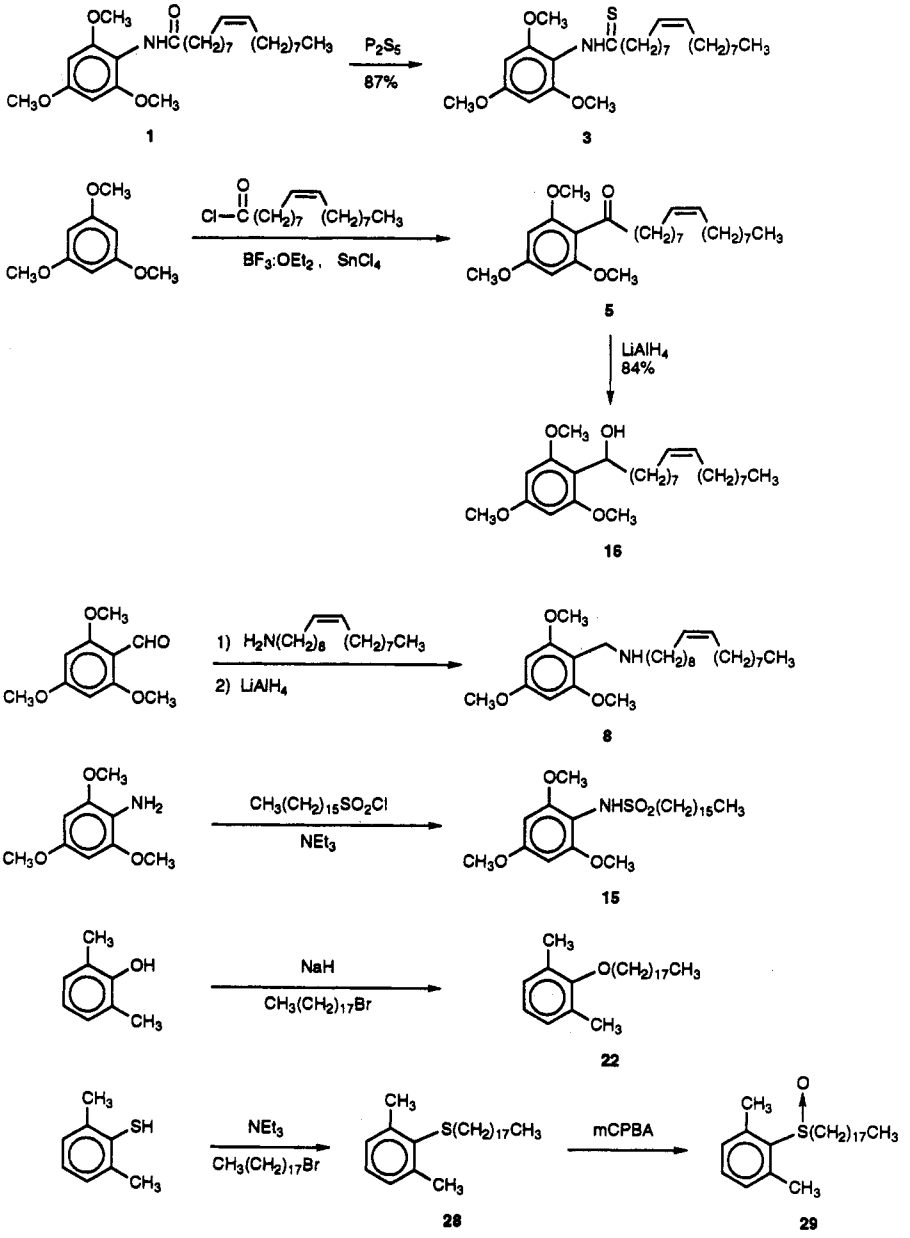
Scheme II



aniline[10] with oleic or stearic acid chloride. Method B involved reaction of the requisite aryl isocyanate or isothiocyanate with a fatty amine, alcohol, or thiol in the presence of triethylamine, or reaction of octadecyl isocyanate with 2,6-dimethylphenol or 2,6-dimethylthiophenol.

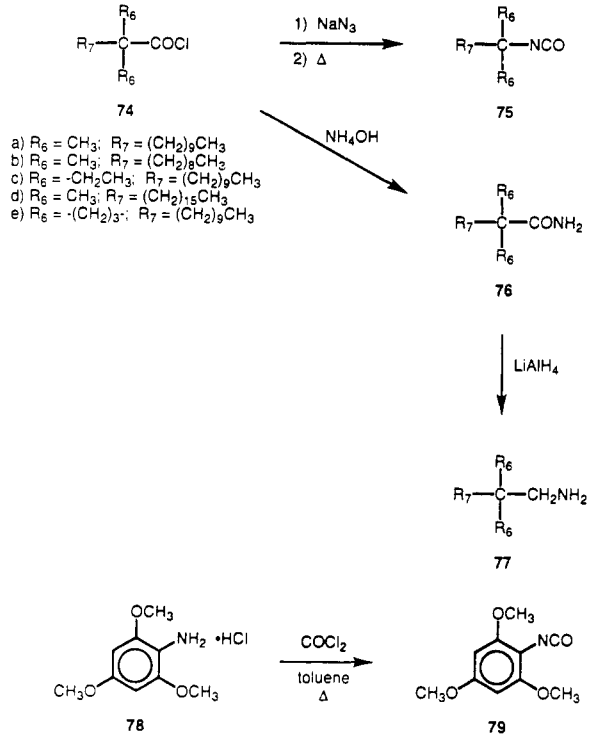
The remaining compounds were prepared by standard techniques (Scheme II). Treatment of **1** with phosphorus pentasulfide gave thioamide **3** in 87% yield. Acylation of 1,3,5-trimethoxybenzene with oleoyl chloride in the presence of boron trifluoride etherate and tin tetrachloride gave ketomethylene analog **5** in 62% yield. Reduction of **5** with lithium aluminum hydride gave hydroxyethylene analog **16** in 84% yield. Reductive amination of 2,4,6-trimethoxybenzaldehyde with oleylamine gave methyleneamino analog **8**. Sulfonamide **15** was prepared by reaction of 2,4,6-trimethoxyaniline with hexadecanesulfonyl chloride in the presence of triethylamine. In the 2,6-dimethyl series, alkylation of 2,6-dimethylphenol and 2,6-dimethylthiophenol with 1-bromooctadecane gave oxymethylene (**22**) and thiomethylene (**28**) analogs, respectively. Oxidation of **28** with *m*-chloroperbenzoic acid gave sulfoxide **29**.

The α,α- and β,β-branched intermediates necessary for the preparation of compounds **65–73** were prepared as shown in Scheme III. The intermediate α,α-branched acids and acid chlorides **74** were prepared as described previously.[7] Reaction of **74** with ammonium hydroxide followed by lithium aluminum hyride reduction gave β,β-disubstituted amine **77**. Branched α,α-disubstituted isocyanate **75** was prepared by reaction of acid chloride **74** with sodium azide followed by thermal rearrangement of the intermediate acyl azide. 2,4,6-Trimethoxyphenyl isocyanate (**79**) was prepared by heating 2,4,6-trimethoxyaniline hydrochloride (**78**) with 12.5% phosgene in toluene.

Results and Discussion

The compounds prepared were evaluated for their ability to inhibit intestinal ACAT in vitro by incubation with [1-^{14}C]oleoyl-CoA and microsomes isolated from the intestinal mucosa of cholesterol-fed rabbits.[5,7] In vivo activity was assessed in rats by admixing the compounds (0.05%) with a diet supplemented with peanut oil (5.5%),

Scheme III

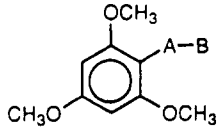


cholesterol (1.5%), and cholic acid (0.3%).[7,11] After 1 week of ad libitum feeding, the animals were sacrificed in the fed state. Plasma total cholesterol (TC) was measured, and the percent change vs control was determined.

The 2,4,6-trimethoxy and 2,6-dimethyl bioisosteric analogs and their biological activities are listed in Tables I and II, respectively. For a given isostere and aniline, oleic acid yielded slightly more potent compounds than stearic acid (compare 1 vs 7, 6 vs 9, and 18 vs 24). As indicated in Tables I and II, of the bioisosteres prepared, none displayed the in vitro potency exhibited by the parent amides. However, the urea analogs 6 and 18 retained considerable inhibitory activity. Although 4-fold less potent in vitro than its amide counterpart 17, urea 18 was more potent in vivo and lowered total cholesterol by 55% when dosed at 0.05% in the diet in cholesterol-fed rats compared with a 38% decrease in total cholesterol with the amide at the same dose. This finding is consistent with the activity reported for a series of trisubstituted ureas.[12] Apparently, bioisosteric substitutions having both hydrogen donor and acceptor functionalities are required to maintain potent inhibitory activity in vitro as exemplified by the sulfonamide 15, thioamide 3, retroamides 2 and 25, ureas 6, 9, 18, and 24, carbamates 10, 14, and 21, and thiocarbamates 11 and 30. The location of the H-bond donor and acceptor was also important since the retroamides were considerably less potent than the amides. In contrast, those bioisosteric analogs having zero or one hydrogen bond donor/acceptor functionality were uniformly inactive (e.g., the esters 13, 23, and 31, oxymethylene 22, thiomethylene 28, methyleneamino 8, sulfoxide 29, ketomethylene 5, and hydroxyethylene 16).

Having identified the urea moiety as an acceptable bioisosteric replacement for amide, we focused our attention on modifications of the phenyl ring combined with variations of the aliphatic chain. Several conclusions could be drawn from this investigation (Table III). First, compounds lacking bulky substituents in both the 2 and 6 positions of the phenyl ring (compounds 32–41, 47, 50, 65) demonstrated reduced potency at inhibiting ACAT in vitro. Second, increasing potency in vitro was observed with compounds having increasing steric bulk in the 2 and 6 positions. With R_1 = oleyl, the rank order of potency for ACAT inhibition in vitro per the 2,6-phenyl ring substitution was Cl < CH_3 < CH_2CH_3 < $CH(CH_3)_2$ (compounds 42, 18, 44, 46). In general, 2,6-diisopropyl was the optimal substitution pattern in the phenyl ring.

Table I. In Vitro and in Vivo Activity of Bioisosteric Analogs of 2,4,6-Trimethoxy Anilides of Oleic and Stearic Acid

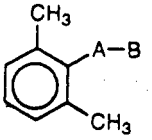


compd	A	B	method	yield,[a] %	mp, °C	IC_{50},[b] μM	% change TC[c,d]	bioisostere
1	NHCO	$C_{17}H_{33}$	A	96	94–95	0.044	−38*	amide
2	CONH	$C_{18}H_{35}$	A	38	wax	9.4		retroamide
3	NHCS	$C_{17}H_{33}$		87	wax	0.9	−22	thioamide
4	CH_2NHCO	$C_{17}H_{33}$	A	62	70–75	6.8		amide homologue
5	$COCH_2$	$C_{16}H_{31}$		62	oil	>50		ketomethylene
6	NHCONH	$C_{18}H_{35}$	B	38	133–140	0.32	−25*	urea
7	NHCO	n-$C_{17}H_{35}$	A	66	105–106	0.052	−11	amide
8	CH_2NH	$C_{18}H_{35}$		18	oil	>100		methyleneamino
9	NHCONH	n-$C_{18}H_{37}$	B	49	125–132	0.90		urea
10	$NHCO_2$	n-$C_{18}H_{37}$	B	66	74–77	7.1		carbamate
11	NHCOS	n-$C_{18}H_{37}$	B	63	75–78	5.7		thiocarbamate
12	$CH_2NHCONH$	n-$C_{18}H_{37}$	B	66	139–142	>5		urea homologue
13	CO_2	$C_{18}H_{35}$	A	48	oil	>5		ester
14	$NHCO_2$	$C_{18}H_{35}$	B	78	oil	0.86		carbamate
15	$NHSO_2$	n-$C_{16}H_{33}$	A	13	86–88	0.69		sulfonamide
16	$-CH(OH)CH_2-$	$C_{16}H_{31}$		84	oil	6% at 10^{-6} M		hydroxyethylene

$C_{16}H_{31}$ = $-(CH_2)_6$–CH=CH–$(CH_2)_7CH_3$ $C_{17}H_{33}$ = $-(CH_2)_7$–CH=CH–$(CH_2)_7CH_3$ $C_{18}H_{35}$ = $-(CH_2)_8$–CH=CH–$(CH_2)_7CH_3$

[a] Analytical results are within ±0.4% of theoretical unless otherwise noted. [b] ACAT inhibition in vitro in intestinal microsomes isolated from cholesterol-fed rabbits. Each determination performed in triplicate. See refs 5 and 7. [c] Denotes percent change in total cholesterol in cholic acid (0.3%)–cholesterol (1.5%)–peanut oil (5.5%) fed rats. All compounds were dosed at 0.05% of the diet for 1 week. In a typical experiment, TC and HDL-C in cholesterol-fed controls would range from 150 to 250 mg/dL and 15 to 20 mg/dL, respectively. All animals gained weight normally. See refs 7 and 11. [d] Compounds with no change indicated were not tested. * Significantly different from rats treated with vehicle alone (unpaired, two-tailed t-test, $p < 0.05$).

Table II. In Vitro and in Vivo Activity of Bioisosteric Analogs of 2,6-Dimethyl Anilides of Oleic and Stearic Acid



compd	A	B	method	yield,[a] %	mp, °C	IC_{50},[b] μM	% change TC[c,d]	bioisostere
17	NHCO	$C_{17}H_{33}$	A	94	51–53	0.043	−38*	amide
18	NHCONH	$C_{18}H_{35}$	B	48	wax	0.16	−55*	urea
19	NHCSNH	$C_{18}H_{35}$	B	96	oil	5.4	−17*	thiourea
20	NHCO-O	n-$C_{18}H_{37}$	B	26	77–80	>10		carbamate
21	OCONH	n-$C_{18}H_{37}$	B	68	80–83	3.1		carbamate
22	OCH_2	n-$C_{17}H_{35}$		15	35–36	>10		oxymethylene
23	OCO	n-$C_{17}H_{35}$	A	60	50–54	>10		ester
24	NHCONH	n-$C_{18}H_{37}$	B	66	109–111	0.61		urea
25	CONH	$C_{18}H_{35}$	A	66	oil	9.8		retroamide
26	CONH	n-$C_{18}H_{37}$	A	23	63–66	>10		retroamide
27	NHCOS	n-$C_{18}H_{37}$	B	55	63–67	>10		thiocarbamate
28	SCH_2	n-$C_{17}H_{35}$		79	31–33	>10		thiomethylene
29	$SOCH_2$	n-$C_{17}H_{35}$		100	45–48	>10		sulfoxide
30	SCONH	n-$C_{18}H_{37}$	B	77	74–77	2.46		thiocarbamate
31	OCO	$C_{17}H_{33}$	A	16	oil	>10		ester

$C_{16}H_{31}$ = —$(CH_2)_6$–CH=CH–$(CH_2)_7CH_3$ $C_{17}H_{33}$ = —$(CH_2)_7$–CH=CH–$(CH_2)_7CH_3$ $C_{18}H_{35}$ = —$(CH_2)_8$–CH=CH–$(CH_2)_7CH_3$

[a] Analytical results are within ±0.4% of theoretical unless otherwise noted. [b] ACAT inhibition in vitro in intestinal microsomes isolated from cholesterol-fed rabbits. Each determination performed in triplicate. See refs 5 and 7. [c] Denotes percent change in total cholesterol in cholic acid (0.3%)–cholesterol (1.5%)–peanut oil (5.5%) fed rats. All compounds were dosed at 0.05% of the diet for 1 week. In a typical experiment, TC and HDL-C in cholesterol-fed controls would range from 150 to 250 mg/dL and 15 to 20 mg/dL, respectively. All animals gained weight normally. See refs 7 and 11. [d] Compounds with no change indicated were not tested. * Significantly different from rats treated with vehicle alone (unpaired, two-tailed t-test, $p < 0.05$).

Interestingly, the 2,4,6-trimethoxyphenyl substitution pattern that was found optimal in the fatty acid amide series is much less active in the ureas. It is also noteworthy that the substitution patterns found optimal in the trisubstituted ureas reported previously[12] resulted in compounds (**38**, **39**) with relatively weak activity in this series. Whether this is related to overall lipophilicity or to some other factor is unclear. By keeping 2,6-diisopropylphenyl constant, it was observed that ACAT inhibition in vitro was relatively insensitive to changes in the alkyl portion of the molecule, with all compounds approximately equipotent, even with variation in chain length from 6 to 18 carbons and branching at either the α or β carbon. A similar result was observed in the fatty acid amides with 2,4,6-trimethoxyphenyl substitution.[7] Alkyl chains with 10–14 carbons coupled with the 2,6-diisopropylphenyl moiety yielded the most efficacious compounds in vivo (compounds **61**, **67**, **73**). While considerable lipophilicity is apparently necessary for optimal inhibition of ACAT in vitro, too much lipophilicity apparently results in diminished cholesterol lowering in vivo, possibly due to decreased absorption into intestinal mucosal cells and/or liver uptake.[13]

In conclusion, bioisosteric analogs of 2,4,6-trimethoxy- and 2,6-dimethylanilides of oleic and stearic acid ACAT inhibitors were prepared with the urea bioisostere being the only amide replacement that retained ACAT inhibitory activity in vitro and cholesterol-lowering activity in vivo. As an extension of this finding, we prepared a series of *N*-phenyl-*N'*-alkylureas that potently inhibit ACAT in vitro with IC_{50}s in the 20 to 60 nM range. Unlike the fatty acid amides, in which 2,4,6-trimethoxyphenyl substitution is optimal for ACAT inhibition in vitro and cholesterol lowering in vivo or trisubstituted ureas where 2,4-difluoro- and 2,4-dimethylphenyl were found optimal, 2,6-diisopropylphenyl substitution is optimal in this series of ureas. These compounds are also potent hypocholesterolemic agents, lowering plasma total cholesterol by 60% in cholesterol-fed rats when dosed at 0.05% in the diet. Further structure–activity studies of urea ACAT inhibitors will be the subject of future publications from this laboratory.

Experimental Section

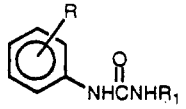
Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet MX-1 FT-IR spectrometer. Nuclear magnetic resonance spectra were determined on either a Varian EM-390 or a Varian XL-200 spectrometer. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Elemental analyses were determined on a Perkin-Elmer 2400C elemental analyzer and were within ±0.4% unless otherwise noted. Yields are unoptimized. Spectral data are consistent with the assigned structures.

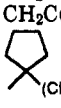
Method A. Typically, acylation conditions for alcohols and amines were interchangeable. To a mixture of amine or alcohol and triethylamine in ethyl acetate or THF at 0–25 °C was added the acid chloride. The whole mixture was stirred at room temperature from 1 h to several days. The resulting mixture was concentrated to dryness, redissolved in ethyl acetate, washed with 1 M aqueous HCl, 10% aqueous K_2CO_3, and saturated NaCl, and dried ($MgSO_4$), filtered, and concentrated. The residue was purified by trituration or chromatography on silica gel (70–230 mesh) using hexane/ethyl acetate mixtures as eluant.

Method B. A mixture of isocyanate or isothiocyanate and amine, alcohol, or thiol was reacted in ethyl acetate in the presence of triethylamine with heating as needed. The resulting ureas, thioureas, carbamates, or thiocarbamates were collected by filtration or were purified by chromatography on silica gel (70–230 mesh) using hexane/ethyl acetate mixtures as eluant.

(*Z*)-*N*-(2,4,6-Trimethoxyphenyl)-9-octadecenamide (1). Method A. To a mixture of 2,4,6-trimethoxyaniline (8.45 g, 46.1 mmol) and triethylamine (11 mL, 79 mmol) in THF (500 mL) at room temperature was added technical-grade oleoyl chloride (18.50 g, 61 mmol) in portions. The reaction mixture was stirred overnight at room temperature, filtered, concentrated, and redissolved in ethyl acetate, and the ethyl acetate solution was washed with 1 N HCl, 10% aqueous K_2CO_3, and saturated aqueous

Table III. In Vitro and in Vivo Activity of *N*-Phenyl-*N'*-alkylureas



compd	R	R_1	mp, °C	IC_{50},[a] μM	% change TC[b,c]
32	H	oleyl	47–50	>5	
33	4-OC_6H_5	oleyl	84–87	>5	
34	2,4,6-$(CH_3)_3$	oleyl	100–104	2.9	
35	2,4-$(OCH_3)_2$	oleyl	98–100	5.4	
36	2,4,6-Cl_3	oleyl	89–92	1.2	
37	2,5-$(CH_3)_2$	oleyl	83–85	4.5	
38	2,4-$(CH_3)_2$	oleyl	76–79	>5	
39	2,4-F_2	oleyl	65–68	4.7	
40	2-OCH_3	oleyl	76–80	3.3	
41	2-CH_2CH_3	oleyl	60–66	0.98	−28*
42	2,6-Cl_2	oleyl	100–107	0.4	−47*
43	2-Cl, 6-CH_3	oleyl	105–107	0.18	−42*
44	2,6-$(CH_2CH_3)_2$	oleyl	wax	0.043	−26*
45	2-CH_2CH_3, 6-$CH(CH_3)_2$	oleyl	wax	0.037	−41*
46	2,6-$(CH(CH_3)_2)_2$	oleyl	wax	0.028	−45*
47	2-Cl, 6-CH_3	$(CH_2)_{17}CH_3$	118–121	1.1	
48	2,6-$(CH_3)_2$	$(CH_2)_{17}CH_3$	109–111	0.61	
49	2,6-$(CH(CH_3)_2)_2$	$(CH_2)_{17}CH_3$	99–103	0.048	−7
50	3-CF_3	$(CH_2)_{13}CH_3$	73–76	>2.5	
51	2,6-Br_2, 4-CH_2CH_3	$(CH_2)_{13}CH_3$	133–134	0.52	
52	2,4-F_2	$(CH_2)_{13}CH_3$	103–104	0.34	
53	2,4,6-F_3	$(CH_2)_{13}CH_3$	117–118	0.25	
54	2-Cl, 6-CH_3	$(CH_2)_{13}CH_3$	125–128	0.07	0
55	2,6-CH_2CH_3	$(CH_2)_{13}CH_3$	114–116	0.021	−38*
56	2-CH_2CH_3, 6-$CH(CH_3)_2$	$(CH_2)_{13}CH_3$	85–87	0.018	−41*
57	2-CH_3, 6-$CH(CH_3)_2$	$(CH_2)_9CH_3$	75–76	0.21	
58	2-CH_3, 6-$C(CH_3)_3$	$(CH_2)_9CH_3$	107–109	0.20	
59	2,6-$(CH_2CH_3)_2$	$(CH_2)_9CH_3$	98–100	0.1	
60	2-CH_2CH_3, 6-$CH(CH_3)_2$	$(CH_2)_9CH_3$	94–97	0.087	−61*
61	2,6-$(CH(CH_3)_2)_2$	$(CH_2)_9CH_3$	138–141	0.056	−64*
62	2,6-$(CH(CH_3)_2)_2$	$(CH_2)_5CH_3$	196–198	0.099	
63	2,6-$(CH(CH_3)_2)_2$	$(CH_2)_3CH_3$	208–210	0.63	
64	2,6-$(CH(CH_3)_2)_2$	CH_3	239–240	>5	
65	2-CH_2CH_3	$C(CH_3)_2(CH_2)_9CH_3$	89–90	0.5	
66	2,6-$(CH_2CH_3)_2$	$C(CH_3)_2(CH_2)_9CH_3$	60–62	0.052	−60*
67	2,6-$(CH(CH_3)_2)_2$	$C(CH_3)_2(CH_2)_9CH_3$	oil	0.045	−60*
68	2,6-$(CH(CH_3)_2)_2$	$CH_2C(CH_3)_2(CH_2)_8CH_3$	98–100	0.046	
69	2,6-$(CH(CH_3)_2)_2$	$CH_2C(CH_3)_2(CH_2)_9CH_3$	87–89	0.047	
70	2,6-$(CH(CH_3)_2)_2$	$CH_2C(CH_2CH_3)_2(CH_2)_9CH_3$	100–102	0.05	
71	2,6-$(CH(CH_3)_2)_2$	$CH_2C(CH_3)_2(CH_2)_{15}CH_3$	79–81	0.073	
72	2,6-CH_2CH_3		82–85	0.11	
73	2,6-$(CH(CH_3)_2)_2$		105–107	0.046	−53*

[a] ACAT inhibition in vitro in intestinal microsomes isolated from cholesterol-fed rabbits. Each determination performed in triplicate. See refs 5 and 7. [b] Denotes percent change in total cholesterol in cholic acid (0.3%)–cholesterol (1.5%)–peanut oil (5.5%) fed rats. All compounds were dosed at 0.05% of the diet for 1 week. In a typical experiment, TC and HDL-C in cholesterol-fed controls would range from 150 to 250 mg/dL and 15 to 20 mg/dL, respectively. All animals gained weight normally. See refs 7 and 11. [c] Compounds with no change indicated were not tested. * Significantly different from rats treated with vehicle alone (unpaired, two-tailed, *t*-test, $p < 0.05$).

NaCl, dried ($MgSO_4$), filtered, and concentrated to a brown oil. The brown oil was flash chromatographed on silica gel (70–230 mesh) eluting with hexane–ethyl acetate (1:1) to give the title compound as an off-white solid: 19.89 g (96%); IR (KBr) ν 2854, 1609, 1530, 1505, 1152, 1055, 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 3H), 1.1–1.9 (m, 22H), 2.01 (m, 4H), 2.37 (br t, 2H), 3.80 (s, 9H), 5.35 (m, 2H), 6.15 (s, 2H), 6.43 (br s, 1H). Anal. ($C_{27}H_{45}NO_4$) C, H, N.

(*Z*)-*N*-(2,4,6-Trimethoxyphenyl)-9-octadecenethioamide (3). A mixture of 1 (3.0 g, 6.7 mmol) and phosphorus pentasulfide (1.62 g, 7.3 mmol) in THF (200 mL) was heated to 50 °C and was then stirred for 2 h at room temperature. The reaction mixture was concentrated and the residue flash chromatographed on silica gel (70–230 mesh) using hexane–ethyl acetate (1:1) as eluant. The title compound was obtained as a waxy solid: 2.72 g (87%); IR (film) ν 2926, 2855, 1596, 1513, 1344, 1156, 1132 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 3H), 1.19–1.46 (m, 20H), 1.67 (m, 1H), 1.84–2.03 (m, 5H), 2.40 (t, 1H), 2.84 (t, 1H), 3.80–3.84 (m, 9H), 5.34 (m, 2H), 6.14 (s, 1H), 6.17 (s, 1H), 8.0 (bs, $^1/_2$H), 8.65 (bs, $^1/_2$H). Anal. ($C_{27}H_{45}NO_3S$) C, H, N, S.

(*Z*)-1-(2,4,6-Trimethoxyphenyl)-9-octadecen-1-one (5). To a mixture of 1,3,5-trimethoxybenzene (3.36 g, 20 mmol), oleic acid chloride (6.0 g, 20 mmol), and diethyl ether (150 mL) at room temperature was added boron trifluoride etherate (3.0 mL, 24 mmol). The reaction mixture was allowed to stand for 0.5 h at room temperature, at which time CH_2Cl_2 (100 mL) and tin tetrachloride (3.0 mL, 25.6 mmol) were added. The mixture was boiled for 0.5 h and then poured into ice-cold 1 N HCl. Ethyl acetate was added, and the layers were separated. The organic layer was washed with 10% aqueous K_2CO_3 (emulsion formed) and saturated aqueous NaCl, dried ($MgSO_4$), filtered, and concentrated. The residual yellow oil was chromatographed on silica gel (70–230 mesh) using hexane–ethyl acetate (4:1) as eluant. The title compound was obtained as a clear oil: 5.35 g (62%); IR (film) ν 2933, 2853, 1701, 1608, 1467, 1437, 1206 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (bt, 3 H), 1.13–1.46 (m, 20H), 1.5–1.7 (m,

2H), 2.0 (m, 4H), 2.74 (t, 2H), 3.77 (s, 6H), 3.82 (s, 3H), 5.34 (m, 2H), 6.09 (s, 2H). Anal. ($C_{27}H_{44}O_4$) C, H.

2,4,6-Trimethoxyphenyl Isocyanate (79). A slurry of 78 (10.0 g, 46 mmol) and 12.5% phosgene in toluene (75 mL) was heated on the steam bath for 3 h, at which time 12.5% phosgene in toluene (50 mL) was added and the mixture was heated an additional 3 h. The reaction mixture was allowed to stand overnight at room temperature. The reaction mixture was warmed on the steam bath, filtered, and concentrated to an oil which crystallized, 9.10 g (96%). This material was used in the next step without further purification.

(*Z*)-*N*-9-Octadecenyl-*N'*-(2,4,6-trimethoxyphenyl)urea (6). Method B. To a solution of 79 (2.49 g, 12 mmol) in ethyl acetate (100 mL) at room temperature was added oleylamine (3.75 g, 14 mmol), and the mixture was allowed to stand at room temperature for 1 h. The reaction mixture was diluted with ethyl acetate, washed with 1 N NaOH, 1 N HCl, and saturated aqueous NaCl, dried ($MgSO_4$), filtered, and concentrated to give an off-white solid. The solid was chromatographed on silica gel (70–230 mesh) using hexane–ethyl acetate (1:1) and then ethyl acetate as eluant. The title compound was obtained as a white solid: 3.57 g (38%); IR (KBr) ν 2924, 2853, 1582, 1523, 1471, 1226, 1133 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.1–1.6 (m, 24H), 1.99 (m, 4H), 3.18 (q, 2H), 3.82 (s, 9H), 4.70 (t, 1H), 5.35 (m, 2H), 5.51 (s, 1H), 6.17 (s, 2H). Anal. ($C_{28}H_{48}N_2O_4$) C, H, N.

(*Z*)-*N*-9-Octadecenyl-2,4,6-trimethoxybenzenemethanamine (8). A mixture of 2,4,6-trimethoxybenzaldehyde (1.96 g, 10 mmol), oleylamine (2.67 g, 10 mmol), and $BaSO_4$ (15 g) in toluene (50 mL) was heated on the steam bath for 1 h. The reaction mixture was filtered and concentrated to a yellow oil. The oil was taken up in tetrahydrofuran (200 mL), and lithium aluminum hydride (1.0 g, 26 mmol) was added at such a rate to keep the reaction under control. The reaction was quenched after 1 h by carefully adding saturated aqueous $NaHSO_4$. The resulting mixture was filtered through Celite, diluted with ethyl acetate, washed with saturated NaCl solution, dried ($MgSO_4$), filtered, and concentrated to a yellow oil. The oil was chromatographed on silica gel (70–230 mesh) using ethyl acetate as eluant. The title compound was obtained as a yellow oil: 0.76 g (18%); IR (film) ν 2924, 2853, 1653, 1596, 1465, 1417, 1205 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.2–1.5 (m, 24H), 1.9–2.1 (m, 4H), 2.54 (t, 2H), 3.76 (s, 2H), 3.80 (s, 9H), 5.34 (t, 2H), 6.12 (s, 2H). Anal. ($C_{28}H_{49}NO_4 \cdot {}^1/_4H_2O$) C, H, N.

(*Z*)-(±)-α-(8-Heptadecenyl)-2,4,6-trimethoxybenzenemethanol (16). Ketone 5 (1.09 g, 2.5 mmol) was added to lithium aluminum hydride (0.60 g, 16 mmol) in diethyl ether (100 mL) at room temperature. The reaction mixture sat at room temperature overnight and was quenched by adding saturated aqueous $NaHSO_4$ solution until no further reaction was observed. The ether layer was decanted, and the gray globular aqueous layer was washed with ethyl acetate. The organic layers were combined, dried ($MgSO_4$), filtered, and concentrated to give an oil. The oil was purified by chromatography on silica gel (70–230 mesh) using 4:1 hexane/ethyl acetate as eluant. The product alcohol 16 was obtained as a clear oil: 0.92 g (84%); IR (film) ν 2924, 1610, 1594, 1457, 1205, 1122 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (bt, 3H), 1.1–1.9 (m, 24H), 1.99 (m, 4H), 3.48 (bs, 1H), 3.81 (s, 9H), 5.02 (bs, 1H), 5.34 (bt, 2H), 6.13 (s, 2H). Anal. ($C_{27}H_{46}O_4$) C, H.

Octadecyl *N*-(2,6-Dimethylphenyl)carbamate (20). 1-Octadecanol (5.4 g, 20 mmol) was dissolved in THF (100 mL), sodium hydride (0.8 g, 60% in oil, 20 mmol) was added, and the mixture was stirred 2 h at room temperature. Then 2,6-dimethylphenyl isocyanate (2.94 g, 20 mmol) was added, and the mixture was stirred for 5 days at room temperature. The reaction mixture was concentrated and the residue partitioned between ethyl acetate and aqueous K_2CO_3. The layers were separated, and the organic layer was washed with 10% aqueous citric acid, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by chromatography on silica gel (70–230 mesh) using 1:1 hexane/ethyl acetate as eluant: 2.18 g (26%); IR (KBr) ν 2919, 2850, 1700, 1256, 1073 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (bt, 3H), 1.1–1.9 (m, 32H), 2.39 (s, 6H), 4.13 (t, 2H), 5.94 (bs, 1H), 7.05 (s, 3H). Anal. ($C_{27}H_{47}NO_2$) C, H, N.

1,3-Dimethyl-2-(octadecyloxy)benzene (22). Sodium hydride (0.87 g, 60% in oil, 22 mmol) was added to 2,6-dimethylphenol (2.42 g, 20 mmol) in THF (100 mL) at room temperature. When no further reaction was evident, 1-bromooctadecane (6.55 g, 20 mmol) was added, and the reaction mixture was stirred for 1 week at room temperature. Water and ethyl acetate were added to the reaction mixture, and the resulting layers were separated. The organic layer was washed with 1 N NaOH(aq), 10% aqueous citric acid, and saturated aqueous NaCl. The organic layer was dried ($MgSO_4$), filtered, and concentrated. The residue was chromatographed on silica gel (70–230 mesh) using hexane and then ethyl acetate as eluant. Hexane eluted mineral oil and 1-bromooctadecane. Ethyl acetate eluted the product: 1.13 g (15%); IR (film) δ 2954, 2918, 2850, 1464, 765 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, 3 H), 1.1–1.7 (m, 30H), 1.82 (m, 2H), 2.29 (s, 6H), 3.77 (t, 2H), 6.88–7.05 (m, 3H). Anal. ($C_{26}H_{46}O$) C, H.

1,3-Dimethyl-2-(octadecylthio)benzene (28). A mixture of 2,6-dimethylthiophenol (0.55 g, 4.0 mmol), 1-bromooctadecane (1.38 g, 4.1 mmol), and excess triethylamine in a 1:1 mixture of diethyl ether/ethyl acetate was heated for several hours on the steam bath and then allowed to stand for 2 days at room temperature. The reaction mixture was then heated an additional 24 h on the steam bath. The resulting brown oil was chromatographed on silica gel (70–230 mesh) using hexane as eluant. The product was obtained as an oil which crystallized on standing: mp 31–33 °C; 1.23 g (79%); IR (KBr) ν 2916, 2849, 1471, 1375, 1163, 766 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.2–1.46 (m, 30H), 1.55 (m, 2H), 2.54 (s, 6H), 2.63 (t, 2H), 7.09 (s, 3H). Anal. ($C_{26}H_{46}S$) C, H.

1,3-Dimethyl-2-(octadecylsulfinyl)benzene (29). A mixture of 28 (0.62 g, 1.6 mmol) and *m*-chloroperoxybenzoic acid (0.40 g, 1.9 mmol) in dichloromethane was heated on the steam bath for 1 h and then allowed to stand overnight at room temperature. The reaction mixture was diluted with ethyl acetate, washed with aqueous $NaHSO_3$, K_2CO_3, and NaCl solutions, dried ($MgSO_4$), filtered, and concentrated to an oil which crystallized: mp 45–48 °C; 0.67 g (100%); IR (KBr) ν 2922, 2917, 1464, 1459, 1046 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.1–1.9 (m, 30H), 1.77 (m, 2H), 2.58 (s, 6H), 2.80 (m, 1H), 3.28 (m, 1H), 7.02–7.26 (m, 3H). Anal. ($C_{26}H_{46}OS$) C, H.

2-Isocyanato-2-methyldodecane (75a). A solution of 2,2-dimethyldodecanoyl chloride (73.95 g, 0.3 M) in 100 mL of dry acetone was added dropwise to a vigorously stirred solution of sodium azide (29.35 g, 0.45 M) in 100 mL of water. The temperature was maintained between 13 and 16 °C by use of an ice bath. The reaction mixture was stirred for 20 min. The layers were separated, and the aqueous layer was washed once with hexane. The hexane solution was combined with the organic layer. The combined organic layers were washed once with saturated NaCl(aq), dried ($MgSO_4$), and concentrated to yield 75.9 g of a clear oil. This oil was taken up in benzene (100 mL), and the resulting solution was added dropwise to vigorously stirred benzene (300 mL) at 60 °C. The reaction mixture was then stirred an additional 2 h at 70 °C and concentrated in vacuo, and the product was distilled to yield 58.0 g (bp 77–80 °C (0.05 mmHg)) of 2-isocyanato-2-methyldodecane. This was used directly in the next step.

***N*-(2,6-Diethylphenyl)-*N'*-(1,1-dimethylundecyl)urea (66).** 2-Isocyanato-2-methyldodecane (2.25 g, 0.01 M) and 2,6-diethylaniline (1.5 g, 0.01 M) were mixed and heated on a steam bath for 16 h. The cooled reaction mixture was taken up in ether. The ether solution was washed with 2 N HCl, saturated sodium bicarbonate, and brine and dried over $MgSO_4$. The ether solution was concentrated in vacuo to yield 3.2 g of the crude product, which was purified by chromatography on silica gel, eluting with 8/2, v/v, hexane/ethyl acetate to yield 1.4 g of 66 as an oil which solidified on standing: mp 60–62 °C; IR (film) ν 3346, 2926, 2856, 1639, 757, 722, 670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.03–1.8 (m, 30H), 2.68 (q, 4H), 3.79 (bs, 1H), 5.84 (bs, 1H), 7.16 (m, 3H). Anal. ($C_{24}H_{42}N_2O$) C, H, N.

2,2-Dimethyldodecanamide (76a). A solution of 2,2-dimethyldodecanoyl chloride[7] (2.75 g, 11 mmol) in ether (50 mL) was added in portions to concentrated ammonium hydroxide (50 mL) at 0 °C. The reaction mixture was stirred for 1 h at room temperature, and then the ether was removed in vacuo and the resulting white precipitate collected by filtration. The solid was washed with water and air-dried to afford 2.29 g (90%) of 76a: mp 80–81 °C; IR (KBr) ν 3213, 3000, 1656, 1469, 1405, 1379, 1108,

722 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.19 (s, 6H), 1.25–1.33 (bs, 16), 1.49 (m, 2H), 5.69 (bs, 2H). Anal. ($C_{14}H_{29}NO$) C, H, N.

2,2-Dimethyldodecanamine (77a). A solution of **76a** (36.5 g, 0.16 M) in THF (400 mL) was added as rapidly as reflux would permit to a suspension of lithium aluminum hydride (12.14 g, 0.32 M) in ether (500 mL). The reaction mixture was refluxed an additional 4 h. The reaction mixture was cooled, and the excess lithium aluminum hydride was quenched by careful addition of H_2O (12.8 mL) and then 20% aqueous NaOH (9.6 mL) and H_2O (44.8 mL). The resulting white solid was filtered and washed with ether, and the ether solution was dried ($MgSO_4$), filtered, and concentrated to yield 35.6 g of product. The product was distilled, bp 133–136 °C (5.25 mmHg) to give 27.4 g (80.4%) of the amine. A small sample was converted to the hydrochloride salt for analysis: mp 128–129 °C; IR (KBr) ν 2924, 2854, 1469, 1402, 723 cm^{-1}; ^1H NMR (D_2O) δ 0.88 (t, 3H), 1.03 (s, 6H), 1.1–1.3 (m, 18H), 2.82 (s, 2H). Anal. ($C_{14}H_{31}N$·HCl) C, H, N.

***N*-[2,6-Bis(1-methylethyl)phenyl]-*N'*-(2,2-dimethyldodecyl)urea (69).** A mixture of **77a** (2.13 g, 0.01 M) and 2,6-diisopropylphenyl isocyanate (2.03 g, 0.01 M) in ethyl acetate (50 mL) was stirred for 12 h at room temperature. The reaction mixture was concentrated to give a clear oil. The oil was taken up in ether and washed with 2 N HCl, saturated $NaHCO_3$, and brine. The ether solution was dried ($MgSO_4$), filtered, and concentrated to give an oil which solidified on scratching; 3.8 g (91%) of **69** was obtained: mp 87–89 °C; IR (KBr) ν 2962, 2855, 1636, 1576, 1468, 1256, 1061 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.76 (s, 6H), 0.80–1.40 (m, 33H), 2.98 (d, 2H), 3.28 (m, 2H), 4.12 (bs, 1H), 5.90 (bs, 1H), 7.1–7.3 (m, 3H). Anal. ($C_{27}H_{48}N_2O$) C, H, N.

Biological Methods. The in vitro ACAT assay and in vivo cholesterol-fed rat assay have been described previously.[7]

Acknowledgment. We thank Dr. Gary McClusky and staff for spectral and analytical determinations and Ms. Patty Elka, Cari Canon, and Beth McMillan for manuscript preparation.

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