Inhibitors of Acyl-CoA:Cholesterol *O*-Acyltransferase. Synthesis and Pharmacological Activity of (±)-2-Dodecyl-α-phenyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-acetamide and Structurally Related Tetrazole Amide Derivatives

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A series of tetrazole amide derivatives of (±)-2-dodecyl-α-phenyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-acetamide (**1**) was prepared and evaluated for their ability to inhibit acyl-CoA:cholesterol *O*-acyltransferase (ACAT) *in vitro* and to lower plasma total cholesterol *in vivo*. For this series of compounds, our objective was to systematically replace substituents appended to the amide and tetrazole moieties of **1** with structurally diverse functionalities and assess the effect that these changes have on biological activity. The ensuing structure−activity relationship (SAR) studies identified aryl (**7b**) and heteroaryl (**7f**,**g**) replacements for 2,4,6-trimethoxyphenyl that potently inhibit liver microsomal and macrophage ACAT *in vitro* and exhibit good cholesterol lowering activity (56−66% decreases in plasma total cholesterol at 30 mg/kg), relative to **1**, when compared in the acute rat model of hypercholesterolemia. Replacement of the α-phenyl moiety with electron-withdrawing substituents (**13e**−**h**), however, significantly reduced liver microsomal ACAT inhibitory activity (IC_{50} > 1 μM). This is in contrast to electron-donating substituents (**13i**,**j**,**m**−**q**), which produce IC_{50} values ranging from 5 to 75 nM in the hepatic microsomal assay. For selected tetrazole amides (**1**, **7b**, **13n**,**o**), reversing the order of substituents appended to the 2- and 5-positions in the tetrazole ring (**36a**−**d**), in general, improved macrophage ACAT inhibitory activity and provided excellent cholesterol-lowering activity (ranging from 65% to 77% decreases in plasma total cholesterol at 30 mg/kg) in the acute rat screen. The most potent isomeric pair in this set of unsubstituted methylene derivatives (**13n** and **36a**) caused adrenocortical cell degeneration in guinea pigs treated with these inhibitors. In contrast, adrenal glands taken from guinea pigs treated with the corresponding α-phenyl-substituted analogs (**7b** and **36c**) were essentially unchanged compared to untreated controls. Subsequent evaluation of **7b** and **36c** in a rabbit bioassay showed that both compounds and/or their metabolites were present in plasma after oral dosing. Unlike **7b** and **36c**, compound **1** and related 2,4,6-trimethoxyanilides (**13j**, **30c**,**d**) showed poor oral activity in the rabbit bioassay. Nevertheless, in cholesterol-fed rabbits, both systemically available (**7b**, **36c**) and poorly absorbed inhibitors (**1**, **36d**) were more effective in lowering plasma total cholesterol than the fatty acid amide CI-976.

Introduction

Epidemiological studies have shown that elevated plasma total and low-density lipoprotein cholesterol (LDL-C) levels are associated with increased risk for premature coronary heart disease (CHD).[1] Currently, the most effective approach for regulating plasma cholesterol concentrations in hypercholesterolemic patients is by inhibiting cholesterol biosynthesis with HMG-CoA reductase (HMGR) inhibitors. Recent clinical studies have demonstrated that reducing LDL-C with HMGR inhibitors alone, or in combination with bile acid sequestrants, translate into a decreased incidence of cardiovascular events.[2] However, the clinical success of HMGR inhibitors has overshadowed the considerable research into other potential lipid-regulating targets, in particular, inhibition of acyl-CoA:cholesterol *O*-acyltransferase (ACAT). ACAT catalyzes the intracellular esterification of cholesterol in most mammalian tissues by using CoA-activated fatty acids to produce cholesteryl ester.[3] Experimental evidence suggests that the ACAT-mediated esterification of cholesterol plays a key role in intestinal cholesterol absorption, hepatic production of very low density lipoproteins (VLDL), and the unregulated accumulation of cholesteryl esters within the arterial wall.[4,5] In animal models, the fatty acid amide ACAT inhibitor CI-976 has been shown to inhibit intestinal cholesterol absorption, reduce the hepatic cholesteryl ester content necessary for VLDL synthesis and secretion, and prevent the accumulation of cholesteryl ester-rich foam cells within the arterial wall, with or without a reduction in plasma total cholesterol.[6,7] Despite impressive lipid-lowering activity in animal models, the hypocholesterolemic effects of ACAT inhibitors in human trials have been disappointing.[8] This

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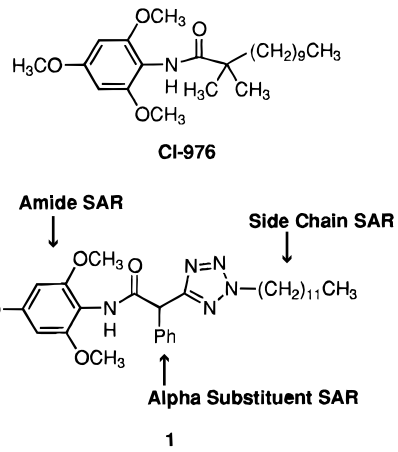


Figure 1. Structures of some known amide ACAT inhibitors.

result, coupled with the recent success of HMGR inhibitors, contributed to the evolution of ACAT inhibitors from nonabsorbed hypocholesterolemic agents to systemically available agents capable of inhibiting the enzyme in the liver and artery wall and thus directly affecting the atherosclerotic process.[9]

The first bioavailable ACAT inhibitor reported to show direct antiatherosclerotic effects was CI-976 (Figure 1).[7] This result prompted extensive SAR studies based on CI-976, from which structurally novel di- and trisubstituted ureas, amino acid anilides, malondiamides, malonester amides, and β-keto amides emerged as potent ACAT inhibitors.[9] Further modification of the fatty acid side chain led to the discovery of (±)-2-dodecyl-α-phenyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-acetamide (**1**), a potent inhibitor of rabbit intestinal and rat liver ACAT *in vitro* (IC_{50} = 8 and 23 nM, respectively), that is significantly more efficacious in lowering non HDL-C in a cholesterol-fed (ED_{50} = 6.4 vs 19 mg/kg) rat model *in vivo*, compared to CI-976. This improvement in efficacy may in part be attributed to the ability of **1** to inhibit cholesterol absorption, since in the lymph fistula model, **1** reduced cholesterol absorption, i.e. the appearance of lymph cholesteryl esters, by 79% at 10 mg/kg, whereas with CI-976 the lowest effective dose is 30 mg/kg.[6] Subsequent evaluation of the individual stereoisomers showed that the majority of biological activity observed for **1** resides in the (+)-enantiomeric form. In this study, the 2-regioisomer (**1**) was shown to be 3 times more potent *in vitro* than the corresponding 1-regioisomer.[10]

As an extension of these studies, we investigated the SAR around the amide moiety of **1** (Figure 1), utilizing (±)-2-phenyl-(2-dodecyl-2*H*-tetrazol-5-yl)acetic acid and a variety of aryl- and heteroaryl-substituted amines. A second approach modified the electronic and steric environment about the tetrazole moiety, varying substituents in either the 2- or 5-positions on the heterocyclic ring. In this paper, we assess the effects these structural changes have on inhibiting hepatic and macrophage ACAT *in vitro*, hypocholesterolemic activity *in vivo*, and, for selected compounds, oral bioactivity *ex vivo*.

Chemistry

The synthetic strategies used to prepare the compounds employed in this study are illustrated in Schemes 1−7. Our first synthetic targets, amides **7a**−**j**, were synthesized using reaction conditions previously described (method A, Scheme 1). The penultimate carboxylic acid derivative **6** was synthesized in three steps utilizing commercially available ethyl phenylcyanoacetate **2**. Amides **7a**−**j** were then prepared by coupling appropriately substituted amines with **6** using dicyclohexylcarbodiimide (DCC) or carbonyldiimidazole (CDI) as the coupling agent.[10]

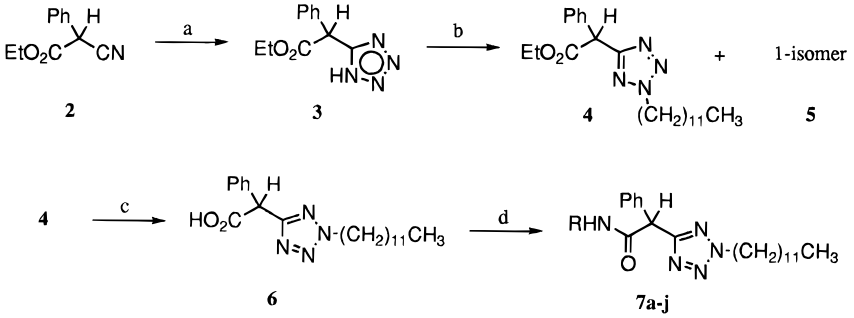
Several methods were used to vary substituents positioned α with respect to the amide moiety (method B, Scheme 2, and method C, Scheme 3). The methylene analogs, **13n** and **13o** (Scheme 2), were synthesized from ethyl cyanoacetate **8n** (R_1 = R_2 = H) using the reaction conditions previously described for amides **7a**−**j**. Alternatively, treatment of **8n** with NaH and a stoichiometric amount of an appropriate electrophile gave substituted cyanoacetate derivatives **8i**,**j**,**p**,**q**, which, like **8n**, were converted to the corresponding amides **13i**,**j**,**p**,**q** utilizing reaction conditions previously described in Scheme 1. Similarly, deprotonation of an α hydrogen atom in **10n** with lithium hexamethyldisilazane at −20 °C, followed by quenching the resulting anion with *N*-fluoro-*O*-benzenedisulfonimide,[11] gave **10e**, an intermediate used in the synthesis of **13e**. The cyclohexyl derivative, **13m**, was prepared by catalytic hydrogenation of **1** over 10% Rh/C.

Commercially available acetonitrile derivatives **14a**−**d**,**f**−**h**,**k**,**l** were also instrumental in functionalizing the α position (Scheme 3). Refluxing the starting nitriles with tri-*n*-butyltin azide, followed by alkylation of the resulting tetrazole with 1-bromododecane, gave a mixture of 1- and 2-regioisomers, from which the 2-regioisomers, **15a**−**d**,**f**−**h**,**k**,**l**, were isolated pure by silica gel chromatography. Compounds **15a**−**d**,**f**−**h**,**k**,**l** were deprotonated in tetrahydrofuran using *n*-butyllithium, with subsequent quenching of the anion with 2,4,6-trimethoxyphenyl or 2,6-diisopropylphenyl isocyanates to give amides **13a**−**d**,**f**,**k** and **13g**,**l**, respectively. For **13g**, treatment with tri-*n*-butyltin azide in refluxing *p*-dioxane yielded the corresponding tetrazole derivative **13h**.

As shown in Scheme 4 (method D), the tetrazoles derived from ethyl cyanoformate **16a** and the methyl ester of 3-cyanopropanoic acid **16b** provided variation in the linkage between the amide and tetrazole moieties. Compound **16a** was converted to the carbethoxy-substituted tetrazole **17a**, when treated with sodium azide in a mixture of pyridine/trifluoroacetic acid at 60 °C. Alkylation of **17a** gave a 1.3:1 mixture of regioisomers **18a** and **19a**, both of which were separated by silica gel chromatography. The 2-regioisomer, **18a**, was hydrolyzed with KOH in ethanol and then coupled in refluxing tetrahydrofuran with 2,4,6-trimethoxyaniline and 2,6-diisopropylaniline, using CDI as the coupling agent, to give compounds **20** and **21**, respectively. Reaction conditions employed for amides **22** and **23** were similar to those described for **20** and **21**.

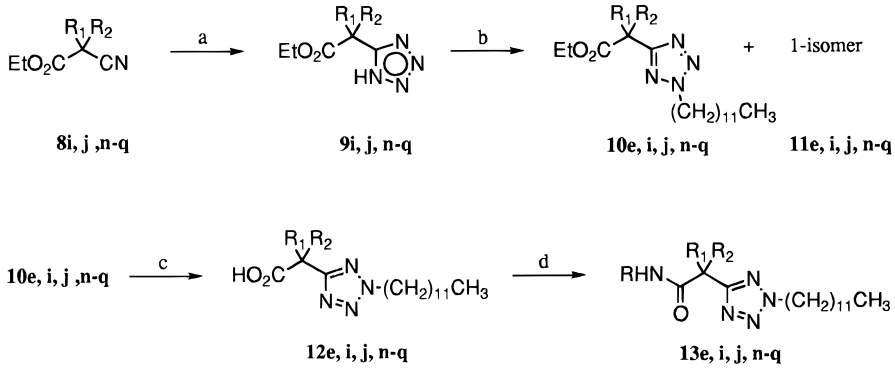
The C_{12} functionality in **1** was replaced with a variety of lipophilic side chains. Side chains incorporating unsaturation were prepared as shown in Scheme 5. The mesylate of 11-dodecen-1-ol (**28**) was synthesized in five steps via homologation of commercially available 10-undecen-1-ol (**24**). Alcohol **24** was activated with methanesulfonyl chloride to give mesylate **25** which underwent nucleophilic displacement to nitrile **26** upon

Scheme 1. Method A[a]



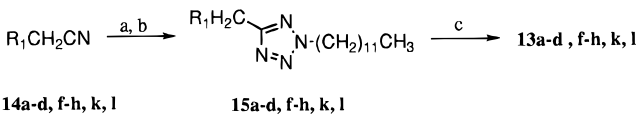
[a] (a) $(n\text{-Bu})_3SnN_3$, *p*-dioxane, reflux; (b) 1-bromododecane, NEt_3, CH_3CN, reflux; (c) NaOH, EtOH; (d) RNH_2, CDI/THF or DCC/CH_2Cl_2.

Scheme 2. Method B[a]



[a] (a) $(n\text{-Bu})_3SnN_3$, *p*-dioxane, reflux; (b) 1-bromododecane, NEt_3, CH_3CN, reflux; (c) NaOH, EtOH; (d) RNH_2, CDI/THF or DCC/CH_2Cl_2.

Scheme 3. Method C[a]



[a] (a) $(n\text{-Bu})_3SnN_3$, *p*-dioxane, reflux; (b) 1-bromododecane, NEt_3, CH_3CN, reflux; (c) RNCO, *n*-BuLi, THF.

treatment with potassium cyanide in dimethyl sulfoxide. Hydrolysis of **26** using aqueous NaOH in refluxing methanol afforded the carboxylic acid intermediate **27**, which reduced to the corresponding alcohol upon treatment with lithium aluminum hydride (LAH) in tetrahydrofuran. The alcohol was then activated with methanesulfonyl chloride in dichloromethane to give mesylate **28** (Scheme 5). Compounds **25** and **28**, and the commercially available saturated alkyl halides were then utilized (R_3) in the synthesis of amides **30a**−**d** as shown in Scheme 6 (method E). These were coupled to tetrazole **3** using the standard conditions previously described. The 2-isomers, **29a**−**d**, were hydrolyzed to the carboxylic acids, activated with DCC, and coupled with 2,4,6-trimethoxyaniline to give amides **30a**−**d**.

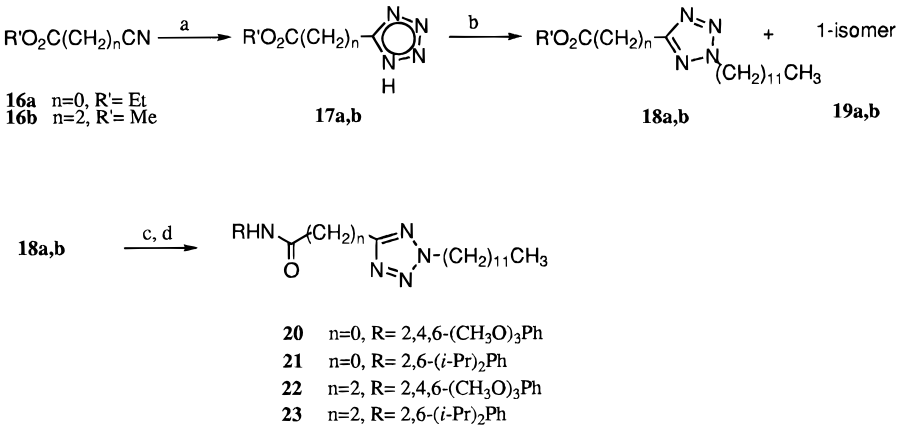
In order to further explore SAR around the 2- and 5-positions of the tetrazole ring, a series of reverse tetrazole amides (i.e. the C_{12} in the 5-position and the acetamide coupled to the 2-position) were prepared utilizing the 2,6-diisopropyl- and the 2,4,6-trimethoxyanilide substitution patterns shown in Scheme 7 (method F). Lauryl cyanide **31** was converted to 5-dodecyltetrazole **32** by cycloaddition with tri-*n*-butyltin azide in refluxing dioxane. The resulting tetrazole was alkylated with the appropriately substituted α-bromoacetate to give a mixture of regioisomers **33a**,**c** and **34a**,**c**, from which **33a**,**c** were isolated pure using silica gel chromatography. Employing reaction conditions previously described, the esters **33a**,**c** were hydrolyzed to the corresponding acids **35a**,**c** and coupled with 2,4,6-trimethoxy- or 2,6-diisopropylaniline to give amides **36a**−**d**.

Results and Discussion

Compounds prepared were initially tested for ACAT inhibition in hepatic microsomes isolated from cholesterol-fed rats and in a cell culture assay (see the Experimental Section) using murine IC-21 macrophages (Tables 1−4).[6] The primary *in vivo* assay was performed in rats given a single dose of test compound by gavage suspended in carboxymethylcellulose (CMC) and Tween-20 in water, followed by a single high-fat, high-cholesterol meal. This model measures the ability of the test compound to inhibit the overnight rise in plasma total cholesterol (Tables 1−4).[12] In the chronic cholesterol-fed rat model (Table 5), rats were fed the same diet as above but for 14 days. During the second week, the test compound was administered daily by gavage using the CMC/Tween vehicle. Efficacy in this model is defined as the ability to reduce plasma total cholesterol (TC), lower the amount of cholesterol in apoB-containing lipoproteins (non HDL-C), and to elevate the diet-induced low levels of HDL cholesterol (HDL-C).[12]

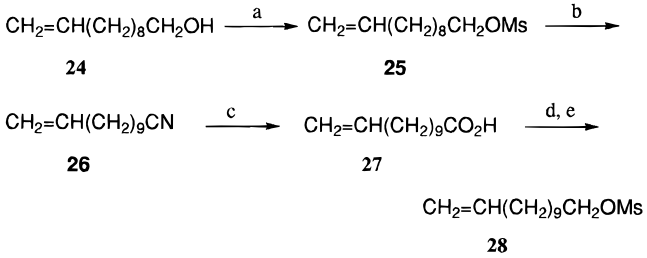
The most potent and efficacious tetrazole amides were evaluated for adrenal and liver toxicity in chow-fed guinea pigs. The test compounds were dosed (100 mg/kg) orally by gavage (oleic acid vehicle) over a 2-week period, followed by the microscopic examination of hepatocytes and adrenal cortical cells for drug-related pathologic alterations, compared to the untreated controls.[13] Compounds classed as nontoxic in this model were further evaluated for oral bioactivity in chow-fed

Scheme 4. Method D[a]



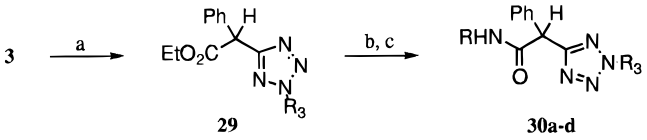
[a] (a) NaN_3, TFA, pyridine, 60 °C, or NaN_3, NH_4Cl, DMF, 100 °C; (b) 1-bromododecane, NEt_3, CH_3CN, reflux; (c) KOH, EtOH; (d) RNH_2, CDI, THF, 60 °C.

Scheme 5[a]



[a] (a) MsCl, NEt_3, CH_2Cl_2, 0 °C; (b) KCN, DMSO, 80 °C; (c) NaOH, H_2O/CH_3OH, reflux; (d) LAH, THF; (e) MsCl, NEt_3, CH_2Cl_2, 0 °C.

Scheme 6. Method E[a]



[a] (a) R_3OMs or R_3Br, NEt_3, CH_3CN, reflux; (b) NaOH, EtOH; (c) RNH_2, DCC, CH_2Cl_2, 0 °C.

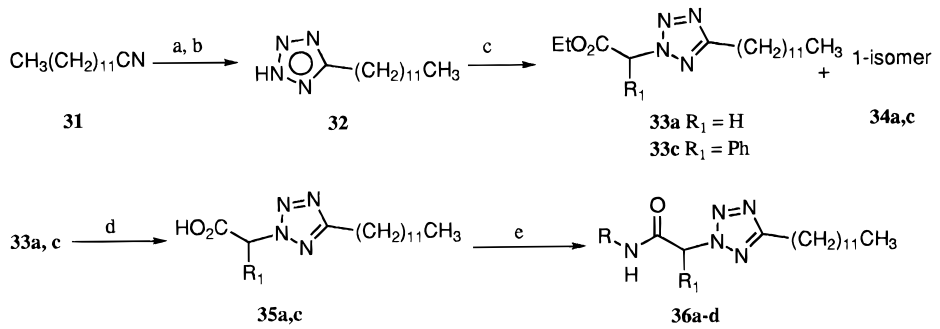
rabbits (see the Experimental Section for the experimental protocol). In this bioassay, systemic availability was determined *ex vivo* utilizing the hepatic microsomal assay previously described.[6]

The biological activity for amides derived from (±)-2-phenyl-(2-dodecyl-2*H*-tetrazol-5-yl)acetic acid is shown in Table 1. Consistent with previous fatty acid amide SAR,[14] optimal *in vitro* and *in vivo* activity was observed for compounds bearing phenyl (**1**, **7b**) or heterocyclic rings (**7f**,**g**) disubstituted with alkyl or alkoxy substituents flanking the amide moiety. For this series of compounds, the steric bulk is required for good microsomal activity, since the unsubstituted anilide **7a** and the heterocyclic derivatives having a nitrogen atom occupying one of the ortho positions in the heteroaryl ring (**7d**,**e**,**h**), in general, are less active at inhibiting hepatic ACAT activity. However, weak inhibitors in the microsomal assay (**7a**,**h**) demonstrated potency comparable to **1** in the cellular assay. The most potent ACAT inhibitor in the cellular assay, **7g**, provided the best overall activity in our preliminary *in vitro* and *in vivo* biological screens. Other modifications such as replacing the 2,4,6-trimethoxyphenyl moiety of **1** with *tert*-butyl (**7j**) or cyclopropyl (**7i**) functionalities failed to provide increases in potency or efficacy.

In order to further define the structural features necessary for potent inhibition of ACAT *in vitro* and efficacy *in vivo*, we systematically replaced the α-phenyl moiety of **1** with substituents capable of affecting the electronic and steric environment about the methylene bridge connecting the amide carbonyl and the tetrazole ring (Table 2). For the 4-substituted phenyl analogs (**13a**−**d**), only the 4-fluoro derivative (**13a**) was as effective as **1** in the biological screens. Electron-donating groups, **13b**−**d**, were at least 10-fold less potent in inhibiting hepatic ACAT *in vitro*, but **13b** was more potent than **1** in the cellular assay. Replacement of the α-phenyl moiety with smaller electron-withdrawing substituents (i.e. F, CN, tetrazolyl), reduced potency in both microsomal (IC_{50} > 1 μM) and cellular assays for both the 2,4,6-trimethoxy- and 2,6-diisopropylanilide derivatives (**13e**−**h**), whereas the 2-pyridyl analogs, **13k**,**l**, maintained potent inhibitory activity. In contrast, electron-donating substituents (**13i**,**j**,**m**−**q**) varying in size and lipophilicity were generally well tolerated in the α-position. Among these, the benzyl derivative, **13j**, potently inhibited macrophage ACAT *in vitro* and maintained excellent hypocholesterolemic activity *in vivo*. Substituting hydrogen for phenyl yielded the most potent inhibitor of hepatic ACAT activity, **13n** (IC_{50} = 5 nM), which unlike **1** does not possess an asymmetric center. The *in vitro* activity for other achiral analogs, however, decreased as the bulk of the substituents increased (compare **13o**−**q**).

Additional SAR studies focused on substituent effects in both the 2- and 5-positions of the tetrazole ring. From Table 3, it can be seen that coupling the amide carbonyl directly to the 5-position of the heterocylic ring (**20**, **21**) significantly reduced *in vitro* and *in vivo* activity compared to the corresponding acetamides, **13n**,**o**. Homologation of the methylene linkage connecting the amide and tetrazole moieties (**22** and **23**) also reduced potency and efficacy in the respective biological screens, albeit to a lesser extent than that observed for carboxamides **20** and **21**. In order to evaluate the role of the dodecyl side chain appended to the 2-position in the tetrazole ring, we replaced the C_{12} functionality in **1** with a variety of saturated (C_8, C_{16}) and unsaturated (C_{11}, C_{12}) aliphatic chains and assessed the effects these changes have on biological activity. As shown in Table 3, inhibiton of hepatic ACAT activity decreased on the order of C_{12} > C_{16} > C_8 for the saturated side chains

Scheme 7. Method F[a]



[a] (a) $(n\text{-Bu})_3SnN_3$, *p*-dioxane, reflux; (b) HCl, Et_2O; (c) ethyl 2-bromophenylacetate or ethyl bromoacetate, NEt_3, CH_3CN, reflux; (d) NaOH, $EtOH/H_2O$; (e) RNH_2, DCC, CH_2Cl_2, 0 °C.

(compounds **1**, **30b**,**a**), which correlates well with the decrease in hypocholesterolemic activity seen in the acute cholesterol-fed rat model. Of the compounds prepared, only the unsaturated C_{12} derivative, **30d**, exhibited comparable *in vitro* and *in vivo* activity, relative to **1**, in our preliminary pharmacological screens. However, **30b** and **30c** are more potent at inhibiting macrophage ACAT than **1** and **30d**.

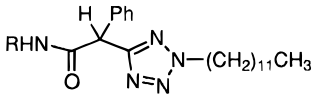
Having identified methylene as the optimal linkage connecting the amide carbonyl and the tetrazole ring, we further investigated substituent effects influencing the α-position by appending the acetamide, substituted with α-phenyl or unsubstituted, to the nitrogen in the 2-position of the heterocyclic ring (Table 4). Despite these changes, the biological activity for these compounds (**36a**−**d**) parallels that previously observed for the parent tetrazole amide derivatives (**1**, **7b**, **13n**,**o**). Once again, the 2,6-disopropylanilides (**36a** and **36c**) potently inhibit ACAT *in vitro*, compound **36a** being equipotent to **13n** in the microsomal assay with an IC_{50} of 6 nM. Interestingly, compounds **36b**−**d** are more potent than the parent tetrazole amides (**13o**, **7b**, **1**) in inhibiting macrophage ACAT activity. When dosed (30 mg/kg) to cholesterol-fed rats, both 2,4,6-trimethoxy- and 2,6-diisopropylanilides significantly reduce the overnight rise in plasma total cholesterol (−65% to −77%).

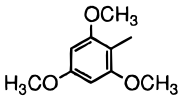
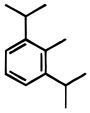
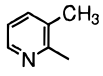
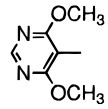
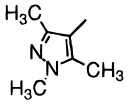
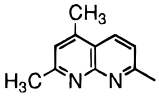
Compounds demonstrating potency comparable to **1** for inhibiting hepatic microsomal and macrophage ACAT *in vitro* and cholesterol-lowering activity *in vivo* were selected for further evaluation in the chronic cholesterol-fed rat model (Table 5). This model more directly mimics the situation found in the clinic, i.e. preestablished hypercholesterolemia, than the acute screen previously described. The α-phenyl-2,6-diispropylanilide derivatives (**7b**, **36c**) previously shown to be as efficacious as the methylene analogs (**13n**, **36a**) in the acute rat screen were considerably less effective at lowering non HDL-C in the chronic screen (−40% and −27% for **7b** an **36c**, respectively, vs −63% and −81% for **13n** and **36a**, respectively). The most efficacious of these, **36a**, also elevated HDL-C by 165%, an increase nearly 2 times that produced by compound **1** (+83%). The opposite effect was observed for the 2,4,6-trimethoxyanilide pair, **1** and **13o**, the α-phenyl derivative providing superior *in vivo* activity (−68% vs −30%). Replacing the dodecyl side chain of **1** with the unsaturated C_{11} hydrocarbon chain (**30c**) further enhanced hypocholesterolemic activity (−68% vs −77%). Other analogs designed to replace the anilide moiety (**7g**) or modify the α-position (**13a**,**j**) failed to attain the efficacy levels established by **1**, **30c**, and **36a** in this biological screen.

Having identified structural features necessary for potent inhibition of ACAT *in vitro* and excellent lipid-regulating activity *in vivo*, we further assessed the most potent and efficacious compounds for adrenal toxicity, since this toxicity has been reported for urea and imidazole-containing ACAT inhibitors.[13,15] In this study, the 2,6-diisopropylanilides substituted with phenyl (**7b**, **36c**) or unsubstituted (**13n**, **36a**) in the α-position were selected for further evaluation in the *in vivo* guinea pig tox assay. Similarly, the corresponding 2,4,6-trimethoxyanilides (**1**, **36d**, **13o**, **36b**) were also tested in this screen. After 2 weeks of oral dosing (100 mg/kg) of compound **13n** using an oleic acid vehicle to facilitate absorption, increased coarse vacoulation of the zona fasciculata cortical cells with adrenocortical cell degeneration was observed in the drug-treated animals. Similar drug-related pathologic alterations to the adrenal gland were observed for all α-methylene unsubstituted anilides (**36a**, **13o**, **36b**), the toxicity being less severe for the 2,4,6-trimethoxyphenyl derivatives. Interestingly, the adrenal glands taken from guinea pigs treated with tetrazole amides bearing an α-phenyl moiety (**1**, **7b**, **36c**, **36d**) were essentially unchanged compared to those of controls. Neither the methylene nor the α-phenyl-substituted anilides caused drug-related alterations in the livers of the treated guinea pigs.

To ensure that the nontoxic compounds **1**, **7b**, and **36c**,**d** (or bioequivalents thereof) were systemically available to peripheral tissues, we assessed the bioactivity upon oral dosing to New Zealand white rabbits utilizing the bioassay (Table 6). Employing CI-976 as the reference standard, compounds were dosed to rabbits (25 mg/kg) using an oil vehicle. Plasma samples were taken at time zero (before drug), 1, 2, and 4 h postdrug meal. The plasma was extracted and evaluated for ACAT inhibitory activity in the microsomal assay. For CI-976, optimal inhibition was observed at the 4-h timepoint (45%). Suprisingly, compound **1** was inactive, even at a dose as high as 50 mg/kg. The corresponding 2,6-diisopropylanilide (**7b**), however, was very effective in this biological screen (96% at 25 mg/kg). This was consistent with the results obtained for the reverse tetrazoles **36c** and **36d** (91% vs 0%). To assess whether other efficacious trimethoxyanilides were inactive, compounds **13j** and **30c**,**d** were screened. Similar to **1**, the higher homolog, **13j**, was also shown

Table 1. Physical and Biological Properties of Analogs of **1** Bearing a Variety of Amide Substituents



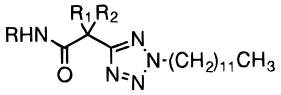
no.	R	method	formula[a]	mp (°C)[b]	ACAT Inhibition liver IC_{50}(uM)[c]	ACAT Inhibition macrophage (% inh., 0.1μM)[d]	% Change in TC[e]
1		A	$C_{30}H_{43}N_5O_4$	119-120	0.023	40	-63*
7a		A	$C_{27}H_{37}N_5O$	74-76	0.480	46	-38
7b		A	$C_{33}H_{49}N_5O$	122-123	0.010	47	-66*
7c		A	$C_{27}H_{35}F_2N_5O$	oil	0.031	1	-70*
7d		A	$C_{26}H_{36}N_6O$	51-52	0.480	8	-22
7e		A	$C_{27}H_{38}N_6O$	68-69	0.085	4	-52*
7f		A	$C_{27}H_{39}N_7O_3$	ND	0.013	39	-56*
7g[f]		A	$C_{27}H_{41}N_7O$	68-70	0.013	66	-62*
7h		A	$C_{31}H_{41}N_7O$	148-149	1.537	49	-8
7i		A	$C_{24}H_{37}N_5O$	86-87	0.244	10	-62*
7j		A	$C_{25}H_{41}N_5O$	84-85	3.346	1	+1

[a] Analytical results are within 0.4% of the theoretical values unless otherwise noted. [b] Melting points are uncorrected. [c] ACAT inhibition in vitro, liver microsomes isolated from cholesterol-fed rats. Each determination performed in triplicate. See ref 6 for the complete protocol. [d] Cell culture assay using murine IC-21 macrophages. See the Experimental Section for the complete protocol. [e] Denotes percent change in total cholesterol in cholic acid (0.5%)−cholesterol (1.5%)−peanut oil (5.5%)-fed rats. See ref 12 for the complete protocol. The standard dose was 30 mg/kg of inhibitor. [f] Anal. C: calcd, 67.61; found, 67.17. An asterisk (*) denotes significantly different from control, $p < 0.05$ using analysis of variance followed by Fisher's multiple range test. ND denotes not determined.

to be inactive in this assay. For the unsaturated side chains, **30c** and **30d**, ACAT was inhibited by 26% and 37%, respectively, when dosed at 50 mg/kg, considerably more effective than **1**, but significantly less active compared to the 2,6-diisopropylanilide derivatives.

In preparation for evaluation in a rabbit model of atherosclerosis, an additional efficacy study was carried out in cholesterol-fed rabbits for selected nonadrenotoxic tetrazole amide derivatives. In this model, we compared the hypocholesterolemic activity for **1** and **36d**, compounds previously shown to be inactive in the bioassay, to that of their bioactive 2,6-diisopropylphenyl-substituted counterparts, **7b** and **36c**. As shown in Table 7, all of the inhibitors significantly lowered rabbit plasma total cholesterol (−55% to −78%) after 2 weeks of oral dosing at 5 and 25 mg/kg compared to the untreated progression controls. However, the tetrazole amides **1** and **7b** were the most efficacious in lowering preestablished hypercholesterolemia (−38% and −41% at 25 mg/kg, respectively) 24-h postdrug meal (T_0). When compared to CI-976 (Figure 2) in a dose−response experiment, **1** was the most potent compound by far, having a calculated ED_{50} (dose required to lower plasma total cholesterol 50%) value of 0.1 mg/kg, 218 times more potent than CI-976. The 2,6-diisopropylanilide **7b** was 18 times more potent than CI-976 (ED_{50} 1.2 vs 21.8 mg/kg) in this pharmacological screen.

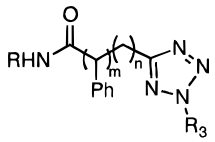
Table 2. Physical and Biological Properties of Tetrazole Amides Bearing α-Substituents Other Than Phenyl



							ACAT inhibition		
no.	R[f]	R_1	R_2	method	formula[a]	mp (°C)[b]	liver (IC_{50}, μM)[c]	macrophage (% inhibn, 0.1 μM)[d]	% change in TC[e]
1	TMP	Ph	H	A	$C_{30}H_{43}N_5O_4$	119−120	0.023	40	−63*
13a	TMP	4-FPh	H	C	$C_{30}H_{42}FN_5O_4$	124−125	0.036	46	−68*
13b[g]	TMP	4-CH_3OPh	H	C	$C_{31}H_{45}N_5O_5$	131−133	0.200	61	−57*
13c	TMP	4-$(NMe)_2$Ph	H	C	$C_{32}H_{48}N_6O_4$	106−107	0.659	0	−62*
13d	TMP	*p*-biphenyl	H	C	$C_{36}H_{47}N_5O_4$	135−137	0.221	10	−43*
13e	TMP	F	H	B	$C_{24}H_{38}FN_5O_4$	oil	1.76	ND	−44*
13f	TMP	CN	H	C	$C_{25}H_{38}N_6O_4$	112−113	13.6	7	−27
13g	DIP	CN	H	C	$C_{28}H_{44}N_6O$	72−75	>5.0	7	−75*
13h	DIP	5-tetrazolyl	H	C	$C_{28}H_{45}N_9O$	147−148	>5.0	8	−37*
13i	TMP	CH_3	H	B	$C_{25}H_{41}N_5O_4$	113−114	0.031	49	−55*
13j	TMP	CH_2Ph	H	B	$C_{31}H_{45}N_5O_4$	86−87	0.049	77	−73*
13k[h]	TMP	2-pyridyl	H	C	$C_{29}H_{42}N_6O_4$	70−72	0.059	23	−60*
13l	DIP	2-pyridyl	H	C	$C_{32}H_{48}N_6O$	70−71	0.026	24	−64*
13m	TMP	cyclohexyl	H	A	$C_{30}H_{49}N_5O_4$	140−141	0.028	8	−61*
13n	DIP	H	H	B	$C_{27}H_{45}N_5O$	75−79	0.005	91	−64*
13o	TMP	H	H	B	$C_{24}H_{39}N_5O_4$	108−109	0.024	66	−60*
13p	TMP	CH_3	CH_3	B	$C_{26}H_{43}N_5O_4$	oil	0.033	34	−69*
13q	TMP	$-(CH_2)_4-$		B	$C_{28}H_{45}N_5O_4$	oil	0.063	16	−56*

[a−e,*] Refer to the footnotes in Table 1. [f] TMP = 2,4,6-trimethoxyphenyl; DIP= 2,6-diisopropylphenyl. ND denotes not determined. [g] Anal. C: calcd, 65.58; found, 66.14. [h] Anal. C: calcd, 64.66; found, 64.20.

Table 3. Modification of Substituents Appended to the 2- and 5-Positions in the Tetrazole Ring



								ACAT inhibition		
no.	R[f]	R_3	m	n	method	formula[a]	mp (°C)[b]	liver (IC_{50}, μM)[c]	macrophage (% inhibn, 0.1 μM)[d]	% change in TC[e]
1	TMP	$(CH_2)_{11}CH_3$	1	0	A	$C_{30}H_{43}N_5O_4$	119−120	0.023	40	−63*
20	TMP	$(CH_2)_{11}CH_3$	0	0	D	$C_{23}H_{37}N_5O_4$	83−85	35.58	ND	−21
21	DIP	$(CH_2)_{11}CH_3$	0	0	D	$C_{26}H_{43}N_5O$	39−43	1.057	33	NC
22[g]	TMP	$(CH_2)_{11}CH_3$	0	2	D	$C_{25}H_{41}N_5O_4$	86−88	0.480	9	−21
23	DIP	$(CH_2)_{11}CH_3$	0	2	D	$C_{28}H_{47}N_5O$	41−43	0.030	17	−47*
30a	TMP	$(CH_2)_7CH_3$	1	0	E	$C_{26}H_{35}N_5O_4$	113−116	0.270	13	−39*
30b	TMP	$(CH_2)_{15}CH_3$	1	0	E	$C_{34}H_{50}N_5O_4$	134−135	0.053	55	−47*
30c	TMP	$(CH_2)_9CH{=}CH_2$	1	0	E	$C_{29}H_{39}N_5O_4$	115−116	0.054	54	−56*
30d	TMP	$(CH_2)_{10}CH{=}CH_2$	1	0	E	$C_{30}H_{41}N_5O_4$	114−115	0.025	37	−63*

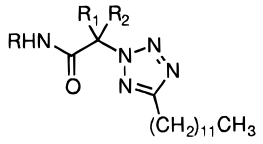
[a,b,c,d,e,*] Refer to footnotes in Table 1. [f] Refer to footnote in Table 2. ND denotes not determined. NC denotes no change. [g] Anal. H: calcd, 8.69; found, 9.12.

Conclusion

In summary, we have examined the structure−activity relationship for a series of tetrazole amides by systematically replacing substituents appended to the amide and tetrazole moieties of **1**. For this series of compounds, the highest potency for both microsomal and cellular ACAT inhibition is obtained with 2,6-diisopropyl substitution in the anilide ring. We have shown that the methylene bridge connecting the amide carbonyl and tetrazole ring is required for good activity, since the higher and lower homologs are considerably less active in our preliminary biological screens. The best profile of *in vivo* activity was found with **36a**, a reverse tetrazole amide which lowered non HDL-C 81% and elevated HDL-C 165% in rats with pre-established hypercholesterolemia. Compound **36a** and other methylene-substituted tetrazole amides (**13n**,**o**, **36b**), however, exhibited drug-related adrenal toxicity in guinea pigs following oral administration at a dose of 100 mg/kg. For the corresponding α-phenyl derivatives (**1**, **7b**, **36c**,**d**), histopathologic alterations to the adrenal or liver of guinea pigs were not observed. Further evaluation of these compounds showed that only the 2,6-diisopropylanilides **7b** and **36c** (or bioactive metabolites) were present in the plasma of rabbits dosed orally with these inhibitors. Compared to CI-976, both 2,4,6-trimethoxyphenyl- and 2,6-diisopropylphenyl-substituted tetrazole amides (**1**, **7b**) displayed superior efficacy in cholesterol-fed rabbits (ED_{50} = 21.8, 0.1, 1.2 mg/kg, respectively).

In conclusion, the cumulative data from this series of compounds reveals two drug profiles, one represented by **7b** and **36c** which would be expected to have direct antiatherosclerotic activity by virtue of their ability to reach the arterial wall to inhibit macrophage ACAT while being nontoxic to steroidogenic tissues and one represented by **1** which preferentially targets intestinal ACAT, thus reducing plasma cholesterol, but yet is intrinsically nontoxic due to the lack of systemic expo-

Table 4. Physical and Biological Properties of Reverse Tetrazole Amides



							ACAT inhibition		
no.	R[f]	R_1	R_2	method	formula[a]	mp (°C)[b]	liver (IC_{50}, μM)[c]	macrophage (% inhibn, 0.1 μM)[d]	% change inTC[e]
36a	DIP	H	H	F	$C_{27}H_{45}N_5O$	91−93	0.006	95	−68*
36b	TMP	H	H	F	$C_{24}H_{39}N_5O_4$	144−146	0.056	61	−69*
36c	DIP	Ph	H	F	$C_{33}H_{49}N_5O$	93−95	0.015	67	−77*
36d	TMP	Ph	H	F	$C_{30}H_{43}N_5O_4$	141−145	0.021	52	−65*

[a−f,*] Refer to the footnotes in Tables 1 and 2.

Table 5. Efficacy Data for Selected Tetrazole Amides in the Chronic Cholesterol-Fed Rat Model[a]

no.	% change in TC	% change in HDL	% change in non-HDL
1	−60*	+83*	−68*
7b	−38*	+27	−40*
7g	−38	+28	−41
13a	−47*	+12	−53*
13j	−48*	+27	−50*
13n	−42*	+82*	−63*
13o	−29	−9	−30
30b	−8	+16	−9
30c	−72*	+102*	−77*
30d	−52*	+98*	−58*
36a	−72*	+165*	−81*
36b	−44*	+3	−46*
36c	-25	+50*	−27
36d	-42	−3	−44

[a] Compounds were dosed at 30 mg/kg. For experimental details, see ref 12. An asterisk (*) denotes significantly different from control, $p < 0.05$ using analysis of variance followed by Fisher's multiple range test.

Table 6. Oral Bioactivity for Selected Tetrazole Amides in the Rabbit Bioassay[a]

no.	dose (mg/kg)	% inhibition 4 h postdose (mean)
CI-976	25	45
1	25	0
1	50	4
7b	25	96
13j	25	0
30c	50	26
30d	50	37
36c	25	91
36d	25	0

[a] See the Experimental Section for the complete protocol.

sure. The latter may have indirect antiatherosclerotic activity due to reduction of plasma cholesterol, but as with other poorly absorbed ACAT inhibitors, may not be efficacious in humans.[8] Studies in atherosclerotic lesion models to differentiate these structurally-related but pharmacokinetically distinct potent inhibitors of ACAT will be the subject of future reports from these laboratories.

Experimental Section

Unless otherwise noted, the starting materials were obtained from commercial suppliers and used without further purification. All organic extracts were dried over $MgSO_4$, except when otherwise noted. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were determined on either a Varian EM-390 or a Varian XL-200 spectrometer. Chemical shifts (δ) are expressed in ppm, relative to internal tetramethylsilane. Mass spectra were obtained on a VG Masslab Trio-2A mass spectrometer. Elemental analyses were determined on a Perkin-Elmer 240C elemental analyzer and were within ±0.4% unless otherwise noted.

Table 7. Efficacy Data for Selected Tetrazole Amides in the Chronic Cholesterol-Fed Rabbit Model[a]

no.	dose (mg/kg)	% change in TC vs T_0	% change in TC vs PC
CI-976	25	+19**	−54*
	5	+96**	−28*
1	25	−38**	−78*
	5	−14	−71*
7b	25	−43**	−73*
	5	−14**	−61*
36c	25	−20**	−68*
	5	+18	−55*
36d	25	−20	−68*
	5	−11**	−63*

[a] Progression control (PC) are the animals maintained on the cholesterol diet (see Experimental Section) without drug intervention. *Significantly different from untreated hypercholesterolemic control at $p < 0.05$. Time zero (T_0) is the time at which drug intervention begins (plasma cholesterol determined 24 h postdrug meal.). **Significantly different from initiation of drug administration at $p < 0.05$.

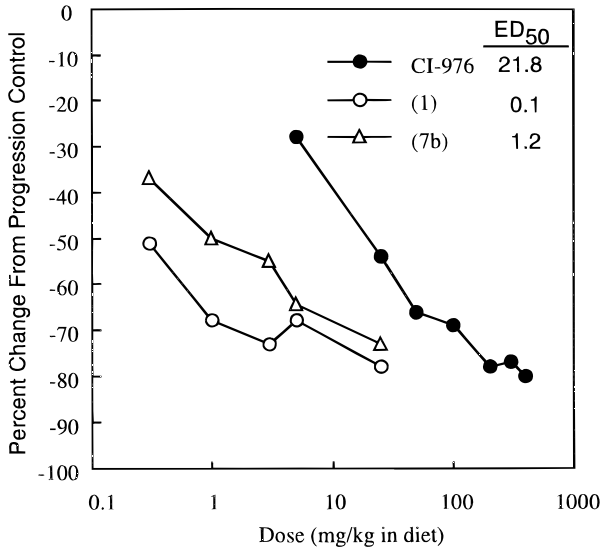


Figure 2. Dose responses for CI-976, **1**, and **7b** in cholesterol-fed rabbits. Each point (dose) represents the mean reduction of plasma cholesterol ($n = 8$ animals/dose). The ED_{50} values were calculated using these mean responses at each dose and the median-effect equation (BIOSOFT Software, Cambridge, U.K.).

General Procedure for Preparing α-Phenyl-Substituted Tetrazole Acetamides. Method A: Synthesis of (±)-2-Dodecyl-α-phenyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-acetamide (1). (a) (±)- Phenyltetrazole-5-acetic acid (3). To a solution of ethyl phenylcyanoacetate **2** (100 g, 0.52 mol) in *p*-dioxane (1.8 L) was added tri-*n*-butyltin azide (191 g, 0.57 mol) in one portion. The solution was refluxed for 16 h, cooled to room temperature, and concentrated *in vacuo*. The resulting liquid was diluted with ethyl ether (2 L), followed by the addition of gaseous HCl over 45 min. The solvent was evaporated and the residue triturated with hexane to give **3** (84 g, 69%) as a white solid: mp 104−105 °C; ^{1}H NMR (DMSO-d_6) δ 7.3 (s, 5H), 5.7 (s, 1H), 4.2 (q, 2H), 1.1 (t, 3H) ppm; CI-MS *m*/*e* 233 (M + 1)$^+$. Anal. ($C_{11}H_{12}N_4O_2$) C, H, N.

(b) (±)-2-Dodecyl-α-phenyl-2*H*-tetrazole-5-acetic acid, Ethyl Ester (4). The tetrazole **3** (55 g, 0.23 mol) was dissolved in a solution of triethylamine (23.8 g, 0.23 mol) and acetonitrile (700 mL). The solution was heated to reflux, followed by the dropwise addition of 1-bromododecane (58.8 g, 0.23 mol). Upon completion, the stirred solution was refluxed for 20 h, then cooled, and concentrated *in vacuo*. The residue was triturated with ethyl acetate (400 mL) and filtered, and the filtrate was evaporated to dryness. The crude liquid containing a mixture of the 2- and 1-regioisomers (3:1) was purified using silica gel chromatography (elution with 75% hexane/25% ethyl acetate) to give **4** (55.4 g, 60%) as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 7.5 (d, 2H), 7.3 (m, 3H), 5.3 (s, 1H), 4.5 (t, 2H), 4.2 (m, 2H), 2.0 (m, 2H), 1.2 (s, 18H), 0.8 (t, 3H) ppm; CI-MS *m*/*e* 401 (M + 1)$^+$. Anal. ($C_{23}H_{36}N_4O_2$) C, H, N.

The 1-regioisomer **5** (17 g, 18%) was isolated pure as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 7.4−7.2 (m, 5H), 5.3 (s, 1H), 4.2 (q, 2H), 4.0 (t, 2H), 1.5 (m, 2H), 1.2 (s, 18H), 0.8 (t, 3H) ppm.

(c) (±)-2-Dodecyl-α-phenyl-2*H*-tetrazole-5-acetic Acid (6). To a solution of **4** (45.3 g, 0.11 mol) in absolute ethanol (400 mL) was added sodium hydroxide (8.8 g, 0.22 mol) in one portion. The solution was stirred at room temperature for 16 h and then concentrated *in vacuo*. The resulting residue was dissolved in water (600 mL) and acidified with concentrated HCl (pH = 1.0), and the product was partitioned between ethyl acetate (400 mL) and aqueous HCl. The organic layer was separated, washed with brine, dried, and concentrated to dryness to give a colorless liquid. Trituration of the liquid with hexane provided **6** (36.6 g, 89%) as a white crystalline solid: mp 55−57 °C; ^{1}H NMR (DMSO-d_6) δ 7.4 (d, 2H), 7.3 (m, 3H), 5.4 (s, 1H), 4.6 (t, 2H), 1.8 (m, 2H), 1.2 (m, 18H), 0.8 (t, 3H) ppm; CI-MS *m*/*e* 373 (M + 1)$^+$. Anal. ($C_{21}H_{32}N_4O_2$) C, H, N.

(d) (±)-2-Dodecyl-α-phenyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-acetamide (1). Compound **6** (20 g, 0.053 mol) was dissolved in tetrahydrofuran (300 mL) and treated with CDI (8.69 g, 0.053 mol) in one portion. The solution was stirred for 1 h at room temperature, followed by the dropwise addition of 2,4,6-trimethoxyaniline (9.8 g, 0.053 mol). After 16 h of stirring, ethyl acetate (1 L) was added, and the solution was washed with aqueous HCl (1 M), aqueous NaOH (1 M), and brine and dried. The solution was concentrated *in vacuo* leaving a lavendar solid. The crude product was purified using silica gel chromatography (elution with 97% chloroform/3% methanol) to give **1** (19.3 g, 67%) as a white solid: mp 119−120 °C; ^{1}H NMR ($CDCl_3$) δ 7.7 (bs, 1H), 7.6 (d, 2H), 7.3 (m, 3H), 6.1 (s, 2H), 5.4 (s, 1H), 4.6 (t, 2H), 3.7 (d, 9H), 2.0 (m, 2H), 1.3 (s, 18H), 0.9 (t, 3H) ppm; CI-MS *m*/*e* 538 (M + 1)$^+$. Anal. ($C_{30}H_{43}N_5O_4$) C, H, N.

Compounds **7a**−**j** were synthesized utilizing similar reaction conditions.

(±)-2-Dodecyl-α-cyclohexyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-acetamide (13m). Compound **1** (0.86 g, 1.5 mmol) was hydrogenated in a Parr shaker containing 10% Rh/C suspended in acetic acid (5 mL) and THF (100 mL). The reaction vessel was shaken for 48 h under 50 psi, followed by filtration of the catalyst. The filtrate was concentrated *in vacuo*, and the residue was recrystallized from ethyl acetate to give **13m** (0.38 g, 48%) as a white solid: mp 140−141 °C; ^{1}H NMR ($CDCl_3$) δ 7.7 (s, 1H), 6.1 (s, 2H), 4.6 (t, 2H), 3.7 (d, 9H), 3.8 (d, 1H), 2.2 (m, 1H), 2.0 (m, 3H), 1.6 (m, 6H), 1.2 (s, 20H), 1.1 (m, 3H), 0.9 (m, 3H) ppm; CI-MS *m*/*e* 544 (M + 1)$^+$. Anal. ($C_{30}H_{49}N_5O_4$) C, H, N.

General Procedure for Varying Substituents Positioned α with Respect to the Amide Moiety. Method B: Synthesis of 2-Dodecyl-α,α-dimethyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-acetamide (13p). (a) Ethyl 2,2-Dimethylcyanoacetate (8p). A solution of ethyl cyanoacetate **8n** (20 g, 0.17 mol) in tetrahydrofuran (350 mL) was cooled to −10 °C followed by the addition of NaH (7.2 g, 0.17 mol) in several portions. The suspension was stirred at −10 °C for 10 min before iodomethane (23.3 g, 0.17 mol) was added. The ice bath was removed and the solution gradually warmed to 20 °C for over 45 min. The solution was then recooled to −10 °C, and a second equivalent of NaH (7.2 g, 0.17 mol) was added. After 10 min of stirring, iodomethane (23.3 g, 0.17 mol) was added, and the solution gradually warmed to room temperature over a 2-h period. The solution was diluted with water (350 mL), followed by the addition of diethyl ether (500 mL). The layers were separated, and the organic portion was washed with brine, dried, and concentrated *in vacuo*. Distillation of the crude product gave **8p** as a colorless liquid (16.9 g, 68%): bp 82−85 °C, 15 mm Hg; ^{1}H NMR ($CDCl_3$) δ 4.3 (q, 2H), 1.6 (s, 6H), 1.3 (t, 3H) ppm; CI-MS *m*/*e* 142 (M + 1)$^+$.

(b) α,α-Dimethyltetrazole-5-acetic Acid, Ethyl Ester (9p). A solution of tri-*n*-butyltin azide (27.3 g, 0.082 mol) in *p*-dioxane (240 mL) was added to ethyl 2,2-dimethylcyanoacetate **8p** (11.6 g, 0.082 mol) in one portion. The solution was refluxed overnight, cooled to room temperature, and concentrated *in vacuo*. The resulting liquid was diluted with ethyl ether (500 mL) and treated with gaseous HCl continuously for 15 min. Evaporation of the solvent yielded a viscous liquid which solidified when triturated with warm hexane. The solid was filtered and recrystallized from hexane/ethyl acetate to give **9p** (8.4 g, 55%) as a cream-colored solid: ^{1}H NMR ($CDCl_3$) δ 12.2 (bs, 1H), 4.2 (q, 2H), 1.8 (s, 6H), 1.3 (t, 3H) ppm; CI-MS *m*/*e* 185 (M + 1)$^+$.

(c) 2-Dodecyl-α,α-dimethyl-2*H*-tetrazole-5-acetic Acid, Ethyl Ester (10p). The tetrazole (4.0 g, 0.021 mol) **9p** was dissolved in acetonitrile (50 mL) containing 1 equiv of triethylamine (2.3 g, 0.021 mol). The solution was heated to reflux, followed by the dropwise addition of 1-bromododecane (5.6 g, 0.022 mol). The solution was refluxed for 16 h, cooled to room temperature, and then concentrated *in vacuo*. The residue was triturated with ethyl acetate (250 mL) and filtered, and the filtrate was washed with aqueous HCl (1 M) and brine, dried, and filtered. Concentration of the filtrate *in vacuo* left a viscous liquid containing both the 1- and 2-regioisomers. Only the latter isomer **10p** (4.5 g, 59%) was obtained, as a colorless liquid, utilizing silica gel chromatography (elution with 90% hexane/10% ethyl acetate): ^{1}H NMR ($CDCl_3$) δ 4.5 (t, 2H), 4.1 (q, 2H), 1.9 (m, 2H), 1.7 (s, 6H), 1.2 (s, 18H), 0.9 (t, 3H) ppm; CI-MS *m*/*e* 353 (M + 1)$^+$.

(d) 2-Dodecyl-α,α-dimethyl-2*H*-tetrazole-5-acetic Acid (12p). Hydrolysis of **10p** was achieved by stirring the ester (3.2 g, 9.0 mmol) in a solution of sodium hydroxide (0.38 g, 9.5 mmol) and ethanol (40 mL) at room temperature for 16 h. The solution was concentrated *in vacuo*, and the product was partitioned between ethyl acetate (50 mL) and aqueous HCl (50 mL, pH = 1.0). The layers were separated, and the organic portion was washed with brine, dried, and filtered. The filtrate was concentrated *in vacuo* to give **12p** (2.0 g, 69%) as a colorless liquid, which gradually solidified to a wax on standing: ^{1}H NMR ($CDCl_3$) δ 4.5 (t, 2H), 2.0 (m, 2H), 1.7 (s, 6H), 1.2 (s, 18H), 0.9 (t, 3H) ppm; CI-MS *m*/*e* 325 (M + 1)$^+$. Anal. ($C_{17}H_{32}N_4O_2$) C, H, N.

(e) 2-Dodecyl-α,α-dimethyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-acetamide (13p). To a stirred solution of **12p** (2.0 g, 6.1 mmol) in tetrahydrofuran (50 mL) was added CDI (1.0 g, 6.1 mmol) in one portion. The solution was stirred for 1 h at room temperature, followed by the dropwise addition of 2,4,6-trimethoxyaniline (1.1 g, 6.1 mmol). After stirring at room temperature for 5 days, the solution was diluted with ethyl acetate (100 mL) and washed with aqueous HCl (1 M), aqueous NaOH (1 M), and brine. The solution was dried and filtered, and the filtrate was concentrated *in vacuo* leaving a maroon-colored liquid. The crude product was purified using

silica gel chromatography (elution with 75% hexane/25% ethyl acetate) to give **13p** as a pale yellow liquid: ^{1}H NMR ($CDCl_3$) δ 7.2 (bs, 1H), 6.1 (s, 2H), 4.6 (t, 2H), 3.7 (d, 9H), 2.1 (m, 2H), 1.7 (s, 6H), 1.3 (s, 18H), 0.9 (t, 3H) ppm; CI-MS *m/e* 490 $(M + 1)^+$. Anal. ($C_{26}H_{43}N_5O_4$) C, H, N.

Compounds **13e**,**i**,**j** were synthesized in this manner. Utilizing steps b−e, anilides **13n**,**o** were prepared.

Alternative Procedure for Varying Substituents Positioned α with Respect to the Amide Moiety. Method C: Preparation of (±)-*N*-[2,6-Bis(1-methylethyl)phenyl]-2-dodecyl-α-2-pyridinyl-2*H*-tetrazole-5-acetamide (13l). (a) 5-(2-Pyridylmethyl)-1*H*-tetrazole. To a solution of 2-pyridylacetonitrile **14l** (24.8 g, 0.21 mol) in *p*-dioxane (500 mL) was added tri-*n*-butyltin azide (100 g, 0.30 mol) in one portion. The solution was refluxed for 20 h, cooled to room temperature, and then concentrated *in vacuo*. Dilution of the resulting liquid with ethyl ether (500 mL), followed by the addition of gaseous HCl over 15 min, provided a maroon-colored precipitate. The solid was collected by filtration and recrystallized from ethanol to give the tetrazole hydrochloride (22.4 g, 54%) as amber-colored needles: ^{1}H NMR (DMSO-d_6) δ 10.4 (bs, 1H), 8.9 (d, 1H), 8.4 (t, 1H), 7.9 (t, 2H), 4.8 (s, 2H) ppm; CI-MS *m/e* 162 $(M + 1)^+$.

(b) 4-(2-Pyridylmethyl)-2-dodecyl-2*H*-tetrazole (15l). The salt (22.4 g, 0.11 mol) obtained in step a was diluted with a solution of acetonitrile (300 mL) containing 2 equiv of triethylamine (22.9 g, 0.22 mol). The solution was heated to reflux, followed by the dropwise addition of 1-bromododecane (28.2 g, 0.11 mol). After 16 h of reflux, the solution was cooled to room temperature and concentrated *in vacuo*. Ethyl acetate was added (300 mL), the insoluble salts were filtered, and the filtrate was concentrated *in vacuo*. The resulting brown liquid was purified by silica gel chromatography (elution with hexane/ethyl acetate (1:1)), isolating only the faster eluting 2-isomer **15l** (15.9 g, 44%) as an orange-colored liquid: ^{1}H NMR ($CDCl_3$) δ 8.5 (d, 1H), 7.7 (t, 1H), 7.3 (d, 1H), 7.2 (m, 1H), 4.5 (t, 2H), 4.4 (s, 2H), 1.9 (m, 2H), 1.3 (s, 18H), 0.9 (t, 3H) ppm; CI-MS *m/e* 330 $(M + 1)^+$. Anal. ($C_{19}H_{31}N_5$) C, H, N.

(c) (±)-*N*-[2,6-Bis(1-methylethyl)phenyl]-2-dodecyl-α-2-pyridinyl-2*H*-tetrazole-5-acetamide (13l). A solution of **15l** (10.7 g, 0.032 mol) in dry tetrahydrofuran (200 mL) was cooled to −20 °C and treated dropwise with *n*-butyllithium (24.4 mL, 0.039 mol). The solution was stirred for 30 min at −20 °C and was delivered by cannula to a solution of 2,6-diisopropylphenyl isocyanate (7.9 g, 0.039 mol) in tetrahydrofuran (50 mL), also cooled to −20 °C. After warming to room temperature, the yellow solution was diluted with ethyl acetate (250 mL) and water (100 mL). The organic phase was separated, dried, and filtered, and the filtrate was concentrated *in vacuo* leaving a yellow liquid. The crude product was purified by silica gel chromatography (elution with hexane/ethyl acetate (1:1)) to give a pale yellow liquid. The liquid obtained crystallized from pentane to give the title compound **13l** (11.9 g, 69%) as a yellow solid: mp 70−71 °C; ^{1}H NMR ($CDCl_3$) δ 9.2 (s, 1H), 8.6 (d, 1H), 7.8 (t, 1H), 7.6 (d, 1H), 7.3 (m, 2H), 7.1 (d, 2H), 5.6 (s, 1H), 4.6 (t, 2H), 2.9 (bs, 2H), 2.0 (m, 2H), 1.3 (s, 20H), 1.1 (d, 12H), 0.8 (t, 3H) ppm; CI-MS *m/e* 533 $(M + 1)^+$. Anal. ($C_{32}H_{48}N_6O$) C, H, N.

Compounds **13a**−**d**,**f**,**g**,**k** were also synthesized from the appropriately substituted acetonitrile utilizing similar reaction conditions.

***N*-(2,6-diisopropylphenyl)-2-(2-dodecyl-2*H*-tetrazol-5-yl)-2-(1*H*-tetrazol-5-yl)acetamide (13h).** To a solution of **13g** (1.4 g, 2.8 mmol) in *p*-dioxane (20 mL) was added, in one portion, tri-*n*-butyltin azide (1.1 g, 3.3 mmol). The solution was refluxed overnight, cooled, and concentrated *in vacuo*. The resulting viscous liquid was diluted with diethyl ether, followed by the addition of gaseous HCl for 15 min. The ethereal solution was concentrated *in vacuo* leaving a viscous liquid, which solidified upon treatment with hexane/ethyl acetate (4:1). The crude product was recrystallized from hexane/ethyl acetate to give **13h** (0.9 g, 62%) as a white solid: mp 147−148 °C; ^{1}H NMR (DMSO-d_6) δ 7.3 (t, 1H), 7.1 (d, 2H), 6.2 (s, 1H), 4.6 (t, 2H), 2.8 (m, 2H), 2.0 (m, 2H), 1.3 (s, 20H), 1.1 (dd, 12H), 0.8 (t, 3H) ppm; CI-MS *m/e* 524 $(M + 1)^+$. Anal. ($C_{28}H_{45}N_8O$) C, H, N.

General Procedure for Preparing Tetrazole Carboxamides. Method D: Synthesis of 2-Dodecyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-carboxamide (20). (a) 1*H*-Tetrazole-5-carboxylic Acid, Ethyl Ester (17a). To a stirred solution of ethyl cyanoformate **16a** (19.6 g, 0.19 mol) in pyridine (78 mL) was added a cold solution (+5 °C) of trifluoroacetic acid (33 mL, 0.43 mol) in pyridine (128 mL). The mixture was heated at 60 °C for 48 h, cooled to room temperature, and then poured over ice (400 g) containing concentrated HCl (156 mL). The aqueous acid was washed with several portions of ethyl acetate. The extracts were combined, dried, and concentrated to an oil. The liquid was filtered through silica gel (elution with ethyl acetate/methanol (15:1)), and fractions containing the product were combined and concentrated *in vacuo*. The oil obtained was diluted with dichloromethane and extracted with aqueous NaOH (2M). The basic solution was washed with dichloromethane, then acidified to pH = 1 with concentrated HCl. Ethyl acetate was added, the layers were separated, the organic portion was dried and filtered, and the filtrate was concentrated *in vacuo* to give **17a** (9.3 g, 33%) as a white solid: mp 87.5−91.5 °C; ^{1}H NMR (DMSO-d_6) δ 4.4 (q, 2H), 1.3 (t, 3H) ppm; EI-MS *m/e* 143 (M^+). Anal. ($C_4H_6N_4O_2$) C, H, N.

(b) 2-Dodecyl-2*H*-tetrazole-5-carboxylic Acid, Ethyl Ester (18a). The tetrazole **17a** (8.9 g, 0.063 mol) was added to a solution of triethylamine (9.6 mL, 0.069 mol) in acetonitrile (175 mL). The suspension was heated to 72 °C and treated with 1-bromododecane (16.5 mL, 0.068 mol) in one portion. The mixture was refluxed for 24 h, cooled, and filtered. Concentration of the filtrate *in vacuo* gave a mixture regioisomers (1:1), from which the 2-isomer was separated by silica gel chromatography (elution with 90% hexane/10% ethyl acetate) to give **18a** (9.2 g, 46%) as a white solid: mp 41−45 °C; ^{1}H NMR ($CDCl_3$) δ 4.7 (t, 2H), 4.5 (q, 2H), 2.0 (m, 2H), 1.5 (t, 2H), 1.33−1.25 (m, 19H), 0.9 (t, 3H) ppm; EI-MS *m/e* 311 (M^+). Anal. ($C_{16}H_{30}N_4O_2$) C, H, N.

(c) 2-Dodecyl-2*H*-tetrazole-5-carboxylic Acid. To a stirred solution of potassium hydroxide (1.9 g, 0.035 mol) in absolute ethanol (280 mL) was added **18a** (8.7 g, 0.028 mol) in one portion. The suspension was stirred at room temperature for 24 h, at which time the precipitate was collected by filtration and washed with ethanol. The filter cake was partitioned between ethyl acetate (160 mL) and 1 M HCl (66 mL). The organic phase was washed with brine, dried, and filtered, and the filtrate was concentrated *in vacuo* to give 2-dodecyl-2*H*-tetrazole-5-carboxylic acid (7.7 g, 98%) as a white solid: mp 122−124 °C; ^{1}H NMR (DMSO-d_6) δ 14.2 (bs, 1H), 4.7 (t, 2H), 1.9 (m, 2H), 1.2 (m, 18H), 0.9 (t, 3H) ppm; EI-MS *m/e* 283 (M^+). Anal. ($C_{14}H_{26}N_4O_2$) C, H, N.

(d) 2-Dodecyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-carboxamide (20). The acid (1.68 g, 5.95 mmol) prepared in step c was dissolved in tetrahydrofuran (35 mL) and treated with CDI (1.0 g, 6.17 mmol) in one portion. The solution was stirred at room temperature for 1 h, followed by the dropwise addition of a solution of 2,4,6-trimethoxyaniline (1.15 g, 6.28 mmol) in tetrahydrofuran (30 mL). The mixture was stirred at room temperature for 72 h and then refluxed for an additional 168 h. The suspension was cooled, filtered, and concentrated to an oil. Ethyl acetate (40 mL) was added, and the solution was washed with aqueous NaOH (1 M), aqueous HCl (1 M), water, and brine. The organic phase was dried and filtered, and the filtrate was concentrated *in vacuo* to an oil. The crude product was purified using silica gel chromatography (elution with petroleum ether/ethyl acetate (4:1)) to give **20** as a white solid: mp 83−85 °C; ^{1}H NMR (DMSO-d_6) δ 9.6 (s, 1H), 6.3 (s, 2H), 4.7 (t, 2H), 3.8 (s, 3H), 3.7 (s, 6H), 1.9 (m, 2H), 1.2 (m, 18H), 0.9 (t, 3H) ppm; EI-MS *m/e* 447 (M^+). Anal. ($C_{23}H_{37}N_5O_4$) C, H, N.

Compound **21** in Table 3 was synthesized in a similar manner.

General Procedure for Preparing Tetrazole Propanamide Derivatives. Method E: Synthesis of 2-Dodecyl-*N*-[2,6-bis(1-methylethyl)phenyl]-2*H*-tetrazole-5-propan-

amide (23). (a) 1*H*-Tetrazole-5-propanoic Acid, Methyl Ester (17b). To a stirred solution of 3-cyanopropanoic acid, methyl ester (**16b**) (109 g, 0.96 mol) in *N*,*N*-dimethylformamide (1 L) was added ammonium chloride (51.5 g, 0.96 mol) and sodium azide (62.7 g, 0.96 mol), respectively. The suspension was stirred for 6 h at 100 °C, then cooled, filtered, and concentrated *in vacuo*, leaving a yellow liquid. Water (500 mL) was added, and the solution was cooled to 5 °C and acidified with concentrated HCl (170 mL). The product was extracted with several portions of ethyl acetate (400 mL), then combined and washed with brine, dried, and concentrated to give **17b** (102 g, 68%) as a viscous oil: ^{1}H NMR (DMSO-d_6) δ 3.6 (s, 3H), 3.5 (bs, 1H), 3.1 (t, 2H), 2.8 (t, 2H) ppm; CI-MS *m*/*e* 157 $(M + 1)^+$. Anal. ($C_5H_8N_4O_2$) C, H, N.

(b) 2-Dodecyl-2*H*-tetrazole-5-propanoic Acid, Ethyl Ester (18b). The tetrazole **17b** (29.2 g, 0.18 mol) was diluted with acetonitrile (500 mL) and treated with 1 equiv of triethylamine (18.1 g, 0.018 mol). The solution was heated to reflux, followed by the addition of 1-bromododecane (49.5 mL, 0.20 mol). After 24 h of reflux, the solution was cooled and filtered. Evaporation of the filtrate gave a viscous liquid, from which the 2-isomer **18b** (12.0 g, 20%) was isolated as a white solid using silica gel chromatography (elution with petroleum ether/ethyl acetate (15:1)): mp 39−42 °C; ^{1}H NMR (DMSO-d_6) δ 4.7 (t, 2H), 3.6 (s, 3H), 3.1 (t, 2H), 2.8 (t, 2H), 1.9 (m, 2H), 1.2 (m, 18H), 0.9 (t, 3H) ppm; EI-MS *m*/*e* 325 (M^+). Anal. ($C_{17}H_{32}N_4O_2$) C, H, N.

(c) 2-Dodecyl-2*H*-tetrazole-5-propanoic Acid. The ester **18b** (11.5 g, 0.035 mol) was dissolved in a solution containing potassium hydroxide (2.5 g, 0.045 mol) and absolute ethanol (210 mL). The solution was stirred for 72 h at room temperature and then concentrated *in vacuo*. The residue was dissolved in water (300 mL), cooled to 5 °C, and acidified with concentrated HCl (10 mL). Dichloromethane was added, the layers were separated, and the organic phase was dried, filtered, and concentrated to give the title compound as a white solid (10.6 g, 96%): mp 63−65 °C; ^{1}H NMR (DMSO-d_6) δ 4.6 (t, 2H), 3.0 (t, 2H), 2.7 (t, 2H), 1.8 (m, 2H), 1.2 (m, 18H), 0.9 (t, 3H) ppm.

(d) 2-Dodecyl-*N*-[2,6-bis(1-methylethyl)phenyl]-2*H*-tetrazole-5-propanamide (23). A portion of the acid (1.2 g, 3.87 mmol) prepared in step c was dissolved in tetrahydrofuran (35 mL), followed by the addition of CDI (0.65 g, 3.98 mmol). The mixture was stirred at room temperature for 1 h, before 2,6-diisopropylaniline (0.70 g, 3.98 mmol) was added in one portion. After 168 h of reflux, the suspension was cooled and filtered, and the filtrate was concentrated to dryness. The resulting oil was diluted with ethyl acetate (40 mL) and washed with aqueous NaOH (1 M), aqueous HCl (1 M), water, and brine, respectively. The organic phase was dried and concentrated to an oil. The crude product was purified using silica gel chromatography (elution with 75% petroleum ether/25% ethyl acetate) to give **23** as a tan solid: mp 41−43 °C; ^{1}H NMR (DMSO-d_6) δ 9.3 (s, 1H), 7.2−7.1 (m, 3H), 4.6 (t, 2H), 3.1 (t, 2H), 2.9 (m, 2H), 2.8 (t, 2H), 1.9 (m, 2H), 1.2 (s, 18H), 1.0 (m, 12H), 0.9 (t, 3H) ppm; EI-MS *m*/*e* 469 (M^+). Anal. ($C_{28}H_{47}N_5O$) C, H, N.

Similar conditions were used for preparing **22** in Table 3.

11-Dodecenyl methanesulfonate (28) was utilized in the synthesis of **30d**. **(a) 10-Undecenyl Methanesulfonate (25).** Alcohol **24** (30.4 g, 0.18 mol) was diluted with dichloromethane (360 mL), cooled to 0 °C, and treated with triethylamine (18.1 g, 0.18 mol) in one portion. Methanesulfonyl chloride (22.4 g, 0.19 mol) was then added at such a rate so as to maintain a solution temperature of 0 °C. After stirring at 0 °C for 3 h, the product was partitioned between dichloromethane and brine. The layers were separated, and the organic phase was dried and filtered, and the filtrate was concentrated *in vacuo*, leaving **25** as a viscous yellow liquid (35.9 g, 81%): ^{1}H NMR ($CDCl_3$) δ 5.8 (m, 1H), 4.9 (m, 2H), 4.2 (t, 2H), 3.0 (s, 3H), 2.1 (m, 2H), 1.8 (m, 2H), 1.3 (bs, 12H) ppm.

(b) 11-Dodecenenitrile (26). To a solution of mesylate **25** (20.5 g, 0.082 mol) in dimethyl sulfoxide (250 mL) was added potassium cyanide (16.0 g, 0.24 mol). The solution was stirred at 80 °C for 10 min, resulting in the formation of a gelatinous precipitate. After the mixture was cooled to room temperature, water (500 mL) was added and the product was extracted with several portions of ethyl ether (2 × 150 mL). The extracts were combined, washed with brine, dried, and filtered. Evaporation of the filtrate gave **26** (14.5 g, 98%) as a viscous orange liquid: ^{1}H NMR ($CDCl_3$) δ 5.8 (m, 1H), 4.9 (m, 2H), 2.3 (t, 2H), 2.0 (m, 2H), 1.6 (m, 2H), 1.3 (bs, 12H) ppm.

(c) 11-Dodecenoic Acid (27). A solution of **26** (14.4 g, 0.080 mol) in methanol (150 mL) was treated with 25% aqueous NaOH (40 g) in one portion. The solution was refluxed for 24 h and cooled to room temperature, and the methanol was concentrated *in vacuo*. The aqueous concentrate was diluted with water (150 mL), washed with ethyl ether (1 × 100 mL), and neutralized (pH = 6.0) with concentrated HCl. The product was exracted with ethyl acetate (150 mL), washed with brine, dried, and filtered. Evaporation of the filtrate gave **27** (10.6 g, 67%), isolated in the form of a viscous liquid which gradually solidified to a wax on standing: ^{1}H NMR ($CDCl_3$) δ 5.8 (m, 1H), 4.9 (m, 2H), 2.3 (m, 2H), 2.0 (m, 2H), 1.6 (m, 2H), 1.3 (s, 12H) ppm.

(d) 11-Dodecen-1-ol. Lithium aluminum hydride (2.2 g, 0.058 mol) was suspended in dry tetrahydrofuran (50 mL) and cooled to 0 °C. The suspension was treated dropwise with a solution of **27** (10.6 g, 0.053 mol) in dry tetrahydrofuran (160 mL). The reaction mixture gradually warmed to room temperature and was stirred for 16 h. Excess hydride was cautiously quenched with aqueous HCl (0.1 M), followed by the addition of ethyl acetate (200 mL). The suspension was filtered through Celite, and the filtrate was dried and concentrated to dryness, isolating the title compound as a yellow liquid (5.9 g, 60%): ^{1}H NMR ($CDCl_3$) δ 5.8 (m, 1H), 4.9 (m, 2H), 3.6 (t, 2H), 2.0 (m, 2H), 1.9 (bs, 1H), 1.6 (m, 2H), 1.3 (s, 12H) ppm.

(e) 11-Dodecenyl Methanesulfonate (28). Following the procedure previously described for **25**, the alcohol (5.9 g, 0.032 mol) prepared from step d yielded mesylate **28** (8.27 g, 99%) as an orange liquid: ^{1}H NMR ($CDCl_3$) δ 5.8 (m, 1H), 4.9 (m, 2H), 4.2 (t, 2H), 3.0 (s, 3H), 2.0 (m, 2H), 1.7 (m, 2H), 1.3 (bs, 12H) ppm. The product was used in the coupling with **3** without further purification.

General Procedure for Varying the Tetrazole Side Chain. Method F: (±)-2-(11-Dodecenyl)-α-phenyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-acetamide (30d). (a) (±)-2-(11-Dodecenyl)-α-phenyl-2*H*-tetrazole-5-acetic Acid, Ethyl Ester (29). The mesylate of 11-dodecen-1-ol **24** (8.2 g, 0.032 mol) was added dropwise to a stirred solution containing **3** (7.4 g, 0.032 mol), triethylamine (3.3 g, 0.032 mol), and acetonitrile (100 mL). The solution was refluxed for 16 h, cooled, and concentrated *in vacuo*. The residue was triturated with ethyl acetate and filtered, and the filtrate was washed with aqueous HCl (1 M) and brine, dried, and filtered. Evaporation of the filtrate gave a viscous liquid containing a 2.5:1 mixture of the 2- and 1-regioisomers, respectively. Only the faster eluting 2-isomer **29** was isolated from the mixture as a pale yellow liquid (4.6 g, 36%) using silica gel chromatography (elution with 75% hexane/25% ethyl acetate): ^{1}H NMR ($CDCl_3$) δ 7.5 (m, 2H), 7.3 (m, 3H), 5.8 (m, 1H), 5.3 (s, 1H), 4.9 (m, 2H), 4.6 (t, 2H), 4.2 (q, 2H), 2.0 (m, 4H), 1.4−1.3 (m, 14H), 1.2 (t, 3H) ppm.

(b) (±)-2-(11-Dodecenyl)-α-phenyl-2*H*-tetrazole-5-acetic Acid. Using the same procedure as that for compound **4**, hydrolysis of **29** (4.5 g, 0.011 mol) yielded the title compound (4.1 g, 98%) as a yellow liquid: ^{1}H NMR ($CDCl_3$) δ 7.5 (m, 2H), 7.3 (m, 3H), 5.8 (m, 1H), 5.4 (s, 1H), 4.9 (m, 2H), 4.6 (t, 2H), 2.0 (m, 4H), 1.3 (m, 14H) ppm.

(c) (±)-2-(11-Dodecenyl)-α-phenyl-*N*-(2,4,6-trimethoxyphenyl)-2*H*-tetrazole-5-acetamide (30d). The acid (4.1 g, 0.011 mol) prepared in step b was dissolved in a solution containing 2,4,6-trimethoxyaniline (2.0 g, 0.011 mol) and dichloromethane (75 mL), cooled to 0 °C, and treated with DCC (2.3 g, 0.011 mol) in one portion. The suspension was stirred at room temperature for 16 h, then diluted with ethyl acetate, and filtered. The filtrate was washed with aqueous HCl (1 M), aqueous NaOH (1 M), and brine and dried. The solution was concentrated *in vacuo*, and the residue was purified using silica gel chromatography (elution with 90% hexane/10% ethyl

acetate). The solid obtained was recrystallized from hexane/ethyl acetate to give **30d** (3.2 g, 53%) as a white solid: mp 114−115 °C; ^{1}H NMR ($CDCl_3$) δ 7.6 (m, 3H), 7.3 (m, 3H), 6.1 (s, 2H), 5.8 (m, 1H), 5.5 (s, 1H), 4.9 (m, 2H), 4.6 (t, 2H), 3.8 (d, 9H), 2.0 (m, 4H), 1.3 (bs, 14H) ppm; CI-MS *m/e* 536 $(M + H)^+$. Anal. ($C_{30}H_{41}N_5O_4$) C, H, N.

The tetrazole side chains for compounds **30a**−**c** were synthesized from **3** using similar reaction conditions.

General Procedure for Preparing Reverse Tetrazole Amides. Method G: Synthesis of *N*-[2,6-bis(1-methylethyl)phenyl]-5-dodecyl-2*H*-tetrazole-2-acetamide (36a). Synthesis of 5-Dodecyl-1*H*-tetrazole (32). A mixture of lauryl cyanide (**31**, 147.1 g, 0.753 mol) and tri-*n*-butyltin azide (375.0 g, 1.13 mol) in 900 mL of dioxane was heated to reflux overnight. The reaction mixture was concentrated *in vacuo* and redissolved in 1 L of diethyl ether. HCl gas was passed through the ether solution for 1 h. The resulting precipitate was collected by filtration to give 115.5 g of product as a white solid. Additional ether was added to the filtrate, and HCl gas was again passed through this solution to give a second crop of product which was washed with pentane to give an additional 30.3 g of product: total yield 145.8 g (81%); mp 68−70 °C; ^{1}H NMR ($CDCl_3$) δ 14.57 (bs, 1H), 3.14 (t, 2H), 1.86−1.94 (m, 2H), 1.18−1.28 (m, 18H), 0.86 (t, 3H) ppm. Anal. ($C_{13}H_{26}N_4$) C, H, N.

Synthesis of Ethyl 5-Dodecyl-2*H*-tetrazole-2-acetate (33a) and Ethyl 5-Dodecyl-1*H*-tetrazole-1-acetate (34a). A solution of 5-dodecyl-1*H*-tetrazole (**32**, 40.4 g, 0.168 mol), ethyl bromoacetate (30.8 g, 0.185 mol), and triethylamine (25.8 mL, 0.185 mol) in 400 mL of acetonitrile was heated to reflux for 4 h. This was then cooled to room temperature and stirred overnight. The solution was concentrated *in vacuo* and the residue partitioned between water and dichloromethane. The organic layer was dried, filtered, and concentrated to leave a yellow oil. Chromatography (10% ethyl acetate/hexanes) on silica gel gave 20.16 g (37%) of ethyl 5-dodecyl-2*H*-tetrazole-2-acetate **33a** as a clear oil which solidified upon standing: mp 38−40 °C; ^{1}H NMR ($CDCl_3$) δ 5.36 (s, 2H), 4.26 (q, 2H), 2.91 (t, 2H), 1.76−1.83 (m, 2H), 1.25−1.37 (m, 21H), 0.88 (t, 3H) ppm. Anal. ($C_{17}H_{32}N_4O_2$) C, H, N.

Also isolated 21.38 g (39%) of ethyl 5-dodecyl-1*H*-tetrazole-1-acetate (**34a**) as a white solid: mp 48−50 °C; ^{1}H NMR ($CDCl_3$) δ 5.08 (s, 2H), 4.27 (q, 2H), 2.78 (t, 2H), 1.78−1.88 (m, 2H), 1.25−1.37 (m, 21H), 0.87 (t, 3H) ppm. Anal. ($C_{17}H_{32}N_4O_2$) C, H, N.

Synthesis of 5-Dodecyl-2*H*-tetrazole-2-acetic Acid (35a). Ethyl 5-dodecyl-2*H*-tetrazole-2-acetate (**33a**, 1.96 g, 0.006 mol) was dissolved in 50 mL of ethanol, and 9 mL of 1 M NaOH was added. After 2 h, the reaction was concentrated *in vacuo*, and the residue was partitioned between water and diethyl ether. The aqueous layer was acidified with concentrated HCl and extracted with diethyl ether. The ether extracts were dried over magnesium sulfate, filtered, and concentrated to give 1.02 g (57%) of 5-dodecyl-2*H*-tetrazole-2-acetic acid (**35a**) as a white solid: mp 89−91 °C; ^{1}H NMR ($CDCl_3$) δ 10.18 (bs, 1H), 5.43 (s, 2H), 2.94 (t, 2H), 1.73−1.83 (m, 2H), 1.20−1.33 (m, 18H), 0.87 (t, 3H) ppm. Anal. ($C_{15}H_{28}N_4O_2$) C, H, N.

Synthesis of *N*-[2,6-Bis(1-methylethyl)phenyl]-5-dodecyl-2*H*-tetrazole-2-acetamide (36a). *N*,*N*-Dicyclohexylcarbodiimide (9.14 g, 0.044 mol) was added in one portion to a solution of 5-dodecyl-2*H*-tetrazole-2-acetic acid (**35a**, 12.5 g, 0.042 mol) and 2,6-diisopropylaniline (7.48 g, 0.042 mol) in 300 mL of dichloromethane at −15 °C. This was allowed to warm to room temperature and stirred overnight. The mixture was then concentrated *in vacuo* and partitioned between water and ethyl acetate. The organic layer was dried, filtered, and concentrated to leave an orange oil. Trituration with hexanes gave 15.16 g (79%) of *N*-[2,6-bis(1-methylethyl)phenyl]-5-dodecyl-2*H*-tetrazole-2-acetamide (**36a**) as an off-white solid: mp 91−93 °C; ^{1}H NMR ($CDCl_3$) δ 7.14−7.37 (m, 4H), 5.46 (s, 2H), 2.81−2.96 (m, 4H), 1.74−1.86 (m, 2H), 1.13−1.38 (m, 30H), 0.88 (t, 3H) ppm. Anal. ($C_{27}H_{45}N_5O_1$) C, H, N.

Biological Methods. *In Vitro* Macrophage Assay. IC-21 mouse macrophages (American Type Culture Collection, Rockville, MD) were preincubated for 16 h in serum-free medium (Dulbecco's modified Eagle medium, DMEM) containing albumin (20 μM) and phosphatidylserine/cholesterol vesicles. The latter were prepared by combining phosphatidylserine (27.4 μmol) and cholesterol (26 μmol) in chloroform and then evaporating the chloroform in order to disperse the dried lipids into 5 mL of 150 mM NaCl, 5 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), and 1 mM EDTA (pH 7.4). This lipid suspension was sonicated to form vesicles, which were then sterilized by filtration (0.45 μM filter). Following the preincubation, the media with vesicles was replaced with serum-free DMEM containing the test compound in DMSO and incubated for 1 h, after which [^{14}C]sodium oleate was added for an additional 4 h. The media was removed, and cells were washed and extracted with hexane/2-propanol (3:2, v/v) containing internal standard ([^{14}C]cholesterol). Neutral lipids were separated by TLC followed by isotopic scanning (PhosphorImager, Molecular Dynamics, Sunnyvale, CA). Cell protein concentration was determined by the method of Lowry et al.[16]

ACAT Bioassay. Male, New Zealand white rabbits (1.2−1.5 kg) were conditioned to meal-feeding for 1 week and then given a meal containing the drug in an oil vehicle (3% peanut oil/3% coconut oil) at the appropriate test dose level. Blood samples were obtained at time zero (before drug) and 1-, 2-, and 4-h postdrug meal. The plasma samples (0.2 mL) were extacted into hexane, dried, and then taken up into chloroform/methanol (2:1, v/v) for transfer to tubes used for the microsomal liver ACAT assay. Standards were prepared for each test compound by direct addition to rabbit plasma in DMSO. A reference control was also included in each assay (i.e. the ACAT inhibitor, CI-976 at 2.5 μM). These "spiked" plasmas were extracted together with the test samples.

***In Vivo* Cholesterol-Fed Rabbit Assay.** Male, New Zealand rabbits (1−1.5 kg) were adapted to a meal-feeding regimen (50 g/day). The diet consisted of normal rabbit chow supplemented with cholesterol (0.5%), coconut oil (3%), and peanut oil (3%). After 1 week the compounds were added to the diet for a period of 2 weeks at the indicated doses. Blood was obtained from the heart of fasted animals after nitrogen asphyxiation for determining plasma total cholesterol concentrations. Efficacy is expressed as a percentage change from the cholesterol-fed controls.

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