Influence of Various Central Moieties on the Hypolipidemic Properties of Long Hydrocarbon Chain Diols and Diacids

Daniela C. Oniciu,*,† Jean-Louis H. Dasseux,† Jing Yang,‡ Ralf Mueller,‡ Emil Pop,‡ Anna Denysenko,‡ Caiming Duan,‡ Tian-Bao Huang,‡ Lianhao Zhang,‡ Brian R. Krause,† Sandra L. Drake,† Narendra Lalwani,† Clay T. Cramer,† Brian Goetz,† Michael E. Pape,† Andrew McKee,† Gregory J. Fici,† Janell M. Lutostanski,† Stephen C. Brown,† and Charles L. Bisgaier†

Esperion Therapeutics, A Division of Pfizer Global Research and Development, 3621 South State Street, 695 KMS Place, Ann Arbor, Michigan 48108, and Alchem Laboratories Corporation, 13305 Rachael Boulevard, Alachua, Florida 32615

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A series of long (11−15) hydrocarbon chain diols and diacids with various central functional groups and terminal *gem*-dimethyl or -methyl/aryl substituents was synthesized and evaluated in both in vivo and in vitro assays for its potential to favorably alter lipid disorders including metabolic syndrome. Compounds were assessed for their effects on the de novo incorporation of radiolabeled acetate into lipids in primary cultures of rat hepatocytes, as well as for their effects on lipid and glycemic variables in obese female Zucker fatty rats, Crl:(ZUC)-faBR. The most active compounds were hydroxyl-substituted symmetrical diacids and diols with a 13-atom chain and terminal *gem*-dimethyl substituents. Furthermore, biological activity was enhanced by central substitution with O, C═O, S, S═O compared to the methylene analogues and was diminished for compounds with central functional groups such as carbamate, ester, urea, acetylmethylene, and hydroxymethylene.

Introduction

We have recently identified several series of functionalized long chain hydrocarbon derivatives as possessing lipid-regulating activity in an animal model of diabetic dyslipidemia (i.e., the obese female Zucker fatty rat).[1−3] The work was focused on the design of long hydrocarbon chain ether[1] and keto[2,3] diols and diacids with *gem*-dialkyl,[1−3] alkyl/aryl,[1,2] and cycloalkyl[3] substitution to the terminal acid or hydroxymethylene functions. The in vivo pharmacologic activity of these compounds is reflected in serum triglyceride reduction, decreases in non-HDL cholesterol (non-HDL-C), and increases in HDL cholesterol (HDL-C). In cell assays (in vitro), activity was also demonstrated by inhibiting both fatty acid and cholesterol syntheses in cultured liver cells at micromolar concentrations and by increasing fatty acid oxidation. Moreover, we reported earlier that this class of compounds form CoA derivatives in vivo and inhibit fatty acid synthesis (FAS) at acetyl-CoA-carboxylase (ACC), the rate-limiting step for FAS, via an allosteric mechanism.[4] The inhibition of fatty acid synthesis produced by such a mechanism of action (MOA) is consistent with the lowering of serum triglycerides and enhancement of β-oxidation. We have also shown that these compounds rapidly block de novo cholesterol synthesis at a step prior to the formation of mevalonic acid.[4] Thus, they are dual inhibitors of lipid synthesis. Statins (HMG-CoA reductase inhibitors), which only inhibit cholesterol synthesis, are distinct from these compounds, which also possess FAS inhibitory activity. When tested in animals, these compounds indeed manifest biological effects related to both cholesterol and FAS inhibition,[4] but in addition they possess biological activities that may be secondary to the direct targets (anti-atherosclerosis, anti-diabetic).[1c,1d,2b] Multiple mechanisms of actions have been reported earlier for compounds presenting HDL-elevating properties in various animal models.[5]

In our earlier studies,[1−3] we have found a chain length of 13−15 atoms to be optimal for biological activity. In addition, we established that the introduction of an oxygen atom either as an ether (**1**) or as a ketone (**2**) inside the chain increased the effect of lowering triglycerides and HDL elevation in the Zucker animal model compared to the methylene analogues. Similarly, Bisgaier et al. reported that ether-containing diacids were biologically active lipid regulators in vivo.[6,7] Ether diacids of 13−15 atom chain lengths proved to be the most active compounds. Our findings showed that active compounds displayed symmetrical or unsymmetrical structures with four to five methylene groups separating the central ether or ketone functionalities and the *gem*-dimethyl, -methyl/aryl, or -cyclopropyl substituents.[1−3] Bisgaier et al. similarly reported biological activity of symmetrical or unsymmetrical ether diacids,[6,7] the compound gemcabene having a 13-atom chain length showing activity in humans.[8] Furthermore, biological activity was found to be greatest in both in vivo and in vitro assays for *gem*-tetramethyl-substituted 5,5-keto-diacids and -diols, while bis(aryl-methyl) derivatives were shown to be the least active.[2]

Therefore, the purpose of the current study has been to examine the role of other central moieties for biological functions. To achieve this goal, we have explored the effects of various substituents having different bulk, electronic, and inductive effects and hydrogen bonding as exemplified in Figure 1 on in vivo and in vitro activity.

Results and Discussion

Chemistry. Long hydrocarbon chain compounds with diverse central moieties (Z, Figure 1) and terminal hydroxyl (X = H_2) or carboxyl (X = O) groups were synthesized. The side chains connected to the central moiety varied both in the number of methylene spacer units (n = 3 or 4) and in the attached geminal modifying groups (R = Me or Ph). The majority of target compounds fell in the category of sulfur derivatives (Scheme 1 and Table 1), namely, sulfides (**4a**−**d** and **8d**),[9] sulfoxides (**6a**−

* To whom correspondence should be addressed. Current address: Department of Chemistry, University of Florida, Gainesville, FL 32611-7200. E-mail: oniciu@yahoo.com.

† Esperion Therapeutics, A Division of Pfizer Global Research and Development.

‡ Alchem Laboratories Corporation.

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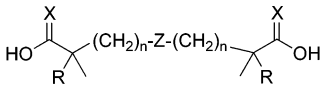
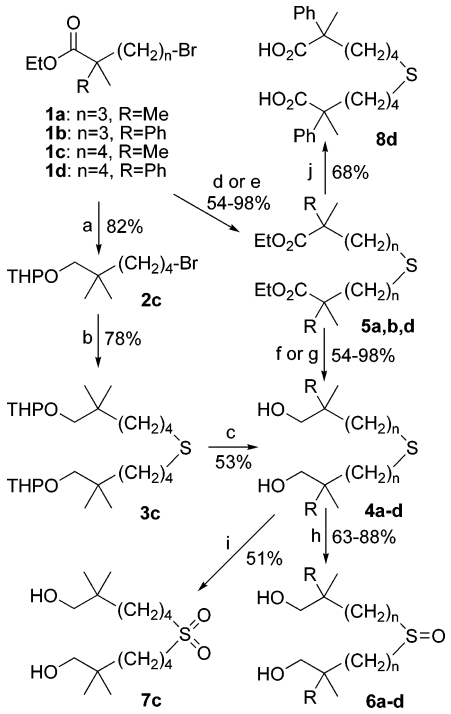


Z = S, SO, SO_2, CHOH, $CHCH_2OH$, CH-$COCH_3$, NH, N-OH, NH-C=O, O-C=O, O-P(O)OH-O, NH-C=O-O, CH_2, CO, O, NH-CO-NH
X = O, H_2; n = 3 - 4; R = CH_3, Ph

Figure 1. Overview of compounds.

Scheme 1. Synthesis of Sulfides, Sulfoxides, and Sulfones[a]



[a] Reagents: (a) $LiBH_4$, MeOH, CH_2Cl_2, then 3,4-dihydro-2*H*-pyran, *p*TosOH, CH_2Cl_2, described in lit. ref 2; (b) Na_2S nonahydrate, water/EtOH; (c) concd HCl, Δ, MeOH; (d) thiourea, KOH, EtOH; (e) Na_2S nonahydrate, water/EtOH; (f) $LiAlH_4$, Et_2O; (g) $LiBH_4$, MeOH, CH_2Cl_2; (h) aq H_2O_2, HOAc; (i) MCPBA, $CHCl_3$; (j) KOH, water/EtOH.

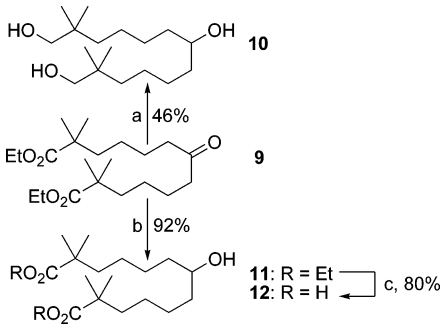
Table 1. Synthesis of Sulfides, Sulfoxides, and Sulfones

no.	*n*	R	yield (%)
3c	4	Me	78
4a	3	Me	98[a]
4b	3	Ph	54[b]
4c	4	Me	77 (53)[c]
4d	4	Ph	74[a]
5a	3	Me	89[d]
5b	3	Ph	85[e]
5d	4	Ph	85[d]
6a	3	Me	67
6b	3	Ph	69
6c	4	Me	63
6d	4	Ph	73
7c	4	Me	51
8d	4	Ph	68

[a] Prepared according to method f in Scheme 1. [b] Prepared according to method g in Scheme 1. [c] After distillation. [d] Prepared according to method d in Scheme 1. [e] Prepared according to method e in Scheme 1.

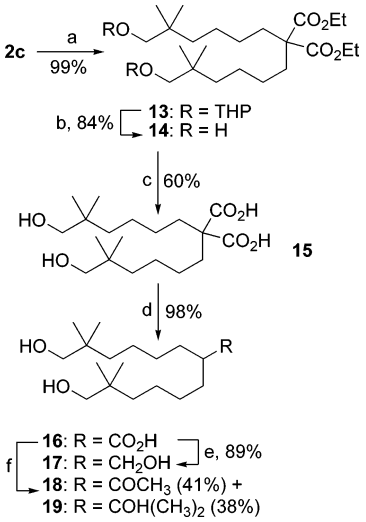
d),[10] and a sulfone (**7c**). Some alcohols (**10**, **12**, and **17**, Schemes 2 and 3),[11,12] a methyl ketone (**18**, Scheme 3),[13] amine derivatives (**21** and **23**, Scheme 4),[14,15] an amide (**30**, Scheme 5),[14,15] an ester **33**,[14] a phosphoric acid ester **35**,[14] and a carbamate **37**[14] (Scheme 6) were prepared and investigated as well. Previously prepared ether **38**,[1] ketones **39** and **41**,[2] and hydrocarbon analogue **40** (Figure 2)[1,2] are included in this work

Scheme 2. Synthesis of Alcohols **10** and **12**[a]



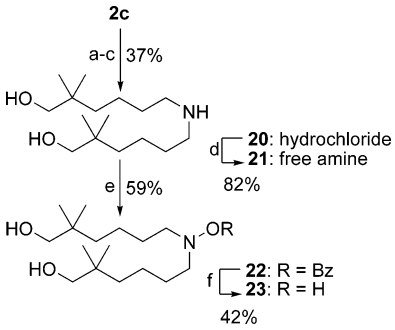
[a] Reagents: (a) $LiAlH_4$, MTBE; (b) $NaBH_4$, MeOH; (c) NaOH, water/EtOH.

Scheme 3. Synthesis of Alcohol **17** and Methyl Ketone **18**[a]



[a] Reagents: (a) diethyl malonate, NaH, TBAI, DMSO; (b) concd HCl, aq EtOH; (c) KOH, aq EtOH; (d) 200 °C; (e) $LiAlH_4$, THF; (e) MeLi, THF/Et_2O.

Scheme 4. Synthesis of Amine **21** and Hydroxylamine **23**[a]

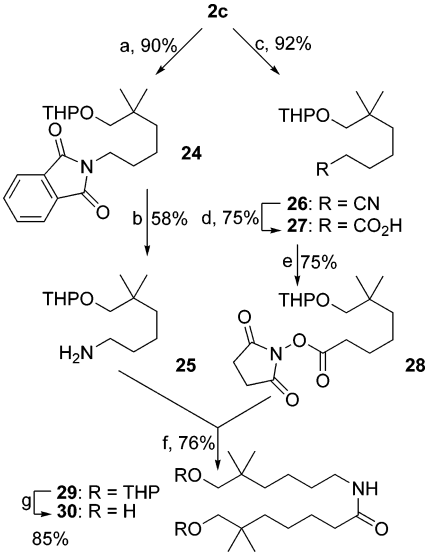


[a] Reagents: (a) *p*-toluenesulfonamide, NaOH, TBAI, 70−80 °C, C_6H_6/H_2O; (b) Na, naphthalene, DME; (c) concd HCl, MeOH; (d) aq NaOH, CH_2Cl_2; (e) benzoyl peroxide, Na_2HPO_4, 45 °C, MTBE; (f) NaOMe, MeOH.

for comparison. Urea derivative **42** was also prepared for this study (Scheme 7).[16]

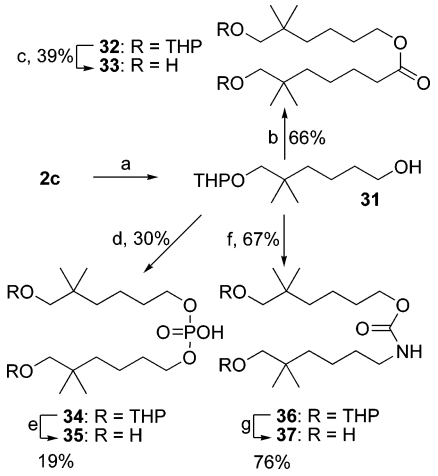
Sulfides **4a**−**d** were prepared by two slightly different pathways starting from bromo esters **1a**−**d** (Scheme 1).[1] For compounds with $n = 4$ and R = Me, bromo ester **1c** was reduced with $LiBH_4$ and MeOH[1] and the formed alcohol (not shown) was subsequently protected as tetrahydropyranyl (THP) ether **2c** (82%). Reaction of **2c** with Na_2S nonahydrate (0.5 equiv) in aqueous EtOH[17] (first at room temperature, then at reflux) gave sulfide **3c** in 78% yield. Removal of the THP protective groups in **3c** by treatment with concd HCl in MeOH

Scheme 5. Synthesis of Amide **30**[a]



[a] Reagents: (a) potassium phthalimide, 80−95 °C, DMF; (b) aq hydrazine, 78 °C, EtOH; (c) NaCN, 90 °C, DMSO; (d) NaOH, Δ, aq EtOH; (e) NHS, DCC, CH_2Cl_2; (f) CH_2Cl_2; (g) concd HCl, Δ, MeOH.

Scheme 6. Synthesis of Ester **33**, Phosphoric Acid Ester **35**, and Carbamate **37**[a]



[a] Reagents: (a) K_2CO_3, Δ, DMSO/water, described in lit. ref 1; (b) **27**, DCC, DMAP, CH_2Cl_2; (c) Δ, HOAc/THF/water; (d) $POCl_3$, NEt_3, Et_2O, then $KHCO_3$, aq CH_3CN; (e) concd HCl, MeOH; (f) CDI, DMAP, CH_2Cl_2, then **25**, CH_3CN; (g) Δ, HOAc/THF/water.

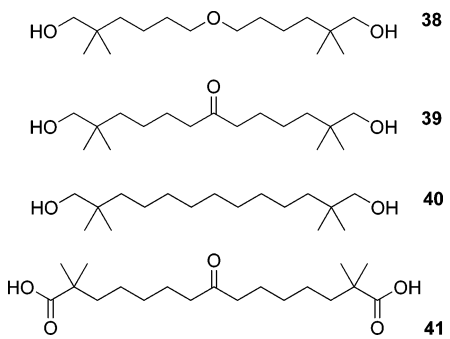
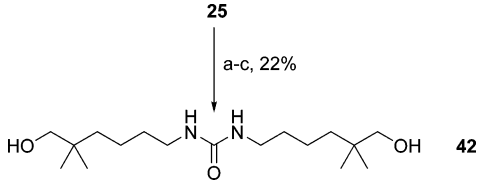


Figure 2. Ether **38**, ketone **39**, hydrocarbon analogue **40**, and ketodiacid **41**.

at reflux[18] furnished sulfide **4c** (77%, 53% after distillation). For all other sulfur compounds, a shorter approach was chosen. Accordingly, bromo esters **1a** and **1d** were reacted with thiourea and KOH in EtOH to give sulfides **5a** and **5d** in 89% and 85%

Scheme 7. Synthesis of Urea **42**[a]



[a] Reagents: (a) CO_2, pyridine, $(PhO)_3P$, 40−55 °C; (b) aq HCl, MeOH, Δ; (c) flash chromatography.

yield,[19] respectively, whereas reaction of **1b** with Na_2S afforded **5b** in 85% yield. Reduction of esters **5a**, **5b**, and **5d** with either $LiAlH_4$ or $LiBH_4$/MeOH gave the corresponding alcohols **4a**, **4b**, and **4d** in varying yields (54−98%). Sulfides **4a**−**d** were oxidized with H_2O_2 in acetic acid[20] to furnish sulfoxides **6a**−**d** (63−73%). With 3-chloroperoxybenzoic acid (MCPBA) used as an oxidizer,[21] sulfoxide **7c** was obtained from **4c** in 51% yield. The only diacid in the sulfur series, compound **8d**, was produced by saponification of **5d** with KOH in aqueous EtOH (68%).

Alcohols **10** and **12** were synthesized from keto diester **9** (Scheme 2).[11] Complete reduction of both ketone and ester functionalities in **9** was achieved by treatment with $LiAlH_4$ to give triol **10** in 46% yield. Use of $NaBH_4$ in MeOH as reducing agent led to selective reduction of the ketone group in **9** to alcohol diester **11** (92%), which was then saponified under standard conditions (NaOH, aqueous EtOH) to afford alcohol diacid **12** in 80% yield.

The synthetic sequence leading to alcohol **17** and methyl ketone **18** (Scheme 3) started with the dialkylation of diethyl malonate with alkyl bromide **2c** under action of NaH and tetrabutylammonium iodide (TBAI) in DMSO to afford compound **13** (99%). Removal of the THP protective groups in **13** with concentrated HCl in aqueous EtOH gave **14** (84%), which was then hydrolyzed (KOH, aqueous EtOH, 60%) to furnish malonic acid derivative **15**. Heating of **15** to 200 °C resulted in decarboxylation[22] to **16** (98%) and subsequent reduction of this compound with $LiAlH_4$ gave triol **17** in 89% yield. When carboxylic acid **16** was treated with an excess of methyllithium at 0 °C,[23] a mixture of methyl ketone **18** (41%) and tertiary alcohol **19** (38%) was formed, which was separated by column chromatography.

Bromide **2c** was also used as a building block for the synthesis of amine **21** and hydroxylamine **23** (Scheme 4). Amine hydrochloride **20** was prepared in 37% yield by 2-fold alkylation of *p*-toluenesulfonamide with **2c** under phase-transfer conditions (TBAI, aqueous NaOH/benzene, 70−80 °C)[24] and subsequent removal of the *N*-tosyl protecting group with sodium naphthalenide in DME[25] and of the THP groups by acid treatment (concentrated HCl/MeOH). The free amine **21**, obtained by extraction of **20** with aqueous NaOH solution and CH_2Cl_2 (82%), was further reacted with benzoyl peroxide and Na_2HPO_4 to give *O*-benzoylhydroxylamine **22** in 59% yield, as described for similar compounds.[26] Debenzoylation of **22** was achieved by treatment with sodium methoxide in MeOH to furnish hydroxylamine **23** in 42% yield after purification by chromatography and crystallization.

Amine **25**, the synthetic precursor of amide **30** (Scheme 5), was prepared by Gabriel synthesis[27] from **2c** via **24** in 52% yield. Substitution of bromide in **2c** by cyanide ion affected by treatment with NaCN in DMSO gave nitrile **26** (92%), which was subsequently hydrolyzed (NaOH in aqueous EtOH)[28] to carboxylic acid **27** in 75% yield. After conversion to NHS-ester **28** by reaction with *N*-hydroxysuccinimide (NHS) and 1,3-

dicyclohexylcarbodiimide (DCC) (75%),[29] this building block was condensed with the previously prepared amine **25** to give the THP-protected amide **29**. Removal of the THP-protective groups in **29** under acidic conditions (concd HCl/MeOH) resulted in formation of amide **30** in 85% yield.

The mono-THP protected diol **31**, prepared by hydrolysis of bromide **2c** with K_2CO_3 in a solvent mixture of DMSO and water,[1] was a common building block for the synthesis of compounds **33**, **35**, and **37** (Scheme 6). Coupling of **31** with **27** by means of DCC and 4-(dimethylamino)pyridine (DMAP) in CH_2Cl_2[30] led to intermediate **32**, which was subsequently deprotected under mildly acidic conditions (acetic acid/THF/water)[31] at 45 °C to furnish ester **33** in 26% yield over both steps. Condensation of alcohol **31** with $POCl_3$ and NEt_3 in Et_2O followed by treatment with $KHCO_3$ in aqueous acetonitrile[32] gave dialkyl phosphate **34** (30%). The THP-protective groups in **34** were removed with concentrated HCl in MeOH at reflux to afford phosphoric acid ester **35** in 19% yield. For the synthesis of carbamate **37**, alcohol **31** was condensed with 1,1′-carbonyldiimidazole (CDI) and DMAP in CH_2Cl_2[33] to form an intermediate imidazole-1-carboxylic acid ester (not shown) that was further reacted with amine **25** in acetonitrile to give protected carbamate **36** in 67% yield. Subsequent deprotection of the hydroxyl groups in a solvent mixture of acetic acid, THF, and water at 45 °C[31] afforded carbamate **37** in 76% yield.

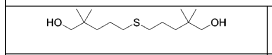
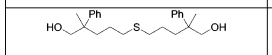
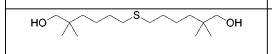
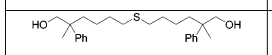
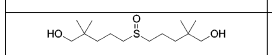
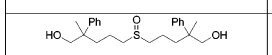
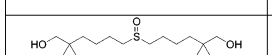
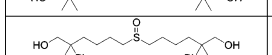
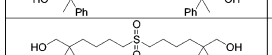
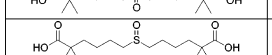
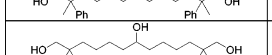
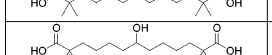
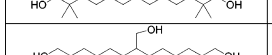
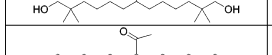
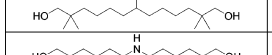
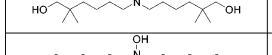
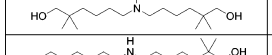
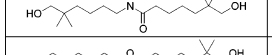
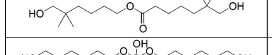
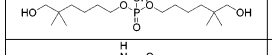
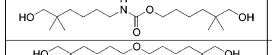
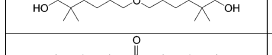
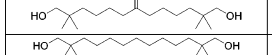
For synthesis of urea **42**, amine **25** was first condensed with CO_2 using triphenyl phosphite as a condensing agent in pyridine (Scheme 7).[9] The obtained THP-protected intermediate (not shown) was then deprotected with concd HCl in MeOH to give urea **42**, which was purified by flash chromatography, in 22% yield over both steps.

Biological Activity. To evaluate the effect of the central moiety on biological activity, we largely limited the SAR to those compounds containing the terminal hydroxymethylene moiety. In vivo and in vitro tests were used to establish the SAR for the central substitution series. The hepatic lipid synthesis assay (HLS) tests the compound effects on the incorporation of ^{14}C-acetate into total cellular lipids of primary rat hepatocytes (Table 2). The obese female Zucker rat, an animal model of diabetic dyslipidemia, was used to test compound effects on serum lipid variables. Compounds were administered by oral gavage over a 2-week period at a single daily dose of 30 or 100 mg/kg (Table 3).

Effect on Lipid Synthesis In Vitro. Compounds in this study display terminal *gem*-dimethyl-hydroxymethylene (e.g., **4a**, **6c**) and *gem*-dimethylcarboxylic groups (e.g., **12**), or methylphenyl-hydroxymethylene groups (e.g., **4b**, **6b**). In vitro HLS studies demonstrated that dimethyl-substituted derivatives were the most active, while most of methylphenyl-substituted derivatives had reduced activity. Therefore, we restricted further SAR studies to compounds having terminal *gem*-dimethyl-hydroxymethylene groups. Replacement of the central oxygen, sulfur, or carbon atoms with nitrogen resulted in little (**23**) or no activity (**21**, **30**). Upon introduction of a phosphate group (**35**) or an amide group (**30**), the activity was lost. A central carbamate moiety (**37**) or a urea (**42**) showed similar activity to ketone **39**. Replacing the central carbonyl with hydroxylamine resulted in activity reduction, for example, hydroxylamine **23** was 40-fold less active than ketone **39**.

Therefore, the activity in the in vitro HLS assay decreased in the series NH−C(O)−O > CO ≈ CH(OH) ≈ NH−C(O)−NH > SO_2 ≈ S ≈ O > SO (**37** > **39** > **12** ≈ **7c** ≈ **38** ≈ **4a** > **6d**).

Table 2. Effect on Lipid Synthesis in Primary Rat Hepatocytes

Compound		IC_{50} (μM)	95% Confidence Interval		R^2
			Lower	Upper	
	**4a**	15	8.5	29.3	0.961
	**4b**	41	17.9	95.2	0.945
	**4c**	18	10.0	33.8	0.965
	**4d**	64	26.4	159	0.952
	**6a**	NA[a]			
	**6b**	NA[a]			
	**6c**	NA[a]			
	**6d**	81	63.3	108	0.994
	**7c**	8.9	4.3	18.4	0.968
	**8d**	26	12.0	56.6	0.959
	**10**	6.4	3.7	11.1	0.998
	**12**	4.7	0.9	24.0	0.903
	**17**	15	12.6	18.8	0.998
	**18**	46	36.6	59.1	0.995
	**21**	NA[a]			
	**23**	63	49.3	81.1	0.997
	**30**	NA[a]			
	**33**	13	5.4	30.9	0.986
	**35**	NA[a]			
	**37**	0.80	0.4	1.5	0.984
	**38**[b]	10.7	5.5	40.6	0.967
	**39**	1.5	0.2	11.4	0.967
	**40**[c]	4.2 58	2.6 35.1	6.9 98.2	0.990 0.978
	**42**	3.4	2.9	3.9	0.999

[a] Not active; inhibition of ^{14}C-acetate incorporation into total lipids is less than 50% at 300 μM. [b] Test compound was assayed multiple times, the values represent the average. [c] Test compound was assayed twice, and both results are displayed.

Based on the in vitro data, carbamate **37** (IC_{50} 0.8), ketone **39** (IC_{50} 1.5), urea **42** (IC_{50} 3.4), alcohols **10** (IC_{50} 6.4) and **12** (IC_{50} 4.7), and hydrocarbon analogue **40** (IC_{50} 4.2) were likely to possess in vivo activity as well. In addition, ether **38** (IC_{50} 10.7), sulfide **4a** (IC_{50} 15), and sulfone **7c** (IC_{50} 8.9) showed good lipid synthesis inhibition. In contrast, sulfoxide **6a** was not active (although it proved to be active in vivo), while sulfone **7c** showed good activity (IC_{50} 8.9).

Effect on Lipid Variables in the Obese Female Zucker Rat. To test the lipid regulating activity of these compounds, we used the obese Zucker fatty rat, Crl:(Zuc)-faBR, as a model of diabetic dyslipidemia, as described in previous articles of the same group.[1−3] The Zucker rat has a mutation in the leptin receptor that leads to a metabolic disorder similar to human non-insulin-dependent diabetes mellitus (NIDDM) or type II

Table 3. Effect in Female Obese Zucker Rats[a]

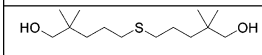
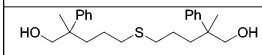
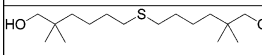
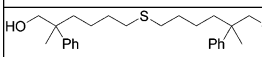
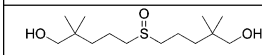
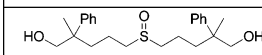
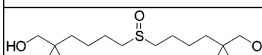
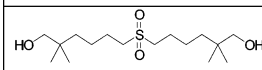
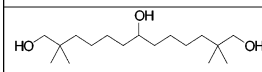
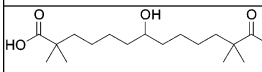
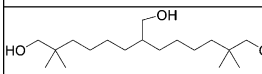
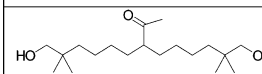
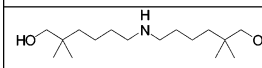
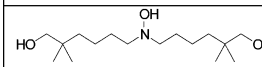
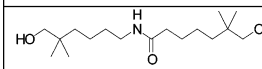
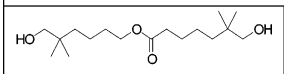
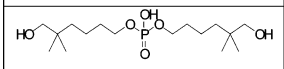
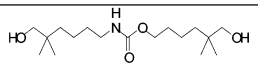
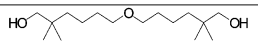
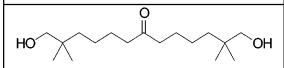
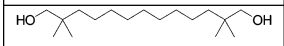
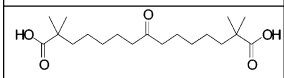
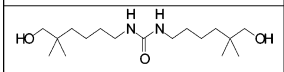
Compound		Serum Variables Percent Change from Pre-Treatment										
				Non-HDL-Cholesterol (Week)			HDL-Cholesterol (Week)			TG (Week)		
		Dose	n	Pre	1	2	Pre	1	2	Pre	1	2
	**4a**	30	4	100 (24 ± 6)	-40*	-14	100 (43 ± 4)	54*	40*	100 (849 ±167)	-51*	-34*
	**4b**	100	3	100 (31 ± 12)	-26	8	100 (39 ± 8)	9	2	100 (1237 ±403)	-18	1
	**4c**	100	5	100 (21 ± 7)	-11	9	100 (38 ± 9)	141*	153*	100 (861 ±247)	-37	-56
	**4d**	ND										
	**6a**	100	4	100 (37 ± 32)	40	12	100 (26 ± 10)	54	93*	100 (821 ± 267)	-36	-5
	**6b**	80	3	100 (25 ± 1)	68	81	100 (58 ± 2)	-23	-36	100 (667 ± 279)	18	41
	**6c**	100	3	100 (13 ± 6)	56	64	100 (31 ± 10)	127*	233*	100 (642 ± 399)	-37	-35
	**7c**	100	3	100 (37 ± 7)	-46*	-34*	100 (32 ± 9)	15	-13	100 (1104 ± 199)	-34	-19
	**10**	100	2	100 (54)	-61	-15	100 (28)	219	222	100 (1509)	-70	-46
	**12**	100	4	100 (26 ± 10)	-6	-41	100 (43 ± 9)	57	50	100 (819 ± 337)	-32	-59*
	**17**	100	3	100 (27 ± 6)	-43*	-12	100 (49 ± 8)	2	-2	100 (992 ± 196)	-52*	-23*
	**18**	30	5	100 (20 ± 6)	15	52	100 (44 ± 7)	-14*	-19*	100 (715 ± 266)	-6	22
	**21**	53	2	100 (21)	78	-10	100 (32)	-23	16	100 (940)	-24	-32
	**23**	30	3	100 (27 ± 12)	-40	-5	100 (85 ± 10)	27	19	100 (951 ± 230)	-26	13
	**30**	30	4	100 (23 ± 7)	-21	-7	100 (41 ± 2)	2	20	100 (821 ± 217)	-20	-10

Table 3 (Continued)

Compound		Serum Variables Percent Change from Pre-Treatment										
				Non-HDL-Cholesterol (Week)			HDL-Cholesterol (Week)			TG (Week)		
		Dose	n	Pre	1	2	Pre	1	2	Pre	1	2
	**33**	100	3	100 (19 ± 1)	-25	-5	100 (38 ± 6)	18	23*	100 (711 ± 129)	-20	7
	**35**	80	2	100 (23)	-29	-17	100 (29)	27	8	100 (756)	-10	-13
	**37**	100	3	100 (22 ± 5)	10	84*	100 (47 ± 6)	60*	65*	100 (758 ± 219)	-52	-4
	**38**	100	4	100 (23 ± 8)	-38	11	100 (59 ± 12)	234*	366*	100 (746 ± 243)	-77*	-71*
	**39**	100	4	100 (41 ± 20)	-23	-10	100 (32 ± 15)	111*	126	100 (1415 ± 749)	-54	-29
	**40**	100	4	100 (39 ± 14)	-67*	-75*	100 (49 ± 23)	189*	137	100 (1130 ± 472)	-83*	-82*
	**41**	100	4	100 (35 ± 3)	-98*	-99*	100 (47 ± 13)	72*	168*	100 (1159 ± 152)	-93*	-94*
	**42**	100	4	100 (28 ± 4)	-33*	-5*	100 (39 ± 8)	66*	92*	100 (1057 ± 99)	-42*	-31*
Control		0	66	100 (28±19)	102 (29±19)	97 (27±10)	100 (47±15)	92 (43±19)	90 (42±20)	100 (1034±485)	100 (1038±49)	98 (1012±375)

[a] ∗ indicates $p < 0.05$ (paired Student's t-test); for compounds where $n < 3$, no statistics were performed. Percent change represents the change of individual animals averaged. The pretreatment "pre" values are expressed as 100%. Absolute data are shown as mean mg/dL ± standard deviations in parentheses.

diabetes and causes an age-dependent progression of hypertriglyceridemia, VLDL cholesterol (VLDL-C) elevation, HDL-C reduction, impaired insulin sensitivity, hyperphagia, and marked weight gain leading to obesity. Characteristic for this rodent model is that the non-HDL-C is mainly VLDL-C with essentially no LDL-C with markedly elevated plasma triglycerides. Thus the lipoproteins in this model are distinct from humans, where most of the non-HDL-C is carried in the LDL fraction.

The lipid regulating activities of the target compounds in this model were assessed by administering a single dose of 30 mg/(kg·day) or 100 mg/(kg·day) every day for up to two weeks. Compounds were evaluated for their ability to reduce non-HDL-C, elevate HDL-C, and reduce triglycerides (TG), namely, a less atherogenic serum lipid profile. Because the disease rapidly progresses in control animals, the non-HDL-C and TG fractions increase, while the HDL is reduced over the two-week experimental period. In our animal model, effective test agents should suppress both non-HDL-C and TG levels, while elevating HDL. Table 3 summarizes those serum lipid changes induced by the tested compounds.

Compounds with *gem*-methylphenyl substitution were not active, regardless of the central moiety (S for **4b**, SO for **6b**), as described earlier for their carbonyl[2] and ether[1] analogues, which infers that dimethyl substitution is desirable. It is possible that the bulk of the methylphenyl moiety does not allow for inhibition of FAS. The compounds may be unable to form xenobiotic CoA's or the ones that are formed are unable to inhibit ACC and may block compound metabolism required for the allosteric ACC inhibition.[4] The central synthon might also influence the ability of the compounds to fit into the pocket and may allow for an easier oxidation of the terminal diol to the corresponding acid with the eventual formation of the xenobiotic CoA. We can speculate that the nature of the central moieties could influence the incorporation of the compounds

in other lipids, such as diglycerides, TG, phospholipids, or cholesteryl esters, or their regulation of the synthesis of fatty acids.

From analysis of the results presented in Table 3, it is obvious that the number of atoms between the quaternary substitutions should be nine or higher. The increasing order of activity for compounds in terms of HDL-C elevation and TG lowering after two weeks of treatment was **38** (O) > **6c** (S═O) > **10** (CH(OH)) > **4c** (S) > **39** (C═O) > **40** (CH_2). The sulfur substitution in both sulfide **4c** and sulfoxide **6c** favorably altered non-HDL-C, HDL-C, and triglycerides in the Zucker rat model, despite the in vitro results. Sulfur derivative **4a** showed reasonable HDL-C elevation and TG lowering properties, although the chain consisted only of seven atoms. Perhaps since the sulfur atom has a larger atomic radius than either carbon or oxygen, the compound topology could sterically better mimic a nine-atom chain, resulting in biological activity.

A central hydroxyl group as in compound **10** altered positively the lipid profile compared to the hydrocarbon analogue **40** and even to the ketone **39**. Hydroxyl derivative **10** showed a better profile than the corresponding diacid **12**, which may be due to absorption, metabolism, distribution, and excretion (ADME) factors.

In the ketone series, compound **39** showed good activity, while compound **18** displaying the double bond in a side chain showed no activity. Other ketone derivatives have been extensively discussed in earlier publications.[2,3]

When the central atom in the hydrocarbon chain was replaced with nitrogen or hydroxylamine, the lipid lowering activity was weakened or lost compared to the keto, sulfur, and hydroxyl analogues. Among other central moieties tested, urea **42** and carbamate **37** positively altered the lipid profile, although less than sulfur, ketone, and hydroxyl derivatives. The internal conjugation in the urea functionality and the difference in length for the C−C−C and C−N−C(═O) bonds definitely induce steric and topologic modifications compared to the above-mentioned derivatives, with implications in binding to active sites and additional hydrogen bonding or hydrogen donation for the urea derivatives. Since compound **41** with the same number of carbons in the chain as **42** is extremely active, one can infer that the steric and electronic changes introduced by the nitrogen atoms in the chain are detrimental to the activity.

Pharmacokinetics and Drug Metabolism of 1,13-Dihydroxy-2,2,12,12-tetramethyl-tridecan-7-one (39). In a species comparison study, primary hepatocytes from rats, dogs, monkeys, and humans were incubated with compound **39** (parent) to determine the metabolite profile of the test article. Suspensions of cryo-preserved (dog, monkey, and human) or freshly isolated (rat) hepatocytes were incubated with 1 or 10 μM compound **39** in 4-h time-course studies. Cell viability controls and cell-free incubation controls were also evaluated; the drug had no effect on viability at these concentrations. Cells and media were periodically assayed for the parent compound and metabolites by mass spectrometry (LC/MS). Analytical ranges of 5 to 50 000 ng/mL for all species were readily attained. Deuterated standards, in which eight of the internal hydrogens were substituted to >95% by deuterium, were prepared for use as internal standards for further quantitation. Deutero (d_8) derivatives were prepared by the same reaction sequence as their nondeuterated analogues[2,11,13] using commercially available 1,4-dibromo-2,2,3,3-tetradeuteriobutane. The deuterated standards were used to identify and confirm the metabolites of compound **39** by mass spectroscopy based on retention time and fragmentation pattern.

Table 4. Metabolites Observed in Hepatocytes of Multiple Species

metabolite name[a]	possible biotransformation	rat	dog	monkey	human
43	monocarboxylic acid of **39**	X	X	X	X
M2	glucuronide of **39**	X	X	X	X
10	reduction of **39**	X	X	X	X
44	monocarboxylic acid of **10**	X	X		X
M5	glucuronide of **10**	X	X	X	X
12	dicarboxylic acid of **10**	X			X
M7	double glucuronide of **10**		X		
M8	glucuronide of **44**		X		
M9	glucuronide of **44**	X	X		
M10	glucuronide of **43**		X		
M11	glucuronide of **43**	X	X		
45	dicarboxylic acid of **39**				
39	parent				

[a] Measurement of parent and metabolites consisted of organic solvent, protein precipitation of suspension hepatocyte cultures, C18 reversed-phase HPLC analyses using standard methods, followed by positive ion ESI-mass spectral analysis using an LCQ ion trap or TSQ triple quad mass spectrometer. Sample aliquots were injected and desalted using standard column switching methods, then eluted under conditions where the metabolites of interest demonstrated retention times from 7 to 9 min. The only significant fragmentation observed for all molecules was dehydration, so ESI source temperatures were kept to ~220 °C. Molecules were identified by retention time, molecular ion, and observation of dehydration.

These studies identified compound **39** and putative metabolites in the hepatocyte cultures, including a monocarboxylic acid (**43**), the central secondary alcohol of compound **39** (**10**), and a mono- and dicarboxylic acid of the secondary alcohol (**44**, **12**). Compound **39** was extensively metabolized by all species, leaving only 6% or less of the parent compound remaining at the end of the incubation period for both starting concentrations (Table 4). The non-glucuronide metabolites were detectable in all species with the exception of compounds **12** in dog and monkey and **44** in monkey. The only glucuronide species detected in human cells were adducts of the test compound **39** and triol metabolite **10**. Since oxidation of compound **39** is a major pathway in hepatocyte metabolism, the prediction is that metabolites **12**, **45**, or both will achieve high levels in plasma or serum after oral dosing of compound **39** in preclinical and clinical studies.

The major metabolic processes observed were oxidation of the primary alcohols to carboxylic acids and reduction of the ketone to a secondary alcohol. Several glucuronide species have been observed as secondary metabolites but were not extensively characterized. Other potential secondary metabolites, such as sulfates or amino acid amides, were not observed. The metabolism of compound **39** is depicted in Figure 3, based on the in vitro hepatocyte studies detailed above and from rat plasma analyses after oral and intravenous drug administration.

A study was conducted to identify compound **39** and its metabolites in male Wistar rats at multiple time points after a single 100 mg/kg oral (Figure 4) or 10 mg/kg intravenous dose (Figure 5). Analytical techniques used to identify parent and metabolites in serum were similar to those described above for hepatocytes.

Parent compound levels are rapidly removed from circulation, achieving extremely low or nondetectable levels when compared to the administered drug (Table 4). Of the predominant metabolites, the dicarboxylic acid **45** and reduced metabolite **12** exhibited the highest $T_{1/2}$, C_{max}, and AUC values. C_{max} values for **12** achieved 40.6 μg/mL in this study (Table 5). These

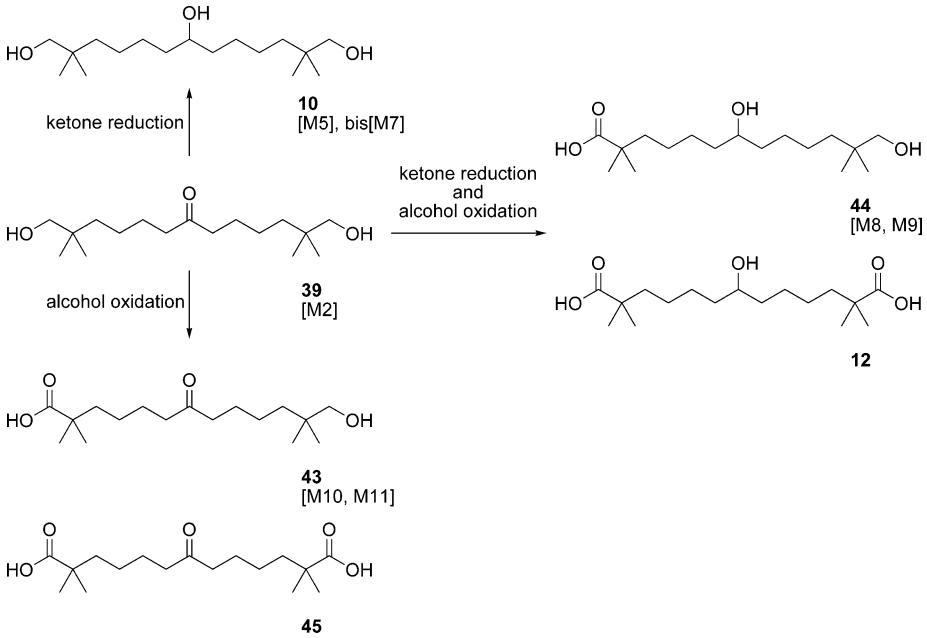


Figure 3. Diagram of compound **39** metabolism. "M#" indicates the metabolite ID number of corresponding glucuronide adducts, indicated by closed brackets, for example, "[**M2**]".

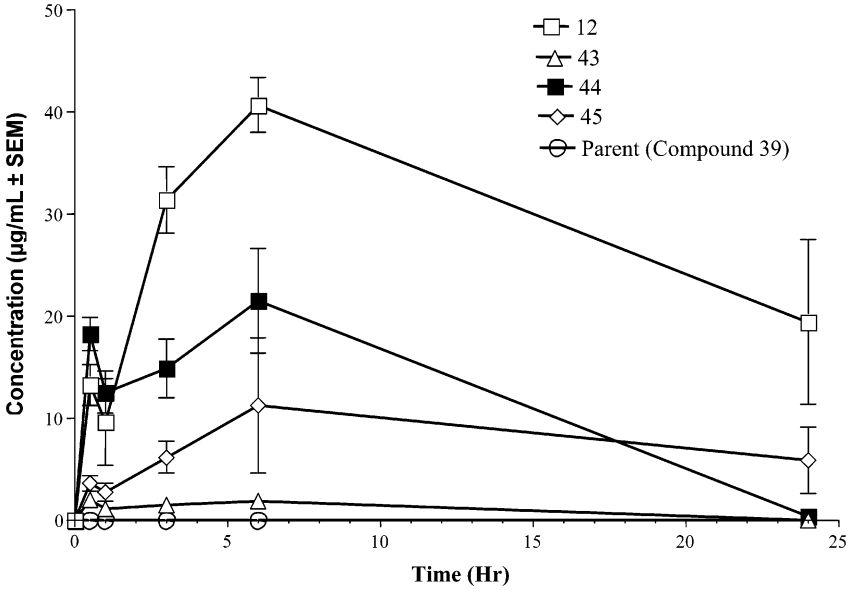


Figure 4. Pharmacokinetics of compound **39** and its major metabolites in serum after oral administration at 100 mg/kg. Each data point represents the average value from three animals ± standard error of the mean, with the exception of **12** at 6 h where $n = 2$. Note that one of the three data points for **12** at 6 h was three times the average of the other two values and was eliminated in the graph shown. If this data point was included, the mean value for **12** at 6 h would be 69.1 ± 28.5 μg/mL.

exploratory data suggest high oral bioavailability of compound **39** and rapid conversion to metabolites.

Preliminary Toxicology Data. a. Genetic Toxicology. Mutagenicity was evaluated by AMES assay for compounds **38** and **39**. *Salmonella typhimurium/Escherichia coli* plate incorporation/preincubation mutation assay was used in the presence and absence of induced rat liver S-9. *Salmonella typhimurium* tester strains, TA98, TA100, TA1535, and TA1537, and *E coli* strain WP2 uvrA were used and results demonstrated that both **38** and **39** were nonmutagenic with and without metabolic activation.

b. Safety and Tolerability in Rats. Safety and tolerability of compounds **4c**, **38**, and **39** were evaluated in rats through adminstration of compounds by oral gavage as a single dose or daily doses for 2 weeks. Compound **39** was well tolerated as a single dose at 2000 mg/kg in both sexes. Upon 2-week dosing, reduced body weights were observed in males at 1000 mg/kg. Dose-related increases in liver weights at all doses and upon histological evaluation hepatocyte hypertrophy were observed in animals treated with ≥200 mg/kg. Compound **39** was well tolerated for 2 weeks at doses of 1000 mg/kg, and 200 mg/kg was considered minimal effect dose. Compound **38** was well tolerated at 2000 mg/kg with mild liver effects. Following a 2-week treatment, body weight gain suppression was observed. Dose-dependent increases in liver weights and slightly decreased red blood cell parameters (red blood cell count, hemoglobin, and hematocrit) were observed following 2-week treatment. Compound **4c** was well tolerated following a single dose administration of 5000 mg/kg. Following a 2-week administration with doses of 50, 100, and 500 mg/kg, decreased body weight gains and increased liver weights were observed. All clinical chemistry parameters measured were within normal

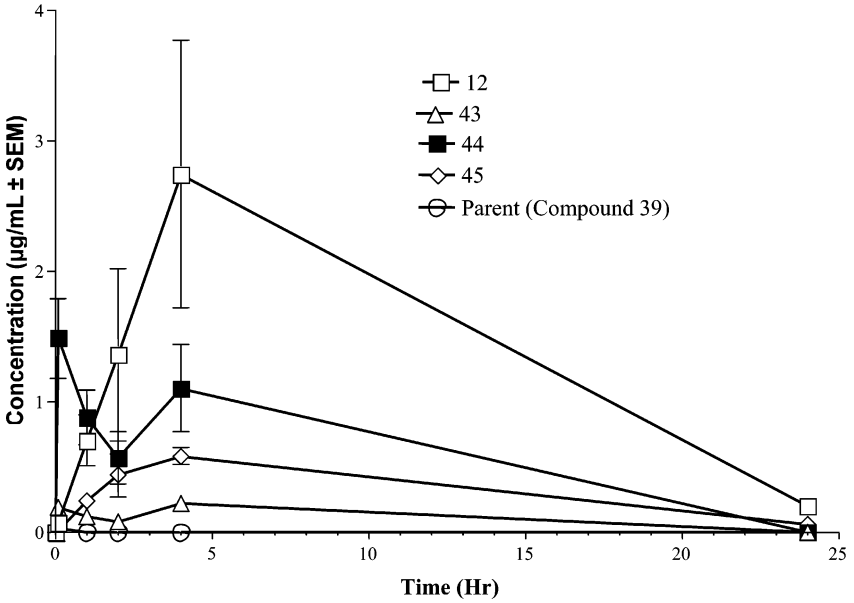


Figure 5. Pharmacokinetics of compound **39** and its major metabolites in serum after intravenous administration at 10 mg/kg. Each data point represents the average value from three to five animals ± standard error of the mean, with the exception of 0 h (pretreatment) where $n = 2$.

Table 5. Pharmacokinetics of Compound **39** Following Single Oral Dose Administration (100 mg/kg) in Male Wistar Rats

variable	**39** (parent)	**43**	**44**	**45**	**12**
$T_{1/2}$ (h)	*a*	2.5	3.1	13.5	16.9
C_{max} (μg/mL)	*a*	2.0	21.4	14.9	40.6
T_{max} (h)	*a*	0.5	6.0	6.0	6.0
$AUC_{(0-t)}$ (μg·h/mL)	*a*	26.7	289.9	230.3	703.3
AUC_{∞} (μg·h/mL)	*a*	26.8	291.6	344.9	1176.1
volume of distribution (mL/kg)	*a*	13670.9	1533.2	5631.9	2072.1
clearance (mL/h)	*a*	747.9	69.0	86.8	28.4

[a] Variables for parent compound **39** could not be determined because most data points were below quantifiable limits. $T_{1/2}$ denotes half-life; C_{max} denotes maximal plasma concentrations; T_{max} denotes time of maximum plasma concentration; AUC denotes area under the curve. Concentrations were determined using co-injected authentic (d_8) internal standards.

range including liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT). In summary, all compounds tested were well tolerated in the preliminary drug safety evaluations performed.

Conclusions

The compounds tested showed a large variation of central moieties and a narrow variation of the length of the chain (11−15 atoms). The SAR showed that introducing oxygen, sulfur, carbonyl, and hydroxyl in the center of the molecules favored a good lipid profile. Compounds with nitrogen or phosphorus atoms included in the central moieties showed either reduced or no activity. Compounds containing more than one heteroatom also had reduced activity, with the exception of urea **42** and carbamate **37**.

These biological observations imply that the chemical features influencing the activity are the following:

•Steric factors—tetramethyl substitution is favored over arylmethyl substitution (e.g., **4b**, **6b**).

•Chain length and geometry—the desirable chain length should be 11−15 atoms.

•Nature of heteroatoms—heteroatoms may inflict interactions with other sites than the desired ones by their ability to form hydrogen bonds or by having a different molecular geometry that does not allow interaction with the required active sites (e.g., **21**, **23**, **30**, and **35**).

•Position of the central moiety—hydroxyl and ketone functionalities should be directly connected to the main chain. Their presence in the lateral chain diminished the activity (e.g., **17**, **18**).

•Electronic effects—inductive and electron-donor properties that lead to conjugation in the center of the molecule may influence the accessibility of the active sites or lead to deactivation of the molecule by weak interactions with other substrates. This may explain why compounds **30**, **33**, **37**, and **42** showed a lower activity as compared to compound **41**, having similar chain length and a central ketone moiety.

Compound **39** was selected for pharmacokinetics and drug metabolism studies because it was considered the most illustrative of this series. Results showed that compound **39** is metabolized rapidly to more polar species and their conjugates with glucuronic acid such that major exposures of compounds **12** and **45** result from oral and intravenous administration of **39**. Another example previously published by our group[4] showed the metabolism of the central hydroxyl form of dicarboxylic acid **41** to its biologically formed CoA ester. This biologically formed CoA ester, but not its precursor, was shown to be the active agent causing allosteric inhibition of acyl-CoA-carboxylase (ACC), the rate-limiting enzyme in fatty acid synthesis. Since CoA esters are only formed within living cells, it was necessary to invoke a cell- or animal-based assay to screen for biological activity.

Therefore, the SAR was developed in hepatocyte and whole animal systems wherein test agents can be subject to absorption, distribution, metabolism, and elimination considerations. Thus, the biological activity and hence the observed SAR likely result from combined effects of the test agent and its metabolites.

Experimental Section

Chemistry. Chemical reagents from Sigma-Aldrich, Acros, or Lancaster were used without further purification. Silica gel for column chromatography (0.035−0.070 mm, pore diameter ca. 6 nm) was obtained from Acros Organics. ACS grade solvents from Fisher Scientific or Mallinckrodt were routinely used for chro-

matographic purifications and extractions. Melting points (uncorrected) were determined on either a Thomas-Hoover capillary or Haake-Buchler melting point apparatus. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra at 75 MHz and ambient temperature on Varian NMR spectrometers. Chemical shifts for proton NMR are given in parts per million downfield from an internal tetramethylsilane standard, and ^{13}C chemical shifts are calibrated on the $CDCl_3$ resonance at 77.23 ppm, unless otherwise specified. Coupling constants (*J*) are given in hertz. The purity of target compounds was analyzed using Shimadzu HPLC systems equipped with UV or RI detection or both.

Bis-(5,5-dimethyl-6-tetrahydropyranyloxyhexyl)-sulfide (3c). A solution of **2c** (14.9 g, 50.8 mmol) in EtOH (100 mL) was added dropwise over 30 min to a solution of Na_2S nonahydrate (6.1 g, 25.4 mmol) in water (10 mL) at room temperature under N_2 atmosphere. The reaction mixture was stirred for 18 h and then heated to reflux for 3.5 h. The solution was concentrated in vacuo, 5% aqueous NaOH solution (100 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (200 mL). The organic layer was dried over $MgSO_4$, concentrated in vacuo, and dried in high vacuum to give **3c** (9.2 g, 78%) as a slightly yellowish oil. ^{1}H NMR ($CDCl_3$): δ 4.54 (t, 2H, $J = 2.9$), 3.83 (m, 2H), 3.48 (m, 2H), 3.45 (d, 2H, $J = 9.2$), 2.98 (d, 2H, $J = 9.2$), 2.50 (t, 4H, $J = 7.3$), 1.82 (m, 2H), 1.75−1.44 (m, 16H), 1.42−1.18 (m, 10H), 0.89 (s, 6H), 0.89 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 98.90, 76.26, 61.68, 38.77, 34.04, 32.00, 30.51, 25.44, 24.42, 24.36, 23.20, 19.28. HRMS: calcd for $C_{26}H_{51}SO_4$ (MH^+) 459.3508; found 459.3504.

5-(5-Hydroxy-4,4-dimethylpentylsulfanyl)-2,2-dimethylpentan-1-ol (4a). Under N_2-atmosphere, $LiAlH_4$ (1.0 g, 26 mmol) was added to anhydrous Et_2O (100 mL) and stirred for 10 min. To this solution was added a solution of **5a** (3.10 g, 8.90 mmol) in Et_2O (50 mL). The reaction mixture was heated to reflux for 3 h and stirred at room temperature overnight. The excess of $LiAlH_4$ was decomposed by addition of EtOAc (20 mL). The sludge was dissolved in aqueous HCl (5 N, 5 mL) and water (50 mL). The mixture was extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with saturated NH_4Cl solution (20 mL), dried over $MgSO_4$, concentrated in vacuo, and dried in high vacuum to give **4a** (2.3 g, 98%) as an oil. ^{1}H NMR ($CDCl_3$): δ 3.31 (s, 4H), 2.72 (br, 2H), 2.50 (t, 4H, $J = 7.3$), 1.62−1.50 (m, 4H), 1.37−1.25 (m, 4H), 0.87 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 71.61, 37.86, 35.16, 33.21, 24.36, 24.02. HRMS: calcd for $C_{14}H_{31}O_2S$ (MH^+) 263.2045; found 263.2044. HPLC: 92.7% pure.

5-(5-Hydroxy-4-methyl-4-phenylpentylsulfanyl)-2-methyl-2-phenylpentan-1-ol (4b). Under N_2-atmosphere, MeOH (2.96 g, 92.6 mmol) was added dropwise to a stirred suspension of $LiBH_4$ (2.04 g, 92.6 mmol) in anhydrous CH_2Cl_2 (160 mL) at room temperature. A solution of **5b** (14.5 g, 30.9 mmol) in anhydrous CH_2Cl_2 (140 mL) was added dropwise. The reaction mixture was heated to reflux overnight. The reaction mixture was cooled to 5 °C, and saturated NH_4Cl solution (200 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (3 × 300 mL). The combined organic layers were washed with water (2 × 200 mL), dried over $MgSO_4$, and concentrated in vacuo to yield the crude product (12.5 g). Purification by column chromatography (silica gel, EtOAc/heptane = 1/9 to 1/3) furnished **4b** (6.39 g, 54%) as a colorless, viscous oil. ^{1}H NMR ($CDCl_3$): δ 7.29−7.19 (m, 10H), 3.64 (d, $J = 9.9$, 2H), 3.51 (m, 2H), 2.32 (t, $J = 6.9$, 4H), 1.81−1.77 (m, 2H), 1.58−1.37 (m, 6H), 1.31 (s, 6H), 1.27−1.21 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 144.5, 128.5, 126.7, 126.2, 72.5, 43.4, 37.6, 32.8, 24.0, 21.7. HRMS: calcd for $C_{24}H_{35}O_2S$ (MH^+) 387.2358; found 387.2350. HPLC: 95.6% pure. Anal. ($C_{24}H_{34}O_2S$) C, H, S.

6-(5,5-Dimethyl-6-hydroxyhexylsulfanyl)-2,2-dimethylhexan-1-ol (4c). A solution of **3c** (9.2 g, 20.0 mmol) in MeOH (100 mL) and concd HCl (10 mL) was heated to reflux for 1.5 h under N_2 atmosphere. The reaction mixture was cooled to room temperature, concentrated in vacuo, diluted with CH_2Cl_2 (250 mL), and extracted with saturated $NaHCO_3$ solution (2 × 100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give **4c** (4.5 g, 77%) as a malodorous oil. ^{1}H NMR ($CDCl_3$): δ 3.30 (s, 4H), 2.52 (t, 4H, $J = 7.1$), 2.24 (s, 2H), 1.57 (m, 4H), 1−1.18 (m, 8H), 0.86 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 71.43, 37.88, 34.86, 31.90, 30.20, 23.78, 22.93. HRMS: calcd for $C_{16}H_{35}SO_2$ (MH^+) 291.2358; found 291.2353. Anal. ($C_{16}H_{34}O_2S$): C, H, S. On a larger scale, deprotection of **3c** (265.0 g, 0.58 mol) as above gave crude **4c** (152.0 g), which was distilled in high vacuum, affording **4c** (90.0 g, 53%). Bp 175−180 °C, 0.1−0.2 mm. HPLC: 99.2% pure.

6-(6-Hydroxy-5-methyl-5-phenylhexylsulfanyl)-2-methyl-2-phenylhexan-1-ol (4d). Under N_2-atmosphere, MeOH (1.41 g, 43.98 mmol) was added dropwise to a stirred suspension of $LiBH_4$ (0.97 g, 43.98 mmol) in anhydrous CH_2Cl_2 (80 mL) at room temperature. A solution of **5d** (7.3 g, 14.66 mmol) in anhydrous CH_2Cl_2 (60 mL) was added dropwise. The reaction mixture was heated to reflux overnight. The mixture was cooled to 5 °C, and ice (30 g) and saturated NH_4Cl solution (100 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with water (2 × 100 mL), dried over $MgSO_4$, and concentrated in vacuo to yield the crude product (6.7 g). Purification by column chromatography (silica gel, EtOAc/heptane =1/10 to 1/2) furnished **4d** (4.5 g, 74% yield) as a colorless viscous oil. ^{1}H NMR ($CDCl_3$): δ 7.35−7.18 (m, 10H), 3.68 (d, $J = 11.1$, 2H), 3.52 (d, $J = 11.1$, 2H), 2.37 (t, $J = 7.5$, 4H), 1.82−1.69 (m, 2H), 1.55−1.42 (m, 8H), 1.33−1.22 (m, 2H), 1.33 (s, 6H), 1.07−1.03 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 144.7, 128.5, 126.7, 126.2, 72.4, 43.5, 38.2, 32.1, 30.5, 23.4, 21.8. HRMS: calcd for $C_{26}H_{39}O_2S$ (MH^+) 415.2671; found 415.2670. Anal. ($C_{26}H_{38}O_2S$): C, H, S.

5-(4-Ethoxycarbonyl-4-methylpentylsufanyl)-2,2-dimethylpentanoic Acid Ethyl Ester (5a). A solution of **1a** (9.10 g, 38.4 mmol), KOH (85%, 2.75 g, 41.7 mmol), and thiourea (3.65 g, 48.0 mmol) in EtOH (100 mL) was stirred at room temperature for 20 min and then heated to 40−45 °C for 1 h. The solution was cooled, and ice (100 g), concd aqueous HCl (100 mL), and water (100 mL) were added. The solution was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic phases were washed with 5% $NaHCO_3$ solution (2 × 200 mL) and saturated NH_4Cl solution (300 mL), then dried over Na_2SO_4 and concentrated in vacuo to **5a** (6.52 g, 89%) as a clear, pale-yellow oil. ^{1}H NMR ($CDCl_3$): δ 4.11 (q, 4H, $J = 7.1$), 2.47 (t, 4H, $J = 6.9$), 1.70−1.46 (m, 8H), 1.25 (t, 6H, $J = 7.1$), 1.17 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 177.5, 60.1, 41.9, 39.7, 32.3, 25.00, 24.95, 14.1. HRMS: calcd for $C_{18}H_{35}O_4S$ (MH^+) 347.2256; found 347.2261.

5-(4-Ethoxycarbonyl-4-phenylpentylsulfanyl)-2-methyl-2-phenylpentanoic Acid Ethyl Ester (5b). A solution of Na_2S nonahydrate (2.65 g, 11 mmol) and **1b** (6.49 g, 22 mol) in water (50 mL) and EtOH (5 mL) was stirred at 40 °C for 20 h and heated to reflux for 1 h. The solution was concentrated in vacuo and 5% aqueous NaOH solution (50 mL) was added. The mixture was extracted with CH_2Cl_2 (3 × 50 mL); the combined organic layers were washed with water (2 × 100 mL) and dried over anhydrous $MgSO_4$. The solution was filtered through basic alumina (100 g), eluting with CH_2Cl_2 (250 mL). The filtrate was concentrated and dried (100 °C, 1 mmHg) to produce **5b** (4.18 g, 85%) as a colorless, viscous oil, which was used without further purification in the next step. ^{1}H NMR ($CDCl_3$): δ 7.32−7.19 (m, 10H), 4.11 (q, 4H, $J = 6.9$), 2.46−2.40 (m, 4H), 2.09−1.98 (m, 4H), 1.54 (s, 6H), 1.48 (m, 4H), 1.17 (t, 6H, $J = 6.9$). ^{13}C NMR ($CDCl_3$): δ 176.08, 143.78, 128.47, 126.79, 126.07, 60.92, 50.14, 38.64, 32.54, 24.95, 22.89, 14.25.

6-(5-Ethoxycarbonyl-5-phenylhexylsulfanyl)-2-methyl-2-phenylhexanoic acid ethyl ester (5d). According to the procedure given for **5a**, **1d** (18.5 g, 59.1 mmol) was reacted with thiourea (7.0 g, 92.0 mmol) and KOH (85%, 6.1 g, 92.4 mmol) in EtOH (200 mL). After workup, the residue was purified by column chromatography (silica gel, hexanes/EtOAc = 10/1) to give **5d** (12.5 g, 85%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.42−7.15 (m, 10H), 4.20−4.05 (q, 4H, $J = 7.1$), 2.44 (t, 4H, $J = 7.6$), 2.15−1.95 (m, 2H), 1.95−1.75 (m, 2H), 1.62−1.40 (m, 10H), 1.35−1.10 (m, 4H), 1.18 (t, 6H, $J = 7.1$). ^{13}C NMR ($CDCl_3$): δ 176.06, 143.89, 128.25,

126.52, 125.84, 60.65, 50.04, 38.77, 31.80, 30.11, 24.06, 22.61, 14.01. HRMS: calcd for $C_{30}H_{43}O_4S_1$ (MH^+) 499.2882; found 499.2868.

5-(5-Hydroxy-4,4-dimethylpentyl-1-sulfinyl)-2,2-dimethylpentan-1-ol (6a). To a solution of **4a** (10.0 g, 38.0 mmol) in glacial acetic acid (50 mL) was added H_2O_2 (50 wt. % in water, 2.64 g, 38.0 mmol) under cooling in an ice−water bath. The reaction mixture was stirred for 4 h and stored in a refrigerator overnight. The reaction mixture was diluted with water (500 mL) and extracted with $CHCl_3$ (2 × 250 mL). The combined organic phases were washed with saturated $NaHCO_3$ solution (2 × 300 mL) and saturated NaCl solution (200 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue (12.8 g) was dissolved in hot EtOAc (ca. 20 mL) and filtered. Hexanes was slowly added to this solution until a slight turbidity was produced, which was cleared by addition of a small portion of EtOAc. The solution was allowed to cool to room temperature and stored at −5 °C overnight to give **6a** (7.1 g, 67%) as a white, crystalline solid. Mp 53−54 °C. ^{1}H NMR ($CDCl_3$): δ 3.31 (m, 4H), 2.78−2.50 (m, 4H), 2.45 (br, 2H), 1.67−1.80 (m, 4H), 1.50−1.25 (m, 4H), 0.87 (s, 6H), 0.86 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 70.82, 52.82, 37.35, 35.16, 24.16, 23.86, 17.43. HRMS: calcd for $C_{14}H_{31}O_3S_1$ (MH^+) 279.1994; found 279.2015. HPLC: 96.7% pure. Anal. ($C_{14}H_{30}O_3S$) C, H, S.

5-(5-Hydroxy-4-methyl-4-phenylpentylsulfinyl)-2-methyl-2-phenylpentan-1-ol (6b). To a solution of **4b** (4.3 g, 11.14 mmol) in glacial acetic acid (120 mL) was added H_2O_2 (50 wt % in water, 0.79 mL, 11.14 mmol). The reaction mixture was stirred at room temperature for 4 h, then diluted with water (500 mL) and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic phases were washed with saturated $NaHCO_3$ solution (3 × 150 mL) and brine (150 mL), dried over $MgSO_4$, and concentrated in vacuo to yield the crude product (4.2 g). Purification by column chromatography (silica gel, EtOAc/CH_2Cl_2 = 1/1) furnished **6b** (3.1 g, 69%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.33−7.21 (m, 10H), 3.70−3.50 (m, 4H), 2.60−2.32 (m, 4H), 2.00−1.80 (m, 4H), 1.80−1.52 (m, 4H), 1.52−1.31 (m, 2H), 1.35 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 144.3, 128.7, 126.6, 126.5, 72.0, 71.7, 53.0, 52.9, 43.5, 37.7, 37.5, 22.2, 21.8, 18.0, 17.8. HRMS: calcd for $C_{24}H_{35}O_3S_1$ (MH^+) 403.2307; found 403.2295. HPLC: 99.4% pure.

6-(5,5-Dimethyl-6-hydroxyhexane-1-sulfinyl)-2,2-dimethylhexan-1-ol (6c). To a solution of **4c** (12.0 g, 41.3 mmol) in glacial acetic acid (100 mL) was added H_2O_2 (50 wt % in water, 2.7 g, 39.7 mmol) under cooling with an ice bath. The reaction mixture was kept in the refrigerator overnight, then diluted with water (400 mL) and extracted with $CHCl_3$ (2 × 150 mL). The combined organic phases were subsequently washed with saturated $NaHCO_3$ solution (3 × 200 mL) and saturated NaCl solution (200 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue (12.0 g) was dissolved in warm Et_2O (ca. 20 mL), and some pentane was added slowly until the solution became turbid. The turbidity was cleared by addition of a small portion of Et_2O, and the solution was cooled to room temperature, then to −5 °C to give a cream-white, crystalline solid. Additional crystallization from warm EtOAc (ca. 10 mL) and some pentane gave **6c** (8.0 g, 63%) as a white, crystalline solid. Mp 43−44 °C. ^{1}H NMR ($CDCl_3$): δ 3.29 (d, 2H, J = 12.2), 3.27 (d, 2H, J = 12.2), 2.70 (m, 4H), 2.48 (s br, 2H), 1.76 (m, 4H), 1.44 (m, 4H), 1.30 (4H), 0.87 (s, 6H), 0.86 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 71.39, 52.20, 38.08, 35.15, 24.21, 24.03, 23.66, 23.26. HRMS: calcd for $C_{16}H_{35}SO_3$ (MH^+) 307.2307; found 307.2309. HPLC: 98.9% pure.

6-(6-Hydroxy-5-methyl-5-phenylhexylsulfinyl)-2-methyl-2-phenylhexan-1-ol (6d). To a solution of **4d** (3.8 g, 9.2 mmol) in glacial acetic acid (100 mL) was added hydrogen peroxide (50 wt % in water, 0.64 mL, 9.2 mmol). The reaction mixture was stirred at room temperature for 4 h, then diluted with water (500 mL) and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic phases were washed with saturated $NaHCO_3$ solution (3 × 150 mL) and brine (150 mL), dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (silica gel, EtOAc/CH_2Cl_2 = 1/1) furnished **6d** (2.9 g, 73%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.31−7.21 (m, 10 H), 3.70 (d, J = 10.5 Hz, 2H), 3.57 (m, 2H), 2.52 (m, 4H), 1.80−1.53 (m, 10 H), 1.33 (s, 6H), 1.33−1.23 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 144.60, 128.61, 126.69, 126.34, 72.47, 72.26, 52.33, 43.55, 43.50, 38.15, 23.51, 22.06, 21.79. HRMS: calcd for $C_{26}H_{39}O_3S_1$ (MH^+) 431.2620; found 431.2611. HPLC: 93.0% pure. Anal. ($C_{26}H_{38}O_3S_1$) C, H, S.

6-(5,5-Dimethyl-6-hydroxyhexane-1-sulfonyl)-2,2-dimethylhexan-1-ol (7c). To a solution of **4c** (7.6 g, 26.2 mmol) in $CHCl_3$ (300 mL) was added a solution of MCPBA (17.6 g, 78.6 mmol) in $CHCl_3$ (150 mL) over 1 h at room temperature. The reaction mixture was stirred for 17 h at room temperature and then washed with saturated $NaHCO_3$ solution (3 × 200 mL) and saturated NaCl solution (200 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (silica; $CHCl_3$/MeOH = 95:5) to yield **7c** (4.3 g, 51%) as an oil, which solidified upon standing. ^{1}H NMR ($CDCl_3$): δ 3.30 (s, 4H), 2.97 (m, 4H), 2.32 (s br, 2H), 1.82 (m, 4H), 1.40 (m, 4H), 1.26 (m, 4H), 0.86 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 71.49, 52.66, 37.95, 35.16, 24.11, 23.03, 22.87. HRMS: calcd for $C_{16}H_{35}SO_4$ (MH^+) 323.2256; found 323.2261. HPLC: 97.8% pure.

6-(5-Carboxy-5-phenyl-hexylsulfanyl)-2-methyl-2-phenylhexanoic Acid (8d). A solution of **5d** (6.0 g, 12.0 mmol) and NaOH (3.1 g, 74.0 mmol) in EtOH (40 mL) and deionized water (4 mL) was heated to reflux for 2 h. The EtOH was evaporated in vacuo, and the residue was dissolved in water (20 mL) and extracted with Et_2O (20 mL). The aqueous layer was acidified with 2 N aqueous HCl (15 mL) to pH 2−3 and extracted with Et_2O (4 × 50 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue (4.5 g) was purified by column chromatography (silica gel, hexanes/EtOAc = 10/1) to give **8d** (3.6 g, 68%) as a yellowish oil. ^{1}H NMR ($CDCl_3$): δ: 7.42−7.20 (m, 10H), 2.45 (t, 4H, J = 7.3), 2.15−1.80 (m, 4H), 1.65−1.45 (m, 4H), 1.56 (s, 6H), 1.40−1.15 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 182.43, 142.89, 128.41, 126.91, 126.05, 50.00, 38.53, 31.80, 30.00, 24.03, 22.40. HRMS: calcd for $C_{26}H_{35}O_4S_1$ (MH^+) 443.2256; found 443.2231. HPLC: 91.9% pure.

2,2,12,12-Tetramethyltridecane-1,7,13-triol (10). Under Ar atmosphere, methyl *tert*-butyl ether (MTBE, 50 mL) was added to $LiAlH_4$ (2.56 g, 67.5 mmol), and the gray suspension was stirred under cooling with an ice bath. A solution of **9** (87% pure by GC, 10.0 g, 27.0 mmol) in MTBE (50 mL) was added dropwise, followed by additional MTBE (100 mL). After 2 h at 0 °C, the reaction mixture was quenched by addition of EtOAc (30 mL) and allowed to warm to room temperature overnight. The mixture was cooled with an ice-bath and carefully hydrolyzed by addition of crushed ice (47 g) and water (50 mL). The pH was adjusted to 1 by addition of 2 N H_2SO_4 (140 mL), and the solution was stirred at room temperature for 15 min. The layers were separated, and the aqueous layer was extracted with MTBE (100 mL). The combined organic layers were washed with deionized water (100 mL), saturated $NaHCO_3$ solution (100 mL), and saturated NaCl solution (100 mL), dried over $MgSO_4$, concentrated in vacuo, and dried in high vacuum. The residue (6.6 g) was crystallized from hot CH_2Cl_2 (65 mL). The crystals were filtered, washed with ice-cold CH_2Cl_2 (3 × 10 mL), and dried in high vacuum to give **10** (3.57 g, 46%) as an off-white solid. Mp 70 °C. ^{1}H NMR (CD_3OD): δ 3.50 (m, 1H), 3.22 (s, 4H), 1.55−1.15 (m, 16H), 0.84 (s, 12H). ^{13}C NMR (CD_3OD): δ 72.38, 72.01, 39.97, 38.47, 35.98, 27.85, 25.09, 24.64, 24.60. HRMS: calcd for $C_{17}H_{37}O_3$ (MH^+) 289.2743; found 289.2756. HPLC: 95.0% pure. Anal. ($C_{17}H_{36}O_3$) C, H.

7-Hydroxy-2,2,12,12-tetramethyltridecanedioic acid diethyl ester (11). To a solution of **10** (9.2 g, 24.8 mmol) in MeOH (200 mL) was added $NaBH_4$ (0.95 g, 25.1 mmol) under cooling with an ice−water bath. After 2 h, another portion of $NaBH_4$ (0.95 g, 25.1 mmol) was added and stirring was continued for 2 h. The reaction mixture was hydrolyzed with water (200 mL) and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to give **11** (8.5 g, 92%) as an oil. ^{1}H NMR ($CDCl_3$): δ 4.11 (q, 4H, J = 7.0), 3.60−3.50 (m, 1H), 1.66−1.32 (m, 11H), 1.24 (t, 12H, J = 7.0), 1.15 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 178.0, 71.7, 60.1, 42.1, 40.6, 37.3, 26.0,

25.1, 24.9, 14.2. HRMS: calcd for $C_{21}H_{41}O_5$ (MH^+) 373.2954; found 373.2936.

7-Hydroxy-2,2,12,12-tetramethyltridecanedioic Acid (12). A mixture of **11** (91.6 g, 0.246 mol) and NaOH (24.6 g, 0.62 mol) in EtOH (60 mL) and water (19 mL) was refluxed for 2.5 h. The mixture was diluted with water (150 mL) and extracted with MTBE (2 × 100 mL). The aqueous solution was acidified with 6 M H_2SO_4 (ca. 100 mL) to pH 2 and extracted with MTBE (2 × 200 mL). The combined organic layers were washed with water (200 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue (77.0 g) was purified by column chromatography (silica gel, heptane/EtOAc = 9:1, 4:1, 2:1, 3:2) to yield two fractions of **12** (10.0 g, 96.3% pure by HPLC; 51.8 g, 97.9% pure by HPLC. Combined yield 80%), both as oils. ^{1}H NMR ($CDCl_3$): δ 8.10 (br, 3H), 3.58 (br, 1H), 1.62−1.22 (m, 16H), 1.18 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 184.3, 71.8, 42.1, 40.5, 36.9, 25.9, 25.0, 24.9. HRMS: calcd for $C_{17}H_{33}O_5$ (MH^+) 317.2328; found 317.2330. Anal. ($C_{17}H_{32}O_5$) C, H.

7-Hydroxymethyl-2,2,12,12-tetramethyltridecane-1,13-diol (17). Under N_2 atmosphere, to a solution of $LiAlH_4$ (1.09 g, 28.8 mmol) in anhydrous THF (100 mL) was added dropwise a solution of **16** (3.64 g, 11.5 mmol) in THF (40 mL) at room temperature. The reaction mixture was heated to reflux for 5 h and kept at room temperature overnight. Water (100 mL) was added carefully to the reaction mixture under cooling with a water bath. The pH of the mixture was adjusted to 1 with 2 M aqueous HCl. The product was extracted with Et_2O (3 × 60 mL). The combined organic layers were washed with water (3 × 50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue (3.2 g) was purified by chromatography (silica, EtOH) to give **17** (3.10 g, 89%) as a yellow oil. ^{1}H NMR (CD_3OD): δ 4.88 (s, 3H), 3.44 (d, 2H, J = 4.8), 1.50−1.10 (m, 17H), 0.85 (s, 12H). ^{13}C NMR (CD_3OD): δ 72.0, 65.7, 41.7, 40.0, 36.0, 32.2, 29.0, 25.4, 24.7, 24.6. HRMS: calcd for $C_{18}H_{39}O_3$ (MH^+) 303.2899; found 303.2901. HPLC: 94.6% pure. Anal. ($C_{18}H_{38}O_3$): C, H.

9-Hydroxy-3-(6-hydroxy-5,5-dimethylhexyl)-8,8-dimethylnonan-2-one (18) and 7-(1-Hydroxy-1-methylethyl)-2,2,12,12-tetramethyltridecan-1,13-diol (19). A solution of **16** (1.0 g, 3.16 mmol) in THF (40 mL) was cooled in an ice−water bath and methyllithium (1.4 M in Et_2O, 27 mL, 37.8 mmol) was added. The mixture was kept at 0 °C for 2 h, and then was poured into diluted HCl (5 mL concd HCl/60 mL water). The organic layer was separated, and the aqueous layer was extracted with Et_2O (2 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The residue (1.0 g) was purified by column chromatography (hexanes/EtOAc = 80/20, then 50/50) to give **18** (0.41 g, 41%) as an oil and **19** (0.40 g, 38%) as a white solid. Compound **18.** ^{1}H NMR ($CDCl_3$): δ 3.26 (s, 4H), 2.45−2.30 (m, 1H), 2.08 (s, 3H), 1.86 (br, 2H), 1.62−1.30 (m, 4H), 1.30−1.05 (m, 12H), 0.82 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 213.4, 71.7, 53.2, 38.3, 34.9, 31.6, 28.7, 28.3, 23.8. HRMS: calcd for $C_{19}H_{39}O_3$ (MH^+) 315.2899; found 315.2866. HPLC: 90.5% pure. Anal. ($C_{19}H_{38}O_3$) C, H. Compound **19.** Mp 72−74 °C. ^{1}H NMR ($CDCl_3$): δ 3.24 (s, 4H), 2.59 (br, 3H), 1.55−0.95 (m, 17H), 1.11 (s, 6H), 0.81 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 74.0, 71.5, 49.6, 38.4, 34.9, 31.2, 30.3, 27.1, 24.3, 23.9, 23.8. HRMS: calcd for $C_{20}H_{43}O_3$ (MH^+) 331.3212; found 331.3205. HPLC: 96.4% pure.

6-(6-Hydroxy-5,5-dimethylhexylamino)-2,2-dimethylhexan-1-ol Hydrochloride (20). A mixture of **2c** (15.2 g, 51.8 mmol), *p*-toluenesulfonamide (4.43 g, 25.9 mmol), NaOH (2.60 g, 64.75 mmol), TBAI (480 mg, 1.30 mmol), benzene (175 mL), and water (50 mL) was stirred vigorously and heated to 70 °C under N_2 atmosphere. Additional TBAI (400 mg, 1.08 mol) was added after 20 h and stirring was continued at 80 °C. After 24 h, the mixture was cooled to room temperature, the layers were separated, and the organic layer was washed with water (100 mL). The organic layer was dried over $MgSO_4$, concentrated, and dried in vacuo to furnish *N*,*N*-bis-(5,5-dimethyl-6-tetrahydropyranyloxyhexyl)-*p*-toluenesulfonamide (14.5 g) as a colorless, viscous oil. Under N_2 atmosphere, anhydrous DME (75 mL) was added to a mixture of Na (2.15 g, 93.6 mmol) and naphthalene (14.8 g, 115.5 mmol). The reaction mixture was stirred for 2 h at room temperature to give a dark-green solution of sodium naphthalenide. A portion of this solution (ca. 40 mL) was added dropwise to a solution of *N*,*N*-bis-(5,5-dimethyl-6-tetrahydropyranyloxyhexyl)-*p*-toluenesulfonamide (7.0 g, 11.7 mmol) in anhydrous DME (200 mL) at −78 °C until the greenish color persisted. After 15 min, the reaction mixture was hydrolyzed with saturated $NaHCO_3$ solution (20 mL) and warmed to room temperature. K_2CO_3 (100 g) was added, and the reaction mixture was stirred for 1.5 h. The solids were removed by filtration and washed with Et_2O (2 × 200 mL). The filtrate was dried over Na_2SO_4 and concentrated to give bis-(5,5-dimethyl-6-tetrahydropyranyloxyhexyl)-amine (13.5 g) as an oil. This oil (13.5 g) was dissolved in MeOH (100 mL), concd HCl (10 mL) was added, and the reaction mixture was heated to reflux under N_2 atmosphere for 2 h. After the mixture cooled to room temperature, water (200 mL) was added, and the non-salts were removed by extraction with CH_2Cl_2 (3 × 100 mL). The pH of the aqueous layer was adjusted to 11 with solid Na_2CO_3. The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to give **21** as a red oil. This oil was dissolved in EtOH (20 mL) and acidified with concd HCl (2 mL) to pH 1. The solvents were removed in high vacuum, affording **20** (1.45 g, 37% over three steps) as a reddish glass. ^{1}H NMR (CD_3OD): δ 3.24 (s, 4H), 3.00 (m, 4H), 1.70 (m, 4H), 1.48−1.22 (m, 8H), 0.88 (s, 12H). ^{13}C NMR (CD_3OD): δ 71.66, 49.06, 39.34, 36.02, 28.25, 24.65, 22.25. HRMS: calcd for $C_{16}H_{36}NO_2$ (MH^+) 274.2746; found 274.2746. GC: 95.5% pure.

6-(6-Hydroxy-5,5-dimethylhexylamino)-2,2-dimethylhexan-1-ol (21). Compound **20** (7.68 g, 24.78 mmol) was extracted with 10% aqueous NaOH solution (100 mL) and CH_2Cl_2 (80 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 80 mL). The combined organic layers were washed with saturated NaCl solution (50 mL), dried over Na_2SO_4, concentrated in vacuo, and dried in high vacuum to afford **21** (5.55 g, 82%) as an orange, viscous oil. ^{1}H NMR ($CDCl_3$): δ 3.27 (s, 4H), 3.1−2.4 (br, OH, NH), 2.60 (t, 4H, J = 7.1), 1.48 (m, 4H), 1.25 (m, 8H), 0.85 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 71.15, 49.62, 38.05, 35.13, 30.35, 24.29, 21.36. HRMS: calcd for $C_{16}H_{36}NO_2$ (MH^+) 274.2746; found 274.2746. Anal. ($C_{16}H_{35}NO_2$) C, H, N.

6-[Benzoyloxy-(6-hydroxy-5,5-dimethylhexyl)-amino]-2,2-dimethylhexan-1-ol (22). Under Ar atmosphere, to a stirred suspension of **21** (2.62 g, 9.58 mmol) and Na_2HPO_4 (6.95 g, 48.93 mmol) in MTBE (50 mL) was added dropwise over 45 min a solution of benzoyl peroxide (2.55 g, 10.54 mmol) in MTBE (90 mL) at room temperature. The mixture was heated to 45 °C for 17 h, cooled to room temperature, diluted with MTBE (100 mL), and extracted with 10% Na_2CO_3 solution (2 × 100 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (silica, hexanes/EtOAc = 50/50) to afford **22** (2.24 g, 59%) as a viscous, slightly yellowish oil. ^{1}H NMR ($CDCl_3$): δ 8.01 (m, 2H), 7.97 (m, 1H), 7.44 (m, 2H), 3.29 (s, 4H), 2.98 (t, 4H, J = 7.3), 2.62 (s, 2H), 1.56 (m, 4H), 1.42−1.16 (m, 8H), 0.83 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 165.92, 133.17, 129.56, 129.17, 128.51, 71.20, 59.33, 37.97, 35.04, 27.46, 24.20, 21.28. HRMS: calcd for $C_{23}H_{40}NO_4$ (MH^+) 394.2957; found 394.2954.

6-[Hydroxy-(6-hydroxy-5,5-dimethylhexyl)-amino]-2,2-dimethylhexan-1-ol (23). Under Ar atmosphere, to a solution of **22** (2.0 g, 5.08 mmol) in anhydrous MeOH (20 mL) was added a solution of NaOMe in anhydrous MeOH (0.5 M, 20.4 mL, 10.16 mmol) at room temperature. The mixture was stirred for 4 h, diluted with saturated NH_4Cl solution (200 mL), and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with saturated NaCl solution (100 mL), dried over $MgSO_4$, concentrated in vacuo, and dried in high vacuum. The residue (1.6 g) was purified by flash chromatography (silica, hexanes/EtOAc = 25/75) to afford **23** (710 mg, 48%) as a white solid. Crystallization from MTBE/hexanes (10 mL, 50/50) at − 5 °C furnished the product (620 mg, 42%) in the form of white crystals. Mp 73 °C. ^{1}H NMR ($CDCl_3$): δ 3.30 (s, 4H), 2.67 (t, 4H, J = 7.1), 1.58 (m, 4H), 1.27 (m, 4H), 0.85 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 71.59, 60.57, 38.31, 35.21,

27.85, 24.29, 21.68. HRMS: calcd for $C_{16}H_{36}NO_3$ (MH^+) 290.2695; found 290.2676. Anal. ($C_{16}H_{35}NO_3$) C, H, N.

6,6-Dimethyl-7-(tetrahydropyran-2-yloxy)-heptanoic Acid [5,5-Dimethyl-6-(tetrahydropyran-2-yloxy)-hexyl]-amide (29). To a solution of **25** (1.0 g, 4.36 mmol) in CH_2Cl_2 (600 mL) was added dropwise a solution of **28** (1.55 g, 4.36 mmol) in CH_2Cl_2 (200 mL). The mixture was stirred for 48 h at room temperature. The solvent was removed in vacuo. The residue was dissolved in Et_2O, and the solution was filtered to remove leftover DCU from the previous step. The filtrate was concentrated in vacuo and purified by flash chromatography (silica gel, hexanes/EtOAc = 2/1) to furnish **29** (1.56 g, 76%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 5.50 (br, 1H), 4.50 (m, 2H), 3.75 (m, 2H), 3.40 (m, 4H), 3.15 (m, 2H), 2.95 (d, 2H, J = 9.2), 2.12 (t, 2H, J = 7.5), 1.90−1.10 (m, 24H), 0.85 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 173.17, 99.27, 99.20, 76.50, 76.43, 62.12, 62.01, 39.44, 38.97, 38.85, 36.85, 34.20, 30.72, 30.52, 26.83, 25.60, 24.54, 23.70, 21.29, 19.60, 19.53. HRMS: calcd for $C_{27}H_{52}O_5N$ (MH^+) 470.3845; found 470.3839.

7-Hydroxy-6,6-dimethylheptanoic Acid (6-Hydroxy-5,5-dimethylhexyl)-amide (30). To a solution of **29** (1.41 g, 3.0 mmol) in MeOH (50 mL) was added concd aqueous HCl (9 mL). The mixture was refluxed for 2 h, cooled to room temperature, diluted with water (50 mL), and concentrated in vacuo to a volume of ca. 60 mL. The solution was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (3 × 100 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residual oil was purified by flash chromatography (silica, EtOAc/hexanes = 1/2, then CH_2Cl_2/MeOH = 10/1) to furnish **30** (0.77 g, 85%) as a yellowish oil. ^{1}H NMR ($CDCl_3$): δ 6.05 (m, 1 H), 3.28 (s, 4 H), 3.25 (m, 2 H), 2.58 (br, 2 H), 2.19 (t, 2 H, J = 7.0), 1.61 (m, 2 H), 1.48 (m, 2 H), 1.25 (m, 8 H), 0.85 (s, 12 H). ^{13}C NMR ($CDCl_3$): δ 173.80, 71.35, 71.15, 39.10, 37.99, 37.82, 36.60, 35.17, 30.55, 26.69, 24.40, 24.30, 23.41, 20.80. HRMS: calcd for $C_{17}H_{36}NO_3$ (MH^+) 302.2695; found 302.2723. HPLC: 97.0% pure.

6,6-Dimethyl-7-(tetrahydropyran-2-yloxy)-heptanoic Acid 5,5-Dimethyl-6-(tetrahydropyran-2-yloxy)-hexyl Ester (32). A mixture of **31** (8.1 g, 35.2 mmol), **27** (10.0 g, 38.7 mmol), DCC (8.8 g, 0.7 mmol), and DMAP (1.1 g, 9.0 mmol) in CH_2Cl_2 (600 mL) under N_2 atmosphere was stirred at room temperature for 18 h. The precipitated DCU was removed by filtration. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (silica, hexanes/EtOAc = 5/1), affording **32** (11.0 g, 66%) as an oil, which was used without further purification for the next step. ^{1}H NMR ($CDCl_3$): δ 4.50 (m, 2H), 4.08 (t, 2H, J = 7.5), 3.75 (m, 2H), 3.40 (m, 4H), 2.95 (m, 2H), 2.25 (t, 2H, J = 7.5), 1.90−1.10 (m, 24H), 0.85 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 174.46, 99.64, 77.00, 64.90, 62.43, 39.57, 35.46, 34.96, 31.21, 30.10, 26.93, 25.99, 24.26, 24.12, 20.94, 19.99. HPLC: 56.7% pure.

7-Hydroxy-6,6-dimethylheptanoic Acid 6-Hydroxy-5,5-dimethylhexyl Ester (33). A solution of **32** (10.0 g, 21.2 mmol) in a solvent mixture of acetic acid, THF, and water (4/2/1, 440 mL) was heated to 45 °C for 4 h. The solution was concentrated in vacuo. Et_2O (300 mL) was added, and the solid (DCU) was removed by filtration. The filtrate was concentrated in vacuo and purified by flash chromatography (silica, EtOAc/hexanes = 1/2) to furnish **33** (2.5 g, 39%) as an oil. ^{1}H NMR ($CDCl_3$): δ 4.08 (t, 2H, J = 6.5), 3.29 (s, 4H), 2.29 (m, 2H), 1.61 (m, 4H), 1.26 (m, 8H), 0.87 (s, 6H), 0.86 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 174.22, 71.71, 64.39, 38.26, 35.10, 34.40, 29.62, 25.92, 23.98, 23.50, 20.38. HRMS (LSIMS, gly): calcd for $C_{17}H_{35}O_4$ (MH^+) 303.2535; found 303.2528. HPLC: 97.6% pure. Anal. ($C_{17}H_{34}O$) C, H.

Phosphoric Acid Bis-[5,5-dimethyl-6-(tetrahydropyran-2-yloxy)-hexyl]-ester (34). $POCl_3$ (4.06 g, 2.5 mL, 26.48 mmol) was added dropwise to a solution of **31** (12.2 g, 52.97 mmol) and NEt_3 (5.36 g, 7.4 mL, 52.97 mmol) in anhydrous Et_2O (200 mL) at room temperature under N_2 atmosphere. The reaction mixture was stirred for 17 h. The ammonium salts were removed by filtration and washed with Et_2O (100 mL). The filtrate was concentrated in vacuo. To a solution of the residue (15.0 g) in water (100 mL) and acetonitrile (100 mL) was added $KHCO_3$ (13.3 g, 133 mmol), and the reaction mixture was stirred at room temperature for 3.5 h. The reaction mixture was diluted with water (250 mL) and extracted with Et_2O (250 mL). The aqueous layer was acidified with concd HCl (7 mL) to pH 1 and then extracted with Et_2O (2 × 250 mL). The combined organic phases were washed with saturated NaCl solution (100 mL), dried over $MgSO_4$, concentrated in vacuo, and dried in high vacuum to give **34** (4.1 g, 30%) as a viscous oil, which was used for the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 4.54 (t, 2H, J = 3.3), 4.02 (m, 4H), 3.82 (m, 2H), 3.50 (m, 2H), 3.45 (d, 2H, J = 9.2), 2.98 (d, 2H, J = 9.2), 1.92−1.20 (m, 24H), 0.89 (s, 6H), 0.88 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 99.33, 76.64, 67.76 (J = 6), 62.14, 39.00, 34.40, 31.28 (J = 7), 30.82, 25.72, 24.67, 20.10, 19.61.

Phosphoric Acid Bis-(5,5-dimethyl-6-hydroxyhexyl)-ester (35). A solution of **34** (4.0 g, 7.65 mmol) in MeOH (100 mL) and concd HCl (10 mL) was refluxed for 2 h. The solution was diluted with water (200 mL) and concentrated under reduced pressure to a volume of ca. 100 mL. The solution was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were extracted with saturated $NaHCO_3$ solution (2 × 50 mL), then acidified with concd HCl (10 mL) to pH 1 and extracted with CH_2Cl_2 (3 × 75 mL). The combined organic layers were dried over $MgSO_4$, concentrated in vacuo, and dried in high vacuum to give **35** (1.73 g, 64%) as a viscous oil. ^{1}H NMR ($CDCl_3$): δ 6.18 (br, 3H), 4.05 (m, 4H), 3.33 (s, 4H), 1.67 (m, 4H), 1.48−1.22 (m, 8H), 0.87 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 71.15, 67.29 (J = 6), 37.70, 35.14, 30.94 (J = 7), 24.38, 19.76. HRMS: calcd for $C_{16}H_{36}PO_6$ (MH^+) 355.2250; found 355.2245.

[5,5-Dimethyl-6-(tetrahydropyran-2-yloxy)-hexyl]-carbamic Acid 5,5-Dimethyl-6-(tetrahydropyran-2-yloxy)-hexyl Ester (36). To a solution of **31** (4.00 g, 17.4 mmol) and CDI (3.52 g, 21.7 mmol) in anhydrous CH_2Cl_2 (200 mL) was added DMAP (0. g, 3.4 mmol) at room temperature under N_2 atmosphere. The reaction mixture was stirred for 2 h and concentrated in vacuo. A portion of the residue (2.7 g of 5.0 g) was dissolved in anhydrous CH_3CN (160 mL), and **25** (8.25 g, 36.00 mmol) in anhydrous CH_3CN (20 mL) was added dropwise. The reaction mixture was stirred for 24 h at room temperature, then washed with 15% aqueous citric acid solution (2 × 75 mL) and 1% aqueous HCl (100 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to afford **36** (3.05 g, 67%) as an oil, which was used without further purification for the next step. ^{1}H NMR ($CDCl_3$): δ 4.75 (br, 1H), 4.48 (m, 2H), 3.99 (t, 2H, J = 6.5,), 3.77 (m, 2H), 3. (m, 4H), 3.11 (m, 2H), 2.94 (d, 2H, J = 9.0), 1.78−1.46 (m, 16H), 1.22 (m, 8H), 0.83 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 156.94, 99.38, 99.16, 76.63, 76.45, 64.98, 62.28, 62.05, 40.98, 39.17, 38.90, 34.39, 30.85, 30.14, 25.72, 24.79, 24.68, 21.18, 20.51, 19.71, 19.60. HRMS: calcd for $C_{27}H_{52}NO_6$ (MH^+) 486.3795; found 486.3775.

(6-Hydroxy-5,5-dimethylhexyl)-carbamic Acid 6-Hydroxy-5,5-dimethylhexyl Ester (37). A solution of **36** (7.35 g, 15.13 mmol) in acetic acid/THF/water (236 mL/118 mL/59 mL) was heated to 45 °C for 24 h. The reaction mixture was poured into ice−water (200 g) and extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were washed with saturated $NaHCO_3$ solution (2 × 100 mL) and brine (150 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, hexanes, then EtOAc/hexanes = 1/10, 1/2, 1/1), affording **37** (3.65 g, 76%) as an oil. ^{1}H NMR ($CDCl_3$): δ 4.95 (br, 1H), 4.08 (t, 2H, J = 6.5), 3.25 (s, 4H), 3.15 (m, 2H), 2.17 (br, 2H), 1.53 (m, 2H), 1.41 (m, 2H), 1.21 (m, 8H), 0.81 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 157.29, 71.61, 71.40, 64.83, 40.71, 38.22, 37.99, 35.19, 30.97, 30.07, 24.21, 24.12, 20.92, 20.31. HRMS: calcd for $C_{17}H_{36}NO_4$ (MH^+) 318.2644; found 318.2663. HPLC: 99.0% pure. Anal. ($C_{17}H_{35}NO_4$) C, H, N.

1,3-Bis(6-hydroxy-5,5-dimethylhexyl)urea (42). To a solution of **25** (14.9 g, 64.96 mmol) in pyridine (200 mL) was added triphenyl phosphite (10.1 g, 32.48 mmol). The solution was heated to 40−50 °C, and CO_2 gas was introduced for ca. 8 h through a gas immersion tube. The reaction mixture was cooled to room temperature after 18 h, and a sample was taken for monitoring of the reaction progress by NMR spectroscopy (ratio P/SM ≫ 1/2).

Additional triphenyl phosphite (10.2 g, 32.80 mmol) was added, CO_2 was introduced for ca. 18 h, and the reaction mixture was heated to 55 °C for 23 h. The solvent was removed in vacuo, and the reaction mixture concentrated in high vacuum to give crude 1,3-bis[5,5-dimethyl-6-(tetrahydropyran-2-yloxy)-hexyl]urea (53 g) as a yellow oil. This crude intermediate was dissolved in MeOH (200 mL) and concd. HCl (20 mL) and heated to reflux for 3 h. The solution was cooled to room temperature, diluted with water (200 mL), and concentrated in vacuo to a volume of ca. 300 mL. The solution was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with 10% NaOH solution (2 × 100 mL) and saturated NaCl solution (2 × 100 mL), dried over $MgSO_4$, concentrated in vacuo, and dried in high vacuum. The residual oil was purified by flash chromatography (silica; hexanes/EtOAc = 40/60, then 20/80, followed by $CHCl_3$/MeOH = 95/5) to furnish **42** (2.24 g, 22% over two steps) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 3.26 (s, 4H), 3.11 (t, 4H, J = 6.8), 1.51−1.38 (m, 4H), 1.36−1.18 (m, 8H), 0.85 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 161.50, 71.97, 41.08, 39.67, 36.07, 32.49, 24.59, 22.33. HRMS: calcd for $C_{17}H_{37}N_2O_3$ (MH^+) 317.2804, found 317.2793.

Biological Methods. In Vitro Measurement of Lipid Synthesis in Isolated Hepatocytes. Compounds were tested for inhibition of lipid synthesis in primary cultures of rat hepatocytes. Male Sprague−Dawley rats were anesthetized with intraperitoneal injection of sodium pentobarbital (80 mg/kg). Rat hepatocytes were isolated essentially as described by the method of Seglen,[34] which includes hepatic exposure to dexamethasone. Hepatocytes were suspended in Dulbecco's modified Eagle's medium containing 25 mM D-glucose, 14 mM HEPES, 5 mM l-glutamine, 5 mM leucine, 5 mM alanine, 10 mM lactate, 1 mM pyruvate, 0.2% bovine serum albumin, 17.4 mM nonessential amino acids, 20% fetal bovine serum, 100 nM insulin, 100 nM dexamethasone, and 20 μg/mL gentamycin and plated at a density of 1.5 × 10^5 cells/cm^2 on collagen-coated 96-well plates. Four hours after plating, media was replaced with the same media without serum. Cells were grown overnight to allow formation of monolayer cultures. Lipid synthesis incubation conditions were initially assessed to ensure the linearity of [1-^{14}C]-acetate incorporation into hepatocyte lipids for up to 4 h. Hepatocyte lipid synthesis inhibitory activity was assessed during incubations in the presence of 0.25 μCi of [1-^{14}C]-acetate/well (final radiospecific activity in assay is 1 Ci/mol) and 0, 1, 3, 10, 30, 100, or 300 μM compounds for 4 h. At the end of the 4-h incubation period, medium was discarded, and cells were washed twice with ice-cold phosphate-buffered saline and stored frozen prior to analysis. To determine total lipid synthesis, 170 μL of MicroScint-E and 50 μL of water was added to each well to extract and partition the lipid-soluble products to the upper organic phase containing the scintillant. Lipid radioactivity was assessed by scintillation spectroscopy in a Packard TopCount NXT. Lipid synthesis rates were used to determine the IC_{50}'s of the compounds.

In Vivo Effects on Lipid Variables in Female Obese Zucker Fatty Rats. Ten- to twelve-week-old (400−500 g) female Zucker fatty rats, Crl:(Zuc)-faBR, were obtained from Charles River Laboratories. Animals were acclimated to the laboratory environment for 7 days. During the acclimation and study period, animals were housed by group in shoebox polycarbonate cages on Cellu-Dri bedding. The temperature and humidity in the animals' quarters (68−78 °F; 30−75% RH) were monitored, and the airflow in the room was sufficient to provide several exchanges per hour with 100% fresh filtered air. An automatic timing device provided an alternating 12-hour cycle of light and dark. Rats received pelleted Purina Laboratory Rodent Chow (5001) prior to and during the drug intervention period except for a 6-hour phase prior to blood sampling. Fresh water was supplied ad libitum via an automatic watering system. Compounds were suspended by mixing in a dosing vehicle consisting of 20% ethanol and 80% poly(ethylene glycol)-200 [v/v]. Dose volume of vehicle or vehicle plus each compound was set at 0.25% of body weight to deliver the appropriate dose. Doses were administered daily by oral gavage, approximately between 8 and 10 AM. Regarding blood sampling, animals were fasted for 6 h prior to all blood collections. Prior to and after 7 days of dosing, a 1.0−2.0-mL sample of blood was collected by administering O_2/CO_2 anesthesia and bleeding from the orbital venous plexus. Following 14 days of dosing, blood was collected by cardiac puncture after euthanasia with CO_2. All blood samples were processed for separation of serum and stored at −80 °C until analysis. Commercially available kits were used to determine serum triglycerides (Roche Diagnostic Corporation, kit no. 148899, or Boehringer Mannheim, kit no. 1488872), total cholesterol (Roche Diagnostic Corporation, kit no. 450061), non-esterified fatty acids (Wako Chemicals, kit no. 994-75409), and β-hydroxybutyrate (Wako Chemicals, kit no. 417-73501, or Sigma, kit. no. 310-0) on a Hitachi 912 automatic analyzer (Roche Diagnostic Corporation). In some instances, an in-house cholesterol reagent was used to determine total serum cholesterol levels. Serum lipoprotein cholesterol levels were determined by lipoprotein profile analysis. Lipoprotein profiles were analyzed using gel-filtration chromatography on a Superose 6HR (1 × 30 cm^2) column equipped with on-line detection of total cholesterol as described by Kieft et al.[35] The total cholesterol content of each lipoprotein was calculated by multiplying the independent values determined for serum total cholesterol by the percent area of each lipoprotein in the profile.

Pharmacokinetics and Drug Metabolism of 1,13-Dihydroxy-2,2,12,12-tetramethyl-tridecan-7-one (39). Primary hepatocytes from male donors were isolated and suspended in cold sHMM medium (Williams' E media supplemented with 0.1 μM insulin, 0.1 μM dexamethasone, 50 μg/mL gentamycin, and 50 ng/mL amphotericin B) to a final concentration of ∼0.8 × 10^6 cells/mL. Cell viability was assessed by exclusion of trypan blue. Test article was diluted from a concentrated stock solution into sHMM medium such that residual organic solvent was <0.5% at final working solutions of 2 and 20 μM drug. Cell suspensions (0.25 mL, ∼200 000 cells) were incubated in 24-well culture plates in a 37 °C water-jacketed incubator with 95% air/5% CO_2 for 30 min before addition of drug. Equal volumes of cell suspensions and test article solutions were gently mixed in triplicate at 37 °C. Negative controls were done in parallel, containing every experimental component except cells. Positive controls were also done in parallel, using 20 μM bufuralol to demonstrate viability of both primary and secondary metabolism characteristics typical of each species. Incubations were quenched at 0, 0.5, 1, 2, and 4 h by addition of ice-cold acetonitrile (0.25 mL), then submitted to LCMS analyses. Quenched cell suspensions were centrifuged (13 000 rpm, 5 °C, 15 min), and 200 μL of supernatant was transferred to an HPLC injection vial. Thirty microliters was injected onto a loading column and desalted, then transferred by loop injection onto a Zorbax XDB C18 (4.6 × 30 mm^2, 3.5 μ d_P) column and eluted at 1 mL/min using a shallow gradient (50%-75% B, 12 min; solvent A 0.2% acetic acid in water; solvent B methanol) into a Finnigan LCQ ion trap mass spectrometer. Column effluent was electrosprayed after a 1:4 split, and positive ions were detected in full scan mode (m/z = 200−800). Total ion current was monitored, as well as XIC of characteristic $[M + H]^{+1}$ ions (compound **39**, 287 m/z (t_R 7.52 min); compound **10**, 289 m/z (t_R 8.62 min); compound **45**, 315 m/z (t_R 7.17 min); compound **12**, 317 m/z (t_R 7.92 min)). Retention times and mass spectral fingerprints were verified by co-injection of authentic standards for these compounds. Intermediate metabolites (compounds **43** and **44**) were inferred from retention times and mass spectra. Multiple dehydrations were the principle in-source fragmentations observed for both parent and primary metabolites. Relative quantities of parent and metabolites were estimated assuming equal detector sensitivities for all species and reported as percentages of the integrated ion intensities of the parent (**39**) at incubation time 0. For in vivo experiments, structures of all primary metabolites were verified and quantitated using co-injected deuterated (d_8) internal standards and reported as mg/mL of serum.

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Supporting Information Available: Details on the syntheses of intermediates **13**−**16** and **24**−**28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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