Articles

Reduction in Glucose Levels in STZ Diabetic Rats by 4-(2,2-Dimethyl-1-oxopropyl)benzoic Acid: A Prodrug Approach for Targeting the Liver

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The overproduction of glucose by the liver in NIDDM patients markedly contributes to their fasting hyperglycemia and is a direct consequence of the increased oxidation of excess free fatty acids (FFA) being released from the adipocyte. 2-(1,1-Dimethylethyl)-2-(4-methylphenyl)-[1,3]dioxolane (SAH51-641, **1**) has previously been demonstrated to reduce glucose levels in animal models of diabetes by reducing fatty acid oxidation and hence depriving the system of the energy and cofactors necessary for gluconeogenesis. However, attempts at lowering glucose levels in vivo with **1** have been associated with toxicity in other organs such as the testes. An approach was developed utilizing the natural processing of triglyceride-like intermediates as a basis for selectively targeting the absorption, processing, and delivery of a prodrug to the liver. Compounds were identified by this method which lowered glucose levels in vivo without releasing toxic amounts of the active metabolites of **1** into circulation.

Introduction

Diabetes mellitus may be categorized into two subclasses: type I, known as insulin-dependent diabetes mellitus (IDDM); and type II, non-insulin-dependent diabetes mellitus (NIDDM). IDDM accounts for about 10% of all diabetes and results from autoimmune-mediated destruction of insulin-secreting β-cells of the pancreas. In contrast, NIDDM is a chronic and progressive metabolic disorder of carbohydrate and lipid metabolism and accounts for the remaining 90% of diabetes mellitus.[1] It is the fourth leading cause of death in developed countries and affects more than 5% of the world's population (and one in four people over the age of 60). Fewer than half of all diabetics receive treatment, and of these only a very small proportion achieve a level of glucose control which is sufficient to avoid the morbidity associated with the disease, namely macrovascular (coronary artery disease, stroke) and microvascular (retinopathy, neuropathy, nephropathy, other microangiopathies) complications. In the 10-year Diabetes Control and Complication Trial (DCCT)[2] study it was found that tight control of blood glucose levels reduced the incidence and progression of neuropathy, retinopathy, and nephropathy, each by more than 50% in IDDM patients. Because therapeutic improvements were continuously related to incremental reductions in glycemia (toward normal levels), a new treatment goal of normoglycemia has been established for both IDDM and NIDDM patients. The initial therapy for newly diagnosed NIDDM patients has conventionally been diet and exercise. Although this leads to a marginal improvement in insulin sensitivity and a corresponding reduction in hyperglycemia, this treatment usually fails within a few months due to both a further progression of the disease as well as a problem with compliance and an oral antidiabetic agent is prescribed. The standard regimen has consisted of treatment with a member of the class of sulfonylurea drugs, which reduce glycemia by inducing the β-cell to release more insulin. However, undesired consequences of prolonged use of sulfonylureas include hypoglycemic episodes and ultimate exhaustion of the β-cell, as well as the long-term angiogenic side effects which are a result of chronic 24-h exposure to increased insulin levels.[3−5] Agents which reduce the glycemia without these above side effects are highly desirable and have been the aim of many research groups over the past 20 years.

Approach

NIDDM is characterized by excessive hepatic glucose production, impaired glucose disposal in the periphery, impaired lipid metabolism, and an inability of the β-cell to secrete sufficient insulin in a timely manner in order to achieve normalization of glucose and lipid homeostasis. The overproduction of glucose by the liver significantly contributes to fasting hyperglycemia in these individuals and is a direct result of the increased oxidation of the excess circulating free fatty acids (FFAs) being released from the adipocyte.[6−10] Clinical studies

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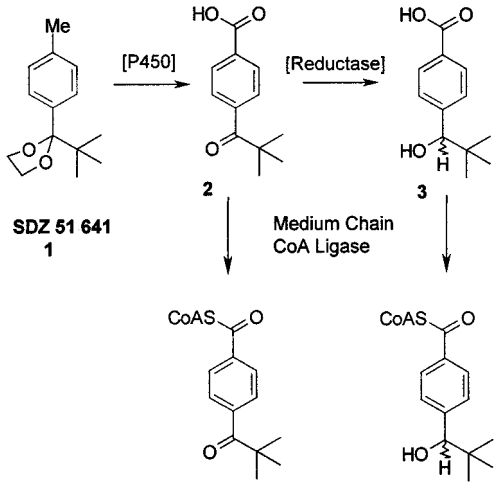
Scheme 1. Metabolic Activation of **1**



have shown that decreasing the supply of fatty acids or inhibiting fatty acid oxidation (FAO) per se results in a reduction in fatty-acid-driven gluconeogenesis.[11,12] One method which has been found useful in limiting FAO has been through control of the transport of fatty acids into the mitochondria where FAO takes place. Whereas octanoate passively diffuses across the inner mitochondrial membrane, long-chain fatty acids (LCFA), which are by far the major source of energy stored as fat, require active transport via the carnitine palmitoyl transferase (CPT) system, which includes CPT1, a translocase, and CPT2. Administration of 2-(1,1-dimethylethyl)-2-(4-methylphenyl)[1,3]dioxolane **(1)** has been previously shown to reduce β-oxidation (FAO) in normal fasted animals by actively sequestering the intramitochondrial CoA required for the transport of fatty acids into the mitochondria. **1** has also resulted in reduced glucose levels when administered to diabetic animal models.[13] It has been shown that **1** is converted by means of metabolic oxidation to **2** followed by subsequent reduction into its active form, **3** (Scheme 1). **3** then acts as a substrate for the medium-chain fatty acyl CoA ligase with the result of interfering in the FAO process by sequestering the available CoA. However, sequestration of CoA, which results in the inhibition of FAO and consequently glucose production in the liver, has proven detrimental to tissues other than the liver, which also depend on the processing of acyl CoA intermediates, and has produced undesired toxicity. If the active CoA-sequestering agent **3**, derived from **1** by metabolic transformation, could be localized to the liver and prevented from reaching general circulation, this should help to eliminate the observed peripheral toxicities (Figure 1). A prodrug approach was employed to provide a compound which was absorbed, sequestered by the liver, converted by the liver into the active agent where its effects could be manifested, and then ultimately conjugated and cleared by the liver thereby avoiding undesired concentrations of the active agent in general circulation. This strategy involves protection of the acid functionality in such a way that hepatic cleavage by either nonspecific esterases or P-450 enzymes is necessary for the biotransformation of the prodrug to **3**. Both methods require that the agent: (1) not be cleaved in the intestine, the enterocyte, or the blood stream; (2) be taken up by the liver preferentially;

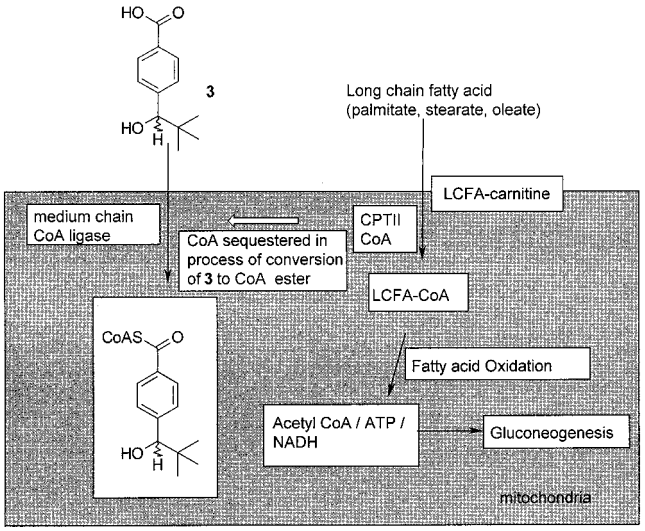


Figure 1. Mechanism of CoA sequestration.

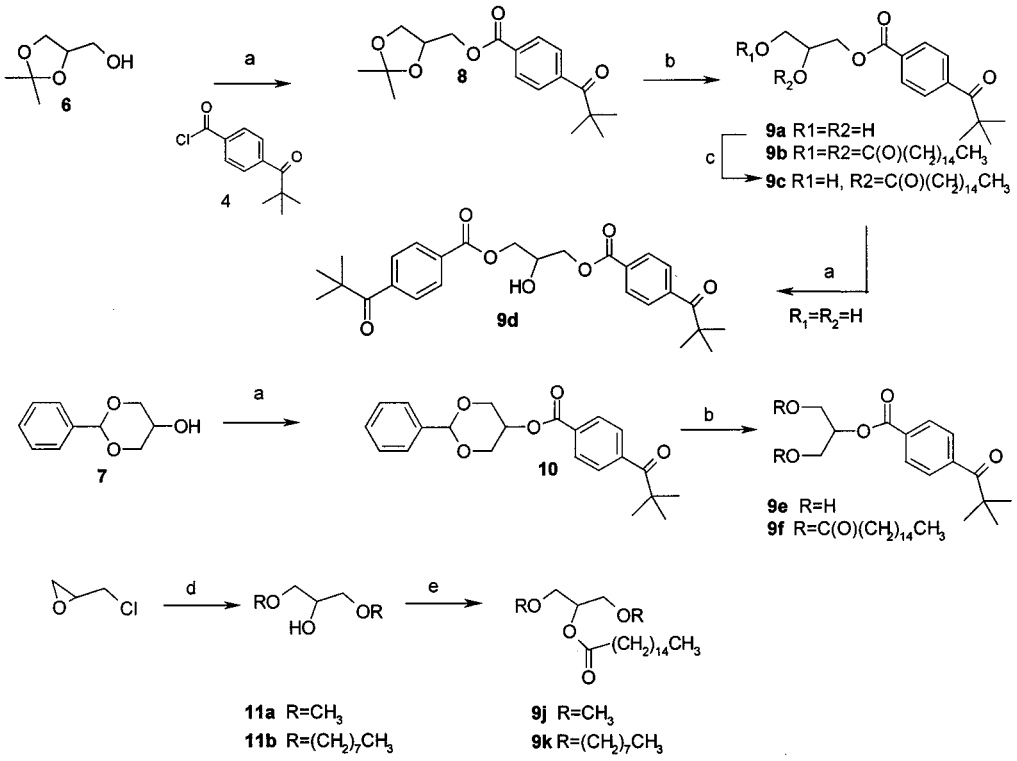
(3) be cleaved in the liver to the active form at a rate sufficient to inhibit FAO; and (4) not escape the liver into the blood stream once activated but be eliminated preferentially through the bile. Glycerol ester prodrugs have been previously utilized to enhance absorption and stability of various biological agents as well as provide selective delivery of these agents to the liver[14−19] (see Discussion section for further explanation). This technique was applied in this setting using various polyol esters and ethers of ketone **2** to modulate the targeting and release of the active pharmacophore **3**.

Chemistry

The key carboxylic acid **2** was prepared as previously described and converted to its acid chloride **4** using thionyl chloride.[20] Bromide **5** was prepared by treating 2,2,4′-trimethylpropiophenone with bromine.[21] Solketal (**6**), 1,3-benzylidene glycerol (**7**), pentaerythritol (**13**), pivaloyl chloride, and oleic acid were available commercially as were the alcohols for the preparation of compounds **18**−**24**. Triethyl 1,1,2-ethanetricarboxylate was reduced with LAH using standard conditions to provide the triol used in the preparation of **12a**.

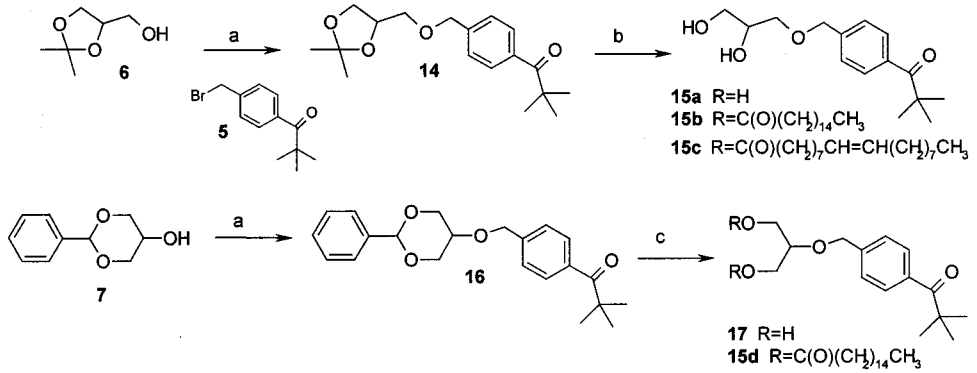
The general strategy used for the preparation of differentially substituted glycerol adducts **9a**−**g** utilized protected glycerols as exemplified by **6** and **7**. Acylation under standard conditions followed by deprotection of the glycerol fragment provided cleanly 1- or 2-acylated glycerols. If desired, the subsequent diol may be further derivatized with a fatty acid (i.e. palmitic acid) to generate pre-formed triglyceride analogues. As glycerols acylate preferentially on the 1-position, a more lengthy procedure was required to generate the 2,3-differentially disubstituted glyceride **9c** involving silylation of the primary position of **9a** followed by acylation of the 2-position and subsequent desilylation. In addition as 2-acylated glycerols have a tendency to migrate to the primary 1- or 3-position of glycerol, **9c** was unstable and converted slowly to the 1,3-disubstituted adduct. **9g** was prepared in a manner similar to **9f** except with substitution of 4-(2,2-dimethyl-1-oxopropyl)benzenepropanoyl chloride in the initial acylation of **7**. Complete acylation of glycerol (**9**) or other polyols (**12** or **13**) using additional equivalents of **4** provides the corresponding polyacylated

Scheme 2. Preparation of Acylated Analogues[a]



[a] Conditions: (a) **4**, Et_3N, THF; (b) 0.5 N HCl, THF, water, $CH_3(CH_2)_{14}COCl$, Et_3N, THF; (c) TBDPSCl, imidazole, DMF, $CH_3(CH_2)_{14}COCl$, Et_3N, THF, *n*-Bu_4NF, THF, AcOH; (d) Na, methanol (octanol); (e) $CH_3(CH_2)_{14}COCl$, Et_3N, THF.

Scheme 3. Preparation of Ether Analogues[a]



[a] Conditions: (a) **5**, NaH, THF; (b) 0.5 N HCl, THF, water, $CH_3(CH_2)_{14}COCl$, Et_3N, THF, $CH_3(CH_2)_7CH{=}CH(CH_2)_7COCl$, Et_3N, THF; (c) 0.5 N HCl, THF, water, $CH_3(CH_2)_{14}COCl$, Et_3N, THF.

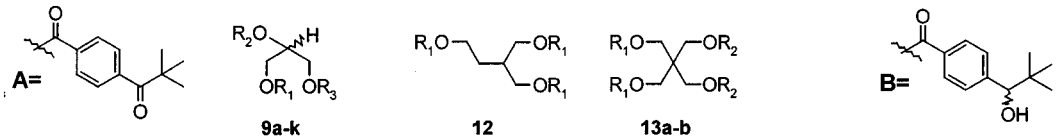
products. The three ketones of **9h** were reduced to the corresponding alcohols with $NaBH_4$ to provide **9i**. Reaction of epichlorohydrin with sodium alcoholates resulted in glycerol analogues **11a,b** with the 1- and 3-positions of glycerol converted to methyl or octyl ether adducts, respectively. Subsequent acylation under normal conditions (method A) provides **9j,k** (Scheme 2 and Table 1).

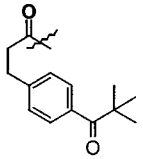
Alkylation of **6**, **7**, or **15a** with bromide **5** using NaH provided the substituted glycerol adducts. If required, removal of the protecting groups followed by acylation was performed in a similar manner as for the acylated adducts (vide supra) to provide **15a**−**e**. Alkylation of other alcohols and diols using NaH provided **18**−**24**. (See Scheme 3 and Table 2.)

Results and Discussion

The process of FAO requires the movement of FFAs across the mitochondrial membrane with subsequent β-oxidation of their CoA esters (Figure 1). Whereas octanoate passively diffuses across the inner mitochondrial membrane, LCFAs, which are by far the major source of energy stored as fat, require active transport via the CPT system, which includes CPT1, a translocase, and CPT2. Once inside the mitochondria, FFAs are then oxidized to produce ATP, acetyl-CoA, and NADH which are necessary cofactors for the process of gluconeogenesis. **1** had previously been demonstrated a potent hypoglycemic agent[13] whose effects are manifested through inhibition of the oxidation of LCFAs, thereby reducing gluconeogenesis. This requires activation of **1** by a suspected P-450 process to provide **2** followed subsequently by the reaction of a reductase to generate **3** as the major CoA-sequestering agent (Scheme 1). Both **2** and **3** are postulated to enter the mitochondria where they are converted into their respective CoA esters, via the medium-chain CoA ligase. After CoA ester forma-

Table 1. Biological Data for Ester Prodrugs



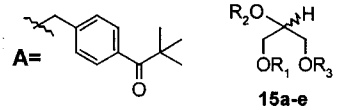
				Normal 18h-fasted rat Model [a]		Chronic STZ diabetic rat model [b]		AUC_{0-48h} in normal rats [c]
Compound #	R_1	R_2	R_3	β-HBA % of control	Glucose % of control	8.25 Days % efficacy	11.25 Days % efficacy	μg•h/mL
1				12**	58*	52**	64**	1513
9a	A	H	H	13.6**	56.1**	41.6	55.1*	1446
9b	A	$CH_3(CH_2)_{14}CO$	$CH_3(CH_2)_{14}CO$	20.5**	63.0**	75.7**	90.2**	1456
9c	A	$CH_3(CH_2)_{14}CO$	H	22.7*	60.7**	69.8**	64.5*	645
9d	A	H	A	11.9**	58.4**	99.5**	111.1**	1785
9e	H	A	H	10.9**	59.3**	67.4**	88.1**	1479
9f	$CH_3(CH_2)_{14}CO$	A	$CH_3(CH_2)_{14}CO$	23.6*	60.7*	62.5	84.7*	885
9g	$CH_3(CH_2)_{14}CO$		$CH_3(CH_2)_{14}CO$	24.6**	71.6**	84.0**	97.5**	930
9h	A	A	A	22.9** ED_{50}=88[#]	82.2	103.7**	103.6**	128
9i	B	B	B	32.5**	57.0**	49.8	61.0**	1165
9j	CH_3	A	CH_3	15.5*	69.4**	75.8**	54.9	1126
9k	$CH_3(CH_2)_7$	A	$CH_3(CH_2)_7$	20.1*	82.9*			
12a	A			28.8** ED_{50}=230[#]	98.6			
13a	A	A		96.2	109.4			
13b	A	H		14.3** ED_{50}=3.4[#]	68.9**			

[a] Acute screen: animals dosed at 100 μmol/kg, data reported as percent of control at 3 h postdose. [b] Chronic screen: animals dosed at 70 μmol/kg in STZ-treated diabetic rats, data reported as percent efficacy (where 100% efficacy is lowering of blood glucose levels to normal levels − 60 mg/dL). [c] Area under the curve of active metabolite **3** in blood plasma after dosing orally at 300 μmol/kg (see Experimental Section for description of in vivo models). *p < 0.01, **p < 0.001. [#]ED_{50} values are reported in mg/kg/day.

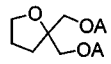
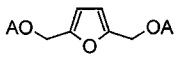
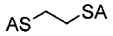
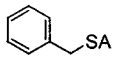
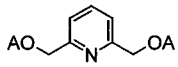
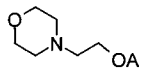
tion, the second step in the liver, under normal conditions, would be the xenobiotic elimination of these foreign aromatic acids through formation of their corresponding glucuronidate or hippurate from the corresponding CoA ester. As this second step is rate-limiting for aromatic acids such as **3**, formation of their CoA esters effectively sequesters the intramitochondrial supply of CoA. Lower levels of intramitochondrial CoA would then have a direct effect on CPT2 thereby limiting the respective transport of LCFA into the mitochondria. In addition, lower levels of intramitochondrial CoA would also have a direct effect on the long-chain acyl dehydrogenase (LCAD) thereby limiting the oxidation of LCFA within the mitochondria. Diminished FAO results in lower acetyl CoA levels and a reduced supply of ATP and NADH required for enzymes in the pathway of gluconeogenesis, such as pyruvate carboxylase, the first step of gluconeogenesis. The immediate outcome would be reduced liver glucose synthesis and thus reduced serum glucose levels.

In normal 18-h fasted rats, glycogen levels are exhausted and gluconeogenesis maintains blood glucose levels in the normal range. Fatty acids are released from the adipocyte, and their subsequent metabolism provides the energy and cofactors necessary for glucose production in the liver. Concurrent with an increase in FAO is an increase in circulating ketone levels (β-hydroxybutyrate, β-HBA) as a consequence of the oxidation of LCFAs. CoA-sequestering agents, such as **3**, have been shown to acutely reduce the levels of β-HBA in these animals and consequently have also reduced glucose levels. It has been observed in normal fasted rats, by either reducing the dose of certain analogues or by selecting analogues with reduced ability to be converted to the active entity **3**, that acute reductions in β-HBA levels (i.e. reduction in FAO) could be accomplished with only marginal reductions in glucose levels. Although the pharmacological effect on glucose levels would be expected to lag behind the primary effect on FAO through CoA sequestration, this delay was considered constant for our purposes based on achieved concentrations of active agent. Consequently, any further separation between the effects on β-HBA levels and glucose levels may be due to other pharmacokinetic effects, such as absorption rate and route (portal versus lymphatic) or metabolic activation rate of the prodrug to **3.** This separation of effects on β-HBA versus glucose levels was examined as a potential marker for those agents most likely to be activated at a rate sufficient for their effects to be manifested in the liver without being activated too rapidly so as to elicit subsequent effects in other tissues. It should be kept in mind,

Table 2. Biological Data for Ether Prodrugs



15a-e

				Normal 18h-fasted rat Model [a]		Chronic STZ diabetic rat model [b]		AUC_{0-48h} in normal rats [c]
Compound #	R_1	R_2	R_3	β-HBA % of control	Glucose % of control	8.25 Days % efficacy	11.25 Days % efficacy	μg•h/mL
1				12**	58*	52**	64**	1513
15a	A	H	H	33.5**	77.6	5.7	4.3	
15b	A	$CH_3(CH_2)_{14}CO$	$CH_3(CH_2)_{14}CO$	31.2**	97.8	0.4	-46.4	
15c	A	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$	20.7**	95.1	9.5	21.8	
15d	$CH_3(CH_2)_{14}CO$	A	$CH_3(CH_2)_{14}CO$	57.2* ED_{50}=127#	111.6	24.8	35.5	92
15e	A	A	A	19.6**	74.1	25.9	3.8	
18				12.7**	78.1*	45.6	40.1	
19				15.0**	88.1	1.4	-5.3	
20				17.3**	79.7**	6.2	-3.9	
21				82.6	79.7			
22				59.5	68.9			
23				32.5**	98.2	18.2	-6.8	
24				23.8*	98.2	-13.1*	-5.9	0

[a] Acute screen: animals dosed at 100 μmol/kg, data reported as percent of control at 3 h postdose. [b] Chronic screen: animals dosed at 70 μmol/kg in STZ-treated diabetic rats, data reported as percent efficacy (where 100% efficacy is lowering of blood glucose levels to normal levels − 60 mg/dL). [c] Area under the curve of active metabolite **3** in blood plasma after dosing orally at 300 μmol/kg (see Experimental Section for description of in vivo models). *p < 0.01, **p < 0.001. #ED_{50} values are reported in mg/kg/day.

however, that in normal animals, counter-regulatory measures would also prevent hypoglycemia thus limiting the maximum glucose lowering that one could achieve.

The testes have proven to be the primary organ of toxicity for this class of CoA-sequestering agents. Evidence from the literature has demonstrated that agents such as *p-tert*-butylbenzoic acid,[22−25] a known CoA-sequestering agent, reduce aspermatogenesis in the testes. Whereas **1** is effective at inhibiting FAO in the liver, it was also found to escape the liver after conversion into **3** and thereby also result in undesired effects on other tissues including the testes. It was hypothesized that localizing the active CoA-sequestering agent to the liver, the desired organ of pharmacological action, should help reduce the observed peripheral toxicities. The goal of this research was therefore to generate the active CoA-sequestrating agent **3** only in the liver where its effects would be desired and at such a rate that its effects in the liver would be complete without release of the agent into general circulation.

Early on in the development process, 28-day safety studies were employed to demonstrate the testicular toxicity associated with these agents. As it was impossible to perform full 28-day safety studies on each candidate, an alternative method for the rapid identification of potentially undesirable compounds was desired. An interesting observation was noted between the incidence and severity of testicular toxicity in these 28-day safety studies and the corresponding blood levels of the active agent **3**, following a single oral dose. This is illustrated for compounds **1**, **9h**, and **12a** in Table 3. It is clear that **1**, which releases active pharmacophore **3** into circulation as confirmed by its high AUC_{0-48h} value, has altered body, testes, and liver weights, whereas **9h** and **12a** had values which were much closer to control. This finding allowed the AUC_{0-48h} (area under the curve) of **3** in blood (collected at seven time points following a single oral dose) to serve as a preliminary assessment of toxicity prior to a full 28-

Table 3. Comparison of Organ and Body Weights to AUC Values

compd	body wt[a]	heart wt[b]	testes wt[b]	liver wt[b]	AUC_{0-48h}[c]
1	76%**	97%	46%*	215%*	1513
9h	95%	92%	102%	113%*	128
12a	101%	100%	100%	101%	0

[a] Body weights are presented as percent of control values. [b] Organ weights were calculated as follows: [organ weight (treated)/body weight (treated)]/[organ weight (control)/body weight (control)]. [c] Area under the curve of active metabolite **3** in blood plasma after dosing orally at 300 μmol/kg (see Experimental Section for description of in vivo models). *p < 0.05, **p < 0.01. Compounds **9h**, **12a,** and **1** were evaluated in a 28-day safety study in normal Sprague−Dawley rats at 380, 550, and 700 μmol/kg/day, respectively (10× ED_{50} for glucose lowering in STZ-treated diabetic animals).

day safety study. More importantly, the AUC_{0-48h} was used as a guide for directing the focus of the chemistry efforts before extensive pharmacology was practical. AUC_{0-48h} values of less than 500 μg·h/mL have not shown toxicity to the testes in comparable 28-day safety studies.[26]

The search for a development candidate required a very specific selection profile in terms of rates of absorption, delivery, metabolism, and clearance. The desired absorption should preferably be over an extended period of time so that the peak concentration of the agent in any given tissue or fluid, such as intestine, blood stream, and even the liver would not be more than the liver's ability to sequester, unmask, and be inhibited by the active agent. An additional aid in controlling the rate of delivery would be a design which promoted lymphatic transport, a mechanism which would slow the rate of delivery to the blood stream thereby avoiding exceeding the capacity of the liver to dispose of the agent as well as providing a longer ultimate duration of action. However the delivery of drugs through the lymphatic system may be neither timely nor sufficient to produce the desired biological effect. As the primary site of action will be the liver, portal delivery and first-pass uptake by the liver is in this approach a desirable event. The physical characteristics of the prodrug should be designed such that its selective uptake by the liver would be nearly complete. The unmasking of the prodrug to the active agent in the liver should be at such a rate as not to overload the liver's capacity to dispose of the agent and allow metabolites to leak into circulation, but also not so slow that the agent is eliminated before the active portion of the prodrug can be unmasked and find its target. The ultimate elimination of the agent should preferably be through the bile so as to avoid a second pass through the blood stream, and the method of elimination should be irreversible so that enterohepatic recirculation does not occur. Whereas one can design certain of these characteristics into potential drug candidates, other factors are more tenuous as the following results, the ultimate effect in animal models, clearly support.

The compounds described herein were all evaluated first for their ability to inhibit oleate-dependent gluconeogenesis in freshly prepared hepatocytes from 18-h fasted rats.[13,27] In general, most compounds significantly decreased glucose production with IC_{50} values of less than 100 μM (data not shown). However it was found that due to the particular nature of each prodrug's environment, even compounds which exhibited no activity in hepatocytes could provide significant activity when placed in an in vivo setting.

Acylated Analogues: Each of the mono-, di-, and triglyceride equivalents of **3** (**9a**−**h**) significantly decreased β-HBA levels to less than 25% of control levels in a 18-h fasted normal rat model indicating pronounced inhibition of β-oxidation (FAO) (Table 1). Glucose levels were also reduced to only 56−82% of control in this model as expected and consistent with the effects on β-HBA preceding and being more pronounced than the corresponding effects on glucose levels. Agents with a profile of significantly reducing β-HBA while having only modest effects on glucose levels were selected as the most promising to elicit the desired glucose-lowering effect in the chronic diabetic model without eliciting peripheral toxicities and were further evaluated for AUC_{0-48h}. Acylation of the remaining sites on glycerol with palmitate or another LCFA should allow the compounds to more closely resemble true triglycerides and so be absorbed and processed as triglycerides. Each of the acylated triglyceride analogues (**9a**−**h**) was active in the chronic STZ diabetic rat model at 6 h after oral dosing with values ranging from 42% to 100% efficacy (100% efficacy being indicative of a return of glucose levels to that of a normal nondiabetic rat). Based on the normal processing of triglycerides by pancreatic-gut lipases, substitution at the 2-position of glycerol by a specific acyl group should allow passage through the intestinal wall without hydrolysis from its glycerol carrier. Therefore, placement of the masked pharmacophore at the 2-position as in compounds **9e**,**f** (palmitoylated **9e**) should provide a better selectivity profile without exhibiting higher AUC_{0-48h} values than 1- and/or 3-acylated glycerol analogues. However, as can be inferred from the AUC_{0-48h} data, both proforms **(9a,e)** produced **3** at levels that would be predictive of toxicity. It was noteworthy that **9h,** a triacylated glycerol analogue, would have been predicted to be toxic, based on normal processing of triglycerides resulting in cleavage of the acyl group from the 1- or 3- position of glycerol during processing and absorption from the gut. However **9h** produced a very low AUC_{0-48h} of 128 μg·h/mL predictive of no toxicity. As the abilities of the gut lipases to cleave benzoyl esters are poor, **9h** likely passes through the gut intact and proceeds to the liver before being unmasked to the active agents. Indeed it has been shown that medium-chain triglycerides, which contain C6−C12 fatty acids and are similar in size to **9h**, can be absorbed from the gut intact and are not partitioned into the lipoprotein fraction but proceed directly to the liver via the portal vein.[28] If **9h** had been cleaved in the gut or enterocyte to any significant rate, then **3** would have entered general circulation and resulted in toxicity.

Ethers **9j**,**k** were effective in the 18-h fasted normal rat model at lowering both β-HBA as well as glucose levels. However, profound differences were apparent in the chronic STZ diabetic rat model where the dimethyl ether **9j** was efficacious while the dioctyl ether **9k** exhibited no activity. From cursory examination, one might argue that some physicochemical parameter is responsible for the difference as **9k** (cLog $P = 9.7$) is much more lipophilic than **9j** (cLog $P = 2.3$) and may require metabolic cleavage or activation in order to be absorbed. This, however does not explain why both **9j**,**k** were active in the 18-h fasted normal rat model, suggesting similar bioavailability and processing, while **9k** was not active in the chronic STZ diabetic rat model. A more reasonable explanation would be that the chronic STZ diabetic model is different perhaps in one or more pharmacokinetic parameters such as absorption or metabolic activation, and this leads to the lack of activity with **9k** in this model. This will be discussed further below.

Whereas the activity of the various intestinal lipases on glycerol analogues is well-documented and quite efficient, even modest changes to the glycerol backbone produce structures which are remarkably resistant to the activity of these lipases. Thus, whereas esters of

methanol, ethylene glycol, and glycerol are cleaved by pancreatic lipase at 63, 200, and 1900 μmol FFA released/min/mg protein, respectively, hydrolysis of erythritol, adonitol, and other higher sugars does not occur at measurable rates.[29] In addition, as it had become apparent that even highly lipophilic structures such as **9h** were able to be absorbed intact without cleavage in the gut and be sequestered by the liver as evident from a significant effect on β-HBA in comparison to a low AUC_{0-48h} value, other backbones were investigated for their potential as prodrug carriers which would be resistant to hydrolysis. For two such compounds, **12a** and **13a,** sequential derivatizable alcohol units were added. Interestingly, **12a** was an effective FAO inhibitor in both normal (lowering β-HBA) and diabetic animals and exhibited an undetectable AUC_{0-48h} for the active species **3**. A 28-day safety study performed in normal rats with **12a** administered at 10× the ED_{50} (for glucose lowering in STZ diabetic rats) produced no detectable histological effects in the testes thereby supporting the predictability of the AUC_{0-48h} data.[24] This would imply that prodrug **12a** was absorbed intact, delivered to the liver, unmasked to **3**, subsequently inhibited FAO in the liver, and ultimately cleared. Structure **12a** therefore fulfilled the objectives of the program. A tetraacyl isomer, **13a**, on the other hand, showed no activity in vitro (data not shown) or in vivo. Since **13b** (the diacyl isomer of **13a)** produced significant activity in all assays, this suggests that **13a** apparently is neither absorbed intact nor activated to any significant extent by pancreatic-gut lipases which would allow for its absorption.

Ether Analogues: As ether analogues are unlikely to be cleaved by lipases or esterases in the gut, enterocyte, or serum, they possess the advantage of being delivered to the liver prior to any unfortuitous metabolic activation, given that the enterocyte possesses at best minor P-450-metabolizing activity. The position on the glycerol backbone should not be critical in this case for ultimate delivery to the liver. The use of the glycerol/triglyceride unit could, in addition, capitalize on slower delivery via the lymphatic system and minimize the rate of release of **3** into circulation. Many of the compounds (see Table 2) produced marked reductions in β-HBA levels in the 18-h fasted normal rat model as had been observed before with the acylated analogues. There were only modest changes in glucose levels in this model, which was initially perceived to be in line with the desire for agents with slower onset of action. The lack of any significant effect of the ether analogues in the chronic STZ diabetic rat model was, however, puzzling. As most compounds provided a significant effect on β-HBA levels in the 18-h fasted normal rat model, this would indicate they are absorbed and unmasked to the active agent **3.** Bioavailability should be comparable to or even better in the chronic versus the normal rat models as lipophilic agents have routinely shown enhanced bioavailability in models where ad libitum food is present[30] (as in the chronic STZ diabetic rat model). This would suggest that in the chronic STZ diabetic rat model something is quite different. For the acylated prodrugs, the reduction in glucose in the chronic model correlates well with the reduction in β-HBA and glucose in the normal 18-h fasted normal rat model. This suggests the liver and intestinal esterases and lipases responsible for generating active pharmacophore **3** are functioning in similar fashions in each model. However, the lack of glucose-lowering activity of the ether glycerol analogues in the chronic model, while at the same time showing significant lowering of β-HBA in the acute model, suggests the enzymes or factors available to unmask the active pharmacophore in the normal animal are evidently not available in the STZ diabetic animal. It has already been shown that **1** is readily converted to **2** and **3** in both normal as well as STZ diabetic animals indicating that the oxidases (P-450) and reductases necessary for these conversions are available in both models. There have been some reports with respect to differences in the amounts of various P-450 isozymes[31] which are available for metabolic transformation in diseased animals such as the STZ rat. This may account for lower levels of **3** or possibly different metabolic products altogether. Whereas it was satisfying that the ether glycerol prodrugs provided significant amounts of active agent **3** and subsequently pronounced activity in the 18-h fasted normal rat model, this series has thus far proven disappointing in terms of their activity in the chronic STZ diabetic rat model.

The ether linkage was also utilized in a number of other alcohol and diol units to determine whether they could also be prodrug carriers (**18**−**24**). Each of these compounds reduced β-HBA levels in normal animals with minor to modest reductions in glucose levels. When utilizing the sulfide linker as in **21** and **22**, one which is generally considered metabolically unstable, it was interesting that no reduction of β-HBA was observed. It is apparent that oxidation of sulfur must generate intermediates (perhaps sulfoxides) which are not as readily converted to **3**.

Conclusion

It has been shown that CoA sequestration by these agents provides reductions in β-HBA levels as a marker for the reduction in FAO. Reduction in FAO results in a corresponding reduction in fatty acid-driven gluconeogenesis and ultimately glucose levels. In the 18-h fasted normal rat model many compounds produced a significant effect on β-HBA levels without showing dramatic effects on glucose production. This profile was desired in our attempt to maximize delivery and activity to the liver target while minimizing peripheral circulation of **3** and its corresponding toxicity. In the case of the STZ diabetic rat model, the portion of glucose production driven by the high levels of circulating FFAs is a significant contributor to the undesired hyperglycemia. This overproduction of glucose is reduced upon treatment with these agents, while the basal gluconeogenesis necessary to sustain basal glucose levels should be unaffected. The prodrug stategies employed herein provided two agents, **9g** and **12a**, which inhibited FAO and reduced glucose levels without inducing toxicity via release of active CoA-sequestering agents into circulation. Agents employing the ether linkage however provided attachment of **3** which was apparently too resistant to activation by the liver of the STZ diabetic animals to provide meaningful amounts of active CoA-sequestering agent **3**. However, it should be recognized that the ability of the liver to effectively mobilize a

prodrug via cleavage and oxidation of an ethereal linkage to a carboxylic acid was clearly demonstrated with 10 out of 12 of these analogues significantly reducing β-HBA levels in 18-h fasted normal rats. On the other hand, the ester approach provided 13 out of 14 compounds which were delivered to and activated by the liver at a rate sufficient to produce significant reductions in β-HBA levels in 18-h fasted normal rats. Of these analogues, 11 exhibited significant reductions in glucose levels in the chronic STZ diabetic rat model. Compound **12a** provided significant glucose lowering in this model (ED_{50} = 39 mg/kg/day) and in conjunction with its undetectable AUC_{0-48h} was selected as a development candidate. It is envisioned that the application of ester prodrugs should be species-independent and correlate well to humans as the complement of intestinal lipases and their respective activities appear similar across species.[32] The application, however, of ether prodrugs and compounds which require more extensive modification may experience some species or model differences as observed above which would affect the tuned prodrug activation rate demonstrated here for the rat and so need to be further evaluated in the clinic. The goal of defining an agent which was activated in the liver without releasing the active pharmacophore **3** into general circulation, reduced FAO-driven gluconeogenesis and most importantly achieved this without the subsequent toxic effects to the testes, was thereby achieved.

Experimental Section

Acute Inhibition of FAO: 18-h Fasted Normal Rat Model. The ability to acutely inhibit FAO was assessed in normal male 18-h fasted Sprague−Dawley rats. Animals were dosed orally (po) with vehicle (0.5% carboxymethylcellulose and 0.2% Tween-80 in water) or compound in vehicle (N = 5 animals/group). Serum glucose and β-hydroxybutrate (β-HBA) levels were determined from blood obtained via cardiac puncture at 3 h postdose. Comparisons between metabolite levels of control and treated animals were made using a Student's t-test and expressed as percent of control values. Standard values for levels of metabolites in control animals are ketones, 11.7 mg/dL; glucose, 115 mg/dL.

Chronic Glucose Lowering in Diabetic Rats: Chronic STZ Diabetic Rat Model. Sprague−Dawley rats fed a high fat diet were rendered diabetic with a single intravenous injection of streptozotocin (40 mg/kg) into the tail vein. After 5 days, animals with blood glucose levels >200 mg/dL were fasted for 18 h and challenged with an oral glucose load (dextrose 1.35 g/kg). Animals with blood glucose between 40 and 80 mg/dL at 3 h were used in the study. These low-dose STZ rats on a high-fat diet have been shown to approximate NIDDM with fed glucose levels between 175 and 260 mg/dL. Animals were dosed orally once per day for 11 days with vehicle (0.5% carboxymethylcellulose and 0.2% Tween-80 in water) or compound in vehicle (N = 9 animals/group). Blood glucose levels were determined from blood obtained from the tip of the tail at 0 and 6 h postdose on days 1, 4, 8, and 11. Food was removed at 0 h and returned after the 6 h measurement on each day. The results presented in Tables 1 and 2 are from 6 h postdose on days 8 and 11 and are presented as percent efficacy where 100% efficacy is defined as normalization of glucose to nondiabetic control levels (60 mg/dL).

Area Under the Curve Exposure Over Time (AUC_{0-48h}) of 3. Normal Sprague−Dawley rats were dosed orally with compound (300 μmol/kg) in vehicle (N = 5 animals/group; vehicle same as above). Blood samples were obtained from the tip of the tail at 2, 4, 7, 8, 24, 36, and 48 h postdose. Blood samples were centrifuged after collection at each time point. Plasma samples from each group were pooled to provide sufficient sample for analysis by HPLC. Animals were allowed ad libitum access to food and water during the study. Results are presented in μg·h/mL. Compounds with values <500 μg·h/mL are considered nontoxic in terms of effects on the testes as verified with historical 28-day safety studies.

General. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR were recorded on a Bruker AC 300 MHz NMR or a JEOL 200 MHz NMR using tetramethylsilane (TMS) as an internal standard and are reported in ppm (δ). Mass spectra were performed on a Finnigan Mat 4600 spectrometer. Elemental analysis was performed with a Carlo Erba CHNS-O EA 1108 elemental analyzer. Data are within 0.4% of theoretical values unless otherwise indicated. All compounds were routinely checked by TLC with Macherey-Nagel Polygram SIL G/UV_{254} plates. Yields are of purified products and were not optimized.

Method A: General Procedure for Acylation of Alcohols Using Acid Chloride 4. To the starting alcohol (0.10 mol) and DMAP or Et_3N (0.34 mol) in anhydrous THF or methylene chloride (800 mL) at 0 °C was added acid chloride **4** (0.11 mol equiv for each alcohol unit) portionwise over 30 min. The reaction was allowed to warm to room temperature and stirred for 15 h. The crude reaction mixture was evaporated and then partitioned between CH_2Cl_2(100 mL) and water (50 mL). Subsequent extraction with 2 portions of CH_2Cl_2 (100 mL) followed by drying the combined organic layer over $MgSO_4$, filtering and evaporating resulted in crude product. Compounds were purified by chromatography.

For Compounds such as 8 or 10 That Have an Acid-Sensitive Protecting Group. The crude material was deprotected using 0.5 N HCl in THF at room temperature for 3 h. Ether (100 mL) was added and the mixture partitioned between ether and water. The aqueous layer was extracted with ether (2 × 50 mL) and the combined organic layer was washed with aqueous $NaHCO_3$ (satd), brine, dried over $MgSO_4$ and concentrated to afford crude product. Chromatography over silica gel afforded pure product.

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 2,3-dihydroxypropyl ester (9a): acylation of **6** by method A − chromatographed on silica gel ($CHCl_3$ to 6% MeOH/$CHCl_3$) to afford **9a** in 72% yield; mp 74−75.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9H), 2.62 (bs, 1H), 3.07 (bs, 1H), 3.75 (bm, 2H), 4.09 (bs, 1H), 4.43 (m, 2H), 7.64 (d, J = 8.5 Hz, 2H), 8.06 (d, J = 8.5 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 281 (100), 298 (36); ^{13}C NMR ($CDCl_3$) δ 27.7, 44.4, 63.5, 66.0, 70.3, 127.4, 129.5, 131.2, 143.4, 166.2, 209.6. Anal. ($C_{15}H_{20}O_5$) C, H.

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (9e): acylation of **7** by method A − chromatography on alumina (neutral $CHCl_3$ followed by EtOAc) provided the title compound which was crystallized from ether to give white crystals (77%); mp 65−67 °C; ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 9H), 2.74 (bs, 2H), 3.95 (d, J = 4.7 Hz, 4H), 5.18 (m, 1H), 7.63 (d, J = 8.2 Hz, 2H), 8.07 (d, J = 8.2 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 298 (100); ^{13}C NMR ($CDCl_3$) δ 27.6, 44.4, 62.3, 76.0, 127.3, 129.6, 131.3, 143.4, 165.9, 209.8. Anal. ($C_{15}H_{20}O_5$) C, H.

Method B: General Procedure for Acylation of Glycerol Analogues with Long-Chain Acid Chlorides. The glycerol analogue (7.72 mmol) was dissolved in anhydrous CH_2Cl_2 (75 mL) and DMAP (1.1 g, 9.0 mmol) was added followed by palmitoyl chloride (2.6 mL, 8.5 mmol per alcohol unit). After stirring for 1 h the reaction was partitioned between water (30 mL) and CH_2Cl_2 (75 mL). Subsequent extraction with 2 portions of CH_2Cl_2 (75 mL) followed by drying the combined organic layer over $MgSO_4$, filtration and evaporation resulted in crude product. Chromatography on silica gel ($CHCl_3$) and recrystallization from ethanol if appropriate afforded the title compound in 70−95% yield.

Benzenepropanoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 2-[(1-oxohexadecyl)oxy]-1-[[(1-oxohexadecyl)oxy]methyl]ethyl ester (9g): acylation of **7** using method A substituting **4** with 4-(2,2-dimethyl-1-oxopropyl)benzenepropanoyl chlo-

ride provided a 2-acylated diol; subsequent acylation according to method B − chromatography on silica gel ($CHCl_3$) afforded **9g** in 92% yield; mp 44 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 6H), 1.25 (bs, 48H), 1.35 (s, 9H), 1.60 (bm, 4H), 2.30 (t, J = 7.5 Hz, 4H), 2.67 (t, J = 7.8 Hz, 2H), 2.98 (t, J = 7.8 Hz, 2H), 4.21 (bm, 4H), 5.26 (bm, 1H), 7.25 (d, J = 8.2 Hz, 2H), 7.68 (d, J = 8.2 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 802 (22) 376 (100); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 24.9, 28.1, 29.1, 29.3, 29.4, 29.5, 29.6, 29.66, 29.70, 30.5, 31.9, 34.0, 35.2, 44.1, 62.0, 69.4, 127.9, 128.5, 136.4, 143.5, 171.7, 173.3, 208.3. Anal. ($C_{49}H_{84}O_8$) C, H.

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 2,3-bis[(1-oxohexadecyl)oxy]propyl ester (9b): acylation of **9a** using method B − chromatography on silica gel ($CHCl_3$) and recrystallization from ethanol afforded the title compound (91%); mp 51−52 °C; ^{1}H NMR ($CDCl_3$) δ 0.88, (m, 6H), 1.25 (bs, 46H), 1.33 (s, 12H), 1.63 (m, 3H), 2.33 (m, 4H), 4.40 (bm, 4H), 5.44 (m, 1H), 7.66 (d, J = 8.5 Hz, 2H), 8.04 (d, J = 8.5 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 774 (100); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 24.88, 24.93, 27.7, 29.08, 29.13, 29.3, 29.4, 29.5, 29.6, 29.67, 29.7, 31.9, 34.1, 34.2, 44.4, 62.1, 63.2, 68.8, 127.4, 129.5, 131.0, 143.3, 165.3, 172.9, 173.3, 209.4. Anal. ($C_{47}H_{80}O_7$) C, H.

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 2-[(1-oxohexadecyl)oxy]-1-[[(1-oxohexadecyl)oxy]methyl]ethyl ester (9f): acylation of **9e** using method B − crystallization from ethanol provided pure product as white crystals (4.13 g, 96.9%); mp 42−43 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 6H), 1.25 (bs, 46H), 1.33 (s, 9H), 1.60 (bm, 6H), 2.32 (t, J = 7.5 Hz, 4H), 4.38 (m, 4H), 5.52 (bm, 1H), 7.67 (d, J = 8.5 Hz, 2H), 8.05 (d, J = 8.5 Hz, 2H); MS (DCI, ammonia) m/z (rel intensity) 774 (100); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 24.8, 27.6, 29.0, 29.1, 29.25, 29.33, 29.5, 29.55, 29.58, 31.8, 33.9, 44.3, 61.9, 70.1, 127.3, 129.4, 131.0, 143.2, 164.8, 173.2, 209.2. Anal. ($C_{47}H_{80}O_7$) C, H.

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 3-hydroxy-2-[(1-oxohexadecyl)oxy]propyl ester (9c). To **9a** (7.0 g, 25 mmol) in 125 mL of anhydrous DMF was added imidazole (4.26 g, 62.5 mmol) followed by *tert*-butyldiphenylsilyl chloride (7.23 g, 26.3 mmol) and the mixture stirred for 16 h at room temperature. The crude reaction was evaporated and then partitioned between $CHCl_3$ (100 mL) and water (50 mL). Subsequent extraction with 2 portions of $CHCl_3$ (100 mL) followed by drying the combined organic layer over $MgSO_4$, filtering and evaporating resulted in crude product. Chromatography on silica gel (5% EtOAc/hexane to 15% EtOAc/hexane) provided **benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 3-[(1,1-dimethylethyl)diphenylsilanyl]oxy-2-hydroxypropyl ester** (14.7 g, 79%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 1.1 (s, 9H), 1.37 (s, 9H), 2.6 (m, 1H), 3.8 (m, 2H, 4.13 (m, 1H)m 4.45 (d, J = 5.5 Hz, 2H), 7.4 (m, 6H), 7.7 (m, 6H), 8.05 (d, J = 8.2 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 536 (24), 242 (100), 291 (44).

The above material was acylated according to method B − chromatography on silica gel (5% EtOAc/hexane to 10% EtOAc/hexane) provided **benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 3-[(1,1-dimethylethyl)diphenylsilyl]oxy-2-[(1-oxohexadecyl)oxy]propyl ester** as a clear oil (94.6%): ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 5.5 Hz, 3H), 1.07 (s, 9H), 1.25 (s, 9H), 1.3 (bs, 24H), 1.60 (m, 2H), 2.28 (m, 2H), 3.85 (m, 2H), 4.50 (dd, J = 12.5, 6.2 Hz, 1H), 4.62 (dd, J = 12.5, 3.1 Hz, 1H), 5.33 (m, 1H), 7.39 (m, 6H), 7.61 (m, 6H), 7.97 (d, J = 8.1 Hz, 2H).

To the above material (18.1 mmol, 13.7 g) in anhydrous THF (175 mL) at 0 °C was added AcOH (1.81 mL, 31.6 mmol) and 1 M tetrabutylammonium fluoride (22.6 mmol, 22.6 mL in THF). After stirring for 2 h, the reaction was evaporated and then partitioned between $CHCl_3$ (150 mL) and pH 7 buffer (100 mL). Subsequent extraction with 2 portions of $CHCl_3$ (100 mL) followed by drying the combined organic layer over $MgSO_4$, filtering and evaporating resulted in crude product. Chromatography on silica gel (5% EtOAc/hexane to 20% EtOAc/hexane) provided **9c** as a clear oil (8.5 g, 90.5%): ^{1}H NMR ($CDCl_3$) δ 0.90 (m, 3H), 1.25 (bs, 24H), 1.32 (s, 9H), 1.63 (bm, 3H), 2.10 (bm, 1H), 2.36 (t, J = 7.5 Hz, 2H), 3.86 (bm, 2H), 4.57 (m, 1H), 5.33 (m, 1H), 7.66 (d, J = 8.6 Hz, 2H), 8.07 (d, J = 8.6 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 536 (24), 242 (100), 291 (44); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.0, 27.7, 29.1, 29.2, 29.3, 29.4, 29.7, 29.9, 31.9, 34.1, 34.3, 61.4, 61.5, 62.1, 63.1, 72.1, 73.5, 127.4, 129.5, 209.3. Anal. ($C_{31}H_{50}O_6$) C, H. *This material is thermally unstable and rearranges to the 1,3-diacyl material if stored at room temperature for prolonged periods.*

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 2-hydroxy-1,3-propanediyl ester (9d). To **9a** (2.0 g, 7.14 mmol) in anhydrous THF (100 mL) at 0 °C was added Et_3N (1.12 mL, 8.0 mmol) followed by the acid chloride **4** (1.68 g, 7.50 mmol) portionwise over 45 min. After stirring for an additional 2 h, the reaction was evaporated and then partitioned between $CHCl_3$ (50 mL) and water (25 mL). Subsequent extraction with 2 portions of $CHCl_3$ (50 mL) followed by drying the combined organic layer over $MgSO_4$, filtration and evaporation resulted in crude **9d**. Chromatography on silica gel ($CHCl_3$) provided pure product which was crystallized from ether/heptane to give the title compound (12.95 g, 82%) as white crystals: mp 87−88 °C; ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 18H), 2.88 (bs, 1H), 4.40 (bs, 1H), 4.55 (m, 4H), 7.65 (d, J = 8.5 Hz, 4H), 8.08 (d, J = 8.5 Hz, 4H); MS (DCI, NH_3) m/z (rel intensity) 469 (32), 486 (100); ^{13}C NMR ($CDCl_3$) δ 27.7, 44.4, 66.0, 68.4, 127.4, 129.5, 131.1, 143.4, 165.9, 209.5. Anal. ($C_{27}H_{32}O_7$) C, H.

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 1,2,3-propanetriyl ester (9h): acylation of glycerol with 3.3 equiv of acid chloride **4** according to method A − chromatography on silica gel (1% MeOH/$CHCl_3$ to 2% MeOH/$CHCl_3$) provided the title compound which was crystallized from methanol to give **9h** as white crystals (87%); mp 96−97 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9H), 1.33 (s, 18H), 4.76 (bm, 4H), 5.86 (bm, 1H), 7.65 (d, J = 8.2 Hz, 6H), 8.05 (m, 6H); MS (DCI, NH_3) m/z (rel intensity) 469 (72) 486 (25); ^{13}C NMR ($CDCl_3$) δ 27.7, 44.4, 63.1, 70.1, 127.5, 129.5, 129.6, 130.9, 143.5, 143.6, 165.0, 165.3, 209.4. Anal. ($C_{39}H_{44}O_9$) C, H.

Benzoic acid, 4-(1-hydroxy-2,2-dimethylpropyl)-, 1,2,3-propanetriyl ester (9i). 9h (6.0 g, 9.14 mmol) was dissolved in 2-propanol (100 mL) and acetic acid (100 mL) and cooled to 0 °C before the addition of $NaBH_4$ (1.0 g, 26 mmol) portionwise. The reaction was stirred for 2 h and evaporated to a crude residue which was partitioned between CH_2Cl_2 and water, dried and concentrated to a crude oil. Chromatography on silica gel (CH_2Cl_2 to 1% MeOH/CH_2Cl_2) afforded 6.05 g of **9i** as an amorphous solid (71%): ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 27H), 2.14 (bs, 3H), 4.43 (s, 3H), 4.73 (bm, 4H), 5.81 (m, 1H), 7.37 (m, 6H), 7.97 (bm, 6H); MS (DCI, NH_3) m/z (rel intensity); ^{13}C NMR ($CDCl_3$) δ 25.7, 35.6, 62.9, 69.6, 81.7, 127.6, 128.26, 128.31, 128.9, 147.6, 147.8, 165.5, 165.9 Anal. ($C_{39}H_{50}O_9$) C, H.

1,3-Dimethoxy-2-propanol (11a) and 1,3-Bis(octyloxy)-2-propanol (11b). Sodium metal (8.8 g, 0.38 mol) was added to anhydrous methanol (200 mL) over 30 min and then stirred for an additional 30 min. Epichlorohydrin (15.0 mL, 0.19 mol) was added over 45 min and the reaction mixture refluxed for 2 h. The reaction was evaporated and then partitioned between ether (100 mL) and water (50 mL). Subsequent extraction with 2 portions of ether (100 mL) followed by drying the combined organic layer over $MgSO_4$, filtering and evaporating resulted in crude 1,3-dimethoxypropan-2-ol in 53% yield. The material was used without further purification in the subsequent reaction. Similar conditions were used to prepare 1,3-bis-(octyloxy)propan-2-ol (2.1 equiv of octanol and sodium to epichlorohydrin−toluene as solvent).

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 2-methoxy-1-methoxymethylethyl ester (9j): acylation of **11a** by method A − chromatography on silica gel ($CHCl_3$) afforded **9j** in 33% yield; ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 9H), 3.40 (s, 6H), 3.69 (d, J = 5.0 Hz, 4H), 5.41 (m, 1H), 7.65 (d, J = 8.4 Hz, 2H), 8.09 (d, J = 8.4 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 760 (100); ^{13}C NMR ($CDCl_3$) δ 27.7, 44.4, 59.3, 71.3, 72.3, 127.4, 129.6, 131.8, 143.1, 165.4, 209.5. Anal. ($C_{17}H_{24}O_5$) C, H.

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 2-octyloxy-1-(octyloxy)methylethyl ester (9k): acylation of **11b** by method A − chromatography on silica gel ($CHCl_3$) afforded

9k in 69% yield; ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 6H), 1.25 (bs, 18H), 1.33 (s, 9H), 1.56 (bm, 6H), 3.49 (m, 4H), 3.70 (d, J = 5.1 Hz, 4H), 5.38 (bm, 1H), 7.65 (d, J = 8.5 Hz, 2H), 8.08 (d, J = 8.5 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 309 (100); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 26.0, 27.6, 29.2, 29.3, 29.5, 31.7, 44.3, 69.2, 71.6, 72.7, 127.2, 129.3, 129.4, 131.9, 142.9, 165.3, 209.3. Anal. ($C_{31}H_{52}O_5$) C, H.

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 1,2,4-butanetriyl ester (12a): acylation of 2-hydroxymethyl-1,4-butanediol[33] according to method A − chromatography on silica gel (1% MeOH/$CHCl_3$ to 2% MeOH/$CHCl_3$) afforded **12a** in 92% yield; mp 99−100 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 27H), 2.10 (m, 2H), 2.59 (m, 1H), 4.53 (m, 6H), 7.63 (d, J = 8.3 Hz, 2H), 7.64 (d, J = 8.2 Hz, 4H), 8.03 (d, J = 8.3 Hz, 2H), 8.04 (d, J = 8.2 Hz, 4H); MS (DCI, ammonia) m/z (rel intensity) 702 (100); ^{13}C NMR ($CDCl_3$) δ 27.7, 27.9, 35.3, 44.3, 62.8, 64.8, 127.4, 129.3, 131.3, 143.3, 165.6, 209.3. Anal. ($C_{41}H_{48}O_9$) C, H.

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 2,2-bis[[4-(2,2-dimethyl-1-oxopropyl)benzoyloxy]methyl]-1,3-propanediyl ester (13a): acylation of pentaerythritol according to method A − chromatography on silica gel (1% MeOH/$CHCl_3$ to 2% MeOH/$CHCl_3$) afforded **13a** in 49% yield; mp 168−170 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 36H), 4.72 (s, 8H), 7.62 (d, J = 8.2 Hz, 8H), 8.03 (d, J = 8.2 Hz, 8H); MS (DCI, ammonia) m/z (rel intensity) 906 (100); ^{13}C NMR ($CDCl_3$) δ 27.7, 43.2, 44.4, 63.6, 127.5, 129.4, 130.7, 143.6, 165.3, 209.2. Anal. ($C_{53}H_{60}O_{12}$) C, H.

Benzoic acid, 4-(2,2-dimethyl-1-oxopropyl)-, 2,2-bis-(hydroxymethyl)-1,3-propanediyl ester (13b): acylation of 2,2-dimethyl-1,3-dioxane-5,5-dimethanol[34] according to method A − acetonide in 98% yield; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 18H), 1.47 (s, 6H), 3.92 (s, 4H), 4.51 (s, 4H), 7.63 (d, J = 8.1 Hz, 4H), 8.05 (d, J = 8.1 Hz, 4H); MS (DCI, NH_3) m/z (rel intensity) 570 (100); ^{13}C NMR ($CDCl_3$) δ 23.6, 27.7, 38.0, 44.4, 62.5, 64.2, 98.9, 127.5, 129.3, 131.2, 143.3, 165.5, 209.3. The crude material from above was deprotected using 0.5 N HCl in THF at room temperature for 3 h. Ether (100 mL) was added and the mixture partitioned between ether and water. The aqueous layer was extracted with ether (2 × 50 mL) and the combined organic layer was washed with aqueous $NaHCO_3$ (satd), brine, dried over $MgSO_4$ and concentrated to afford **13b** in 98% yield: mp 104−105 °C; ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 18H), 2.76 (bs, 2H), 3.79 (s, 4H), 4.52 (s, 4H), 7.65 (d, J = 8.5 Hz, 4H), 8.04 (d, J = 8.5 Hz, 4H); MS (DCI, NH_3) m/z (rel intensity) 513 (7), 530 (100); ^{13}C NMR ($CDCl_3$) δ 27.7, 44.4, 45.6, 62.8, 63.4, 127.5, 129.5, 130.9, 143.6, 166.3, 209.4. Anal. ($C_{29}H_{36}O_8$) C, H.

Method C: Alkylation of Glycerols To Prepare Ether Analogues of 5. Sodium hydride (26 mmol 1.15 equiv per alcohol equiv) was washed with pentane (2 × 10 mL) and then suspended in anhydrous THF (35 mL) and cooled to 0 °C. An alcohol (22.5 mmol) was added and stirred for 15 min. To this solution **5** (22.5 mmol) was added and the resulting mixture stirred for 4 h while warming to ambient temperature. The reaction was concentrated and the solids were diluted with ether (50 mL), acidified to pH 1 with 2 N HCl, extracted with 4 × 30 mL of ether, dried over $MgSO_4$ and concentrated to a crude solid. The crude material was either triturated with hexane or chromatographed to afford pure product.

For Compounds such as 14 or 16 That Have an Acid-Sensitive Protecting Group. The crude material was deprotected using 0.5 N HCl in THF at room temperature for 3 h. Ether (100 mL) was added and the mixture partitioned between ether and water. The aqueous layer was extracted with ether (2 × 50 mL) and the combined organic layer was washed with aqueous $NaHCO_3$ (satd), brine, dried over $MgSO_4$ and concentrated to afford crude product. Chromatography over silica gel afforded pure product.

1-Propanone, 1-[4-[(2,3-dihydroxypropoxy)methyl]-phenyl]-2,2-dimethyl- (15a): alkylation of **6** using method C − purified on silica gel (20% MeOH/CH_2Cl_2 to 80% MeOH/CH_2Cl_2) to provide **15a** in 92% yield; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9H), 2.33 (bs, 1H), 2.77 (bs, 1H), 3.58 (m, 2H), 3.66 (m, 2H), 3.92 (m, 1H), 4.58 (s, 2H), 7.35 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 284 (100); ^{13}C NMR ($CDCl_3$) δ 28.1, 28.2, 44.3, 64.2, 70.8, 72.2, 73.1, 127.2, 127.9, 128.3, 138.1, 140.8, 209.0. Anal. ($C_{15}H_{22}O_4$) C, H.

1-Propanone, 1-[4-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]phenyl]-2,2-dimethyl- (17): alkylation of **7** using method C − purified on silica gel (20% MeOH/CH_2Cl_2 to 80% MeOH/CH_2Cl_2) to provide the product (82%); ^{1}H NMR (CD_3OD) δ 1.33 (s, 9H), 3.5 (m, 1H), 3.7 (m, 4H), 4.75 (s, 2H), 7.4 (d, J = 8.1 Hz, 2H), 7.70 (d, J = 8.1 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 284 (100).

Hexadecanoic acid 2-[[4-(2,2-dimethyl-1-oxopropyl)-phenyl]methoxy]-, 1,3-propanediyl ester (15d): acylation of **17** according to method B − chromatography (silica gel/gradient hexane to 66% hexane/ether) then provided a 74% yield of **15d**; mp 31−33 °C; ^{1}H NMR (C_6D_6) δ 0.83 (t, J = 6.4 Hz, 6H), 1.13 (s, 9H), 1.24 (s, 48H), 1.51 (m, 4H), 2.07 (t, J = 7.4 Hz, 4H), 3.55 (m, 1H), 4.09 (dd, J = 11.7, 5.3 Hz, 2H), 4.22 (dd, J = 11.7, 4.8 Hz, 2H), 4.32 (s, 2H), 7.15 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 8.1 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 760 (100); ^{13}C NMR (C_6D_6) δ 14.2, 23.0, 25.2, 28.0, 29.3, 29.6, 29.7, 29.8, 29.9, 30.0, 30.04, 32.3, 34.1, 43.9, 62.7, 71.2, 75.5, 127.0, 128.5, 138.0, 141.4, 172.7, 206.5. Anal. ($C_{47}H_{82}O_6$) C, H.

Hexadecanoic acid, 1-[[[4-(2,2-dimethyl-1-oxopropyl)-phenyl]methoxy]methyl]-1,2-ethanediyl ester (15b): acylation of **15a** according to method B − chromatography on silica gel (hexane to 66% ether/hexane) then afforded a 71% yield of **15b**; mp 32−34 °C; ^{1}H NMR (C_6D_6) δ 0.71 (t, J = 6.9 Hz, 6H), 1.01 (s, 9H), 1.13 (s, 48H), 1.40 (m,4H), 2.03 (m, 4H), 3.22 (m, 2H), 4.00 (d, J = 3 Hz, 2H), 4.05 (dd, J = 11.8,6.5 Hz,1H), 4.29 (dd, J = 11.9,3.5 Hz, 1H), 5.27 (m, 1H), 6.94 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 760 (100); ^{13}C NMR (C_6D_6) δ 14.4, 23.2, 25.3, 25.4, 28.2, 29.5, 29.6, 29.8, 29.9, 30.17, 30.21, 30.22, 30.26, 32.4, 34.3, 34.6, 44.1, 63.1, 69.2, 70.5, 72.8, 127.1, 128.7, 138.2, 141.3, 172.7, 172.9, 206.7. Anal. ($C_{47}H_{82}O_6$) C, H.

9-Octadecenoic acid, 1-[[[4-(2,2-dimethyl-1-oxopropyl)-phenyl]methoxy]methyl]-1,2-ethanediyl ester ((9*Z*)-15c). To **15a** (2.0 g, 7.5 mmol) in CH_2Cl_2 (30 mL) at 0 °C was added DMAP (0.3 g, 3 mmol) and oleic acid (4.3 g, 15.2 mmol) followed by DCC (3.29 g, 16.0 mmol) and the mixture stirred for 36 h at ambient temperature. The reaction mixture was diluted with ether, washed with 10% HCl, satd sodium bicarbonate, dried over $MgSO_4$ and evaporated to an oil. Chromatography on silica gel (hexane to 30% ether/hexane) afforded 5.73 g (96% yield) of **15c** as a clear oil: ^{1}H NMR (C_6D_6) δ 0.72 (t, J = 6.4 Hz, 6H), 1.01 (s, 9H), 1.02 (bs, 40H), 1.39 (m, 4H), 1.90 (t, J = 6.8 Hz, 8H), 2.01 (m, 4H), 3.22 (m,2H), 4.01 (d, J = 2.8 Hz, 2H), 4.05 (dd, J = 11.9,6.5 Hz, 1H), 4.29 (dd, J = 11.9, 3.4 Hz, 1H), 5.30 (m, 5H), 6.95 (d, J = 8.1 Hz, 2H), 7.50 (d, J = 8.1 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 812 (100); exact mass M + Na 817.6336 (817.6322 theoretical); ^{13}C NMR (C_6D_6) δ 14.2, 23.0, 25.2, 27.5, 27.6, 28.0, 29.3, 29.4, 29.5, 29.6, 29.9, 30.0, 30.1, 32.2, 34.1, 34.4, 43.9, 62.9, 68.9, 70.3, 72.5, 126.9, 128.6, 130.0, 130.1, 138.0, 141.1, 172.5, 172.7, 206.5. Anal. ($C_{51}H_{86}O_6$) C, H.

1-Propanone], 1,1′,1″-[1,2,3-propanetriyltris(oxymethylene)-4,1-phenylene]tris[2,2-dimethyl- (15e). Sodium hydride (0.52 g, 10.8 mmol) was washed with pentane (2 × 10 mL) and then suspended in anhydrous THF (35 mL) and cooled to 0 °C. **15a** (2.5 g, 9.4 mmol) was added in THF (5 mL) and the mixture stirred for 30 min before adding 1-[4-(bromomethyl)phenyl]-2,2-dimethyl-1-propanone (**5**; 2.5 g, 9.5 mmol). The reaction was allowed to warm to room temperature over 15 h and then cooled to 0 °C before addition of an additional portion of sodium hydride (0.56 g, 11.6 mmol). After 50 min an additional amount of **5** (2.6 g, 9.9 mmol) was added and the reaction was warmed to room temperature over 4 h before being heated to 60 °C for 24 h. The reaction was cooled and partitioned between methyl *tert*-butyl ether and pH 4 buffer. Subsequent extraction with methyl *tert*-butyl ether (2 × 50 mL) was followed by drying the combined organic layer over $MgSO_4$. Chromatography on silica gel (10% EtOAc/hexane to 30% EtOAc/hexane) afforded **15e** as a gummy solid (3.4 g,

69%): ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 27H), 3.67 (d, J = 6 Hz, 4H), 3.85 (m, 1H), 4.58 (s, 4H), 4.75 (s, 2H), 7.35 (d, J = 8.0 Hz, 4H), 7.38 (d, J = 8.0 Hz, 2H), 7.68 (d, J = 8.0 Hz, 2H), 7.70 (d, J = 8.0 Hz, 4H); MS (DCI, NH_3) m/z (rel intensity) 632 (100); ^{13}C NMR ($CDCl_3$) δ 28.0, 44.2, 70.7, 71.8, 72.9, 77.7, 126.9, 127.0, 128.1, 128.13, 137.7, 137.8, 141.2, 141.7, 208.7. Anal. ($C_{39}H_{50}O_6$) C, H.

The following were all prepared using method C and the appropriate alcohol.

1-Propanone], 1,1′-[(tetrahydrofuran-2-ylidene)bis-(methyleneoxymethylene)-4,1-phenylene]bis[2,2-dimethyl-(18): chromatography on silica gel (hexane to 30% diethyl ether/hexane) afforded **18** in 40% yield; ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 18H), 1.9 (bm, 4H), 3.52 (q, J = 9 Hz, 4H), 3.90 (t, J = 6.8 Hz, 2H), 4.63 (abq, LS = 15.1, 5.2 Hz, 4H), 7.37 (d, J = 8.1 Hz, 4H), 7.71 (d, J = 8.1 Hz, 4H); MS (DCI, NH_3) m/z (rel intensity) 481 (14), 498 (100); exact mass M + Na 503.2761 (503.2773 theoretical); ^{13}C NMR ($CDCl_3$) δ 26.0, 28.1, 30.8, 44.2, 68.7, 72.9, 73.0, 84.0, 126.8, 128.1, 137.5, 141.6, 208.7. Anal. ($C_{30}H_{40}O_5$) C, H.

1-Propanone], 1,1′-[(2-butene-1,4-diyl)bis(oxymethylene)-4,1-phenylene]bis[2,2-dimethyl- ((2*Z*)-19): chromatography on silica gel (hexane to 30% diethyl ether/hexane) afforded **19** in 37% yield; ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 18H), 4.11 (d, J = 5.6 Hz, 4H), 4.72 (s, 4H), 5.82 (m, 2H), 7.34 (d, J = 8.2 Hz, 4H), 7.69 (d, J = 8.2 Hz, 4H); MS (DCI, NH_3) m/z (rel intensity) 437 (15), 454 (100); ^{13}C NMR ($CDCl_3$) δ 28.0, 44.2, 66.1, 71.7, 127.1, 128.2, 129.4, 137.8, 141.1, 208.8. Anal. ($C_{28}H_{36}O_4$) C, H.

1-Propanone], 1,1′-[(2,5-furandiyl)bis(methyleneoxymethylene)-4,1-phenylene]bis[2,2-dimethyl- (20): chromatography on silica gel (hexane to 30% diethyl ether/hexane) afforded **20** in 64% yield; ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 18H), 4.50 (s, 4H), 4.58 (s, 4H), 6.30 (s, 2H), 7.37 (d, J = 8.2 Hz, 4H), 7.68 (d, J = 8.2 Hz, 4H); MS (DCI, NH_3) m/z (rel intensity) 494 (69), 302 (100); ^{13}C NMR ($CDCl_3$) δ 28.0, 44.2, 64.3, 71.4, 110.3, 127.2, 128.1, 137.8, 140.9, 151.9, 208.8. Anal. ($C_{30}H_{36}O_5$) C, H.

1-Propanone], 1,1′-[(1,2-ethanediyl)bis(thiomethylene)-4,1-phenylene]bis[2,2-dimethyl- (21): crude product was crystallized from hexane to afford **21** in 90% yield; mp 81−82 °C; ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 18H), 2.58 (s, 4H), 3.72 (s, 4H), 7.30 (d, J = 8 Hz, 4H), 7.68 (d, J = 8 Hz, 4H); MS (DCI, NH_3) m/z (rel intensity) 443 (17), 460 (100); ^{13}C NMR ($CDCl_3$) δ 28.1, 31.1, 36.0, 44.2, 96.1, 128.4, 128.5, 137.2, 141.3, 208.4. Anal. ($C_{26}H_{34}O_2S_2$) C, H, S.

1-Propanone, 2,2-dimethyl-1-[4-[[(phenylmethyl)thio]-methyl]phenyl]- (22): chromatography on silica gel (hexane to 30% diethyl ether/hexane) afforded **22** in 83% yield; ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 9H), 3.61(s, 4H), 7.30 (m, 7H), 7.68 (d, J = 8 Hz, 2H); MS (DCI, NH_3) m/z (rel intensity) 299 (11), 316 (100); ^{13}C NMR ($CDCl_3$) δ 28.1, 31.1, 36.0, 44.2, 96.1, 128.4, 128.5, 137.2, 141.3, 208.4 Anal. ($C_{19}H_{22}OS$) C, H, S; C: calcd, 76.47; found, 76.05.

1-Propanone], 1,1′-[2,6-pyridinediylbis(methyleneoxymethylene)-4,1-phenylene]bis[2,2-dimethyl- (23): crude product was crystallized from hexane to afford **23** in 70% yield; mp 49−50 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 18H), 4.68 (s, 4H), 4.70 (s, 4H), 7.42 (d, J = 8.2 Hz, 6H), 7.70, (d, J = 8.2 Hz, 4H), 7.74, (m, 1H); MS (DCI,NH_3) m/z (rel intensity) 488 (100); ^{13}C NMR ($CDCl_3$) δ 28.0, 44.2, 72.4, 73.3, 120.2, 127.1, 128.2, 137.5, 137.9, 141.0, 157.7, 208.7. Anal. ($C_{31}H_{37}NO_4$) C, H, N.

1-Propanone, 2,2-dimethyl-1-[4-[[2-(4-morpholinyl)-ethoxy]methyl]phenyl]- hydrochloride (24): chromatography on silica gel (hexane to 30% EtOAc/hexane) followed by conversion to the HCl salt afforded **24** in 70% yield; mp 151−152 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9H), 3.00 (m, 2H), 3.27 (bs, 2H), 3.57 (bd, J = 12.1 Hz, 2H), 3.95 (dd, J = 12.9, 2.8 Hz, 2H), 4.11 (s, 2H), 4.32 (bt, J = 12.2 Hz, 2H), 4.59 (s, 2H), 7.34 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H), 13.24 (bs, 1H); MS (DCI, NH_3) m/z (rel intensity) 306 (100); ^{13}C NMR ($CDCl_3$) δ 28.0, 44.2, 53.0, 57.5, 63.7, 65.1, 72.9, 127.3, 128.2, 138.4, 139.7, 208.8. Anal. ($C_{18}H_{28}NO_3Cl$) C, H, N, Cl.

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