Application of a Flexible Synthesis of (5*R*)-Thiolactomycin To Develop New Inhibitors of Type I Fatty Acid Synthase

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Fatty acid synthase (FAS) catalyzes the synthesis of palmitate from the sequential condensation of an acetyl primer with two carbon units added from malonyl-CoA. Inhibition of the β-ketoacyl synthase domain of mammalian FAS leads to selective cytotoxicity to various cancer cell lines in vitro and in vivo. Also, inhibitors of FAS can cause reduced food intake and body weight in mice. Naturally occurring thiolactomycin (TLM) was used as a template to develop a new class of type I FAS inhibitors. Using a flexible synthesis, families of TLM structural analogues were obtained that possess selective FAS activity and display anticancer and weight loss effects. Compounds **13a** and **13d** inhibit pure FAS (ZR-75-1 breast cancer, $IC_{50} = \leq 20$ μg/mL), are nontoxic (MCF-7, $IC_{50} = >50$ μg/mL), and display effective weight loss in BalbC mice (>5%). Another subclass of TLM derivatives (**23b**−**d**, **31a**) exhibits FAS activity ($IC_{50} = \leq 15$ μg/mL), causes weight loss (>5%), and is cytotoxic to cancer cells ($IC_{50} < 38$ μg/mL). Finally, a third subclass (**16b**, **29**, **30**) is also active against FAS ($IC_{50} = \leq 20$ μg/mL), is cytotoxic to cancer cells ($IC_{50} < 25$ mg/mL), and does not cause weight loss in BalbC mice. These studies identify thiolactomycin as a promising template for the development of new selective cancer and obesity treatments.

Introduction

Human fatty acid synthase (FAS) is emerging as an important therapeutic target for carcinomas of the breast, prostate, endometrium, ovary, and colon.[1,2] In addition, inhibition of FAS, notably in the hypothalamus, and the coupled stimulation of fatty acid degradation in the adipose tissue have been demonstrated to coordinately mediate profound weight loss in animals.[3] FAS is encoded by a single locus in the human genome.[4] The design and synthesis of small molecule inhibitors of this exquisitely evolved polydomainal protein with the goal to achieve selectivity in their cytotoxic or weight loss effects is a significant challenge. We address this question through consideration of reaction mechanism, X-ray crystal structures, and molecular modeling. Structural diversification of a natural product template has been undertaken to achieve both crossover through a fundamental prokaryotic/eukaryotic activity barrier, and successful differentiation of cytotoxic and weight loss effects.

Vastly higher levels of FAS (FAS, EC 2.3.1.85) are expressed in many human cancers and tumor cells[1,2] than in normal tissues in which FAS activity is significantly down-regulated and compensated with dietary fat.[4] This difference in expression and activity of FAS between normal and cancer cells provides an attractive approach to cancer therapy having the potential for a large therapeutic index.

Inhibition of mammalian FAS (multifunctional, type I; in contrast to the dissociable, bacterial type II FAS) displays selective cytotoxicity in various cancer cell lines. For example, administration of cerulenin (**1**) or C75 (**2**) (Figure 1) to MCF7 and SKBR3 breast cancer cell lines show FAS inhibition, followed by apoptosis.[2] Systemic treatment of MCF7 breast cancer xenografts in nude mice with **2** also shows FAS inhibition, apoptosis and reduction in tumor size. Despite its cytotoxicity to cancer cells, C75 displayed no adverse histological effects, but caused reversible weight loss in test animals.[2a,b]

Recent reports demonstrate that C75 acts as a malonyl-CoA mimetic and exerts its effects both centrally to reduce neuropeptide Y (NPY) expression[3b] and peripherally as a CPT-1 agonist[3a] causing reduced food intake and body weight in mice.[3] Furthermore, diminished adipose tissue and fatty liver in OB/OB mice is also observed despite elevated levels of malonyl-CoA (a known inhibitor of CPT-1) produced by FAS inhibition.

Both naturally occurring cerulenin (e.g. *Cephalosporium caerulens*) and synthetic C75 inactivate bacterial (type II) and mammalian (type I) FAS systems.[2,5] Nature has also produced a unique thiolactone-containing molecule that is a selective and reversible inhibitor of the KAS enzymes in type II bacterial FAS systems.[6] Thiolactomycin (TLM, e.g. *Nocardia* sp.) exhibits broad-spectrum antibiotic activity in vitro against Gram-positive and Gram-negative bacteria,[6] *Mycobacterium tuberculosis*,[7a,b] the malaria parasite, *Plasmodium falciparum,*[8a−e] and African trypanosomes.[8a,d] Intrigued by TLM's reported selectivity for type II FAS systems, the challenge to develop TLM structural analogues that show inhibitory activity against type I FAS systems and

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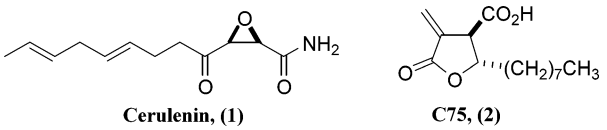


Figure 1. Inhibitors of fatty acid synthase.

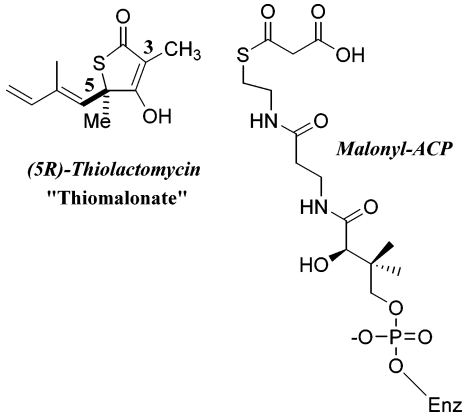


Figure 2. Thiolactomycin: a "thiomalonate isostere".

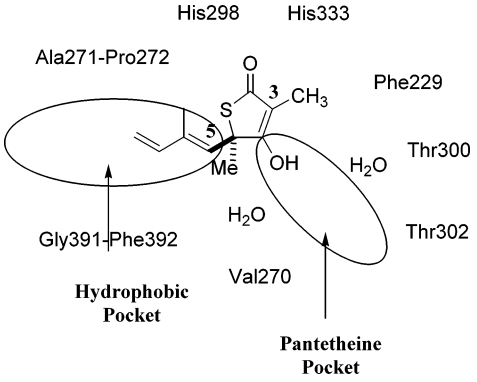


Figure 3. TLM cocrystallized in KAS I of *E. coli*.

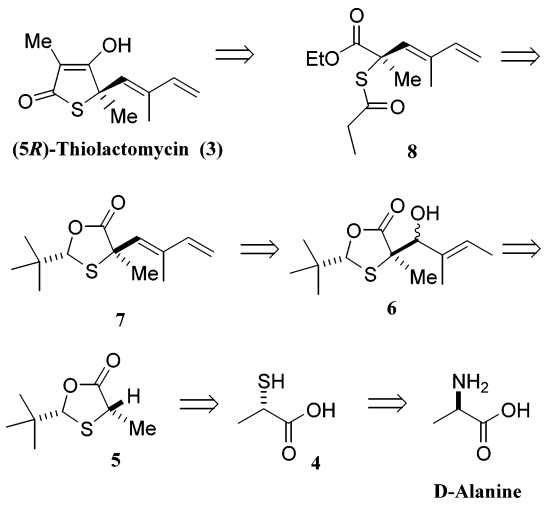
act as anticancer and/or weight loss agents became a compelling avenue to explore (Figure 2).

Thiolactomycin has been cocrystallized with KAS I (FabB) from *E. coli*.[9] The structure reveals that TLM occupies a different region in the active site than cerulenin and likely binds in the malonyl-ACP pocket on the basis of kinetic evidence (Figure 2).[9] Furthermore, the crystal structure provides insight into the architecture of the KAS active site and identifies the presence of hydrophobic and pantetheine binding pockets which are both suboptimally filled by TLM (Figure 3).[9]

Reports show that TLM analogues with an extended C-5 hydrocarbon chain are more effective inhibitors of pea (*Pisum sativum*) FAS[10a] and displayed improved activity against mycobacteria.[7a,b] Enhanced activity was also observed against *Staphylococcus aureus* and *Pasteurella multocida* by appending both the C-3 (acetyl) and C-5 (aryl or alkyl functionality) of the thiolactone skeleton.[10b]

We have recently described an efficient asymmetric synthesis of (5*R*)-thiolactomycin which was conceived to allow the medicinal potential of this template to be explored.[11] This flexible route has been applied to the preparation of several subclasess of thiolactomycin structural analogues that are varied at the C-3, C-5, and C-4 enol loci. Modification of the functionality around

Scheme 1



the thiolactone ring was successful in generating TLM analogues that are inhibitors of type I human FAS. Moreover, several of these compounds display effective anticancer activity, while others have been identified that generally minimize cytotoxicity and cause weight loss.

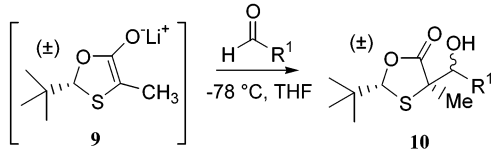
Chemistry

The asymmetric synthesis of TLM developed earlier in this laboratory employs Seebach's self-regeneration of chirality method using amino acids as the chiral building blocks (Scheme 1). The key steps involve a thio-Dieckman reaction of **8** to provide (5*R*)-thiolactomycin (**3**) and a sulfenate-sulfoxide [2,3]-sigmatropic rearrangement accompanied by a thermal *syn*-elimination to provide diene **7** to achieve almost exclusive trans stereochemistry at the C-1′ alkene. Finally, optically pure oxathiolanone **5** can be synthesized from (2*S*)-thiolactic acid, which is readily prepared from D-alanine (Scheme 1).[11]

First, several derivatives of TLM modified at C-5 were synthesized to probe the effects of alkyl, alkenyl and aryl substitutents in this hydrophobic pocket poorly filled by the short, unsaturated side chain of TLM (Figure 3). Extended hydrocarbon chains with and without the 1,3-diene at C-5 were prepared. Formation of the lithium-enolate of racemic **9**[12] was achieved with LDA at −78 °C and addition of tiglic aldehyde, 2-methyl-2-pentenal, *trans*-2-hexenal, and *trans*-2-octenal to the *re*-face gave 2:1 mixtures of the diastereomeric alcohols **10a** (81%), **10b** (71%), **10c** (88%), and **10d** (81%, Scheme 2.

Treatment of these alcohols with 2,4-dinitrobenzenesulfenyl chloride and NEt_3 in refluxing dichloroethane provided **11a** (75%), **11b** (73%), **11c** (72%), and **11d** (75%, Scheme 3). This conversion to the 1,3-diene was advantageous in our synthesis of thiolactomycin since the [2,3]-sigmatropic rearrangement and elimination provided the trans stereochemistry at the C-1′ alkene predominantly (14:1, trans:cis).[11,13] This configurational bias toward trans selectivity was also observed with **11b** (14:1, trans:cis) but less so with **11c** and **11d** (4:1 trans:cis). Previously, it was reported that the [2,3]-sigmatropic rearrangement of the allyl sulfenate to the allyl sulfoxide is concerted and reversible with the equilibrium lying on the side of the sulfoxide.[14]

Scheme 2



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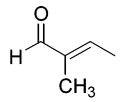
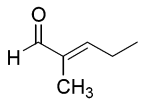
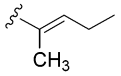
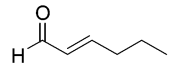
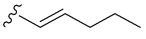
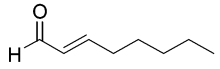
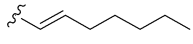
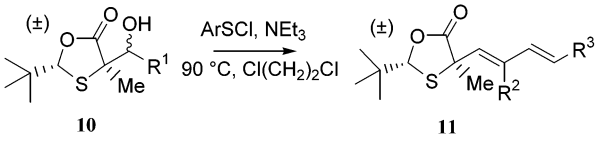
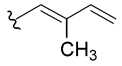
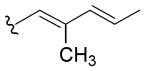
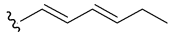
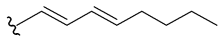
a			81%
b			71%
c			88%
d			81%

Scheme 3



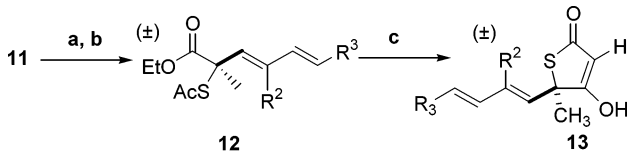
entry	Diene	Yield	*trans/cis*
a		75%	14:1
b		73%	14:1
c		72%	4:1
d		75%	4:1

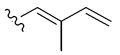
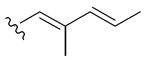
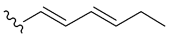
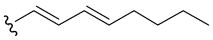
In our case, each diastereomeric allylic sulfenate can give both the *E*- and *Z*-sulfoxides. Presumably, the presence of the 2-methyl group in tiglic aldehyde and 2-methyl-2-pentenal introduces additional steric interactions in the *Z*-sulfoxides, making them higher in energy [calcd $\Delta G = 1.9$ kcal (at 90 °C), 14:1 trans:cis] than their *E* counterparts. Thus, dienes **11c**,**d**, which lack this 2-methyl group, lost their high trans selectivity as a consequence of a decrease in energy difference between the *E*- and *Z*-sulfoxides [calcd $\Delta G = 1.0$ kcal (at 90 °C), 4:1 trans:cis].

The oxathiolanone ring of **11a**−**d** was opened with Cs_2CO_3 in EtOH and immediately acylated with acetyl chloride to yield thioesters **12a**−**d** (63−77%, Scheme 4). Finally, thio-Dieckman condensation of **12a**−**d** gave **13a** (53%), **13b** (49%), **13c** (60%), and **13d** (41%, Scheme 4).

Thiolactomycin analogues containing a C-5 saturated hydrocarbon chain were obtained by a slight modification of the above procedure. Addition of octyl or hexyl triflate to **9** provided **14a** (83%) and **14b** (81%) (Scheme 5). The oxathiolanone ring was cleaved with NaOEt/EtOH, and the released thiol was acylated with acetyl,

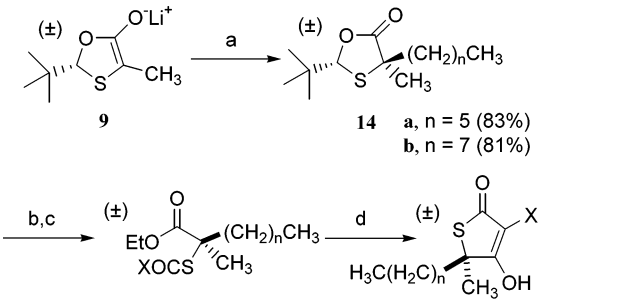
Scheme 4[a]



entry	Diene	Yield (12)	Yield (13)
a		61%	53%
b		77%	49%
c		63%	60%
d		59%	41%

[a] Reagents and conditions: (a) Cs_2CO_3, EtOH; (b) AcCl, NEt_3, CH_2Cl_2; (c) LiHMDS/THF, −78 °C to −5 °C.

Scheme 5[a]



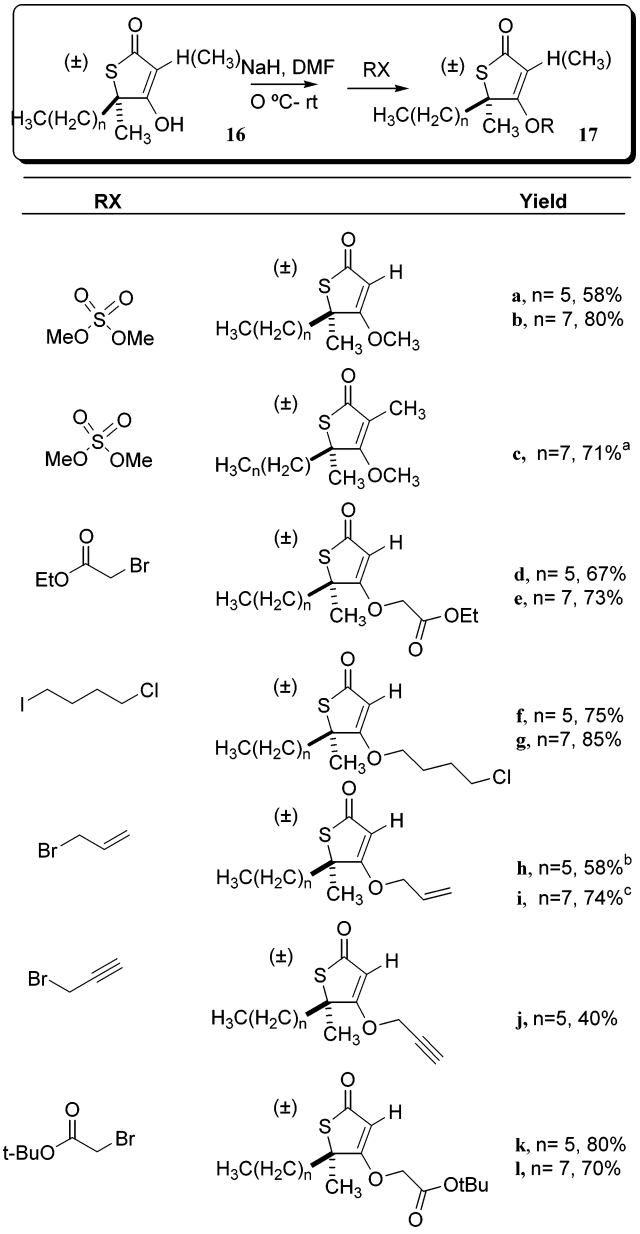
[a] Reagents and conditions: (a) $CH_3(CH_2)_nOTf$, $n = 5, 7$; (b) NaOEt/EtOH; (c) XCOCl, NEt_3, CH_2Cl_2; (d) LiHMDS, toluene, −78 °C to −5 °C. (e) NaHMDS was used instead LiHMDS in this case.

propionyl, and 4-pentenoyl chloride to give **15a** (77%), **15b** (79%), **15c** (75%), and **15d** (86%). Enolate formation and Dieckman condensation provided thiotetronic derivatives **16a** (69%), **16b** (73%), **16c** (70%), and **16d** (57%).

Next, preferential O-alkylation of **16b** was achieved using NaH in DMF to append the C-4 enol with various functional groups including alkenes, alkynes, alkyl halides, and esters (Scheme 6). In the crystal structure of TLM bound to KAS I, the C-4 enol appears to be directed into the probable pantethine recognition pocket of malonyl-ACP (vide infra). Therefore, TLM analogues with extended polar functionality at the C-4 enol were thought to further stablize ligand/enzyme binding (Figure 3).

In a few cases, C-3 and C-2 O-alkylation was observed. Methylation of **16c** provided both C-4,C-2 O-alkylated products as an inseparable mixture **17c/17m** (2.2:1, Scheme 7). Addition of allyl bromide to the sodium enolate of **16c** gave predominately C-alkylated (**17n**, 70%, Scheme 7) and C-4,C-2 O-alkylated products **17o/17p** (30%, 2.6:1 inseparable mixture) in an 82% overall yield. Alkylation of **16d** provided C-alkylated

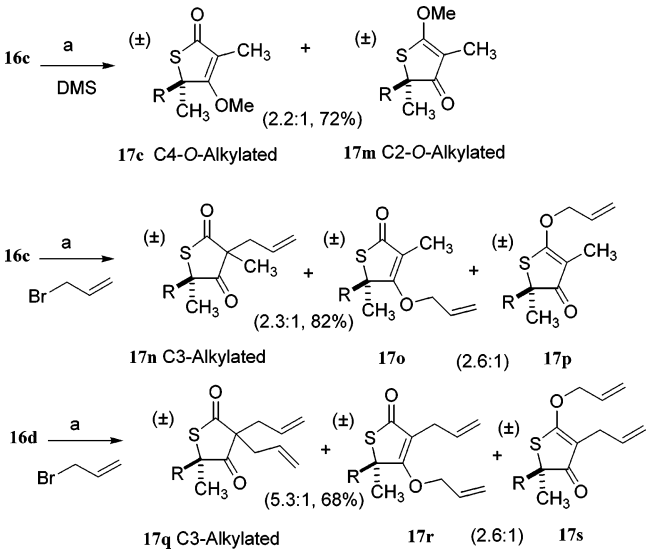
Scheme 6



[a] Mixture of C-4 *O*-methyl (**17c**): C-2 *O*-methyl (**17m**) 2:2:1. [b]Mixture of C-4 *O*-allyl (**17h**): C-3-dialkylated (**17t**) (3:1). [c]Mixture of C-4 *O*-alkyl (17i): C-3 dialkylated (**17q**) (3:5:1).

17q as the major product and C-4 O/C-2 O-alkylated **17r/s** (**17q**:**17r/s**, 5.3:1, 68% overall yield, Scheme 7). Perhaps, C-2 O- and C-alkylation is favored in **16c** due to the steric congestion of both the adjacent C-5 quaternary center and the C-3 methyl group. Recently, a study showed that both C-2 and C-4 O-alkylation (C-2 O:C-4 O, 1:2.3−1:9.7) of 3,5-dimethylthiotetronic acid was observed using various alkyl electrophiles.[15] Contrary to the reactivity of C-3 methylated **16c**, C-4 O-alkylation was the only or predominant route observed in most cases for **16a** and **16b** bearing a C-3 hydrogen. C-3 Dialkylated **17q** and **17t** were formed in minor amounts using allyl bromide as the electrophile in the alkylation of **16a** and **16b** (Scheme 6). C-4 O-Alkylation was determined through direct comparison of ^{13}C NMR carbonyl carbons with those of natural product thiolactomycin (196.7 ppm, C-2; 179.2 ppm, C-4)

Scheme 7[a]



[a] Reagents and conditions: (a) NaH, DMF, R = $(CH_2)_7CH_3$.

C-3 dialkylated **17n** (214.6 ppm, C-4; 204.5 ppm, C-2), **17q** (213.5 ppm, C-4; 203.9 ppm, C-2), and C-4,C-2 O-alkylated **17c** (195.9 ppm, C-2; 180.2 ppm, C-4)/**17q** (202.7 ppm, C-4; 184.9 ppm, C-2).

Acid hydrolysis of **17k**−**l** provided acids **18a** (98%) and **18b** (89%, Scheme 8). Coupling reactions of **18** with EDC, or tris(2,3-dihydro-2-oxobenzoxazol-3-yl)phosphine oxide[16] and the corresponding amines, provided **19** (80%), **21** (68%), and **22** (66%). Significantly, the methyl ester **19** was hydrolyzed instead of the thiolactone ring in high yield to give **20** (80%, Scheme 8). This result suggests that the thiolactone moiety in all of these analogues should be stable at physiological pH.

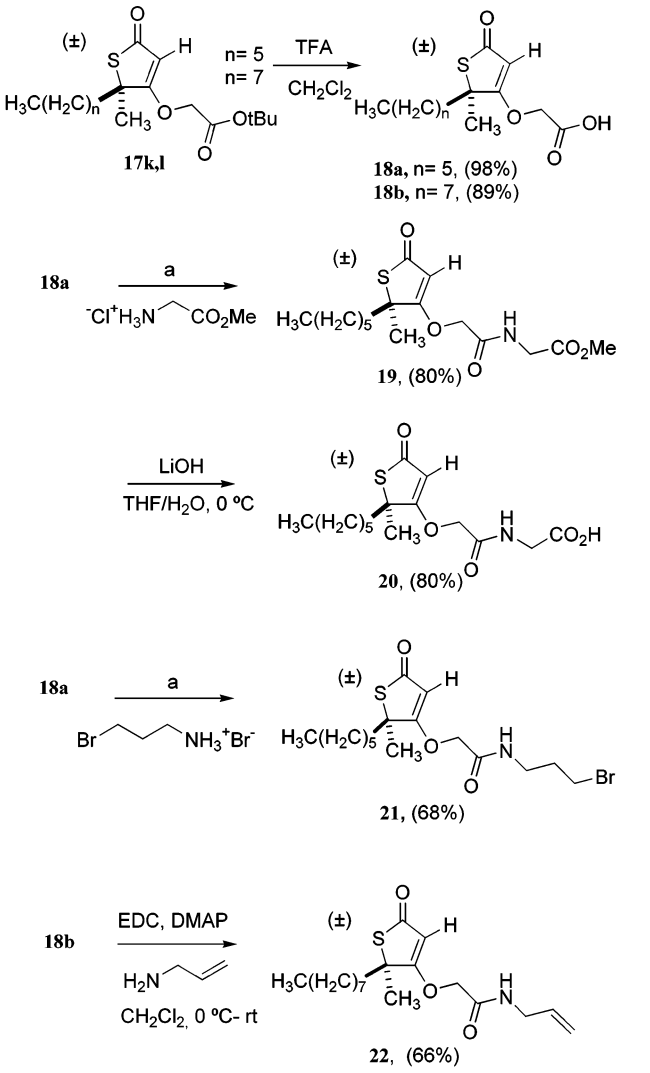
Enolate formation of **16b** with LiHMDS/THF (−78 °C−rt) and addition of methyl, ethyl, and allyl chloroformate provided O-acylated (confirmed by HMQC NMR) **23a** (47%), **23b** (70%), **23c** (91%), and **23d** (67%) (Scheme 9).

Additionally, C-5 aryl derivatives were also prepared to ascertain their affinity for the hydrophobic pocket. Alkaline cleavage (NaOEt/EtOH) and acylation of benzylated **24** yielded **25** (76%). Thio-Dieckman condensation of **25** gave **26** (45%, Scheme 10). O-Methylation to give **27** (74%) was achieved with dimethyl sulfate in DMF (Scheme 10).

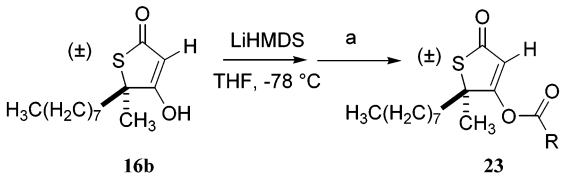
Finally, examination of the KAS I/TLM crystal structure revealed that the C-3 methyl group of thiolactomycin likely occupies the region where the carboxyl group of malonyl-ACP binds. Also, close inspection of the crystal structure reveals the presence of two threonines that lie 5.2 Å (Thr302) and 3.9 Å (Thr300) from the C-3 methyl group of TLM.[9] We anticipated that additional hydrogen bond acceptors/donors will favorably interact with these two threonines or the catalytic His333 (Figure 4).

Attempts to prepare molecules containing these features were made using a Mukaiyama aldol approach.[17] Indeed, silyl enol ether **28** was formed quantitatively from **16b** with TMSCl/benzene. Addition of $TiCl_4$ at −78 °C and acetaldehyde provided a mixture of diastereomers **29** and **30,** which was separated by column chromatography (50% overall yield, Scheme 11).

Scheme 8



Scheme 9[a]

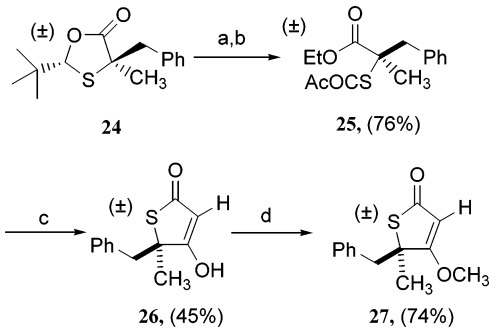


entry	R	Yield
a	CH_2CH_3	47%
b	OCH_3	70%
c	OCH_2CH_3	91%
d	OCH_2CHCH_2	67%

[a] Reagents and conditions: (a) ClCOR, R = CH_2CH_3, OCH_3, OCH_2CH_3, OCH_2CHCH_2.

Unfortunately, addition of acid chlorides, chloroformates, and anhydrides in the presence of $TiCl_4$ or other Lewis acids (e.g. $ZnCl_2$, $BiCl_3$/NaI, $MgCl_2$, $AlCl_3$) was unsuccessful.

Scheme 10[a]



[a] Reagents and conditions: (a) NaOEt/EtOH; (b) AcCl, NEt_3, CH_2Cl_2; (c) LiHMDS, −78 °C to −5 °C; (d) DMS, DMF.

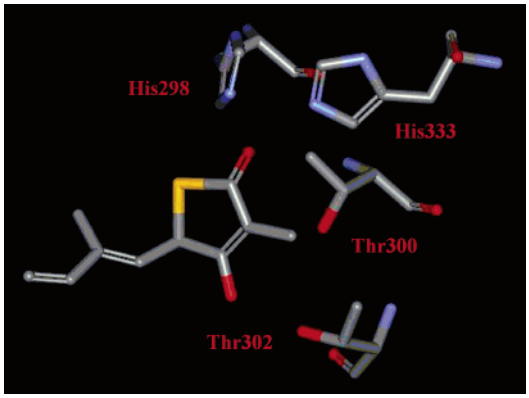
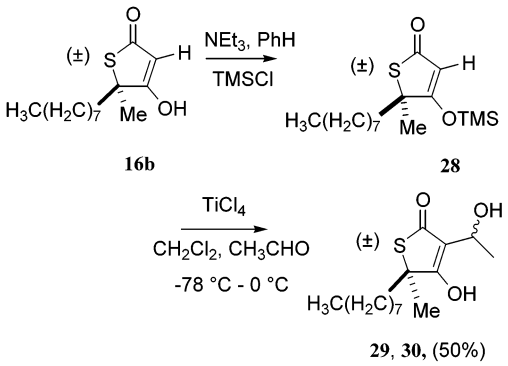


Figure 4. (5*R*)-TLM and nearby residues in *E. coli* KAS I.

Scheme 11

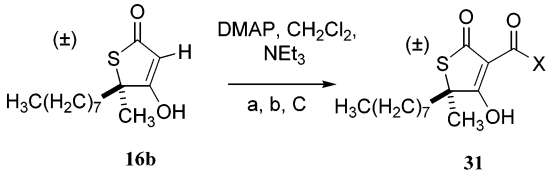


Nomura et al. reported an efficient protocol for the C-3 acylation of tetronic acids.[18] This method involves initial formation of the kinetic O-alkylated product in the presence of base and a anhydride. Then, catalytic DMAP-mediated transfer of the acyl group to the C-3 of the tetronic acid occurs over time to provide the C-alkylated thermodynamic product. Using these conditions **31a** (78%), **31b** (86%), and **31c** (59%, 79% based on recovered starting material, **16b**) were obtained from **16b** and the corresponding anhydrides or chloroformates in good yields (Scheme 12).

Biological Results and Discussion

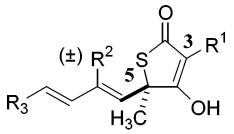
The biological activity of these thiolactomycin analogues was screened for (i) inhibition of fatty acid synthesis activity in whole cells, (ii) inhibition of purified human FAS (ZR-75-1 human breast cancer cells), (iii)

Scheme 12[a]



[a] Reagents and conditions: (a)$CH_3CO_2COCH_3$; (b) $CF_3CO_2COCF_3$; (c) $ClCO_2Me$.

Table 1. TLM Analogues with the C-5 1,3-Dienyl Unit



IC_{50} μg/mL

compd	R^1	R^2	R^3	MCF-7, ^{14}C[a]	ZR-75-1[b,c]	MCF-7, XTT[d]	wt loss (%)[e]
TLM	CH_3	CH_3	H	41.8	30	>80	2.6
{5*R*}-TLM	CH_3	CH_3	H	38.4	21.1	>80	
13a	H	CH_3	H	40.3	8.4	>80	7.8
13b	H	CH_3	CH_3	17.3	neg	>80	4.1
13c	H	H	CH_2CH_3	16.9	neg	>80	3.2
13d	H	H	$(CH_2)_3CH_3$	22.6	8.7	60	11.0

[a] Measure ^{14}C acetate incorporation into total lipids in MCF-7 breast cancer cells; average of duplicate runs. [b] Measure of FAS activity (overall reaction) by following oxidation of NADPH at 340 nm from purified ZR-5−1 breast cancer cells; neg = IC_{50} > 50 μg/mL. [c] Error = <±1. [d] Measure cytotoxicity using XTT assay (nonradioactive assay); average of duplicate runs. [e] Maximum amount of weight loss with one 60 mg/kg, ip dose (measured over 7 days).

cytotoxicity against cultured MCF-7 human breast cancer cells (possess high levels of FAS and FAS activity), and (iv) selected compounds were tested for weight loss in Balb/C mice. Table 1 displays the inhibition data of TLM analogues containing the isoprenyl unit at C-5. Despite previous reports that (+)-thiolactomycin is not active against type I rat FAS,[19] both (±) TLM (IC_{50} = 30 μg/mL) and (5*R*)-TLM (IC_{50} = 21 μg/mL) showed considerable activity against purified human FAS from breast cancer cells (Table 1). Significantly, **13a** (IC_{50} = 8.4 μg/mL) in which the C-3 methyl group of TLM is replaced with hydrogen was 3.4 times more potent than TLM against purified human FAS and relatively noncytotoxic (IC_{50} > 80 μg/mL). A similar potency/cytotoxicity profile was observed with the extended C-5 diene **13d** [IC_{50} = 8.7 μg/mL (ZR-75-1); IC_{50} = 60 μg/mL (MCF-7, XTT)]. Shorter-chain dienes (**13b**, **13c**) displayed lower FAS inhibition. These unsaturated analogues were also screened for weight loss in Balb/C mice. The compounds were diluted in DMSO at 10 mg/mL and the mice were injected intraperitoneally (ip) with 60 mg/kg in 100 μL of DMSO or with vehicle alone. The mice were observed and weighed daily, and the experiment was continued until treated animals reached their pretreatment weights. The most effective FAS inhibitors in this unsaturated class, **13a** and **13d** (both comparatively noncytotoxic) displayed considerable weight loss with one 60 mg/kg dose (**13a**, 7.8%; **13b**, 11.0%), in contrast to the less active analogues (TLM, **13b**, **13c**; IC_{50} > 20 μg/kg ZR-75-1), which displayed less weight loss (2−4%). These data demonstrate a second class of compounds derived from the TLM scaffold that are FAS inhibitors with potential antiobesity applications.

In the crystal structure of TLM/KAS I, the 1,3-diene of TLM resides between two peptide bonds (Gly391-Phe392 and Ala271-Pro272) and is possibly stabilized by π-stacking interactions (Figure 3).[9] Also, an edge-to-face interaction is apparent between the C-1′ alkene and Phe392.[9] To test the importance of π-stacking interactions in TLM binding, benzyl and C-5 alkyl derivatives (hexyl vs octyl) were prepared and their biological activity was profiled (Table 2).

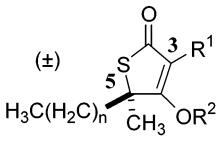
TLM analogue **16b** was also an effective inhibitor of purified FAS (IC_{50} = 4.0 μg/mL), suggesting that the 1,3-dienyl group is not essential for effective TLM binding. Surprisingly, in contrast to the compound series above, **16b** is relatively cytotoxic against MCF-7 cancer cells (IC_{50} = 17.6 μg/mL) and does not cause significant weight loss (2%, 60 mg/kg, ip). Replacement of the C-3 hydrogen in **16b** with a methyl group was quite detrimental to FAS inhibition **16c** (IC_{50} = 49.2 μg/mL), yet incorporation of an allyl branch at C-3 displayed FAS inhibition (**16d**, IC_{50} = 2.8 μg/mL). Perhaps the allyl substituent in **16d** is stabilized by π-stacking with the conserved aromatic residue (Phe229, *E. coli*; Tyr229, human/rat) present in the β-ketoacyl synthase. It has been suggested that Phe229 (*E. coli*) helps to promote decarboxylation of malonyl-ACP.[9]

Indeed, the hydrophobic pocket appears to be flexible and accommodates extended C-5 hydrocarbon units. C-5 Benzylated analogues (**26**, **27**), however, were not active against FAS, suggesting that aryl groups are not tolerated in this pocket.

Attempts to mimic the pantetheine arm and optimize interactions in its proposed binding pocket consisted of appending to the C-4 OH a range of structural elements (Figure 3). Several trends were recognized from the data in Table 2. First, substitution of the C-4 OH with various acetyl [i.e. acetal (**18b**); ethyl acetyl- (**17d**,**e**); *N*-acetyl derivatives of glycinate (**19**,**20**), 3-bromopropane (**21**), and *N*-allyl (**22**)] and alkyl groups (**17a**,**b**,**f**−**i**)] were inactive against purified FAS under the experimental conditions (The measurement of enzymatic activity was performed in 10 min. intervals; therefore, it is possible that these analogues may be slow-binding inhibitors as was found for C75). Yet, several of these derivatives conferred inhibition against whole-cell FAS. Unsaturated analogues **17h**,**i** and **22** were cytotoxic [**17h** (IC_{50} = 9.0 μg/mL); **17i** (IC_{50} = 14.5 μg/mL); and **22** (IC_{50} = 12.1 μg/mL)], but inactive against whole-cell and purified FAS. Propargyl analogue **17j** was slightly active against whole-cell FAS (IC_{50} = 21.9 μg/mL) and also very cytotoxic (IC_{50} = 8.9 μg/mL). The C-4 OH of TLM in the crystal structure is stabilized by hydrogen bonding with the carbonyl of Val270 and the amide of Gly305 through a network of water molecules. The inability for these acetyl derivatives to inhibit purified FAS [ZR-75-1 cells (Table 2)] suggests that this hydrogen bonding network is important for TLM binding and stabilization.

High activity against both whole-cell and purified FAS was achieved by directly attaching the carbonyl moiety to the C-4 OH to provide carbonates **23b**−**d** (IC_{50}

Table 2. Substitution at C3, C4-OR, and C5 of TLM



				IC$_{50}$, μg/mL			
compd	*n*	R^1	R^2	MCF-7 ^{14}C[a]	ZR-75-1[b]	MCF-7, XTT[c]	wt loss (%)[d]
C75	–	–	–	10.8	neg	10.8	11[e]
16a	5	H	H	16.5	neg	>80	
16b	7	H	H	12.6	4.0	17.6	2
16c	7	CH_3	H	16.5	49.2	48	0
16d	7	CH_2CHCH_2	H	34.8	2.8	44.4	
17a	5	H	CH_3	14.0	neg	9.4	
17b	7	H	CH_3	neg	neg	16.4	
17c/m	7	CH_3	CH_3	neg	neg	17.3	
17d	5	H	CH_2CO_2Et	14.2	neg	39.6	
17e	7	H	CH_2CO_2Et	10.8	neg	35.3	2.7
17f	5	H	$(CH_2)_4Cl$	8.6	neg	20.8	
17 g	7	H	$(CH_2)_4Cl$	neg	neg	35.3	
17h	5	H	CH_2CHCH_2	neg	neg	9.0	
17i	7	H	CH_2CHCH_2	neg	neg	14.5	
17j	5	H	CH_2CCH	21.9	neg	8.9	
17n	7	CH_3, CH_2CHCH_2	–	neg	neg	40.5	
17o/p	7	CH_3	CH_2CHCH_2	neg	neg	34.7	
17q	7	$(CH_2CHCH_2)_2$	–	neg	neg	40.5	8.0
18b	7	H	CH_2CO_2H	13.8	neg	50.3	
19	5	H	$CH_2CONHCH_2CO_2Me$	9.8	neg	40.5	
20	5	H	$CH_2CONHCH_2CO_2H$	6.6	neg	>80	3.5
21	5	H	$CH_2CONH(CH_2)_3Br$	6.7	neg	21	
22	7	H	$CH_2CONHCH_2CHCH_2$	neg	neg	12.1	2.4
23a	7	H	$COCH_2CH_3$	22.6	neg	26.8	
23b	7	H	CO_2CH_3	10.7	2.1	21.6	6.1
23c	7	H	$CO_2CH_2CH_3$	14.5	4.6	15.1	7.1
23d	7	H	$CO_2CH_2CHCH_2$	14.2	6.8	37.3	3.4
29	7	$C(OH)CH_3$	H	17.6	5.7	23.9	3.5
30	7	$C(OH)CH_3$	H	21.7	3.3	21.0	0.2
31a	7	$COCH_3$	H	12.0	neg	12.5	8.2
31b	7	$COCF_3$	H	14.7	41	18.4	
31c	7	CO_2CH_3	H	18.7	neg	47.2	
26	benzyl[f]	H	H	neg	neg	>80	1.6
27	benzyl[f]	H	CH_3	neg	neg	61.3	

[a] Measure ^{14}C acetate incorporation into total lipids in MCF-7 breast cancer cells: neg = IC$_{50}$ > 50 μg/mL; average of duplicate runs. [b] Measure of FAS activity (overall reaction) by following oxidation of NADPH at 340 nm from purified ZR-75−1 breast cancer cells; neg = IC$_{50}$ > 50 μg/mL, error = <±1. [c] Measure of cytotoxicity using XTT assay (nonradioactive assay); average of duplicate runs. [d] Maximum amount of weight loss with one 60 mg/kg ip dose (measured over 7 days). [e] 20 mg/kg ip dose. [f] C-5 = benzylated.

= 2.1, 4.6, and 6.8 μg/mL). Furthermore, linking **16b** with a carbonyl moiety at the C-4 OH provided derivatives that also caused significant weight loss (**23b** (6.1%); **23c** (7.1%); **23d** (3.4%). We anticipated that this slight modification at the C-4 OH would place the carbonate moiety closely aligned with first *N*-acetylpantetheinyl amide of malonyl-CoA in our structural model (Figure 2), and that these analogues would be more structurally similar to C75. Indeed the FAS inhibition, cytotoxicity and weight loss observed by the carbonates (**23b**−**d**) supports this rationale. These data suggest that appending the TLM skeleton to become more of a malonyl-CoA mimetic influences the proportion of the observed weight loss. Finally, the loss of FAS inhibition by propyl analogue **23a** suggests that the presence of the carbonate carbonyl alone does not confer activity.

We anticipated that incorporation of hydrogen bonding donors/acceptors at C-3 could interact with the active site histidine and/or either of the two threonines (Figure 4). Recently, a detailed kinetic analysis of the β-ketoacyl synthase domain of multifunctional fatty acid synthase provides an alternative mechanism to those previously reported.[20] Smith and co-workers present evidence for an activated water molecule in the active site that either protonates the malonyl carboxylate, prior to its release as bicarbonate, or attacks the malonyl carboxylate to provide a negatively charged tetrahedral transition state which is stabilized by His 293 (His 298 in *E. coli*).[20] Then, bicarbonate rather than CO_2 is released directly upon breakdown of the tetrahedral transition state to provide the acetyl enolate.[20] Thus, the active site of β-ketoacyl synthase has to stablize the formation of three tetrahedral transition states: (i) the transfer of the acyl-primer to the active site cysteine reside; (ii) the release of bicarbonate by attack of water; (iii) the condensation of the acetyl-ACP enolate and the extended acyl chain. Since thiolactomycin acts as a thiomalonate mimetic, analogues containing C-3 acetyl or alcohol units could be stabilized by the active site histidines or the residues that reside in the oxyanion holes. Alcohol derivatives **29** and **30** demonstrated good activity against whole-cell (**29**, IC$_{50}$ = 17.6 μg/mL; **30**, IC$_{50}$ = 21.7 μg/mL) and purified FAS (**29**, IC$_{50}$ = 5.7 μg/mL; **30**, IC$_{50}$ = 3.3 μg/mL). Trifluoroacetyl analogue **31b** displayed modest purified FAS

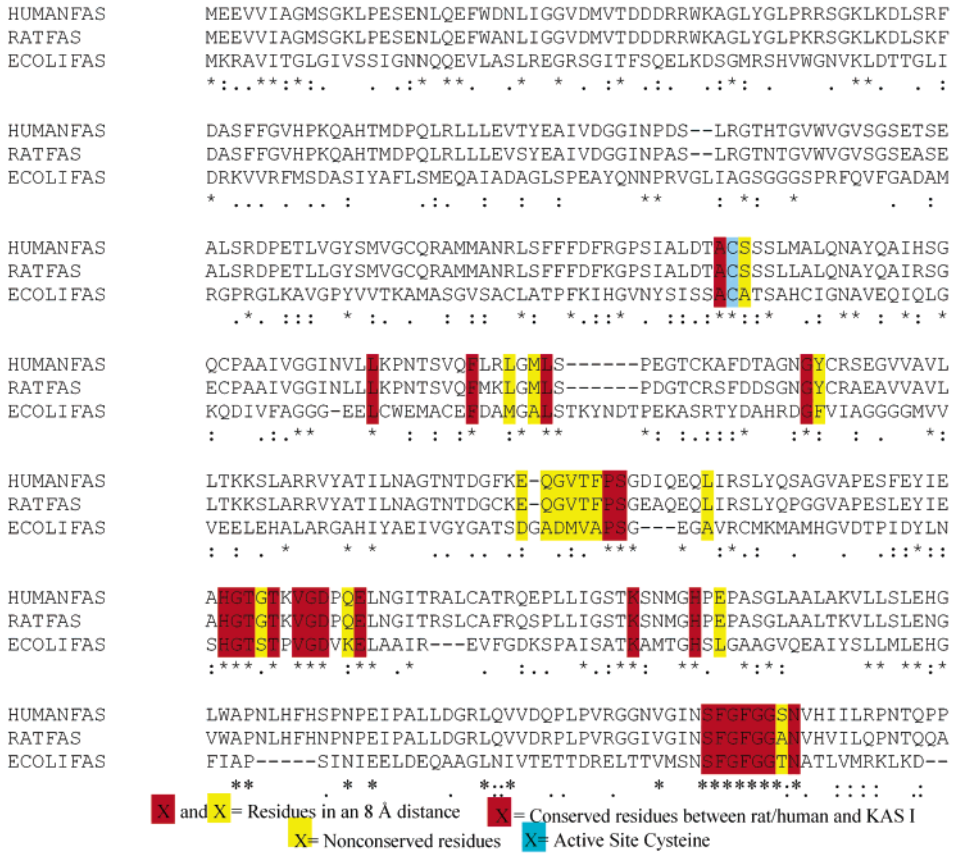


Figure 5. CLUSTAL W multiple sequence alignment of the β-keto acyl synthase of human/rat FAS with KAS I.

inhibition ($IC_{50} = 41$ μg/mL). All five C-3 analogues (**29**–**31c**, $IC_{50} = 14-21.7$ μg/mL) were effective against whole-cell FAS. Also, consistent with the correlation of malonyl-CoA mimetics displaying weight loss, the most active derivative, **31a**, caused significant weight loss (8.2%). Indeed, **31a** would most likely be the best hydrogen bond acceptor among the C-3 carbonyl-bearing substituents and therefore be more stabilized by the active site histidines or threonines present in the vicinity.

These structure–activity studies identify a fine line between properties that molecules must possess to display antiobesity vs anticancer activity. As shown with C75, inhibition of fatty acid synthase in cancer cells can be a route to develop new anticancer agents.[1,2] Researchers have proposed that cancer cells overexpress FAS to maintain redox balance to compensate for the hypoxic conditions that malignant cellular phenotypes exhibit.[21] High oxidative stress is counterbalanced by the continuous supply of $NAD^+/NADP^+$ furnished by dramatic upregulation of FAS.[21] One can imagine that disrupting this pathway using FAS inhibitors will destablize the redox balance of cancer cells causing apoptosis and cell death. We have successfully developed a TLM subclass of type I FAS inhibitors that displays anticancer activity with little or no weight loss. Furthermore, we have identified a second subclass that also inhibits FAS, but is minimally cytotoxic and causes substantial weight loss (>5.0%, 60 mg/kg). It appears that fine-tuning the TLM skeleton to more closely resemble malonyl-CoA is the determinant of the observed weight loss in several derivatives including **13a**, **13d**, **23b**–**d**, and **31a**. Questions still remain whether and to what extent these analogues cause weight loss through both peripheral and central mechanisms as C75 does. These studies and their effects against CPT-1 and NPY are currently underway.

Structural Modeling of TLM Analogues. Several compounds described in Tables 1 and 2 were effective inhibitors of type I human FAS. TLM analogue **13a**, which is virtually identical to TLM with the exception that the C-3 methyl group is replaced with a hydrogen, is a more potent ($IC_{50} = 8.8$ mg/mL) inhibitor of human cancer FAS than (±) TLM ($IC_{50} = 30$ μg/mL). Also, the C-5 saturated version **16b** is 11 times more potent than **16c** containing a C-3 methyl group. These data suggest that the presence of a C-3 methyl group in TLM analogues hinders activity against type I systems and perhaps is the principal determinant making naturally occurring thiolactomycin selective for bacterial type II FAS systems. In an attempt to understand this difference in observed activity between the methyl-containing natural product (TLM) and unmethylated **13a** and **16b**, we have carried out modeling experiments using the crystal structure of thiolactomycin bound in KAS I of *E. coli*. The crystal structure of KAS I contains four identical, independent subunits in one asymmetric unit, which pair around two, 2-fold axes to form a pair of dimers.[22] Each subunit contains 406 amino acids which fold into an α–β–α domain which, upon dimer formation, gives an α–β–α–β–α structure.[22]

A BLASTP pair alignment[23] was carried out with KAS I and the β-ketoacyl synthase regions of human and rat FAS. Both rat and human FAS are 26% identical and 42% and 43% similar to KAS I, respectively. The InsightII molecular modeling program was

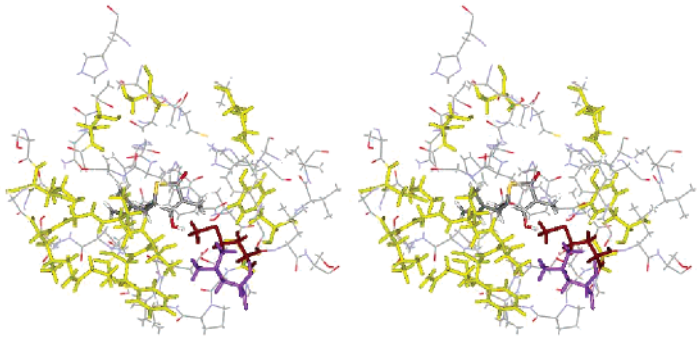


Figure 6. TLM−human/rat chimera.

used to identify residues within an 8 Å diameter of the bound ligand in KAS I. Forty residues were identified in this region. A multiple CLUSTALW alignment[24] was carried out to compare the identity/similarity of these 40 residues in KASI with the β-ketoacyl synthase regions of rat and human FAS. Twenty-five of the 40 are identical between human/rat FAS and KAS I (Figure 5). Using InsightII, the other 15 nonidentical residues were mutated in one subunit of the KAS I structure to correspond to those residues in human/rat FAS. This human/rat chimera containing TLM was minimized using Discover. Careful inspection of the minimized chimeric structure showed that TLM maintains the important hydrogen bonding interactions that are present in the KAS I crystal structure. The Phe229Tyr and Leu335Glu mutations in human/rat incorporate a hydrogen-bonding donor/acceptor pair. Rock and co-workers propose that the presence of the aromatic moiety (Phe229, KAS I) promotes decarboxylation of the malonyl-ACP through electronic repulsion between the π-electrons of the aromatic ring and the incoming carboxylate of malonyl-ACP.[9]

Several of the mutations are clustered in the hydrophobic binding pocket (Figure 6). It has been shown that slight modifications in this pocket can confer TLM resistance.[25] Recently, six independent TLM-resistant clones of strain ANS1 were isolated and all expressed a mutant protein, FabB(F390V). Structural modeling of this mutant predicted that the valine side chain interfered with positioning of the C-2′ methyl group on the diene of TLM. Our biological data suggest that the changes in the hydrophobic pocket between the human and bacterial β-ketoacyl synthase do not effect binding of the isoprenoid-like side chain since **13a** is still active. Yet, saturated alkyl analogue, **16b** (without the C-2′-methyl) is slightly more active than **13a.** A more probable explanation for the enhanced activity of **13a** and **16b** compared to TLM and **16c** is a steric effect involving the C-3 methyl group. The minimized structure of the TLM-chimera reveals two mutations that are in close proximity to the C3 methyl and can possibly impede TLM binding (Figure 6).

Ala206Met is located between the C-3 methyl of TLM and Met204Leu. Rotation of the Cγ-S-methyl group of Met206 is restricted due to unfavorable interactions with the C-3 methyl of TLM and the isobutyl group of Leu204. Therefore, it is possible that this added steric congestion in the human protein can interfere with TLM binding. Thus, substitution of the C-3 methyl group in TLM for a smaller and less sterically demanding hydrogen atom enhanced the binding affinity of these analogues and conferred them with inhibitory activity against type I FAS systems as was observed in Tables 1 and 2.

Conclusions

In summary, we have demonstrated the flexibility of our previously described total synthesis to prepare TLM analogues. The γ-thiolactone skeleton was modified at C-5, C-3, and C-4 OH to provide structural analogues that both encompass and successfully partition weight loss and cytotoxic activities. Several of these derivatives including **13a**, **13d**, carbonates (**23b**−**d**), and C-4 enol analogues (**16b**, **29**, **30**) are potent inhibitors of human FAS ($IC_{50} \leq 20$ μg/mL). **13a** and **13d** are nontoxic to cancer cells ($IC_{50} > 50$ mg/mL) and cause considerable weight loss with one 60 mg/kg ip dose in BalbC mice (>5%). **23b**−**d** and **31a** display biological activity similar to C75. Both weight loss (>5%) and cytotoxicity ($IC_{50} <$ 40 mg/kg) toward cancer cells are observed. Other TLM compounds including **16b**, **29**, **30** show effective anticancer activity ($IC_{50} <$ 25 mg/kg) but do not cause weight loss. Indeed, a direct correlation between FAS inhibition and cell death in cancer cells overexpressing FAS has been shown by us and others.[1,2] Evidence suggests that FAS inhibition and partition into the hypothalamus and brain in conjunction with a second or possibly several metabolic pathways (i.e. NPY or CPT1 or an unknown route) lead to the weight loss.[3] Current investigations into distinguishing between these outcomes and developing molecules that exert selective activity for each are underway. As an important first step we have shown that the γ-thiolactone skeleton can be used as a template to develop agents to treat obesity and diabetes-related diseases, or new therapeutics to treat cancer. Furthermore, we have also demonstrated how one can synthetically evolve a type II FAS inhibitor (TLM) into an effective inhibitor of the higher order type I multifunctional FAS systems.

Experimental Section

Biological Methods. The inhibition of fatty acid synthesis activity in whole cells;[26a−c] inhibition of purified FAS (ZR-75-1, human breast cancer cells);[26d,e] cytotoxicity against cultured MCF-7 human breast cancer cells;[26f] and weight loss in Balb/C mice[3b] were assayed as described previously.

Inhibition of FAS from ZR-75-1 Breast Cancer Cells. Using a 96-well plate and adapting a procedure from Dils et al.[26d] and Arslanian et al.,[26e] FAS activity (the overall reaction) was determined with a Molecular Devices Spectramax Plus spectrophotometer at 37 °C by following the malonyl CoA dependent oxidation of NADPH at 340 nm (at 10 s intervals for 5 min). The reaction mixture contained 100 mM potassium

phosphate buffer, pH 6.5, acetyl-CoA (61.8 uM), malonyl-CoA (67.4 uM), NADPH (187.5 uM), DTT (1 mM), and 2 μg of FAS (purified from ZR-75-1 cells[26c]) in a total volume 100 uL.

Structural Modeling. All molecular modeling was performed on an SGI Octane 2XR10000 work station. The β-ketoacyl synthase (KAS I) from *E. coli* was downloaded from the PDB data bank (http://www.rcsb.org/pdb/; PDB ID: 1FJ4). An 8 Å diameter subset around the TLM ligand containing 40 amino acid residues was identitified using InsightII 1997. The 15 nonidentical residues were mutated using InsightII 1997 Builder to correspond to those residues in the β-ketoacyl synthase regions of human and rat FAS. This human/rat chimera was energy minimized with Insight 1997 Discover. Initially the CVFF force field, Steepest Descent algorithm for 1000 iterations was used to minimize large steric interactions. Then the chimera was energy minimized using the CVFF force field, conjugate gradients until the maximum RMS derivative was less than 0.001 kcal/Å. This final structure was compared with the energy minimized wildtype β-ketoacyl synthase following the same procedure as above.

General Methods. Air- and moisture-sensitive reactions were run under inert atmosphere (Ar or N_2) using flame-dried glassware. Diisopropylamine, CH_2Cl_2, and triethylamine were distilled from CaH_2. THF was distilled from sodium benzophenone ketyl. *n*-Butyllithium in hexanes (nominally 1.6 M) was purchased from Aldrich and titrated[27] before use. All other solvents were used as received or dried by standard procedures.[28] Elemental analyses were carried out by Atlantic Microlab, Inc. (Norcross, GA). Low- and high-resolution data were obtained on a VG Instruments 70-S GC/MS at 70 eV and are tabulated as *m/z* (intensity expressed as percent of base peak) or obtained from the Lab for Mass Spectrometry, Ohio State University. ^{1}H and ^{13}C NMR were acquired on a Bruker AMX 300 MHz or a Varian Unity^{plus} 400 MHz spectrometer. Chemical shifts are reported relative to residual $CHCl_3$ (7.25 ppm, ^{1}H; 77.0 ppm ^{13}C). IR spectra were obtained on a Bruker Vector 22 spectrophotometer. Melting points were determined in a Thomas-Hoover melting point apparatus and are uncorrected. Purity for new target compounds was established by ^{1}H NMR and/or elemental analyses.

General Procedure A. (±)-2-(*tert*-Butyl)-5-(1-hydroxy-2-methyl-2-pentenyl)-5-methyl-1,3-oxathiolan-4-one (10b). To a mixture of diisopropylamine (0.6 mL, 4.6 mmol) in THF (8.0 mL) at −78 °C was added *n*-BuLi (3.69 mL, 1.2 M in *n*-hexane), and the resulting solution was stirred for 30 min at 0 °C and then cooled to −78 °C. Then (±)-**9**[12] (800 mg, 4.6 mmol) in THF at −78 °C was added dropwise by cannula and the resulting solution stirred for 30 min at −78 °C. *trans*-2-Methyl-2 pentenal (0.58 mL, 5.1 mmol) in THF (1.4 mL), at −78 °C was then added via cannula. After stirring at −78 °C for 1.5 h, 1 N HCl (25 mL) was added and the solution was extracted with Et_2O (3 × 30 mL). The combined organics were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (10% EtOAc/hexanes) gave **10b** (884 mg, 71%) as a 1.8:1 mixture of diastereomers at the C1′. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93−0.99 (m, 12 H), 1.40 (s, 3 H), 1.68 (s, 3 H), 2.01−2.06 (m 2 H), 4.33 (d, J = 6.9 Hz, 1 H), 5.24 (s, 1 H), 5.48−5.54 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.6, 13.8, 20.9, 21.1, 24.8, 35.4, 60.6, 81.8, 87.9, 132.6, 133.9, 178.3. IR (NaCl) 2961, 1767 cm^{-1}. Anal. ($C_{14}H_{24}O_3S$) C, H.

(±)-2-(*tert*-Butyl)-5-(1-hydroxy-2-hexenyl)-5-methyl-1,3-oxathiolan-4-one (10c). From (±)-**9** (800 mg, 4.59 mmol) and 2-*trans*-hexenal (0.56 mL, 4.8 mmol) following general procedure, **10c** (1.3 g, 88%) was obtained after flash chromatography (10% EtOAc/hexanes) as a 2.4:1 mixture of diastereomers at C1′. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 7.3 Hz, 3 H), 0.99 (s, 9 H), 1.38−1.45 (m, 2 H), 1.41 (s, 3 H), 2.02 (q, J = 6.5 Hz, 2 H), 4.26−4.31 (m, 1 H), 5.27 (s, 1 H), 5.45−5.63 (m, 1 H), 5.74−5.83 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.6, 21.6, 24.1, 24.9, 35.2, 37.2, 61.2, 78.5, 87.9,127.3, 137.3, 179.1. IR (NaCl) 2960 1765 cm^{-1}. Anal. ($C_{14}H_{24}O_3S$), C, H.

(±)-2-(*tert*-Butyl)-5-(1-hydroxy-2-octenyl)-5-methyl-1,3-oxathiolan-4-one (10d). From (±)-**9** (800 mg, 4.59) and 2-*trans*-octenal (0.75 mL, 5.0 mmol) following general procedure A, **10d** was obtained (1.1 g, 81%) after flash chromatography (10% EtOAc/hexanes) as a 1.2:1 mixture of diastereomers at C1′. ^{1}H NMR (300 MHz, $CDCl_3$) major diastereomer δ 0.85 (t, J = 7.2 Hz, 3 H), 0.97 (bs, 9 H), 1.18−1.35 (m, 6 H), 1.56 (s, 3 H), 2.00−2.08 (m, 2 H), 2.38 (d, J = 5.0 Hz, 1 H), 4.15−4.19 (m, 1 H), 5.13 (s, 1 H), 5.45−5.59 (dd, J = 7.7, 15.3 Hz, 1 H), 5.72−5.77 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 22.3, 24.7, 28.5, 31.3, 32.1, 35.2, 60.6, 78.8, 87.4, 127.2, 136.5, 175.7. ^{1}H NMR (300 MHz, $CDCl_3$) minor diastereomer ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 7.2 Hz, 3 H), 0.97 (s, 9 H), 1.18−1.35 (m, 6 H), 1.40 (s, 3 H), 2.00−2.07 (m, 2 H), 2.31 (d, J = 5 Hz, 1 H), 4.25−4.30 (m, 1 H), 5.27 (s, 1 H), 5.45−5.59 (dd, J = 7.7, 15.3 Hz, 1 H), 5.79−5.83 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 22.3, 23.9, 24.8, 28.5, 31.2, 32.1, 35.3, 61.1, 78.3, 87.8, 127.2, 137.2, 177.0. IR (NaCl) 2959, 1765 cm^{-1}. Anal. ($C_{16}H_{28}O_3S$) C, H.

General Procedure B. (±)-2-(*tert*-Butyl)-5-(2-methyl-penta-1,3-dienyl)-5-methyl-1,3-oxathiolan-4-one (11b). To a solution of **10b** (500 mg, 1.84 mmol) in $Cl(CH_2)_2Cl$ (17 mL) cooled to 0 °C were added NEt_3 (0.6 mL, 4.4 mmol) and 2,4-dinitrobenzenesulfenyl chloride (969 mg, 4.1 mmol). The solution was warmed to room temperature for 30 min or until TLC indicated complete formation of the diastereomeric sulfenate esters. The mixture was then refluxed at 90 °C for 4 h or until complete conversion of the sulfenate ester was indicated by TLC. After cooling to 0 °C, pentane (50 mL) was added and this mixture was filtered through Celite and evaporated. Flash chromatography (2% EtOAc/hexanes) gave pure **11b** (342 mg, 73%, 14:1 trans:cis at C1′). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (s, 9 H), 1.70 (s, 3 H), 1.75 (d, J = 6.6 Hz, 3 H), 1.85 (s, 3 H), 5.18 (s, 1 H), 5.57 (s, 1 H), 5.75 (dq, J = 6.6, 15.5 Hz, 1 H), 5.97 (d, J = 15.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) d 13.0, 18.0, 25.2, 27.4, 34.8, 53.8, 87.4, 125.4, 129.3, 135.5, 137.8, 176.3. IR (NaCl) 2961, 1770 cm^{-1}. HRMS (EI) *m/z* calculated for $C_{14}H_{22}O_2S$ (M^+) 254.1341, obsd 254.1309.

(±)-2-(*tert*-Butyl)-5-(hexa-1,3-dienyl)-5-methyl-1,3-oxathiolan-4-one (11c). From (±)-**10c** (690 mg, 2.53 mmol) following general procedure B, **11c** (461 mg, 72%, 4:1 trans:cis at C-1′) was obtained after flash chromatography (2% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95−1.01 (m, 12 H), 1.61 (s, 3 H), 2.07−2.12 (m, 2 H), 5.05 (s, 1 H), 5.58 (d, J = 15.2 Hz, 1 H), 5.81 (dt, J = 6.0, 15.2 Hz, 1 H), 6.00−6.05 (m, 1 H), 6.15−6.24 (dd, J = 10.0, 15.2 Hz, 1 H); ^{13}C (75 MHz, $CDCl_3$) δ 13.3, 24.8, 25.3, 25.7, 34.5, 56.1, 87.2, 127.4, 129.4, 130.0, 138.9, 175.1. IR (NaCl) 2966, 1771 cm^{-1}. HRMS (ES) *m/z* calculated for $C_{14}H_{22}O_2SNa^+$ ($M + Na^+$) 277.1232, obsd 277.1237.

(±)-2-(*tert*-Butyl)-5-(octa-1,3-dienyl)-5-methyl-1,3-oxathiolan-4-one (11d). From (±)-**10d** (306 mg, 1.00 mmol) following general procedure B, **11d** (212 mg, 75%, 4:1 trans:cis at C-1′) was obtained after flash chromatography (2% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) trans isomer δ 0.84−0.89 (m, 3 H), 1.01 (s, 9 H), 1.22−1.38 (m, 4 H), 1.61 (s, 3 H), 2.04−2.11 (m, 2 H), 5.03 (s, 1 H), 5.58 (d, J = 15.1 Hz, 1 H), 5.64−5.78 (m, 1 H), 0.96−6.05 (m, 1 H), 6.19 (dd, J = 10.1, 15.1 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) trans isomer δ 13.6, 22.0, 22.5, 25.2, 31.2, 32.1, 34.6, 55.9, 87.0, 128.5, 129.6, 130.2, 137.2, 174.7. IR (NaCl) 2959, 1772 cm^{-1}; HRMS (EI) *m/z* calculated for $C_{16}H_{26}O_2S$ (M^+) 282.1653, obsd 282.1681.

General Procedure C. (±)-2-Thioacetyl-2,4-dimethyl-hexa-3,5-dienoic Acid Ethyl Ester (12a). Cesium carbonate (609 mg, 1.6 mmol) was added directly to a solution of **11a** (380 mg, 1.6 mmol) in EtOH (6.0 mL). After 20 min this mixture was poured into NH_4Cl(sat.)/1 N HCl (15 mL, 3:1) and extracted with Et_2O (3 × 20 mL) and then water (3 × 20 mL). The combined organics were dried ($MgSO_4$), filtered, evaporated, and redissolved in CH_2Cl_2 (12 mL). To this precooled solution at 0 °C were added NEt_3 (0.22 mL, 1.0 mmol) and acetyl chloride (0.11 mL, 1.6 mmol). After 40 min NH_4Cl (sat) (20 mL) was added, and this mixture was extracted with CH_2Cl_2 (3 × 15 mL). The combined organics were dried ($MgSO_4$), filtered, and evaporated. Flash chromatograpy (5% EtOAc/hex) gave pure **12a** (230 mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, J = 7.1 Hz, 3 H), 1.84 (s, 3 H), 1.87 (s, 3 H), 2.24 (s, 3

H), 4.21 (q, J = 7.1 Hz, 2 H), 5.03 (d, J = 10.6 Hz, 1 H), 5.21 (d, J = 17.3 Hz, 1 H), 5.74 (s, 1 H), 6.26−6.35 (dd, J = 10.6, 17.3 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.9, 13.9, 25.9, 30.1, 55.8, 62.0, 113.3, 131.3, 138.3, 141.3, 182.3, 194.6. IR (NaCl) 2982, 1735, 1692 cm^{-1}.

(±)-2-Thioacetyl-2,4-dimethyl-hepta-3,5-dienoic Acid Ethyl Ester (12b). From **11b** (369 mg, 1.5 mmol) and acetyl chloride (0.10 mL, 1.5 mmol) following general procedure C gave **12b** (271 mg, 77%) after flash chromatography (5% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (t, J = 7.1 Hz, 3 H), 1.74 (d, J = 6.6 Hz, 3 H), 1.81 (s, 3 H), 1.85 (s, 3 H), 2.25 (s, 3 H), 4.17 (q, J = 7.1 Hz, 2 H), 5.56 (s, 1 H), 5.65−5.73 (dq, J = 6.6, 15.5 Hz, 1 H), 5.99 (d, J = 15.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 14.1, 18.2, 26.2, 30.5, 55.6, 62.0, 125.2, 128.3, 135.7, 138.5, 172.2, 194.8. IR (NaCl) 2926, 1737, 1694 cm^{-1}; HRMS (EI) *m/z* calculated for $C_{13}H_{20}O_3S$ (M^+) 256.1133 obsd 256.1118.

(±)-2-Thioacetyl-2-methyl-octa-3,5-dienoic Acid Ethyl Ester (12c). From **11c** (567 mg, 2.2 mmol) and acetyl chloride (174 μL, 2.5 mmol) following general procedure C gave **12c** (374 g, 63%) after flash chromatography (5% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, J = 7.3 Hz, 3 H), 1.24 (t, J = 7.1 Hz, 3 H), 1.72 (s, 3 H), 2.03−2.17 (m, 2 H), 2.25 (s, 3 H), 4.17 (q, J = 7.1 Hz, 2 H), 5.72−5.81 (m, 2 H), 5.95−6.04 (dd, J = 10.2, 15.3 Hz, 1 H), 6.18−6.27 (dd, J = 10.2, 15.3 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.2, 13.9, 22.8, 25.6, 30.2, 56.1, 61.9, 128.2, 128.4, 132.1, 138.5, 171.6, 194.8. IR (NaCl) 2929, 1736, 1693 cm^{-1}; HRMS (ES) *m/z* calculated for $C_{13}H_{20}O_3SNa^+$ ($M + Na^+$) 279.1025 obsd 279.1032.

(±)-2-Thioacetyl-2-methyl-deca-3,5-dienoic Acid Ethyl Ester (12d). From **11d** (200 mg, 0.71 mmol) and acetyl chloride (55μL, 0.78 mmol) following general procedure C gave **12d** (119 g 59%) after flash chromatography (5% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84−0.89 (m, 3 H), 1.23 (t, J = 7.1 Hz, 3 H), 1.28−1.38 (m, 4 H), 1.71 (s, 3 H), 2.01−2.08 (m, 2 H), 2.23 (s, 3 H), 4.18 (q, J = 7.1 Hz, 2 H), 5.66−5.76 (m, 2 H), 5.89−6.03 (m, 1 H), 6.20 (dd, J = 10.3, 15.3 Hz, 1 H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 13.9, 22.2, 22.8, 29.9, 31.2, 32.3, 56.1, 61.9, 128.4, 129.2, 132.2, 137.1, 171.6, 194.6. IR (NaCl) 2930, 1737, 1694 cm^{-1}. HRMS (ES) *m/z* calculated for $C_{15}H_{24}O_3SNa^+$ ($M + Na^+$) 307.1338 obsd 307.1339.

General Procedure D. (±)-4-Hydroxy-5-methyl-5-(2-methyl-buta-1,3-dienyl)-5*H*-thiophen-2-one (13a). To **12a** (263 mg, 1.09 mmol) in THF (16.4 mL) at −78 °C was added LiHMDS (1.8 mL, 1.8 mmol, 1.0 M in THF), and the solution was allowed to slowly warm to −5 °C. The solution was then poured into 1 N HCl (25 mL) and extracted with EtOAc (3 × 15 mL). The combined organics were dried ($MgSO_4$), filtered, and evaporated. This crude mixture was taken up in $NaHCO_3$ (sat, 15 mL) and extracted with Et_2O (3 × 10 mL). The aqueous layer was then acidified to pH 3 (pH paper) with 1 N HCl and extracted with Et_2O (3 × 10 mL) and EtOAc (2 × 10 mL). The combined organics were dried ($MgSO_4$), filtered, and evaporated to provide pure **13a** (114 mg, 53%). ^{1}H NMR (300 MHz, $CDCl_3$) (keto tautomer) δ 1.78 (s, 3 H), 1.86 (s, 3 H), 3.43 (d, J = 5.6 Hz, 2 H), 5.12 (d, J = 10.6 Hz, 1 H), 5.27 (d, J = 17.3 Hz, 1 H), 5.83 (s, 1 H), 6.27−6.37 (dd, J = 10.6, 17.3 Hz, 1 H). ^{1}H NMR (300 MHz, MeOD) (enol tautomer) δ 1.79 (s, 3 H), 1.84 (s, 3 H), 5.04 (d, J = 10.7 Hz, 1 H), 5.25 (d, J = 17.3 Hz, 1 H), 5.66 (s, 1 H), 6.36 (dd, J = 10.7, 17.3 Hz, 1 H); ^{13}C NMR (75 MHz, MeOD) δ 12.6, 30.4, 59.0, 102 (m), 116.9, 131.4, 140.6, 142.3, 189.9, 197.3. HRMS (EI) *m/z* calculated for $C_{10}H_{12}O_2S^+$ (M^+) 196.0552 obsd 196.0552. Anal. Calcd for $C_{10}H_{12}O_2S$: C, 61.2; H, 6.16; Found: C, 59.5; H, 6.26.

(±)-4-Hydroxy-5-methyl-5 (2-methyl-penta-1,3-dienyl)-5*H*-thiophen-2-one (13b). From **12b** (226 mg, 0.9 mmol) following general procedure D, **13b** (95 mg, 49%) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) (keto-tautomer) δ 1.75 (s, 3 H), 1.77 (d, J = 3.2 Hz, 3 H), 1.84 (s, 3 H), 3.42 (d, J = 1.5 Hz, 2 H), 5.66 (bs, 1 H), 5.78 (m, 1 H), 6.04 (d, J = 15.4 Hz, 1 H); ^{1}H NMR (300 MHz, MeOD) (enol tautomer) δ 1.80−1.85 (m, 6 H), 1.90 (s, 3 H), 5.59 (s, 1 H), 5.80−5.95 (dq, J = 6.6, 15.5 Hz, 1 H), 6.17 (d, J = 14.9 Hz, 1 H); ^{13}C NMR (75 MHz, MeOD) (enol tautomer) δ 13.4, 18.4, 30.7, 59.2, 101.2 (m) 126.2, 128.4, 136.9, 140.6, 190.2, 197.6. IR (NaCl) 2929, 1593 cm^{-1}; Found: C, 61.8; H, 6.83. HRMS (ES) *m/z* calculated for $C_{11}H_{14}O_2SNa^+$ ($M + Na^+$) 233.0607 obsd 233.0597; Anal. Calcd for $C_{11}H_{14}O_2S$: C, 62.8; H, 6.71.

(±)-4-Hydroxy-5-methyl-hexa-1,3-dienyl-5*H*-thiophen-2-one (13c). From **12c** (364 mg, 0.46 mmol) following general procedure D, **13c** was obtained (180 mg, 60%). ^{1}H (300 MHz, $CDCl_3$, exists as a mixture 2.3:1 of the keto:enol tautomer) keto tautomer: δ 1.00 (t, J = 7.4 Hz, 3 H); 1.76 (s, 3 H); 2.09−2.16 (m, 2 H); 3.21 (d, J = 21.1 Hz, 1 H); 3.52 (d, J = 21.1 Hz, 1 H); 5.70 (d, J = 15.1 Hz, 1 H); 5.86 (dt, J = 15.2, 6.4 Hz, 1 H), 6.02 (dd, J = 10.2, 15.1 Hz, 1 H), 6.38 (dd, J = 15.1, 10.1 Hz, 1 H); ^{1}H NMR (300 MHz, MeOD) enol tautomer δ 1.09 (t, J = 7.4 Hz, 3 H), 1.87 (s, 3 H), 2.14−2.29 (m, 2 H), 5.78 (d, J = 15.1 Hz, 1 H), 5.87 (dt, J = 15.2, 6.5 Hz, 1 H), 6.09−6.18 (dd, J = 10.2, 15.1, 1 H), 6.38 (dd, J = 10.2, 15.2 Hz, 1 H); ^{13}C NMR (75 MHz, MeOD) enol tautomer δ 14.1, 25.2, 26.9, 61.0, 101 (m), 129.7, 131.7, 132.7, 138.9, 188.9, 197.1. IR (NaCl) 2965, 1592 cm^{-1}; Found: C, 62.0; H, 6.94. HRMS (ES) *m/z* calculated for $C_{11}H_{14}O_2SNa^+$ ($M + Na^+$) 233.0607, obsd 233.0626. Anal. Calcd for $C_{11}H_{14}O_2S$: C, 62.8; H, 6.71; Found: C, 62.0; H, 6.94.

(±)-4-Hydroxy-5-methyl-5-octa-1,3-dienyl-5*H*-thiophen-2-one (13d). From **12d** (62 mg, 0.22 mmol) following general procedure D, **13d** was obtained (21 mg, 41%). ^{1}H NMR (300 MHz, $CDCl_3$) (keto tautomer) δ 0.88 (t, J = 6.9 Hz, 3 H), 1.19−1.41 (m, 4 H), 1.75 (s, 3 H), 2.03−2.19 (m, 2 H), 3.22 (d, J = 21.1 Hz, 1 H), 3.51 (d, J = 21.1 Hz, 1 H), 5.67 (d, J = 15.1 Hz, 1 H), 5.80 (dt, J = 6.9, 14.5 Hz, 1 H), 6.02 (dd, J = 10.2, 15.1 Hz, 1 H), 6.37 (dd, J = 10.2, 15.1 Hz, 1 H). ^{1}H NMR (300 MHz, MeOD) enol tautomer δ 0.97−1.03 (m, 3 H), 1.36−1.53 (m, 4 H), 1.87 (s, 3 H), 2.15−2.22 (m, 2 H), 5.78 (d, J = 15.4 Hz, 1 H), 5.82−5.90 (m, 1 H), 6.10−6.19 (m, 1 H), 6.38 (dd, J = 10.3, 15.4 Hz, 1 H); ^{13}C (75 MHz, MeOD) enol tautomer δ 14.4, 23.3, 25.2, 32.6, 33.4, 60.9, 102.1 (m), 130.7, 131.7, 132,7, 137.5, 188.9, 196.9. IR (NaCl) 2927, 1588 cm^{-1}; HRMS (ES) calculated for $C_{13}H_{18}O_2SNa^+$ ($M + Na^+$) 261.0911; obsd 261.0912; Anal. Calcd for $C_{13}H_{18}O_2S$: C, 65.5; H, 7.61; Found: C, 64.7; H, 7.68.

(±)-5-Benzyl-4-hydroxy-5-methyl-5*H*-thiophen-2-one (26). From **25** (1.4 gm, 5.0 mmol) following general procedure D, **26** (500 mg, 45%) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.71 (s, 3 H), 2.89 (ab q, J = 21.7 Hz, 2 H), 3.17 (ab q, J = 13.6 Hz, 2 H), 7.26 (m, 5 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 46.6, 48.5, 67.9, 127.7, 128.6, 130.6, 134.9, 195.3, 207.3. HRMS (ES) calculated for $C_{12}H_{12}O_2SNa^+$ ($M + Na^+$) 243.0450; obsd 243.0433.

General Procedure E. (±)-2-*tert*-Butyl-5-methyl-5-hexyl-[1,3]-oxathiolan-4-one (14a). To a mixture of LiHMDS (6.2 mL, 6.20 mmol, 1 M in THF) in THF (9.7 mL) at −78 °C was added (±)-**9** (1.00 g, 5.75 mmol) in THF (9.60 mL) by cannula dropwise, and the resulting solution was stirred for 30 min at −78 °C. Then, hexyl triflate (1.1 g, 4.7 mmol) in THF (4 mL) at −78 °C was added via cannula. After stirring at −78 °C for 2 h, 1 N HCl (10 mL) was added and the solution was extracted with Et_2O (3 × 15 mL). The combined organics were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (2% EtOAc/hexanes) gave pure **14a** as a 2:1−6:1 mixture of separable diastereomers (1.00 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 6.5 Hz, 3 H), 0.99 (s, 9 H), 1.24−1.29 (m, 8 H), 1.54 (s, 3 H), 1.72−1.80 (m, 2 H), 5.13 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.5, 24.9, 24.9, 25.1, 25.9, 29.1, 31.6, 41.2, 55.3, 86.7, 177.8. HRMS (EI) *m/z* calculated for $C_{14}H_{26}O_2S^+$ (M^+) 258.1654 obsd 258.1654; Anal. ($C_{14}H_{26}O_2S$) C, H.

(±)-2-*tert*-Butyl-5-methyl-5-octyl-[1,3]-oxathiolan-4-one (14b). From (±)-**9** (1.00 g, 5.8 mmol) and octyl triflate (1.6 g, 6.20 mmol) following general procedure E, **14b** (1.33 mg, 81%) was obtained (2% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.5 Hz, 3 H), 0.99 (s, 9 H), 1.24−1.26 (m, 12 H), 1.54 (s, 3 H), 1.72−1.84 (m, 2 H), 5.13 (s, 1 H); ^{13}C NMR (75 MHz,$CDCl_3$) δ 13.9, 22.6, 24.9, 25.1, 25.9, 29.2, 29.3, 29.5, 31.8, 35.2, 41.2, 55.3, 86.5, 177.7. IR (NaCl) 3443,

2929, 1829, 1769 cm^{-1}; HRMS (EI) *m/z* calculated for $C_{16}H_{30}O_2S^+$ (M^+) 286.1967 obsd 286.1969; Anal. ($C_{16}H_{30}O_2S$) C, H.

General Procedure F. (±)-2-Acetylsulfanyl-2-methyl-octanoic Acid Ethyl Ester (15a). To **14a** (940 mg, 3.6 mmol) in EtOH (14.6 mL) was added NaOEt (2.1 M, 2.3 mL, 4.72 mmol) [freshly prepared from Na metal (200 mg, 8.3 mmol) in EtOH (4.0 mL)], and the solution was allowed to stir at room temperature. After 2 h, the solution was poured into $NH_4Cl_{(sat)}$/1 N HCl (25 mL, 3:1) and extracted with Et_2O (3 × 20 mL). The combined organics were then washed with H_2O (3 × 25 mL), dried ($MgSO_4$), filtered, evaporated, and redissolved in CH_2Cl_2 (26 mL). To this precooled solution (0 °C) were added NEt_3 (0.5 mL, 3.6 mmol) and acetyl chloride (0.26 mL, 3.6 mmol). After 40 min at 0 °C, $NH_4Cl_{(sat)}$ (30 mL) was added and the solution was extracted with CH_2Cl_2. The combined organics were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (5% EtOAc/hexanes) gave pure **15a** (943 mg, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) d 0.86 (t, *J* = 6.9 Hz, 3 H), 1.22−1.27 (m, 11 H), 1.61 (s, 3 H), 1.75−1.79 (m, 2 H), 2.25 (s, 3 H), 4.17 (q, *J* = 7.1 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 14.1, 22.4, 23.4, 24.4, 29.3, 30.3, 31.5, 38.4, 55.7, 61.5 173.0, 194.7. IR (NaCl) 3449, 1736, 1694 cm^{-1}; Anal. ($C_{13}H_{24}O_3S$) C, H.

(±)-2-Acetylsulfanyl-2-methyl-decanoic Acid Ethyl Ester (15b). From **14b** (904 mg, 3.2 mmol) and acetyl chloride (0.2 mL, 3.3 mmol) following general procedure F, **15b** (725 mg, 80%) was obtained after flash chromatography (5% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.9 Hz, 3 H),; 1.22−1.27 (m, 15 H), 1.61 (s, 3 H), 1.75−1.84 (m, 2 H), 2.26 (s, 3 H), 4.18 (q, *J* = 7.1 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 14.1, 22.6, 23.4, 24.4, 29.1, 29.2, 29.6, 30.3, 31.8, 38.3, 55.8, 61.5, 173.1, 195.8. IR (NaCl) 3430, 1868, 1693, 1644 cm^{-1}; Anal. ($C_{15}H_{28}O_3S$) C, H.

(±)-2-Propionylsulfanyl-2-methyldecanoic Acid Ethyl Ester (15c). From **14b** (613 mg, 2.14 mmol) and propionyl chloride (0.19 mL, 2.14 mmol) following general procedure F, **15c** (484 mg, 75%) was obtained after flash chromatography (5% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, *J* = 6.9 Hz, 3 H), 1.10 (t, *J* = 7.5 Hz, 3 H), 1.19−1.24 (m, 15 H), 1.58 (s, 3 H), 1.72−1.77 (m, 2 H), 2.48 (q, *J* = 7.5 Hz, 2 H), 4.17 (q, *J* = 7.1 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.45, 14.1, 14.1, 22.6, 23.5, 24.5, 29.1, 29.3, 29.7, 31.8, 36.9, 38.5, 55.5, 61.4, 173.2, 199.2. Anal. ($C_{16}H_{30}O_3S$) C, H.

(±)-2-(4-Pentenoyl)sulfanyl-2-methyldecanoic Acid Ethyl Ester (15d). From **14b** (1.3 g, 4.54 mmol) and 4-pentenoyl chloride (0.65 mL, 5.90 mmol) following general procedure F, **15d** (1.29 g, 86%) was obtained after flash chromatography (5% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) d 0.86 (t, *J* = 6.9 Hz, 3 H), 1.23 (m, 15 H), 1.60 (s, 3 H), 1.76−1.78 (m, 2 H), 2.34−2.36 (m, 2 H), 2.53−2.59 (m, 2 H), 4.16 (q, *J* = 7.2 Hz, 2 H), 4.98 (d, *J* = 10.3 Hz, 1 H), 5.01 (d, *J* = 17.6 Hz, 1 H), 5.77 (ddt, *J* = 10.3, 17.6, 6.3 Hz, 1 H).

(±)-2-Acetylsulfanyl-2-methyl-3-phenyldecanoic Acid Ethyl Ester (25). From 5-benzyl-2-*tert*-butyl-5-methyl-[1,3]-oxathiolan-4-one[11] (1.2 g, 4.7 mmol) following general procedure F, **25** (954 mg, 76%) was obtained after flash chromatography (5% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (t, *J* = 7.2 Hz, 3 H), 1.55 (s, 3 H), 2.26 (s, 3 H), 3.13 (q, *J* = 11.9 Hz, 2 H), 4.13 (q, *J* = 7.2 Hz, 2 H), 7.1 (m, 2 H), 7.2 (m, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 23.1, 30.3, 43.6, 56.3, 61.7, 127.2, 128.1, 130.7, 135.4, 172.8, 194.8. HRMS (ES) calculated for $C_{14}H_{18}O_3SNa^+$ ($M + Na^+$) 289.0869; obsd 289.0892.

General Procedure G. (±)-4-Hydroxy-5-methyl-5-hexyl-5*H*-thiophen-2-one (16a). To **15a** (715 mg, 2.8 mmol) in toluene (43 mL) at −78 °C was added LiHMDS (6.3 mL, 6.3 mmol, 1.0 M in THF), and the solution was allowed to slowly warm to −5 °C. The solution was then poured into 1 N HCl (40 mL) and extracted with EtOAc (3 × 25 mL). The combined organics were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (20% EtOAc/2% CH_3CO_2H/hexanes) gave **16a** (402 mg, 69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (keto tautomer) 0.86 (t, *J* = 6.8 Hz, 3 H), 1.27 (bs, 8 H), 1.68 (s, 3 H), 1.94−2.26 (m, 2 H), 3.35 (s, 2 H). ^{1}H NMR (300 MHz, MeOD) (enol tautomer) δ 0.89 (t, *J* = 6.5 Hz, 3 H), 1.21−1.36 (m, 7 H), 1.46−1.54 (m, 1 H), 1.64 (s, 3 H), 1.80−1.90 (m, 2 H); ^{13}C NMR (75 MHz, MeOD) δ 14.6, 23.8, 26.3, 27.1, 30.5, 32.9, 39.8, 61.3, 103.5 (m), 189.8, 197.8. Anal. ($C_{11}H_{18}O_2S$) C, H.

(±)-4-Hydroxy-5-methyl-5-octyl-5*H*-thiophen-2-one (16b). From **15b** (500 mg, 1.7 mmol) following general procedure G, **16b** (308 mg, 73%) was obtained after flash chromatography (20% EtOAc/2% CH_3CO_2H/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) (keto-tautomer) δ 0.86 (t, *J* = 6.7 Hz, 3 H), 1.19−1.24 (m, 10 H), 1.48−1.53 (m, 2 H), 1.65 (s, 3 H), 1.77−1.85 (m, 1 H), 1.94−2.01 (m, 1 H), 3.36 (s, 2 H); ^{1}H NMR (300 MHz, MeOD) (enol tautomer) 0.87−0.89 (m, 3 H), 1.29 (m, 10 H), 3.29 (s, 3 H), 1.81−1.87 (m, 2 H); ^{13}C NMR (75 MHz, MeOD) (enol tautomer) δ 14.7, 23.8, 26.4, 27.1, 30.5, 30.6, 30.8, 33.2, 39.8, 61.3, 103.1 (m), 189.8, 197.8. IR (NaCl) 3422, 1593 cm^{-1}; Anal. ($C_{13}H_{22}O_2S$), C, H.

(±)-4-Hydroxy-3,5-dimethyl-5-octyl-5*H*-thiophen-2-one (16c). From **15c** (469 mg, 1.55 mmol) and NaHMDS (3.87 mL, 3.87 mmol, 1.0 M in THF) following general procedure G, **16c** (397 mg, 70%) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) (enol tautomer) δ 0.86 (t, *J* = 6.8 Hz, 3 H), 1.23 (s, 11 H), 1.30−1.45 (m, 1 H), 1.59 (s, 3 H), 1.74 (s, 3 H), 1.84−1.88 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.48, 14.0, 22.6, 25.2, 25.9, 29.2, 29.4, 29.6, 31.8, 38.5, 58.2, 110.5, 180.9, 198.0. IR (NaCl) 2927, 1601 cm^{-1}. HRMS (ES) calculated for $C_{14}H_{24}O_2SNa^+$ ($M + Na^+$) 279.1389; obsd 279.1380; Anal. ($C_{14}H_{24}O_2S$), C, H.

(±)-4-Hydroxy-3-(2-propenyl)-5-methyl-5-octyl-5*H*-thiophen-2-one (16d). From **15d** (1.29 g, 6.04 mmol) following general procedure G, **16d** (629 mg, 57%) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, *J* = 6.9 Hz, 3 H), 1.24 (m, 12 H), 1.65 (s, 3 H), 1.81−1.86 (m, 2 H), 3.02 (d, *J* = 6.4 Hz, 2 H), 5.12 (dq, *J* = 10.6, 1.5 Hz, 1 H), 5.20 (dq, *J* = 17.3, 1.5 Hz, 1 H), 5.84 (ddt, *J* = 10.6, 17.3, 6.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 25.2, 26.1, 26.9, 29.1, 29.3, 29.5, 31.8, 38.5, 57.5, 111.5, 117.4, 134.4, 180.8, 195.4.

General Procedure H. (±)-4- Methoxy-5-methyl-5-hexyl-5*H*-thiophen-2-one (17a). To **16a** (40 mg, 0.19 mmol) in DMF (0.8 mL) cooled to −40 °C was added NaH (11 mg, 0.26 mmol, 60% in mineral oil), and the solution was allowed to warm and stir at 0 °C for 30 min. Dimethyl sulfate (35 μL, 0.38 mmol) was then added directly and the mixture was allowed to warm and stir for 2.5 h at room temperature. $NH_4Cl_{(sat)}$/1 N HCl (3:1, 10 mL) was added and the solution was extracted with Et_2O (3 × 10 mL). The combined organics were washed with H_2O (3 × 15 mL), dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (15% EtOAc/hexanes) gave pure **17a** (24.7 mg, 58%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.7 Hz, 3 H), 1.08−1.13 (m, 1 H), 1.24 (s, 6 H), 1.35−1.39 (m, 1 H), 1.61 (s, 3 H), 1.75−1.82 (m, 2 H), 3.81 (s, 3 H), 5.30 (s, 1 H); ^{13}C NMR (75 Mz, $CDCl_3$) δ 14.0, 22.5, 25.1, 26.4, 29.2, 31.5, 38.9, 59.4, 59.4, 101.3, 187.3, 193.8. HRMS (ES) calculated for $C_{12}H_{20}O_2SNa^+$ ($M + Na^+$) 251.1076; obsd 251.1076; Anal. ($C_{12}H_{20}O_2S$), C, H

(±)-4-Methoxy-5-methyl-5-octyl-5*H*-thiophen-2-one (17b). From **16b** (70 mg, 0.27 mmol) and dimethyl sulfate (50 μL, 0.55 mmol) following general procedure H, **17b** (59 mg, 80%) was obtained was obtained after flash chromatography (15% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, *J* = 7.1 Hz, 3 H); 1.07−1.18 (m, 1 H), 1.23 (s, 10 H), 1.43−1.49 (m, 1 H), 1.61 (s, 3 H), 1.74−1.81 (m, 2 H), 3.81 (s, 3 H), 5.29 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.6, 25.1, 26.4, 29.1, 29.3, 29.5, 31.8. 38.8, 59.3, 59.4, 101.3, 187.3, 193.8. IR (NaCl) 2927, 1682, 1608 cm^{-1}. Anal. ($C_{14}H_{24}O_2S$) C, H.

(±)-4-Methoxy-3,5-dimethyl-5-octyl-5*H*-thiophen-2-one (17c); (±)-5-Methoxy-2,4-dimethyl-2-octyl-2,3-dihydro-3-thiophenone (17m). From **16c** (50 mg, 0.17 mmol), and dimethyl sulfate (28 μL, 0.30 mmol) following general procedure H, **17c/17m** (2.2:1, 39 mg, 72%) was obtained ^{1}H NMR (300 MHz, $CDCl_3$) (**17c**) δ 0.86 (t, *J* = 6.3 Hz, 3 H), 1.06−1.09 (m, 1 H), 1.24 (bs, 10 H), 1.41−1.48 (m, 1 H), 1.55 (s, 3 H), 1.71−1.79 (m, 2 H), 1.98 (s, 3 H), 4.09 (s, 3 H); (17m) d 0.86 (t, *J* = 6.3 Hz, 3 H), 1.06−1.09 (m, 1 H), 1.24 (bs, 10 H), 1.41−1.48 (m, 1H), 1.48 (s, 3H), 1.71−1.79 (m, 2 H), 1.66 (s, 3 H),

4.03 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) (17c) δ 9.59, 14.1, 22.6, 25.1, 26.5, 29.2, 29.3, 29.5, 31.8, 38.9, 57.3, 59.8, 111.3, 180.1, 195.8. (**17m**) d 7.72, 9.57, 14.1, 22.6, 25.1, 25.3, 29.2, 29.5, 29.6, 38.9, 58.8, 64.8, 107.4, 184.9, 202.7. (**17c/17m** mixture): IR (NaCl) 2927, 1676, 1631, 1582 cm^{-1}. HRMS (ES) calculated for $C_{15}H_{26}O_2SNa^+$ (M + Na^+) 293.1545; obsd 293.1552. Anal. ($C_{15}H_{26}O_2S$) C, H.

(±)-5-Benzyl-4-methoxy-5-methyl-5*H*-thiophen-2-one (27). From **26** (50 mg, 0.23 mmol), and dimethyl sulfate (44 μL, 0.45 mmol) following general procedure H, **27** (38 mg, 74%) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.65 (s, 3 H), 3.1 (q, J = 7.1 Hz, 2 H), 3.84 (s, 3 H), 5.19 (s, 1 H), 7.21 (m, 5 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0, 45.0, 59.3, 59.9, 101.9, 127.2, 128.0, 130.4, 135.9, 186.5, 192.9. HRMS (ES) calcd for $C_{13}H_{14}O_2SNa^+$ (M + Na^+) 257.0606; obsd 257.0615; Anal. ($C_{13}H_{14}O_2S$) C, H

(±)- 5-Methyl-5-hexyl-2-oxo-thiophen-4-yloxy)acetic Acid Ethyl Ester (17d). From **16a** (20 mg, 0.09 mmol) and ethyl bromoacetate (20 μL, 0.2 mmol) following general procedure H, **17d** (18 mg, 67%) was obtained after flash chromatography (15% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) d 0.86 (t, J = 6.8 Hz, 3 H), 1.24−1.27 (m, 7 H), 1.32 (t, J = 7.1 Hz, 3 H), 1.47−1.48 (m, 1 H), 1.68 (s, 3 H), 1.84−1.88 (m, 2 H); 4.25 (q, J = 7.1 Hz, 2 H), 4.54 (s, 2 H), 5.21 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.1, 22.5, 25.1, 26.4, 29.2, 31.6, 38.9, 59.7, 61.9, 68.0, 102.3, 166.2, 185.3, 193.3. IR (NaCl) 2932, 1762, 1682, 1612 cm^{-1}. HRMS (ES) calcd for $C_{15}H_{24}O_4SNa^+$ (M + Na^+) 323.1287; obsd 323.1286; Anal. ($C_{15}H_{24}O_4S$) C, H.

(±)-5-Methyl-5-octyl-2-oxo-thiophen-4-yloxy)acetic Acid Ethyl Ester (17e). From **16b** (39 mg, 0.16 mmol) and ethyl bromoacetate (36 μL, 0.32 mmol) following general procedure H, **17e** (39 mg, 73%) was obtained after flash chromatography (15% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.6 Hz, 3 H), 1.24 (s, 11 H), 1.29 (t, J = 7.2 Hz, 3 H), 1.47−1.48 (m, 1 H), 1.68 (s, 3 H), 1.85−1.88 (m, 2 H), 4.25 (q, J = 7.1 Hz, 2 H), 4.54 (s, 2 H), 5.20 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.1, 22.6, 25.1, 26.4, 29.2, 29.3, 29.5, 31.8, 38.8, 59.7, 61.9, 67.9, 102.3 166.2, 185.3, 193.4. IR (NaCl) 2928, 1762, 1682, 1612 cm^{-1}. Anal. ($C_{17}H_{28}O_4S$), C, H.

(±)-4-(4-Chlorobutoxy)-5-methyl-5-hexyl-5*H*-thiophen-2-one (17f). From **16a** (36 mg, 0.17 mmol) and 3-iodo-1-chlorobutane (40 μL, 0.34 mmol) following general procedure H, **17f** (32 mg, 75%) was obtained after flash chromatography (20% EtOAc/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, J = 5.1 Hz, 3 H), 1.09−1.14 (m, 1 H), 1.25 (s, 6 H), 1.44−1.53 (m, 1 H), 1.63 (s, 3 H), 1.77−1.85 (m, 2 H), 1.90−2.00 (m, 4 H), 3.59 (t, J = 4.5 Hz, 2 H), 3.95−3.99 (m, 2 H), 5.28 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 22.3, 25.1, 26.1, 26.4, 29.1, 29.1, 31.5, 39.0, 43.9, 59.5, 71.6, 101.5, 185.9, 192.9. IR (NaCl) 2927, 1683, 1607 cm^{-1}. Anal. ($C_{15}H_{25}ClO_2S$), C, H.

(±)-4-(4-Chlorobutoxy)-5-methyl-5-octyl-5*H*-thiophen-2-one (17g). From **16b** (47 mg, 0.18 mmol) and 3-iodo-1-chlorobutane (40 μL, 0.36 mmol) following general procedure H, **17g** (46 mg, 85%) was obtained after flash chromatography (20% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 7.3 Hz, 3 H), 1.07−1.27 (m, 1 H), 1.24 (s, 10 H) 1.48−1.51 (m, 1 H), 1.62 (s, 3 H), 1.75−1.82 (m, 2 H), 1.89−1.98 (m, 4 H), 3.59 (t, J = 5.9 Hz, 2 H), 3.95−3.98 (m, 2 H), 5.28 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.6, 25.1, 26.0, 26.5, 29.0, 29.2, 29.3, 29.5, 29.7, 31.8, 44.1, 59.6, 71.7, 101.6, 186.1, 193.8. IR (NaCl) 2926, 1682, 1608 cm^{-1}. Anal. ($C_{17}H_{29}ClO_2S$) C, H.

(±)-4-Allyloxy-5-methyl-5-hexyl-5*H*-thiophen-2-one (17h). From **16a** (270 mg, 1.30 mmol) and allyl bromide (0.2 mL, 2.52 mmol) following general procedure H, **17h** and C-3 dialkylated **17t** were obtained (205 mg, 58%) as a 3:1 (**17h:17t**) mixture which could be separated and purified using flash chromatography (15% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) (**17h**) (O-alkylation) δ 0.84 (t, J = 6.9 Hz, 3 H), 1.09−1.17 (m, 1 H), 1.23 (s, 6 H), 1.40−1.51 (m, 1 H), 1.62 (s, 3 H), 1.73−1.83 (m, 2 H), 4.46 (d, J = 5.6 Hz, 2 H), 5.33 (d, J = 10.0 Hz, 1 H), 5.38 (d, J = 17.3 Hz, 1 H), 5.28 (s, 1 H), 5.87−5.98 (ddd, J = 5.6, 10.2, 17.3 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.5, 25.1, 26.5, 29.2, 31.6, 38.9, 59.7, 72.8, 101.9, 119.6, 130.7, 185.8, 193.9. HRMS (ES) calcd for $C_{14}H_{22}O_2SNa^+$ (M + Na^+) 277.1232; obsd 277.1241; Anal. ($C_{14}H_{22}O_2S$) C, H. (**17t**): ^{1}H NMR (300 MHz, $CDCl_3$) d 0.86 (t, J = 6.8 Hz, 3 H), 1.24 (bs, 8 H), 1.54 (s, 3 H), 1.81−1.84 (m, 2 H), 2.42−2.48 (m, 2 H), 5.05−5.10 (m, 4 H), 5.56−5.67(m, 2 H).

(±)-4-Allyloxy-5-methyl-5-octyl-5*H*-thiophen-2-one (17i). From **16b** (197 mg, 0.91 mmol) and allyl bromide (140 μL, 1.62 mmol) following general procedure H, **17i** and C-3 dialkylated **17q** (173 mg, 76%) were obtained as a 3.5:1 mixture which could be separated and purified using flash chromatography (15% EtOAc/hexanes). (**17i**) (O-alkylation) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.8 Hz, 3 H), 1.12−1.17 (m, 1 H), 1.24 (s, 10 H), 1.45−1.49 (m, 1 H), 1.64 (s, 3 H), 1.77−1.84 (m, 2 H), 4.47 (d, J = 5.6 Hz, 2 H), 5.29 (s, 1 H), 5.31 (d, J = 10.4 Hz, 1 H), 5.39 (d, J = 16.9 Hz, 1 H), 5.90−5.99 (ddd, J = 5.6, 10.4, 16.9 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.6, 25.1, 26.5, 29.2, 29.3, 29.5, 31.8, 38.9, 59.7, 72.8, 102.0, 119.5, 130.8, 185.8, 193.8. IR (NaCl) 3441, 1682, 1608 cm^{-1}. Anal. ($C_{16}H_{26}O_2S$) C, H.

(±)-3-(2-Propenyl)-3,5-Dimethyl-5-octyl-thiophene-2,4-dione (17n); (±)-4-Allyloxy-3,5-dimethyl-5-octyl-5*H*-thiophen-2-one (17o)/5-Allyloxy-2,4-dimethyl-2-octyl-2,3-dihydro-3-thiopenone (17p). From **16c** (70.1 mg, 0.27 mmol) and allyl bromide (47 μL, 0.55 mmol) following general procedure H, **17n** and **17o,p** (**17o**:**17p**, 2.6:1) (82% overall) were obtained as a 2.3:1 mixture. **17n** was easily separated from the C-4 and C-2 O-alkylated mixture (**17o**, **17p**) using flash chromatography (20% EtOAc/hexanes).

(**17n**). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.6 Hz, 3 H), 1.16−1.47 (m, 15 H), 1.57 (s, 3 H), 1.74−1.96 (m, 2 H), 2.42−2.46 (m, 2 H),. 5.04−5.10 (m, 2 H), 5.53−5.67 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.2, 22.6, 25.5, 26.2, 29.1, 29.2, 29.5, 31.7, 41.3, 42.0, 58.4, 65.1, 120.2, 131.4, 204.5, 214.6. IR (NaCl) 2928, 1742, 1698 cm^{-1}. Anal. Calcd for $C_{17}H_{28}O_2S$: C, 68.9; H, 9.52; Found: C, 69.8; H, 9.85

(**17o/17p** mixture) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.3 Hz, 3 H), 1.06−1.48 (m, 12 H), 1.58 (s, 3 H), 1.71−1.82 (m, 2 H), 1.94 (s, 3 H), 4.80−4.82 (m, 2 H), 5.28−5.40 (m, 2 H), 5.89−6.03 (m, 1 H); (17p) d 0.86 (t, J = 6.3 Hz, 3 H), 1.06−1.48 (m, 12 H), 1.49 (s, 3 H), 1.71−1.82 (m, 2 H), 1.69 (s, 3 H), 4.73−4.75 (m, 2 H), 5.32−5.46 (m, 2 H), 5.89−6.03 (m, 1 H). (**17o**) ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.65, 14.0, 22.6, 25.2, 26.6, 29.2, 29.3, 29.6, 31.8, 39.0, 57.5, 72.5, 111.8, 118.2, 132.6, 179.4, 195.7. (**17p**) 9.65, 14.0, 22.6, 25.2, 26.6, 29.2, 29.3, 29.6, 31.8, 39.0, 64.9, 75.5, 107.9, 119.5, 131.3, 185.1, 202.8. (17o/17p mixture): IR (NaCl) 2855, 1676, 1628, 1580 cm^{-1}; Anal. ($C_{17}H_{28}O_2S$) C, H.

(±)-5-Methyl-5−4-prop-2-ynyloxy-5*H*-thiophen-2-one (17j). From **16a** (45 mg, 0.21 mmol) and propargyl bromide (37 μL, 0.21 mmol) following general procedure H, **17j** (21 mg, 40%) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) d 0.86 (t, J = 6.9 Hz, 3 H), 1.11−1.20 (m, 1 H), 1.24 (s, 6 H), 1.41−1.49 (m, 1 H), 1.63 (s, 3 H), 1.76−1.86 (m, 2 H), 2.59 (t, J = 2.5 Hz, 1 H), 4.62 (d, J = 3.7 Hz, 1 H), 4.63 (d, J = 3.7 Hz, 1 H), 5.43 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 22.5, 24.8, 26.4, 29.0, 31.5, 38.7, 59.3, 59.5, 75.6, 77.4, 102.9, 184.4,193.6. IR (NaCl) 2130, 1676, 1607 cm^{-1}.

(±)-5-Methyl-5-hexyl-2-oxo-thiophen-4-yloxy)acetic Acid *tert*-Butyl Ester (17k). From **16a** (169 mg, 0.79 mmol) and *tert*-butyl bromoacetate (0.23 mL, 1.58 mmol) following general procedure H, **17k** (206 mg, 80%) was obtained after flash chromatography (15% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (t, J = 6.8 Hz, 3 H), 1.21 (s, 8 H), 1.47 (s, 9 H), 1.64 (s, 3 H), 1.78−1.83 (m, 2 H), 4.41 (s, 2 H), 5.15 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.5, 25.1, 26.3, 28.0, 29.1, 31.5, 38.9, 59.6, 68.4, 83.4, 102.1, 165.2, 185.5, 193.4. HRMS (ES) calcd for $C_{17}H_{28}O_4SNa^+$ (M + Na^+) 351.1600; obsd 351.1612.

(±)-5-Methyl-5-octyl-2-oxo-thiophen-4-yloxy)acetic Acid *tert*-Butyl Ester (17l). From **16b** (60 mg, 0.25 mmol) and *tert*-butyl bromoacetate (73 μL, 0.49 mmol) following general procedure H, **17l** (62 mg, 70%) was obtained after flash chromatography (15% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.9 Hz, 3 H), 1.24 (s, 12 H), 1.49 (s, 9 H),

1.68 (s, 3 H), 1.83−1.86 (m, 2 H), 4.43 (s, 2 H), 5.19 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.6, 25.2, 26.3, 28.1, 29.2, 29.3, 29.5, 31.8, 38.9, 59.7, 68.5, 83.4, 102.1, 165.2, 185.5, 193.4. Anal. ($C_{19}H_{32}O_4S$) C, H.

(±)-3,3-Diallyl-5-methyl-5-octyltetrahydro-2,4-thiophenedione (17q). 3-Allyl-4-allyoxyl-5-methyl-5-octyl-2,5-dihydro-2-thiophenone (17r); 4-Allyl-5-allyloxy-2-methyl-2-octyl-2,3-dihydro-3-thiophenone (17s). From **16d** (374 mg, 1.33 mmol) and allyl bromide (184 μL, 2.13 mmol) following general procedure H, **17q** and **17r/17s** (2.6:1) (68%, 5.3:1 **17q:17r/17s**) was obtained. **17q** was separated from **17r/17s** mixture after flash chromatography (15% EtOAc/hexanes). (**17q**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.5 Hz, 3 H), 1.25 (m, 11 H), 1.43−1.47 (m, 1 H), 1.54 (s, 3 H), 1.79−1.84 (m, 2 H), 2.43−2.47 (m, 4 H), 5.05−5.11 (m, 4 H), 5.57−5.69 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 25.1, 25.8, 29.1, 29.2, 29.5, 31.8, 40.2, 40.7, 41.3, 62.8, 64.8, 120.3, 120.4, 131.2, 131.2, 203.9, 213.5.

General Procedure I. (±)-5-Methyl-5-hexyl-2-oxo-thiophen-4-yloxy)acetic Acid (18a). To **17k** (177 mg, 0.54 mmol) dissolved in CH_2Cl_2 (3.9 mL) was added trifluoroacetic acid (TFA) (2.6 mL), and the solution was stirred at room temperature for 4 h. The solvents were evaporated, and the crude material was chromatographed (20% EtOAc/2% CH_3CO_2H/hexanes) to give pure **18a** (144 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.8 Hz, 3 H), 1.24 (s, 7 H), 1.44−1.47 (m, 1 H), 1.68 (s, 3 H), 1.84−1.91 (m, 2 H), 4.62 (s, 2 H), 5.33 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.6, 25.1, 26.1, 29.2, 31.6, 38.9, 60.3, 67.7, 102.4, 169.8, 185.9, 196.1. HRMS (ES) calcd for $C_{13}H_{20}O_4SNa^+$ ($M + Na^+$) 295.0974; obsd 295.0950.

(±)-5-Methyl-5-octyl-2-oxo-thiophen-4-yloxy)acetic Acid (18b). To **17l** (65 mg, 0.18 mmol) and trifluoroacetic acid (TFA) (0.7 mL) following general procedure I, **18b** (48 mg, 89%) was obtained after flash chromatography (20% EtOAc/2% CH_3CO_2H/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.9 Hz, 3 H), 1.24 (s, 11 H), 1.47−1.48 (m, 1 H), 1.68 (s, 3 H), 1.84−1.88 (m, 2 H), 4.62 (s, 2 H), 5.31 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.6, 25.1, 26.1, 29.2, 29.3, 29.5, 31.8, 38.9, 60.1, 67.7, 102.4, 169.8, 185.8, 195.4. IR (NaCl) 3442, 1645 cm^{-1}; Anal. ($C_{15}H_{24}O_4S$) C, H.

General Procedure J. (±)−(5-Methyl-5-hexyl-2-oxo-thiophen-4-yloxy)acetylmethyl Glycinate (19). To a solution of **18a** (42.4 mg, 0.15 mmol) in CH_3CN (0.86 mL) were added tris(2-oxo-3-oxazolinyl)phosphine oxide[16] (91 mg, 0.20 mmol), methyl glycinate hydrochloride (19.7 mg, 0.16 mmol), and NEt_3 (43 μL, 0.31 mmol), and the solution was allowed to stir at room temperature for 20 min. The mixture was poured into a solution of $NH_4Cl_{(sat)}$/1 N HCl (10 mL) and extracted with Et_2O (3 × 10 mL). The combined organics were dried ($MgSO_4$), filtered, evaporated, and chromatographed (40−50% EtOAc/hexanes) to give pure **19** (43 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.8 Hz, 3 H), 1.23−1.26 (m, 7 H), 1.49−1.55 (m, 1 H), 1.65 (s, 3 H), 1.84−1.90 (m, 2 H), 3.79 (s, 3 H), 4.11 (d, J = 5.0 Hz, 1 H), 4.02−4.09 (m, 2 H), 4.47 (s, 2 H), 5.36 (s, 1 H), 6.76 (bs, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 22.4, 25.2, 26.2, 29.0, 31.4, 38.9, 40.8, 52.4, 59.3, 70.1, 103.1, 165.6, 169.6, 184.2, 192.7; IR (NaCl) 1752, 1689, 1612, 1535 cm^{-1}; Anal. ($C_{16}H_{25}NO_5S$) C, H.

(±)-(5-Methyl-5-hexyl-2-oxo-thiophen-4-yloxy)acetyl Glycinate (20). To **18a** (22 mg, 0.06 mmol) dissolved in THF/H_2O (0.5 mL, 3:1), cooled to 0 °C, was added LiOH (3 mg, 0.07 mmol), and this solution was allowed to stir for 45 min. Then, the mixture was poured into a solution of HCl (10 mL, 1 N) and extracted with Et_2O (3 × 10 mL). The combined organics were dried ($MgSO_4$), filtered, and evaporated to give crude **20**. Flash chromatography (50% EtOAc/2%CH_3CO_2H/hexanes) gave pure **20** (19 mg, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.7 Hz, 3 H), 1.25 (s, 7 H), 1.48−1.52 (m, 2 H), 1.68 (s, 3 H), 2.08−2.10 (m, 2 H), 4.05 (s, 2 H), 4.56 (s, 2 H), 5.41 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.5, 25.3, 25.9, 29.1, 31.5, 38.8, 40.9, 59.9, 69.9, 103.2, 166.8, 171.9, 184.9, 194.9; Anal. Calcd for $C_{15}H_{23}NO_5S$: C, 54.6; H, 7.16; Found: C, 53.4; H, 7.16.

(±)-*N*-(4-Bromobutyl)(5-methyl-5-hexyl-2-oxo-thiophen-4-yloxy)acetamide (21). To **18a** (61 mg, 0.22 mmol) and 1-aminopropanol hydrobromide (50 mg, 0.23 mmol) following general procedure J, **21** (65 mg, 74%) was obtained after flash chromatography (50% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.9 Hz, 3 H), 1.12−1.15 (m, 1 H), 1.23−1.28 (s, 6 H), 1.46−1.53 (m, 1 H), 1.69 (s, 3 H), 1.82−1.88 (m, 2 H), 2.14 (quint. J = 6.3 Hz, 2 H), 3.42 (m, 2 H), 3.54 (q, J = 6.3 Hz, 2 H), 4.43 (s, 2 H), 5.35 (s, 1H) 6.45 (bs, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.5, 25.3, 25.3, 26.4, 29.2, 30.7, 31.5, 31.7, 38.1, 39.0, 59.4, 70.4, 103.3, 167.9, 184.2, 192.9. IR (NaCl) 1666, 1607, 1543 cm^{-1}. Anal. ($C_{16}H_{26}NO_3BrS$) C, H.

(±)-*N*-Allyl-(5-methyl-5-octyl-2-oxo-thiophen-4-yloxy)-acetamide (22). To a cooled solution (0 °C) of **18b** (64 mg, 0.21 mmol) in CH_2Cl_2 (1.1 mL) were added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) (49 mg, 0.25 mmol), DMAP (3 mg, 0.02 mmol), and allylamine (18 μL, 0.25 mmol), and the mixture was allowed to warm to room temperature and stir for 12 h. The solution was poured into a solution of 1 N $HCl_{(sat)}$ (1:3) and extracted with Et_2O (3 × 10 mL). The combined organics were dried ($MgSO_4$), filtered, and evaporated to give crude **22**. Flash chromatography (50% EtOAc/hexanes) gave pure **22** (50 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.9 Hz, 3 H), 1.12−1.22 (m, 1 H), 1.24 (s, 10 H), 1.41−1.51 (m, 1 H), 1.68 (s, 3 H), 1.82−1.87 (m, 2 H), 3.98 (app t, J = 5.7 Hz, 2 H), 4.50 (s, 2 H), 5.20 (d, J = 10.2 Hz, 1 H), 5.22 (d, J = 17.3 Hz, 1 H), 5.35 (s, 1 H), 5.80−5.90 (ddt, J = 5.4, 10.2, 17.3 Hz, 1 H), 6.19 (bs, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.6, 25.3, 26.5, 29.2, 29.4, 29.5, 31.8, 39.1, 41.6, 59.3, 70.3, 103.4, 117.2, 133.2, 165.3, 183.9, 192.8. IR (NaCl) 2957, 1692, 1605, 1539 cm^{-1}. Anal. ($C_{18}H_{29}NO_3S$) C, H.

General Procedure K. (±)-4-Propionyl-5-methyl-5-octyl-5*H*-thiophen-2-one (23a). To a solution of **16b** (40 mg, 0.17 mmol) in THF (0.78 mL) cooled to −78 °C was added LiHMDS (0.24 mL, 0.25 mmol, 1 M in THF), and the solution was allowed to stir for 30 min at −78 °C. Propionyl chloride (20 μL, 0.62 mmol) was then added, and the mixture was transferred to an ice bath and allowed to slowly warm to room temperature. After 1 h at room temperature, the mixture was poured into a solution of HCl (1 N)/$NH_4Cl_{(sat)}$ (10 mL) and extracted with Et_2O (3 × 10 mL). The combined organics were dried ($MgSO_4$), filtered, evaporated, and chromatographed (15% EtOAc/hexanes) to give pure **23a** (23.1 mg, 47%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.9 Hz, 3 H), 1.12−1.25 (m, 13 H), 1.42−1.49 (m, 2 H), 1.64 (s, 3 H), 1.78−1.84 (m, 2 H), 2.57 (q, J = 7.5 Hz, 2 H), 6.39 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.71, 14.0, 22.6, 25.1, 25.9, 27.9, 29.1, 29.3, 29.5, 31.8, 38.6, 60.4, 113.8, 169.1, 177.0, 179.9. IR (NaCl) 2928, 1787, 1688 cm^{-1}; Anal. ($C_{16}H_{26}O_3S$) C, H.

(±)-4-Carbonic Acid Methyl Ester, 5-Methyl-5-octyl-5*H*-thiophen-2-one (23b). From **16b** (73 mg, 0.30 mmol) and methyl chloroformate (37 μL, 0.48 mmol) following general procedure K, **23b** (63 mg, 70%) was obtained after flash chromatography (20% EtOAc/hexanes).^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.4 Hz, 3 H), 1.15−1.21 (m, 1 H), 1.22 (s, 10 H), 1.41−1.51 (m, 1 H), 1.66 (s, 3 H), 1.81 (d, J = 9.0 Hz, 1 H), 1.83 (d, J = 9.0 Hz, 1 H), 3.92 (s, 3 H), 6.39 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.6, 25.2, 25.9, 29.2, 29.3, 29.4, 31.8, 38.4, 56.2, 60.2, 112.9, 150.9, 175.5, 194.1. IR (NaCl) 3382, 1626, 1560, 1542 cm^{-1}. Anal. ($C_{15}H_{24}O_4S$) C, H.

(±)-4-Carbonic Acid Ethyl Ester, 5-Methyl-5-octyl-5*H*-thiophen-2-one (23c). From **16b** (95 mg, 0.39 mmol) and ethyl chloroformate (60 μL, 0.32 mmol) following general procedure K, **23c** (111 mg, 91%) was obtained after flash chromatography (15% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.9 Hz, 3 H), 1.12−1.17 (m, 11 H), 1.38 (t, J = 7.1 Hz, 3 H), 1.42−1.50 (m, 1 H), 1.67 (s, 3 H), 1.82 (d, J = 9.0 Hz, 1 H), 1.85 (d, J = 9.0 Hz, 1 H), 4.33 (q, J = 7.1 Hz, 2 H), 6.38 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.0, 22.6, 25.2, 25.8, 29.1, 29.2, 30.4, 31.8, 38.4, 60.1, 66.0, 112.8, 150.2, 175.6, 193.9. IR (NaCl) 2928, 1782, 1690, 1625 cm^{-1}. Anal. ($C_{16}H_{26}O_4S$) C, H.

(±)-4-Carbonic Acid Allyl Ester, 5-methyl-5-octyl-5*H*-thiophen-2-one (23d). From **16b** (51.5 mg, 0.21 mmol) and allyl chloroformate (33 μL, 0.32 mmol) following general procedure K, **23d** (46.3 mg, 67%) was obtained after flash chromatography (15% EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.9 Hz, 3 H), 1.16−1.23 (bs, 10 H), 1.41−1.51 (m, 2 H), 1.67 (s, 3 H), 1.81−1.87 (m, 2 H), 4.74 (app dt, J = 6.1, 1.3 Hz, 2 H), 5.37 (app dq, J = 10.3, 1.0 Hz, 1 H), 5.44 (app dq, J = 15.9, 1.0 Hz, 1 H), 5.90−6.0 (m, 1 H), 6.39 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) 14.0, 22.6, 25.2, 25.8, 29.1, 29.2, 29.4, 31.8, 38.4, 60.1, 70.2, 112.9, 120.6, 130.2, 150.0, 175.5, 193.7. IR (NaCl) 2927, 1782, 1691, 1606 cm^{-1}. Anal. ($C_{17}H_{26}O_4S$) C, H.

4-Hydroxy-3-(1-hydroxyethyl)-5-methyl-5-octyl-5*H*-thiophen-2-one (29, 30). To **16b** (247 mg, 1.02 mmol) dissolved in hexanes were added triethylamine (0.23 mL, 1.68 mmol) and trimethylsilyl chloride (0.21 mL, 1.64 mmol), and the solution was allowed to stir at room temperature for 4 h. The mixture was filtered over Celite and evaporated to provide 5-methyl-5-octyl-4-trimethylsilanyloxy-5*H*-thiophen-2-one. To a solution of $TiCl_4$ (0.7 mL, 0.7 mmol) in CH_2Cl_2 (1.95 mL) at −78 °C was added acetaldehyde (54 μL, 0.97 mmol), and this solution was allowed to stir for 5 min at −78 °C. Then, 5-methyl-5-octyl-4-trimethylsilanyloxy-5*H*-thiophen-2-one dissolved in CH_2Cl_2 (0.4 mL) was cannulated into $TiCl_4$/acetaldehyde solution giving a bright orange color. This mixture was allowed to warm and stir for 20 min at 0 °C. The mixture was poured into $NH_4Cl_{(sat)}$ (15 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The organics were combined, dried ($MgSO_4$), filtered, and evaporated. Flash chromatography (10% EtOAc/hexanes) provided pure **29** (34 mg) and **30** (24 mg) (50%). (**29**) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.9 Hz, 3 H), 1.05−1.08 (m, 1 H), 1.24 (bs, 11 H), 1.49 (d, J = 6.5 Hz, 3 H, rotamer) 1.55 (d, J = 5.2 Hz, 3 H, rotamer), 1.62 (s, 3 H), 1.78−1.82 (m, 2 H), 4.68 (q, J = 6.5 Hz, 1 H, rotamer), 5.04 (q, J = 5.2 Hz, 1 H, rotamer). HRMS (ES) *m/z* calculated for $C_{16}H_{28}O_3SNa^+$ (M + CH_2 + Na^+) 323.1660 obsd 323.1660.

(**30**) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.9 Hz, 3 H), 1.24 (bs, 12 H), 1.47 (d, J = 6.6 Hz, 3 H, rotamer), 1.54 (d, J = 5.4 Hz, 3 H, rotamer), 1.59 (s, 3 H), 1.76−1.82 (m, 2 H), 4.65 (q, J = 6.3 Hz, 1 H), 5.06 (q, J = 5.4 Hz, 1 H). HRMS (ES) *m/z* calculated for $C_{16}H_{28}O_3SNa^+$ (M + CH_2 + Na^+) 323.1660 obsd 323.1660.

General Procedure L. 3-Acetyl-4-hydroxy-5-methyl-5-octyl-5*H*-thiophen-2-one. (31a). To **16b** (94 mg, 0.38 mmol) in CH_2Cl_2 (1.9 mL) at 0 °C was added NEt_3 (58 μL, 0.42 mmol), (dimethylamino)pyridine (DMAP) (19 mg, 0.15 mmol) and acetic anhydride (43 μL, 0.47 mmol). The solution stirred at 0 °C for 15 min then was allowed to warm and stir at room temperature for 2−14 h or until TLC indicated completion of the reaction. The mixture was poured into NH$_4$Cl(sat)/HCl (1 N) (3:1, 8 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organics were combined, dried ($MgSO_4$), filtered, and evaporated to give crude **31a**. Flash chromatography 30%EtOAc/2%AcOH/hex (R_f = 0.44) gave pure **31a** (83 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (m, 3 H), 1.22 (bs, 10 H), 1.48 (m, 2 H), 1.65 (s, 3 H), 1.77−1.92 (m, 2 H), 2.55 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.6, 23.8, 25.1, 26.3, 29.1, 29.2, 29.5, 31.7, 39.4, 59.7, 109.7, 190.5, 195.5, 204.9. Anal. Calcd for $C_{15}H_{24}O_3S$: C, 63.3; H, 8.51; Found: C, 59.5; H, 7.83. HRMS (EI) *m/z* calculated for $C_{15}H_{24}O_3S^+$ (M^+) 284.1441 obsd 284.1414.

4-Hydroxy-5-methyl-5-octyl-3-(2,2,2-trifluoro-acetyl)-5*H*-thiophen-2-one (31b). From **16b** (90 mg, 0.37 mmol), trifluoroacetic anhydride (114 μL, 0.81 mmol), (dimethylamino)pyridine (DMAP) (18 mg, 0.15 mmol), and NEt_3 (108 μL, 0.77 mmol) following General Procedure L, **31b** (107 mg, 86%) was obtained after flash chromatography (40%Hex/10% THF/2%AcOH/EtOAc). ^{1}H NMR (300 MHz, MeOD) δ 0.85 (t, J = 6.9 Hz, 3 H), 1.09 (m, 1 H), 1.21 (bs, 11 H), 1.38 (s, 3 H), 1.51−1.60 (m, 1 H), 1.65−1.71 (m, 1 H). ^{13}C NMR (100 MHz, MeOD) d 14.6, 23.8, 26.3, 27.8, 30.5, 30.7, 30.9, 33.1, 41.2, 61.2, 105.7, 152.9 (q, J = 57 Hz), 174.3, 196.1, 203.5. Anal. Calcd for $C_{15}H_{21}F_3O_3S$; C, 53.2; H, 6.26; Found: C, 52.0; H, 6.15. HRMS (EI) *m/z* calculated for $C_{15}H_{21}F_3O_3S^+$ (M^+) 338.1158 obsd 338.1171.

4-Hydroxy-5-methyl-5-octyl-2-oxo-2,5-dihydro-thiophene-3-carboxylic Acid Methyl Ester (31c). From **16b** (91 mg, 0.37 mmol), methyl chloroformate (63 μL, 0.81 mmol), (dimethylamino)pyridine (DMAP) (23 mg, 0.18 mmol), and NEt_3 (108 μL, 0.77 mmol) following General Procedure L, **31c** (66 mg, 59%, 79% based on recovered starting material) was obtained after flash chromatography (30% EtOAc/2%AcOH/hexanes−10%THF/2%AcOH/EtOAc). ^{1}H NMR (300 MHz, MeOD) δ 0.86 (t, J = 6.9 Hz, 3 H), 1.20 (bs, 12 H), 1.35 (s, 3 H), 1.55 (m, 1 H), 1.71−1.75 (m, 1 H), 3.59 (s, 3 H); ^{13}C NMR (100 MHz, MeOD) δ 13.9, 22.1, 24.6, 27.3, 28.5, 28.6, 28.8, 29.2, 31.3, 50.9, 58.4, 97.1, 168.4, 187.9, 203.2. HRMS (EI) *m/z* calculated for $C_{15}H_{24}O_4S^+$ (M^+) 300.1389 obsd 300.1375.

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Supporting Information Available: Copies of ^{1}H NMR spectra for compounds **13a−d**, **16a−d**, **17a−c/m**, **17d−j**, **17n**, **17o/p**, **17q**, **18b**, **19**, **20**, **21**, **22**, **23a−d**, **26**, **27**, **29**, **30**, **31a−c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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