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Practical, Catalytic, Asymmetric Synthesis of β-Lactones via a Sequential Ketene Dimerization/Hydrogenation Process: Inhibitors of the Thioesterase Domain of Fatty Acid Synthase

Vikram C. Purohit,[†] Robyn D. Richardson,[‡] Jeffrey W. Smith,*[,‡] and Daniel Romo*[,†]

Department of Chemistry, Texas A & M University, P.O. Box 30012, College Station, Texas 77842-3012 and Cell Adhesion and Extra Cellular Matrix Biology, Burnham Institute for Medical Research, Infectious and Inflammatory Disease Center, La Jolla, California 92037

romo@mail.chem.tamu.edu; jsmith@burnham.org

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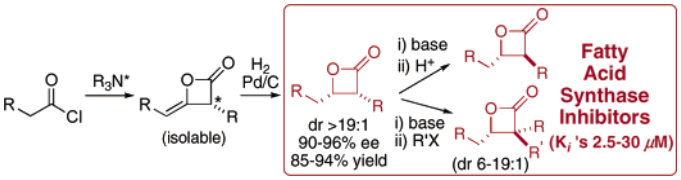

The recent finding that the FDA-approved antiobesity agent orlistat (tetrahydrolipstatin, Xenical) is a potent inhibitor of the thioesterase domain of fatty acid synthase (FAS) led us to develop a concise and practical asymmetric route to pseudosymmetric 3,4-dialkyl-*cis*-β-lactones. The well-documented up-regulation of FAS in cancer cells makes this enzyme complex an interesting therapeutic target for cancer. The described route to 3,4-dialkyl-β-lactones is based on a two-step process involving Calter's catalytic, asymmetric ketene dimerization of acid chlorides followed by a facial-selective hydrogenation leading to *cis*-substituted-β-lactones. Importantly, the ketene dimer intermediates were found to be stable to flash chromatography, enabling opportunities for subsequent transformations of these optically active, reactive intermediates. Subsequent α-epimerization and α-alkylation or acylation led to *trans*-β-lactones and β-lactones bearing α-quaternary carbons, respectively. Several of the ketene dimers and β-lactones displayed antagonistic activity (apparent K_i in the low micromolar range) in competition with a fluorogenic substrate toward a recombinant form of the thioesterase domain of fatty acid synthase. The best antagonist, a simple phenyl-substituted *cis*-β-lactone **3d**, displayed an apparent K_i (2.5 ± 0.5 μM) of only ~10-fold lower than that of orlistat (0.28 ± 0.06 μM). In addition, mechanistic studies of the ketene dimerization process by ReactionView infrared spectroscopy support previous findings that ketene formation is rate determining.

Introduction

The development of synthetic processes that deliver optically active products from achiral starting materials in a practical and catalytic manner continues to be a vital area of research in organic synthesis.[1] In the area of β-lactone synthesis, the Wynberg process continues to be a benchmark for further developments in this area.[2] Several groups have recently developed various catalytic, asymmetric routes to β-lactones involving nucleophile-catalyzed aldol−lactonizations and Lewis-acid-catalyzed [2+2] cycloadditions.[3] With regard to the former route, we recently reported an intramolecular, nucleophile-catalyzed aldol−lactonization process building on the work of Wynberg that effectively joins catalytic, asymmetric carbocycle synthesis with β-lactone synthesis employing organocatalysis.[4] This was the first example of this process with highly electrophilic (e.g., non-α-chlorinated) aldehydes. More recently, this

* To whom correspondence should be addressed.
[†] Texas A & M University.
[‡] Burnham Institute for Medical Research.

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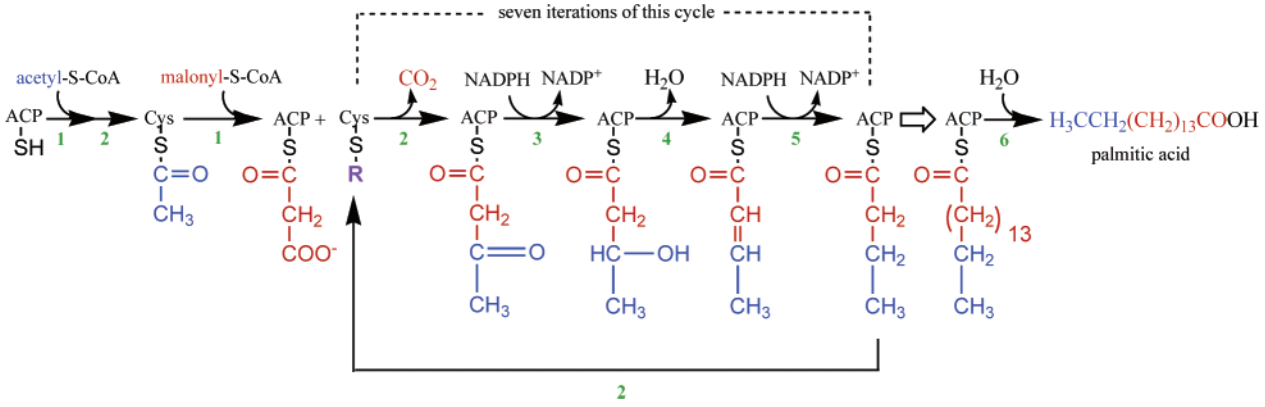
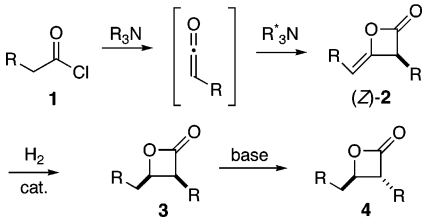


FIGURE 1. Schematic diagram of FAS showing enzymatic steps involved in fatty acid biosynthesis leading to palmitic acid. FAS is comprised of six enzymatic domains and an acyl-carrier protein (ACP). The steps in fatty acid biosynthesis are as follows. (i) The malonyl/acetyl transferase domain (1) transfers an acetyl group onto the ACP. It is then translocated to the active-site cysteine by α-ketoacyl synthase (2). This position, marked "R", also serves as the loading position for the growing acyl chain in subsequent iterations. (ii) The malonyl/acetyl transferase domain (1) then transfers a malonyl group to the ACP, and the two are condensed (2) into a four-carbon product bound to the enzyme through the thiol of the ACP. (iii) The ketoacyl reductase (3) reduces the ketone at C-3 to an alcohol. (iv) The dehydrase (4) further reduces the alcohol to an alkene. (v) The enoyl reductase domain (5) further reduces the alkene bond to an alkane, and the ACP-bound chain is translocated back to the active-site cysteine (2). Steps ii−v are then repeated six times to yield a 16-carbon, fully saturated palmitic acid bound to the ACP. (vi) The palmitate is released from FAS by the enzyme's intrinsic thioesterase domain (FAS TE, 6), which is the target of orlistat and the *β*-lactones generated in this study.

SCHEME 1. Strategy toward Pseudosymmetric *cis*-3,4-Dialkyl-*β*-lactones 3 via a Ketene Dimerization/ Hydrogenation Process from Acid Chlorides 1 and Epimerization to *trans*-*β*-Lactones 4



limitation was overcome by use of Lewis-acid additives in the Wynberg process by Nelson and Calter, allowing access to *cis*-*β*-lactones in high enantiopurity.[5] In a related process, the Calter group described a novel application of asymmetric organocatalysis for the dimerization of methylketene[6] and more recently several *in situ* generated ketenes leading to pseudosymmetric, chiral 3-alkyl-4-methylene-2-oxetanones **2** (Scheme 1, **1** → **2**).[7] These homodimers were typically not isolated but directly ring-opened with lithiated secondary amines to provide enolates that could undergo subsequent aldol reactions leading to polypropionate fragments and ultimately to polyketide natural products.[8] Important to the process described herein, the geometry of the olefin in dimer **2** was determined to be *Z* by virtue of the stereochemical outcome of the subsequent aldol process. In this article, we describe hydrogenation of isolated, optically active ketene dimers **2** leading to *cis*-*β*-lactones **3** and subsequent α-epimerization and alkylations leading to *trans*-*β*-lactones **4** and α,α-disubstituted-*β*-lactones, respectively (Scheme 1). This process provides a practical (two steps from acid chlorides) and complimentary approach to simplified pseudosymmetric dialkyl-*β*-lactones **3**.

Our long-standing interest in the development of enantioselective methodologies for *β*-lactone synthesis and our recent discovery that tetrahydrolipstatin (orlistat) potently inhibits fatty acid synthase (FAS)[9] prompted us to explore the potential of optically active ketene dimers as precursors to pseudosymmetric *trans*-3,4-dialkyl-*β*-lactones **4** and study the ability of these simplified *β*-lactones to inhibit the thioesterase domain of fatty acid synthase (FAS TE) (Figure 1). Human fatty acid synthase (FAS) is the enzyme responsible for cellular synthesis of palmitate. FAS contains seven separate enzymatic pockets that function sequentially to condense acetyl-CoA with malonyl-CoA to form a four-carbon intermediate. Six additional turns of the enzyme's cycle convert this intermediate to palmitate, which is then liberated from FAS by the action of the thioesterase domain.[10] FAS is attracting great interest as a drug target in oncology because it is up-regulated in most solid tumors, including those of the breast,[11] prostate,[12] and ovary.[13] Furthermore, a number of studies show that a pharmacologic blockade of FAS can be cytostatic and cytotoxic to tumor cells.[14] For example, the fungal product cerulenin[15] and a synthetic

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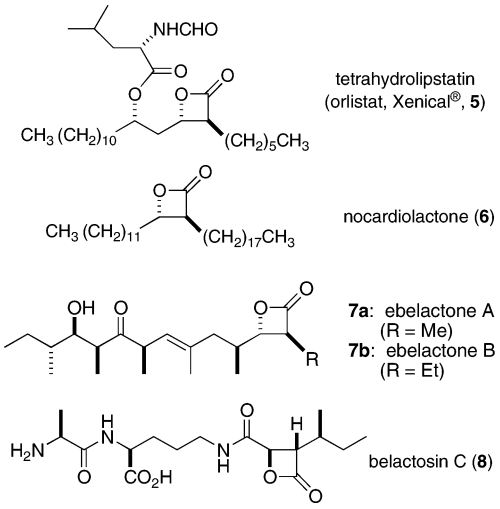


FIGURE 2. Structures of representative, bioactive natural products possessing *trans*-3,4-disubstituted-β-lactones.

derivative, c75,[16] were found to inhibit the ketosynthase (KS) domain of FAS and thus shut down tumor cell proliferation and in some cases induce apoptosis.[17] We recently showed that orlistat, which bears a β-lactone and is a drug approved for treating obesity, is a potent inhibitor of FAS TE and that this natural product derivative is cytotoxic and cytostatic to tumor cells in vitro and can inhibit tumor growth in vivo.[9] However, orlistat has poor solubility and poor bioavailability, so there is a great need to develop new β-lactones that overcome these problems and that can be deployed as potential antitumor drugs. In addition, simplified derivatives that are readily prepared would also ultimately be attractive from the standpoint of process development.

A particularly attractive feature of the described strategy is its practicality since β-lactones **4**, which are simplified but structurally analogous to orlistat, could potentially be obtained in three steps from acid chlorides in optically active form. Following ketene dimerization and hydrogenation, α-epimerization would allow access to the thermodynamically favored *trans*-β-lactones **4** (Scheme 1) corresponding to the relative stereochemistry found in orlistat (**5**) and also commonly observed in β-lactone-containing natural products such as nocardiolactone (**6**), the ebelactones (**7a**,**b**), and most recently the belactosins (e.g., belactosin C (**8**), (Figure 2)). Furthermore, given the potential that β-lactones are showing currently as inhibitors of several enzymes including proteases,[18] HMG Co-A synthase,[19] esterases, and also the proteasome,[20] the ketene dimerization/hydrogenation process described herein could also find wider application.

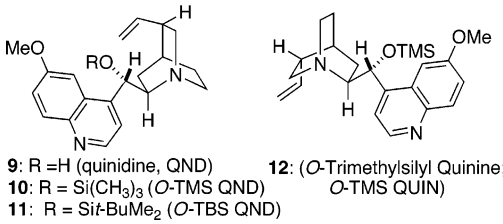
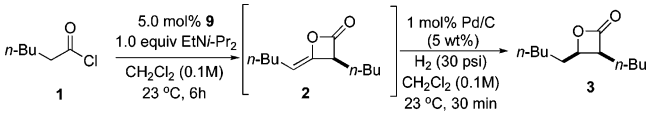


FIGURE 3. *Cinchona* alkaloids and derivatives **9**−**11** employed in the ketene dimerization.

SCHEME 2. Attempted One-Pot, Two-Step Catalytic Asymmetric β-Lactone Synthesis via a Ketene Dimerization/Hydrogenation Sequence



Results and Discussion

Development of a One-Pot Ketene Dimerization/Hydrogenation Sequence. Initial studies focused on the possibility of developing a one-pot, two-step ketene dimerization/hydrogenation process employing hexanoyl chloride **1a** to access β-lactone **3a** (Scheme 2). Following ketene dimerization by the method of Calter[7] with quinidine (**9**, Figure 3) as nucleophilic catalyst and simple filtration to remove amine hydrochloride salts, the reaction mixture was transferred to a hydrogenation vessel and pressurized to 30 psi of H_2. This procedure provided only modest overall yields of β-lactone **3a** due to apparent degradation of the ketene dimers in the presence of traces of dissolved quaternary ammonium salts, a process with precedent in the literature.[21] Despite careful filtration of the amine salts prior to hydrogenation, extensive degradation of the dimer was always observed. More significantly, the enantiopurity of the ketene dimer (97% → 78% ee at 40% conversion) and thus the enantiopurity of the β-lactone (97% → 74% ee) were found to erode during the hydrogenation, thus rendering this method ineffective for obtaining highly enantiopure β-lactones. The stereochemistry of β-lactone **3a** was assigned based on coupling constant analysis ($J_{cis} \approx 6$ Hz, J_{trans} 4−4.5 Hz)[22] and subsequently confirmed by X-ray analysis of the cyclohexyl-containing β-lactone **3c** (vide infra).

Isolation and Purification of Ketene Dimers. To avoid erosion of optical purity and degradation during the hydrogenation step, we studied the stability of ketene dimers **2** toward isolation and purification. Previous large-scale synthesis and purification of racemic ketene dimers bearing long alkyl chains (>13 carbons) relied on acidic extraction to remove alkylam-

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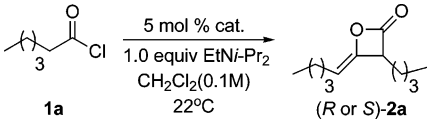
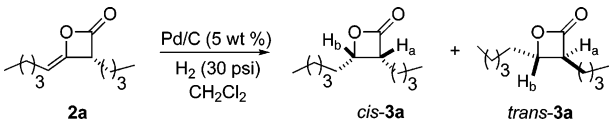
TABLE 1. Effect of Catalyst Structure and Reaction Conditions on Efficiency of Ketene Dimerization Leading to Dimer 2a



entry	catalyst	time (h)	% yield[a]	% ee[c]	config[d]
1	QND (**9**)	24	58	98	S
2	*O*-TBS QND (**11**)	24	54	ND	S
3	*O*-TMS QND (**10**)	24	55	ND	S
4	*O*-TMS QUIN (**12**)	6	62	ND	R
5	*O*-TMS QUIN (**12**)	6	75[b]	96	R

[a] Yields refer to isolated, purified dimer. [b] Freshly, doubly-distilled acid chloride was used. [c] ND = not determined. [d] Absolute configurations are based on the precedent of Calter (ref 5).

TABLE 2. Effect of Catalyst Loading, Base, and Reaction Time on Diastereoselectivity and Enantioselectivity[a]



entry	mol % Pd/C	mol % Et_3N	time (h)	% yield	dr[b]
1	5	0	24	88	4/1
2	5	100	24	92	17/1
3	5	0	0.5	89	17:1
4	1	0	0.5	90	>19:1[c]

[a] Reactions were conducted at 0.1 M in CH_2Cl_2. [b] Ratios estimated by ^1H NMR (500 MHz) integration of H_a and H_b in crude reaction mixtures. [c] Enantiomeric excess of *cis*-**3a** was determined by GC analysis to be 98% ee.

monium salts followed by vacuum distillation, and this could potentially be applied to the asymmetric process described herein.[23] However, in efforts to develop a procedure that would be tolerant of acid-sensitive functionality and avoid extensive heating, we studied the stability of ketene dimers to silica gel chromatography. Indeed, we were able to purify all ketene dimers by normal silica gel flash chromatography. Ketene dimer **2a** possessed sufficient stability to allow determination of its enantiomeric purity by gas chromatography using cyclodextrin bis-*O*TBS as chiral stationary phase.[24] β-Lactones (*R*)-**2a** and (*S*)-**2a** were obtained in high optical purity by use of either QND (**9**, >98% ee) or *O*-TMS QUIN (**12**, >96% ee), respectively (Table 1, entries 1 and 5). The enantiopurity of other dimers was determined following hydrogenation to the *cis*-β-lactones (vide infra). Silylated alkaloids gave comparable yields of ketene dimer **3a** compared to *in situ* generated, acetylated derivatives (Table 1, entry 1 vs 2).[25] A slight improvement in yield was realized when shorter reaction times were employed (6 vs 24 h), consistent with our finding that the ketene dimer is unstable to prolonged exposure to trialkylammonium salts (Table 1, entries 1−3 vs 4). The yield of ketene dimer could be improved further by use of doubly-distilled acid chloride (75% vs 62% yield, Table 1, entry 4 vs 5). While our work was in progress, Calter reported that ketene dimers derived from propanoyl chloride and malonyl chloride half-ester could be purified by rapid filtration through silica gel.[7] The ability to isolate and purify optically active ketene dimers **2** greatly extends the potential utility of these intermediates for β-lactone synthesis, allowing for subsequent transformations including epoxidation as reported by us recently, leading to novel spiro-epoxy-β-lactones.[26]

Hydrogenation of Ketene Dimers. We next studied the hydrogenation of the isolated ketene dimers. Prior hydrogenation studies of diketenes have primarily focused on the parent diketene, 4-methylene-2-oxetanone, in both racemic and asymmetric fashion, as a means to obtain the corresponding 4-methyl-2-oxetanone, a commodity chemical utilized on ton scale for polymer applications.[27] Several catalysts have been utilized for hydrogenation of enol ethers;[28] however, for simplicity and practicality, palladium on carbon was studied initially with racemic ketene dimer **2a**. At the outset, we expected high facial selectivity for the hydrogenation due to the proximity of the alkene to the α-stereogenic carbon at C3 of the β-lactone. However, hydrogenation of dimer **2a** employing 5 mol % Pd/C (5 wt %) resulted in high yield but low diastereoselectivity, providing a mixture of *cis*- and *trans*-β-lactones **3a** (4:1 favoring *cis*) after a 24 h reaction time (entry 1, Table 2). Addition of 100 mol % triethylamine relative to Pd catalyst, in an attempt to reduce the activity of the catalyst, improved the diastereoselectivity to 17:1 with the same reaction time (entry 2, Table 2). However, repeating these conditions with optically active dimer **2a** (98% ee) indicated that racemization was occurring under these conditions necessarily at the dimer stage, providing *cis*-β-lactone **3a** with reduced enantiopurity (71% ee). Shortening the reaction time using 5 mol % catalyst without added Et_3N also gave high diastereoselectivity (entry 3, Table 2). Taken together, these results suggest that both longer reaction times and the absence of catalyst poison leads to erosion in diastereoselectivity. Optimal conditions that prevented racemization and maintained high diastereoselectivity were eventually realized by decreasing the amount of Pd/C to 1 mol % and reducing the reaction time to 30 min under 30 psi of H_2 pressure without Et_3N. Under these conditions, no racemization or epimerization was observed for either the ketene dimer or the major diastereomer isolated, *cis*-β-lactone **3a**. The latter could be isolated in 90% yield as a >19:1 mixture of *cis*/*trans* diastereomers **3a** as determined by coupling constant analysis (**3a**, $J_{Ha,Hb}$ = 6.3 Hz) in 96% ee (Table 2, entry 4).[29] In this manner, hydrogenation of a series of ketene dimers **2a**−**f** gave consistently high yields of the corresponding β-lactones **3a**−**f** with high enantiomeric purities (Table 3). Enantiomer ratios were determined by chiral GC analysis following hydrogenation. Racemic β-lactone **3c** was crystalline, and thus, X-ray analysis verified the *cis* stereochemistry obtained during hydrogenation and suggested by coupling constant analysis for the entire series (Figure 4).

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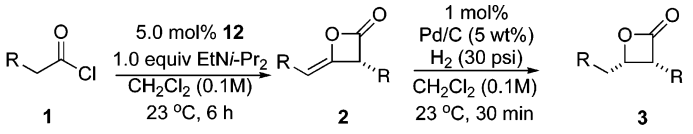
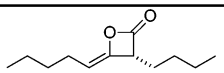
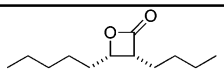
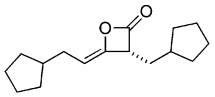
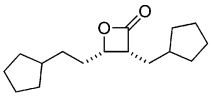
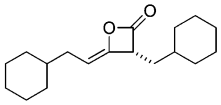
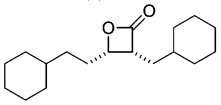
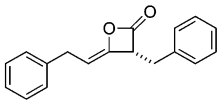
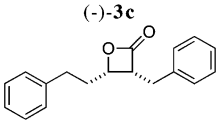
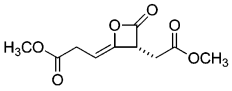
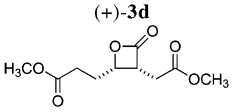
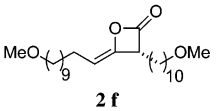
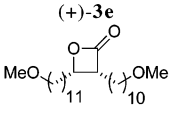
(29) Samples taken at 2, 4, 6, 12, and 18 h revealed no erosion of enantiomeric purity by chiral GC analysis for both ketene dimer **2a** and *cis*-β-lactone **3a**.

TABLE 3. Catalytic Asymmetric β-Lactone Synthesis via a Sequential, Two-Step Ketene Dimerization/Hydrogenation Sequence[a]



1 2 3

entry	R	ketene dimer (**2**)	% yield (**2**)[b]	β-lactone (**3**)	% yield (**3**)[c]	% ee (**3**)[d]
1	*n*-butyl	 **2 a**	75	 **3 a**	90	96
2	cyclopentyl	 **2 b**	54	 (-)-**3b**	89	94
3	cyclohexyl	 **2 c**	55	 (-)-**3c**	89	90
4	benzyl	 **2 d**	48	 (+)-**3d**	85	96
5	CH_2CO_2Me	 **2 e**	60[e]	 (+)-**3e**	94	92
6	11-methoxy nonyl	 **2 f**	62[e]	 (-)-**3f**	94[f]	ND[g]

[a] All reactions were carried out at 0.1 M (final concentration) with freshly distilled acid chloride. [b] Refers to isolated, purified yields. [c] Enantiomeric excess was determined by chiral GC analysis. [d] Absolute configuration of the major enantiomer is depicted and based on the previously determined absolute configuration of ketene dimers (ref 7). [e] Reaction time was 3 h at 0 °C. [f] Reaction was performed with 5 mol % Pd/C for 3.5 h. [g] ND = not determined.

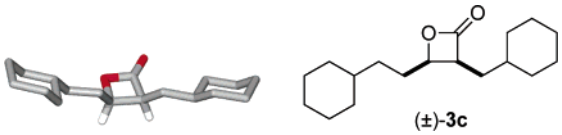
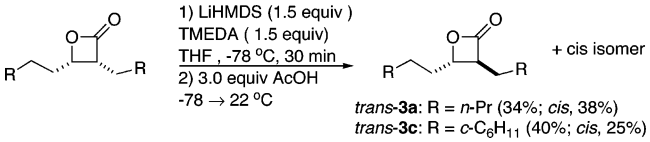


FIGURE 4. X-ray crystal structure (POV Chem rendering) of (±)-*cis*-β-lactone **3c**.

SCHEME 3. Epimerization of *cis*-β-Lactones to the *trans*-Isomers



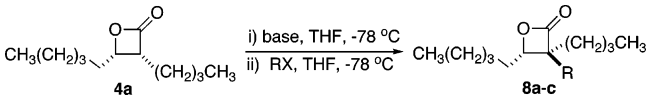
α-Epimerization of *cis*- to *trans*-β-Lactones. As described above, the majority of naturally occurring β-lactones possess *trans*-β-lactone stereochemistry including orlistat (see Figure 2). Thus, we explored conditions to epimerize to the thermodynamically preferred *trans*-β-lactones. Deprotonation leading to β-lactone enolates is possible since β-elimination in these systems is a symmetry forbidden process.[30] Competing intermolecular Claisen condensations can be precluded provided there is an α-substituent; however, in some cases, low yields of α-monoalkylated systems have been obtained using inverse addition with highly reactive electrophiles.[31] Numerous thermodynamic conditions to effect epimerization were studied including (a) triethylamine/ammonium acetate buffer system in dichloromethane (~10−15% conversion by ^{1}H NMR analysis after prolonged stirring for 48 h at 23 °C), (b) *t*-BuOK in *t*-BuOH, (c) DABCO, (d) DBU, 1,2-dichlorobenzene, or acetonitrile, (e) HMDS, THF, (f) catalytic KHMDS/18-crown-6/THF, (g) *t*-BuOK/HMDS/THF, (h) *t*-BuOK/HMDS/18-crown-6/THF. However, all these conditions gave low conversion to the *trans*-isomers along with decomposition. Ultimately, an unsatisfying but functional procedure was developed involving kinetic deprotonation and quenching to provide a mixture of *cis*- and *trans*-β-lactones, which could then be separated by silica gel chromatography to deliver pure *trans*-isomers **3a** and **3c** (Scheme 3).

Alkylation/Acylation of *cis*-β-Lactones. Enolization with lithium hexamethyldisilazide (LiHMDS) followed by addition of various electrophiles proved more fruitful and allowed access to β-lactones bearing α-quaternary centers. Alkylation proceeded with high diastereoselectivity when benzyl bromide was employed (entries 2 and 3, Table 4), whereas the smaller electrophile, methyl iodide, provided only moderate diastereoselectivity (entry 1, Table 4), as previously observed for β-lactones with moderately sized β-substituents.[32] The relative stereochemistry of the major diastereomer of benzyl-β-lactone **8b** was determined by nOe analysis.[33] In the case of acylation with benzyl

(30) Mulzer, J.; Zippel, M.; Bruentrup, G.; Segner, J.; Finke, J. *Liebigs Ann. Chem.* **1980**, 1108.

(31) (a) Parsons, P. J.; Cowell, J. *Synlett* **2000**, 107. (b) Hanessian, S.; Tehim, A.; Chen, P. *J. Org. Chem.* **1993**, *58*, 7768.

TABLE 4. Diastereoselective Alkylations and Acylations of *cis*-β-Lactone 4a Leading to Quaternary Carbon Bearing β-Lactones 8a−c



entry	R	cmpd no.	base	% yield[a]	dr[b]
1	CH_3	**8a**	LiHMDS	73	6:1
2	Bn	**8b**	LiHMDS	88	19:1
3	CO_2Bn	**8c**	LiHMDS	68	8:1
			NaHMDS	74	19:1

[a] Refers to isolated, purified yield. [b] Diastereomeric ratio was determined by integration (^{1}H NMR 500 MHz) of crude reaction mixtures.

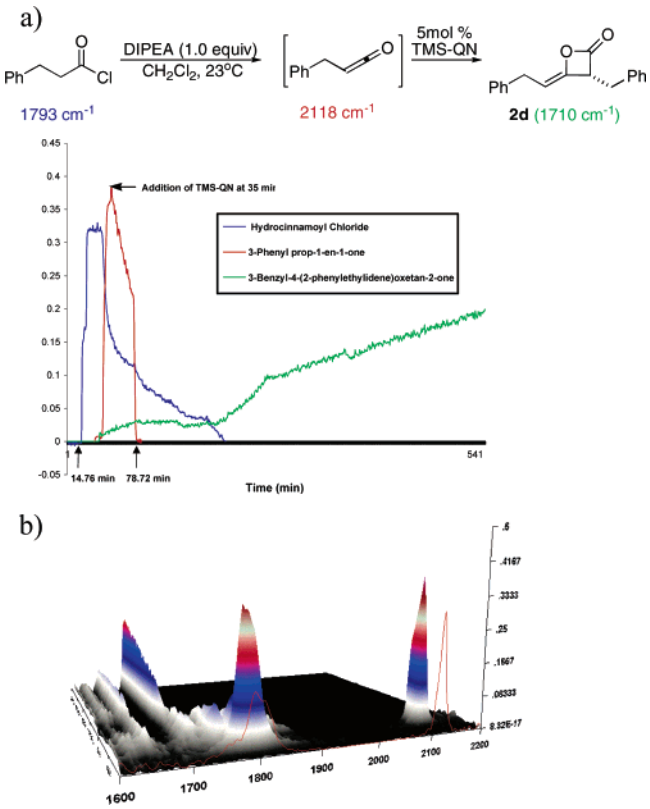


FIGURE 5. Reaction View IR monitoring (1600−2200 cm^{-1}, t = 0−540 min) of the ketene dimerization process leading to ketene dimer **2d** under Reaction Condition I from hydrocinnamoyl chloride: (a) normalized Lorentz curve fit for absorbance vs time (min) for three distinct species (acid chloride, ketene, and ketene dimer **2d**) for the dimerization reaction of hydrocinnamoyl chloride. (b) Mid-IR ReactionView spectrum for dimerization of 3-phenyl prop-1-en-1-one.

chloroformate, the use of sodium hexamethyldisilazide (NaHMDS) gave improved yields and diastereoselectivities relative to LiHMDS (entry 3, Table 4).

Monitoring Ketene Formation and Dimerization of Hydrocinnamoyl Chloride by ReactionView in Situ Mid-Infrared (IR) Spectroscopy. The formation and dimerization of 3-phenyl-1-propen-2-one derived from hydrocinnamoyl chloride by the action of Hünig's base was monitored in efforts to detect intermediates by mid-IR spectroscopy and follow the

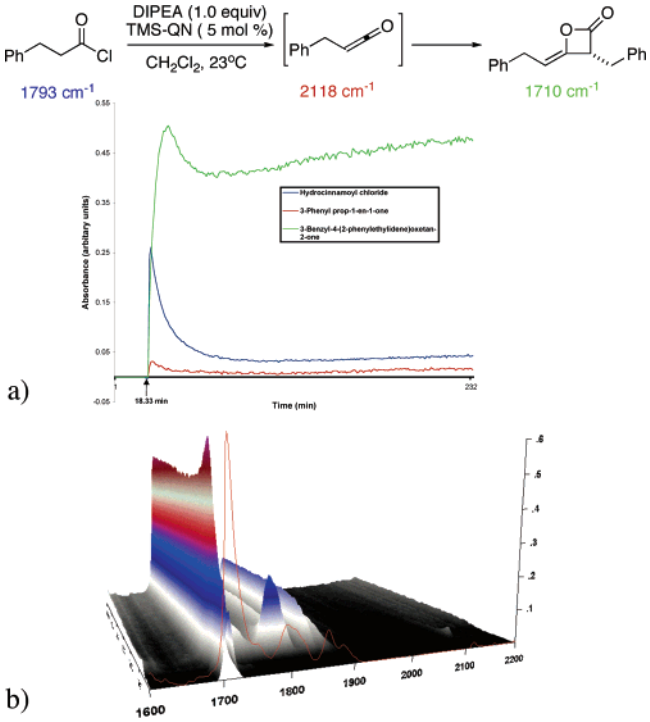


FIGURE 6. ReactionView IR monitoring (1600−2200 cm^{-1}, t = 0−232 min) of the ketene dimerization process leading to ketene dimer **2d** under Reaction Condition II from hydrocinnamoyl chloride: (a) normalized Lorentz curve fit for absorbance vs time (min) for three distinct species. (b) Mid-IR ReactionView spectrum for dimerization of 3-phenyl prop-1-en-1-one.

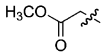
overall dimerization process. Figures 5 and 6 show results of ReactionView IR monitoring for the overall dimerization reaction of hydrocinnamoyl chloride in CH_2Cl_2 (0.1 M) leading to ketene dimer **2d**. Under Reaction Conditions I, the nucleophilic catalyst, TMS-QND (**10**), is added 5 min after the addition of Et*N*i-Pr$_2$ (Hünig's, DIPEA) base at 30 min (Figure 5). Under these conditions, three distinct species (i) hydrocinnamoyl chloride (1793 cm^{-1}), (ii) 3-phenyl prop-1-en-1-one (2118 cm^{-1}), and (iii) 3-benzyl-4-(2-phenylethylidene)oxetan-2-one (**2d**, 1710 cm^{-1}) were detected and monitored over a period of 540 min. The data are plotted as absorbance versus time in a two-dimensional format (Figure 5a) and three-dimensional format (Figure 5b) following peak fitting (ReactionView software). Upon addition of 1.0 equiv of Hünig's base at t = 30 min to the hydrocinnamoyl chloride, ketene generation is immediately initiated, and after 5 min, a 50% decrease in absorbance for hydrocinnamoyl chloride is observed. Separate experiments revealed that ketene was stable at this temperature for at least 15 min. Subsequent addition of TMS-QND (**10**) at t = 35 min completely consumes the ketene after 44 min. However, a brief induction period is observed wherein ketene dimer formation is retarded until hydrocinnamoyl chloride is almost consumed at t =160 min. It is clear that under these conditions the formed ketene is rapidly consumed, and following this time, presumably an undetectable amount of ketene is produced slowly from acid chloride, leading to complete conversion to ketene dimer **2d**.

In a second experiment the order of addition of reagents was altered (Reaction Conditions II, Figure 6). Addition of hydrocinnamoyl chloride at t = 18.3 min to a mixture of Hünig's base and TMS-QND immediately produced ketene dimer along with a trace amount of ketene. Under these reaction conditions

(32) For a review on the utility of β-lactones and their application as intermediates in natural product synthesis, see: Wang, Y.; Tennyson, R. L.; Romo, D. *Heterocycles* **2004**, *64*, 605.
(33) See Supporting Information for details.

TABLE 5. Antagonistic Activity of Ketene Dimers 2 and β-Lactones 3 and 8 toward Recombinant FAS TE Compared to Orlistat (1)

entry	cmpd.	R	apparent K_i (μM)
	2a-d		
1	**2a**		17± 3
2	**2b**		13 ± 1
3	**2c**		>100
4	**2d**		5.0 ± 2.1
	trans-**3a-d**		
5	*trans*-**3a**		4.0 ± 1.9
6	*trans*-**3b**		6.7 ± 1.0
7	*trans*-**3c**		6.3 ± 0.7
8	*trans*-**3d**		3.3 ± 0.2

entry	cmpd.	R	apparent K_i (μM)
	cis-**3a-f**		
9	orlistat (**1**)		0.28 ± 0.06
10	*cis*-**3a**		23 ± 4
11	*cis*-**3b**		14 ± 8
12	*cis*-**3c**		7.7 ± 0.4
13	*cis*-**3d**		2.5 ± 0.5
14	*cis*-**3e**		>100
15	*cis*-**3f**		35 ± 5
	8a-c		
16	**8a**	CH_3	15 ± 4
17	**8b**	$PhCH_2$	19 ± 1
18	**8c**	CO_2Bn	10 ± 3

it appears that the rate of ketene consumption exceeds that of ketene generation in accordance with the results above and also those observed by Calter,[7] and thus, the ketene resonance is fleeting on the time scale of mid-IR measurements. These ReactionView studies provide further evidence for a rate-determining ketene generation step followed by rapid ketene dimerization as found previously by Calter during rate studies of the ketene dimerization process.[7a]

Enzyme Inhibition Studies. A recombinant form of the thioesterase domain of fatty acid synthase was used in a substrate-based screen to measure the apparent K_i's of ketene dimers **2a**−**d** and both *cis*-*β*-lactone and *trans*-isomers of the derived simplified orlistat derivatives. Importantly, orlistat is presumed to be a covalent inhibitor of the FAS TE domain, in analogy to the adduct formed with the active site serine of pancreatic lipase.[34] We presume that all of the *β*-lactones examined in this study function via the same mechanism. Therefore, the results are reported as apparent inhibition constants (app K_i), as the term K_i is usually used for reversible inhibitors. 4-Methylumbelliferyl heptanoate (4-MUH) was utilized as a substrate for FAS TE as it was found to provide the best signal-to-noise ratio and an acceptable turnover rate. The product of this substrate, 4-methylumbelliferone, fluoresces at 450 nM (excitation at 350 nM) and provides a convenient readout of thioesterase activity. In this assay, the hydrolysis of 4-MUH is blocked by orlistat, our lead antagonist with an apparent K_i of 0.21 μM. The ability of the various *β*-lactones and some ketene dimer precursors described above to act as antagonists of 4-MUH toward recombinant FAS TE was

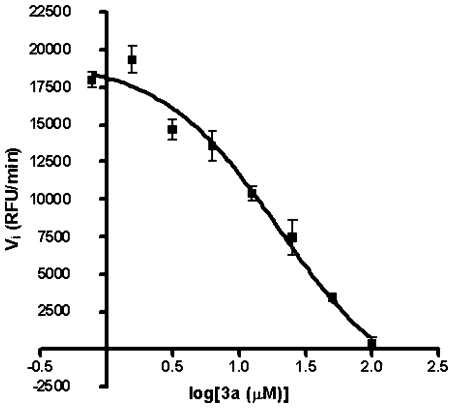


FIGURE 7. Dose−response curve illustrating the inhibition of FAS TE by *trans*-*β*-lactone **3a** in a fluorogenic assay.

(34) (a) Borgstrom, B. *Biochim. Biophys. Acta* **1988**, *962*, 308. (b) Hadvary, P.; Lengsfeld, H.; Wolfer, H. *J. Biol. Chem.* **1988**, *256*, 357. (c) Hadvary, P.; Sidler, W.; Meister, W.; Vetter, W.; Wolfer, H. *Biochem. J.* **1991**, *266*, 2021.

measured by this assay (Table 5). The β-lactones were tested across a concentration range of 1−100 μM, and the dose−response curve for *trans*-β-lactone **3a** is shown as a representative example (Figure 7).

Ketene dimers were tested and showed moderate activity (5−16 μM) with the exception of dimer **2c** which showed no activity (Table 5, entries 1−4). The most potent ketene dimer tested was phenyl derivative **2d** with an app K_i of ∼5.0 μM, which is approximately 25-fold less potent than orlistat.

In the *cis*-isomeric series (Table 5, entries 9−15), addition of an ester substituent in proximity to the β-lactone nucleus led to a drastic reduction in activity (Table 5, entry 14). However, a remote methoxy substituent as in β-lactone *cis*-**3f** (entry 15) restored activity comparable to that observed for the *n*-butyl-substituted β-lactone, *cis*-**3a** (entry 10).[35] As expected, the *trans*-isomers, in general, were found to possess greater inhibitory activity compared to the *cis*-isomers with the same substituents (entries 5−8 vs 10−13). β-Lactones **8a**−**c** bearing α-quaternary carbons showed moderate activity (10−19 μM) but presumably also lead to covalent adducts suggestive of the possibility that the thioesterase domain is not selective for only 3,4-disubstituted-β-lactones.

In the case of the phenyl-substituted ketene dimer **2d** (5.0 μM), a ∼2-fold increase in activity to 2.5 μM was observed following hydrogenation to give the phenyl-substituted *cis*-β-lactone **3d** (Table 5, entry13). Surprisingly, little variation in activity is observed (3.3 μM, entry 8) when this derivative is epimerized to *trans*-β-lactone **3d** possessing the relative and absolute stereochemistry corresponding to that found in orlistat.

Although none of the simple 3,4-dialkyl-β-lactones described herein were found to be as potent as orlistat, the findings are significant since these are highly simplified β-lactones devoid of the *N*-formyl aminoester side chain. These polar substituents are expected to serve as additional recognition sites via potential hydrogen-bonding and polar−polar interactions to FAS TE. In addition, they should also contribute to water solubility. However, a promising lead compound for further optimization is the phenyl-substituted derivative *trans*-**3d**.

In summary, we developed a scaleable process to prepare *cis*-3,4-disubstituted β-lactones using a ketene dimerization/hydrogenation sequence from readily available acid chlorides in good overall yields and high enantioselectivity. This reaction could be run as a single-pot, two-step process, but higher overall yields and optical purities were obtained upon isolation of the ketene dimer by silica gel chromatography and subsequent hydrogenation of the purified ketene dimer at moderate pressures. Several ketene dimers were isolable, and their enantiomeric purity could be determined by chiral GC analysis. A current limitation in this process is the accessibility of only pseudosymmetric ketene dimers **2** via the Calter procedure leading to pseudosymmetric β-lactones **3**. Enolization followed by alkylation and acylation of the *cis*-β-lactones provided ready access to α,α-disubstituted β-lactones with high diastereoselectivities. *trans*-β-Lactones could also be obtained by α-epimerization in low yield; however, a general, practical solution for this process remains elusive. These highly simplified dialkyl-β-lactones are analogous to the natural products norcardiolactone, valilactone, and orlistat and were found to exhibit moderate to good inhibitory activity toward recombinant FAS TE as measured by enzymatic activity using recombinant protein with a fluorogenic substrate. The best antagonist, *cis*-β-lactone **3d**, displayed an app K_i of only ∼10-fold less than that of orlistat. In addition, analysis of the ketene dimerization process by ReactionView IR spectroscopy further substantiates findings by Calter that ketene formation is rate determining in the catalyzed ketene dimerization process. Further transformations of these optically pure ketene homodimers are being explored as a means to provide practical routes to β-lactones more structurally analogous to orlistat and expected to have higher affinity for FAS TE. In addition, the use of hetero-ketene dimers will expand the β-lactone structures accessible by this strategy.

Experimental Section

General Experimental Procedure for Dimerization As Described for (*R*,*Z*)-3-Butyl-4-pentylideneoxetan-2-one (2a). This procedure is slightly modified from the method of Calter.[7a] To a flame-dried 1 L round-bottomed flask was added 764 mg (5 mol %, 1.926 mmol) of TMS-quinine, 385 mL of CH_2Cl_2 (0.1 M), and 6.86 mL (1.0 equiv, 38.53 mmol) of Hünig's base under nitrogen atmosphere at 22 °C. To this colorless solution, 5 mL (5.18 g, 38.53 mmol) of freshly double-distilled, hexanoyl chloride was added over 15 min via syringe. After 6 h the dark yellow solution was concentrated down to 100 mL (one-fifth original volume) in vacuo and 250 mL of pentanes was added to precipitate the ammonium salts. Filtration through Whatmann filter paper (#1, qualitative grade), concentration in vacuo, and purification by flash column chromatography on deactivated SiO_2 (10% H_2O) (2.5 cm × 35.0 cm column, 15 cm pad) eluting with 0−20% Et_2O:hexanes gave 2.83 g (75%) of **2a** as a colorless oil (96% ee, chiral GC analysis). R_f 0.54 (15% Et_2O:hexanes); $[\alpha]_D^{26}$ +5.3 (c = 0.51, $CHCl_3$); IR (thin film) 1865 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.70 (dt, J = 1.3, 6.3 Hz, 1H), 3.94 (dt, J = 1.0, 7.0 Hz, 1H), 2.13 (app q, 2H), 1.75−1.83 (m, 2H), 1.28−1.53 (m, 8H), 0.88−0.96 (m, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.5, 14.6, 22.9, 23.1, 25.1, 28.0, 29.2, 32.3, 54.45, 102.4, 146.4, 170.6; ESI LRMS calcd for $C_{12}H_{20}O_2$ [M + Li], 202; found, 202.

Racemic ketene dimers were initially prepared in a similar manner using Hünig's base. Subsequently, 5 mol % quinuclidine hydrochloride with 1.0 equiv of Hünig's base gave optimal results in terms of reaction rate and yield; therefore, this method was subsequently used for preparation of racemic ketene dimers.

Representative Procedure for Hydrogenation As Described for *cis*-(3*R*,4*S*)-3-Butyl-4-pentyloxetan-2-one (3a). The purified *n*-butyl ketene dimer **2a** (5.1 mmol, 1.0 g) was dissolved in 40 mL of dichloromethane (0.1 M) and transferred to a Parr bomb apparatus under a N_2 atmosphere using an additional 10 mL of dichloromethane as wash solvent. Pd/C (107 mg, 1 mol %, 5 wt %) was then added at one time under N_2 atmosphere. The Parr bomb was then subjected to three consecutive evacuation−saturation cycles of hydrogen gas and then pressurized to 30 psi hydrogen gas pressure. Hydrogenation with shaking (Parr shaker) was continued for 30 min at this pressure, and then the heterogeneous slurry was vacuum filtered through a plug of Celite and concentrated, yielding a colorless oil. Flash chromatography with gradient elution (5−15% diethyl ether/hexanes; 2.5 × 35.0 × 5 cm pad) gave 3-butyl-4-pentyl-oxetan-2-one (**3a**, 897 mg, 90%) as a

(35) For previous studies of the impact of β-lactone stereochemistry and substitution on enzyme inhibitory activity, see: (HMG−CoA synthase) (a) Tomoda, H.; Ohbayashi, N.; Kumagai, H.; Hashizume, H.; Sunazuka, T.; Omura, S. *Biochem. Biophys. Res. Commun.* **1999**, *265*, 536. (b) Romo, D.; Harrison, P. H. M.; Jenkins, S. I.; Riddoch, R. W.; Park, K.; Yang, H. W.; Zhao, C.; Wright, G. D. *Bioorg. Med. Chem.* **1998**, *6*, 1255. (c) Tomoda, H.; Kumagai, H.; Ogawa, Y.; Sunazuka, T.; Hashizume, H.; Nagashima, H.; Omura, S. *J. Org. Chem.* **1997**, *62*, 2161. (d) Mayer, R. J.; Louis-Flamberg, P.; Elliott, J. D.; Fisher, M.; Leber, J. *Biochem. Biophys. Res. Commun.* **1990**, *169*, 610. (e) Romo, D.; Harrison, P. H. M.; Jenkins, S. I.; Riddoch, R. W.; Park, K.; Yang, H. W.; Zhao, C.; Wright, G. D. *Bioorg. Med. Chem.* **1998**, *6*, 1255. (proteasome) (f) Macherla, V. R.; Mitchell, S. S.; Manam, R. R.; Reed, K. A.; Chao, T. H.; Nicholson, B.; Deyanat-Yazdi, G.; Mai, B.; Jensen, P. R.; Fenical, W. F.; Neuteboom, S. T. C.; Lam, K. S.; Palladino, M. A.; Potts, B. C. M. *J. Med. Chem.* **2005**, *48*, 3684.

colorless oil (>96% ee, chiral GC): $[\alpha]_D^{26}$ −27.1 (c = 0.5, $CHCl_3$); R_f 0.47 (15% Et_2O:hexanes); IR (thin film) 1824 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.54 (ddd, J = 2.1, 3.6, 5.7 Hz, 1H), 3.59 (ddd, J = 4.5, 5.4, 8.4 Hz, 1H), 1.46−1.84 (m, 6H), 1.31−1.44 (m, 8H), 0.88−0.94 (m, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 14.5, 14.7, 23.2, 23.2, 24.4, 26.0, 30.5, 30.9, 32.2, 53.3, 76.5, 173.1; ESI LRMS calcd for $C_{12}H_{22}O_2$ [M + Li], 205; found, 205.

The enantiomeric purity of ketene dimer **2a** and β-lactone **3a** was determined to be >96% ee by chiral GC analysis. Column type, chiral bis-OTBS-cyclodextrin; retention time, t_{dimer} 16.96 (major) and 17.16 (minor), $t_{\beta-lactone}$ 26.23 (major) and 26.44 (minor). Conditions: make up flow, 25 mL/min; H_2 flow, 30 mL/min; air flow, 300 mL/min; injector temperature, 200 °C, pressure, 5 psi (hold time 30 min); oven temperature gradient, 100 →140 °C (hold time 30 min); detector temperature, 250 °C.

***trans*-(3*R*,4*R*)-3-Butyl-4-pentyloxetan-2-one (*trans*-3a).** To a −78 °C solution of 100 mg (0.51 mmol) of **3a** in 5 mL of THF was added 760 μL of LiHMDS (1.5 equiv, 1.0 M in THF), and this was allowed to stir for 1 h. Tetramethylenediamine (TMEDA, 120 μL, 1.5 equiv, 0.76 mmol) was then added at −78 °C and allowed to stir for an additional 30 min, after which the solution was quenched with glacial acetic acid (130 μL, 3.0 equiv, 2.27 mmol) and warmed to 22 °C. After extraction with diethyl ether (2 × 6 mL), the combined organics were washed with 2 mL of pH 7.0 buffer and 2 mL of brine and then dried over Na_2SO_4. Concentration in vacuo gave a colorless oil, which upon purification by flash chromatography on SiO_2 (15% Et_2O:hexanes) gave *cis*-**3a** and *trans*-**3a** (72 mg, 72% yield) as a 1:1 mixture of diastereomers. Further purification by gravity column chromatography (2×, 5% Et_2O:hexanes) delivered 34 mg (34%) of *trans*-**3a** and 38 mg of *cis*-**3a** (38%): R_f 0.38 (10% Et_2O:hexanes; *cis*-**3a** R_f 0.29); IR (thin film) 1824 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.22 (ddd, J = 4.2, 6.0, 7.5 Hz, 1H), 3.16 (ddd, J = 3.9, 6.6, 9.0 Hz, 1H), 1.67−1.93 (m, 4H), 1.26−1.49 (m, 10H), 0.91 (bs, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) 14.5, 14.7, 23.1, 23.2, 25.4, 28.3, 29.9, 32.1, 35.2, 56.9, 78.9, 172.5; ESI LRMS calcd for $C_{12}H_{22}O_2$ [M + Li], 205; found, 205.

***trans*-(3*S*,4*S*)-4-(2-Cyclohexylethyl)-3-(cyclohexylmethyl)oxetan-2-one (*trans*-3c).** To a −78 °C solution of 30 mg (0.108 mmol) of *cis*-β-lactone **3c** in 3 mL of THF was added 162 μL of LiHMDS (1.5 equiv, 1.0 M in THF), and this was stirred for 1 h. Tetramethylenediamine (TMEDA, 25 μL, 1.5 equiv, 0.162 mmol) was then added at −78 °C and allowed to stir for an additional 30 min, after which the solution was quenched with glacial acetic acid (18 μL, 3.0 equiv, 0.323 mmol) and warmed to 22 °C. After extraction with diethyl ether (2 × 3 mL), the combined organics were washed with 2 mL of pH 7.0 buffer and 2 mL of brine and then dried over Na_2SO_4. Concentration in vacuo gave a colorless oil that was purified by flash chromatography on SiO_2 (10% Et_2O: hexanes) to give a mixture of *cis*- and *trans*-**3c** (19.8 mg, 66% yield). Further purification by gravity column chromatography (10% Et_2O:hexanes) delivered 12 mg (40%) of *trans*-**3c** and 7.6 mg of *cis*-**3c** (25%): R_f 0.47 (10% Et_2O:hexanes; *cis*-**3c**, R_f 0.40); IR (thin film) 1824 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.17 (ddd, J = 4.2, 6.0, 7.4 Hz, 1H), 3.24 (ddd, J = 4.0, 6.5, 9.0 Hz, 1H), 1.79−1.89 (m, 1H), 1.59−1.78 (m, 12H), 1.30−1.39 (m, 1H), 1.07−1.30 (m, 10H), 0.82−0.99 (m, 4H); ^{13}C NMR (300 MHz, $CDCl_3$) 15.4, 26.1, 26.2, 26.3, 26.4, 26.6, 29.8, 31.9, 32.6, 32.9, 33.2, 33.3, 35.9, 37.4, 54.2, 66.0, 79.4, 172.3; ESI LRMS calcd for $C_{18}H_{30}O_2$ [M + Li], 285; found, 285.

(3*R*,4*S*)-3-Butyl-3-methyl-4-pentyloxetan-2-one (8a). A solution of β-lactone **3a** (36.3 mg, 0.1835 mmol) in 1.9 mL of THF was cooled to −78 °C, and 370 μL of LiHMDS (0.367 mmol, 2.0 equiv, 1.0 M solution in THF) was added under a nitrogen atmosphere. After 1.5 h, 23 μL (0.367 mmol, 2.0 equiv) of iodomethane was added and the reaction warmed to −40 °C and stirred for an additional 45 min. The reaction mixture was concentrated in vacuo and purified by flash chromatography (0−15% Et_2O:hexanes) to give β-lactone **8a** (28 mg, 73%) as a colorless oil and a mixture of cis/trans diastereomers (dr, 6:1). Data provided for major diastereomer: R_f (15% Et_2O:hexanes) 0.64; IR (thin film) 1824 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.18 (dd (major diast.), J = 6.0, 8.5 Hz, 1H), 1.63−1.78 (m, 2H), 1.45−1.53 (m, 2H), 1.39 (s, 3H), 1.24−1.39 (m, 10H), 0.89−0.94 (m, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) 14.1, 14.2, 20.0, 22.1, 25.5, 25.6, 26.5, 30.3, 31.7, 35.9, 56.8, 84.5, 175.5; ESI LRMS calcd for $C_{13}H_{24}O_2Li$ [M + Li], 219; found, 219.

(3*S*,4*S*)-3-Benzyl-3-butyl-4-pentyloxetan-2-one (8b). To a −78 °C solution of β-lactone **3a** (153 mg, 0.76 mmol) dissolved in 7.5 mL of THF was added 1.52 mL of LiHMDS (1.52 mmol, 2.0 equiv, 1.0 M solution in THF) under nitrogen atmosphere. After 1.5 h, 180 μL (1.52 mmol, 2.0 equiv) of benzyl bromide was added and the reaction warmed to −40 °C and stirred for an additional 45 min. The reaction mixture was concentrated in vacuo and purified by flash chromatography (0−5% Et_2O:hexanes) to give β-lactone **8b** (192 mg, 88%) as a mixture of cis/trans diastereomers (>19:1). Data provided for major diastereomer: R_f 0.55(5% Et_2O:hexanes); IR (thin film) 1813 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.30−7.34 (m, 2H), 7.25−7.28 (m, 1H),7.16−7.17 (m, 2H), 4.34 (dd, J = 4.5, 9.5 Hz, 1H), 3.13, 2.88 (AB q, J = 14.5 Hz, 2H), 1.68−1.78 (m, 2H), 1.45−1.61 (m, 4H), 1.18−1.39 (m, 8H), 0.94 (t, J = 3.5, 3H), 0.87 (t, J = 3.5, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) 13.87, 13.89, 22.4, 23.2, 25.2, 26.3, 28.7, 29.7, 31.4, 38.2, 61.3, 80.3, 127.1(2C), 128.7(2C), 129.8, 135.8, 174.2; ESI LRMS calcd for $C_{19}H_{28}O_2$ [M + Li], 295; found, 295.

(3*S*,4*S*)-Benzyl 3-butyl-2-oxo-4-pentyloxetane-3-carboxylate (8c). To a −78 °C solution of NaHMDS (1.2 equiv, 0.91 mmol, 45 μL, 2M in THF) in 5 mL of THF was added 150 mg (0.73 mmol) of β-lactone **3a** dissolved in 2.5 mL of THF. After 1.5 h, benzylchloroformate (1.1 equiv, 0.833 mmol, 120 μL) was added at one time and stirred for an additional 3 h. This solution was warmed to 23 °C over 1 h and worked up as described above for β-lactone **8b**. Flash chromatography on SiO_2 (5% Et_2O:hexanes) gave β-lactone **8c** (185 mg, 74% yield) as a colorless oil: R_f 0.48 (15% Et_2O:hexanes); IR (thin film) 1824, 1757, 1716 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40 (m, 5H), 5.27, 5.22 (AB q, J = 12.0, 2H), 4.52 (dd, J = 4.8, 8.4 Hz, 1H), 2.50−2.56 (m, 1H), 2.22−2.30 (m, 2H), 1.71−1.85 (m, 1H), 1.44−1.60 (m, 4H), 1.15−1.39 (m, 8H), 0.81−0.92 (m, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) 14, 14.6, 14.7, 23.1, 23.3, 23.6, 24.9, 25.8, 26.9, 29.4, 30.4, 31.1, 31.9, 32.3, 40.4, 71.6, 79.8, 121.7, 129.2, 129.4, 129.5, 129.8, 133.1, 152.7, 163.7, 167.3; ESI LRMS calcd for $C_{20}H_{28}LiO_4^+$ [M + Li], 339; found, 338.

General Experimental Procedure for in Situ Mid-IR Spectroscopy with a RemSpec ReactionView System. A RemSpec Reaction View system was fitted with a double-pass liquid transmission head that was placed into a flame-dried two-necked 20 × 2.3 cm reaction tube equipped with a spin vane stir bar, and the reaction flask was placed under a nitrogen atmosphere. Following addition of 30 mL of CH_2Cl_2, a background spectrum was obtained for 15 min and then automatic data collection was initiated and provided 45 scans/min for both experiments at 23 °C. The data collected for hydrocinnamoyl chloride, ketene, and ketene dimer for both experiments were peak fitted with a Lorentzian function on Grams/AI software and normalized on Microsoft Excel to obtain absorbance versus time curves.

Reaction Condition I. Sequential addition of hydrocinnamoyl chloride (0.45 mL, 2.97 mmol; t = 14.5 min) and 565 μL of Hünig's base (2.97 mmol; t = 30 min) gave rise to expected acid chloride and ketene absorbances. After complete consumption of acid chloride, 5 mol % TMS-QN (22 mg) was added as a solution in 1 mL of CH_2Cl_2 in one portion at t = 35 min, and data was collected for an additional 540 min.

Reaction Condition II. In this procedure 5 mol % TMS-QN (22 mg) and 565 μL of Hünig's base was added to 30 mL of CH_2Cl_2. At t = 18.3 min, hydrocinnamoyl chloride (0.45 mL, 2.97 mmol) was added over a period of 5 min and data was collected for an additional 230 min.

Fluorogenic Assay for Detection of Enzyme Inhibition. Expression of the recombinant thioesterase domain of FAS was performed as described previously,[9] and large-scale expression was performed by Invitrogen Corp. (Madison, WI). The synthetic fluorogenic substrate, 4-methylumbelliferyl heptanoate (4-MUH), was purchased from Sigma (St. Louis, MO). The reaction mixture consisted of 45 μL of 500 nM FAS TE in buffer A (100 mM Tris-HCl, 50 mM NaCl at pH 7.4) which was preincubated with 2.5 μL of stock solutions of test β-lactones dissolved in DMSO at final concentrations of 0.32−100 μM at 37 °C for 30 min. The reaction was initiated by addition of 5 μL of 1.25 mM 4-MUH in 1:1 DMSO: buffer A. The resulting fluorescence from liberated 4-methylumbelliferone was measured every 5 min at 350/450 nm for 40−60 min. Results are the average of triplicate time points in which the typical standard deviation was <5%. Each compound was tested at least twice, yielding essentially identical results.

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Supporting Information Available: General procedures for dimerization, hydrogenation, and subsequent transformations with characterization data (including ^{1}H and ^{13}C NMR spectra) for ketene dimers **2a**−**d**, **2f**, β-lactones *cis*-**3a**−**f**, *trans*-**3a**, **3c**, and **8a**−**8c** and nOe data for β-lactone **8b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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