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## Synthesis of Novel $\beta$ -Lactone Inhibitors of Fatty Acid Synthase

Robyn D. Richardson<sup>†</sup>, Gil Ma<sup>‡</sup>, Yatsandra Oyola<sup>‡</sup>, Manuel Zancanella<sup>‡</sup>, Lynn M. Knowles<sup>†</sup>, Piotr Cieplak<sup>†</sup>, Daniel Romo<sup>\*‡</sup>, and Jeffrey W. Smith<sup>\*†</sup>

NCI Cancer Center, The Burnham Institute for Medical Research, 10901 North Torrey Pines Road, La Jolla, California 92037, Department of Chemistry, Texas A & M University, P.O. Box 30012, College Station, Texas 77842

### Abstract

Fatty acid synthase (FAS) is necessary for growth and survival of tumor cells and is a promising drug target for oncology. Here, we report on the syntheses and activity of novel inhibitors of the thioesterase domain of FAS. Using the structure of orlistat as a starting point, which contains a  $\beta$ -lactone as the central pharmacophore, 28 novel congeners were synthesized and examined. Structural features such as the length of the  $\alpha$ - and  $\beta$ -alkyl chains, their chemical composition, and amino ester substitutions were altered and the resulting compounds explored for inhibitory activity toward the thioesterase domain of FAS. Nineteen congeners show improved potency for FAS in biochemical assays relative to orlistat. Three of that subset, including the natural product valilactone, also display an increased potency in inducing tumor cell death and improved solubility compared to orlistat. These findings support the idea that an orlistat congener can be optimized for use in a preclinical drug design and for clinical drug development.

### Introduction

Most human carcinomas, including those of the breast and prostate, overexpress fatty acid synthase (FAS<sup>a</sup>), the sole enzyme responsible for de novo biosynthesis of fatty acids.<sup>1–6</sup> In the vast majority of cases, FAS is required for tumor cell survival and it also seems to play a role in conferring chemoresistance.<sup>7,8</sup> In contrast, most normal cells utilize dietary fats and therefore FAS is not required for survival. Consequently, FAS is a promising drug target for the treatment of human carcinomas.

Orlistat is a pancreatic lipase inhibitor that is currently marketed for the treatment of obesity. In the gut, orlistat forms a covalent, but reversible, bond with the active site serine residue of pancreatic lipases, rendering them unable to hydrolyze dietary fat into free fatty acids and thereby reducing the absorption of dietary fat.<sup>9</sup> Previously, we showed that orlistat is also a potent inhibitor of the thioesterase activity of FAS and that it has antitumor activity in vitro and in vivo.<sup>10</sup> The three-dimensional structure of orlistat bound to FAS shows that the compound forms a covalent adduct with the enzyme's active site serine, the same mechanism by which it inhibits pancreatic lipase.<sup>11</sup>

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\*To whom correspondence should be addressed. For J.W.S.: phone, 858-646-3100; fax, 858-795-5221; jsmith@burnham.org.. For D.R.: phone, 979-845-9571; fax, 979-862-4880; romo@mail.chem.tamu.edu..

<sup>†</sup>NCI Cancer Center, The Burnham Institute for Medical Research.

<sup>‡</sup>Department of Chemistry, Texas A & M University.

**Supporting Information Available:** Purity data for synthesized compounds is available online. This material is available free of charge via the Internet at <http://pubs.acs.org>.

<sup>a</sup>Abbreviations FAS, fatty acid synthase; TE, thioesterase; MUH, methylumbelliferyl heptanoate; TMAL, tandem Mukaiyama aldol-lactonization.

Despite its ability to inhibit FAS and elicit tumor cell death, there are a number of challenges that prevent the deployment of orlistat as an antitumor drug: it has poor solubility and bioavailability and it lacks sufficient potency. Here we sought to take the first step toward the synthesis of an orlistat derivative suitable for use as an antitumor drug. The specific objectives of the present study were to (1) synthesize derivatives of orlistat with increased solubility, (2) determine the structural alterations that can be made to orlistat without loss of activity toward FAS, and (3) identify orlistat derivatives with increased potency toward FAS and increased cytotoxicity toward tumor cells. Twenty-eight novel congeners of orlistat were synthesized, most having increased solubility and inhibitory activity compared to orlistat. The  $\alpha$ - and  $\beta$ -side chains extending from the  $\beta$ -lactone were shown to be amenable to optimization, and alkenyl bonds can be incorporated into their structure without loss of activity. The amino ester can be changed without substantial loss of activity toward FAS. Reversal of chirality at  $C_\alpha$  and  $C_\delta$  from *S* to *R* is tolerated, but compounds with *R*- $C_\beta$  chirality had substantially reduced activity. The results of the study reveal several new FAS inhibitors that are more suitable for preclinical drug development and also provide the foundation for a more detailed structure-based drug design centered on the  $\beta$ -lactone pharmacophore.

## Results and Discussion

### Synthesis of Orlistat Congeners

Twenty-eight congeners of orlistat were synthesized to identify the structural components important for activity toward FAS (Table 1). Four structural features of orlistat were altered in the test set: the  $\alpha$ - and  $\beta$ -side chains, the amino ester, and chirality (Figure 1). The described  $\beta$ -lactones were synthesized employing the tandem Mukaiyama aldol-lactonization (TMAL) process previously developed in our group as the key step.<sup>12</sup> Previously we reported general strategies for the synthesis of orlistat derivatives employing the TMAL process in conjunction with coupling reactions that enable facile modification of both the  $\alpha$ - and  $\delta$ -side chain including cuprate addition, simple alkylation of ethylacetoacetate for subsequent Noyori reduction, and an olefin metathesis/hydrogenation sequence.<sup>13</sup> These methods were also employed to prepare the derivatives studied herein.

The TMAL process employs readily available thiopyridyl ketene acetals **3** and optically active aldehydes **8** and **12**. A series of thiopyridyl ketene acetals **3** were obtained according to previously reported methods involving generation of thiopyridyl esters **2** from the corresponding acid chlorides **1** and an enolization/silylation sequence (Scheme 1).

One route studied for the preparation of optically active  $\beta$ -hydroxy aldehydes **8** made use of the chiral pool and involved cuprate addition to a common tosylate **6** derived from malic acid to install the  $\delta$ -chain, which would enable its facile variation by appropriate choice of cuprate reagent (Scheme 2). Thus, (*S*)-malic acid was converted to tosylate ester **6** in a four-step sequence by following literature procedures (the synthesis of aldehyde **8a** was described previously; see supporting documentation in ref 13). After some experimentation, suitable cuprate conditions were identified and the addition of the octyl Gilman reagent gave ester **7** in 78% yield. However, this process was not readily extended to other cuprates, so an alternative route to optically active aldehydes was studied (vide infra, Scheme 3). Following ester half-reduction, aldehyde **8a** could be obtained in 86% yield.

In an alternative route to optically active aldehydes **8**, simple alkylation of ethylacetoacetate was employed to provide several  $\beta$ -ketoesters **10** and subsequent Noyori reduction, alcohol protection, and ester half-reduction provided aldehydes **8b–g**. This enabled variation of the  $\delta$ -chain of orlistat derivatives in a lengthier but high-yielding route (Scheme 3).

Treatment of various aldehydes **8** in a dichloromethane slurry of freshly fused ZnCl<sub>2</sub> and thioketeneacetals **3** delivered the desired β-lactones as mixtures of *anti/syn* diastereomers (7–9:1, dr) with complete selectivity for the *trans*-β-lactone. The β-lactones were directly desilylated to afford the more readily separable γ-hydroxy β-lactones **13** and **14** (Scheme 4). The *trans* relative stereochemistry of the β-lactone core was verified by analysis of coupling constants ( $J_{\text{Ha,Hb}} \sim 4.0$  Hz) as described previously, whereas the relative stereochemistry of the major diastereomers with respect to the γ-stereocenter (C5) is consistent with Evans' model for additions to β-silyloxy aldehydes as previously observed for these TMAL reactions.<sup>14</sup> Further verification came following completion of the synthesis of orlistat and valilactone and comparison to literature data (*vide infra*). To obtain a *cis*-β-lactone to determine the effect of β-lactone relative stereochemistry on FAS inhibition, we employed our stereocomplimentary approach to β-lactones, which employs SnCl<sub>4</sub> as Lewis acid for the aldol-lactonization process.<sup>15</sup> Reaction of aldehyde **8f** and ketene acetal **3d** under these conditions gave a mixture of diastereomeric *cis*-β-lactones from which the major β-lactone *cis*-**13** could be isolated.

To obtain the required *syn* relative stereochemistry with respect to the β- and γ-stereocenters as found in orlistat, the major diastereomeric hydroxy-β-lactones **13**, possessing the 6*R* configuration, were subjected to Mitsunobu conditions to invert this stereocenter (Scheme 5). This was accomplished with *N*-formyl amino acids and proceeded with the expected inversion of configuration, providing the 3*S*,4*S*,6*S*-diastereomers **15**. To probe the effect of stereochemistry on FAS inhibition, the minor 3*R*,4*R*-diastereomers **14** obtained in the TMAL process were also converted to the 3*R*,4*R*,6*R*-diastereomers **16** via the Mitsunobu reaction. In addition, retention of stereochemistry was possible by simple acylation of β-lactones **13** with *N*-formyl amino acids to provide 3*S*,4*S*,6*R*-diastereomers **17** in high yield.

Finally, we also studied olefin cross metathesis as a possible tactic for modification of the δ-side chain from olefin precursors **18** involving two routes that differ only in reaction order, olefin metathesis, and introduction of the α-amino ester moiety (methods A and B, Scheme 6). In one example, cross-metathesis with excess *n*-nonene in the presence of the first generation Grubb's catalyst gave low conversion (20% yield, unoptimized) of intermediate **20a** (~4:1 *E/Z*).<sup>16</sup> Subsequent hydrogenation of the derived olefinic derivatives under standard conditions afforded saturated δ-alkyl substituted derivatives **22** in nearly quantitative yield. This overall process provides an expedient route to modify the δ-side chain of orlistat. However, while this strategy successfully provided olefinic orlistat derivatives **21a–c** (~4:1 *E/Z* with exception of **21c**, which gave exclusively *E*), generally, this route suffered from low conversion in the olefin metathesis step.

### Effect of Novel β-Lactones on the Thioesterase Activity of FAS

The thioesterase domain of FAS cleaves palmitate from the acyl carrier protein once its synthesis is complete.<sup>17</sup> Compounds were compared for their ability to inhibit the activity of the recombinant thioesterase using methods we have previously described.<sup>18,19</sup> Four structural components of the orlistat molecule were modified to test for effect on potency: the α- and β-side chains, the amino ester, and both relative and absolute stereochemistry (Figure 1). The results of this analysis are summarized below:

**(i) Modifications to the α-Chain**—In general, the inhibitory activity toward FAS is tolerant to changes in the length of the α-chain. An α-chain of just two carbons appears to be sufficient for good potency. Compounds **17a** (Table 2, entry 2), **15f**, and **17b** (Table 3, entries 14 and 18, respectively) all have α-chains less than the six carbons found in orlistat, and each has an IC<sub>50</sub> equal to or less than that of orlistat. In fact, **17a**, with an ethyl α-chain, has an IC<sub>50</sub> of 0.23 μM, which is greater than 6-fold more potent than orlistat (IC<sub>50</sub>) 1.35

$\mu\text{M}$ ). The reduction of the  $\alpha$ -chain to a methyl group, however, generally resulted in reduced potency. Compound **15e** (Table 4, entry 7), which has the same structure as orlistat with the exception of having a methyl group in the  $\alpha$ -chain position, is approximately 8-fold less potent than orlistat.

We have also observed that ebelactone B, which contains an ethyl  $\alpha$ -chain, is roughly 10 times more potent than ebelactone A, which contains a methyl  $\alpha$ -chain<sup>20</sup> (data not shown). Altogether these data establish that an  $\alpha$ -chain of just two carbons is sufficient for retention of good potency toward FAS. This observation is consistent with the recent finding from the crystal structure of orlistat bound to the FAS thioesterase which shows the  $\alpha$ -chain packs against the histidine residue of the catalytic triad.<sup>11</sup> On the basis of this structure, an ethyl moiety is expected to maintain such packing where a methyl group would be insufficient to extend to this histidine.

**(ii) Modifications to the  $\beta$ -Chain**—Optimization of the  $\beta$ -chain would seem to present a greater challenge because it binds within the putative palmitate binding pocket of the FAS thioesterase, helping to properly position the  $\beta$ -lactone in the vicinity of the catalytic triad.<sup>11,21</sup> Therefore, truncation or other alteration of the  $\beta$ -chain could lead to decreased potency if the conjugate bond cannot be formed. In a direct head-to-head comparison, the reduction of the  $\beta$ -chain from 11 carbons (**17c**, Table 4, entry 2) to just four carbons (**23b**, Table 3, entry 2) resulted in a 15-fold increase in potency against FASTE in the biochemical assay, suggesting that the full length  $\beta$ -chain is not necessary to position the  $\beta$ -lactone at the catalytic triad. Even though they have additional structural changes, compounds **17d** and **15f** (Table 3, entries 12 and 14, respectively) also have  $\beta$ -chains of reduced length and have increased potency compared to orlistat, providing further support that the full length chain is not necessary for activity.

Other alterations to the  $\beta$ -chain were also well-tolerated. The incorporation of a single alkenyl bond into the  $\beta$ -chain (**21a**, Table 3, entry 3) increased the potency of the compound 10-fold compared to orlistat. Several other olefinic compounds showed good potency toward FASTE, i.e., **15j** (Table 2, entry 3), **21a–c** (Table 3, entries 3, 8, and 10, respectively), and **15k** (Table 3, entry 15) even though they were not part of direct head-to-head comparisons. Even the placement of an alkene at the terminal position of the  $\beta$ -chain is tolerated as in compounds **15i** (Table 3, entry 17) and **17f** (Table 3, entry 11).

A small set of compounds were synthesized with  $\beta$ -chains other than simple hydrocarbons. Two of these compounds, **17e** and **21c** (Table 3, entries 7 and 10, respectively), were at least as active as orlistat in the biochemical assay, providing additional support for the idea that significant structural variability within the  $\beta$ -chain can be accommodated by FASTE.

**(iii) Modifications to the Amino Ester Side Chain**—Orlistat contains an *N*-formyl-*l*-leucine moiety branching from the  $\delta$  carbon (C6) of the lactone. Interactions between the enzyme and the amino ester may contribute to positioning orlistat at the active site.<sup>11</sup> Interestingly, the substitution of glycine for leucine at this position (**15g**, Table 3, entry 16) had little effect on potency toward the FAS thioesterase. Results from other head-to-head comparisons of compounds with long  $\beta$ -chains, i.e., **15b** (Table 3, entry 5) versus **15c** (Table 3, entry 6), were similar, indicating such substitutions are tolerated but provide no significant enhancement in potency by themselves. Importantly, though, in compounds with much shorter  $\beta$ -chains that contain an alkenyl bond, substitution of the amino ester yielded a greater than 10-fold increase in potency as with **15i** (Table 3, entry 17) versus **15h** (Table 4, entry 8).

Overall, 12 of the congeners we present here contain an *N*-formyl-glycine moiety that lends greater conformational flexibility than the *N*-formyl-leucine moiety and significantly decreases the compounds' overall hydrophobicity. Nine of these compounds had significantly improved inhibitory activity toward FASTE compared to orlistat. A small set of congeners (8) containing valine, phenylalanine, and histidine at the amino ester position were also synthesized and tested. These compounds also exhibited good potency toward the enzyme, suggesting a wide range of substitutions at this position are accommodated by the enzyme.

**(iv) Modifications in Chirality**—Several compounds (11) with differing relative and absolute stereochemistry at C<sub>α</sub>, C<sub>β</sub>, and/or C<sub>δ</sub> were also synthesized and tested. While no head-to-head comparisons were made, initial indications suggest that stereochemical changes at C<sub>β</sub> (*R* versus *S*) abrogates inhibitory activity toward the FASTE such as with compounds **16a** (Table 4, entry 6; IC<sub>50</sub>) 9.74 μM) and **16b** (Table 4, entry 9; IC<sub>50</sub>) 45.11 μM). However, changes to chirality at C<sub>α</sub> appear to be tolerated, i.e., **17i** (Table 3, entry 4; IC<sub>50</sub>) 0.18 μM), as long as the *S*-configuration is retained at the β-chain. Somewhat surprisingly, inverted stereochemistry at C<sub>δ</sub>, the carbon from which the amino ester side chain branches off the main β-chain, was also tolerated. In fact, compound **17a** (Table 2, entry 2), with increased potency both toward FASTE and in cell-based assays, has inverted stereochemistry at C<sub>δ</sub>. The vast majority of compounds with this inverted stereochemistry contained an *N*-formyl-glycine moiety that allows for greater conformational flexibility; however, we acknowledge the possibility of an alternate binding mode to the enzyme.

### Summary of All Orlistat Derivatives Synthesized

In Table 1, we summarize all orlistat derivatives prepared in this study with yields and diastereoselectivity of the key TMAL reaction process as well as yields for subsequent amino ester introduction via Mitsunobu or acylation chemistry.

### Effect of Compounds on Tumor Cell Apoptosis

Each compound was also tested in MDA-MB-231 breast carcinoma cells and Hs58.Fs foreskin fibroblasts to distinguish mechanism-based cell death elicited by inhibition of FAS from general cytotoxicity. To establish the degree to which each cell type depends on FAS for survival, we treated them with siRNA targeting FAS or with 25 μM orlistat (Figure 2). Both treatments induced death in the cancer cells but had only minimal effect on the fibroblasts. Importantly, the absolute level of apoptosis was essentially equivalent in both cell lines when they were treated with staurosporine, a compound known to induce apoptosis via an unrelated cAMP-mediated mechanism<sup>22</sup> (data not shown). These results establish that the cancer cells are dependent on FAS for survival but the fibroblasts are not.

Three compounds (**17a**, **15j**, and valilactone) were identified that had increased potency toward the thioesterase of FAS and in cell-based assays and that showed selective toxicity to the cancer cells compared to fibroblasts (Table 2). The enhanced potency of this subset of compounds toward cells could be due to their increased affinity for the thioesterase domain, their enhanced aqueous solubility (cLogP values of less than 5 vs 8.6 for orlistat), or some combination of both. One other compound, **15g** (Table 3, entry 16) had better potency than orlistat in the cell-based assays, even though its performance against the recombinant thioesterase was not significantly different from orlistat. The increase in cellular potency of this compound is probably due to a single structural variation, the substitution of a glycine at the amino ester position, which makes the compound less hydrophobic and reduces its cLogP.



The remainder of the compounds either had a reduction in potency toward tumor cells or lost selectivity for tumor cells over the fibroblasts. Most of the compounds having good potency toward the recombinant thioesterase exhibited an  $IC_{50}$  in tumor cells greater than that of orlistat. There are two possibilities to explain this observation. First, it is conceivable that the FAS holoenzyme, in the context of the cellular environment, displays a thioesterase with a catalytic cleft that is structurally distinct from that of the recombinant thioesterase. Second, and probably more likely, is the possibility that these compounds simply have reduced ability to traverse the plasma membrane. Some of the compounds also lost selectivity for tumor cells over fibroblasts, which may be indicative of the reactivity of these compounds for other enzymes, especially other serine hydrolases that may be important for survival of all cells.

## Conclusion

Synthesis of 28 orlistat congeners led to the identification of molecular features that can be optimized to generate  $\beta$ -lactones with improved potency toward the thioesterase domain of FAS. Three of these compounds had an improved affinity for the thioesterase, an increased potency toward tumor cells, and greater selectivity for tumor cells over fibroblasts. Interestingly, these three compounds show considerable structural diversity. Each includes a unique amino ester substitution and a unique  $\beta$ -chain. Additionally, compound **17a** (Table 2, entry 2) has a truncated  $\alpha$ -chain and opposite chirality (*R* versus *S*) at the  $\delta$  carbon. These results provide an important foundation for structure-based design of  $\beta$ -lactones with optimal cytotoxicity profiles and the heterogeneity of the most promising compounds suggests a larger, more diverse study is warranted. The data presented here also underpin the idea that an orlistat congener can be optimized for use in preclinical drug design and for clinical drug development for the treatment of carcinomas.

## Materials and Methods

### Chemical Synthesis and Characterization

All reactions were carried out under nitrogen atmosphere in flame-dried glassware. Dichloromethane, acetonitrile, methanol, tetrahydrofuran, and ethyl ether were purified by passage through activated molecular sieves. Hünig's base and triethylamine were distilled from potassium hydroxide prior to use. All other commercially obtained reagents were used as received.  $^1H$  NMR chemical shifts are reported as  $\delta$  values in ppm relative to  $CDCl_3$  (7.27 ppm) and coupling constants (*J*) are reported in hertz (Hz). Unless indicated otherwise, deuterio-chloroform ( $CDCl_3$ ) served as an internal standard (77.23 ppm) for all  $^{13}C$  spectra. Flash column chromatography was performed using 60 Å silica gel (Silicycle, 230–400 mesh) as stationary phase. Mass spectra were obtained at the Mass Spectrometry Application and Collaboration Facility (Texas A&M University). Thin layer chromatography (TLC) was performed using glass-backed silica gel 60<sub>F254</sub> (Silicycle, 250  $\mu$ m thickness).  $^1H$  and  $^{13}C$  NMR spectra were acquired using Varian spectrometers at the frequency indicated. Solvents are indicated for each compound. Chemical shifts are expressed in ppm referenced to the residual nondeuterated solvent as an internal standard.  $^1H$  NMR coupling constants (*J*) are reported in hertz (Hz) and multiplicities are abbreviated as follows: app = apparent, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, br = broadband signal. For representative procedures for synthesis of ketene acetals **3a–d** and aldehydes **8a–g**, please see ref 22.

**Representative Procedure for Synthesis of  $\beta$ -Lactones 13/14 via the TMAL Reaction/Desilylation, as Described for (3S,4S)-3-Hexyl-4-((R)-2-hydroxytridecyl)-oxetan-2-one (13) [reproduced from ref 22]. TMAL Reaction**

ZnCl<sub>2</sub> (398 mg, 2.92 mmol) was fused under vacuum and then allowed to cool to room temperature under a flow of nitrogen. Then 7.5 mL of dry CH<sub>2</sub>Cl<sub>2</sub> was then added via syringe, followed by a solution of aldehyde **8f** (500 mg, 1.46 mmol) in 2.5 mL of CH<sub>2</sub>Cl<sub>2</sub> and ketene acetal **3d** (719 mg, 2.04 mmol). The reaction mixture was stirred for 60 h at ambient temperature. Then 5 mL of pH 7 buffer were added, and the mixture was stirred vigorously for 30 min, filtered through a pad of Celite, and washed with CH<sub>2</sub>Cl<sub>2</sub>. The organic filtrate was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by flash chromatography on SiO<sub>2</sub> (10:1, hexanes:EtOAc) to provide the mixture of silyloxy- $\beta$ -lactone diastereomers as a pale-yellow oil. <sup>1</sup>H NMR (500 MHz) analysis of the mixture of diastereomers indicated a diastereomeric ratio of 8:1. Without further purification, the mixture was used directly in the next step.

**Desilylation**

To a stirred solution of the mixture of silyloxy- $\beta$ -lactones in 24 mL of CH<sub>3</sub>CN cooled to 0 °C, 2.4 mL of HF (48%) was added dropwise. The mixture was stirred at 0 °C for 2 h, allowed to warm to ambient temperature, and stirred for an additional 5 h. The reaction mixture was diluted with 100 mL of Et<sub>2</sub>O, quenched carefully with cold saturated NaHCO<sub>3</sub>, and washed with brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The residue was purified by flash chromatography on SiO<sub>2</sub> (10:1, hexanes:EtOAc) to provide the hydroxy- $\beta$ -lactone **13** (190 mg, 44%) and a mixture of the two diastereomers (112 mg, 14%) as white solids (58% overall, 2 steps). Spectroscopic data are reported for the major diastereomeric  $\beta$ -lactone **13**: *R*<sub>f</sub> = 0.54 (20% EtOAc/hexanes); [ $\alpha$ ]<sub>D</sub><sup>23</sup> -30.9 (*c* 0.7, CHCl<sub>3</sub>). IR (thin film) 1819 cm<sup>-1</sup>. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  4.51 (dt, *J* = 4.3, 8.5 Hz, 1H), 3.79–3.86 (m, 1H), 3.27 (ddd, *J* = 4.0, 7.0, 8.5 Hz, 1H), 1.93 (ddd, *J* = 3.0, 8.5, 14.5 Hz, 1H), 1.72–1.87 (m, 3H), 1.24–1.56 (m, 28H), 0.891 (t, *J* = 6.0 Hz, 3H), 0.889 (t, *J* = 6.0 Hz, 3H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  171.8, 75.8, 68.7, 56.8, 42.0, 38.3, 32.1, 31.7, 29.9, 29.83, 29.77 (2), 29.7, 29.6, 29.2, 27.9, 27.0, 25.6, 22.9, 22.7, 14.3, 14.2. LRMS (ESI) calcd for C<sub>22</sub>H<sub>42</sub>O<sub>3</sub> [M + Li], 361; found, 361.

**Representative Mitsunobu Reaction Procedure as Described for (S)-2-Formylamino-4-methyl-pentanoic Acid 1-((2S,3S,4S)-3-Hexyl-4-oxo-oxetan-2-ylmethyl)-tetradecyl Ester (15a)**

To a solution of  $\beta$ -lactone **13a** (30.0 mg, 0.0784 mmol) in THF (1.0 mL) at 0 °C was added triphenylphosphine (66.6 mg, 0.254 mmol), *N*-formyl-L-leucin (47.3 mg, 0.296 mmol), and DIAD (49  $\mu$ L, 0.25 mmol). The reaction was monitored by TLC. After the reaction was complete, solvent was removed in vacuo and purified by flash chromatography, eluting with Hexanes/EtOAc (4:1), to give  $\beta$ -lactone **15a** (33 mg, 80%) as a colorless oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.22 (s, 1H), 5.97 (d, *J* = 8.1 Hz, 1H), 4.99–5.07 (m, 1H), 4.69 (dt, *J* = 5.1, 9.0 Hz, 1H), 4.30 (quint, *J* = 4.5 Hz, 1H), 3.22 (dt, *J* = 4.2, 7.8 Hz, 1H), 2.17 (dt, *J* = 7.8, 14.7 Hz, 1H), 2.00 (dt, *J* = 4.8, 14.7 Hz, 1H), 1.25–1.82 (m, 37H), 0.86–0.98 (m, 12H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  172.2, 171.0, 160.8, 75.0, 73.0, 57.2, 49.8, 41.8, 38.9, 34.3, 32.1, 31.7, 29.89, 29.86(3), 29.7, 29.64, 29.56, 29.5, 29.2, 27.8, 26.9, 25.3, 25.1, 23.1, 22.9, 22.7, 22.0, 14.3, 14.2. LRMS HRMS (ESI) calcd for C<sub>31</sub>H<sub>57</sub>NO<sub>5</sub> [M + Li], 530.4397; found, 530.4282.

**(2S,3S,4S)-Formylamino-acetic Acid 1-(3-Hexyl-4-oxo-oxetan-2-ylmethyl)-tetradecyl Ester (15b)**

Prepared according to the representative Mitsunobu reaction procedure using  $\beta$ -lactone **13a** (20.0 mg, 0.0523 mmol), triphenylphosphine (44.4 mg, 0.169 mmol), *N*-formylglycine (20.4 mg, 0.197 mmol), and DIAD (33  $\mu$ L, 0.169 mmol) in 0.7 mL of THF. Purification by flash chromatography on SiO<sub>2</sub> (4:1, hexanes:EtOAc) gave  $\beta$ -lactone **15b** (17.8 mg, 73%). <sup>1</sup>H NMR (300 MHz, C<sub>6</sub>D<sub>6</sub>)  $\delta$  7.72 (s, 1H), 5.16 (br, 1H), 4.94–5.01 (m, 1H), 4.06 (dt, *J* = 4.8, 7.5 Hz, 1H), 3.78 (dd, *J* = 6.0, 18.0 Hz, 1H), 3.69 (dd, *J* = 5.7, 18.0 Hz, 1H), 2.74 (dt, *J* = 4.2, 7.5 Hz, 1H), 1.72 (dt, *J* = 8.1, 15.0 Hz, 1H), 1.15–1.47 (m, 35H), 0.88–0.94 (m, 6H). <sup>13</sup>C NMR (75 MHz, C<sub>6</sub>D<sub>6</sub>)  $\delta$  170.7, 169.6, 160.9, 74.8, 72.8, 57.6, 40.5, 39.2, 34.8, 32.7, 32.2, 30.53 (2), 30.51, 30.5, 30.4, 30.3, 30.2, 30.1, 29.7, 28.1, 27.3, 25.9, 23.5, 23.3, 14.7, 14.6.

**(S)-2-Formylamino-3-phenyl-propionic Acid 1-((2S,3S,4S)-3-Hexyl-4-oxo-oxetan-2-ylmethyl)-tetradecyl Ester (15c)**

Prepared according to the representative Mitsunobu reaction procedure using  $\beta$ -lactone **13a** (20.0 mg, 0.0523 mmol), triphenylphosphine (44.4 mg, 0.169 mmol), *N*-formyl-L-leucine (47.3 mg, 0.296 mmol), and DIAD (49  $\mu$ L, 0.25 mmol) in 1.0 mL of THF. Purification by flash chromatography on SiO<sub>2</sub> (4:1, hexanes:EtOAc) gave  $\beta$ -lactone **15c** (33 mg, 80%). <sup>1</sup>H NMR (300 MHz, C<sub>6</sub>D<sub>6</sub>)  $\delta$  7.57 (s, 1H), 6.96–7.12 (m, 5H), 5.08 (d, *J* = 8.1 Hz, 1H), 4.92–4.98 (m, 1H), 4.87 (q, *J* = 7.5 Hz, 1H), 3.93–3.99 (m, 1H), 3.01 (dd, *J* = 6.3, 14.1 Hz, 1H), 2.84 (dd, *J* = 7.2, 14.1 Hz, 1H), 2.70 (dt, *J* = 4.2, 7.8 Hz, 1H), 1.73 (dt, *J* = 7.5, 15.0 Hz, 1H), 1.14–1.54 (m, 35H), 0.88–0.95 (m, 6H). HRMS (ESI) calcd for C<sub>34</sub>H<sub>55</sub>NO<sub>5</sub> [M + Li], 564.4240; found, 564.4166.

**(S)-2-Formylamino-4-methyl-pentanoic Acid 1-((2S,3S,4S)-3-Methyl-4-oxo-oxetan-2-ylmethyl)-dodecyl Ester (15d)**

Prepared according to the representative Mitsunobu reaction procedure using  $\beta$ -lactone **13a** (40.0 mg, 0.105 mmol), triphenylphosphine (88.9 mg, 0.339 mmol), *N*-formyl-L-histidine (72.3 mg, 0.395 mmol), and DIAD (66  $\mu$ L, 0.34 mmol) in 3 mL of THF. Purification by flash chromatography on SiO<sub>2</sub> (2:1, hexanes:EtOAc) gave  $\beta$ -lactone **15d** (10 mg, 17%). <sup>1</sup>H NMR (300 MHz, C<sub>6</sub>D<sub>6</sub>)  $\delta$  7.91 (s, 1H), 7.78 (s, 1H), 6.97 (s, 1H), 6.82 (d, *J* = 7.5 Hz, 1H), 4.93–4.99 (m, 2H), 4.82 (quint, *J* = 6.6 Hz, 1H), 4.22 (dt, *J* = 3.9, 6.3 Hz, 1H), 2.99 (dd, *J* = 5.1, 14.1 Hz, 1H), 2.86 (dd, *J* = 5.4, 15.0 Hz, 1H), 2.78–2.82 (m, 1H), 1.88 (dt, *J* = 6.9, 14.7 Hz, 1H), 1.16–1.63 (m, 31H), 0.87–0.96 (m, 10H).

**(S)-2-Formylamino-4-methyl-pentanoic Acid 1-((2S,3S,4S)-3-Methyl-4-oxo-oxetan-2-ylmethyl)-dodecyl Ester (15e)**

Prepared according to the representative Mitsunobu reaction procedure using the mixture of  $\beta$ -lactones **13e** and **14e** (11.9 mg, 0.0418 mmol), triphenylphosphine (32.8 mg, 0.125 mmol), *N*-formylleucine (23.2 mg, 0.146 mmol), and DIAD (25  $\mu$ L, 0.13 mmol) in 1.0 mL of THF. Purification by flash chromatography on SiO<sub>2</sub> (3:2, hexanes: EtOAc) followed by a second purification by flash chromatography on SiO<sub>2</sub> (0.4:1:3 THF:CHCl<sub>3</sub>:hexanes) gave the desired  $\beta$ -lactone as a mixture of diastereomers. The two diastereomers could be separated by MPLC (65:35, hexanes: EtOAc), affording  $\beta$ -lactone **15e** (6.8 mg, 38%) and diastereomeric  $\beta$ -lactone **16b** (4.4 mg, 24%). Major diastereomer (**15e**): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.23 (s, 1H), 5.92 (d, *J* = 8.7 Hz, 1H), 4.98–5.10 (m, 1H), 4.69 (td, *J* = 4.8, 8.4, 1H), 4.23 (ddd, *J* = 8.4, 4.2, 4.2 Hz, 1H), 3.28 (dq, *J* = 4.2, 7.8 Hz, 1H), 1.96–2.26 (m, 2H), 1.51–1.75 (m, 5H), 1.40 (d, *J* = 7.8 Hz, 3H), 1.26 (br. s, 18H), 0.98 (d, *J* = 6.0 Hz, 6H), 0.89 (t, *J* = 6.6 Hz, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  172.2, 171.4, 160.8, 76.5, 72.8, 51.8,



49.9, 41.8, 38.7, 34.4, 32.1, 29.92, 29.83, 29.75, 29.65, 29.56, 29.51, 25.3, 25.1, 23.1, 22.9, 22.0, 14.3, 12.6.

**(S)-((R)-1-((2S,3S)-3-Ethyl-4-oxooxetan-2-yl)tridecan-2-yl) 2-Methanamido-4-methylpentanoate (15f)**

To a mixture of hydroxy  $\beta$ -lactone **13d** (17.7 mg, 0.0594 mmol), *N*-formylleucine (33.4 mg, 0.209 mmol), and triphenylphosphine (53.4 mg, 0.203 mmol) in a 10 mL round-bottom flask was added 0.5 mL of xylene, and the mixture was azeotroped in vacuo. After 1.0 h, THF (1.4 mL) was added under  $N_2$  and the solution was cooled to 0 °C. Next, DIAD (37.9  $\mu$ L, 0.1947 mmol) was added and the reaction mixture was stirred at 0 °C for 10 min. The reaction was allowed to warm to room temperature and stirred overnight. The mixture was concentrated in vacuo and purified twice by chromatography ( $SiO_2$ , 10  $\rightarrow$  30% EtOAc:hexanes) to afford  $\beta$ -lactone **15f** (6 mg, 25%).  $R_f$  = 0.26 (30% EtOAc/hexanes);  $[\alpha]^{22}_D = -3.68$  ( $c$  0.4,  $CHCl_3$ ). IR (thin film) 2926, 2856, 1828, 1740, 1671  $cm^{-1}$ .  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  8.23 (br s, 1H), 5.90 (d,  $J$  = 8.5 Hz, 1H), 5.04 (app quint,  $J$  = 5.5 Hz, 1H), 4.69 (ddd,  $J$  = 5.0, 8.5, 13.0 Hz, 1H), 4.30–4.33 (m, 1H), 3.18–3.22 (m, 1H), 2.19 (app quint,  $J$  = 7.0 Hz, 1H), 2.00 (dt,  $J$  = 4.5, 19.0 Hz, 1H), 1.64–1.70 (m, 2H), 1.54–1.60 (m, 19H) 1.22–1.32 (m, 6H), 1.042 (dppt,  $J$  = 7.5 Hz, 3H) 0.97 (dd,  $J$  = 1.5, 4.5 Hz, 4H), 0.90 (appt,  $J$  = 7.5 Hz, 3H).  $^{13}C$  NMR (125 HMz,  $CDCl_3$ )  $\delta$  172.2, 169.5, 161.2, 74.7, 73.3, 58.6, 40.4, 39.1, 34.4, 32.2, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 25.4, 22.9, 21.1, 14.4, 11.3. HRMS (ESI) calcd for  $_{38}C_{21}H_{45}NO_5$  [M + H], 384.2744; found, 384.2786.

**(2S,3S,4S)-Formylamino-acetic Acid 1-(3-Hexyl-4-oxo-oxetan-2-ylmethyl)-dodecyl Ester (15g)**

Prepared according to the representative Mitsunobu reaction procedure using  $\beta$ -lactone **13b** (40.0 mg, 0.113 mmol), triphenylphosphine (74.1 mg, 0.283 mmol), *N*-formylglycine (40.7 mg, 0.395 mmol), and DIAD (55  $\mu$ L, 0.28 mmol) in 2 mL of THF. Purification by flash chromatography on  $SiO_2$  (4:1, hexanes:EtOAc) gave  $\beta$ -lactone **15g** (43 mg, 87%).  $^1H$  NMR (300 MHz,  $C_6D_6$ )  $\delta$  7.79 (s, 1H), 5.45 (br, 1H), 4.95–5.02 (m, 1H), 4.08 (dt,  $J$  = 5.1, 7.2 Hz, 1H), 3.81 (dd,  $J$  = 5.7, 18.0 Hz, 1H), 3.73 (dd,  $J$  = 5.7, 18.3 Hz, 1H), 2.78 (dt,  $J$  = 4.2, 7.8 Hz, 1H), 1.75 (dt,  $J$  = 8.1, 15.0 Hz, 1H), 1.17–1.52 (m, 31H), 0.87–0.94 (m, 6H).  $^{13}C$  NMR (75 MHz,  $C_6D_6$ )  $\delta$  170.8, 169.7, 161.1, 74.9, 72.8, 57.6, 40.6, 39.2, 34.8, 32.7, 32.2, 30.47, 30.45, 30.40, 30.3, 30.2, 30.1, 29.7, 28.2, 27.4, 25.9, 23.5, 23.3, 14.7, 14.6.

**2-Formylamino-3-methyl-butyric Acid (2>S,2R,3S,4S)-1-(3-Hexyl-4-oxo-oxetan-2-ylmethyl)-hex-4-enyl Ester (15h)**

Prepared according to the representative Mitsunobu reaction procedure using  $\beta$ -lactone **13h** (20.0 mg, 0.0745 mmol), triphenylphosphine (27.4 mg, 0.104 mmol), *N*-formyl-L-valine (21.6 mg, 0.149 mmol), DIAD (20  $\mu$ L, 0.10 mmol) in 1 mL of THF. Purification by flash chromatography on  $SiO_2$  (9:1, hexanes:EtOAc) gave  $\beta$ -lactone **15h** (13.3 mg, 45%) as a colorless oil as a *E/Z*-mixture, only peaks for major olefin isomer are assigned.  $R_f$  = 0.24 (30% EtOAc/hexanes);  $[\alpha]^{22}_D = -7.95$  (0.9,  $CHCl_3$ ). IR (thin film) 1823, 1737, 1670  $cm^{-1}$ .  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  8.28 (s, 1H), 6.05 (d,  $J$  = 8.0 Hz, 1H), 5.32–5.50 (m, 2H), 5.03–5.08 (m, 1H), 4.64 (ddd,  $J$  = 4.5, 7.5, 16.5 Hz, 1H), 4.30 (dt,  $J$  = 4.0, 8.0 Hz, 1H), 3.23 (dt,  $J$  = 4.5, 7.0 Hz, 1H), 2.06–2.26 (m, 3H), 2.02 (dt,  $J$  = 5.5, 14.5 Hz, 2H), 1.64–1.85 (m, 6H), 1.28–1.48 (m, 9H), 1.01 (d,  $J$  = 6.5 Hz, 3H), 0.93 (d,  $J$  = 7.0 Hz, 3H), 0.89 (t,  $J$  = 6.6 Hz, 3H).  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$  171.2, 170.9, 161.0, 129.4, 126.6, 74.8, 72.5, 57.3, 56.1, 38.8, 33.9, 31.7, 31.2, 29.2, 28.4, 27.9, 26.9, 22.7, 19.5, 18.11, 17.7, 14.2. HRMS (ESI) calcd for  $C_{22}H_{37}NO_5$  [M + H], 396.2744; found, 396.2833.

**(S)-2-Formylamino-3-phenyl-propionic Acid 1-((2S,3S,4S)-3-Hexyl-4-oxo-oxetan-2-ylmethyl)-hex-4-enyl Ester (15i)**

Prepared according to the representative Mitsunobu reaction procedure using  $\beta$ -lactone **13h** (20.0 mg, 0.0745 mmol), triphenylphosphine (27.4 mg, 0.104 mmol), *N*-formyl-L-phenylalanine (28.8 mg, 0.149 mmol), and DIAD (20  $\mu$ L, 0.10 mmol) in 2 mL of THF. Purification by flash chromatography on SiO<sub>2</sub> (4:1, hexanes:EtOAc) gave  $\beta$ -lactone **15i** (yield not determined) as a *E/Z*-mixture; only peaks for major olefin isomer are assigned. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.18 (s, 1H), 7.17–7.32 (m, 5H), 5.99 (d, *J* = 7.8 Hz, 1H) 5.30–5.47 (m, 2H), 4.97–5.05 (m, 1H), 4.91 (q, *J* = 6.9 Hz, 1H), 4.28 (dt, *J* = 5.1, 7.8 Hz, 1H), 3.06–3.24 (m, 3H), 1.26–2.10 (m, 19H), 0.90 (t, *J* = 6.6 Hz, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  171.0, 170.9, 160.6, 135.7, 129.5, 129.4, 128.9, 127.6, 126.5, 74.7, 72.7, 57.3, 52.3, 38.8, 38.0, 33.9, 31.7, 29.2, 28.3, 27.8, 26.9, 22.7, 18.1, 14.3. HRMS (ESI) calcd for C<sub>26</sub>H<sub>37</sub>NO<sub>5</sub> [M + H], 444.2744; found, 444.2730.

**(S)-2-Formylamino-4-methyl-pentanoic Acid 1-((2S,3S,4S)-3-Hexyl-4-oxo-oxetan-2-ylmethyl)-pent-4-enyl Ester (15j)**

Prepared according to the representative Mitsunobu reaction procedure using  $\beta$ -lactone **13g** (80.0 mg, 0.315 mmol), triphenylphosphine (116 mg, 0.441 mmol), *N*-formylleucin (126 mg, 0.788 mmol), and DIAD (85  $\mu$ L, 0.44 mmol) in 5.0 mL of THF, purified by flash chromatography on SiO<sub>2</sub> (4:1, hexanes:EtOAc) gave  $\beta$ -lactone **15j** (85 mg, 68%). <sup>1</sup>H NMR (300 MHz, C<sub>6</sub>D<sub>6</sub>)  $\delta$  7.99 (s, 1H), 5.94 (d, *J* = 8.1 Hz, 1H), 5.64–5.78 (m, 1H), 4.94–5.09 (m, 3H), 4.75 (dt, *J* = 5.1, 9.6 Hz, 1H), 4.10 (dt, *J* = 4.8, 8.1 Hz, 1H), 2.79 (dt, *J* = 3.9, 7.5 Hz, 1H), 1.96–2.05 (m, 1H), 1.17–1.83 (m, 18H), 1.05 (d, *J* = 6.0 Hz, 3H), 0.86–0.91 (m, 6H). <sup>13</sup>C NMR (75 MHz, C<sub>6</sub>D<sub>6</sub>)  $\delta$  172.6, 170.8, 161.3, 137.9, 116.0, 74.8, 72.2, 57.6, 50.3, 41.5, 39.2, 33.9, 32.2, 30.0, 29.7, 28.1, 27.3, 25.4, 23.35, 23.27, 21.9, 14.6. HRMS (ESI) calcd for C<sub>22</sub>H<sub>37</sub>NO<sub>5</sub> [M + Li], 402.2832; found, 402.2830.

**(S)-2-Formylamino-3-phenyl-propionic Acid 1-((2S,3S,4S)-3-Hexyl-4-oxo-oxetan-2-ylmethyl)-pent-4-enyl Ester (15k)**

Prepared according to the representative Mitsunobu reaction procedure using  $\beta$ -lactone **13g** (40.0 mg, 0.157 mmol), triphenylphosphine (57.6 mg, 0.220 mmol), *N*-formyl-L-phenylalanine (70.0 mg, 0.362 mmol), and DIAD (43  $\mu$ L, 0.220 mmol) in 3.0 mL of THF. Purification by flash chromatography on SiO<sub>2</sub> (4:1, hexanes:EtOAc) gave  $\beta$ -lactone **15k** (31 mg, 46%). <sup>1</sup>H NMR (300 MHz, C<sub>6</sub>D<sub>6</sub>)  $\delta$  7.56 (s, 1H), 6.94–7.12 (m, 5H), 5.60–5.75 (m, 1H), 4.82–5.06 (m, 4H), 3.90–3.92 (m, 1H), 2.99 (dd, *J* = 6.3, 14.4 Hz, 1H), 2.82 (dd, *J* = 6.9, 14.4 Hz, 1H), 2.67 (dt, *J* = 3.9, 7.5 Hz, 1H), 1.91 (q, *J* = 6.9 Hz, 1H), 1.22–1.68 (m, 16H), 0.89 (t, *J* = 6.9 Hz, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  170.9 (2), 160.7, 137.1, 135.7, 129.4, 128.9, 127.6, 115.9, 74.6, 72.6, 57.3, 52.3, 38.9, 38.0, 33.2, 31.7, 29.5, 29.2, 27.8, 26.9, 22.7, 14.3.

**(S)-2-Formylamino-4-methyl-pentanoic Acid 1-((2S,3S,4S)-3-Octyl-4-oxo-oxetan-2-ylmethyl)-decyl Ester (15l)**

Prepared as previously reported.<sup>23</sup>

**(S)-2-Formylamino-4-methyl-pentanoic Acid 1-((2S,3R,4R)-3-Hexyl-4-oxo-oxetan-2-ylmethyl)-tetradecyl Ester (16a)**

Prepared according to the representative Mitsunobu reaction procedure using  $\beta$ -lactone **14a** (20.0 mg, 0.052 mmol), triphenylphosphine (44.4 mg, 0.169 mmol), *N*-formylleucin (31.6 mg, 0.197 mmol), and DIAD (33  $\mu$ L, 0.17 mmol) in 1.0 mL of THF. Purification by flash chromatography on SiO<sub>2</sub> (4:1, hexanes:EtOAc) gave  $\beta$ -lactone **16a** (10 mg, 17%). <sup>1</sup>H NMR (300 MHz, C<sub>6</sub>D<sub>6</sub>)  $\delta$  7.80 (s, 1H), 5.05–5.13 (m, 2H), 4.79 (dt, *J* = 4.2, 8.7 Hz, 1H), 4.05

(quint,  $J = 4.5$  Hz, 1H), 2.81 (ddd,  $J = 4.2, 4.9, 11.1$  Hz, 1H), 1.12–1.71 (m, 39H), 0.83–0.92 (m, 12H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{C}_6\text{D}_6$ )  $\delta$  172.6, 170.4, 160.7, 74.2, 72.6, 57.2, 50.1, 41.9, 39.4, 34.9, 32.7, 32.2, 30.54 (2), 30.52, 30.5, 30.4, 30.3, 30.2, 30.1, 29.7, 28.2, 27.4, 25.8, 25.3, 23.5, 23.31, 23.27, 22.0, 14.7, 14.6.

**(S)-2-Formylamino-4-methyl-pentanoic Acid 1-((2S,3R,4R)-3-Methyl-4-oxo-oxetan-2-ylmethyl)-dodecyl Ester (16b)**

Prepared according to the representative Mitsunobu reaction procedure using the mixture of  $\beta$ -lactones **13e** and **14e** (11.9 mg, 0.042 mmol), triphenylphosphine (32.8 mg, 0.125 mmol), *N*-formylleucine (23.2 mg, 0.146 mmol), and DIAD (25  $\mu\text{L}$ , 0.13 mmol) in 1.0 mL of THF. Purification by flash chromatography on  $\text{SiO}_2$  (3:2, hexanes: EtOAc) followed by a second purification by flash chromatography on  $\text{SiO}_2$  (0.4:1:3 THF: $\text{CHCl}_3$ :hexanes) gave the desired  $\beta$ -lactone as a mixture of diastereomers. The two diastereomers could be separated by MPLC (65:35, hexanes: EtOAc), affording the  $\beta$ -lactone diastereomer **16b** (4.4 mg, 24%).  $^1\text{H}$  NMR minor diastereomer (**16b**) (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.23 (s, 1H),  $\delta$  5.92 (d,  $J = 8.7$  Hz, 1H),  $\delta$  5.08–4.98 (m, 1H),  $\delta$  4.71 (td,  $J = 8.4, 4.8$ , 1H),  $\delta$  4.27–4.19 (m, 1H),  $\delta$  3.29 (qd,  $J = 7.5, 3.9$  Hz, 1H),  $\delta$  2.12–2.05 (m, 2H),  $\delta$  1.75–1.50 (m, 5H),  $\delta$  1.41 (d,  $J = 7.5$  Hz, 3H),  $\delta$  1.26 (br s, 18H),  $\delta$  0.98 (dd,  $J = 6.3, 2.4$  Hz, 6H),  $\delta$  0.89 (t,  $J = 6.6$  Hz, 3H). HRMS (ESI) calcd for  $\text{C}_{24}\text{H}_{43}\text{NO}_5$  [ $\text{M} + \text{Li}$ ], 432.3301; found, 432.3309.

**Representative Procedure for Acylation as Described for (R)-1-((2S,3S)-3-Ethyl-4-oxooxetan-2-yl)tridecan-2-yl-2-methanamidoethanoate (17a)**

A solution of hydroxy  $\beta$ -lactone **13d** (11.0 mg, 0.0369 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (9.20 mg, 0.048 mmol), and 4-dimethylaminopyridine (5.45 mg, 0.044 mmol) in 0.5 mL of xylene was azeotroped in vacuo for 1.0 h. The residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (1.0 mL), and *N*-formyl glycine (5.71 mg, 0.055 mmol) was added. After 12 h, the mixture was extracted with water ( $3 \times 1$  mL) and dichloromethane. The organic extract was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated in vacuo. The residue was purified by chromatography ( $\text{SiO}_2$ , 20% EtOAc:hexanes) to afford  $\beta$ -lactone **17a** (17.4 mg, 99%).  $R_f = 0.22$  (50% EtOAc/hexanes);  $[\alpha]_D^{22} = -9.4$  (c 0.4,  $\text{CHCl}_3$ ). IR (thin film) 2926, 2855, 1823, 1738, 1651.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.25 (br s, 1H), 6.22 (br s, 1H), 5.05–5.09 (m, 1H), 4.30–4.27 (m, 1H), 4.08 (d,  $J = 5.5$  Hz, 2H), 3.21 (dq,  $J = 1.0, 4.5$  Hz, 1H), 2.05–2.09 (m, 2H), 1.75–1.87 (m, 2H), 1.59–1.68 (m, 2H), 1.25–1.33 (m, 19H), 1.04 (app t,  $J = 7.0$  Hz, 3H), 0.88 (app t,  $J = 6.5$  Hz, 3H).  $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  171.0, 169.4, 161.3, 74.1, 73.2, 58.3, 40.4, 39.1, 34.5, 32.2, 30.0 (2), 29.9, 29.8, 29.7, 29.6, 25.4, 23.0, 21.2 (2), 14.5, 11.4.

**(R)-1-((2S,3S)-3-Butyl-4-oxooxetan-2-yl)tridecan-2-yl-2-methanamidoethanoate (17b)**

Prepared according to the representative acylation procedure, hydroxy  $\beta$ -lactone **13c** (8.5 mg, 0.026 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (6.5 mg, 0.034 mmol), 4-dimethylaminopyridine (3.8 mg, 0.031 mmol), and *N*-formyl glycine (4.0 mg, 0.039 mmol), purified twice by chromatography ( $\text{SiO}_2$ , 40% EtOAc:hexanes) to afford  $\beta$ -lactone **17b** (7.4 mg, 69%).  $R_f = 0.24$  (40% EtOAc/hexanes);  $[\alpha]_D^{22} = -19.5$  (c 0.8,  $\text{CHCl}_3$ ). IR (thin film) 3386, 2926, 2855, 1825, 1745, 1681, 1519  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.27 (br s, 1H), 6.09 (br s 1H), 5.09 (app quint,  $J = 6.5$  Hz, 1H), 4.26–4.30 (m, 1H), 4.13 (dd,  $J = 1.0, 7.0$  Hz, 1H), 4.10 (d,  $J = 5.0$  Hz, 2H), 3.22–3.26 (m, 1H), 2.07 (app t,  $J = 6.5$  Hz, 1H), 2.05 (d,  $J = 1.00$  Hz, 1H), 1.55–1.87 (m, 7H), 1.26–1.38 (m, 20H), 0.93 (app t,  $J = 7.5$  Hz, 2H), 0.89 (app t,  $J = 7.5$  Hz, 2H).  $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  171.0, 169.3, 161.145, 74.5, 73.1, 56.9, 40.3, 39.0, 34.4, 32.1, 29.84, 29.83, 29.75, 29.67, 29.7, 29.53 29.2, 27.6, 25.3, 22.9, 22.6, 14.4, 14.0. HRMS (ESI) calcd for  $\text{C}_{23}\text{H}_{41}\text{NO}_5$  [ $\text{M} + \text{Li}$ ], 418.3145; found, 418.3324.

**(2R,3S,4S)-Formylamino-acetic Acid 1-(3-Hexyl-4-oxo-oxetan-2-ylmethyl)-dodecyl Ester (17c)**

Prepared according to the representative acylation procedure using  $\beta$ -lactone **13b** (15.0 mg, 0.0423 mmol), *N*-formylglycin (4.8 mg, 0.047 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (10.5 mg, 0.0550 mmol), and 4-dimethylaminopyridine (5.2 mg, 0.042 mmol). Purification by flash chromatography on SiO<sub>2</sub> (4:1, hexanes:EtOAc) gave  $\beta$ -lactone **17c** (18 mg, 97%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.28 (s, 1H), 6.15 (br, 1H), 5.06–5.11 (m, 1H), 4.27–4.30 (m, 1H), 4.03–4.14 (m, 2H), 3.22–3.26 (m, 1H), 2.07 (t, *J* = 3.9 Hz, 2H), 1.26–1.85 (m, 30H), 0.87–0.91 (m, 6H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  170.9, 169.3, 161.1, 74.4, 73.0, 56.9, 40.3, 39.0, 34.4, 32.1, 31.7, 29.8 (2), 29.7, 29.6, 29.54, 29.52, 29.1, 27.9, 27.0, 25.3, 22.9, 22.7, 14.3, 14.2. LRMS (ESI) calcd for C<sub>25</sub>H<sub>45</sub>NO<sub>5</sub> [M + H], 440; found, 440.

**(R)-1-((2S,3S)-3-Hexyl-4-oxooxetan-2-yl)propan-2-yl-2-methanamidoethanoate (17d)**

Prepared according to the representative acylation procedure using hydroxy  $\beta$ -lactone **13i** (10.9 mg, 0.051 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (12.6 mg, 0.066 mmol), 4-dimethylaminopyridine (7.5 mg, 0.061 mmol), and *N*-formyl glycine (7.9 mg, 0.077 mmol). Purification by chromatography (SiO<sub>2</sub>, 10  $\rightarrow$  40% EtOAc:hexanes) afforded  $\beta$ -lactone **17d** (7.9 mg, 52%). *R<sub>f</sub>* = 0.11 (40% EtOAc/hexanes); [ $\alpha$ ]<sub>D</sub><sup>22</sup> = -30.9 (*c* 0.60, CHCl<sub>3</sub>). IR (thin film) 3376, 2931, 1820, 1747, 1685 cm<sup>-1</sup>. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.23 (br s, 1H), 6.07 (brs, 1H), 5.10–5.17 (m, 1H), 4.30 (app quint, *J* = 4.0 Hz, 1H), 4.09 (dd, *J* = 0.5, 5.0 Hz, 1H), 3.25 (ddd, *J* = 1.5, 4.0, 6.5 Hz, 1H), 2.10–2.15 (m, 1H), 2.01–2.06 (m, 1H), 1.80–1.87 (m, 1H), 1.70–1.77 (m, 1H), 1.30–1.47 (m, 14H), 0.89 (app t, *J* = 3.5 Hz, 3H). <sup>13</sup>C (125 MHz, CDCl<sub>3</sub>)  $\delta$  170.9, 169.1, 161.2, 74.3, 59.8, 57.0, 40.8, 40.3, 31.7, 29.1, 27.9, 27.0, 22.7, 20.5, 14.2. HRMS (ESI) calcd for C<sub>16</sub>H<sub>27</sub>NO<sub>5</sub> [M + Li], 306.1983; found, 306.1987.

**(S)-1-((2S,3S)-3-Hexyl-4-oxooxetan-2-yl)-3-(tosyloxy)propan-2-yl-2-methanamidoethanoate (17e)**

Prepared according to the representative acylation procedure using hydroxy  $\beta$ -lactone **13j** (13.0 mg, 0.034 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (8.50 mg, 0.044 mmol), 4-dimethylaminopyridine (5.0 mg, 0.041 mmol), and *N*-formyl glycine (5.26 mg, 0.051 mmol). Purification by chromatography (SiO<sub>2</sub>, 20% EtOAc:hexanes) afforded  $\beta$ -lactone **17e** (9.2 mg, 58%). *R<sub>f</sub>* = 0.22 (40% EtOAc/hexanes); [ $\alpha$ ]<sub>D</sub><sup>22</sup> = -6.5 (*c* 0.4, CHCl<sub>3</sub>). IR (thin film) 2955, 2925, 2855, 1738, 1607, 1516, 1466. <sup>1</sup>H NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  8.27 (br s, 1H), 7.77 (d, *J* = 7 Hz, 2H), 7.38 (d, *J* = 8 Hz, 2H), 6.07 (br s, 1H), 5.14–5.19 (m, 1H), 4.29 (app t, *J* = 12 Hz, 1H), 4.05–4.16 (m, 4H), 3.23–3.27 (m, 1H), 2.47 (s, 3H), 2.26–2.31 (m, 2H), 1.99–2.05 (m, 2H), 1.72–1.86 (m, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  170.3, 168.3, 161.2, 145.8, 132.7, 130.3 (2), 128.12 (2), 73.7, 69.7, 57.2, 40.1, 35.6, 31.6, 29.1, 27.8, 27.0, 22.7, 21.9, 14.3. HRMS (ESI) calcd for C<sub>22</sub>H<sub>31</sub>NO<sub>8</sub>S, 476.1930; found, 476.1955.

**(R)-1-((2S,3S)-3-Hexyl-4-oxooxetan-2-yl)hex-5-en-2-yl-2-methanamidoethanoate (17f)**

Prepared according to the representative acylation procedure using hydroxy  $\beta$ -lactone **13g** (15.0 mg, 0.059 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (14.8 mg, 0.077 mmol), 4-dimethylaminopyridine (8.71 mg, 0.071 mmol), and *N*-formyl glycine (9.12 mg, 0.089 mmol). Purification by chromatography (SiO<sub>2</sub>, 10  $\rightarrow$  40% EtOAc:hexanes) afforded  $\beta$ -lactone **17f** (10.8 mg, 55%). *R<sub>f</sub>* = 0.33 (40% EtOAc/hexanes); [ $\alpha$ ]<sub>D</sub><sup>22</sup> = -5.1 (*c* 1.8, CHCl<sub>3</sub>). IR (thin film) 3418, 2925, 2854, 1819, 1740, 1667 cm<sup>-1</sup>. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.27 (br s, 1H), 6.10 (br s, 1H), 5.73–5.81 (m, 1H), 5.07–5.14 (m, 1H), 5.02 (dt, *J* = 1.5, 10.5 Hz, 2H), 4.27–4.30 (m, 1H), 4.09 (dt, *J* = 0.5, 5.0 Hz, 2H), 3.21–3.25 (m, 1H), 2.09 (app t, *J*

= 7.0 Hz, 4H), 1.64–1.85 (m, 4H), 1.24–1.45 (m, 8H), 0.89 (app t,  $J = 7.0$  Hz, 3H).  $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  170.9, 169.3, 161.1, 137.0, 116.0, 74.4, 72.5, 57.0, 40.3, 39.1, 33.5, 31.7, 29.5, 29.2, 27.9, 27.0, 22.7, 14.3. HRMS (ESI) calcd for  $\text{C}_{18}\text{H}_{29}\text{NO}_5$  [ $\text{M} + \text{Li}$ ], 346.2206; found, 346.2276.

### **(R)-1-((2S,3S)-3-Hexyl-4-oxooxetan-2-yl)hept-5-en-2-yl-2-methanamidoethanoate (17g)**

Prepared according to the representative acylation procedure using hydroxy  $\beta$ -lactone **13h** (15.6 mg, 0.058 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (14.4 mg, 0.075 mmol), 4-dimethylaminopyridine (8.7 mg, 0.070 mmol), and *N*-formyl glycine (9.0 mg, 0.087 mmol). Purification by chromatography ( $\text{SiO}_2$ , 10 $\rightarrow$ 40% EtOAc:hexanes) afforded  $\beta$ -lactone **17g** (11.7 mg, 57%).  $R_f = 0.38$  (40% EtOAc/hexanes);  $[\alpha]_D^{22} = -22$  ( $c$  1.1,  $\text{CHCl}_3$ ). IR (thin film) 2926, 2855, 1823, 1712  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.27 (br s, 1H), 6.11 (br s, 1H), 5.42–5.48 (m, 1H), 5.34–5.39 (m, 1H), 5.10 (app quint,  $J = 5.0$  Hz, 1H), 4.26–4.29 (m, 1H), 4.09 (d,  $J = 5.5$  Hz, 2H), 3.22–3.60 (m, 1H), 1.99–2.10 (m, 4H), 1.64–1.85 (m, 4H), 1.25–1.45 (m, 8H), 0.89 (app t,  $J = 9.0$  Hz, 6H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  170.9, 169.3, 161.1, 129.5, 126.6, 74.4, 72.5, 56.9, 40.3, 39.0, 34.2, 31.7, 29.2, 28.4, 27.0, 22.7, 18.1, 14.3. HRMS (ESI) calcd for  $\text{C}_{19}\text{H}_{31}\text{NO}_5$  [ $\text{M} + \text{Li}$ ], 360.2362; found, 360.2367.

### **2-Formylamino-3-methyl-butyric Acid (2S,2R,3S,4S)-1-(3-Hexyl-4-oxo-oxetan-2-ylmethyl)-hex-4-enyl Ester (17h)**

Prepared according to the representative acylation procedure using  $\beta$ -lactone **13h** (20.0 mg, 0.0745 mmol), *N*-formyl-L-valine (11.9 mg, 0.0820 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (18.6 mg, 0.0969 mmol), and 4-dimethylaminopyridine (9.2 mg, 0.0745 mmol). Purification by flash chromatography on  $\text{SiO}_2$  (0.4:1:3 THF/ $\text{CHCl}_3$ /hexane) gave  $\beta$ -lactone **17h** as a *E/Z*-mixture; only peaks for major olefin isomer are assigned.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.28 (s, 1H), 6.08 (br, 1H), 5.34–5.50 (m, 2H), 5.02–5.10 (m, 1H), 4.63–4.68 (m, 1H), 4.28 (dt,  $J = 3.9, 8.1$  Hz, 1H), 3.20–3.28 (m, 1H), 2.18–2.26 (m, 1H), 1.30–2.08 (m, 19H), 1.01 (d,  $J = 6.9$  Hz, 3H), 0.93 (d,  $J = 6.9$  Hz, 3H), 0.89 (t,  $J = 6.9$  Hz, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.3 (2), 161.1, 129.4, 126.6, 74.4, 72.3, 57.0, 55.9, 39.2, 34.3, 31.7, 31.4, 29.1, 28.3, 27.8, 27.0, 22.7, 19.4, 18.1, 17.6, 14.2.

### **Mukaiyama Aldol-Lactonization Process using $\text{SnCl}_4$ as Described for (R)-1-((2S,3R)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl-2-methanamidoethanoate (17i)**

To a solution of aldehyde **8f** (0.189 g, 0.55 mmol) and thiopyridyl ketene acetal **3d** (0.21 g, 1.10 mmol) in  $\text{CH}_2\text{Cl}_2$  (4 mL) at  $-78$  °C was added  $\text{SnCl}_4$  (0.66 mL, 0.66 mmol, 1.0 M in  $\text{CH}_2\text{Cl}_2$ ) via syringe pump over 2 h. After 10 h, the reaction was warmed to  $-50$  °C for additional 6 h. Next, the reaction was quenched with pH 7 buffer (10 mL) at  $-50$  °C and warmed to 23 °C with vigorous stirring for 20 min, and the resulting mixture was filtered through a small pad of celite. After the filtrate was dried over  $\text{Na}_2\text{SO}_4$  and filtered, the volume of  $\text{CH}_2\text{Cl}_2$  was adjusted to a final concentration of 0.15 M.  $\text{CuBr}_2$  (0.26 g, 1.10 mmol) was added, and the mixture was stirred for 2 h. The resulting suspension was filtered through celite and washed with 10% aqueous  $\text{K}_2\text{CO}_3$  ( $2 \times 10$  mL) solution and brine ( $2 \times 20$  mL). The organic phase was separated and dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated in vacuo. The residue was purified by chromatography ( $\text{SiO}_2$ , 30% EtOAc in hexanes) to afford a mixture of *cis*- $\beta$ -lactone diastereomers, which was taken on directly to desilylation (*dr* = 3:1).

### **Representative Procedure for Desilylation of Crude Silyloxy- $\beta$ -Lactones from the Aldol-Lactonization Process**

To a solution of the diastereomeric mixture of silyloxy- $\beta$ -lactones (obtained from the TMAL process) in dry  $\text{CH}_3\text{CN}$  (20 mL) at 0 °C was added 48% aqueous HF (0.43 mL). The



reaction was stirred at 0 °C for 2 h and warmed to room temperature. After stirring for additional 2 h, the reaction mixture was diluted with 15 mL of ether. The organic layer was separated, washed with a saturated solution of NaHCO<sub>3</sub> (2 × 20 mL) with caution, followed with brine (2 × 20 mL). The residue was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated in vacuo. The mixture was purified by flash chromatography (2–10% EtOAc in hexanes). The diastereomers were partially separated via MPLC on SiO<sub>2</sub> (10% EtOAc in hexanes). Only 12 mg of the major diastereoisomer *cis*-**13j** (dr >19:1) was separated completely.

### β-Lactone **17i**

Acylation was performed according to the representative procedure using hydroxy β-lactone *cis*-**13** (12.0 mg, 0.032 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (7.8 mg, 0.041 mmol), 4-dimethylamino-pyridine (54.7 mg, 0.038 mmol), and *N*-formyl glycine (5.0 mg, 0.048 mmol). Purification by chromatography (SiO<sub>2</sub>, 20% EtOAc:hexanes) to afford β-lactone **17i** (7.2 mg, 51%). *R*<sub>f</sub> = 0.32 (40% EtOAc/hexanes); [α]<sub>D</sub><sup>22</sup> = −8.8 (*c* 0.45, CHCl<sub>3</sub>). IR (thin film) 2925, 2854, 1823, 1748, 1824, 1748 1654 cm<sup>−1</sup>. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.27 (br s, 1H), 6.09 (br s, 1H), 5.11–5.15 (m, 1H), 4.58–4.67 (m, 1H), 4.12–4.13 (m, 2H), 3.67 (app d, *J* = 7.0 Hz, 1H), 1.90–2.05 (m, 2H), 1.70–1.80 (m, 8H), 1.20–1.40 (m, 25H), 0.99 (m, 7H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 171.6, 169.3, 161.2, 73.1, 71.8, 53.2, 40.2, 34.9, 34.5, 32.1, 31.7, 29.9, 29.85 (2), 29.83, 29.77, 29.7, 29.57, 29.56, 29.2, 27.6, 25.3, 24.3, 22.9, 22.8, 14.4, 14.3. LRMS (ESI) calcd for C<sub>27</sub>H<sub>49</sub>NO<sub>5</sub> [M + Li], 446.68; found, 446.35.

### Representative Cross-Metathesis Procedure as Described for 2-Formylamino-4-methyl-pentanoic Acid 1-(3-Hexyl-4-oxooxetan-2-ylmethyl)-dodec-4-enyl Ester (**21a**)

To a solution of β-lactone **15j** (23.5 mg, 0.0594 mmol) and *n*-nonene (51 μL, 0.297 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.2 mL) at rt was added a solution of Grubb's catalyst (4.9 mg, 0.00594 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.1 mL). The mixture was heated to 45 °C for 24 h and directly purified using flash chromatography (4:1, hexanes:EtOAc) gave β-lactone **21a** (yield not determined). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.23 (s, 1H), 5.90 (d, *J* = 8.5 Hz, 1H), 5.31–5.47 (m, 2H), 5.04–5.08 (m, 1H), 4.70 (dt, *J* = 4.0, 8.5 Hz, 1H), 4.30 (quint, *J* = 4.5 Hz, 1H), 3.20–3.25 (m, 1H), 1.95–2.20 (m, 6H), 1.57–1.82 (m, 8H), 1.25–1.46 (m, 17H), 0.87–0.99 (m, 12H). HRMS (ESI) calcd for C<sub>29</sub>H<sub>51</sub>NO<sub>5</sub> [M + Li], 500.3927; found, 500.3734.

### 2-Formylamino-3-phenyl-propionic Acid 1-(3-Hexyl-4-oxooxetan-2-ylmethyl)-dodec-4-enyl Ester (**21b**)

Prepared according to the representative cross-metathesis procedure using β-lactone **15i** (10 mg, 0.023 mmol), *n*-nonene (20 μL, 0.12 mmol), and Grubb's catalyst (3.8 mg, 0.0047 mmol). Purification by flash chromatography (10:1, hexanes:EtOAc) gave desired β-lactone **21b** (5.5 mg, 45%) (3.8 mg, 38%) as a E/Z-mixture; only peaks for major olefin isomer are assigned. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.20 (s, 1H), 7.20–7.30 (m, 5H), 6.02 (d, *J* = 7.2 Hz, 1H), 5.30–5.50 (m, 2H), 5.00–5.08 (m, 1H), 4.94 (q, *J* = 6.9 Hz, 1H), 4.17–4.23 (m, 1H), 3.13–3.21 (m, 4H), 1.30–2.12 (m, 27H), 0.89–0.93 (m, 6H). HRMS (ESI) calcd for C<sub>32</sub>H<sub>49</sub>NO<sub>5</sub> [M + Li], 534.3771; found, 534.3659.

### 2-Formylamino-4-methyl-pentanoic Acid 5-(4-Fluoro-phenyl)-1-(3-hexyl-4-oxo-oxetan-2-ylmethyl)-pent-4-enyl Ester (**21c**)

Prepared according to the cross-metathesis procedure using β-lactone **15j** (15.0 mg, 0.0379 mmol), Grubb's catalyst (3.1 mg, 0.0038 mmol), and 4-fluorostyrene (18 μL, 0.15 mmol). Purification by flash chromatography (4:1, hexanes:EtOAc) gave β-lactone **21c** (yield not determined). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.15 (s, 1H), 7.30–7.34 (m, 2H), 6.98–17.04 (m, 2H), 6.40 (d, *J* = 15.6 Hz, 1H), 6.10 (dt, *J* = 6.6, 15.6 Hz, 1H), 5.90 (d, *J* = 8.1 Hz, 1H),

5.10–5.20 (m, 1H), 4.72 (dt,  $J = 5.1, 9.3$  Hz, 1H), 4.30–4.36 (m, 1H), 3.22–3.29 (m, 1H), 1.25–2.70 (m, 19H), 0.89–1.01 (m, 9H). HRMS (ESI) calcd for  $C_{23}H_{40}FNO_5$  [ $M + Na$ ], 512.2788; found, 512.2828.

### **(R)-1-((2S,3S)-3-Hexyl-4-oxooxetan-2-yl)hexan-2-yl-2-methanamidoethanoate (23b)**

$\beta$ -lactone **17f** (6.2 mg, 0.018 mmol) and 5 wt % palladium on carbon (5 mg) in 2 mL of  $CH_2Cl_2$  was stirred at ambient temperature for 12 h under  $H_2$  atmosphere. Filtration and chromatography ( $SiO_2$ , 40% EtOAc:hexanes) gave the desired  $\beta$ -lactone **23b** (5.8 mg, 95%).  $R_f = 0.24$  (40% EtOH/hexanes);  $[\alpha]_D^{22} = -28.9$  ( $c$  0.8,  $CHCl_3$ ). IR (thin film) 2927, 2858, 1819, 1745, 1675  $cm^{-1}$ .  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  8.27 (br s, 1H), 6.09 (br s, 1H), 5.06–5.12 (m, 1H), 4.27–4.30 (m, 1H), 4.10 (dd,  $J = 2.0, 5.0$  Hz, 2H), 3.22–3.27 (m, 1H), 2.07 (dt,  $J = 1.5, 6.5$  Hz, 2H), 1.53–1.87 (m, 8H), 1.23–1.47 (m, 10H), 0.90 (dd,  $J = 7.0, 15.0$  Hz, 4H).  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )  $\delta$  171.0, 169.3, 161.2, 74.5, 73.0, 56.9, 40.3, 39.0, 34.1, 31.7, 29.2, 27.9, 27.4, 27.0, 22.7, 22.6, 14.3, 14.1. HRMS (ESI) calcd for  $C_{18}H_{31}NO_5$  [ $M + Li$ ], 348.2362; found, 348.2311.

### **Fluorogenic Assay for Detection of FASTE Inhibition**

The synthetic fluorogenic substrate, 4-methylumbelliferyl heptanoate (4-MUH), was purchased from Sigma (St. Louis, MO). The reaction mixture consisted of 500 nM FASTE in buffer (100 mM Tris-HCl, 50 mM NaCl at pH 7.4), which was preincubated with 2.5  $\mu$ M of test compounds dissolved in DMSO at final concentrations of 0.32–100  $\mu$ M and/or 0.08–10  $\mu$ M at 37 °C for 30 min. The reaction was initiated by addition of 5  $\mu$ L of 1.25 mM 4-MUH in 1:1 DMSO:buffer. The resulting fluorescence from liberated 4-methylumbelliferone was measured every 5 min at 350/450 nm for 40–60 min. 4-MUH incubated without enzyme served as a background control. Results are the average of triplicate data points.

### **Cell Viability Assays for Determination of Test Compound Potencies**

MB-MDA-231 or human fibroblast cells were plated in 96-well plates in appropriate media and incubated overnight at 37 °C and 5%  $CO_2$ . Cells were treated with test compounds (0.1–100  $\mu$ M) or vehicle in triplicate, with a final percentage of DMSO not exceeding 1% (v/v). At 48 h, the medium was aspirated and replaced with complete MEM, containing 333  $\mu$ g/mL [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and 25  $\mu$ M phenazine methosulfate (PMS), using the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega). Plates were incubated for 2 h and absorbance was assayed at 490 nm. Background levels of formazan formation were measured in medium alone.  $IC_{50}$  values were derived from dose–response curves.

### **Cell Lines**

The MDA-MB-231 cell line and Hs 58.Fs cells were purchased from American Type Culture Collection (Manassas, VA). MDA-MB-231 tumor cells were maintained in minimum essential medium Eagle (MEM) with Earl's Salts (Mediatech, Inc., Herndon, VA) supplemented with 10% fetal bovine serum (FBS; Irvine Scientific, Santa Ana, CA), 2 mM L-glutamine (Invitrogen Life Technologies, Inc., Carlsbad, CA), MEM vitamins (Invitrogen Life Technologies, Inc.), nonessential amino acids (Mediatech, Inc.), and antibiotics (Omega Scientific, Inc., Tarzana, CA). Human fibroblast (Hs 58.Fs) cells were maintained in Dulbecco's modified Eagle's medium (Irvine Scientific) supplemented with 10% FBS and antibiotics. All cells were grown at 37 °C under a humidified, 5%  $CO_2$  atmosphere.

## RNA Interference

siRNA was purchased from Dharmacon (Lafayette, CO). Cells were plated in 96-well plates and grown for 48 h prior to transfection with 100 nM FAS SMARTpool (M-003954-02) or nonsilencing control (D-001206-13) siRNA using LipofectAMINE 2000 reagent (Invitrogen) as previously described.<sup>24</sup>

## Inhibition of Fatty Acid Synthase with Orlistat

Orlistat (Xenical) was extracted from capsules (Roche Applied Science, Indianapolis, IN) as previously described.<sup>24</sup> Cells were plated in 96-well plates for 48 h, followed by treatment with 25  $\mu$ M orlistat or vehicle control for 72 h.

## DNA Fragmentation Assay

Apoptosis was monitored by measuring the level of DNA fragments present following 72 h exposure to FAS siRNA or orlistat using the Cell Death Detection ELISA<sup>plus</sup> kit (Roche) according to manufacturer instructions.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

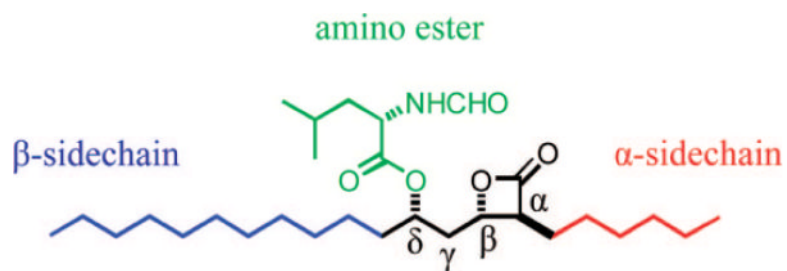
## Acknowledgments

We thank the NIH (NCI CA 10658, D.R. and J.W.S.; CA81713, J.W.S.) and the Welch Foundation (D.R.) for support of these investigations. We thank Dr. Wei Zhang for assistance with manuscript preparation and Ms. Li Yang for technical assistance with purity chromatograms. Helpful conversations with Dr. Adam Richardson and Dr. Russell Dahl are gratefully acknowledged.

## References

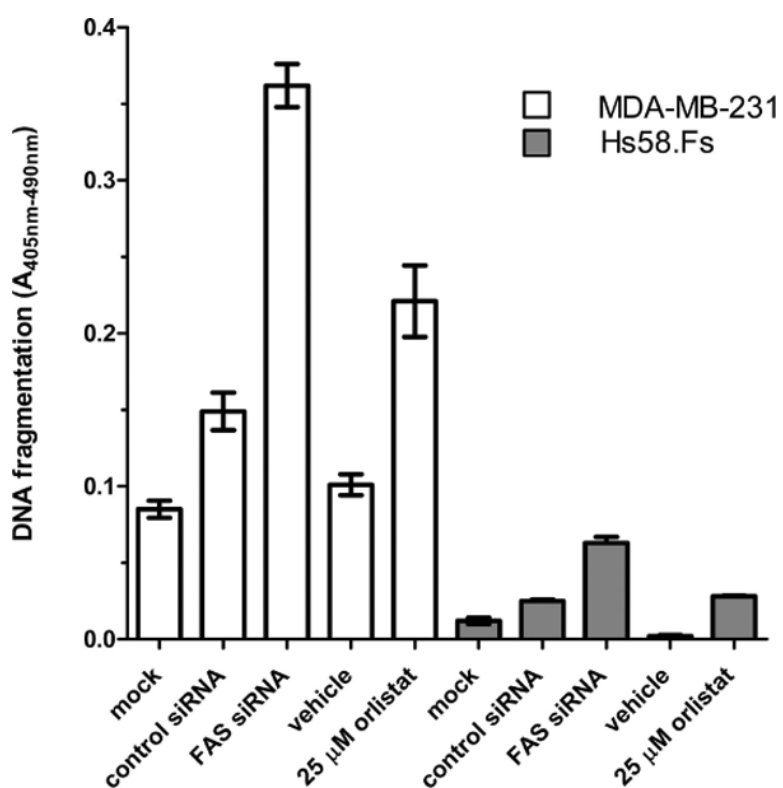
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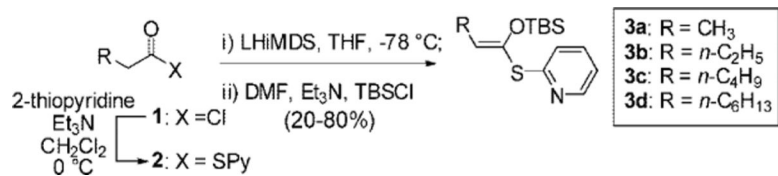


**Figure 1.** Molecular structure of tetrahydrolipstatin (orlistat) and the structural features modified in this study. Modifications were made to reduce the overall hydrophobicity of the compounds and then tested for retention of activity. Several compounds with variation at one or a combination of these features were shown to have increased potency toward FASTE in a fluorogenic biochemical assay.

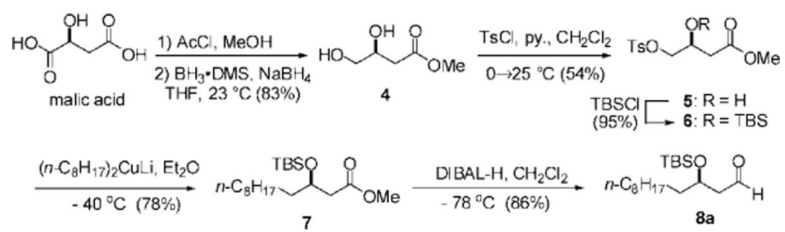




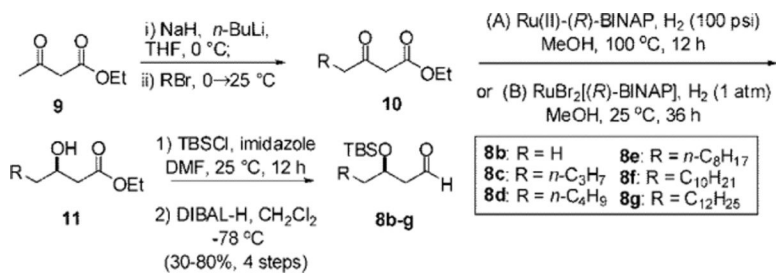
**Figure 2.** Differential cytotoxicity in MDA-MB-231 and Hs58.Fs cells are a model platform for screening FAS inhibitors, as shown by the pharmacological and siRNA inhibition of FAS. MDA-MB-231 tumor cells and primary cultures of Hs58.Fs fibroblasts were exposed to 100 nM FAS siRNA or nonsilencing control siRNA and 25 μM orlistat or vehicle control for 72 h. Cellular cytotoxicity was monitored by measuring DNA fragmentation. Values are means ± SE of four samples per treatment group.



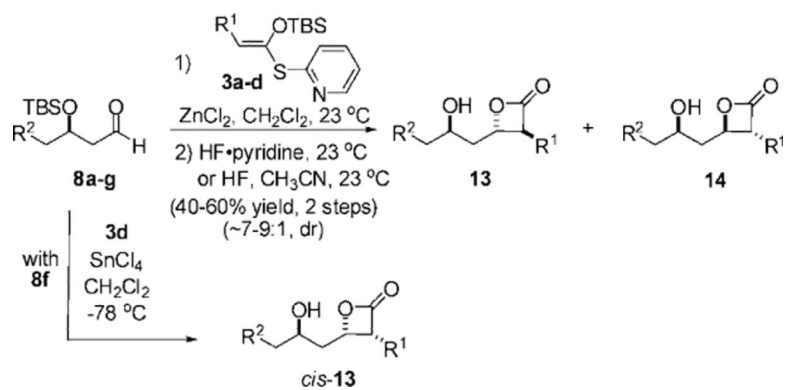
**Scheme 1.**  
Syntheses of the Thiopyridyl Ketene Acetals



**Scheme 2.**  
 Synthesis of the Chiral Aldehyde **8a** from Malic Acid Involving Cuprate Addition

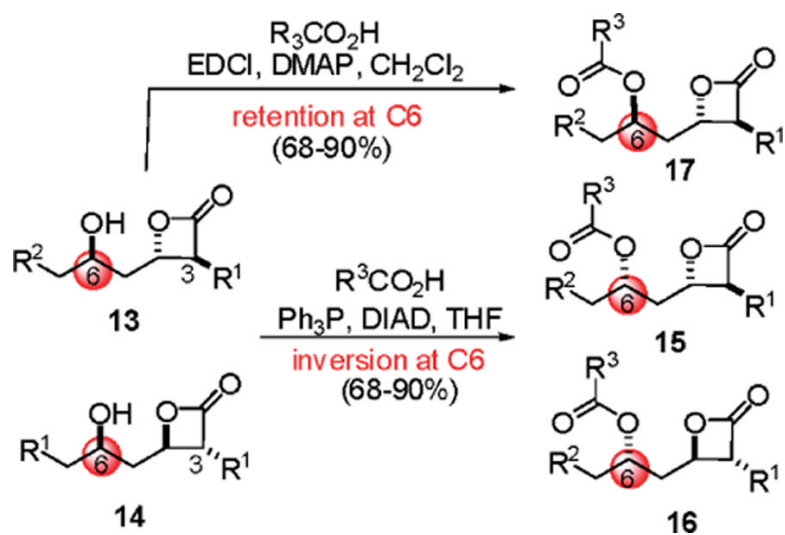


**Scheme 3.**  
 Syntheses of Chiral Aldehydes **8b–g** via Noyori Reduction

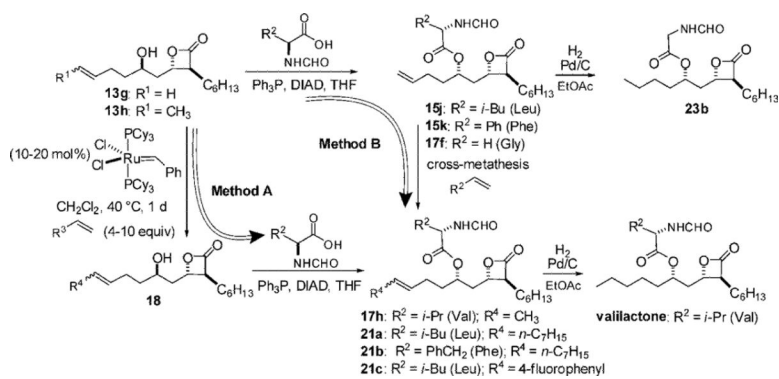


**Scheme 4.**  
 Tandem Mukaiyama Aldol-Lactonization Process and Deprotection Delivering  $\beta$ -Lactones  
**13/14**



**Scheme 5.**

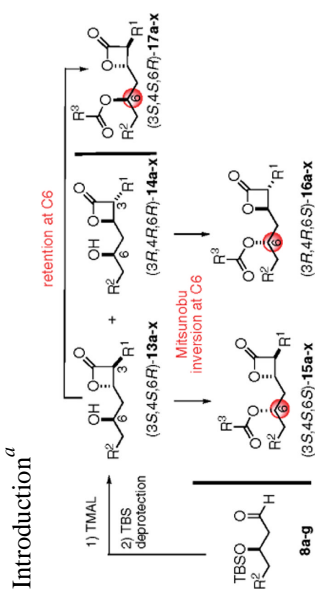
Introduction of the  $\alpha$ -Amino Ester Moiety via Mitsunobu Reaction (with Inversion) or Acylation (with Retention) at C6, Providing Orlistat Derivatives **15-17**



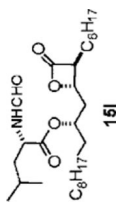
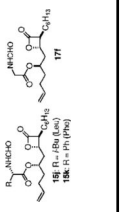
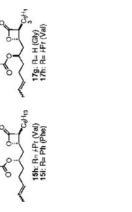
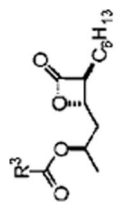
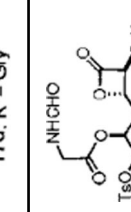
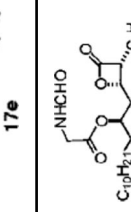
**Scheme 6.**  
 Alternative Strategy for Modification of the  $\delta$ -Side Chain Involving Olefin Cross-Metathesis and Hydrogenation

Table 1

Orlistat Derivatives Prepared by the TMAL Process Followed by Either Inversion or Retention of C6 Stereochemistry during Amino Ester side Chain Introduction<sup>a</sup>



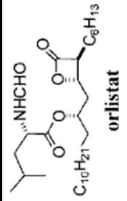
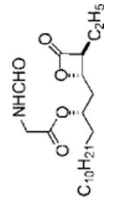
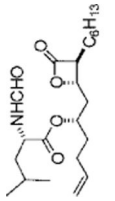
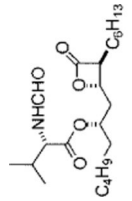
entry	R <sub>1</sub>	R <sub>2</sub>	TMAL % yield <sup>b</sup>	TMAL ratio (13:14) <sup>a</sup>	% yield Mitsunobu/acylation	Orlistat derivatives 15–17
1	C <sub>6</sub> H <sub>13</sub>	C <sub>12</sub> H <sub>25</sub>	58	13a: 14a = 6:1	80 (15a) 73 (15b) 80 (15c) 17 (15d) 17 (16a)	 15a: R <sup>3</sup> = Me, R <sup>4</sup> = H; 15b: R <sup>3</sup> = Me, R <sup>4</sup> = Me; 15c: R <sup>3</sup> = Me, R <sup>4</sup> = Ph; 15d: R <sup>3</sup> = Me, R <sup>4</sup> = t-Bu; 16a: R <sup>3</sup> = Me, R <sup>4</sup> = t-Bu
2	C <sub>6</sub> H <sub>13</sub>	C <sub>10</sub> H <sub>21</sub>	58	13b: 14b = 8:1	87 (15g) 97 (17c)	 15g: R <sup>3</sup> = Me, R <sup>4</sup> = Me; 17c: R <sup>3</sup> = Me, R <sup>4</sup> = t-Bu
3	C <sub>4</sub> H <sub>9</sub>	C <sub>10</sub> H <sub>21</sub>	49	13c: 14c = 7.7:1	52 (17d)	 17d: R <sup>3</sup> = Me, R <sup>4</sup> = t-Bu
4	C <sub>2</sub> H <sub>5</sub>	C <sub>10</sub> H <sub>21</sub>	58	13d: 14d = 6:1	25 (15f) 99 (17a)	 15f: R <sup>3</sup> = Me, R <sup>4</sup> = Me; 17a: R <sup>3</sup> = Me, R <sup>4</sup> = t-Bu
5	Me <i>E/Z</i>	C <sub>10</sub> H <sub>21</sub>	20	13e: 14e = 1.5:1	38 (15e) 24 (16b)	 15e: R <sup>3</sup> = Me, R <sup>4</sup> = Me; 16b: R <sup>3</sup> = Me, R <sup>4</sup> = t-Bu

entry	R <sub>1</sub>	R <sub>2</sub>	TMAL % yield <sup>b</sup>	TMAL ratio (13:14) <sup>a</sup>	% yield Mitsunobu/acylanon	Orlistat derivatives 15–17
6	C <sub>8</sub> H <sub>17</sub>	C <sub>8</sub> H <sub>17</sub>	42	13f:14f = 8.4:1	ref. 22	 16i
7	C <sub>6</sub> H <sub>13</sub>	allyl	60	13g:14g = ND	68 (15j) 46 (15k) 55 (17f)	
8	C <sub>6</sub> H <sub>13</sub>	<i>trans</i> -crotyl	62	13h:14h = 9:1	45 (15h) ND (15i) 57 (17g) ND (17h)	
9	C <sub>6</sub> H <sub>13</sub>	H	42	13i:14i = 7.1:1	52 (17d)	 17d: R <sup>2</sup> = Gly
10	C <sub>6</sub> H <sub>13</sub>	TsO	42	13j:14j = 7.7:1	58 (17e)	 17e
11	C <sub>6</sub> H <sub>13</sub>	C <sub>10</sub> H <sub>21</sub>	ND <sup>a</sup>	13j:14j = 3:1	51 (17i)	 17i

<sup>a</sup>ND = not determined.<sup>b</sup>Includes yield for TBS deprotection step.

Table 2

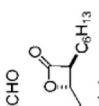
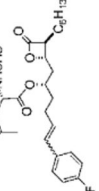
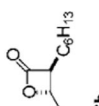
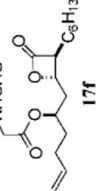
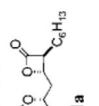
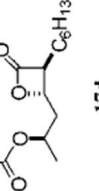
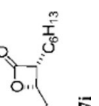
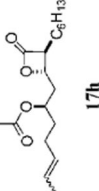
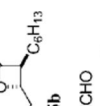
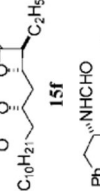
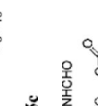
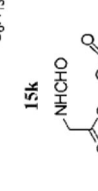

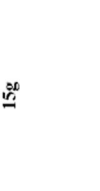
Compounds that Displayed Enhanced Inhibitory Activity in the Biochemical Assay as Well as Better Selectivity for Lipogenic Cells Relative to Orlistat<sup>a</sup>

Entry	Structure	FASTE/ <sup>1</sup> IC <sub>50</sub> (μM)	MDA-MB 231 <sup>2</sup> IC <sub>50</sub> (μM)	Hs58.Fs <sup>2</sup> IC <sub>50</sub> (μM)	Ratio <sup>3</sup>	cLogP <sup>4</sup>
1	 orlistat C <sub>10</sub> H <sub>21</sub>	1.35±0.34	16.8±0.5	70.2±15.9	4.2	8.609
2	 C <sub>10</sub> H <sub>21</sub>	0.23±0.02	1.3±0.1	10.0±2.4	7.7	4.727
3	 15j C <sub>8</sub> H <sub>13</sub>	0.28±0.04	3.3±0.6	29.6±2.3	9.0	4.422
4	 valilactone C <sub>4</sub> H <sub>9</sub>	0.30±0.07	10.5±2.5	>100	>9.5	4.906

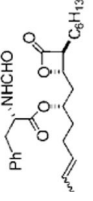
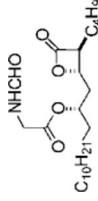
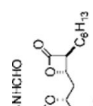
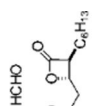
<sup>a</sup> (1) Results are presented as mean and 95% CI. (2) Results are presented as the mean ± SD of at least two independent experiments. (3) Ratio of IC<sub>50</sub> values (Hs58.Fs/MDA-MB-231). (4) cLogP values were calculated with ChemDraw Ultra 10.0 software (CambridgeSoft).

Table 3

Displayed Enhanced Inhibitory Activity Relative to Orlistat but Less Cellular Selectivity<sup>a</sup>

Structure	FASTE/IC <sub>50</sub> (μM)	MDA-MB-231 <sup>2</sup> IC <sub>50</sub> (μM)	Hs58.Fs <sup>2</sup> IC <sub>50</sub> (μM)	Ratio <sup>3</sup>	cLogP <sup>4</sup>	Entry	Structure	FASTE/IC <sub>50</sub> (μM)	MDA-MB-231 <sup>2</sup> IC <sub>50</sub> (μM)	Hs58.Fs <sup>2</sup> IC <sub>50</sub> (μM)	Ratio <sup>3</sup>	cLogP <sup>4</sup>
	1.35±0.34	16.8±0.5	70.2±15.9	4.2	8.609	10		0.50±0.12	14.1	32.9	2.3	6.163
	0.10±0.03	48.0±5.7	52.2±3.9	1.1	3.140	11		0.50±0.18	54.0±9.4	>100	>1.9	2.656
	0.72±0.29	>100	43.9±13.9	<0.4	8.125	12		0.52±0.14	>100	>100	ND	1.553
	0.18±0.01	>100	>100	ND	7.901	13		0.68±0.12	56.9±19.0	>100	>1.8	4.422
	0.28±0.04	26.9±12.2	37.3-0.3	1.4	7.901	14		0.79±0.12	88.4±7.3	>100	>1.1	4.727
	0.29±0.04	>100	>100	ND	9.628	15		0.90±0.10	24.5±0.8	73.6±2.0	3.0	4.383
	0.37±0.06	>100	>100	ND	2.508	16		1.02±0.38	4.8±0.5	19.9±4.4	4.2	6.843

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Structure	FASTE/IC <sub>50</sub> (μM)	MDA-MB-231 <sup>2</sup> IC <sub>50</sub> (μM)	Hs58.Fs <sup>2</sup> IC <sub>50</sub> (μM)	Ratio <sup>3</sup>	cLogP <sup>4</sup>	Entry	Structure	FASTE <sup>1</sup> IC <sub>50</sub> (μM)	MDA-MB-231 <sup>2</sup> IC <sub>50</sub> (μM)	Hs58.Fs <sup>2</sup> IC <sub>50</sub> (μM)	Ratio <sup>3</sup>	cLogP <sup>4</sup>
	0.40±0.05	>100	24.9±0.1	<0.3	8.086	17		1.04±0.33	38.3±0.9	61.2±3.0	1.6	4.912
	0.45±0.10	37.1 ±11.8	15.8±3.4	0.4	7.227	18		1.35±0.07	35.7±1.3	31.1±3.2	0.9	5.785

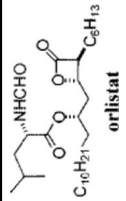
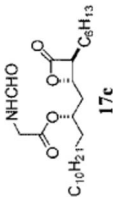
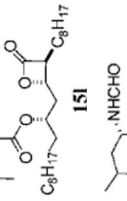
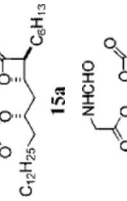
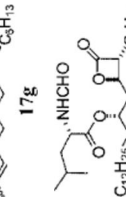
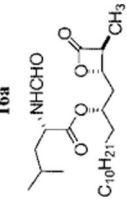
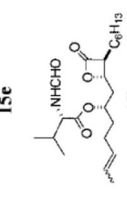

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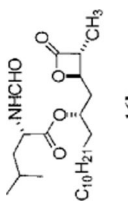
Results are presented as the mean ± SD of at least two independent experiments. (3) Ratio of IC<sub>50</sub> values (Hs58.Fs/MDA-MB-231). (4) cLogP values determined using ChemDraw Ultra 10.0 software (CambridgeSoft). ND = not determined.



Table 4

Compounds with Structural Changes that Proved Deleterious to the Biochemical Inhibition of PASTE<sup>a</sup>

Entry	Structure	FASTE/ IC <sub>50</sub> (μM)	MDA-MB-231 <sup>2</sup> IC <sub>50</sub> (μM)	Hs58.Fs <sup>2</sup> IC <sub>50</sub> (μM)	Ratio <sup>3</sup>	cLogP <sup>4</sup>
1	 orlistat C <sub>10</sub> H <sub>21</sub> C <sub>8</sub> H <sub>13</sub>	1.35±0.34	16.5±0.5	70.2±15.9	4.2	8.609
2	 17e C <sub>10</sub> H <sub>21</sub> C <sub>8</sub> H <sub>13</sub>	1.72±0.09	11.7±0.9	46.8±0.3	4.0	6.843
3	 15l C <sub>8</sub> H <sub>17</sub> C <sub>8</sub> H <sub>13</sub>	3.00±2.21	>100	87.2±10.5	<1.0	8.609
4	 15a C <sub>12</sub> H <sub>25</sub> C <sub>8</sub> H <sub>13</sub>	3.67±0.89	>100	>100	ND	9.667
5	 17g C <sub>8</sub> H <sub>13</sub> C <sub>8</sub> H <sub>13</sub>	6.24±0.46	ND	ND	ND	3.185
6	 16a C <sub>12</sub> H <sub>25</sub> C <sub>8</sub> H <sub>13</sub>	9.74±6.04	ND	ND	ND	9.667
7	 15e C <sub>10</sub> H <sub>21</sub> CH <sub>3</sub>	10.81 ± 1.66	ND	ND	ND	5.964
8	 15h C <sub>8</sub> H <sub>13</sub> C <sub>8</sub> H <sub>13</sub>	13.54±2.54	ND	ND	ND	4.422

Entry	Structure	FASTE/ IC <sub>50</sub> (μM)	MDA-MB-231 <sup>2</sup> IC <sub>50</sub> (μM)	Hs58.Fs <sup>2</sup> IC <sub>50</sub> (μM)	Ratio <sup>3</sup>	cLogP <sup>4</sup>
9	 <chem>CCCC(=O)Nc1ccc(cc1)C(C)CC</chem> <b>16b</b>	45.1 1±11.39	ND	ND	ND	5.964

<sup>a</sup> Compounds with an IC<sub>50</sub> > 5 μM in the FASTE biochemical assay were not tested in cells. (1) Results are presented as mean and 95% CI. (2) Results are presented as mean ± SD of at least two independent experiments. (3) Ratio of IC<sub>50</sub> values (Hs58.Fs/MDA-MB-231). (4) cLogP values were calculated with ChemDraw Ultra 10.0 software (Cambridge-Soft). ND = not determined.