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Total Synthesis and Comparative Analysis of Orlistat, Valilactone, and a Transposed Orlistat Derivative: Inhibitors of Fatty Acid Synthase

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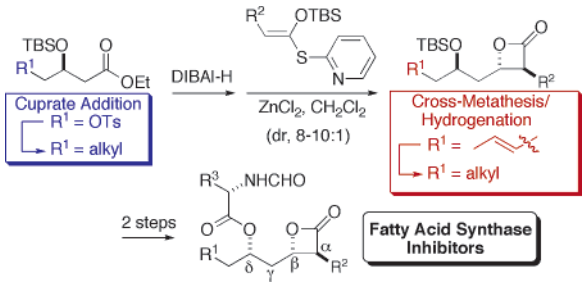
ABSTRACT



Concise syntheses of orlistat (Xenical), a two-carbon transposed orlistat derivative, and valilactone are described that employ the tandem Mukaiyama aldol–lactonization (TMAL) process as a key step. This process allows facile modification of the α-side chain. Versatile strategies for modifying the *δ*-side chain are described, involving cuprate addition and olefin metathesis. Comparative antagonistic activity of these derivatives toward a recombinant form of the thioesterase domain of fatty acid synthase is reported along with comparative activity-based profiling.

Tetrahydrolipstatin (orlistat), a reduced form of the natural product lipstatin, is an antiobesity agent marketed under the tradename of Xenical that was recently approved by the FDA as the first over-the-counter weight-loss medication (Figure 1). This *β*-lactone-containing, natural product derivative inhibits gastric and pancreatic lipases by blocking hydrolysis of triglycerides and thus uptake of fatty acids from the diet.[1] The mechanism of inhibition involves covalent but reversible modification of the active site serine via acylation by the *β*-lactone. Renewed interest in tetrahydrolipstatin stems from our recent finding that it is also a specific inhibitor of the thioesterase domain of fatty acid synthase (FAS-TE), a validated drug target for anticancer therapy.[2]

We previously developed the tandem Mukaiyama aldol−lactonization (TMAL) process mediated by $ZnCl_2$ which delivers *trans*-*β*-lactones with high diastereoselectivity.[3] We also developed a stereocomplimentary process mediated by $SnCl_4$ that delivers *cis*-*β*-lactones.[4] The former procedure was applied to the synthesis of okinonellin,[5] brefeldin A,[6] and, most pertinent to the present work, panclicin D, a naturally

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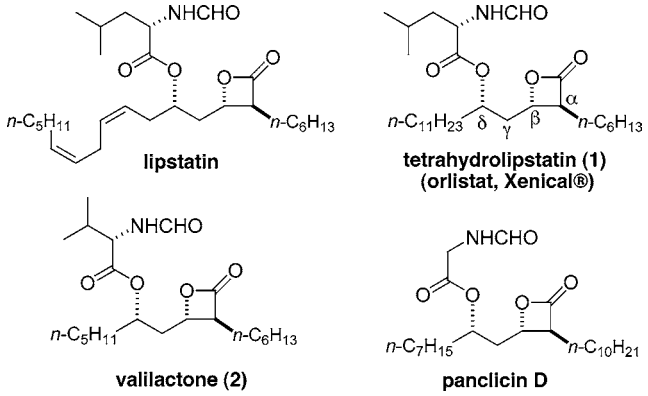

Figure 1. Structures of β-lactone-containing natural products and derivatives.

occurring derivative of orlistat with 2-fold higher potency toward pancreatic lipase.[7]

We now report the development of several strategies for the systematic study of orlistat congeners for exploration of structure−activity relationships with respect to the thioesterase domain of FAS[8] using the TMAL process as a key step. The utility of these strategies is demonstrated by enantioselective syntheses of tetrahydrolipstatin (THL), valilactone, and a 2-carbon transposed THL derivative. The antagonistic activity of the title compounds toward FAS-TE was measured as inhibition of turnover of a fluorogenic substrate by a recombinant form of the enzyme.[9] A comparison of selectivity toward various hydrolyases in cell lysates for the transposed orlistat derivative vs orlistat and ebelactone A by activity-based profiling is also reported.

Numerous total syntheses of orlistat have been reported owing to its interest as an antiobesity agent and also as a showcase for new methods in polyketide synthesis. The majority of strategies has relied on late-stage lactonization to access the β-lactone moiety.[10] Alternative strategies to synthesize this pharmacophore are quite scarce and include [2+2] cycloadditions of ketenes and aldehydes,[11] a bromolactonization strategy,[12] and one example employing the TMAL process.[13] There are two syntheses of valilactone reported to date.[14]

At the beginning of our studies aimed at mapping the active site of FAS-TE in conjunction with structural analysis,[15] we wondered what role the position of the β-lactone moiety and *N*-formylleucine ester along the hydrocarbon backbone might have on the inhibitory activity toward FAS-TE. It is intriguing and perhaps not surprising that the β-lactone carbonyl carbon is located at C16′ in orlistat and that the end product of FAS is the C16 carboxylic acid, palmitic acid (Figure 2). In previous studies, we found that

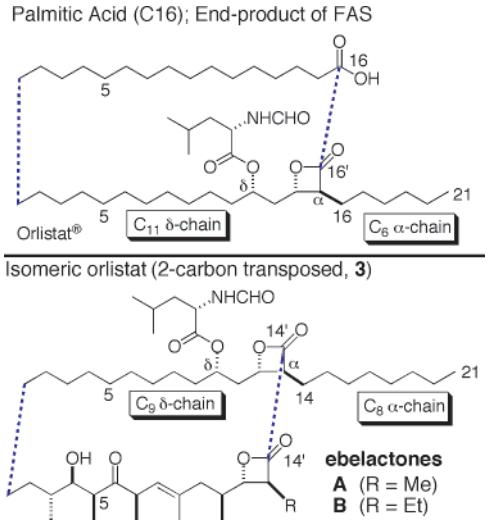

Figure 2. Structural comparison of palmitic acid (the end product of FAS), orlistat, and ebelactones A and B. Design of a 2-carbon transposed derivative related to ebelactones.

the ebelactones are also antagonists of FAS; however, these agents were not as potent nor as selective for FAS in cell lysates as orlistat.[2a] It is noteworthy that in the ebelactones the β-lactone carbonyl carbon is located at C14′ rather than at C16′. To test whether the reduced activity was a consequence of the transposed β-lactone in ebelactone vs orlistat (C16′→C14′), we synthesized orlistat derivative **3**, in which both functionalities are shifted by two carbons (Figure 2).

The synthesis of (−)-tetrahydrolipstatin began with a TMAL reaction between known aldehyde **4**[16] and thiopyridyl ketene acetal **5**[7] which delivered the desired β-lactone as an 8:1 mixture of *anti*/*syn* diastereomers with complete *trans*

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(13) While our work was in progress, the TMAL process developed in our laboratory was employed in a synthesis of orlistat; see: Yin, J.; Yang, X. B.; Chen, Z. X.; Zhang, Y. H. *Chin. Chem. Lett.* **2005**, *16*, 1448.

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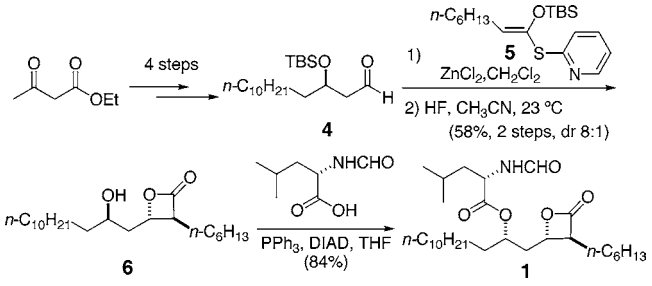
(16) Aldehydes **4** and **7** are available in four steps from ethylacetoacetate involving (a) alkylation, (b) Noyori reduction, (c) alcohol silyl protection, and (d) ester half-reduction. See Supporting Information for details.

Scheme 1. Total Synthesis of Orlistat



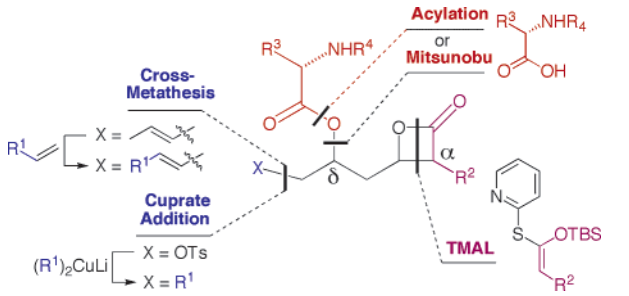


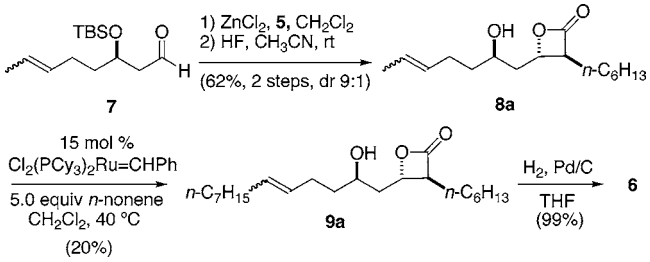
Figure 3. Overall strategies for orlistat derivative synthesis.

stereocontrol with respect to the β-lactone (Scheme 1). The *trans* stereochemical arrangement of the β-lactone core was verified by analysis of the coupling constant ($J_{Ha,Hb} = 4.0$ Hz) as described previously,[3] whereas the relative stereochemistry with respect to the γ-stereocenter was proven after completion of the synthesis. The relative stereochemistry is consistent with Evans' model for additions to β-silyloxy aldehydes[17] as previously observed for these TMAL reactions.[7b] It should be noted that the TMAL process expediently provides the *trans*-β-lactone stereochemistry found in all naturally occurring 3,4-disubstituted-β-lactones reported to date and also obviates the sometimes problematic ring closure of hydroxy acid precursors.[10k] Subsequent desilylation afforded the more readily separable diastereomeric γ-hydroxy-β-lactones, and the major diastereomer **6** was isolated pure in 44% yield (dr > 19:1) plus 14% of a mixture of diastereomers (58% overall, two steps). A Mitsunobu reaction employing *N*-formyl leucine proceeded with the expected inversion of configuration providing synthetic orlistat (**1**), which displayed spectroscopic and physical properties that matched those of the natural product ($[\alpha]^{20}_D$ −31.2 (*c* 0.65, $CHCl_3$); lit. $[\alpha]^{20}_D$ −33 (c 0.65, $CHCl_3$)).[18] This constitutes one of the most concise syntheses of orlistat reported to date (seven steps from ethylacetoacetate, 29% overall yield).

Although efficient, this approach would not be suitable to expediently generate a collection of orlistat derivatives for SAR studies of FAS-TE, as a different γ-siloxy aldehyde (i.e., **4**) would have to be prepared anew from ethylacetoacetate in each case. We therefore sought to develop several strategies that would allow for the concise and facile diversification of the α- and δ-side chains. As a variety of ketene acetals can be readily prepared from commercially available acids or acid halides, the TMAL reaction offered a convenient solution for the α-chain (Figure 3), whereas for the δ-side chain, we envisioned olefin cross metathesis[19] or cuprate addition as viable tactics from olefin or tosylate precursors, respectively. A subsequent Mitsunobu reaction or simple acylation would allow for the diversification of the δ-hydroxy moiety with complete control over the relative stereochemistry with respect to the β-lactone core. In this manner, a number of orlistat congeners could be prepared in seven to nine steps from malic acid or ethylacetoacetate.

To demonstrate the olefin cross-metathesis route, we targeted the synthesis of the hydroxy-β-lactone **6** which would constitute a formal synthesis of orlistat. Aldehyde **7** was prepared by a sequence similar to that for aldehyde **4** using crotyl bromide. Subjecting aldehyde **7** (∼4−5:1, *E*/*Z*) to typical TMAL reaction conditions with ketene acetal **5** provided intermediate silyloxy-β-lactones (not shown) as a 9:1 mixture of *syn*/*anti* diastereomers (∼4−5:1, *E*/*Z*). Direct desilylation allowed isolation of the *anti*-β-lactone **8a** (33% yield; ∼4−5:1, *E*/*Z*) plus 29% of a mixture of diastereomers (62% overall yield, two steps, Scheme 2). Subsequent cross

Scheme 2. Cross-Metathesis Strategy to Orlistat Derivatives: Formal Synthesis of Orlistat



metathesis with excess *n*-nonene in the presence of the first-generation Grubbs catalyst[20] gave low conversion (20% yield, unoptimized) of intermediate **9a** (∼4:1, *E*/*Z*). Finally, hydrogenation of **9a** under standard conditions afforded hydroxy-β-lactone **6** in nearly quantitative yield constituting a formal synthesis of orlistat. This overall process provides an expedient route to append a variety of δ-side chains to the orlistat core structure. Further utility of hydroxy-β-lactone **8a** was demonstrated by facile conversion to valilactone (**2**) (Scheme 3). Mitsunobu reaction with *N*-formyl valine followed by hydrogenation delivered valilactone (**2**) in eight steps from ethylacetoacetate.

For the synthesis of the 2-carbon transposed orlistat derivative **3**, we explored an alternative route for the

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(18) Barbier, P.; Schneider, F.; Widmer, U. *Helv. Chim. Acta* **1987**, *70*, 1412.

(19) We previously demonstrated the applicability of cross-metathesis to a related β-lactone substrate in our synthesis of brefeldin A. See ref 6.

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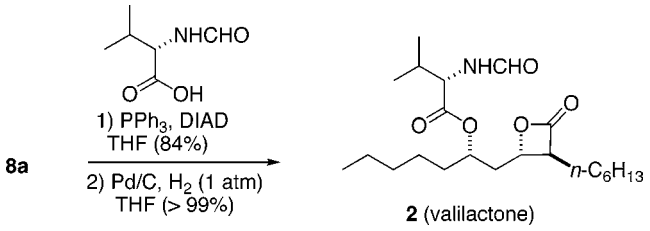
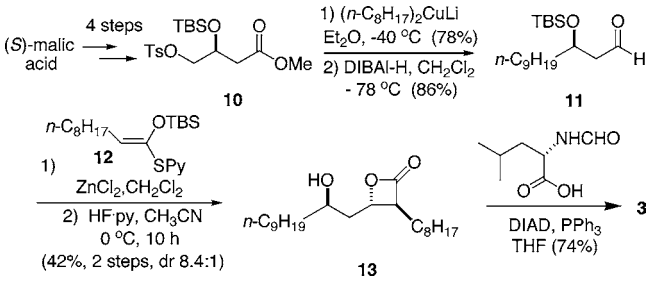
Scheme 3. Total Synthesis of Valilactone



installation of the δ-chain involving cuprate addition to a tosylate. Thus, (*S*)-malic acid was converted to tosylated ester **10** in a four-step sequence by literature procedures.[21] After some experimentation, suitable cuprate conditions were identified, and following ester half-reduction, aldehyde **11** could be obtained in 67% yield over the two steps. TMAL reaction with ketene acetal **12** followed by desilylation afforded *anti*- and *syn*-hydroxy-β-lactones (dr, 8.4:1), and the major diastereomer, *anti*-δ-hydroxy-β-lactone **13**, was isolated in 42% yield (over two steps). Finally, Mitsunobu reaction with *N*-formylleucine delivered the 2-carbon transposed β-lactone **3** (Scheme 4).

Scheme 4. Total Synthesis of a 2-Carbon Transposed Orlistat Derivative (**3**)



For enzyme inhibition studies, a recombinant form of the thioesterase domain of fatty acid synthase was used in a substrate-based assay to measure the apparent K_i values of **1**−**3** (Table 1).[9] Synthetic orlistat (**1**) and valilactone (**2**) showed similar activity compared to commercial orlistat (0.21 μM), whereas the 2-carbon transposed orlistat derivative **3** displayed 3-fold lower potency than that of orlistat which is consistent with activity for ebelactones A and B (10.8 ± 2.3 and 0.84 ± 0.19, respectively) bearing a carbonyl at C14′ (cf. Figure 1). We also performed comparative analysis of these derivatives in their ability to compete with a fluorophosphonate probe in activity-based profiling.[2] Bands labeled by the probe in mammary carcinoma (MDA-MB-435) cell lysates were visualized on a fluorescence scanner (Figure 4). The labeling of FAS (filled arrow) by the activity-based probe is inhibited to an approximately equal extent by the ebelactones and the 2-carbon transposed derivative **3** (lanes 3−5) as expected but most potently and selectively by orlistat (lane 2). Derivative **3** also inhibits labeling of a 28 kDa band (open arrow) but shows greater specificity overall than the ebelactones (lanes 3 and 4; *cf.* the ~85 kDa band).

Table 1. Antagonistic Activity of **1**, **2**, and **3** toward Recombinant FAS-TE

entry	compound	apparent K_i (μM)
1	**1** (orlistat)	0.28 ± 0.05
2	**2** (valilactone)	0.27 ± 0.05
3	**3** (2-C transposed)	0.70 ± 0.02

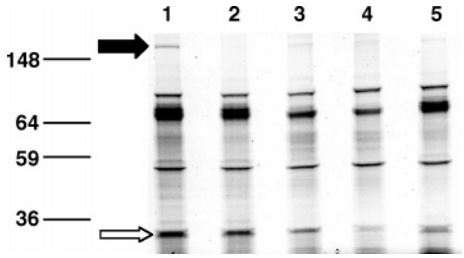


Figure 4. Activity-based profiling of MDA-MB-435 mammary carcinoma cells with a TAMRF probe in competition with various β-lactones. Cell lysates were incubated with vehicle DMSO (lane 1) and 100 μM synthetic orlistat **1** (lane 2), ebelactone A (lane 3), ebelactone B (lane 4), or 2-carbon transposed THL **3** (lane 5).

In summary, we have developed highly concise total syntheses of orlistat and valilactone and versatile strategies to prepare derivatives employing the TMAL process. Olefin metathesis enables facile modification of the δ-side chain, and this strategy could also be utilized to modify the α-side chain through the use of an appropriate alkene-bearing ketene acetal. These strategies are enabling delineation of the structural requirements for inhibition of FAS-TE and also providing improvements to solubility, potency, and selectivity. The orlistat derivatives inhibited a recombinant form of the thioesterase domain of FAS (<1 μM). A comparison of their ability to compete with an activity-based probe vs ebelactones demonstrated that the 2-carbon transposed derivative **3** also blocks the active site of an unidentified 28 kDa serine hydrolase while maintaining much of the specificity of orlistat, pointing to the importance of the relative positioning of the β-lactone carbonyl at C16′.

Acknowledgment. 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.

Supporting Information Available: Selected experimental procedures and characterization data (including ^{1}H and ^{13}C) for compounds **1**−**3**, **4**, **6**, **7**, **8a**, **9a**, **11**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) See Supporting Information for details.