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Combining cross-metathesis and activity-based protein profiling: New β-lactone motifs for targeting serine hydrolases

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ABSTRACT

β-Lactones are a privileged structural motif as enzyme inhibitors and chemical probes, particularly for the inhibition of enzymes from the serine hydrolase class. Herein, we demonstrate that cross-metathesis (CM) of α-methylene-β-lactones offers rapid access to structurally diverse, previously unexplored β-lactones. Combining this approach with competitive activity-based protein profiling (ABPP) identified lead β-lactone inhibitors/probes for several serine hydrolases, including disease-associated enzymes and enzymes of uncharacterized function. The structural diversity afforded by the α-methylene-β-lactone scaffold thus expands the landscape of serine hydrolases that can be targeted by small-molecule inhibitors and should further the functional characterization of enzymes from this class through the optimization of target-selective probes.

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β-Lactones are a versatile class of electrophilic small molecules that have found use as biological research probes and therapeutic agents largely due to their capacity to react with and inhibit enzymes from the serine/threonine hydrolase class.[1] More recently, β-lactones have emerged as attractive probes for activity-based protein profiling (ABPP) of the larger serine hydrolase class directly in native biological systems.[2] Many of the β-lactone inhibitors and probes are 3,4-disubstituted, designed around the natural product-based β-lactone, tetrahydrolipstatin (THL or Orlistat®-Fig. 1), a pancreatic lipase inhibitor used to treat obesity.[3] While there are many stereoselective approaches to such β-lactones,[4] we believe that cross-metathesis (CM) of α-methylene-β-lactones **1** offers rapid access to structurally diverse, previously unexplored 3,4-disubstituted-β-lactones.

In investigating strained heterocycles with exocyclic unsaturation, we found that α-methylene-β-lactones **1** participated in CM reactions with Type I[5] alkenes, with couplings proceeding in high yields with excellent *Z*-stereoselectivities (Fig. 2).[6] This methodology is attractive for making focused libraries of β-lactones for several reasons. First, the α-alkylidene-β-lactones **4** themselves represent a new class of probes. In addition, metathesis reactions tend to be highly tolerant of a broad range of functionality, thus allowing for the preparation of diverse compounds from a single template. Moreover, it should be possible to selectively access either *cis*- or *trans*-β-lactones **5** from the α-alkylidene-β-lactones. *cis*-Lactones would be the expected products from hydrogenation

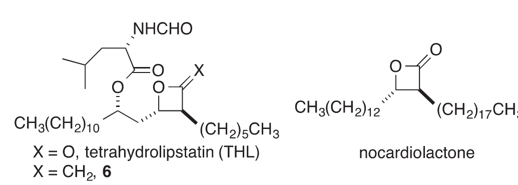

Figure 1. Examples of biologically active β-lactones.

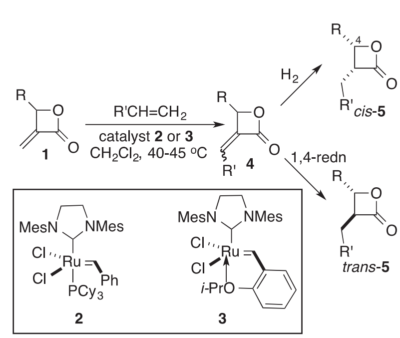

Figure 2. Cross-metathesis approach to β-lactone libraries.

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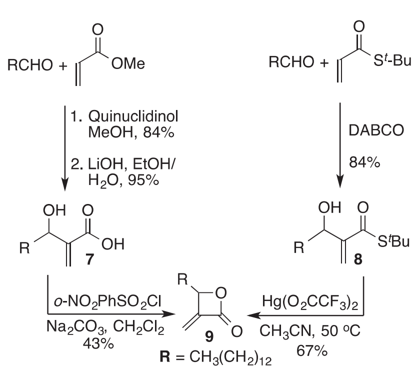
reactions. Limited literature precedent with exocyclic α,β-unsaturated lactones suggests that 1,4-reductions can provide *trans*-diastereomers under the right conditions (***vide infra***). Most monocyclic β-lactone natural products and close analogs (see examples in Fig. 1) that have been explored as drugs or probes are *trans* diastereomers.[1d,2b,7] *cis*-β-Lactones, however, have been shown to be as potent as their *trans*-isomers in some cases.[7b,c] Overall, then, CM with α-methylene-β-lactones would provide a new class of β-lactone probes (α-alkylidene-β-lactones), while subsequent stereoselective reduction would lead to previously unexplored *cis*- or *trans*-β-lactones. Moreover, an opportunity to examine the potential of 2-methyleneoxetanes (see **11b**/**11d**) in competitive ABPP is offered. We have previously shown that the 2-methyleneoxetane analog **6** (see Fig. 1) of the anti-obesity drug, THL, was nearly as potent an inhibitor of porcine pancreatic lipase (PPL) as THL.[8]

Herein, the development of this straightforward CM approach to β-lactone probes, highlighted by a four-step synthesis of (±)-nocardiolactone (Fig. 1),[9] is described. Nineteen probes, including nocardiolactone, were prepared from a single α-methylene-β-lactone scaffold **9**. The probes include *E*- and *Z*-α-alkylidene-β-lactones, *cis*- and *trans*-3,4-disubstituted-β-lactones, and 2-methyleneoxetanes. The utility of the probes in competitive ABPP was demonstrated by assaying these compounds in native cell (COLO205 human colon cancer cell line) and tissue (mouse brain) proteomes to assess their in vitro inhibitory activity against serine hydrolases. A combination of competitive gel- and MS-based ABPP methods identified novel β-lactone probes that target diverse members of the serine hydrolase family, including uncharacterized enzymes that lack selective inhibitors.

As previously noted, an attractive feature of our planned CM approach to monocyclic β-lactone analogs was that a single template could be used to access a broad range of lactones. The first important choice was the identity of the substituent at C4, since this would be a part of the initial set of analogs. THL has been shown to inhibit the thioesterase domain of fatty acid synthase,[10] forming a covalent bond with an active site serine. Crystallographic studies show the C4 chain to be buried in a hydrophobic channel.[11] While this channel may not be a universal motif, we and others have found that THL interacts with a variety of serine hydrolases.[12] Böttcher and Sieber also took inspiration from the aliphatic chains of β-lactone natural products in the design of β-lactone ABPPs.[1e] In the initial series, for ease of synthesis we elected to use a simple alkyl chain at C4 and chose the chain length based on nocardiolactone, which has the same number of carbons in its alkyl chain as THL, giving us the opportunity to develop the methodology around a straightforward synthesis of (±)-nocardiolactone. Thus, the key intermediate for its synthesis and for diversification was α-methylene-β-lactone **9**.

We previously reported the synthesis of α-methylene-β-lactones via lactonization of readily accessible, hydrolyzed



Scheme 1.

Morita–Baylis–Hillman (MBH) adducts like **7** (Scheme 1).[13] While α-methylene-β-lactone **9** was readily prepared from tetradecanal by this approach, a more direct sequence involved MBH reaction between tetradecanal and *t*-butyl thioacrylate, followed by mercury-mediated cyclization of **8**[13] to **9**. For nocardiolactone the remaining steps were CM and reduction.

CM between **9** and 1-nonadecene, catalyzed by Grubbs second-generation catalyst **2** under conditions we had previously developed for α-methylene-β-lactones,[6] was straightforward, yielding mainly the *Z*-diastereomer (*Z*-**4a**) (Table 1). The enoate diastereomers were separable, providing two probes to evaluate. While it was anticipated that conversion to the *cis*-isomer of nocardiolactone could be readily achieved using hydrogenation, selective conversion to the *trans*-isomer was more challenging. Romo and co-workers have used epimerization of *cis*-3,4-disubstituted-β-lactones to access the *trans*-isomers,[7c] but the yields were relatively low (35–40%). Our preference was to avoid this extra, material depleting transformation. 1,4-Reduction of enoates **4** represented an alternative pathway that might be manipulated to provide the *trans*-isomers. We found no examples in the literature of 1,4-reduction of α-alkylidene-β-lactones; however, there were a number of examples involving α-alkylidene-γ-lactones.[14] Although simple steric arguments would suggest that *cis*-diastereomers should dominate from protonation from the less hindered face of intermediate enolates, the literature cited showed that other factors play a role. Consequently, a variety of 1,4-reduction conditions were explored.

Iwasaki and co-workers had examined the use of transition metals (Co, Ni, Cu) in conjunction with $NaBH_4$ to effect the reduction of α-alkylidene-γ-lactones with high *trans*-selectivity.[14c] They demonstrated that the protonation of the enoate was not reversible under the reaction conditions, making it the determinant of the relative stereochemistry. The high stereoselectivities were rationalized on the basis of conformational effects from 1,3-allylic strain. While our substituents were different, we reasoned that it might also be possible to use ligands to bias the outcome. Focusing first on cobalt(II) additives with $NaBH_4$ as the reductant, $CoCl_2$ (with MeOH as the proton source) (Table 1, entry 1) gave more *cis*-**5a**.

The stereoselectivity improved with the use of $Co(acac)_2$ (entry 2), with a further improvement with a slightly more sterically hindered proton source, *i*-PrOH (Entry 4). Using phosphine-containing $CoCl_2(Ph_3P)_2$ provided additional improvement in stereoselectivity (entry 5). No further enhancement was seen with *i*-PrOH (entry 7) or by using alternative phosphine ligands (not shown). Separating the product from phosphine related byproducts proved problematic, but this was resolved by cutting back on the additive (entry 6). It is noteworthy that running the reaction at higher temperatures resulted in considerable quantities of methyl esters (entry 3). Although $NiCl_2$ initially looked more promising than the corresponding $CoCl_2$ (entry 8 vs 1), this was not the case with the optimal phosphine ligand (entry 9). Copper(II) reagents gave very little conversion to product (not shown). Alternative reductants, such as L-Selectride[14a] or Mg,[14b] that had been reported for the stereoselective reduction of α-alkylidene-γ-lactones (entries 10 and 11) did not provide significant quantities of product. Although other 1,4-reduction conditions, such as those shown in entries 12[15] and 13,[16] did lead to product, none of these provided higher stereoselectivities than the use of $NaBH_4$ with catalytic $CoCl_2(Ph_3P)_2$ as an additive. While we continue to explore other conditions, the optimized 1,4-reduction conditions identified thus far (entry 6) do provide access to both *cis*- and *trans*-β-lactones **5**, and these diastereomers are separable by column chromatography.

With α-methylene-β-lactone scaffold **9** in hand, the initial diversification was undertaken. Using four additional alkene partners (**10b**–**e**) that varied in chain length, with three (**10c-e**)

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