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Natural product derivatization with β-lactones, β-lactams and epoxides toward ‘infinite’ binders

Morgan Jouanneau, Sreekumar Vellalath, Guowei Kang, Daniel Romo*

Department of Chemistry and Biochemistry, Baylor University, Waco, TX 76798, USA

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β-Lactones, β-lactams and epoxides are privileged structural motifs found in both therapeutics and natural products. Herein we report several strategies for annulation of these motifs onto natural products that are not known to covalently modify their cellular targets. These strategies can facilitate identification of previously unidentified cellular targets or identify novel cellular targets of these natural products. The reported strategies include telescoped synthesis of β-lactones from allylic alcohols, nucleophile-catalyzed Michael aldol-β-lactonizations, and [2 + 2] β-lactam annulations with complex, commercially available alkene-containing natural products as substrates. A novel method for the tagging of phenolic natural products with epoxides is also reported.

1. Introduction

Given the rich history of natural products as enduring leads for drug discovery and their inherent ability to interact with protein folds, the application of β-lactone or β-lactam annulation methods for the conversion of non-covalent bioactive natural products (NPs) to covalent (‘infinite’) inhibitors [1] presents new opportunities in cellular probe synthesis. The annulation of relatively small-sized β-lactones, epoxides, and β-lactams onto alkenes are expected to exert minimal steric and conformational perturbations to the overall structure of the NP, while introducing useful electrophilic moieties for activity-based proteome profiling [2]. We have therefore investigated methods for annulation of β-lactones from simple alkene-containing substrates [3]. Herein, we describe application of this methodology, our previously described organocatalytic β-lactone annulation methods [4], and known β-lactam forming methods for a different purpose; namely, to convert non-covalent bioactive NPs to covalent (‘infinite’) inhibitors.

β-Lactone-containing natural products and derivatives have attracted significant attention in cell biology due to their ability to covalently modify proteins [5] and despite their covalent mechanism of action, typically considered a red flag for drug development, continue to be exploited in the clinic. Nature extensively exploits ring strain in the generation of covalent warheads. The most prevalent mechanism of action involves the covalent acylation of nucleophilic residues found in the active sites of enzymes by the β-lactone to produce inactive acyl-enzyme adducts [6]. Naturally occurring lipstatin (**1**) inspired the anti-obesity drug and lipase inhibitor tetrahydrolipstatin, (Orlistat®, Xenical®, Allí®), acylates an active site serine in pancreatic lipase and this drug also served as a lead for inhibitors of fatty acid synthase as potential anticancer leads [7]. Salinosporamide A (**2**), a potent cytotoxic agent, leads to acylation of threonine at all three proteolytic sites of the 20S proteasome [8]. Salinosporamide A, marketed as Marizomib®, recently received orphan drug designation for treatment of malignant glioma in combination with Avastin® and is also in various clinical trials for the treatment of multiple myeloma [9]. Thus, enzymes possessing active-site, nucleophilic residues, such as serine, lysine, histidine, and cysteine are particularly susceptible to inactivation through covalent modification by β-lactone-containing NPs [10]. Interestingly, the Porco group recently reported the lactonization of diastereomeric hydroxy acids derived from methyl rocaglate (**7**), an inhibitor of elongation initiation factor eIF4A, to generate a β-lactone that covalently modifies serine hydrolases and serine peptidases (Fig. 1) [11].

Some of the oldest and most intensely studied electrophilic moieties found in natural products are β-lactams [12] and are also known to engage in covalent protein modification through

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* Corresponding author.
E-mail address: Daniel_Romo@baylor.edu (D. Romo).

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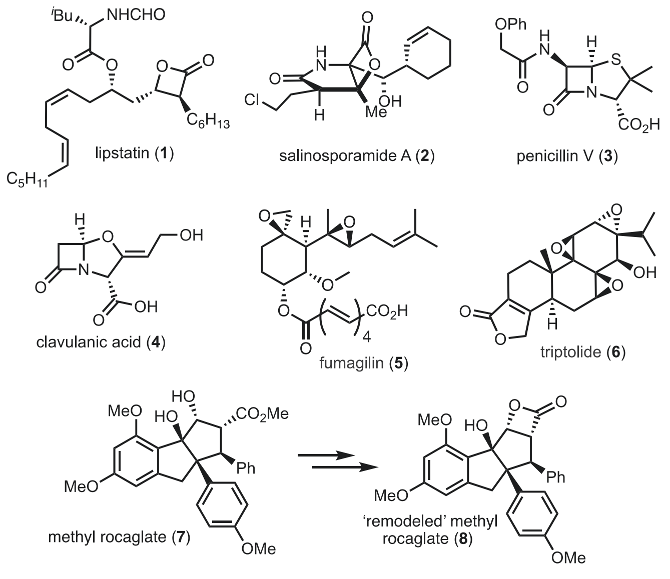


Fig. 1. β-Lactone, β-lactam and epoxide containing NPs and derivatives that have been used for proteomics profiling.

acylation [13]. Similar to β-lactones, the electrophilic carbonyl group embedded in the strained β-lactam ring is the reactive moiety of β-lactam antibiotics such as penicillin V (**3**) and clavulanic acid (**4**) [14], with the latter functioning as a mechanism-based β-lactamase inhibitor.

The ability of epoxides, including those found in natural products, to serve as electrophilic moieties leading to covalent inhibition of cellular protein targets is also well recognized and exploited [15]. The natural product, fumagillin (**5**), is known to target methionine aminopeptidase leading to inhibition of angiogenesis [16]. The diterpene triepoxy triptolide (**6**), which covalently modifies a subunit of the transcription factor TFIIH [17], has long been used in traditional Chinese medicine to treat a host of diseases including inflammation and derivatives have entered clinical trials for the treatment of cancer [18].

The methods described herein for annulation of electrophilic moieties onto natural products include a recently developed method for *in situ* generation of allylzinc reagents followed by CO_2 capture and bromo-lactonization (Fig. 2a),[3] [2 + 2] cycloadditions of chlorosulfonyl isocyanate with alkenes delivering β-lactams (Fig. 2b) [19], an application of our nucleophile-catalyzed Michael-aldol-lactonization (NCMAL) for cyclopentyl β-lactone annulation onto unsaturated carboxylic acids (Fig. 2b) [20], and a simple oxidation-cyclization sequence of a 1,3-diol (Fig. 2c) [21]. We also report the tagging of natural products with epoxides through an acylation-cyclization sequence of phenols with γ-bromo-β-lactone **9** (Fig. 2d).

2. Results and discussion

We began our study of β-lactone annulations of natural products with commercially available perillyl alcohol (**10**), a naturally occurring dietary monoterpene which exhibits *in vitro* and *in vivo* antitumor activity [22]. The annulation sequence began by conversion of the alcohol to the corresponding allylic bromide **11** through an Appel reaction [23]. Subsequent conversion to the β,γ-unsaturated acids **13,** as a 1:1 mixture of diastereomers, was achieved through our previously described Zn^0 mediated carboxylation utilizing CO_2.[3] Finally, bromo-β-lactonization was achieved at low temperature through a selective bromination with NBS in the presence of $(DHQD)_2$PHAL as catalyst [24], to afford the derived bicyclic-β-lactone **14** from only a single diastereomer of the starting carboxylic acids **13** (Scheme 1).

Annulation of cyclopentyl β-lactones onto natural products bearing an α,β-unsaturated acid can be achieved through our previously described nucleophile (Lewis base)-catalyzed Michael-aldol-lactonization (NCMAL) which generates two new C—C bonds and a C—O bond in a single operation through the intermediacy of an *in situ* generated unsaturated acylammonium salt [20]. Application of this reaction to perillic acid (**15**) with ketomalonate **16** as Michael donor delivered the complex β-lactone **18** in low yield (25%) but as a single diastereomer. Cyclic α,β-disubstituted unsaturated acid substrates are known to be challenging substrates for the NCMAL process. Ghosez's reagent **17** was employed for *in situ* conversion of perillic acid (**15**) to the corresponding acid chloride and 9-azajulolidine was employed as the Lewis base in the NCMAL process (Scheme 2).

In a similar reaction sequence, caffeic acid **19** was converted to β-lactone **22** following permethylation and ester hydrolysis to deliver unsaturated acid **21** in 94% yield. Treatment of acid **21** with Ghosez's reagent **17** and subsequent NCMAL reaction with ketomalonate **16** delivered the caffeic acid derived β-lactone **22** in 49% yield as a single diastereomer (Scheme 3).

Toward addition of an additional electrophilic moiety to the immunomodulatory natural product, andrographolide **23**, we converted the 1,3-diol moiety to a fused β-lactone. First, the 1,3-diol

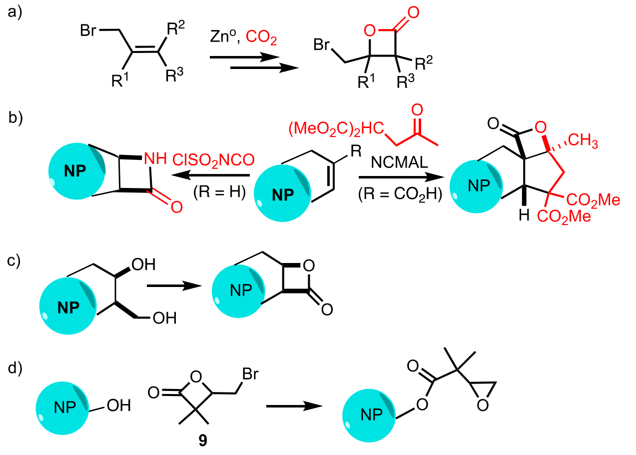
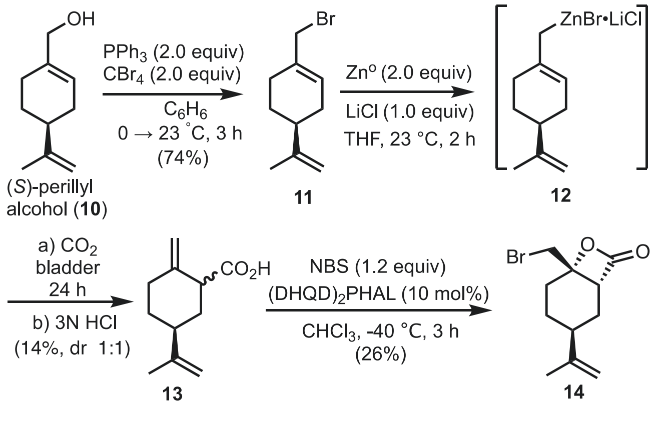
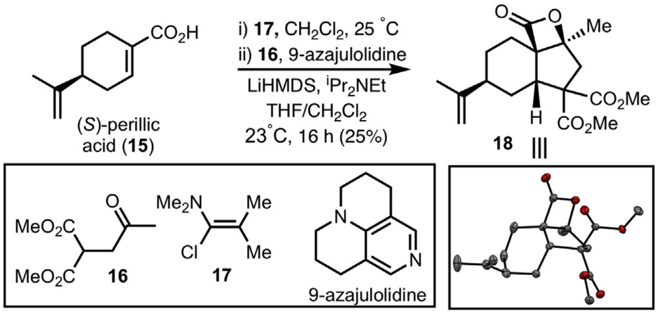


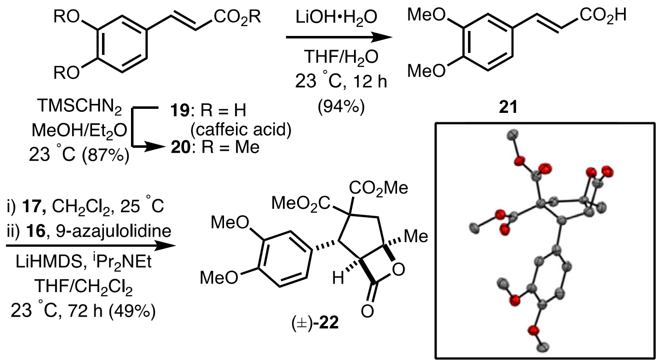
Fig. 2. Described strategies for conversion of natural products to 'infinite binders'.



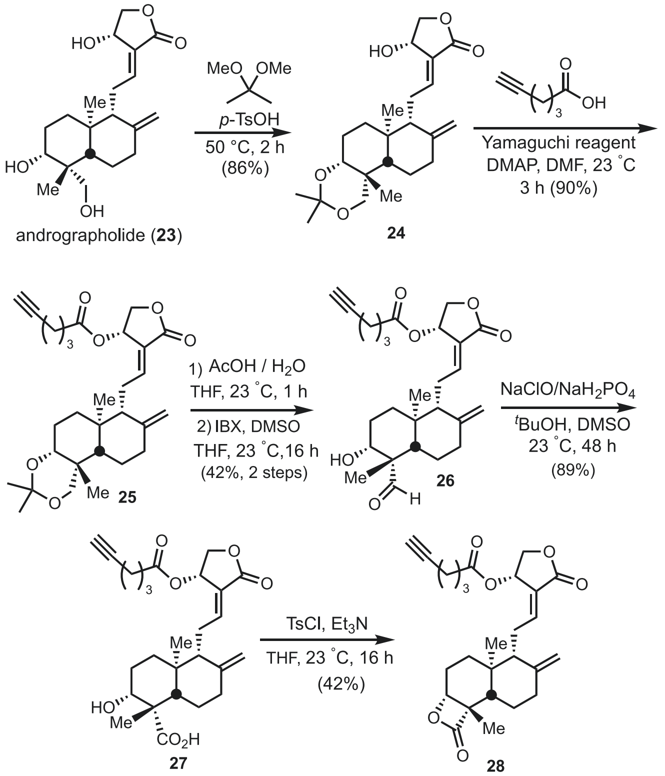
Scheme 1. Annulation of a β-lactone onto the allylic alcohol-containing natural product, perillyl alcohol (**10**).



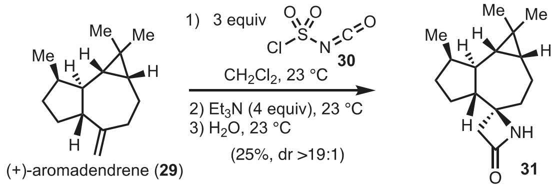
Scheme 2. Application of the nucleophile-catalyzed Michael aldol-β-lactonization (NCMAL) to perillic acid (**15**). (inset single-crystal X-ray structure of -lactone **18**).



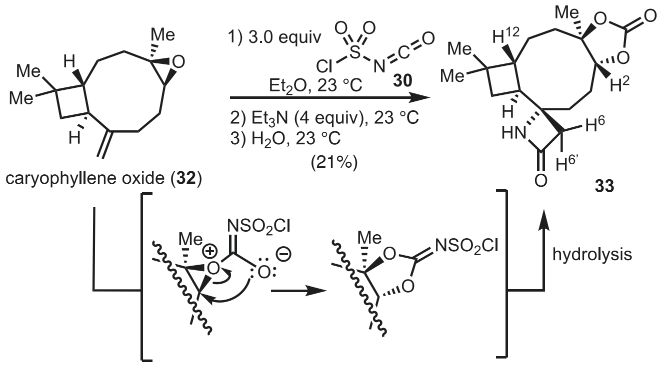
Scheme 3. Application of the nucleophile (Lewis base)-catalyzed Michael aldol-β-lactonization (NCMAL) to caffeic acid (**19**). (inset is a single crystal X-ray structure of **22**).



Scheme 4. Conversion of andrographolide (**23**) to a β-lactone-bearing proteomics probe **28**.



Scheme 5. Annulation of a spiro-β-lactam onto (+)-aromadendrene (**29**).



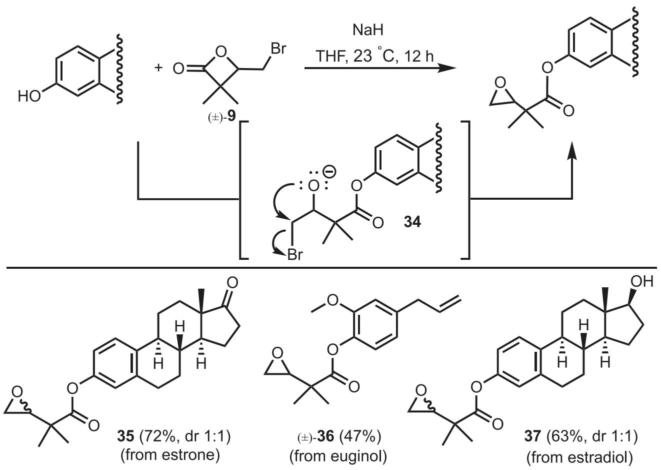
Scheme 6. Annulation of a spiro-β-lactam onto caryophyllene oxide (**32**) with concomitant ring expansion of the epoxide to a cyclic carbonate following hydrolysis through a presumed stereoinvertive, disallowed 5-endo-tet cyclization.

was protected as an acetonide and the secondary alcohol was converted to alkyne ester **25** through Yamaguchi esterification [25] with hex-5-ynoic acid toward synthesis of a proteomics probe. Deprotection of the acetonide followed by oxidation delivered aldehyde **26** in 42% yield over two steps. The aldehyde was then oxidized to acid **27** and lactonization of this β-hydroxy acid delivered β-lactone **28** using a modified Adam procedure [26] in 42% yield (Scheme 4).

β-Lactam annulations onto alkene-bearing natural products were also investigated through a net [2 + 2] cycloaddition with chlorosulfonyl isocyanate [19]. Reaction of aromadendrene (**29**), a sesquiterpene with a fused dimethylcyclopropane on a hydroazulene skeleton, with chlorosufonyl isocyanate delivered spiro-β-lactam **31** in 25% yield with high diastereoselectivity (Scheme 5). This ring annulation proceeds with high facial selectivity likely controlled by the adjacent allylic stereocenter working in concert with overall topology of the tricyclic natural product including the fused gem-dimethyl cyclopropane.

Application of this [2 + 2] cycloaddition strategy to caryophyllene oxide (**32**) led to simultaneous β-lactam annulation and ring expansion of the epoxide to a cyclic carbonate providing spiro-β-lactam **33** (Scheme 6). Cyclic carbonate formation likely results from initial *O*-acylation with the isocyanate, epoxide ring opening, recyclization, and subsequent hydrolysis of the resulting imino ketene acetal aryl-substituted epoxides [27]. The relative stereochemistry of the β-lactam is based on anticipated facial selectivity and nOe data. The relative stereochemistry of the cyclic carbonate is assigned based on nOe data showing correlations between H2/H6, H2/H12, H6’/H12 (see SI for full details), the proposed mechanism for the ring expansion (‘S_N2-like’ through a disallowed 5-endo-tet cyclization) [28], and literature precedent for this transformation including with 8-membered rings [29].

Finally, we studied derivatization of alcohol-containing natural products with the racemic γ-bromo-β-lactone (±)-**9**, available from carboxylation and bromo-β-lactonization of allyl alcohol [3], that



Scheme 7. Tagging of phenol-containing natural products with epoxide (±)-**9**.

could proceed through either *O*-alkylation at the bromide-bearing carbon or *O*-acylation with the β-lactone. We first studied the steroid estrone bearing a phenol and upon deprotonation with NaH and addition of bromide (±)-**9**, this delivered the epoxide **35** in 72% yield as an expected 1:1 mixture of diastereomers (Scheme 7). This adduct presumably arises from initial *O*-acylation to deliver an intermediate alkoxide **34** that can then cyclize to the epoxide. While various bases and reaction conditions (*e.g.* $NaHCO_3$/DMSO, LiHMDS/THF) were also studied, use of NaH as stoichiometric base in THF was found to be optimal. This transformation also proceeded with the essential oil eugenol to deliver the corresponding racemic epoxide **36** in 47% yield. Selective reaction with only the phenolic alcohol in the case of estradiol delivered epoxide **37** while the secondary alcohol was found to be unreactive even in the presence of excess base. This may be a consequence of the sterics associated with the gem-dimethyl β-lactone (±)-**9** leading to selective reaction with sterically less encumbered phenolic alkoxides.

3. Conclusion

In summary, we demonstrated the utility of various methods for β-lactone and β-lactam, annulations to deliver natural products that have the potential to lead to covalent modification of previously described or novel cellular protein targets. Furthermore, the utility of a γ-bromo-β-lactone (±)-**9** for the tagging of phenolic natural products with an epoxide was also demonstrated. The described strategies for conversion of non-covalent natural products into ‘infinite’ binders pave the way for subsequent proteomics studies following attachment of suitable reporter tags (*e.g.* alkynes) as in the case of andrographolide. The biological screening of the described natural product derivatives and others available from the described electrophilic ring annulation strategies are underway.

4. Experimental section

4.1. General

All non-aqueous reactions were performed under an argon atmosphere in oven-dried glassware. Tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), dimethyl formamide (DMF) and diethyl ether was used from (solvent purification system). Diisopropylethylamine (DIPEA) and triethyl amine (Et_3N) were distilled from potassium hydroxide prior to use. Other solvents and reagents were used as received from commercially available sources. Deuterated solvents were purchased from either Aldrich or Cambridge Isotopes and used as received. 1H NMR spectra were measured at 600 MHz, 500 MHz, 400 MHz and 300 MHz and referenced relative to residual chloroform (7.26 ppm) and is reported in parts per million (δ). Coupling constants (*J*) are reported in Hertz (Hz), with multiplicity reported following usual convention: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; dt, doublet of triplets; ddd, doublet of doublet of doublets; m, multiplet; br, broad; app, apparent. ^{13}C NMR spectra were measured at 150 MHz, 125 MHz, 100 MHz and 75 MHz and referenced relative to residual chloroform (77.23 ppm) and was reported in parts per million (ppm). Flash column chromatography was performed on an automated flash chromatography system (EtOAc/hexanes or Et_2O/hexanes as eluent unless indicated otherwise). High resolution mass data (ESI) was obtained on instrument in the Baylor University Mass Spectrometry Center and the Laboratory for Biological Mass Spectrometry (Texas A&M University). Thin Layer Chromatography (TLC) was performed using glass-backed silica gel F_{254} (250 µm thickness). Visualization of developed plates was performed by fluorescence quenching or by using iodine, CAM and vanillin stains. Fourier Transform Infrared (FT-IR) spectra were recorded as thin films on NaCl plates. CO_2 (99.99%) was purchased from Praxair. The heavy-duty rubber balloon, actually a punching bag bladder, was purchased from Amazon.

4.1.1. Synthesis of β,γ-unsaturated acid **13**

To an oven-dried, 50 mL round-bottomed flask, equipped with a stirring bar, was added LiCl (169 mg, 4.0 mmol, 2.0 equiv) and Zn dust (392 mg, 6.0 mmol, 3.0 equiv). The flask was heated under vacuum to 450 °C for 10 min with a heat gun, followed by the addition of 5 mL THF. The flask was then evacuated and refilled with argon. 1,2-dibromoethane (20 µL) and Me_3SiCl (20 µL) were added sequentially to activate the Zn powder. Then, a solution of the allyl bromide **11** (428 mg, 2.0 mmol, 1.0 equiv) in 2 mL THF was added dropwise. The resulting mixture was allowed to stir for 18 h at 23 °C. After the formation of the allyl zinc bromide, the flask was evacuated and refilled with CO_2 using a rubber bladder. The reaction mixture was allowed to stir for additional 24 h at the same temperature. Excess zinc was removed through filtration and the reaction was quenched with 2 mL of 2 M HCl. An additional 10 mL H_2O was added. The aqueous phase was extracted with ethyl acetate (15 mL x 3), dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The crude mixture was purified by an automated flash chromatography system (0 → 50%, EtOAc/hexanes) to provide the β,γ-unsaturated acid **13** as a mixture of diastereomers (52 mg, 14%, dr 1:1). TLC (Hexane: EtOAc 1:1), R_f 0.40; NMR data reported for 1:1 mixture of inseparable diastereomers of unsaturated acids **13**.^{1}H NMR (600 MHz, $CDCl_3$) δ 4.92 (s, 1H), 4.88 (s, 1H), 4.85 (s, 1H), 4.72 (s, 3H), 4.70 (s, 1H), 3.45–3.37 (m, 1H), 3.17–3.10 (m, 1H), 2.46 (dt, $J = 13.5$, 3.4 Hz, 1H), 2.38–2.33 (m, 2H), 2.32–2.24 (m, 2H), 2.17–2.10 (m, 2H), 2.06–2.02 (m, 1H), 1.91–1.84 (m, 2H), 1.73 (s, 5H), 1.67–1.60 (m, 1H),1.55–1.51 (m, 1H), 1.40–1.32 (m, 1H), 1.31–1.24 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 179.1, 178.8, 149.3, 148.9, 145.3, 145.3, 112.6, 109.6, 109.3, 108.1, 49.7, 48.7, 44.3, 40.9, 36.0, 35.3, 34.5, 33.0, 32.7, 21.2, 20.9; IR (thin film): 3473, 2935, 1706, 1442; HRMS (ESI): *m/z* calcd for $C_{11}H_{16}O_2Na$ $[M + Na]^+$ 203.1048, found 203.1042.

4.1.2. Synthesis of γ-bromo-β-lactone **14**

To a screw-top vial equipped with a stir bar was added **13** (14.7 mg, 0.082 mmol, 1.0 equiv), $(DHQD)_2PHAL$ (6.4 mg, 8.2 µmol, 0.1 equiv) and 0.5 mL $CHCl_3$. The vial was put in −40 °C, followed by the addition of NBS (17.4 mg, 0.098 mmol, 1.2 equiv). After the full consumption of the starting material (1.5 h, monitored by TLC), the reaction mixture was purified by an automated flash chromatography system (0 → 10%, EtOAc/hexanes) to provide the β-lactone **14** as a single diastereomer (5.5 mg, 26%.). TLC (Hexanes/EtOAc 3:1), R$_f$ 0.58; ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.77 (s, 1H), 4.72 (s, 1H), 3.71 (dd, *J* = 6.8, 2.3 Hz, 1H), 3.66 (d, *J* = 10.7 Hz, 1H), 3.62 (d, *J* = 10.7 Hz, 1H), 2.32–2.16 (m, 3H), 2.15–2.08 (m, 1H), 1.98–1.89 (m, 1H), 1.74 (s, 3H), 1.71–1.61 (m, 1H), 1.53–1.43 (m, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ170.3, 148.2, 110.1, 77.1, 53.4, 38.2, 38.0, 29.9, 26.9, 25.4, 20.8; IR (thin film): 2940, 1827; HRMS (ESI): *m/z* calcd for $C_{11}H_{15}BrNaO_2$ [M + Na]$^+$ 281.0153, found 281.0996.

4.1.3. *Synthesis of tricyclic-β-lactone* ***18***

To a solution of S-perillic acid (100 mg, 0.60 mmol, 1 equiv) in dry CH_2Cl_2 (4 mL) at 23 °C was added dropwise 1-chloro-N,N-2-trimethylpropenylamine (0.08 mL, 0.60 mmol, 1 equiv). The reaction was stirred at room temperature for 30 min. In the meantime, to a solution of dimethylketomalonate (113 mg, 0.60 mmol, 1 equiv) in THF (4 mL) at −78 °C was added dropwise a 1 M solution in THF of LiHMDS (0.60 mL, 0.60 mmol, 1 equiv) and the yellow solution was stirred for 10 min at −78 °C and allowed to warm to 0 °C for 10 min. A solution of 9-azajulolidine (104 mg, 0.60 mmol, 1 equiv) in CH_2Cl_2 (1 mL) and DIPEA (0.1 mL, 0.60 mmol, 1 equiv) were added to this yellow solution at 0 °C. Finally, the acid chloride solution was added over 1 h with a syringe pump at 0 °C and the reaction mixture was allowed to warm up to 23 °C and stirred for 16 h. The reaction mixture was quenched with saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed three times with saturated aqueous solution of Na_2CO_3, to remove unreactive perillic acid, and dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (0 → 33%, EtOAc/hexanes). β-Lactone was obtained (50 mg, 25%) as a white solid and the relative stereochemistry was confirmed by X-ray analysis (CCDC: 1897092). TLC (EtOAc:hexanes, 1:2 v/v): R$_f$ 0.41; ^{1}H NMR (500 MHz, CDCl$_3$): 4.95 (br d, *J* = 1.5 Hz, 1H), 4.81 (br s, 1H), 3.71 (s, 3H), 3.70 (s, 3H), 3.36 (dd, *J* = 13.4, 5.3 Hz, 1H), 2.87 (d, *J* = 15.6 Hz, 1H), 2.64 (d, *J* = 15.6 Hz, 1H), 2.33 (br s, 1H), 1.85–1.94 (m, 2H), 1.64–1.74 (m, 2H), 1.68 (s, 3H), 1.57 (s, 3H), 1.27–1.34 (m, 1H), 1.13 (td, *J* = 13.7, 5.4 Hz, 1H); ^{13}C NMR (125 MHz; CDCl$_3$): 172.6, 170.6, 169.1, 144.1, 112.4, 89.5, 65.2, 62.9, 53.3, 53.1, 42.1, 39.8, 36.7, 26.8, 23.5, 22.2, 19.4, 19.1; IR (thin film): 3078, 2952, 1820, 1737, 1643, 1436, 1386, 1267, 1226, 1145 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{18}H_{24}O_6$ [M + Na]$^+$ 319.1470, found 359.1482.

4.1.4. *Synthesis of bicyclic-β-lactone* ***22***

To a solution of carboxylic acid **21** (40 mg, 0.19 mmol, 1 equiv) in dry CH_2Cl_2 (2 mL) at 23 °C was added dropwise 1-chloro-N,N-2-trimethylpropenylamine (0.03 mL, 0.21 mmol, 1.1 equiv). The reaction was stirred at room temperature for 30 min. In the meantime, to a solution of dimethylketomalonate (50 mg, 0.29 mmol, 1.5 equiv) in THF (2 mL) at −78 °C was added dropwise a 1 M solution in THF of LiHMDS (0.30 mL, 0.29 mmol, 1.5 equiv) and the yellow solution was stirred for 10 min at −78 °C and allowed to warm up to 0 °C for 10 min. A solution of 9-Azajulolidine (50 mg, 0.29 mmol, 1.5 equiv) in CH_2Cl_2 (1 mL) and DIPEA (0.05 mL, 0.29 mmol, 1.5 equiv) were added to this yellow solution at 0 °C. Finally, the acid chloride solution was added over 1 h with a syringe pump at 0 °C and the reaction mixture was allowed to warm up to 23 °C and stirred for 3 days. The reaction mixture was quenched with saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc (20 mL x 3). The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. Solvents were removed under reduced pressure and the crude product was purified by flash column chromatography (0 → 50%, EtOAc/hexanes). Bicyclic-β-lactone **22** was obtained (35 mg, 49%) as a white solid and the relative stereochemistry was assigned based on X-ray analysis (CCDC: 1897091). TLC (EtOAc:hexanes, 1:1 v/v): R$_f$ 0.55; ^{1}H NMR (500 MHz, CDCl$_3$): 6.76 (d, *J* = 8.3 Hz, 1H), 6.52 (br d, *J* = 2.0 Hz, 1H), 6.43 (dd, *J* = 8.3, 2.2 Hz,1H), 4.56 (s, 1H), 3.83 (s, 6H), 3.78 (s, 3H), 3.65 (s, 1H), 3.35 (s, 3H), 2.89 (s, 2H), 1.87 (s, 3H); ^{13}C NMR (125 MHz; CDCl$_3$): 170.9, 168.0, 167.9, 148.9, 148.7, 130.9, 119.7, 111.9, 111.3, 87.3, 66.9, 65.9, 56.1, 56.0, 53.7, 52.9, 50.8, 42.2, 21.7; IR (thin film): 3043, 2955, 1831, 1731, 1592, 1519, 1438 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{19}H_{22}O_8$ [M + Li]$^+$ 385.0687, found 385.0683.

4.1.5. *Synthesis of tricyclic-β-lactone* ***28***

To a solution of 14-hexynoyl-andrographolide-19-oic acid **27** (25 mg, 0.054 mmol, 1 equiv) in THF (1 mL) at 0 °C were added TsCl (52 mg, 0.273 mmol, 5 equiv) and Et_3N (36 μL, 0.273 mmol, 5 equiv). The reaction mixture was stirred at 0 °C for 10 min and allowed to warm to 23 °C and stirred for 16 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with Et_2O (20 mL x 3). The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. The solvents were removed under reduced pressure. The crude product was purified by flash column chromatography 25 → 50%, EtOAc/hexanes. The desired tricyclic-β-lactone **28** was obtained (10 mg, 42%) as a colorless oil. TLC (EtOAc:hexanes, 1:1): R$_f$ 0.52; ^{1}H NMR (500 MHz, CDCl$_3$): 6.99 (app br dt, *J* = 6.6, 1.2 Hz, 1H), 5.92 (br d, *J* = 6.1 Hz, 1H), 4.96 (br s, 1H), 4.56 (br s, 1H) overlap with 4.55 (dd, *J* = 11.3, 6.1 Hz, 1H), 4.40 (br dd, *J* = 9.2, 3.2 Hz, 1H), 4.21 (br dd, *J* = 11.2, 2.0 Hz, 1H), 2.51 (t, *J* = 7.6 Hz, 2H) overlap with 2.49-2.30 (m, 4H), 2.26 (td, *J* = 6.8, 2.7 Hz, 2H), 2.11 (ddd, *J* = 6.9, 3.2 Hz, 1H), 2.00 (dd, *J* = 13.2, 4.9 Hz, 1H), 1.96 (br t, *J* = 2.6 Hz, 1H), 1.84 (t, *J* = 7.1 Hz, 2H) overlap with 1.90 (m, 3H), 1.66 (qd, *J* = 13.2, 4.2 Hz, 1H), 1.49 (s, 3H), 1.38 (dd, *J* = 12.7, 2.9 Hz, 1H), 1.22 (td, *J* = 13.6, 6.4 Hz, 1H), 0.81 (s, 3H); ^{13}C NMR (125 MHz; CDCl$_3$): 175.0, 172.9, 169.2, 150.3, 146.3, 124.2, 110.5, 82.8, 77.3, 71.8, 69.9, 67.9, 55.9, 53.9, 52.3, 38.9, 37.4, 34.0, 32.7, 25.6, 24.6, 24.5, 23.5 (x2), 17.9, 12.5; IR (thin film): 3285, 2941, 2361, 1812, 1736, 1683; HRMS (ESI): *m/z* calcd for $C_{26}H_{32}O_6$ [M + H]$^+$ 441.2277, found 441.2271.

4.1.6. *Synthesis of spiro-β-lactam* ***31***

To an oven-dried 25 mL round-bottomed flask was charged with (+) aromadendrene **29** (112 μL, 0.5 mmol, 1.0 equiv) and 3 mL CH_2Cl_2, followed by the addition of **30** (124 μL, 0.55 mmol, 1.1 equiv). After stirring for 2 h at 23 °C, Et_3N (278 μL, 2.0 mmol, 4.0 equiv) was added. The reaction was allowed to stir for additional 3 h. Then, H_2O (5 mL) was added and the resulting mixture was allowed to stir for 24 h. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (5 mL x 3). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated under vacuum. The residue was purified by an automated flash chromatography system (0 → 50%, EtOAc/hexanes) to provide the spiro-β-lactam **31** with 25% yield. TLC (EtOAc), R$_f$ 0.68; ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.87 (s, 1H), 2.65 (d, *J* = 14.6 Hz, 1H), 2.45 (dd, *J* = 14.6, 1.4 Hz, 1H), 2.13–1.99 (m, 2H), 1.95–1.67 (m, 5H), 1.44–1.20 (m, 3H), 1.01 (s, 3H), 0.98 (s, 3H), 0.93 (d, *J* = 7.1 Hz, 3H), 0.66–0.50 (m, 2H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 168.2, 59.8, 53.2, 42.9, 41.3, 41.3, 36.7, 34.7, 28.9, 28.3, 26.6, 26.3, 21.4, 20.1, 16.3, 15.9; IR (thin film): 3240, 1745, 1454; HRMS (ESI): *m/z* calcd for $C_{16}H_{25}NONa$ [M + Na]$^+$ 270.1834, found 270.1829.

4.1.7. *Synthesis of spiro-β-lactam* ***33***

To an oven-dried 50 mL round-bottomed flask was charged with (−)-caryophyllene oxide **32** (441 mg, 2.0 mmol, 1.0 equiv) and 6 mL Et_2O, followed by the addition of **30** (522 μL, 6.0 mmol, 3.0 equiv). After stirring for 27 h at 23 °C, Et_3N (2.78 mL, 20 mmol, 10 equiv) was added. The reaction was allowed to stir for additional 2 h. Then, H_2O (10 mL) was added and the resulting mixture was allowed to stir for 17 h. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (10 mL x 3). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated under vacuum. The residue was purified by an automated flash chromatography system (0 → 50%, EtOAc/hexanes) to provide the spiro-β-lactam **33** with 21% yield. TLC (EtOAc), R_f 0.34; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (s, 1H), 4.18 (dd, $J = 7.1$, 2.8 Hz, 1H), 3.09 (d, $J = 14.6$ Hz, 1H), 2.73 (d, $J = 14.3$ Hz, 1H), 2.39–2.28 (m, 1H), 2.26-2.16 (m, 1H), 2.15–2.04 (m, 1H), 2.01–1.83 (m, 3H), 1.82–1.73 (m, 2H), 1.65–1.56 (m, 2H), 1.46 (t, $J = 10.8$ Hz, 1H), 1.34 (s, 3H), 1.31–1.21 (m, 1H), 1.00 (s, 3H), 0.99 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 154.2, 86.4, 79.6, 57.4, 52.9, 44.6, 44.3, 40.2, 36.2, 34.3, 34.3, 29.8, 25.2, 23.4, 22.3, 18.7; IR (thin film): 3261, 1795,1751; HRMS (ESI): m/z calcd for $C_{17}H_{25}NO_4Na$ $[M + Na]^+$ 330.1681, found 330.1677.

*4.1.8. General procedure for the tagging of phenol-containing natural products as described for estrone-derived epoxide **35***

Into an oven-dried, 10 mL round-bottomed flask containing NaH (60% suspension in mineral oil, 13 mg, 0.3 mmol, 1.0 equiv) in THF (1 mL) was added slowly a solution of estrone (81 mg, 0.3 mmol, 1.0 equiv) in THF (1 mL). After gas evolution had ceased, a solution of (±)-**9** (95.5 mg, 0.5 mmol, 1.7 equiv) in THF (1 mL) was slowly added at 23 °C and stirred for 12 h. Upon completion (as judged by TLC), the reaction mixture was diluted with Et_2O (5 mL) quenched with saturated NH_4Cl (10 mL). The organic layer was separated and the aqueous layer was extracted with Et_2O (2 × 20 mL) and dried over anhydrous Na_2SO_4. The filtrate was concentrated by rotary evaporation, and purified by an automated flash chromatography system (0 → 60% EtOAc/hexanes) to afford estrone-derived epoxide **35** (83.0 mg, 72% yield) as white solid. TLC (EtOAc:hexanes, 3:7 v/v): R_f 0.33; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.30 (d, $J = 8.46$ Hz, 1H), 6.86 (dd, $J = 8.5$, 2.6 Hz, 1H), 6.82 (d, $J = 2.5$ Hz, 1H), 3.32 (dd, $J = 4.0$, 2.8 Hz, 1H), 2.94–2.91 (m, 2H), 2.82–2.76 (m, 2H), 2.54–2.50 (m, 1H), 2.44–2.40 (m, 1H), 2.32–2.28 (m, 1H), 2.19–2.13 (m, 1H), 2.09–1.95 (m, 3H), 1.70–1.41 (m, 6H), 1.36 (s, 3H), 1.30 (s, 3H), 0.93 (s, 3H); ^{13}C NMR (150 MHz; $CDCl_3$): δ 220.7, 174.6, 148.6, 138.0, 137.4, 126.3, 121.4, 118.5, 56.3, 50.4, 47.9, 44.14, 44.0, 42.8, 38.0, 35.8, 31.5, 29.3, 26.3, 25.7, 21.59, 21.26, 20.5, 13.8; IR (thin film): 2929, 1729, 1469, 1368, 1254, 1219, 1149, 1108, 1094, 899 cm^{-1}; HRMS (ESI) m/z calcd for $C_{24}H_{30}O_4$ $[M+H]^+$: 383.2144, found: 383.2282.

*4.1.8.1. Eugenol-derived epoxide **36**.* Prepared according to the general procedure using NaH (60% suspension in mineral oil, 10 mg, 0.26 mmol, 1.0 equiv), euginol (43 mg, 0.26 mmol, 1.0 equiv), (±)-**9** (50 mg, 0.26 mmol, 1.0 equiv) in THF (3 mL). Upon completion (as judged by TLC), the crude product was purified by automated flash chromatography (0 → 40%, EtOAc/hexanes) to afford (34.0 mg, 47% yield) clear liquid. TLC (EtOAc:hexanes, 3:7 v/v): R_f 0.5; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.96 (d, $J = 7.9$ Hz, 1H), 6.85–6.73 (m, 2H), 6.01–5.95 (m, 1H), 5.20–5.05 (m, 2H), 3.82 (s, 3H), 3.40 (d, $J = 6.7$ Hz, 2H), 3.36 (t, $J = 3.4$ Hz, 1H), 2.81 (d, $J = 3.4$ Hz, 2H), 1.40 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (150 MHz; $CDCl_3$): δ 174.0, 150.7, 138.98, 138.08, 137.0, 122.34, 120.6, 116.1, 112.7, 56.32, 55.8, 44.1, 42.8, 40.0, 21.6, 20.0; IR (thin film): 2920, 1757, 1637, 1605, 1541, 1508, 1465, 1419, 1267, 1200, 1149, 912 cm^{-1}; HRMS (ESI) m/z calcd for $C_{16}H_{20}O_4$ $[M+H]^+$: 277.1362, found: 277.1477.

*4.1.8.2. Estradiol-derived epoxide **37**.* Prepared according to the general procedure using NaH (60% suspension in mineral oil, 30 mg, 0.75 mmol, 2.5 equiv), estradiol (272 mg, 0.3 mmol, 1.0 equiv), (±)-**9** (115 mg, 0.6 mmol, 1.0 equiv) in THF (3 mL). Upon completion (as judged by TLC), the crude product was purified by automated flash chromatography (0 → 80%, EtOAc/hexanes) to afford (73.0 mg, 63% yield) of estradiol-derived epoxide **37** as a white solid. TLC (EtOAc:hexanes, 3:7 v/v): R_f 0.18; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.31 (d, $J = 8.4$ Hz, 1H), 6.86 (dd, $J = 8.5$, 2.6 Hz, 1H), 6.81 (d, $J = 2.4$ Hz, 1H), 3.77–3.74 (m, 1H), 3.33 (dd, $J = 4.0$, 2.7 Hz, 1H), 2.90–2.87 (m, 2H), 2.84–2.77 (m, 2H), 2.37–2.30 (m, 1H), 2.27–2.21 (m, 1H), 2.19–2.09 (m, 1H), 2.0–1.95 (m, 1H), 1.93–1.87 (m, 1H), 1.75–1.69 (m, 1H), 1.59–1.41 (m, 3H), 1.42–1.33 (m, 5H), 1.34–1.27 (m, 5H), 1.25–1.19 (m, 1H), 0.80 (s, 3H); ^{13}C NMR (150 MHz; $CDCl_3$): δ 174.5, 148.4, 138.28, 138.05, 126.3, 121.3, 118.3, 81.8, 56.3, 50.0, 44.14, 44.10, 43.2, 42.8, 38.4, 36.6, 30.5, 29.5, 27.0, 26.1, 23.1, 21.3, 20.4, 11.0; IR (thin film): 2924, 2360, 1745, 1609, 1493, 1469, 1379, 1251, 1119, 1055, 912 cm^{-1}; HRMS (ESI) m/z calcd for $C_{24}H_{32}O_4$ $[M+H]^+$: 385.2301, found: 385.2373.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.tet.2019.04.068.

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