Concise Total Synthesis of (±)-Salinosporamide A, (±)-Cinnabaramide A, and Derivatives via a Bis-cyclization Process: Implications for a Biosynthetic Pathway?

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Gil Ma, Henry Nguyen, and Daniel Romo*

Department of Chemistry, Texas A&M University, P.O. Box 30012, College Station, Texas 77842-3012

romo@tamu.edu

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4-Alkylidene- β -lactones (hetero ketene dimers) and α -amino acids are useful precursors for total syntheses of the β -lactone-containing proteasome inhibitors salinosporamide A, cinnabaramide A, and derivatives. A key step is a nucleophile-promoted, bis-cyclization of keto acids that simultaneously generates the γ -lactam and β -lactone of these natural products. This reaction sequence may have implications for the biosynthesis of these natural products.

Omuralide (1), derived from lactacystin (2),¹ the salinosporamides, e.g., salinosporamide A (3),² and the recently disclosed cinnabaramides, e.g., cinnabaramide A (4)³ are unique bicyclic β -lactone-containing natural products of bacterial origin that have recently attracted intense interest from both synthetic and biological perspectives (Figure 1). Several synthetic efforts including numerous total syntheses of omuralide¹ and three syntheses of salinosporamide A⁴ attest to the interest in these novel proteasome inhibitors due to their highly functionalized [3.2.0] bicyclic core and due to the validation of this therapeutic target for cancer.⁵ Recent crystallographic studies have elucidated fascinating details regarding inhibition of the 20S proteasome by salinosporamide A involving acylation of the active site threonine by

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Figure 1. Structures of proteasome inhibitors and a possible biosynthetic origin for the γ -lactam-fused β -lactone core.

the β -lactone with concomitant cyclization of the incipient alkoxide with the C13 chloro substituent leading to a tetrahydrofuran.⁶ Salinosporamide is currently in phase I human clinical studies for multiple myeloma.

We previously reported a catalytic, asymmetric intramolecular, nucleophile catalyzed aldol-lactonization (NCAL) process employing aldehyde acids that allows access to carbocycle-fused β -lactones,⁷ and this process was recently extended to keto acid substrates.⁸ This methodology was initially inspired by omuralide which contains such a bicyclic β -lactone core. Regarding the biosynthesis of these metabolites, one could speculate the joining of an appropriate amino acid **5** with an activated β -keto ester **6** followed by either an aldol-lactonization sequence⁹ or a [2 + 2] cycloaddition via a ketene intermediate, a mechanism commonly invoked for related bis-cyclizations (Figure 1).¹⁰

Building on our work with carbocycle-fused β -lactones, we envisioned a concise synthetic strategy to the bicyclic core of these natural products by simultaneous formation of the C–C and C–O bonds from a keto acid precursor **10** via

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Figure 2. Retrosynthetic analysis of salinosporamide A, cinnabaramide A, and derivatives.

an intramolecular bis-cyclization process (Figure 2, 10 \rightarrow 9). Attachment of the cyclohexenyl moiety, or other side chains, would rely on the strategy of Corey developed in the course of their salinosporamide synthesis on simpler aldehyde γ -lactam precursors.¹¹ This would entail addition of a cyclohexenyl zinc reagent to the aldehyde derived from benzyl ether 9; however, the success of this process and subsequent manipulations was not guaranteed given the presence of the β -lactone.¹² The keto acid substrate 10 could be derived from coupling of an α -amino acid 11 and a ketene dimer 12, the latter serving as a suitable latent equivalent for a β -ketoester.

Ultimately, we sought the development of an asymmetric strategy. However, one difficulty to be overcome was the potential for enolization of the substrate β -ketoamide rendering the ketone nonelectrophilic and most importantly, the possibility of rapid racemization at C2. However, due to the known conformationally controlled acidity of β -ketoamides owing to A^{1,3} strain,¹³ retention of optical activity appeared plausible. Herein, we describe the implementation of the first goal of this strategy; namely, the bis-cyclization process which has led to concise total syntheses of *rac*-salinosporamide A (**3**), *rac*-cinnabaramide A (**4**), and derivatives.

We began our studies with simple C2-unsubstituted substrates which were readily prepared by coupling of racemic ketene homodimer $14a^{14}$ with *N*-PMB-glycine benzyl ester (13a) by the method of Calter,¹⁵ which proceeded efficiently to provide keto acid substrate 17a following hydrogenolysis. We were pleased to find that bis-cyclization employing conditions similar to those developed for carbocycles,⁸ using 4-pyrrolidinopyridine (4-PPY) as a nucleophilic promoter, proceeded efficiently to give bicyclic- β -lactones 19a-d (Table 1). However, the C2-unsubstituted ketoamide 17d gave only 25% yield (entry 4). Without the C2-substitutent, facile enolization of the ketoamide likely leads to diminished rates of the initial aldol step. Interestingly, increased dia-

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 Table 1.
 Synthesis of Simplified, C4-Unsubstituted

 Salinosporamide/Cinnabaramide Derivatives
 19a-d

PMBHN 13 + C R ¹ 14a	CO ₂ Bn	15 N OH IF, 50 °C PMB 0 N 0 R ¹ 0 R ¹ 16a-d: R ² 17a-d: R ²	$\begin{array}{c} & & & \\ & & & \\ & & \\ & & \\ & & \\ & & \\$	equiv) 18 PMB A
entry	\mathbb{R}^1	$\%$ yield $({\bf 17})^{a,b}$	% yield $(19)^b$	$\mathrm{d}\mathbf{r}^{c}$
1	$CyCH_2$	84 (17a)	93 (19a)	2.2:1
2	<i>n</i> -hexyl	80 (17b)	90 (19b)	2.2:1
3	$PhCH_2$	72(17c)	85(19c)	$2.5:1 (>19:1)^d$
4	Н	77 (17d)	25~(19d)	

^{*a*} Yield is for two steps. ^{*b*} Yields refer to isolated, purified (SiO₂) product. ^{*c*} Determined by ¹H NMR analysis of crude reaction mixtures. ^{*d*} Observed diastereomeric ratio (dr) if reaction is allowed to proceed at 25 °C for 1.5 d (54% yield). PMB = *p*-methoxybenzyl, 4-PPY = 4-pyrrolidinopyridine, Cy = cyclohexyl.

stereoselectivity is obtained during bis-cyclization if the reaction is performed at 25 °C for extended times (1.5 d) by selective degradation of the minor diastereomer (confirmed by ¹H NMR reaction monitoring).

A likely mechanistic pathway for this bis-cyclization building on our related work with carbocycle-fused β -lactones⁸ involves initial activation of the carboxylic acid as pyridone ester 20 with modified Mukaiyama reagent 18. Following transacylation with 4-PPY, deprotonation by Hünig's base leads to ammonium enolate 21. Subsequent net aldol-lactonization via aldolate 22 then provides the γ -lactam-fused β -lactone **19a** with concomitant regeneration of the nucleophilic promoter, 4-PPY. However, a [2 + 2]cycloaddition mechanism via an intermediate ketene has not been excluded at this time.¹⁶ The relative configuration of the major diastereometric β -lactone **19a** was confirmed by X-ray analysis following cleavage of the PMB group with ceric ammonium nitrate (CAN) to provide amide 23a (Scheme 1). Importantly, the relative configuration corresponds to that found in the salinosporamides and the cinnabaramides.



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We next studied the impact of a C2 substituent during the bis-cyclization by targeting the synthesis of cinnabaramide A (4). The synthesis commenced by reductive amination of commercially available O-benzyl-L-serine with p-anisalde-hyde (Scheme 2). Subsequent esterification provided the



protected serine derivative 11a in 58% overall yield (two steps). The required unsymmetrical ketene dimer 12a was obtained by heterodimerization of acetyl and octanoyl chlorides.^{14a} Coupling of ketene dimer **12a** with L-N-PMBserine 11a gave diastereomeric esters 25 (dr, 1:1), and subsequent Sn-mediated hydrolysis¹⁷ provided acids 26 in good overall yield. The key bis-cyclization provided diastereomeric β -lactones 27/28 in 45% yield with moderate diastereoselectivity (dr 3.3:1); however, the major diastereomer corresponded to that found in the natural product as verified by NOE analysis.¹⁸ Deprotection of the benzyl ether enabled separation of the major alcohol diastereomer 29 (79%, dr > 19:1), and this was followed by Parikh–Doering oxidation¹⁹ to give an intermediate aldehyde which was used directly in the next step. Applying the method developed by Corey, addition of zinc reagent **30**^{4a} gave alcohol **31** in 57% yield (two steps, dr 4.7:1). Finally, oxidative cleavage of the PMB group gave *rac*-cinnabaramide A (4), which could be isolated diastereomerically pure in 48% yield. Spectral data for the synthetic material correlated with the published data.³ Further verification of relative configuration was accomplished by X-ray analysis.18

⁽¹⁶⁾ In addition to previous studies with carbocycle-fused β -lactones which clearly point to participation of the nucleophile in these bis-cyclization reactions, lower conversions were obtained with less nucleophilic promoters e.g., dimethylaminopyridine, suggestive of nucleophile involvement in the rate-determining or prior step.

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To further validate the mildness of this strategy, we targeted the synthesis of salinosporamide A bearing the required chloro substituent in the keto acid substrate. In this case, *N*-PMB serine allyl ester **11b**, available in two steps from serine, was utilized to enable mild ester deprotection since presumed cyclopropane formation occurred during attempted saponification of the corresponding keto methyl ester (not shown, cf. **25**) (Scheme 3). Amine **11b** was coupled



with heteroketene dimer **12b**, readily available in gram quantities from heterodimerization of acetyl chloride and commercially available 4-chlorobutanoyl chloride,¹⁸ to provide keto acids **33** following Pd-mediated ester deprotection. Bis-cyclization provided bicyclic- β -lactones **34/35** in 25–35% yield (dr 2–3:1) favoring the relative configuration found in salinosporamide A.²⁰ Deprotection of the benzyl

⁽²⁰⁾ Lower yields in this bis-cyclization and that leading to cinnabaramide A ($26 \rightarrow 27, 28$) in comparison with C4-unsubstituted substrates (Table 1) are clearly a result of increased steric issues during the bis-cyclization. While no starting material is recovered, two major byproducts have been identified, A and B (20–30% combined yield).



ether enabled enrichment of the major diastereomer to 6-10:1 upon purification. Modified Moffatt²¹ oxidation using 1-(1,3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDCI)²² and dichloroacetic acid²³ followed by addition of zinc reagent **30** gave predominantly two diastereomeric alcohols **37** (dr 3.5:1) in 33% yield (two steps).²⁴ Final deprotection of the PMB group enabled isolation of diastereomerically pure *rac*-salinosporamide A, which correlated with the published data and the relative configuration was further confirmed by X-ray analysis.¹⁸

In summary, we have developed concise synthetic routes to rac-salinosporamide A, rac-cinnabaramide A, and simplified derivatives. This strategy is unique in enabling simultaneous construction of both the γ -lactam and fused- β -lactone found in these metabolites via a bis-cyclization process. The β -lactone in these systems and the chloro-substituent in salinosporamide precursors is shown to be tolerant to several transformations which contributes to the brevity of the sequence.^{1c} The described bis-cyclization process points to a logical biosynthetic origin for these intriguing natural products and raises the interesting question of whether such a bis-cyclization might be involved in the biosynthesis of these natural products.9 Further optimization of this process including mechanistic studies and extension to an enantioselective strategy premised on A1,3-strain in keto acid precursors, e.g., keto acids 25 and 33, constitute our ongoing efforts in this area.

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Supporting Information Available: General procedures for ketene-dimerizations, bis-cyclizations, and subsequent transformations with characterization data (including ¹H and ¹³C NMR spectra) for β -lactones 3, 4, 19a-d, 23a, 27, 29, 31, 34, 36, and 37 and products 11a,b, 12a,b, 14b, 16a-c, 17a-d, 25, 26, 32, and 33. This material is available free of charge via the Internet at http://pubs.acs.org.

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⁽²⁴⁾ Yields in this two-step process are lower due to incomplete oxidation and inability to purify the aldehyde due to some sensitivity of this intermediate. Diastereoselectivity is considerably lower than that reported previously (see refs 4a–c); however, this appears to be highly substrate dependent given that Danishefsky observed reduced diastereoselectivity with *N*-unprotected substrates (ref 4c).