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Total Synthesis and Absolute Stereochemistry of the Proteasome Inhibitors Cystargolides A and B

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Abstract

The absolute stereochemistry of the cystargolides was determined by total synthesis. Evaluation of synthetic cystargolides and derivatives showed that the natural (2*S*,3*R*) stereochemistry is essential for activity. Moreover, benzyl esters (–)-10 and (–)-15 were found to be about 100 times more potent, and to selectively kill MCF-7 cancerous cells.

Proteasome inhibitors^{1–4} have attracted much attention in recent years due to their potential to regulate cellular protein homeostasis. The inhibition of the proteasome function has been clinically validated with the approval of bortezomib⁵ for the treatment of multiple myeloma. Bortezomib belongs to the boronic acid class of proteasome inhibitors. Other classes of proteasome inhibitors include epoxyketones such as epoxomicin,⁶ and β -lactones such as the belactosins A and C,⁷ and salinosporamide A.^{8,9}

Recently, Kerr et al.¹⁰ isolated two novel β -lactone containing natural products, cystargolides A and B (**1** and **2**, Fig. 1) from the actinomycete *Kitasatospora cystarginea*.¹⁰ The structures of the cystargolides were elucidated using a combination of NMR, tandem mass spectrometry experiments, and chemical derivatization. Based on analysis of the ³*J*_{HH} values for H-2'' and H-3'', the relative stereochemistry at the β -lactone fragment was established as *trans*, however, the absolute stereochemistry remained unassigned.¹⁰

Both cystargolides A and B exhibited activity as 20S proteasome inhibitors, with potency comparable to that of the belactosins A and C.⁷ Several belactosin analogs have been synthesized,¹¹ and structure-activity relationship studies have established that the β -lactone moiety is responsible for inhibiting the proteasome activity. Inhibition of the proteasome has also a potential impact on the regulation of the NF- κ B pathway, an essential mechanism of homeostasis involved in the control of the inflammatory response.¹² Based on these precedents, and because of their relatively simple structure, the cystargolides represent an attractive starting point for the development of ubiquitin-proteasome inhibitors with potential applications in cancer or anti-inflammatory therapies.

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Before any optimization studies can be carried out, the correct and complete structure of the cystargolides must be known. We decided to embark in a project aimed at determining the absolute stereochemistry of the cystargolides by total synthesis. The approach had to be flexible to access either enantiomer of the β -lactone fragment, which could be incorporated into a protected dipeptide corresponding to cystargolide A or B. Herein, we disclose our results culminating into the assignment of the complete absolute stereochemistry for both natural products.

Beta-lactones are common motifs found in bioactive natural products, and are useful synthetic intermediates.¹³ Several methods for the stereoselective synthesis of β -lactones are known in the literature.^{14–17} We chose to use a one-pot chlorination-lactonization approach described by Barlaam,¹⁸ and showcased by Armstrong,¹⁹ in his synthesis of belactosin A.

Our synthesis started by alkylating the known imide (–)-**3**²⁰ (Scheme 1) under Evans conditions²¹ to deliver (–)-**4** as the only detectable diastereomer (¹H NMR). Cleavage of the chiral auxiliary under standard conditions efficiently yielded the known succinic acid derivative (–)-**5**, which was taken into the one-pot chlorination-lactonization step to give (–)-**6**.

The stereochemical outcome of this sequence is rationalized based on Armstrong's model,¹⁹ in which deprotonation of (–)-**5** is expected to produce enolate **5a**. Electrophilic attack of CCl₄ from the less hindered side produced a beta-chloride carboxylate with the correct stereochemistry to generate *trans*- β -lactone (–)-**6** via intramolecular displacement of the chloride by the carboxylate. The *trans*- relationship of the substituents was confirmed by analysis of the ³J_{HH} values^{22, 23} for H-2'' and H-3'' (cystargolide numbering), and supported by NOE studies. Cleavage of the *t*-Butyl group of (–)-**6** was achieved by treating (–)-**6** with TFA at low temperature forming the acid (–)-**7** in quantitative yield. The same synthetic sequence yielded (+)-**7** starting from imide (+)-**3**.

The synthesis of the dipeptide fragment of the cystargolides started by union of L-isoleucine benzyl ester (–)-**8**²⁴ with *N*-Boc valine,²⁵ using standard amino acid coupling conditions²⁶ to give (–)-**9** (Scheme 2). The Boc protecting group was then cleaved under acidic conditions, and the resulting dipeptide trifluoroacetate salt was used directly in the coupling reaction with carboxylic acids (–)-**7** and (+)-**7** separately to give (–)-**10** and (–)-**11**, respectively.

Finally, hydrogenolysis of the benzyl ester in (–)-**10** and (–)-**11** afforded diastereomeric (–)-**1** and (–)-**12**. Comparison of the ¹H NMR spectra of both lactones with the data for natural cystargolide A revealed subtle differences between the signals corresponding to H-2'' of the β -lactone, which suggested diastereomer (–)-**1** to be a better match. Analysis of the ¹³C NMR data, particularly at the methyl group region, was more informative, and supported the same conclusions, with diastereomer (–)-**1** showing a closer match in all signals (δ 0.5 ppm, see supporting information). Together, these results establish the absolute stereochemistry at the 2'' and 3'' positions of cystargolide A as (2*R*,3*S*). Of note, the same configuration is found in the lactone portion of the belactosins.⁷

The synthesis of cystargolide B followed a similar pathway starting with the coupling of L-valine benzyl ester **13**²⁷ and *N*-Boc valine (Scheme 3).

As before, the Boc protecting group in the obtained dipeptide (–)-**14** was cleaved under acidic conditions, and the corresponding trifluoroacetate salt was used directly in the coupling with acids (–)-**7** and (+)-**7** separately. Hydrogenolysis afforded diastereomers **2** and (–)-**17**. Again, comparison of the ¹H and ¹³C NMR data with that of natural cystargolide B (see supporting information) revealed diastereomer **2** to be a better match, and consequently the 2'' and 3'' positions of the lactone portion also possess (2*R*,3*S*) stereochemistry in the natural product.

Both synthetic cystargolides A and B, their corresponding (2*S*,3*R*) diastereomers and benzyl ester precursors were evaluated as 20S proteasome inhibitors, by determining the degree of proteolytic cleavage of the substrate Suc-LLVY-AMC in lysed Jurkat cells at different inhibitor concentrations (see supplementary information for details). Cystargolide A (**1**) exhibited an IC₅₀ value of 0.97 ± 0.19 μM and cystargolide B (**2**) exhibited an IC₅₀ of 0.9 ± 0.11 μM, correlating with the results observed by Kerr et al.¹⁰ Benzyl esters (–)-**10** and (–)-**15** exhibited IC₅₀ values of 9.2 ± 0.5 nM and 9.0 ± 1.4 nM respectively, representing a ~100-fold increase in potency with respect to their natural product analogs (Fig. 2).

Compounds (–)-**11**, (–)-**12**, (–)-**16** and (–)-**17**, possessing the unnatural (2*S*,3*R*) stereochemistry were much less active, with IC₅₀ >10 μM. These results highlight the importance of the (2*R*,3*S*) stereochemistry for activity, and further support the stereochemical assignment on the natural products.

Cytotoxicity assays were also conducted on MCF-7 and MCF10A cell lines as models, for breast cancer and normal breast tissue respectively, using a standard MTT colorimetric assay (see supplementary information for details). Both the proteasome inhibition and the cytotoxicity results are shown in table 1.

Benzyl ester (–)-**15** exhibited significant cytotoxicity towards MFC-7 cell lines with an IC₅₀ value of 7.0 ± 1.2 μM. Benzyl ester (–)-**10** displayed similar behavior with IC₅₀ values of 11.4 ± 1.7 μM in MFC-7 cancer cells. Remarkably, neither (–)-**15** nor (–)-**10** showed any cytotoxicity towards the MCF10A normal breast epithelial cells up to 100 μM concentrations. Although the work presented here is limited only to these two cell lines, such selective cytotoxicity towards the tumorigenic MCF7 cell line, but not towards the “normal”, non-tumorigenic MCF 10A cell line, makes these benzyl ester analogs particularly promising leads for anti-cancer drug discovery.

In conclusion, a one-pot halogenation/lactonization strategy on chiral succinic acid derivatives generated the enantiomeric β-lactone acids (–)-**7** and (+)-**7**. Coupling of each acid to dipeptides (–)-**9** and (–)-**14** separately, followed by deprotection yielded **1**, **2** and their corresponding (2*S*,3*R*) diastereomers. Comparison of the spectroscopic data with that of the natural cystargolides established the absolute stereochemistry of the lactone portion of the natural products as (2*R*,3*S*). Evaluation of the proteasome inhibition activity of the synthetic cystargolides, as well as their diastereomeric analogs and benzyl ester precursors

revealed that the (2*R*,3*S*) stereochemistry at the lactone fragment is essential for activity, and that esterification with a benzyl group produces analogs that are about 100 times more potent than the natural products. Overall, the cystargolides and related structural congeners possess interesting proteasome inhibition activity that is comparable to other naturally occurring β -lactones, representing an attractive starting point for the development of proteasome inhibitors with potential applications in cancer or anti-inflammatory therapies. Efforts toward these goals are currently underway and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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R = Et, Cystargolide A (**1**)
R = Me, Cystargolide B (**2**)

Figure 1.
Cystargolides A and B

Figure 2.
Proteasome inhibition of cystargolides and benzyl derivatives

Scheme 1.
Synthesis of (-)-7 and (+)-7

Scheme 2.
Synthesis of cystargolide A (1) and (-)-12

Scheme 3.
Synthesis of cystargolide B and (-)-17

Table 1

Proteasome Inhibition and Cytotoxicity Results.

Compound	IC ₅₀ (μM)		
	Proteasome Inhibition	MCF-7	MCF10A
(-)- 1	0.97 ± 0.19 ^a	58.0 ± 9.5	>100
(-)- 2	0.90 ± 0.11 ^a	84.7 ± 18.6	>100
(-)- 10	0.0092 ± 0.0005 ^a	11.4 ± 1.7	>100
(-)- 11	~50 ^b	41.2 ± 3.5	29.3 ± 3.3 ^c
(-)- 12	>100 ^b	>100	>100 ^c
(-)- 15	0.0090 ± 0.0014 ^a	7.0 ± 1.2	>100
(-)- 16	~30 ^b	23.1 ± 3.3	24.2 ± 1.4 ^c
(-)- 17	>100 ^b	>100	>100 ^c

^a Measured in biological triplicate and confirmed by technical triplicate.^b Estimated from biological singlicate and confirmed by technical duplicate.^c Measured in biological quadruplicate, as a technical singlicate.

Remaining values represent means of quadruplicates measured in technical duplicate.