Bioorganic & Medicinal Chemistry Letters 23 (2013) 6905–6910

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

(*S*)-4-Trimethylsilyl-3-butyn-2-ol as an auxiliary for stereocontrolled synthesis of salinosporamide analogs with modifications at positions C2 and C5

Landy K. Blasdel [a,†], DongEun Lee [b], Binyuan Sun [c,†], Andrew G. Myers [b,*]

[a] *The Pennsylvania State University, PA, USA*
[b] *Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA*
[c] *Merck Research Laboratories, 33 Avenue Louis Pasteur, Boston, MA 02115, USA*

ARTICLE INFO

Article history:
Received 4 September 2013
Accepted 23 September 2013
Available online 30 September 2013

Keywords:
Salinosporamide A
Proteasome inhibitor
Mukaiyama aldol reaction
Chiral auxiliary

ABSTRACT

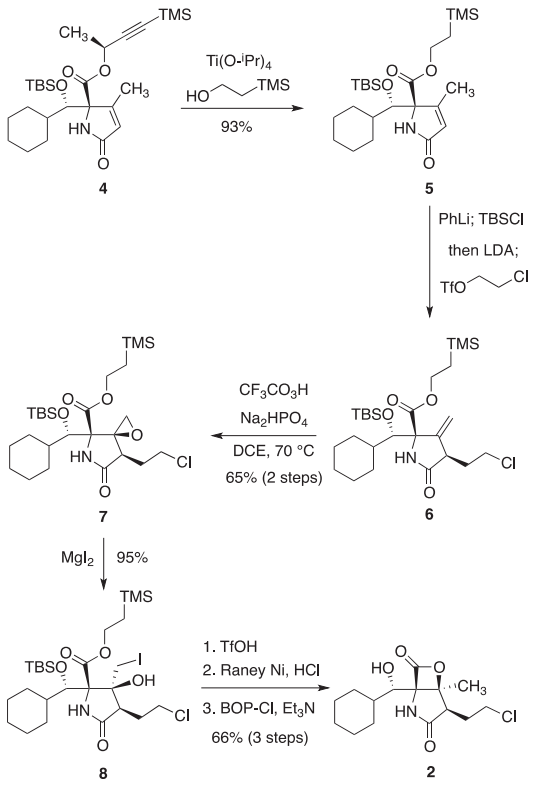
Analogs of salinosporamide A with variations of the C2 and C5 substituents are prepared in 8–10 steps using as the first and key transformation a diastereoselective Mukaiyama aldol reaction between the chiral 5-*tert*-butyldimethylsiloxy-3-methyl-1*H*-pyrrole-2-carboxylic ester depicted and various aldehyde substrates, promoted by *tert*-butyldimethylsilyl triflate. In this transformation, the 4-trimethylsilyl-3-butyn-2-ol ester functions to direct the formation of predominantly one of four possible diastereomeric aldol products. Introduction of the C2 appendage by a later-stage, stereocontrolled alkylation reaction permits the construction of analogs variant at this position. Results from in vitro and cell-based assays of proteasomal inhibition are reported. Mass spectrometric studies provide mechanistic details of proteasomal modification by salinosporamide A and analogs.

Salinosporamide A (**1**, Table 2) is a marine natural product with antiproliferative effects in human cancer cells grown in culture.[1] These effects correlate with and are thought to derive from the ability of salinosporamide A to inhibit the chymotryptic-like subunit of the proteasome.[2] In light of the potential therapeutic benefits of proteasomal inhibitors for the treatment of multiple myeloma and other cancers, many laboratories have sought to develop chemistry to access analogs of the densely functionalized, stereochemically complex salinosporamide natural product family. Corey and co-workers[3] reported the first laboratory synthetic route to salinosporamide A subsequent to their pioneering achievements in the lactacystin–omuralide class.[4] Since then, an extraordinary diversity of innovative developments in many laboratories have permitted access to **1**,[5] dihydrosalinosporamide A[2b] (**2**, Scheme 1) and structural analogs.[2b,6] In this work we provide details of a new process that permits stereocontrolled synthesis of salinosporamide analogs with structural variations at positions C2 and C5. The key step in the sequence establishes the contiguous stereogenic centers C4, a quarternary carbon center, and C5 by a diastereoslective Mukaiyama-type aldol addition reaction between 5-*tert*-butyldimethylsilyloxy-3-methyl-1*H*-pyrrole-2-carboxylic esters and different aldehyde substrates. A strategically related bond formation was earlier demonstrated by Baldwin and co-workers[7] in the key step of their synthetic route to lactacystin, depicted in Figure 1. The present work differs from that precedent in that the pyrrole ester we employ as substrate bears a C3-methyl substituent (present in salinosporamide A but not lactacystin–omuralide) but no C2 substituent, carries C15 in its proper oxidation state, and most significantly, uses a simple chiral alcohol in ester linkage at that same position to direct stereoselective aldol bond formation at C4–C5, rather than a cyclic scaffolding element.

The basis for the present route was the preliminary finding that methyl 5-*tert*-butyldimethylsilyloxy-3-methyl-1*H*-pyrrole-2-carboxylate (1.0 equiv) and cyclohexanecarboxaldehyde (1.5 equiv) react in the presence of *tert*-butyldimethylsilyl triflate (2.0 equiv) in dichloromethane at −78 °C to afford a racemic mixture of Mukaiyama aldol addition products that substantially favors the C4–C5 anti stereoisomer over the syn diastereoisomer (20:1, 67% combined yield, Table 1, entry 1). As salinosporamide A is a C4–C5 anti stereoisomer, we were encouraged that a route to various salinosporamide analogs might be developed provided that the issue of absolute stereochemical control could be addressed. To enable an enantio- as well as diastereocontrolled synthesis of salinosporamide analogs we investigated Mukaiyama aldol addition reactions of cyclohexane-carboxaldehyde with a number of esters derived from 5-*tert*-butyldimethylsilyloxy-3-methyl-1*H*-pyrrole-2-carboxylic acid and simple chiral alcohols (Table 1). We observed that a preponderance of one of the four possible diastereoisomeric Mukaiyama aldol addition products was formed in each case, on the basis of ^{1}H NMR analysis of the crude reaction

* Corresponding author. Tel.: +1 617 495 5718; fax: +1 617 495 4976.
E-mail address: myers@chemistry.harvard.edu (A.G. Myers).
† Present address.

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.09.066



Scheme 1. Synthesis of dihydrosalinosporamide A (**2**).

mixtures, although differing proportions of the three alternative diastereomeric addition products were also evident (Table 1). While we believe that in each case the major product was likely an anti diastereomer, we did not rigorously establish this, but instead focused on identifying the most useful substrate in terms of the net diastereoselectivity of the aldol addition reaction and the ease of purification of the major product. This proved to be the 4-trimethylsilyl-3-butyn-2-ol ester substrate of entry 10. The parent chiral alcohol used to prepare this substrate is readily available in gram amounts in both enantiomeric forms by asymmetric hydrogenation of 4-trimethylsilyl-3-butyn-2-one using the Noyori protocol.[8]

In one larger-scale implementation of the transformation of entry 10 (Table 1), we added *tert*-butyldimethylsilyl trifluoromethanesulfonate (9.6 mL, 42 mmol, 2.0 equiv) to a solution of the pyrrole ester substrate **3** (7.9 g, 21 mmol, 1 equiv) and cyclohexanecarboxaldehyde (3.8 mL, 31 mmol, 1.5 equiv) in dichloromethane (104 mL) at −78 °C. After 6 h, triethylamine was added, and the product mixture was isolated by extraction. Diastereomerically pure anti aldol addition product **4** (4.4 g, 43% yield) was obtained by flash-column chromatography, then trituration with hexanes. X-ray crystallographic analysis (Fig. 2) established that the product we obtained was the (4*S*,5*S*)-anti stereoisomer.

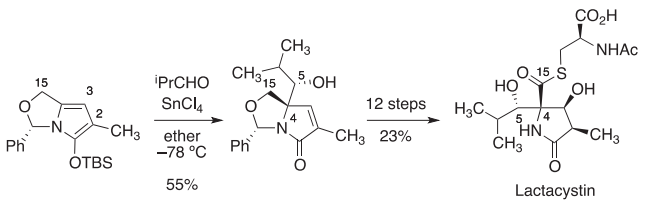


Figure 1. A diastereoselective Mukaiyama aldol reaction featured in a prior synthesis of lactacystin,[7] numbered to correspond with salinosporamide A.

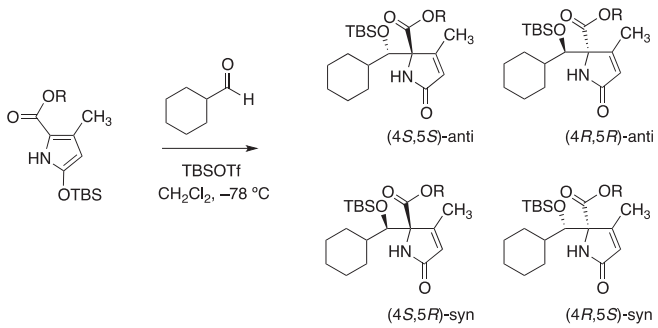
To transform the aldol addition product **4** into enantiomerically pure dihydrosalinosporamide A (**2**), we developed the 7-step sequence shown in Scheme 1. The sequence began with transesterification of **4** in the presence of 2-trimethylsilylethanol and titanium isopropoxide[9] (recovery of the volatile chiral alcohol by-product was not attempted) affording the 2-trimethylsilylethyl ester **5** in 93% yield. Alkylative introduction of the C2 substitutent was then achieved in one operation by in situ *N*-*tert*-butyldimethylsilylation (PhLi; TBSCl) followed by formation of an extended enolate at −78 °C with LDA and trapping with 2-chloroethyl triflate. The alkylation reaction was position- and stereo-specific, providing exclusively the α-adduct **6**. Stereoselective epoxidation then occurred in the presence of trifluoroperacetic acid to provide the epoxide **7** in 65% yield (two steps, from **5**). Epoxide opening with MgI_2[10] gave rise to the iodohydrin **8**. Exposure of the latter product to triflic acid led to cleavage of the 2-(trimethylsilyl)ethyl ester function as well as the *tert*-butyldimethylsilyl ether; selective deiodination of the resulting iodo acid then occurred in the presence of Raney nickel.[11] Lastly, the β-lactone ring was formed in the presence of BOP-Cl and triethylamine,[4] completing the synthesis of dihydrosalinosporamide A (**2**, 7 steps from **4**, 38% yield).

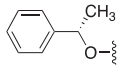
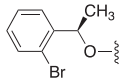
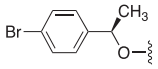
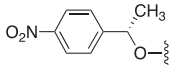
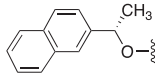
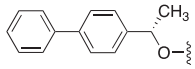
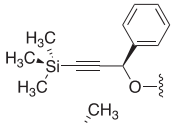
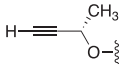
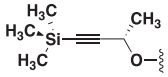
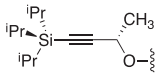
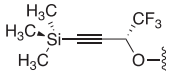
Salinosporamide analogs with 5-cyclopropyl and 5-benzyl substituents in place of cyclohexyl were synthesized using cyclopropanecarboxaldehyde and 2-(cyclohexa-2,5-dien-1-yl)acetaldehyde,[12] respectively, as alternative substrates in the Mukaiyama aldol coupling with the pyrrole ester substrate **3** (Scheme 2). Although the yields of the diastereomerically pure anti-aldol products were only modest (due to need for extensive purification), the transformations nevertheless provided sufficient material to permit further processing of these products by sequences analogous to Scheme 1 to produce the corresponding salinosporamide analogs (compounds **17** and **18**, respectively, Table 2) for evaluation of proteasomal inhibition (8–9 steps, 6–10% yield, see Supplementary data for details).

In addition to variation of the aldehyde coupling partner, we employed alternative electrophiles for enolate trapping and so produced analogs with different C2-substituents. For example, use of 3-chloropropyl triflate as the electrophile provided access to the homologated dihydrosalinosporamide analog **16** (Scheme 3 and Table 2) whereas allyl bromide provided access to the aldehyde and ester analogs **19** and **20** by selective oxidation of the allyl side-chain (**11** → **12**, Scheme 4). The latter α-allylation reaction employed a different protocol for in situ *N*-*tert*-butyldimethylsilylation, using *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide, which allowed us to isolate and vacuum-dry the *N*-protected lactam prior to adding LDA. All three analogs (**16**, **19**, and **20**, see Table 2) were prepared with the goal of exploring alternative modes of cyclization of the hydroxyl group that is liberated upon opening of the β-lactone function by the N-terminal threonine residue of the chymotryptic subunit of the proteasome (see Fig. 3), which has been suggested to be an important factor in the functioning of salinosporamide A.[2b,6e,13]

To evaluate the ability of each salinosporamide analog to inhibit the chymotryptic site of the 20S proteasome, an in vitro assay was conducted using purified human 20S proteasome and a commercial fluorogenic substrate[14] (assays conducted by Dr. Sridevi Ponduru and Mr. Ronald Paranal, laboratory of Prof. James Bradner, Dana Farber Cancer Institute). The IC_{50} values that were obtained are listed in Table 2. Dihydrosalinosporamide A was found to be somewhat less potent (one to threefold) than an authentic sample of salinosporamide A (in a prior study, dihydrosalinosporamide A (**2**) was found to be ~eightfold less potent than **1** in an assay of the chymotryptic activity of rabbit 20S proteasome).[2b] Interestingly, we found that the 5-cyclopropyl analog **17** and **1** displayed similar potencies while the 2-(3-chloropropyl) analog **16** was two to threefold less potent than **1**.[15] In contrast, the 5-benzyl

Table 1
Mukaiyama aldol addition reactions of various pyrrole ester substrates and cyclohexanecarboxaldehyde



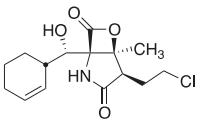
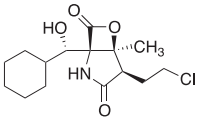
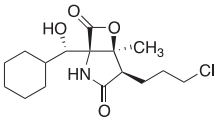
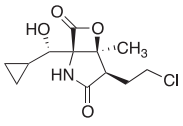
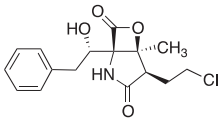
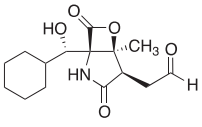
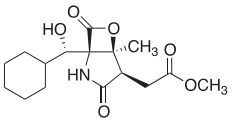
Entry	Chiral ester	Isomer ratio[a]
1		20:1
2		13:1.9:1.2:1
3		16.8:6.4:1.4:1
4		10.7:5.8:1.9:1
5		18.7:6.8:1.2:1
6		9.2:2.3:1.7:1
7		6.8:1.3:1:1
8		5.4:3.5:1:1
9		20.9:4.6:1.1:1
10		**19:2:1:1**
11		19.1:2.4:1.1:1
12		16.3:5.4:1.2:1

[a] All ratios were established by integration of ^{1}H NMR spectra of unpurified product mixtures. Only the stereochemical assignments of the major products of entries 1 and 10 were rigorously established.

salinosporamide analog and the analogs bearing aldehyde and methyl ester functions on the C2 side-chain were >10-fold less potent than salinosporamide A. To evaluate our synthetic analogs in a cell-based assay, an image-based screening method[16] was employed that assayed proteasomal inhibition by fluorescence readout from a stably transfected HEK cell line expressing a GFP-tagged proteasomal substrate. The 5-cyclopropyl analog **17** failed to perform as well in this assay as it did in the prior extracellular assay, whereas dihydrosalinosporamide A (**2**) and the 2-(3-chloropropyl) dihydrosalinosporamide analog **16** functioned to inhibit the proteasome in both the cell-based assay and the in vitro proteasomal inhibition assay.

In light of the fact that the 2-(3-chloropropyl) dihydrosalinosporamide analog **16** was active in both the in vitro and cell-based assays of proteasomal inhibition, we were curious to learn if the chloropropyl side-chain underwent cyclization to form a tetrahydropyran ring after acylation of the N-terminal threonine residue of the chymotryptic site (in analogy to the tetrahydrofuran ring formation that occurs with salinosporamide A)[17] and if so, at what rate. To address these questions, we developed a protocol to monitor the acylation reactions by mass spectrometry. This is possible because the unmodified proteasome, its direct acylation product (without secondary cyclization), and the putative fully cyclized acylated product all have different molecular weights. We were

Table 2
Assays evaluating proteasomal inhibition by salinosporamide analogs

Analog	IC_{50} (nM) Chymotryptic site	EC_{50} (nM) HEK 293
 **1**	22[b]	ND
 **2**	23[a], 77[b]	10[a]
 **16**	50[a], 67[b]	90[a]
 **17**	28[a]	360[a]
 **18**	500[a]	332[a]
 **19**	330[b]	ND
 **20**	500[b]	ND

[a] Assays performed by Dr. Sridevi Ponduru, Bradner Laboratory.
[b] Assays performed by Mr. Ronald Paranal, Bradner Laboratory.

successful in developing two different mass spectrometric protocols to monitor each step of proteasomal modification by salinosporamide A and its analogs (results are summarized in Figs. 3 and 4). Briefly, purified human 20S proteasome (1.4 μM) was incubated with a 10-fold molar excess of salinosporamide A or an analog in pH 7.5 buffer solution (10 mM Tris/HCL, 1 mM EDTA, 1 mM DTT, <1% DMSO) at 23 °C for varying times before a denaturing quench and sample cleaning using a C4 'ZipTip' (Millipore, Billerica, MA), eluting with 70:30:0.1 acetonitrile–water–formic acid. In the first method static nanospray experiments were conducted using a glass capillary emitter (NewObjective GlassTip, model BG12-94-4-N, Woburn, MA) and a solariX 12T FTMS (Bruker Daltonics, Billerica, MA). Due to the complexity of the sample, continuous accumulation of selected ions (CASI) from m/z 1000–1200 was performed, and ions were detected from m/z 150–3000, averaging 200 scans per sample. Maximum Entropy deconvolution was performed on the complex mass spectrum using DataAnalysis 4.0 (Bruker Daltonics, Billerica, MA). Later, in a second method, we replicated these experiments using a glass insert with sample introduction into an ESI-TOF (Agilent) by electrospray. Maximum Entropy deconvolution was performed on the complex mass spectrum using Mass Hunter.

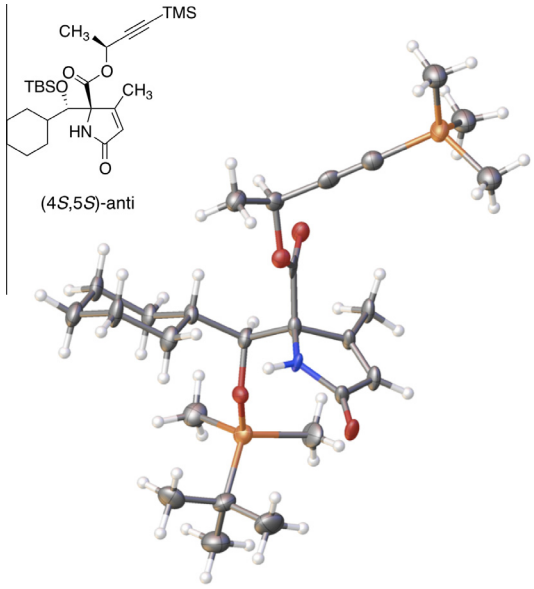
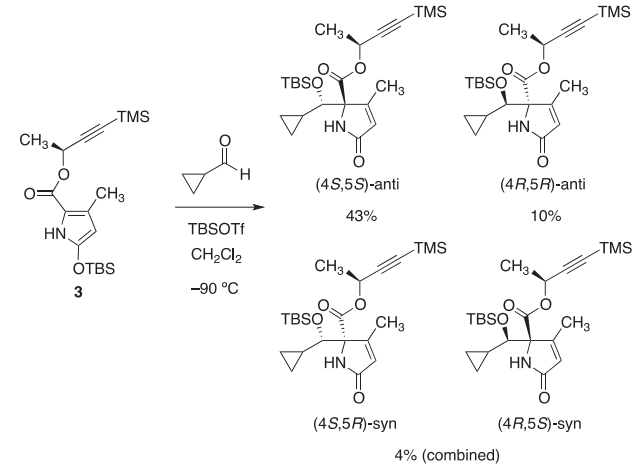
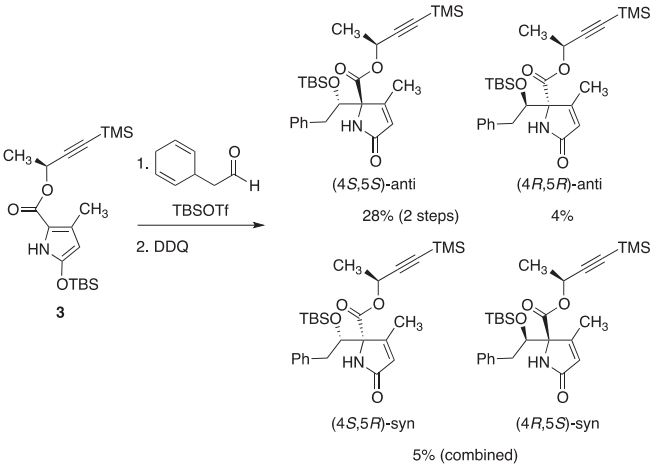
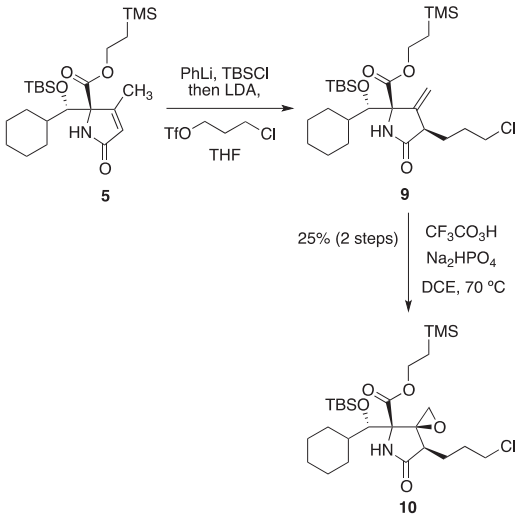


Figure 2. A 3-dimensional representation of the solid-state structure of the major diasteromeric Mukaiyama aldol addition product, based on X-ray crystallographic analysis.

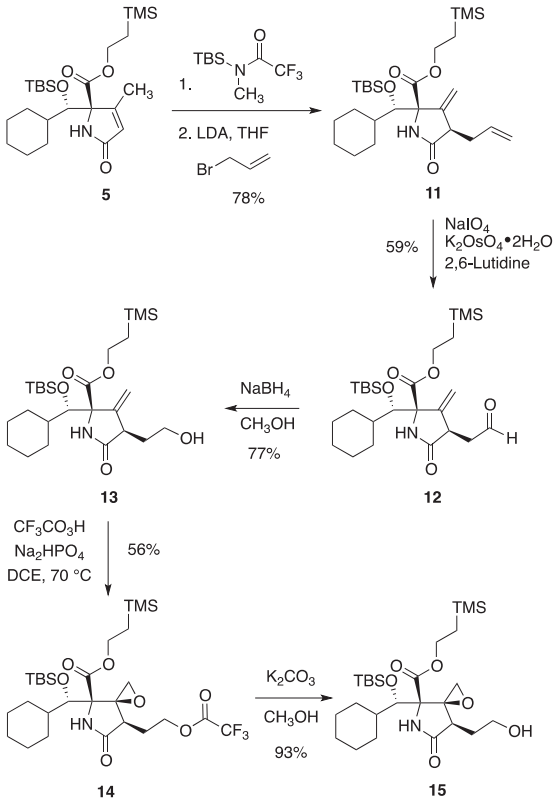




Scheme 2. Synthesis of intermediates to 5-cyclopropyl and 5-benzyl analogs of salinosporamide A (compounds **17** and **18**, respectively, Table 2).



Scheme 3. Synthesis of an intermediate to the 2-(3-chloropropyl) analog **16**.



Scheme 4. Synthesis of a common intermediate (alcohol **15**) to aldehyde and methyl ester analogs **19** and **20**.

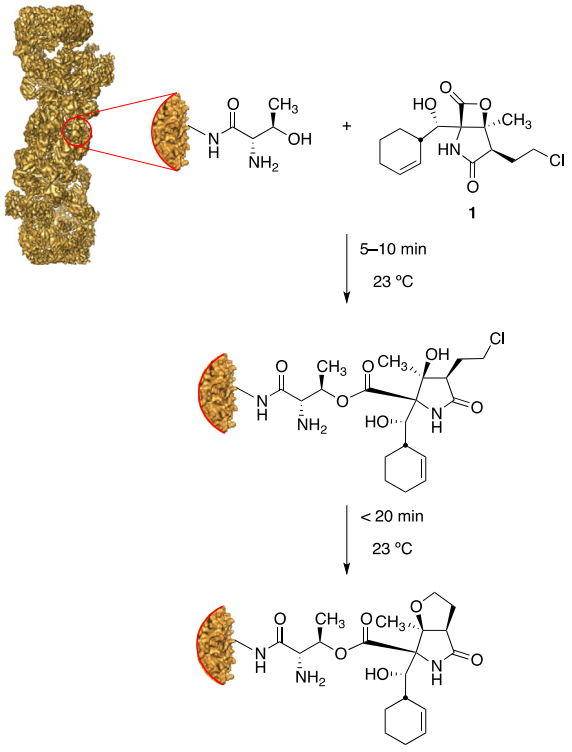
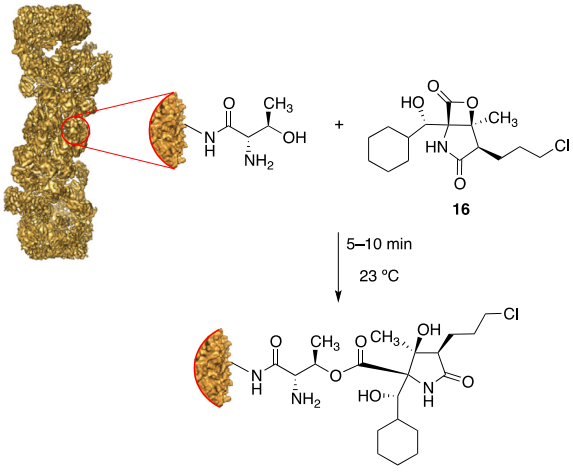


Figure 3. Inhibition of the 20S proteasome by salinosporamide A (**1**).



No evidence of futher reaction (within 48 h), cyclization or hydrolysis

Figure 4. Inhibition of the 20S proteasome by 2-(3-chloropropyl) dihydrosalinosporamide A (**16**).

Both methods allowed us to identify the unmodified proteasomal subunit (β5), the immediate (non-cyclized) product of its acylation by salinosporamide A (**1**) or an analog, and the fully cyclized acylated protein product (when this occurred). By varying incubation times, we observed that β-lactone ring-opening of **1** by the proteasome is relatively rapid under the conditions of our experiment; complete reaction occurred within ~5–10 min at 23 °C. Cyclization of the resulting adduct to form a tetrahydrofuran ring was nearly as rapid, and therefore competitive; complete reaction occurred in <20 min. Results for dihydrosalinosporamide A (**2**) were quite similar to those obtained with **1**. In marked contrast, we found that while β-lactone opening of the 2-(3-chloropropyl) analog **16** was rapid, no evidence for any subsequent second cyclization was observed even after 48 h. Furthermore, and importantly, no evidence for hydrolysis of the acylated, non-cyclized product was observed within 48 h.

Our mass spectrometry studies confirm the established mechanism of inhibition of the proteasome by salinosporamide A (**1**, Fig. 3) and provide for the first time detail concerning the relative rates of the first and second steps, which are comparable under the conditions of our experiment. Interestingly, the 2-(3-chloropropyl) analog **16** rapidly acylates the proteasome, but shows no evidence for second-step cyclization nor hydrolysis, which would re-activate the proteasome. In this regard, analog **16** behaves much like

omuralide[2a] and non- or slowly cyclizing analogs of salinosporamide such as 2-(2-fluoroethyl) and 2-*n*-propyl derivatives reported by Potts and co-workers.[6e,17]

Acknowledgments

Financial support from the NIH (CA-047148) is gratefully acknowledged. We acknowledge Stona Jackson for his contribution of entry 1, Table 1. We wish to thank Dr. Shao-Liang Zheng for X-ray crystallographic analyses and Dr. Sridevi Ponduru, Mr. Ronald Paranal and Professor James Bradner for their assistance with proteasomal inhibition assays. We also express our gratitude to Dr. Jeremy Wolff and Dr. Sunia Trauger for their mass spectrometry expertise and Bruker Daltonics for use of their 12T FTMS. We would also like to thank Prof. E. J. Corey for his contribution in providing an authentic sample of salinosporamide A.

Supplementary data

Supplementary data associated (experimental procedures and spectroscopic data for all intermediates) with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.09.066.

References and notes

1. Feling, R. H.; Buchanan, G. O.; Mincer, T. J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Angew. Chem., Int. Ed.* **2003**, *42*, 355.
2. (a) Fenteany, G.; Standaert, R. F.; Lane, W. S.; Choi, S.; Corey, E. J.; Schreiber, S. L. *Science* **1995**, ***268***, 726; (b) Macherla, V. R. et al *J. Med. Chem.* **2005**, *48*, 3684.
3. Reddy, L. R.; Saravanan, P.; Corey, E. J. *J. Am. Chem. Soc.* **2004**, *126*, 6230.
4. Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677.
5. (a) Endo, A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2005**, *127*, 8298; (b) Mulholland, N. P.; Pattenden, G.; Walters, I. A. S. *Org. Biomol. Chem.* **2006**, *4*, 2845; (c) Caubert, V.; Langlois, N. *Tetrahedron Lett.* **2006**, *47*, 4473; (d) Ling, T.; Macherla, V. R.; Manam, R. R.; McArthur, K. A.; Potts, B. C. M. *Org. Lett.* **2007**, *9*, 2289; (e) Ma, G.; Nguyen, H.; Romo, D. *Org. Lett.* **2007**, *9*, 2143; (f) Takahashi, K.; Midori, M.; Kawano, K.; Ishihara, J.; Hatakeyama, S. *Angew. Chem., Int. Ed.* **2008**, *47*, 6244; (g) Fukuda, T.; Sugiyama, K.; Arima, S.; Harigaya, Y.; Nagamitsu, T.; Omura, S. *Org. Lett.* **2008**, *10*, 4239; (h) Margalef, I. V.; Rupnicki, L.; Lam, H. W. *Tetrahedron* **2008**, *64*, 7896; (i) Mosey, R. A.; Tepe, J. J. *Tetrahedron Lett.* **2009**, *50*, 295; (j) Struble, J. R.; Bode, J. W. *Tetrahedron* **2009**, *65*, 4957; (k) Momose, T.; Kaiya, Y.; Hasegawa, J.; Sato, T.; Chida, N. *Synthesis* **2009**, *17*, 2983; (l) Nguyen, H.; Ma, G.; Romo, D. *Chem. Commun.* **46, 2010**, 4803; (m) Kaiya, Y.; Hasegawa, J.; Momose, T.; Sato, T.; Chida, N. *Chem. Asian J.* **2011**, *6*, 209; (n) Satoh, N.; Yokoshima, S.; Fukuyama, T. *Org. Lett.* **2011**, *13*, 3028.
6. (a) Reddy, L. R.; Fournier, J.-F.; Reddy, B. V. S.; Corey, E. J. *J. Am. Chem. Soc.* **2005**, *127*, 8974; (b) Reddy, L. R.; Fournier, J.-F.; Reddy, B. V. S.; Corey, E. J. *Org. Lett.* **2005**, *7*, 2699; (c) Hogan, P. C.; Corey, E. J. *J. Am. Chem. Soc.* **2005**, *127*, 15386; (d) Eustaquio, A. S.; Moore, B. S. *Angew. Chem., Int. Ed.* **2008**, *47*, 3936; (e) Manam, R. R. et al *J. Med. Chem.* **2008**, *51*, 6711; (f) Nett, M.; Gulder, T. A. M.; Kale, A. J.; Hughes, C. C.; Moore, B. S. *J. Med. Chem.* **2009**, *52*, 6163; (g) Chen, Z.-H.; Wang, B.-L.; Kale, A. J.; Moore, B. S.; Wang, R.-W.; Qing, F.-L. *J. Fluorine Chem.* **2012**, *136*, 12.
7. Uno, H.; Baldwin, J. E.; Russell, A. T. *J. Am. Chem. Soc.* **1994**, *116*, 2139.
8. Matsumura, K.; Hashiguchi, S.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1997**, *119*, 8738.
9. Seebach, D.; Hungerbühler, E.; Naef, R.; Schnurrenberger, P.; Weidmann, B.; Züger, M. *Synthesis* **1982**, 138.
10. Otsubo, K.; Inanaga, J.; Yamaguchi, M. *Tetrahedron Lett.* **1987**, *28*, 4435.
11. Barrero, A. F.; Alvarez-Manzaneda, E. J.; Chahboun, R.; Meneses, R.; Romera, J. L. *Synlett* **2001**, 485.
12. Phenylacetaldehyde was not a suitable substrate in the Mukaiyama aldol reaction as it underwent preferential enolsilylation. Use of 2-(cyclohexa-2,5-dien-1-yl)acetaldehyde followed by oxidation with DDQ provided a functional equivalent.
13. Groll, M.; Huber, R.; Potts, B. C. M. *J. Am. Chem. Soc.* **2006**, *128*, 5136.
14. Promega Corporation. Proteasome-Glo™ Assay Systems Protocol. http://www.promega.com/~/media/Files/Resources/Protocols/Technical%20Bulletins/101/Proteasome-Glo%20Assay%20Systems%20Protocol.pdf (accessed Aug 2013).
15. Romo and co-workers synthesized homosalinosporamide A and evaluated it as an inhibitor for each of the three active sites of the human 20S proteasome. They report that it is of comparable potency to **1** except at the trypsin-like site, where it showed 3-fold lower potency Nguyen, H.; Ma, G.; Gladysheva, T.; Fremgen, T.; Romo, D. *J. Org. Chem.* **2011**, *76*, 2.
16. Rickardson, L.; Wickström, M.; Larsson, R.; Lövborg, H. *J. Biomol. Screen.* **2007**, *12*(2), 203.
17. Groll, M.; McArthur, K. A.; Macherla, V. R.; Manam, R. R.; Potts, B. C. *J. Med. Chem.* **2009**, *52*, 5420.