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A Concise Total Synthesis of Largazole, Solution Structure, and Some Preliminary Structure Activity Relationships

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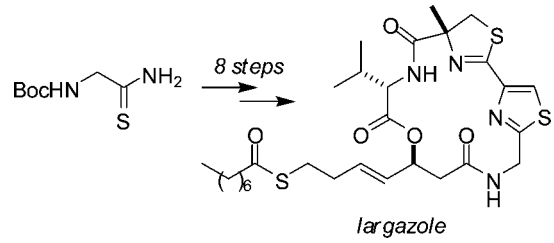
ABSTRACT



largazole

A total synthesis of largazole that proceeds in 8 steps from commercial materials is reported, along with some structure−activity relationships. A combination of NMR studies and molecular modeling have also provided a preliminary picture of the conformation of largazole.

As part of an investigation into the natural products chemistry of the marine cyanobacterium *Symploca* sp. collected in the Florida Keys, Leusch and co-workers recently described the isolation and structure elucidation of the depsipeptide largazole (**1**, Figure 1).[1] Largazole's structure is defined by the presence of an α-methylcysteine-derived thiazoline coupled to a thiazole embedded within a 16-membered macrocycle and the presence of a caprylic acid-derived thioester. Largazole's structural simplicity belies its potent antiproliferative activity against a number of cancer cell-lines including MDA-MB-231 mammary cells (GI_{50} 7.7 nM), U2OS fibroblastic osteosarcoma cells (GI_{50} 55 nM), HT29 colon cells (GI_{50} 12 nM), and IMR-32 neuroblastoma cells (GI_{50} 16 nM).

In broad connection with our interest in the synthesis of marine natural products,[2,3] we were attracted to largazole on the basis of biological activity and potential as a small-molecule natural product lead for the development of new therapeutics or cellular probes. Given these considerations, our overall synthesis plan was predicated on considerations of brevity and in particular a minimalistic protecting group strategy (Figure 1). In accord with this, introduction of the potentially delicate thioester by cross-metathesis as the final step of the synthesis and this analysis led ultimately to three fragments of comparable size: olefin **2**, ester **4**, and thiazoline-thiazole **5**. As well as providing plans for a very direct approach to largazole, this strategy is also amenable to the systematic variation of structure in search of improved activity. It should be noted that contemporaneous with our studies, Luesch and Hong also developed a total synthesis

(1) Kanchan, T.; Paul, V. J.; Luesch, H. *J. Am. Chem. Soc.* **2008**, *130*, 1806.

(2) (a) Hart, A. C.; Phillips, A. J. *J. Am. Chem. Soc.* **2006**, *128*, 1094. (b) O'Neil, G. W.; Phillips, A. J. *J. Am. Chem. Soc.* **2006**, *128*, 5340. (c) Chandler, C. L.; Phillips, A. J. *Org. Lett.* **2005**, *7*, 3493.

(3) For recent reviews of marine natural products synthesis see the following, as well as earlier installments in this series: Morris, J. C.; Phillips, A. J. *Nat. Prod. Rep.* **2008**, *25*, 95.

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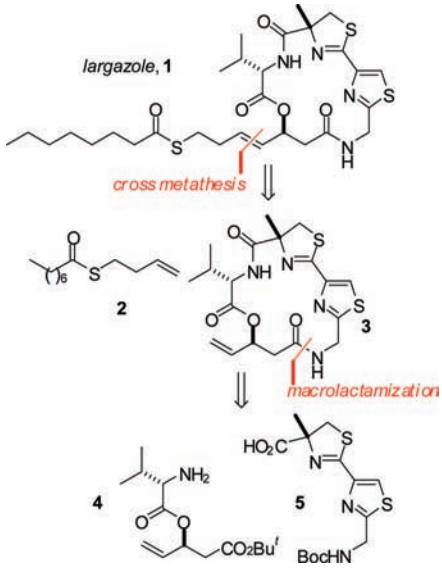
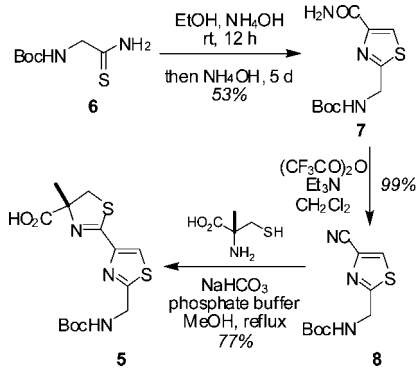


Figure 1. Largazole and overview of the synthesis plan.

that shares strategic similarities, although notably the macrocyclization occurs at different positions.[4]

The synthesis commenced with commercially available Boc-protected gylcine thioamide **6**, which was converted to thiazolyl amide **7** in 53% yield by a two-step, one-pot protocol consisting of a Hantzsch thiazole synthesis[5] with ethyl α-bromopyruvate followed by aminolysis of the ester with aqueous ethanolic ammonia (Scheme 1).[6] Dehydration

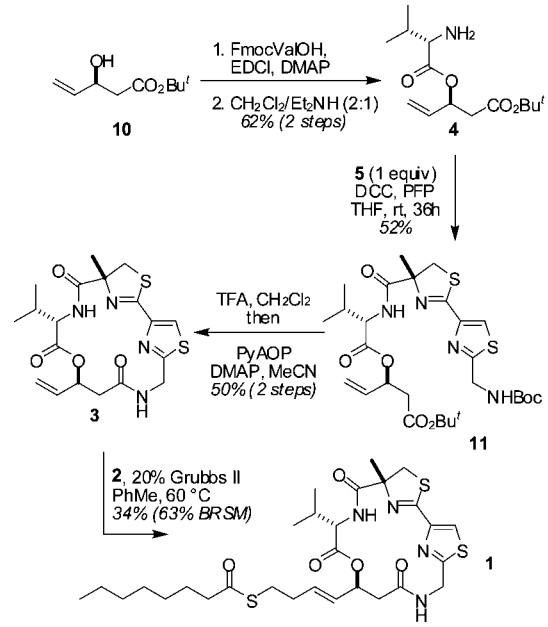
Scheme 1. Synthesis of the Thiazolylthiazoline Domain



of the amide with trifluoroacetic anhydride produced nitrile **8** in essentially quantitative yield,[7] and it proved possible to engage this nitrile directly with commercially available α-methylcysteine **9** to give thiazolylthiazoline **5** in 77% yield for these two steps.[8]

β-Hydroxy ester **10** (readily available by enzymatic resolution of the corresponding racemic aldol adduct[9]) served as the departure point for the valine-containing fragment and completion of the synthesis (Scheme 2). EDCI-mediated

Scheme 2. Completion of the Synthesis



coupling with FmocValOH and subsequent Fmoc removal with diethylamine gave **4** in 62% yield for the two steps. Coupling of this amine with thiazolylthiazoline **5** was accomplished with 1.4 equiv of **4**, using standard DCC/pentafluorophenol conditions to give **11** in 52% yield. A two-step protocol consisting of TFA-mediated removal of the Boc carbamate and subsequent macrolactamization with PyAOP[10] and DMAP in acetonitrile gave the macrocycle **3** in 50% yield for these two steps. A variety of other coupling reagents proved less successful: e.g., BOP 24% of **3**, PyBOP 30%, DEPBT 17%; HATU, HBTU, and DPPA all <5%; DCC/DMAP or EDCI/DMAP gave appreciable amounts of β-elimination of the acyloxy group. Subjecting a solution of **3** and **2** to 20 mol % of Grubbs' second-generation catalyst[11] resulted in smooth cross metathesis to directly yield largazole in 34% yield (63% yield based on recovered **3**). Synthetic largazole had spectroscopic data in accord with both the reported data and also an authentic sample. This route proceeds in 8 steps, the longest linear sequence from the commercially available thioamide **6**.

(4) Ying, Y.; Kanchan, T.; Kim, H.; Hong, J.; Luesch, H. *J. Am. Chem. Soc.* **2008**, *130*, 8455. The Hong-Luesch synthesis proceeds in 12 steps from commercial materials.

(5) Hantzsch, A. *Chem. Ber.* **1890**, *23*, 2339.

(6) Pfeiffer, T. *Ger. Offen. DE 19933861, Feb. 1*, 2001.

(7) Houssin, R.; Bernier, J.-L.; Hénichart, J.-P. *Synthesis* **1988**, 259.

(8) Bergeron, R. J.; Wiegand, J.; McManis, J. S.; Bharti, N. *J. Med. Chem.* **2006**, *49*, 7032.

(9) Tan, C.-H.; Holmes, A. B. *Chem. Eur. J.* **2001**, *7*, 1845.

(10) PyAOP = (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate. See: Carpino, L.; ElFaham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201.

(11) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953.

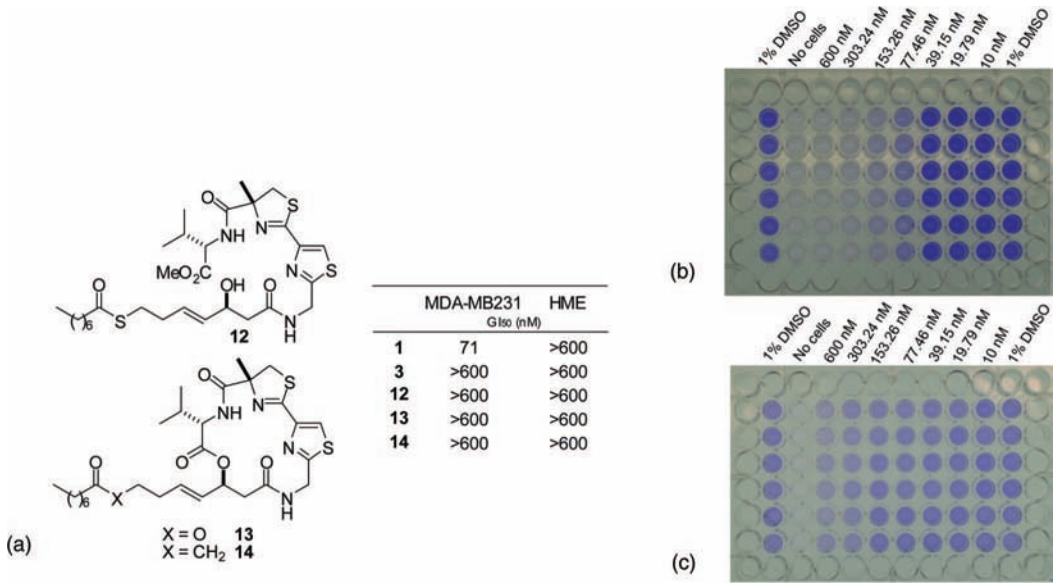


Figure 2. (a) Synthesized analogues and GI_{50} values against MDA-MB231 and HME cells; (b) MDA-MB231 cells + largazole; (c) HME cells + largazole. For details of the cellular proliferation assay see the Supporting Information.

With a route to largazole secured, it was possible to evaluate both the original claim of differential growth inhibition for transformed vs. nontransformed breast cells and also the effect of variation of the side chain on antiproliferative activity. Readily accessible analogues, such as **3**, *seco*-ester **12**, ester **13**, and ketone **14**, as well as synthetic largazole were tested against MDA-MB231 cells and nontransformed human mammary epithelial cells (HME). We choose the MDA-MB231 cell line in light of the data published in the isolation paper describing largazole's remarkable differential activity against transformed vs. nontranformed cells. We expected that the comparison between tumorigenic human breast cancer cells and non-transformed human mammary epithelial cells should be more meaningful than the original comparison utilizing mouse mammary epithelial cells (NMuMG). As can be seen in Figure 2, largazole inhibits the growth of MDA-MB231 cells with the GI_{50} of 71 nM. In contrast, the same compound had little effect on proliferation of HME cells (GI_{50} values of >600 nM). Even though the GI_{50} value of largazole on MDA-MB231 is higher than what was observed in the previous studies (7.7 nM), our results support the notion that largazole preferentially acts on tumor cells and this unique property of largazole could be exploited in antibreast cancer therapies in the future.

Of further interest is the degree to which the importance of the thioester/thiol domain is underscored by the testing of analogues **3**, **13**, and **14** in our cell proliferation assay. These data are in full accord with the studies by Luesch and Hong and support the notion that the cellular target of largazole is histone deacetylase. Also of note is the lack of activity of the *seco*-ester **12**. This presumably reflects the importance of the overall topology of the depsipeptide in terms of targeting, and also suggests a potential site for modifications to improve hydrolytic stability.

In light of the loss of activity of the *seco*-ester, we set out to study the conformation of largazole in $CDCl_3$ by NMR as a starting point for developing a three-dimensional picture of largazole's pharmacophore. NOESY spectra were collected at a variety of mixing times ranging from 150 to 700 ms.[12] Key transannular and long-range correlations that were used for Monte−Carlo conformational searching are shown in Figure 3.[13] The structures generated for largazole with these data all depict a relatively rigid and flat macrocycle, with the thiol-ester side chain and Val residue residing on opposite faces. In keeping with our observations above, cleavage of the ester (or any of the amides) could be expected to significantly change the overall molecular topology. Although it seems not likely to be relevant to the actual active compound, the acyl side chain assumes a position across the periphery of the macrocycle and shows some conformational mobility within the constraints provided.

(12) A 10.8 mg sample of largazole was dissolved in 700 μL of $CDCl_3$ and was rigorously degassed. Spectra were recorded at 20 °C on a Varian Inova 500 NMR spectrometer operating at 500.369 MHz for ^{1}H observation, using an inverse triple-resonance probe optimized for ^{1}H detection. The standard NOESY experiment (Bodenhausen, G.; Kogler, H.; Ernst, R. R. *J. Magn. Reson.* **1984**, *58*, 370) was used, with the addition of the use of pulsed field gradients, both to eliminate residual, unrelaxed magnetiztiion between transients, and also in the center of the mixing time to minimize unwanted artifacts due to incomplete phase-cycling to eliminate COSY-type cross-peaks (Wagner, R.; Berter, S. *J. Magn. Reson. Ser. A* **1996**, *123*, 119). The following parameters were used: data were aquired in phase-sensitive (States-TPPI) mode, using 90° excitation pulses = 9.0 μs, relaxation delay = 1.25 s, spectral width (f_1 and f_2) = 4203.7 Hz, mixing times ranging from 150 to 700 ms were used, acquisition time = 0.244 s in T2 (1024 complex points in the acquisition dimension), 128 increments were acquired in t_1, and linear-predicted (4×) to 512 points. The final spectrum consisted of 1024 complex points in both dimensions, after application of optimized gaussian apodiztion functions in t_1 and t_2.

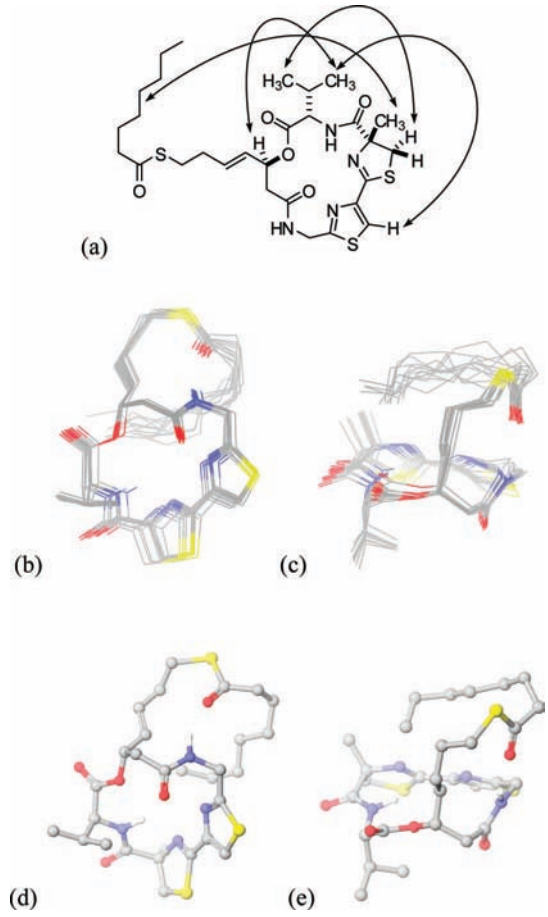


Figure 3. (a) Key transannular and long-range NOESY correlations and conformations for largazole based on NOESY constraints and Monte−Carlo conformational searching. Overlays of structures within 10 kJ mol^{-1} of the global minimum are shown in parts b and c, and the global minimum structure is illustrated in parts d and e.

In summary, we have described a very concise synthesis of largazole A that proceeds in 8 steps from commercial materials. Initial biological studies indicate the importance of the thioester, which is in accord with Luesch and Hong's studies which invoke cleavage of the thioester to produce a thiol that is the active inhibitor of HDACs. We have also been able to verify the differential activity of largazole against breast cancer cells, and a preliminary picture of largazole's conformation in $CDCl_3$ has been provided.

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Supporting Information Available: Experimental procedures, characterization data, and copies of spectra for compounds **2**→**8**, **10**→**14**, and largazole. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) All modeling was performed with MacroModel [Version 9.6, Schrodinger Inc, New York, NY, 2008]. Monte−Carlo searching [10 000 iterations with a 20 kJ/mol window for structure retention] and minimizations were run with the constraints shown in Figure 3. NOE data were used to provide qualitative restraints only (i.e., protons showing an NOE were constrained to be not greater than 5 Å apart). The Schrodinger OPLS_2005 force field, an enhanced version of Jorgensen's OPLS force field (Kaminski, G.A.; Friesner, R.A.; Tirado-Rives, J.; Jorgensen, W. J. *J. Phys. Chem. B* **2001**, *105*, 6474), was used along with GB/SA solvation, using the chloroform parameters for $CDCl_3$.