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Total Synthesis of Syringolin A

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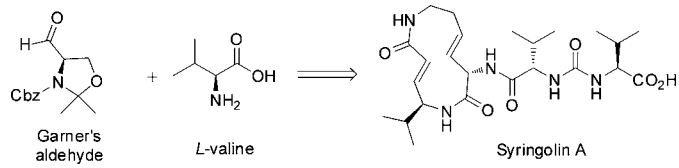
ABSTRACT



A convergent, efficient synthesis of syringolin A has been accomplished in 13 steps from commercially available materials, Garner's aldehyde and L-valine. The unnatural 3,4-dehydrolysine fragment was prepared using successive Johnson−Claisen/Curtius rearrangement reactions. The macrolactamization and late-stage introduction of the side chain will provide convenient access to analogues of this promising proteasome inhibitor.

Syringolin A was isolated from strains of the plant pathogen *Pseudomonas synringae pv Syringae* (*Pss*) in 1998.[1] It possesses a unique 12-membered dipeptide ring structure containing two (*E*)-configured double bonds and a urea side chain. It was initially reported to induce death of hypersensitive cells colonized by powdery mildew in wheat and other cereals[2] and more recently to inhibit cell proliferation and induce apoptosis in neuroblastoma and ovarian cancer cells.[3] Studies revealed that syringolin A inhibited both p53 wild-type NB cell lines SK-N-SH and the p53 mutant NB cell line LAN-1 with IC_{50} values between 20 and 25 μM. Furthermore, it was shown to selectively inhibit all three catalytic activities of eukaryotic proteasomes by covalent modification of a threonine residue in the active site.[4] The proteasome is essential for protein degradation[5,6] and has recently been validated in the clinic as a biological target for the treatment of multiple myeloma with the launch of bortezomib by Millennium Pharmaceuticals (now Takeda Oncology).[7] Unfortunately, expansion of the clinical utility of bortezomib beyond multiple myeloma has yet to be achieved, and its use has been limited by poor pharmacological properties, a requirement for intravenous administration, neurotoxicity, and selectivity for only one of the three enzymatic functions of the 20S proteasome.[8] Therefore, the development of new proteasome inhibitors that display improved activity and improved properties has been under intense investigation. Given the promising bioactivities and unique structure of syringolin A, it has drawn considerable attention to investigate its synthesis along with evaluation of the biological activity of its analogues.[9,10]

To synthesize syringolin A and its analogues for biological evaluation, we sought a convergent and flexible approach utilizing commercially available amino acids to introduce all of the stereocenters (Figure 1). Meanwhile, we also

(1) (a) Wäspi, U.; Blanc, D.; Winkler, T.; Ruedi, P.; Dudler, R. *Mol. Plant-Microbe Interact.* **1998**, *11*, 727. (b) Wäspi, U.; Hassa, P.; Staempfli, A. A.; Molleyres, L. P.; Winker, T.; Dudler, R. *Microbiol. Res.* **1999**, *154*, 89.

(2) Wäspi, U.; Schweizer, P.; Dudler, R. *Plant Cell* **2001**, *13*, 153.

(3) Coleman, C. S.; Rocetes, J. P.; Park, D. J.; Wallick, C. J.; Warn-Cramer, B. J.; Michel, K.; Dudler, R.; Bachmann, A. S. *Cell Proliferation* **2006**, *39*, 599.

(4) Groll, M.; Schellenberg, B.; Bachmann, A. S.; Archer, C. R.; Huber, R.; Powell, T. K.; Lindow, S.; Kaiser, M.; Dudler, R. *Nature* **2008**, *452*, 755.

(5) (a) Ciechanover, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 5944. (b) Goldberg, A. L. *Biochem. Soc. Trans.* **2007**, *35*, 12.

(6) For a discussion of the proteasome as a target for cancer chemotherapy, see: Almond, J. B.; Cohen, G. M. *Leukemia* **2002**, *16*, 433.

(7) (a) Adams, J. *Cancer Cell* **2004**, *5*, 417. (b) Kyle, R. A.; Rajkumar, S. V. *N. Engl. J. Med.* **2004**, *351*, 1860. (c) Rajkumar, S. V.; Richardson, P. G.; Hideshima, T.; Anderson, K. C. *J. Clin. Oncol.* **2005**, *23*, 630. (d) Borissenko, L.; Groll, M. *Chem. Rev.* **2007**, *107*, 687.

(8) Chauhan, D.; Hideshima, T.; Anderson, K. C. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 465.

(9) (a) Clerc, J.; Groll, M.; Illich, D. J.; Bachmann, A. S.; Huber, R.; Schellenberg, B.; Dudler, R.; Kaiser, M. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 6507. (b) Clerc, J.; Schellenberg, B.; Groll, M.; Bachmann, A. S.; Huber, R.; Dudler, R.; Kaiser, M. *Eur. J. Org. Chem.* **2010**, published online May 19, 2010; DOI: 10.1002/ejoc.201000317.

(10) Pirrung, M. C.; Biswas, G.; Ibarra-Rivera, T. R. *Org. Lett.* **2010**, *12*, 2402.

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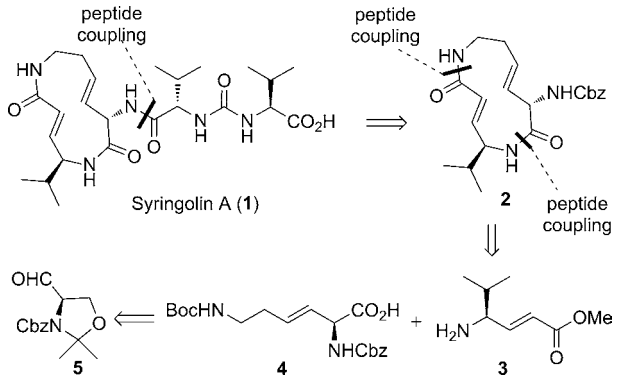


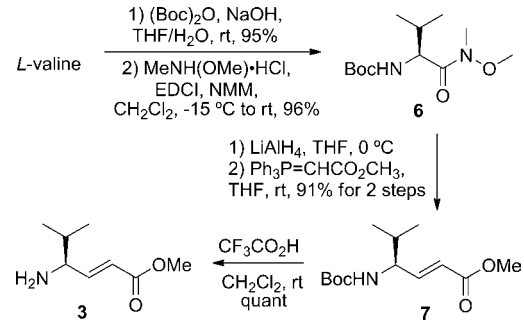
Figure 1. Retrosynthetic analysis of syringolin A.

envisioned introduction of the side chain at a late stage, which would expedite the synthesis of other analogues. The key 12-membered macrocycle core **2** would be formed through an intramolecular peptide coupling at the less hindered position. Horner−Wadsworth−Emmons olefination would provide the α,*β*-unsaturated ester **3**. The (*E*)-olefin of **4** could be furnished by Johnson−Claisen rearrangement, and its precursor could be generated from easily accessible Garner's aldehyde **5**.[11] Herein, we report the successful execution of this strategy to the enantioselective synthesis of syringolin A.

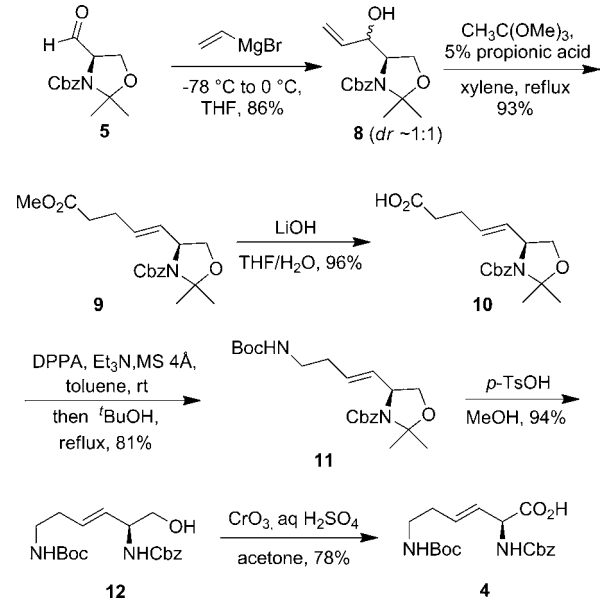
The synthesis of the α,*β*-unsaturated ester **3** began with Boc protection of L-valine, followed by coupling with *N*,*O*-dimethylhydroxylamine to afford Weinreb amide **6**.[12] Reduction of the amide to the corresponding aldehyde with $LiAlH_4$, followed by immediate subjection of the crude material to Horner−Wadsworth−Emmons olefination provided the (*E*)-olefin **7** in 91% yield and >99% ee.[13,14] Removal of the *N*-Boc protecting group provided amine **3** in quantitative yield (Scheme 1).

To access the 3,4-dehydrolysine fragment,[15] Garner's aldehyde **5**, prepared from D-serine according to the literature procedure,[11] was treated with vinyl magnesium bromide in THF for 30 min at −78 °C and then for 2 h at room temperature to afford vinyl alcohol **8** in 86% yield. Johnson−Claisen rearrangement employing excess $CH_3C(OMe)_3$ in xylene at reflux provided the desired α,*β*-unsaturated ester **9** in high yield and excellent stereoselectivity (*E*/*Z* > 95:5).[16] Hydrolysis led to the carboxylic acid **10**, which was subjected to Curtius rearrangement using DPPA, Et_3N, and *tert*-butanol in the presence of activated 4 Å molecular sieves. The use of sieves was found to be crucial in this transformation to avoid the formation of the corresponding symmetrical urea. Intermediate **11** was obtained with 81% yield and then selectively deprotected using *p*-TsOH to afford alcohol **12**. Subsequent oxidation to corresponding acid using two-step protocols proved to be problematic because of the instability of the aldehyde intermediate.[17,18] Gratifyingly, after extensive studies on the oxidation conditions, we found that the Jones reagent efficiently converted alcohol **12** to the desired amino acid **4** in 1 h at 0 °C in 78% yield (86% brsm) without any observable epimerization of the stereogenic center (Scheme 2).[19]

Scheme 1. Synthesis of Fragment **3**



Scheme 2. Synthesis of Fragment **4**



With both coupling fragments **3** and **4** in hand, the first peptide bond formation was accomplished using EDCI,

(11) Garner, P.; Park, J. M. *J. Org. Chem.* **1987**, *52*, 2361.

(12) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.

(13) The stereochemical integrity of the α-stereogenic center and % ee of **7** were determined by HPLC analysis.

(14) For seminal studies on the preparation and utility of vinylogous amino acids, see: (a) Hagihara, M.; Anthony, N. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6568. (b) Hagihara, M.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6570.

(15) During the preparation of this manuscript, Kaiser and coworkers described a similar approach to fragment **4**; see ref 9b.

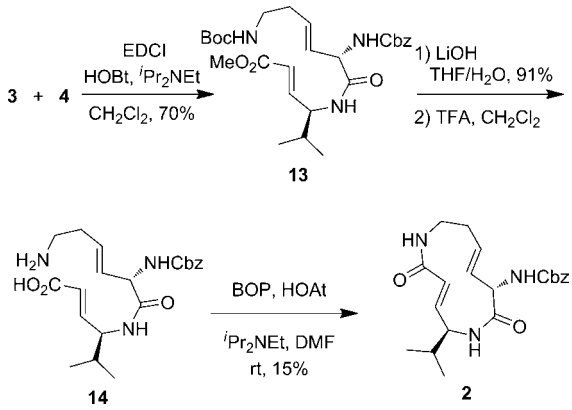
(16) *E*/*Z* selectivity of compound **9** was determined by analysis of the crude ^1H NMR.

(17) Decomposition and epimerization were always observed when handling the aldehyde. In contrast, the carboxylic acid was found to be quite stable and did not undergo noticeable epimerization .

(18) For an example of the lability of the products of oxidation of vinylglycinol derivatives, see: Campbell, A. D.; Taylor, R. J. K.; Raynham, T. M. *Chem. Commum.* **1999**, *24*, 245.

HOBt, and $^{i}Pr_2NEt$ in dichloromethane over 12 h to afford **13** in 70% yield. Removal of the C- and N-terminal protecting groups generated the macrolactamization precursor **14**, which was then treated with peptide coupling reagents BOP and HOAt in DMF for 48 h, providing the macrocycle **2**[20] in 15% yield (Scheme 3).[21] Although the yield of this

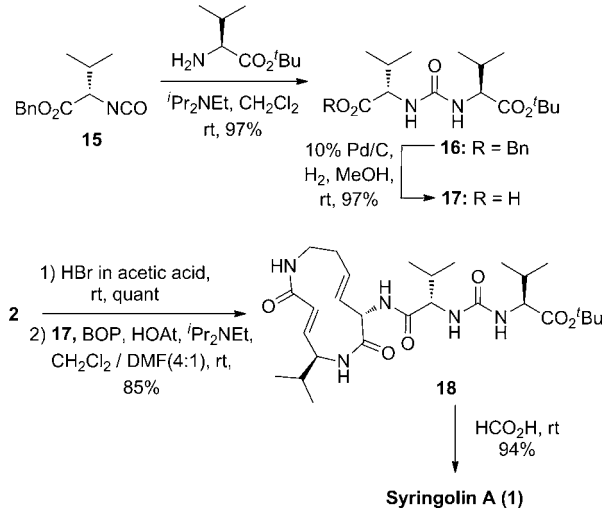
Scheme 3. Synthesis of Macrolactam **2**



macrolactamization is low, this approach provides the most straightforward approach to syringolin A and its analogues. Similar macrolactamization approaches to syringolin A have previously been reported to be unsuccessful.[9] However, the macrolactamization containing a lysine residue in place of 3,4-dehydrolysine has been reported to provide the macrolactam of syringolin B, in 30% yield.[9]

The final stage of the synthesis required introduction of the urea side chain **17**, which was obtained by treatment of benzyl (*S*)-(−)-2-isocyanato-3-methylbutyrate[22] with *tert*-butyl valine in the presence of $^{i}Pr_2NEt$,[23] followed by benzyl deprotection via hydrogenolysis with 10% Pd/C in methanol. Removal of Cbz group in macrocycle core **2** with HBr in acetic acid afforded the corresponding free amine in quantitative yield, which was then coupled with side chain **17** using BOP, HOAt, and $^{i}Pr_2NEt$ to afford **18** in 85% yield. The *tert*-butyl protecting group was cleaved under mild acidic condition using formic acid, affording syringolin A in 94% isolated yield (Scheme 4). The data for synthetic syringolin

Scheme 4. Completion of the Synthesis



A were identical in all respects with those previously reported for the natural product.[24]

In summary, utilizing commercially available amino acids, we have developed a convergent synthesis of the proteasome inhibitor syringolin A in 13 steps from Garner's aldehyde. In addition, it demonstrates that the macrolactamization of the 12-membered cycle core can be accomplished via peptide coupling with reasonable yield, which will allow for facile modification of both the macrocycle and side chain. Studies directed toward the preparation of analogues of syringolin A and their evaluation as proteasome and protease inhibitors are currently underway and will be reported in due course.

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Supporting Information Available: Experimental procedures and ^{1}H and ^{13}C NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) The enantiomeric purity of compound **4** was determined by its coupling with (*S*)-(−)-α-methylbenzylamine (EDCI, HOBt) and analysis of the crude ^{1}H NMR as compared with the coupling of **4** with (±)-α-methylbenzylamine. See Supporting Information for further details.

(20) This intermediate was also prepared in the recently reported synthesis by Pirrung and coworkers using a Horner−Wadsworth−Emmons macrocyclization. See ref 10 for details.

(21) Analysis of the crude UPLC-MS data indicated that dimeric and trimeric products are also formed during the formation of the strained 12-membered macrocycle.

(22) The isocyanate was prepared using triphosgene; see: Sunder-Plassmann, N.; Sarli, V.; Gartner, M.; Utz, M.; Seiler, J.; Huemmer, S.; Mayer, T. U.; Surrey, T.; Giannis, A. *Biorg. Med. Chem.* **2005**, *13*, 6094.

(23) Majer, P.; Randad, R. S. *J. Org. Chem.* **1994**, *59*, 1937.

(24) Data for synthetic syringolin A (**1**). R_f (85:15 CH_2Cl_2/MeOH, 2% v/v AcOH): 0.28; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.5 (br s, 1 H), 7.98−8.06 (m, 2 H), 7.47 (t, J = 7.2 Hz, 1 H), 6.68 (dd, J = 15.5, 5.5 Hz, 1 H), 6.29 (d, J = 8.6 Hz, 1 H), 6.25 (d, J = 9.0 Hz, 1 H), 6.09 (d, J = 15.2 Hz, 1 H), 5.59 (dt, J = 15.8, 7.6 Hz, 1 H), 5.41 (dd, J = 15.6, 7.8 Hz, 1 H), 4.85 (m, 1 H), 4.02−4.12 (m, 2 H), 3.94 (dd, J = 8.8, 4.9 Hz, 1 H), 3.08−3.25 (m, 2 H), 2.23−2.32 (m, 1 H), 1.86−2.02 (m, 3 H), 1.68−1.78 (m, 1 H), 0.94 (d, J = 6.7 Hz, 3 H), 0.90 (d, J = 6.7 Hz, 3 H), 0.82−0.87 (m, 9 H), 0.77 (d, J = 6.9 Hz, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.5, 171.6, 168.9, 166.3, 157.6, 143.3, 133.0, 126.1, 121.5, 58.2, 57.6, 55.5, 53.6, 42.5, 35.0, 31.5, 30.9, 30.6, 19.8, 19.4, 19.3, 19.2, 17.8, 17.6; HRMS (ESI) *m/z* calcd for $C_{24}H_{39}N_5O_6$ $[M + H]^+$ 494.2979, found 494.3003.