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A total synthesis of the ammonium ionophore, (−)-enniatin B

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ABSTRACT

A nine-step (longest linear) batch total synthesis of the cyclic hexadepsipeptide (−)-enniatin B is described. The synthesis minimizes precipitation during reaction conditions for adaptability to flow synthesis. The route was used to prepare >100 mg of the natural product.

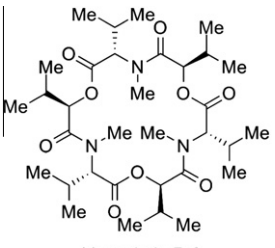
(−)-Enniatin B (**1**, Fig. 1) is a cyclohexadepsipeptide fungal product known to possess various antibiotic,[1] cytotoxic,[2] and ion-transport[3] activities. It has also been investigated for its potential as an anti-HIV[4] and anti-cancer agent,[5] the latter due to its recently discovered new mechanisms of pro-apoptotic activity.[6] Additionally, as a chemically stable mycotoxin, enniatin B serves as an indicator of fungal contamination of food grains,[7] and is thus required as an analytical standard worldwide.[8] Small quantities of enniatin B for such diverse research purposes are commercially available through fermentation, albeit though at high cost (~$200 USD per milligram). A simple chemical synthesis of enniatin B to provide both material and potential analogs is therefore desirable.

In our laboratories, we have been investigating methods for the flow chemical synthesis of natural products.[9–11] Despite their simple appearance, the syntheses of cyclodepsipeptides such as enniatin B are known to be difficult to automate with solid-phase chemistry:[12] depsipeptide bonds require several equivalents of the expensive hydroxyester component to drive on-resin chain extension to completion,[13] *N*-methyl amines are known to be very poor nucleophilic coupling partners,[14] and *N*-methyl amides are known to cause difficulties such as epimerization during chain extension due to the rapid formation of oxazolones.[14] We therefore wished to apply newly developed flow technologies to automate the synthesis of enniatin-type cyclodepsipeptides in the solution phase. To study the application of our flow technologies, we first needed to define a batch synthesis that would minimize the risk of in-line precipitation events throughout the sequence.[15–17]

Previous syntheses of enniatin B were completed simultaneously in 1963 by Shemyakin[18,19] and Plattner[20] using PCl_5 and HBr-mediated couplings. Another synthesis of enniatin B was reported in 2006 employing Mitsunobu reactions to form the required ester bonds and a silver-mediated amide trimethylation process for the final step.[21] Neither route is ideally suited for flow chemistry, due to precipitation and difficult intermediate purification steps. We have therefore developed an improved batch synthesis, disclosed herein.

In initial studies, several attempts to couple fragments **2** and **3** failed to provide desired tetramer **4**, evidently due to rapid formation of oxazolonium cation **5** (Scheme 1).

We thus opted to reverse the order of bond formation to prevent amide-induced deactivation of the necessary acid coupling components. EDCI coupling of acid **8**[22] and alcohol **7** cleanly provided depsipeptide **9** (Scheme 2). In the coupling step, it was important to use stoichiometric DMAP to minimize the formation



(-)-enniatin B **1**

Figure 1. (−)-Enniatin B (**1**), a fungal cyclohexadepsipeptide.

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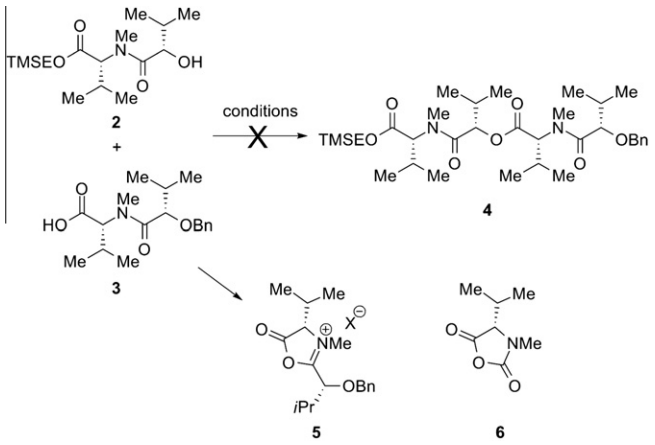
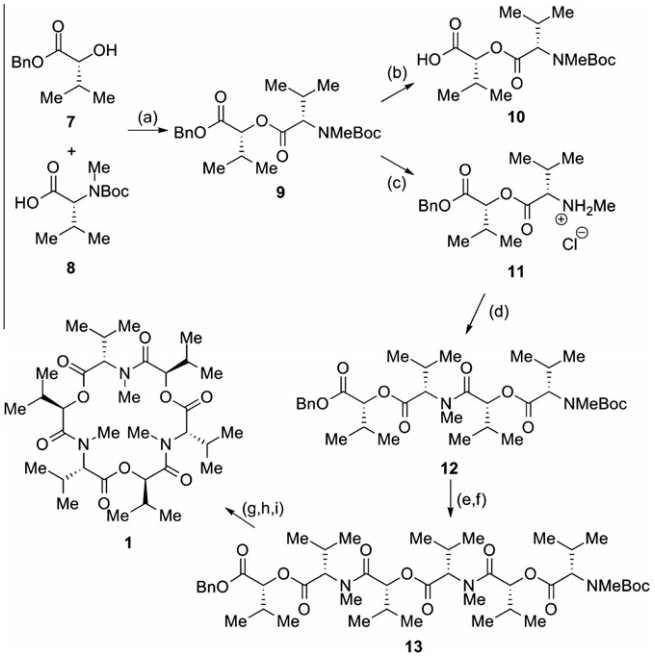


Scheme 1. Initial coupling partners **2** and **3** failed to provide product **4** due to rapid formation of oxazalonium cation **5**; anhydride **6** resulted from a similar interaction with the Boc-protecting group in acid **8.**



Scheme 2. Total synthesis of (−)-enniatin B. Reagents and conditions: (a) EDCI, DMAP (1.3 equiv), CH_2Cl_2 (82%); (b) H_2, Pd/C, EtOAc (100%) (c) 4 M HCl/1,4-dioxane (100%), then vacuum; (d) **10,** Ghosez's reagent, DIPEA, CH_2Cl_2 (91%); (e) H_2, Pd/C, EtOAc (100%); (f) **11**, Ghosez's reagent, DIPEA, CH_2Cl_2 (69%, 86% based on recovered starting material); (g) H_2, Pd/C, EtOAc (88%); (h) 4 M HCl/1,4-dioxane, then vacuum (100%); (i) Ghosez's reagent, then DIPEA, 0.005 M CH_2Cl_2 (56%). Ghosez's reagent = 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine, DMAP = 4-dimethylaminopyridine, DIPEA = diisopropylethylamine.

of anhydride **6** (Scheme 1). Hydrogenolysis and acid-catalyzed deprotection of **9** provided acid **10** and amine salt **11** respectively, which were then coupled using Ghosez's chloroenamine reagent[23,24] to provide tetradepsipeptide **12**. Tetradepsipeptide **12** was subjected to hydrogenolysis and coupled to a second equivalent of amine salt **11** to provide hexadepsipeptide **13**. Global deprotection of **13** by hydrogenolysis and acidification provided a linear amino acid HCl salt[25] which was cyclized with Ghosez's reagent with the assistance of a tertiary amine base to provide synthetic (−)-**1**, which was identical in all respects to a sample of enniatin B.[26]

In summary, a convenient batch total synthesis of (−)-enniatin B from (2*R*)-2-hydroxy-3-methylbutanoic acid has been described (nine steps, longest linear, 15% yield overall). Notably, only two chromatography steps were necessary in the entire sequence beginning from L-valine and no precipitation from reaction solution was observed during the reaction sequence. Our efforts to harness this sequence using flow technology and biological investigations on the natural product will be disclosed in due course.

Acknowledgments

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Supplementary data

Supplementary data (detailed experimental procedures, compound characterization, and original ^1H and ^{13}C NMR spectra of all compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.05.110.

References and notes

1. (a) Shemyakin, M. M.; Ovchinnikov, Y. A.; Ivanov, V. T.; Kiryushkin, A. A.; Zhdanov, G. L.; Ryabova, I. D. *Experientia* **1963**, *19*, 566–568; (b) Bahdury, P.; Mohammad, B. T.; Wright, P. C. *J. Ind. Microbiol. Biot.* **2005**, *33*, 325–337; (c) Meca, G.; Soriano, J. M.; Gaspari, A.; Ritieni, A.; Moretti, A.; Manes, J. *Toxicon* **2010**, *56*, 480–485.
2. (a) Ivanova, L.; Skjerve, E.; Eriksen, G. S.; Uhlig, S. *Toxicon* **2006**, *47*, 868–876; (b) Tonshin, A. A.; Teplova, V. V.; Andersson, M. A.; Salkinoja-Salonen, M. S. *Toxicology* **2010**, *276*, 49–57; (c) Meca, G.; Sospedra, I.; Valero, M. A.; Manes, J.; Font, G.; Ruiz, M. *J. Toxicol. Mech. Method* **2011**, *21*, 503–512; (d) Fornelli, F.; Minervini, F.; Logrieco, A. *J. Invertebr. Pathol.* **2004**, *85*, 74–79.
3. (a) Haynes, D. H.; Wiens, T.; Pressman, B. C. *J. Membr. Biol.* **1974**, *18*, 23–38; (b) Benz, R. *J. Membr. Biol.* **1978**, *43*, 367–394.
4. McKee, T. C.; Bokesch, H. R.; McCormick, J. L.; Rashid, M. A.; Spielvogel, D.; Gustafson, K. R.; Alavanja, M. M.; Cardellina, J. H.; Boyd, M. R. *J. Nat. Prod.* **1997**, *60*, 432–438.
5. Meca, G.; Font, G.; Ruiz, M. *J. Food Chem. Toxicol.* **2011**, *49*, 2464–2469. and references cited therein..
6. (a) Watjen, W.; Debbab, A.; Hohlfeld, A.; Chouvolou, Y.; Kampkotter, A.; Edrada, R. A.; Ebel, R.; Hakiki, A.; Mosaddak, M.; Totzke, F.; Kubbutat, M. H. G.; Proksch, P. *Mol. Nutr. Food Res.* **2008**, *53*, 431–440; (b) Dornetshuber, R.; Heffeter, P.; Kamyar, R.; Berger, W.; Lemmens-Gruber, R. *Toxicol. Lett.* **2006**, *164S*, S250.
7. Sorensen, J. L.; Nielsen, K. F.; Rasmussen, P. H.; Thrane, U. *J. Agric. Food Chem.* **2008**, *56*, 10439–10443.
8. Italy: (a) Castoria, R.; Lima, G.; Ferracane, R.; Ritieni, A. *J. Food Protect.* **2005**, *68*, 416–420; Norway: (b) Uhlig, S.; Torp, M.; Heier, B. T. *Food Chem.* **2006**, *94*, 193–201; Nigeria: (c) Adejumo, T. O.; Hettwer, U.; Karlovsky, P. *Food Addit. Contam.* **2007**, *24*, 993–1000; Denmark: (d) Sorensen, J. L.; Nielsen, K. F.; Rasmussen, P. H.; Thrane, U. *J. Agric. Food Chem.* **2008**, *56*, 10439–10443; Germany: (e) Goertz, A.; Zuehlke, S.; Spiteller, M.; Steiner, U.; Dehne, H. W.; Waalwijk, C.; Vries, I.; Oerke, E. *Eur. J. Plant. Pathol.* **2010**, *128*, 101–111; Spain: (f) Meca, G.; Zinedine, A.; Blesa, J.; Font, G.; Manes, J. *Food Chem. Toxicol.* **2010**, *48*, 1412–1416; Czech Rep.: (g) Malachova, A.; Dzuman, Z.; Veprikova, Z.; Vaclavikova, M.; Zachariasova, M.; Hajslova, J. *J. Agric. Food Chem.* **2011**, *59*, 12990–12997; Morocco: (h) Sifou, A.; Meca, G.; Serrano, A. B.; Mahnine, N.; El Abidi, A.; Manes, J.; El Azzouzi, M.; Zinedine, A. *Food Control* **2011**, *22*, 1826–1830.
9. Baxendale, I. R.; Griffiths-Jones, C. M.; Ley, S. V.; Tranmer, G. K. *Synlett* **2006**, 427–430.
10. Baxendale, I. R.; Deeley, J.; Griffiths-Jones, C. M.; Ley, S. V.; Saaby, S.; Tranmer, G. K. *Chem. Commun.* **2006**, 2566–2568.
11. (a) Baumann, M.; Baxendale, I. R.; Brasholz, M.; Hayward, J. J.; Ley, S. V.; Nikbin, N. *Synlett* **2011**, 1375–1380; (b) Hodgkinson, J. T.; Galloway, W. R. J. D.; Saraf, S.; Baxendale, I. R.; Ley, S. V.; Ladlow, M.; Welch, M.; Spring, D. R. *Org. Biomol. Chem.* **2011**, *9*, 57–61.
12. Scherkenbeck, J.; Lüttenberg, S.; Ludwig, M.; Brücher, K.; Kotthaus, A. *Eur. J. Org. Chem.* **2012**, 1546–1553.
13. Albericio, F.; Burger, K.; Ruiz-Rodriguez, J.; Spengler, J. *Org. Lett.* **2005**, *7*, 597–600. and references cited therein..
14. Teixido, M.; Albericio, F.; Giralt, E. *J. Pept. Res.* **2005**, *65*, 153–166.
15. Slurries and bulk solids can be handled in laboratory flow reactors with agitating reactors or ultrasound, (see Ref.[16]) or monoliths (see Ref.[17]); precipitation events which generate small particles out of homogenous solution, however, bypass filters, damage pumps, and cause clogging in thin flow lines during multi-step synthesis.
16. (a) Sedelmeier, J.; Ley, S. V.; Baxendale, I. R.; Baumann, M. *Org. Lett.* **2010**, *12*, 3618–3621; (b) Noel, T.; Naber, J. R.; Hartman, R. L.; McMullen, J. P.; Jensen, K. F.; Buchwald, S. L. *Chem. Sci.* **2011**, *2*, 287–290; (c) Browne, D. L.; Deadman, B. J.; Ashe, R.; Baxendale, I. R.; Ley, S. V. *Org. Proc. Res. Dev.* **2011**, *15*, 693–697.

17. Baumann, M.; Baxendale, I. R.; Ley, S. V.; Nikbin, N.; Smith, C. D. *Org. Biomol. Chem.* **2008**, *6*, 1587–1593.
18. Shemyakin, M. M.; Ovchinnikov, Y. A.; Kiryushkin, A. A.; Ivanov, V. T. *Izv. Akad. Nauk SSSR: Ser. Khim.* **1963**, *9*, 1623–1630.
19. Shemyakin, M. M.; Ovchinnikov, Y. A.; Kiryushkin, A. A.; Ivanov, V. T. *Tetrahedron. Lett.* **1963**, *14*, 885–890.
20. Plattner, P. A.; Vogler, K.; Studer, R. O.; Quitt, P.; Keller-Schierlein *Helv. Chim. Acta* **1963**, *98*, 927–935.
21. Nakatsu, S.; Yamada, K.; Oku, H.; Katakai, R. Proceedings of the International Conference of the 43rd Japanese Peptide Symposium and 4th Peptide Engineering Meeting 2006, 66–67.
22. Kogan, T. P.; Somers, T. C.; Venuti, M. C. *Tetrahedron* **1990**, *46*, 6623–6632.
23. Ghosez, L.; Haveuaux, B.; Viehe, H. G. *Angew. Chem., Int. Ed.* **1969**, *8*, 454–455.
24. Ghosez, L.; George-Koch, I.; Patiny, L.; Houtekie, M.; Bovy, P.; Nshimyumukiza, P.; Phan, T. *Tetrahedron* **1998**, *54*, 9207–9222.
25. The use of the linear amino acid's HCl salt in the macrocyclization step was important in protecting the N-terminus from premature reaction with Ghosez's reagent.
26. One milligram from Sigma–Aldrich Corporation, from Gnomonia errabundia; see Supplementary data.