ORGANIC LETTERS

2006
Vol. 8, No. 24
5601−5604

Verticilide: Elucidation of Absolute Configuration and Total Synthesis

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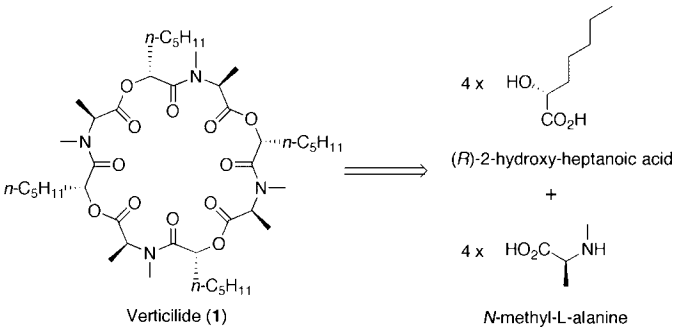
Received September 22, 2006

ABSTRACT



Verticilide (1) is a 24-membered cyclic depsipeptide isolated from the culture broth of *Verticillium* sp. FKI-1033. It inhibits ryanodine binding to ryanodine receptor (RyR) and has insecticidal activity. The stereochemistry of 2-hydroxyheptanoic acid in verticilide was elucidated by chiral HPLC analysis of the degradation product 6 and synthetic (+) and (–)-6. We also describe the practical total synthesis of verticilide.

A plant alkaloid, ryanodine, is an agonist of ryanodine receptor (RyR) and exhibits insecticidal activity. RyR is a Ca^{2+}-release channel of the sarcoplasmic reticulum. There are three isoforms, RyR1, RyR2, and RyR3, in mammalian RyR. However, insects have a single but distinct RyR. Therefore, insect RyR is a potential target for new insecticides.[1] In the course of screening for ryanodine binding inhibitors, verticilide **1** was isolated from the culture broth of *Verticilium* sp. FKI-1033[2] (Figure 1). Verticilide **1** is a new 24-membered cyclic depsipeptide consisting of four 2-hydroxyheptanoic acids and four *N*-methyl-L-alanines. Verticilide **1** selectively inhibited the binding of ryanodine to insect RyR with an IC_{50} value of 4.2 μM (mammalian RyR IC_{50} = 54 μM). Verticilide **1** is expected to be a potential lead compound for insecticides with a new mode

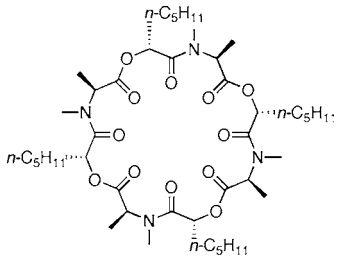


Figure 1. Structure of verticilide (**1**).

(1) (a) Fill, M.; Copello, J. A. *Physiol. Rev.* **2002**, *82*, 893−922. (b) Schmitt, M.; Turberg, A.; Londershausen, M; Dorn, A. *Pestic. Sci.* **1996**, *48*, 375−378. (c) Xu, X.; Bhat, M. B.; Nishi, M.; Takeshima, H.; Ma, J. *Biophys. J.* **2000**, *78*, 1270−1281.

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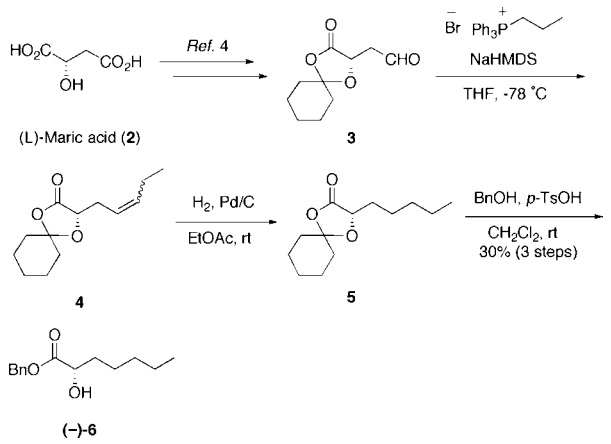
10.1021/ol0623365 CCC: $33.50
Published on Web 10/31/2006

of action. However, the stereochemistry of 2-hydroxyheptanoic acid in **1** could not be elucidated by spectroscopic analysis because of the small value of $[\alpha]_D$.[3]

A practical method for synthesis of **1** is required to allow further biological evaluation, and the synthesis of its analogs is impotant for the study of the structure−activity relationships. Herein we report the elucidation of stereochemistry of 2-hydroxyheptanoic acid by chiral HPLC analysis and an efficient total synthesis of verticilide **1**, which can be applied for scaled-up production of verticilide.

In investigating the stereochemistry of 2-hydroxyheptanoic acid and to confirm the absolute configulation of **1**, we synthesized the enantiomers of 2-hydroxyheptanoic acid, which can be prepared from L- and D-malic acids, respectively, and compared them with a fragment obtained by degradation of natural product directly. Synthesis of (*S*)-2-hydroxyheptanoic acid benzyl ester **6** is summarized in Scheme 1.

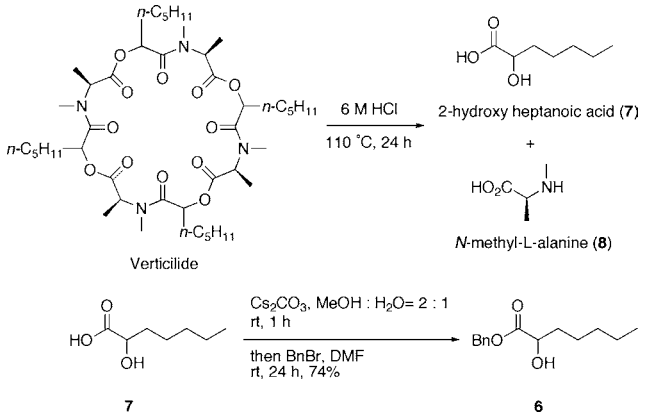
Scheme 1. Preparation of (*S*)-2-Hydroxyheptanoic Acid **(−)-6**



l-Malic acid **2** was converted into aldehyde **3** using a method reported by Dutton.[4] Wittig olefination of **3** with *n*-propyltriphenylphosphonium bromide[5] and NaHMDS gave **4**, which was used without further purification because of its instability. Hydrogenation of **4** followed by transesterification of **5** with benzyl alcohol and *p*-TsOH condition afforded (*S*)-2-hydroxyheptanoic acid benzyl ester (−)-**6**. Furthermore, we synthesized enantiomer (+)-**6** from D-malic acid using the same procedure.

Degradation of **1** in 6 M HCl at 110 °C afforded 2-hydroxyheptanoic acid **7** and *N*-methyl-L-alanine **8**, which was determined by amino acid analysis. Then, **7** was treated with Cs_2CO_3 and BnBr to provide benzyl ester **6** (Scheme 2). ^1H and ^{13}C NMR and MS spectral data of **6** from the natural product were found to be identical to those of synthetic **6**. The result of chiral HPLC analysis is shown in Figure 2.

Scheme 2. Resolution of Verticilide into Two Building Blocks



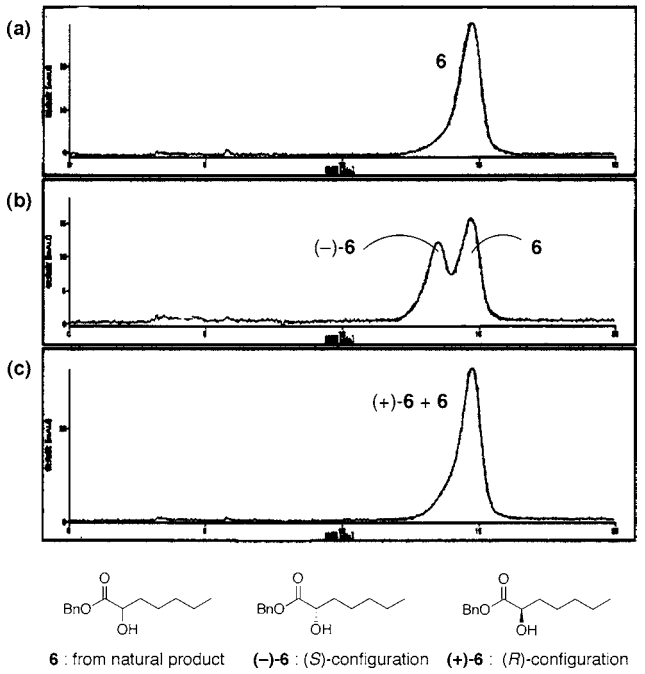


Figure 2. HPLC analysis of 2-hydroxyheptanoic acid. Column, Daicel OD10C; column temperature, 10 °C; eluent, *n*-hexane/2-propanol = 400:15; detection, UV 210 nm; flow rate, 1 mL/min. (a) Fragment **6** from natural product; (b) 1:1 mixture of fragment **6** and synthetic (−)-**6**; (c) 1:1 mixture of fragment **6** and synthetic (+)-**6**.

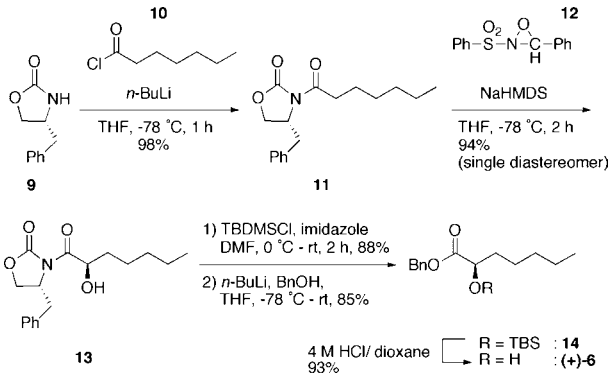
We could achieve separation of synthetic enantiomers (+)-**6** and (−)-**6** by using chiral HPLC. After several attempts, enantiomers (+)-**6** and (−)-**6** were separated by

(3) (a) Nugteren, D. H.; Van Dorp, D. A.; Bergstrom, S.; Hamburg, M.; Samuelsson, B. *Nature* **1966**, *212*, 38. (b) Hauser, F. M.; Coleman, M. L.; Huffman, R. C.; Carroll, F. I. *J. Org. Chem.* **1974**, *39*, 3426. (c) Townsend, C. A.; Christensen, S. B. *Tetrahedron Lett.* **1986**, *27*, 887−888.

(4) Dutton, F. E.; Lee, B. H.; Johnson, S. S.; Coscarelli, E. M.; Lee, P. H. *J. Med. Chem.* **2003**, *46*, 2057−2073.

(5) (a) Ainai, T.; Matsuumi, M.; Kobayashi, Y. *J. Org. Chem.* **2003**, *68*, 7825−7832. (b) Stockman, R. A.; Sinclair, A.; Arini, L. G.; Szeto, P.; Hughes, D. L. *J. Org. Chem.* **2004**, *69*, 1598−1602.

Scheme 3. Preparation of (*R*)-2-Hydroxyheptanoic Acid Benzyl Ester **(+)-6**

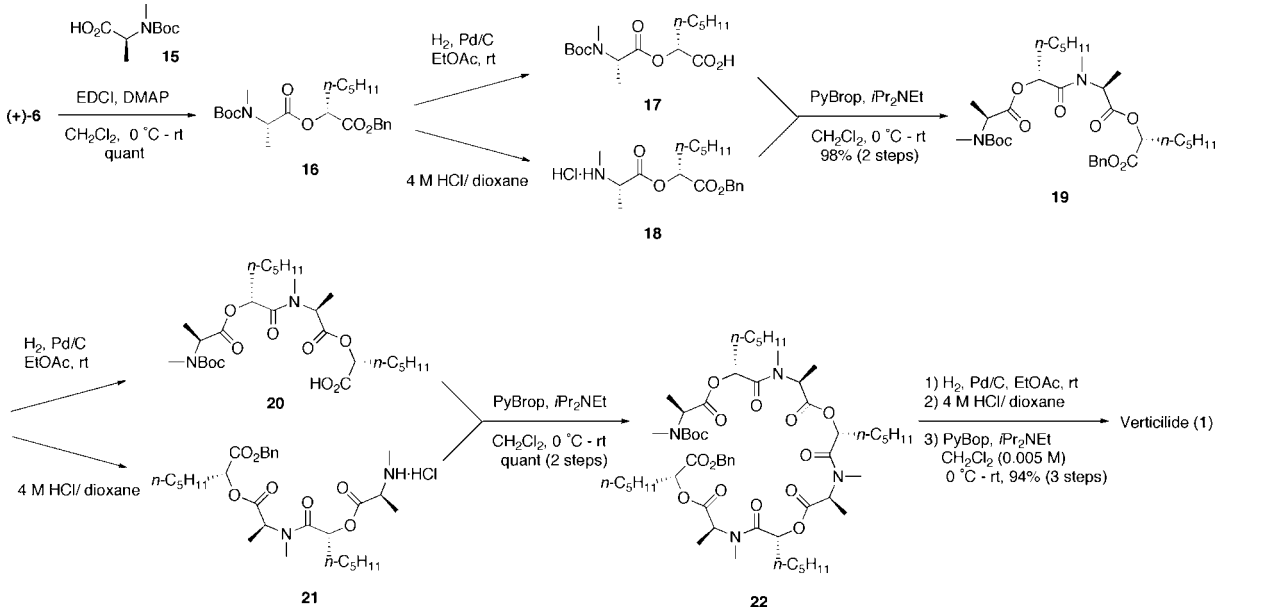


Daicel OD10C column with an eluent of *n*-hexane/2-propanol (400:15). A 1:1 mixture of fragment **6** and synthetic (+)-**6** was subjected to the chiral HPLC analysis and produced duplicate peaks (Figure 2c). This result indicated that fragment **6** was identical with synthetic (+)-**6**. Therefore we confirmed that the absolute configuration at C2 in 2-hydroxyheptanoic acid is *R*. However, this synthetic route of (*R*)-2-hydroxyheptanoic acid appeared to be inadequate for practical synthesis of verticilide, because the overall yield of (+)-**6** was very low and D-malic acid was expensive as a starting material. Accordingly, we employed diastereoselective oxidation reported by Evans[6] for an effective synthesis of (+)-**6** (Scheme 3).

Condensation of (*R*)-oxazolidinone **9** and heptanoyl chloride **10**, followed by diastereoselective oxidation of the imide with NaHMDS and Davis reagent **12** gave **13** in 94% yield. After protection of the hydroxyl group by TBDMSCl and imidazole, transesterification with BnOH[7] afforded **14** in excellent yield. Finally, deprotection of the TBDMS group by 4 M HCl/dioxane gave (*R*)-2-hydroxyheptanoic acid benzyl ester (+)-**6** efficiently. As the synthetic route of (+)-**6** was developed, we turned to total synthesis of verticilide **1**. Esterification of (+)-**6** and alanine **15**[8] with EDCI in the presence of DMAP gave the key intermediate **16** in quantitative yield. A benzyl group of one **16** was deprotected and a Boc group of another **16** was deprotected by 4 M HCl/dioxane. Condensation of **17** and **18** with PyBrop and *i*-Pr_2NEt produced tetradepsipeptide **19** in 98% yield for two steps. Use of other coupling reagents instead of PyBrop reduced the yields. Octadepsipeptide **22** was prepared in similar manner using **19** in quantitative yield. Finally, deprotection of the benzyl and Boc group, followed by the cyclization of linear depsipeptide in highly diluted CH_2Cl_2 by PyBop and *i*-Pr_2NEt, provided verticilide (**1**) in 94% yield for 3 steps (Scheme 4). Its spectral properties were identical in all respects (^{1}H and ^{13}C NMR, IR, HR-FABMS, optical rotation, TLC, HPLC, and bioactivity) to those of the natural product.

In conclusion, the elucidation of the stereochemistry for 2-hydroxyheptanoic acid and the total synthesis of verticilide were completed. The longest linear synthetic sequence for the synthesis of verticilide comprised 13 steps and proceeded in 66% overall yield (corresponding to a 97% average yield per step). Our synthesis used a diastereoselective Davis oxidation for the effective synthesis of 2-hydroxyheptanoic acid. We have synthesized 10 g of verticilide by using this synthetic route for further biological evaluations. Studies on the mode of action and the structure−activity relationships of verticilide are currently under way.

Scheme 4. Total Synthesis of Verticilide



Acknowledgment. This work was supported by a Grant from the 21st Century COE Program, Ministry of Education, Culture, Sports, Science and Technology. We also thank Ms. A. Nakagawa, Ms. C. Sakabe, and Ms. N. Sato (School of Pharmaceutical Sciences, Kitasato University) for the various instrumental analysis.

(6) Evans, D. A.; Morrissey, M. M.; Dorow, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4346−4348.
(7) Ghosh, A. K.; Liu, C. *J. Am. Chem. Soc*. **2003**, *125*, 2374−2375.
(8) Schutkowski, M.; Mrestani-Klaus, C.; Neubert, K. *Int. J. Peptide Protein Res.* **1995**, *45*, 257−265.

Supporting Information Available: Experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0623365