Communications to the Editor

The Oxidative Macrocyclization of Phenolic Peptides. A Biomimetic Approach to the Synthesis of the Vancomycin Family of Antibiotics

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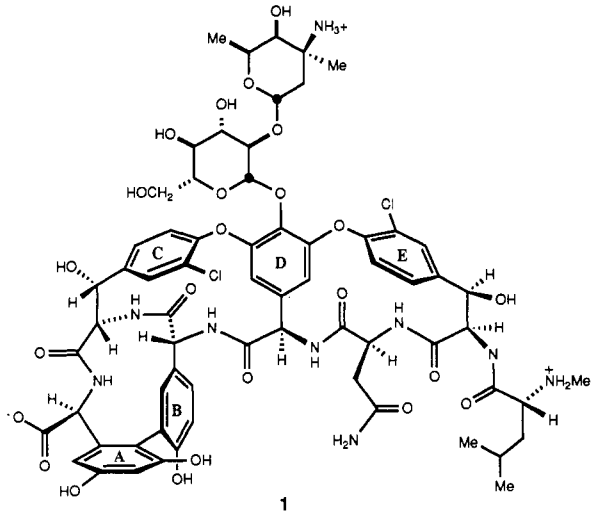
The family of glycopeptide antibiotics, as represented by vancomycin (**1**), is widely used in the treatment of staphylococcal infections. These important compounds express their antibiotic activity by inhibiting bacterial cell wall biosynthesis by selectively binding to the C terminal D-Ala-D-Ala residues of peptidoglycan precursors. The specific mode of binding of these compounds is under investigation by a number of research groups;[1] however, due to the architectural complexity of these molecules, only the syntheses of simple analogues have been accomplished to date.[1e,f] In accord with our objectives, which have been directed at the development of general approaches to the synthesis of vancomycin and other members of this family of antibiotics,[2] we report our preliminary investigations on a biomimetic oxidative macrocyclization approach to the bicyclic C, D, E phenyl ether fragment of vancomycin.



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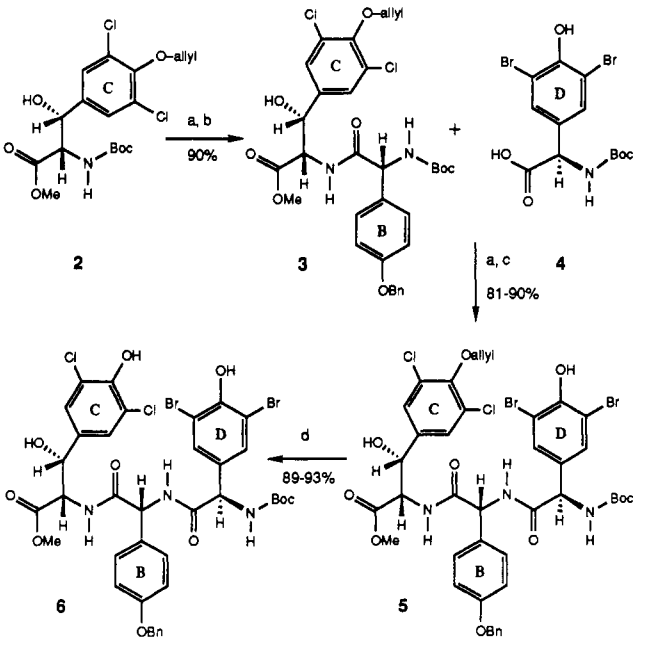
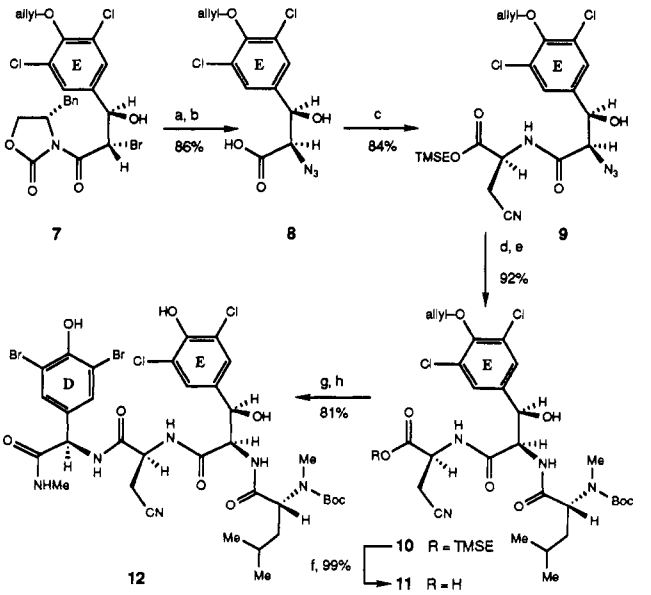
In initial studies, we chose to construct two model peptides, one with which to assess the C–D ring macrocyclization and the other with which to assess the analogous D–E ring closure. The synthesis of the C–D ring cyclization precursor began with the *N*-Boc methyl ester **2**, prepared according to previously described methodology from this laboratory (Scheme I).[3] Removal of the Boc protecting group from **2** with trifluoroacetic acid followed by coupling to the

Scheme I[a]



[a] (a) TFA, thioanisole; (b) *N*-Boc-*O*-benzyl-4-hydroxyphenylglycine, diisopropylcarbodiimide, hydroxybenzotriazole (HOBt); (c) 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), HOBt; (d) Bu_3SnH, Pd(II).

Scheme II[a]



[a] (a) NaN_3, DMSO; (b) LiOOH; (c) β-cyanoalanine (trimethylsilyl)ethyl ester, EDC, HOBt; (d) $SnCl_2$, MeOH; (e) *N*-Boc-*N*-methylleucine, EDC, HOBt; (f) *n*-Bu_4NF; (g) *N*-Boc-3,5-dibromo-4-hydroxyphenylglycine *N*-methyl amide, EDC, HOBt; (h) Bu_3SnH, Pd(II).

(1) (a) Waltho, J. P.; Williams, D. H.; Stone, D. J. M.; Skelton, N. J. *J. Am. Chem. Soc.* **1988**, *110*, 5638–5643. (b) Nagarajan, R.; Schabel, A. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1306–1307. (c) Harris, C. M.; Kannan, R.; Kopecka, H.; Harris, T. M. *J. Am. Chem. Soc.* **1985**, *107*, 6652–6658. (d) Mueller, L.; Heald, S. L.; Hempel, J. C.; Jeffs, P. W. *J. Am. Chem. Soc.* **1989**, *111*, 496–505. (e) Pant, N.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 2002–2003. (f) Hobbs, D. W.; Still, W. C. *Tetrahedron Lett.* **1987**, *28*, 2805–2808.

(2) (a) Evans, D. A.; Weber, A. E.; Britton, T. C.; Sjogren, E. B. In *Peptides: Chemistry and Biology: Proceedings of the 10th American Peptide Symposium*; Marshall, G. R., Ed.; ESCOM Science Publishers: Leiden, The Netherlands, 1988; pp 143–149. (b) Evans, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 1063–1072.

(3) (a) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1987**, *109*, 7151–7157. (b) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1986**, *108*, 6757–6761.

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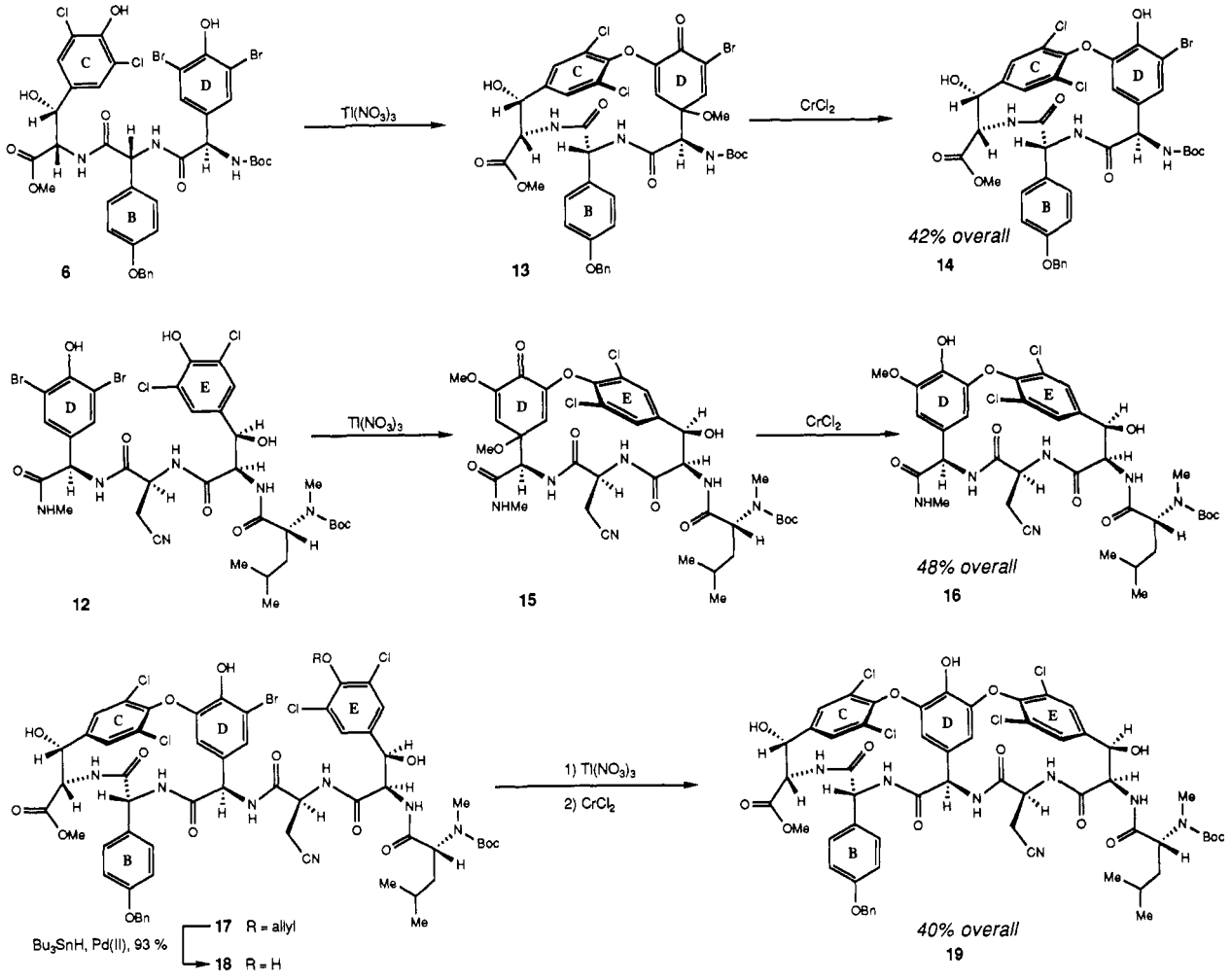
Scheme III



racemization-prone D-*N*-Boc-*O*-benzyl-4-hydroxyphenylglycine[4] provided dipeptide **3** in 90% overall yield with less than 1% racemization as determined by HPLC analysis. After removal of the Boc protecting group of dipeptide **3** followed by base extraction, the resulting free amine was coupled to the D-*N*-Boc-phenylglycine **4**[5] to yield tripeptide **5** in 81–90% yield with less than 4% racemization as determined by HPLC analysis. Treatment of tripeptide **5** with Bu_3SnH and $Pd(PPh_3)_2Cl_2$[6] as catalyst removed the allyl protecting group without any concomitant hydrodehalogenation, to provide the tripeptide cyclization precursor **6** in 89–93% yield.[7]

The tetrapeptide that was used to model the D–E ring closure was synthesized from the α-bromo carboximide **7**, prepared according to methodology previously established in this laboratory (Scheme II).[8] Reaction of **7** with sodium azide in dimethyl sulfoxide followed by removal of the oxazolidinone by treatment with lithium hydrogen peroxide[9] provided the α-azido acid **8** (86%), which was then coupled to the (trimethylsilyl)ethyl (TMSE) ester of L-β-cyanoalanine[10] to give the dipeptide **9** in 84% yield.[11,12] Reduction of the azide with stannous chloride in methanol[13] followed by coupling to D-*N*-Boc-*N*-methylleucine then provided tripeptide **10** in 92% yield. The removal of the (trimethylsilyl)ethyl protecting group was accomplished in 99% yield by treatment with tetrabutylammonium fluoride in DMF.[14] Coupling the resulting acid **11** with the *N*-methyl amide allyl ether of the D-phenylglycine **4**[15] followed by removal of the allyl protecting groups with Bu_3SnH and $Pd(PPh_3)_2Cl_2$ afforded the D–E cyclization precursor **12** in 81% overall yield.[16]

(4) Prepared as described by Kamiya et al.: Kamiya, T.; Hashimoto, M.; Nakaguchi, O.; Oku, T. *Tetrahedron* **1979**, *35*, 323–328.

(5) Prepared in 87% overall yield by bromination of D-hydroxyphenylglycine with bromine in acetic acid, followed by protection with *tert*-butyl pyrocarbonate in dioxane/water at pH 11. HPLC analysis (Pirkle column) of the dimethylated derivative (prepared by treatment with diazomethane) revealed that less than 1% racemization had occurred during the above reaction sequence.

(6) Guibe, F.; Four, P. *Tetrahedron Lett.* **1982**, *23*, 1825–1828.

(7) Established methods for the removal of the allyl group, such as employing $RhPPh_3Cl$ to isomerize the allyl ether to the enol ether, proved unsuccessful due to the unreactivity of the resultant enol ether toward acidic hydrolysis or mercuric acetate oxidation.

(8) Evans, D. A.; Sjogren, E. B.; Weber, A. E.; Conn, R. E. *Tetrahedron Lett.* **1987**, *28*, 39–42.

(9) Evans, D. A.; Britton, T. C.; Ellman, J. E. *Tetrahedron Lett.* **1987**, *28*, 6141–6144.

(10) Prepared in 96% yield by treatment of Cbz-β-cyanoalanine with dicyclohexylcarbodiimide and (dimethylamino)pyridine in the presence of (trimethylsilyl)ethanol, followed by hydrogenolysis of the Cbz protecting group with Pd/C as catalyst. The enantiomeric purity of the resulting amino ester was determined to be 96% by GC analysis of the α-methoxy-α-(trifluoromethyl)phenylacetamide derivative.

(11) We chose to employ β-cyanoalanine as a protected derivative of asparagine in order to avoid the potential side reactions during activation and peptide coupling that are inherent to asparagine-containing peptides. The nonpolar nature of β-cyanoalanine also greatly facilitated the manipulation and purification of the peptides that contained this amino acid.

(12) Previous studies have shown that the α-azido acids do not racemize under standard peptide coupling conditions (ref 2b).

(13) Maiti, S. N.; Singh, M. P.; Micetich, R. G. *Tetrahedron Lett.* **1986**, *27*, 1423–1424.

(14) Carpino, L. A.; Sali, A. C. *J. Chem. Soc., Chem. Commun.* **1979**, 514–515.

(15) Prepared by coupling the pentafluorophenol ester of **4** (DICI, pentafluorophenol) with *N*-methylamine. The enantiomeric purity was determined to be 93% by HPLC analysis of the α-methoxy-α-(trifluoromethyl)-phenylacetamide methyl phenyl ether derivative.

Our optimized conditions for promoting the oxidative cyclization of substrates **6** and **12** (Scheme III) are derived from a related transformation initially reported by Yamamura[17] and later modified by Inoue.[18,19] We have found that the optimal protocol for the cyclization of **6** is oxidation with 10 equiv of thallium(III) nitrate trihydrate (TTN) (excess TTN is necessary to ensure complete reaction) in 5:1 THF/methanol at 1 mM concentration with 3 equiv of pyridine/equiv of TTN to serve as an acid scavenger. Increasing the ratio of THF to methanol results in incomplete reaction, while increasing the ratio of methanol to THF results in a lower yield. The reduction of the resulting para-quinol **13** is accomplished in situ by the addition of excess $CrCl_2$.[20] We have found these conditions to be superior to the zinc/acetic acid reduction described by Yamamura[19] as they avoid the isolation of the unstable intermediate para-quinol methyl ether. Under the conditions described above, the cyclic product **14** is isolated in 42% overall yield from the cyclization precursor **6**.

Model peptide **12** was cyclized and subsequently reduced under analogous conditions except that 1:1 CH_2Cl_2/methanol was employed as the solvent. When these conditions were employed, the macrocyclic diphenyl ether **16** was obtained in 48% overall yield. One notable difference between the two macrocyclizations is the displacement of bromine by methoxide in the formation of para-quinol **15**.[21] Presumably, this substitution occurs in the cyclization of **12** and *not* in the cyclization of **6** due to a more sterically crowded environment at the para position of the intermediate leading to **15**. *These observations thus dictate the order of assemblage of the macrobicyclic diether* **19**.

In order to evaluate the oxidative coupling strategy to provide the C, D, E bicyclic phenyl ether vancomycin synthon, the monocyclic diphenyl ether **14** was treated with trifluoroacetic acid to remove the Boc protecting group, and the resulting amine was coupled to tripeptide **11** with diisopropylcarbodiimide and hydroxybenzotriazole to provide the hexapeptide **17** in 72-78% overall yield (Scheme III).[16] The allyl group was then removed as described previously in 92-93% yield to provide hexapeptide **18**. The optimal conditions for the cyclization of **18** were found to be 5 equiv of TTN in 30:1 methylene chloride/methanol at 1 mM concentration (4 h, -23 °C). After in situ reduction of the resulting para-quinol ether with excess $CrCl_2$, the dicyclic compound **19** was obtained in 40% overall yield.

These studies clearly demonstrate the feasibility of pursuing a total synthesis of vancomycin and related antibiotics via biomimetic oxidative phenolic coupling.

Acknowledgment. Support has been provided by the National Institutes of Health, Eli Lilly, and Merck. The NIH BRS Shared Instrumentation Grant Program 1 S10 RR01748-01A1 is acknowledged for providing NMR facilities. J.A.E. and K.M.D. acknowledge support as NSF Predoctoral Fellows.

Supplementary Material Available: Spectral data for all compounds and detailed experimental procedures for the oxidative macrocyclizations as well as for the syntheses of **2-6**, **17**, and **18** (15 pages). Ordering information is given on any current masthead page.

(16) A diastereomeric compound, 7%, was also produced in this coupling reaction.

(17) Nishiyama, S.; Nakamura, K.; Suzuki, Y.; Yamamura, S. *Tetrahedron Lett.* **1986**, *27*, 4481-4484.

(18) Inaba, T.; Umezawa, I.; Yuasa, M.; Inoue, T.; Mihashi, S.; Itokawa, H.; Ogura, K. *J. Org. Chem.* **1987**, *52*, 2957-2958.

(19) More recently, Yamamura has employed these conditions in the synthesis of OF4949-III (Nishiyama, S.; Suzuki, Y.; Yamamura, S. *Tetrahedron Lett.* **1988**, *29*, 559-562) and in the synthesis of K-13 (Nishiyama, S.; Suzuki, Y.; Yamamura, S. *Tetrahedron Lett.* **1989**, *30*, 379-382).

(20) Hanson, J. R.; Mehta, M. S. *J. Chem. Soc., Chem. Commun.* **1969**, 2349-2350.

(21) In the oxidation of an analogue of **6** containing the Cbz protecting group rather than the Boc protecting group, approximately 5-10% of the methoxy-substituted derivative of **14** was also observed. In the limited number of systems that we have studied, increasing the ratio of THF to methanol and addition of pyridine increases the ratio of the bromo- to the methoxy-substituted cyclic products.

Aristolochene Biosynthesis and Enzymatic Cyclization of Farnesyl Pyrophosphate

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Aristolochene (**1**) is a bicyclic sesquiterpene belonging to the eremophilane group of hydrocarbons. The (-) enantiomer of **1** was first isolated in 1970 by Govindachari et al. from the plant *Aristolochia indica*.[1] It is also reported to occur in *Bixa orellana* leaf oil and in the defensive secretions of *Syntermes* soldier termites.[2,3] The (+) enantiomer **1** was recently isolated in our laboratory from the mycelial extracts of the fungus *Aspergillus terreus*.[4] The (+) enantiomer is also the probable biosynthetic precursor of PR toxin produced by *Penicillium roquefortii*.[5] Recently, Hohn and co-workers have isolated aristolochene synthase from *P. roquefortii*[6] and purified the enzyme to homogeneity.[7]

The proposed mechanism for the formation of aristolochene from farnesyl pyrophosphate (FPP) (**2**), the universal precursor of sesquiterpenes,[10] is shown in Scheme I. Cell-free extracts of *A. terreus* prepared from mycelia harvested between 45 and 60 h after inoculation showed terpenoid cyclase activity.[11] Preparative incubation of [1-^{3}H]FPP (**2a**)[12] with crude cell-free extracts produced radioactive hydrocarbon **1a**,[13] which was found to cochromatograph with synthetic (±)-aristolochene[14] on TLC (SiO_2, $AgNO_3$-SiO_2) as well as by radio-GC analysis (FFAP). Dilution with carrier (±)-aristolochene and oxidation with MCPBA followed by hydrolysis with $HClO_4$ gave the corresponding diol **3a**, which was recrystallized to constant activity, thereby confirming

(1) Govindachari, T. R.; Mohamed, P. A.; Parthasarathy, P. C. *Tetrahedron* **1970**, *26*, 615.

(2) Lawrence, B. M.; Hogg, J. W. *Phytochemistry* **1973**, *12*, 2995.

(3) Baker, R.; Cole, H. R.; Edwards, M.; Evans, D. A.; Howse, P. E.; Walmsley, S. *J. Chem. Ecol.* **1981**, *7*, 135.

(4) (a) Cane, D. E.; Rawlings, B. J.; Yang, C.-C. *J. Antibiot.* **1987**, *40*, 1331. (b) Cane, D. E.; Salaski, E. J.; Prabhakaran, P. C. *J. Antibiot.*, submitted for publication.

(5) (a) Moreau, S.; Lablache-Combier, A.; Biguet, J. *Phytochemistry* **1981**, *20*, 2339. (b) Gorst-Allman, C. P.; Steyn, P. S. *Tetrahedron Lett.* **1982**, *23*, 5359. (c) Chalmers, A. A.; DeJesus, A. E.; Gorst-Allman, C. P.; Steyn, P. S. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2899.

(6) Hohn, T. M.; Plattner, R. D. *Arch. Biochem. Biophys.* **1989**, *272*, 137.

(7) The epimeric sesquiterpene, (+)-5-epiaristolochene, has been demonstrated to be an intermediate[8a] in the biosynthesis of the phytoalexins capsidiol and debneyol.[8a] It has recently been shown that treatment of tissue cultures of *Nicotiana tabacum* with various elicitors results in appearance of a cyclase capable of converting FPP to a sesquiterpene hydrocarbon identified as (+)-5-epiaristolochene.[9]

(8) (a) Threlfall, D. R.; Whitehead, I. M. *Phytochemistry* **1988**, *27*, 2567. (b) Brooks, C. J. W.; Watson, D. G.; Rycroft, D. S.; Freer, I. M. *Phytochemistry* **1987**, *26*, 2243. (c) Hoyano, Y.; Stoessl, A.; Stothers, J. B. *Can. J. Chem.* **1980**, *58*, 1894. (d) Baker, F. C.; Brooks, C. J. W. *Phytochemistry* **1976**, *15*, 689. (e) Baker, F. C.; Brooks, C. J. W.; Hutchinson, S. A. *J. Chem. Soc., Chem. Commun.* **1975**, 293.

(9) (a) Whitehead, I. M.; Threlfall, D. R.; Ewing, D. F. *Phytochemistry* **1989**, *28*, 775. (b) Vogeli, U.; Chappel, J. *Plant Physiol.* **1988**, *88*, 1291. (c) Whitehead, I. M.; Prabhakaran, P. C.; Ewing, D. F.; Cane, D. E.; Threlfall, D. R. *Phytochemistry*, in press.

(10) (a) Cane, D. E. *Acc. Chem. Res.* **1985**, *18*, 220. (b) Cane, D. E. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; John Wiley: New York, 1981; Vol. 1, p 284.

(11) Croteau, R.; Cane, D. E. *Methods Enzymol.* **1985**, *110*, 383.

(12) Prepared as previously described: Cane, D. E.; Iyengar, R.; Shiao, M.-S. *J. Am. Chem. Soc.* **1981**, *103*, 914.

(13) The mycelia from 500 mL of 45-h culture were used to prepare the cell-free extract[11] in 190 mL of buffer (10 mM Tris, 5 mM $MgCl_2 \cdot 6H_2O$, 5 mM β-mercaptoethanol, and 15% v/v glycerol adjusted to pH 7.8 with 6 N HCl). [1-^{3}H]FPP (8.8×10^5 dpm, 7 mmol) was incubated with 2 mL of crude extract at 30 °C for 2 h, and the resulting radioactive hydrocarbon (4.0×10^4 dpm) was extracted into pentane, passed through a small SiO_2 column, and concentrated by Vigreux distillation.

(14) Piers, E.; Geraghty, M. B. *Can. J. Chem.* **1973**, *51*, 2166.

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