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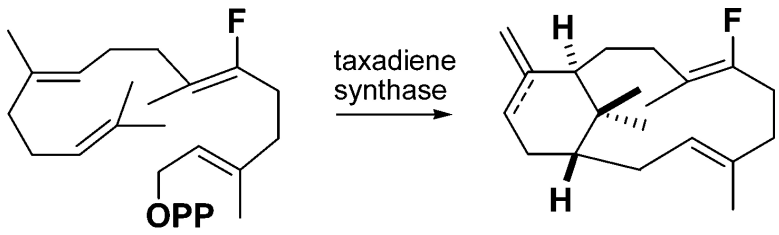

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Taxadiene Synthase-Catalyzed Cyclization of 6-Fluorogeranylgeranyl Diphosphate to 7-Fluoroverticillenes

Yinghua Jin,[†] David C. Williams,[‡] Rodney Croteau,[‡] and Robert M. Coates*,[†]

Contribution from the Department of Chemistry, University of Illinois, 600 South Mathews Avenue, Urbana, Illinois 61801, and the Institute of Biological Chemistry, Washington State University, Pullman, Washington, 99164-6340

Received January 28, 2005; E-mail: coates@scs.uiuc.edu

Abstract: The mechanism of the taxadiene synthase-catalyzed cyclization of (*E*,*E*,*E*)-geranylgeranyl diphosphate (GGPP, **7**) to taxadiene (**5**) is proposed to proceed through a verticillen-12-yl carbocation intermediate (**8**) that undergoes an 11 → 7 proton transfer leading to formation of the C ring. The substrate analogue 6-fluoroGGPP (**17**) was synthesized to elucidate the stereochemistry of the putative verticillenyl intermediate. It was expected that the inductive electron-withdrawing effect of the fluoro substituent would prevent the critical proton transfer to the Δ^7 double bond and thereby derail the cyclization at the bicyclic stage. Incubation of the fluoro analogue with recombinant taxadiene synthase yielded a mixture of three major and two minor fluoro diterpenes according to GC/MS analyses. The three major products were identified as the exocyclic, endocyclic, and 4(20)-methylene 7-fluoroverticillenes, i.e., $\Delta^{3,7,12\,(18)}$, $\Delta^{3,7,12}$, and $\Delta^{4(20),7,11}$ isomers (**22**, **23**, and **24**) on the basis of ^{1}H NMR analyses and comparisons with the parent bicyclic diterpenes. The H1β, H11α (1*S*,11*R*) configurations at the bridgehead positions of **22** were established by means of NOE experiments and CD spectra. The absolute configuration of (+)-verticillol (**4**) was revised after the anomalous dispersion X-ray analysis of (+)-verticillol *p*-iodobenzoate. Of particular note, all absolute configurations of verticillane diterpenes in the literature should be reversed. This work affords compelling evidence supporting the H11α (11*R*) stereochemistry of the verticillen-12-yl$^+$ ion intermediate in the taxadiene synthase-catalyzed reaction and illustrates the capability of vinyl fluoro analogues to intercept complex cyclization cascades.

Introduction

The taxanes constitute a large family of highly functionalized diterpenes based on the tricyclo[9.3.1.0^{3,8}]pentadecane carbon framework (**1**, Figure 1).[1] A prominent example is the antitumor agent taxol, **6**. The structure of the *O*-cinnamoyl taxicin-I triacetate (**2a**) was first established by Harrison and Lythgoe through chemical degradations and NMR correlations.[2] The apparent biogenetic progenitor of **2a** lacking the bridgehead hydroxyl, *O*-cinnamoyl taxicin-II triacetate (taxinine, **2b**), was isolated from *T. cuspidata* by Nakanishi and co-workers, and its structure and absolute stereochemistry were determined unambiguously by chemical degradations, NMR data, CD and ORD spectra, and X-ray analyses.[3] The constitution of taxol was confirmed by total syntheses[4−9] and X-ray crystallography.[10]

Harrison and Lythgoe postulated that the novel tricyclic ring system of the taxanes might arise from (*E*,*E*,*E*)-geranylgeranyl diphosphate (GGPP, **7**) by initial bridging cyclization to form a bicyclo[9.3.1]pentadeca-3,7-decadiene precursor (e.g., verticillene, **3**).[2d] Further proton-induced closure of the fused six-membered C ring would generate the taxane nucleus. The then tentative structure of the macrocyclic diterpene alcohol verticillol (**4**)[11] was cited as possible precedent for this biogenetic hypothesis. Although the structure of (+)-verticillol was later confirmed by an X-ray crystal analysis of its 3,4:7,8-diepoxide,[12] the absolute configuration assigned (*ent*-**4** with H1α) on the basis of the positive Cotton effect of a norketo diepoxide derivative was opposite to the H1β stereochemistry of the taxanes.[12] In this paper, we revise the earlier absolute configuration by X-ray crystallographic analysis of verticillol *p*-

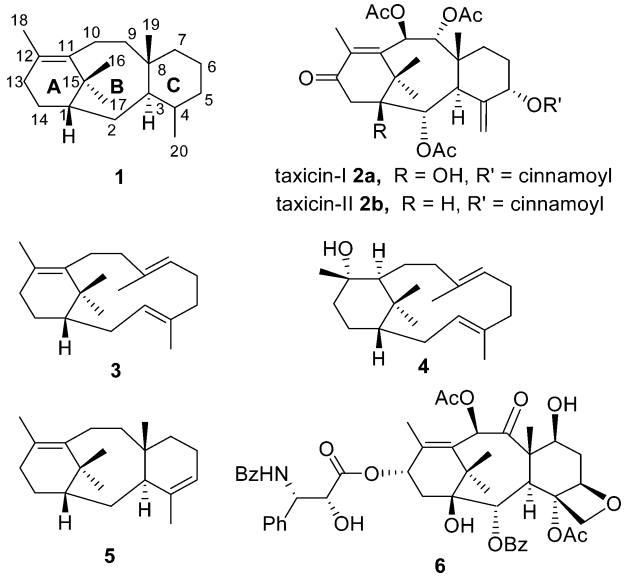

Figure 1. Structures of taxane and verticillane diterpenes.

[†] University of Illinois.
[‡] Washington State University.

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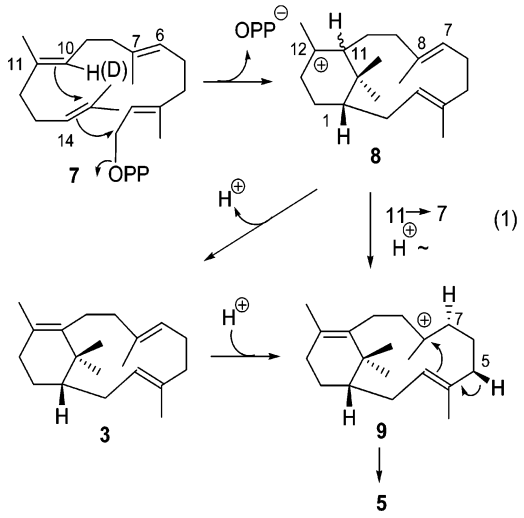
iodobenzoate to the enantiomer (**4**) with H1β configuration now consistent with taxane stereochemistry. Unsuccessful attempts to effect biomimetic cyclizations of synthetic (±)-verticillene and its 7,8-epoxides to taxanes provided further reason to question the biogenetic relationship of these diterpene classes.[13]

The isolation of a diterpene synthase from Pacific yew that produces taxa-4(5),11(12)-diene opened the way to investigations on the biosynthesis of the tricyclo[9.3.1.$0^{3,8}$]pentadecane framework in vitro.[14] The structure of the taxadiene product (**5**) established by NMR analysis was confirmed by total synthesis of the diterpene in racemic form.[15] Isotope dilution experiments with [^{3}H]-**5** demonstrated the presence of taxadiene in an extract from the bark of the Pacific yew. However, the failure of inhibition, trapping, and direct conversion experiments with deuterium-labeled verticillene and taxadiene synthase ruled out verticillene as a free intermediate in the enzyme-catalyzed reaction.[16] Conversion of [10-^{2}H$_1$]-GGPP to taxadiene bearing deuterium at C7 established that the reaction takes place with rearrangement of the label from C11 to C7. Subsequent NMR analysis of the resulting [7-^{2}H$_1$]taxadiene proved that the label has the 7α configuration.[17] Enzyme-catalyzed cyclizations of GGPPs labeled at C1, C16, and C4 revealed inversion at C1, antiperiplanar bondings across the 14,15 double bond, and elimination of H5β in forming the C-ring double bond.[17,18] A mechanism involving a transannular proton transfer that converts a verticillen-12-yl carbocation to verticillen-8-yl$^+$ isomer (**8** → **9**) and final cyclization to form the C ring was proposed (eq 1). Although molecular modeling indicated the feasibility of an intramolecular 11α → 7α proton migration, no direct experimental evidence was available to support the structure and stereochemistry of the proposed verticillen-12-yl$^+$ intermediate **8**.[17]



The objectives of the present work were to intercept the enzyme-catalyzed cyclization prior to the 11 → 7 proton transfer by means of a suitable substrate analogue and to establish the structure and stereochemistry of the putative verticillene intermediate. In this paper we report the synthesis of 6-fluoroGGPP (**17**) and its enzymatic cyclization to 7-fluoroverticillenes.

Fluorinated analogues of enzyme substrates have proven quite useful in mechanistic studies,[19] especially in the case of enzymes associated with isoprenoid biosynthesis. The special utility of fluoro analogues is attributed to the slight perturbation of the size and shape of the modified substrate so that binding affinity is not greatly affected, while at the same time the fluoro substituent exerts a strong influence on the electronic environment at the site of replacement. The electrophilic nature of isoprenoid chain extensions catalyzed by prenyl transferases and of cyclizations mediated by monoterpene synthases was demonstrated by kinetic and inhibition experiments with fluoro

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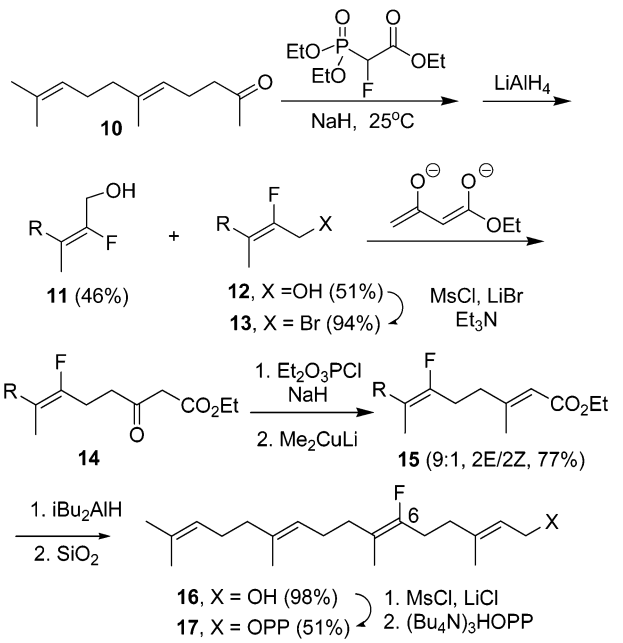
Scheme 1



analogues of geranyl and linaloyl diphosphates.[20] 10-Fluorofarnesyl diphosphate proved to be a potent inhibitor of trichodiene synthase (K_i = 16 nM ~ 20% K_m^{FPP}).[21] Polyene cyclizations catalyzed by squalene-hopene cyclase were intercepted when 11- and 14-fluoro derivatives of (*S*)-oxidosqualene were used as substrates.[22] We considered that the presence of a fluoro substituent on the 6,7 double bond of the GGPP substrate for taxadiene synthase would be likely to block the proton-transfer step and thereby might terminate the multistep mechanism at the verticillene stage.

Synthesis of 6-FluoroGGPP Substrate and Reference Compounds. The modified substrate, 6-fluoroGGPP (**17**), was synthesized from the known[23] 2-fluorofarnesol (**12**) by Weiler's isoprenoid chain extension method (Scheme 1).[24,25] Condensation of geranylacetone (**10**) with triethyl fluorophosphonoacetate[26] followed by $LiAlH_4$ reduction afforded the chromatographically separable 2-fluorofarnesol isomers, **11** and **12**. The trans (Z) configuration of the 2,3 double bond in the more polar isomer was established by comparison of the ^{1}H NMR chemical shifts of the C3−CH_3 groups of the corresponding 2-fluorofarnesals (cis δ_H 1.95; trans δ_H 2.11) obtained by Swern oxidations of **11** and **12**.[27] The slightly larger 4-bond HF couplings with the C3 methyl observed in the NMR spectra of the cis isomers (J_{HF} = 3.43 and 3.71 Hz for the alcohol and aldehyde, respectively) compared to those of the trans isomers (J_{HF} = 3.03 and 3.14 Hz) are consistent with this assignment.[28]

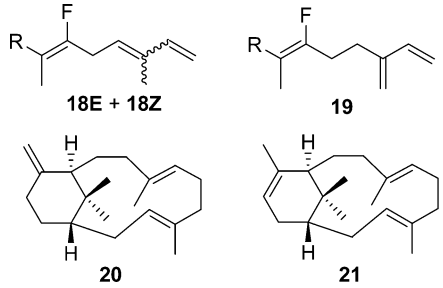


Figure 2. Structures of authentic standards of 6-fluorogeranylocimenes (**18E** + **18Z**), 6-fluoro-geranylmyrene (**19**), *exo*-verticillene (**20**), and *endo*-verticillene (**21**); R = homogeranyl.

Alkylation of lithio-sodio acetoacetate dianion (3 equiv, THF, 0 °C) with bromide **13** (from **12**; MsCl, LiBr, Et_3N, THF, −45 → 0°C)[29] provided fluoro β-keto ester **14** (~100%). Conversion to the trans enol phosphate as a single isomer (NaH, $(EtO)_2P(=O)Cl$, ether, 0 °C) followed by vinylic coupling with lithium dimethylcuprate (2 equiv, ether, −78 °C, 3.5 h) gave a 9:1 mixture of trans (**15**) and cis isomers (77%),[30] along with some recovered enol phosphate (7%). Pure 2,3-trans 6-fluoro GGOH (**16**, 98%) was obtained after hydride reduction (*i*-Bu_2AlH, toluene, −78 °C) and chromatography on silica gel to remove the cis isomer (6%). Conversion to 6-fluoroGGPP (**17**) was accomplished by activation as the allylic chloride (MsCl, LiCl, *s*-collidine, DMF, 0 °C, 3 h),[31] diphosphate displacement ($HOPP(NBu_3)_4$, CH_3CN, rt, 24 h), and anion exchange to the NH_4^+ salt.[32]

The real possibility that the enzyme-catalyzed reaction might lead to the simple elimination products, i.e., fluoro analogues of the geranylocimenes or geranylmyrcene,[33] prompted us to synthesize the acyclic fluoro diterpenes as GC reference compounds. Dehydration of **16** (PPTS, 1,2-dichloroethane, 155 °C, 10 min)[34] afforded a mixture of fluoropentaenes **18*E*** + **18*Z*** + **19** in a 1.5:1:2.3 ratio (Figure 2). Specimens of authentic *exo*- and *endo*-verticellenes **20** and **21** were obtained by dehydration of (+)-verticillol[11,12] with $POCl_3$.[13] The proton NMR data and assignments and the CD curves for (+)-verticillol and *exo*-verticillene **20** are presented in Table 1 and Figure 5, and the revised absolute stereochemistry is discussed below.

Enzymatic Cyclization of 6-FluoroGGPP to 7-Fluoroverticillenes. Preparative-scale incubation of 6-fluoroGGPP NH_4^+ salt with recombinant taxadiene synthase[14b] (pH 8.0, 31 °C, 36 h) afforded a mixture of three major and two minor fluoro

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(30) Interestingly, the trans/cis ratio varied with the reaction temperature. Higher selectivity (up to trans/cis, 93:7) was achieved at lower temperature (−78 °C), while ratios of 3:1 to 2:1 were typically obtained at higher temperatures (−45 to 0 °C). Evidently the remote fluoro substituent has a pronounced effect on the stereoeselectivity of the cuprate coupling since Weiler and others reported consistently high stereoselectivity (~98:2) for similar substrates lacking the fluoro substituent at −45 to 0 °C. (a) Sum, F. W.; Weiler, L. *J. Am. Chem. Soc.* **1979**, *101*, 4401−4403. (b) Brown, R. C. D.; Hughes, R. M.; Keily, J.; Kenney, A. *Chem. Commun.* **2000**, 1735−1736. (c) Jin, Y.; Coates, R. M. *Org. Synth.* Submitted for publication.
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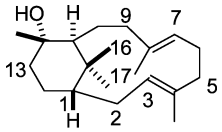
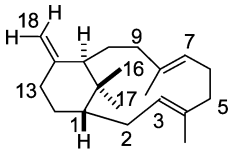
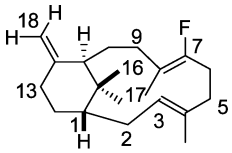
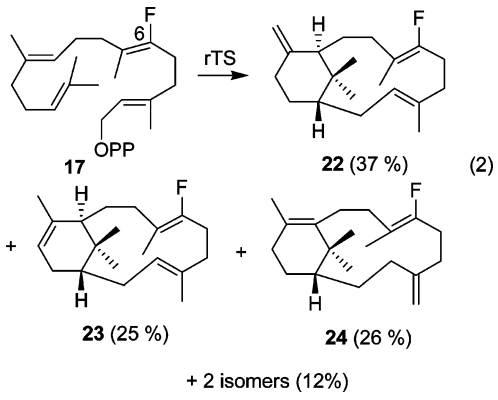
Table 1. ^{1}H NMR Spectral Data and Assignments for (+)-Verticillol (**4**), *exo*-Verticillene (**20**), and the Enzymatic Cyclization Product, *exo*-7-Fluoroverticillene (**22**)

C#	verticillol **4**[a]	*exo*-verticillene **20**[a]	*exo*-7-fluoro-verticillene **22**[b]
	 **4**	 **20**	 **22**
1	δ 1.25, m	δ 1.36, m	δ 1.35, m
2α	δ 1.82−1.83, m	δ 1.89, br d, J = 15.0	δ 1.84−1.90, m
2β	δ 2.64, dddd, J = 14.8, 12.7, 6.1, 1.0 Hz	δ 2.68, dddd, J = 14.5, 12.7, 4.4, 1.6 Hz	δ 2.67, dddd, J = 14.3, 13.7, 4.3, 1.5 Hz
3	δ 5.71, br d, J = 12.7 Hz	δ 5.61, br d, J = 12.1 Hz	δ 5.81, br d, J = 12.6 Hz
4			
5α	δ 2.04, dd, J = 12.4, 3.2 Hz	δ 1.97, td, J = 12.8, 3.4 Hz	δ 2.47, td, J = 13.0, 4.2 Hz
5β	δ 1.82−1.84, m	δ 2.08−2.15, m	δ 1.84−1.90, m
6α	δ 1.98, br d, J = 13.6 Hz	δ 1.93, br d, J = 13.1 Hz	δ 1.99, br d, J = 13.8 Hz
6β	δ 2.29−2.37, m	δ2.35, dddd, J = 13.5, 12.7, 11.6, 3.3 Hz	δ2.34, dtd, J = 40.0, 14.1, 4.1 Hz
7	δ 5.02, br d, J = 12.1 Hz	δ 4.71, br d, J = 11.6 Hz	
8			
9α	δ 2.58, td, J = 12.9, 4.4 Hz	δ 2.04−2.11, m	δ 2.86, t, J = 13.5 Hz
9β	δ 2.07−2.11, m	δ 2.04−2.11, m	δ 1.44−1.45, m
10α	δ 1.20, tdd, J = 13.9, 3.4, 1.2 Hz	δ 1.44−1.51, m	δ 1.50, br t, J = 12.1 Hz
10β	δ 1.42−1.45, m	δ 1.25, td, J = 12.9, 4.2 Hz	δ 1.18, td, J = 13.7, 2.3 Hz
11	δ 2.27, d, J = 7.0 Hz	δ 2.96, d, J = 10.6 Hz	δ 2.62, d, J = 10.5 Hz
12			
13α	δ 1.78−1.83, m	δ 2.45, td, J = 14.0, 6.1 Hz	δ 2.59, td, J = 13.8, 6.5 Hz
13β	δ 2.07−2.11, m	δ 2.30, dd, J = 12.7, 6.1 Hz	δ 2.32, dd, J = 14.2, 4.7 Hz
14α	δ 1.55, dd, J = 8.5, 2.7 Hz	δ 1.55, br dd, J = 13.9, 6.2 Hz	δ 1.55, br dd, J = 14.1, 6.2 Hz
14β	δ 1.42−1.45, m	δ 2.04−2.11, m	δ 2.06, tt, J = 13.5, 6.7 Hz
15			
16	δ 0.73, s	δ 0.85, s	δ 0.83, s
17	δ0.66, s	δ 0.82, s	δ 0.84, s
18*Z*	δ1.14, s	δ 4.70, q, J = 1.7 Hz	δ 4.74, q, J = 1.8 Hz
18*E*		δ 4.98, q, J = 1.8 Hz	δ 5.00, q, J = 1.6 Hz
19	δ1.48, t, J = 1.3 Hz	δ 1.47, br s	δ 1.32, br s
20	δ1.46, t, J = 1.5 Hz	δ 1.48, t, J = 1.3 Hz	δ 1.43, br s

[a] 500 MHz, C_6D_6. [b] 600 MHz, C_6D_6.

diterpenes according to GC (Figure 3) and MS analyses (**a**−**e**, 7:26:25:37:5 ratio in GC elution order, *m*/*z* 290, total yield 468 μg, ~11%).

All five products differed in GC retention behavior from the acyclic fluoro diterpenes standards **18E**, **18Z**, and **19**. The three major products were separated or obtained in enriched fractions by silica gel chromatography and assigned structures corresponding to *exo*-7-fluoroverticillene, *endo*-7-fluoro-verticillene, and 7-fluoroverticilla-4(20),7(8),11(12)-triene (**22**, **23**, and **24**) as shown in eq 2 on the basis of extensive NMR and optical data presented below and comparisons with the known verticillene isomers.



Most of the resonances in the proton NMR spectra of *exo*-7-fluoro-verticillene **22** could be assigned by chemical shifts, coupling analysis, COSY, HMQC, and NOE methods, and the assignments were strengthened by correlations with the corresponding peaks in the spectra of (+)-verticillol and *exo*-verticillene (Table 1). The appearance of signals for C=CH_2 (δ_H 4.74, 5.00), C=C−H (δ_H 5.81), geminal methyls C(CH_3)$_2$ (δ_H 0.83, 0.84), two vinyl methyls C=C−CH_3 (δ_H 1.32, 1.43), and vinyl fluoro C=C−F group (δ_F −115.1, d, J = 39.9 Hz) in the spectrum of the major product are consistent with cyclization across the terminal double bond of the fluoro substrate to form bicyclic structure **22** with an exocyclic double bond and retention of the fluoro double bond. The chemical shift values match closely those in the spectrum of *exo*-verticillene (**20**) within ca. ±0.05 ppm except those for protons in the vicinity of the fluoro-bearing double bond. The larger

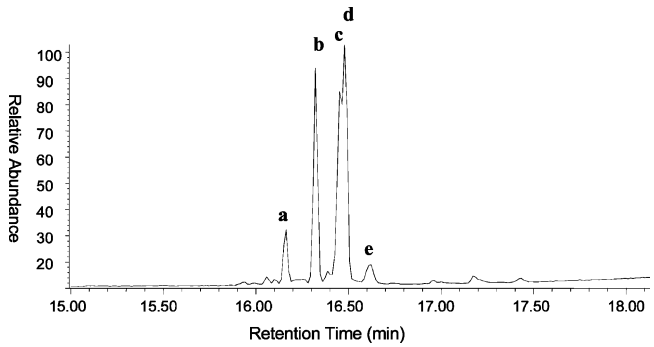


Figure 3. GC/MS analysis of the diterpene olefin products **a**−**e** formed in incubation of 6-fluoro GGPP (**17**) with the recombinant TS.

Table 2. NOE Enhancements in the 500 MHz ^{1}H NMR Spectrum of *exo*-Verticillene (**20**) in C_6D_6 after Irradiation at 2.96, 4.98, and 5.61 ppm

irradiation at 2.96 ppm (H11)		irradiation at 4.98 ppm (H18*E*)		irradiation at 5.61 ppm (H3)	
shift (ppm)	NOE (%)	shift (ppm)	NOE (%)	shift (ppm)	NOE (%)
0.82 (H17)	2.13	2.30 (H13β)	3.94	1.89(H2α)	3.21
1.25 (H10β)	1.59	4.71 (H18Z)	17.39	1.97(H5α)	6.34
2.02 (H9α)	1.73			2.45(H13α)	9.46
2.45 (H13α)	1.87			2.96(H11)	3.77
4.71 (H7)	5.08			4.71(H7)	1.04
5.61 (H3)	3.29				

chemical shift deviations were observed for H3 ($\Delta\delta$ −0.20), H5α and H5β ($\Delta\delta$ −0.50 and +0.24), H9α and H9β ($\Delta\delta$ −0.82 and +0.60), and 19 CH_3 ($\Delta\delta$ +0.15).

The less extensive NMR data acquired for component **c** (25%) from an enriched fraction (**b** + **c**) are nevertheless sufficient to assign the structure as 7-fluoroverticilla-3(4),7(8),12(13)-triene (**23**) that would be expected to accompany the exocyclic isomer. Two vinyl protons at δ_H 5.79 and 5.48 indicate the presence of two trisubstituted double bonds, and five 3H singlets (δ_H 0.80, 0.87, 1.30, 1.45, 2.01) are attributed to two quaternary and three vinyl methyl groups. The similarity of the spectrum and data to those of the parent diterpene *endo*-verticillene (**21**) obtained from the verticillol dehydration support this assignment (see SI).

The appearance of typical signals for C=CH_2 (δ_H 4.80−4.81, 2 overlapping H), two C−CH_3 (δ_H 0.93, 1.03), and two C=C−CH_3 (δ_H 1.39, 1.82) in the proton NMR spectrum for component **b** (26%), together with the absence of a peak in the region expected for a vinyl hydrogen on a trisubstituted double bond, point to 7-fluoroverticilla-4(20),7(8),11(12)-triene (**24**). The parent diterpene is in fact known,[35] and the reported NMR data match well with those of this fluoro product.

Stereochemistry and Conformation of *exo*-Fluoroverticillene (22). The NMR couplings (Table 1) and NOE values (Figure 4) observed in the spectra of *exo*-fluoroverticillene (**22**) provide conclusive evidence for the H1−H11 trans stereochemistry of the bridgehead hydrogens, and a chair conformation for the methylenecyclohexane A ring of the bicyclo[9.3.1]-pentadecane nucleus with H1 equatorial and H11 axial as shown in the Figure 4. The 180° dihedral relationship between H13α and H14β is apparent from the large vicinal coupling constant (av $J_{13\alpha/14\beta}$ = 13.6 Hz) for these trans diaxial hydrogens and reduced couplings for their equatorial H13β and H14α partners.[36] The large NOE (20%) observed at the superimposed 11α and 13α protons upon irradiation of the H3 vinyl proton shows that the 3,4 double bond is folded underneath the six-membered ring. This position requires that the exocyclic C1−C2 bond be axial, confirming the trans relationship of the equatorial proton at C1 and the axial proton at C11. Inspection of molecular models for the H1−H11 cis stereoisomer of *exo*-7-fluoroverticillene shows that no conformations of the nonadienyl bridge can be attained that bring H3, H11, and H13α into close proximity as required by the NOE results.

The proton NMR assignments were confirmed by the COSY map of coupling interactions and other NOEs determined for

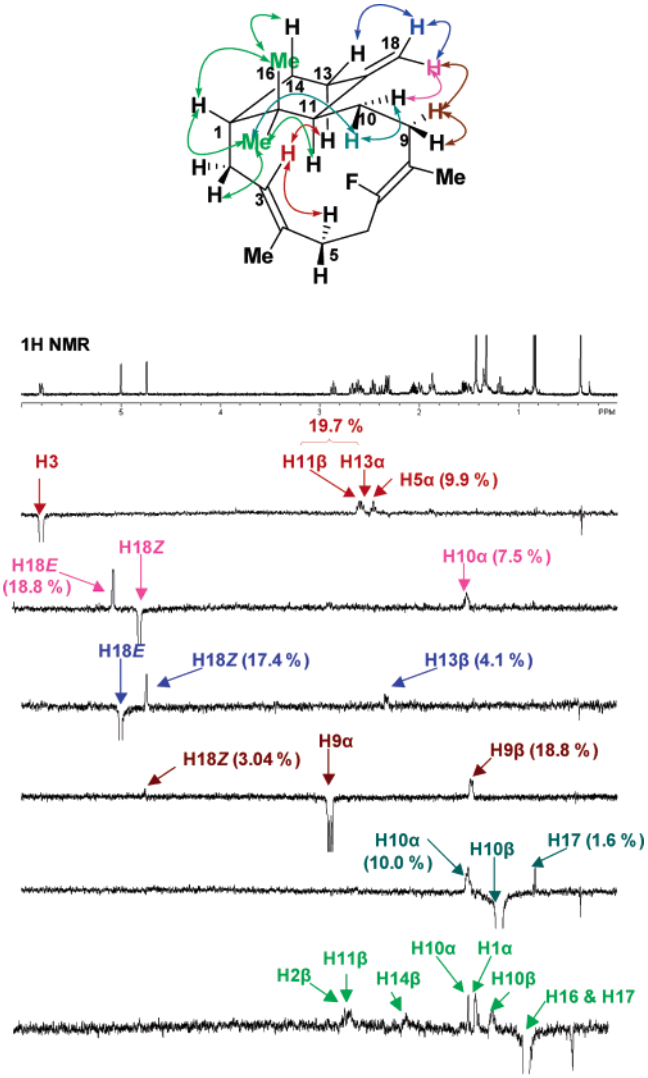


Figure 4. NOE enhancements and correlations in the 600 MHz ^{1}H NMR spectrum of *exo*-7-fluoroverticillene (**22**) in C_6D_6.

exo-fluoroverticillene (**22**). For example, the COSY shows cross-peaks for the following coupling interactions on the A ring: H13β (2.32) ↔ H13α (2.60) ↔ H14β (2.06) ↔ H14α (1.55) ↔ H1 (1.35). The spatial proximity of H18E (5.00) and H13β (2.32) was verified by the 4.1% NOE observed upon irradiation of the former. The 39.9 Hz coupling between the vinyl fluoro substituent and H6β indicates an antiperiplanar disposition of the C−F and C−H bonds.[37] The very similar coupling interactions and NOEs determined for *exo*-verticillene (**20**) and (+)-verticillol **4** (Tables 1 and 2 and Supporting Information) point to very similar conformations of these diterpenes. The unusually low chemical shift for the H7 vinyl proton (4.71) of *exo*-verticillene is attributed to its position just below the exocyclic double bond and the resulting shielding influence of the π electrons. The X-ray crystal structure of verticillol diepoxide previously established the trans relationship of the bridgehead protons.[12]

The H1β absolute stereochemistry of the fluoroverticillene products **22**−**24** is assumed to be the same as that of taxadiene (**5**), the product of the enzyme-catalyzed cyclization of GGPP

(35) Basar, S.; Koch, A.; Konig, W. A. *Flavour Fragrance J.* **2001**, *16*, 315−318.
(36) Grenier-Loustalot, M. F.; Lectard, A.; Lichanot, A.; Metras, F. *Org. Magn. Reson.* **1977**, *10*, 86−91.
(37) Emsley, J. W.; Phillips, L.; Wray, V. *Prog. Nucl. Magn. Reson. Spectrosc.* **1976**, *10*, 83−756.

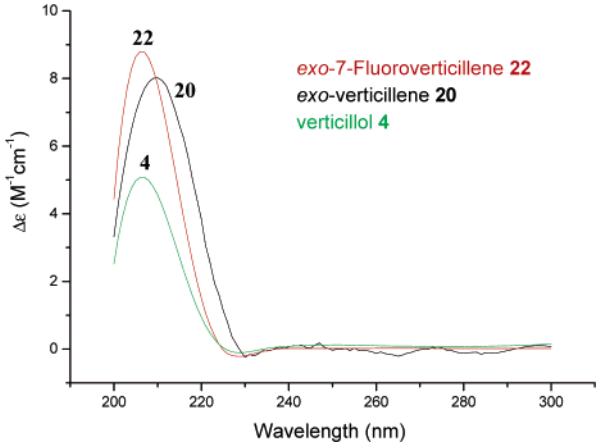


Figure 5. CD spectra of (+)-verticillol (**4**), *exo*-verticillene (**20**), and *exo*-fluoroverticillene (**22**).

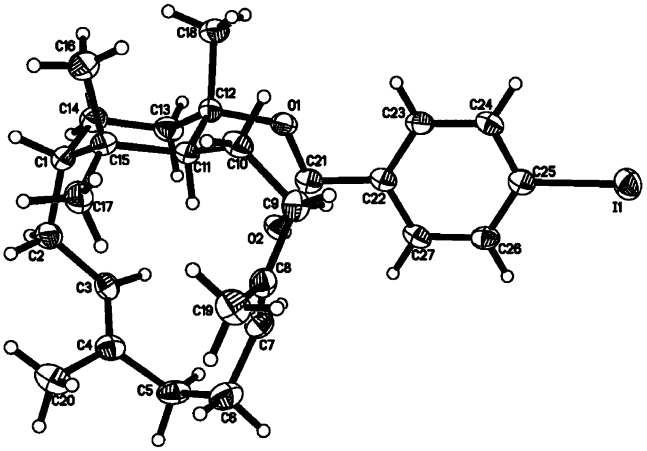


Figure 6. ORTEP drawing of the *p*-iodobenzoate derivative of (+)-verticillol (**4**), depicting the revised absolute configuration (1*S*,11*S*).

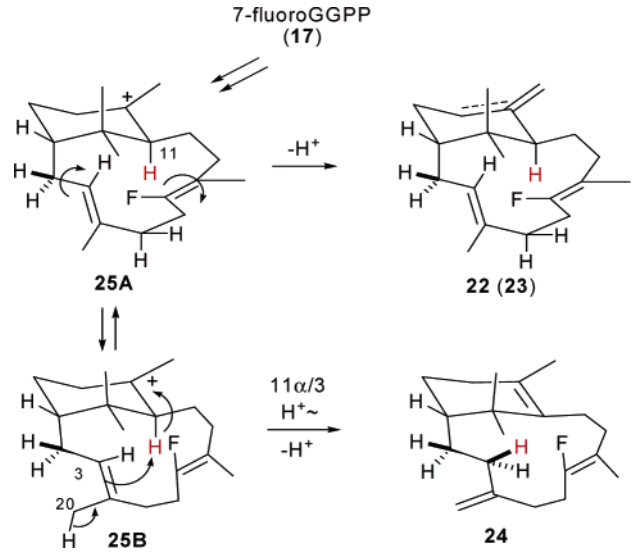
(**7**). It was therefore surprising to find that the circular dichroism curves of *exo*-fluoroverticillene (**22**), *exo*-verticillene **(20)**, and (+)-verticillol (**4**) are all positive in sign and have similar shapes and amplitudes (Figure 5). The absolute configuration of (+)-verticillol was assigned in 1978 to be enantiomeric to the taxanes on the basis of a positive Cotton effect observed for the 18-norverticillan-18-one diepoxide, and the predicted dominance of the diepoxy-nonane atoms in a positive octant.[12] The structure and relative stereochemistry of the related verticillol diepoxide were established by X-ray analysis.

In the meantime a number of other verticillane diterpenes having the same absolute configuration[35] and the enantiomeric stereochemistry, including (−)-verticillol itself, have been reported.[38,39] However, to our knowledge no independent evidence about absolute configuration has been presented for any of these natural products, with the exception of the related phomactatriene,[40] the stereochemistry of which is reasonably assumed to be the same as that of phomactin.[41]

The absolute stereochemistry of (+)-verticillol was reinvestigated by X-ray analysis. For this purpose, the *p*-iodobenzoyl derivative was prepared and crystallized from methanol. The structure was solved by the direct method and refined by full-matrix least-squares on F^2. The refinement converged with a wR2 value of 0.0592 using all data and an R1 value of 0.0325 for observed reflections. The configurations (1*S*,11*S*) were established by anomalous scattering of the iodine atom with the absolute structure parameter −0.016(16) (Figure 6).

Additionally, the revision of the absolute configuration for (+)-verticillol (**4**) leads inevitably to the conclusion that all absolute configurations for verticillane diterpenes in the literature should be reversed. That is, (+)-verticillol from *Sciadopitys verticillata*[12] must have the same H1β configuration as the taxanes, and (−)-verticillol from the Japanese liverwort *Jackiella javanicai* must have H1α at the bridgehead position.[39b]

Scheme 2



Discussion

This work has established that 6-fluoroGGPP undergoes enzyme-catalyzed cyclization in the presence of taxadiene synthase to a series of fluorobicyclo[9.3.1]pentadeca-3,7-decatrienes, i.e., fluoroverticillenes **22**, **23**, and **24** (eq 2). The major product was identified as *exo*-7-fluoroverticillene **22**. The fluoro substituent on the 6,7 double bond of the acyclic substrate analogue (**7** vs **17**) evidently decreases the π basicity sufficiently to prevent the proton reincorporation at this position and thereby blocks further cyclization that normally forms the tricyclo-[9.3.1.$0^{3,8}$]pentadecane nucleus of the taxanes. Thus the 7-fluoroverticillen-12-yl$^+$ ion intermediate **25A** has freedom to undergo other reactions including exocyclic and endocyclic eliminations and competing proton transfer from H11α to the 3,4 double bond setting the stage for elimination into the C20 methyl group (Scheme 2).

The effectiveness of the fluoro analogue in blocking the cyclization leading to the C-ring is presumably associated with the inductive electron-withdrawing influence of this highly

(38) (a) Shi, Q.-W.; Oritani, T.; Sugiyama, T. *Nat. Prod. Lett*. **1999**, *13*, 81−88. (b) Shi, Q.-W.; Oritani, T.; Sugiyama, T. *Planta Med.* **1999**, *65*, 356−359. (c) Duh, C.-Y.; El-Gamal, Ali Ali H.; Wang, S.-K.; Dai, C.-F. *J. Nat. Prod*. **2002**, *65*, 1429−1433.

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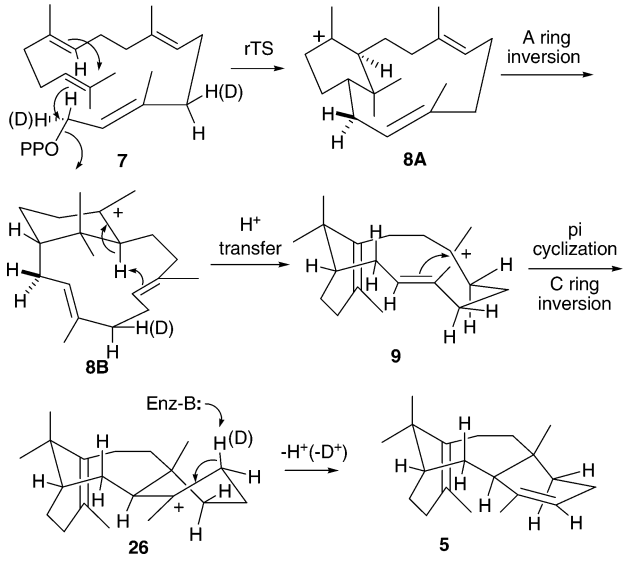
(40) (a) Chu, M.; Truumees, I.; Gunnarsson, I.; Bishop, W. R.; Kreutner, W.; Horan, A. C.; Patel, M. G.; Gullo, V. P.; Puar, M. S. *J. Antibiot.* **1993**, *46*, 554−563. (b) Tokiwano, T.; Fukushi, E.; Endo, T.; Oikawa, H. *Chem. Commun.* **2004**, 1324−1325.

(41) (a) Sugano, M.; Sato, A.; Iijima, Y.; Oshima, T.; Furuya, K.; Kuwano, H.; Hata, T.; Hanzawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 5463−5464. (b) Sugano, M.; Sato, A.; Iijima, Y.; Furuya, K.; Haruyama, H.; Yoda, K.; Hata, T. *J. Org. Chem.* **1994**, *59*, 564−569.

Scheme 3



electronegative group on the electron density in the Δ^7 double bond. The substantial rate-retarding effect of allylic and vinyl fluoro substituents on the solvolytic reactivity of various fluoro geranyl methanesulfonates is clearly documented.[20a,b,42] However, it is also well-known that the fluoro group is an ortho−para director (σ^+ −0.07) in electrophilic aromatic substitution[43] and is a useful stabilizing group for propagating carbocation polyene cyclizations.[44] In the present case, the activation energy for the proton transfer to the Δ^7 double bond bearing the fluoro group must be substantially increased, allowing time for slower vicinal proton eliminations and the alternative 11 → 3 proton transfer to occur (Scheme 2). Proton transfer to the double bond bearing the fluoro substituent would produce a 7-fluoro derivative of verticillenyl$^+$ ion **8**, which would be destabilized by the inductive effect of the adjacent C−F bond. Alternatively, the conformations of the fluoroverticillen-12-yl$^+$ ion **25A** and **25B** in the TS active site might well be altered to disfavor an intramolecular or an intermolecular proton transfer to C7.

The trans bridgehead stereochemistry of *exo*-7-fluoroverticillene **(22)** and the H1β absolute configuration of the taxanes are assumed to be imposed by the shape of the taxadiene synthase active site. It follows that the normal cyclization mechanism proceeds through a 1β,11α-verticillen-12-yl carbocation (**8**) intermediate as proposed previously.[17] A plausible mechanism is illustrated in Scheme 3. Deuterium-labeling results[18] prove that C1 of GGPP undergoes inversion and antiperiplanar addition occurs across the 14,15 double bond in forming the verticillen-12-yl$^+$ ion intermediate initially with ring A in a boat conformation (**8A**). Conformational inversion of the six-membered ring to a chair form (**8B**) with H11α axially disposed would allow an intramolecular proton transfer to the *re*,*re* face of the 7,8 double bond. The NMR coupling and NOE data for *exo*-7-fluoroverticillene (**22**) support a conformation in which H11α is situated close to the 7,8 double bond (Figure 4). Bond rotations of the nonadienyl bridge would enable close approach of the 3,4 double bond to C8 and C−C bond formation to occur on the *re* face of C3, as required by the formal syn relationship of the proton transferred and C3−C8 interannular bond. Inversion of the C ring of the taxen-4-yl$^+$ intermediate (**26**) to a twist boat conformation would be followed by stereoelectronically favorable, although ostensibly higher energy, elimination of H5β as illustrated in Scheme 3.

Intramolecular proton transfers have also been documented to occur in the cyclization mechanisms leading to the sesquiterpene pentalenene and the diterpene abietadiene by similar isotope labeling experiments.[45] In all three known cases, the H$^+$ transfer could occur by geometrically feasible intramolecular paths, i.e., 1,2, 1,4, and 1,5 hydrogen-bridged transition states. However, there is at present no evidence that would exclude mechanisms in which the proton is transferred to an acceptor on the cyclase active site interiors and then is later reincorporated at a different position, thus continuing the cyclization or rearrangement process. Perhaps the simpler mechanism of direct, intramolecular proton migration should be favored until further evidence is available. We hope that X-ray crystallographic structures of these unique cyclases may provide evidence for or against true intramolecular proton transfers or enzyme-mediated mechanisms.

The formation of 7-fluoroverticilla-4(20),7(8),11(12)-triene (**24**) is intriguing and probably of biosynthetic significance. This isomer could be formed by an intramolecular 11α → C3 proton transfer followed by elimination into the C20 methyl group as illustrated above in Scheme 2. The H$^+$ transfer could take place through a 1,5 hydrogen-bridged transition state analogous to the one depicted in Scheme 3. Thus rotations about the 2,3-, 6,7-, and 8,9- bonds would bring C3 into the proximity of the axial 11α hydrogen. However, once again an indirect mechanism in which the proton is transferred to the protein interior and back again to C3 would lead to the same outcome. It seems reasonable to propose that the known verticillane diterpenes[35,38c] having the unusual exocyclic 4(20) double bond arise by similar mechanisms.

The same positive sign and similar shapes of the CD curves for *exo*-fluoroverticillene **(22)**, *exo*-verticillene (**20**), and (+)-verticillol together with the close correspondence of their NMR spectral data led us to surmise that all three compounds probably have similar conformations and the same H1β-H11α absolute stereochemistry. The revised absolute configuration of (+)-verticillol was verified by X-ray crystallographic analysis.[46] Since the absolute configurations of all known verticillane diterpenes have evidently been assigned by reference to (+)-**4**, their stereochemistry needs to be reevaluated.

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(43) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3*rd* ed.; Harper and Row: New York, 1987; p 144.
(44) (a) Johnson, W. S.; Chenera, B.; Tham, F. S.; Kullnig, R. K. *J. Am. Chem. Soc.* **1993**, *115*, 493−497. (b) Johnson, W. S.; Fletcher, V. R.; Chenera, B.; Bartlett, W. R.; Tham, F. S.; Kullnig, R. K. *J. Am. Chem. Soc.* **1993**, *115*, 497−504.
(45) (a) Cane, D. E.; Weiner, S. W. *Can. J. Chem.* **1994**, *72*, 118−127. (b) Ravn, M. M.; Coates, R. M.; Jetter, R.; Croteau, R. B. *Chem. Commun.* **1998**, 21−22. (c) Ravn, M. M.; Coates, R. M.; Flory, J. E.; Peters, R. J.; Croteau, R. *Org. Lett.* **2000**, *2*, 573−576.
(46) The reasons behind the original incorrect absolute configurational assignment (*ent*-**4**) for (+)-verticillol based on the strong positive Cotton effect in the ORD of its keto diepoxide derivative and the Octant Rule[12] are unclear at this time. The probable conformation of the enantiomeric keto diepoxide derivative (C1−C2 and C10−C11 exocyclic bonds axial and equatorial, respectively, as in Figure 6) should have the mobile diepoxy nonane macrocycle in a positive octant, albeit with uncertainty in the exact atomic positions. The anticipated positive contribution from the macrocycle should be offset by the levorotatory effects of the rigid axial and equatorial methyl groups on the cyclohexanone ring. It seems plausible that an anomolaous rotatory contribution might be engendered by a through-space interaction of the n electrons of the 2,3-epoxide with the cyclohexanone carbonyl during the n → π^* excitation.

Conclusion

The mechanism of the enzyme-catalyzed cyclization of GGPP to taxadiene was studied by incubation of the substrate analogue 6-fluoro GGPP with recombinant taxadiene synthase. The electron-withdrawing effect of the fluoro substituent effectively retarded the 11 → 7 proton transfer that normally occurs and led to formation of three major, partially cyclized fluoroverticillene products. The stereochemistry of *exo*-7-fluoroverticillene (**22**) with the H1β,H11α (1*S*,11*R*) configurations at the bridgehead positions provides evidence for a verticillen-12-yl carbocation intermediate **8** with an 11*R* stereocenter. The H11α stereochemistry of this bicyclic intermediate would allow close approach of the bridgehead proton to the 7,8 double bond, consistent with a direct, intramolecular 1,5 proton transfer from C11 to C7. Vinyl fluoro analogues of isoprenoid diphosphate substrates for terpenyl cyclases offer an attractive approach to intercept multistep polyene cyclizations.

Experimental Section

Representative preparative procedures and characterization data for 6-fluoro-GGPP (**17**), taxadiene synthase incubation products, and verticillol *p*-iodobezoate are given below. General experimental aspects, as well as procedures and characterization data for the other compounds, are available in Supporting Information.

(2*E*,6*Z*,10*E*)-6-Fluoro-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-ol (16, X = OH). The procedure reported by Sum and Weiler was followed.[24,30c] A solution of ester **15** (56 mg, 9:1 mixture of trans and cis isomers, 0.16 mmol) in toluene (1 mL) was stirred and cooled at −78 °C, as DIBAL-H (479 μL, 0.48 mmol, 1.0 M in hexane) was added. TLC showed that the ester was all consumed after 30 min. The reaction was quenched by the addition of MeOH (200 μL). The mixture was allowed to warm to room temperature and diluted with saturated NH_4Cl (10 mL). Aqueous HCl (1 M, 10 mL) was added to break the emulsion. The organic layer was separated, and the aqueous layer was extracted with ether (3 × 20 mL). The combined ether extracts were washed with saturated NaCl (3 × 50 mL), dried over Na_2SO_4, and concentrated to give the crude alcohol as a light yellow oil. Purification by flash column chromatography (10% EtOAc in hexane) afforded the cis isomer (3 mg) and the pure fluoro alcohol **16** (43 mg, 98%). Data for the cis isomer: TLC R_f 0.41 (30% EtOAc in hexane); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.57 (d, 3H, J = 2.5 Hz, CH_3), 1.61 (s, 6H, CH_3), 1.69 (s, 3H, CH_3), 1.77 (s, 3H, CH_3), 1.97−2.00 (m, 2H, CH_2), 2.05−2.09 (m, 6H, CH_2), 2.26−2.37 (m, 4H, CH_2), 4.12 (br d, 2H, J = 7.0 Hz, CH_2OH), 5.11 (t of septet, 1H, J = 7.1, 1.4 Hz, vinyl *H*) 5.13 (br t, 1H, J = 6.9 Hz, vinyl *H*), 5.48 (br t, 1H, J = 7.2, vinyl *H*); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 15.8 (d, J = 6.3 Hz), 16.2,17.9, 23.5, 25.9 26.4, 26.9, 27.5 (d, J = 30.4 Hz), 29.1, 29.9 (d, J = 7.6 Hz), 39.9, 59.2, 111.8 (d, J = 16.6 Hz), 124.0, 124.6, 125.4, 131.6, 135.7, 139.0, 153.9 (d, J = 242.1 Hz); ^{19}F NMR ($CDCl_3$, 470 MHz) δ −113.4 (t, J = 21.1 Hz). Data for **16**: TLC R_f 0.34 (30% EtOAc in hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.58 (d, 3H, J = 2.7 Hz, CH_3), 1.61 (s, 6H, CH_3), 1.69 (s, 3H, CH_3), 1.71 (d, 3H, J = 0.9 Hz, CH_3), 1.97−2.00 (m, 2H, CH_2), 2.05−2.09 (m, 6H, CH_2), 2.19−2.22 (m, 2H, CH_2), 2.30−2.38 (m, 2H, CH_2), 4.16 (br d, 2H, J = 7.1 Hz, CH_2OH), 5.11 (t of septet, 1H, J = 7.3, 1.5 Hz, vinyl *H*) 5.13 (br t, 1H, J = 6.7 Hz, vinyl *H*), 5.44 (t of sextet, 1H, J = 7.0, 1.2 Hz, vinyl *H*); ^{13}C NMR ($CDCl_3$, 127 MHz) δ 15.7 (d, J = 6.0 Hz), 16.2, 16.4, 17.9, 25.9, 26.4, 26.9, 27.6 (d, J = 29.6 Hz), 29.9 (d, J = 7.3 Hz), 36.7, 39.9, 59.6, 111.8 (d, J = 17.1 Hz), 124.1, 124.2, 124.6, 131.5, 135.6, 139.0, 154.1 (d, J = 242.7 Hz); ^{19}F NMR ($CDCl_3$, 470 MHz) δ −113.2 (t, J = 22.9 Hz); LR-MS (EI) *m*/*z* 308.2; HR-MS (EI) calcd for $C_{20}H_{33}FO$ 308.2504, found 308.2502.

(2*E*,6*Z*,10*E*)-1-Chloro-6-fluoro-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraene (16, X = Cl). Alcohol **16** was converted to the corresponding allylic chloride using the general procedure of Meyers.[31] A solution of alcohol **16** (19 mg, 0.062 mmol) containing LiCl (26 mg, 0.62 mmol) and *s*-collidine (75 mg, 0.62 mmol) in DMF (1.5 mL) was stirred and cooled at 0 °C. Methanesulfonyl chloride (21 mg, 0.19 mmol) was added slowly, and the mixture was stirred for 3.5 h at 0 °C. Ice water (30 mL) was added, and the mixture was extracted with cold pentane (3 × 30 mL). The combined organic extracts were washed with saturated $Cu(NO_3)_2$ (3 × 50 mL), brine (50 mL), saturated $NaHCO_3$ (50 mL), and brine (50 mL), and dried over anhydrous Na_2SO_4. Removal of the solvent in vacuo gave the chloride (20 mg, ∼100%) as a yellow oil, which was used without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.58 (d, 3H, J = 2.9 Hz, CH_3), 1.62 (s, 6H, CH_3), 1.70 (d, 3H, J = 1.3 Hz, CH_3), 1.76 (d, 3H, J = 1.3 Hz, CH_3), 1.97−2.00 (m, 2H, CH_2), 2.04−2.11 (m, 6H, CH_2), 2.22−2.25 (m, 2H, CH_2), 2.31−2.39 (m, 2H, CH_2), 4.10 (d, 2H, J = 8.1 Hz, CH_2-Cl), 5.11 (t of septet, 1H, J = 6.9, 1.4 Hz, vinyl *H*), 5.13 (br t, 1H, J = 7.0 Hz, vinyl *H*), 5.48 (t of sextet, 1H, J = 7.8, 1.3 Hz, vinyl *H*); ^{13}C NMR ($CDCl_3$, 127 MHz) δ 15.7 (d, J = 6.0 Hz), 16.2, 16.4, 17.9, 25.9, 26.4, 26.9, 27.6 (d, J = 29.6 Hz), 29.87, 29.92, 36.7, 39.9, 59.6, 111.8 (d, J = 17.1 Hz), 124.1, 124.2, 124.6, 131.5, 135.6, 139.0, 154.1 (d, J = 242.7 Hz); ^{19}F NMR ($CDCl_3$, 470 MHz) δ −113.6 (t, J = 23.1 Hz).

(2*E*,6*Z*,10*E*)-6-Fluoro-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl Diphosphate, Triammonium Salt (17). The procedure by Poulter was followed.[32] A suspension of powdered 3 Å molecular sieves (135 mg), HOPP(NBu_4)$_3$ (119 mg, 0.12 mmol), and the above chloride (20 mg, 0.06 mmol) in CH_3CN (2 mL) was stirred at room temperature for 24 h. The suspension was diluted with CH_3CN (10 mL). The solids were filtered and washed with CH_3CN (40 mL), and the filtrate was washed with pentane (3 × 50 mL). The CH_3CN layer was concentrated to give the Bu_4N salt of diphosphate **17** as an orange oil (140 mg, calibrated yield ∼52%), which was contaminated by inorganic pyrophosphate (1.6:1 mixture of PPi and organic diphosphate). A 49-mg portion was converted to the corresponding triammonium salt by ion exchange chromatography using cation-exchange resin (Aldrich, Dowex AG 50 W-X8, 100−200 mesh) and buffer solution (30 mL, 5:1 v/v 2 M $(NH_4)_2CO_3$/1-propanol). The buffer solution was lyophilized to give a white solid containing inorganic phosphate and probably some $(NH_4)_2CO_3$. Most of the inorganic diphosphate and $(NH_4)_2CO_3$ was removed by washing the crude product with MeOH (4 × 6 mL). The triammonium salt of diphosphate **17** was obtained as a white solid with a little contamination (6 mg, 14:1 mixture of organic diphosphate and PPi, overall 51%). NMR data for **17**: ^{1}H NMR (CD_3OD, 400 MHz) δ 1.55 (d, 3H, J = 2.7 Hz, CH_3), 1.57 (br s, 6H, 2 CH_3), 1.63 (d, 3H, J = 0.8 Hz, CH_3), 1.68 (s, 3H, CH_3), 1.91−1.95 (m, 2H, CH_2), 2.01−2.06 (m, 6H, 6 CH_2), 2.12−2.16 (m, 2H, CH_2), 2.26−2.36 (m, 2H, CH_2), 4.49 (t, 2H, J = 6.4 Hz, CH_2OPP), 5.06 (t of septet, 1H, J = 7.1, 1.3 Hz, vinyl H), 5.08 (m, 1H, vinyl H), 5.42 (t of sextet, 1 H, J = 6.7, 1.1 Hz, vinyl H); ^{31}P NMR (CD_3OD, 162 MHz) δ −8.12 (d, J = 18.7 Hz), −8.78 (d, J = 19.1 Hz); ^{19}F NMR (CD_3OD, 376 MHz) δ −114.3 (t, J = 23.1 Hz); LR-MS (ESI) *m*/*z* 467.4; HR-MS (ESI) calcd for $C_{20}H_{34}FP_2O_7$ 467.1764, found 467.1749.

Incubation of 6-FluoroGGPP with rTS. The enzyme incubation was carried out as described by Croteau.[14b] Taxadiene synthase solution (3.6 mL, 3.0 mg protein) was diluted to 300 mL with standard assay buffer consisting of 25 mM tris-HCl, 5 mM dithiothreitol, 1 mM $MgCl_2$, and 10% (v/v) glycerol. The assay solution was gently mixed as 6-fluoroGGPP triammonium salt **17** (8 mg, 0.015 mmol, 50 μM) was added followed by a pentane overlay (5 mL). After incubation for 36 h at 31 °C, the olefin products were extracted into pentane (3 × 200 mL). The pentane extract was evaporated under a nitrogen stream until ∼ 5 mL of solvent remained, which was passed through a pipet column of silica gel, overlayed by anhydrous $MgSO_4$, prior to GC and GC/MS analysis. GC/MS (Figure 3) showed products **a**−**e** (t_R 16.16 min, 7%; t_R 16.32 min, 26%; t_R 16.45 min, 25%; t_R 16.48 min, 37%; t_R 16.61 min, 5%), all of which displayed a molecular ion peak at *m*/*z* 290. The

total yield of **a**−**e** was 468 μg (∼11%) based on GC calibration using *ent*-kaurene as an internal standard. The olefin fractions were repeatedly purified by flash column chromatography (in pipet columns) on silica gel using 100% pentane as eluent. Component **b**, the least polar major product (GC t_R 16.32 min, TLC R_f 0.50) was separated almost cleanly (∼90%) and identified as 7-fluoroverticilla-4(20),7(8),11(12)-triene (**24**): ^{1}H NMR (C_6D_6, 600 MHz) δ 0.93 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.35−1.46 (m, 3H), 1.39 (br d, 3H, J = 1.4 Hz), 1.67−1.61 (m, 1H), 1.82 (s, 3H, CH_3), 1.86−1.76 (m, 2H), 2.32−2.07 (m, 9H), 2.82 (dd, 1H, J = 15.3, 11.3 Hz), 3.34 (td, 1H, J = 13.3, 4.5 Hz), 4.80−4.81 (m, 2H). Component **c** (GC t_R 16.45 min, TLC R_f 0.42) was obtained as a 2:1 mixture with product **24** and identified as *endo*-7-fluoroverticillene (**23**): ^{1}H NMR (C_6D_6, 500 MHz) δ 0.80 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 1.20 (td, 1H, J = 14.1, 4.9 Hz), 1.30 (br s, 3H), 1.45 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 1.30−2.71 (m, 12H), 2.82 (tdd, 1H, J = 13.7, 5.0, 1.5 Hz), 5.48 (br s, 1H), 5.59 (br d, 1H, J = 11.9 Hz). (See also SI for ^{1}H NMR spectra of **23** and **24**). Component **d** (GC t_R 16.48 min, TLC R_f 0.33 in 100% pentane) was identified as *exo*-7-fluoroverticillene (**22**). ^{1}H NMR (see Table 1); CD $\Delta\epsilon$ = +8.0 (210 nm, pentane, c 8.2 × 10^{-5}) (Figure 5).

(+)-Verticillol *p*-Iodobenzoate. A solution of (+)-verticillol (20 mg, 0.067 mmol) in THF (1 mL) was stirred and cooled at 0 °C as *n*-BuLi (76 μL, 1.0 M in hexane, 0.076 mmol) was added dropwise. After 40 min at 0 °C, *p*-iodobenzoyl chloride (21 mg, 0.080 mmol) in THF (1 mL) was added. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. The reaction was quenched by adding saturated NH_4Cl (3 mL). The mixture was extracted with ether (3 × 10 mL). The combined ethereal extracts were washed with brine (1 × 15 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. Purification by flash column chromatography on silica gel (gradient elution with 100% hexane → 10% EtOAc in hexane) provided verticillol *p*-iodobenzoate as a white solid (23 mg, 94%, corrected for recovered **4**) and unreacted verticillol **4** (6 mg, 70% conversion). Product data: TLC R_f 0.67 (30% EtOAc in hexane); $[\alpha]^{23}_D$ +102 (c 0.2, benzene); ^{1}H NMR (C_6D_6, 500 MHz) δ 0.65 (s, 3H, CH_3), 0.73 (s, 3H, CH_3), 1.20−1.28 (m, 2H), 1.42−1.56 (m, 2H), 1.46 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.72 (s, 3H, CH_3), 1.77−1.87 (m, 2H), 2.00−2.16 (m, 4H), 2.24−2.40 (m, 2H), 2.44 (td, 1H, J = 12.9, 4.2 Hz), 2.62 (dddd, 1H, J = 15.0, 12.7, 6.8, 1.1 Hz), 2.68 (br d, 1H, J = 7.0 Hz), 2.72 (dt, 1H, J = 13.1, 3.8 Hz), 5.20 (br d, 1H, J = 11.5 Hz), 5.84 (br d, 1H, J = 12.6 Hz), 7.35−7.39 (m, 2H), 7.72−7.75 (m, 2H); ^{13}C NMR ($CDCl_3$, 127 MHz) δ 15.76, 16.77, 21.73, 21.80, 27.29, 27,32, 28.26, 28.97, 34.59, 36.04, 38.12, 40.21, 41.63, 43.47, 43.95, 91.93, 100.23, 127.93, 131.11, 131.44, 133.35, 133.41, 133.57, 138.20, 165.97.

X-ray Crystallography and Absolute Stereochemistry (Figure 6). Crystals of (+)-verticillol *p*-iodobenzoate for X-ray analysis were obtained by recrystallization from methanol (1 mL) at room temperature. Crystal data: $C_{27}H_{37}IO_2$, orthorhombic form, space group P 21 21 21, and cell dimensions a = 7.510(3) Å, b = 15.122(6) Å, c = 21.737(9) Å, and Z = 4.

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Supporting Information Available: Experimental procedures, characterization data, reproductions of NMR spectra, and X-ray analysis data; X-ray crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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