Studies on Taxadiene Synthase: Interception of the Cyclization Cascade at the Isocembrene Stage with GGPP Analogs.

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SUPPORTING INFORMATION

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1. General Remarks

Chemicals and reagents used were of reagent grade, purchased from general suppliers, and used without further purification, unless noted otherwise. Deionized, doubly-distilled water (ddH$_2$O) was used for the preparation of GGPP, GGPP analogs, enzymes and buffer. Anhydrous THF and diethyl ether were obtained by distillation from sodium/ benzophenone. Anhydrous acetonitrile, dichloromethane, chloroform, DMF were purchased from Aldrich, and used without further purification.

Water-sensitive reactions were performed in either flame-dried or oven-dried (oven-dried, 95°C) glassware cooled to room temperature in an argon blanket. Butyl lithium was titrated with 0.1 M N-benzylbenzamide in anhydrous THF at -40°C. The endpoint was reached when the mixture remained deep blue for more than twenty seconds.

TLC was performed on UV-254 TLC plates, coated with silica gel on polyester. Column chromatography was performed on silica gel 60-200 mesh. TLC plates were visualized under UV light at 254 nm, or stained with aqueous KMnO$_4$, 10% phosphomolybdic acid in ethanol, or anisaldehyde solution.

NMR spectra were recorded in CDCl$_3$, D$_2$O or d$_6$-acetone at 500 MHz for $^1$H, 125 MHz for $^{13}$C, and 202 MHz for $^{31}$P. $^{13}$C chemical shifts were referenced to 77.0 (CDCl$_3$) or 0.00 (DSS in D$_2$O); $^1$H chemical shifts were referenced to 7.26 (residual CHCl$_3$ in CDCl$_3$) or 0.00 (DSS in D$_2$O); $^{31}$P chemical shifts were referenced to external phosphoric acid (δ 0.00) in D$_2$O.

Preparative GC was performed on a 12-feet, 4 mm internal diameter column packed with 3% OV 101 on Chromosorb 750 (100-120 mesh), operating with a nitrogen
flow rate of 90 mL/min at approximately 200 °C, adjusted to obtain optimal resolution of peaks. GCMS analyses were performed using a 30M, 0.25 mm ID, 0.25 μM DB-5MS column, programmed 120 °C for 2.00 min, to 280 °C at 5 °C/min, final time 15 min.

2. Experimental Procedure

\[
\begin{align*}
14, \ X = \text{Br} \\
15, \ X = \text{I} \\
16, \ X = \text{SO}_2\text{Tol}
\end{align*}
\]

To a solution of 14 (1.00 g, 4.6 mmol) in 35 mL acetone was added NaI (0.75 g, 5.0 mmol). The reaction was sealed, and stirred overnight in the dark. Hexane (~35 mL) was added, and the product mixture was concentrated to about 5 mL. Hexane (~50 mL) was added again, and the product mixture was filtered. The filtrate was transferred to a dry flask, and was concentrated in vacuo to give 1.13 g citronellyl iodide 15 (4.3 mmol, 93%). Citronellyl iodide 15 was dissolved in 10 mL dry DMF and sodium toluenesulfinate (0.89 g, 5.0 mmol) was added, and the reaction was stirred under an argon blanket at room temperature for 48 hours. Extractive workup with Et₂O and water gave a mixture of two compounds, as analyzed by TLC (2:8 EtOAc: Hexane, UV visualization). The desired product was the major product with the lower Rᵣ, and was purified by column chromatography to give 1.20 g (4.1 mmol, 89 % over two steps) clear liquid. \(^{13}\)C NMR (CDCl₃) δ 144.2, 136.0, 131.1, 129.6, 127.7, 123.9, 54.1, 36.1, 31.1, 28.9, 25.4, 24.9, 21.3, 18.8, 17.3. \(^{1}\)H NMR (CDCl₃) δ 7.77 (d, J=8.3 Hz, 2H), 7.35 (d, J=8.1 Hz, 2H), 5.01 (t, J=7.1 Hz, 1H), 3.05 (m, 2H), 2.44 (s, 3H), 1.89 (m, 2H), 1.74-1.45 (m, 3H), 1.65 (s, 3H), 1.54 (s, 3H), 1.25 (m, 1H), 1.13 (m, 1H), 0.84 (d, J=6.4 Hz, 3H).
HRMS (ESI, M+Li) caled for C_{17}H_{26}O_{2}SLi 301.1814, found 301.1843. [α]_D= +12.2 (c 0.50 CHCl₃)

In a flame-dried flask, sulfone 16 (1.90 g, 6.5 mmol) was dissolved in anhydrous THF (35 mL), and cooled to -40°C using a dry ice/ acetonitrile bath. Butyl lithium (7.1 mmol, titrated) was added dropwise, the reaction turned yellow immediately, and was allowed to stir at -40°C for 30 min. Bromide 17 (2.08 g, 6.5 mmol) dissolved in 10 mL anhydrous THF was added, and the cooling bath was removed. The reaction was allowed to warm to room temperature, and stirred overnight. The reaction was quenched with 5 mL methanol for 1 minute, followed by 35 mL sat. NH₄Cl and extraction with 3 × 50 mL diethyl ether. The organic layers were combined, dried, and concentrated. If unreacted bromide 17 was seen in the TLC (2:8 EtOAc: hexane), then the crude product was subjected to column chromatography to remove (17). Otherwise, the crude coupling product 18 (2.54 g) was used in the next step without purification.

In a 500 mL three neck round bottom flask equipped with a dry-ice condenser (connected to an argon balloon to equalize pressure), 1.4 g (200 mmol) of lithium granules (high
sodium grade) and a stirbar were cooled to -78°C with dry ice/acetone, and ammonia gas was allowed to condense into the flask until approximately 250 mL of blue solution was obtained. The cooling bath was removed, and the mixture was allowed to stir and reflux at room temperature (condenser temperature kept at -78 °C throughout) for 30 min to ensure good dissolution of the metal. The flask was cooled to -78 °C again, and crude compound 18 (2.54 g) dissolved in 10 mL dry THF was injected into the reaction. The cooling bath was removed, and the reaction was allowed to reflux at room temperature for 30 min. The reaction was cooled to -78 °C once again, and solid NH₄Cl, followed by wash ethanol were added very slowly and carefully over 10-20 minutes to quench the reaction. The dry ice bath and the condenser were removed, and the flask was placed in a warm water bath (~ 40°C) to accelerate evaporation of ammonia gas. After most of the ammonia has evaporated the contents of the flask were transferred to a regular one-neck round bottom flask, was concentrated in vacuo. The remaining slurry was dissolved in water (100 mL) and extracted with 3 × 100 mL Et₂O. The organic layers were combined, dried, and concentrated to give a crude yellow oil. The crude product was subjected to column chromatography to give the desired product 19 (0.62 g, 2.1 mmol, 32% over 2 steps). ¹³C NMR is in complete agreement with data by Kato et al.,¹ restated here:

(CDCl₃) δ 139.7, 135.7, 130.9, 125.0, 123.5, 123.3, 59.3, 39.9, 39.5, 37.1, 36.5, 32.2, 26.2, 25.7, 25.5, 25.3, 19.5, 17.6, 16.2, 15.8. ¹H NMR is in agreement with the literature¹ except for minor discrepancies at 2.13-1.00 ppm. ¹H NMR (CDCl₃) δ 5.40 (t, J=6.5 Hz, 1H), 5.09 (m, 2H), 4.15 (d, J=6.9 Hz, 2H), 2.13-1.00 (m, 15H; not stated in the literature),

1.68 (s, 6H), 1.60 (s, 3H), 1.58 (s, 3H), 0.86 (d, J=6.6 Hz, 3H). [α]D=+5.64 (c 10.0 MeOH) for 10,11R-dihydroGGOH 19, and [α]D= -5.82 (c 10.0 MeOH) for 10,11S-dihydroGGOH (synthesized separately), literature [α]D= -3.2 (c 1.00 MeOH) for 10,11S-dihydroGGOH.¹

The procedures for the preparation of compounds 20 and 10 are shown in the article, under the Experimental Section.
10,11S-dihydroGGPP [(Bu$_4$N)$_2$NH$_4$ salt by $^1$H NMR] 11 was synthesized R-citronellyl bromide 21 using the identical procedure described for compound 10. The final product 11 was identical to its enantiomer 10 by $^1$H, $^{13}$C and $^{31}$P NMR.

The multistep preparation of 23 from 22 reaction was performed using the procedure by Joullie et al.$^2$ for a similar compound. To a stirred solution of farnesyl benzyl ether 22 (13.2 g, 42.3 mmol) in t-butanol (210 mL) and water (29 mL), was added N-bromosuccinimide (8.00 g, 45.0 mmol) in one portion. The reaction was allowed to stir for one hour, and TLC (2:8 EtOAc: hexane) showed that the starting material (R$_f$ 0.8) was no longer present, and a new spot (R$_f$ 0.4) corresponding to the bromohydrin S1 was seen, along with byproducts at R$_f$ 0.7. A solution of KOH (5.0 g in 14 mL water) was added, and the reaction was stirred for at least another hour at room temperature. TLC (2:8 EtOAc: hexane) showed that the bromohydrin was no longer present, and a new spot

(R_f 0.6) corresponding to the epoxide S2 was seen. The reaction was concentrated in vacuo to remove t-butanol, and the crude product was redissolved in 100 mL Et_2O and washed with 2x200 mL water to remove KBr. The organic layer was concentrated in vacuo, and the crude product was redissolved in 150 mL THF. Dilute aq. H_2SO_4 (5% v/v, 25 mL) was added to the reaction, and additional water was added as necessary, to make the reaction homogeneous. After stirring for 2 hours, TLC (2:8 EtOAc: hexane) showed that the epoxide spot was no longer present, and a new spot corresponding to the diol product was seen at R_f 0.1 (2:8 EtOAc: hexane). Solid NH_4Cl was added to the reaction to allow phase separation, and the THF layer was separated, dried, and concentrated in vacuo. The crude product was purified by column chromatography to give 4.1 g (11.8 mmol, 28% yield from S2) of the desired diol S3. ^13C NMR (CDCl_3) δ 140.1, 138.2, 134.9, 128.3, 127.8, 127.5, 124.6, 120.8, 77.7, 72.9, 72.0, 66.5, 39.4, 36.5, 29.3, 26.3, 25.9, 23.0, 16.3, 15.8. ^1H NMR (CDCl_3) δ 7.28 (m, 5H), 5.39 (t, J=6.8 Hz, 1H), 5.18 (t, J=6.8 Hz, 1H), 4.51 (s, 2H), 4.01 (d, J=6.7 Hz, 2H), 3.31 (dd, J=10.5 Hz, J=1.5 Hz, 1H), 2.39 (br, 2H, hydroxyls), 2.08 (m, 8H), 1.63 (s, 3H), 1.60 (s, 3H), 1.16 (s, 3H), 1.12 (s, 3H). HRMS (ESI, M+Li) calcd for C_{22}H_{34}O_3Li 353.2668, found 353.2765.

Diol S3 was converted to aldehyde 23 using the procedure by Joullie for a similar compound. To a solution diol S3 (5.19 g, 15.0 mmol) in 75 mL THF was added 30 mL water and NaIO_4 (4.28 g, 20.0 mmol). The mixture was stirred for 2 hours, and white precipitates formed. TLC analysis (2:8 EtOAc: Hexane) indicated that diol S3 was fully consumed, and a new spot corresponding to the desired product was seen at R_f ~0.7. The reaction was diluted with 200 mL Et_2O and washed with sat. aq. NH_4Cl (3×100 mL). The organic layer was dried with MgSO_4, and concentrated in vacuo to give 4.71 g (13.6
mmol, 91%) of aldehyde 23. $^{13}$C NMR (CDCl$_3$) δ 202.6, 139.9, 138.4, 133.2, 128.2, 127.7, 127.4, 124.8, 120.9, 71.9, 66.4, 42.0, 39.2, 31.7, 26.0, 16.4, 16.0. $^1$H NMR (CDCl$_3$) δ 9.71 (t, J=1.8 Hz, 1H), 7.33 (m, 5H), 5.39 (t, J=6.8 Hz, 1H), 5.14 (t, J=6.8 Hz, 1H), 4.50 (s, 2H), 4.02 (d, J=6.7 Hz, 2H), 2.49 (dt, J=7.6 Hz, J=1.8 Hz, 2H), 2.30 (t, J=7.5 Hz, 2H), 2.14-2.02 (m, 4H), 1.63 (s, 3H), 1.60 (s, 3H). HRMS (ESI, M+Li) calcld for C$_{19}$H$_{26}$O$_2$Li 293.2093, found 293.2161.

The Wittig reagent used in this reaction was synthesized as follows: to a solution of triphenylphosphine (13.1 g, 50.0 mmol) in 100 mL EtOAc, was added ethyl-2-bromoacetate (8.85 g, 53.0 mmol), and the mixture was refluxed overnight. The resulting precipitate was collected by filtration and redissolved in a minimal amount of CH$_2$Cl$_2$. With stirring, fresh EtOAc (200 mL) was added dropwise using a funnel to reprecipitate the product. The precipitate was collected by filtration, and washed with 50 mL fresh EtOAc. The precipitate was then dissolved in 200 mL fresh CH$_2$Cl$_2$, and transferred to a separatory funnel. Aqueous KOH (5.6 g in 200 mL water) was added, and the separatory funnel was shaken vigorously to ensure complete mixing. During this step, the CH$_2$Cl$_2$ layer developed a bright yellow color, while the aqueous layer became cloudy white. The CH$_2$Cl$_2$ layer was collected, dried with MgSO$_4$, filtered, and evaporated to give the desired Wittig reagent as a yellow solid (12.2 g, 35.0 mmol, 70%), which could be stored in the dark at room temperature in a closed container.
Unsaturated ester 24 was prepared from aldehyde 23 using the procedure by Coates et al. for a similar compound.³ To a refluxing solution of the Wittig reagent (12.2 g, 35.0 mmol) in 35 mL CH₂Cl₂, aldehyde 23 (7.78 g, 27.2 mmol) was added dropwise. The reaction was allowed to reflux for 4 hours, and TLC (2:8 EtOAc : Hexane) indicated that aldehyde 23 (Rᵣ~0.4) was fully consumed, and a new spot corresponding to the product was seen at Rᵣ~0.5. Solvent was evaporated, and the residue was redissolved in a small amount of CH₂Cl₂, and loaded onto a silica column. Elution with 2:8 EtOAc:Hexane, gave the desired product (8.80 g, 24.7 mmol, 91%). ¹³C NMR (CDCl₃) δ 166.5, 148.7, 140.0, 138.4, 133.6, 128.2, 127.6, 127.4, 124.8, 121.2, 120.8, 71.8, 66.4, 59.9, 39.3, 37.7, 30.6, 26.1, 16.3, 15.8, 14.1. ¹H NMR (CDCl₃) δ 7.31 (m, 5H), 6.93 (dt, J=15.6 Hz, J=6.9 Hz, 1H), 5.80 (d, J=15.6 Hz, 1H), 5.40 (t, J=6.7 Hz, 1H), 5.14 (t, J=6.2 Hz, 1H), 4.49 (s, 2H), 4.16 (q, J=7.1 Hz, 2H), 4.02 (d, J=6.8 Hz, 2H), 2.30-2.00 (m, 8H), 1.64 (s, 3H), 1.60 (s, 3H), 1.27 (t, J=7.1 Hz, 3H). HRMS (ESI, M+Li) calcd for C₂₃H₃₂O₃Li 363.2511, found 363.2620.

In a flame-dried flask, ester 24 (8.80 g, 24.7 mmol) was dissolved in 200 mL anhydrous Et₂O, and the cooled to 0°C. Commercial DiBAL-H solution (54.0 mmol, 1.0 M in hexane) was added using a syringe, and the reaction was allowed to stir at room temperature for two hours. A small volume of the reaction mixture was removed, diluted with hexane, and hydrolyzed for TLC analysis (2:8 EtOAc: hexane). If the starting

material remained, additional DiBAL-H was added at 0°C, and the reaction was stirred for another two hours at room temperature. When all the starting material had reacted, the reaction mixture was cooled to 0°C, and saturated aqueous NH₄Cl was added dropwise using a funnel. During this quenching step, the reaction may boil gently (exothermic liberation of H₂) at 0°C for a short time, and white precipitates would form. Addition of NH₄Cl was stopped when precipitation had stopped. The reaction mixture was diluted with Et₂O, and the precipitate was filtered away. The organic layer was collected, dried with MgSO₄, filtered, and concentrated in vacuo to give the desired product (7.20 g, 22.9 mmol, 93%). ¹³C NMR (CDCl₃) δ 140.2, 138.4, 134.5, 132.6, 129.0, 128.3, 127.8, 127.5, 124.2, 120.7, 71.8, 66.4, 63.5, 39.4, 39.0, 30.6, 26.0, 16.4, 15.9. ¹H NMR (CDCl₃) δ 7.33 (m, 5H), 5.63 (m, 2H), 5.39 (t, J=6.8Hz, 1H), 5.12 (t, J=6.8Hz, 1H), 4.50 (s, 2H), 4.03 (m, 4H), 2.15-2.00 (m, 8H), 1.64 (s, 3H), 1.59 (s, 3H). HRMS (ESI, M+Li) calcd for C₂₁H₃₀O₂Li 321.2406, found 321.2398.

In a flame-dried flask, alcohol 25 (2.36 g, 7.5 mmol) was dissolved in anhydrous CH₂Cl₂ (60 mL). Dry Et₃N (9.8 mmol, 1.4 mL, dried with solid KOH) was added, and the mixture was cooled to -40°C in a dry ice/ acetonitrile bath. Methanesulfonyl chloride (9 mmol, 0.7 mL) was added, and the reaction was stirred at -40°C for one hour. LiBr (18.8 mmol, 1.63 g, kept in 95°C oven) was dissolved in anhydrous THF (60 mL) in a separate dry flask, and this solution was added to the reaction using a cannula. The
reaction was allowed to warm to 0°C, and stirred at 0°C for another hour. The reaction was quenched with 100 mL half-saturated aq. NH₄Cl, and extracted with 3 × 100 mL hexane. The organic layers were combined, dried, and evaporated to give the desired product (2.78 g, 7.4 mmol, 99%). The product was used in the next step without any further purification.

\[ \text{OH} \quad \rightarrow \quad \text{Br} \quad \rightarrow \quad \text{SO}_2\text{Tol} \]

A solution of prenyl alcohol (0.86 g, 10.0 mmol) in 50 mL ether was cooled to 0°C, and PBr₃ (1.37 g, 5.0 mmol, 0.47 mL) was added via a calibrated plastic pipette. The solution was allowed to stir at 0°C for 30 min, and 50 mL saturated NaCl was added to quench the reaction. The ether layer was separated, dried, and concentrated in vacuo to give prenyl bromide (1.40 g, 9.5 mmol, 95%). The product was used immediately in the subsequent step without further purification.

Prenyl bromide was converted to sulfone 27 in 85% yield, by treatment with sodium toluenesulfinate, as described above for the preparation of 16. Here, the reaction time was shortened to 24 hours, because the allylic bromide presented a better leaving group. Data for 27: m.p. 78-80 °C. $^{13}$C NMR (CDCl₃) $\delta$ 144.3, 142.6, 135.5, 129.4, 128.2, 110.3, 56.0, 25.7, 21.5, 17.6. $^1$H NMR (CDCl₃) $\delta$ 7.70 (d, J=8.2 Hz, 2H), 7.30 (d, J=8.1 Hz, 2H), 5.15 (t, J=7.9 Hz, 1H), 3.74 (d, J=7.9 Hz, 2H), 2.41 (s, 3H), 1.69 (s, 3H), 1.30 (s, 3H). HRMS (ESI, M+Li) calcd for C₁₂H₁₆O₂SLi 231.1031, found 231.1065.
Compound 28 was prepared using the similar procedure described for the synthesis of 18. The use of slight excess (1.1-1.2 eq) of sulfone 27 ensured complete consumption of bromide 26. The coupling product 28 was used without purification or analysis.

Alcohol 29 was prepared from 28 using the similar procedure described for the synthesis of 19. The overall yield of alcohol 29 from 25 (three steps) was 44%. $^{13}$C NMR is in complete agreement with the data by Coates, and is restated here: (CDCl$_3$) δ 139.6, 135.1, 131.5, 130.0, 129.9, 124.1, 123.8, 123.3, 59.3, 39.7, 39.5, 32.8, 31.2, 28.2, 26.2, 25.7, 17.7, 16.2, 16.0. $^1$H NMR is in agreement with the literature except for minor discrepancies at 5.40 ppm and 5.11 ppm. $^1$H NMR (CDCl$_3$) δ 5.40 (m, 3H observed; m, 2H literature), 5.11 (m, 2H observed; m, 3H literature), 4.14 (d, J=6.9Hz, 2H), 2.12-2.00 (m, 12H), 1.68 (two singlets, 6H), 1.59 (s, 6H). HRMS (ESI, M+Li) calcd for C$_{19}$H$_{32}$OLi 283.2613, found 283.2449.
11-desmethyl GGPP 12 [1.88 g, 1.5 mmol, 46% (Bu₄N)₃ salt by ¹H NMR] was prepared from the corresponding alcohol 29 (0.91 g, 3.3 mmol) using the modified Poulter procedure as described for the synthesis of 10 (see the Experimental Section of the article). Data for 12: Solvent suppressed ¹H NMR (D₂O) 5.40 (m, 3H), 5.14 (br t, J=6.8 Hz, 1H), 5.10 (br t, J=6.8 Hz, 1H), 4.46 (br t, 4.5 Hz, 2H), 3.17 (m, 24H), 2.15-1.93 (m, 12H), 1.74-1.54 (m, 36H), 1.36 (sextet, J=7.3 Hz, 24H), 0.94 (t, J=7.5 Hz, 36H). ¹³C NMR (D₂O) δ 143.2, 137.1, 133.8, 132.7, 132.3, 127.1, 126.6, 123.3 (d, ³J_P,C=9.8 Hz), 64.9 (d, ³J_P,C=4.4 Hz), 60.7 (Bu₄N), 42.24, 42.15, 35.3, 33.8, 30.7, 29.0, 28.0, 25.7 (Bu₄N), 21.7 (Bu₄N), 20.0, 18.5, 18.2, 15.5 (Bu₄N). ³¹P NMR (D₂O) δ -8.8 (d, J=19.4 Hz), -10.8 (d, J=19.3 Hz). ESIHRMS calcd for C₁₉H₃₃O₇P₂ 435.1702, found 435.1708.

The phosphonate ester (CF₃CH₂O)₂POCHMeCO₂Et shown above was synthesized using the Still-Gennari procedure, and was distilled at 125°C (~25 µmHg) as a clear liquid. ¹³C NMR (CDCl₃) δ 168.3 (d, ²J_P,C=2.7 Hz), 122.4 (dq, ¹J_F,C=277 Hz, ³J_P,C=3.4 Hz), 122.3 (dq, ¹J_F,C=277 Hz, ³J_P,C=2.7 Hz), 62.4 (dq, ²J_F,C=38 Hz, ²J_P,C=5.8 Hz), 61.9, 39.3 (d, ¹J_P,C=140 Hz), 13.5, 11.2 (d, ²J_P,C=6.3 Hz). ¹H NMR (CDCl₃) δ 4.35 (m, 4H), 4.12 (m, 2H), 3.10 (dq, ²J_H,H=22.8 Hz, ³J_H,H=7.4 Hz, 1H), 1.40 (dd, ³J_H,H=19.3 Hz, ³J_H,H=7.5 Hz), 1.19 (t, J=7.2 Hz, 3H). HRMS (ESI, M+Li) calcd for C₉H₁₃F₆O₆PLi 353.0565, found 353.0570.
A solution of \((\text{CF}_3\text{CH}_2\text{O})_2\text{POCHMeCO}_2\text{Et}\) (4.42 g, 12.8 mmol) and 18-crown-6 (6.75 g, 25.5 mmol) in dry THF (175 mL) was cooled to -78°C, and KHMDS (12.8 mmol, 0.5 M solution in toluene) was added, followed by aldehyde 23 (11.6 mmol). After stirring at -78°C for 30 min, the reaction mixture was poured into a rapidly stirring sat. NH4Cl to give 4.05 g (10.9 mmol, 94%) product 31 upon extractive workup. 1H NMR analysis showed that the newly created double bond was predominantly the desired Z-product (≥79:1 cis:trans). If desired, column chromatography was used to obtain 100% pure Z-product. 1H NMR (CDCl₃) δ 7.27 (m, 5H), 5.82 (t, J=7.2 Hz, 1H), 5.33 (t, J=6.8 Hz, 1H), 5.06 (t, J=6.8 Hz, 1H), 4.43 (s, 2H), 4.12 (q, J=7.1 Hz, 2H), 3.96 (d, J=6.7 Hz, 2H), 2.54 (q, J=8.2 Hz, 2H), 2.2-2.0 (m, 6H), 1.81 (s, 3H), 1.57 (s, 3H), 1.53 (s, 3H), 1.23 (t, J=7.1 Hz, 3H). 13C NMR (CDCl₃) δ 168.1, 142.5, 140.4, 138.5, 134.5, 128.3, 127.8, 127.5, 127.0, 124.4, 120.8, 72.0, 66.5, 60.0, 39.5, 39.1, 27.9, 26.2, 20.6, 16.5, 15.8, 14.3. HRMS (ESI, M+Li) calcd for C₂₄H₃₄O₃Li 377.2668, found 377.2759.

Compounds 32, 33, 34, 35, 36, and 13 were synthesized using similar procedures described above for compounds 25, 26, 28, 29, 30, and 12 respectively.

Compound 32: 1H NMR (CDCl₃) δ 7.35 (m, 5H), 5.40 (t, J=6.8 Hz, 1H), 5.27 (t, J=7.3 Hz, 1H), 5.11 (t, J=6.8 Hz, 1H), 4.51 (s, 2H), 4.10 (s, 2H), 4.02 (d, J=6.7 Hz, 2H), 2.15-1.97 (m, 8H), 1.79 (s, 3H), 1.65 (s, 3H), 1.60 (s, 3H). 13C NMR (CDCl₃) δ 140.3,
138.5, 134.8, 134.3, 128.3, 128.2, 127.8, 127.5, 124.4, 120.8, 72.0, 66.6, 61.6, 39.8, 39.5, 26.2 (two overlapping signals), 21.3, 16.5, 16.1. HRMS (ESI, M+Li) calcd for C_{22}H_{32}O_{2}Li 335.2562, found 335.2543.

Compound 33: this compound was used directly in the subsequent step.

Compound 34: the use of slight excess (1.1-1.2 eq) of sulfone 27 ensured complete consumption of bromide 33. The coupling product 34 was used without purification or analysis.

Compound 35: \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 5.42 (dt, J = 6.9 Hz, 1.1 Hz, 1H), 5.10 (m, 3H), 4.14 (d, J = 6.9 Hz, 2H), 2.13-1.96 (m, 12H), 1.68 (overlapping singlets, 9H), 1.61-1.60 (overlapping singlets, 6H). \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 139.7, 135.3, 135.0, 131.5, 124.9, 124.3, 123.7, 123.3, 59.3, 39.9, 39.5, 31.9, 26.6, 26.4, 26.2, 25.7, 23.3, 17.6, 16.2, 15.9. HRMS (ESI, M+Li) calcd for C_{20}H_{34}OLi 297.2770, found 297.2746.

Compound 36: this compound was used immediately in the subsequent step without purification or spectroscopic analysis.

Compound 13 [1.70g, 1.45 mmol, 51\%, (Bu\(_4\)N)\(_3\) salt by \(^1\)H NMR] was prepared from the corresponding alcohol 35 (0.83g, 2.9 mmol) as described for the synthesis of 10 (see the Experimental Section of the article). Solvent suppressed \(^1\)H NMR (D\(_2\)O) 5.41 (br t, J=6.5 Hz, 1H), 5.14 (br t, J=6.5 Hz, 1H), 5.10 (m, 2H), 4.46 (m, 2H), 3.18 (m, 24H), 2.15-1.92 (m, 15H), 1.73-1.55 (m, 39H), 1.36 (sextet, J=7.4 Hz, 24H), 0.95 (t, J=7.3 Hz, 36H). \(^{13}\)C NMR (D\(_2\)O) 142.9, 136.9, 136.7, 133.5, 127.6, 127.0, 126.6, 123.2 (d, \(^3\)J\(_{P,C}=10.0\) Hz), 64.8 (d, \(^2\)J\(_{P,C}=5.6\) Hz), 60.6 (Bu\(_4\)N), 42.5, 42.1, 34.3, 29.0 (overlap of 3 CH\(_2\)), 27.9 (CH\(_3\)), 25.62 (Bu\(_4\)N), 25.60 (CH\(_3\)), 21.6 (Bu\(_4\)N), 19.7 (CH\(_3\)), 18.4 (CH\(_3\)), 18.0 (CH\(_3\)), 15.4 (Bu\(_4\)N). The \(^{13}\)C peaks at 25.62 and 25.60 were resolved by DEPT 135.
experiment (not shown). $^{31}$P NMR (D$_2$O) $\delta$ -8.9 (d, J=19.8 Hz), -10.8 (d, J=19.8 Hz).

HRMS (ESI, M) calcd for C$_{20}$H$_{35}$O$_7$P$_2$ 449.1858, found 449.1879.

Incubations reactions of 11, 12 and 13 with taxadiene synthase were performed using the procedure described for the incubation of 10, described in the Experimental section of the article. The stoichiometry of each reaction is shown in Table 1, and the NMR data for compounds 38-40 are shown in Table 2.

Data for 41: $^1$H NMR (δ, CDCl$_3$) 6.38 (dd, J=17.5, 10.8 Hz, 1H), 5.25 (d, J=17.5 Hz, 1H), 5.15 (t, J=7.30 Hz, 1H), 5.10 (t, J=7.30 Hz, 1H), 5.06 (d, J=10.8 Hz, 1H), 5.01 (br, 1H), 5.00 (br, 1H), 2.30-1.85 (m, 8H), 1.68 (s, 3H), 1.60 (s, 3H), 1.59 (s, 3H), 1.45-1.00 (m, 7H), 0.86 (d, J=6.8 Hz, 3H). A water peak was also seen at δ 1.56 ppm.

GCEIMS: m/z 274.
Spectra of Selected Compounds

\[ \text{H NMR of compound 10 } \left[ (\text{Bu}_4\text{N})_2\text{NH}_4 \text{ salt} \right] \]
$^{13}$C NMR of compound 10 [(Bu$_4$N)$_2$NH$_4$ salt]
$^{31}\text{P NMR of compound 10 [(Bu}_4\text{N)}_2\text{NH}_4\text{ salt]}}$
$^1$H NMR of compound 12 [(Bu$_4$N)$_3$ salt]
$^{13}$C NMR of compound 12 [(Bu$_4$N)$_3$ salt]
$^{31}$P NMR of compound 12 [(Bu$_4$N)$_3$ salt]
$^1$H NMR of compound 13 [(Bu$_4$N)$_3$ salt]
$\text{^{13}C NMR of compound 13 [(Bu}_4\text{N)}_3\text{ salt]}$
\(^{31}\)P NMR of compound 13 [(Bu\(_4\)N)\(_3\) salt]
$^1$H NMR of compound 37
$^{13}$C NMR of compound 37
DEPT 90 (top) and DEPT 135 (bottom) NMR of compound 37
HSQC spectrum of compound 37
HMBC spectrum of compound 37.
COSY spectrum of compound 37
TOCSY spectrum of compound 37
GCEIMS of compound 37
$^1$H NMR of compound 38
$^{13}$C NMR of compound 38
DEPT 90 (top) and DEPT 135 (bottom) NMR of compound 38
HSQC spectrum of compound 38
HMBC spectrum of compound 38
COSY spectrum of compound 38
GCEIMS of compound 38
$^1$H NMR of compound 39
$^{13}$C NMR of compound 39
DEPT 90 (top) and DEPT 135 (bottom) NMR of compound 39
HSQC spectrum of compound 39
HMBC spectrum of compound 39
COSY spectrum of compound 39
TOCSY spectrum of 39
GCEIMS of compound 39
\[ ^1H \text{NMR of compound 40} \]
$^{13}$C NMR of compound 40
DEPT 90 (top) and DEPT 135 (bottom) NMR of compound 40
HSQC spectrum of compound 40
HMBC spectrum of compound 40
COSY spectrum of compound 40
TOCSY spectrum of compound 40
GCEIMS of compound 40
$\text{H NMR of compound 41}$
GCEIMS of compound 41
4. Complete Citations


