STRUCTURAL ELUCIDATION OF CISOID AND TRANSOID CYCLIZATION PATHWAYS OF A SESQUITERPENE SYNTHASE USING 2-FLUOROFARNESYL DIPHOSPHATES

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SUPPORTING INFORMATION

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Preparation and Characterization of (2-cis, 6-trans)-2-Fluorofarnesyl Diphosphate

General Aspects:

$^1$H and $^{13}$C NMR spectra were recorded in CDCl$_3$ ($^1$H, 7.26; $^{13}$C, 77.0) or CD$_3$OD [ $^1$H, 3.31 (quintet); $^{13}$C, 49.2 (septet)] with U400 and U500 spectrometers in SCS NMR Spectroscopy Facility at the University of Illinois. Chemical shifts are in ppm and coupling constants are in Hertz. The abbreviation ‘app’ is used to describe the apparent multiplicity of the peak and may or may not be a valid first-order analysis.

All chemical reactions were performed in flame-dried glassware under nitrogen. THF and Et$_2$O were dried and distilled from Na/benzophenone; benzene and CH$_2$Cl$_2$ were dried and distilled from CaH$_2$. Hexane and ethyl acetate were freshly distilled from CaH$_2$. DMF, acetonitrile, and CDCl$_3$ were dried over molecular sieves (4 Å) prior use. TLC analyses were performed on silica gel 60 F254 precoated-plates 250 µm. All retention factors (R$_f$) are on silica gel TLC plates until otherwise noted. TLC visualizations were performed with 5% phosphomolybdic acid (0.2 M in 2.5% concd. H$_2$SO$_4$/EtOH (v/v)), I$_2$ vapor, or UV light. Commercial reagents were used without further purification unless specifically noted. Column chromatography was performed according to Still’s procedure$^1$ using 100-700 times excess 32-64 µm grade silica gel. Products separated by chromatography are specified in elution order.

\[
\text{OH} \quad \text{LiCl/MsCl} \quad \text{Collidine} \quad \text{DMF} \quad 2.5 \text{h} \quad \text{F} \quad \text{Cl}
\]

(2Z, 6E)-1-Chloro-3,7,11-trimethylundeca-2,6,10-triene ((2-cis, 6-trans)-2-Fluorofarnesyl Chloride) (2-cis, 6-trans)-2-Fluorofarnesol$^2$ was converted to the allylic chloride under Meyers’ conditions$^3$ as previously described for (2-trans, 6-trans)-2-fluorofarnesol.$^4$ Reaction of the
alcohol (44 mg, 0.18 mmol) with LiCl (77 mg, 1.8 mmol), s-collidine (222 mg, 1.8 mmol), and MsCl (67 mg, 0.54 mmol) in dry DMF provided the chloride as a yellow oil (47 mg, 99%). The chloride was converted to the diphosphate directly without purification. Product characterization data: TLC $R_f$ 0.83 (15% EtOAc in hexane); $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 5.09 (m, 2H, vinyl $H$), 4.18 (dd, 2H, $J = 22.5, 0.5$ Hz, $CH_2Cl$), 1.95-2.18 (m, 8H, 4$CH_2$), 1.72 (app d, 3H, $J_{app} = 3.5$ Hz, $CH_3$), 1.68 (d, 3H, $J = 1.0$ Hz, $CH_3$), 1.60 (s, 6H, 2$CH_3$); $^{19}$F NMR (CDCl$_3$, 376 MHz) $\delta$ –116.7 (td, $J = 23.2, 2.8$ Hz).

(2E, 6E)-2-Fluoro-3,7,11-trimethyl undeca-2,6,10-trien-1-yl Diphosphate, Trisammonium Salt (2b, (2-cis, 6-trans)-2-Fluorofarnesyl Diphosphate).

The diphosphorylation was carried out as previously described for the trans,trans isomer$^4$ using Poulter's methodology.$^5$ The reaction of the chloride (47 mg, 0.18 mmol), HOPP(NBu$_4$)$_3$ (320 mg, 0.36 mmol) and 3 Å molecular sieves (400 mg) in CH$_3$CN (2.0 mL) provided the crude tetrabutylammonium diphosphate as a yellow oil (366 mg). Based on the $^{31}$P NMR spectrum, it was a 1: 0.81 mixture of inorganic pyrophosphate and organic diphosphate (corrected yield 91%). Ion exchange chromatography on BioRad (NH$_4$)$^+$cation exchange resin (40 mL of 25 mM NH$_4$HCO$_3$ in 2% v/v 1-propanol/D.I. water) and lyophilization followed by washing with MeOH (3 x 5 mL) to remove the inorganic pyrophosphate afforded the (NH$_4$)$^+$ salt of diphosphate 2b as a white solid (51 mg, 68 %): $^1$H NMR (CD$_3$OD, 400 MHz) $\delta$ 5.17-5.11 (m, 1H, vinyl $H$), 5.11-5.05 (m, 1H, vinyl $H$), 4.59 (dd, 2H, $J = 23.3, 5.4$ Hz, $CH_2OPP$), 2.16-2.11 (m, 4H, $CH_2$), 2.09-2.04 (m, 2H, $CH_2$), 2.00-1.95 (m, 2H, $CH_2$), 1.68 (d, 3H, $J = 3.5$ Hz, $CH_3$), 1.66 (q, 3H, $J = 1.2$ Hz,
$\text{CH}_3$, 1.61 (d, 3H, $J = 1.2$ Hz, $\text{CH}_3$), 1.60 (br d, 3H, $J = 0.6$ Hz, $\text{CH}_3$); $^{31}\text{P}$ NMR (CD$_3$OD, 162 MHz) $\delta$ -7.99 (br d, $J = 14.9$ Hz), -9.30 (br d, $J = 14.1$ Hz); $^{19}\text{F}$ NMR (CD$_3$OD, 376 MHz) $\delta$ -118.9 (td, $J = 23.2$, 3.5 Hz).

**References**

(1) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* 1978, 43, 2923-2925.

(2) Jin, Y.; Williams, D. C.; Croteau, R.; Coates, R. M. *J. Am. Chem. Soc.*, 2005, 127, 7834-7842.

(3) Collington, E.W.; Meyers, A. I. *J. Org. Chem.* 1971, 36, 3044-3045.

(4) Shishova, E. Y.; Yu, F.; Miller, D. J.; Faraldos, J. A.; Zhao, Y.; Coates, R. M.; Allemann, R. K.; Cane, D. E.; Christianson, D. W. *J. Biol. Chem.* 2008, 283, 15431-15439. (ms ref 17).

(5). Woodside, A. B.; Huang, Z.; Poulter, C. D. *Org. Synth.* 1993, *Coll. Vol.* 8, 616-620.
Table S1. Global comparison of TEAS wt and M4 crystal structures.

|                       | M4 TEAS·cis-2F-FPP | M4 TEAS·trans-2F-FPP | wt TEAS·cis-2F-FPP | wt TEAS·trans-2F-FPP |
|-----------------------|--------------------|----------------------|--------------------|----------------------|
| M4 TEAS·cis-2F-FPP    | -                  | -                    | -                  | -                    |
| M4 TEAS·trans-2F-FPP  | 0.242              | -                    | -                  | -                    |
| wt TEAS·cis-2F-FPP    | 0.282              | 0.321                | -                  | -                    |
| TEAS wt-trans-2F-FPP  | 0.29               | 0.328                | 0.219              | -                    |
| 5EAT                  | 0.334              | 0.369                | 0.294              | 0.335                |

Global comparisons were performed by superposing all C-alpha carbons to derive root mean square deviation (rmsd) values expressed in the unit angstroms.
Figure S1. Annotation of global structure using B-factors reveals a similar pattern of dynamically accessible polypeptide segments. All structures were colored according to their refined isotropic by B-factors, with the corresponding color values of the blue to red gradient shown in the legend at the bottom right.
Figure S2. Disorder in the J-K loop of experimental crystal structures. An active site model for the wild-type TEAS trans-2F-FPP is shown as a van der Waals surface clipped to reveal the bound substrate analogue and helices J and K with the intervening loops. All experimental structures are overlaid on the original TEAS-FHP structure (pdb id 5eat) shown in a grey semitransparent trace. Each structure is colored as indicated in the legend below, with the omitted J-K loop regions highlighted in grey.
Figure S3. Spatial distribution of M4 mutations and closest distances to the farnesyl chain. a. The global structure of M4 TEAS with bound cis-2F-FPP ligand modeled into the active site and the protein backbone is depicted as rainbow colored ribbons. Distances from the active sited center to the side-chains of the M4 mutations are shown as dashed lines.
Figure S4. Farnesyl chain topology of wild-type TEAS from fluorofarnesyl analogues. a. Observable electron density from the wild-type complex with cis-2F-FPP reveals a U-shaped curl (left panel) possibly contributed to by four distinct binding modes of the farnesyl chain (right panel). b. Calculated electron density contoured at 1σ in the SIGMAA-weighted 2Fo-Fc map with the modeled trans-2F-FPP shown with a plane passing through the U-shape curl of the farnesyl chain (left panel). An overlay of trans-2F-FPP (silver chain) with farnesylhydroxy phosphonate (FHP, white chain) in the calculated electron density for the trans-2F-FPP ligand from the left panel.
Figure S5. Spatial depiction of mutational effects in M4 TEAS on the active site contour and substrate-binding mode in the trans-2F-FPP and cis-2F-FPP complexes. The ribbon and active site surface (cream) of wild-type TEAS wild is superimposed on the corresponding M4 TEAS 2F-FPP complex, with ribbons and side chains rendered with rainbow coloration (as in Fig. 3a and 4a). The ligand from wild-type TEAS (cyan) and M4 TEAS (gray) is overlaid and electron density from the SIGMAA-weighted 2Fo-Fc electron density maps at 1σ is shown for Y520 and I516 for the M4 TEAS structures.
**Computational Details**

As noted in the article Hong and Tantillo\(^1\) concurrently carried out computational studies on the conversion of (6S)-\(\alpha\)-bisabolyl cation to \(\alpha\)-cedrene. In Figure 2a of the current article, the pathway reported by Hong and Tantillo proceeds via the curved blue arrow, avoiding the formation of the \(\alpha\)-acorenyl cation. In contrast to this, we located an alternative pathway (red curved arrow via transition structure 14) that instead avoids the formation of the (7R)-\(\beta\)-bisabolyl cation. The question then arises how might two pathways exist that both lead from (6S)-\(\alpha\)-bisabolyl cation to \(\alpha\)-cedrene (3). It was found that these two pathways lead from two different conformers, a and b of (6S)-\(\alpha\)-bisabolyl cation (Figure S7):

![Figure S6](image)

**Figure S6.**

Note that the location of the side-chain (\(R\)) in these two conformers is quite different and might very well affect the course of any reaction they might undergo. The enzyme could utilize this by it “freezing” one of these conformers, which would direct the course of the ensuing reaction. The red pathway (via transition structure 14) shown in Figure 2 in the article leads from

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\(^1\) Hong, Y. J.; Tantillo, D. J. *J. Am. Chem. Soc.* **2009**, *131*, 7999-8015.
conformer a, and the blue pathway from b. A transition structure was also located that leads from (1R, 4S, 5S)-α-acorenyl cation to the carbocation precursor to (-)-α-cedrene (2).²

² An analogous pathway was also located that leads from (6R)-α-bisabolycation to the epimer of the (-)-α-cedrene (2) shown if Figure 2a in the article (the methyl group in the five-membered ring is cis to the CH₂ group of the adjacent six-membered ring:

This “epimeric” pathway was essentially indistinguishable from that reported here with the exception of this methyl group placement.
Geometries and Energies of All Stationary Points

(6S)-α-bisabolyl cation

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Charge = 1 Multiplicity = 1

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|   | 6  | 0   | -2.267409 | -1.296044 | -0.667234 |
|---|----|-----|-----------|-----------|-----------|
| 2 | 6  | 0   | -0.931583 | -0.651595 | -0.274241 |
|   |   |   |           |           |           |
|---|---|---|----------|----------|----------|
| 3 | 6 | 0 | -3.472203 | -0.657890 | -0.011413 |
| 4 | 6 | 0 | -1.070916 |  0.887354 | -0.320722 |
| 5 | 6 | 0 |  0.280379 |  2.960189 | -0.824636 |
| 6 | 6 | 0 |  0.109652 |  1.706628 | -0.054776 |
| 7 | 6 | 0 |  1.106677 |  1.357899 |  0.956191 |
| 8 | 6 | 0 |  2.570046 |  1.102027 |  0.333108 |
| 9 | 6 | 0 |  2.692825 | -0.153128 | -0.466951 |
|10 | 6 | 0 | -2.110623 |  1.308237 |  0.819681 |
|11 | 6 | 0 | -3.376071 |  0.506208 |  0.643636 |
|12 | 6 | 0 | -4.779301 | -1.387881 |  0.162530 |
|13 | 6 | 0 |  3.305882 | -1.295070 | -0.096742 |
|14 | 6 | 0 |  3.952077 | -1.536144 |  1.243213 |
|15 | 6 | 0 |  3.401521 | -2.452853 | -1.058017 |
|16 | 1 | 0 | -2.397633 | -1.269287 | -1.759619 |
|17 | 1 | 0 | -2.231792 | -2.362243 | -0.407043 |
|18 | 1 | 0 | -0.137070 | -0.984027 | -0.948244 |
|19 | 1 | 0 | -0.651203 | -0.968618 |  0.736385 |
|20 | 1 | 0 |  0.958984 |  3.678137 | -0.359563 |
|21 | 1 | 0 |  0.703741 |  2.679048 | -1.805987 |
|22 | 1 | 0 | -0.684040 |  3.428566 | -1.051122 |
|23 | 1 | 0 | -1.528362 |  1.196295 | -1.268170 |
|24 | 1 | 0 |  0.822947 |  0.489097 |  1.550370 |
|25 | 1 | 0 |  1.239970 |  2.229924 |  1.612201 |
|26 | 1 | 0 |  2.831023 |  1.981033 | -0.266330 |
|27 | 1 | 0 |  3.231840 |  1.099656 |  1.200990 |
|28 | 1 | 0 |  2.265744 | -0.114605 | -1.470631 |
|29 | 1 | 0 | -1.653515 |  1.133183 |  1.802130 |
|30 | 1 | 0 | -2.329939 |  2.380017 |  0.752328 |
|31 | 1 | 0 | -4.263711 |  0.959200 |  1.082507 |
|32 | 1 | 0 | -5.607047 | -0.841996 |  0.298428 |
|33 | 1 | 0 | -5.020866 | -1.543664 | -1.222342 |
|34 | 1 | 0 | -4.726805 | -2.383115 |  0.297402 |
|35 | 1 | 0 |  5.023817 | -1.735837 |  1.115700 |
|36 | 1 | 0 |  3.848707 | -0.707346 |  1.947325 |
|37 | 1 | 0 |  3.526626 | -2.432041 |  1.713078 |
|38 | 1 | 0 |  4.451567 | -2.707980 | -1.250850 |
|39 | 1 | 0 |  2.938499 | -3.352814 | -0.632175 |
|40 | 1 | 0 |  2.922803 | -2.238201 | -2.017890 |

SCF Done:  E(RB+HF-LYP) =  -586.365866023     A.U. after  1 cycles
Frequencies --    26.6249             33.7822                50.7079
Zero-point correction=                    0.365327 (Hartree/Particle)
Thermal correction to Energy=                    0.383301
Thermal correction to Enthalpy=                  0.384245
Thermal correction to Gibbs Free Energy=         0.318499
Sum of electronic and zero-point Energies=           -586.000539
Sum of electronic and thermal Energies=              -585.982565
Sum of electronic and thermal Enthalpies=            -585.981621
Sum of electronic and thermal Free Energies=         -586.047367
mpw1pw91/6-311+G(2d,p)//B3LYP/6-31G*

DFT = -586.379668
DFT + ZPE = -586.01434

14 – transition structure linking (6S)-α-bisabolyl cation with (1R,4S,5S)-a-acorenyl cation

Charge = 1 Multiplicity = 1

1  6  0  -1.561819  3.011953  0.199064
2  6  0  -0.596343  2.165354 -0.649350
3  6  0  -2.431453  2.176256  1.111275
4  6  0  0.091963  1.084510  0.154049
5  6  0  2.393059  1.672747 -0.799609
6  6  0  1.486149  0.827695  0.068211
7  6  0  2.138178 -0.374764  0.705617
8  6  0  2.483062 -1.448940 -0.383437
9  6  0  1.298949 -1.940581 -1.174043
10  6  0  -0.869282  0.190987  0.917631
11  6  0  -2.109742  0.909054  1.396193
12  6  0  -3.655058  2.849425  1.671574
13  6  0  0.656720 -3.115696 -1.044314
14  6  0  0.998249 -4.177927 -0.029528
15  6  0  -0.485330 -3.471675 -1.964993
16  1  0  -0.999005  3.752783  0.787173
17  1  0  -2.189736  3.598399 -0.482284
18  1  0  0.100635  2.796731 -1.201382
19  1  0  -1.178502  1.608784 -1.399054
20  1  0  3.430557  1.582026 -0.471868
21  1  0  2.330674  1.299891 -1.829799
22  1  0  2.124383  2.729935  0.816763
23  1  0  0.928205  1.617355  1.007487
24  1  0  1.502022 -0.837615  1.460181
25  1  0  3.067941 -0.056961  1.190061
26  1  0  3.237527 -1.032528 -1.062356
27  1  0  2.976104 -2.262889  0.154360
28  1  0  0.962630 -1.274966 -1.971925
29  1  0 -1.122656 -0.634451  0.233308
30  1  0 -0.373715 -0.290790  1.766411
31  1  0 -2.754215  0.318550  2.042978
32  1  0 -4.203245  2.193005  2.352534
33  1  0 -3.384259  3.760710  2.221018
34  1  0 -4.336274  3.157503  0.867811
35  1  0  1.274130 -5.111618 -0.536127
36  1  0  1.818163 -3.906551  0.639523
37  1  0  0.122301 -4.412376  0.589339
38  1  0 -0.253591 -4.382164 -2.532694
39  1  0 -1.399150 -3.688531 -1.395948
40  1  0 -0.704741 -2.674623 -2.682017

SCF Done: E(RB+HF-LYP) =  -586.350482839  A.U. after 1 cycles
Frequencies --  -522.5457  29.3134  40.5082
Zero-point correction=                    0.363077 (Hartree/Particle)
Thermal correction to Energy=                    0.380476
Thermal correction to Enthalpy=                  0.381421
Thermal correction to Gibbs Free Energy=         0.317184
Sum of electronic and zero-point Energies=           -585.987406
Sum of electronic and thermal Energies=              -585.970006
Sum of electronic and thermal Enthalpies=            -585.969062
Sum of electronic and thermal Free Energies=         -586.033299

mpw1pw91/6-311+G(2d,p)//B3LYP/6-31G*

DFT = -586.374902
DFT + ZPE = -586.01183

(1R, 4S, 5S)-α-acorenyl cation (lower energy conformer – product of IRC)
#6-31g* b3lyp nopop freq guess=read geom=check

Charge = 1 Multiplicity = 1

|   |   |   |    |    |    |
|---|---|---|----|----|----|
| 1 | 6 | 0 | -2.530442 | -0.063980 | -0.165330 |
| 2 | 6 | 0 | -1.246163 | -0.923381 | -0.118245 |
| 3 | 6 | 0 | -2.453121 | 1.187110  | 0.679010  |
| 4 | 6 | 0 | -3.745432 | 1.926885  | 0.896253  |
| 5 | 6 | 0 | -1.285146 | 1.613336  | 1.180740  |
| 6 | 6 | 0 |  0.031777 | 0.894658  | 1.002754  |
| 7 | 6 | 0 |  0.001588 | -0.043136 | -0.222096 |
| 8 | 6 | 0 |  0.170697 | 0.734686  | -1.568419 |
| 9 | 6 | 0 |  1.654787 | 1.134138  | -1.619467 |
|10 | 6 | 0 | -0.239891 | -0.047562 | -2.825913 |
|11 | 6 | 0 |  2.434080 | -0.100514 | -1.114052 |
|12 | 6 | 0 |  1.434277 | -0.993549 | -0.339366 |
|13 | 6 | 0 |  1.768755 | -1.481120 |  0.965501 |
|14 | 6 | 0 |  2.762256 | -0.810667 |  1.838525 |
|15 | 6 | 0 |  1.139525 | -2.713373 |  1.496425 |
|16 | 1 | 0 | -2.758400 |  0.219338 | -1.202616 |
|17 | 1 | 0 | -3.378461 | -0.681328 |  0.158652 |
|18 | 1 | 0 | -1.275635 | -1.675743 | -0.912876 |
|19 | 1 | 0 | -1.236684 | -1.462091 |  0.836792 |
|20 | 1 | 0 | -3.599295 |  2.838311 |  1.482412 |
|21 | 1 | 0 | -4.474976 |  1.295348 |  1.420283 |
|22 | 1 | 0 | -4.205337 |  2.206262 | -0.061026 |
|23 | 1 | 0 | -1.253469 |  2.524924 |  1.773841 |
|24 | 1 | 0 |  0.244132 |  0.315453 |  1.916039 |
|25 | 1 | 0 |  0.841752 |  1.629255 |  0.924570 |
|26 | 1 | 0 | -0.463077 |  1.626675 | -1.493443 |
|27 | 1 | 0 |  1.962765 |  1.423325 | -2.628139 |
|28 | 1 | 0 |  1.848493 |  1.997339 | -0.975365 |
|29 | 1 | 0 | -0.087798 |  0.583683 | -3.707270 |
|30 | 1 | 0 | -1.293240 | -0.339528 | -2.817493 |
SCF Done:  E(RB+HF-LYP) =  -586.375831628     A.U. after    1 cycles
Frequencies --    48.4539                78.0842               109.4122
Zero-point correction=                           0.368243 (Hartree/Particle)
Thermal correction to Energy=                    0.384587
Thermal correction to Enthalpy=                  0.385531
Thermal correction to Gibbs Free Energy=         0.326229
Sum of electronic and zero-point Energies=           -586.007589
Sum of electronic and thermal Energies=              -585.991245
Sum of electronic and thermal Enthalpies=            -585.990300
Sum of electronic and thermal Free Energies=         -586.049603

mpw1pw91/6-311+G(2d,p)//B3LYP/6-31G*

DFT =  -586.396703994
DFT + ZPE = -586.02846

(1R, 4S, 5S)-α-acorenyl cation (higher energy conformer)
#6-31g* b3lyp nopop freq guess=read geom=check

Charge = 1 Multiplicity = 1

SCF Done:  E(RB+HF-LYP) = -586.366027117  A.U. after 1 cycles

Frequencies --  27.1277  42.9804  79.6402
Zero-point correction= 0.367584  (Hartree/Particle)
Thermal correction to Energy= 0.384217
Thermal correction to Enthalpy = 0.385162
Thermal correction to Gibbs Free Energy = 0.324108
Sum of electronic and zero-point Energies = -585.998443
Sum of electronic and thermal Energies = -585.981810
Sum of electronic and thermal Enthalpies = -585.980865
Sum of electronic and thermal Free Energies = -586.041919

mpw1pw91/6-311+G(2d,p)//B3LYP/6-31G*
DFT = -586.386818366
DFT + ZPE = -586.01923

(1R, 4R, 5S)-a-acorenyl cation

-----------------------------------------------
#6-31g* b3lyp nopop freq geom=check guess=read
-----------------------------------------------
Charge = 1 Multiplicity = 1

S20
|      | 1    | 0    |                  |                  |                  |                  |
|------|------|------|-----------------|-----------------|-----------------|-----------------|
| 18   | 1    | 0    | 1.932639        | -1.047006       | 1.964268        |
| 19   | 1    | 0    | -0.479926       | -0.845206       | 2.336423        |
| 20   | 1    | 0    | -0.547277       | -2.251280       | 1.280197        |
| 21   | 1    | 0    | -0.818816       | -0.290670       | -1.938036       |
| 22   | 1    | 0    | -1.260701       | -1.839733       | -1.279695       |
| 23   | 1    | 0    | -3.050710       | 0.939420        | -1.384345       |
| 24   | 1    | 0    | -3.481831       | 1.781216        | 0.094014        |
| 25   | 1    | 0    | -1.415124       | 2.971081        | -0.062560       |
| 26   | 1    | 0    | -0.995654       | 2.024761        | -1.483293       |
| 27   | 1    | 0    | -2.560580       | 0.115967        | 1.497846        |
| 28   | 1    | 0    | -3.132636       | -2.181422       | 0.647659        |
| 29   | 1    | 0    | -4.407634       | -0.992261       | 0.383357        |
| 30   | 1    | 0    | -3.401908       | -1.500575       | -0.970655       |
| 31   | 1    | 0    | 3.856110        | -1.078465       | 0.512034        |
| 32   | 1    | 0    | 3.590303        | -0.670984       | -1.206649       |
| 33   | 1    | 0    | 3.544632        | -2.342665       | -0.686461       |
| 34   | 1    | 0    | -0.591838       | 1.341759        | 1.475052        |
| 35   | 1    | 0    | 1.492790        | 2.662901        | -1.281804       |
| 36   | 1    | 0    | 2.716753        | 1.406137        | -1.185860       |
| 37   | 1    | 0    | 1.123272        | 1.075511        | -1.940894       |
| 38   | 1    | 0    | 1.545113        | 1.472527        | 2.346535        |
| 39   | 1    | 0    | 2.967665        | 1.608350        | 1.262049        |
| 40   | 1    | 0    | 1.787171        | 2.912334        | 1.355131        |

SCF Done: E(RB+HF-LYP) = -586.381341674   A.U. after 1 cycles

Frequencies -- 79.8947   82.1013   101.8161

Zero-point correction= 0.369611 (Hartree/Particle)

Thermal correction to Energy= 0.385474

Thermal correction to Enthalpy= 0.386419

Thermal correction to Gibbs Free Energy= 0.328742

Sum of electronic and zero-point Energies= -586.011730

Sum of electronic and thermal Energies= -585.995867

Sum of electronic and thermal Enthalpies= -585.994923

Sum of electronic and thermal Free Energies= -586.052600

mpw1pw91/6-311+G(2d,p)//B3LYP/6-31G*

DFT = -586.403228493

DFT + ZPE = -586.03362

Tertiary carbocation precursor to (+)-2-epi-prezizaene
#6-31g* b3lyp nopop freq guess=read geom=check

Charge =  1 Multiplicity = 1

|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| 1 | 6 | 0 | -0.517646 | -1.839934 | 1.112556 |
| 2 | 6 | 0 | 0.806011  | -1.046329 | 1.189215 |
| 3 | 6 | 0 | -0.980237 | -1.698530 | -0.449070|
| 4 | 6 | 0 | 0.368955  | -1.437998 | -1.137265|
| 5 | 6 | 0 | -1.861305 | -0.567182 | -0.237711|
| 6 | 6 | 0 | -3.316075 | -0.851440 | -0.161030|
| 7 | 6 | 0 | -1.338491 | 0.833152  | -0.065420|
| 8 | 6 | 0 | -2.113601 | 1.723041  | -1.097871|
| 9 | 6 | 0 | 0.195754  | 0.919587  | -0.369974|
|10 | 6 | 0 | -1.720950 | 1.352317  | 1.358257 |
|11 | 6 | 0 | 1.001535  | -0.398121 | -0.207549|
|12 | 6 | 0 | 2.434495  | 0.092165  | -0.530635|
|13 | 6 | 0 | 2.516224  | 1.491991  | 0.145160 |
|14 | 6 | 0 | 3.589340  | -0.844209 | -0.171066|
|15 | 6 | 0 | 1.052091  | 1.978108  | 0.358472 |
|16 | 1 | 0 | -0.403509 | -2.920266 | 1.245595 |
|17 | 1 | 0 | -1.269960 | -1.520389 | 1.840877 |
|18 | 1 | 0 | 1.622665  | -1.743438 | 1.396722 |
|19 | 1 | 0 | 0.799067  | -0.316699 | 2.003306 |
|20 | 1 | 0 | -1.514554 | -2.595564 | -0.767935|
|21 | 1 | 0 | 0.253006  | -1.086339 | -2.167914|
|22 | 1 | 0 | 0.929191  | -2.379762 | -1.166638|
|23 | 1 | 0 | -3.907758 | -0.923800 | 0.349089 |
|24 | 1 | 0 | -3.512842 | -1.842057 | 0.261258 |
|25 | 1 | 0 | -3.663905 | -0.901920 | -1.207920|
|26 | 1 | 0 | -1.678349 | 2.725840  | -1.050251|
|27 | 1 | 0 | -3.178638 | 1.805030  | -0.866678|
|28 | 1 | 0 | -1.997450 | 1.356854  | -2.122832|
|29 | 1 | 0 | 0.268053  | 1.152562  | -1.441874|
|30 | 1 | 0 | -1.462979 | 2.412451  | 1.413649 |
|31 | 1 | 0 | -1.179671 | 0.830436  | 2.150484 |
|   |   |   |   |   |
|---|---|---|---|---|
| 32 | 1 | 0 | -2.792265 | 1.264141 | 1.553838 |
| 33 | 1 | 0 | 2.458775  | 0.242754 | -1.619753 |
| 34 | 1 | 0 | 3.035965  | 1.420923 | 1.106886  |
| 35 | 1 | 0 | 3.093713  | 2.188443 | -0.469259 |
| 36 | 1 | 0 | 4.536461  | -0.428466| -0.530618 |
| 37 | 1 | 0 | 3.475215  | -1.833141| -0.631201 |
| 38 | 1 | 0 | 3.687837  | -0.979109| 0.912074  |
| 39 | 1 | 0 | 0.882627  | 2.985382 | -0.034758 |
| 40 | 1 | 0 | 0.822708  | 2.015012 | 1.427440  |

SCF Done: $E(RB+HF-LYP) = -586.385770487$ A.U. after 6 cycles

Frequencies -- 65.1480 75.6308 102.3592

Zero-point correction= 0.371457 (Hartree/Particle)
Thermal correction to Energy= 0.386534
Thermal correction to Enthalpy= 0.387479
Thermal correction to Gibbs Free Energy= 0.331410

Sum of electronic and zero-point Energies= -586.014313
Sum of electronic and thermal Energies= -585.999236
Sum of electronic and thermal Enthalpies= -585.998292
Sum of electronic and thermal Free Energies= -586.054361

**mpw1pw91/6-311+G(2d,p)//B3LYP/6-31G***

DFT = -586.413165107
DFT + ZPE = -586.04171

Precursor tertiary carbocation to (-)-α-cedrene (higher energy conformer, “product of irc”)
#6-31g* b3lyp noppop freq guess=read geom=check

Charge = 1 Multiplicity = 1

| 1 | 6 | 0 | -1.986145 | -0.560007 | -1.114734 |
| 2 | 6 | 0 | -0.781513 | 0.086170  | -1.838199 |
| 3 | 6 | 0 | -2.217552 | -0.185175 | 0.297395  |
| 4 | 6 | 0 | -3.503960 | -0.541928 | 0.925710  |
| 5 | 6 | 0 | -1.185848 | 0.486173  | 1.053205  |
| 6 | 6 | 0 | -0.406203 | 1.477554  | 0.162920  |
| 7 | 6 | 0 | 0.301039  | 0.549500  | -0.844947 |
| 8 | 6 | 0 | 1.559341  | 1.212918  | -1.478450 |
| 9 | 6 | 0 | 2.643086  | 0.963292  | -0.403848 |
|10 | 6 | 0 | 1.967247  | 0.640631  | -2.845117 |
|11 | 6 | 0 | 2.367337  | -0.464735 | 0.104850  |
|12 | 6 | 0 | 0.822117  | -0.642151 | 0.039375  |
|13 | 6 | 0 | 0.016973  | -0.622800 | 1.389883  |
|14 | 6 | 0 | 0.758609  | -0.028554 | 2.602065  |
|15 | 6 | 0 | -0.500501 | -2.011915 | 1.788301  |
|16 | 1 | 0 | -2.930420 | -0.466980 | -1.671180 |
|17 | 1 | 0 | -1.855232 | -1.659683 | -1.050131 |
|18 | 1 | 0 | -1.136859 | 0.941965  | -2.424643 |
|19 | 1 | 0 | -0.378699 | -0.636733 | -2.552875 |
|20 | 1 | 0 | -3.397150 | -0.765693 | 1.992025  |
|21 | 1 | 0 | -4.040986 | -1.337414 | 0.402254  |
|22 | 1 | 0 | -4.131464 | 0.367131  | 0.870340  |
|23 | 1 | 0 | -1.569396 | 0.875362  | 1.999477  |
|24 | 1 | 0 | 0.292214  | 2.037442  | 0.789229  |
|25 | 1 | 0 | -1.058101 | 2.210797  | -0.324916 |
|26 | 1 | 0 | 1.381796  | 2.288895  | -1.605625 |
|27 | 1 | 0 | 3.656736  | 1.077380  | -0.800267 |
|28 | 1 | 0 | 2.543771  | 1.687553  | 0.413951  |
|29 | 1 | 0 | 2.891249  | 1.119387  | -3.184750 |
|30 | 1 | 0 | 1.207561  | 0.824774  | -3.611888 |
|31 | 1 | 0 | 2.153104  | -0.439438 | -2.808477 |
|32 | 1 | 0 | 2.793615  | -0.648743 | 1.093574  |
33 1 0 2.829825 -1.194593 -0.567190
34 1 0 0.602618 -1.596704 -0.452522
35 1 0 0.089492 0.053443 3.464510
36 1 0 1.196624 0.953479 2.410095
37 1 0 1.571446 -0.703100 2.889735
38 1 0 -1.058526 -2.514408 0.989941
39 1 0 -1.131896 -1.973863 2.683114
40 1 0 0.354508 -2.654094 2.025771

SCF Done: E(RB+HF-LYP) = -586.390371153 A.U. after 1 cycles
Frequencies -- 68.7704  79.1418  130.8598
Zero-point correction= 0.370146 (Hartree/Particle)
Thermal correction to Energy= 0.385171
Thermal correction to Enthalpy= 0.386115
Thermal correction to Gibbs Free Energy= 0.330378
Sum of electronic and zero-point Energies= -586.020225
Sum of electronic and thermal Energies= -586.005200
Sum of electronic and thermal Enthalpies= -586.004256
Sum of electronic and thermal Free Energies= -586.059993

mpw1pw91/6-311+G(2d,p)//B3LYP/6-31G*
DFT = -586.41745987
DFT + ZPE = -586.04731

Precursor tertiary carbocation to (-)-α-cedrene (lower energy conformer)

#6-31g* b3lyp nopop freq guess=read geom=check
Charge = 1 Multiplicity = 1

1 6 0 0.821187 0.289456 0.500298


SCF Done:  E(RB+HF-LYP) =  -586.391511674     A.U. after    1 cycles
Frequencies --    63.1134                79.6270               139.0493
Zero-point correction=                           0.370440 (Hartree/Particle)
Thermal correction to Energy=                    0.385371
Thermal correction to Enthalpy=                  0.386315
Thermal correction to Gibbs Free Energy=         0.330835
Sum of electronic and zero-point Energies=           -586.021071
Sum of electronic and thermal Energies=              -586.006140
Sum of electronic and thermal Enthalpies=            -586.005196

---------------------------------------------------------------------
Sum of electronic and thermal Free Energies = -586.060677

mpw1pw91/6-311+G(2d,p)//B3LYP/6-31G*

DFT = -586.422749615
DFT + ZPE = -586.05231

15 - Transition state linking (1R, 4R, 5S)-a-acorenyl cation to the tertiary carbocation precursor to (+)-2-epi-prezizaene

#6-31g* b3lyp nopop freq guess=read geom=check

Charge = 1 Multiplicity = 1

| 1  | 6  | 0   | -0.075792 | -0.059851 | 2.022135 |
| 2  | 6  | 0   | 0.135954  | -1.986766 | 0.648942 |
| 3  | 6  | 0   | 1.285919  | -1.393393 | -0.032680 |
| 4  | 6  | 0   | 1.058069  | 0.101099  | -0.234890 |
| 5  | 6  | 0   | 0.925973  | 0.743102  | 1.171389 |
| 6  | 6  | 0   | 1.235805  | 2.031866  | -1.715148 |
| 7  | 6  | 0   | -0.273333 | 1.692351  | -1.531922 |
| 8  | 6  | 0   | 2.046731  | 0.815643  | -1.186074 |
| 9  | 6  | 0   | 3.418703  | 1.177587  | -0.612730 |
| 10 | 6  | 0   | -0.894200 | -1.138354 | 1.156151 |
| 11 | 6  | 0   | -1.952040 | -1.860305 | 1.994654 |
| 12 | 6  | 0   | -0.296337 | 0.242909  | -0.988298 |
| 13 | 6  | 0   | -1.511909 | -0.266034 | -0.157045 |
| 14 | 6  | 0   | -2.412993 | 0.848329  | 0.392092 |
| 15 | 6  | 0   | -2.377840 | -1.223571 | -0.998424 |
| 16 | 1  | 0   | 0.417704  | -0.594241 | 2.839042 |
| 17 | 1  | 0   | -0.845327 | 0.566078  | 2.480791 |
| 18 | 1  | 0   | 0.101730  | -3.070760 | 0.791232 |
| 19 | 1  | 0   | 1.478815  | -1.952873 | -0.965237 |
|   |   |   |           |           |           |
|---|---|---|-----------|-----------|-----------|
| 20| 1 | 0 | 2.176810  | -1.636461 | 0.583700  |
| 21| 1 | 0 | 0.603024  | 1.783226  | 1.072875  |
| 22| 1 | 0 | 1.900232  | 0.764701  | 1.668548  |
| 23| 1 | 0 | 1.494455  | 2.933971  | -1.149814 |
| 24| 1 | 0 | 1.487426  | 2.39505   | -2.758841 |
| 25| 1 | 0 | -0.835386 | 1.769361  | -2.467263 |
| 26| 1 | 0 | -0.734494 | 2.395321  | -0.833987 |
| 27| 1 | 0 | 2.207591  | 0.128426  | -2.030149 |
| 28| 1 | 0 | 3.951596  | 0.298473  | -0.229133 |
| 29| 1 | 0 | 4.047750  | 1.619772  | -1.392110 |
| 30| 1 | 0 | 3.343192  | 1.911075  | 0.197556  |
| 31| 1 | 0 | -2.426549 | -2.675109 | 1.440877  |
| 32| 1 | 0 | -2.734762 | -1.176075 | 2.328275  |
| 33| 1 | 0 | -1.493590 | -2.287856 | 2.892930  |
| 34| 1 | 0 | -0.215176 | -0.420945 | -1.860822 |
| 35| 1 | 0 | -2.890288 | 1.343936  | -0.460815 |
| 36| 1 | 0 | -3.216723 | 0.451658  | 1.017735  |
| 37| 1 | 0 | -1.883865 | 1.620874  | 0.953943  |
| 38| 1 | 0 | -1.808853 | -2.085468 | -1.365879 |
| 39| 1 | 0 | -3.248192 | -1.592476 | -0.448681 |
| 40| 1 | 0 | -2.753385 | -0.681777 | -1.873688 |

SCF Done:  E(RB+HF-LYP) =  -586.367407599  A.U. after  1 cycles
Frequencies -- -248.5415  70.6111  90.0784
Zero-point correction=  0.369393 (Hartree/Particle)
Thermal correction to Energy=  0.383950
Thermal correction to Enthalpy=  0.384894
Thermal correction to Gibbs Free Energy=  0.330413
Sum of electronic and zero-point Energies=  -586.998014
Sum of electronic and thermal Energies=  -585.983457
Sum of electronic and thermal Enthalpies=  -585.982513
Sum of electronic and thermal Free Energies=  -586.036995

mpw1pw91/6-311+G(2d,p)//B3LYP/6-31G*

DFT = -586.394419128
DFT + ZPE = -586.02503

transtion state linking (1R, 4S, 5S)-α-acorenyl cation to the tertiary carbocation precursor to (-)-α-cedrene
#6-31g* b3lyp nopop freq(noraman) guess=read geom=check

Charge =  1 Multiplicity = 1

|   |   |   |         |         |         |
|---|---|---|---------|---------|---------|
| 1 | 6 | 0 | -2.381569 | -0.765704 | -0.505685 |
| 2 | 6 | 0 | -1.221983 | -0.759414 | 0.507604  |
| 3 | 6 | 0 | -2.465895 | 0.584853  | -1.186299 |
| 4 | 6 | 0 | -3.826515 | 1.140148  | -1.500233 |
| 5 | 6 | 0 | -1.321463 | 1.223172  | -1.464169 |
| 6 | 6 | 0 | 0.036284  | 0.581207  | -1.287873 |
| 7 | 6 | 0 | 0.131497  | -0.498174 | -0.163500 |
| 8 | 6 | 0 | 0.880849  | -1.765932 | -0.699003 |
| 9 | 6 | 0 | 2.338205  | -1.304540 | -0.874395 |
|10 | 6 | 0 | 0.778753  | -3.036591 | 0.155571  |
|11 | 6 | 0 | 2.660327  | -0.441080 | 0.370295  |
|12 | 6 | 0 | 1.313614  | -0.084241 | 1.041780  |
|13 | 6 | 0 | 1.087479  | 1.236292  | 1.547910  |
|14 | 6 | 0 | 1.806320  | 2.428771  | 1.041206  |
|15 | 6 | 0 | 0.118136  | 1.480465  | 2.643024  |
|16 | 1 | 0 | -2.257955 | -1.569703 | -1.246353 |
|17 | 1 | 0 | -3.311699 | -0.989663 | 0.029079  |
|18 | 1 | 0 | -1.190118 | -1.690330 | 1.079792  |
|19 | 1 | 0 | -1.452482 | 0.040418  | 1.217279  |
|20 | 1 | 0 | -3.765496 | 2.114785  | -1.993047 |
|21 | 1 | 0 | -4.432644 | 1.249048  | -0.590879 |
|22 | 1 | 0 | -4.379365 | 0.460037  | -2.161992 |
|23 | 1 | 0 | -1.336675 | 2.196860  | -1.949847 |
|24 | 1 | 0 | 0.802032  | 1.354268  | -1.183351 |
|25 | 1 | 0 | 0.296797  | 0.085510  | -2.234277 |
|26 | 1 | 0 | 0.432681  | -1.989236 | -1.676572 |
|27 | 1 | 0 | 3.023800  | -2.150415 | -0.973113 |
|28 | 1 | 0 | 2.444422  | -0.703642 | -1.782884 |
|29 | 1 | 0 | 1.372668  | -3.827489 | -0.313858 |
|30 | 1 | 0 | -0.246694 | -3.408836 | 0.231986  |
|31 | 1 | 0 | 1.169396  | -2.908115 | 1.172616  |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 32 | 1 | 0 | 3.243024 | 0.442651 | 0.099417 |
| 33 | 1 | 0 | 3.260190 | -0.989549 | 1.103852 |
| 34 | 1 | 0 | 1.039896 | -0.813690 | 1.809422 |
| 35 | 1 | 0 | 2.711561 | 2.536120 | 1.665477 |
| 36 | 1 | 0 | 1.227881 | 3.348303 | 1.170185 |
| 37 | 1 | 0 | 2.158820 | 2.330112 | 0.012860 |
| 38 | 1 | 0 | -0.244661 | 0.573467 | 3.126192 |
| 39 | 1 | 0 | -0.735782 | 2.046921 | 2.236685 |
| 40 | 1 | 0 | 0.566153 | 2.146771 | 3.393924 |

SCF Done:  \( E(\text{RB+HF-LYP}) = -586.366331328 \) A.U. after 1 cycles

Frequencies -- -55.6163  23.9536  113.6646
Zero-point correction= 0.367854 (Hartree/Particle)
Thermal correction to Energy= 0.383462
Thermal correction to Enthalpy= 0.384406
Thermal correction to Gibbs Free Energy= 0.326406
Sum of electronic and zero-point Energies= -585.998477
Sum of electronic and thermal Energies= -585.982870
Sum of electronic and thermal Enthalpies= -585.981926
Sum of electronic and thermal Free Energies= -586.039925

mpw1pw91/6-311+G(2d,p)//B3LYP/6-31G*

DFT = -586.387200021
DFT + ZPE = -586.01935
**Supplemental Methods**

*Protein expression and purification*

pH9GW expression vectors (an in-house Gateway destination vector) were transformed into *E. coli* BL21(λDE3) and plated on LB agar containing 50 μg/mL kanamycin for selection. Colonies were transferred to 100 mL of liquid media (LB with kanamycin) followed by 16-h growth with shaking at 37 °C at 275 rpm. Cultures were diluted 50-fold into 1 L of Terrific Broth with kanamycin, followed by growth with shaking at 37 °C at 275 rpm until cultures reached OD600 ≥ 1.5. Protein expression was induced by addition of isopropyl β-D-thiogalactoside (IPTG) to 0.1 mM followed by growth with shaking at 20 °C at 275 rpm for 5 h. Cells were harvested by centrifugation and cell pellets frozen at -20 °C. Frozen pellets were re-suspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 20 mM imidazole, pH 8.0, 10% [v/v] glycerol, 10 mM β-mercaptoethanol, and 1% [v/v] Tween-20) containing 1 mg/mL lysozyme followed by stirring at 4 °C for 1 h. After sonication and centrifugation, the clarified supernatant was passed over a column of Ni^{2+}–NTA resin (Qiagen), washed with 10 bed volumes of lysis buffer and 10 bed volumes of wash buffer (50 mM Tris–HCl, pH 8.0, 500 mM NaCl, 20 mM imidazole, pH 8.0, 20 mM β-mercaptoethanol, and 10% [v/v] glycerol), and the His-tagged protein was eluted with elution buffer (50 mM Tris–HCl, pH 8.0, 500 mM NaCl, 250 mM imidazole, pH 8.0, 20 mM β-mercaptoethanol, and 10% [v/v] glycerol). N-terminal His-tags were removed via proteolysis with thrombin as follows: thrombin was added to a ratio of 1:1,000 [w/w] directly to the eluted protein fraction and dialyzed against two changes of buffer (50 mM Tris–HCl, pH 8.0, 100 mM NaCl, and 10 mM β-mercaptoethanol) over 24 h at 4 °C. Following digestion, samples were passed over a column containing 0.5 mL Benzamidine Sepharose to remove thrombin and 0.5 mL Ni^{2+}-NTA resin to capture undigested protein. The resulting protein solutions were collected and
concentrated to approximately 10 mg/mL or greater by centrifugation using 30,000 Da molecular weight cut-off concentrators (Millipore, Bedford, MA). Concentrated samples were injected onto a Sephacryl S-200 column equilibrated with buffer (25 mM Tris–HCl, pH 8.0, 50 mM NaCl and 1 mM DTT). Fractions corresponding to digested protein were verified by SDS-PAGE, pooled and concentrated (as described above) to approximately 20 mg/mL and aliquoted for freezing at -80 °C. Samples were judged to be ~99% pure by Coomassie stained SDS-PAGE gels.

Kinetic measurement

Kinetic characterization of purified wild-type and M4 mutant TEASs were conducted as previously described (1). Briefly, 500-µL scale reactions using a 3-component buffer system (25 mM 2- (N-morpholino)ethanesulfonic acid (MES), 50 mM Tris, and 25 mM 3-(cyclohexylamino)propanesulfonic acid (CAPS) at pH 7.0 with 10 mM MgCl₂) were conducted in triplicate at room temperature (25 °C) with 15 nM protein and variable concentrations of (cis,trans)-FPP. Reaction products were analyzed using a Hewlett-Packard 6890 gas chromatograph (GC) coupled to a 5973 mass selective detector (MSD) equipped with an HP-5MS capillary column (0.25 mm i.d. 30 m with 0.25 µm film thickness) (Agilent Technologies). Product quantification was performed using SIM mode, set to detect ions with m/z = 91, 133, and 189. The GC was operated at a He flow rate of 2 mL/min, and the MSD was operated at 70 eV. Split-less injections (2 µL) were performed with an inlet temp of 250 °C, a temp that drives the Cope rearrangement of germacrene A (11) to completion. The GC was programmed with an initial oven temp of 50 °C (5-min hold), which was then increased 10 °C/min up to 180 °C (4-min hold), followed by a 100 °C/min ramp until 240 °C (1-min hold). A solvent delay of 8.5 min was allowed prior to the acquisition of the MS data. (+)-2-Epi-prezizaene (2) was quantified by integration of peak areas using Enhanced Chemstation (version B.01.00, Agilent Technologies). The GC–MS instrument was calibrated with an authentic (+)-2-epi-prezizaene standard(16).
Corrected velocity data (Table 1) were fitted to the Michaelis–Menten equation using GraphPad Prism (version 4.00 for Windows, GraphPad Software).

References

1. O'Maille, P. E., Chappell, J. & Noel, J. (2004) A single-vial analytical and quantitative gas chromatography-mass spectrometry assay for terpene synthases. *Anal Biochem* 335, 210-217.