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

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SUPPORTING INFORMATION

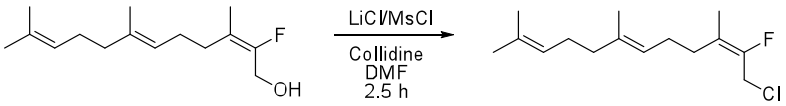
Table of Contents

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

General Aspects:

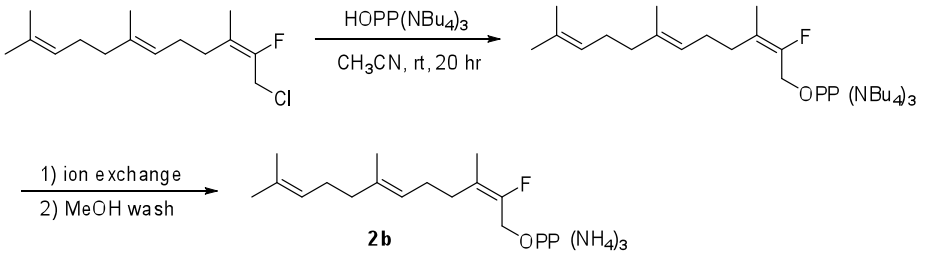
^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ (^{1}H, 7.26; ^{13}C, 77.0) or CD_3OD [^{1}H, 3.31 (quintet); ^{13}C, 49.2 (septet)] with U400 and U500 spectrometers in SCS NMR Spectroscopy Facility at the University of Illinois. Chemical shifts are in ppm and coupling constants are in Hertz. 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_2O were dried and distilled from Na/benzophenone; benzene and CH_2Cl_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_2SO_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.



(2*Z*, 6*E*)- 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) δ 5.09 (m, 2H, vinyl *H*), 4.18 (dd, 2H, *J* = 22.5, 0.5 Hz, C*H*$_2$Cl), 1.95-2.18 (m, 8H, 4C*H*$_2$), 1.72 (app d, 3H, J_{app} = 3.5 Hz, C*H*$_3$), 1.68 (d, 3H, *J* = 1.0 Hz, C*H*$_3$), 1.60 (s, 6H, 2C*H*$_3$); ^{19}F NMR ($CDCl_3$, 376 MHz) δ –116.7 (td, *J* = 23.2, 2.8 Hz).



(2*E*, 6*E*)-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_3CN (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_4HCO_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_3OD, 400 MHz) δ 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, C*H*$_2$OPP), 2.16-2.11 (m, 4H, C*H*$_2$), 2.09-2.04 (m, 2H, C*H*$_2$), 2.00-1.95 (m, 2H, C*H*$_2$), 1.68 (d, 3H, *J* = 3.5 Hz, C*H*$_3$), 1.66 (q, 3H, *J* = 1.2 Hz,

CH_3), 1.61 (d, 3H, *J* = 1.2 Hz, CH_3), 1.60 (br d, 3H, *J* = 0.6 Hz, CH_3); ^{31}P NMR (CD_3OD, 162 MHz) δ -7.99 (br d, *J* = 14.9 Hz), -9.30 (br d, *J* = 14.1 Hz); ^{19}F NMR (CD_3OD, 376 MHz) δ -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 superpositioning all C-alpha carbons to derive root mean square deviation (rmsd) values expressed in the unit angstroms.

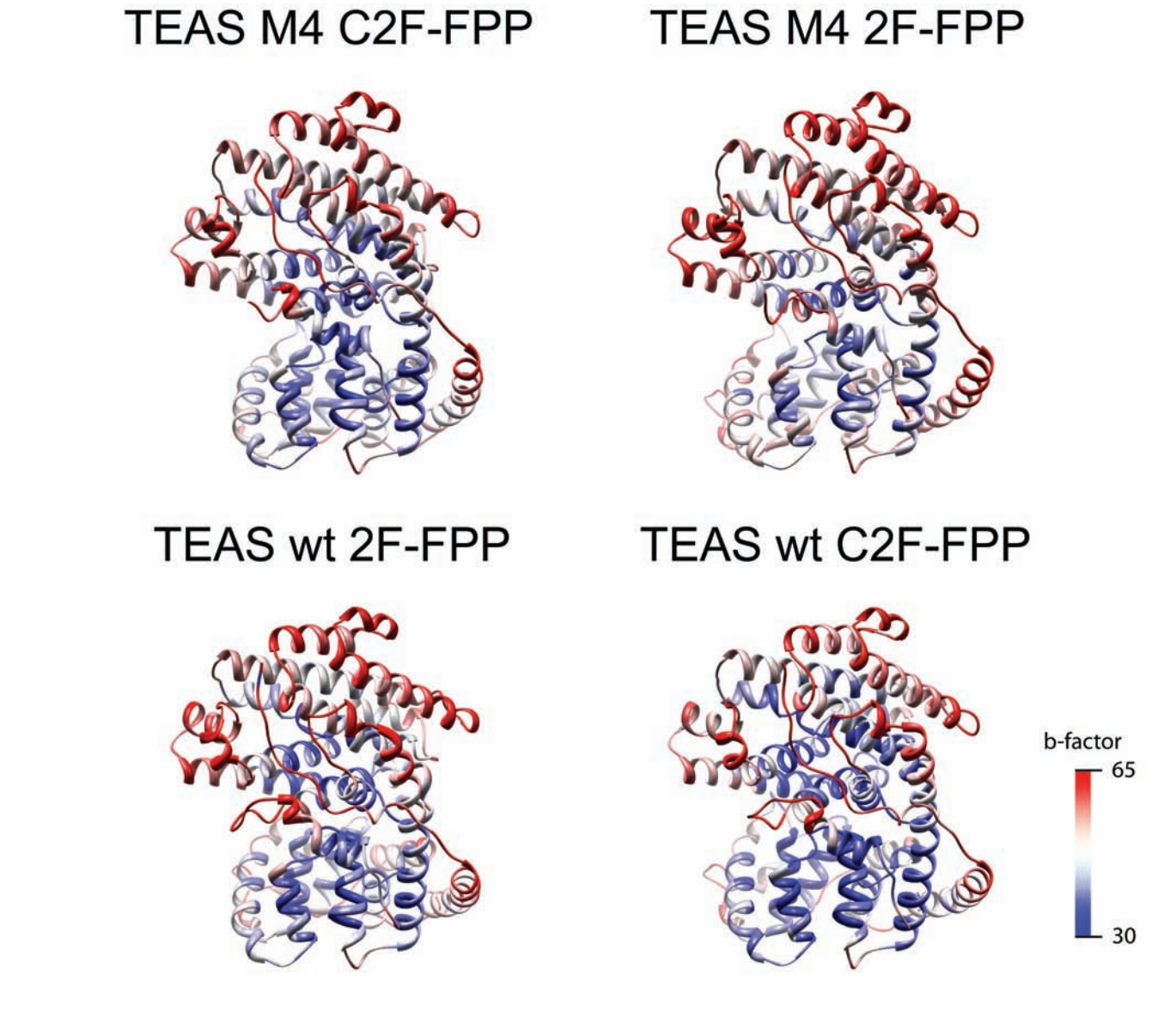


Figure S1. Annotation of global structure using B-factors reveals a similar pattern of dynamically accessible polypeptide segments. All structures were colored according to their refined isotropic by B-factors, with the corresponding color values of the blue to red gradient shown in the legend at the bottom right.

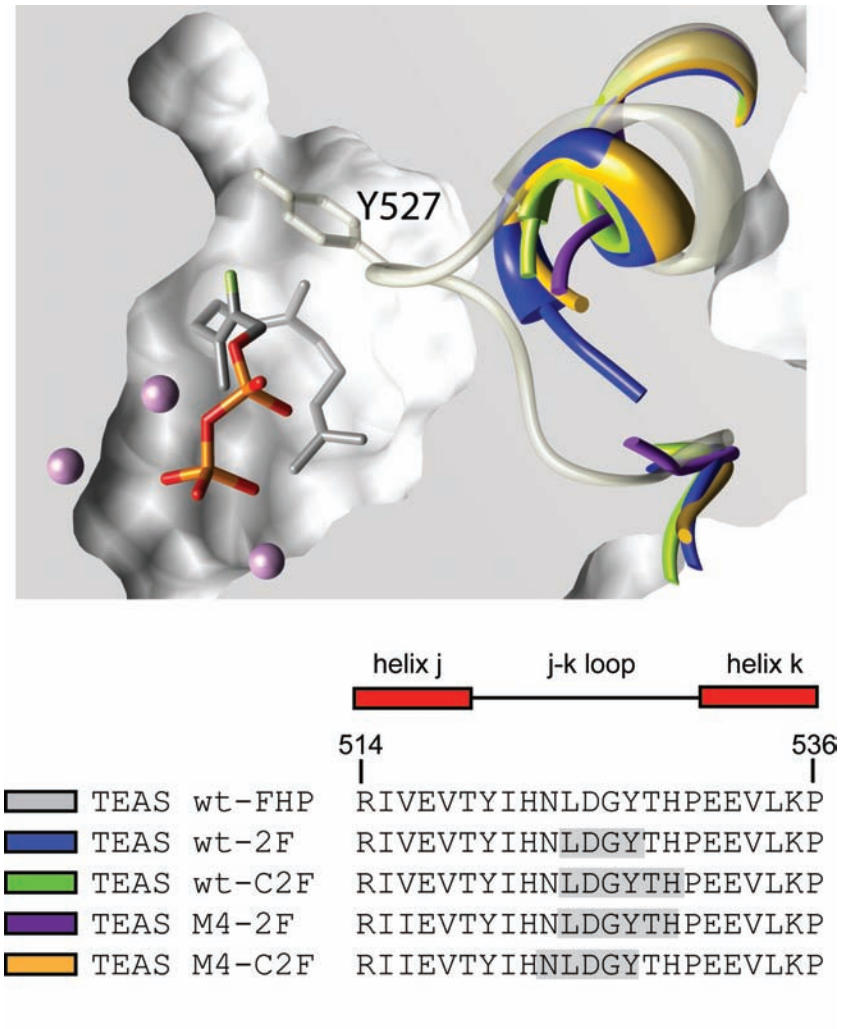


Figure S2. Disorder in the J-K loop of experimental crystal structures. An active site model for the wild-type TEAS *trans*-2F-FPP is shown as a van der Waals surface clipped to reveal the bound substrate analogue and helices J and K with the intervening loops. All experimental structures are overlaid 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.

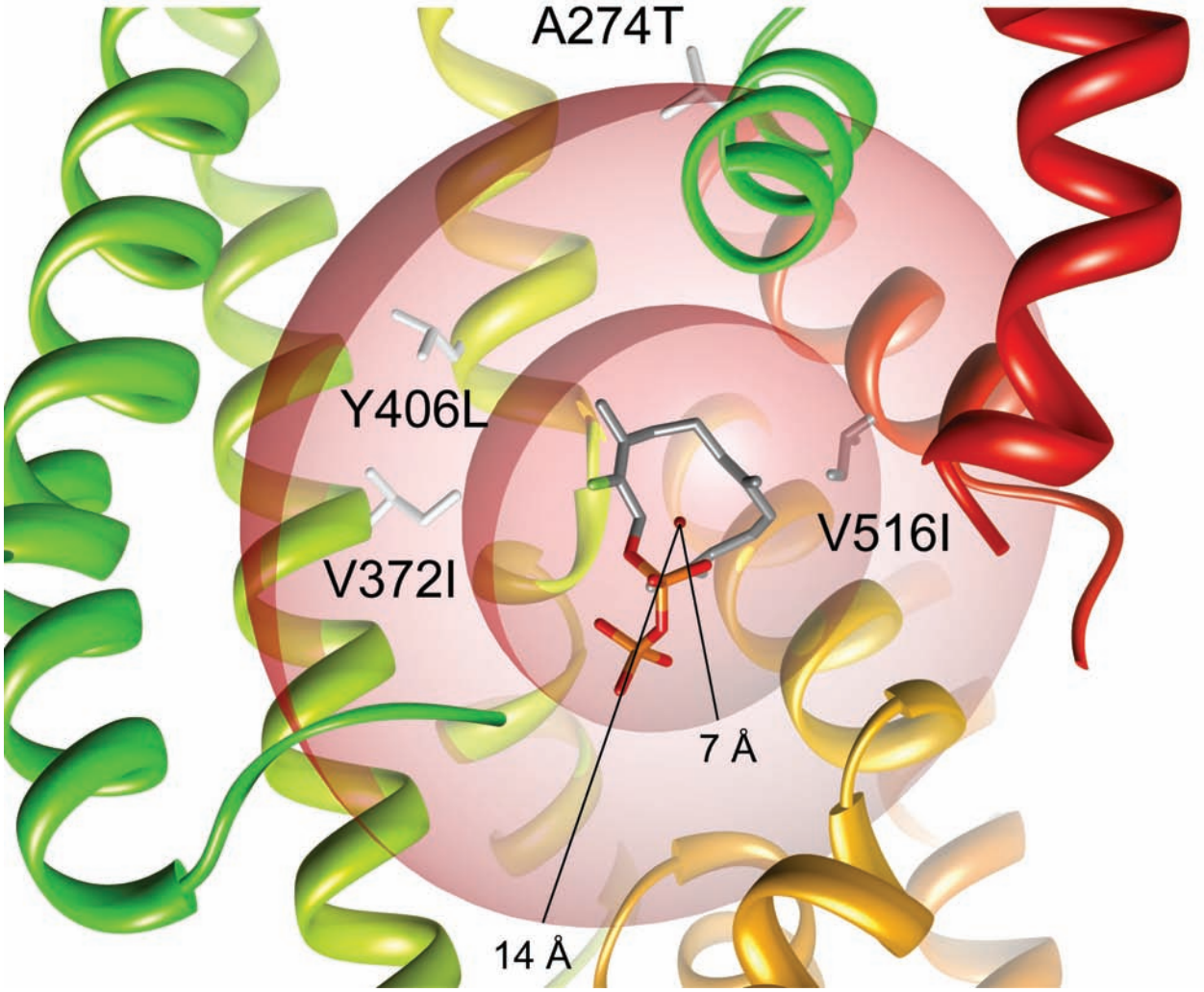


Figure S3. Spatial distribution of M4 mutations and closest distances to the farnesyl chain. a. The global structure of M4 TEAS with bound *cis*-2F-FPP ligand modeled into the active site and the protein backbone is depicted as rainbow colored ribbons. Distances from the active sited center to the side-chains of the M4 mutations are shown as dashed lines.

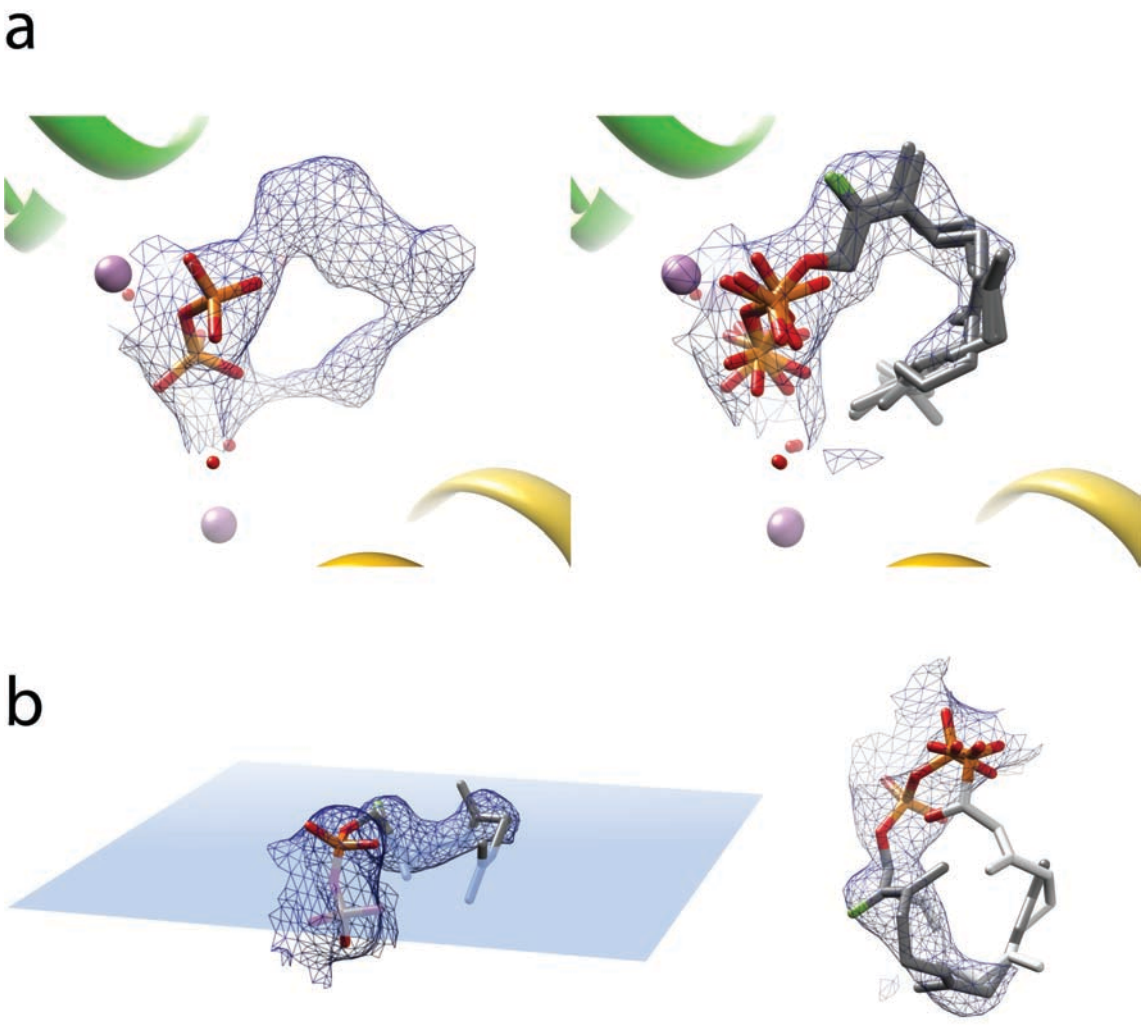


Figure S4. Farnesyl chain topology of wild-type TEAS from fluorofarnesyl analogues. a. Observable electron density from the wild-type complex with cis-2F-FPP reveals a U-shaped curl (left panel) possibly contributed to by four distinct binding modes of the farnesyl chain (right panel). b. Calculated 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.

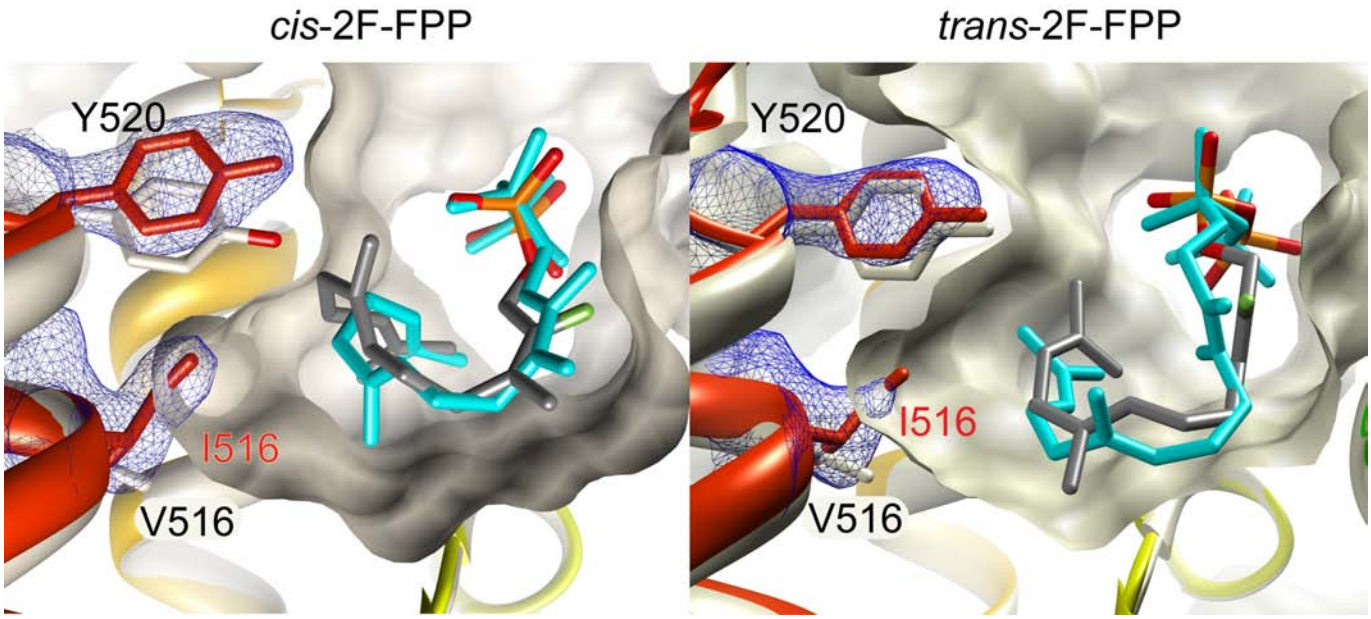
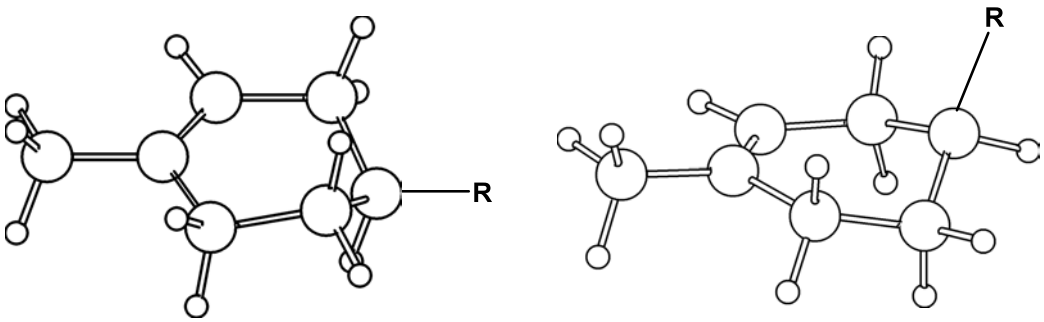


Figure S5. Spatial depiction of mutational effects in M4 TEAS on the active site contour and substrate-binding mode in the *trans*-2F-FPP and *cis*-2F-FPP complexes. The ribbon and active site surface (cream) of wild-type TEAS wild is superimposed on the corresponding M4 TEAS 2F-FPP complex, with ribbons 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 $2F_o$-F_c 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 (6*S*)-α-bisabolyl cation to α-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 α-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 (7*R*)-β-bisabolyl cation. The question then arises how might two pathways exist that both lead from (6*S*)-α-bisabolyl cation to α-cedrene (**3**). It was found that these two pathways lead from two different conformers, **a** and **b** of (6*S*)-α-bisabolyl cation (Figure S7):



a **b**

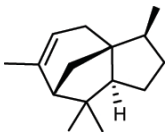
Figure S6.

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

[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 (1*R*, 4*S*, 5*S*)-α-acorenyl cation to the carbocation precursor to (-)-α-cedrene (**2**).[2]

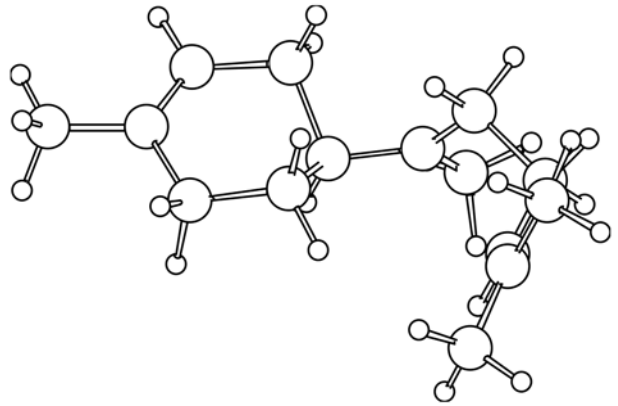
[2] An analogous pathway was also located that leads from (6R)-α-bisabolycation to the epimer of the (-)-α-cedrene (**2**) shown if Figure 2a in the article (the methyl group in the five-membered ring is cis to the CH_2 group of the adjacent six-membered ring:



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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5	6	0	0.280379	2.960189	-0.824683
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
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Thermal correction to Gibbs Free Energy= 0.318499
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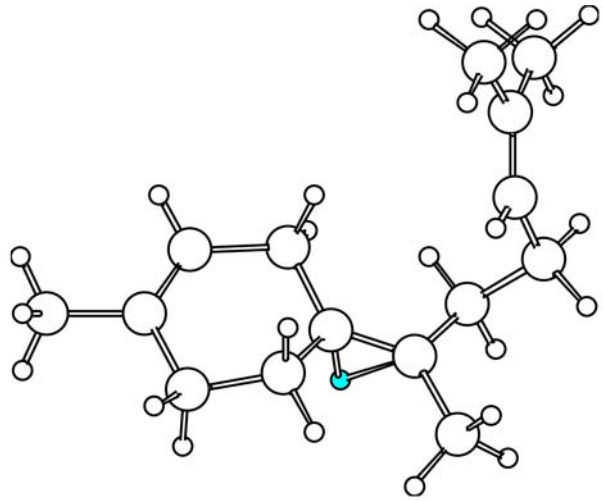
14 – transition structure linking (6S)-α-bisabolyl cation with (1*R*,4*S*,5*S*)-a-acorenyl cation



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21	1	0	2.330674	1.299891	-1.829799
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27	1	0	2.976104	-2.262889	0.154360
28	1	0	0.962630	-1.274966	-1.971925
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SCF Done: E(RB+HF-LYP) = -586.350482839 A.U. after 1 cycles
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Thermal correction to Energy= 0.380476
Thermal correction to Enthalpy= 0.381421
Thermal correction to Gibbs Free Energy= 0.317184
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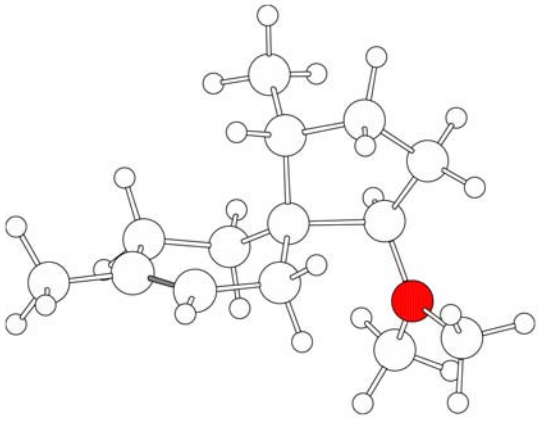
(1*R*, 4*S*, 5*S*)-α-acorenyl cation (lower energy conformer – product of IRC)



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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

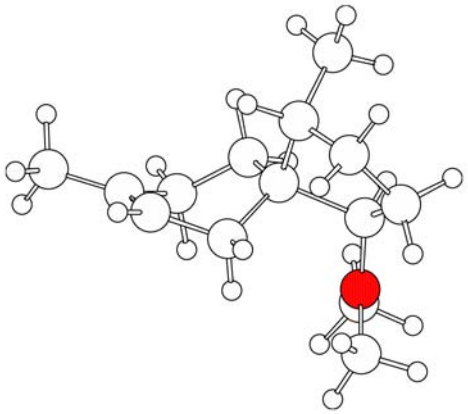
31	1	0	0.359228	-0.954407	-2.976291
32	1	0	3.298356	0.188203	-0.511318
33	1	0	2.827943	-0.695197	-1.944290
34	1	0	1.065235	-1.820237	-0.953070
35	1	0	3.749773	-1.230248	1.576510
36	1	0	2.598978	-1.023326	2.898444
37	1	0	2.831556	0.265299	1.664168
38	1	0	0.465558	-3.203869	0.794160
39	1	0	0.611269	-2.490670	2.435437
40	1	0	1.936043	-3.417963	1.783968

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

(1*R*, 4*S*, 5*S*)-α-acorenyl cation (higher energy conformer)



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

Charge = 1 Multiplicity = 1

1	6	0	-2.124372	0.019614	-1.202851
2	6	0	-2.053748	-0.095947	1.218347
3	6	0	-0.638883	-0.574688	1.011135
4	6	0	0.121180	0.390155	0.058788
5	6	0	1.650869	-0.285408	-0.407580
6	6	0	-0.757695	0.736032	-1.172907
7	6	0	2.608148	0.093232	0.748982
8	6	0	1.860023	1.082476	1.671400
9	6	0	0.689637	1.625102	0.834505
10	6	0	1.140540	2.810336	-0.034329
11	6	0	1.476731	-1.661541	-0.762730
12	6	0	-2.811955	0.157302	0.139989
13	6	0	-4.253510	0.569065	0.189691
14	6	0	1.706441	-2.770378	0.197004
15	6	0	1.121070	-2.046016	-2.151060
16	1	0	-1.999445	-1.047579	-1.447229
17	1	0	-2.730467	0.444922	-2.009431
18	1	0	-2.441847	-0.005710	2.229278
19	1	0	-0.115837	-0.690376	1.963534
20	1	0	-0.676436	-1.575184	0.553788
21	1	0	1.848342	0.305055	-1.306259
22	1	0	-0.965730	1.808538	-1.142317
23	1	0	-0.221417	0.577308	-2.114333
24	1	0	3.493428	0.553970	0.298918
25	1	0	2.968743	-0.776901	1.302818
26	1	0	1.499058	0.569363	2.567559
27	1	0	2.516123	1.885398	2.018544
28	1	0	-0.125533	1.970128	1.484855
29	1	0	0.322391	3.260280	-0.602674
30	1	0	1.930823	2.539066	-0.745528
31	1	0	1.547459	3.592919	0.613602
32	1	0	-4.624715	0.627387	1.216741
33	1	0	-4.400900	1.547580	-0.286912
34	1	0	-4.882376	-0.142797	-0.361566
35	1	0	2.760327	-3.080324	0.081396
36	1	0	1.097223	-3.649191	-0.031493
37	1	0	1.582013	-2.474973	1.239944
38	1	0	0.837079	-1.202469	-2.781014
39	1	0	0.353844	-2.827967	-2.174730
40	1	0	2.020990	-2.504454	-2.597797

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)

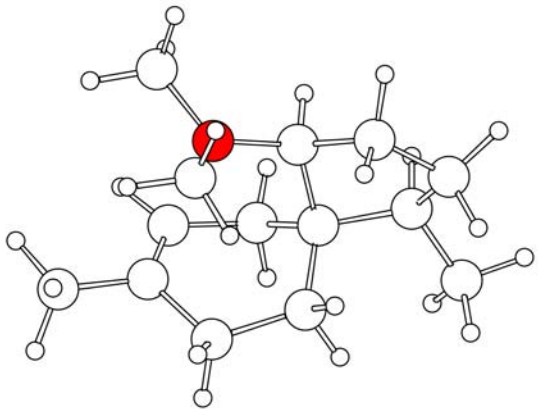
Thermal correction to Energy= 0.384217

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

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



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

Charge = 1 Multiplicity = 1

1	6	0	0.878929	-1.519695	-1.308397
2	6	0	1.265495	-1.126303	1.108581
3	6	0	-0.214638	-1.203371	1.335926
4	6	0	-0.907431	-0.374892	0.241594
5	6	0	-0.580158	-0.997002	-1.136562
6	6	0	-2.698209	1.180231	-0.375570
7	6	0	-1.346478	1.950563	-0.450360
8	6	0	-2.430908	-0.117818	0.431028
9	6	0	-3.386347	-1.266814	0.099220
10	6	0	1.795946	-1.251214	-0.143130
11	6	0	3.275078	-1.321832	-0.382065
12	6	0	-0.376852	1.104628	0.426142
13	6	0	1.084859	1.294592	0.229833
14	6	0	1.640231	1.580747	-1.125363
15	6	0	1.899574	1.813942	1.372526
16	1	0	0.862714	-2.610715	-1.444803
17	1	0	1.330924	-1.135558	-2.229926

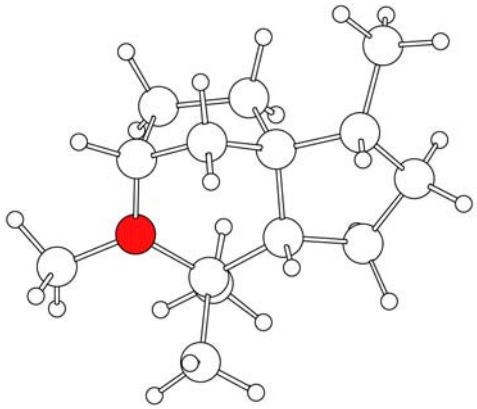
18	1	0	1.932639	-1.047006	1.964268
19	1	0	-0.479926	-0.845206	2.336423
20	1	0	-0.547277	-2.251280	1.280197
21	1	0	-0.818816	-0.290670	-1.938036
22	1	0	-1.260701	-1.839733	-1.279695
23	1	0	-3.050710	0.939420	-1.384345
24	1	0	-3.481831	1.781216	0.094014
25	1	0	-1.415124	2.971081	-0.062560
26	1	0	-0.995654	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	-0.092380	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

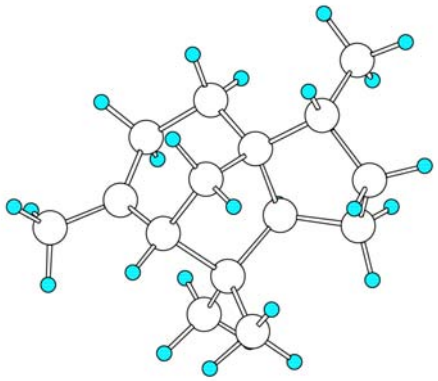
32	1	0	-2.792265	1.264141	1.553838
33	1	0	2.458775	0.242754	-1.619753
34	1	0	3.035965	1.420923	1.106886
35	1	0	3.093713	2.188443	-0.469259
36	1	0	4.536461	-0.428466	-0.530618
37	1	0	3.475215	-1.833141	-0.631201
38	1	0	3.687837	-0.979109	0.912074
39	1	0	0.882627	2.985382	-0.034758
40	1	0	0.822708	2.015012	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 nopop 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

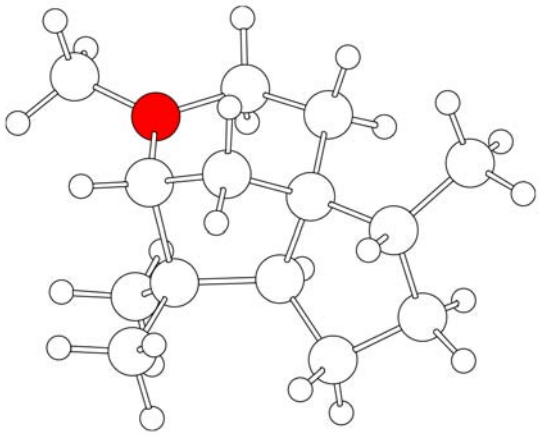
33	1	0	2.829825	-1.194593	-0.567190
34	1	0	0.602618	-1.596704	-0.452522
35	1	0	0.089492	0.053443	3.464510
36	1	0	1.196624	0.953479	2.410095
37	1	0	1.571446	-0.703100	2.889735
38	1	0	-1.058526	-2.514408	0.989941
39	1	0	-1.131896	-1.973863	2.683114
40	1	0	0.354508	-2.654094	2.025771

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

2	6	0	0.364616	-0.936578	-0.372277
3	6	0	2.061042	-0.207464	1.320939
4	6	0	-0.421672	0.625114	1.344176
5	6	0	1.088959	1.515098	-0.396968
6	6	0	3.200193	0.791402	1.543670
7	6	0	2.489910	-1.502350	0.596856
8	6	0	1.161502	-2.145010	0.168687
9	6	0	-0.151810	1.894869	-1.237343
10	6	0	-1.576245	0.496919	0.325213
11	6	0	-1.199763	-0.985364	-0.373557
12	6	0	-1.828821	-2.023515	0.572190
13	6	0	-1.791504	-1.202568	-1.772715
14	6	0	-1.476407	1.486735	-0.718913
15	6	0	-2.686647	2.077676	-1.321974
16	1	0	0.697982	-0.779866	-1.406489
17	1	0	1.685056	-0.502276	2.311104
18	1	0	-0.583019	-0.095277	2.150127
19	1	0	-0.378271	1.618289	1.805344
20	1	0	1.372971	2.374244	0.220211
21	1	0	1.925935	1.329945	-1.077592
22	1	0	3.988349	0.326722	2.145155
23	1	0	2.866672	1.685399	2.083127
24	1	0	3.658684	1.112529	0.601638
25	1	0	3.097346	-1.257758	-0.285792
26	1	0	3.095320	-2.156211	1.232607
27	1	0	0.673815	-2.588065	1.043725
28	1	0	1.281619	-2.938455	-0.575611
29	1	0	-0.183423	2.961091	-1.505434
30	1	0	-0.119226	1.400124	-2.229148
31	1	0	-2.587335	0.435816	0.735506
32	1	0	-1.537602	-3.024717	0.235694
33	1	0	-2.921487	-1.968839	0.537306
34	1	0	-1.511980	-1.921582	1.612424
35	1	0	-2.884754	-1.129005	-1.772807
36	1	0	-1.538762	-2.214294	-2.108058
37	1	0	-1.395887	-0.514010	-2.527796
38	1	0	-2.975215	2.915585	-0.660214
39	1	0	-3.535336	1.386688	-1.317227
40	1	0	-2.518684	2.497405	-2.317481

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

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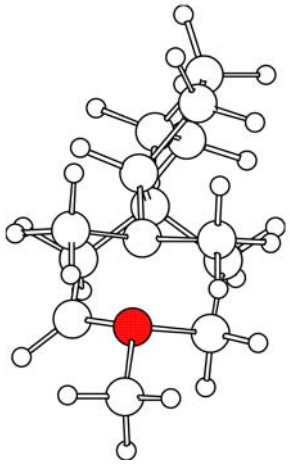
15 - Transition state linking (1*R*, 4*R*, 5*S*)-a-acorenyl cation to the tertiary carbocation precursor to (+)-2-epi-prezizaene



#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

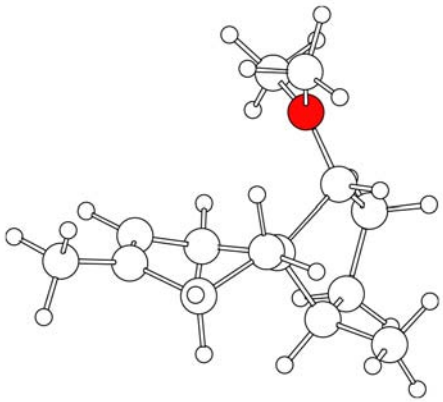
20	1	0	2.176810	-1.636461	0.583700
21	1	0	0.603024	1.783226	1.072875
22	1	0	1.900232	0.764701	1.668548
23	1	0	1.494455	2.933971	-1.149814
24	1	0	1.487426	2.239505	-2.758841
25	1	0	-0.835386	1.769361	-2.467263
26	1	0	-0.734494	2.395321	-0.833987
27	1	0	2.207591	0.128426	-2.030149
28	1	0	3.951596	0.298473	-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= -585.998014
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 (1*R*, 4*S*, 5*S*)-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(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 ^{o}C at 275 rpm. Cultures were diluted 50-fold into 1 L of Terrific Broth with kanamycin, followed by growth with shaking at 37 ^{o}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 ^{o}C at 275 rpm for 5 h. Cells were harvested by centrifugation and cell pellets frozen at -20 ^{o}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 ^{o}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 ^{o}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 $MgCl_2$) were conducted in triplicate at room temperature (25 ^{o}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.