Effect of Isotopically Sensitive Branching on Product Distribution for Pentalenene Synthase – Support for a Mechanism Predicted by Quantum Chemistry

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Contents

Enzyme assays

To simplify purification of pentalenene synthase (PS), 6×His tags were added to the wild-type and H309A mutant. This was accomplished by PCR based sub-cloning, from the originally reported expression constructs,[1, 2] first into pENTR/SD/D-TOPO via directional topoisomerization, with verification by complete gene sequencing, followed by directional recombination into pDEST17, which adds an N-terminal 6×His tag and linker (all vectors and molecular biology reagents were obtained from Invitrogen).

Expression was carried out in the C41 OverExpress strain of *E. coli* (Lucigen), which was transformed with the pDEST17/PS or pDEST17/PS:H309A constructs. These were then grown in 100 mL liquid NZY media in an incubator shaker at 37 °C to OD_{600} ~0.6, then the temperature reduced to 16 °C, and the cultures induced with 0.5 mM IPTG one hour later. Following fermentation overnight (~16 h), the cells were harvested by centrifugation (20 min. × 6,000*g*), resuspended in lysis buffer (50 mM Tris-HCl, pH 6.8, 150 mM KCl, 10 mM $MgCl_2$, 10 % (v/v) glycerol), and lysed by sonication. The resulting lysate was clarified by centrifugation (20 min. × 8,000*g*), and pentalene synthase purified using Ni-resin following the manufacturers directions (Themo Scientific). The purified protein was then dialyzed against storage buffer (20 mM Tris-HCl, pH 7.8, 150 mM KCl, 10% (v/v) glycerol).

Assays were performed in triplicate, using 1 mL the previously defined assay buffer (50 mM Tris-HCl, pH 8.2, 5 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA, 0.2 mM PMSF, 20% (v/v) glycerol)[1] in capped 13×100 mm glass tubes with 3 μM pentalenene synthase (wild-type or H309A mutant), initiated by the addition of 20 μM FPP (unlabeled or [6-^{2}H] labeled), and overlaid with 0.5 mL hexane. These reactions were incubated overnight (~16 h) at 30 °C, then terminated by the addition of 0.1 mL 100 mM EDTA (pH 8.0). The resulting sesquiterpenes were extracted by vortexing the quenched reaction with its hexane overlay, with removal of the hexane to a clean glass tube, and subsequent re-extraction with another 1 mL of hexane. The pooled extracts were then dried under nitrogen gas and resuspended in 0.1 mL of hexane for analysis by gas chromatography with mass spectrometry detection (GC-MS). This was carried out on a Varian (Palo Alto, CA) 3900 GC with Saturn 2100 ion trap mass spectrometer (MS) in electron ionization (70 eV) mode. Separation of the injected sample (1 μl; split injection with an injector port temperature of 250 °C) was achieved using an HP-5 column with a H_2 flow rate of 2 mL/min with a temperature program of 3 min. at 50 °C, followed by a gradient from 50 to 300 °C at 14 °C/min. with a 3 min. hold at 300 °C, with collection of mass spectra data from *m/z* from 90 to 650.

Pentalenene was easily identified as the sole enzymatic product from incubations of wild-type PS (with either unlabeled or [6-^{2}H]-FPP as substrate), as well as by comparative searches of its mass spectra against the MassFinder database. As previously reported,[2] the H309A mutant produces only three sesquiterpenes (Figure 1), pentalenene (**1**), protoilludene (**2**), and germacrene A, which was detected as the Cope rearrangement product β-elemene, with identification of the latter two confirmed by both comparative searches of their mass spectra using MassFinder (Figure S1), as well as self-consistent retention indexes – e.g., the retention time (RT) for pentalenene is 11.02 min., with reported retention index (RI) of 1343; the peak corresponding to β-elemene has RT = 11.53 min. and reported RI = 1389; such that the RT of 11.41 min. for the protoilludene peak leads to a calculated RI of 1378, very similar to the reported RI of 1382.

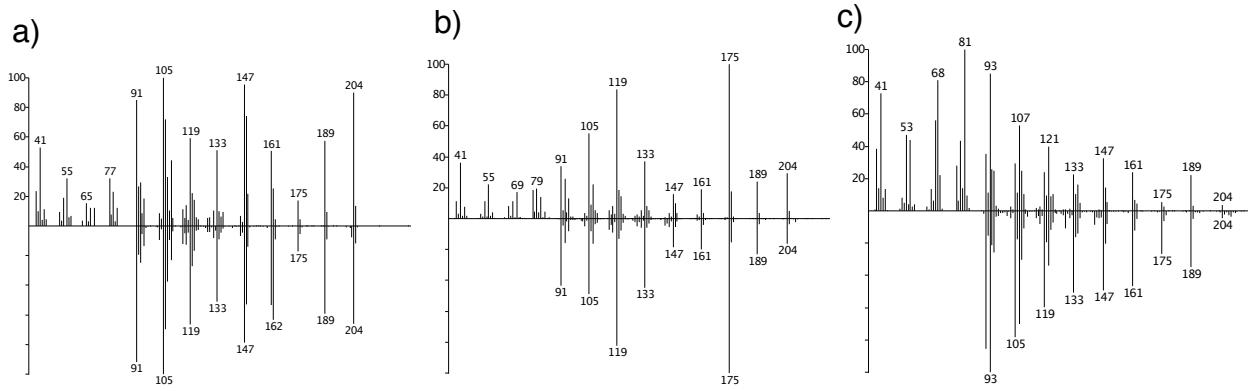

Figure S1: Comparison of mass spectra for authentic sesquiterpene standards (top) and enzymatic product peaks (bottom) from PS:H309A reacting with unlabeled FPP (those with [6-^{2}H]-FPP show the expected increase in parent *m/z* from 204 to 205). a) Pentalenene and peak at RT = 11.02 min. b) Protoilludene and peak at RT = 11.41 min. c) β-elemene and peak at RT = 11.53 min.

Enzyme assay references

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2. M. Seemann, G. Zhai, K. Umezawa and D. Cane, *J. Am. Chem. Soc.*, 1999, **121**, 591-592.

Computational Methods

All calculations were performed with Gaussian03.[G03] Geometries were optimized in the gas phase without symmetry constraints using the B3LYP/6-31+G(d,p) method.[B3LYP] Frequency calculations at the same level of theory were used to characterize each stationary point as a minimum or first order transition state structure. Single point calculations on the computed geometries were performed with the mPW1PW91/6-31+G(d,p) method[mpw1pw91] and reported energies include zero point correction from B3LYP frequency calculations at 298.15 K. Intrinsic Reaction Coordinate (IRC) calculations[IRC] were performed for each reaction step. Some of these calculations were unsuccessful, particularly where the potential energy surface appears to be relatively flat. In these cases, the transition state structures were linked to their corresponding minima by perturbing the structures in both directions along the imaginary frequency coordinate and minimizing the resulting structure. Structural images were produced with *Ball and Stick* version 4.0.[B&S]

Calculation of k_H/k_D kinetic isotope effects (KIEs)

Predicted KIE values for each step of the pathway leading to protoilludene and pentalenene were computed using the Bigeleisen and Mayer method, as implemented in *Quiver*.[quiver] For this analysis, the computed frequencies were scaled by 0.9648[scaled] and a temperature of 303 K was specified in accord with the experimental conditions. The table below summarizes the computed KIE values for each step.

Reaction Step	Computed KIE value
A→B	1.01
B→C1	1.05
C1→protoilludene	1.79
C2→protoilludene	1.55
C1→C2	0.90
C2→C3	1.02
C3→C4	0.94
C4→D’	1.10
D’→E’	1.01
E’→F	0.99
F→pentalenene	1.02, 1.04

C1-C4 refer to different conformers of this structure present along the computed pathway.

For the deprotonation steps leading to protoilludene and pentalenene, $H_2PO_4^-$ was utilized as a model for the pyrophosphate. Note that conformers C1 and C2 can both lead to protoilludene, resulting in a predicted KIE range of 1.55-1.79. Two unique transition state structures from the same reactant were located for the deprotonation leading to pentalenene. From the branch point of conformer C1, a net KIE range of 0.96 - 0.98 is predicted for pentalenene. Thus we do not see evidence that the small KIE values for this branch constitute a significant effect when taken together.

Computational references:

G03

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c. A modified version of *Quiver* provided by Prof. Daniel Singleton (Texas A&M) was utilized.

scaled

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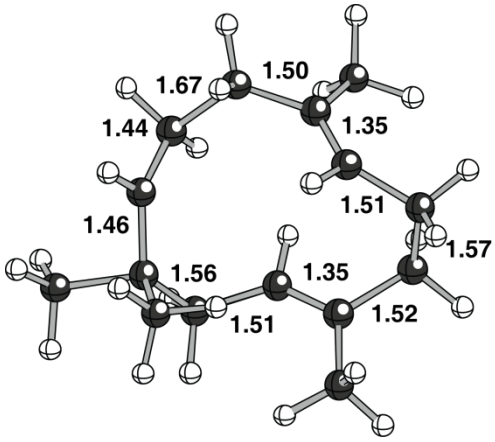
Computed Energies and Coordinates

Distances are shown in angstroms

Structure A
B3LYP electronic energy: -586.3694874 H
B3LYP zero-point energy: 0.364004 H
mPW1PW91 electronic energy: -586.2301285 H

Coordinates (from last standard orientation):

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	2.252808	0.874856	1.359109
2	1	2.841119	1.779050	1.182616
3	1	1.255413	1.171165	1.688914
4	1	2.734403	0.309743	2.162904
5	6	2.170796	0.059468	0.041802
6	6	1.705979	-1.297628	0.311392
7	6	0.804367	-2.180111	-0.378614
8	1	1.269554	-3.170116	-0.457324
9	6	-0.498235	-2.461103	0.621686
10	1	-0.729248	-3.510899	0.427877
11	6	-1.653524	-1.556786	0.315496
12	6	-1.800409	-0.421891	1.028106
13	6	-2.743795	0.723223	0.765118
14	1	-3.763899	0.372438	0.575817
15	1	-2.793985	1.355816	1.656423
16	6	-2.288686	1.581728	-0.466676
17	6	-0.793683	1.857710	-0.479376
18	6	1.512103	0.865786	-1.116989
19	1	1.975816	1.857880	-1.099982
20	1	1.812096	0.412891	-2.069494
21	6	-2.536431	-1.970165	-0.836106
22	1	-2.943744	-2.974462	-0.669814
23	1	-3.377923	-1.290054	-0.976499
24	1	-1.982834	-2.009264	-1.784507
25	1	2.164875	-1.740942	1.200087
26	6	3.673790	-0.310647	-0.380504
27	1	4.136602	0.653749	-0.615196
28	1	3.714381	-0.938840	-1.273209
29	1	4.236686	-0.776961	0.430591
30	1	-1.077005	-0.249307	1.826306
31	1	-0.168932	-2.378817	1.659786
32	1	0.458269	-1.829898	-1.348611
33	6	0.005805	0.955105	-1.078886
34	1	-0.499906	0.152616	-1.610492
35	6	-0.334066	3.100598	0.237133
36	1	-0.814656	3.981708	-0.204658
37	1	-0.631564	3.088192	1.294034
38	1	0.745936	3.253885	0.191833
39	1	-2.860575	2.515935	-0.463967
40	1	-2.561795	1.043640	-1.379580



Structure TSA→B

B3LYP electronic energy: -586.3572043 H

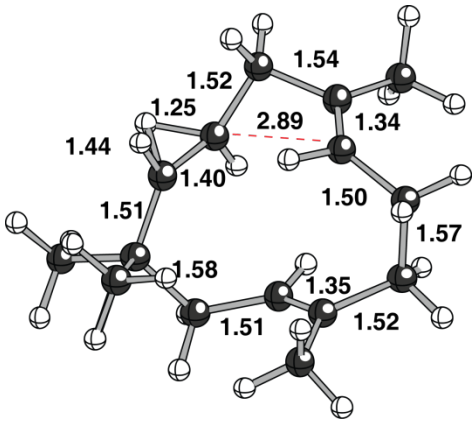
B3LYP zero-point energy: 0.361589 H

mPW1PW91 electronic energy: -586.2210695 H

Imaginary frequency: -616.6836 cm^{-1}

Coordinates (from last standard orientation):

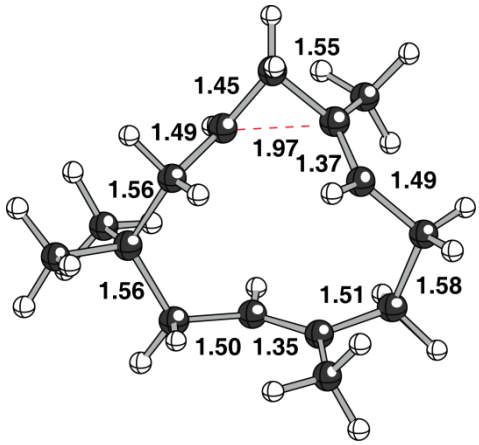
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	2.685396	0.428530	1.264900
2	1	3.468088	1.140639	0.990701
3	1	1.846191	0.993921	1.676503
4	1	3.085305	-0.222967	2.048598
5	6	2.279702	-0.387271	0.005599
6	6	1.217913	-1.340583	0.495699
7	6	0.138118	-1.906193	-0.202301
8	1	1.133226	-2.651583	-0.096305
9	6	-1.113575	-2.438508	0.467400
10	1	-1.462566	-3.348310	-0.026301
11	6	-2.124988	-1.289018	0.281105
12	6	-1.860499	-0.118417	0.885208
13	6	-2.502713	1.226977	0.681612
14	1	-3.569313	1.144066	0.453214
15	1	-2.423818	1.807675	1.606614
16	6	-1.812624	2.008887	-0.493887
17	6	-0.292723	1.968303	-0.445290
18	6	1.789090	0.572127	-1.149598
19	1	2.459581	1.437634	-1.117597
20	1	1.988993	0.081032	-2.108699
21	6	-3.257987	-1.555728	-0.673793
22	1	-3.862077	-2.403435	-0.328194
23	1	-3.918396	-0.693334	-0.778190
24	1	-2.889886	-1.819121	-1.674695
25	1	1.276418	-1.621385	1.550898
26	6	3.517510	-1.195856	-0.475705
27	1	4.303002	-0.492047	-0.765605
28	1	3.278915	-1.812857	-1.348107
29	1	3.919018	-1.838454	0.314192
30	1	-0.981898	-0.089109	1.533006
31	1	-0.929871	-2.667009	1.521899
32	1	0.063514	-1.688491	-1.265200
33	6	0.335386	0.992412	-1.129794
34	1	-0.311409	0.386006	-1.760994
35	6	0.376767	3.009008	0.414711
36	1	0.064656	4.009606	0.092514
37	1	0.077070	2.920602	1.467612
38	1	1.466667	2.970420	0.366709
39	1	-2.175535	3.042583	-0.472884
40	1	-2.154521	1.565886	-1.434888



Structure B
B3LYP electronic energy: -586.3909783 H
B3LYP zero-point energy: 0.364782 H
mPW1PW91 electronic energy: -586.2558043 H

Coordinates (from last standard orientation):

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-3.651895	0.102213	-0.657405
2	1	-4.310590	0.791420	-0.120607
3	1	-3.502391	0.504410	-1.665806
4	1	-4.177402	-0.854983	-0.747703
5	6	-2.311397	-0.056295	0.094697
6	6	-1.396903	-0.940404	-0.810200
7	6	-0.325110	-1.798711	-0.233896
8	6	0.657185	-2.530620	-1.007293
9	1	0.821277	-3.574121	-0.737791
10	6	1.631692	-1.609626	-0.233194
11	6	1.873402	-0.360529	-0.730097
12	6	2.843409	0.648165	-0.216798
13	1	3.683304	0.180660	0.303204
14	1	3.245914	1.198860	-1.073399
15	6	2.135315	1.666072	0.761399
16	6	0.752119	2.036282	0.270496
17	6	-1.722686	1.375601	0.309695
18	1	-1.812581	1.931699	-0.628707
19	1	-2.379583	1.882808	1.029693
20	6	2.320885	-2.207528	0.968508
21	1	3.188881	-2.791436	0.639111
22	1	2.663590	-1.449129	1.674207
23	1	1.663680	-2.896422	1.507709
24	1	-2.044708	-1.739000	-1.230398
25	6	-2.587104	-0.744690	1.446399
26	1	-3.355000	-0.190883	1.994396
27	1	-1.706805	-0.791895	2.096300
28	1	-2.963612	-1.765587	1.308700
29	1	1.283405	-0.048027	-1.589198
30	1	0.634188	-2.376423	-2.085094
31	1	-0.426913	-2.080808	0.811404
32	6	-0.303187	1.385291	0.793496
33	1	-0.101993	0.742692	1.651498
34	6	0.697728	3.039580	-0.854906
35	1	1.107735	3.999077	-0.516508
36	1	1.311626	2.730773	-1.712105
37	1	-0.313870	3.227786	-1.217408
38	1	2.793522	2.536768	0.851298
39	1	2.069010	1.199075	1.748500
40	1	-1.051397	-0.396109	-1.696700



Structure TSB→C1

B3LYP electronic energy: -586.3816593 H

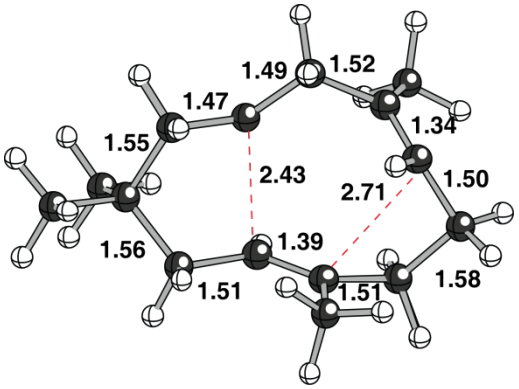
B3LYP zero-point energy: 0.363422 H

mPW1PW91 electronic energy: -586.059676 H

Imaginary frequency: -105.6230 cm^{-1}

Coordinates (from last standard orientation):

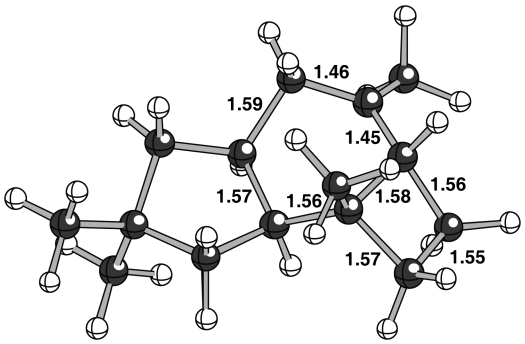
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-3.918111	0.294797	-0.669089
2	1	-4.496513	1.030688	-0.101479
3	1	-3.710114	0.721910	-1.656184
4	1	-4.552608	-0.586604	-0.806899
5	6	-2.622208	-0.061908	0.078604
6	6	-1.766906	-1.019794	-0.797509
7	6	-0.439504	-1.334596	-0.243915
8	6	0.713596	-1.816982	-1.058023
9	1	0.597100	-2.918782	-1.067437
10	6	2.019796	-1.400985	-0.394220
11	6	2.523091	-0.231478	-0.809506
12	6	3.246189	0.768514	0.047205
13	1	4.016691	0.337608	0.691499
14	1	3.726685	1.541823	-0.557386
15	6	2.123688	1.382598	0.968015
16	6	0.795786	1.560702	0.271519
17	6	-1.745813	1.208593	0.266819
18	1	-1.740016	1.773305	-0.669874
19	1	-2.222914	1.855482	1.015828
20	6	2.468700	-2.184399	0.809970
21	1	2.681004	-3.222594	0.526556
22	1	3.379100	-1.772701	1.249373
23	1	1.707702	-2.226711	1.601170
24	1	-2.287703	-1.995895	-0.848220
25	6	-2.980104	-0.703426	1.433797
26	1	-3.599205	-0.017435	2.020006
27	1	-2.107602	-0.948830	2.048092
28	1	-3.557001	-1.623126	1.290986
29	1	2.102788	0.188132	-1.724000
30	1	0.643493	-1.472769	-2.093819
31	6	-0.339811	0.897093	0.709313
32	1	-0.236507	0.396581	1.671006
33	6	0.763781	2.567416	-0.842968
34	1	0.434978	3.533410	-0.432656
35	1	1.751880	2.728225	-1.278768
36	1	0.062680	2.309423	-1.639770
37	1	2.454685	2.367295	1.329827
38	1	1.995592	0.735587	1.839907
39	1	-1.686009	-0.658081	-1.828705
40	1	-0.411202	-1.632809	0.800881



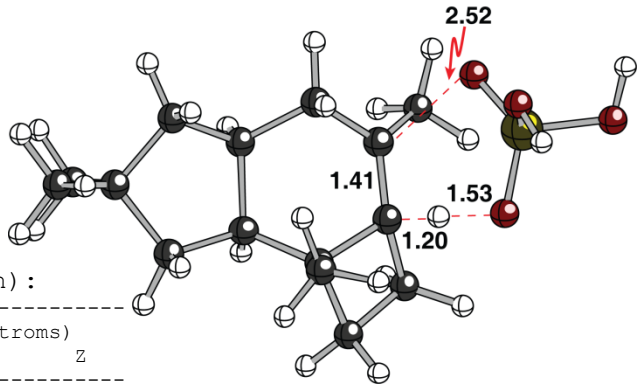
Structure C1
B3LYP electronic energy: -586.4104289 H
B3LYP zero-point energy: 0.366171 H
mPW1PW91 electronic energy: -586.2884495 H

Coordinates (from last standard orientation):

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	3.896209	-0.297180	-0.598995
2	1	4.421415	-1.114877	-0.093397
3	1	3.674210	-0.621979	-1.621795
4	1	4.586103	0.551525	-0.656195
5	6	2.616808	0.080010	0.162408
6	6	1.809099	1.170606	-0.592689
7	6	0.389601	1.055696	-0.002787
8	6	-0.726904	1.590090	-1.005685
9	1	-0.600612	2.658691	-1.189983
10	6	-1.982001	1.242180	-0.350684
11	6	-2.256391	-0.181422	-0.234386
12	6	-2.822485	-0.837028	1.065314
13	1	-2.581788	-0.261028	1.962815
14	1	-3.887683	-1.072335	1.071515
15	6	-1.838477	-2.013021	0.810710
16	6	-1.024485	-1.172313	-0.230389
17	6	1.593815	-1.087197	0.196907
18	1	1.630717	-1.603895	-0.766294
19	1	1.834322	-1.827596	0.966605
20	6	-2.841107	2.268073	0.263720
21	1	-3.732304	1.866767	0.746120
22	1	-2.248010	2.832776	1.001520
23	1	-3.112713	3.014473	-0.496379
24	1	2.231593	2.174109	-0.479688
25	6	2.986107	0.543310	1.586208
26	1	3.531213	-0.245987	2.114206
27	1	2.109606	0.794903	2.193810
28	1	3.632601	1.426815	1.553508
29	1	-2.916091	-0.412925	-1.092485
30	1	-0.606602	1.024792	-1.936386
31	6	0.199111	-0.443706	0.420810
32	1	-0.004287	-0.433610	1.498311
33	6	-0.740482	-1.894109	-1.549091
34	1	-0.113976	-2.774605	-1.380394
35	1	-1.672680	-2.247015	-2.002190
36	1	-0.227987	-1.263804	-2.283891
37	1	-2.339072	-2.862323	0.338010
38	1	-1.280574	-2.369319	1.680209
39	1	1.796099	0.933808	-1.664190
40	1	0.325998	1.688694	0.888414



Structure C1→protoilludene (2)
B3LYP electronic energy: -1230.2109293 H
B3LYP zero-point energy: 0.402858 H
mPW1PW91 electronic energy: -1230.0080479 H
Imaginary frequency: -177.2334 cm^{-1}



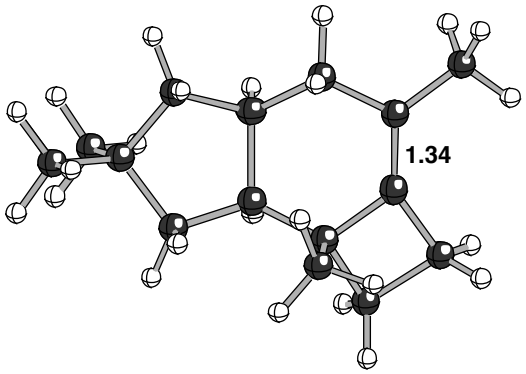
Coordinates (from last standard orientation):

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-4.748037	-1.582803	-1.117344
2	1	-5.533172	-0.985531	-1.596274
3	1	-4.120191	-2.008486	-1.908567
4	1	-5.237492	-2.412728	-0.593761
5	6	-3.917446	-0.728922	-0.147978
6	6	-2.738714	-1.526898	0.463973
7	6	-1.756945	-0.454303	0.969051
8	6	-0.289993	-0.949363	1.126826
9	1	-0.192442	-1.662495	1.949106
10	6	0.575277	0.248422	1.321432
11	6	0.567891	1.170895	0.250654
12	6	0.542043	2.719996	0.355335
13	1	0.081074	3.088429	1.277880
14	1	1.498887	3.226730	0.210720
15	6	-0.420525	2.717703	-0.869141
16	6	-0.619261	1.165948	-0.770714
17	6	-3.165316	0.417724	-0.876433
18	1	-2.853736	0.052733	-1.859478
19	1	-3.810064	1.286585	-1.051798
20	6	1.166902	0.557072	2.646432
21	1	0.346571	0.898052	3.300257
22	1	1.608714	-0.335901	3.091270
23	1	1.908740	1.356055	2.590995
24	1	-3.056694	-2.219061	1.252649
25	6	-4.842553	-0.171885	0.953771
26	1	-5.651478	0.421605	0.512035
27	1	-4.310599	0.474472	1.660016
28	1	-5.301497	-0.985845	1.527353
29	1	1.577805	0.885766	-0.322594
30	1	0.027436	-1.457735	0.211502
31	6	-1.925526	0.768356	-0.008011
32	1	-2.172948	1.637203	0.614751
33	6	-0.379591	0.419231	-2.087408
34	1	-1.082143	0.752185	-2.859138
35	1	0.634805	0.610252	-2.447268
36	1	-0.490381	-0.666023	-1.982525
37	1	0.092911	3.003149	-1.791719
38	1	-1.328025	3.321218	-0.768304
39	1	-2.260494	-2.126277	-0.322846
40	1	-2.088262	-0.133390	1.964481
41	15	3.421873	-0.656601	-0.278642
42	8	2.619439	-1.167819	0.894069
43	8	4.946642	-0.255467	0.168138
44	8	3.645518	-1.909786	-1.310380
45	8	2.881501	0.549127	-1.047919
46	1	4.038248	-1.606454	-2.141760
47	1	5.312290	-0.920983	0.768710

Protoilludene (2)
B3LYP electronic energy: -586.0667102 H
B3LYP zero-point energy: 0.355483 H
mPW1PW91 electronic energy: -585.9763966 H

Coordinates (from last standard orientation):

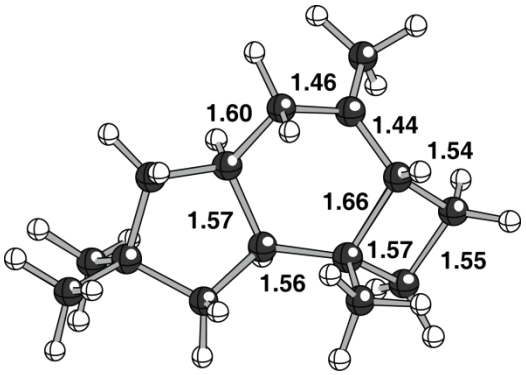
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-3.913011	-0.185094	0.812772
2	1	-4.465598	-1.077744	0.494764
3	1	-3.614000	-0.330636	1.857601
4	1	-4.607907	0.662997	0.778108
5	6	-2.691469	0.060456	-0.084584
6	6	-1.842758	1.263404	0.395549
7	6	-0.441102	1.067880	-0.220592
8	6	0.707450	1.731193	0.578221
9	1	0.649574	2.819839	0.451564
10	6	2.073120	1.218675	0.141097
11	6	2.158622	-0.104867	-0.009486
12	6	3.100836	-1.148367	-0.579595
13	1	3.652909	-0.879780	-1.486963
14	1	3.819282	-1.545476	0.148632
15	6	1.848134	-2.071361	-0.759661
16	6	1.005319	-1.088458	0.135831
17	6	-1.655235	-1.088574	0.010400
18	1	-1.597625	-1.407750	1.056925
19	1	-1.950669	-1.968271	-0.574816
20	6	3.189757	2.195183	-0.108258
21	1	4.102419	1.691900	-0.441767
22	1	2.908008	2.934530	-0.870723
23	1	3.431741	2.761311	0.801727
24	1	-2.292916	2.228567	0.132082
25	6	-3.164440	0.263151	-1.538778
26	1	-3.710317	-0.618593	-1.895266
27	1	-2.329493	0.434979	-2.225831
28	1	-3.838111	1.125228	-1.613050
29	1	0.564797	1.547711	1.653591
30	6	-0.303927	-0.491697	-0.454714
31	1	-0.225143	-0.637609	-1.537897
32	6	0.878520	-1.625060	1.573554
33	1	0.242837	-2.517036	1.613072
34	1	1.863332	-1.904603	1.963610
35	1	0.456135	-0.877216	2.253494
36	1	1.942293	-3.099573	-0.394919
37	1	1.498319	-2.094235	-1.796422
38	1	-1.770386	1.232000	1.492286
39	1	-0.428387	1.540685	-1.210335



Structure TSC2→C3
B3LYP electronic energy: -586.4082177 H
B3LYP zero-point energy: 0.366745 H
mPW1PW91 electronic energy: -586.2856326 H
Imaginary frequency: -56.3246 cm^{-1}

Coordinates (from last standard orientation):

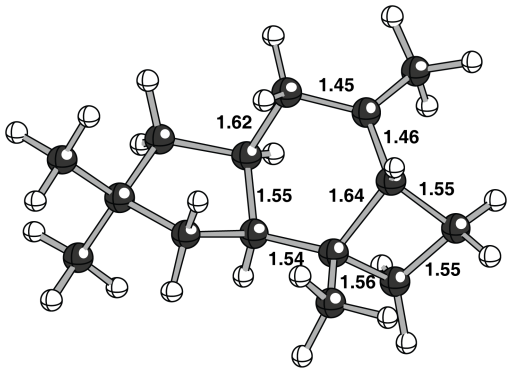
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	3.912814	-0.405615	-0.637052
2	1	4.405099	-1.258939	-0.157883
3	1	3.677090	-0.689297	-1.668968
4	1	4.636553	0.415773	-0.669482
5	6	2.650450	0.000086	0.137796
6	6	1.894579	1.155285	-0.574038
7	6	0.453770	1.068386	-0.021428
8	6	-0.612045	1.573852	-1.097554
9	1	-0.435273	2.631577	-1.302430
10	6	-1.864522	1.301162	-0.404230
11	6	-2.297635	-0.076048	-0.454028
12	6	-3.216716	-0.750495	0.580922
13	1	-3.628651	-0.100014	1.355453
14	1	-4.047694	-1.289312	0.121768
15	6	-2.042057	-1.658660	1.040345
16	6	-1.074767	-1.123557	-0.068859
17	6	1.574277	-1.115283	0.117152
18	1	1.556497	-1.551672	-0.886138
19	1	1.795712	-1.926913	0.818124
20	6	-2.515815	2.329780	0.430789
21	1	-3.513183	2.515437	-0.002442
22	1	-2.716074	1.964597	1.444607
23	1	-1.967850	3.271999	0.462969
24	1	2.346587	2.136885	-0.401288
25	6	3.036736	0.390904	1.578326
26	1	3.510227	-0.453221	2.090424
27	1	3.752590	1.219535	1.575568
28	1	-2.550106	-0.285499	-1.503465
29	1	-0.486323	0.972209	-2.000984
30	6	0.226076	-0.418414	0.437312
31	1	0.125371	-0.378902	1.527025
32	6	-0.873996	-2.138359	-1.194830
33	1	-0.389825	-1.703648	-2.075557
34	1	-0.252328	-2.968535	-0.845391
35	1	-1.833008	-2.561561	-1.510002
36	1	-2.236631	-2.732158	0.977679
37	1	-1.696477	-1.427354	2.051287
38	1	1.898806	0.974109	-1.655928
39	1	0.365220	1.726826	0.848498
40	1	2.176765	0.699918	2.182812



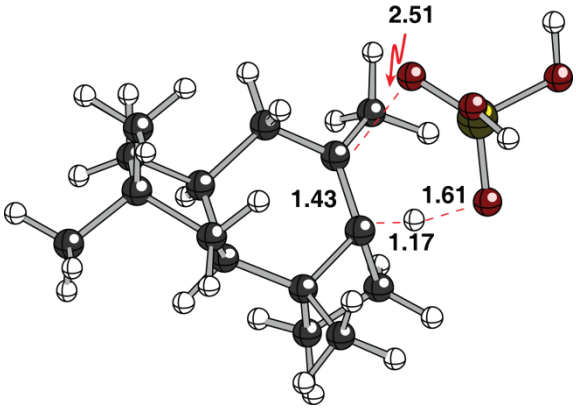
Structure C2
B3LYP electronic energy: -586.4109987 H
B3LYP zero-point energy: 0.366456 H
mPW1PW91 electronic energy: -586.289115 H

Coordinates (from last standard orientation):

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	3.646390	0.364914	-1.085006
2	1	4.135460	-0.538319	-1.465361
3	1	3.157159	0.862144	-1.930846
4	1	4.430819	1.031883	-0.711622
5	6	2.641369	0.018002	0.025856
6	6	1.889144	1.294751	0.544237
7	6	0.375795	0.945734	0.571548
8	6	-0.352855	1.655144	-0.688287
9	1	-0.146467	2.725440	-0.679967
10	6	-1.742217	1.322717	-0.443067
11	6	-2.139819	-0.061089	-0.660214
12	6	-3.264748	-0.676449	0.203271
13	1	-3.969505	0.005182	0.682491
14	1	-3.838869	-1.418483	-0.353701
15	6	-2.173268	-1.312808	1.104811
16	6	-1.094274	-1.138038	-0.010489
17	6	1.502045	-0.896346	-0.498417
18	1	1.280726	-0.655859	-1.547010
19	1	1.784560	-1.952391	-0.473820
20	6	-2.650425	2.308394	0.182317
21	1	-3.636437	2.265423	-0.298619
22	1	-2.837309	2.022932	1.228933
23	1	-2.259057	3.325505	0.157178
24	1	2.220255	1.570572	1.550439
25	6	3.386618	-0.663775	1.191502
26	1	3.892617	-1.572863	0.849477
27	1	2.713450	-0.943695	2.009981
28	1	4.148023	0.004637	1.607081
29	1	-2.195148	-0.203344	-1.752243
30	1	0.060374	1.182430	-1.583083
31	6	0.286597	-0.586925	0.401492
32	1	0.493180	-1.023048	1.389887
33	6	-1.006095	-2.397313	-0.877604
34	1	-0.467572	-2.221567	-1.812571
35	1	-0.478234	-3.183468	-0.327143
36	1	-1.998641	-2.784921	-1.124868
37	1	-2.341943	-2.338986	1.441563
38	1	-1.958772	-0.685441	1.977292
39	1	2.087179	2.160997	-0.094405
40	1	-0.101392	1.303762	1.489230



Structure TSC2→protoilludene (2)
B3LYP electronic energy: -1230.211706 H
B3LYP zero-point energy: 0.403588 H
mPW1PW91 electronic energy: -1230.008554 H
Imaginary frequency: -130.9728 cm^{-1}



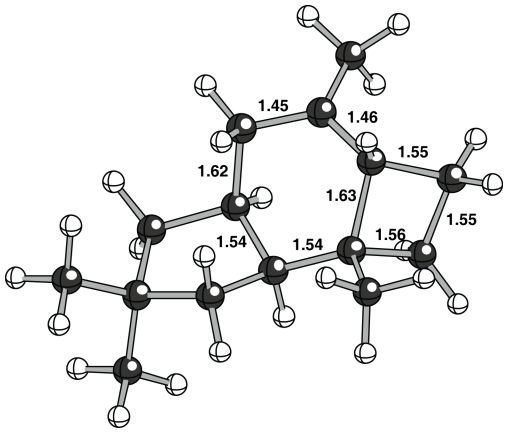
Coordinates (from last standard orientation):

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-3.063609	-2.766450	0.111437
2	1	-3.144454	-3.264773	-0.861923
3	1	-2.078475	-3.007132	0.526425
4	1	-3.822804	-3.201829	0.772356
5	6	-3.258359	-1.247096	-0.029621
6	6	-3.061119	-0.501699	1.339542
7	6	-1.923031	0.527758	1.140097
8	6	-0.560143	-0.046640	1.614823
9	1	-0.576471	-0.269810	2.686628
10	6	0.562788	0.874243	1.290492
11	6	0.613723	1.485078	0.002718
12	6	0.791747	3.036178	-0.112393
13	1	0.999989	3.582729	0.810622
14	1	1.543551	3.305295	-0.857171
15	6	-0.664945	3.094381	-0.641775
16	6	-0.668902	1.548324	-0.898679
17	6	-2.180902	-0.619287	-0.960679
18	1	-1.264040	-1.221430	-0.940842
19	1	-2.513694	-0.591189	-2.003004
20	6	1.471289	1.319740	2.378285
21	1	1.741607	0.477473	3.017653
22	1	2.370390	1.807728	1.998452
23	1	0.914879	2.047509	2.992501
24	1	-3.984687	0.012913	1.627677
25	6	-4.670791	-0.969068	-0.582511
26	1	-4.823901	-1.475092	-1.542998
27	1	-4.840166	0.102927	-0.737450
28	1	-5.440052	-1.328232	0.111566
29	1	1.511875	0.936494	-0.506700
30	1	-0.348072	-0.994640	1.107468
31	6	-1.909767	0.787720	-0.386225
32	1	-2.782278	1.414921	-0.625768
33	6	-0.359633	1.250383	-2.371604
34	1	-0.106194	0.199125	-2.528908
35	1	-1.224369	1.499478	-2.998535
36	1	0.492867	1.841189	-2.721079
37	1	-0.848094	3.723596	-1.519021
38	1	-1.366280	3.376235	0.151961
39	1	-2.828924	-1.201485	2.150541
40	1	-2.124964	1.447399	1.704244
41	15	2.923478	-1.045112	-0.236409
42	8	2.043072	-1.126980	0.992901
43	8	4.510418	-1.077779	0.175782
44	8	2.719140	-2.445601	-1.066786
45	8	2.748438	0.144074	-1.171488
46	1	3.141460	-2.391226	-1.936326
47	1	4.670526	-1.742586	0.861066

Structure TSC2→C3
B3LYP electronic energy: -586.4109976 H
B3LYP zero-point energy: 0.366338 H
mPW1PW91 electronic energy: -586.2891035 H
Imaginary frequency: -10.0160 cm^{-1}

Coordinates (from last standard orientation):

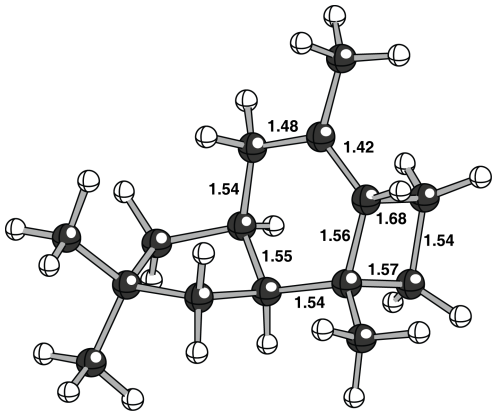
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	3.627040	0.402432	-1.095084
2	1	4.113276	-0.488504	-1.506689
3	1	3.122638	0.919492	-1.919927
4	1	4.414738	1.062856	-0.716990
5	6	2.640784	0.022842	0.022034
6	6	1.887445	1.282040	0.581342
7	6	0.374752	0.933284	0.595364
8	6	-0.348115	1.659369	-0.655738
9	1	-0.143962	2.729972	-0.635322
10	6	-1.742598	1.325115	-0.438433
11	6	-2.133909	-0.061822	-0.651315
12	6	-3.260993	-0.676250	0.212018
13	1	-3.956721	0.007229	0.701619
14	1	-3.843952	-1.409183	-0.347776
15	6	-2.168509	-1.326316	1.102337
16	6	-1.092162	-1.142471	-0.014634
17	6	1.501267	-0.889126	-0.507347
18	1	1.273382	-0.637719	-1.551963
19	1	1.787766	-1.944334	-0.497279
20	6	-2.669302	2.314458	0.152020
21	1	-3.668604	2.216530	-0.288935
22	1	-2.807302	2.081001	1.220260
23	1	-2.308109	3.339636	0.065667
24	1	2.216136	1.521602	1.597614
25	6	3.407467	-0.680378	1.160674
26	1	3.918027	-1.575443	0.789597
27	1	4.167491	-0.015245	1.584036
28	1	-2.199742	-0.195820	-1.744674
29	1	0.073080	1.199585	-1.554135
30	6	0.288966	-0.596338	0.402317
31	1	0.500123	-1.047713	1.382953
32	6	-1.004565	-2.396280	-0.890048
33	1	-0.467481	-2.214019	-1.824661
34	1	-0.476020	-3.186427	-0.346120
35	1	-1.997633	-2.781921	-1.138767
36	1	-2.339897	-2.355813	1.427290
37	1	-1.949642	-0.709659	1.981279
38	1	2.087596	2.170575	-0.025324
39	1	-0.107436	1.277913	1.515669
40	1	2.746837	-0.987947	1.979536



Structure C3
B3LYP electronic energy: -586.417119 H
B3LYP zero-point energy: 0.366927 H
mPW1PW91 electronic energy: -586.295644 H

Coordinates (from last standard orientation):

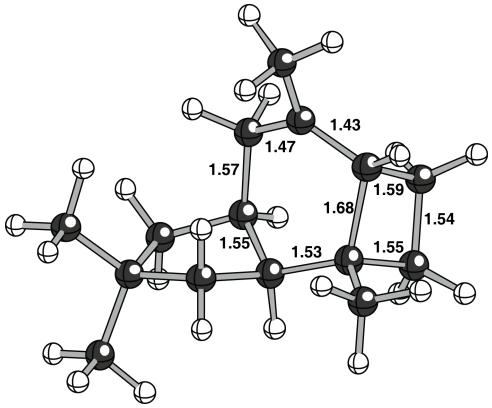
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	3.150603	1.185791	-0.958695
2	1	3.535442	0.763650	-1.893570
3	1	2.426104	1.966472	-1.223566
4	1	3.986563	1.673805	-0.446171
5	6	2.522772	0.091266	-0.077176
6	6	1.869961	0.686037	1.224896
7	6	0.350118	0.431334	1.120393
8	6	-0.368867	1.610403	0.430581
9	1	-0.502413	2.462293	1.108823
10	6	-1.625438	1.316426	-0.297361
11	6	-2.015338	-0.023421	-0.565239
12	6	-2.874149	-0.256714	0.855984
13	1	-2.692361	0.477382	1.643320
14	1	-3.939534	-0.284135	0.627930
15	6	-2.100350	-1.580095	1.007245
16	6	-1.140514	-1.256071	-0.187619
17	6	1.338499	-0.617798	-0.810397
18	1	0.936050	0.027254	-1.605049
19	1	1.660717	-1.541759	-1.298199
20	6	-2.480971	2.443876	-0.752724
21	1	-3.472015	2.120604	-1.077293
22	1	-2.560189	3.235460	-0.002001
23	1	-1.980540	2.892929	-1.625471
24	1	2.258970	0.172771	2.109845
25	6	3.609409	-0.935536	0.297900
26	1	4.084499	-1.349752	-0.597969
27	1	4.392799	-0.469754	0.905703
28	1	-2.702175	-0.148902	-1.403801
29	1	0.268528	2.019173	-0.377128
30	6	0.273386	-0.865479	0.278687
31	1	0.627738	-1.687468	0.915668
32	6	-1.136769	-2.331789	-1.274902
33	1	-0.621601	-1.994548	-2.179076
34	1	-0.629838	-3.232028	-0.911371
35	1	-2.156724	-2.617227	-1.553969
36	1	-2.737684	-2.445635	0.810873
37	1	-1.617353	-1.709212	1.978681
38	1	2.103086	1.748821	1.356032
39	1	-0.097868	0.300471	2.112509
40	1	3.195704	-1.770043	0.875823



Structure TSC3→C4
B3LYP electronic energy: -586.4155732 H
B3LYP zero-point energy: 0.366225 H
mPW1PW91 electronic energy: -586.2931957 H
Imaginary frequency: -56.7561 cm^{-1}

Coordinates (from last standard orientation):

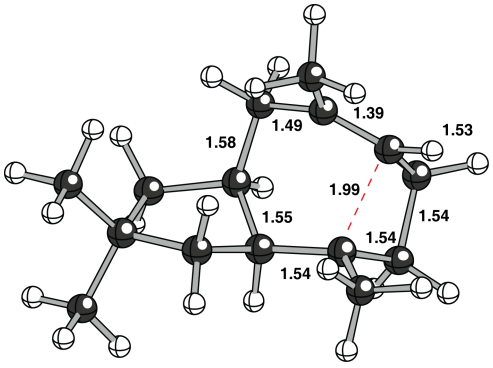
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-3.151828	1.111276	0.663199
2	1	-3.473235	0.915589	1.692187
3	1	-2.507543	1.999137	0.680873
4	1	-4.044419	1.363316	0.080850
5	6	-2.431091	-0.111857	0.069522
6	6	-1.873321	0.172310	-1.373522
7	6	-0.333480	0.097600	-1.281926
8	6	0.271187	1.497393	-0.920239
9	1	0.740070	1.917572	-1.830391
10	6	1.339185	1.510815	0.097467
11	6	2.187116	0.373343	0.247047
12	6	2.856465	-0.272640	-1.040320
13	1	2.499074	0.183347	-1.964664
14	1	3.942247	-0.172786	-0.994063
15	6	2.274076	-1.664885	-0.755264
16	6	1.340670	-1.078501	0.336389
17	6	-1.161640	-0.480450	0.901789
18	1	-0.808719	0.395803	1.466784
19	1	-1.369998	-1.257671	1.642631
20	6	1.466199	2.685956	0.990393
21	1	2.472519	2.801637	1.398078
22	1	1.130055	3.607162	0.504756
23	1	0.783734	2.526723	1.843029
24	1	-2.225103	-0.593784	-2.072176
25	6	-3.408782	-1.302859	0.031051
26	1	-3.815219	-1.511334	1.026553
27	1	-4.251979	-1.092304	-0.635652
28	1	2.894811	0.474148	1.070389
29	1	-0.497490	2.228959	-0.650202
30	6	-0.106280	-0.914671	-0.131504
31	1	-0.393398	-1.902678	-0.520951
32	6	1.496029	-1.693762	1.720381
33	1	0.989509	-1.113040	2.495973
34	1	1.058691	-2.700156	1.709699
35	1	2.549627	-1.798572	1.995237
36	1	3.008870	-2.363457	-0.348381
37	1	1.766527	-2.149641	-1.592629
38	1	-2.217610	1.133013	-1.771539
39	1	0.103833	-0.249734	-2.221849
40	1	-2.921876	-2.215654	-0.331324



Structure C4
B3LYP electronic energy: -586.417315 H
B3LYP zero-point energy: 0.367208 H
mPW1PW91 electronic energy: -586.2930655 H

Coordinates (from last standard orientation):

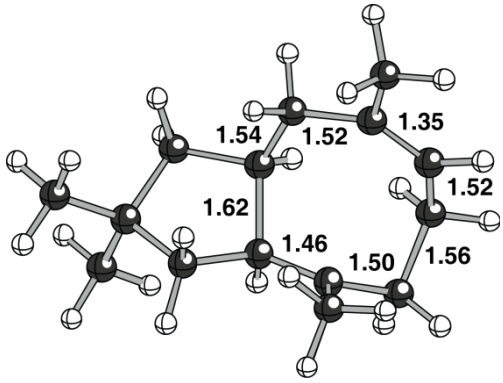
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-3.247250	1.097204	0.559197
2	1	-3.597287	0.936258	1.584829
3	1	-2.640878	2.010801	0.552106
4	1	-4.127793	1.280040	-0.065536
5	6	-2.454547	-0.117902	0.048316
6	6	-1.863420	0.126026	-1.389372
7	6	-0.325309	0.041959	-1.276793
8	6	0.333494	1.454977	-1.039327
9	1	0.768023	1.785165	-1.993347
10	6	1.406161	1.456851	0.000891
11	6	2.443445	0.536370	-0.023829
12	6	2.839042	-0.382007	-1.176159
13	1	2.366707	-0.097103	-2.117685
14	1	3.920169	-0.397748	-1.327116
15	6	2.259860	-1.681095	-0.585342
16	6	1.306982	-1.033052	0.440080
17	6	-1.201469	-0.388222	0.939850
18	1	-0.884084	0.534223	1.440908
19	1	-1.411356	-1.118668	1.726954
20	6	1.313850	2.449949	1.114368
21	1	2.018069	2.252672	1.925271
22	1	1.562699	3.436524	0.693615
23	1	0.298217	2.538867	1.510751
24	1	-2.220488	-0.645206	-2.079517
25	6	-3.375650	-1.354285	0.034896
26	1	-3.799106	-1.540904	1.027602
27	1	-4.208756	-1.208565	-0.660796
28	1	3.174857	0.615373	0.778681
29	1	-0.429760	2.188821	-0.772036
30	6	-0.115187	-0.866106	-0.038322
31	1	-0.375315	-1.889281	-0.362699
32	6	1.522109	-1.354412	1.891393
33	1	1.010988	-0.661426	2.562779
34	1	1.094222	-2.353817	2.065787
35	1	2.581103	-1.408543	2.152549
36	1	3.022728	-2.277269	-0.077929
37	1	1.738167	-2.323999	-1.300623
38	1	-2.182842	1.085417	-1.808753
39	1	0.110200	-0.400035	-2.176329
40	1	-2.841147	-2.259137	-0.278556



Structure C4→D’
B3LYP electronic energy: -586.4109204 H
B3LYP zero-point energy: 0.365872 H
mPW1PW91 electronic energy: -586.281245 H
Imaginary frequency: -50.0778 cm^{-1}

Coordinates (from last standard orientation):

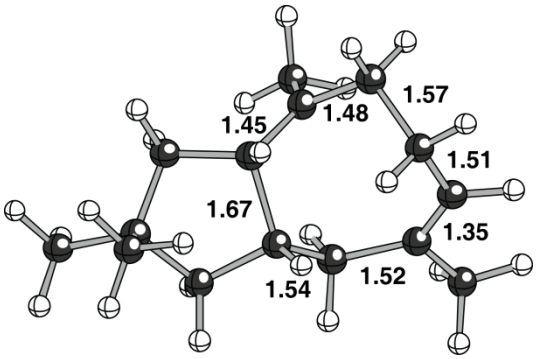
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-3.591960	1.063297	0.793556
2	1	-4.173784	0.484032	1.518737
3	1	-3.046927	1.840744	1.340731
4	1	-4.302618	1.560176	0.124940
5	6	-2.637721	0.157807	-0.000984
6	6	-1.759693	0.972108	-1.006112
7	6	-0.289206	0.462928	-0.949504
8	6	0.653174	1.538803	-0.392224
9	1	0.623323	2.372931	-1.110006
10	6	2.110004	1.247951	-0.086290
11	6	2.854212	0.190295	-0.475845
12	6	2.453000	-1.023913	-1.300261
13	1	1.796393	-0.760391	-2.129995
14	1	3.341089	-1.480777	-1.744715
15	6	1.779519	-2.092061	-0.390868
16	6	0.824703	-1.386250	0.524504
17	6	-1.581635	-0.500307	0.927026
18	1	-1.255738	0.226122	1.680593
19	1	-1.976381	-1.373353	1.458011
20	6	2.753988	2.337302	0.744877
21	1	3.814957	2.147153	0.920097
22	1	2.663822	3.308009	0.242018
23	1	2.256952	2.443429	1.717413
24	1	-2.154667	0.890206	-2.023042
25	6	-3.461526	-0.916956	-0.740603
26	1	-4.022930	-1.535721	-0.032358
27	1	-4.183276	-0.444938	-1.414725
28	1	3.888985	0.180418	-0.136803
29	1	0.207845	1.958763	0.520610
30	6	-0.426946	-0.857385	-0.017392
31	1	-0.763519	-1.613782	-0.741701
32	6	1.100773	-1.372870	1.968350
33	1	0.664057	-0.517246	2.487655
34	1	0.572605	-2.264644	2.362891
35	1	2.157739	-1.497152	2.205576
36	1	2.539349	-2.614779	0.195572
37	1	1.240616	-2.829166	-0.999641
38	1	-1.775510	2.035128	-0.744427
39	1	0.046552	0.146044	-1.935775
40	1	-2.843039	-1.585702	-1.351041



Structure D’
B3LYP electronic energy: -586.4144409 H
B3LYP zero-point energy: 0.366323 H
mPW1PW91 electronic energy: -586.2842826 H

Coordinates (from last standard orientation):

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-3.902872	0.702996	0.754199
2	1	-4.652609	-0.094694	0.783024
3	1	-3.608151	0.928208	1.785288
4	1	-4.387317	1.593894	0.341621
5	6	-2.695353	0.291684	-0.101318
6	6	-1.578865	1.356356	-0.087234
7	6	-0.231897	0.684175	-0.442357
8	6	0.937870	1.383935	0.271313
9	1	0.774376	2.453829	0.080855
10	6	2.395367	1.106357	-0.075808
11	6	2.914745	0.059261	-0.742878
12	6	2.216690	-1.139647	-1.348549
13	1	1.422766	-0.854707	-2.044609
14	1	2.934855	-1.718477	-1.934326
15	6	1.641770	-2.130594	-0.278020
16	6	0.575026	-1.498385	0.530087
17	6	-1.950576	-0.917683	0.521810
18	1	-1.866572	-0.766636	1.602850
19	1	-2.467522	-1.869959	0.362309
20	6	3.315113	2.191069	0.442875
21	1	4.366297	1.949784	0.272102
22	1	3.101361	3.148600	-0.048203
23	1	3.172492	2.352179	1.518913
24	1	-1.772994	2.179102	-0.785477
25	6	-3.165609	-0.014740	-1.537958
26	1	-3.876134	-0.847966	-1.545006
27	1	-3.673873	0.855750	-1.964522
28	1	3.994891	0.057464	-0.872485
29	1	0.817202	1.298480	1.361802
30	6	-0.564799	-0.928358	-0.154511
31	1	-0.636356	-1.369247	-1.151119
32	6	0.767498	-1.425772	1.986632
33	1	0.005687	-0.872463	2.531315
34	1	0.799300	-2.461968	2.364309
35	1	1.772387	-1.031375	2.195679
36	1	2.448987	-2.512768	0.350963
37	1	1.184731	-2.975038	-0.817372
38	1	-1.506309	1.794225	0.916227
39	1	-0.048409	0.712080	-1.518565
40	1	-2.343740	-0.272222	-2.215267

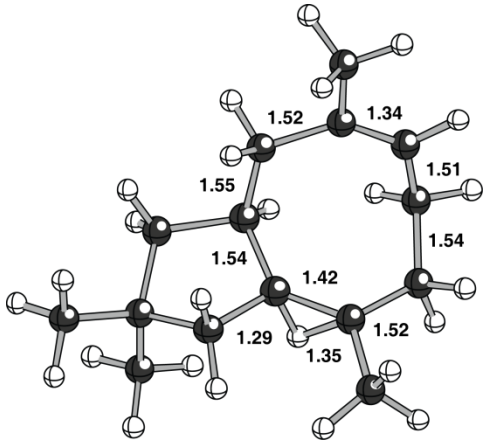


Structure D'→E'

B3LYP electronic energy: -586.4034256 H
B3LYP zero-point energy: 0.363823 H
mPW1PW91 electronic energy: -586.2760572 H
Imaginary frequency: -360.9702 cm^{-1}

```
Coordinates (from last standard orientation):
  31 -   16    H    C distance =     1.348
  31 -   30    H    C distance =     1.294
Valence > maximum for atom=31 type=H valence=2 max=1
```

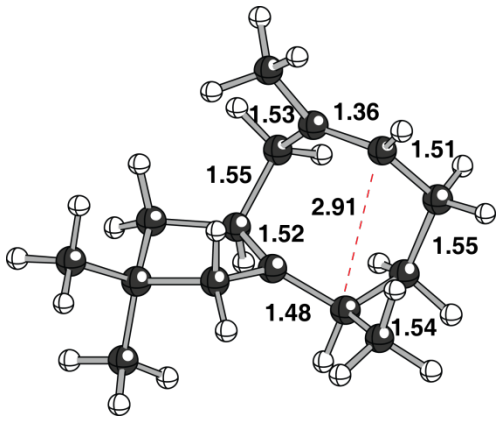
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	3.652729	-1.213123	0.669103
2	1	4.329360	-0.556807	1.226937
3	1	3.215517	-1.928817	1.373825
4	1	4.258344	-1.776821	-0.047678
5	6	2.566932	-0.407126	-0.057382
6	6	1.564077	-1.315823	-0.823272
7	6	0.151113	-0.656111	-0.736607
8	6	-0.860983	-1.653519	-0.110114
9	1	-0.851880	-2.538608	-0.762432
10	6	-2.305167	-1.273975	0.148572
11	6	-2.971345	-0.215940	-0.342146
12	6	-2.470804	0.889881	-1.243941
13	1	-1.864349	0.522102	-2.073918
14	1	-3.334877	1.366891	-1.717371
15	6	-1.744878	2.004370	-0.472829
16	6	-0.465798	1.713944	0.297286
17	6	1.638268	0.330621	0.942736
18	1	1.365785	-0.338163	1.772659
19	1	2.091436	1.219435	1.383846
20	6	-3.015788	-2.277826	1.030165
21	1	-4.069930	-2.024984	1.162728
22	1	-2.962603	-3.284727	0.597280
23	1	-2.549871	-2.337189	2.021973
24	1	1.871334	-1.467109	-1.861803
25	6	3.225479	0.608990	-1.011596
26	1	3.860290	1.315000	-0.465365
27	1	3.854907	0.092490	-1.742579
28	1	-4.015147	-0.119495	-0.048406
29	1	-0.434463	-2.010392	0.837957
30	6	0.363621	0.574051	0.159693
31	1	0.556819	1.621174	-0.575841
32	6	-0.144615	2.779628	1.321391
33	1	0.909009	2.844286	1.588149
34	1	-0.492766	3.758872	0.985838
35	1	-0.703402	2.524690	2.231054
36	1	-2.428513	2.404628	0.286939
37	1	-1.532799	2.861547	-1.128883
38	1	1.529817	-2.305496	-0.356404
39	1	-0.192407	-0.362876	-1.726684
40	1	2.493342	1.192132	-1.588161



Structure E’
B3LYP electronic energy: -586.4208055 H
B3LYP zero-point energy: 0.366256 H
mPW1PW91 electronic energy: -586.2929108 H

Coordinates (from last standard orientation):

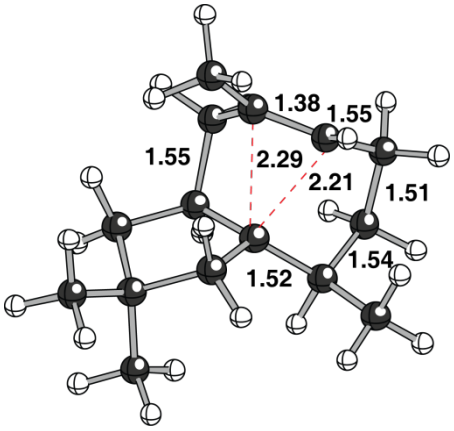
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	3.509953	0.490900	-0.931436
2	1	3.911585	-0.233373	-1.647804
3	1	3.134052	1.352916	-1.493264
4	1	4.342852	0.834358	-0.309520
5	6	2.415543	-0.136203	-0.058165
6	6	1.771211	0.880308	0.918075
7	6	0.333301	0.368235	1.192779
8	6	-0.764285	1.465413	1.246432
9	1	-1.547609	1.210190	1.964078
10	6	-1.352436	1.628028	-0.154251
11	6	-2.353078	0.830555	-0.606933
12	6	-3.113025	-0.224627	0.155645
13	1	-3.769942	0.252951	0.895886
14	1	-3.778740	-0.736728	-0.543902
15	6	-2.243399	-1.276287	0.897799
16	6	-0.985475	-1.642323	0.089580
17	6	1.173278	-0.564980	-0.910531
18	1	0.960057	0.237530	-1.638753
19	1	1.288742	-1.484378	-1.489609
20	6	-0.791378	2.729238	-1.012589
21	1	-1.255038	2.756711	-2.001390
22	1	-0.961709	3.699572	-0.528020
23	1	0.295183	2.643789	-1.141144
24	1	2.343800	0.988947	1.843339
25	6	2.978839	-1.357392	0.694690
26	1	3.331568	-2.122881	-0.004404
27	1	3.828633	-1.059023	1.316312
28	1	-2.683567	0.999984	-1.630671
29	1	-0.313985	2.400487	1.591900
30	6	0.040895	-0.582638	0.039367
31	1	-0.414904	-2.362285	0.726076
32	6	-1.295040	-2.353951	-1.238704
33	1	-0.409032	-2.804503	-1.691124
34	1	-2.014783	-3.156648	-1.060448
35	1	-1.731971	-1.661290	-1.963653
36	1	-2.833841	-2.182483	1.060775
37	1	-1.959183	-0.917428	1.889633
38	1	1.733715	1.869026	0.449073
39	1	0.314166	-0.225159	2.119093
40	1	2.240529	-1.825951	1.356996



Structure TSE'→F
B3LYP electronic energy: -586.4193086 H
B3LYP zero-point energy: 0.367606 H
mPW1PW91 electronic energy: -586.2951401 H
Imaginary frequency: -129.3186 cm^{-1}

Coordinates (from last standard orientation):

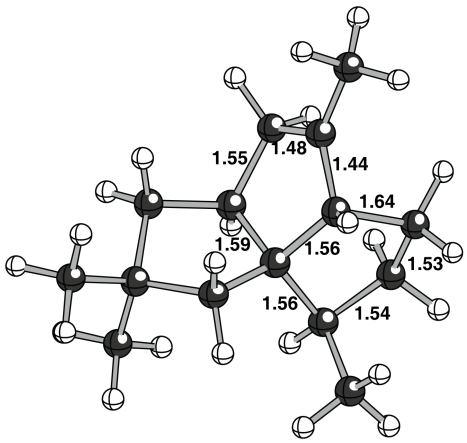
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	3.470197	0.271116	-0.791717
2	1	3.793404	-0.427778	-1.570352
3	1	3.249019	1.231789	-1.270213
4	1	4.316208	0.422049	-0.113235
5	6	2.256040	-0.273363	-0.024518
6	6	1.740735	0.726293	1.045359
7	6	0.233161	0.429517	1.240771
8	6	-0.698907	1.668765	1.259527
9	1	-1.573950	1.516038	1.898068
10	6	-1.079590	1.715712	-0.204277
11	6	-1.934589	0.761838	-0.720802
12	6	-2.987143	-0.017642	0.036766
13	1	-3.565285	0.654894	0.680417
14	1	-3.686679	-0.431932	-0.692667
15	6	-2.350739	-1.154065	0.877612
16	6	-1.066753	-1.589131	0.156482
17	6	1.012537	-0.416569	-0.967005
18	1	1.000874	0.440079	-1.650333
19	1	1.037486	-1.313595	-1.588453
20	6	-0.396020	2.701126	-1.090965
21	1	-0.579042	2.520602	-2.152185
22	1	-0.800788	3.693360	-0.840766
23	1	0.680251	2.764330	-0.900030
24	1	2.291729	0.641664	1.986403
25	6	2.619686	-1.628268	0.615113
26	1	2.880882	-2.366781	-0.150081
27	1	3.486118	-1.514335	1.274154
28	1	-1.998839	0.730408	-1.807020
29	1	-0.188880	2.574438	1.593729
30	6	-0.174219	-0.379539	-0.037184
31	1	-0.472811	-2.206206	0.855656
32	6	-1.345046	-2.429752	-1.099175
33	1	-0.431897	-2.845584	-1.531108
34	1	-1.990929	-3.272076	-0.836729
35	1	-1.851388	-1.852604	-1.879109
36	1	-3.040852	-1.993487	0.994522
37	1	-2.120112	-0.795328	1.884624
38	1	1.885753	1.752673	0.690078
39	1	0.076168	-0.191800	2.129028
40	1	1.807451	-2.047039	1.219682



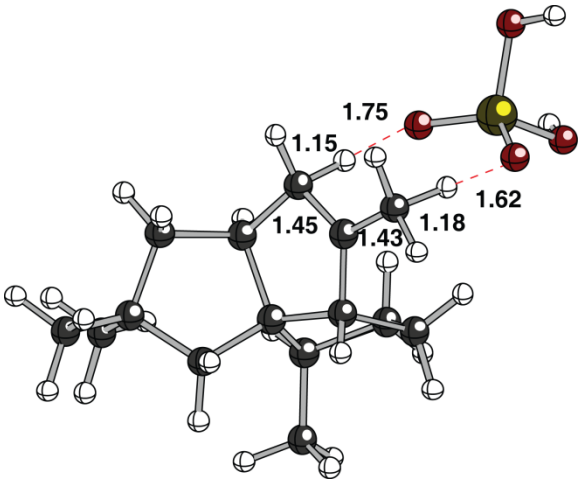
Structure F
B3LYP electronic energy: -586.4378871 H
B3LYP zero-point energy: 0.366841 H
mPW1PW91 electronic energy: -586.3173747 H

Coordinates (from last standard orientation):

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-3.388392	-1.042122	-0.955218
2	1	-3.897902	-0.339085	-1.623029
3	1	-2.949420	-1.834064	-1.573046
4	1	-4.150991	-1.498888	-0.315447
5	6	-2.320772	-0.325983	-0.114121
6	6	-1.521513	-1.310261	0.771756
7	6	-0.186764	-0.601072	1.094963
8	6	1.023946	-1.561842	1.134376
9	1	1.766477	-1.290814	1.908061
10	6	1.707477	-1.413573	-0.168441
11	6	1.373742	-0.150370	-0.775622
12	6	2.592985	0.774112	-0.183894
13	1	3.438592	0.209380	0.221077
14	1	2.966025	1.322775	-1.051407
15	6	1.905272	1.663146	0.858368
16	6	0.454725	1.838805	0.369735
17	6	-1.194294	0.265135	-1.001741
18	1	-0.994400	-0.438669	-1.820778
19	1	-1.487898	1.205877	-1.472521
20	6	2.594137	-2.429984	-0.752341
21	1	1.920500	-3.145778	-1.258539
22	1	3.267000	-2.031725	-1.514764
23	1	3.135746	-3.006488	0.003368
24	1	-2.062546	-1.610141	1.675245
25	6	-2.997433	0.763228	0.743032
26	1	-3.489187	1.506898	0.106971
27	1	-2.291310	1.294612	1.388755
28	1	-3.763487	0.321468	1.388780
29	1	1.434175	-0.134361	-1.865944
30	1	0.787790	-2.613060	1.345909
31	6	0.070424	0.401917	-0.111041
32	1	-0.187858	2.134436	1.205591
33	6	0.352894	2.925189	-0.713651
34	1	-0.683194	3.117561	-1.001573
35	1	0.761238	3.867574	-0.335784
36	1	0.905012	2.667174	-1.625246
37	1	2.441396	2.613243	0.955511
38	1	1.931828	1.188584	1.845740
39	1	-1.329703	-2.227877	0.196846
40	1	-0.266499	-0.056579	2.039257



Structure TSF→pentalenene (1) (transition state structure #1)
B3LYP electronic energy: -1230.2355925 H
B3LYP zero-point energy: 0.402952 H
mPW1PW91 electronic energy: -1230.0337765 H
Imaginary frequency: -194.0440 cm^{-1}



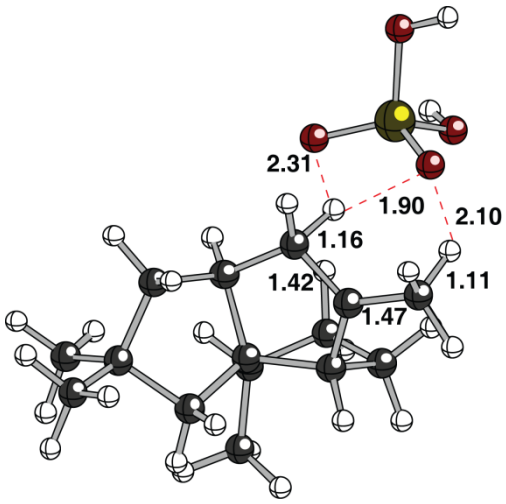
Coordinates (from last standard orientation):

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	4.849937	-1.663734	0.237254
2	1	5.681283	-1.025035	0.558792
3	1	4.512931	-2.238830	1.107648
4	1	5.244738	-2.372302	-0.500351
5	6	3.705597	-0.827099	-0.355756
6	6	2.484671	-1.695601	-0.738557
7	6	1.260618	-0.749206	-0.728810
8	6	-0.011747	-1.380444	-0.141718
9	1	-0.956620	-0.959906	-0.648872
10	6	-0.193718	-0.867673	1.199062
11	6	0.568785	0.397441	1.384878
12	6	-0.375494	1.650534	1.201897
13	1	-1.429192	1.422476	1.380050
14	1	-0.072026	2.409797	1.928777
15	6	-0.095928	2.106547	-0.237205
16	6	1.411765	1.852941	-0.445995
17	6	3.080445	0.119581	0.699169
18	1	3.030788	-0.423693	1.652704
19	1	3.696905	1.003801	0.883218
20	6	-1.146957	-1.373414	2.139751
21	1	-1.326424	-2.448706	2.075738
22	1	-1.027683	-1.015147	3.163406
23	1	-2.146430	-0.898368	1.736634
24	1	2.612119	-2.205060	-1.700657
25	6	4.234865	-0.048541	-1.577448
26	1	5.043743	0.631399	-1.285226
27	1	3.457449	0.549293	-2.062158
28	1	4.635477	-0.739032	-2.328643
29	1	1.000909	0.413948	2.392244
30	1	-0.131059	-2.463125	-0.252496
31	6	1.637273	0.456382	0.231630
32	1	1.634325	1.784167	-1.517080
33	6	2.260294	2.999281	0.129895
34	1	3.324398	2.876228	-0.092269
35	1	1.944745	3.950839	-0.311652
36	1	2.157865	3.090746	1.217616
37	1	-0.371442	3.155262	-0.392884
38	1	-0.693802	1.507238	-0.932167
39	1	2.353765	-2.479588	0.020610
40	1	1.061659	-0.382991	-1.740192
41	15	-3.650609	-0.202610	-0.404891
42	8	-3.485878	-0.239883	1.109137
43	8	-4.712819	-1.367369	-0.862862
44	8	-4.440753	1.198217	-0.731452
45	8	-2.442633	-0.328134	-1.311405
46	1	-4.419581	1.376953	-1.682402
47	1	-5.425478	-1.445834	-0.212649

Structure TSF→pentalenene (1) (transition state structure #1)
B3LYP electronic energy: -1230.2259929 H
B3LYP zero-point energy: 0.403172 H
mPW1PW91 electronic energy: -1230.0239357 H
Imaginary frequency: -178.2716 cm^{-1}

Coordinates (from last standard orientation):

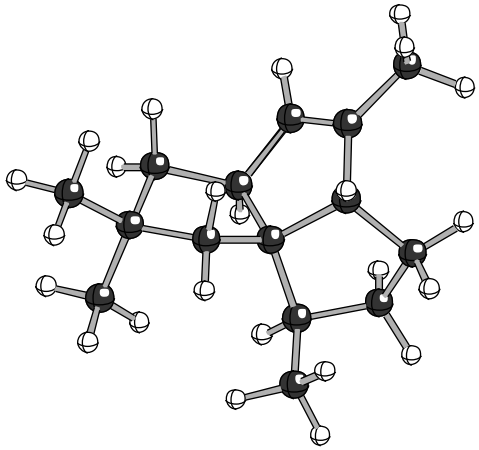
Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	4.599783	-1.794157	0.085897
2	1	5.503919	-1.173823	0.101078
3	1	4.420041	-2.150866	1.106971
4	1	4.810283	-2.667818	-0.541981
5	6	3.394938	-1.005789	-0.451790
6	6	2.093882	-1.844501	-0.430594
7	6	0.919724	-0.837242	-0.335523
8	6	-0.140739	-1.237836	0.692041
9	1	-1.205534	-0.864551	0.405454
10	6	-0.073742	-0.368938	1.817937
11	6	0.701365	0.850743	1.496966
12	6	-0.274220	2.047957	1.135549
13	1	-1.295783	1.884740	1.487766
14	1	0.106500	2.953388	1.616864
15	6	-0.192254	2.132708	-0.397875
16	6	1.255202	1.734660	-0.750220
17	6	3.015838	0.169066	0.484387
18	1	3.110025	-0.182676	1.521605
19	1	3.694914	1.020955	0.387028
20	6	-0.867403	-0.552555	3.038693
21	1	-0.791899	-1.573858	3.425191
22	1	-0.651915	0.181893	3.816008
23	1	-1.914439	-0.441232	2.685269
24	1	2.005067	-2.502551	-1.301814
25	6	3.713790	-0.521117	-1.880943
26	1	4.575299	0.156938	-1.880650
27	1	2.873507	0.005551	-2.341361
28	1	3.959684	-1.372510	-2.525792
29	1	1.307983	1.131543	2.367152
30	1	-0.347482	-2.297074	0.867848
31	6	1.532082	0.525572	0.203742
32	1	1.301093	1.404465	-1.793483
33	6	2.225118	2.917053	-0.586995
34	1	3.238317	2.670386	-0.917777
35	1	1.882903	3.762557	-1.193432
36	1	2.291064	3.262741	0.451921
37	1	-0.449670	3.135206	-0.756947
38	1	-0.898157	1.430894	-0.853575
39	1	2.104173	-2.493291	0.456202
40	1	0.439006	-0.698660	-1.306754
41	15	-3.362248	-0.379786	-0.586472
42	8	-2.250596	-0.405667	-1.602190
43	8	-4.263036	0.993674	-0.733496
44	8	-4.432662	-1.568800	-0.968734
45	8	-3.004136	-0.480574	0.892170
46	1	-5.063699	-1.693849	-0.245937
47	1	-4.388222	1.205483	-1.669407



Pentalenene (1)
B3LYP electronic energy: -586.0984116 H
B3LYP zero-point energy: 0.356593 H
mPW1PW91 electronic energy: -585.9433723 H

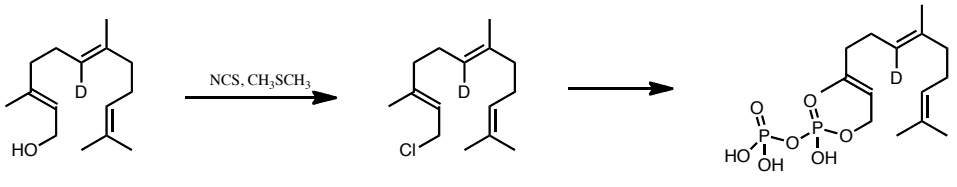
Coordinates (from last standard orientation):

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-0.132282	-0.745202	1.074140
2	1	-0.106694	-0.263852	2.062081
3	6	1.010875	-1.712484	0.921183
4	1	1.104245	-2.602569	1.540965
5	6	1.860308	-1.390887	-0.064669
6	6	3.058491	-2.180201	-0.507428
7	1	3.982528	-1.593107	-0.428766
8	1	3.184401	-3.087785	0.090703
9	1	2.965265	-2.476743	-1.560978
10	6	1.481702	-0.080775	-0.730934
11	1	1.396518	-0.218378	-1.818207
12	6	2.471099	1.075553	-0.416527
13	1	3.486875	0.720131	-0.214153
14	1	2.538577	1.760529	-1.269650
15	6	1.838669	1.786172	0.790239
16	1	2.044981	1.215022	1.704018
17	1	2.223638	2.800365	0.946584
18	6	0.324006	1.762308	0.486466
19	6	0.100039	0.315906	-0.076869
20	6	-0.070724	2.903573	-0.466220
21	1	-1.152301	2.948333	-0.624375
22	1	0.237766	3.866992	-0.044059
23	1	0.402545	2.806543	-1.450078
24	6	-1.119162	0.097956	-1.007426
25	1	-1.399057	0.984864	-1.584755
26	1	-0.859846	-0.679749	-1.737997
27	6	-2.285761	-0.439742	-0.141092
28	6	-3.004657	0.690074	0.624535
29	1	-3.812490	0.279568	1.242069
30	1	-2.329496	1.237093	1.288538
31	1	-3.451674	1.412580	-0.069024
32	6	-1.535875	-1.377829	0.838204
33	1	-2.091454	-1.530762	1.771127
34	6	-3.320081	-1.195542	-0.990705
35	1	-3.806649	-0.524859	-1.709600
36	1	-2.851204	-2.009747	-1.555389
37	1	-4.105798	-1.631815	-0.361995
38	1	-1.415811	-2.363586	0.371253
39	1	-0.244751	1.897656	1.414303



Synthesis of [6-^{2}H]-FPP

[6-^{2}H]-FPP is a known compound, as is the alcohol starting material shown below (see, Cane, D. E.; Tandon, M. *Tetrahedron Lett.* **1994**, *35*, 5355-5358.). Additional details are given here, however, for the conversion of the latter to the former.



References:

Organic Syntheses, *Coll. Vol*. *8*, 616, **1993**.

JACS, *132*, 4281–4289, **2010**.

To a stirred solution of *N*-chlorosuccinimide (80 mg, 0.6 mmol; recrystallized from benzene before use) in anhydrous dichloromethane (5 mL) in a 25 mL round bottom flask (flame dried before use) under N_2 at -40 ^oC (dry ice–acetonitrile bath) was added dimethyl sulfide (44 uL, 0.6 mmol; filtered through basic aluminum oxide before use). After being stirred at -40 ^oC for 15 min, the mixture was allowed to warm to 0 ^oC in an ice–water bath. The mixture was cooled to -40 ^oC again, and the deuterated alcohol (110 mg, 0.5 mmol) in 1 mL anhydrous dichloromethane was added. The resulting mixture was then stirred at 0 ^oC for 2 hours and then at room temperature overnight. The reaction was diluted with hexanes (50 mL), and washed with brine (50 mL). The organics were separated, and dried over sodium sulfate. Solvents were removed to afford the crude product, which was used for next step without further purification.

The crude chloride dissolved in anhydrous acetonitrile (2 mL) was added to a well–stirred solution of tris(tetrabutylammonium) hydrogen pyrophosphate (900 mg, 1.0 mmol) in anhydrous acetonitrile (3 mL) containing molecular sieves. The mixture was stirred at room temperature overnight. The mixture was filtered through a pad of celite, and concentrated to furnish the crude product. The crude product was dissolved in 2 mL ion exchange buffer (1g ammonium bicarbonate dissolved in 500 mL of 2% (v/v) isopropanol/water), and loaded onto a DOWEX-50W–X8 cation–exchange resin (NH_4^+ form, 100–200 mesh) column (1cm diameter, 10 cm high). The column was eluted with 2 column volumes of ion exchange buffer. The combined eluant was washed with ether, and then concentrated to furnish a crude white powder, which was extracted with methanol (5mL X 6). The methanol extracts were concentrated, and re-dissolved in 4 mL methanol. To the solution was added 12 mL ethyl ether to precipitate the product, which was collected after centrifugation (85 mg, 39%).

DOWEX-50W–X8 cation–exchange resin (NH_4^+ form) column preparation:

1. The column (1cm diameter) was packed with DOWEX-50W–X8 cation–exchange resin.

2. The column was washed with 2 column volumes of ammonium hydroxide.

3. The column was washed with distilled water to pH=7-8.

4. The column was washed with 2 column volumes of ion exchange buffer. At this point, the height of the column is about 10 cm.