Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Infromation

Catalytic Promiscuity of Non-native FPP Substrate in TEAS enzyme: Nonnegligible Flexibility of the Carbocation Intermediate

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Table S1; Figure S1-S7

Table S1. Summary of available crystal structures (with published literature) for TEAS.

PDB Entry	Ligand	Resolution(Å)	Mutant	Missing Residues
5EAS	/	2.25	/	1-23 522-532
5EAT	FHP	2.80	/	1-16
5EAU	Trifluoro-FPP	2.15	/	1-20 524-528
1HX9	FHP	3.50	W273S	1-20 98-102
1HXA	FHP	2.32	W273S	1-20
1HXC	FHP	2.25	C440W	1-20 521-532
1HXG	/	2.90	W273S/C440W	1-20 521-532
3LZ9	(2-trans,6-trans)-2-fluoro-FPP	2.28	A274T/V372I/Y406L/V516I	524-530
3M00	(2-cis,6-trans)-2-fluoro-FPP	2.10	A274T/V372I/Y406L/V516I	524-528
3M01	(2-trans,6-trans)-2-fluoro-FPP	2.60	/	523-527
3M02	(2-cis,6-trans)-2-fluoro-FPP	2.50	/	1-13
4DI5	1GA	2.30	/	1-13
4RNQ	1GA	2.47	/	1-20
5DHI	/	2.25	W273E	1-12
5DHK	FAR	2.43	W273E	1-12
5IK0	FPP	2.20	/	1-12
5IK6	PPI&GA	2.30	/	1-12
5IK9	FMP	2.23	/	1-12
5IKA	PPI	2.45	/	1-12
5IKH	(-)-premnaspirodiene	2.10	A274T/V372I/Y406L/V516I	1-12

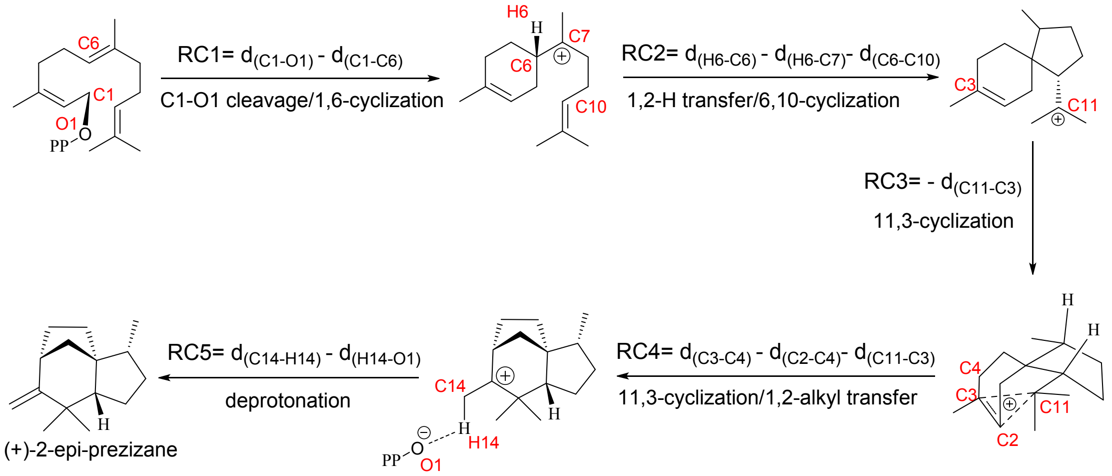

Figure S1. The defined reaction coordinates (RC) for biosynthesis of (+)-2-epi-prezizaene by TEAS-catalyzed (*cis,trans*)-FPP cyclizations.

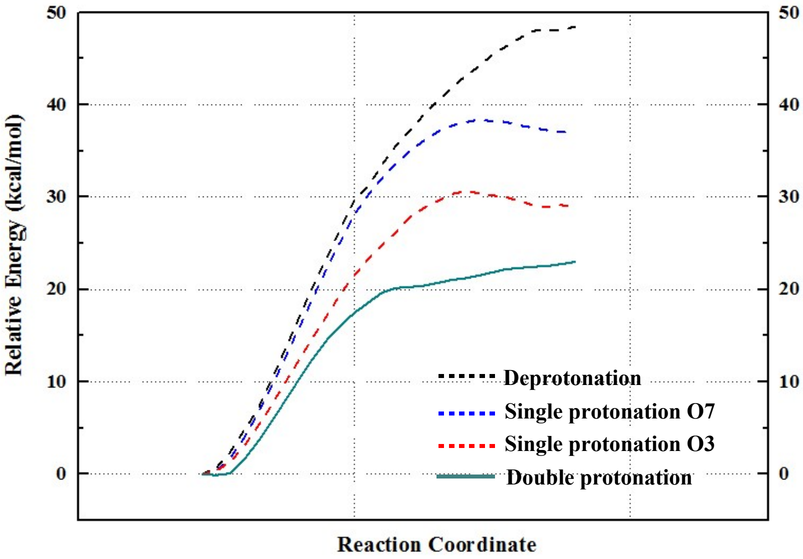

Figure S2. The relative energy profiles of (cis,trans)-FPP PPi cleavage for different protonation states in TEAS. All these profiles were obtained after minimizing the energy pathway forward and backward several times.

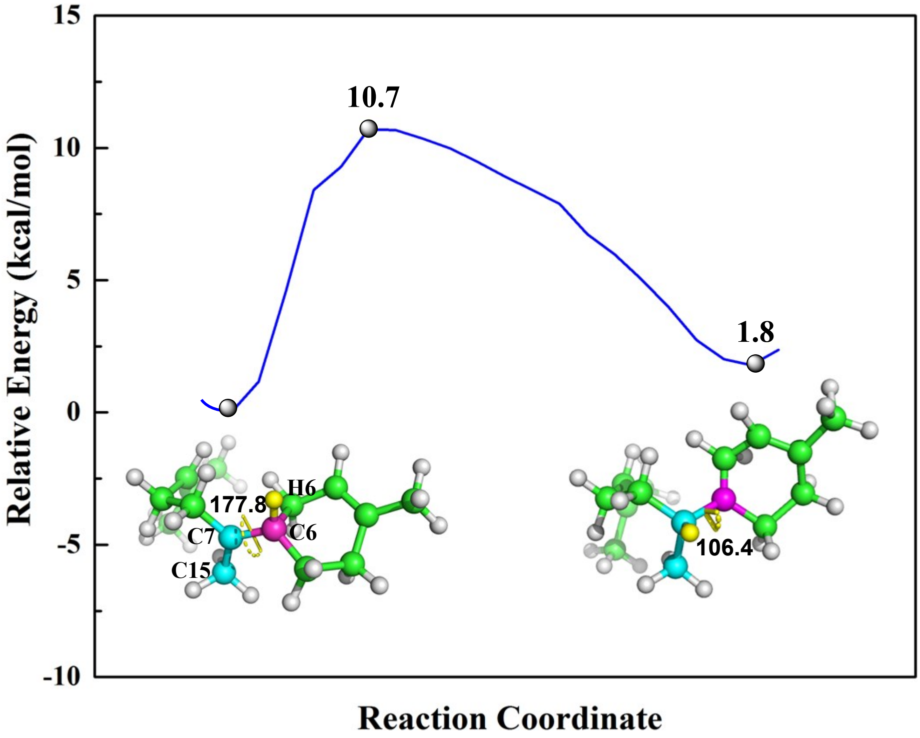

Figure S3. The relative energy profiles of direct 1,2-hydride transfer from bisabolyl cation A1 conformation, as well as the predicted structures of the reactant and product state. One hand, the energy barrier is ~11 kcal/mol, disagreed with the observations of previous sesquiterpene synthases computational studies in which an appropriate hydride transfer often appear as low barrier and exothermic steps.[23, 26] The other hand, the dihedral of labeled atoms (H6-C6-C7-C15) is measured, indicating the rotation of C-6-C7 is necessary to promote the 1,2-hydride transfer. Therefore, direct 1,2-hydride transfer is excluded and the concerted mechanism is considered as discussed in main text.

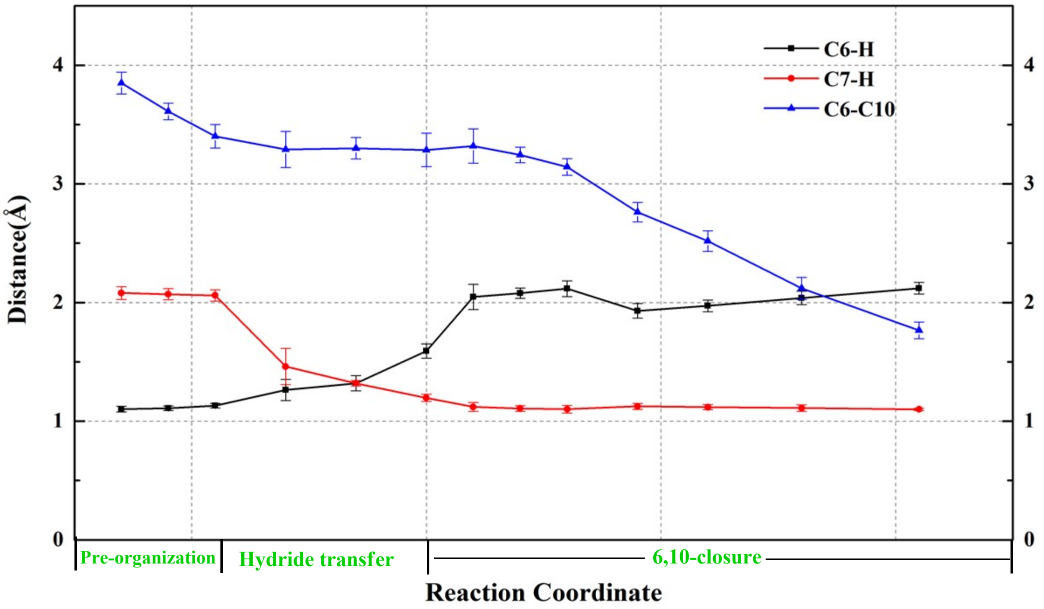

Figure S4. The C6-H/C7-H/C6-C10 distance evolutions during concerted hydride transfer and 6,10-closure.

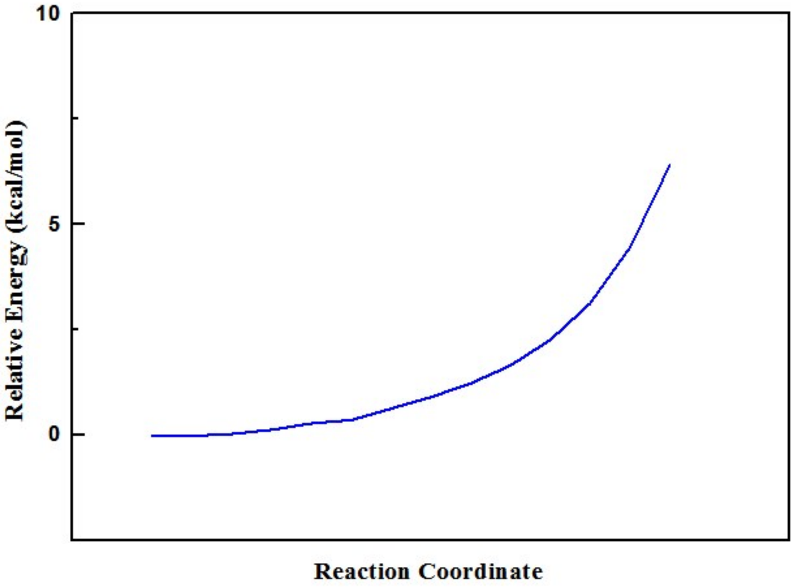

Figure S5. The relative energy profile of 11,2-closure from C1’.

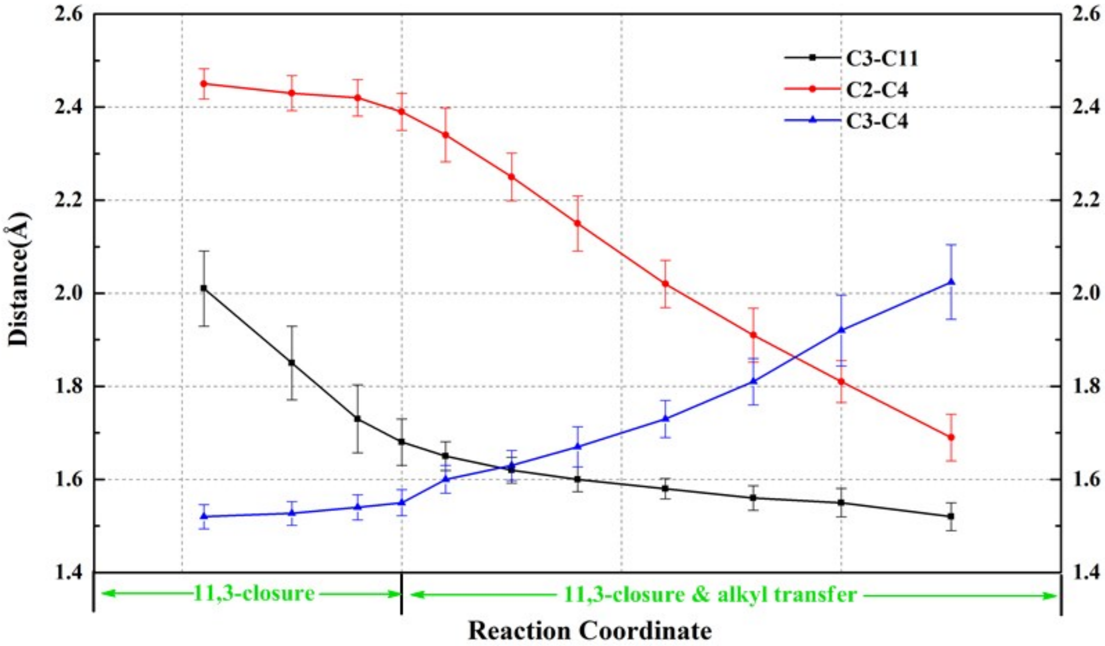

Figure S6. The C3-C11/C2-4/C3-C4 distance evolutions during concerted 11,3-closure and 1.2-alkyl transfer.

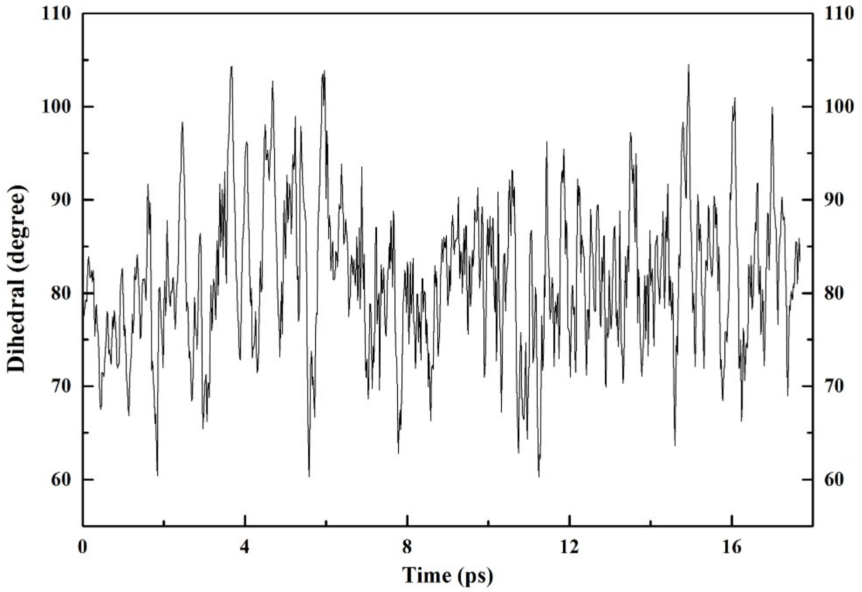

Figure S7. The C1-C6-C7-C15 dihedral distribution alone the simulation timescale of bisabolyl cation in TEAS. The lower dihedral indicate the H5 approaching the PPi group, as shown in Figure 8.