Supplementary Information

QM/MM calculations suggest a novel intermediate following the proton abstraction catalyzed by thymidylate synthase

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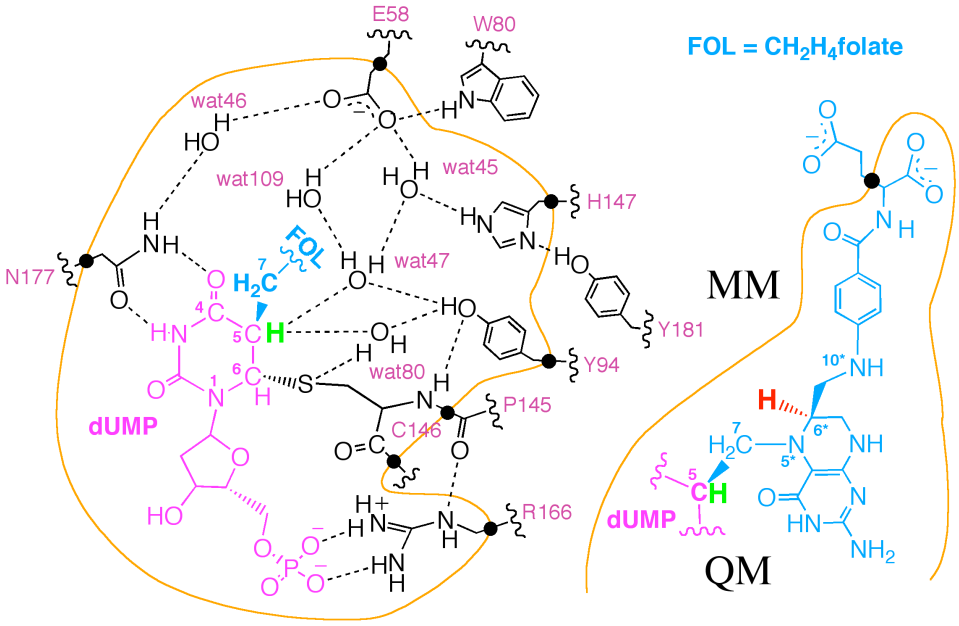

Figure S1. The initial AM1/MM PES calculations in this work used a large QM region containing all the residues involved in the network of H-bonds at the active site. This QM region included 157 atoms (within the yellow curve) and 8 hydrogen link atoms (black dots) at the boundary between the QM and MM regions. Further studies examined the effects of various QM regions on the reaction energy profiles, and the results suggested that the smaller QM region (57 atoms) in Figure 1 in the main text was sufficient for calculations on the proposed mechanisms (Scheme 3).

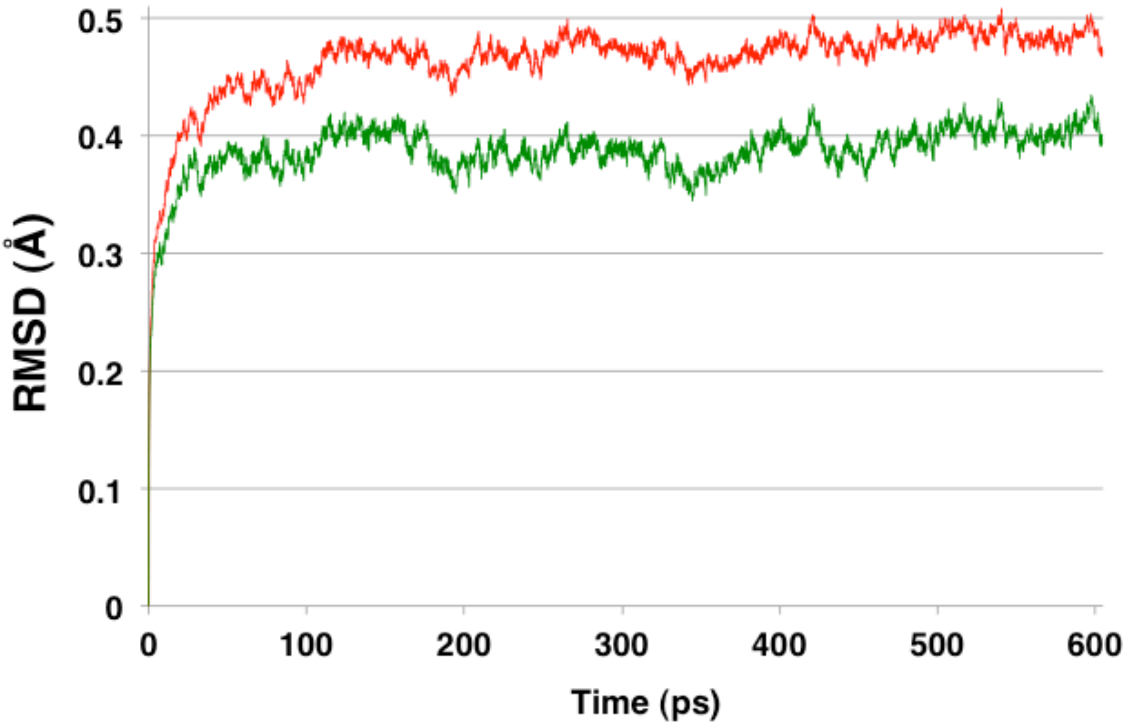

Figure S2. Time evolution of the root mean square deviation (RMSD) of the atomic positions during the 600 ps of AM1/MM MD simulation of the WT ecTSase reaction intermediate (Intermediate C in Scheme 2 in the main text). The green curve corresponds to the RMSD measured only on the backbone of the protein while the red curve corresponds to the RMSD measured on all the non-hydrogen atoms of the protein. The time evolutions of the RMSD for the examined mutants are similar to this plot.

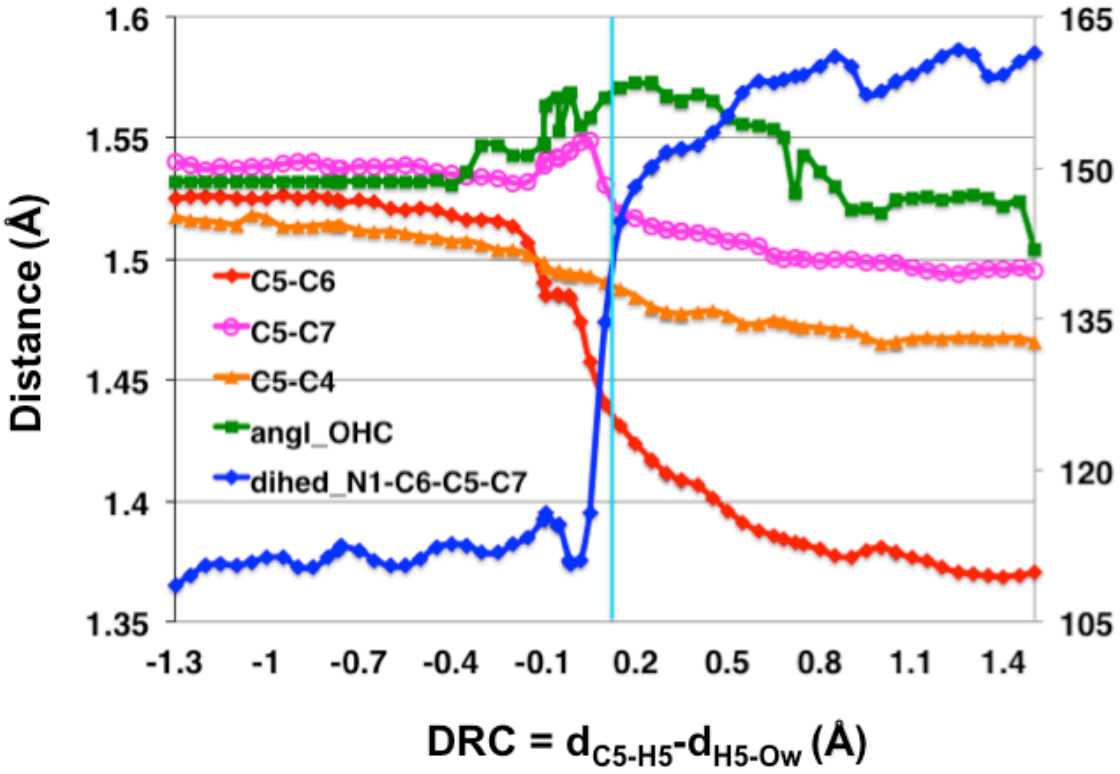

Figure S3. Changes in the geometric parameters near C5 of dUMP during the deprotonation of C5, analyzed from the 1D AM1/MM PMF calculation results (Figure 3 in the main text). The C5-C6 bond length decreases from 1.53 Å to 1.37 Å, and the dihedral of N1-C6-C5-C7 increases from 106˚ to 164˚, both suggesting that the deprotonation leads to a double bond formation on C5-C6 that is concomitant with cleavage of the C6-S bond (see the main text). Subtle decreases are also observed for the C5-C7 and C5-C4 bond lengths. The angle of C5-H5-O_{wat47} (agl_OHC, green) reaches the maximum near the TS (vertical cyan line).

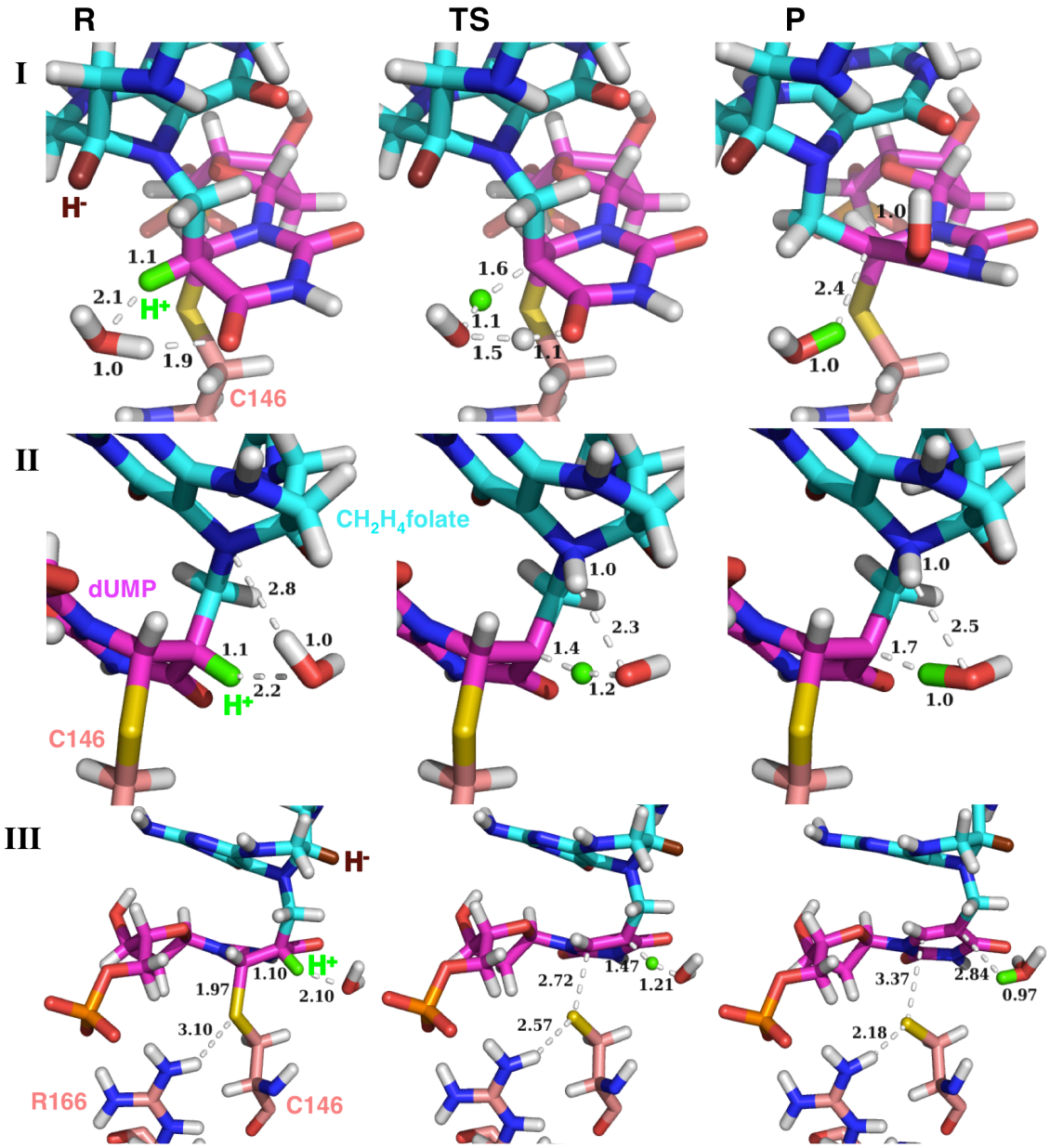

Figure S4. Optimized structures with the B3LYP/MM method for the reactant (R), transition state (TS), and product (P) of the deprotonation of C5 in Mechanisms I, II, and III (Scheme 3). Although the specific geometric parameters are different from the corresponding structures calculated with AM1/MM (e.g. compare Table S1 and Table S2), the energy barriers calculated with the B3LYP/MM method (Figure 2) substantiate the conclusion that the deprotonation of C5 is concerted with the cleavage of C6-S bond (Mechanism III), which is assisted by the strong H-bond formed between R166 and C146.

Table S1. Comparison of key distances (Å), angle (°) and dihedral (°) in the reactant state (R), several different TSs (TSA, TSB, and TSC), a pseudo-product state along the reaction path (D), and product state (P) of the proton transfer step calculated with the 2D AM1/MM PMF at 298 K (Figure 4 in the main text).

Geometric Variable	R	TS			D	P
		TSA	TSB	TSC		
C5(dUMP)-H5(dUMP)	1.14 ± 0.03	1.40 ± 0.03	1.40 ± 0.03	1.78 ± 0.04	2.61 ± 0.04	2.78 ± 0.04
H5(dUMP)-Ow(wat47)	2.49 ± 0.04	1.27 ± 0.03	1.31 ± 0.03	1.07 ± 0.03	1.04 ± 0.03	1.05 ± 0.03
C5(dUMP)-Ow(wat47)	3.30 ± 0.09	2.62 ± 0.06	2.60 ± 0.06	2.78 ± 0.07	3.59 ± 0.07	3.73 ± 0.09
C6(dUMP)-S(C146)	2.09 ± 0.03	4.50 ± 0.03	3.40 ± 0.03	2.19 ± 0.04	4.51 ± 0.03	4.41 ± 0.03
S(C146)-H(R166)	4.6 ± 0.3	2.1 ± 0.1	2.1 ± 0.2	5.0 ± 0.3	2.1 ± 0.1	5.2 ± 0.3
S(C146)-H(Y94)	3.4 ± 0.2	3.8 ± 0.2	4.3 ± 0.1	2.5 ± 0.2	3.9 ± 0.2	1.9 ± 0.1
C5(dUMP)-C6(dUMP)	1.52 ± 0.02	1.44 ± 0.02	1.45 ± 0.02	1.43 ± 0.03	1.38 ± 0.02	1.38 ± 0.02
C4(dUMP)-O4(dUMP)	1.24 ± 0.02	1.24 ± 0.01	1.25 ± 0.02	1.26 ± 0.02	1.26 ± 0.01	1.25 ± 0.01
Angle C5-H5-Ow	127 ± 6	157 ± 10	149 ± 8	154 ± 9	158 ± 9	152 ± 12
Dihedral N1-C6-C5-C7	115 ± 5	150 ± 6	136 ± 6	130 ± 5	161 ± 5	171 ± 5

Table S2. Comparison of key distances (Å), angle (˚) and dihedral (˚) in the structures of the reactant, TS, and product of the proton transfer step calculated with B3LYP/MM (Scheme 3, step III.1 in main text). The structures are presented in Figure S4, III.

Geometric Variable	R	TS	P
C5(dUMP)-H5(dUMP)	1.10	1.47	2.84
H5(dUMP)-Ow(wat47)	2.10	1.21	0.97
C5(dUMP)-Ow(wat47)	3.09	2.64	3.20
C6(dUMP)-S(C146)	1.97	2.72	3.37
S(C146)-H(R166)	3.10	2.57	2.18
S(C146)-H(Y94)	4.57	4.56	4.75
C5(dUMP)-C6(dUMP)	1.54	1.44	1.37
C4(dUMP)-O4(dUMP)	1.22	1.23	1.27
Angle C5-H5-Ow	148.2	159.4	103.1
Dihedral N1-C6-C5-C7	95.3	123.4	155.9