Electronic Supplementary Information

Theoretical Study on Substrate Recognition and Catalytic Mechanism of Gephyronic Acid Dehydratase DH1

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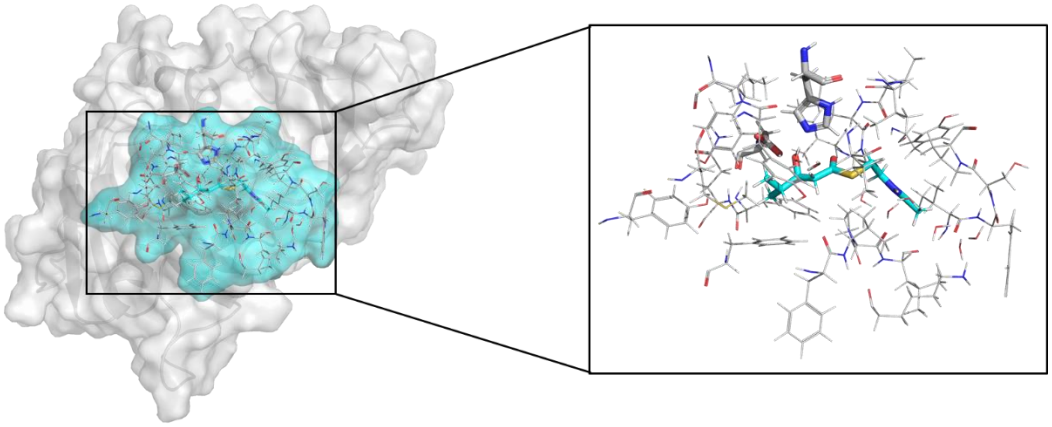

Fig. S1 The whole system (large model, gray) and the truncated system (small model, blue) for QM/MM calculations.

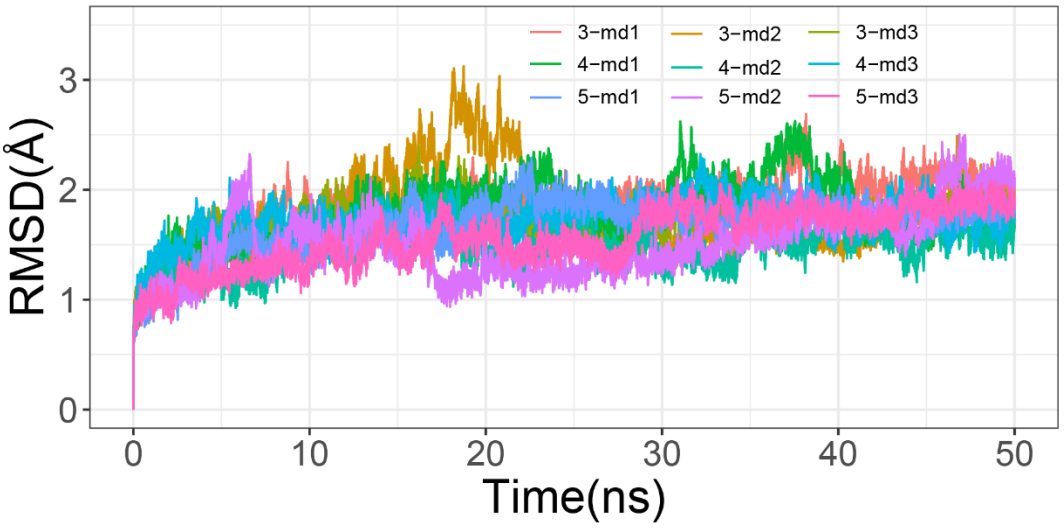

Fig.S2 RMSD comparisons between sub3, sub4, sub5 systems.

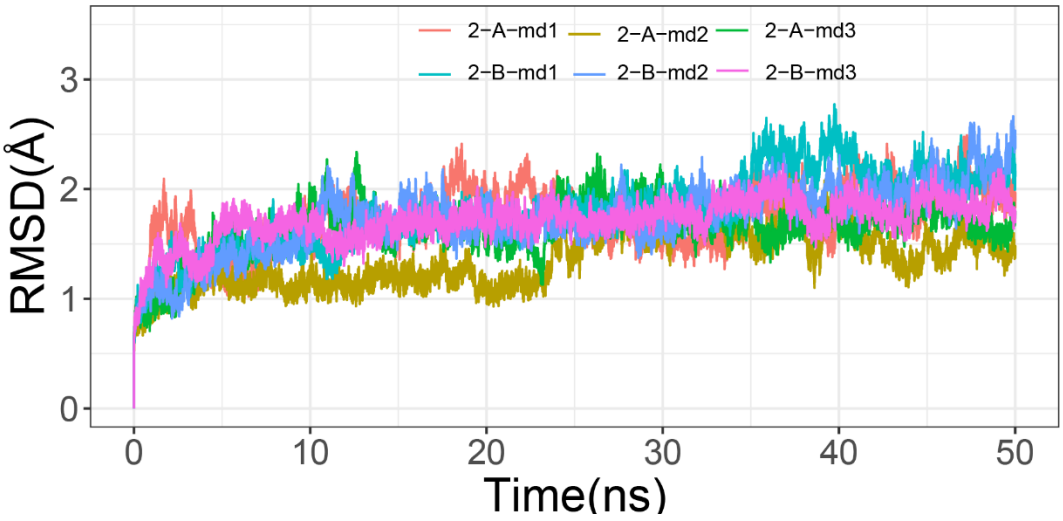

Fig. S3 RMSD comparisons between 2-A and 2-B systems.

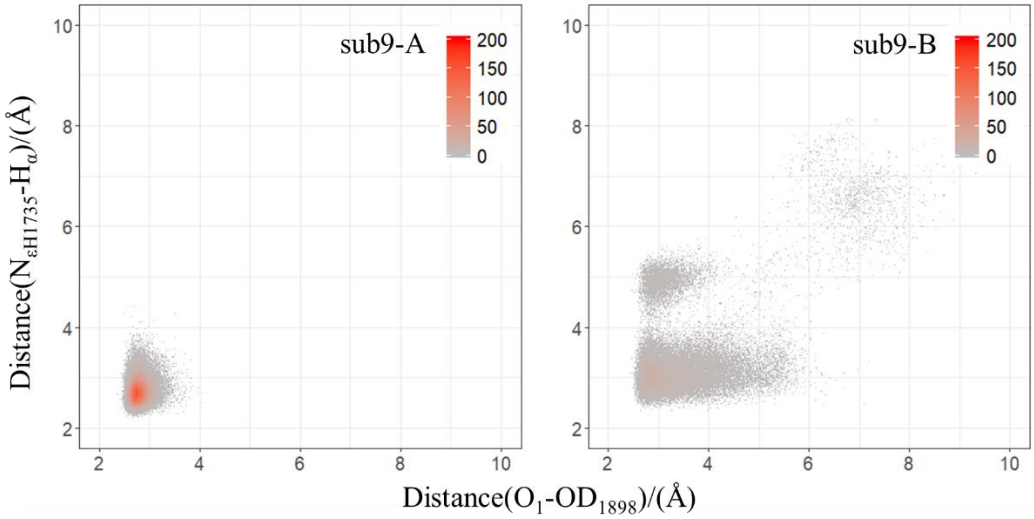

Fig. S4 The distributions of d(O_1-O_{D1898}) and d($N_{\varepsilon H1735}$-H_{α}/H_{γ}) in two systems.

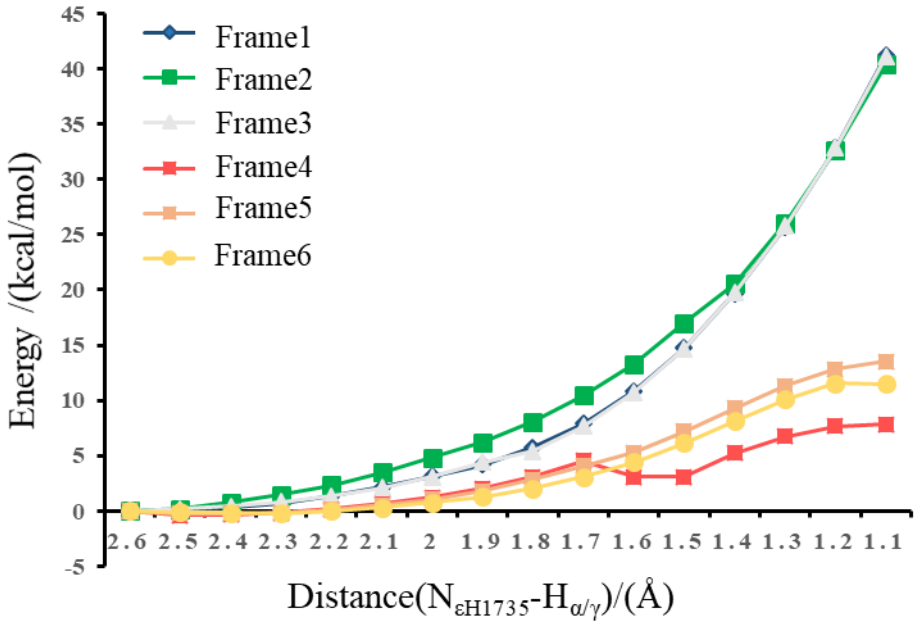

Fig. S5 The enengy profiles for the transfer of H_α/ H_γ to $N_{\varepsilon H1735}$ in sub9-A and sub9-B. Frames (1-3) were obtained form sub9-B system while Frames (4-6) were from sub9-A system.

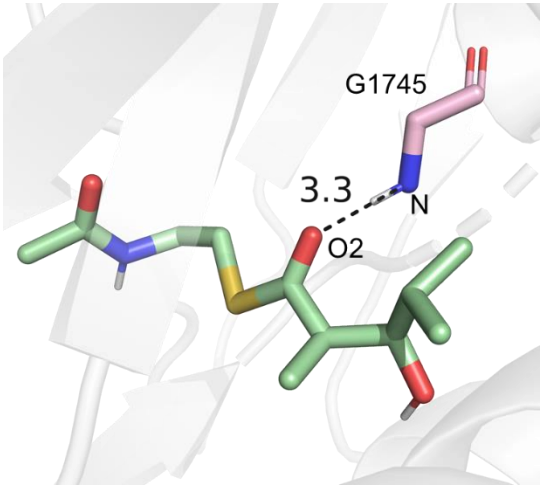

Fig. S6 The hydrogen bond formed by residue Gly1745.

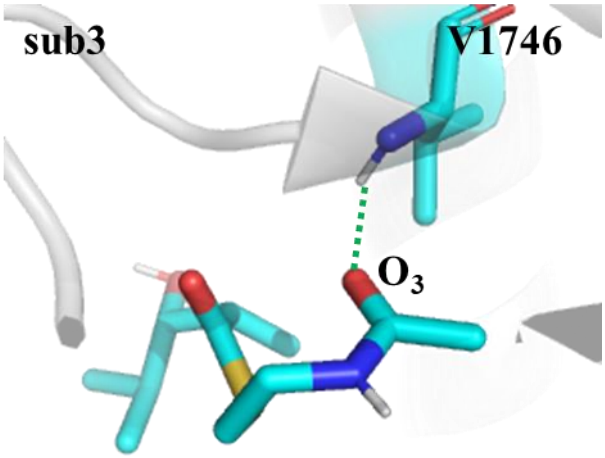

Fig. S7 A hydrogen bond between O_3 atom and NH group of V1746 in sub3 system.

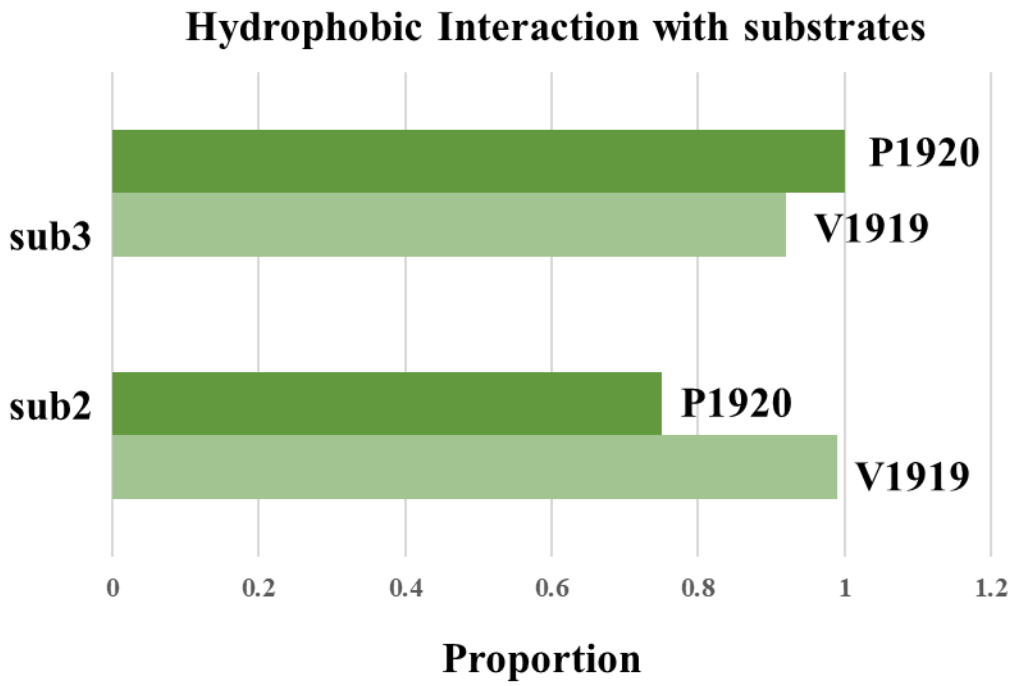

Fig. S8 The proportion of hydrophobic interaction between substrate and residues (V1919, P1920) of GphF DH1 in sub2 and sub3 systems.

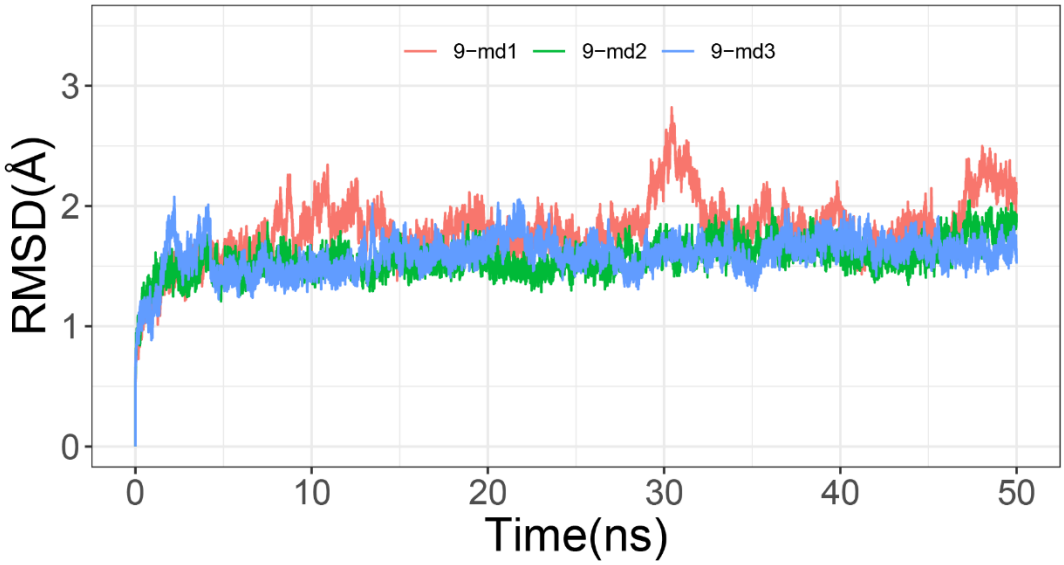

Fig. S9 RMSD values in sub9 system.

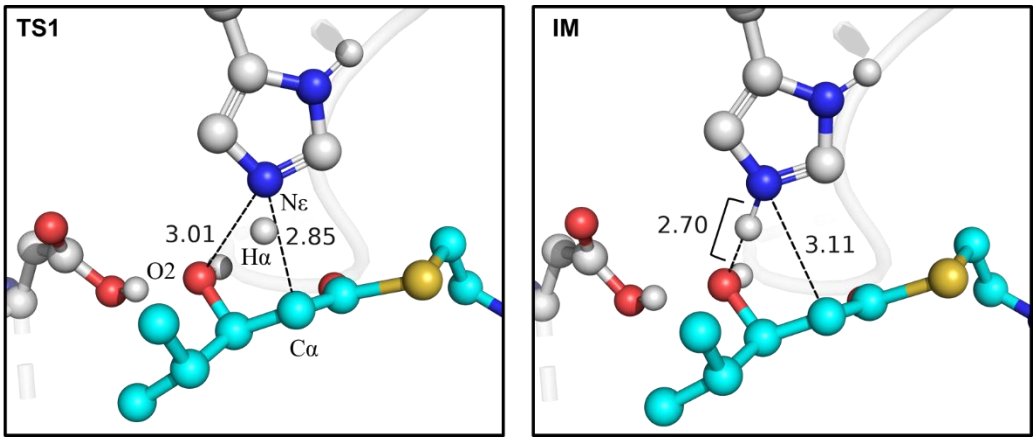

Fig. S10 The structures of TS1 and IM in Path1 calculated by small model QM/MM calculations.

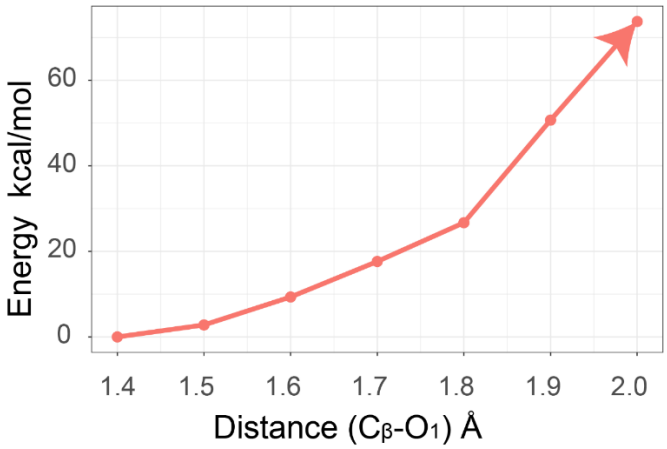

Fig. S11 The energy profile of step1 for Path2 calculated by large model QM/MM calculations.

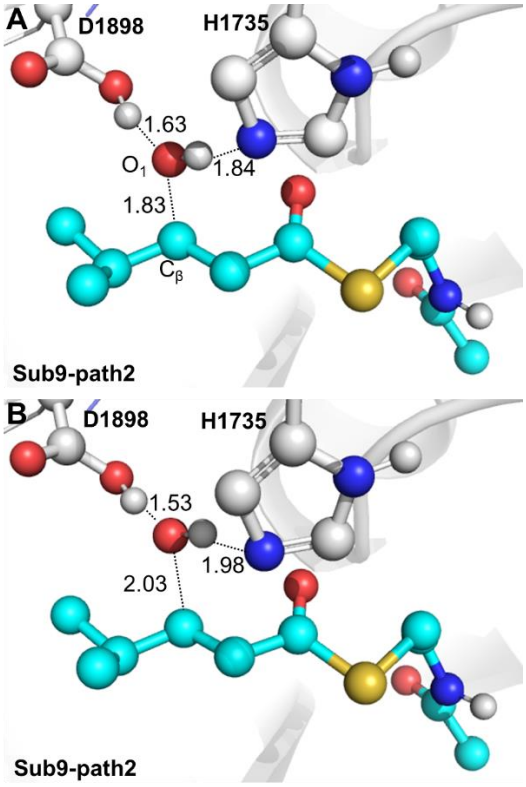

Fig. S12 Structures along the reaction for Path2.

Table S1. Key distances and angles in reactant, transition, intermediate, and product in dehydration reaction

Replica	Key distances and angles	R	TS1	IM	IM'	TS2	P
Replica1	$d(N_\varepsilon\text{-}H_\alpha)$	2.29 Å	1.08 Å	1.06 Å	1.07 Å	1.63 Å	1.84 Å
	$d(C_\alpha\text{-}H_\alpha)$	1.09 Å	1.82 Å	2.49 Å	-	-	-
	$d(O2\text{-}H_\alpha)$	-	-	1.65 Å	1.54 Å	1.02 Å	0.99 Å
	$d(C_\beta\text{-}O2)$	-	-	1.48 Å	1.45 Å	1.74 Å	3.11 Å
	$a(N_\varepsilon\text{-}H_\alpha\text{-}C_\alpha)$	148.9°	156.8°	-	-	-	-
	$a(N_\varepsilon\text{-}H_\alpha\text{-}O2)$	-	-	168.6°	162.6°	171.2°	-
Replica2	$d(N_\varepsilon\text{-}H_\alpha)$	2.25 Å	1.07 Å	1.06 Å	1.07 Å	1.65 Å	1.83 Å
	$d(C_\alpha\text{-}H_\alpha)$	1.10 Å	1.82 Å	2.62 Å	-	-	-
	$d(O2\text{-}H_\alpha)$	-	-	1.71 Å	1.56 Å	1.02 Å	0.98 Å
	$d(C_\beta\text{-}O2)$	-	-	1.47 Å	1.43 Å	1.74 Å	2.82 Å
	$a(N_\varepsilon\text{-}H_\alpha\text{-}C_\alpha)$	163.1°	152.7°	-	-	-	-
	$a(N_\varepsilon\text{-}H_\alpha\text{-}O2)$	-	-	162.2°	171.3°	173.0°	-
Replica3	$d(N_\varepsilon\text{-}H_\alpha)$	2.23 Å	1.07 Å	1.05 Å	1.07 Å	1.58 Å	1.88 Å
	$d(C_\alpha\text{-}H_\alpha)$	1.10 Å	1.87 Å	2.76 Å	-	-	-
	$d(O2\text{-}H_\alpha)$	-	-	1.68 Å	1.53 Å	1.03 Å	0.99 Å
	$d(C_\beta\text{-}O2)$	-	-	1.45 Å	1.43 Å	1.76 Å	3.26 Å
	$a(N_\varepsilon\text{-}H_\alpha\text{-}C_\alpha)$	151.6°	160.4°	-	-	-	-
	$a(N_\varepsilon\text{-}H_\alpha\text{-}O2)$	-	-	157.0°	167.5°	173.0°	-

Table S2. The energies in ONIOM calculations (Default units are Hartree).

Replica		R	TS1	IM	IM'	TS2	P
Replica1	MM_{model}	0.011694	0.120685	0.024064	0.038753	1.529047	-0.000218
	MM_{real}	-0.344988	-0.233319	-0.328874	-0.307664	1.180232	-0.353744
	$MM_{environment}$	-0.356682	-0.354004	-0.352938	-0.346417	-0.348815	-0.353526
	$\Delta MM_{environment}$ (kcal/mol)	0	1.7	2.3	6.4	4.9	2.0
	QM	-1642.119631	-1642.091329	-1642.099264	-1642.100689	-1642.084264	-1642.113623
	ΔQM (kcal/mol)	0	17.8	12.8	11.9	22.2	3.8
Replica2	MM_{model}	0.013446	0.067314	0.016418	0.032563	1.507303	0.000916
	MM_{real}	-0.377271	-0.321603	-0.372251	-0.350717	1.123474	-0.383723
	$MM_{environment}$	-0.390717	-0.388917	-0.388669	-0.383280	-0.383829	-0.384638
	$\Delta MM_{environment}$ (kcal/mol)	0.0	1.1	1.3	4.7	4.3	3.8
	QM	-1642.120503	-1642.093159	-1642.09893	-1642.10018	-1642.08669	-1642.11357
	ΔQM (kcal/mol)	0	17.2	13.5	12.8	21.2	4.3
Replica3	MM_{model}	0.034219	0.092564	0.225716	0.037539	1.494310	0.021319
	MM_{real}	-0.371426	-0.313281	-0.181108	-0.360256	1.096301	-0.379182
	$MM_{environment}$	-0.405645	-0.405844	-0.406824	-0.397795	-0.398009	-0.400500
	$\Delta MM_{environment}$ (kcal/mol)	0.0	-0.1	-0.7	4.9	4.8	3.2
	QM	-1642.120827	-1642.095914	-1642.10073	-1642.0982	-1642.08336	-1642.11752
	ΔQM (kcal/mol)	0	15.6	12.6	14.2	23.5	2.1

Table S3. Energetic corrections (kcal/mol) at different levels with M062X method sub9 system

Replica	Different levels	R	TS1	IM	IM’	TS2	P
Replica 1	M062X/6-31G*	0	26.2	23.6	23.1	28.4	9.4
	M062X/6-31G*(SMD)	0	21.8	18.0	22.1	31.7	12.2
	M062X/6-311+G**	0	24.1	21.0	20.1	25.2	2.8
	M062X/6-311+G**(SMD)	0	19.4	15.1	18.3	27.1	5.8
Replica 2	M062X/6-31G*	0	29.0	26.9	23.7	29.5	9.5
	M062X/6-31G*(SMD)	0	21.1	17.7	19.6	29.0	13.1
	M062X/6-311+G**	0	25.6	23.6	20.8	25.7	3.9
	M062X/6-311+G**(SMD)	0	18.3	14.8	17.4	25.5	8.2

Replica 3	M062X/6-31G*	0	25.2	23.2	25.4	31.4	10.1
	M062X/6-31G*(SMD)	0	18.1	14.6	21.6	31.9	10.4
	M062X/6-311+G**	0	22.4	22.4	22.7	27.7	4.7
	M062X/6-311+G**(SMD)	0	15.5	11.9	19.1	28.3	5.3

Table S4. Key distances in reactant, transition, intermediate, and product in sub10 system

	R	TS1	IM	TS2	P
d(N_ε-H_γ)	2.1 Å	1.2 Å	1.1 Å	1.1 Å	2.1 Å
d(C_γ-H_γ)	1.1 Å	1.5 Å	3.2 Å	-	-
d(C_α-H_γ)	-	-	1.9 Å	1.6 Å	1.1 Å
a(N_ε-H_γ-C_γ)	133.5°	167.2°	-	-	-
a(N_ε-H_γ-C_α)	-	-	154.4°	162.3°	-

Table S5. Energetic corrections (kcal/mol) at different levels with M062X method sub10 system

	R	TS1	IM	TS2	P
M062X/6-31G*(SMD)	0	19.4	11	13.4	-5.4
M062X/6-311+G**	0	20.6	17.1	17.8	-1.1
M062X/6-311+G**(SMD)	0	17.2	8.4	11.2	-6.1