

Supporting Information

Benchmarking QM/MM Methods on the Thymidylate Synthase Catalyzed Hydride Transfer

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Figure S1. Evolution of key distances along the RM1/MM 1D-PMF for the hydride transfer step of the TSase catalyzed reaction computed with the QM-MM partitioning used in the manuscript (red dashed line) and with an enlarged QM region where full H4folate was treated quantum mechanically. Calculations were carried out with the OPLS and TIP3P force fields, at 293 K.

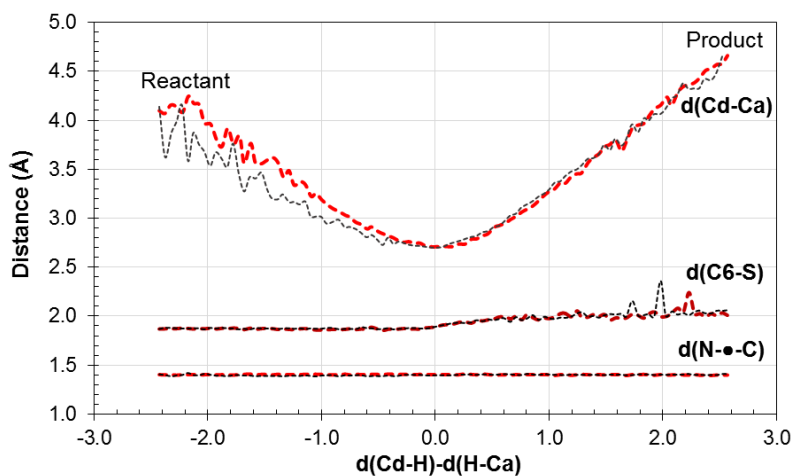


Figure S2. Time-dependent evolution of the RMSD of those atoms belonging to the protein backbone.

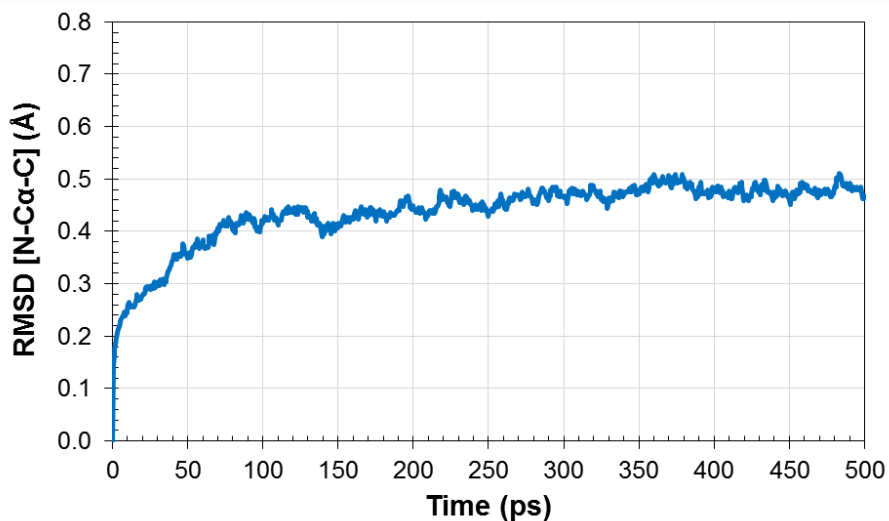


Figure S3. Comparison of the QM/MM classical mechanical PMF, W^{CM} , for the hydride transfer step of the TSase catalyzed reaction computed with the SRP/MM methods with those obtained with the standard AM1/MM, PM3/MM, PDDG/MM and RM1/MM methods. Results obtained at 293 K.

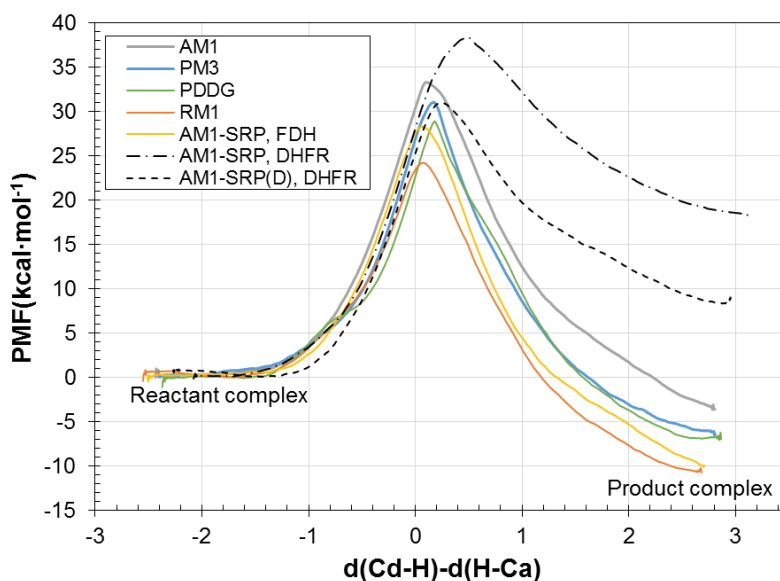


Figure S4. Evolution of the hybridization states of the carbon atom involved in the C6(dUMP)–S(Cys146) breaking bond along the reaction coordinate (in Å). Results from the geometries selected from the reaction free energy path obtained at AM1/MM (black dashed lines) and RM1/MM (orange dashed lines) from the corresponding 2D PMFs performed at 303 K. The hybridization value of 2 on the carbon atom corresponds to a sp^2 molecule, while a value of 3 represents a sp^3 hybridization. Positions of the TSs are indicated as crosses.

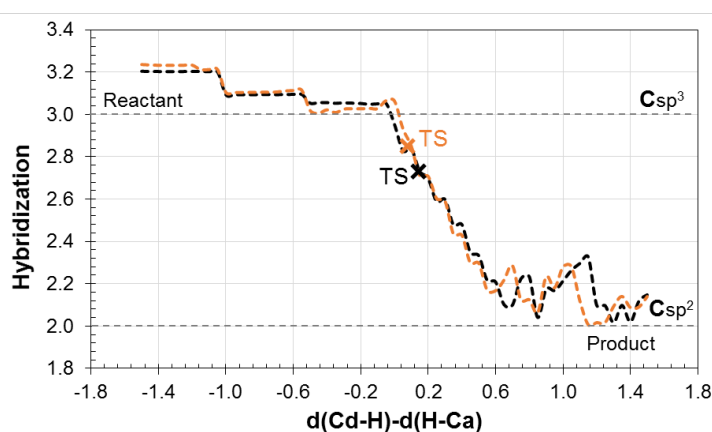
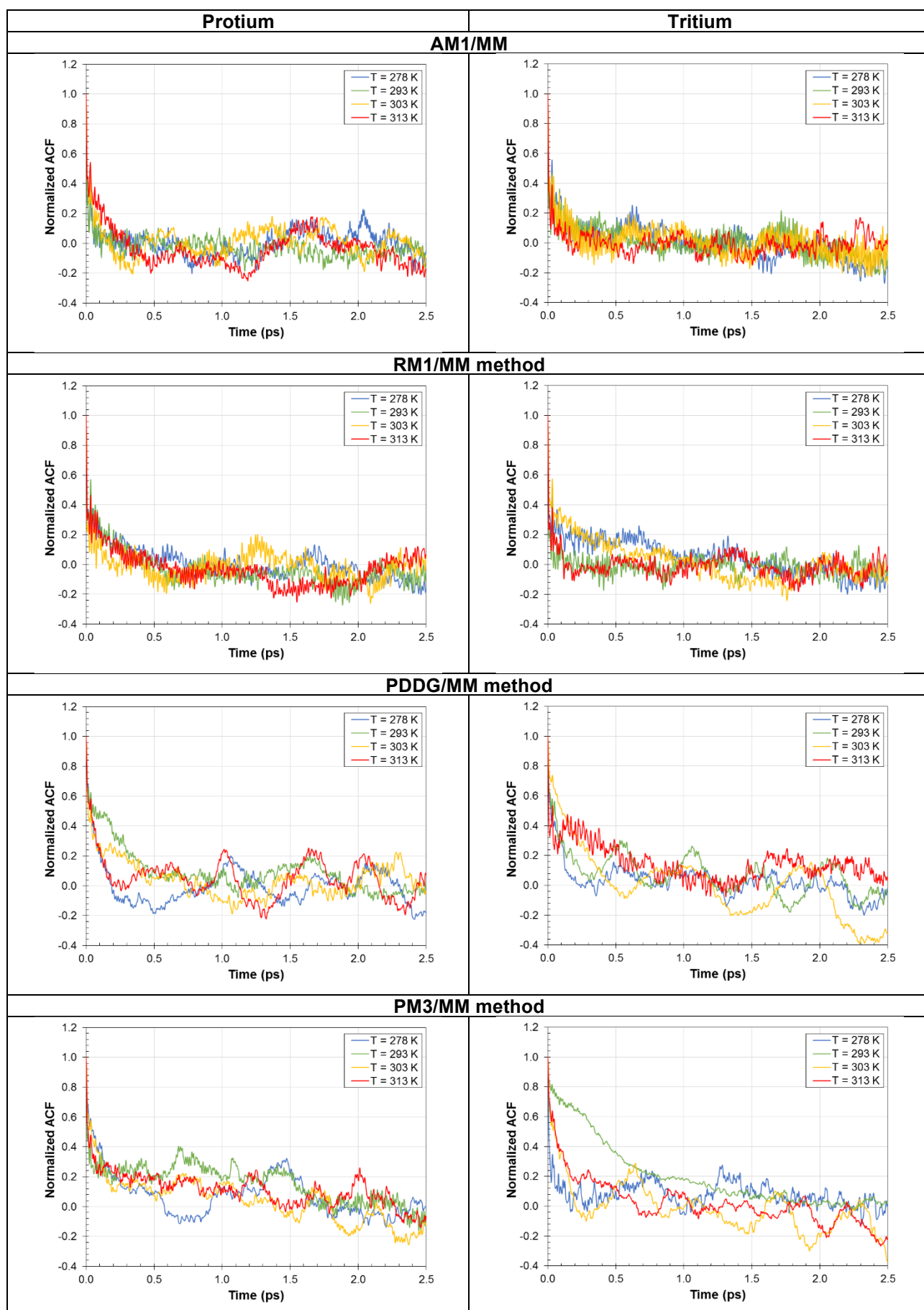


Figure S5. Normalized autocorrelation functions of the forces acting on the reaction coordinate at the transition states for protium and tritium.



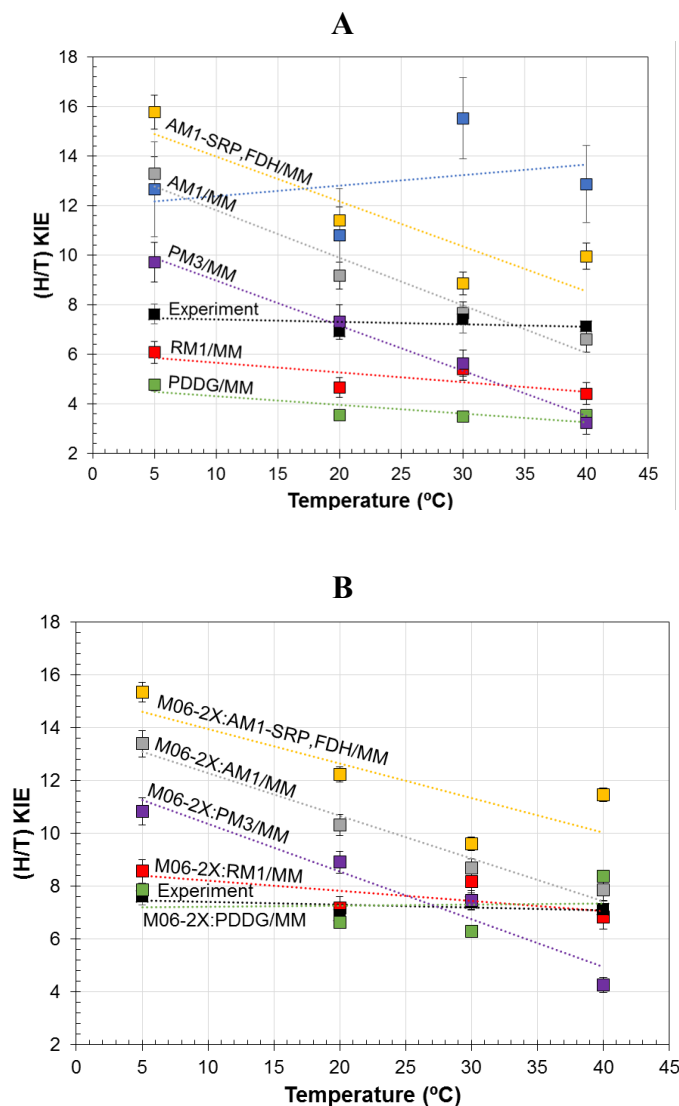


Figure S6. A) Temperature dependence of QM/MM KIEs obtained with different semiempirical methods: RM1 (red squares), AM1 (grey squares), PM3 (purple squares), PDDG (green squares) and AM1-SRP,FDH (yellow squares); and **B)** the corresponding values after correction at M06-2X DFT level. Experimental KIEs (from reference 25 of the text) are reported as black squares and previous theoretical results (from reference 77 of the text) as blue squares in panel A. Experimental errors and theoretical standard deviations are reported as bars on each point. Linear regressions on each set of data are presented for comparison purposes.

Table S1. Averaged Interatomic Distances for Reactants, TS and Products for the Hydride Transfer Between H₄folate and dUMP, Derived from the 1D-PMFs Computed at 293 K, that are Displayed in Fig. 1, at Different Levels of Theory. Optimizes Structures Computed at M06-2X/MM Level are also Reported. All Values are Reported in Å.

d (Å)	RM1/MM	AM1/MM	PM3/MM	PDDG/MM	AM1-SRP,FDH/MM	M06-2X/MM
reactants						
d(Cd-H)	1.13 ± 0.03	1.14 ± 0.03	1.13 ± 0.04	1.13 ± 0.03	1.14 ± 0.03	1.09
d(Ca-H)	2.82 ± 0.05	2.76 ± 0.04	2.93 ± 0.04	2.93 ± 0.04	2.93 ± 0.04	2.25
d(Cd-Ca)	3.56 ± 0.14	3.65 ± 0.10	3.58 ± 0.13	3.68 ± 0.11	3.71 ± 0.13	3.31
d(S-C6)	1.87 ± 0.05	1.91 ± 0.05	1.87 ± 0.04	1.85 ± 0.04	1.92 ± 0.05	1.87
transition state						
d(Cd-H)	1.59 ± 0.03	1.63 ± 0.04	1.59 ± 0.04	1.58 ± 0.04	1.41 ± 0.04	1.45
d(Ca-H)	1.23 ± 0.03	1.26 ± 0.03	1.32 ± 0.04	1.28 ± 0.03	1.36 ± 0.04	1.26
d(Cd-Ca)	2.79 ± 0.06	2.87 ± 0.07	2.88 ± 0.07	2.83 ± 0.06	2.75 ± 0.07	2.70
d(S-C6)	1.96 ± 0.06	2.03 ± 0.07	1.93 ± 0.05	1.91 ± 0.05	1.95 ± 0.05	1.92
products						
d(Cd-H)	3.69 ± 0.04	3.82 ± 0.05	3.81 ± 0.04	3.81 ± 0.04	3.76 ± 0.04	3.67
d(Ca-H)	1.12 ± 0.03	1.13 ± 0.03	1.11 ± 0.03	1.11 ± 0.03	1.14 ± 0.03	1.10
d(Cd-Ca)	4.66 ± 0.10	4.76 ± 0.09	4.78 ± 0.10	4.63 ± 0.17	4.77 ± 0.11	3.75
d(S-C6)	2.01 ± 0.07	2.18 ± 0.11	1.96 ± 0.06	1.94 ± 0.05	2.04 ± 0.08	1.98

Table S2. Temperature Dependence of the Classical Mechanical Free Energies of Activation, W^{CM} , Computed at Different Levels of Theory. Values in kcal·mol⁻¹.

T (K)	RM1	AM1	PM3	PDDG	AM1-SRP,FDH
278	23.8	32.3	31.1	30.5	27.7
293	24.1	33.0	31.1	28.9	28.3
303	23.8	34.2	30.5	29.1	28.4
313	24.1	31.9	31.6	30.0	28.9

Table S3. Temperature Dependence of the Recrossing Transmission Coefficient, γ , for the Protium and Tritium Transfer Computed at Different Levels of Theory.

T(K)	AM1		RM1		PM3		PDDG		AM1-SRP, FDH	
	H	³ H	H	³ H	H	³ H	H	³ H	H	³ H
278	0.62 ± 0.04	0.79 ± 0.07	0.57 ± 0.03	0.83 ± 0.08	0.33 ± 0.02	0.56 ± 0.03	0.47 ± 0.02	0.75 ± 0.05	0.56 ± 0.03	0.80 ± 0.07
293	0.55 ± 0.03	0.79 ± 0.07	0.53 ± 0.03	0.85 ± 0.08	0.21 ± 0.01	0.41 ± 0.02	0.34 ± 0.02	0.60 ± 0.03	0.55 ± 0.03	0.84 ± 0.08
303	0.53 ± 0.03	0.79 ± 0.07	0.60 ± 0.04	0.76 ± 0.06	0.31 ± 0.02	0.64 ± 0.04	0.46 ± 0.02	0.74 ± 0.04	0.52 ± 0.03	0.87 ± 0.09
313	0.56 ± 0.04	0.85 ± 0.08	0.58 ± 0.04	0.83 ± 0.08	0.16 ± 0.01	0.53 ± 0.03	0.47 ± 0.02	0.53 ± 0.03	0.61 ± 0.04	0.79 ± 0.07

Table S4. Temperature Dependence of the Tunneling Transmission Coefficient, κ , for the Protium and Tritium Transfer Computed at Different Levels of Theory.

T(K)	AM1		RM1		PM3		PDDG		AM1-SRP, FDH	
	H	³ H	H	³ H	H	³ H	H	³ H	H	³ H
278	21.07 ± 3.71	10.50 ± 1.70	11.91 ± 2.27	8.23 ± 1.93	32.04 ± 10.51	14.32 ± 2.72	13.71 ± 0.99	9.11 ± 0.50	42.14 ± 5.62	16.22 ± 3.40
293	14.09 ± 2.20	8.09 ± 1.81	8.74 ± 1.46	6.63 ± 1.50	21.66 ± 6.08	10.55 ± 1.71	9.81 ± 0.56	7.21 ± 0.36	26.20 ± 3.20	11.90 ± 2.32
303	11.30 ± 1.63	6.98 ± 1.49	7.37 ± 1.14	5.86 ± 1.30	17.27 ± 4.39	8.86 ± 1.30	8.15 ± 0.41	6.31 ± 0.32	20.13 ± 2.32	10.02 ± 1.87
313	9.33 ± 1.25	6.13 ± 1.25	6.36 ± 0.91	5.26 ± 1.14	14.11 ± 3.25	7.60 ± 1.02	6.93 ± 0.31	5.61 ± 0.28	16.44 ± 2.02	8.60 ± 1.54

Table S5. Temperature Dependence of the Contribution of the Quasi-Classical Primary Tritium KIEs, KIE^{QC} , Computed at Different Levels of Theory.

T (K)	RM1	AM1	PM3	PDDG	AM1-SRP,FDH	M06-2X
278	5.87 ± 0.11	8.20 ± 0.13	7.42 ± 0.28	5.00 ± 0.13	8.50 ± 0.18	8.27
293	5.44 ± 0.09	7.47 ± 0.11	6.89 ± 0.29	4.49 ± 0.45	7.82 ± 0.15	8.40
303	5.16 ± 0.10	6.93 ± 0.22	5.92 ± 0.17	4.36 ± 0.33	7.23 ± 0.11	7.85
313	4.95 ± 0.07	6.45 ± 0.17	5.81 ± 0.18	3.23 ± 0.31	6.66 ± 0.13	7.67

Table S6. Temperature Dependence of the Contribution of the Recrossing Transmission Coefficients to the Primary Tritium KIEs, KIE_{γ} , Computed at Different Levels of Theory.

T (K)	RM1	AM1	PM3	PDDG	AM1-SRP, FDH
278	0.69 ± 0.10	0.79 ± 0.12	0.59 ± 0.07	0.63 ± 0.07	0.70 ± 0.10
293	0.62 ± 0.09	0.69 ± 0.11	0.52 ± 0.06	0.58 ± 0.06	0.65 ± 0.10
303	0.79 ± 0.11	0.67 ± 0.10	0.49 ± 0.06	0.62 ± 0.06	0.60 ± 0.09
313	0.70 ± 0.12	0.66 ± 0.11	0.30 ± 0.03	0.88 ± 0.10	0.77 ± 0.11

Table S7. Temperature Dependence of the Quantum Tunneling Contribution to the Primary Tritium KIEs, KIE_{κ} , Computed at Different Levels of Theory.

T (K)	RM1	AM1	PM3	PDDG	AM1-SRP,FDH
278	1.50 ± 0.23	2.05 ± 0.25	2.22 ± 0.45	1.51 ± 0.17	2.65 ± 0.40
293	1.38 ± 0.20	1.78 ± 0.18	2.04 ± 0.35	1.36 ± 0.13	2.24 ± 0.30
303	1.32 ± 0.19	1.65 ± 0.16	1.94 ± 0.30	1.29 ± 0.11	2.04 ± 0.26
313	1.27 ± 0.18	1.55 ± 0.14	1.85 ± 0.26	1.24 ± 0.10	1.94 ± 0.29

Table S8. Total Primary Tritium KIEs for Hydride Transfer with the QM Part Described at Different Semiempirical Levels (A), and with Correction of the Quasi-Classical KIE at M06-2X/MM Level of Theory (B).

A

T (K)	AM1	RM1	PM3	PDDG	AM1-SRP,FDH
278	13.28 ± 0.50	6.08 ± 0.44	9.72 ± 0.80	4.76 ± 0.37	15.77 ± 0.68
293	9.17 ± 0.40	4.65 ± 0.41	7.31 ± 0.70	3.54 ± 0.64	11.39 ± 0.55
303	7.66 ± 0.48	5.38 ± 0.43	5.63 ± 0.53	3.49 ± 0.50	8.85 ± 0.46
313	6.60 ± 0.42	4.40 ± 0.44	3.22 ± 0.47	3.52 ± 0.51	9.95 ± 0.53

B

T (K)	M06-2X: AM1	M06-2X: RM1	M06-2X: PM3	M06-2X: PDDG	M06-2X: AM1-SRP,FDH	Exp. ^a
278	13.39 ± 0.33	8.56 ± 0.37	10.83 ± 0.52	7.87 ± 0.50	15.34 ± 0.50	7.62 ± 0.4
293	10.32 ± 0.29	7.19 ± 0.29	8.91 ± 0.41	6.63 ± 0.19	12.23 ± 0.40	6.91 ± 0.05
303	8.68 ± 0.30	8.19 ± 0.26	7.46 ± 0.36	6.28 ± 0.17	9.61 ± 0.35	7.40 ± 0.55
313	7.85 ± 0.30	6.82 ± 0.25	4.26 ± 0.29	8.37 ± 0.20	11.46 ± 0.40	7.12 ± 0.17

Table S9. Equilibrium and reaction frequencies (in cm^{-1}) computed for the protium and tritium at the different levels of theory and at four different temperatures (in K).

AM1/MM				
T (K)	Hydride		Tritium	
	ω^{eq}	ω^{r}	ω^{eq}	ω^{r}
278	3244.51	2019.04	3490.49	2682.13
293	3191.83	1715.64	3315.94	2572.96
303	3385.95	1750.92	3359.45	2635.09
313	3438.13	1886.92	3418.52	2880.98
RM1/MM				
T (K)	Hydride		Tritium	
	ω^{eq}	ω^{r}	ω^{eq}	ω^{r}
278	3673.54	2110.36	3427.90	2855.02
293	3855.78	2049.58	3504.51	2992.75
303	3507.93	2114.79	3688.29	2813.17
313	3357.81	1941.52	3327.93	2755.71
PM3/MM				
T(K)	Hydride		Tritium	
	ω^{eq}	ω^{r}	ω^{eq}	ω^{r}
278	4380.32	1197.15	4714.10	1905.14
293	5254.41	862.96	5024.83	1432.35
303	4809.18	1094.76	5159.55	2341.09
313	4637.23	524.42	5059.41	1757.39
PDDG/MM				
T (K)	Hydride		Tritium	
	ω^{eq}	ω^{r}	ω^{eq}	ω^{r}
278	4727.87	2240.39	4699.24	3534.56
293	4950.61	1700.87	4712.21	2807.35
303	4225.50	1946.16	4771.41	3532.00
313	4171.92	1944.58	4339.72	2296.82
AM1-SRP, FDH/MM				
T (K)	Hydride		Tritium	
	ω^{eq}	ω^{r}	ω^{eq}	ω^{r}
278	3610.92	2021.62	3746.62	3016.62
293	3813.46	2094.27	3790.94	3202.05
303	3842.14	2013.30	3743.95	3236.73
313	3562.16	2172.06	3452.63	2746.48

Table S10. Cartesian coordinates of the QM atoms of the TS localized at M06-2X/6-31+G(d,p)/MM level starting from structures obtained from four different 1D-PMF computed at AM1/MM level at 278, 293, 303 and 313 K.

T = 278 K	$\nu = -852.530 \text{ cm}^{-1}$			T = 293 K	$\nu = -842.469 \text{ cm}^{-1}$		
Atoms	x	y	z	Atoms	x	y	z
S	11.19931	8.227758	3.465541	S	10.717367	8.225342	2.864226
C	10.71002	6.550885	2.892722	C	10.31032	6.537396	2.259997
H	10.67437	6.548381	1.799504	H	10.148598	6.551513	1.179427
H	11.46087	5.817332	3.206641	H	11.15175	5.867699	2.474864
N	12.65481	9.059573	1.229113	N	12.554539	9.078268	0.942566
C	12.71726	8.211529	0.171751	C	12.66705	8.229544	-0.107987
N	13.05124	6.899038	0.49201	N	12.931076	6.905535	0.232987
C	13.85319	6.52511	1.603303	C	13.608407	6.524598	1.421994
C	13.86001	7.471576	2.653039	C	13.456858	7.432498	2.489335
C	12.86689	8.579886	2.586419	C	12.516403	8.563067	2.297186
O	12.48751	8.540222	-0.99911	O	12.547675	8.558662	-1.294567
O	14.45729	5.436159	1.544812	O	14.263038	5.466629	1.427359
C	12.44496	10.50078	0.965855	C	12.40917	10.528124	0.691127
C	11.13378	10.85901	0.26874	C	11.036845	10.974176	0.198682
C	10.90436	12.29647	0.73578	C	10.999772	12.429319	0.661099
C	11.49237	12.28474	2.148372	C	11.802204	12.408728	1.973396
O	11.6245	13.2444	-0.05984	O	11.692655	13.272766	-0.270021
O	12.38409	11.15149	2.211168	O	12.587157	11.188833	1.917257
H	9.847499	12.56538	0.756816	H	9.987131	12.79823	0.816273
H	13.11446	6.279545	-0.30673	H	13.083778	6.291624	-0.558263
C	14.834	7.391503	3.695998	C	14.306557	7.307653	3.628926
H	12.10328	13.18579	2.288801	H	12.509062	13.249186	1.950795
H	11.18685	10.76461	-0.81578	H	10.915366	10.864292	-0.88155
H	10.34647	10.20295	0.652122	H	10.274267	10.385928	0.71433
H	13.30802	10.86294	0.387088	H	13.197626	10.802065	-0.025939
H	13.20321	9.428856	3.178186	H	12.760422	9.391516	2.959098
H	11.2467	13.23255	-0.95079	H	11.237603	13.216802	-1.124102
H	15.24243	6.398869	3.888888	H	14.626358	6.292394	3.87028
H	14.5982	7.934232	4.615499	H	14.015198	7.886687	4.507045
N	15.45244	12.14672	1.10426	N	15.320769	12.04317	1.090153

C	15.76143	12.31904	-0.16292	C	15.68504	12.222453	-0.159449
N	15.40248	13.435	-0.85189	N	15.419509	13.374042	-0.836398
N	16.51666	11.42054	-0.86278	N	16.412824	11.294492	-0.854545
C	17.00622	10.22175	-0.33933	C	16.7699	10.045546	-0.353363
O	17.68985	9.459941	-1.02081	O	17.482066	9.276282	-0.998816
C	16.63047	10.03723	1.020929	C	16.24111	9.819701	0.946
N	17.07316	8.892719	1.693145	N	16.538766	8.612304	1.584426
C	17.06511	8.764137	3.029191	C	16.63534	8.479959	2.915497
C	16.68943	10.04176	3.760633	C	16.424527	9.792003	3.659562
N	15.67211	10.79778	3.027867	N	15.414863	10.612624	2.982717
C	15.92025	11.01487	1.68958	C	15.648501	10.850857	1.645513
C	18.24516	7.934028	3.60993	C	17.777111	7.518656	3.359856
N	18.96339	7.133522	2.669206	N	18.386302	6.795579	2.272801
H	18.5943	6.198143	2.48544	H	17.993338	5.871429	2.088302
H	14.6339	13.94061	-0.43016	H	14.649994	13.892874	-0.43101
H	15.40174	13.35653	-1.90716	H	15.413112	13.277674	-1.89695
H	16.74984	11.62862	-1.82472	H	16.745606	11.521292	-1.783021
H	15.29236	11.60888	3.503141	H	15.073105	11.422612	3.487706
H	17.62036	10.61377	3.872795	H	17.402009	10.297931	3.696906
H	16.32398	9.818625	4.765476	H	16.112308	9.611705	4.689707
H	15.89937	7.978787	3.366298	H	15.429863	7.806589	3.360324
H	18.90797	8.668963	4.092966	H	18.507717	8.137124	3.900024
H	17.87824	7.298171	4.418109	H	17.401859	6.802305	4.094895
H	17.60747	8.21209	1.153823	H	17.002952	7.901466	1.020668
H	19.5761	7.491127	1.964431	H	19.023263	7.173244	1.600752
H	10.7786	12.21095	2.844852	H	11.272745	12.415316	2.821706
H	9.813541	6.333796	3.278987	H	9.489815	6.247998	2.752968

T = 303 K		$\nu = -816.687 \text{ cm}^{-1}$		T = 313 K		$\nu = -833.941 \text{ cm}^{-1}$	
Atoms	x	y	z	Atoms	x	y	z
S	11.11708	7.927989	3.105034	S	11.154209	8.066984	3.151737
C	10.58245	6.32563	2.38424	C	10.609214	6.468213	2.442461
H	10.42341	6.425707	1.306613	H	10.493158	6.566968	1.35831
H	11.36061	5.56978	2.544616	H	11.377256	5.708325	2.631673

N	12.68898	8.846715	1.014783	N	12.723769	8.93162	1.026004
C	12.7794	8.045085	-0.07518	C	12.775788	8.144512	-0.076498
N	13.12713	6.727813	0.199987	N	13.088415	6.811342	0.155409
C	13.92236	6.342584	1.317162	C	13.850091	6.36473	1.264575
C	13.82782	7.210094	2.421822	C	13.792829	7.207802	2.392481
C	12.82105	8.300787	2.355393	C	12.864862	8.368792	2.359591
O	12.57411	8.418737	-1.23853	O	12.564391	8.539174	-1.229193
O	14.61523	5.314152	1.220486	O	14.500612	5.311125	1.150858
C	12.52928	10.30032	0.823448	C	12.543581	10.389766	0.852774
C	11.18911	10.78493	0.28442	C	11.230509	10.840222	0.2218
C	11.19075	12.23711	0.768303	C	11.135137	12.279538	0.732008
C	11.88925	12.14854	2.134239	C	11.7694	12.193925	2.126995
O	11.90754	13.08801	-0.13136	O	11.923508	13.164069	-0.072179
O	12.65557	10.91452	2.081339	O	12.536211	10.965689	2.135685
H	10.19205	12.65669	0.844645	H	10.109594	12.645411	0.784988
H	13.23878	6.141022	-0.61841	H	13.194594	6.256703	-0.685601
C	14.76369	7.092378	3.499282	C	14.681463	6.947684	3.478896
H	12.61226	12.97076	2.210719	H	12.476075	13.025224	2.246472
H	11.09726	10.70457	-0.79919	H	11.231305	10.776714	-0.866424
H	10.39442	10.2136	0.770336	H	10.416925	10.229578	0.623484
H	13.35212	10.60457	0.152264	H	13.400336	10.755723	0.264723
H	13.11713	9.122343	3.003646	H	13.226573	9.162141	3.00991
H	12.77131	12.69173	-0.30889	H	11.550656	13.16289	-0.965434
H	15.19422	6.098402	3.626041	H	14.955697	5.900321	3.61632
H	14.4636	7.550359	4.446397	H	14.455522	7.462324	4.416156
N	15.28514	12.00239	1.095349	N	15.496586	11.780651	1.551712
C	15.62051	12.20639	-0.16014	C	15.764786	12.09406	0.296887
N	15.25414	13.32257	-0.83799	N	15.305929	13.225874	-0.28643
N	16.39234	11.33081	-0.87521	N	16.561456	11.312651	-0.50003
C	16.90856	10.14284	-0.3601	C	17.052228	10.064176	-0.130196
O	17.64231	9.414959	-1.02906	O	17.752742	9.390613	-0.886574
C	16.50075	9.921092	0.980587	C	16.654597	9.699276	1.182947
N	16.97394	8.780273	1.63021	N	17.063227	8.458312	1.672691
C	16.95694	8.605351	2.956268	C	17.056765	8.125308	2.971517

C	16.49816	9.826264	3.73856	C	16.760247	9.308412	3.888526
N	15.5078	10.59806	2.987656	N	15.747969	10.186522	3.289336
C	15.76241	10.86466	1.667302	C	15.969696	10.587103	1.985845
C	18.14975	7.782261	3.499333	C	18.163213	7.108473	3.36711
N	18.82684	7.002643	2.496289	N	18.829379	6.469202	2.256891
H	18.34477	6.128793	2.283022	H	18.367789	5.651265	1.878522
H	14.62018	13.94892	-0.36197	H	14.527958	13.658285	0.197905
H	15.27351	13.28038	-1.8986	H	15.266158	13.211279	-1.344364
H	16.67384	11.58084	-1.81423	H	16.786197	11.629604	-1.435801
H	15.00857	11.32845	3.480494	H	15.37972	10.918952	3.884942
H	17.39617	10.41602	3.965465	H	17.708497	9.837965	4.062619
H	16.06595	9.522141	4.69438	H	16.400976	8.953236	4.85701
H	15.79967	7.73287	3.248176	H	15.825185	7.409342	3.224017
H	18.83208	8.499414	3.976567	H	18.883057	7.647272	3.997973
H	17.81258	7.116136	4.297756	H	17.742097	6.330559	4.00708
H	17.52732	8.13668	1.067984	H	17.550743	7.852443	1.016852
H	19.44764	7.36188	1.799467	H	19.45012	6.927626	1.620867
H	11.28282	12.14754	2.92938	H	11.097182	12.181285	2.867241
H	9.734337	6.085737	2.856641	H	9.73926	6.228641	2.873488