Electronic Supplementary Information

A mechanistic study supports a two-step mechanism for peptide bond formation on the ribosome

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Full Reference 24: M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *GAUSSIAN 09 (Revision A.02)*, Gaussian, Inc., Wallingford, CT, 2009.

Detailed methods for calculations

All density functional calculations were carried out using Gaussian 09.[24] GaussView[28] was used in editing all structures. Based on the results of ammonolysis of 1-*O*-formyl-1,2-ethanediol,[29] the initial structures of all TSs for the aminolysis of Ac-Rib by $MeNH_2$ (the small model system) were generated, where the puckering of ribose was initially assigned to be C2′-endo as found in the X-ray structures of TS analogs complexed with the 50S subunit.[4] The *S* chirality was assigned to each carbonyl carbon of the acetyl group with the tetrahedral geometry in TS, as found in experimental results.[4,11] The feasible pathways for the aminolysis of Ac-Rib by $MeNH_2$ are depicted in supplementary Figure S2 of Supporting Information. These initial TS structures were optimized at the B3LYP/6-31+G(d) level of theory and followed by optimizations using the meta-hybrid M06-2X functional[22] and the 6-31+G(d) basis set in the gas phase.

In the uncatalyzed aminolysis of Ac-Rib by Ala-Rib (the large model system), both the concerted and stepwise pathways with the four- and six-membered TSs were also considered, as done for the small model system. The initial TS structures for the large system were generated

by the combination of the optimized TS structures for the small model system at the M06-2X/6-31+G(d) level of theory and the structure of the A-site ribose moiety in the X-ray structure of the complex of the 50S subunit and the TS analog DCSN (PDB entry 1VQL).[4] Then, these initial TS structures were optimized at the M06-2X/6-31+G(d) level of theory in the gas phase.

For the catalyzed PT reactions, the quaternary ribosome model system was constructed by Ac-Rib (P-site substrate), Phe-Rib (A-site substrate), two water molecules W1 and W2 found in the PTC of X-ray structures of the complex of the 50S subunit and the TS analog DCSN (PDB entry 1VQL),[4] and adenosine (as a ribosomal A2451). Based on the observed kinetics results,[9–11] only the optimized structures of six-membered TSs for the concerted and stepwise pathways of Ac-Rib and Ala-Rib at the M06-2X/6-31+G(d) level of theory were used in generating the initial TS structures for the catalyzed reaction of Ac-Rib and Phe-Rib. The initial locations of two water molecules (W1 and W2) and adenosine were taken from those in the X-ray structure of the complex of the 50S subunit and the TS analog RAP (PDB entry 1VQP).[4] In addition, the initial structure of the eight-membered TS with a water molecule W1 (denoted by TS_{C8}) for the concerted pathway was constructed using the TS_{C8} structure of the small model located at the B3LYP/6-311G(d,p) level of theory.[19] However, the initial structure of the eight-membered TS1 for the stepwise pathway cannot be generated because the hydrogen of the 2′-OH of Ac-Rib points to its carbonyl oxygen and cannot be provided to the water molecule W1 to form a bifurcated hydrogen bond with its O2′ and O3′. All initial TS structures for the three quaternary ribosome model systems were optimized at the M06-2X/6-31G(d) level of theory in the gas phase because this model system contains 93 atoms.

Each TS can be checked by the intrinsic reaction coordinate (IRC) method[30,31] whether it connects the reactants and products. However, the IRC calculation does not step all the way to

the minimum on either side of the path in most cases, especially for large systems.[32] Further optimizations are required by starting from the reactants and products obtained by the IRC method to reach the two minima that the TS connects. In the present study, we used this IRC-optimization procedure to locate the local minima (reactant or product complexes) or intermediates connected by each TS at the same levels of theory as for the TS optimizations in the gas phase.

All local minima and TSs in the gas phase were re-optimized using the SMD method[23] at the same levels of theory in acetonitrile and water. As a test, the IRC-optimization calculations were carried out for all TSs of the small model system only in acetonitrile, since all the IRC calculations even for the small system were terminated without moving far from their initial TS structures in water. It was found that their local minima are the same as those obtained by optimizing the gas-phase local minima in acetonitrile. Vibrational frequencies were calculated for all local minima and TSs at the same levels of theory for optimizations in acetonitrile and water. In particular, each TS was confirmed by checking whether it has one imaginary frequency. The scale factors used for vibrational frequencies are 0.9440 and 0.9262 at the M06-2X/6-31+G(d) and M06-2X/6-31G(d) levels of theory, respectively, that were chosen to reproduce experimental frequency for the amide I band of *N*-methylacetamide in Ar and N_2 matrixes.[33] The zero-point and thermal energy corrections were employed in calculating the Gibbs free energy of each structure at 25 °C and 1 atm. Here, the ideal gas, rigid rotor, and harmonic oscillator approximations were used for the translational, rotational, and vibrational contributions to the Gibbs free energy, respectively.[34]

Single-point energies were calculated using the double-hybrid functional B2PLYP-D with empirical dispersion terms[25] and the 6-311++G(d,p) basis set for all local minima and TSs

located in acetonitrile and water. The bond overlaps of the optimized C–O3′, C–N, C=$O_{P\text{-site}}$, and N–$H_{A\text{-site}}$ bonds and the natural charges along the stepwise S6 pathway of the quaternary model system in water were calculated at the M06-2X/6-311++G(d,p) level of theory using the natural bond orbital (NBO) method[35] implemented in Gaussian 09. The Wiberg bond index[36] obtained by the sums of squares of off-diagonal density matrix elements of each bond is used as a measure of a bond overlap.

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Table S1 Thermodynamic quantities of the catalyzed PT reactions for the quaternary model system in acetonitrile and water[a]

		Acetonitrile			Water		
Pathway[b]	Species[c]	ΔE_e	ΔH	ΔG	ΔE_e	ΔH	ΔG
C6	RC	4.78	4.50	2.06	2.70	2.94	0.78
	TS_{C6}	35.18	31.38	31.74 (29.68)[d]	35.87	32.61	32.10 (31.32)[d]
	PC	–5.54	–6.40	–8.10	–5.58	–5.82	–9.02
C8	RC	6.60	6.27	4.63	9.39	9.55	7.79
	TS_{C8}	40.47	35.10	37.14 (32.51)[d]	40.77	36.12	38.19 (30.40)[d]
	PC	–0.95	–1.41	–2.98	–1.40	–0.98	–3.30
S6	RC	0.00	0.00	0.00	0.00	0.00	0.00
	$TS1_{S6}$	20.17	17.36	20.90	17.79	15.80	20.84
	$I1_{S6}$	8.17	7.83	10.40	9.68	9.90	12.15
	$I2_{S6}$	8.48	8.67	13.31	6.29	6.84	10.09
	$TS2_{S6}$	22.66	20.18	25.20 (11.89)[e]	23.04	21.48	23.43 (13.34)[e]
	PC	–19.14	–19.12	–14.78	–15.35	–15.83	–13.88

[a] All values (kcal mol^{-1}) are calculated relative to the reactant complex for the S6 pathway at the B2PLYP-D/6-311++G(d,p)//SMD M06-2X/6-31G(d) level of theory at 25 °C and 1 atm in solution. [b] C6 and C8 denote the concerted pathways with the six- and eight-membered TSs, respectively, whereas S6 the stepwise pathway with the six-membered TS. [c] RC, TS, I, and PC indicate the reactant complex, transition state, intermediate, and product complex, respectively. [d] Barrier $\Delta G^{\ddagger}$ to RC for C6 and C8 pathways. [e] Barrier $\Delta G^{\ddagger}_2$ to $I2_{S6}$ for the S6 pathway.

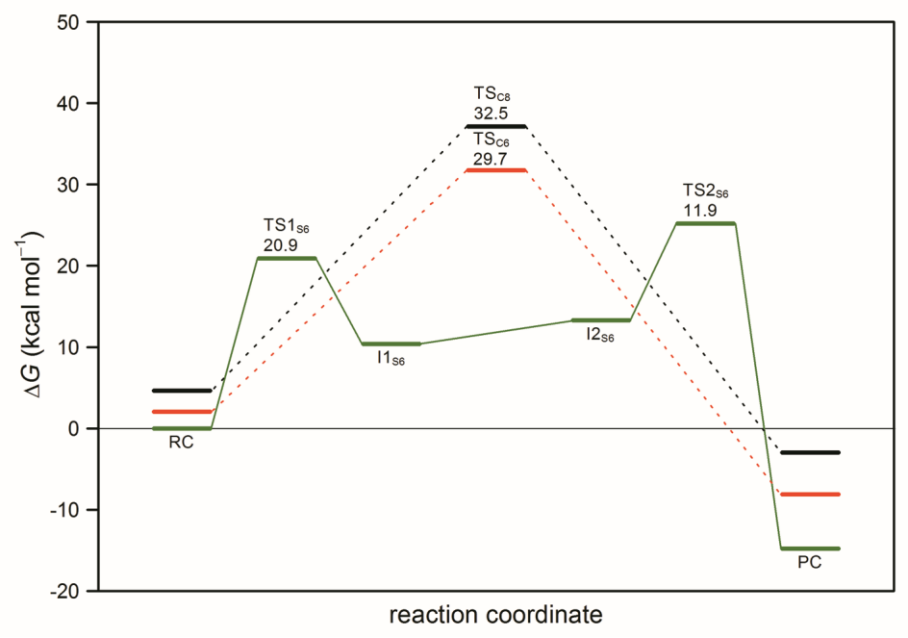

Fig. S1 Free energy profiles along the reaction coordinate for the catalyzed PT reaction for the quaternary model system in acetonitrile. TS_{C6} and TS_{C8} denote six- and eight-membered TSs for the concerted pathways, respectively. In the stepwise pathway, the first and second six-membered TSs are denoted by $TS1_{S6}$ and $TS2_{S6}$, respectively. The calculated barriers (kcal mol^{-1}) of three pathways are also shown on profiles. All free energies were calculated at the B2PLYP-D/6-311++G(d,p)//SMD M06-2X/6-31G(d) level of theory at 25 °C and 1 atm in acetonitrile.

Table S2 Calculated and observed activation parameters of the PT reactions[a]

	Solvent	Ester	Amine	$\Delta G^{\ddagger}$	$\Delta H^{\ddagger}$	$T\Delta S^{\ddagger}$
Calculated						
Uncatalyzed	CH_3CN	Ac-Rib	Ala-Rib	23.4	17.9	–5.4
	Water	Ac-Rib	Ala-Rib	19.5	15.7	–3.8
Catalyzed	CH_3CN	Ac-Rib	Phe-Rib	20.9	17.4	–3.5
	Water	Ac-Rib	Phe-Rib	20.8	15.8	–5.0
Observed						
Uncatalyzed[b]	Buffer	fMet-tRNA^{fMet}	Tris	22.7	16.2	–6.5
Ribosome[c]	Buffer	fMet-Phe-tRNA^{Phe}	Puromycin	14.0	16.0	2.0
Ribosome[d]	Buffer	fMet-tRNA^{fMet}	Phe-codon	15.1	17.0	2.0

[a] Activation parameters (kcal mol^{-1}) are at 25 °C and 1 atm. Thermodynamic quantities of the uncatalyzed aminolysis of Ac-Rib by Ala-Rib are taken from Table S3. The catalyzed ones are for the quaternary system composed of Ac-Rib (P-site substrate), Phe-Rib (A-site substrate), two water molecules, and an adenosine. [b] Data from ref. 14. [c] Data from ref. 26. [d] Data from ref. 27.

Table S3 Thermodynamic quantities of the uncatalyzed PT reactions for the large model system in acetonitrile and water[a]

		Acetonitrile			Water		
Pathway[b]	Species[c]	ΔE_e	ΔH	ΔG	ΔE_e	ΔH	ΔG
C4	RC	–8.07	–7.80	–6.86	–6.74	–6.75	–7.01
	TS_{C4}	26.04	23.41	25.93 (32.79)[d]	23.06	21.45	23.21 (30.22)[d]
	PC	–15.47	–15.28	–16.89	–15.90	–15.80	–16.42
C6	RC	–0.55	–0.93	–4.45	–1.97	–2.16	–4.44
	TS_{C6}	30.98	27.78	29.28 (33.73)[d]	27.60	25.63	26.16 (30.60)[d]
	PC	–10.30	–10.34	–12.32	–12.45	–12.58	–14.87
S4	RC	–0.07	–0.29	–0.71	–1.58	–1.74	–3.31
	$TS1_{S4}$	41.54	38.76	41.82 (42.53)[e]	37.19	34.48	37.16 (40.47)[e]
	$I1_{S4}$	12.78	12.90	16.32	9.78	10.09	12.48
	$I2_{S4}$	8.80	9.10	12.18	6.25	6.89	10.41
	$TS2_{S4}$	29.67	28.03	30.88 (18.70)[f]	23.20	21.92	23.51 (13.10)[f]
	PC	–16.14	–15.67	–15.12	–16.62	–16.56	–17.30
S6	RC	0.00	0.00	0.00	0.00	0.00	0.00
	$TS1_{S6}$	20.40	17.94	23.38	17.20	15.70	19.49
	$I1_{S6}$	7.15	7.54	9.48	6.15	6.60	7.92
	$I2_{S6}$	9.47	9.97	11.70	6.60	7.35	9.38
	$TS2_{S6}$	29.40	26.83	28.18 (16.48)[f]	24.99	23.74	24.68 (15.30)[f]
	PC	–8.30	–8.41	–10.43	–12.12	–12.10	–13.92

[a] The large model system is composed of Ac-Rib and Ala-Rib. All values (kcal mol^{-1}) are calculated relative to the reactant complex for the S6 pathway at the B2PLYP-D/6-311++G(d,p)//SMD M06-2X/6-31+G(d) level of theory at 25 °C and 1 atm in solution. [b] C4 and C6 denote the concerted pathways with the four- and six-membered TSs, respectively, whereas S4 and S6 the stepwise pathways with the four- and six-membered TSs, respectively. [c] RC, TS, I, and PC indicate the reactant complex, transition state, intermediate, and product complex, respectively. [d] Barrier $\Delta G^{\ddagger}$ to RC for C4 and C6 pathways. [e] Barrier $\Delta G^{\ddagger}_1$ to RC for the S4 pathway. [f] Barrier $\Delta G^{\ddagger}_2$ to I2 for the S4 and S6 pathways.

Table S4 Distances and Wiberg bond indices of the C–O3′, C–N, C=$O_{P\text{-site}}$, and N–$H_{A\text{-site}}$ bonds along the stepwise S6 pathway of the quaternary model system in water

	Distance (Å)[a]				Wiberg bond index[b]			
Species	C–O3′	C–N	C=$O_{P\text{-site}}$	N–$H_{A\text{-site}}$	C–O3′	C–N	C=$O_{P\text{-site}}$	N–$H_{A\text{-site}}$
RC	1.34		1.22	1.02	1.0507		1.6724	0.8281
$TS1_{S6}$	1.40	1.52	1.38	1.17	0.9163	0.8411	0.9733	0.4623
$I1_{S6}$	1.41	1.47	1.40		0.8883	0.9385	0.9307	
$I2_{S6}$	1.44	1.44	1.40		0.8167	1.0004	0.9415	
$TS2_{S6}$	2.22	1.32	1.32		0.1870	1.3524	1.1292	
PC		1.35	1.24			1.2258	1.5443	

[a] Optimized at the SMD M06-2X/6-31G(d) level of theory in water. [b] Calculated at the M06-2X/6-311++G(d,p)//SMD M06-2X/6-31G(d) level of theory. The Wiberg bond index obtained by the sums of squares of off-diagonal density matrix elements of each bond is a measure of a bond overlap.[36]

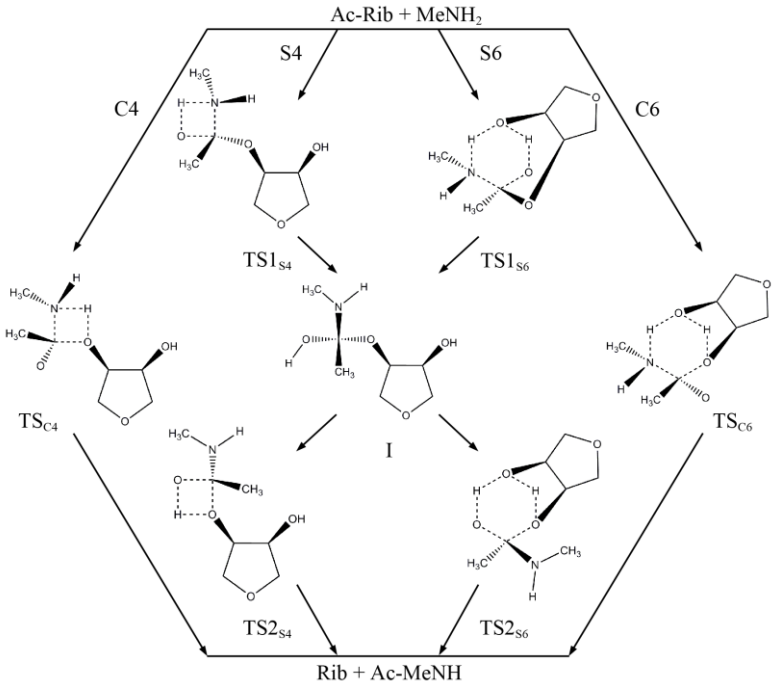

Fig. S2 Feasible pathways of the small model system. Based on the results of ammonolysis of 1-*O*-formyl-1,2-ethanediol,[29] the initial structures of all TSs for the aminolysis of Ac-Rib by $MeNH_2$ (the small model system) were generated, where the puckering of ribose was initially assigned to be C2′-endo as found in the X-ray structures of TS analogs complexed with the 50S subunit.[4] The *S* chirality was assigned to each carbonyl carbon of the acetyl group with the tetrahedral geometry in TS, as found in experimental results.[4,11]

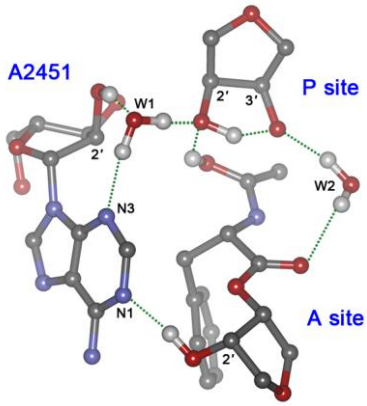

Fig. S3 Structure of the second transition state $TS2_{S6}$ for the catalyzed peptide bond formation in the stepwise S6 pathway of the quaternary model system optimized at the SMD M06-2X/6-31G(d) level of theory in water. A proton originated from the A-site amino group lies in-between the O3′ and O2′ of A76 and the latter simultaneously accepts its proton donated to the carbonyl oxygen and a proton from W1. W1 accepts a proton from the 2′-OH of A2451 and donates two protons to the O2′ of A76 and the N3 of A2451. W2 donates two protons to the O3′ of A76 and the carbonyl oxygen of the A site.

Cartesian coordinates of $TS1_{S6}$ and $TS2_{S6}$ structures of the quaternary model system optimized at the SMD M06-2X/6-31G(d) level of theory in water

(1) $TS1_{S6}$

E = -2512.283924 Hartrees
H = -2511.515106 Hartrees
G = -2511.649656 Hartrees

N	1.741894	-0.530052	-1.577905
C	1.367774	0.887904	-1.343874
C	1.631066	1.205765	0.123734
O	1.081686	0.624846	1.034432
C	-0.122676	1.056646	-1.650928
C	3.510906	3.915485	1.634432
O	4.509658	3.892996	2.654694
C	2.959253	2.493274	1.583899
O	2.508093	2.195704	0.260219
C	4.183818	1.663787	1.982424
O	5.073121	1.637313	0.883771
C	4.719192	2.544359	3.101120
C	3.195316	-0.916296	-1.340658
O	3.408925	-0.836583	0.023503
C	4.169878	-0.020203	-2.062315
C	3.242722	-4.591719	-1.798333
O	1.894083	-4.946614	-2.146526
C	3.169632	-3.330024	-0.954400
O	3.296997	-2.216045	-1.859728
C	1.753901	-3.438116	-0.359344
C	0.994211	-4.011502	-1.555608
H	1.232783	-1.279690	-0.845113
H	1.972372	1.534639	-1.982801
H	-0.670777	0.389245	-0.982715
H	2.144565	2.346259	2.294703
H	2.747625	4.655891	1.886349
H	3.962952	4.176127	0.669948
H	3.923134	0.655218	2.318819
H	5.558635	0.781652	0.912154
H	4.177065	2.371299	4.039266
H	5.788023	2.388856	3.265696
H	3.961582	-3.275485	-0.202676
H	3.688104	-5.412153	-1.227110
H	3.831707	-4.428205	-2.705874
H	1.804524	-4.213035	0.421730
O	1.229123	-2.251943	0.163028
H	0.077036	-4.533533	-1.267609
H	0.742167	-3.212342	-2.266256
H	-0.301751	0.721960	-2.678365
H	5.172589	-0.423577	-1.900659
H	3.951273	-0.009261	-3.132527
H	4.128460	0.993283	-1.658989
H	2.674150	-1.364206	0.455968
H	1.496615	-0.787020	-2.541005
C	-3.511619	-3.230716	1.086260
O	-2.893780	-2.426888	0.060602
C	-2.881318	-2.788521	2.424048
O	-2.661772	-3.863070	3.309763
C	-1.583055	-2.111497	1.968422
O	-0.667434	-3.150434	1.710001
C	-2.019470	-1.488654	0.645913
N	-2.699071	-0.202048	0.811941
C	-3.012913	0.502203	1.952588
N	-3.685505	1.602631	1.728825
C	-3.835454	1.625337	0.356590
C	-4.356602	2.593228	-0.525804
N	-4.933572	3.728962	-0.084427
N	-4.294021	2.360500	-1.848762
C	-3.717468	1.227165	-2.267958
N	-3.180620	0.249429	-1.542775
C	-3.242341	0.514070	-0.226792
O	-1.376008	-1.852175	-2.450106
H	-3.253780	-4.278841	0.897441
H	-3.535173	-2.079132	2.938726
H	-1.904798	-4.353864	2.942649
H	-1.180166	-1.381387	2.678926
H	0.092633	-2.766467	1.174330
H	-1.180294	-1.347681	-0.037838
H	-2.708223	0.165363	2.933917
H	-4.752450	4.011531	0.870522
H	-3.689054	1.086594	-3.345439
H	-1.663863	-2.574967	-1.869008
H	-1.991830	-1.124222	-2.214203
H	-5.060707	4.473513	-0.759058
O	6.079800	-0.918748	0.815451
H	6.587658	-0.912788	-0.011550
H	5.172977	-1.147881	0.528804
C	-0.607134	2.474687	-1.471087
C	-0.996758	2.940340	-0.210596
C	-0.690677	3.338529	-2.566546
C	-1.472673	4.240833	-0.051485
H	-0.952798	2.270791	0.647042
C	-1.166278	4.638423	-2.410193
H	-0.392612	2.981276	-3.549359
C	-1.563418	5.090590	-1.152185
H	-1.794249	4.580130	0.929259
H	-1.234482	5.295304	-3.272108
H	-1.945982	6.099562	-1.031156
C	-5.014318	-3.071079	1.028494
H	-5.383488	-3.417403	0.055078
H	-5.454104	-3.706508	1.809260
O	-5.332962	-1.702803	1.231092
H	-6.299017	-1.625033	1.242042

(2) $TS2_{S6}$

E = -2512.269248 Hartrees
H = -2511.499748 Hartrees
G = -2511.639223 Hartrees

N	-0.561611	2.087923	1.403050
C	-1.000588	1.187640	0.344103
C	-2.388649	1.657867	-0.066000
O	-2.876418	2.708886	0.297000
C	-0.969271	-0.296945	0.752026
C	-5.333051	1.372103	-0.235041
O	-6.514942	0.712844	-0.694005
C	-4.288264	1.209299	-1.356188
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O	-4.658250	-1.174187	-1.637498
C	-6.352628	0.420456	-2.082307
C	0.677172	2.275820	1.813519
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C	0.924831	3.152216	2.987907
C	3.002183	5.085004	-0.800365
O	4.177030	4.637594	-1.466632
C	2.584755	3.928570	0.122953
O	1.218837	3.796682	0.284126
C	3.131351	2.676364	-0.675222
C	3.914662	3.286722	-1.840052
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H	-0.373652	1.307084	-0.550780
H	-1.307008	-0.861701	-0.117006
H	0.072910	-0.575511	0.944367
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H	-5.546485	2.426300	-0.038213
H	-4.997401	0.893734	0.691866
H	-4.525173	0.130807	-3.257603
H	-3.737891	-1.460350	-1.852263
H	-6.622173	1.287659	-2.699419
H	-6.993109	-0.425990	-2.336344
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H	3.239682	6.016206	-0.281704
H	2.192053	5.263547	-1.524741
H	3.749030	2.025672	-0.053496
O	1.978482	1.955600	-1.097842
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H	3.293748	3.252507	-2.747877
H	0.860790	2.533488	3.889902
H	1.927376	3.575422	2.921322
H	0.178841	3.945304	3.040576
H	1.643269	1.166823	0.572742
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C	3.741407	-1.289250	0.680445
O	4.437788	-0.173239	1.183872
C	3.120070	-1.055838	-0.695925
O	4.069000	-0.364327	-1.474964
C	2.956204	-2.490726	-1.210316
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C	1.345579	-4.027059	0.045116
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C	0.447232	-2.636481	-1.393436
O	2.190225	0.322033	-3.301886
H	5.595362	-2.143566	-0.016386
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H	1.986045	1.036641	-2.660234
H	1.559056	-0.388424	-3.055404
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C	-1.831827	-0.615657	1.945075
C	-3.145009	-1.064039	1.770090
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C	-2.146098	-0.710918	4.346031
H	-0.312043	-0.113853	3.383931
C	-3.457456	-1.148578	4.163846
H	-4.972014	-1.676439	2.725319
H	-1.749535	-0.579813	5.348330
H	-4.086255	-1.358159	5.023824
O	-1.076117	5.010137	1.022889
H	-0.182479	4.719323	0.715544
H	-1.687065	4.546641	0.428099
C	4.947551	-3.357135	1.611111
H	5.562118	-4.213102	1.306220
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O	3.726780	-3.788194	2.189889
H	3.942336	-4.294616	2.988010