SUPPORTING INFORMATION

Quantum mechanical analysis of nonenzymatic nucleotidyl transfer reactions: Kinetic and thermodynamic effects of β−γ bridging groups of dNTP substrates

Zheng Zhang, Josh Eloge and Jan Florián

Department of Chemistry and Biochemistry, Loyola University Chicago, Chicago, IL

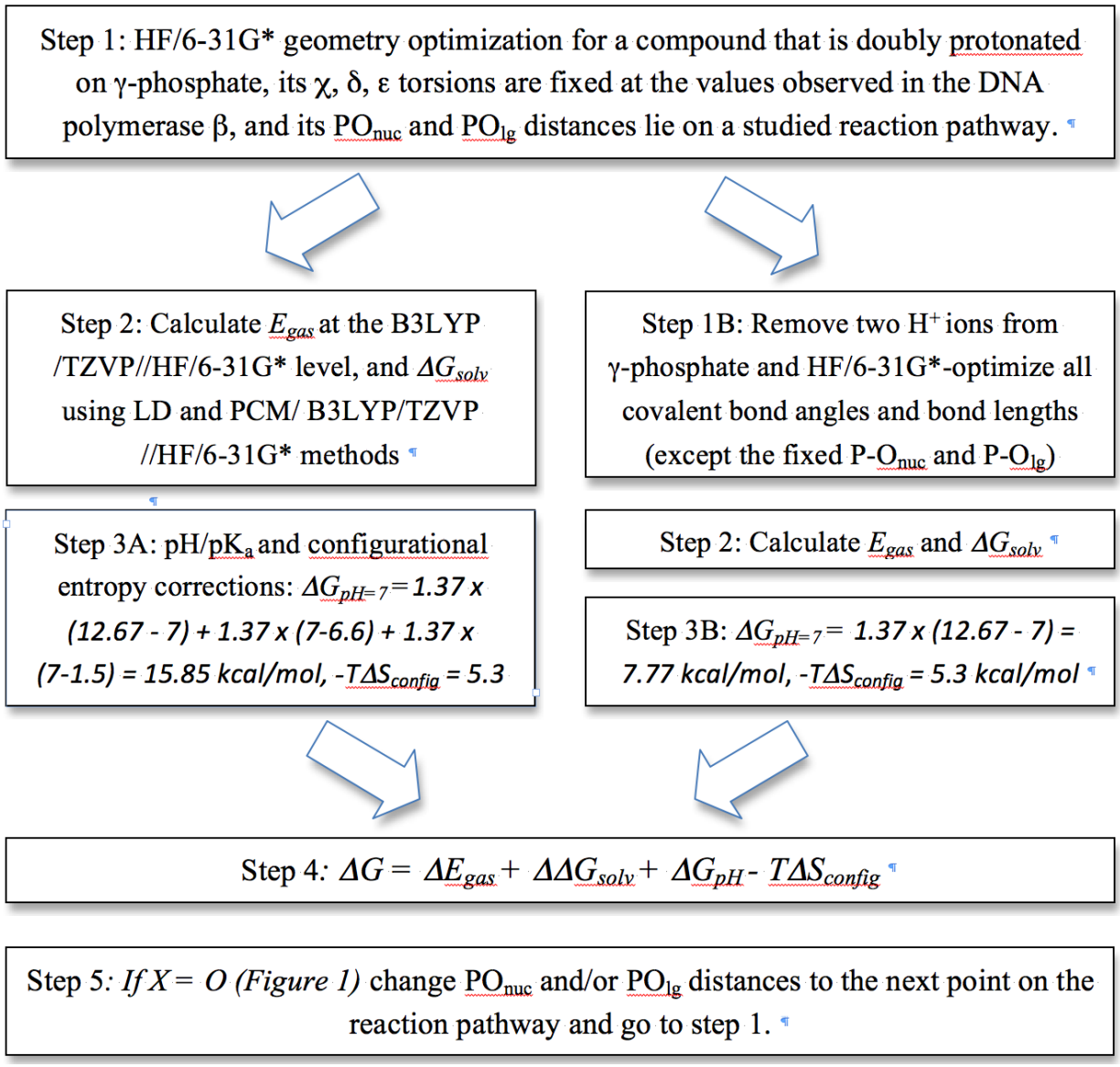

Figure 1S: Methodological flow chart for calculations of free energy surfaces for the reaction 3 using mechanisms 1 (steps 1, 2, 3A, 4, 5) and 2 (steps 1, 1B, 2, 3B, 4). The pKa corrections at pH 7 (steps 3A and 3B) are based on the experimental pK_a for the first protonation of γ-phosphate in deoxyadenosine triphosphate (6.6), for the second protonation of methyl triphosphate (1.6), and for dexyribose (12.67).

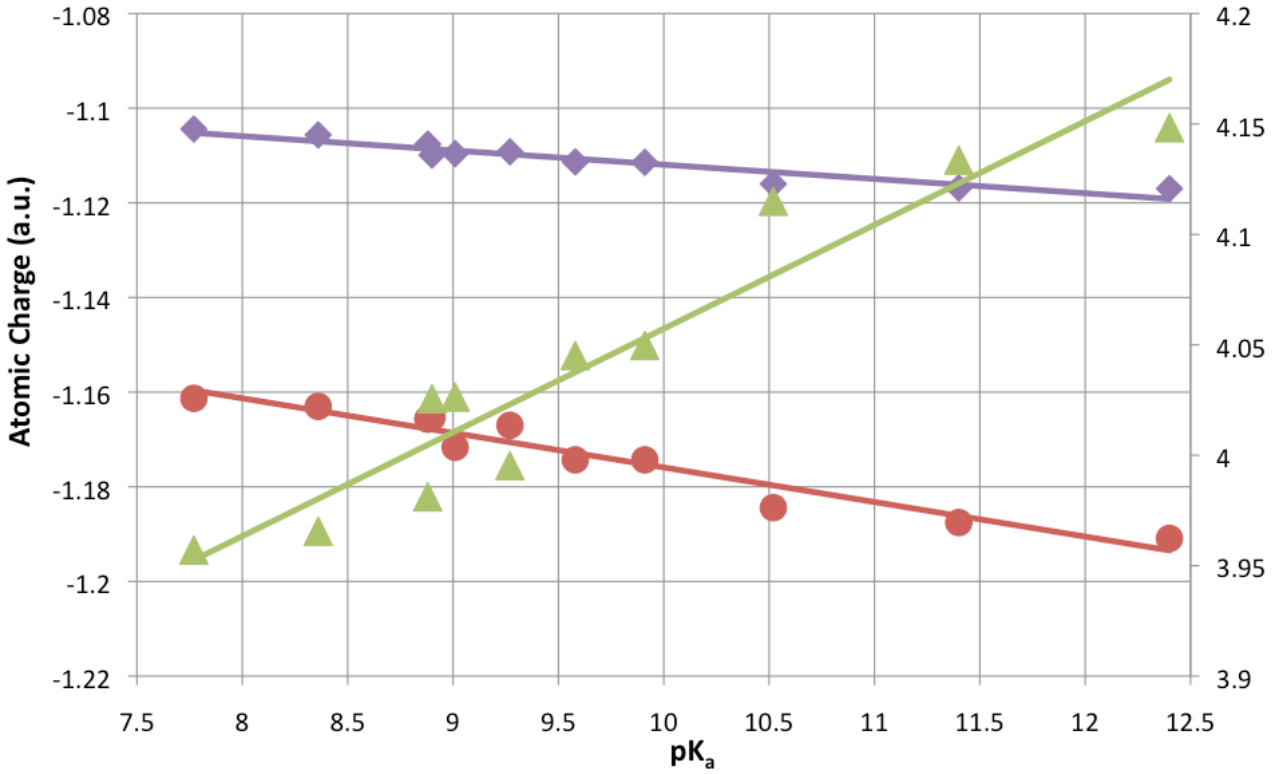

Figure 2S: Relationship between pK_a of the leaving group (PP_i or β–γ-substituted bisphosphonate) and the NBO charge of O_{lg} atom, average charge of O_{45}, or charge of P_β+Xtot+Pγ of methyl triphosphate complexes doubly protonated on the gamma phosphate (Figure 1). The purple diamonds (R=0.95, slope=-0.003), red circles (R=0.97, slope=-0.0073), and green triangles (R=0.95, slope=0.047) represent the O_{lg} charge, average of the charges of the two nonbridging O atoms on b-phosphate (O_{45}), and the charge on P_β, the bridging C (or O for parent O) with substituents and Pγ, for doubly protonated compounds, respectively. The value of the charge on P_β, the bridging C (or O for parent O) with substituents and Pγ correspond to the Y-axis on the right.

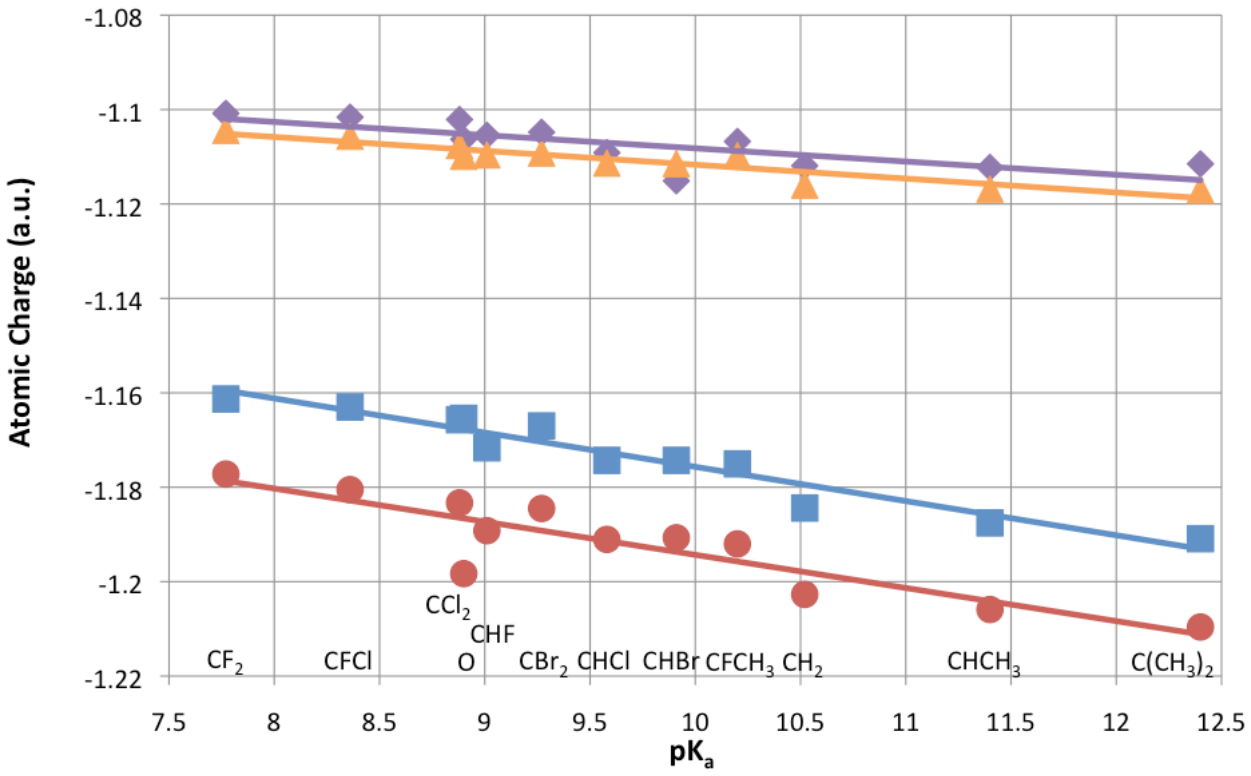

Figure 3S: Relationship between pK_a of the leaving group (PP_i or β–γ-substituted bisphosphonate) and the Mulliken charge of O_{lg} atom (purple and orange points), or average charge of O_{45} and atoms (blue and red points) of methyl triphosphate complexes. The purple diamonds (R=0.78, slope= -0.0028) and orange triangles (R=0.93, slope= -0.0029) represent O_{lg} charges for unprotonated and doubly protonated compounds, respectively. Red circles (R=0.89, slope=-0.007) and blue squares (R=0.96, slope=-0.0072) represent average of the charges of the two nonbridging O atoms on b-phosphate (O_{45}), for unprotonated and doubly protonated compounds, respectively.

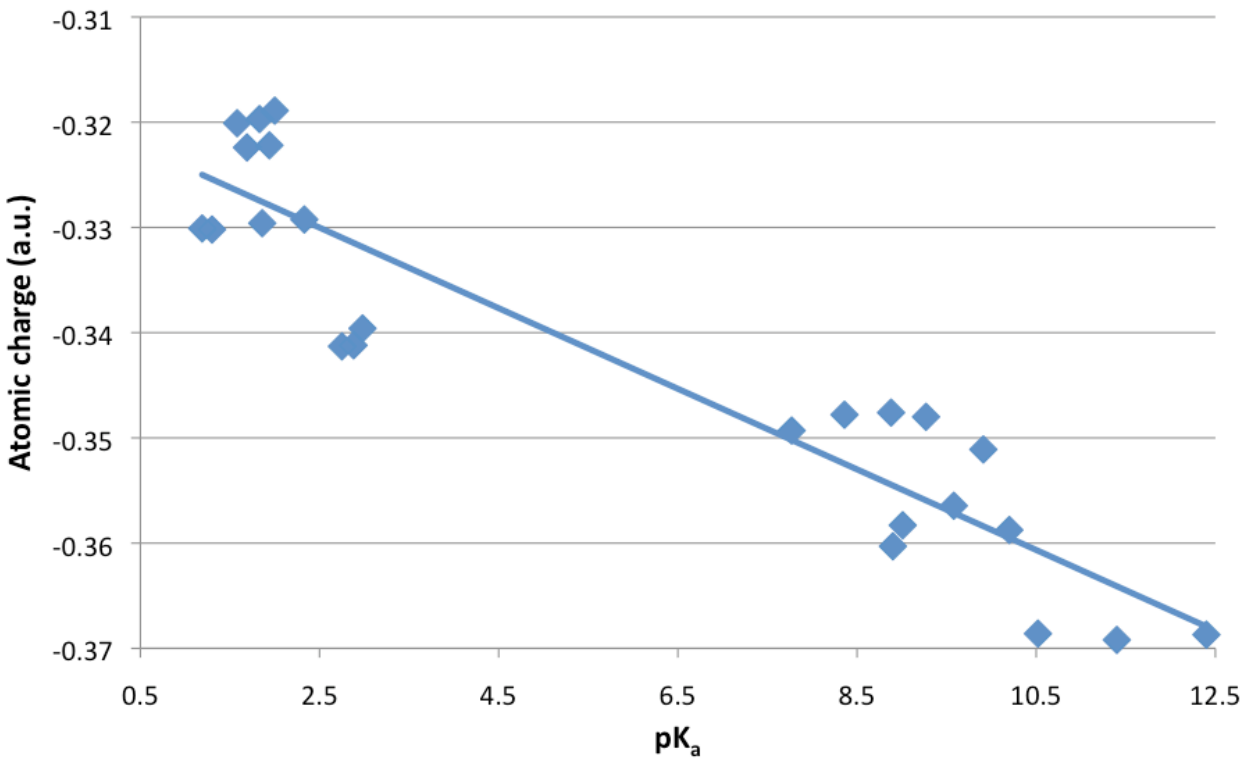

Figure 4S: Atomic charge in the reactant state on P_{α}, O_{12}, $-OCH_3$ (CH_3PO_3) vs pK_{a4} for unprotonated methyl triphosphated analogs and pK_{a2} for doubly protonated methyl triphosphated analogs (R=0.93, slope=-0.0038).

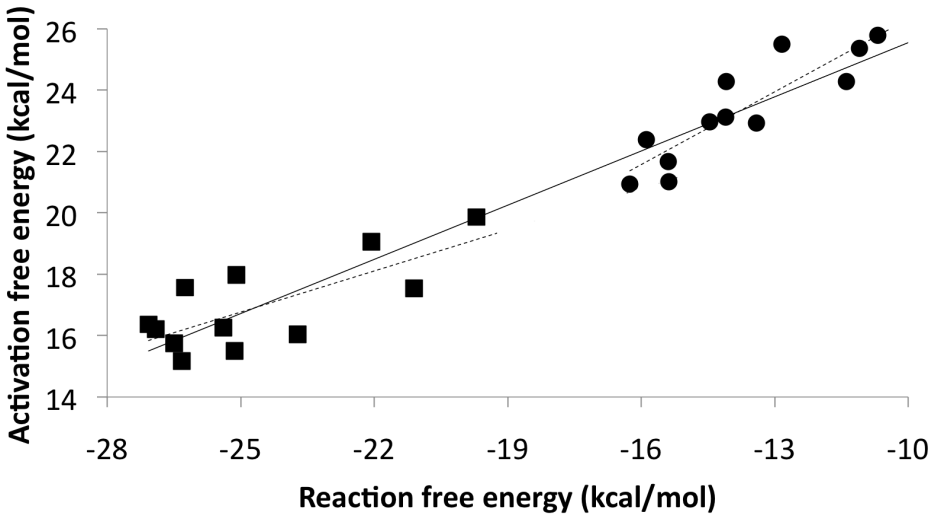

Figure 5S: Relationship between activation free energy and reaction free energy for the reactions 1 (squares) and 2 (circles). The activation free energies for the reaction 1 were evaluated for TS along the dissociative pathway (point D6 in Figure 3). The activation free energies for the reaction 2 were evaluated as the average of activation free energies for the synchronous and dissociative pathways (points S7 and D9 in Figure 3). The slope and correlation coefficient for the linear fit of all points (full line) are 0.59 and R = 0.97, respectively. Slopes for the lines fitted separately using the reaction 1 and reaction 2 points are 0.45 and 0.79, respectively.

Table 1S: Conformational Variability of the Triphosphate chain of dNTP substrates bound to DNA polymerase's active site.

	High resolution structures								
Torsions[j]	pol β/dCTP[a]	T7 pol/dGTP[b]	Taq pol/dCTP[c]	pol η/dACT[d]	BF pol/dCTP·A[e]	pol λ/dGTP·T[f]	dUTPase[i]		
Pα–O_{lg}–Pβ–X	-87.1	-72.7	-81.0	-121.9	-79.2	-127.4	-75.2		
O_{lg}–Pβ–X–Pγ	98.4	108.0	78.4	65.0	118.4	40.8	109.2		
Pβ–X–Pγ–O_6	98.0	93.4	127.8	127.5	97.5	143.9	97.7		
How inhibited	Removed O_{nuc}, Na^+ at catalytic site	Removed O_{nuc}	Removed O_{nuc}	Nitrogen at O3A position	2′,3′-dideoxy-nucleotide	Carbon at O3A position	Nitrogen at O3A position		
	Time resolved structures (time intervals)								
	pol η[g] 0 s	40 s	80 s	140 s	200 s	230 s	pol β[h] 0 s	10 s	20 s
Pα–O_{lg}–Pβ–X	-130.2	-135.3	-138.4	-137.4	-143.6	-137.8	-123.1	-139.0	-130.3
O_{lg}–Pβ–X–Pγ	77.7	76.6	74.9	72.9	74.1	73.5	86.0	70.4	69.8
Pβ–X–Pγ–O_6	113.1	111.3	117.9	116.7	115.2	117.2	122.3	124.5	127.3
How inhibited	Ca^{2+} instead of Mg^{2+}; Mg^{2+} added as reaction progresses.								

[a] PDB code 2FMP. Batra et al. (2006) Structure 14: 757-766.
[b] PDB code 1T7P. Doublie et al. (1998) Nature 391: 251-258.
[c] PDB code 3KTQ. Li et al. (1998) EMBO J. 17: 7514-7525.
[d] PDB code 3MR2. Biertumpfel et al. (2010) Nature 465: 1044-1048.
[e] PDB code 3PX6. Wang et al. (2011) Proc.Natl.Acad.Sci.USA 108:17644-17648.
[f] PDB code 3PML. Bebenek et al. (2011) Proc.Natl.Acad.SCI.USA 108: 1862-1867.
[g] PDB codes 4ECQ, 4ECR, 4ECS, 4ECT, 4ECU, 4ECV. Nakamura et al. (2012) Nature 487: 196-201.
[h] PDB codes 4KLD, 4KLE, 4KLF. Freudenthal et al. (2013) Cell 154: 157-168.
[i] PDB code 2XCE, Garcia-Nafria et al. (2010) Acta Cryst D66: 953-961.
[j] Figure 1

Table 2S: Variation of Mulliken charges of atoms and groups during model nucleotide transfer reactions in aqueous solution.

atom/group[b]	total charge[a] (a.u.)					
	R_∞	$R_{3.6}$	TS_A	TS_S	TS_D	P_∞
			Mechanism 2 [c]			
lg	-1.32	-1.41	-1.66	-1.73	-1.83	-2.00
O_{lg}	-0.47	-0.56	-0.58	-0.62	-0.68	-0.81
nuc	-1.00	-0.91	-0.66	-0.67	-0.71	-0.53
O_{nuc}	-0.99	-0.88	-0.72	-0.73	-0.76	-0.42
CH_3PO_3	-0.68	-0.68	-0.69	-0.60	-0.46	-0.47
P_α	0.93	1.00	1.09	1.10	1.10	0.89
P_β	0.89	0.93	0.90	0.90	0.89	0.89
P_γ	0.85	0.87	0.85	0.85	0.85	0.85
$\overline{O_{1,2}}$	-0.71	-0.71	-0.78	-0.76	-0.71	-0.71
$\overline{O_{4,5}}$	-0.72	-0.74	-0.77	-0.78	-0.79	-0.82
$\overline{O_{6,7,8}}$	-0.78	-0.80	-0.80	-0.81	-0.81	-0.81
Mg	1.44	1.46	1.56	1.56	1.56	1.56
			Mechanism 1 [d]			
lg	0.61	0.48	0.22	0.21	0.11	0.00
O_{lg}	-0.44	-0.50	-0.57	-0.57	-0.63	-0.71
nuc	-1.00	-0.87	-0.50	-0.64	-0.68	-0.60
O_{nuc}	-0.99	-0.90	-0.72	-0.73	-0.76	-0.42
CH_3PO_3	-0.61	-0.61	-0.62	-0.56	-0.42	-0.40
$\overline{O_{1,2}}$	-0.69	-0.69	-0.78	-0.74	-0.71	-0.70
Mg	1.44	1.44	1.66	1.56	1.56	1.56

[a] Atomic charge or the sum of atomic charges for the group of atoms. Mulliken atomic charges that were calculated at the PCM/B3LYP/TZVP//HF/6-31G* level. R_∞, $R_{3.6}$, P_∞, TS_A, TS_S, and TS_D

denote, respectively, infinitely separated reactants (i.e. $THFO^-$ and methyl triphosphate·Mg complex, Figure 1), reactants with the $O_{nuc} - P_\alpha$ distance of 3.6 Å, infinitely separated products (i.e. phosphate diester and PPi·Mg complex), and transition states along the associative, synchronous and dissociative pathways (Figure 3).

[b] The nucleophile (nuc) includes all atoms of the THFO moiety. The leaving group (lg) includes complex of Mg^{2+} ion with three water molecules and β- and γ-phosphate groups. CH_3PO_3 denotes 'Metaphosphate' moiety that consists of P_α, O_1, O_2, O_3 atoms and the methyl group. $\overline{O_{1,2}}$, $\overline{O_{4,5}}$ and $\overline{O_{6,7,8}}$ denote average of atomic charges of non-bridging oxygen atoms on the α-, β-, and γ-phosphate, respectively. For atom numbering, see Figure 1.

[c] See eq 2

[d] See eq 1.

Table 3S: Variation of ESP charges of atoms and groups during model nucleotide transfer reactions in aqueous solution.

atom/group[b]	total charge[a] (a.u.)					
	R_∞	$R_{3.6}$	TS_A	TS_S	TS_D	P_∞
			Mechanism 2 [c]			
lg	-1.35	-1.53	-1.85	-1.89	-2.00	-2.00
O_{lg}	-0.63	-0.74	-0.78	-0.82	-0.90	-1.03
nuc	-1.00	-0.93	-0.63	-0.65	-0.69	-0.71
O_{nuc}	-1.23	-1.17	-1.02	-1.03	-1.07	-0.69
CH_3PO_3	-0.65	-0.54	-0.52	-0.46	-0.31	-0.29
P_α	1.41	1.41	1.38	1.38	1.60	1.26
P_β	1.47	1.51	1.54	1.56	1.60	1.59
P_γ	1.57	1.49	1.44	1.44	1.44	1.41
$\overline{O_{1,2}}$	-0.89	-0.80	-0.80	-0.79	-0.76	-0.74
$\overline{O_{4,5}}$	-0.91	-0.95	-0.98	-0.99	-1.00	-1.01
$\overline{O_{6,7,8}}$	-1.00	-0.98	-0.98	-0.98	-0.99	-0.97
Mg	1.68	1.70	1.62	1.62	1.60	1.59
			Mechanism 1 [d]			
lg	0.57	0.36	0.17	0.16	0.11	0.00
O_{lg}	-0.63	-0.62	-0.61	-0.62	-0.65	-0.89
nuc	-1.00	-0.92	-0.45	-0.49	-0.53	-0.44
O_{nuc}	-1.23	-1.23	-0.86	-0.89	-0.92	-0.69
CH_3PO_3	-0.57	-0.45	-0.71	-0.68	-0.58	-0.56
$\overline{O_{1,2}}$	-0.89	-0.84	-0.78	-0.78	-0.73	-0.72
Mg	1.59	1.75	1.76	1.79	1.81	1.54

[a] Atomic charge or the sum of atomic charges for the group of atoms. ESP atomic charges that were calculated at the PCM/B3LYP/TZVP//HF/6-31G* level. R_∞, $R_{3.6}$, P_∞, TS_A, TS_S, and TS_D denote, respectively, infinitely separated reactants (i.e. $THFO^-$ and methyl triphosphate·Mg complex, Figure

1), reactants with the $O_{nuc} - P_\alpha$ distance of 3.6 Å, infinitely separated products (i.e. phosphate diester and PPi·Mg complex), and transition states along the associative, synchronous and dissociative pathways (Figure 3).

[b] The nucleophile (nuc) includes all atoms of the THFO moiety. The leaving group (lg) includes complex of Mg^{2+} ion with three water molecules and β- and γ-phosphate groups. CH_3PO_3 denotes 'Metaphosphate' moiety that consists of P_α, O_1, O_2, O_3 atoms and the methyl group. $\overline{O_{1,2}}$, $\overline{O_{4,5}}$ and $\overline{O_{6,7,8}}$ denote average of atomic charges of non-bridging oxygen atoms on the α-, β-, and γ-phosphate, respectively. For atom numbering, see Figure 1.

[c] See eq 2

[d] See eq 1.

Table 4S: Mulliken charges of atoms and groups of β–γ substituted [Mg·methyl triphosphate]$^{2-}$ complexes in aqueous solution

atom/group[b]	total charge[a] (a.u.)											
	CF_2	CFCl	CCl_2	O	CHF	CBr_2	CHCl	CHBr	$CFCH_3$	CH_2	$CHCH_3$	$C(CH_3)_2$
X	-0.03	-0.22	-0.41	-0.49	-0.32	-0.36	-0.55	-0.53	-0.18	-0.70	-0.52	-0.32
XA	-0.24	-0.23	-0.14	N/A	0.18	-0.15	0.24	0.23	-0.14	0.17	0.17	-0.03
XB	-0.24	-0.16	-0.13	N/A	-0.30	-0.15	-0.21	-0.23	-0.14	0.17	0.01	-0.03
Lg	-1.34	-1.35	-1.35	-1.32	-1.34	-1.35	-1.34	-1.33	-1.35	-1.32	-1.33	-1.33
O_{lg}	-0.46	-0.46	-0.47	-0.47	-0.48	-0.47	-0.48	-0.47	-0.48	-0.49	-0.49	-0.49
CH_3PO_3	-0.66	-0.65	-0.65	-0.68	-0.66	-0.65	-0.66	-0.67	-0.65	-0.68	-0.67	-0.67
P_α	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.94
P_β	0.87	0.91	0.93	0.89	0.90	0.93	0.94	0.94	0.89	0.93	0.93	0.92
P_γ	0.83	0.88	0.91	0.85	0.85	0.89	0.89	0.89	0.87	0.88	0.88	0.88
$\overline{O_{1,2}}$	-0.70	-0.70	-0.70	-0.71	-0.70	-0.69	-0.70	-0.70	-0.70	-0.71	-0.71	-0.71
$\overline{O_{4,5}}$	-0.70	-0.78	-0.70	-0.72	-0.72	-0.70	-0.72	-0.72	-0.72	-0.74	-0.74	-0.74
$\overline{O_{6,7,8}}$	-0.78	-0.78	-0.77	-0.78	-0.80	-0.77	-0.80	-0.79	-0.80	-0.82	-0.81	-0.81
Mg	1.44	1.44	1.44	1.44	1.45	1.44	1.44	1.43	1.44	1.45	1.44	1.44

X = O, CHF, CHCl, CHBr, CFCl, CFCl, $CHCH_3$, $CFCH_3$, CH_2, CF_2, CCl_2, CBr_2, $C(CH_3)_2$; arrange on horizontal (wide) page format; start with O and then order the substituents according to their pKa; for the bridging group use X, XA, XB abbreviations in the left column (e.g. X = C and XA=Cl and XB = no value for CCl2, for XA provide average over the two Cl atoms)

Table 5S: Atom and group charges at TS_S for the mechanism 2.

atom/group[b]	total charge[a] (a.u.) for Associaitve											
	CF_2	CFCl	CCl_2	O	CHF	CBr_2	CHCl	CHBr	$CFCH_3$	CH_2	$CHCH_3$	$C(CH_3)_2$
X	-0.05	-0.24	-0.42	-0.52	-0.34	-0.37	-0.57	-0.54	-0.19	-0.72	-0.55	-0.33
XA	-0.25	-0.24	-0.17	N/A	0.17	-0.17	0.23	0.22	-0.31	0.16	0.16	-0.04
XB	-0.24	-0.20	-0.15	N/A	-0.31	-0.19	-0.24	-0.25	0.01	0.16	-0.03	-0.06
lg	-1.68	-1.70	-1.68	-1.66	-1.68	-1.71	-1.68	-1.68	-1.69	-1.66	-1.67	-1.68
O_{lg}	-0.57	-0.57	-0.57	-0.58	-0.59	-0.57	-0.59	-0.59	-0.60	-0.61	-0.61	-0.61
Nuc	-0.66	-0.62	-0.65	-0.66	-0.65	-0.61	-0.65	-0.66	-0.65	-0.66	-0.66	-0.66
O_{nuc}	-0.72	-0.70	-0.71	-0.72	-0.72	-0.70	-0.72	-0.72	-0.71	-0.72	-0.71	-0.71
CH_3PO_3	-0.67	-0.68	-0.67	-0.69	-0.67	-0.68	-0.67	-0.67	-0.66	-0.68	-0.68	-0.66
P_α	1.10	1.01	1.10	1.09	1.10	1.06	1.10	1.11	1.09	1.10	1.11	1.11
P_β	0.88	0.91	0.95	0.90	0.92	0.92	0.97	0.95	0.93	0.96	0.96	0.95
P_γ	0.83	0.88	0.91	0.85	0.85	0.90	0.90	0.89	0.86	0.88	0.88	0.87
$\overline{O_{1,2}}$	-0.78	-0.76	-0.78	-0.78	-0.78	-0.79	-0.78	-0.78	-0.78	-0.78	-0.78	-0.77
$\overline{O_{4,5}}$	-0.75	-0.74	-0.75	-0.77	-0.77	-0.75	-0.77	-0.77	-0.78	-0.79	-0.80	-0.79
$\overline{O_{6,7,8}}$	-0.80	-0.79	-0.79	-0.80	-0.82	-0.78	-0.815	-0.815	-0.82	-0.84	-0.84	-0.83
Mg	1.55	1.51	1.55	1.56	1.54	1.55	1.545	1.55	1.54	1.55	1.54	1.54

atom/group[b]	total charge[a] (a.u.) for Synchronous											
	CF_2	CFCl	CCl_2	O	CHF	CBr_2	CHCl	CHBr	$CFCH_3$	CH_2	$CHCH_3$	$C(CH_3)_2$
X	-0.06	-0.24	-0.42	-0.52	-0.35	-0.36	-0.58	-0.54	-0.19	-0.73	-0.55	-0.32
XA	-0.25	-0.24	-0.17	N/A	0.17	-0.18	0.23	0.22	-0.32	0.16	0.16	-0.05
XB	-0.25	-0.20	-0.15	N/A	-0.31	-0.18	-0.24	-0.26	0.01	0.15	-0.04	-0.06
lg	-1.75	-1.75	-1.75	-1.73	-1.75	-1.79	-1.74	-1.75	-1.76	-1.73	-1.74	-1.75
O_{lg}	-0.62	-0.61	-0.61	-0.62	-0.64	-0.59	-0.63	-0.63	-0.63	-0.65	-0.66	-0.65
Nuc	-0.66	-0.67	-0.66	-0.67	-0.67	-0.61	-0.67	-0.67	-0.67	-0.67	-0.67	-0.67
O_{nuc}	-0.73	-0.73	-0.73	-0.73	-0.73	-0.70	-0.73	-0.73	-0.73	-0.73	-0.73	-0.73
CH_3PO_3	-0.58	-0.58	-0.58	-0.60	-0.59	-0.60	-0.59	-0.59	-0.58	-0.60	-0.59	-0.58
P_α	1.11	1.11	1.11	1.10	1.11	0.92	1.11	1.11	1.10	1.11	1.12	1.11
P_β	0.89	0.93	0.95	0.90	0.92	0.90	0.96	0.95	0.92	0.95	0.96	0.95
P_γ	0.83	0.88	0.91	0.85	0.85	0.90	0.89	0.89	0.86	0.88	0.88	0.87
$\overline{O_{1,2}}$	-0.75	-0.76	-0.76	-0.76	-0.76	-0.71	-0.76	-0.76	-0.76	-0.76	-0.76	-0.75
$\overline{O_{4,5}}$	-0.76	-0.76	-0.76	-0.78	-0.78	-0.74	-0.78	-0.78	-0.79	-0.80	-0.80	-0.80
$\overline{O_{6,7,8}}$	-0.80	-0.80	-0.79	-0.81	-0.82	-0.78	-0.82	-0.82	-0.82	-0.84	-0.84	-0.83
Mg	1.55	1.55	1.55	1.56	1.55	1.47	1.55	1.55	1.54	1.56	1.545	1.54

atom/group[b]	total charge[a] (a.u.) for Dissociative											
	CF2	CFCl	CCl2	O	CHF	CBr2	CHCl	CHBr	$CFCH_3$	CH2	$CHCH_3$	$C(CH_3)_2$
X	-0.06	-0.24	-0.42	-0.52	-0.35	-0.37	-0.58	-0.55	-0.21	-0.73	-0.55	-0.33
XA	-0.25	-0.24	-0.17	N/A	0.17	-0.19	0.23	0.22	-0.32	0.15	0.16	-0.05
XB	-0.25	-0.20	-0.16	N/A	-0.31	-0.18	-0.24	-0.26	-0.01	0.15	-0.04	-0.06
lg	-1.85	-1.85	-1.86	-1.83	-1.85	-1.86	-1.85	-1.85	-1.85	-1.83	-1.84	-1.84
O_{lg}	-0.67	-0.68	-0.67	-0.68	-0.70	-0.67	-0.69	-0.69	-0.69	-0.71	-0.71	-0.70
Nuc	-0.70	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71
O_{nuc}	-0.76	-0.76	-0.77	-0.76	-0.76	-0.76	-0.77	-0.77	-0.76	-0.76	-0.76	-0.76
CH_3PO_3	-0.44	-0.44	-0.44	-0.46	-0.45	-0.44	-0.45	-0.44	-0.45	-0.46	-0.46	-0.44
P_α	1.11	1.12	1.12	1.10	1.12	1.12	1.12	1.12	1.12	1.11	1.11	1.11
P_β	0.89	0.94	0.95	0.89	0.92	0.95	0.96	0.96	0.94	0.95	0.96	0.95
P_γ	0.83	0.87	0.90	0.85	0.85	0.89	0.89	0.89	0.86	0.88	0.88	0.87
$\overline{O_{1,2}}$	-0.71	-0.71	-0.71	-0.71	-0.72	-0.71	-0.71	-0.71	-0.71	-0.71	-0.71	-0.70
$\overline{O_{4,5}}$	-0.78	-0.78	-0.77	-0.79	-0.80	-0.77	-0.80	-0.80	-0.80	-0.81	-0.82	-0.81
$\overline{O_{6,7,8}}$	-0.80	-0.80	-0.79	-0.81	-0.82	-0.79	-0.82	-0.82	-0.82	-0.84	-0.84	-0.83
Mg	1.55	1.55	1.55	1.56	1.545	1.55	1.55	1.55	1.55	1.56	1.55	1.54

X = O, CHF, CHCl, CHBr, CFCl, CFCl, $CHCH_3$, $CFCH_3$, CH_2, CF_2, CCl_2, CBr_2, $C(CH_3)_2$; arrange on horizontal (wide) page format; start with O and then order the substituents according to their pKa; for the bridging group use X, XA, XB abbreviations in the left column (e.g. X = C and XA=Cl and XB = no value for CCl2, for XA provide average over the two Cl atoms)

Table 6S: Atom and group charges for $[PPi{\cdot}Mg{\cdot}3H2O]^{2-}$ complex with the bridging oxygen replaced by various functional groups

atom/group[b]	total charge[a] (a.u.) for pyrophosphate											
	CF2	CFCl	CCl2	O	CHF	CBr2	CHCl	CHBr	$CFCH_3$	CH2	$CHCH_3$	$C(CH_3)_2$
X	-0.07	-0.25	-0.44	-0.55	-0.35	-0.38	-0.61	-0.57	-0.22	-0.77	-0.58	-0.35
XA	-0.25	-0.25	-0.18	N/A	0.20	-0.19	0.26	0.25	-0.33	0.17	0.19	-0.06
XB	-0.26	-0.21	-0.18	N/A	-0.33	-0.20	-0.27	-0.29	-0.01	0.17	-0.05	-0.06
lg	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00
O_{lg}	-0.80	-0.77	-0.75	-0.81	-0.83	-0.78	-0.82	-0.81	-0.79	-0.84	-0.84	-0.82
P_{β}	0.88	0.91	0.92	0.89	0.87	0.95	0.95	0.95	0.90	0.94	0.94	0.94
P_{γ}	0.85	0.89	0.90	0.85	0.86	0.91	0.92	0.91	0.87	0.91	0.90	0.89
$\overline{O_{4,5}}$	-0.81	-0.81	-0.80	-0.82	-0.81	-0.81	-0.83	-0.83	-0.83	-0.85	-0.85	-0.85
$\overline{O_{6,7,8}}$	-0.81	-0.80	-0.79	-0.81	-0.82	-0.79	-0.82	-0.82	-0.82	-0.85	-0.85	-0.84
Mg	1.56	1.57	1.58	1.56	1.50	1.57	1.56	1.57	1.57	1.56	1.56	1.55

Table 7S: NBO Atomic Charge vs pK_a and charge difference vs pK_a correlations for substituent compounds.

	Mechanism 2		Mechanism 1	
	Slope	R^2	*Slope*	R^2
	RS			
O_{12}	-0.0017	0.5483	-0.0017	0.5825
O_{45}	-0.0077	0.814	-0.0078	0.9421
O_{lg}	-0.0032	0.6738	-0.0033	0.9385
CH_3PO_3	-0.0056	0.6605	-0.006	0.7516
$P_\beta+X_{tot}+P_\gamma$	0.0529	0.8275	0.0504	0.9275
	$TS_{AVE}(S+D)$		*TS (D)*	
O_{12}	-0.0007	0.2836	-0.0003	0.0909
O_{45}	-0.0074	0.7835	-0.0086	0.8694
O_{lg}	-0.0058	0.706	-0.0062	0.7952
CH_3PO_3	-0.0025	0.4268	-0.0025	0.4346
$P_\beta+X_{tot}+P_\gamma$	0.0536	0.7796	0.0532	0.9058
	$TS_{AVE}(S+D)$-RS[a]		*TS(D)-RS*	
O_{12}	0.001	0.4283	0.0014	0.6516
O_{45}	0.0003	0.0362	-0.0002	0.0373
O_{lg}	-0.0026	0.331	-0.003	0.7973
CH_3PO_3	0.0031	0.7073	0.0042	0.8697
$P_\beta+X_{tot}+P_\gamma$	0.001	0.0378	0.0014	0.1564

[a]: Charge difference between TS and the reactant state, $\Delta Q = Q(TS) - Q(RS)$. Note that for mechanism 2, the TS was averaged over the TS value for synchronous and dissociative pathway as both pathways provide similar activation free energies. For mechanism 1, the TS was evaluated only for the dissociative pathway.

Table 8S: Calculated gas-phase reaction energies ($\Delta E_{gas,0}$), reaction free energies in aqueous solution ($\Delta G_{r,0}$), gas-phase activation free energies ($\Delta E_{gas}^{\ddagger}$), and activation free energies in aqueous solution ($\Delta G^{\ddagger}$) for reaction 1 involving β–γ substituted methyl triphosphate analogs.[a]

X	Enant[c]	pKa_4	ΔE_{gas}	ΔG_r	$\Delta E_{gas}^{\neq}$	$\Delta G^{\neq}$
			kcal/mol			
CF_2		7.8	-12.8	-26.5	-29.4	15.8
CFCl	S	8.4	-14.2	-26.2	-30.3	15.8
CClF	R	8.4	-12.8	-26.5	-30.0	14.5
CCl_2		8.8	-12.6	-26.3	-29.0	17.6
O		8.9	-15.9	-27.1	-27.2	16.4
CHF	S	9.0	-13.1	-24.3	-25.4	15.5
CFH	R	9.0	-11.8	-26.0	-28.4	15.5
CBr_2		9.3	-12.5	-23.5	-30.2	16.2
CHCl	S	9.5	-14.9	-23.7	-23.9	21.1
CClH	R	9.5	-12.7	-27.1	-25.2	11.4
CHBr	S	9.9	-13.5	-24.0	-25.6	20.7
CBrH	R	9.9	-12.4	-26.2	-28.5	15.2
$CFCH_3$	R	10.2	-10.6	-26.9	-25.1	15.9
CCH_3F	S	10.2	-11.9	-23.7	-24.9	16.2
CH_2		10.5	-12.6	-22.1	-23.9	19.1
$CHCH_3$	S	11.6	-12.1	-21.3	-22.8	18.6
CCH_3H	R	11.6	-11.8	-20.9	-25.8	16.5
$C(CH_3)_2$		12.3	-11.5	-19.7	-23.8	19.9
Slope[c]			0.48	1.74	1.51	0.86
R[c]			0.51	0.93	0.81	0.76

[a] Note that the data in this table utilize the reactant and product protonation states shown in eq 1 (reaction 1) to evaluate energy differences.

[b] The R- or S-enantiomers. Atoms bound to the chiral center (bridging C) are ordered with the lowest atomic number atom pointing away and the remaining atoms from P_β to P_γ to the remaining substitutent are ordered clockwise in the R-form. This means that when looking from P_β to bridging C to P_γ, the heavier substituent is on the left in the *R*-form.

[c] Slope and R denote slope of the linear interpolation of energy as a function of pK_a and the correlation coefficient, respectively. Enantiomer energies were averaged.