Transition states of uncatalyzed hydrolysis and aminolysis reactions of a ribosomal P-site substrate determined by kinetic isotope effects†

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Supporting Information

Calculation of Hydrolysis Transition State

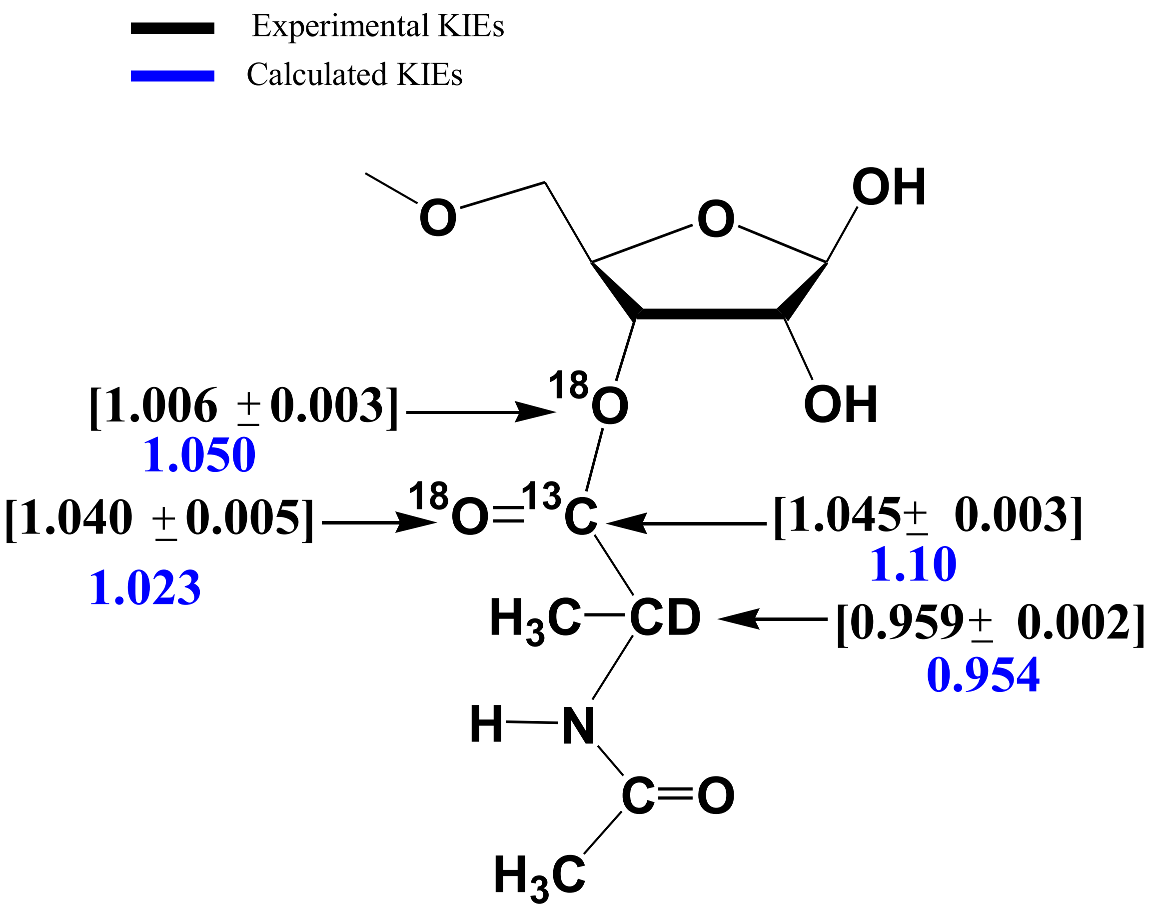
The transition state of the hydrolysis reaction was determined using the model shown in Figure 1b. The initial *ab initio* transition state for the hydrolysis reaction was modeled using hydroxyl as a nucleophile without the application of any external constraints. The optimization gave a transition state structure that has a single imaginary frequency of 249i cm^{-1} corresponding to the reaction coordinate (Supplementary Table 1). The transition state structure corresponding to this imaginary frequency had partial bond orders to the nucleophile as well as to the 3'-oxygen. However, the kinetic isotope effects calculated for this transition state structure were significantly different than the KIEs measured experimentally, suggesting that the transition state in solution is different than the one predicted *in vacuo* by Gaussian03 (Supplementary Figure 1). The small 3'-oxygen-18 experimental KIE of 0.6% for the hydrolysis reaction suggests that the O3'-C bond is fully intact at the transition state and a large oxygen-18 experimental KIE of 4.0% for the carbonyl oxygen indicates full bond order to the nucleophile. No cleavage of the 3'O-C bond and full bonding to the nucleophile suggests that transition state of the hydrolysis reaction resembles a tetrahedral intermediate.

Calculation of Hydroxylaminolysis Transition State

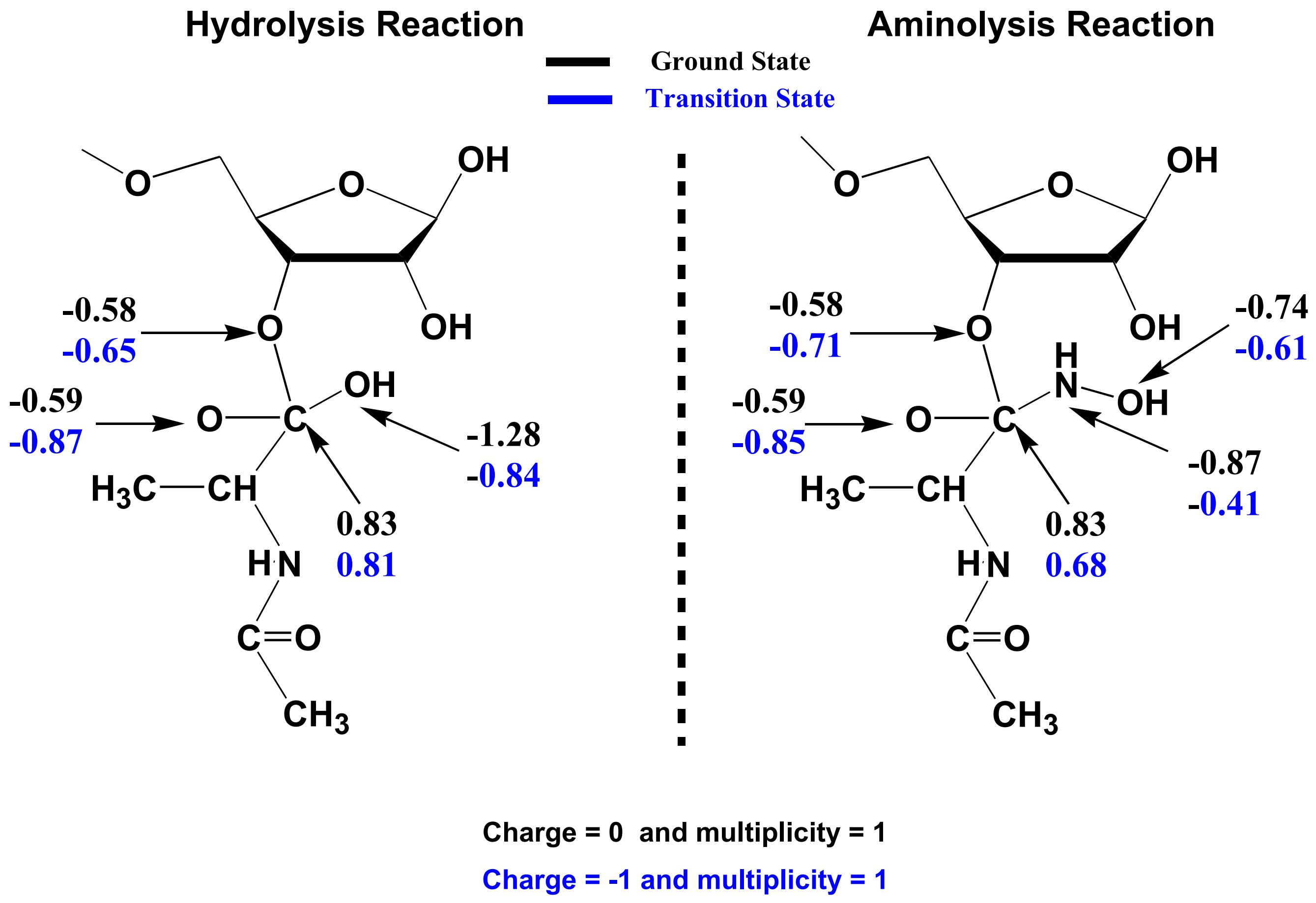
Modeling of the transition state for the aminolysis reaction was initiated by first matching the 3'-oxygen-18 KIE by increasing the 3'O-C bond length. In the absence of nucleophile, the 3'-oxygen-18 KIE of 2.9%

corresponds to a 3'-O-C bond length of 1.55 Å compared with 1.36 Å in the ground state. Matching the 3'-oxygen-18 KIE in the absence of nucleophile however gave a normal carbonyl carbon-13 KIE of 2.1% which was less than the experimental KIE of 2.7% measured experimentally, indicating that some contribution to the carbon-13 KIE comes from increased bonding with the hydroxylamine nucleophile. Also, cleavage of the 3'O-C bond gave a small inverse isotope effect of 0.2% at the carbonyl oxygen due to the strengthening of the carbonyl bond as a result of partial cleavage of the 3'O-C bond. This is significantly different from the large normal KIE of 3.7% measured experimentally, suggesting the carbonyl oxygen-18 KIE must come entirely from increased bonding between the primary carbon and the nucleophile. Subsequent calculations were performed by increasing the bond order between the primary carbon and the nucleophile in small iterative steps until a close match was obtained between the calculated and experimental carbonyl oxygen-18 KIE. The experimental carbonyl oxygen-18 KIE of 3.7% corresponds to a C-N bond length of 1.33 Å suggesting a full bond order between the carbonyl carbon and the hydroxylamine nucleophile. At this step the 3'-O-C bond length was slightly adjusted to 1.64 Å to account for the small contribution to the 3'-oxygen-18 KIE that comes from bond formation with the nucleophile. The carbonyl oxygen-18 KIE of 3.7% corresponds to a carbonyl bond length of 1.37 Å at the transition state relative to 1.21 Å in the ground state. Furthermore, correlating 3'-oxygen-18 and carbonyl oxygen-18 KIES gave a primary carbon-13 KIE of 3.0% which is within the error of the value of 2.7% measured experimentally. The transition state model generated by correlating the 3'-oxygen-18, carbonyl oxygen-18, and carbonyl carbon-13 KIEs gave an α-deuterium KIE of inverse 1.9%, less inverse than the KIE of 3.9% measured experimentally. This suggests some portion of the isotope effect is contributed by the conformational change around the C-H bond influencing its out-of-plane bending modes. Adjusting the C^{methyl}-C^{sec}-N^{amide}-C^{amide} torsional angle from -64.3° in the substrate to -62.0° in the transition state gave a secondary deuterium isotope effect of inverse 3.6%, close to the experimental value.

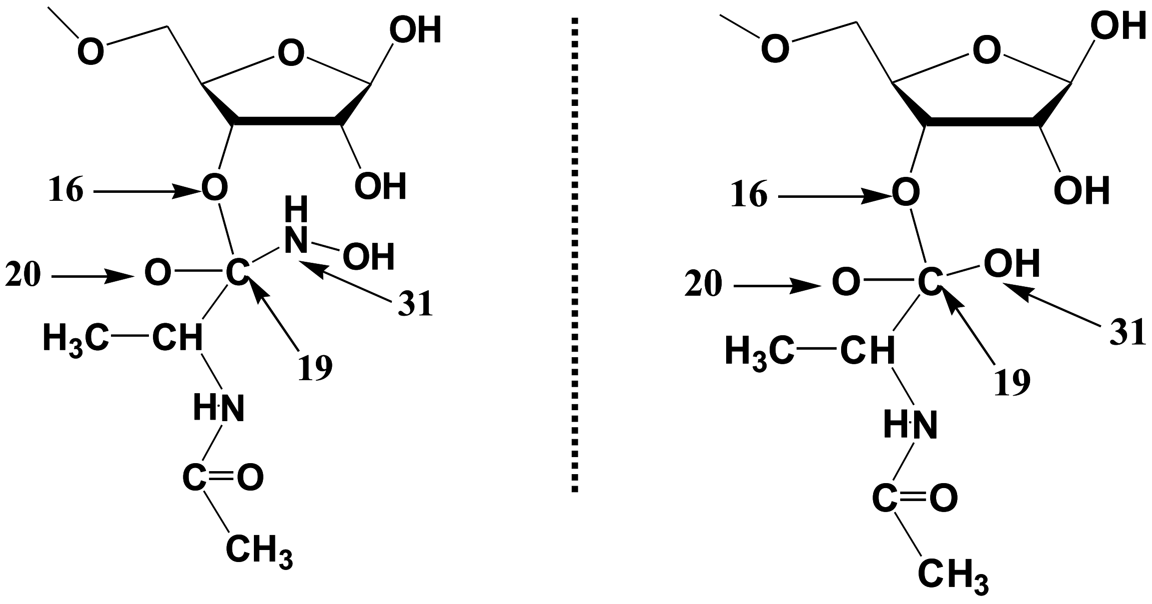
Supplementary Figure 1. Kinetic isotope effects for the hydrolysis transition state calculated without the application of any external constraints and their comparison to the experimental values.



Supplementary Figure 2. Comparison of distribution of natural charges between the ground state and the transition state for the hydrolysis and aminolysis reactions, as calculated using Natural Bond Orbital (NBO) analysis at B3LYP/6-31G** using Gaussian 03.



Supplementary Figure 3. Numbering scheme of atoms used in the Gaussian calculations.



Aminolysis **Hydrolysis**

Supplementary Table 1. Imaginary mode for the unrestrained transition state for the hydrolysis reaction.

Frequencies -- -249.4518
Red. masses -- 7.7138
Frc consts -- 0.2828
IR Inten -- 86.6213

Atom	AN	X	Y	Z	
1	6	0.04	0.01	0.00	
2	1	0.03	0.00	0.00	
3	1	0.03	0.02	0.00	
4	6	0.08	0.02	-0.01	
5	1	0.08	0.02	-0.01	
6	8	0.06	0.02	0.00	
7	6	0.04	0.02	-0.01	
8	1	0.05	0.02	-0.01	
9	8	0.03	0.02	0.00	
10	6	0.06	0.02	-0.04	
11	1	0.06	0.00	-0.05	
12	6	0.06	0.00	0.01	
13	1	0.10	-0.01	0.02	
14	8	0.00	-0.01	0.04	
15	1	-0.30	-0.22	0.02	
16	8	0.21	0.05	-0.10	(3'-Oxygen)
17	8	0.04	-0.01	0.00	
18	1	0.03	0.01	0.00	
19	6	-0.33	0.19	0.39	(Carbonyl Carbon or Primary Carbon)
20	8	-0.08	-0.01	0.01	(Carbonyl Oxygen)
21	6	-0.04	0.03	0.07	
22	1	0.12	-0.02	0.16	
23	6	0.00	-0.01	0.03	
24	1	0.06	-0.06	-0.04	
25	1	-0.08	0.04	-0.03	
26	7	-0.12	0.00	-0.08	
27	1	-0.27	0.03	-0.23	
28	6	-0.06	0.01	-0.01	
29	8	0.00	0.02	0.06	
30	1	0.04	-0.03	0.11	
31	6	0.02	-0.02	0.00	(Hydroxyl Nucleophile)
32	1	0.01	-0.02	0.00	
33	1	0.01	-0.02	0.00	
34	1	0.03	-0.03	0.01	
35	8	-0.01	-0.24	-0.24	
36	1	-0.01	-0.22	-0.19	
37	6	-0.05	0.00	0.01	
38	1	-0.06	0.00	0.03	
39	1	-0.03	0.00	0.00	
40	1	-0.05	0.00	0.02	

Supplementary Table 2. Coordinates for the hydrolysis transition state.

Center	Atomic	Atomic	Coordinates (Angstroms)		
Number	Number	Type	X	Y	Z
1	6	0	0.295568	1.816471	1.489339
2	1	0	1.332399	1.811321	1.138256
3	1	0	-0.284919	2.412281	0.771912
4	6	0	-0.225902	0.358525	1.549033
5	1	0	0.608562	-0.330550	1.442969
6	8	0	-0.872479	0.060163	2.810578
7	6	0	-2.254185	0.233513	2.637335
8	1	0	-2.758658	-0.318256	3.441405
9	8	0	-2.599221	1.613977	2.669613
10	6	0	-1.290031	0.066714	0.457758
11	1	0	-1.476098	0.950850	-0.154404
12	6	0	-2.563875	-0.336475	1.249287
13	1	0	-3.468061	0.125881	0.829819
14	8	0	-2.716613	-1.744244	1.331047
15	1	0	-2.134449	-2.092000	0.635090
16	8	0	-0.929219	-1.007686	-0.395318
17	8	0	0.208716	2.431978	2.784959
18	1	0	-1.804889	2.051096	3.026735
19	6	0	0.017878	-0.600032	-1.380357
20	8	0	0.965246	0.320097	-0.946820
21	6	0	0.572010	-1.876482	-2.054317
22	1	0	-0.281717	-2.315266	-2.597945
23	7	0	1.497581	-1.473444	-3.133441
24	1	0	0.938415	-0.851277	-3.715077
25	6	0	2.675226	-0.798022	-2.768122
26	8	0	3.506463	-1.267389	-2.005156
27	6	0	0.737239	3.736689	2.774641
28	1	0	1.803787	3.742147	2.498725
29	1	0	0.204987	4.395802	2.068552
30	1	0	0.632365	4.144676	3.784986

31	8	0	-0.865990	0.088655	-2.529493
32	1	0	-1.583377	-0.547986	-2.673489
33	6	0	2.893755	0.522847	-3.488224
34	1	0	2.611858	0.472817	-4.546734
35	1	0	2.244064	1.243868	-2.981601
36	1	0	3.938895	0.824337	-3.397368
37	6	0	1.098486	-3.027405	-1.200225
38	1	0	0.313307	-3.378610	-0.524462
39	1	0	1.403753	-3.849324	-1.854860
40	1	0	1.964470	-2.720167	-0.610594

Applied Constraints

16 19 21 23 F

19 21 23 25 F

6 4 1 17 F

19 20 1.390 F

19 31 1.605 F

16 19 1.426 F

20 19 31 32 -171.00 F

37 21 23 25 -71.5 F

Supplementary Table 3. Largest Imaginary mode for the aminolysis reaction (there are more imaginary modes smaller than -50 cm^{-1}).

Frequencies -- -414.1875
Red. Masses -- 1.9292
Frc consts -- 0.1950
IR Intensity -- 154.6929

Atom	AN	X	Y	Z
1	6	0.00	0.00	0.00
2	1	0.00	0.00	0.00
3	1	-0.01	0.00	0.00
4	6	-0.01	0.00	0.00
5	1	-0.01	0.00	0.00
6	8	0.00	0.00	0.00
7	6	0.00	0.00	0.00
8	1	-0.01	0.00	0.00
9	8	0.00	0.00	0.00
10	6	0.00	0.01	0.01
11	1	0.05	0.02	0.00
12	6	-0.01	0.00	0.00
13	1	0.00	-0.01	0.00
14	8	0.00	0.00	-0.01
15	1	0.00	-0.03	0.00
16	8	-0.02	0.00	0.07
17	8	0.00	0.00	0.00
18	1	0.00	0.00	0.00
19	6	0.03	-0.02	-0.01
20	8	0.04	-0.01	-0.04
21	6	0.00	0.00	0.00
22	1	0.00	0.00	0.00
23	7	0.00	0.00	0.00
24	1	0.05	0.03	0.04
25	6	0.00	0.00	0.00
26	8	0.00	0.00	0.00
27	6	0.00	0.00	0.00
28	1	0.00	0.00	0.00
29	1	0.00	0.00	0.00
30	1	0.00	0.00	0.00
31	7	-0.13	0.18	0.01
32	1	0.56	-0.71	-0.08
33	6	0.00	0.00	0.00
34	1	0.00	0.00	0.00
35	1	0.00	-0.01	0.00
36	1	0.01	0.00	0.00
37	8	0.05	-0.08	-0.05
38	1	0.15	-0.13	0.25
39	6	0.00	0.00	0.00
40	1	0.00	0.00	-0.01
41	1	0.00	0.00	0.00
42	1	0.00	0.00	0.00

Supplementary Table 4. Coordinates for the aminolysis transition state.

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---------------------------------------------------------------------
 Center     Atomic      Atomic              Coordinates (Angstroms)
 Number     Number       Type              X           Y           Z
 ---------------------------------------------------------------------
    1          6           0        -0.726499    2.349559    0.407470
    2          1           0        -0.666962    2.320433    1.502805
    3          1           0         0.304620    2.349768    0.038553
    4          6           0        -1.477745    1.098810   -0.116962
    5          1           0        -1.630538    0.406228    0.708222
    6          8           0        -2.779355    1.443658   -0.653611
    7          6           0        -2.717332    1.424256   -2.059662
    8          1           0        -3.737488    1.255430   -2.429466
    9          8           0        -2.211945    2.653709   -2.568981
   10          6           0        -0.724261    0.353433   -1.262964
   11          1           0         0.126449    0.970599   -1.595588
   12          6           0        -1.767525    0.280892   -2.415654
   13          1           0        -1.306791    0.455241   -3.399226
   14          8           0        -2.448713   -0.960503   -2.387976
   15          1           0        -1.836536   -1.522639   -1.872692
   16          8           0        -0.321555   -0.939913   -0.943556
   17          8           0        -1.407159    3.557570    0.015206
   18          1           0        -2.042587    3.193075   -1.773719
   19          6           0         1.012975   -0.928237    0.014745
   20          8           0         0.937360    0.120715    0.892744
   21          6           0         0.846435   -2.350836    0.645711
   22          1           0         0.921058   -3.094198   -0.155039
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23	7	0	1.984419	-2.655966	1.545679
24	1	0	2.810237	-2.859956	0.999049
25	6	0	2.289322	-1.992594	2.716009
26	8	0	1.481716	-1.576874	3.532063
27	6	0	-0.691819	4.705896	0.398319
28	1	0	-0.565549	4.760015	1.492016
29	1	0	0.312229	4.742068	-0.056076
30	1	0	-1.261809	5.579941	0.066949
31	7	0	2.089806	-0.830995	-0.759773
32	1	0	2.310901	0.054547	-1.188573
33	6	0	3.789152	-1.971939	3.051017
34	1	0	3.986898	-1.073623	3.637815
35	1	0	4.043241	-2.841326	3.668590
36	1	0	4.439897	-1.973560	2.169958
37	8	0	2.749607	-1.935521	-1.336649
38	1	0	3.581625	-1.976292	-0.841852
39	6	0	-0.482981	-2.546411	1.370168
40	1	0	-1.302230	-2.510251	0.651868
41	1	0	-0.487227	-3.513166	1.885395
42	1	0	-0.616998	-1.753308	2.106567

Applied Constraints

19 20 1.37 F

19 31 1.33 F

16 19 1.643 F

6 4 1 17 F

16 19 21 23 F

19 21 23 25 F

20 19 31 37 F

25 23 21 39 -62.00 F

31 37 F

20 19 31 32 F

Supplementary Table 5: Sum of Second order Perturbation Contributions Calculated by NBO Basis in the representative models of the substrate and the transition state for the hydrolysis reaction calculated using B3LYP/6-31G**

Hyperconjugation (kcals/mol) [a]

Bond Type	**Reactant State**		**Transition State**	
	σ→	**→σ***	**σ→**	**→σ***
C-OH	-	-	4.84	58.40
C-O	6.62	72.47[b]	3.23	14.90
O3'-C	4.84	40.02[c]	7.24	37.05[d]
C-H	12.94	10.57[e]	17.33	7.41[f]

[a] Sum of second order perturbation contributions calculated by NBO analysis. Lp1 is the sp-type lone pair; and Lp2 is *p*-type lone pair. In the bracket is an atom/bond that contributes maximally to the hyper conjugation stabilization energy: [b] Lp2(O3'), [c,d] Lp2($O^{carbonyl}$); [e,f] Lp2(C=O).

Supplementary Table 6: Sum of Second order Perturbation Contributions Calculated by NBO Basis in the representative models of the substrate and the transition state for the Aminolysis reaction calculated using B3LYP/ 6-31G**

Hyperconjugation (kcals/mol) [a]

Bond Type	**Reactant State**		**Transition State**	
	σ→	→σ*	σ→	→σ*
C-OH	-	-	4.42	38.33
C-O	6.46	69.56[b]	8.58	13.45
O3'-C	4.21	41.44[c]	10.03	88.24[d]
C-H	14.68	5.76[e]	11.18	5.68[f]

[a] Sum of second order perturbation contributions calculated by NBO analysis. Lp1 is the sp-type lone pair; and Lp2 is p-type lone pair. In the bracket is an atom/bond that contributes maximally to the hyper conjugation stabilization energy: [b] Lp2(O3'), [c,d] Lp($O^{carbonyl}$); [e,f] Lp2(C=O).

Supplementary Table 7: Showing the contribution of including imaginary frequencies on the calculated equilibrium isotope effects (EIEs) for the hydrolysis reaction

Position	**[a]EIEs**	**[b]EIEs**
Carbonyl carbon	1.0457	1.0455
Carbonyl oxygen	1.006	1.006
3'-oxygen	1.040	1.040
α-hydrogen	0.959	0.959

[a] EIEs calculated by including the imaginary frequencies in the substrate and the transition state for the hydrolysis reaction.

[b] EIEs calculated by ignoring imaginary frequencies in the substrate and the transition state.

Supplementary Table 8: Showing the influence of imaginary frequencies on kinetic isotope effects (KIEs) and equilibrium isotope effects (EIEs) for the aminolysis reaction..

Position	**[a]KIEs**	**[b]KIEs**	**[c]EIEs**
Carbonyl carbon	1.0306	1.0306	1.035
Carbonyl oxygen	1.0285	1.0251	1.028
3'-oxygen	1.0365	1.0345	1.038
α-hydrogen	0.9644	0.9644	0.963

[a] KIEs in which only the largest imaginary frequency (-414 cm^{-1}) is included in the calculation of isotope effects along with complete set of real frequencies

[b] KIEs calculated by including complete set of frequencies including all the imaginary frequencies in the substrate and the transition state.

[C] EIEs calculated by ignoring all imaginary frequencies of magnitude greater than -50 cm^{-1} in the substrate and the transition state.