Supporting Information to

QM/MM STUDIES OF HAIRPIN RIBOZYME SELF-CLEAVAGE SUGGEST THE FEASIBILITY OF MULTIPLE COMPETING REACTION MECHANISMS

Vojtěch Mlýnský, Pavel Banáš, Nils G. Walter, Jiří Šponer, and Michal Otyepka

1) Parameters of reaction intermediates

Two pentahedral phosphorane intermediates (IN-*pro*-S_PH, IN-*pro*-R_PH) were used as non-standard residues in molecular dynamics (MD) simulations. The partial atomic charges for adenine/guanine (A/G) pentahedral intermediates protonated on *pro*-S_P or *pro*-R_P nonbridging oxygens were obtained by the restrained electrostatic potential (RESP) fit procedure at HF/6-31G(d) level of theory according to the scheme of Cornell *et al.*[1,2] The model compound contained A/G pentahedral intermediate capped by 5'-methylphosphate and 3'-methylphosphate termini (Figure S1). The geometry of the A/G RNA strand carrying the pentahedral phosphorane was taken from our preliminary QM/MM calculations. This model was protonated on *pro*-S_P and *pro*-R_P nonbridging oxygens, respectively, and all hydrogen atoms were geometrically optimized at HF/6-31G(d) level of theory using the Gaussian03 program.[3] All heavy atoms were fixed in their original positions. Subsequently, the partial atomic charges were determined using RESP procedure at HF/6-31G(d) level of theory. New residue was defined for A-1 and G+1 connected by pentahedral phosphorane. The obtained partial charges were used for atoms of sugar-phosphate backbone between A(C2', C3') and G(C4') carrying the pentahedral phosphorane, while the parm99 charges were used for the remaining atoms. The residual fractional charge was spread equally to all atoms of A/G intermediate residue. Non-zero van der Waals parameters (r_0=0.6 Å, σ=0.0157 kcal/mol) of hydrogen atom (HX) bound to non-bridging oxygen of the scissile phosphate together with P-OH-HX angle parameters (k_θ = 100 kcal/molÅ^2, θ_0 = 108.5°) were used to retain a reasonable conformational behavior of protonated nonbridging oxygen in phosphorane intermediates. The other missing bonding parameters were derived by analogy from parm99 force field.

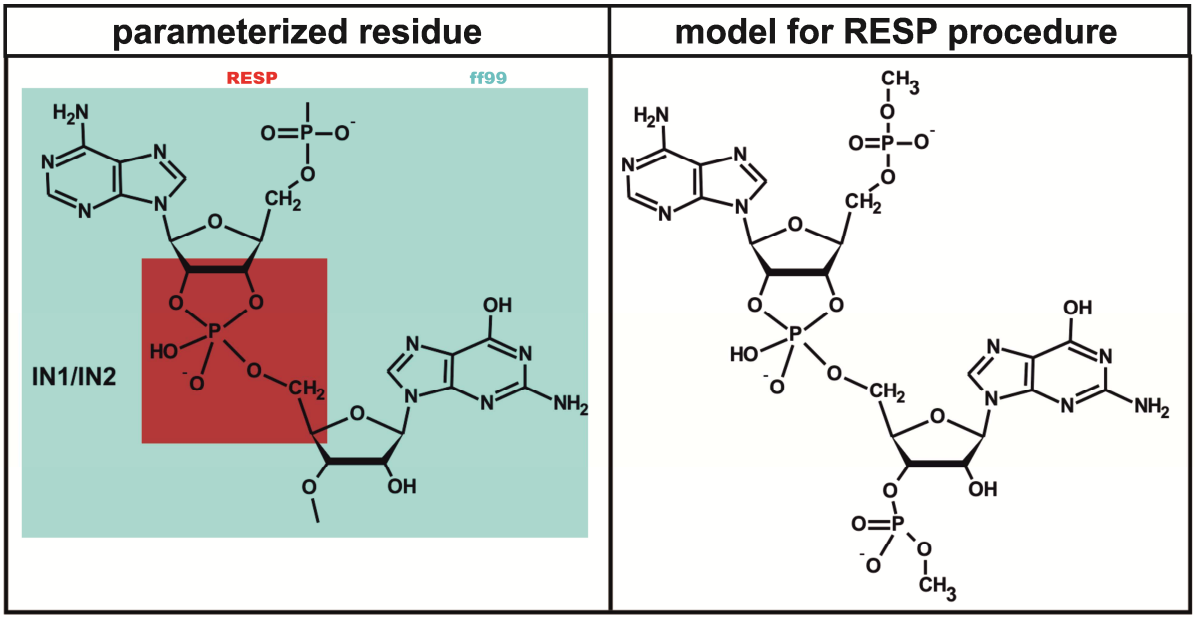

Figure S1: *Left panel shows the structure of the non-standard residue and right panel the model, used for calculation of RESP charges. Whereas the green inset on the left panel illustrates atoms with default parm99 charges, the red inset shows atoms, which partial charges were derived from the RESP procedure.*

a) *AMBER prep file for phosphorane intermediate with protonated pro-S_P (O1P) oxygen (IN-pro-S_PH)*

```
    0    0    2

This is a remark line
molecule.res
IN1   XYZ  0
CHANGE      OMIT DU   BEG
  0.0000
   1   DUMM   DU     M         999.000      999.0      -999.0           .00000
   2   DUMM   DU     M         999.000     -999.0       999.0           .00000
   3   DUMM   DU     M        -999.000      999.0       999.0           .00000
   4   P1     P      M          12.609000    1.116000    1.757000     1.15830
   5   O1P    O2     E          12.953000    2.380000    2.497000    -0.78390
   6   O2P    O2     E          11.729000    0.078000    2.381000    -0.78390
   7   O5'    OS     M          14.020000    0.387000    1.294000    -0.50680
   8   C5'    CT     M          14.082000   -0.919000   -0.052000     0.04790
   9   H5'1   H1     E           1.129000   -2.609000   -1.006000     0.06000
  10   H5'2   H1     E           2.668000   -2.564000   -1.854000     0.06000
  11   C4'    CT     M           2.766000   -1.848000    0.163000     0.09860
  12   H4'    H1     E           2.549000   -2.226000    1.153000     0.10950
  13   O4'    OS     S           4.209000   -1.769000    0.007000    -0.36270
  14   C1'    CT     B          17.346000   -0.004000   -0.468000     0.03150
  15   H1'    H2     E          17.904000   -0.122000   -1.397000     0.19280
  16   N9     N*     S          17.639000    1.363000    0.062000    -0.03300
  17   C8     CK     B          16.831000    2.254000    0.786000     0.19270
  18   H8     H5     E          15.778000    2.054000    0.918000     0.14740
  19   N7     NB     S          17.499000    3.303000    1.171000    -0.61520
  20   C5     CB     S          18.779000    3.188000    0.605000     0.04360
  21   C6     CA     B          20.023000    3.874000    0.619000     0.69300
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  22  N6    N2    B        20.319000    5.031000    1.153000   -0.90980
  23  H61   H     E        21.182000    5.472000    0.869000    0.40360
  24  H62   H     E        19.663000    5.604000    1.665000    0.40360
  25  N1    NC    S        21.098000    3.367000   -0.036000   -0.76940
  26  C2    CQ    B        21.019000    2.136000   -0.589000    0.57960
  27  H2    H5    E        21.933000    1.880000   -1.103000    0.03940
  28  N3    NC    S        19.855000    1.490000   -0.817000   -0.70760
  29  C4    CB    E        18.818000    2.063000   -0.172000    0.29740
  30  C3'   CT    M         2.237000   -0.397000    0.038000    0.01573
  31  H3'   H1    E         1.932000   -0.180000   -0.976000    0.04001
  32  C2'   CT    B         3.424000    0.490000    0.527000    0.30115
  33  H2'1  H1    E         3.586000    1.355000   -0.106000    0.03906
  34  O2'   OX    E         3.104000    0.896000    1.844000   -0.61791
  35  O3'   OS    M         1.075000   -0.183000    0.856000   -0.24735
  36  P2    P     M         1.298000    0.403000    2.441000    0.94910
  37  O1P2  OH    S         1.941000   -0.613000    3.508000   -0.59197
  38  H1P2  HX    E         1.215000   -0.919000    4.043000    0.43897
  39  O2P2  O2    E         0.903000    1.853000    2.824000   -0.67713
  40  O5x   OX    M        -0.540000   -0.205000    2.776000   -0.57352
  41  C5x   CT    M        -1.068000   -1.504000    2.449000   -0.09649
  42  H5x1  H1    E        -0.965000   -2.166000    3.310000    0.06973
  43  H5x2  H1    E        -0.548000   -1.964000    1.617000    0.06973
  44  C4x   CT    M        -2.587000   -1.429000    2.075000    0.41309
  45  H4x   H1    E        -3.084000   -2.285000    2.519000    0.10950
  46  O4x   OS    S        -3.261000   -0.264000    2.559000   -0.36270
  47  C1x   CT    B        -3.993000    0.354000    1.501000    0.01120
  48  H1x   H2    E        -4.958000    0.648000    1.885000    0.19270
  49  N9b   N*    S        -3.293000    1.576000    1.010000    0.04130
  50  C8b   CK    B        -2.203000    2.213000    1.550000    0.12950
  51  H8b   H5    E        -1.662000    1.780000    2.366000    0.15610
  52  N7b   NB    S        -1.760000    3.217000    0.837000   -0.57880
  53  C5b   CB    S        -2.745000    3.381000   -0.143000    0.16650
  54  C6b   C     B        -2.899000    4.363000   -1.188000    0.46910
  55  O6b   O     E        -2.155000    5.293000   -1.522000   -0.56760
  56  N1b   NA    B        -4.077000    4.224000   -1.888000   -0.48660
  57  H1b   H     E        -4.237000    4.929000   -2.574000    0.33450
  58  C2b   CA    B        -5.001000    3.270000   -1.637000    0.75780
  59  N2b   N2    B        -6.074000    3.300000   -2.377000   -0.97510
  60  H21b  H     E        -6.006000    3.653000   -3.305000    0.42850
  61  H22b  H     E        -6.653000    2.494000   -2.287000    0.42850
  62  N3b   NC    S        -4.884000    2.321000   -0.711000   -0.64020
  63  C4b   CB    E        -3.732000    2.429000    0.018000    0.11430
  64  C3x   CT    M        -2.823000   -1.502000    0.563000    0.19430
  65  H3x   H1    E        -2.028000   -1.007000    0.023000    0.05360
  66  C2x   CT    B        -4.130000   -0.717000    0.419000    0.05910
  67  H2x1  H1    E        -4.252000   -0.287000   -0.562000    0.08930
  68  O2x   OH    S        -5.264000   -1.520000    0.717000   -0.62180
  69  HOx2  HO    E        -4.992000   -2.423000    0.601000    0.41070
  70  O3x   OS    M        -2.932000   -2.853000    0.116000   -0.53250

LOOP
 C1'  C2'
 C5   C4
 C4   N9
 O2'  P2
 C1x  C2x
 C4b  C5b
 C4b  N9b

IMPROPER
 C8   C4   N9   C1'
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 C6   H61  N6   H62
 N7   N9   C8   H8
 N1   N3   C2   H2
 C5   N1   C6   N6
 C8b  C4b  N9b  C1x
 C5b  N1b  C6b  O6b
 C6b  C2b  N1b  H1b
 C2b  H21b N2b  H22b
 N7b  N9b  C8b  H8b
 N1b  N3b  C2b  N2b

DONE
STOP
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b) *AMBER prep file for phosphorane intermediate with protonated pro-R_P (O2P) oxygen (IN-pro-R_PH)*

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This is a remark line
molecule.res
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CHANGE     OMIT DU   BEG
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   2  DUMM  DU    M        999.000    -999.0       999.0           .00000
   3  DUMM  DU    M       -999.000     999.0       999.0           .00000
   4  P1     P    M         12.609000    1.116000    1.757000     1.15809
   5  O1P   O2    E         12.953000    2.380000    2.497000    -0.78411
   6  O2P   O2    E         11.729000    0.078000    2.381000    -0.78411
   7  O5'   OS    M         14.020000    0.387000    1.294000    -0.50701
   8  C5'   CT    M         14.082000   -0.919000   -0.052000     0.04769
   9  H5'1  H1    E          1.129000   -2.609000   -1.006000     0.05979
  10  H5'2  H1    E          2.668000   -2.564000   -1.854000     0.05979
  11  C4'   CT    M          2.766000   -1.848000    0.163000     0.09839
  12  H4'   H1    E          2.549000   -2.226000    1.153000     0.10929
  13  O4'   OS    S          4.209000   -1.769000    0.007000    -0.36291
  14  C1'   CT    B         17.346000   -0.004000   -0.468000     0.03129
  15  H1'   H2    E         17.904000   -0.122000   -1.397000     0.19259
  16  N9    N*    S         17.639000    1.363000    0.062000    -0.03321
  17  C8    CK    B         16.831000    2.254000    0.786000     0.19249
  18  H8    H5    E         15.778000    2.054000    0.918000     0.14719
  19  N7    NB    S         17.499000    3.303000    1.171000    -0.61541
  20  C5    CB    S         18.779000    3.188000    0.605000     0.04339
  21  C6    CA    B         20.023000    3.874000    0.619000     0.69279
  22  N6    N2    B         20.319000    5.031000    1.153000    -0.91001
  23  H61   H     E         21.182000    5.472000    0.869000     0.40339
  24  H62   H     E         19.663000    5.604000    1.665000     0.40339
  25  N1    NC    S         21.098000    3.367000   -0.036000    -0.76961
  26  C2    CQ    B         21.019000    2.136000   -0.589000     0.57939
  27  H2    H5    E         21.933000    1.880000   -1.103000     0.03919
  28  N3    NC    S         19.855000    1.490000   -0.817000    -0.70781
  29  C4    CB    E         18.818000    2.063000   -0.172000     0.29719
  30  C3'   CT    M          2.240000   -0.405000    0.047000     0.02332
  31  H3'   H1    E          1.938000   -0.196000   -0.971000     0.02685
  32  C2'   CT    B          3.426000    0.487000    0.527000     0.32830
  33  H2'1  H1    E          3.593000    1.339000   -0.126000     0.01660
  34  O2'   OX    E          3.106000    0.906000    1.839000    -0.62827
  35  O3'   OS    M          1.077000   -0.182000    0.861000    -0.26326
  36  P2    P     M          1.299000    0.420000    2.440000     1.00501
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  37  O1P2  O2    E          1.941000   -0.585000    3.518000    -0.74579
  38  O2P2  OH    S          0.904000    1.875000    2.807000    -0.56120
  39  H2P2  HX    E          0.086000    1.827000    3.292000     0.45277
  40  O5x   OX    M         -0.540000   -0.184000    2.779000    -0.62624
  41  C5x   CT    M         -1.068000   -1.486000    2.465000    -0.10012
  42  H5x1  H1    E         -0.947000   -2.120000    3.337000     0.10206
  43  H5x2  H1    E         -0.541000   -1.945000    1.640000     0.10206
  44  C4x   CT    M         -2.586000   -1.415000    2.089000     0.41103
  45  H4x   H1    E         -3.084000   -2.265000    2.543000     0.10929
  46  O4x   OS    S         -3.260000   -0.244000    2.561000    -0.36291
  47  C1x   CT    B         -3.991000    0.363000    1.495000     0.01099
  48  H1x   H2    E         -4.955000    0.665000    1.874000     0.19249
  49  N9b   N*    S         -3.290000    1.579000    0.993000     0.04109
  50  C8b   CK    B         -2.201000    2.221000    1.527000     0.12929
  51  H8b   H5    E         -1.703000    1.766000    2.350000     0.15589
  52  N7b   NB    S         -1.756000    3.218000    0.804000    -0.57901
  53  C5b   CB    S         -2.741000    3.372000   -0.178000     0.16629
  54  C6b   C     B         -2.894000    4.343000   -1.234000     0.46889
  55  O6b   O     E         -2.149000    5.270000   -1.577000    -0.56781
  56  N1b   NA    B         -4.071000    4.198000   -1.933000    -0.48681
  57  H1b   H     E         -4.226000    4.892000   -2.631000     0.33429
  58  C2b   CA    B         -4.995000    3.246000   -1.673000     0.75759
  59  N2b   N2    B         -6.068000    3.269000   -2.415000    -0.97531
  60  H21b  H     E         -6.011000    3.636000   -3.338000     0.42829
  61  H22b  H     E         -6.651000    2.466000   -2.321000     0.42829
  62  N3b   NC    S         -4.879000    2.306000   -0.738000    -0.64041
  63  C4b   CB    E         -3.729000    2.422000   -0.009000     0.11409
  64  C3x   CT    M         -2.820000   -1.503000    0.578000     0.19409
  65  H3x   H1    E         -2.024000   -1.016000    0.034000     0.05339
  66  C2x   CT    B         -4.127000   -0.720000    0.424000     0.05889
  67  H2x1  H1    E         -4.250000   -0.299000   -0.562000     0.08909
  68  O2x   OH    S         -5.261000   -1.519000    0.730000    -0.62201
  69  HOx2  HO    E         -4.985000   -2.424000    0.634000     0.41049
  70  O3x   OS    M         -2.930000   -2.859000    0.145000    -0.53271

LOOP
 C1'  C2'
 C5   C4
 C4   N9
 O2'  P2
 C1x  C2x
 C4b  C5b
 C4b  N9b

IMPROPER
 C8   C4   N9   C1'
 C6   H61  N6   H62
 N7   N9   C8   H8
 N1   N3   C2   H2
 C5   N1   C6   N6
 C8b  C4b  N9b  C1x
 C5b  N1b  C6b  O6b
 C6b  C2b  N1b  H1b
 C2b  H21b N2b  H22b
 N7b  N9b  C8b  H8b
 N1b  N3b  C2b  N2b

DONE
STOP
```

c) AMBER parm file with non-standard parameters

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# force field modification for RMA, RGM, RGT, RAP, IN-pro-RpH, IN-pro-SpH, and L25 residues
MASS
OX         16.00    0.465  based on OS ether and ester oxygen
HX          1.008   0.135  based on HO hydroxyl group

BOND
CT-OX     320.0     1.410  based on CT-OS JCC,7,(1986),230; NUCLEIC ACIDS
OX-P      230.0     1.965  based on P-OS JCC,7,(1986),230; NA PHOSPHATES with changed distance
HX-OH     553.0     0.960  based on HO-OH JCC,7,(1986),230; SUGARS,SER,TYR
CQ-NA     502.0     1.324  based on CQ-NC JCC,7,(1986),230; ADE

ANGLE
H1-CT-OX   50.0   109.50   based on H1-CT-OS changed based on NMA nmodes
CT-CT-OX   50.0   109.50   based on CT-CT-OS
CT-OX-P   100.0   120.50   based on CT-OS-P
OX-P –OX   45.0   180.00   based on OS-P -OS with 180 degree instead of 45
OS-P –OX   45.0    90.00   based on OS-P -OS with 90 degree instead of 45
O2-P –OX   45.0    90.00   based on O2-P -OS with 90 degree instead of 45
OH-P –OX   45.0    90.00   based on OH-P -OS with 90 degree instead of 45
HX-OH-P   100.0   108.50   based on HO-OH-P  with force constant 100kcal/molA2
CB-CA-NA   70.0   117.30   based on CB-CA-NC
NC-CQ-NA   70.0   129.10   based on NC-CQ-NC
H5-CQ-NA   50.0   115.45   based on H5-CQ-NC
CA-NA-CQ   70.0   118.60   based on CA-NC-CQ
CQ-NA-H    50.0   118.00   based on CA-NA-H changed based on NMA nmodes

DIHEDRAL
H1-CT-OX-P     3    1.15         0.0         3.    based on X-CT-OS-X JCC,7,(1986),230
CT-CT-OX-P     3    1.15         0.0         3.    based on X-CT-OS-X JCC,7,(1986),230
O2-P -OX-CT    1    0.25         0.0        -3.    based on OS-P-OS-CT JCC,7,(1986),230
O2-P -OX-CT    1    1.20         0.0         2.    based on OS-P-OS-CT gg&gt ene.631g*/mp2
OH-P -OX-CT    1    0.25         0.0        -3.    based on OS-P-OS-CT JCC,7,(1986),230
OH-P -OX-CT    1    1.20         0.0         2.    based on OS-P-OS-CT gg&gt ene.631g*/mp2
OS-P -OX-CT    1    0.25         0.0        -3.    based on OS-P-OS-CT JCC,7,(1986),230
OS-P -OX-CT    1    1.20         0.0         2.    based on OS-P-OS-CT gg&gt ene.631g*/mp2
OX-P -OX-CT    1    0.00         0.0         1.    this torsion should be zero in sp3d
OX-P -OS-CT    1    0.25         0.0        -3.    based on OS-P-OS-CT JCC,7,(1986),230
OX-P -OS-CT    1    1.20         0.0         2.    based on OS-P-OS-CT gg&gt ene.631g*/mp2
OX-CT-CT-OS    1    0.144        0.0        -3.    based on OS-CT-CT-OS parm98, TC,PC,PAK
OX-CT-CT-OS    1    1.175        0.0         2.    based on OS-CT-CT-OS Piotr et al.
H1-CT-CT-OX    1    0.25         0.0         1.    based on H1-CT-CT-OS Junmei et al, 1999
OX-CT-CT-OH    1    0.144        0.0        -3.    based on OS-CT-CT-OH parm98, TC,PC,PAK
OX-CT-CT-OH    1    1.175        0.0         2.    based on OS-CT-CT-OH parm98, TC,PC,PAK
X -NA-CQ- X    4    9.60        80.0         2.


NONBON
 OX          1.6837   0.1700            based on OS OPLS ether
 HX          0.6000   0.0157            based on HS W. Cornell CH3SH --> CH3OH FEP
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2) Behavior of Reaction Intermediates

We performed four 50 ns-long MD simulations of all possible combinations of protonation state of the reaction intermediate and A38 adenine (protonated *pro*-R_PH or *pro*-S_PH nonbridging oxygen of phosphorane group with either canonical A38 or protonated A38H$^+$ form). We used the same protocol for setting up MD's as in our previous MD study.[4] MD simulations containing the canonical A38 are not further described as the A38 left the active site within the first ~4 ns of each MD simulation and exhibited the same behavior as reported recently in our MD paper.[4] As a consequence, only two simulations with protonated A38H$^+$ form are discussed below and were further used for preparation of the starting geometries for the following hybrid quantum mechanical/ molecular mechanical QM/MM study. We would like to note, that the bonding force field parameters for pentahedral phosphorane intermediate

residues were not thoroughly tested and therefore the results should be interpreted with care considering potential limitation of the force field used.

G+1(pro-S_PH) intermediate with A38H$^+$ (IN-pro-S_PH/G8/A38H$^+$)

The G8 nucleobase established the 4BPh (base-phosphate) contact with G+1 pentahedral phosphorane within first part of MD simulation (Figure S2C). This contact was weakened just for a limited time (for ~6 ns in overall) by reorientation of G8 within the active site and formation of transient G+1(*pro*-S_PH)…G8(O6) H-bond around ~33 and ~46 ns. The A9 nucleobase formed bifurcated A9(N6H)…A-1(O2')/G+1(*pro*-R_P) H-bond, temporary substituted (from ~38 to 46 ns) by the formation of the A10(N6H)…A-1(O2') H-bond by the neighboring A10 nucleobase (Figures S2A, S2C). The protonated A38H$^+$ nucleobase established two stable A38H$^+$(N6H)…G+1(*pro*-R_P) and A38H$^+$(N1H)…G+1(O5') H-bonds to the pentahedral phosphorane (Figure S2C).

G+1(pro-R_PH) intermediate with A38H$^+$ (IN-pro-R_PH/G8/A38H$^+$)

The A-1(O2') oxygen lost immediately its interaction with G8 and established a new A10(N6H)…A-1(O2') H-bond within the first ns of MD simulation (Figure S2C). Thereby, G8 formed temporary bifurcated G8(N1H/N2H)…G+1(*pro*-S_P) H-bond. The protonated G+1(*pro*-R_PH) group established also bifurcated A38H$^+$(N6H)…G+1(*pro*-R_P)/A9(N6H)…G+1(*pro*-R_P) H-bond (Figure S2C). Interestingly, we observed significant H-bond reorientation and a renewal of G8(N1H)…A-1(O2') and G8(N2H)…G+1(*pro*-R_P) H-bonds in the second part of MD simulation (Figures S2B, S2C).

The hydrogen on protonated G+1(*pro*-R_PH) group was not involved as a proton donor in any H-bond with surrounding bases and pointed either in the direction to the A-1 sugar (~75%), or the G+1 sugar (~25%). We did not observe any water mediated H-bond between G+1(O5') and G+1(*pro*-R_PH). In contrast to the previous IN-*pro*-R_PH/G8/A38H$^+$ MD simulation, the A38H$^+$(N6H)…G+1(*pro*-R_P) H-bond was significantly weakened (after ~25 ns) due to the reorientation of non-bridging oxygens of the pentahedral phosphorane and subsequent formation of the G8(N1H)…A-1(O2') and G8(N2H)…G+1(*pro*-R_P) H-bonds. Nevertheless, an average structure taken at the end of this simulation showed the best agreement with the X-ray structures (Figure S2B) of vanadate transition states analogs of the hairpin ribozyme (PDB code 1M5O, 2P7E).[5,6]

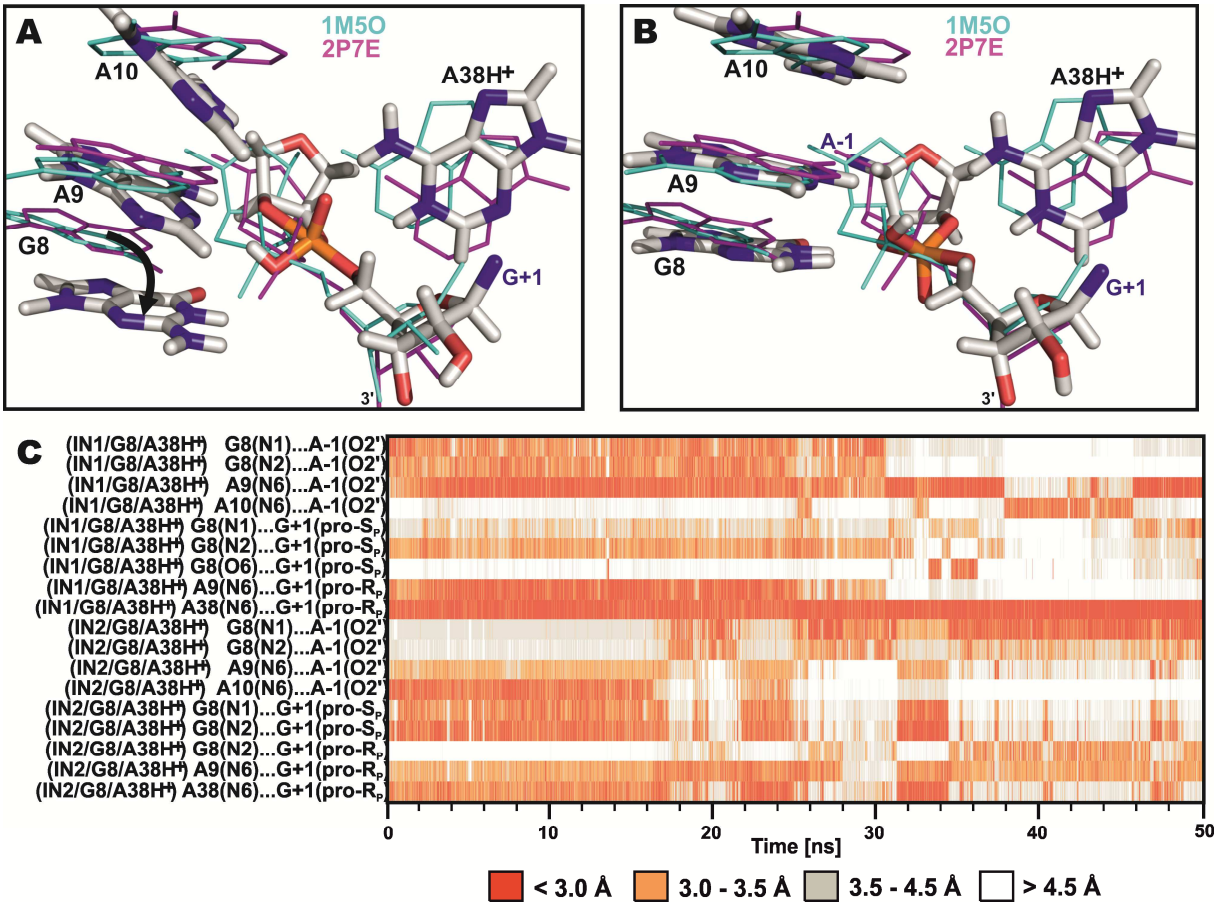

Figure S2: *Last one ns average structures of the active site of IN-pro-R_PH/G8/A38H$^+$ (A) and IN-pro-S_PH/G8/A38H$^+$ (B) MD simulations (in sticks) of the hairpin ribozyme are superimposed with two crystal structures of vanadate TS analogs (1M5O in cyan, 2P7E in magenta lines).* ***(A)*** *The protonated pro-S_P oxygen (pro-S_PH) causes reorientation of G8 (black arrow), A9 and A10 nucleobases in IN-pro-R_PH/G8/A38H$^+$ MD simulation.* ***(B)*** *On the other hand, the MD simulation of IN-pro-S_PH/G8/A38H$^+$ phosphorane intermediate shows the best agreement with the X-ray structures of vanadate TS analogs.* ***(C)*** *Time evolution of interactions of the G+1(O2') oxygen and protonated pro-R_P/pro-S_P oxygens with groups of neighboring nucleobases (IN1 and IN2 denote IN-pro-S_PH and IN-pro-R_PH, respectively).*

3) Starting structures for QM/MM calculations

Snapshots from both IN-*pro*-R_PH/G8/A38H$^+$ and IN-*pro*-S_PH/G8/A38H$^+$ MD simulations were chosen based on geometrical arrangement of the protonated phosphorane with maximalized base-phosphate interaction (BPh)[7] towards canonical G8 and protonated A38H$^+$ residues. Further we reselected only those snapshots, where the protonated G+1(*pro*-R_PH) or G+1(*pro*-S_PH) non-bridging oxygen achieved the best orientation in the direction either of the A-1(O2') or the G+1(O5') oxygen. Finally, we considered 4 different MD snapshots (two with protonated G+1(*pro*-R_P) and other two with protonated G+1(*pro*-S_P) oxygen) that were subsequently used to prepare four starting structures for QM/MM calculations (Figure S3).

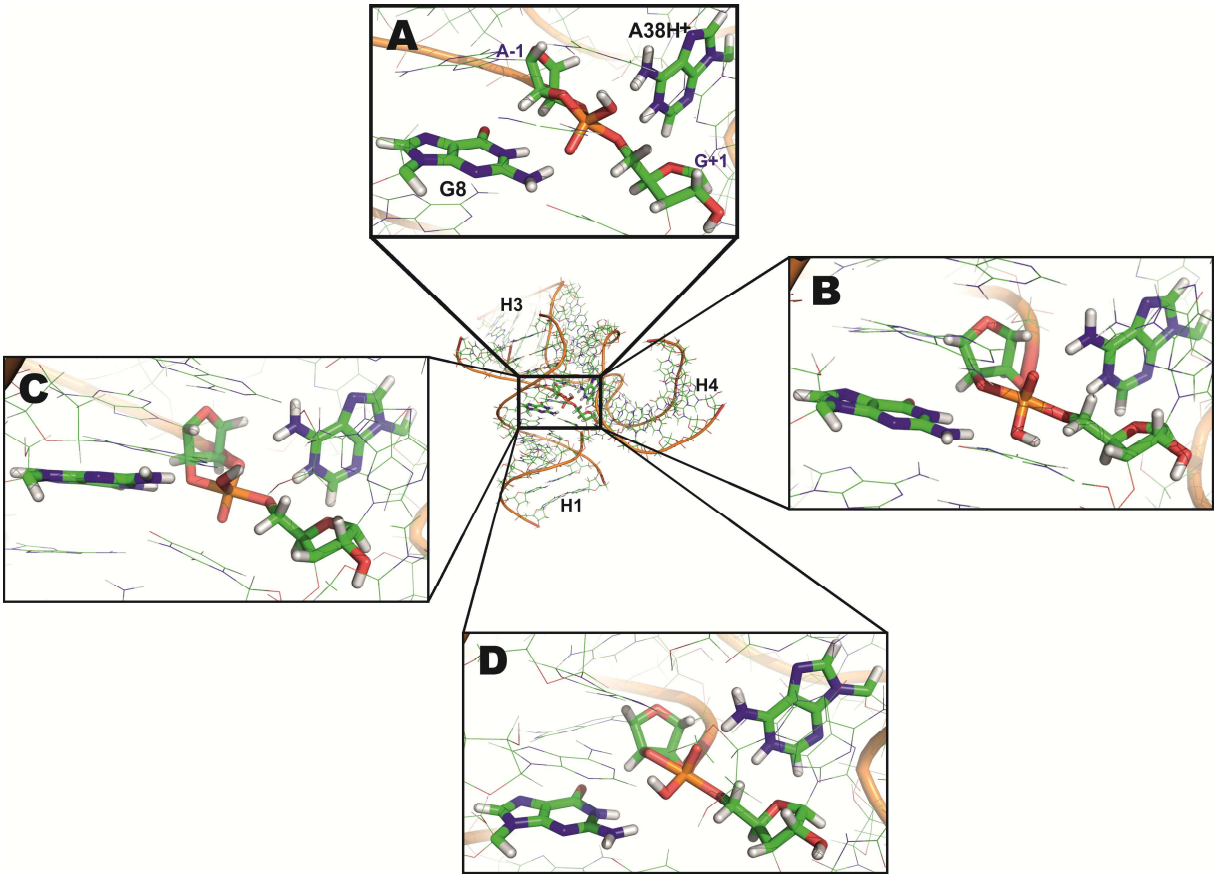

Figure S3: (A) *Starting structure for QM/MM calculations taken from IN-pro-S_PH/G8/A38H$^+$ MD simulation of the hairpin ribozyme. The protonated pentahedral phosphorane and key nucleobases within the active site are highlighted in sticks. Water molecules and counter ions are not shown for clarity.* ***(B,C,D)*** *Detailed view into the active site shows additional conformations of the pentahedral phosphorane and neighboring nucleobases, which were also considered as starting points for the subsequent QM/MM study.*

Supporting tables:

Table S1: *Summarized extrapolated CBS(T)[a] energies (in kcal/mol, related to the reactant state), MPW1K/6-31+G(d,p) solvation energies, Gibbs Energy corrections and total Gibbs energies at the extrapolated CBS(T) level of the endo/exo-3'-(1'-amino-4'-methylribose)-5'-methylphosphodiester cleavage model. All geometries were optimized at CPCM (ε_r= 78.4)/MPW1K/6-31+G(d,p) level (see Methods in main text for details). The solvation term was calculated as the difference between CPCM(ε_r=78.4)/MPW1K/6-31+G(d,p) and gas phase MPW1K/6-31+G(d,p) SCF energies. The corrections to Gibbs energies were calculated at the CPCM(ε_r=78.4)/MPW1K/6-31+G(d,p) level.*

endo (*pro*-R_P)	R	TS_1	IN_1	TS_2	IN_2	TS_3	P	P'
CBS(T)	0.0	28.9	27.1	36.8	30.0	37.4	4.1	19.0
MPW1K solvation energy	0.0	-1.2	-4.5	-4.6	-5.2	-0.6	-1.4	-9.4
MPW1K Gibbs Energy correction	0.0	0.3	1.2	0.4	0.5	-2.2	-4.1	-12.9
CBS(T) Gibbs Energy in water	0.0	28.0	23.8	32.5	25.3	34.6	-1.4	-3.2
exo (*pro*-S_P)	R	TS_1	IN_1	TS_2	IN_2	TS_3	P	P'
CBS(T)	0.0	27.1	21.7	32.4	26.6	37.9	4.1	19.0
MPW1K solvation energy	0.0	0.1	-1.6	-4.1	-3.4	-1.0	-1.4	-9.4
MPW1K Gibbs Energy correction	0.0	0.3	1.9	0.2	0.9	-2.2	-4.1	-12.9
CBS(T) Gibbs Energy in water	0.0	27.5	21.9	28.5	24.1	34.6	-1.4	-3.2

[a] MP2/CBS energies corrected to higher-order correlation effects using CCSD(T) energies (see Methods section).

Table S2: *List of the specific reaction mechanisms studied here and protonation states of key reaction participants G8, A38 and phosphate/phosphorane/cyclic phosphate, along respective reaction pathways (for names see Table 2 in main text). G8t stands for G8 enol tautomer, Ph for phosphate, Phr for phosphorane, cPh for cyclic phosphate.*

Name	General base	General acid	R			IN/TS			P		
			G8	A38	Ph	G8	A38	Phr	G8	A38	cPh
$G^-/A^+/G8^-/A38H^+$	$G8^-$	$A38H^+$	$G8^-$	$A38H^+$	Ph^-	G8	$A38H^+$	Phr^{2-}	G8	A38	cPh^-
pro-R_P/*pro*-R_P/G8/$A38H^+$	G+1(*pro*-R_P)	G+1(*pro*-R_P)	G8	$A38H^+$	Ph^-	G8	$A38H^+$	Phr^-	G8	$A38H^+$	cPh^-
pro-R_P/*pro*-R_P/G8t/$A38H^+$	G+1(*pro*-R_P)	G+1(*pro*-R_P)	G8t	$A38H^+$	Ph^-	G8t	$A38H^+$	Phr^-	G8t	$A38H^+$	cPh^-
pro-R_P/*pro*-R_P/G8/A38	G+1(*pro*-R_P)	G+1(*pro*-R_P)	G8	A38	Ph^-	G8	A38	Phr^-	G8	A38	cPh^-
pro-R_P/A^+/G8/$A38H^+$	G+1(*pro*-R_P)	$A38H^+$	G8	$A38H^+$	Ph^-	G8	$A38H^+$	Phr^-	G8	A38	cPh
pro-S_P/*pro*-S_P/G8/$A38H^+$	G+1(*pro*-S_P)	G+1(*pro*-S_P)	G8	$A38H^+$	Ph^-	G8	$A38H^+$	Phr^-	G8	$A38H^+$	cPh^-
pro-S_P/*pro*-S_P/G8t/$A38H^+$	G+1(*pro*-S_P)	G+1(*pro*-S_P)	G8t	$A38H^+$	Ph^-	G8t	$A38H^+$	Phr^-	G8t	$A38H^+$	cPh^-
pro-S_P/*pro*-S_P/G8/A38	G+1(*pro*-S_P)	G+1(*pro*-S_P)	G8	A38	Ph^-	G8	A38	Phr^-	G8	A38	cPh^-

Table S3a: *The MPW1K/6-31+G(d,p) gas phase energies, solvation and Gibbs Energy corrections (calculated at CPCM(ε_r=78.4)/MPW1K/6-31+G(d,p) level), and total Gibbs Energy profiles of the self-cleavage reaction of the endo/exo-3'-(1'-amino-4'-methylribose)-5'-methylphosphodiester sugar-phosphate backbone model extended by N9-methyl guanine and protonated N9-methyladenine. The proton of 2'-OH hydroxyl was shuttled via pro-R_P (endo path) or pro-S_P (exo path) non-bridging oxygen. All energies and energy corrections are in kcal/mol and are related to reactant state. No pK_a correction (see Methods section) for N1-protonated-N9-methyladenine was included in total Gibbs energies.*

endo (*pro*-R_P)	R	TS_1	IN_1	TS_2	IN_2	TS_3	P
MPW1K/6-31+G(d,p)	0.0	25.6	14.4	26.8	19.4	24.3	-9.1
Solvation energy	0.0	-2.2	2.1	-1.2	0.6	5.0	5.6
Gibbs Energy correction	0.0	0.0	-0.8	-1.5	-0.7	-1.9	-4.2
MPW1K Gibbs Energy in water	0.0	23.4	15.7	24.1	19.3	27.3	-7.7
exo (*pro*-S_P)	R	TS_1	IN_1	TS_2	IN_2	TS_3	P
MPW1K/6-31+G(d,p)	0.0	23.7	3.4	13.5	10.5	16.4	-9.1
Solvation energy	0.0	-3.8	6.6	3.2	1.8	6.7	3.7
Gibbs Energy correction	0.0	-0.8	1.2	0.0	0.5	-2.4	-3.0
MPW1K Gibbs Energy in water	0.0	19.1	11.2	16.7	12.8	20.7	-8.4

Table S3b: *The MPW1K/6-31+G(d,p) gas phase energies, solvation and Gibbs Energy corrections (calculated at CPCM(ε_r=78.4)/MPW1K/6-31+G(d,p) level), and total Gibbs Energy profiles of the self-cleavage reaction of the endo/exo-3'-(1'-amino-4'-methylribose)-5'-methylphosphodiester sugar-phosphate backbone model extended by N1-deprotonated-N9-methyl guanine and N1-protonated-N9-methyladenine. The deprotonated guanine acts as a general base, while the protonated adenine acts as a general acid. All energies and energy corrections are in kcal/mol and are related to reactant state. No pK_a corrections for N1-deprotonated-N9-methyl guanine and N1-protonated-N9-methyladenine were included in total Gibbs energies.*

	R	TS	P
MPW1K/6-31+G(d,p)	0.0	0.2	-41.4
Solvation energy	0.0	13.5	33.8
Gibbs Energy correction	0.0	-0.1	-4.0
MPW1K Gibbs Energy in water	0.0	13.6	-11.6

Table S4a: *The MPW1K/6-31+G(d,p) reaction barriers (in kcal/mol, related to the reactant state) obtained for geometries along various paths representing the proton shuttling and G8⁻ general base reaction mechanisms (for names see Table 2 in main text). No pK_a corrections (for deprotonated G8⁻ and protonated A38H⁺) and Gibbs energy corrections (see Methods section) were applied in this Table.*

	R	TS_1	IN_1	TS_2	IN_2	TS_3	P
pro-R_P/pro-R_P/G8/A38H⁺	0.0	18.9	2.0	9.0	3.3	4.3	-14.8
pro-R_P/A⁺/G8/A38H⁺	0.0	18.9	2.0	9.0	3.3	3.6	-5.3
pro-R_P/pro-R_P/G8t/A38H⁺	0.0	23.6	2.6	10.2	4.3	5.9	-11.1
pro-R_P/pro-R_P/G8/A38	0.0	20.5	4.8	14.4	5.2	14.1	-8.6
pro-S_P/pro-S_P/G8/A38H⁺	0.0	21.7	8.0	12.8	3.0	6.6	-10.7
pro-S_P/pro-S_P/G8t/A38H⁺	0.0	29.0	11.1	14.4	5.6	5.6	-7.6
pro-S_P/pro-S_P/G8/A38	0.0	24.0	14.9	18.1	8.3	13.9	-5.1
G⁻/A⁺/G8⁻/A38H⁺	0.0	15.0					-15.4

Table S4b: *The BLYP/6-31G(d) energies (in kcal/mol, related to the reactant state) obtained from QM/MM calculations representing the proton shuttling and G8⁻ general base reaction mechanisms (for names see Table 2 in main text). No pK_a corrections (for deprotonated bG8⁻ and protonated A38H⁺) and Gibbs energy corrections (see Methods section) were applied in this Table.*

	R	TS_1	IN_1	TS_2	IN_2	TS_3	P
pro-R_P/pro-R_P/G8/A38H⁺	0.0	15.9	1.8	8.2	2.5	3.1	-12.8
pro-R_P/A⁺/G8/A38H⁺	0.0	15.9	1.8	8.2	2.5	2.6	-4.9
pro-R_P/pro-R_P/G8t/A38H⁺	0.0	20.7	1.9	9.0	3.6	3.7	-10.4
pro-R_P/pro-R_P/G8/A38	0.0	17.8	4.2	11.7	4.6	10.2	-8.0
pro-S_P/pro-S_P/G8/A38H⁺	0.0	18.3	7.4	11.2	2.4	4.1	-10.4
pro-S_P/pro-S_P/G8t/A38H⁺	0.0	25.7	10.3	12.9	4.7	4.9	-7.3
pro-S_P/pro-S_P/G8/A38	0.0	20.1	12.0	15.6	7.9	10.6	-4.1
G⁻/A⁺/G8⁻/A38H⁺	0.0	9.7					-15.2

Table S5a: *The MPW1K/6-31+G(d,p) gas phase and extrapolated CBS(T) energies, MPW1K/6-31+G(d,p) solvation energies, Gibbs Energy corrections and total Gibbs energies at the extrapolated CBS(T) level of the endo/exo-3'-(1'-amino-4'-methylribose)-5'-methylphosphodiester cleavage model, where the endo (pro-R_P) non-bridging oxygen was thio-substituted. The proton of 2'-OH hydroxyl was shuttled via pro-R_P sulphur atom (endo path) or pro-S_P non-bridging oxygen (exo path). All energies and energy corrections are in kcal/mol and are related to reactant state. The solvation term was calculated as the difference between CPCM(ε_r=78.4)/MPW1K/6-31+G(d,p) and gas phase MPW1K/6-31+G(d,p) SCF energies. The corrections to Gibbs energies were calculated at the CPCM (ε_r= 78.4)/MPW1K/6-31+G(d,p) level.*

endo (*pro*-R_P)	R	TS_1	IN_1	TS_2	IN_2	TS_3	P	P'
MPW1K/6-31+G(d,p)	0.0	43.6	33.1	41.1	32.7	44.6	1.3	15.0
CBS(T)	0.0	40.7	30.4	38.2	30.2	44.1	3.5	17.6
Solvation energy	0.0	-2.7	-4.5	-4.0	-4.2	-1.0	-1.5	-9.0
Gibbs Energy correction	0.0	-2.6	-0.1	-1.1	-0.6	-3.3	-4.1	-12.5
CBS(T) Gibbs Energy in water	0.0	35.4	25.8	33.2	25.4	39.8	-2.1	-3.8
exo (*pro*-S_P)	R	TS_1	IN_1	TS_2	IN_2	TS_3	P	P'
MPW1K/6-31+G(d,p)	0.0	27.5	19.2	29.4	23.9	36.8	1.3	15.0
CBS(T)	0.0	27.5	20.0	30.2	24.8	38.2	3.5	17.6
Solvation energy	0.0	-0.1	-2.6	-5.1	-4.7	-0.8	-1.5	-9.0
Gibbs Energy correction	0.0	0.6	1.8	1.4	2.1	-1.9	-4.1	-12.5
CBS(T) Gibbs Energy in water	0.0	28.0	19.2	26.4	22.2	35.5	-2.1	-3.8

Table S5b: *The computed energies and corrections of the endo/exo-3'-(1'-amino-4'-methylribose)-5'-methylphosphodiester cleavage model, where the pro-S_P non-bridging oxygen was thio-substituted. The proton of 2'-OH hydroxyl was shuttled via pro-R_P non-bridging oxygen (exo path) or pro-S_P sulphur atom (exo path). The energies and corrections were computed by the same methodology as in Table S5a.*

endo (*pro*-R_P)	R	TS_1	IN_1	TS_2	IN_2	TS_3	P	P'
MPW1K/6-31+G(d,p)	0.0	31.8	25.5	33.9	28.7	38.3	5.7	15.4
CBS(T)	0.0	31.5	26.3	33.6	29.2	39.4	6.9	17.6
Solvation energy	0.0	-1.8	-5.5	-4.9	-6.2	-0.8	-2.3	-8.5
Gibbs Energy correction	0.0	-0.5	1.4	0.2	0.8	-2.9	-5.7	-13.3

CBS(T) Gibbs Energy in water	0.0	29.2	22.2	28.9	23.8	35.8	-1.1	-4.2
exo (*pro*-S_P)	R	TS_1	IN_1	TS_2	IN_2	TS_3	P	P'
MPW1K/6-31+G(d,p)	0.0	42.0	28.2	37.9	32.8	46.0	5.7	15.4
CBS(T)	0.0	39.2	25.6	34.6	30.3	44.8	6.9	17.6
Solvation energy	0.0	-1.2	-1.4	-4.6	-3.5	-1.0	-2.3	-8.5
Gibbs Energy correction	0.0	-3.3	-0.8	-2.7	-1.7	-4.0	-5.7	-13.3
CBS(T) Gibbs Energy in water	0.0	34.7	23.4	27.3	25.1	39.8	-1.1	-4.2

Table S5c: *The computed energies and corrections of the endo/exo-3'-(1'-amino-4'-methylribose)-5'-methylphosphodiester cleavage model, where both pro-R_P and pro-S_P non-bridging oxygens were thio-substituted. The proton of 2'-OH hydroxyl was thereby shuttled via pro-R_P (exo path) or pro-S_P (exo path) sulphur atoms. The energies and corrections were computed by the same methodology as in Table S5a.*

endo (*pro*-R_P)	R	TS_1	IN_1	TS_2	IN_2	TS_3	P	P'
MPW1K/6-31+G(d,p)	0.0	45.0	31.4	39.3	31.3	43.7	4.3	13.3
CBS(T)	0.0	43.0	29.4	37.0	29.3	43.6	5.9	16.3
Solvation energy	0.0	-4.6	-5.3	-4.6	-5.2	-1.8	-1.9	-7.7
Gibbs Energy correction	0.0	-2.3	0.0	-0.9	-0.7	-2.8	-4.5	-13.0
CBS(T) Gibbs Energy in water	0.0	36.1	24.0	31.6	23.5	39.1	-0.5	-4.4
exo (*pro*-S_P)	R	TS_1	IN_1	TS_2	IN_2	TS_3	P	P'
MPW1K/6-31+G(d,p)	0.0	41.4	25.1	34.4	30.1	46.8	4.3	13.3
CBS(T)	0.0	39.4	23.5	31.6	28.3	46.2	5.9	16.3
Solvation energy	0.0	-2.5	-2.1	-3.8	-4.4	-3.5	-1.9	-7.7
Gibbs Energy correction	0.0	-2.4	-0.1	-1.3	-1.1	-2.9	-4.5	-13.0
CBS(T) Gibbs Energy in water	0.0	34.5	21.3	26.5	22.7	39.9	-0.5	-4.4

Supporting figures:

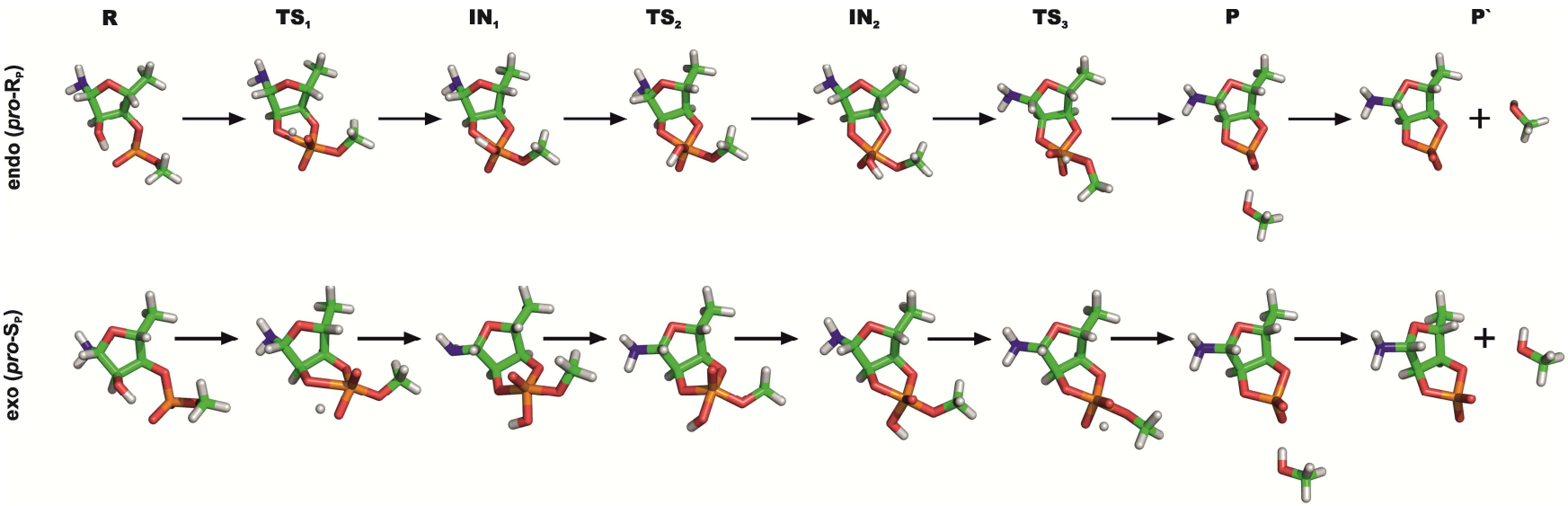

Figure S4: *The structures along the reaction path of the endo/exo-3'-(1'-amino-4'-methylribose)-5'-methylphosphodiester self-cleavage reaction.*

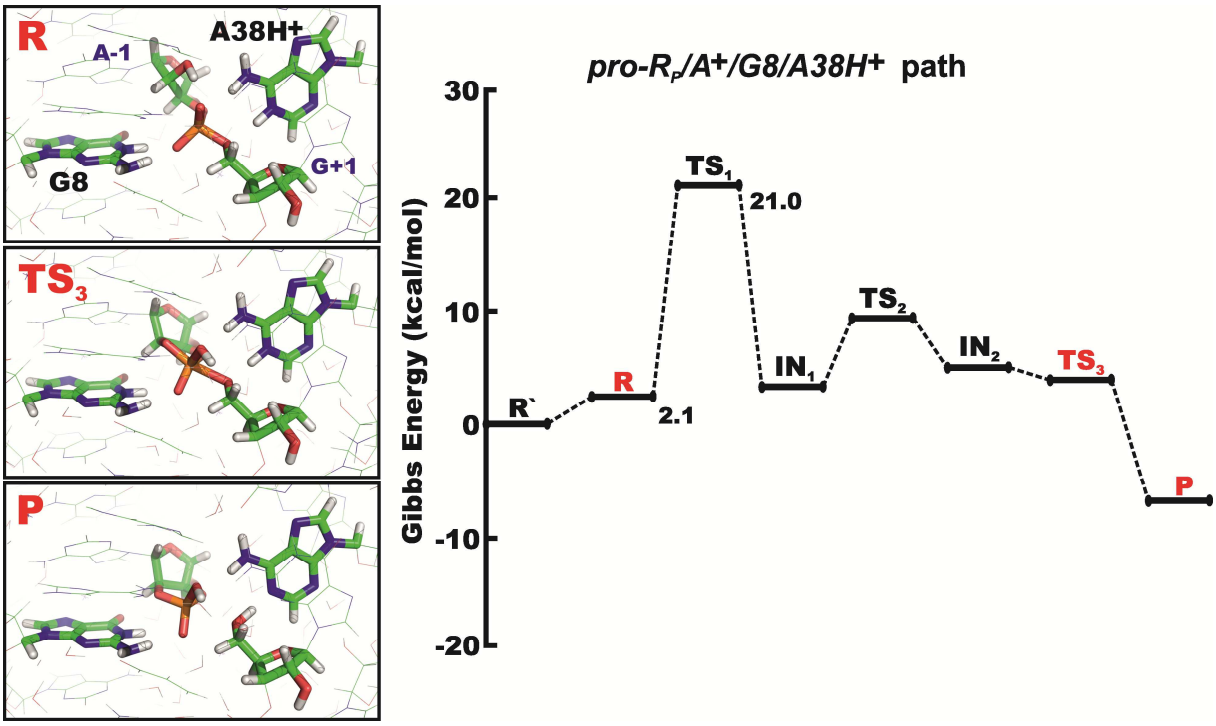

Figure S5: *The Gibbs Energy profile of the pro-R_P/A^+/G8/$A38H^+$ mechanism with $A38H^+$ acting as a general acid. Structures in boxes show detailed view into the active site (QM core is highlighted in sticks) of the reactant, transition and product states (R, TS_3 and P) along the reaction pathway. All energies are relative to the R' state that represents the reactant with the dominant protonation states of G8 and A38 at pH ~7, i.e. both nucleobases in canonical forms.*

References:

(1) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Kollman, P. A. *J. Am. Chem. Soc.* **1993**, *115*, 9620-9631.

(2) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179-5197.

(3) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C. et al. Gaussian 03; Gaussian, Inc.: Pittsburgh, 2003.

(4) Mlynsky, V.; Banas, P.; Hollas, D.; Reblova, K.; Walter, N. G.; Sponer, J.; Otyepka, M. *J. Phys. Chem. B* **2010**, *114*, 6642-6652.

(5) Rupert, P. B.; Massey, A. P.; Sigurdsson, S. T.; Ferre-D'Amare, A. R. *Science* **2002**, *298*, 1421-1424.

(6) Torelli, A. T.; Krucinska, J.; Wedekind, J. E. *RNA* **2007**, *13*, 1052-1070.

(7) Zirbel, C. L.; Sponer, J. E.; Sponer, J.; Stombaugh, J.; Leontis, N. B. *Nucleic Acids Res.* **2009**, *37*, 4898-4918.