



Supporting Information

for

Thermophilic phosphoribosyltransferases *Thermus thermophilus* HB27 in nucleotide synthesis

Ilja V. Fateev, Ekaterina V. Sinitina, Aiguzel U. Bikanasova, Maria A. Kostromina, Elena S. Tuzova, Larisa V. Esipova, Tatiana I. Muravyova, Alexei L. Kayushin, Irina D. Konstantinova and Roman S. Esipov

Beilstein J. Org. Chem. **2018**, *14*, 3098–3105. doi:10.3762/bjoc.14.289

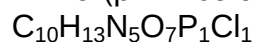
Detailed analysis of mass spectrometry and NMR data

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1. Mass spectra of nucleotides were measured on Agilent 6224, ESI-TOF, LC/MS (USA) in positive ion mode (ESI), LCQ Fleet ion trap mass spectrometer (Thermo Electron, USA).

1.1. 9-(β -D-ribofuranosyl)-2-chloroadenine 5'-monophosphate



Calc. $[\text{M}+\text{H}]^+ = 382.0315$

Exp. $[\text{M}+\text{H}]^+ = 382.0353$, $[\text{2M}+\text{H}]^+ = 763.0606$, $[\text{Base}+\text{H}]^+ = 170.0244$

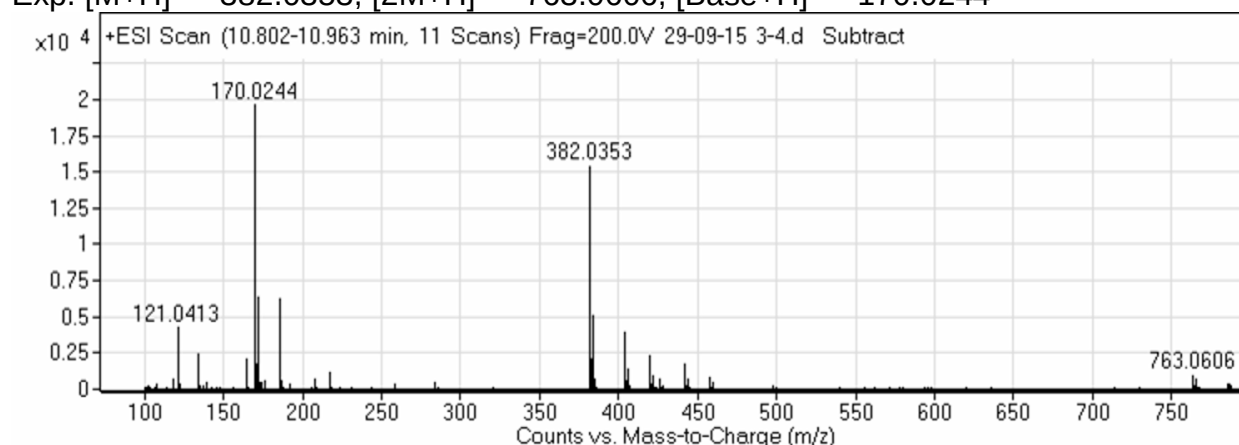
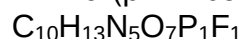


Figure S1

1.2. 9-(β -D-ribofuranosyl)-2-fluoroadenine 5'-monophosphate



Calc. $[\text{M}+\text{H}]^+ = 366.0611$

Exp. $[\text{M}+\text{H}]^+ = 366.0564$, $[\text{2M}+\text{H}]^+ = 731.1034$, $[\text{Base}+\text{H}]^+ = 154.0511$

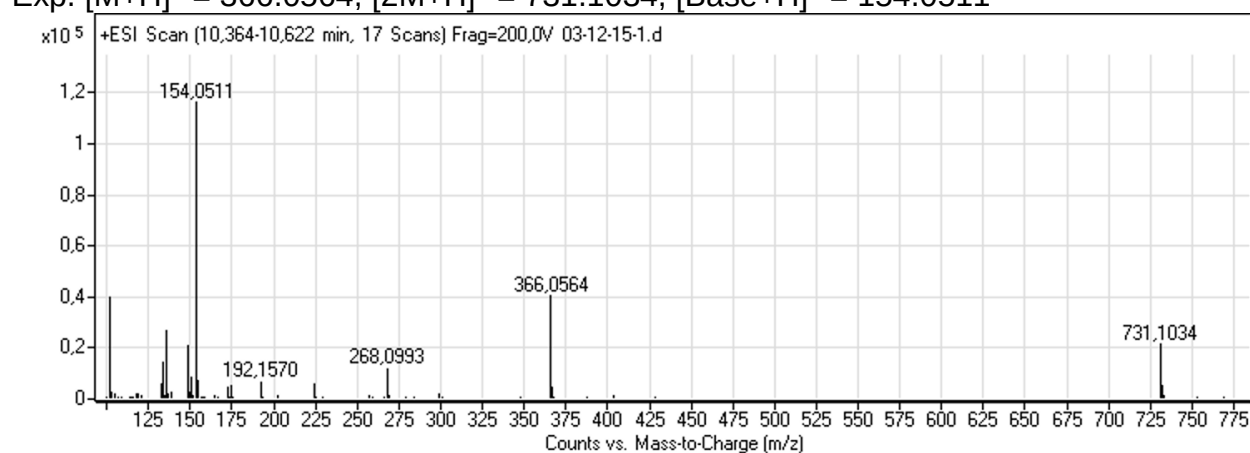
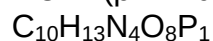


Figure S2

1.3. 1-(β -D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine-4-one 5'-monophosphate



Calc. $[\text{M}+\text{H}]^+ = 349.0545$

Exp. $[\text{M}+\text{H}]^+ = 349.0520$, $[\text{2M}+\text{H}]^+ = 697.0952$, $[\text{3M}+\text{H}]^+ = 1045.1374$, $[\text{Base}+\text{H}]^+ = 137.0453$

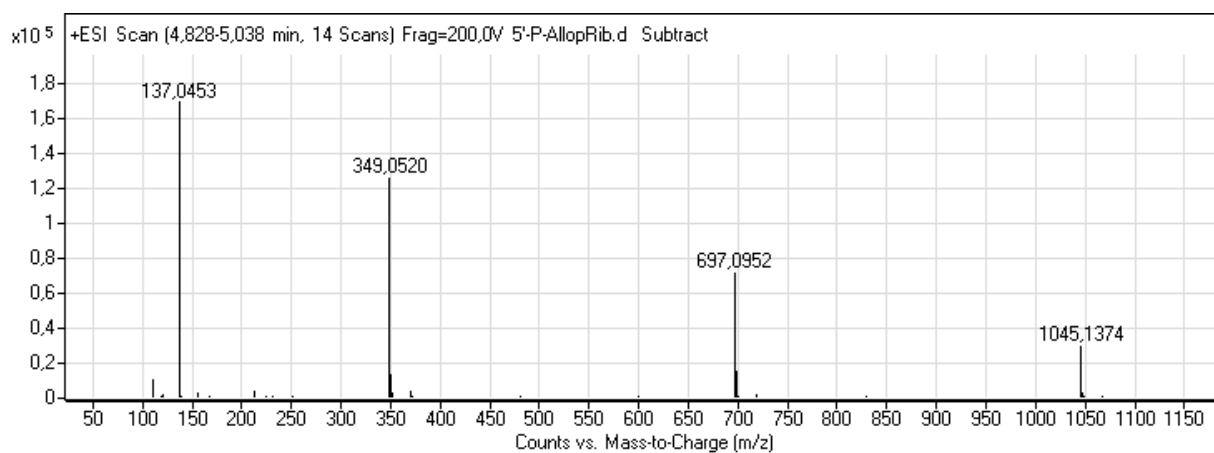
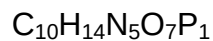


Figure S3

1.4. Adenosine 5'-monophosphate



Calc. $[M+H]^+ = 348.0705$

Exp. $[M+H]^+ = 348.0677$, $[2M+H]^+ = 695.1258$, $[Base+H]^+ = 136.0607$

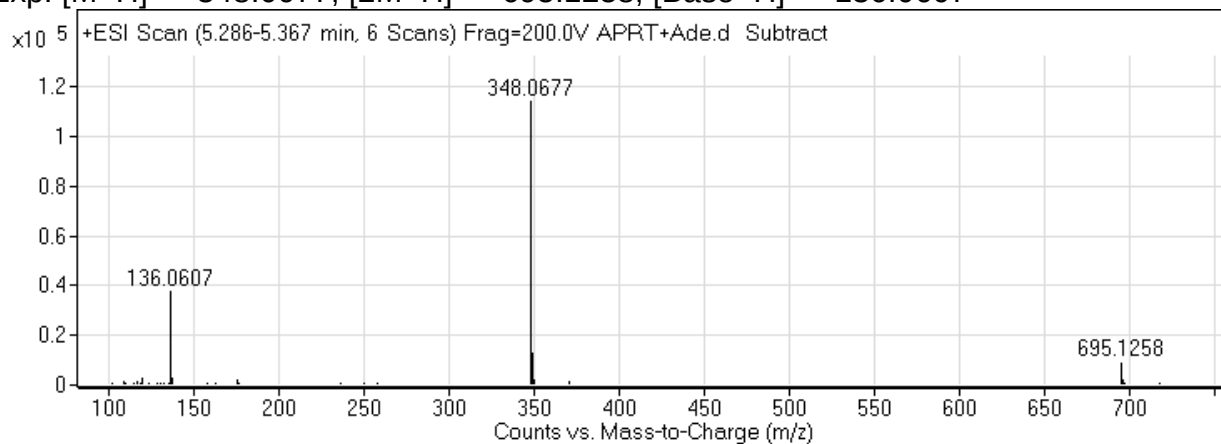
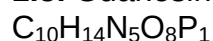


Figure S4

1.5. Guanosine 5'-monophosphate



Calc. $[M+H]^+ = 364.0654$

Exp. $[M+H]^+ = 364.0614$, $[2M+H]^+ = 727.1128$, $[Base+H]^+ = 152.0557$

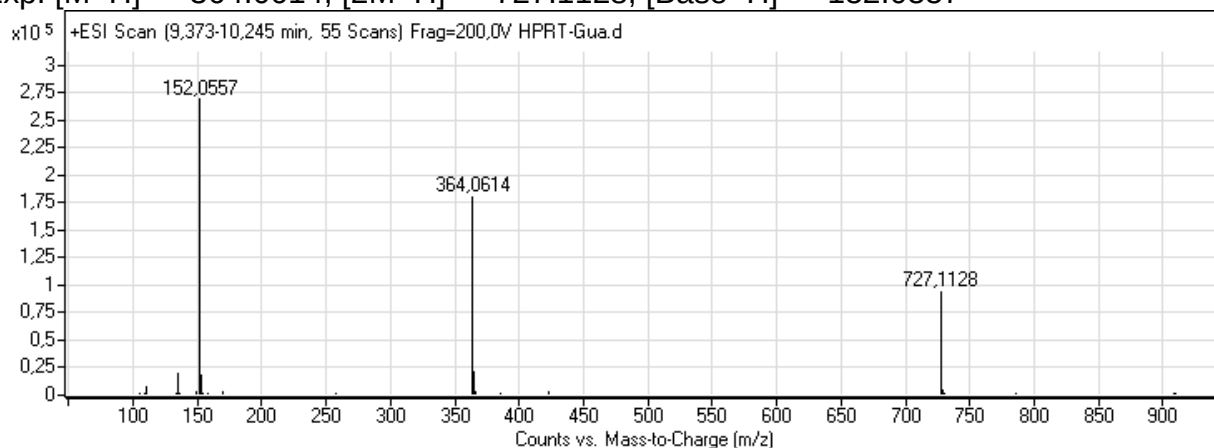


Figure S5

2. Mass spectra were measured on Agilent 1100 LC/MSD VL (Agilent Technologies) equipped APCI and ESI source in negative mode of ionization, 1100 DAD and ELSD PL-ELS 1000 (Polymer Laboratories).

2.1. 9-(β -D-ribofuranosyl)-6-mercaptopurine 5'-monophosphate

$C_{10}H_{13}N_4O_7P_1S_1$

Calc. $[M]^- = 363.0$

Exp. $[M]^- = 362.8$

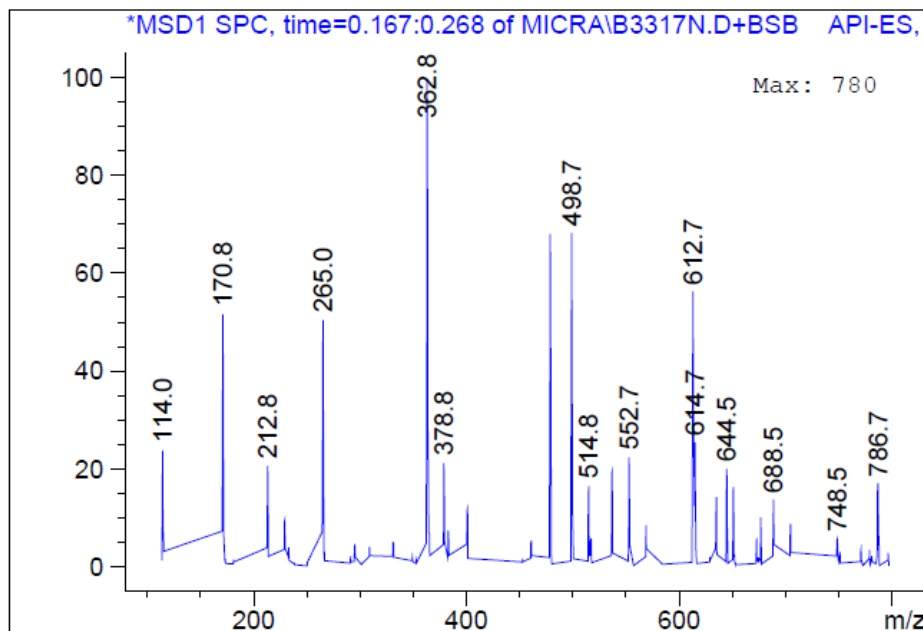


Figure S6

2.2. 5-Amino-3-(β -D-ribofuranosyl)-1,2,3-triazolo[4,5-d]pyrimidin-7-one 5'-monophosphate

$C_9H_{13}N_6O_8P_1$

Calc. $[M]^- = 363.0$

Exp. $[M]^- = 362.8$

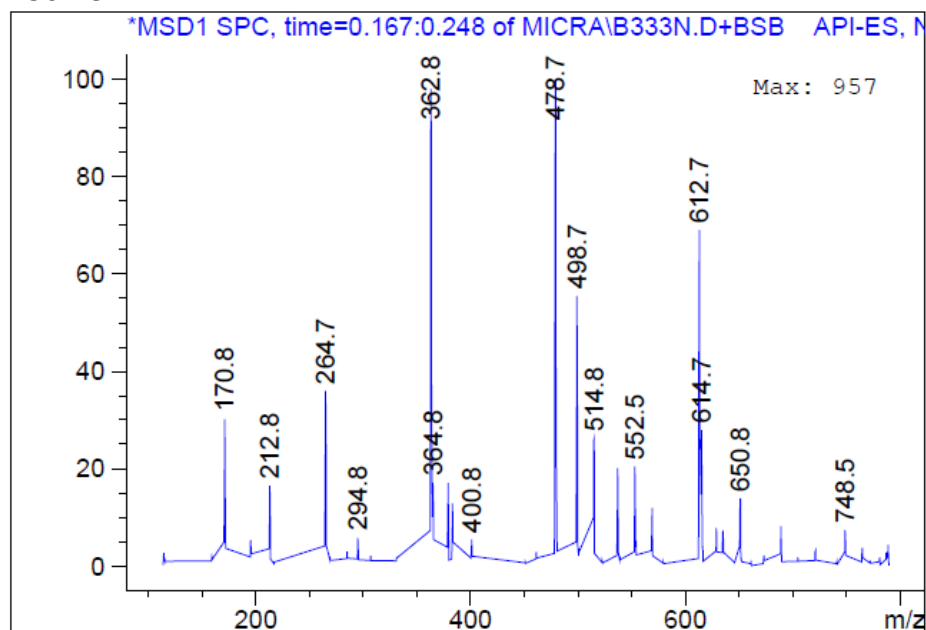


Figure S7

2.3. Inosine 5'-monophosphate

$C_{10}H_{13}N_4O_8P_1$

Calc. $[M]^- = 347.0$

Exp. $[M]^- = 347.0$

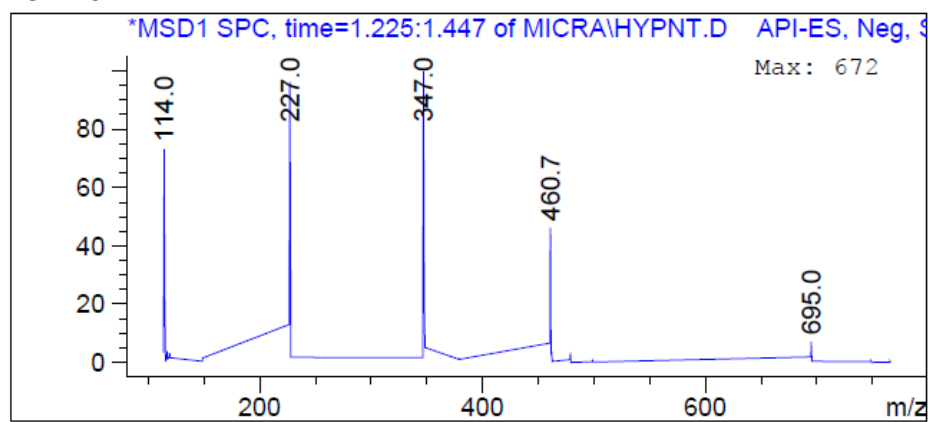


Figure S8

3. NMR data of 2-Cl-AMP

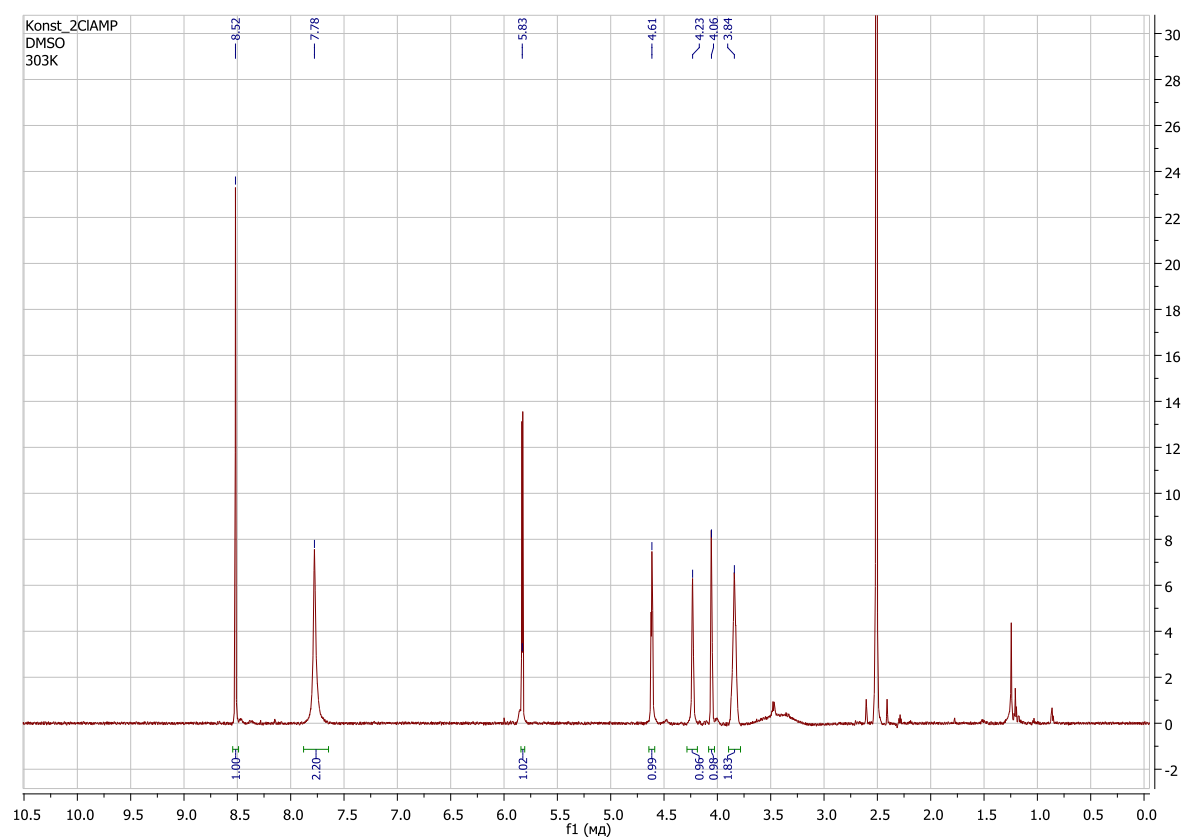


Figure S9. 1H NMR spectrum of 2-Cl-AMP

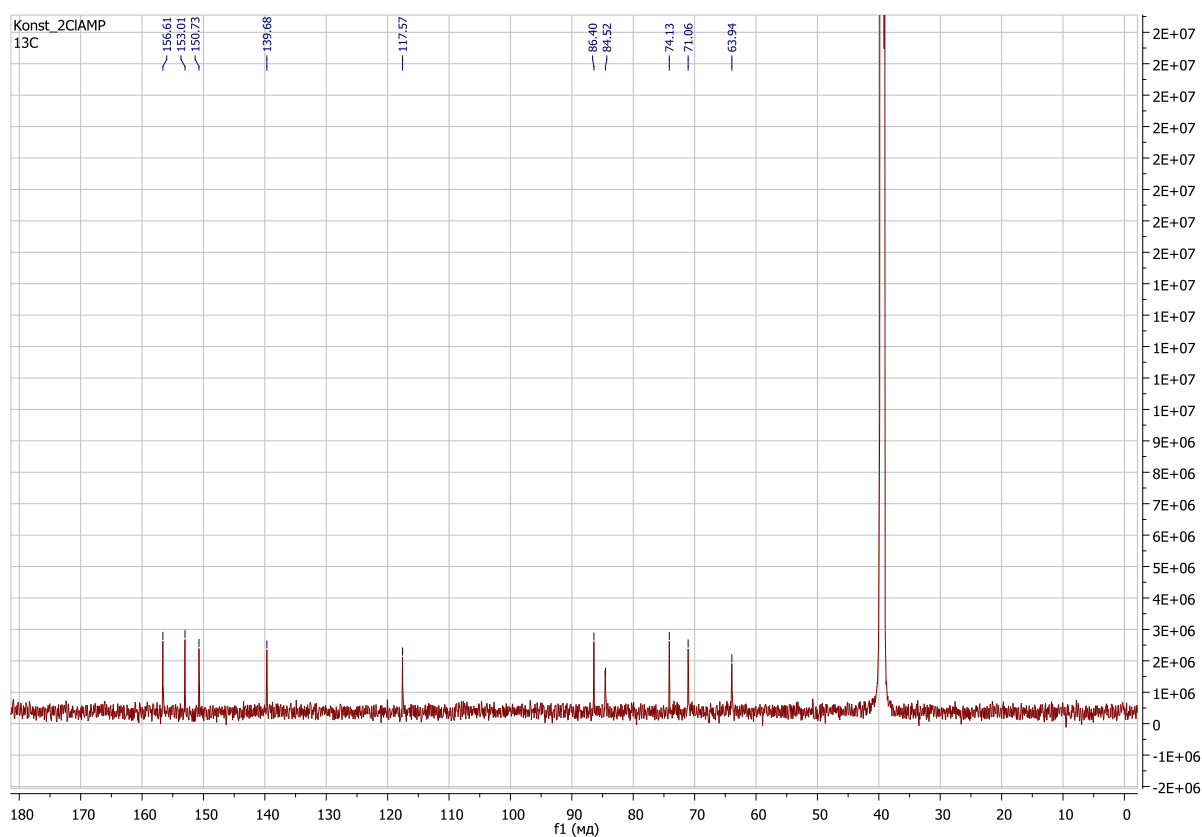


Figure S10. ^{13}C NMR spectrum of 2-Cl-AMP



Figure S11. ^1H , ^{13}C -HSQC spectrum of 2-Cl-AMP

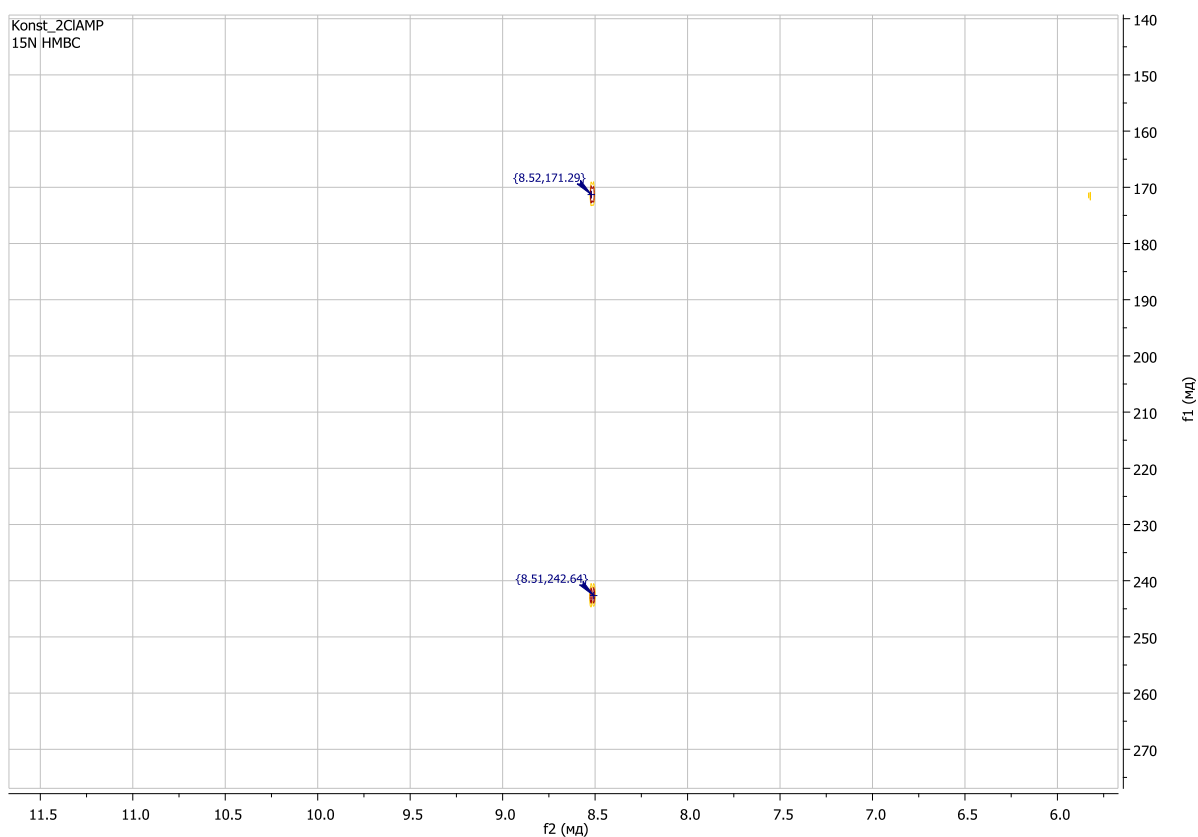


Figure S12. ^1H , ^{15}N -HMBC spectrum of 2-Cl-AMP

4. NMR data of Allop-MP

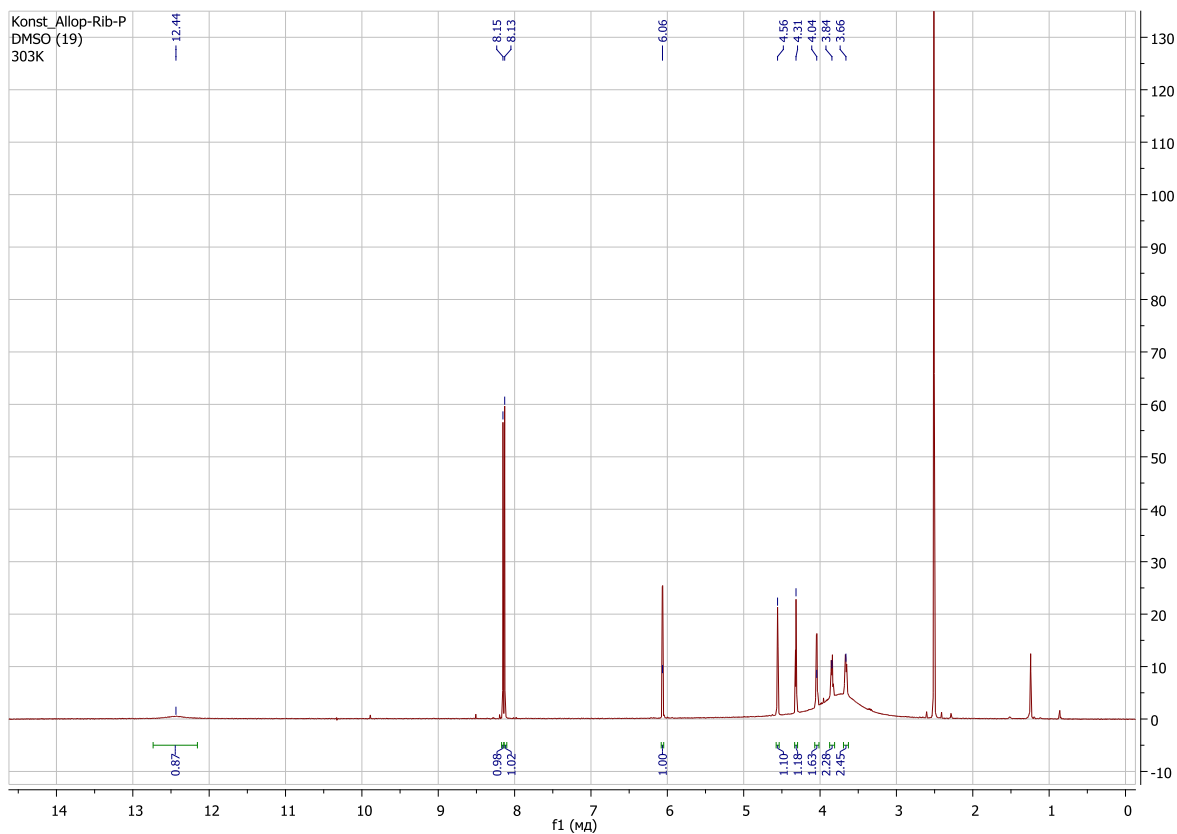


Figure S13. ^1H NMR spectrum of Allop-MP

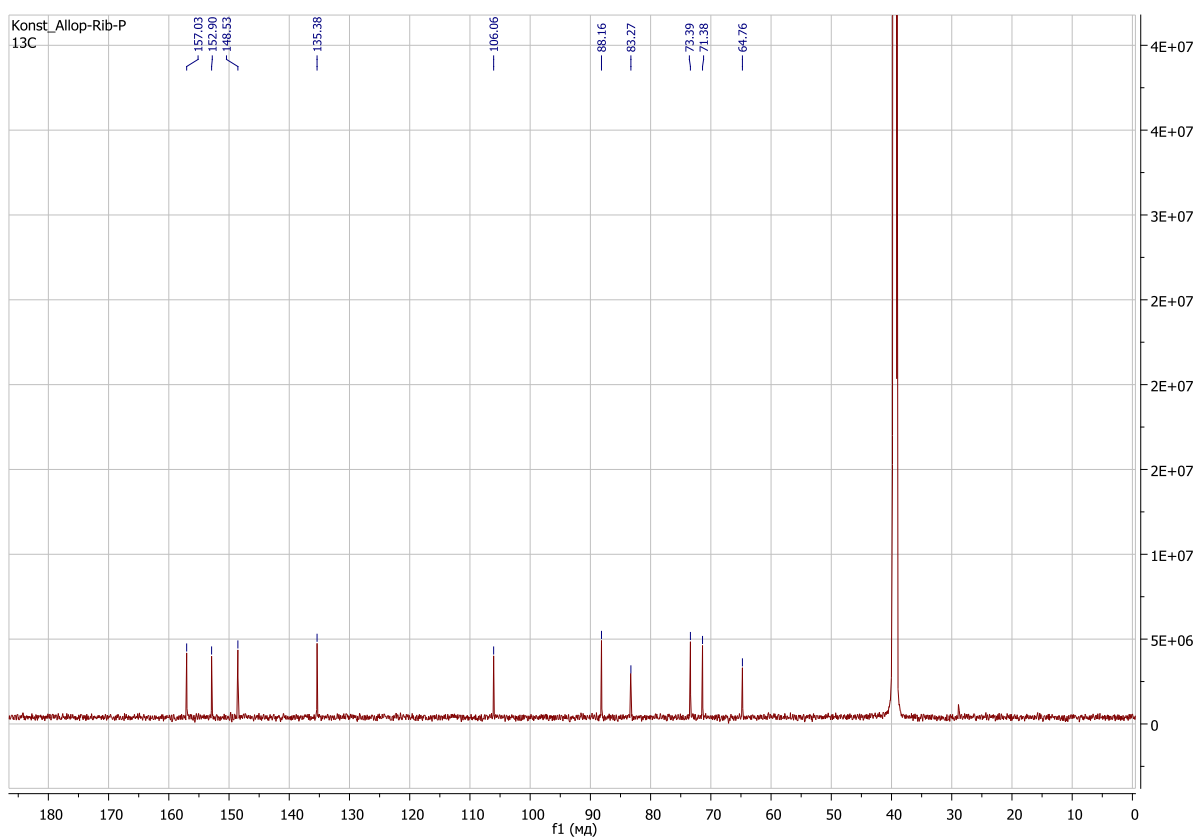


Figure S14. ^{13}C NMR spectrum of Allop-MP

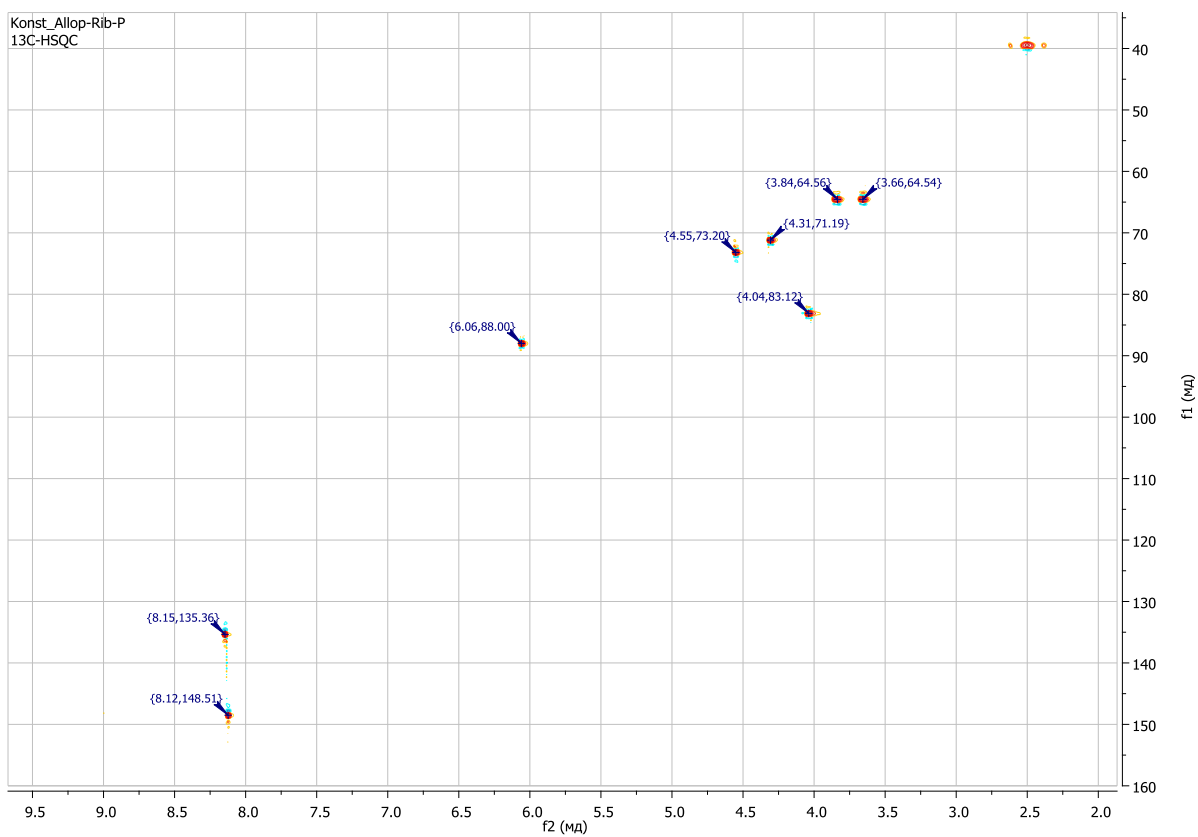


Figure S15. ^1H , ^{13}C -HSQC spectrum of Allop-MP

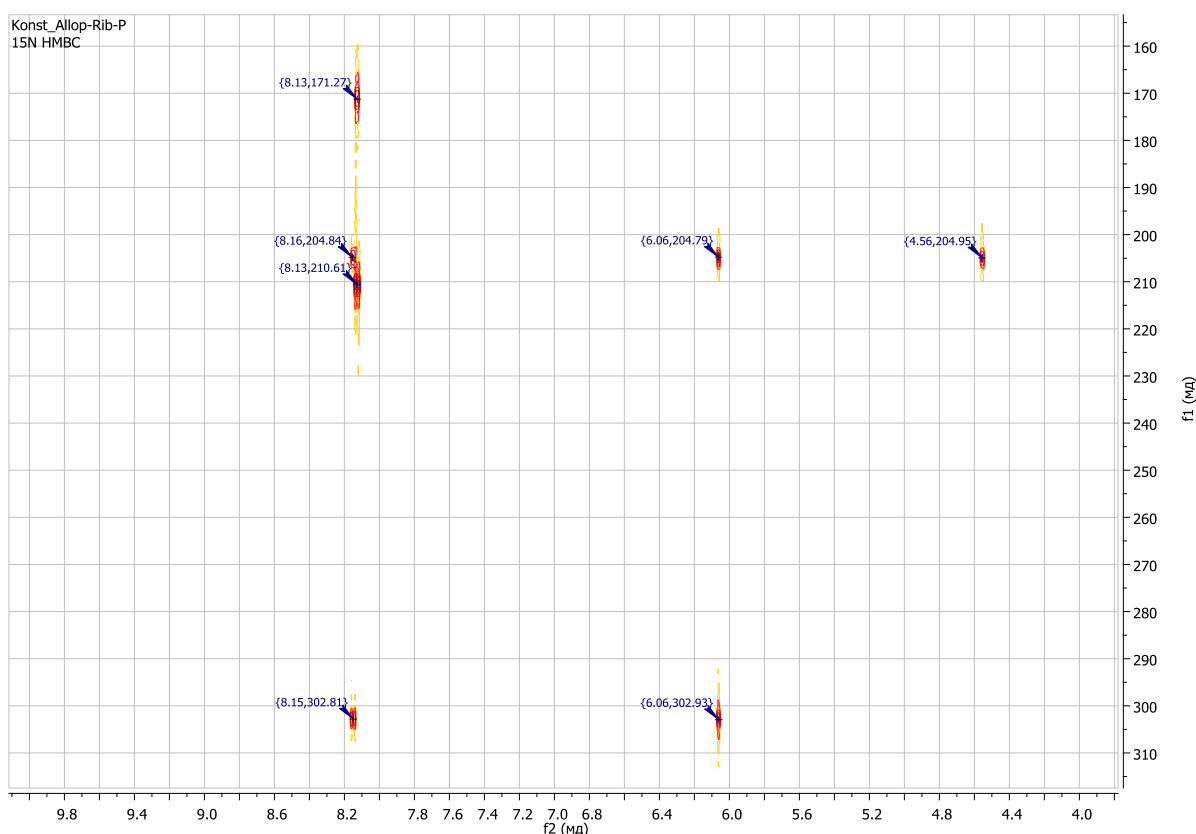


Figure S16. ^1H , ^{15}N -HMBC spectrum of Allop-MP

5. HPLC examples for nucleosides formation.

Waters 1525, column Ascentis Express C18, 2.7 μm , 3.0 x 75 mm, eluent A 0.1 % aqueous TFA, eluent B 0.1 % TFA / 70 % acetonitrile in water, detection at 254 nm, Waters 2489

Method 1: linear gradient elution from 0 to 10 % eluent B in eluent A, 20 min, flow rate 0.5 mL/min.

Method 2: elution 3 % eluent B in eluent A, flow rate 0.3 mL/min.

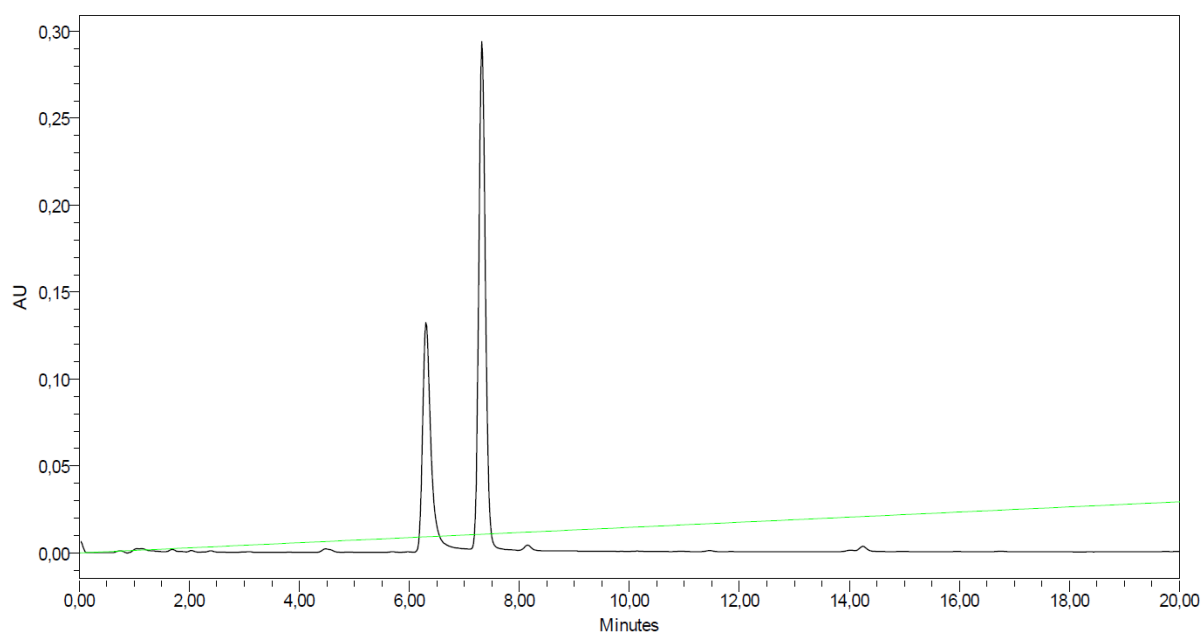


Figure S17. HPLC results for conversion 2-chloroadenine (RT = 6.3 min) to 2-Cl-AMP (RT = 7.4 min) at the presence of *Tth*APRT Method 1.

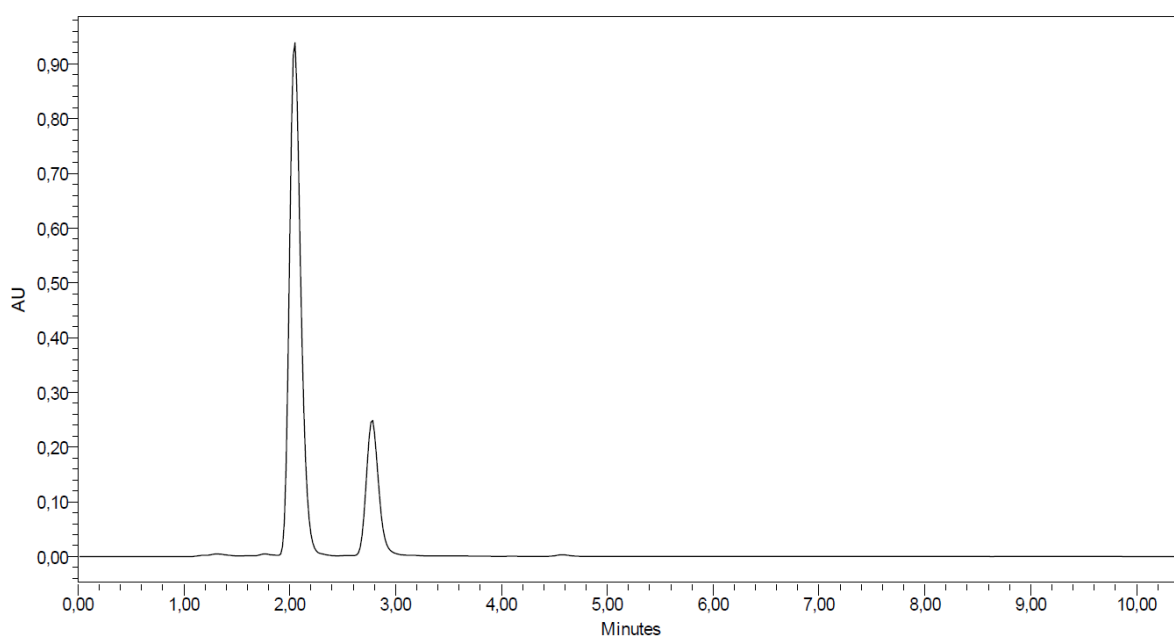


Figure S18. HPLC results for conversion 8-azaguanine (RT = 2.8 min) to 5-amino-3-(β-D-ribofuranosyl)-1,2,3-triazolo[4,5-d]pyrimidin-7-one 5'-monophosphate (RT = 2.0 min) at the presence of *Tth*HPRT Method 2.

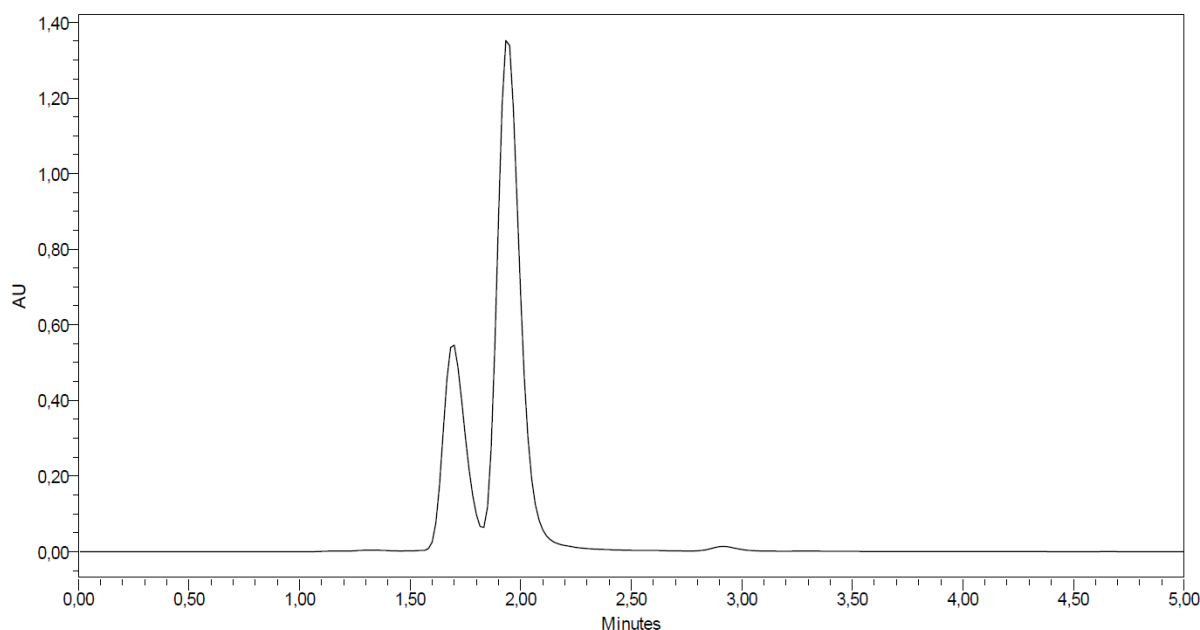


Figure S19. HPLC results for conversion adenine (RT = 2.0 min) to adenosine 5'-monophosphate (RT = 1.7 min) at the presence of *Tth*APRT Method 2.

6. Nucleotide sequence and corresponding amino acid sequence of the gene encoding the *Tth*HGPRT

ATGAAGGGCATGTTACGCGGGGCAACGGACCCGTGCAGATCAGCGCCGAGG
 CCATAAAGAAGCGGGTGGAGGAGCTGGGG**GGG**GAGATCGCCCGGGACTACCA
 GGGCAAGACCCCTCACCTGATCTGCGTCCTGAACGGGGCCTTTATCTTCATGG
 CCGACCTGGTGCGGGCCATCCCCCTGCCCTCACCATGGACTTCATCGCCATC
 AGCTCCTACGGGAACGCCTTCAAGTCCAGCGGGGAAGTGGAGCTTTTGAAGGA
 CCTCCGCCTTCCCATCCACGGCCGGGACGTGATCGTGGTGGAGGACATCGTGG
 ACACGGGGCTCACCTCTCCTACCTTCTGGACTACCTCGAGGCCCGGAAGCCC
 GCCTCCGTCCGCGTGGCCGCCCTCCTCTCCAAGCCCAGCCGCCGCCAGGTGG
 AGGTGCCCATCCACTACCTGGGCTTTGAGATTGAGGACGCCTACGTCTACGGC
 TACGGCCTGGACCGGGCCCAGTTTGACCGCAACCTGCCCTTCATCACCTCCAT
 CCGCCCGGAGGAGGAATAA

MKGMFTPGNGPVQISAEAIKKRVEELG**G**EIARDYQGKTPHLICVLNGAFIFMADLVR
 AIPLPLTMDFIAISSYGNAFKSSGEVELLKDLRLPIHGRDVIVVEDIVDTGLTLSYLLDY
 LEARKPASVRVAALLSKPSRRQVEVPIHYLGFEIEDAYVYGYGLDRAQFDRNLPFITS
 IRPEEEAAALEHHHHHHH

The mutation (codone AGG → GGG) corresponding to amino-acid substitution Arg27Gly is boldfaced

Nucleotide sequence and corresponding amino acid sequence of the gene encoding the *Tth*APRT

ATGGTGAGGACCTACCCCGTTGAGATCGCCGGCGTGCGCAGGGAGCTCCCCAT
 CGTCCAAGTGGGGCCGGGCGTGGCCGTGGCCCTGCTCAACCTCTTGGGGGAC
 ACCGAGCTCACCGAGGCGGGCCGCGAGGCGCTGGCCAAGCGGCTTCCCCCGG
 AGGTGGAGGTCCTGGTCACCCCCGAGGTCAAGGCCGTGCCCCTGGCCACGC
 CCTCTCCCGCATCACGGGCAAGCCCTACGTGGTGGCCCGCAAGACGGAGAAG
 CCCTACATGATCAACCCCGTGAGCCGCCAGGTGCTCTCCATCACCACGGGGAA
 GCCCCAGCTTCTGGTGCTGGACGGGGCCGACATCCCCCGGGTTCTGGGGCAAG
 AAGGTGGCCATCGTGGACGACGTGGTGTCCACCGGCTCCACCCTGGCGGGGC
 TGAGGGAGCTCATTGAGAGCGTGGGGGGTGAGGTGGTCGCCGTCTCGCCGT
 CTTACCGAGGGCACGCCCCGCCAGGACGTCGTGGCCCTCGGCCACCTCCCC
 CTCTTCAAGCCGGAGTAGTAA

MVRTYPVEIAGVRRELPVQVGPVAVALLNLLGDTELTEAAAEALAKRLPPEVEVLV
 TPEVKAVPLAHLASRITGKPYVVARKTEKPYMINPVSRQVLSITTGKPQLLVLDGADI
 PRVRGKKVAIVDDVVSTGSTLAGLRELIESVGGEVVAVLAVFTEGTPRQDVVALGHL
 PLFKPE

7. SDS-PAGE examples

Purification of the *Tth*HGPRT (SDS-PAGE documentation)

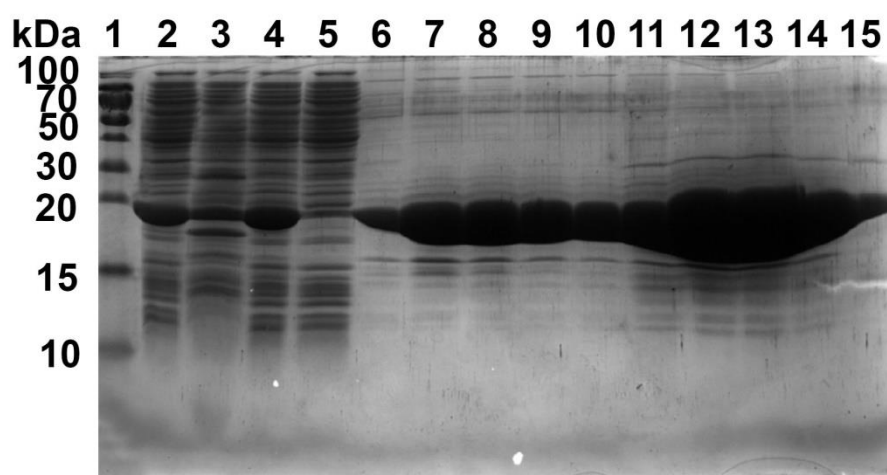


Figure S20. Protein fractions during immobilized metal affinity chromatography on Ni-IDA Sepharose column. 15% -SDS-PAGE. lane 1. molecular mass standards «PageRuler Broad Range Unstained Protein Ladder» (# 26630); lane 2, clarified cell supernatant; lane 3, denatured proteins after heating of clarified cell supernatant at 65°C for 10 min; lane 4, clarified cell supernatant after heating at 65°C for 10 min; lane 5, flow-through fraction; lanes 6 – 15, fractions after chromatography.

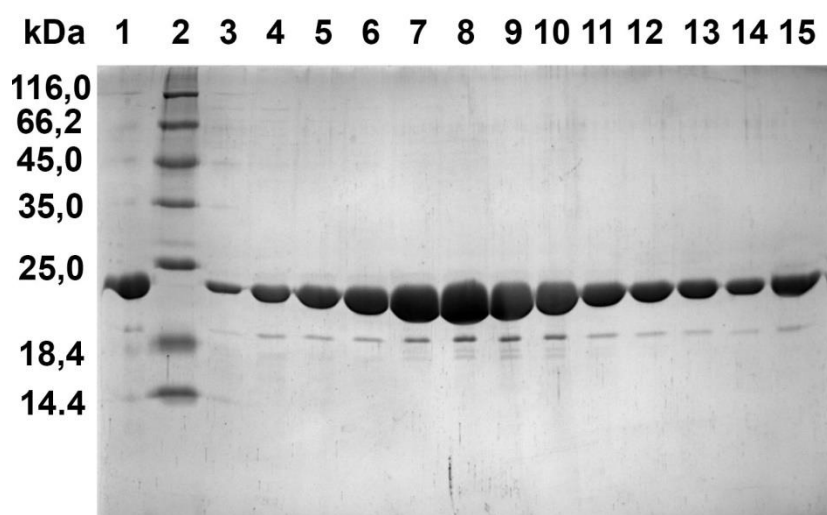


Figure 21. Protein fractions during size-exclusion chromatography on HiLoad 16/60 Superdex 75pg column. 15% -SDS-PAGE. lane 1, the combined fractions after immobilized metal affinity chromatography; lane 2, molecular mass standards «PageRuler Broad Range Unstained Protein Ladder» (# 26610); lane 3 – 15, fractions after chromatography.

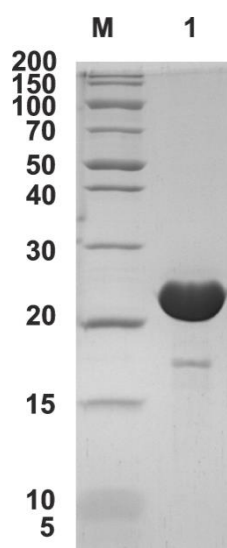


Figure S22. Purified *TthHGPRT*. 15% -SDS-PAGE. lane M, molecular mass standards «PageRuler Broad Range Unstained Protein Ladder» (# 26630); lane 1, purified *TthHGPRT*, 5 mkg

Purification of the *Tth*APRT (SDS-PAGE documentation)

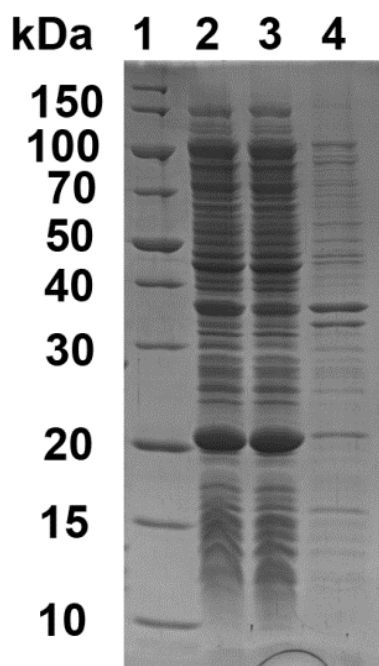


Figure S23. Protein fractions during hydrophobic chromatography on Phenyl Sepharose HP column. 15% -SDS-PAGE. . lane 1. molecular mass standards «PageRuler Broad Range Unstained Protein Ladder» (# 26630); lane 2, total cell lysate of the *E. coli* cells; lane 3, clarified cell supernatant; lane 4, the *E. coli* cells debris.

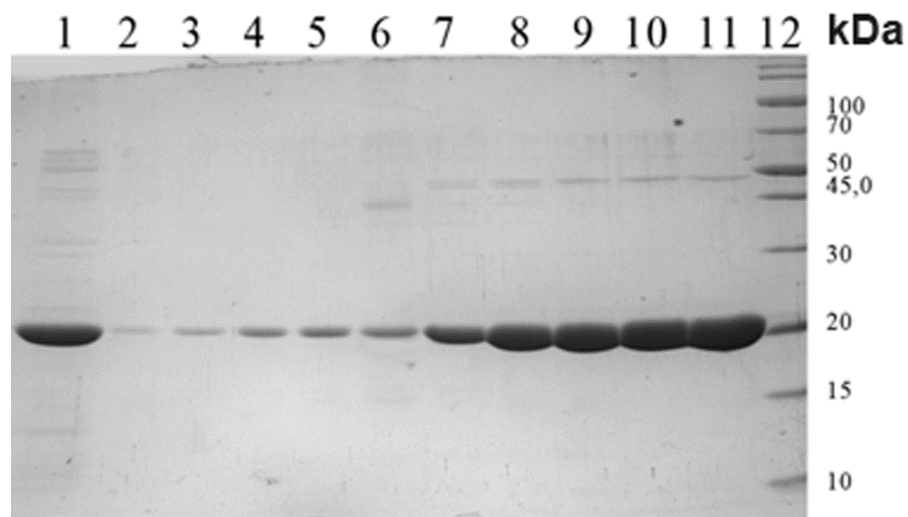


Figure S24. Protein fractions during hydrophobic chromatography on Phenyl Sepharose HP column. 15% -SDS-PAGE. lane 1, clarified cell supernatant after heating at 65°C for 10 min; lane 2 – 11, fractions after chromatography; lane 12 molecular mass standards «PageRuler Broad Range Unstained Protein Ladder» (# 26630).

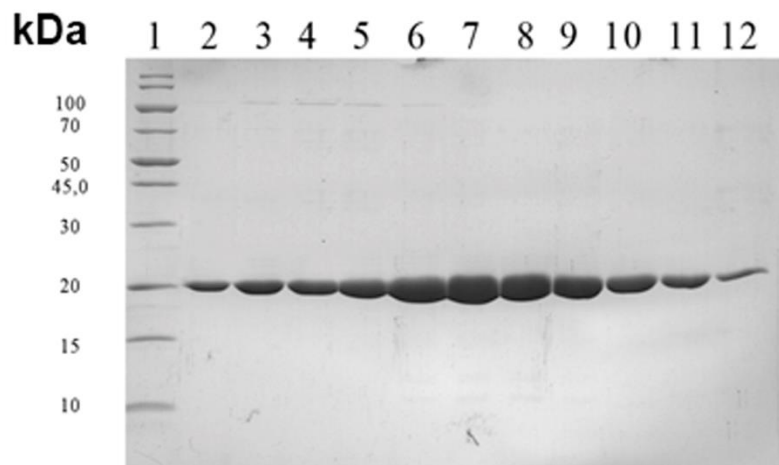


Figure S25. Protein fractions during size-exclusion chromatography on HiLoad 16/60 Superdex 200 pg column. 15% -SDS-PAGE. lane 1, molecular mass standards «PageRuler Broad Range Unstained Protein Ladder» (# 26630); lane 2 – 12, fractions after chromatography.

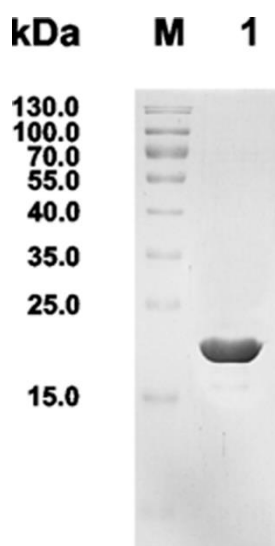


Figure S26. Purified *Tth*APRT. 15% -SDS-PAGE. lane 1, molecular mass standards «PageRuler Broad Range Unstained Protein Ladder» (# 26616). lane 2, purified *Tth*APRT, 5 mkg