Supporting Information

for

Thermophilic phosphoribosyltransferases Thermus thermophilus HB27 in nucleotide synthesis

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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
C_{10}H_{13}N_{5}O_{7}P_{1}Cl_{1}
Calc. [M+H]^+ = 382.0315

1.2. 9-(β-D-ribofuranosyl)-2-fluoroadenine 5'-monophosphate
C_{10}H_{13}N_{5}O_{7}P_{1}F_{1}
Calc. [M+H]^+ = 366.0611

1.3. 1-(β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine-4-one 5'-monophosphate
C_{10}H_{13}N_{4}O_{8}P_{1}
Calc. [M+H]^+ = 349.0545
1.4. Adenosine 5'-monophosphate
C_{10}H_{14}N_{5}O_{7}P_{1}
Calc. [M+H]^+ = 348.0705

1.5. Guanosine 5'-monophosphate
C_{10}H_{14}N_{5}O_{8}P_{1}
Calc. [M+H]^+ = 364.0654
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](image)

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](image)
2.3. Inosine 5’-monophosphate
\[ \text{C}_{10}\text{H}_{13}\text{N}_{4}\text{O}_{8}\text{P}_{1} \]
Calc. [M] = 347.0
Exp. [M] = 347.0

![Figure S8](image)

3. NMR data of 2-Cl-AMP

![Figure S9](image)
Figure S10. $^{13}$C NMR spectrum of 2-Cl-AMP

Figure S11. $^1$H, $^{13}$C-HSQC spectrum of 2-Cl-AMP
Figure S12. $^1$H, $^{15}$N-HMBC spectrum of 2-Cl-AMP

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

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

Figure S15. $^1$H, $^{13}$C-HSQC 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 S16. $^1$H, $^{15}$N-HMBC spectrum of Allop-MP
Figure S17. HPLC results for conversion 2-chloroadenine (RT = 6.3 min) to 2-Cl-AMP (RT = 7.4 min) at the presence of TthAPRT 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 TthHPRT 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*HGPR**

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ATGAAGGGCATGTTCAACGCAGGGAAGCAAACCGGTGCAGATCACGCAGCGCCGAG
CCATAAAGAAGCGGTGAGAGCTGGGGGAGGAGATCCCGCGGACTACCA
GGGCAAGACCCCTTCCTGATCTGGGTCTTGAACGCGCCGCGCTTTATCTTCATGG
CCGACCTGCTGCCGGCCATCCCTCCTGCCCTCCCCTACATGAGACTTCATCGCCATC
ACGTCCTACCGGAAAGCGGAGGAAAGTTGAGACTTTTGAAGGA
CCTCCTCCTCCATCCACCGCCCGGAGCGTATGTCGTGAGAGCATCAGTGG
ACACGGGCTCACCCTCTCTACCCCTTCTGGAATACCTGAGGCCCCGAAGCCC
GCTCCGTGCCTGGCTCCGCTGCCCTCTCCATTCCACCAGCCACGCGCCGCGCCGACGTGG
AGGTGCCCATCCACCATCGGCTTTTGAATGAGGACGCTACGTCTACGCC
TACGGCCTGGACCGGCCGAGGAGTTGAGACCAGCTCCCTACCTACCCAT
CCGCCCGGGAGAGAATAA
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MKGMFTPGNGPVQISAEAIKRRVEELGGEIARDYQGKTPLHTLCVLNQAFIFMADLVR
AIPPLTMDFIAISSYFAGKSSGHEVAKLRLPHGRDVIVVEDIVDGLTLPSYLLDY
LEARKPASVRVAALLSKPSRRQVEVPIHYLFEIedayVYGLDRAQFDRLPFITS
IRPEEEAAALEHHHHHH
```

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**
ATGGTGAGGACCTACCCGTTGAGATCGCCGGCGTGCGCAGGGAGCTCCCCATCGTCCAAGTGCCGGCCGGCGTGGCCGTGGCCCTGCTCAACCTTGCTGGGGGACACCGAGCTCACCGAGGCGGCCGCCGAGGCGCTGGCCAAGCGGCTTCCCCCGGAGGTGGAGGTCCTGGTCACCCCCGAGGTCAAGGCCGTGCCCCTGGCCCACGCCTCTCCCGCATAACGGGCAAGCCTACGTGAGCTCCATCATACACCACCGGGGAAAGCCCGAGCTCTTTGTGCTGGACGGGGCCGACATCCCGGGTTCGGGGCAAGAAGGTGGCCATCGTGGACGACGTGGTGTCCACCGCCTCCACCCTGGCGGGGTAGGGAGCTCATTGAGAGCGTGGGGGGTGAGGTGGTCGCCGTCCCTCGCCGCTTCACCGAGGCGGCCGCCAGGACGTCGTGGCCCTCGGCCACCTCCCCCTCTTCAAGGCCGGAGTAGTAA

MVRTYPVEIAGVRRELPIVQVPGPGVAVALLNLGDTELTEEAAEALAKRLPPEVEVLVTPEVKAVPLAHALSRTGKYVARKTEKPYMINPVRSQVLSITGGKPLLVLGDADI

7. SDS-PAGE examples

Purification of the *TthHGPR*T (SDS-PAGE documentation)

![SDS-PAGE Image](image)

**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 *T*thHGPRT. 15% -SDS-PAGE. lane M, molecular mass standards «PageRuler Broad Range Unstained Protein Ladder» (# 26630); lane 1, purified *T*thHGPRT, 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 \textit{Tlh}APRT. 15% -SDS-PAGE. lane 1, molecular mass standards «PageRuler Broad Range Unstained Protein Ladder» (# 26616). lane 2, purified \textit{Tlh}APRT, 5 mkg