

Enzymatic Synthesis of Modified Nucleoside 5'-Monophosphates

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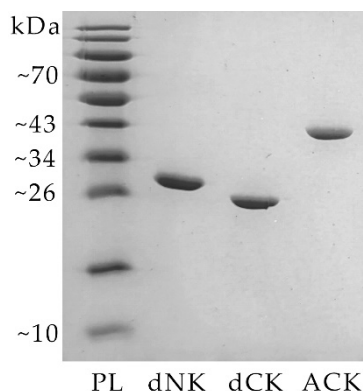


Figure S1. SDS-PAGE of the purified enzymes. PL – protein ladder; dNK – *D. melanogaster* deoxynucleoside kinase (29.1 kDa); dCK – *B. subtilis* deoxycytidine kinase (25.4 kDa); ACK – *E. coli* acetate kinase (43.3 kDa).

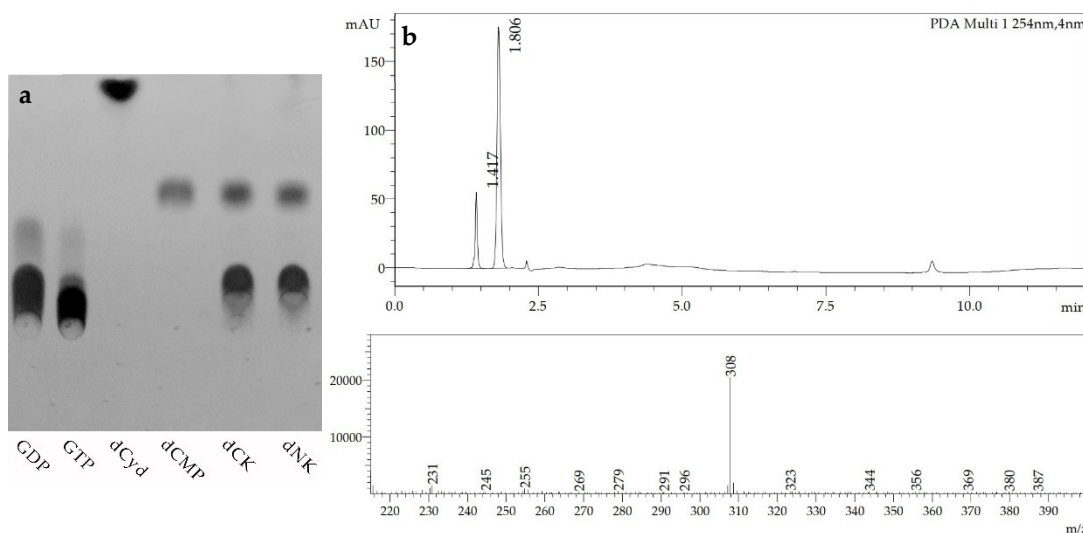


Figure S2. (a) Catalytic verification of dCK and dNK using TLC analysis; (b) HPLC-MS analyses of 2'-deoxycytidine phosphorylation by dNK. HPLC chromatogram is above. 2'-Deoxycytidine retention time (r.t.) is at around 1.4 s, 2'-deoxycytidine 5'-monophosphate r.t. is at around 1.8 s. MS spectra (below) displays positive ionization of dCMP (m/z 308 $[M+H]^+$) which represents the formed nucleotide at r.t. of 1.8 s with a molecular mass of 307 g/mol.

Reagents

Isocytidine, 2'-deoxyisocytidine, 2'-deoxycytidine, 2'-deoxyadenosine, 5-fluorocytidine, 5-methylcytidine, pseudouridine, 2-thiouridine, 4-thiouridine, 2',5'-dideoxyuridine, 5-hydroxy-2'-deoxyuridine, 2'-deoxy-4-thiouridine, 2'-O-methyl-5-methyluridine, 3'-O-benzoyl-2'-deoxyuridine, 2'-amino-2'-deoxyuridine, and 2',3'-dideoxyuridine were purchased from Carbosynth (Compton, UK). Thymidine, 2'-deoxyuridine, 2'-deoxyguanosine, guanosine, adenosine, and 2'-O-methyluridine were purchased from Alfa Aesar (Kandel, Germany). Uridine and *N*⁴-benzoyl-2'-deoxycytidine were purchased from Sigma Aldrich (Burlington, MA, USA). Cytidine and 2',3'-O-isopropylideneuridine were purchased from Acros Organics (Geel, Belgium). 3'-O-Methyluridine, 2'-(O-allyl)-uridine, 3'-(O-allyl)-uridine, and 3'-O-acetyluridine were purchased from Jena Bioscience (Jena, Germany). *N*⁴-Acetyl-2'-deoxycytidine and *N*⁴-isobutyryl-2'-deoxycytidine were purchased from Combi-Blocks (San Diego, CA, USA). *N*⁴-Hydroxycytidine was purchased from BLD Pharm (Kaiserslautern, Germany). *N*¹-Methylpseudouridine was purchased from Boc Sciences (Shirley, NY, USA). 5-Fluoro-5'-deoxyuridine was purchased from Apollo Scientific (Cheshire, UK). *N*-(1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)isonicotinamide (**17**) was synthesized as described previously[1]. (S)-2-((1-((2*R*,4*S*,5*R*)-4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)propenamide (**19**), *tert*-butyl ((S)-1-((1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-1-oxopropan-2-yl)carbamate (**20**), 2-((1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)acetamide (**21**), *tert*-butyl (2-((1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-2-oxoethyl)carbamate (**22**), 2-((1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-3-(4-hydroxyphenyl)propenamide (**23**), *tert*-butyl (1-((1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (**24**), *tert*-butyl (1-((1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**25**), and *tert*-butyl (1-((1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamate (**26**) were synthesized as described in our previous article[2]. 4-(*sec*-Butylamino)-1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1*H*)-one (**27**), 1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-(((*R*)-1-phenylethyl)amino)pyrimidin-2(1*H*)-one (**28**), 1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-(((*S*)-1-phenylethyl)amino)pyrimidin-2(1*H*)-one (**29**), 1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-(phenylamino)pyrimidin-2(1*H*)-one (**30**), 1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-((2,3,4,5,6-pentahydroxyhexyl)amino)pyrimidin-2(1*H*)-one (**31**), *N*⁴-decyl-2'-deoxycytidine (**32**), 5-fluoro-4-methylthiouridine, 4-methylthiouridine, 5-fluoro-4-ethylthiouridine, 4-ethylthiouridine, 5-fluoro-4-benzylthiouridine, 4-benzylthiouridine, and 2'-*N*-acetyl-2'-amino-2'-deoxyuridine were synthesized as described previously[3].

Acetylphosphate

75 mL 1 M aqueous solution.

³¹P NMR (D₂O, 162 Hz): δ = -2.02 (s).

Synthesized nucleoside 5'-monophosphates

2. '-Deoxycytidine 5'-monophosphate

Yield 17 mg (4%), white solid.

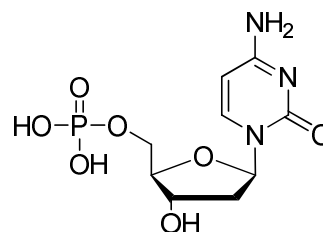
MS (ESI⁺): m/z 307.75 [M+H]⁺, 305.75 [M-H]⁻. UV (H₂O)

λ_{\max} 279 nm. $\epsilon_{279}=12100 \text{ M}^{-1}\text{cm}^{-1}$.

¹H NMR (D₂O, 400 MHz): δ = 2.22–2.30 (m, 1H, CH₂), 2.31–2.39 (m, 1H, CH₂), 3.92 (h, J = 7.2 Hz, 2H, CH₂), 4.11 (q, J = 3.9 Hz, 1H, CH), 4.49 (dt, J = 6.9, 3.6 Hz, 1H, CH), 6.06 (d, J = 7.5 Hz, 1H, CH=CH), 6.27 (t, J = 6.7 Hz, 1H, CH), 7.95 (d, J = 7.6 Hz, 1H, CH=CH).

¹³C NMR (D₂O, 101 MHz): δ = 39.38, 63.98, 71.06, 85.97, 96.62, 129.37, 141.97, 157.60, 166.17.

³¹P NMR (D₂O, 162 Hz): δ = 2.05 (s, P _{α}).



N⁴-Acetyl-2'-deoxycytidine 5'-monophosphate

Yield 11 mg (5%), white solid.

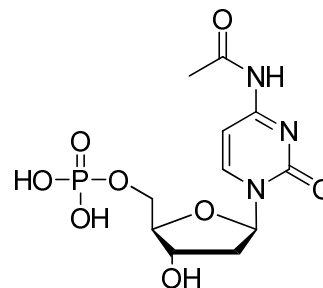
MS (ESI⁺): m/z 349.75 [M+H]⁺, 347.85 [M-H]⁻. UV (H₂O)

λ_{\max} 243; 296 nm. $\epsilon_{243}=11200 \text{ M}^{-1}\text{cm}^{-1}$, $\epsilon_{296}=6500 \text{ M}^{-1}\text{cm}^{-1}$.

¹H BMR (D₂O, 400 MHz): δ = 2.14 (s, 3H, CH₃), 2.20–2.31 (m, 1H, CH₂), 2.51–2.64 (m, 1H, CH₂), 3.66–3.86 (m, 2H, CH₂), 4.14 (q, J = 4.2 Hz, 1H, CH), 4.55 (tt, J = 7.8, 4.4 Hz, 1H, CH), 6.17 (t, J = 6.3 Hz, 1H, CH), 7.26 (d, J = 7.5 Hz, 1H, CH=CH), 8.26 (d, J = 7.5 Hz, 1H, CH=CH).

¹³C BMR (D₂O, 101 MHz): δ = 23.66, 39.40, 61.17, 72.60, 86.81, 87.39, 98.12, 145.68, 157.07, 162.62, 174.10.

³¹P BMR (D₂O, 162 Hz): δ = 3.34 (s, P _{α}).



2-Thiouridine 5'-monophosphate

Yield 16 mg (6%), white solid.

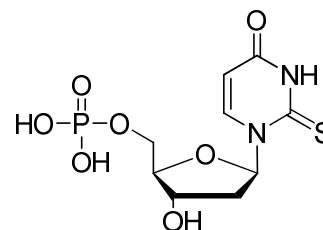
MS (ESI⁺): m/z 340.70 [M+H]⁺, 338.75 [M-H]⁻. UV (H₂O)

λ_{\max} 257 nm. $\epsilon_{257}=13500 \text{ M}^{-1}\text{cm}^{-1}$.

¹H BMR (D₂O, 400 MHz): δ = 4.13 (d, J = 13.2 Hz, 1H, CH), 4.29 (d, J = 11.9 Hz, 1H, CH), 4.40 (d, J = 5.8 Hz, 1H, CH), 4.43–4.54 (m, 2H, CH₂), 6.39 (d, J = 8.2 Hz, 1H, CH=CH), 6.69 (s, 1H, CH), 8.29 (d, J = 8.1 Hz, 1H, CH=CH).

¹³C BMR (D₂O, 400 MHz): δ = 24.29, 68.95, 74.79, 77.93, 83.46, 118.64, 142.36, 163.16, 181.96.

³¹P BMR (D₂O, 162 Hz): δ = 2.50 (s, P _{α}).



References

1. Jakubovska, J.; Tauraitė, D.; Birštonas, L.; Meškys, R. N⁴-Acyl-2'-Deoxycytidine-5'-Triphosphates for the Enzymatic Synthesis of Modified DNA. *Nucleic Acids Res.* **2018**, *46*, 5911–5923, doi:10.1093/nar/gky435.
2. Koplūnaitė, M.; Butkutė, K.; Meškys, R.; Tauraitė, D. Synthesis of Pyrimidine Nucleoside and Amino Acid Conjugates. *Tetrahedron Lett.* **2020**, *61*, 152598, doi:10.1016/j.tetlet.2020.152598.
3. Meškys, R.; Urbelienė, N.; Tiškus, M.; Preitkaitė, V.; Tauraitė, D. Hydrolases and Uses Thereof. Lithuanian patent application No. LT2022 514, 2022.