

Supplementary Materials

Multienzymatic cascades in the synthesis of modified nucleosides: comparison of the thermophilic and mesophilic pathways.

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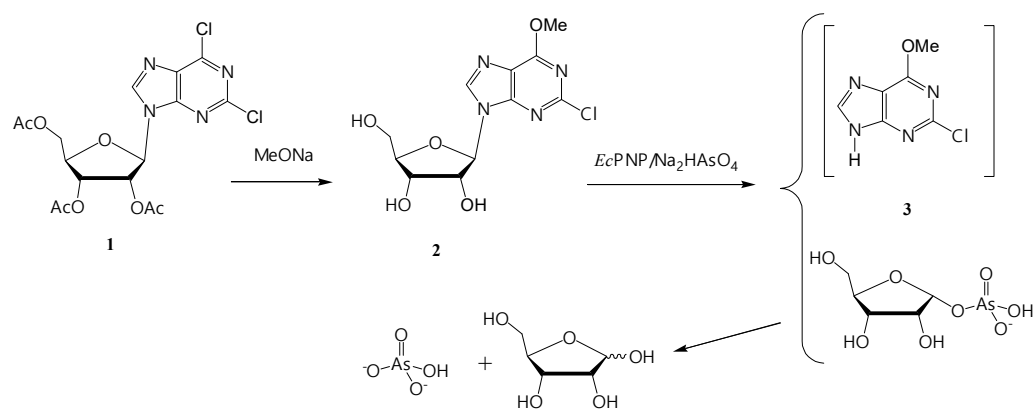


Figure S1. Synthesis of 2-chloro-6-methoxypurine (3)

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine (1)

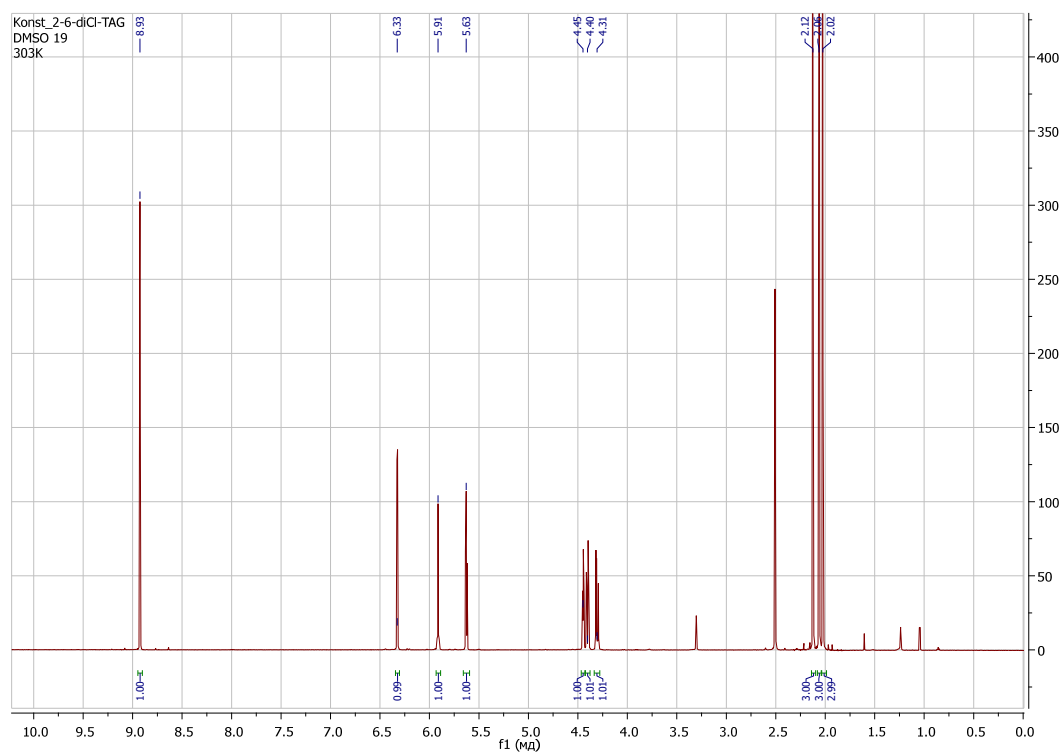


Figure S2. The ¹H NMR spectrum of compound 1

¹H NMR (700 MHz, DMSO-d₆, 30 °C): δ 8.93 (s, 1 H, H-8), 6.33 (d, $J_{H1,H2} = 5.0$ Hz, 1 H, H-1'), 5.91 (t, $J = 5.5$ Hz, 1 H, H-2'), 5.63 (t, $J = 5.6$ Hz, 1 H, H-3'), 4.45 (m, 1 H, H-4'), 4.40 (dd, $J = 3.7, 12.12$ Hz, 1 H, H-5'a), 4.31 (dd, $J = 5.5, 12.1$ Hz, 1 H, H-5'b), 2.12, 2.06, 2.02 (3 s, 9 H, 3 MeAc).^{1, 2}

2-Chloro-6-methoxy-9-(β -D-ribofuranosyl)purine (2).

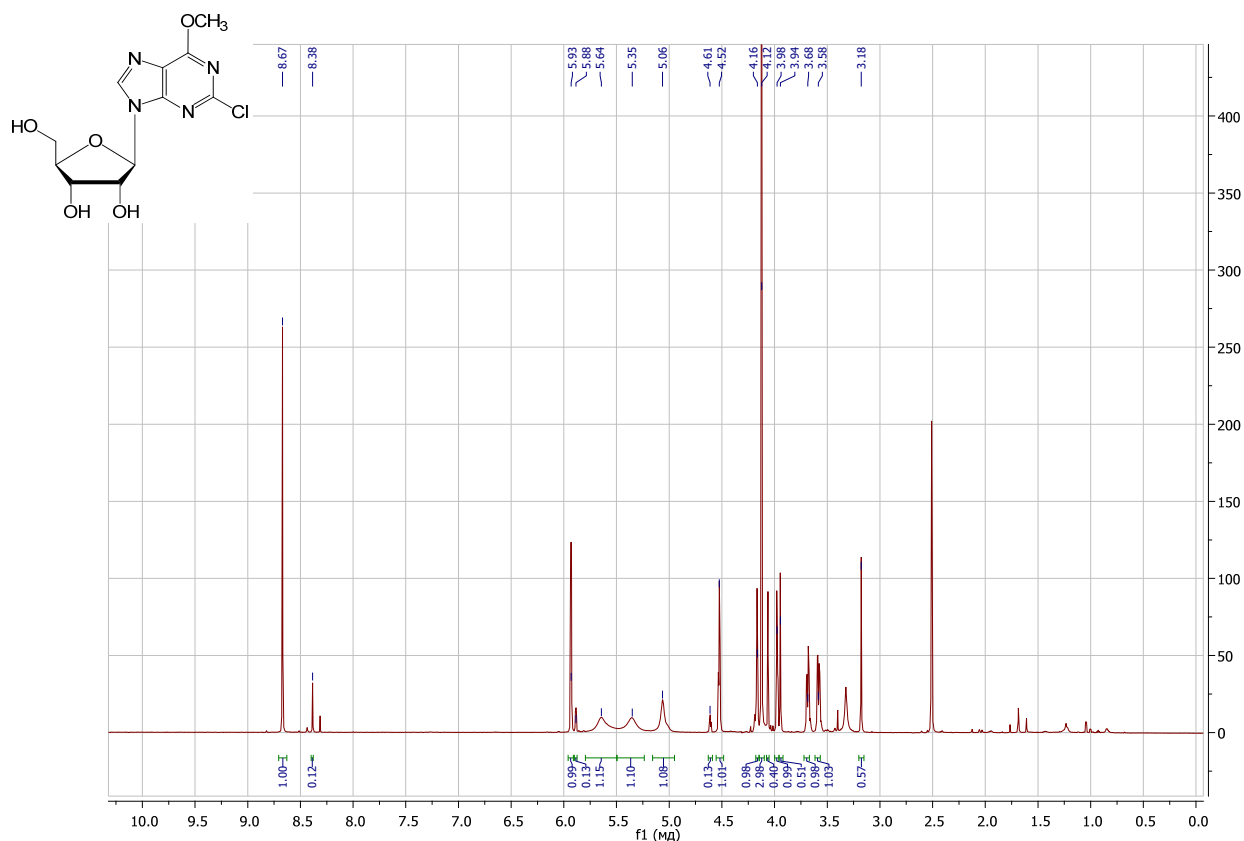


Figure S3. The ^1H NMR spectrum of compound 2

^1H NMR (700 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ =8.67 (s, 1 H, H-8), 5.93 (d, J = 5.5 Hz, 1 H, H-1'), 5.64 and 5.35 (br. s, 2 H, OH-2' and OH-3'), 5.06 (m, 1 H, OH-5'), 4.52 (t, J = 5.1 Hz, 1 H, H-2'), 4.16 (t, J = 4.2 Hz, 1 H, H-3'), 4.12 (s, 3 H, OMe), 3.98 (m, 1 H, H-4'), 3.68 (dd, J = 4.0, 12.2 Hz, 1 H, H-5'a), 3.58 (dd, J = 3.7, 12.0 Hz, 1 H, H-5'b).

^{13}C NMR (176 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ =160.78 (C-6), 152.84 (C-4), 151.43 (C-2), 142.72 (C-8), 120.19 (C-5), 87.59 (C-1'), 85.63 (C-4'), 73.85 (C-2'), 70.05 (C-3'), 61.00 (C-5'), 54.89 (OCH₃).

^{15}N NMR (71 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ =241.3 (N-7), 172.47 (N-9).

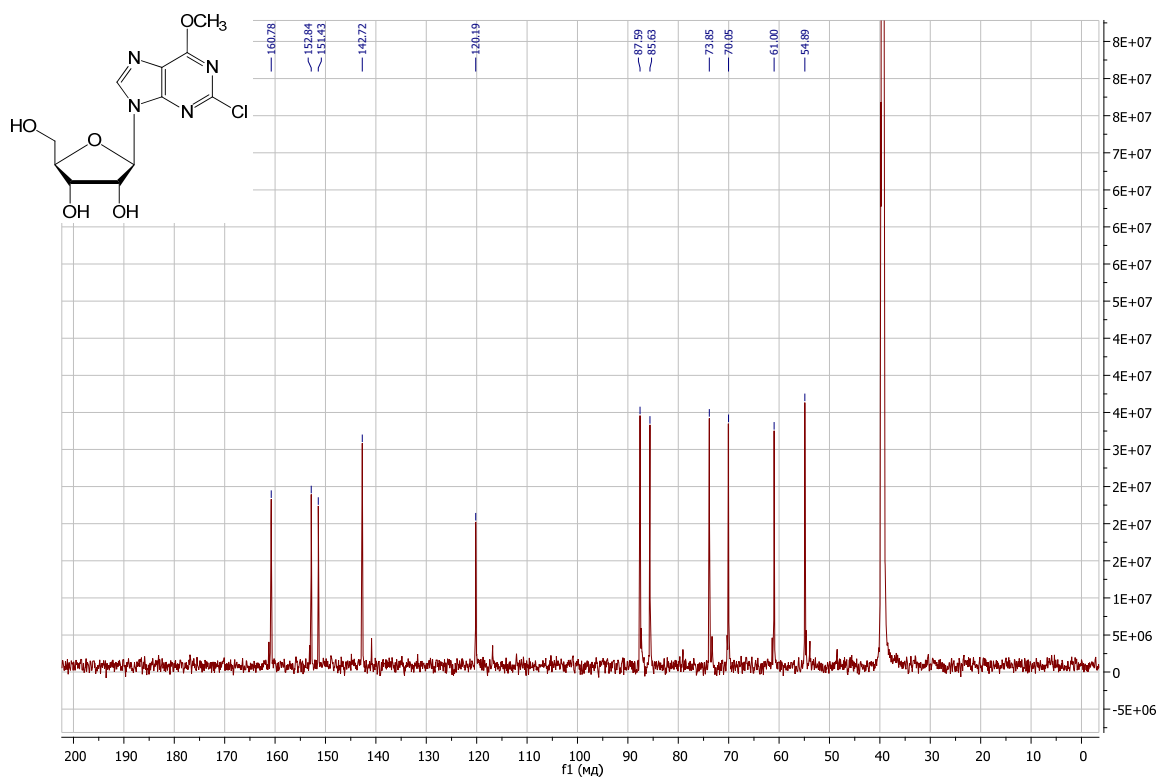


Figure S4. The ¹³C NMR spectrum of compound **2**

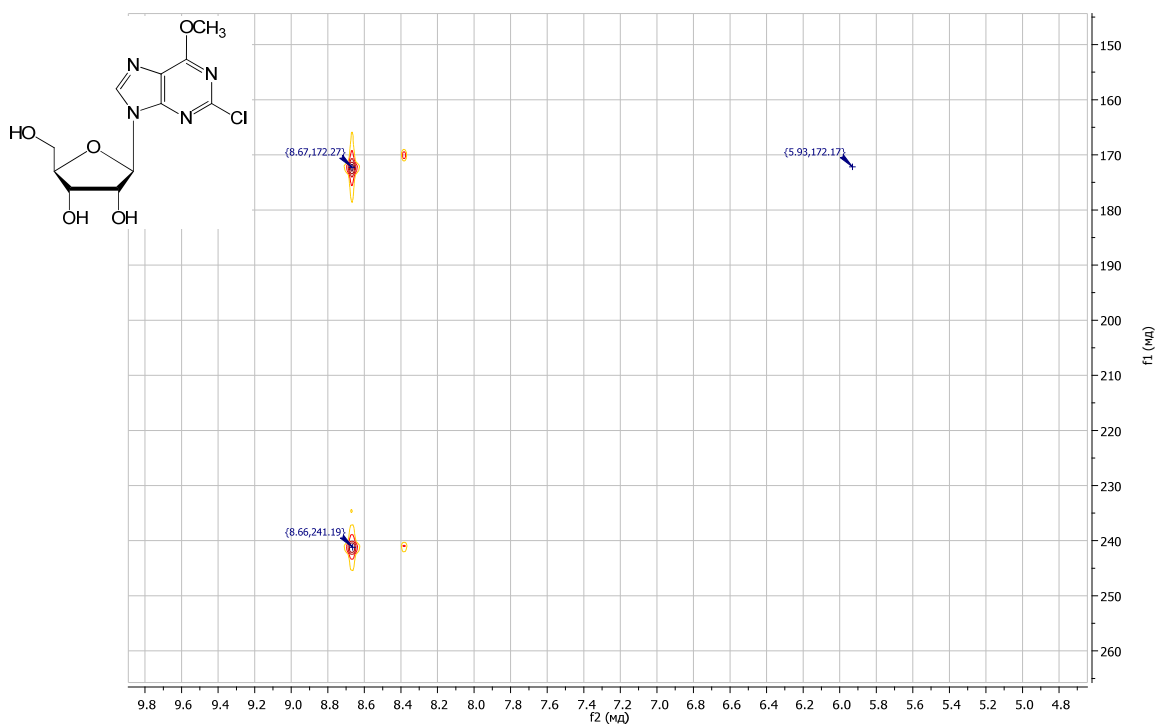


Figure S5. The fragment of ¹H-¹⁵N-HMBC NMR spectrum of nucleoside **2**

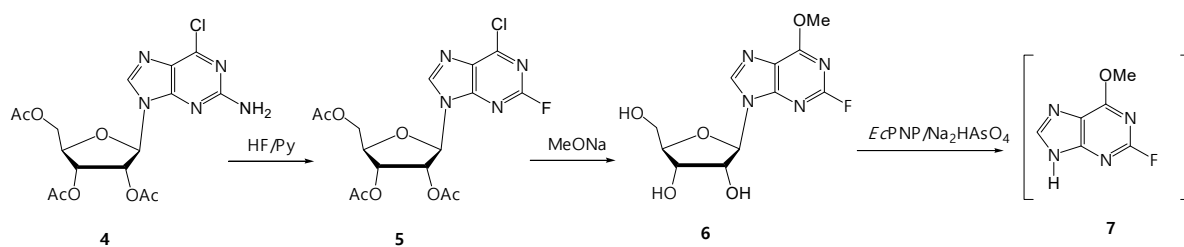


Figure S6. Synthesis of 2-fluoro-6-methoxypurine (**7**)

9-(2,3,5-Tri-*O*-acetyl- β -D-ribofuranosyl)-2-amino-6-chloropurine (4**)**

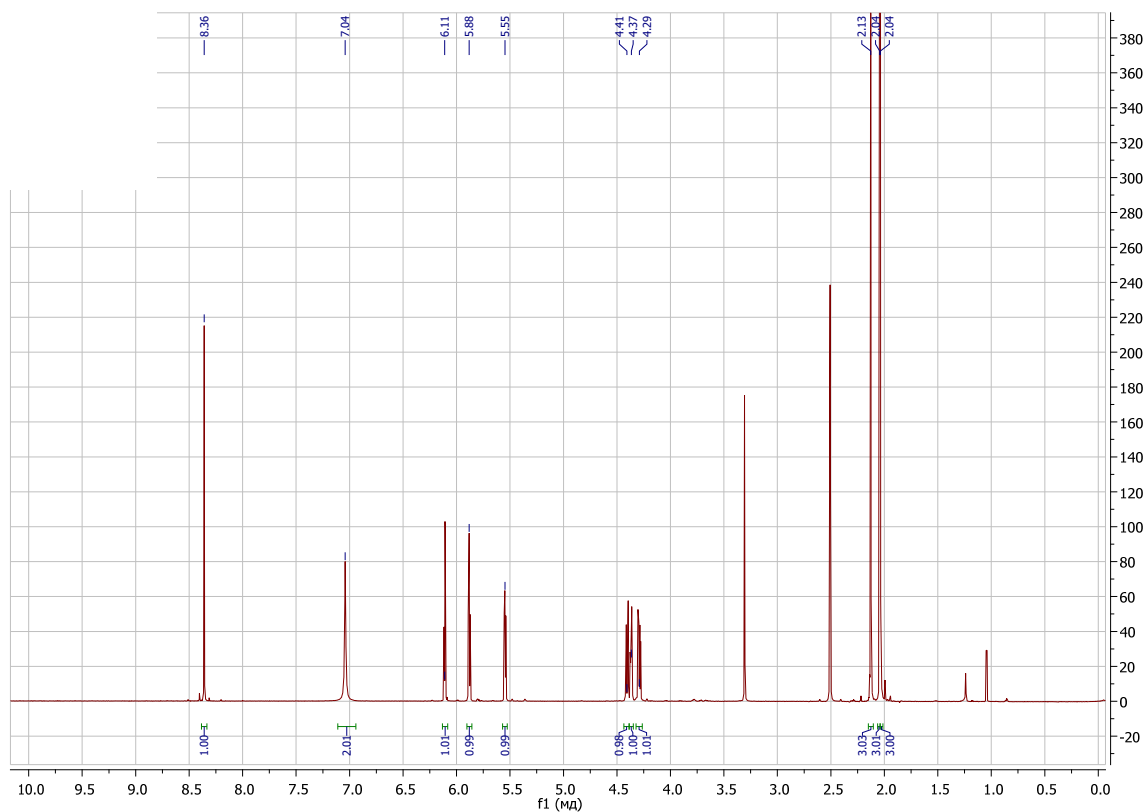


Figure S7. The ^1H NMR spectrum of compound **4**

^1H NMR (700 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ 8.36 (s, 1 H, H-8), 7.04 (s, 2 H, NH_2), 6.11 (d, $J_{\text{H1,H2}}$ = 5.8 Hz, 1 H, H-1'), 5.88 (t, J = 5.9 Hz, 1 H, H-2'), 5.55 (dd, J = 4.7, 5.7 Hz, 1 H, H-3'), 4.37 (m, 1 H, H-4'), 4.41 (dd, J = 4.1, 11.8 Hz, 1 H, H-5'a), 4.29 (dd, J = 5.8, 11.8 Hz, 1 H, H-5'b), 2.13, 2.043, 2.037 (3 s, 9 H, 3 MeAc).

9-(2,3,5-Tri-*O*-acetyl- β -D-ribofuranosyl)-2-fluoro-6-chloropurine (5)

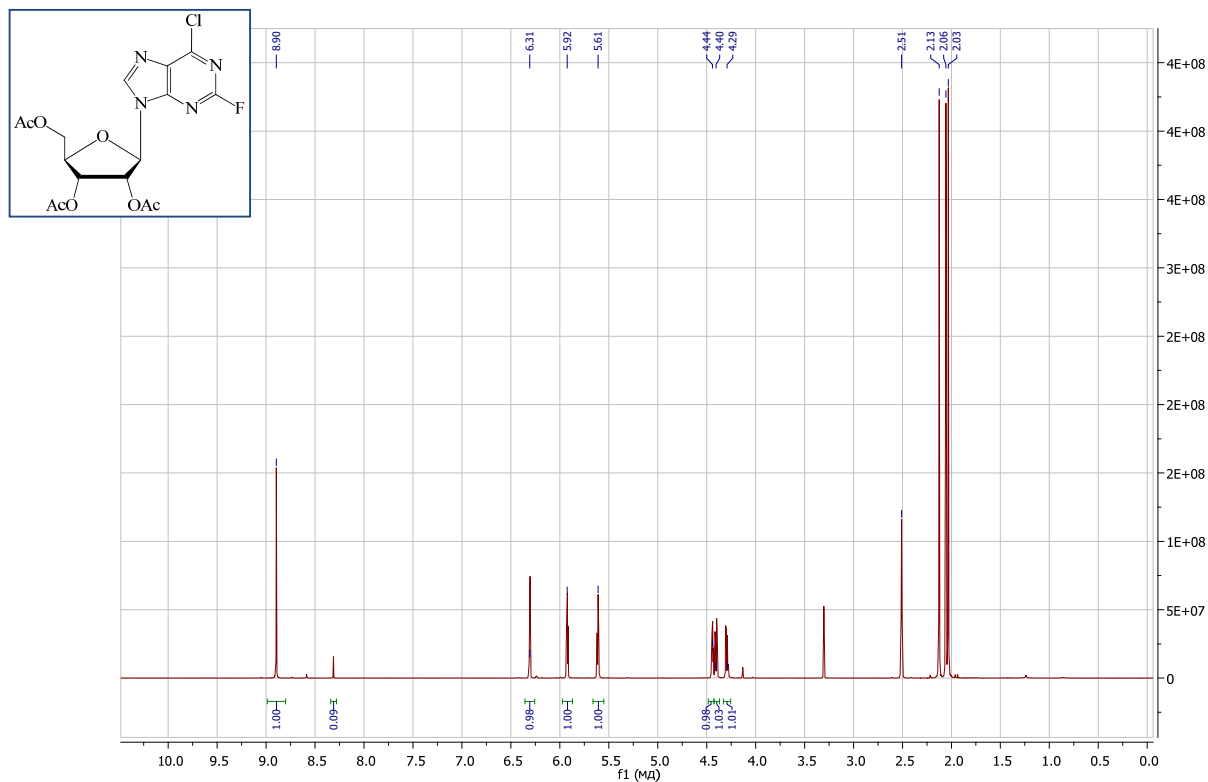


Figure S8. The ^1H NMR spectrum of compound 5

^1H NMR (700 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ = 8.90 (s, 1 H, H-8), 6.31 (d, $J_{\text{H1,H2}}$ = 5.2 Hz, 1 H, H-1'), 5.92 (t, J = 5.5 Hz, 1 H, H-2'), 5.61 (t, J = 5.6 Hz, 1 H, H-3'), 4.44 (m, 1 H, H-4'), 4.40 (dd, J = 3.4, 12.2 Hz, 1 H, H-5'a), 4.29 (dd, J = 5.4, 12.2 Hz, 1 H, H-5'b), 2.13 (s, 3 H, MeAc3'), 2.06 (s, 3 H, MeAc2'), 2.03 (s, 3 H, MeAc5').

^{13}C NMR (176 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ = 169.88 (C=O_{Ac5'}), 169.24 (C=O_{Ac3'}), 169.09 (C=O_{Ac2'}), 156.01 (d, $J_{\text{C2,F2}}$ = 215.1 Hz, C-2), 153.12 (d, $J_{\text{C4,F2}}$ = 16.6 Hz, C-4), 150.95 (d, $J_{\text{C6,F2}}$ = 17.3 Hz, C-6), 146.79 (C-8), 130.65 (C-5), 86.09 (C-1'), 79.70 (C-4'), 72.01 (C-2'), 69.63 (C-3'), 62.51 (C-5'), 20.29 (C_{MeAc5'}), 20.22 (C_{MeAc3'}), 20.07 (C_{MeAc2'}).

^{15}N NMR (71 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ = 243.03 (N-7), 169.79 (N-9).

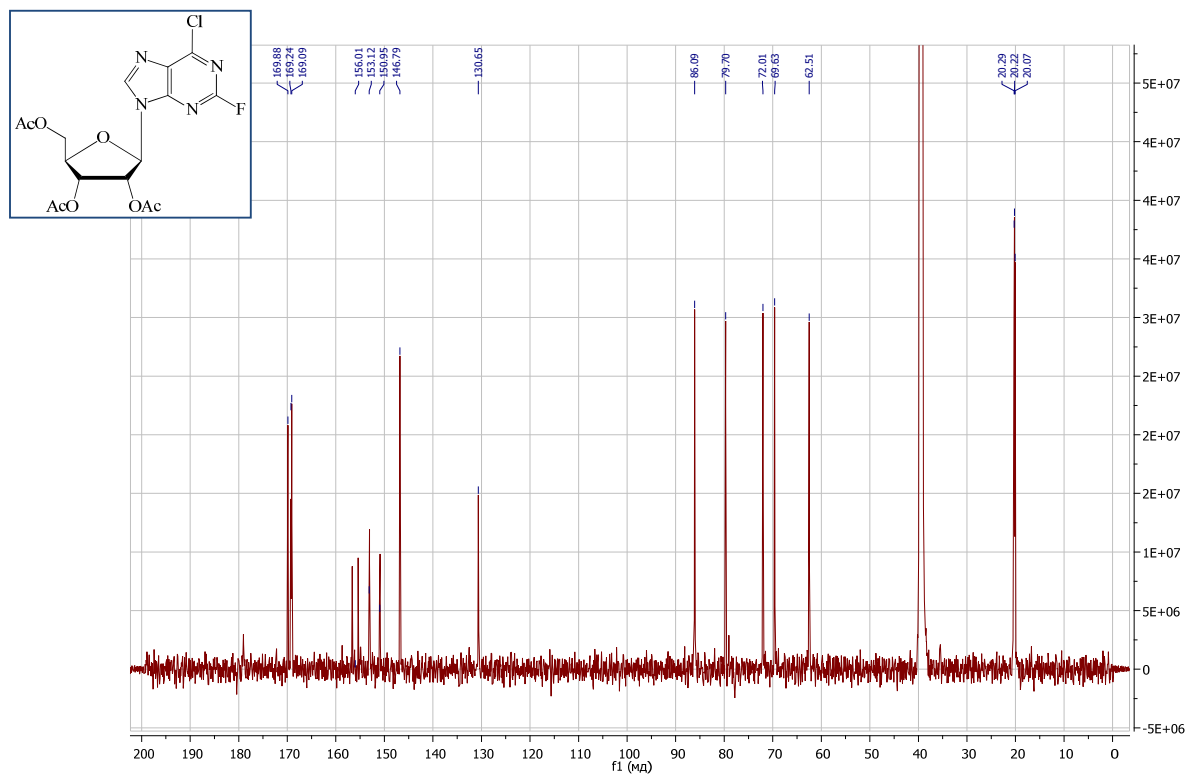


Figure S9. The ^{13}C NMR spectrum of compound 5

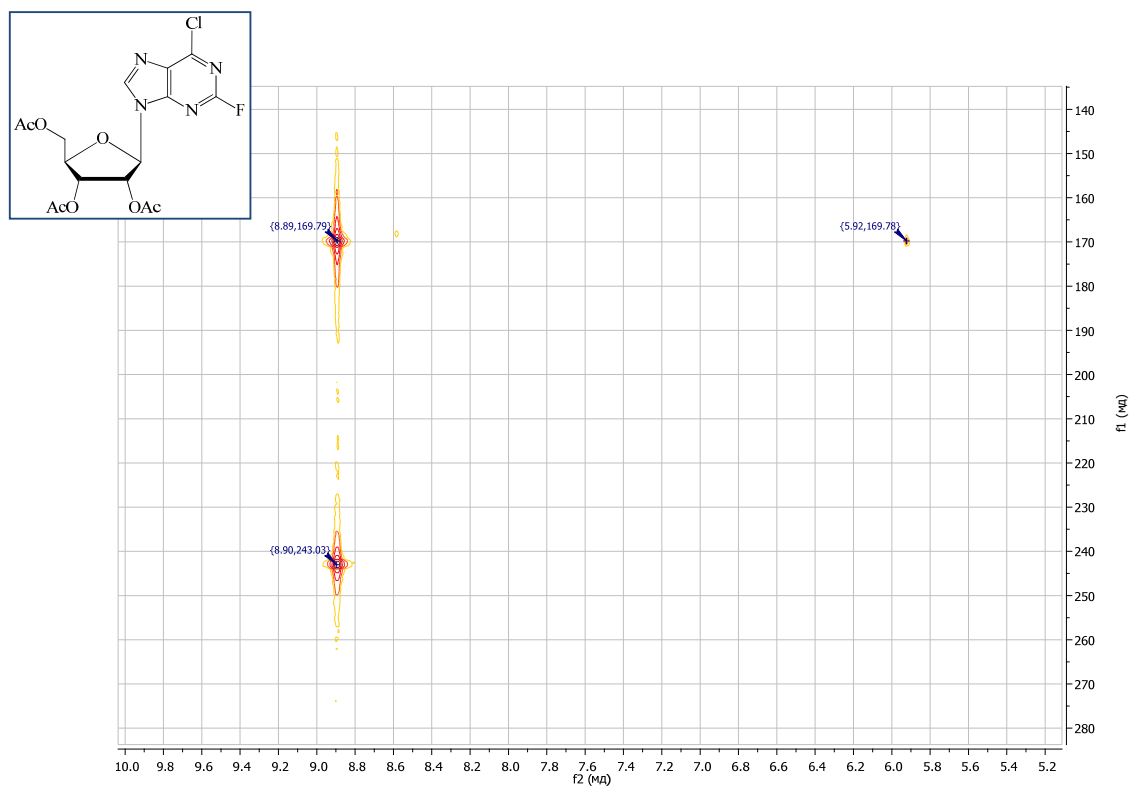


Figure S10. The fragment of ^1H - ^{15}N -HMBC NMR spectrum of nucleoside 5

2-Fluoro-6-methoxy-9-(β -D-ribofuranosyl)purine (6).

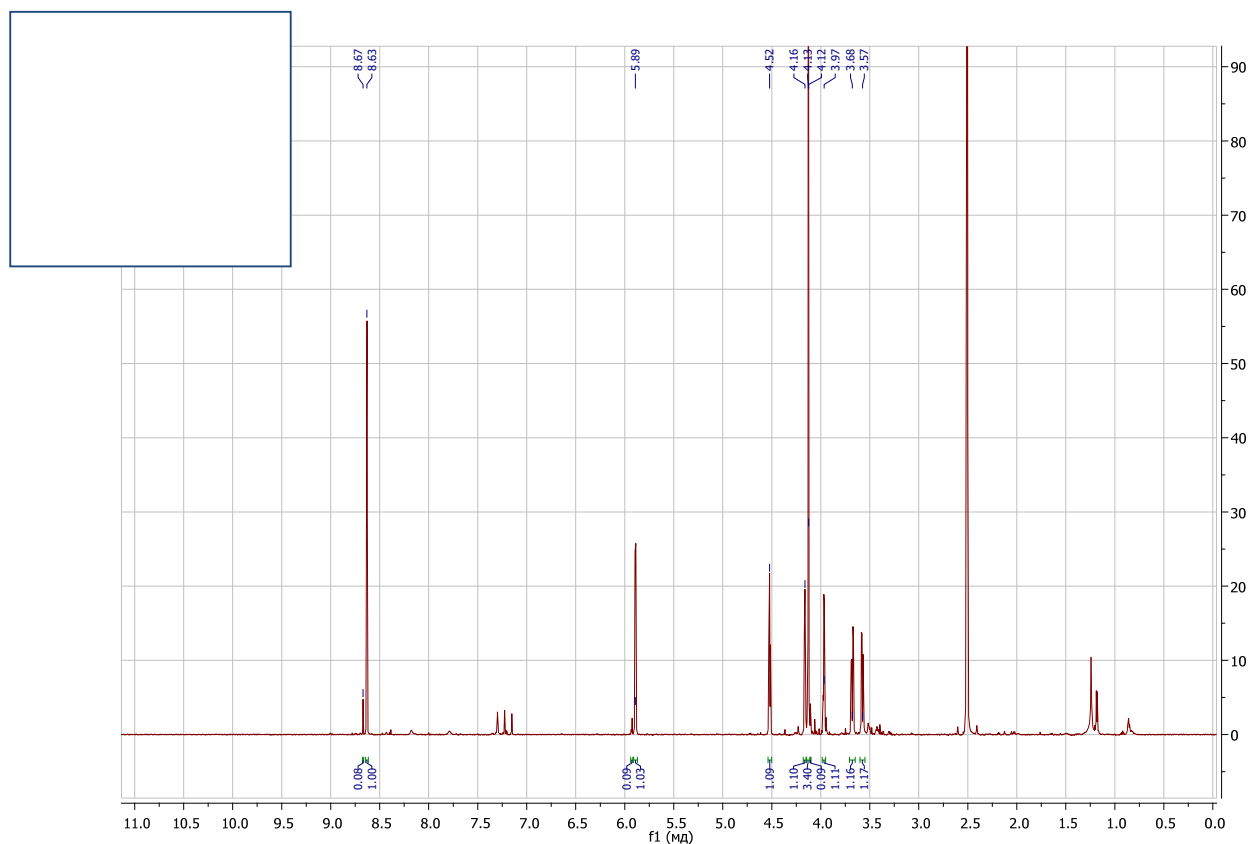


Figure S11. The ^1H NMR spectrum of compound **6**

^1H NMR (700 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ =8.63 (s, 1 H, H-8), 5.89 (d, $J_{\text{H1,H2}}$ = 5.5 Hz, 1 H, H-1'), 4.52 (t, J = 5.3 Hz, 1 H, H-2'), 4.16 (t, J = 4.3 Hz, 1 H, H-3'), 4.13 (s, 3 H, OMe), 3.97 (m, 1 H, H-4'), 3.68 (dd, J = 4.1, 12.0 Hz, 1 H, H-5'a), 3.57 (dd, J = 4.1, 12.0 Hz, 1 H, H-5'b).

^{13}C NMR (176 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ =162.16 (d, $J_{\text{C6,F}}$ = 18.0, C-6), 156.97 (d, $J_{\text{C2,F}}$ = 210.5, C-2), 152.89 (d, $J_{\text{C4,F}}$ = 18.8, C-4), 142.53 (d, $J_{\text{C8,F}}$ = 2.0, C-8), 119.35 (d, $J_{\text{C5,F}}$ = 5.0, C-5), 87.57 (C-1'), 85.54 (C-4'), 73.73 (C-2'), 70.01 (C-3'), 60.98 (C-5'), 54.96 (OCH₃).

^{15}N NMR (71 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ =241.76 (N-7), 172.78 (N-9).

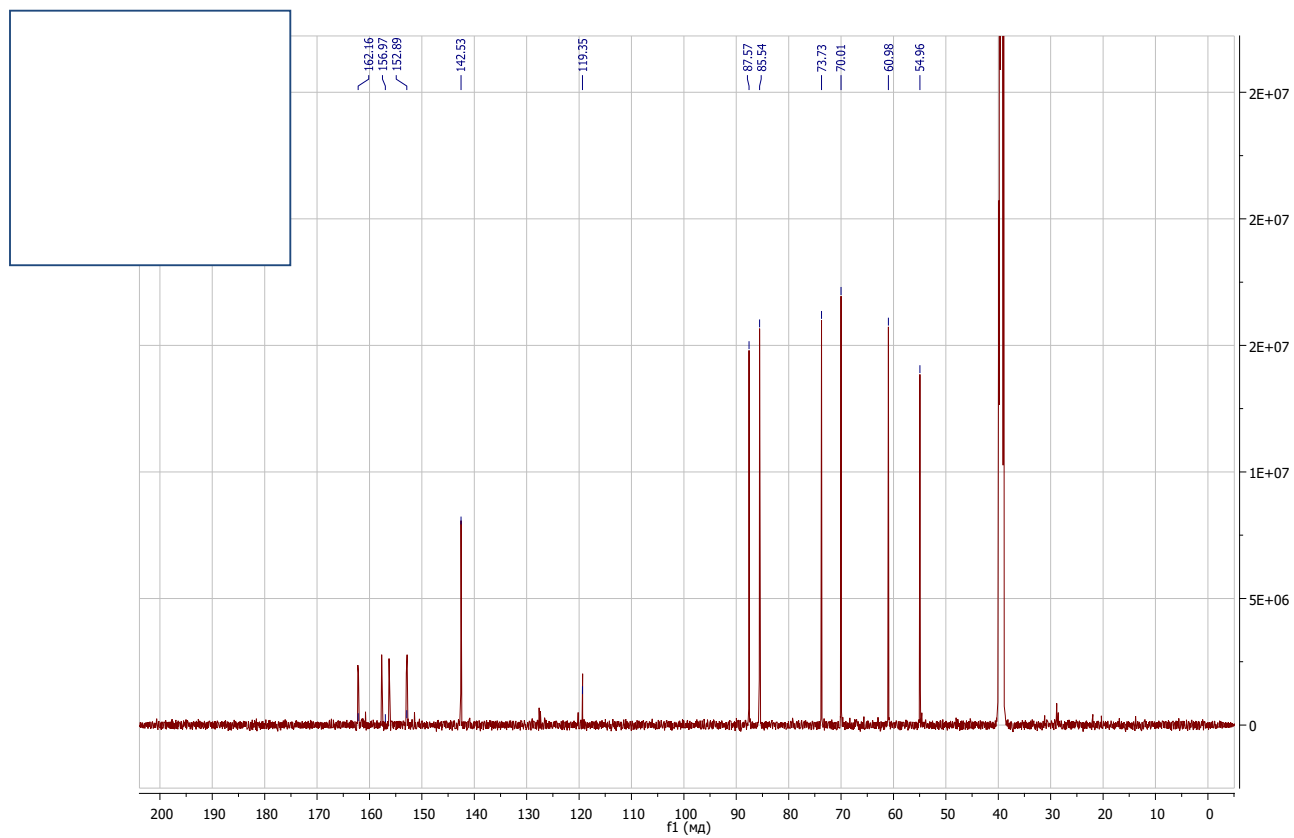


Figure S12. The ^{13}C NMR spectrum of compound 6



Figure S13. The fragment of ^1H - ^{15}N -HMBC NMR spectrum of nucleoside 6

5-Amino-3-(β -D-ribofuranosyl)triazolo[4,5-d]pyrimidin-7-one (8).

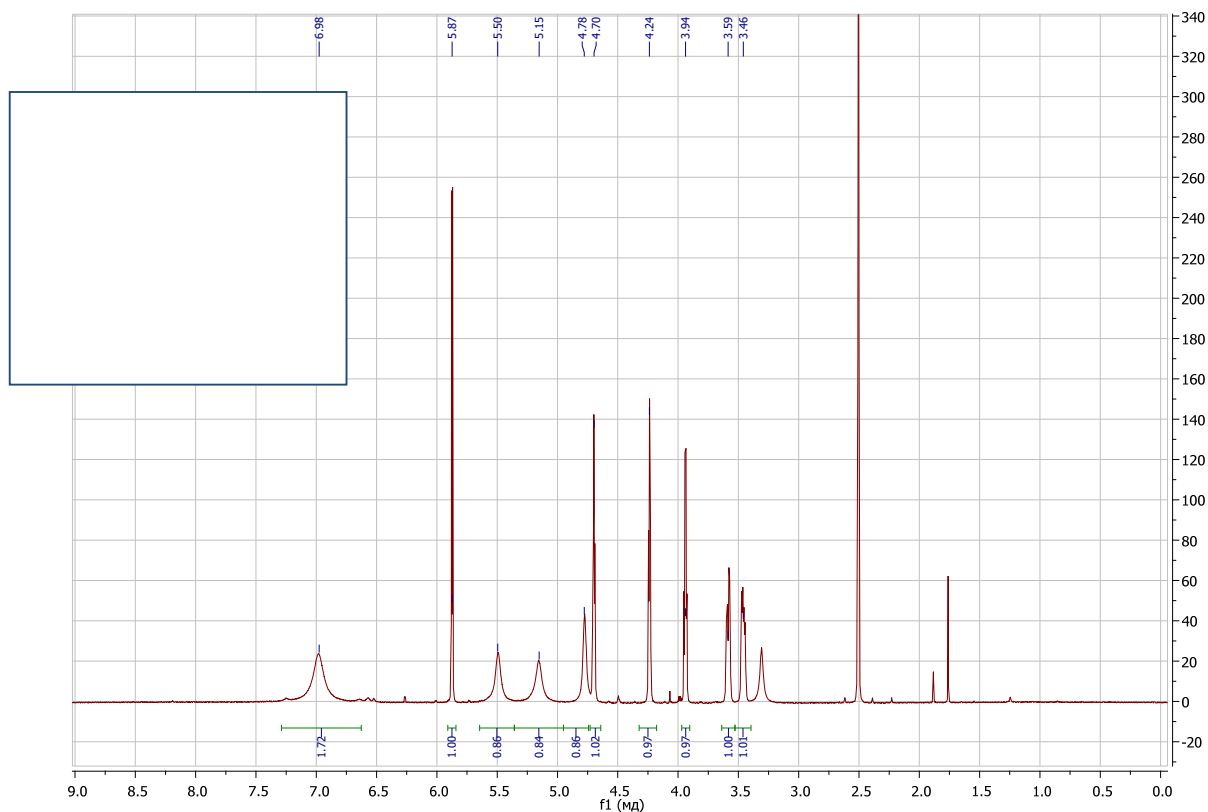


Figure S14. The ^1H NMR spectrum of compound **8**

^1H NMR (700 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ =6.98 (br.s, 2 H, NH_2), 5.87 (d, J = 4.8 Hz, 1 H, H-1'), 5.50 (s, 1 H, OH-2'), 5.15 (br. s, 1 H, OH-3'), 4.78 (m, 1 H, OH-5'), 4.70 (t, J = 4.9 Hz, 1 H, H-2'), 4.24 (t, J = 4.8 Hz, 1 H, H-3'), 3.94 (m, 1 H, H-4'), 3.59 (dd, J = 4.6, 11.9 Hz, 1 H, H-5'a), 3.46 (dd, J = 5.9, 11.7 Hz, 1 H, H-5'b).

^{13}C NMR (176 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ =151.73 (C-4), 87.75 (C-1'), 85.28 (C-4'), 72.47 (C-2'), 70.30 (C-3'), 61.62 (C-5').

^{15}N NMR (71 MHz, DMSO- d_6 , 30 $^\circ\text{C}$): δ =234.90, 156.95.

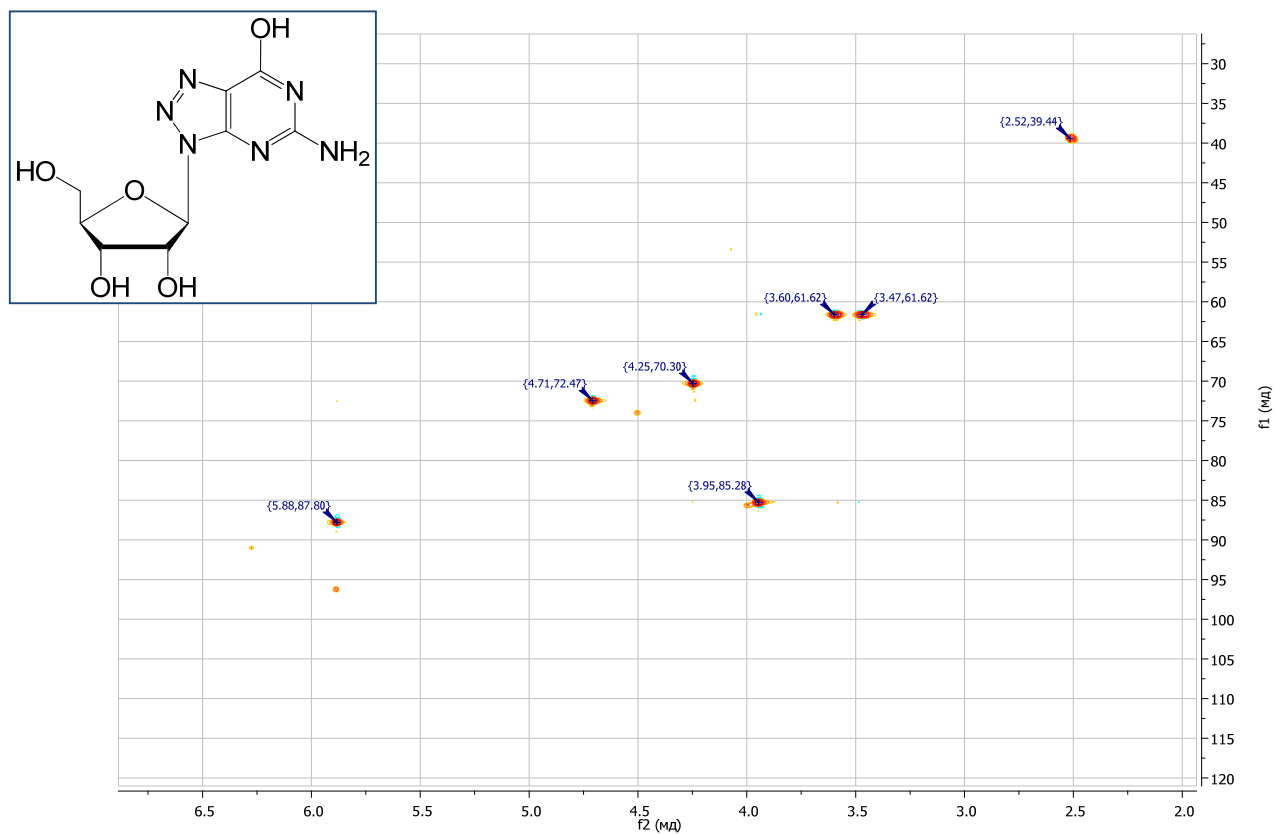


Figure S15. The fragment of ^1H - ^{13}C -HSQC NMR spectrum of nucleoside 8

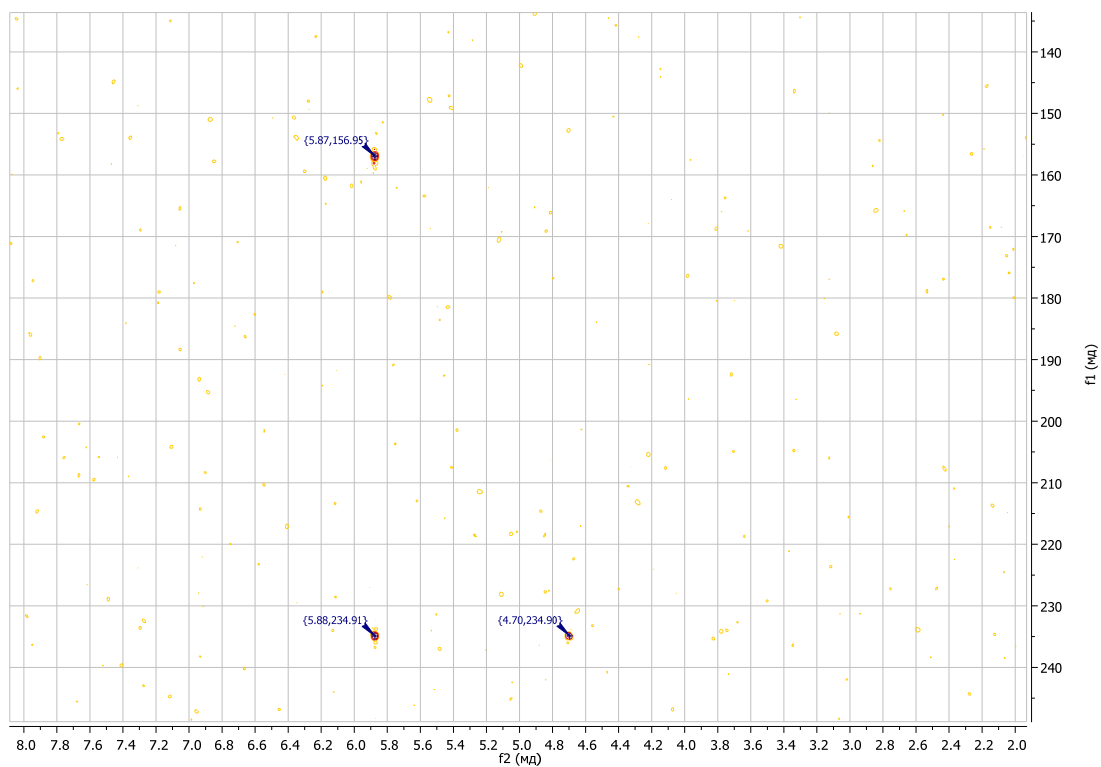


Figure S16. The fragment of ^1H - ^{15}N -HMBC NMR spectrum of nucleoside 8

1-(β -D-Ribofuranosyl)pyrazolo[3,4-d]pyrimidine-4-one (9).

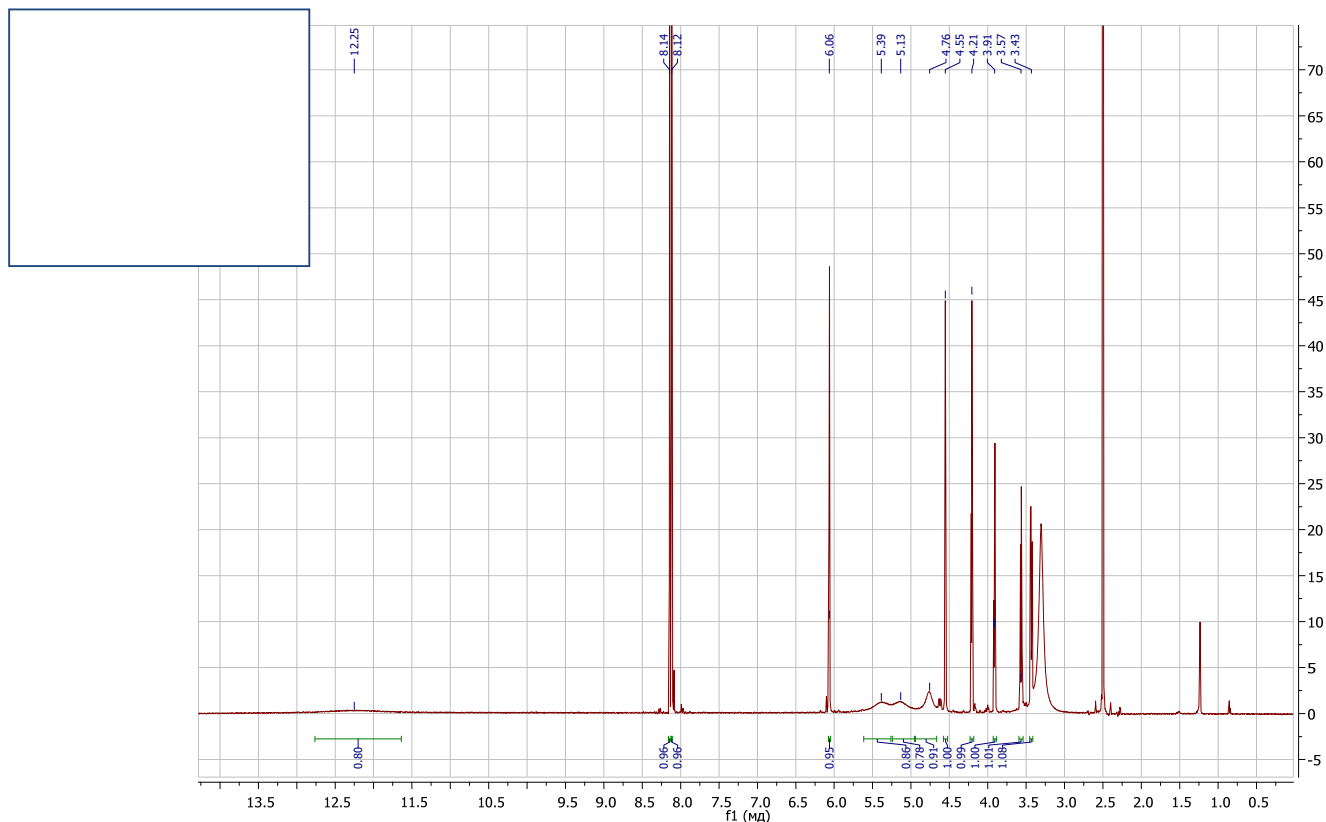


Figure S17. The ^1H NMR spectrum of compound **9**

^1H NMR (700 MHz, DMSO- d_6): δ = 12.25 (br. s, 1 H, NH), 8.14 (s, 1 H, H-3), 8.12 (s, 1 H, H-6), 6.06 (d, J = 4.5 Hz, 1 H, H-1'), 5.39 (br. sign, 1H, OH-2'), 5.13 (br. sign, 1 H, OH-3'), 4.76 (br. sign, 1 H, OH-5'), 4.55 (dt, J = 4.6; < 0.5 Hz, 1 H, H-2'), 4.21 (t, J = 4.8 Hz, 1 H, H-3'), 3.91 (m, 1 H, H-4'), 3.58 (dd, J = 4.6, 11.7 Hz, 1 H, H-5'a), 3.43 (dd, J = 6.0, 11.8 Hz, 1 H, H-5'b).

^{13}C NMR (176 MHz, DMSO- d_6): δ = 157.45 (C-4), 153.98 (C-7a), 148.86 (C-6), 135.37 (C-3), 106.23 (C-4a), 88.38 (C-1'), 85.25 (C-4'), 73.31 (C-2'), 70.81 (C-3'), 62.27 (C-5').

^{15}N NMR (71 MHz, DMSO- d_6): δ = 302.20 (N-2), 210.47 (N-7), 204.80 (N-1), 173.67 (N-5).

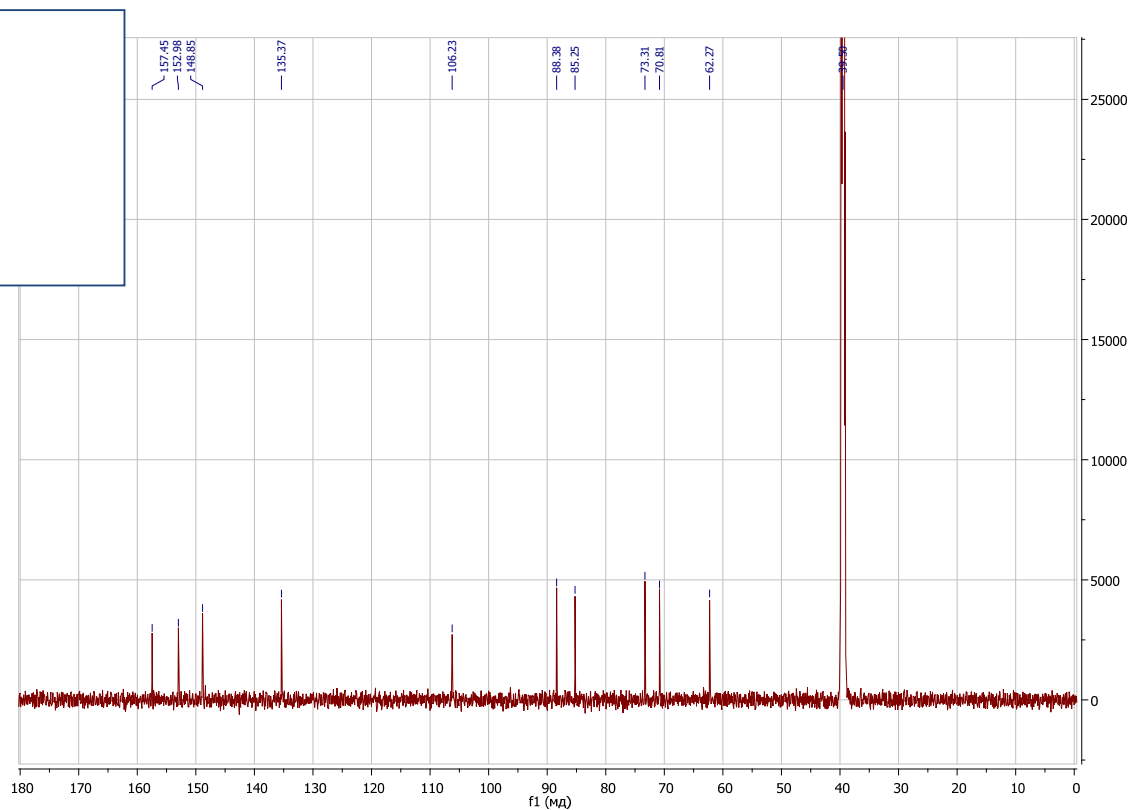


Figure S18. The ¹³C NMR spectrum of compound 9

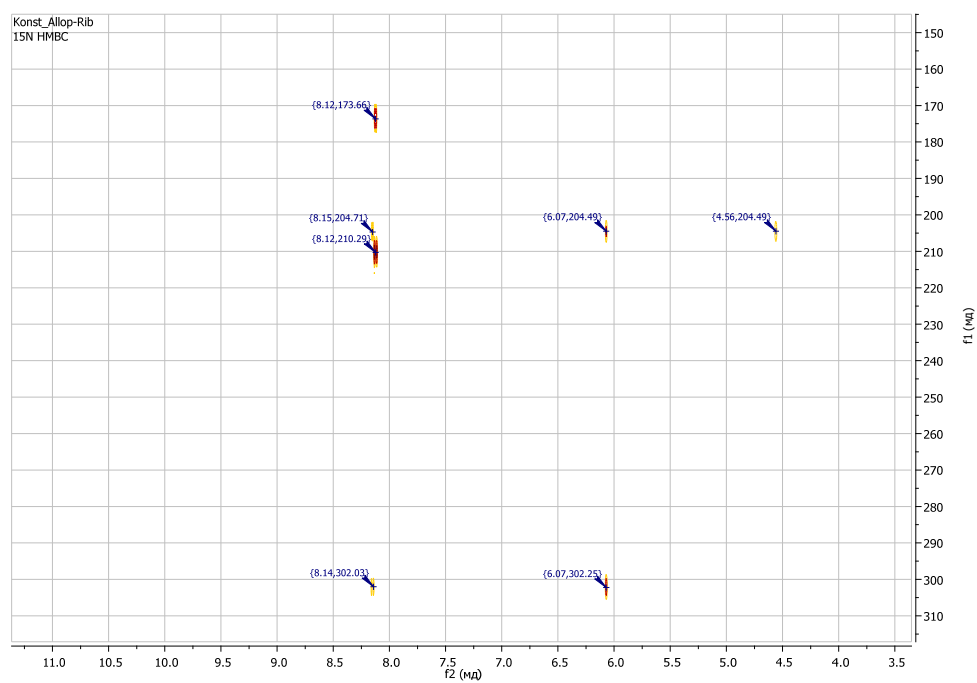


Figure S19. The fragment of ¹H-¹⁵N-HMBC NMR spectrum of nucleoside 9

9-(β -D-Arabinofuranosyl)-2-chloro-6-methoxypurine (10).

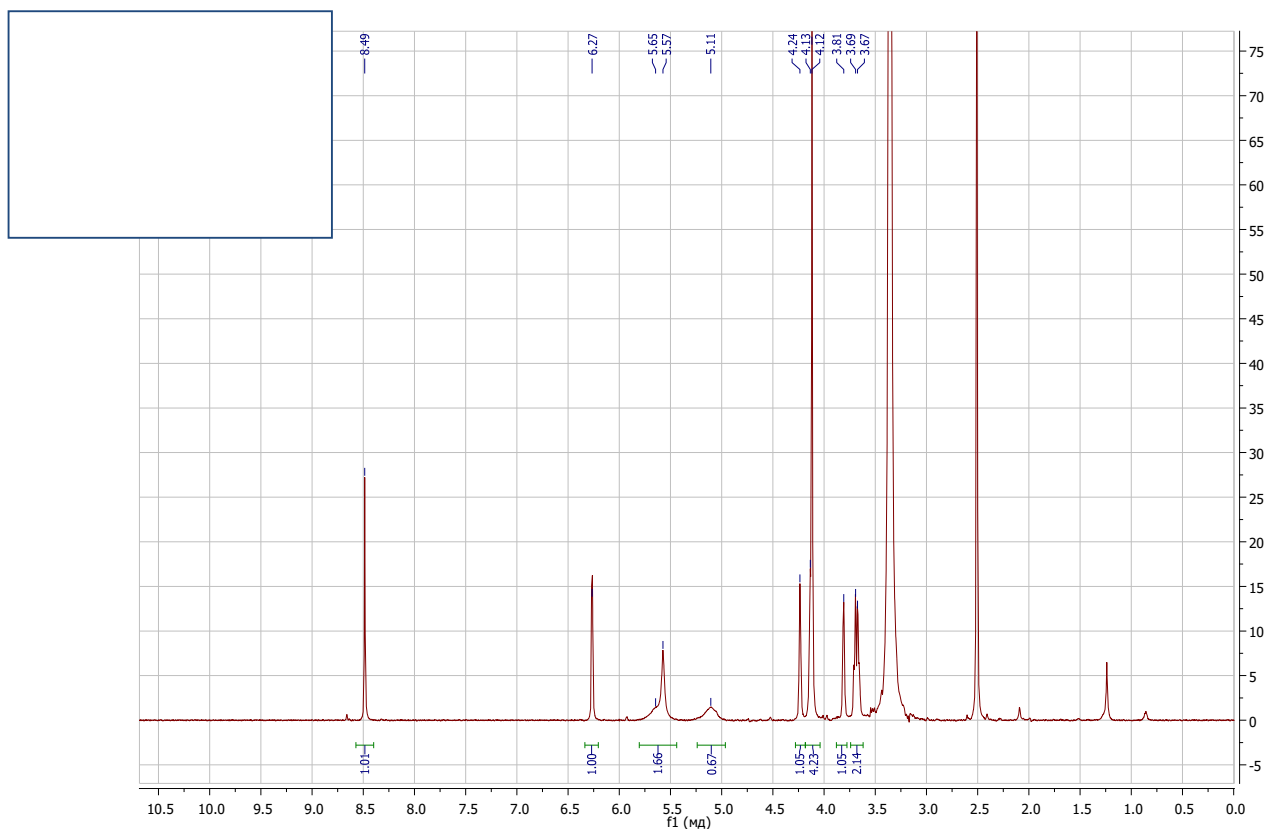


Figure S20. The ^1H NMR spectrum of compound 10

^1H NMR (700 MHz, $\text{DMSO}-d_6$, 30 °C): δ =8.49, (s, 1 H, H-8), 6.27 (d, J = 4.8 Hz, 1 H, H-1'), 5.65 (br. s., 1 H, OH-2'), 5.57 (br. s, 1 H, OH-3'), 5.11 (br. s, 1 H, OH-5'), 4.24 (m, 1 H, H-2'), 4.13 (m, 1 H, H-3'), 4.12 (s, 3 H, O-CH₃), 3.81 (m, 1 H, H-4'), 3.69 (m, 1 H, H-5'a), 3.67 (m, 1 H, H-5'b).

^{13}C NMR (176 MHz, $\text{DMSO}-d_6$, 30 °C): δ = 160.59 (C-6), 152.86 (C-4), 151.19 (C-2), 143.53 (C-8), 119.44 (C-5), 84.08 (C-4'), 84.06 (C-1'), 75.52 (C-2'), 74.19 (C-3'), 60.40 (C-5'), 54.83 (O-CH₃).

^{15}N ЯМР (71 МГц, $\text{DMSO}-d_6$, 30 °C): δ =239.34 (N-7), 170.3 (N-9).

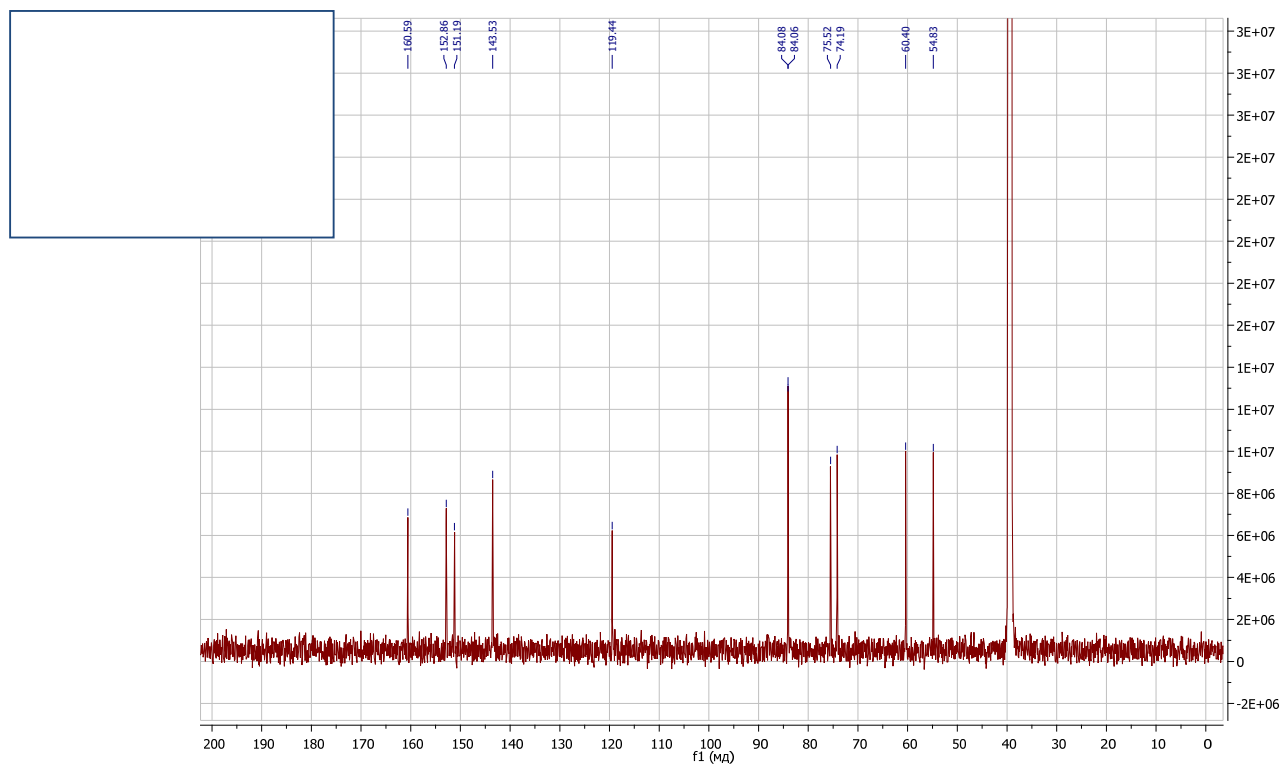


Figure S21. The ¹³C NMR spectrum of compound 10

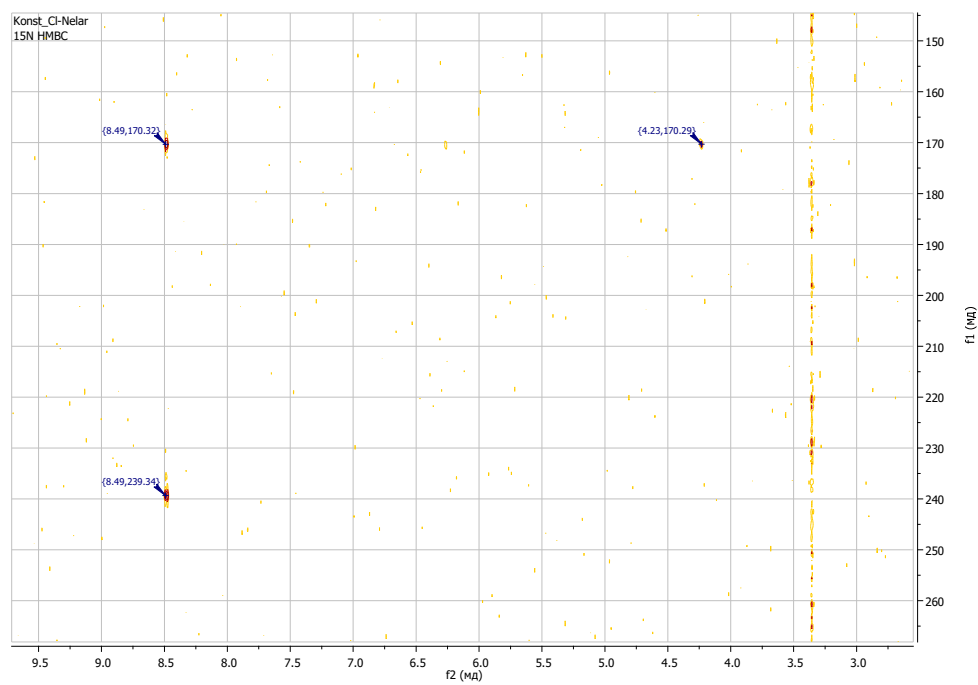


Figure S22. The fragment of ¹H-¹⁵N-HMBC NMR spectrum of nucleoside 10

9-(β -D-Arabinofuranosyl)-2-fluoro-6-methoxypurine (**11**).

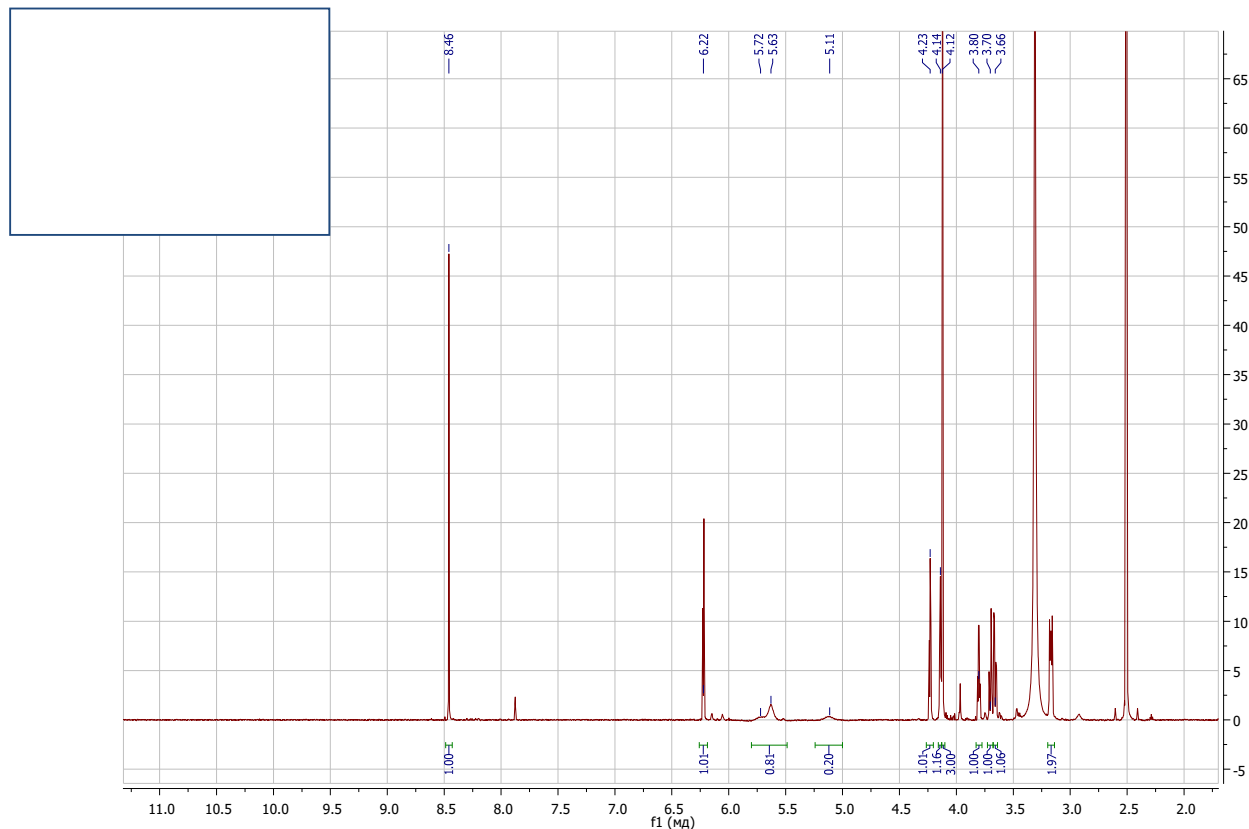


Figure S23. The ^1H NMR spectrum of compound **11**

^1H NMR (700 MHz, $\text{DMSO}-d_6$, 30 $^\circ\text{C}$): δ =8.46, (s, 1 H, H-8), 6.22 (d, J = 5.4 Hz, 1 H, H-1'), 5.72 (br. s., 1 H, OH-2'), 5.63 (br. s, 1 H, OH-3'), 5.11 (br. s, 1 H, OH-5'), 4.23 (t, J = 5.2 Hz, 1 H, H-2'), 4.14 (t, J = 5.4 Hz, 1 H, H-3'), 4.12 (s, 3 H, O-CH₃), 3.80(m, 1 H, H-4'), 3.70 (dd, J = 3.7, 12.0 Hz, 1 H, H-5'a), 3.66 (dd, J = 4.8, 12.0 Hz, 1 H, H-5'b).

^{13}C NMR (176 MHz, $\text{DMSO}-d_6$, 30 $^\circ\text{C}$): δ = 161.98 (d, $J_{\text{C6,F}}$ = 17.0 Hz, C-6), 156.98 (d, $J_{\text{C2,F}}$ = 210.8 Hz, C-2), 153.00 (d, $J_{\text{C4,F}}$ = 18.8 Hz, C-4), 143.38 (C-8), 118.64 (d, $J_{\text{C5,F}}$ = 4.5 Hz, C-5), 84.09 (C-4'), 84.05 (C-1'), 75.59 (C-2'), 74.18 (C-3'), 60.44 (C-5'), 54.97 (O-CH₃).

^{15}N ЯМР (71 МГц, $\text{DMSO}-d_6$, 30 $^\circ\text{C}$): δ =239.9 (N-7), 200.11 (N-3), 170.71 (N-9).

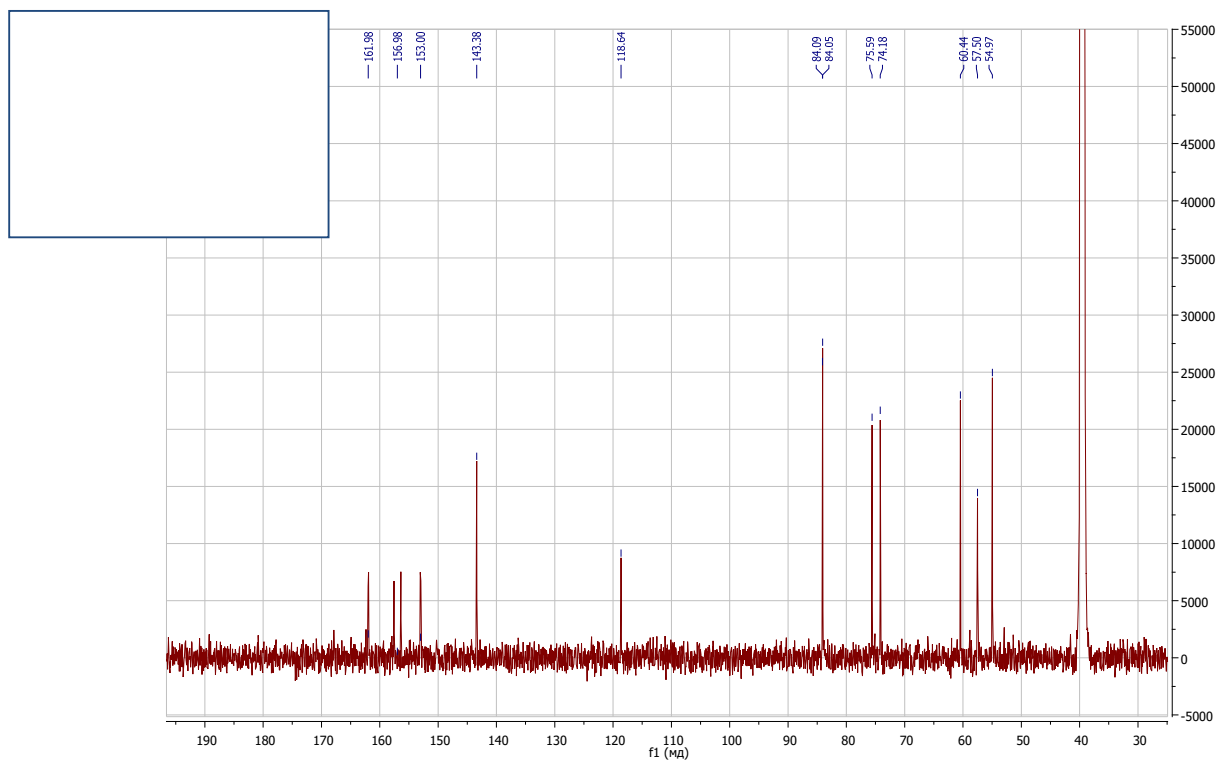


Figure S24. The ¹³C NMR spectrum of compound **11**

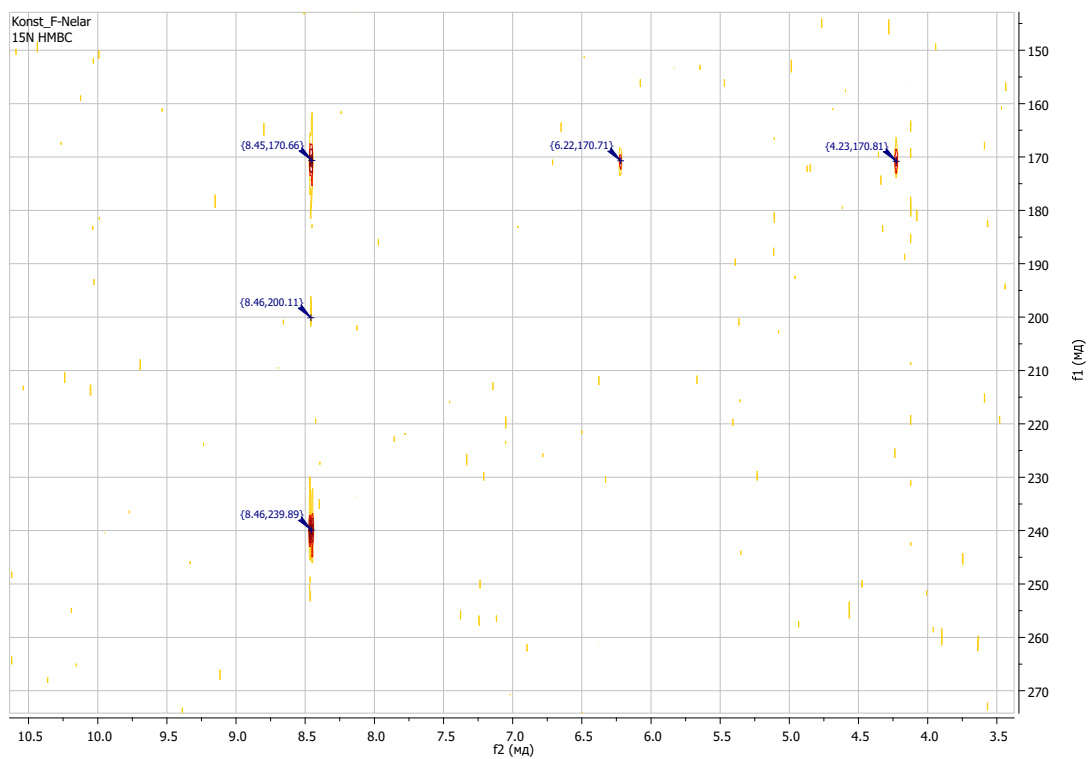


Figure S25. The fragment of ¹H-¹⁵N-HMBC NMR spectrum of nucleoside **11**

References

1. Robins, M. J.; Uznański, B., Nucleic acid related compounds. 33. Conversions of adenosine and guanosine to 2, 6-dichloro, 2-amino-6-chloro, and derived purine nucleosides. *Canadian Journal of Chemistry* **1981**, 59 (17), 2601-2607.
2. Robins, M. J.; Zou, R.; Hansske, F.; Madej, D.; Tyrrell, D. L. J., Synthesis, Transformation Chemistry, and Biological Activity of Guanine Nucleosides and Analogues. *Nucleosides & Nucleotides* **1989**, 8 (5-6), 725-741.