

Electronic Supporting information for

Enzymatic synthesis of base-modified RNA by T7 RNA polymerase. A systematic study and comparison of 5-substituted pyrimidine and 7-substituted 7-deazapurine nucleoside triphosphates as substrates

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I Complete experimental part

Synthetic methods

General chemistry

NMR spectra were measured on a 500 MHz (499.8 or 500 MHz for ^1H , 202.3 or 202.4 MHz for ^{31}P , 125.7 MHz for ^{13}C) or a 600 MHz (600.1 MHz for ^1H , 150.9 MHz for ^{13}C) spectrometers in D_2O (referenced to dioxane as internal standard, $\delta_{\text{H}} = 3.75$ ppm, $\delta_{\text{C}} = 69.30$ ppm); in CD_3OD (referenced to solvent signal, $\delta_{\text{H}} = 3.31$ ppm, $\delta_{\text{C}} = 49.00$ ppm); or $\text{DMSO}-d_6$ (referenced to solvent signal, $\delta_{\text{H}} = 2.50$ ppm, $\delta_{\text{C}} = 39.70$ ppm). Chemical shifts are given in ppm (δ scale), coupling constants (J) in Hz. Complete assignment of all NMR signals was achieved by using a combination of H,H-COSY , H,C-HSQC and H,C-HMBC experiments. Mass spectra and high resolution mass spectra were measured by ESI ionization technique. The separation of nucleoside was performed by preparative flash chromatography on reverse phase (C18 RediSep column on a CombiFlash Teledyne ISCO system). Semi-preparative separation of nucleoside triphosphate was performed by HPLC on a column packed with $10\ \mu\text{m}$ C18 reversed phase (Phenomenex, Luna C18). Syntheses and characterization data for nucleosides **A**¹, **G**¹, **U**³ and 2',3',5'-tris-(*O*-benzoyl)-*N*²-pivaloyl-7-iodo-7-deazaguanosine, **A**^E, **C**^E, **U**^E, **G**^{Ph}, **G**^{BF} and **G**^{TMS} and for the triphosphates **A**^ITP, **A**^{Ph}TP, **C**^ITP, **U**^ITP, **U**^{Ph}TP and **U**^{BF}TP were previously reported. **C**^I nucleoside was purchased from commercial supplier (Carbosynth Ltd.).

Synthesis of modified nucleosides:

7-Methyl-7-deazaadenosine (**A**^{Me})

7-Iodo-7-deazaadenosine (300 mg, 0.76 mmol) and ammonium sulfate (0.076 mmol, 10 mg) were coevaporated with hexamethyldisilazane (7.6 mmol, 1.5 mL) at 40°C . Argon purged solution of $\text{Pd}(\text{PPh}_3)_4$ (0.03 mmol, 44 mg) in 5 mL of anhydrous THF was rapidly added to the residue, followed by the addition of AlMe_3 (2M solution in hexanes) (9.1 mmol, 4.5 mL). The reaction mixture was refluxed at 80°C for 2h. After cooling down to room temperature, the reaction mixture was slowly poured to saturated ice-cold solution of NaH_2PO_4 . Resulting suspension was evaporated *in vacuo*, resuspended in methanol, coevaporated with silica gel and purified by high performance reverse phase flash chromatography (0 $\rightarrow$ 100% MeOH in water) using C18RediSep column on a CombiFlash Teledyne ISCO system. Product was isolated as a white solid (130 mg, 60%). ^1H NMR (500.0 MHz, $\text{DMSO}-d_6$): 2.33 (s, 3H, CH_3); 3.47 (m, 1H, H-5'b); 3.58 (m, 1H, H-5'a); 3.86 (m, 1H, H-4'); 4.05 (dd, 1H, $J_{3',2'} = 5.3$, $J_{3',4'} = 2.6$, H-3'); 4.36 (dd, 1H, $J_{2',1'} = 7.1$, $J_{2',3'} = 5.3$, H-2'); 5.07 (d, 1H, $J = 4.7$, OH-2'); 5.21 (bs, 2H, OH-3' and OH-5'); 5.97 (d, 1H, $J_{1',2'} = 7.1$, H-1'); 6.60 (bs, 2H, - NH_2); 7.08 (s, 1H, H-6); 8.01 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $\text{DMSO}-d_6$): 12.38 (CH_3); 62.35 (CH_2 -5'); 71.19 (CH -3'); 73.97 (CH -2'); 85.33 (CH -4'); 87.23 (CH -1'); 103.33 (C-4a); 110.19 (C-5); 120.13 (CH -6); 151.14 (C-7a); 151.83 (C-2); 158.38 (C-4). MS (ESI) m/z (%): 302 (100) [$\text{M}+\text{Na}$], 281 (65) [$\text{M}+\text{H}$]. HRMS-ESI [$\text{M}+\text{H}$]⁺ calcd for $\text{C}_{12}\text{H}_{17}\text{O}_4\text{N}_4$: 281.12439, found: 281.12443.

7-Methyl-7-deazaguanosine (**G**^{Me})

A solution of protected 2',3',5'-tris-(*O*-benzoyl)-*N*²-pivaloyl-7-iodo-7-deazaguanosine (1 g, 1.2 mmol), CsOAc (700 mg, 3.6 mmol), DABCO (136 mg, 1.2 mmol) and triethylamine (0.5 mL, 3.6 mmol) in anhydrous DMF (10 mL) was stirred at room temperature for 2 hours. The reaction

mixture was then poured into water (100 mL) and the resulting suspension was extracted with chloroform. Organic phase was dried over MgSO_4 and purified by column chromatography (0 $\rightarrow$ 30% MeOH in CHCl_3). The product (700 mg, 72%) was obtained as a white foam. Spectral data are in accordance with those reported in literature.⁴ Protected oxo derivative obtained in the previous step (500 mg, 0.6 mmol) and ammonium sulfate (8 mg, 0.06 mmol) were coevaporated with hexamethyldisilazane (10 mL) and THF (10 mL) at 40°C. Argon purged solution of $\text{Pd}(\text{PPh}_3)_4$ (0.03 mmol, 35 mg) in 5 mL of anhydrous THF was rapidly added to the dry residue, followed by the addition of AlMe_3 (2M solution in hexanes) (4 mL, 7.4 mmol). The reaction mixture was refluxed at 80°C for 2h. After cooling down to room temperature, the reaction mixture was slowly poured to saturated ice-cold solution of methanol. Resulting suspension was evaporated *in vacuo*, resuspended in methanol, coevaporated with silica gel and purified on SiO_2 column (0 $\rightarrow$ 30%, ethyl acetate in petroleum ether). 200 mg of an inseparable mixture of methylated product and the dehalogenated derivative were obtained in the form of white foam. The crude mixture was subsequently dissolved in 10 mL of 1M solution of NaOMe in MeOH and left stirring over night at 80°C and then neutralized with 0.5 mL of glacial AcOH. After several coevaporations with water, the residue was dissolve in 3:1 mixture of water/methanol and purified by HPLC (C-18 column, 0 $\rightarrow$ 100% MeOH in water). The product was obtained as a white solid (45 mg, 17% overall yield). ^1H NMR (500.0 MHz, MeOD): 2.35 (d, 3H, $J_{\text{CH}_3,6} = 1.2$, CH_3); 3.51 (m, H-5'b); 3.60 (m, H-5'a); 3.86 (m, 1H, H-4'); 4.06 (dd, 1H, $J_{3',2'} = 5.3$, $J_{3',4'} = 2.6$, H-3'); 4.60 (dd, 1H, $J_{2',1'} = 7.1$, $J_{2',3'} = 5.3$, H-2'); 5.07 (d, 1H, $J = 4.7$, OH-2'); 5.21 (bs, 2H, OH-3' and OH-5'); 5.82 (d, 1H, $J = 6.4$, H-1'), 6.17 (bs, 2H, $-\text{NH}_2$); 6.63 (s, 1H, H-6). ^{13}C NMR (125.7 MHz, MeOD) 10.11 (CH_3); 62.05 (CH_2 -5'); 70.97 (CH-3'); 73.89 (CH-2'); 84.91 (CH-4'); 88.25 (CH-1'); 100.53 (C-4a); 114.52 (CH-6); 116.07 (C-5); 151.19 (C-7a); 152.29 (C-2); 160.96 (C-4). MS (ESI) m/z (%): 319 (100) $[\text{M}+\text{Na}]$, 297 (95) $[\text{M}+\text{H}]$. HRMS-ESI $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{12}\text{H}_{17}\text{O}_5\text{N}_4$: 297.11498, found: 297.11935.

General procedure A: Suzuki cross-coupling on iodinated nucleosides

Water/ACN mixture (2:1, 15 mL) was added through septum to an argon purged vial containing iodinated nucleoside (0.2 mmol), corresponding boronic acid and Cs_2CO_3 (399 mg, 1.2 mmol), followed by the addition of the degassed solution of $\text{Pd}(\text{OAc})_2$ (7 mg, 0.02 mmol) and TPPTS ligand (69 mg, 0.12 mmol) in water/ACN (2:1, 10 mL). After argon/vacuum exchange, the reaction mixture was left stirring at 100°C overnight. The mixture was cooled to the room temperature, coevaporated with silica gel and purified by high performance reverse phase flash chromatography (0 $\rightarrow$ 100% MeOH in water)

7-(Dibenzofuran-4-yl)-7-deazaguanosine (G^{DB})

Prepared from G^{I} (80 mg) and dibenzofuran-4-ylboronic acid (390 mg, 1.8 mmol) according to general procedure A. The product was isolated as a white solid (58 mg, 67%). ^1H NMR (500.0 MHz, MeOD): 4.12 (m, 1H, H-5'b); 4.22 (m, 1H, H-5'a); 4.36 (m, 1H, H-4'); 4.58 (m, 1H, H-3'); 4.77 (dd, 1H, $J_{2',1'} = 6.8$, $J_{2',3'} = 5.5$, H-2'); 6.04 (d, 1H, $J_{1',2'} = 6.8$, H-1'); 7.38 (m, 1H, H-1-dibenzofuryl and H-2-dibenzofuryl); 7.44-7.50 (m, 2H, H-8-dibenzofuryl and H6); 7.60 (m, 1H, H7-dibenzofuryl); 7.71 (m, 1H, H-6-dibenzofuryl); 7.96 (m, 1H, H-3-dibenzofuryl); 8.03 (m, 1H, H-9-dibenzofuryl). ^{13}C NMR (125.7 MHz, MeOD): 62.05 (CH_2 -5'); 71.16 (CH-3'); 74.57 (CH-2'); 85.08 (CH-4'); 86.77 (CH-1'); 98.31 (C-4a) 112.30 (C-5); 114.41 (CH-6-dibenzofuryl); 118.71 (C-4-dibenzofuryl); 119.04 (CH-3-dibenzofuryl); 119.73 (CH-9-dibenzofuryl); 121.42 (CH-6);

123.25 (CH-8-dibenzofuryl); 123.46 (CH-2-dibenzofuryl); 123.74 (C-9b-dibenzofuryl); 124.29 (C-9a-dibenzofuryl); 127.61 (CH-7-dibenzofuryl); 129.19 (CH-1-dibenzofuryl) 152.71 (C-4a-dibenzofuryl); 152.88 (C-7a); 153.22 (C-2); 155.60 (C-5a-dibenzofuryl); 159.33 (C-4). MS (ESI) m/z (%): 471 (100) [M+Na], 449 (65) [M+H]. HRMS-ESI [M+H]⁺ calcd for C₂₃H₂₁O₆N₄: 449.14572, found: 449.14556.

5-(Benzofuran-2-yl)-uridine (U^{BF}) (a known compound previously prepared by the Stille coupling - ref.¹¹)

Prepared according to the general procedure from U^I (80 mg) and benzofuran-2-yl boronic acid (87 mg, 0.4 mmol). Product was isolated as a white solid (55 mg, 70%). ¹H NMR (500.0 MHz, DMSO): 3.74 (m, 1H, H-5'b+H-5'a); 3.96 (d, $J = 4.8$ Hz, H-4'); 4.13 (m, 2H, H-2' and H-3'); 5.89 (d, 1H, $J = 4.3$ Hz, H-1'); 7.22 (m, 2H, H-5,6-benzofuryl); 7.35 (m, 1H, H-7-benzofuryl); 7.59 (m, 1H, H-4-benzofuryl); 8.80 (s, 1H, H6). 60.67 (CH₂-5'); 69.92 (CH-3'); 74.77 (CH-2'); 85.01 (CH-4'); 89.32 (CH-1'); 104.12 (C-5); 105.14 (CH-3-benzofuryl); 111.19 (CH-7-benzofuryl); 121.35 (CH-4-benzofuryl); 123.39 (CH-5-benzofuryl); 124.55 (CH-6-benzofuryl); 129.33 (C-3a-benzofuryl); 137.64 (CH-6); 149.98 (C-2-benzofuryl); 150.83 (C-7a-benzofuryl); 153.44 (C-2); 161.81 (C-4). MS (ESI) m/z (%): 383 (100) [M+Na], 361 (2) [M+H] HRMS-ESI [M+Na]⁺ calcd for C₁₇H₁₆O₇N₂Na: 383.08524, found: 383.08497.

General procedure B: phosphorylation of modified nucleosides

Modified nucleoside (1 eq.) was dried at 60°C overnight under vacuum. It was then suspended in PO(OMe)₃, stirred at room temperature for 15 minutes, followed by cooling to 0°C and addition of POCl₃ (1.2 eq.). The reaction mixture was left stirring at 0°C (1.5 - 24h) and then ice cold solution of (NHBu₃)₂H₂P₂O₇ (5 eq.) and tributyl amine (5 eq.) in anhydrous DMF (1-5 mL) was added. The reaction mixture was stirred at 0°C for another hour. Then aqueous solution of TEAB (2M, 2 mL, 4 mmol) was added and the mixture was evaporated under reduced pressure. The residue was coevaporated several times with water. The product was purified by chromatography on DEAE Sephadex column (0→1.2M aq. TEAB) and then by HPLC (C-18 column, 0.1M TEAB in water to 0.1M TEAB in 50% aq. MeOH), it was coevaporated several times with water and, where possible, converted to the sodium salt form (Dowex 50 in Na⁺ cycle).

7-Methyl-7-deazaadenosine 5'-O-triphosphate sodium salt (A^{Me}TP)

A^{Me}TP was prepared according to general procedure B by dissolving A^{Me} (30 mg, 0.11 mmol) in PO(OMe)₃ (300 μl) and stirring with POCl₃ (14 μL, 0.14mmol) for 1.5h, followed by the addition of solution of (NHBu₃)₂H₂P₂O₇ (293 mg, 0.54 mmol) and tributyl amine (110 μL, 0.48 mmol) in anhydrous DMF (1 mL) and stirring for another hour. Triphosphate (25 mg, 30 %) was obtained as a white lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 2.42 (d, 3H, $J_{CH3,6} = 1.2$, CH₃); 4.11 (ddd, 1H, $J_{gem} = 11.6$, $J_{H,P} = 4.5$, $J_{5'b,4'} = 3.2$, H-5'b); 4.24 (ddd, 1H, $J_{gem} = 11.6$, $J_{H,P} = 6.5$, $J_{5'a,4'} = 2.9$, H-5'a); 4.31 (m, 1H, H-4'); 4.57 (dd, 1H, $J_{3',2'} = 5.3$, $J_{3',4'} = 2.6$, H-3'); 4.70 (dd, 1H, $J_{2',1'} = 7.1$, $J_{2',3'} = 5.3$, H-2'); 6.22 (d, 1H, $J_{1',2'} = 7.1$, H-1'); 7.32 (q, 1H, $J_{6,CH3} = 1.2$, H-6); 8.10 (s, 1H, H-2). ¹³C NMR (125.7 MHz, D₂O): 13.75 (CH₃); 68.19 (d, $J_{C,P} = 5.7$, CH₂-5'); 73.43 (CH-3'); 76.10 (CH-2'); 86.31 (d, $J_{C,P} = 9.1$, CH-4'); 87.93 (CH-1'); 106.27 (C-4a); 115.56 (C-5); 122.00 (CH-6); 153.28 (C-7a); 153.84 (C-2); 160.48 (C-4). ³¹P NMR (202.3 MHz, D₂O): -22.39 (t, $J = 19.8$, P_b); -11.23 (d, $J = 19.8$, P_a); -7.43 (bd, $J = 19.8$, P_g). MS (ESI) m/z (%): 439 (100) [M-H₂PO₃-H], 461

(65) [M-H₂PO₃-2H+Na], 519 (12) [M-H], 541 (23) [M-2H+Na], 563 (23) [M-3H+2Na]. HRMS-ESI [M-2H+Na]⁺ calcd for C₁₂H₁₇O₁₃N₄NaP₃: 540.99032, found: 540.99081.

7-Ethynyl-7-deazaadenosine 5'-O-triphosphate sodium salt (A^ETP)

A^ETP was prepared according to general procedure B by dissolving A^E (30 mg, 0.10 mmol) in PO(OMe)₃ (300 μl) and stirring with POCl₃ (14 μL, 0.14mmol) for 1.5h, followed by the addition of solution of (NHBu₃)₂H₂P₂O₇ (284 mg, 0.5 mmol) and tributyl amine (110 μL, 0.48mmol) in anhydrous DMF (1 mL) and stirring for another hour. Triphosphate (5 mg, 8 %) was obtained as a white lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 3.70 (s, 1H, HC≡C-); 4.16 (ddd, 1H, J_{gem} = 11.6, J_{H,P} = 4.8, J_{5'b,4'} = 3.2, H-5'b); 4.27 (ddd, 1H, J_{gem} = 11.6, J_{H,P} = 6.6, J_{5'a,4'} = 3.1, H-5'a); 4.35 (m, 1H, H-4'); 4.56 (dd, 1H, J_{3',2'} = 5.3, J_{3',4'} = 2.7, H-3'); 4.67 (dd, 1H, J_{2',1'} = 6.7, J_{2',3'} = 5.3, H-2'); 6.22 (d, 1H, J_{1',2'} = 6.7, H-1'); 7.82 (s, 1H, H-6); 8.13 (s, 1H, H-2). ¹³C NMR (125.7 MHz, D₂O): 68.11 (d, J_{C,P} = 5.6, CH₂-5'); 73.19 (CH-3'); 76.58 (CH-2'); 78.99 (-C≡CH); 84.53 (HC≡C-); 86.47 (d, J_{C,P} = 9.0, CH-4'); 88.57 (CH-1'); 98.78 (C-5); 105.82 (C-4a); 129.78 (CH-6); 151.91 (C-7a); 155.08 (C-2); 160.08 (C-4). ³¹P NMR (202.3 MHz, D₂O): -21.47 (t, J = 19.6, P_b); -10.40 (d, J = 19.6, P_a); -7.03 (d, J = 19.6, P_g). MS (ESI) m/z (%): 449 (100) [M-H₂PO₃-H], 471 (50) [M-H₂PO₃-2H+Na], 529 (10) [M-H], 551 (26) [M-2H+Na], 573 (34) [M-3H+2Na]. HRMS-ESI [M-2H+Na]⁺ calcd for C₁₃H₁₅O₁₃N₄NaP₃: 550.97559, found: 550.97516.

5-Ethynylcytidine 5'-O-triphosphate triethylammonium salt (C^ETP)

C^ETP was prepared according to general procedure B by dissolving C^E (40 mg, 0.14 mmol) in PO(OMe)₃ (500 μl) and stirring with POCl₃ (20 μL, 0.22mmol) for 1.5h, followed by the addition of solution of (NHBu₃)₂H₂P₂O₇ (524 mg, 0.95 mmol) and tributyl amine (220 μL, 0.95 mmol) in anhydrous DMF (1 mL) and stirring for another hour. Triphosphate (10 mg, 9 %) was obtained as a glassy lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 1.28 (t, 9H, J_{vic} = 7.3, CH₃CH₂N); 3.20 (q, 6H, J_{vic} = 7.3, CH₃CH₂N); 3.60 (s, 1H, HC≡C-); 4.27 (m, 3H, H-4' and H-5'); 4.40 (m, 2H, H-3' and H-4'); 5.97 (d, 1H, J_{1',2'} = 4.9, H-1'); 8.24 (s, 1H, H-6). ¹³C NMR (125.7 MHz, D₂O): 10.48 (CH₃CH₂N); 49.38 (CH₃CH₂N); 67.39 (d, J_{C,P} = 5.9 Hz; CH₂-5'); 71.67 (CH-3'); 76.69 (CH-2'); 77.05 (-C≡CH); 85.42 (d, J_{C,P} = 9.1, CH-4'); 87.99 (HC≡C-); 92.23 (CH-1'); 94.62 (C-5); 148.08 (CH-6); 158.85 (C-2); 167.83 ppm (C-4). ³¹P NMR (202.3 MHz, D₂O): -23.06 (bs, P_b); -11.45 (d, J = 19.9, P_a); -10.59 (d, J = 19.9, P_g). MS (ESI) m/z (%): 426 (100) [M-H₂PO₃-H], 448 (21) [M-H₂PO₃-2H+Na], 506 (26) [M-H], 527 (17) [M-2H+Na]. HRMS-ESI [M-H]⁺ calcd for C₁₁H₁₅O₁₄N₃P₃: 505.97610, found: 505.97723.

5-Ethynyluridine 5'-O-triphosphate triethylammonium salt (U^ETP)

U^ETP was prepared according to general procedure B by dissolving U^E (40 mg, 0.19 mmol), proton sponge (80 mg, 0.38 mmol) in PO(OMe)₃ (500 μl) and stirring with POCl₃ (20 μL, 0.22mmol) for 1.5h, followed by the addition of solution of (NHBu₃)₂H₂P₂O₇ (524 mg, 0.95 mmol) and tributyl amine (220 μL, 0.95 mmol) in anhydrous DMF (1 mL) and stirring for another hour. Triphosphate (20 mg, 18 %) was obtained as a glassy lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 1.28 (t, 20H, J_{vic} = 7.3, CH₃CH₂N); 3.20 (q, 13H, J_{vic} = 7.3, CH₃CH₂N); 3.60, s, 1H, (HC≡C-); 4.27 (m, 3H, H-4' and H-5'); 4.40 (m, 2H, H-3' and H-4'); 5.97 (d, 1H, J_{1',2'} = 4.9, H-1'); 8.24 (s, 1H, H-6). ¹³C NMR (125.7 MHz, D₂O): 10.95 (CH₃CH₂N); 49.38 (CH₃CH₂N); 67.59 (d, J_{C,P} = 5.5, CH₂-5'); 72.09 (CH-3'); 76.57 (CH-2'); 79.96 (-C≡CH); 86.06 (d, J_{C,P} = 9.1, CH-4'); 86.21 (HC≡C-); 91.40 (CH-1'); 101.71 (C-5); 147.98 (CH-6); 153.49 (C-2); 167.38 (C-4). ³¹P NMR (202.3 MHz, D₂O): -23.06 (t, J = 19.9, P_b); -11.59 (d, J = 19.9, P_a); -9.92 (bs, P_g). MS (ESI) m/z (%): 427 (100)

[M-H₂PO₃-H], 449 (50) [M-H₂PO₃-2H+Na], 507 (13) [M-H], 529 (30) [M-2H+Na], 551 (17) [M-3H+2Na]. HRMS-ESI [M-2H+Na]⁻ calcd for C₁₁H₁₃O₁₅N₃NaP₃: 528.94311, found: 528.94319.

7-Methyl-7-deazaguanosine- 5'-O-triphosphate sodium salt (G^{Me}TP)

G^{Me}TP was prepared according to general procedure B by dissolving **G^{Me}** (20 mg, 0.06 mmol) in trimethyl phosphate (300 µl) and stirring with POCl₃ (12 µL, 0.12 mmol) overnight followed by the addition of solution of (NHBU₃)₂H₂P₂O₇ (184 mg, 0.33 mmol) and tributyl amine (70 µL, 0.28 mmol) in anhydrous DMF (1 mL) and stirring for another four hours. Triphosphate (25 mg, 62 %) was obtained as a white lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 2.27 (d, 3H, J_{CH₃,6} = 1.2, CH₃); 4.11 (ddd, 1H, J_{gem} = 11.6, J_{H,P} = 5.0, J_{5'b,4'} = 3.6, H-5'b); 4.22 (ddd, 1H, J_{gem} = 11.6, J_{H,P} = 6.7, J_{5'a,4'} = 3.3, H-5'a); 4.27 (m, 1H, H-4'); 4.54 (dd, 1H, J_{3',2'} = 5.3, J_{3',4'} = 2.7, H-3'); 4.64 (dd, 1H, J_{2',1'} = 7.0, J_{2',3'} = 5.3, H-2'); 6.00 (d, 1H, J_{1',2'} = 7.0, H-1'); 6.91 (q, 1H, J_{6,CH₃} = 1.2, H-6). ¹³C NMR (125.7 MHz, D₂O): 13.39 (CH₃); 68.14 (d, J_{C,P} = 5.4, CH₂-5'); 73.25 (CH-3'); 75.77 (CH-2'); 86.08 (d, J_{C,P} = 9.1, CH-4'); 88.04 (CH-1'); 103.00 (C-4a); 118.33 (CH-6); 118.63 (C-5); 154.90 (C-7a); 155.42 (C-2); 164.65 (C-4). ³¹P NMR (202.3 MHz, D₂O): -21.25 (t, J = 19.6, P_b); -10.38 (d, J = 19.6, P_a); -5.50 (bd, J = 19.6, P_g). MS (ESI) m/z (%): 455 (100) [M-H₂PO₃-H], 477 (50) [M-H₂PO₃-2H+Na], 535 (13) [M-H], 557 (15) [M-2H+Na], 579 (20) [M-3H+2Na]. HRMS-ESI [M-2H+Na]⁻ calcd for C₁₂H₁₇O₁₄N₄P₃: 556.98547, found: 556.98573.

7-Ethynyl-7-deazaguanosine 5'-O-triphosphate triethylammonium salt (G^ETP)

G^ETP was prepared according to general procedure B by dissolving **G^{TMS}** (40 mg, 0.11 mmol), proton sponge (68 mg, 0.33 mmol) in PO(OMe)₃ (400 µl) and stirring with POCl₃ (freshly distilled!, 12 µL, 0.12 mmol) for 4h, followed by the addition of solution of (NHBU₃)₂H₂P₂O₇ (290 mg, 0.53 mmol) and tributyl amine (120 µL, 0.53 mmol) in anhydrous DMF (1 mL) and stirring for another hour. Triphosphate (5 mg, 8 %) was obtained as a yellowish glassy lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 1.26 (t, 9H, J_{vic} = 7.3, CH₃CH₂N); 3.18 (q, 6H, J_{vic} = 7.3, CH₃CH₂N); 3.52 (s, 1H, HC≡C-); 4.15 (ddd, 1H, J_{gem} = 11.6, J_{H,P} = 4.5, J_{5'b,4'} = 3.4, H-5'b); 4.22 (ddd, 1H, J_{gem} = 11.6, J_{H,P} = 6.2, J_{5'a,4'} = 3.4, H-5'a); 4.30 (m, 1H, H-4'); 4.50 (dd, 1H, J_{3',2'} = 5.3, J_{3',4'} = 2.7, H-3'); 4.64 (dd, 1H, J_{2',1'} = 7.0, J_{2',3'} = 5.3, H-2'); 6.01 (d, 1H, J_{1',2'} = 7.0, H-1'); 7.46 (s, 1H, H-6). ¹³C NMR (125.7 MHz, D₂O): 10.95 (CH₃CH₂N); 49.38 (CH₃CH₂N); 67.61 (d, J_{C,P} = 5.5, CH₂-5'); 72.09 (CH-3'); 76.10 (CH-2'); 79.42 (-C≡CH); 83.08 (HC≡C-); 86.37 (d, J_{C,P} = 9.1, CH-4'); 88.66 (CH-1'); 101.10 (C-5); 103.00 (C-4a); 127.41 (CH-6); 154.17 (C-7a); 156.11 (C-2); 163.60 (C-4). ³¹P NMR (202.3 MHz, D₂O): -22.36 (bs, P_b); -10.59, -10.05 (2 × bs, P_{a,b}). MS (ESI) m/z (%): 465 (100) [M-H₂PO₃-H], 485 (16) [M-H₂PO₃-2H+Na], 545 (28) [M-H], 565 (5) [M-2H+Na]. HRMS-ESI [M-2H+Na]⁻ calcd for C₁₃H₁₆O₁₄N₄P₃: 544.98832, found: 544.98813.

7-Phenyl-7-deazaguanosine 5'-O-triphosphate sodium salt (G^{Ph}TP)

G^{Ph}TP was prepared according to general procedure B by dissolving **G^{Ph}** (30 mg, 0.11 mmol), in PO(OMe)₃ (400 µl) and stirring with POCl₃ (9 µL, 0.10 mmol) for 1h, followed by the addition of solution of (NHBU₃)₂H₂P₂O₇ (183 mg, 0.33 mmol) and tributyl amine (84 µL, 0.35 mmol) in anhydrous DMF (1 mL) and stirring for another hour. Triphosphate (17 mg, 30 %) was obtained as a white lyophilizate (water). Triphosphate (17 mg, 30 %) was obtained as a white lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 4.16 (ddd, 1H, J_{gem} = 11.5, J_{H,P} = 5.1, J_{5'b,4'} = 3.6, H-5'b); 4.24 (ddd, 1H, J_{gem} = 11.5, J_{H,P} = 6.3, J_{5'a,4'} = 3.4, H-5'a); 4.32 (dddd, 1H, J_{4',5'} = 3.6, J_{4',3'} = 2.6, J_{H,P} = 2.1, H-4'); 4.54 (dd, 1H, J_{3',2'} = 5.4, J_{3',4'} = 2.6, H-3'); 4.73 (dd, 1H, J_{2',1'} = 7.0, J_{2',3'} = 5.4, H-2'); 6.11 (d, 1H, J_{1',2'} = 7.0, H-1'); 7.31 (s, 1H, H-6); 7.35 (m, 1H, H-*p*-Ph); 7.45 (m, 2H, H-*m*-Ph); 7.74

(m, 2H, H-*o*-Ph). ^{13}C NMR (125.7 MHz, D_2O): 68.30 (d, $J_{\text{C,P}} = 5.7$, $\text{CH}_2\text{-5'}$); 73.36 (CH-3'); 75.91 (CH-2'); 86.20 (d, $J_{\text{C,P}} = 9.1$, CH-4'); 88.42 (CH-1'); 100.88 (C-4a); 118.99 (CH-6); 124.11 (C-5); 129.76 (CH-*p*-Ph); 131.09 (CH-*o*-Ph); 131.20 (CH-*m*-Ph); 136.03 (C-*i*-Ph); 155.61 (C-2,7a); 163.86 (C-4). ^{31}P NMR (202.3 MHz, D_2O): -22.26 (t, $J = 19.9$, P_b); -10.66 (d, $J = 19.9$, P_a); -9.39 (bd, $J = 19.9$, P_g). MS (ESI) m/z (%): 517 (100) [$\text{M-H}_2\text{PO}_3\text{-H}$], 539 (45) [$\text{M-H}_2\text{PO}_3\text{-2H+Na}$], 597 (12) [M-H], 619 (15) [M-2H+Na], 641 (11) [M-3H+2Na]. HRMS-ESI [M-2H+Na] $^-$ calcd for $\text{C}_{17}\text{H}_{19}\text{O}_{14}\text{N}_4\text{NaP}_3$: 619.00151, found: 619.00138.

7-(Benzofuran-2-yl)-7-deazaguanosine 5'-O-triphosphate sodium salt ($\text{G}^{\text{BF}}\text{TP}$)

$\text{G}^{\text{BF}}\text{TP}$ was prepared according to general procedure B by dissolving G^{BF} (30 mg, 0.07 mmol), in $\text{PO}(\text{OMe})_3$ (200 μL) and stirring with POCl_3 (8 μL , 0.09 mmol) overnight, followed by the addition of solution of $(\text{NHBu}_3)_2\text{H}_2\text{P}_2\text{O}_7$ (165 mg, 0.30 mmol) and tributylamine (75 μL , 0.30 mmol) in anhydrous DMF (1 mL) and stirring for another hour. Triphosphate (14 mg, 26 %) was obtained as a white lyophilizate (water). ^1H NMR (500.0 MHz, D_2O): 4.19 (ddd, 1H, $J_{\text{gem}} = 11.6$, $J_{\text{H,P}} = 5.3$, $J_{5'b,4'} = 3.9$, H-5'b); 4.28 (ddd, 1H, $J_{\text{gem}} = 11.6$, $J_{\text{H,P}} = 6.8$, $J_{5'a,4'} = 3.5$, H-5'a); 4.34 (dddd, 1H, $J_{4',5'} = 3.9$, 3.5, $J_{4',3'} = 3.0$, $J_{\text{H,P}} = 1.7$, H-4'); 4.61 (dd, 1H, $J_{3',2'} = 5.4$, $J_{3',4'} = 3.0$, H-3'); 4.75 (dd, 1H, $J_{2',1'} = 6.8$, $J_{2',3'} = 5.4$, H-2'); 6.08 (d, 1H, $J_{1',2'} = 6.8$, H-1'); 7.27 (td, 1H, $J_{5,4} = J_{5,6} = 7.5$, $J_{5,7} = 1.2$, H-5-benzofuryl); 7.33 (ddd, 1H, $J_{6,7} = 8.3$, $J_{6,5} = 7.5$, $J_{6,4} = 1.3$, H-6-benzofuryl); 7.48 (d, 1H, $J_{3,7} = 0.8$, H-3-benzofuryl); 7.57 (ddt, 1H, $J_{7,6} = 8.3$, $J_{7,5} = 1.2$, $J_{7,3} = J_{7,4} = 0.8$, H-7-benzofuryl); 7.63 (ddd, 1H, $J_{4,5} = 7.5$, $J_{4,6} = 1.3$, $J_{4,7} = 0.8$, H-4-benzofuryl); 7.66 (s, 1H, H-6). ^{13}C NMR (125.7 MHz, D_2O): 68.16 (d, $J_{\text{C,P}} = 4.9$, $\text{CH}_2\text{-5'}$); 73.07 (CH-3'); 75.91 (CH-2'); 86.22 (d, $J_{\text{C,P}} = 9.0$, CH-4'); 88.70 (CH-1'); 100.08 (C-4a); 106.46 (CH-3-benzofuryl); 113.53 (C-5); 113.57 (CH-7-benzofuryl); 119.81 (CH-6); 123.61 (CH-4-benzofuryl); 125.65 (CH-5-benzofuryl); 126.87 (CH-6-benzofuryl); 131.98 (C-3a-benzofuryl); 153.43 (C-2-benzofuryl); 155.70 (C-7a); 155.95 (C-2); 156.73 (C-7a-benzofuryl); 163.66 (C-4). ^{31}P NMR (202.3 MHz, D_2O): -21.64 (t, $J = 19.4$, P_b); -10.33 (d, $J = 19.4$, P_a); MS (ESI) m/z (%): 557 (100) [$\text{M-H}_2\text{PO}_3\text{-H}$], 579 (46) [$\text{M-H}_2\text{PO}_3\text{-2H+Na}$], 637 (5) [M-H], 659 (8) [M-2H+Na], 681 (10) [M-3H+2Na]. HRMS-ESI [M-2H+Na] $^-$ calcd for $\text{C}_{19}\text{H}_{19}\text{O}_{15}\text{N}_4\text{P}_3$: 658.99561, found: 658.99629.

7-(Dibenzofuran-4-yl)-7-deazaguanosine 5'-O-triphosphate sodium salt ($\text{G}^{\text{DB}}\text{TP}$)

$\text{G}^{\text{DB}}\text{TP}$ was prepared according to general procedure B by dissolving G^{DB} (30 mg, 0.06 mmol), in $\text{PO}(\text{OMe})_3$ (200 μL) and stirring with POCl_3 (7 μL , 0.08 mmol) overnight, followed by the addition of solution of $(\text{NHBu}_3)_2\text{H}_2\text{P}_2\text{O}_7$ (147 mg, 0.28 mmol) and tributylamine (67 μL , 0.28 mmol) in anhydrous DMF (1 mL) and stirring for another hour. Triphosphate (5 mg, 10 %) was obtained as a white lyophilizate (water). ^1H NMR (500.0 MHz, D_2O , ref(dioxane) = 3.75 ppm): 4.12 (ddd, 1H, $J_{\text{gem}} = 11.6$, $J_{\text{H,P}} = 5.2$, $J_{5'b,4'} = 3.6$, H-5'b); 4.22 (ddd, 1H, $J_{\text{gem}} = 11.6$, $J_{\text{H,P}} = 6.9$, $J_{5'a,4'} = 3.8$, H-5'a); 4.36 (dddd, 1H, $J_{4',5'} = 3.8$, 3.6, $J_{4',3'} = 3.2$, $J_{\text{H,P}} = 1.6$, H-4'); 4.58 (dd, 1H, $J_{3',2'} = 5.5$, $J_{3',4'} = 3.2$, H-3'); 4.77 (dd, 1H, $J_{2',1'} = 6.8$, $J_{2',3'} = 5.5$, H-2'); 6.04 (d, 1H, $J_{1',2'} = 6.8$, H-1'); 7.38 (m, 1H, H-1-dibenzofuryl and H-2-dibenzofuryl); 7.44-7.50 (m, 2H, H-8-dibenzofuryl and H6); 7.60 (m, 1H, H7-dibenzofuryl); 7.71 (m, 1H, H-6-dibenzofuryl); 7.96 (m, 1H, H-3-dibenzofuryl); 8.03 (m, 1H, H-9-dibenzofuryl). ^{13}C NMR (125.7 MHz, D_2O , ref(dioxane) = 69.3 ppm): 68.16 (d, $J_{\text{C,P}} = 4.9$, $\text{CH}_2\text{-5'}$); 73.07 (CH-3'); 75.91 (CH-2'); 86.22 (d, $J_{\text{C,P}} = 9.0$, CH-4'); 88.70 (CH-1'); 101.92 (C-4a); 114.56 (C-5); 114.36 (CH-6-dibenzofuryl); 119.91 (C-4-dibenzofuryl); 122.76 (CH-3-dibenzofuryl); 123.70 (CH-9-dibenzofuryl); 124.23 (CH-6); 125.92 (CH-8-dibenzofuryl); 126.15 (CH-2-dibenzofuryl); 126.44 (C-9b-dibenzofuryl); 126.95 (C-9a-dibenzofuryl); 130.37 (CH-7-dibenzofuryl); 130.77 (CH-1-dibenzofuryl); 155.22 (C-4a-dibenzofuryl); 155.73 (C-7a); 156.04 (C-

2); 158.39 (C-5a-dibenzofuryl) 163.71 (C-4). ³¹P NMR (202.3 MHz, D₂O): -21.64 (t, *J* = 19.4, P_b); -10.33 (d, *J* = 19.4, P_a); -6.65 (bs, P_g). MS (ESI) *m/z* (%): 607 (100) [M-H₂PO₃-H], 629 (34) [M-H₂PO₃-2H+Na], 687 (5) [M-H], 709 (10) [M-2H+Na], 731 (15) [M-3H+2Na]. HRMS-ESI [M-2H+Na]⁻ calcd for C₂₁H₂₀O₁₆N₄NaP₃: 709.01062, found: 709.01194.

General procedure C: Suzuki cross-coupling on iodinated triphosphates:

Degassed solution of Pd(Ac)₂ (0.5 mg, 0.002 mmol) and TPPTS (6 mg, 0.01 mmol) in water/ACN (2 mL, 2:1) was added to the water/ACN (5 mL, 2:1) solution of N^ITP (30 mg, 0.04 mmol), corresponding boronic acid (0.11 mmol) and Cs₂CO₃ (70 mg, 0.2 mmol). The reaction mixture was left stirring for 30 min at 100°C. After cooling to the room temperature, the mixture was concentrated under reduced pressure and the product was purified by HPLC (C-18 column, 0.1M TEAB in water to 0.1M TEAB in 50% aq. MeOH), coevaporated several times with water and, where possible, converted to a sodium salt form (Dowex 50 in Na⁺ cycle).

7-(Benzofuran-2-yl)-7-deazaadenosine 5'-O-triphosphate sodium salt (A^{BF}TP)

Prepared from A^ITP (30 mg) and benzofuran-2-ylboronic acid (17 mg, 0.11 mmol) according to general procedure C. Triphosphate (17 mg, 58 %) was obtained as a white lyophilizate (water). Triphosphate (17 mg, 58 %) was obtained as a white lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 4.20 (ddd, 1H, *J*_{gem} = 11.6, *J*_{H,P} = 4.8, *J*_{5'b,4'} = 3.3, H-5'b); 4.31 (ddd, 1H, *J*_{gem} = 11.6, *J*_{H,P} = 6.5, *J*_{5'a,4'} = 3.1, H-5'a); 4.35 (dddd, 1H, *J*_{4',5'} = 3.3, 3.1, *J*_{4',3'} = 3.2, *J*_{H,P} = 2.2, H-4'); 4.63 (dd, 1H, *J*_{3',2'} = 5.3, *J*_{3',4'} = 3.2, H-3'); 4.74 (dd, 1H, *J*_{2',1'} = 6.5, *J*_{2',3'} = 5.3, H-2'); 6.19 (d, 1H, *J*_{1',2'} = 6.5, H-1'); 7.08 (s, 1H, H-3-benzofuryl); 7.16 – 7.28 (m, 2H, H-5,6-benzofuryl); 7.46 (m, 1H, H-7-benzofuryl); 7.54 (m, 1H, H-4-benzofuryl); 7.99 (s, 1H, H-2); 8.00 (s, 1H, H-6). ¹³C NMR (125.7 MHz, D₂O): 68.04 (d, *J*_{C,P} = 5.3, CH₂-5'); 73.10 (CH-3'); 76.51 (CH-2'); 86.32 (d, *J*_{C,P} = 8.9, CH-4'); 88.64 (CH-1'); 102.57 (C-4a); 104.80 (CH-3-benzofuryl); 110.37 (C-5); 113.47 (CH-7-benzofuryl); 123.55 (CH-4-benzofuryl); 123.69 (CH-6); 126.07 (CH-5-benzofuryl); 126.66 (CH-6-benzofuryl); 131.41 (C-3a-benzofuryl); 152.74 (C-2-benzofuryl); 153.15 (C-7a); 154.34 (CH-2); 156.46 (C-7a-benzofuryl); 159.78 (C-4). ³¹P NMR (202.3 MHz, D₂O): -21.06 (dd, *J* = 20.0, 19.6, P_b); -10.35 (d, *J* = 19.6, P_a); -6.65 (d, *J* = 20.0, P_g). MS (ESI) *m/z* (%): 541 (100) [M-H₂PO₃-H], 563 (56) [M-H₂PO₃-2H+Na], 621 (5) [M-H], 643 (23) [M-2H+Na], 665 (20) [M-3H+2Na]. HRMS-ESI [M-2H+Na]⁻ calcd for C₁₉H₁₉O₁₄N₄NaP₃: 643.00082, found: 643.00138.

7-(Dibenzofuran-4-yl)-7-deazaadenosine 5'-O-triphosphate sodium salt (A^{DB}TP)

Prepared from A^ITP (30 mg) and dibenzofuran-4-ylboronic acid (22 mg, 0.11 mmol) according to general procedure C. Triphosphate (14 mg, 45 %) was obtained as a white lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 4.12 (ddd, 1H, *J*_{gem} = 11.6, *J*_{H,P} = 5.2, *J*_{5'b,4'} = 3.6, H-5'b); 4.22 (ddd, 1H, *J*_{gem} = 11.6, *J*_{H,P} = 6.9, *J*_{5'a,4'} = 3.8, H-5'a); 4.36 (dddd, 1H, *J*_{4',5'} = 3.8, 3.6, *J*_{4',3'} = 3.2, *J*_{H,P} = 1.6, H-4'); 4.58 (dd, 1H, *J*_{3',2'} = 5.5, *J*_{3',4'} = 3.2, H-3'); 4.77 (dd, 1H, *J*_{2',1'} = 6.8, *J*_{2',3'} = 5.5, H-2'); 6.28 (d, 1H, *J*_{1',2'} = 6.8, H-1'); 7.28 (m, 1H, H-2-dibenzofuryl); 7.40 (m, 1H, H-1-dibenzofuryl); 7.44 (m, 1H, H-8-dibenzofuryl); 7.49 – 7.54 (m, 2H, H-6,7-dibenzofuryl); 7.61 (s, 1H, H-6); 7.71 (m, 1H, H-3-dibenzofuryl); 8.01 (m, 1H, H-9-dibenzofuryl); 8.08 (s, 1H, H-2). ¹³C NMR (125.7 MHz, D₂O): 68.11 (d, *J*_{C,P} = 5.4, CH₂-5'); 73.04 (CH-3'); 75.97 (CH-2'); 86.17 (d, *J*_{C,P} = 8.8, CH-4'); 88.45 (CH-1'); 104.29 (C-4a); 114.32 (C-5); 114.36 (CH-6-dibenzofuryl); 119.91 (C-4-dibenzofuryl); 122.76 (CH-3-dibenzofuryl); 123.70 (CH-9-dibenzofuryl); 124.23 (CH-6); 125.92 (CH-8-dibenzofuryl); 126.15 (CH-2-dibenzofuryl); 126.44 (C-9b-dibenzofuryl); 126.95 (C-9a-dibenzofuryl); 130.37 (CH-7-dibenzofuryl); 130.77 (CH-1-dibenzofuryl); 152.96 (C-7a); 154.16

(CH-2); 155.62 (C-4a-dibenzofuryl); 158.32 (C-5a-dibenzofuryl); 159.72 (C-4). ³¹P NMR (202.3 MHz, D₂O): -21.13 (dd, *J* = 19.9, 19.3, *P_b*); -10.32 (d, *J* = 19.3, *P_a*); -5.50 (d, *J* = 19.9, *P_g*). MS (ESI) *m/z* (%): 591 (100) [M-H₂PO₃-H], 613 (86) [M-H₂PO₃-2H+Na], 671 (6) [M-H], 693 (26) [M-2H+Na], 715 (27) [M-3H+2Na]. HRMS-ESI [M-2H+Na]⁻ calcd for C₂₃H₂₁O₁₄N₄NaP₃: 693.01611, found: 693.01703.

5-Phenylcytidine 5'-O-triphosphate triethylammonium salt (C^{Ph}TP)

Prepared from C^ITP (15 mg) and phenylboronic acid (13 mg, 0.11 mmol) according to general procedure C. Triphosphate (9 mg, 64 %) was obtained as a glassy lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 1.28 (t, 9H, *J_{vic}* = 7.3, CH₃CH₂N); 3.20 (q, 6H, *J_{vic}* = 7.3, CH₃CH₂N); 4.07 (ddd, 1H, *J_{gem}* = 11.5, *J_{H,P}* = 5.1, *J_{5'b,4'}* = 3.6, H-5'b); 4.13 (ddd, 1H, *J_{gem}* = 11.5, *J_{H,P}* = 6.3, *J_{5'a,4'}* = 3.4, H-5'a); 4.18 (m, H-4'); 4.33 (m, 2H, H-3' and H-2'); 5.97 (d, *J_{1',2'}* = 5.0, 1H, H-1'); 7.37 (m, 2H, H-*o*-Ph); 7.41 (m, 1H, H-*p*-Ph); 7.47 (m, 2H, H-*m*-Ph); 7.69 (s, 1H, H-6). ¹³C NMR (125.7 MHz, D₂O): 10.95 (CH₃CH₂N); 49.35 (CH₃CH₂N); 67.71 (d, *J_{C,P}* = 5.7, CH₂-5'); 72.18 (CH-3'); 76.48 (CH-2'); 85.82 (d, *J_{C,P}* = 9.1, CH-4'); 91.64 (CH-1'); 113.76 (C-5); 131.53 (CH-*p*-Ph); 132.07 (CH-*m*-Ph); 132.09 (CH-*o*-Ph); 134.71 (C-*i*-Ph); 142.31 (CH-6); 160.13 (C-2); 167.47 (C-4). ³¹P NMR (202.3 MHz, D₂O): -22.89 (t, *J* = 19.6, *P_b*); -11.64 (d, *J* = 19.6, *P_a*); -7.98 (bd, *J* = 19.6, *P_g*). MS (ESI) *m/z* (%): 478 (70) [M-H₂PO₃-H], 540 (2) [M-H₂PO₃-2H+Na], 558 (100) [M-H]. HRMS-ESI [M-2H+Na]⁻ calcd for C₁₅H₁₈O₁₄N₃NaP₃: 579.98956, found: 579.99048.

5-(Benzofuran-2-yl)-cytidine 5'-O-triphosphate sodium salt (C^{BF}TP)

Prepared from C^ITP (30 mg) and benzofuran-2-ylboronic acid (17 mg, 0.11 mmol) according to general procedure C. Triphosphate (11 mg, 37 %) was obtained as a white lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 4.25 – 4.36 (m, 3H, H-4',5'); 4.45 (dd, 1H, *J_{3',2'}* = 5.2, *J_{3',4'}* = 4.8, H-3'); 4.49 (dd, 1H, *J_{2',3'}* = 5.2, *J_{2',1'}* = 4.6, H-2'); 6.04 (d, 1H, *J_{1',2'}* = 4.6, H-1'); 7.24 (d, 1H, *J_{3,7}* = 1.0, H-3-benzofuryl); 7.33 (td, 1H, *J_{5,4}* = *J_{5,6}* = 7.6, *J_{5,7}* = 1.1, H-5-benzofuryl); 7.38 (ddd, 1H, *J_{6,7}* = 8.3, *J_{6,5}* = 7.6, *J_{6,4}* = 1.5, H-6-benzofuryl); 7.62 (dq, 1H, *J_{7,6}* = 8.3, *J_{7,3}* = *J_{7,4}* = *J_{7,5}* = 1.1, H-7-benzofuryl); 7.74 (ddd, 1H, *J_{4,5}* = 7.6, *J_{4,6}* = 1.5, *J_{4,7}* = 1.1, H-4-benzofuryl); 8.31 (s, 1H, H-6). ¹³C NMR (125.7 MHz, D₂O): 67.47 (d, *J_{C,P}* = 5.4, CH₂-5'); 71.87 (CH-3'); 77.0 (CH-2'); 85.90 (d, *J_{C,P}* = 9.1, CH-4'); 92.23 (CH-1'); 103.36 (C-5); 107.60 (CH-3-benzofuryl); 114.04 (CH-7-benzofuryl); 124.28 (CH-4-benzofuryl); 126.09 (CH-5-benzofuryl); 127.50 (CH-6-benzofuryl); 131.02 (C-3a-benzofuryl); 143.73 (CH-6); 151.66 (C-2-benzofuryl); 157.18 (C-7a-benzofuryl); 159.40 (C-2); 165.72 (C-4). ³¹P NMR (202.4 MHz, D₂O): -22.19 (t, *J* = 19.8, *P_b*); -11.36 (d, *J* = 19.8, *P_a*); -6.81 (bd, *J* = 19.8, *P_g*). MS (ESI) *m/z* (%): 518 (100) [M-H₂PO₃-H], 540 (76) [M-H₂PO₃-2H+Na], 598 (8) [M-H], 620 (30) [M-2H+Na], 642 (45) [M-3H+2Na]. HRMS-ESI [M-2H+Na]⁻ calcd for C₁₇H₂₀O₁₅N₃NaP₃: 619.98520, found: 619.98539.

5-(Dibenzofuran-4-yl)-cytidine 5'-O-triphosphate sodium salt (C^{DB}TP)

Prepared from C^ITP (30 mg) and dibenzofuran-4-ylboronic acid (22 mg, 0.11 mmol) according to general procedure C. Triphosphate (11 mg, 37 %) was obtained as a white lyophilizate (water). ¹H NMR (500.0 MHz, D₂O): 4.16 – 4.21 (m, 2H, H-5'); 4.29 (dtd, 1H, *J_{4',3'}* = 5.1, *J_{4',5'}* = 3.4, *J_{H,P}* = 1.7, H-4'); 4.39 (t, 1H, *J_{3',2'}* = *J_{3',4'}* = 5.1, H-3'); 4.43 (dd, 1H, *J_{2',3'}* = 5.1, *J_{2',1'}* = 4.9, H-2'); 6.06 (d, 1H, *J_{1',2'}* = 4.9, H-1'); 7.44 (ddd, 1H, *J_{8,9}* = 7.8, *J_{8,7}* = 7.3, *J_{8,6}* = 1.1, H-8-dibenzofuryl); 7.50 – 7.54 (m, 2H, H-1,2-dibenzofuryl); 7.55 (ddd, 1H, *J_{7,6}* = 8.5, *J_{7,8}* = 7.3, *J_{7,9}* = 1.3, H-7-dibenzofuryl);

7.62 (ddd, 1H, $J_{6,7} = 8.5$, $J_{6,8} = 1.1$, $J_{6,9} = 0.7$, H-6-dibenzofuryl); 7.96 (s, 1H, H-6); 8.10 (ddd, 1H, $J_{9,8} = 7.8$, $J_{9,7} = 1.3$, $J_{9,6} = 0.7$, H-9-dibenzofuryl); 8.14 (dd, 1H, $J_{3,2} = 6.5$, $J_{3,1} = 2.5$, H-3-dibenzofuryl). ^{13}C NMR (125.7 MHz, D_2O): 67.80 (d, $J_{\text{C,P}} = 5.5$, $\text{CH}_2\text{-5'}$); 72.75 (CH-3'); 76.60 (CH-2'); 85.68 (d, $J_{\text{C,P}} = 8.9$, CH-4'); 92.23 (CH-1'); 108.25 (C-5); 114.45 (CH-6-dibenzofuryl); 118.57 (C-4-dibenzofuryl); 123.83 (CH-9-dibenzofuryl); 124.58 (CH-3-dibenzofuryl); 126.02 (CH-8-dibenzofuryl); 126.43 (C-9a-dibenzofuryl); 126.68 (CH-2-dibenzofuryl); 127.14 (C-9b-dibenzofuryl); 130.52 (CH-7-dibenzofuryl); 131.83 (CH-1-dibenzofuryl); 143.99 (CH-6); 156.22 (C-4a-dibenzofuryl); 158.41 (C-5a-dibenzofuryl); 160.06 (C-2); 167.08 (C-4). ^{31}P NMR (202.4 MHz, D_2O): -22.82 (t, $J = 19.7$, P_b); -11.59 (d, $J = 19.7$, P_a); -9.54 (bd, $J = 19.7$, P_g). MS (ESI) m/z (%): 568 (100) $[\text{M-H}_2\text{PO}_3\text{-H}]$, 590 (47) $[\text{M-H}_2\text{PO}_3\text{-2H+Na}]$, 648 (10) $[\text{M-H}]$, 670 (25) $[\text{M-2H+Na}]$, 692 (17) $[\text{M-3H+2Na}]$. HRMS-ESI $[\text{M-2H+Na}]^-$ calcd for $\text{C}_{21}\text{H}_{20}\text{O}_{15}\text{N}_3\text{NaP}_3$: 670.00190, found: 670.00104.

5-(Dibenzofuran-4-yl)-uridine 5'-O-triphosphate triethylammonium salt ($\text{U}^{\text{DB}}\text{TP}$)

Prepared from $\text{U}^{\text{I}}\text{TP}$ (30 mg) and dibenzofuran-4-ylboronic acid (22 mg, 0.11 mmol) according to general procedure C. Triphosphate (10 mg, 33 %) was obtained as a white lyophilizate (water). ^1H NMR (500.0 MHz, D_2O): 1.28 (t, 9H, $J_{\text{vic}} = 7.3$, $\text{CH}_3\text{CH}_2\text{N}$); 3.20 (q, 6H, $J_{\text{vic}} = 7.3$, $\text{CH}_3\text{CH}_2\text{N}$); 4.27 (m, 3H, H-4' and H-5'); 4.40 (m, 2H, H-3' and H-4'); 5.97 (d, 1H, $J_{1',2'} = 4.9$, H-1'); 7.34 (m, 2H, H-1-dibenzofuryl and H-2-dibenzofuryl); 7.46 (m, 2H, H-8-dibenzofuryl and H-7-dibenzofuryl); 7.52 (m, 1H, H-6-dibenzofuryl); 7.95-7.98 (m, 3H, H-3-dibenzofuryl and H-9-dibenzofuryl and H-6). ^{13}C NMR (125.7 MHz, D_2O): 10.95 ($\text{CH}_3\text{CH}_2\text{N}$); 49.34 ($\text{CH}_3\text{CH}_2\text{N}$); 68.13 (d, $J_{\text{C,P}} = 5.5$, $\text{CH}_2\text{-5'}$); 72.65 (CH-3'); 76.22 (CH-2'); 86.14 (d, $J_{\text{C,P}} = 9.1$, CH-4'); 91.83 (CH-1'); 114.16 (C-5); 114.49 (CH-6-dibenzofuryl); 118.78 (C-4-dibenzofuryl); 123.72 (CH-9-dibenzofuryl); 124.00 (CH-3-dibenzofuryl); 126.00 (CH-8); 126.22 (CH-9a-dibenzofuryl); 126.38 (CH-2-dibenzofuryl); 126.82 (C-9b-dibenzofuryl); 130.50 (CH-7-dibenzofuryl); 131.18 (CH-1-dibenzofuryl); 143.18 (CH-6); 154.13 (C-2); 155.98 (C-4a-dibenzofuryl); 158.28 (C-5a-dibenzofuryl); 166.27 (C-4). ^{31}P NMR (202.3 MHz, D_2O): -20.68 (t, $J = 19.9$, $\text{P}_\square$); -9.12 (d, $J = 19.9$, $\text{P}_\square$); -8.30 (d, $J = 19.9$, $\text{P}_\square$). MS (ESI) m/z (%): 649 (100) $[\text{M-H}]$, 569 $[\text{M-H}_2\text{PO}_3\text{-H}]$. HRMS-ESI $[\text{M-H}]^-$ calcd for $\text{C}_{21}\text{H}_{20}\text{O}_{16}\text{N}_2\text{P}_3$: 670.00312, found: 670.00378.

Biochemistry

In vitro transcription with modified NTPs

A solution of template oligonucleotides (100 μ M each) in annealing buffer [Tris (10 mM), NaCl (50 mM), EDTA (1 mM), pH 7.8] was heated to 95°C for 5 minutes and slowly cooled to 25°C over a period of 45 minutes. The resulting DNA (50 μ M) was used as a template for transcription reactions. *In vitro* transcription reactions were performed in the total volume of 20 μ L in 40 mM Tris buffer (pH 7.9) containing modified NTP (2 mM), three natural NTPs (2 mM), DTT (10 mM), MgCl₂ (25 mM), Ribolock RNase inhibitor (1U/ μ L), Triton X-100 (0.1%), dsDNA template (0.625 μ M), T7 RNA polymerase (2U/ μ L, Thermoscientific) and [α -³²P]-GTP (111 TBq/mmol, 370 MBq/mL, 0.4 μ L) or [α -³²P]-ATP (111 TBq/mmol, 370 MBq/mL, 0.4 μ L) if transcription is done with modified GTPs. In the negative control experiment, water was used instead of the solution of modified NTP, and in the positive control the natural NTP (2 mM) was used instead. The transcription reactions were performed at 37°C for 2-4 h. The samples (2 μ L) were mixed with RNA loading dye (2 μ L, Thermoscientific), heated to 75°C for 10 minutes and cooled on ice. The samples were then analyzed by gel electrophoresis on 12.5% denaturing polyacrylamide gel containing 1xTBE buffer (pH 8) and urea (7 M) at 42 mA for 45 minutes. The gels were dried (85°C, 75 minutes), autoradiographed and visualized by phosphorimager (Typhoon 9410, Amersham Biosciences). Transcription efficiencies were determined densitometrically from three independent experiments using QuantityOne software. Transcriptions with modified adenosines were performed according to this general condition, with 2h transcription and twofold excess modified ATPs or natural ATP in positive control (4 mM). RNA_{3A}^R was transcribed with reduced concentration of MgCl₂ (15 mM) to reduce misincorporations.

Modified cytidines and uridines were transcribed according to the protocol for 3h and 4h respectively.

In vitro transcription with modified GTPs in the presence of GMP

A solution of template oligonucleotides (100 μ M each) in annealing buffer [Tris (10 mM), NaCl (50 mM), EDTA (1 mM), pH 7.8] was heated to 95°C for 5 minutes and slowly cooled to 25°C over a period of 45 minutes. The resulting DNA (50 mM) was used as a template for transcription reactions. *In vitro* transcription reactions were performed in the total volume of 20 μ L in 40 mM Tris buffer (pH 7.9) containing modified GTP (2 mM), GMP (30 mM), CTP (2 mM), UTP (2 mM), ATP (2 mM), DTT (10 mM), MgCl₂ (25 mM), Ribolock RNase inhibitor (1U/ μ L), Triton X-100 (0.1%), dsDNA template (0.625 μ M), T7 RNA polymerase (3U/ μ L, Thermoscientific) and [α -³²P]-ATP (111 TBq/mmol, 370 MBq/mL, 0.4 μ L). In the negative control experiment, water was used instead of the solution of modified GTP, and in the positive control the natural GTP (2 mM) was used instead. The transcription reactions were performed at 37°C for 4h. The samples (2 μ L) were mixed with RNA loading dye (2 μ L, Thermoscientific), heated to 75°C for 10 minutes and cooled on ice. The samples were then analyzed by gel electrophoresis on 12.5% denaturing polyacrylamide gel containing 1xTBE buffer (pH 8) and urea (7 M) at 42 mA for 45 minutes. The gels were dried (85°C, 75 minutes), autoradiographed and visualized by phosphorimager (Typhoon

9410, Amersham Biosciences). Transcription efficiency was determined densitometrically from three independent experiments using QuantityOne software.

MALDI-TOF analysis of modified RNAs

A solution of template oligonucleotides (100 μ M each) in annealing buffer [Tris (10 mM), NaCl (50 mM), EDTA (1 mM), pH 7.8] was heated to 95°C for 5 minutes and slowly cooled to 25°C over a period of 45 minutes. The resulting DNA (50 μ M) was used as a template for transcription reactions. *In vitro* transcription reactions were performed in the total volume of 50 μ L in 40 mM Tris buffer (pH 7.9) containing modified NTP (2 mM or 4 mM for modified CTPs), three natural NTPs (2 mM), DTT (10 mM), MgCl₂ (25 mM), Ribolock RNase inhibitor (1U/ μ L), Triton X-100 (0.1%), dsDNA template (1.25 μ M), T7 RNA polymerase (3U/ μ L, Thermoscientific). The transcription reactions were performed at 37°C for 2-4h. Then, DNase I (2U, Thermoscientific) was added and samples were incubated for further 15 minutes. The samples were then purified on NucAway spin columns (Ambion, elution done in DEPC treated water) as per supplier's protocol. Purified samples were analyzed by MALDI-TOF mass spectrometry. The MALDI-TOF spectra were measured on a MALDI-TOF/TOF mass spectrometer with 1 kHz smartbeam II laser. The measurements were done in reflectron mode by droplet technique, with the mass range up to 30 kDa. The matrix consisted of 3-hydroxypicolinic acid (HPA)/picolinic acid (PA)/ ammonium tartrate in ratio 9/1/1. The matrix (1 μ L) was applied on the target (ground steel) and dried down at room temperature. The sample (1 μ L) and matrix (1 μ L) were mixed and added on the top of the dried matrix preparation spot and dried at room temperature.

II Sequences of DNA templates and RNA transcripts
Table S1. Sequences of ds DNA template and RNA transcripts

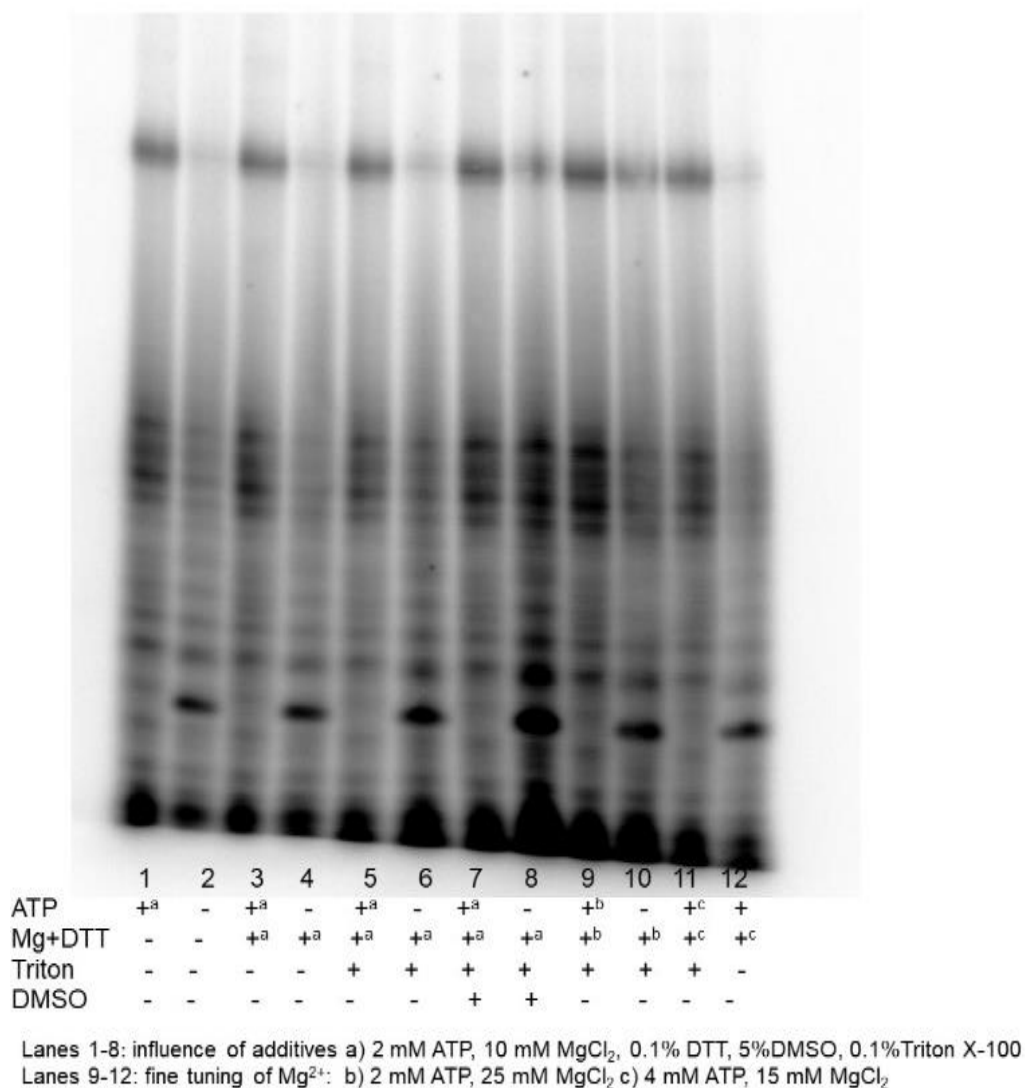
DNA_1A^R	5' TAATACGACTCACTATAGGGCCCCTATTGTCTCTCTCTTCTCTGCTGTTTCC3' 3' <u>ATTATGCTGAGTGATAT</u> CCCGGGGATAAACAGAGAGAGAAGAGACGACAAAG (2' MeO) G5'
RNA_1A^R	pppGGGCCCCUAUUGUCUCUCUCUUCUCUGCUGUUUCC
DNA_3A^R	5' TAATACGACTCACTATAGGGCCCGTATGTTACTTGCTCTTATCGTCTCTCGC3' 3' <u>ATTATGCTGAGTGATAT</u> CCCGGGCATACAATGAACGAGAAATAGCAGAGAGC (2' MeO) G5'
RNA_3A^R	pppGGGCCCCUAUGUUACUUGCUCUUACUGUCUCUCGC
DNA_7A^R	5' TAATACGACTCACTATAGGGCTTGACAGTGAATCGCTCTTAATGGATCGCGA3' 3' <u>ATTATGCTGAGTGATAT</u> CCCGAACGTGCACTTAGCGAGAAATTACCTAGCGC (2' MeO) T5'
RNA_7A^R	pppGGGCUUGCACGUGAAUCGCUCUUAAUGGAUCGCGA
DNA_1C^R	5' TAATACGACTCACTATAGGGAGGGACTGTGAGTGGAGATTGTAGGATTGAGG3' 3' <u>ATTATGCTGAGTGATAT</u> CCCTCCCTGACACTCACCTCTAACATCCTAACTC (2' MeO) C5'
RNA_1C^R	pppGGGAGGGAUGUGAGUGGAGAUUGUAGGAUUGAGG
DNA_3C^R	5' TAATACGACTCACTATAGGGAGGATCAGTACAGAGGTATGCTGGGATAGGGA3' 3' <u>ATTATGCTGAGTGATAT</u> CCCTCCTAGTCATGTCTCCATACGACCCTATCCC (2' MeO) T5'
RNA_3C^R	pppGGGAGGAUCAGUACAGAGGUAUGCUGGGAUAGGGA
DNA_7C^R	5' TAATACGACTCACTATAGGGATTGACAGTGCCTAGATATTCCGGGCTAGGGC3' 3' <u>ATTATGCTGAGTGATAT</u> CCCTAACTGTCACGGATCTATAAGGCCGATCCC (2' MeO) G5'
RNA_7C^R	pppGGGAUUGACAGUGCCUAGAUUUCCGGGCUAGGGC
DNA_1U^R	5' TAATACGACTCACTATAGGGCCAGCTCAAGCCACGCACCCACGGCACACAC3' 3' <u>ATTATGCTGAGTGATAT</u> CCCGGTTCGAGTTCGGTGCGTGGGGTGCCGTGTGT (2' MeO) G5'
RNA_1U^R	pppGGGCCAGCUCAAGCCACGCACCCACGGCACACAC
DNA3U^R3gG^R	5' TAATACGACTCACTATAGGGCCAGCTCACATCAAAGACACTACGACACACAC3' 3' <u>ATTATGCTGAGTGATAT</u> CCCGGTTCGAGTGTAGTTTCTGTGATGCTGTGTGT (2' MeO) G5'
RNA_3U^R	pppGGGCCAGCUCAUCAAAAGACACUACGACACACAC
RNA_3gG^R	pppGGGCCAGCUCACAUCAAAGACACUACGACACACAC
DNA_7U^R	5' TAATACGACTCACTATAGGGCCAGCTCAAGTTACGCACCCTTCGGTACAAAT3' 3' <u>ATTATGCTGAGTGATAT</u> CCCGGTTCGAGTTCAAATGCGTGGGAAGCCATGTTT (2' MeO) A5'
RNA_7U^R	pppGGGCCAGCUCAAGUUACGCACCCUUCGGUACAAAU
DNA_1aG^R	5' TAATACGACTCACTATTAAACCAATGCACCCTCCTCATCCTCCACCACACCA3' 3' <u>ATTATGCTGAGTGATAA</u> TTTGGTTACGTGTGGGAGGAGTAGGAGGTGGTGTGG (2' MeO) T5'
RNA_1aG^R	pppAAACCAAUGCACCCUCCUAUCCUCCACCACACCA
DNA_3aG^R	5' TAATACGACTCACTATTAAACCAATGCACAGCACTCCTCCGACACCACACCA3' 3' <u>ATTATGCTGAGTGATAA</u> TTTGGTTACGTGTCTGTGAGGAGGCTGTGGTGTGG (2' MeO) T5'
RNA_3aG^R	pppAAACCAAUGCACAGCACUCCUCCGACACCACACCA
DNA_7aG^R	5' TAATACGACTCACTATTAAACCAATGCACAGGACTCATCCGGCAGACCACCG3' 3' <u>ATTATGCTGAGTGATAA</u> TTTGGTTACGTGTCTGTGAGTAGGCCGTTCTGTGG (2' MeO) C5'
RNA_7aG^R	pppAAACCAAUGCACAGGACUCAUCCGGCAGACCACCG
DNA_8G^R	5' TAATACGACTCACTATAGCAAACCAGTCCAGGAACATCAGGTACAGTCTCTG3' 3' <u>ATTATGCTGAGTGATAT</u> CGTTTGGTCAGGTCCTTGTAGTCCATGTGAGAG (2' MeO) A (2' MeO) C5'
DNA_8G^R	pGCAAACCAGUCCAGGAACAUCAGGUACAGUCUCUG

Promoter regions in the antisense strand are underlined. The one or two 5'-terminal nucleotides in the antisense strand was 2'-MeO ribonucleotide to minimize non-templated nucleotide addition.¹²

The first base after the promoter region is marked in magenta, while those that are directing transcription of modified bases are marked in blue, whereas the modified bases in the transcripts are marked in red. In the case of template **DNA_3U^RgG^R** which is used either for synthesis of RNA containing modified Us or Gs, when it is used to yield RNA with modified uridines complementary adenines are marked green, while when used for the incorporation of modified guanosines, the complementary cytosines are marked blue.

III Copies of PAGE GELS

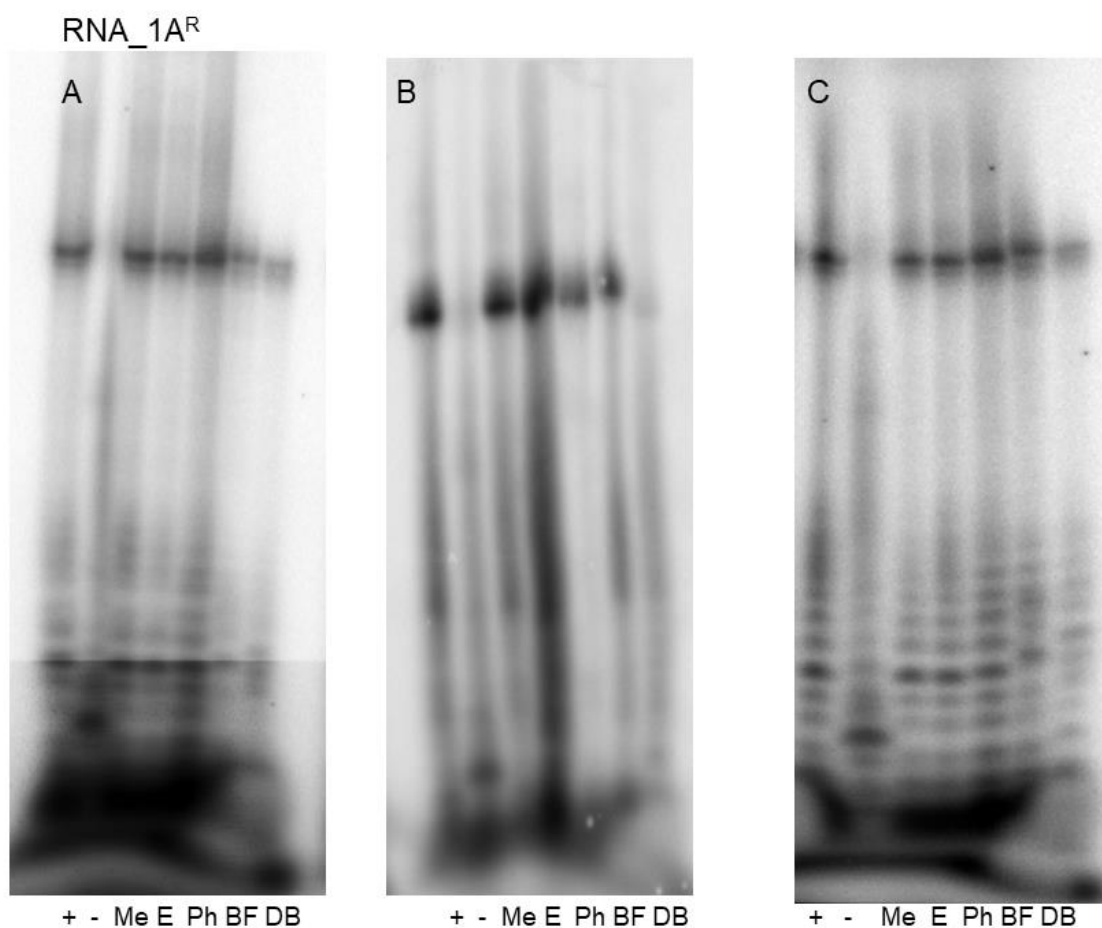
Figure S1. Optimization of conditions for modified ATPs. Template: DNA_3A^R



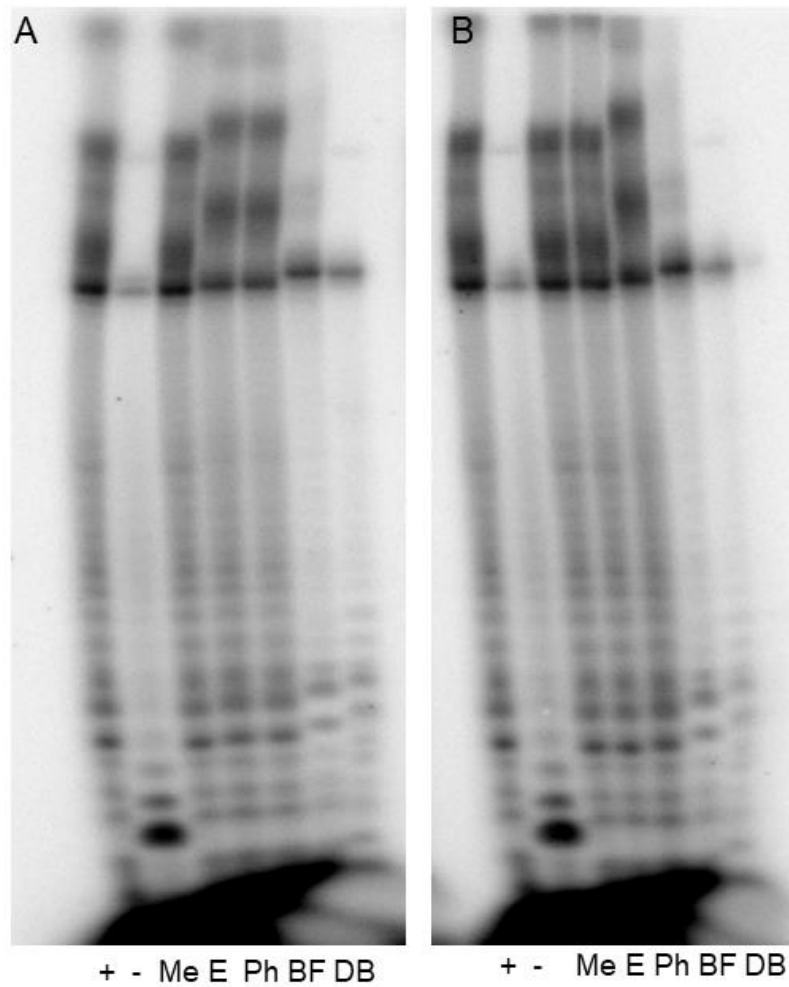
In vitro transcription reactions were performed in the total volume of 20 μ L in 40 mM Tris buffer (pH 7.9) containing modified ATP, three natural NTPs (2 mM), Ribolock RNase inhibitor (1U/ μ L), dsDNA template (0.625 μ M), T7 RNA polymerase (2U/ μ L, ThermoFisher) and [α -³²P]-GTP (111 TBq/mmol, 370 MBq/mL, 0.4 μ L). In the negative control experiment, water was used instead of the solution of modified A^RTP, and in the positive control the natural ATP was used instead. Reactions were incubated at 37°C for 2 h. The use of additives gives the optimal yields while keeping the misincorporations minimal for most of the templates and these conditions

were used as standard for majority of experiments. Transcription of DNA_3A^R under these conditions gives higher amount of RNA but misincorporations are also significant (lanes 9,10), so this template was also transcribed in presence of additives but with lower concentration of Mg²⁺ to reduce misincorporations (lanes 11, 12)

Figure S2. Transcription with modified ATPs



RNA_{3A}^R



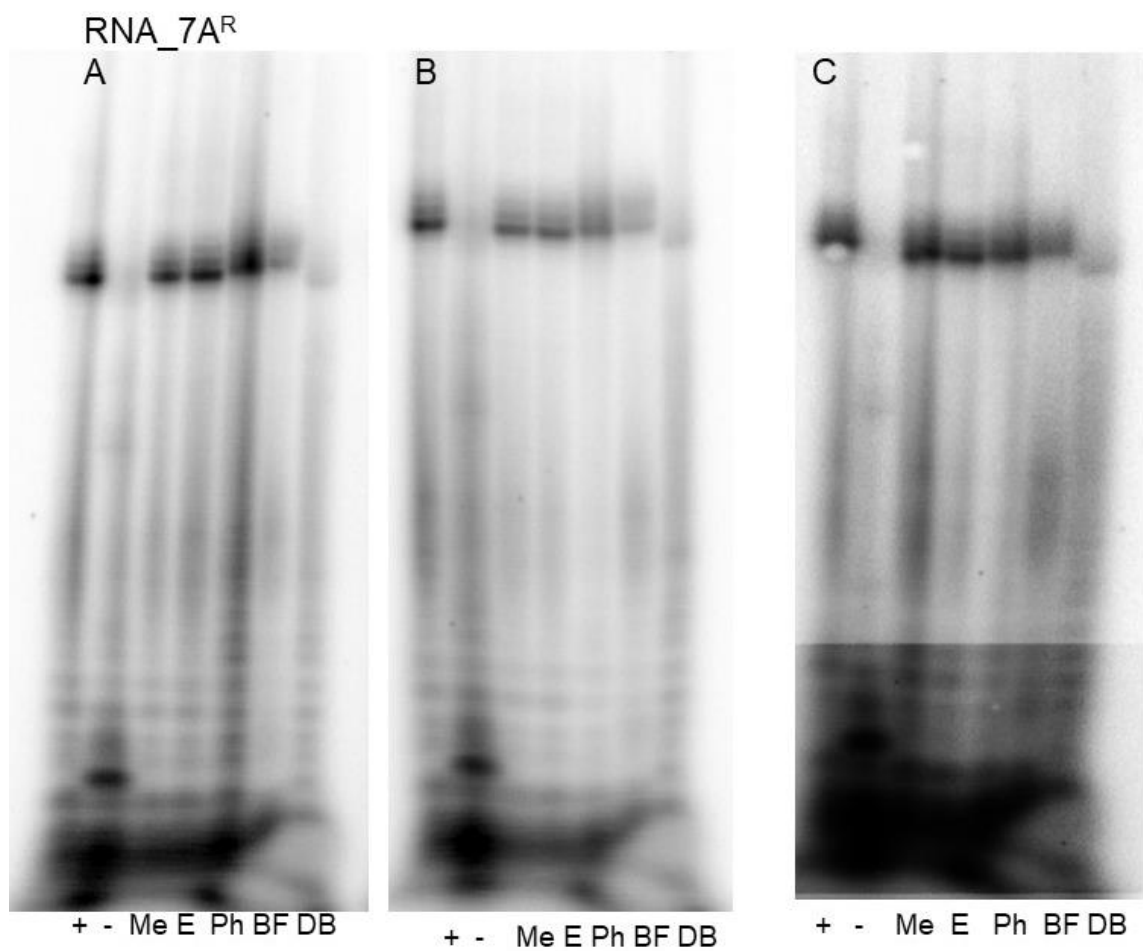
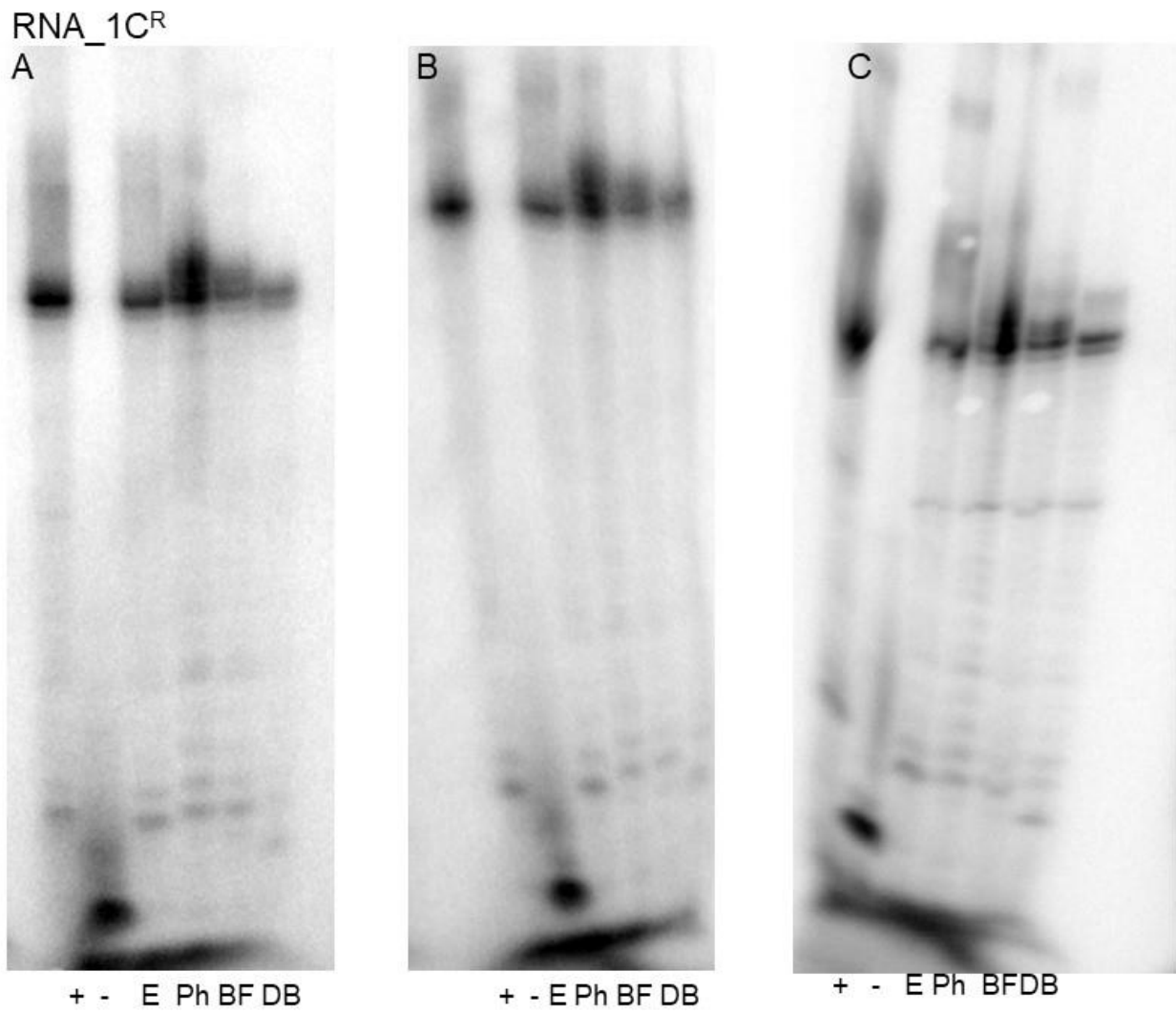
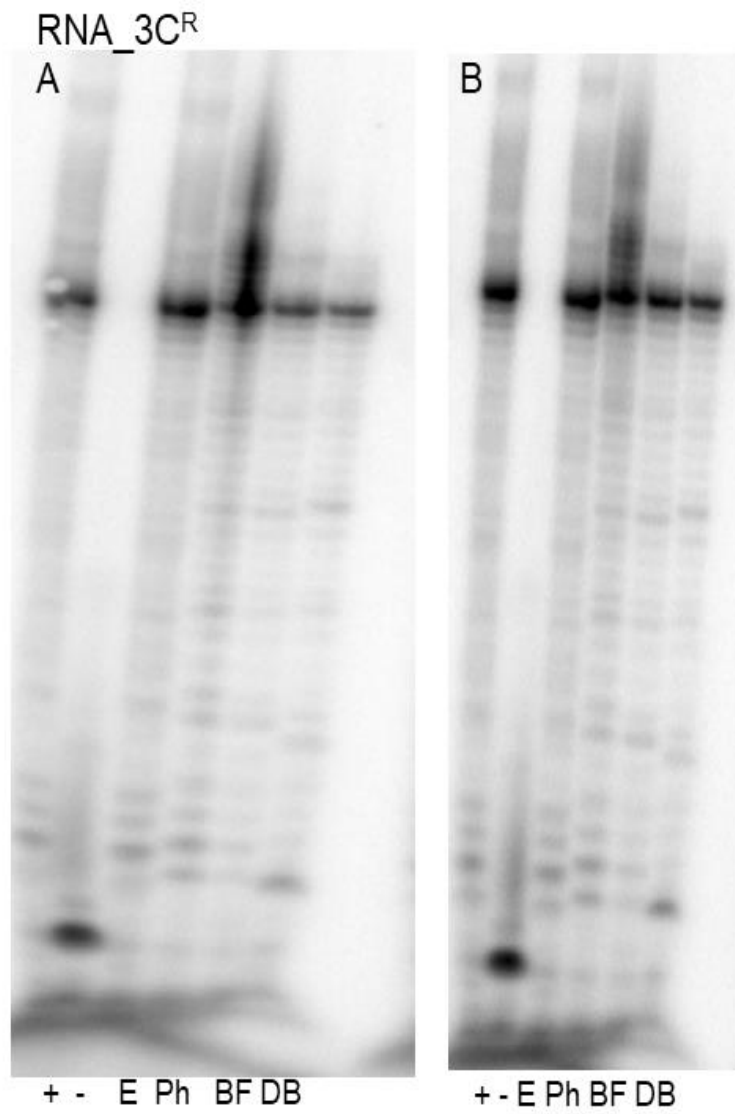


Figure S3. Transcription with modified CTPs





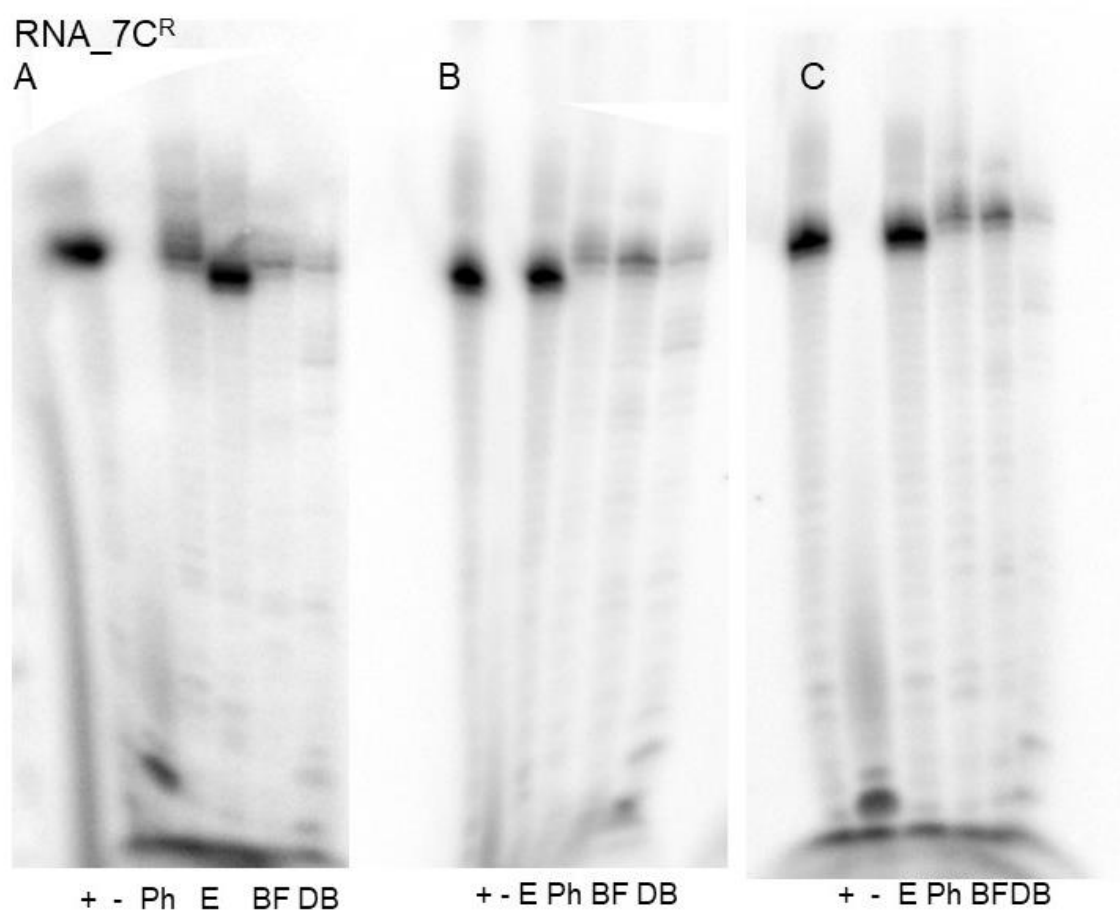
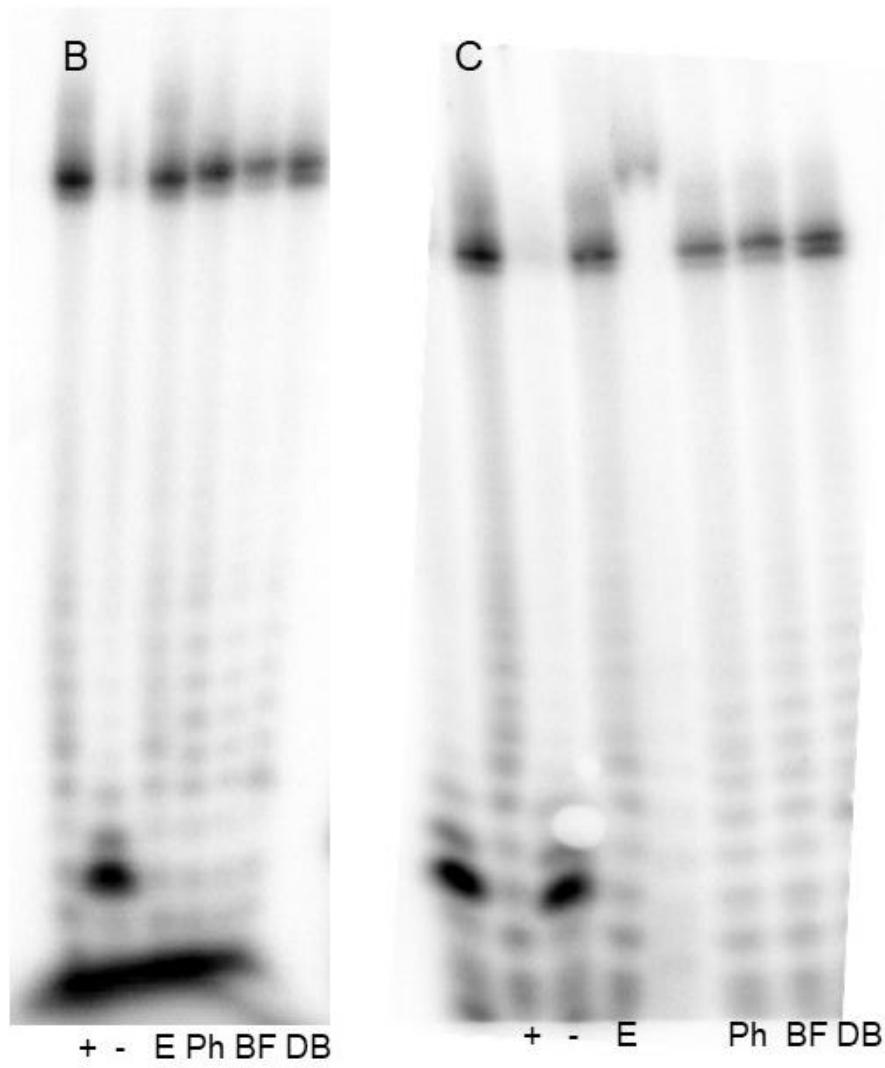


Figure S4. Transcription with modified UTPs



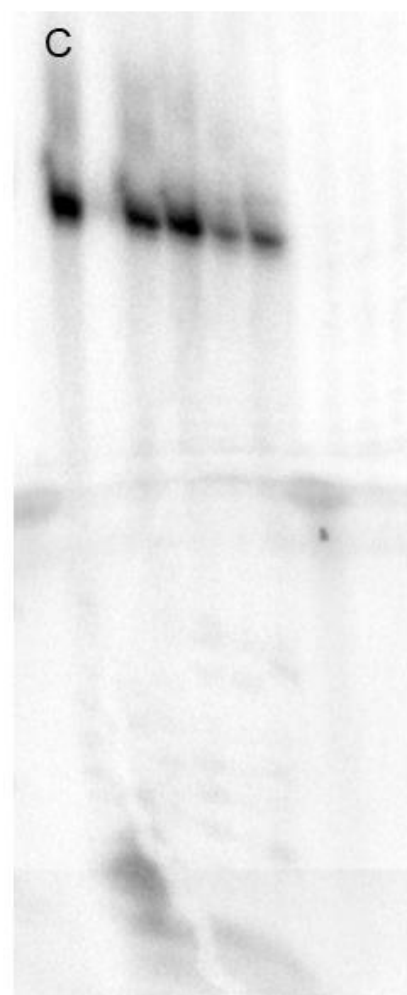
RNA_3U^R



+ - E Ph BFDB



+ - E Ph BF DB



+ - E Ph BFDB

RNA_7U^R

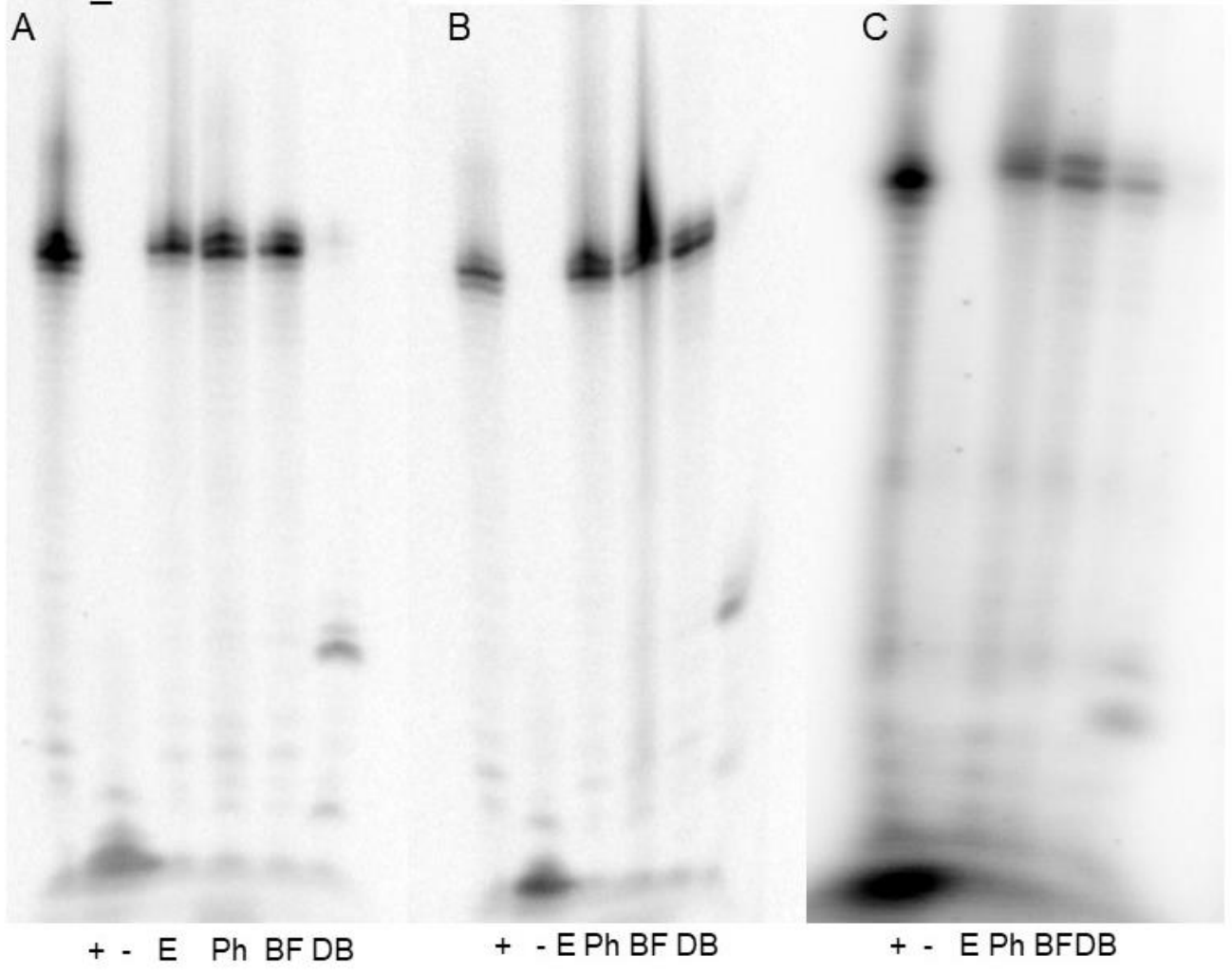


Figure S5. Transcription with modified GTPs

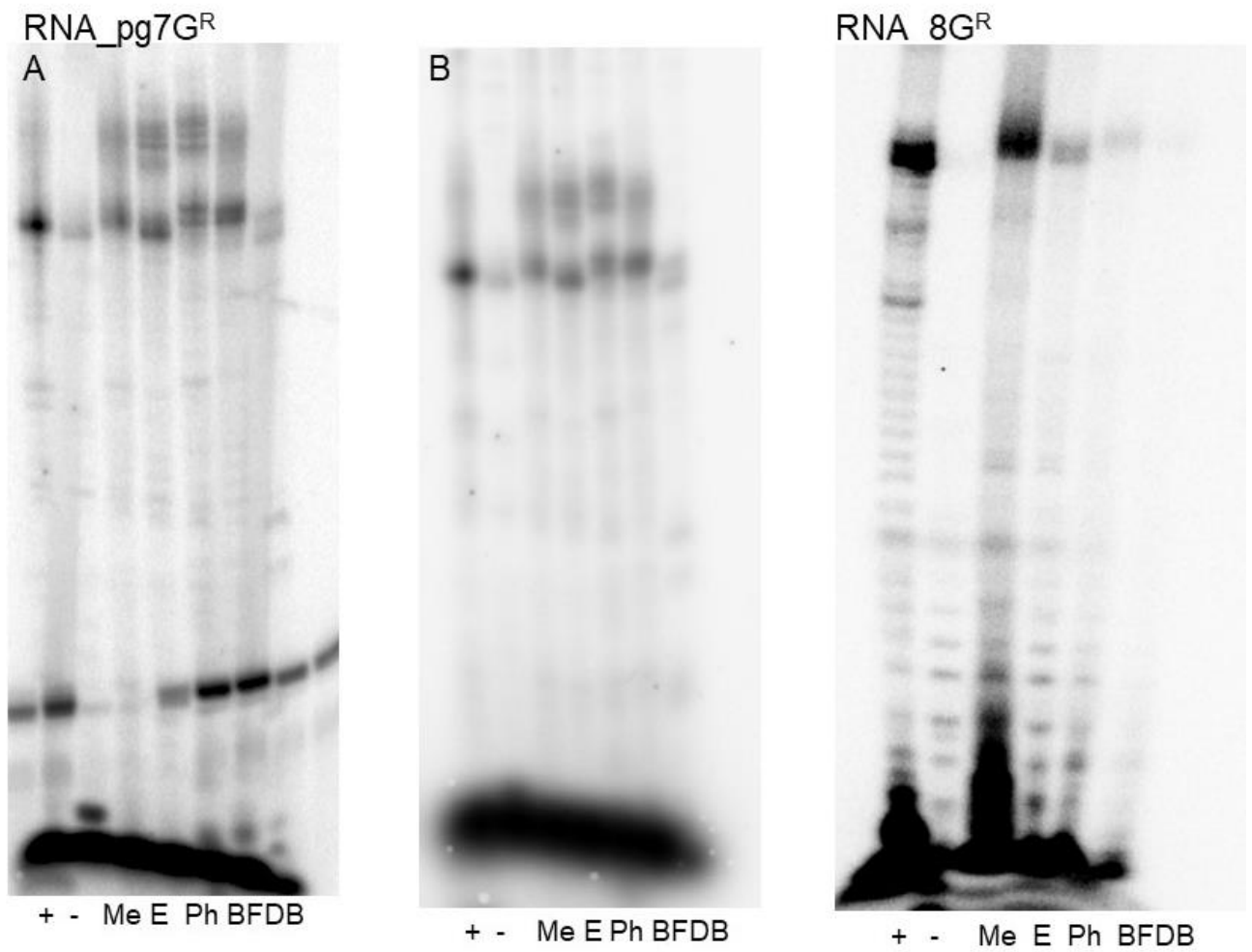


Table S2. Densitometric quantification of RNA transcripts

Transcript	Rel.yield (%)	SD
RNA_1A ^{Me}	107	8
RNA_1A ^E	89	6
RNA_1A ^{Ph}	75	4
RNA_1A ^{BF}	42	11
RNA_3A ^{Me}	84	2
RNA_3A ^E	77	6
RNA_3A ^{Ph}	81	3
RNA_3A ^{BF}	70	5
RNA_7A ^{Me}	90	13
RNA_7A ^E	86	10
RNA_7A ^{Ph}	70	10
RNA_7A ^{BF}	28	4
RNA_1C ^E	70	4
RNA_1C ^{Ph}	91	10
RNA_1C ^{BF}	50	3
RNA_1C ^{DB}	35	6
RNA_3C ^E	98	1
RNA_3C ^{Ph}	89	8
RNA_3C ^{BF}	89	3
RNA_3C ^{DB}	64	2
RNA_7C ^E	97	11
RNA_7C ^{Ph}	21	6
RNA_7C ^{BF}	21	8
RNA_7C ^{DB}	5	1
RNA_1U ^E	80	12
RNA_1U ^{Ph}	72	4
RNA_1U ^{BF}	52	1
RNA_1U ^{DB}	54	7
RNA_3U ^E	59	9
RNA_3U ^{Ph}	66	10
RNA_3U ^{BF}	43	9
RNA_3U ^{DB}	30	12
RNA_7U ^E	55	6
RNA_7U ^{Ph}	50	15
RNA_7U ^{BF}	53	15
RNA_8G ^{Me}	46	11
RNA_8G ^E	54	11
RNA_8G ^{Ph}	32	1
RNA_8G ^{BF}	48	1

IV Copies of MALDI spectra

Table S3. Molecular masses of obtained transcripts (MALDI)

Transcript	M calculated	M observed
RNA_1A ^{Me}	11151.3	11152.8
RNA_1A ^E	11161.3	11161.7
RNA_1A ^{Ph}	11213.4	11214.1
RNA_1A ^{BF}	11253.4	11252.3
RNA_3A ^{Me}	11303.6	11646.9 (M+A ^{Me} MP)
RNA_3A ^E	11333.5	11684.7 (M+A ^E MP)
RNA_3A ^{Ph}	11489.8	11864.0 (M+ A ^{Ph} MP)
RNA_3A ^{BF}	11609.8	12055 (M+ A ^{BF} MP)
RNA_7A ^{Me}	11568.0	11567.1
RNA_7A ^E	11637.9	11989.5 (M+A ^E MP)
RNA_7A ^{Ph}	12002.5	12406.1(M+A ^{Ph} MP)
RNA_7A ^{BF}	12282.6	12726 (M + A ^{BF} MP)
RNA_1C ^E	11803.9	12131.1 M (+AMP)
RNA_1C ^{Ph}	11856.0	12183.1 (M+AMP)
RNA_1C ^{BF}	11896.0	11895.8
RNA_1C ^{DB}	11946.1	11943.0
RNA_3C ^E	11818.0	12143.6 (M+AMP)
RNA_3C ^{Ph}	11974.2	12302.4 (M+AMP)
RNA_3C ^{BF}	12094.3	12421.2 (M+AMP)
RNA_3C ^{DB}	12244.5	12.571 (M+AMP)
RNA_7C ^E	11723.9	11724.0
RNA_7C ^{Ph}	12088.4	12086.4
RNA_7C ^{BF}	12366.6	12748 (M+GMP+K-H)
RNA_7C ^{DB}	12719.0	12715.7
RNA_1U ^E	11421.1	11418.8
RNA_1U ^{Ph}	11473.0	11472.1
RNA_1U ^{BF}	11513.0	11511.9
RNA_1U ^{DB}	11563.0	11562.0
RNA_3U ^E	11486.1	11486.0
RNA_3U ^{Ph}	11643.0	11641.1
RNA_3U ^{BF}	11760.6	11759.1
RNA_3U ^{DB}	1907.6	11909.5
RNA_7U ^E	11570.	11898.9 (M+AMP)
RNA_7U ^{Ph}	11929.6	11930.4
RNA_7U ^{BF}	12215.1	12211.7
RNA_8G ^{Me}	11380.1	11380.0
RNA_8G ^E	11450.1	11471 (M+2Na-1H)
RNA_8G ^{Ph}	11814.6	11816.1
RNA_8G ^{BF}	12094.7	12094.1

Figure S6. MALDI-TOF spectrum of RNA_{1A}^{Me}

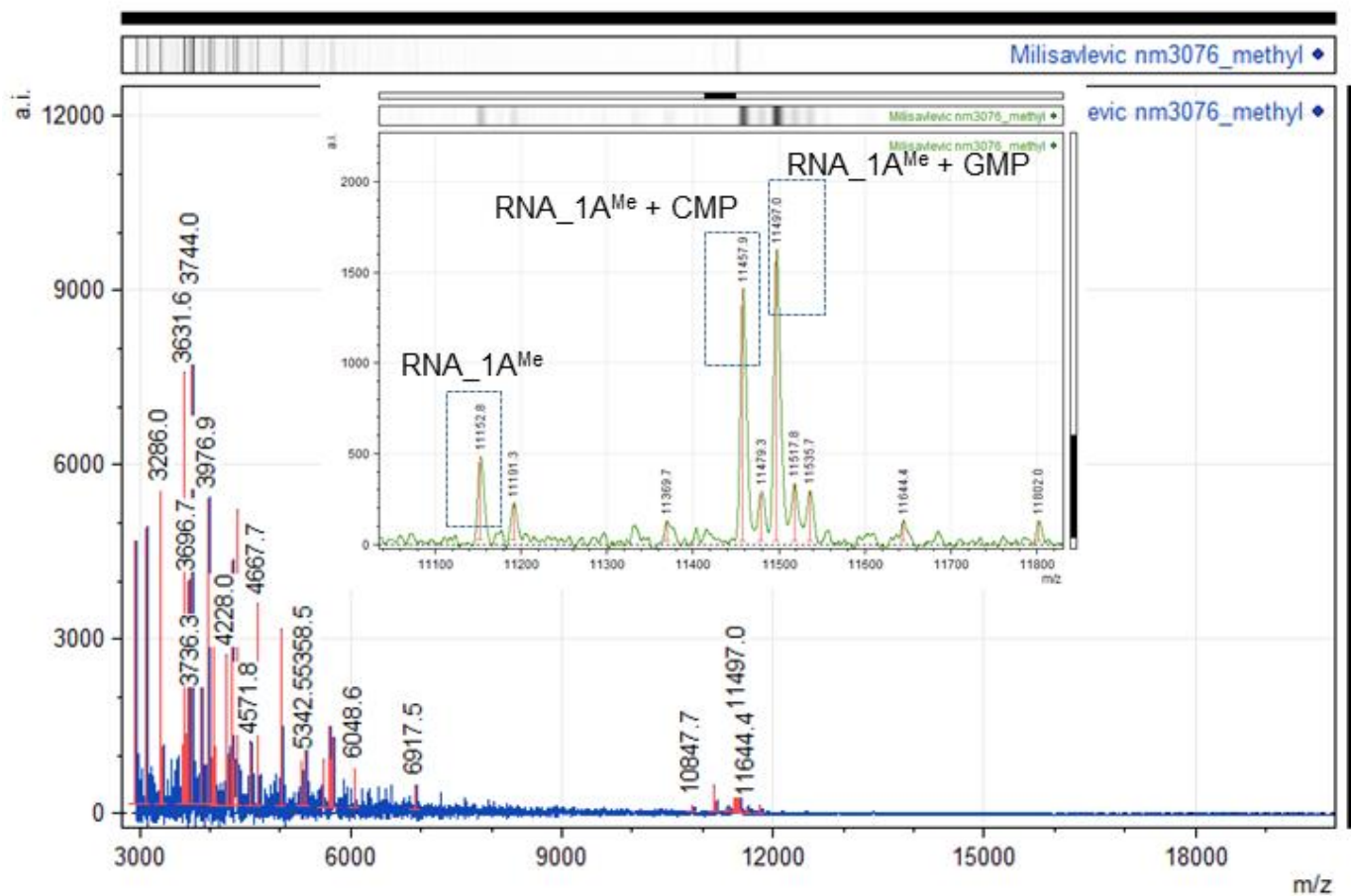


Figure S7. MALDI-TOF spectrum of RNA_{1A}^E

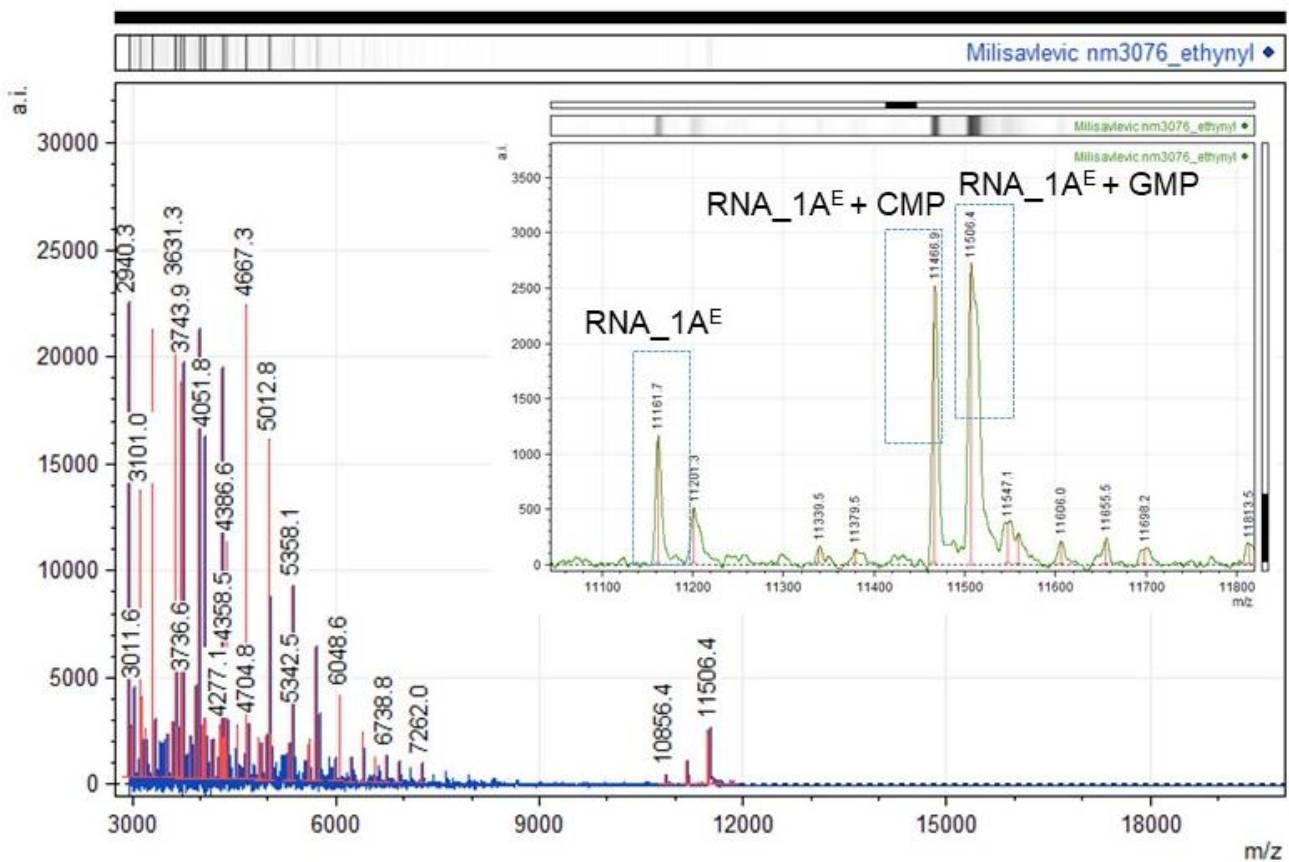


Figure S8. MALDI-TOF spectrum of RNA_1A^{Ph}

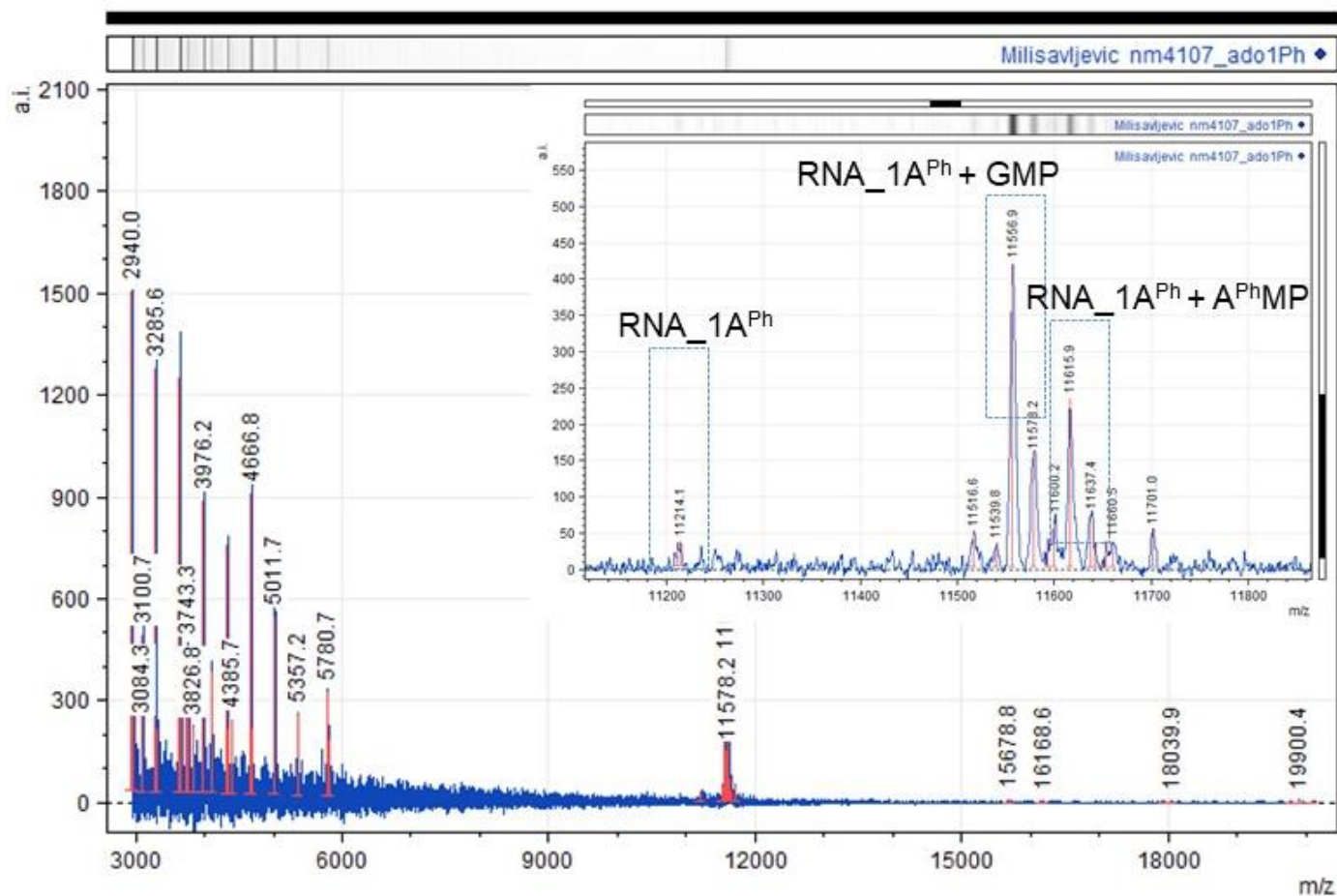


Figure S9. MALDI-TOF spectrum of RNA_1A^{BF}

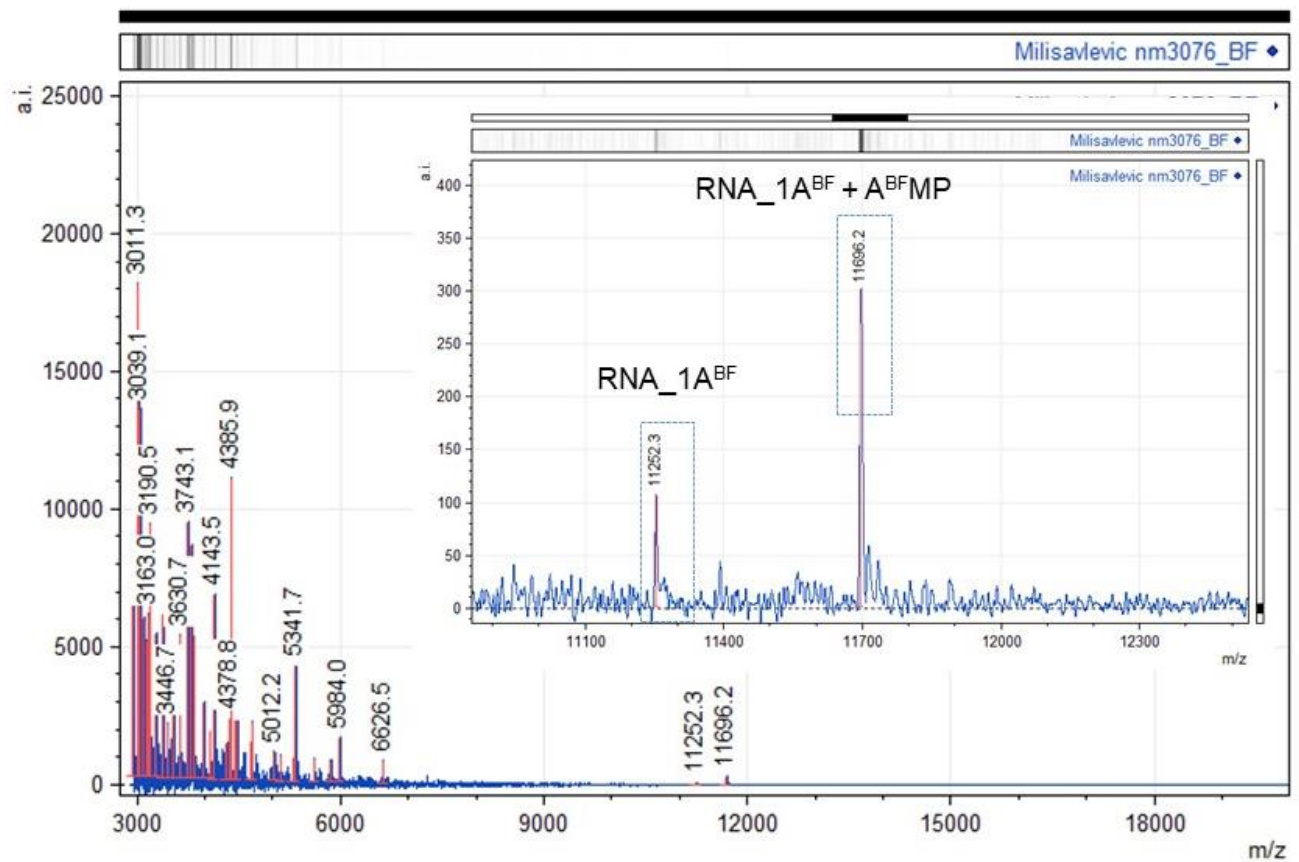


Figure S10. MALDI-TOF spectrum of **RNA_3A^{BF}**

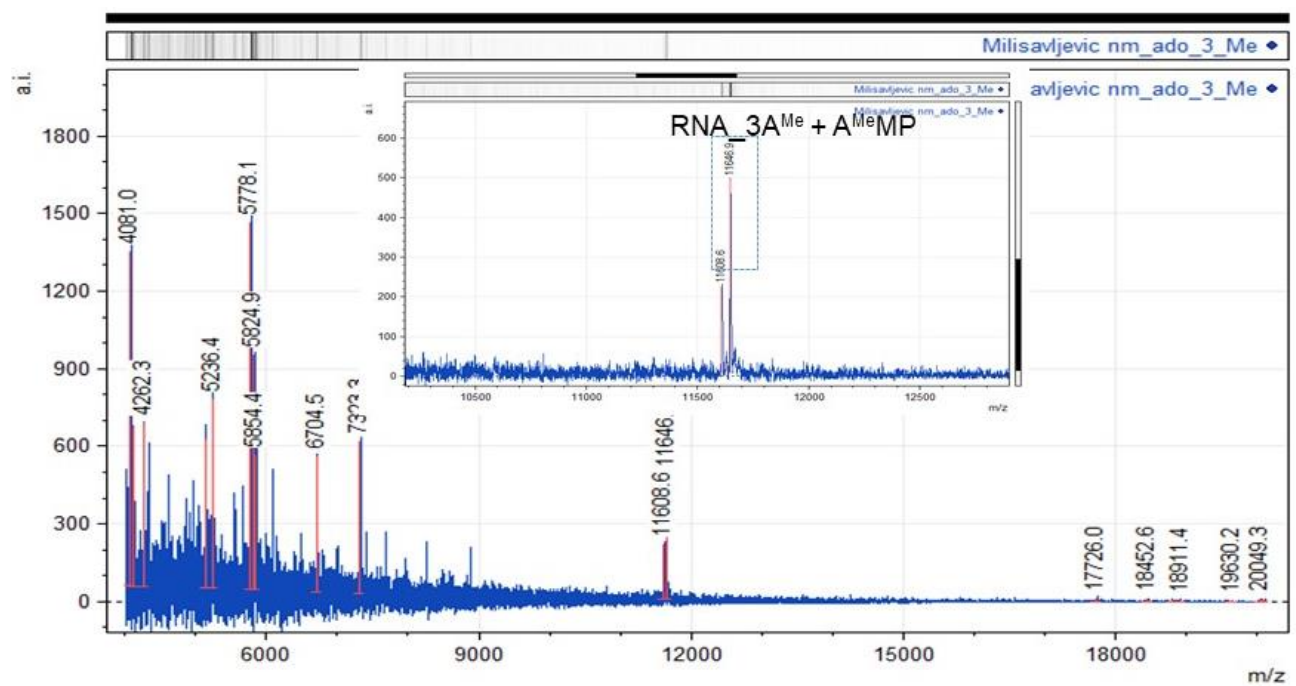


Figure S11. MALDI-TOF spectrum of RNA_{3A}^E

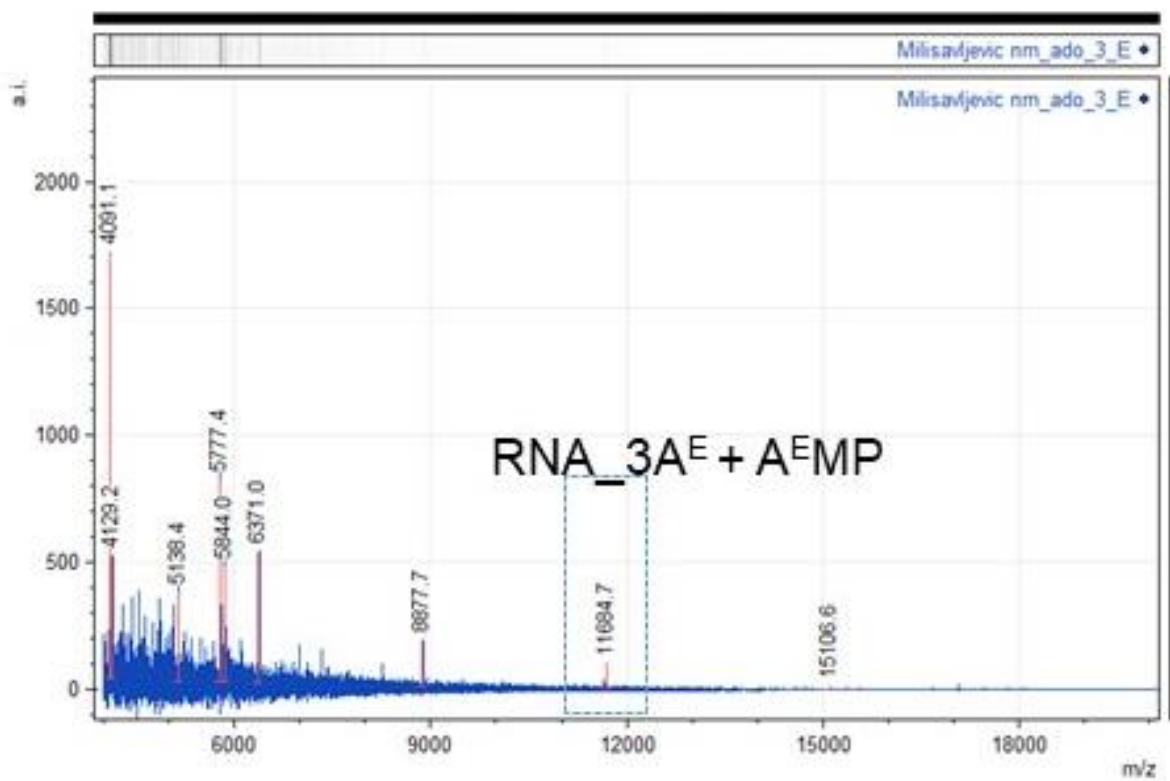


Figure S12. MALDI-TOF spectrum of RNA_3A^{Ph}

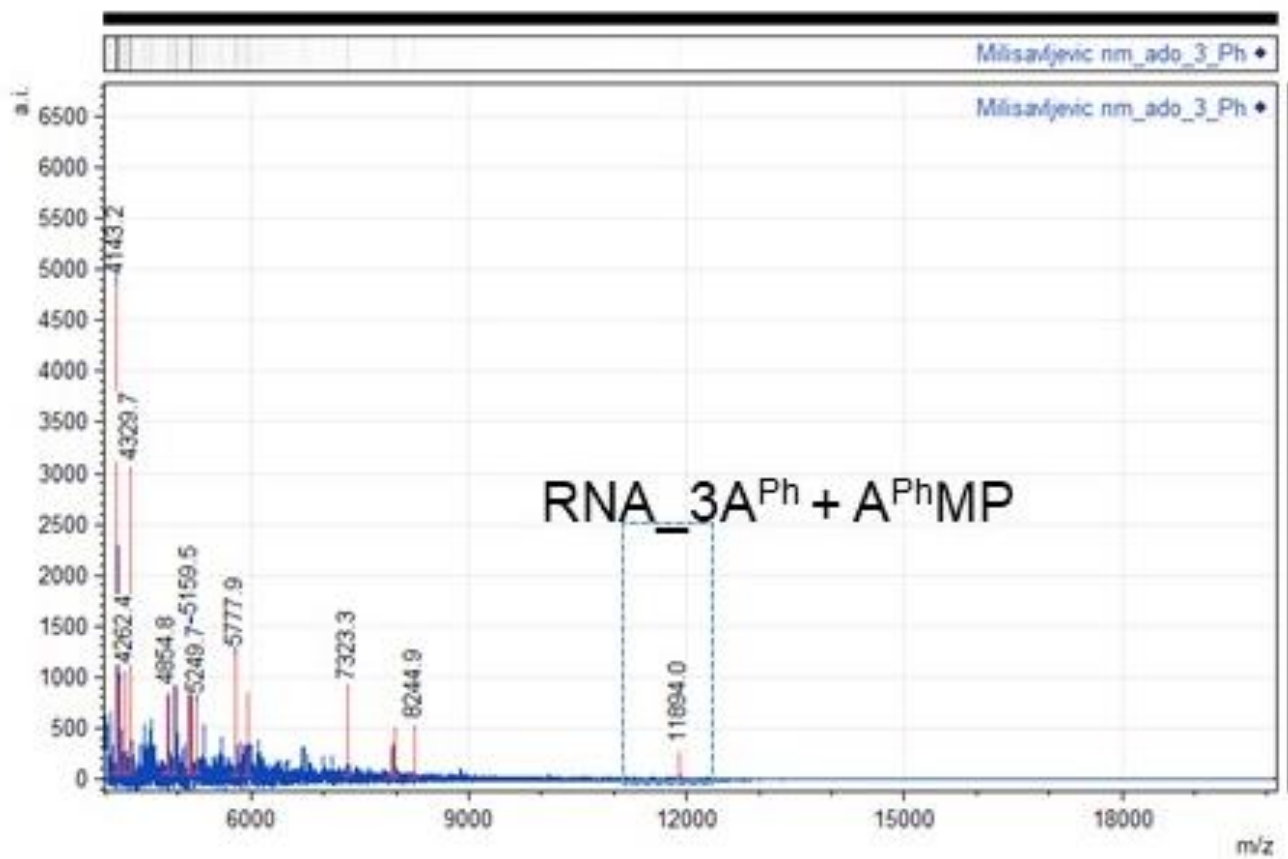


Figure S13. MALDI-TOF spectrum of **RNA_3A^{BF}**

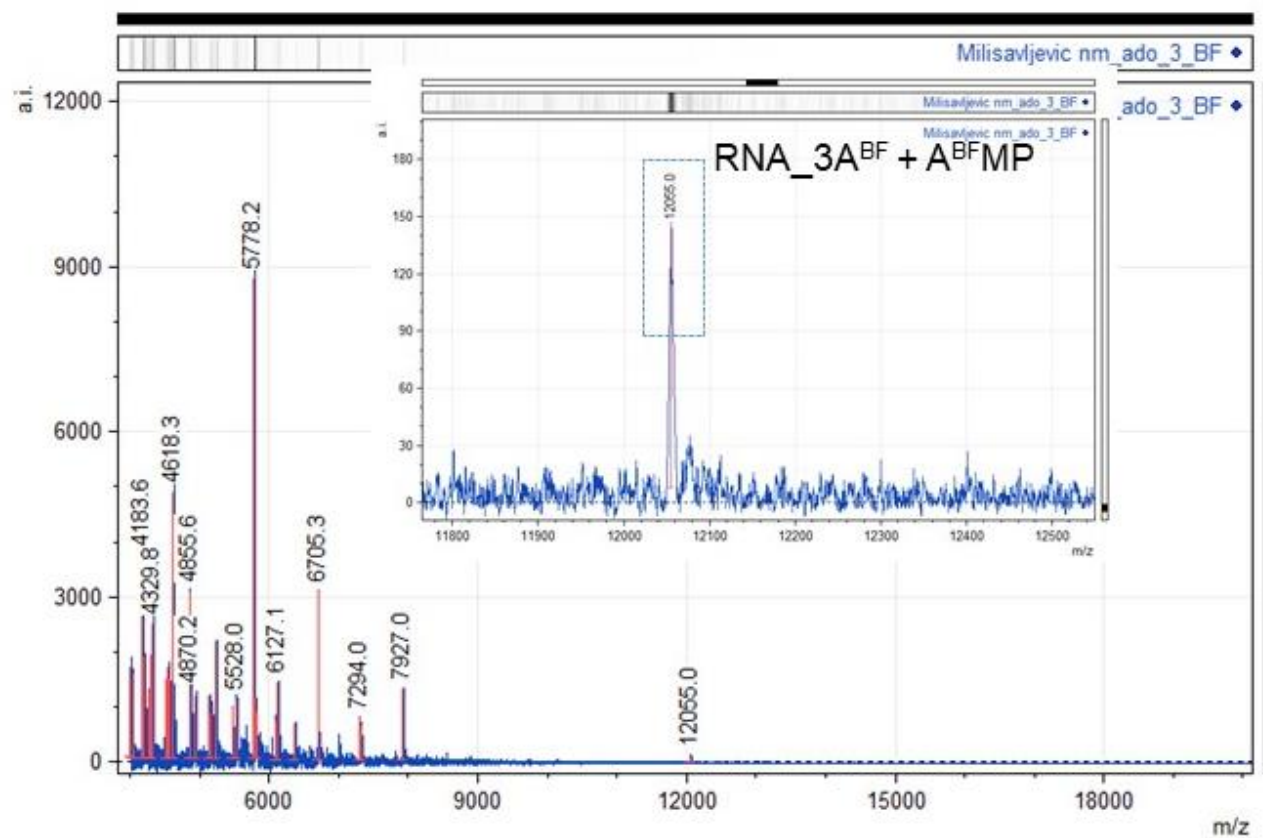


Figure S14. MALDI-TOF spectrum of RNA_7A^{Me}

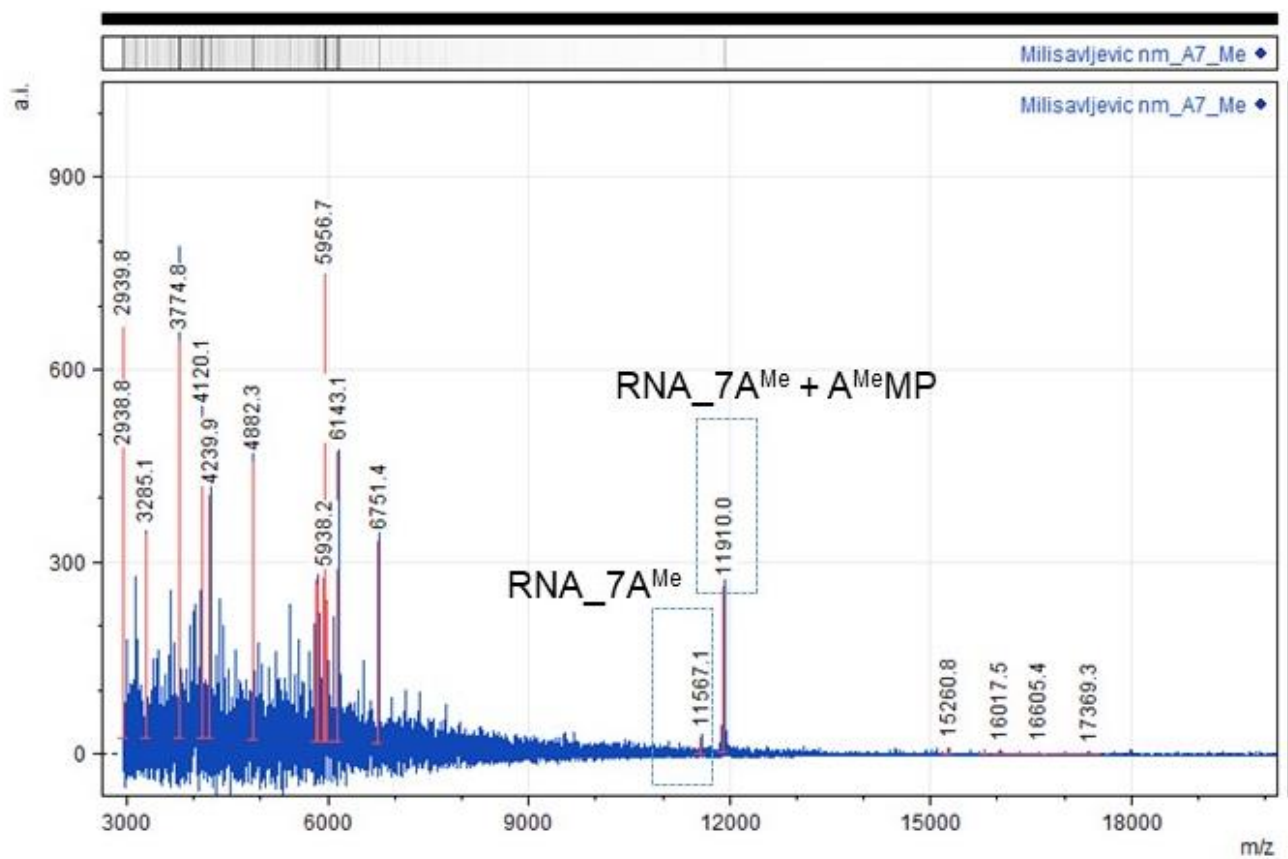


Figure S15. MALDI-TOF spectrum of RNA_{7A}^E

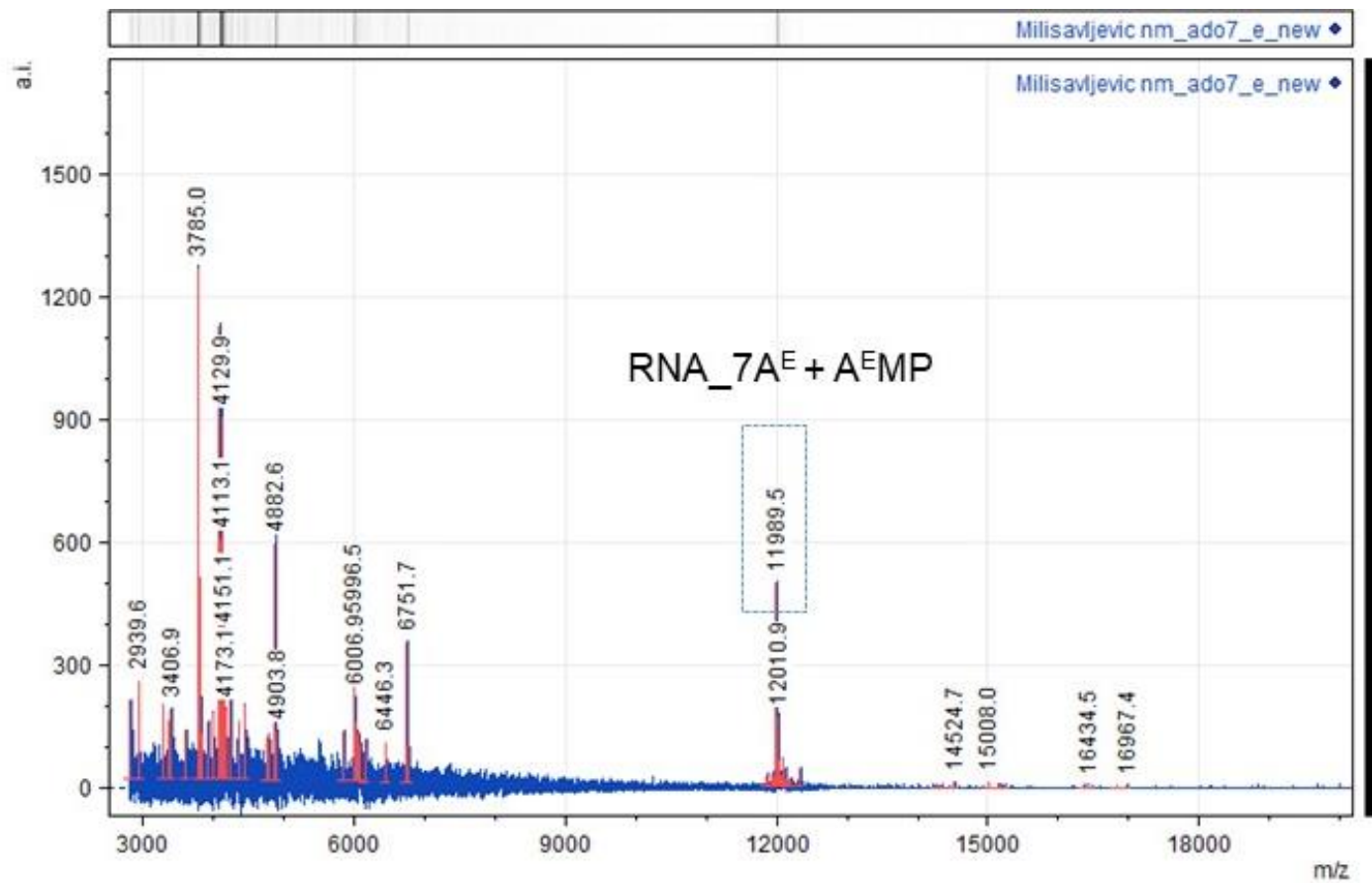


Figure S16. MALDI-TOF spectrum of RNA_7A^{Ph}

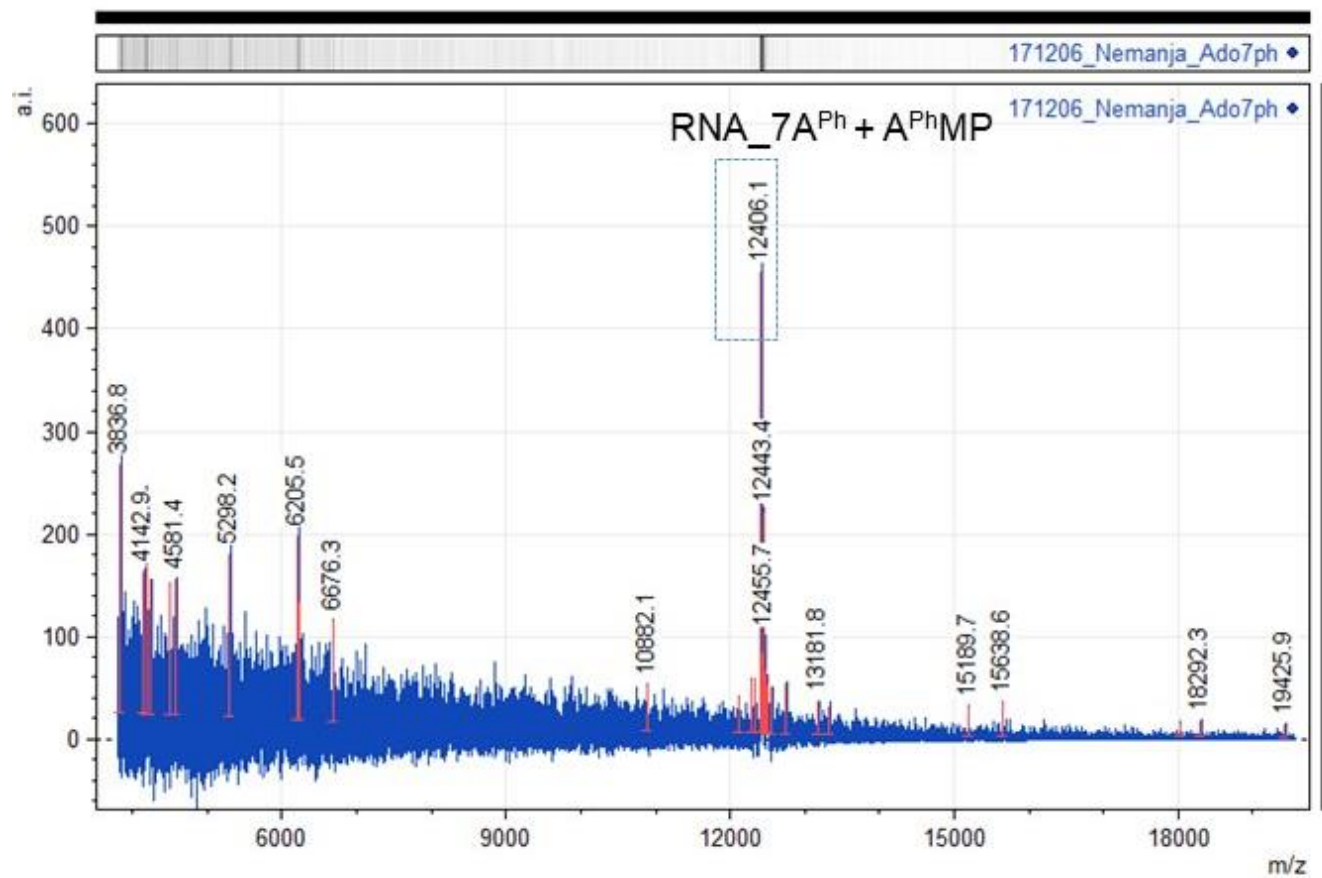


Figure S17. MALDI-TOF spectrum of **RNA_7A^{BF}**

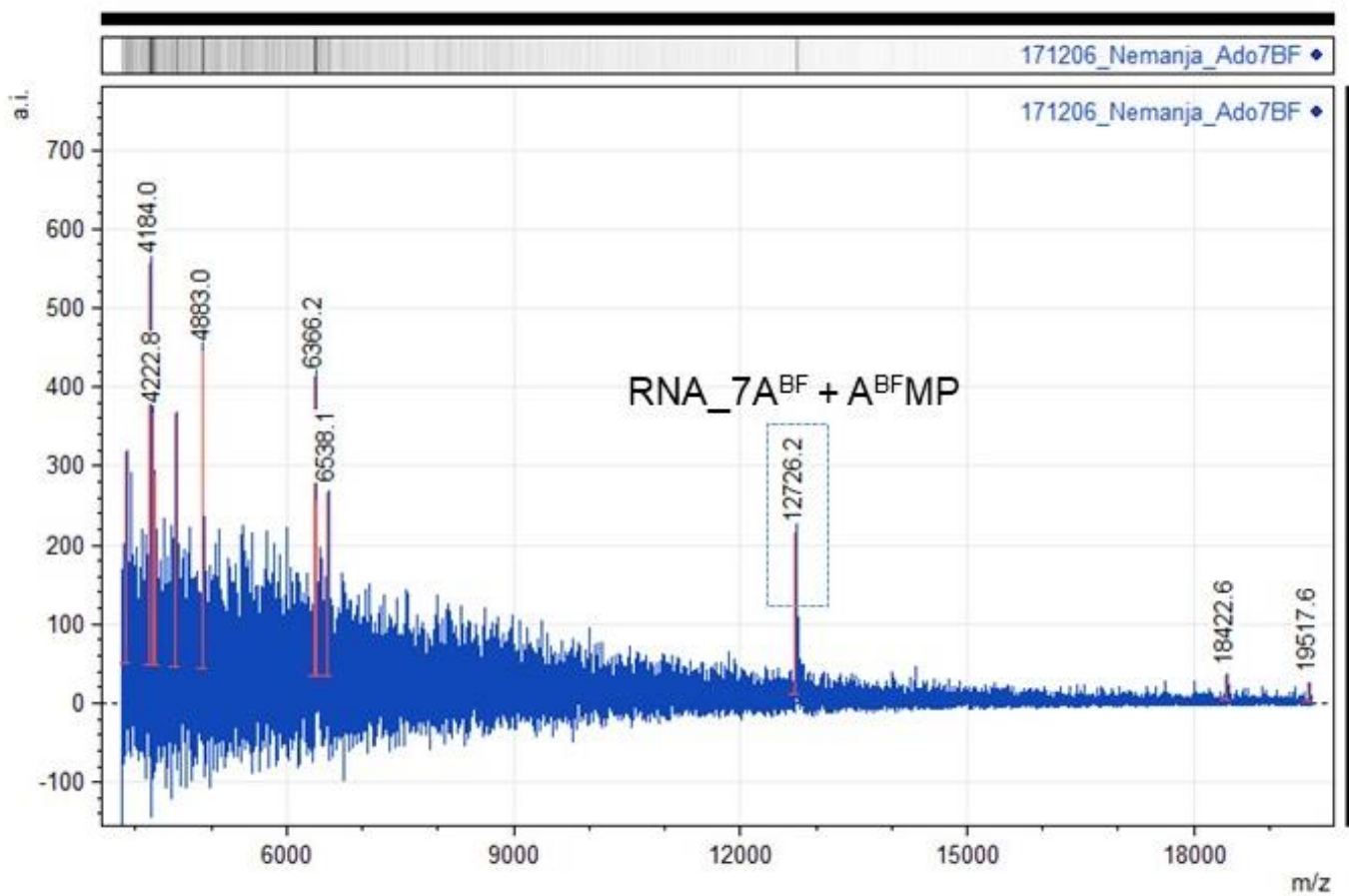


Figure S18. MALDI-TOF spectrum of RNA₁C^E

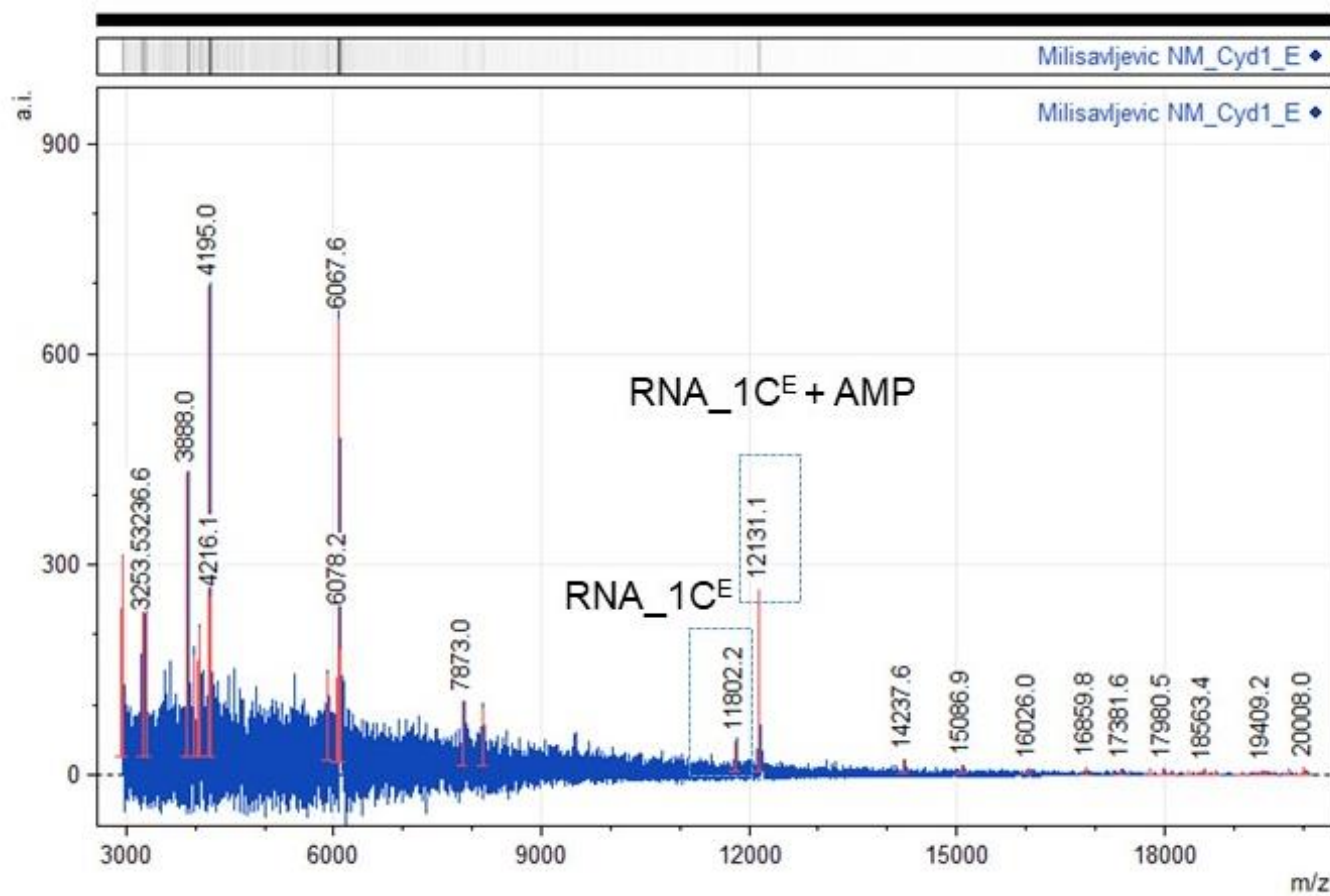


Figure S19. MALDI-TOF spectrum of RNA_1C^{Ph}

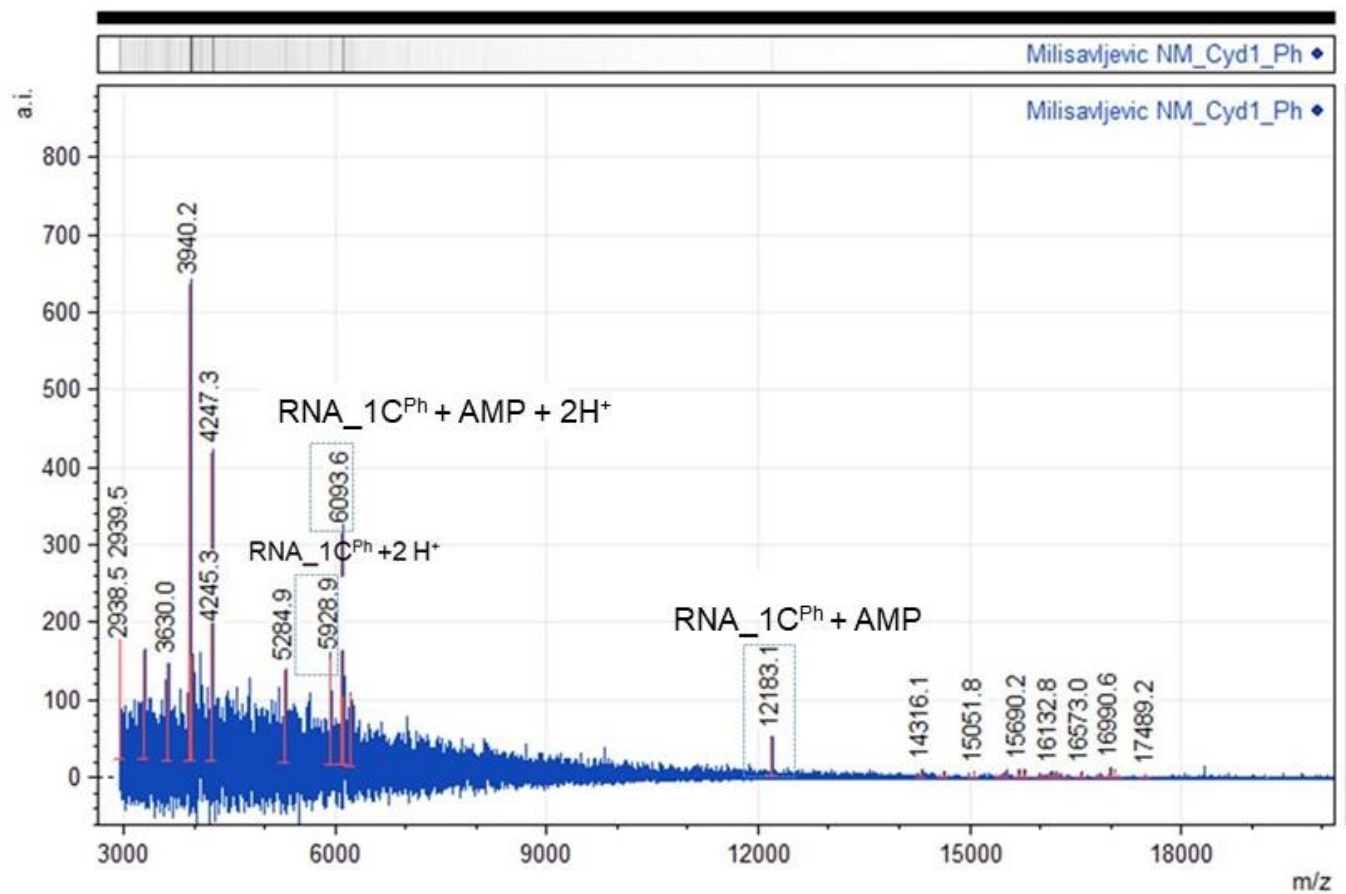


Figure S20. MALDI-TOF spectrum of **RNA_1C^{BF}**

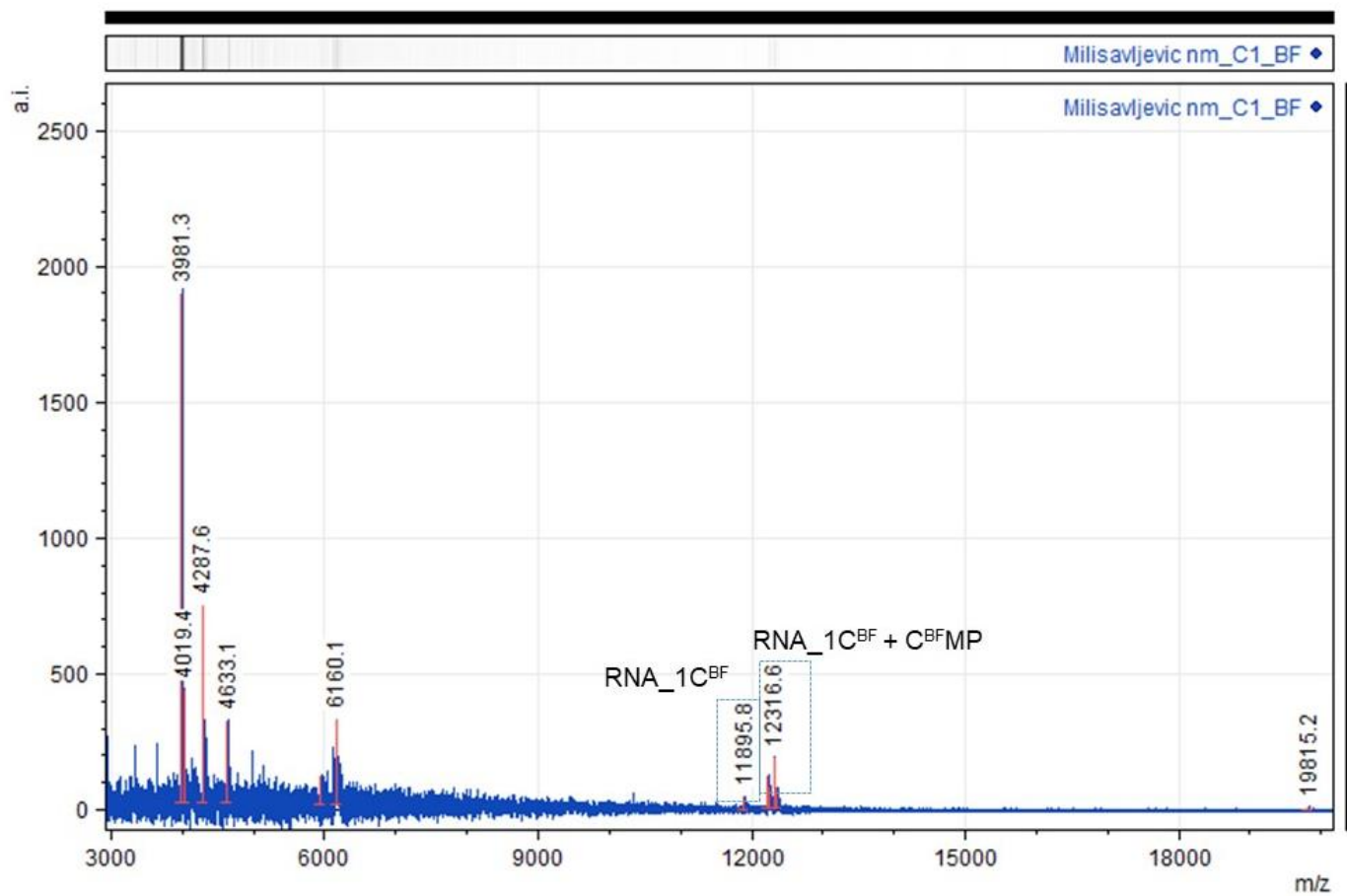


Figure S21. MALDI-TOF spectrum of **RNA_1C^{DB}**

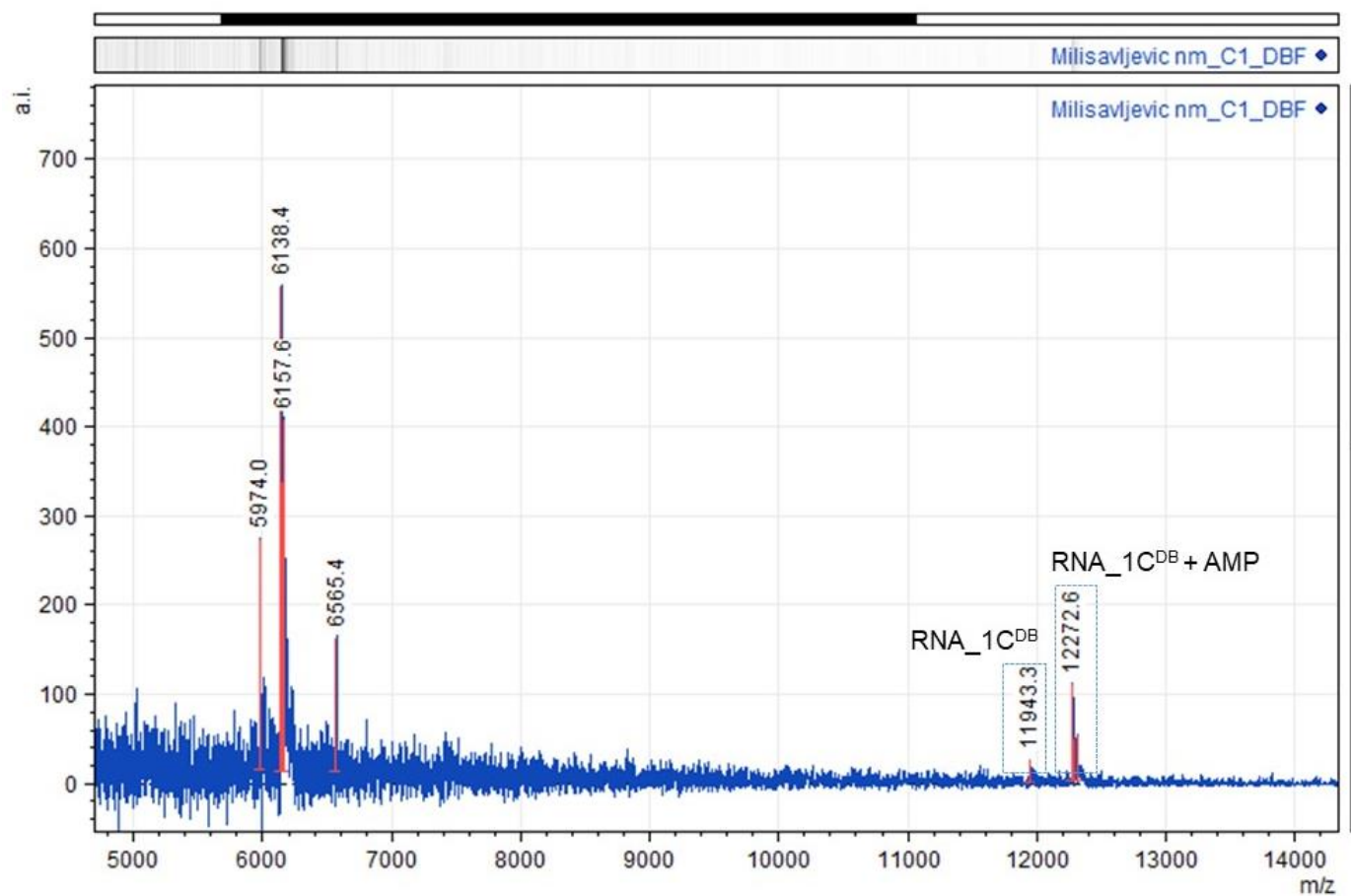


Figure S22. MALDI-TOF spectrum of **RNA_{3C}^E**

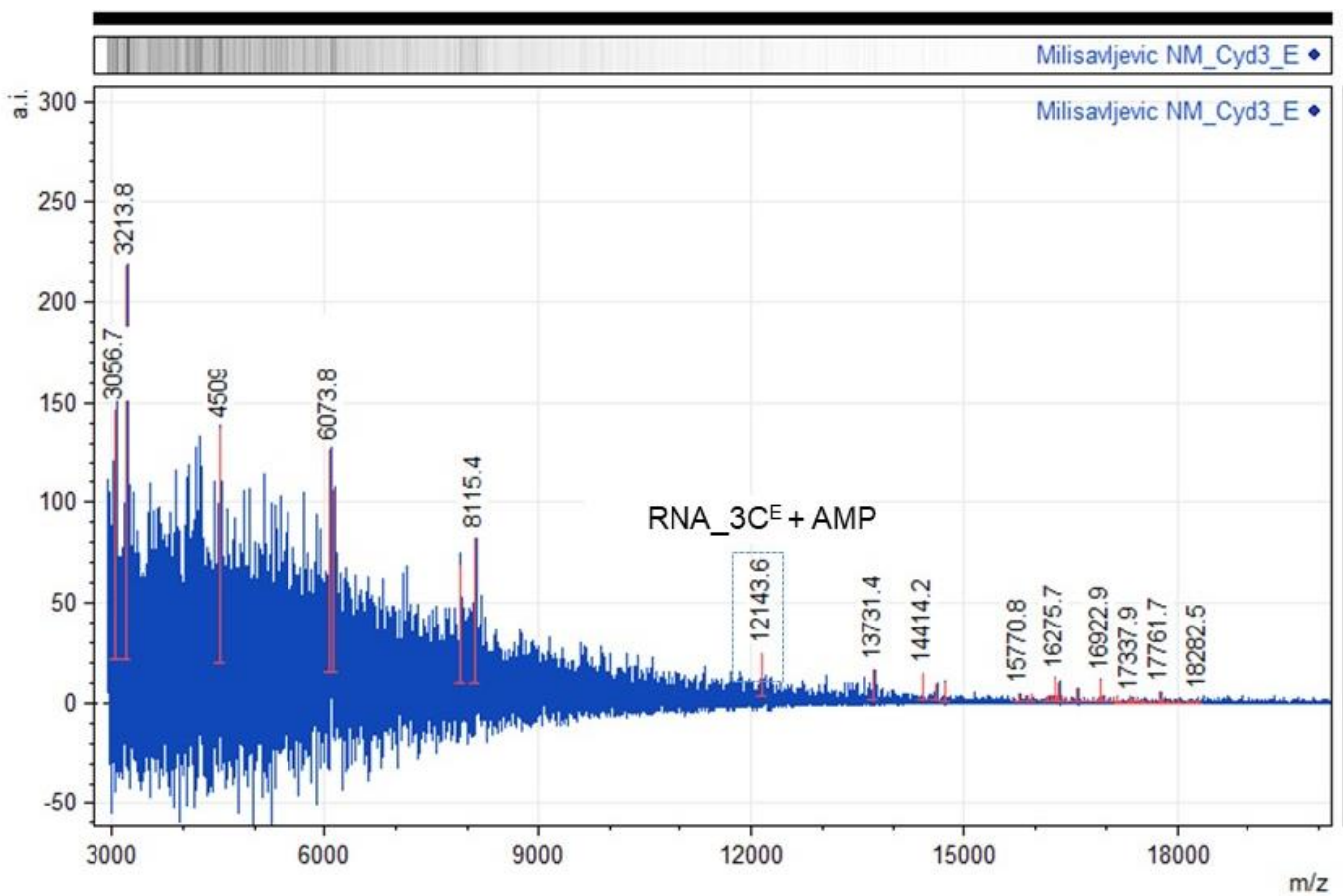


Figure S23. MALDI-TOF spectrum of RNA_3C^{Ph}

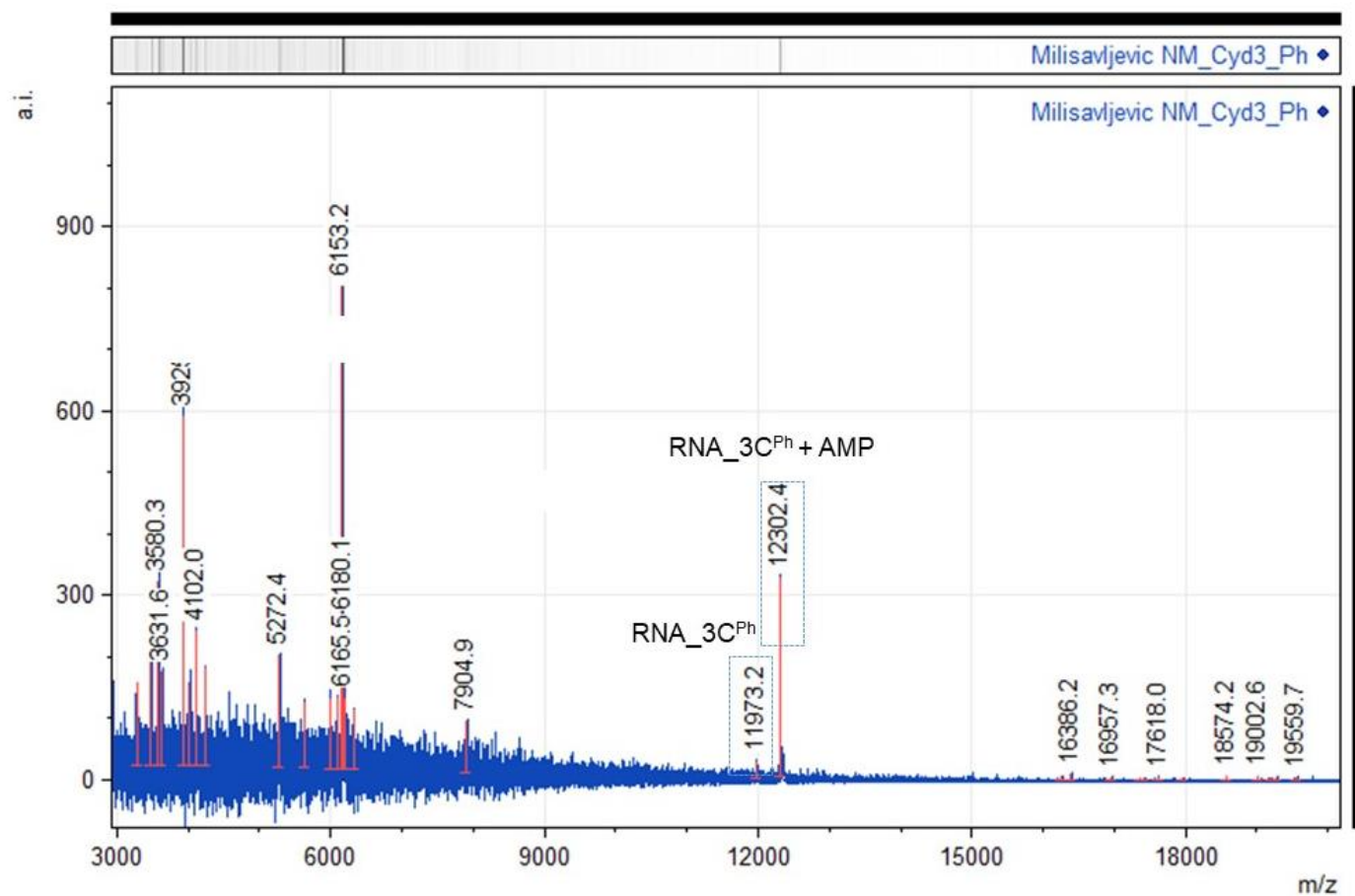


Figure S24. MALDI-TOF spectrum of RNA_{3C}^{BF}

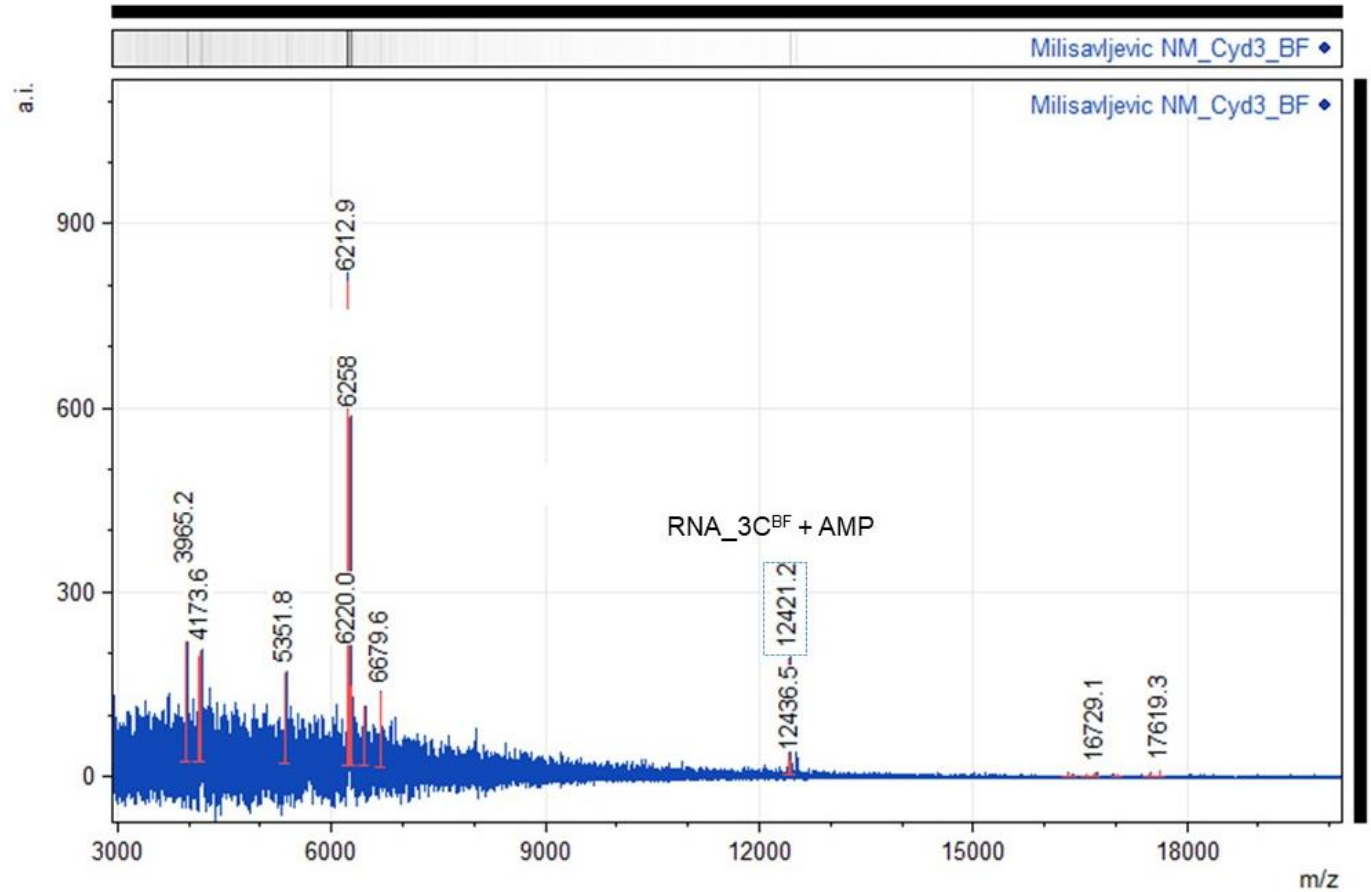


Figure S25. MALDI-TOF spectrum of RNA_{3C^{DB}}

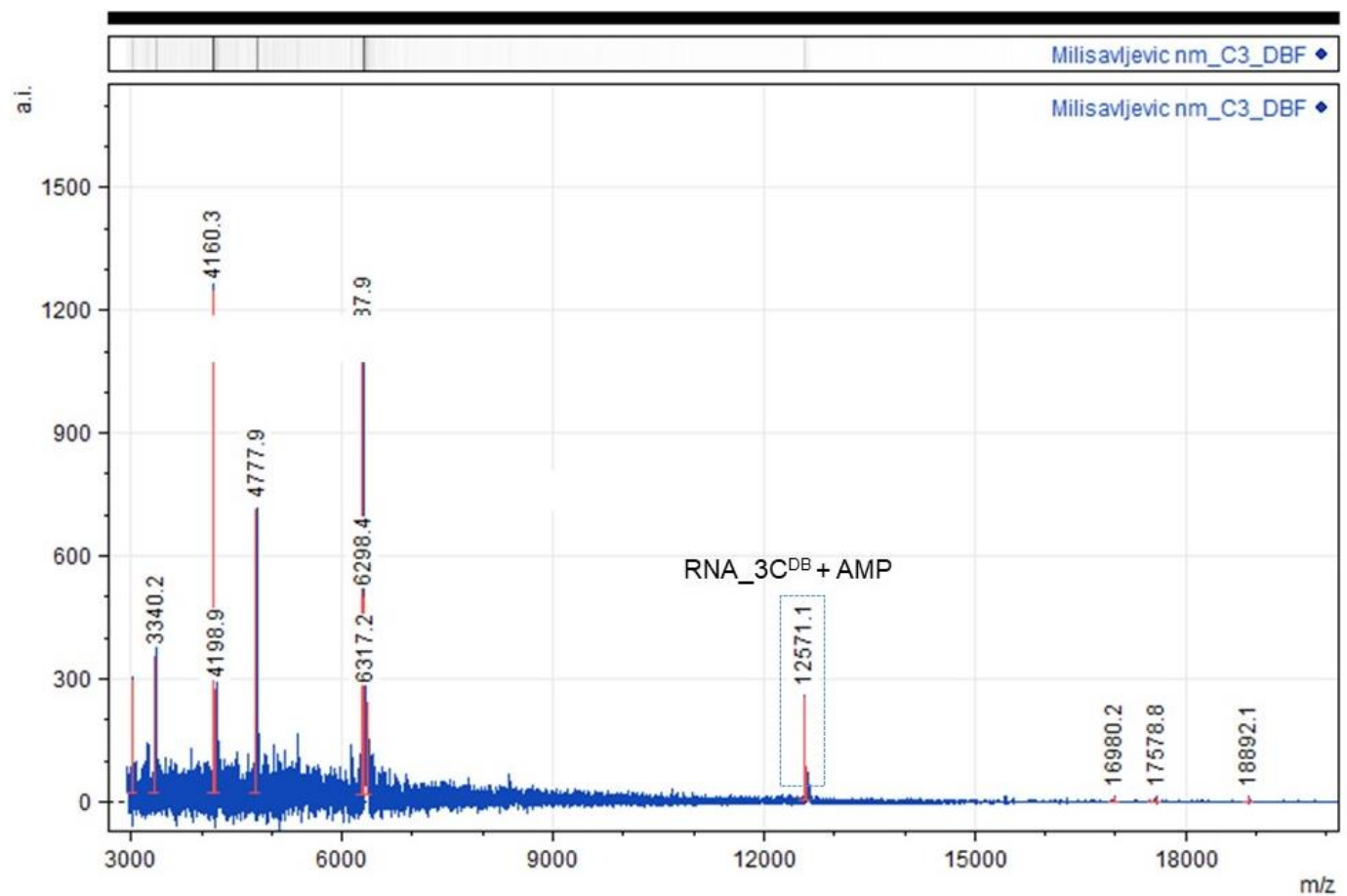


Figure S26. MALDI-TOF spectrum of RNA_{7C^E}

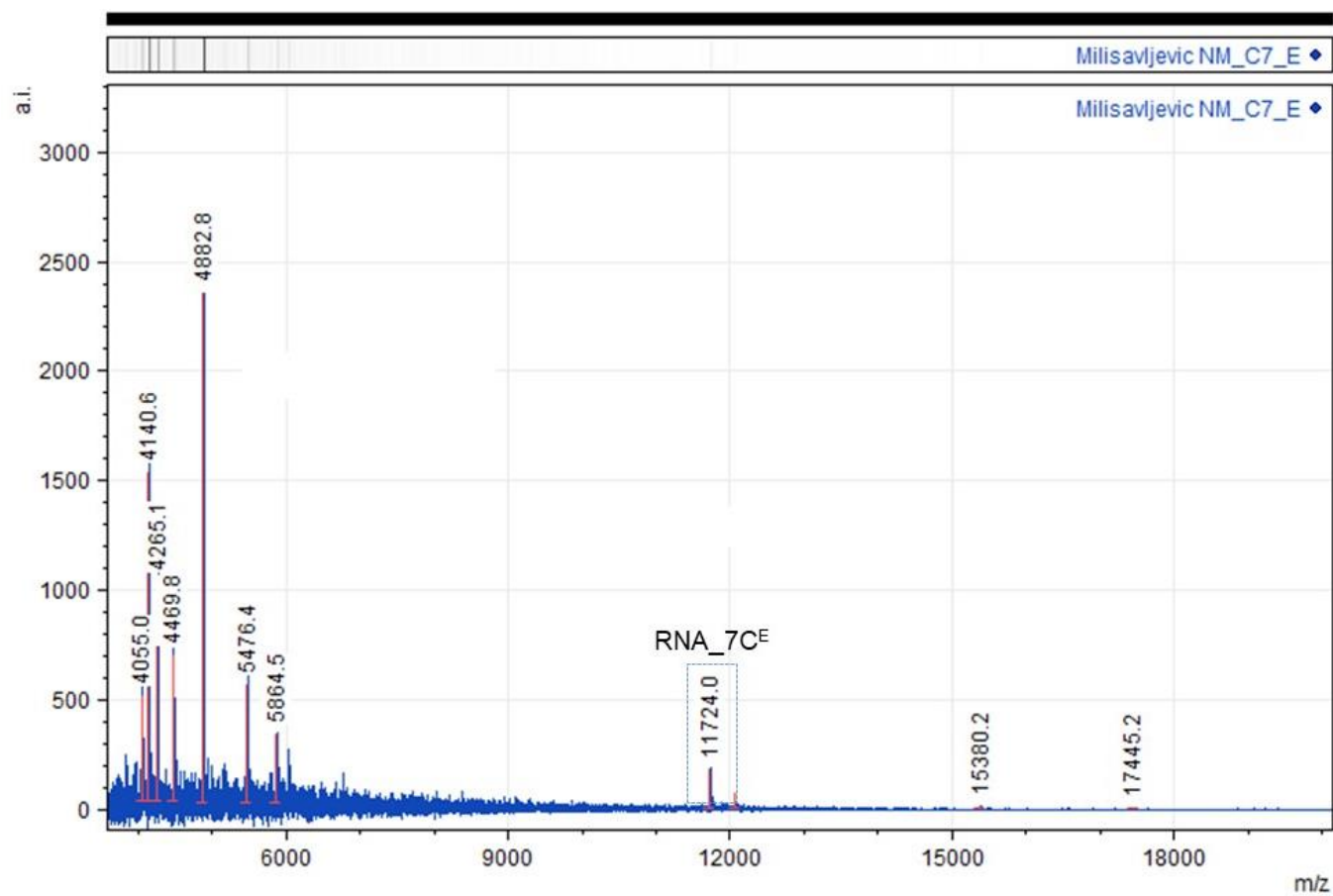


Figure S27. MALDI-TOF spectrum of RNA_7C^{Ph}

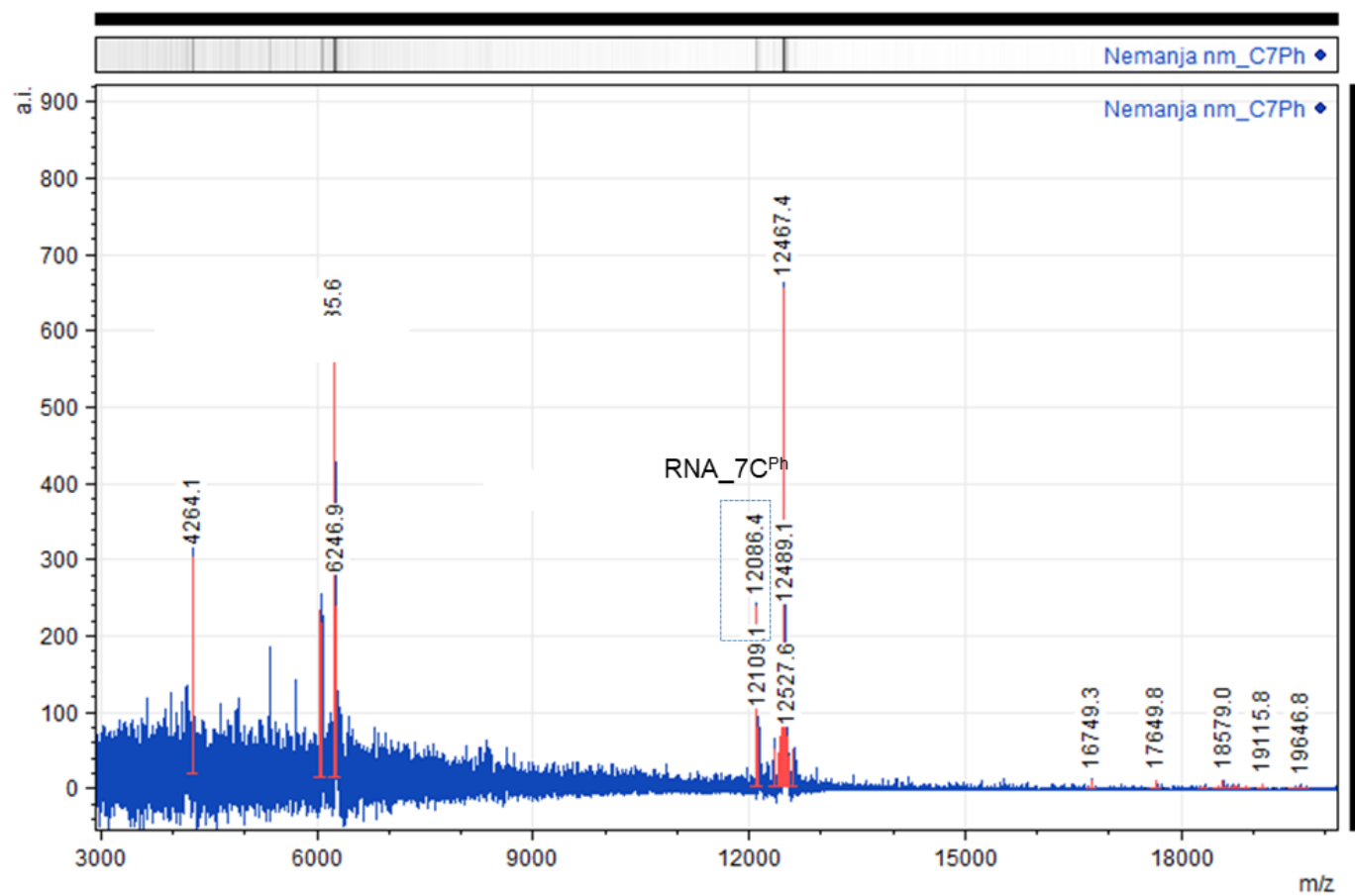


Figure S28. MALDI-TOF spectrum of **RNA_7C^{BF}**

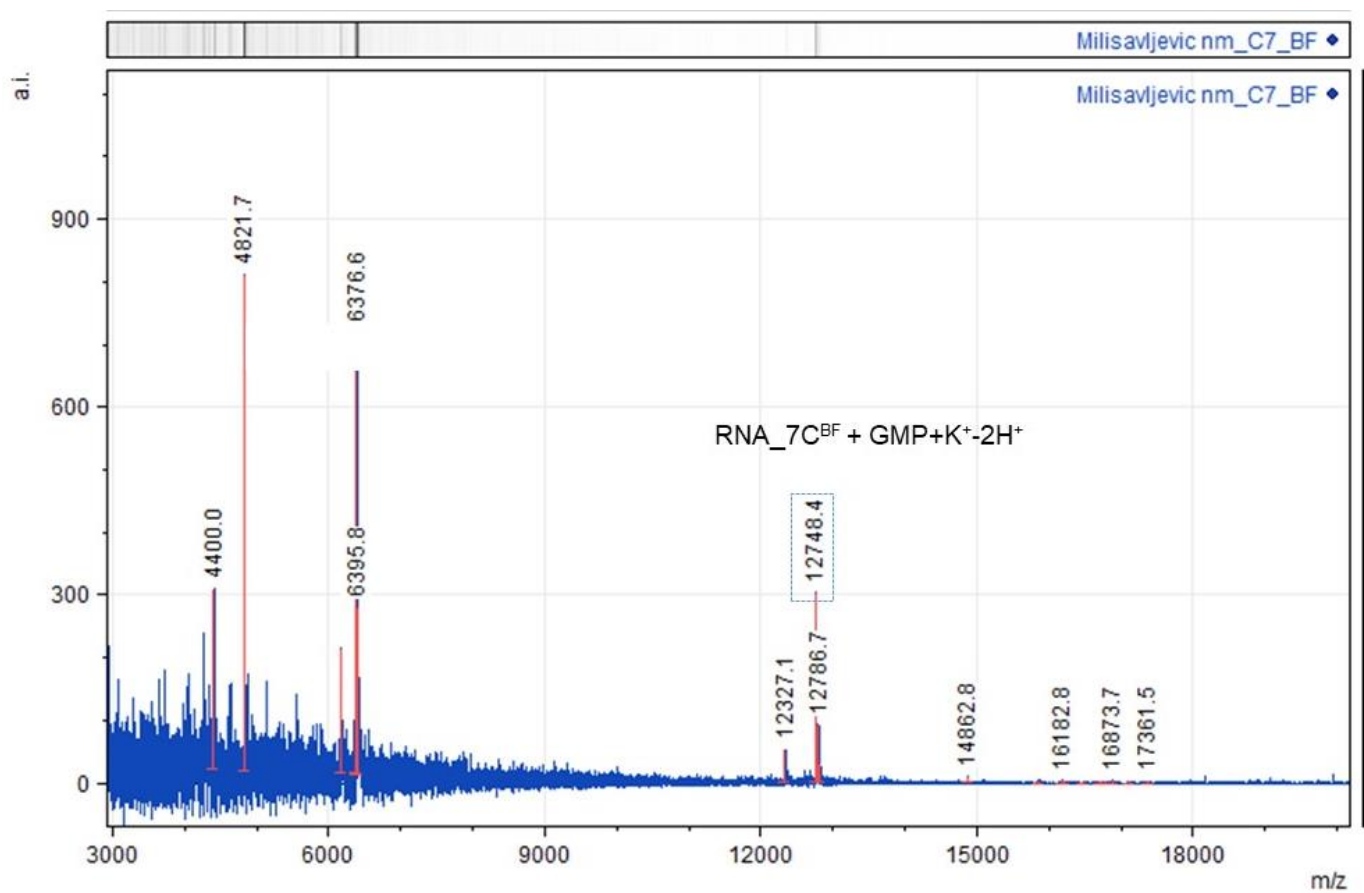


Figure S29. MALDI-TOF spectrum of **RNA_7C^{DB}**

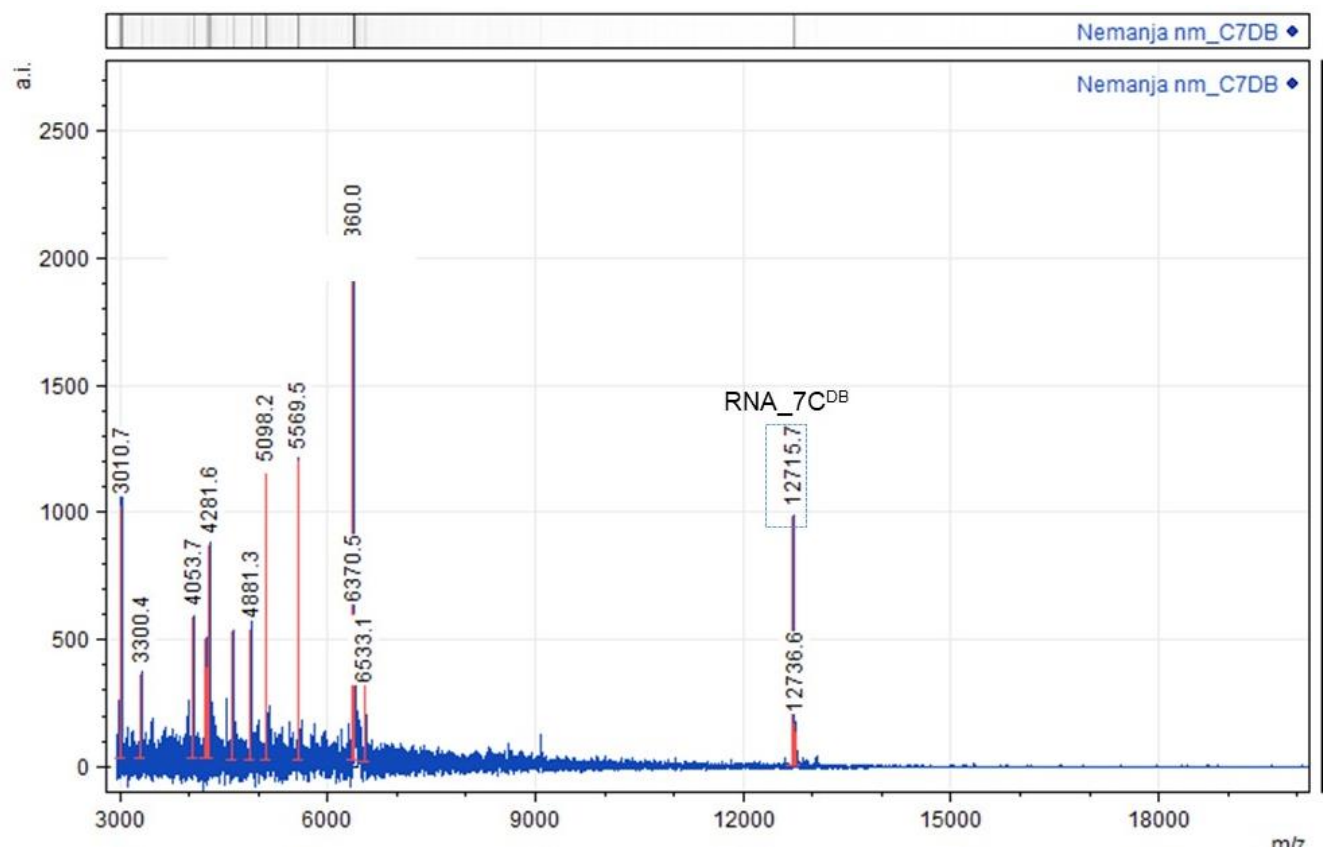


Figure S30. MALDI-TOF spectrum of RNA_{1U^E}

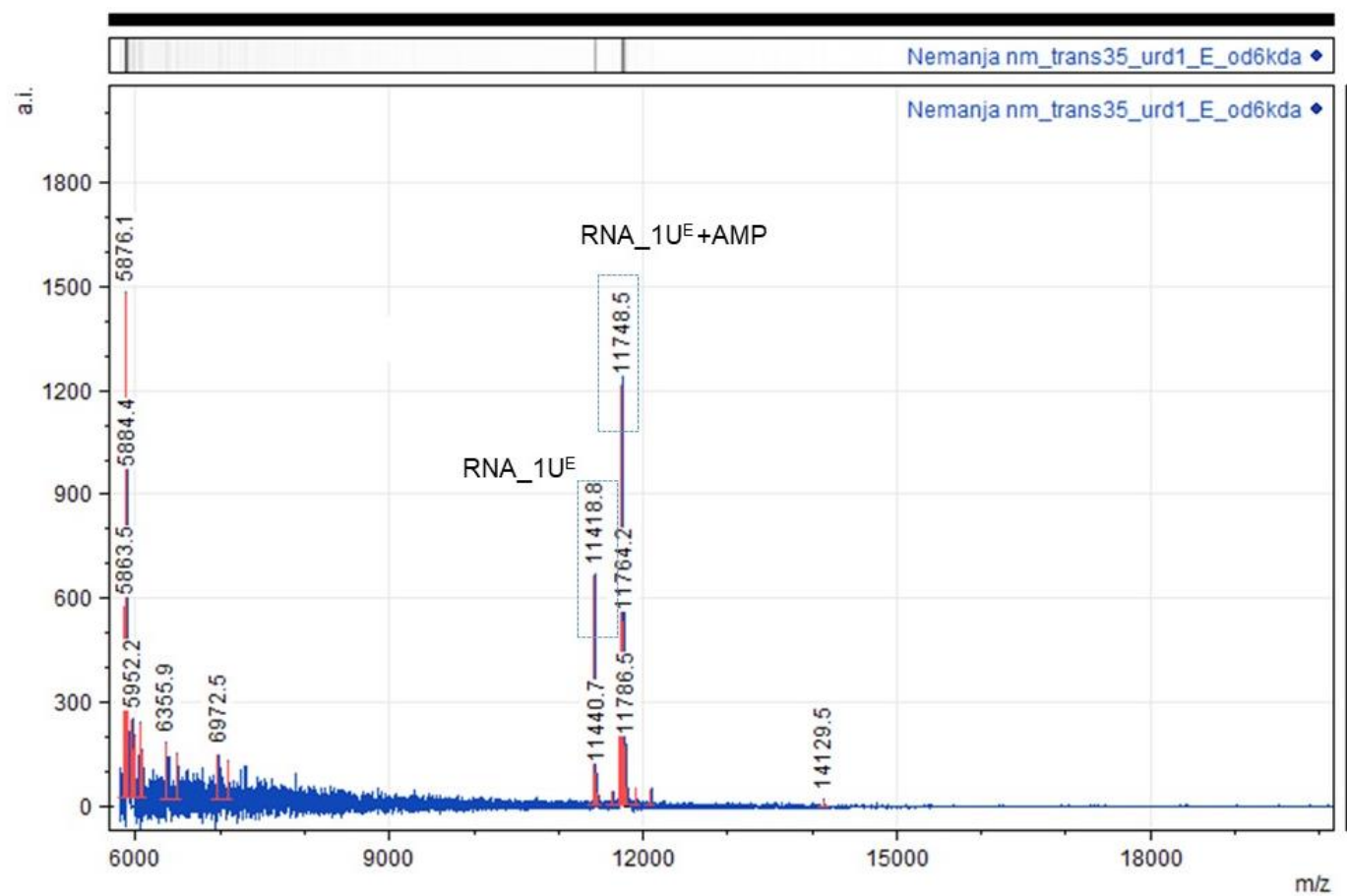


Figure S31. MALDI-TOF spectrum of RNA₁U^{Ph}

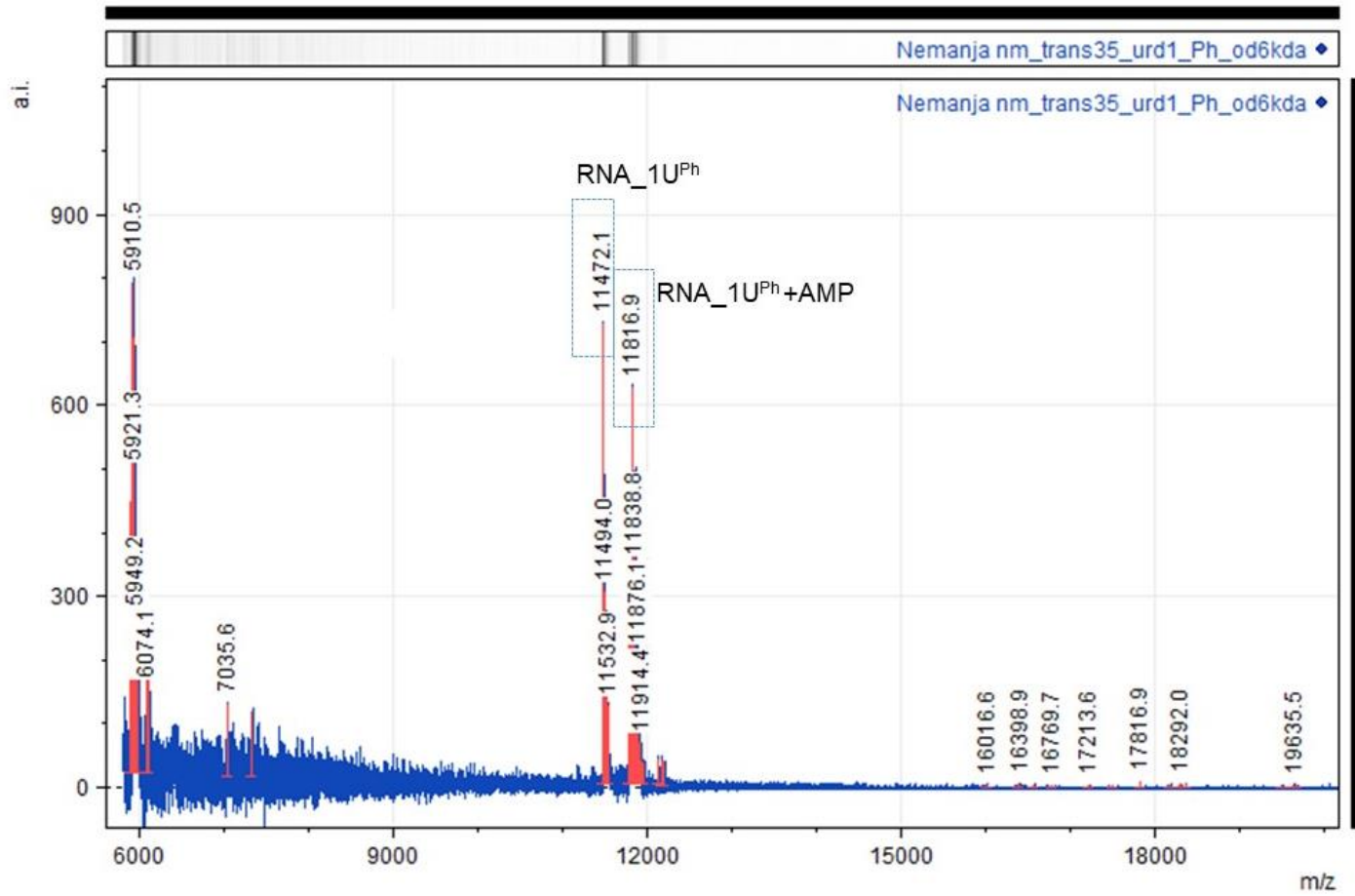


Figure S32. MALDI-TOF spectrum of **RNA_1U^{BF}**

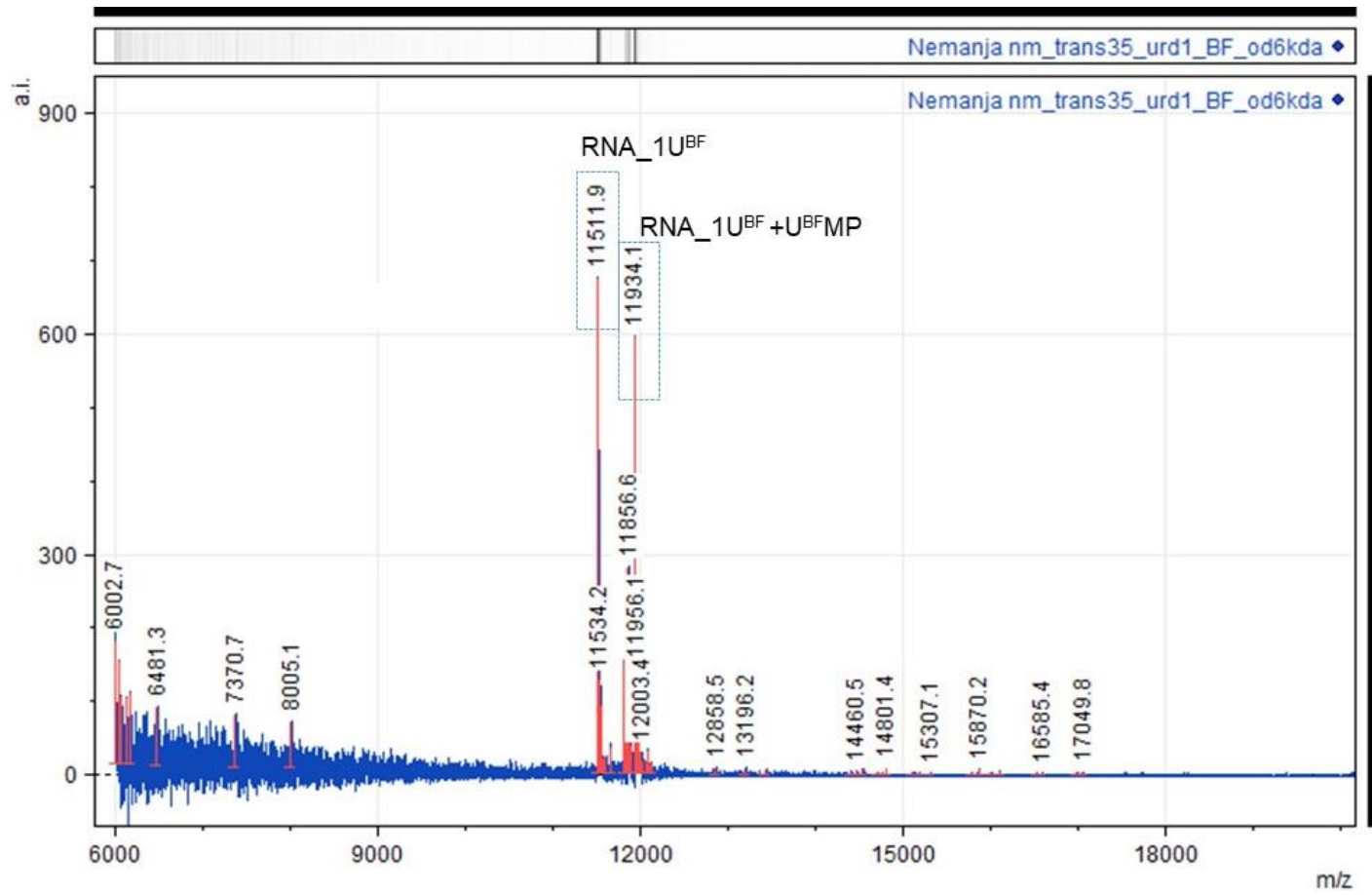


Figure S33. MALDI-TOF spectrum of **RNA_1U^{DB}**

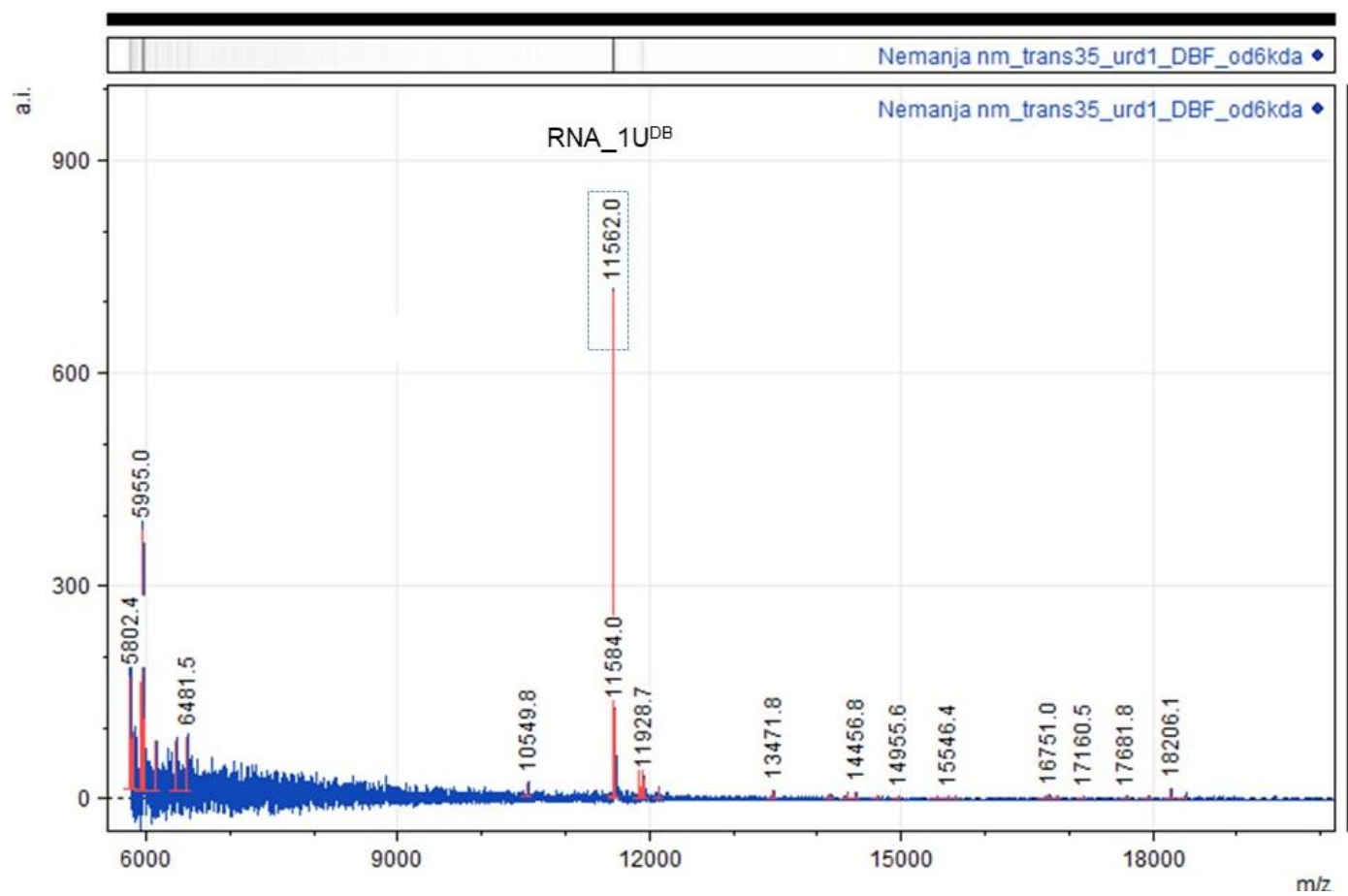


Figure S34. MALDI-TOF spectrum of RNA_{3U^E}

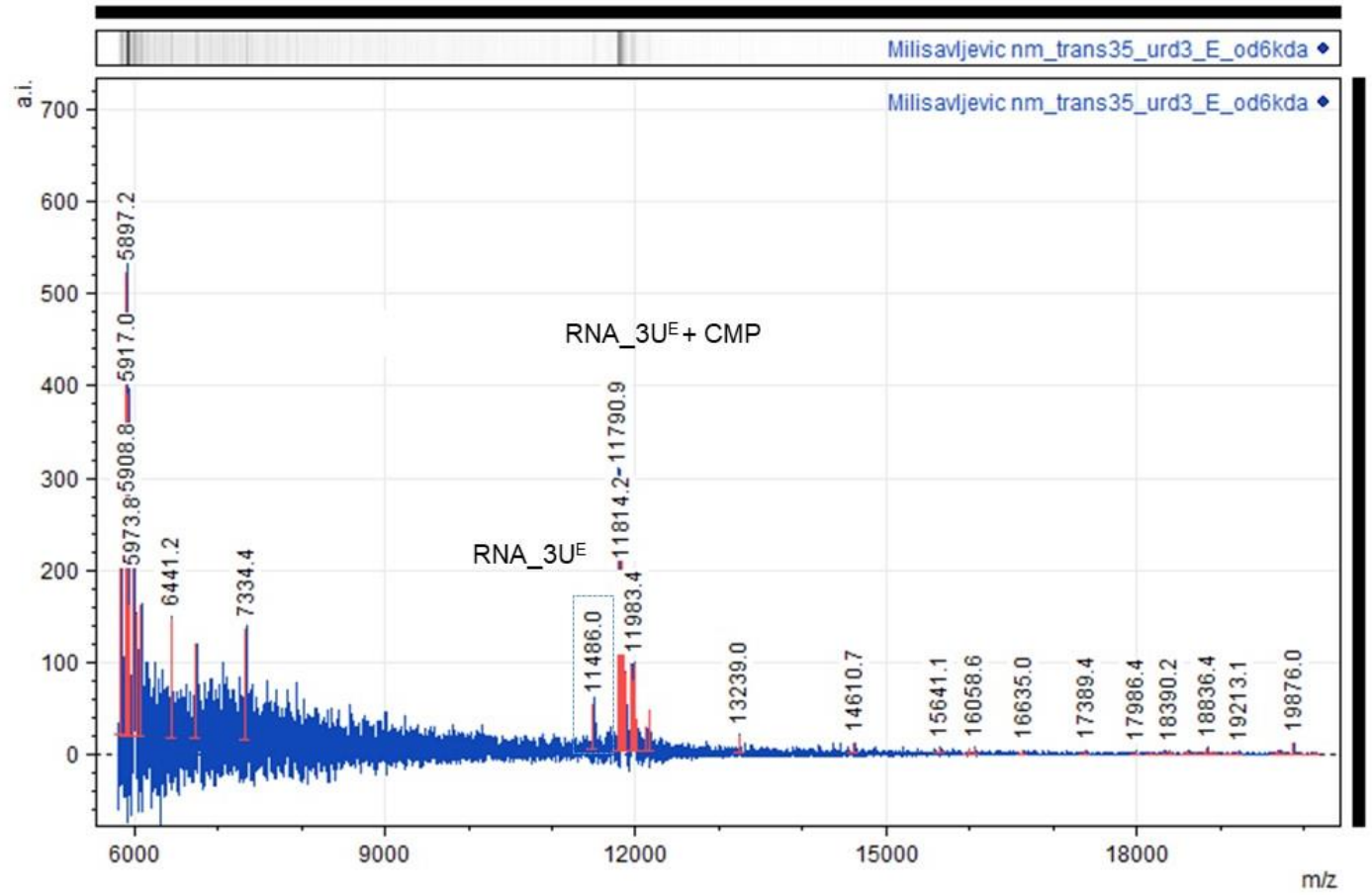


Figure S35. MALDI-TOF spectrum of RNA₃U^{Ph}

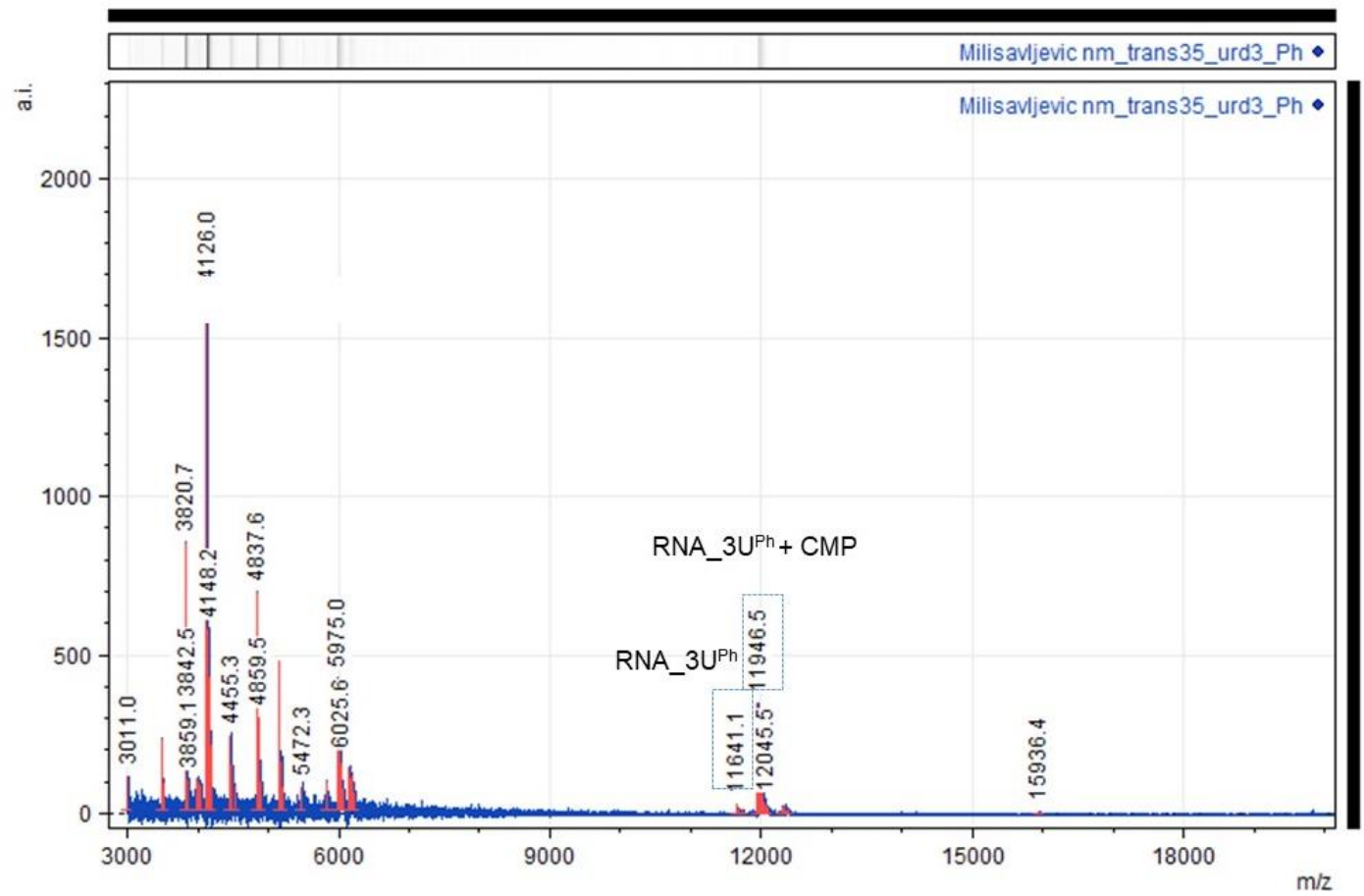


Figure S36. MALDI-TOF spectrum of **RNA_3U^{BF}**

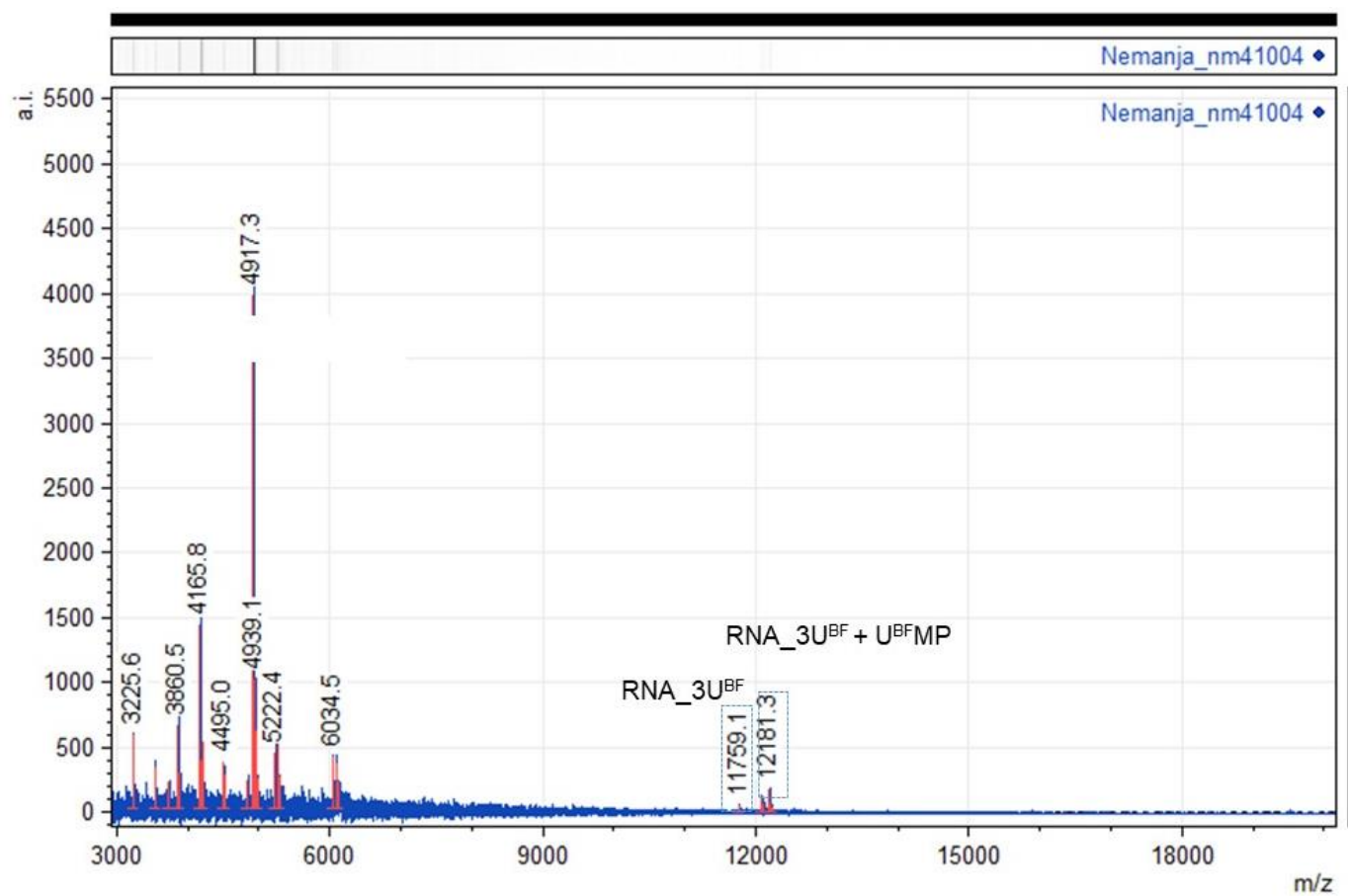


Figure S37. MALDI-TOF spectrum of RNA_{3U}^{DB}

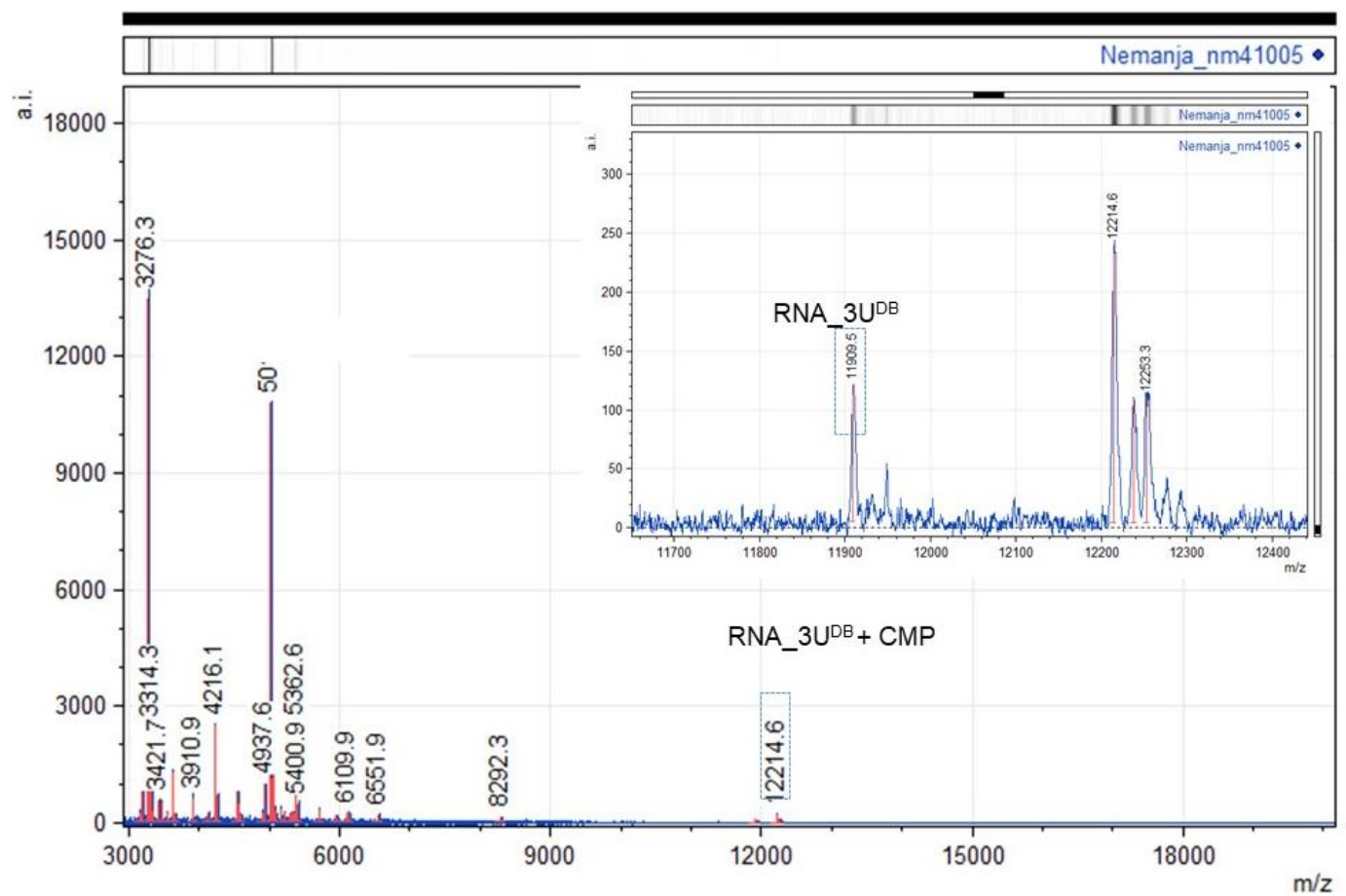


Figure S38. MALDI-TOF spectrum of RNA_{7U}^E

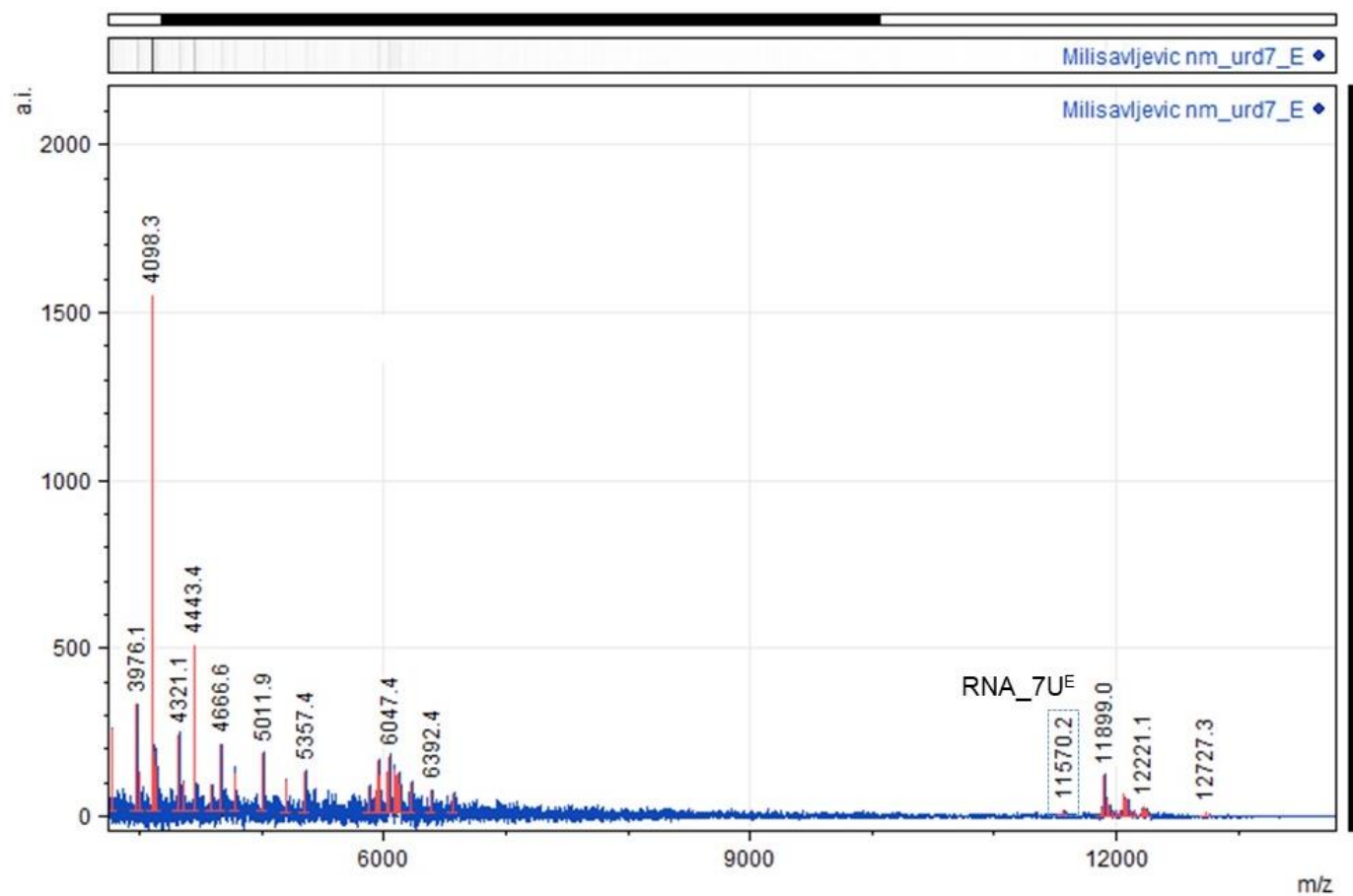


Figure S39. MALDI-TOF spectrum of RNA_7U^{Ph}

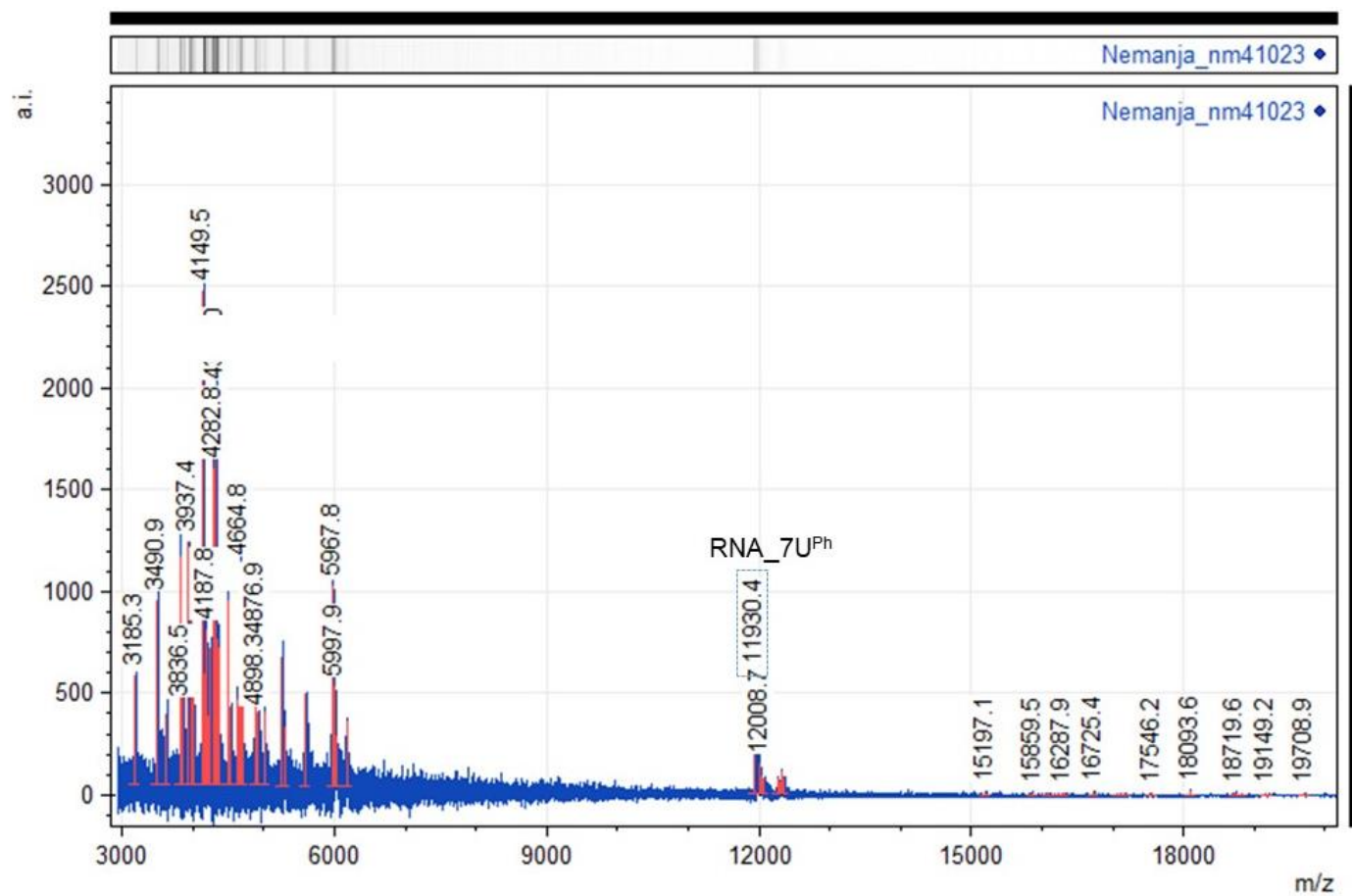


Figure S40. MALDI-TOF spectrum of **RNA_7U^{BF}**

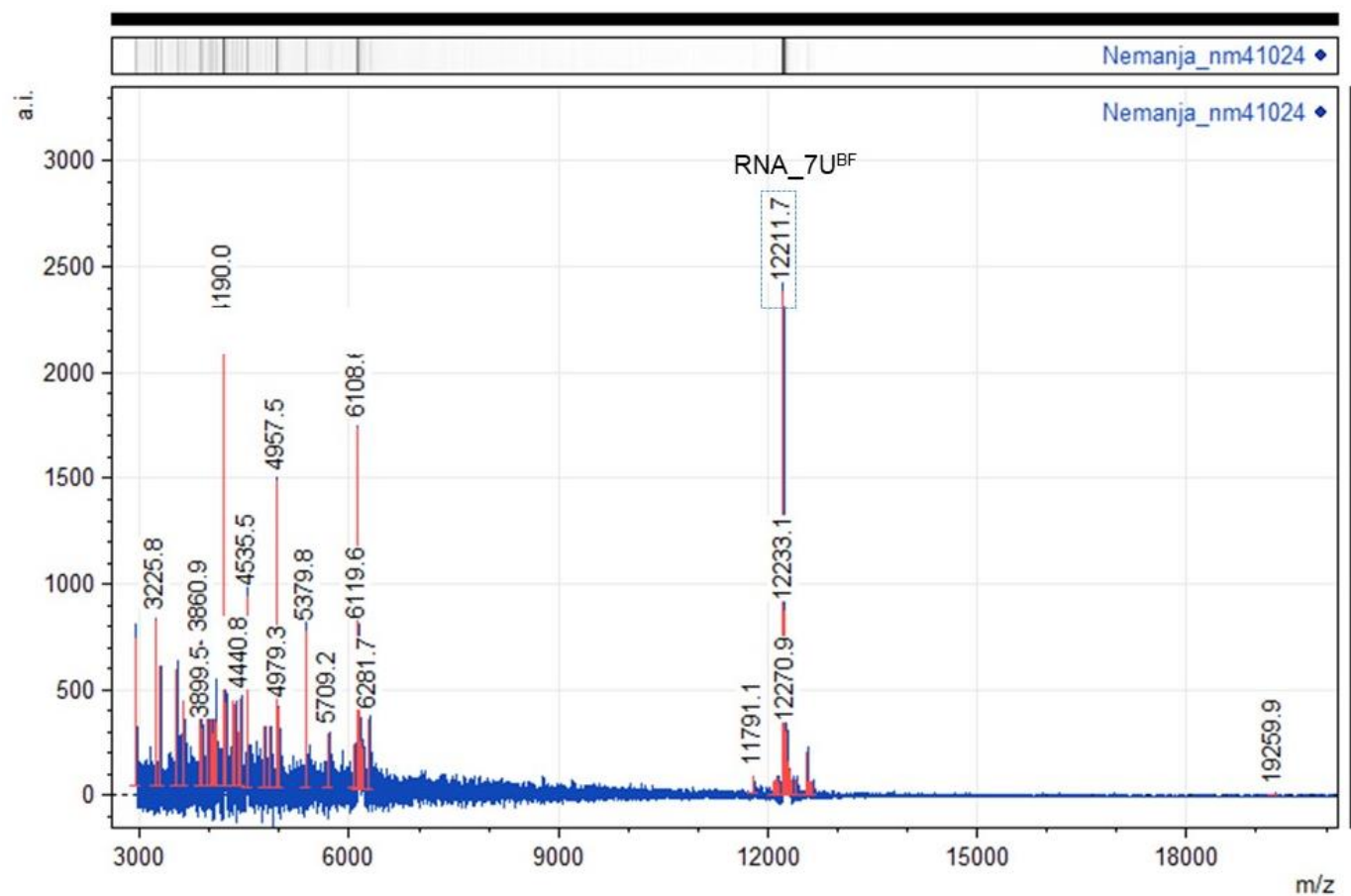


Figure S41. MALDI-TOF spectrum of **RNA_8G^{Me}**

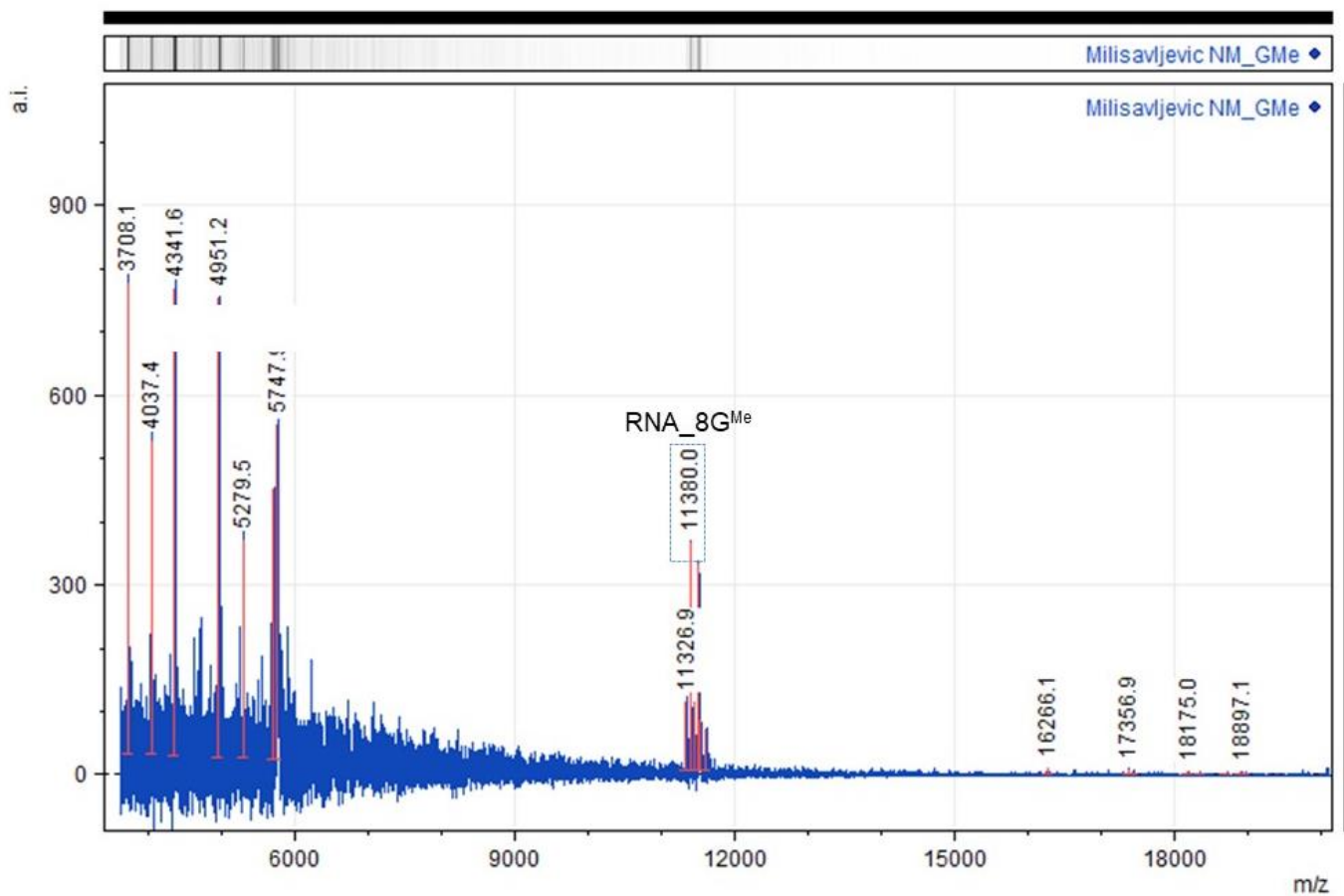


Figure S42. MALDI-TOF spectrum of RNA₈G^E

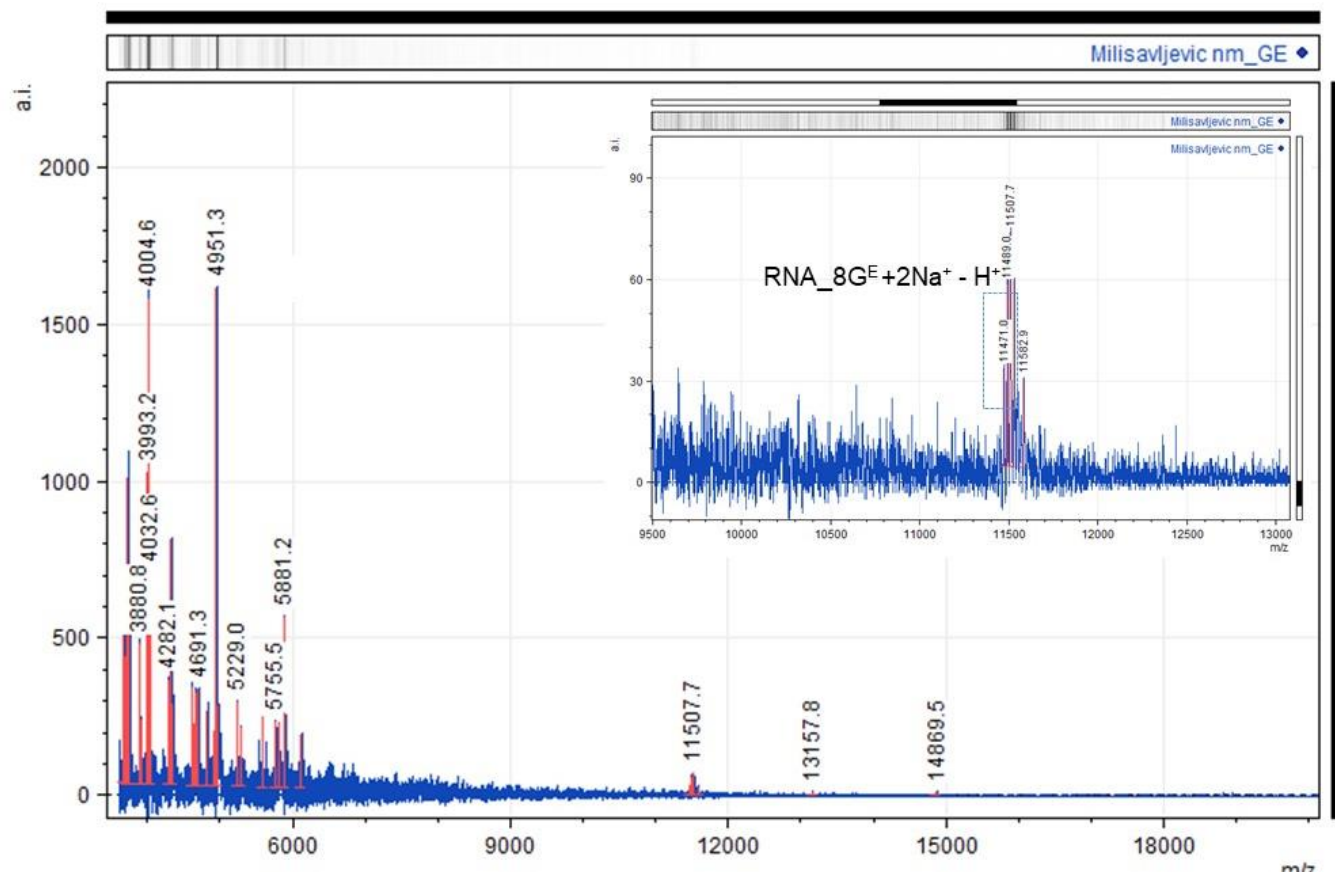


Figure S43. MALDI-TOF spectrum of **RNA_8G^{Ph}**

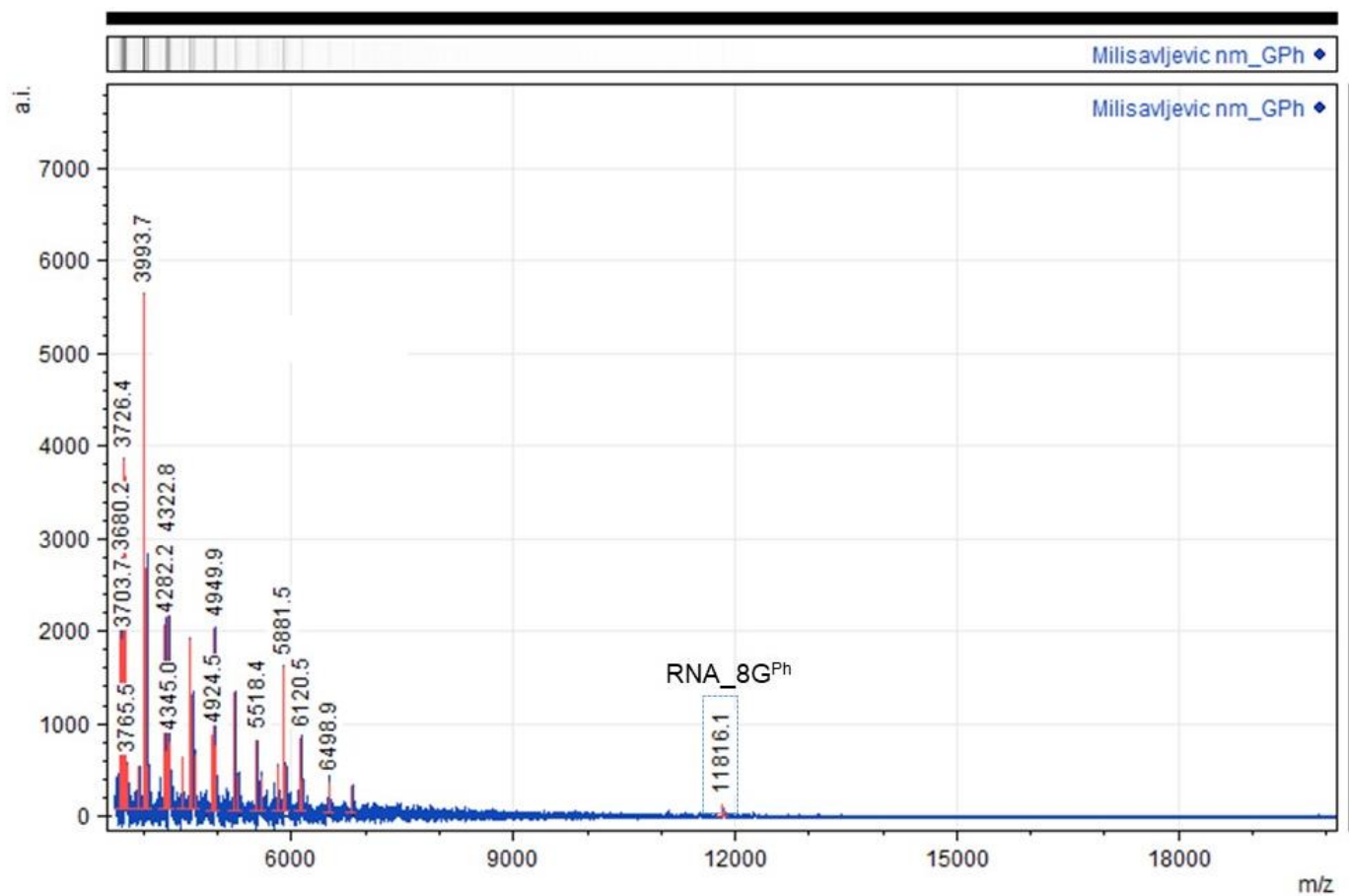
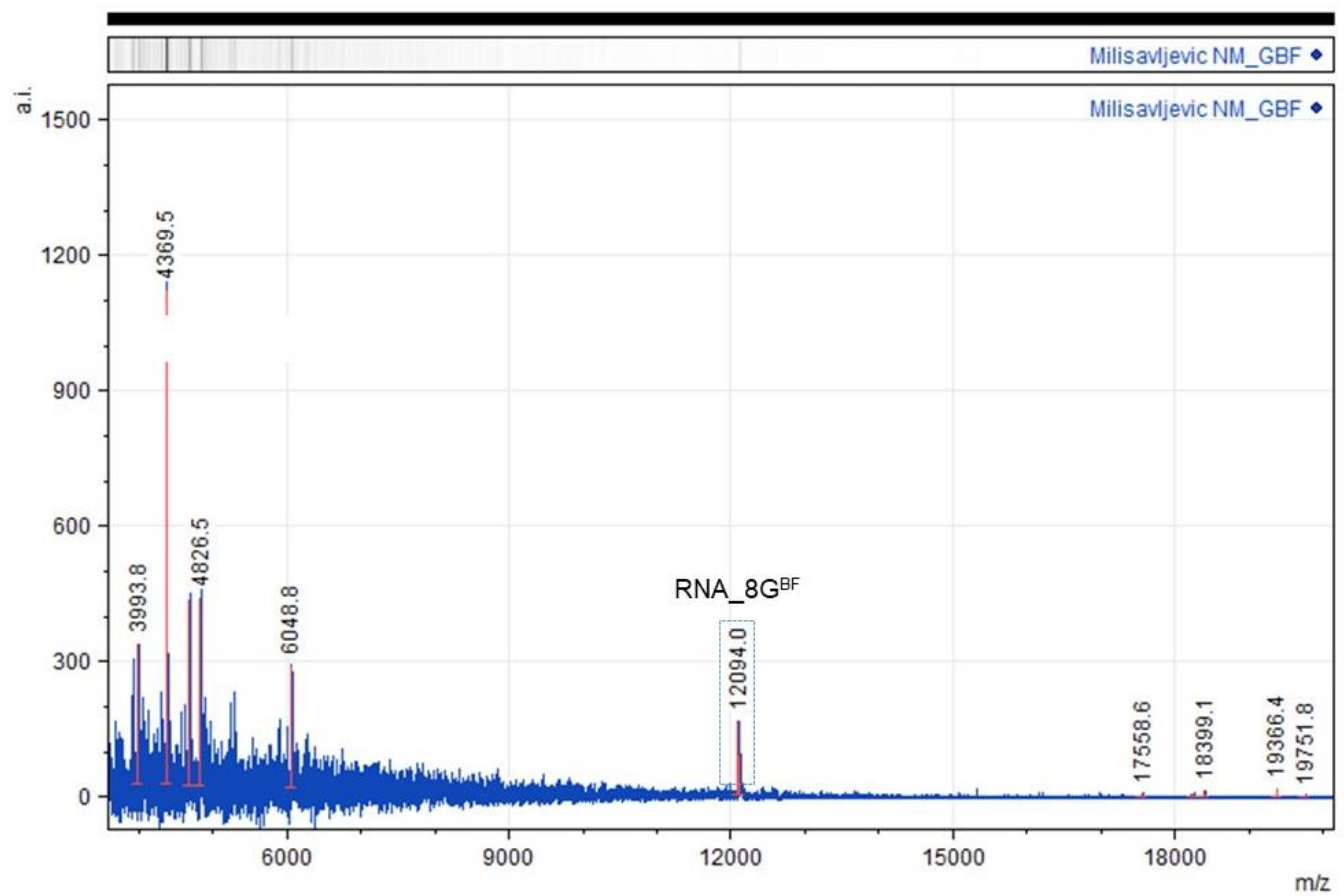
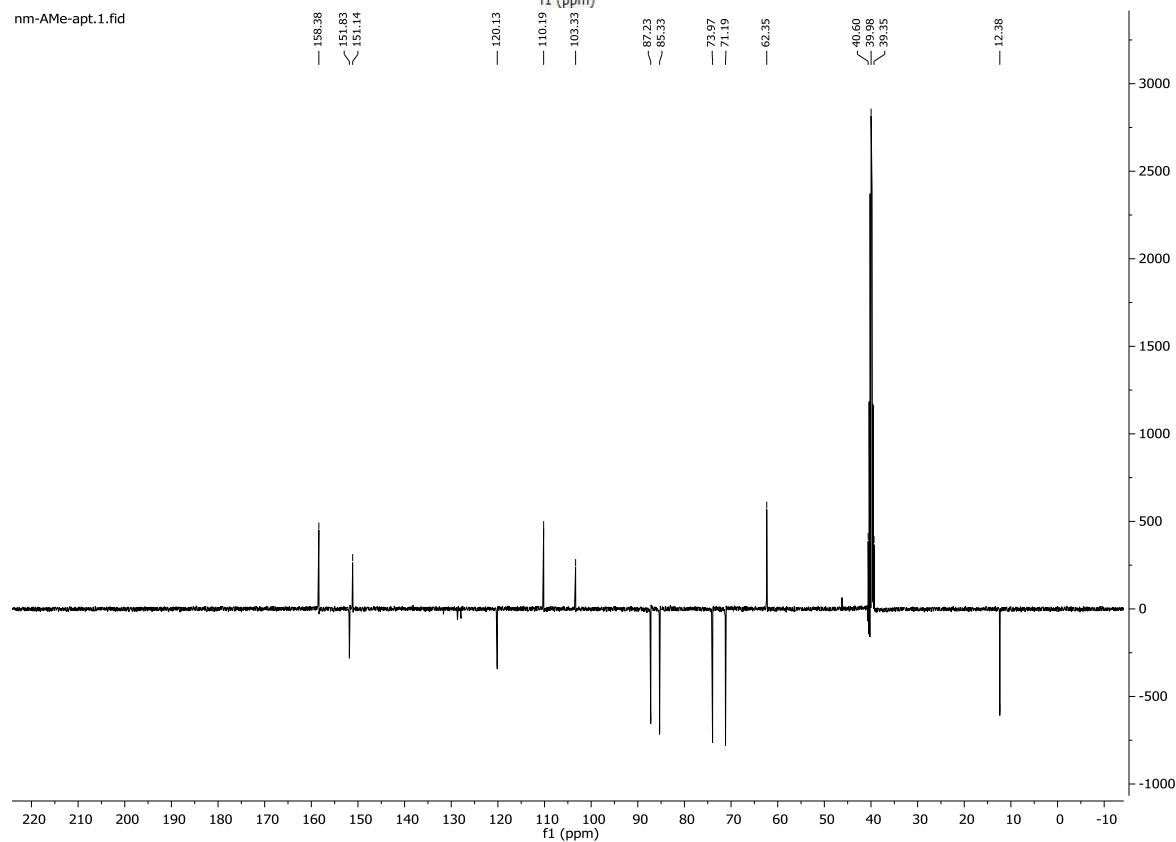
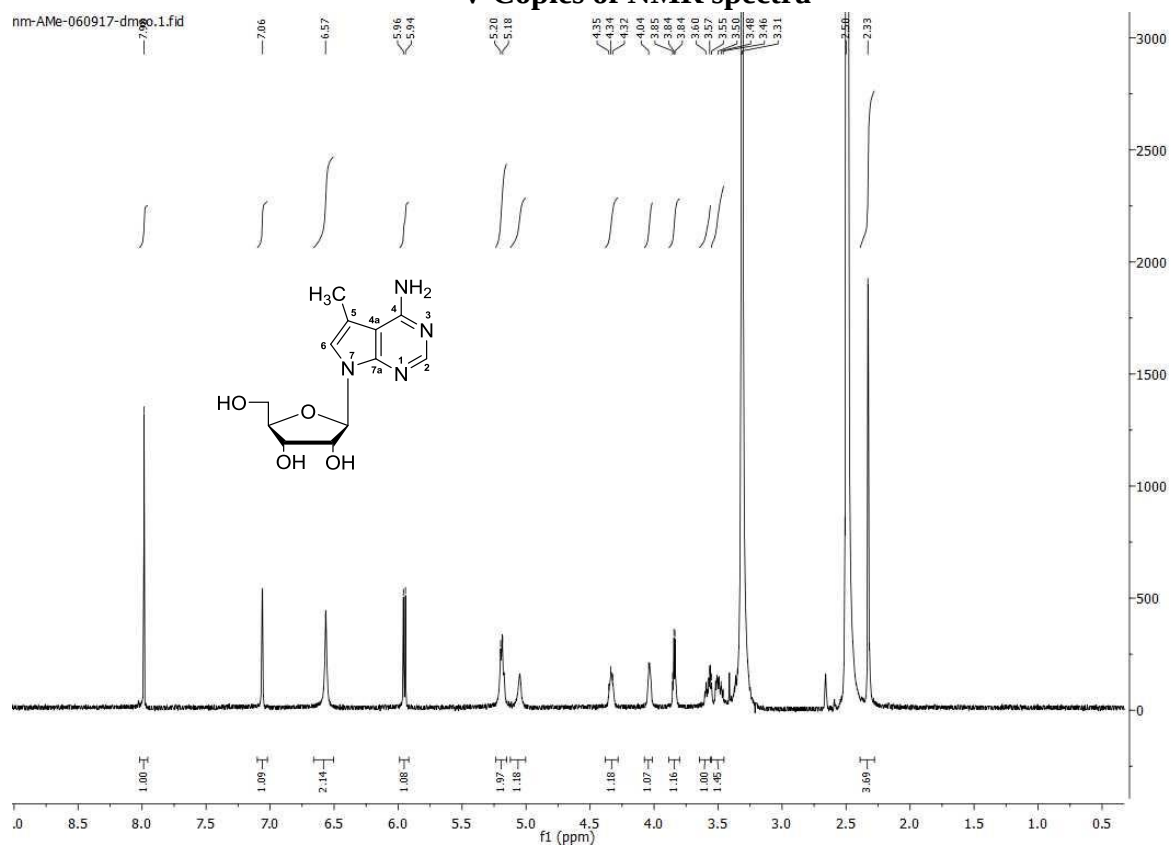
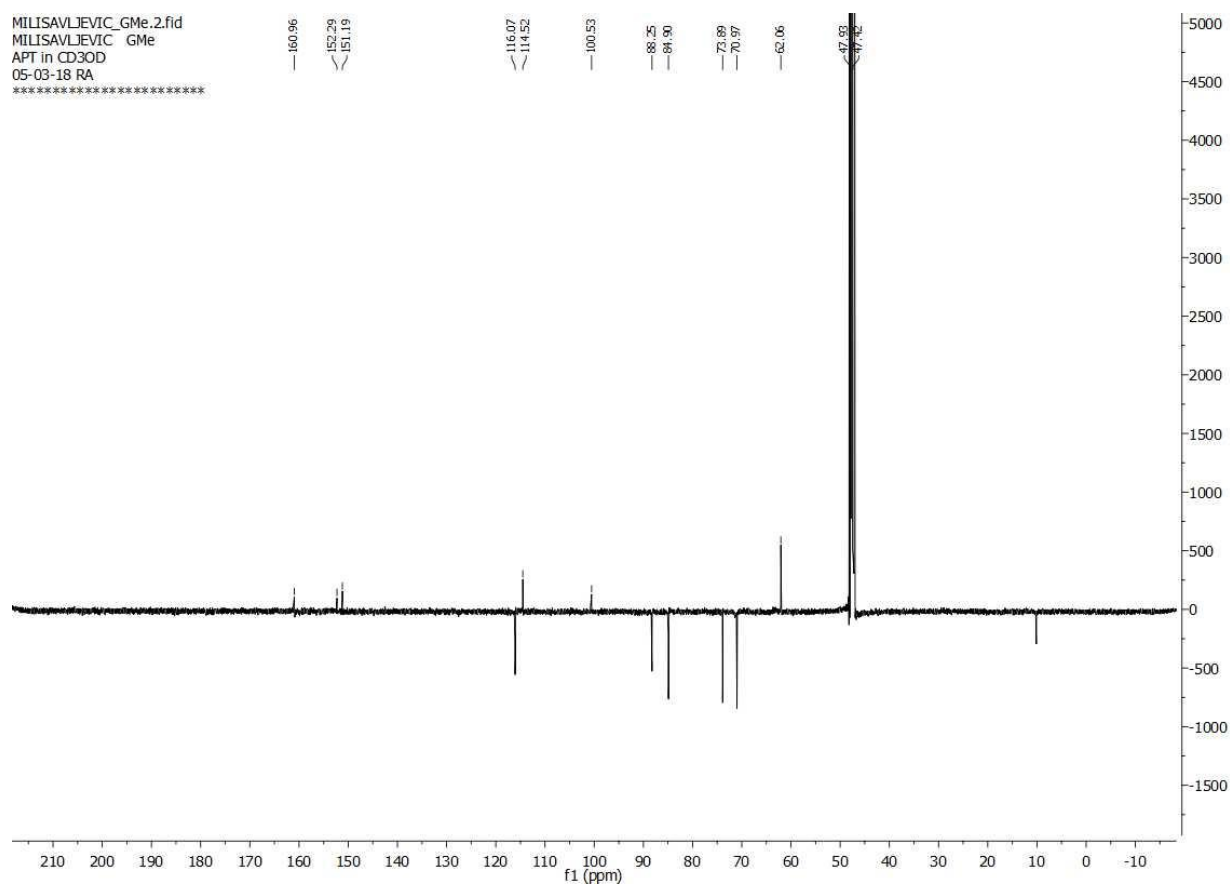
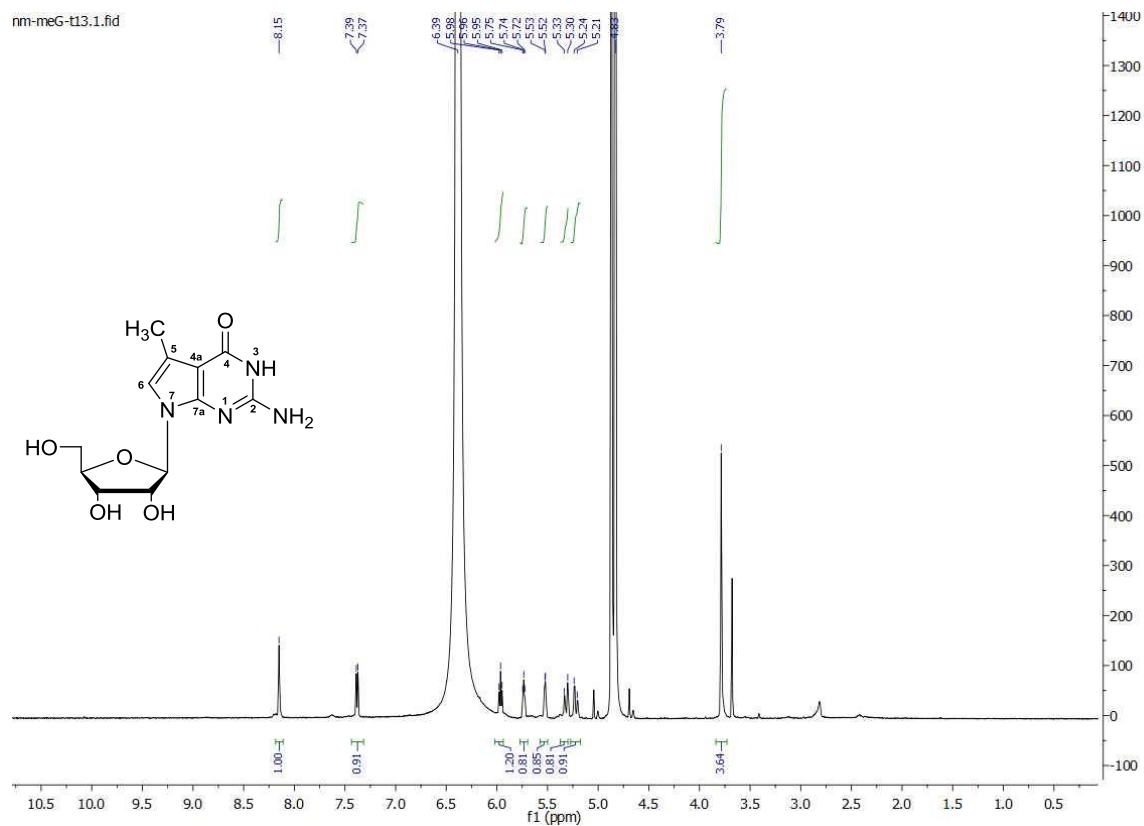


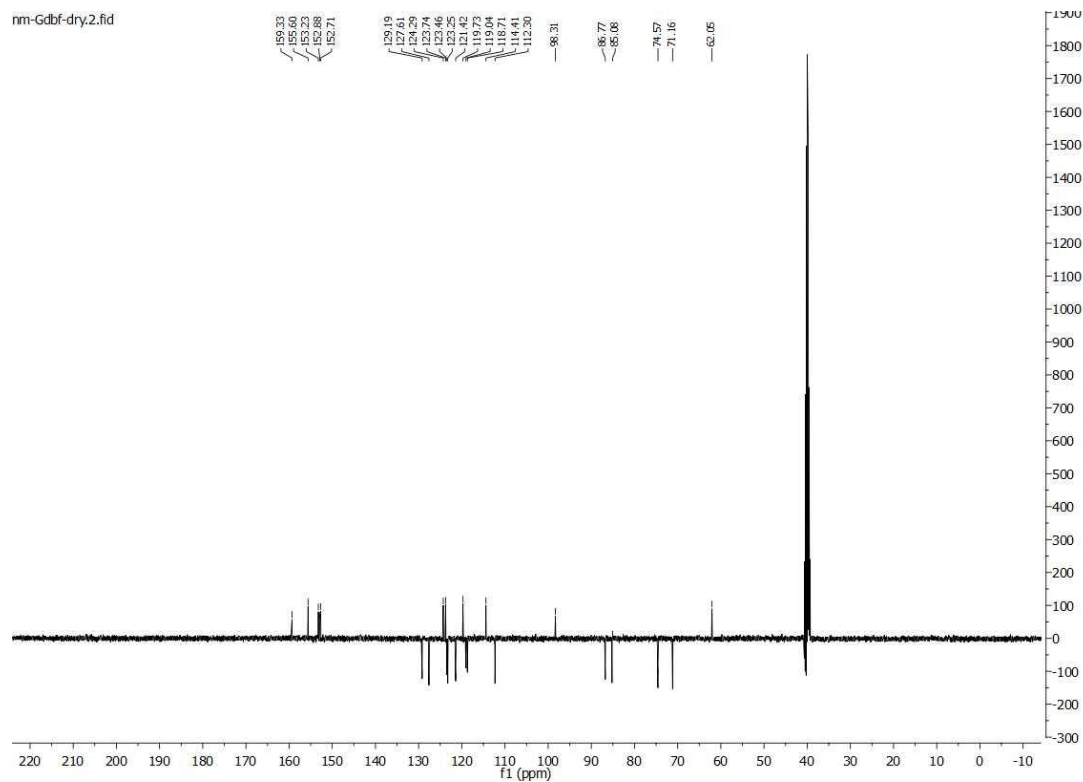
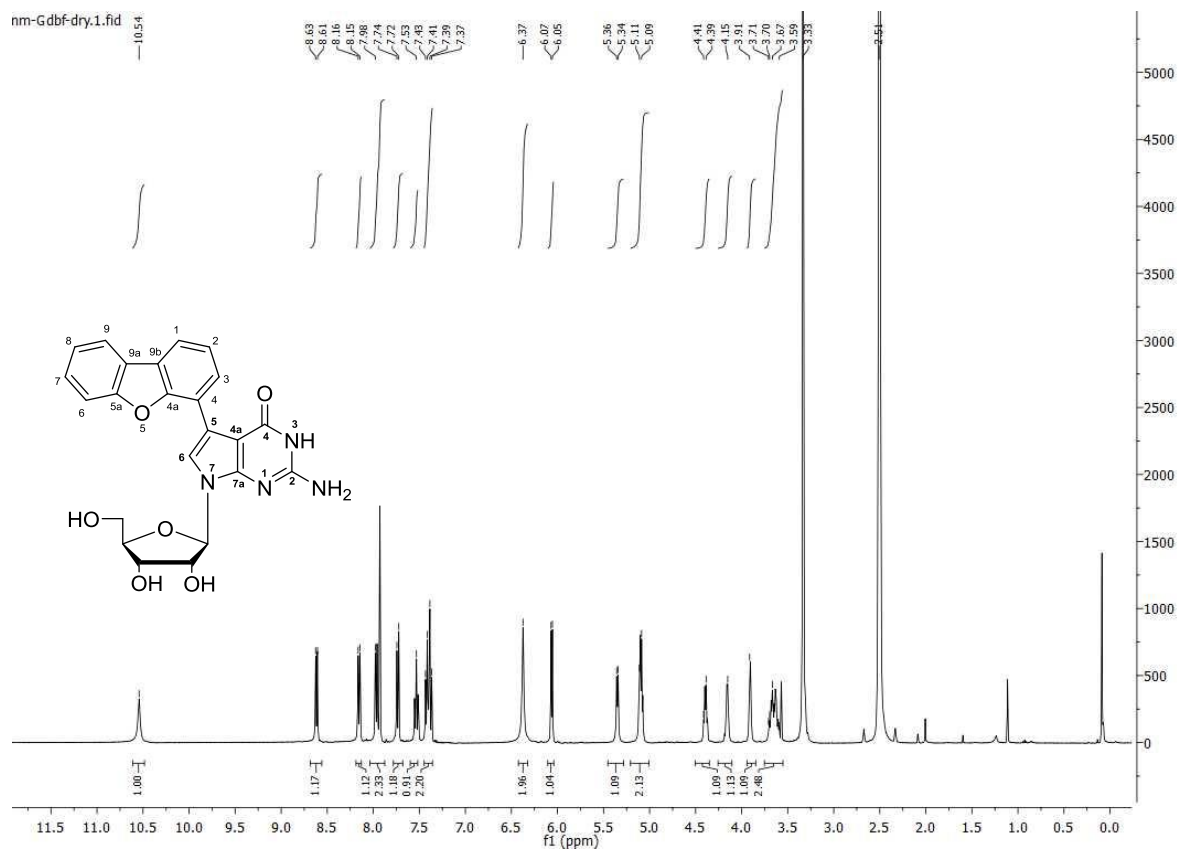
Figure S44. MALDI-TOF spectrum of **RNA_8G^{BF}**



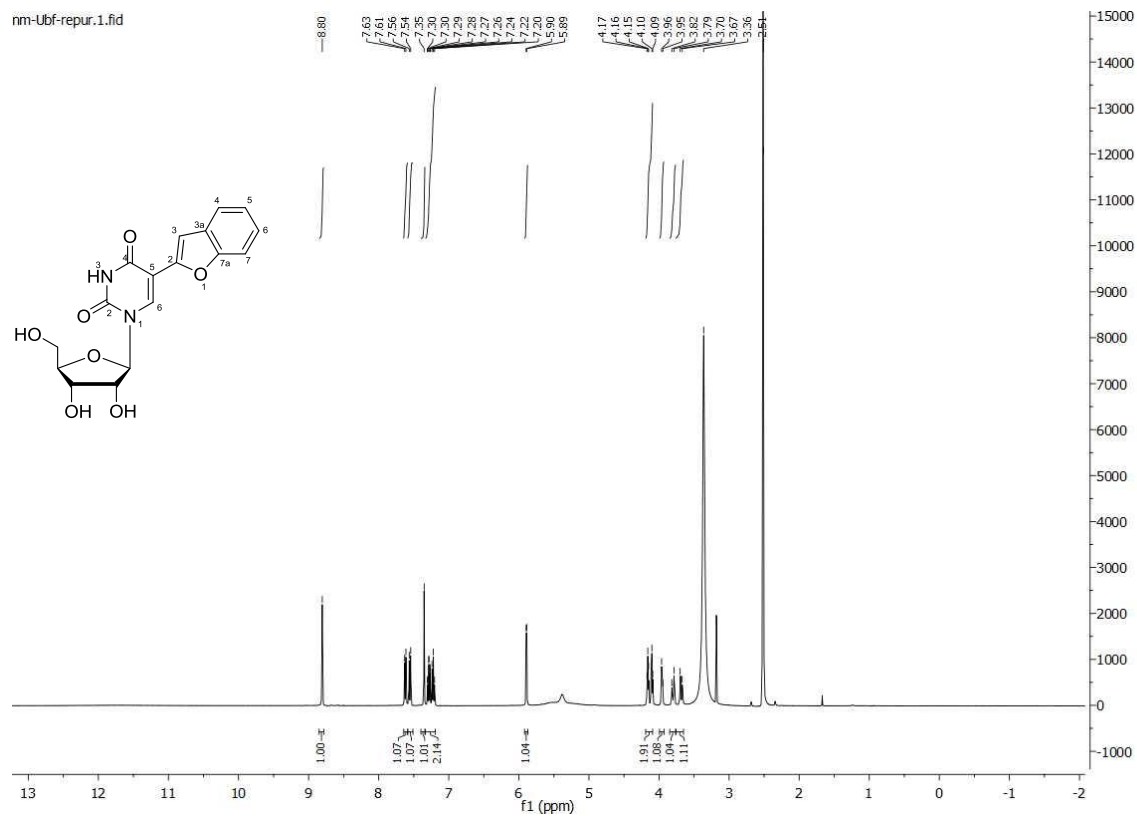
V Copies of NMR spectra



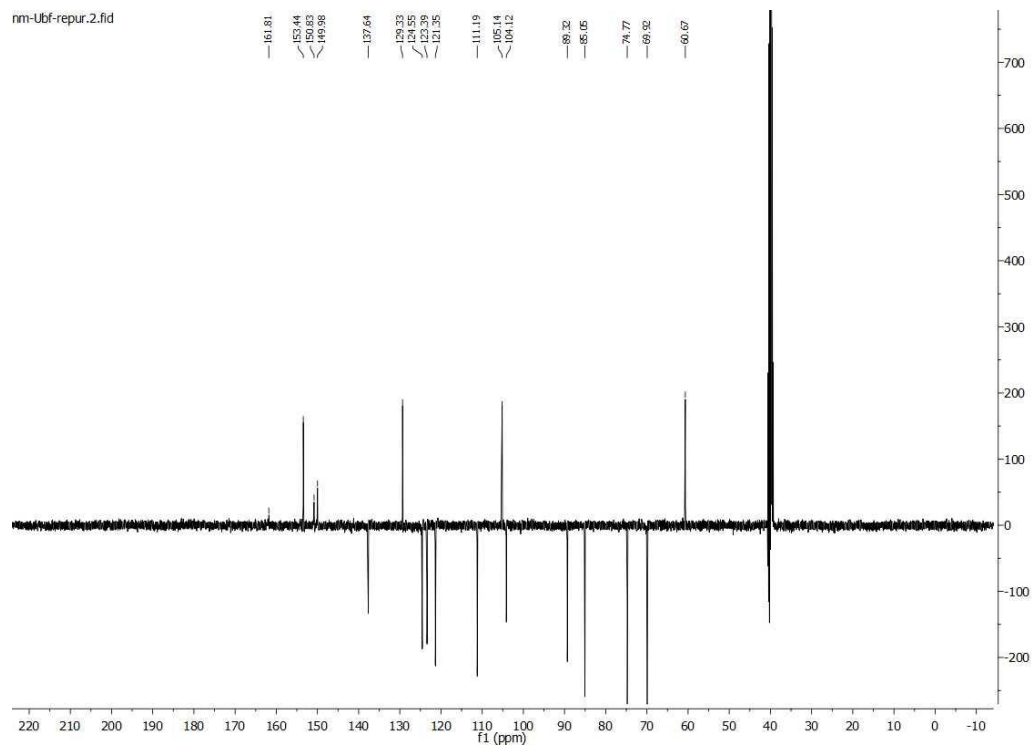




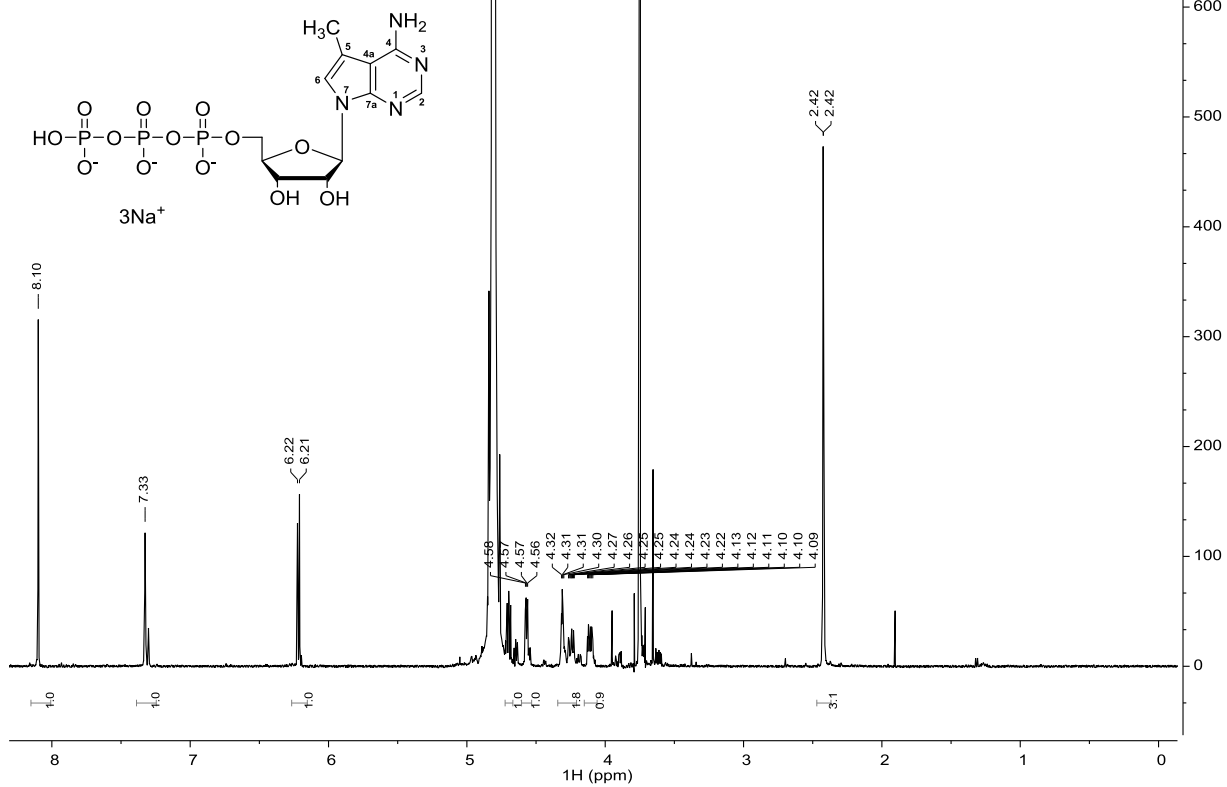
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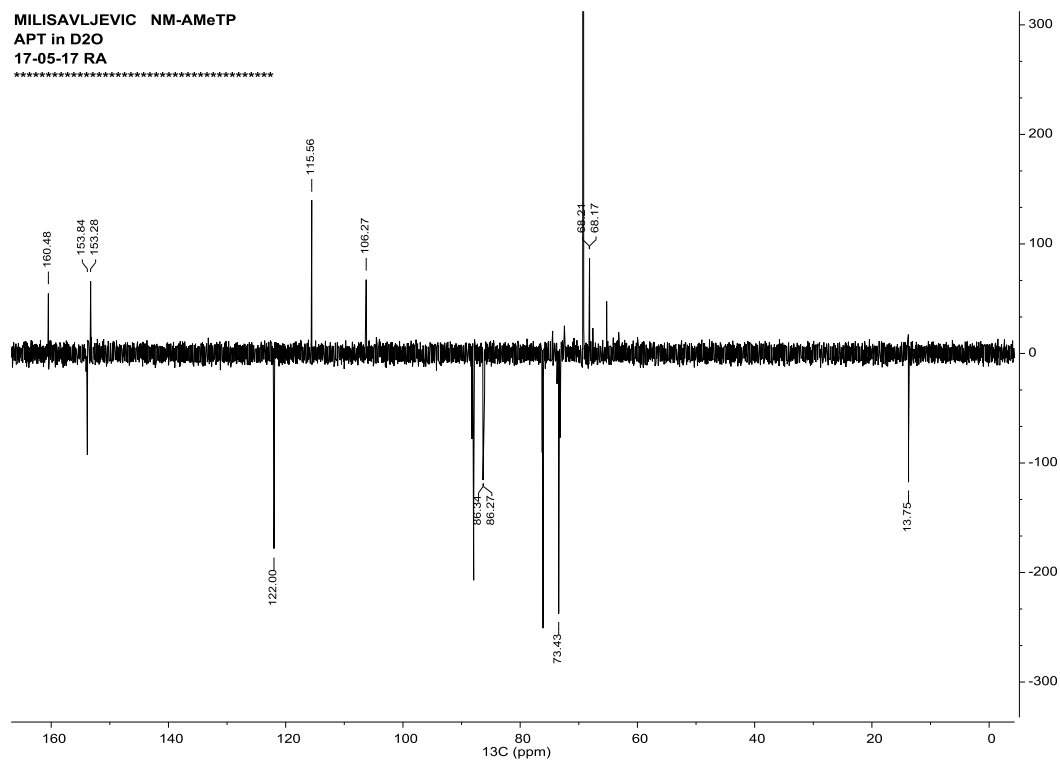
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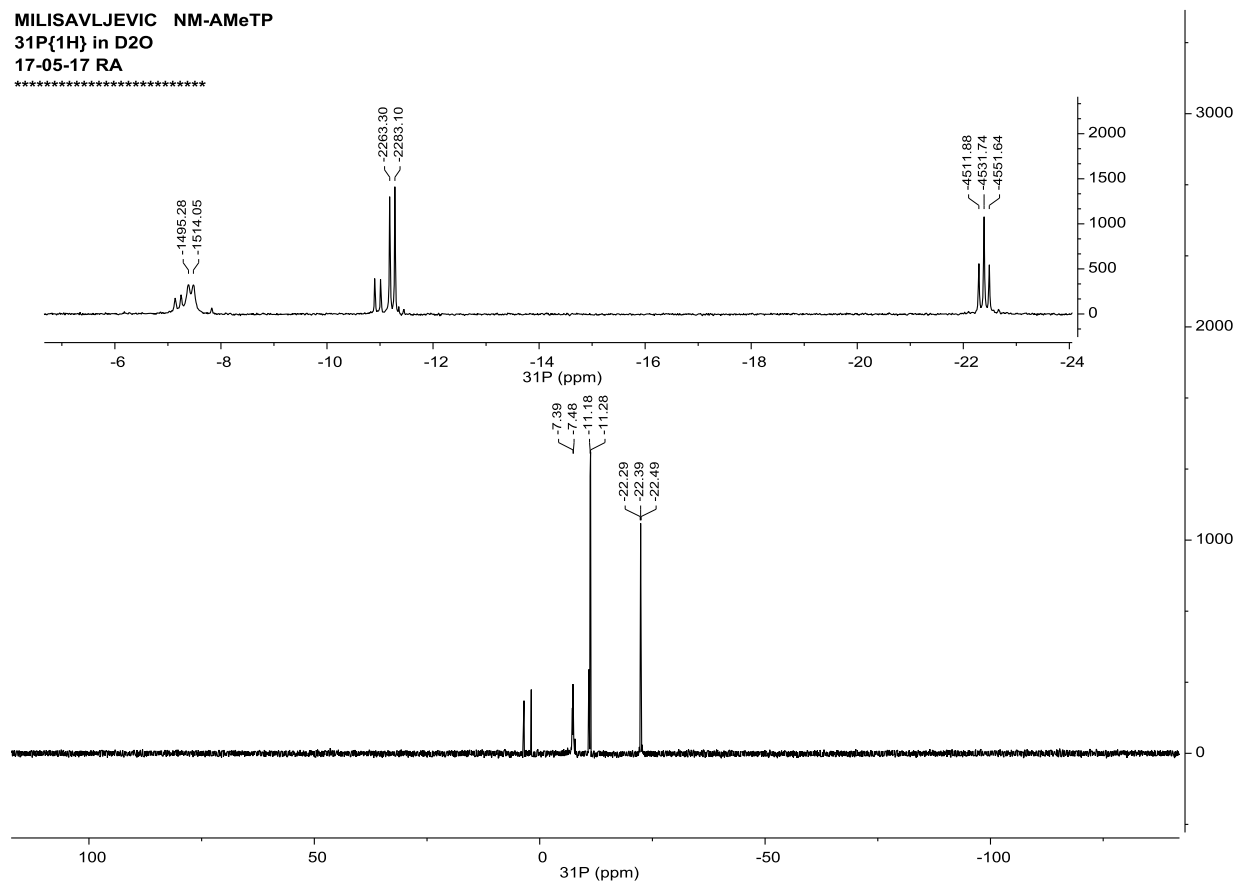
MILISAVLJEVIC NM-AMeTP
 1H NMR in D2O
 17-05-17 RA



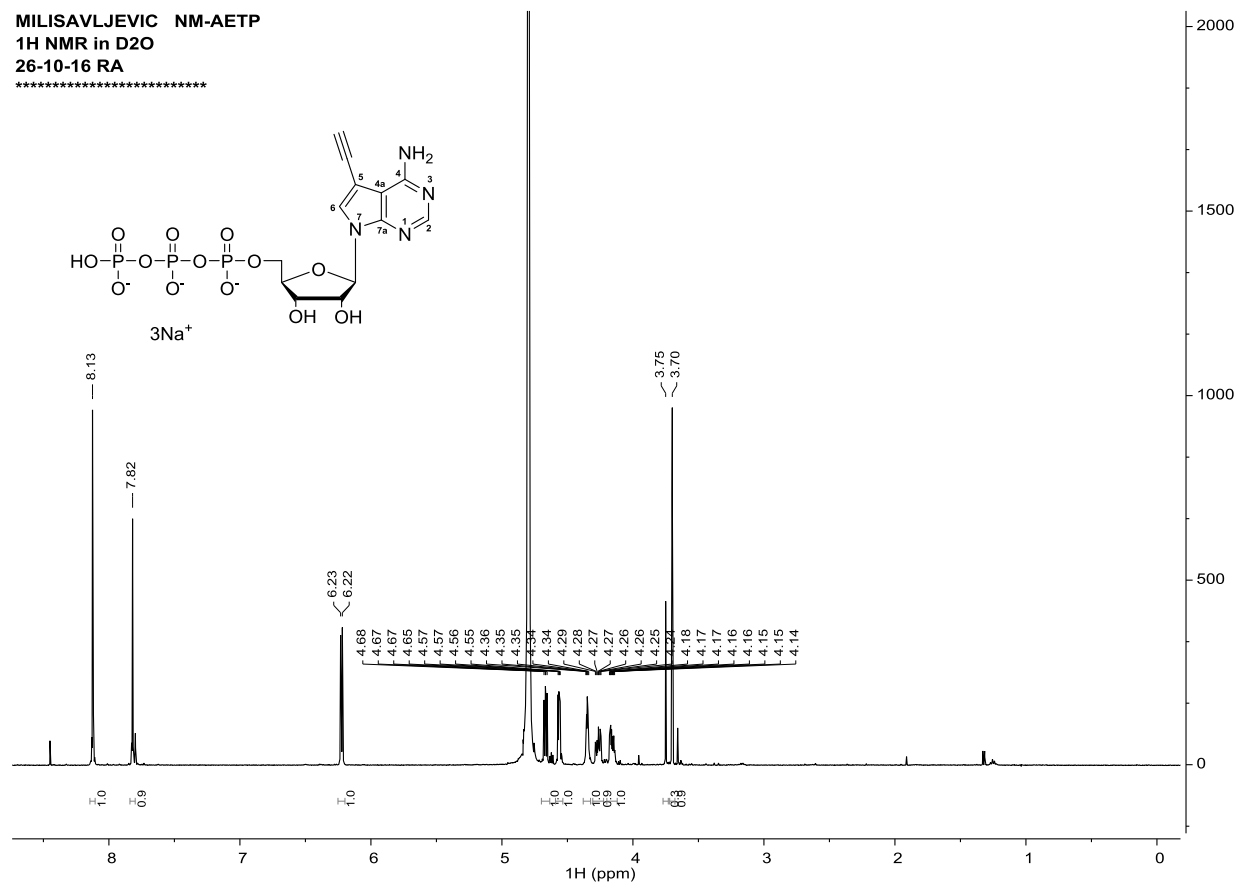
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APT in D2O
17-05-17 RA



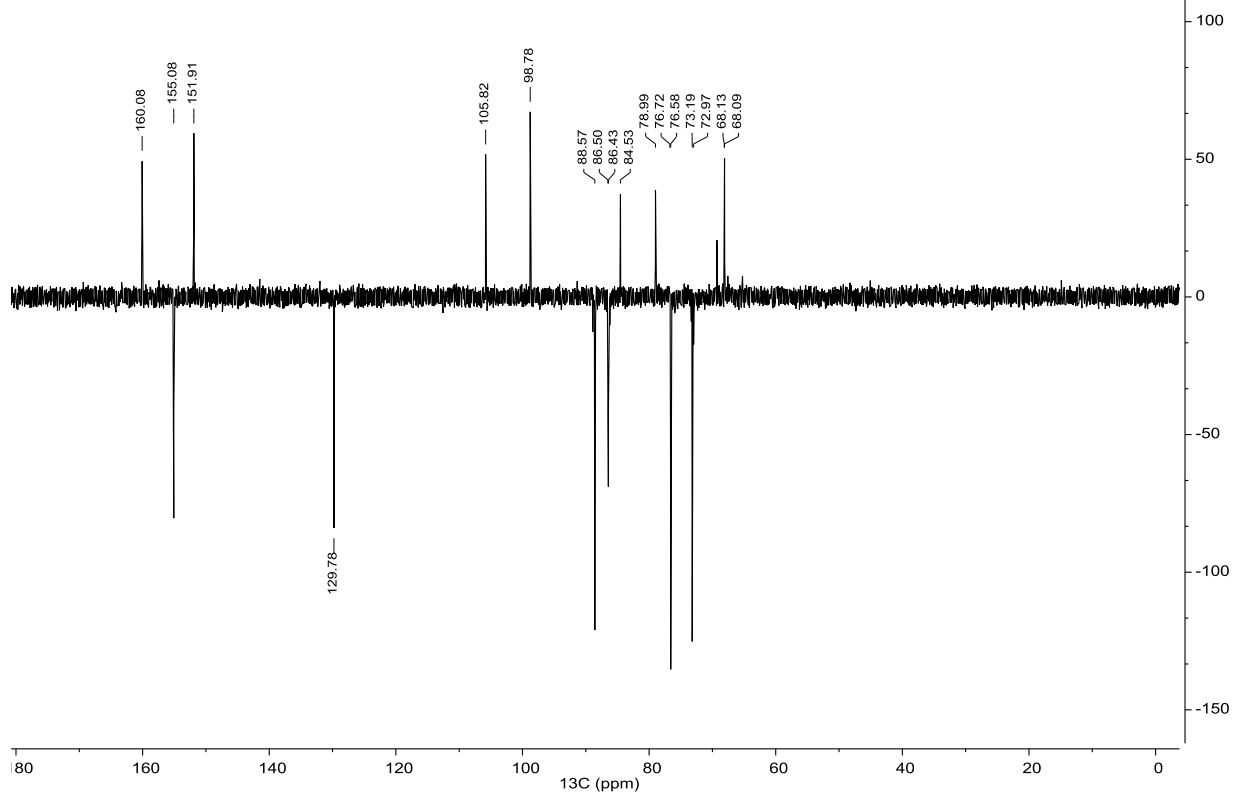
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31P{1H} in D2O
17-05-17 RA



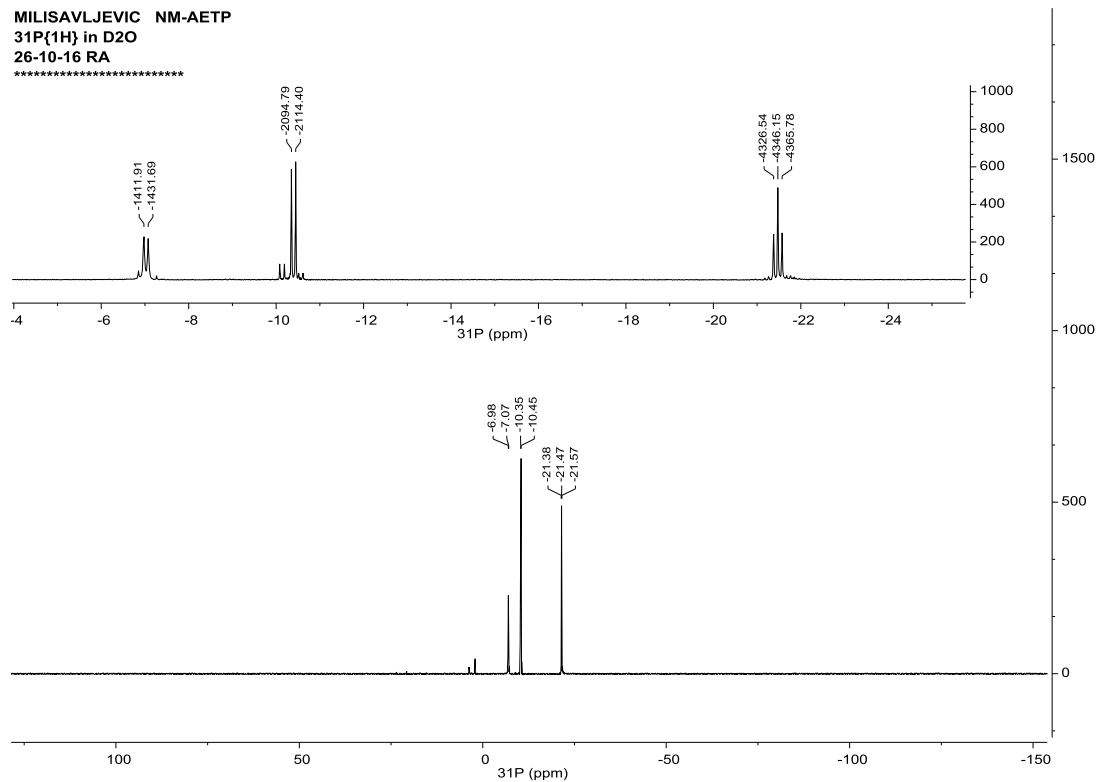
26-10-16 RA



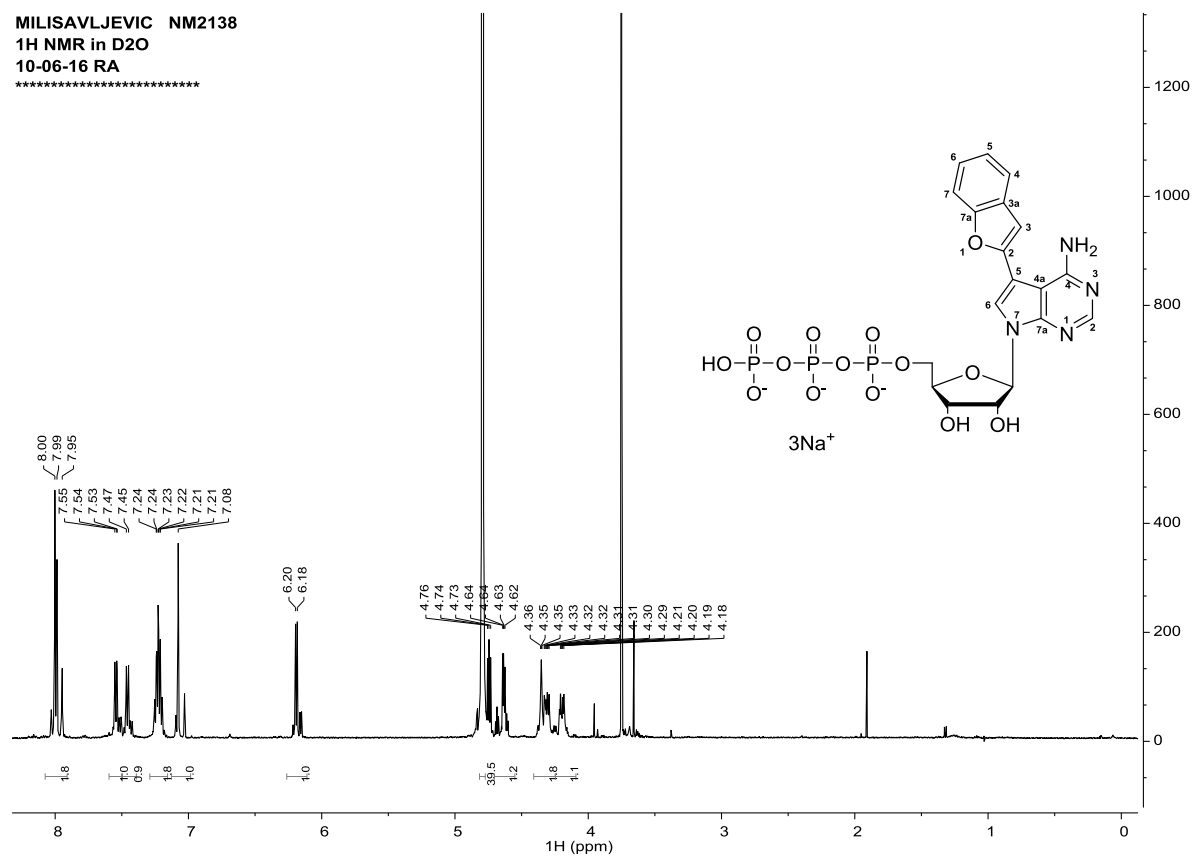
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APT in D2O
26-10-16 RA



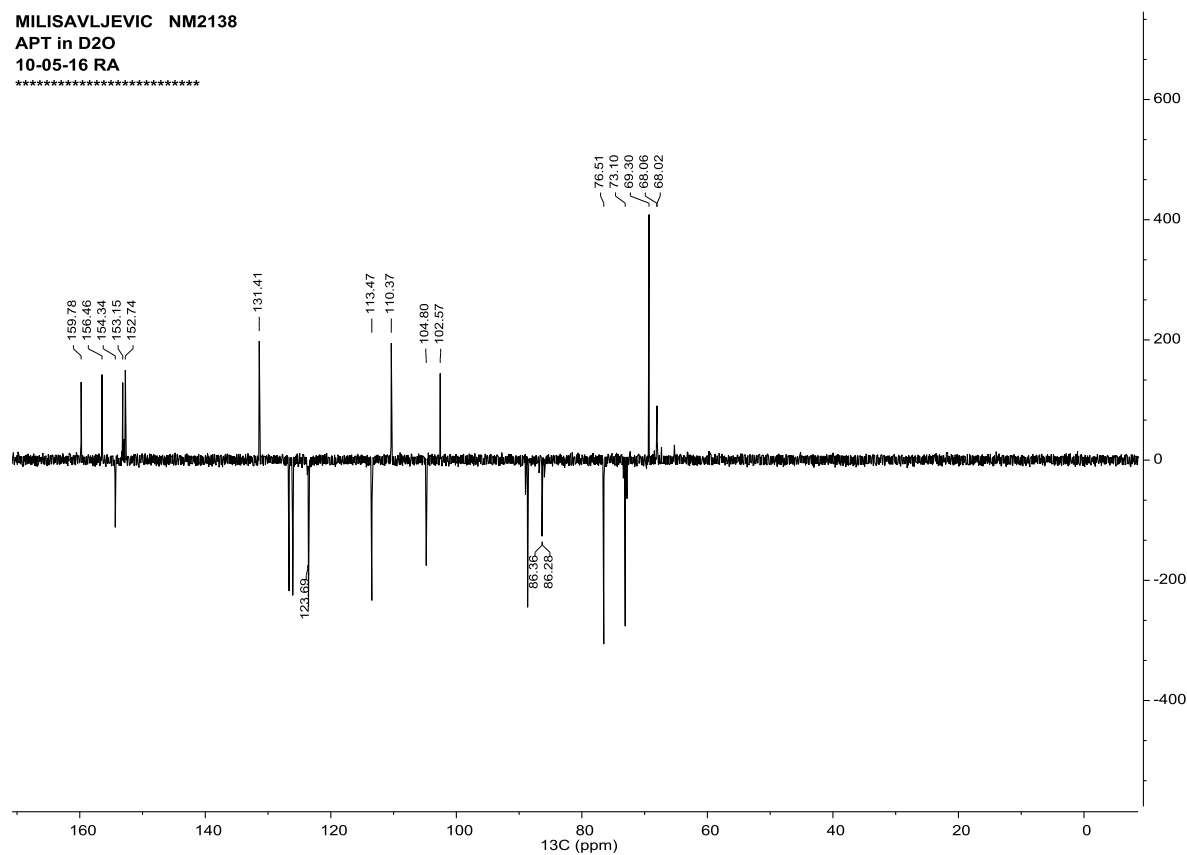
MILISAVLJEVIC NM-AETP
31P{1H} in D2O
26-10-16 RA



MILISAVLJEVIC NM2138
 1H NMR in D2O
 10-06-16 RA



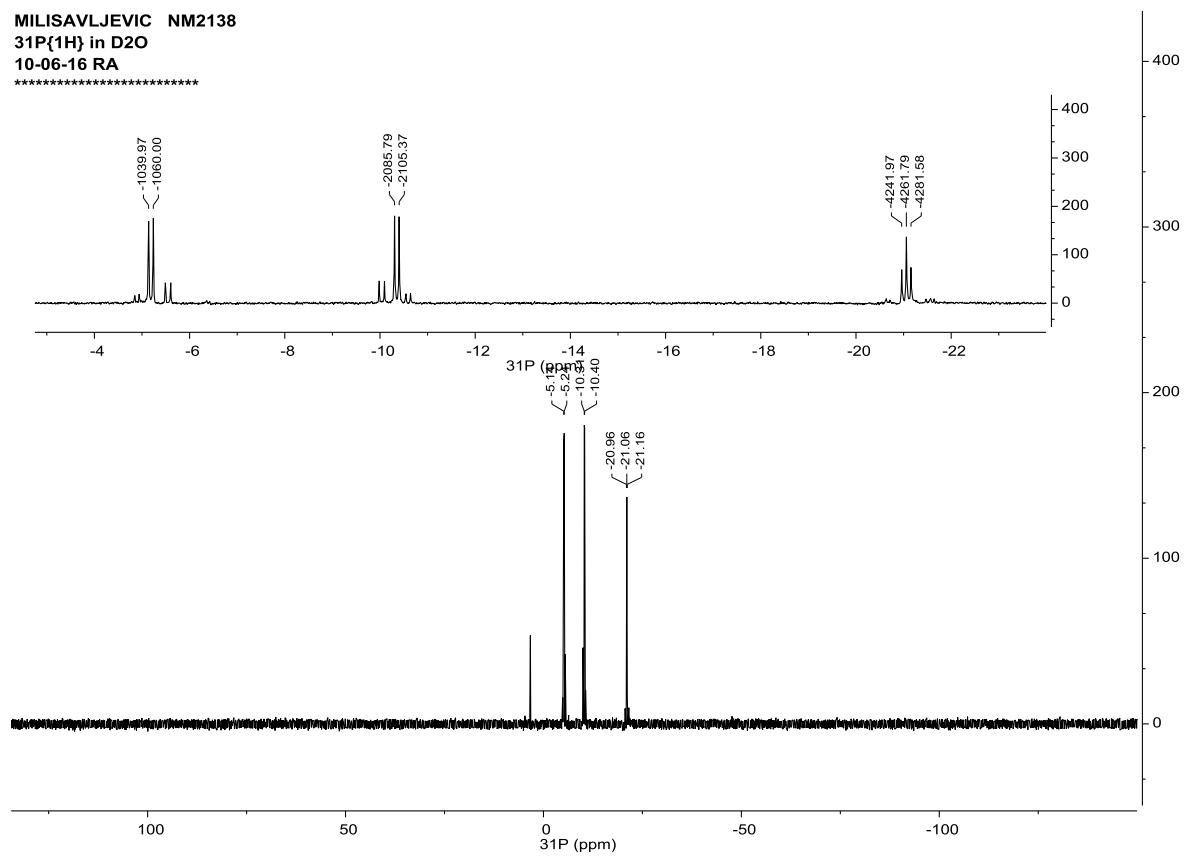
MILISAVLJEVIC NM2138
 APT in D2O
 10-05-16 RA



MILISAVLJEVIC NM2138

$^{31}\text{P}\{^1\text{H}\}$ in D_2O

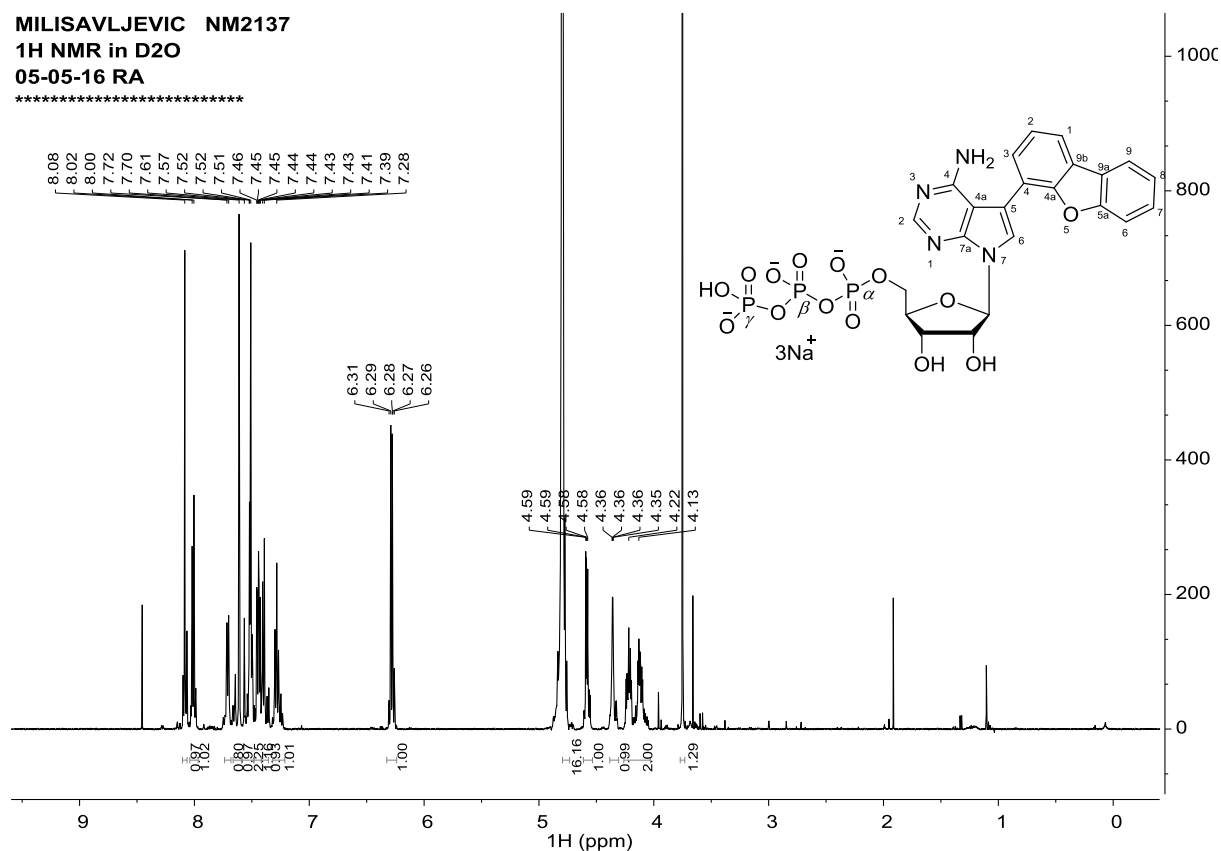
10-06-16 RA



MILISAVLJEVIC NM2137

1H NMR in D2O

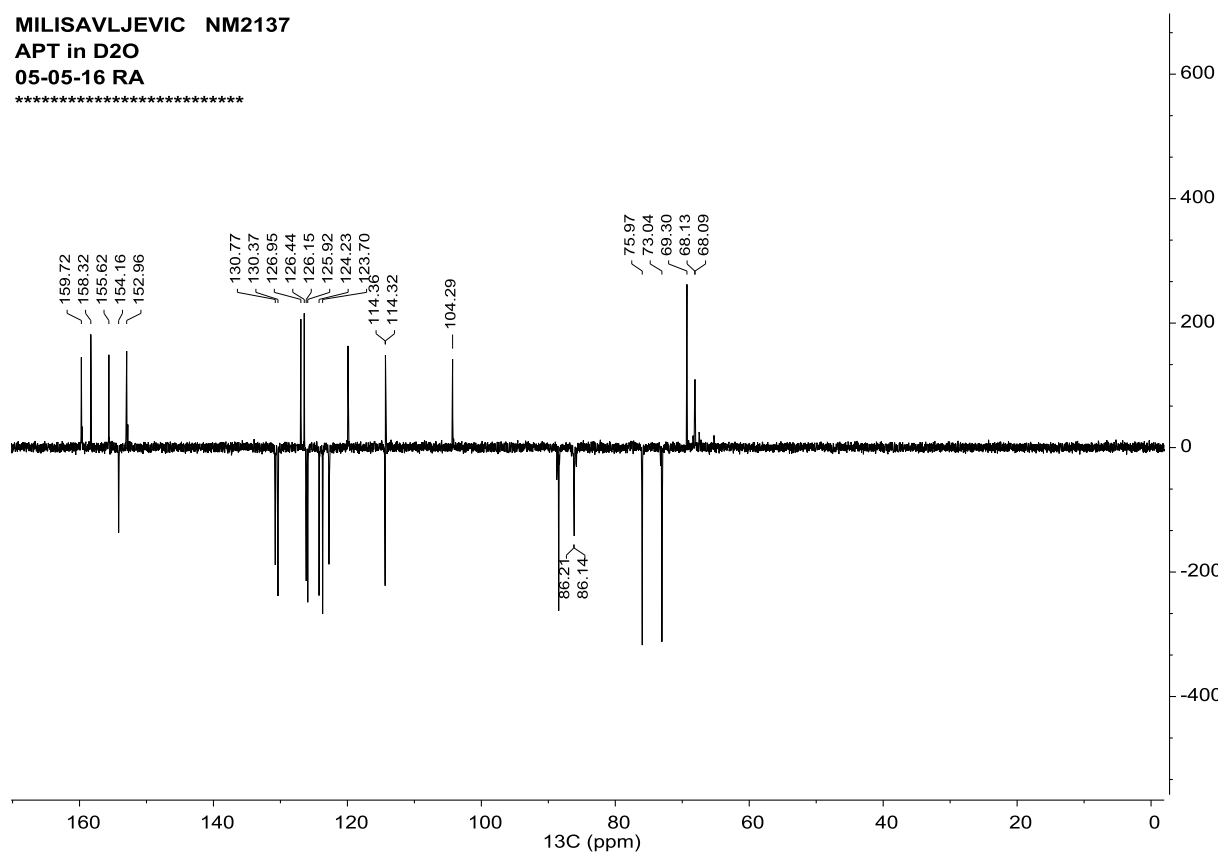
05-05-16 RA



MILISAVLJEVIC NM2137

APT in D2O

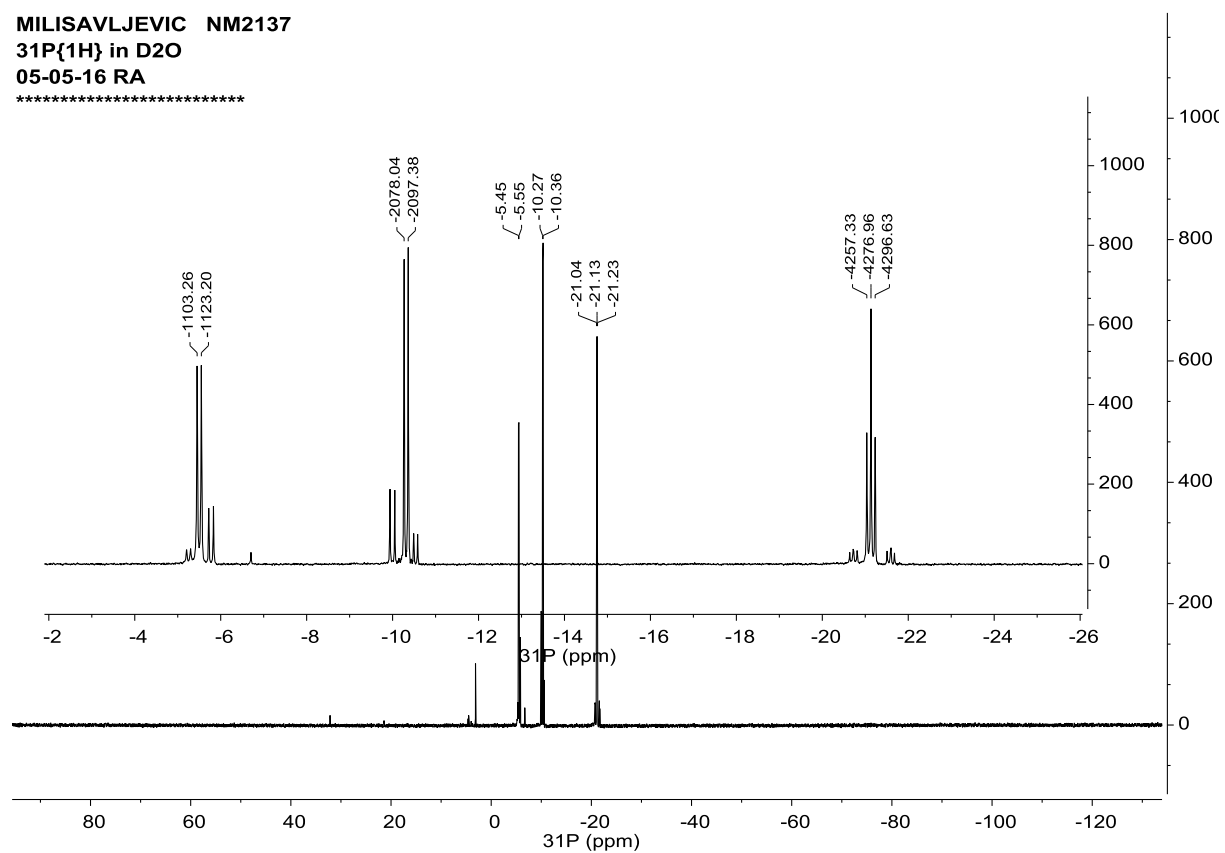
05-05-16 RA



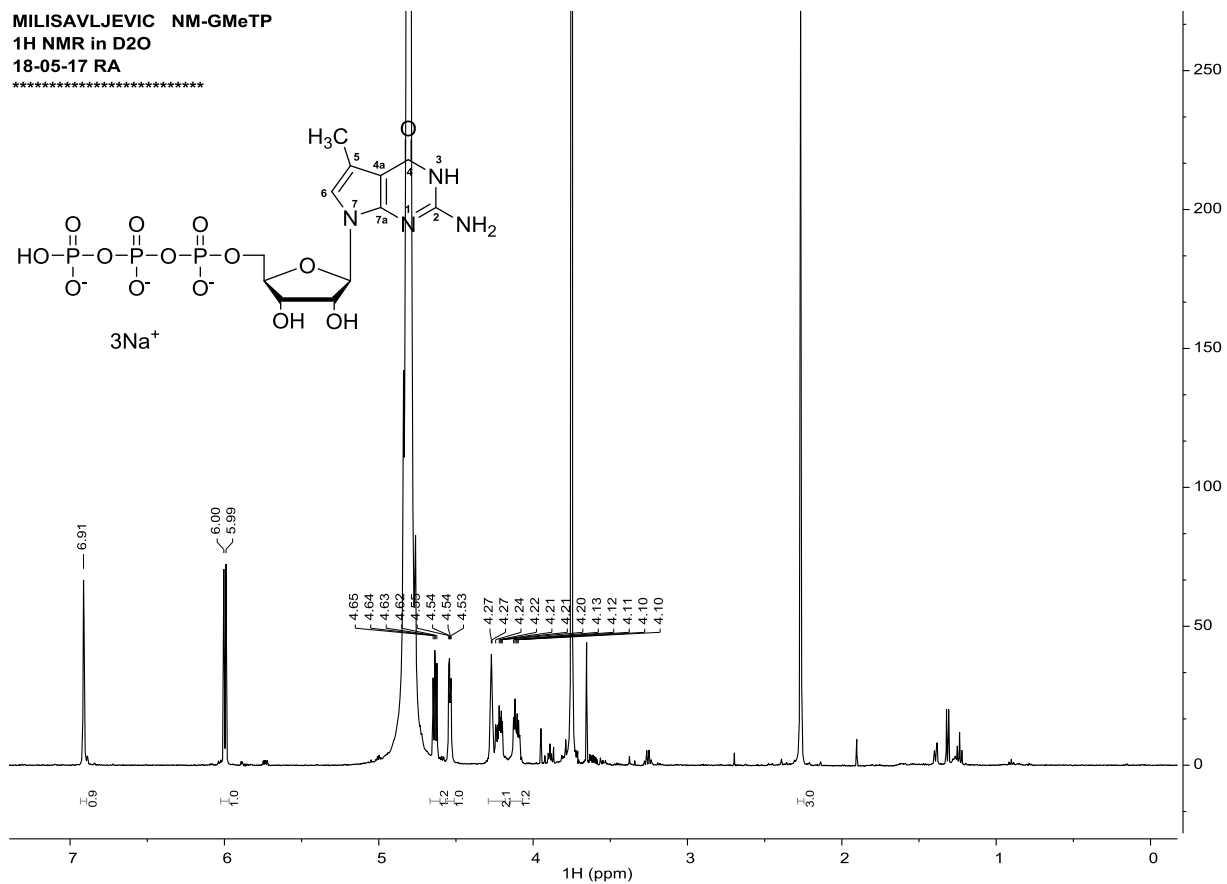
MILISAVLJEVIC NM2137

$^{31}\text{P}\{^1\text{H}\}$ in D_2O

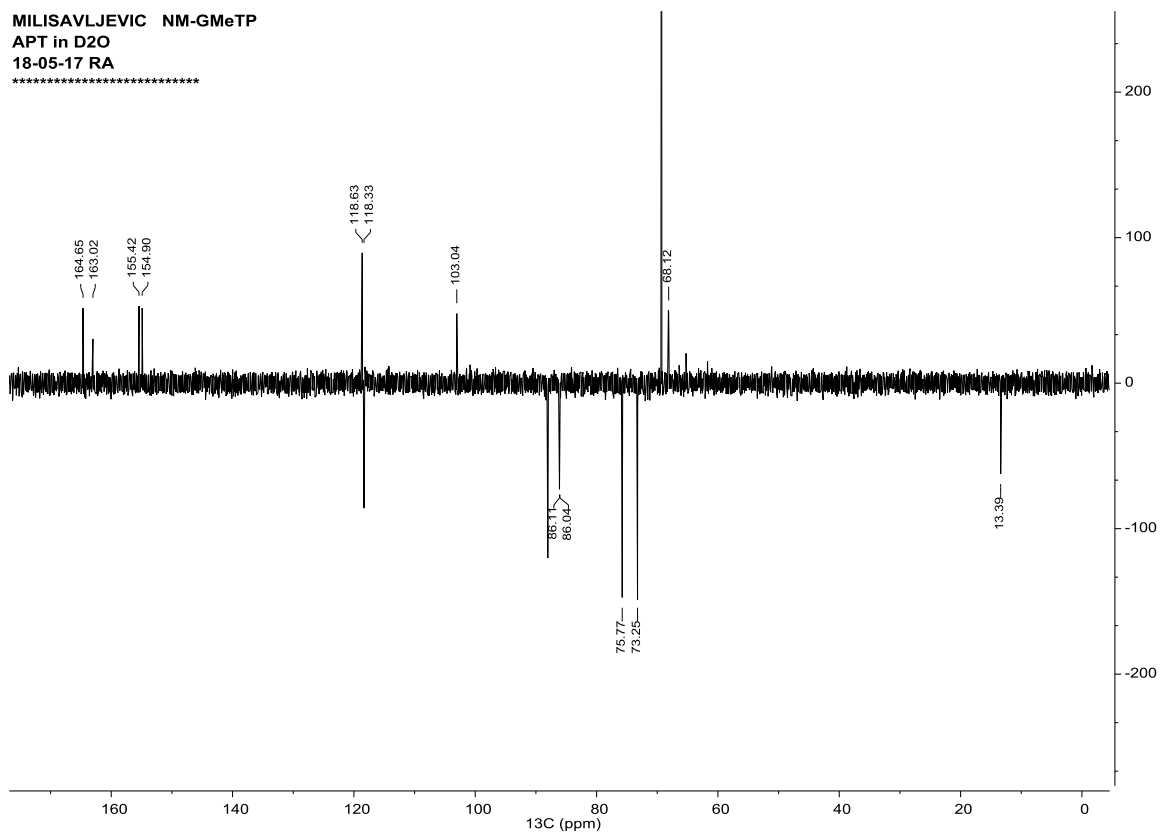
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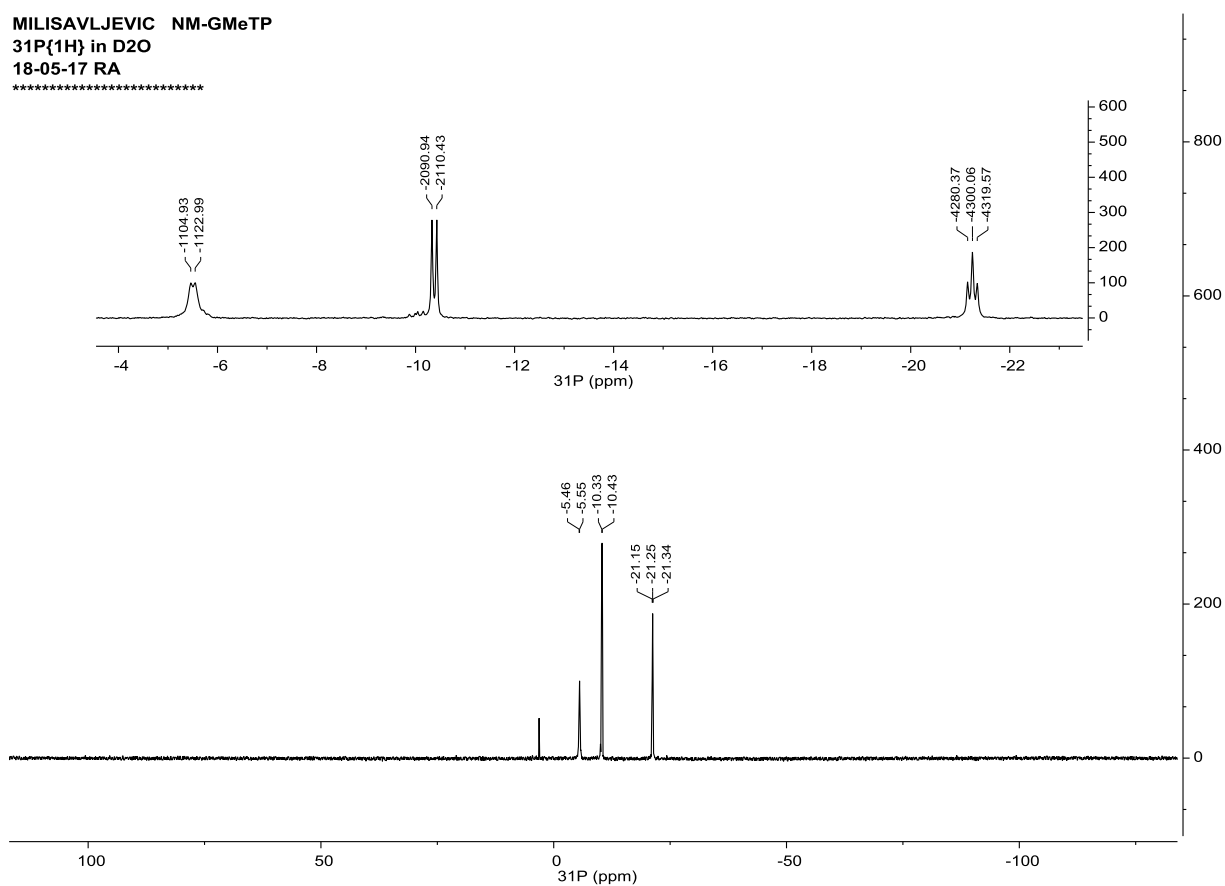
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 18-05-17 RA



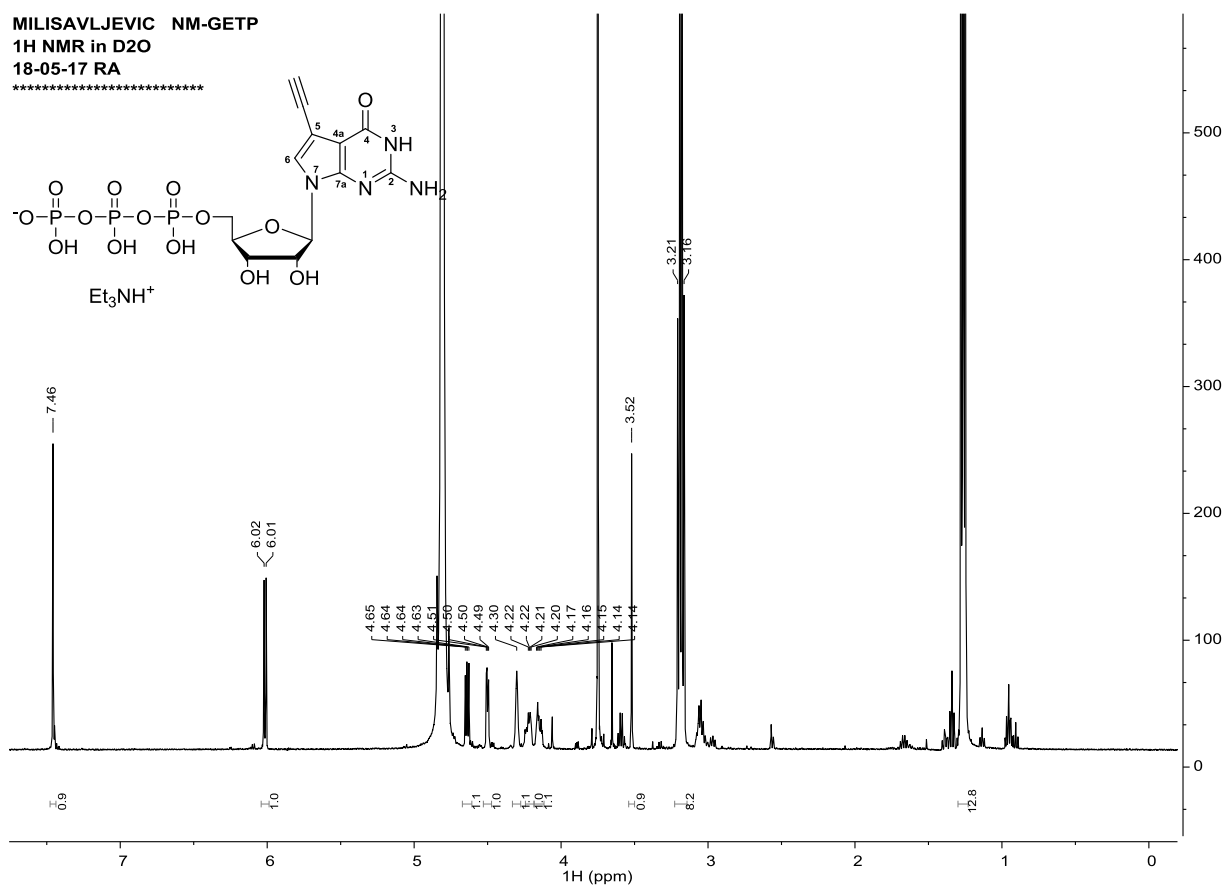
MILISAVLJEVIC NM-GMeTP
APT in D2O
18-05-17 RA



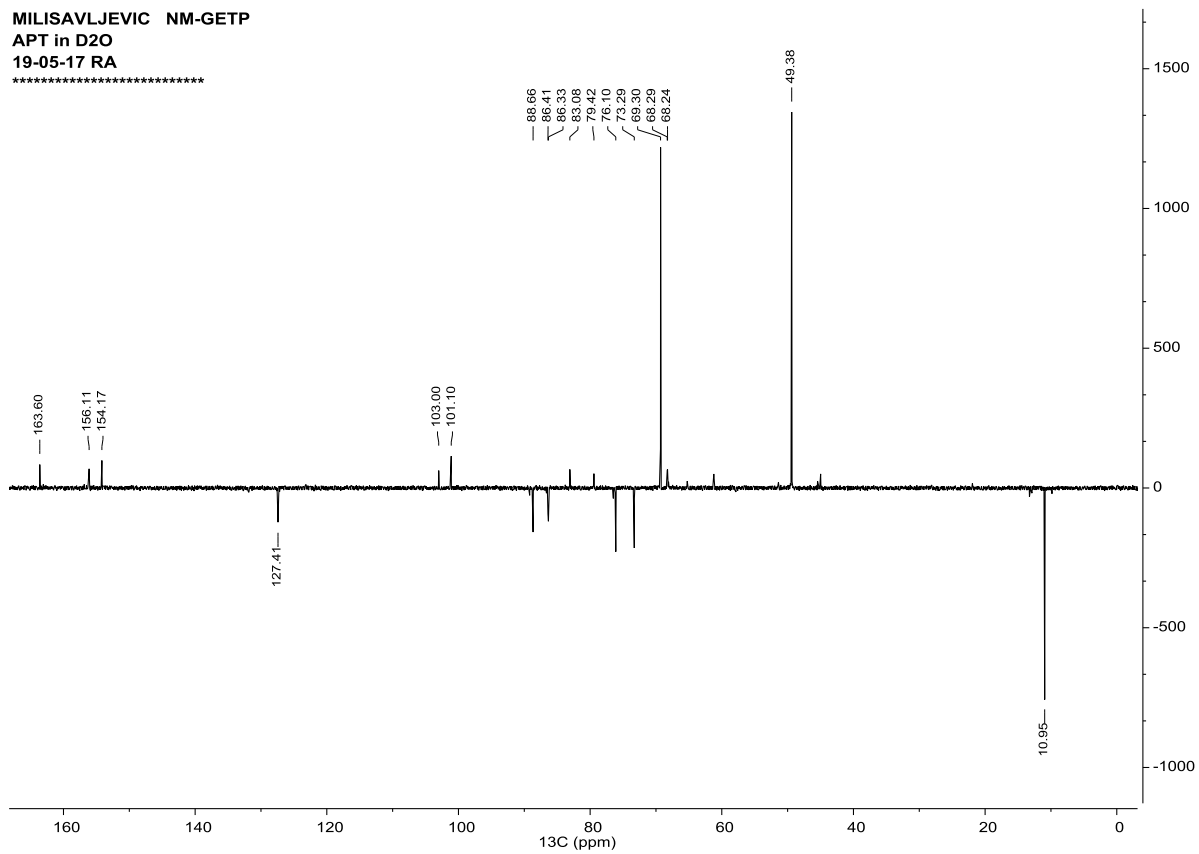
MILISAVLJEVIC NM-GMeTP
31P{1H} in D2O
18-05-17 RA



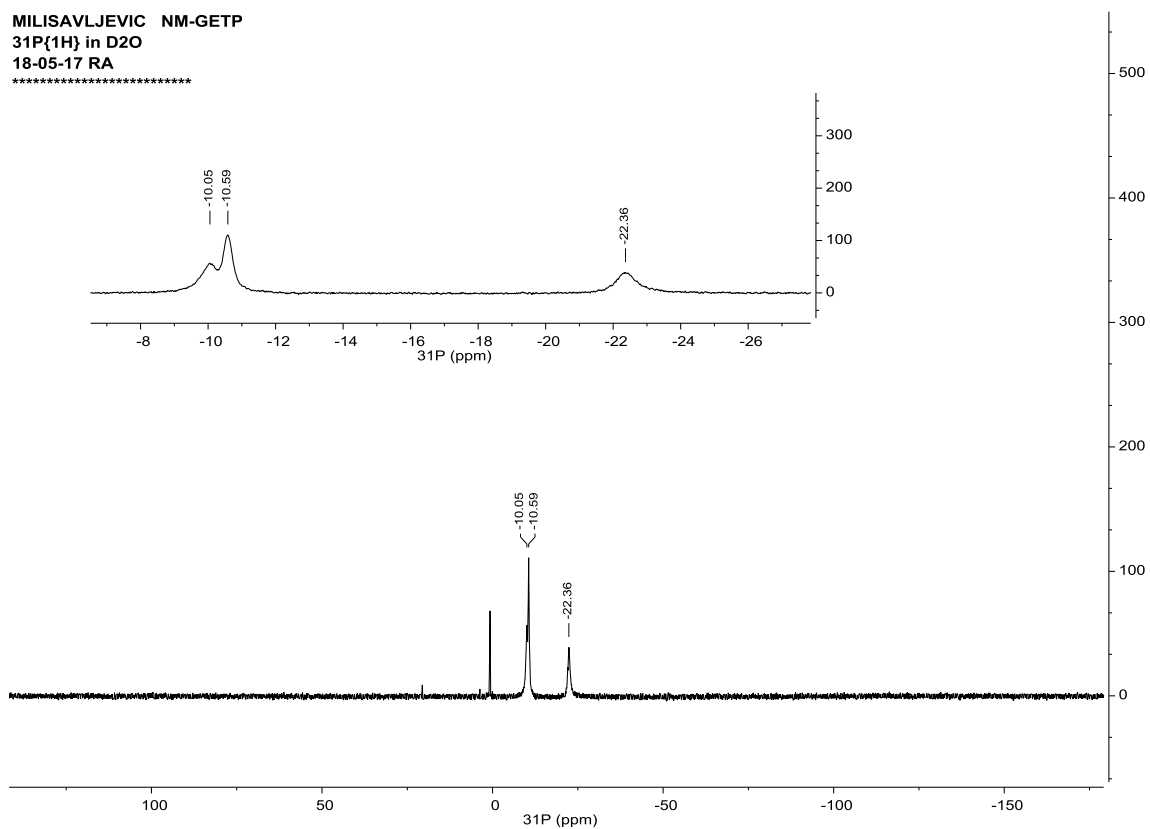
MILISAVLJEVIC NM-GETP
 1H NMR in D2O
 18-05-17 RA



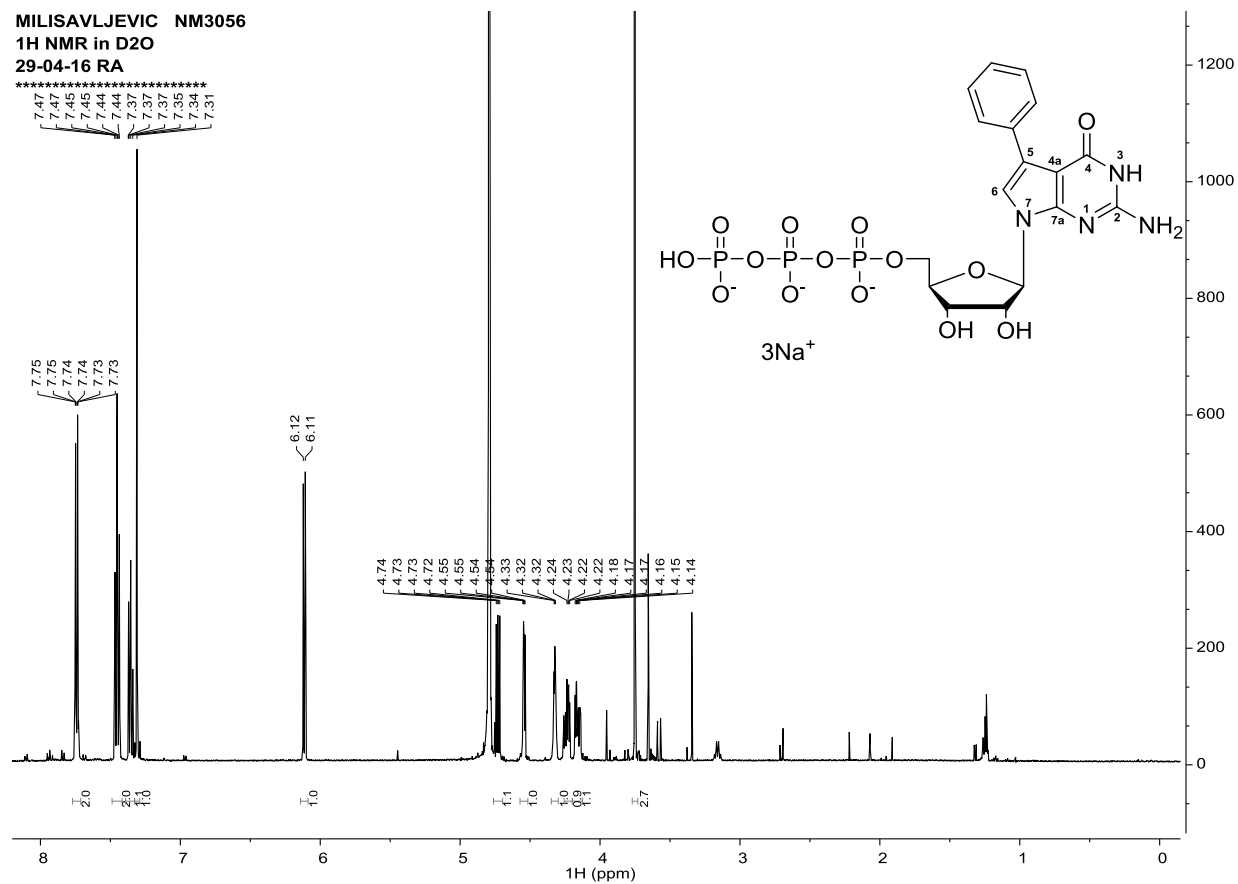
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APT in D2O
19-05-17 RA



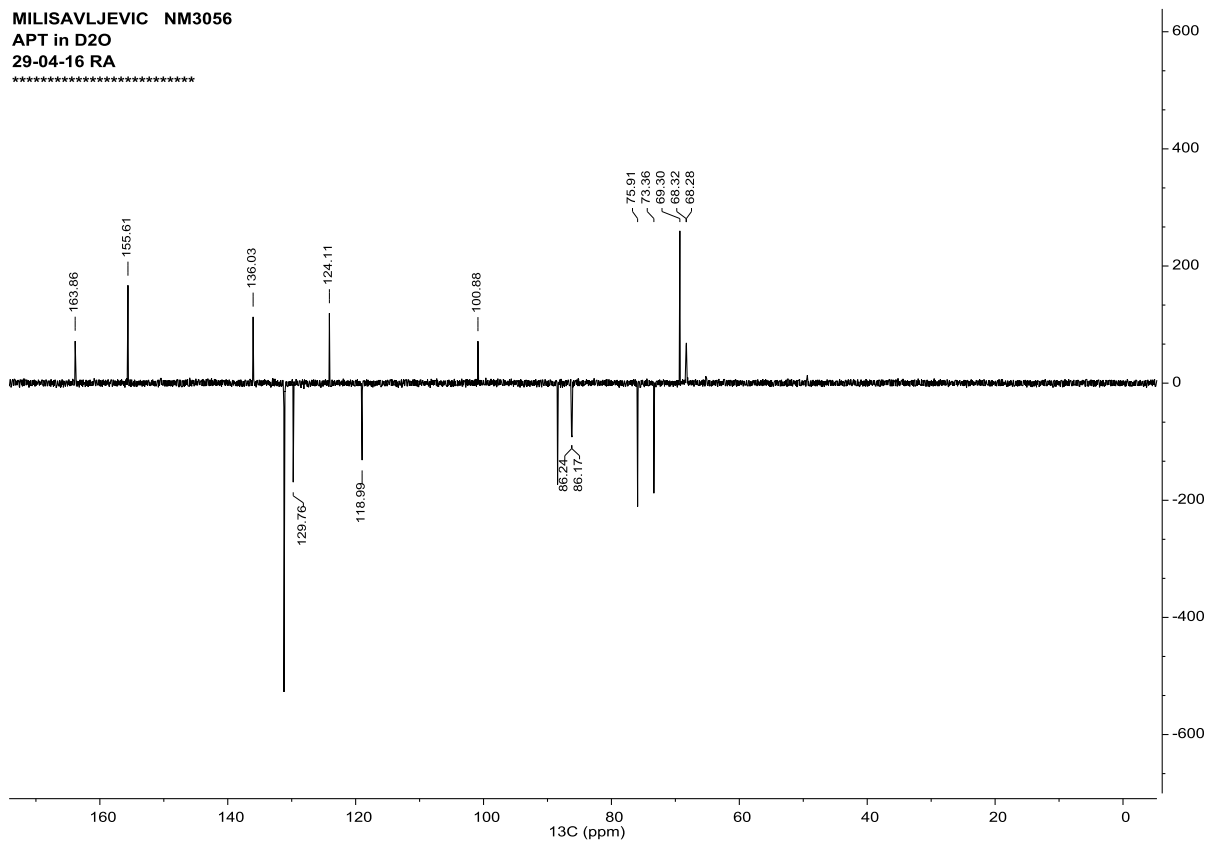
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31P{1H} in D2O
18-05-17 RA



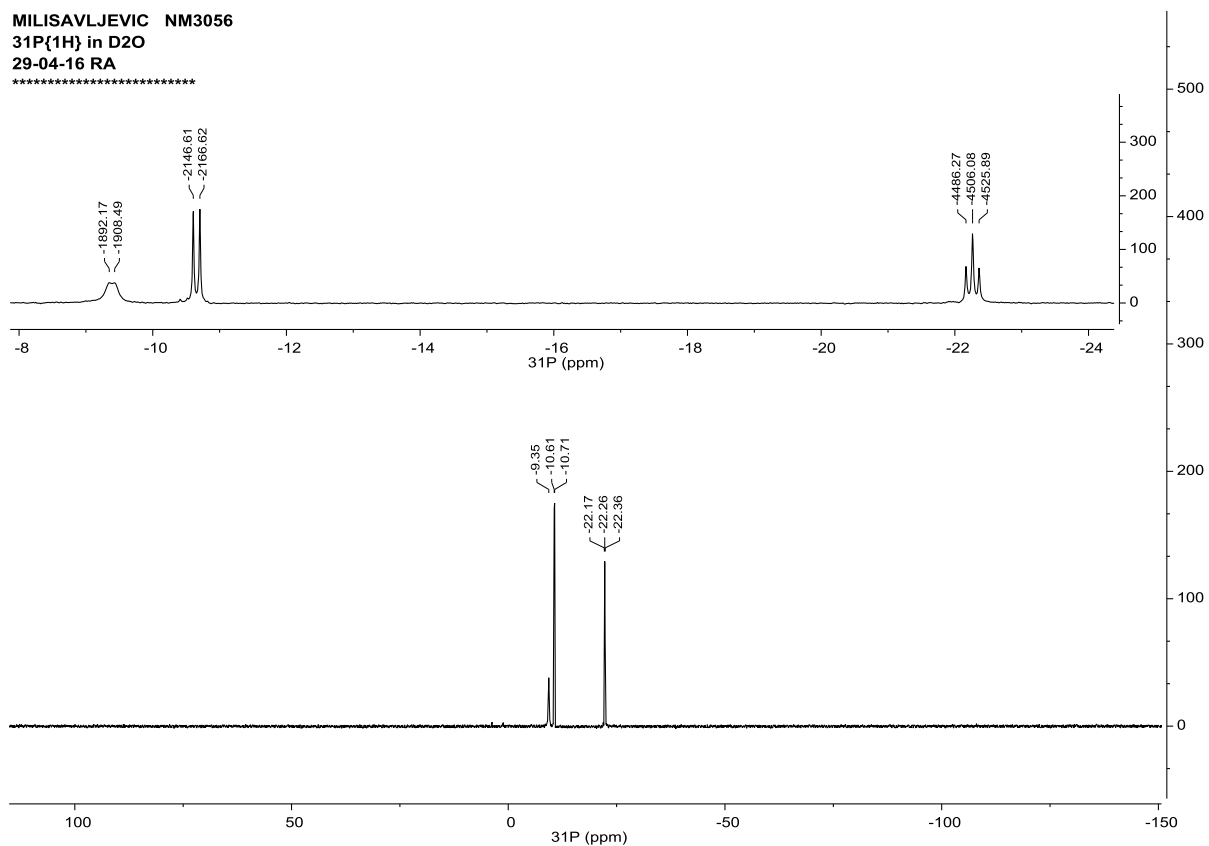
29-04-16 RA



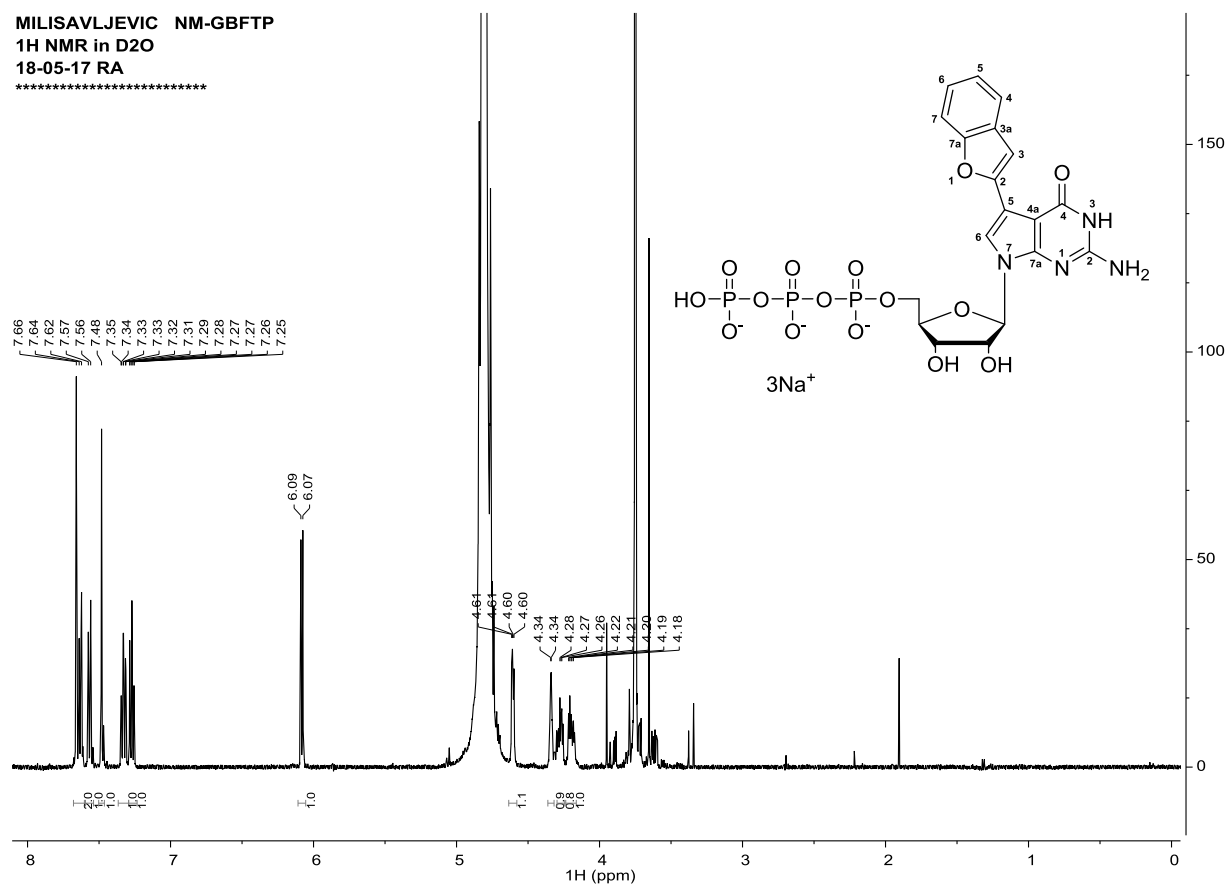
MILISAVLJEVIC NM3056
APT in D2O
29-04-16 RA



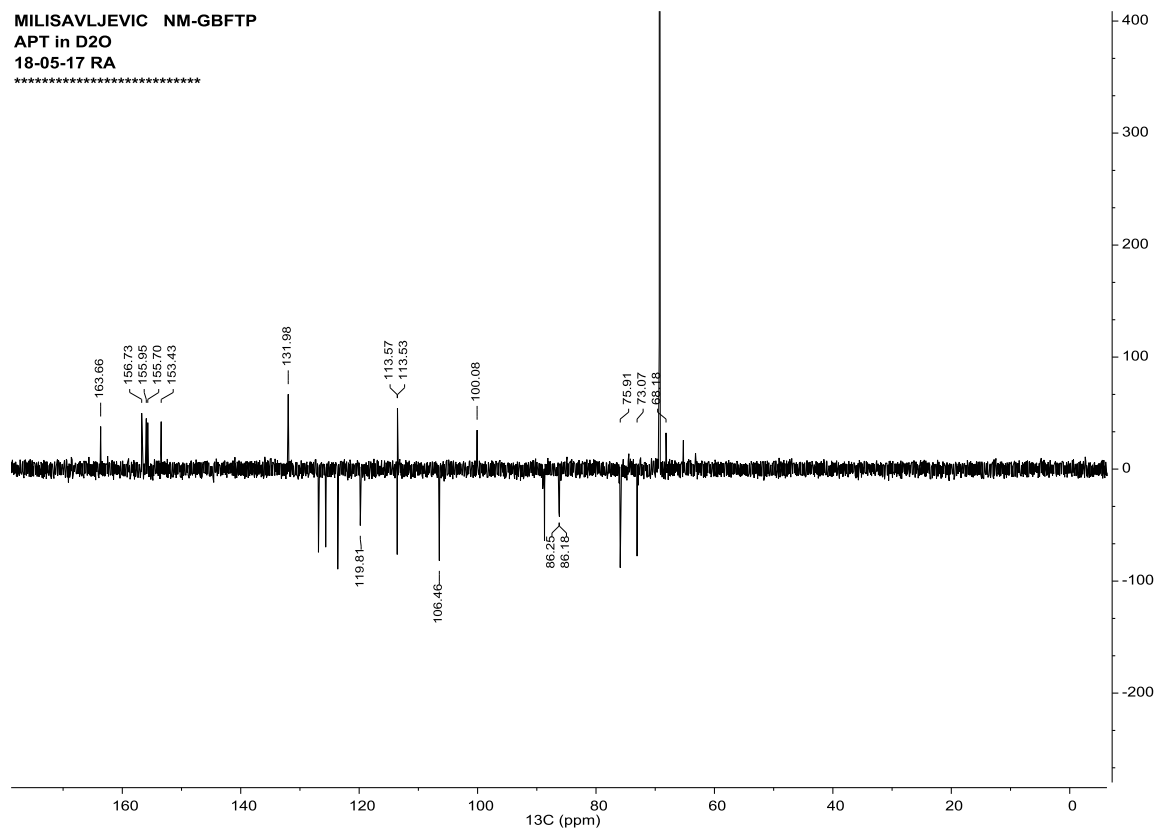
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31P{1H} in D2O
29-04-16 RA



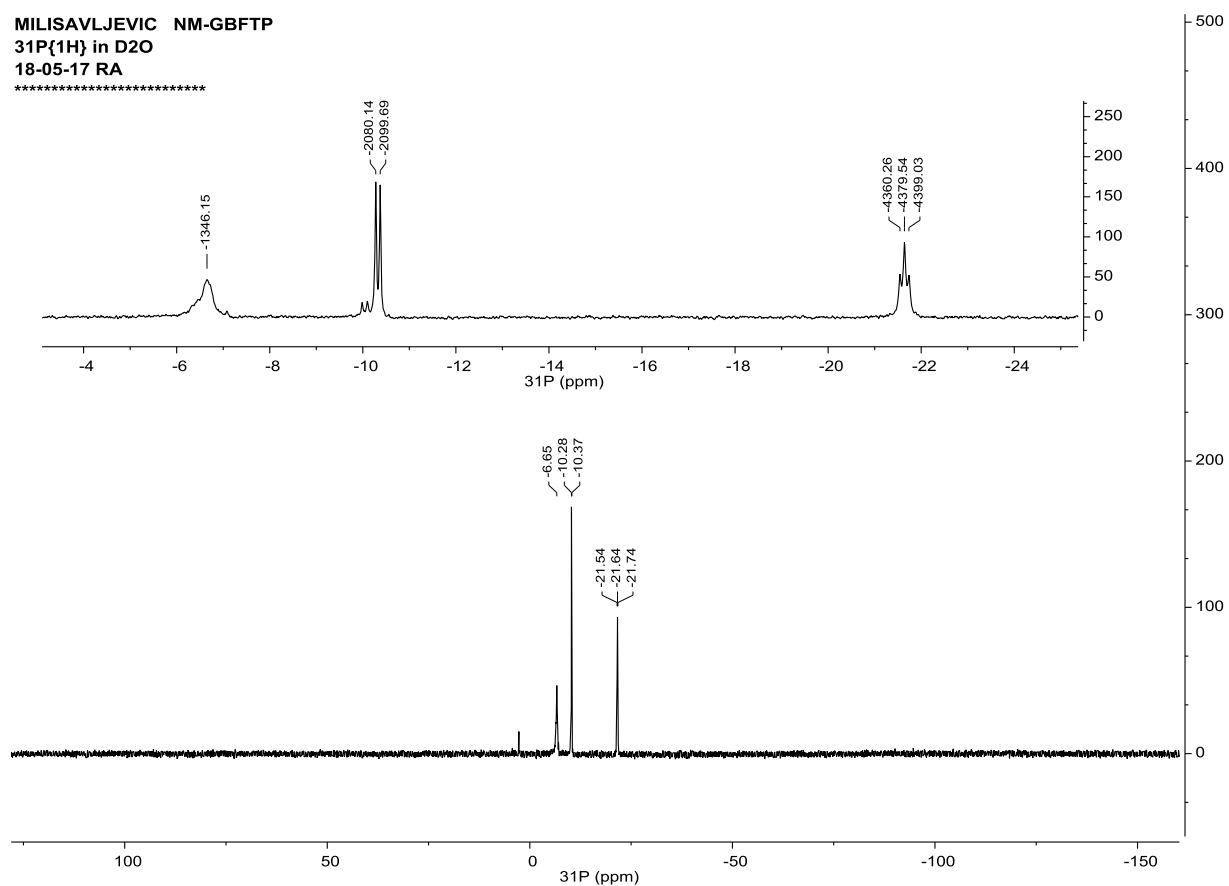
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 1H NMR in D2O
 18-05-17 RA

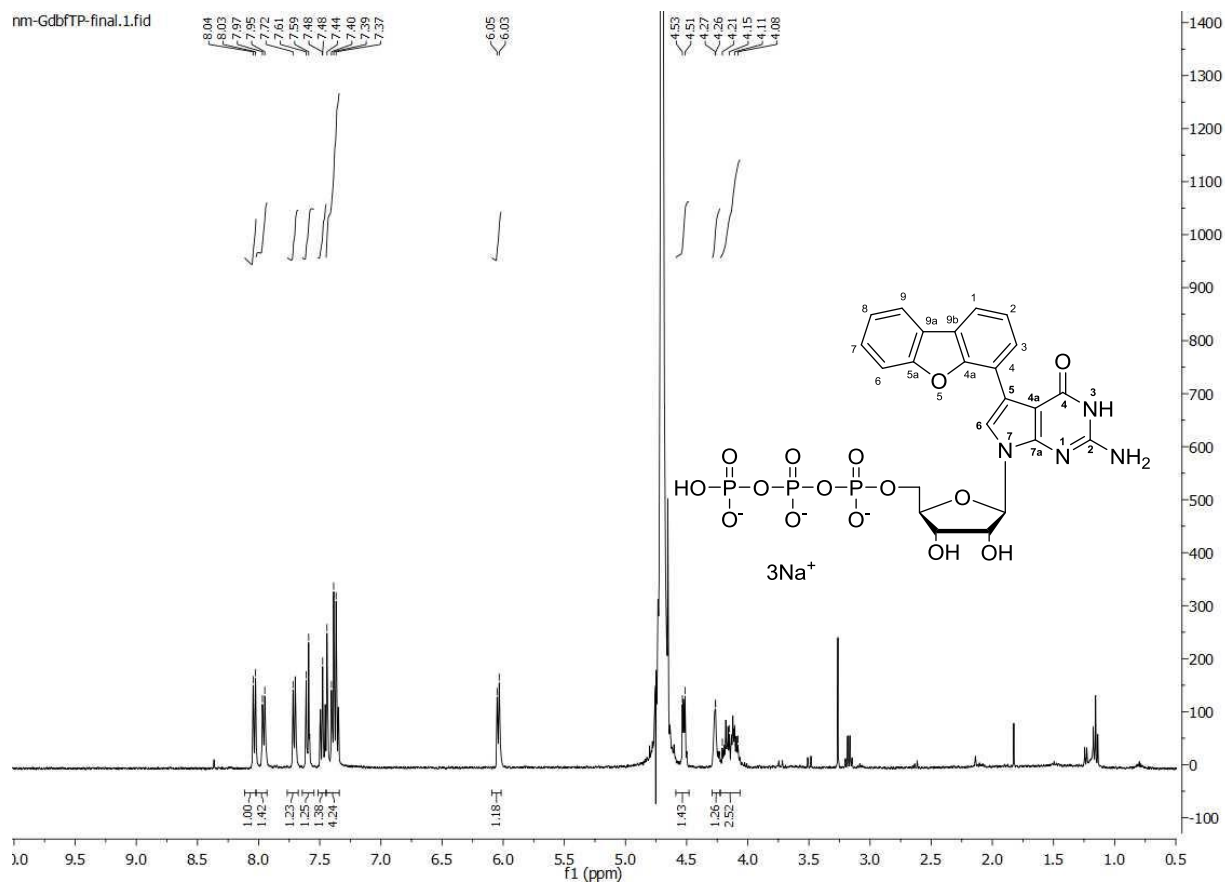


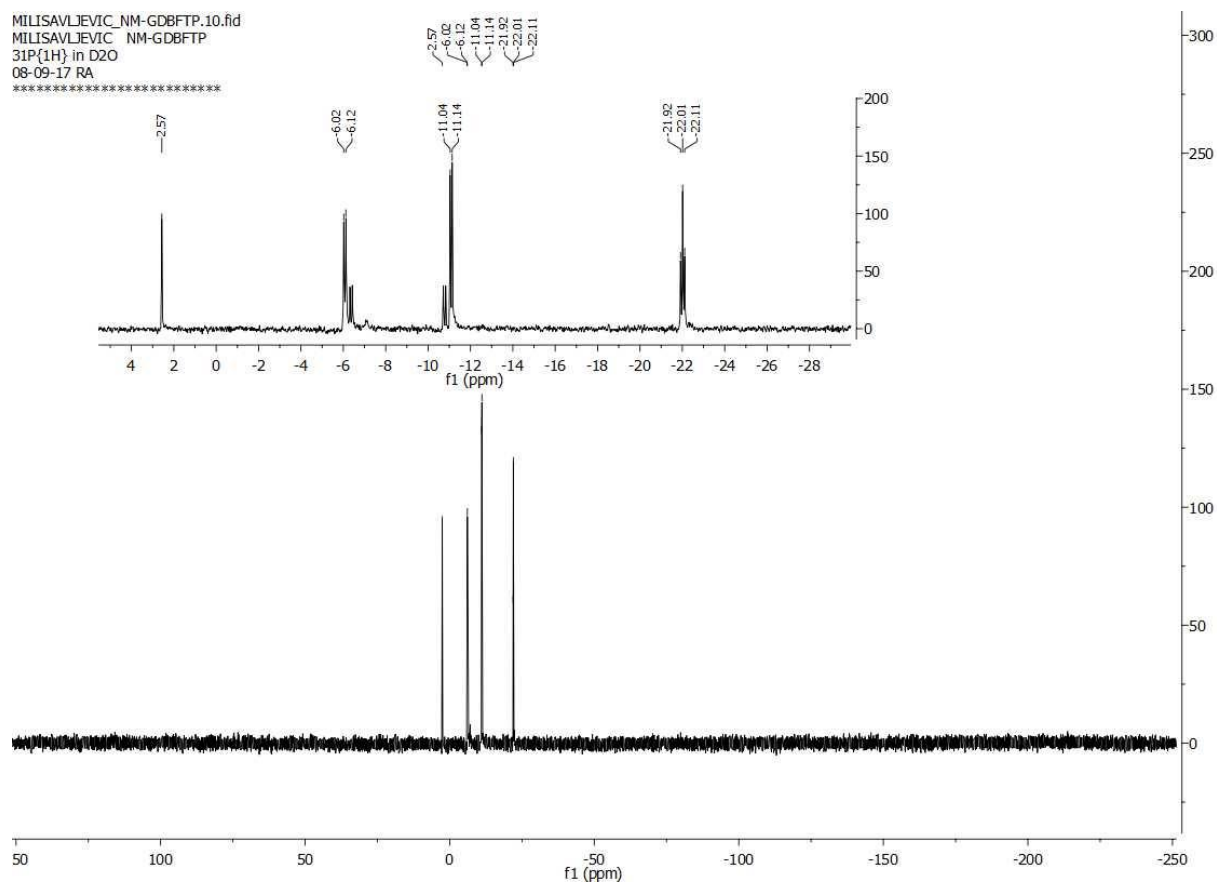
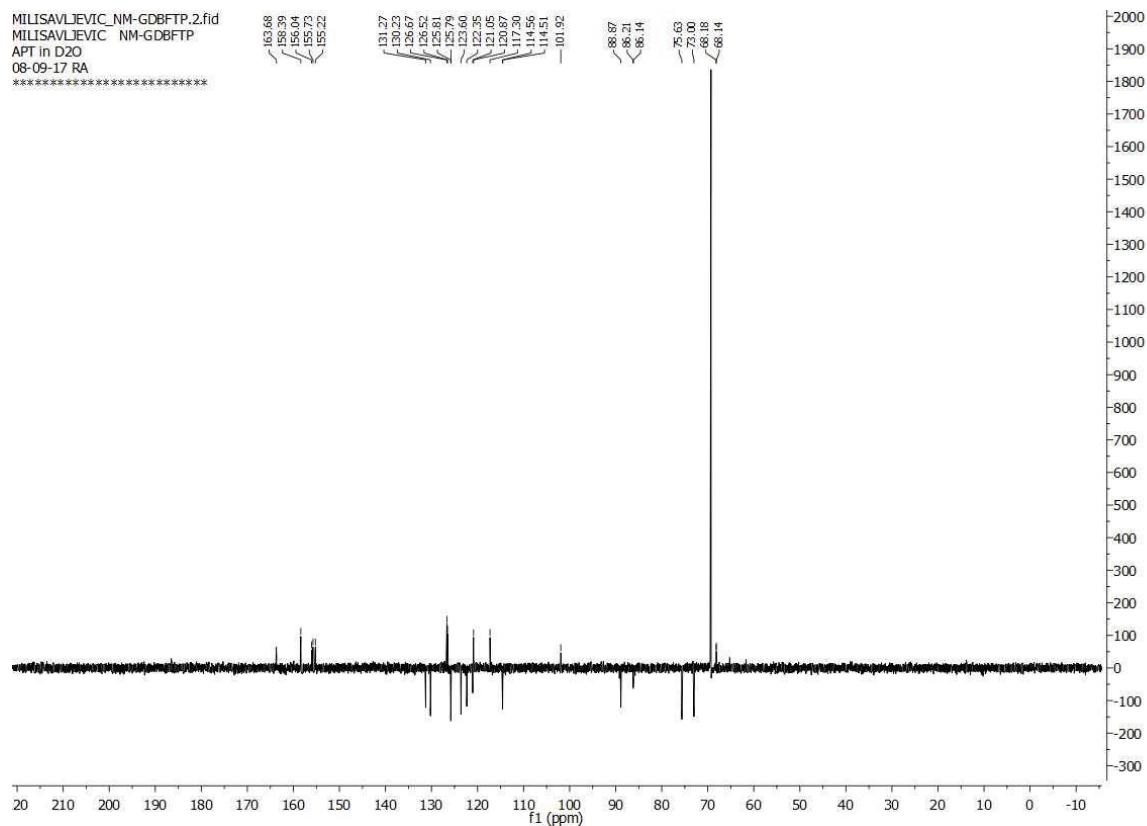
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APT in D2O
18-05-17 RA

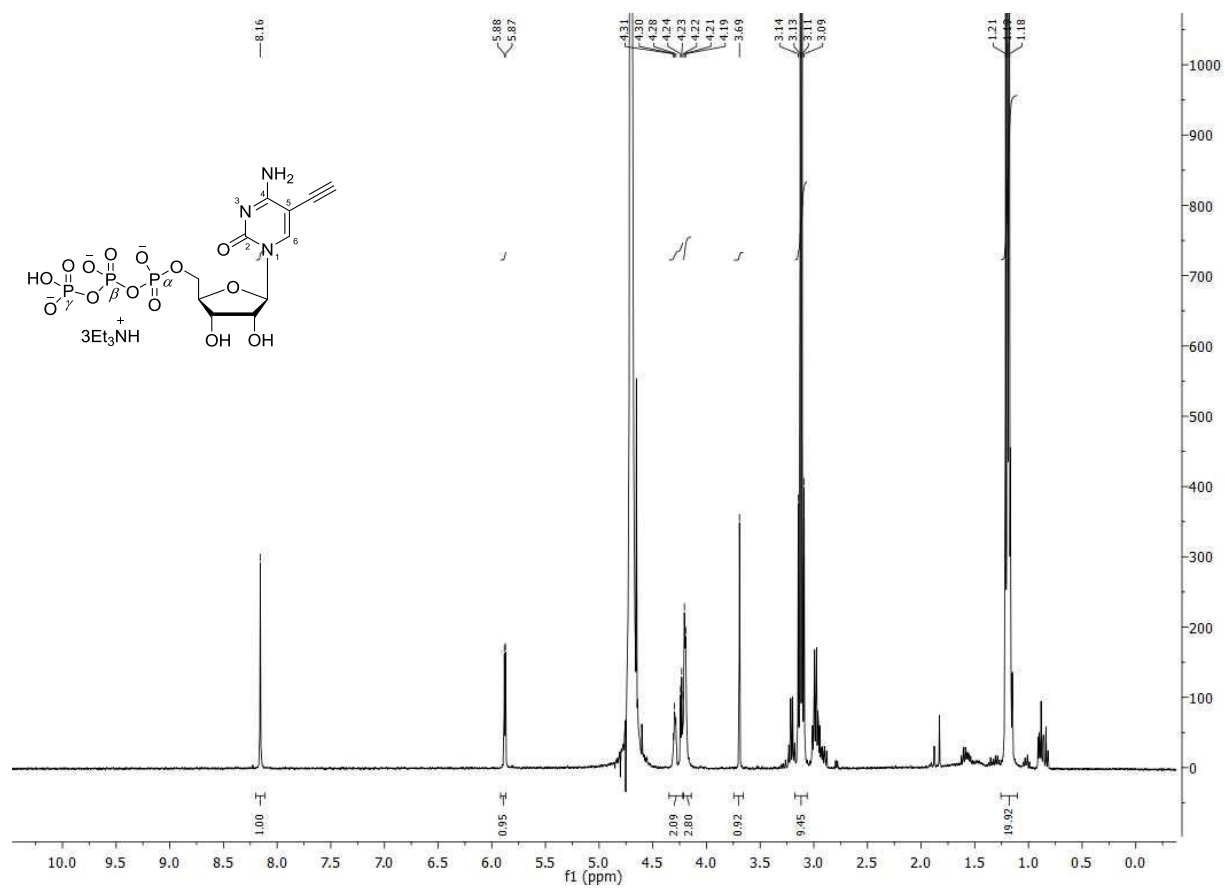


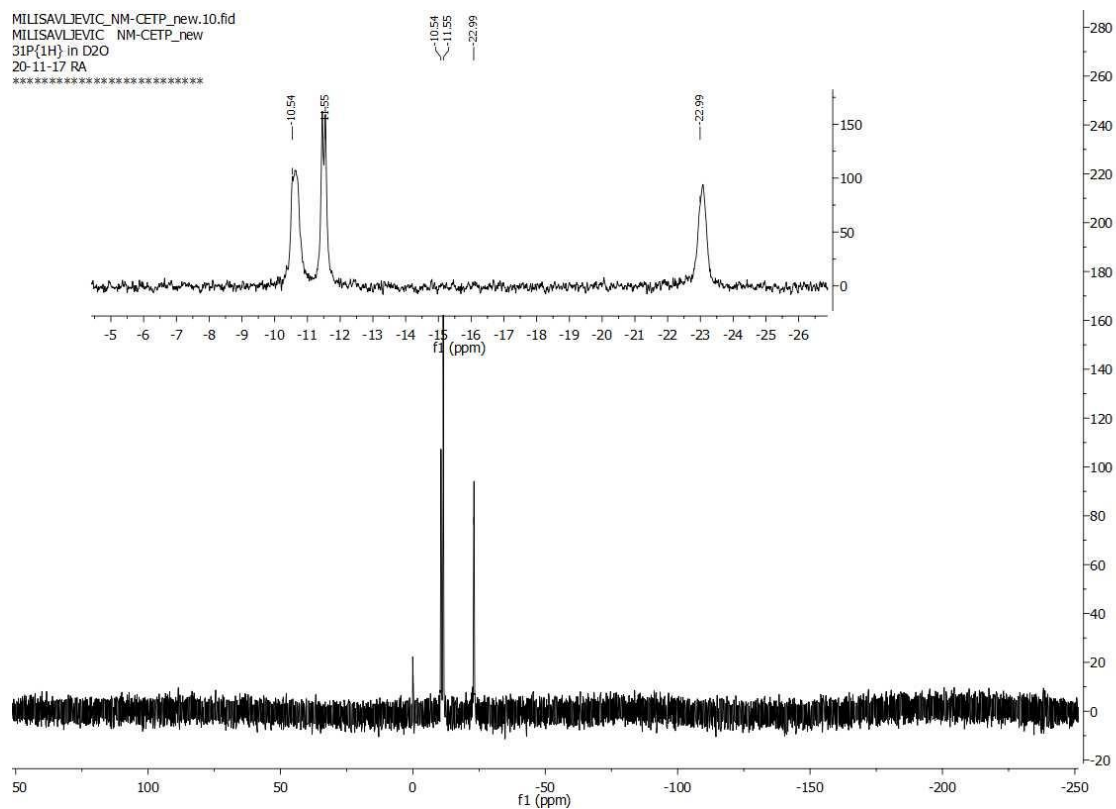
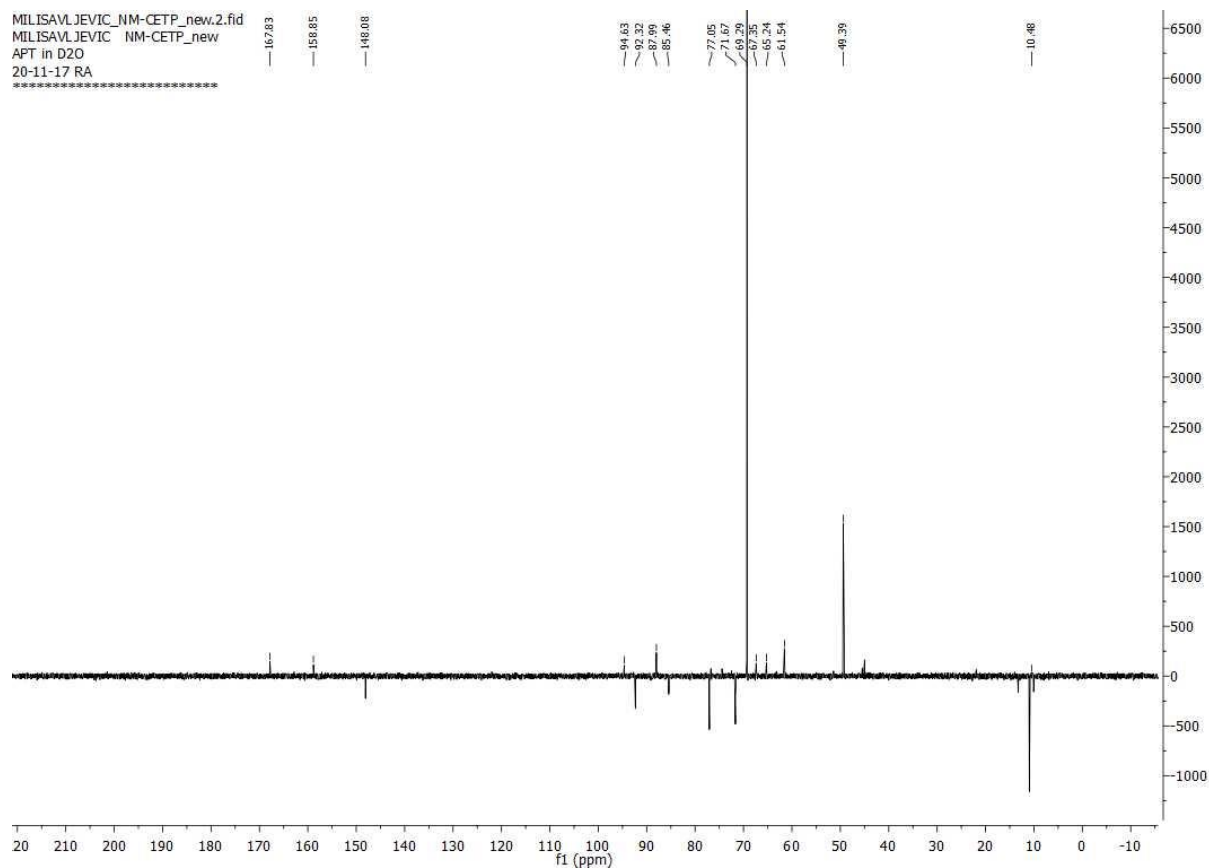
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31P{1H} in D2O
18-05-17 RA

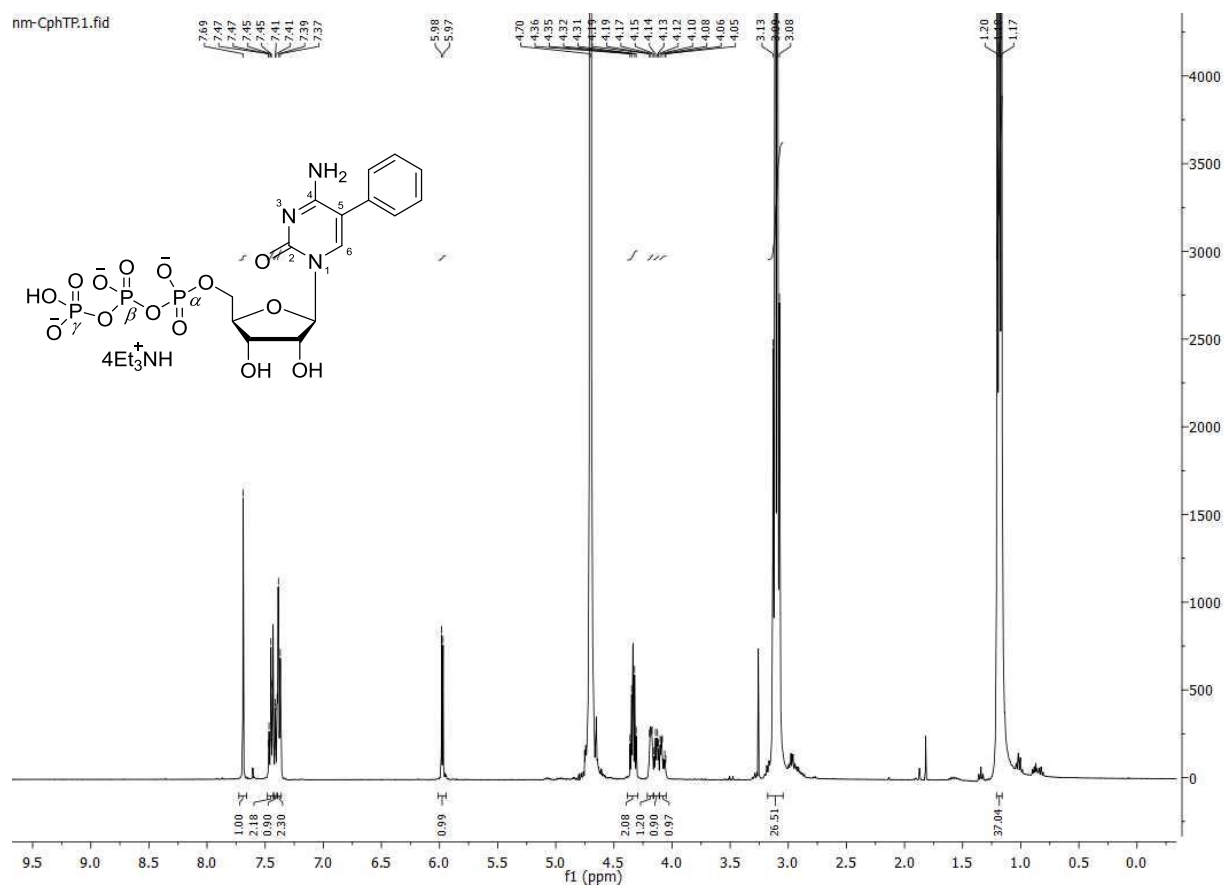


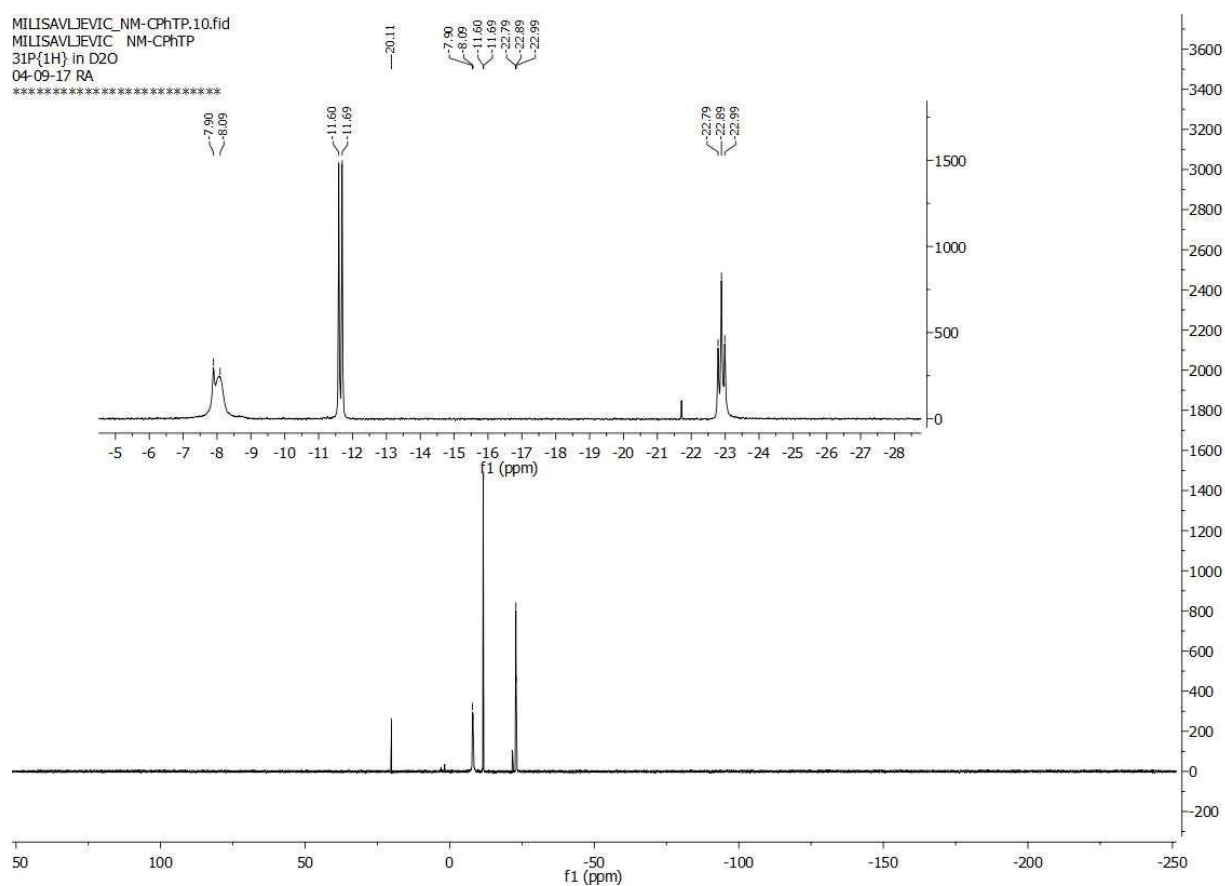
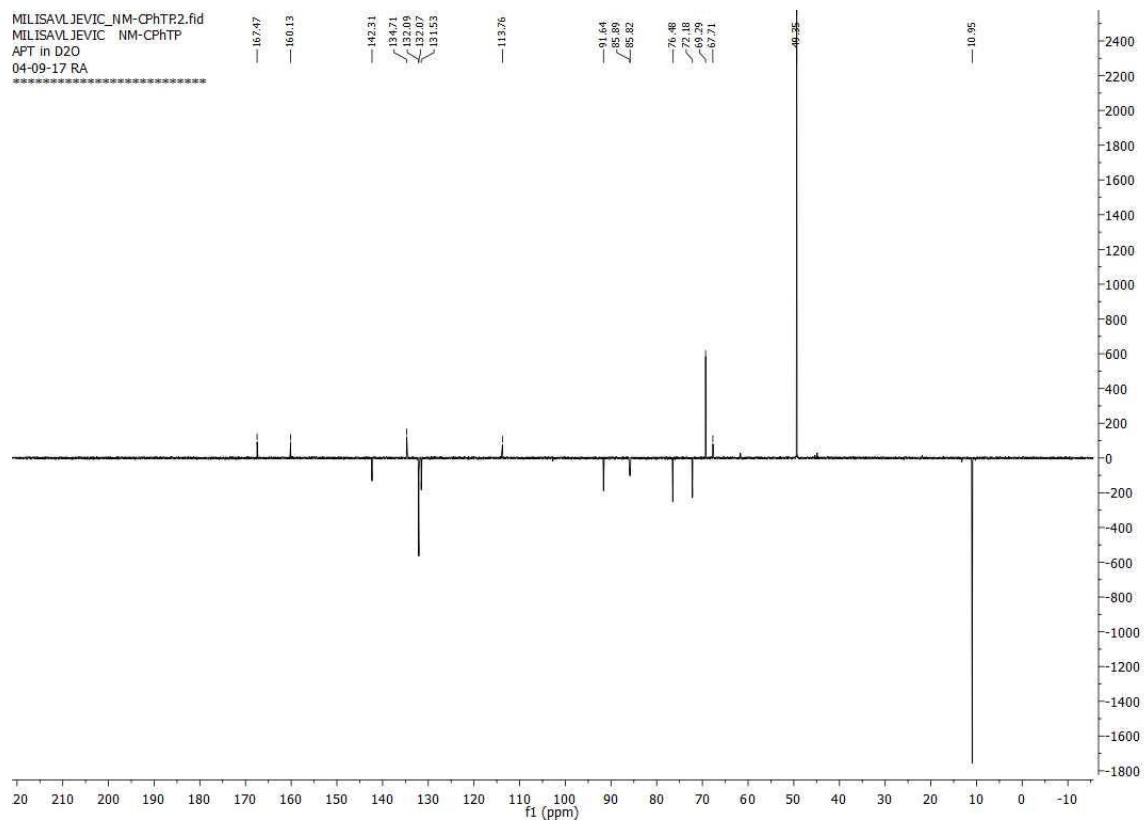




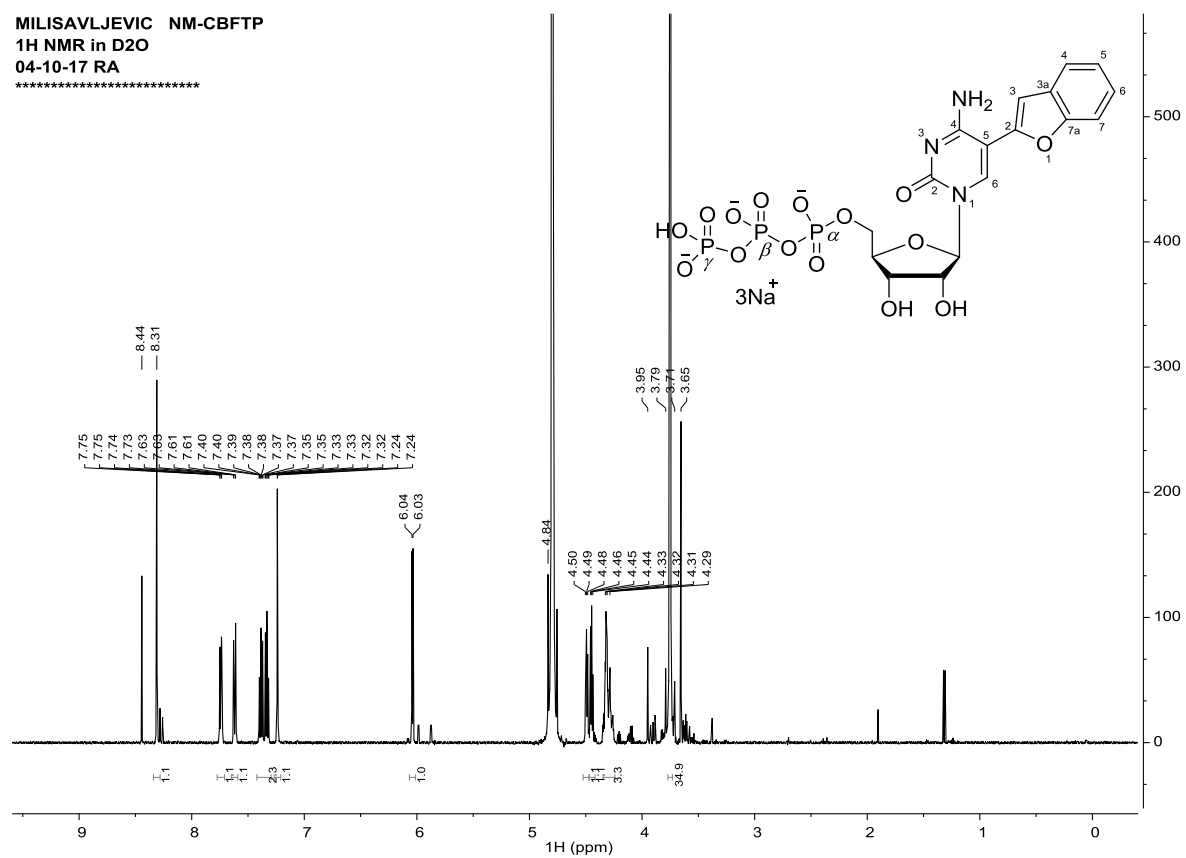




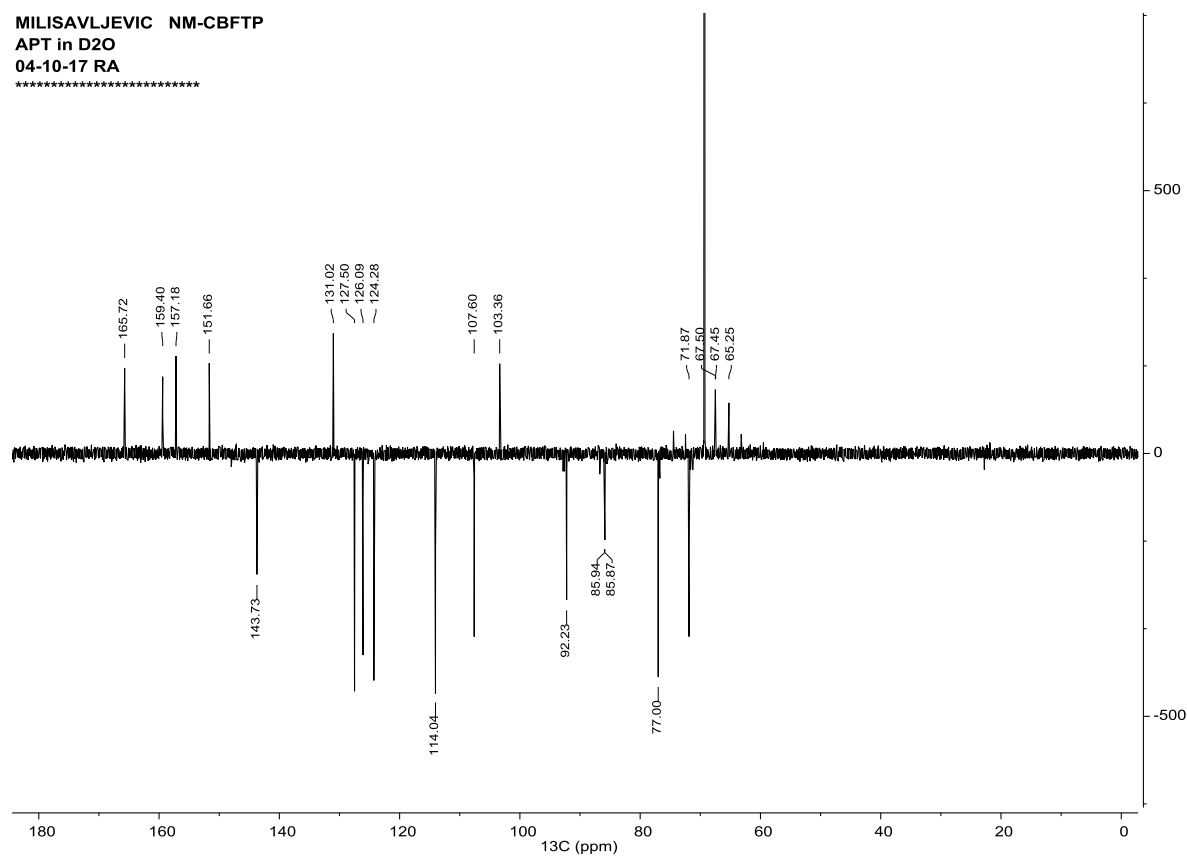


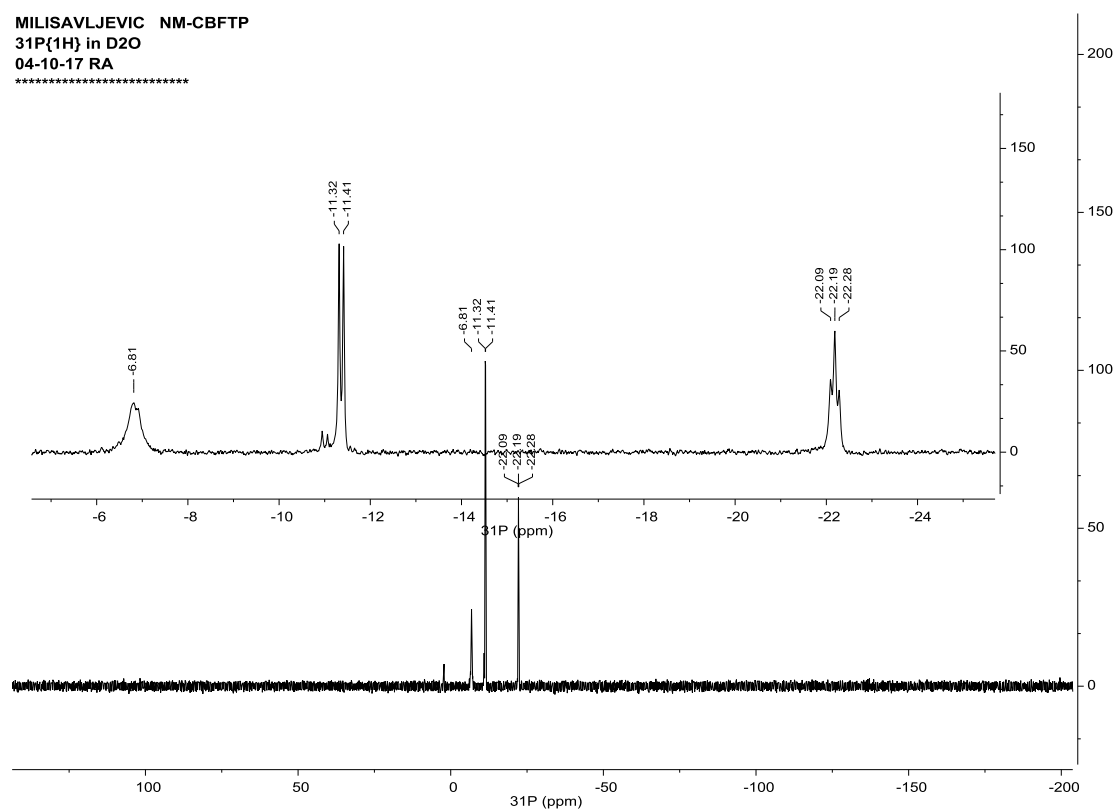


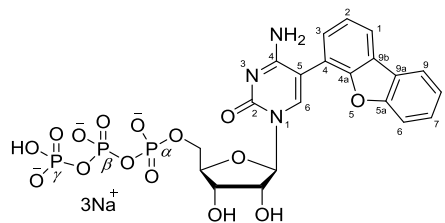
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 1H NMR in D2O
 04-10-17 RA



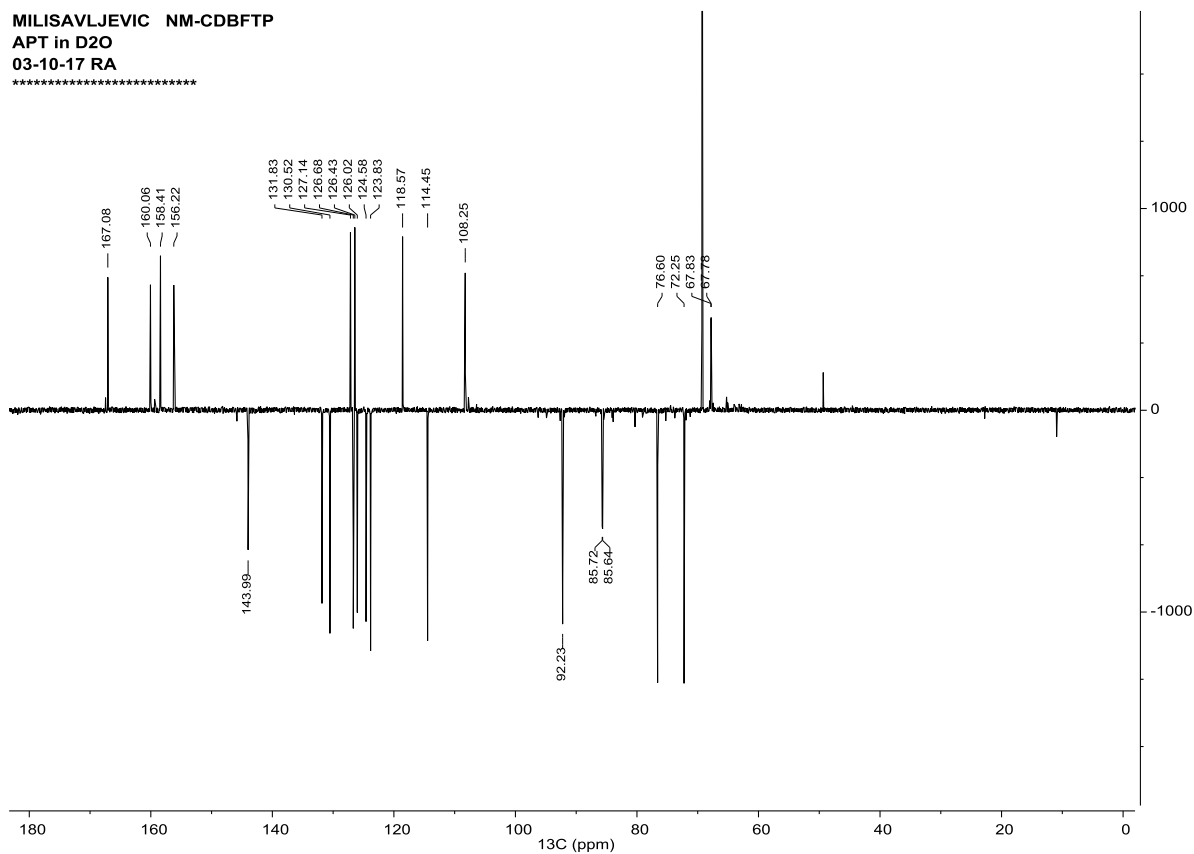
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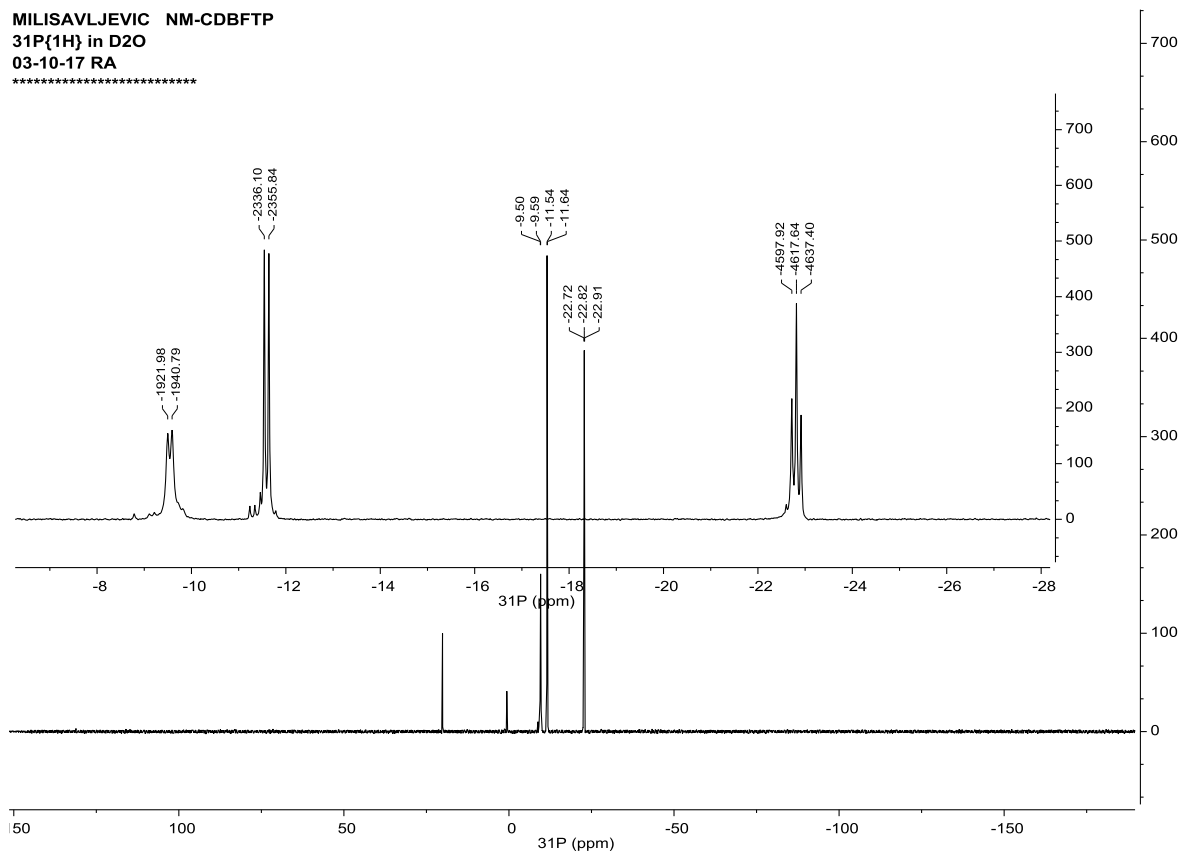




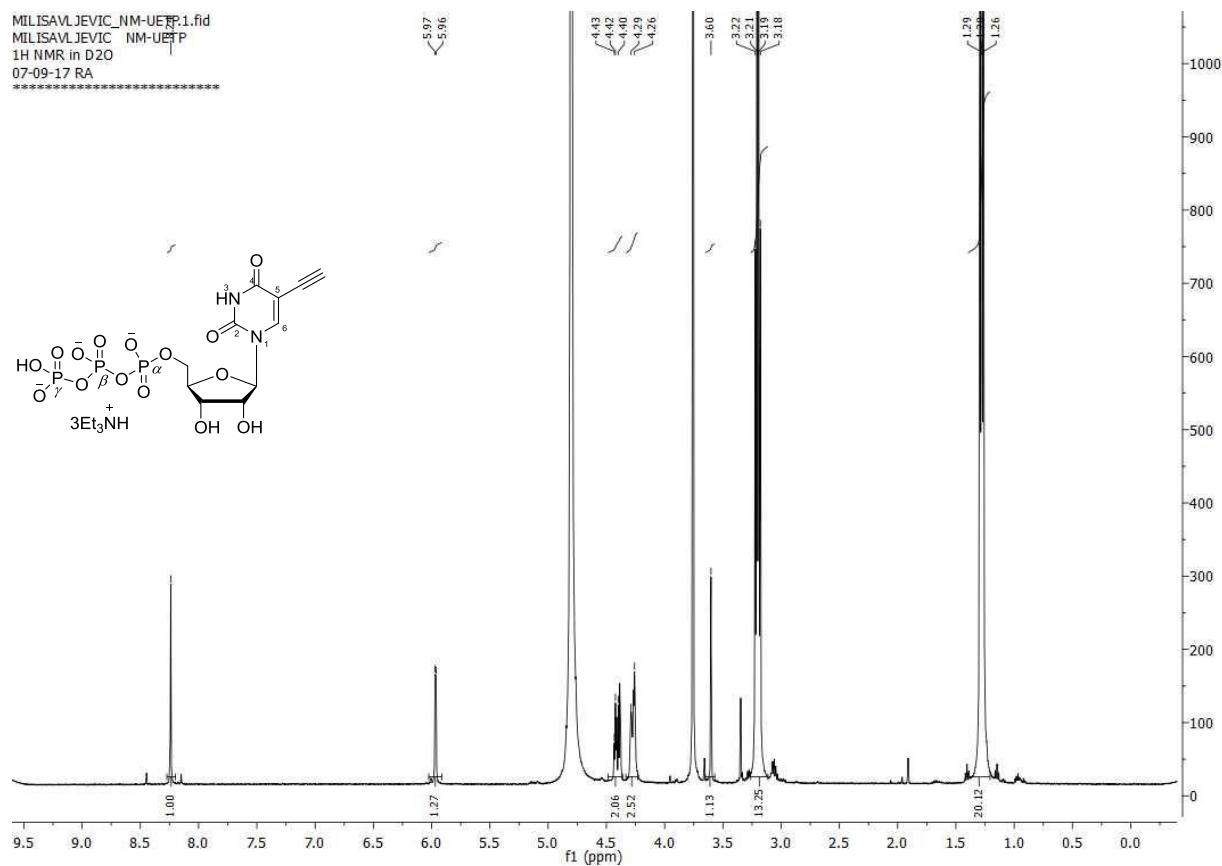
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APT in D2O
03-10-17 RA



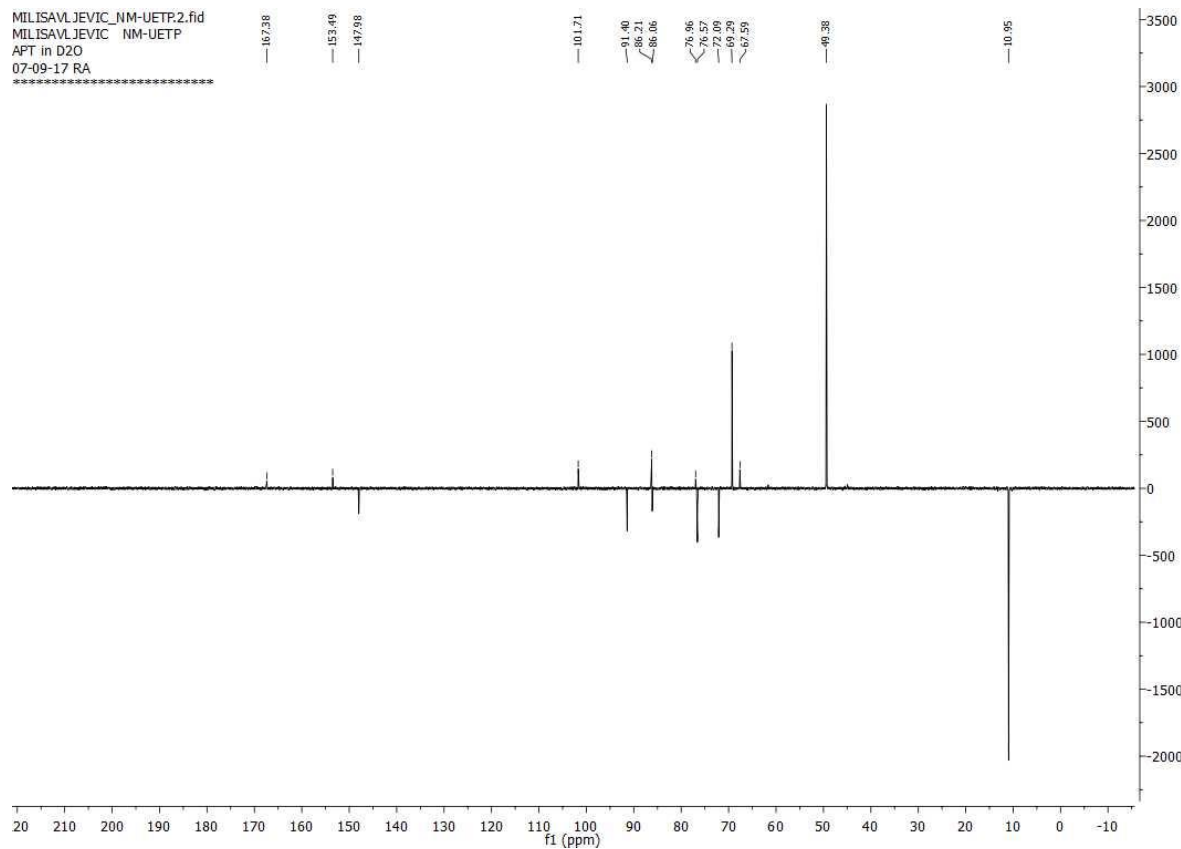
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31P{1H} in D2O
03-10-17 RA



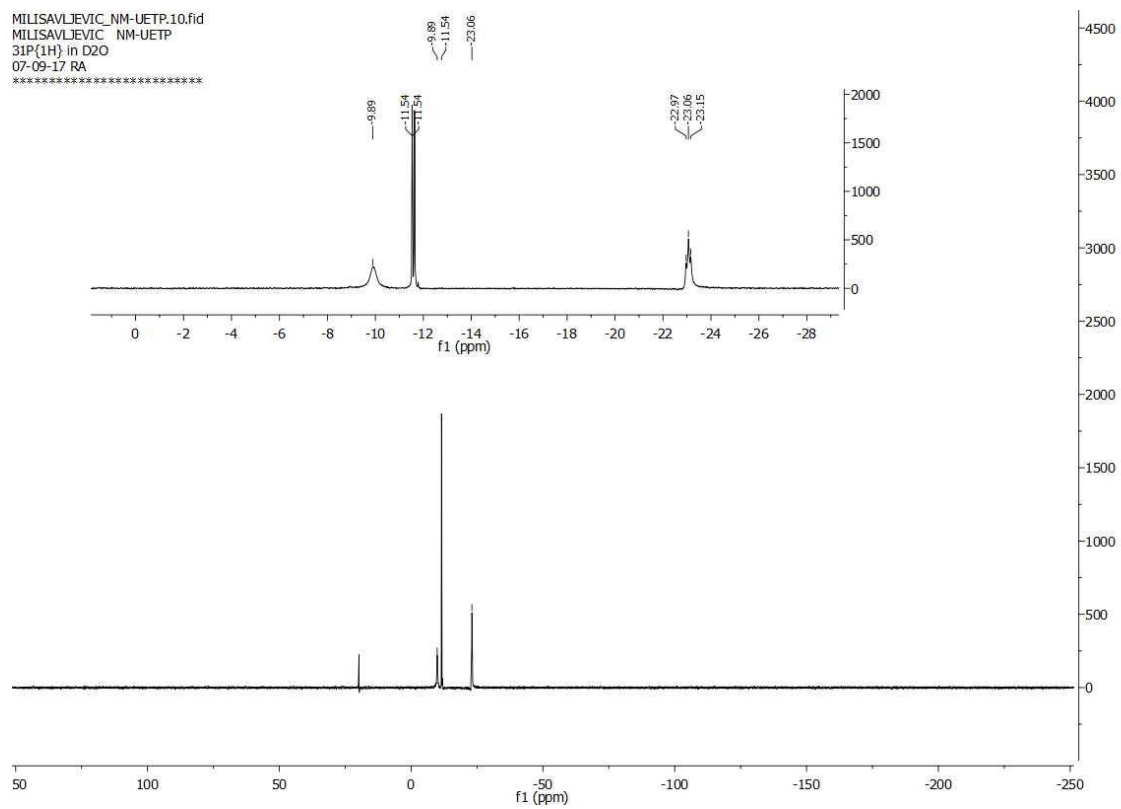
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1H NMR in D2O
07-09-17 RA



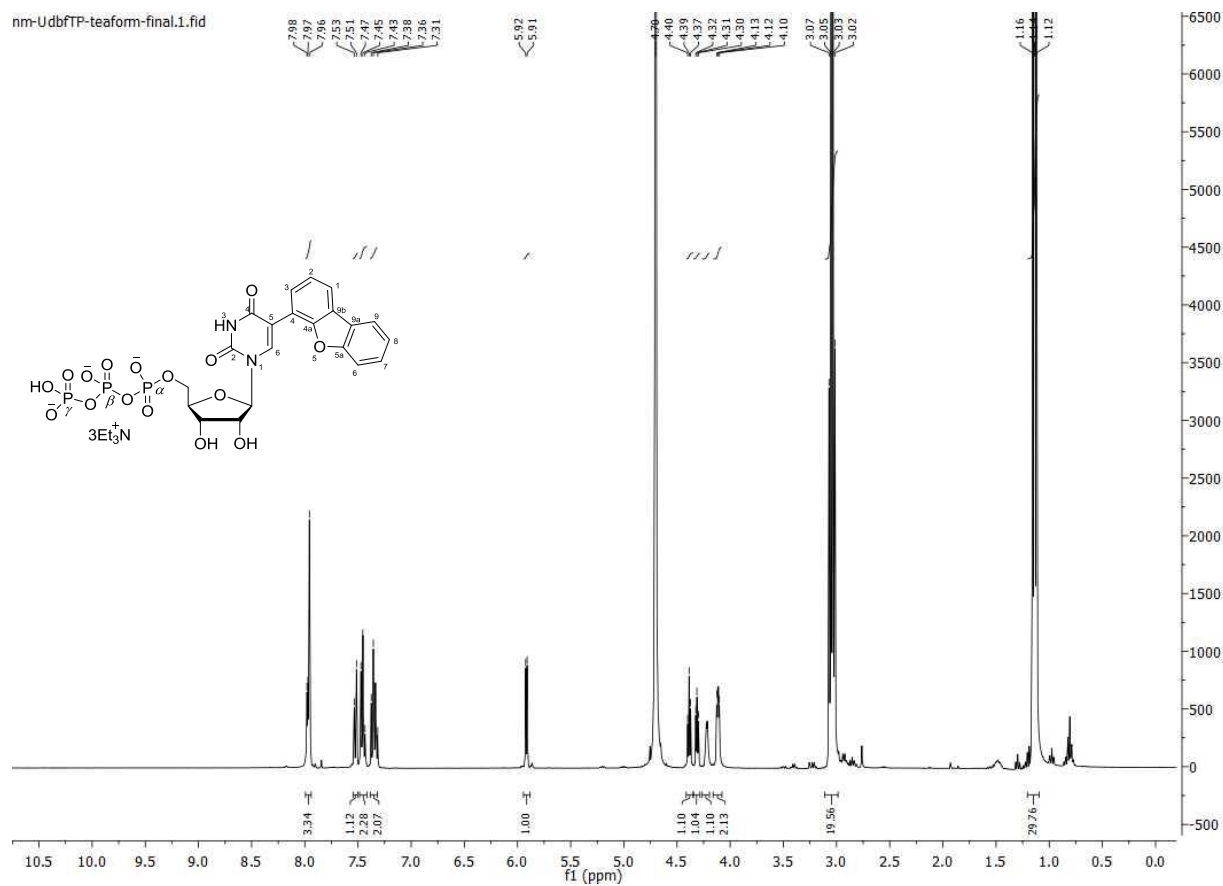
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APT in D2O
07-09-17 RA



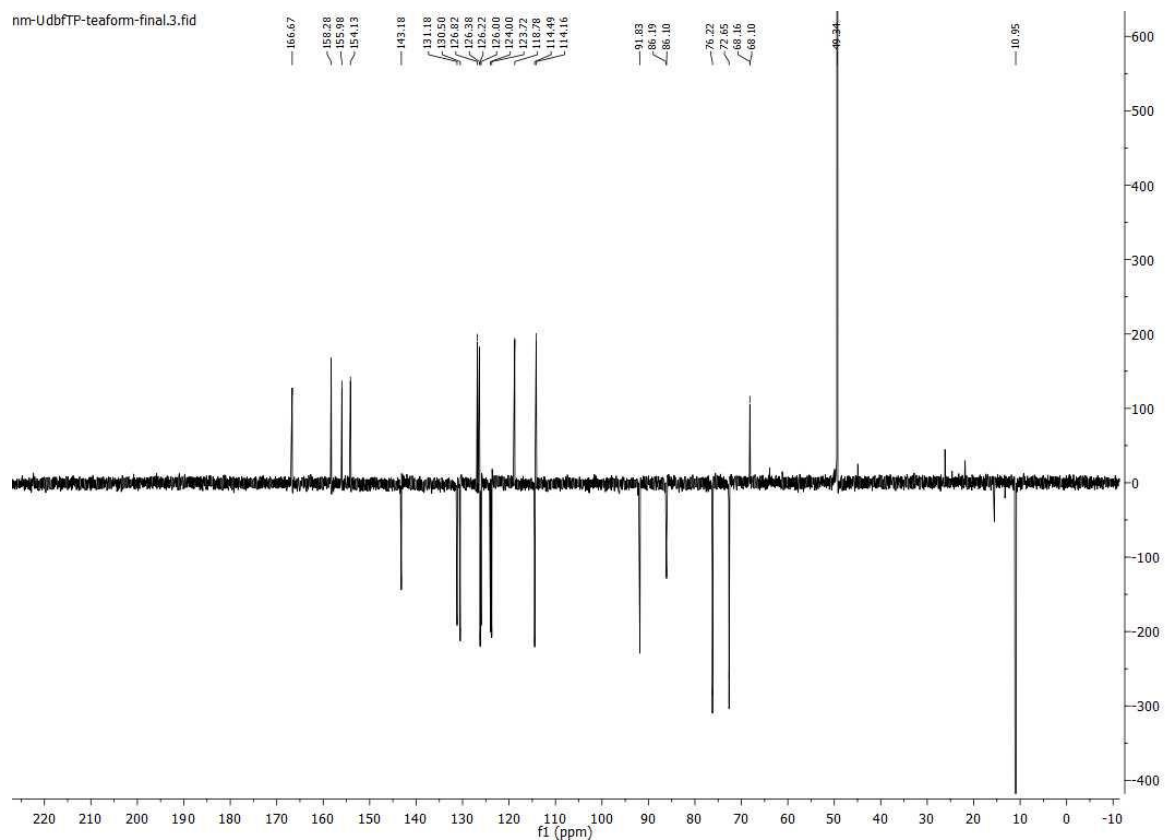
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31P{1H} in D2O
07-09-17 RA



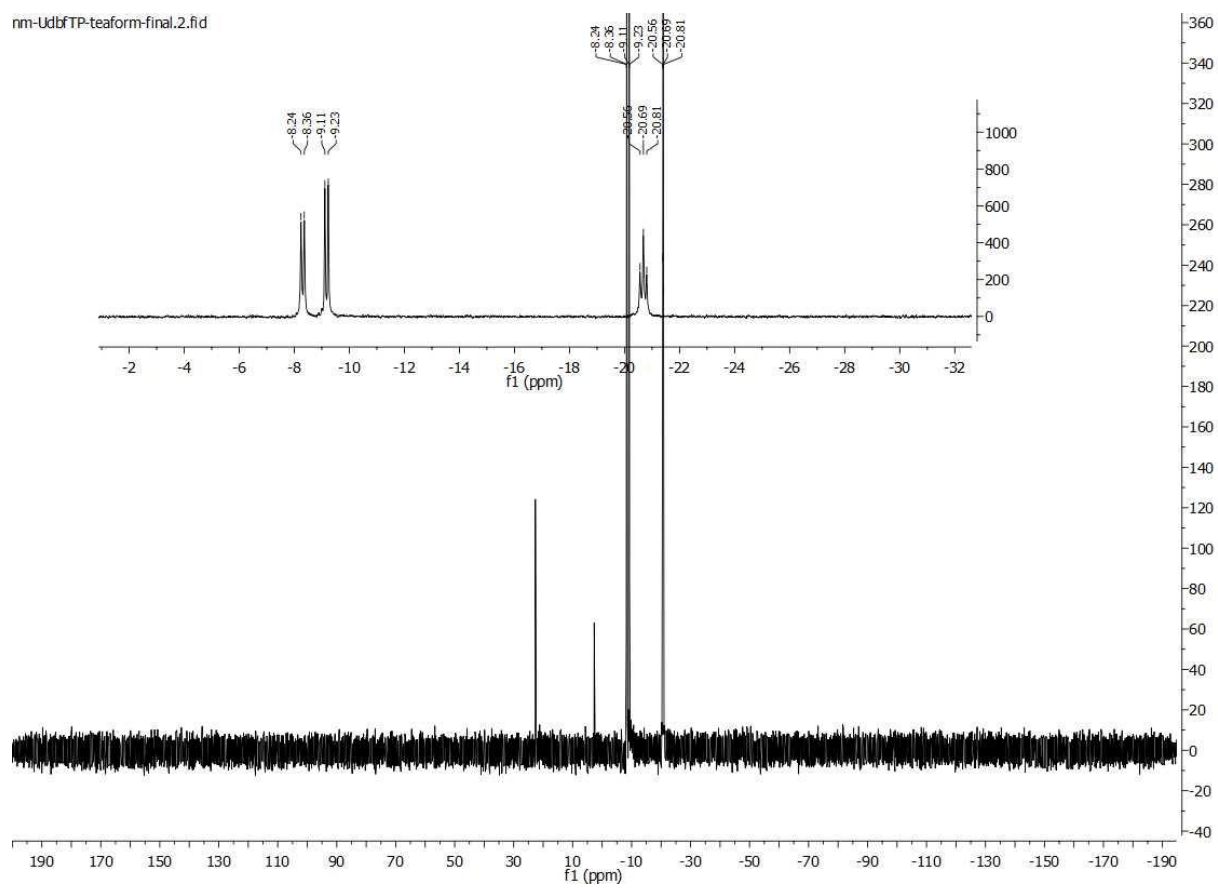
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nm-UdbfTP-teaform-final.3.fid



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