Supplementary data

1. Analytical data

Analytical data for newly described compounds are presented.

Analytical HPLC was performed on Agilent Tech. Series 1200 using Supelcosil LC-18-T RP column (4.6 x 250 mm, flow rate 1.3 mL/min) with a linear gradient 0–25% of methanol in 0.05 M ammonium acetate buffer (pH 5.9) in 15 min, UV-detection at 260 nm and fluorescence detection (excitation at 280 nm and detection at 337 nm). ¹H NMR and ³¹P NMR spectra were recorded at 25°C on a Varian UNITY-plus spectrometer at 399.94 MHz and 161.90 MHz respectively. ¹H NMR chemical shifts were reported to sodium 3-trimethylsilyl-[2,2,3,3-D4]-propionate (TSP) in D₂O as an internal standard. ³¹P NMR chemical shifts were reported to 20% phosphorus acid in D₂O as an external standard. Data obtained as a FID sygnals were processed using SpinWorks 3.0.0.1. All ¹H NMR spectras contain signals from HDO (4.8 ppm)and also spectras of compounds obtained as TEA salt contains sygnals from triethylamine (around 1.2 and 3.1 ppm). Mass spectra were recorded on Micromass QToF 1 MS spectrometer.



a. P1-(7-methylguanosin-5´-yl) P4-guanosin-5´-yl 1,2-methylenetetraphosphate (1) m^{7} GpCH₂pppG ¹H NMR spectrum



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m⁷GpCH₂pppG ESI MS spectrum and HPLC profile of purified compound 11:10:46



b. P1-(7-methylguanosin-5´-yl) P4-guanosin-5´-yl 2,3-methylenetetraphosphate (2) $m^{7}GppCH_{2}ppG^{-1}H$ NMR spectrum

m⁷GppCH₂ppG ³¹P NMR spectrum

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m⁷GppCH₂ppG ESI MS spectrum and HPLC profile of purified compound 11:07:45



c. P1-(7-methylguanosin-5´-yl) P4-guanosin-5´-yl 3,4-methylenetetraphosphate (**3**) m⁷GpppCH₂pG ¹H NMR spectrum









d. P1-(7, 2'-O-dimethylguanosin-5'-yl) P4-guanosin-5'-yl 1,2-methylenetetraphosphate (4) $\underline{m_2}^{7,2'-O}$ GpCH₂pppG ¹H NMR spectrum



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e. P1-(7, 2'-O-dimethylguanosin-5'-yl) P4-guanosin-5'-yl 2,3-methylenetetraphosphate (5) $m_2^{7,2'-O}GppCH_2ppG^{-1}H NMR spectrum$











m₂^{7,2'-O}GpppCH₂pG ³¹P NMR spectrum





g. P1-guanosin-5´-yl 2,3-methylenetriphosphate (**9**) GppCH₂p ¹H NMR spectrum

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GppCH₂p ³¹P NMR spectrum





h. P1-guanosin-5´-yl 1,2-methylenetriphosphate (**13**) GpCH₂pp ¹H NMR spectrum



GpCH₂pp ³¹P NMR spectrum

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i. P1-(7, 2'-O-dimethylguanosin-5'-yl) 1,2-methylenediphosphate (14)  $m_2^{7,2'-O}$ GpCH₂p ¹H NMR spectrum  $m_2^{7,2'-O}$ GpCH₂p ³¹P NMR spectrum















f. P1-guanosin-5´-yl 1,2-methylenediphosphate P2- imidazolide (**31**) GpCH₂p-Im HPLC profile



#### 7-methylguanosine (22)

¹H NMR (400 MHz, D₂O, 25°C, TSP):  $\delta$  (ppm) 6.00 (1H, d, J_{1'-2'}=4.7 Hz; H1'); 4.71 (1H, t, J_{1'-2'}=J_{2'-3'})=4.7 Hz; H2'); 4.39 (1H, ~dd, J_{2'-3'}=4.7, J_{3'-4'}=5.0 Hz; H3'), 4.30 (1H, m, H4'); 4.09 (3H, s; CH₃), 3.97 (1H, ~dd, J_{5'-5''}=13.0, J_{4'-5'}=2.7 Hz; H5'), 3.85 (1H, ~dd, J_{5'-5''}=13.0, J_{4'-5''}=3.5 Hz; H5'');

### 7,2'-O-dimethylguanosine (**23**)

¹H NMR (400 MHz, D₂O, 25°C, TSP):  $\delta$  (ppm) 6.15 (1H, d, J_{1'-2'}=3.5 Hz; H1'); 4.51 (1H, ~dd, J_{2'-3'}=5.1, J_{3'-4'})=5.8 Hz; H3'); 4.40 (1H, dd, J_{1'-2'}=3.5, J_{2'-3'}=5.1 Hz; H2'); 4.27 (1H, m,; H4'); 4.12 (3H, s; NCH₃); 3.98 (1H, ~dd, J_{5'-5''}=12.9, J_{4'-5'}=2.7 Hz; H5'), 3.87 (1H, ~dd, J_{5'-5''}=12.9, J_{4'-5'}=4.0 Hz; H5''); 3.59 (3H, m; s; OCH₃);

### 7-methylguanosine 5'-monophosphate (25)

¹H NMR (400 MHz, D₂O, 25°C, TSP):  $\delta$  (ppm) 6.06 (1H, d, J_{1'-2'}=4.0 Hz; H1'); 4.72 (1H, ~dd, J_{1'-2'}=4.0 Hz J_{2'-3'})=5.1 Hz; H2'); 4.58 (1H, ~dd, J_{2'-3'}=5.1, J_{3'-4'}=4.6 Hz; H3'), 4.41 (1H, m, H4'); 4.30 (2H, m; H5', H5'), 4.13 (1H, s; CH₃), ³¹P NMR (162 MHz, D₂O, 25°C, ext. 20% H₃PO₄):  $\delta$  (ppm) 3.91 (1P, s)

#### 7-methylguanosine 5'-diphosphate (**29**)

¹H NMR (400 MHz, D₂O, 25°C, TSP):  $\delta$  (ppm) 6.07 (1H, d, J_{1'-2'}=3.0 Hz; H1'); 4.69 (1H, ~dd, J_{1'-2'}=3.0; J_{2'-3'})=4.1 Hz; H2'); 4.53 (1H, ~dd, J_{2'-3'}=4.1, J_{3'-4'}=6.2 Hz; H3'), 4.40 (1H, m, H4'); 4.27 (1H, m; H5'), 4.14 (1H, m; H5''); 4.12 (1H, s; CH₃), ³¹P NMR (162 MHz, D₂O, 25°C, ext. 20% H₃PO₄):  $\delta$  (ppm) -10.4 (1P, d, J=20.5 Hz); -11.0 (1P, d, J=20.5 Hz)

### 7,2'-O-dimethylguanosine monophosphate (26)

¹H NMR (400 MHz, D₂O, 25°C, TSP):  $\delta$  (ppm) 6.17 (1H, d, J_{1'-2'}=3.3 Hz; H1'); 4.63 (1H, ~t, J_{2'-3'}=J_{3'-4'})=5.1 Hz; H3'); 4.40 (1H, dd, J_{2'-3'}=3.3, J_{3'-4'}=5.1 Hz m; H2'); 4.37 (1H, m; H4'); 4.32 (1H, m; H5'); 4.19 (1H, m; H5''); 4.16 (3H, s; NCH₃); 3.61 (3H, s; OCH₃); ³¹P NMR (162 MHz, D₂O, 25°C, ext. 20% H₃PO₄):  $\delta$  (ppm) 2.47 (1P, s)

#### 7,2'-O-dimethylguanosine diphosphate (30)

¹H NMR (400 MHz, D₂O, 25°C, TSP):  $\delta$  (ppm) 6.16 (1H, d, J_{1'-2'}=3.0 Hz; H1'); 4.63 (1H, ~t, J_{2'-3'}=J_{3'-4'})=5.5 Hz; H3'); 4.38 (2H, overlapped m; H2', H4'); 4.27 (1H, m; H5'); 3.13 (1H, m; H5''); 4.12 (3H, s; NCH₃); 3.60 (3H, m; s; OCH₃); ³¹P NMR (162 MHz, D₂O, 25°C, ext. 20% H₃PO₄):  $\delta$  (ppm) -8.42 (1P, d, J= 21.0 Hz) -10.8 (1P, d, J= 21.0 Hz)