Supplementary data

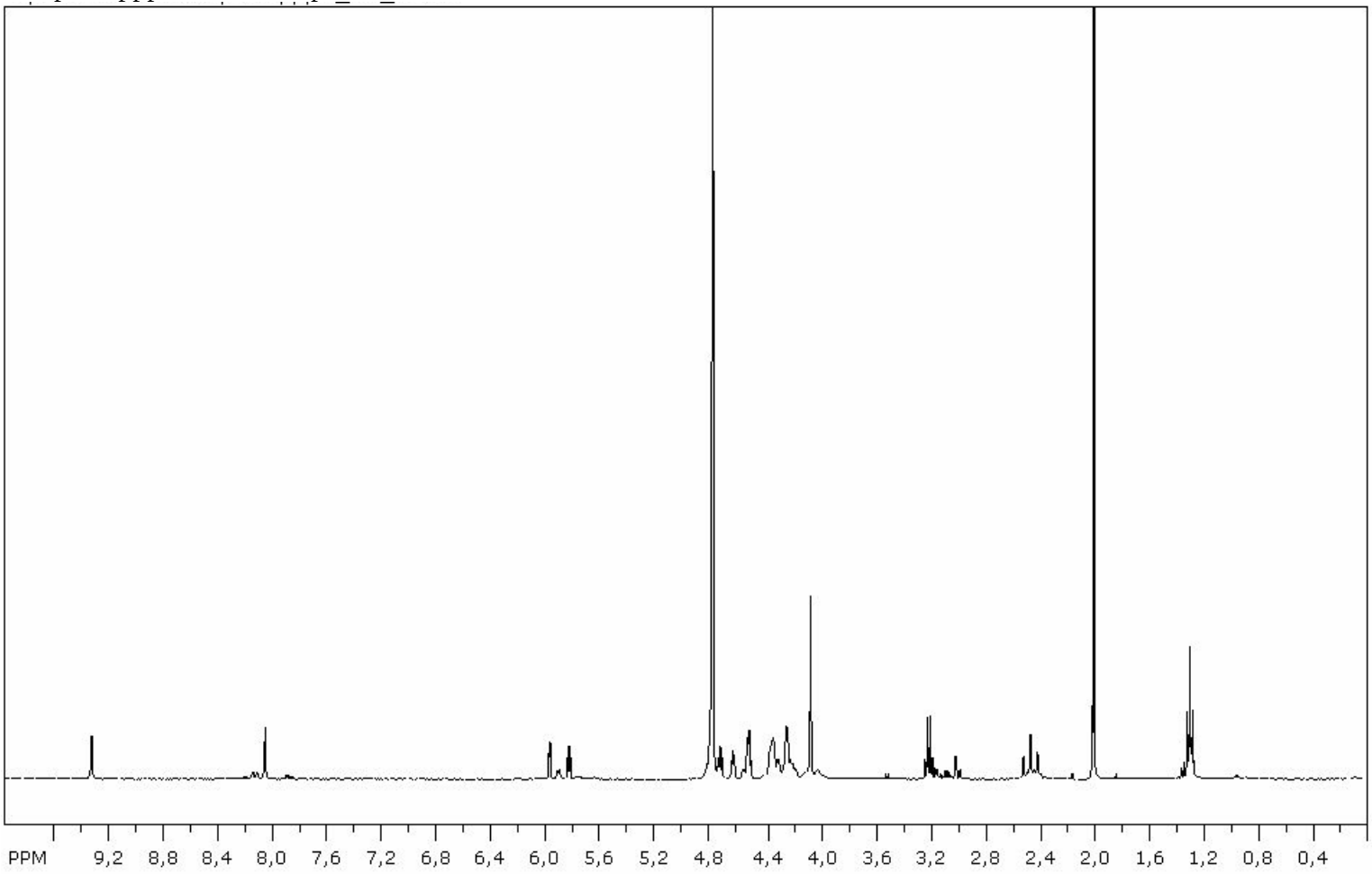
1. Analytical data

Analytical data for newly described compounds are presented.

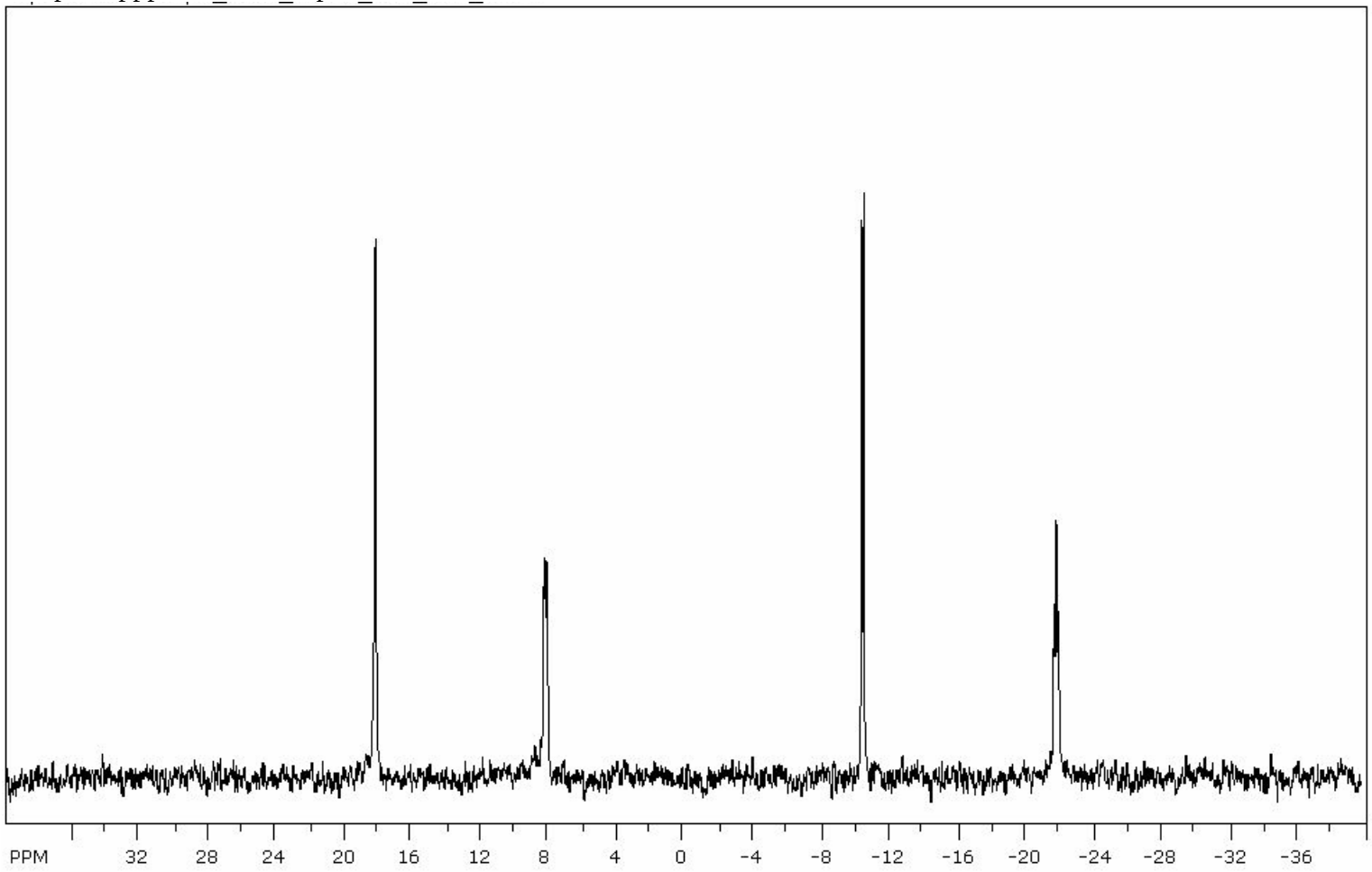
Analytical HPLC was performed on Agilent Tech. Series 1200 using Supelcosil LC-18-T RP column (4.6 x 250 mm, flow rate 1.3 mL/min) with a linear gradient 0–25% of methanol in 0.05 M ammonium acetate buffer (pH 5.9) in 15 min, UV-detection at 260 nm and fluorescence detection (excitation at 280 nm and detection at 337 nm). ^{1}H NMR and ^{31}P NMR spectra were recorded at 25°C on a Varian UNITY-plus spectrometer at 399.94 MHz and 161.90 MHz respectively. ^{1}H NMR chemical shifts were reported to sodium 3-trimethylsilyl-[2,2,3,3-D4]-propionate (TSP) in D_2O as an internal standard. ^{31}P NMR chemical shifts were reported to 20% phosphorus acid in D_2O as an external standard. Data obtained as a FID sygnals were processed using SpinWorks 3.0.0.1. All ^{1}H 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.

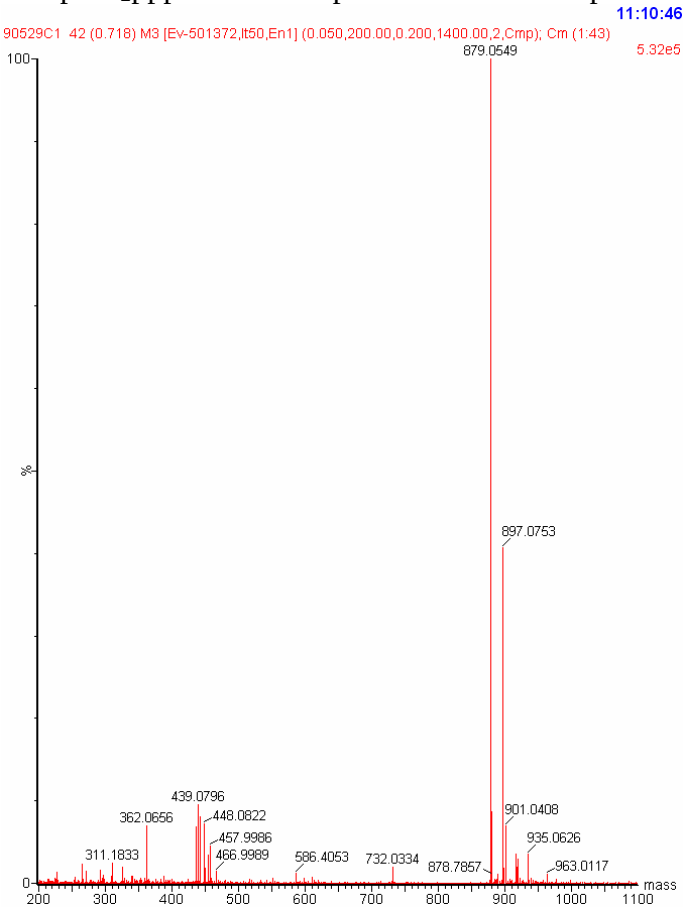
a. P1-(7-methylguanosin-5´-yl) P4-guanosin-5´-yl 1,2-methylenetetraphosphate (**1**)
m^7GpCH_2pppG ^1H NMR spectrum

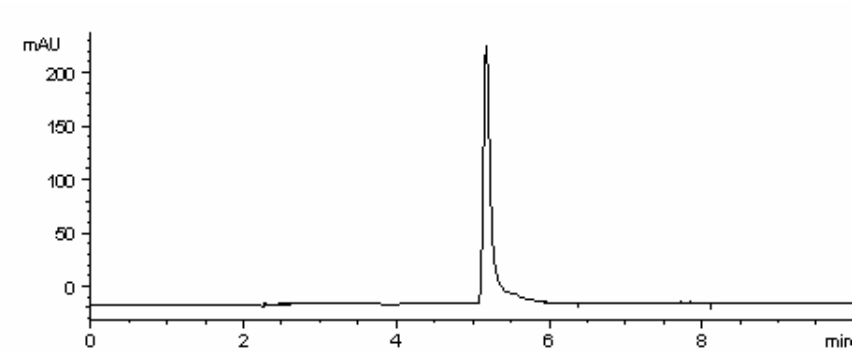


m^7GpCH_2pppG ^{31}P NMR spectrum

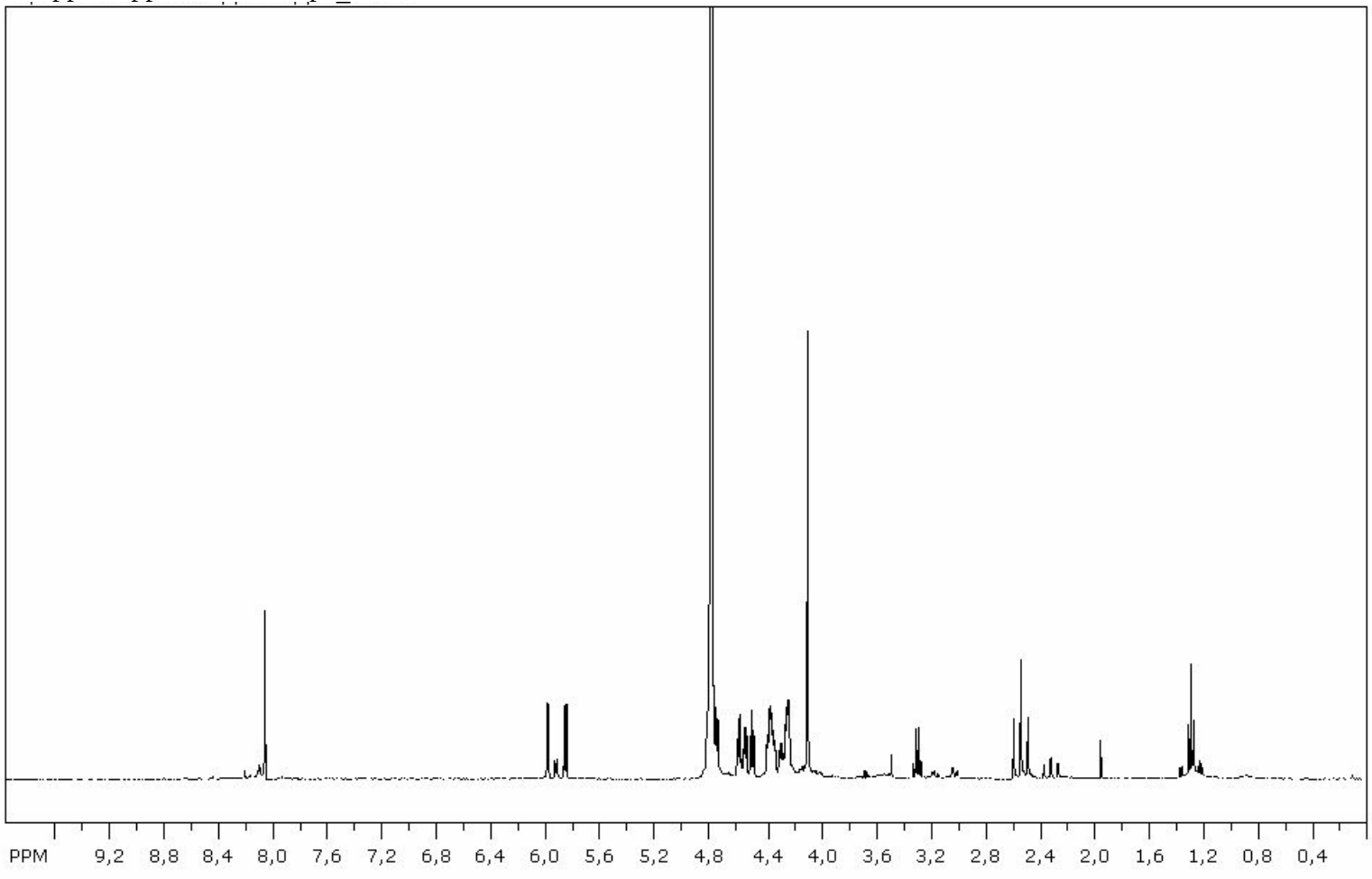


m^7GpCH_2pppG ESI MS spectrum and HPLC profile of purified compound

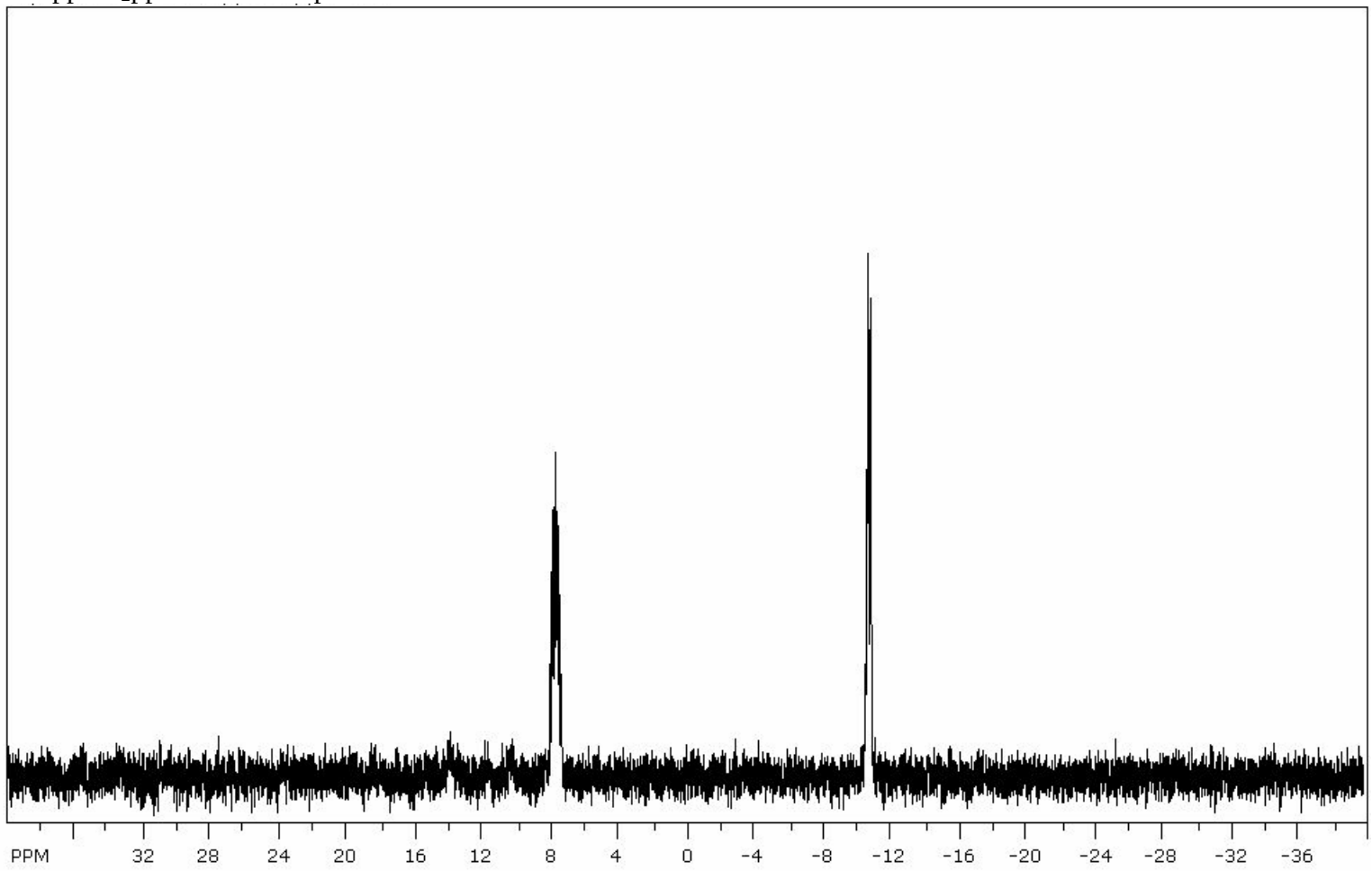




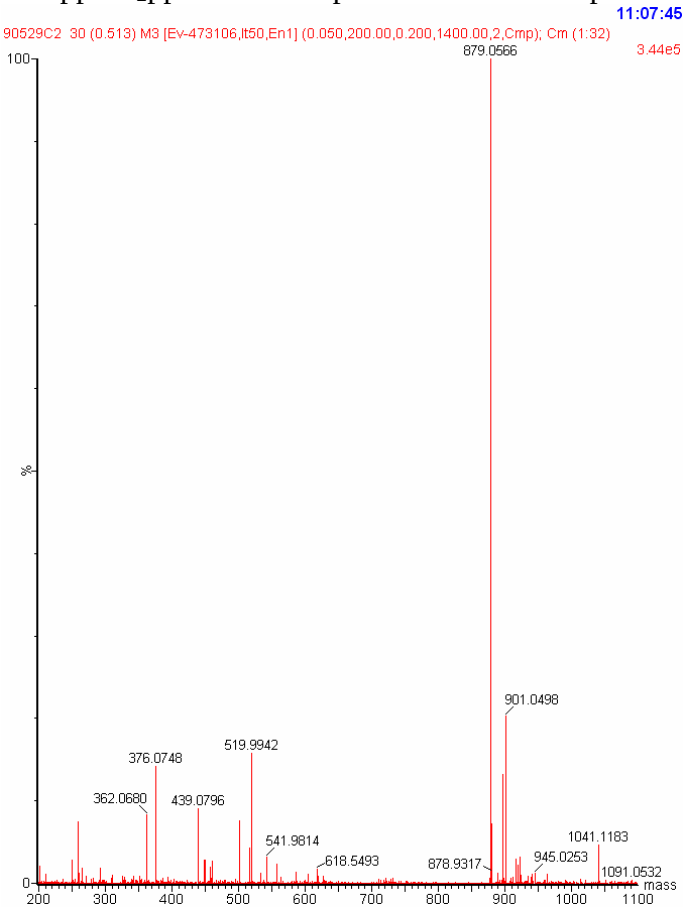
b. P1-(7-methylguanosin-5´-yl) P4-guanosin-5´-yl 2,3-methylenetetraphosphate (**2**)
m^7GppCH_2ppG ^1H NMR spectrum

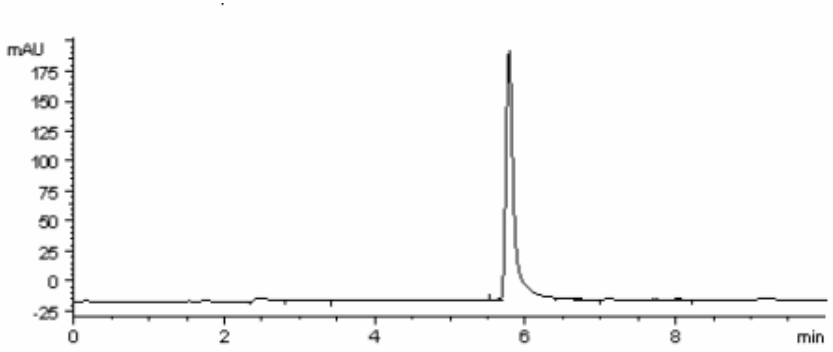


m^7GppCH_2ppG ^{31}P NMR spectrum

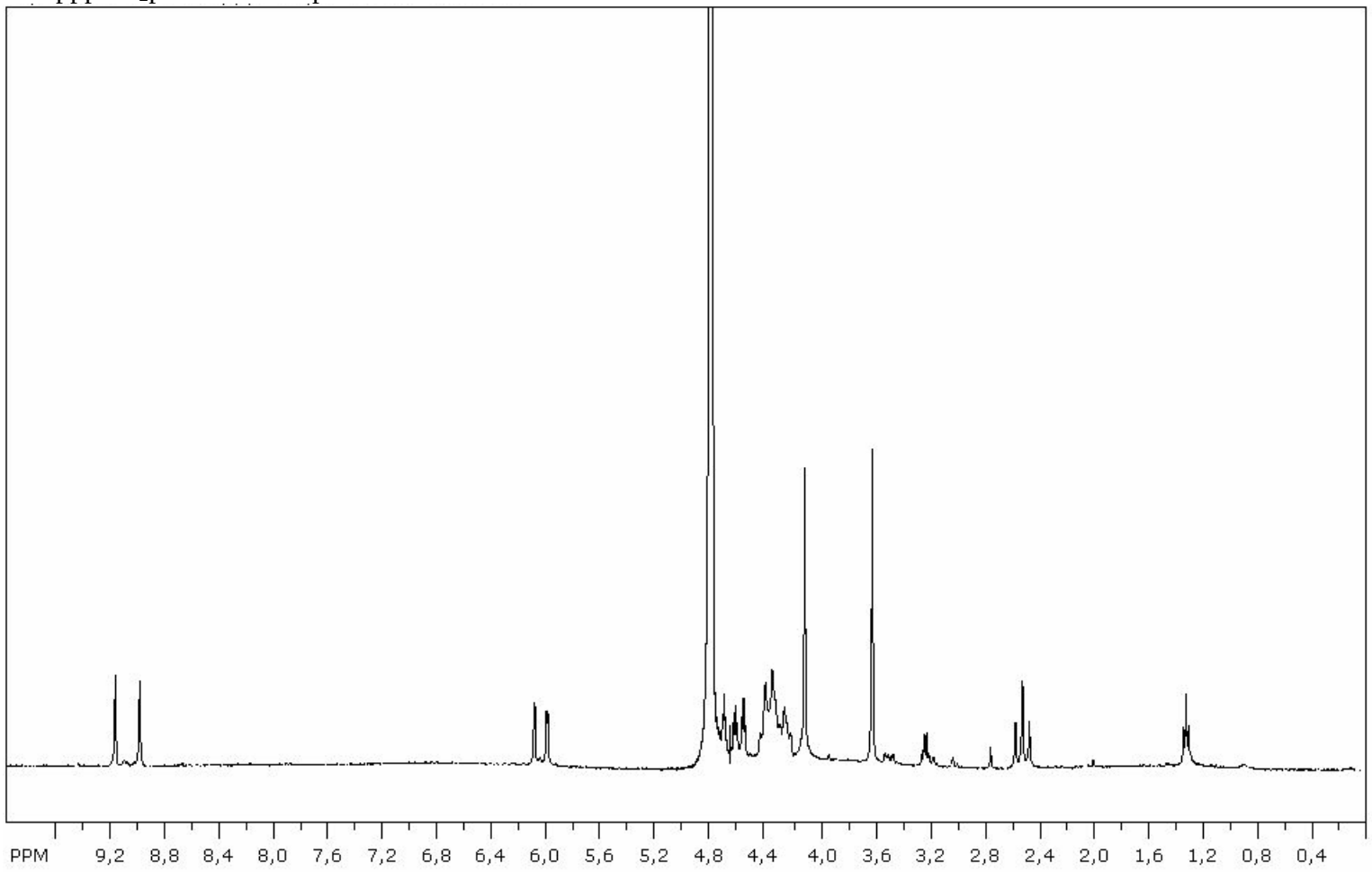


m^7GppCH_2ppG ESI MS spectrum and HPLC profile of purified compound

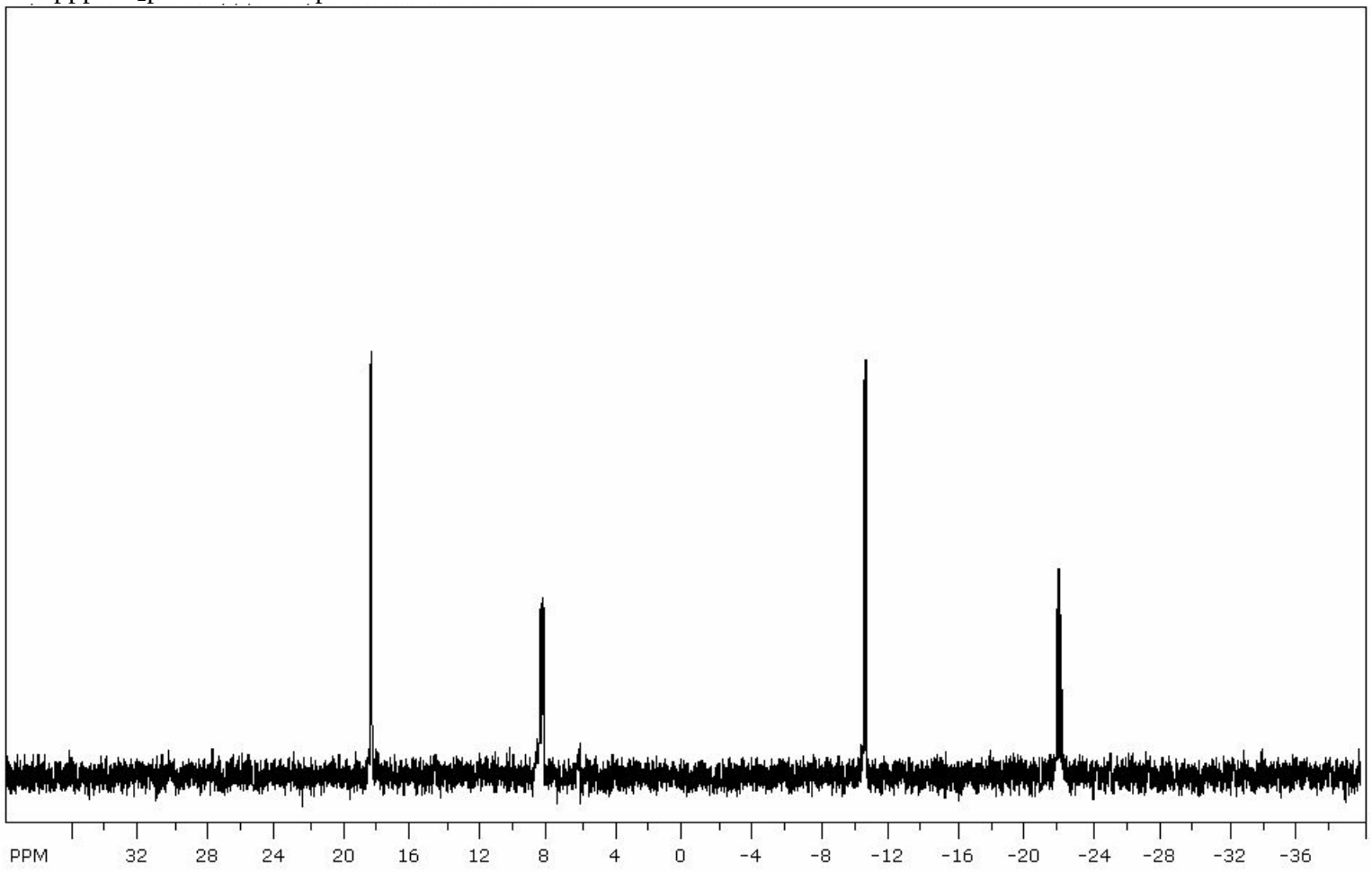




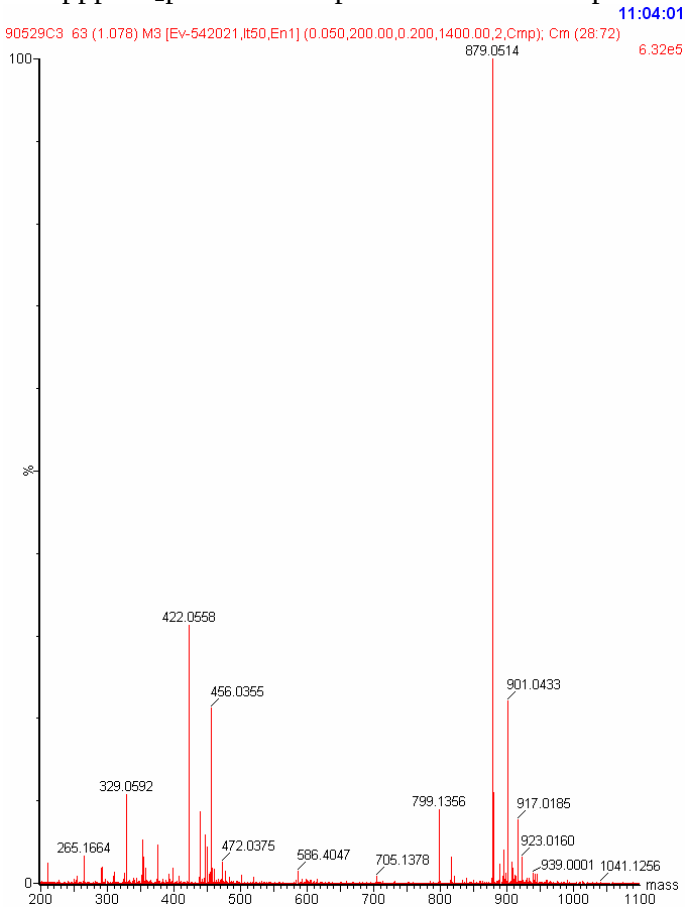
c. P1-(7-methylguanosin-5´-yl) P4-guanosin-5´-yl 3,4-methylenetetraphosphate (**3**)
$m^7GpppCH_2pG$ ^1H NMR spectrum

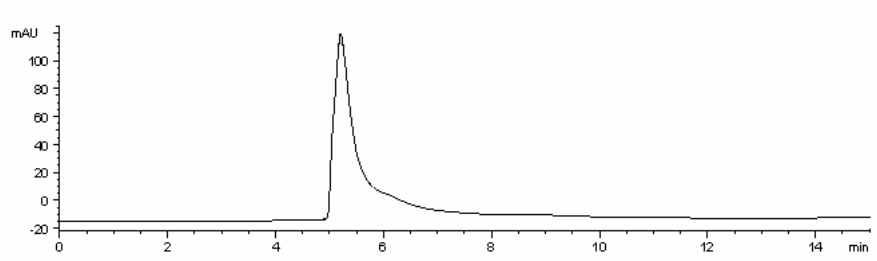


$m^7GpppCH_2pG$ ^{31}P NMR spectrum

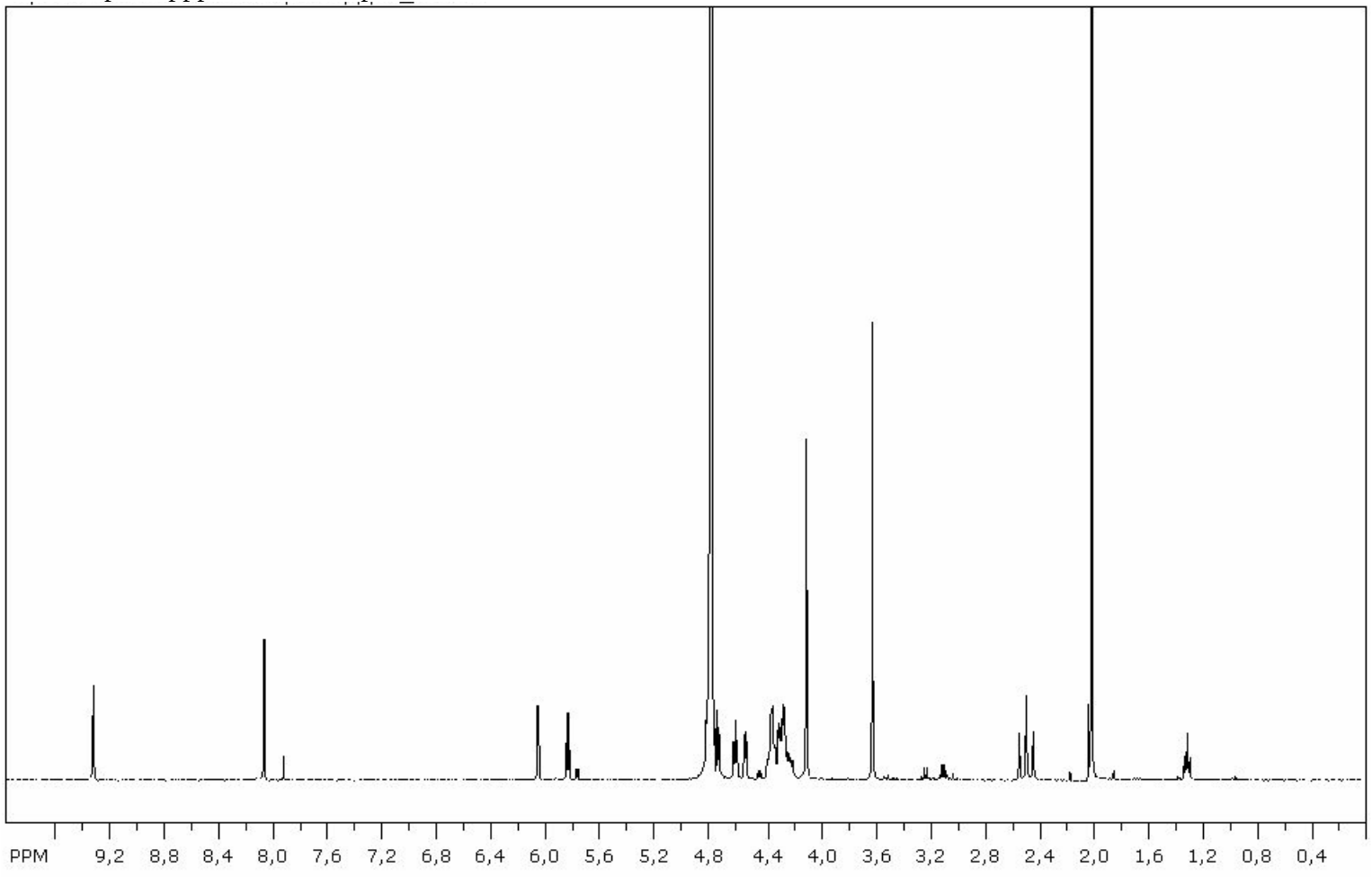


$m^7GpppCH_2pG$ ESI MS spectrum and HPLC profile of purified compound

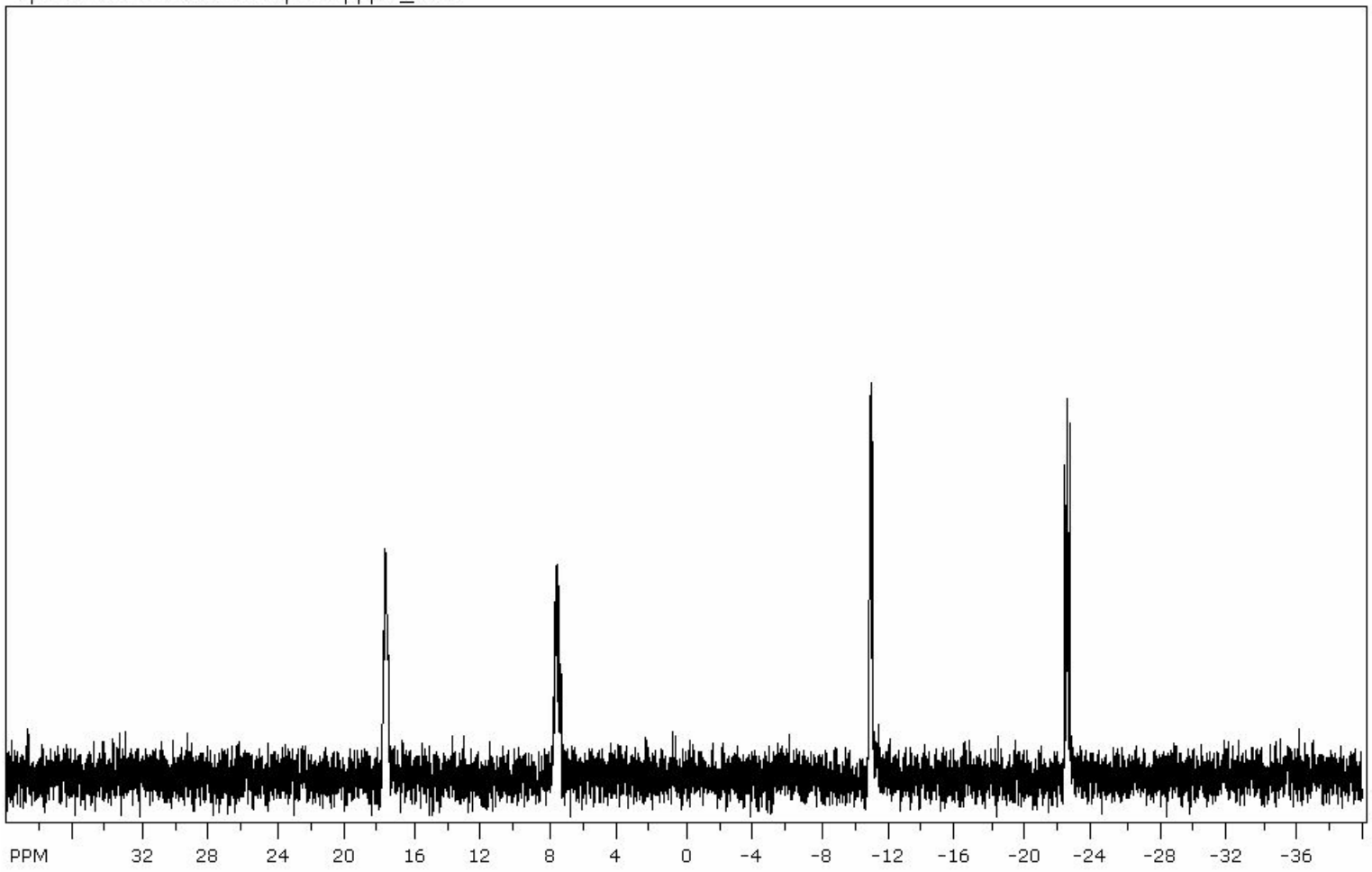




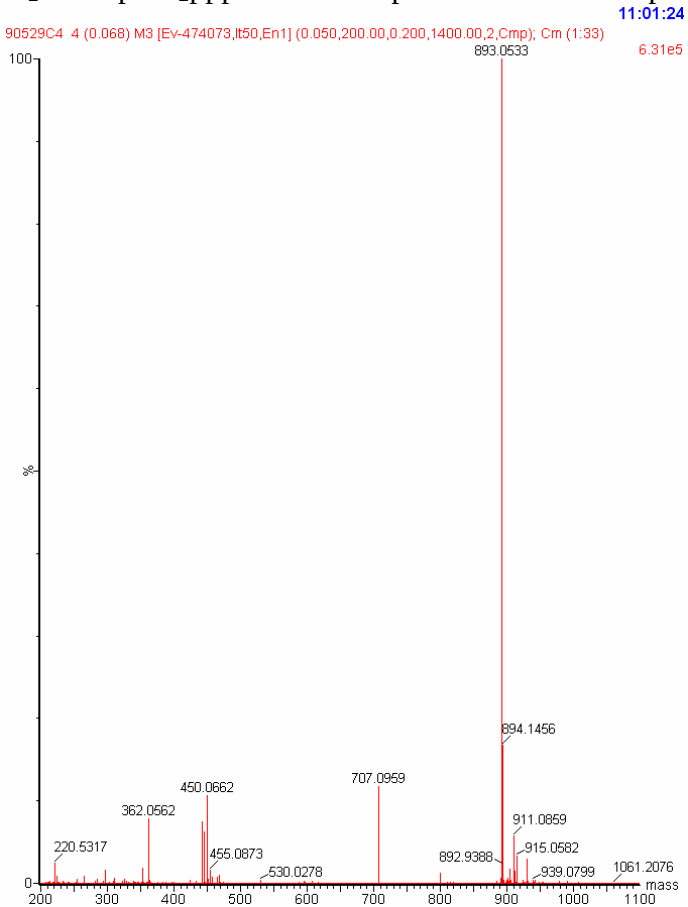
d. P1-(7, 2'-O-dimethylguanosin-5´-yl) P4-guanosin-5´-yl 1,2-methylenetetraphosphate (**4**)
$m_2^{7,2'\text{-}O}GpCH_2pppG$ ^1H NMR spectrum

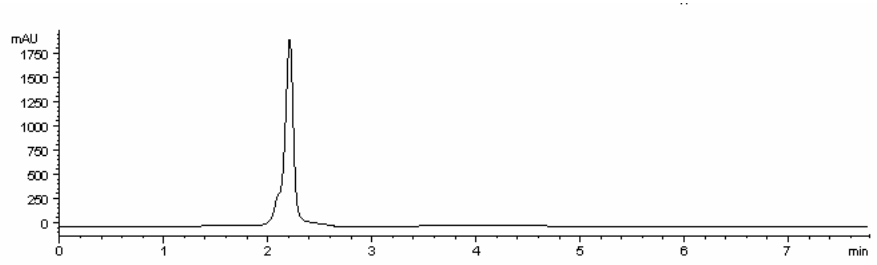


$m_2^{7,2'\text{-}O}GpCH_2pppG$ ^{31}P NMR spectrum

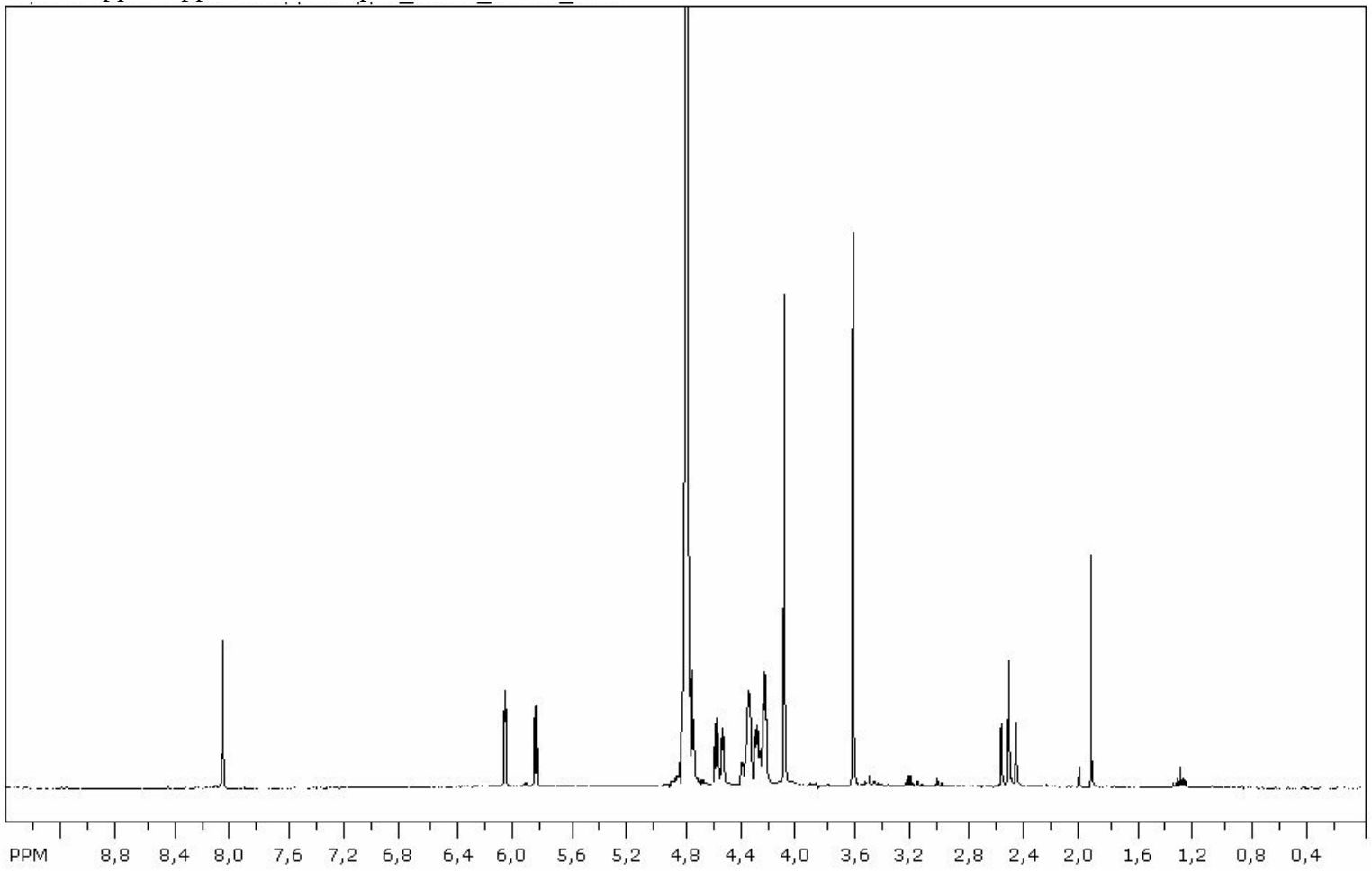


$m_2^{7,2'\text{-}O}GpCH_2pppG$ ESI MS spectrum and HPLC profile of purified compound

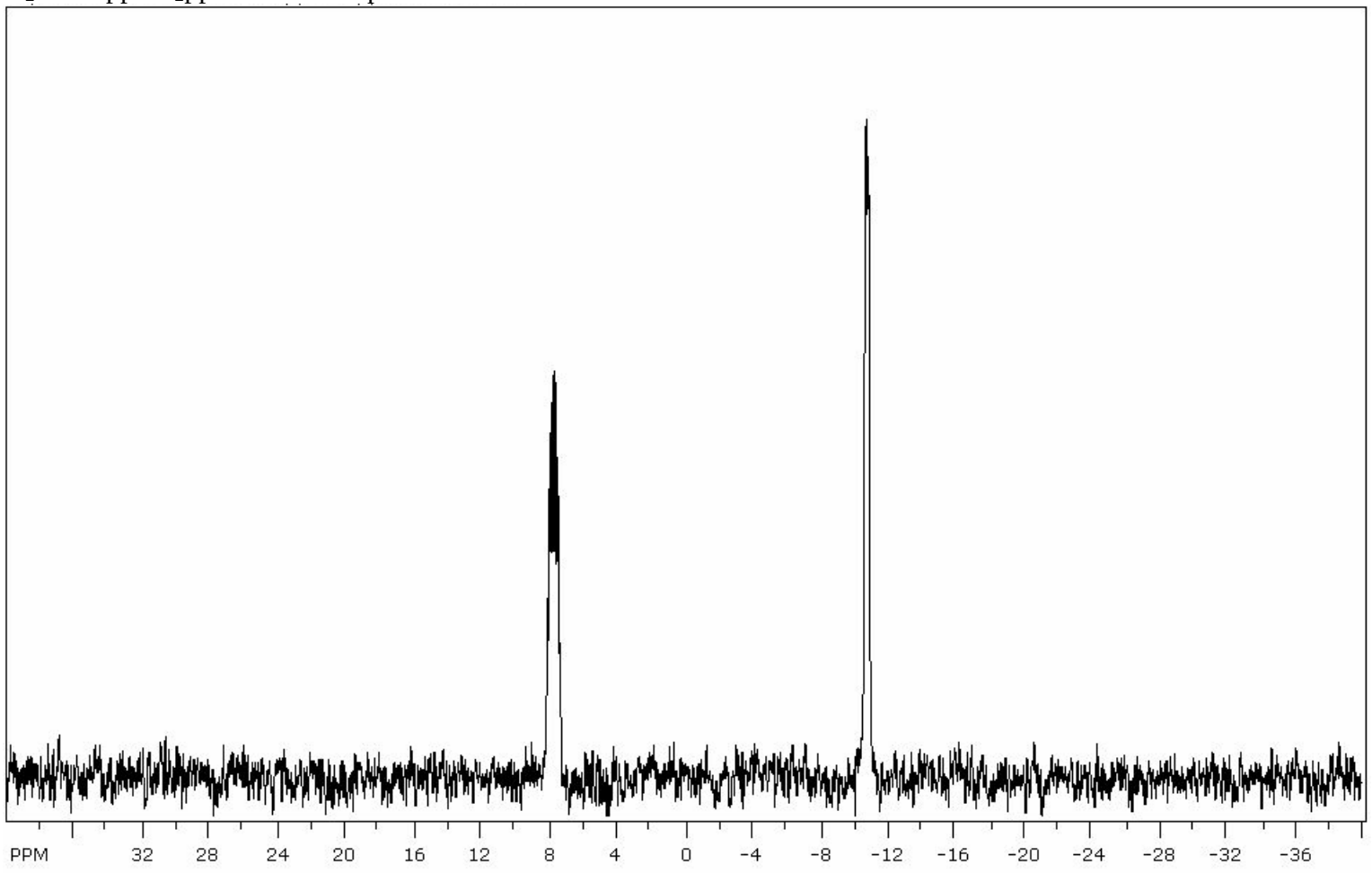




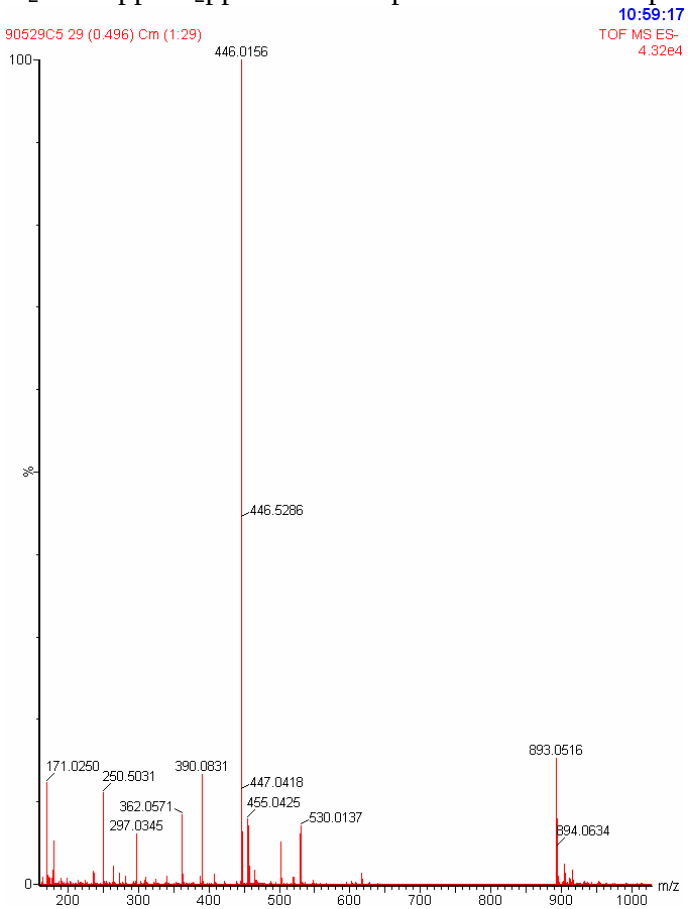
e. P1-(7, 2'-O-dimethylguanosin-5´-yl) P4-guanosin-5´-yl 2,3-methylenetetraphosphate (**5**)
$m_2^{7,2'\text{-}O}GppCH_2ppG$ ^1H NMR spectrum

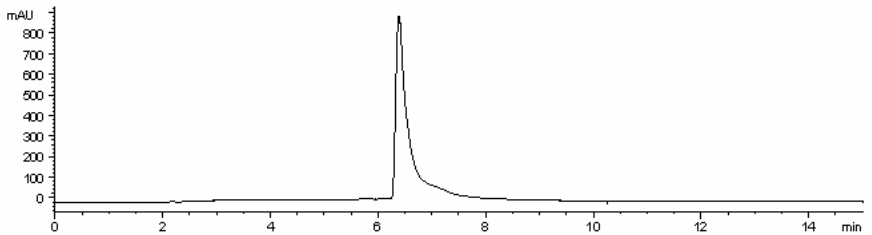


$m_2^{7,2'\text{-}O}GppCH_2ppG$ ^{31}P NMR spectrum

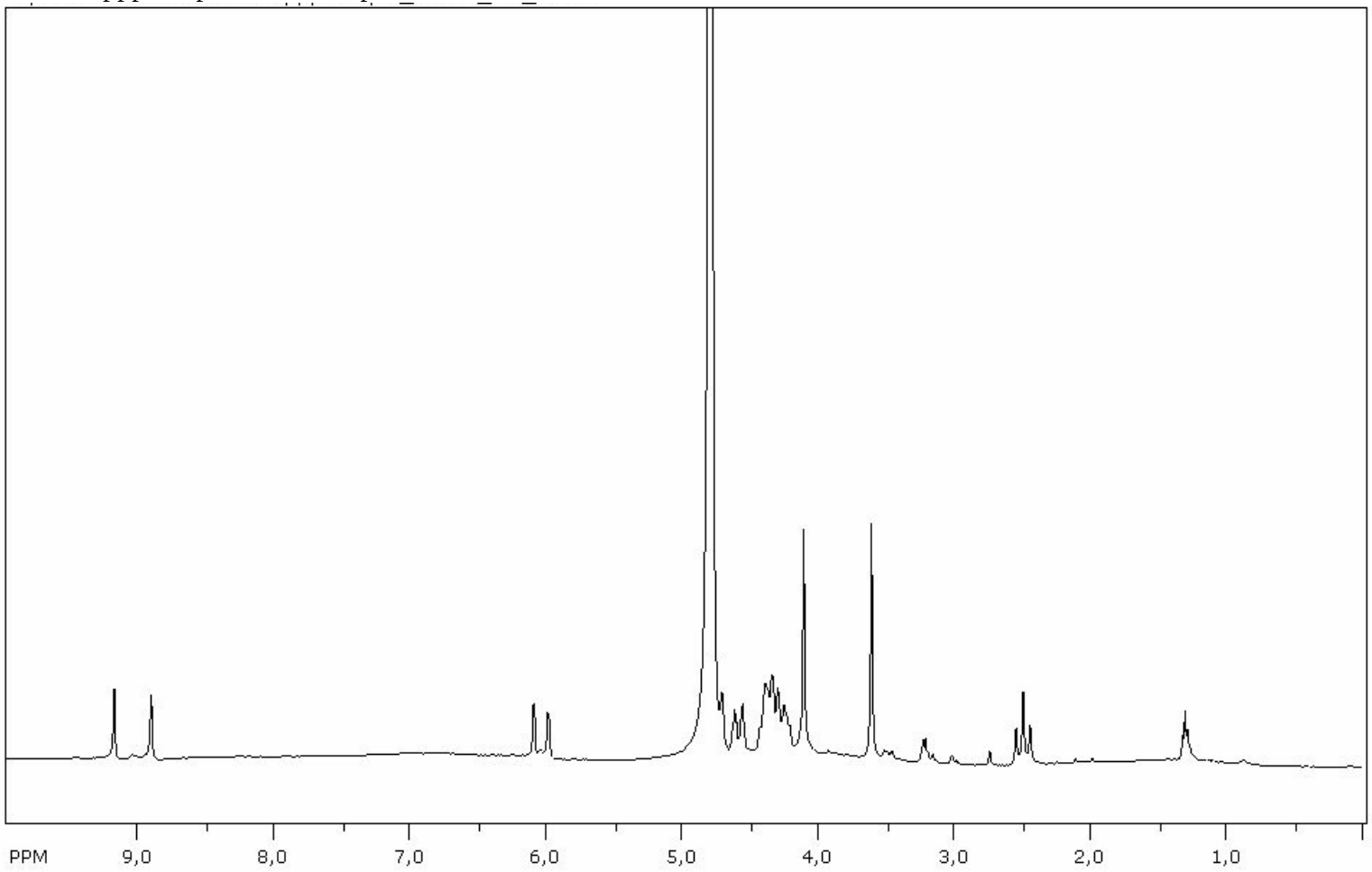


$m_2^{7,2'\text{-}O}GppCH_2ppG$ ESI MS spectrum and HPLC profile of purified compound

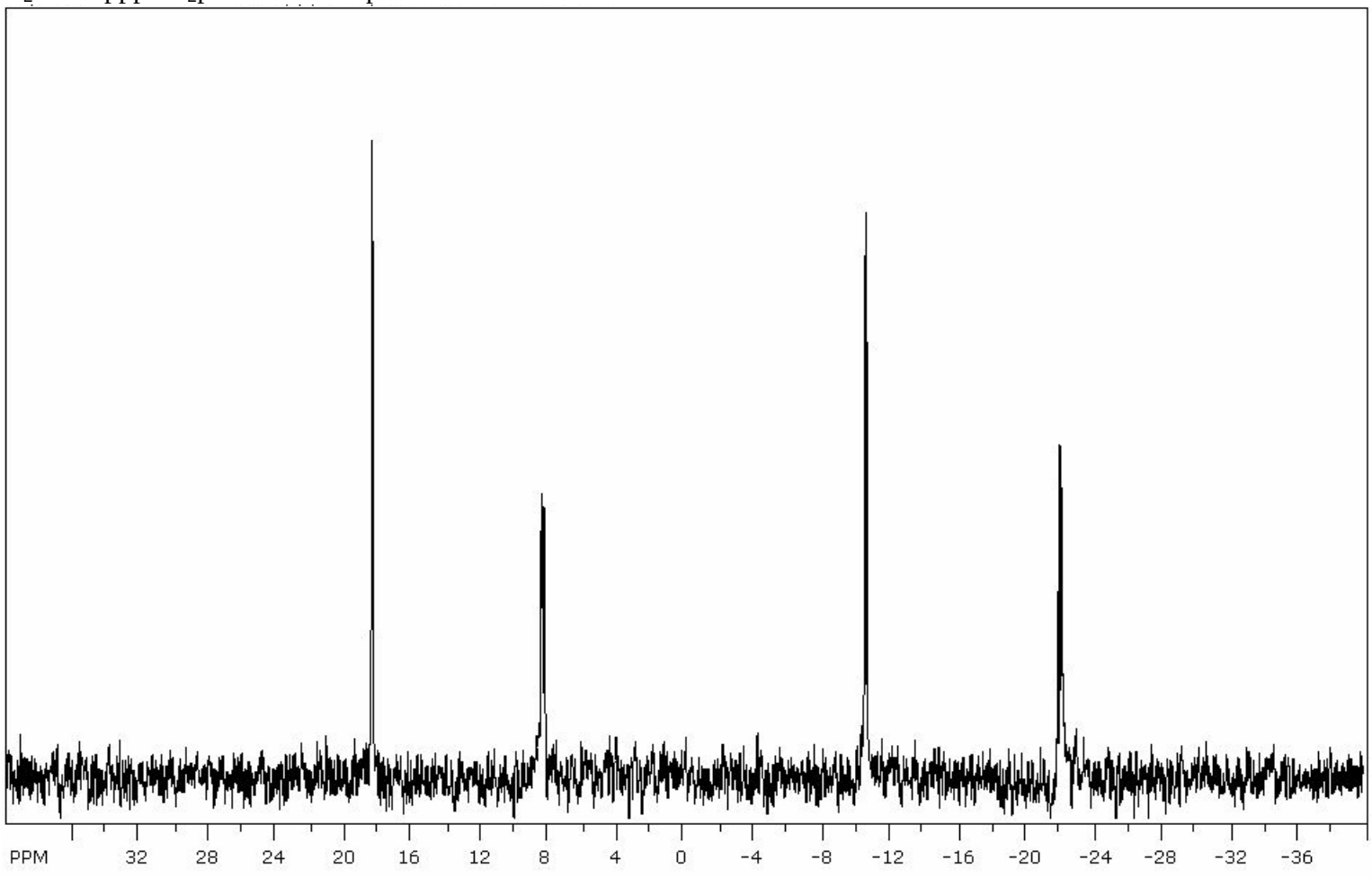




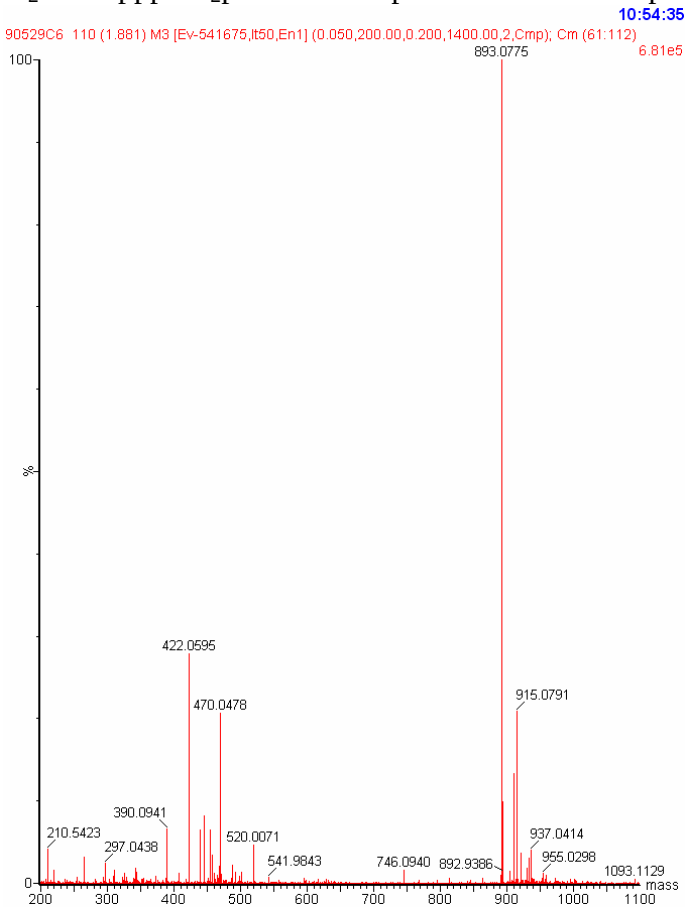
f. P1-(7, 2'-O-dimethylguanosin-5´-yl) P4-guanosin-5´-yl 3,4-methylenetetraphosphate (**6**)
$m_2^{7,2'\text{-}O}GpppCH_2pG$ ^1H NMR spectrum

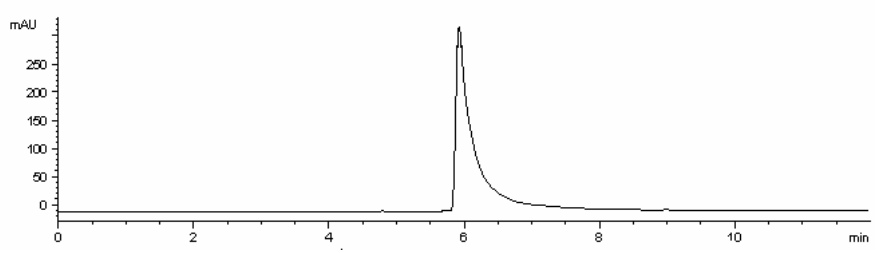


$m_2^{7,2'\text{-O}}GpppCH_2pG$ ^{31}P NMR spectrum

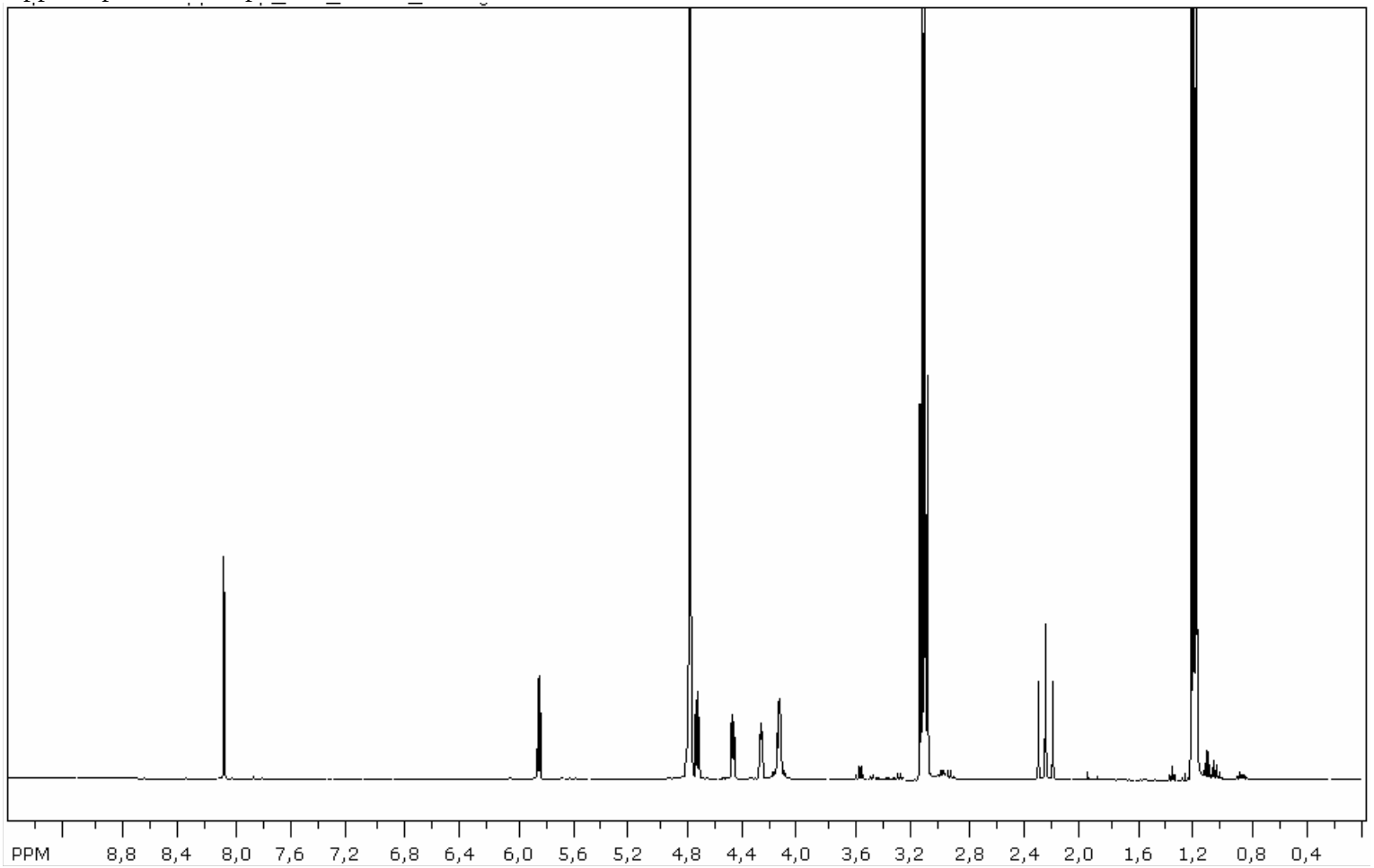


$m_2^{7,2'\text{-}O}GpppCH_2pG$ ESI MS spectrum and HPLC profile of purified compound

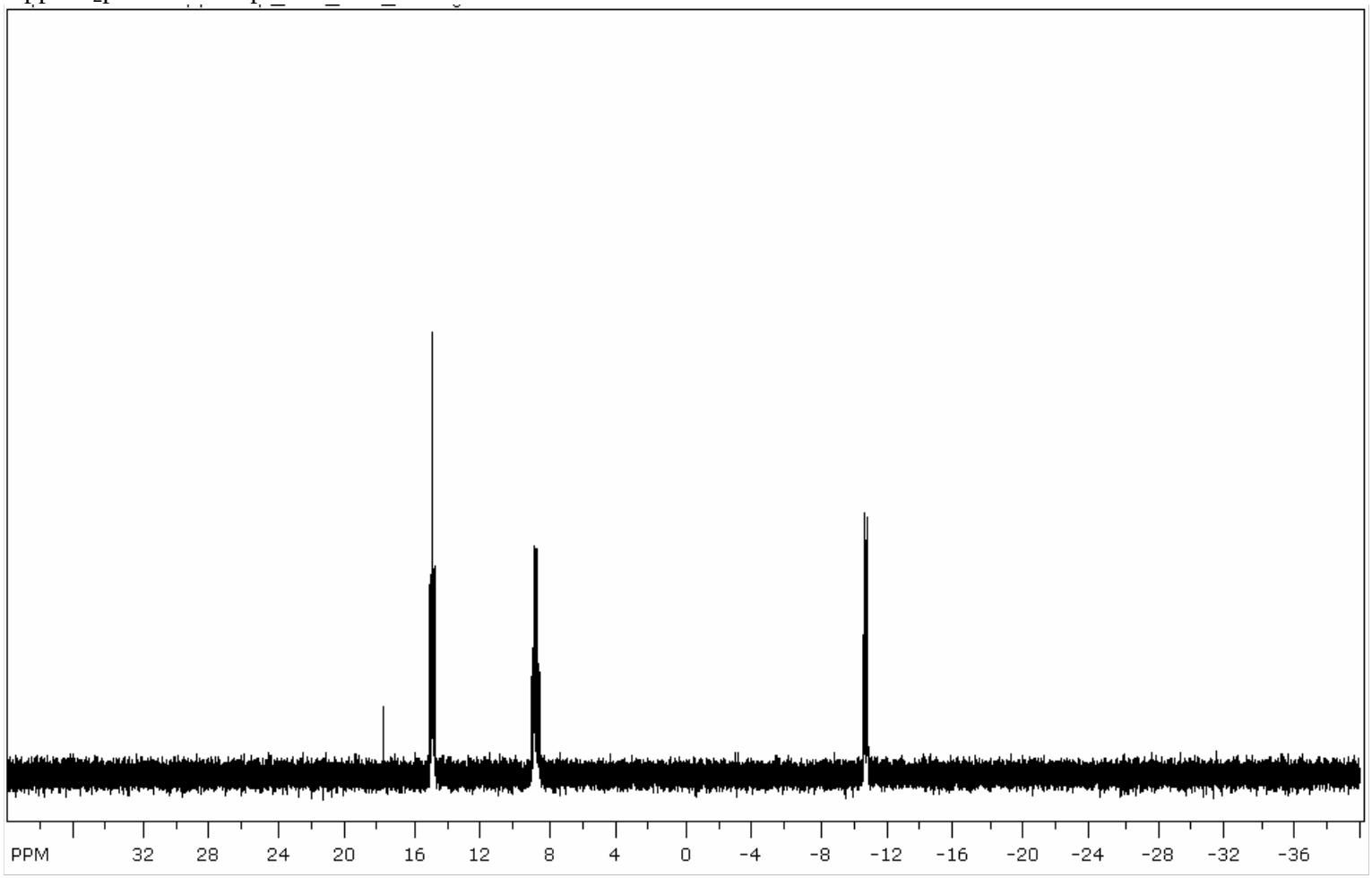




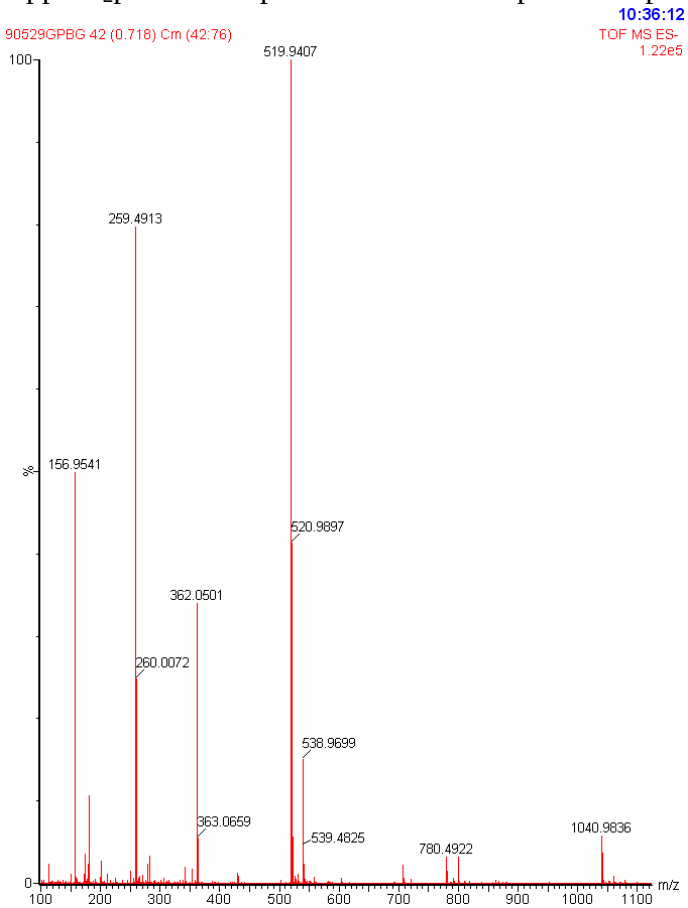
g. P1-guanosin-5´-yl 2,3-methylenetriphosphate (**9**)
GppCH$_2$p ^{1}H NMR spectrum

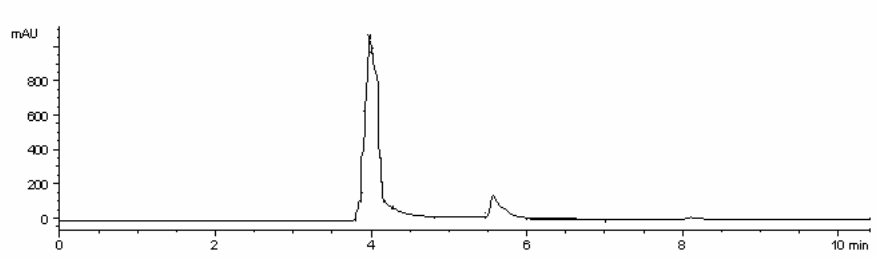


GppCH$_2$p ^{31}P NMR spectrum

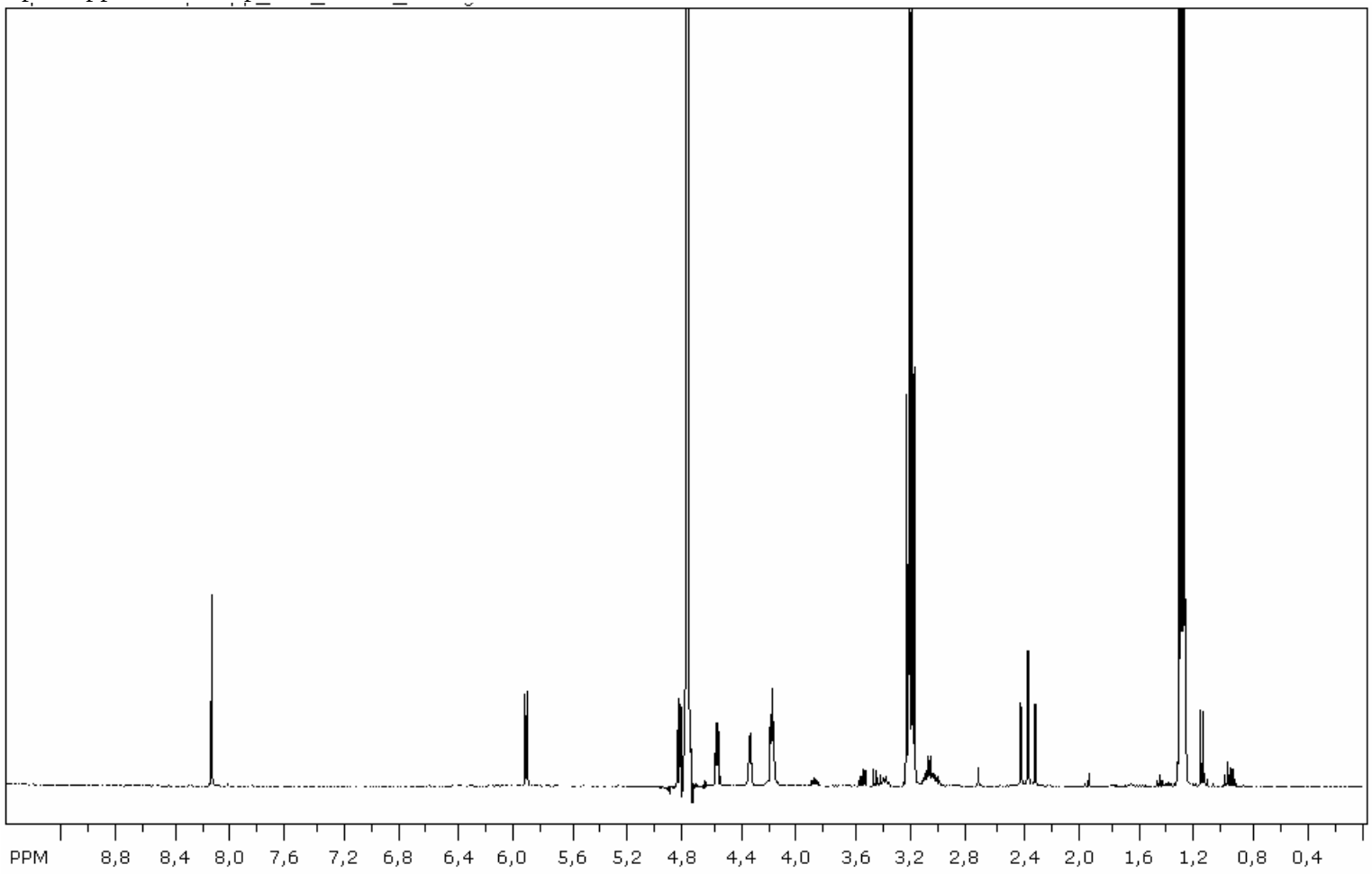


GppCH$_2$p ESI MS spectrum and HPLC profile of purified compound

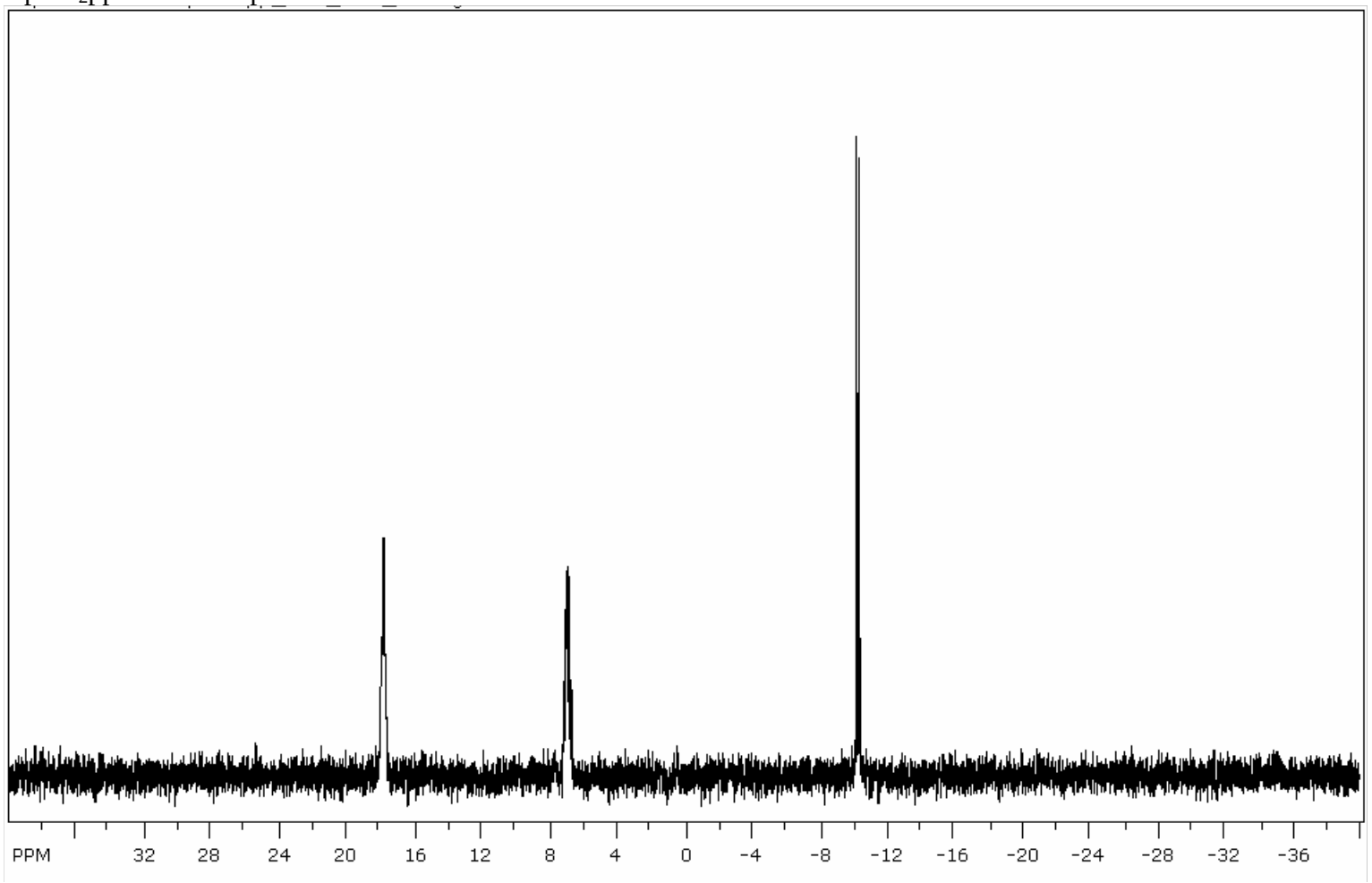




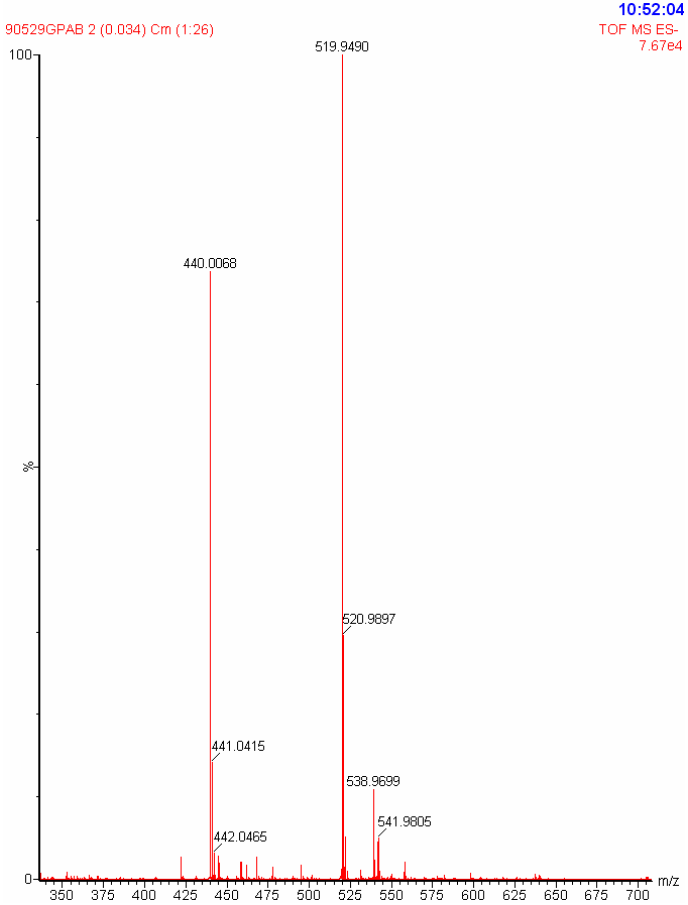
h. P1-guanosin-5´-yl 1,2-methylenetriphosphate (**13**)
$GpCH_2pp$ ^1H NMR spectrum

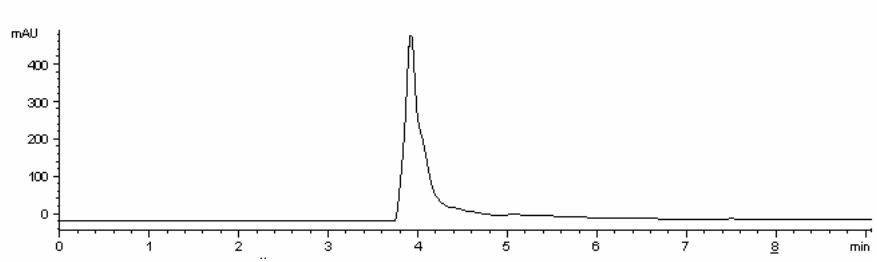


GpCH$_2$pp ^{31}P NMR spectrum



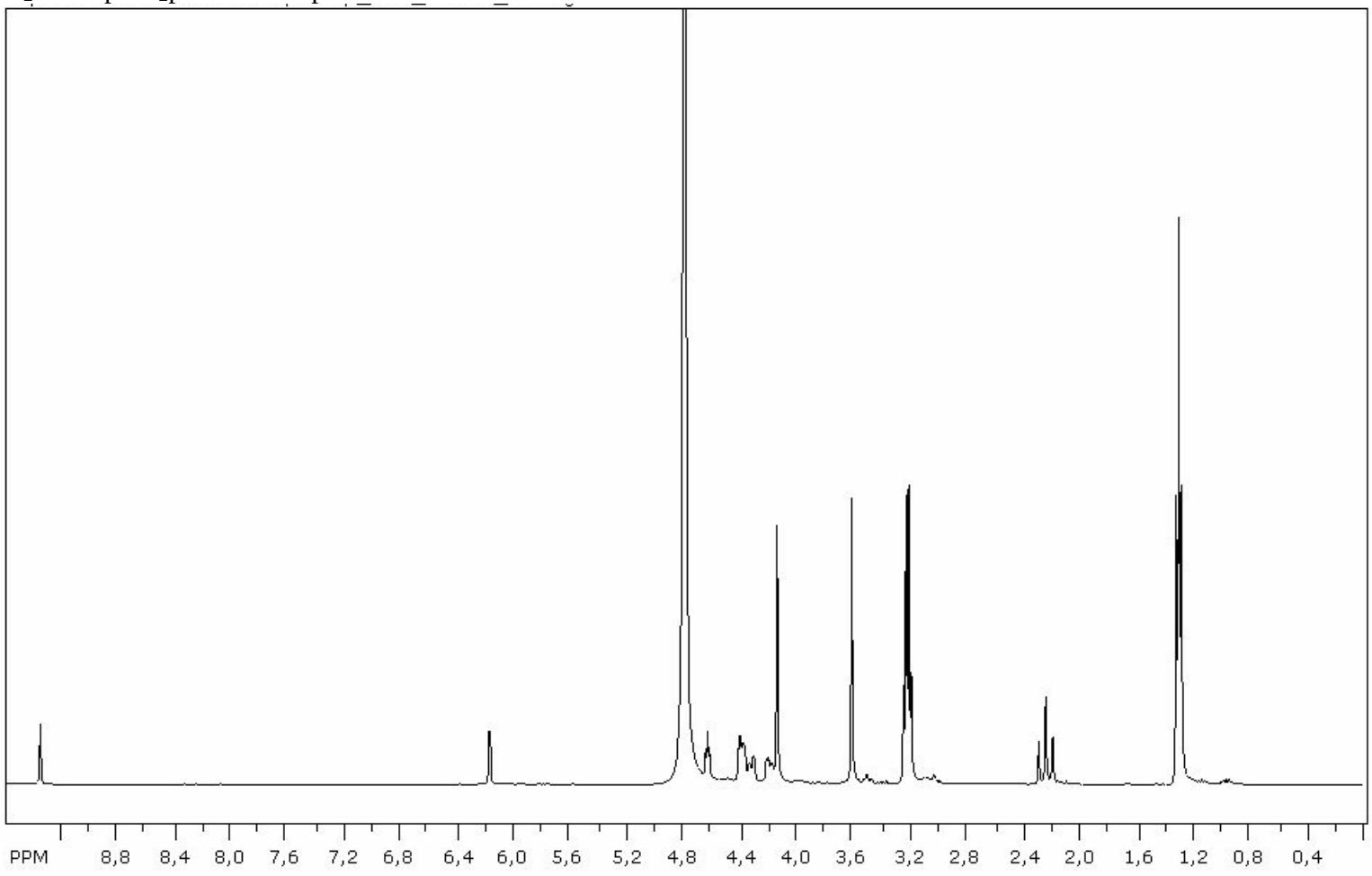
$GpCH_2pp$ ESI MS spectrum and HPLC profile of purified compound



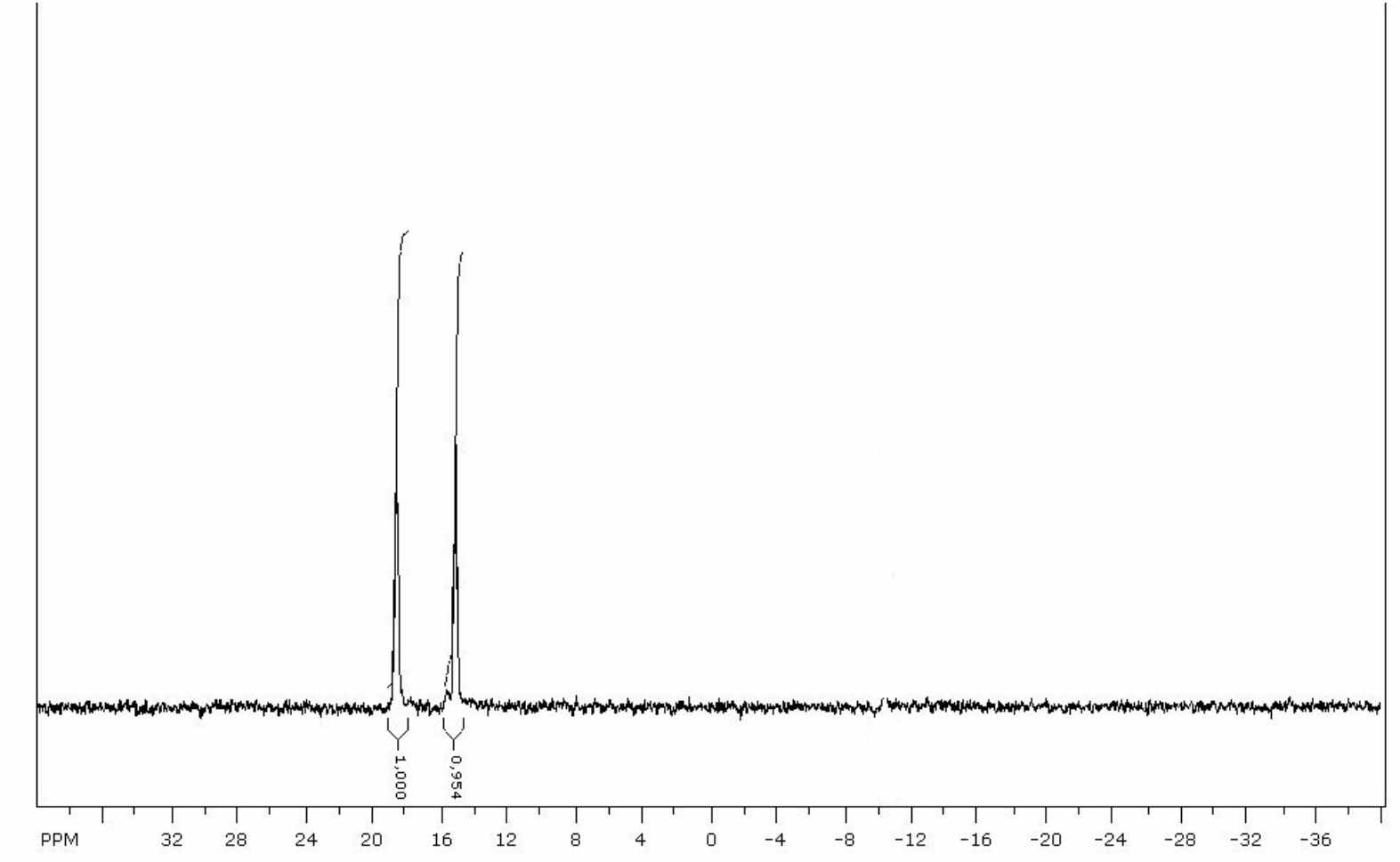


i. P1-(7, 2'-O-dimethylguanosin-5´-yl) 1,2-methylenediphosphate (**14**)

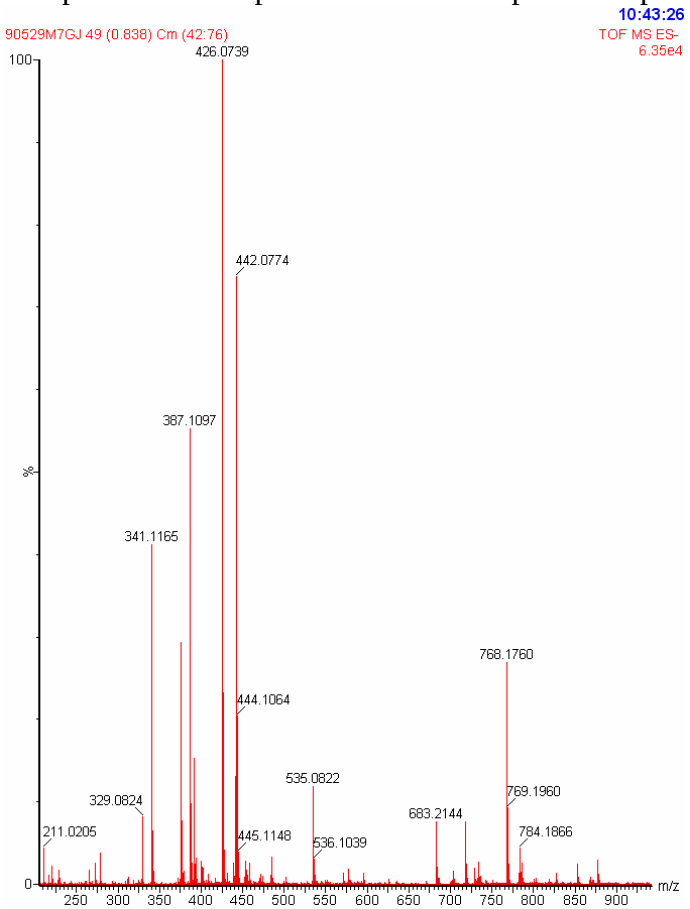
$m_2^{7,2'\text{-}O}GpCH_2p$ ^1H NMR spectrum

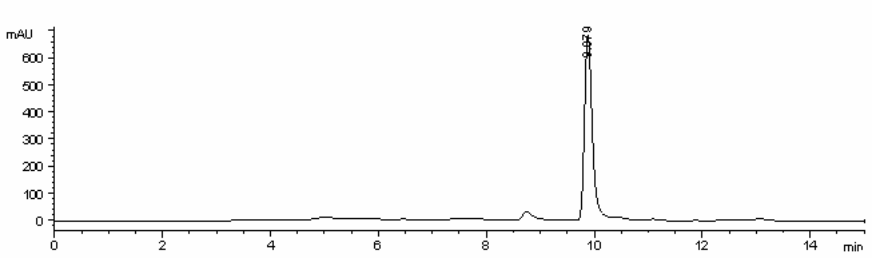


$m_2^{7,2'\text{-O}}GpCH_2p$ ^{31}P NMR spectrum

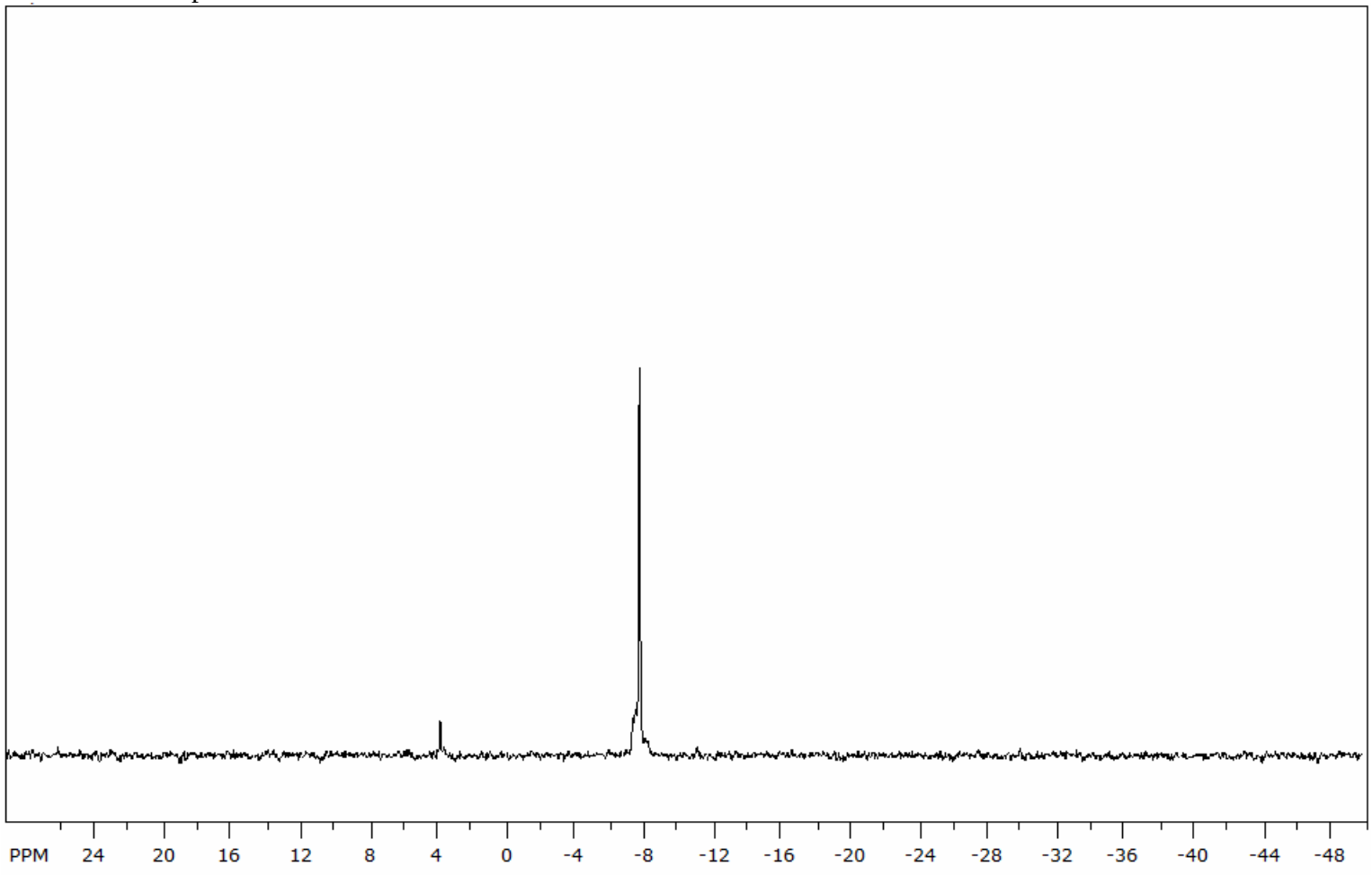


j. 7-methylguanosin-5´-yl monophosphate P-imidazolide (**10**)
m^7Gp-Im ESI MS spectrum and HPLC profile of purified compound

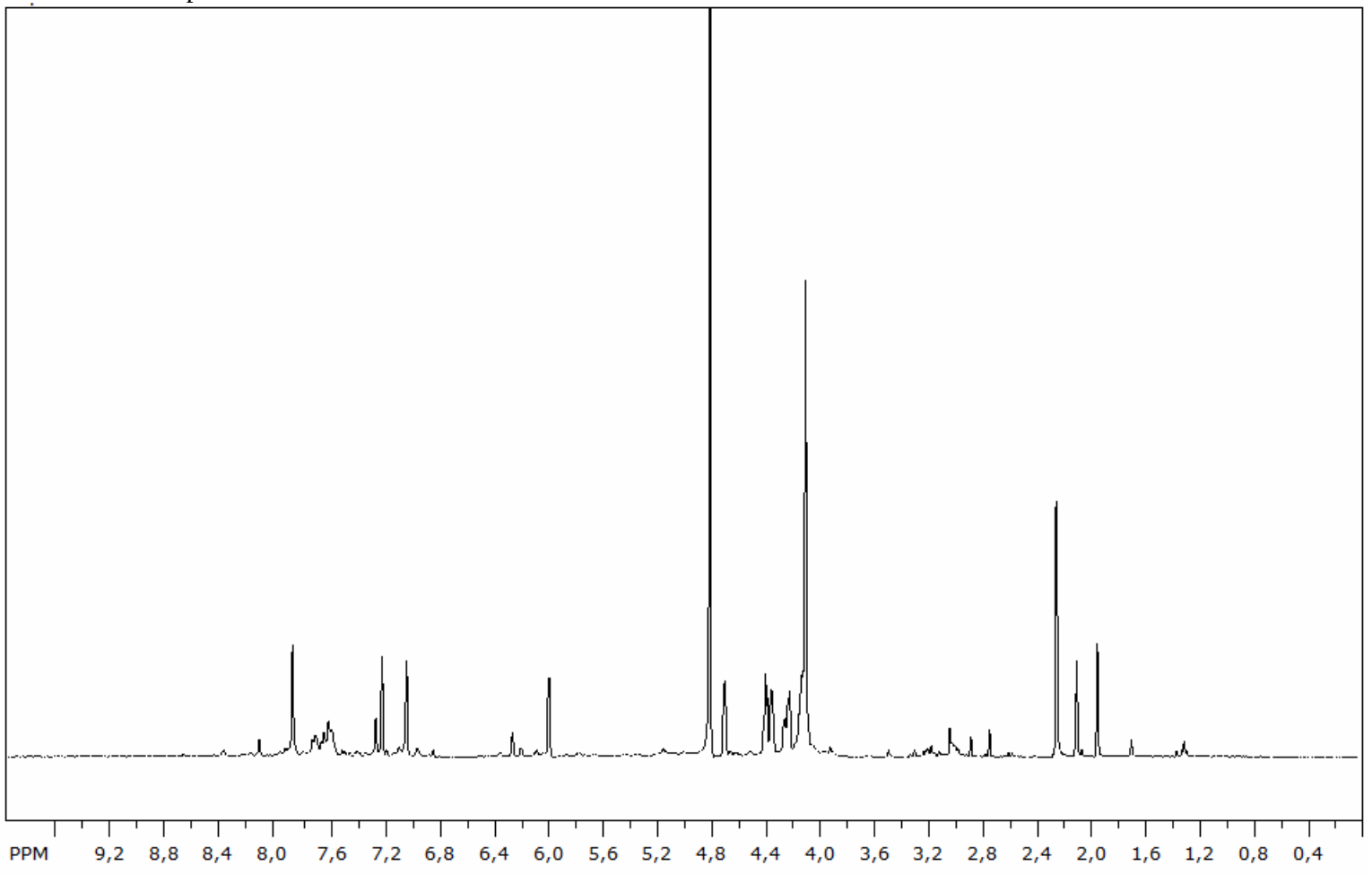




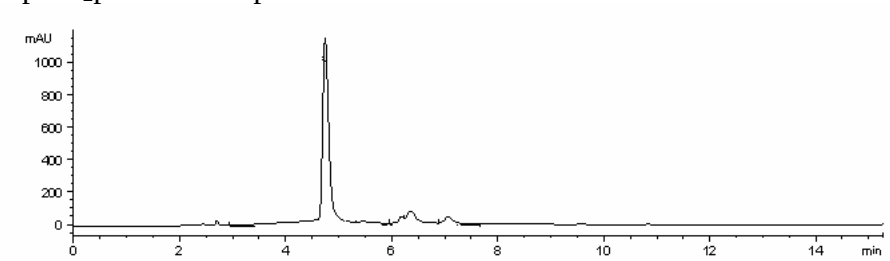
^{31}P NMR m^{7}Gmp-Im



^{1}H NMR m^{7}Gmp-Im



f. P1-guanosin-5´-yl 1,2-methylenediphosphate P2- imidazolide (**31**)
$GpCH_2p$-Im HPLC profile



7-methylguanosine (**22**)
^{1}H NMR (400 MHz, D_2O, 25ºC, TSP): δ (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; CH_3), 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**)
^{1}H NMR (400 MHz, D_2O, 25ºC, TSP): δ (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; NCH_3); 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; OCH_3);

7-methylguanosine 5'-monophosphate (**25**)
^{1}H NMR (400 MHz, D_2O, 25ºC, TSP): δ (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; CH_3),
^{31}P NMR (162 MHz, D_2O, 25ºC, ext. 20% H_3PO_4): δ (ppm) 3.91 (1P, s)

7-methylguanosine 5'-diphosphate (**29**)
^{1}H NMR (400 MHz, D_2O, 25ºC, TSP): δ (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; CH_3),
^{31}P NMR (162 MHz, D_2O, 25ºC, ext. 20% H_3PO_4): δ (ppm) -10.4 (1P, d, J= 20.5 Hz); -11.0 (1P, d, J= 20.5 Hz)

7,2'-O-dimethylguanosine monophosphate (**26**)
^{1}H NMR (400 MHz, D_2O, 25ºC, TSP): δ (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; NCH_3); 3.61 (3H, s; OCH_3);
^{31}P NMR (162 MHz, D_2O, 25ºC, ext. 20% H_3PO_4): δ (ppm) 2.47 (1P, s)

7,2'-O-dimethylguanosine diphosphate (**30**)
^{1}H NMR (400 MHz, D_2O, 25ºC, TSP): δ (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; NCH_3); 3.60 (3H, m; s; OCH_3);
^{31}P NMR (162 MHz, D_2O, 25ºC, ext. 20% H_3PO_4): δ (ppm) -8.42 (1P, d, J= 21.0 Hz) -10.8 (1P, d, J= 21.0 Hz)