Preparation of synthetically challenging nucleotides using cyanoethyl P-imidazolides and microwaves

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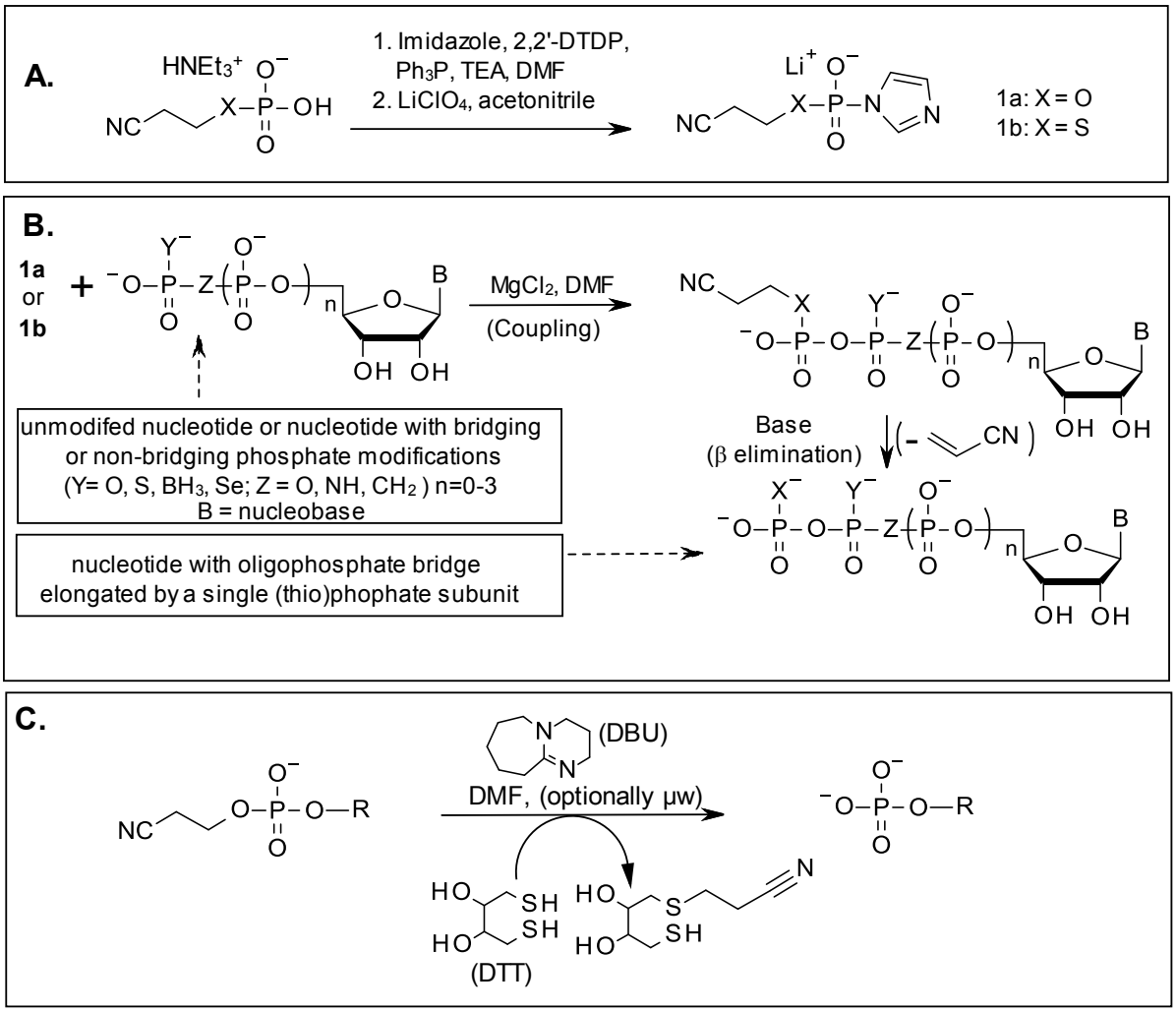
Supporting Information

List of content

I. Supplementary figures



Scheme S1. **(A)** Synthesis of **1a** and **1b**. **(B)** Application of **1a** and **1b** to nucleoside oligophosphate synthesis. **(C)** CE removal reaction in the presence of DTT

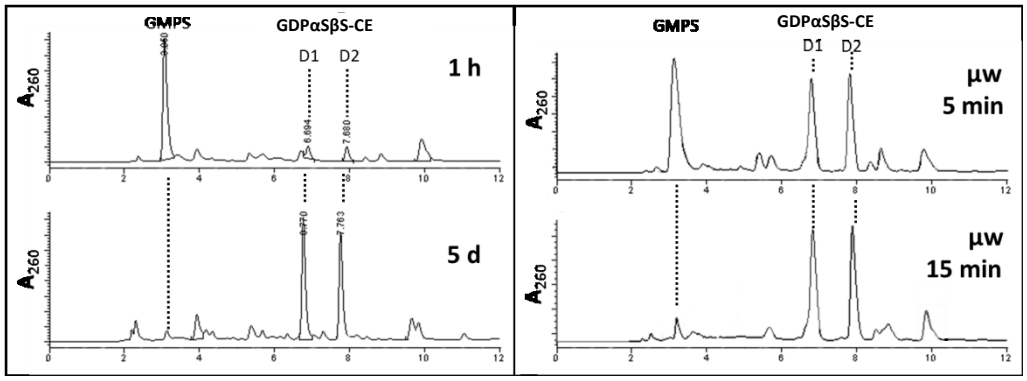


Fig. S1. Comparison of exemplary modified pyrophosphate bond formation reactions carried out under conventional conditions (RT) and in the presence of microwave irradiation. Reaction conditions are given in paragraph 5.9.

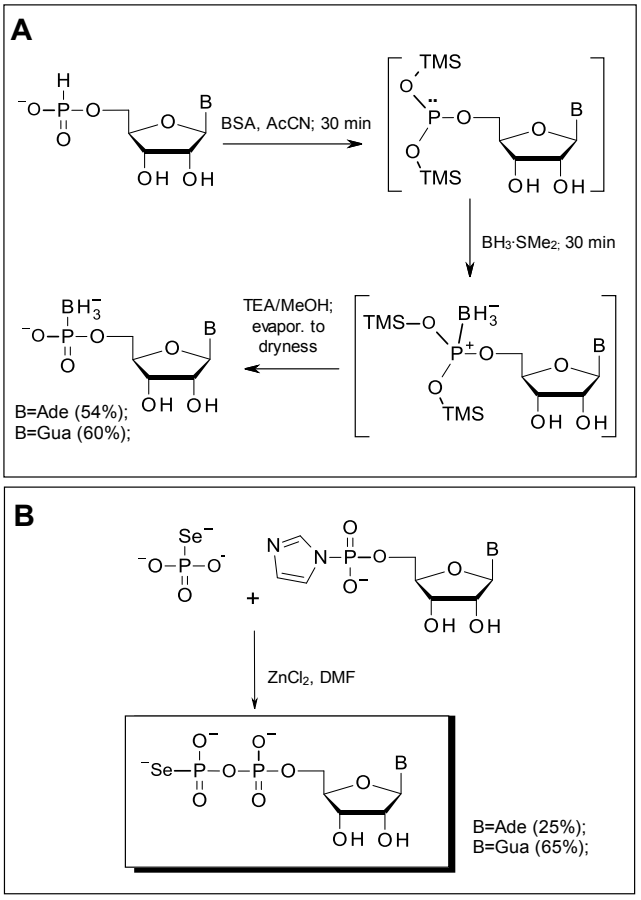


Fig. S2. Synthesis of starting materials reported for the first time. A) Synthesis of nucleoside boranophosphates (paragraph 3.3) – starting materials for the preparation of nucleoside 1-boranodiphosphates (**3** and **5**) and **B)** nucleoside 2-selenodiphosphates (paragraph 3.4) – starting materials for the preparation of nucleoside 2-selenotriphosphates (**15** and **16**).

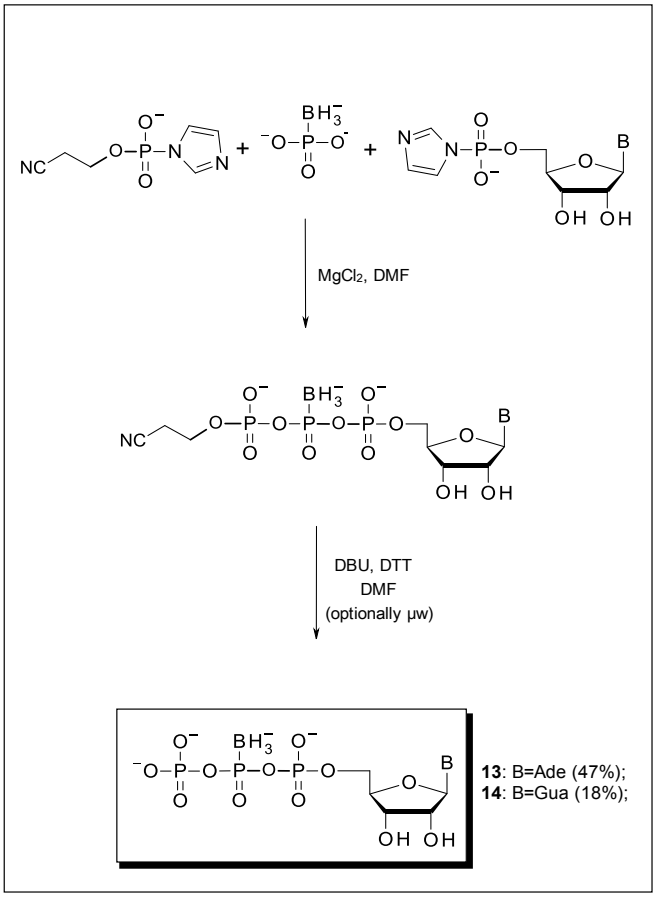


Fig. S3. One-pot synthesis of nucleoside 2-boranotriphosphates from corresponding 5'-phosphorimidazolides

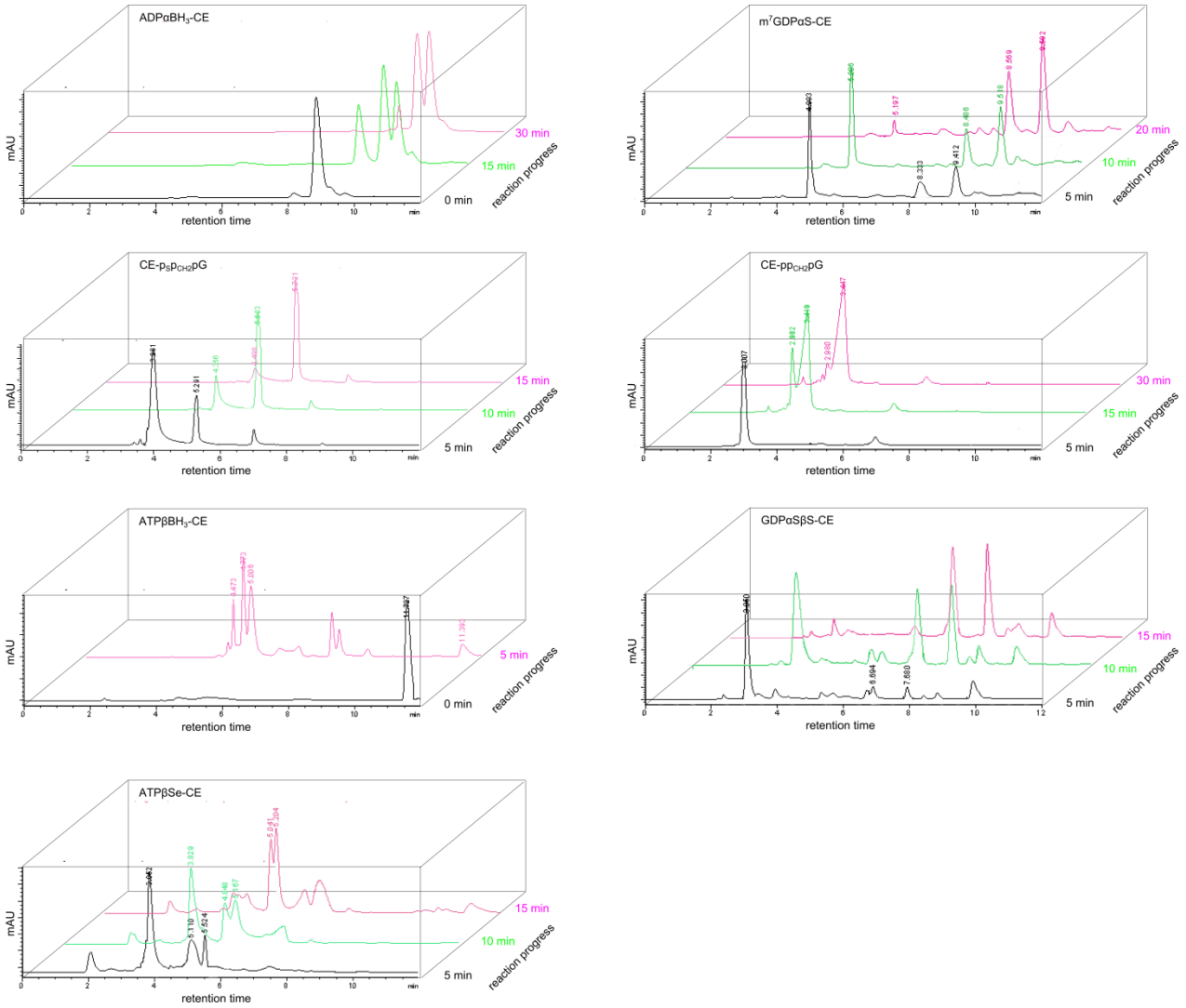
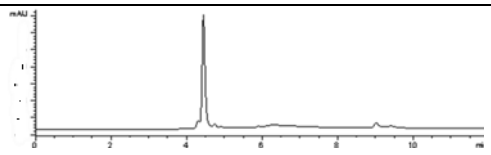
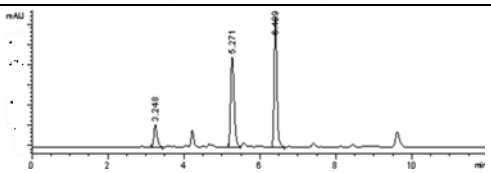
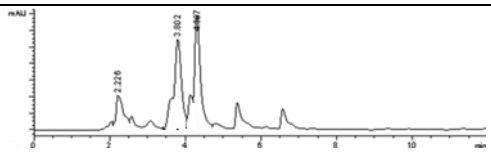
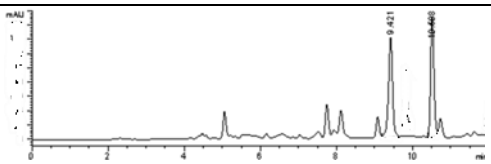
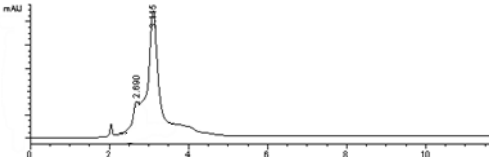
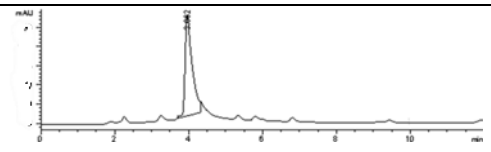
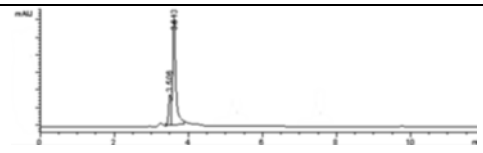


Fig. S4. Exemplary RP HPLC profiles from microwave-assisted oligophosphate chain elongation reactions by means of reagents 1a and 1b at different time points.

a) Synthesis of ADPαBH_3-CE from adenosine 5'-boranophophate (AMPBH_3) and **1a**
b) Synthesis of m^7GDPαS-CE from 7-methylguanosine 5'-thiophosphate and **1a**
c) Synthesis of CE-p_SpCH_2pG from guanosine 5'-methylenebisphosphonate and **1b**
d) Synthesis of CE-ppCH_2pG from guanosine 5'-methylenediphosphate and **1a**
e) Synthesis of ATPβBH_3-CE from adenosine 5'-(2-selenodiphosphate) and **1a**
f) Synthesis of GDPαSβS-CE from guanosine 5'- thiophosphate and **1b**
g) Synthesis of ATPβSe-CE from adenosine 5'-(2-selenodiphosphate) and **1a**

II. Supplementary table.

Table S1. Microwave-assisted synthesis of selected nucleotides performed without Sephadex isolation of the final product.

Reaction product	Starting materials	Coupling time[a] / HPLC conversion into CE-nucleotide	HPLC profile after coupling reaction	CE removal time[b]
GDP	**GMP + 1a**	**15 min/100%**		**15 min**
GDPαS (2)	**GMPS + 1a**	**30 min/91%**		**20 min**
UDPαS (6)	**UMPS + 1a**	**30 min/86%**		**30 min**
ADPαSβS (9)	**AMPS +1b**	**30 min/88%**		**30 min**
ppCH$_2$pA (17)	**pCH$_2$pA + 1a**	**30 min/82%**		**30 min**
ppNHpA (19)	**pNHpA + 1a**	**30 min/95%**		**15 min**
p$_4$A (21)	**ATP + 1a**	**30 min/86%**		**15 min**

[a] reaction conditions: 8 equiv $MgCl_2$, DMF
[b] reaction conditions: 10–15% DBU; DMF, DTT;. μw: 40 °C, closed vessel, dynamic power max. 10 W.; reaction progress was followed by RP HPLC equipped with UV-VIS detector and coupled with electrospray ionization (ESI) quadrupole mass spectrometer.

III. Experimental procedures

1. General information.

Synthesized nucleotides were purified by ion-exchange chromatography on DEAE-Sephadex A-25 (HCO_3^- form) column. A column was loaded with reaction mixture and washed through with excess of water to remove metal (II) salt/EDTA complex. Then, the nucleotides were eluted using a linear gradient of triethylammonium bicarbonate (TEAB) in deionized water. After evaporation under reduced pressure with repeated additions of ethanol to decompose TEAB, compounds were isolated as triethylammonium (TEA) salts. Yields were calculated on the basis of either sample weight or (preferably) optical milliunits (opt.mu) of the product. Optical unit measurements were performed in 0.1 M phosphate buffer (pH =7 or pH =6 for m^7G nucleotides) at 260 nm.

Analytical HPLC was performed on Agilent Tech. Series 1200 using Supelcosil LC-18-T HPLC 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). Semi-preparative HPLC was performed on the same apparatus equipped with Discovery RP Amide C-16 HPLC column (25cm x 21.2 mm, 5µm, flow rate 5.0 mL/min) with linear gradients of acetonitrile in 0.05 M ammonium acetate buffer (pH 5.9) and UV-detection at 260 nm. The structure and homogeneity of each final product was confirmed by RP HPLC, high resolution mass spectrometry HRMS (ESI^-) and ^1H NMR and ^{31}P NMR spectroscopy. Intermediate products were characterized by low resolution MS (ESI^-) or NMR. Mass spectra were recorded on Thermo Scientific LTQ Orbitrap Velos (high resolution) and AB Sciex API 3200 (low resolution) spectrometers. ^1H NMR and ^{31}P NMR spectra were recorded at 25ºC 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 D_2O as an internal standard. ^{31}P NMR chemical shifts were reported to 20% phosphorus acid in D_2O as an external standard.

The reactions involving microwave irradiation were conducted in heavy-walled glass 10-mL pressurized vials and CEM's proprietary "snap-on" caps. The microwave heating was performed in a CEM Discover single-mode microwave cavity, using dynamic power mode with maximum power of 10 W and at 45 ± 1 °C and at 2450 MHz. The reaction mixtures were stirred with a magnetic stir bar during the irradiation. The temperature, pressure and power were monitored during the course of reactions using the provided software (standard infrared temperature sensor).

Solvents and chemical reagents were purchased from Sigma-Aldrich and used without any pre-treatment unless otherwise stated.

2. Preparation and characterization of phosphorylating reagents 1a and 1b.

(2-Cyanoethyl) phosphate TEA salt was obtained from commercially available (2-cyanoethyl) phosphate barium salt dihydrate (Sigma-Aldrich). A suspension of 5 g (15.5 mmol) of the barium salt in 100 mL of water was passed through Dowex 50 W x 8 (200–400 mesh) resin in H^+ form and eluted with water (~500 mL). The eluate was collected into a round bottom-flask containing a solution of TEA (3.2 mL) in ethanol (100 mL), evaporated and dried in vacuum over P_4O_{10}. (2-Cyanoethyl) thiophosphate sodium salt was prepared from trisodium thiophosphate dodecahydrate according to procedures described by Burgers *et al.*[1] and converted into TEA following analogous procedure as for (2-cyanoethyl) phosphate. Thereafter, an appropriate starting material, (2-cyanoethyl) phosphate or (2-cyanoethyl) thiophosphate TEA salt, was mixed with imidazole (10 equiv., 10.54 g, 0.155 mol), 2,2′-dithiodipyridine (3 equiv., 10.13 g, 46 mmol), DMF (25 mL), triethylamine (3 equiv., 4 mL, 30 mmol) and triphenylphosphine (3 equiv., 12.0 g, 46 mmol), and the mixture was stirred for 6–8 h. The product was precipitated from the reaction mixture with a solution of anhydrous $LiClO_4$ (4 equiv., 6.58 g, 61.9 mmol) in dry acetonitrile (250 mL). After cooling at 4°C, the precipitate was filtered off, washed repeatedly with cold, dry acetonitrile, and dried in vacuum over P_4O_{10}. Reagents **1a** and **1b** can be stored at 4 °C in a closed vessel for several months. Yields: **1a** – 2.86 g (13.8 mmol, 89%) from 5g (15.5 mmol) of (2-cyanoethyl) phosphate barium salt dihydrate; **1b** – 2.51 g (11.25 mmol, 89%) from 5g (12.6 mmol) of commercial trisodium thiophosphate dodecahydrate.

1a: ^1H NMR (400 MHz, D_2O, 25 °C) δ = 7.99 (s, 1 H), 7.37 (s, 1 H), 7.18 (s, 1 H), 4.07 (td, *J* = 5.8, 6.6 Hz, 2 H), 2.81 (t, *J* = 5.8 Hz, 32 H); ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = -7.65 (t, *J* =6.6 Hz, 1 P); MS (ESI^-) Calcd m/z for $C_6H_7N_3O_2P^-$ ($M\text{-}H^+$) 200.0, found 200.0.

1b:). ^1H NMR (400 MHz, D_2O, 25 °C) δ = 8.00 (s, 1 H), 7.38 (s, 1 H), 7.14 (br. s., 1 H), 2.93 (td, *J* = 6.8, 16.9 Hz, 2 H), 2.65 (t, *J* = 6.8 Hz, 2 H); ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 11.29 (t, *J* =16.9 Hz, 1 P); MS (ESI^-) Calcd m/z for $C_6H_7N_3O_2PS^-$ ($M\text{-}H^+$) 216.0, found 216.0.

3. Preparation of starting materials.

Thiophosphate, selenophosphate and boranophosphate TEA salts were obtained as described earlier (Kowalska et al. 2007, Kowalska et al. 2009, Nahum and Fischer 2004). ATP TEA salt was obtained from commercial ATP sodium salt by passing through Dowex 50 W x 8 (200–400 mesh) in TEA form. m^7GppCH_2p TEA salt was obtained as described earlier (Rydzik et al. 2009). 7-methylguanosine was prepared

from guanosine by methylation with CH_3I similar to previously reported procedure.[5] Nucleoside phosphorimidazolides, AMP-Im, GMP-Im, were prepared according to Mukaiyama and Hashimoto as described in general procedure **3.1**. Guanosine and adenosine 5'-*O*-(H-phosphonates) were prepared as described by Yoshikawa et al. (1970). Modified nucleoside 5'-*O*-monophosphates and 5'-*O*-diphosphates were prepared as described below (general procedures 3.1–3.5).

3.1. Synthesis of nucleoside phosphorimidazolides (AMP-Im, GMP-Im).

The appropriate starting material (nucleotide TEA salt) (0.010 mol, 1equiv.), imidazole (6.8 g, 0.10 mol, 10 equiv.), and 2,2′-dithiodipyridine (6.6 g, 0.030 mol, 3 equiv.) were mixed in 30 mL DMF. Triethylamine (4.3 mL, 3.0 g, 0.030 mol, 3 equiv.) and triphenylphosphine (7.9 g, 0.030 mol, 3 equiv.) were added, and the mixture was stirred for 6–8 h. The product was precipitated from reaction mixture with anhydrous $NaClO_4$ (0.040 mol, 4.9 g) solution in dry acetone (300 mL). After cooling at 4°C, the precipitate was filtered, washed repeatedly with cold, dry acetone, and dried in vacuum over P_4O_{10}.

3.2. Thiophosphorylation of nucleosides.

An appropriate nucleoside (1 mmol) dried overnight in vacuum over P_4O_{10} was suspended in trimethyl phosphate (10 mL) and placed on ice-bath. After cooling to 0 °C, 2,6-lutidine (4 mmol, 465 μL) and freshly distilled $PSCl_3$ (3 mmol, 305 μL) was added into the mixture and reaction was stirred at 0 °C until the disappearance of the starting material as determined by HPLC profile (1–6 h). Then reaction could by stopped by addition of 0.7 M TEAB (pH =7) to obtain 5'-*O*-monothiophosphates or continued until 5'-*O*-monothiophosphate was converted into nucleoside 2',3'-*O*-cyclothiophosphate-5'-*O*-(thiophosphate) and then stopped the same way. The solution was washed 3 times with ethyl acetate and then products were purified by DEAE-Sephadex and isolated as TEA salts. Products containing some inorganic contamination after chromatography, were additionally precipitated with acetonitrile, centrifuged and freeze-dried.

Guanosine 5'-*O*-monothiophosphate, GMPS. GMPS (428 mg, 9032 opt.mu, 0.750 mmol, 76%; TEA salt) was obtained starting from guanosine (315 mg, 0.980 mmol) following the general procedure of thiophosphorylation of nucleosides. Reaction time: 3 h. R_t= 3.0 min; ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 44.09 (s, 1P); MS (ESI^-) Calcd m/z for $C_{10}H_{13}N_5O_7PS^-$ ($M\text{-}H^+$) 378.0, found 378.0; Exemplary previous synthesis: Eckstein F., *JACS.* **1970**, 92, 4718–4723.

7-methylguanosine 5'-*O*-monothiophosphate, m^{7}GMPS. m^{7}GMPS (190 mg, 4000 opt.mu, 0.350 mol, 70%; TEA salt) was obtained starting from guanosine (170 mg, 0.51 mmol) following the general procedure of thiophosphorylation of nucleosides. Reaction time: 3 h. R_t= 5.5 min; ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 46.80.(s, 1P); MS (ESI^-) Calcd m/z for $C_{11}H_{15}N_5O_7PS^-$ ($M\text{-}H^+$) 392.0, found 392.0. Exemplary previous synthesis: Kowalska J. et al. *RNA* **2008**, 14, 1–13.

Adenosine 5'-*O*-monothiophosphate, AMPS. AMPS (371 mg, 10280 opt.mu, 0.685 mol, 61%; TEA salt) was obtained starting from adenosine (300 mg, 1.123 mmol) following the general procedure of thiophosphorylation of nucleosides. Reaction time: 5 h. R_t = 4.6 min; ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 47.45 (s, 1P); MS (ESI^-) Calcd m/z for $C_{10}H_{13}N_5O_6PS^-$ ($M\text{-}H^+$) 362.0, found 362.0. Exemplary previous synthesis: Eckstein F., *JACS.* **1970**, 92, 4718–4723.

Uridine 5'-*O*-monothiophosphate, UMPS. UMPS (650 mg, 11780 opt.mu 1.220 mmol, 75%; TEA salt) was obtained starting from uridine (500 mg, 1.640 mmol) following the general procedure of thiophosphorylation of nucleosides. Reaction time: 2 h. R_t = 2.4 min; ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 45.75 (s, 1P); MS (ESI^-) Calcd m/z for $C_9H_{12}N_2O_8PS^-$ ($M\text{-}H^+$) 339.0, found 339.0. Exemplary previous synthesis: Eckstein F., *JACS.* **1970**, 92, 4718–4723.

Uridine 2',3'-*O*-cyclothiophosphate 5'-*O*-thiophosphate, $^{2'3'\text{-}cyc}$*P*$_S$-UMPS. $^{2'3'\text{-}cyc}$*P*$_S$-UMPS. (360 mg, 4850 opt.mu, 0.502 mmol, 51%; TEA salt) was obtained starting from uridine (300 mg, 0.984 mmol) following the general procedure of thiophosphorylation of nucleosides. Reaction time: 8 h. R_t = 2.3 min; ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 76.10 (t, *J* =6.3 Hz, 1P), 49.44 (s, 1P); MS (ESI^-) Calcd m/z for $C_9H_{11}N_2O_9P_2S_2^-$ ($M\text{-}H^+$) 417.0, found 417.0; Exemplary previous synthesis: Eckstein F., *JACS.* **1970**, 92, 4718–4723.

3.3. Preparation of nucleoside 5'-*O*-boranophosphates.

Nucleoside-5'-*O*-(H-phosphonate) was placed in a rubber septum capped round-bottom flask under argon atmosphere and suspended in acetonitrile (30 mL/100 mg) Then, N,O-bis(trimethylsilyl) acetamide (BSA, 15–20 equiv.) was added through a syringe and after 30 min of stirring, $BH_3{\cdot}SMe_2$ (2 equiv.) was added and the mixture was left for 30 min. An adequate product was released after addition of TEA/MeOH solution. The solution was evaporated to dryness and 3 times re-evaporated from methanol. Obtained crude products were used for the reaction with **1a** without further purification. For the purpose of NMR characterization, the products were purified on DEAE-Sephadex and isolated as TEA salts and the preparative yields are given for these syntheses.

Adenosine 5'-*O*-monoboranophosphate, AMPBH$_3$; AMPBH$_3$ (305 mg, 9720 opt.mu, 0.65 mmol, 54%; TEA salt) was obtained starting from adenosine H-phosphonate triethylammonium salt (540 mg, 18,000 opt.mu, 1.2 mmol mmol) following the general procedure. R_t = 7.5 min; ^{1}H NMR (400 MHz, D_2O, 25 °C) δ = 8.54 (s, 1 H), 8.23 (s, 1 H), 6.14 (d, *J* = 5.7 Hz, 1 H), 4.76 (dd, *J* = 5.2, 5.7 Hz, 1 H), 4.50 (dd, *J* = 3.2, 5.2 Hz, 1 H), 4.39 (q, *J* = 3.2 Hz, 1 H), 4.16–

4.05 (m, 2 H), 3.20 (q, *J* = 7.5 Hz, 6 H), 1.28 (t, *J* = 7.5 Hz, 9 H), 0.36 (q, *J* = 88.0 Hz, 3 H); ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 88.14 (q, *J* =143 Hz, 1P) MS (ESI$^-$) Calcd m/z for $C_{10}H_{16}BN_5O_6P^-$ (M-H$^+$) 344.1, found 344.1.

Guanosine 5'-*O*-monoboranophosphate, GMPBH$_3$; GMPBH$_3$ (650 mg, 16560 opt.mu, 1.4 mmol, 60%; TEA salt) was obtained starting from guanosine H-phosphonate (1030 mg, 27600 opt.mu, 2.3 mmol) following the general procedure. R_t = 5.5 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ = 8.19 (s, 1 H), 5.93 (d, *J* = 6.0 Hz, 1 H), 4.76 (dd, *J* = 4.8, 6.0 Hz, 1 H), 4.47 (dd, *J* = 3.1, 4.8 Hz, 1 H), 4.32 (q, *J* = 3.1 Hz, 1 H), 4.07–4.00 (m, 1 H), 3.20 (q, *J* = 7.5 Hz, 6 H), 1.28 (t, *J* = 7.5 Hz, 9 H), 0.76–-0.14 (m, 3 H) ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 85.80 (q, *J* =146 Hz, 1P); MS (ESI$^-$) Calcd m/z for $C_{10}H_{16}BN_5O_7P^-$ (M-H$^+$) 360.1, found 360.1.

3.4. Preparation of nucleoside 5'-*O*-(2-thiodiphosphates) and 5'-*O*-(2-selenodiphosphates)

Generally, these compounds were synthesized following several previously published procedures (Kowalska et al. 2007; Kowalska et al. 2009). An appropriate nucleoside 5'-*O*-monophosphate phosphorimidazolide (1 mmol, 1 equiv.), either thiophosphate or selenophosphate TEA salt (2 mmol, 2 equiv.) and anhydrous $ZnCl_2$ (8 mmol, 8 equiv.) were dissolved in DMF and stirred at RT. After 30 min, the reaction was quenched by addition of solution of EDTA (8 mmol, 8 equiv.) and $NaHCO_3$ (18 equiv.) in water. Products were purified on DEAE-Sephadex and isolated as TEA salts.

Adenosine 5'-*O*-(2-thiodiphosphate), ADPβS; ADPβS (160 mg, 5460 opt.mu, 0.364 mmol, 70%; TEA salt) was obtained starting from AMP-Im (200 mg, 7790 opt.mu, 0.520 mmol, sodium salt) following the general procedure. R_t = 4.8 min; ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 30.40 (d, *J* =29.8 Hz, 1 P), -11.58 (d, *J* =29.8 Hz, 1P); MS (ESI$^-$) Calcd m/z for $C_{10}H_{14}N_5O_9P_2S^-$ (M-H$^+$) 442.0, found 442.0. Exemplary previous synthesis: Eckstein F. et al., *Biochemistry,* **1976**, 15, 1685–91.

Guanosine 5'-*O*-(2-thiodiphosphate), GDPβS; GDPβS (180 mg, 2820 opt.mu, 0.230 mmol, 79%; TEA salt) was obtained starting from GMP-Im (150 mg, 3570 opt.mu, 0.300 mmol, sodium salt) following the general procedure. R_t = 3.0 min; ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 34.69 (d, *J* =31.4 Hz, 1 P), -11.57 (d, *J* =31.4 Hz, 1 P); MS (ESI$^-$) Calcd m/z for $C_{10}H_{14}N_5O_{10}P_2S^-$ (M-H$^+$) 458.0, found 458.0. Exemplary previous synthesis: Eckstein F. et al., *Biochemistry,* **1976**, 15, 1685–91.

Adenosine 5'-*O*-(2-selenodiphosphate), ADPβSe; ADPβSe (145 mg, 2900 opt.mu, 0.193 mmol, 25%; TEA salt) was obtained starting from AMP-Im (360 mg, 11680 opt.mu, 0.779 mmol, sodium salt) following the general procedure. After quenching with EDTA/$NaHCO_3$ aqueous solution, the mixture was extracted several times with CH_2Cl_2 to to remove elemental selenium. R_t = 3.9 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ = 8.57 (s, 1 H), 8.25 (s, 1 H), 6.11 (d, *J* =5.5 Hz, 2 H), 4.78 (dd, *J* =5.5, 4.9 Hz, 1 H), 4.63 (dd, *J* =5.0, 3.0 Hz, 1 H), 4.35–4.41 (m, 1 H), 4.16–4.31 (m, 2 H), 3.20 (q, *J* = 7.6 Hz, 12 H), 1.28 (t, *J* = 7.6 Hz, 18 H); ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 21.68 (d, *J* =35.2 Hz, 1 P) -12.28 (d, *J* =35.2 Hz, 1 P) MS (ESI$^-$) Calcd m/z for $C_{10}H_{14}N_5O_9P_2Se^-$ (M-H$^+$) 489.9, found 489.9.

Guanosine 5'-*O*-(2-selenodiphosphate), GDPβSe; GDPβSe (232 mg, 5720 opt.mu, 0.470 mmol, 65%; TEA salt) was obtained starting from GMP-Im (360 mg, 8820 opt.mu, 0.840 mmol; sodium salt) following the general procedure. After quenching with EDTA/$NaHCO_3$ aqueous solution, the mixture was extracted several times with CH_2Cl_2 to remove elemental selenium. R_t = 2.4 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ = 8.06–7.98 (m, 1 H), 5.84 (d, *J* = 5.2 Hz, 1 H), ~ 4.82–4.78 (overlapped with water, m, 1 H), 4.56–4.51 (m, 1 H), 4.32–4.27 (m, 1 H), 4.23–4.15 (m, 2 H), 3.19 (q, *J* = 7.5 Hz, 12 H), 1.27 (t, *J* = 7.5 Hz, 18 H); ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 26.53 (d, *J* =37.0 Hz, 1 P) -12.11 (d, *J* =37.0 Hz, 1 P); MS (ESI$^-$) Calcd m/z for $C_{10}H_{15}N_5O_{13}P_2Se^-$ (M-H$^+$) 505.9, found 505.9.

3.5. Preparation of nucleoside 5'-*O*-imidodiphosphates

Generally, these compounds were synthesized following a previously published procedure (Tomasz, J., et al. 1988). Nucleoside (1 mmol) was suspended in 8 mL of trimethyl phosphate and cooled to -8°C. Then, $Cl_3PNP(O)Cl_2$ (3 mmol, 3 equiv.) was added under vigorous stirring. Reaction was maintained at -8°C and was stopped after 2 h by addition of 0.7 M TEAB to pH =7 and diluted with water. The product was purified on DEAE-Sephadex and isolated as TEA salt.

Adenosine 5'-*O*-imidodiphosphate, pNHpA; pNHpA (366 mg, 13000 opt.mu, 0.86 mmol, 44%) was obtained starting from 500 mg (28140 opt.mu, 1.82 mmol) of adenosine following the general procedure. R_t = 5.2 min; ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 0.95 (m, 1 P) -0.61 (d, *J* =5.8 Hz, 1 P); MS (ESI$^-$) Calcd m/z for $C_{10}H_{15}N_6O_9P_2^-$ (M-H$^+$) 425.0, found 425.0; Exemplary previous synthesis: Tomasz J. et al., *Nucl. Acids Res.* **1988**, 16, 8645–8664.

Guanosine 5'-*O*-imidodiphosphate, pNHpG; pNHpG (931 mg, 14280 opt.mu, 134 mmol, 56%) was obtained starting from 600 mg (25400 opt.mu, 2.12 mmol) guanosine following the general procedure. R_t = 4.1 min; ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 0.96 (m, 1P), -0.64 (d, 1P, *J* =5.5 Hz); MS (ESI$^-$) Calcd m/z for $C_{10}H_{15}N_6O_{10}P_2^-$ (M-H$^+$) 441.0, found 441.0; Exemplary previous synthesis: Tomasz J.et al, *Nucl. Acids Res.* **1988**, 16, 8645–8664.

3.6. Preparation of nucleoside 5'-O-methylenebisphosphonates

Generally, these compounds were synthesized following a previously published procedure (Kalek, M. et al. 2005). Solution of methylenebis(phosphonic dichloride) (300 mg, 1.2 mmol, 1.2 equiv.) in 8 mL of trimethyl phosphate cooled to 0 °C was added to a suspension of nucleoside (1 mmol, 1 equiv.) in 8 mL of trimethyl phosphate at 0 °C. The reaction mixture was stirred at 0 °C. After 1 h, 0.7 M aqueous TEAB (to achieve pH 7.0) was added. The product was purified on DEAE-Sephadex and isolated as TEA salt.

Adenosine 5'-*O*-methylenediphosphate, pCH_2pA; pCH_2pA (376 mg, 10260 opt.mu, 0.86 mmol, 61%; TEA salt) was obtained starting from 300 mg (16880 opt.mu, 1.12 mmol) adenosine following the general procedure. R_t = 4.4 min; ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 19.32 (m, 1P) 15.59 (m, 1P); MS (ESI^-) Calcd m/z for $C_{11}H_{16}N_5O_9P_2^-$ ($M-H^+$) 424.0, found 424.0; Exemplary previous synthesis: Kalek M., Jemielity J., Stepinski J., Stolarski R., Darzynkiewicz E., *Tetrahedron Lett.,* **2005***,* 46, 2417–2421.

Guanosine 5'-*O*-methylenediphosphate, pCH_2pG; pCH_2pG (502 mg, 13700 opt.mu, 1.14 mmol, 65%) was obtained starting from 600 mg (25400 opt.mu,, 2.12 mmol) guanosine following the general procedure. R_t = 3.3 min; ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 19.27 (m, 1P) 15.70 (dt J =19.9, 9.8 Hz 1 P); MS (ESI^-) Calcd m/z for $C_{11}H_{16}N_5O_{10}P_2^-$ ($M-H^+$) 440.0378, found 440.0; Exemplary previous synthesis: Kalek M., Jemielity J., Stepinski J., Stolarski R., Darzynkiewicz E., *Tetrahedron Lett.,* **2005***,* 46, 2417–2421.

4. General procedures for the phosphorylation reactions by means of 1a and 1b (synthesis of 2-24)

4.1. Conventional synthesis (2–14, 16–23).

An appropriate nucleotide (0.30 mmol, 1 equiv., TEA salt) was suspended in 3 mL of DMF. Then, **1a** (186 mg, 0.90 mmol, 3 equiv.) (syntheses **2–8**, **11–24**) or **1b** (201 mg, 0.90 mmol, 3 equiv.) (syntheses **9–10**, **24**) and anhydrous $MgCl_2$ (228 mg, 2.4 mmol, 8 equiv.) were added to the suspension. In the case of syntheses of **14** and **15** starting materials were generated in situ (due to difficulties in their synthesis and isolation) from nucleoside 5'-phosphoridimidazolides NMP-Im (0.300 mmol, 1 equiv.) and triethylammonium boranophosphate (352 mg, 1.8 mmol, 6 equiv.) mixed together with **1a** (186 mg, 0.90 mmol, 3 equiv.) and anhydrous $MgCl_2$ (228 mg, 2.4 mmol, 8 equiv.) in 3 mL DMF. The mixture was stirred at room temperature until the starting material disappeared, as determined by HPLC. Next, the product was subjected to cyanoethyl group removal (one-pot reaction): The solution was diluted with 3 mL of DMF and 1.1 mL of DBU was added. The reaction was carried out at 50 °C in an open flask protected from moisture (capped with drying tube packed with anhydrous calcium chloride) or alternatively DTT (138 mg, 0.9 mmol, 3 equiv.) was added to the mixture. The reaction was stopped by addition of 1% acetic acid to pH =7, diluted with water and washed 3 times with ethyl acetate. Finally, products were purified on DEAE-Sephadex and isolated as TEA salts. Exact amounts used for particular reactions, yields and reaction times are provided in section 5.

4.2. Microwave-assisted synthesis (compounds 5, 7, 10, 13, 15, 18, 24).

An appropriate nucleotide (0.1 mmol, TEA salt) was placed in a 10 ml microwave tube and suspended in 1 mL of DMF. Then, **1a** (62 mg, 0.300 mmol, 3 equiv.) (syntheses **5, 7, 13, 18**) or **1b** (67 mg, 0.300 mmol, 3 equiv.) (syntheses **10**, **24**) and anhydrous $MgCl_2$ (76 mg, 0.8 mmol, 8 equiv.) were added to the suspension. The tube was heated for 10–30 min in the microwave oven using dynamic power mode (parameters: P_{max} = 10 W and T_{max} = 45 ± 1 °C). Then, 1 mL of DMF, 0.35 mL DBU and DTT (3 equiv.) were added to the mixture to remove the 2-cyanoethyl group. The tube was again heated in the microwave oven using the same parameters as given above. The reaction was stopped by addition of 1% acetic acid to pH =7, diluted with water and washed with ethyl acetate. Products were purified on DEAE-Sephadex and isolated as TEA salts. Exact amounts used for particular reactions, yields and reaction times are provided in section 5.

5. Synthesis and characterization data for the compounds 2–24.

5.1. Guanosine 5'-*O*-(1-thiodiphosphate); GDPαS (2)

2 (93 mg, 1625 opt.mu, 0.135 mmol, 37%; TEA salt) was obtained starting from 200 mg (4400 opt.mu, 0.365 mmol) of GMPS (TEA salt) following the general procedure **4.1.** Coupling time: 3 d; deprotection time: 5 h. Diastereoisomeres D1**(2a)** and D2 **(2b)** were later separated on semi-preparative RP HPLC and after repeated freeze-drying, were isolated as ammonium salts.

(2a) R_t = 3.6 min; ^{1}H NMR (400 MHz, D_2O, 25 °C) δ = 8.24 (s, 1 H), 5.96 (d, *J* = 6.0 Hz, 1 H), 4.82 (dd, *J* = 5.0, 6.0 Hz, 1 H), 4.57 (dd, *J* = 3.2, 5.0 Hz, 1 H), 4.42–4.37 (m, 1 H), 4.32–4.23 (m, 2 H); ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 82.50 (m, 1 P) -10.16 (d, *J* =28.8 Hz, 1 P); HRMS (ESI^-) Calcd m/z for $C_{10}H_{14}N_5O_{10}P_2S^-$ ($M-H^+$) 457.9942, found 457.9928; **(2b)** R_t = 4.1 min; ^{1}H NMR (400 MHz,D_2O, 25 °C) δ = 8.19 (s, 1 H), 5.94 (d, *J* = 6.0 Hz, 1 H), 4.82 (dd, *J* = 4.5, 6.0 Hz, 1 H), 4.56 (dd, *J* = 2.5, 4.5 Hz, 1 H), 4.41–4.36 (m, 1 H), 4.29–4.23 (m, 2 H); ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 82.50 (m, 1 P) -10.16 (d, *J* =28.8 Hz, 1 P); HRMS (ESI^-) Calcd m/z for $C_{10}H_{14}N_5O_{10}P_2S^-$ ($M-H^+$) 457.9942, found 457.9927; Exemplary previous synthesis: Connolly B. et al, *Biochemistry,* **1982,** 21**,** 1983**–**1989.

5.2. Guanosine 5'-*O*-(1-boranodiphosphate); GDPαBH$_3$ (3)

3 (125 mg, 2280 opt.mu, 0.19 mmol, 62%, TEA salt) was obtained starting from crude $GMPBH_3$ (3720 opt.mu, 0.31

mmol, TEA salt) following the general procedure **4.1.** Coupling time: 24 h; deprotection time: 5 h. Diastereoisomeres D1 **(3a)** and D2 **(3b)** were later separated on semi-preparative RP HPLC and after repeated freeze-drying, were isolated as ammonium salts.

(3a) R_t = 4.8 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ = 8.21 (s, 1 H), 5.96 (d, *J* = 6.0 Hz, 1 H), 4.77 (dd, *J* = 4.8, 6.0 Hz, 1 H), 4.57 (dd, *J* = 3.4, 4.8 Hz, 1 H), 4.37 (dt, *J* = 2.7, 3.0 Hz, 1 H), 4.25–4.12 (m, 2 H), 1.11–-0.14 (m, 3 H); ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 41.32 (dt, *J* =40.04, 6.10 Hz, 1 P) 34.37 (d, *J* =40.00 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{10}H_{17}BN_5O_{10}P_2^-$ (M-H$^+$) 440.0549, found 440.0555 **(3b)** R_t = 5.5 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ = 8.18 (s, 1 H), 5.95 (d, *J* = 6.0 Hz, 1 H), 4.78 (dd, *J* = 5.2, 6.0 Hz, 1 H), 4.51 (dd, *J* = 3.5, 5.2 Hz, 1 H), 4.37 (td, *J* = 3.0, 3.5 Hz, 1 H), 4.28–4.13 (m, 2 H), 0.89–-0.05 (m, 3 H) ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ =; HRMS (ESI$^-$) Calcd m/z for $C_{10}H_{17}BN_5O_{10}P_2^-$ (M-H$^+$) 440.0549, found 440.0556. Exemplary previous synthesis: Li P. et al., *JACS*, **2005**, 127, 16782–16783.

5.3. Adenosine 5'-*O*-(1-thiodiphosphate); ADPαS (4).

4 (127 mg, 2670 opt.mu, 0.178 mmol, 61%; TEA salt) was obtained starting from 228 mg (4350 opt.mu, 0.290. mmol) of AMPS (TEA salt) following the general procedure **4.1.** Coupling time: 3 d; deprotection time: 5 h. Diastereoisomeres D1**(4a)** and D2 **(4b)** were later separated on semi-preparative RP HPLC and after repeated freeze-drying, were isolated as ammonium salts.

5.3.1. Microwave assisted synthesis of (4).

7 (143 mg, 3000 opt.mu, 0.200 mmol, 63%; TEA salt) was obtained starting from 250 mg of AMPS (TEA salt, 4770 opt.mu, 0.318 mmol) following the general procedure **4.2.** Coupling time: 30 min; deprotection time: 20 min.

(4a) R_t = 5.3 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ = 8.66 (s, 1 H), 8.26 (s, 1 H), 6.16 (d, *J* = 5.7 Hz, 1 H), 4.82–4.81 (m, /overlapped by solvent/ 1 H), 4.59 (dd, *J* = 4.1, 4.9 Hz, 1 H), 4.45–4.40 (m, 1 H), 4.28–4.21 (m, 1 H);^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 43.40 (d, *J* =27.0 Hz, 1 P), -9.51 (d, *J* =27.0 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{10}H_{14}N_5O_9P_2S^-$ (M-H$^+$) 441.9993, found 441.9980; **(4b)** R_t = 6.3 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ = 8.61 (s, 1 H), 8.27 (s, 1 H), 6.16 (d, *J* = 6.0 Hz, 1 H), 4.80 (dd, *J* = 4.9, 6.0 Hz, 1 H), 4.57 (dd, *J* = 3.5, 4.9 Hz, 1 H), 4.45–4.41 (m, 1 H), 4.30–4.25 (m, 2 H); ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 43.40 (d, *J* =27.0 Hz, 1 P), -9.51 (d, *J* =27.0 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{10}H_{14}N_5O_9P_2S^-$ (M-H$^+$) 441.9993, found 441.9985; Exemplary previous synthesis: Li P. et al., *JACS*, **2005**, 127, 16782–16783.

5.4. Adenosine 5'-*O*-(1-boranodiphosphate); ADPαBH$_3$ (5).

5 (2340 opt.mu, 0.16 mmol, 65%, TEA salt) was obtained starting from crude AMPBH$_3$ (TEA salt, 3580 opt.mu, 0.24 mmol) following the general procedure **4.1.** Coupling time: 24 h; deprotection time: 3 h**.**

5.4.1. Microwave assisted synthesis of (5).

5 (5460 opt.mu, 0.364 mmol, 58%; TEA salt) was obtained starting from crude AMPBH$_3$ (TEA salt, 9420 opt.mu, 0.63 mmol) following the general procedure **4.2.** Coupling time: 30 min; deprotection time: 20 min.

(5a) R_t = 6.0 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ = 8.57 (s, 1 H), 8.22 (s, 1 H), 6.12 (d, *J* = 5.7 Hz, 1 H), 4.76 (dd, *J* = 5.0, 5.7 Hz, 1 H), 4.58 (dd, *J* = 3.2, 5.0 Hz, 1 H), 4.40 (td, *J* = 3.0, 3.2 Hz, 1 H), 4.30–4.13 (m, 2 H), 3.19 (q, *J* = 7.5 Hz, 12 H), 1.27 (t, *J* = 7.5 Hz, 18 H), 0.87–-0.02 (m, 3 H); ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 82.50 (m, 1 P), -10.68 (d, *J* =30.3 Hz, 1 P); HRMS (ESI$^+$) Calcd m/z for $C_{10}H_{19}BN_5O_{10}P_2^+$ (M+H$^+$) 426.0746 found 426.0742. **(5b)** R_t= 7.1 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ = 8.56 (s, 1 H), 8.22 (s, 1 H), 6.12 (d, *J* = 5.7 Hz, 1 H), 4.76 (dd, *J* = 5.0, 5.7 Hz, 1 H), 4.52 (dd, *J* = 3.4, 5.0 Hz, 1 H), 4.40 (td, *J* = 3.0, 3.4 Hz, 2 H), 4.30–4.13 (m, 2 H), 0.87–-0.02 (m, 3 H); ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 82.50 (m, 1 P), -10.68 (d, *J* =30.3 Hz, 1 P); HRMS (ESI$^+$) Calcd m/z for $C_{10}H_{19}BN_5O_{10}P_2^-$ (M+H$^+$) 426.0746, found 426.0744. Exemplary previous synthesis: Li P. et al. *JACS*, **2005**, 127, 16782–16783.

5.5. Uridine 5'-*O*-(1-thiodiphosphate); UDPαS (6).

6 (48 mg, 650 opt.mu, 0.067 mmol, 65%; TEA salt) was obtained starting from 53 mg (1000 opt.mu, 0.104 mmol) of UMPS (TEA salt) following the general procedure **4.1.** Coupling time: 3 d; deprotection time: 5 h

(6a) R_t = 4.7 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ = 8.09 (d, *J* = 8.2 Hz, 1 H), 6.00 (d, *J* = 4.3 Hz, 1 H), 5.98 (d, *J* = 8.2 Hz, 1 H), 4.43–4.37 (m, 2 H), 4.35–4.24 (m, 3 H), 3.19 (q, *J* = 7.5 Hz, 12 H), 1.27 (t, *J* = 7.5 Hz, 18 H); ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 42.69 (dt, *J* =28.6, 6.3 Hz, 1 P) -11.34 (d, *J* =28.6 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_9H_{13}N_2O_{11}P_2S^-$ (M-H$^+$) 418.9721, found 418.9713; **(6b)** R_t = 5.6 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ =8.06 (d, *J* = 8.2 Hz, 1 H), 6.00 (d, *J* = 4.3 Hz, 1 H), 5.98 (d, *J* = 8.2 Hz, 1 H), 4.43–4.37 (m, 2 H), 4.35–4.24 (m, 3 H), 3.19 (q, *J* = 7.5 Hz, 12 H), 1.27 (t, *J* = 7.5 Hz, 18 H); ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 42.53 (dt, *J* =28.6, 6.3 Hz, 1 P) -11.24 (d, *J* =28.6 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_9H_{13}N_2O_{11}P_2S^-$(M-H$^+$) 418.9721, found 418.9713; Exemplary previous synthesis: Sheu K.R. et al., *Biochemistry*, **1979**, 18, 5548–5556.

5.6. N^7-methylguanosine 5'-*O*-(1-thiodiphosphate) m^{7}GDPαS (7).

7 (250 mg, 2560 opt.mu, 0.225 mmol, 39%; TEA salt) was obtained starting from 310 mg (6525 opt.mu, 0.572 mmol) of

m^7GMPS (TEA salt) following the general procedure **4.1.** Coupling time: 3 d; deprotection time: 6 h. Diastereoisomeres D1 **(7a)** and D2 **(7b)** were later separated on semi-preparative RP HPLC and after repeated freeze-drying, were isolated as ammonium salts.

5.6.1. Microwave assisted synthesis of (7).

7 (42 mg, 430 opt.mu, 0.038 mmol, 60%; TEA salt) was obtained starting from 34 mg of m^7GMPS (TEA salt, 720 opt.mu, 0.063 mmol) following the general procedure **4.2.** Coupling time: 20 min; deprotection time: 20 min.

(7a) R_t = 5.0 min ^{1}H NMR (400 MHz, D_2O, 25 °C) δ = 9.30 (s, 1 H), 6.09 (d, *J* = 3.5 Hz, 1 H), 4.72 (dd, *J* = 3.5, 4.7 Hz, 1 H), 4.54 (dd, *J* = 4.7, 5.5 Hz, 1 H), 4.46–4.42 (m, 1 H), 4.37 (ddd, *J* = 2.2, 4.7, 12.0 Hz, 1 H), 4.28 (dd, *J* = 6.8, 12.0 Hz, 2 H), 4.16 (s, 3 H); ^{31}P NMR (162 MHz D_2O, 25 °C) δ = 43.73 (d, *J* =28.0 Hz, 1 P), -10.66 (d, *J* =28.0 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{11}H_{16}N_5O_{10}P_2S^-$ (M-H$^+$) 472.0098, found 472.00826; **(7b)** R_t = 6.4 min;^{1}H NMR (400 MHz, D_2O, 25 °C) δ = 9.29 (s, 1 H), 6.08 (d, *J* = 3.2 Hz, 1 H), 4.71 (dd, *J* = 3.2, 5.0 Hz, 1 H), 4.53 (t, *J* = 5.0 Hz, 1 H), 4.46–4.42 (m, 1 H), 4.40 (dd, *J* = 5.3, 12.0 Hz, 1 H), 4.27 (dd, *J* = 6.0, 12.0 Hz, 1 H), 4.15 (s, 3 H); ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 43.45 (d, *J* =26.0 Hz, 1 P), -10.54 (d, *J* =26.0 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{11}H_{16}N_5O_{10}P_2S^-$ (M-H$^+$) 472.0098, found 472.0084.

5.7. Uridine 2',3'-*O*,*O*-cyclothiophosphate 5'-*O*-(1-thiodiphosphate); $^{2'3'\text{-}cyc}$-P_S-UDPαS (8).

8 (70 mg, 1015 opt.mu, 0.106 mmol, 59%; TEA salt) was obtained starting from 92 mg (1730 opt.mu 0.180 mmol) of $^{2'3'\text{-}cyc}$-P_S-**UMPS** (TEA salt) following the general procedure **4.1.** Coupling time: 5 d; deprotection time: 5 h. Diastereoisomeres D1**(8a)** and D2 **(8b)** were later separated on semi-preparative RP HPLC and after repeated freeze-drying, were isolated as ammonium salts.

(8a) R_t = 2.6 min; ^{1}H NMR (400 MHz, D_2O, 25 °C) δ = 7.89 (d, *J* = 8.1 Hz, 1 H), 6.09 (d, *J* = 2.2 Hz, 1 H), 5.94 (d, *J* = 8.1 Hz, 1 H), 5.22 (dd, *J* = 2.2, 4.2 Hz, 1 H), 5.19 (dd, *J* = 1.6, 4.2 Hz, 1 H), 4.64–4.58 (m, 1 H), 4.29 (dd, *J* = 3.4, 6.3 Hz, 2 H); ^{31}P NMR (162 MHz, D_2O, 25 °C), δ = 76.90 (t, *J* =10.3 Hz, 1 P), 43.64 (dt, *J* =27.8, 6.3 Hz, 1 P), -10.49 (d, *J* =27.8 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_9H_{12}N_2O_{12}P_3S_2^-$ (M-H$^+$) 496.9050, found 496.9034; **(8b)** R_t = 2.8 min; ^{1}H NMR (400 MHz, D_2O, 25 °C) δ = 7.90 (d, *J* = 8.0 Hz, 1 H), 6.10 (br. s, 1 H), 5.95 (d, *J* = 8.0 Hz, 1 H), 5.24–5.21 (m, 1 H), 5.21–5.18 (m, 1 H), 4.65–4.58 (m, 1 H), 4.33–4.24 (m, 2 H); ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 76.92 (t, *J* =10.0 Hz, 1 P), 43.64 (dt, *J* =27.8, 6.3 Hz, 1 P), -10.48 (d, *J* =27.8 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_9H_{12}N_2O_{12}P_3S_2^-$ (M-H$^+$) 496.9050, found 496.9036.

5.8. Adenosine 5'-*O*-(1,2-dithiodiphosphate) ADPαSβS (9)

9 (48 mg, 840 opt.mu, 0.056 mmol, 20%, TEA salt) was obtained starting from 190 mg (4300 opt.mu, 0.287 mmol) of AMPS (TEA salt) following the general procedure **4.1.** Coupling time: 5 d; deprotection time: 5 h. D1 **(9a)** and D2 **(9b)** were later separated on semi-preparative RP HPLC and after repeated freeze-drying, were isolated as ammonium salts.

(9a) R_t = 4.5 min; ^{1}H NMR (400 MHz, D_2O, 25 °C) δ = 8.70 (s, 1 H), 8.24 (s, 1 H), 6.13 (d, *J* = 5.5 Hz, 1 H), 4.82 (dd, *J* = 5.0, 5.5 Hz, 1 H), 4.63 (dd, *J* = 4.0, 5.0 Hz, 1 H), 4.42–4.37 (m, 1 H), 4.36–4.28 (m, 1 H), 4.27–4.20 (m, 1 H); ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 41.21 (d, *J* =43.2 Hz, 1 P) 33.80 (d, *J* =43.2 Hz, 1 P); Calcd m/z for $C_{10}H_{14}N_5O_8P_2S_2^-$ (M-H$^+$) 457.9764, found **(9a)** 457.9766; **(9b)** R_t = 5.2 min; ^{1}H NMR (400 MHz, D_2O, 25 °C) δ = 8.63 (s, 1 H), 8.24 (s, 1 H), 6.12 (d, *J* = 5.7 Hz, 1 H), 4.81 (dd, *J* = 5.0, 5.7 Hz, 1 H), 4.64 (dd, *J* = 3.5, 5.0 Hz, 1 H), 4.42–4.38 (m, 1 H), 4.36–4.29 (m, 1 H), 4.28–4.20 (m, 1 H); ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 40.66 (dt, *J* =41.0, 6.5 Hz, 1P) 33.75 (d, *J* =41.0 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{10}H_{14}N_5O_8P_2S_2^-$ (M-H$^+$) 457.9764, found 457.9768; Exemplary previous synthesis: Ahmadibeni J. et al., *Org.Lett*., **2005**, 7 (25), 5589–5592.

5.9. Guanosine 5'-*O*-(1,2-dithiodiphosphate) GDPαSβS (10).

10 (85 mg, 1460 opt.mu, 0.121 mmol, 36%, TEA salt) was obtained starting from 182 mg (4000 opt.mu, 0.332 mmol) of GMPS (TEA salt) following the general procedure **4.1.** Coupling time: 5 d; deprotection time: 5 h.

5.9.1. Microwave assisted synthesis of (10).

10 (25 mg, 430 opt.mu, 0.036 mmol, 30%; TEA salt) was obtained starting from 55 mg of GMPS (TEA salt, 1445 opt.mu, 0.120 mmol) following the general procedure **4.2.** Coupling time: 15 min; deprotection time: 30 min.

(10a) R_t= 2.0 min; ^{1}H NMR (400 MHz, D_2O, 25 °C) δ = 8.25 (s, 1 H) 5.91 (d, *J* =6.10 Hz, 1 H) 4.82 (dd, *J* =6.10, 5.20 Hz, 1 H) 4.62 (dd, *J* =5.20, 3.00 Hz, 1 H) 4.34–4.38 (m, 1 H) 4.23–4.32 (m, 2 H); Calcd m/z for $C_{10}H_{14}N_5O_9P_2S_2^-$ (M-H$^+$) 473.9714, found 473.9716**;(10b)** R_t= 3.6 min; ^{1}H NMR (400 MHz, D_2O, 25 °C) δ = 8.25 (s, 1 H), 5.90 (d, *J* = 6.0 Hz, 1 H), 4.80 (dd, *J* = 5.0, 6.0 Hz, 1 H), 4.61 (dd, *J* = 3.5, 5.0 Hz, 1 H), 4.37–4.33 (m, 1 H), 4.30–4.23 (m, 2 H) ^{31}P NMR (162 MHz, D_2O, 25 °C) δ = 41.26 (dt, *J* =40.00, 6.10 Hz, 1 P) 33.81 (d, *J* =40.00 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{10}H_{14}N_5O_9P_2S_2^-$ (M-H$^+$) 473.9714, found 473.9701. Exemplary previous synthesis: Ludwig J. et al., *JOC***, 1991**, 56, 5860–5865.

5.10. Adenosine 5'-*O*-(2-thiotriphosphate); ATPβS (11).

11 (39 mg, 1012 opt.mu, 0.067 mmol, 62%; TEA salt) was obtained starting from 70 mg (1630 opt.mu, 0.109 mmol) of

ADPβS (TEA salt) following the general procedure **4.1.** Coupling time: 2 h; deprotection time: 12 h.

R_t= 3.9 min; ^{1}H NMR (400 MHz,D_2O, 25 ^oC) δ = 8.61 (s, 1 H), 8.32 (s, 1 H), 6.17 (d, *J* = 6.2 Hz, 1 H), 4.86 (dd, *J* = 4.5, 6.2 Hz, 1 H), 4.68–4.64 (m, 1 H), 4.46–4.41 (m, 1 H), 4.44–4.29 (m, 1 H), 4.29–4.22 (m, 1 H); **(11a)** ^{31}P NMR (162 MHz, D_2O, 25 ^oC) δ = 30,72 (m, 1 P), -10.69 (d, *J* = 22.0 Hz, 1 P), -11.31 (d, *J* =25.9 Hz,1 P) **(11b)** ^{31}P NMR, (162 MHz, D_2O, 25 ^oC): δ = 30.72 (m, 1 P), -10.69 (d, *J* = 22,0 Hz, 1P), -11.31 (d, *J* =25,9 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{10}H_{15}N_5O_{12}P_3S^-$ (M-H$^+$) 521.9656; found 521.9640. Exemplary previous synthesis: Goody R. S. & Eckstein F., *Biochemistry,* **1976**, 15, 1685–91.

5.11. Guanosine 5'-*O*-(2-thiotriphosphate); GTPβS (12).

12 (59 mg, 1290 opt.mu, 0.107 mmol, 73%) was obtained starting from 67 mg (1750 opt.mu, 0.145 mmol) of GDPβS (TEA salt) following the general procedure **4.1.** Coupling time: 24 h; deprotection time: 5 h.

R_t = 2.1 min; ^{1}H NMR (400 MHz,D_2O, 25 ^oC) δ = 8.18;8.17* (s, 1 H), 5.95 (d, *J* =6.5 Hz, 1 H), 4.87 (m, 1 H), 4.64 (m, 1 H), 4.38 (m, 1H), 4.31 (m, 1H), 4.24 (m, 1H); **(12a)** ^{31}P NMR (162 MHz, D_2O, 25 ^oC) δ =30.51 (t, *J* =26.9 Hz, 1P), -10.72 (d, *J* = 26.9 Hz, 1P), -11,34 (d, *J* =26.9 Hz, 1P); **(12b)** ^{31}P NMR (162 MHz, D_2O, 25 ^oC) δ = 30.54 (t, *J* =26.9 Hz, 1P), -10,72 (d, *J* = 26.9 Hz,1P), -11.34 (d, *J* =26.9 Hz, 1P); HRMS (ESI$^-$) Calcd m/z for $C_{10}H_{15}N_5O_{13}P_3S^-$ (M-H$^+$) 537.9605; found 537.9590. Exemplary previous synthesis: Connolly B. et al, *Biochemistry,* **1982,** 21*,* 1983–1989.

5.12. Adenosine 5'-*O*-(2-boranotriphosphate); ATPβBH_3 (13).

13 (65.5 mg, 1600 opt.mu, 0.107 mmol, 47%; TEA salt) was obtained starting from 100 mg of AMP-Im/Na^+ (3400 opt.mu, 0.240 mmol) and boranophosphate (TEA salt, 280 mg, 1.44 mmol) following the general procedure **4.1.** Coupling time: 5 h; deprotection time: 2 h. **13** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

5.12.1. Microwave assisted synthesis of (13).

13 (33 mg, 800 opt.mu, 0.054 mmol, 20%; TEA salt) was obtained starting from 150 mg of AMP-Im/Na^+ (4000 opt.mu, 0.267 mmol) and boranophosphate (TEA salt, 314 mg, 1.60 mmol) following the general procedure **4.2.** Coupling time: 5 min; deprotection time: 5 min.

R_t = 3.6 min;^{1}H NMR (400 MHz, D_2O, 25 ^oC) δ = 8.44 (s, 1 H), 8.13 (s, 1 H), 6.03 (d, *J* = 6.0 Hz, 1 H), 4.69 (dd, *J* = 5.0, 6.0 Hz, 1 H), 4.51 (dd, *J* = 3.0, 5.0 Hz, 1 H), 4.35–4.31 (m, 1 H), 4.17 (s, 2 H), 4.19–4.14 (m, 2 H), 0.81–-0.03 (m, 3 H); ^{31}P NMR (162 MHz, D_2O, 25 ^oC) δ = 72.39–76.45 (m, 1 P) -10.81 (d, *J* =30.8 Hz, 1 P) -11.29 (d, *J* =32.5 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{10}H_{18}BN_5O_{12}P_3^-$ (M-H$^+$) 504.0263; found 504.02494.

5.13. Guanosine 5'-*O*-(2-boranotriphosphate); GTPβBH_3 (14).

14 (37.1 mg, 1002 opt.mu, 0.083 mmol, 18%; TEA salt) was obtained starting from GMP-Im/Na^+ 235 mg (5640 opt.mu, 0.470 mmol) and boranophosphate (TEA salt, 553 mg, 2.82 mmol) following the general procedure **4.1.** Coupling time: 5 h; deprotection time: 2 h. **14** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

R_t = 2.6 min; ^{1}H NMR (400 MHz, D_2O, 25 ^oC) δ = 8.17 (s, 1 H) 5.97 (d, *J* =6.5 Hz, 1 H) 4.85 (dd, *J* =6.5, 5.0 Hz, 1 H) 4.62 (s, 1 H) 4.60–4.64 (m, 1 H) 4.38–4.43 (m, 1 H) 4.24–4.30 (m, 2 H); ^{31}P NMR (162 MHz, D_2O, 25 ^oC) δ = 74.60 (s, 1 P) 65.23–76.25 (m, 1 P) -10.47 (d, *J* =26.4 Hz, 1 P) -11.26 (d, *J* =32.5 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{10}H_{18}BN_5O_{13}P_3^-$ (M-H$^+$) 520.0212; found 520.0196.

5.14. Adenosine 5'-*O*-(2-selenotriphosphate) ATPβSe (15) (microwave assisted synthesis)

15 (21.2 mg, 560 opt.mu, 0.037 mmol, 43%) was obtained starting from 42.6 mg (1305 opt.mu, 0.086 mmol) of ADPβSe (TEA salt) following the general procedure **4.2.** Coupling time: 30 min; deprotection time: 5 min. **15** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

R_t = 3.8 min; ^{1}H NMR (400 MHz, D_2O,25 ^oC) δ = 8.54 (s, 1 H), 8.22 (s, 1 H), 6.11 (d, *J* = 5.8 Hz, 1 H), 4.81 (dd, *J* = 5.0, 5.8 Hz, 1 H), 4.64 (s, 1 H), 4.67–4.62 (m, 1 H), 4.38 (s., 1 H), 4.41–4.36 (m, 1 H), 4.33–4.15 (m, 2 H) **(15a)** ^{31}P NMR (162 MHz, D_2O, 25 ^oC) δ = 16.35 (dd, *J* =31.7, 30.8 Hz, 1 P) -11.99 (d, *J* =30.8 Hz, 1 P) -12.57 (d, *J* =31.7 Hz, 1 P) **(15b)** ^{31}P NMR (162 MHz, D_2O, 25 ^oC) δ = 16.30 (dd, *J* =31.7, 30.8 Hz, 1 P) -11.99 (d, *J* =30.8 Hz, 1 P) -12.57 (d, *J* =31.7 Hz, 1 P) HRMS (ESI$^-$) Calcd m/z for $C_{10}H_{15}N_5O_{12}P_3Se^-$ (M-H$^+$) 569.9101 found 569.9088.

5.15. Guanosine 5'-*O*-(2-selenotriphosphate) GTPβSe (16).

16 (11.1 mg, 230 opt.mu, 0.019 mmol, 15%; TEA salt) was obtained starting from 85.2 mg (1530 opt.mu, 0.127 mmol) of GDPβSe (TEA salt) following the general procedure **4.1.** Coupling time: 24 h; deprotection time: 2 h. **16** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

R_t = 2.6 min. ^{1}H NMR (400 MHz, D_2O, 25 ^oC) δ = 8.13 (s, 1 H), 5.90 (d, *J* = 6.1 Hz, 1 H), 4.81 (dd, *J* = 5.1, 6.1 Hz, 1 H), 4.62–4.58 (m, 1 H), 4.36–4.31 (m, 1 H), 4.30–4.17 (m, 2 H) ^{31}P NMR (162 MHz, D_2O, 25 ^oC) δ = 16.56 (s., 1 P) -11.97 (d, *J* =30.5 Hz, 1 P) -12.67 (d, *J* =30.5 Hz, 1 P) HRMS (ESI$^-$

) Calcd m/z for $C_{10}H_{15}N_5O_{13}P_3Se^-$ (M-H$^+$) 585.9050 found 585.90584.

5.16. Adenosine 5'-*O*-(1,2-methylenetriphoshate); ppCH$_2$pA (17).

17 (68 mg, 1263 opt.mu, 84 mmol, 77%; TEA salt) was obtained starting from 68 mg (1640 opt.mu, 0.109 mmol) of ppCH$_2$pA (TEA salt) following the general procedure **4.1.** Coupling time: 1.5 h; Deprotection time: 4 h. **17** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

R_t = 4.0 min; ^{1}H NMR (400 MHz, D$_2$O, 25 ^{o}C) δ = 8.56 (s, 1 H), 8.26 (s, 1H) 6.14 (d, *J* = 5.7 Hz, 1 H), 4.85 – 4.80 (m, 1 H), 4.58 (t, *J* = 4.7, 4.2 Hz 1 H), 4.46–4.42 (m, 1 H), 4.40–4.36 (m, 1 H), 4.27 (dd, *J* = 4.0, 8.2 Hz, 1 H), 2.38 (t, *J* = 20.4 Hz, 2 H) ^{31}P NMR (162 MHz, D$_2$O, 25 ^{o}C) δ = 18.67 (td, *J* =21.5, 9.3 Hz, 1 P) 7.29 (dtd, *J* =24.9, 21.5, 21.5, 9.5 Hz, 1 P) -8.80 (d, *J* =24.9 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{11}H_{16}N_5O_{12}P_3^-$ (M-H$^+$) 504.0092, found 504.0077. Exemplary previous synthesis: Spelta V., et al. *Br. J. Pharmacol.* **2003,** 140(6), 1027–34.

5.17. Guanosine 5'-*O*-(1,2-methylenetriphoshate), ppCH$_2$pG (18).

18 (64 mg, 930 opt.mu, 0.078 mmol, 78%; TEA salt) was obtained starting from 65 mg (1200 opt.mu, 0.100 mmol) of pCH$_2$pG (TEA salt) following the general procedure **4.1.** Coupling time: 1.5 h; Deprotection time: 4 h. **18** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

5.17.1. Microwave assisted synthesis of (18).

18 (32 mg, 470 opt.mu, 0.039 mmol, 80%; TEA salt) was obtained starting from 30 mg of pCH$_2$pG (TEA salt) (590 opt.mu, 0.049 mmol) following the general procedure **4.2.** Coupling time: 30 min; Deprotection time: 20 min.

R_t= 2.6 min;^{1}HNMR (400 MHz, D$_2$O, 25 ^{o}C) δ = 8.15 (s, 1 H) 5.93 (d, *J* =6.2 Hz, 1 H) 4.83 (dd, *J* =6.2, 5.0 Hz, 1 H) 4.57 (dd, *J* =5.0, 3.4 Hz, 1 H) 4.32–4.37 (m, 1 H) 4.16–4.21 (m, 2 H) 2.37 (t, *J* =20.4 Hz, 2 H); ^{31}PNMR (162 MHz, D$_2$O, 25 ^{o}C) δ = 18.37 (td, J = 20.4, 8.5 Hz, 1 P) 7.53 (dtd, J = 24.7, 20.4, 20.4, 8.5 Hz, 1P) -9.90 (d, J = 24.9 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{11}H_{17}N_5O_{13}P_3^-$ (M-H$^+$) 520.0041, found 520.0024. Exemplary previous synthesis: Rydzik A.M. et al., *J. Org. Biomol. Chem*. **2009**, 7, 4763–76.

5.18. Adenosine 5'-*O*-(1,2-imidotriphosphate) ppNHpA (19).

20 (52 mg, 969 opt.mu, 0.064 mmol, 76%; TEA salt) was obtained starting from 53 mg (1260 opt.mu, 0.084 mmol) of pNHpA (TEA salt) following the general procedure **4.1.** Coupling time: 2 h; Deprotection time: 4 h. **19** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

R_t = 3.4 min; ^{1}H NMR (400 MHz, D$_2$O, 25 ^{o}C) δ 8.57 (s, 1 H), 8.26 (s, 1 H), 6.15 (d, *J* =5.7 Hz, 1 H), 4.82 (dd, *J* =5.7, 5.0 Hz, 1 H), 4.61 (dd, *J* =5.0, 3.5 Hz, 1 H), 4.39–4.43 (m, 1 H), 4.18–4.25 (m, 1 H), 4.12–4.18 ppm (m, 1 H); ^{31}PNMR (162 MHz, D$_2$O) δ = 0.68 (dt, *J* =6.3, 5.7 Hz, 1 P) -9.51 (d, *J* =21.0 Hz, 1 P) -11.16 (dd, *J* =21.0, 6.3 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{11}H_{17}N_5O_{12}P_3^-$ (M-H$^+$) 505.0092, found 505.0027. Exemplary previous synthesis: Ma Q. et al., *JACS*, **1988**, 110, 4060 – 4061.

5.19. Guanosine 5'-*O*-(1,2-imidotriphosphate) ppNHpG (20).

20 (108 mg, 1600 opt.mu, 0.134 mmol, 72%; TEA salt) was obtained starting from 158 mg (2230 opt.mu, 0.185 mmol) of pNHpG (TEA salt) following the general procedure **4.1.** Coupling time: 1.5 h; Deprotection time: 4 h. **20** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

R_t = 4.0 min;^{1}H NMR (400 MHz, D$_2$O, 25 ^{o}C) δ = 8.29 (s, 1 H), 5.95 (d, *J* = 6.0 Hz, 1 H), 4.81 (dd, *J* = 5.0, 6.0 Hz, 1 H), 4.57 (dd, *J* = 3.7, 5.0 Hz, 1 H), 4.39–4.34 (m, 1 H), 4.21–4.14 (m, 2 H); ^{31}PNMR (162 MHz, D$_2$O, 25 ^{o}C) δ = 0.62 (dt, *J* =6.0, 5.9 Hz, 1 P) -9.74 (d, *J* =20.5 Hz, 1 P) -11.10 (dd, *J* =20.5, 6.1 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{11}H_{17}N_5O_{13}P_3^-$ (M-H$^+$) 520.9994, found 520.9988.

5.20. Adenosine 5'-*O*-tetraphosphate ppppA (21).

21 (90 mg, 1500 opt.mu, 0.1 mmol, 82% TEA salt) was obtained starting from ATP (97 mg, 1830 opt.mu, 0.12 mmol, TEA salt) following the general procedure **4.1.** Coupling time: 3 h; Deprotection time: 3 h. **21** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

R_t = 3.0 min;^{1}H NMR (400 MHz, D$_2$O, 25 ^{o}C) δ = 8.44 (s, 1 H), 8.12 (s, 1 H), 6.06 (d, *J* = 6.1 Hz, 1 H), 4.72 (dd, *J* = 5.0, 6.1 Hz, 1 H), 4.55 (dd, *J* = 3.0, 5.0 Hz, 1 H), 4.40–4.35 (m, 1 H), 4.30–4.15 (m, 2 H); ^{31}PNMR (162 MHz, D$_2$O, 25 ^{o}C) δ = -10.12 (d, *J* =18.6 Hz, 1 P) -11.07 (d, *J* =18.6 Hz, 1 P) -22.68 (m, 2 P); HRMS (ESI$^+$) Calcd m/z for $C_{10}H_{18}N_5O_{16}P_4$ (M+H$^+$) 587.9699, found 587.9690.

5.21. N^7-methylguanosine 5'-*O*-(2,3-methylenetetraphosphate); ppCH$_2$pp(m^{7}G) (22).

23 (45 mg, 500 opt.mu, 0.044 mmol, 56%; TEA salt) was obtained from pCH$_2$pp(m^{7}G) 85 mg (890 opt.mu, 0.078 mmol, TEA salt) following the general procedure **4.1.** Coupling time: 3.5 h; deprotection time: 2 h; **22** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

R_t = 3.5 min. ^{1}H NMR (400 MHz, D$_2$O, 25 ^{o}C) δ = 6.08 (d, *J* =3.7 Hz, 1 H), 4.74 (dd, *J* =5.0, 3.7 Hz, 1 H), 4.60 (dd, *J* =5.0, 4.0 Hz, 1 H), 4.38–4.43 (m, 1 H), 4.28–4.36 (m, 1 H),

4.18–4.25 (m, 1 H), 4.15 (s, 3 H), 2.46 (t, *J* =20.0 Hz, 2 H); ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 17.66 (td, *J* =20.0, 9.0 Hz, 1 P), 8.50 (dtd, *J* =21.0, 20.0, 9.0 Hz, 1 P), -10.51 (d, *J* =21.0 Hz, 1 P), -22.74 (t, *J* =21.0 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{12}H_{20}N_5O_{16}P_4^-$ (M-H$^+$) 613.9861; found 613.9871.

5.22. N^7-methylguanosine 5'-*O*-(1,2-methylenetetraphosphate); pppCH$_2$p(m^{7}G) (23).

23 (52 mg, 580 opt.mu, 0.051 mmol, 51%; TEA salt) was obtained starting from ppCH$_2$p(m^{7}G) (102 mg, 1140 opt.mu, 0.10 mmol, TEA salt) following the general procedure **4.1.** Coupling time: 4h; Deprotection time: 2 h. **23** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

R_t = 3.5 min; ^{1}H NMR (400 MHz, D_2O, 25 ^{o}C) δ = 6.03 (d, *J* = 3.7 Hz, 1 H), 4.67 (dd, *J* = 3.7, 5.0 Hz, 1 H), 4.51 (dd, *J* = 4.0, 5.0 Hz, 1 H), 4.39–4.35 (m, 1 H), 4.33–4.27 (m, 1 H), 4.25–4.19 (m, 1 H), 4.09 (s, 3 H), 2.45 (t, *J* = 20.8 Hz, 2 H); ^{31}P NMR (162 MHz, D_2O, 25 ^{o}C) δ = 8.28 (dtd, *J* =25.5, 20.8, 9.0 Hz, 1 P), 6.35 (dtd, *J* =25.5, 20.8, 9.0 Hz, 1 P), -9.85 (d, *J* =25.5 Hz, 1 P), -11.12 (d, *J* =25.5 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{12}H_{20}N_5O_{16}P_4^-$ (M-H$^+$) 613.9861; found 518.9869.

5.23. Guanosine 5'-*O*-(1,2-methylene-3-thiotriphosphate); p$_S$pCH$_2$pG (24) (microwave assisted synthesis)

24 (28 mg, 530 opt.mu, 0.047 mmol, 70%; TEA salt) was obtained starting from 44 mg (800 opt.mu, 0.067 mmol) of pCH$_2$pG (TEA salt) following the general procedure **4.2**. Coupling time: 30 min; deprotection time: 30 min. **24** was later converted into ammonium salt on semi-preparative RP HPLC and repeatedly freeze-dried.

R_t = 3.9 min; ^{1}HNMR (400 MHz, D_2O, 25 ^{o}C) δ 8.29 (s, 1 H), 5.97 (d, *J* = 5.9 Hz, 1 H), 4.84 (dd, *J* = 4.5, 5.9 Hz, 1 H), 4.61 (dd, *J* = 3.5, 4.5 Hz, 1 H), 4.40–4.35 (m, 1 H), 4.26–4.21 (m, 2 H), 2.52 (t, *J* = 20.1 Hz, 2 H); ^{31}PNMR (162 MHz, D_2O, 25 ^{o}C) δ = 33.23 (d, *J* =32.0 Hz, 1 P), 19.86 (tdt, *J* =20.1, 7.6, 5.0, Hz, 1 P), 7.02 (dtd, *J* =32.0, 20.1, 7.6 Hz, 1 P); HRMS (ESI$^-$) Calcd m/z for $C_{11}H_{18}N_5O_{16}P_4^-$ (M-H$^+$) 535.9704; found 535.9820.

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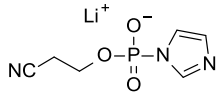
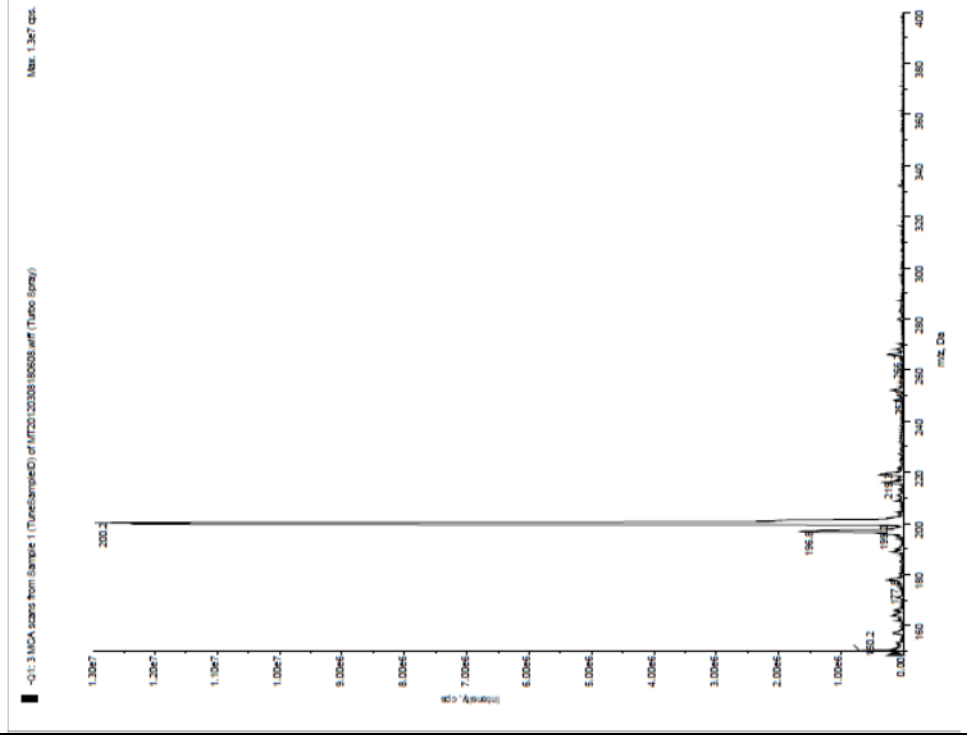
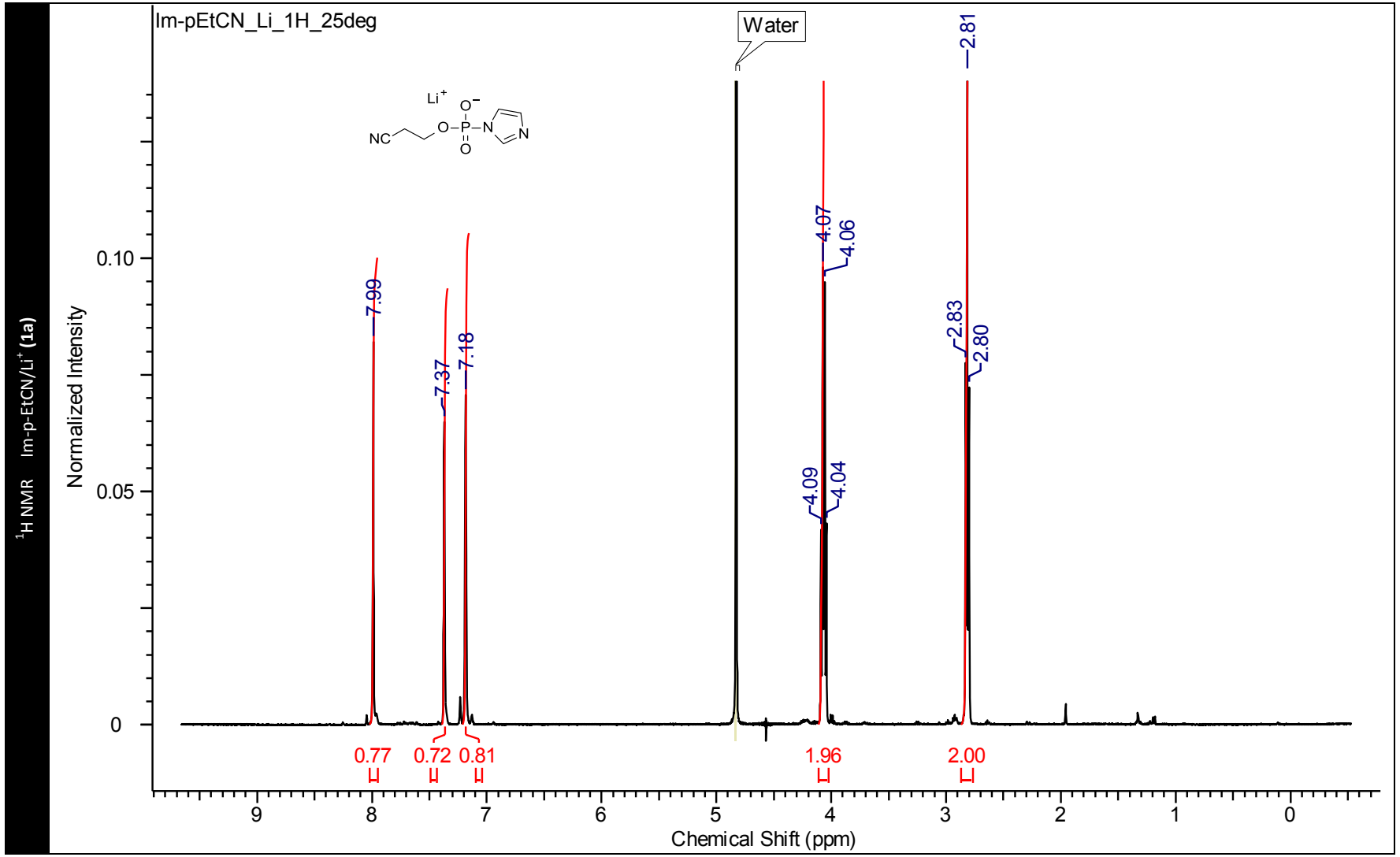
IV. HPLC profiles, HRMS and NMR spectra

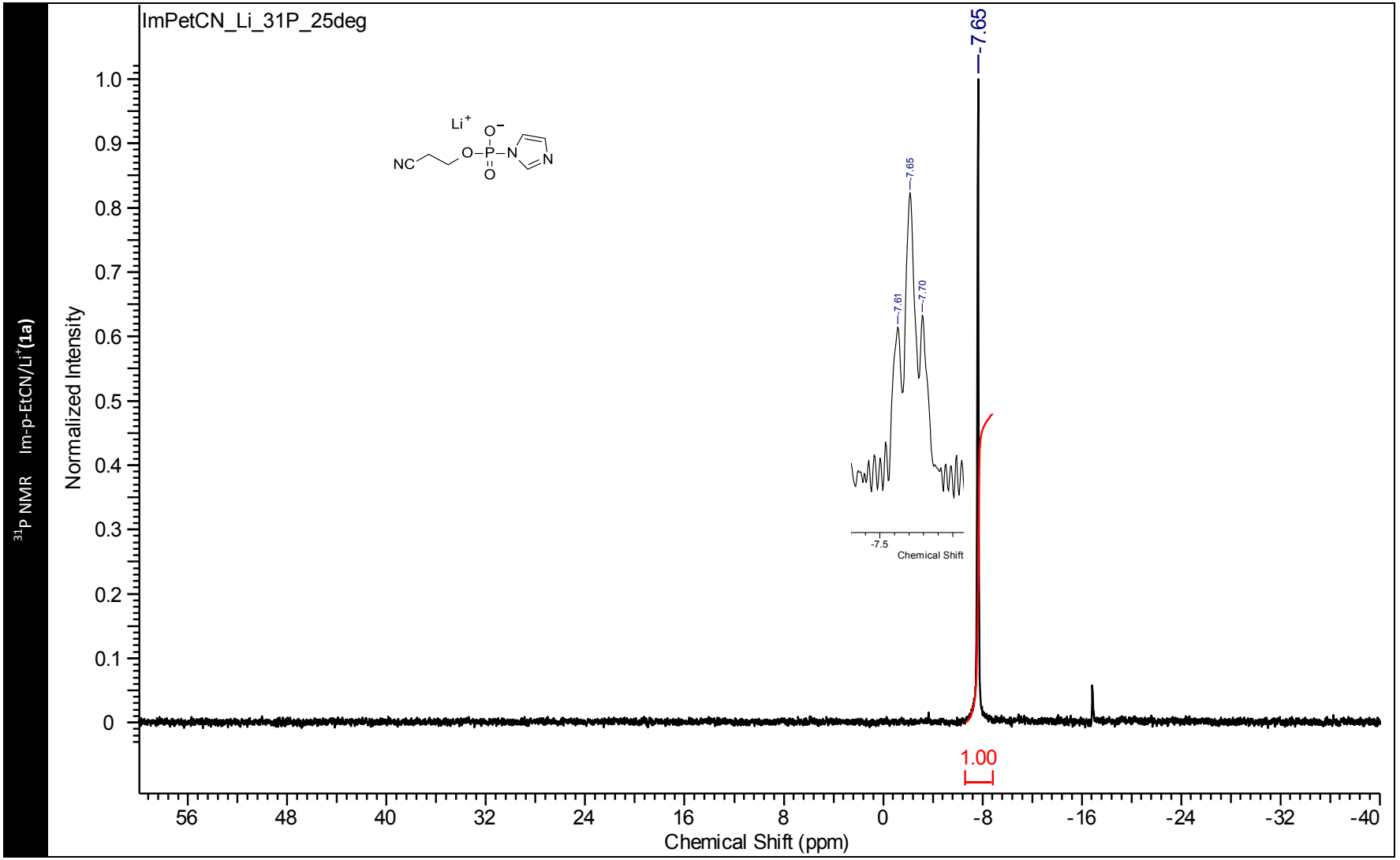
(**1a**) Im-p-EtCN/Li^+



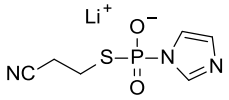
Im-p-EtCN/Li^+ (**1a**) MS (-)ES (Calc. $[M-H]^-$ $C_6H_7N_3O_2P^-$ 200.0



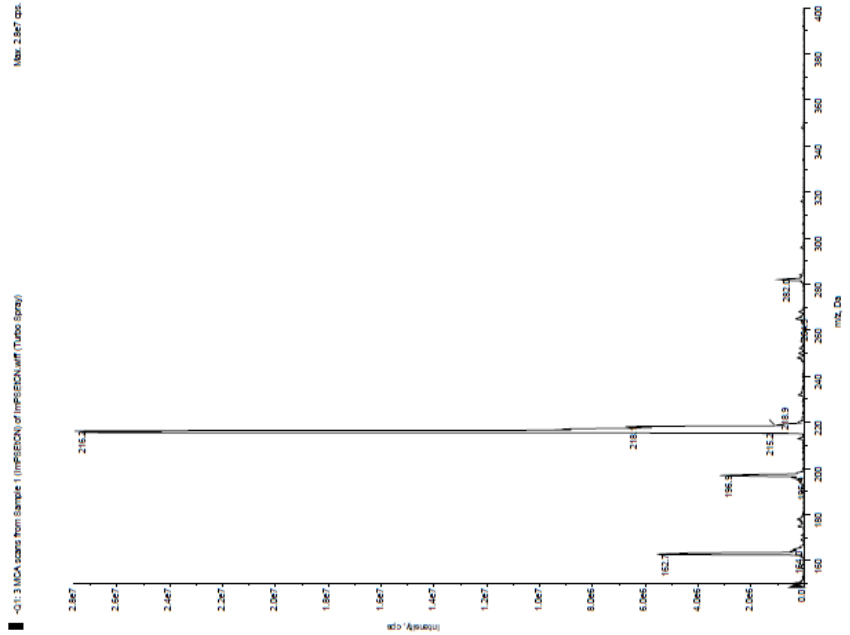

^{1}H NMR Im-p-EtCN/Li$^+$ (**1a**)
Im-pEtCN_Li_1H_25deg
Water
Li+
NC
O
P
N
Normalized Intensity
0.10
0.05
0
7.99
7.37
7.18
4.07
4.06
4.09
4.04
2.83
2.80
2.81
0.77
0.72
0.81
1.96
2.00
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

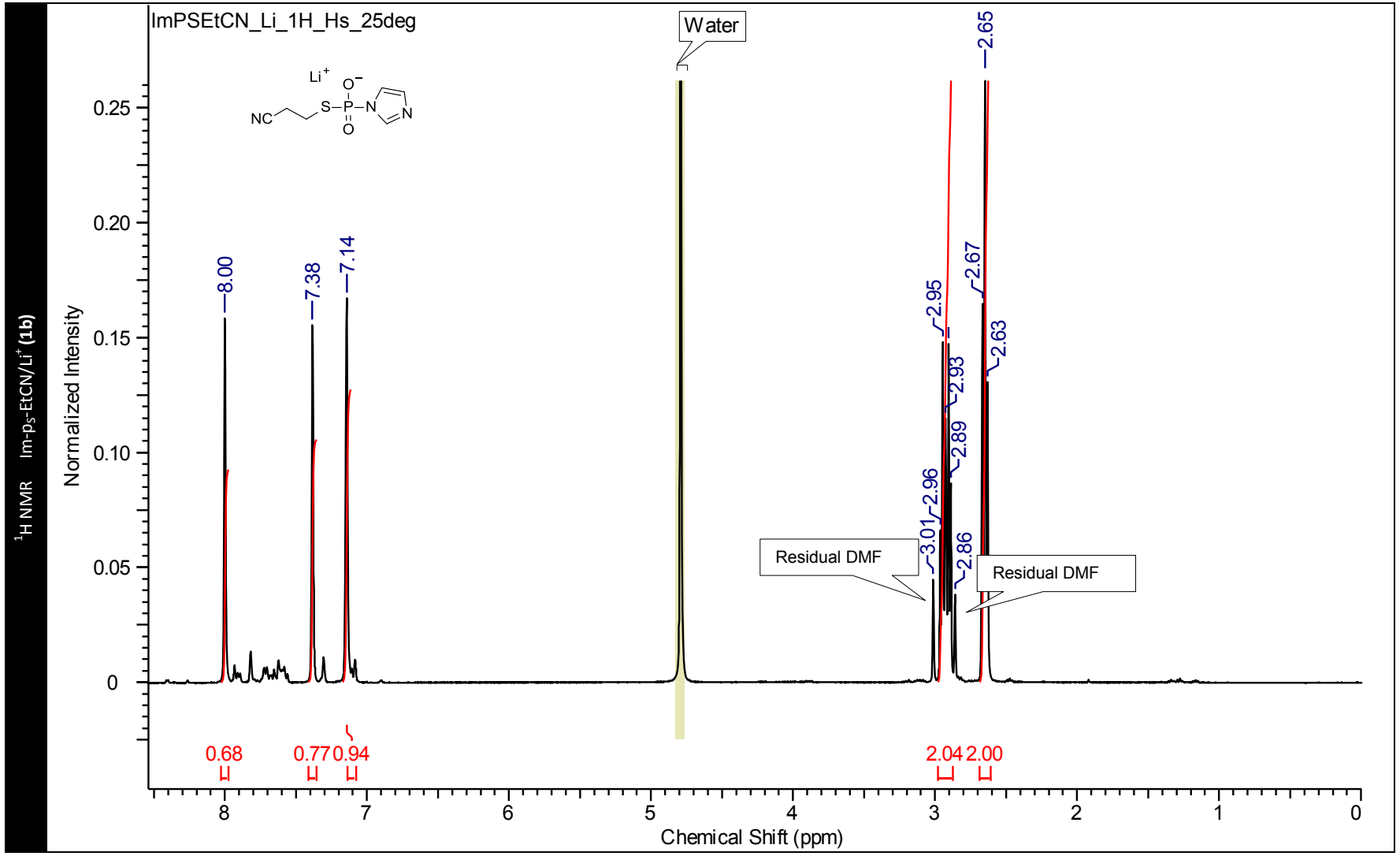

ImPetCN_Li_31P_25deg
^{31}P NMR Im-p-EtCN/Li^{+}(**1a**)
Normalized Intensity
Chemical Shift (ppm)
-7.65
-7.61
-7.70
1.00
Li+
NC
O
P
N

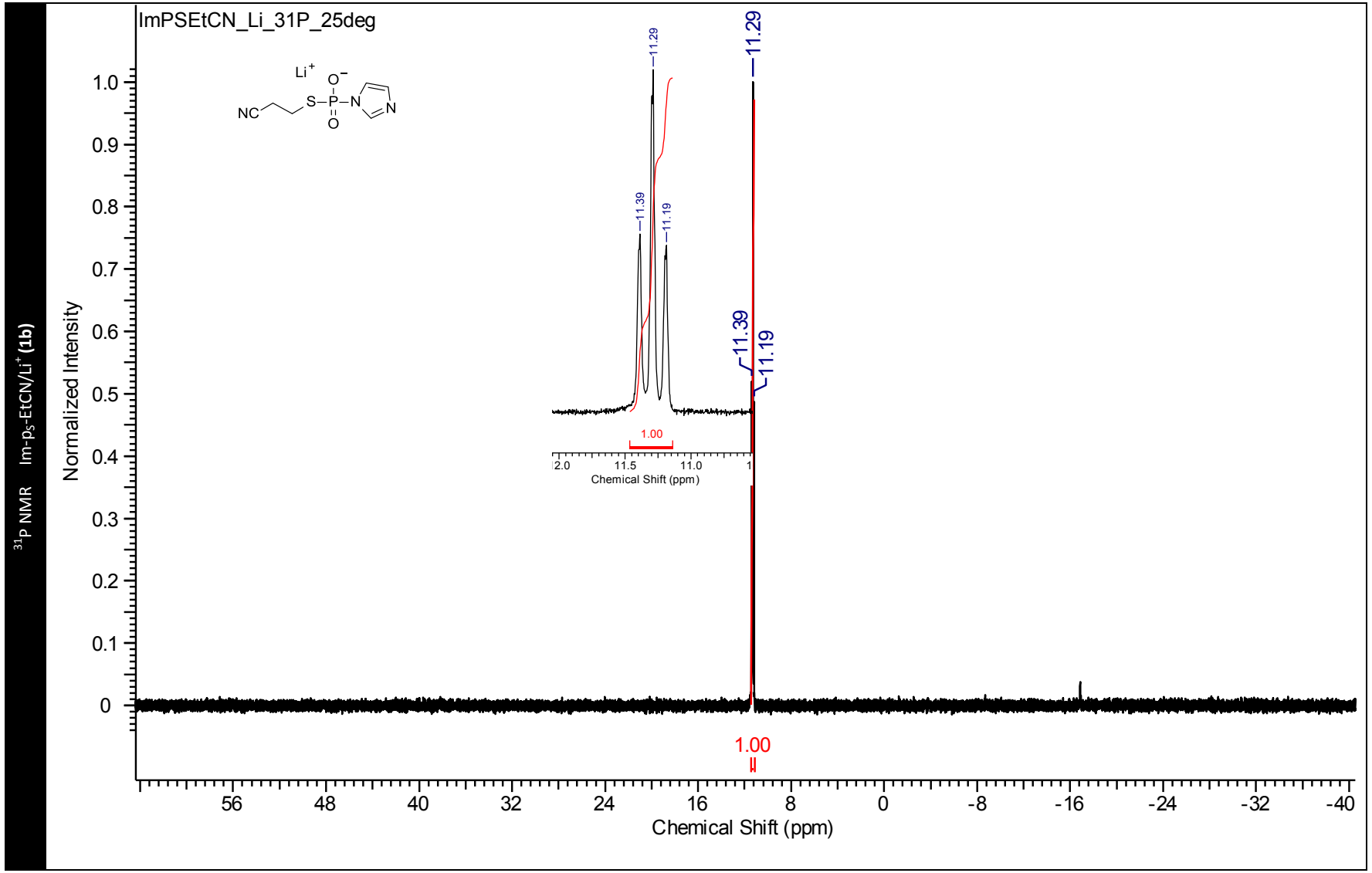
(**1b**) Im-p_S-EtCN/Li^+



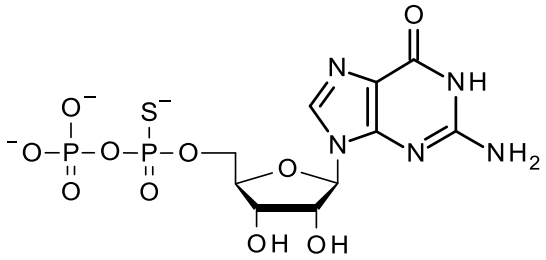
Im-p_S-EtCN/Li^+ (**1b**) MS (-)ES (Calc. $[M-H]^-$ $C_6H_7N_3O_2P_S^-$ 216.0002



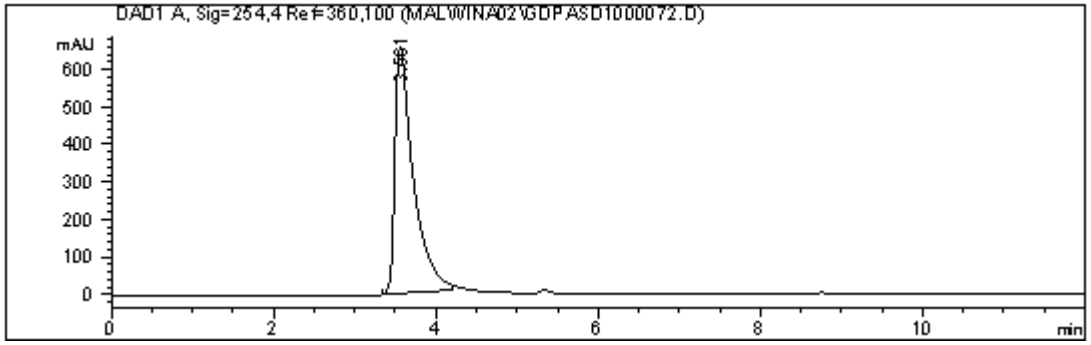

^{1}H NMR Im-ρ$_S$-EtCN/Li$^+$ (1b)
ImPSEtCN_Li_1H_Hs_25deg
Normalized Intensity
Chemical Shift (ppm)
Water
Residual DMF
Residual DMF
8.00
7.38
7.14
2.95
2.67
2.65
2.63
2.93
2.89
2.96
3.01
2.86
0.68
0.77
0.94
2.04
2.00

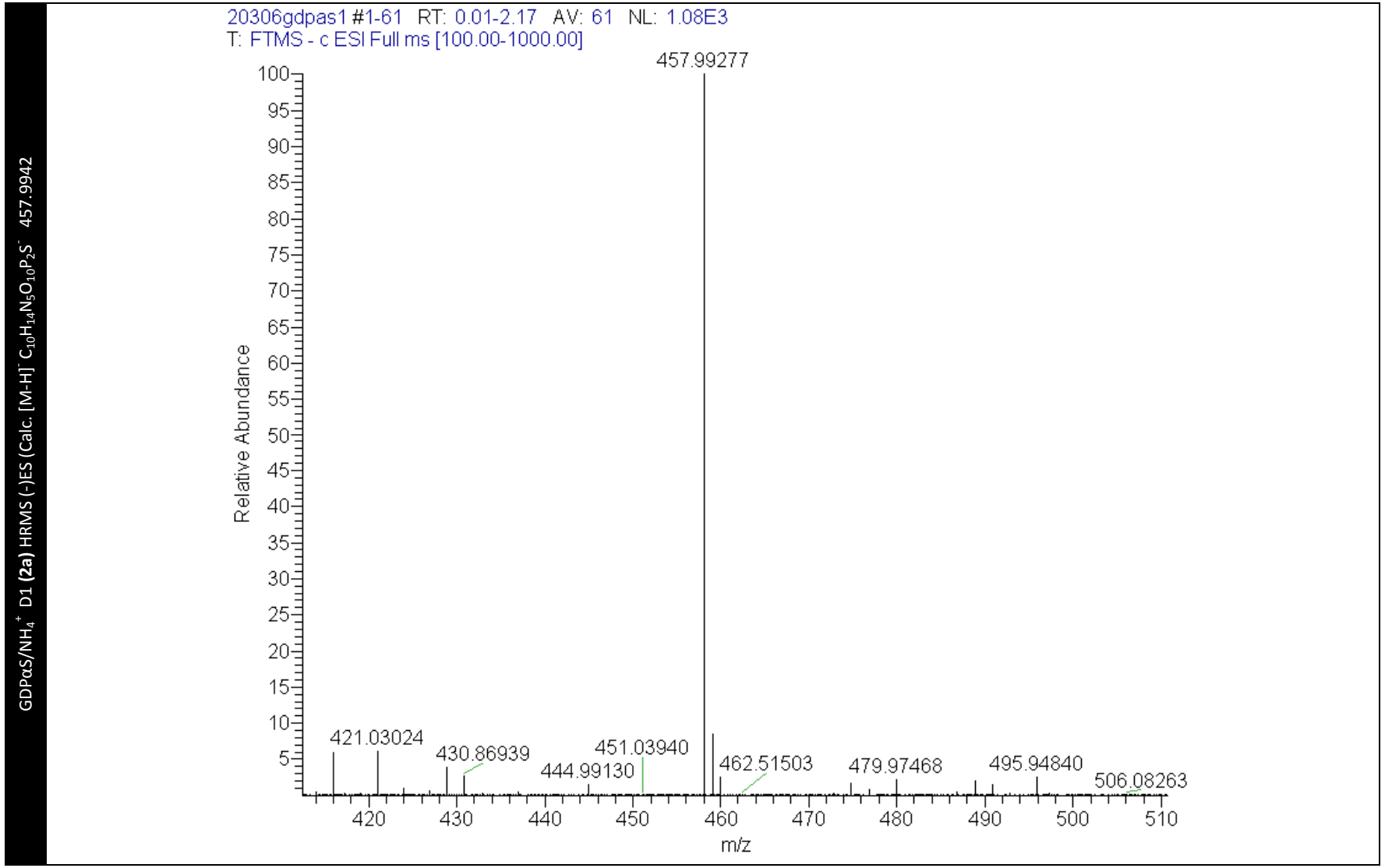

ImPSEtCN_Li_31P_25deg
^{31}P NMR Im-p$_S$-EtCN/Li$^+$ (1b)
Normalized Intensity
Chemical Shift (ppm)
11.29
11.39
11.19
1.00

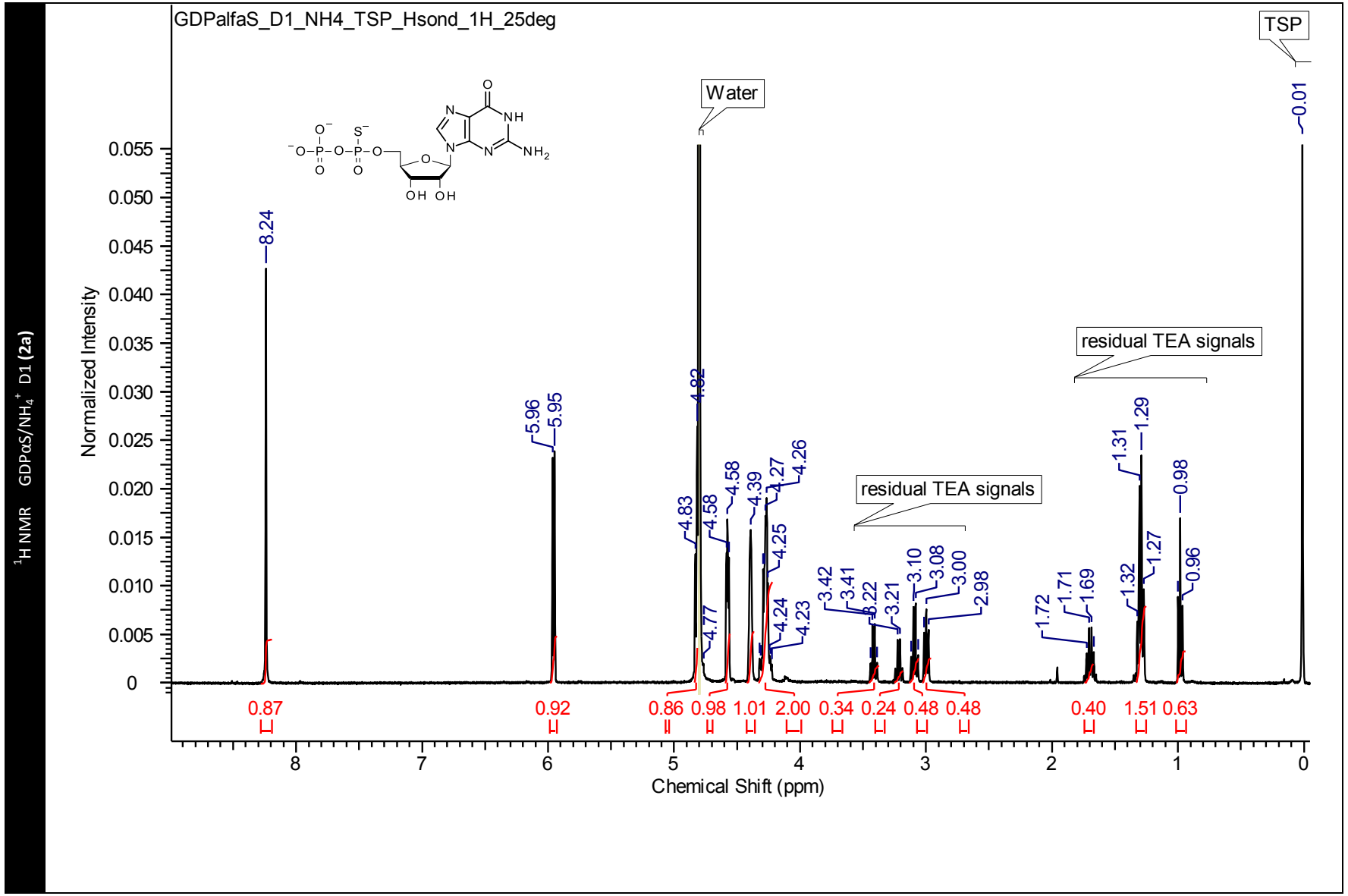
(**2a**) GDPαS/NH_4^+ D1

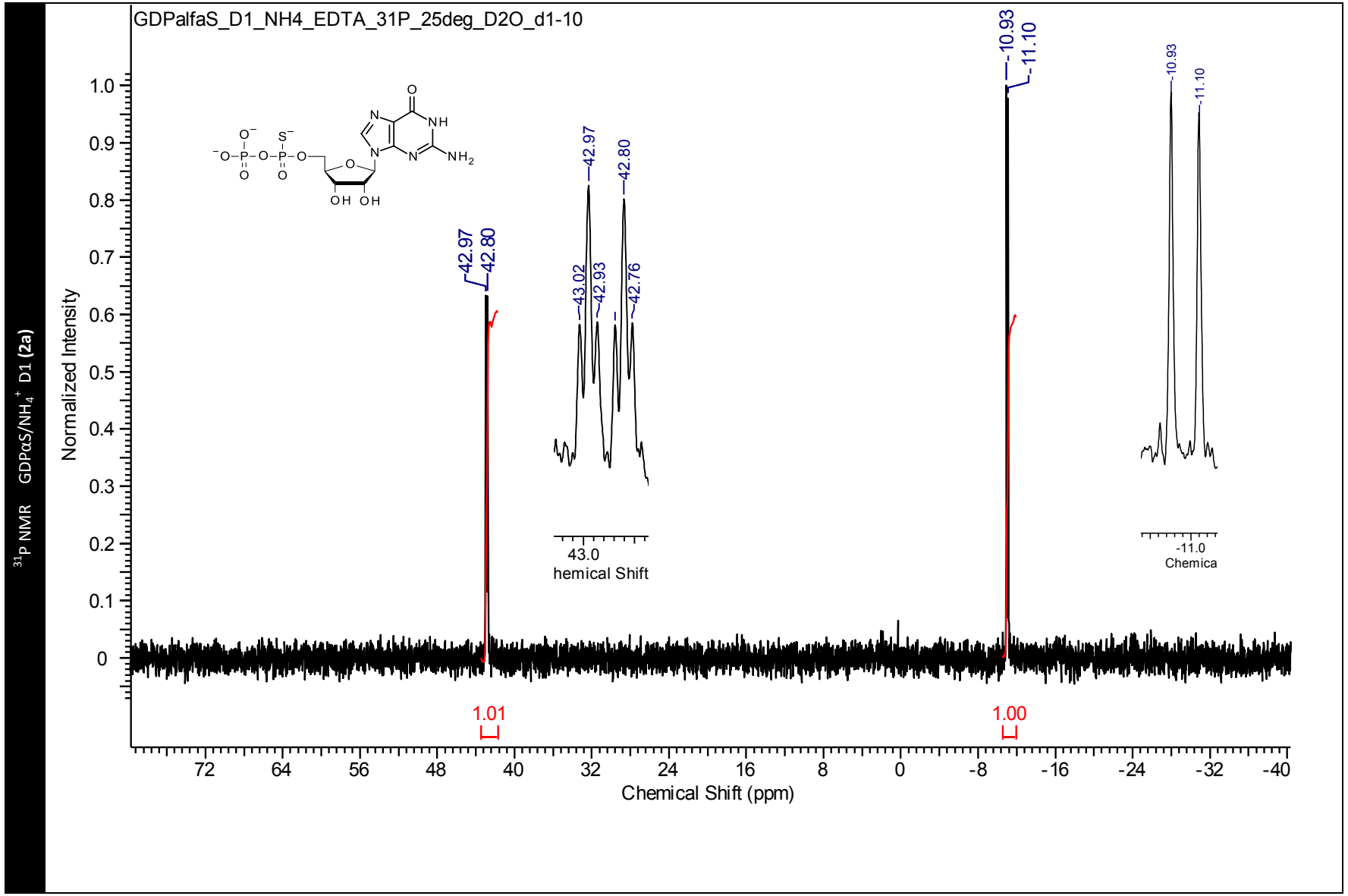


GDPαS/NH_4^+ D1 **(2a)** HPLC

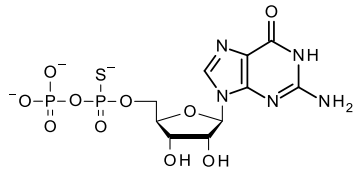



GDPαS/NH4+ D1 (2a) HRMS (-)ES (Calc. [M-H]- C10H14N5O10P2S- 457.9942
20306gdpas1 #1-61 RT: 0.01-2.17 AV: 61 NL: 1.08E3
T: FTMS - c ESI Full ms [100.00-1000.00]
457.99277
421.03024
430.86939
444.99130
451.03940
462.51503
479.97468
495.94840
506.08263
Relative Abundance
m/z

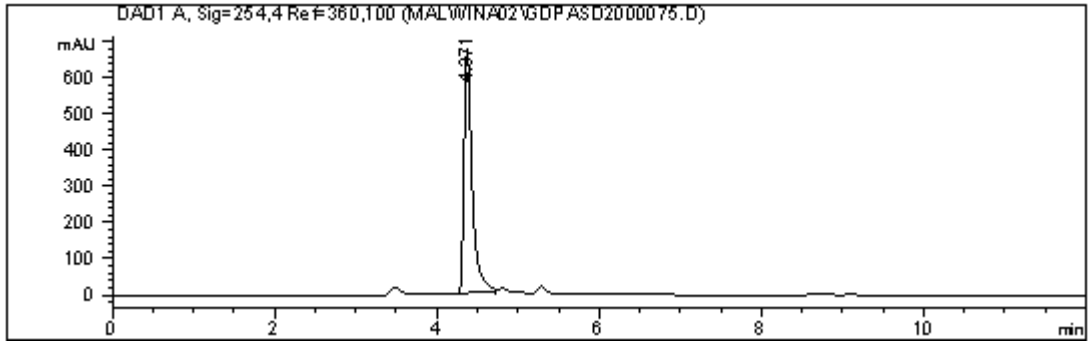

^{1}H NMR GDPαS/NH$_4^+$ D1 (2a)
GDPalfaS_D1_NH4_TSP_Hsond_1H_25deg
Normalized Intensity
Chemical Shift (ppm)
Water
TSP
residual TEA signals
residual TEA signals
8.24
5.96
5.95
4.92
4.83
4.58
4.58
4.39
4.27
4.26
4.25
4.24
4.23
4.77
3.42
3.41
3.22
3.21
3.10
3.08
3.00
2.98
1.72
1.71
1.69
1.32
1.31
1.29
1.27
0.98
0.96
0.01
0.87
0.92
0.86
0.98
1.01
2.00
0.34
0.24
0.48
0.48
0.40
1.51
0.63

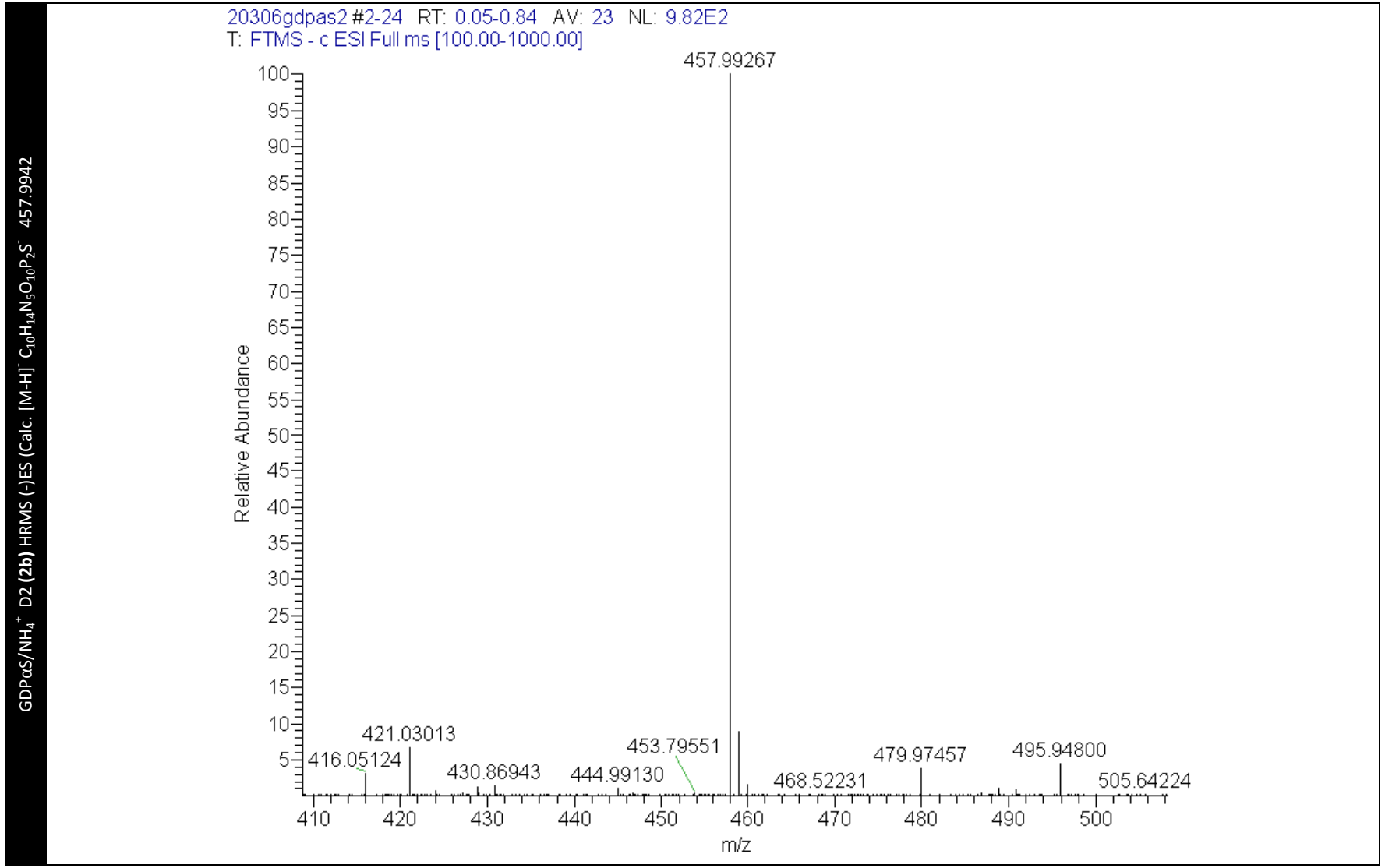

^{31}P NMR GDPαS/NH_4^+ D1 (2a)
GDPalfaS_D1_NH4_EDTA_31P_25deg_D2O_d1-10
Normalized Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
42.97
42.80
43.02
42.93
42.76
43.0
hemical Shift
-10.93
-11.10
-11.0
Chemica
1.01
1.00
72
64
56
48
40
32
24
16
8
0
-8
-16
-24
-32
-40
Chemical Shift (ppm)

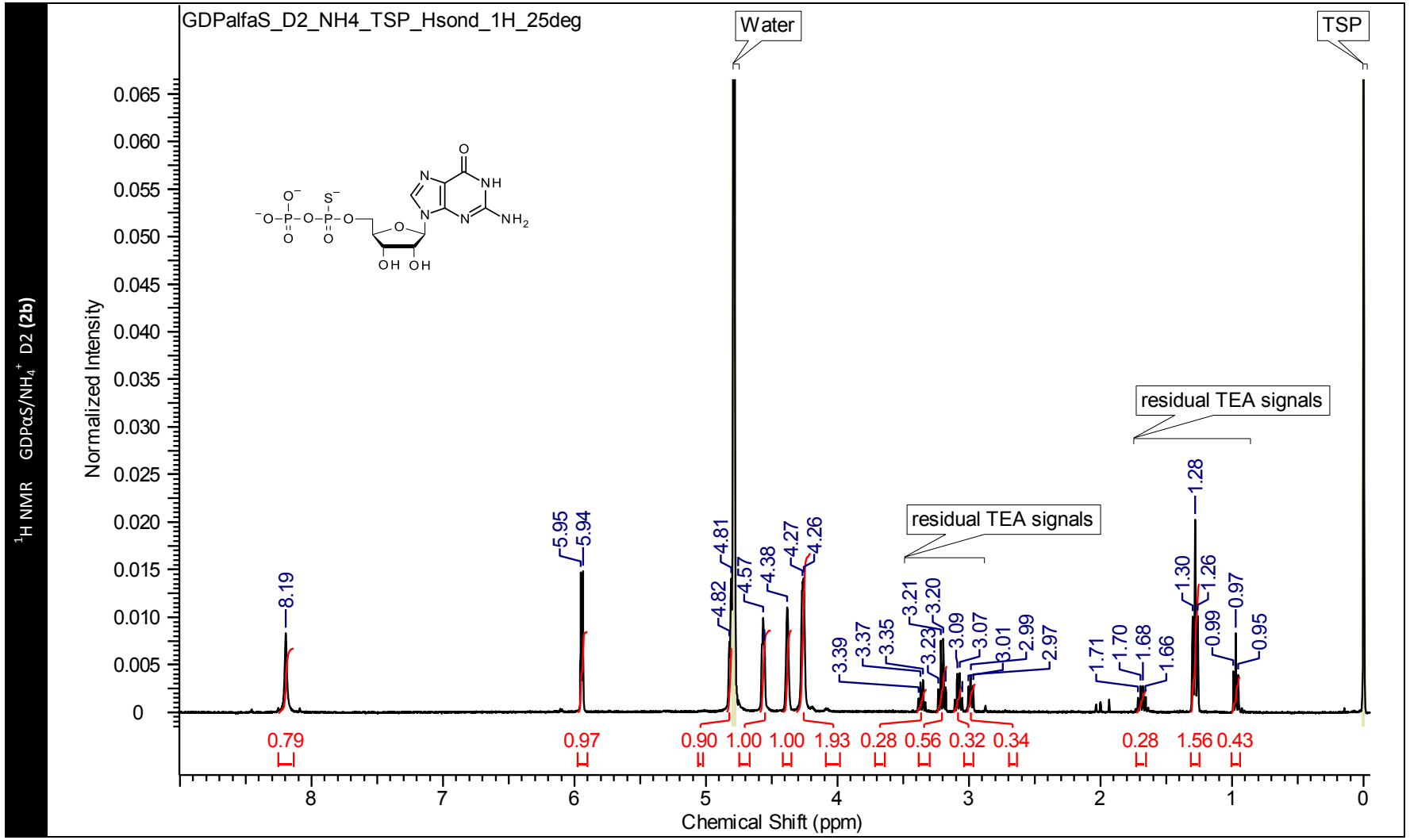
(**2b**) GDPαS/NH_4^+ D2

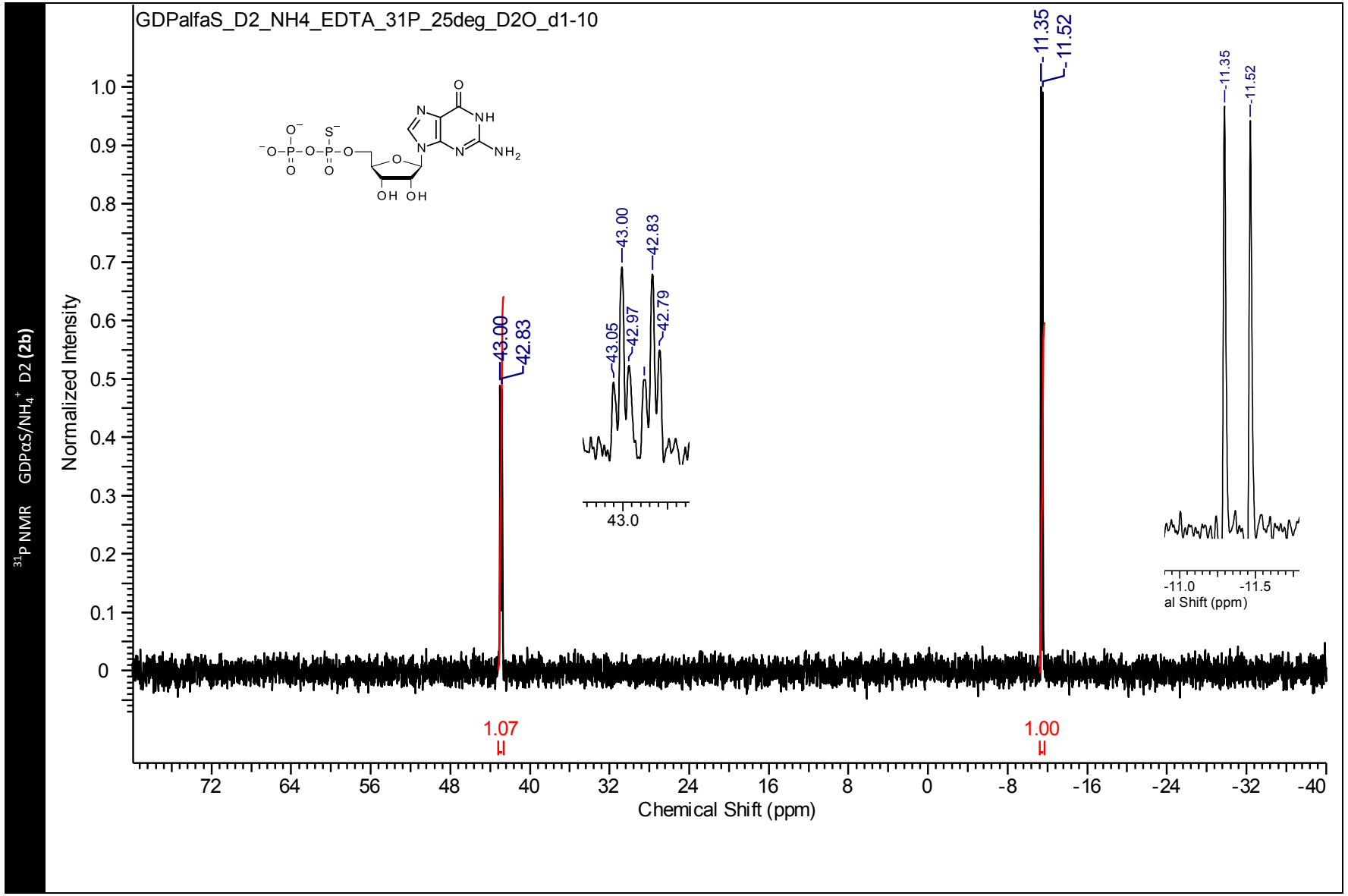


GDPαS/NH_4^+ D2 **(2b)** HPLC

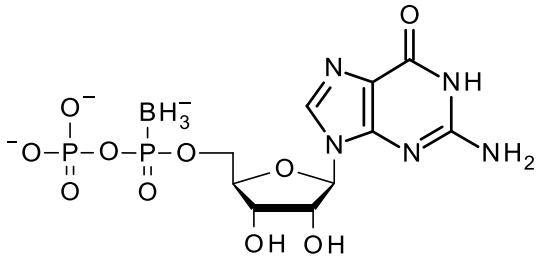



GDPαS/NH4+ D2 (2b) HRMS (-)ES (Calc. [M-H]- C10H14N5O10P2S- 457.9942
20306gdpas2 #2-24 RT: 0.05-0.84 AV: 23 NL: 9.82E2
T: FTMS - c ESI Full ms [100.00-1000.00]
457.99267
421.03013
416.05124
430.86943
444.99130
453.79551
468.52231
479.97457
495.94800
505.64224
Relative Abundance
m/z

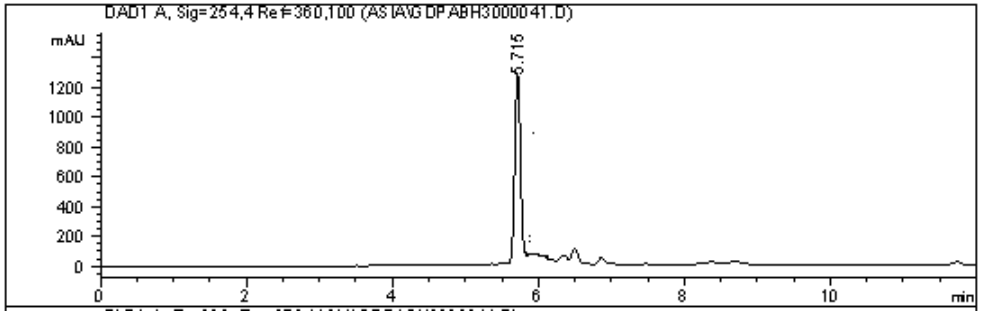

^{1}H NMR GDPαS/NH$_4^+$ D2 (2b)
GDPalfaS_D2_NH4_TSP_Hsond_1H_25deg
Water
TSP
residual TEA signals
residual TEA signals
Normalized Intensity
Chemical Shift (ppm)
8.19
5.95
5.94
4.82
4.81
4.57
4.38
4.27
4.26
3.39
3.37
3.35
3.23
3.21
3.20
3.09
3.07
3.01
2.99
2.97
1.71
1.70
1.68
1.66
1.30
1.28
1.26
0.99
0.97
0.95
0.79
0.97
0.90
1.00
1.00
1.93
0.28
0.56
0.32
0.34
0.28
1.56
0.43


^{31}P NMR GDPαS/NH_4^+ D2 (2b)
GDPalfaS_D2_NH4_EDTA_31P_25deg_D2O_d1-10
Normalized Intensity
Chemical Shift (ppm)
43.00
42.83
43.05
42.97
42.79
43.0
-11.35
-11.52
-11.0
-11.5
1.07
1.00

(3a) GDPαBH$_3$/NH$_4^+$ D1

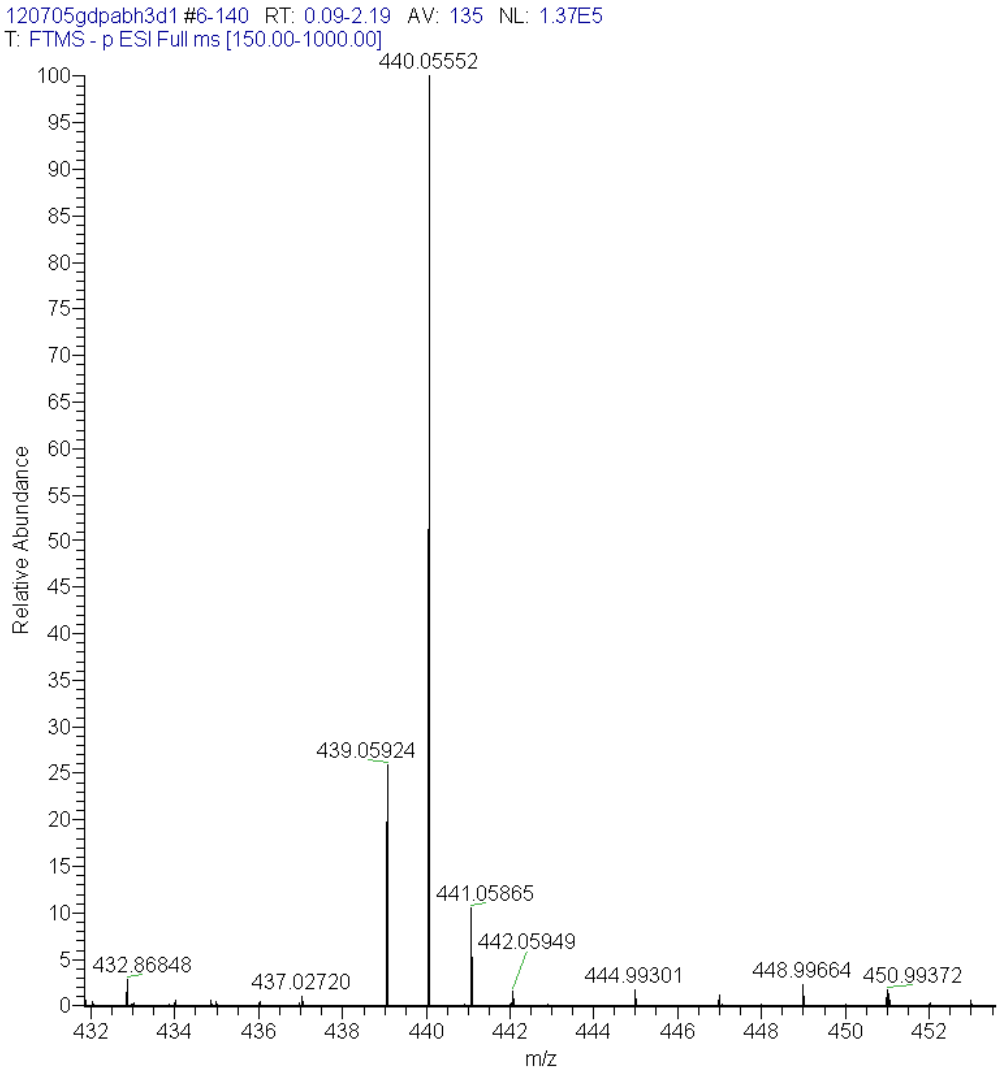


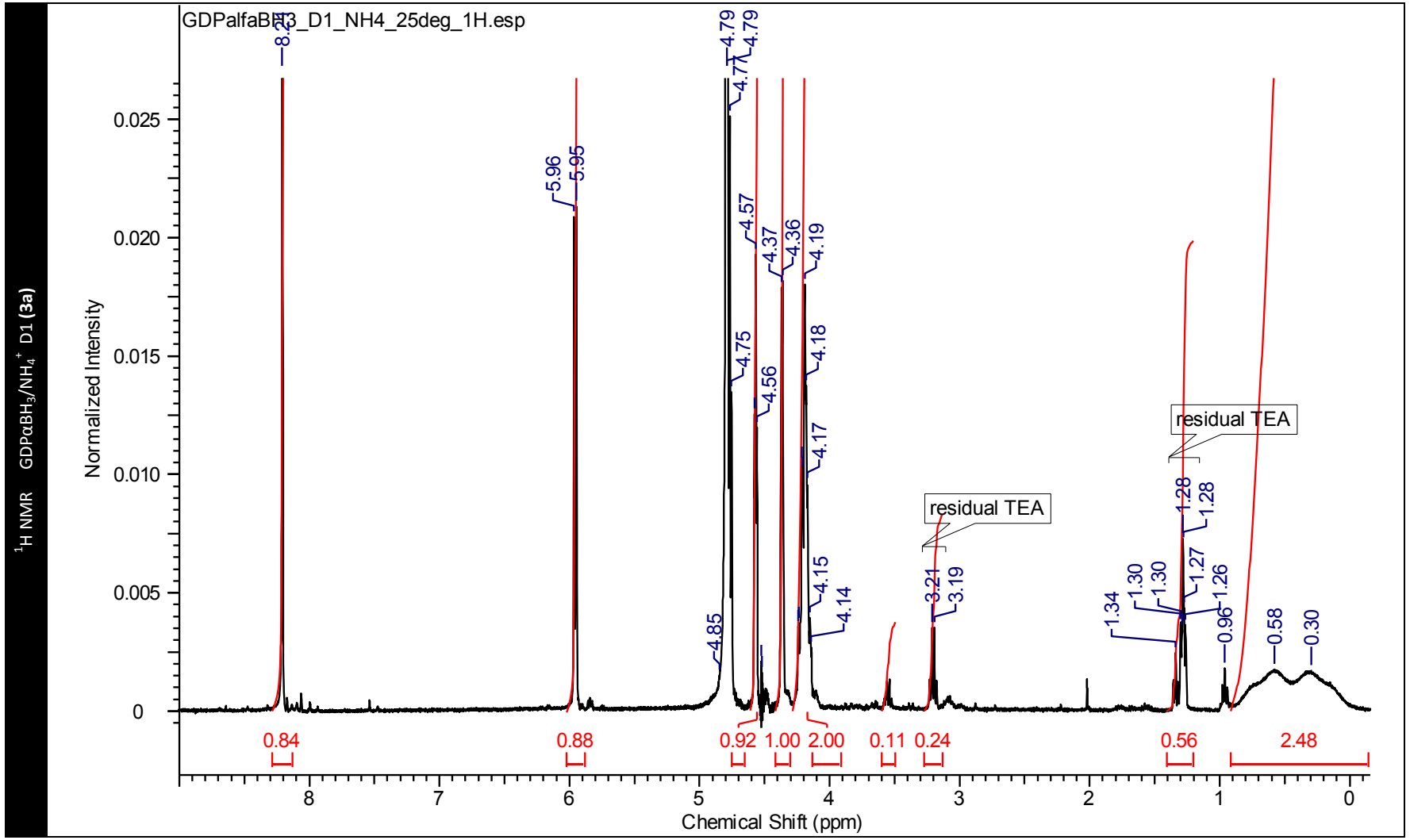
GDPαBH$_3$/NH$_4^+$ D1 **(3a)** HPLC

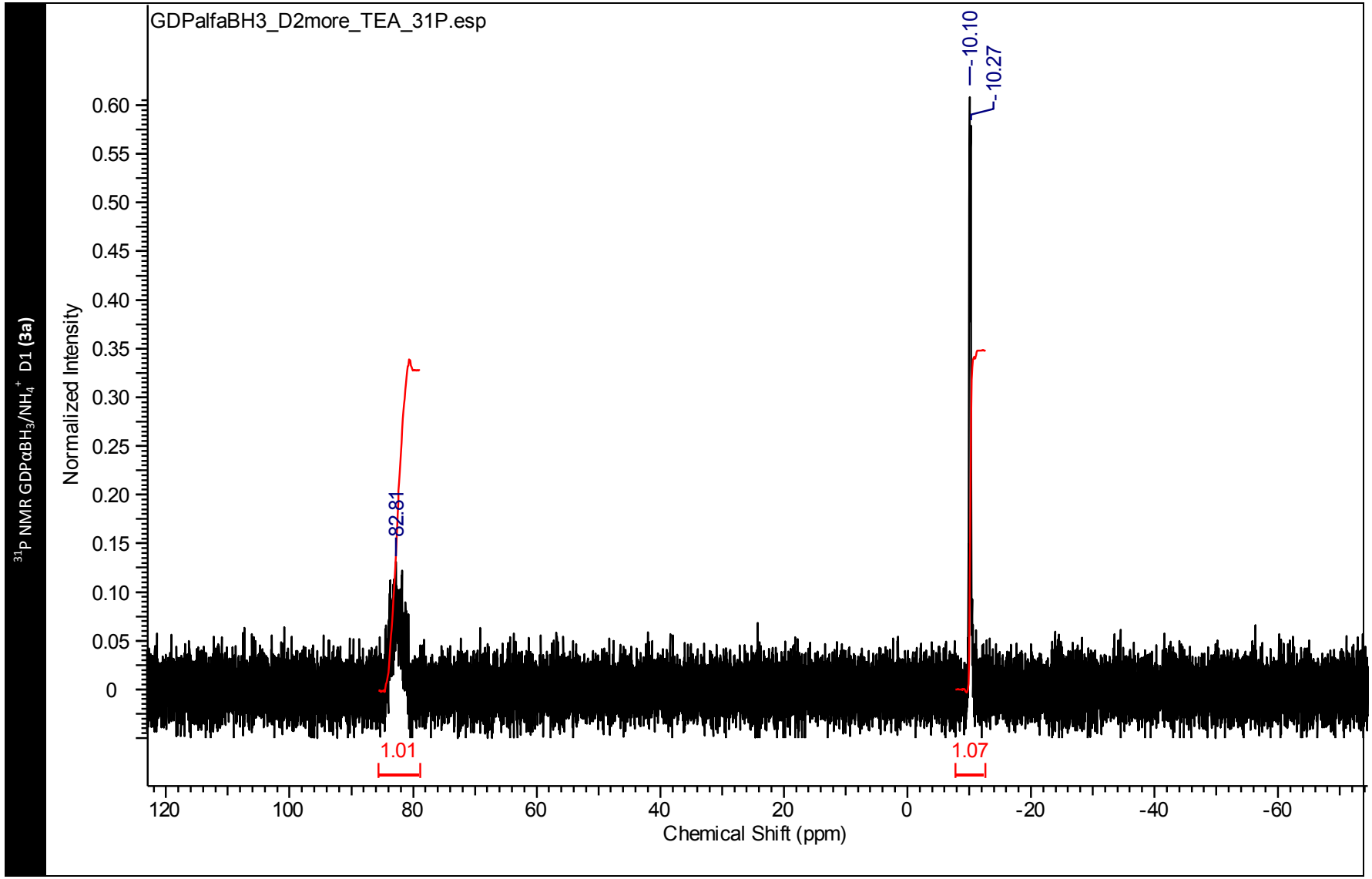


MS

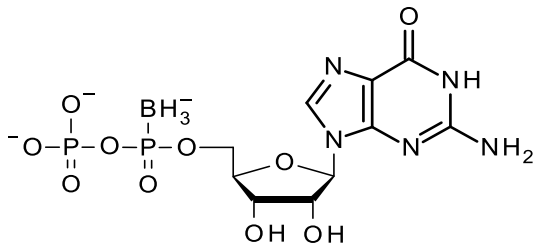
GDPαBH$_3$/NH$_4^+$ D1 **(3a)** MS (-)ES (Calc. [M-H]$^-$ $C_{10}H_{17}BN_5O_{10}P_2^-$ 440.0549



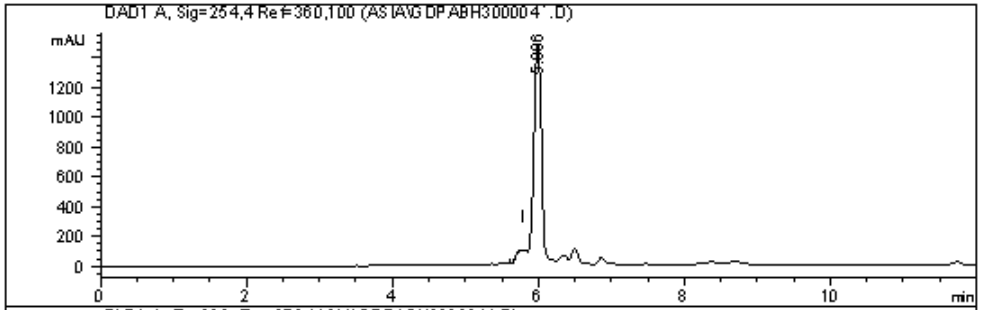

^{1}H NMR GDPαBH$_3$/NH$_4^+$ D1 (3a)
GDPalfaBH3_D1_NH4_25deg_1H.esp
Normalized Intensity
Chemical Shift (ppm)
residual TEA
residual TEA
0.84
0.88
0.92
1.00
2.00
0.11
0.24
0.56
2.48

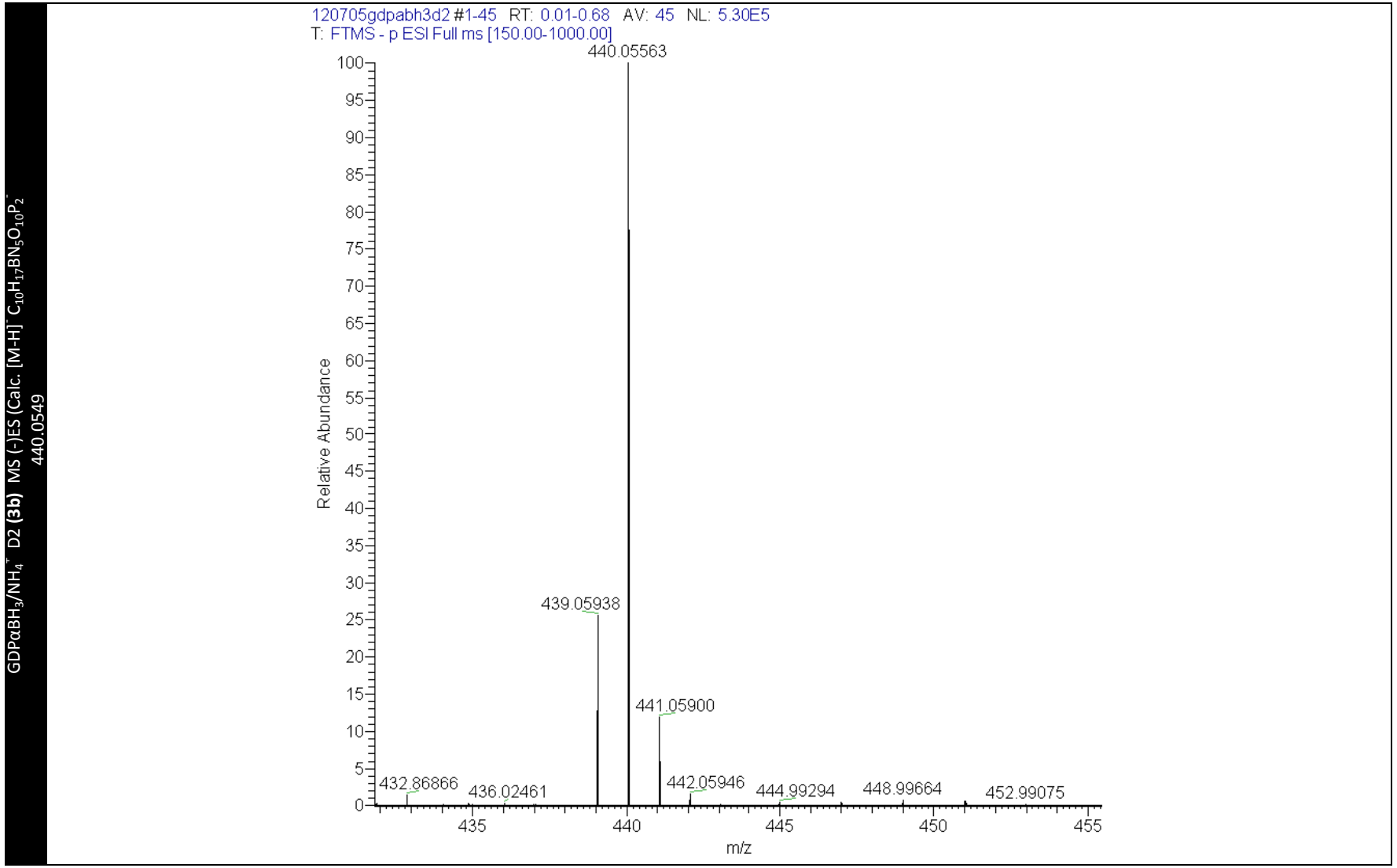

^{31}P NMR GDPαBH$_3$/NH$_4^+$ D1 (3a)
GDPalfaBH3_D2more_TEA_31P.esp
Normalized Intensity
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
82.81
10.10
10.27
1.01
1.07
120
100
80
60
40
20
0
-20
-40
-60
Chemical Shift (ppm)

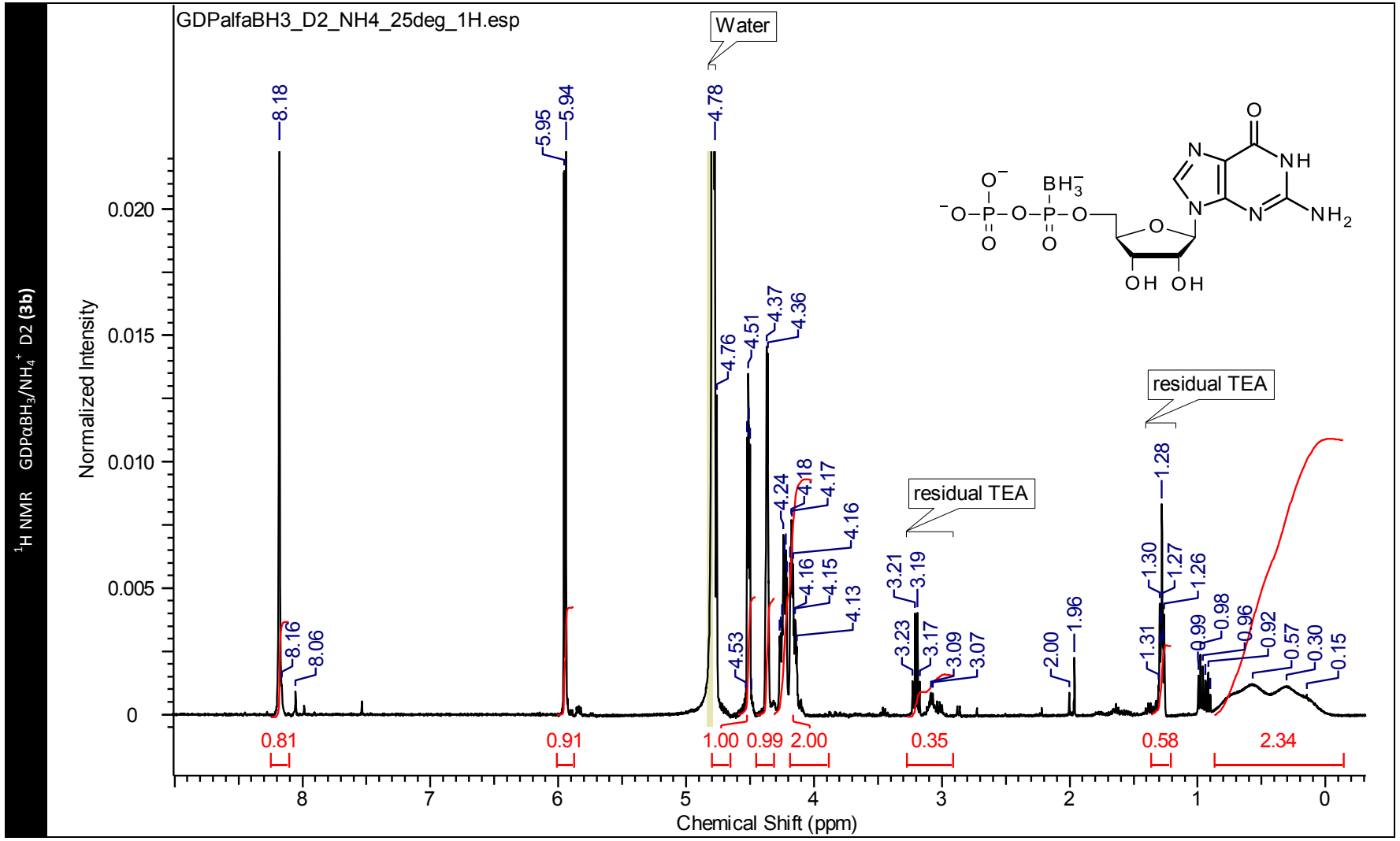
(**3b**) GDPαBH$_3$/NH$_4^+$ D2

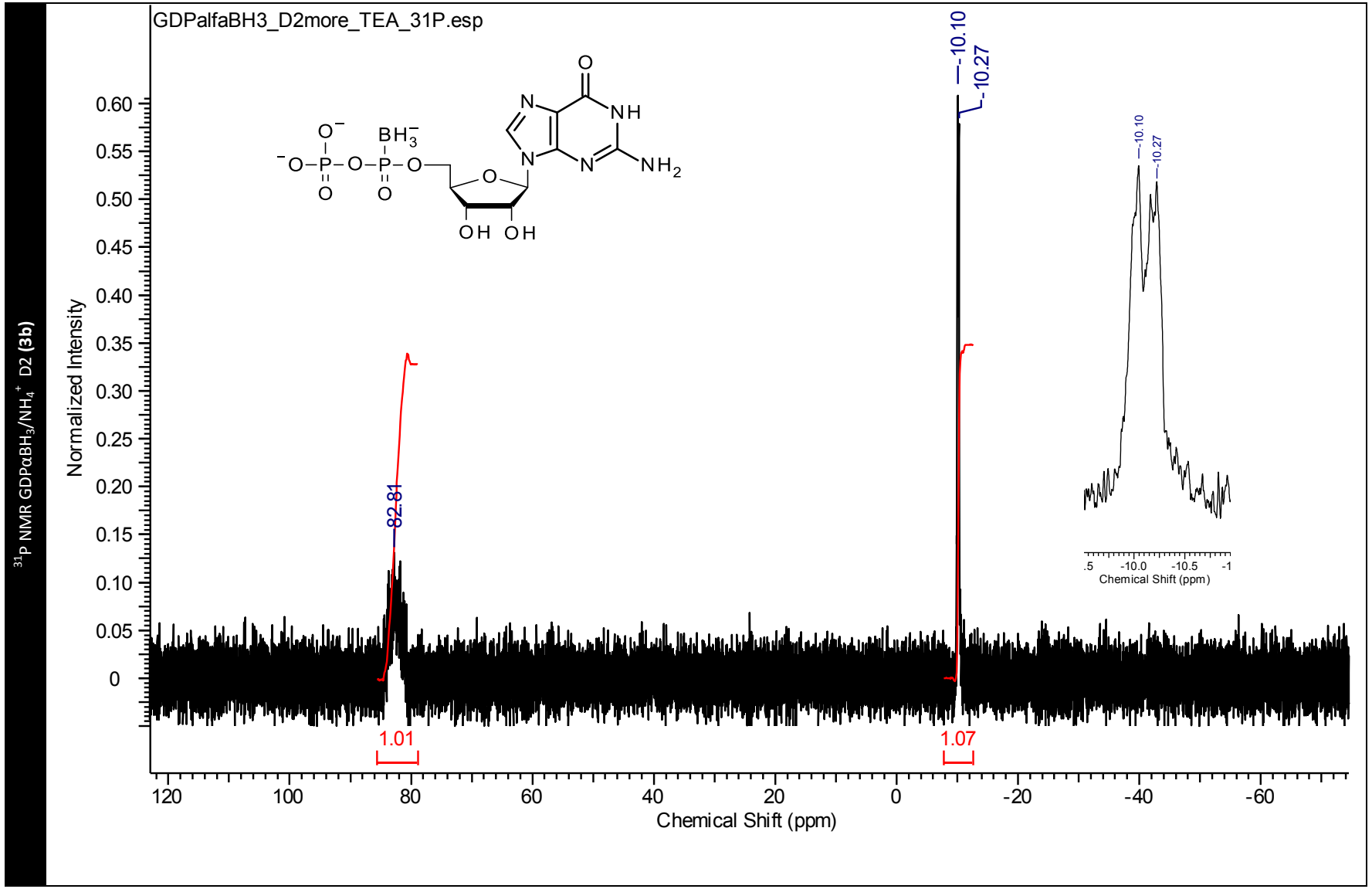


GDPαBH$_3$/NH$_4^+$ D2 **(3b)** HPLC

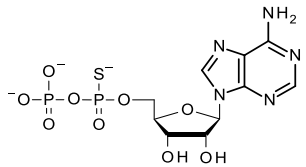



GDPαBH3/NH4+ D2 (3b) MS (-)ES (Calc. [M-H]- C10H17BN5O10P2- 440.0549
120705gdpabh3d2 #1-45 RT: 0.01-0.68 AV: 45 NL: 5.30E5
T: FTMS - p ESI Full ms [150.00-1000.00]
440.05563
439.05938
441.05900
432.86866
436.02461
442.05946
444.99294
448.99664
452.99075
Relative Abundance
m/z
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
435
440
445
450
455

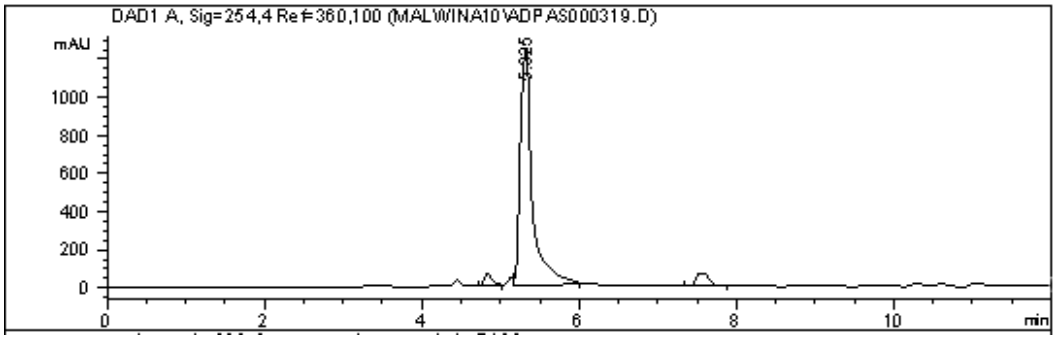

^{1}H NMR GDPαBH$_3$/NH$_4^+$ D2 (3b)
GDPalfaBH3_D2_NH4_25deg_1H.esp
Water
residual TEA
residual TEA
Normalized Intensity
Chemical Shift (ppm)
0.81
0.91
1.00
0.99
2.00
0.35
0.58
2.34

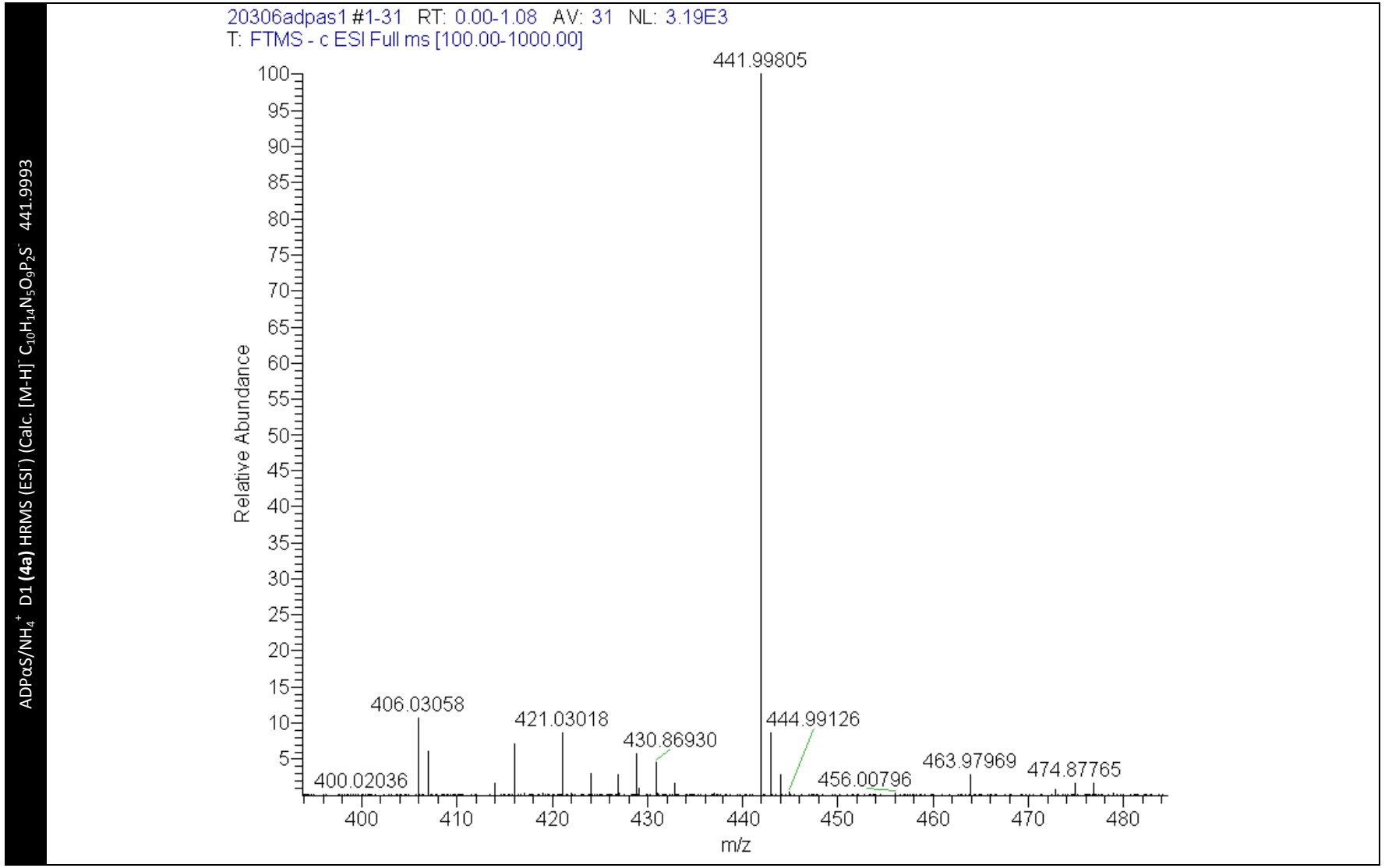

^{31}P NMR GDPαBH$_3$/NH$_4^+$ D2 (3b)
GDPalfaBH3_D2more_TEA_31P.esp
-10.10
-10.27
82.81
1.01
1.07
Normalized Intensity
Chemical Shift (ppm)
Chemical Shift (ppm)

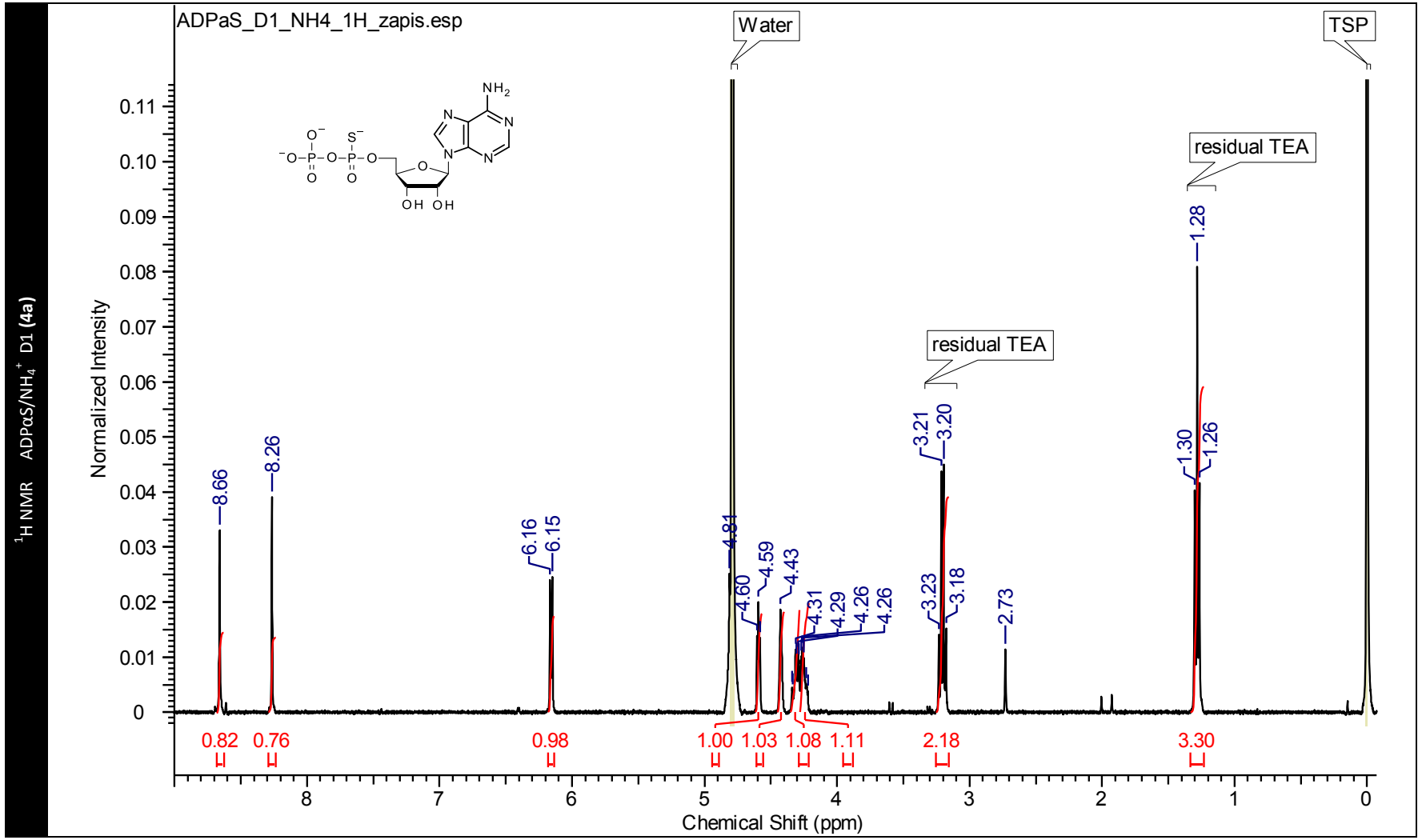
(**4a**) ADPαS/NH_4^+ D1

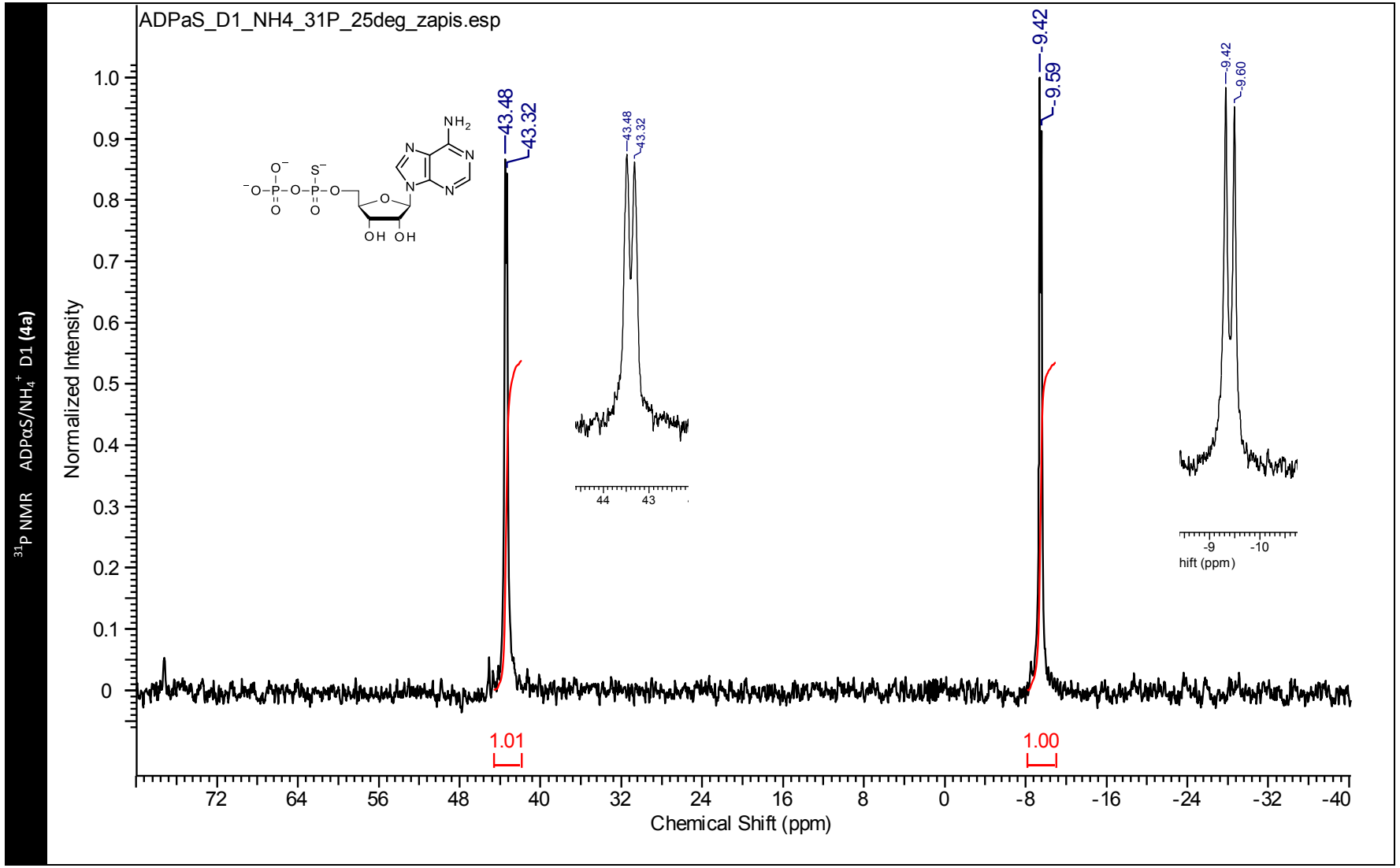


ADPαS/NH_4^+ D1 **(4a)** HPLC

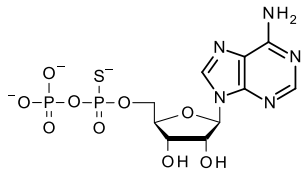



ADPαS/NH4+ D1 (4a) HRMS (ESI-) (Calc. [M-H]- C10H14N5O9P2S- 441.9993
20306adpas1 #1-31 RT: 0.00-1.08 AV: 31 NL: 3.19E3
T: FTMS - c ESI Full ms [100.00-1000.00]
441.99805
406.03058
421.03018
430.86930
444.99126
400.02036
456.00796
463.97969
474.87765
Relative Abundance
m/z

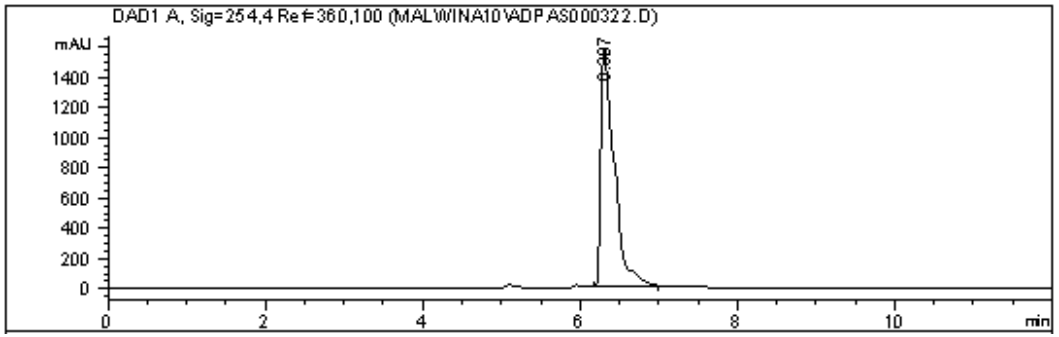

^{1}H NMR ADPαS/NH$_4^+$ D1 (4a)
ADPaS_D1_NH4_1H_zapis.esp
Water
TSP
residual TEA
residual TEA
Normalized Intensity
Chemical Shift (ppm)
8.66
8.26
6.16
6.15
4.81
4.60
4.59
4.43
4.31
4.29
4.26
4.26
3.23
3.21
3.20
3.18
2.73
1.30
1.28
1.26
0.82
0.76
0.98
1.00
1.03
1.08
1.11
2.18
3.30

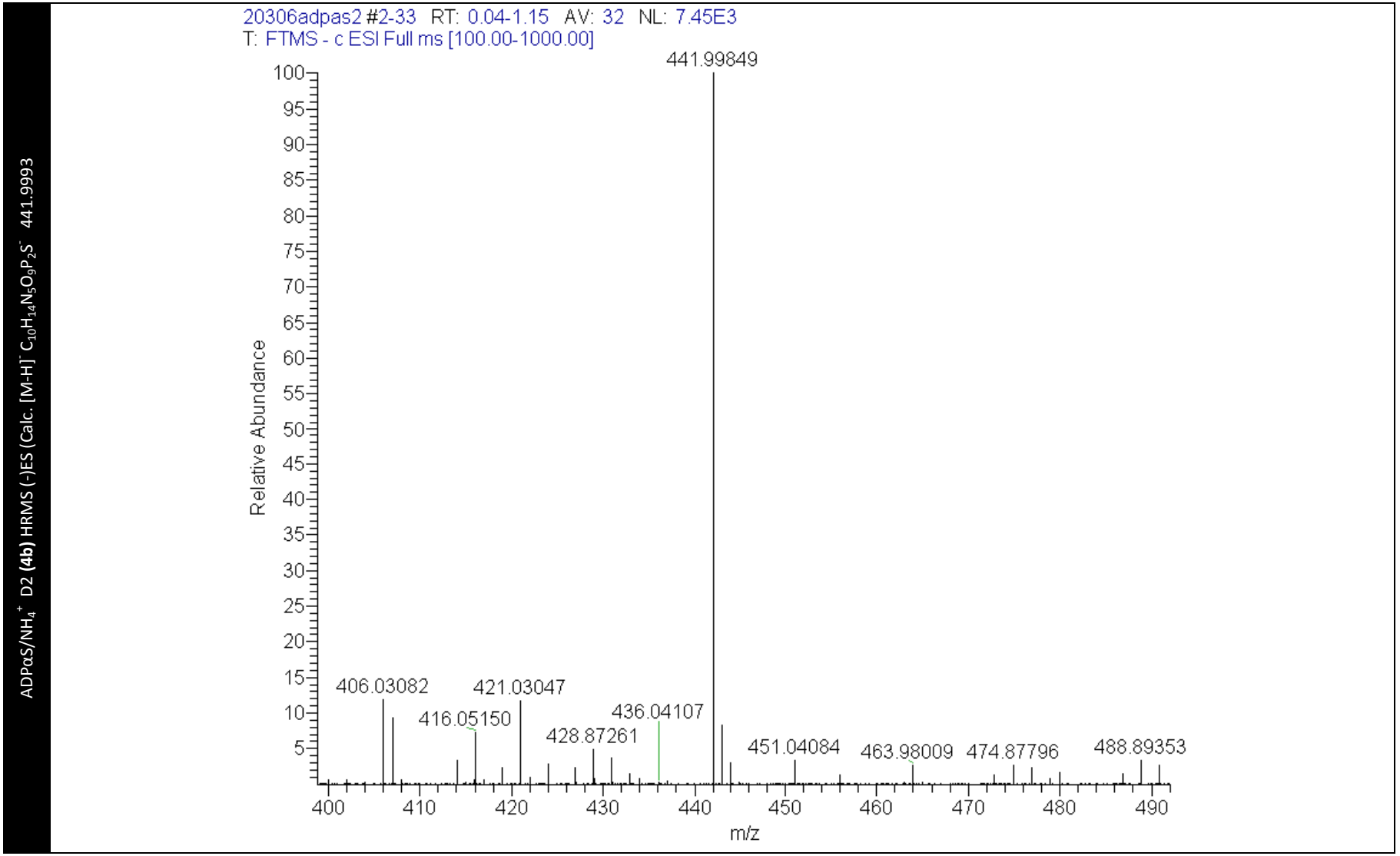

^{31}P NMR ADPαS/NH$_4^+$ D1 (4a)
ADPaS_D1_NH4_31P_25deg_zapis.esp
Normalized Intensity
Chemical Shift (ppm)
43.48
43.32
-9.42
-9.59
-9.60
1.01
1.00

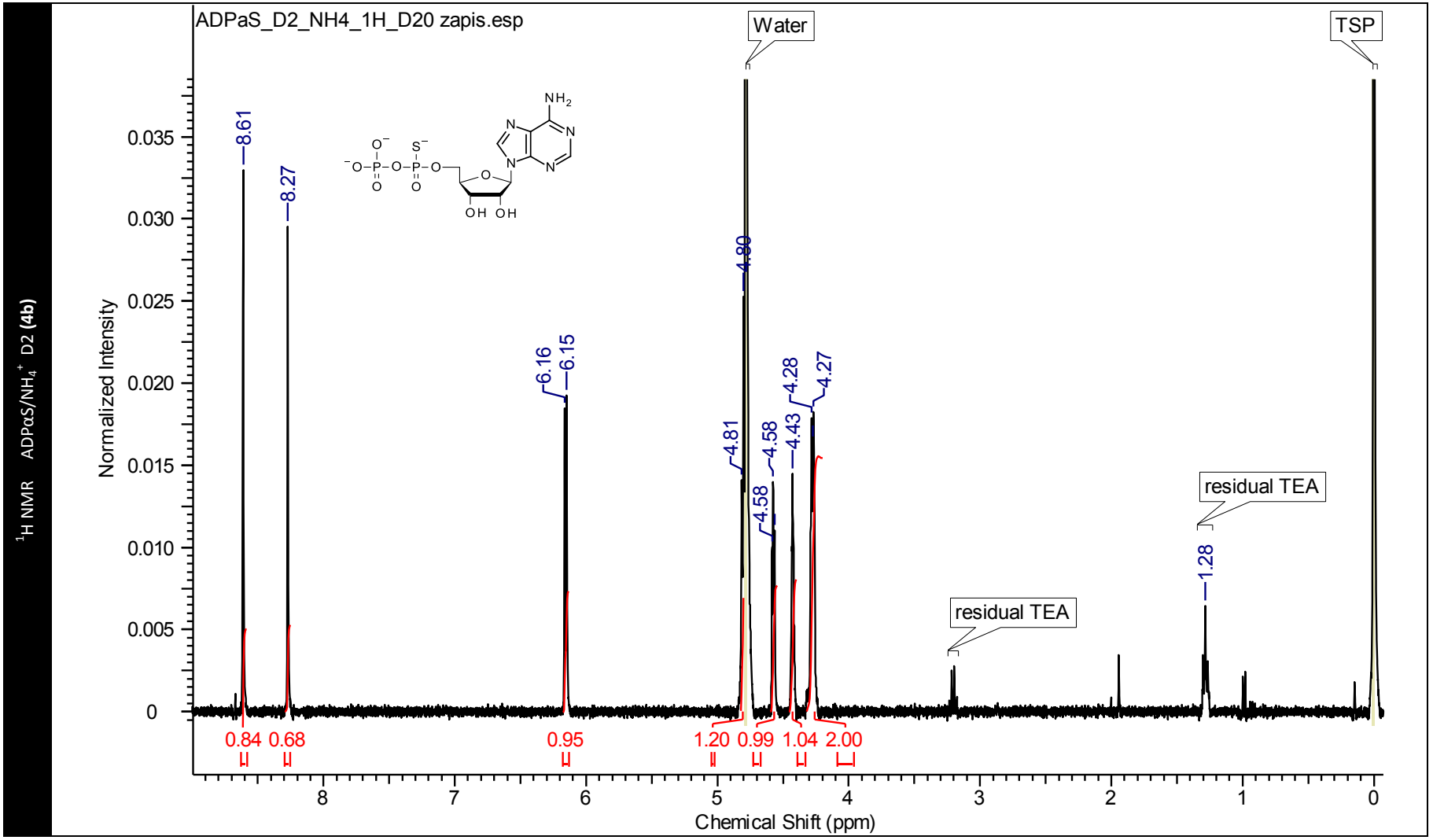
(**4b**) ADPαS/NH_4^+ D2

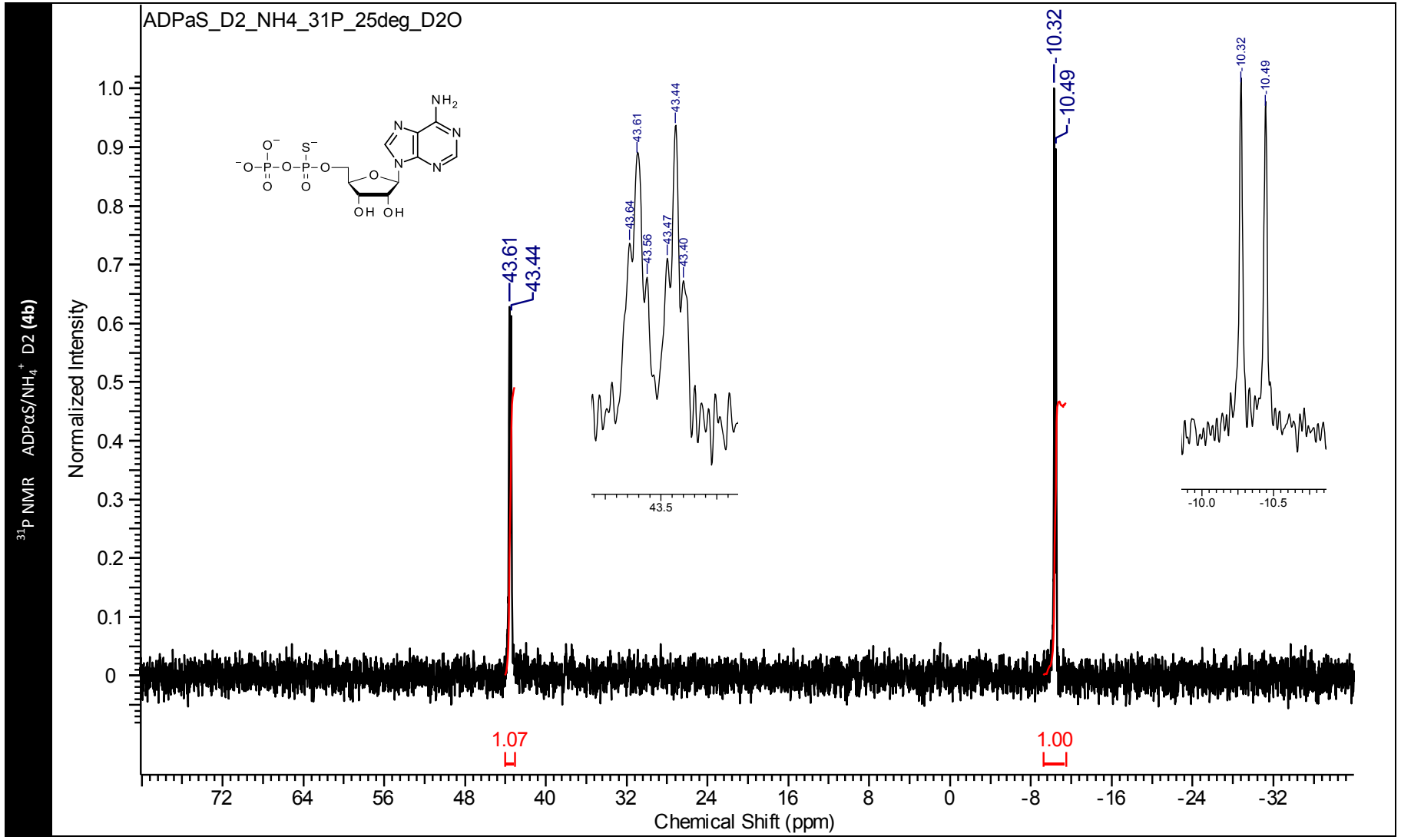


ADPαS/NH_4^+ D2 (**4b**) HPLC

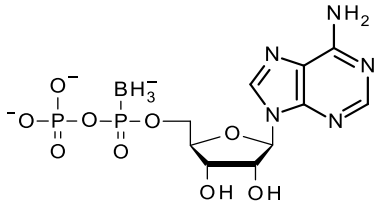



ADPαS/NH4+ D2 (4b) HRMS (-)ES (Calc. [M-H]- C10H14N5O9P2S- 441.9993
20306adpas2 #2-33 RT: 0.04-1.15 AV: 32 NL: 7.45E3
T: FTMS - c ESI Full ms [100.00-1000.00]
Relative Abundance
m/z
441.99849
406.03082
421.03047
416.05150
436.04107
428.87261
451.04084
463.98009
474.87796
488.89353

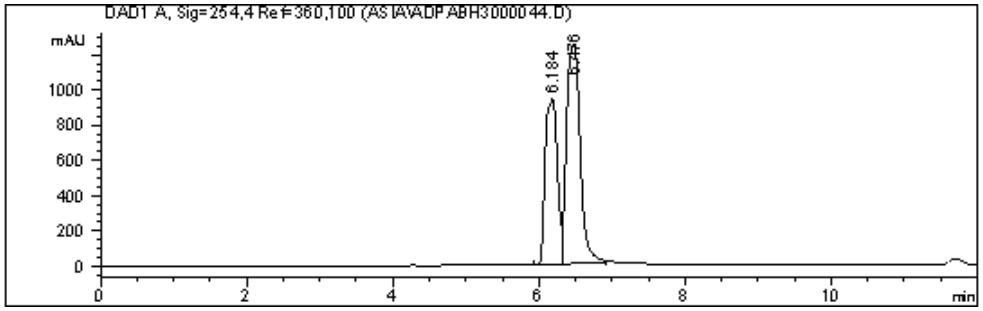

^{1}H NMR ADPαS/NH_4^+ D2 **(4b)**
ADPaS_D2_NH4_1H_D20 zapis.esp
Water
TSP
residual TEA
residual TEA
8.61
8.27
6.16
6.15
4.90
4.81
4.58
4.58
4.43
4.28
4.27
1.28
0.84
0.68
0.95
1.20
0.99
1.04
2.00
Normalized Intensity
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

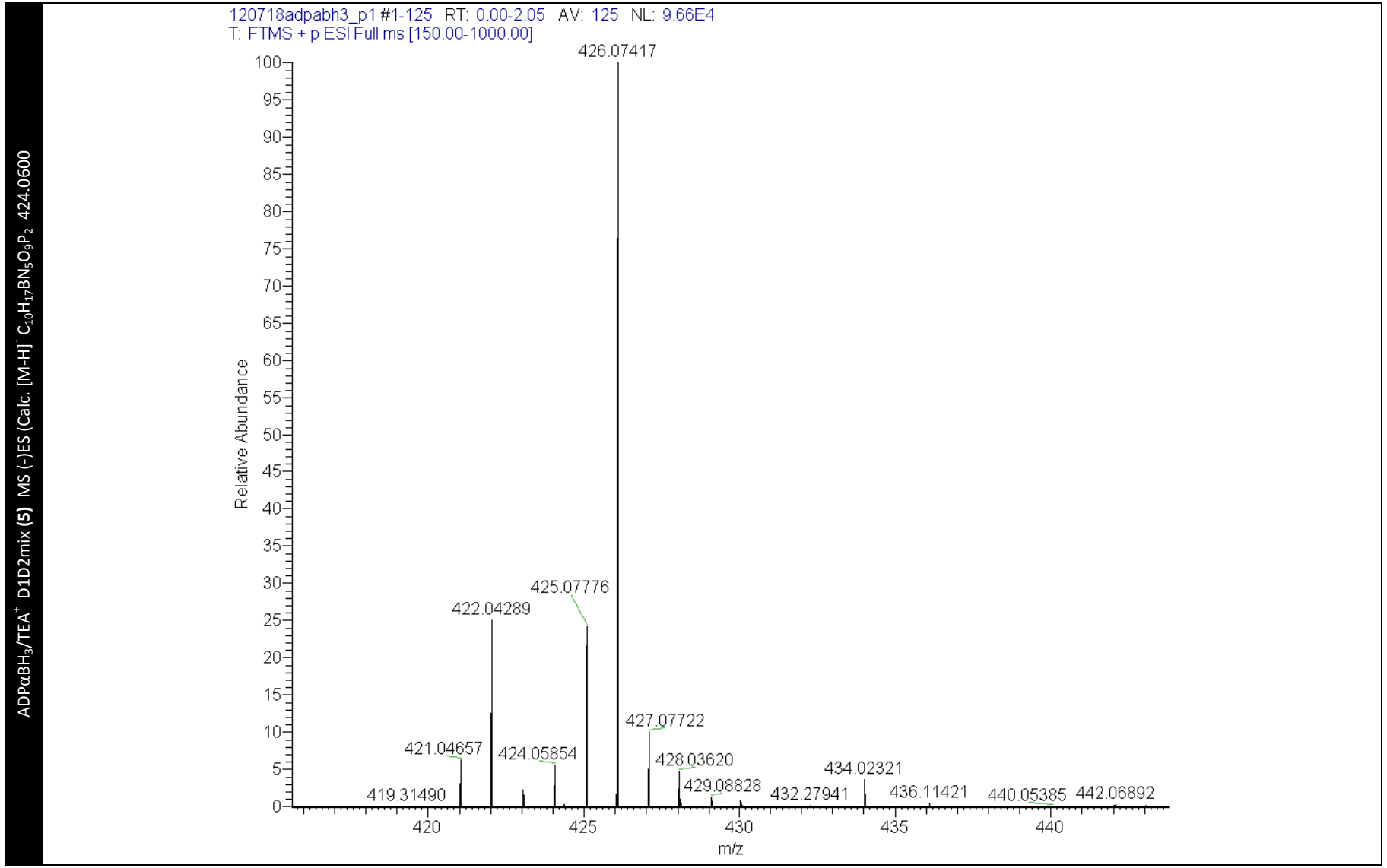

^{31}P NMR ADPαS/NH_4^+ D2 (4b)
ADPaS_D2_NH4_31P_25deg_D2O
Normalized Intensity
Chemical Shift (ppm)
43.61
43.44
43.64
43.56
43.47
43.40
-10.32
-10.49
1.07
1.00

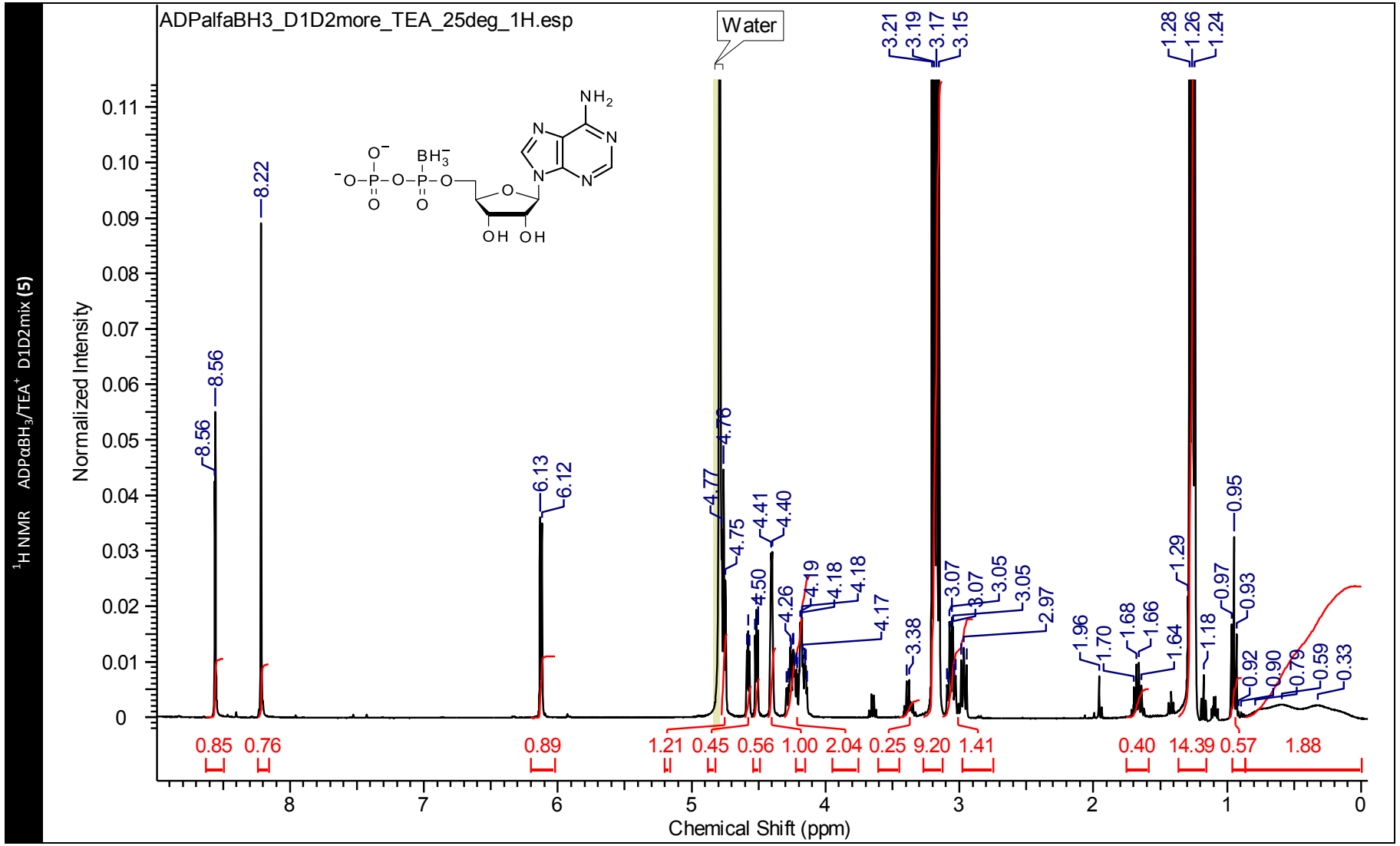
(**5**) ADPαBH$_3$/TEA$^+$ D1D2mix

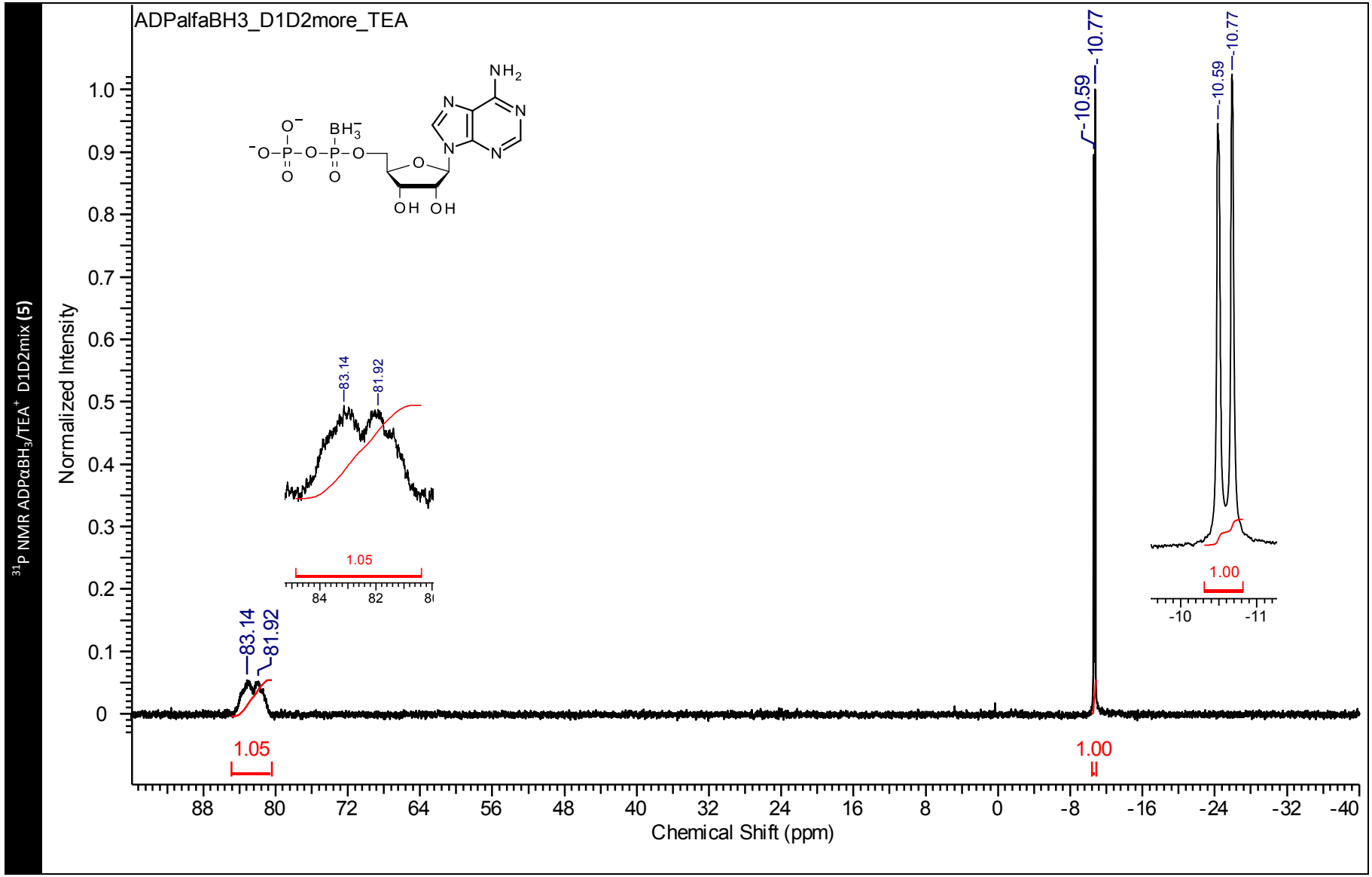


ADPαBH$_3$/TEA$^+$ D1D2mix **(5)** HPLC

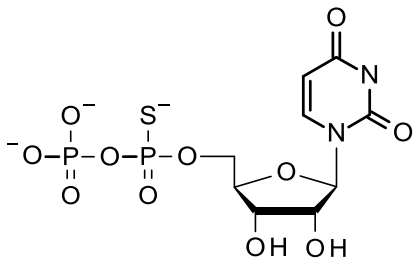



ADPαBH3/TEA+ D1D2mix (5) MS (-)ES (Calc. [M-H]- C10H17BN5O9P2 424.0600
120718adpabh3_p1 #1-125 RT: 0.00-2.05 AV: 125 NL: 9.66E4
T: FTMS + p ESI Full ms [150.00-1000.00]
Relative Abundance
m/z
426.07417
425.07776
422.04289
427.07722
421.04657
424.05854
428.03620
429.08828
434.02321
419.31490
432.27941
436.11421
440.05385
442.06892

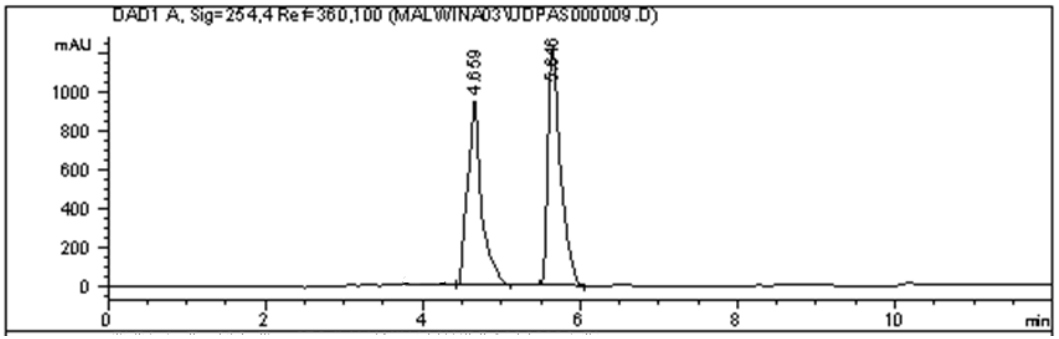

^{1}H NMR ADPαBH$_3$/TEA$^+$ D1D2mix (5)
ADPalfaBH3_D1D2more_TEA_25deg_1H.esp
Water
Normalized Intensity
Chemical Shift (ppm)
0.85 0.76 0.89 1.21 0.45 0.56 1.00 2.04 0.25 9.20 1.41 0.40 14.39 0.57 1.88


ADPalfaBH3_D1D2more_TEA
^{31}P NMR ADPαBH$_3$/TEA$^+$ D1D2mix **(5)**
Normalized Intensity
Chemical Shift (ppm)
83.14
81.92
10.59
10.77
1.05
1.00
NH$_2$
BH$_3^-$
OH OH

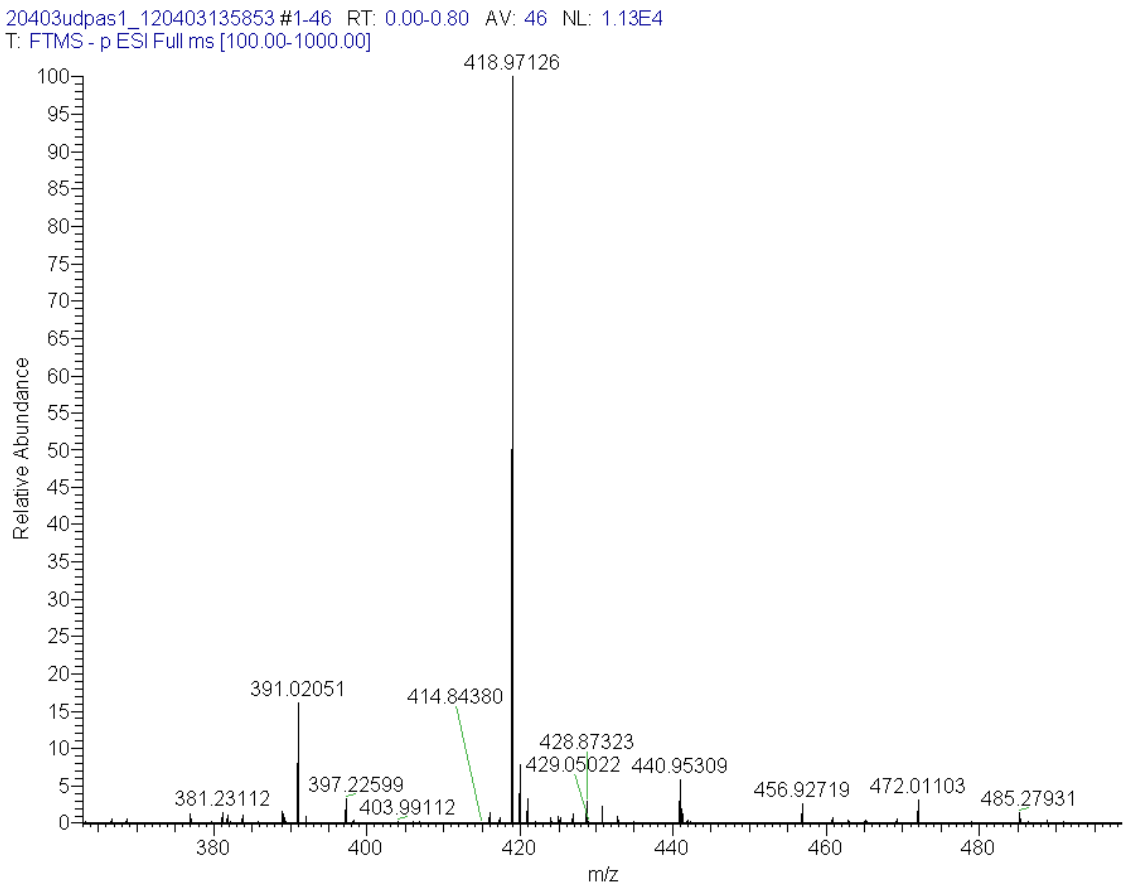
(**6**) UDPαS/TEA$^+$ D1D2mix



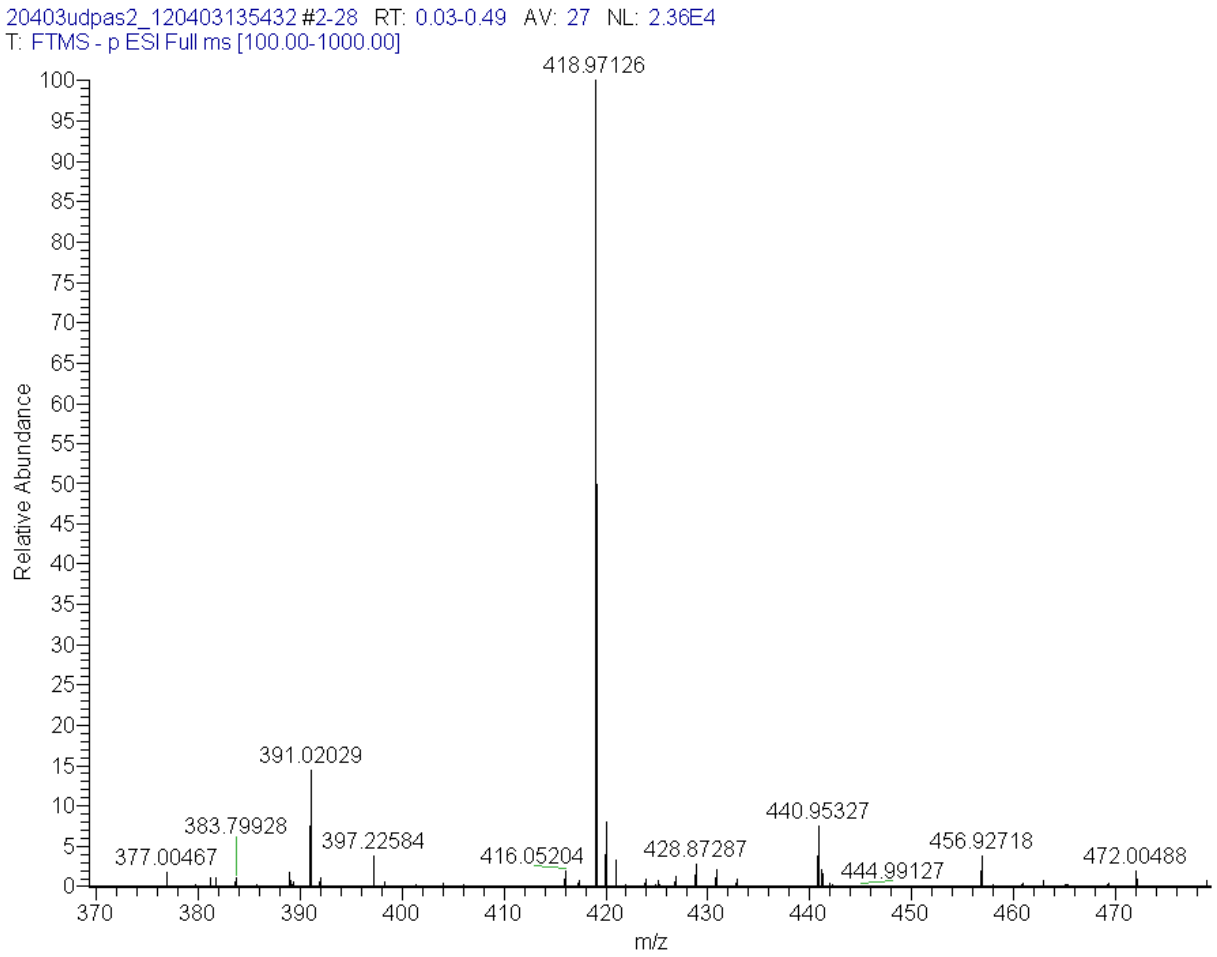
UDPαS/TEA$^+$ D1D2mix **(6)** HPLC

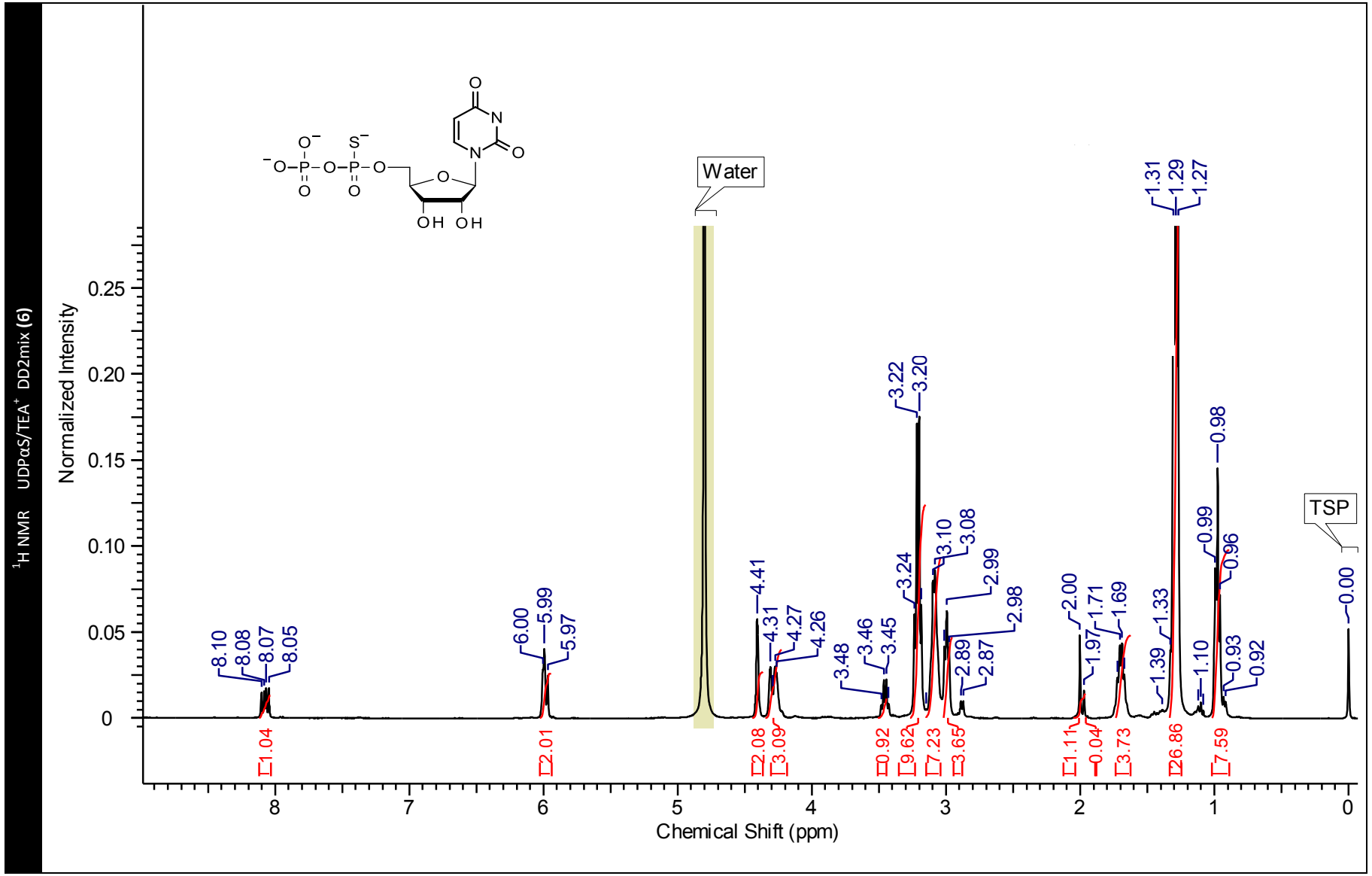


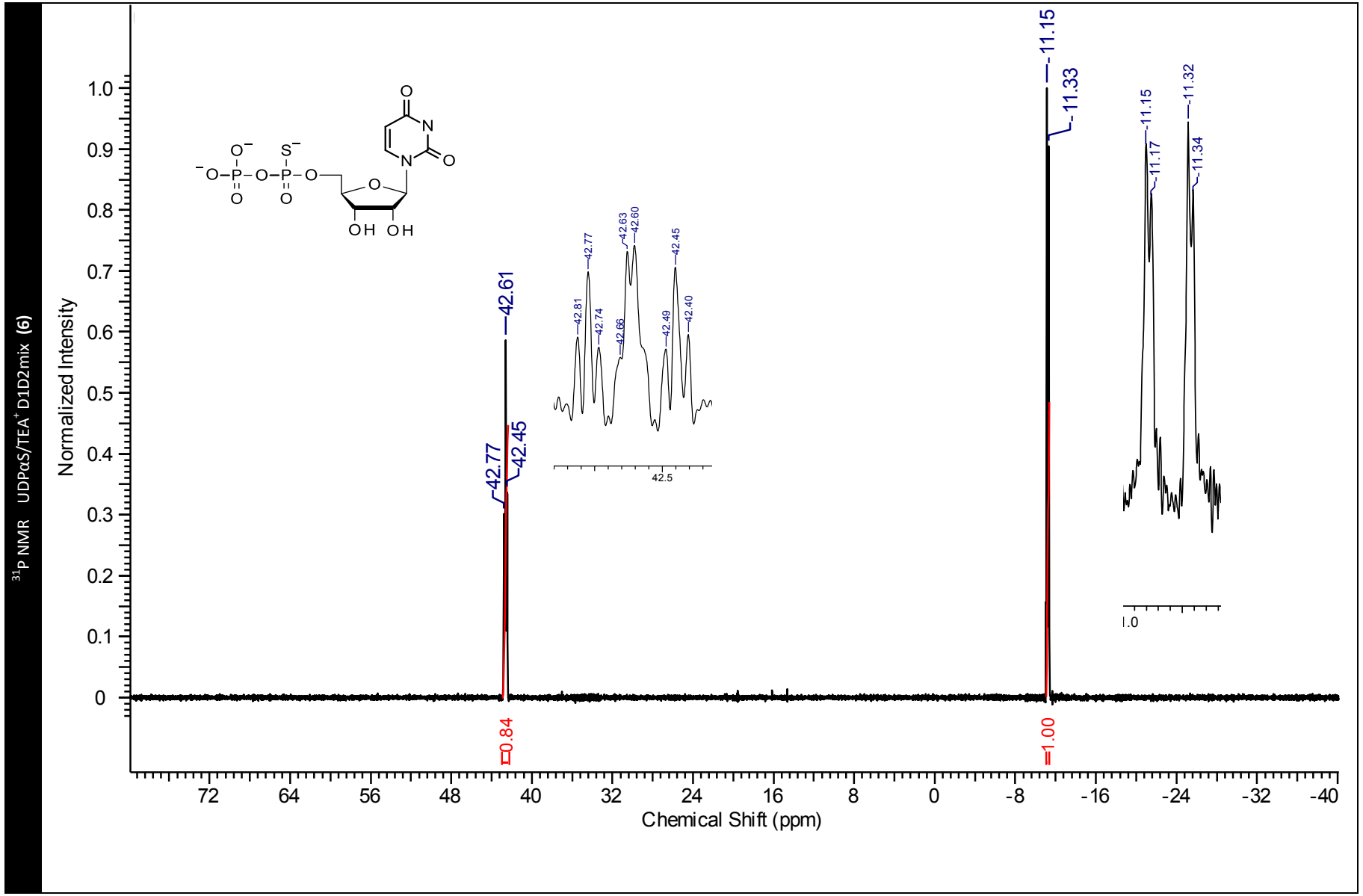
UDPαS/TEA **D1(6)** MS (-)ES (Calc. $[M-H]^-$ $C_9H_{13}N_2O_{11}P_2S^-$ 418.9721



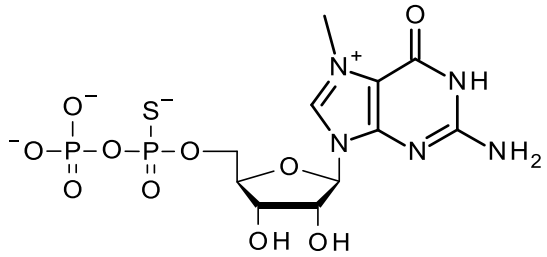
UDPαS/TEA **(6)** MS (-)ES (Calc. [M-H] $C_9H_{13}N_2O_{11}P_2S$ 418.9721



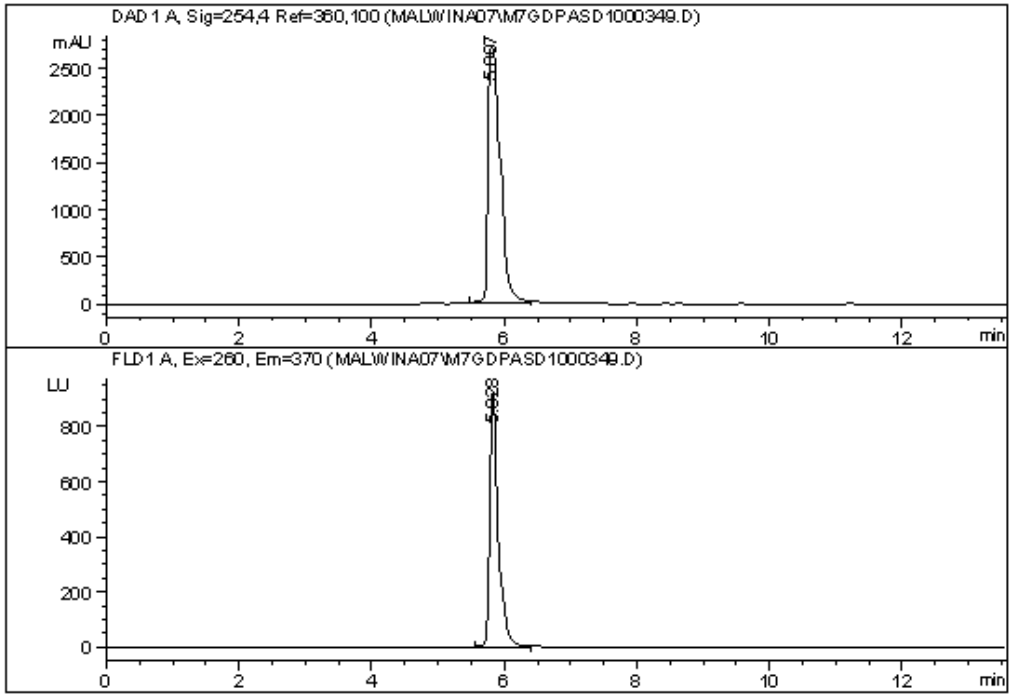

^{1}H NMR UDPαS/TEA$^+$ DD2mix (6)
Normalized Intensity
Chemical Shift (ppm)
Water
TSP
8.10
8.08
8.07
8.05
6.00
5.99
5.97
4.41
4.31
4.27
4.26
3.48
3.46
3.45
3.24
3.22
3.20
3.10
3.08
2.99
2.98
2.89
2.87
2.00
1.97
1.71
1.69
1.39
1.33
1.31
1.29
1.27
1.10
0.99
0.98
0.96
0.93
0.92
0.00
1.04
2.01
2.08
3.09
0.92
9.62
7.23
3.65
1.11
0.04
3.73
26.86
7.59

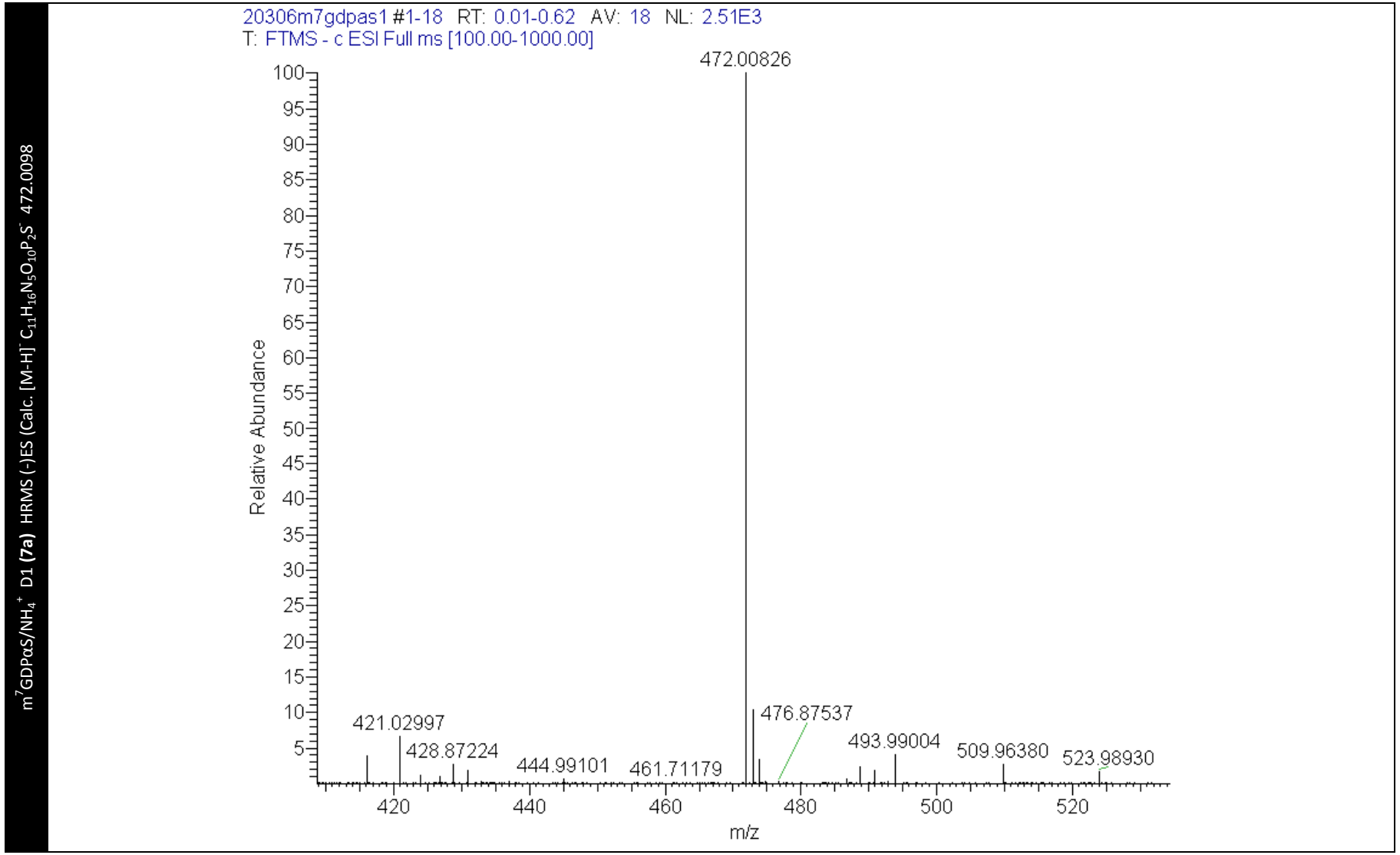

^{31}P NMR UDPαS/TEA^{+} D1D2mix **(6)**
Normalized Intensity
Chemical Shift (ppm)
42.61
42.77
42.45
11.15
11.33
11.15
11.17
11.32
11.34
0.84
1.00

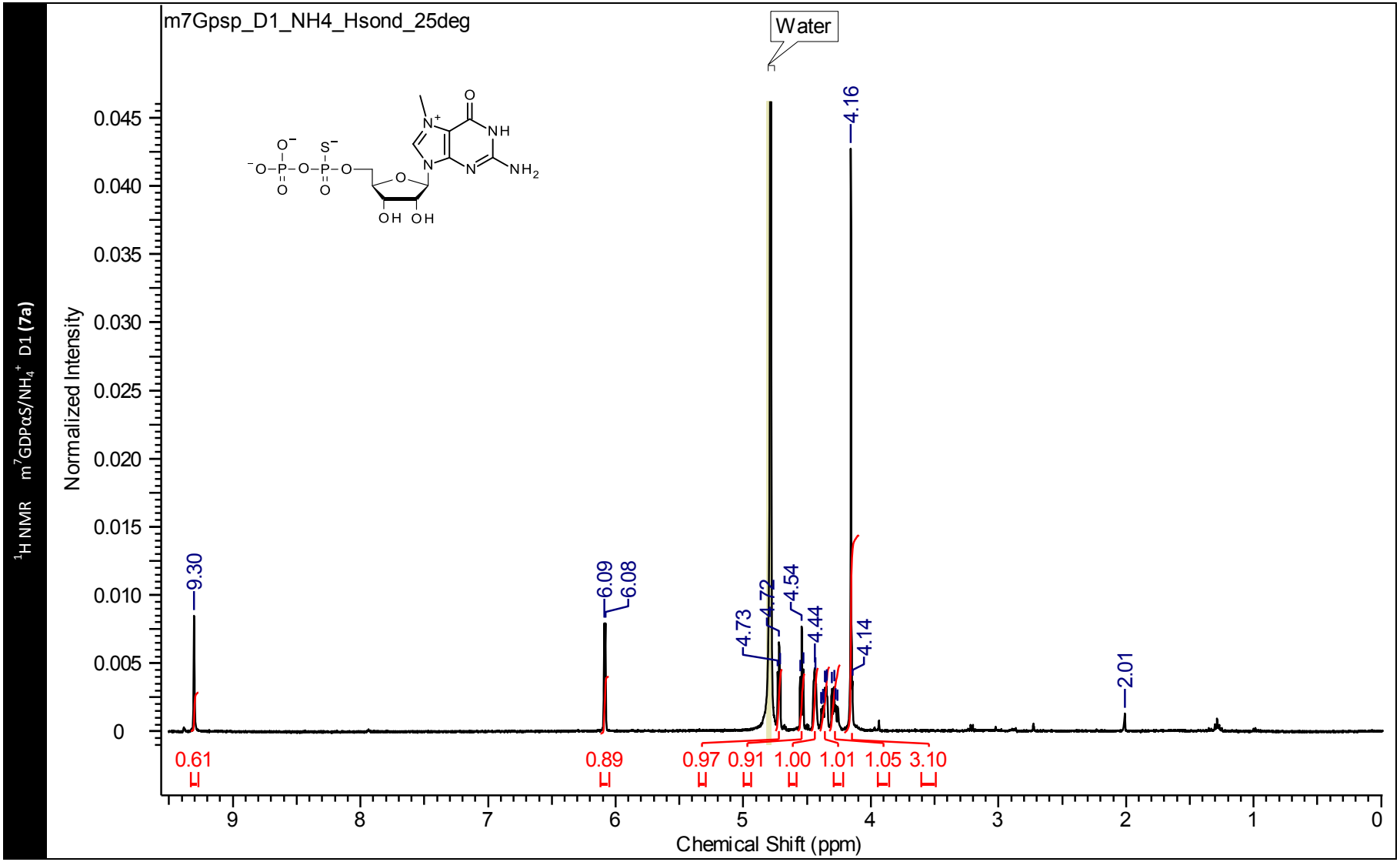
(**7a**) $m^7GDP\alpha S/NH_4^+$ D1

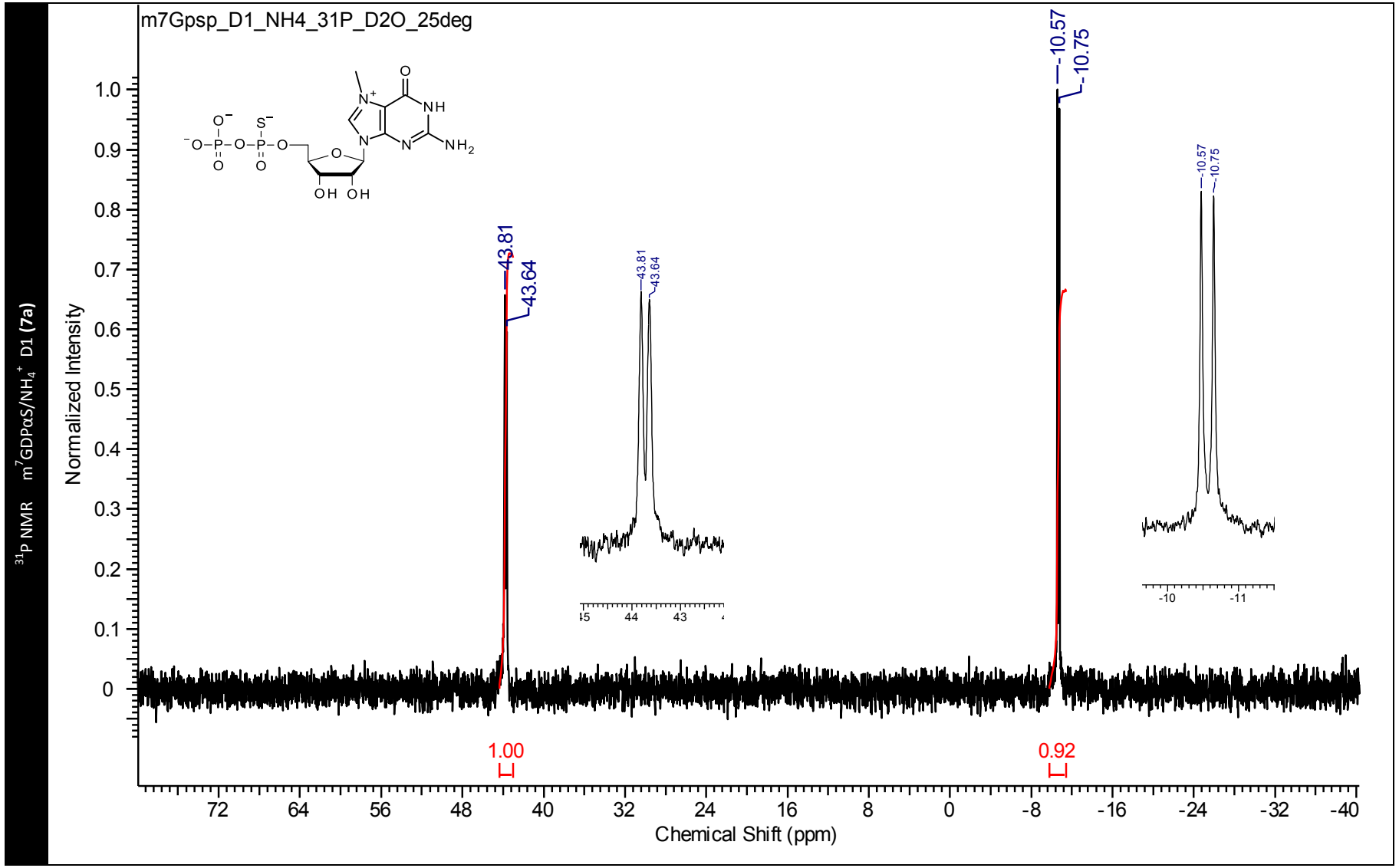


$m^7GDP\alpha S/NH_4^+$ D1 (**7a**) HPLC

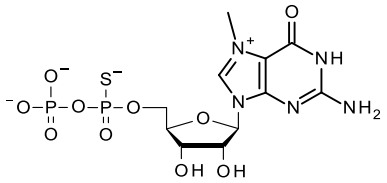



m7GDPαS/NH4+ D1 (7a) HRMS (-)ES (Calc. [M-H]- C11H16N5O10P2S- 472.0098
20306m7gdpas1 #1-18 RT: 0.01-0.62 AV: 18 NL: 2.51E3
T: FTMS - c ESI Full ms [100.00-1000.00]
Relative Abundance
m/z
472.00826
476.87537
421.02997
428.87224
444.99101
461.71179
493.99004
509.96380
523.98930

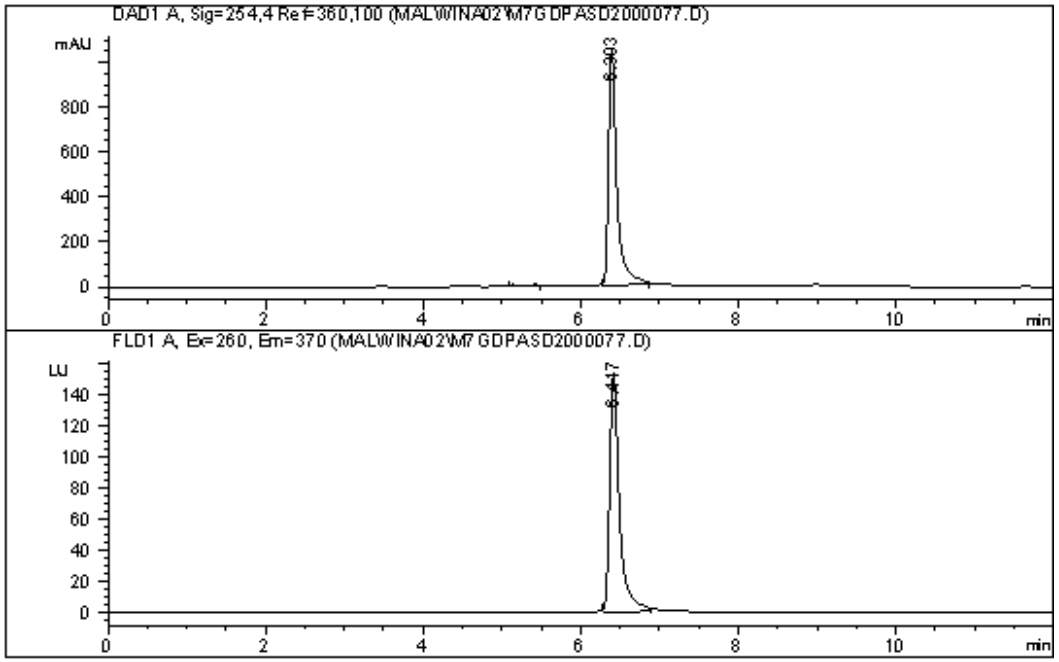

^{1}H NMR m^{7}GDPαS/NH$_4^+$ D1 (7a)
m7Gpsp_D1_NH4_Hsond_25deg
Water
Normalized Intensity
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0
9.30
6.09
6.08
4.73
4.72
4.54
4.44
4.16
4.14
2.01
0.61
0.89
0.97
0.91
1.00
1.01
1.05
3.10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

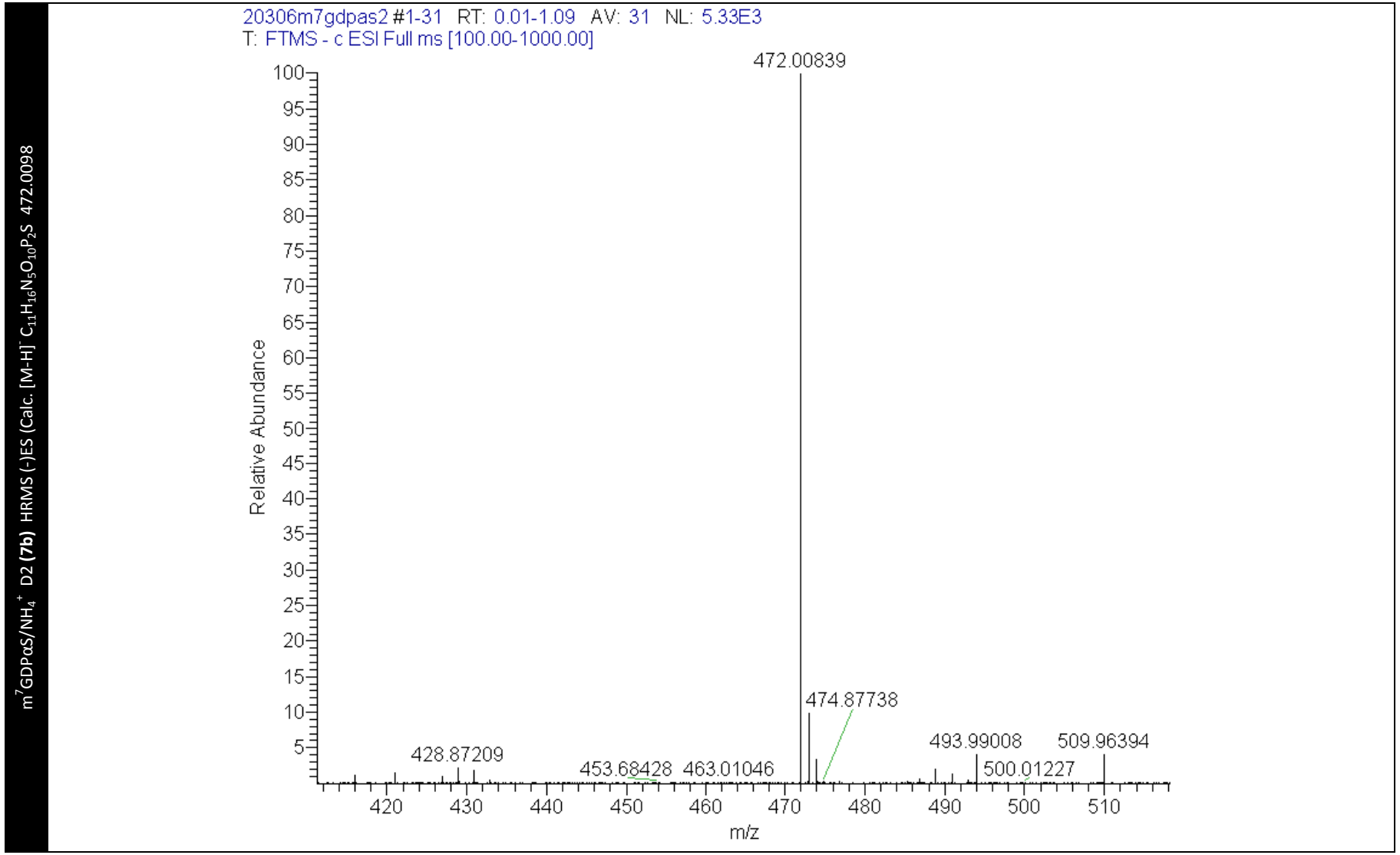

^{31}P NMR m^{7}GDPαS/NH$_4^+$ D1 (7a)
m7Gpsp_D1_NH4_31P_D2O_25deg
Normalized Intensity
Chemical Shift (ppm)
43.81
43.64
-10.57
-10.75
1.00
0.92

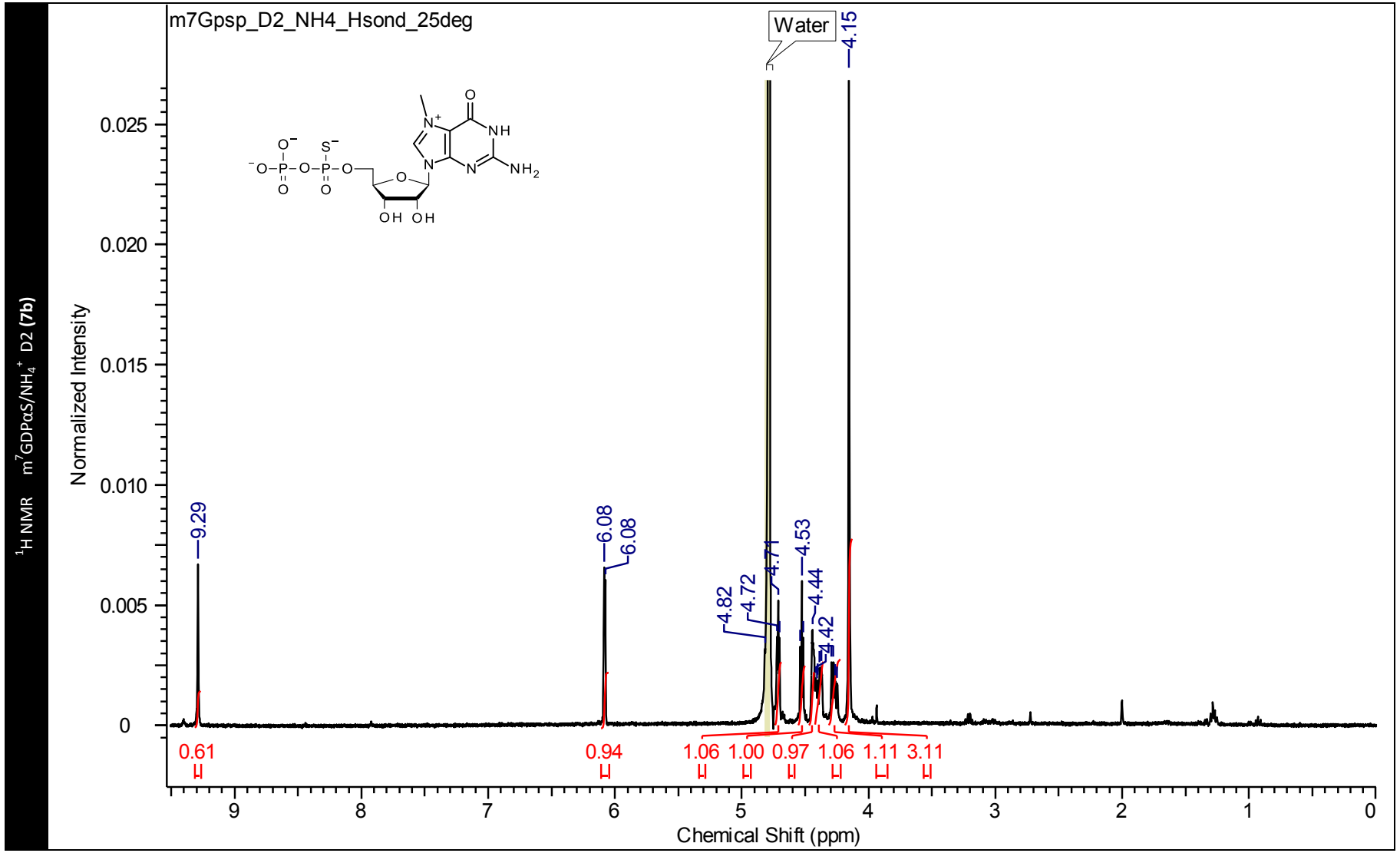
(**7b**) $m^7GDP\alpha S/NH_4^+$ D2

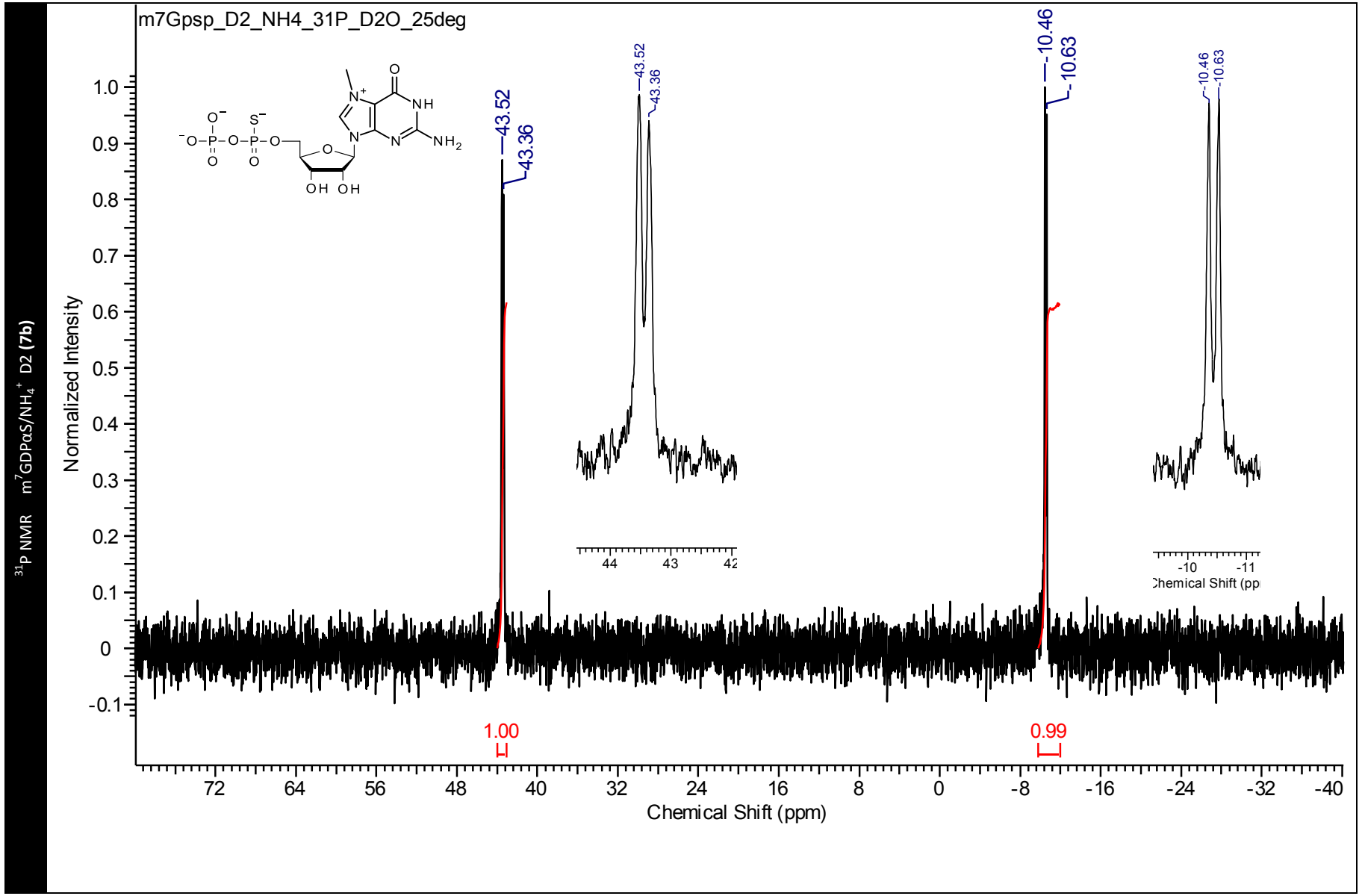


$m^7GDP\alpha S/NH_4^+$ D2 **(7b)** HPLC

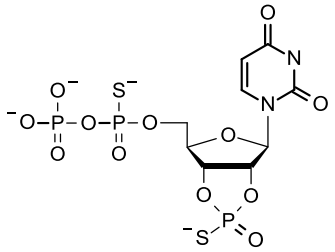



m7GDPαS/NH4+ D2 (7b) HRMS (-)ES (Calc. [M-H]- C11H16N5O10P2S 472.0098
20306m7gdpas2 #1-31 RT: 0.01-1.09 AV: 31 NL: 5.33E3
T: FTMS - c ESI Full ms [100.00-1000.00]
472.00839
474.87738
428.87209
453.68428
463.01046
493.99008
500.01227
509.96394
Relative Abundance
m/z

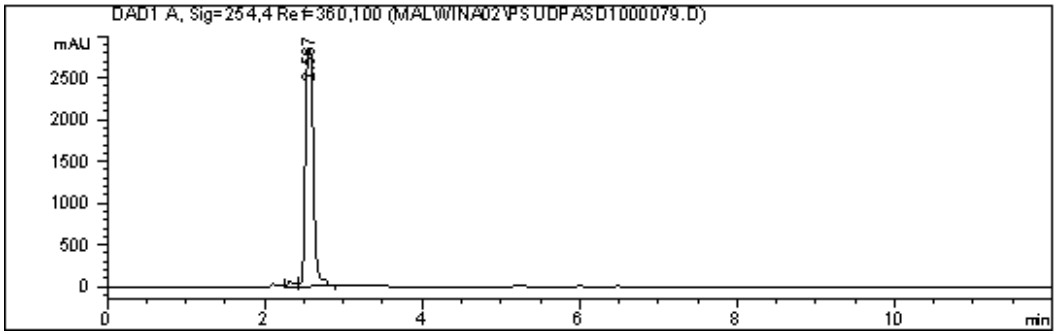

m7Gpsp_D2_NH4_Hsond_25deg
^{1}H NMR m^{7}GDPαS/NH$_4^+$ D2 (7b)
Water
4.15
9.29
6.08
6.08
4.82
4.72
4.71
4.53
4.44
4.42
0.61
0.94
1.06 1.00 0.97 1.06 1.11 3.11
Normalized Intensity
Chemical Shift (ppm)

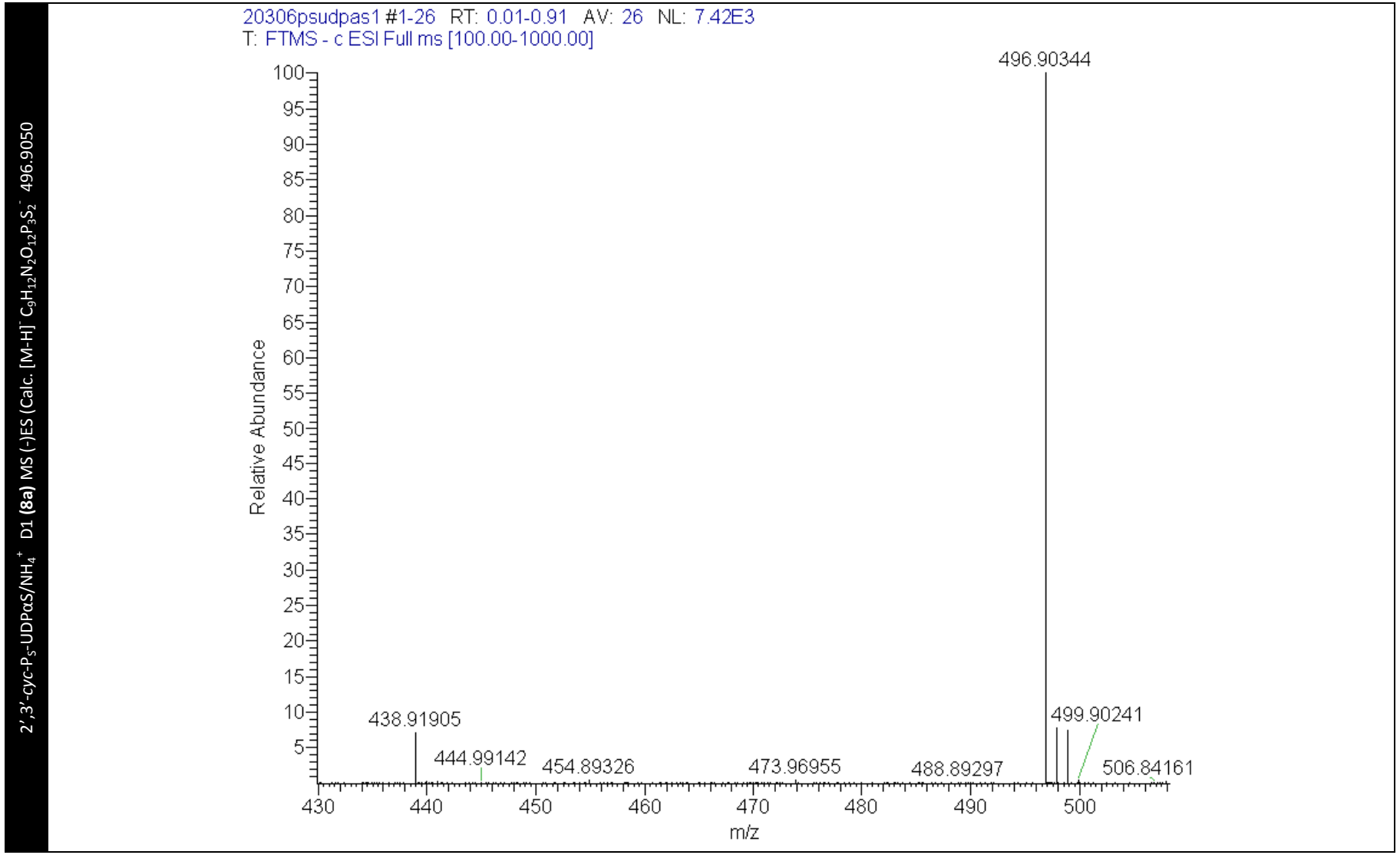

^{31}P NMR m^{7}GDPαS/NH$_4^+$ D2 (7b)
m7Gpsp_D2_NH4_31P_D2O_25deg
Normalized Intensity
Chemical Shift (ppm)
43.52
43.36
-10.46
-10.63
1.00
0.99

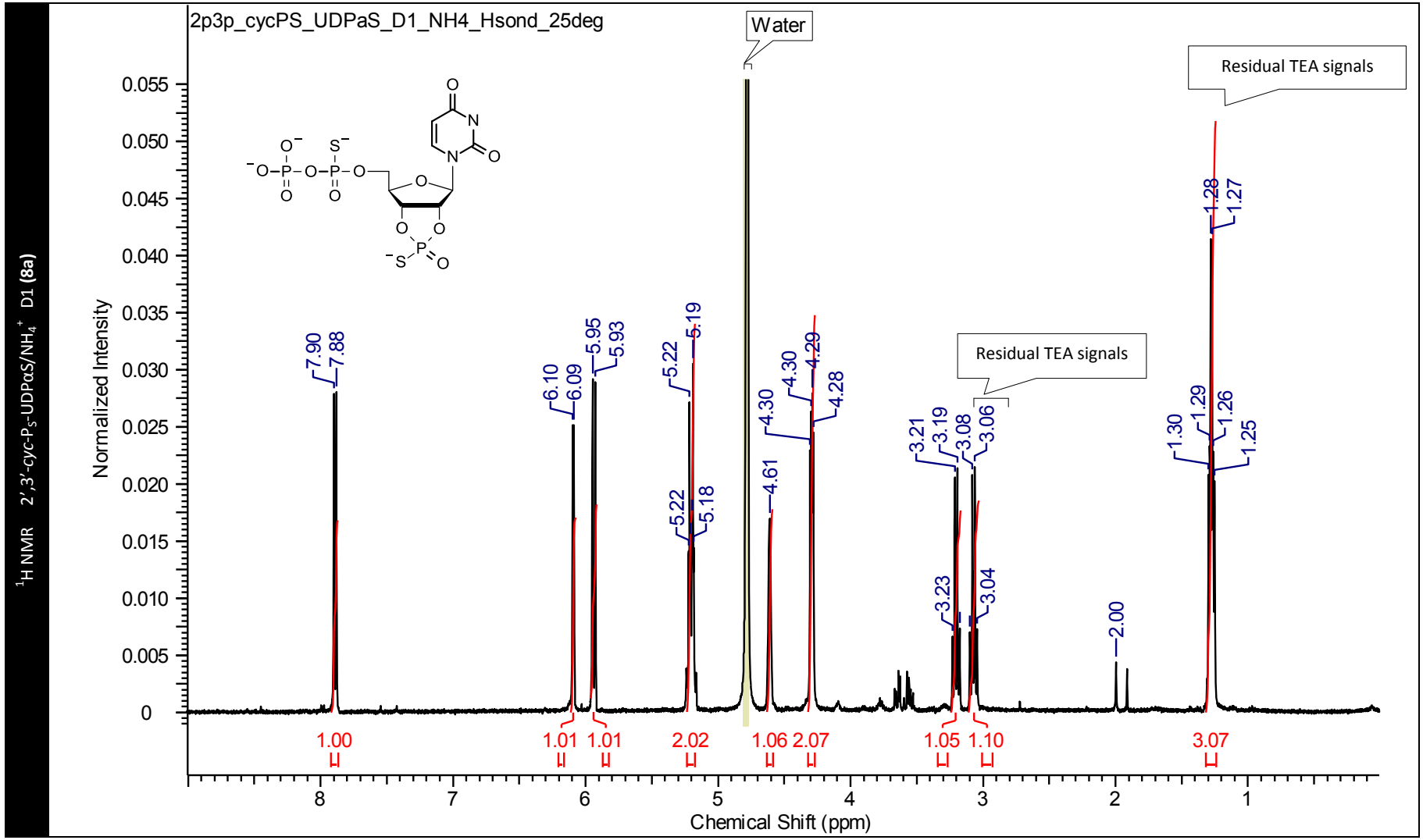
(**8a**) 2',3'-*cyc*-P_S-UDPαS/NH_4^+ D1

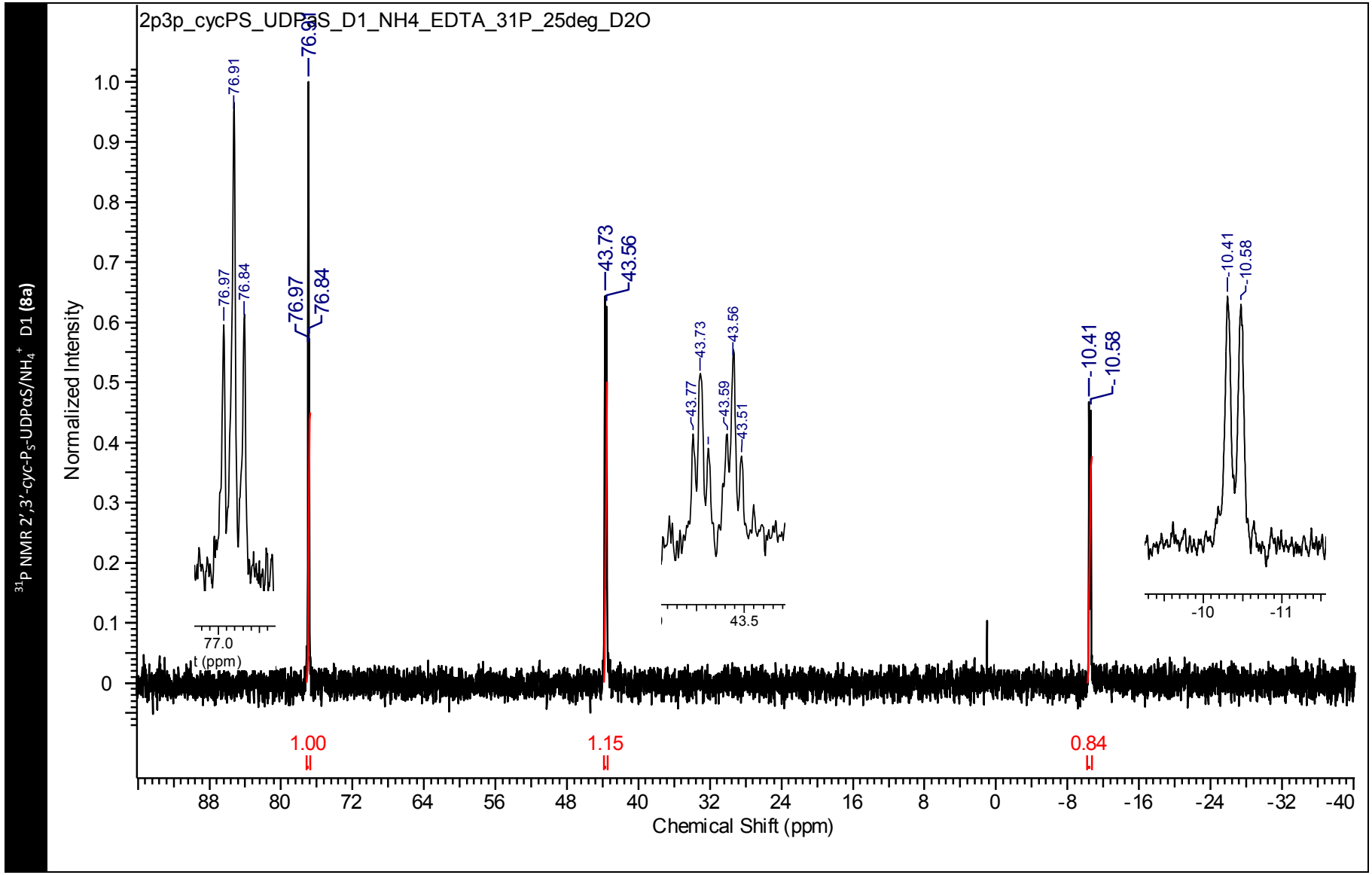


2',3'-*cyc*-P_S-UDPαS/NH_4^+ D1 (**8a**) HPLC

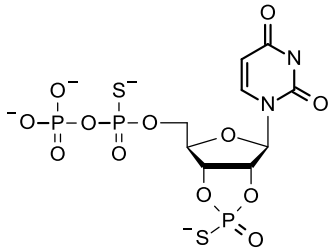



2',3'-cyc-P$_S$-UDPαS/NH$_4^+$ D1 **(8a)** MS (-)ES (Calc. [M-H]$^-$ $C_9H_{12}N_2O_{12}P_3S_2^-$ 496.9050
20306psudpas1 #1-26 RT: 0.01-0.91 AV: 26 NL: 7.42E3
T: FTMS - c ESI Full ms [100.00-1000.00]
Relative Abundance
m/z
496.90344
438.91905
444.99142
454.89326
473.96955
488.89297
499.90241
506.84161

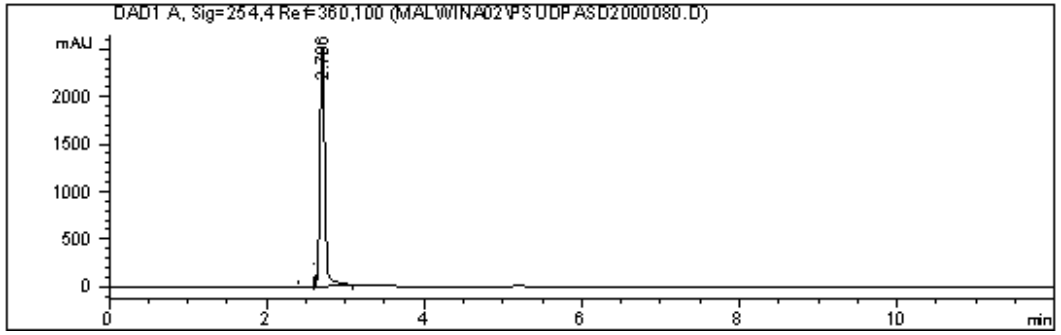

^{1}H NMR 2′,3′-cyc-P$_S$-UDPαS/NH$_4^+$ D1 (8a)
2p3p_cycPS_UDPaS_D1_NH4_Hsond_25deg
Water
Residual TEA signals
Residual TEA signals
Normalized Intensity
Chemical Shift (ppm)
7.90
7.88
6.10
6.09
5.95
5.93
5.22
5.19
5.22
5.18
4.61
4.30
4.29
4.28
4.30
3.21
3.19
3.08
3.06
3.23
3.04
2.00
1.30
1.29
1.28
1.27
1.26
1.25
1.00
1.01
1.01
2.02
1.06
2.07
1.05
1.10
3.07

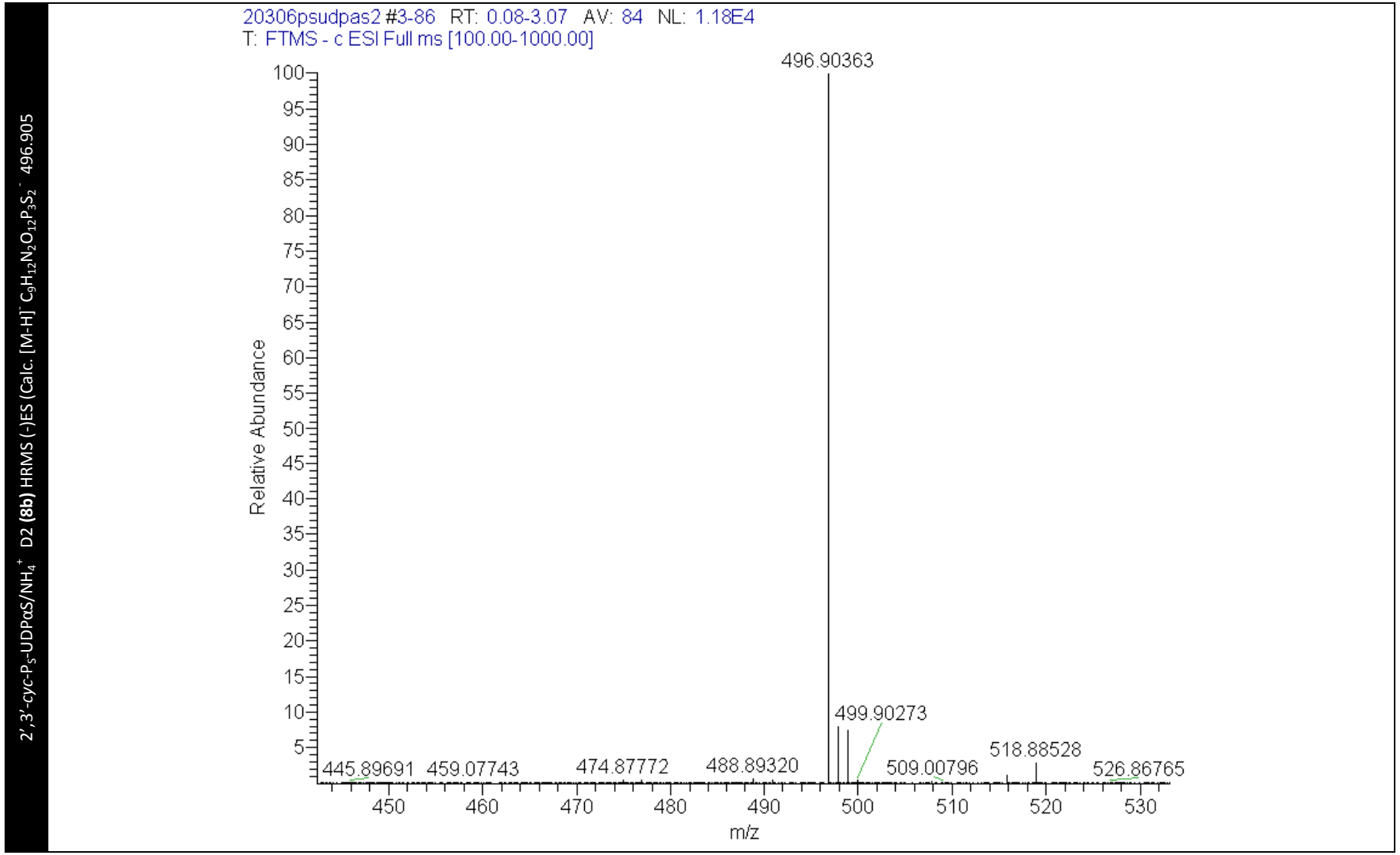

^{31}P NMR 2′,3′-*cyc*-P$_S$-UDPαS/NH$_4^+$ D1 (8a)
2p3p_cycPS_UDPaS_D1_NH4_EDTA_31P_25deg_D2O
Normalized Intensity
Chemical Shift (ppm)
76.91
76.97
76.84
43.73
43.56
43.77
43.59
43.51
-10.41
-10.58
1.00
1.15
0.84

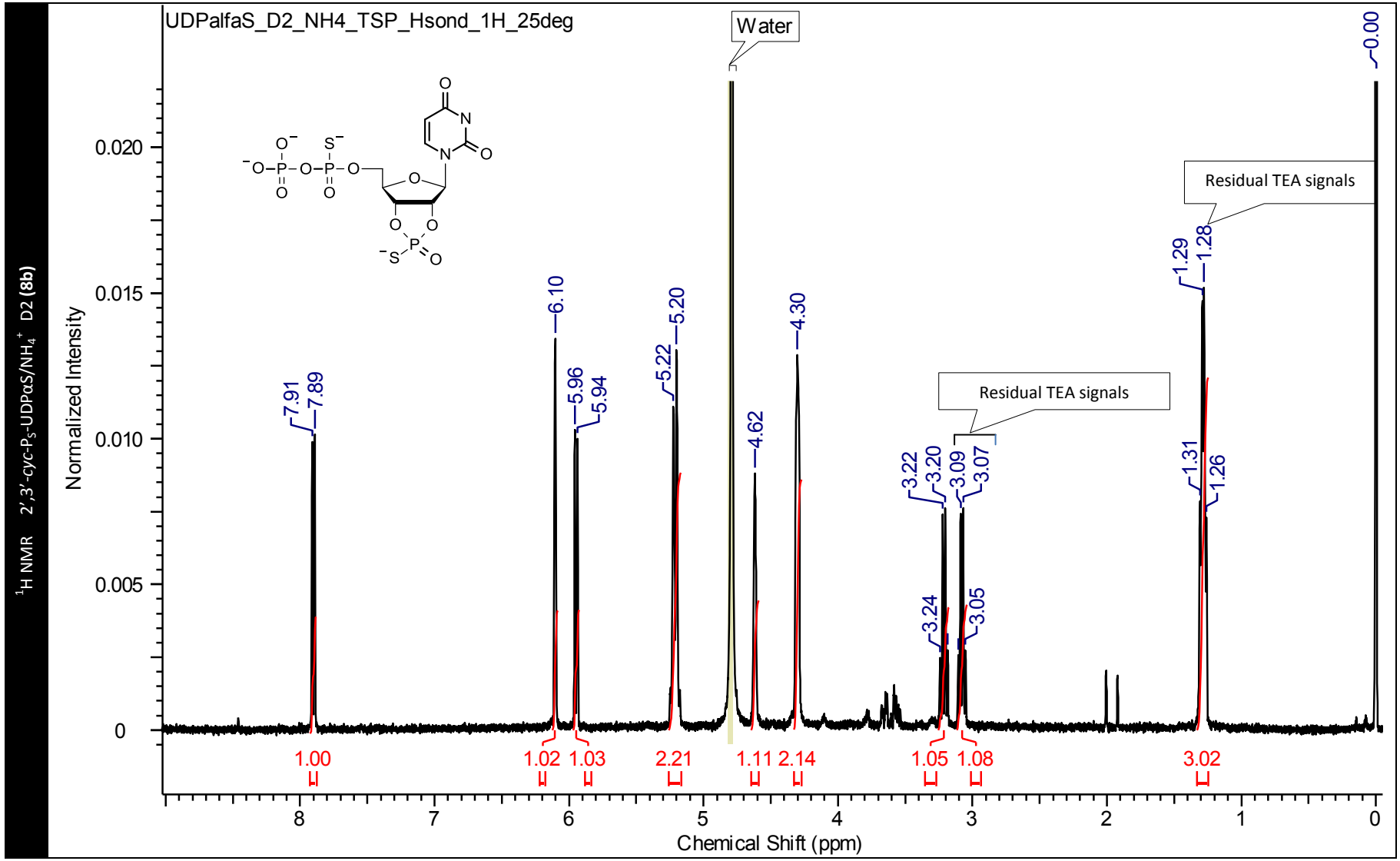
(**8b**) 2',3'-*cyc*-P_S-UDPαS/NH_4^+ D2

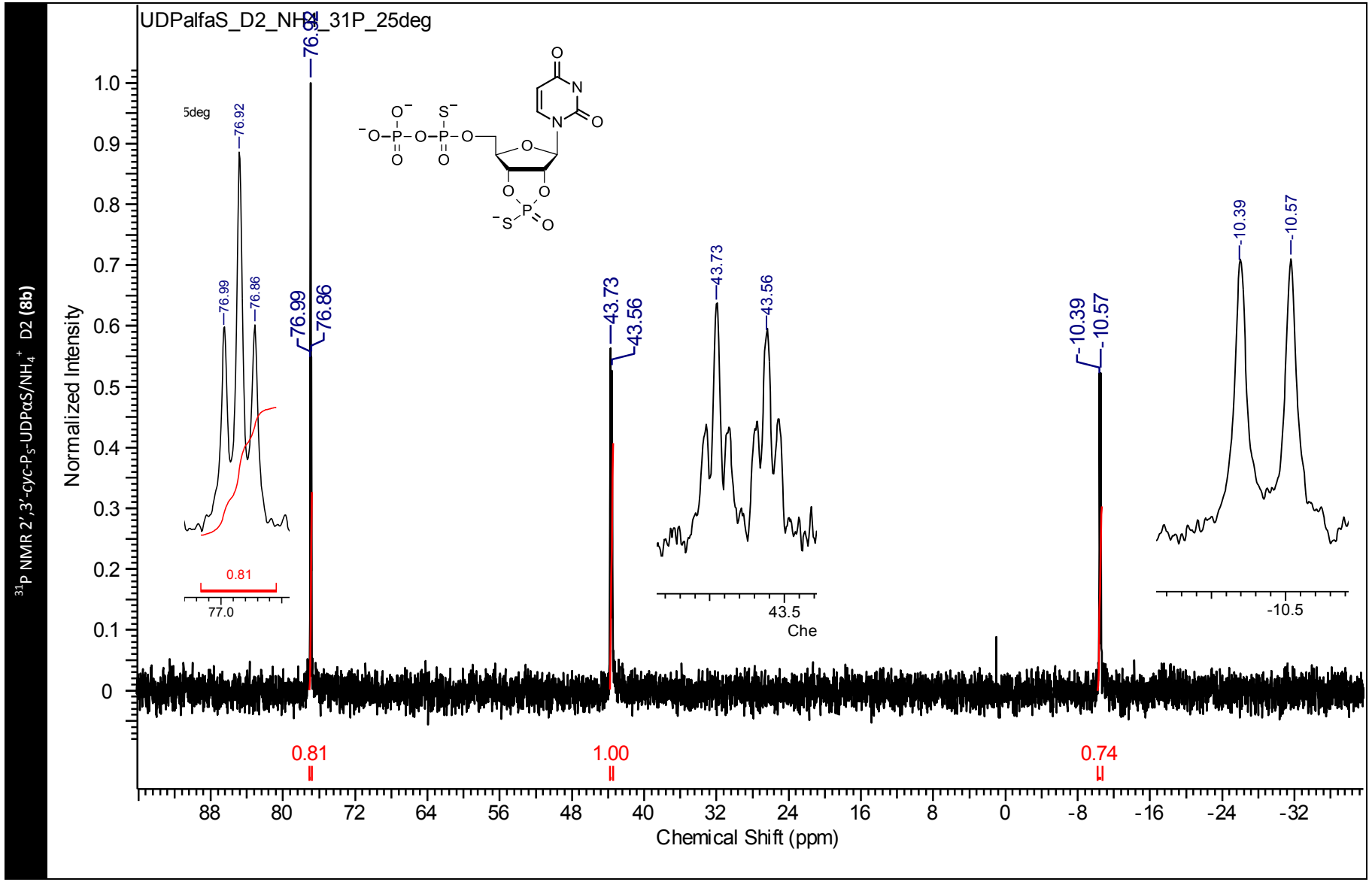


2',3'-*cyc*-P_S-UDPαS/NH_4^+ D2 (**8b**) HPLC

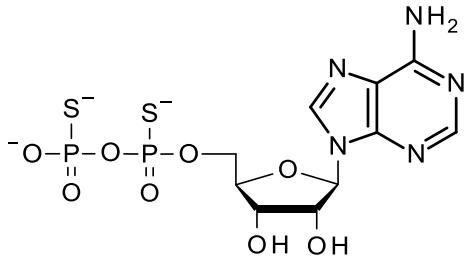



2',3'-cyc-P$_S$-UDPαS/NH$_4^+$ D2 (8b) HRMS (-)ES (Calc. [M-H]$^-$ $C_9H_{12}N_2O_{12}P_3S_2^-$ 496.905
20306psudpas2 #3-86 RT: 0.08-3.07 AV: 84 NL: 1.18E4
T: FTMS - c ESI Full ms [100.00-1000.00]
Relative Abundance
m/z
496.90363
499.90273
445.89691
459.07743
474.87772
488.89320
509.00796
518.88528
526.86765

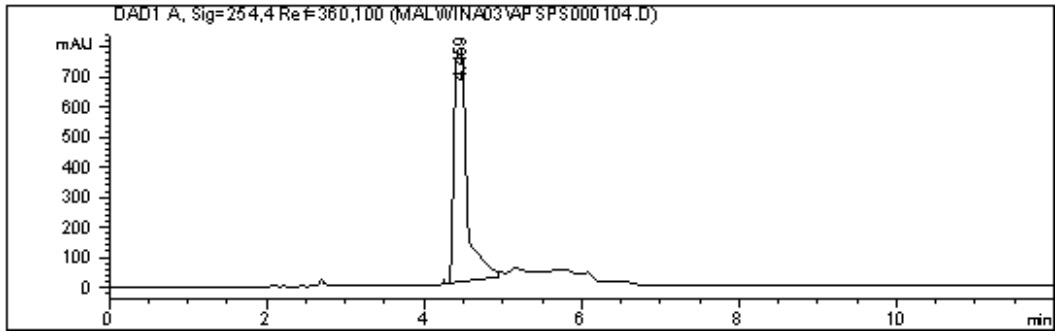

^{1}H NMR 2',3'-cyc-P$_S$-UDPαS/NH$_4^+$ D2 (8b)
UDPalfaS_D2_NH4_TSP_Hsond_1H_25deg
Water
Residual TEA signals
Residual TEA signals
Normalized Intensity
Chemical Shift (ppm)
0.020
0.015
0.010
0.005
0
8
7
6
5
4
3
2
1
0
7.91
7.89
6.10
5.96
5.94
5.22
5.20
4.62
4.30
3.22
3.20
3.09
3.07
3.24
3.05
1.29
1.28
1.31
1.26
0.00
1.00
1.02
1.03
2.21
1.11
2.14
1.05
1.08
3.02

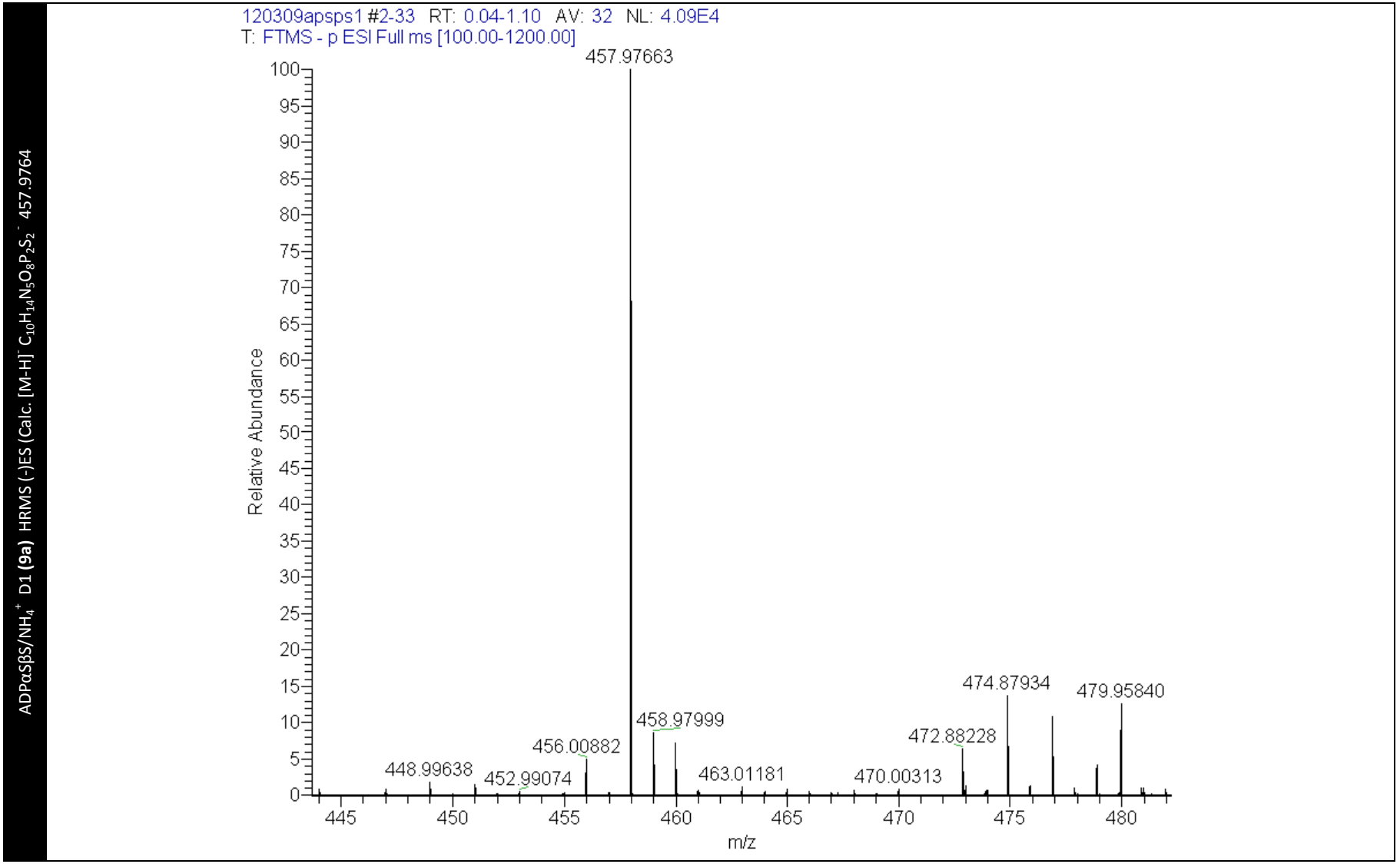

^{31}P NMR 2',3'-*cyc*-P$_S$-UDPαS/NH$_4^+$ D2 **(8b)**
UDPalfaS_D2_NH4_31P_25deg
Normalized Intensity
Chemical Shift (ppm)
76.99
76.86
43.73
43.56
-10.39
-10.57
0.81
1.00
0.74
Che

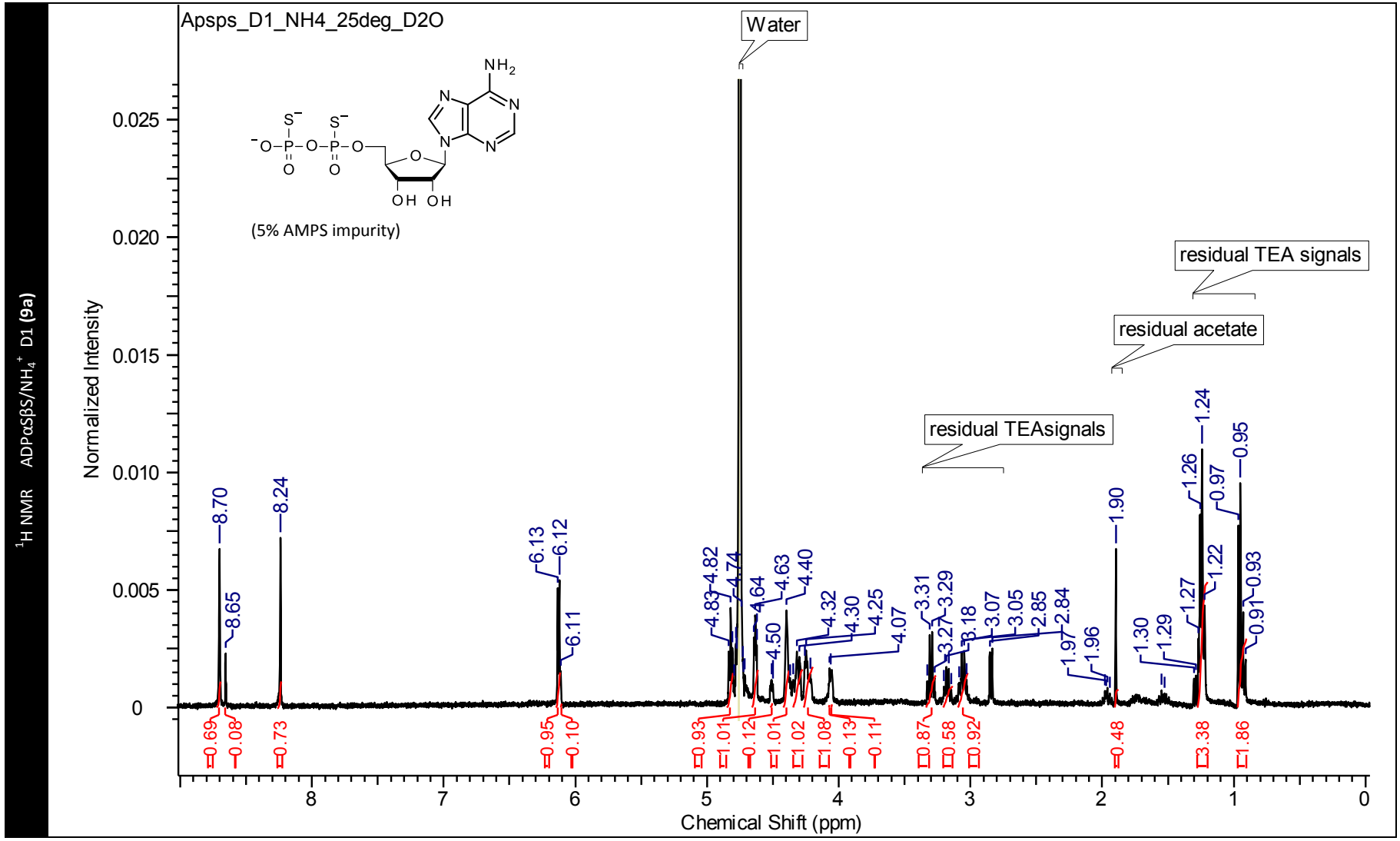
(**9a**) ADPαSβS/NH_4^+ D1

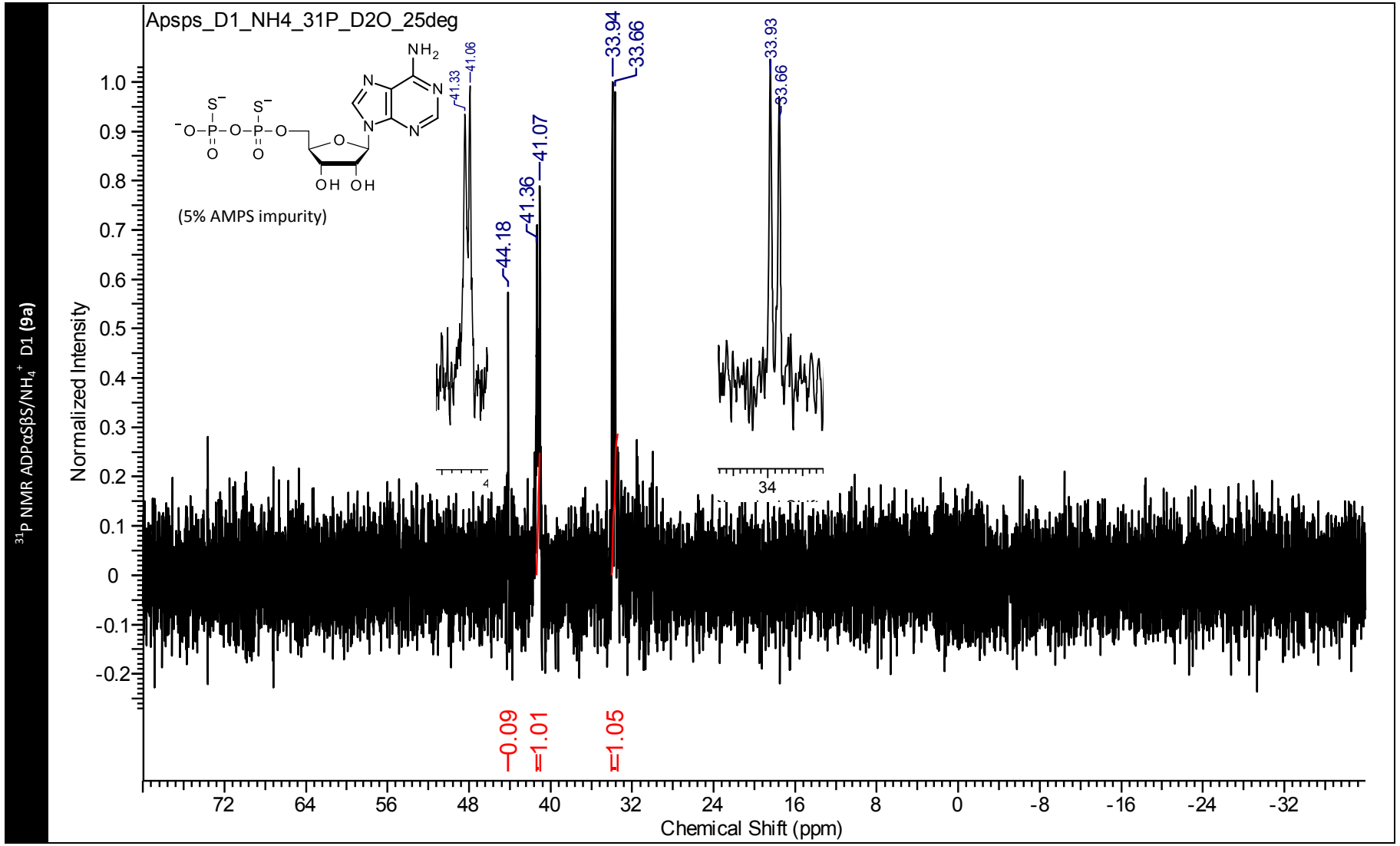


ADPαSβS/TNH4+ D1 (**9a**) HPLC

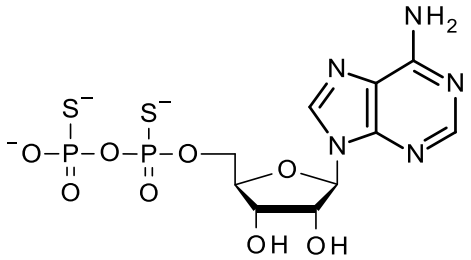



ADPαSβS/NH4+ D1 (9a) HRMS (-)ES (Calc. [M-H]- C10H14N5O8P2S2- 457.9764
120309apsps1 #2-33 RT: 0.04-1.10 AV: 32 NL: 4.09E4
T: FTMS - p ESI Full ms [100.00-1200.00]
Relative Abundance
m/z
457.97663
458.97999
456.00882
448.99638
452.99074
463.01181
470.00313
472.88228
474.87934
479.95840

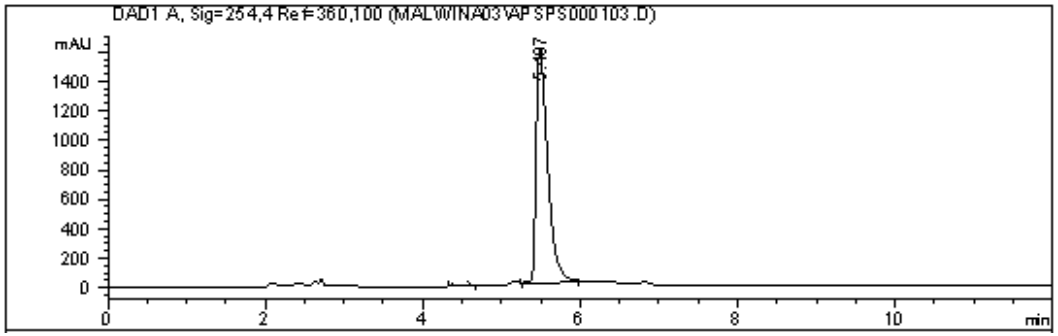

^{1}H NMR ADPαSβS/NH$_4^+$ D1 (9a)
Apsps_D1_NH4_25deg_D2O
Water
residual TEA signals
residual acetate
residual TEAsignals
(5% AMPS impurity)
Normalized Intensity
Chemical Shift (ppm)
8.70
8.65
8.24
6.13
6.12
6.11
4.83
4.82
4.74
4.64
4.63
4.50
4.40
4.32
4.30
4.25
4.07
3.31
3.29
3.27
3.18
3.07
3.05
2.85
2.84
1.97
1.96
1.90
1.30
1.29
1.27
1.26
1.24
1.22
0.97
0.95
0.93
0.91

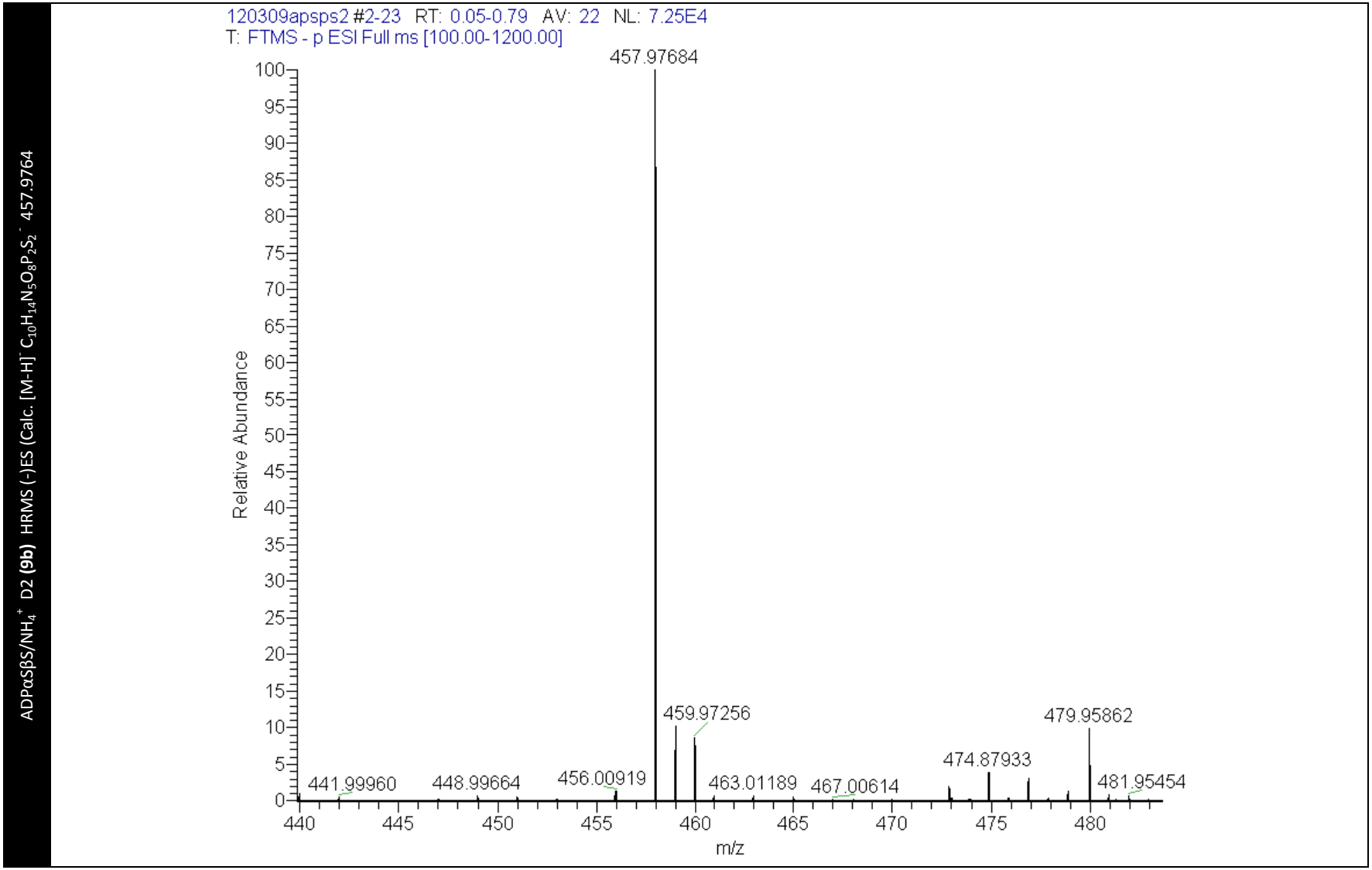

^{31}P NMR ADPαSβS/NH_4^+ D1 (9a)
Apsps_D1_NH4_31P_D2O_25deg
(5% AMPS impurity)
Normalized Intensity
Chemical Shift (ppm)
44.18
41.36
41.07
41.33
41.06
33.94
33.66
33.93
33.66
0.09
1.01
1.05

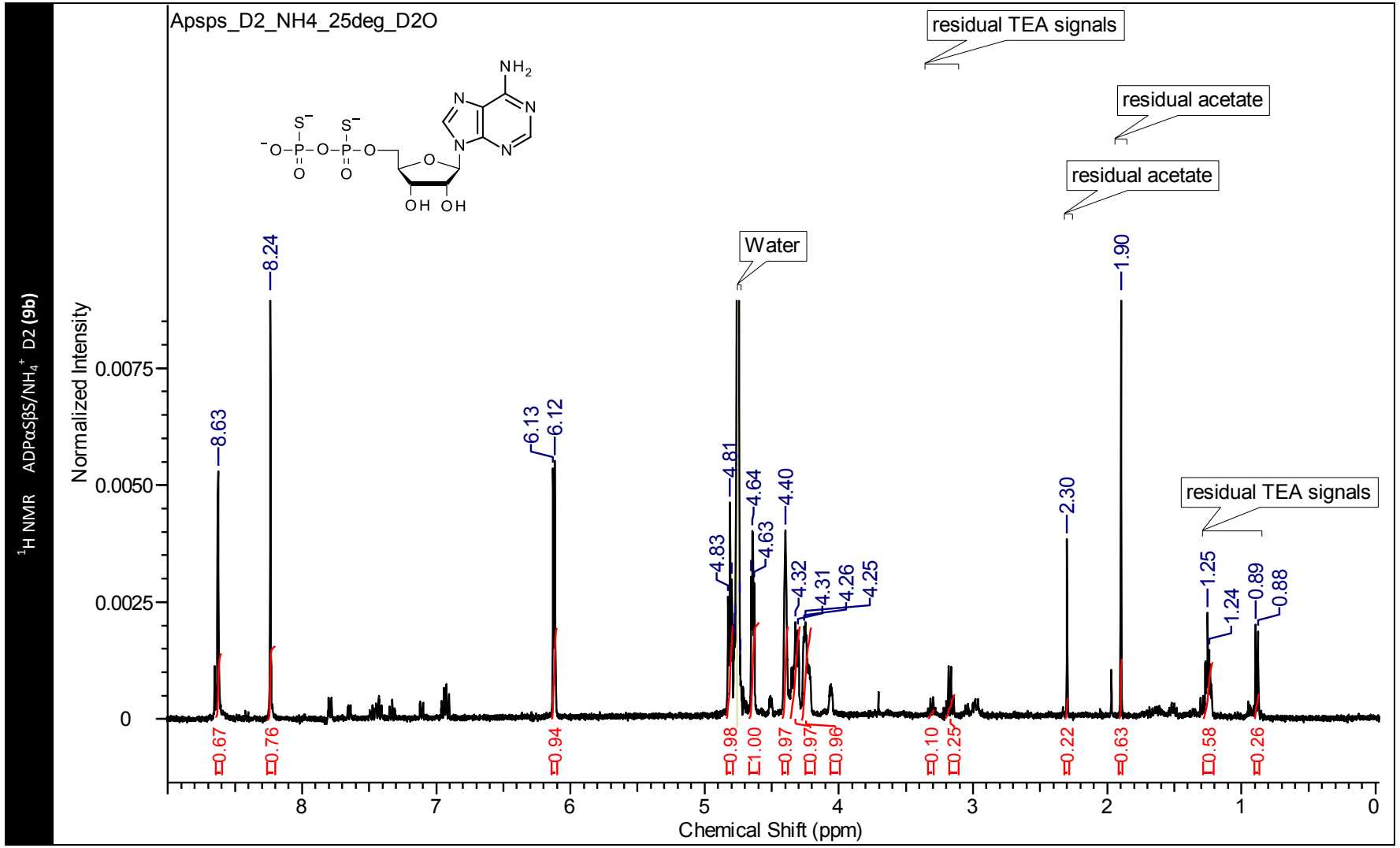
(**9b**) ADPαSβS/NH_4^+ D2

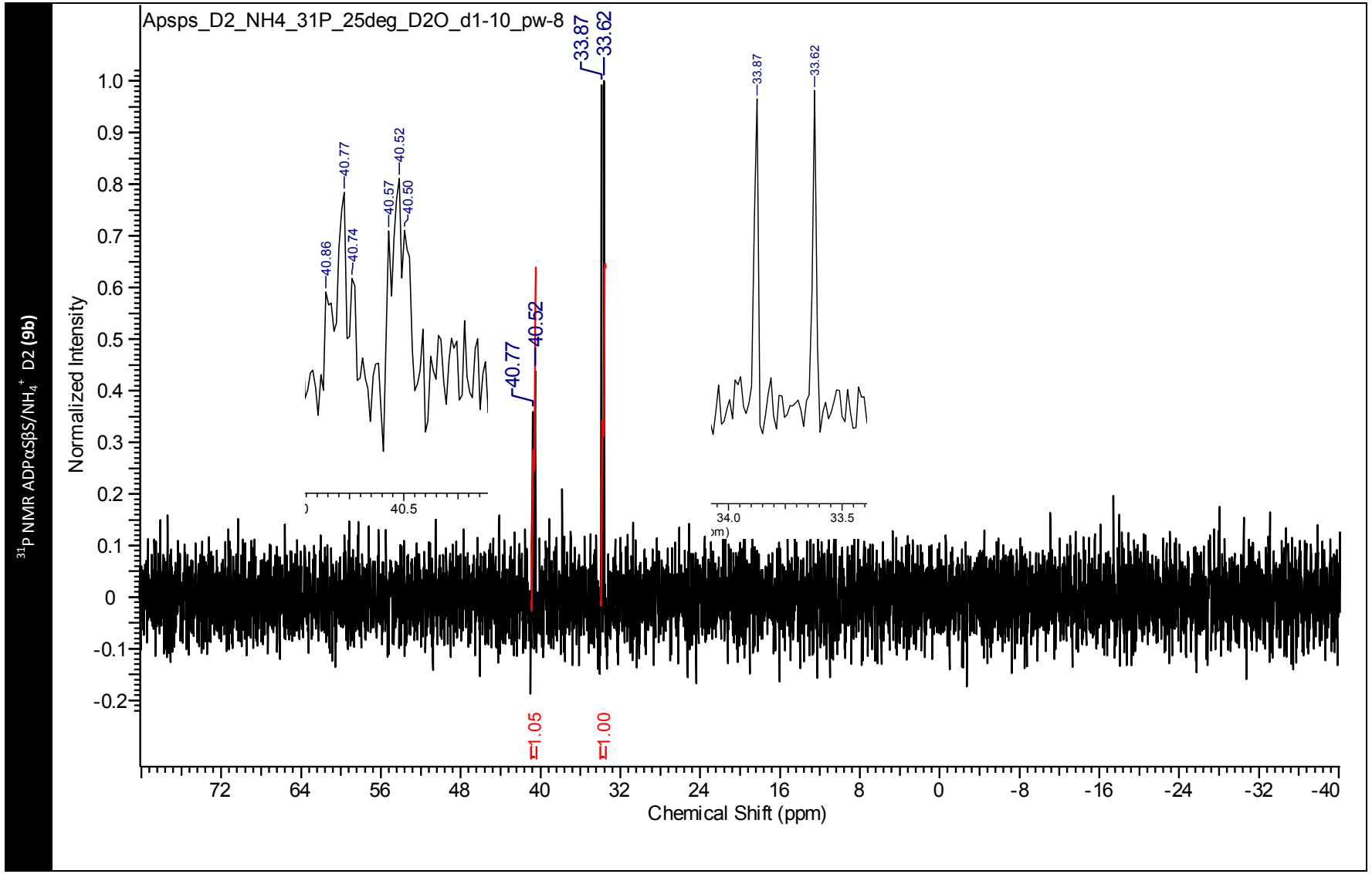


ADPαSβS/TNH_4^+ D2 **(9b)** HPLC

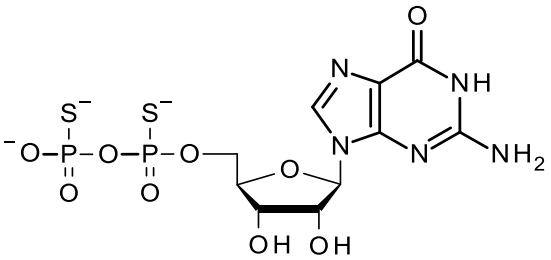



ADPαSβS/NH4+ D2 (9b) HRMS (-)ES (Calc. [M-H]- C10H14N5O8P2S2- 457.9764
120309apsps2 #2-23 RT: 0.05-0.79 AV: 22 NL: 7.25E4
T: FTMS - p ESI Full ms [100.00-1200.00]
Relative Abundance
m/z
457.97684
459.97256
479.95862
474.87933
441.99960
448.99664
456.00919
463.01189
467.00614
481.95454

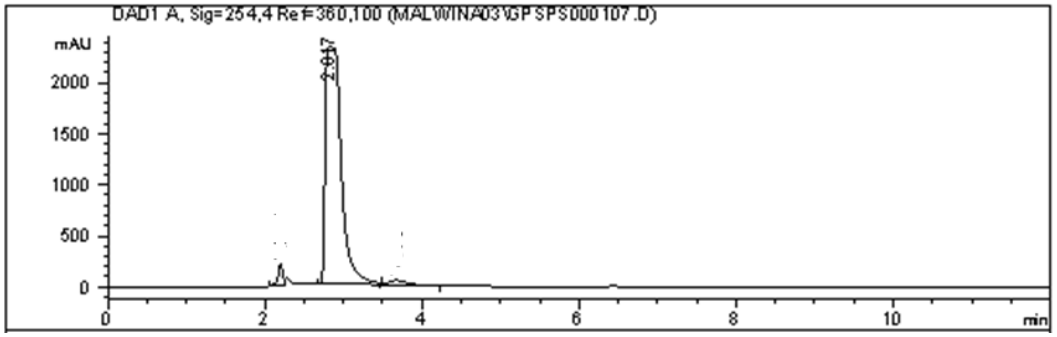

^{1}H NMR ADPαSβS/NH$_4^+$ D2 (9b)
Apsps_D2_NH4_25deg_D2O
residual TEA signals
residual acetate
residual acetate
Water
residual TEA signals
8.24
1.90
8.63
6.13
6.12
4.81
4.64
4.40
4.83
4.63
4.32
4.31
4.26
4.25
2.30
1.25
1.24
0.89
0.88
0.67
0.76
0.94
0.98
1.00
0.97
0.97
0.96
0.10
0.25
0.22
0.63
0.58
0.26
Normalized Intensity
0.0075
0.0050
0.0025
0
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

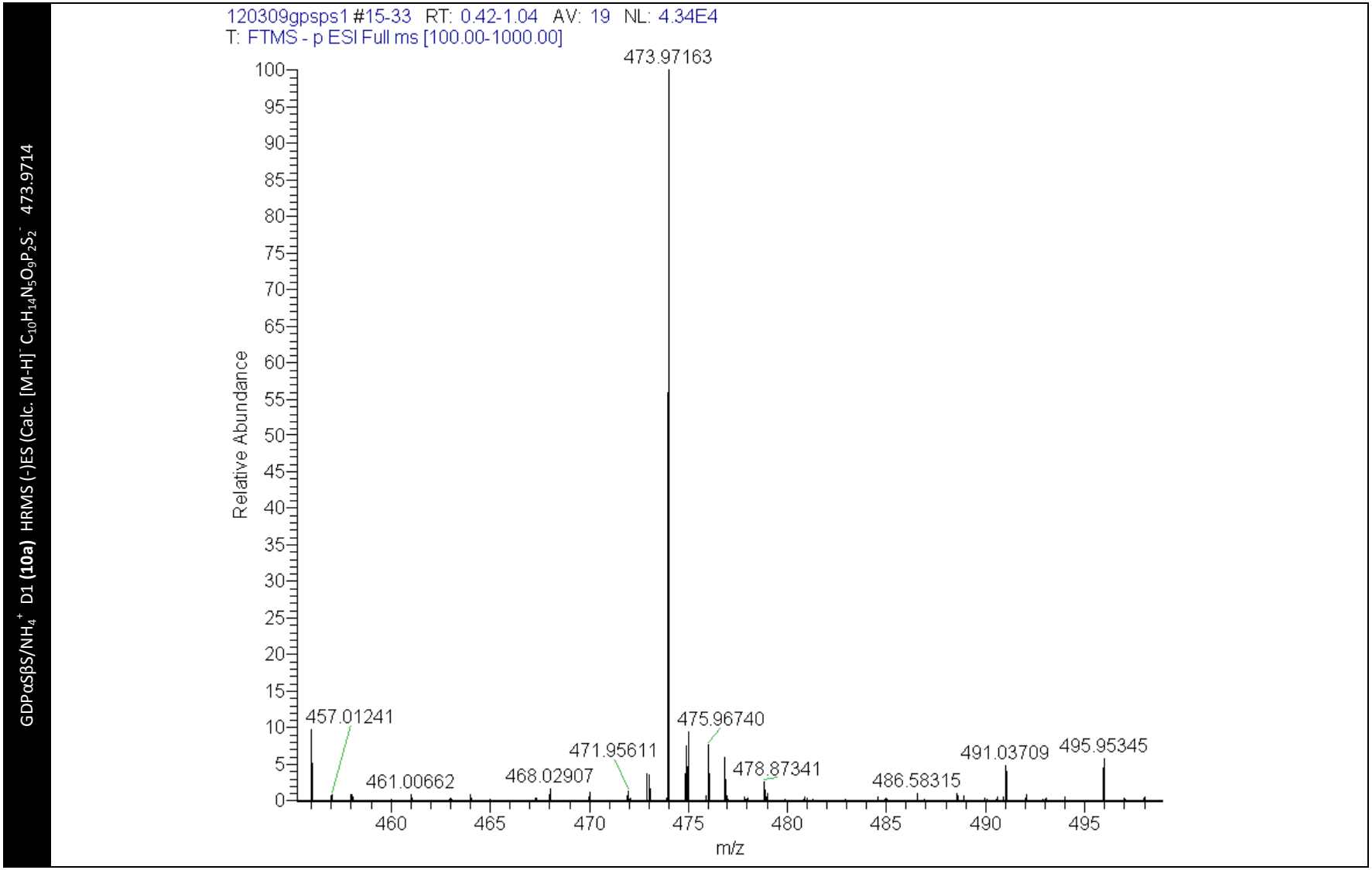

^{31}P NMR ADPαSβS/NH_4^+ D2 (9b)
Apsps_D2_NH4_31P_25deg_D2O_d1-10_pw-8
Normalized Intensity
Chemical Shift (ppm)
33.87
33.62
40.77
40.52
40.86
40.74
40.57
40.50
1.05
1.00

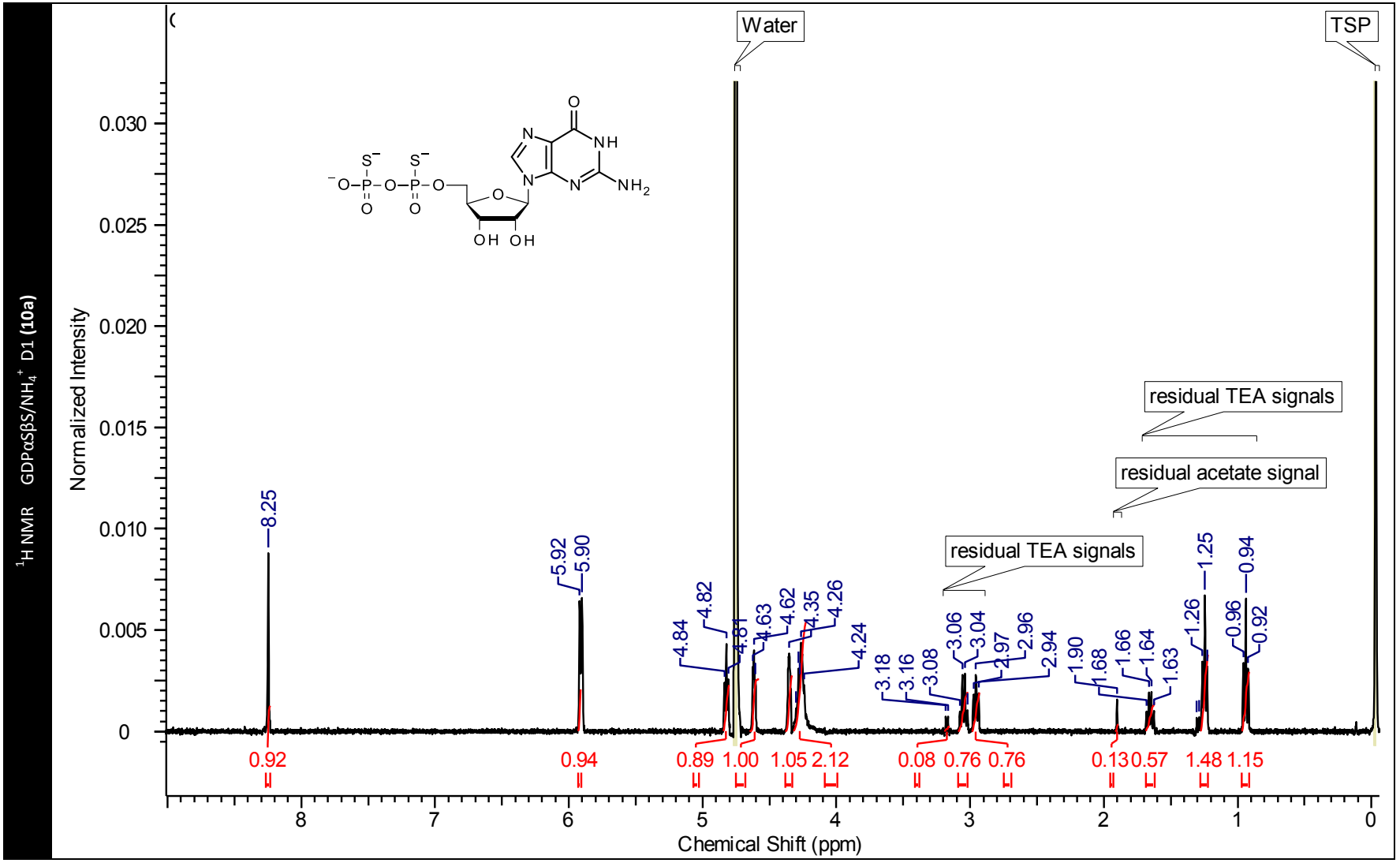
(**10a**) GDPαSβS/NH_4^+ D1

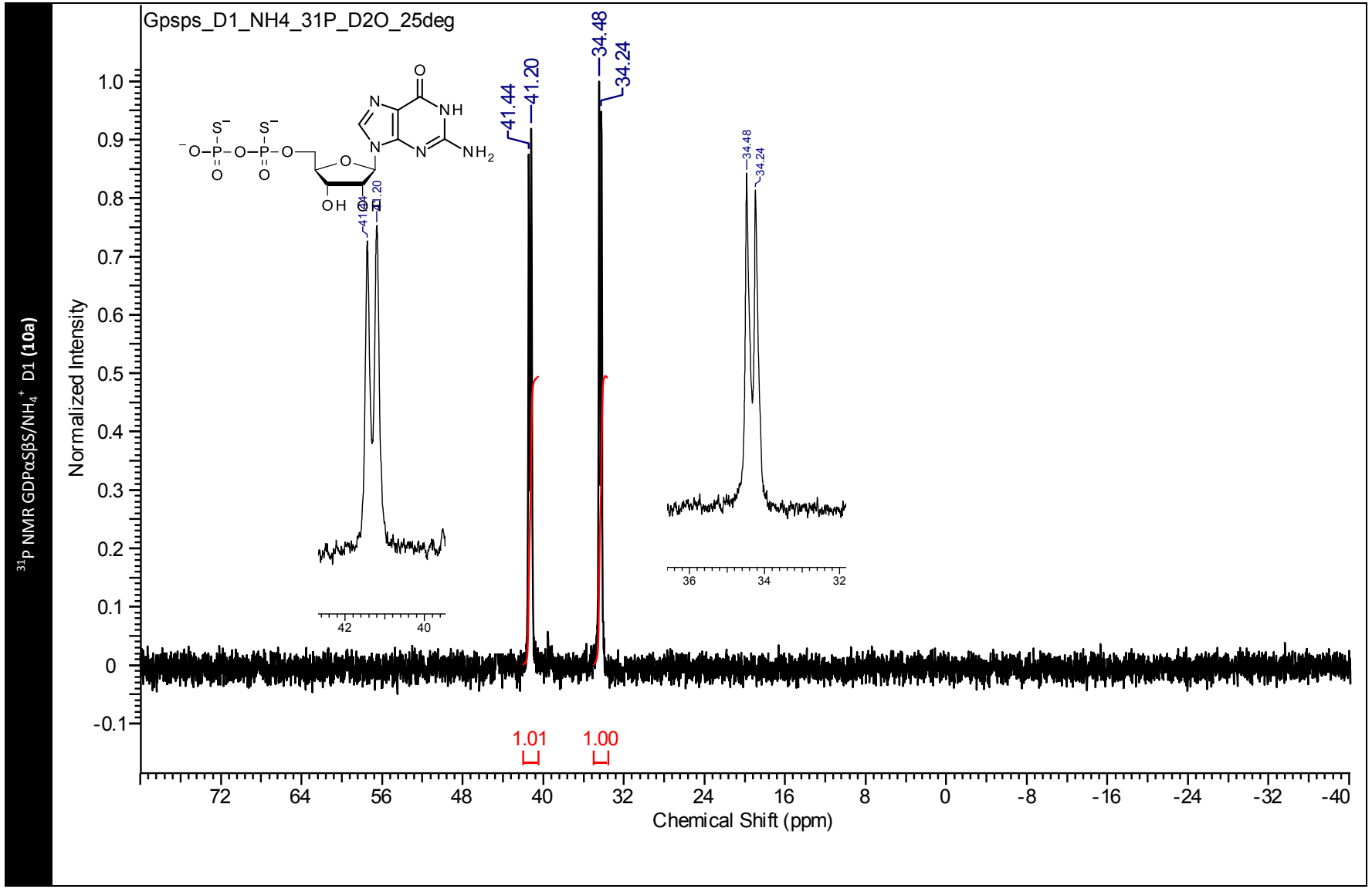


GDPαSβS/NH_4^+ D1 (**10a**) HPLC

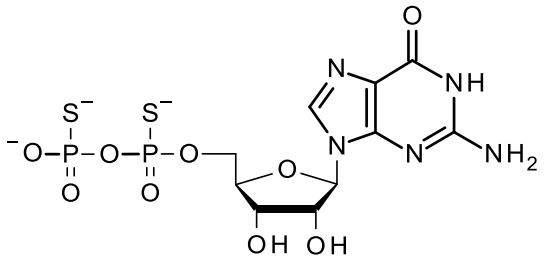



GDPαSβS/NH4+ D1 (10a) HRMS (-)ES (Calc. [M-H]- C10H14N5O9P2S2- 473.9714
120309gpsps1 #15-33 RT: 0.42-1.04 AV: 19 NL: 4.34E4
T: FTMS - p ESI Full ms [100.00-1000.00]
Relative Abundance
m/z
473.97163
457.01241
461.00662
468.02907
471.95611
475.96740
478.87341
486.58315
491.03709
495.95345

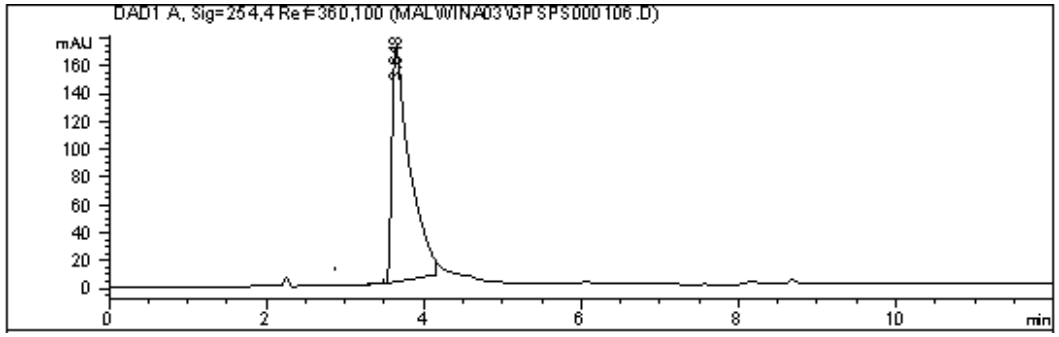

^{1}H NMR GDPαSβS/NH$_4^+$ D1 (10a)
Normalized Intensity
0.030
0.025
0.020
0.015
0.010
0.005
0
Water
TSP
residual TEA signals
residual acetate signal
residual TEA signals
8.25
5.92
5.90
4.84
4.82
4.81
4.63
4.62
4.35
4.26
4.24
3.18
3.16
3.08
3.06
3.04
2.97
2.96
2.94
1.90
1.68
1.66
1.64
1.63
1.26
1.25
0.96
0.94
0.92
0.92
0.94
0.89
1.00
1.05
2.12
0.08
0.76
0.76
0.13
0.57
1.48
1.15
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

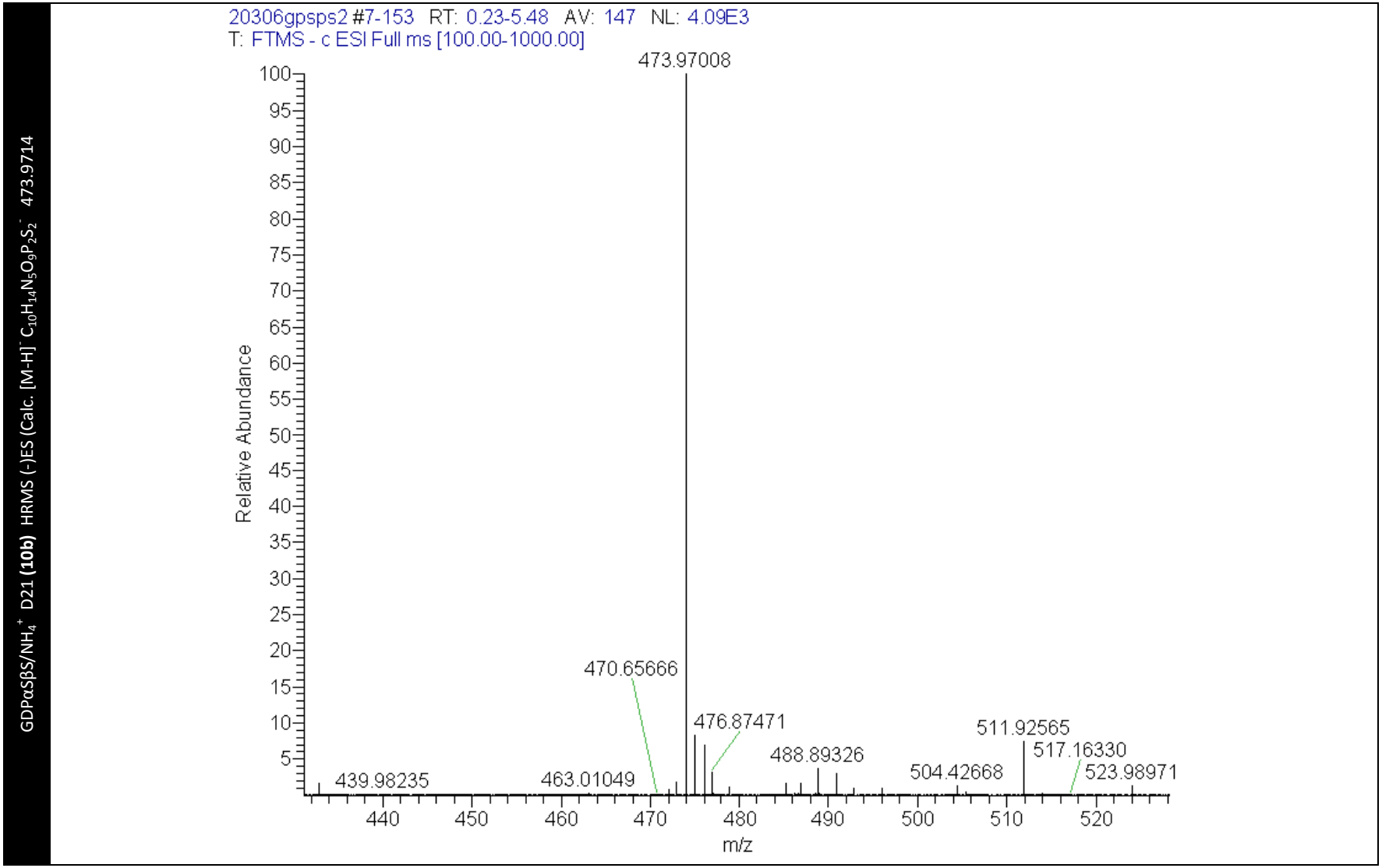

^{31}P NMR GDPαSβS/NH$_4^+$ D1 (10a)
Gpsps_D1_NH4_31P_D2O_25deg
Normalized Intensity
Chemical Shift (ppm)
41.44
41.20
34.48
34.24
1.01
1.00

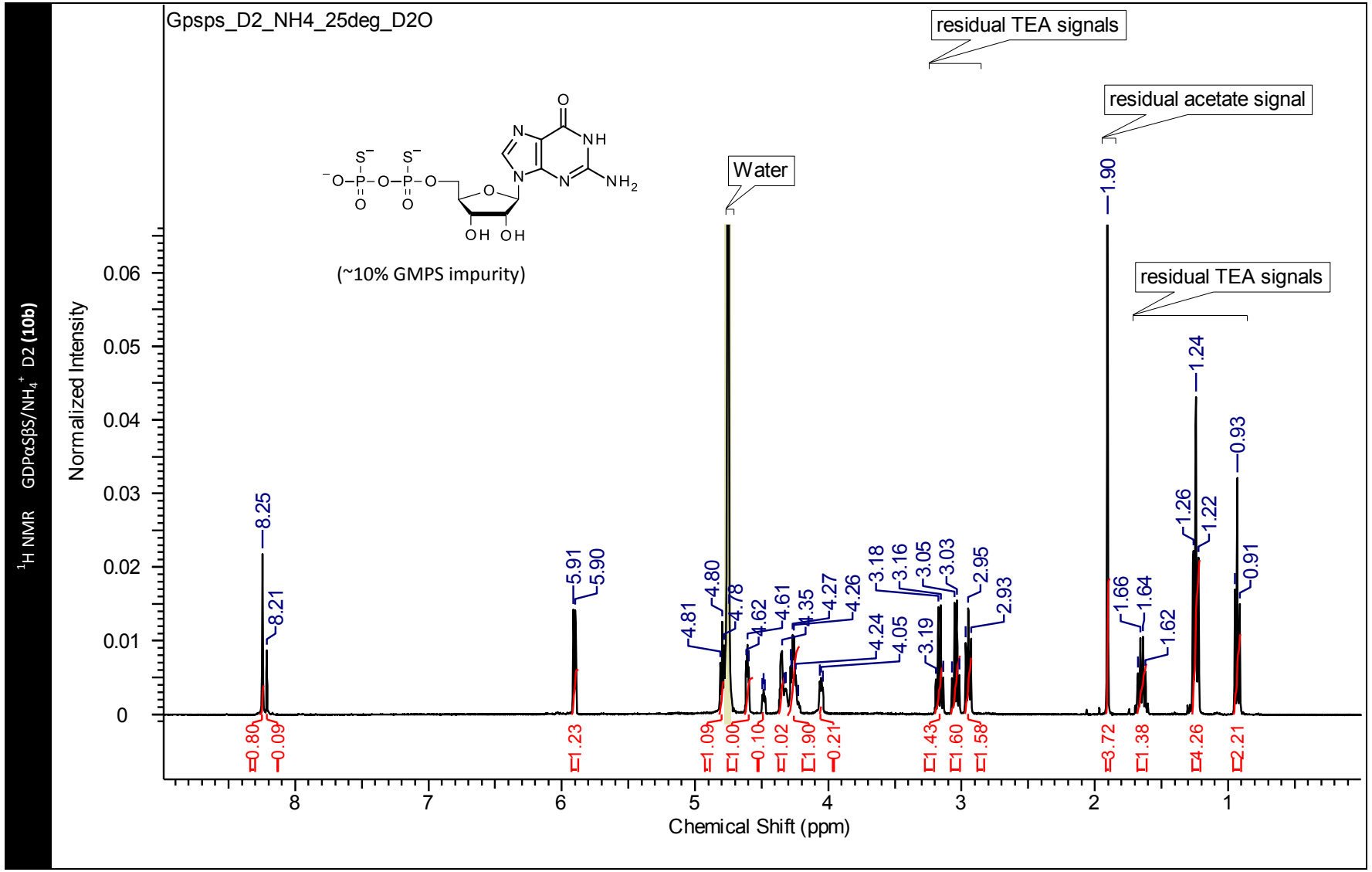
(10b) GDPαSβS/NH_4^+ D2

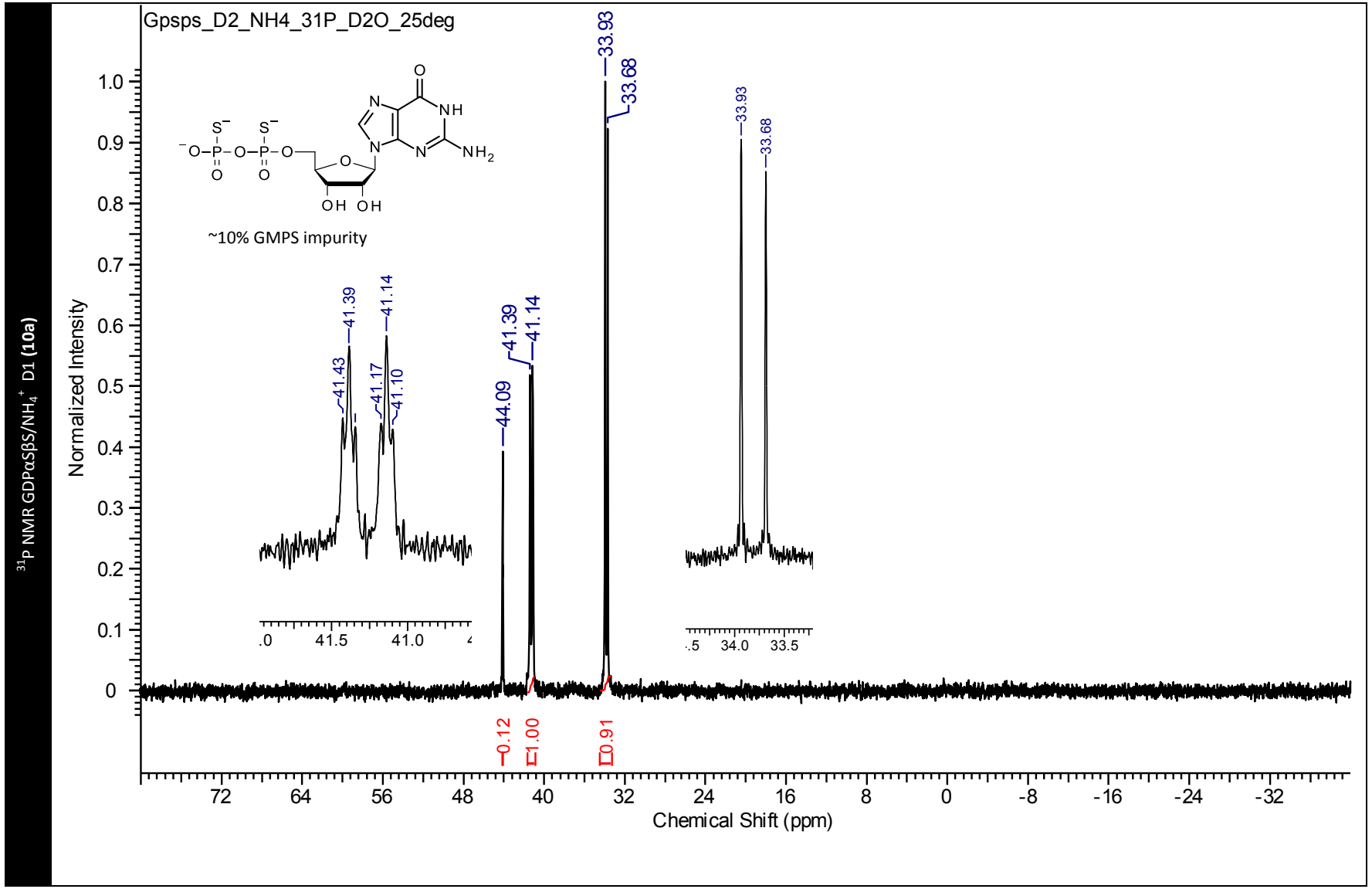


GDPαSβS/NH_4^+ D2 **(10b)** HPLC

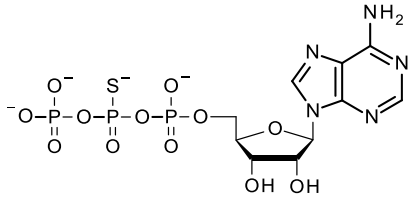



GDPαSβS/NH4+ D21 (10b) HRMS (-)ES (Calc. [M-H]- C10H14N5O9P2S2- 473.9714
20306gpsps2 #7-153 RT: 0.23-5.48 AV: 147 NL: 4.09E3
T: FTMS - c ESI Full ms [100.00-1000.00]
Relative Abundance
m/z
473.97008
470.65666
476.87471
488.89326
511.92565
517.16330
523.98971
439.98235
463.01049
504.42668

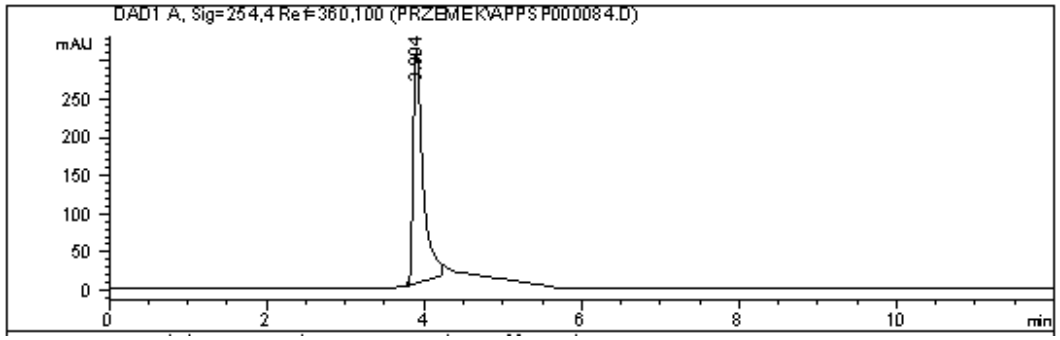

^{1}H NMR GDPαSβS/NH_4^+ D2 (10b)
Gpsps_D2_NH4_25deg_D2O
residual TEA signals
residual acetate signal
residual TEA signals
Water
(~10% GMPS impurity)
Normalized Intensity
Chemical Shift (ppm)
8.25
8.21
5.91
5.90
4.81
4.80
4.78
4.62
4.61
4.35
4.27
4.26
4.24
4.05
3.19
3.18
3.16
3.05
3.03
2.95
2.93
1.90
1.66
1.64
1.62
1.26
1.24
1.22
0.93
0.91
0.80
0.09
1.23
1.09
1.00
0.10
1.02
1.90
0.21
1.43
1.60
1.58
3.72
1.38
4.26
2.21

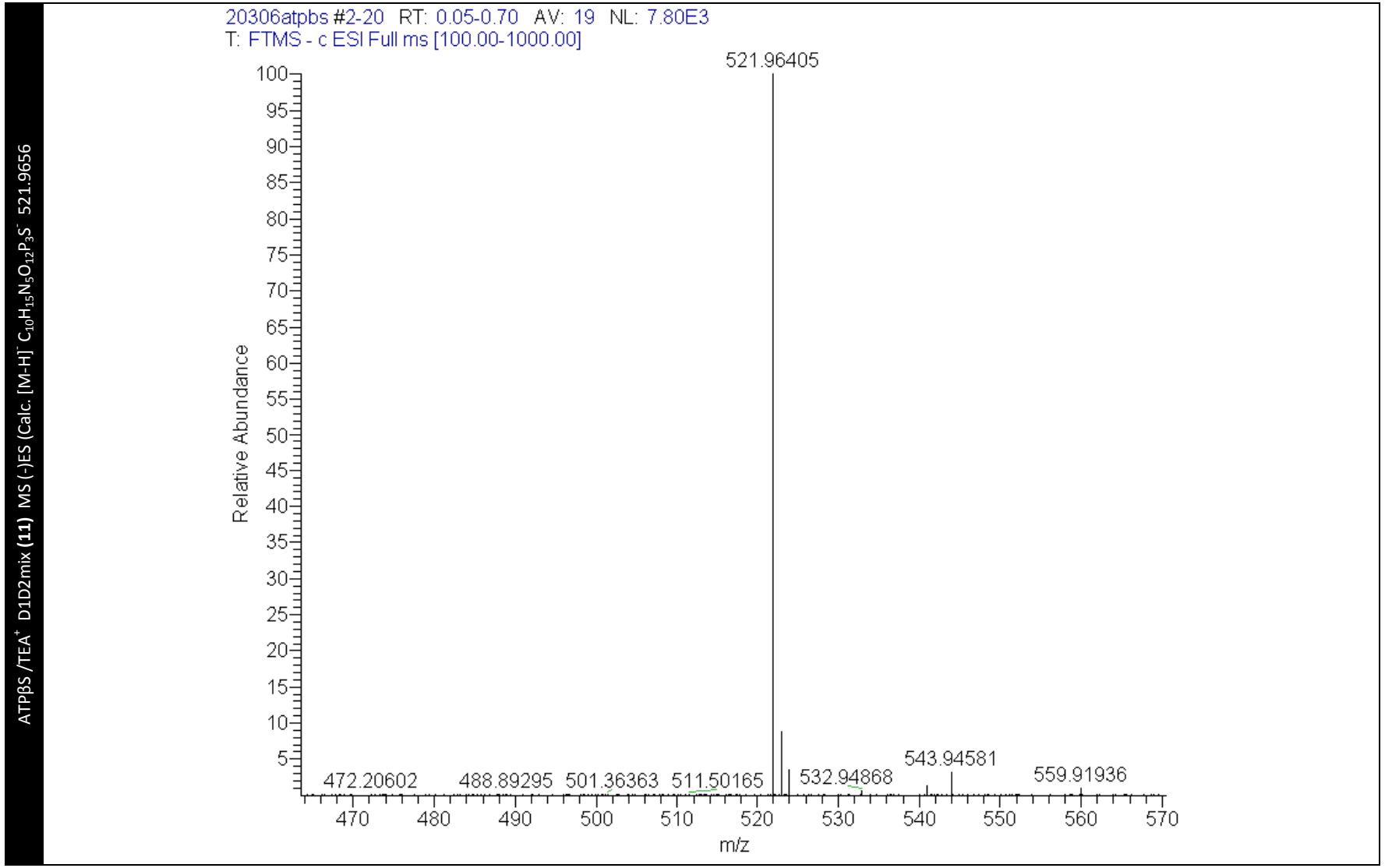

^{31}P NMR GDPαSβS/NH_4^+ D1 (10a)
Gpsps_D2_NH4_31P_D2O_25deg
~10% GMPS impurity
Normalized Intensity
Chemical Shift (ppm)
33.93
33.68
41.39
41.14
44.09
41.43
41.17
41.10
0.12
1.00
0.91

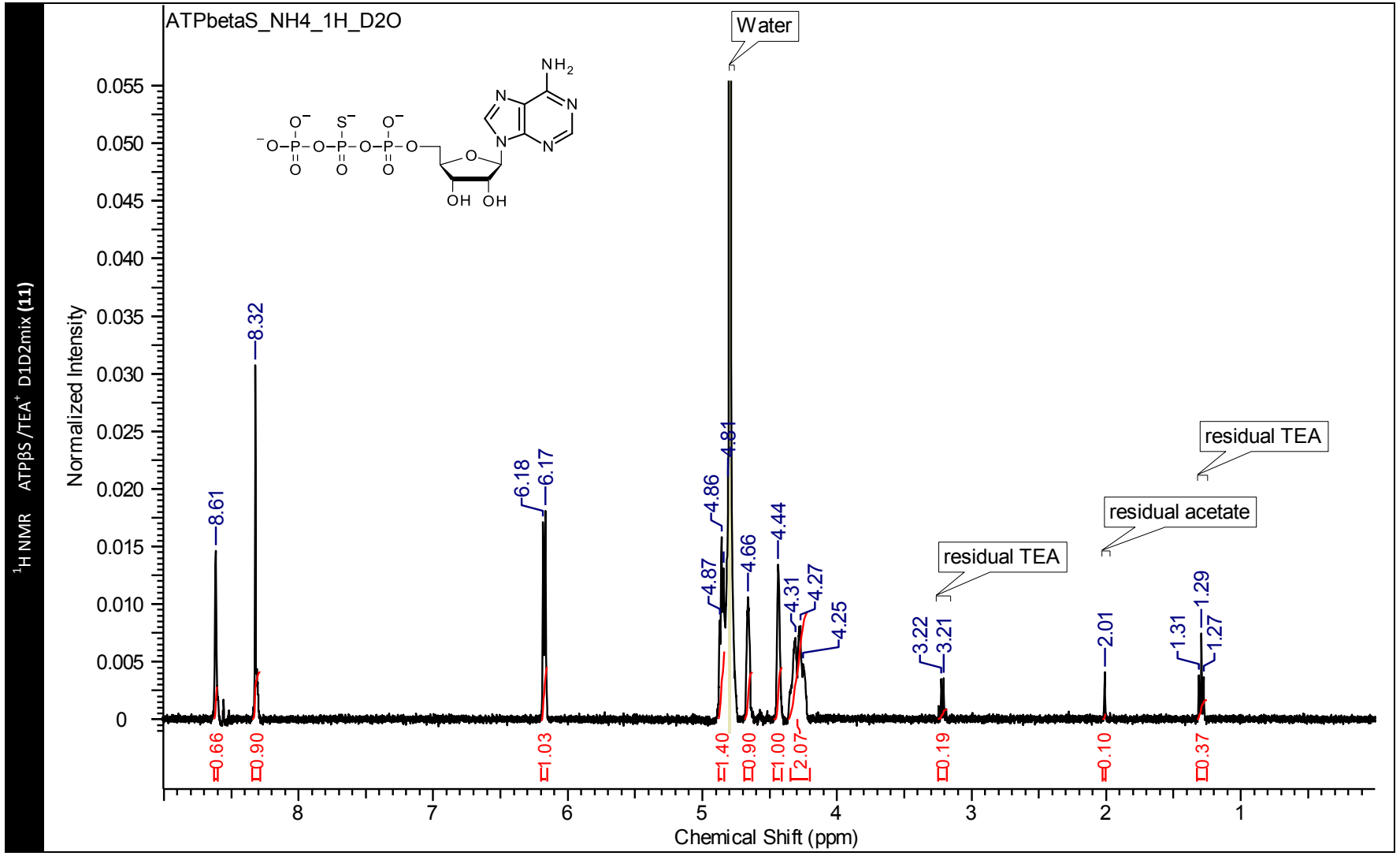
(**11**) ATPβS/TEA$^+$ D1D2mix

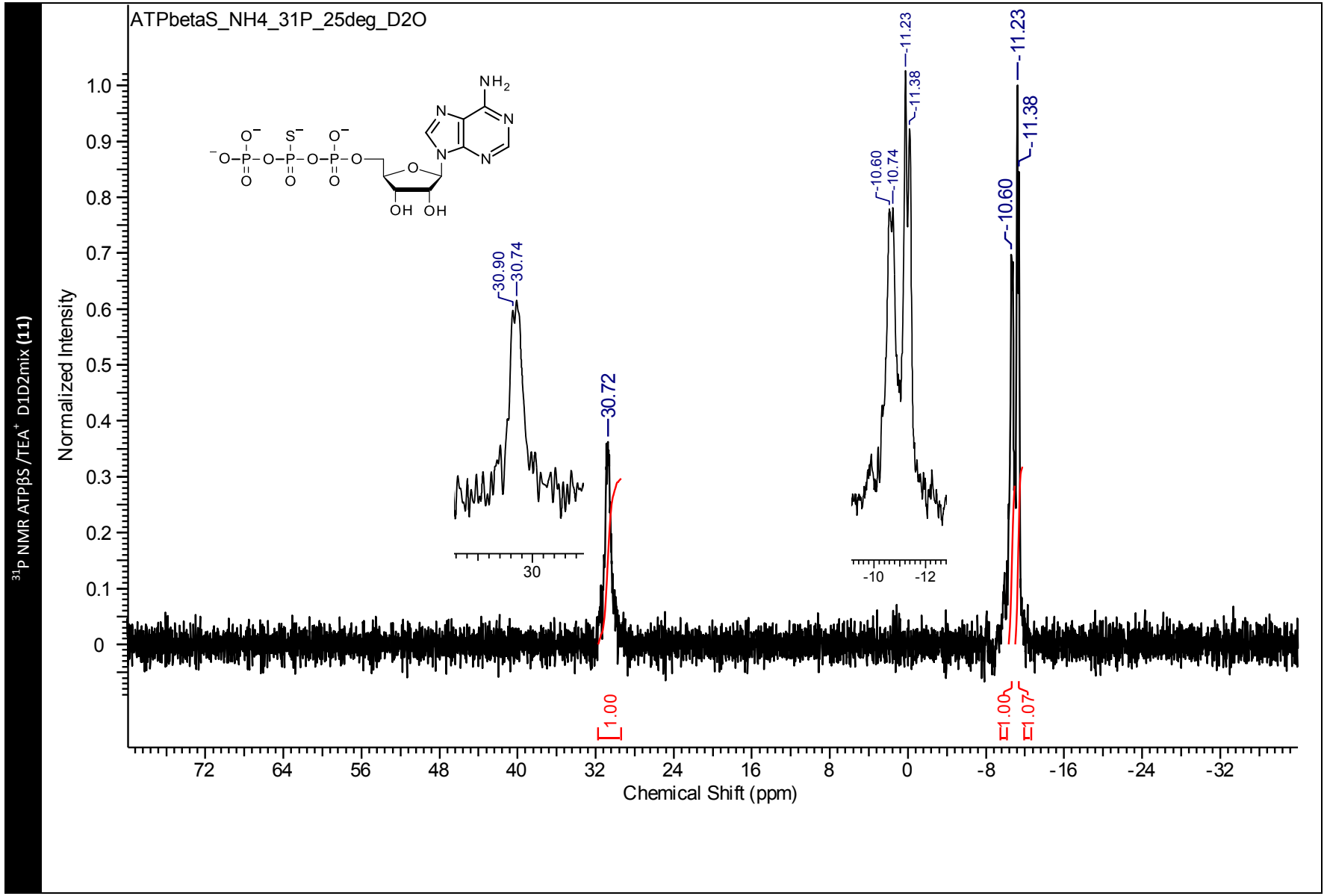


ATPβS S/TEA$^+$ D1D2mix **(11)** HPLC

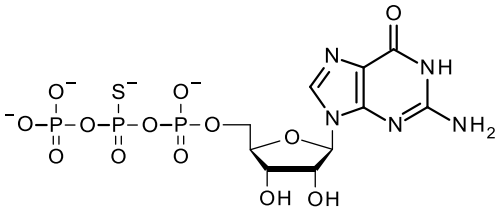



ATPβS /TEA+ D1D2mix (11) MS (-)ES (Calc. [M-H]- C10H15N5O12P3S- 521.9656
20306atpbs #2-20 RT: 0.05-0.70 AV: 19 NL: 7.80E3
T: FTMS - c ESI Full ms [100.00-1000.00]
521.96405
472.20602
488.89295
501.36363
511.50165
532.94868
543.94581
559.91936
Relative Abundance
m/z

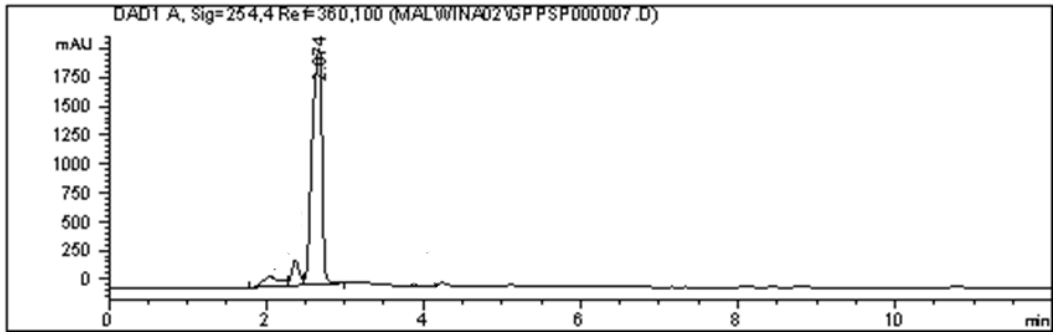

^{1}H NMR ATPβS /TEA$^+$ D1D2mix (11)
ATPbetaS_NH4_1H_D2O
Water
residual TEA
residual acetate
residual TEA
Normalized Intensity
Chemical Shift (ppm)
8.61
8.32
6.18
6.17
4.86
4.84
4.87
4.66
4.44
4.31
4.27
4.25
3.22
3.21
2.01
1.31
1.29
1.27
0.66
0.90
1.03
1.40
0.90
1.00
2.07
0.19
0.10
0.37

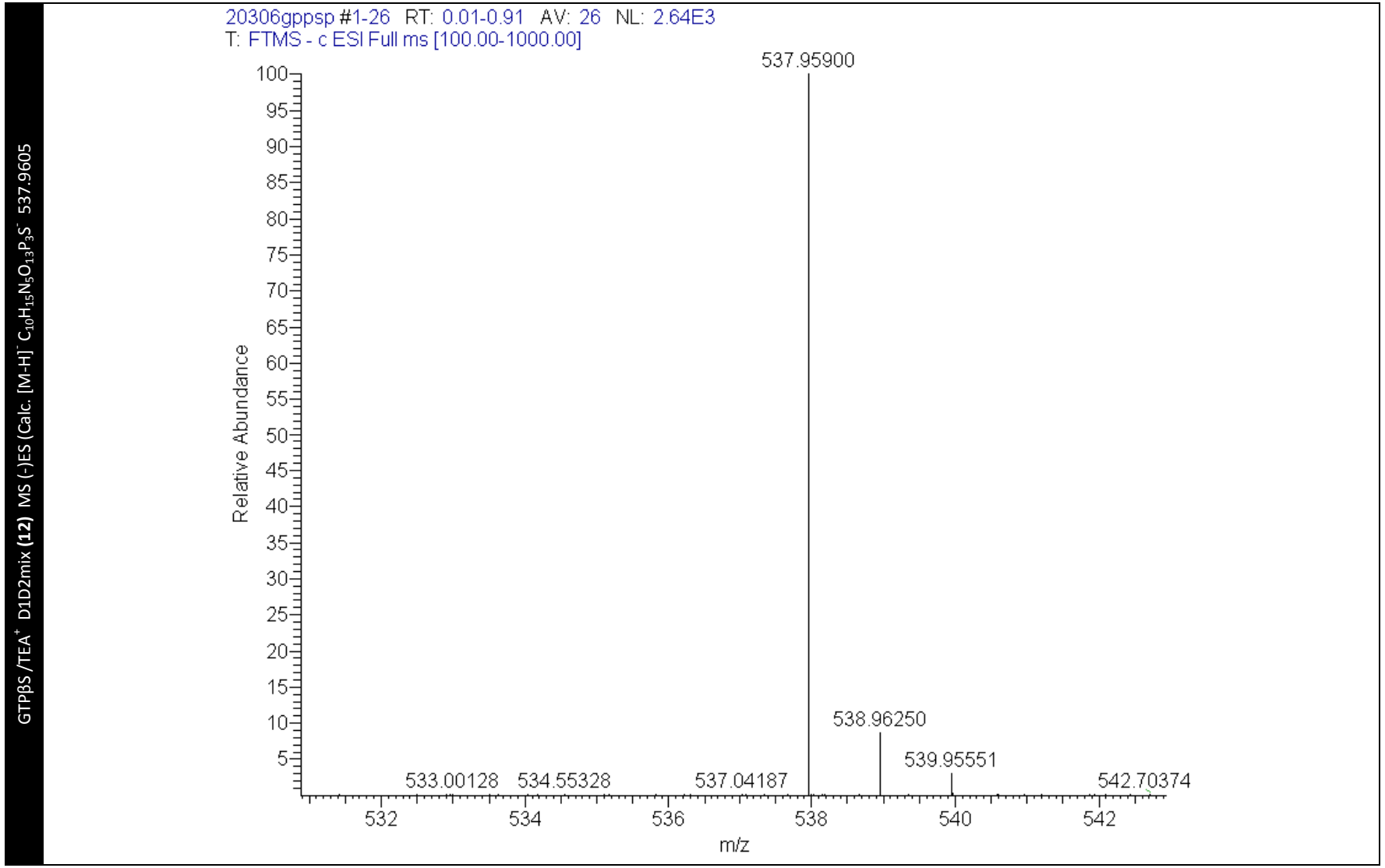

^{31}P NMR ATPβS /TEA^{+} D1D2mix **(11)**
ATPbetaS_NH4_31P_25deg_D2O
Normalized Intensity
Chemical Shift (ppm)
30.90
30.74
30.72
10.60
10.74
11.38
11.23
1.00
1.00
1.07

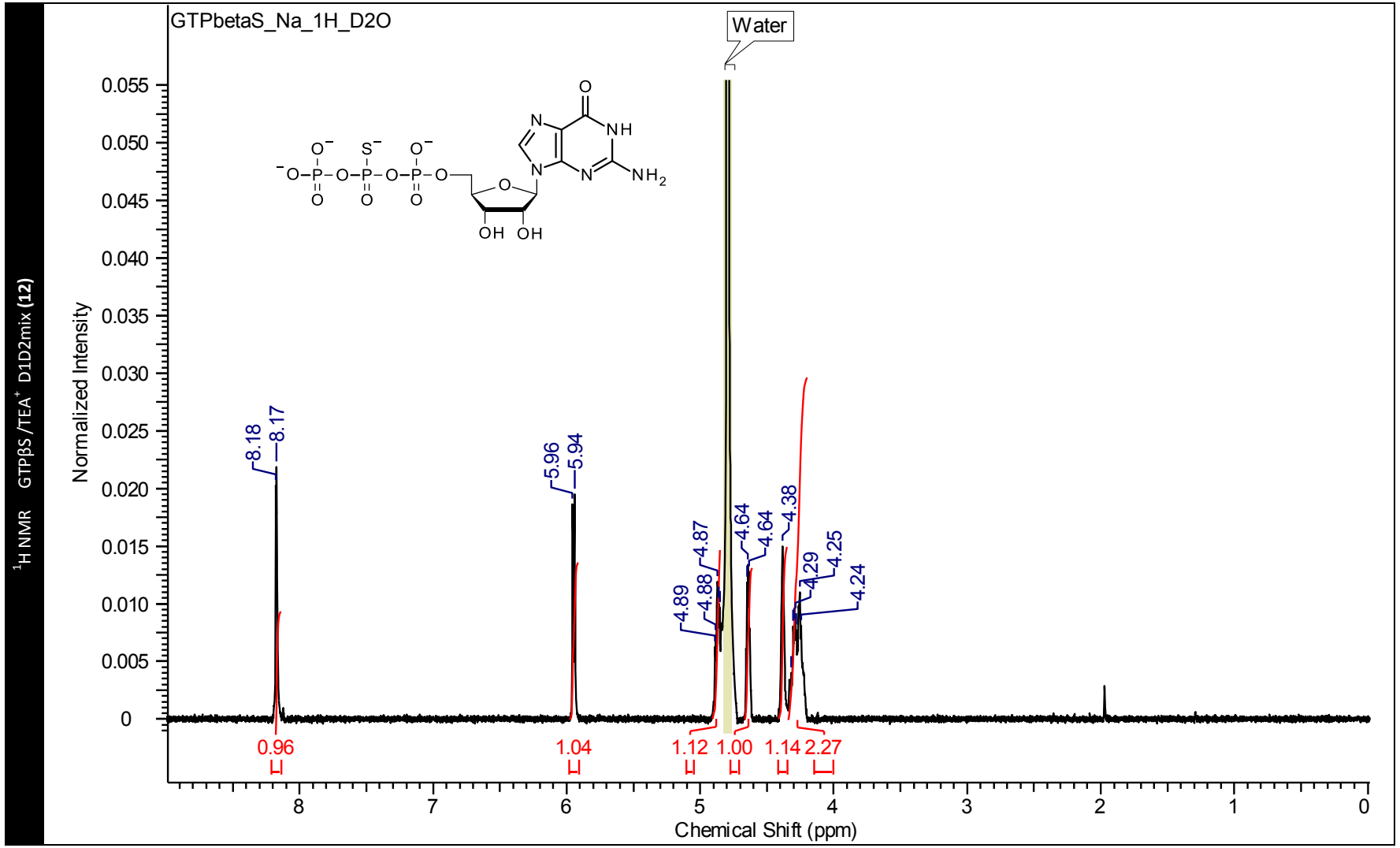
(**12**) GTPβS/TEA$^+$ D1D2mix

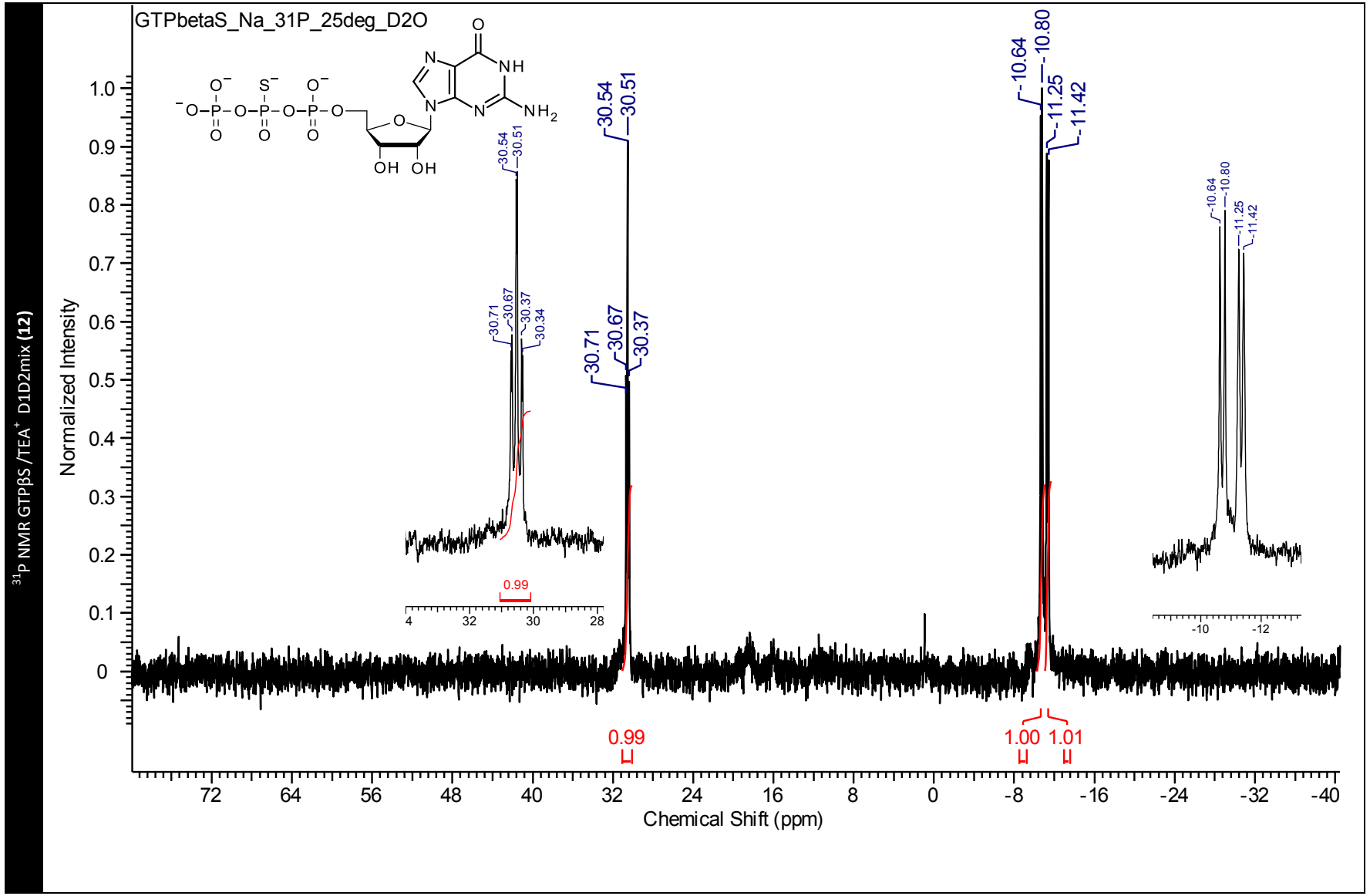


GTPβS S/TEA$^+$ D1D2mix **(12)** HPLC

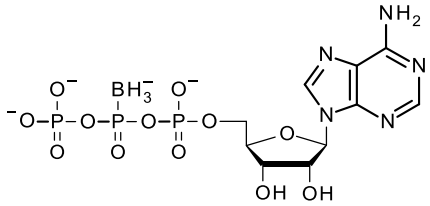



GTPβS /TEA+ D1D2mix (12) MS (-)ES (Calc. [M-H]- C10H15N5O13P3S- 537.9605
20306gppsp #1-26 RT: 0.01-0.91 AV: 26 NL: 2.64E3
T: FTMS - c ESI Full ms [100.00-1000.00]
537.95900
538.96250
539.95551
533.00128
534.55328
537.04187
542.70374
Relative Abundance
m/z
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
532
534
536
538
540
542

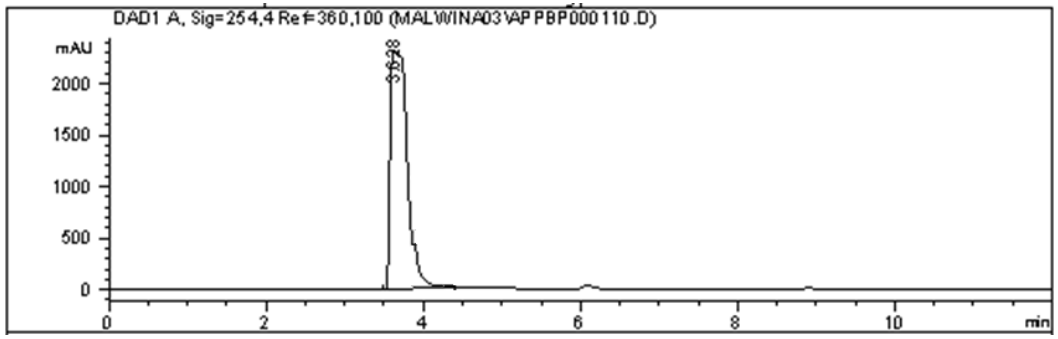

^{1}H NMR GTPβS /TEA$^+$ D1D2mix **(12)**
GTPbetaS_Na_1H_D2O
Water
Normalized Intensity
Chemical Shift (ppm)
8.18
8.17
5.96
5.94
4.89
4.88
4.87
4.64
4.64
4.38
4.29
4.25
4.24
0.96
1.04
1.12
1.00
1.14
2.27

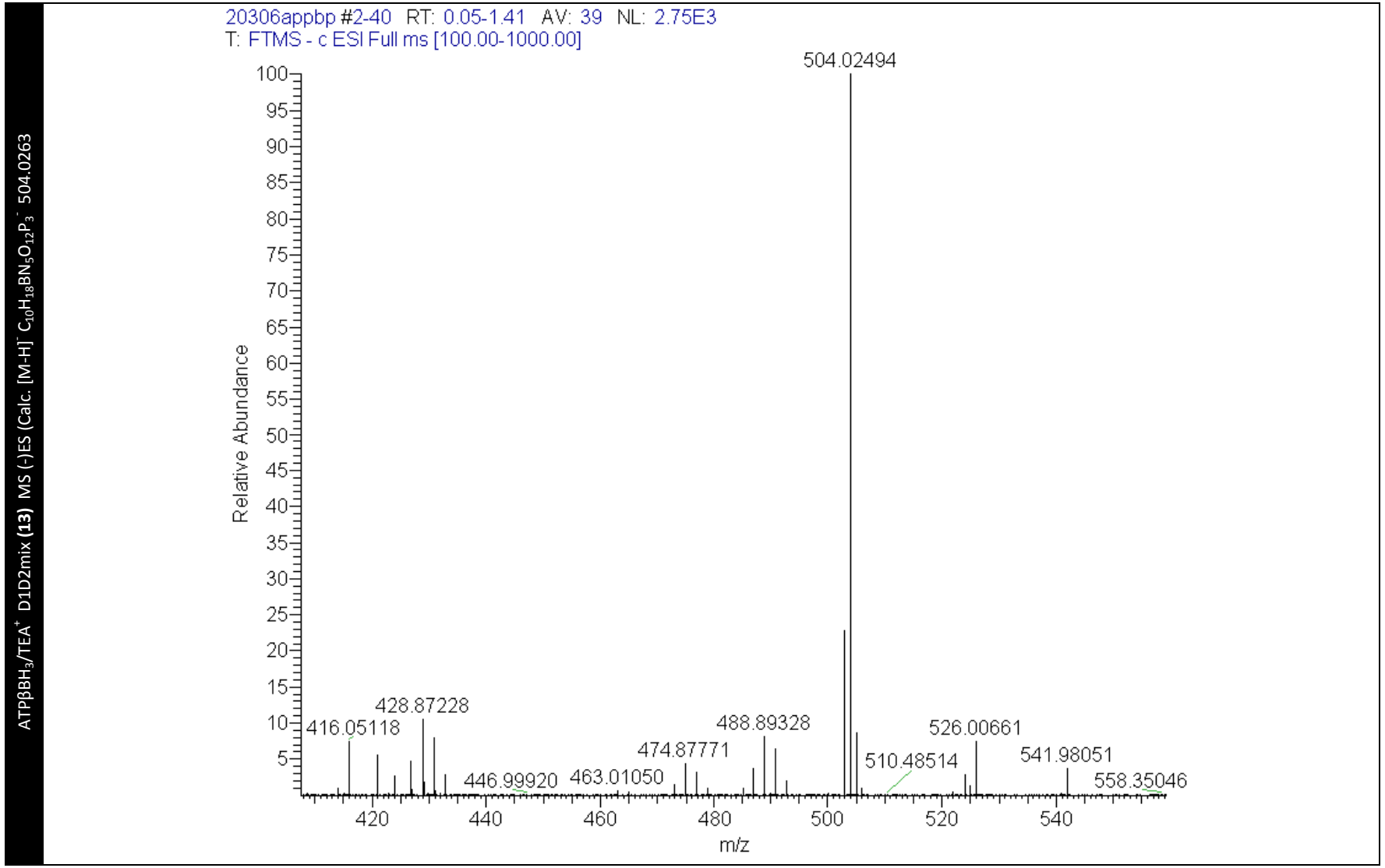

^{31}P NMR GTPβS /TEA^{+} D1D2mix **(12)**
GTPbetaS_Na_31P_25deg_D2O
Normalized Intensity
Chemical Shift (ppm)
30.54
30.51
30.71
30.67
30.37
30.34
10.64
10.80
11.25
11.42
0.99
1.00
1.01

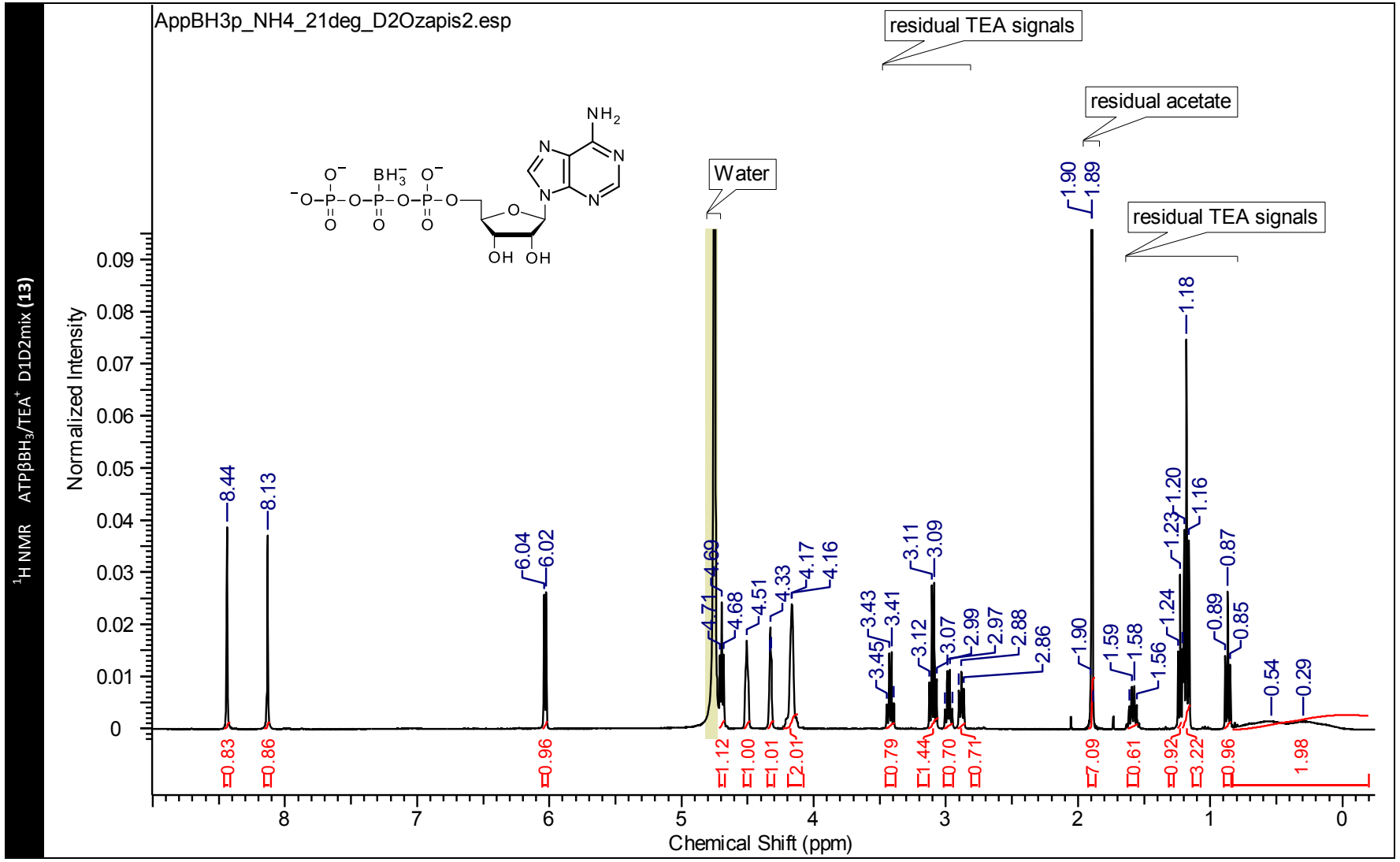
(**13**) ATPβBH$_3$/TEA$^+$ D1D2mix

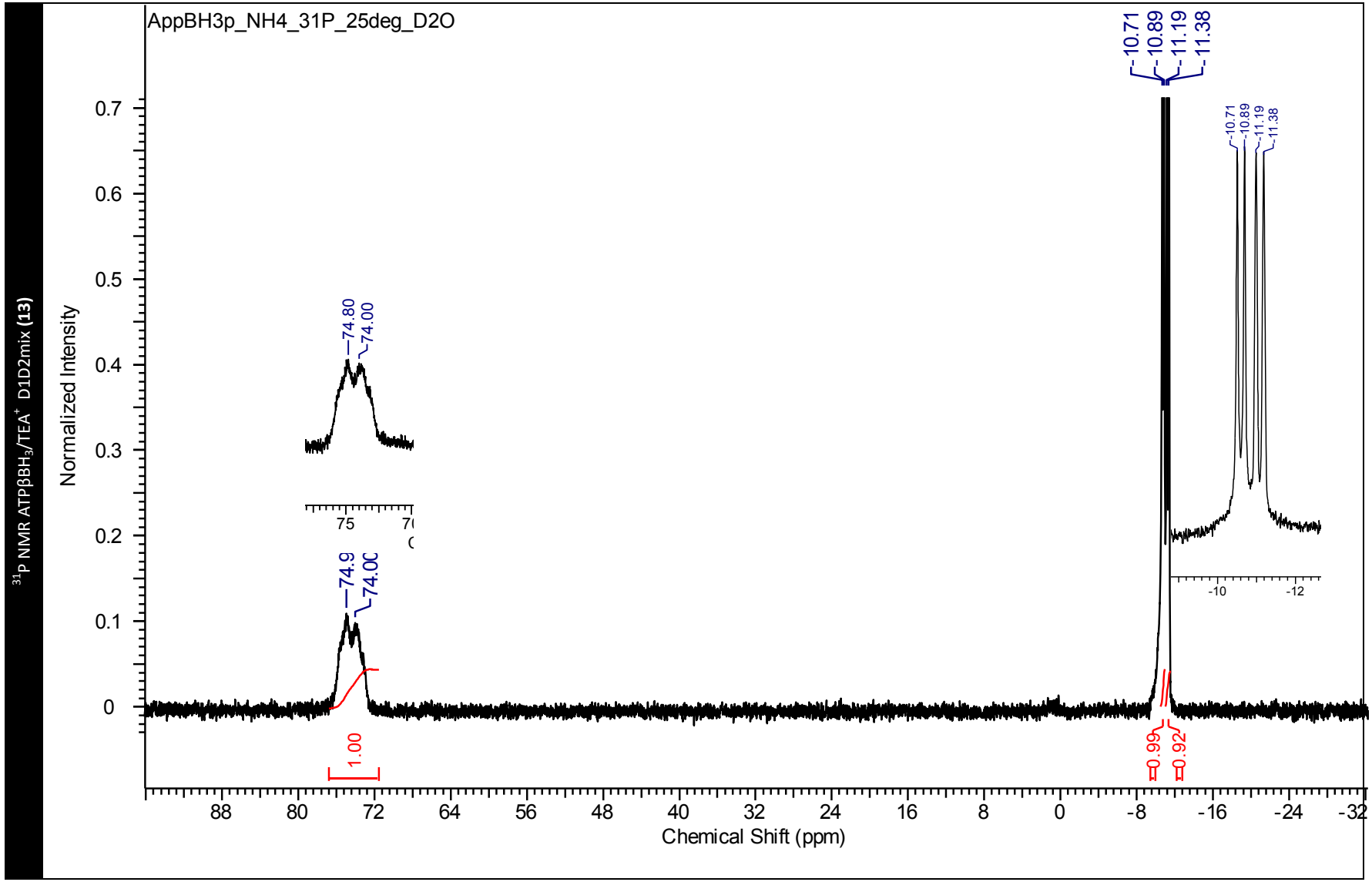


ATPβBH$_3$/TEA+ D1D2mix **(13)** HPLC

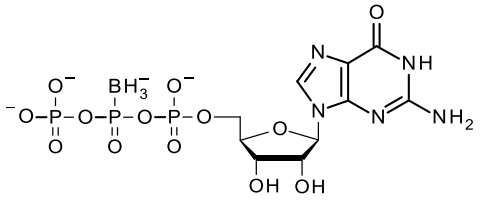



ATPβBH3/TEA+ D1D2mix (13) MS (-)ES (Calc. [M-H]- C10H18BN5O12P3- 504.0263
20306appbp #2-40 RT: 0.05-1.41 AV: 39 NL: 2.75E3
T: FTMS - c ESI Full ms [100.00-1000.00]
Relative Abundance
m/z
504.02494
428.87228
416.05118
488.89328
474.87771
526.00661
510.48514
541.98051
446.99920
463.01050
558.35046

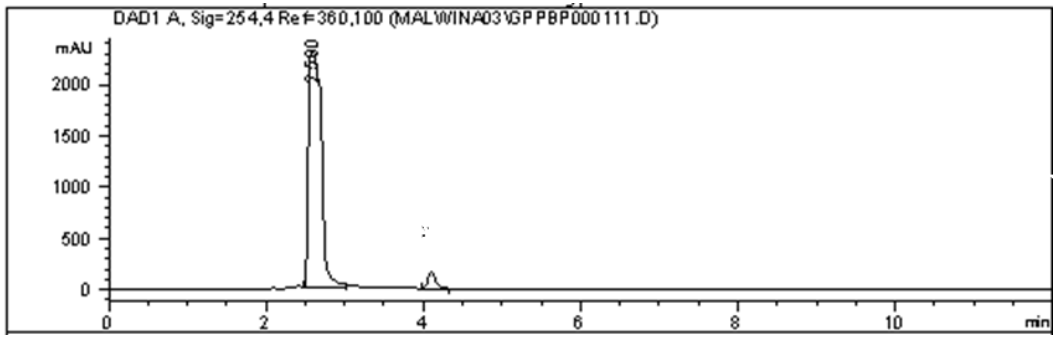

^{1}H NMR ATPβBH$_3$/TEA$^+$ D1D2mix (13)
AppBH3p_NH4_21deg_D2Ozapis2.esp
residual TEA signals
residual acetate
Water
residual TEA signals
Normalized Intensity
Chemical Shift (ppm)
8.44
8.13
6.04
6.02
4.71
4.69
4.68
4.51
4.33
4.17
4.16
3.45
3.43
3.41
3.12
3.11
3.09
3.07
2.99
2.97
2.88
2.86
1.90
1.89
1.59
1.58
1.56
1.24
1.23
1.20
1.18
1.16
0.89
0.87
0.85
0.54
0.29
0.83
0.86
0.96
1.12
1.00
1.01
2.01
0.79
1.44
0.70
0.71
7.09
0.61
0.92
3.22
0.96
1.98

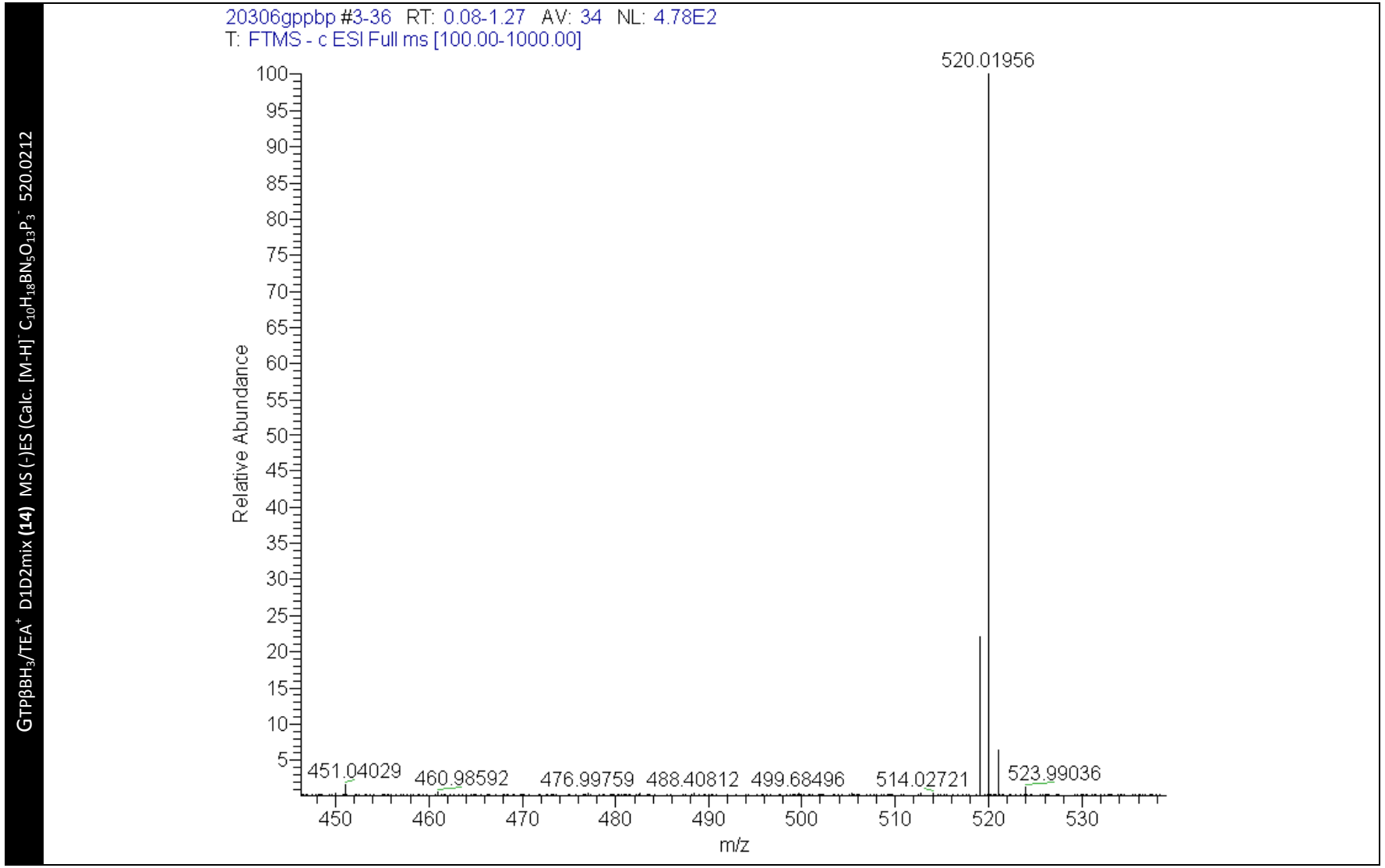

^{31}P NMR ATPβBH$_3$/TEA$^+$ D1D2mix (13)
AppBH3p_NH4_31P_25deg_D2O
Normalized Intensity
Chemical Shift (ppm)
74.80
74.00
10.71
10.89
11.19
11.38
1.00
0.99
0.92

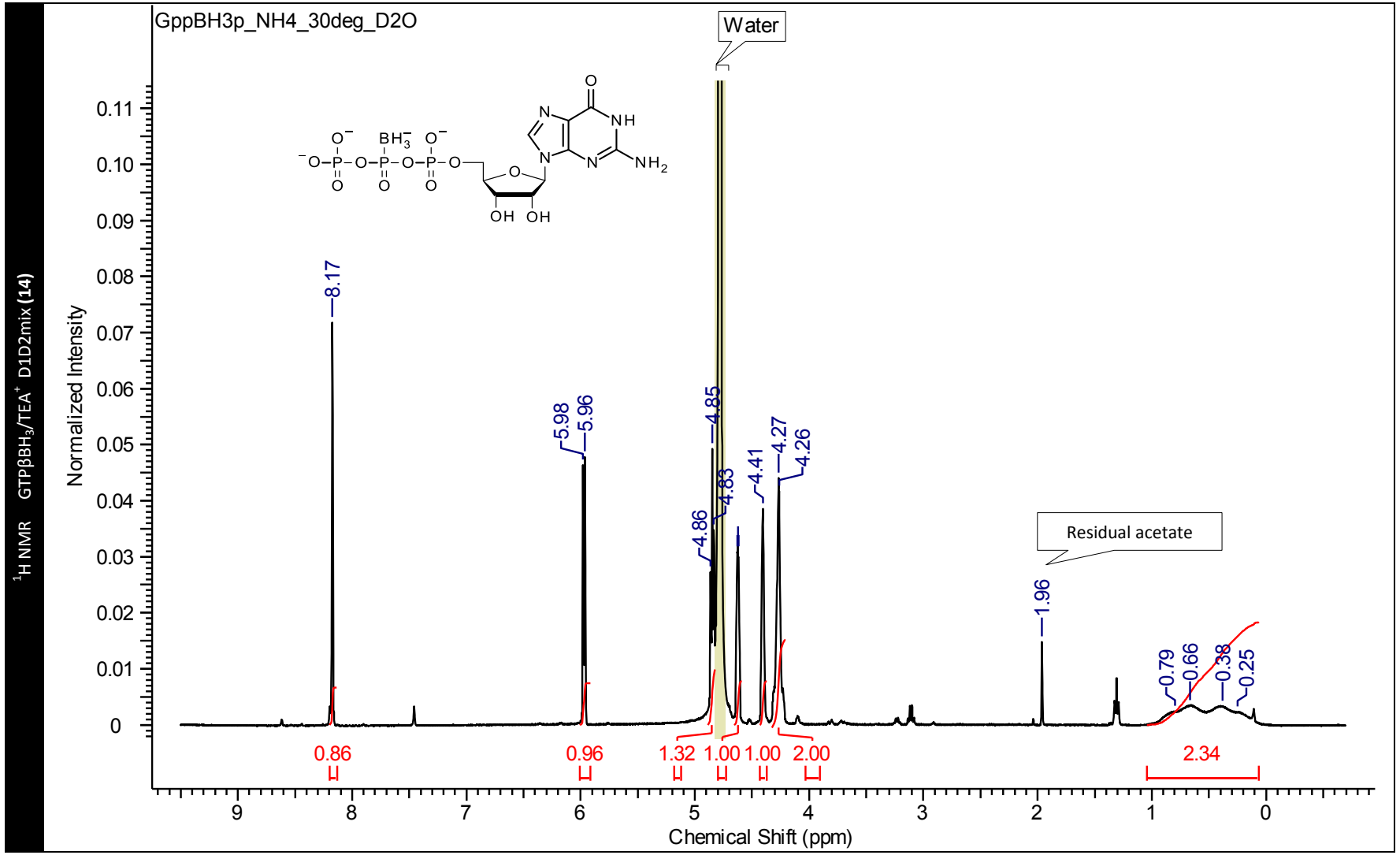
(**14**) GTPβBH$_3$/TEA$^+$ D1D2mix

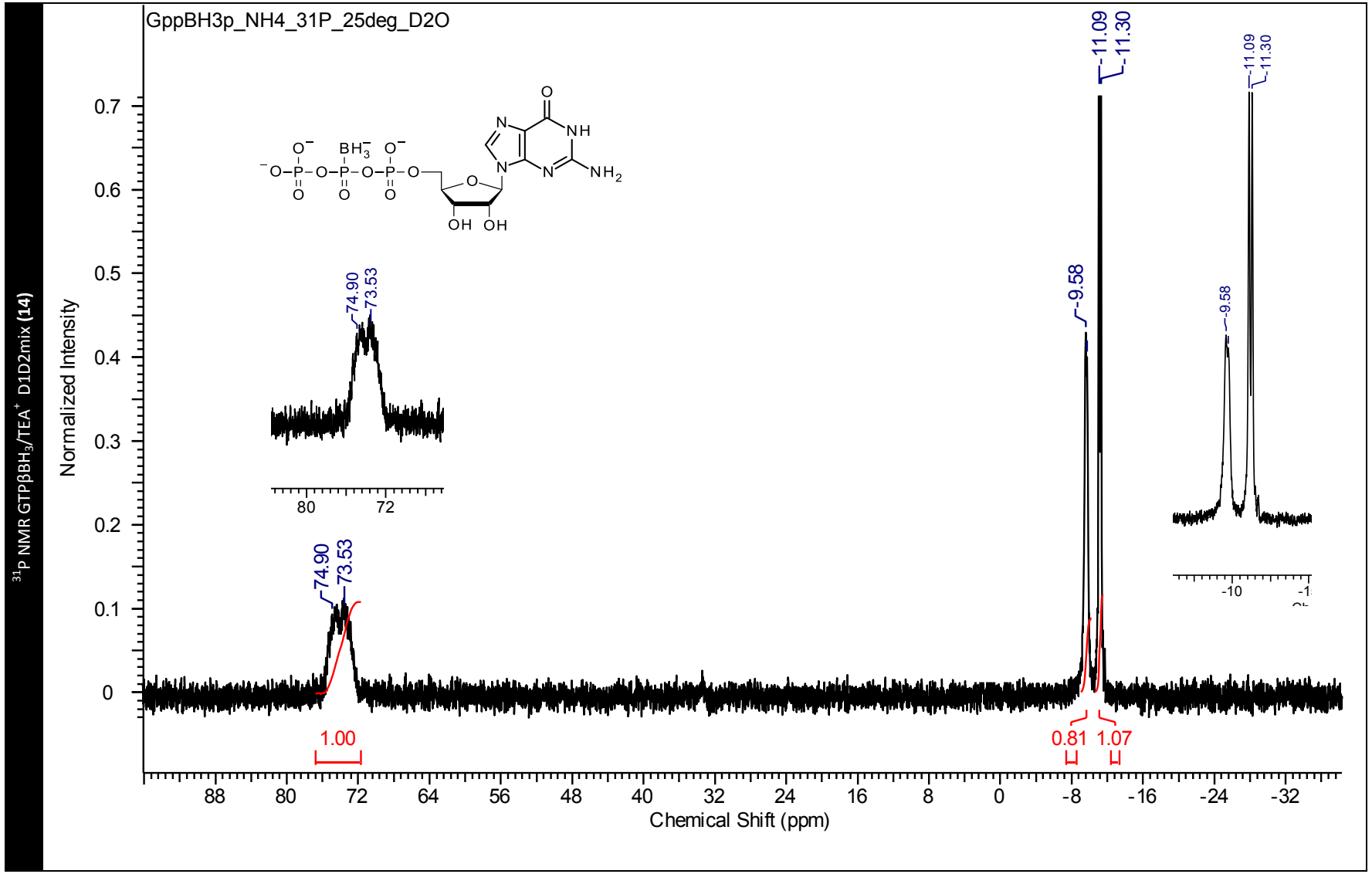


GTPβBH$_3$/TEA+ D1D2mix **(14)** HPLC

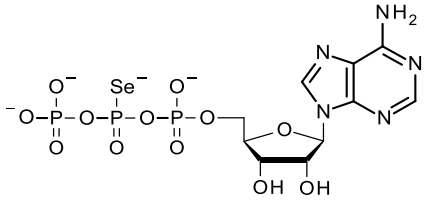



GTPβBH3/TEA+ D1D2mix (14) MS (-)ES (Calc. [M-H]- C10H18BN5O13P3- 520.0212
20306gppbp #3-36 RT: 0.08-1.27 AV: 34 NL: 4.78E2
T: FTMS - c ESI Full ms [100.00-1000.00]
520.01956
451.04029
460.98592
476.99759
488.40812
499.68496
514.02721
523.99036
Relative Abundance
m/z

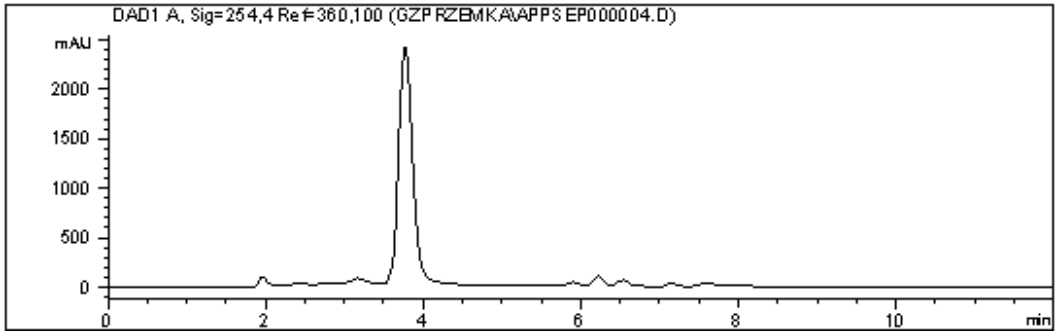

^{1}H NMR GTPβBH$_3$/TEA$^+$ D1D2mix (14)
GppBH3p_NH4_30deg_D2O
Water
Residual acetate
Normalized Intensity
Chemical Shift (ppm)
8.17
5.98
5.96
4.85
4.86
4.83
4.41
4.27
4.26
1.96
0.79
0.66
0.38
0.25
0.86
0.96
1.32
1.00
1.00
2.00
2.34


GppBH3p_NH4_31P_25deg_D2O
^{31}P NMR GTPβBH$_3$/TEA$^+$ D1D2mix (**14**)
Normalized Intensity
Chemical Shift (ppm)
74.90
73.53
-9.58
-11.09
-11.30
1.00
0.81
1.07

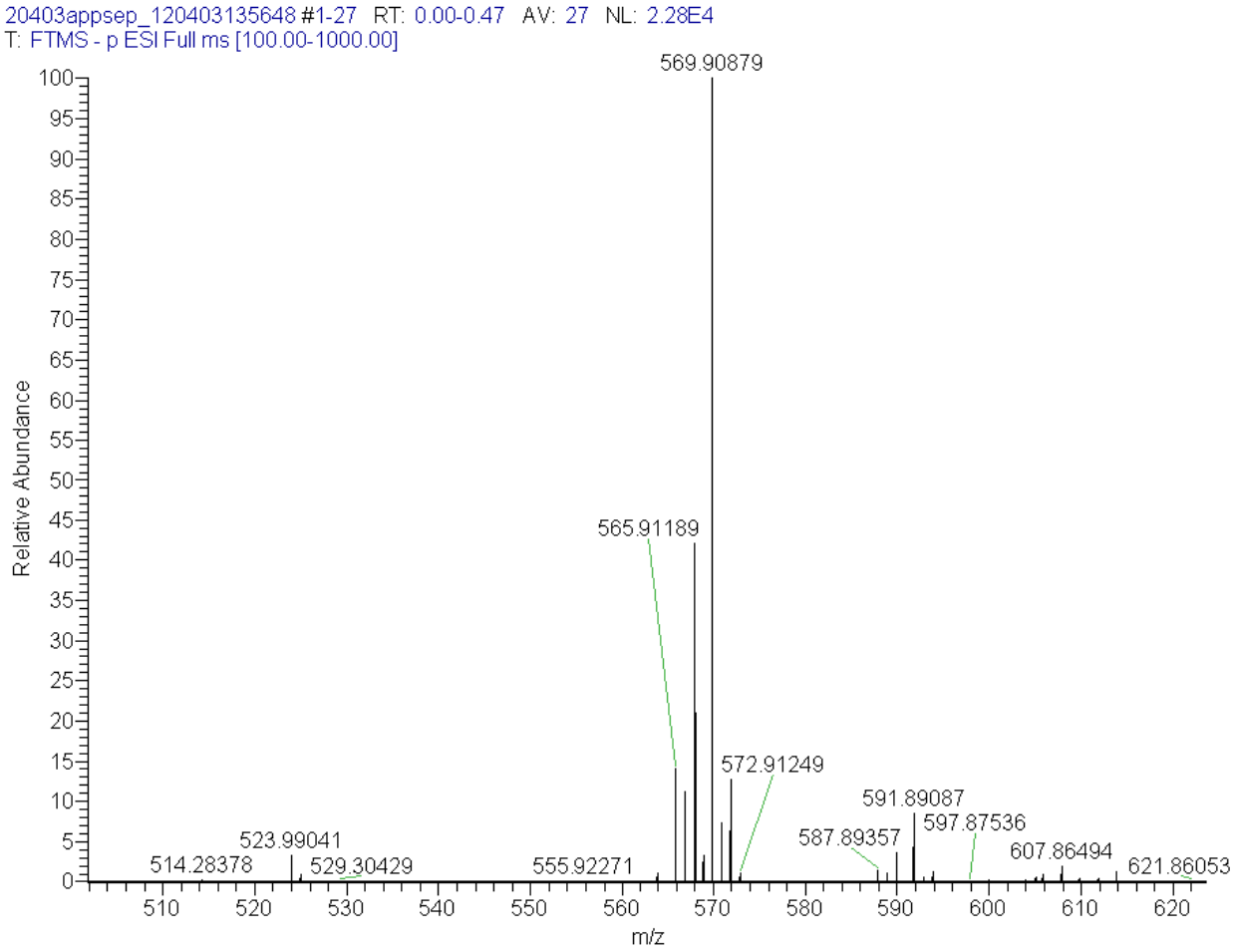
(**15**) ATPβSe/TEA^{+} D1D2mix

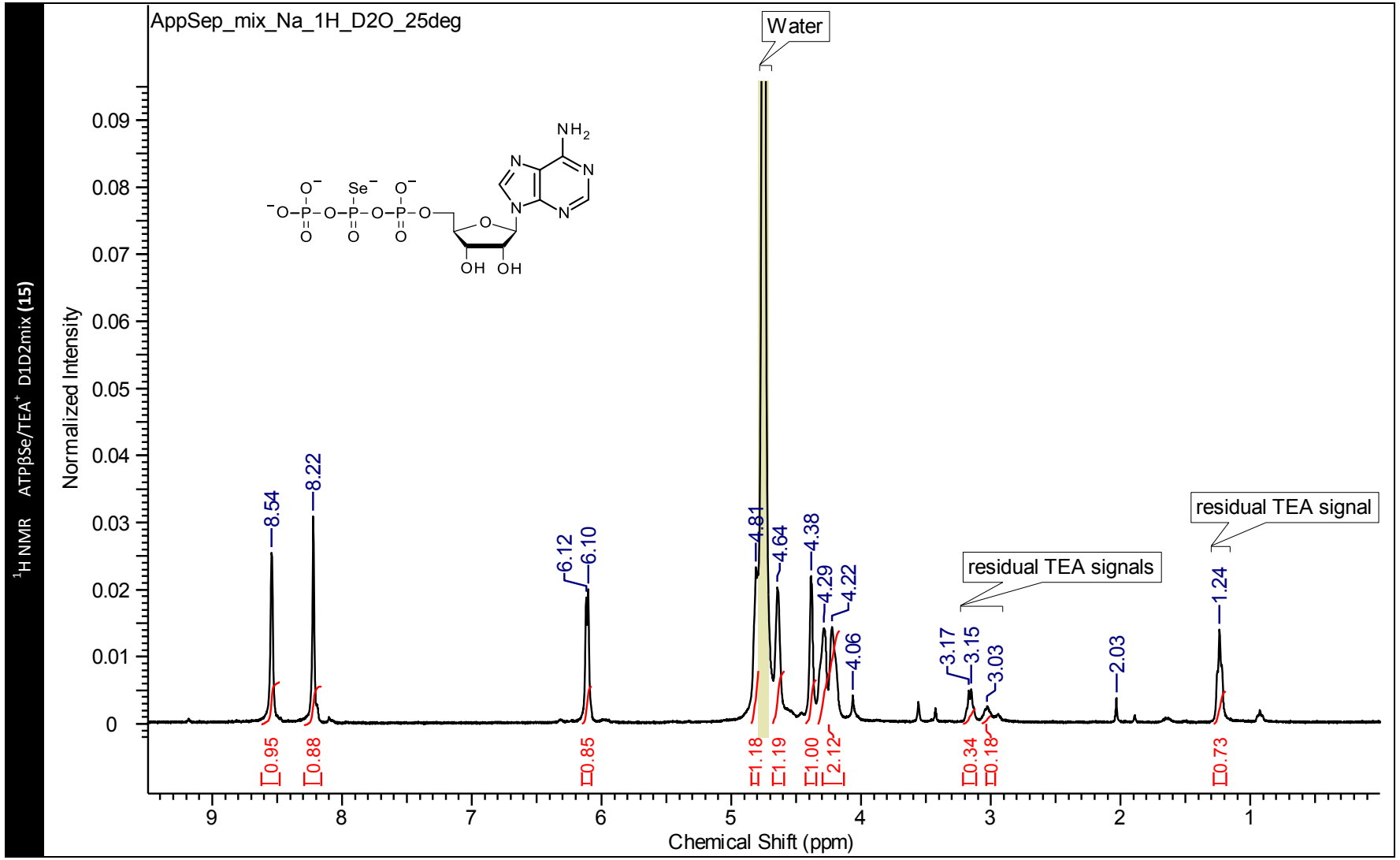


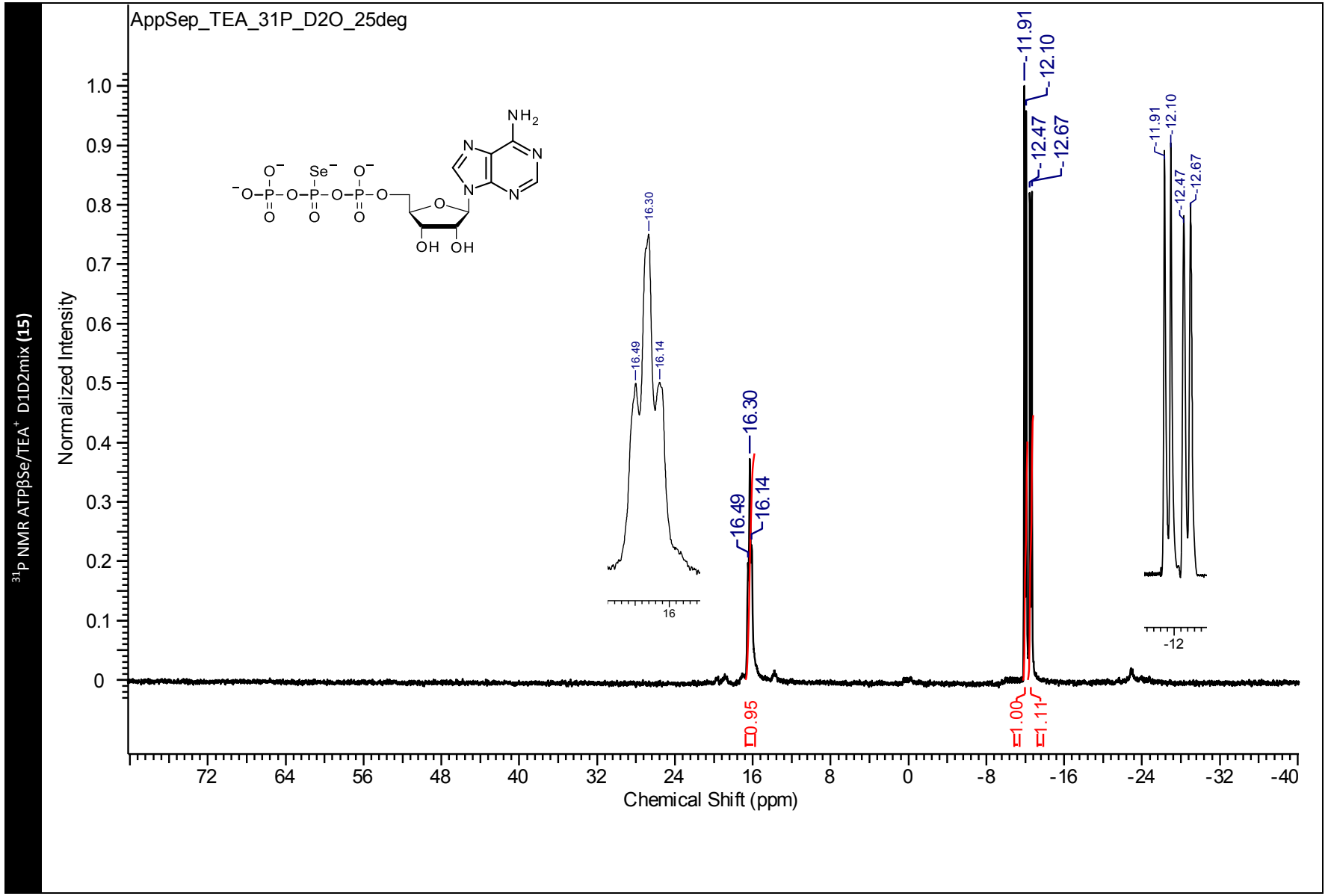
ATPβSe/TEA+ D1D2mix **(15)** HPLC



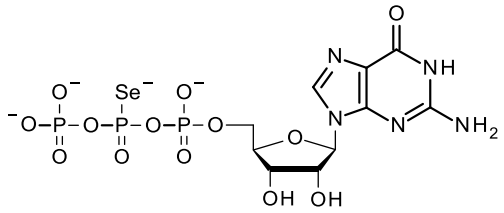
ATPβSe/TEA+ D1D2mix **(15)** MS (-)ES (Calc. $[M-H]^-$ $C_{10}H_{15}N_5O_{12}P_3Se^-$ 569.9101



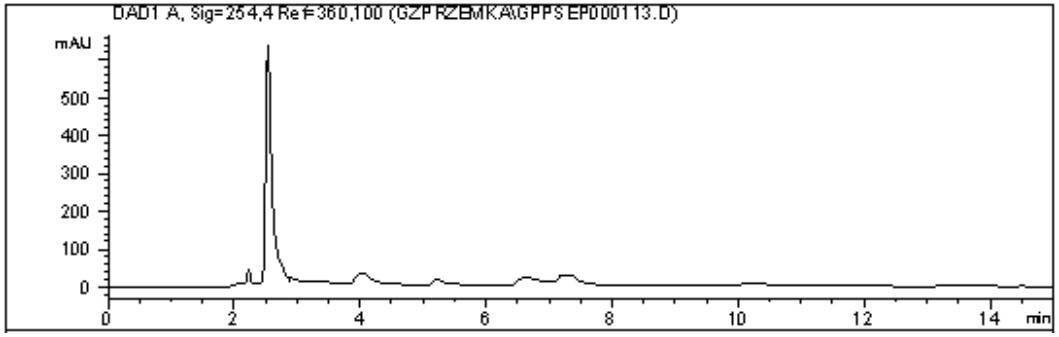

^{1}H NMR ATPβSe/TEA$^+$ D1D2mix (15)
AppSep_mix_Na_1H_D2O_25deg
Water
residual TEA signals
residual TEA signal
Normalized Intensity
Chemical Shift (ppm)
8.54
8.22
6.12
6.10
4.81
4.64
4.38
4.29
4.22
4.06
3.17
3.15
3.03
2.03
1.24
0.95
0.88
0.85
1.18
1.19
1.00
2.12
0.34
0.18
0.73


^{31}P NMR ATPβSe/TEA^{+} D1D2mix **(15)**
AppSep_TEA_31P_D2O_25deg
Normalized Intensity
Chemical Shift (ppm)
16.49
16.30
16.14
11.91
12.10
12.47
12.67
0.95
1.00
1.11

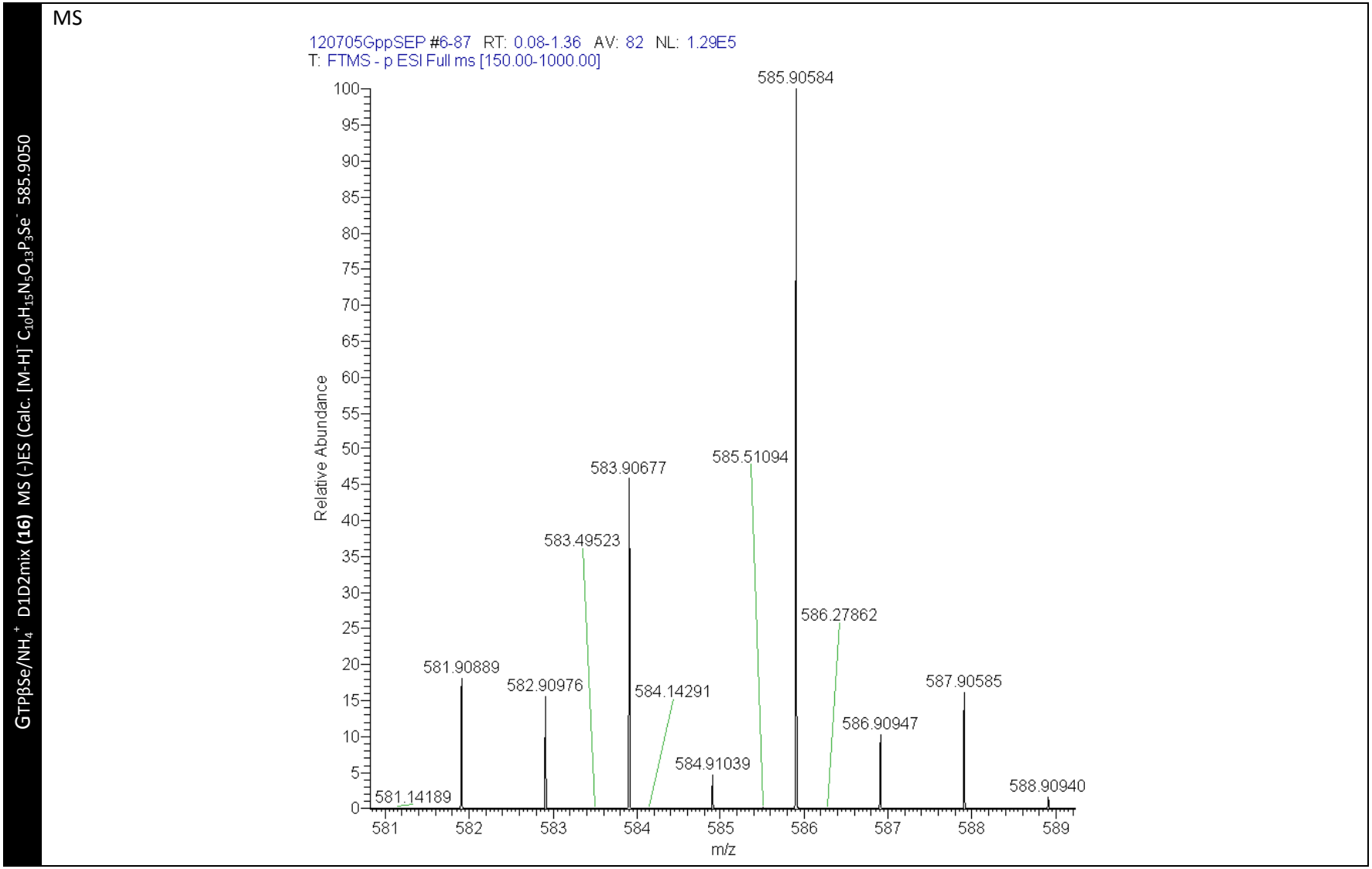
(**16**) GTPβSe/NH_4^+ D1D2mix

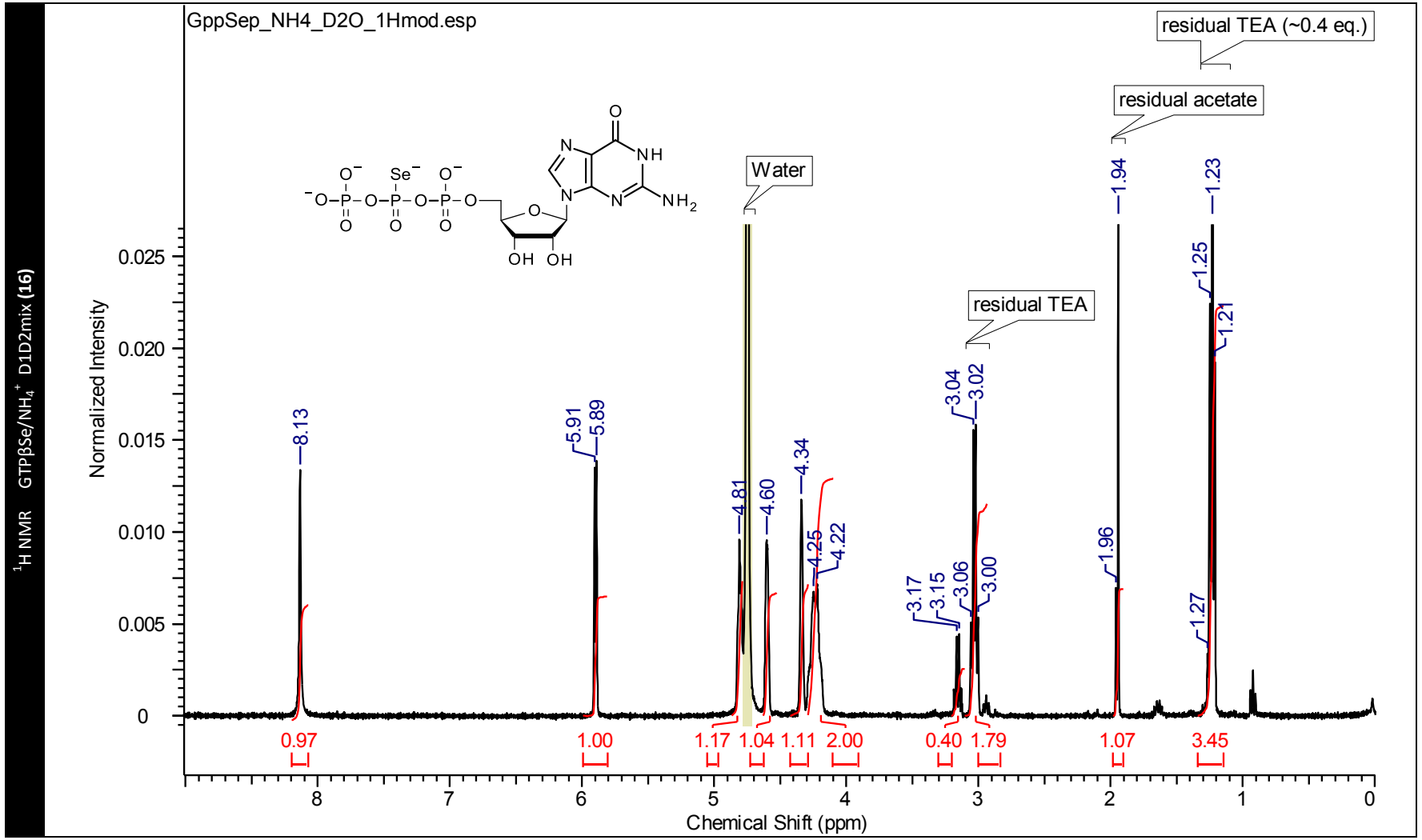


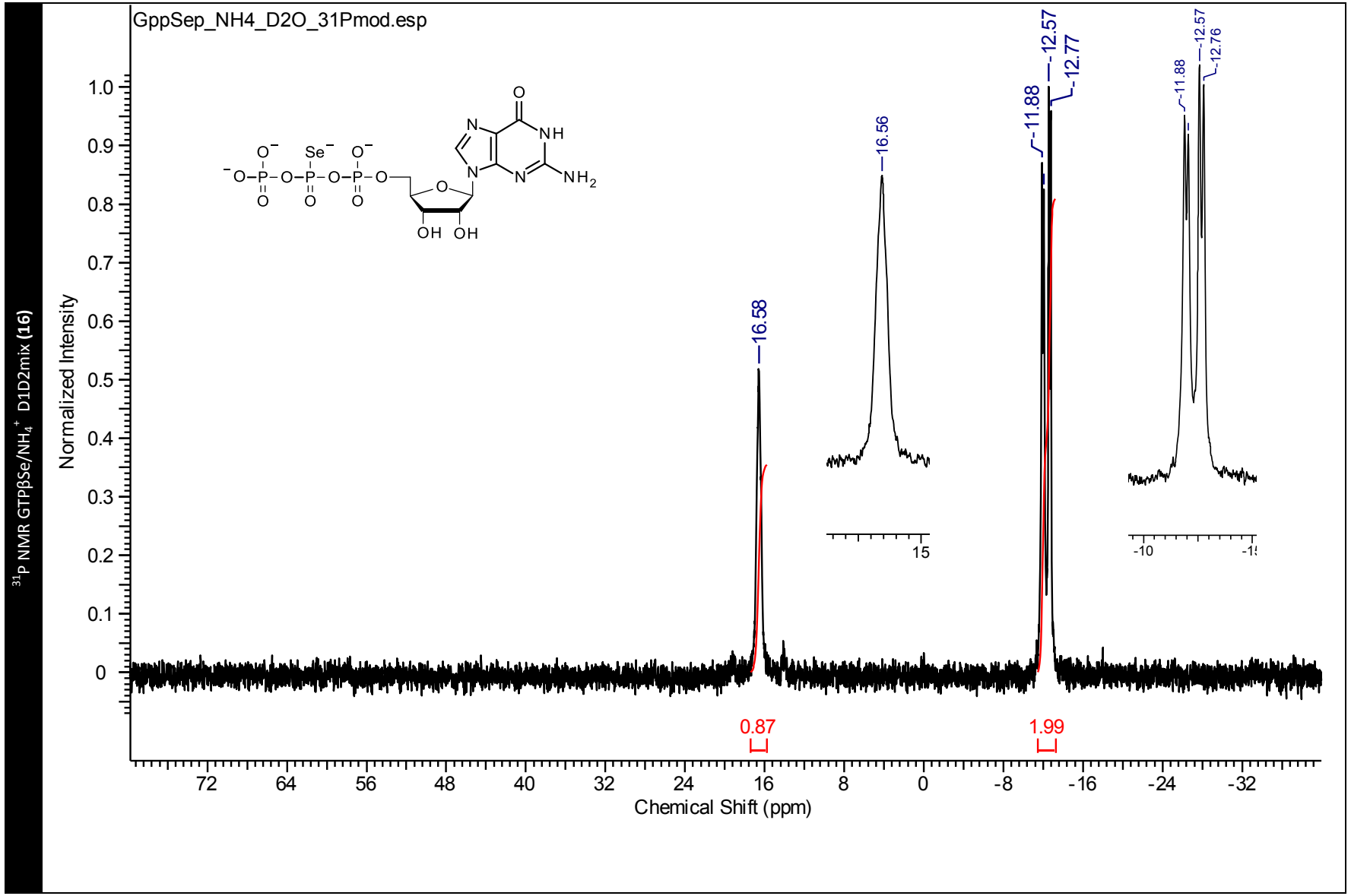
GTPβSe/NH_4^+ D1D2mix (**16**) HPLC



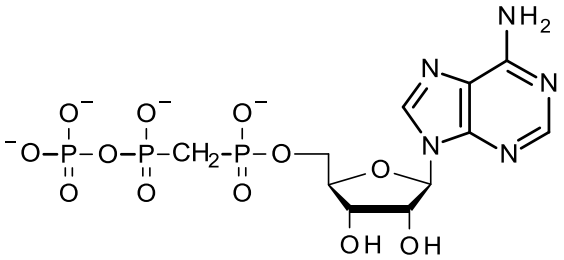
MS


GTPβSe/NH4+ D1D2mix (16) MS (-)ES (Calc. [M-H]- C10H15N5O13P3Se- 585.9050
120705GppSEP #6-87 RT: 0.08-1.36 AV: 82 NL: 1.29E5
T: FTMS - p ESI Full ms [150.00-1000.00]
Relative Abundance
m/z
585.90584
585.51094
583.90677
583.49523
586.27862
581.90889
582.90976
584.14291
587.90585
586.90947
584.91039
588.90940
581.14189

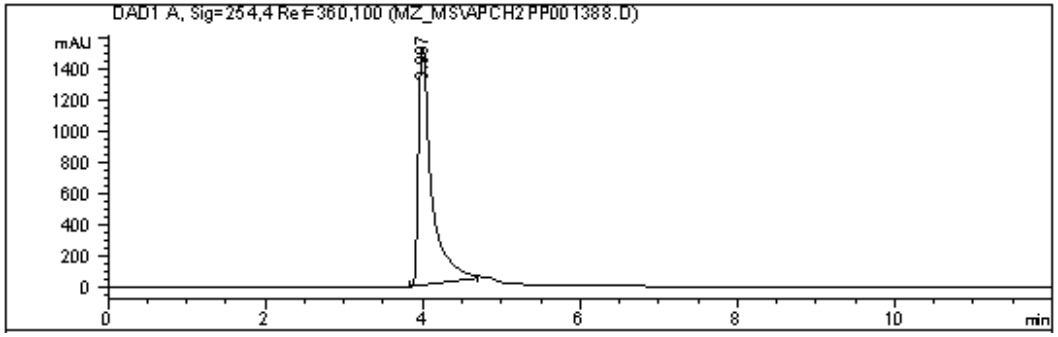

^{1}H NMR GTPβSe/NH$_4^+$ D1D2mix **(16)**
GppSep_NH4_D2O_1Hmod.esp
residual TEA (~0.4 eq.)
residual acetate
Water
residual TEA
Normalized Intensity
Chemical Shift (ppm)
8.13
5.91
5.89
4.81
4.60
4.34
4.25
4.22
3.17
3.15
3.06
3.04
3.02
3.00
1.96
1.94
1.27
1.25
1.23
1.21
0.97
1.00
1.17
1.04
1.11
2.00
0.40
1.79
1.07
3.45

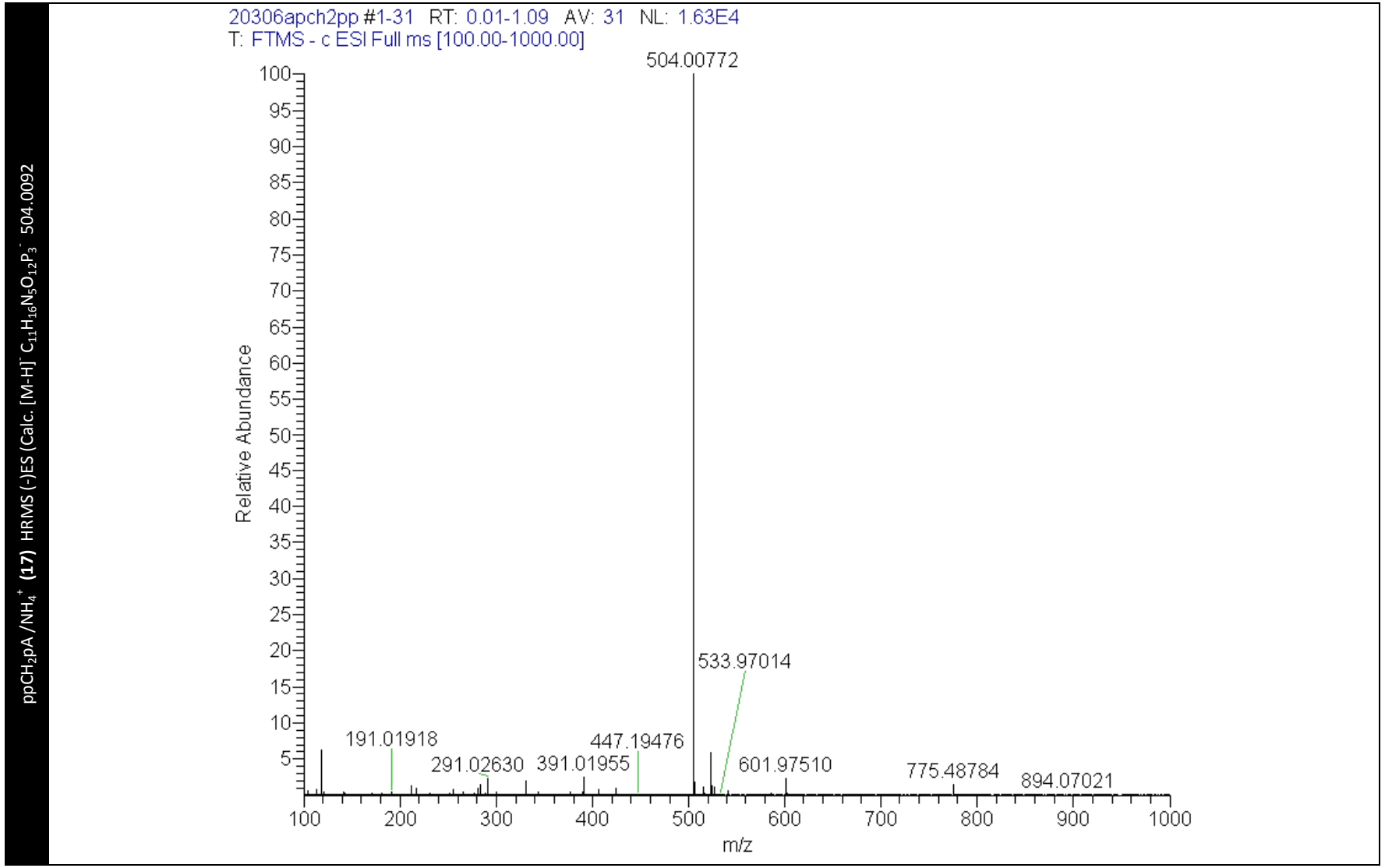

GppSep_NH4_D2O_31Pmod.esp
^{31}P NMR GTPβSe/NH_4^+ D1D2mix **(16)**
Normalized Intensity
Chemical Shift (ppm)
16.58
16.56
11.88
12.57
12.77
11.88
12.57
12.76
0.87
1.99

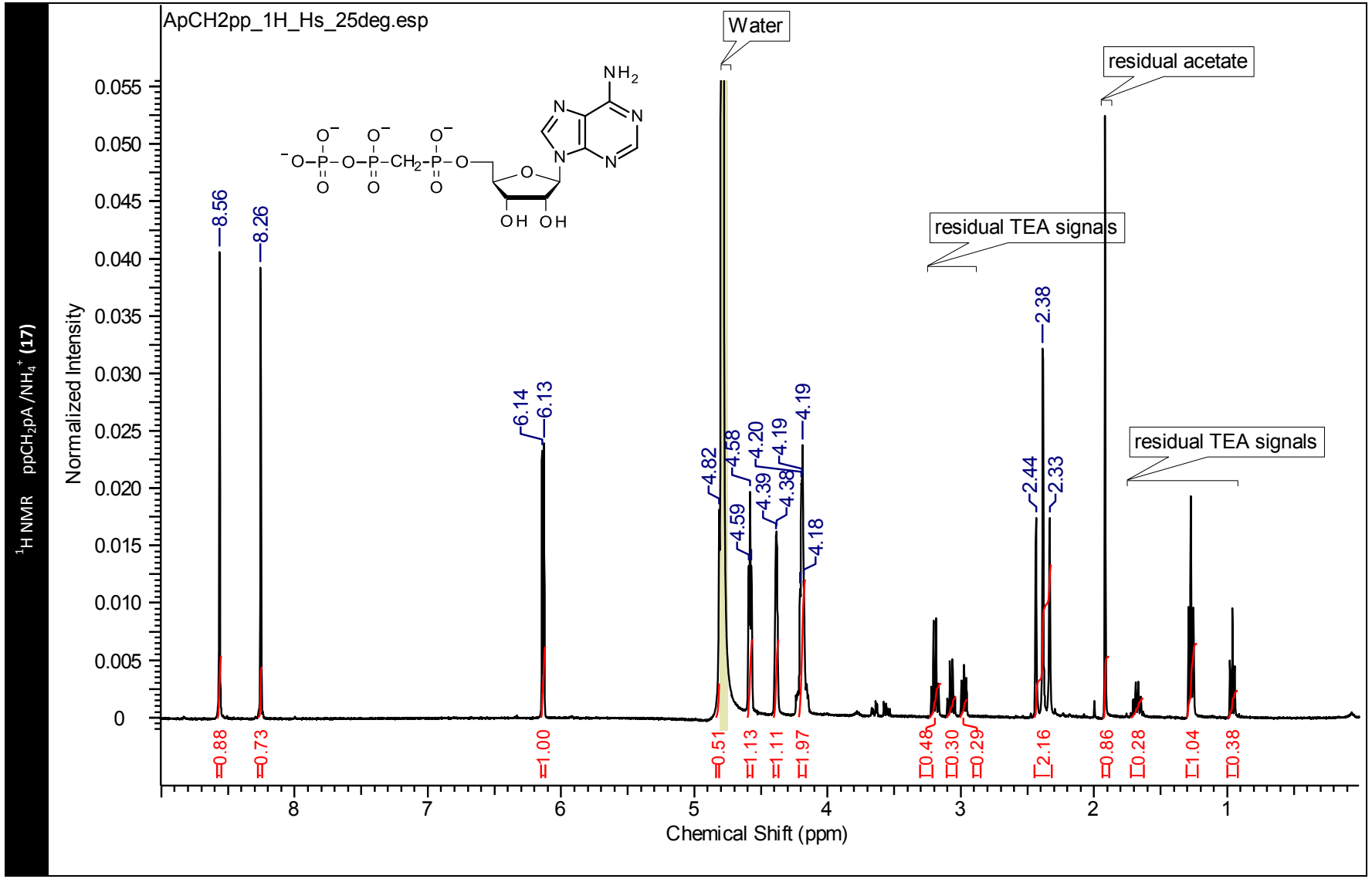
(**17**) $ppCH_2pA/NH_4^+$

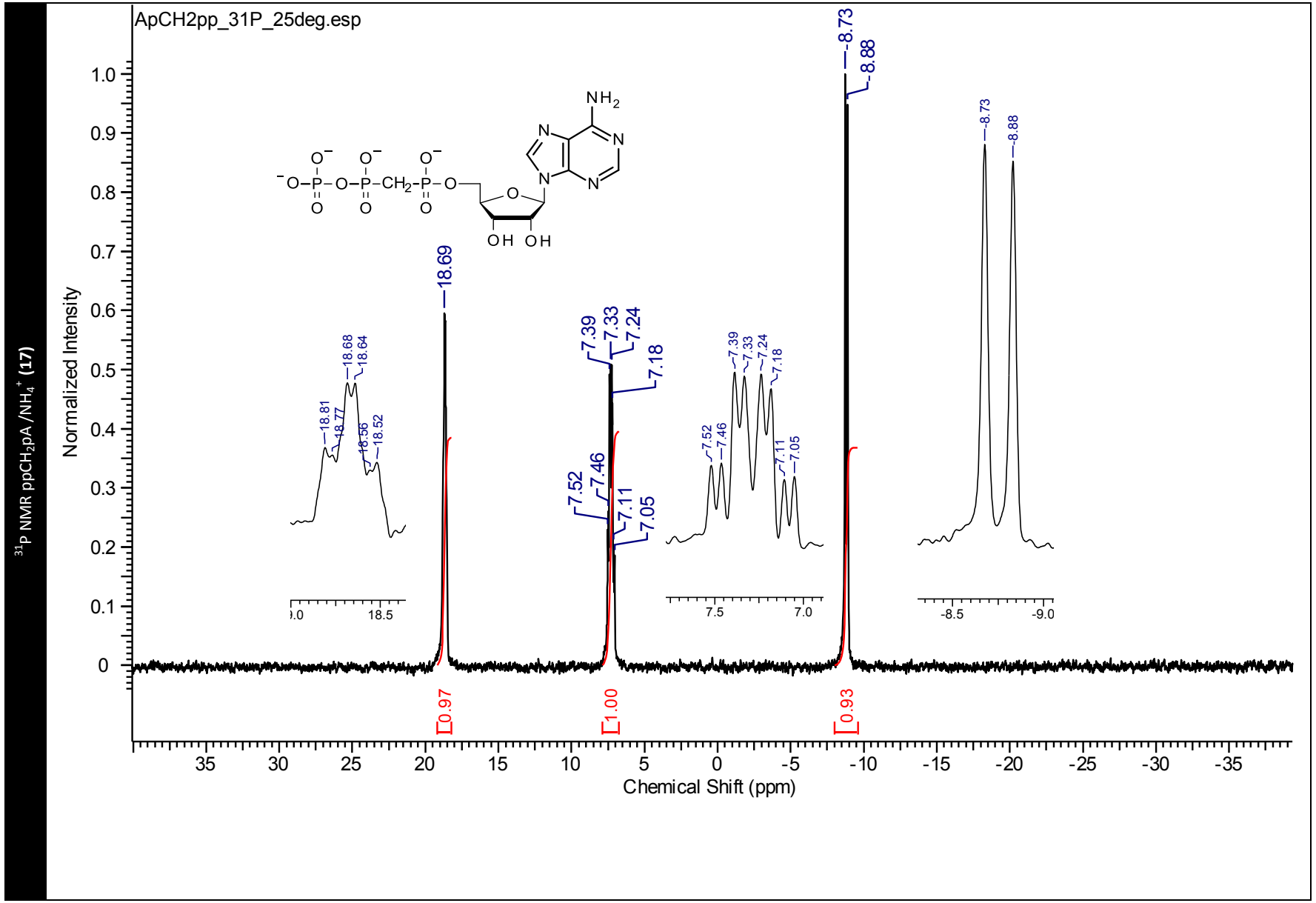


ppCH$_2$pA /NH$_4^+$ (**17**) HPLC

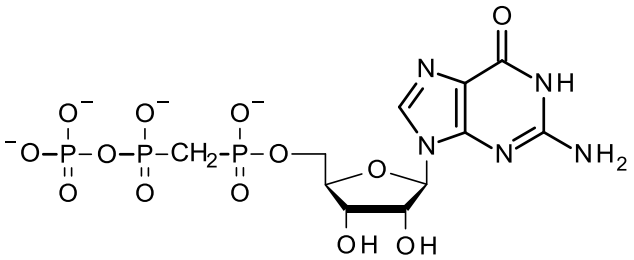



ppCH2pA /NH4+ (17) HRMS (-)ES (Calc. [M-H]- C11H16N5O12P3- 504.0092
20306apch2pp #1-31 RT: 0.01-1.09 AV: 31 NL: 1.63E4
T: FTMS - c ESI Full ms [100.00-1000.00]
504.00772
533.97014
191.01918
291.02630
391.01955
447.19476
601.97510
775.48784
894.07021
Relative Abundance
m/z

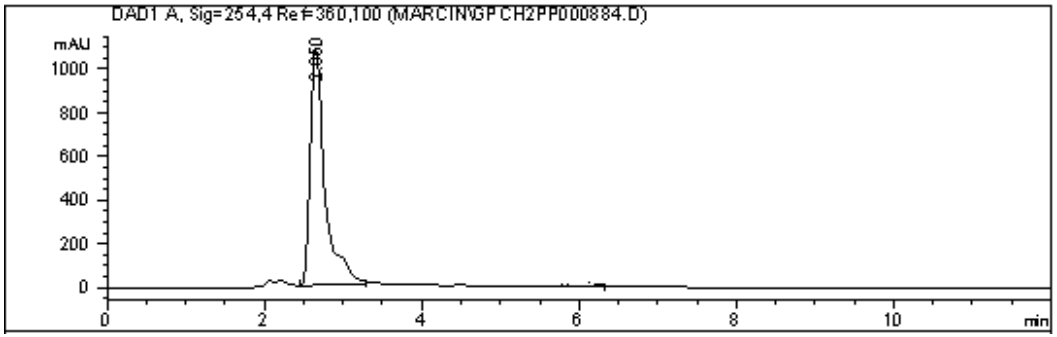

^{1}H NMR ppCH$_2$pA /NH$_4^+$ (17)
ApCH2pp_1H_Hs_25deg.esp
Normalized Intensity
Chemical Shift (ppm)
Water
residual acetate
residual TEA signals
residual TEA signals
8.56
8.26
6.14
6.13
4.82
4.59
4.58
4.39
4.38
4.20
4.19
4.19
4.18
2.44
2.38
2.33
0.88
0.73
1.00
0.51
1.13
1.11
1.97
0.48
0.30
0.29
2.16
0.86
0.28
1.04
0.38

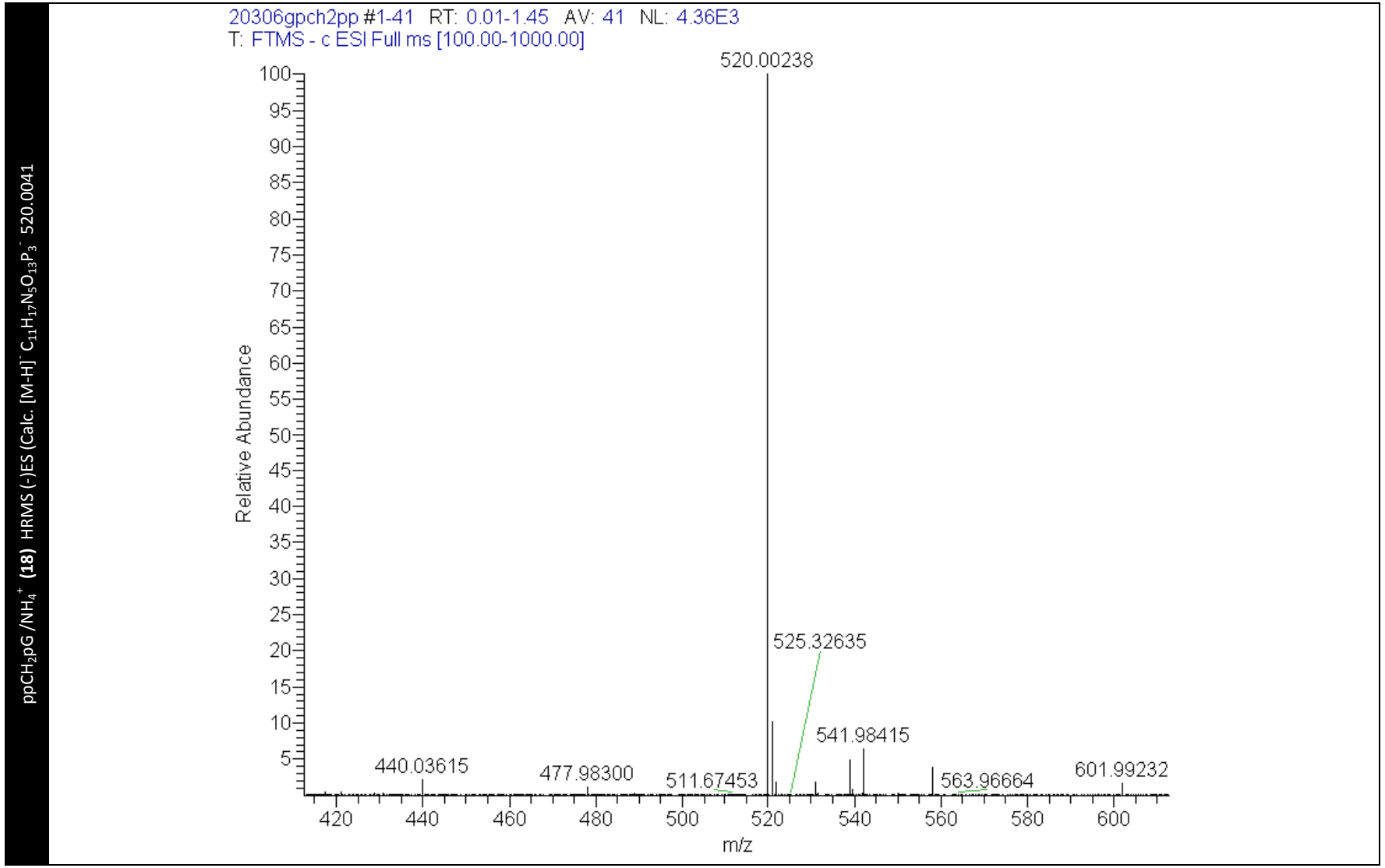

^{31}P NMR ppCH$_2$pA /NH$_4^+$ (17)
ApCH2pp_31P_25deg.esp
Normalized Intensity
Chemical Shift (ppm)
18.69
7.39
7.33
7.24
7.18
7.52
7.46
7.11
7.05
8.73
8.88
0.97
1.00
0.93

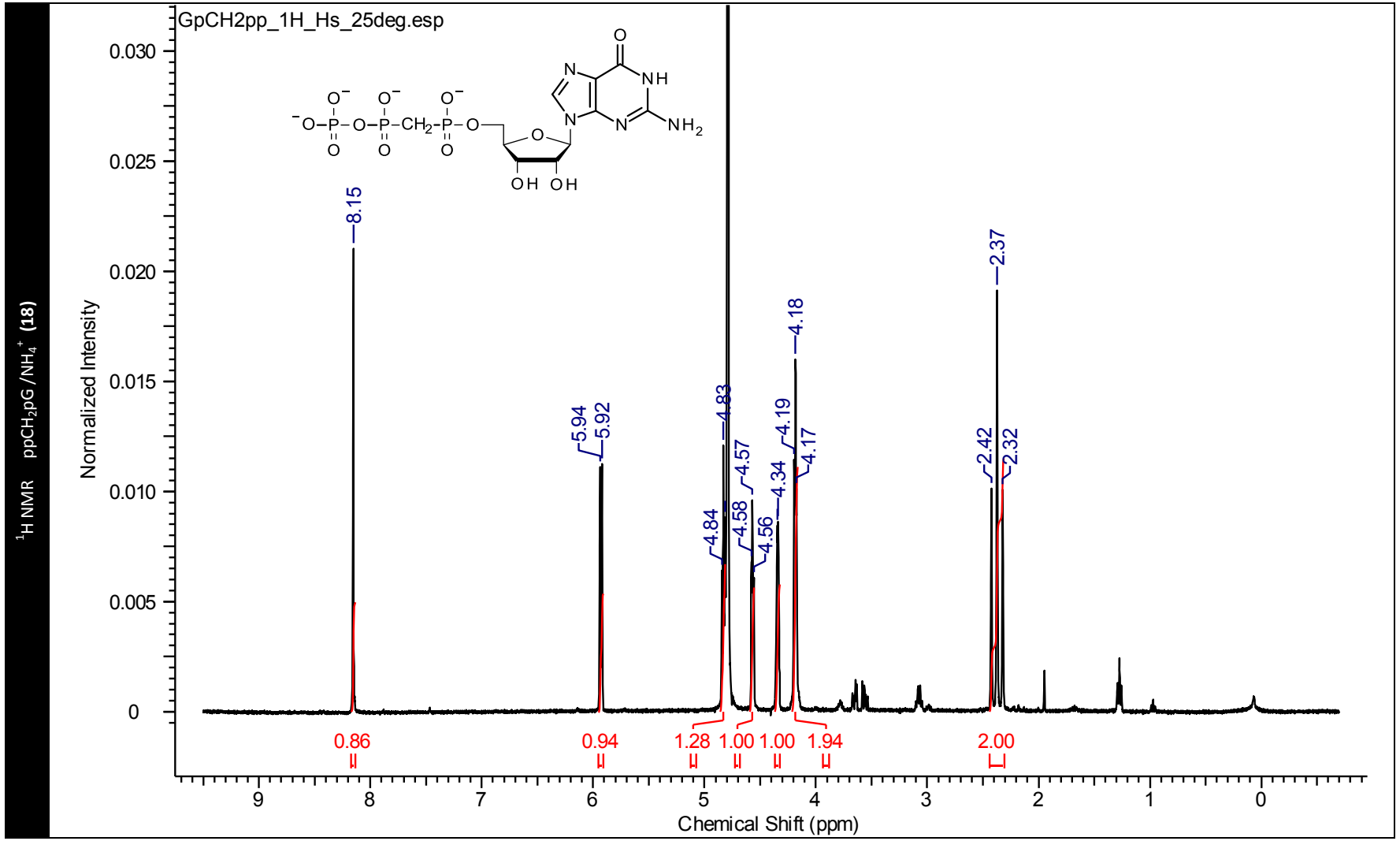
(18) $ppCH_2pG\ /NH_4^+$

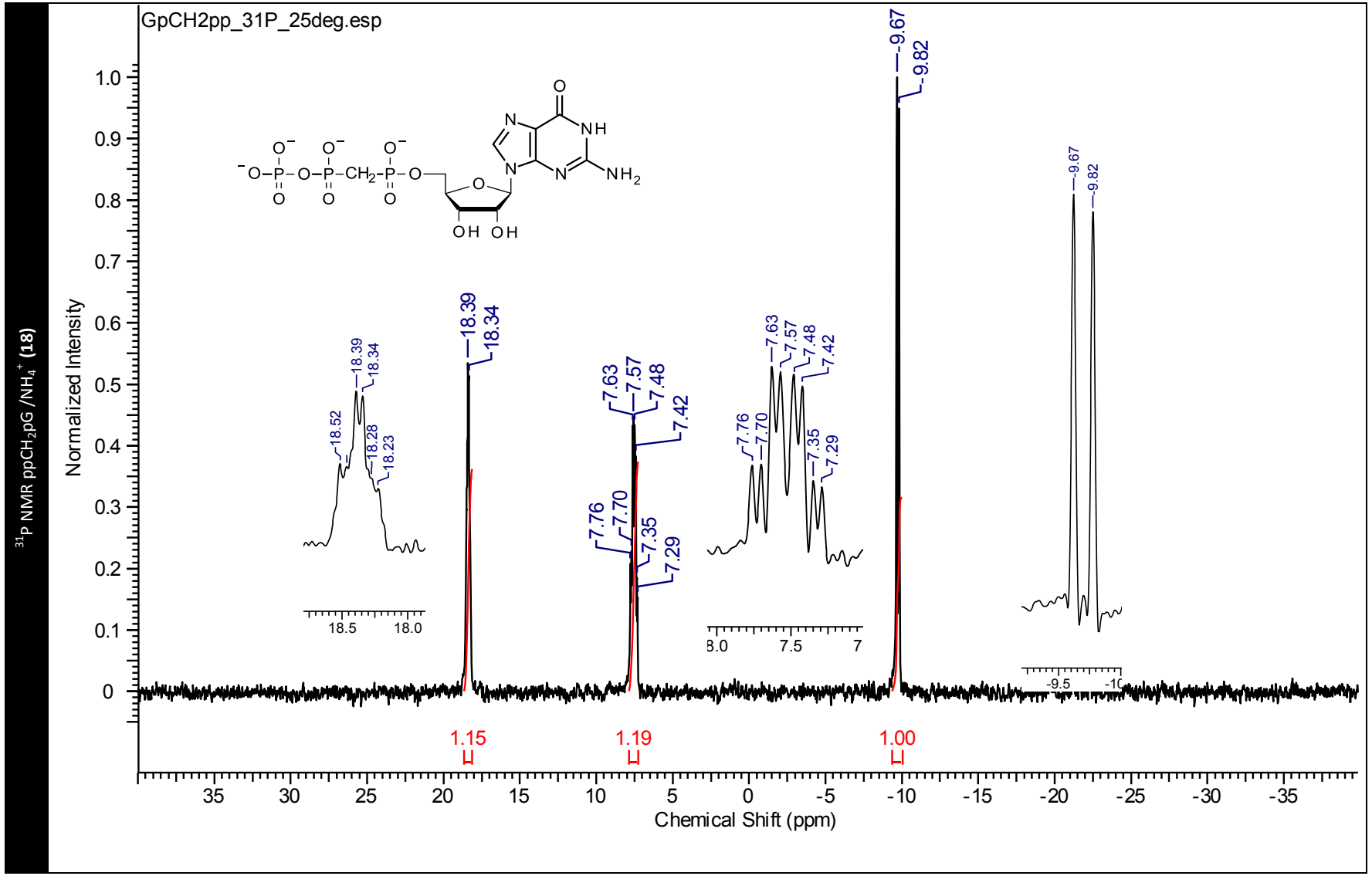


$ppCH_2pG\ /NH_4^+$ **(18)** HPLC

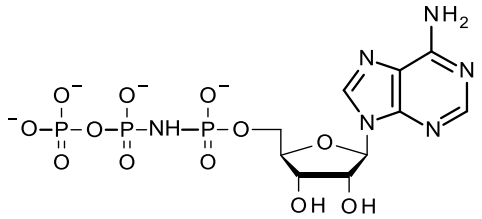



ppCH2pG /NH4+ (18) HRMS (-)ES (Calc. [M-H]- C11H17N3O13P3- 520.0041
20306gpch2pp #1-41 RT: 0.01-1.45 AV: 41 NL: 4.36E3
T: FTMS - c ESI Full ms [100.00-1000.00]
Relative Abundance
m/z
520.00238
525.32635
541.98415
440.03615
477.98300
511.67453
563.96664
601.99232

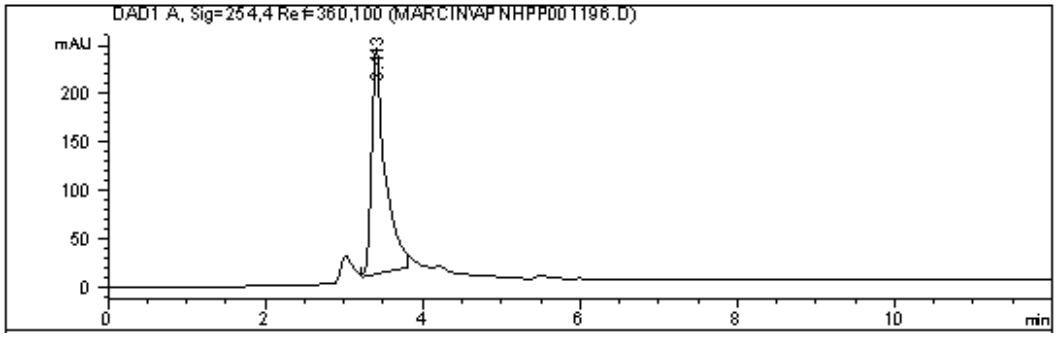

GpCH2pp_1H_Hs_25deg.esp
^{1}H NMR ppCH$_2$pG /NH$_4^+$ (18)
Normalized Intensity
Chemical Shift (ppm)
8.15
5.94
5.92
4.84
4.83
4.58
4.57
4.56
4.34
4.19
4.18
4.17
2.42
2.37
2.32
0.86
0.94
1.28
1.00
1.00
1.94
2.00

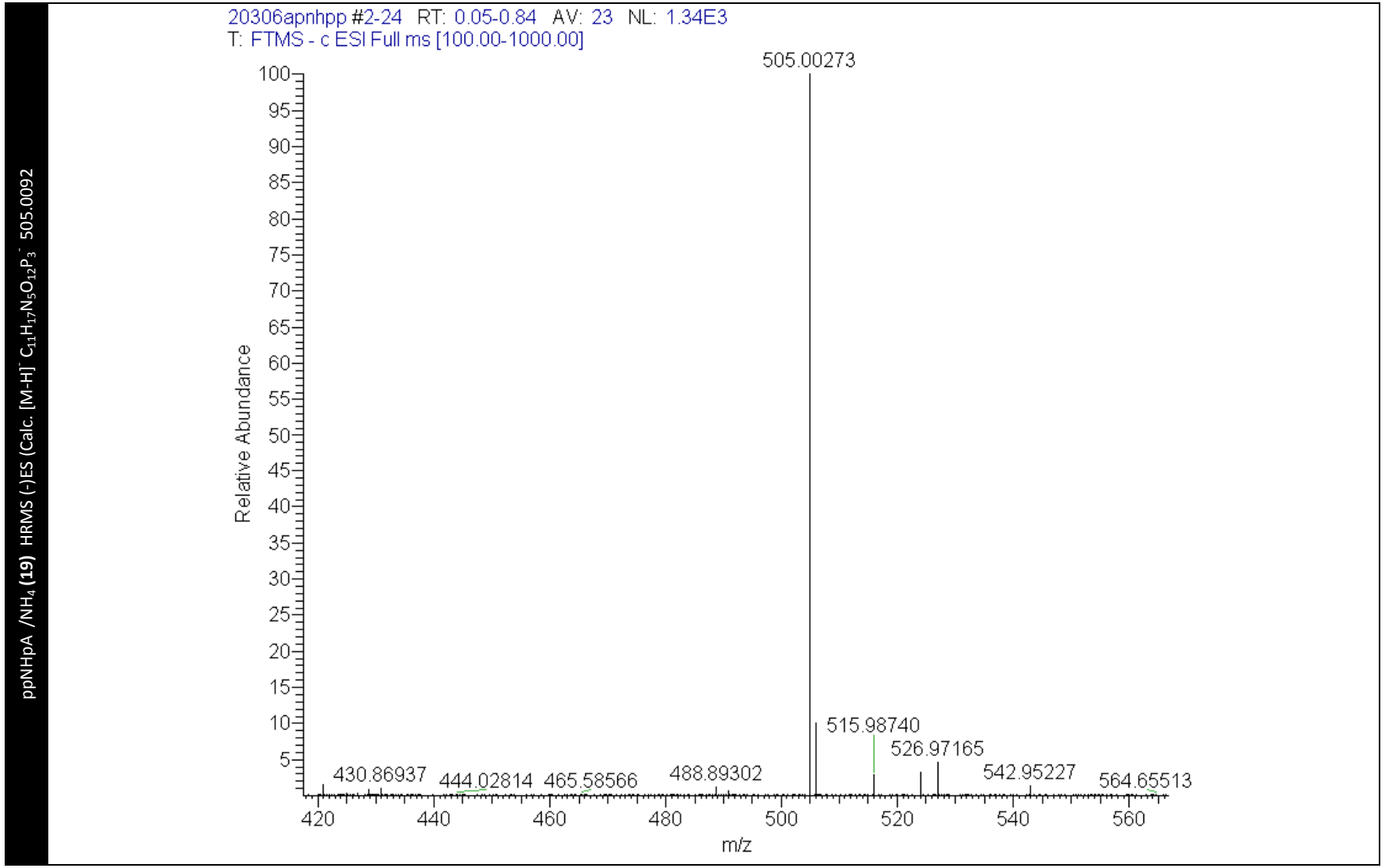

^{31}P NMR ppCH$_2$pG /NH$_4^+$ **(18)**
GpCH2pp_31P_25deg.esp
Normalized Intensity
Chemical Shift (ppm)
18.52
18.39
18.34
18.28
18.23
7.76
7.70
7.63
7.57
7.48
7.42
7.35
7.29
9.67
9.82
1.15
1.19
1.00

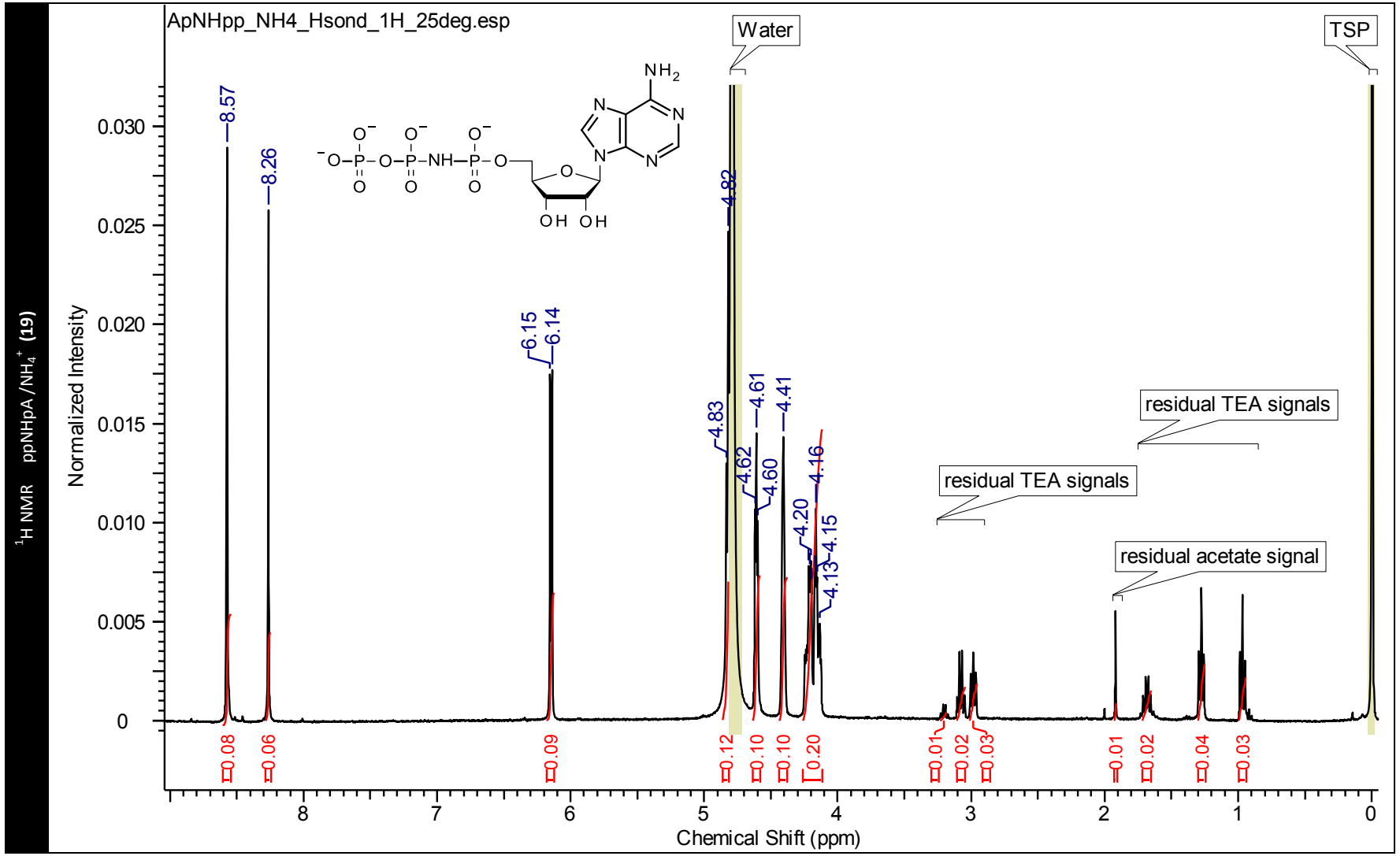
(**19**) ppNHpA /NH_4^+

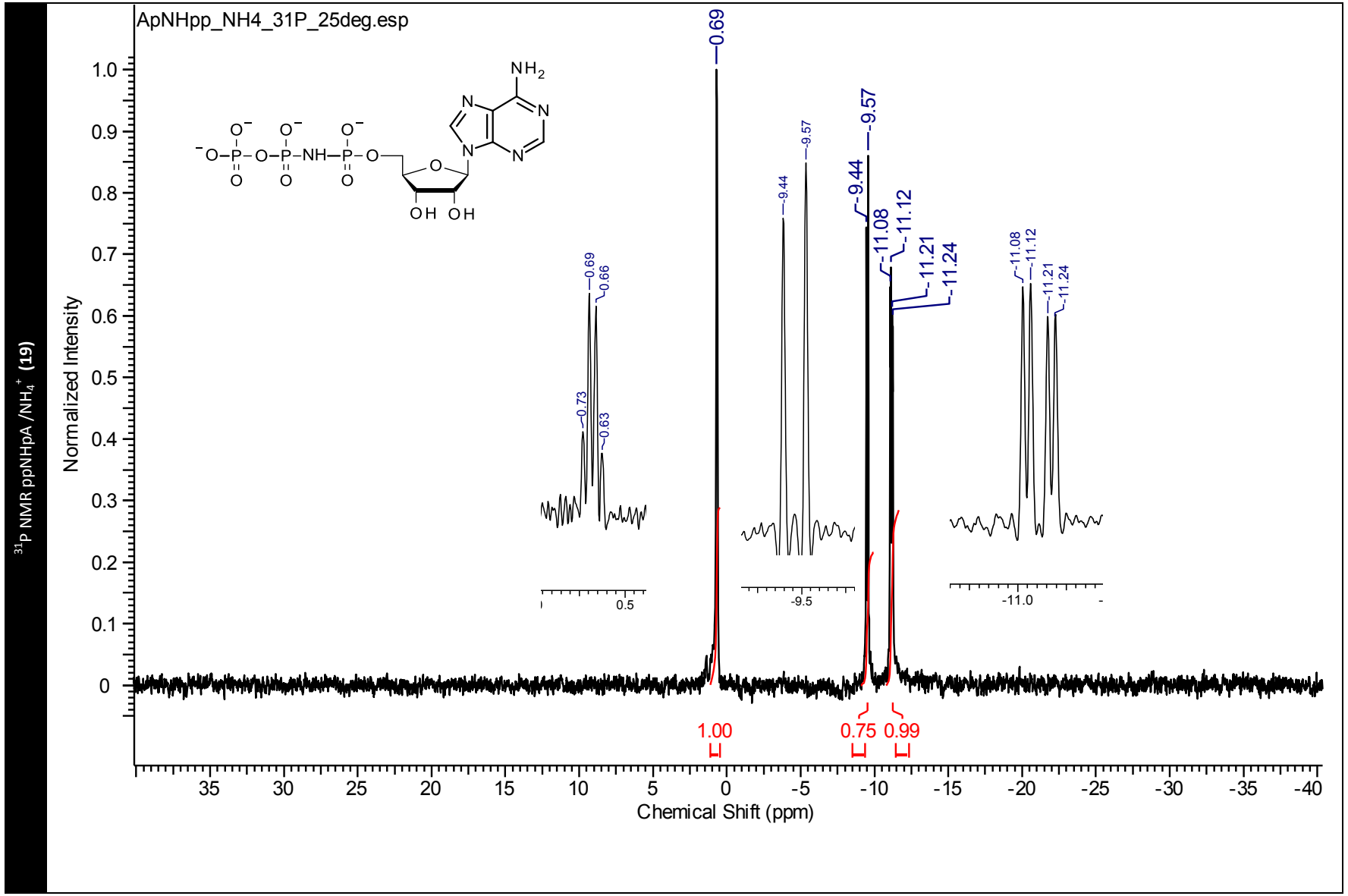


ppNHpA /NH_4 (**19**) HPLC

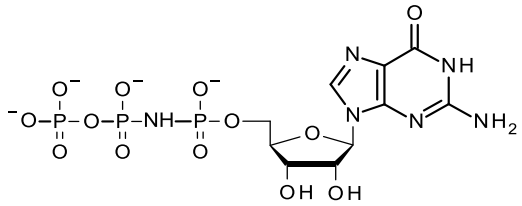



ppNHpA /NH4 (19) HRMS (-)ES (Calc. [M-H]- C11H17N5O12P3- 505.0092
20306apnhpp #2-24 RT: 0.05-0.84 AV: 23 NL: 1.34E3
T: FTMS - c ESI Full ms [100.00-1000.00]
505.00273
515.98740
526.97165
542.95227
564.65513
430.86937
444.02814
465.58566
488.89302
Relative Abundance
m/z
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
420
440
460
480
500
520
540
560

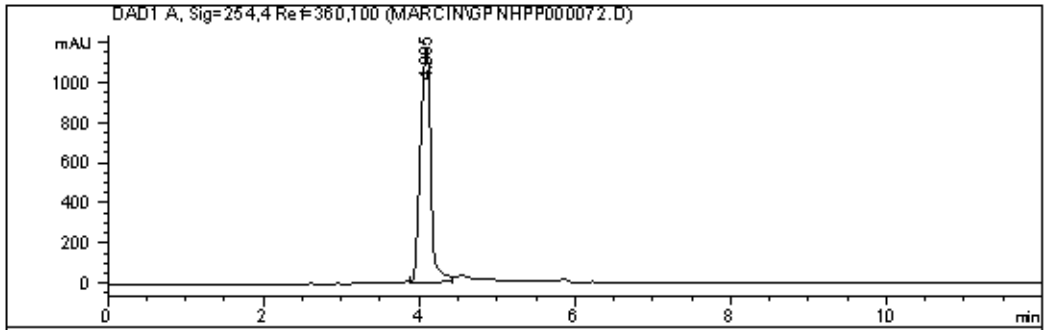

^{1}H NMR ppNHpA /NH_4^+ (19)
ApNHpp_NH4_Hsond_1H_25deg.esp
Water
TSP
residual TEA signals
residual TEA signals
residual acetate signal
8.57
8.26
6.15
6.14
4.83
4.82
4.62
4.61
4.60
4.41
4.20
4.16
4.15
4.13
0.08
0.06
0.09
0.12
0.10
0.10
0.20
0.01
0.02
0.03
0.01
0.02
0.04
0.03
Normalized Intensity
0.030
0.025
0.020
0.015
0.010
0.005
0
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

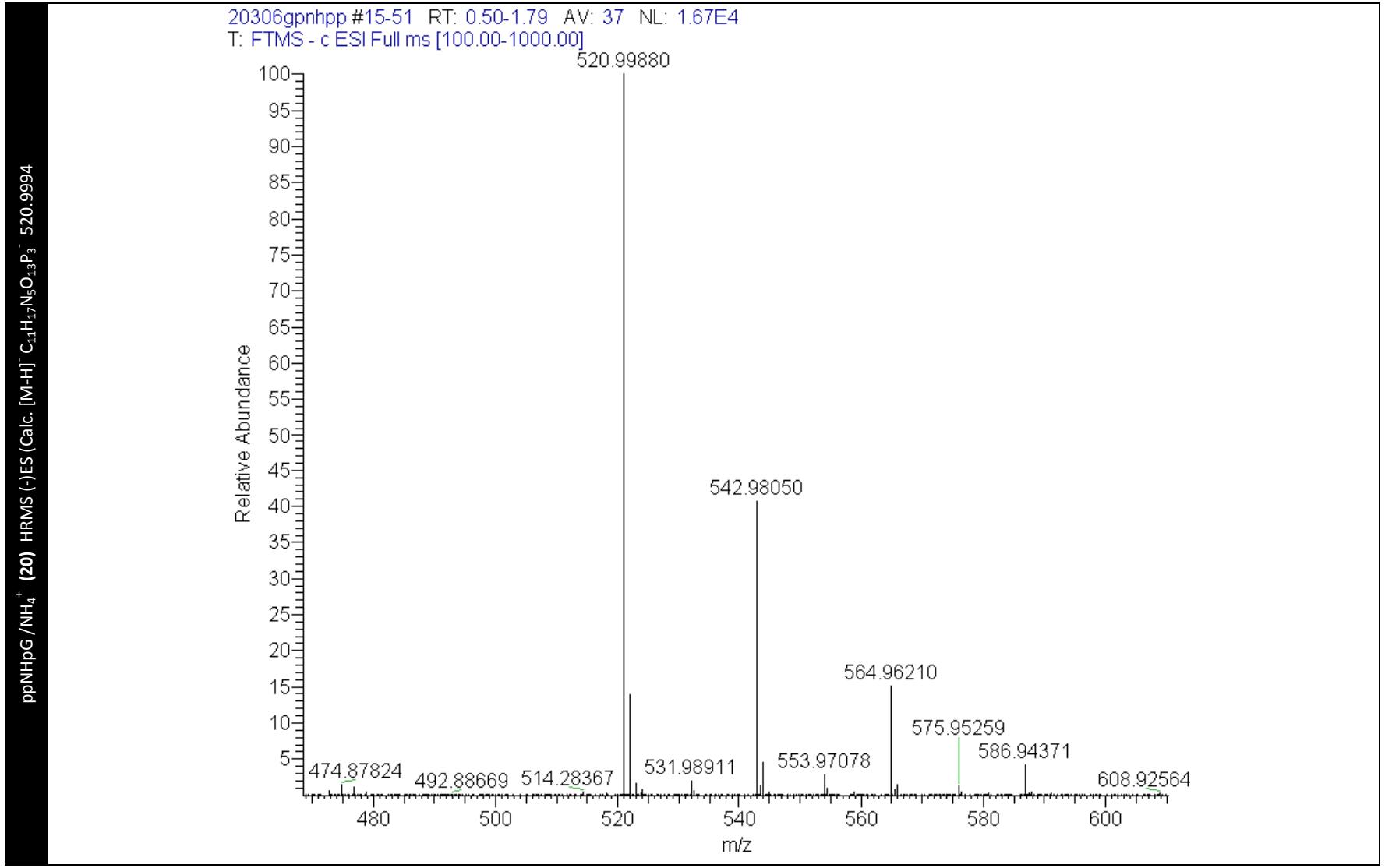

^{31}P NMR ppNHpA /NH_4^+ (19)
ApNHpp_NH4_31P_25deg.esp
Normalized Intensity
Chemical Shift (ppm)
-0.69
-9.44
-9.57
-11.08
-11.12
-11.21
-11.24
1.00
0.75
0.99

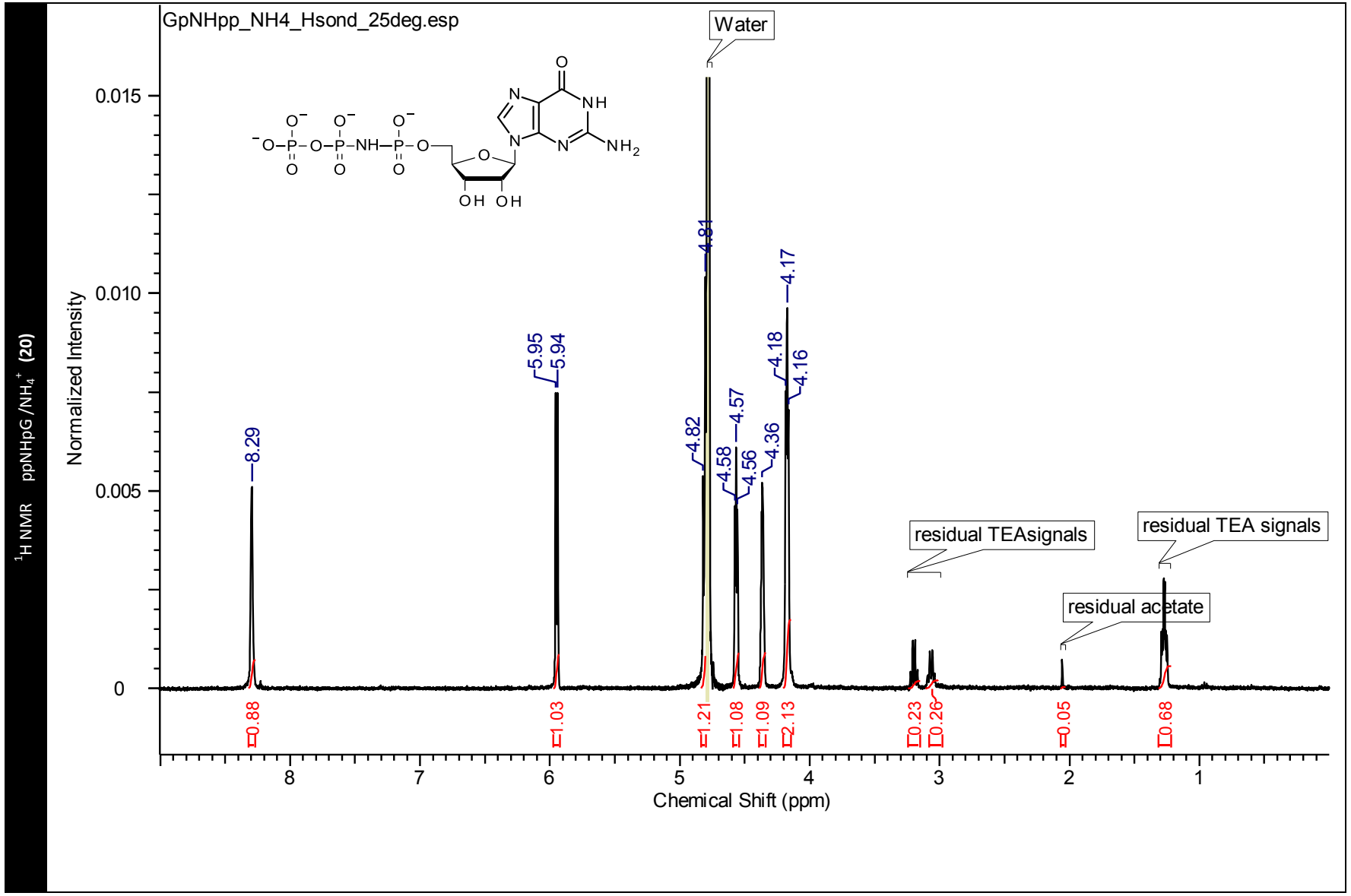
(**20**) ppNHpG /NH_4^+

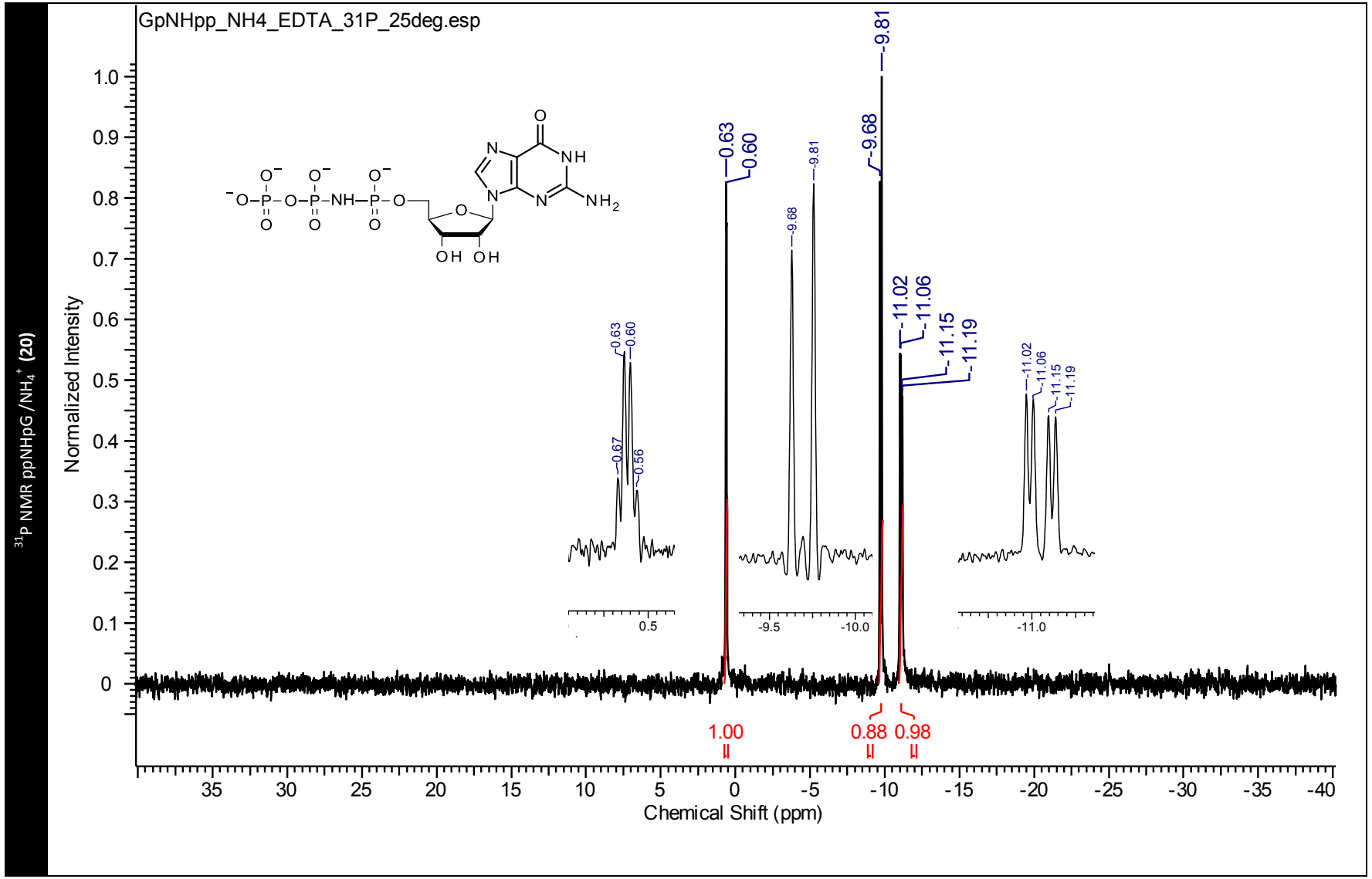


ppNHpG /NH_4^+ (**20**) HPLC

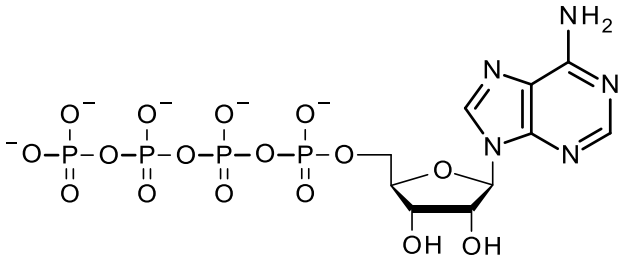



ppNHpG /NH4+ (20) HRMS (-)ES (Calc. [M-H]- C11H17N5O13P3- 520.9994
20306gpnhpp #15-51 RT: 0.50-1.79 AV: 37 NL: 1.67E4
T: FTMS - c ESI Full ms [100.00-1000.00]
Relative Abundance
m/z
520.99880
542.98050
564.96210
575.95259
586.94371
608.92564
474.87824
492.88669
514.28367
531.98911
553.97078

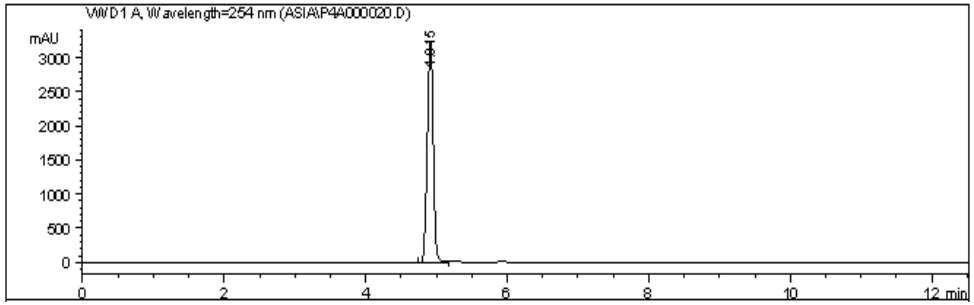

^{1}H NMR ppNHpG /NH_4^+ (20)
GpNHpp_NH4_Hsond_25deg.esp
Water
Normalized Intensity
0.015
0.010
0.005
0
8.29
5.95
5.94
4.82
4.81
4.58
4.57
4.56
4.36
4.18
4.17
4.16
residual TEAsignals
residual TEA signals
residual acetate
0.88
1.03
1.21
1.08
1.09
2.13
0.23
0.26
0.05
0.68
8
7
6
5
4
3
2
1
Chemical Shift (ppm)

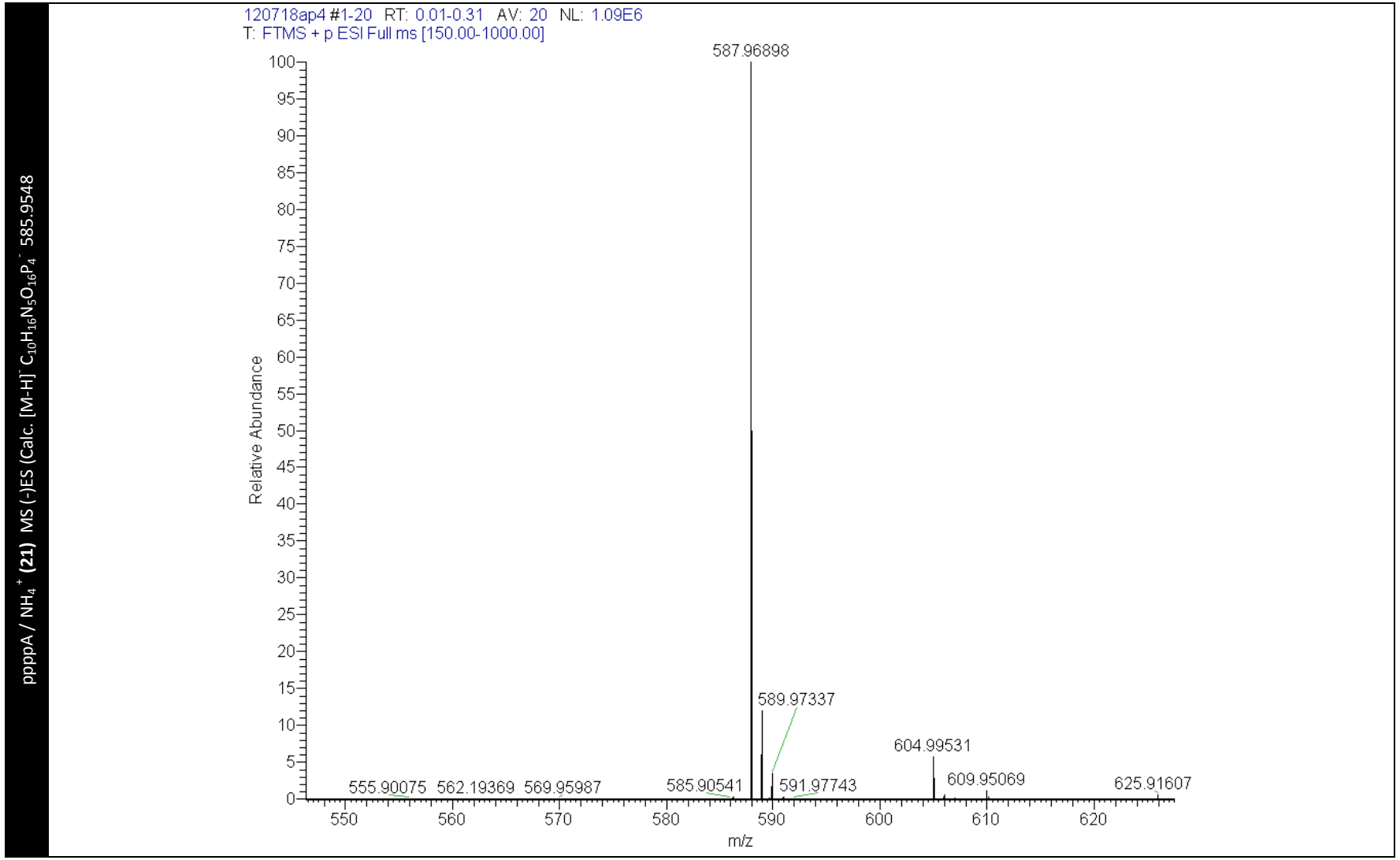

GpNHpp_NH4_EDTA_31P_25deg.esp
^{31}P NMR ppNHpG /NH_4^+ (20)
Normalized Intensity
Chemical Shift (ppm)
-0.63
-0.60
-9.81
-9.68
-11.02
-11.06
-11.15
-11.19
-0.67
-0.56
1.00
0.88
0.98

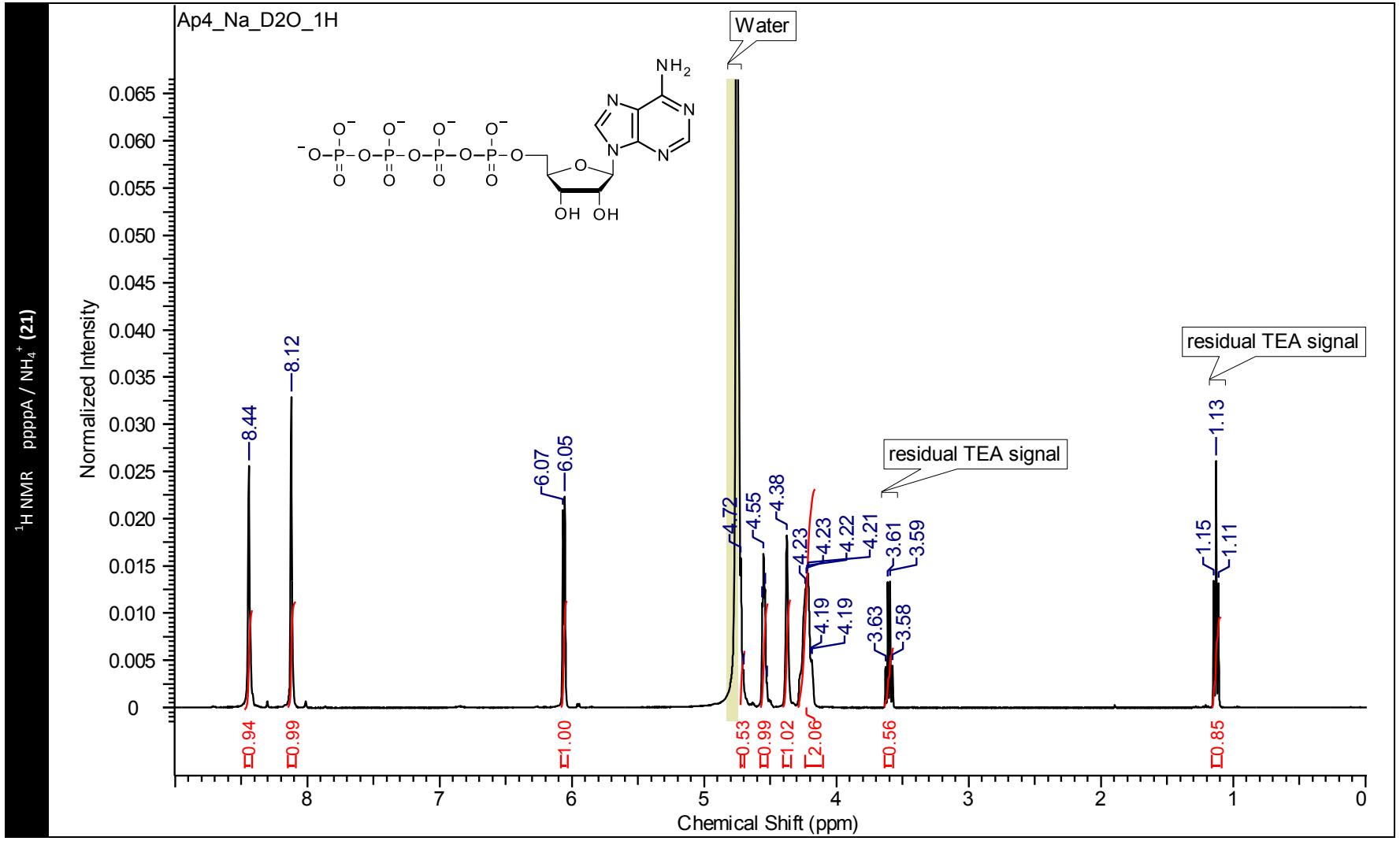
(**21**) ppppA /NH_4^+

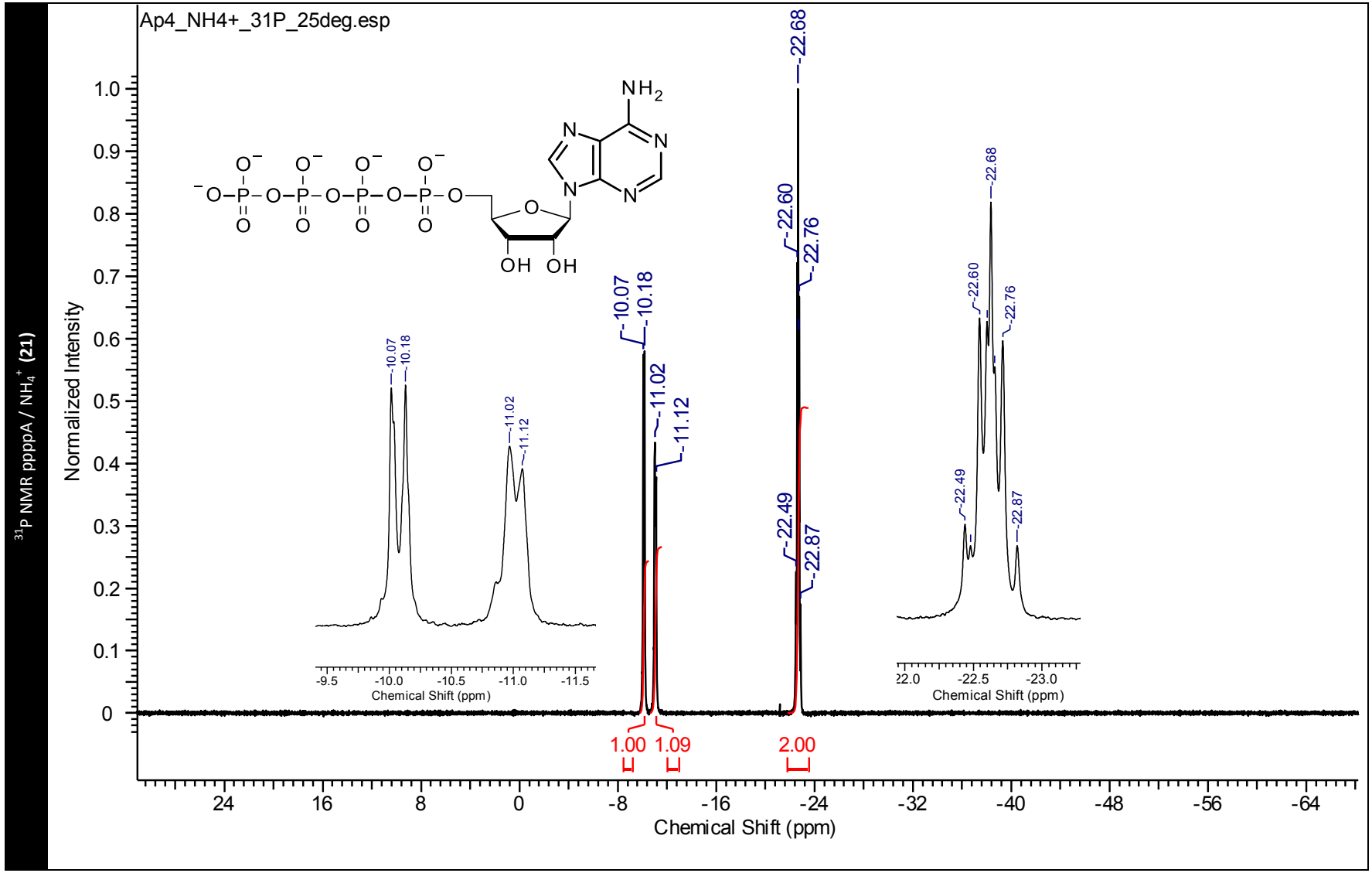


pppA) / NH_4^+ **(21)** HPLC

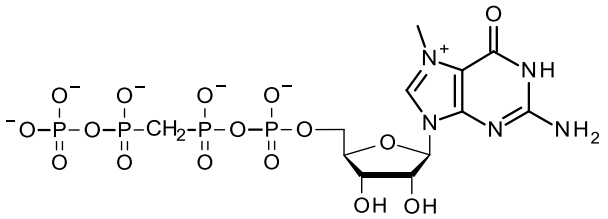



pppA / NH4+ (21) MS (-)ES (Calc. [M-H]- C10H16N5O16P4- 585.9548
120718ap4 #1-20 RT: 0.01-0.31 AV: 20 NL: 1.09E6
T: FTMS + p ESI Full ms [150.00-1000.00]
587.96898
589.97337
604.99531
555.90075
562.19369
569.95987
585.90541
591.97743
609.95069
625.91607
Relative Abundance
m/z

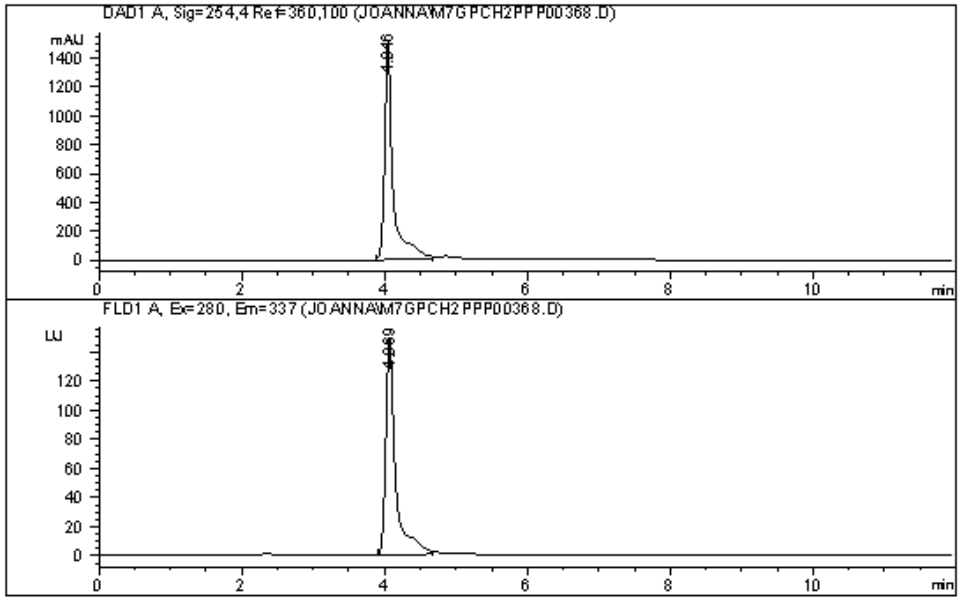

^{1}H NMR pppA / NH_4^+ (21)
Ap4_Na_D2O_1H
Water
residual TEA signal
residual TEA signal
Normalized Intensity
Chemical Shift (ppm)
8.44
8.12
6.07
6.05
4.72
4.55
4.38
4.23
4.22
4.21
4.19
4.19
3.63
3.61
3.59
3.58
1.15
1.13
1.11
0.94
0.99
1.00
0.53
0.99
1.02
2.06
0.56
0.85

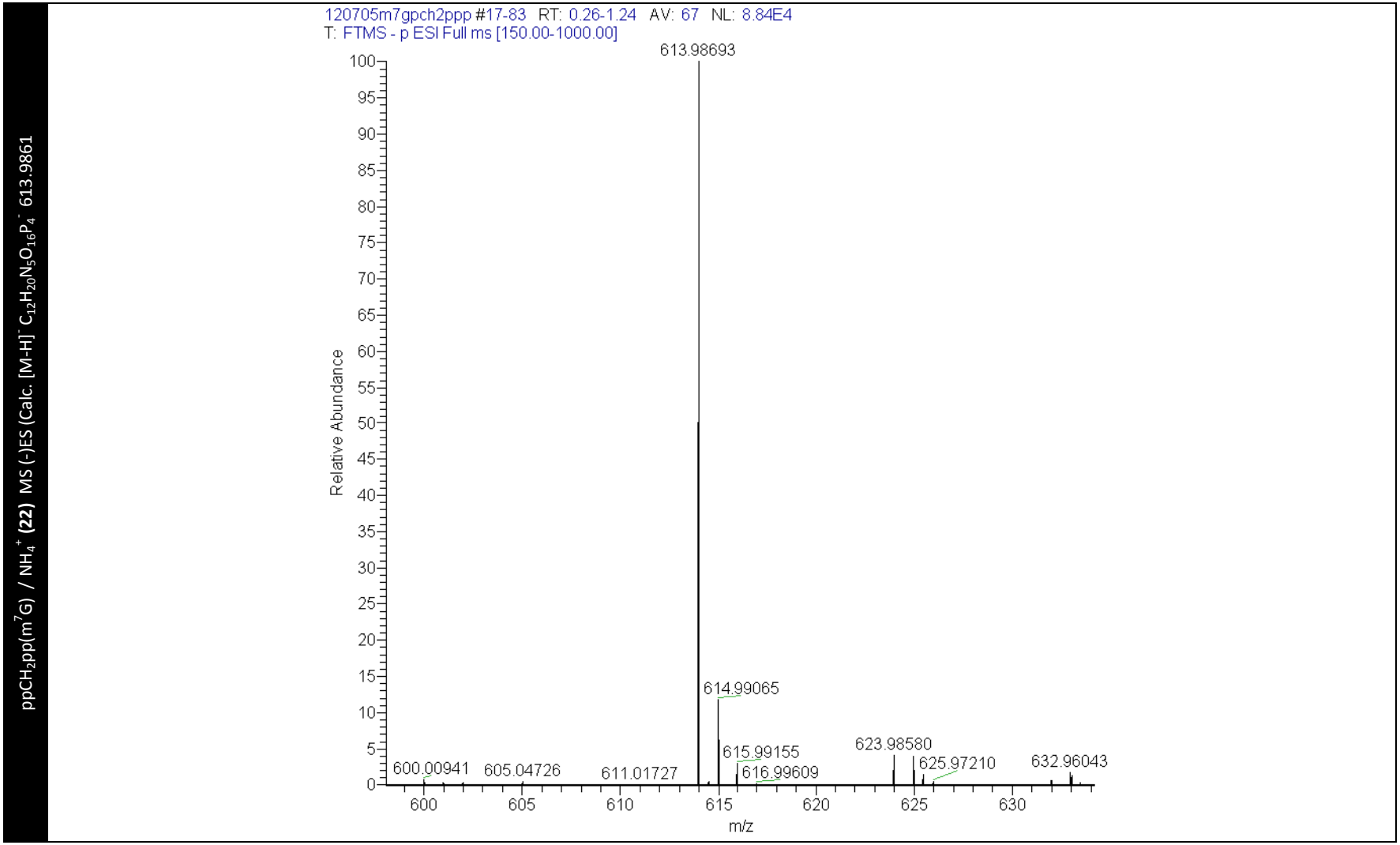

^{31}P NMR ppppA / NH_4^+ (21)
Ap4_NH4+_31P_25deg.esp
Normalized Intensity
Chemical Shift (ppm)
-10.07
-10.18
-11.02
-11.12
-22.68
-22.60
-22.76
-22.49
-22.87
1.00
1.09
2.00

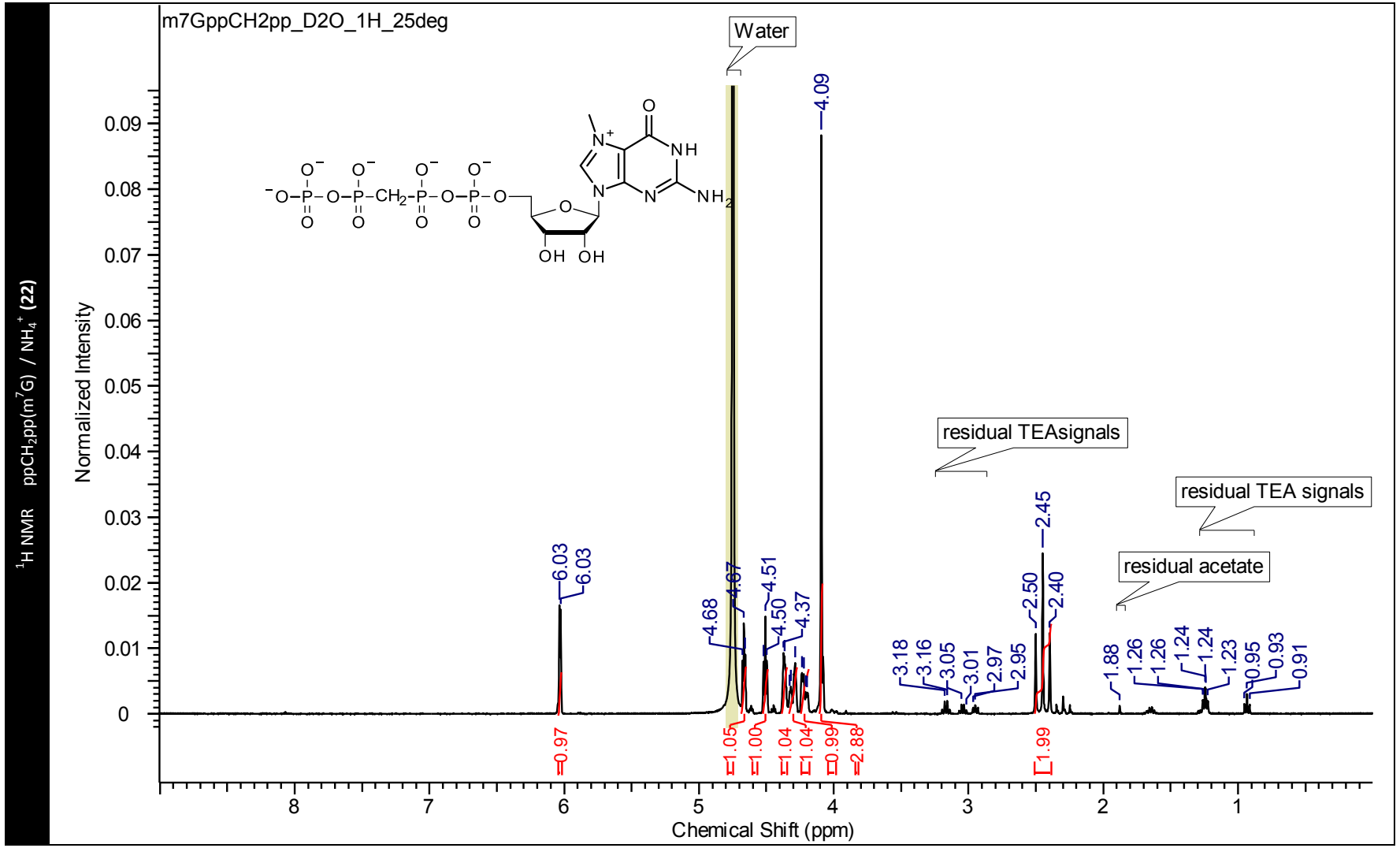
(**22**) $ppCH_2pp(m^7G) / NH_4^+$

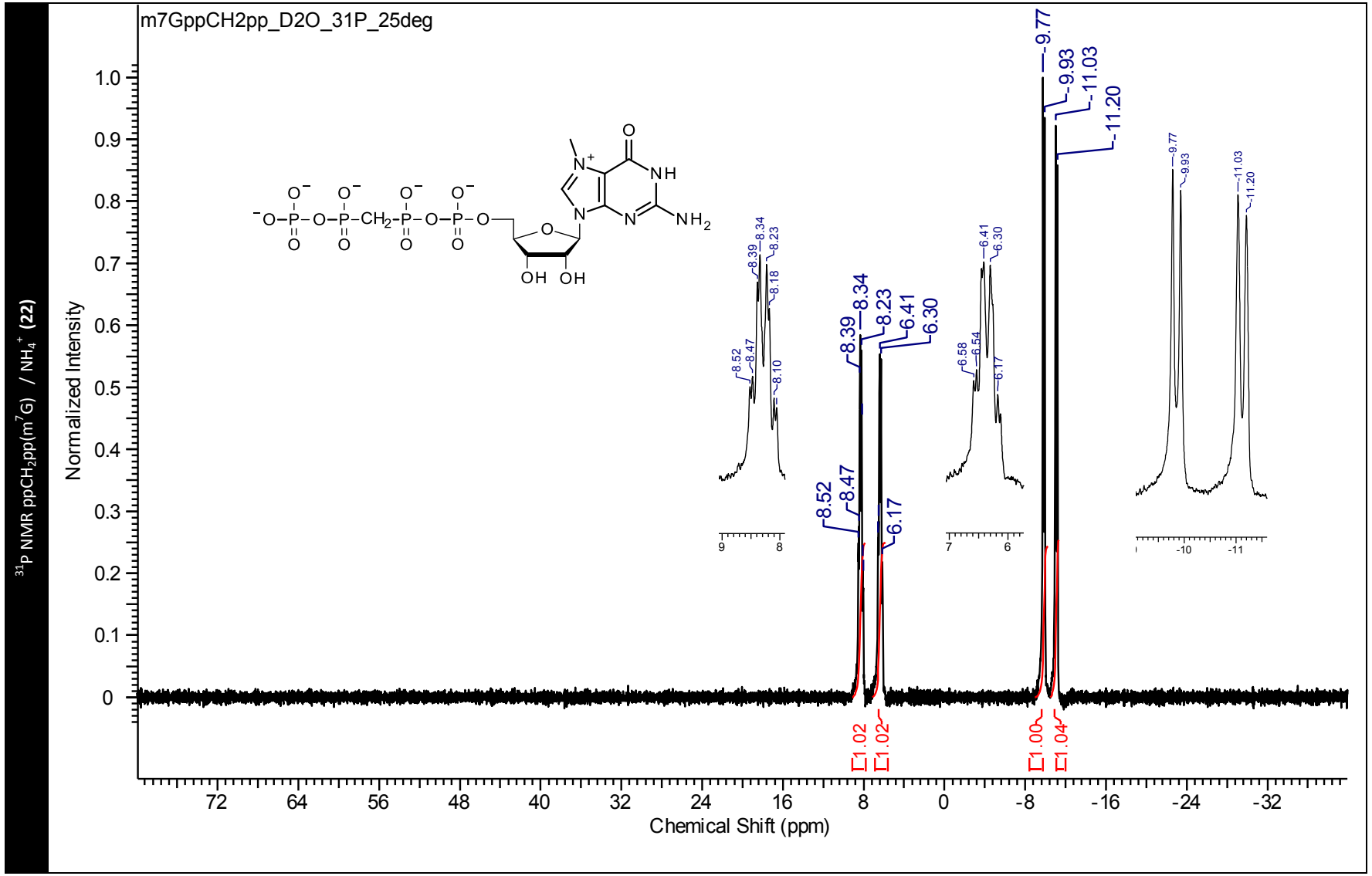


ppCH$_2$pp(m^{7}G) / NH$_4^+$ **(22)** HPLC

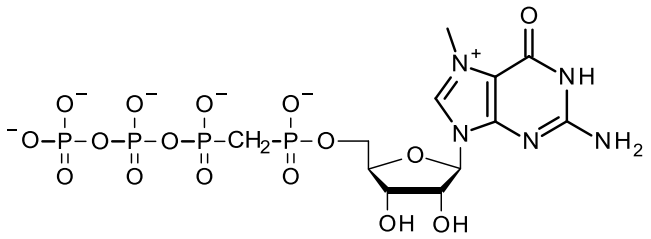



ppCH2pp(m7G) / NH4+ (22) MS (-)ES (Calc. [M-H]- C12H20N5O16P4- 613.9861
120705m7gpch2ppp #17-83 RT: 0.26-1.24 AV: 67 NL: 8.84E4
T: FTMS - p ESI Full ms [150.00-1000.00]
Relative Abundance
m/z
613.98693
614.99065
615.99155
616.99609
600.00941
605.04726
611.01727
623.98580
625.97210
632.96043

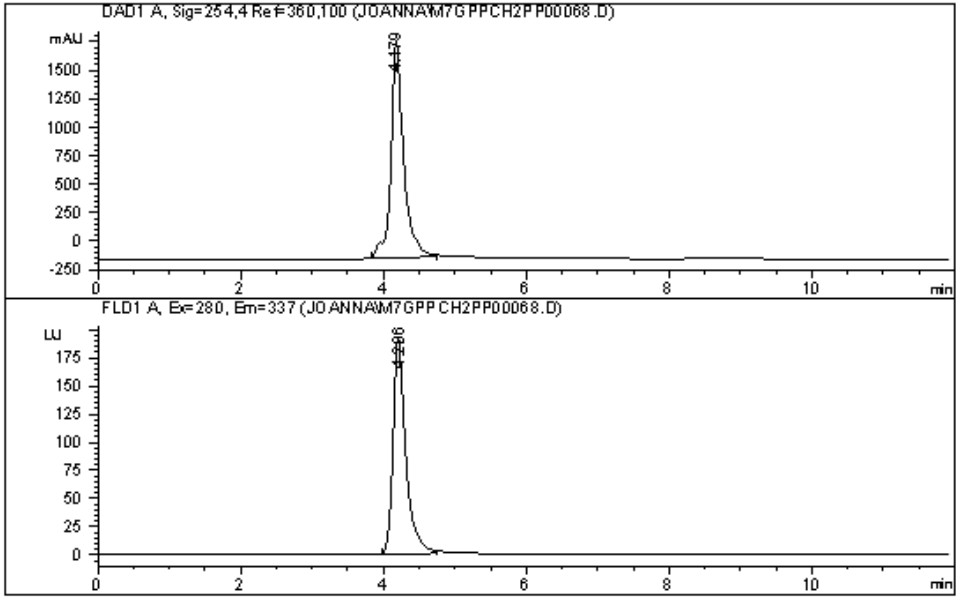

m7GppCH2pp_D2O_1H_25deg
^{1}H NMR ppCH$_2$pp(m^{7}G) / NH$_4^+$ (22)
Water
residual TEAsignals
residual TEA signals
residual acetate
Normalized Intensity
Chemical Shift (ppm)
6.03
6.03
4.68
4.67
4.51
4.50
4.37
4.09
3.18
3.16
3.05
3.01
2.97
2.95
2.50
2.45
2.40
1.88
1.26
1.26
1.24
1.24
1.23
0.95
0.93
0.91
0.97
1.05
1.00
1.04
1.04
0.99
2.88
1.99

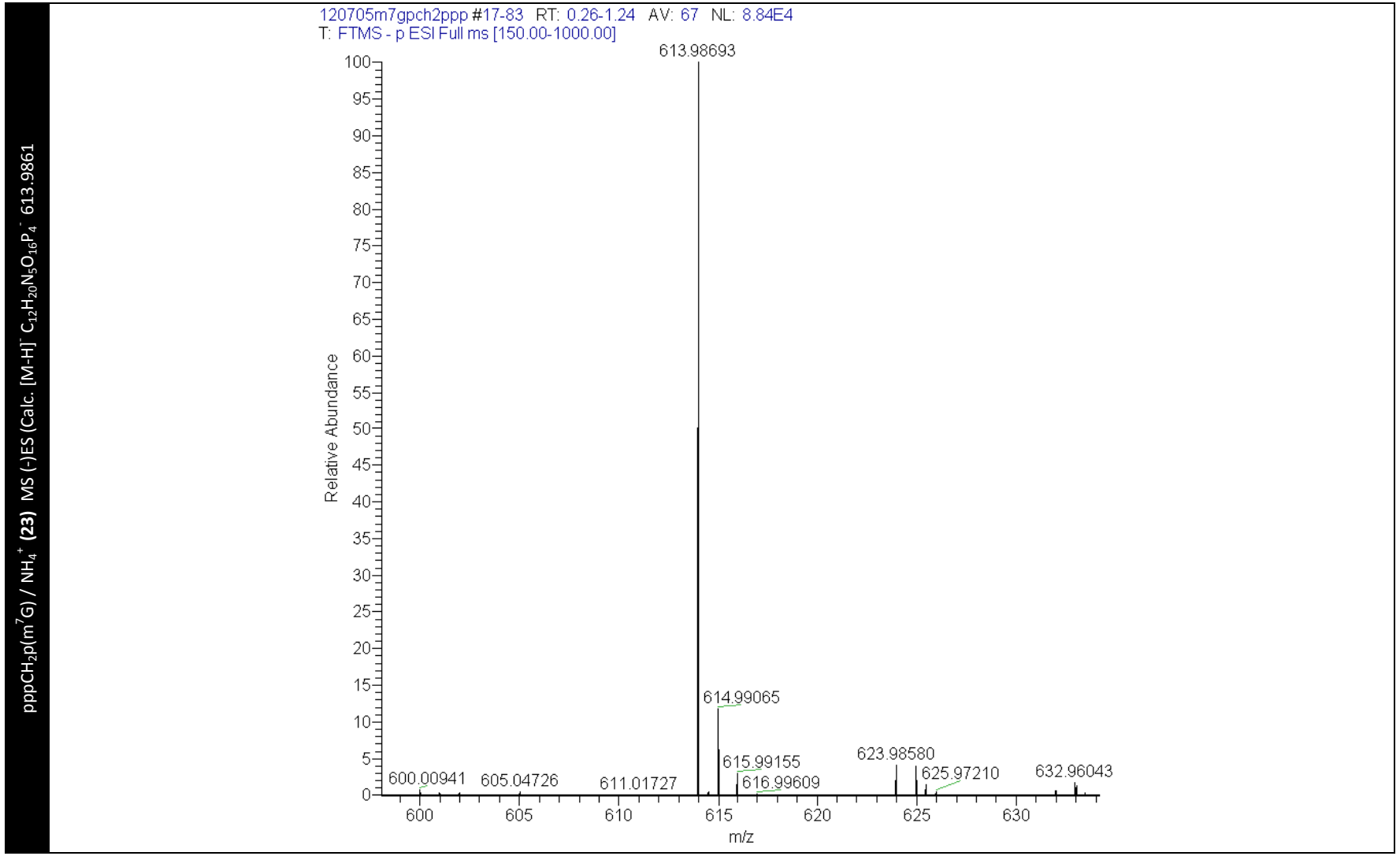

m7GppCH2pp_D2O_31P_25deg
^{31}P NMR ppCH$_2$pp(m^{7}G) / NH$_4^+$ (22)
Normalized Intensity
Chemical Shift (ppm)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
72
64
56
48
40
32
24
16
8
0
-8
-16
-24
-32
-9.77
-9.93
-11.03
-11.20
8.39
8.34
8.23
6.41
6.30
8.52
8.47
6.17
1.02
1.02
1.00
1.04
OH OH
NH
NH$_2$

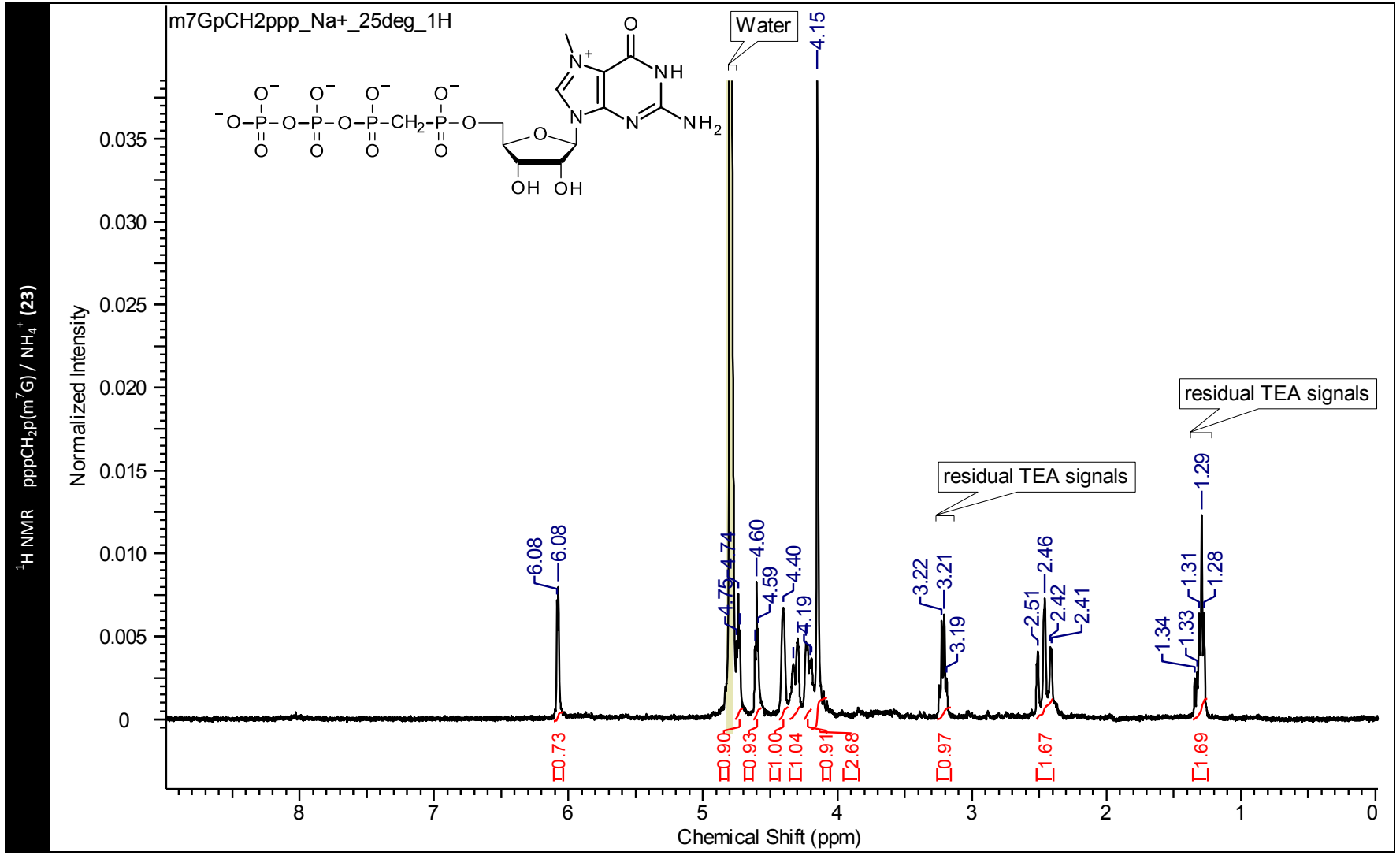
(**23**) pppCH$_2$p(m^{7}G) / NH$_4^+$

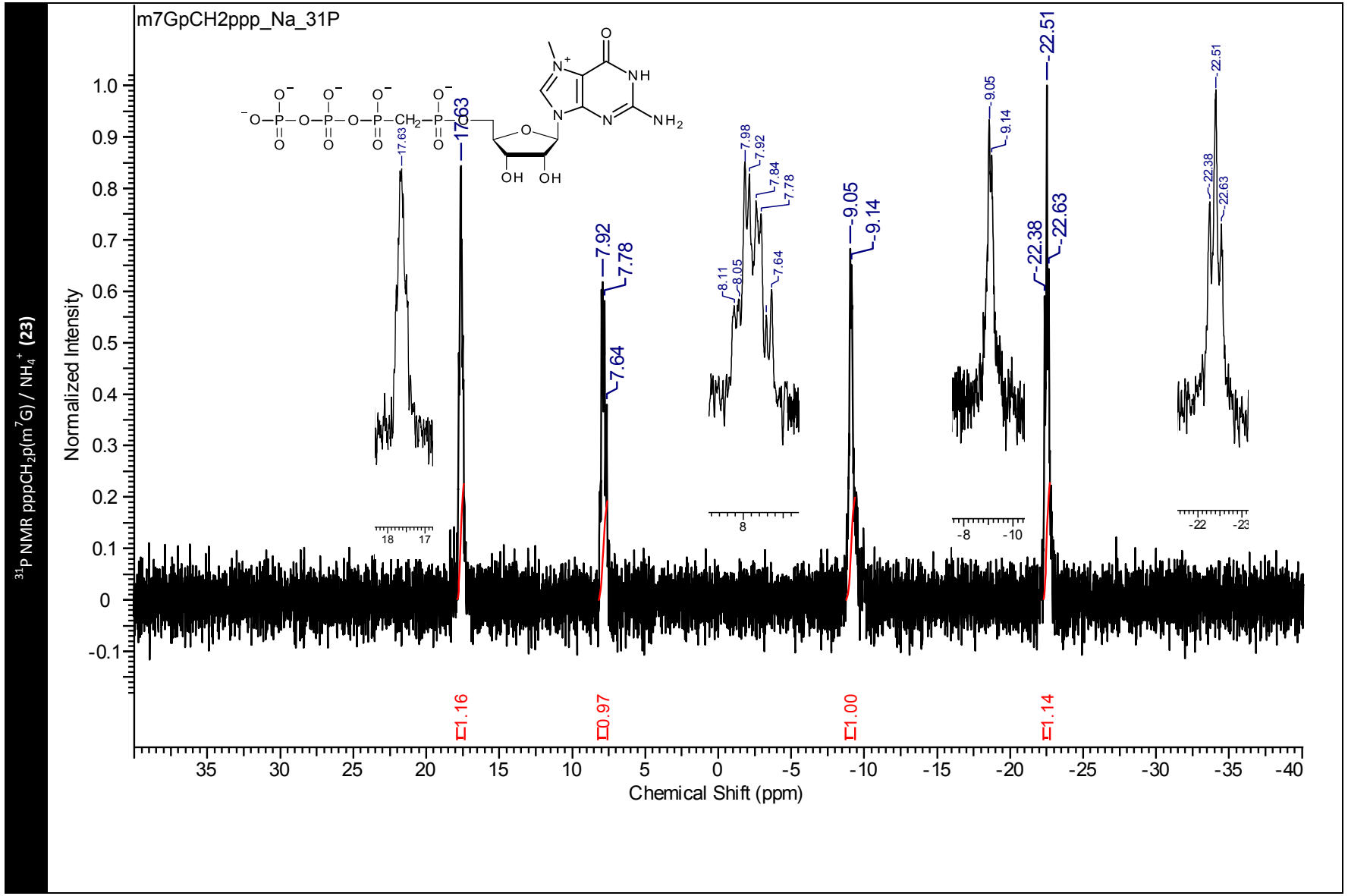


pppCH$_2$p(m^{7}G) / NH$_4^+$ **(23)** HPLC

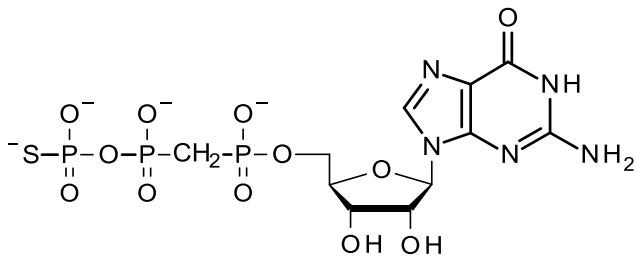



pppCH2p(m7G) / NH4+ (23) MS (-)ES (Calc. [M-H]- C12H20N5O16P4- 613.9861
120705m7gpch2ppp #17-83 RT: 0.26-1.24 AV: 67 NL: 8.84E4
T: FTMS - p ESI Full ms [150.00-1000.00]
613.98693
614.99065
615.99155
616.99609
600.00941
605.04726
611.01727
623.98580
625.97210
632.96043
Relative Abundance
m/z

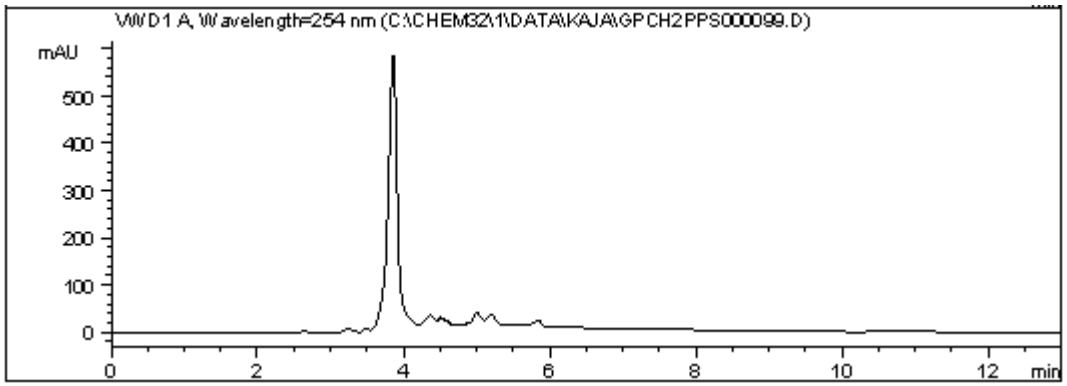

m7GpCH2ppp_Na+_25deg_1H
^{1}H NMR pppCH$_2$p(m^{7}G) / NH$_4^+$ (23)
Water
residual TEA signals
residual TEA signals
Normalized Intensity
Chemical Shift (ppm)
6.08
6.08
4.75
4.74
4.60
4.59
4.40
4.19
4.15
3.22
3.21
3.19
2.51
2.46
2.42
2.41
1.34
1.33
1.31
1.29
1.28
0.73
0.90
0.93
1.00
1.04
0.91
2.68
0.97
1.67
1.69

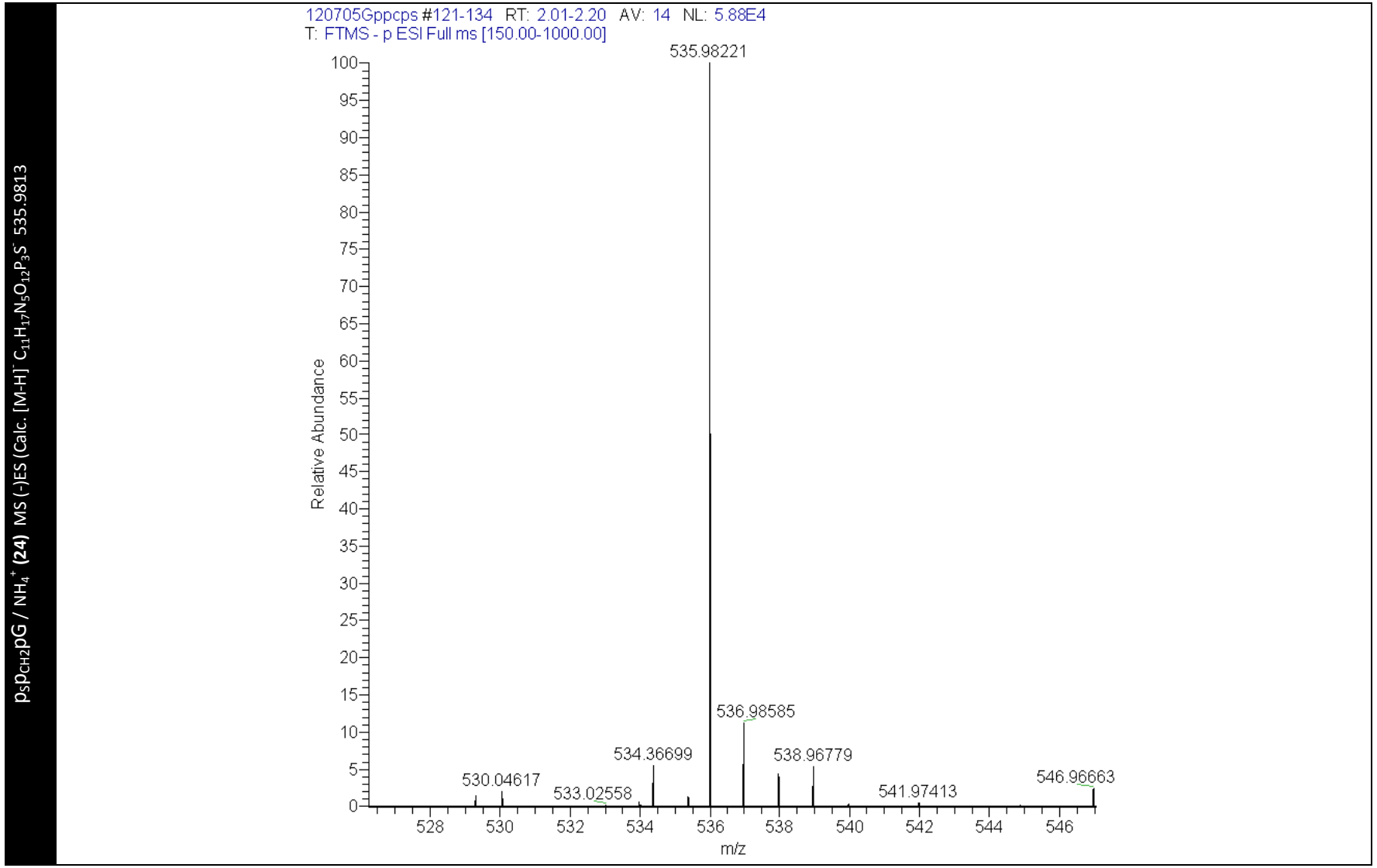

^{31}P NMR pppCH$_2$p(m^{7}G) / NH$_4^+$ (23)
m7GpCH2ppp_Na_31P
Normalized Intensity
Chemical Shift (ppm)
17.63
7.92
7.78
7.64
9.05
9.14
22.51
22.38
22.63
1.16
0.97
1.00
1.14

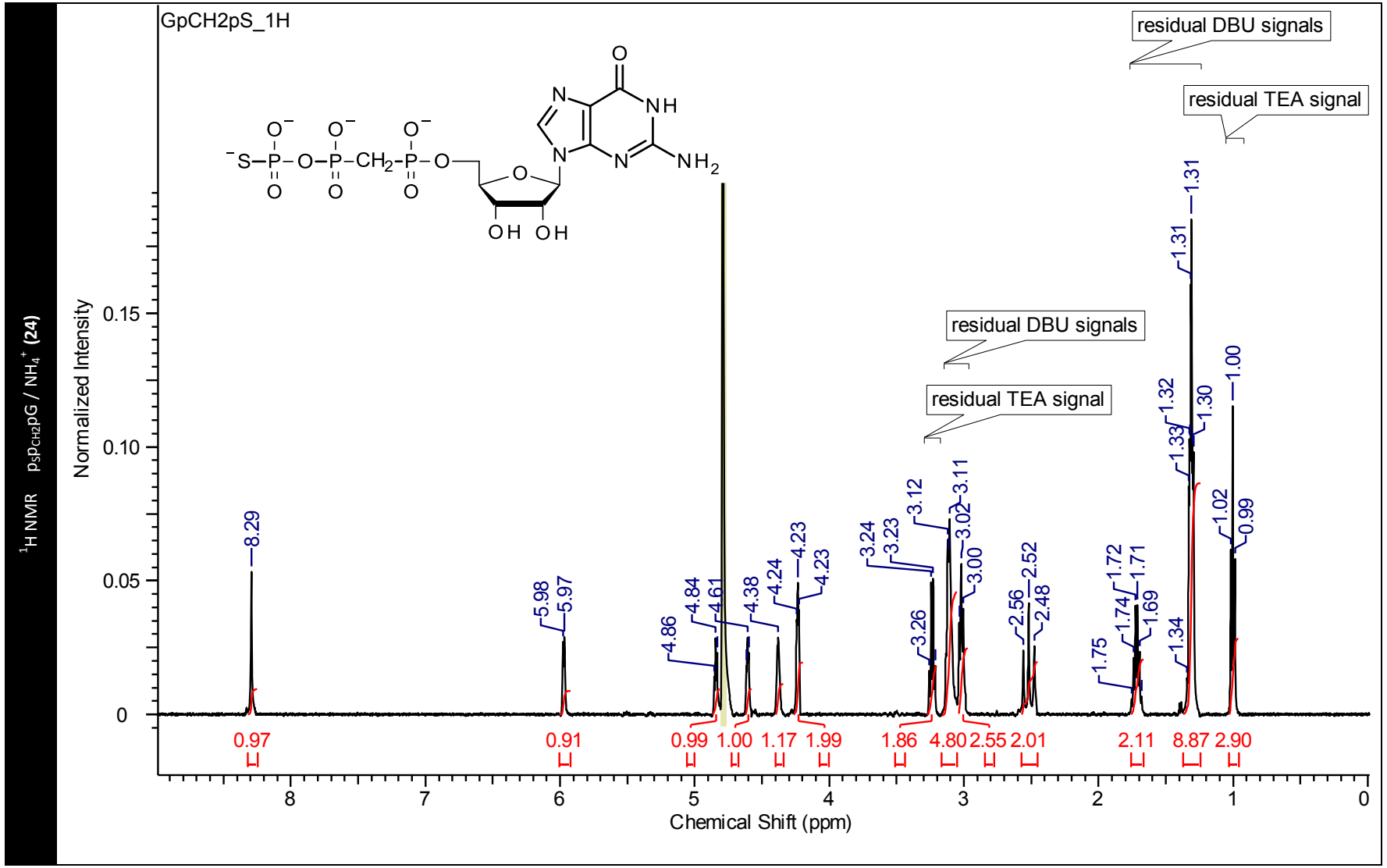
(24) $p_Sp_{CH_2}pG / NH_4^+$

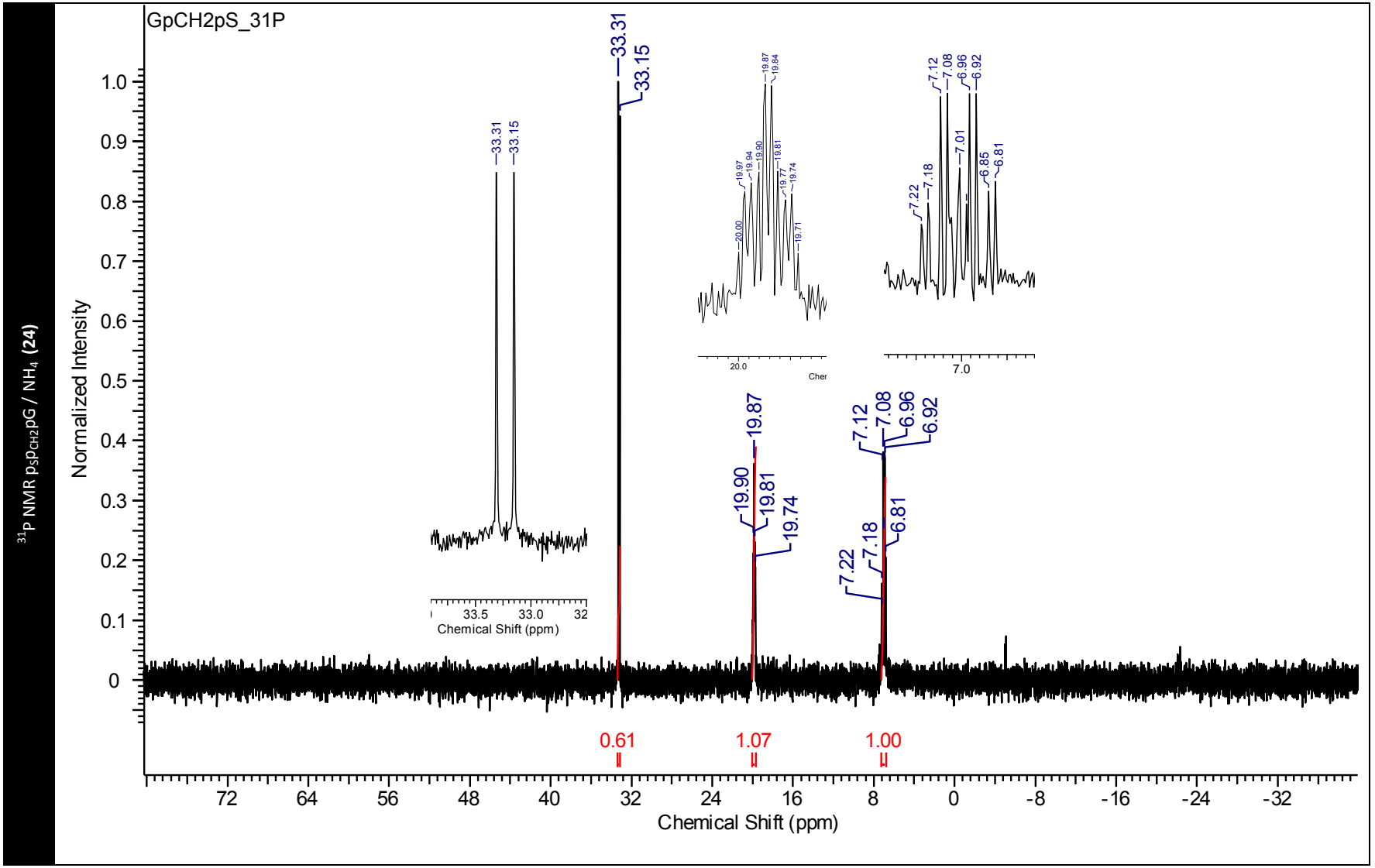


$p_Sp_{CH_2}pG / NH_4^+$ **(24)** HPLC




pSpCH2pG / NH4+ (24) MS (-)ES (Calc. [M-H]- C11H17N5O12P3S- 535.9813
120705Gppcps #121-134 RT: 2.01-2.20 AV: 14 NL: 5.88E4
T: FTMS - p ESI Full ms [150.00-1000.00]
Relative Abundance
m/z
535.98221
536.98585
534.36699
538.96779
530.04617
533.02558
541.97413
546.96663


GpCH2pS_1H
^{1}H NMR pSpCH2pG / NH4+ (24)
residual DBU signals
residual TEA signal
residual DBU signals
residual TEA signal
Normalized Intensity
Chemical Shift (ppm)
8.29
5.98
5.97
4.86
4.84
4.61
4.38
4.24
4.23
4.23
3.24
3.23
3.26
3.12
3.11
3.02
3.00
2.56
2.52
2.48
1.75
1.72
1.71
1.74
1.69
1.34
1.33
1.32
1.31
1.31
1.30
1.02
1.00
0.99
0.97
0.91
0.99
1.00
1.17
1.99
1.86
4.80
2.55
2.01
2.11
8.87
2.90


^{31}P NMR pSpCH2pG / NH4 (24)
GpCH2pS_31P
Normalized Intensity
Chemical Shift (ppm)
33.31
33.15
19.87
19.90
19.81
19.74
7.12
7.08
6.96
6.92
7.22
7.18
6.81
0.61
1.07
1.00