# Synthesis of modified nucleoside oligophosphates simplified: Fast, pure, and protecting group free

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## Abbreviation

| | | |
|---|---|---|
| TBA | : | Tetrabutylammonium |
| ETT | : | 5-(Ethylthio)-1*H*-tetrazole |
| DCI | : | Dicyanoimidazole |
| *m*CPBA | : | *meta*-Chloroperbenzoic acid |
| EtOAc | : | Ethyl acetate |
| $Et_2O$ | : | Diethyl ether |
| $Et_3N$ | : | Triethylamine |
| DIPEA | : | Diisopropylethylamine |
| MeCN | : | Acetonitrile |
| DMF | : | Dimethylformamide |
| $PP_i$ | : | Pyrophosphate |
| PCP | : | Methylene diphosphate |
| d4T | : | Stavudine |
| AZT | : | Azidothymidine |
| $NH_4HCO_3$ | : | Ammonium bicarbonate |
| $NaClO_4$ | : | Sodium perchlorate |
| TEAA | : | Triethylammonium Acetate |

## General remarks

**Reagents** were purchased from commercial suppliers (Acros, Sigma Aldrich, Fluka, TCI, Chem Genes Corp.) and used without further purification, unless noted otherwise.

**Starting precursors** were obtained from Sigma Aldrich, Fluka, or TCI as sodium salts and converted into their tetrabutylammonium (TBA) salts by ion exchange on Dowex 50WX8 ($H^+$) followed by neutralization with TBA hydroxide and subsequent lyophilization. The accurate amounts of TBA counterions in the obtained salts were determined by $^{1}$H-NMR and $^{31}$P-NMR by the use of tetramethylphosphonium bromide as internal standard. Furthermore, all the polyPs for e.g. pyrophosphate, methylenediphosphate, difluoromethylenediphosphate, dichloromethylenediphosphate and imidodiphosphate were stored over molecular sieves (3Å) in anhydrous DMF or MeCN.

**Adenosine triphosphate** and fluoromonophosphate was obtained from Sigma Aldrich stored over molecular sieves (4Å) in DMF.

**Solvents** were obtained in analytical grade and used as received.

**Dry solvents** were purchased in analytical grade and used without further purification.

Oxidation and hydrolysis sensitive reactions were performed with dry solvents which were stored under an argon (Ar) atmosphere. Diethyl ether ($Et_2O$), dichloromethane (DCM) and tetrahydrofuran (THF) were purified using *Braun Solvent Purification System 800* and stored under Ar atmosphere.

Acetonitrile (MeCN) was purchased from Acros (*Acetonitril, 99.9%, Extra dry over Molecular Sieve, AcroSeal$^{TM}$*) and stored over molecular sieves (3 Å) under Ar atmosphere.

*N,N*-Dimethylformamide (DMF) was purchased from Sigma-Aldrich (*N,N-Dimethylformamide, anhydrous, 99.8%*) and it was further dried by storing over activated molecular sieve (3Å) under Ar atmosphere.

*N,N*-Diisopropylethylamine (DIPEA) and triethylamine ($Et_3N$) was distilled under Ar atmosphere and was stored over activated molecular sieve (3Å) and Ar atmosphere.

**Deuterated solvents** for NMR and reactions were obtained from Aldrich and deutero Germany, in the indicated purity grade and used as received for NMR spectroscopy.

**Reactions** were carried out under Ar or $N_2$ atmosphere, the gasses were pass through drying column ($P_2O_5$) and NMR tubes were preheated prior to use and samples were prepared under inert atmosphere.

**Anion exchange chromatography** was performed using Q Sepharose® Fast Flow or DEAE Sepharose® Fast Flow (GE Healthcare). Crude products were loaded with water and were eluted using ammonium bicarbonate buffer by stepwise increase of the buffer concentration. Fractions were lyophilised and analyzed by $^{31}$P NMR.

**Medium pressure liquid chromatography** (MPLC) was performed using PuriFlash® from interchim®.

**Lyophilizations** were done with Alpha 1-4 LD plus Freeze Dryer from Christ.

**$^{1}$H-NMR spectra** were recorded on Bruker 300 MHz, Bruker 400 MHz and Bruker 500 MHz spectrometers in the indicated deuterated solvent. Data are reported as follows: chemical shift (δ, ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; $m_c$, centered multiplet; br, broad signal), coupling constant(s) (J, Hz), integration. All signals were referenced to the internal solvent signal as standard ($CDCl_3$, δ 7.26; $D_2O$, δ 4.79; $CD_3OD$, δ 3.31; DMSO-$d_6$, δ 2.50).

**$^{13}$C{$^{1}$H}-NMR** spectra were recorded on Bruker 101 MHz (with cryoprobe) and Bruker 126 MHz (without cryoprobe) spectrometers at 298 K in the indicated deuterated solvent.

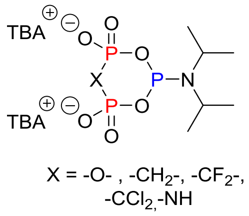
**$^{31}$P{$^{1}$H}-NMR** spectra and **$^{31}$P-NMR spectra** were recorded on Bruker 162 MHz (equipped with a cryo platform) or 202 MHz spectrometers at 298 K in the indicated deuterated solvent. All signals were referenced to an internal standard phosphate (PPP).

**$^{19}$F-NMR** spectra were recorded on Bruker 377 MHz (equipped with a cryo platform) at 298K in the indicated deuterated solvent.

**Mass Spectrometer** were recorded by Analytical department of the institute of Organic Chemistry at ALU-Freiburg using a Thermo LCQ Advantage [spray voltage: 2.5–4.0 kV, spray current: 5 μA; ion transfer tube: 250 (150) °C, Vapourizer temperature: 50–400 °C] and Exactive with Orbitrap-Analysator, Thermo Scientific used for HRMS.

# 1 Synthesis of P-amidite derivative (*c*-PyPA)

## 1.1 General procedure for preparation of *c*-PyPA

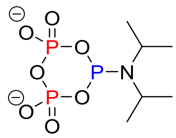



**(A)**

$PxP_i$ × 1.6 - 2.2 TBA (1.0 eq.) was coevaporated with dry MeCN (3 × 4 ml), then dissolved in appropriate amount of dry MeCN or in dry DMF under an Ar atmosphere. Distilled $Et_3N$ or DIPEA (2.0 - 3.0 eq.) stored over molecular sieves (3Å) was added and the mixture was cooled to -4ºC. Afterwards, distilled $(iPr)_2N\text{-}PCl_2$ (1.0 eq.) was added dropwise into the reaction mixture, which was then stirred at -4ºC to -10ºC for 5 - 10 mins and the reaction was followed by $^{31}$P-NMR. The reaction mixture was stored at -20ºC under Ar atmosphere for up to four weeks and added directly for coupling reactions without further manipulations.

**Note-:** Additionally, activated molecular sieves (3Å) could be added after the coevaporation step in order to further prevent hydrolysis within the formation of c-PyPA.

### 1.1.1 Cyclic pyrophosphoryl-P-amidite (*c*-PyPA, -O-, $A_1$)

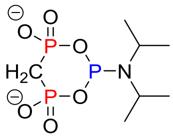



**($A_1$)**

$PP_i$ × 2.0 TBA (2.09 g, 3.02 mmol, 1.0 eq.) was coevaporated with dry MeCN (2 × 20 ml) and then dissolved in dry MeCN (40 ml) under an Ar atmosphere. Additionally, activated molecular sieves (3Å) was added in the above flask. $Et_3N$ (1.50 ml, 10.5 mmol, 3.5 eq.) was added and the mixture was cooled to -10ºC. Previously distilled $(iPr)_2N\text{-}PCl_2$ (520 µl, 3.02 mmol, 1.0 eq.) was then slowly added into the mixture and it was stirred at -10ºC. After 5 mins of stirring, $^{31}$P-NMR of an aliquot of the reaction mixture in dry $CDCl_3$ confirmed full conversion. The reaction mixture was stored at -20ºC under Ar atmosphere for up to four weeks and added directly without further manipulations for coupling reactions. The analytical data is consistent with the literature.[1]

**$^{31}$P{$^1$H} NMR** (162 MHz, aliquot of the reaction mixture in dry $CDCl_3$, δ/ppm): 130.9 (t, J = 22.8 Hz, 1P), -17.4 (d, J = 22.8 Hz, 2P). **$^{31}$P NMR** (162 MHz, aliquot of the reaction mixture in dry $CDCl_3$, δ/ppm): 130.9 (tt, J = 21.9, 11.6 Hz, 1P), -17.4 (d, J = 22.7 Hz, 2P).

### 1.1.2 Cyclic methylenediphosphoryl-P-amidite (*c*-Py$_{CH2}$PA, A$_2$)




2 TBA

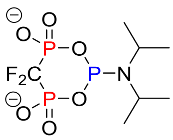
**(A$_2$)**

Methylenediphosphonic acid × 1.9 TBA (1.00 g, 1.57 mmol, 1.0 eq.) was coevaporated with dry MeCN (4 × 2 ml), then dissolved in dry MeCN (20 ml) under an Ar atmosphere. Additionally, activated molecular sieves (3Å) was added in the above flask. $Et_3N$ (670 µl, 4.71 mmol, 3.0 eq.) was added and the mixture was cooled to -4°C. Previously distilled (*i*Pr)$_2$N-$PCl_2$ (250 µl, 1.57 mmol, 1.0 eq.) was then slowly added into the mixture and it was stirred at -4°C. After 5 mins of stirring, $^{31}$P-NMR of an aliquot of the reaction mixture in dry $CDCl_3$ confirmed full conversion. The reaction mixture was stored at -20°C under an Ar atmosphere for up to two weeks and added directly without further manipulations for coupling reactions.

**$^{31}$P{$^1$H} NMR** (122 MHz, aliquot of the reaction mixture in dry $CDCl_3$, δ/ppm): 125.7 (t, J = 9.0 Hz, 1P), 6.25 (d, J = 9.0 Hz, 2P).**$^{31}$P NMR** (162 MHz, aliquot of the reaction mixture in dry $CDCl_3$, δ/ppm): 125.7 (p, J = 10.1 Hz, 1P), 6.25 (td, J = 18.6, 8.7 Hz, 2P). **$^1$H NMR** (400 MHz, aliquot of the reaction mixture in dry $CDCl_3$, δ/ppm contains1.9 TBA salt): 3.25 – 3.18 (m, 2H), 2.92 – 2.40 (m, 16H), 1.16 (tt, J = 8.3, 5.9 Hz, 16H), 0.93 (h, J = 7.3 Hz, 16H), 0.79 – 0.65 (m, 14H), 0.56 (t, J = 7.3 Hz, 24H).

**HRMS** (ESI) m/z for $[C_7H_{17}NO_6P_3]^-$: calcd 304.0274, found 304.0273.

### 1.1.3 Cyclic difluoromethylenediphosphoryl-P-amidite (*c*-Py$_{CF2}$PA, A$_3$)




2 TBA

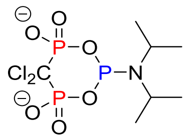
**(A$_3$)**

Difluoromethylenediphosphonic acid × 1.95 TBA (500 mg, 0.73 mmol, 1.0 eq.) was coevaporated with dry MeCN (2 × 2 mL), then dissolved in dry MeCN (10 ml) under an Ar atmosphere. Additionally, activated molecular sieves (3Å) was added in the flask. $Et_3N$ (310 µl, 2.20 mmol, 3.0 eq.) was added and the mixture was cooled to -4°C. Previously, distilled (*i*Pr)$_2$N-$PCl_2$ (135 µl, 0.730 mmol, 1.0 eq.) was then slowly added into the mixture and it was stirred at -4°C. After 5 mins of stirring, $^{31}$P-NMR of an aliquot of the reaction mixture in dry $CD_3CN$ confirmed the reaction was completed by total consumption of starting material, but due to the instability of reagent, NMR was variable. The reaction mixture was stored at -20ºC under Ar atmosphere for up to three weeks and added directly without further manipulations for coupling reactions.

**$^{31}P\{^1H\}$ NMR** (162 MHz, aliquot of the reaction mixture in dry $CD_3CN$, δ/ppm): 128.8 – 126.8 (m, 1P), -5.48 (ddd, J = 82.3, 77.6, 13.0 Hz, 2P). **$^{31}P$ NMR** (162 MHz, aliquot of the reaction mixture in dry $CD_3CN$, δ/ppm): 130.1 – 125.7 (m, 1P), -5.49 (ddd, J = 82.2, 77.4, 13.0 Hz, 2P).**$^{31}P\{^{19}F\}$ NMR** (162 MHz, aliquot of the reaction mixture in dry $CD_3CN$, δ/ppm): 127.7 (m, 1P), -5.50 (d, J = 13.0 Hz, 2P).

**HRMS** (ESI) m/z for $[C_7H_{15}F_2NO_6P_3]^-$ : calcd 340.0086, found 340.0082.

### 1.1.4 Cyclic dichloromethylenediphosphoryl-P-amidite (*c*-Py$_{CCl2}$PA, A$_4$)




2 TBA

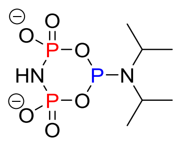
**(A$_4$)**

Dichloromethylenediphosphonic acid × 2.0 TBA (1.00 g, 1.38 mmol, 1.0 eq.) was coevaporated with dry MeCN (5 × 2 ml), then dissolved in dry MeCN (20 ml) under Ar atmosphere. Additionally, activated molecular sieves (3Å) was added in the flask. $Et_3N$ (580 µl, 4.13 mmol, 3.0 eq.) was added and the mixture was cooled to -4°C. Previously distilled (*i*Pr)$_2$N-$PCl_2$ (250 µl, 1.38 mmol, 1.0 eq.) was then slowly added into the mixture and it was stirred at -4°C. After 5 mins of stirring, $^{31}$P-NMR of an aliquot of the reaction mixture in dry $CDCl_3$ confirmed full conversion. The reaction mixture was stored at -20ºC under Ar atmosphere for up to three weeks and added directly without further manipulations for coupling reactions.

**$^{31}P\{^1H\}$ NMR** (122 MHz, aliquot of the reaction mixture in dry $CD_3CN$, δ/ppm): 129.0 (t, J = 9.0 Hz, 1P), -1.33 (d, J = 9.5 Hz, 2P).**$^{31}P$ NMR** (162 MHz, aliquot of the reaction mixture in dry $CD_3CN$, δ/ppm): 131.2 – 126.6 (m, 1P), -1.33 (d, J = 9.2 Hz, 2P).

**HRMS** (ESI) m/z for $[C_7H_{15}Cl_2NO_6P_3]^-$ : calcd 371.9495, found 371.9491.

### 1.1.5 Imidodiphosphoryl-P-amidite (*c*-Py$_{NH}$PA, A$_5$)




2 TBA

**(A$_5$)**

Imidodiphosphonic acid × 2.0 TBA (1.00 g, 1.52 mmol, 1.0 eq.) was coevaporated with dry MeCN (5 × 2 mL), then dissolved in dry MeCN (20 ml) under an Ar atmosphere. Additionally, activated molecular sieves (3Å) was added in the flask. $Et_3N$ (650 µl, 1.52 mmol, 3.0 eq.) was added and the mixture was cooled to -4°C. Previously distilled ($i$Pr)$_2$N-PCl$_2$ (270 μl, 1.52 mmol, 1.0 eq.) was then slowly added into the mixture and it was stirred at -4°C. After 5 mins of stirring, $^{31}$P-NMR of an aliquot of the reaction mixture in dry $CDCl_3$ confirmed total consumption of the starting material, but due to the instability of the product, $^{31}$P-NMR was variable. Nevertheless, a triplet at +128 ppm confirmed the formation of P-amidite (**A$_5$**). The reaction mixture was stored at -20ºC under Ar atmosphere and added directly without further manipulations for coupling reactions.

**$^{31}$P{$^1$H} NMR** (122 MHz, aliquot of the reaction mixture in dry $CD_3CN$, δ/ppm): 128.7 (t, J = 9.0 Hz, 1P).

**HRMS** (ESI) m/z for $[C_6H_{16}N_2O_6P_3]^-$ : calcd 305.0227, found 305.0224.

**Note -**: The preparation method of the imidodiphosphonic acid was another reason for the lower purity of this P-amidite (A$_5$) since the imidodiphosphosphate is acid labile. It was partially decomposed while passing through Dowex 50WX8 ($H^+$). The column was performed according to the reported literature.[2] However, it was employed without any further manipulation proceeded for coupling reaction.

# 2 Synthesis of triphosphates

## 2.1 General procedure

**Procedure 1**

**Note -**: The coevaporation worked better in Oven-dried pear shaped flask.

Alcohol (1.2 - 1.5 eq.) and ETT (3.0 - 5.0 eq.) were coevaporated together in an oven-dried pear shaped flask with dry MeCN (2 × 2 ml). Under an Ar atmosphere, a reaction mixture containing *c*-PyPA (**A**) (0.075 M, 1.0 eq. in MeCN) was added to the dried solids. The mixture was stirred at r.t. for 5-10 mins and the reaction was followed by $^{31}$P NMR (complete conversion of the reaction was monitored by $^{31}$P NMR, shifting a triplet from +130 ppm to +100 ppm). Afterwards; the reaction mixture was cooled to 0°C or - 4°C, *m*CPBA (1.5 - 2.0 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P NMR confirmed complete oxidation (a formation of triplet at -22 ppm which is diagnostic signal for the cyclotriphosphate **B**). The cyclic-intermediates were ring-opened by adding the reaction mixture to nucleophiles stirred well for 5 mins at r.t. (in solution) and then linearized products were isolated and purified by methods as given in purification section respectively.

**Procedure 2**

Nucleoside (1.2 - 1.5 eq.) and DCI (3.0 - 5.0 eq.) were coevaporated together in an oven-dried pear shaped flask with dry MeCN (2 × 2 ml). Under an Ar atmosphere, both solids were dissolved in dry DMF (1 mL) and a reaction mixture containing *c*-PyPA (**A**) (0.075 M or 0.5 M, 1.0 eq. in MeCN or DMF) was added. The mixture was stirred at r.t. for 5-10 mins and the reaction was followed by $^{31}$P NMR (complete conversion of the reaction was monitored by $^{31}$P-NMR, shifting a triplet from +130 ppm to +100 ppm). Upon cooling to 0°C or -4°C, *m*CPBA (1.5 - 2.0 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P NMR confirmed complete oxidation (a formation of triplet at -22 ppm which is diagnostic signal for the cyclotriphosphate **B**). The cyclic-intermediates were ring-opened by adding the reaction mixture to nucleophiles stirred well for 5 mins at r.t. (in solution) and the linearized products were isolated and purified.

## 2.2 Purification

**Method A -:** The product was precipitated from the reaction mixture by $NaClO_4$ sol. (-20°C, 0.5 M in acetone), it was stand at -4°C for 10 - 20 mins and the resulting precipitate was collected *via* centrifugation. The organic layer was discarded, the precipitate was thoroughly washed twice with acetone and dried under vacuum. The organic layer was stored at r.t. and the residue was again precipitated by the addition of cold $NaClO_4$ sol. (-20°C, 0.5 M in acetone) at the next day.

**Note** -: In some cases crude sample could be contaminated by remaining benzoic acid, which would be completely removed by washing the precipitate with the minimal amount of MeOH and acetone mixture (1:5, v/v).

**Method B** -: Reaction mixture was precipitated by Method A and the crude product was further purified by Strong anion-exchange chromatography (SAX) (Q Sepharose® Fast Flow, increasing concentrations of aqueous $NH_4HCO_3$) and freeze drying of the fractions eluted with specific concentrations of buffer.

**Method C** -: Reaction mixture was precipitated by Method A and the crude product was analyzed by analytical HPLC:

Column: Hypersil GOLD™ aq.,

Dimension: 150 x 3 mm,

Particle size: 3 µm,

Flow rate: 1 ml/min,

Mobile phase: Phase **A**: Water, Phase **B**: 100 mM of TEAA (pH adjusted at 8.5), Phase **C**: Acetonitrile.

Gradient:

1. 0 min : 60% mobile phase A
   40% mobile phase B
2. 5 min : 60% mobile phase A
   40% mobile phase B
3. 16 min : 60% mobile phase B
   40% mobile phase C
4. 21 min : 60% mobile phase A
   40% mobile phase B

The crude product was purified on a preparative scale with the above mentioned method by MPLC. Therefore, the precipitate was dissolved in deionized water and loaded on puriFlash C18 aqueous column:

Column size: F0012 (20 g), Particle size: 30 µm, Flow rate: 10ml/min,

Mobile Phase: Phase **A**: Water, Phase **B**: 100 mM of TEAA (pH adjusted at 8.5), Phase **C**: Acetonitrile.

Gradient:

1. 0 min : 60% mobile phase A
   40% mobile phase B
2. 15 min : 60% mobile phase A
   40% mobile phase B
3. 45 min : 60% mobile phase B

40% mobile phase C

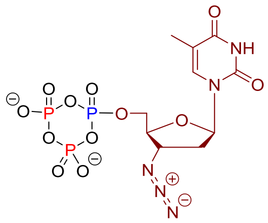
The fractions containing product were concentrated under reduced pressure to the minimal amount of the solvent and then product was precipitated by $NaClO_4$ sol. (0.5M in acetone).The precipitate was washed twice with acetone.

**Note-:** It was found that purification of adenosine triphosphates by Strong anion-exchange chromatography (Method B) is straightforward as they give 2´-3´cyclophosphates as byproducts, which can be easily removed from the product.

In the case of (deoxy)nucleoside such as thymidine, cPyPA is also phosphitylating the 3´OH. The resulting 3´-triphosphate can be removed by RP-LC.

## 2.2.1 Synthesis of triphosphates based on *c*-PyPA ($A_1$)

**3'-Azido-3'-deoxythymidine 5′-cyclotriphosphate (AZT cyclotriphosphate $B_1$)**



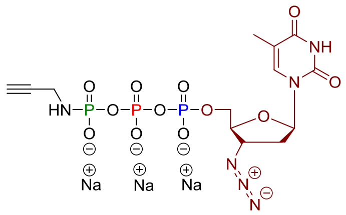
($B_1$)

AZT (50 mg, 0.18 mmol, 1.2 eq.) and ETT (78 mg, 0.60 mmol, 4.0 eq.) were coevaporated with dry MeCN (2 × 1 ml). Under an Ar atmosphere, a reaction mixture containing the *c*-PyPA $\mathbf{A_1}$ (0.075M in MeCN, 2 ml, 0.15 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 39 mg, 0.22 mmol, 1.5 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (formation of triplet at -23 ppm which is diagnostic signal for the cyclotriphosphate $\mathbf{B_1}$).[3]

Aliquots of this reaction mixture were used for the ring opening with various nucleophiles. The AZT cyclotriphosphate ($\mathbf{B_1}$) is stable in reaction mixture at -20°C up to 2 weeks.

**$^{31}$P{$^1$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): -23.2 – -25.2 (m, 1P), -25.8 (d, J = 21.1 Hz, 2P).

**Ring opening by using propargylamine: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-propargylamino triphosphate (1)**



(1)

The reaction mixture of **B1** (250 µL, 18.7 µmol, 1.0 eq.) was added to propargylamine (250 µL) and then $D_2O$ (100 µl) was added (stirred it well for 5 mins). The product **1** was isolated by Method A, affording a colourless solid (8.20 mg, 13.0 µmol, 72%).

__Reaction on large scale (1)__

AZT (344 mg, 1.28 mmol, 1.0 eq.) and ETT (540 mg, 4.15 mmol, 3.2 eq.) were coevaporated with dry MeCN (2 × 4 ml) in oven dried pear shape 25 ml flask. Under an atmosphere of dry Ar, a mixture of *c*-PyPA (**A1**) (0.075M, 17.0 ml, 1.27 mmol, 1.0 eq.) in MeCN was added to the dried solids. The mixture was stirred at r.t. for 10 mins. Upon cooling to -4 °C, *m*CPBA (≤77%, 439 mg, 2.55 mmol, 2.0 eq.) was added and the mixture was stirred for 10 mins until $^{31}$P-NMR confirmed complete oxidation (formation of triplet at -23 ppm which is diagnostic signal for the cyclotriphosphate **B1**). Then propargylamine (520 µl, 8.28 mmol, 6.5 eq.) was added to the reaction mixture at -4°C and the mixture was stirred at r.t. for 15 mins.

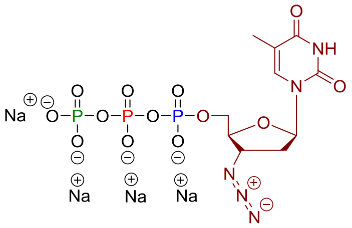
The product **1** was isolated by Method A, affording a colourless solid (712 mg, 1.16 mmol, 91%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.70 (s, 1H), 6.24 (t, J = 6.9 Hz, 1H), 4.53 (s, 1H), 4.17 (m, 3H), 3.64 (dd, J = 9.8, 2.2 Hz, 2H), 2.51 (t, J = 2.5 Hz, 1H), 2.48 – 2.36 (m, 2H), 1.88 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -1.99 (d, J = 20.7 Hz, 1P), -10.5 (d, J = 19.0 Hz, 1P), -20.7 (t, J = 19.9 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -1.99 (dt, J = 19.4, 9.2 Hz, 1P), -10.5 (dt, J = 19.1, 5.6 Hz, 1P), -20.7 (t, J = 19.8 Hz, 1P).**$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 151.8, 137.2, 111.8, 84.8, 82.9 (d, J = 9.3 Hz), 71.3, 65.6 (d, J = 5.6 Hz), 60.8, 36.3, 30.9 (d, J = 3.7 Hz), 11.7.

**HRMS** (ESI) m/z for $[C_{13}H_{18}N_6NaO_{12}P_3]^-$ : calcd. 566.0099, found 566.0088.

AZT triphosphate **2** can also be obtained from **1** by dissolving in water and adjusting the pH with HCl at 2.5 resulted in completed hydrolysis of amidate (P-N) bond. Afterwards, the product was isolated by Method A.

## Ring opening by using aq. sodium hydroxide: 3'-Azido-3'-deoxythymidine 5'-triphosphate (2)



(2)

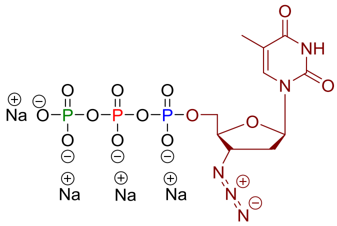
The reaction mixture of **B₁** (250 µL, 18.7 µmol, 1.0 eq.) was added to aq. NaOH (1M, 250 µL) and then $D_2O$ (100 µl) was added (stirred it well for 5 mins). The product **2** was isolated by Method A, affording a colourless solid (7.60 mg, 13.3 µmol, 71%).

The analytical data are consistent with literature.[3]

**Note** -: It was found that use of aq. NaOH as a nucleophile, was a superior way to open the cyclotriphosphate as compared to $H_2O$.

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.63 (s, 1H), 6.36 (t, J = 7.0 Hz, 1H), 4.69 – 4.52 (m, 1H), 4.29 – 4.11 (m, 3H), 2.46 (dd, J = 7.0, 5.1 Hz, 2H), 1.91 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): - 7.02 (d, J = 20.3 Hz, 1P), -11.5 (d, J = 19.8 Hz, 1P), -22.4 (t, J = 20.1 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -7.01 (d, J = 20.8 Hz, 1P), -11.5 (dt, J = 21.7, 4.7 Hz, 1P), -22.4 (t, J = 20.1 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.6, 151.7, 137.2, 111.8, 84.8, 83.0 (d, J = 9.3 Hz), 65.7 (d, J = 5.6 Hz), 60.9, 36.3, 11.6. **HRMS** (ESI): calculated for $[C_{10}H_{14}N_5NaO_{13}P_3]^-$ : calcd. 527.9704 found: 527.9706.

## Ring opening by using water: 3'-Azido-3'-deoxythymidine 5'-triphosphate (2)

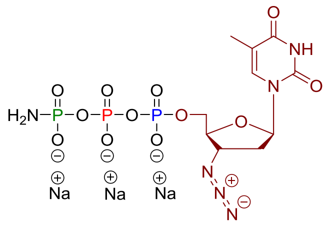


(2)

The reaction mixture of **B₁** (250 µL, 18.7 µmol, 1.0 eq.) was added to $D_2O$ (250 µl) and it was stirred at r.t. for 2.5 hr. The product **2** was isolated by Method A, affording a colourless solid (6.4 mg, 10.7 µmol, 57%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.63 (s, 1H), 6.36 (t, J = 7.0 Hz, 1H), 4.69 – 4.52 (m, 1H), 4.29 – 4.11 (m, 3H), 2.46 (dd, J = 7.0, 5.1 Hz, 2H), 1.91 (s, 3H). **$^{31}$P{$^1$H} NMR** (162 MHz, $D_2O$, δ/ppm): -7.02 (d, J = 20.3 Hz, 1P), -11.5 (d, J = 19.8 Hz, 1P), -22.4 (t, J = 20.1 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -7.01 (d, J = 20.8 Hz, 1P), -11.5 (dt, J = 21.7, 4.7 Hz, 1P), -22.4 (t, J = 20.1 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.6, 151.7, 137.2, 111.8, 84.8, 83.0 (d, J = 9.3 Hz), 65.7 (d, J = 5.6 Hz), 60.9, 36.3, 11.6. **HRMS** (ESI): calculated for $[C_{10}H_{14}N_5NaO_{13}P_3]^-$ : calcd. 527.9704 found: 527.9706.

**Ring opening by using aq. ammonia: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-amino triphosphate (3)**



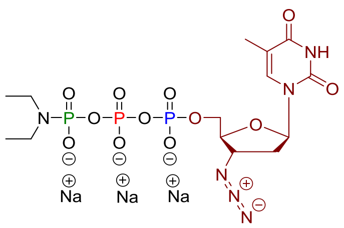
**(3)**

The reaction mixture of **B₁** (250 µL, 18.7 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (250 µL) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **3** was isolated by Method A, affording a colourless solid (7.90 mg, 13.8 µmol, 73%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.67 (d, J = 1.4 Hz, 1H), 6.21 (t, J = 6.9 Hz, 1H), 4.52 (dt, J = 6.3, 3.8 Hz, 1H), 4.22 – 4.01 (m, 3H), 2.84 – 2.25 (m, 2H), 1.85 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -2.71 (d, J = 20.4 Hz, 1P), -11.6 (d, J = 19.2 Hz, 1P), -22.6 (t, J = 19.9 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -1.04 (d, J = 19.1 Hz, 1P), -11.6 (dt, J = 19.6 Hz, 4.7 Hz, 1P), -22.6 (t, J = 19.5 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.6, 151.7, 137.2, 111.8, 84.8, 83.02 (d, J = 9.3 Hz), 65.7 (d, J = 5.6 Hz), 60.9, 36.3, 11.6.

**HRMS** (ESI): for $[C_{10}H_{15}N_6O_{12}P_3]^{2-}$ : calcd. 251.9986, found: 251.9986.

**Ring opening by using diethylamine: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-diethylamino triphosphate (4)**



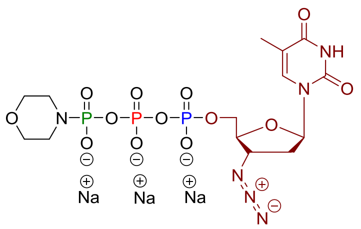
**(4)**

The reaction mixture of **B₁** (250 µL, 18.7 µmol, 1.0 eq.) was added to diethylamine (250 µL) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **4** was isolated by Method A, affording a colourless solid (8.80 mg, 14.0 µmol, 76%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.68 (s, 1H), 6.22 (t, J = 6.9 Hz, 1H), 4.51 (dt, J = 6.4, 3.6 Hz, 1H), 4.25 – 3.94 (m, 3H), 2.96 (dq, J = 11.3, 7.2 Hz, 4H), 2.52 – 2.36 (m, 2H), 1.85 (d, J = 1.1 Hz, 3H), 0.99 (t, J = 7.1 Hz, 6H). **$^{31}$P {$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -0.55 (d, J = 24.2 Hz, 1P), -11.8 (d, J = 19.1 Hz, 1P), -23.0 (dd, J = 24.2, 19.0 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -0.55 (dp, J = 23.9, 11.5 Hz, 1P), -11.8 (dt, J = 18.6, 4.5 Hz, 1P), -23.0 (dd, J = 24.4, 18.8 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.9, 152.0, 137.3, 111.8, 84.8, 83.0 (d, J = 9.5 Hz), 65.6 (d, J = 5.8 Hz), 61.0, 40.5 (d, J = 3.7 Hz), 36.2, 13.8 (d, J = 3.7 Hz), 11.7.

**HRMS** (ESI) m/z for $[C_{14}H_{23}N_6O_{12}P_3]^{2-}$ : calcd. 280.0299, found 280.0298.

**Ring opening by using morpholine: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-morpholino triphosphate (5)**



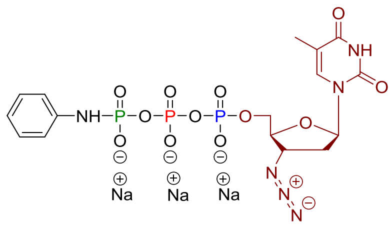
(5)

The reaction mixture of **$B_1$** (250 µL, 18.7 µmol, 1.0 eq.) was added to morpholine (250 µL) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **5** was isolated by Method A, affording a colourless solid (9.60 mg, 14.9 µmol, 80%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.71 (s, 1H), 6.23 (t, J = 6.9 Hz, 1H), 4.61 – 4.52 (m, 1H), 4.17 (d, J = 4.5 Hz, 3H), 3.91 – 3.75 (m, 2H), 3.66 – 3.56 (m, 2H), 3.09 – 2.91 (m, 4H), 2.70 – 2.37 (m, 2H), 1.88 (s, 3H).**$^{31}$P {$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -3.36 (d, J = 23.9 Hz, 1P), -11.7 (d, J = 19.3 Hz, 1P), -22.7 (dd, J = 23.8, 18.9 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -2.98 – -3.77 (m, 1P), -11.7 (dt, J = 18.6, 4.5 Hz, 1P), -22.7 (dd, J = 23.9, 19.3 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 151.9, 137.3, 111.8, 84.9, 83.0 (d, J = 9.1 Hz), 66.9 (d, J = 8.2 Hz), 65.6 (d, J = 5.6 Hz), 64.9, 44.9, 36.9, 11.7.

**HRMS** (ESI) m/z for $[C_{14}H_{21}N_6NaO_{13}P_3]^-$ : calcd.: 597.0283, found 597.0291.

**Ring opening by using aniline: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-anilino triphosphate (6)**



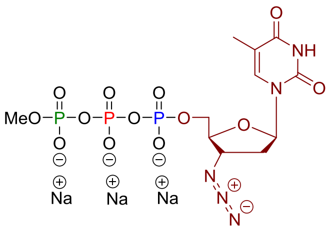
(6)

The reaction mixture of **$B_1$** (250 µL, 18.7 µmol, 1.0 eq.) was added to aniline (250 µL) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **6** was isolated by Method A, affording a colourless solid (9.45 mg, 14.5 µmol, 77%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.57 (s, 1H), 7.16 ($m_c$, 2H), 6.98 ($m_c$, 2H), 6.80 ($m_c$, 1H), 6.12 (t, J = 6.9 Hz, 1H), 4.51 – 4.31 (m, 1H), 4.02 – 3.94 (m, 2H), 3.94 – 3.81 (m, 1H), 2.42 (t, J = 6.5 Hz, 1H), 2.37 – 2.25 (m, 1H), 1.81 (s, 3H). **$^{31}$P {$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -10.1 (d, J = 19.1 Hz, 1P), -11.6 (d, J = 18.5, 1P), -21.4 – -24.0 (m, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -10.1 (d, J = 19.1 Hz, 1P), -11.9 (dt, J = 18.5, 4.5 Hz, 1P), -21.4 – -24.0 (m, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.5, 151.6, 141.9, 137.1, 129.1, 120.1, 117.3 (d, J = 7.3 Hz), 111.8, 84.7, 82.9 (d, J = 9.5 Hz), 65.5 (d, J = 5.4 Hz), 60.9, 36.4, 11.7.

**HRMS** (ESI) m/z for $[C_{16}H_{18}N_6Na_2O_{12}P_3]^-$ : calcd. 624.9996, found 624.9999.

**Ring opening by using NaOMe solution: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-methoxy triphosphate (7)**



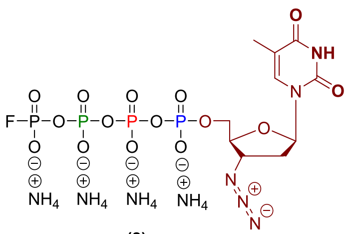
(7)

The reaction mixture of **B₁** (250 µL, 18.7 µmol, 1.0 eq.) was added to NaOMe Solution (0.5M in methanol, 300 µl) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **7** was isolated by Method A, affording a colourless solid (9.00 mg, 15.3 µmol, 81%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.63 (s, 1H), 6.36 (t, J = 7.0 Hz, 1H), 4.69 – 4.52 (m, 1H), 4.29 – 4.11 (m, 3H), 3.48 – 3.45 (m, 3H) 2.46 (dd, J = 7.0, 5.1 Hz, 2H), 1.91 (s, 3H).**$^{31}$P{$^1$H} NMR** (162 MHz, $D_2O$, δ/ppm):-9.63 (d, J = 19.1 Hz, 1P), -11.6 (d, J = 19.4 Hz, 1P), -22.9 (t, J = 19.1 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -9.64 (dq, J = 19.3, 11.8 Hz, 1P), -11.6 (dt, J = 19.1, 5.2 Hz, 1P), -22.9 (t, J = 19.1 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.6, 151.7, 137.2, 111.8, 95.7, 84.8, 83.0 (d, J = 9.3 Hz), 65.7 (d, J = 5.6 Hz), 60.9, 36.3, 11.6.

**HRMS** (ESI): calculated for $[C_{11}H_{17}N_5O_{13}P_3]^-$ : calcd. 520.0041 found: 520.0045.

**Ring opening by using monofluorophosphate: 3'-Azido-3'-deoxythymidine 5'-δ-*P*-fluoro tetraphosphate (8)**



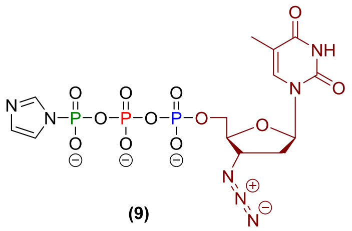
(8)

The reaction mixture of **B₁** (2.00 ml, 0.15 mmol, 1.0 eq.) was added to monofluorophosphate × 1.2 TBA (0.12 g, 0.30 mmol, 2.0 eq.) and subsequently addition of anhydrous $MgCl_2$ (0.08 g, 0.84 mmol, 5.6 eq.) and further addition of dry DMF (1 mL). The reaction mixture was stirred for 1 hr and the crude product was then purified by Method B (the product was eluted at 300-500 mM $NH_4HCO_3$ buffer). The title compound **8** (51.9 mg, 0.07 mmol, 52%) was obtained as a colourless solid.

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.53 (s, 1H), 6.08 (t, J = 6.9 Hz, 1H), 4.37 (m, 1H), 4.06 – 3.98 (m, 3H), 2.39 – 2.13 (m, 2H), 1.73 (s, 3H). **$^{31}$P{$^1$H} NMR** (162 MHz, $D_2O$, δ/ppm): -11.1 – -12.1 (m, 1P), -18.2 (dd, J = 934.0, 16.2 Hz, 1P), -22.9 – -23.9 (m, 2P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -11.7 (m, 1P), -17.7 – -18.4 (m, 1P), -23.0 – -23.9 (m, 2P). **$^{13}$C NMR** (126 MHz, $D_2O$, δ/ppm): 166.7, 165.6, 151.8, 137.1, 111.7, 82.8 (d, J = 9.1 Hz), 65.7 (d, J = 5.5 Hz), 60.8, 36.1, 11.6. **$^{19}$F NMR** (377 MHz, $D_2O$, δ/ppm): -73.1 (d, J = 935.1 Hz, 1P).

**HRMS** (ESI) m/z for $[C_{10}H_{16}FN_5O_{15}P_4]^-$ : calcd. 588.9577, found 588.9583.

**Ring opening by using imidazole: 3'-Azido-3'-deoxythymidine 5'-*γ*-*P*-imidazole triphosphate (9)**



(9)

AZT (50 mg, 0.18 mmol, 1.2 eq.) and ETT (78 mg, 0.60 mmol, 4.0 eq.) were coevaporated together with dry MeCN (2 × 1 mL). Under an Ar atmosphere, a freshly prepared reaction mixture containing the *c*- PyPA $\mathbf{A_1}$ (0.075M in MeCN, 2 mL, 0.15 mmol, 1.0 eq.) was added to the dried solids. The mixture was stirred at r.t. for 10 mins. Upon cooling to 0 °C, *m*CPBA (≤77%, 39 mg, 0.22 mmol, 1.5 eq.) was added and the mixture was stirred for 5 mins.

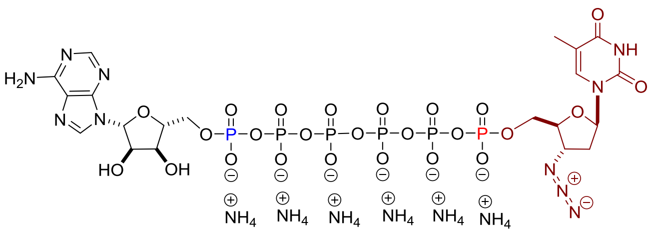
A solution of imidazole in dry DMF (1.47M, 4.00 ml, 5.88 mmol, 39.2 eq.) was added to the cyclotriphosphate ($\mathbf{B_1}$) and reaction was stirred for 15 mins (the reaction progress was followed by $^{31}$P NMR) which results in quantitative conversion to **9**.

**Note** -: Imidazole was dried before using by coevaporation with MeCN and it was further stored of molecular sieve (3Å).

**$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -11.0 (d, J = 19.5 Hz, 1P), -20.5 (d, J =19.1 Hz, 1P), -23.4 (t, J = 19.3 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -11.0 (dt, J = 20.4, 10.1 Hz, 1P), -20.5 (d, J = 19.0 Hz, 1P), -23.4 (t, J = 19.4 Hz, 1P).

**HRMS** (ESI) m/z for $[C_{13}H_{17}N_7O_{12}P_3]^-$: calcd. 556.0154, found 556.0156.

**5′-Adenosyl 3'-azido-3'-deoxy-5'-thymidinyl hexaphosphate (Ap$_6$AZT, P1)**



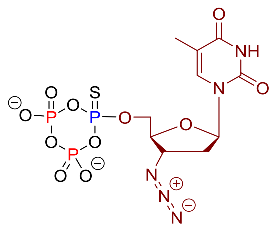
(P1)

Anhydr. $ZnCl_2$ (0.25 g, 1.8 mmol, 20 eq.) and ATP x 2.1 TBA (0.25 g, 0.24 mmol, 2.7 eq.) were added to a freshly prepared solution of **9** (0.09 mmol). The reaction mixture was then diluted with dry DMF: DMSO (v:v, 1:1, 8 ml) in order to increase the solubility and it was stirred at r.t. for 6 hrs. The crude product was purified by Method B and target product was eluted with 400-600 mM conc. of $NH_4HCO_3$ buffer. Purification gave **P1** as a colorless solid (0.03 g, 0.03 mmol, 35%).

**Note -**: The reactions for capped hexapolyPs were not optimized and therefore the yield could still be improved.

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 8.45 (s, 1H), 8.16 (s, 1H), 7.61 (s, 1H), 6.13 (t, J = 6.9 Hz, 1H), 6.04 (d, J = 6.2 Hz, 1H),4.66 – 4.44 (m, 2H), 4.44 – 4.32 (m, 1H), 4.26 – 4.08 (m, 5H), 2.37 (dd, J = 6.9, 5.2 Hz, 2H), 1.81 (s, 3H).The DHO peak overlaps with the signal 4.66 (1H).**$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -11.3 (d, J = 19.8 Hz, 1P), -11.6 (d, J = 19.8 Hz, 1P), -21.4 – -23.4 (m, 4P).**$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -11.2 – -11.5 (br, 1P), -11.5 – -11.7 (br, 1P), -22.1 – -23.6 (m, 4P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.4, 155.6, 152.8, 151.5, 149.1, 139.8, 137.1, 118.5, 111.6, 86.5, 84.8, 84.1 (d, J = 9.3 Hz), 82.9 (d, J = 9.3 Hz), 74.2, 70.4, 65.7 (d, J = 5.7 Hz), 65.4 (d, J = 5.7 Hz), 60.9, 36.3, 11.6. **HRMS** (ESI) m/z for $[C_{20}H_{28}N_{10}O_{25}P_6]^{2-}$ : calcd. 496.9832, found 496.9831.

**3'-Azido-3'-deoxythymidine 5'-α-thiocyclotriphosphate (AZT α-(S)-cyclotriphosphate: B$_2$)**

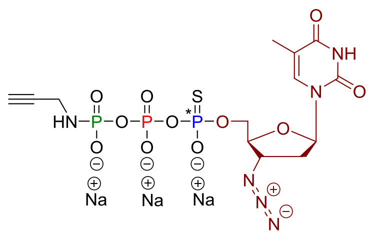


(B$_2$)

AZT (45 mg, 0.17 mmol, 1.5 eq.) and ETT (44 mg, 0.34 mmol, 3.0 eq.) were coevaporated with dry MeCN (2 × 2 mL). Under an Ar atmosphere, a freshly prepared reaction mixture containing the *c*-PyPA **A$_1$** (0.075M in MeCN, 1.5 ml, 0.11 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, Becauge's reagent (45 mg, 0.22 mmol, 2.0 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (the oxidation product shows a triplet at +43 ppm which is diagnostic signal for the α-(S)-cyclotriphosphate **B$_2$**).[4,5]

**$^{31}$P{$^1$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): 42.6 (t, J = 35.0 Hz, 1P), -24.7 (d, J = 35.3 Hz, 2P).

**Ring opening by using propargylamine: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-propargylamino α-thiotriphosphate (10)**



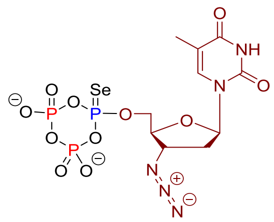
(10)

The reaction mixture of **$B_2$** (250 µL, 18.0 µmol, 1.0 eq.) was added to propargylamine (250 µl) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **10** was isolated by Method A, affording a colourless solid, whereas products were obtained as mixtures of diastereoisomers (**10**, 7.01 mg, 11.2 µmol, 59%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.70 (s, 1H), 6.20 (t, J = 6.8 Hz, 1H), 4.50 (dt, J = 6.6, 4.0 Hz, 1H), 4.23 – 4.09 (m, 3H), 3.67 – 3.51 (m, 2H), 2.56 (m, 1H), 2.52 – 2.43 (m, 2H), 1.87 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 42.7 (m,1P), -2.93 (dd, J = 21.2, 4.1 Hz, 1P), -23.0 – -24.8 (m,1P).**$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 42.8 (m, 1P), -1.73 – -6.95 (m,1P), -24.0 (t, J = 25.3 Hz, 1P).**$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 151.9, 137.3, 111.9, 84.8, 83.0 (d, J = 9.0 Hz), 71.3, 65.6 (d, J = 5.5 Hz), 60.9, 36.3, 31.0, 11.7.

**HRMS** (ESI): calculated for $[C_{13}H_{18}N_6O_{11}P_3S]^-$ : calcd. 558.9973 found: 558.9978.

**AZT α-(Se)-cyclotriphosphate: 3'-Azido-3'-deoxythymidine 5'-α-selenocyclotriphosphate ($B_3$)**

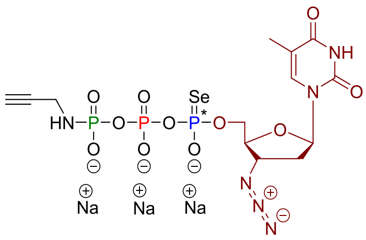


($B_3$)

AZT (115 mg, 0.43 mmol, 1.2 eq.) and ETT (150 mg, 1.15 mmol, 3.2 eq.) were coevaporated with dry MeCN (2 × 4 mL). Under an Ar atmosphere, a freshly prepared reaction mixture containing the *c*-PyPA **$A_1$** (0.075M in MeCN, 5.00 mL, 0.36 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 10 mins. Upon cooling to 0 °C, potassium selenocyanate (650 mg, 4.51 mmol, 12.5 eq.) was added and the mixture was stirred for 15 mins. A colourless precipitate was observed, which was further enhanced by addition of acetone and it was collected *via* centrifugation. The colourless residue was further checked by $^{31}$P-NMR confirmed complete oxidation (the oxidation product shows triplet at +33 ppm for the α- (Se)-cyclotriphosphate **$B_3$**).

**$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 35.8 – 30.6 (m, 1P), -20.8 – -23.5 (m, 1P), -23.7 – -26.1 (m, 1P).
**$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 34.6 – 32.5 (m, 1P), -20.2 – -23.2 (m, 1P), -23.8 – -25.1 (m, 1P).
**HRMS** (ESI) m/z for $[C_{10}H_{15}N_5O_{12}P_3Se]^-$ : calcd.: 569.9101, found 569.9103.

**Ring opening by using propargylamine: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-propargylamino α-selenotriphosphate (11)**



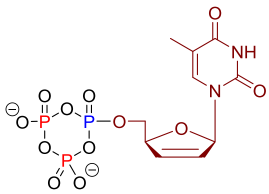
**(11)**

The precipitated product **B3** was further dissolved in $D_2O$ (1 ml) and treated with propargylamine (1 ml) for 15 mins at r.t. The product **11** was obtained by Method B as a colourless solid (the product was eluted at 300-500 mM $NH_4HCO_3$ buffer) (98 mg, 0.14 mmol, 40%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm):7.73 (s, 1H), 6.23 (t, J = 6.9 Hz, 1H), 4.61 – 4.49 (m, 1H), 4.28 – 4.09 (m, 3H), 3.65 (dt, J = 9.8, 2.9 Hz, 2H), 2.50 (t, J = 2.7 Hz, 1H), 2.48 – 2.34 (m, 2H), 1.91 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 33.2 (dd, J =32.4, 19.9 Hz), -2.89 (dd, J = 21.5, 3.0 Hz), -24.5 (ddd, J = 33.1, 21.4, 3.0 Hz). **$^{31}$P NMR** (122 MHz, $D_2O$, δ/ppm): 33.2 – 32.2 (m, 1P), -2.89 – -3.40 (m, 1P), -24.5 – -25.6 (m, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 151.8, 137.2, 111.8, 84.8, 82.9 (d, J = 9.3 Hz), 82.4, 71.3, 65.6 (d, J = 5.6 Hz), 60.8, 30.9, 11.7.

**HRMS** (ESI) m/z for $[C_{13}H_{18}N_6O_{11}P_3Se]^-$ : calcd. 606.9417, found 606.9415.

**2',3'-didehydro-2',3'-dideoxythymidine 5'-cyclotriphosphate (d4T cyclotriphosphate B4)**



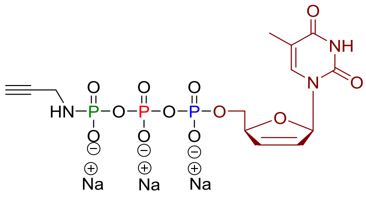
**(B$_4$)**

d4T (70 mg, 0.31 mmol, 1.4 eq.) and ETT (113 mg, 0.87 mmol, 4.0 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an Ar atmosphere, a reaction mixture containing the *c*-PyPA **A1** (0.075M in MeCN, 2.9 ml, 0.22 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 56 mg, 0.32 mmol, 1.5 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (the oxidation product shows triplet at -23 ppm which is diagnostic signal for the cyclotriphosphate **B4**). Aliquot of this reaction mixture were used for the ring opening with various nucleophiles. The d4T cyclotriphosphate **B4** is stable in reaction mixture at -20°C upto 2 weeks.

Ring opening was monitored by $^{31}$P-NMR of the resulting reaction mixtures.

**$^{31}$P{$^1$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): -22.1 – -23.9 (t, 1P), -26.5 (d, J = 21.1 Hz, 2P).

**Ring opening by using propargylamine: 2’,3’-didehydro-2’,3’-dideoxythymidine 5’-γ-*P*-propargylamino triphosphate (12)**



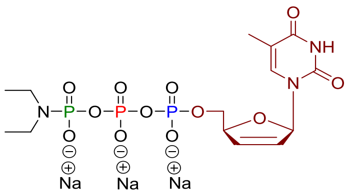
**(12)**

The reaction mixture of **B4** (250 µL, 18.7 µmol, 1.0 eq) was added to propargylamine (250 µL) and then (100 µl) was added afterwards it was stirred for 5 mins. The product **12** was obtained by Method A as a colourless solid (7.70 mg, 13.8 µmol, 74%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.58 (s, 1H), 6.91 (dt, J = 3.3, 1.7 Hz, 1H), 6.48 (dt, J = 6.2, 1.8 Hz, 1H), 5.92 – 5.86 (m, 1H), 5.06 (s, 1H), 4.13 (dt, J = 6.1, 3.1 Hz, 2H), 3.61 (dd, J = 9.9, 2.4 Hz, 2H), 2.49 (t, J = 2.5 Hz, 1H), 1.85 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -2.81 (d, J = 20.9 Hz, 1P), -11.5 (d, J = 19.4 Hz, 1P), -22.5 – -23.4 (m, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -1.97 (dt, J = 19.1, 9.4 Hz, 1P), -10.3 (dt, J = 19.4, 6.0 Hz, 1P), -20.6 (t, J = 19.9). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 167.0, 152.5, 138.2, 134.3, 125.3, 111.6, 89.9, 85.9 (d, J = 8.8 Hz), 83.1, 66.5 (d, J = 6.0 Hz), 30.9, 11.5.

**HRMS** (ESI) m/z for $[C_{13}H_{17}N_3NaO_{12}P_3]^-$: calcd. 522.9928, found 522.9927.

**Ring opening by using diethylamine: 2’,3’-didehydro-2’,3’-dideoxythymidine 5’-γ-*P*-diethylamino triphosphate (13)**



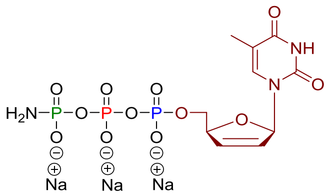
**(13)**

The reaction mixture of **B4** (250 µL, 18.7 µmol, 1.0 eq.) was added to diethylamine (250 µL) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The Product **13** was obtained by Method A as a colourless solid (8.80 mg, 15.0 µmol, 80%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.50 (s, 1H), 6.90 (dt, J = 3.4, 1.7 Hz, 1H), 6.44 (dt, J = 6.2, 1.8 Hz, 1H), 5.86 (dt, J = 5.9, 1.9 Hz, 1H), 5.20 – 4.94 (m, 1H), 4.20 – 3.98 (m, 2H), 2.93 (dq, J =11.3, 7.2 Hz, 4H), 1.81 (s, 3H), 0.97 (t, J = 7.1 Hz, 6H).**$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -0.51 (d, J = 24.7 Hz, 1P), -11.5 (d, J = 19.0 Hz, 1P), -22.9 (dd, J = 24.6, 18.6 Hz, 1P).**$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): - 0.51 (dp, J = 22.9, 11.4 Hz), -11.5 (dt, J = 19.1, 6.5 Hz), -22.9 (dd, J = 24.3, 18.9 Hz). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.0, 152.5, 137.8, 133.9, 125.5, 111.6, 90.0, 85.8 (d, J = 8.5 Hz), 66.6 (d, J = 5.7 Hz), 40.5 (d, J = 3.7 Hz), 13.8 (d, J = 3.7 Hz), 11.9.

**HRMS** (ESI) m/z for $[C_{14}H_{23}N_3O_{12}P_3]^-$: calcd. 518.0500, found 518.0501.

**Ring opening by using aq. ammonia: 2',3'-didehydro-2',3'-dideoxythymidine 5'-γ-*P*-amino triphosphate (14)**



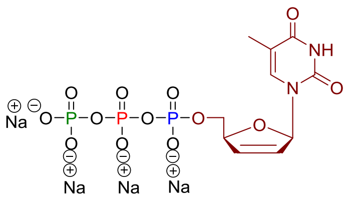
(14)

The reaction mixture of **B4** (250 µL, 18.7 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (250 µL) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **14** was obtained by Method A as a colourless solid (7.73 mg, 14.6 µmol, 79%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.55 (s, 1H), 6.91 (dt, J = 3.3, 1.7 Hz, 1H), 6.48 (dt, J = 6.2, 1.8 Hz, 1H), 5.89 (d, J = 6.3 Hz, 1H), 5.06 (s, 1H), 4.11 (dt, J = 6.3, 3.3 Hz, 2H), 1.84 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -1.08 (d, J = 19.3 Hz, 1P), -11.5 (d, J = 20.1 Hz,1P), -22.7 (t, J = 19.4 Hz,1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -0.33 (d, J = 19.1 Hz, 1P), -10.9 (dt, J = 19.4, 6.5 Hz, 1P), -21.6 (t, J = 19.3 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 152.3, 138.2, 134.4, 125.0, 111.5, 89.9, 86.0 (d, J = 8.5 Hz), 66.4 (d, J = 5.8 Hz), 11.4.

**HRMS** (ESI) m/z for $[C_{10}H_{15}N_3Na_2O_{12}P_3]^+$: calcd. 507.9658, found 507.9660.

**Ring opening by using aq. sodium hydroxide: 2',3'-didehydro-2',3'-dideoxythymidine 5'-triphosphate (15)**



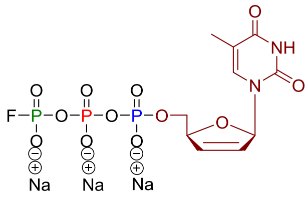
(15)

The reaction mixture of **B4** (250 µL, 18.7 µmol, 1.0 eq.) was added to 1M aq. NaOH (250 µL) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **15** was obtained by Method A as a colourless solid (7.95 mg, 14.9 µmol, 80%).The analytical data are consistent with literature.[6]

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.55 (s, 1H), 6.90 (dt, J = 3.4, 1.7 Hz, 1H), 6.49 (dt, J = 6.2, 1.8 Hz, 1H), 5.87 (dt, J =6.2, 1.9 Hz, 1H), 5.05 (td, J = 3.6, 1.9 Hz, 1H), 4.32 – 3.98 (m, 2H), 1.83 (s, 3H).**$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -10.9 (d, J = 19.6 Hz, 1P), -11.6 (d, J = 20.1 Hz, 1P), -23.4 (t, J = 19.8Hz, 1P).**$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -9.68 (d, J = 18.3 Hz, 1P), -10.7 (dt, J = 19.4, 6.5 Hz, 1P ), -21.6 (m,1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 152.3, 138.2, 134.4, 125.0, 111.5, 89.9, 86.0 (d, J = 8.5 Hz), 66.4 (d, J = 5.8 Hz), 11.5.

**HRMS** (ESI) m/z for $[C_{10}H_{14}N_2O_{13}P_3]^-$: calcd. 462.9714, found 462.9712.

**Ring opening by caesium fluoride: 2’,3’-didehydro-2’,3'-dideoxythymidine 5’-γ-*P*-fluorotriphosphate (16)**

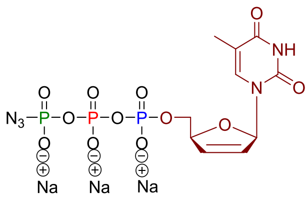


**(16)**

The reaction mixture of **B4** (300 µL, 13.5 µmol, 1.0 eq.) was added to caesium fluoride (100 mg, 661 µmol, 50 eq) and then immediately $D_2O$ (200 µl) was added afterwards it was stirred for 5 mins. The crude product was isolated by Method A and the impurity $CsClO_4$ was co-precipitated which was further removed by dissolving the crude product in water (2 mL). The insoluble residue was removed by filtration (syringe filter, pore size 0.45 µm). Afterwards, the product **16** was isolated from the aq. solution again by Method A and a colourless solid was obtained (5.9 mg, 11.0 µmol, 82%).

**$^{1}$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.53 (s, 1H), 6.97 – 6.81 (m, 1H), 6.57 – 6.40 (m, 1H), 5.95 – 5.81 (m, 1H), 5.04 (s, 1H), 4.08 (dd, J = 6.4, 3.4 Hz, 2H), 1.82 (s, 3H).**$^{31}$P{$^{1}$H} NMR** (122 MHz, $D_2O$, δ/ppm): -11.5 (d, J = 19.2 Hz, 1P), -17.9 (dd, J = 932.9, 17.3 Hz, 1P), -22.3 – -23.5 (m,1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -11.5 (dt, J = 19.3, 6.4 Hz, 1P), -17.9 (dd, J = 932.9, 17.3 Hz, 1P), -22.4 – -24.2 (m, 1P). **$^{31}$P{$^{19}$F} NMR** (162 MHz, $D_2O$, δ/ppm): -11.5 (dt, J = 19.5, 6.4 Hz,1P), -17.9 (d, J = 17.2 Hz,1P), -21.6 – -23.5 (m,1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 152.3, 138.2, 134.4, 125.0, 111.5, 89.9, 86.0 (d, J = 8.5 Hz), 66.4 (d, J = 5.8 Hz), 11.5. **$^{19}$F NMR** (377 MHz, $D_2O$, δ/ppm): -73.1 (d, J = 933.5 Hz, 1F). **HRMS** (ESI) m/z for $[C_{10}H_{13}FN_2O_{12}P_3]^-$: calcd. 464.9671, found 464.9678.

**Ring opening by using lithium azide: 2’,3’-didehydro-2’,3’-dideoxythymidine 5’-γ-P-azidotriphosphate (17)**



**(17)**

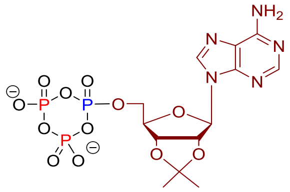
The reaction mixture of **B4** (250 µL, 18.7 µmol, 1.0 eq.) was added to the lithium azide (500 µL) and $D_2O$ 100 µl was added afterwards it was stirred for 5 mins. The product was purified by Method A, affording a colourless solid **17** (9.89 mg, 17.8 µmol, 95%).

**Note -:** As the commercial availability of $LiN_3$ solution is 20% wt. in water which results in $H_2O$ is also acting as a nucleophile, so the product contains 30% of hydrolysed d4T triphosphate **15**, indicated yield does not refer to the pure product.

**$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -10.8 (d, J = 17.2 Hz, 1P), -12.9 (d, J = 19.5 Hz, 1P), -21.5 – -22.2 (m, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -10.9 (dt, J = 17.5 Hz, 1P), -12.9 (d, J = 19.4 Hz, 1P), -21.8 (t, J = 18.4 Hz, 1P).

**HRMS** (ESI) m/z for $[C_{10}H_{13}N_5O_{12}P_3]^-$: calcd. 487.9779, found 487.9785.

**2’,3’-O-Isopropylideneadenosine 5’-cyclotriphosphate ($B_5$)**

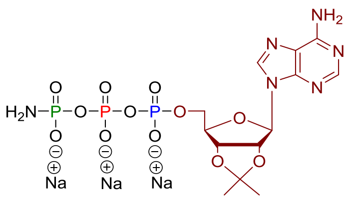


($B_5$)

2',3'-O-Isopropylideneadenosine (70 mg, 0.22 mmol, 1.5 eq.) and ETT (80 mg, 0.61 mmol, 4.0 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an Ar atmosphere, both solids were dissolved in dry DMF (2 mL), a reaction mixture containing the *c*-PyPA **$A_1$** (0.075M in MeCN, 2.0 mL, 0.15 mmol, 1.0 eq.) was added and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 40 mg, 0.23 mmol, 1.5 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (formation of triplet at -22 ppm which is diagnostic signal for the cyclotriphosphate **$B_5$**). The final concentration of cyclotriphosphate **$B_5$** is 0.037M.

**$^{31}$P{$^1$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): -23.2 – -25.2 (m, 1P), -25.8 (d, J = 21.1 Hz, 2P).

**Ring opening by using aq. ammonia: 2’,3’-O-Isopropylideneadenosine 5’-γ-*P*-amino triphosphate (S-1)**



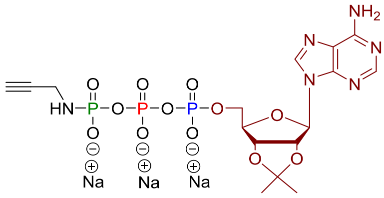
(S-1)

The reaction mixture of **$B_5$** (500 µL, 18.7 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (500 µL) and then $D_2O$ (200 µl) was added afterwards it was stirred for 5 mins. The product **S-1** was isolated by Method A, affording a colourless solid (7.50 mg, 12.5 µmol, 66%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 8.40 (s, 1H), 8.20 (s, 1H), 6.24 (d, J = 3.5 Hz, 1H), 5.36 (dd, J = 6.2, 3.5 Hz, 1H), 5.20 (dd, J = 6.2, 2.2 Hz, 1H), 4.33 – 4.04 (m, 2H), 1.64 (s, 3H), 1.41 (s, 3H). The DHO peak overlaps with the signal 4.50 (1H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -1.04 (d, J = 19.4 Hz, 1P), -11.6 (d, J = 19.7 Hz, 1P), -22.6 (t, J = 19.4 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -0.98 (d, J = 19.1 Hz, 1P), -11.6 (dt, J = 19.7, 5.7 Hz, 1P), -22.5 (t, J = 19.2 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 155.6, 152.9, 148.8, 140.0, 118.7, 114.9, 90.0, 84.5 (d, J = 9.4 Hz), 83.8, 81.3, 65.8 (d, J = 5.6 Hz), 26.1, 24.4.

**HRMS** (ESI) m/z for $[C_{13}H_{20}N_6O_{12}P_3]^-$: calcd. 545.0358, found 545.0359.

**Ring opening by using propargylamine: 2',3'-O-Isopropylideneadenosine 5'-γ-*P*-propargylamino triphosphate (S-2)**



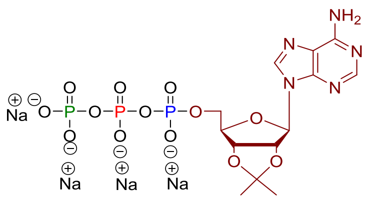
**(S-2)**

The reaction mixture of **B5** (500 µL, 18.7 µmol, 1.0 eq.) was added to propargylamine (500 µL) and then $D_2O$ (200 µl) was added afterwards it was stirred for 5 mins. The product **S-2** was isolated by Method A, affording a colourless solid (9.56 mg, 14.6 µmol, 79%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 8.41 (s, 1H), 8.20 (s, 1H), 6.23 (d, J = 3.6 Hz, 1H), 5.36 (dd, J = 6.2, 3.5 Hz, 1H), 5.20 (dd, J =6.2, 2.2 Hz, 1H), 4.27 – 4.03 (m, 2H), 3.56 (d, J = 9.9 Hz, 2H), 2.51(t, J = 2.5 Hz, 1H) 1.64 (s, 3H), 1.41 (s, 3H). The DHO peak overlaps with the signal 4.55 (1H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -2.76 (d, J = 20.9 Hz, 1P), -11.6 (d, J = 19.2 Hz, 1P), -21.2 – -23.5 (m, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -2.76 (dt, J = 20.4, 10.1 Hz, 1P), -11.6 (d, J = 18.3 Hz, 1P), -22.0 – -23.1 (m, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 155.7, 152.9, 148.9, 140.0, 118.7, 114.9, 89.9, 84.5 (d, J = 9.7 Hz), 83.8, 81.3, 65.8 (d, J = 5.5 Hz), 42.4, 30.9, 26.2, 24.4.

**HRMS** (ESI) m/z for $[C_{16}H_{21}N_6O_{12}P_3]^{2-}$: calcd. 291.5260, found 291.5251.

**Ring opening by using aq. sodium hydroxide: 2',3'-O-Isopropylideneadenosine 5'-triphosphate (S- 3)**



**(S-3)**

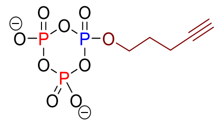
The reaction mixture of **B5** (500 µL, 18.7 µmol, 1.0 eq.) was added to 1M aq. NaOH (500 µL) and then $D_2O$ (200 µl) was added. The product **S-3** was isolated by Method A, affording a colourless solid (9.10 mg, 14.7 µmol, 78%).

The analytical data is consistent with literature.[7]

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 8.41 (s, 1H), 8.20 (s, 1H), 6.23 (d, J = 3.5 Hz, 1H), 5.35 (dd, J = 6.1, 3.6 Hz, 1H), 5.21 (dd, J = 6.1, 2.2, 1H), 4.43 – 4.05 (m, 2H), 1.63 (s, 3H), 1.41 (s, 3H). The DHO peak

overlaps with the signal 4.55 (1H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -5.55 (d, J = 20.1 Hz, 1P), -11.2 (d, J = 18.6 Hz, 1P), -21.5 (t, J = 19.2 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -5.56 (d, J = 20.0 Hz, 1P), -11.2 (d, J = 18.7 Hz, 1P), -21.1 – -21.8 (m, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 155.6, 152.9, 148.9, 140.0, 118.7, 114.9, 90.0, 84.5 (d, J = 9.4 Hz), 83.8, 81.3, 65.8 (d, J = 5.6 Hz), 26.1, 24.4.
**HRMS** (ESI) m/z for $[C_{13}H_{17}N_5Na_4O_{13}P_3]^+$: calcd. 635.9621, found 635.9623.

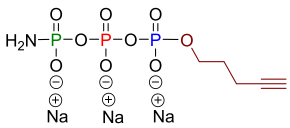
**4-Pentyne-1-cyclotriphosphate ($B_6$)**



($B_6$)

4-Pentyn-1-ol (50 mg, 0.59 mmol, 1.5 eq.) and ETT (118 mg, 0.90 mmol, 2.3 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an atmosphere of dry Ar, a reaction mixture containing the *c*-PyPA **$A_1$** (0.079M in MeCN, 5.0 mL, 0.39 mmol, 1.0 eq.) was added and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 75 mg, 0.43 mmol, 1.1 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (formation of triplet at -21 ppm which is diagnostic signal for the cyclotriphosphate **$B_6$**).
**$^{31}$P{$^1$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): -21.2 – -23.1 (m, 1P), -24.3 (d, J = 20.7 Hz, 2P).

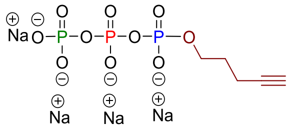
**Ring opening by using aq. ammonia: 4-Pentyne γ-*P* -amino triphosphate (S-4)**



(S-4)

The reaction mixture of **$B_6$** (250 µL, 19.7 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (250 µL) and then $D_2O$ (100 µl) was added. The product **S-4** was isolated by Method A, affording a colourless solid (7.55 mg, 17.9 µmol, 91%).
**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 3.98 (q, J = 6.5 Hz, 2H), 2.26 (t, J = 7.2 Hz, 1H), 2.15 (m, 2H), 1.79 (pent, J = 7.1, 6.7 Hz, 2H). **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): -1.04 (d, J = 19.2 Hz, 1P), -10.8 (d, J = 20.1 Hz, 1P), -22.6 (t, J = 19.4 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -0.70 (d, J = 23.5 Hz, 1P), -10.3 (dt, J = 9.2 Hz, 1P), -18.9 – -22.1 (m, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 85.2, 69.4, 65.3 (d, J = 5.9 Hz), 28.8 (d, J = 7.2 Hz), 14.2.
**HRMS** (ESI) m/z for $[C_5H_{11}NO_9P_3]^-$: calcd. 321.9652, found 321.9652.

## Ring opening by using aq. sodium hydroxide: 4-Pentyne-1-triphosphate (S-5)



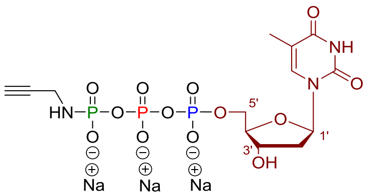
**(S-5)**

The reaction mixture of **B6** (250 µL, 19.7 µmol, 1.0 eq.) was added to aq. NaOH (1M, 250 µL) and then $D_2O$ (100 µl) was added. The crude product was purified by Method A, affording a colourless solid **S-5** (7.80 mg, 18.9 µmol, 95%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 3.99 (dd, J = 7.2, 6.3 Hz, 2H), 2.29 (t, J = 7.2, 1H), 2.19 (m, 2H), 1.82 (p, J = 6.7 Hz, 2H). **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): -4.33 (d, J = 18.0 Hz, 1P), -9.74 (d, J = 17.8 Hz, 1P), -19.7 (t, J = 18.0 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -4.35 (d, J = 18.0 Hz, 1P), -9.75 (dt, J = 17.6, 6.9 Hz, 1P), -19.7 (t, J = 17.9 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 85.1, 69.3, 65.3 (d, J = 5.9 Hz), 28.8 (d, J = 7.1 Hz), 14.2.

**HRMS** (ESI) m/z for $[C_5H_{10}O_{10}P_3]^-$: calcd. 322.9492, found 322.9468.

## Ring opening by using propargylamine: 2'-deoxythymidine 5'-γ-*P*-triphosphate (18)



**(18)**

Thymidine (470 mg, 1.95 mmol, 1.3 eq.) and DCI (700 mg, 6.00 mmol, 4.0 eq.) were coevaporated together with dry MeCN (2 × 5 mL). Under an Ar atmosphere, both solids were dissolved in dry DMF (5 mL) and a reaction mixture containing the *c*-PyPA **A1** (0.075M in MeCN, 20.0 ml, 1.50 mmol, 1.0 eq.) was added. The mixture was stirred for 10 mins at r.t. and the reaction was followed by $^{31}$P-NMR (of an aliquot of the reaction mixture in dry $CDCl_3$) which shows characteristic peak triplet at -100 ppm confirms complete coupling. Upon cooling to -4°C, *m*CPBA (460 mg, 2.70 mmol, 1.8 eq.) was added in portions. The mixture was stirred mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (a triplet at - 22 ppm revealed formation of cyclotriphosphate). Then propargylamine (1.52 ml, 24.0 mmol, 16.0 eq.) was added to the reaction mixture at -4°C and the mixture was stirred another for 15 mins at r.t. The product was further purified by Method C and obtained as a colourless solid, details of the MPLC purification can be found in Supporting figure S3. (**18**, 520 mg, 0.89 mmol, 64%).

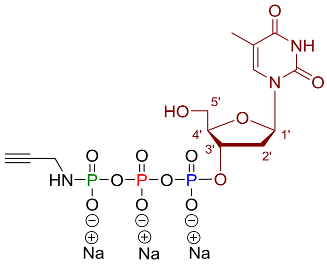
**Fraction 1:** 2'-deoxythymidine 5'-γ-*P*-propargylamino triphosphate

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.69 (s, 1H), 6.28 (t, J = 6.9 Hz, 1H), 4.58 (dt, J = 6.3, 3.4 Hz, 1H), 4.13 (q, J = 4.8, 4.4 Hz, 3H), 3.62 (dd, J = 9.9, 2.5 Hz, 2H), 2.49 (dt, J = 3.5, 1.8 Hz, 1H), 2.37 – 2.23 (m, 2H), 1.86 (s, 3H). **$^{31}$P{$^1$H} NMR** (162 MHz, $D_2O$, δ/ppm): -2.75 (d, J = 20.7 Hz, 1P), -11.5 (d, J = 19.4

Hz, 1P), -22.8 (t, J = 20.1 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -2.75 (dt, J = 20.2, 9.8 Hz, 1P), - 11.5 (dt, J = 19.4, 5.2 Hz, 1P), -22.8 (t, J = 19.9 Hz, 1P). **$^{13}$C NMR** (126 MHz, $D_2O$, δ/ppm): 166.6, 151.7, 137.9, 111.8, 85.4 (d, J = 9.2 Hz), 84.9, 82.8 (d, J = 11.5 Hz), 71.3, 70.8, 65.3 (d, J = 5.7 Hz), 46.6, 23.0 (d, J = 1.3 Hz), 11.6.

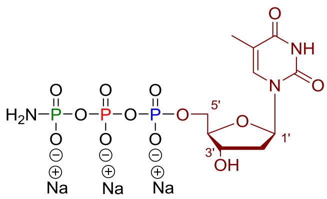
**HRMS** (ESI) m/z for $[C_{13}H_{19}N_3O_{13}P_3]^-$: calcd. 518.0136, found 518.0139.

**Fraction 2:** 2'-deoxythymidine 3'-γ-*P*-propargylamino triphosphate




**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.61 (s, 1H), 6.27 (t, J = 7.0, 1H), 4.85 (m, 1H), 4.26 – 4.08 (m, 1H), 3.78 (m, 2H), 3.62 (d, J = 7.2 Hz, 2H), 2.74 – 2.47 (m, 1H), 2.45 – 2.36 (m, 2H), 1.67 (s, 3H). **$^{31}$P{$^1$H} NMR** (162 MHz, $D_2O$, δ/ppm): -2.61 (d, J = 20.3 Hz, 1P), -12.1 (d, J = 19.0 Hz, 1P), -22.7 (t, J = 19.9 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -2.61 (dt, J = 20.4, 9.9 Hz, 1P), -12.1 (dd, J = 19.0, 7.9 Hz, 1P), -22.7 (t, J = 20.0 Hz, 1P).

**Ring opening by using aq. ammonia: 2'-deoxythymidine 5'-γ-*P*-amino triphosphate (19)**




**(19)**

Thymidine (110 mg, 0.46 mmol, 1.5 eq.) and DCI (120 mg, 1.05 mmol, 3.5 eq) were coevaporated together with dry MeCN (2 × 5 mL). Under an Ar atmosphere, both solids were dissolved in dry DMF (2 mL) and a reaction mixture containing the *c*-PyPA **$A_1$** (0.075M in MeCN, 4.0 ml, 0.30 mmol, 1.0 eq.) was added. The mixture was stirred for 5 mins at r.t. and the reaction was followed by $^{31}$P-NMR (of an aliquot of the reaction mixture in dry $CDCl_3$) which shows characteristic peak triplet at -100 ppm confirms complete coupling. Upon cooling to -4°C to 0°C, *m*CPBA (80 mg, 0.45 mmol, 1.5 eq) was added in portion wise and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (reaction progress was followed by $^{31}$P NMR, and formation of triplet at -22 ppm which is diagnostic signal for the cyclotriphosphate). Then 25% aq. $NH_4OH$ (0.5 ml) was added to the reaction mixture at -4°C and the mixture was stirred another for 15 mins at r.t.

The product was further purified by Method C and obtained as a colourless solid. (**19**, 98 mg, 0.18 mmol, 59%).

**<u>Fraction 1</u>:** 2'-deoxythymidine 5'-γ-*P*-amino triphosphate

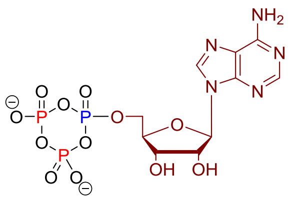
**$^{1}$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.67 (s, 1H), 6.28 (t, J = 7.0 Hz, 1H), 4.62 – 4.51 (m, 1H), 4.27 – 3.94 (m, 3H), 2.62 – 2.19 (m, 2H), 1.86 (s, 3H). **$^{31}$P{$^{1}$H} NMR** (162 MHz, $D_2O$, δ/ppm): -0.97 (d, J = 19.0 Hz, 1P), -11.5 (d, J = 20.0 Hz, 1P), -22.5 (t, J = 19.5 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -0.97 (d, J = 19.0 Hz, 1P), -11.5 (dt, J = 19.4, 5.2 Hz, 1P), -22.5 (t, J = 19.5 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.6, 151.8, 137.7, 111.8, 85.3 (d, J = 9.1 Hz), 84.9, 70.8, 65.4 (d, J = 5.9 Hz), 46.7, 11.3.

**HRMS** (ESI) m/z for $[C_{10}H_{17}N_3Na_2O_{13}P_3]^+$: calcd. 525.9764, found 525.9765.

**<u>Fraction 2</u>:** 2'-deoxythymidine 3'-γ-*P*-amino triphosphate

**$^{31}$P{$^{1}$H} NMR** (162 MHz, $D_2O$, δ/ppm): -0.87 (d, J = 19.1 Hz, 1P), -12.0 (d, J = 19.3 Hz, 1P), -22.4 (t, J = 19.3 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -0.88 (d, J = 19.0 Hz, 1P), -12.0 (dd, J = 19.5, 8.0 Hz, 1P), -22.4 (t, J = 19.2 Hz, 1P).

**Adenosine-5'-cyclotriphosphate ($B_7$)**

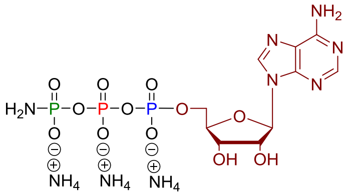


($B_7$)

Adenosine (120 mg, 0.45 mmol, 1.5 eq.) and DCI (94 mg, 0.80 mmol, 2.7 eq.) were coevaporated together with dry MeCN (2 × 5 mL). Under an atmosphere of dry Ar, both solids were dissolved in dry DMF (5.5 ml). A reaction mixture containing the *c*-PyPA **$A_1$** (0.5M in DMF, 0.60 ml, 0.30 mmol, 1.0 eq.) was added to the above flask. The mixture was stirred for 5 mins at r.t. and the reaction was followed by $^{31}$P-NMR (of an aliquot of the reaction mixture in dry $CDCl_3$) which shows characteristic peak triplet at -100 ppm confirms complete coupling. Upon cooling to -4°C, *m*CPBA (100 mg, 0.60 mmol, 2.0 eq.) was added in the mixture and stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (reaction progress was followed by $^{31}$P NMR, and formation of triplet at -22 ppm which is diagnostic signal for the cyclotriphosphate **$B_7$**).

**$^{31}$P{$^{1}$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): -23.0 (t, J = 24.5 Hz, 1P), -24.0 – -25.9 (m, 2P).

**Ring opening by using aq. ammonia: Adenosine 5'-γ-*P*-amino triphosphate (20)**



**(20)**

The reaction mixture of **B7** (500 µl, 25.0 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (100 µl) and $D_2O$ (100 µl) was added. The crude product was purified by Method B and freeze drying of the fractions (2×) eluted at 6.4-9.2% of aq. 1 M $NH_4HCO_3$ buffer afforded the title compound (**20**, 6.50 mg, 12.0 µmol, 47%) as colourless solid.

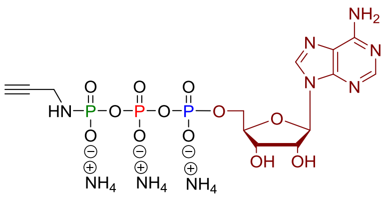
Analytical data are consistent with those reported in the literature (reported in DMSO-$d_6$).[8]

**$^1$H-NMR:** (300 MHz, $D_2O$, δ/ppm): 8.54 (s, 1H), 8.29 (s, 1H), 6.17 (d, J = 6.0 Hz, 1H), 4.60 (dd, J = 5.2, 3.6 Hz, 1H), 4.48-4.40 (m, 1H), 4.35-4.22 (m, 2H). The DHO peak overlaps with the signal 4.88 (1H)

**$^{31}$P{$^1$H}-NMR:** (122 MHz, $D_2O$, δ/ppm): –0.99 (d, J = 19.2 Hz, 1P), –11.4 (d, J = 20.3 Hz, 1P), –22.6 (t, J = 19.5 Hz, 1P).

**HRMS** (ESI) m/z for $[C_{10}H_{16}N_6O_{12}P_3]^-$: calcd. 505.0045, found 505.0046.

**Ring opening by using propargylamine: Adenosine 5'-γ-*P*-propargylamino triphosphate (21)**



**(21)**

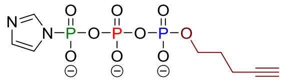
The reaction mixture of **B7** (400 µl, 20.0 µmol, 1.0 eq.) was added to propargylamine (100 µl) and $D_2O$ (100 µl) was added. The crude product was purified by Method B and freeze drying of the fractions (2×) eluted at 8.7-13% of aq. 1 M $NH_4HCO_3$ buffer afforded the title compound (**21**, 4.30 mg, 7.20 µmol, 37%) as colourless solid.

Analytical data are consistent with those reported in the literature.[9]

**$^1$H-NMR:** (300 MHz, $D_2O$, δ/ppm): 8.56 (s, 1H), 8.29 (s, 1H), 6.17 (d, J = 5.8 Hz, 1H), 4.63 – 4.56 (m, 1H), 4.46-4.40 (m, 1H), 4.35-4.23 (m, 2H), 3.67 (d, J = 9.9 Hz, 2H), 2.50 ($m_c$, 1H). The DHO peak overlaps with the signal 4.88 (1H). **$^{31}$P{$^1$H}-NMR:** (122 MHz, $D_2O$, δ/ppm): –2.72 (d, J = 20.7 Hz, 1P), –11.4 (d, J = 19.5 Hz, 1P), –22.8 (t, J = 19.5 Hz, 1P).

**HRMS** (ESI) m/z for $[C_{13}H_{18}N_6O_{12}P_3]^-$: calcd. 543.0201, found 543.0202.

**Ring opening by using imidazole: 4-Pentyne γ-*P* –imidazole triphosphate (S-6)**



**(S-6)**

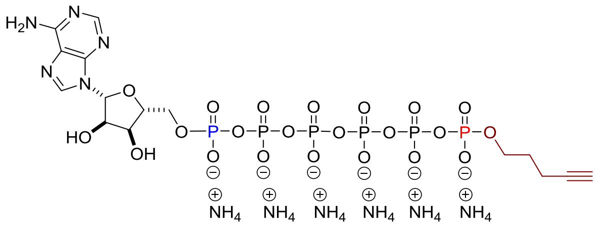
4-Pentyn-1-ol (51 mg, 0.60 mmol, 2.0 eq.) and ETT (156 mg, 1.20 mmol, 4.0 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an atmosphere of dry Ar, a freshly prepared reaction mixture containing the *c*-PyPA **$A_1$** (0.075M, 4.0 mL, 0.30 mmol, 1.0 eq.) in MeCN was added in the above reaction mixture. The mixture was stirred at r.t. for 10 mins. Upon cooling to 0 °C, *m*CPBA (≤77%, 80 mg, 0.47 mmol, 1.5 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (reaction progress was followed by $^{31}$P NMR, and formation of triplet at -23 ppm which is diagnostic signal for the cyclotriphosphate **$B_6$**).

A solution of imidazole in dry DMF (1.47M, 9.00 ml, 13.2 mmol, 45 eq.) was added to the cyclotriphosphate (**$B_6$**) and reaction was stirred for another 20 mins (the reaction progress was followed by $^{31}$P NMR) which results in the quantative conversion to **S-6**.

**Note** -: Imidazole was dried before using by coevaporation with MeCN and it was further stored of molecular sieve (3Å).

**$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -11.1 (d, J = 19.9 Hz, 1P), -20.2 (d, J = 19.3 Hz, 1P), -23.8 (t, J = 19.6 Hz, 1P). **$^{31}$P NMR** (122 MHz, $D_2O$, δ/ppm): -11.1 (dt, J = 19.9, 5.5 Hz, 1P), -20.2 (d, J = 19.3 Hz, 1P), -23.9 (t, J = 19.6 Hz, 1P).

**5'-Adenosyl 4-Pentyne hexaphosphate (P2)**



**(P2)**

Anhydr. $ZnCl_2$ (389 mg, 2.84 mmol, 9.5 eq.) and ATP x 2.1 TBA (390 mg, 0.28 mmol, 1.3 eq.) were added to a freshly prepared solution of **S-6** (0.30 mmol). The reaction mixture was then diluted with dry DMF: DMSO (v:v, 1:1, 16 ml) in order to increase the solubility and it was stirred at r.t. for 48 hrs. The crude product was purified by Method B and target product was eluted with 400-600 mM conc. of $NH_4HCO_3$ buffer. Purification gave **P2** as a colorless solid (0.03 g, 0.03 mmol, 13%).

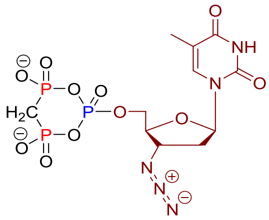
**Note-**: The reactions for capped hexapolyPs were not optimized and therefore the yield could still be improved.

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 8.29 (s, 1H), 8.05 (s, 1H), 5.93 (d, J = 5.8 Hz, 1H), 4.56 (s, 2H), 4.43 – 4.28 (m, 1H), 4.19 (s, 1H), 4.04 (s, 1H), 3.84 (q, J = 6.4 Hz, 2H), 2.25 – 2.08 (m, 3H), 1.64 (p, J = 6.7, 2H). **$^{31}$P{$^{1}$H} NMR** (122 MHz, $D_2O$, δ/ppm): -10.6 – -11.2 (m, 1P), -11.2 – -11.6 (m, 1P), -21.7 – -23.4 (m, 4P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): -10.2 – -11.1 (m, 1P), -11.1 – -11.7 (m, 1P), -20.8 – -23.9 (m, 4P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 155.7, 152.9, 149.2, 139.9, 118.6, 86.6, 85.2, 84.2 (d, J = 9.2 Hz), 74.2, 70.4, 69.5, 65.4 (d, J = 5.6 Hz), 65.04 (d, J = 5.9 Hz), 28.8 (d, J = 7.6 Hz), 14.2 (d, J = 3.9 Hz).

**HRMS** (ESI) m/z for $[C_{15}H_{25}{}^{2}HN_5O_{22}P_6]^+$: calcd. 814.9552, found 814.9548.

### 2.2.2 Synthesis of triphosphates based on *c*-Py$_{CH2}$PA (A$_2$)

**3'-Azido-3'-deoxythymidine 5'-β,γ-methylene cyclotriphosphate (B$_8$)**

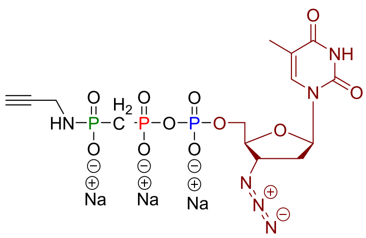



**(B$_8$)**

AZT (50 mg, 0.18 mmol, 1.2 eq.) and ETT (81 mg, 0.62 mmol, 4.0 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an Ar atmosphere, a reaction mixture containing the *c*-Py$_{CH2}$PA **A$_2$** (0.078M in MeCN, 2.0 mL, 0.15 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 40 mg, 0.23 mmol, 1.5 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (a formation of triplet at - 24 ppm which is diagnostic signal for **B$_8$**).

**$^{31}$P{$^{1}$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): 5.06 (d, J = 18.5 Hz, 2P), -24.1 (t, J = 18.6 Hz, 1P). **$^{31}$P NMR** (162 MHz, $CDCl_3$, δ/ppm): 5.06 (q, J = 19.4 Hz, 2P), -23.2 – -24.6 (m, 1P).

**Ring opening by using propargylamine: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-propargylamino β,γ-methylenetriphosphate (22)**




**(22)**

The reaction mixture of **B$_8$** (250 µL, 19.5 µmol, 1.0 eq) was added to propargylamine (250 µL) and $D_2O$ (100 µl) was added. The crude product was purified by Method A, affording a pale yellow solid **22** (9.10 mg, 15.0 µmol, 79%).

**Reaction on large scale (22)**

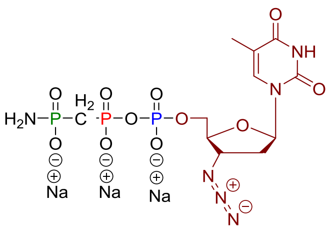
AZT (220 mg, 0.820 mmol, 1.0 eq.) and ETT (325 mg, 2.49 mmol, 3.2 eq.) were coevaporated with dry MeCN (2 × 4 mL). Under Ar atmosphere, a reaction mixture containing the *c*-$Py_{CH2}$PA **$A_2$** (0.078M in MeCN, 10 ml, 0.78 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 10 mins. Upon cooling to -4 C, *m*CPBA (≤77%, 300 mg, 1.74 mmol, 2.2 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (formation of triplet at -24 ppm which is diagnostic signal for the cyclotriphosphate **$B_8$**). Then propargylamine (1.00 ml, 15.6 mmol, 20 eq) was added to the reaction mixture at -4°C and it was stirred at r.t. for 15 mins.

The product **22** was isolated by Method A, affording a colourless solid (411 mg, 0.67 mmol, 85%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.70 (s, 1H), 6.23 (t, J = 6.9 Hz, 1H), 4.52 (q, J = 4.8, 1H), 4.25 – 4.11 (m, 3H), 3.61 (dd, J = 10.1, 2.4 Hz, 2H), 2.54 (t, J = 2.5 Hz, 1H), 2.44 (dd, J = 6.6, 4.6 Hz, 2H), 2.30 (dd, J = 20.6, 18.8 Hz, 2H), 1.89 (d, J = 1.1 Hz, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 17.3 (d, J = 8.6 Hz, 1P), 10.2 (dd, J = 25.0, 8.6 Hz, 1P), -9.95 (d, J = 25.0 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm):17.3 (dq, J = 18.2, 9.0 Hz, 1P), 10.2 (dtd, J = 26.9, 18.2, 6.68 Hz, 1P), -9.96 (dt, J = 26.2, 5.2 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 151.9, 137.3, 111.8, 84.9, 82.9 (d, J = 9.2 Hz), 76.1, 69.9, 65.4 (d, J = 5.5 Hz), 60.9, 36.2, 31.7 – 27.6 (dd, J = 129.2, 107.1 Hz), 11.7.

**HRMS** (ESI) m/z for $[C_{14}H_{20}N_6O_{11}P_3]^-$: calcd. 541.0408, found 541.0409.

**Ring opening by using aq. ammonia: 3’-Azido-3’-deoxythymidine 5’-γ-*P*-amino β,γ-methylene triphosphate (23)**



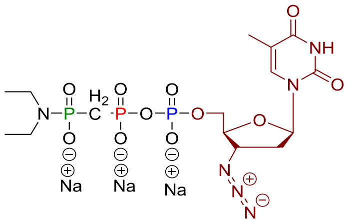
(23)

The reaction mixture of **$B_8$** (250 µL, 19.5 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (250 µL) and then addition of $D_2O$ (100 µl). The product **23** was isolated by Method A, affording a colourless solid (9.54 mg, 16.7 µmol, 86%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.55 (s, 1H), 6.14 (t, J = 6.9 Hz, 1H), 4.41 (td, J =5.7, 5.2, 3.6 Hz, 1H), 4.10 – 3.85 (m, 3H), 2.38 – 2.30 (m, 2H), 2.19 (dd, J =20.6, 19.1 Hz, 2H), 1.77 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 18.2 (d, J = 6.7 Hz, 1P), 9.70 (dd, J = 25.5, 6.7 Hz, 1P), -10.6 (d, J = 25.5 Hz,1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 18.2 (td, J = 18.9, 6.7 Hz, 1P), 9.70 (dtd, J = 26.9, 20.4, 6.7 Hz, 1P), -9.52 – -11.4 (m, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 169.3, 153.8, 136.9, 111.9, 84.9, 82.8 (d, J = 9.3 Hz), 65.4 (d, J = 5.5 Hz), 60.9, 36.2, 31.9 (dd, J = 129.2, 107.1 Hz), 11.9.

**HRMS** (ESI) m/z for $[C_{11}H_{18}N_6O_{11}P_3]^-$: calcd. 503.0252, found 503.0253.

**Ring opening by using diethylamine: 3'-Azido-3'-deoxythymidine 5′-γ-*P*-diethylamino β,γ-methylene triphosphate (24)**



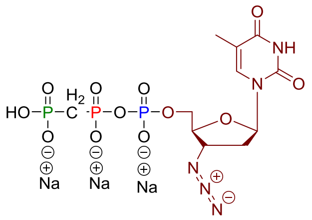
**(24)**

The reaction mixture of **B8** (250 µL, 19.5 µmol, 1.0 eq.) was added to diethylamine (250 µL) and then (100 µl) was added. The product **24** was isolated by Method A, affording a colourless solid (7.10 mg, 11.0 µmol, 59%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.63 (s, 1H), 6.24 (t, J = 6.9 Hz, 1H), 4.66 – 4.40 (m, 1H), 4.24 – 4.03 (m, 3H), 2.90 (dq, J = 9.9, 7.1 Hz, 4H), 2.52 – 2.32 (m, 2H), 2.16 (dd, J =20.6, 19.1 Hz, 2H), 1.85 (d, J = 1.2 Hz, 3H), 0.98 (t, J = 7.1 Hz, 6H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 16.7 (d, J = 9.9 Hz, 1P), 10.2 (dd, J = 26.1, 10.0 Hz, 1P), -10.6 (d, J = 26.2 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 17.3 – 16.3 (m, 1P), 10.1 (dtd, J = 36.0, 20.9, 10.4 Hz, 1P), -10.6 (dt, J = 26.2, 5.2 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.5, 152.9, 137.8, 111.9, 84.7, 83.0 (d, J = 9.5 Hz), 65.6 (d, J = 5.8 Hz), 61.0, 40.5 (d, J = 3.7 Hz), 36.2, 30.9 (dd = 129.4, 107.1 Hz), 13.8 (d, J = 3.7 Hz), 11.7.

**HRMS** (ESI) m/z for $[C_{15}H_{25}N_6O_{11}P_3]^{2-}$: calcd. 279.0403, found 279.0400.

**Ring opening by using aq. sodium hydroxide: 3’-Azido-3’-deoxythymidine 5’-β,γ-methylenetriphosphate (25)**



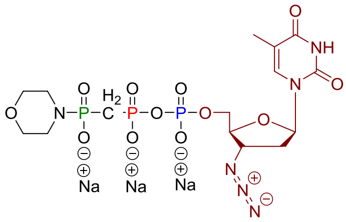
**(25)**

The reaction mixture of **B8** (250 µL, 19.7 µmol, 1.0 eq.) was added to 1M aq. NaOH (250 µL) and then $D_2O$ (100 µl) was added. The crude product was purified by Method A, affording a colourless solid **25** (7.25 mg, 12.0 µmol, 66%). The analytical data are consistent with literature.[10]

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.58 (s, 1H), 6.24 (t, J = 7.0 Hz, 1H), 4.48 (td, J = 5.2, 3.5 Hz, 1H), 4.26 – 4.01 (m, 3H), 2.48 – 2.28 (m, 2H), 2.12 – 1.99 (m, 2H), 1.83 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 13.5 (dd, J = 27.0, 7.0 Hz, 1P), 11.6 (d, J = 6.9 Hz, 1P), -11.1 (d, J = 26.4 Hz). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm):13.5 (dtd, J = 27.9, 21.1, 7.0 Hz, 1P), 11.6 (td, J = 18.7, 7.0 Hz, 1P), -11.2 (dt, J = 27.0, 5.7 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 164.6, 156.5, 136.5, 111.9, 84.8, 82.7 (d, J = 8.9 Hz), 65.4 (d, J = 5.6 Hz), 61.06, 48.8, 30.7 (dd, J = 129.9, 117.4 Hz), 12.4.

**HRMS** (ESI) m/z for $[C_{11}H_{17}N_5O_{12}P_3]^-$: calcd. 504.0092, found 504.0098.

**Ring opening by using morpholine: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-morpholino β,γ-methylene-triphosphate (26)**



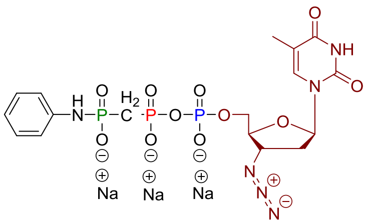
**(26)**

The reaction mixture of **B8** (500 µL, 37.5 µmol, 1.0 eq.) was added to morpholine (500 µL) and then $D_2O$ (200 µl) was added. The product **26** was isolated by Method A, affording a colourless solid (18.2 mg, 28.5 µmol, 76%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.69 (s, 1H), 6.21 (t, J = 6.9 Hz, 1H), 4.56 – 4.42 (m, 1H), 4.23 – 4.03 (m, 3H), 3.60 (m, 4H), 2.98 (m, 4H), 2.50 – 2.34 (m, 2H), 2.24 – 2.00 (m, 2H), 1.86 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 16.4 (d, J = 9.0 Hz, 1P), 9.08 (dd, J = 26.2, 9.1 Hz, 1P), -11.4 (d, J = 26.4 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 16.4 (tdt, J = 17.8, 8.7, 4.4 Hz, 1P), 9.07 (dtd, J = 26.5, 21.2, 9.0 Hz, 1P), -11.4 (dt, J = 27.0, 5.7 Hz, 1P).**$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 151.9, 137.9, 111.8, 84.8, 82.9 (d, J = 9.2 Hz), 67.3 (d, J = 6.7 Hz), 65.4 (d, J = 5.6 Hz), 64.6, 44.4 (d, J = 1.2 Hz), 36.3, 27.9 (dd, J = 130.9, 110.1 Hz), 11.7.

**HRMS** (ESI) m/z for $[C_{15}H_{24}N_6O_{12}P_3]^-$: calcd. 573.0671, found 573.0670.

**Ring opening by using aniline: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-anilino β,γ-methylene triphosphate (27)**



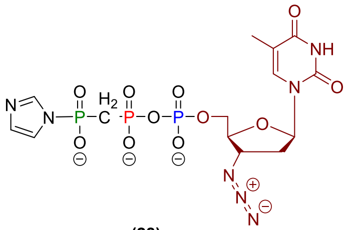
**(27)**

The reaction mixture of **B8** (250 µL, 18.7 µmol, 1.0 eq.) was added to aniline (300 µL) and $D_2O$ (100 µl) was added. The product **27** was isolated by Method A, affording a colourless solid (8.50 mg, 13.1 µmol, 70%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.68 (s, 1H), 7.21 ($m_c$, 2H), 6.92 – 6.76 ($m_c$, 3H), 6.22 (t, J = 6.9 Hz, 1H), 4.57 – 4.45 (m, 1H), 4.24 – 4.06 (m, 3H), 2.48 – 2.39 (m, 2H), 2.27 (t, J = 20.4 Hz, 2H), 1.87 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 14.6 (d, J = 8.4 Hz, 1P), 8.72 (dd, J = 25.6, 8.3 Hz, 1P), -11.3 (d, J = 25.4 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 14.6 (td, J = 19.9, 8.4 Hz, 1P), 8.72 (dtd, J = 29.2, 21.0, 8.5 Hz, 1P), -11.3 (dt, J = 24.8, 5.5 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.5, 151.7, 145.1, 137.3, 129.5, 120.1, 116.8, 111.8, 84.9, 83.0 (d, J = 9.2 Hz), 65.5 (d, J = 5.7 Hz), 60.9, 36.3, 29.6 (dd, J = 130.9, 110.1 Hz), 11.7.

**HRMS** (ESI) m/z for $[C_{17}H_{22}N_6O_{11}P_3]^-$: calcd. 579.0565, found 579.0569.

**Ring opening by using imidazole: 3'-Azido-3'-deoxythymidine 5'-*γ*-*P*-imidazole β,γ-methylene triphosphate (28)**



**(28)**

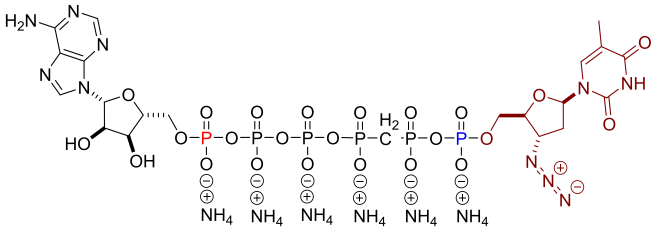
AZT (0.12 g, 0.46 mmol, 1.2 eq.) and ETT (0.15 g, 1.2 mmol, 3.0 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an Ar atmosphere, a reaction mixture containing the $Py_{CH2}PA$ **$A_2$** (0.078M in MeCN, 5.0 mL, 0.39 mmol, 1.0 eq.) was added to the dried solids and the mixture was stirred at r.t. for 10 mins. Upon cooling to -10°C, *m*CPBA (≤77%, 0.13 g, 0.78 mmol, 2.0 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (reaction progress was followed by $^{31}$P NMR, and formation of triplet at -24 ppm which is diagnostic signal for the **$B_8$**).

A solution of imidazole in dry DMF (1.5M, 10 ml, 15 mmol, 37.0 eq.) was added to the cyclotriphosphate **$B_8$** and reaction was stirred for another 20 mins (the reaction progress was followed by $^{31}$P NMR) which results in quantative conversion to **28**.

**Note** -: Imidazole was dried before using by coevaporation with MeCN and it was further stored of molecular sieve (3Å).

**$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 8.33 (d, J = 8.1 Hz, 1P), 4.65 (dd, J = 25.7, 8.0 Hz, 1P), -11.6 (d, J = 25.7 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 8.76 – 7.98 (m, 1P), 4.58 (dtd, J = 26.5, 21.2, 9.0 Hz, 1P), -11.6 (d, J = 26.3, 1P).

**5'-Adenosyl 3'-azido-3'-deoxythymidinyl δ,ε-methylene hexaphosphate (P3)**



**(P3)**

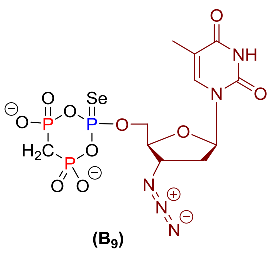
Anhydr. $ZnCl_2$ (0.25 g, 1.81 mmol, 9.3 eq.) and ATP x 2.1 TBA (0.48 g, 0.39 mmol, 2.0 eq.) were added to a freshly prepared solution of **28** (0.19 mmol). The reaction mixture was then diluted with dry DMF: DMSO (v:v, 1:1, 16 ml) in order to increase the solubility and it was stirred at r.t. for 48 hrs. The crude product was purified by Method B and the target product was eluted with 500-600 mM conc. of $NH_4HCO_3$ buffer. Purification gave **P3** as a colorless solid (0.027 g, 0.02 mmol, 13%).

**Note** -: The reactions for capped hexapolyPs were not optimized and therefore the yield could still be improved.

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 8.47 (s, 1H), 8.16 (s, 1H), 7.61 (s, 1H), 6.14 (t, J = 6.9 Hz, 1H), 6.04 (d, J = 6.2 Hz, 1H), 4.57 – 4.41 (m, 2H), 4.33 (t, J = 2.9 Hz, 1H), 4.25 – 4.04 (m, 5H), 2.66 – 2.26 (m, 5H), 1.80 (s, 3H). The DHO peak overlaps with the signal 4.88 (1H).. **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 7.46 (dd, J = 21.3, 12.8 Hz, 2P), -8.27 – -13.6 (m, 2P), -23.02- – -22.4 (m, 2P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 8.78 – 6.84 (m, 2P), -9.62 – -12.9 (m, 2P), -19.5 – -24.9 (m, 2P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.4, 155.6, 152.8, 151.6, 149.1, 139.9, 137.1, 118.5, 111.7, 86.6, 84.8, 84.2 (d, J = 9.3 Hz), 82.9 (d, J = 9.3 Hz), 74.3, 70.4, 65.4 (d, J = 5.6 Hz), 65.4 (d, J = 5.6 Hz), 60.9, 36.2, 27.9 (dd, J = 130.9, 110.1 Hz), 11.6.

**HRMS** (ESI) m/z for $[C_{21}H_{31}N_{10}O_{24}P_6]^-$: calcd. 992.9944, found 992.9942.

**AZT 5′-α-P-Seleno-β,γ-methylenecyclotriphosphate: 3'-Azido-3'-deoxythymidine 5'-α-*P*-seleno-β,γ-methylene 5′-cyclotriphosphate ($B_9$)**



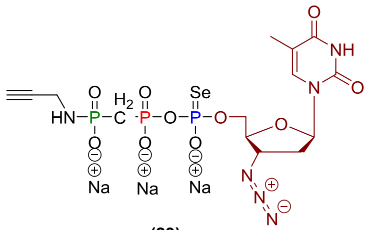
**($B_9$)**

AZT (120 mg, 0.45 mmol, 1.2 eq.) and ETT (147 mg, 1.12 mmol, 3.0 eq.) were coevaporated with dry MeCN (2 × 4 mL). Under an Ar atmosphere, a reaction mixture containing the *c*-Py$_{CH2}$PA **A$_2$** (0.075M in MeCN, 5.00 mL, 0.37 mmol, 1.0 eq.) was added and the mixture was stirred at r.t. for 10 mins. Upon cooling to 0°C, potassium selenocyanate (550 mg, 3.82 mmol, 10.5 eq.) was added and the mixture was stirred for 10 mins. A colourless precipitate was observed and acetone was added to complete the precipitation. The precipitate was then collected *via* centrifugation and a $^{31}$P-NMR confirmed the formation of **B9**. Afterwards, the crude product was purified by Method B, affording the pure product eluted at 150 mM – 250 mM conc. of $NH_4HCO_3$ buffer as a colourless solid **B9** (0.09 g, 0.16 mmol, 42%).

**Note -**: The isolated product **B9** was very unstable.

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.73 (s, 1H), 6.22 (t, J = 6.8, 1H), 4.57 – 4.50 (m, 1H), 4.32 – 4.14 (m, 3H), 2.68 – 2.50 (m, 2H), 2.43 (m, 2H), 1.91 (s, 3H). **$^{31}$P {$^1$H} NMR** (122 MHz, D2O, δ/ppm): 32.2 (dd, J = 35.7, 27.0 Hz, 1P), 7.91 – 7.24 (m, 2P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 32.2 (dd, J = 35.2, 27.7 Hz, 1P), 9.42 – 5.79 (m, 2P). **$^{13}$C NMR** (126 MHz, $D_2O$, δ/ppm): 166.6, 151.7, 137.3, 111.9 (d, J = 2.6 Hz), 84.9 (d, J = 4.4 Hz), 82.8 (t, J = 9.3 Hz), 65.8 (dd, J = 61.3, 6.2), 61.0 (d, J = 44.6 Hz), 36.3 (d, J = 12.4 Hz), 32.4, 11.9.

**Ring opening by using propargylamine: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-propargylamino α-*P*-seleno-β,γ-methylene-triphosphate (29)**



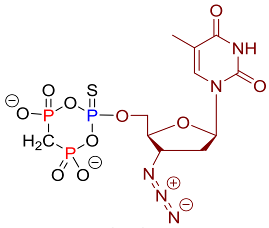
**(29)**

Freshly prepared **B9** was dissolved, after precipitation but without any purification, in $D_2O$ (1 mL). propargylamine (1 mL) was added and the mixture was stirred at r.t. for 15 mins. The product **29** was obtained after purification with Method B, eluted with 300-450 mM conc. of $NH_4HCO_3$ buffer, as colourless solid (111 mg, 0.16 mmol, 45%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.72 (s, 1H), 6.22 (t, J = 6.8, 1H), 4.58 – 4.49 (m, 1H), 4.34 – 4.11 (m, 3H), 3.61 (dd, J = 10.1, 2.5 Hz, 2H), 2.56 – 2.51 (m, 1H), 2.48 – 2.39 (m, 2H), 2.34 – 2.23 (m, 2H), 1.90 (s, 3H). **$^{31}$P {$^1$H} NMR** (122 MHz, D2O, δ/ppm): 32.0 (dd, J = 37.1, 13.7 Hz, 1P), 17.6 (d, J = 7.4 Hz, 1P), 8.4 (ddd, J = 36.7, 7.3, 4.9 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 33.1 – 31.5 (m, 1P), 18.8 – 16.9 (m, 1P), 9.54 – 7.90 (m, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.6, 151.7, 137.3 (d, J = 2.6 Hz), 111.8, 84.8 (d, J = 15.8 Hz), 82.7, 71.5, 65.7 (dd, J = 47.6, 6.0 Hz), 60.9 (d, J = 45.2 Hz), 36.2 (d, J = 8.4 Hz), 30.9, 31.0 – 27.6 (dd, J = 129.9, 117.4), 11.8.

**HRMS** (ESI) m/z for $[C_{14}H_{22}N_6O_{10}P_3Se]^+$: calcd. 606.9770, found 606.9769.

**3'-Azido-3'-deoxythymidine 5'-*α-P*-thio β,γ-methylene cyclotriphosphate ($B_{10}$)**

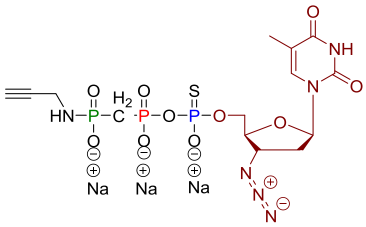


**($B_{10}$)**

AZT (50 mg, 0.18 mmol, 1.2 eq.) and ETT (78 mg, 0.60 mmol, 4.0 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an Ar atmosphere, a reaction mixture containing the $Py_{CH2}PA$ **$A_2$** (0.075M in MeCN, 2 mL, 0.15 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, Becauge's reagent (50 mg, 0.25 mmol, 1.7 eq.) was added and the mixture was stirred for 10 mins until $^{31}$P-NMR confirmed complete oxidation (formation of triplet at +40 ppm which is diagnostic signal for the α-(S)-cyclotriphosphate **$B_{10}$**).

**$^{31}$P {$^1$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): 39.3 (t, J = 30.6 Hz, 1P), 2.38 (d, J = 30.5 Hz, 2P). $^{31}$P NMR (122 MHz, $CDCl_3$, δ/ppm): 40.3 (m, 1P), 2.38 (dt, J = 30.6, 19.5 Hz, 2P).

**Ring opening by using propargylamine: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-propargylamino α-*P*-thio β,γ-methylene triphosphate (30)**

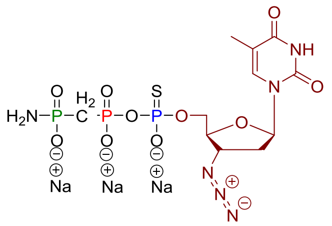


**(30)**

The reaction mixture of **$B_{10}$** (250 µL, 18.7 µmol, 1.0 eq.) was added to propargylamine (250 µL) and $D_2O$ (100 µl) was added. The product **30** was isolated by Method A, affording a colourless solid whereas products were obtained as mixtures of diastereoisomers (8.70 mg, 14.0 µmol, 75%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.74 (s, 1H), 6.23 (t, J = 6.9 Hz, 1H), 4.57 – 4.46 (m, 1H), 4.27 – 4.12 (m, 3H), 3.62 (dd, J = 9.9, 2.3 Hz, 2H), 2.60 – 2.38 (m, 5H), 1.91 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 42.1 (dd, J = 33.3, 23., 1P), 19.0 – 16.6 (m, 1P), 9.28 – 8.47 (m, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 42.7 – 41.5 (m, 1P), 17.9 (td, J = 18.6, 6.1 Hz, 1P), 9.57 – 8.23 (m, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 151.9, 137.3, 111.8, 84.9, 82.9 (d, J = 9.2 Hz), 76.1, 69.9, 65.4 (d, J = 5.5 Hz), 60.9, 36.2, 31.7 – 27.6 (dd, J = 129.2, 107.1 Hz), 11.7.

**Ring opening by using aq. ammonia: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-amino *α*-*P*-thio β,γ-methylene triphosphate (31)**

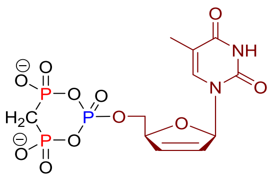


(31)

The reaction mixture of **B10** (250 µL, 18.7 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (250 µL) and of $D_2O$ (100 µl) was added. The product **31** was isolated by Method A, affording a colourless solid whereas products were obtained as mixtures of diastereoisomers (8.50 mg, 14.5 µmol, 80%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.73 (s, 1H), 6.23 (t, J = 6.9 Hz, 1H), 4.65 – 4.47 (m, 1H), 4.46 – 4.20 (m, 3H), 2.57 – 2.41 (m, 2H), 2.33 (m, 2H), 1.90 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 45.8 – 43.2 (m, 1P), 17.2 (d, J = 8.1, 1P), 9.80 (d, J = 31.4, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 45.3 – 43.6 (m, 1P), 18.1 – 16.4 (m, 1P), 10.7 – 9.10 (m, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 169.3, 153.8, 136.9, 111.9, 84.9, 82.8 (d, J = 9.3 Hz), 65.4 (d, J = 5.5 Hz), 60.9, 36.2, 31.9 (dd, J = 129.2, 107.1 Hz), 11.9.

**2',3'-didehydro-2',3'-dideoxythymidine 5'-β,γ-methylene cyclotriphosphate (B11)**

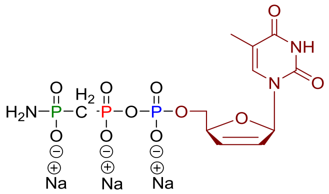


(B11)

d4T (50 mg, 0.22 mmol, 1.5 eq.) and ETT (69 mg, 0.52 mmol, 3.5 eq.) were coevaporated with dry MeCN (2 × 1 mL) and the dried solids were dissolved in dry DMF (1 mL). Under an Ar atmosphere, a reaction mixture containing the $Py_{CH2}PA$ **A2** (0.075M in MeCN, 2.0 mL, 0.15 mmol, 1.0 eq.) was added and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 39 mg, 0.22 mmol, 1.5 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (formation of triplet at -25 ppm which is diagnostic signal for the d4T cyclotriphosphate **B11**).

**$^{31}$P{$^1$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): 5.16 (d, J = 18.5 Hz, 2P), -24.9 (t, J = 18.6 Hz, 1P).

**Ring opening by using aq. ammonia: 2',3'-didehydro-2',3'-dideoxythymidine 5'-γ-*P*-amino β,γ-methylene triphosphate (32)**



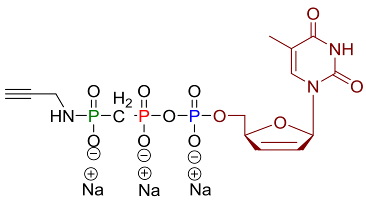
**(32)**

The reaction mixture of **B₁₁** (250 µL, 12.5 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (250 µL) and then $D_2O$ (100 µl) was added. The product **32** was isolated by Method A, affording a colourless solid (5.31 mg, 10.7 µmol, 80%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.53 (s, 1H), 6.89 (m, 1H), 6.45 (m, 1H), 5.87 (m, 1H), 5.03 (d, J = 4.2 Hz, 1H), 4.07 (dt, J = 6.1, 3.1 Hz, 2H), 2.25 (dd, J = 20.6, 19.1 Hz, 2H), 1.82 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 17.9 (d, J = 6.1 Hz, 1P), 9.14 (dd, J = 26.1, 6.1 Hz, 1P), -11.5 (d, J = 26.1 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 17.9 (td, J = 19.1, 6.1 Hz, 1P), 8.91 (dtd, J = 26.4, 20.3, 6.1 Hz, 1P), -11.4 (dt, J = 26.1, 6.6 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 167.3, 152.6, 138.1, 134.2, 125.2, 111.5, 89.9, 85.9 (d, J = 8.7 Hz), 66.3 (d, J = 5.7 Hz), 31.9 (dd, J =129.8, 106.3 Hz), 11.5.

**HRMS** (ESI) m/z for $[C_{11}H_{16}N_3NaO_{11}P_3]^-$: calcd. 481.9901, found 481.9901.

**Ring opening by using propargylamine: 2',3'-didehydro-2',3'-dideoxythymidine 5'-γ-*P*-propargylamino β,γ-methylene triphosphate (33)**



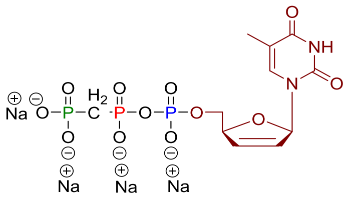
**(33)**

The reaction mixture of **B₁₁** (250 µL, 12.5 µmol, 1.0 eq.) was added to propargylamine (250 µL) and then $D_2O$ (100 µl) was added. The product **33** was isolated by Method A, affording a colourless solid (5.45 mg, 9.64 µmol, 75%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.54 (dd, J = 5.3, 1.4 Hz, 1H), 6.89 (m, 1H), 6.45 (m, 1H), 5.87 (m, 1H), 5.03 (dq, J = 3.7, 1.9 Hz, 1H), 4.07 (dt, J = 5.7, 2.7 Hz, 2H), 3.56 (d, J = 10.1 Hz, 1H), 2.34 – 2.17 (m, 2H), 1.83 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 17.8 (d, J = 7.8 Hz, 1P), 8.53 (dd, J = 26.2, 7.1 Hz, 1P), -11.4 (d, J = 27.1, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 18.6 – 17.4 (m, 1P), 8.5 (dtd, J = 27.5, 20.9, 7.3 Hz, 1P), -11.4 (dt, J = 26.3, 6.3 Hz, 1P).**$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 152.3, 138.1 (d, J = 4.4 Hz), 134.3, 125.2, 111.5, 89.9, 85.9 (dd, J = 8.7, 3.0 Hz),74.7, 66.3 (d, J = 5.6 Hz), 30.4 (d, J = 37.9 Hz), 29.0 (dd, J =129.8, 106.3 Hz), 11.5.

**HRMS** (ESI) m/z for $[C_{14}H_{19}N_3O_{11}P_3]^-$ :calcd. 498.0238, found 498.0238.

**Ring opening by using aq. sodium hydroxide: 2',3'-didehydro-2',3'-dideoxythymidine 5'-β,γ-methylene triphosphate (34)**



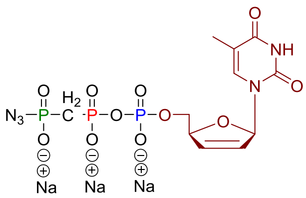
(34)

The reaction mixture of **B11** (250 µL, 12.5 µmol, 1.0 eq.) was added to aq. NaOH (1M, 250 µL) and then $D_2O$ (100 µl) was added. The product **34** was isolated by Method A, affording a colourless solid (4.92 mg, 8.94 µmol, 71%). The analytical data are consistent with literature. [11]

**$^{1}$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.51 (s, 1H), 6.89 (dt, J = 3.4, 1.7 Hz, 1H), 6.44 (dt, J = 6.1, 1.8 Hz, 1H), 5.86 (dt, J = 6.2, 1.9 Hz, 1H), 5.19 – 4.95 (m, 1H), 4.06 (dd, J = 6.6, 3.7 Hz, 1H), 2.09 (dd, J = 21.3, 18.9 Hz, 1H), 1.81 (s, 2H). **$^{31}$P{$^{1}$H} NMR** (122 MHz, D2O, δ/ppm): 13.07 (dd, J = 25.4, 6.6 Hz, 1P), 11.7 (d, J = 6.8 Hz, 1P), -11.1 (d, J = 26.5 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 14.1 – 12.4 (m, 1P), 12.3 – 11.4 (m, 1P), -11.1 (dt, J = 26.7, 6.7 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 168.7, 153.7, 137.9, 134.1, 125.3, 111.6, 90.0, 85.9 (d, J = 8.5 Hz), 66.3 (d, J = 5.5 Hz), 30.6 (dd, J = 129.5, 117.8 Hz), 11.7.

**HRMS** (ESI) m/z for $[C_{11}H_{16}N_2O_{12}P_3]^-$ : calcd. 460.9922, found, 460.9928.

**Ring opening by tetramethyl guanidinium azide: 2',3'-didehydro-2',3'-dideoxythymidine 5'-γ-*P*-azido β,γ-methylene triphosphate (35)**



(35)

The reaction mixture of **B11** (250 µL, 12.5 µmol, 1.0 eq.) was added to tetramethyl guanidinium azide (400 mg, 2.52 mmol, 200 eq.) and then $D_2O$ (200 µl) were added. The crude product was isolated by Method A and the product was precipitated as guanidinium salt which was again by dissolved in water (2 mL).Afterwards, the product **35** was isolated from the aq. solution again by Method A and a colourless solid was obtained (6.40 mg, 11.2 µmol, 92%).

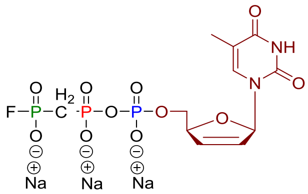
**Note** -: Alternative, lithium azide can also be used as a nucleophile for ring-opening. But, commercially available $LiN_3$ solution is 20% wt. in water which results in $H_2O$ acts as a competitive nucleophile giving rise to lower yield. In this case, simultaneously, $H_2O$ is also acting as a nucleophile the product was obtained as a mixture of **34:35** in 20:80 ratio.

Additionally, it was found that triphosphoazidate **35** is decomposed into the **34** in two weeks which, was further confirmed by spiking experiments (see the NMR supporting).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.45 (s, 1H), 6.90 (m, 1H), 6.44 (m, 1H), 5.86 (m, 1H), 5.19 – 4.95 (m, 1H), 4.06 (dd, J = 6.6, 3.7 Hz, 2H), 2.09 (dd, J = 21.3, 18.9 Hz, 2H), 1.81 (s, 3H).**$^{31}$P{$^1$H} NMR** (162 MHz, $D_2O$, δ/ppm) 16.4 (d, J = 7.8 Hz, 1P), 6.94 (dd, J = 24.1, 8.0 Hz, 1P), -10.5 (d, J = 23.9 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 16.4 (td, J = 19.7, 7.8 Hz, 1P), 8.07 – 5.95 (m, 1P), -10.5 (td, J = 24.4, 6.2 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 167.3, 152.6, 138.1, 134.2, 125.2, 111.5, 89.9, 85.9 (d, J = 8.7 Hz), 66.3 (d, J = 5.7 Hz), 31.9 (dd, J =129.8, 106.3 Hz), 11.5.

**HRMS** (ESI) m/z for $[C_{11}H_{15}N_5O_{11}P_3]^-$ : calcd. 485.9986, found, 485.9987.

**Ring opening by caesium fluoride: 2’,3’-didehydro-2’,3’-dideoxythymidine 5’-γ-*P*-fluoro β,γ-methylene triphosphate (36)**



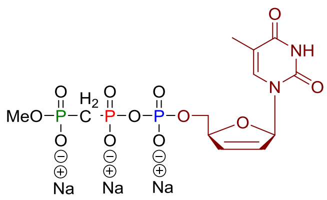
**(36)**

The reaction mixture of **$B_{11}$** (250 µL, 11.2 µmol, 1.0 eq.) was added to caesium fluoride (105 mg, 691 µmol, 58 eq.) and then immediately $D_2O$ (250 µl) was added. The crude product was isolated by Method A and the impurity $CsClO_4$ was co-precipitated which was further removed by dissolving the crude product in water (2 mL). The insoluble residue was removed by filtration (syringe filter, pore size 0.45 µm). Afterwards, the product **36** was isolated from the aq. solution again by Method A and a colourless solid was obtained (5.8 mg, 10.9 µmol, 97%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.51 (s, 1H), 6.89 (dt, J = 3.4, 1.7 Hz, 1H), 6.44 (dt, J = 6.1, 1.8 Hz, 1H), 5.86 (dt, J = 6.2, 1.9 Hz, 1H), 5.19 – 4.95 (m, 1H), 4.06 (dd, J = 6.6, 3.7 Hz, 2H), 2.09 (dd, J = 21.3, 18.9 Hz, 2H), 1.81 (s, 3H).**$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 17.3 (dd, J = 962.3, 7.9 Hz; 1P), 6.06 (m, 1P), -11.1 (d, J = 24.3 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 17.8 (dtd, J = 971.1, 21.2, 9.4 Hz, 1P), 5.98 (qd, J = 21.9, 21.3, 9.2 Hz, 1P), -11.3 (dt, J = 24.9, 6.2 Hz, 1P). **$^{31}$P{$^{19}$F} NMR** (162 MHz, $D_2O$, δ/ppm): 17.8 (td, J = 21.2, 9.3, 1P), 5.98 (dtd, J = 24.9, 20.9, 9.5 Hz, 1P), -11.3 (dt, J = 24.6, 6.0 Hz,1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 168.7, 153.7, 137.9, 134.1, 125.3, 111.6, 90.0, 85.9 (d, J = 8.5 Hz), 66.3 (d, J = 5.5 Hz), 30.6 (dd, J = 129.5, 117.8 Hz), 11.7. **$^{19}$F NMR** (377 MHz, $D_2O$, δ/ppm): -54.5 (d, J = 971.5 Hz, 1F).

**HRMS** (ESI) m/z for $[C_{11}H_{14}FN_2NaO_{11}P_3]^-$ : calcd. 484.9490, found, 484.9479.

**Ring opening by using methanolic sodium methoxide: 2’,3’-didehydro-2’,3’-dideoxythymidine 5′-γ-*P*-methoxy β,γ-methylene triphosphate (37)**



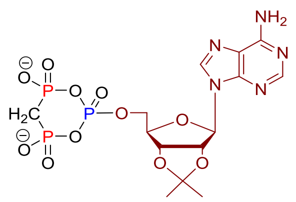
(37)

The reaction mixture of **B11** (250 µL, 11.2 µmol, 1.0 eq.) was added to NaOMe (0.5M in methanol, 1.00 mL, 50.0 µmol, 4.5 eq.) and then $D_2O$ (200 µl) was added. The product **37** was isolated by Method A, affording a colourless solid (4.90 mg, 9.03 µmol, 81%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.52 (s, 1H), 6.88 (s, 1H), 6.44 (d, J = 6.0 Hz, 1H), 5.88 (d, J = 6.2 Hz, 1H), 5.04 (s, 1H), 4.05 (dd, J = 6.4, 3.6 Hz, 2H), 3.50 (d, J = 10.8 Hz, 2H), 3.12 (d, J = 7.6 Hz, 1H), 2.15 (s, 5H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 18.7 (d, J = 8.0 Hz, 1P), 7.97 (dd, J = 25.4, 7.8 Hz, 1P), -11.0 (m, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 19.4 – 18.2 (m, 1P), 7.96 (dtd, J = 25.0, 20.4, 7.7 Hz, 1P), -11.2 (dt, J = 25.3, 6.5 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 168.7, 153.7, 137.9, 134.1, 125.3, 111.6, 90.0, 85.9 (d, J = 8.5 Hz), 66.3 (d, J = 5.5 Hz), 51.7 (d, J = 5.6 Hz), 30.6 (dd, J = 129.5, 117.8 Hz), 11.5.

**HRMS (ESI)** m/z for $[C_{12}H_{18}N_2O_{12}P_3]^-$ : calcd. 475.0078, found, 475.0075.

**2’,3’-O-Isopropylideneadenosine β,γ-methylene cyclotriphosphate (B12)**

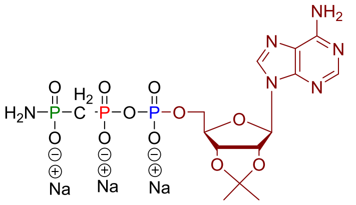


($B_{12}$)

2', 3’-O-Isopropylideneadenosine (59 mg, 0.19 mmol, 1.2 eq.) and ETT (60 mg, 0.49 mmol, 3.0 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an Ar atmosphere, both solids were dissolved in dry DMF (1 mL), a reaction mixture containing the $Py_{CH2}PA$ **A2** (0.078M, 2.0 mL, 0.15 mmol, 1.0 eq.) in MeCN was added and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 47 mg, 0.27 mmol, 1.8 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (formation of triplet at -23 ppm which is diagnostic signal for the cyclotriphosphate **B12**).

**$^{31}$P{$^1$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): 5.79 (d, J = 18.5 Hz, 2P), -25.0 (t, J = 18.6 Hz, 1P).

**Ring opening by using aq. ammonia: 2',3'-O-Isopropylideneadenosine 5'-γ-*P*-amino β,γ-methylenetriphosphate (38)**



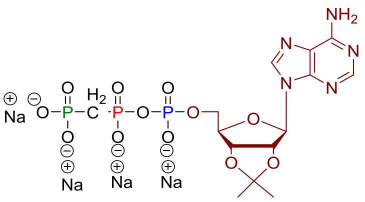
**(38)**

The reaction mixture of **$B_{12}$** (250 μL, 13.0 μmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (250 μL) and then $D_2O$ (100 μl) was added. The product **38** was isolated by Method A, affording a colourless solid (6.80 mg, 11.1 μmol, 85%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 8.37 (s, 1H), 8.18 (s, 1H), 6.21 (d, J = 3.5 Hz, 1H), 5.34 (dd, J = 6.2, 3.5 Hz, 1H), 5.17 (dd, J = 6.2, 2.2 Hz, 1H),4.61 (The DHO peak overlaps with the signal (1H)), 4.09 (m, 2H), 2.25 (dd, J = 20.7, 19.1 Hz, 2H), 1.61 (s, 3H), 1.38 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 18.1 (d, J = 7.1 Hz, 1P), 9.88 (dd, J = 25.0, 7.0 Hz, 1P), -10.5 (d, J = 24.9 Hz, 1P).**$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 18.1 (td, J = 18.8, 6.8 Hz, 1P), 10.4 – 9.39 (m, 1P), -10.5 (dt, J = 24.9, 6.5 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 155.6, 152.9, 148.8, 140.0, 118.7, 114.9, 90.0, 84.5 (d, J = 9.4 Hz), 83.1, 81.3, 65.5 (d, J = 5.4 Hz), 31.9 (dd, J = 129.4, 106.3 Hz), 26.1, 24.3.

**HRMS** (ESI) m/z for $[C_{14}H_{22}N_6O_{11}P_3]^-$: calcd. 543.0565, found, 543.0566.

**Ring opening by using aq. sodium hydroxide: 2',3'-O-Isopropylideneadenosine 5'-β,γ-methylene triphosphate (39)**



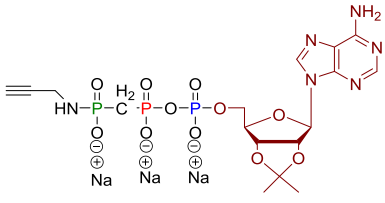
**(39)**

The reaction mixture of **$B_{12}$** (250 μL, 13.0 μmol, 1.0 eq.) was added to 1M aq. NaOH (250 μL) and then $D_2O$ (100 μl) was added. The product **39** was isolated by Method A, affording a colourless solid (5.98 mg, 9.44 μmol, 72%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 8.39 (s, 1H), 8.18 (s, 1H), 6.21 (d, J = 3.5 Hz, 1H), 5.33 (dd, J = 6.1, 3.6 Hz, 1H), 5.17 (dd, J = 6.2, 2.2 Hz, 1H), 4.61 (The DHO peak overlaps with the signal (1H)), 4.28 – 4.02 (m, 2H), 2.20 – 1.99 (m, 2H), 1.61 (s, 3H), 1.38 (s, 3H).**$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 15.1 (dd, J = 23.9, 6.2 Hz, 1P), 12.9 (d, J = 6.6 Hz, 1P), -10.0 (d, J = 23.9 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 15.5 – 14.7 (m, 1P), 12.9 (td, J = 18.4, 6.6 Hz, 1P), -10.0 (dt, J = 24.2, 6.5 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 155.6, 152.9, 148.8, 140.0, 118.7, 114.9, 90.0, 84.5 (d, J = 9.4 Hz), 83.1, 81.3, 65.5 (d, J = 5.3 Hz), 32.0 (dd, J = 128.9, 106.5 Hz), 26.1, 24.3.

**HRMS** (ESI) m/z for $[C_{14}H_{20}N_5NaO_{12}P_3]^-$: calcd. 566.0224, found, 566.022

**Ring opening by propargylamine: 2',3'-*O*-Isopropylideneadenosine 5'-γ-*P*-propargylamino β,γ-methylenetriphosphate (40)**



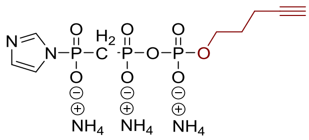
**(40)**

The reaction mixture of $\mathbf{B_{12}}$ (250 µL, 13.0 µmol, 1.0 eq.) was added to propargylamine (250 µL) and then $D_2O$ (100 µl) was added. The product **40** was isolated by Method A, affording a colourless solid (5.54 mg, 8.54 µmol, 65%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 8.39 (s, 1H), 8.18 (s, 1H), 6.21 (d, J = 3.5, 1H), 5.34 (dd, J=6.1, 3.5, 1H), 5.17 (dd, J = 6.1, 2.2 Hz, 1H), 4.61 (The DHO peak overlaps with the signal (1H)), 4.17 – 4.03 (m, 2H), 3.53 (dd, J = 10.1, 2.4 Hz, 2H), 2.42 (t, J = 2.5 Hz, 1H), 2.24 (dd, J = 20.6, 18.9 Hz, 2H), 1.61 (s, 3H), 1.38 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 17.7 (d, J = 7.1 Hz, 1P), 8.70 (dd, J = 26.3, 7.1 Hz, 1P), -11.5 (d, J = 26.2 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 18.9 – 16.9 (m, 1P), 8.70 (dtd, J = 27.5, 20.8, 7.2, 1P), -11.5 (dt, J = 27.5, 5.2 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.6, 155.6, 152.9, 148.8, 140.0, 118.7, 114.9, 90.0, 84.6 (d, J = 9.4 Hz), 83.9, 81.3, 65.6 (d, J = 4.9Hz), 30.6 (dd, J = 128.9, 106.5 Hz), 29.3, 26.1, 24.3.

**HRMS** (ESI) m/z for $[C_{17}H_{22}N_6Na_3O_{11}P_3]^-$: calcd: 648.0252, found, 648.0259.

**Ring opening by using imidazole**: **4'-Pentyne γ-imidazole β,γ-methylene triphosphate (S-7)**



**(S-7)**

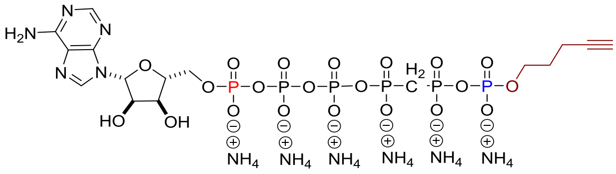
4-Pentyn-1-ol (40 mg, 0.45 mmol, 1.5 eq.) and ETT (98 mg, 0.75 mmol, 2.5 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an atmosphere of dry Ar, a $Py_{CH2}PA$ $\mathbf{A_2}$ (0.075M in MeCN, 4.0 mL, 0.30 mmol, 1.0 eq.) was added in the above reaction mixture. The mixture was stirred at r.t. for 5 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 79 mg, 0.45 mmol, 1.5 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation.

A solution of imidazole in dry DMF (1.47M, 10.0 ml, 14.7 mmol, 49.0 eq.) was added to the cyclotriphosphate and the reaction was stirred for another 15 mins (the reaction progress was followed by $^{31}$P NMR) which results in quantative conversion to **S-7**.

**Note** -: Imidazole was dried before using by coevaporation with MeCN and it was further stored of molecular sieve (3Å).

**$^{31}$P{$^{1}$H} NMR** (122 MHz, D2O, δ/ppm): 8.80 (d, J = 7.8 Hz, 1P), 4.30 (dd, J = 25.8, 8.1 Hz, 1P), -10.9 (d, J = 25.5 Hz, 1P).

**5'-Adenosyl 4-Pentyne δ,ε-methylene hexaphosphate (P4)**




(P4)

Anhydr. $ZnCl_2$ (0.33 g, 2.4 mmol, 11 eq.) and ATP x 2.1 TBA (0.40 g, 0.39 mmol, 1.7 eq.) were added to a freshly prepared solution of **S-7** (0.22 mmol). The reaction mixture was then diluted with dry DMF: DMSO (v:v, 1:1, 16 ml) in order to increase the solubility and it was stirred at r.t. for 20 hrs. The crude product was purified by Method B and the target product was eluted with 550-700 mM conc. of $NH_4HCO_3$ buffer. Purification gave **P4** as a colorless solid (0.03 g, 0.04 mmol, 22%).

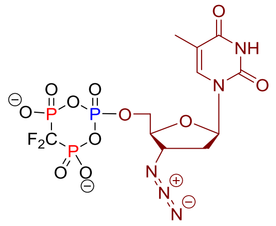
**Note** -: The reaction for capped hexapolyPs were not optimized.

**$^{1}$H NMR** (500 MHz, $D_2O$, δ/ppm): 8.47 (s, 1H), 8.19 (s, 1H), 6.07 (d, J = 6.2 Hz, 1H), 4.74 (t, J = 5.7 Hz, 1H), 4.53 (dd, J = 5.2, 3.3 Hz, 1H), 4.35 (m, J = 2.9 Hz, 1H), 4.22 (dt, J = 6.1, 2.9 Hz, 1H), 4.15 (ddd, J = 11.7, 4.6, 3.0 Hz, 1H), 3.96 (q, J = 6.5 Hz, 2H), 2.44 (t, J = 21.1 Hz, 2H), 2.36 – 2.24 (m, 3H), 1.79 (m, 2H).**$^{31}$P{$^{1}$H} NMR** (202 MHz, $D_2O$, δ/ppm): 8.61 – 7.57 (m, 1P), 7.31 – 6.77 (m, 1P), -10.6 (d, J = 26.1 Hz, 1P), -11.3 (d, J = 22.1 Hz, 1P), -22.5 – -23.8 (m, 2P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 8.37 – 7.59 (m, 1P), 7.59 – 6.29 (m, 1P), -10.6 (dt, J = 26.0, 7.1, 1P), -11.4 (d, J = 16.6, 5.1 Hz, 1P), -21.6 – -23.2 (m, 2P). **$^{13}$C NMR** (126 MHz, $D_2O$, δ/ppm): 155.7, 152.9, 149.2, 139.9, 118.6, 86.6, 85.2, 84.2 (d, J = 9.2 Hz), 74.2, 70.4, 69.5, 65.4 (d, J = 5.6 Hz), 65.0 (d, J = 5.9 Hz), 29.9 (t, J = 131.5 Hz), 28.8 (d, J = 7.6 Hz), 14.2 (d, J = 3.9 Hz).

**HRMS** (ESI) m/z for $[C_{16}H_{27}DN_5O_{21}P_6]^+$calcd 812.9760, found 812.9750.

### 2.2.3 Synthesis of triphosphates based on *c*-Py$_{CF2}$PA (A$_3$)

**3’-Azido-3’-deoxythymidine β,γ-difluoromethylene cyclotriphosphate (B$_{13}$)**



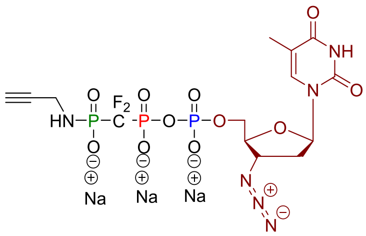
(B$_{13}$)

AZT (44 mg, 0.16 mmol, 1.5 eq.) and ETT (50 mg, 0.38 mmol, 3.5 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an Ar atmosphere, a reaction mixture containing the *c*-Py$_{CF2}$PA **A$_3$** (0.073M in MeCN, 1.5 mL, 0.11 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 24 mg, 0.16 mmol, 1.5 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (a formation of triplet at - 25 ppm which is diagnostic signal for **B$_{13}$**).

**$^{31}$P{$^1$H} NMR** (122 MHz, $CD_3CN$, δ/ppm): -9.21 (dt, J = 87.1, 23.8 Hz, 2P), -25.4 (t, J = 23.6 Hz, 1P).

**$^{31}$P NMR** (162 MHz, $CD_3CN$, δ/ppm):-9.22 (dt, J = 86.8, 23.6 Hz, 2P), -24.5 – -25.9 (m, 1P).

**Ring opening by using propargylamine: 3’-Azido-3’-deoxythymidine 5’-γ-*P*-propargylamine β,γ-difluoromethylene triphosphate (41)**



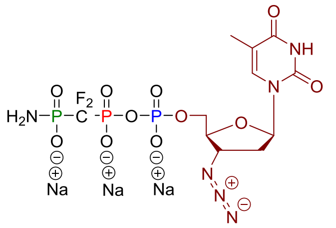
(41)

The reaction mixture of **B$_{13}$** (500 µL, 36.5 µmol, 1.0 eq.) was added to propargylamine (500 µL) and then $D_2O$ (100 µl) was added. The product **41** was isolated by Method A, affording a colourless solid (18.4 mg, 30.8 µmol, 85%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.69 (s, 1H), 6.21 (t, J = 6.9 Hz, 1H), 4.55 – 4.46 (m, 1H), 4.33 – 4.03 (m, 3H), 3.66 (dd, J = 8.7, 2.2 Hz, 2H), 2.47 (t, J = 2.5 Hz, 1H), 2.46 – 2.33 (m, 2H), 1.86 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 7.56 – 5.23 (m, 1P), -5.38 (tdd, J = 87.9, 59.4, 31.4 Hz, 1P), -11.6 (d, J = 31.8 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 7.44 – 5.29 (m, 1P), -5.41 (tdd, J = 87.6, 59.3, 31.3 Hz, 1P), -11.6 (dt, J = 27.5, 5.2 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.7, 151.8, 137.2, 111.9, 84.8, 83.0 (d, J = 9.4 Hz), 70.5, 65.6 (d, J = 5.8 Hz), 60.9, 36.3, 30.9, 11.7. **$^{19}$F NMR** (377 MHz, $D_2O$, δ/ppm): -119.2 (dd, J = 87.8, 78.0 Hz, 2F).

**HRMS** (ESI) m/z for [$C_{14}H_{18}F_2N_6NaO_{11}P_3$]: calcd. 600.0112 found 600.0108.

**Ring opening by using aq. ammonia: 3’-Azido-3’-deoxythymidine 5’-γ-*P*-amino β,γ-difluoromethylene triphosphate (42)**



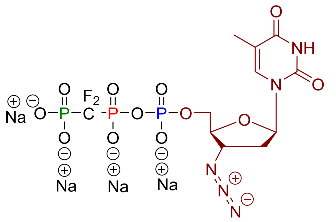
**(42)**

The reaction mixture of **B13** (500 µL, 36.5 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (500 µL) and $D_2O$ (100 µl) was added. The product **42** was isolated by Method A, affording a colourless solid (14.1 mg, 26.7 µmol, 73%).

The analytical data are consistent with literature.[12]

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.66 (s, 1H), 6.21 (t, J = 6.9 Hz, 1H), 4.65 – 4.46 (m, 1H), 4.23 – 3.97 (m, 3H), 2.59 – 2.33 (m, 2H), 1.85 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 8.36 (td, J = 78.7, 59.5 Hz, 1P), -5.18 (tdd, J = 87.9, 59.4, 31.4 Hz, 1P), -11.56 (d, J = 31.2, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 8.36 (td, J = 78.8, 59.3, 1P), -5.19 (tdd, J = 87.6, 59.3, 31.3, 1P), -11.1 – -12.0 (dt, J = 31.6, 5.3, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 168.7, 153.3, 137.0, 111.9, 84.9, 82.9 (d, J = 9.4 Hz), 65.7 (d, J = 5.8 Hz), 61.0, 36.2, 11.9. **$^{19}$F NMR** (377 MHz, $D_2O$, δ/ppm): -121.1 (dd, J = 87.9, 78.1 Hz, 2F). **HRMS** (ESI) m/z for $[C_{11}H_{15}F_2N_6NaO_{11}P_3]^-$ : calcd 560.9883 found 560.9886.

**Ring opening by using aq. sodium hydroxide: 3’-Azido-3’-deoxythymidine 5’-β,γ-difluoromethylene triphosphate (43)**



**(43)**

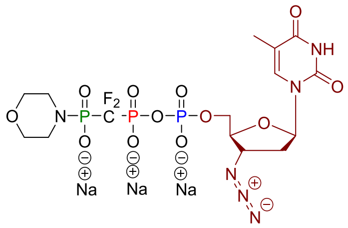
The reaction mixture of **B13** (500 µL, 36.5 µmol, 1.0 eq.) was added to aq. NaOH (1M, 500 µL) and then $D_2O$ (100 µl) was added. The product **43** was isolated by Method A, affording a colourless solid (15.1 mg, 23.8 µmol, 65%).

The analytical data are consistent with literature.[12]

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.65 (s, 1H), 6.22 (t, J = 7.0 Hz, 1H), 4.58 – 4.44 (m, 1H), 4.28 – 4.05 (m, 3H), 2.46 – 2.27 (m, 2H), 1.85 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, D2O, δ/ppm): 3.94 (td, J = 72.3, 56.6 Hz, 1P), -2.70 (tdd, J = 89.5, 56.7, 32.2 Hz, 1P), -11.2 (d, J = 32.2, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 3.94 (td, J = 72.2, 56.9 Hz, 1P), -2.70 (tdd, J = 89.9, 56.9, 32.6 Hz, 1P), -11.2 (dt, J = 31.6, 5.3 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 168.7, 153.3, 137.0, 111.9, 84.8, 82.9 (d, J = 9.4 Hz), 65.6 (d, J = 5.8 Hz), 61.0, 36.2, 11.9. **$^{19}$F NMR** (377 MHz, $D_2O$, δ/ppm): -118.1 (dd, J = 89.9, 72.8 Hz, 2F).

**HRMS** (ESI) m/z for $[C_{11}H_{14}F_2N_5O_{12}P_3]^{2-}$: calcd 269.4915, found 269.4916.

**Ring opening by using morpholine: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-morpholino β,γ-difluoromethylene triphosphate (44)**



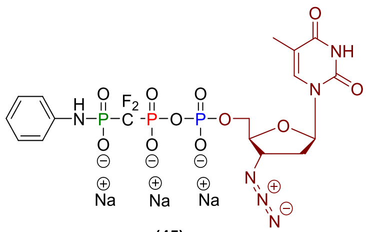
**(44)**

The reaction mixture of **B13** (250 µL, 20.2 µmol, 1.0 eq.) was added to morpholine (250 µL) and then $D_2O$ (100 µl) was added. The product **44** was isolated by Method A, affording a colourless solid (10.2 mg, 15.1 µmol, 74%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.70 (s, 1H), 6.23 (t, J = 6.9 Hz, 1H), 4.55 – 4.49 (m, 1H), 4.16 (d, J = 4.6 Hz, 3H), 3.61 (m, 4H), 3.07 (s, 4H), 2.75 – 2.25 (m, 2H), 1.87 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 5.08 (td, J = 77.4, 58.0 Hz, 1P), -5.51 (tdd, J = 86.9, 58.1, 31.7 Hz, 1P), -11.5 (d, J = 31.6 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 7.96 – 3.69 (m, 1P), -3.35 – -7.45 (m, 1P), -11.5 (dt, J = 32.3, 5.3 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 151.9, 137.3, 111.9, 84.9, 83.0 (d, J = 9.5 Hz), 67.7 (d, J = 3.9 Hz), 65.6 (d, J = 5.5 Hz), 64.9, 44.8 (d, J =2.2 Hz), 36.3, 11.7. **$^{19}$F NMR** (471 MHz, $D_2O$, δ/ppm): -118.1 (ddd, J = 87.6, 77.3, 3.5 Hz, 2F).

**HRMS** (ESI) m/z for $[C_{15}H_{22}F_2N_6NaO_{12}P_3]^-$: calcd 632.0374, found 632.0371.

**Ring opening by using aniline: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-anilino β,γ-difluoromethylene triphosphate (45)**



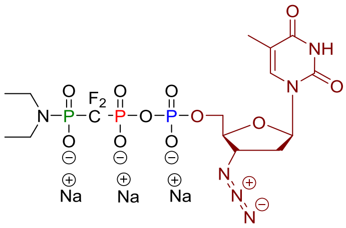
**(45)**

The reaction mixture of **B13** (250 µL, 20.2 µmol, 1.0 eq.) was added to aniline (250 µL) and then $D_2O$ (100 µl) was added. The product **45** was isolated by Method A, affording a colourless solid (11.0 mg, 16.1 µmol, 79%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.55 (s, 1H), 7.30 – 7.00 (m, 4H), 6.83 (tt, J = 7.0, 1.4 Hz, 1H), 6.13 (t, J = 6.9 Hz, 1H), 4.34 (dt, J = 6.9, 3.6 Hz, 1H), 4.11 – 3.97 (m, 3H), 2.36 – 2.18 (m, 2H), 1.80 (s, 3H).**$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 1.44 (td, J = 80.8, 59.1 Hz, 1P), -5.44 (tdd, J = 87.0, 59.2, 30.8 Hz, 1P), -11.4 (d, J = 30.8 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 1.44 (td, J = 80.8, 59.4 Hz, 1P), -3.62 – -6.92 (m, 1P), -11.4 (dt, J = 30.6, 5.3 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.5,

151.6, 141.8, 137.1, 128.8, 120.9, 118.4 (d, J = 5.4 Hz), 111.8, 84.7, 82.9 (d, J = 9.7 Hz), 65.5 (d, J = 5.5 Hz), 60.8, 36.2, 11.6. **$^{19}$F NMR** (471 MHz, $D_2O$, δ/ppm): -119.3 (ddd, *J* = 88.5, 77.7, 11.2 Hz, 2F).
**HRMS** (ESI) m/z for $[C_{17}H_{21}F_2N_6NaO_{11}P_3]^+$: calcd 639.0341, found 639.0337.

**Ring opening by using diethylamine: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-diethylamino β,γ-difluoromethylene triphosphate (46)**

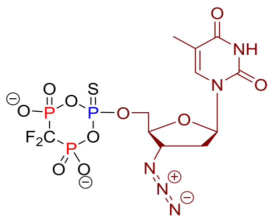


**(46)**

The reaction mixture of **B13** (250 µL, 20.2 µmol, 1.0 eq.) was added to diethylamine (250 µL) and then (100 µl) was added. The product **46** was isolated by Method A, affording a colourless solid (7.40 mg, 11.2 µmol, 55%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.68 (s, 1H), 6.24 (t, J = 6.9 Hz 1H), 4.63 – 4.46 (m, 1H), 4.36 – 4.27 (m, 1H), 4.32 – 4.04 (m, 2H), 3.09 – 2.95 (m, 4H), 2.70 – 2.33 (m, 2H), 1.87 (s, 3H), 1.21 (t, J = 7.3 Hz, 3H), 1.01 (t, J = 7.0 Hz, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 8.32 (td, J = 75.4, 57.6 Hz, 1P), -4.77 (tdd, J = 88.8, 57.6, 32.1 Hz, 1P), -11.4 (d, J = 32.2 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 8.31 (tdt, J = 76.1, 57.4, 9.9 Hz, 1P), -4.78 (tdd, J = 88.8, 57.5, 31.9 Hz, 1P), -11.4 (dd, J = 31.5, 5.7 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.7, 152.0, 137.3, 111.8, 84.8, 83.0 (d, J = 9.5 Hz), 65.6 (d, J = 5.8 Hz), 61.0, 40.5 (d, J = 3.7 Hz), 36.2, 13.8 (d, J = 3.7 Hz), 11.7. **$^{19}$F NMR** (471 MHz, $D_2O$, δ/ppm): -117.7 (dd, J = 88.5, 74.7 Hz, 2F).
**HRMS** (ESI) m/z for $[C_{15}H_{24}F_2N_6O_{11}P_3]^-$: calcd 595.0689, found 595.0683.

**3'-Azido-3'-deoxythymidine 5'-β,γ-difluoromethylene α-*P*-thiocyclotriphosphate ($B_{14}$)**

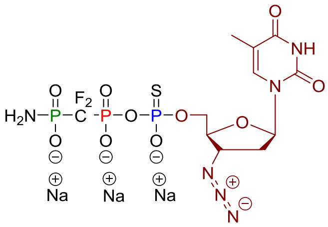


**($B_{14}$)**

AZT (52 mg, 0.19 mmol, 1.2 eq.) and ETT (84 mg, 0.65 mmol, 4.0 eq.) were coevaporated with dry MeCN (2 × 2 mL). Under an Ar atmosphere, a reaction mixture containing the *c*-$Py_{CF2}$PA **$A_3$** (0.081M in MeCN, 2.0 mL, 0.16 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, Becauge's reagent (55 mg, 0.27 mmol, 1.7 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (formation of triplet at +40.0 ppm which is diagnostic signal for the α-(S)-cyclotriphosphate **$B_{14}$**).

**$^{31}P\{^1H\}$ NMR** (122 MHz, $CDCl_3$, δ/ppm): 39.4 (t, J = 35.8 Hz, 1P), -8.72 (td, J = 88.0, 35.9 Hz, 2P).

**Ring opening by using aq. ammonia: 3'-Azido-3'-deoxythymidine 5′-γ-*P*-amino β,γ-difluoromethylene-α-*P*-thiotriphosphate (47)**



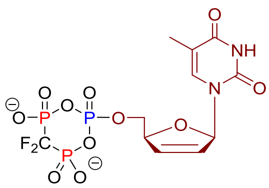
**(47)**

The reaction mixture of **$B_{14}$** (250 µL, 20.2 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (250 µL) and then $D_2O$ (100 µl) was added. The product **47** was isolated by Method A, affording a colourless solid, whereas products were obtained as mixtures of diastereoisomers (8.70 mg, 13.9 µmol, 69%).

**$^1H$ NMR** (300 MHz, $D_2O$, δ/ppm): 7.78 (s, 1H), 6.24 (t, J = 6.9, 1H), 4.54 (m, 1H), 4.22 (m, 3H), 2.64 – 2.31 (m, 2H), 1.90 (s, 3H).**$^{31}P\{^1H\}$ NMR** (122 MHz, $D_2O$, δ/ppm): 42.9 (dd, J = 38.2, 29.2, 1P), 12.0 – 5.88 (m, 1P), -5.60 (tdd, J = 87.7, 58.9, 38.0 Hz, 1P). **$^{31}P$ NMR** (162 MHz, $D_2O$, δ/ppm): 44.2 – 41.7 (m, 1P), 12.5 – 6.70 (m, 1P), -3.77 – -7.02 (m, 1P).**$^{13}C$ NMR** (101 MHz, $D_2O$, δ/ppm): 168.7, 153.3, 137.0, 111.9, 84.9, 82.9 (d, J = 9.4 Hz), 65.7 (d, J = 5.8 Hz), 61.0, 36.2, 11.9. **$^{19}F$ NMR** (377 MHz, $D_2O$, δ/ppm):- 121.0 (ddd, J = 87.7, 78.7, 6.2 Hz, 2F).

**HRMS** (ESI) m/z for $[C_{11}H_{16}F_2N_6O_{10}P_3S]^-$: calcd 554.9835, found 554.9839.

**2',3'-didehydro-2',3'-dideoxythymidine 5′-β,γ-difluoromethylene cyclotriphosphate ($B_{15}$)**

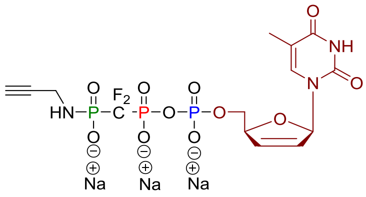


**($B_{15}$)**

d4T (40 mg, 0.17 mmol, 1.2 eq.) and ETT (67 mg, 0.5 mmol, 3.5 eq.) were coevaporated with dry MeCN (2 × 1 mL) and the dried solids were dissolved in dry DMF (1 mL). Under an Ar atmosphere, a reaction mixture containing the *c*-$Py_{CF2}$PA **$A_3$** (0.073M in MeCN, 2.0 mL, 0.15 mmol, 1.0 eq.) was added and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 45 mg, 0.26 mmol, 1.7 eq.) was added and the mixture was stirred for 5 mins until $^{31}P$-NMR confirmed complete oxidation (reaction progress was followed by $^{31}P$ NMR, and formation of triplet at -23 ppm which is diagnostic signal for the d4T cyclotriphosphate **$B_{15}$**).

**$^{31}P\{^1H\}$ NMR** (162 MHz, $CD_3CN$, δ/ppm): -9.21 (dt, J = 87.1, 23.8 Hz, 2P), -25.4 (t, J = 23.6 Hz, 1P).

**Ring opening by using propargylamine: 2’,3’-didehydro-2’,3’-dideoxythymidine 5’-γ-propargylamine β,γ-(difluoromethylene)triphosphate (48)**



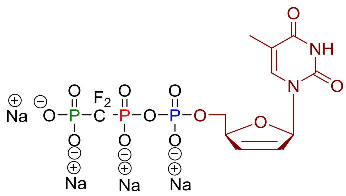
**(48)**

The reaction mixture of **B15** (250 µL, 12.2 µmol, 1.0 eq.) was added to propargylamine (250 µL) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **48** was isolated by Method A, affording a colourless solid. The obtained colourless precipitate was washed with methanol twice and isolated by centrifugation and dried under reduced pressure (6.60 mg, 10.8 µmol, 89%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.55 (s, 1H), 6.90 (dt, J = 3.4, 1.7 Hz, 1H), 6.46 (dt, J = 6.2, 1.8 Hz, 1H), 5.96 – 5.75 (m, 1H), 5.14 – 4.97 (m, 1H), 4.23 – 3.83 (m, 2H), 3.65 (dd, J = 8.8, 2.5 Hz, 2H), 2.48 (t, J = 2.5 Hz, 1H), 1.83 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 6.36 (td, J = 78.1, 58.1 Hz, 1P), -5.24 (tdd, J = 87.6, 58.2, 30.7 Hz, 1P), -11.3 (d, J = 31.0, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 8.26 – 5.58 (m, 1P), -5.25 (tdd, J = 88.1, 58.3, 30.7, 1P), -11.3 (dt, J = 30.8, 6.1 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.9, 152.3, 138.2, 134.3, 125.2, 111.6, 89.9, 87.5, 85.9 (d, J = 9.1 Hz), 66.5 (d, J = 5.9 Hz), 27.0 (d, J = 18.6 Hz), 11.5. **$^{19}$F NMR** (377 MHz, $D_2O$, δ/ppm): -119.7 (dd, J = 87.7, 78.0 Hz, 2F).

**HRMS** (ESI) m/z for $[C_{14}H_{17}F_2N_3Na_2O_{11}P_3]^+$: calcd 579.9834, found 579.9833.

**Ring opening by using aq. sodium hydroxide: 2’,3’-didehydro-2’,3’-dideoxythymidine 5’-β,γ-difluoromethylene triphosphate (49)**



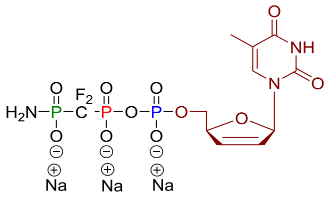
**(49)**

The reaction mixture of **B15** (250 µL, 12.2 µmol, 1.0 eq.) was added to aq. NaOH (1M, 250 µL) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **49** was isolated by Method A, affording a colourless solid (5.30 mg, 9.04 µmol, 74%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.54 (s, 1H), 6.89 (d, J = 3.4 Hz, 1H), 6.47 (d, J = 6.2 Hz, 1H), 5.86 (dt, J = 6.2 Hz, 1H), 5.11 – 4.90 (m, 1H), 4.23 – 4.04 (m, 2H), 1.82 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 3.93 (td, J = 72.5, 56.9 Hz, 1P), -2.87 (tdd, J = 89.5, 57.0, 32.2 Hz, 1P), -11.0 (d, J = 32.2 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 3.93 (td, J = 72.8, 57.0 Hz, 1P), -2.87 (tdd, J = 89.3, 57.0, 32.3 Hz, 1P), -11.0 (dt, J = 32.1, 6.6 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 152.3, 138.2, 134.4, 125.4, 111.5, 89.9, 85.9 (d, J = 8.7 Hz), 66.4 (d, J = 5.9 Hz), 11.5. **$^{19}$F NMR** (377 MHz, $D_2O$, δ/ppm): -118.0 (dd, J = 89.9, 72.8 Hz, 2F).

**HRMS** (ESI) m/z for $[C_{11}H_{14}F_2N_2O_{12}P_3]^-$: calcd 496.9733, found 496.9733.

**Ring opening by using aq. ammonia: 2’,3’-didehydro-2’,3’-dideoxythymidine 5’-γ-*P*-amino β,γ-difluoromethylene triphosphate (50)**



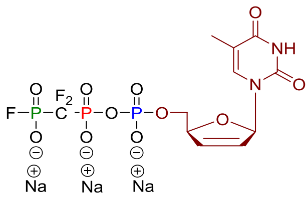

**(50)**

The reaction mixture of **B15** (250 µL, 12.2 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (250 µL) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **50** was isolated by Method A, affording a colourless solid (5.60 mg, 10.1 µmol, 87%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.53 (s, 1H), 6.89 (dt, J = 3.4 Hz, 1H), 6.45 (dt, J = 6.1 Hz, 1H), 5.86 (d, J = 6.2 Hz, 1H), 5.18 – 4.97 (m, 1H), 4.09 (m, 2H), 1.82 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 8.39 (td, J = 78.7, 59.3 Hz, 1P), -5.29 (tdd, J = 87.6, 59.4, 31.2 Hz, 1P), -11.4 (d, J = 31.1 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 8.38 (td, J = 78.7, 59.3 Hz, 1P), -5.29 (tdd, J = 87.5, 59.3, 31.1 Hz, 1P), -11.4 (dt, J = 31.4, 6.4 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 167.2, 152.6, 138.1, 134.3, 125.1, 111.5, 89.9, 85.9 (d, J = 8.6 Hz), 66.5 (d, J = 6.0 Hz), 11.5. **$^{19}$F NMR** (377 MHz, $D_2O$, δ/ppm): - 121.10 (dd, J = 87.4, 78.7 Hz, 2F).

**HRMS** (ESI) m/z for $[C_{11}H_{17}N_3O_{11}P_3]^-$: calcd 460.0081, found 460.0095.

**Ring opening by using caesium fluoride: 2’,3’-didehydro-2’,3’-dideoxythymidine 5’-γ-*P*-fluoro β,γ-difluoromethylene triphosphate (51)**



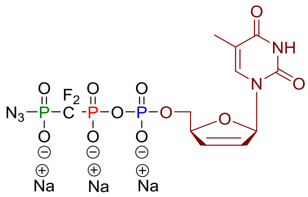

**(51)**

The reaction mixture of **B15** (250 µL, 12.2 µmol, 1.0 eq.) was added to caesium fluoride (105 mg, 691 µmol, 58 eq.) and then immediately addition of $D_2O$ (250 µl) afterwards it was stirred for 5 mins. The crude product was isolated by Method A and the impurity $CsClO_4$ was co-precipitated which was further removed by dissolving the crude product in water (2 mL). The insoluble residue was removed by filtration (syringe filter, pore size 0.45 µm). Afterwards, the product **51** was isolated from the aq. solution again by Method A and a colourless solid was obtained (6.0 mg, 11.3 µmol, 93%).

$^{1}H$ **NMR** (400 MHz, $D_2O$, δ/ppm): 7.52 (s, 1H), 6.89 (dt, J = 3.3, 1.7 Hz, 1H), 6.45 (dt, J = 6.2, 1.8 Hz, 1H), 5.88 (dt, J = 6.1, 2.0 Hz, 1H), 5.04 (d, J = 4.1 Hz, 1H), 4.28 – 4.03 (m, 2H), 1.82 (s, 3H). $^{31}P\{^{1}H\}$ **NMR** (122 MHz, $D_2O$, δ/ppm): 5.82 – -2.25 (m, 1P), -7.52 (tdd, J = 82.2, 64.2, 29.3 Hz, 1P), -11.4 (d, J = 29.1 Hz, 1P). $^{31}P$ **NMR** (162 MHz, $D_2O$, δ/ppm):1.76 (dtd, J = 1027.9, 90.9, 64.2 Hz, 1P), -7.53 (tdd, J = 82.3, 64.2, 29.3 Hz, 1P), -11.4 (dt, J = 29.3, 6.2 Hz, 1P). $^{31}P\{^{19}F\}$ **NMR** (162 MHz, $D_2O$, δ/ppm):1.77 (d, J = 64.1 Hz, 1P), -7.52 (dd, J = 64.2, 29.1 Hz, 1P), -11.4 (dt, J = 29.3, 6.2 Hz, 1P). $^{13}C$ **NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 152.3, 138.1, 134.3, 125.1, 111.5, 89.9, 85.9 (d, J = 8.6 Hz), 66.5 (d, J = 6.0 Hz), 11.5. $^{19}F$ **NMR** (377 MHz, $D_2O$, δ/ppm): -68.7 (d, J = 1027.9 Hz, 1F), -121.4 (ddd, J = 90.9, 82.3, 3.3 Hz, 2F).

**HRMS** (ESI) m/z for $[C_{11}H_{13}F_3N_2O_{11}P_3]^-$: calcd 498.9690, found 498.9689.

**Ring opening by using tetra methylgaunidinium azide: 2’,3’-didehydro-2’,3’-dideoxythymidine 5’-γ-*P*-azido β,γ-difluoromethylene triphosphate (52)**

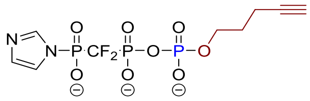


**(52)**

The reaction mixture of **B15** (250 µL, 12.2 µmol, 1.0 eq.) was added to a solution of tetramethyl guanidinium azide (420 mg 2.62 mmol, 220 eq.) in MeCN (1 mL) and $D_2O$ (100 µl) was added subsequently. The crude product was isolated by Method A and the product was precipitated as gaunidinium salt which was again by dissolved in water (2 mL).Afterwards, the product **52** was isolated from the aq. solution again by Method A and a colourless solid was obtained (6.20 mg, 11.2 µmol, 94%).
$^{1}H$ **NMR** (400 MHz, $D_2O$, δ/ppm): 7.53 (s, 1H), 7.03 – 6.76 (m, 1H), 6.45 (d, J=6.1, 1.8, 1H), 5.86 (d, J=6.2, 1.9, 1H), 5.03 (s, 1H), 4.37 – 3.98 (m, 2H), 1.82 (s, 3H). $^{31}P\{^{1}H\}$ **NMR** (162 MHz, D2O, δ/ppm): 3.11 (td, J = 85.4, 63.9 Hz, 1P), -6.35 – -8.09 (m, 1P), -11.2 (d, J = 29.7 Hz, 1P). $^{31}P$ **NMR** (162 MHz, $D_2O$, δ/ppm): 3.11 (td, J = 85.8, 64.1 Hz, 1P), -7.18 (tdd, J = 82.5, 63.9, 29.5 Hz, 1P), -11.2 (dt, J = 29.5, 6.1 Hz, 1P). ). $^{13}C$ **NMR** (101 MHz, $D_2O$, δ/ppm): 167.2, 152.6, 138.1, 134.3, 125.1, 111.5, 89.9, 85.9 (d, J = 8.6 Hz), 66.5 (d, J = 6.0 Hz), 11.5. $^{19}F$ **NMR** (377 MHz, $D_2O$, δ/ppm): -119.2 (dd, J = 87.8, 78.0 Hz, 2F).

**HRMS** (ESI) m/z for $[C_{11}H_{13}F_2N_5O_{11}P_3]^-$: calcd 521.9798, found 521.9801.

**Ring opening by using Imidazole**: **4’-Pentyne γ-*P*-imidazole β,γ-difluoromethylene triphosphate (S- 8)**



**(S-8)**

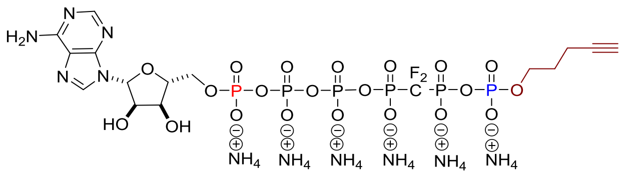
4-Pentyn-1-ol (24 mg, 0.27 mmol, 1.2 eq.) and ETT (102 mg, 0.78 mmol, 3.5 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an Ar atmosphere, a reaction mixture containing the *c*-$Py_{CF2}$PA **A3** (0.075M in MeCN, 3.0 ml, 0.22 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 10 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 24 mg, 0.16 mmol, 1.5 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (reaction progress was followed by $^{31}$P NMR, and formation of triplet at -25 ppm which is diagnostic signal for the cyclotriphosphate).

A solution of imidazole in dry DMF (1.47M, 4.00 ml, 5.88 mmol, 26.0 eq.) was added to the above cyclotriphosphate and reaction was stirred for another 30 mins (the reaction progress was followed by $^{31}$P NMR) which results in quantative conversion to **S-8**.

**Note** -: Imidazole was dried before using by coevaporation with MeCN and it was further stored of molecular sieve (3Å).

**$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -4.94 (td, J = 80.5, 61.7 Hz, 1P), -6.64 – -8.64 (m, 1P), -12.3 (d, J = 27 Hz, 1P).

**5’-Adenosine 4’-Pentyne β,γ-difluoromethylene hexaphosphate (P5)**



**(P5)**

Anhydr. $ZnCl_2$ (0.26 g, 1.9 mmol, 8.4 eq.) and ATP x 2.1 TBA (0.36 g, 0.35 mmol, 1.6 eq.) were added to a freshly prepared solution of **S-8** (0.22 mmol).The reaction mixture was then diluted with dry DMF: DMSO (v:v, 1:1, 16 ml) in order to increase the solubility and it was stirred at r.t. for 24 hrs. The crude product was purified by Method B and the target product was eluted with 500-600 mM conc. of $NH_4HCO_3$ buffer. Compound **P5** was obtained as a colorless solid. (0.02 g, 0.03 mmol, 12%).

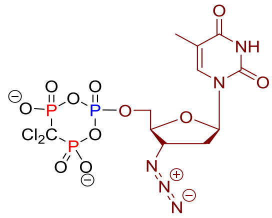
**Note** -: The reaction for capped hexapolyPs were not optimized.

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 8.42 (s, 1H), 8.16 (s, 1H), 6.05 (d, J = 6.0 Hz, 1H), 4.65 (s, 1H), 4.57 – 4.45 (m, 1H), 4.43 – 4.22 (m, 1H), 4.25 – 4.09 (m, 2H), 4.04 – 3.88 (m, 2H), 2.55 – 2.00 (m, 3H), 1.91 – 1.65 (m, 2H).**$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): -5.63 – -7.57 (m, 2P), -10.6 (d, J = 26.4 Hz, 1P), -11.4 (d, J = 15.8 Hz, 1P), -22.0 – -23.9 (m, 2P). **$^{31}$P NMR** (202 MHz, $D_2O$, δ/ppm): -5.69 – -7.97 (m, 2P), - 10.3 – -10.9 (m, 1P), -11.1 – -11.8 (m, 1P), -22.1 – -23.8 (m, 2P). **$^{13}$C NMR** (126 MHz, $D_2O$, δ/ppm): 165.7, 155.7, 152.9, 149.2, 139.8, 118.6, 86.6, 84.1 (d = 8.4 Hz), 74.2, 70.1, 65.3 (d = 6.6 Hz), 65.4, 28.7 (d, J = 7.3 Hz), 14.1. **$^{19}$F NMR** (471 MHz, $D_2O$, δ/ppm): -120.5 (t, J = 83.7, 2F).

**HRMS** (ESI) m/z for $[C_{16}H_{25}DF_2N_5O_{21}P_6]^+$: calcd 848.9571, found 848.9587.

## 2.2.4 Synthesis of triphosphates based on *c*-Py$_{CCl2}$PA (A$_4$)

**3'-Azido-3'-deoxythymidine β,γ-dichloromethylene cyclotriphosphate (B$_{16}$)**

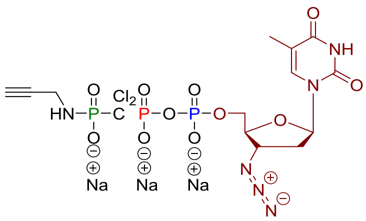


**(B$_{16}$)**

AZT (68 mg, 0.25 mmol, 1.2 eq.) and ETT (0.13 g, 0.99 mmol, 4.8 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an Ar atmosphere, a reaction mixture containing the *c*-Py$_{CCl2}$PA **A$_4$** (0.069M in MeCN, 3.0 mL, 0.20 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 45 mins. Upon cooling to 0°C, *m*CPBA (≤77%, 54 mg, 0.31 mmol, 1.5 eq.) was added and the mixture was stirred for 10 mins until $^{31}$P-NMR confirmed complete oxidation (reaction progress was followed by $^{31}$P NMR, and formation of triplet at -26 ppm which is diagnostic signal for **B$_{16}$**).

**$^{31}$P{$^1$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): -4.54 (d, J = 21.8 Hz, 2P), -26.3 (t, J = 21.7 Hz, 1P).

**Ring opening by using propargylamine: 3'-Azido-3'-deoxythymidine 5'-*γ*-*P*-propargylamino β,γ-dichloromethylene triphosphate (53)**



**(53)**

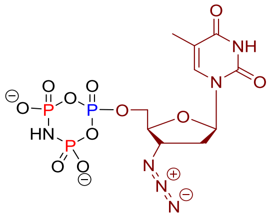
The reaction mixture of **B$_{16}$** (250 µL, 17.2 µmol, 1.0 eq.) was added to propargylamine (250 µL) and then $D_2O$ (100 µl) was added afterwards it was stirred for 5 mins. The product **53** was isolated by Method A, affording a colourless solid (5.8 mg, 8.58 µmol, 50%).

**$^1$H NMR** (400 MHz, $D_2O$, δ/ppm): 7.68 (s, 1H), 6.22 (t, J = 6.9 Hz, 1H), 4.53 (dt, J = 6.5, 3.7 Hz, 1H), 4.18 (m, 3H), 3.76 – 3.51 (m, 2H), 2.53 – 2.36 (m, 3H), 1.86 (s, 3H).**$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 9.94 (d, J = 18.9 Hz, 1P), -0.72 (dd, J = 30.6, 19.0 Hz, 1P), -11.5 (d, J = 30.7 Hz, 1P). **$^{31}$P NMR** (162 MHz, $D_2O$, δ/ppm): 9.92 (dt, J = 19.4, 8.1 Hz, 1P), -0.73 (dd, J = 30.5, 19.3 Hz, 1P), -11.6 (dt, J = 31.4, 6.4 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 151.8, 137.2, 111.8, 84.8, 82.9 (d, J = 9.3 Hz), 71.3, 65.6 (d, J = 5.6 Hz), 60.8, 36.3, 30.9 (d, J = 3.7 Hz), 11.7.

**HRMS** (ESI) m/z for $[C_{14}H_{19}Cl_2N_6O_{11}P_3]^-$: calcd 609.9707, found 609.9695.

### 2.2.5 Synthesis of triphosphates based on *c*-Py$_{NH}$PA (A$_5$)

**3'-Azido-3'-deoxythymidine β,γ-imido cyclotriphosphate (B$_{17}$)**

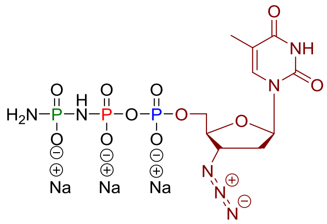


(B$_{17}$)

AZT (52 mg, 0.19 mmol, 1.3 eq.) and ETT (69 mg, 0.52 mmol, 3.5 eq.) were coevaporated with dry MeCN (2 × 1 mL). Under an Ar atmosphere, a reaction mixture containing the *c*-Py$_{NH}$PA **A$_5$** (0.075M in MeCN, 2.0 mL, 0.15 mmol, 1.0 eq.) was added to the dried solids and it was stirred at r.t. for 5 mins. Upon cooling to -4°C, *m*CPBA (≤77%, 39 mg, 0.22 mmol, 1.5 eq.) was added and the mixture was stirred for 5 mins until $^{31}$P-NMR confirmed complete oxidation (a formation of triplet at -23 ppm which is diagnostic signal for **B$_{17}$**).

**$^{31}$P{$^1$H} NMR** (122 MHz, $CDCl_3$, δ/ppm): -13.7 (d, J = 17.9 Hz, 2P), -22.5 (t, J = 19.2 Hz, 1P).

**Ring opening by using aq. ammonia: 3'-Azido-3'-deoxythymidine 5'-γ-*P*-amino β,γ-imidotriphosphate (54)**



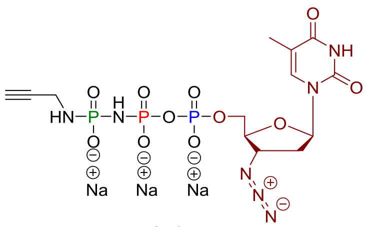
(54)

The reaction mixture of **B$_{17}$** (500 µL, 37.5 µmol, 1.0 eq.) was added to 25% aq. $NH_4OH$ (500 µL) and then $D_2O$ (200 µl) afterwards it was stirred for 5 mins. The product **54** was isolated by Method C, affording a colourless solid (11.9 mg, 20.8 µmol, 55%).

**$^1$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.67 (s, 1H), 6.21 (t, J = 6.9 Hz, 1H), 4.50 (dt, J = 6.1, 4.1 Hz, 1H), 4.30 – 3.89 (m, 3H), 2.54 – 2.31 (m, 2H), 1.85 (s, 3H). **$^{31}$P{$^1$H} NMR** (122 MHz, $D_2O$, δ/ppm): 4.05 (d, J = 5.3 Hz, 1P), -10.5 (dd, J = 20.2, 5.4 Hz, 1P), -11.1 (d, J = 20.2 Hz, 1P). **$^{31}$P NMR** (122 MHz, $D_2O$, δ/ppm): 4.67 (d, J = 6.8 Hz, 1P), -10.6 (dd, J = 21.2, 6.2 Hz, 1P), -11.4 (dt, J = 21.8, 5.8 Hz, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 167.1, 152.1, 137.2, 111.8, 84.8, 82.9 (d, J = 9.0 Hz), 65.4 (d, J =5.5 Hz), 60.8, 36.2, 11.7.

**HRMS** (ESI) m/z for $[C_{10}H_{17}N_7O_{11}P_3]^-$: calcd 504.0204, found 504.0204.

**Ring opening by using propargylamine: 3’-Azido-3’-deoxythymidineb 5’-γ-*P*-propargylamino β,γ-imidotriphosphate (55)**



**(55)**

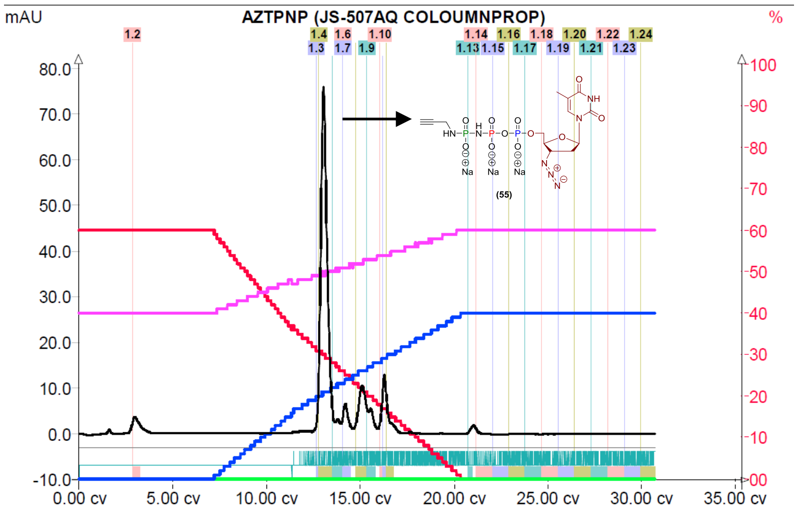
The reaction mixture of **B17** (2.0 mL, 0.15 mmol, 1.0 eq.) was added to propargylamine (3.0 ml, 4.7 mmol, 31 eq) and stirred at r.t. for 15 mins. The product **55** was isolated by Method C (details of the MPLC purification can be found in Supporting figure S1), affording a colourless solid (59.8 mg, 0.09 mmol, 65%).

**$^{1}$H NMR** (300 MHz, $D_2O$, δ/ppm): 7.68 (s, 1H), 6.21 (t, J = 6.8 Hz, 1H), 4.63 – 4.42 (m, 1H), 4.32 – 4.03 (m, 3H), 3.56 (dd, J = 9.7, 2.5, 2H), 2.49 (t, J = 2.5, 1H), 2.42 (td, J = 6.2, 4.8, 2.7, 2H), 1.86 (s, 3H). **$^{31}$P{$^{1}$H} NMR** (122 MHz, $D_2O$, δ/ppm): 4.05 (d, J = 5.3 Hz, 1P), -10.5 (dd, J = 20.2, 5.4 Hz, 1P), -11.1 (d, J = 20.2, 1P). **$^{31}$P NMR** (122 MHz, $D_2O$, δ/ppm): 4.00 (td, J = 9.7, 5.5 Hz, 1P), -10.7 (dd, J = 20.6, 5.6 Hz, 1P), -11.03 – -11.5 (m, 1P). **$^{13}$C NMR** (101 MHz, $D_2O$, δ/ppm): 166.8, 160.2, 151.7, 137.2, 111.8, 84.8, 83.02 (d, J = 9.3 Hz), 71.3, 65.7 (d, J = 5.6 Hz), 60.9, 36.3, 11.6.

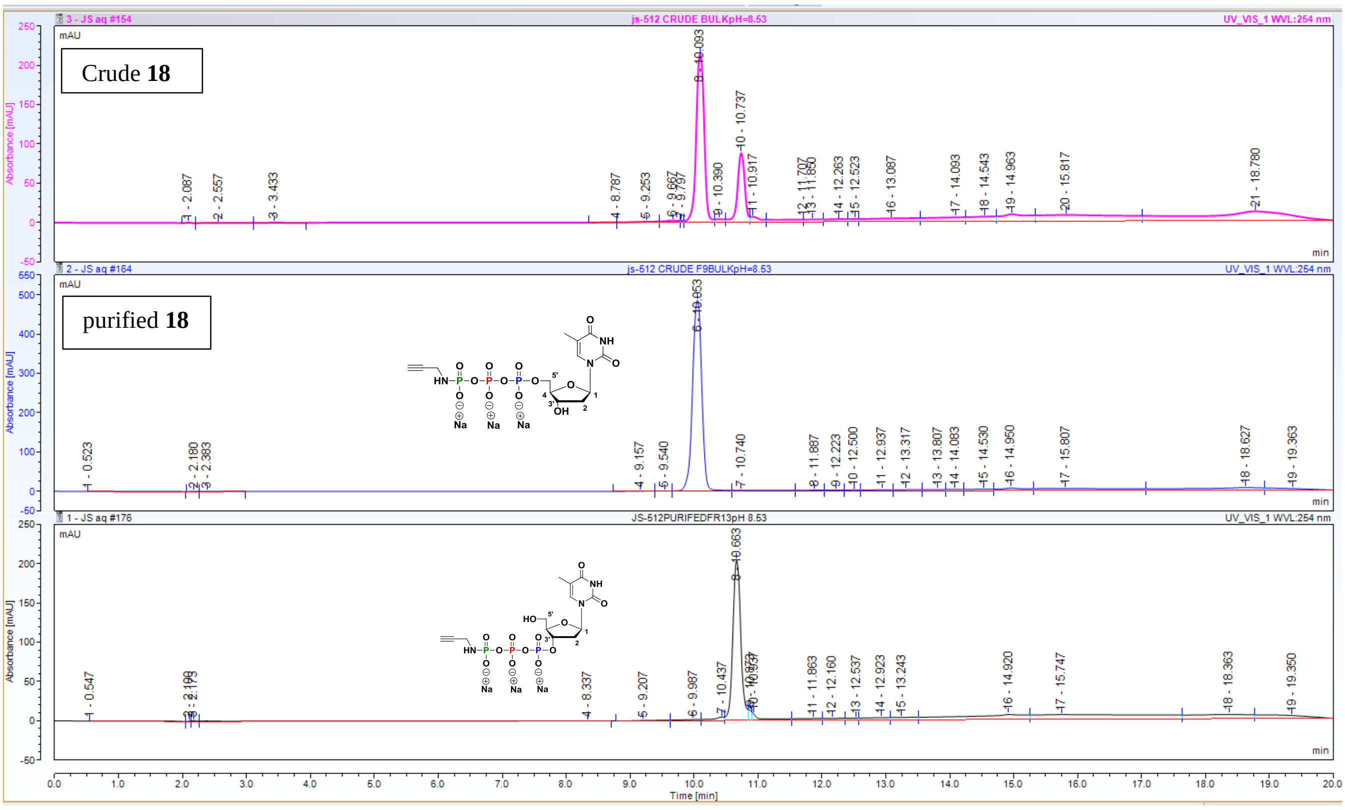
**HRMS** (ESI) m/z for $[C_{13}H_{18}N_7NaO_{11}P_3]^-$: calcd 564.0180, found 564.0182.

## Analytical Data (Chromatogram and IR)

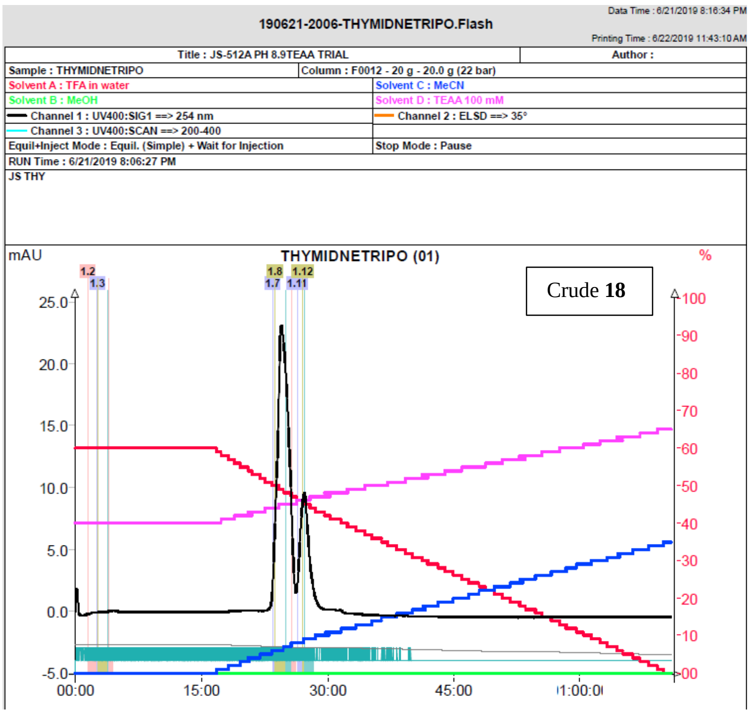
| Title : JS-507AQ COLOUMNPROP | Author : |
|---|---|
| Sample : AZTPNP | Column : F0012 - 20 g - 20.0 g (22 bar) |
| Solvent A : TFA in water | Solvent C : MeCN |
| Solvent B : MeOH | Solvent D : TEAA 100 mM |
| Channel 1 : UV400:SIG1 ==> 254 nm | Channel 2 : ELSD ==> 35° |
| Channel 3 : UV400:SCAN ==> 200-400 | |
| Equil+Inject Mode : Equil. (Simple) + Wait for Injection | Stop Mode : Pause |
| RUN Time : 6/14/2019 4:38:28 PM | |



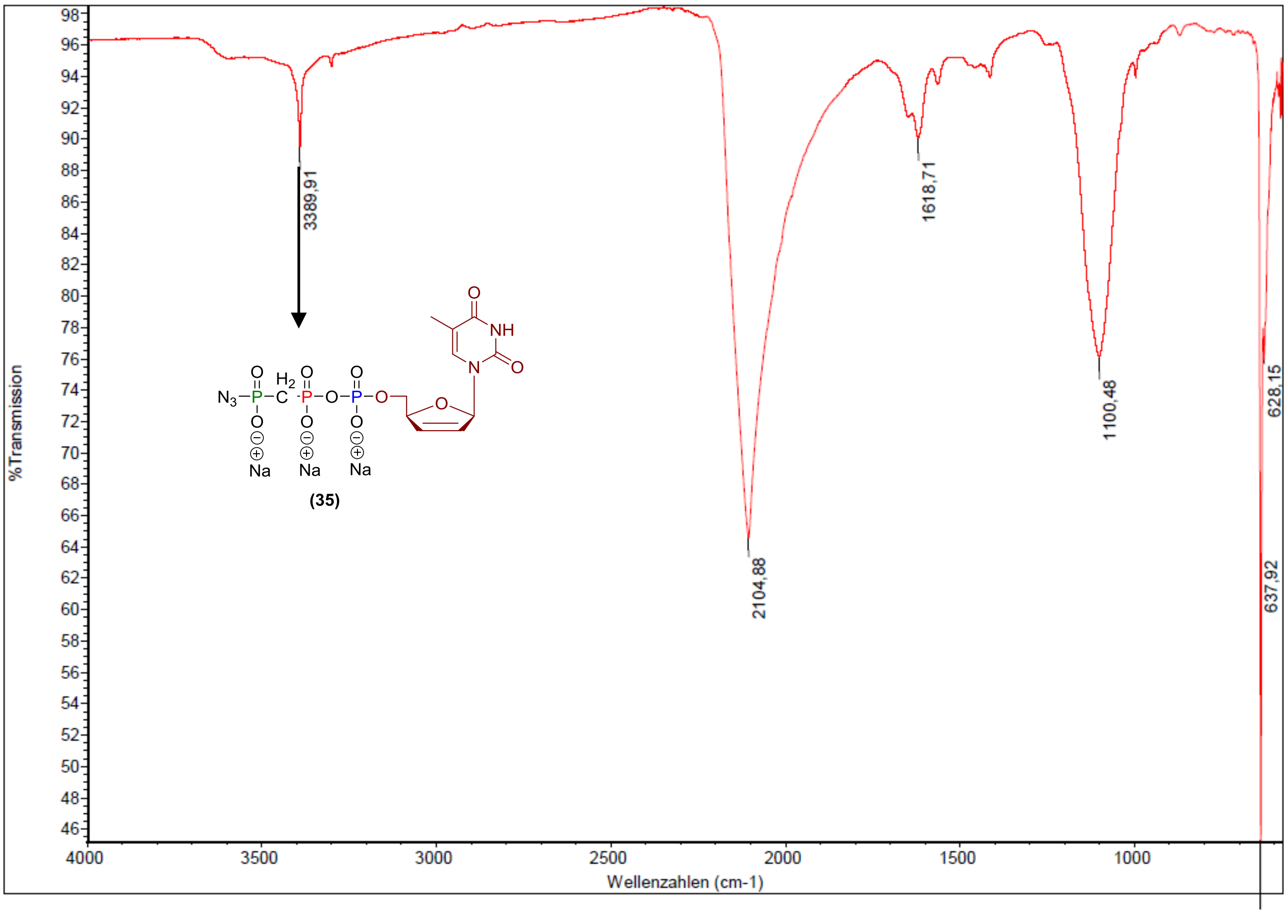

**Supporting Figure S1**: MPLC Chromatogram of crude **55**.100 mM of TEAA buffer was adjusted at pH = 8.55. The crude product was dissolved in deionized water and loaded on puriFlash C18 aqueous column, Column size: F0012 (20 g), Particle size: 30 µm, Flow rate: 10 ml/mins, Mobile Phase: 100 mM of TEAA (pH adjusted at 8.5) and MeCN, Gradient: 40 mM of TEAA for 15 mins then 60 mM of TEAA: 40 mM of MeCN for 45 mins. The target fractions were pooled and concentrated to the minimal amount of the solvent and then product was precipitated by $NaClO_4$ (0.5 m in acetone).The precipitate was washed two times with acetone (10 - 20 ml).




**Supporting Figure S2**: Analytical RP-HPLC Profile of crude product **18**.




**Supporting Figure S3:** MPLC Chromatogram of crude **18**. 100 mM of TEAA buffer was adjusted at pH = 8.5. The crude product was dissolved in deionized water and loaded on puriFlash C18 aqueous column, Column size: F0012 (20 g), Particle size: 30 µm, Flow rate: 10 ml/mins, Mobile Phase: 100 mM of TEAA (pH adjusted at 8.5) and MeCN, Gradient: 40 mM of TEAA for 15 mins then 60 mM of TEAA: 40 mM of MeCN for 45 mins. The target fractions were pooled and concentrated reduced pressure to the minimal amount of the solvent and then product was precipitated by $NaClO_4$ (0.5 m in acetone).The precipitate was washed two times with acetone (10 - 20 ml).




**Supporting Figure S4**: IR Spectrum of crude **35** indicating presence of an azide group.

## 3 Computational Details

Computed structure and property results were carried out using the GAMESS[13] and Gaussian09[21] software packages. The B97-D[14] density functional was used in combination with the Def2-TZVPPD basis set[15] for geometry optimizations, Hessian evaluations, and property calculations. Full geometry optimizations were performed and uniquely characterized via second derivatives (Hessian) analysis to determine the number of imaginary frequencies (0=minima; 1=transition state), and effects of zero point energy. Effects of solvation were taken into account using the COSab method,[16,17] using a dielectric in accord with experiment and solvent radii from Klamt.[18] Visualization and analysis of structural and property results were obtained using Avogadro[19] and WEBMO.[20]

# 4 References

(1) Singh, J.; Steck, N.; De, D.; Hofer, A.; Ripp, A.; Captain, I.; Keller, M.; Wender, P. A.; Bhandari, R.; Jessen, H. J. A Phosphoramidite Analogue of Cyclotriphosphate Enables Iterative Polyphosphorylations. *Angewandte Chemie (International ed. in English)* **2019**, *58*, 3928–3933, DOI: 10.1002/anie.201814366.

(2) Yount, R. G.; Babcock, D.; Ballantyne, W.; Ojala, D. Adenylyl imidodiphosphate, an adenosine triphosphate analog containing a P--N--P linkage. *Biochemistry* **1971**, *10*, 2484–2489, DOI: 10.1021/bi00789a009.

(3) Mohamady, S.; Taylor, S. D. Synthesis of nucleoside 5'-tetraphosphates containing terminal fluorescent labels via activated cyclic trimetaphosphate. *The Journal of organic chemistry* **2014**, *79*, 2308–2313, DOI: 10.1021/jo500051y.

(4) Janos Ludwig and Fritz Eckstein. Rapid and efficient synthesis of nucleoside 5'-0-(1-thiotriphosphates), 5'-triphosphates and 2',3'-cyclophosphorothioates using 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one.

(5) Janos Ludwig and Fritz Eckstein. Synthesis of nucleoside 5'-O-(1,3-dithiotriphosphates) and 5'-O-(1,1-dithiotriphosphates).

(6) Sun, Q.; Liu, S.; Sun, J.; Gong, S.-S. An H-phosphonate strategy for the synthesis of 2′,3′-dideoxynucleoside triphosphates and homodinucleotides. *Chinese Chemical Letters* **2014**, *25*, 427–430, DOI: 10.1016/j.cclet.2013.11.029.

(7) Dal Ben, D.; Buccioni, M.; Lambertucci, C.; Marucci, G.; Spinaci, A.; Marchenkova, A.; Abdelrahman, A.; Nistri, A.; Müller, C. E.; Volpini, R. Investigation on 2',3'-O-Substituted ATP Derivatives and Analogs as Novel P2X3 Receptor Antagonists. *ACS medicinal chemistry letters* **2019**, *10*, 493–498, DOI: 10.1021/acsmedchemlett.8b00524.

(8) Ahmadibeni, Y.; Tiwari, R. K.; Sun, G.; Parang, K. Synthesis of nucleoside mono-, di-, and triphosphoramidates from solid-phase cyclosaligenyl phosphitylating reagents. *Organic letters* **2009**, *11*, 2157–2160, DOI: 10.1021/ol900320r.

(9) Lee, S. E.; Elphick, L. M.; Anderson, A. A.; Bonnac, L.; Child, E. S.; Mann, D. J.; Gouverneur, V. Synthesis and reactivity of novel gamma-phosphate modified ATP analogues. *Bioorganic & medicinal chemistry letters* **2009**, *19*, 3804–3807, DOI: 10.1016/j.bmcl.2009.04.028.

(10) Ahmadibeni, Y.; Dash, C.; Le Grice, S. F. J.; Parang, K. Solid-Phase Synthesis of 5'-O-β,γ-Methylenetriphosphate Derivatives of Nucleosides and Evaluation of Their Inhibitory Activity Against HIV-1 Reverse Transcriptase. *Tetrahedron Letters* **2010**, *51*, 3010–3013, DOI: 10.1016/j.tetlet.2010.04.005.

(11) You, Y. H.; Gong, S. S.; Sun, Q. Practical Synthesis of β,γ-Bridging Oxygen-Modified Stavudine 5’-Triphosphates. *AMR* **2014**, *908*, 207–210, DOI: 10.4028/www.scientific.net/AMR.908.207.

(12) Wang, G.; Boyle, N.; Chen, F.; Rajappan, V.; Fagan, P.; Brooks, J. L.; Hurd, T.; Leeds, J. M.; Rajwanshi, V. K.; Jin, Y. *et al.* Synthesis of AZT 5'-triphosphate mimics and their inhibitory effects on HIV-1 reverse transcriptase. *Journal of medicinal chemistry* **2004**, *47*, 6902–6913, DOI: 10.1021/jm040116w.

(13) Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S. *et al.* General atomic and molecular electronic structure system. *J. Comput. Chem.* **1993**, *14*, 1347–1363, DOI: 10.1002/jcc.540141112.

(14) Grimme, S. Semiempirical GGA-type density functional constructed with a long-range dispersion correction. *Journal of computational chemistry* **2006**, *27*, 1787–1799, DOI: 10.1002/jcc.20495.

(15) Rappoport, D.; Furche, F. Property-optimized gaussian basis sets for molecular response calculations. *The Journal of chemical physics* **2010**, *133*, 134105, DOI: 10.1063/1.3484283.

(16) Klamt, A.; Schüürmann, G. COSMO: A new approach to dielectric screening in solvents with explicit expressions for the screening energy and its gradient. *J. Chem. Soc., Perkin Trans. 2* **1993**, 799–805, DOI: 10.1039/P29930000799.

(17) Baldridge, K.; Klamt, A. First principles implementation of solvent effects without outlying charge error. *The Journal of chemical physics* **1997**, *106*, 6622–6633, DOI: 10.1063/1.473662.

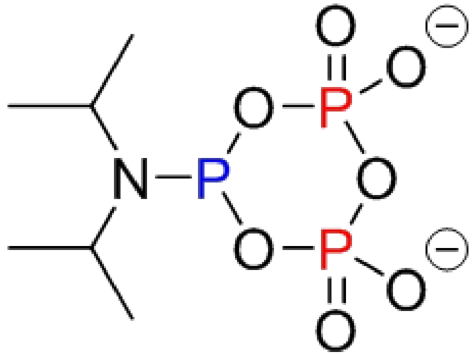
(18) Klamt, A.; Jonas, V.; Bürger, T.; Lohrenz, J. C. W. Refinement and Parametrization of COSMO-RS. *J. Phys. Chem. A* **1998**, *102*, 5074–5085, DOI: 10.1021/jp980017s.

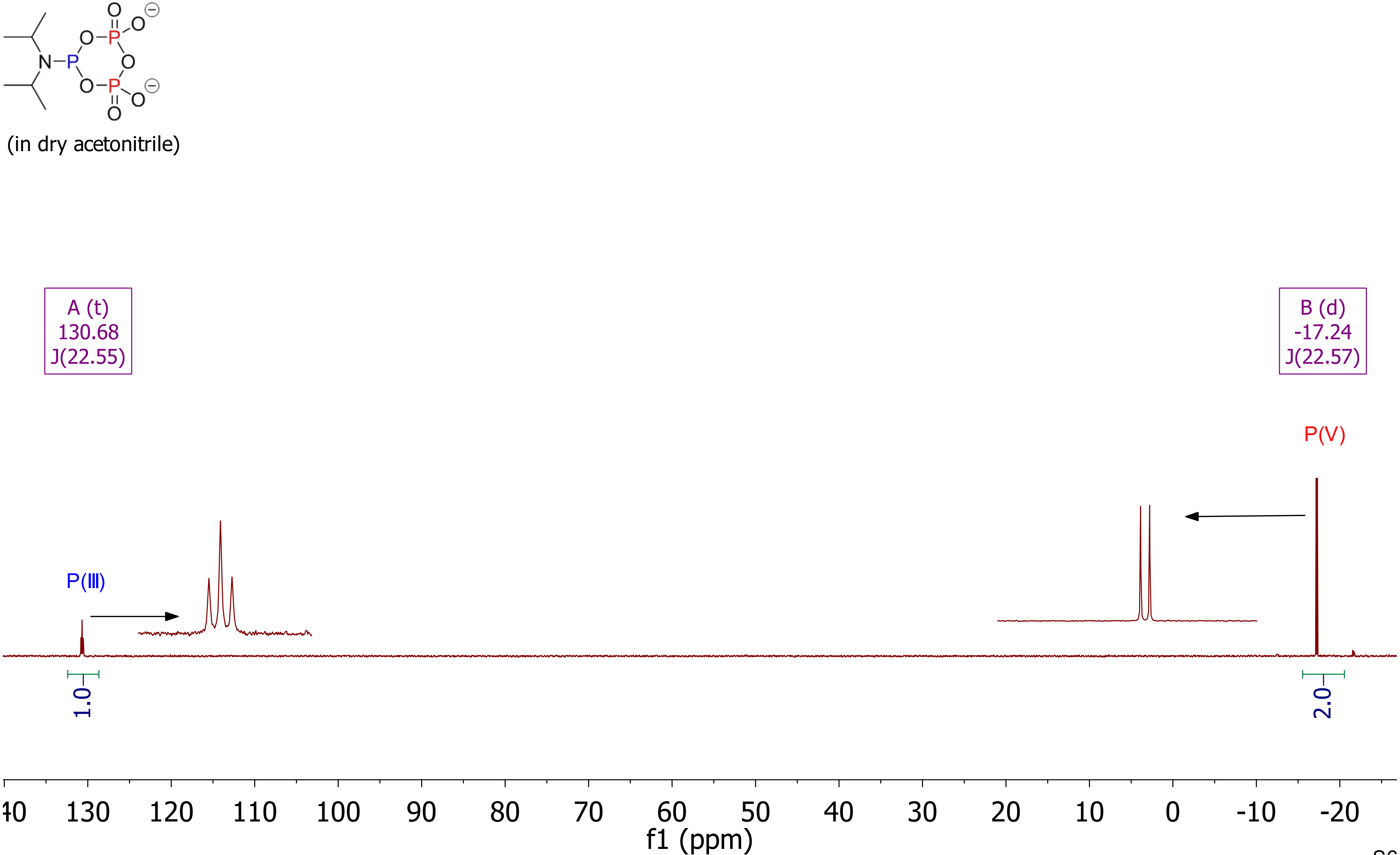
(19) Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E.; Hutchison, G. R. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of cheminformatics* **2012**, *4*, 17, DOI: 10.1186/1758-2946-4-17.

(20) http://www.webmo.net/index.html.

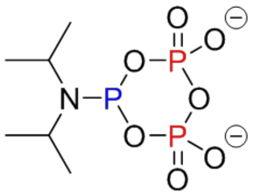
(21) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr., Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision E.01, Gaussian, Inc., Wallingford CT, 2013.

# Triphosphates based on c-PyPA ($A_1$)

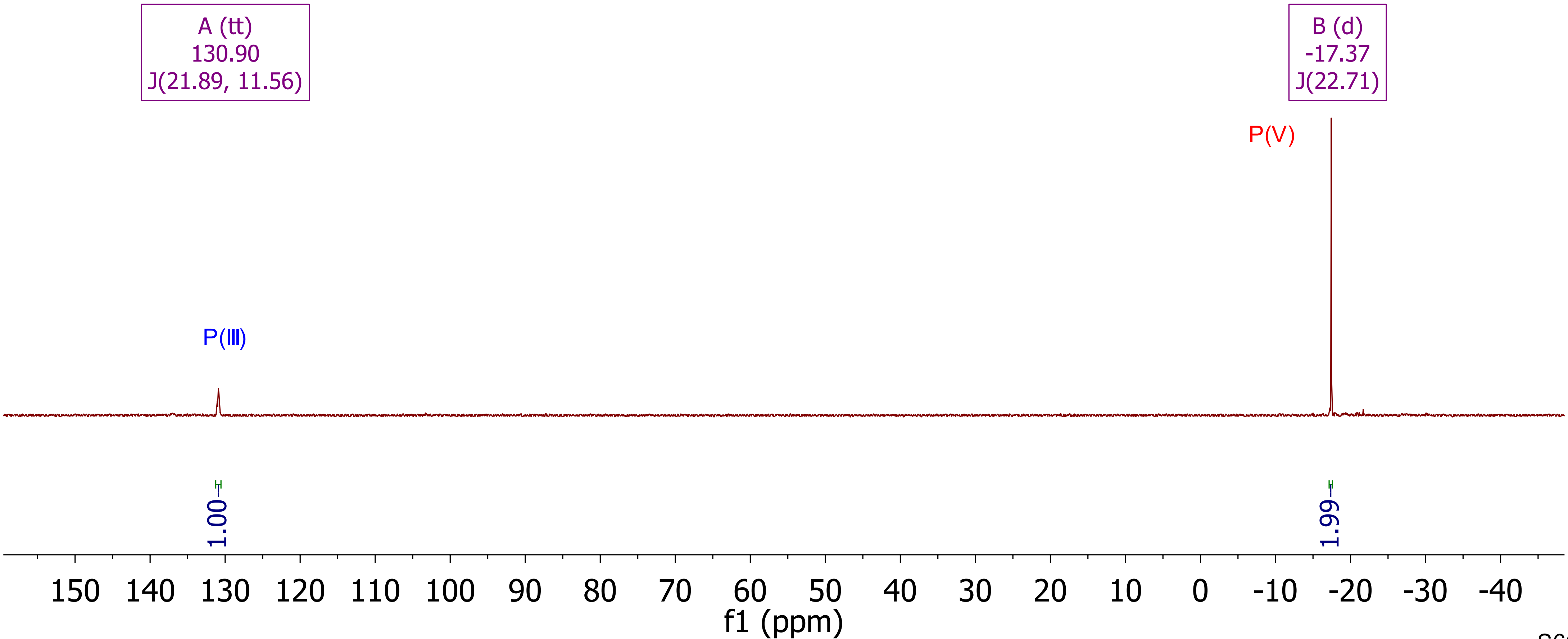




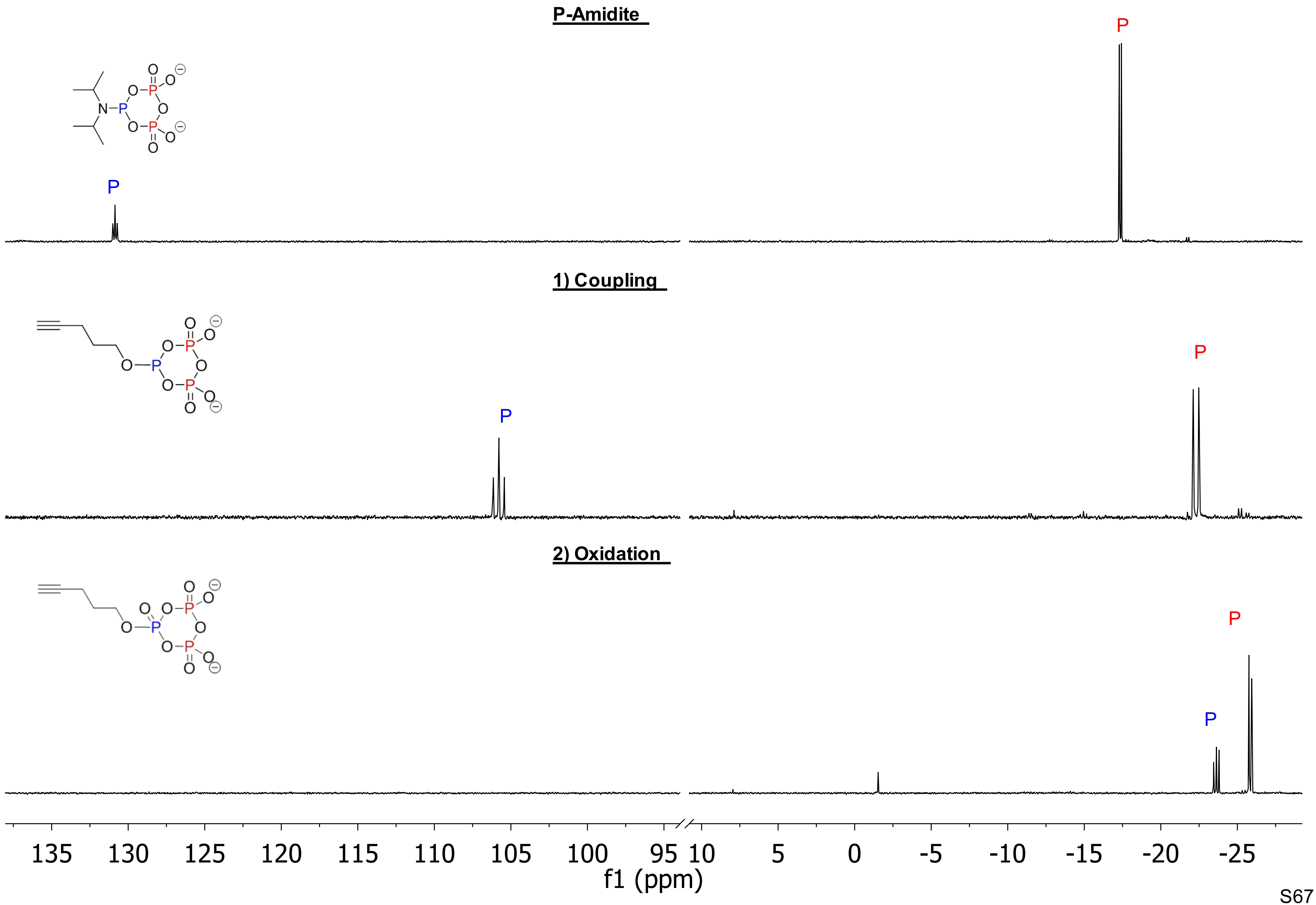
N
P
O
(in dry acetonitrile)
A (t)
130.68
J(22.55)
B (d)
-17.24
J(22.57)
P(V)
P(III)
1.0
2.0
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)

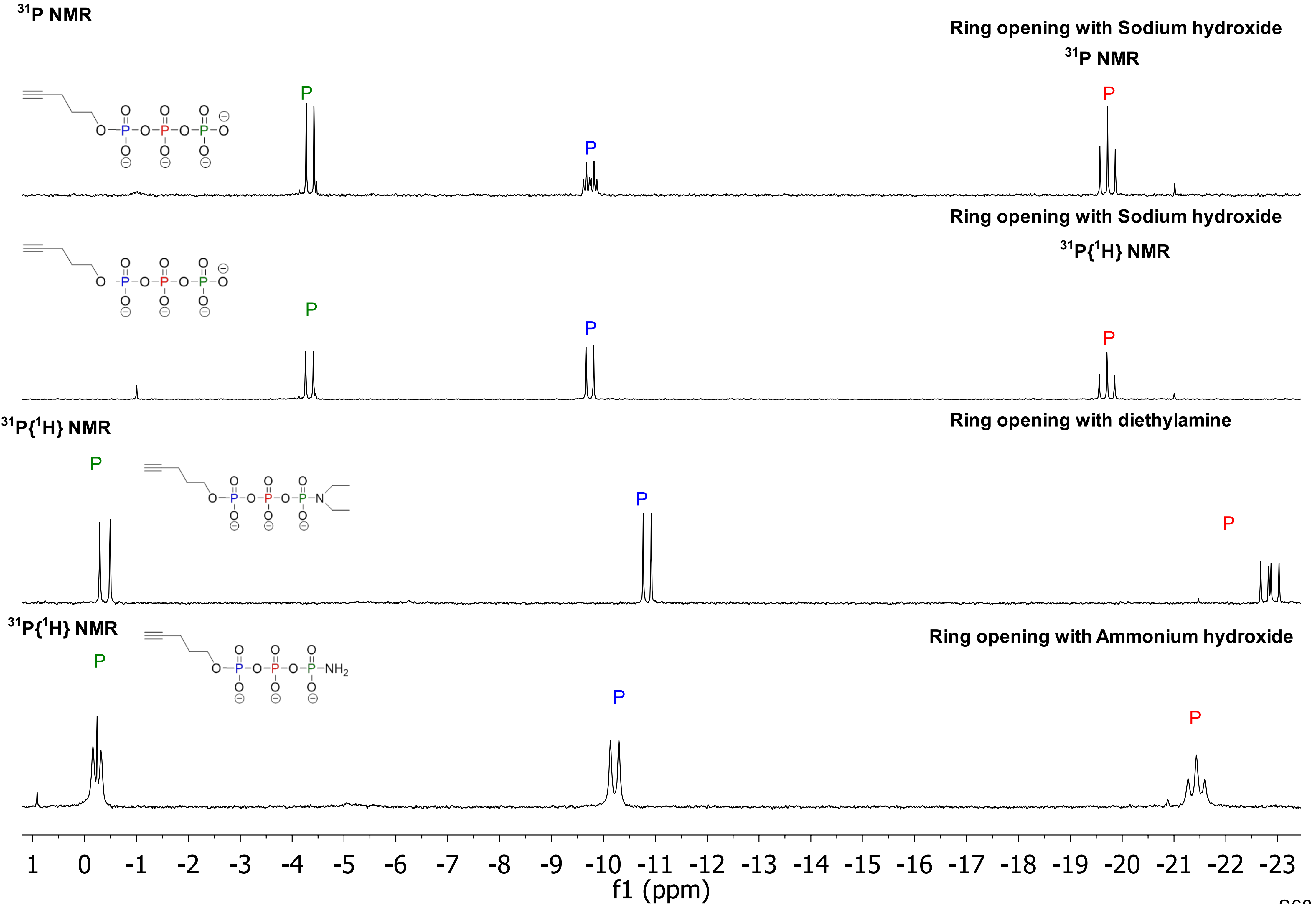


(in dry acetonitrile)

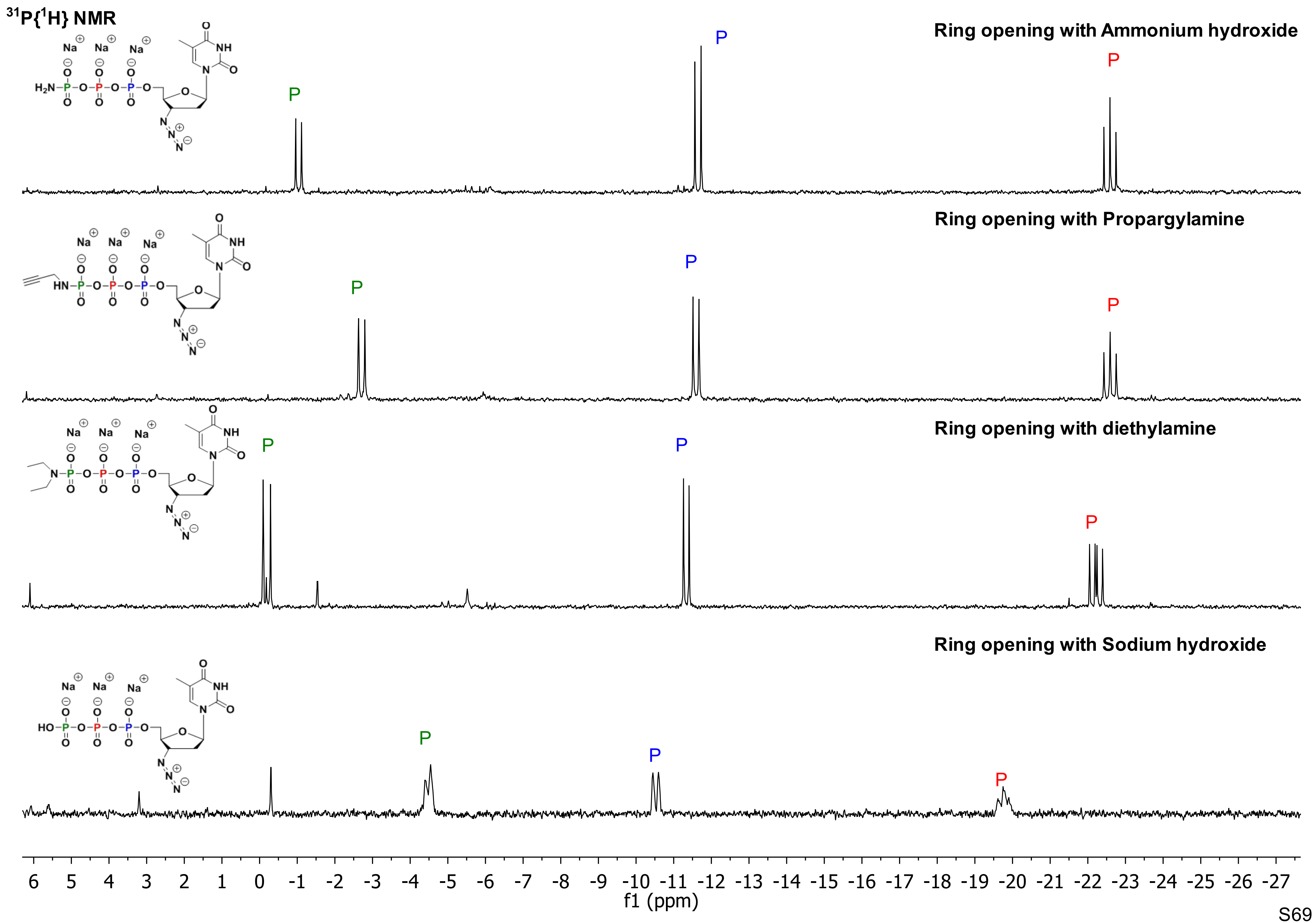


$^{31}P\{^1H\}$ NMR

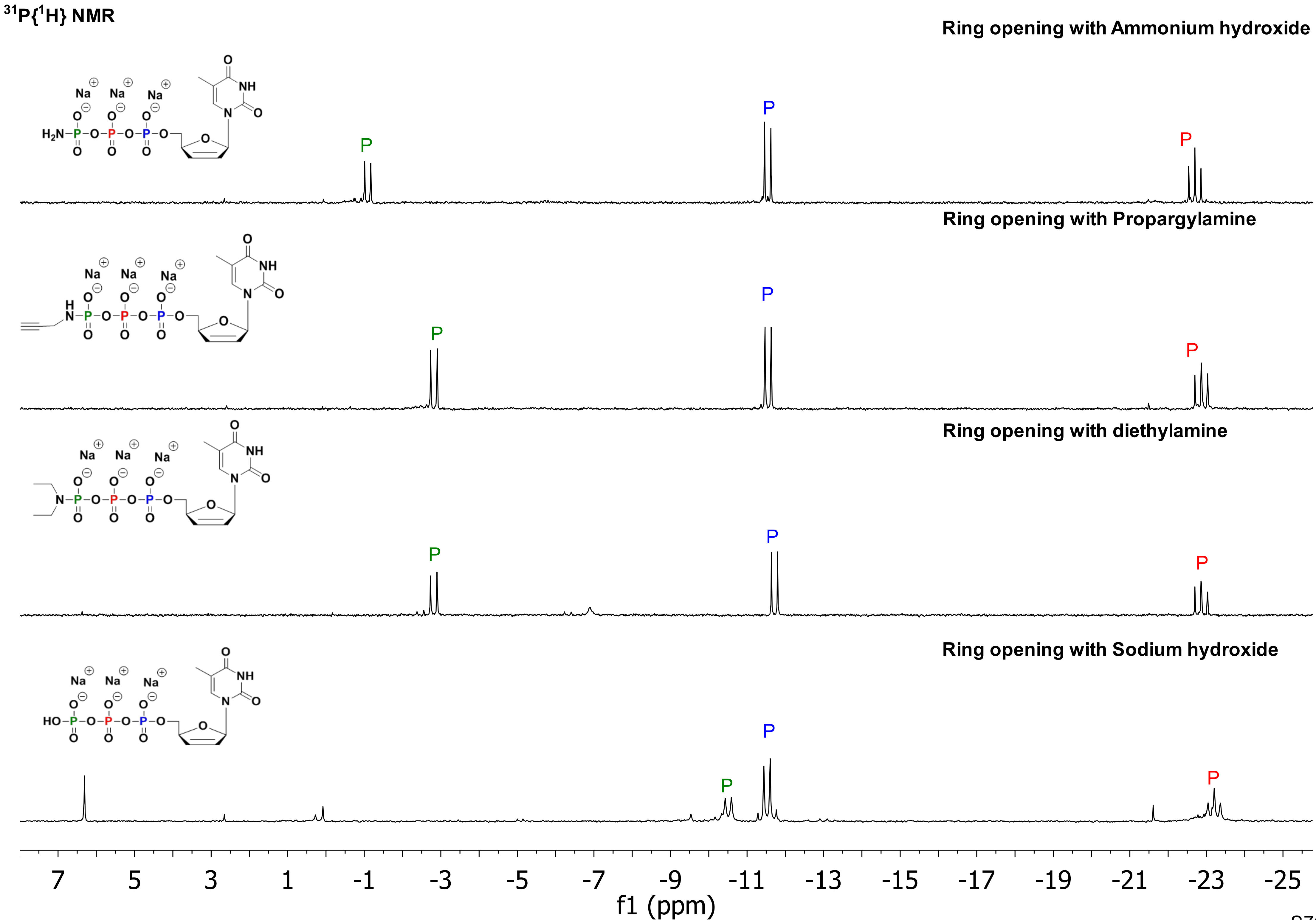

P-Amidite
P
P
1) Coupling
P
P
2) Oxidation
P
P
135 130 125 120 115 110 105 100 95 10 5 0 -5 -10 -15 -20 -25
f1 (ppm)



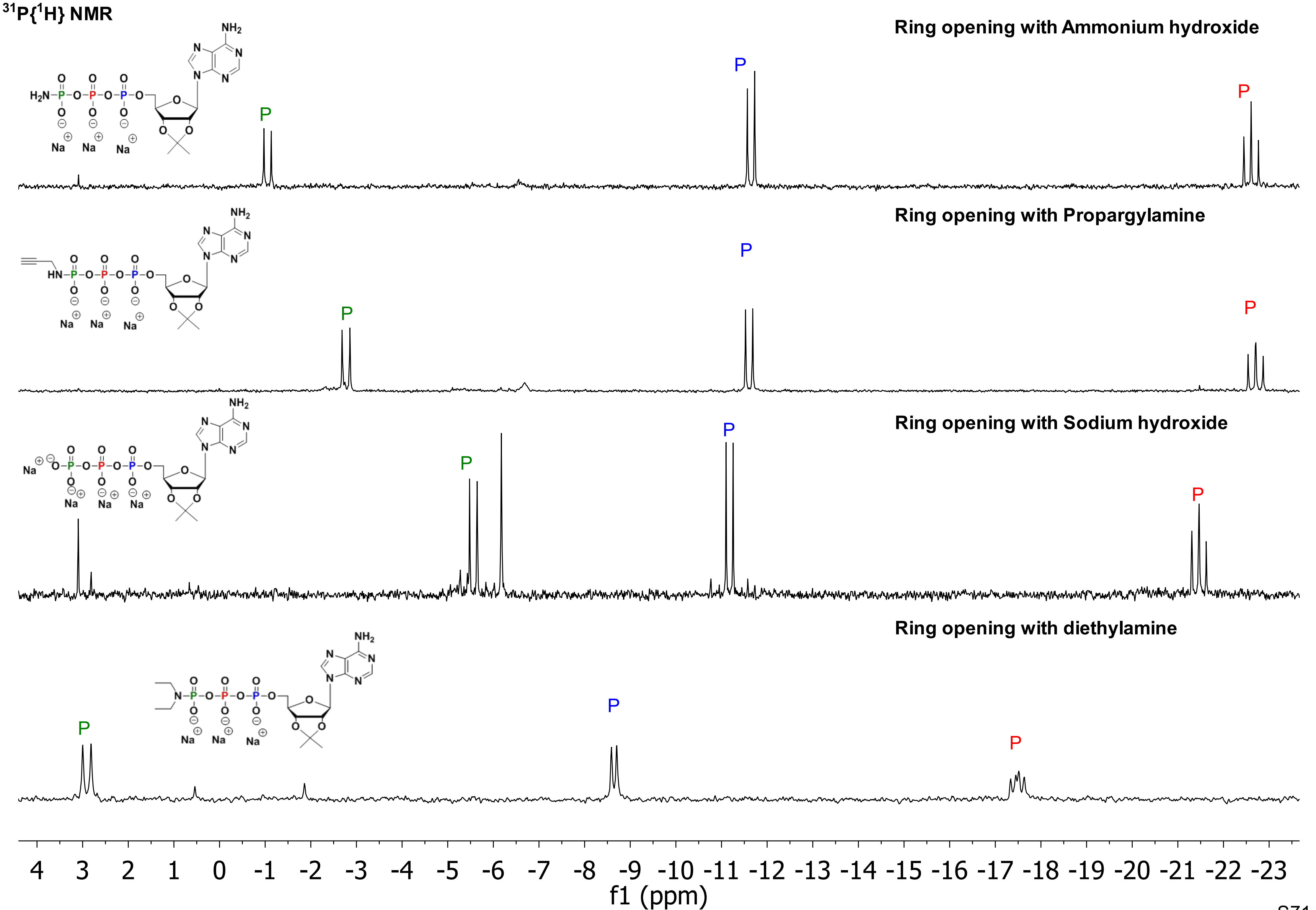
31P NMR
Ring opening with Sodium hydroxide
31P NMR
Ring opening with Sodium hydroxide
31P{1H} NMR
31P{1H} NMR
Ring opening with diethylamine
31P{1H} NMR
Ring opening with Ammonium hydroxide
1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23
f1 (ppm)



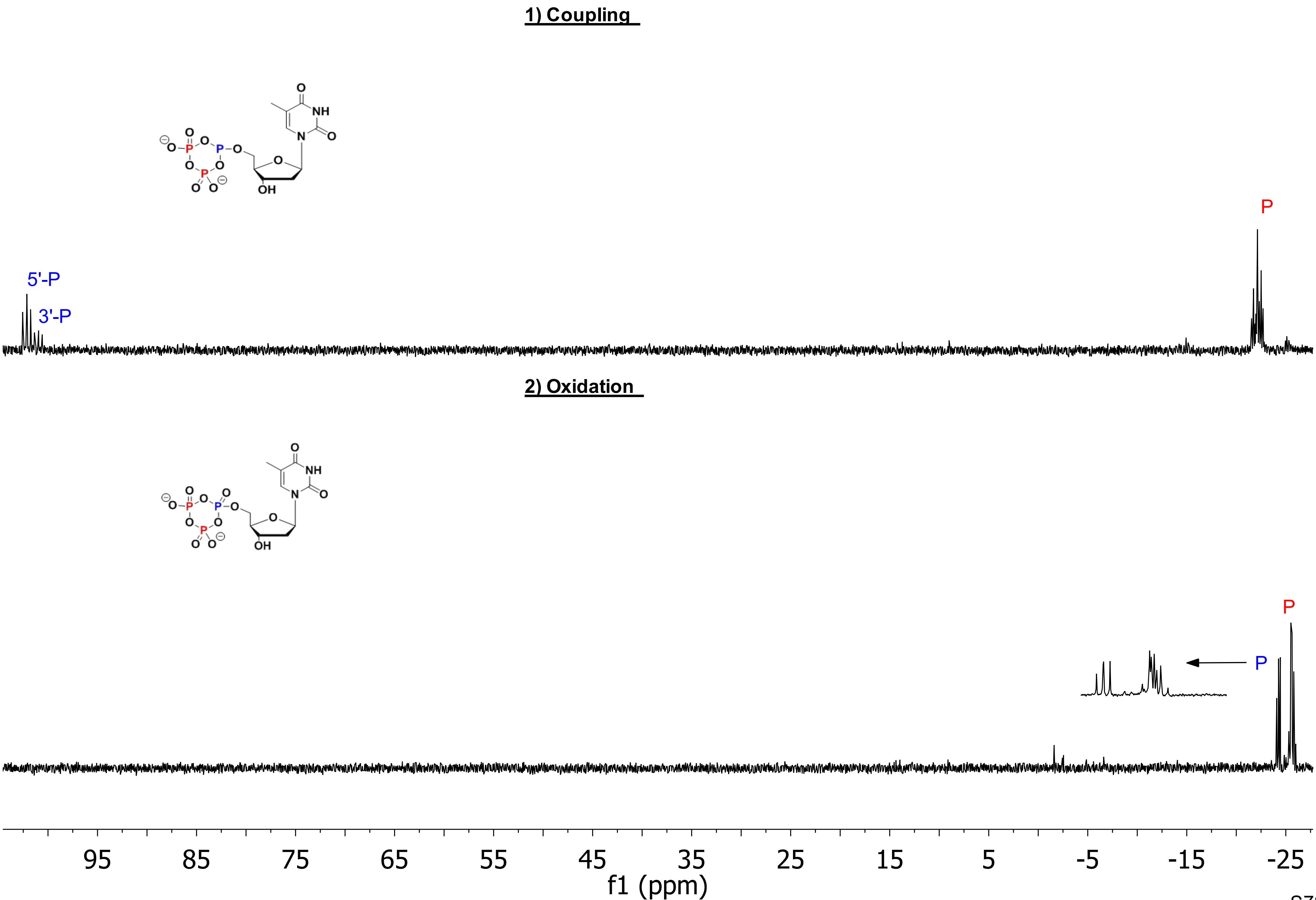
$^{31}P\{^1H\}$ NMR
Ring opening with Ammonium hydroxide
Ring opening with Propargylamine
Ring opening with diethylamine
Ring opening with Sodium hydroxide
f1 (ppm)



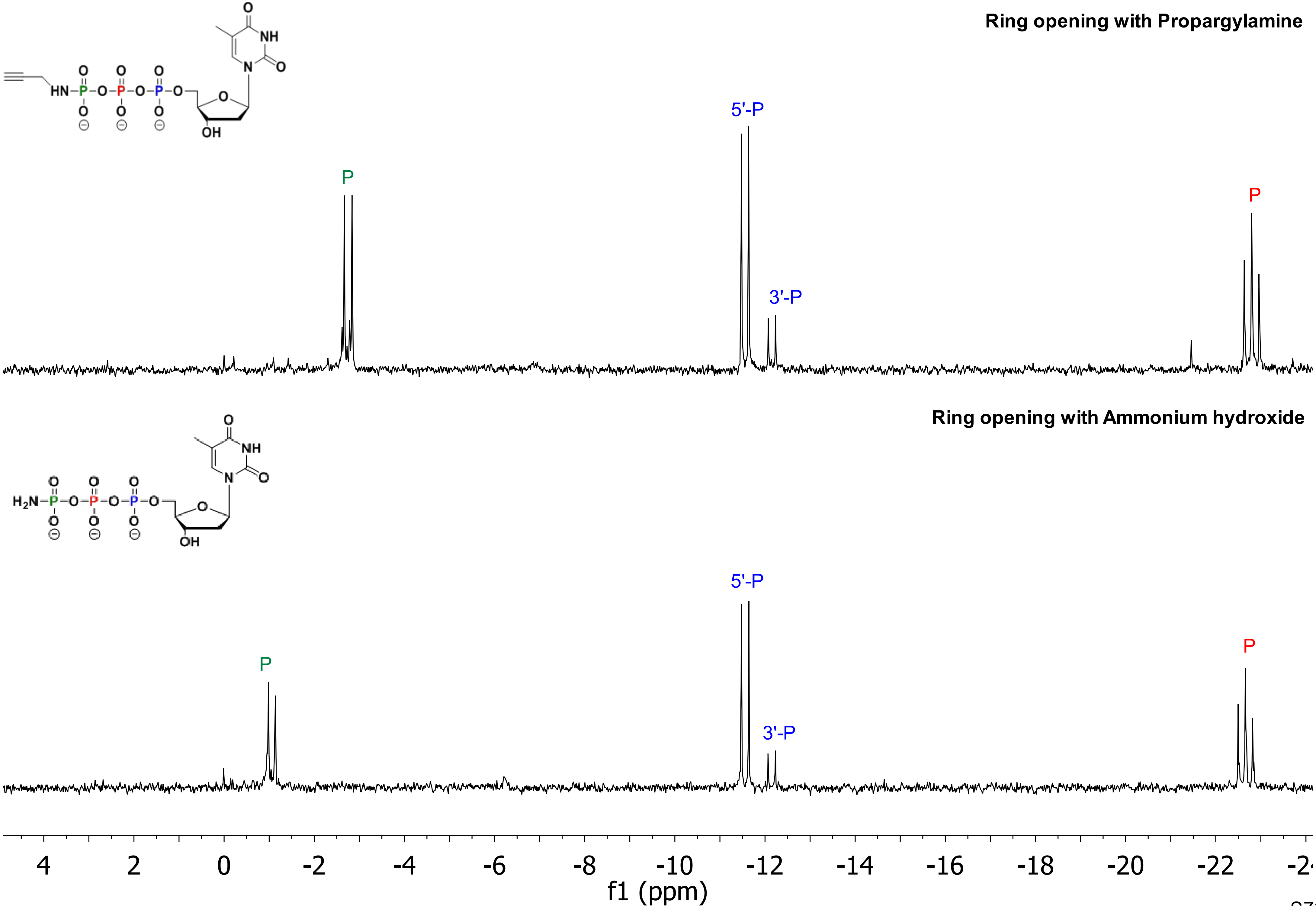
31P{1H} NMR
Ring opening with Ammonium hydroxide
Ring opening with Propargylamine
Ring opening with diethylamine
Ring opening with Sodium hydroxide
7 5 3 1 -1 -3 -5 -7 -9 -11 -13 -15 -17 -19 -21 -23 -25
f1 (ppm)


31P{1H} NMR
Ring opening with Ammonium hydroxide
Ring opening with Propargylamine
Ring opening with Sodium hydroxide
Ring opening with diethylamine
4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23
f1 (ppm)

$^{31}P\{^1H\}$ NMR

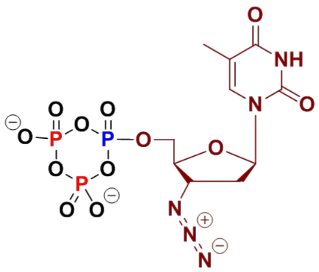

1) Coupling
5'-P
3'-P
P
2) Oxidation
P
P
95
85
75
65
55
45
35
25
15
5
-5
-15
-25
f1 (ppm)

**$^{31}P\{^1H\}$ NMR**

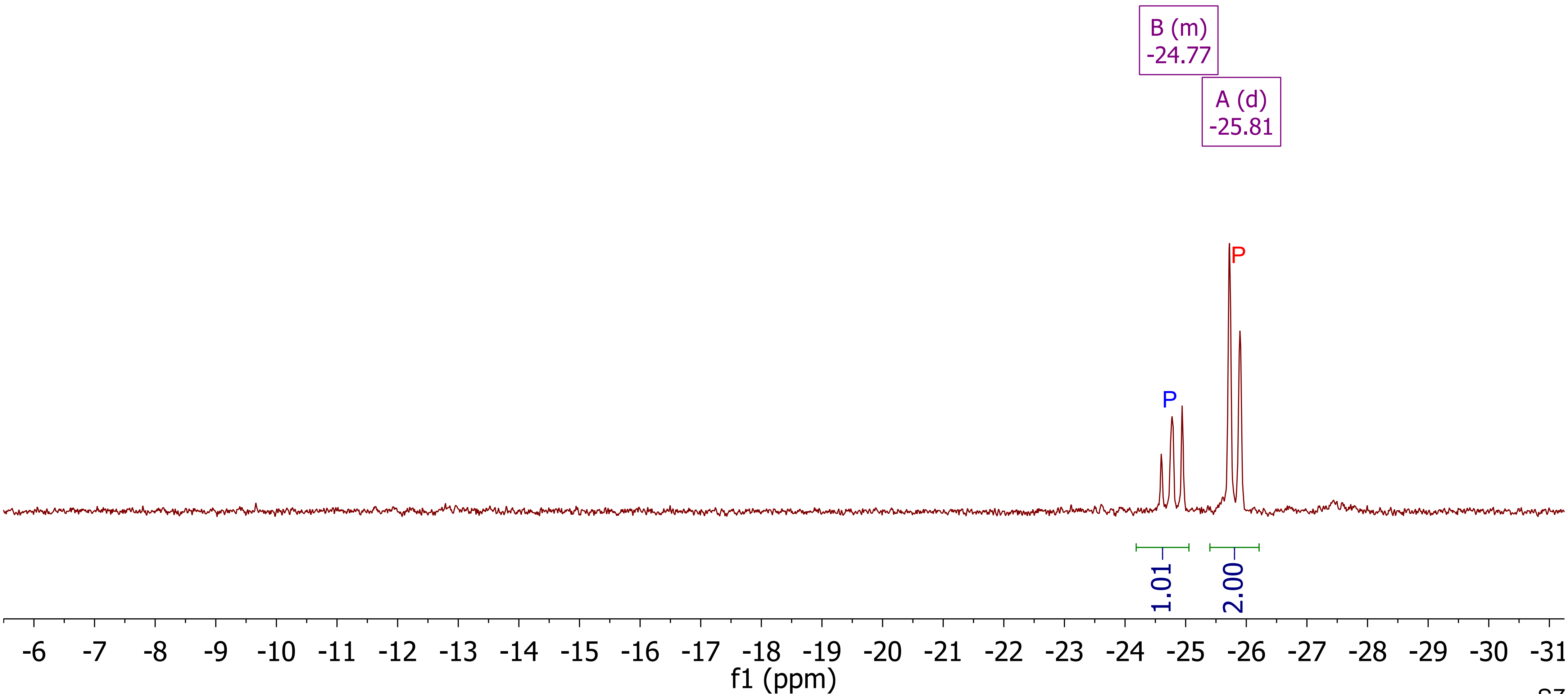


Ring opening with Propargylamine
5'-P
P
3'-P
P
Ring opening with Ammonium hydroxide
5'-P
P
3'-P
P
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
-22
f1 (ppm)

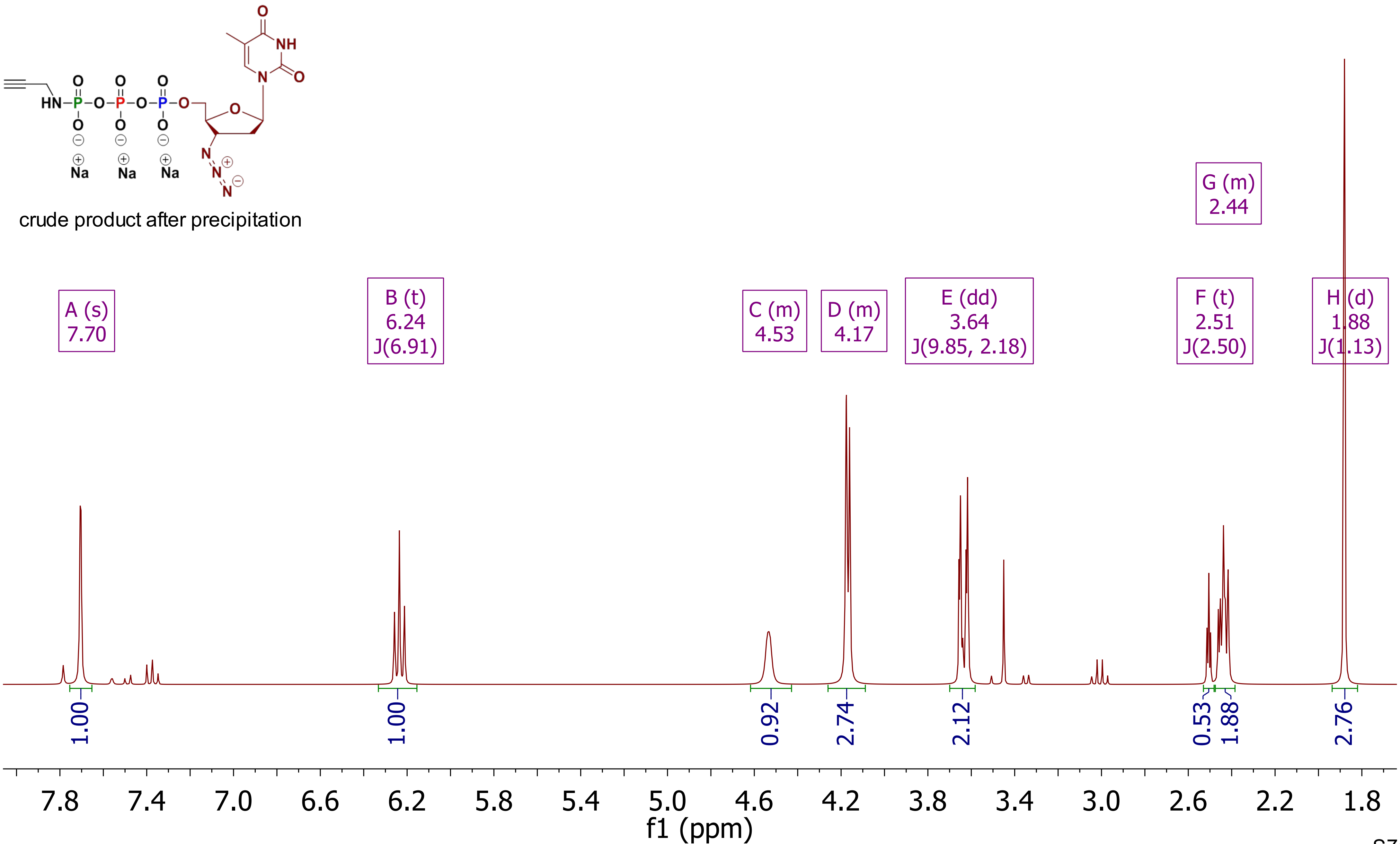
**$^{31}P\{^1H\}$ NMR**

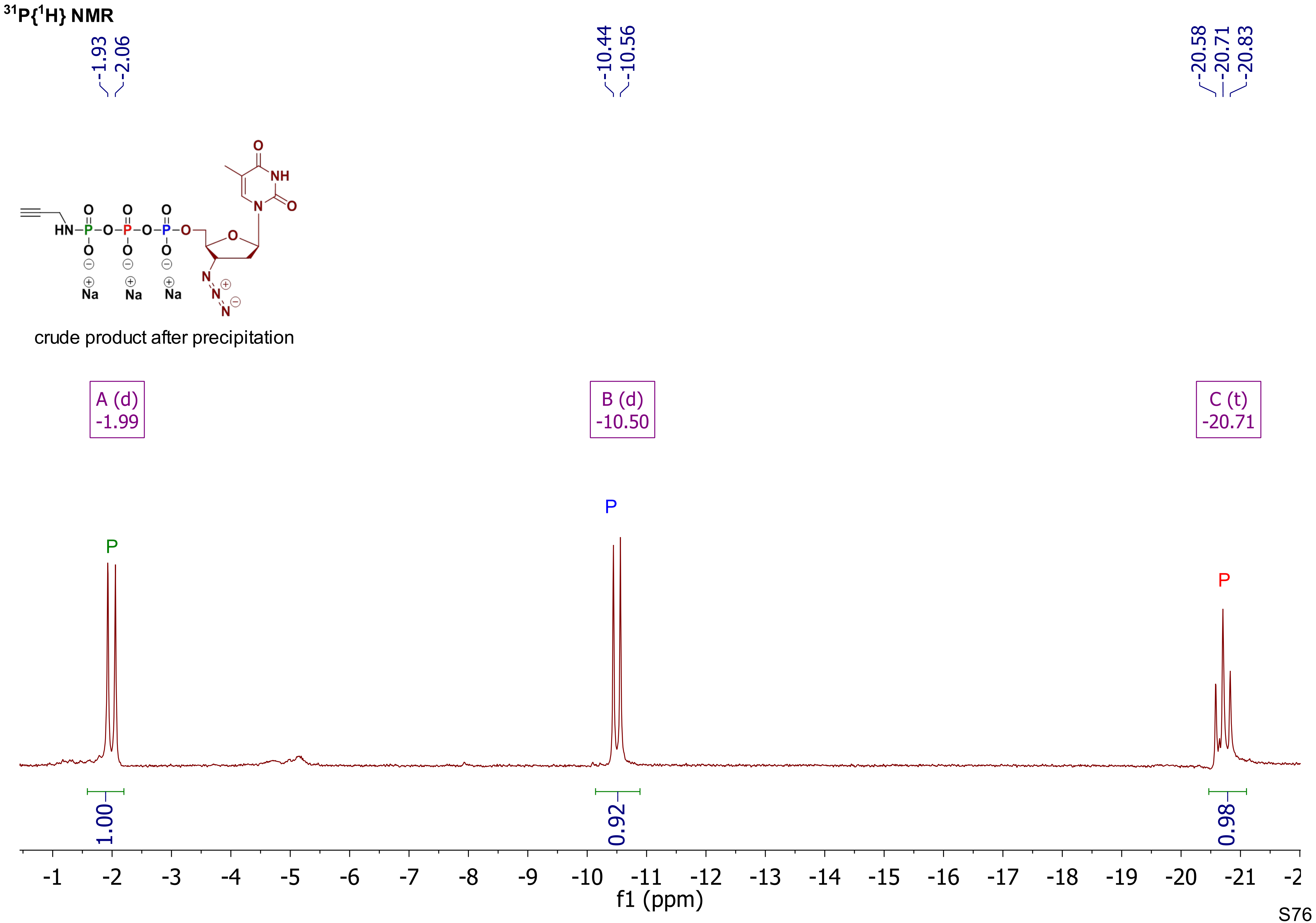


**<u>Oxidation</u>**



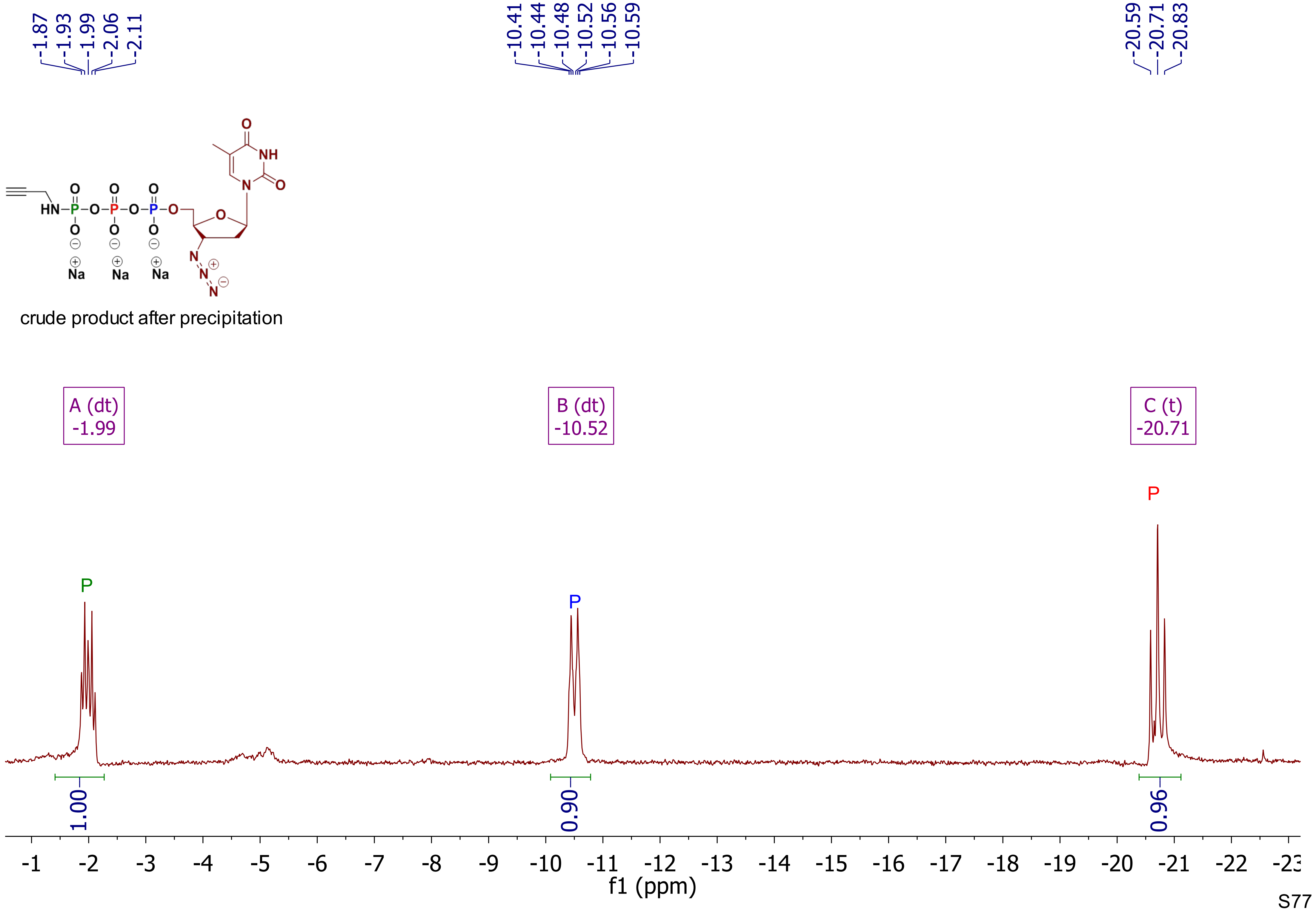
**$^{1}$H NMR**


HN
O
Na
N
NH
crude product after precipitation
A (s)
7.70
B (t)
6.24
J(6.91)
C (m)
4.53
D (m)
4.17
E (dd)
3.64
J(9.85, 2.18)
G (m)
2.44
F (t)
2.51
J(2.50)
H (d)
1.88
J(1.13)
1.00
1.00
0.92
2.74
2.12
0.53
1.88
2.76
7.8 7.4 7.0 6.6 6.2 5.8 5.4 5.0 4.6 4.2 3.8 3.4 3.0 2.6 2.2 1.8
f1 (ppm)



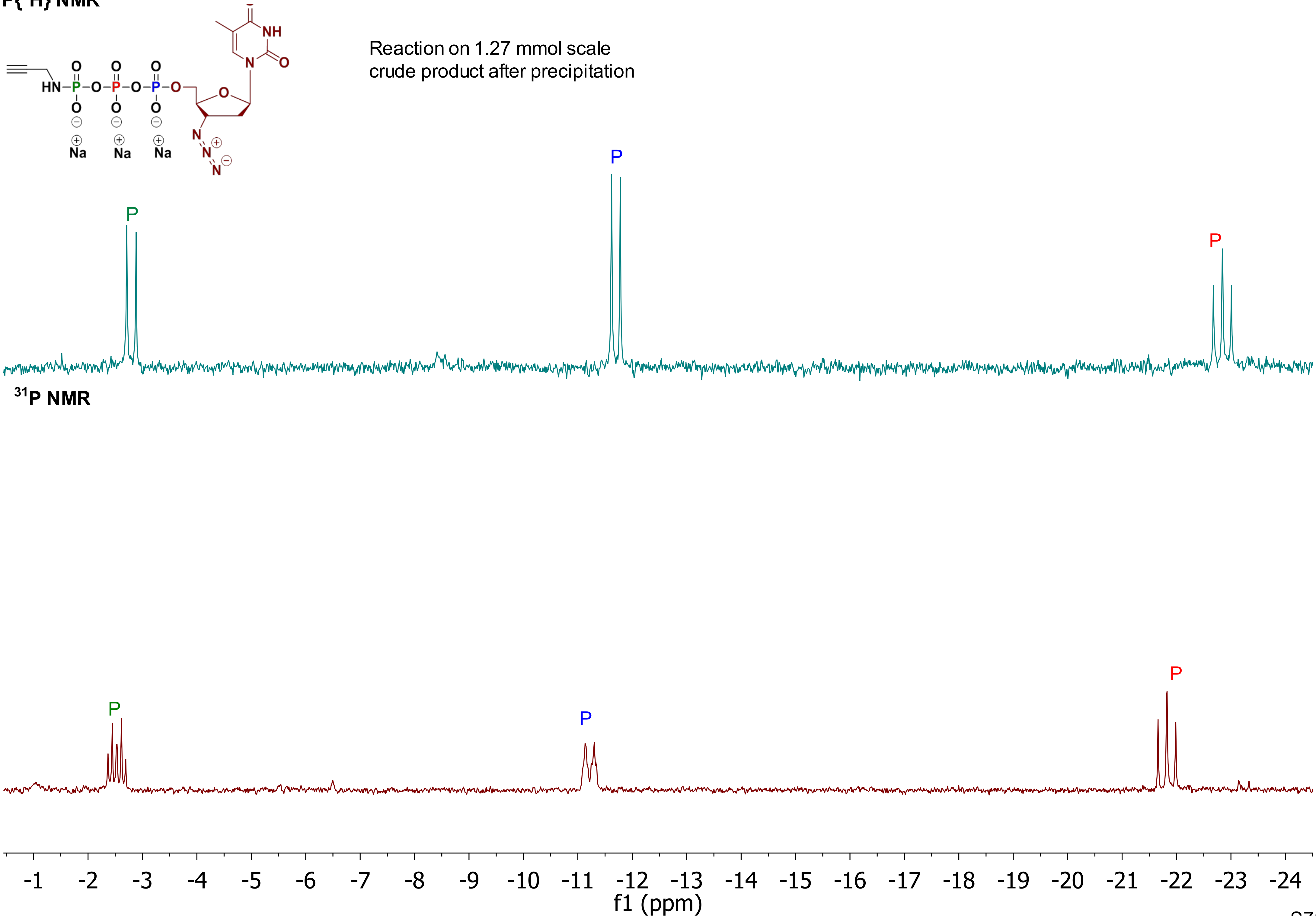
$^{31}P\{^1H\}$ NMR
-1.93
-2.06
-10.44
-10.56
-20.58
-20.71
-20.83
crude product after precipitation
A (d)
-1.99
B (d)
-10.50
C (t)
-20.71
P
P
P
1.00
0.92
0.98
f1 (ppm)

$^{31}$P NMR


-1.87
-1.93
-1.99
-2.06
-2.11
-10.41
-10.44
-10.48
-10.52
-10.56
-10.59
-20.59
-20.71
-20.83
crude product after precipitation
A (dt)
-1.99
B (dt)
-10.52
C (t)
-20.71
P
P
P
1.00
0.90
0.96
f1 (ppm)

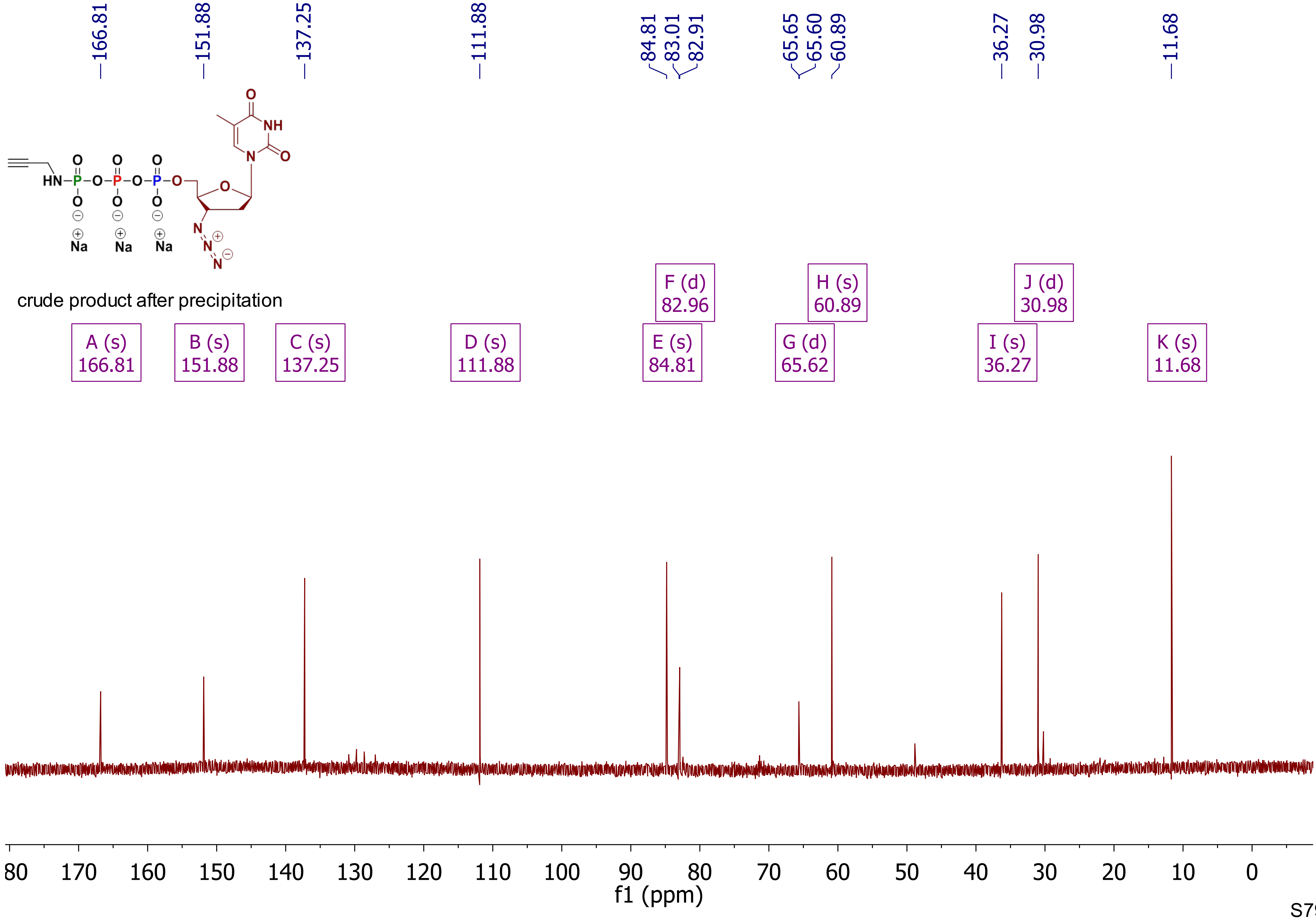
$^{31}P\{^1H\}$ NMR

Reaction on 1.27 mmol scale
crude product after precipitation



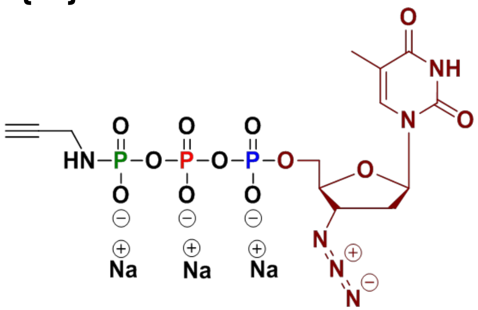

$^{31}P$ NMR

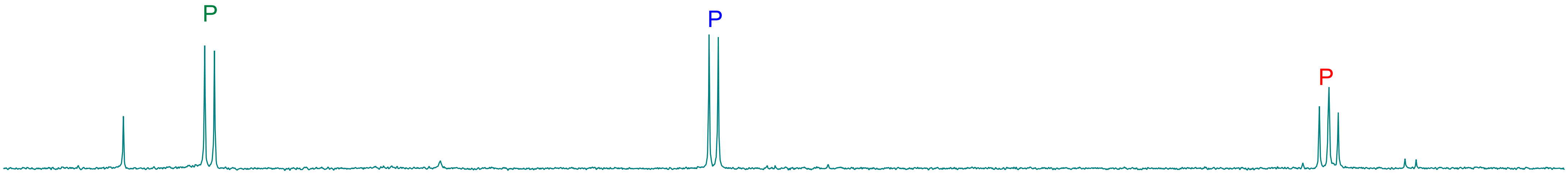
$^{13}$C NMR

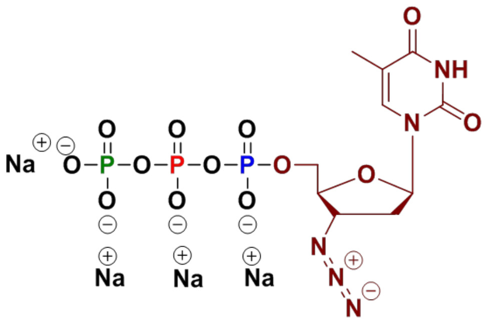


166.81
151.88
137.25
111.88
84.81
83.01
82.91
65.65
65.60
60.89
36.27
30.98
11.68
crude product after precipitation
A (s) 166.81
B (s) 151.88
C (s) 137.25
D (s) 111.88
E (s) 84.81
F (d) 82.96
G (d) 65.62
H (s) 60.89
I (s) 36.27
J (d) 30.98
K (s) 11.68
f1 (ppm)

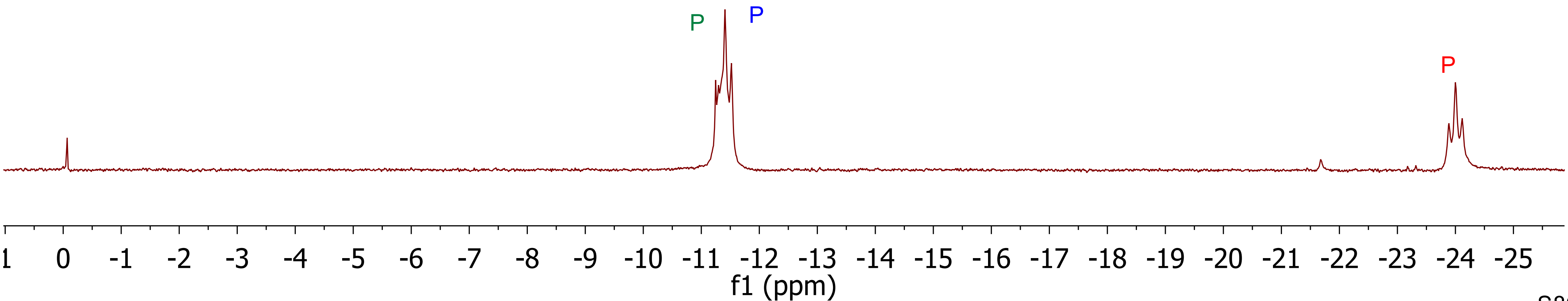
**$^{31}P\{^1H\}$ NMR**

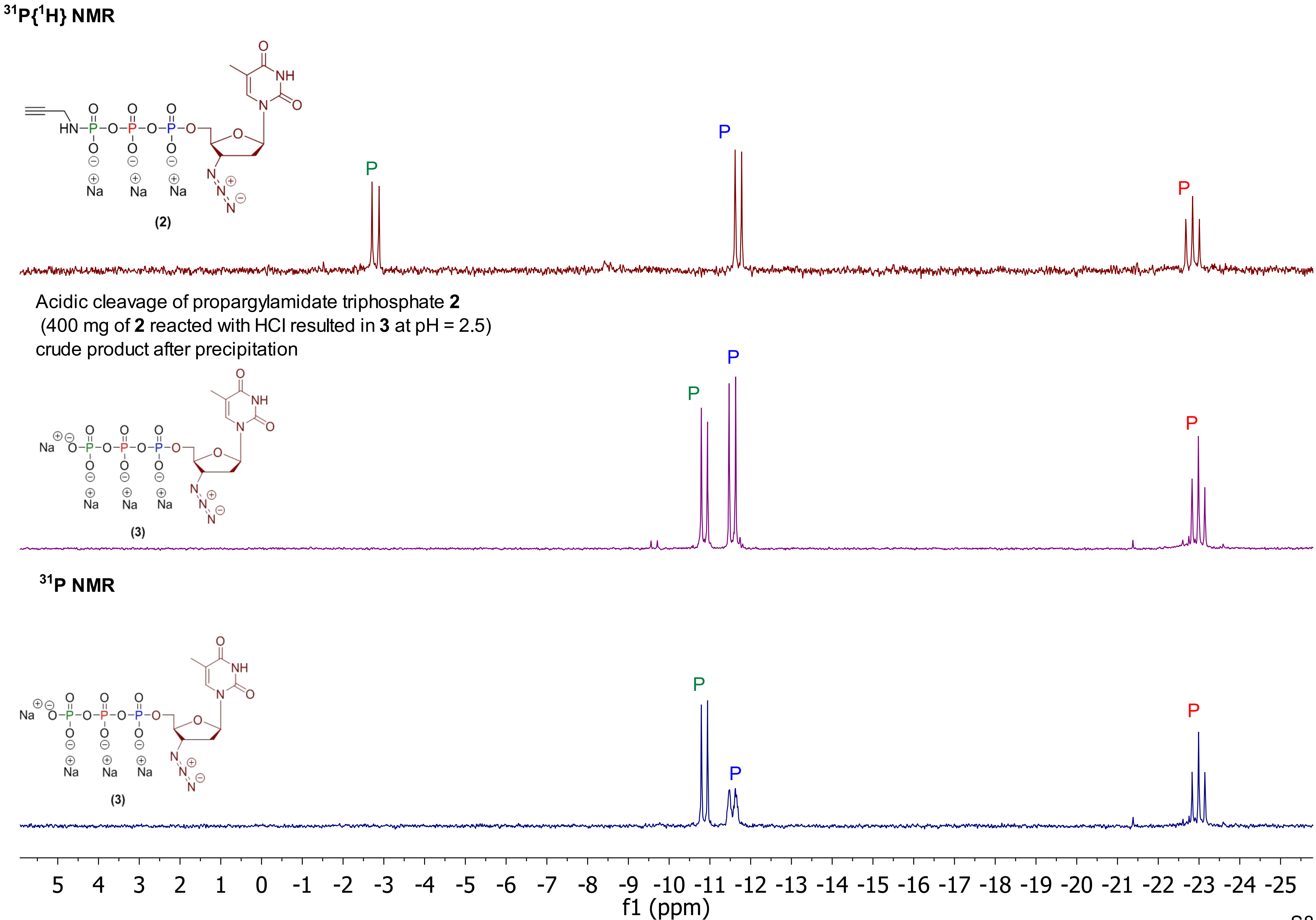


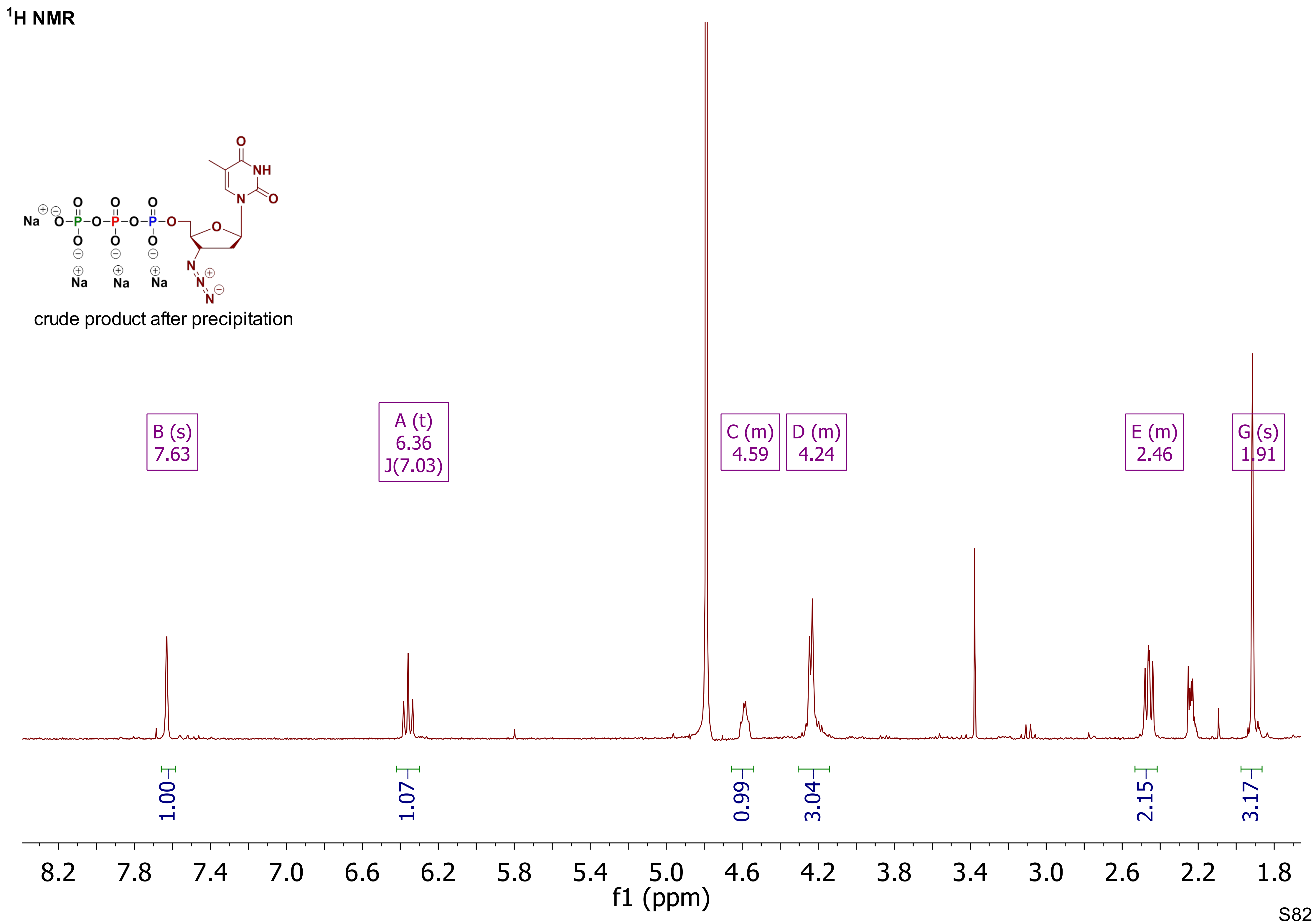


Acidic cleavage of propargyl amine at pH = 4.5

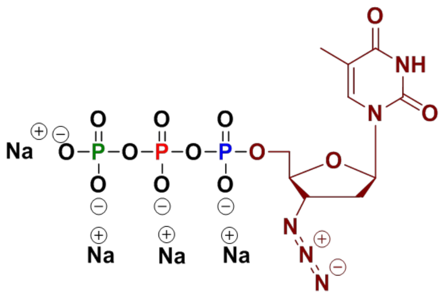



31P{1H} NMR
(2)
Acidic cleavage of propargylamidate triphosphate 2
(400 mg of 2 reacted with HCl resulted in 3 at pH = 2.5)
crude product after precipitation
(3)
31P NMR
(3)
f1 (ppm)

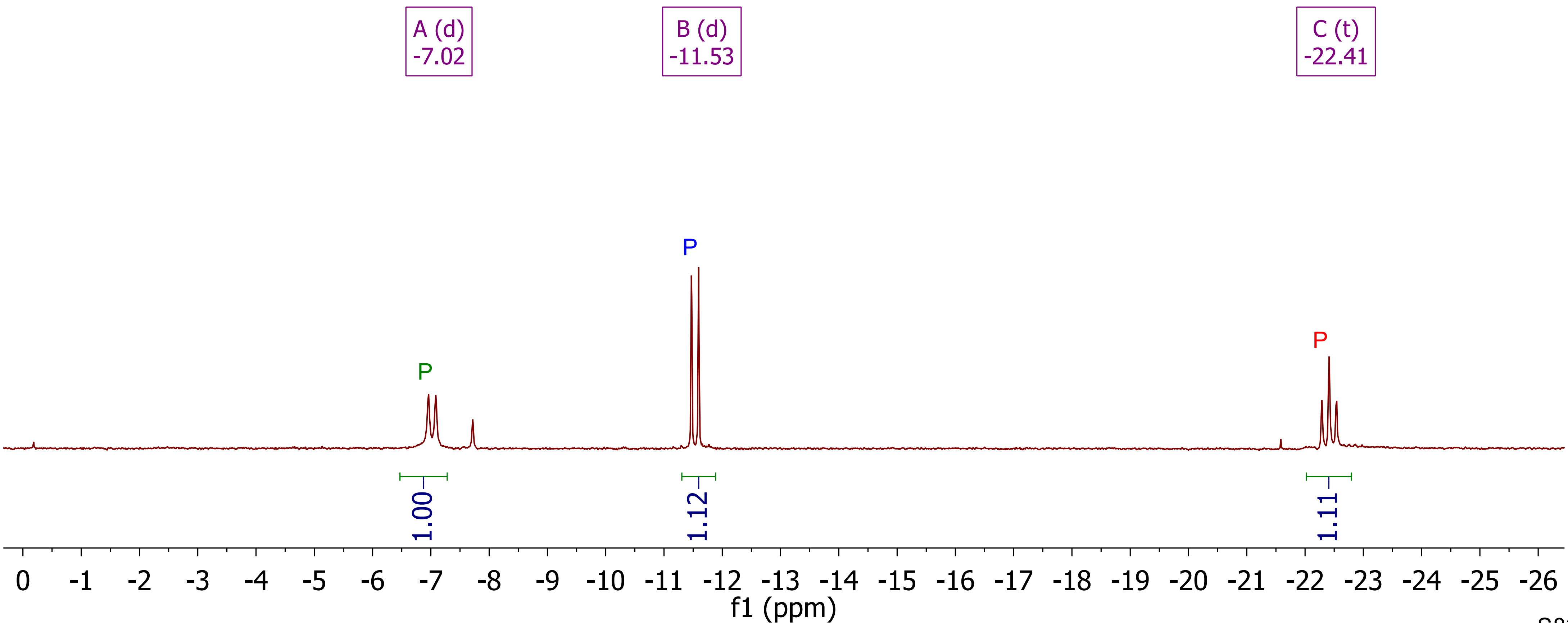


1H NMR
crude product after precipitation
B (s) 7.63
A (t) 6.36 J(7.03)
C (m) 4.59
D (m) 4.24
E (m) 2.46
G (s) 1.91
1.00
1.07
0.99
3.04
2.15
3.17
8.2 7.8 7.4 7.0 6.6 6.2 5.8 5.4 5.0 4.6 4.2 3.8 3.4 3.0 2.6 2.2 1.8
f1 (ppm)

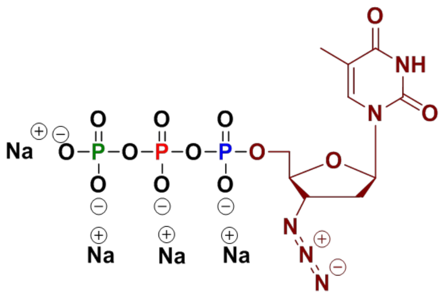
**$^{31}P\{^1H\}$ NMR**



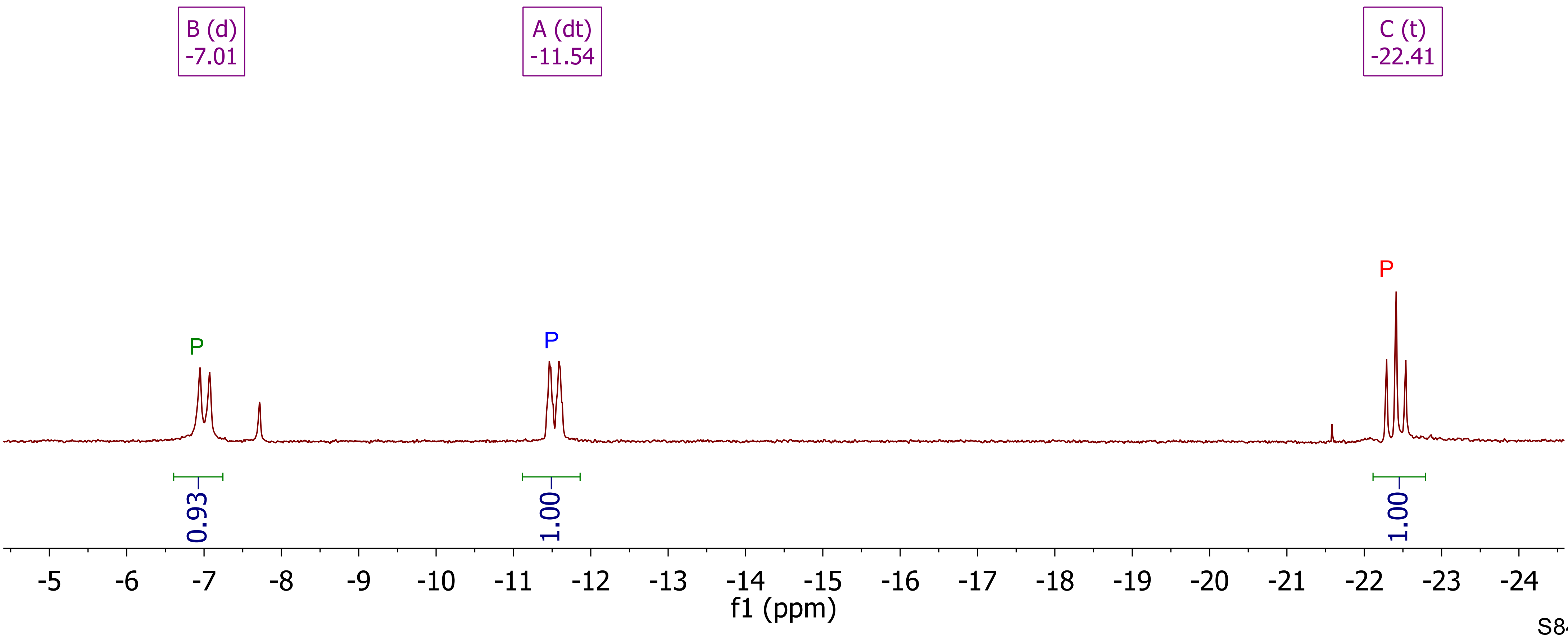
crude product after precipitation



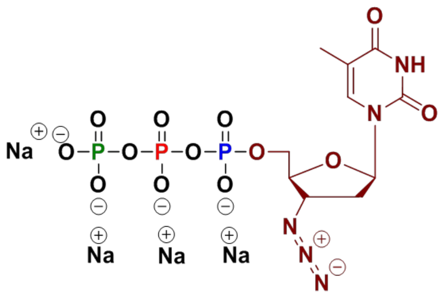
**$^{31}$P NMR**



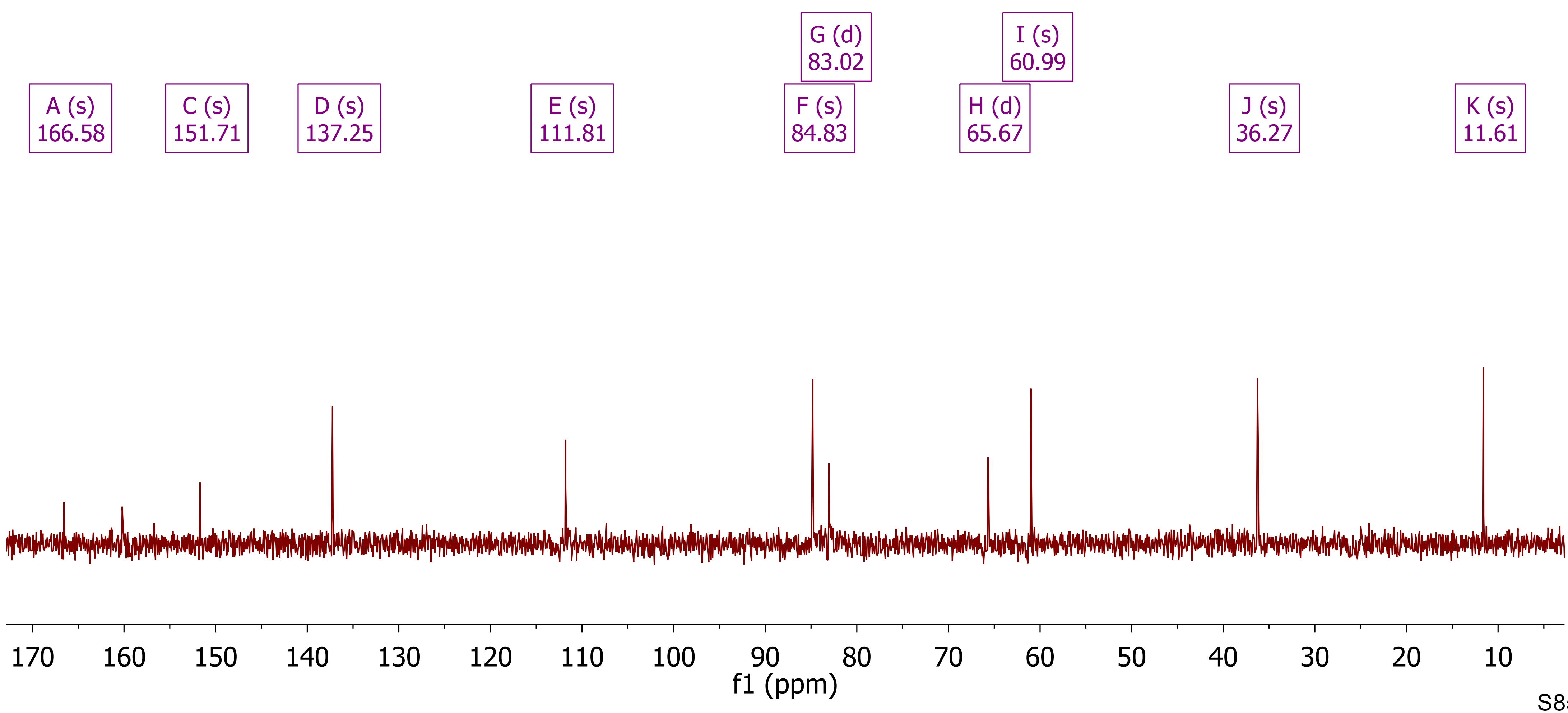
crude product after precipitation



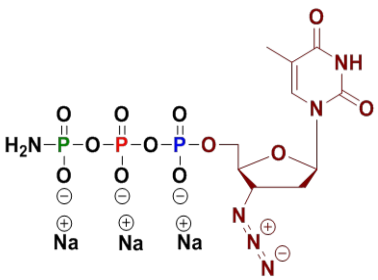
**$^{13}$C NMR**



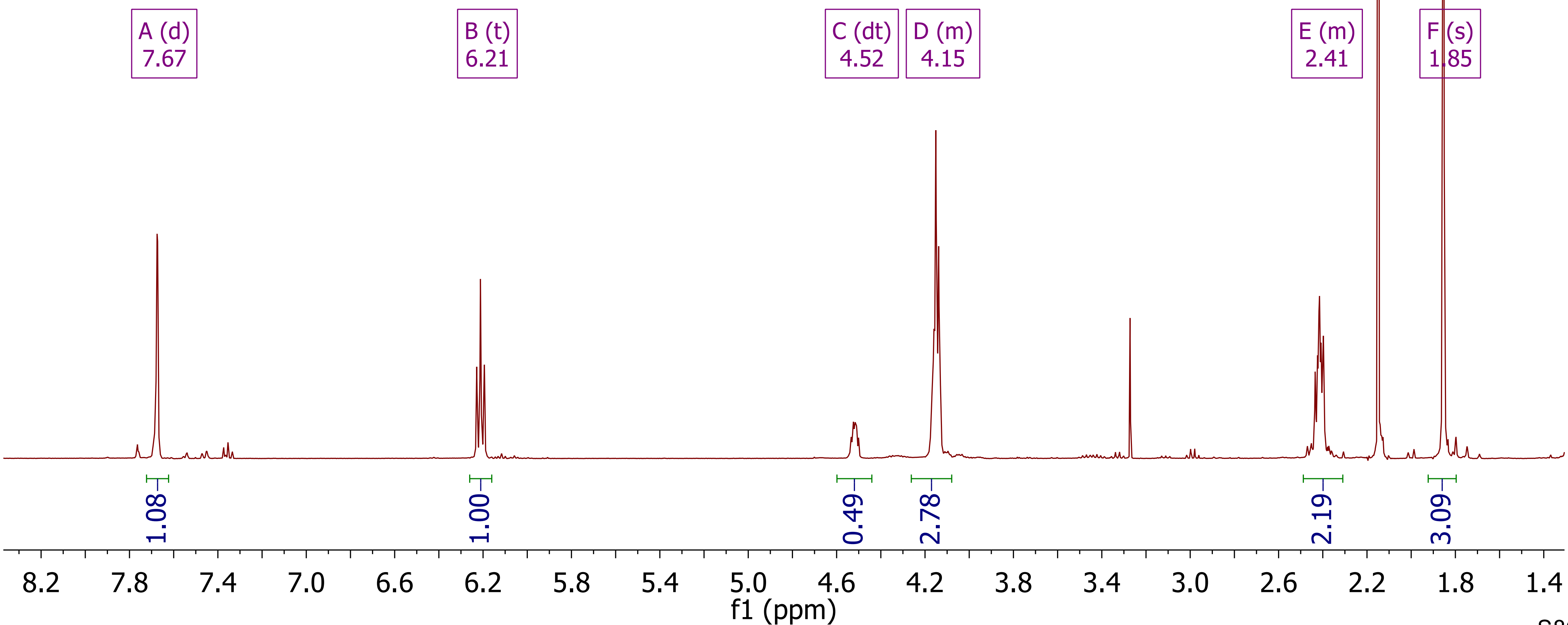

crude product after precipitation



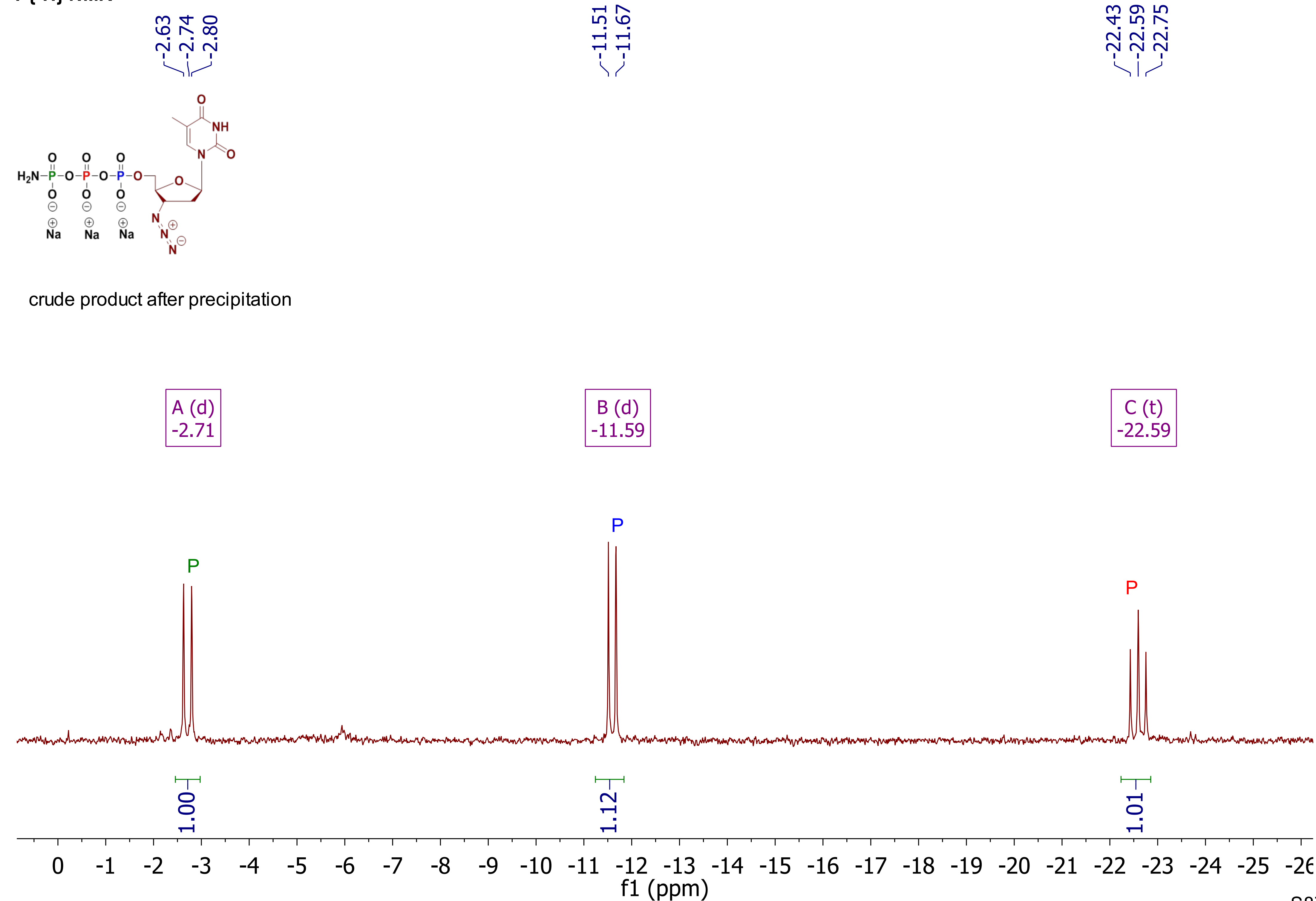
**$^{1}$H NMR**



crude product after precipitation

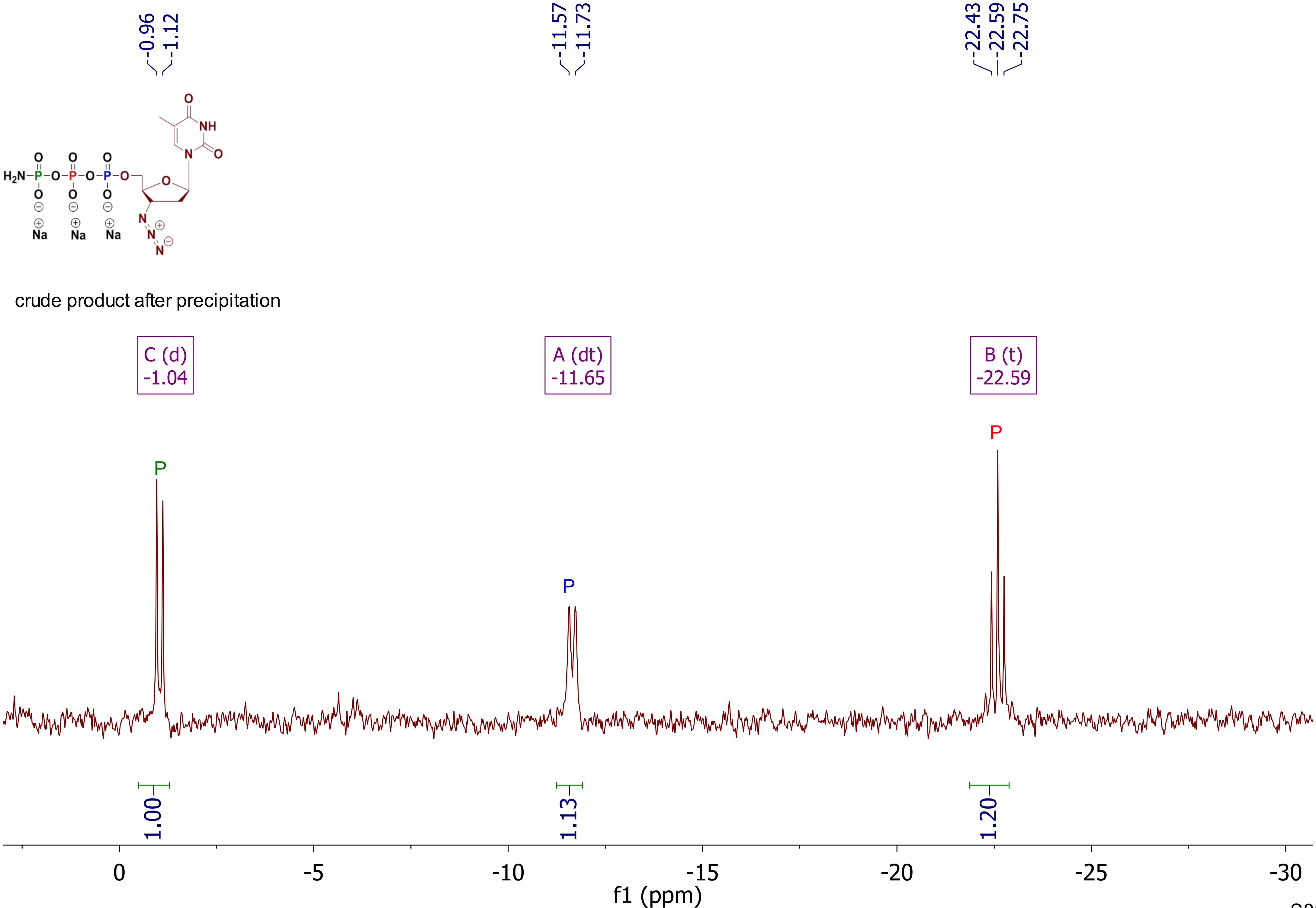


$^{31}P\{^1H\}$ NMR

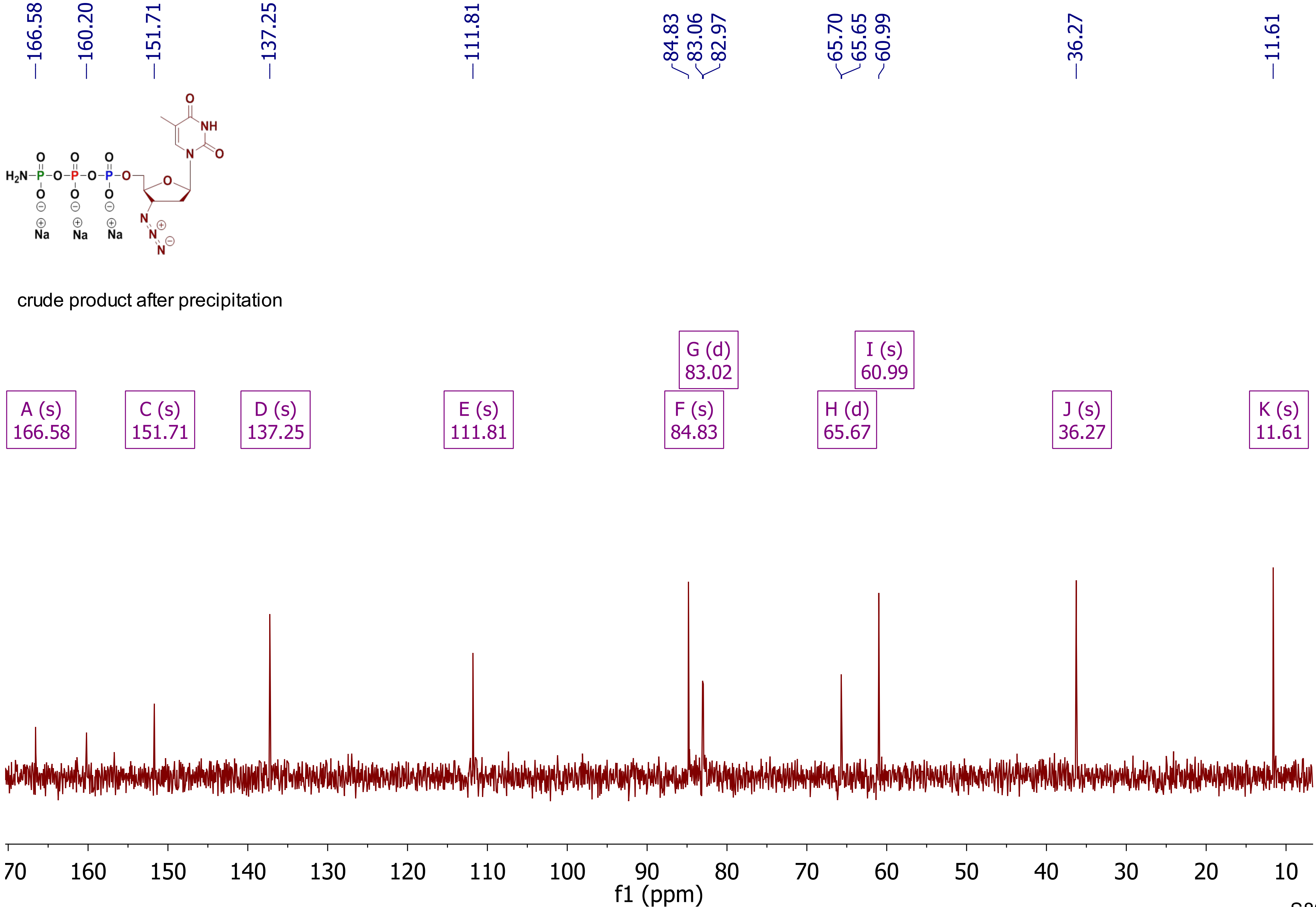


-2.63
-2.74
-2.80
-11.51
-11.67
-22.43
-22.59
-22.75
crude product after precipitation
A (d)
-2.71
B (d)
-11.59
C (t)
-22.59
P
P
P
1.00
1.12
1.01
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
-19
-20
-21
-22
-23
-24
-25
-26
f1 (ppm)

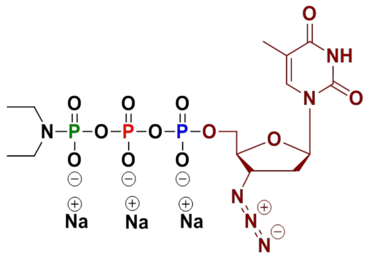
**$^{31}$P NMR**


-0.96
-1.12
-11.57
-11.73
-22.43
-22.59
-22.75
crude product after precipitation
C (d)
-1.04
A (dt)
-11.65
B (t)
-22.59
P
P
P
1.00
1.13
1.20
0
-5
-10
-15
-20
-25
-30
f1 (ppm)

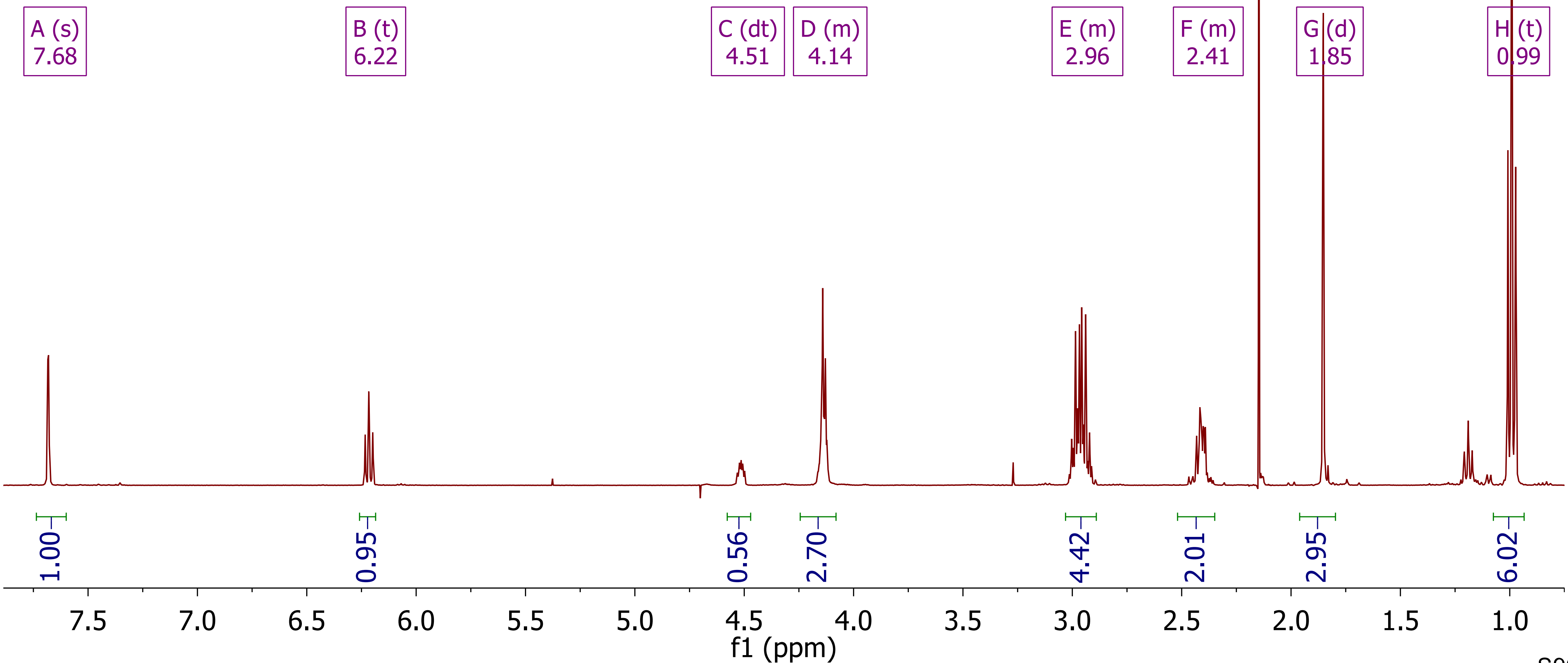
**$^{13}$C NMR**


166.58
160.20
151.71
137.25
111.81
84.83
83.06
82.97
65.70
65.65
60.99
36.27
11.61
crude product after precipitation
G (d) 83.02
I (s) 60.99
A (s) 166.58
C (s) 151.71
D (s) 137.25
E (s) 111.81
F (s) 84.83
H (d) 65.67
J (s) 36.27
K (s) 11.61
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

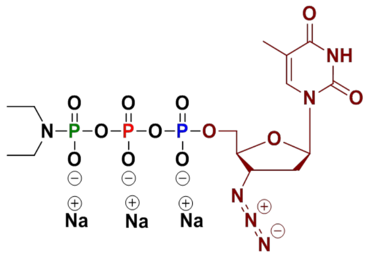
## $^{1}$H NMR



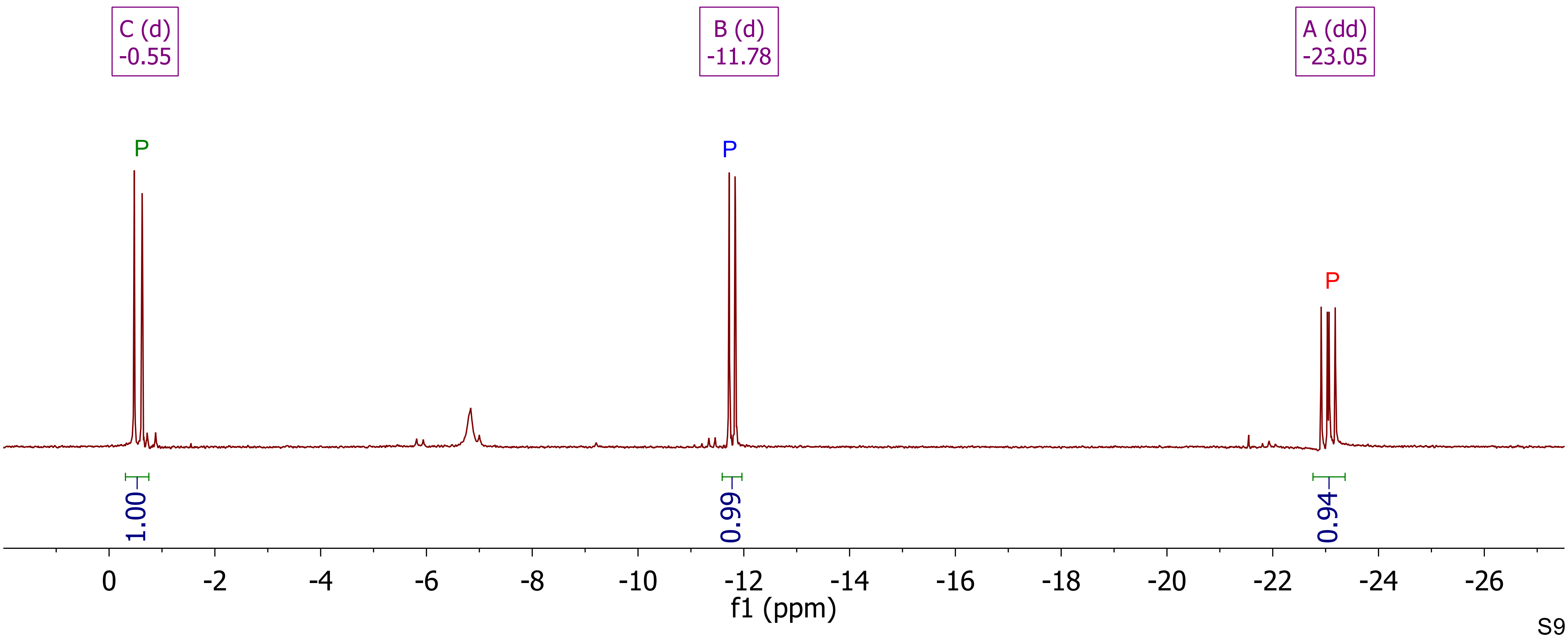
crude product after precipitation



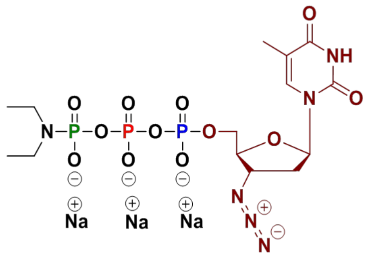
$^{31}P\{^{1}H\}$ NMR



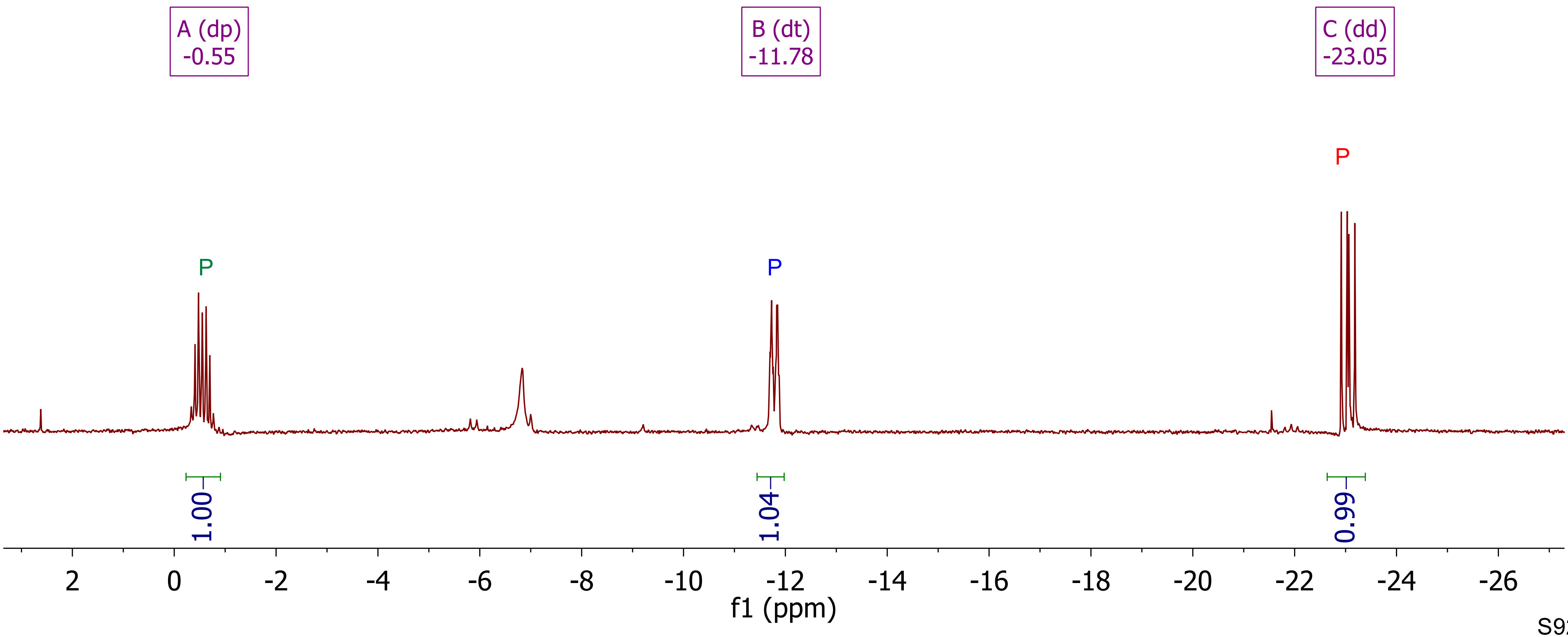

crude product after precipitation



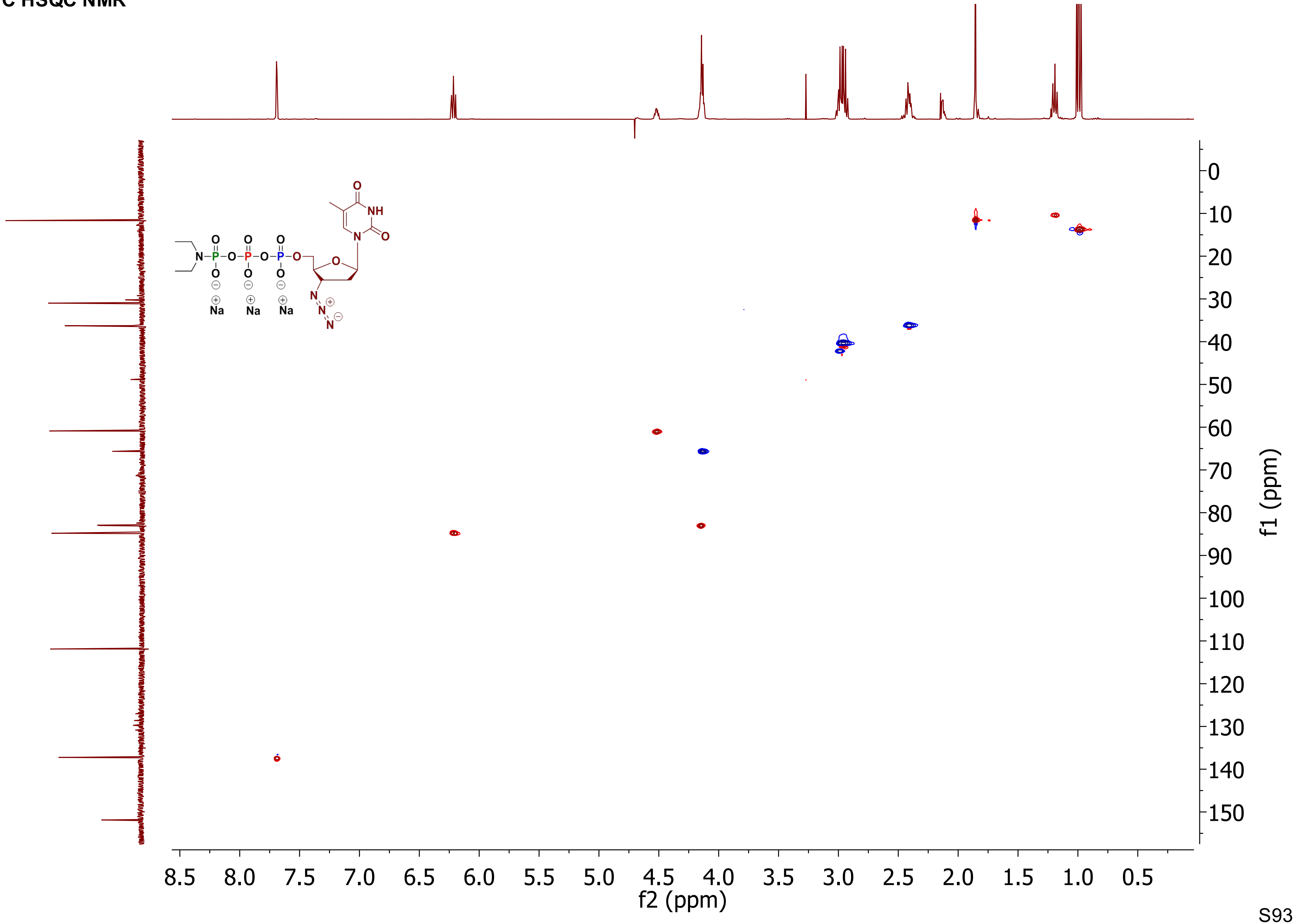
**$^{31}$P NMR**



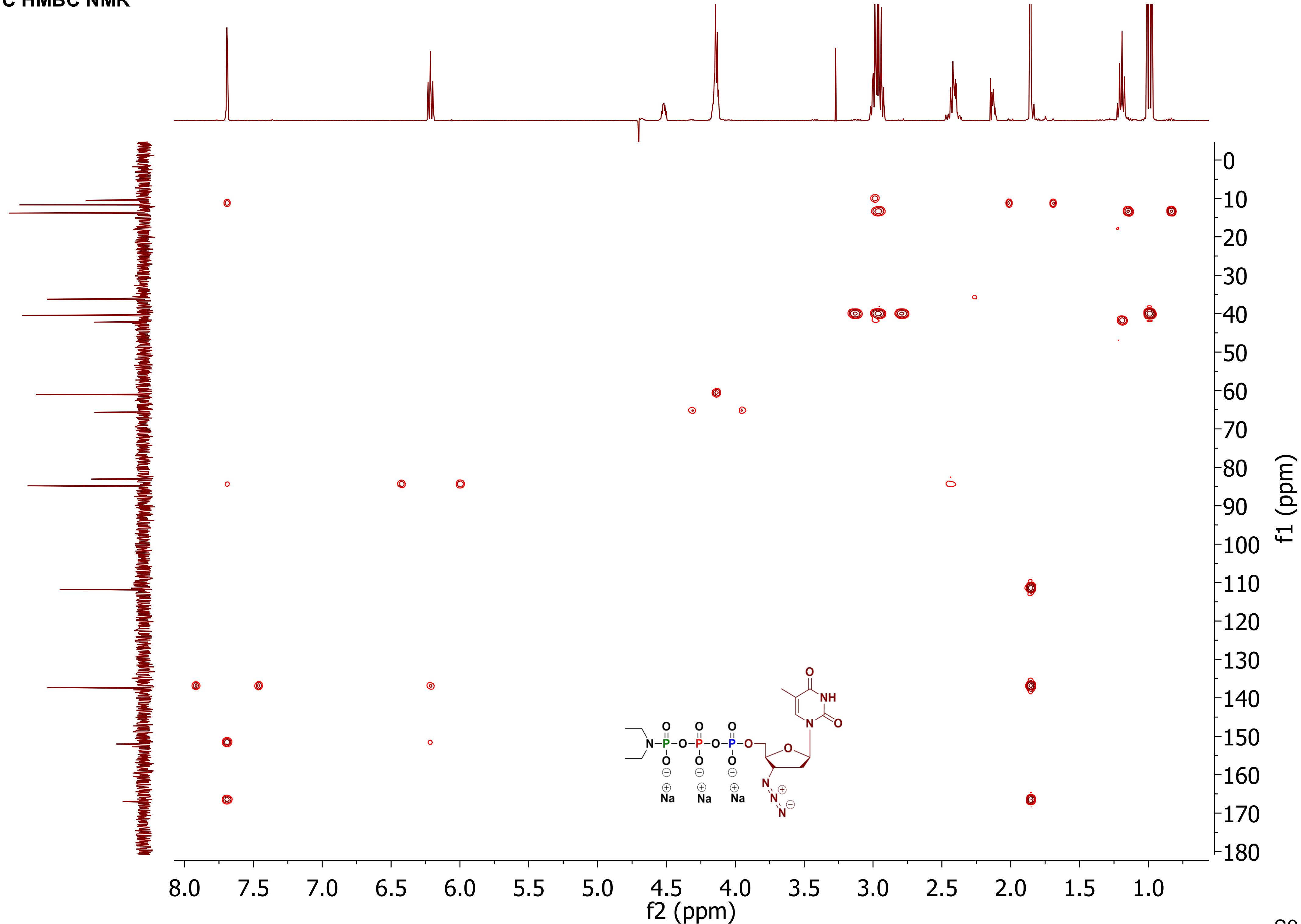
crude product after precipitation



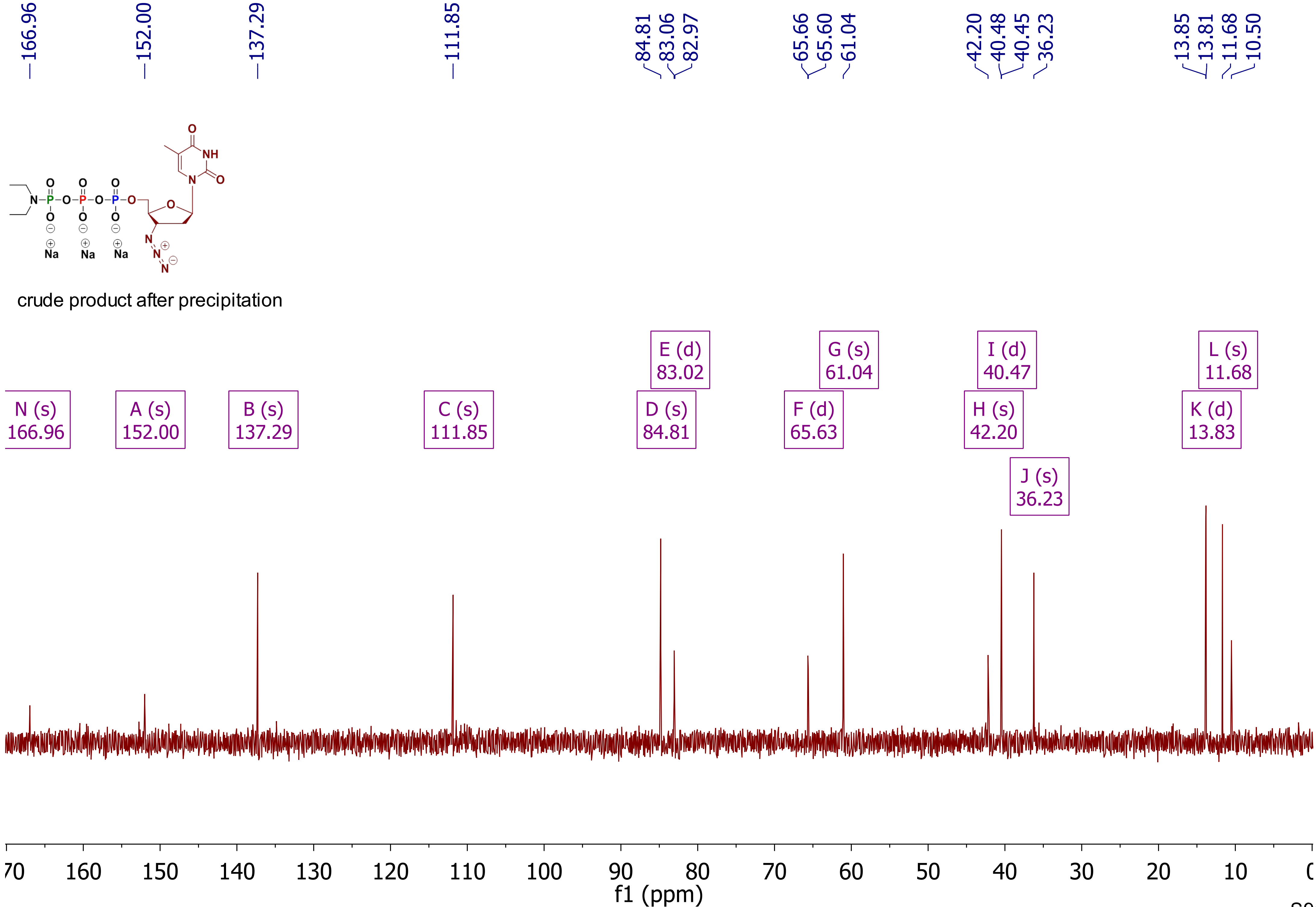
**$^1H$-$^{13}C$ HSQC NMR**



$^1H$-$^{13}C$ HMBC NMR

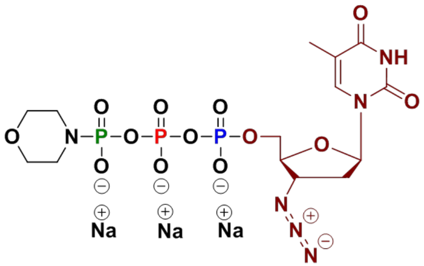


## $^{13}$C NMR

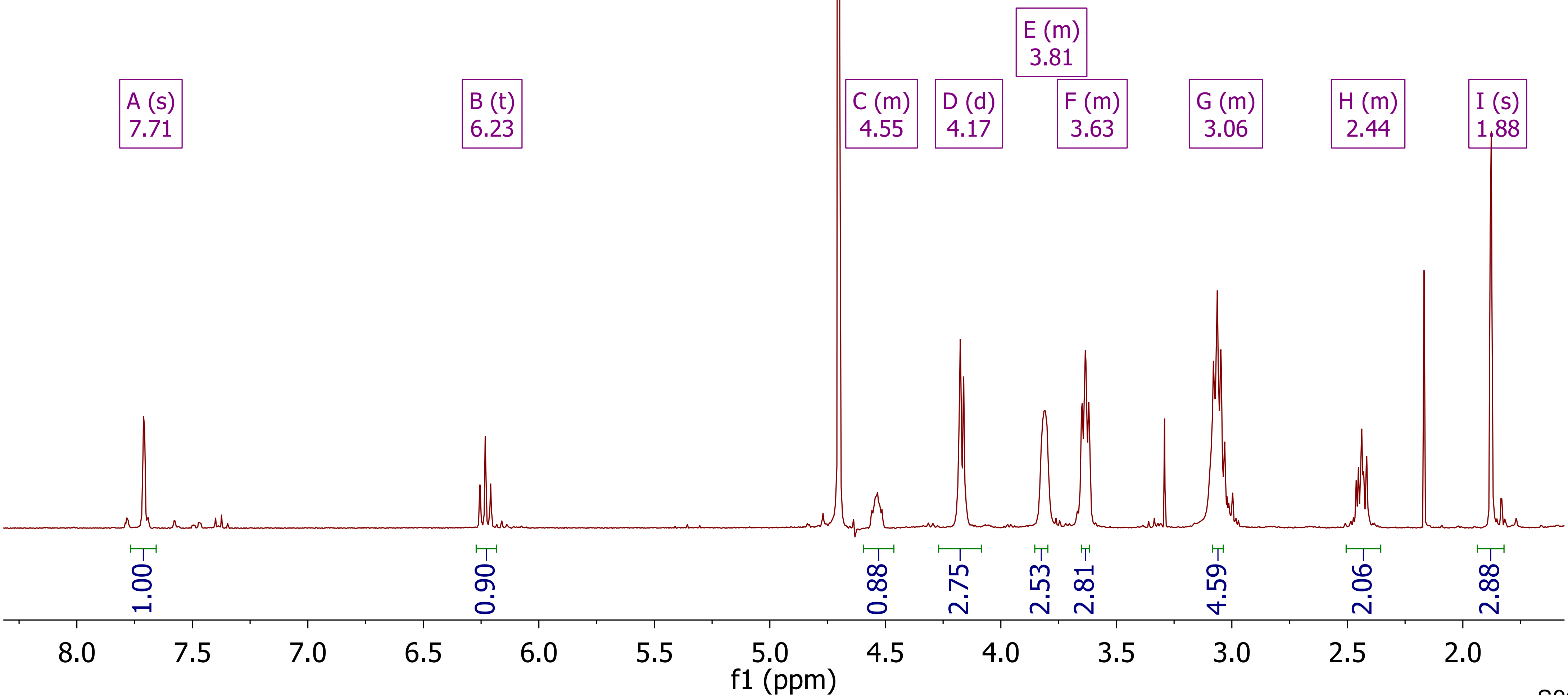


166.96
152.00
137.29
111.85
84.81
83.06
82.97
65.66
65.60
61.04
42.20
40.48
40.45
36.23
13.85
13.81
11.68
10.50
crude product after precipitation
N (s) 166.96
A (s) 152.00
B (s) 137.29
C (s) 111.85
D (s) 84.81
E (d) 83.02
F (d) 65.63
G (s) 61.04
H (s) 42.20
I (d) 40.47
J (s) 36.23
K (d) 13.83
L (s) 11.68
70
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

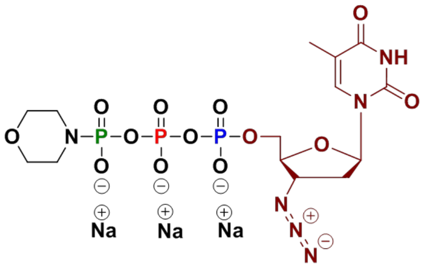
$^1$H NMR



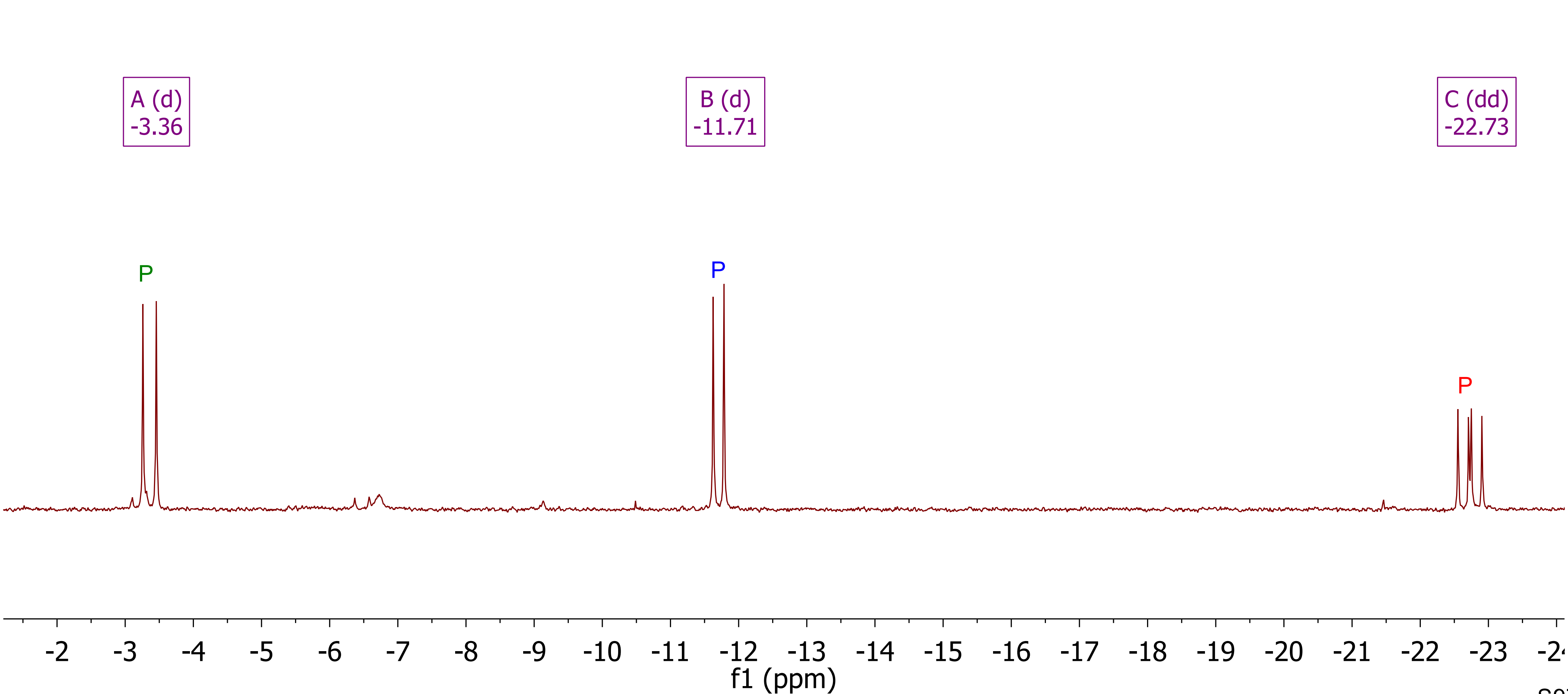

crude product after precipitation



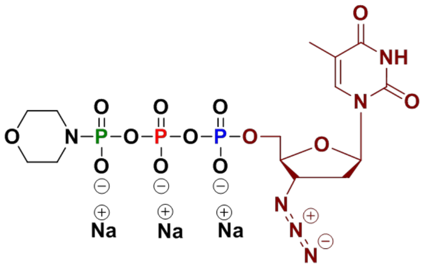
$^{31}P\{^1H\}$ NMR



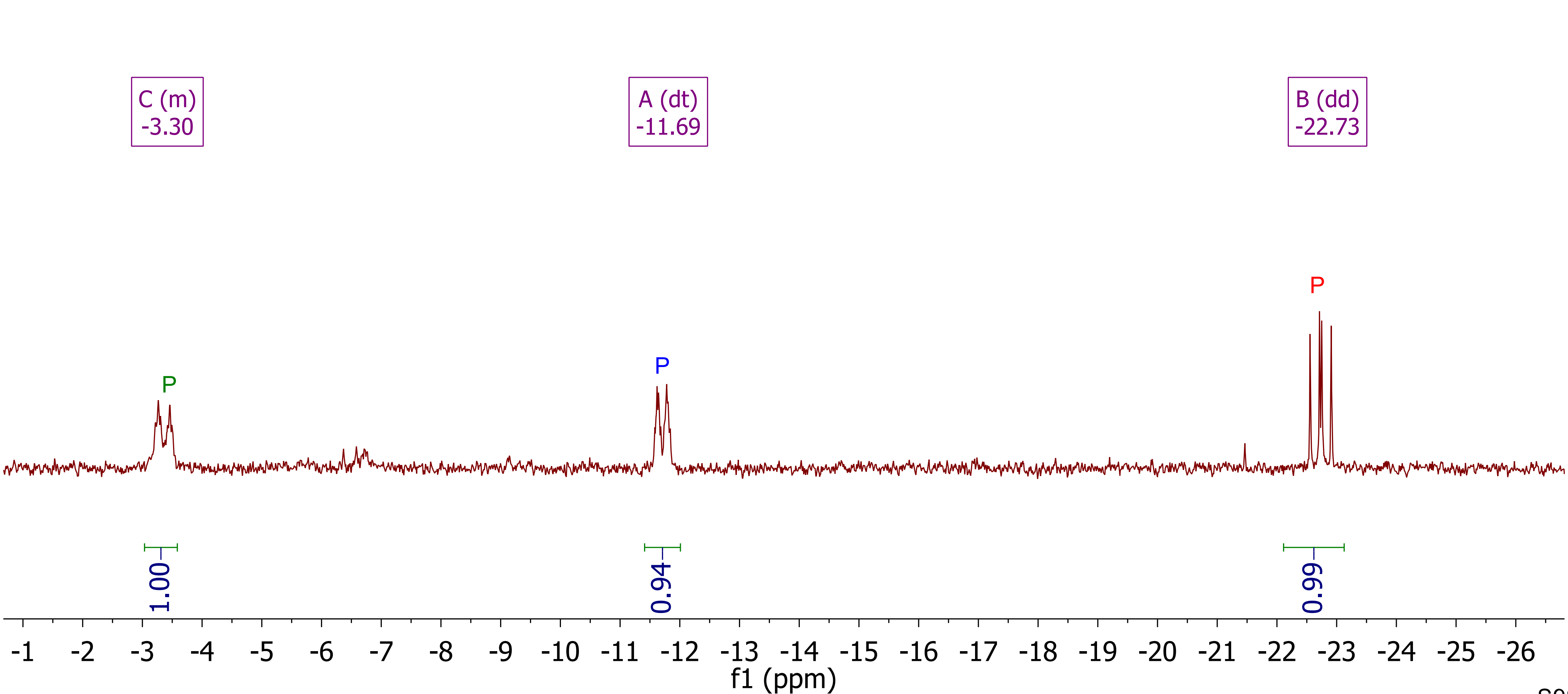

crude product after precipitation



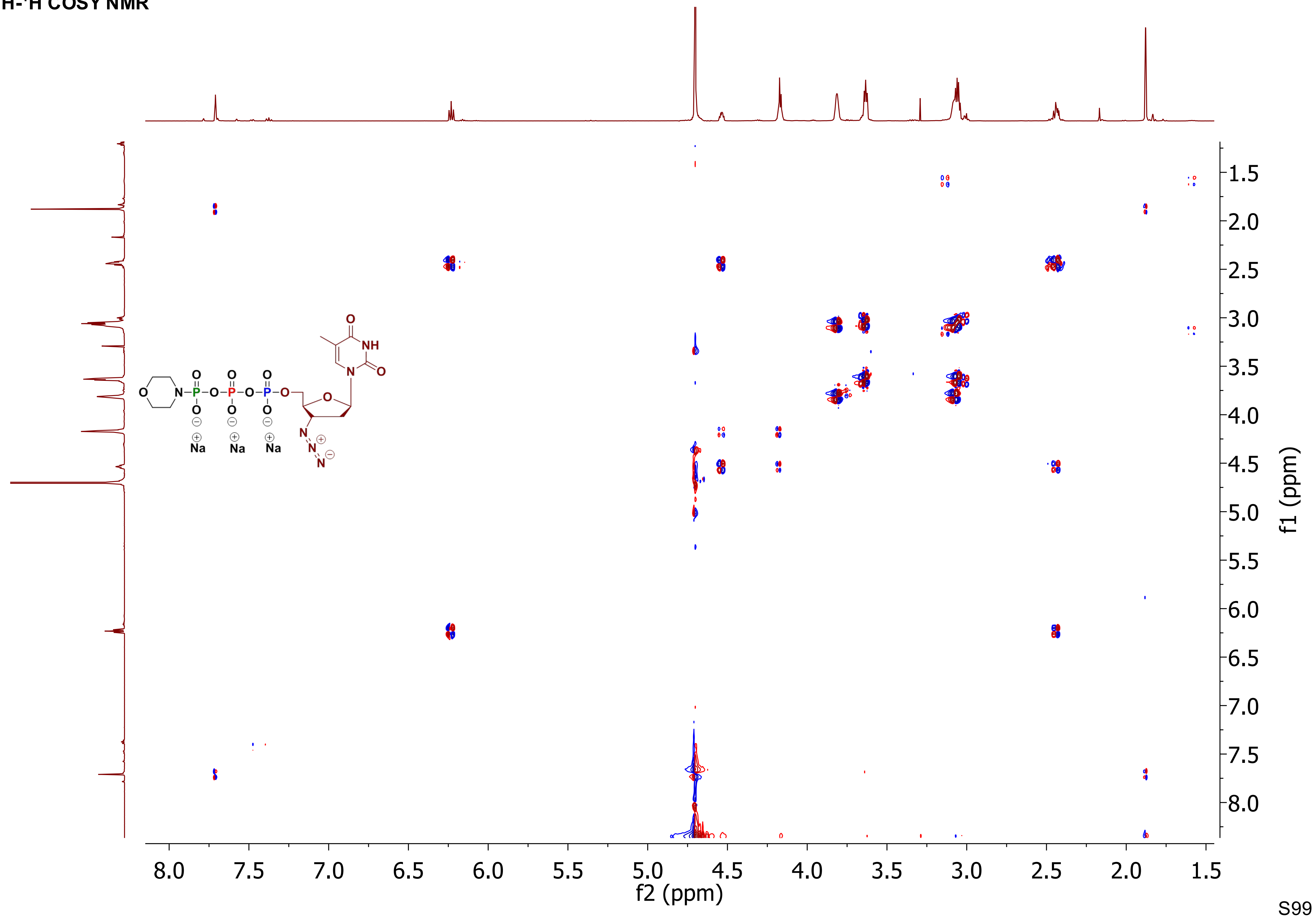
$^{31}$P NMR



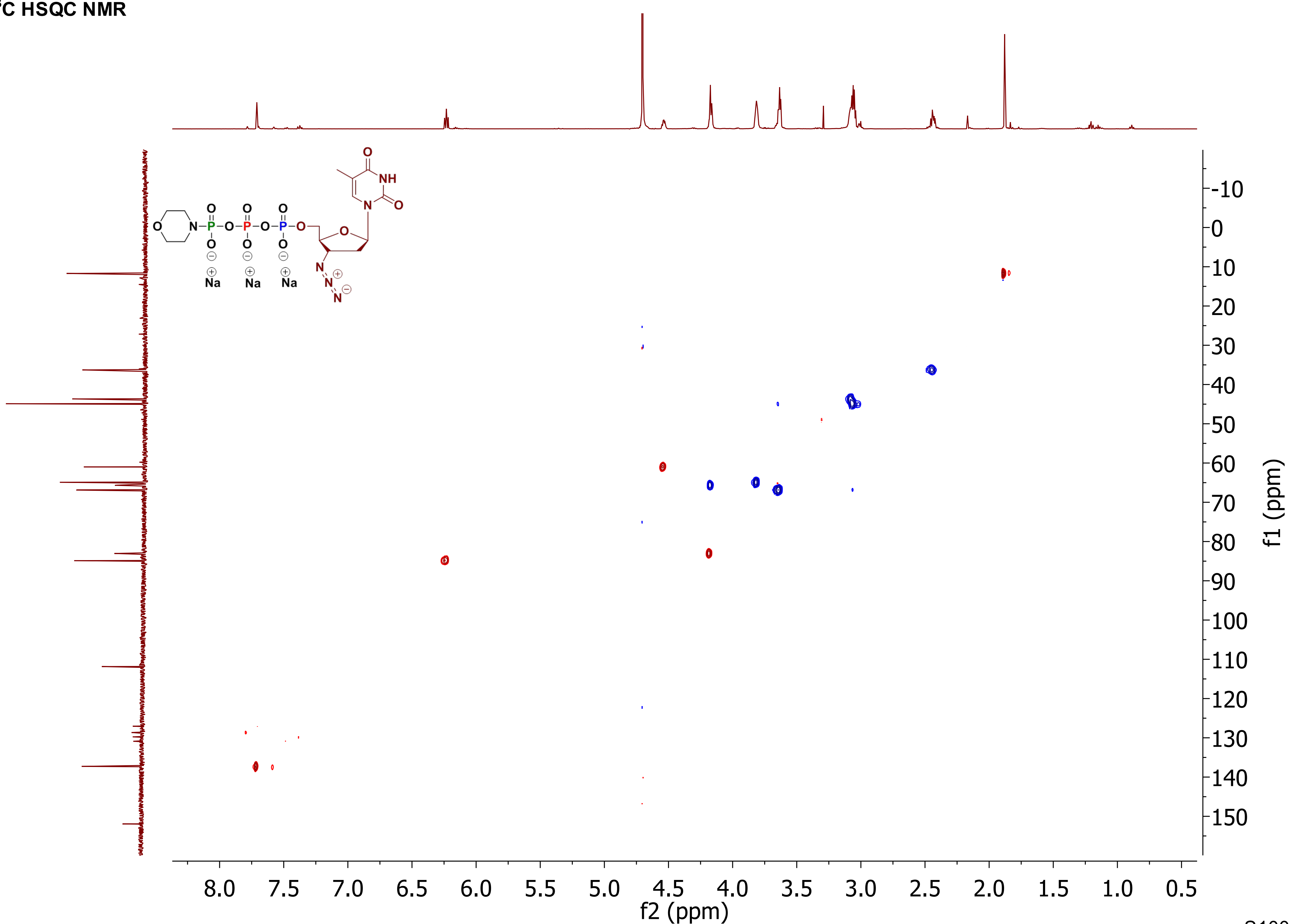
crude product after precipitation



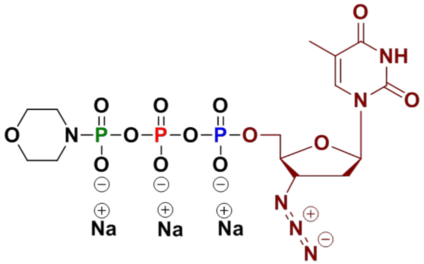
$^1H$-$^1H$ COSY NMR



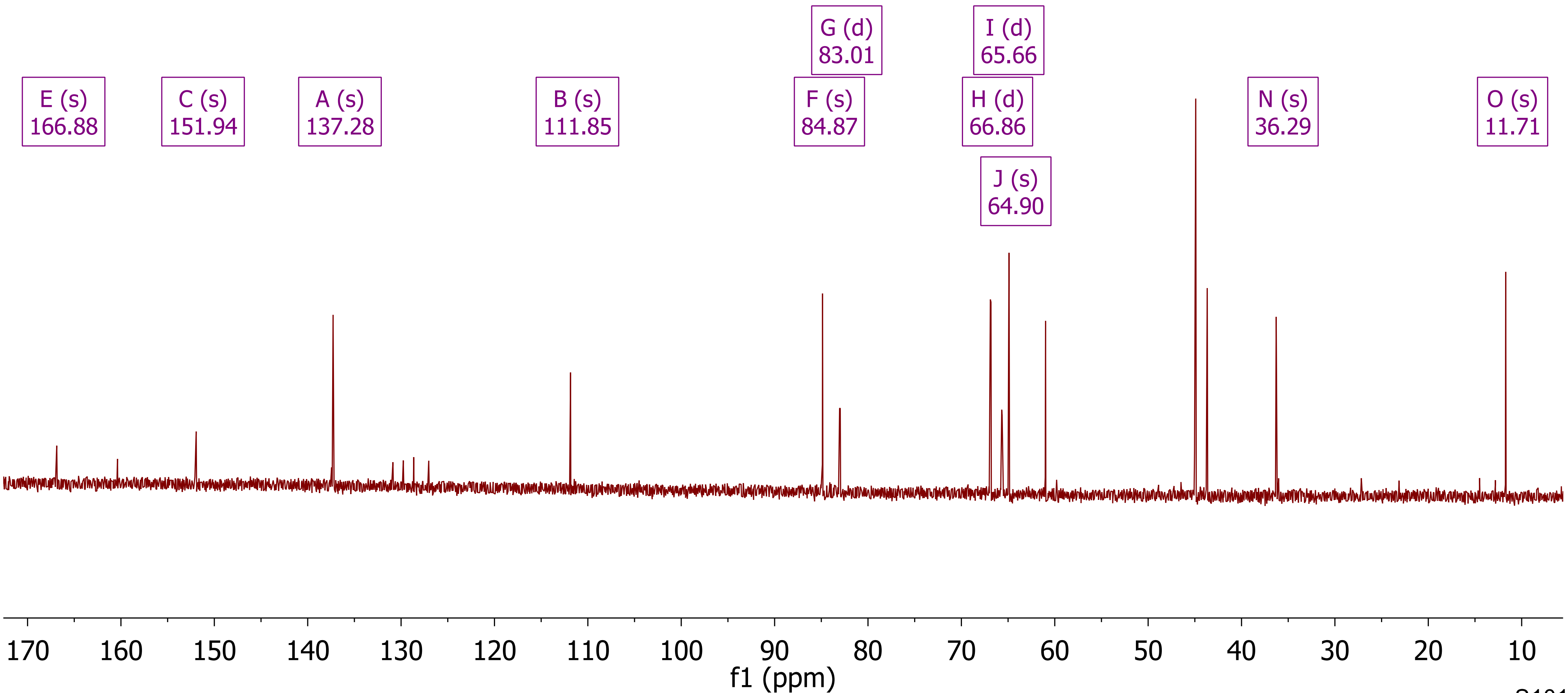
$^1H$-$^{13}C$ HSQC NMR



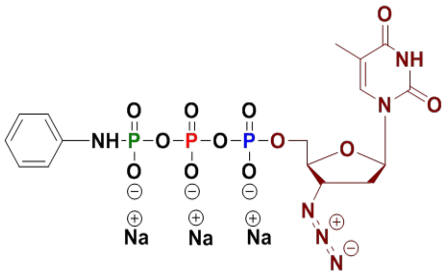
$^{13}$C NMR



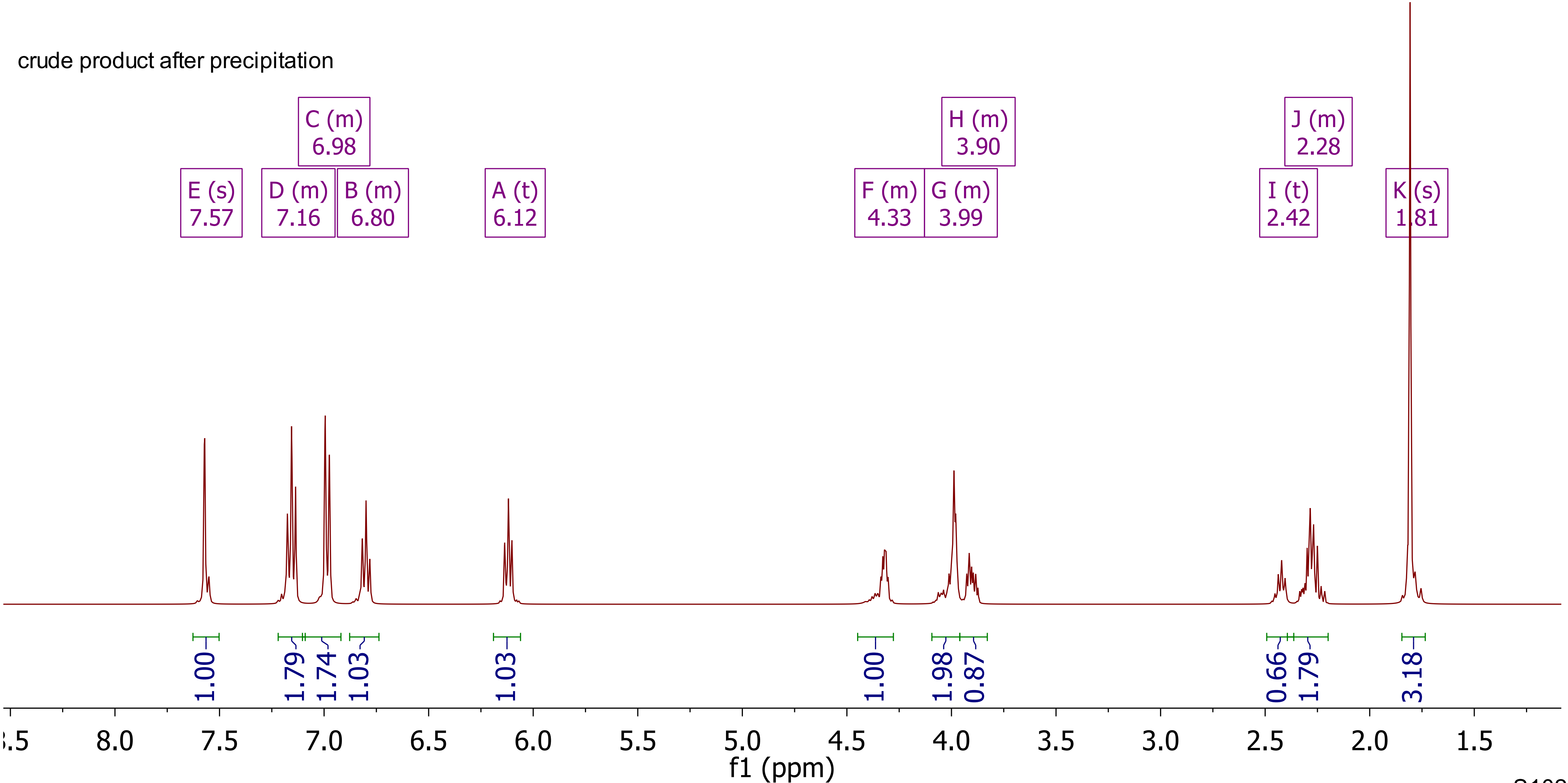
crude product after precipitation



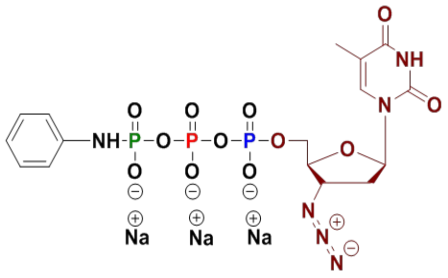
**$^1$H NMR**



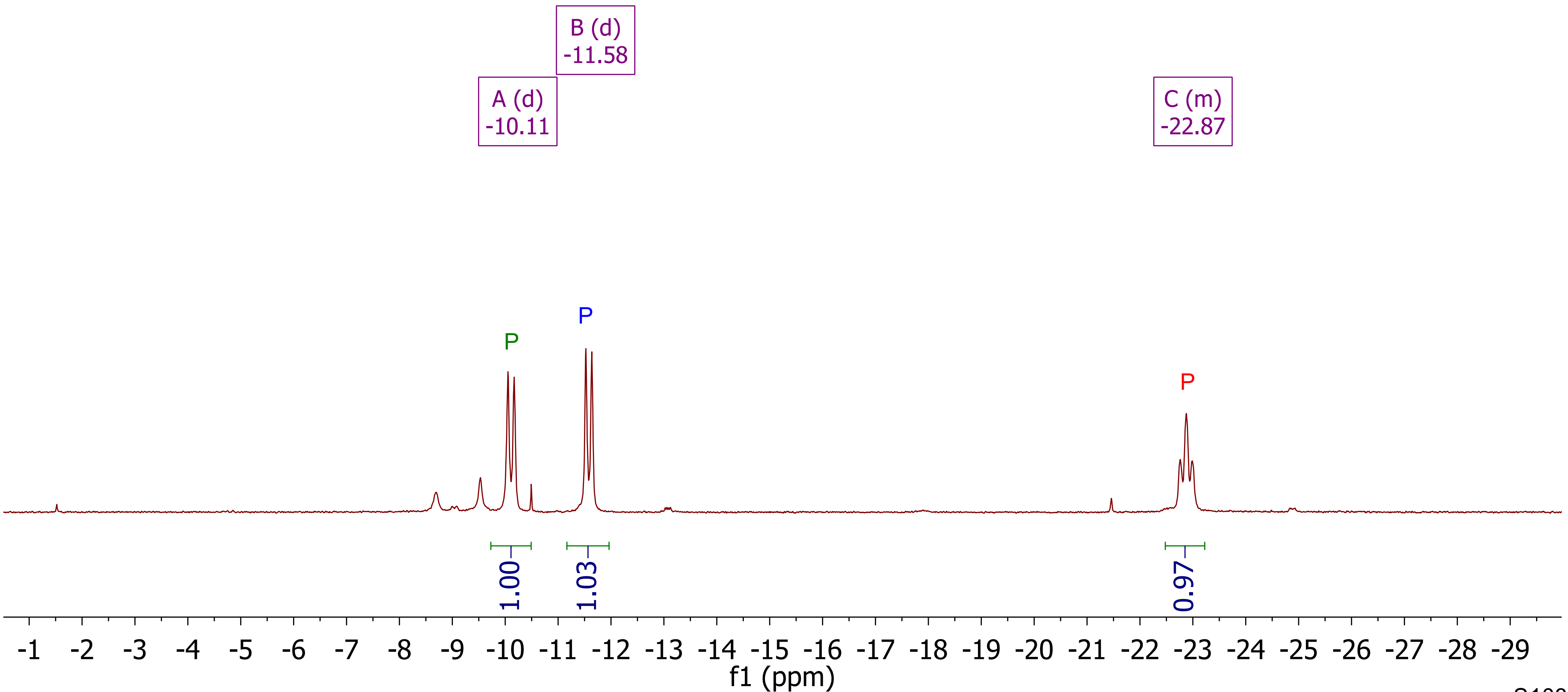
crude product after precipitation



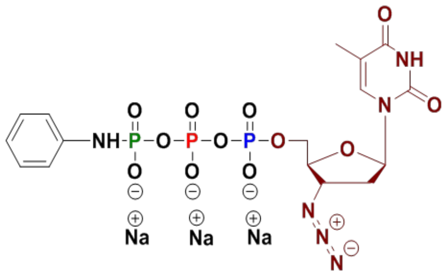
**$^{31}P\{^1H\}$ NMR**



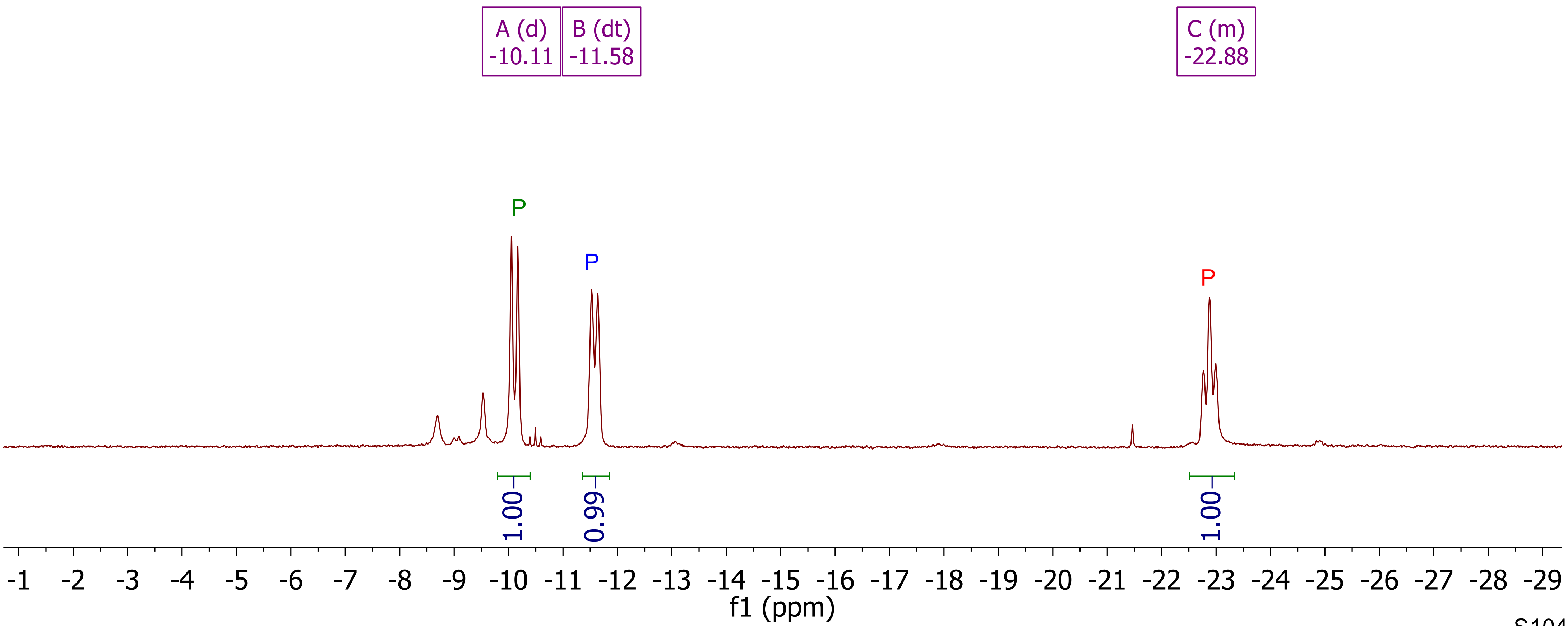

crude product after precipitation



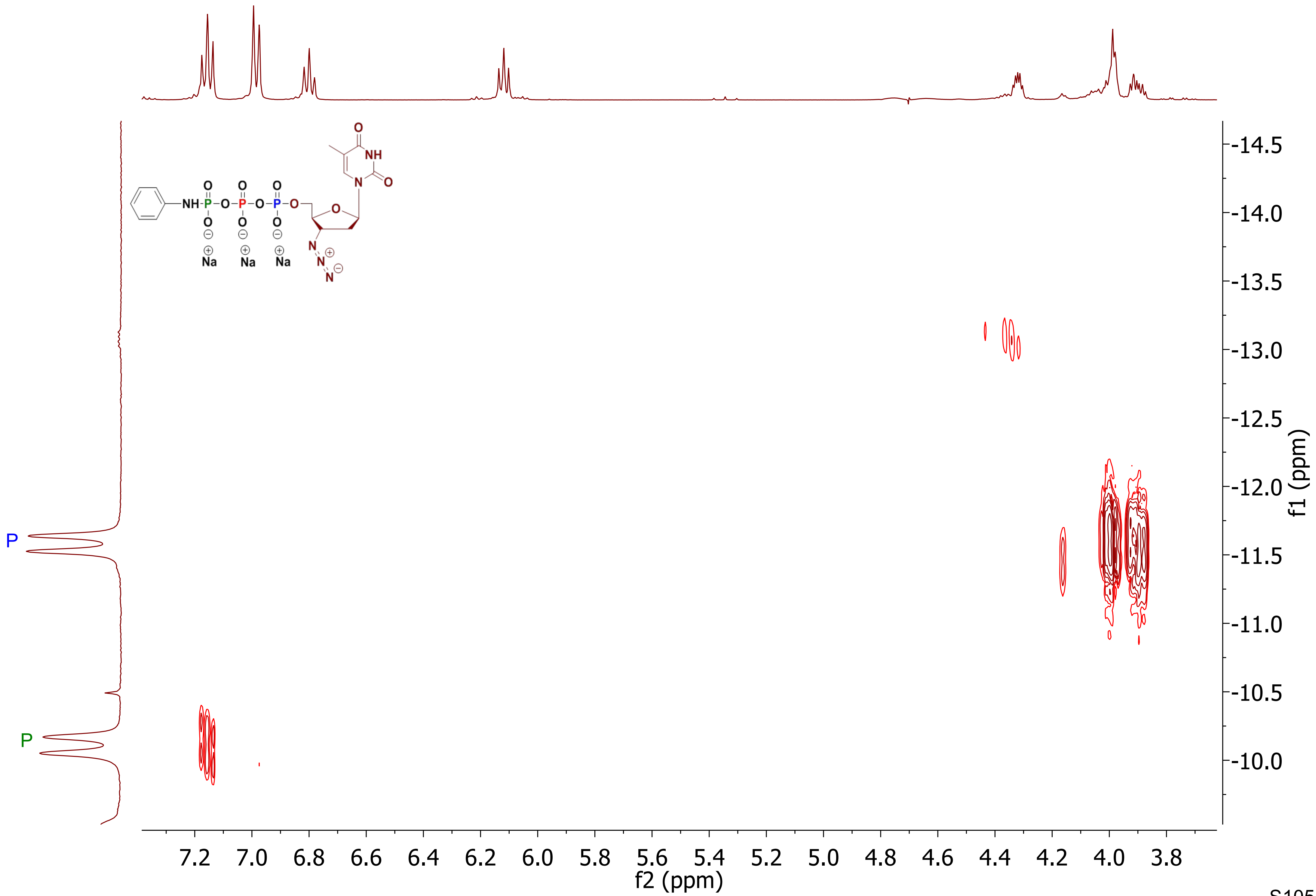
## $^{31}$P NMR



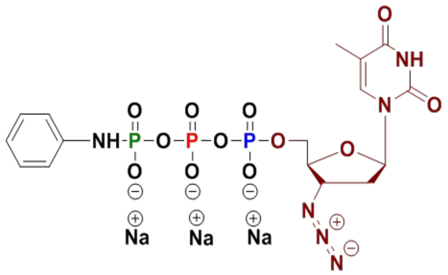
crude product after precipitation



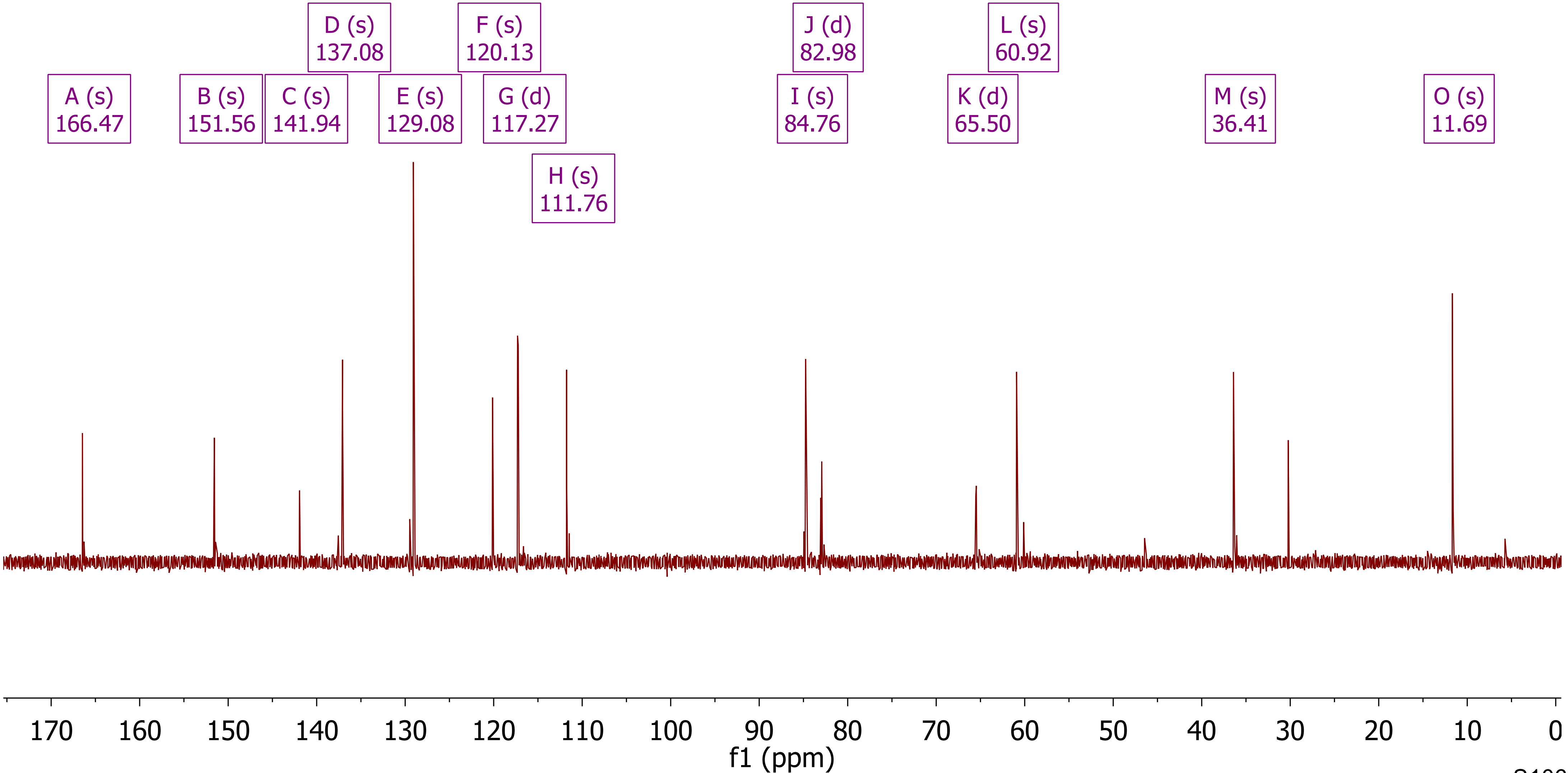
**$^{31}$P-$^{1}$H HMBC NMR**



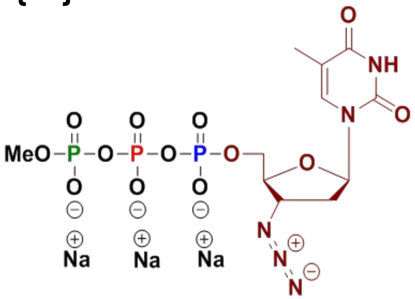
**$^{13}$C NMR**



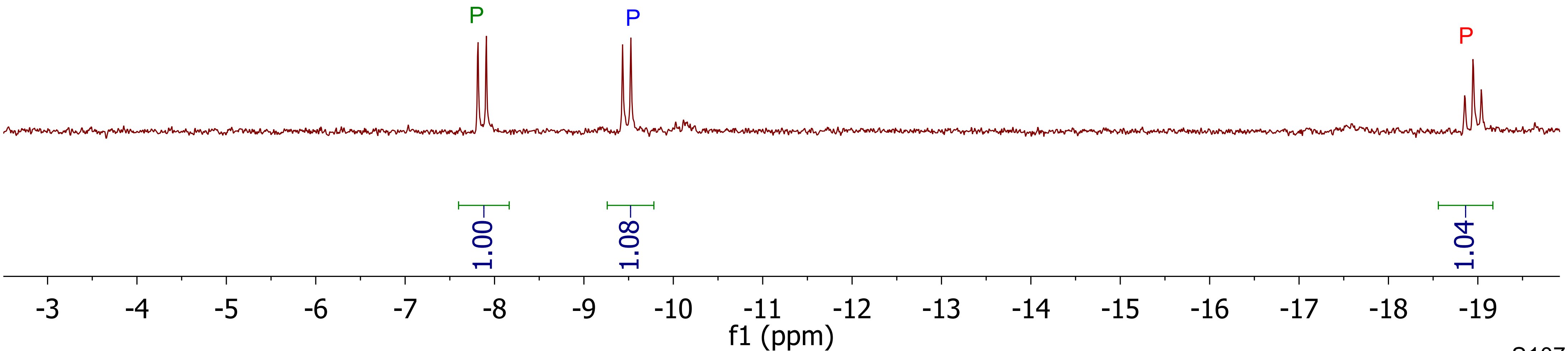

crude product after precipitation



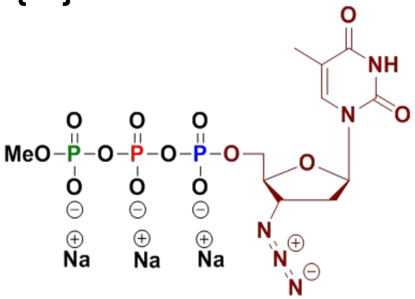
**$^{31}P\{^{1}H\}$-NMR**



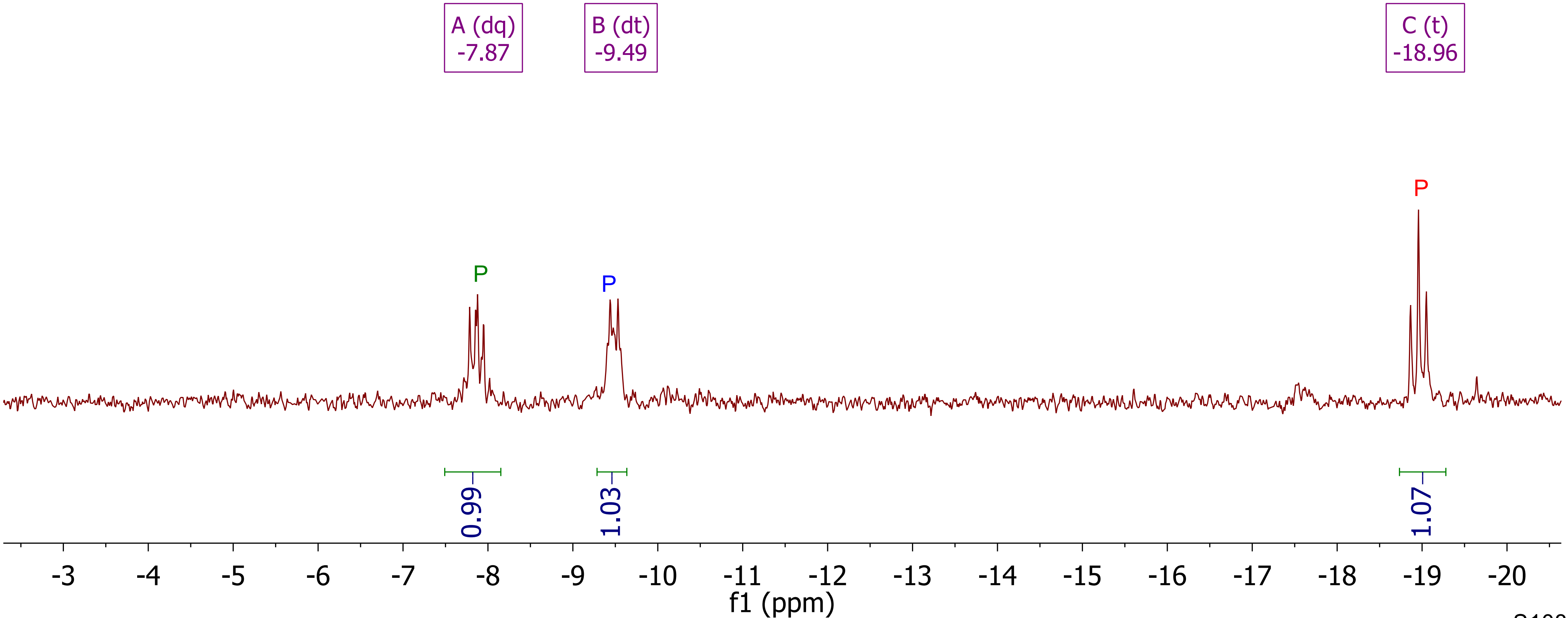

crude product after precipitation



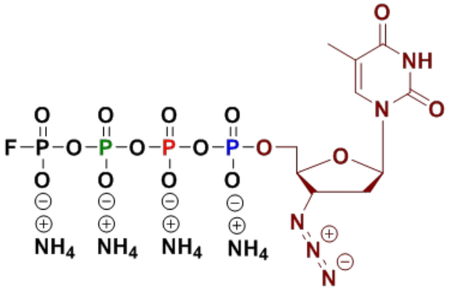
$^{31}P\{^1H\}$-NMR



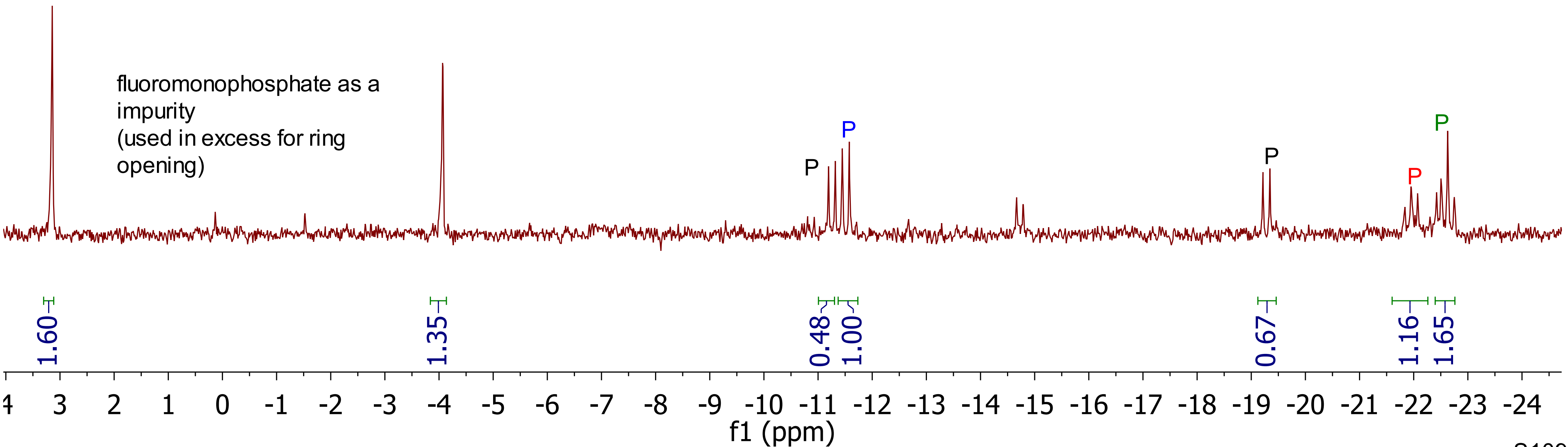
crude product after precipitation



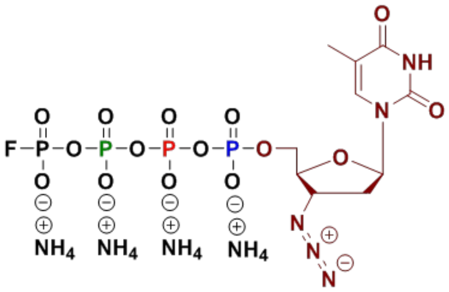
**$^{31}P\{^{1}H\}$-NMR**



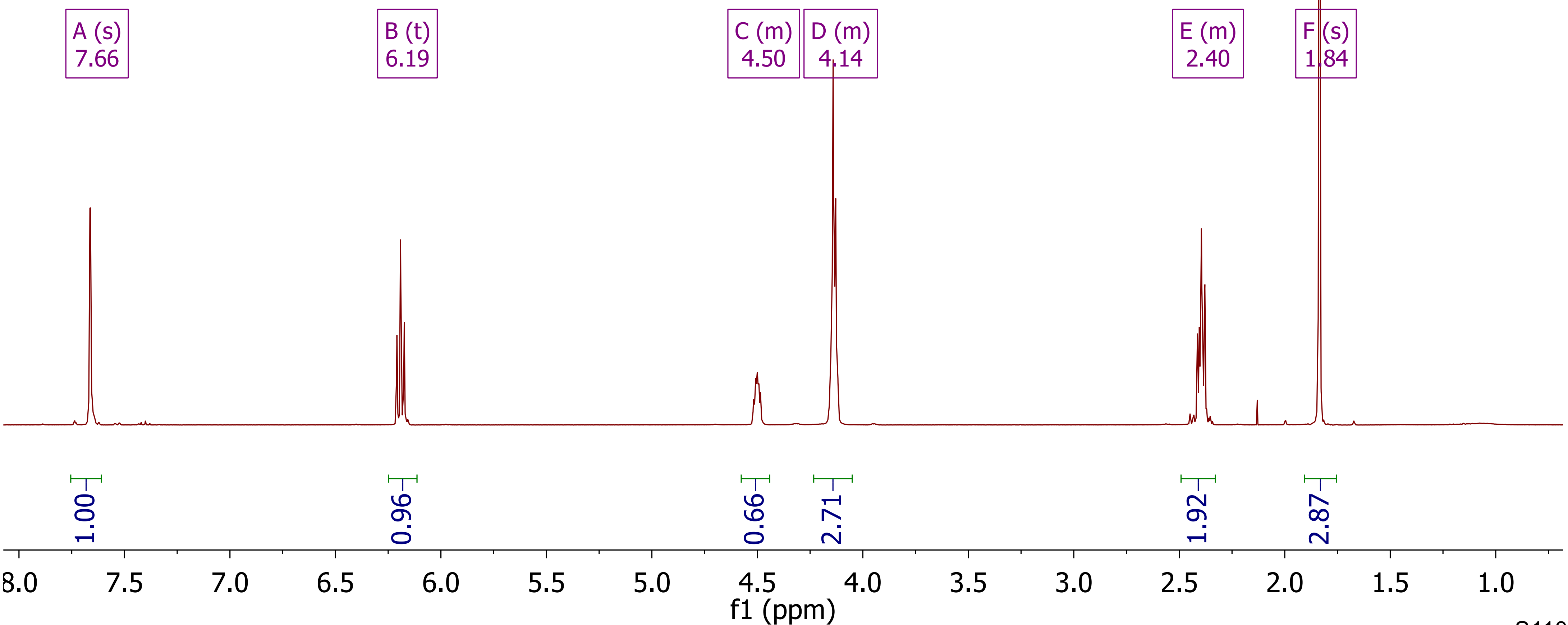

crude product after precipitation



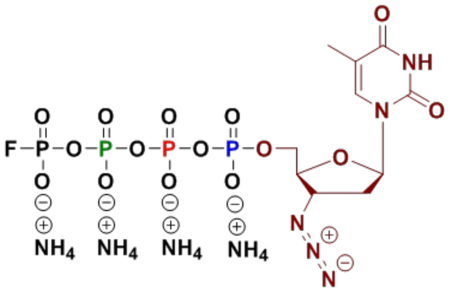
## $^1$H NMR



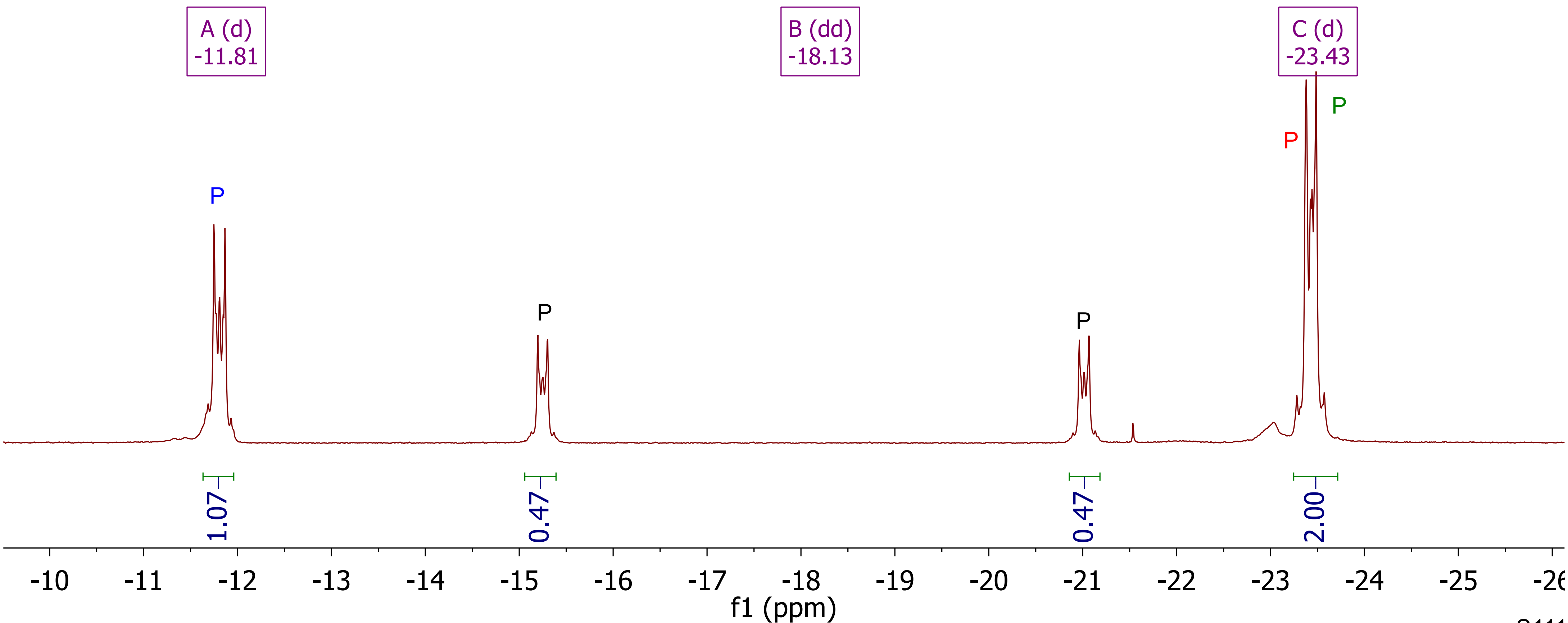
Product after purification by SAX



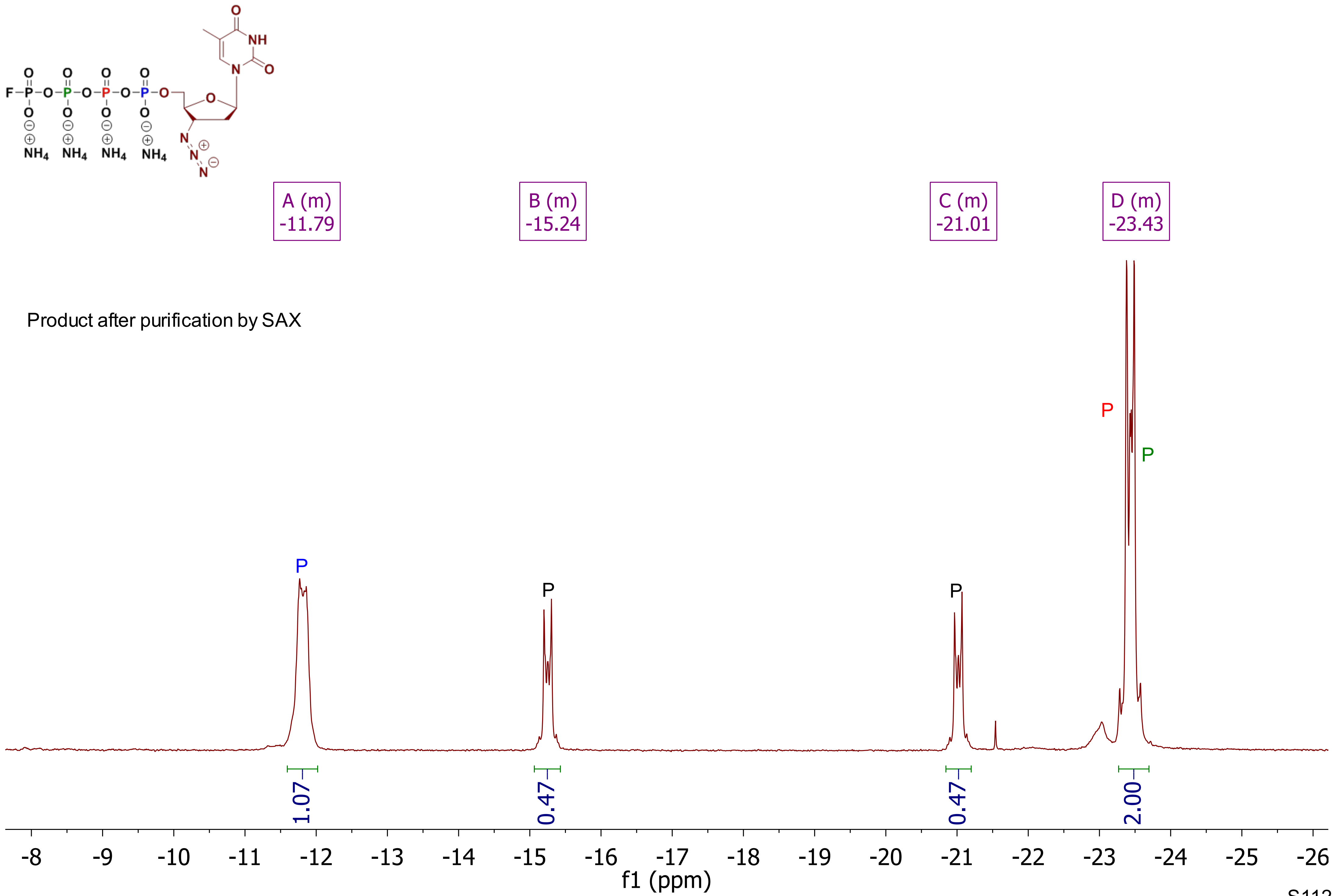
**$^{31}P\{^1H\}$-NMR**




Product after purification by SAX

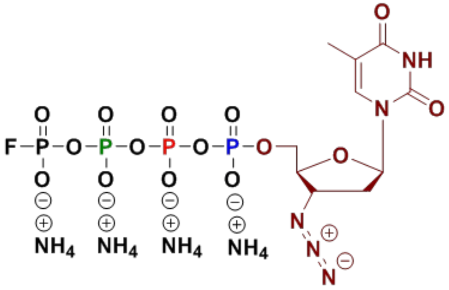


**$^{31}P$-$^{1}H$ NMR**

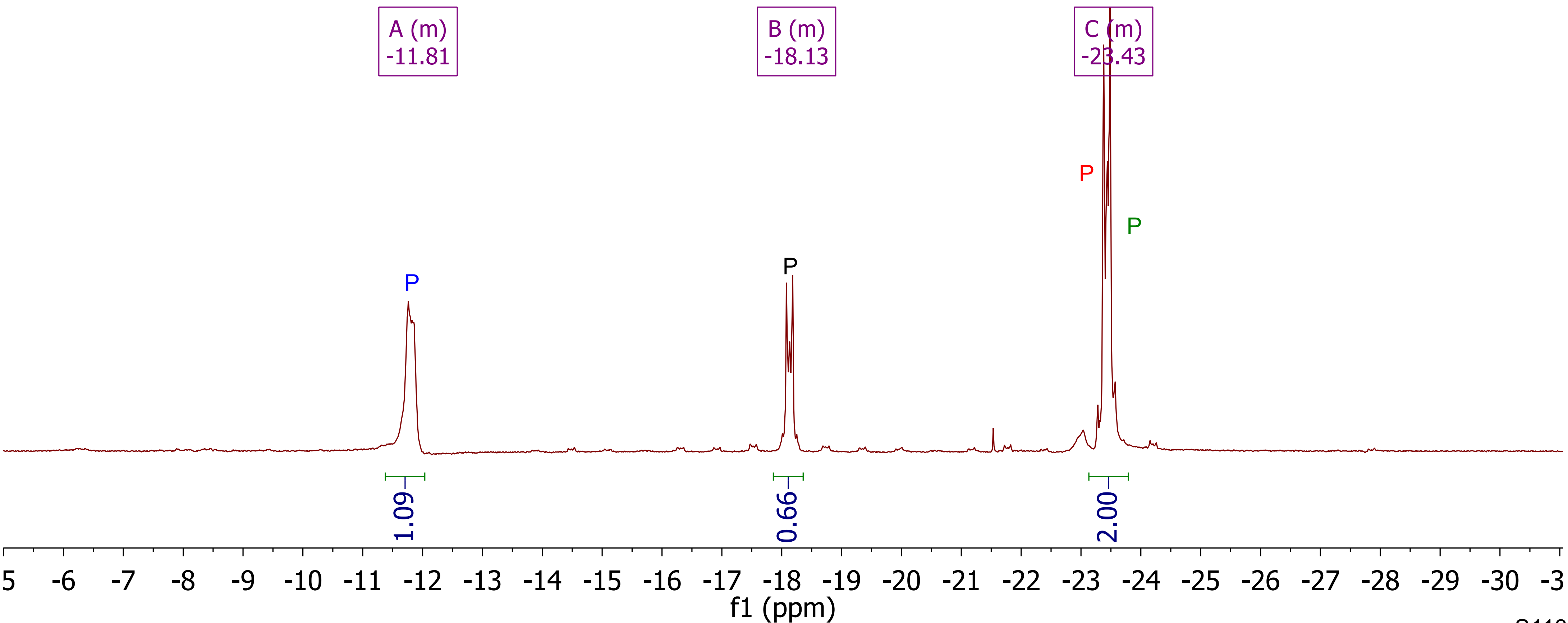


A (m)
-11.79
B (m)
-15.24
C (m)
-21.01
D (m)
-23.43
Product after purification by SAX
P
P
P
P
P
1.07
0.47
0.47
2.00
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
-19
-20
-21
-22
-23
-24
-25
-26
f1 (ppm)

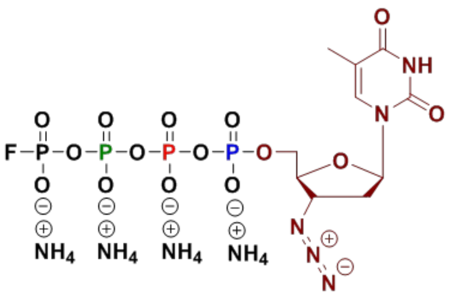
$^{31}P\{^{19}F\}$ NMR



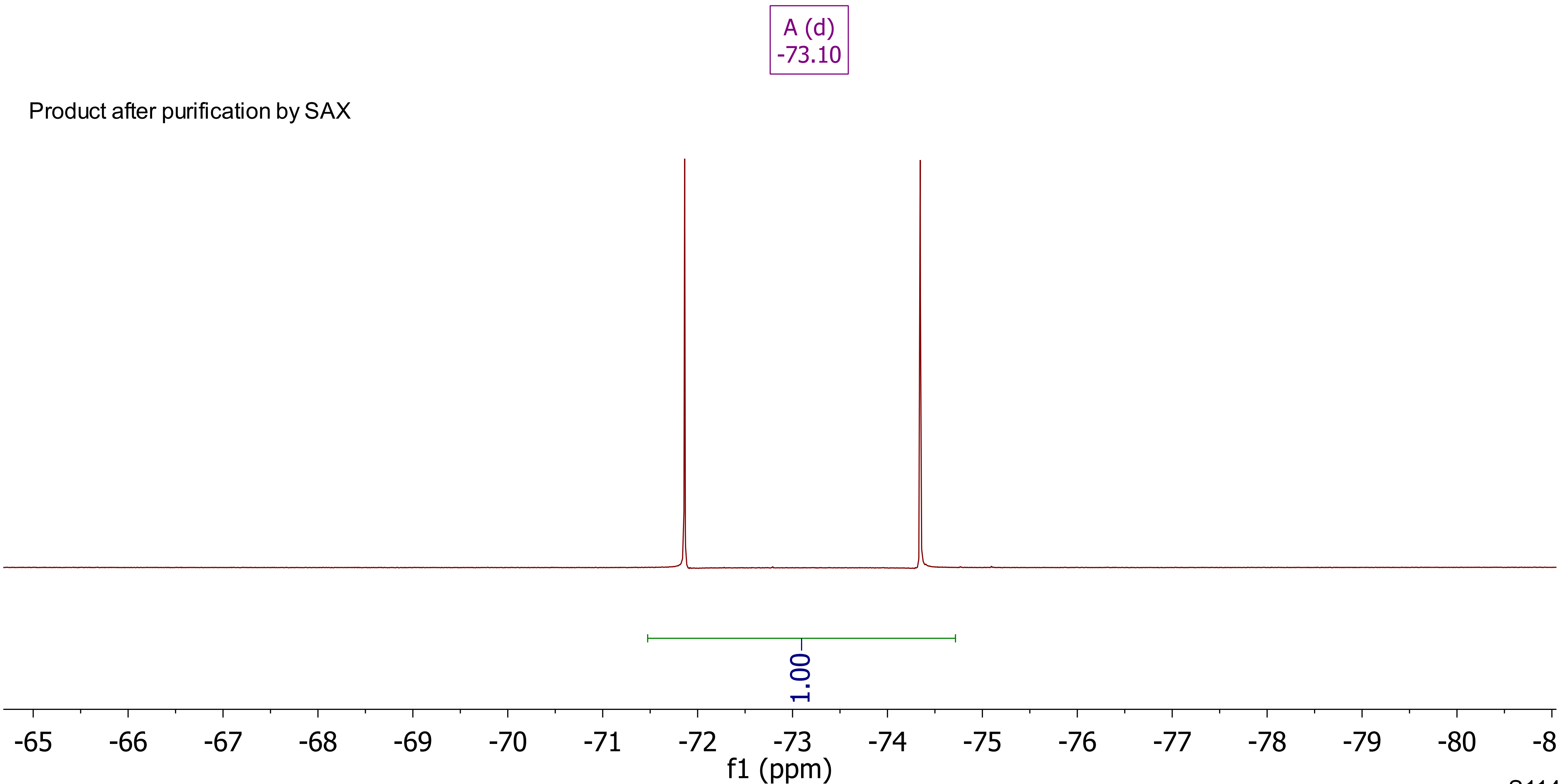
Product after purification by SAX



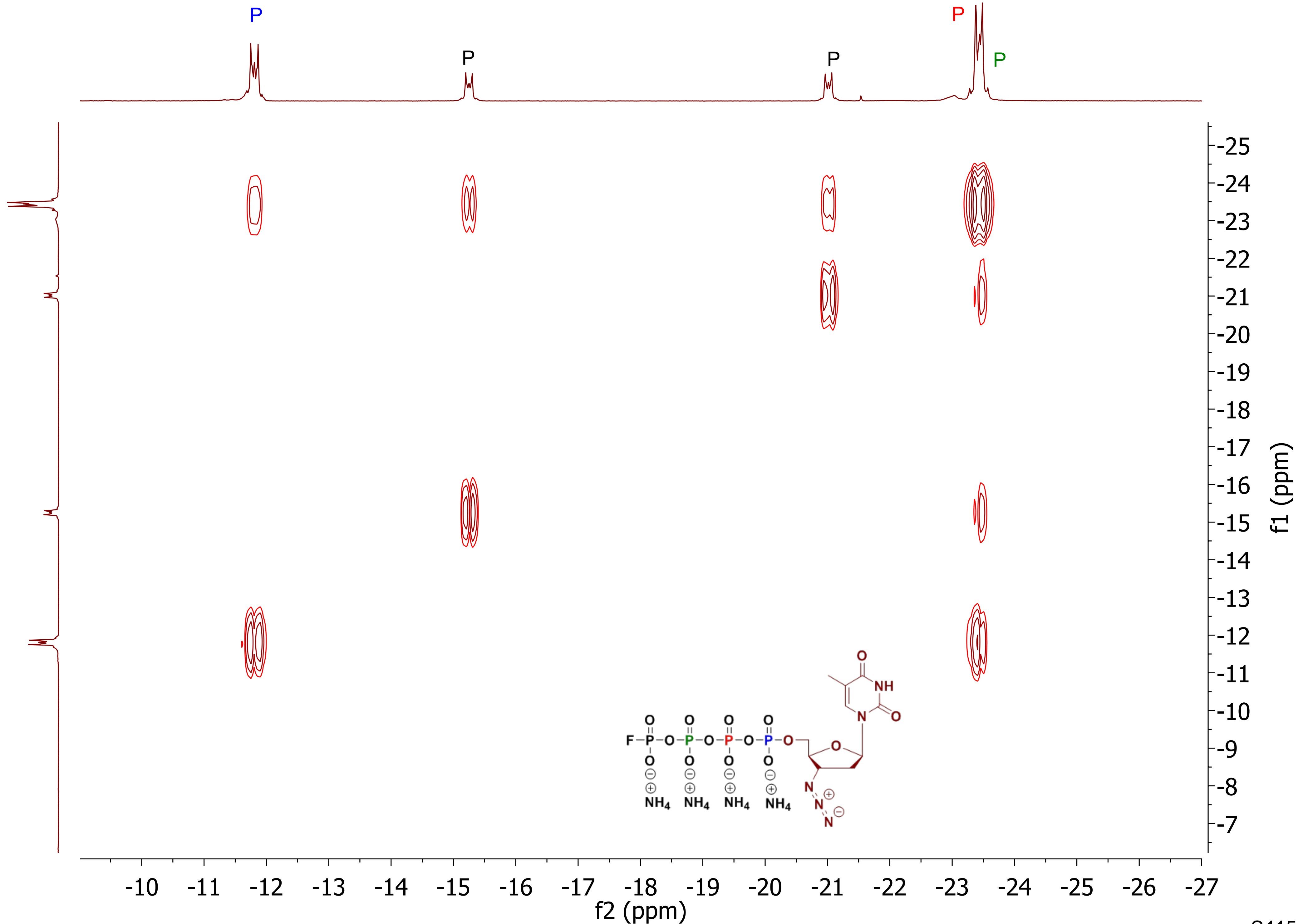
$^{19}$F-NMR



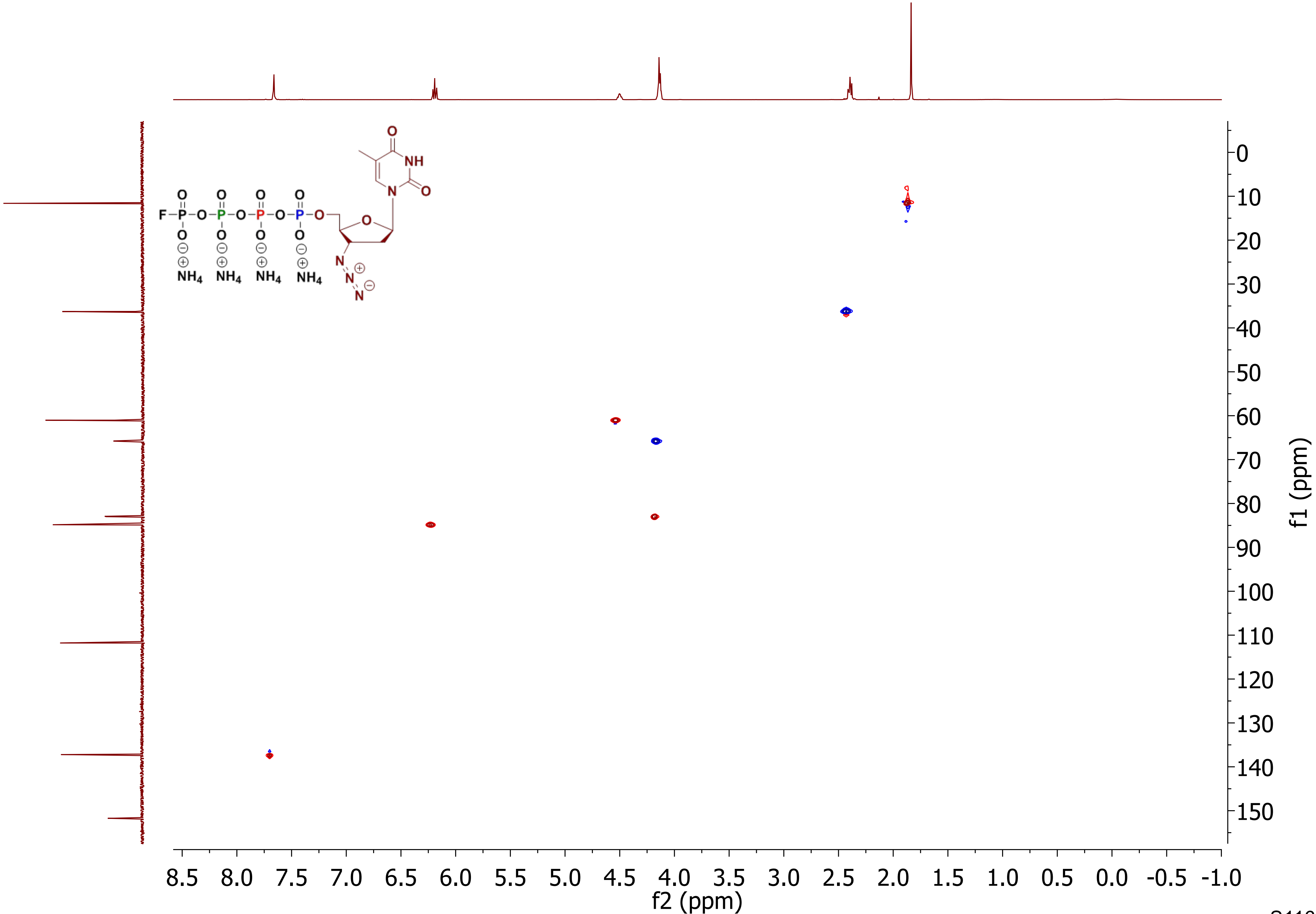
Product after purification by SAX



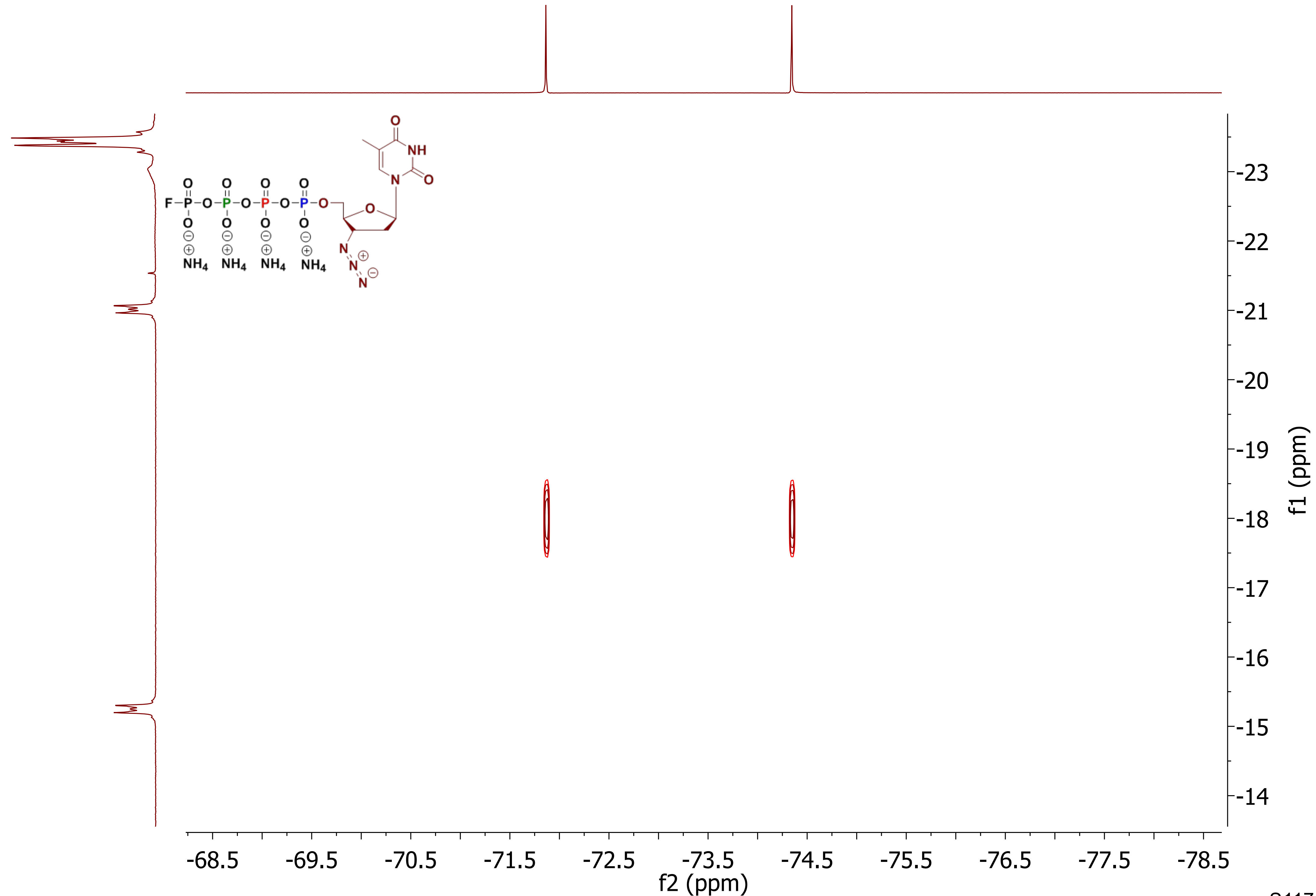
**$^{31}P$-$^{31}P$ Cosy NMR**



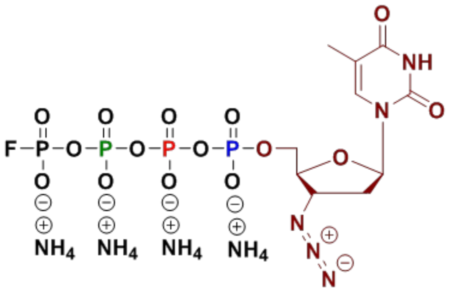
$^{1}H$-$^{13}C$ HSQC NMR



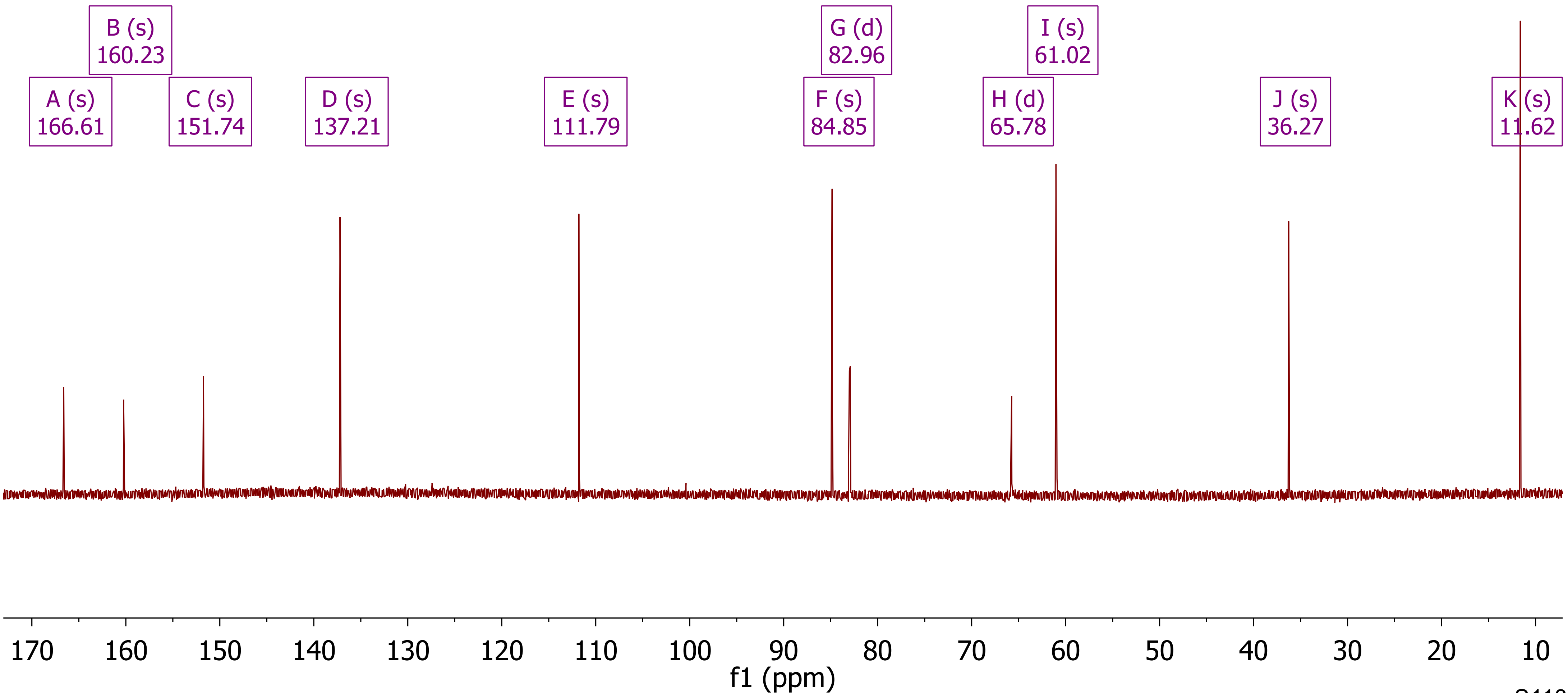
**$^{31}P$-$^{19}F$ Cosy NMR**

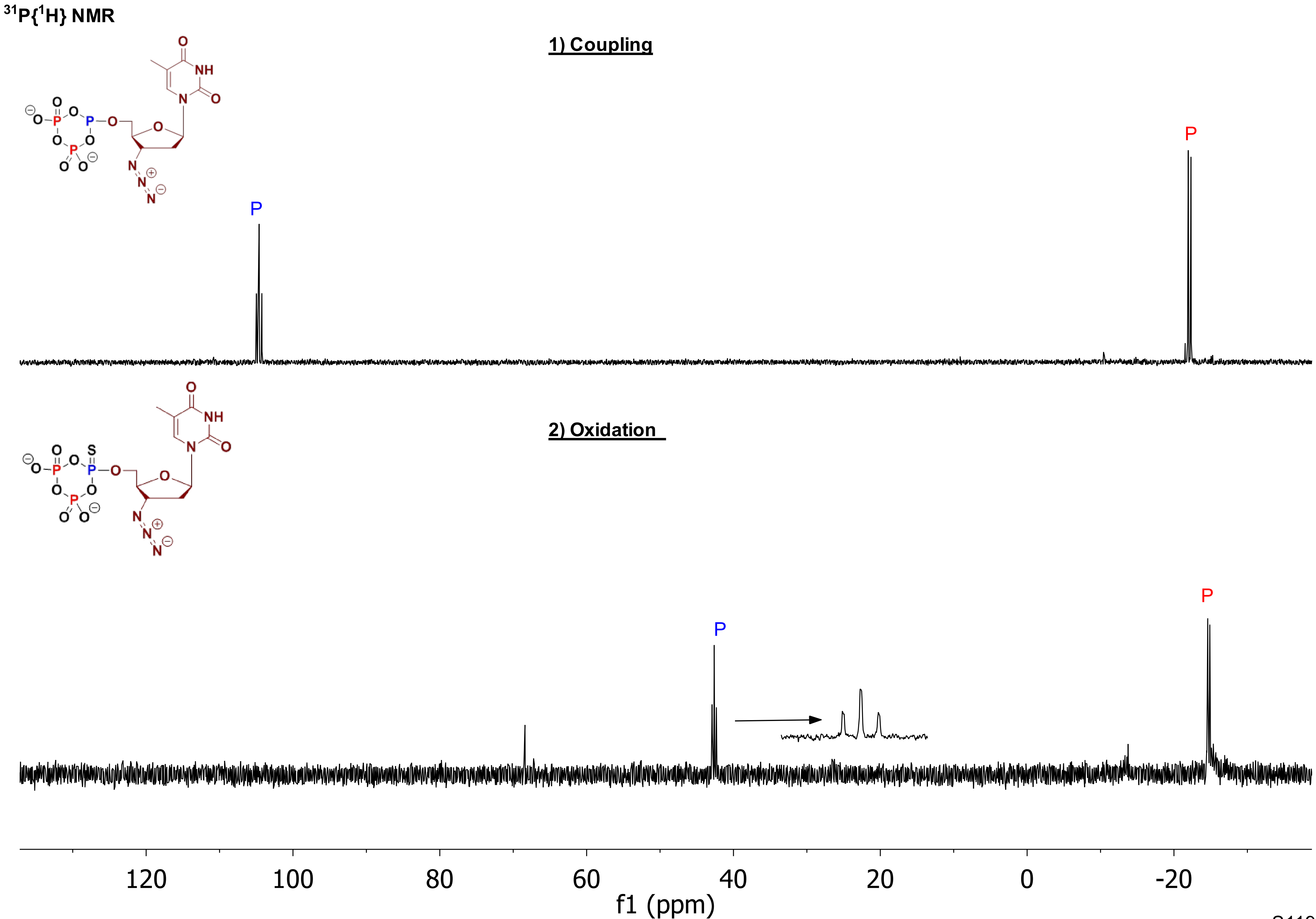


**$^{13}C$ NMR**



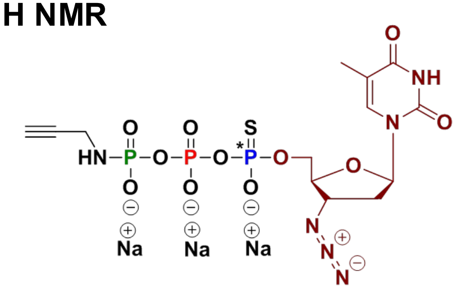
Product after purification by SAX



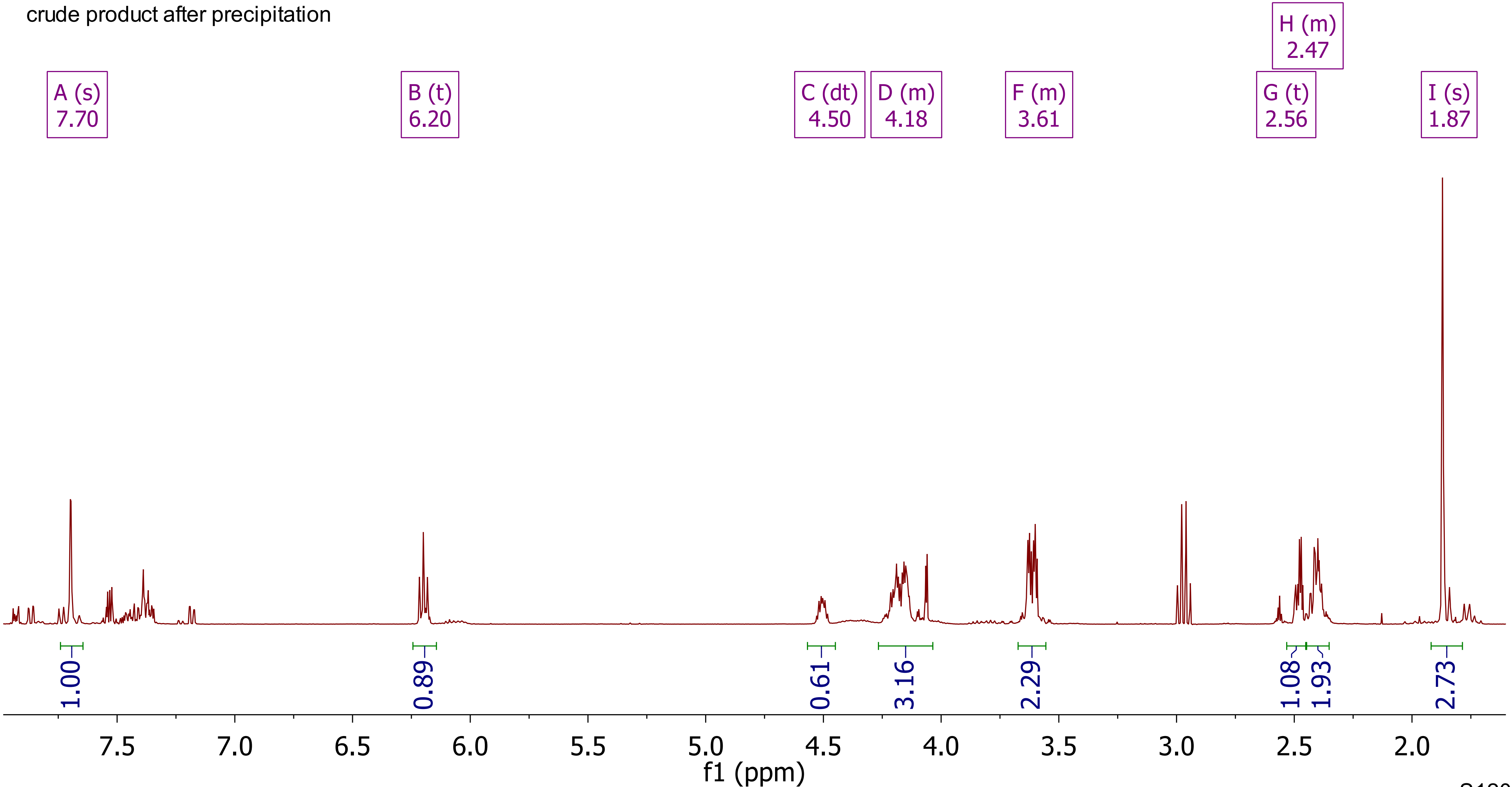


$^{31}P\{^1H\}$ NMR
1) Coupling
2) Oxidation
P
P
P
P
120
100
80
60
40
20
0
-20
f1 (ppm)

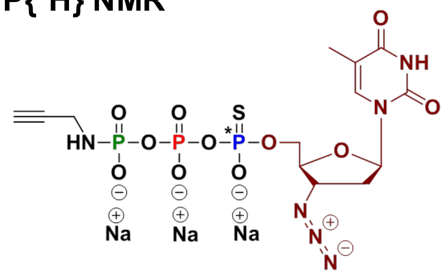
# $^{1}$H NMR



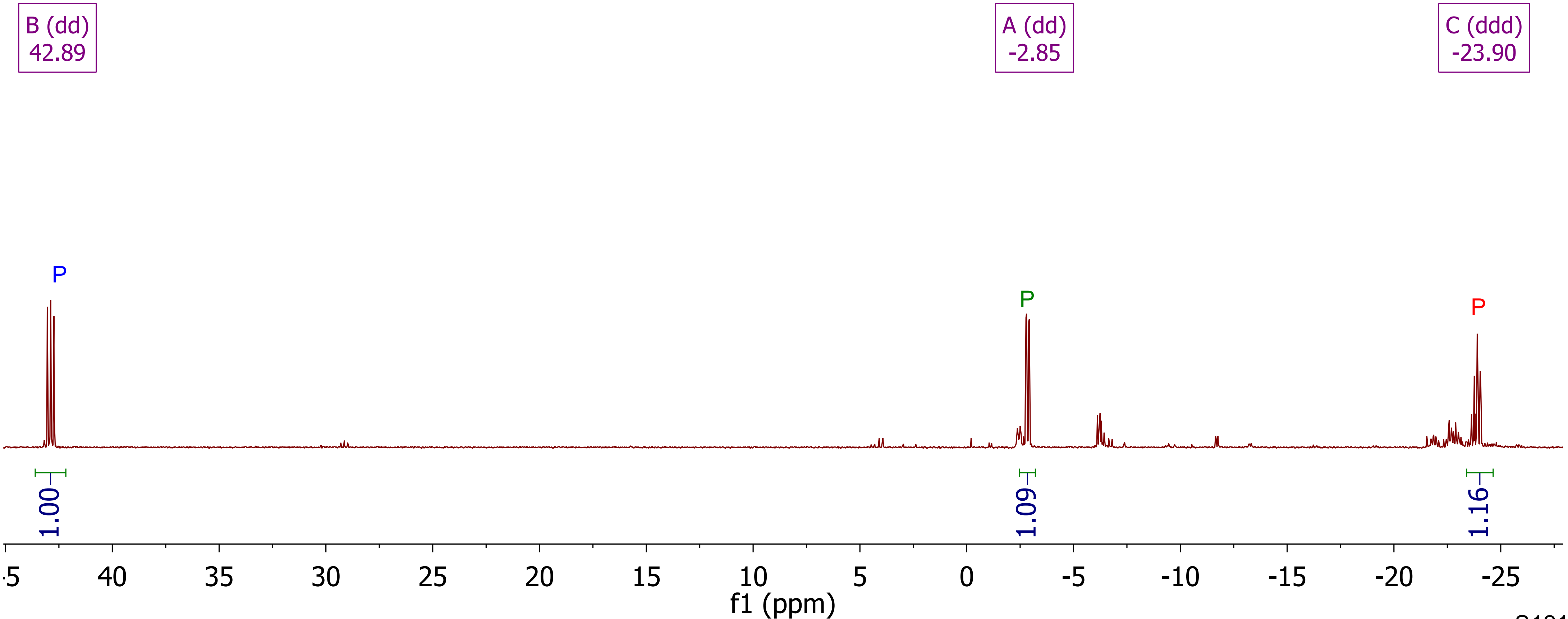
crude product after precipitation



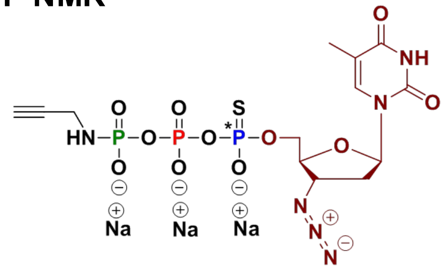
**$^{31}P\{^{1}H\}$ NMR**



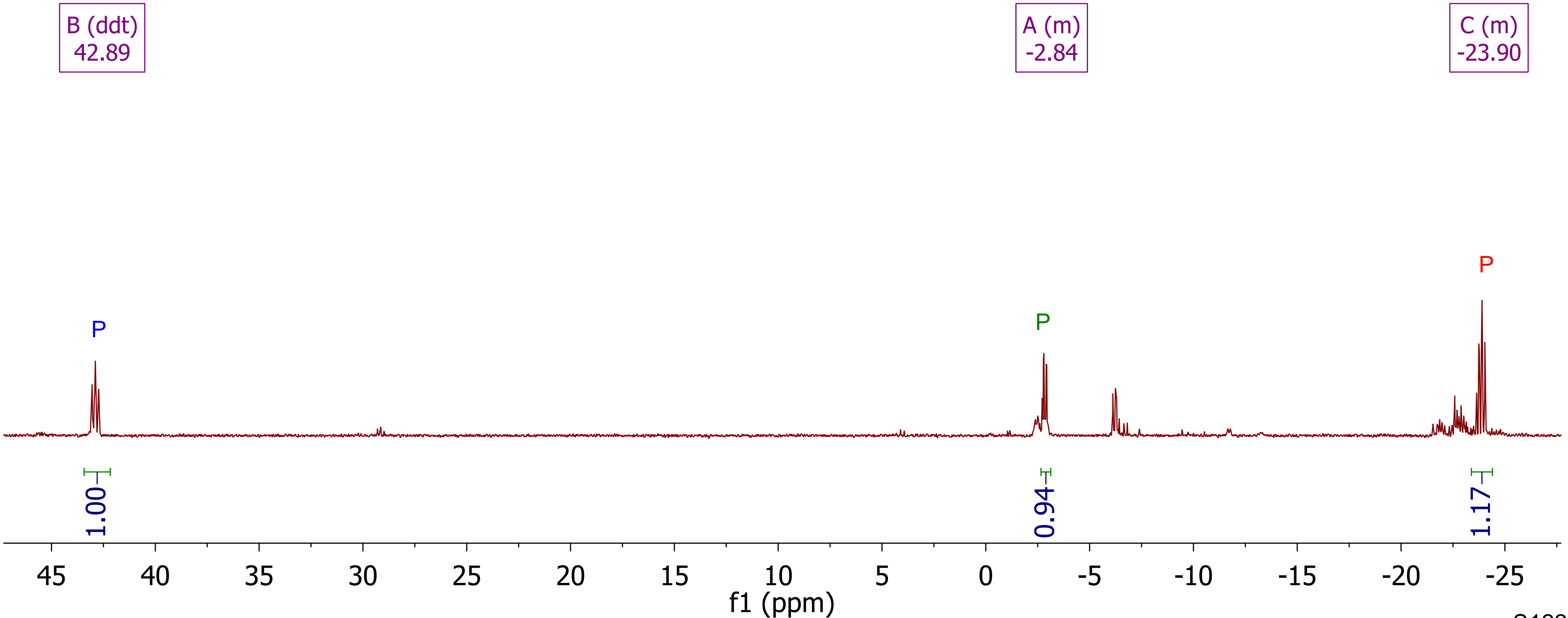
crude product after precipitation



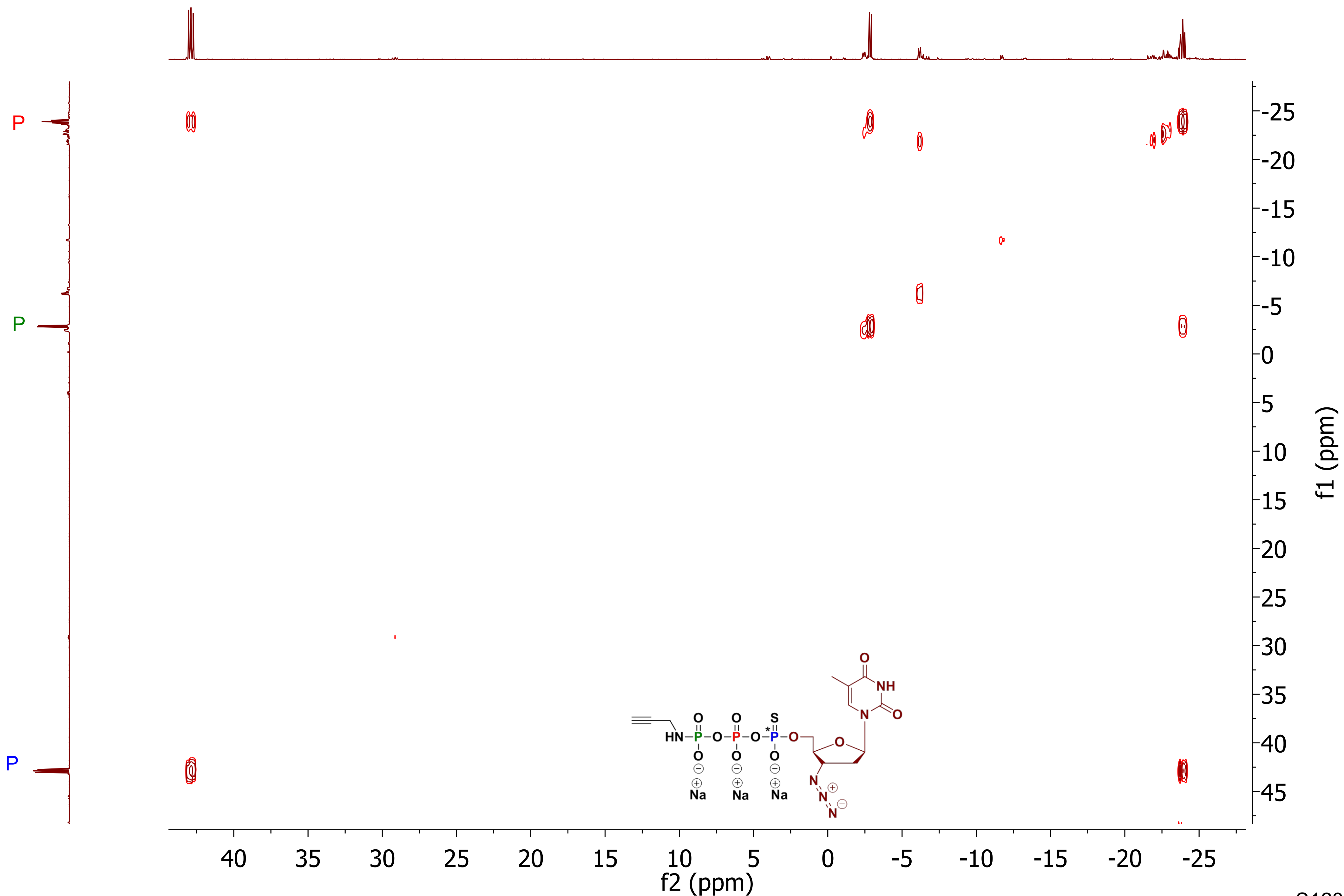
**$^{31}$P NMR**



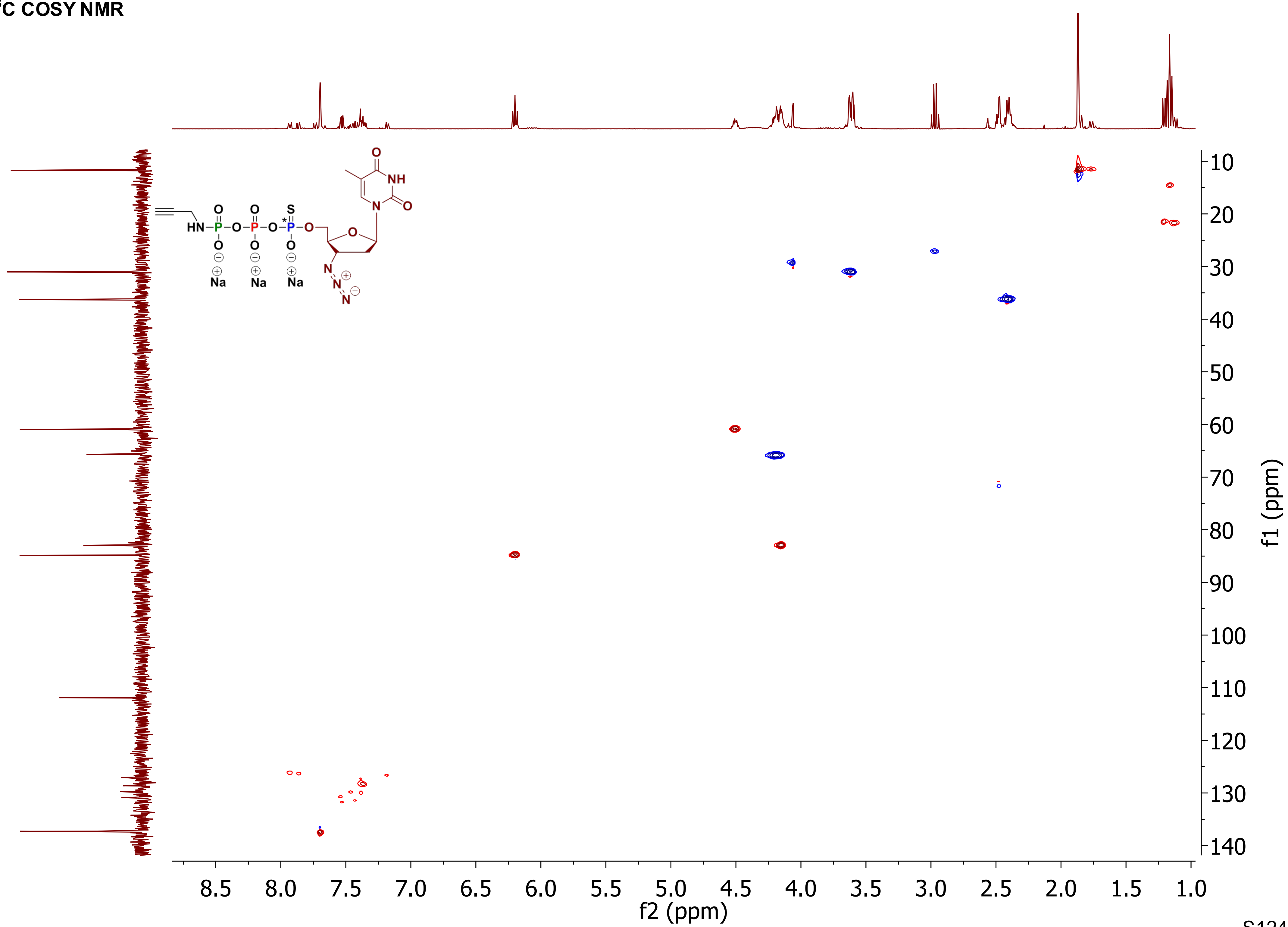
crude product after precipitation



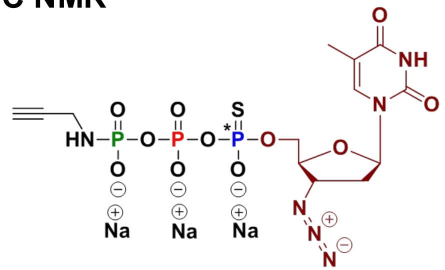
**$^{31}P$-$^{31}P$ COSY NMR**



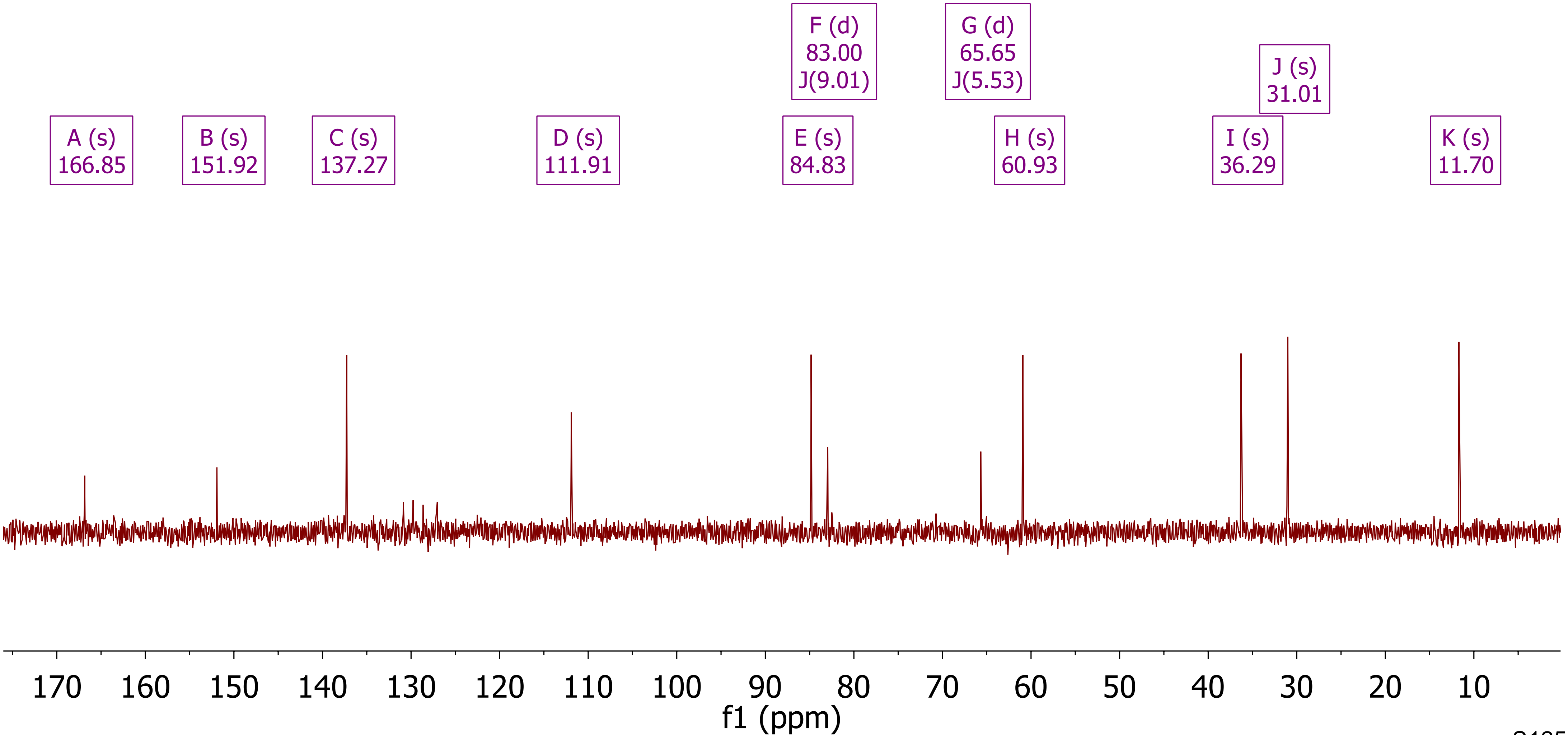
$^{1}H$-$^{13}C$ COSY NMR



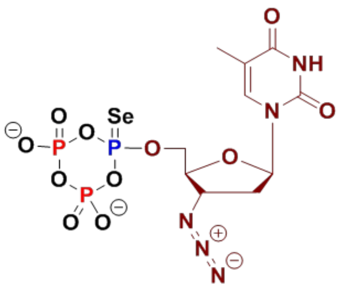
**$^{13}$C NMR**



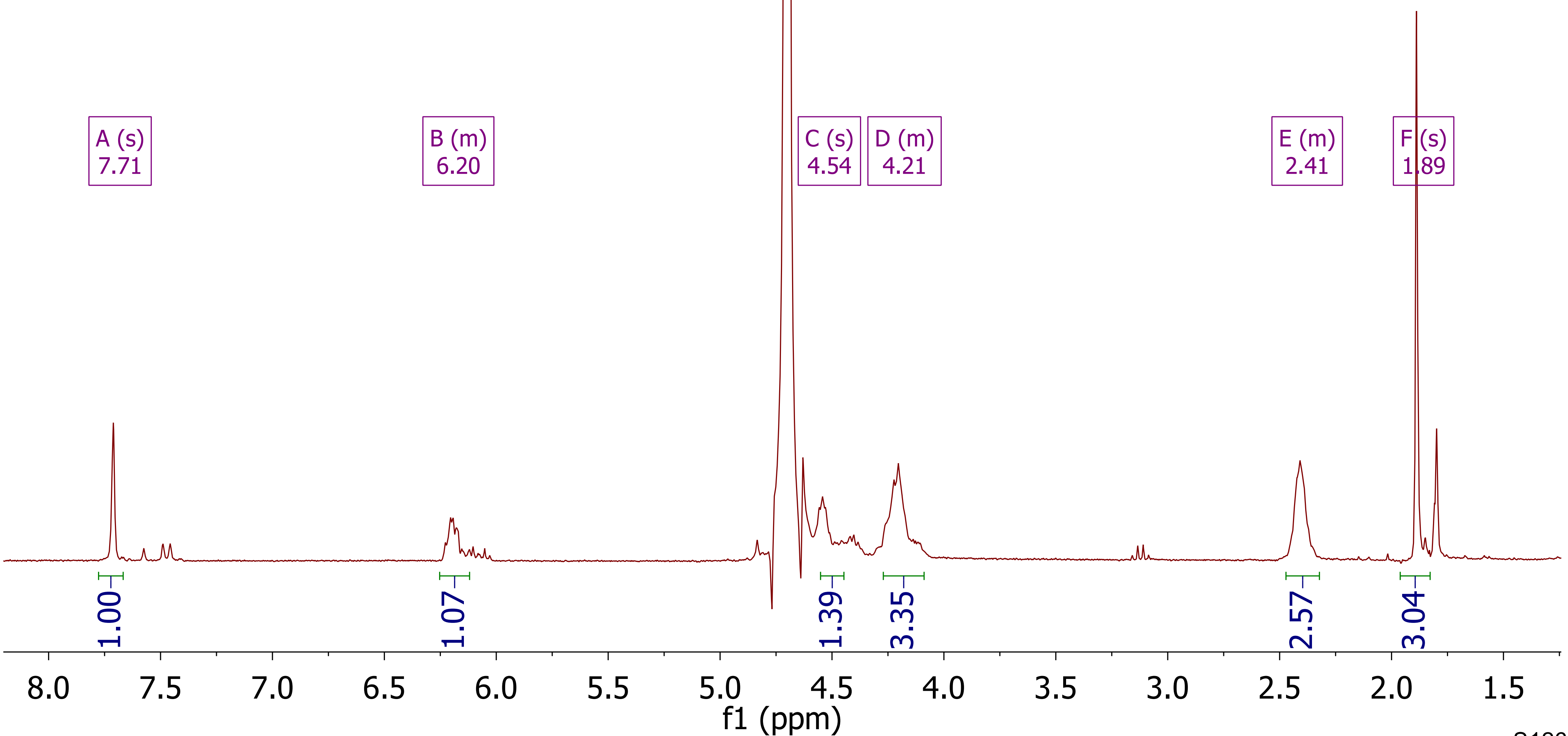

crude product after precipitation



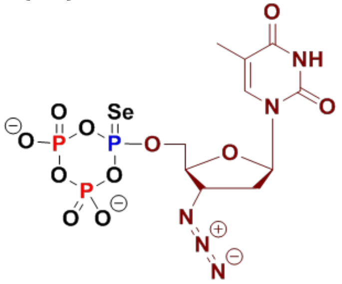
$^1$H NMR



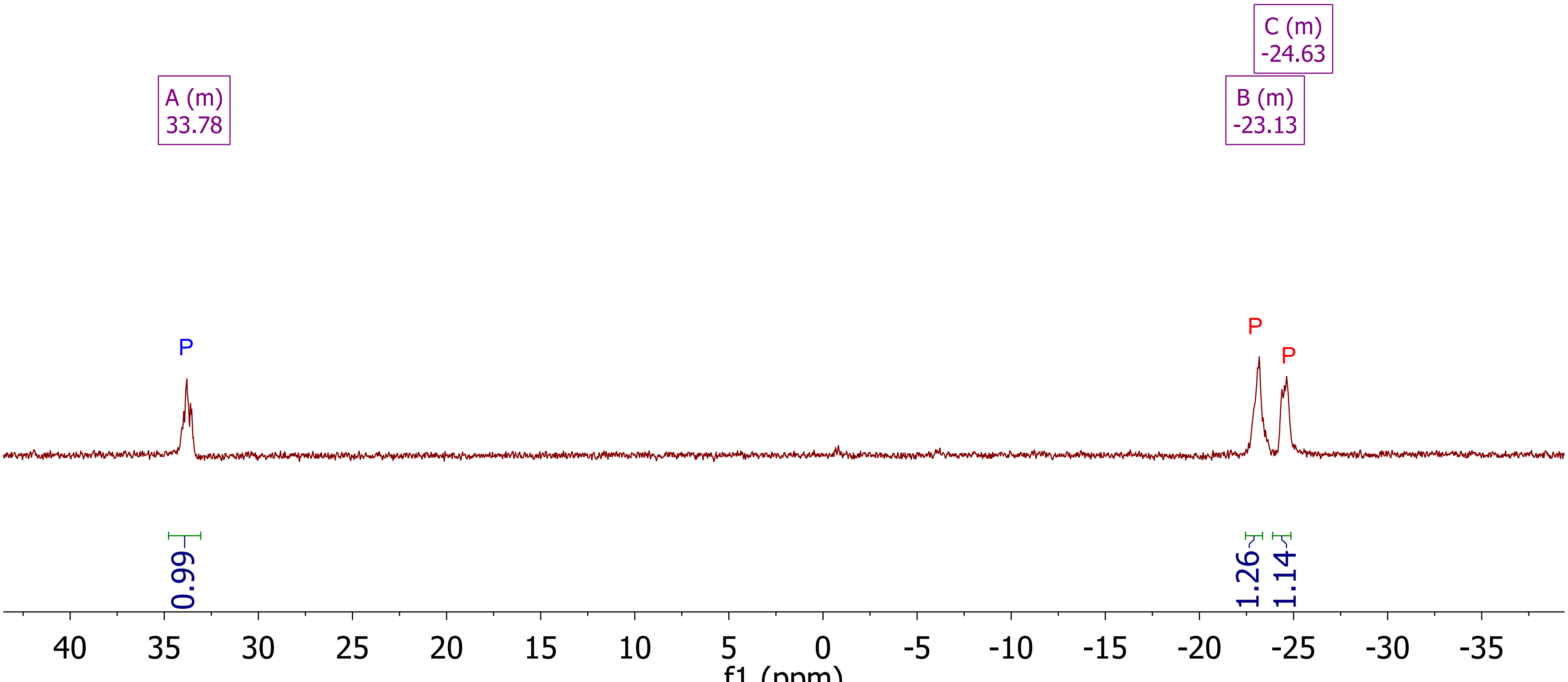
Product after purification by SAX



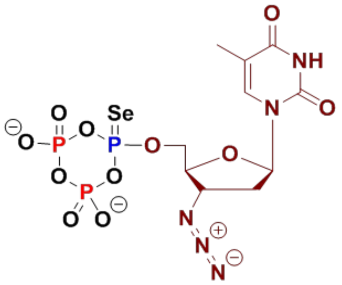
$^{31}P\{^1H\}$ NMR



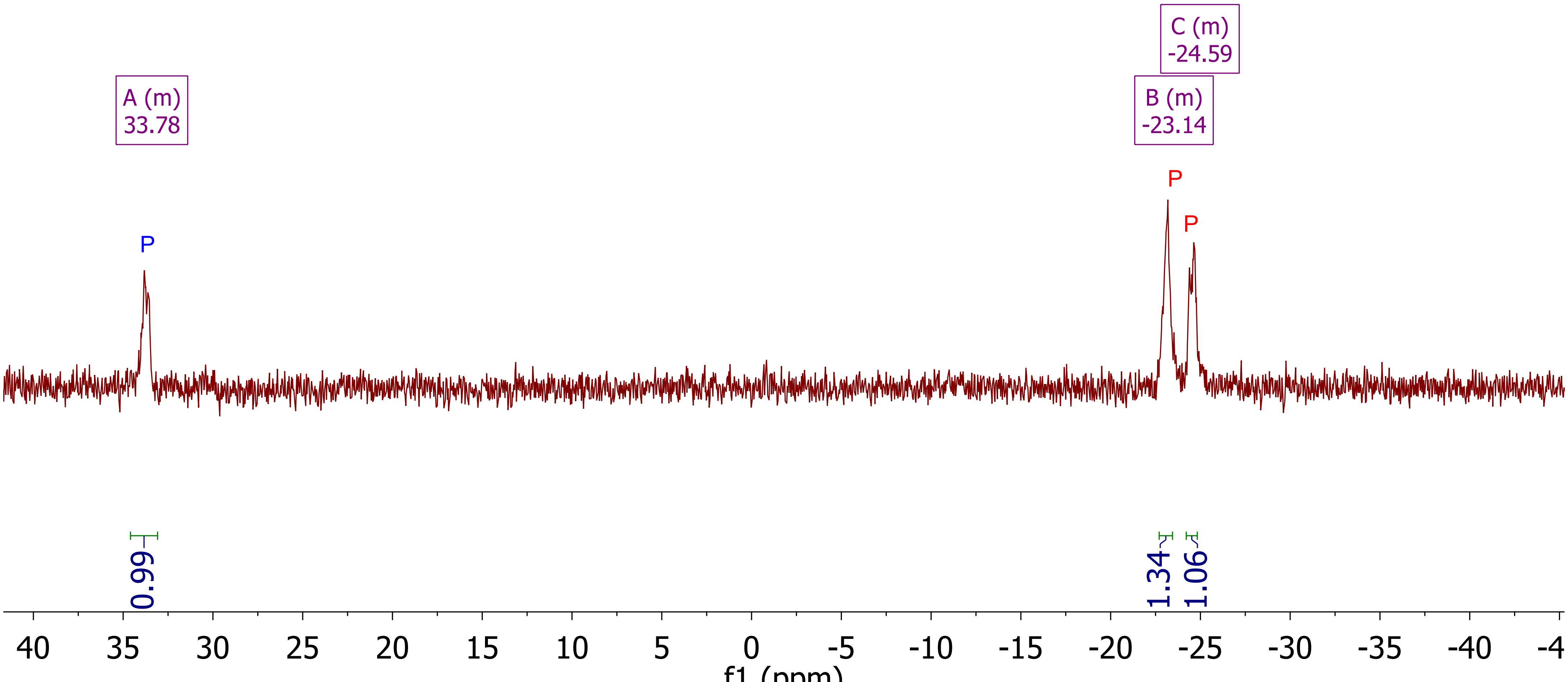
Product after purification by SAX

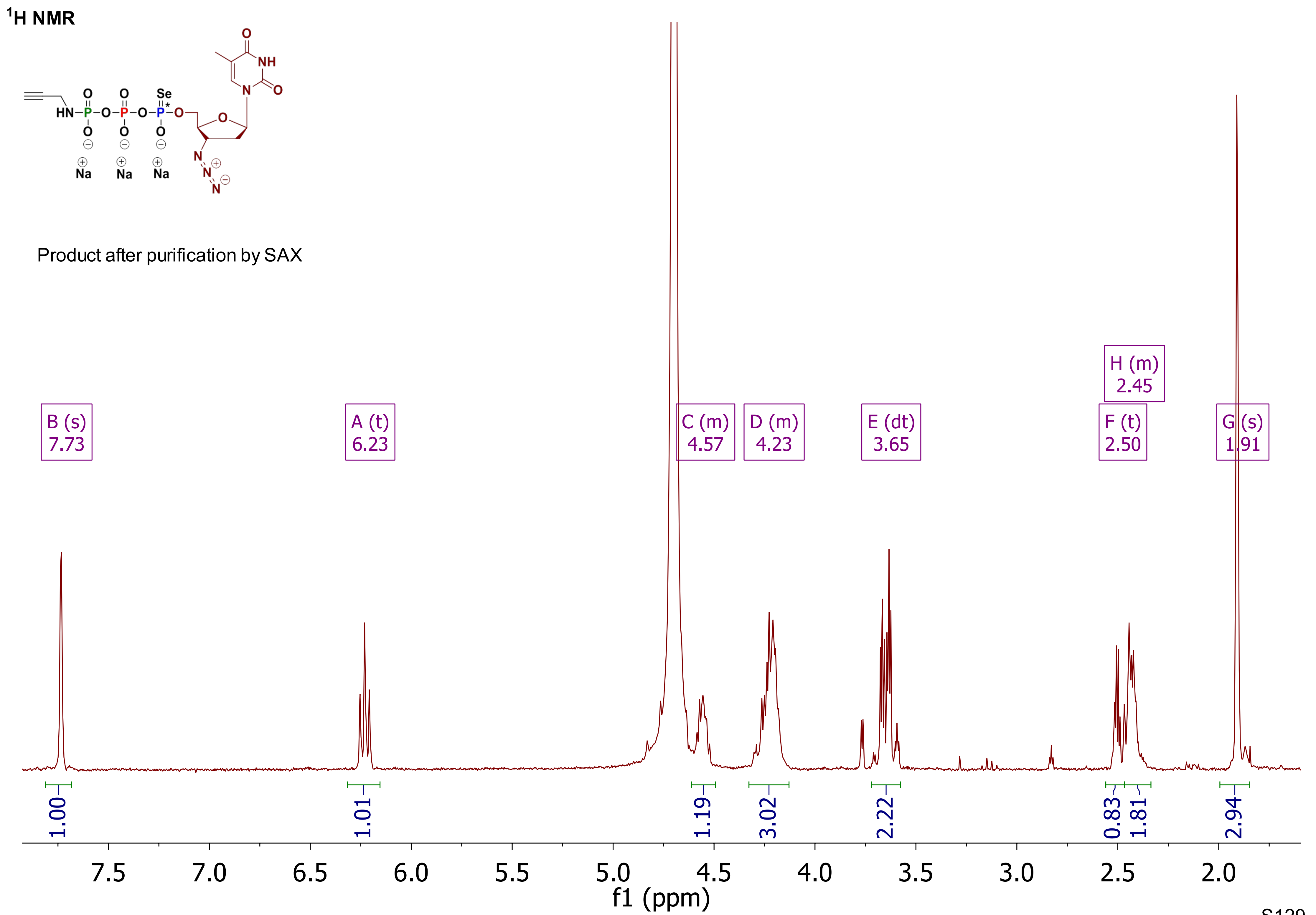


$^{31}$P NMR



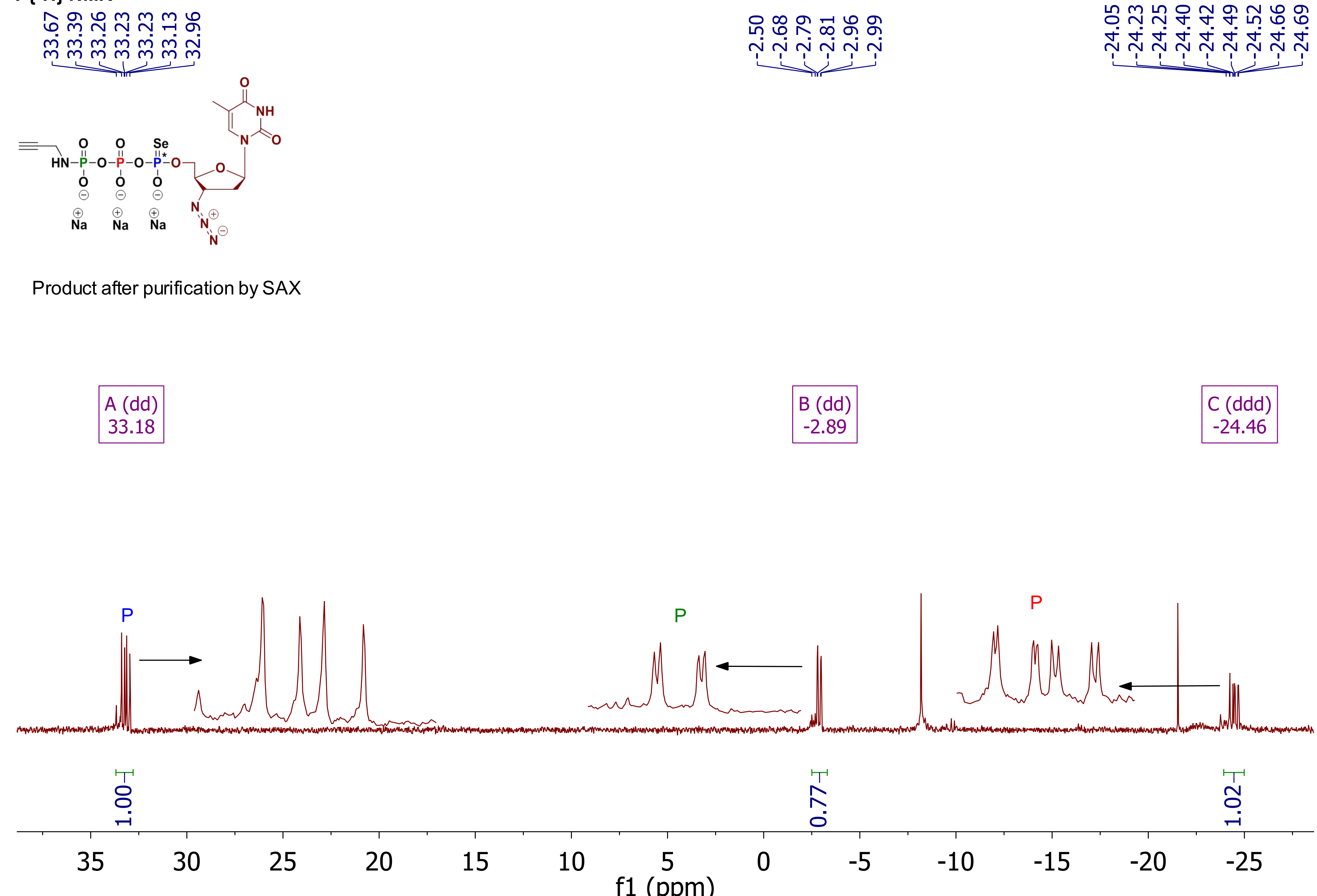
Product after purification by SAX



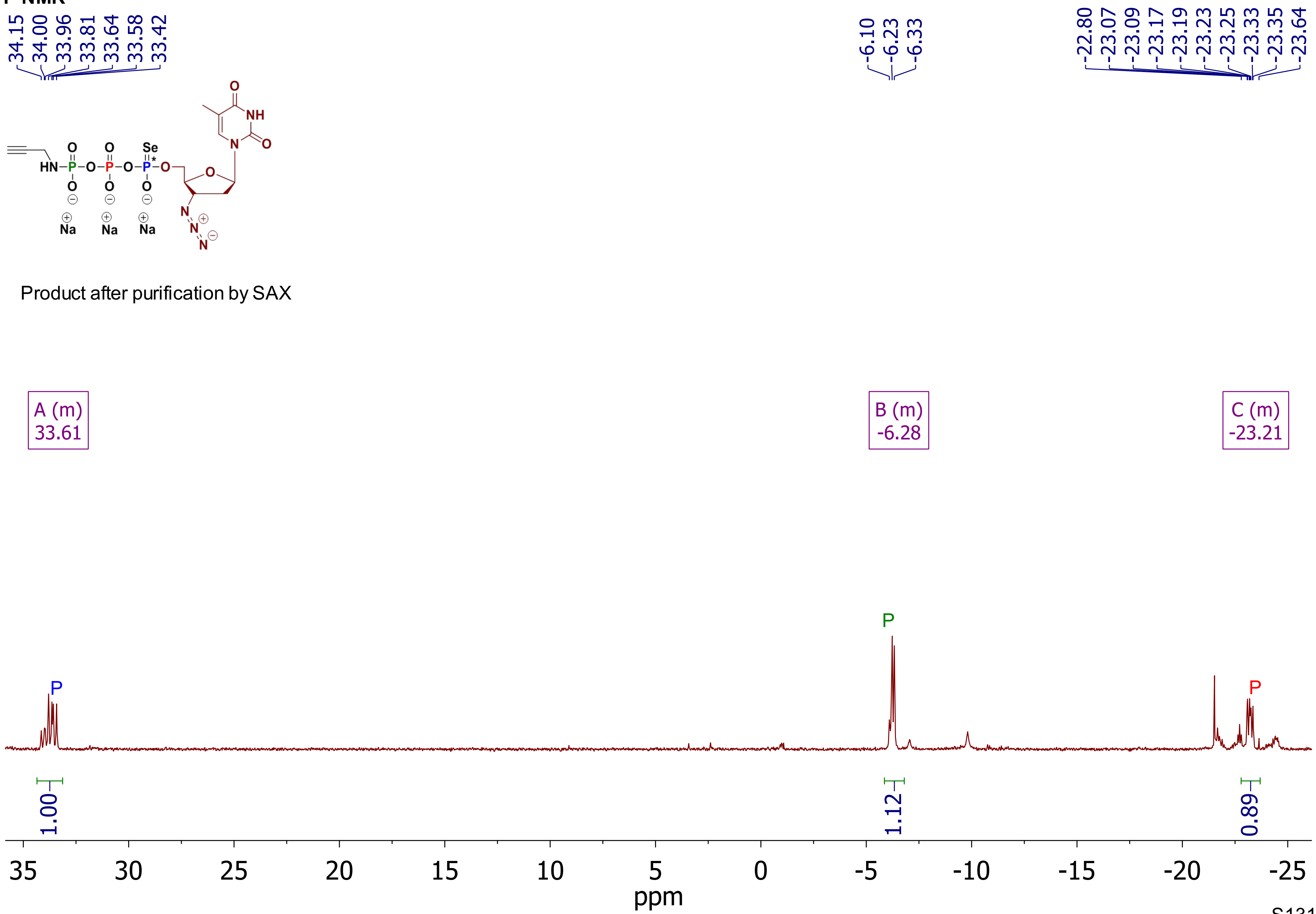


$^1$H NMR
Product after purification by SAX
B (s) 7.73
A (t) 6.23
C (m) 4.57
D (m) 4.23
E (dt) 3.65
H (m) 2.45
F (t) 2.50
G (s) 1.91
1.00
1.01
1.19
3.02
2.22
0.83
1.81
2.94
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

**$^{31}P\{^{1}H\}$ NMR**

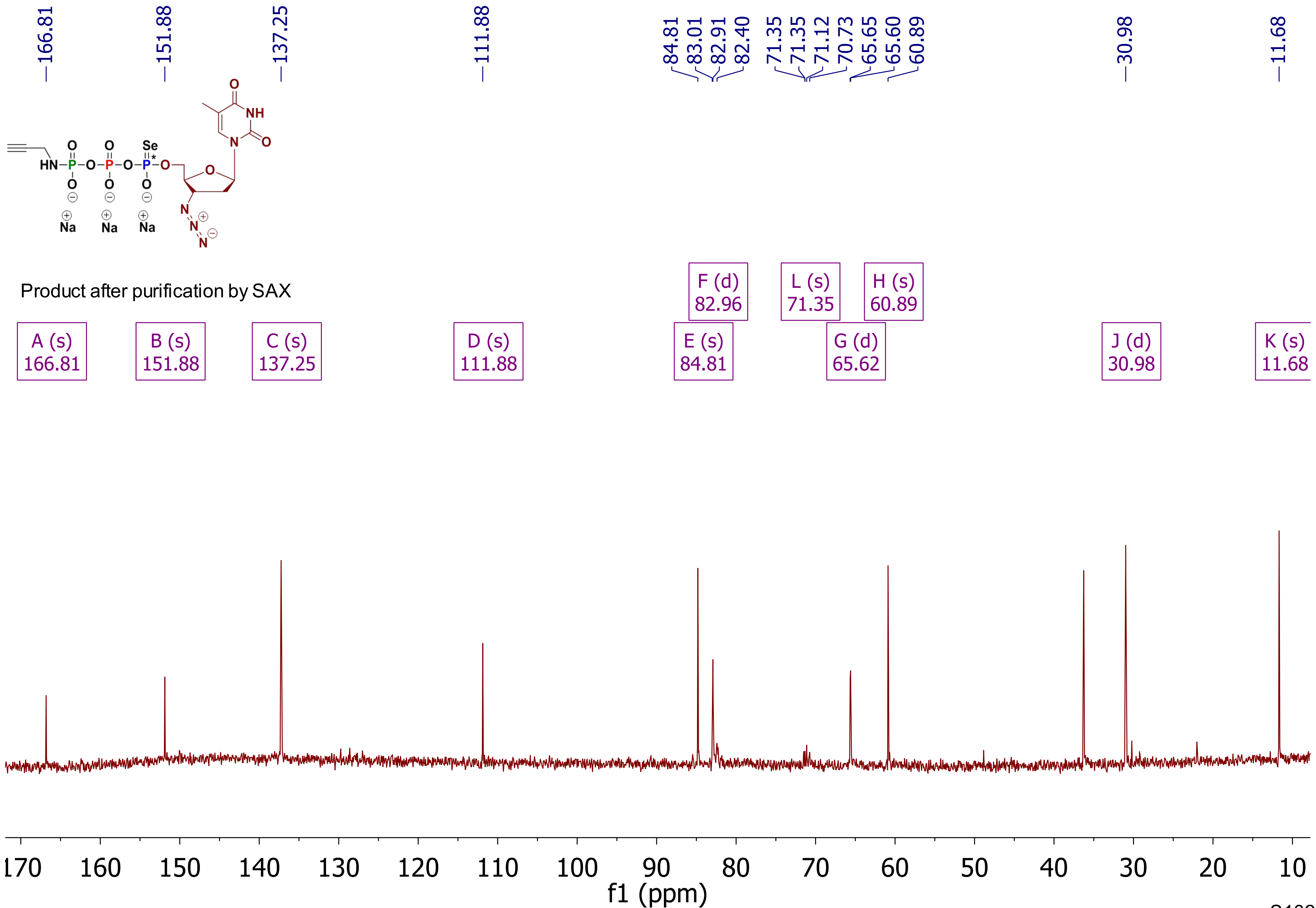

33.67
33.39
33.26
33.23
33.23
33.13
32.96
-2.50
-2.68
-2.79
-2.81
-2.96
-2.99
-24.05
-24.23
-24.25
-24.40
-24.42
-24.49
-24.52
-24.66
-24.69
Se
O
NH
HN
N
Na
Product after purification by SAX
A (dd)
33.18
B (dd)
-2.89
C (ddd)
-24.46
P
P
P
1.00
0.77
1.02
35
30
25
20
15
10
5
0
-5
-10
-15
-20
-25
f1 (ppm)

$^{31}$P NMR



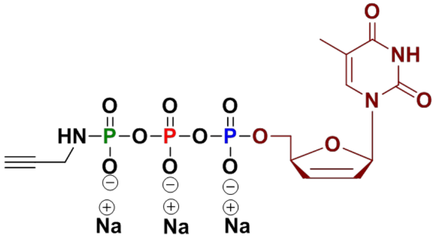
34.15
34.00
33.96
33.81
33.64
33.58
33.42
-6.10
-6.23
-6.33
-22.80
-23.07
-23.09
-23.17
-23.19
-23.23
-23.25
-23.33
-23.35
-23.64
Product after purification by SAX
A (m)
33.61
B (m)
-6.28
C (m)
-23.21
1.00
1.12
0.89
35
30
25
20
15
10
5
0
-5
-10
-15
-20
-25
ppm

$^{13}$C NMR

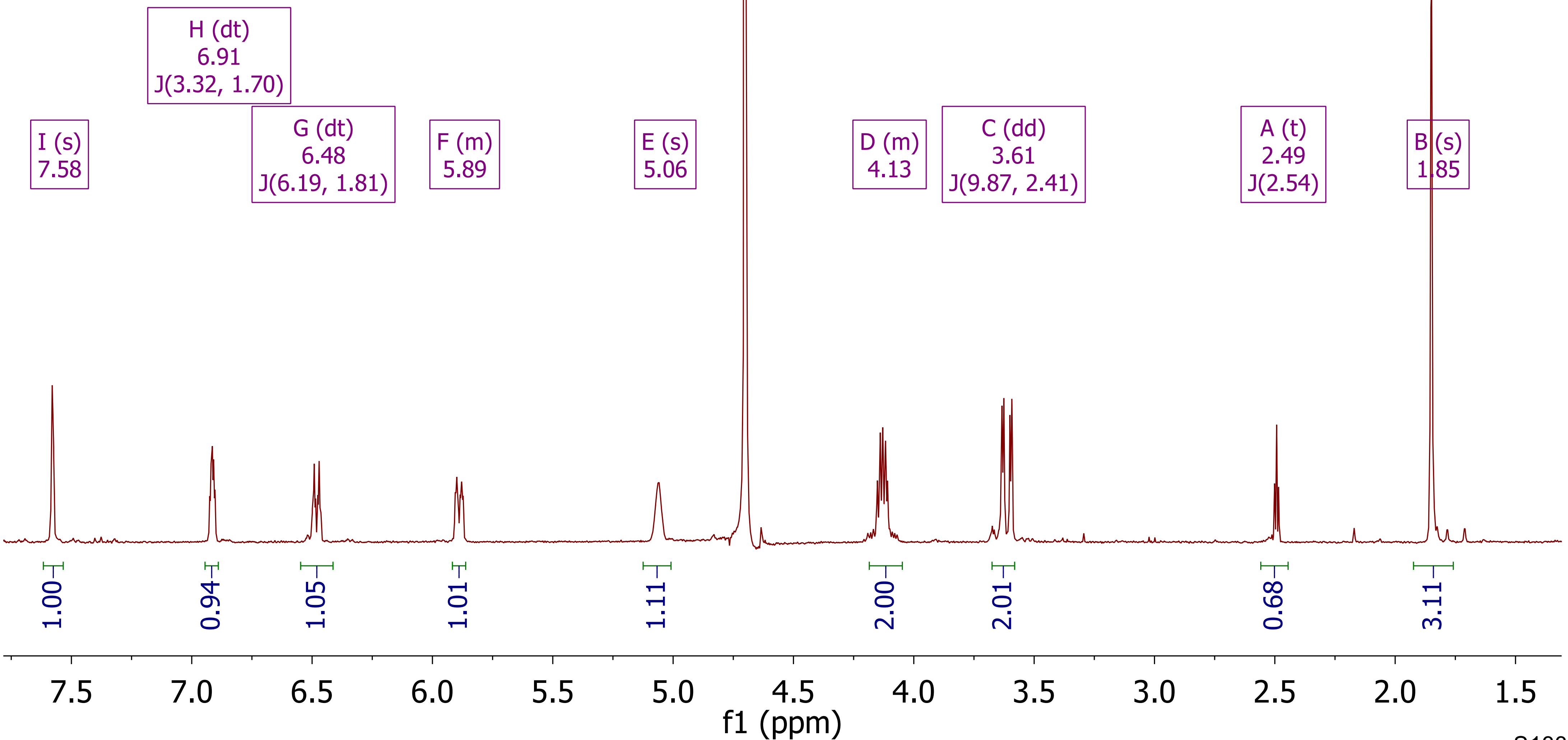


166.81
151.88
137.25
111.88
84.81
83.01
82.91
82.40
71.35
71.35
71.12
70.73
65.65
65.60
60.89
30.98
11.68
Product after purification by SAX
F (d) 82.96
L (s) 71.35
H (s) 60.89
A (s) 166.81
B (s) 151.88
C (s) 137.25
D (s) 111.88
E (s) 84.81
G (d) 65.62
J (d) 30.98
K (s) 11.68
f1 (ppm)

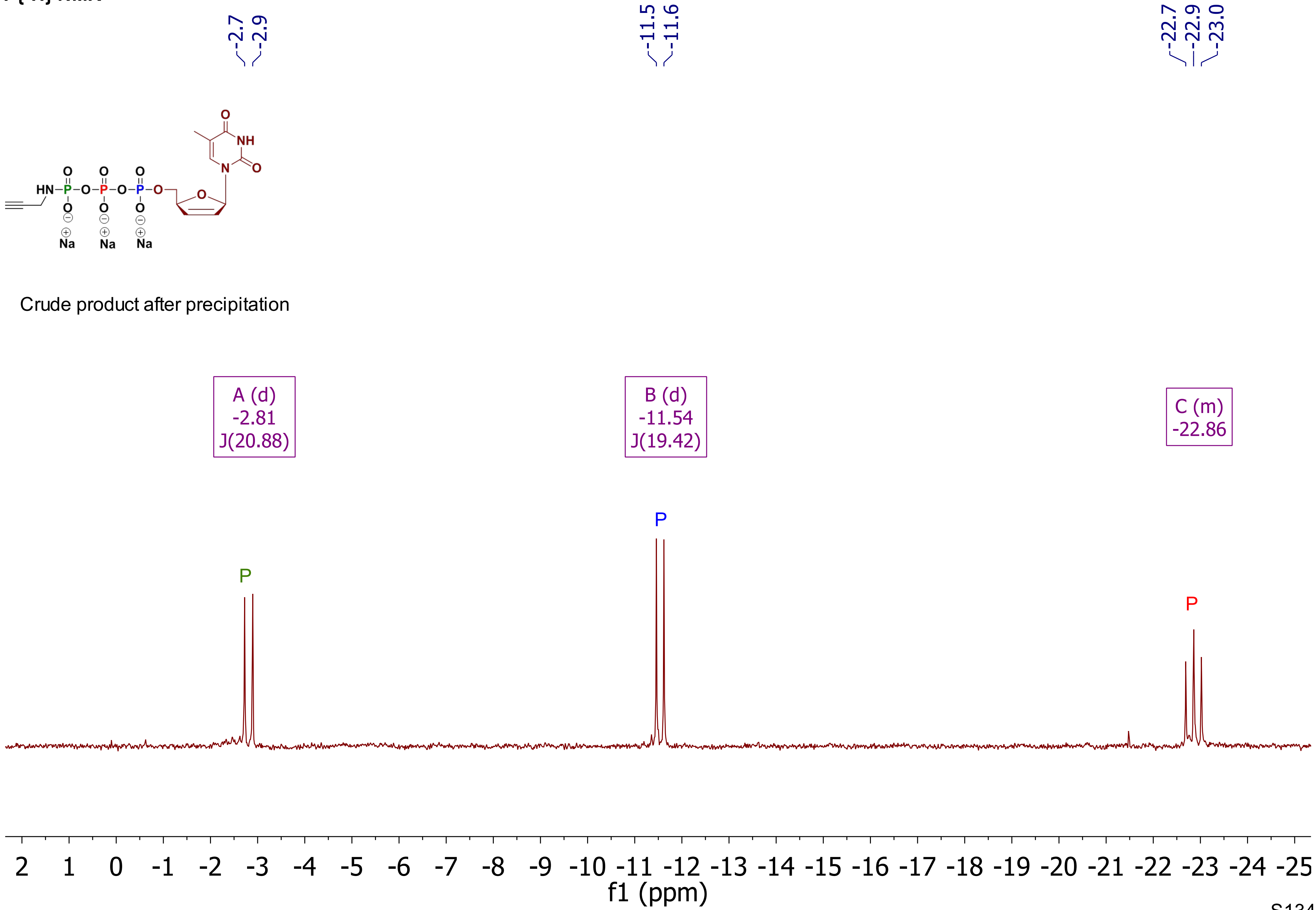
**$^1$H NMR**



Crude product after precipitation

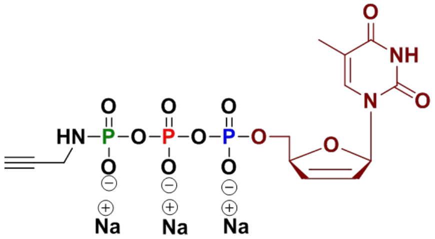


$^{31}P\{^1H\}$ NMR

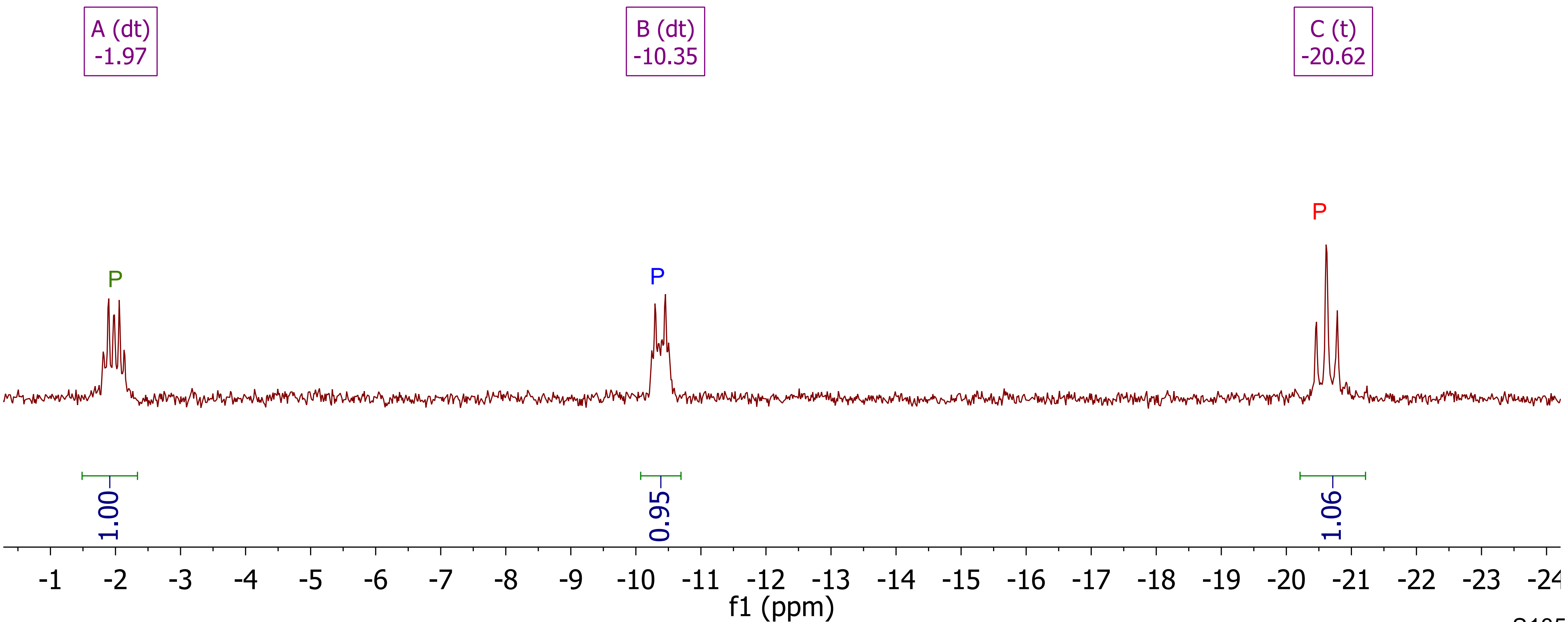


-2.7
-2.9
-11.5
-11.6
-22.7
-22.9
-23.0
HN
P
O
Na
NH
N
Crude product after precipitation
A (d)
-2.81
J(20.88)
B (d)
-11.54
J(19.42)
C (m)
-22.86
P
P
P
2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23 -24 -25
f1 (ppm)

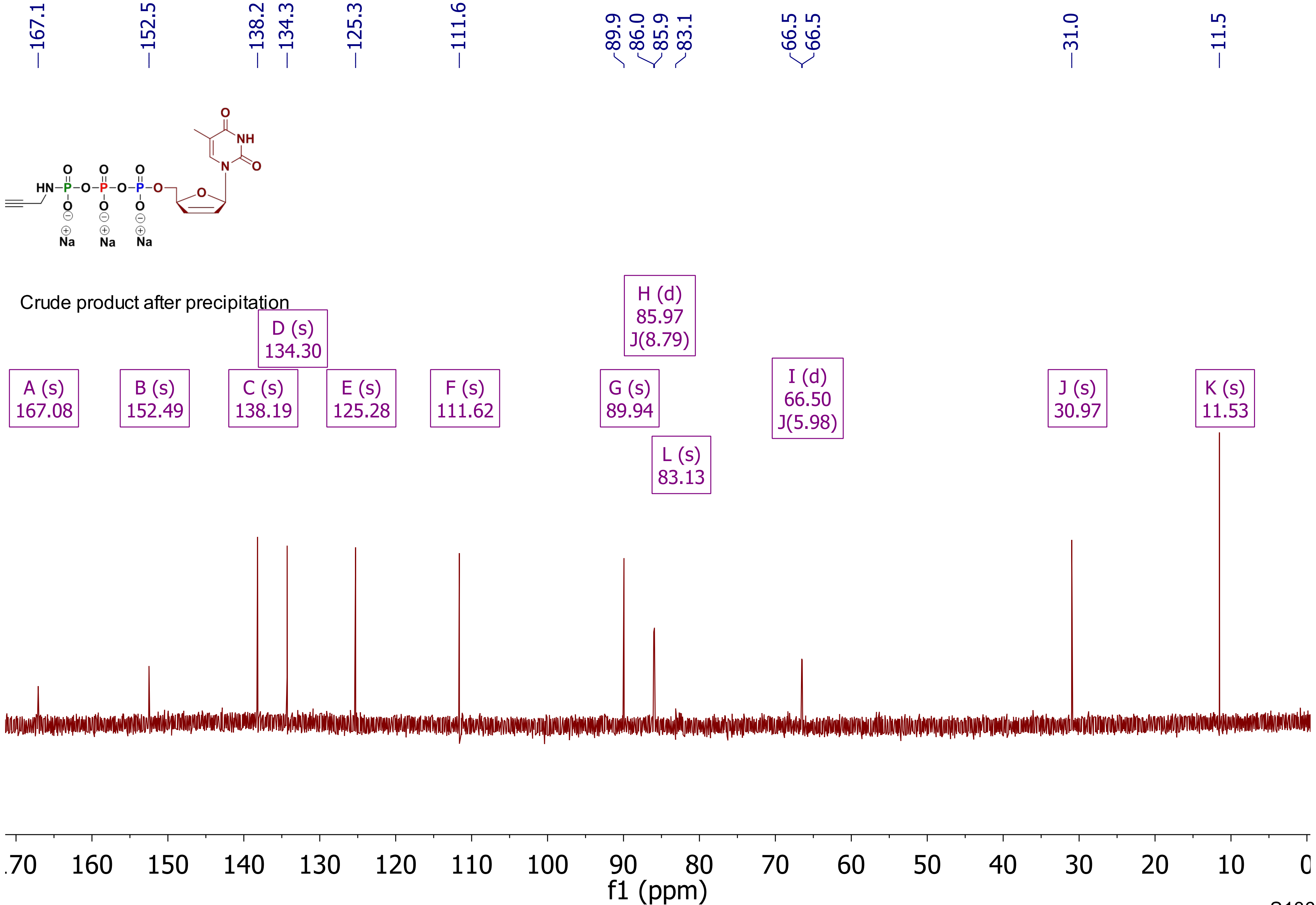
**$^{31}$P NMR**



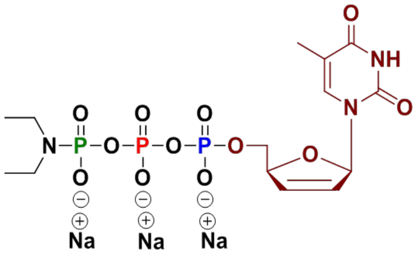
Crude product after precipitation



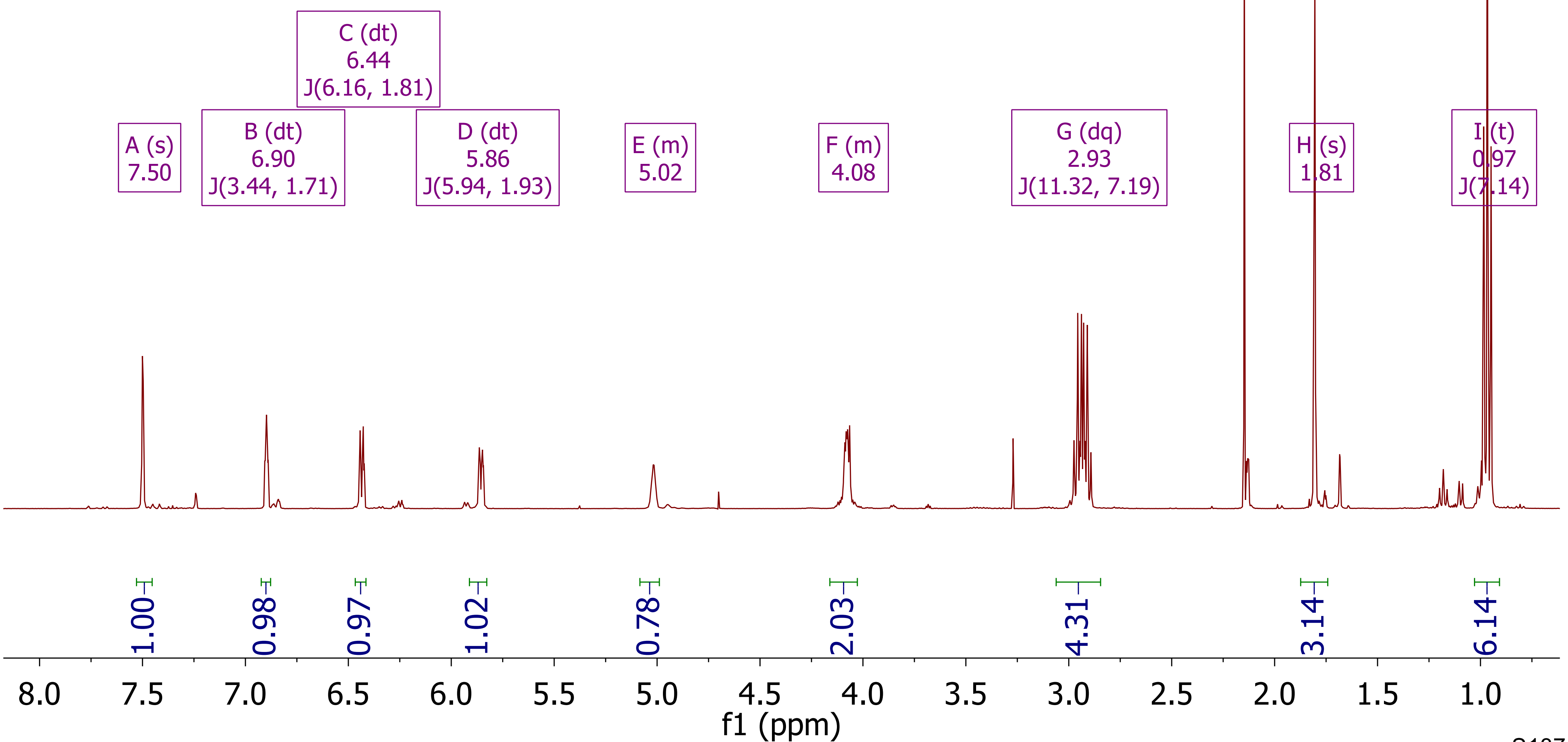
## $^{13}$C NMR


167.1
152.5
138.2
134.3
125.3
111.6
89.9
86.0
85.9
83.1
66.5
66.5
31.0
11.5
Crude product after precipitation
A (s) 167.08
B (s) 152.49
C (s) 138.19
D (s) 134.30
E (s) 125.28
F (s) 111.62
G (s) 89.94
H (d) 85.97 J(8.79)
L (s) 83.13
I (d) 66.50 J(5.98)
J (s) 30.97
K (s) 11.53
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

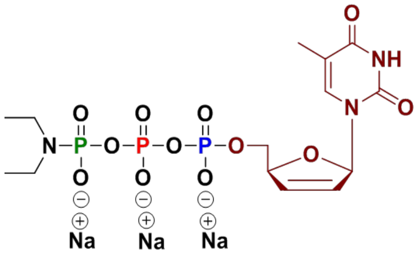
$^{1}$H NMR



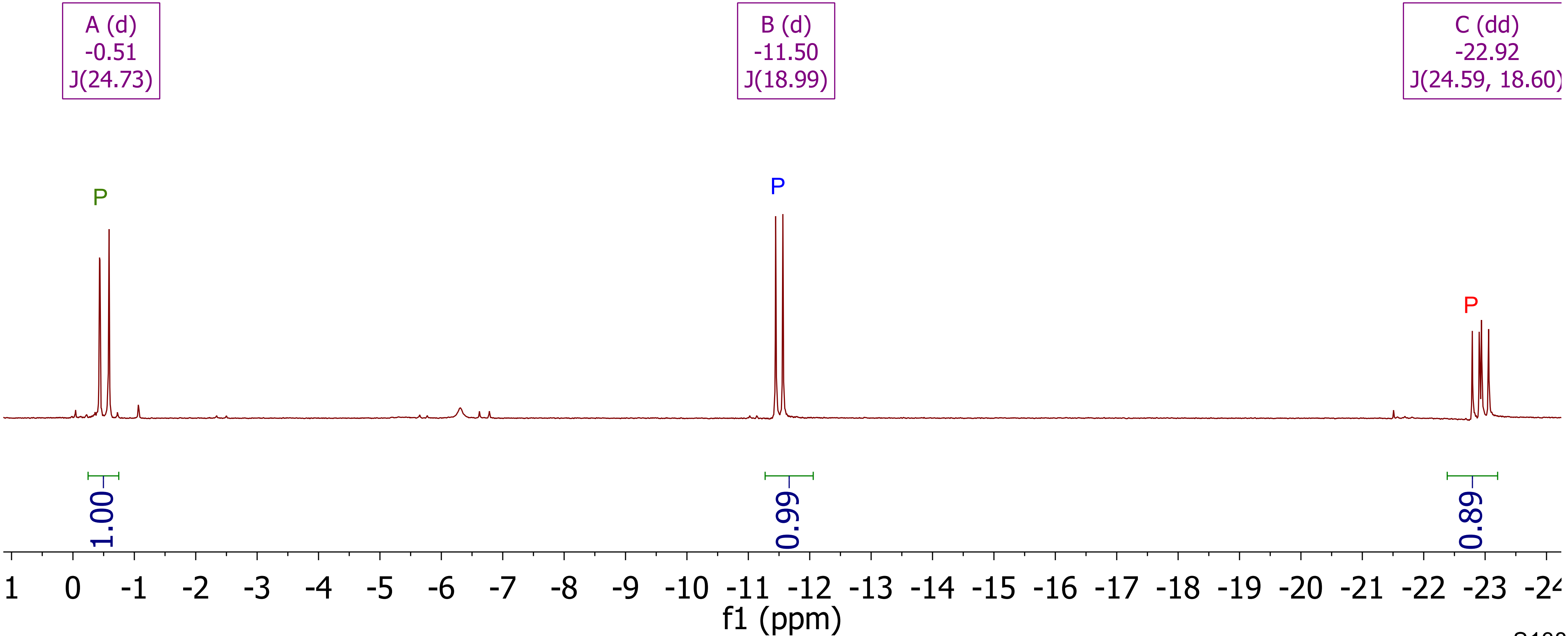
Crude product after precipitation



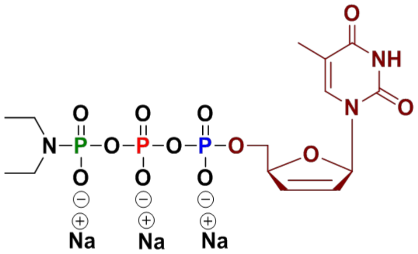
$^{31}P\{^1H\}$ NMR



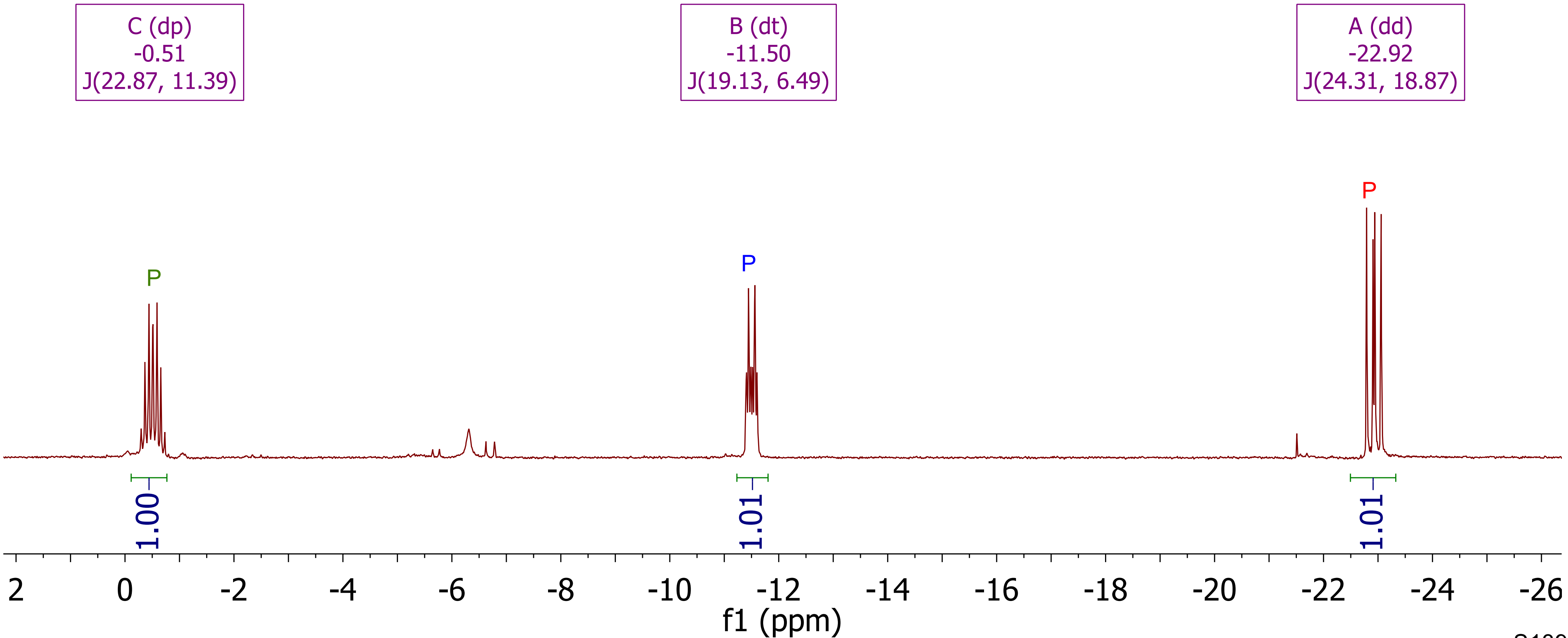

Crude product after precipitation



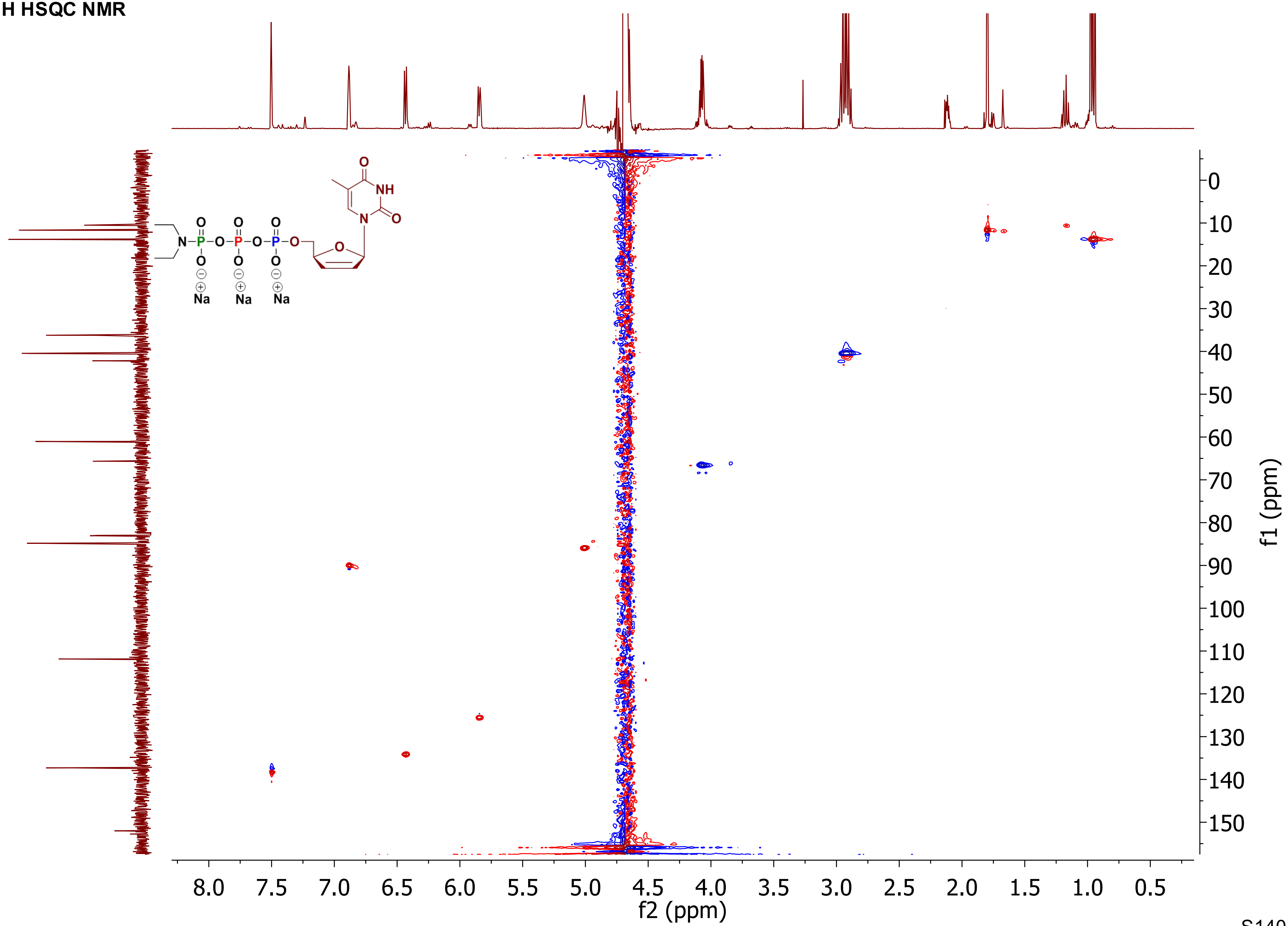
$^{31}$P NMR



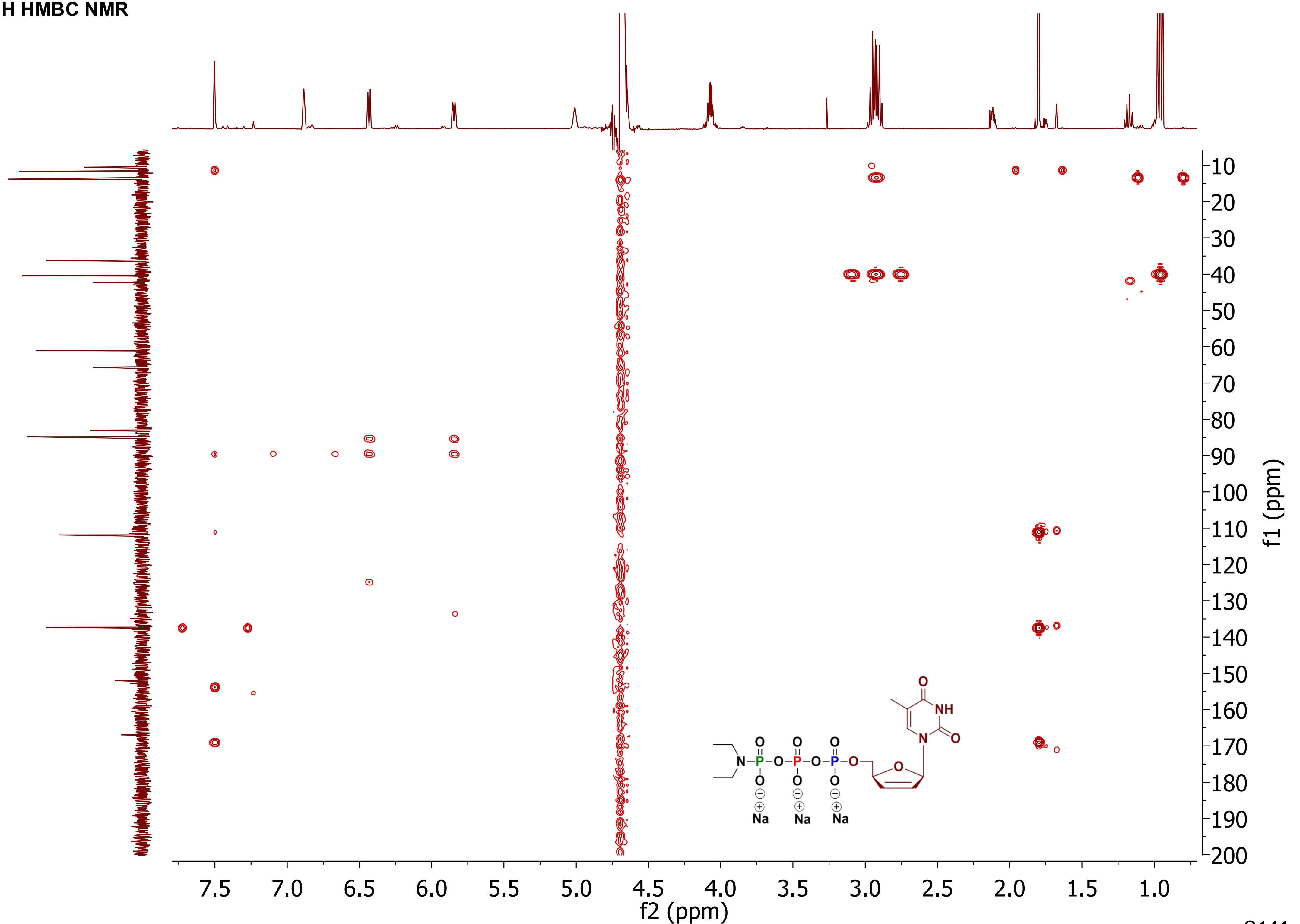
Crude product after precipitation



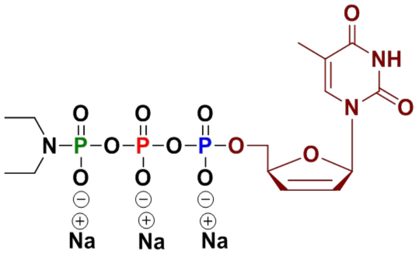
$^{13}C$-$^{1}H$ HSQC NMR



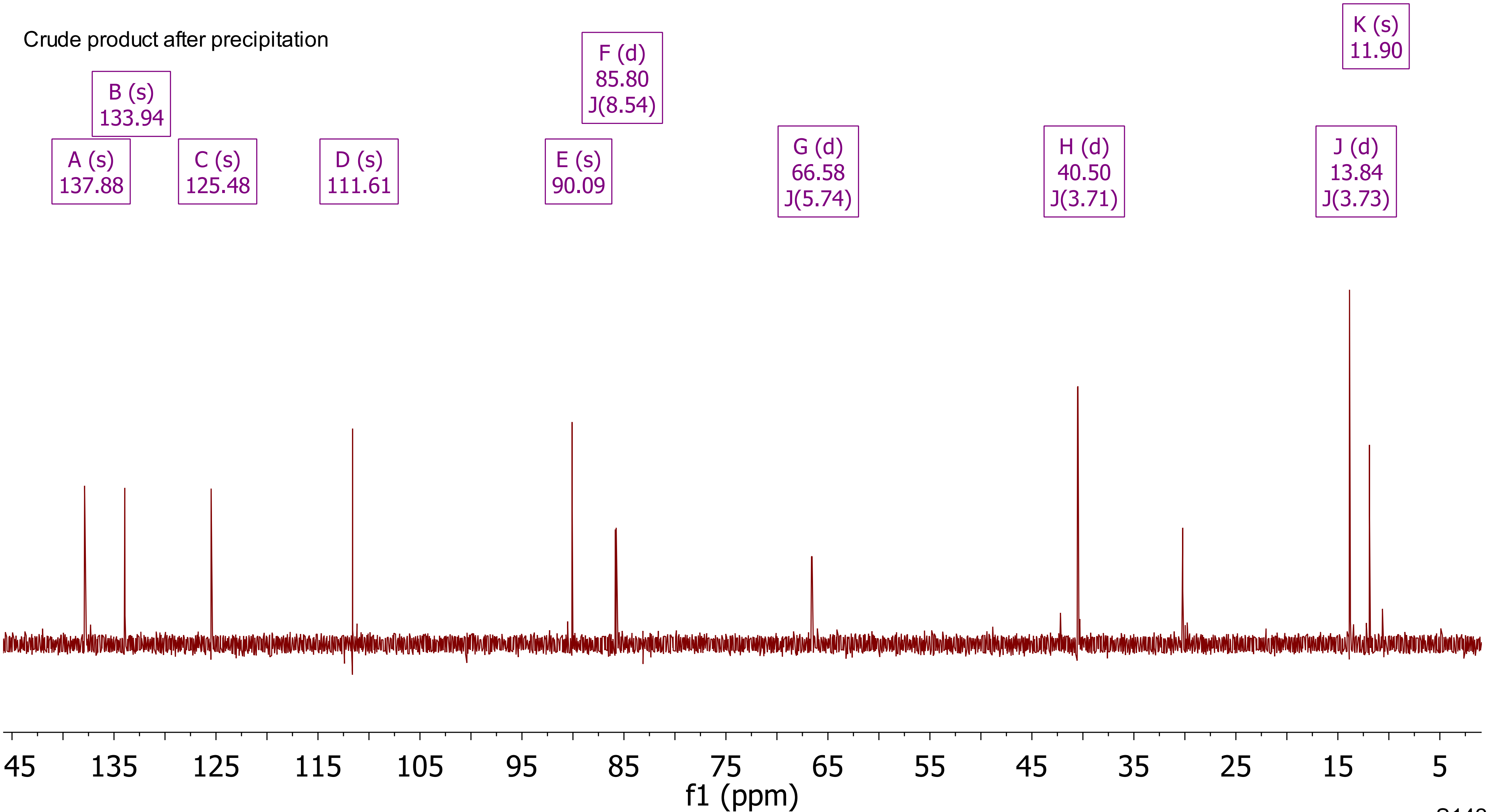
$^{13}$C-$^{1}$H HMBC NMR



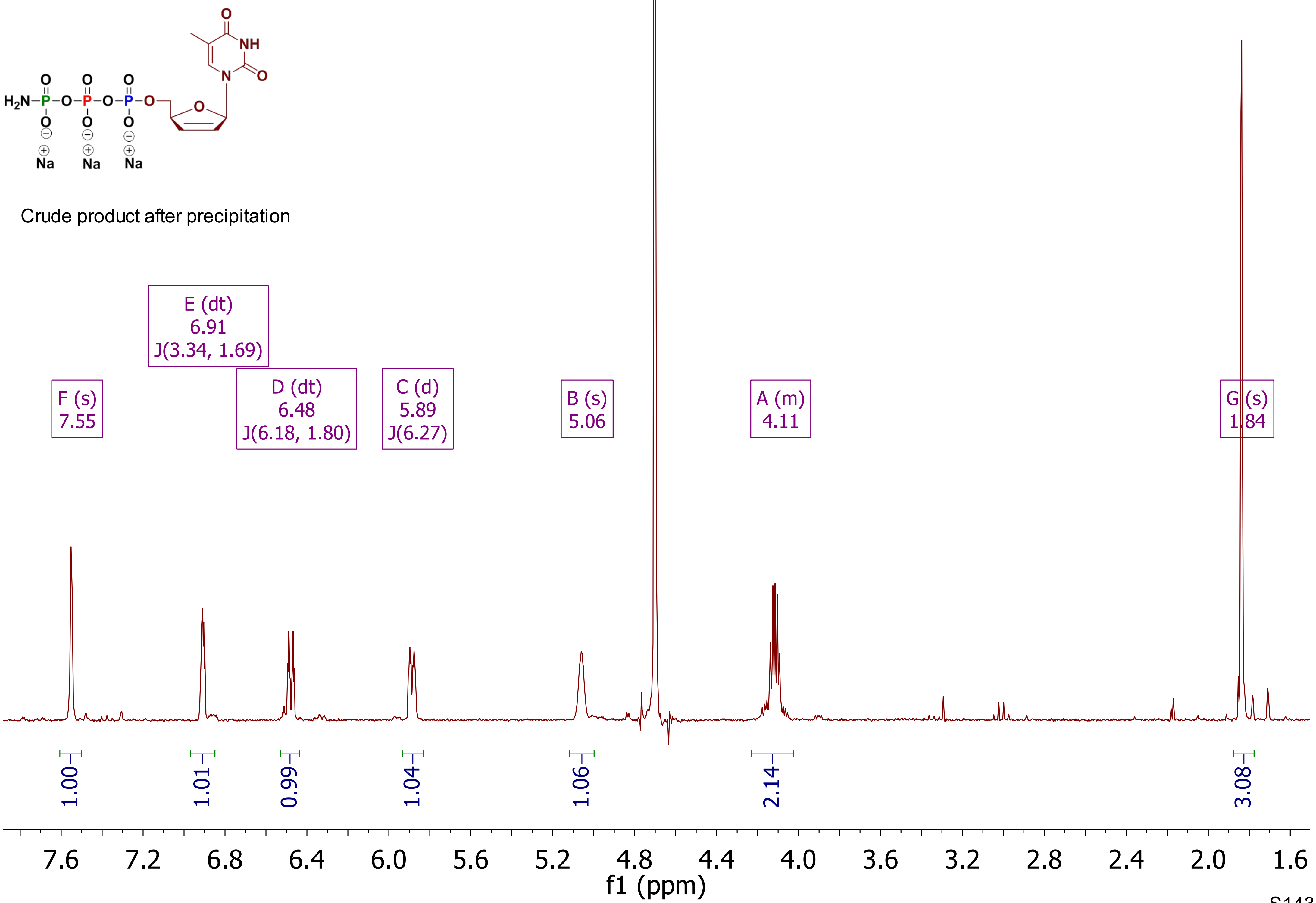
**$^{13}$C NMR**



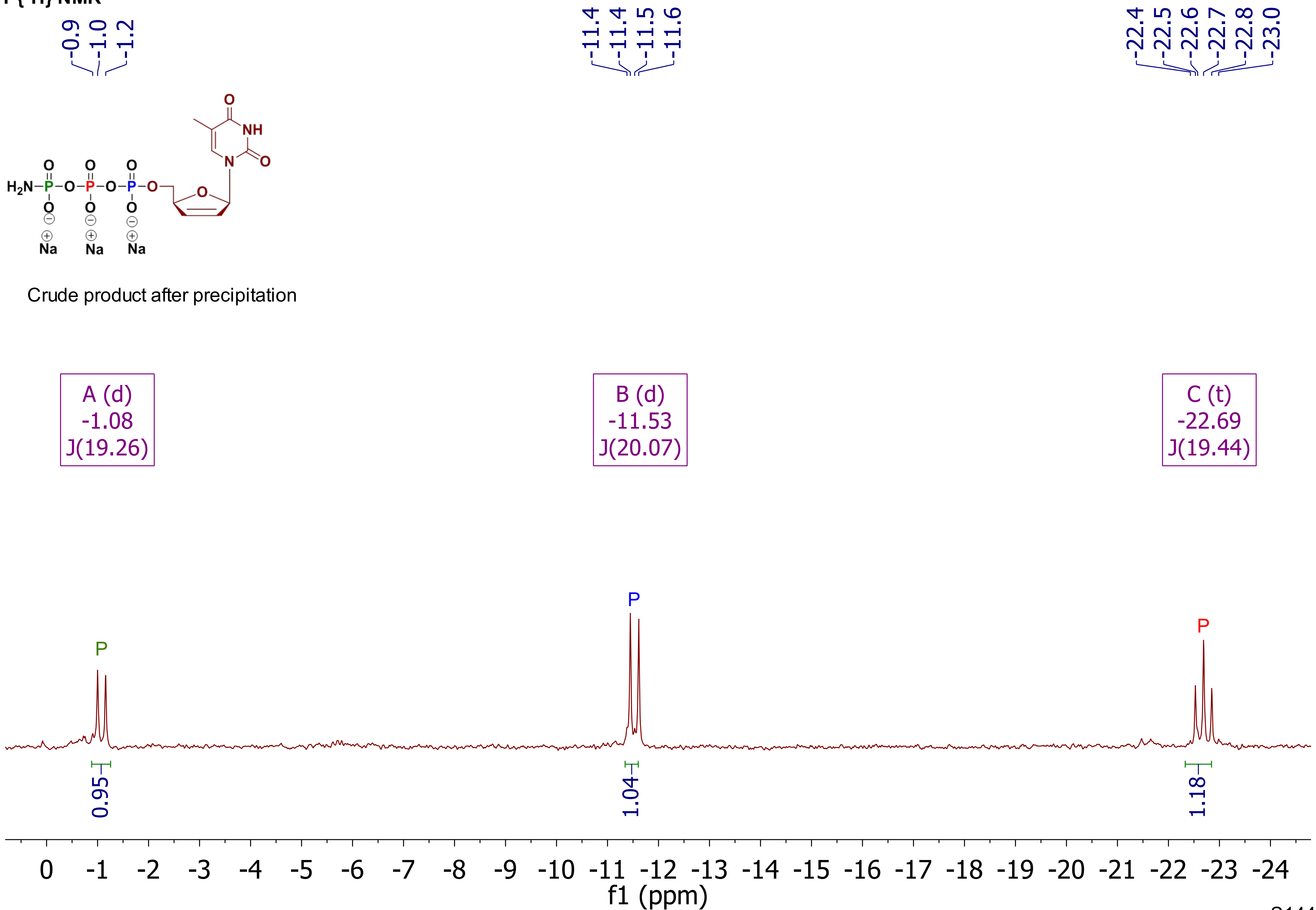

Crude product after precipitation



$^1$H NMR

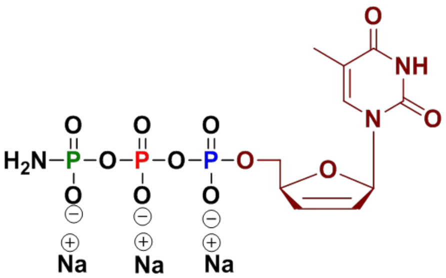

H2N
O
NH
Na
Na
Na
Crude product after precipitation
E (dt)
6.91
J(3.34, 1.69)
F (s)
7.55
D (dt)
6.48
J(6.18, 1.80)
C (d)
5.89
J(6.27)
B (s)
5.06
A (m)
4.11
G (s)
1.84
1.00
1.01
0.99
1.04
1.06
2.14
3.08
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
f1 (ppm)

$^{31}P\{^1H\}$ NMR

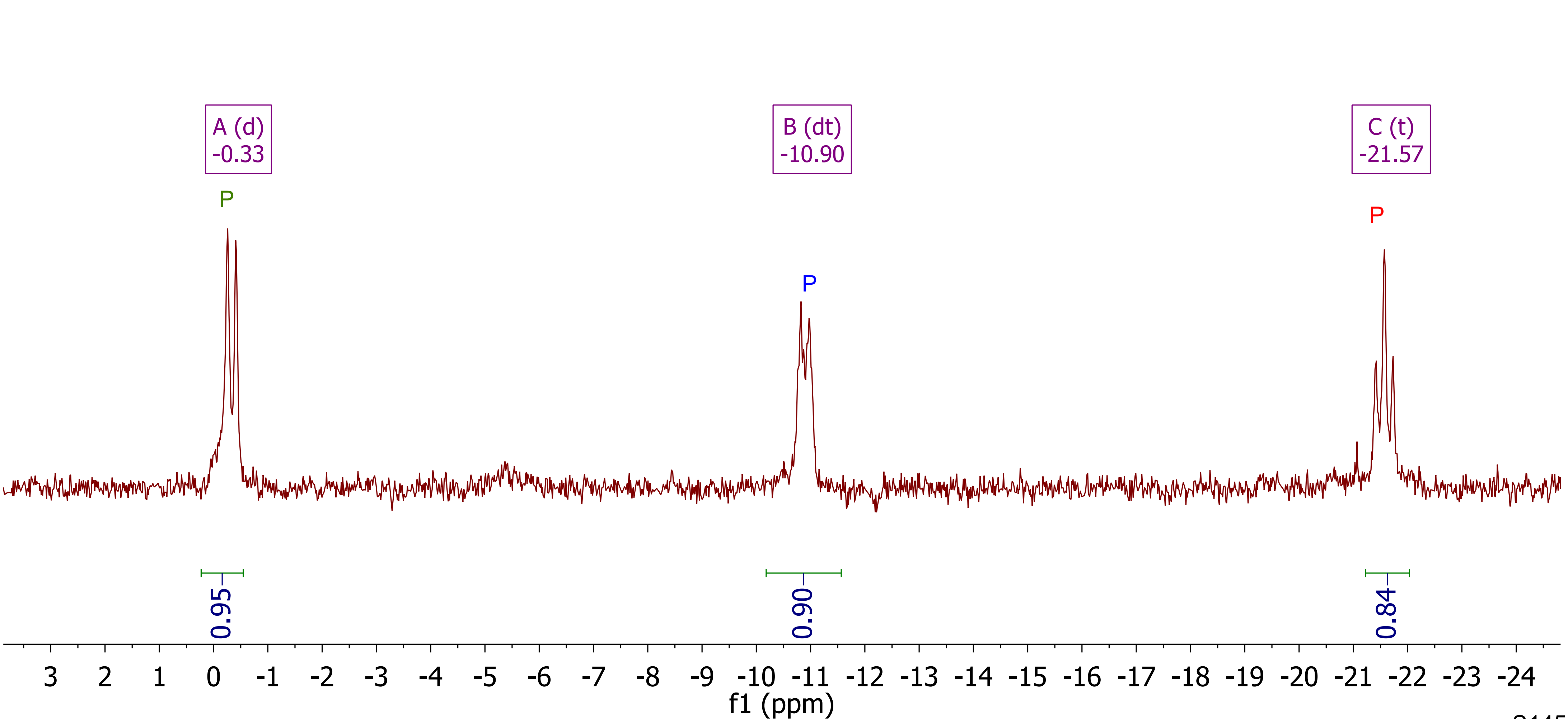



Crude product after precipitation

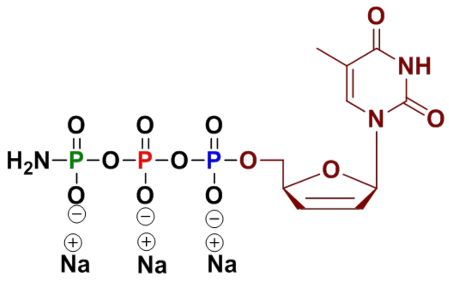
**$^{31}$P NMR**



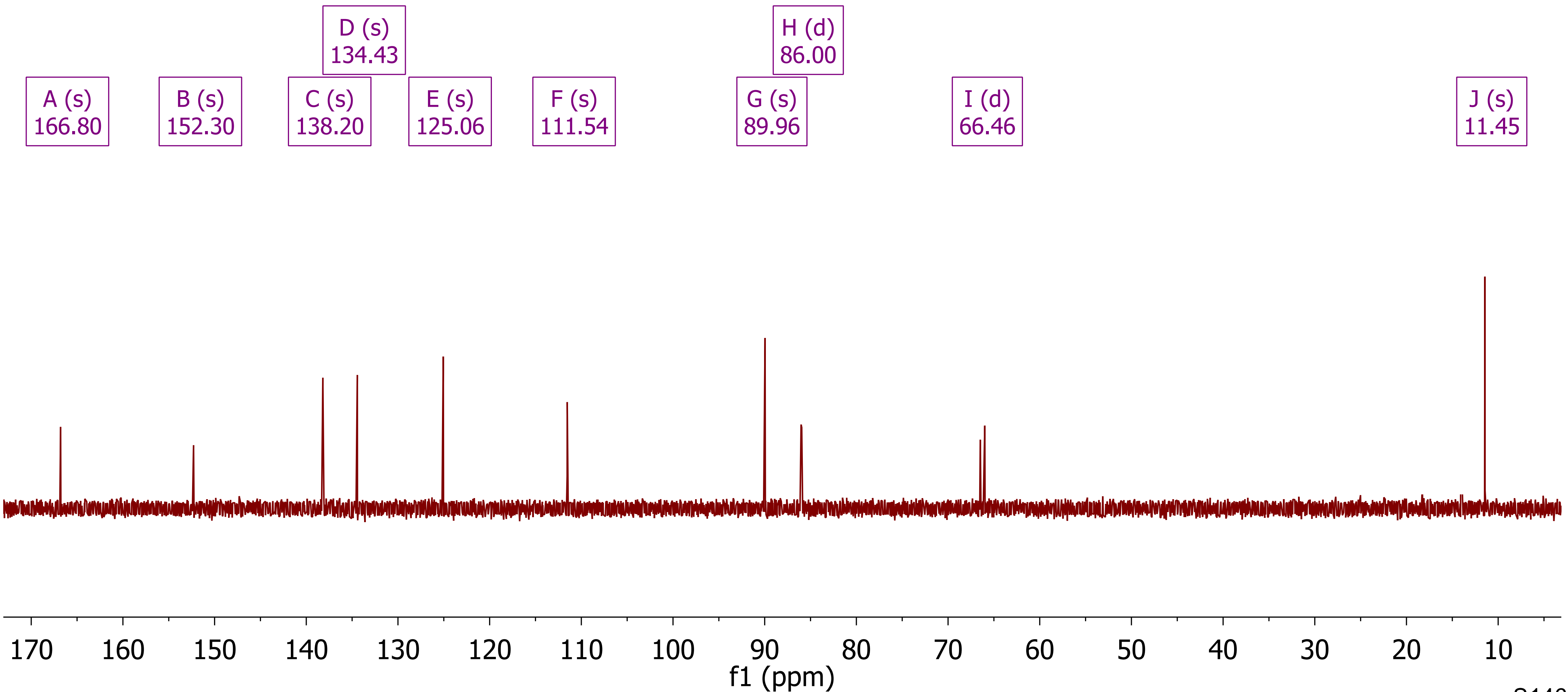

Crude product after precipitation



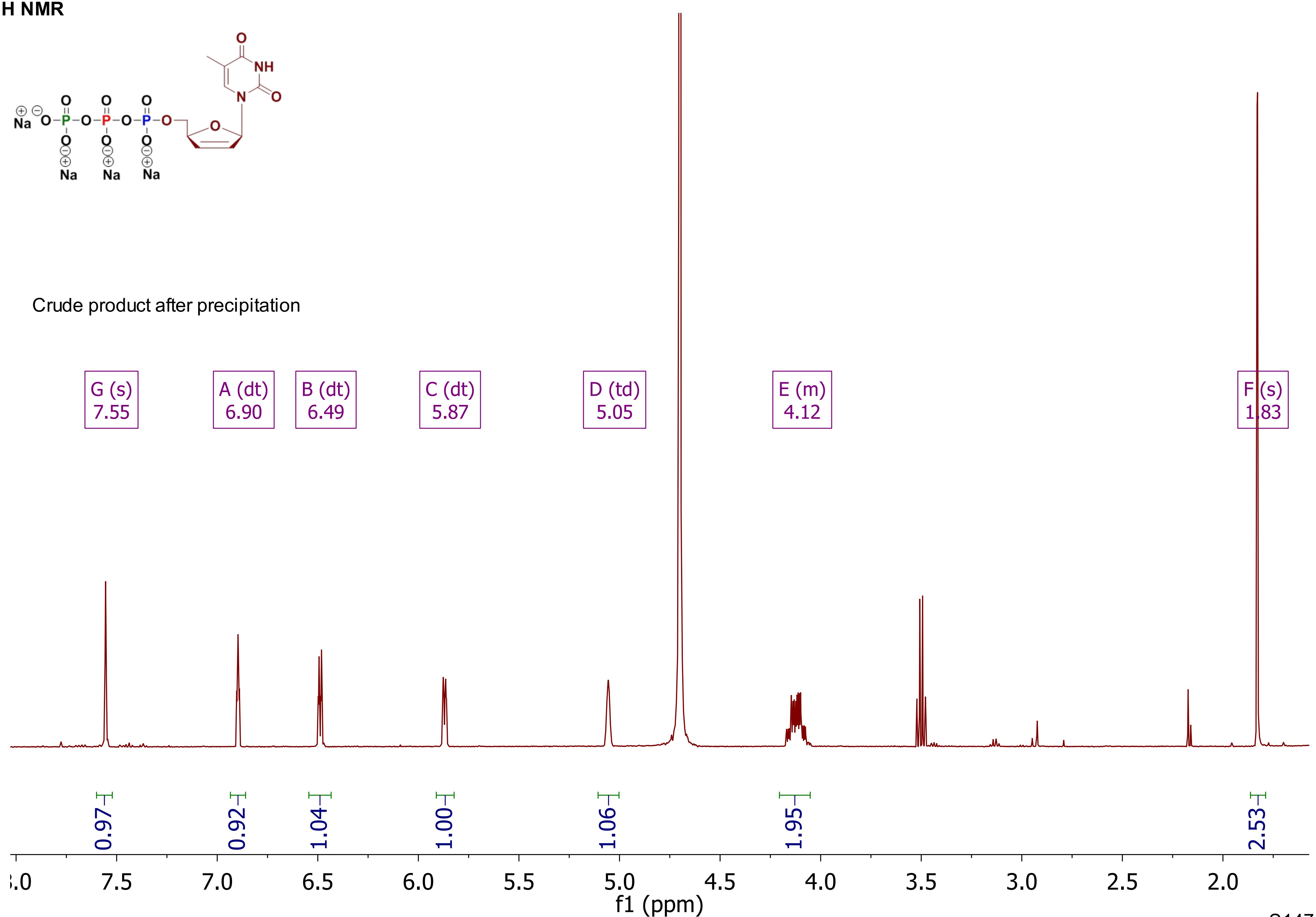
$^{13}$C NMR



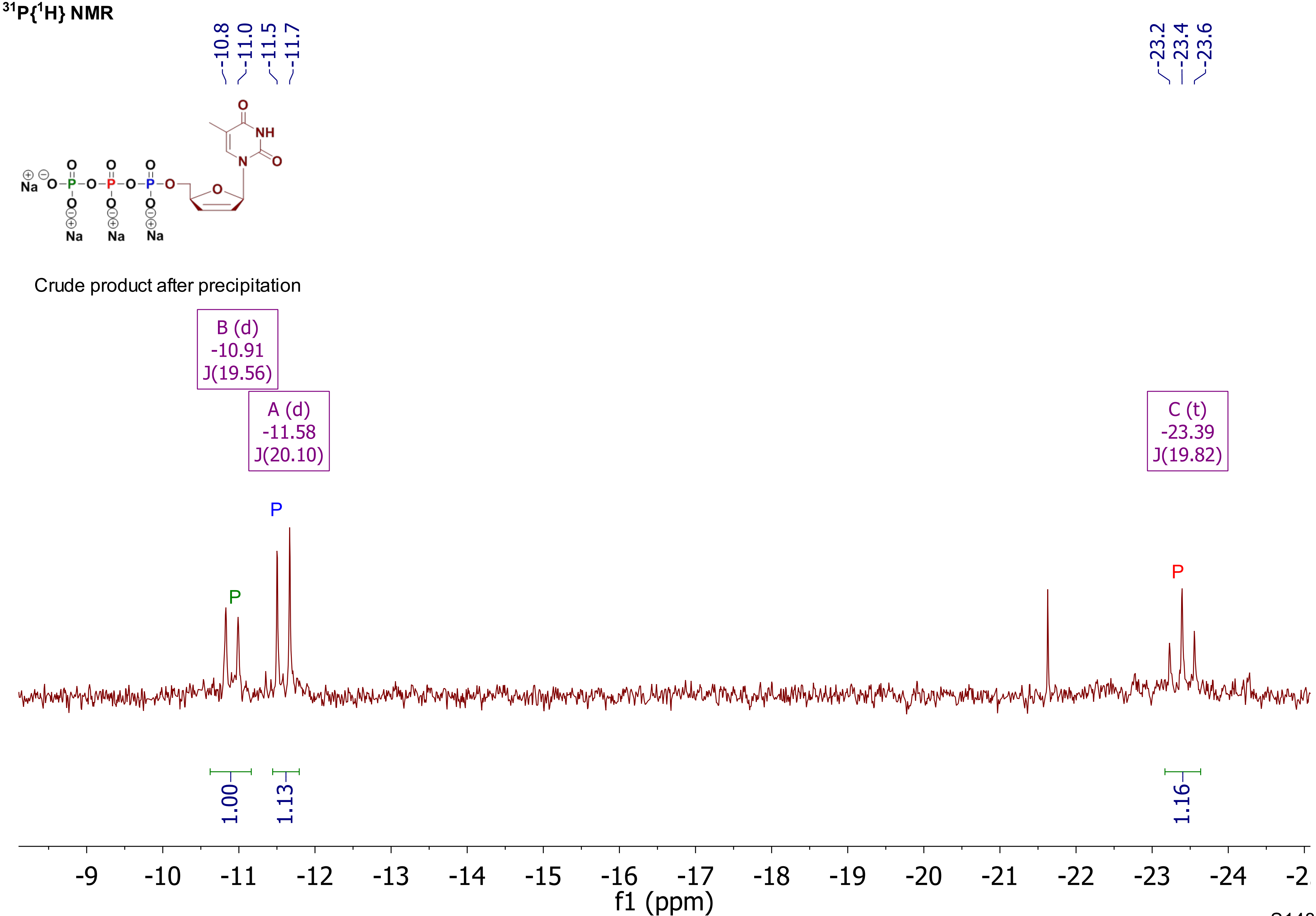
Crude product after precipitation



$^1$H NMR

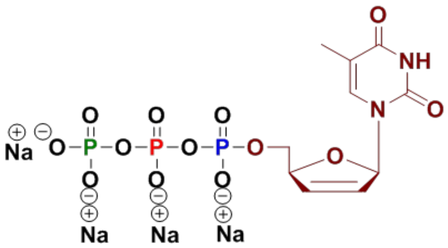


Crude product after precipitation
G (s) 7.55
A (dt) 6.90
B (dt) 6.49
C (dt) 5.87
D (td) 5.05
E (m) 4.12
F (s) 1.83
0.97
0.92
1.04
1.00
1.06
1.95
2.53
f1 (ppm)

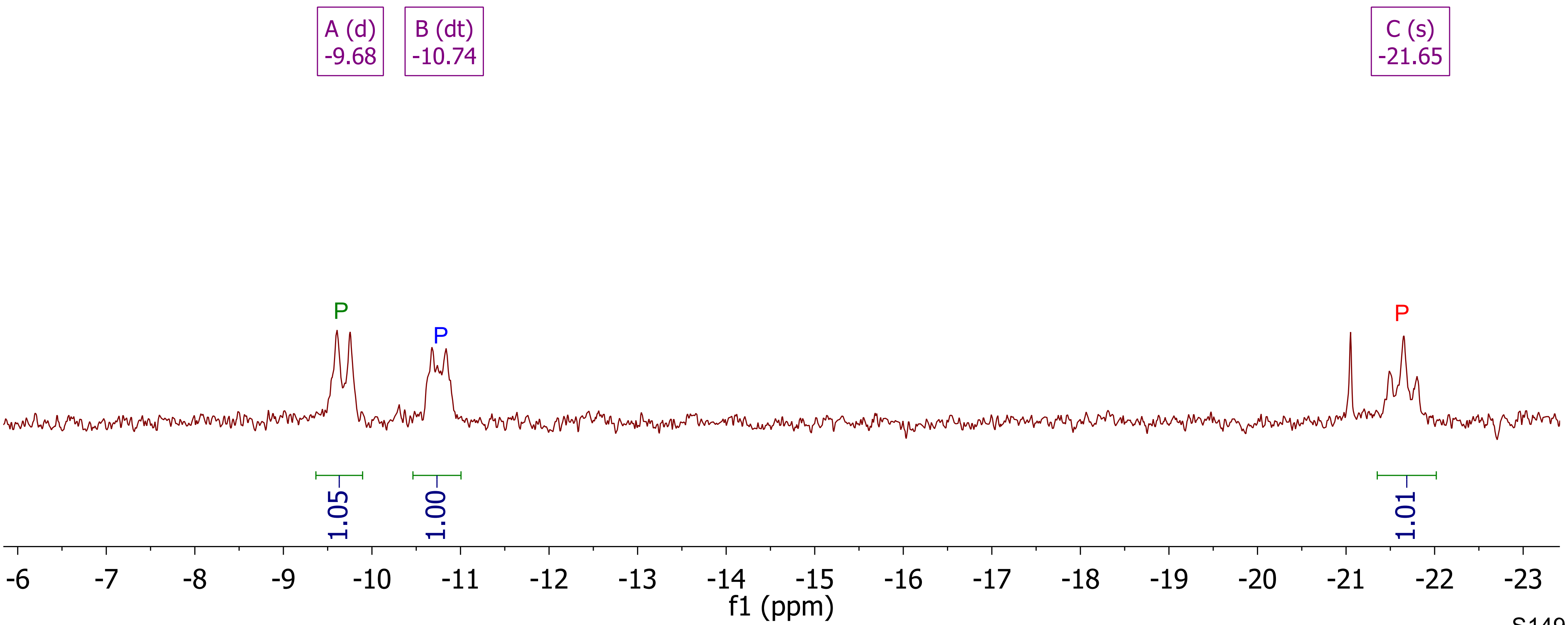


31P{1H} NMR
-10.8
-11.0
-11.5
-11.7
-23.2
-23.4
-23.6
O
NH
N
O
Na
O
P
Crude product after precipitation
B (d)
-10.91
J(19.56)
A (d)
-11.58
J(20.10)
C (t)
-23.39
J(19.82)
P
1.00
1.13
1.16
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
-19
-20
-21
-22
-23
-24
f1 (ppm)

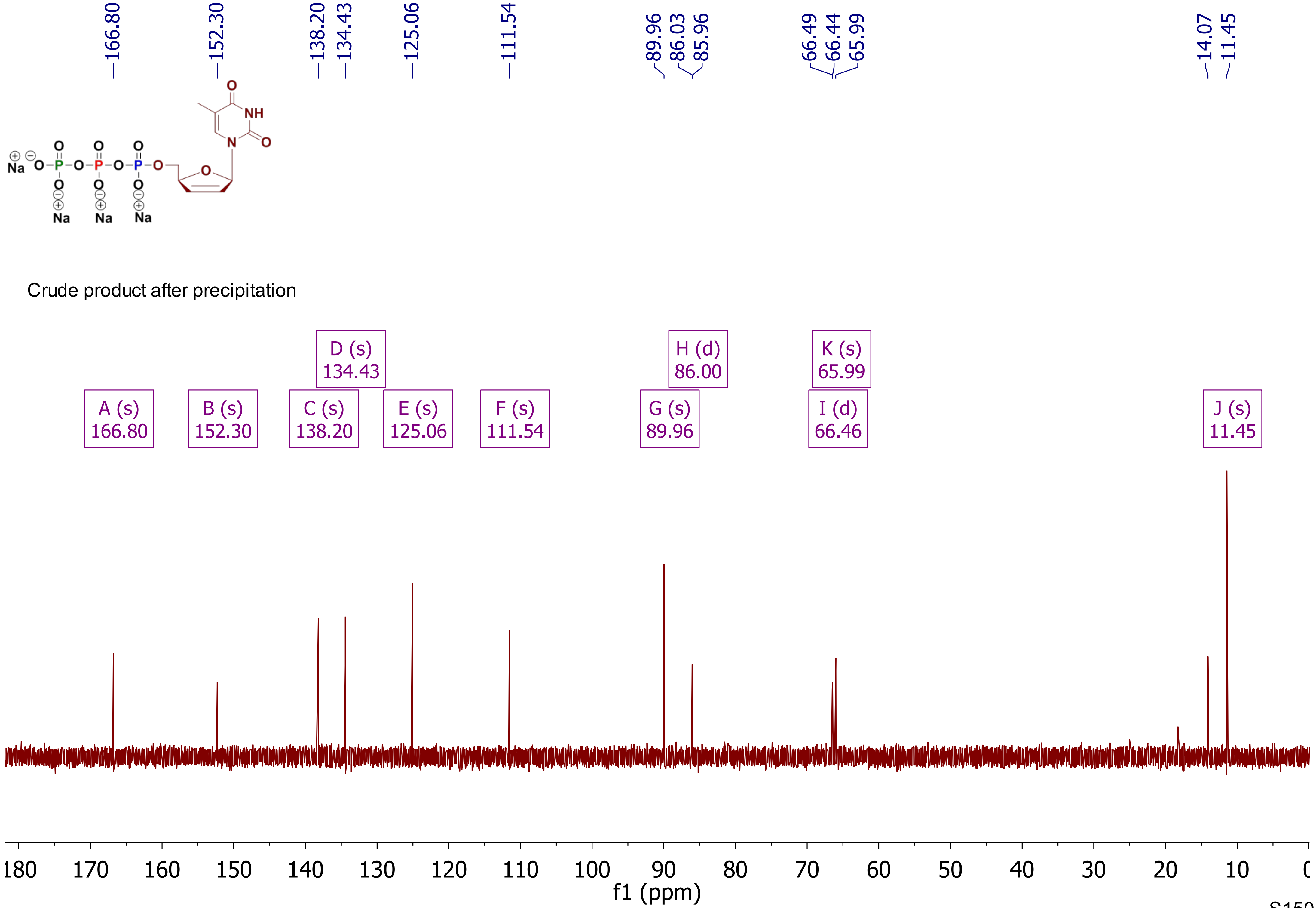
$^{31}$P NMR



Crude product after precipitation

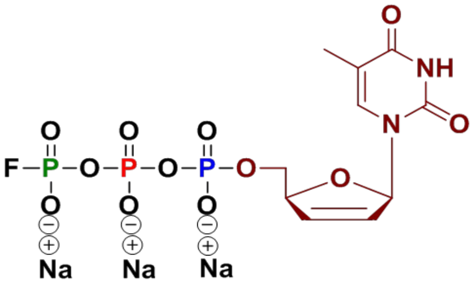


**$^{13}$C NMR**

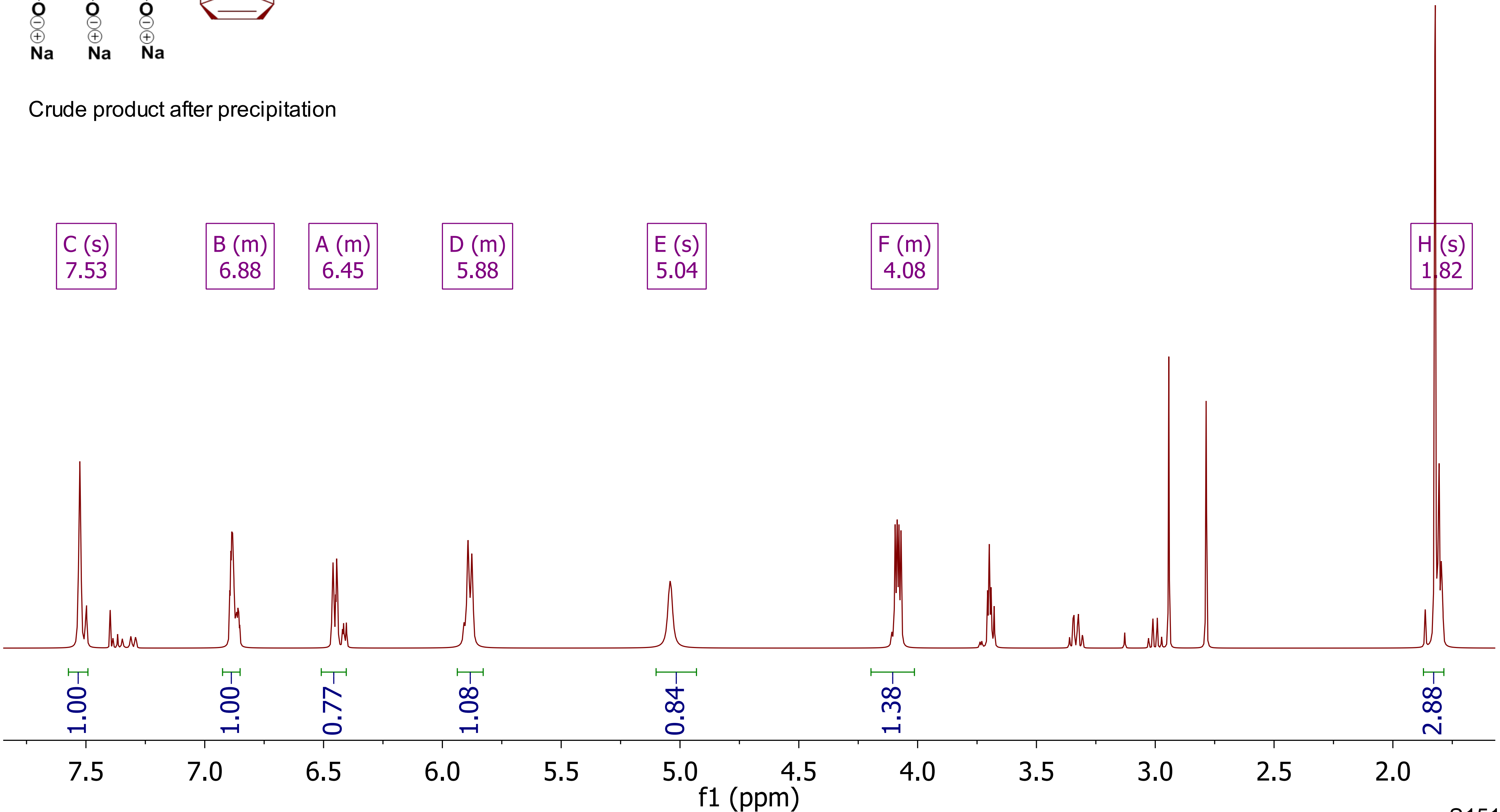


166.80
152.30
138.20
134.43
125.06
111.54
89.96
86.03
85.96
66.49
66.44
65.99
14.07
11.45
Na O P O P O P O O NH N O O
Crude product after precipitation
D (s) 134.43
H (d) 86.00
K (s) 65.99
A (s) 166.80
B (s) 152.30
C (s) 138.20
E (s) 125.06
F (s) 111.54
G (s) 89.96
I (d) 66.46
J (s) 11.45
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

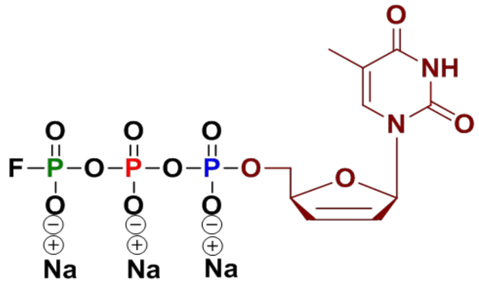
**$^1$H NMR**



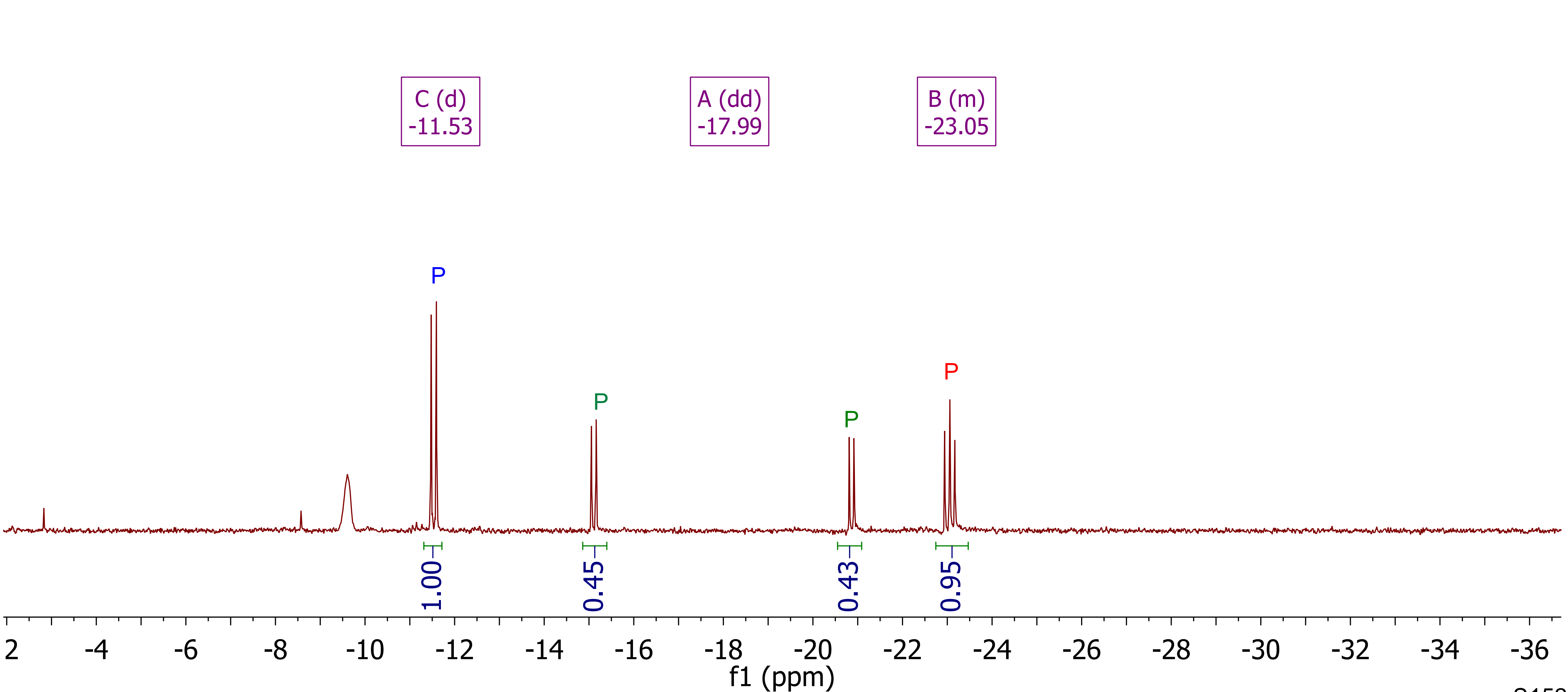
Crude product after precipitation



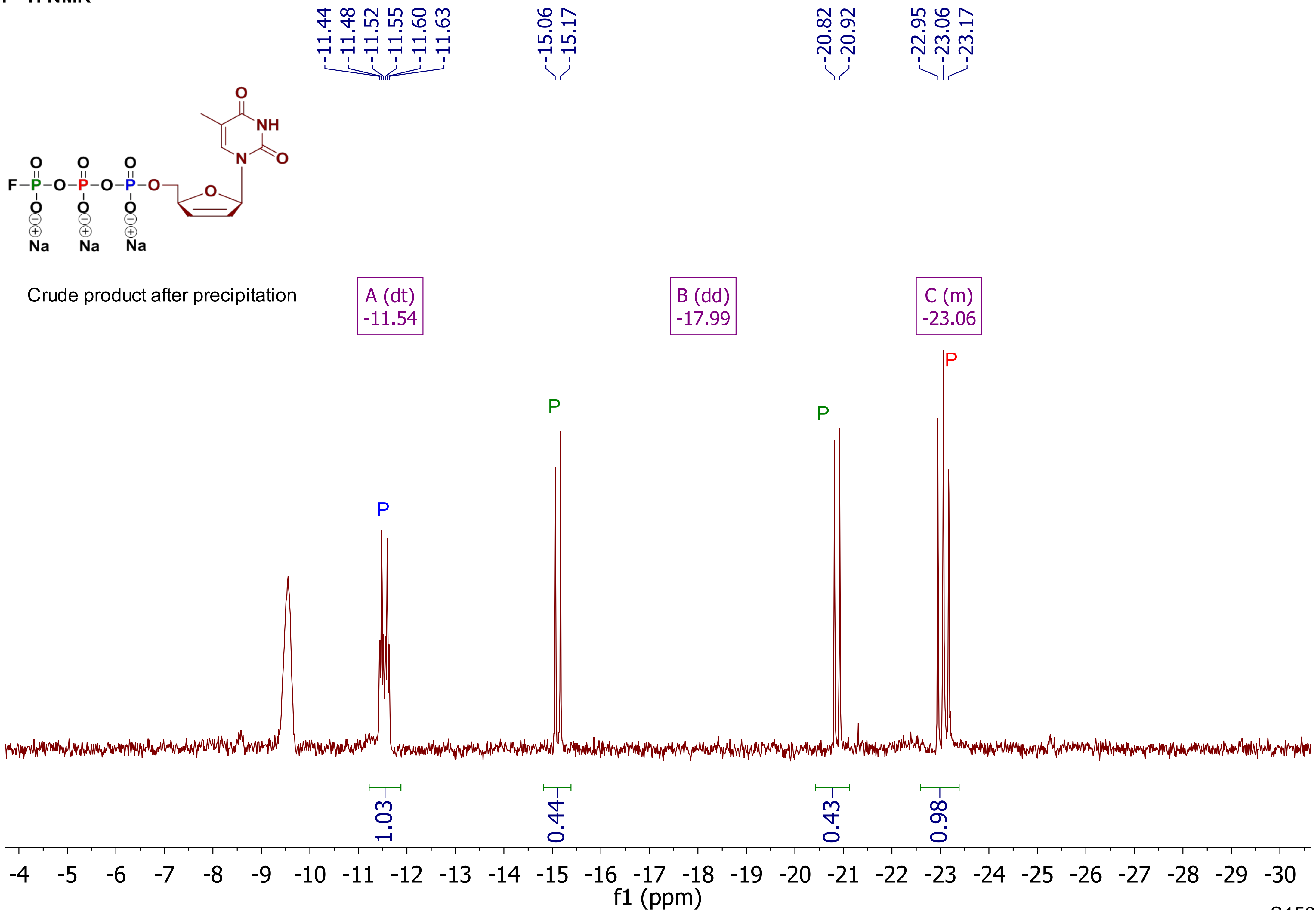
**$^{31}P\{^{1}H\}$-NMR**



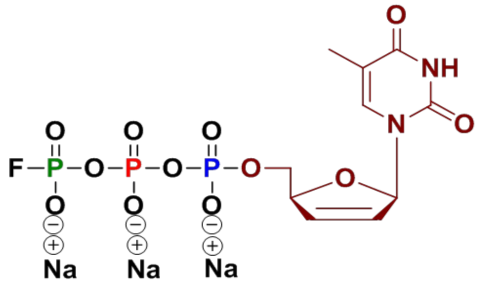

Crude product after precipitation



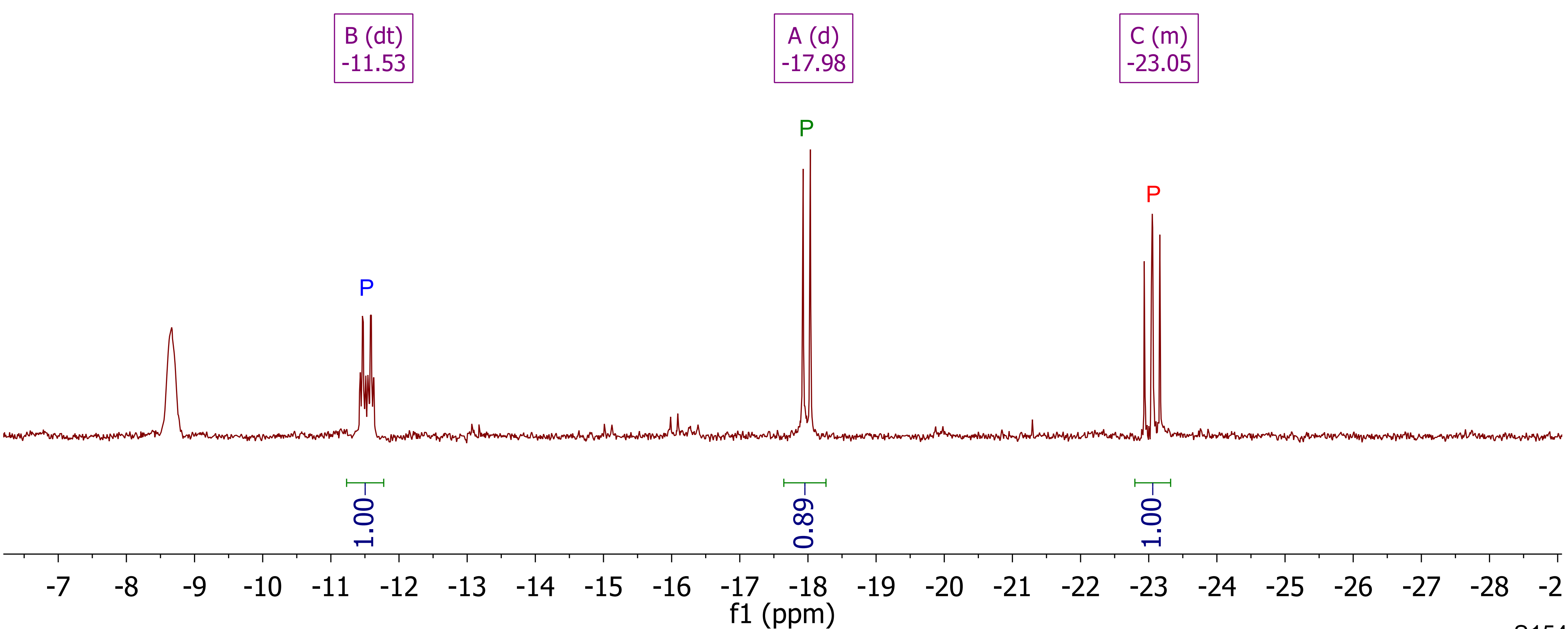
**$^{31}P$-$^{1}H$ NMR**


-11.44
-11.48
-11.52
-11.55
-11.60
-11.63
-15.06
-15.17
-20.82
-20.92
-22.95
-23.06
-23.17
F
P
O
Na
NH
N
Crude product after precipitation
A (dt)
-11.54
B (dd)
-17.99
C (m)
-23.06
P
P
P
P
1.03
0.44
0.43
0.98
-4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23 -24 -25 -26 -27 -28 -29 -30
f1 (ppm)

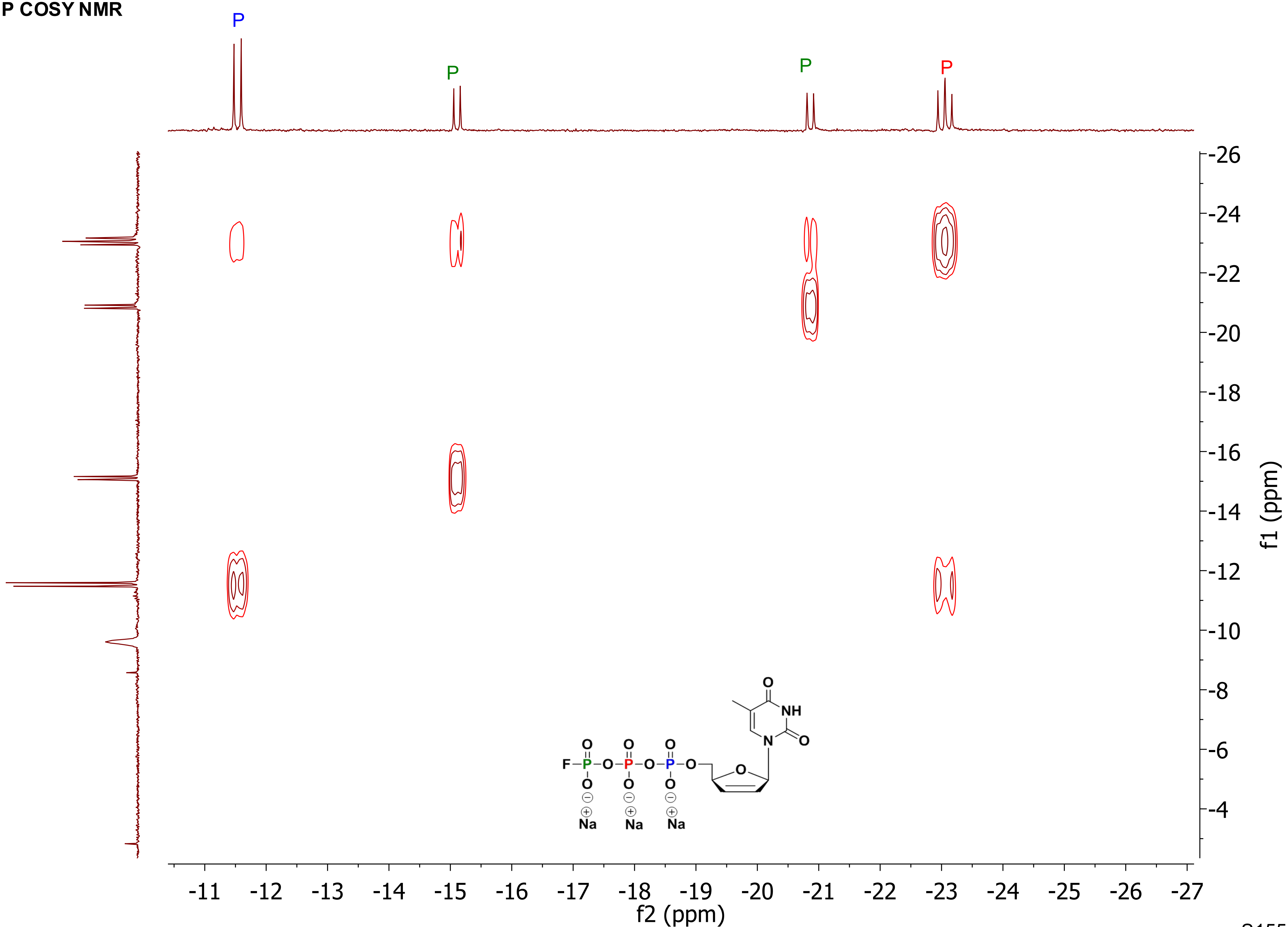
$^{31}P\{^{19}F\}$NMR



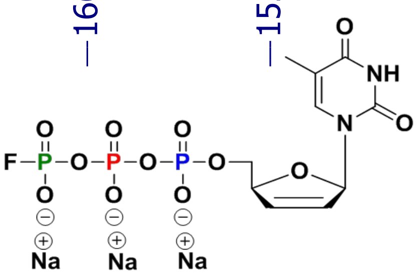
Crude product after precipitation



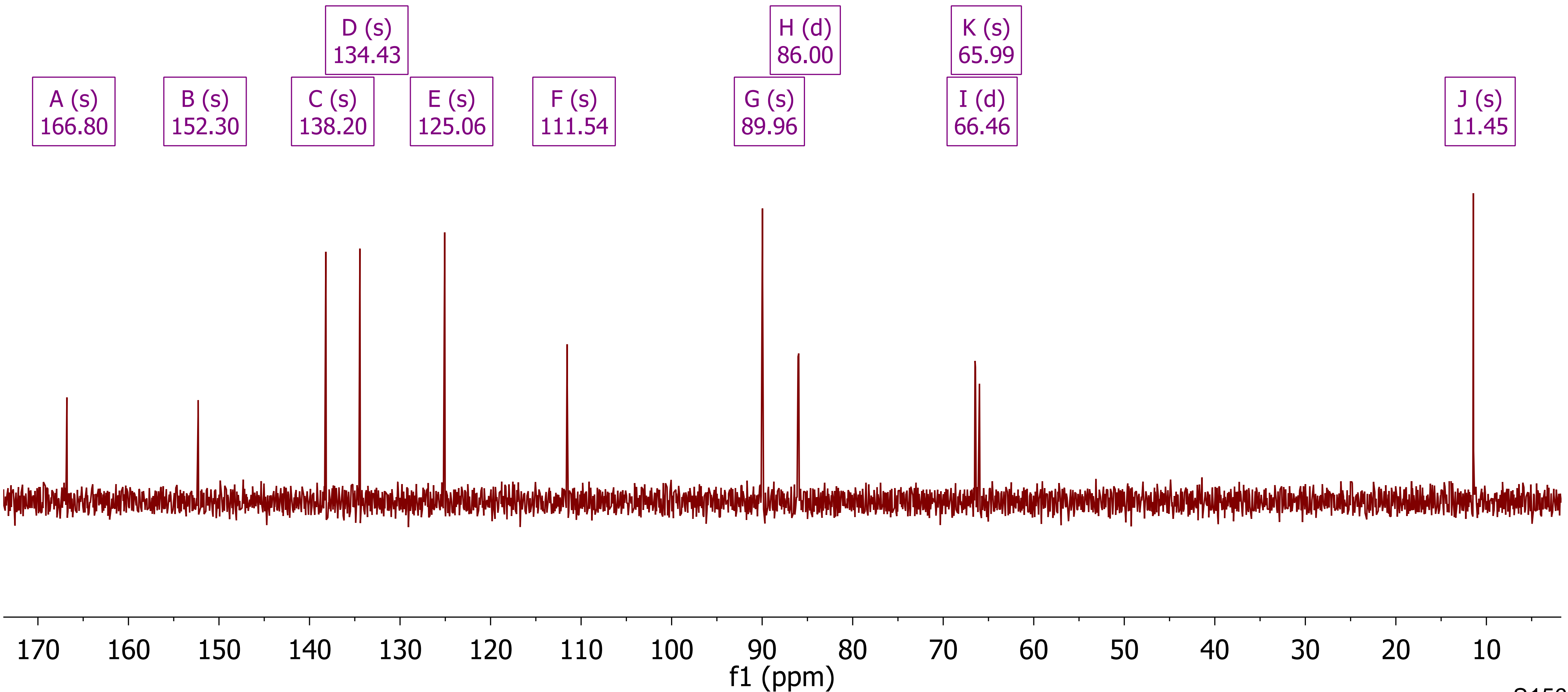
**$^{31}P$-$^{31}P$ COSY NMR**



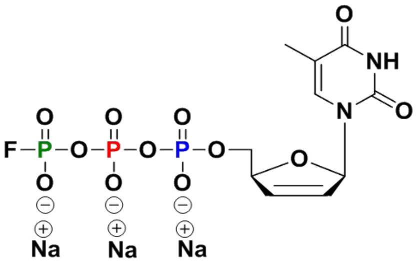
$^{13}C$ NMR

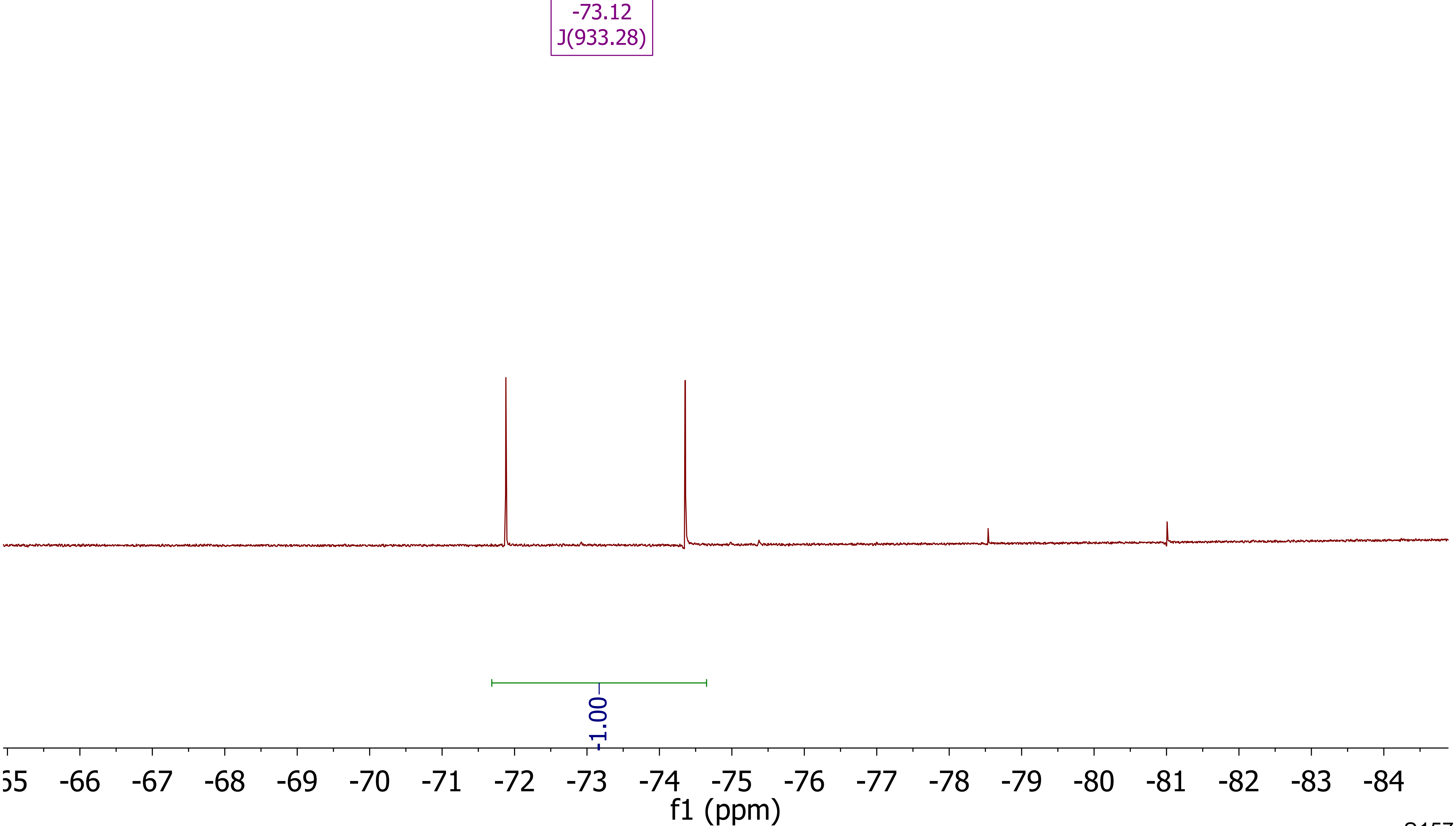


Crude product after precipitation



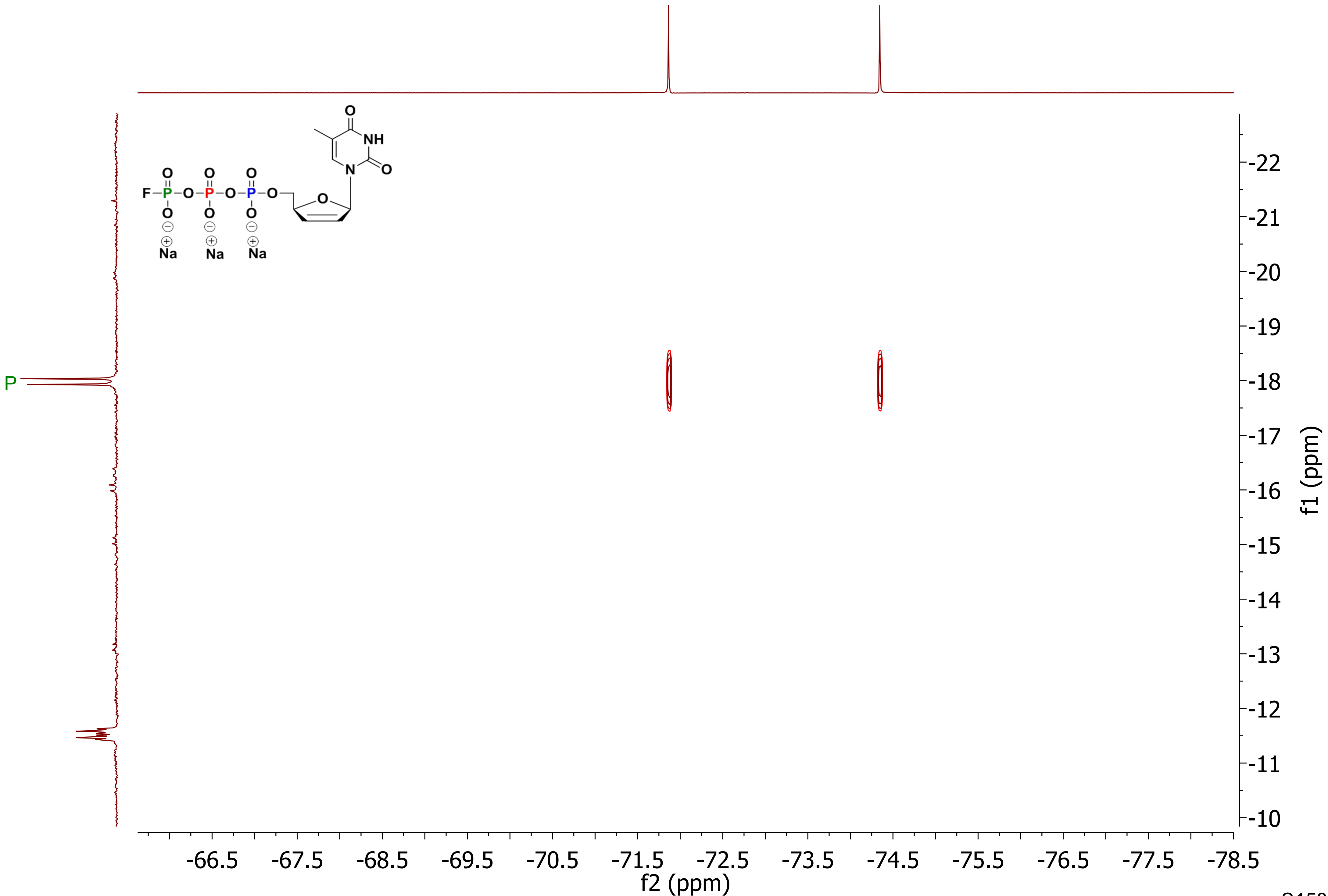
$^{19}$F NMR



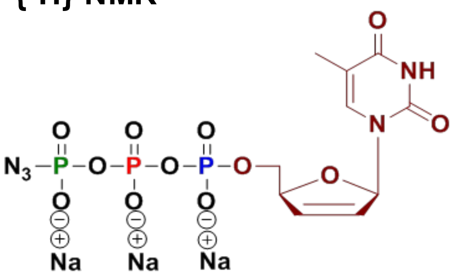


-71.9
-74.4
A (d)
-73.12
J(933.28)
1.00
65
-66
-67
-68
-69
-70
-71
-72
-73
-74
-75
-76
-77
-78
-79
-80
-81
-82
-83
-84
f1 (ppm)

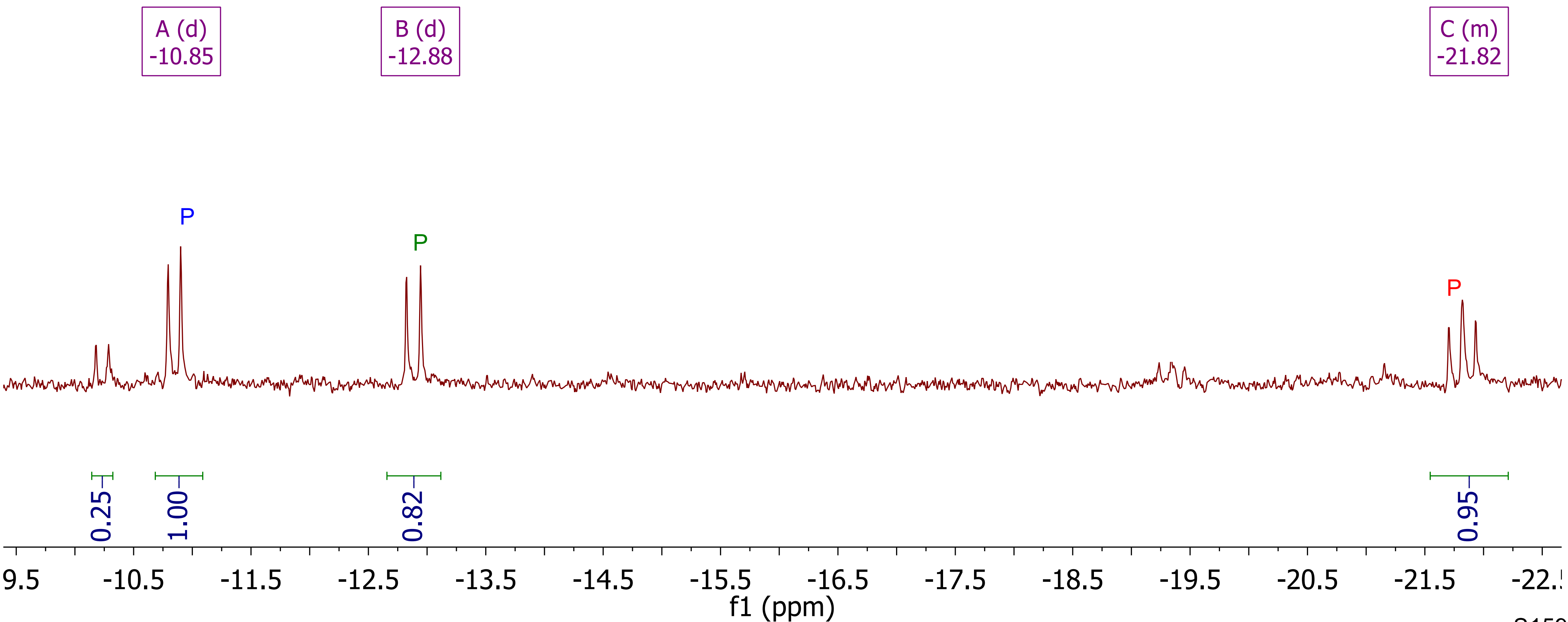
**$^{31}P$-$^{19}F$ HMBC NMR**



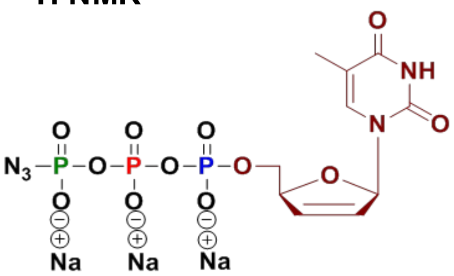
$^{31}P\{^{1}H\}$-NMR



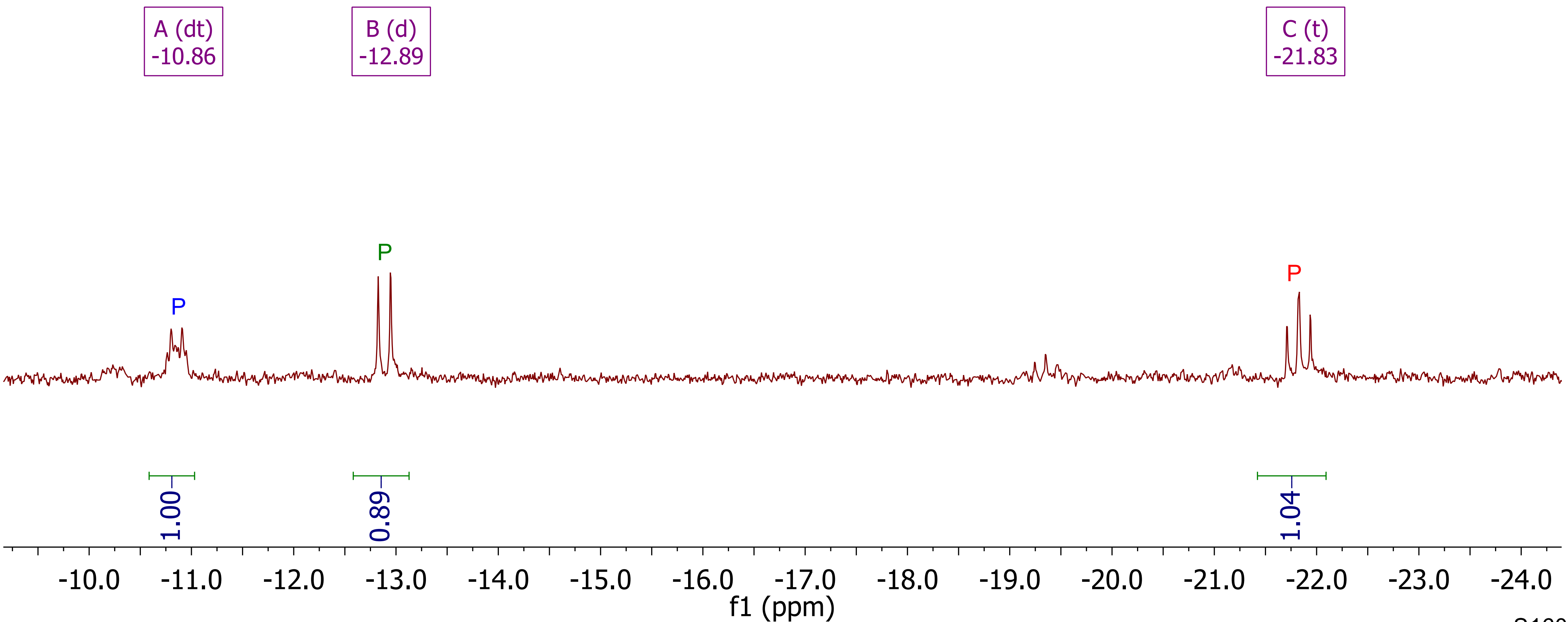
Crude product after precipitation



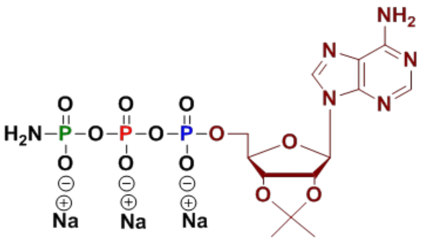
**$^{31}P$-$^{1}H$ NMR**



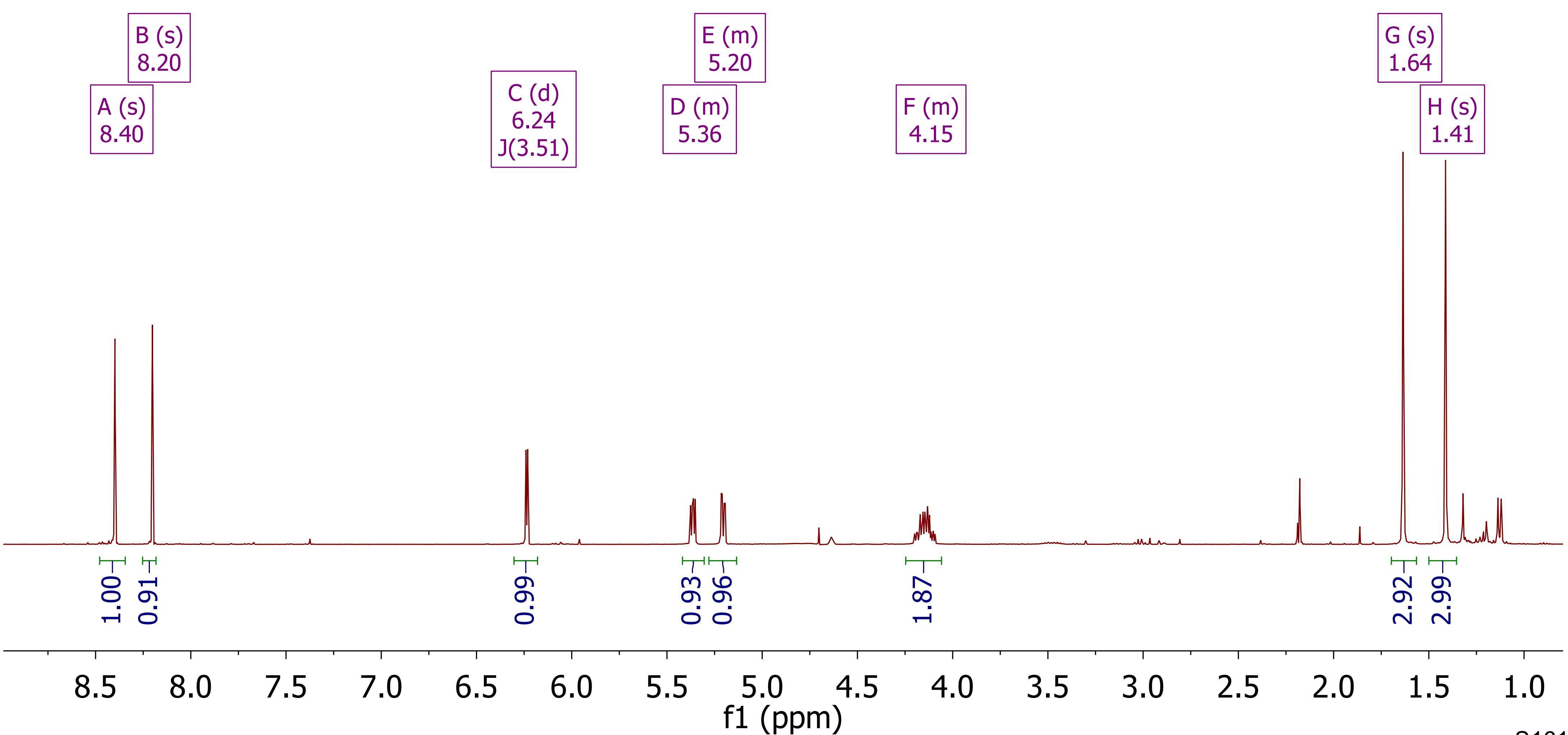
Crude product after precipitation



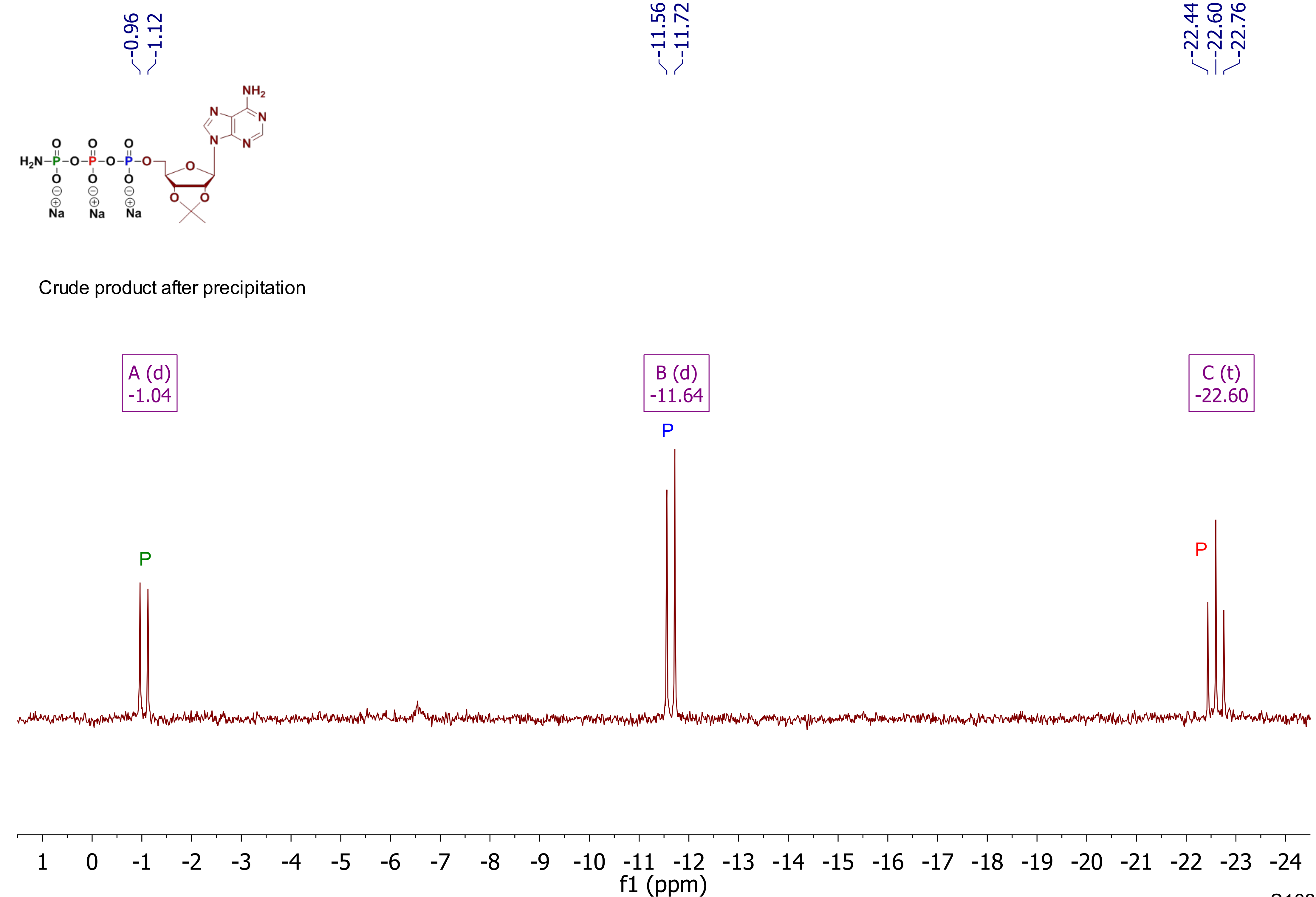
$^1H$ NMR



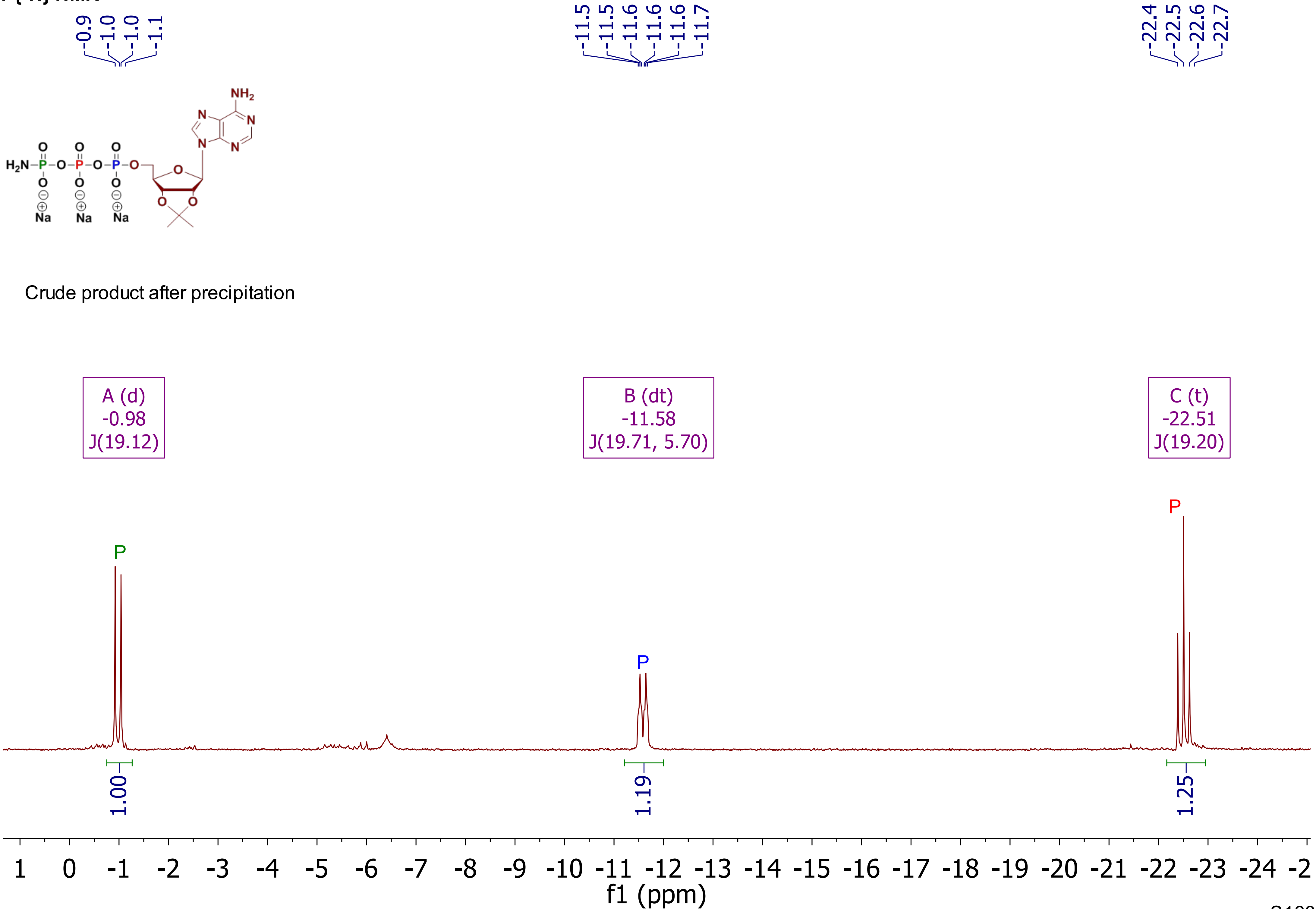
Crude product after precipitation



$^{31}$P NMR

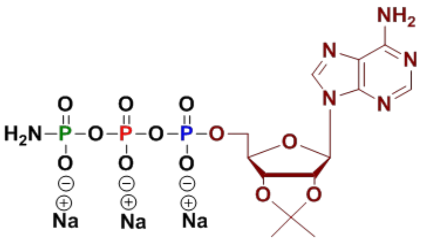

-0.96
-1.12
-11.56
-11.72
-22.44
-22.60
-22.76
Crude product after precipitation
A (d)
-1.04
B (d)
-11.64
C (t)
-22.60
P
P
P
1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23 -24
f1 (ppm)

$^{31}P\{^{1}H\}$ NMR

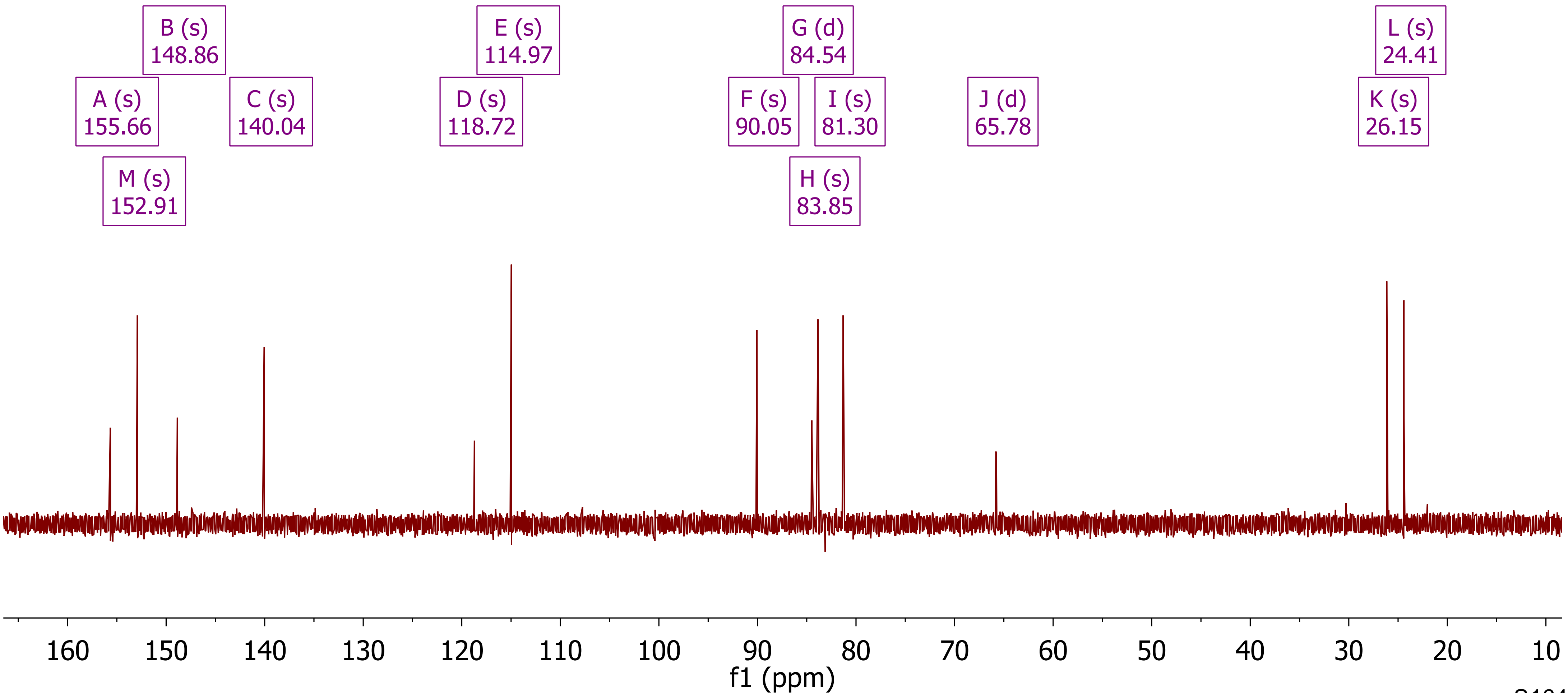


-0.9
-1.0
-1.0
-1.1
-11.5
-11.5
-11.6
-11.6
-11.6
-11.7
-22.4
-22.5
-22.6
-22.7
NH2
N
N
N
N
H2N
P
O
O
Na
Na
Na
Crude product after precipitation
A (d)
-0.98
J(19.12)
B (dt)
-11.58
J(19.71, 5.70)
C (t)
-22.51
J(19.20)
P
P
P
1.00
1.19
1.25
1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23 -24 -2
f1 (ppm)

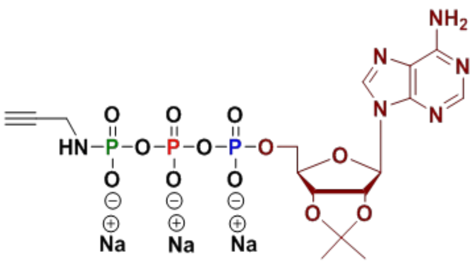
**$^{13}C$ NMR**

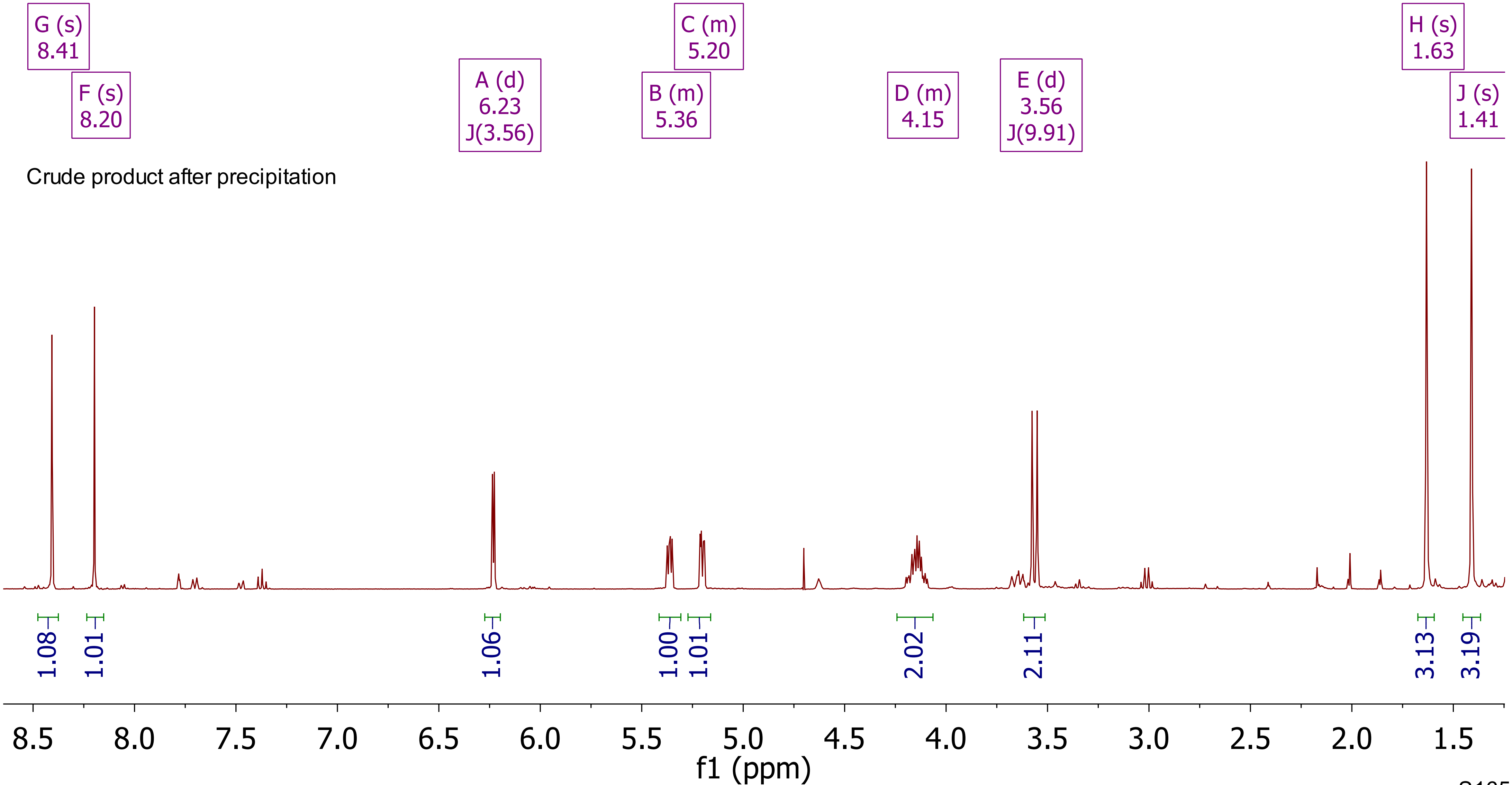



Crude product after precipitation

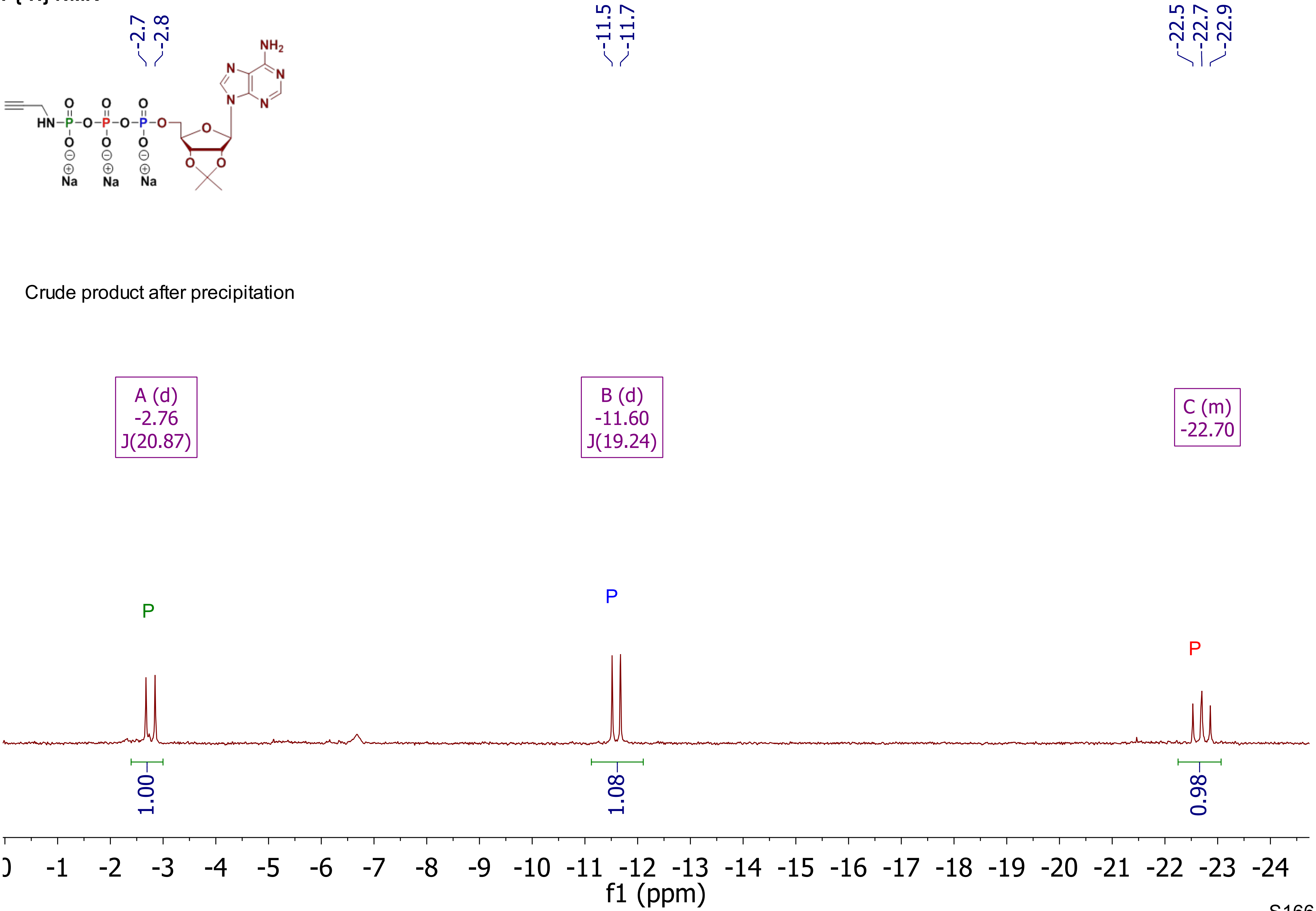


## $^1$H NMR

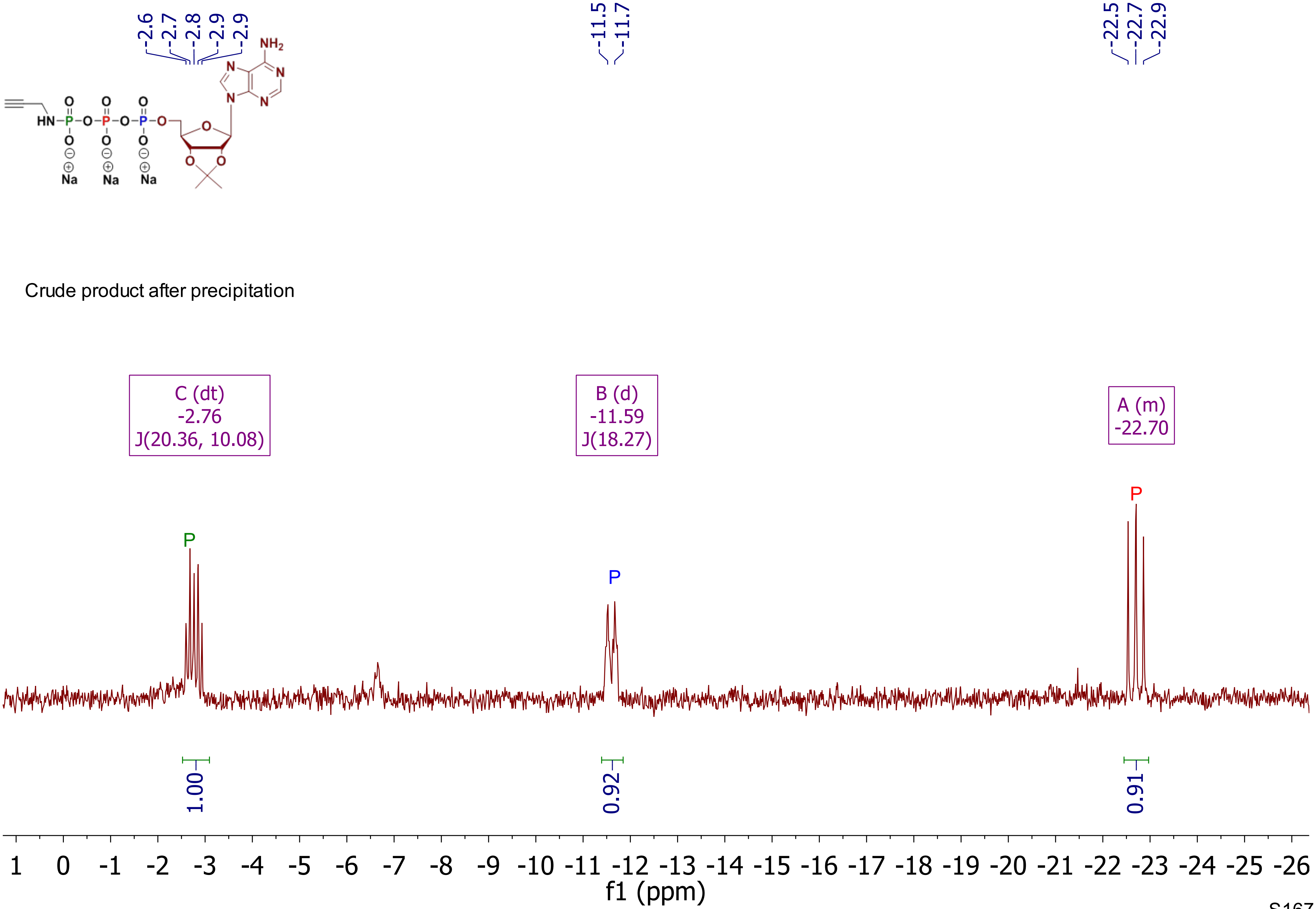



G (s) 8.41
F (s) 8.20
A (d) 6.23 J(3.56)
B (m) 5.36
C (m) 5.20
D (m) 4.15
E (d) 3.56 J(9.91)
H (s) 1.63
J (s) 1.41
Crude product after precipitation
1.08
1.01
1.06
1.00
1.01
2.02
2.11
3.13
3.19
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

$^{31}P\{^1H\}$ NMR

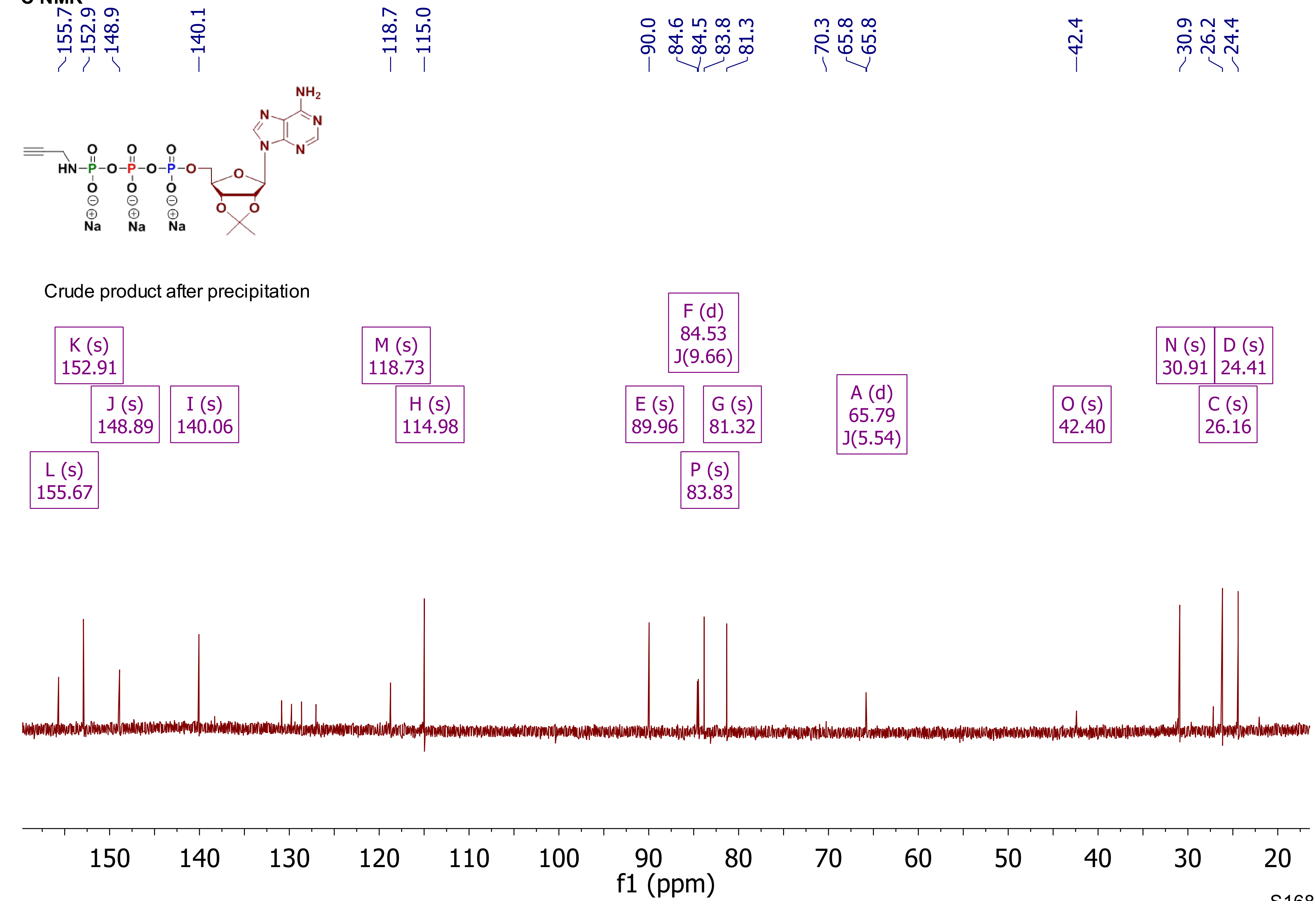

-2.7
-2.8
-11.5
-11.7
-22.5
-22.7
-22.9
NH2
N
HN
O
P
Na
Crude product after precipitation
A (d)
-2.76
J(20.87)
B (d)
-11.60
J(19.24)
C (m)
-22.70
P
P
P
1.00
1.08
0.98
0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23 -24
f1 (ppm)

$^{31}$P NMR



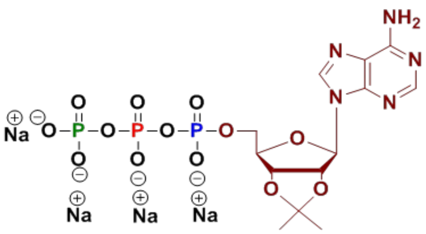
-2.6
-2.7
-2.8
-2.9
-2.9
-11.5
-11.7
-22.5
-22.7
-22.9
NH2
N
HN
O
Na
Crude product after precipitation
C (dt)
-2.76
J(20.36, 10.08)
B (d)
-11.59
J(18.27)
A (m)
-22.70
P
1.00
0.92
0.91
1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23 -24 -25 -26
f1 (ppm)

**$^{13}$C NMR**

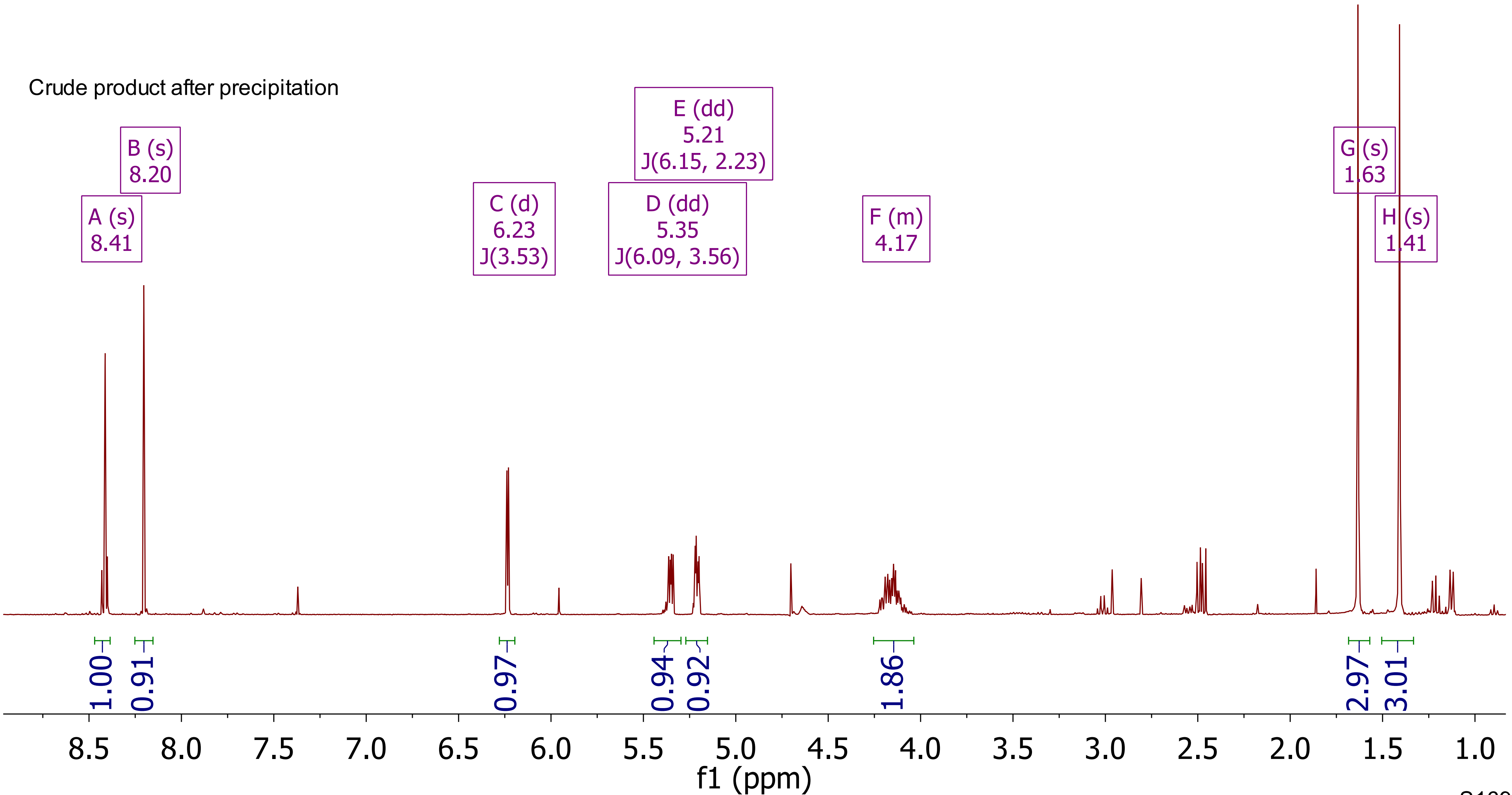


155.7
152.9
148.9
140.1
118.7
115.0
90.0
84.6
84.5
83.8
81.3
70.3
65.8
65.8
42.4
30.9
26.2
24.4
NH2
N
HN
O
P
Na
Crude product after precipitation
K (s) 152.91
M (s) 118.73
F (d) 84.53 J(9.66)
N (s) 30.91
D (s) 24.41
J (s) 148.89
I (s) 140.06
H (s) 114.98
E (s) 89.96
G (s) 81.32
A (d) 65.79 J(5.54)
O (s) 42.40
C (s) 26.16
L (s) 155.67
P (s) 83.83
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

$^1$H NMR

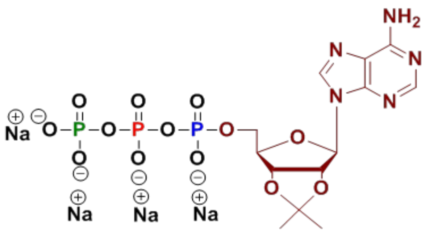


NH2
N
N
N
N
O
O
O
O
O
O
Na
O
P
O
P
O
P
O
Na
Na
Na

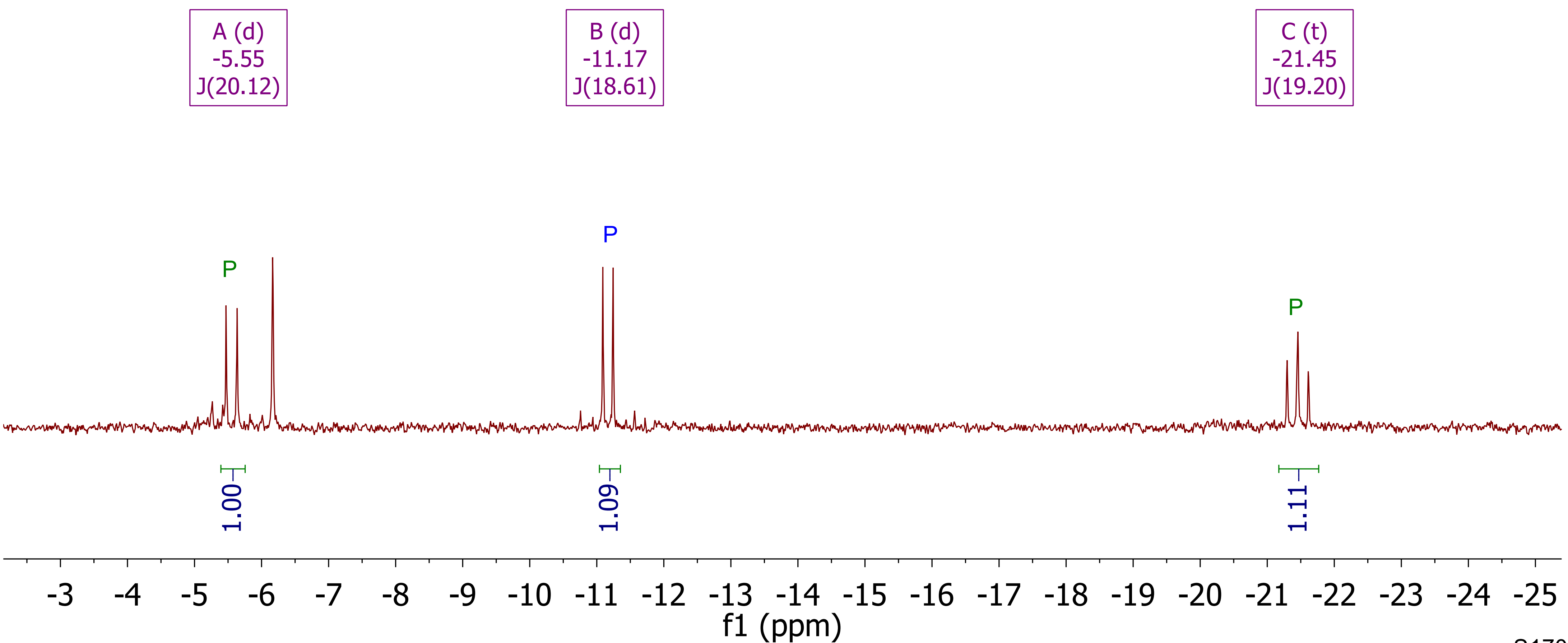



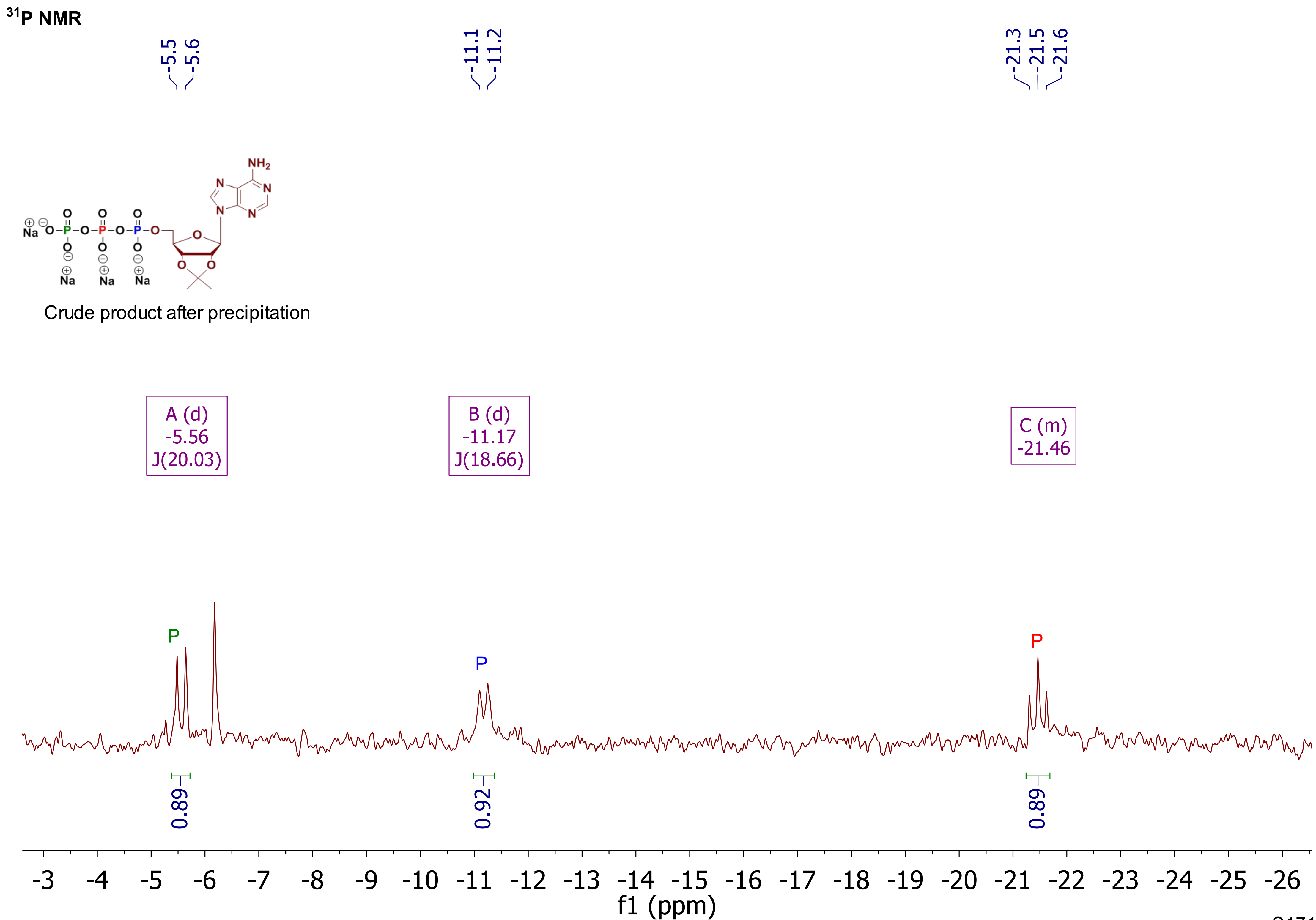
Crude product after precipitation
A (s)
8.41
B (s)
8.20
C (d)
6.23
J(3.53)
D (dd)
5.35
J(6.09, 3.56)
E (dd)
5.21
J(6.15, 2.23)
F (m)
4.17
G (s)
1.63
H (s)
1.41
1.00
0.91
0.97
0.94
0.92
1.86
2.97
3.01
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

$^{31}P\{^1H\}$ NMR



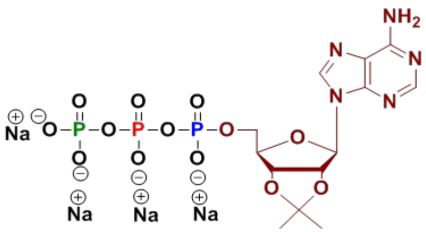
Crude product after precipitation



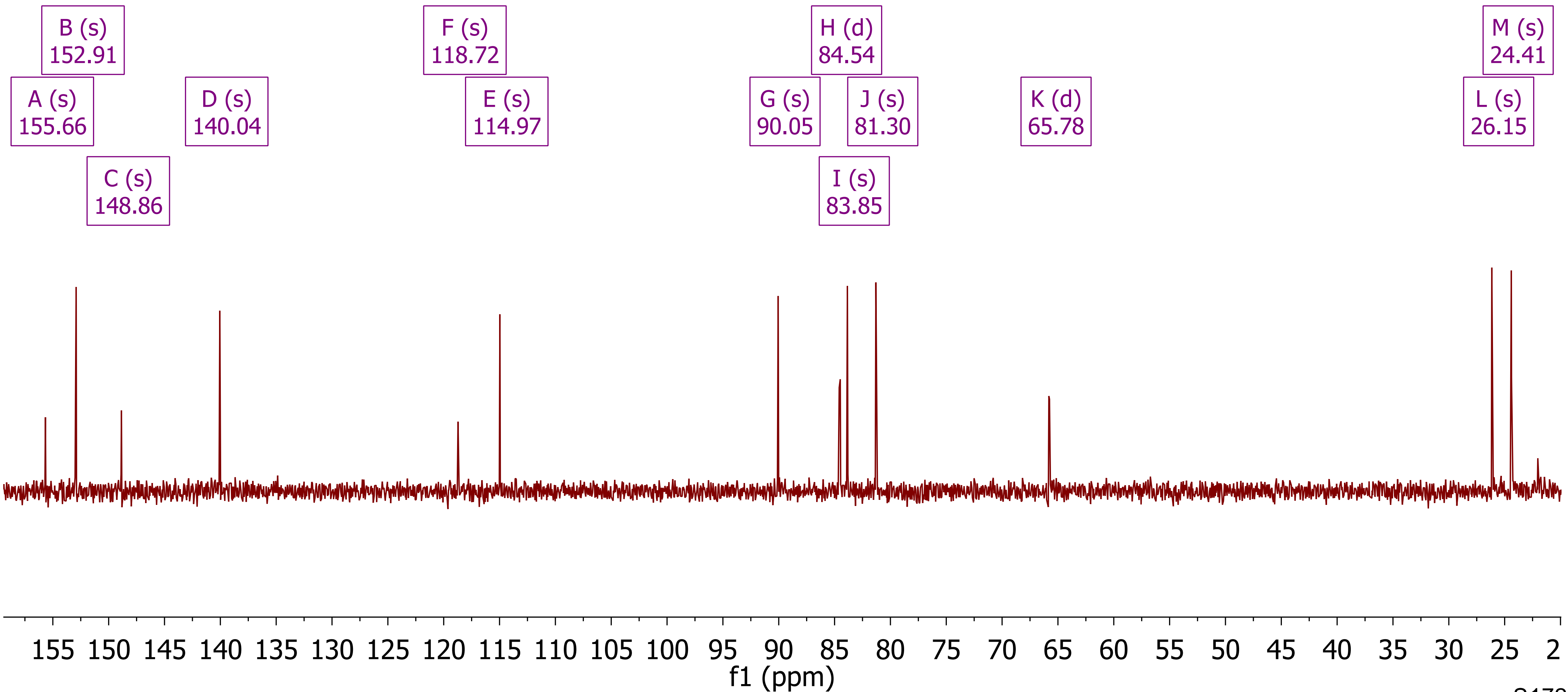


$^{31}$P NMR
-5.5
-5.6
-11.1
-11.2
-21.3
-21.5
-21.6
Crude product after precipitation
A (d) -5.56 J(20.03)
B (d) -11.17 J(18.66)
C (m) -21.46
P
P
P
0.89
0.92
0.89
-3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23 -24 -25 -26
f1 (ppm)

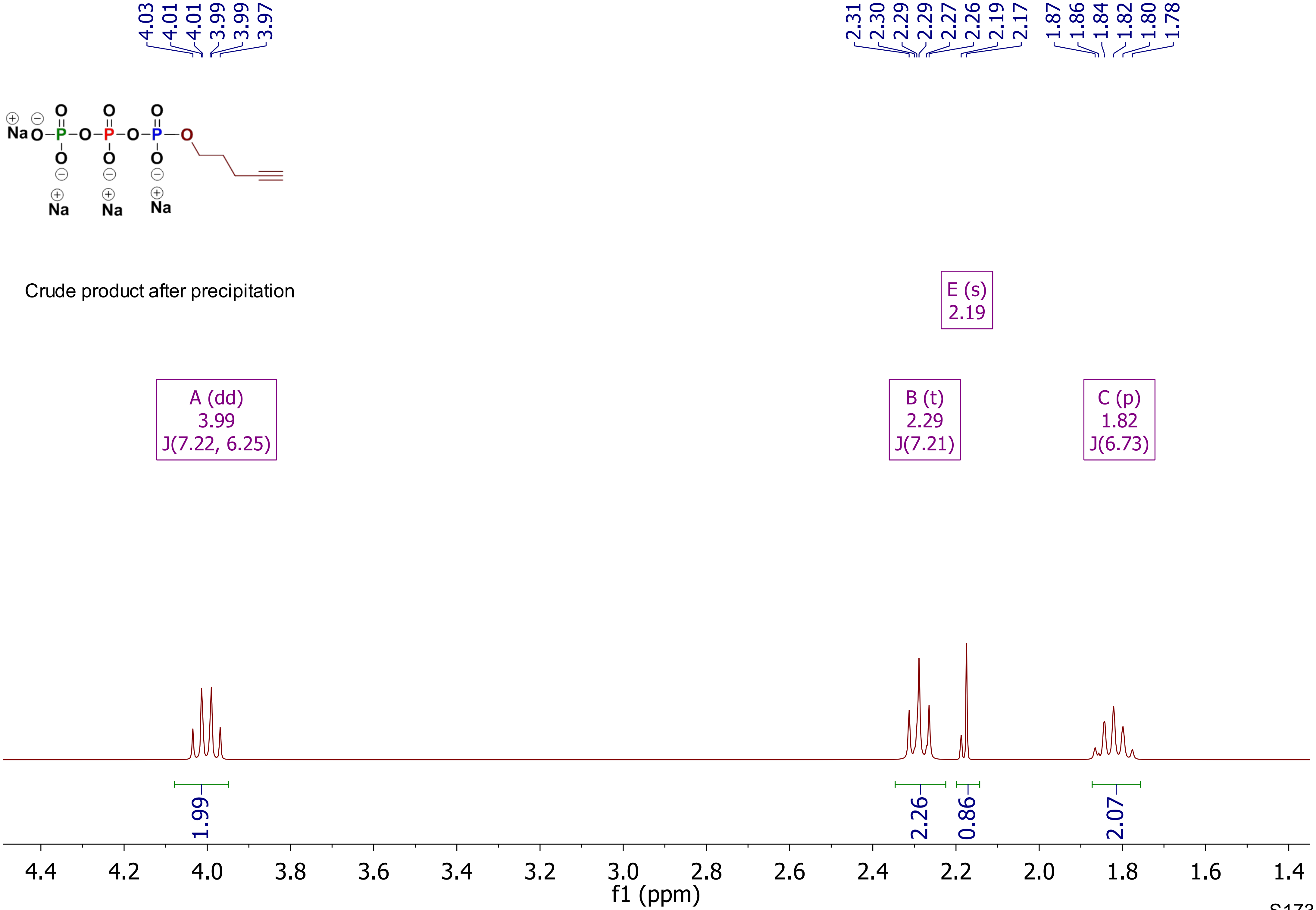
$^{13}$C NMR



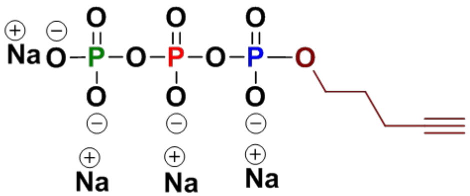
Crude product after precipitation



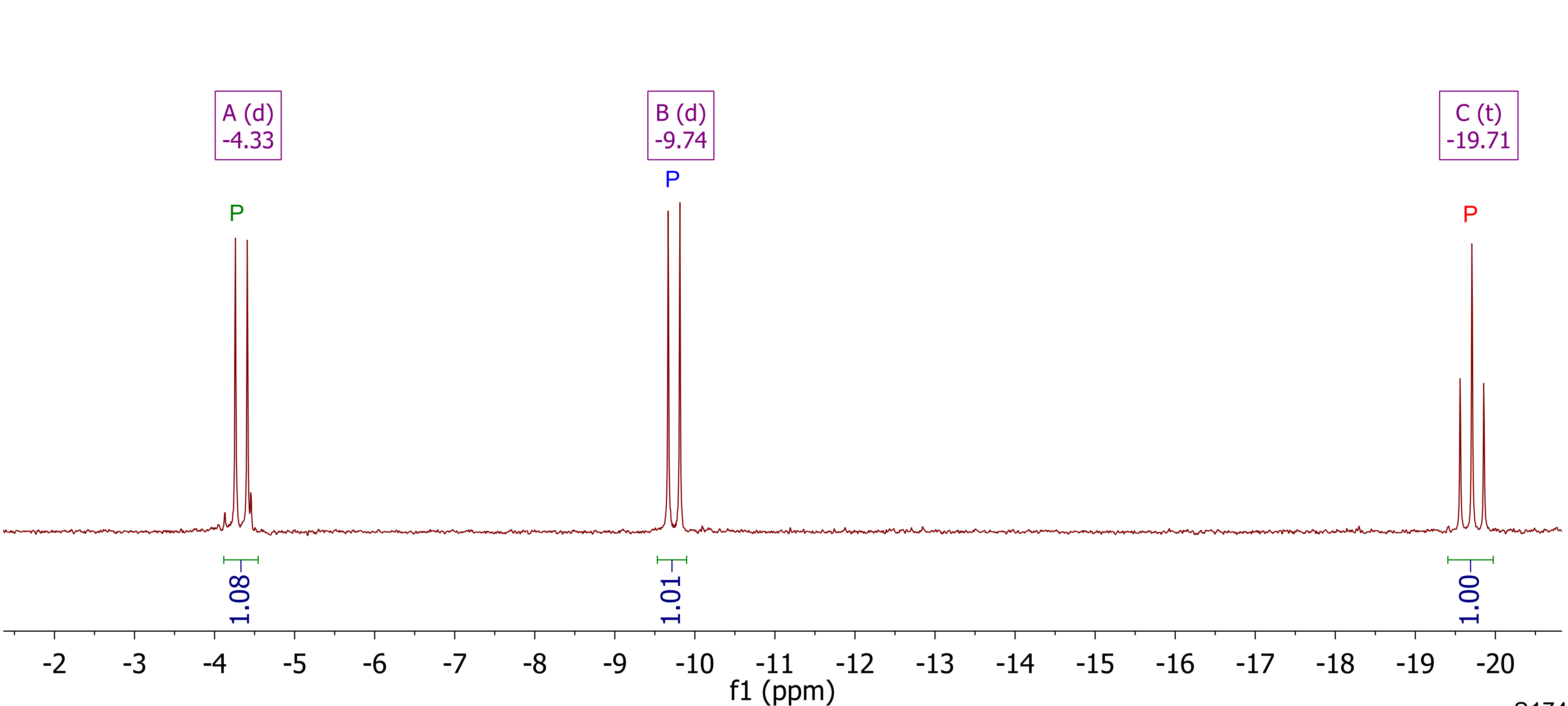
$^1$H- NMR


4.03 4.01 4.01 3.99 3.99 3.97
2.31 2.30 2.29 2.29 2.27 2.26 2.19 2.17
1.87 1.86 1.84 1.82 1.80 1.78
Na O P O P O P O
Na Na Na
Crude product after precipitation
E (s)
2.19
A (dd)
3.99
J(7.22, 6.25)
B (t)
2.29
J(7.21)
C (p)
1.82
J(6.73)
1.99
2.26
0.86
2.07
4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4
f1 (ppm)

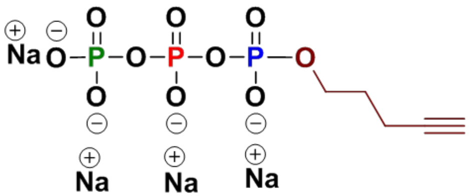
$^{31}P\{^1H\}$ NMR



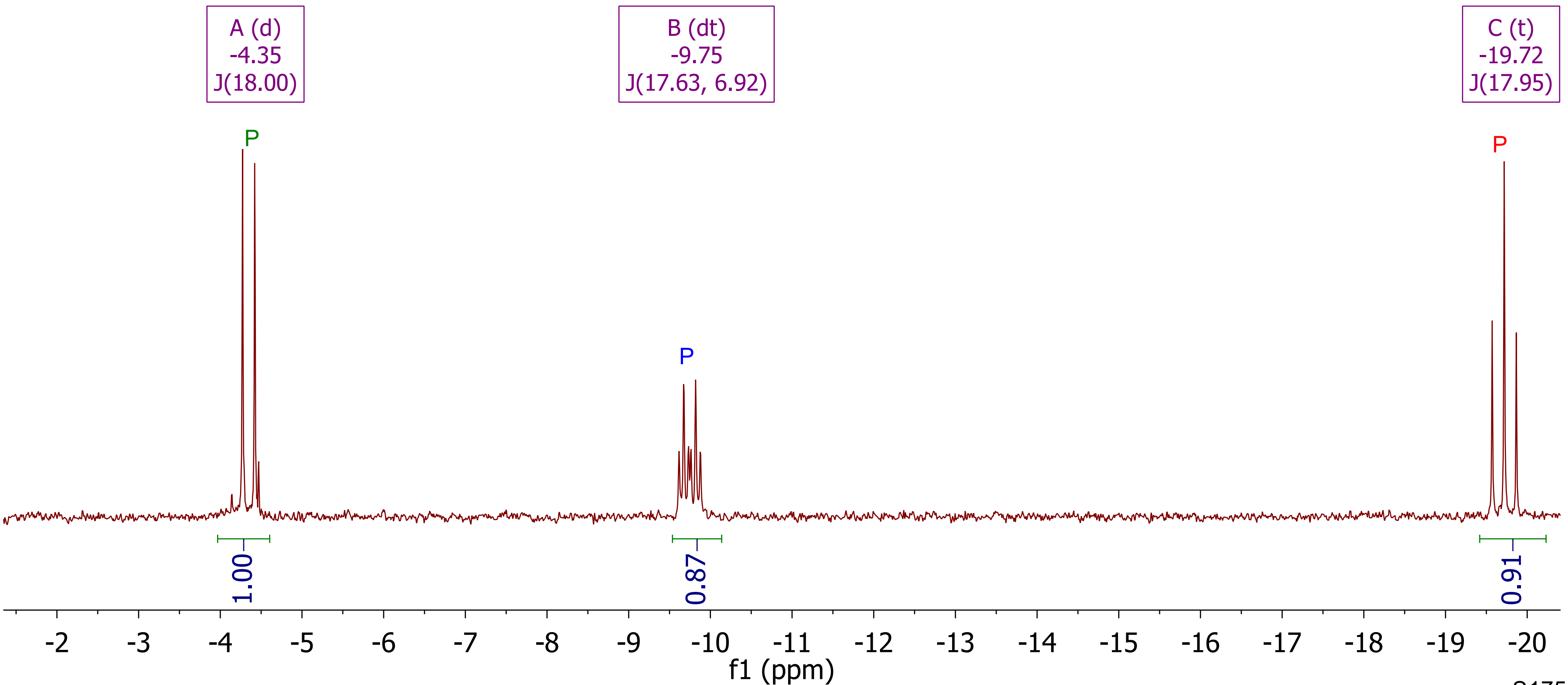

Crude product after precipitation



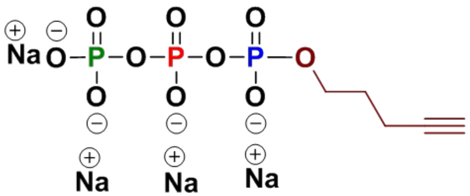
**$^{31}$P NMR**



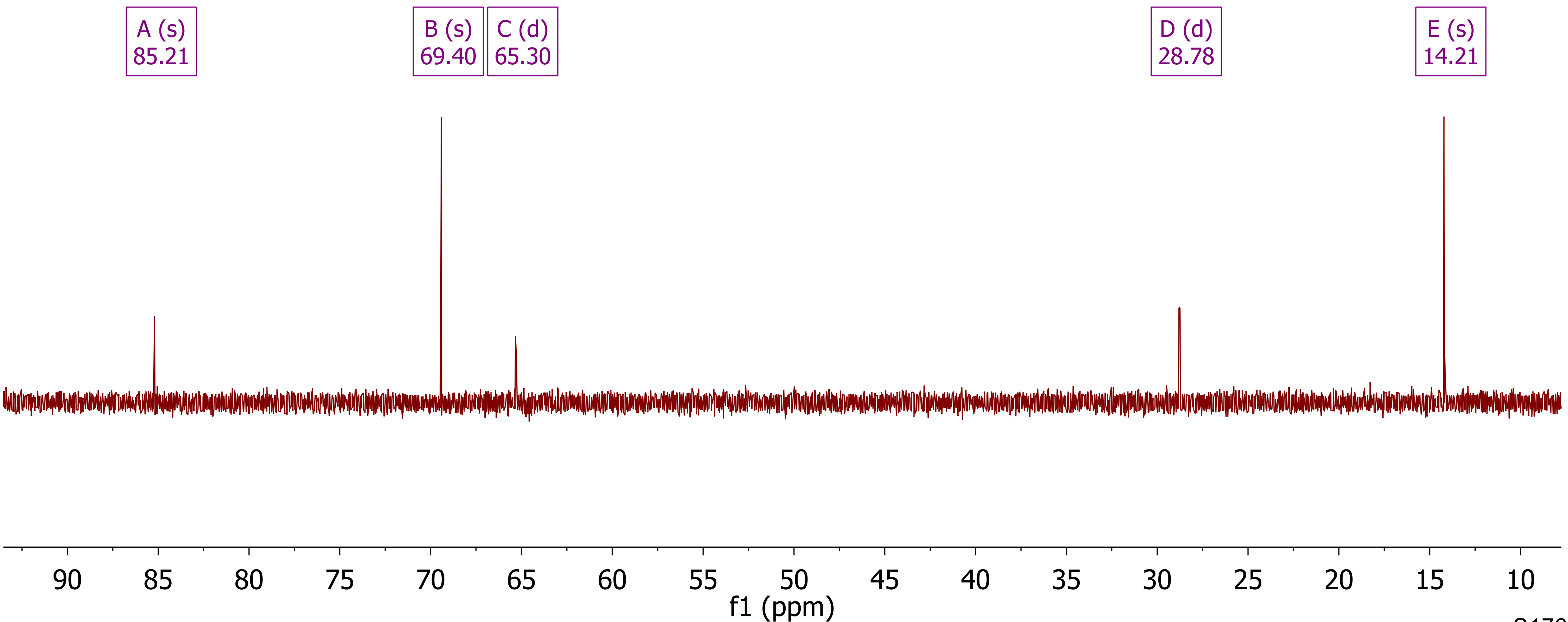
Crude product after precipitation



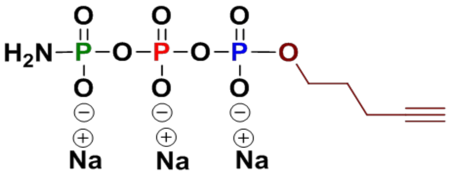
$^{13}$C NMR



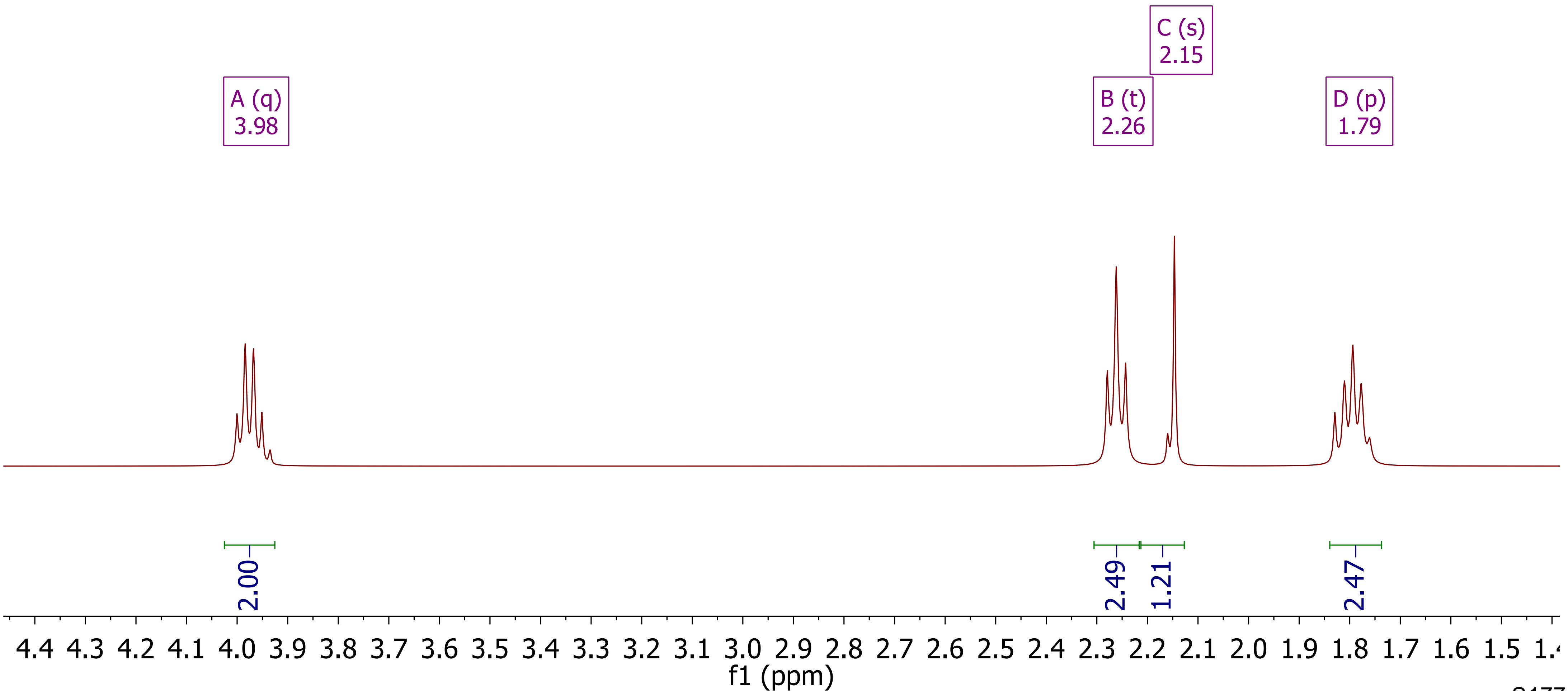
Crude product after precipitation



$^{1}$H NMR

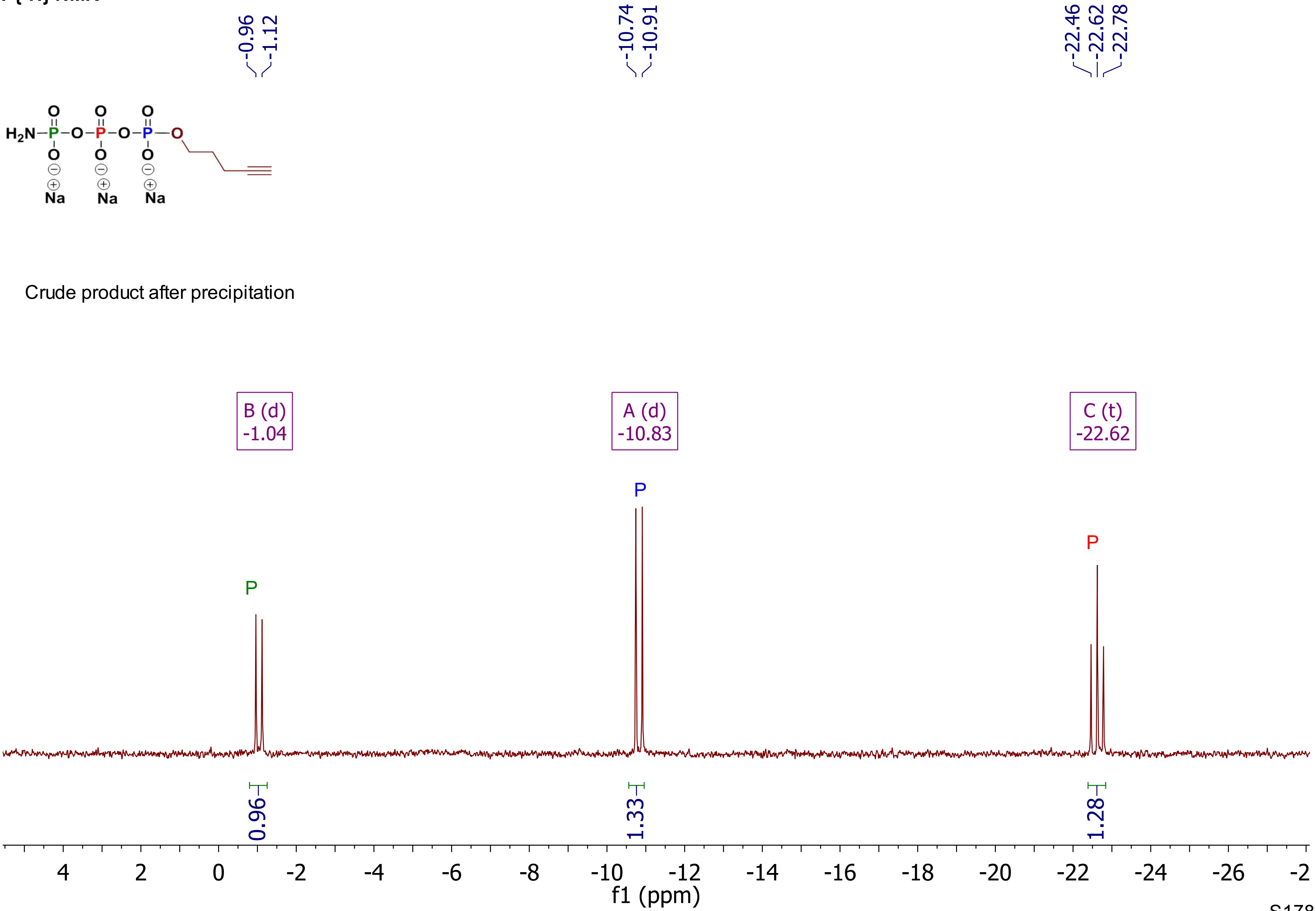



Crude product after precipitation

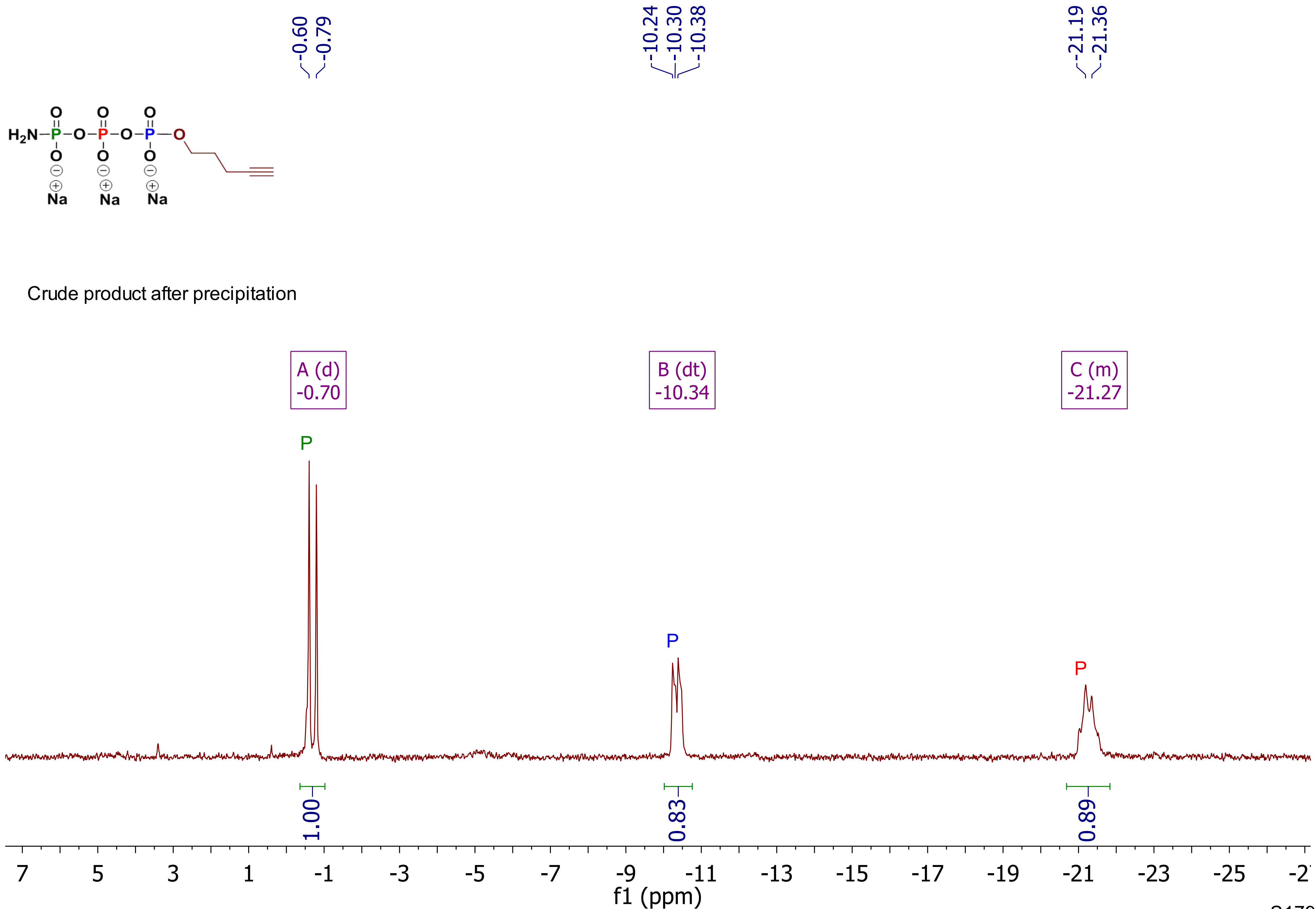


**$^{31}P\{^{1}H\}$ NMR**

Crude product after precipitation

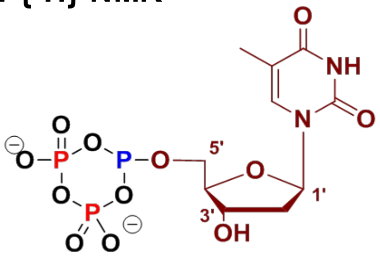


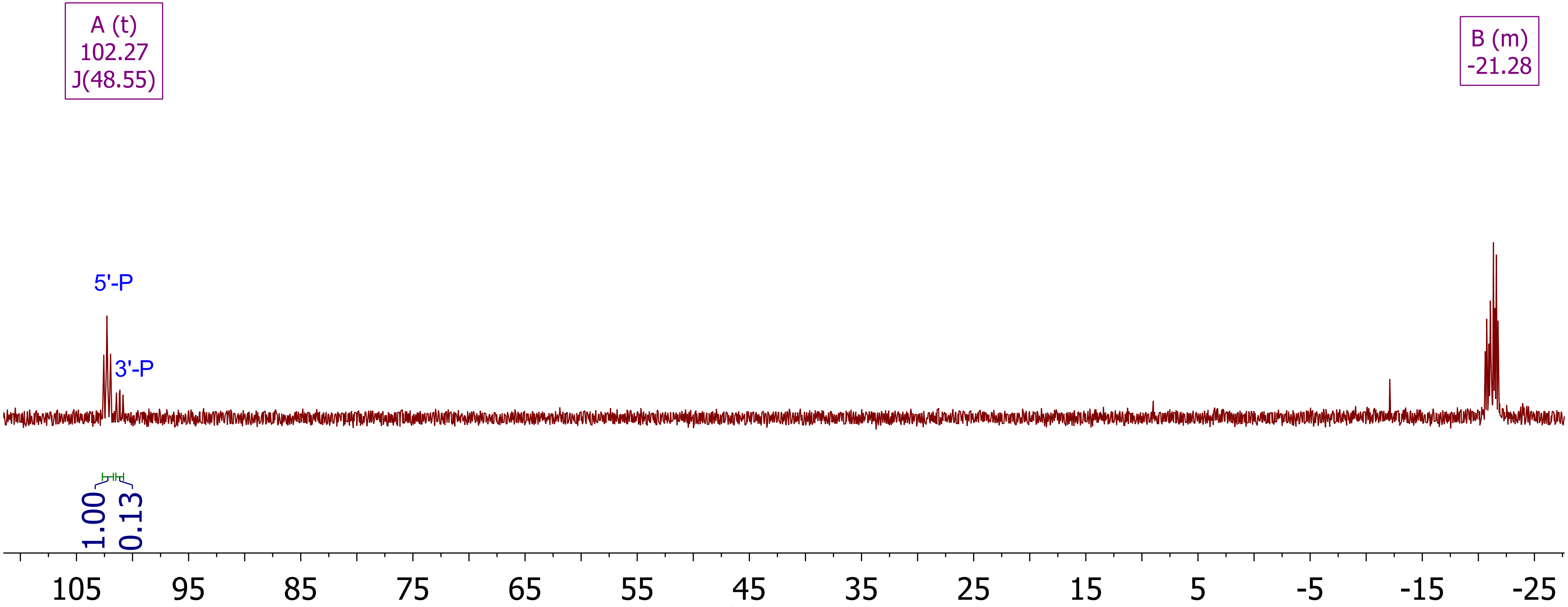
**$^{31}$P NMR**



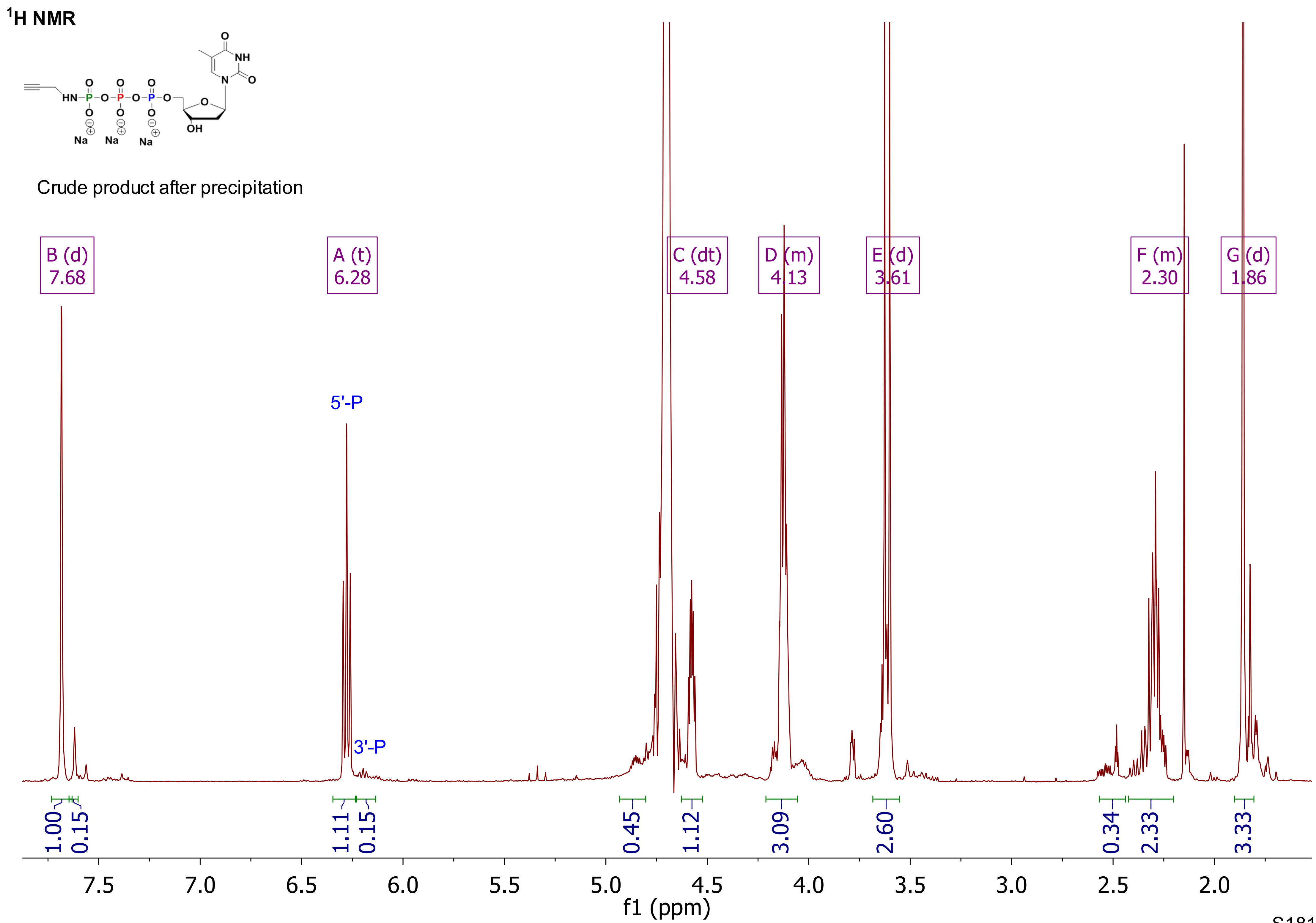
-0.60
-0.79
-10.24
-10.30
-10.38
-21.19
-21.36
Crude product after precipitation
A (d)
-0.70
B (dt)
-10.34
C (m)
-21.27
1.00
0.83
0.89
f1 (ppm)

$^{31}P\{^1H\}$-NMR



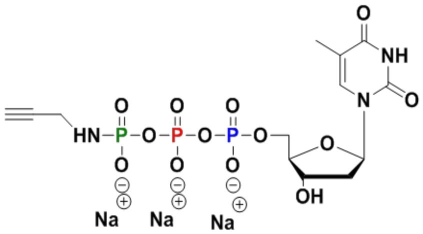


A (t)
102.27
J(48.55)
B (m)
-21.28
5'-P
3'-P
1.00
0.13
105
95
85
75
65
55
45
35
25
15
5
-5
-15
-25
f1 (ppm)

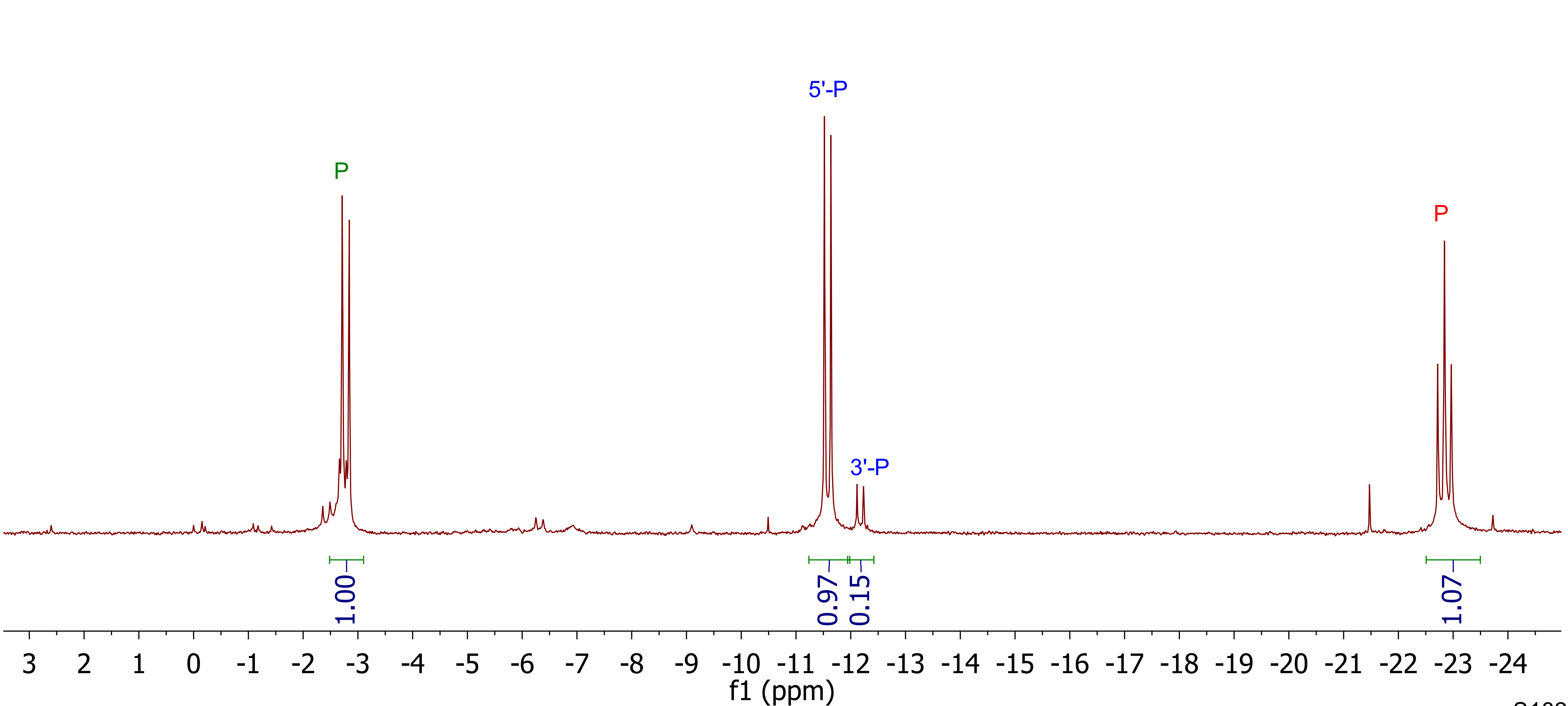


1H NMR
Crude product after precipitation
B (d) 7.68
A (t) 6.28
C (dt) 4.58
D (m) 4.13
E (d) 3.61
F (m) 2.30
G (d) 1.86
5'-P
3'-P
1.00
0.15
1.11
0.15
0.45
1.12
3.09
2.60
0.34
2.33
3.33
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

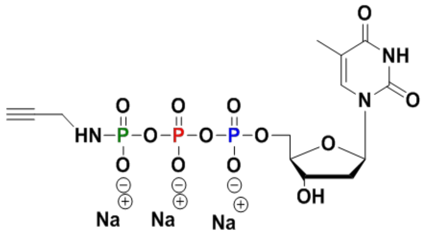
**$^{31}P\{^{1}H\}$ NMR**



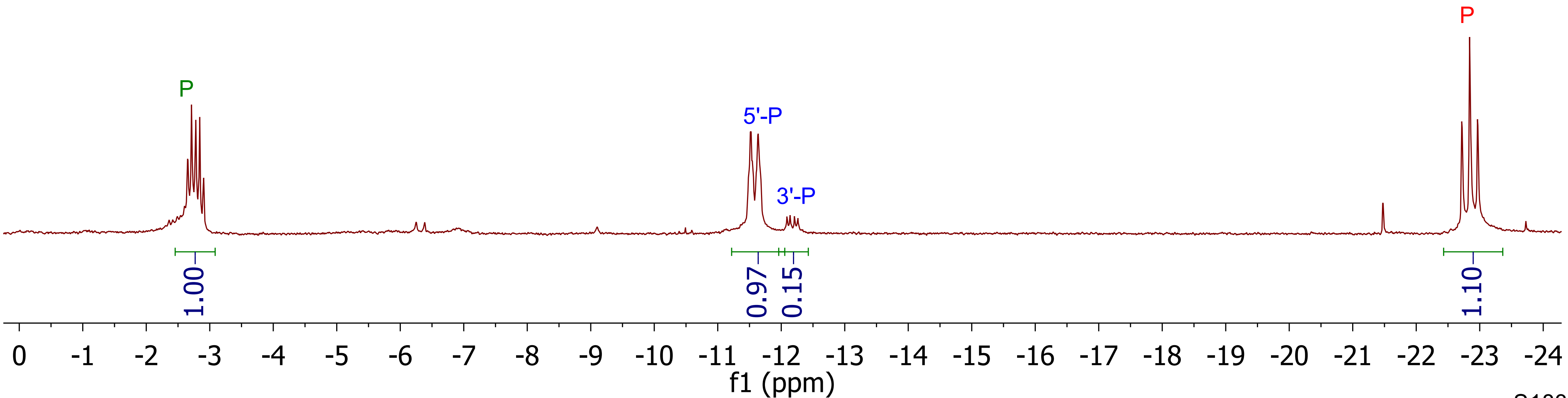

Crude product after precipitation



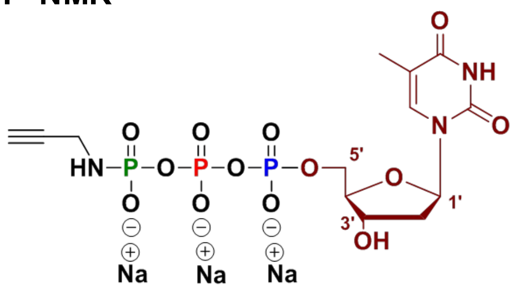
**$^{31}$P NMR**



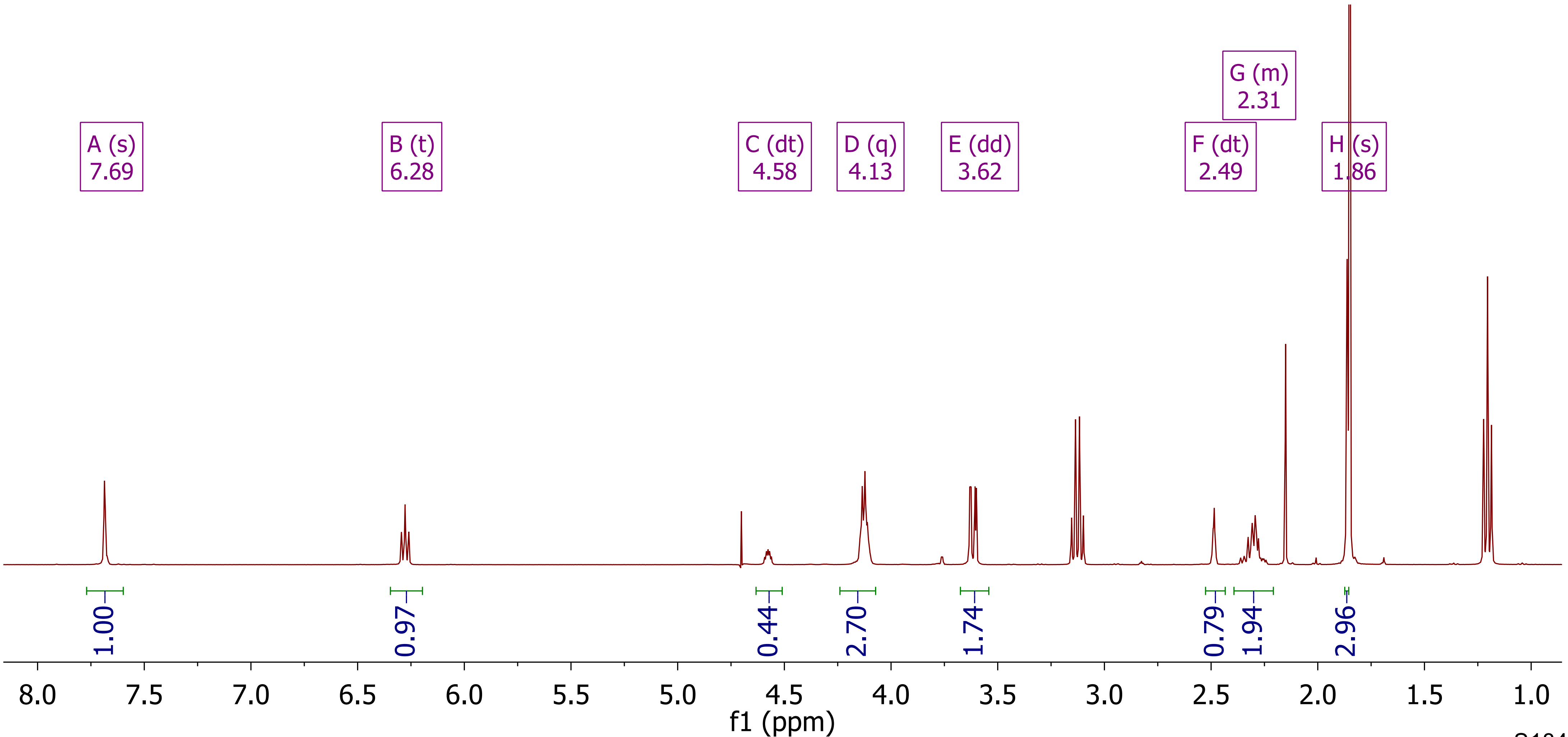
Crude product after precipitation



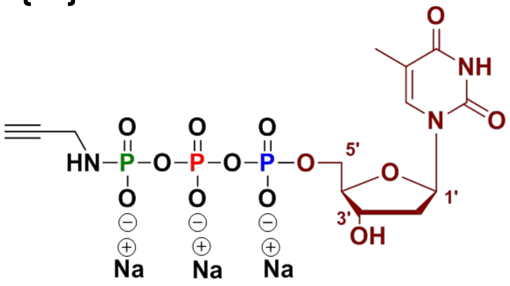
$^1$H - NMR



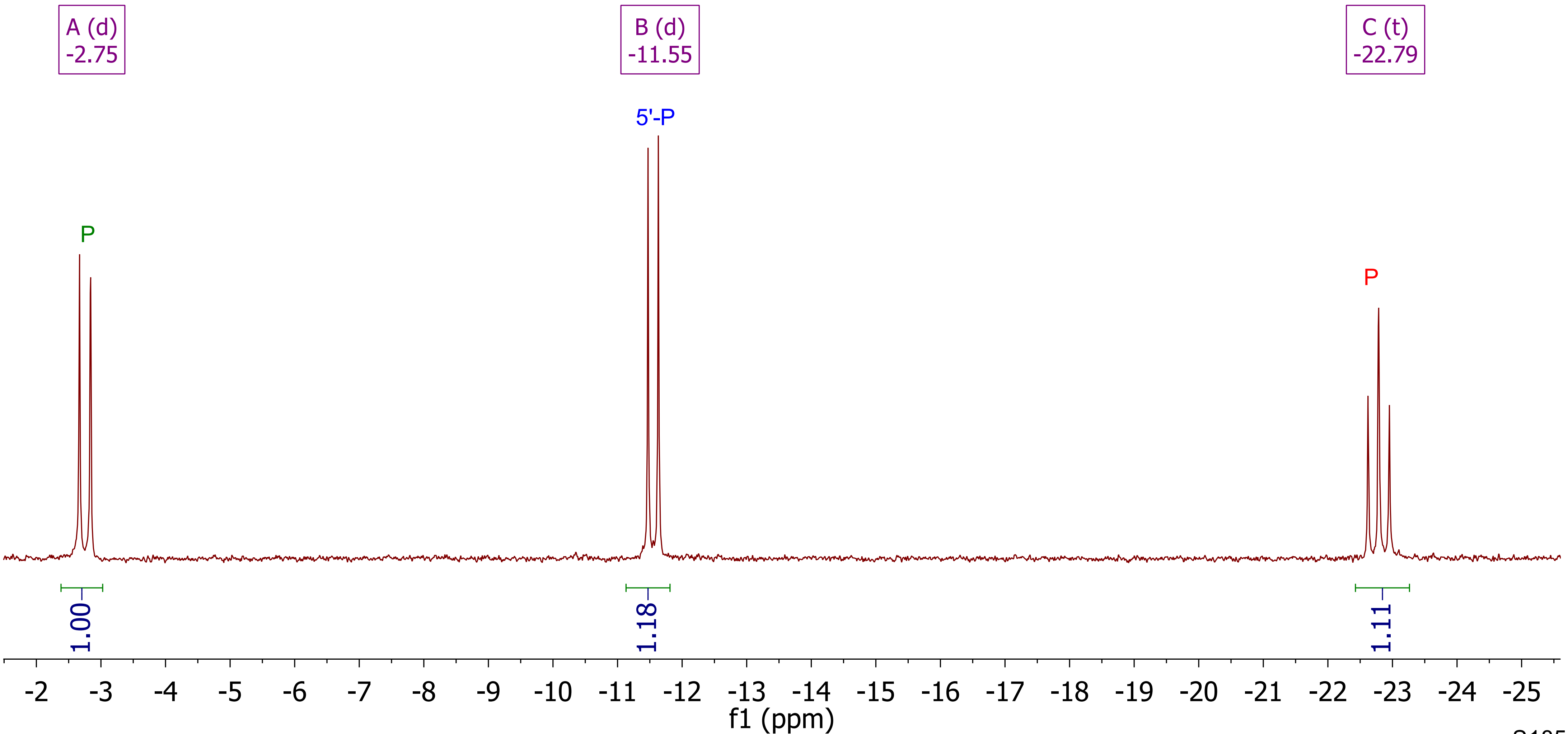
Product after purifucation by MPLC



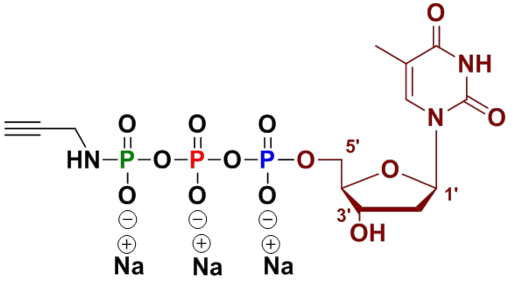
$^{31}P\{^1H\}$-NMR



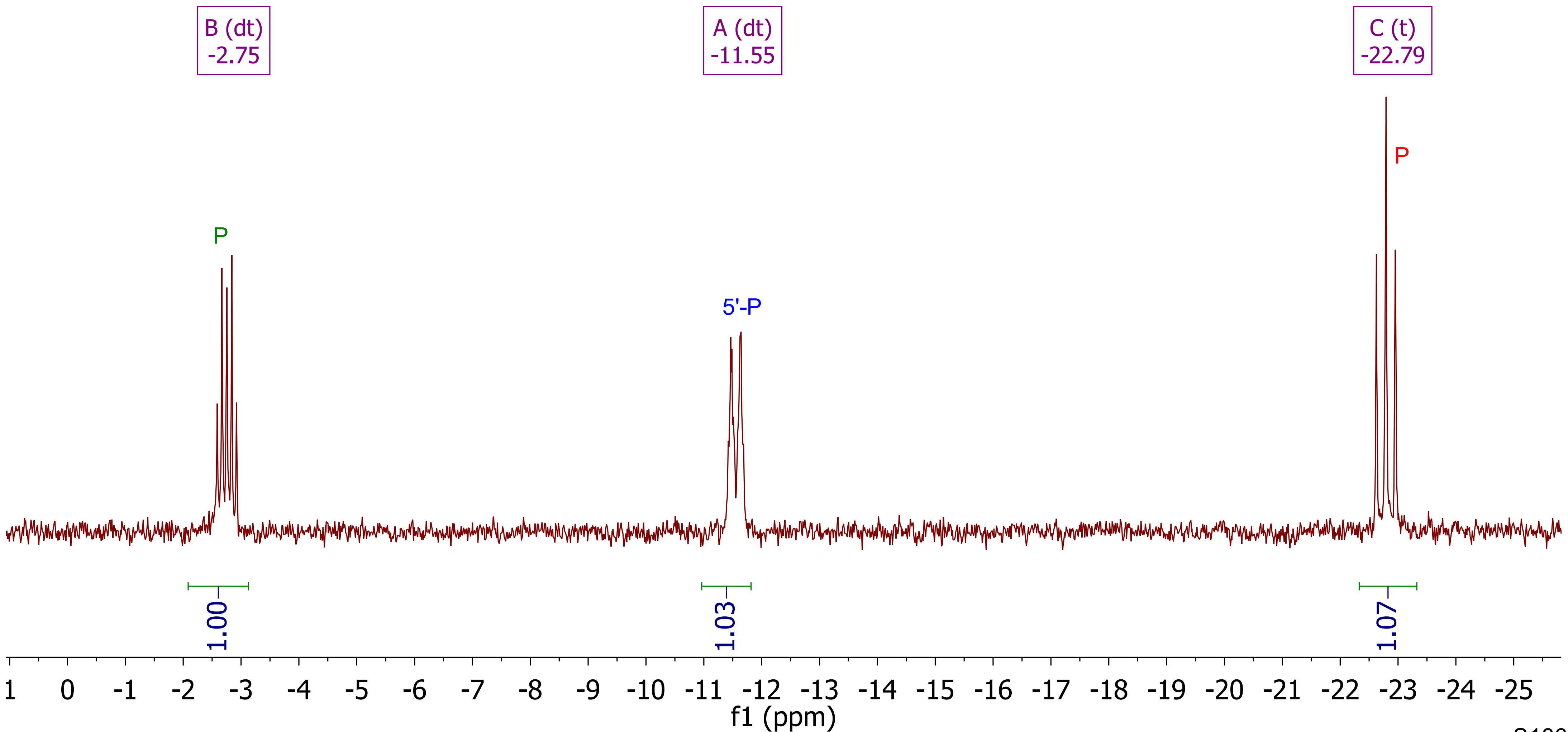
Product after purifucation by MPLC



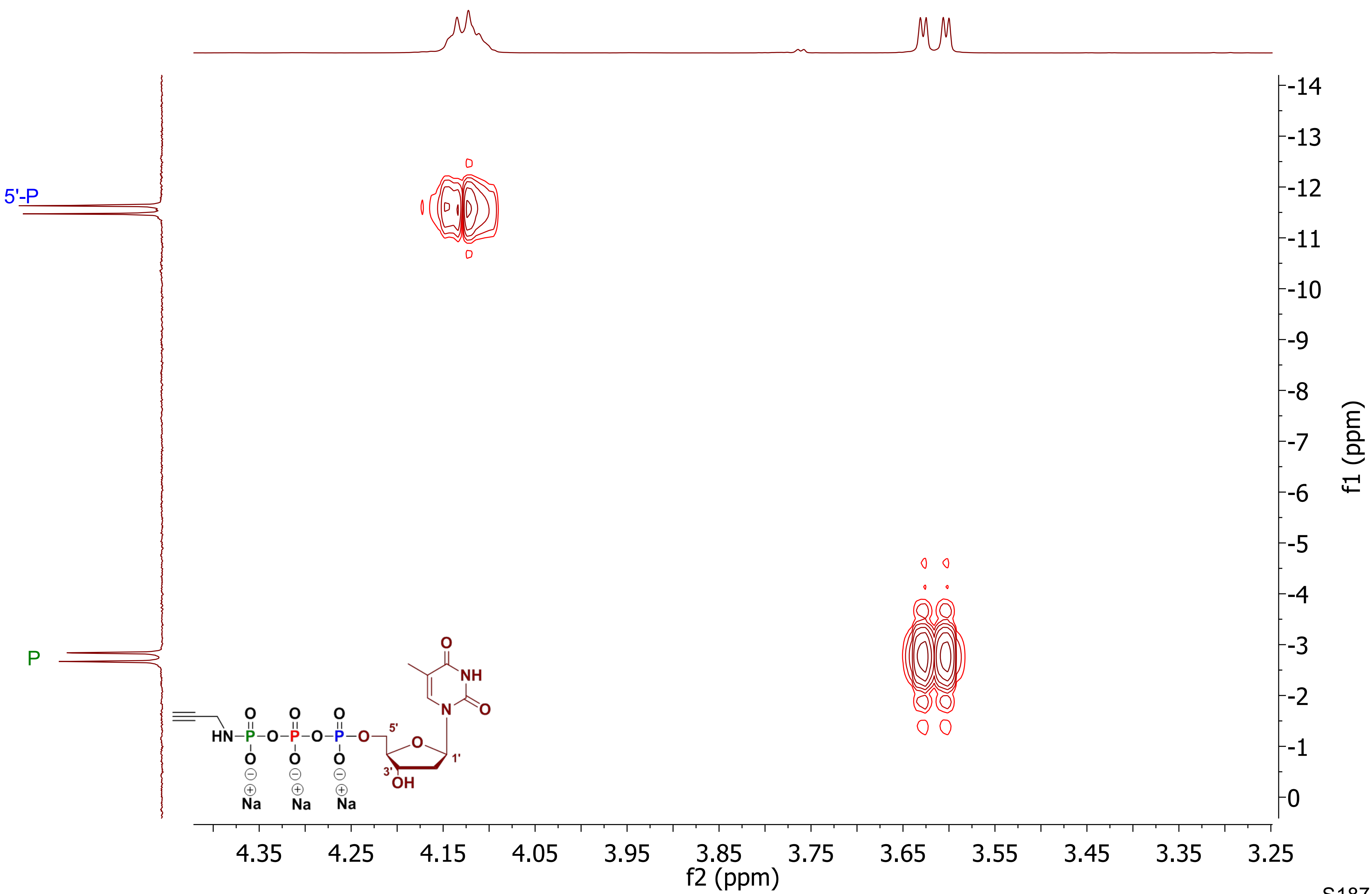
**$^{31}P$-$^{1}H$ NMR**



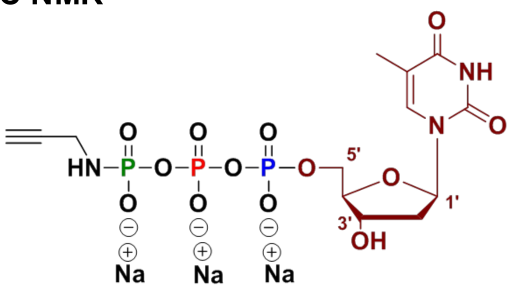

Product after purifucation by MPLC



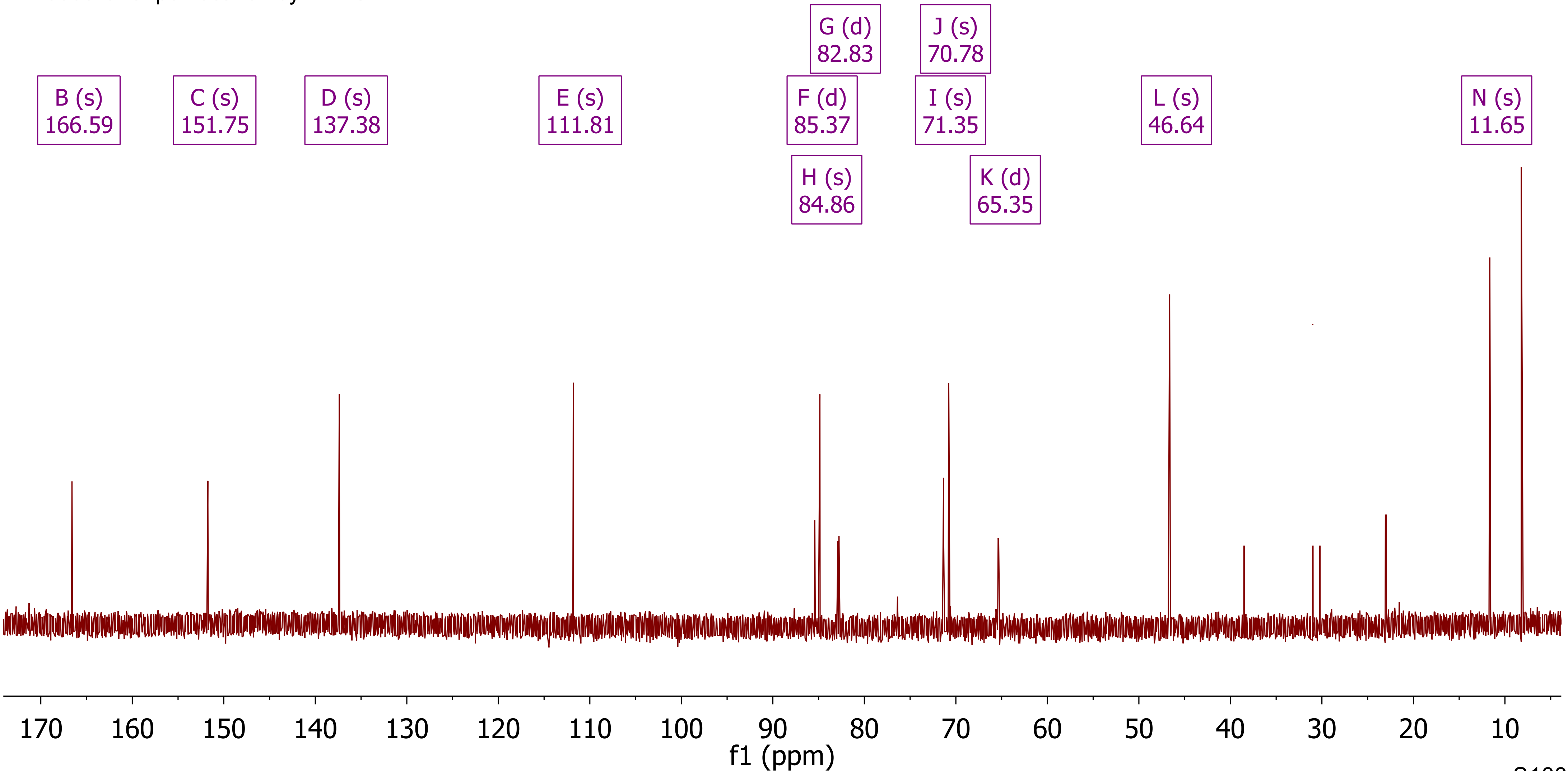
$^{31}P$-$^{1}H$ HMBC NMR



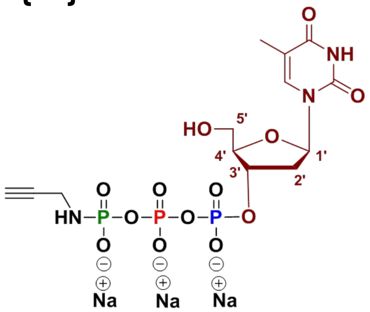
**$^{13}$C NMR**



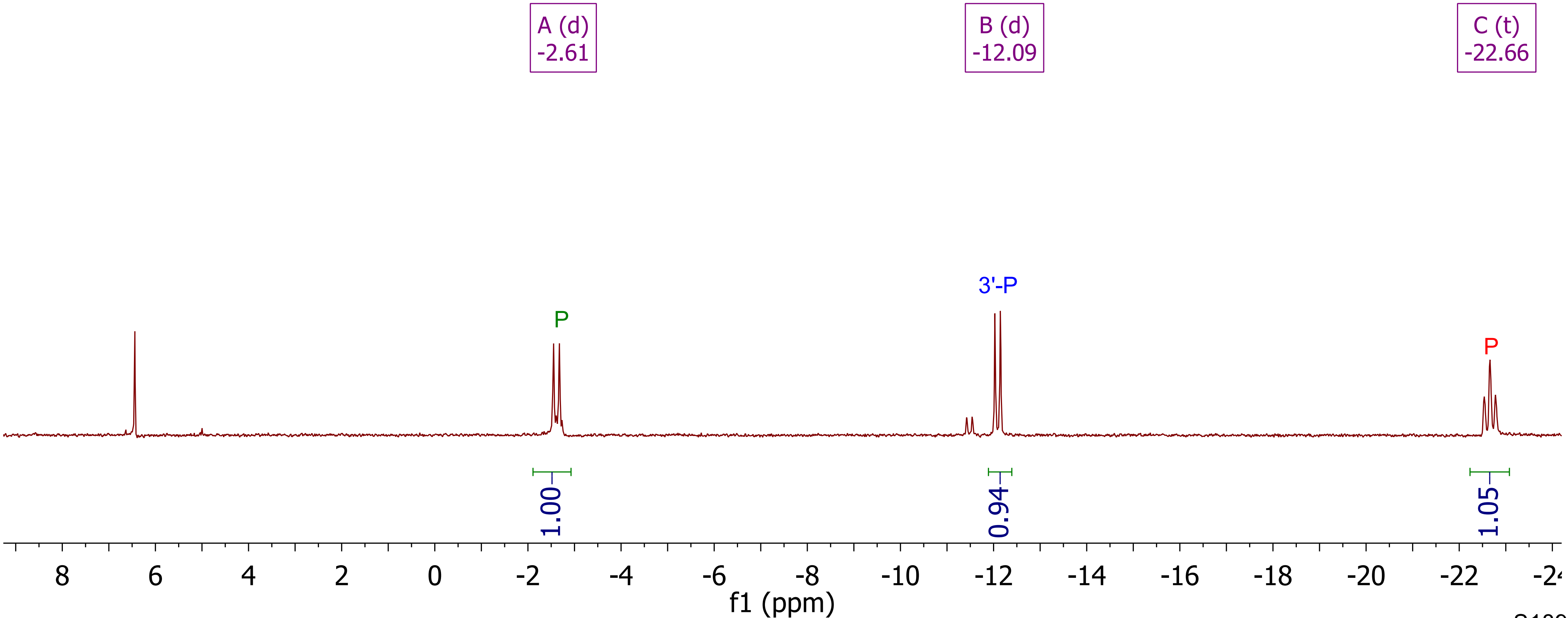

Product after purifucation by MPLC



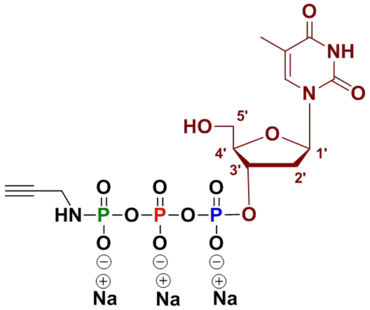
$^{31}P\{^{1}H\}$-NMR



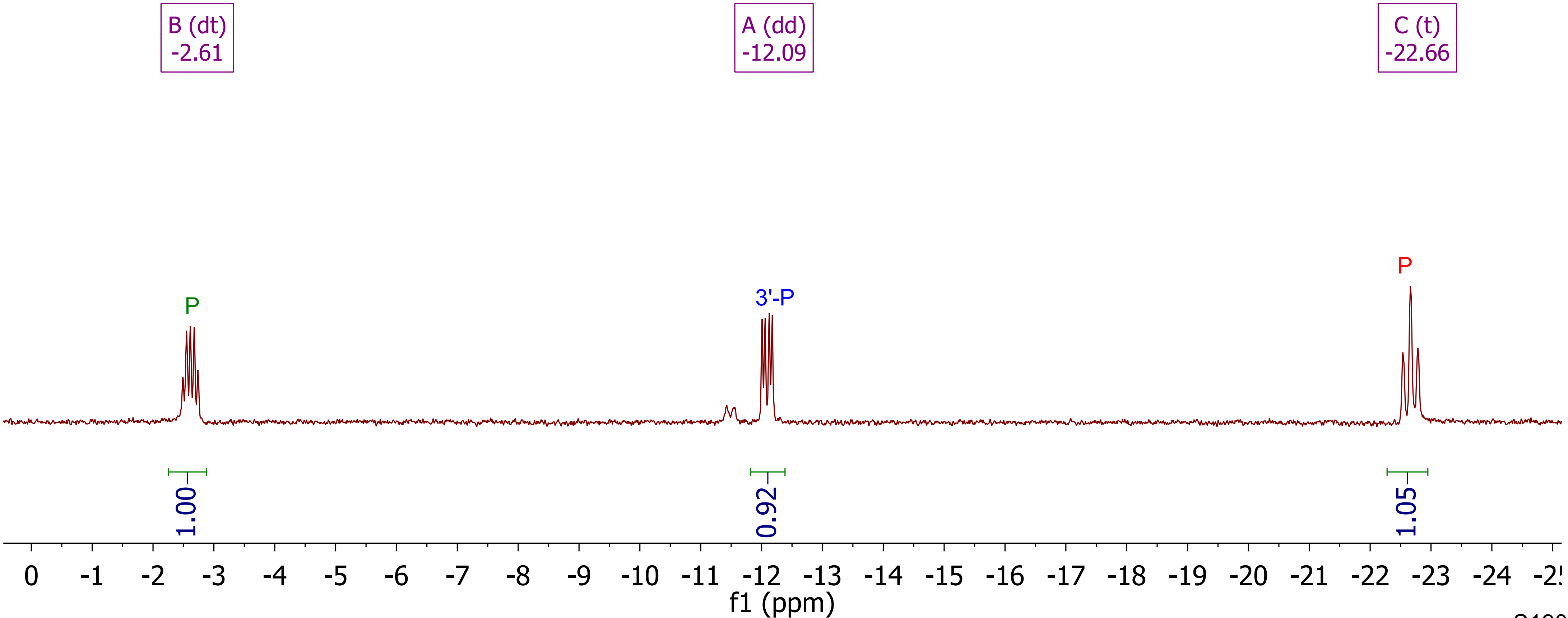
Product after purifucation by MPLC



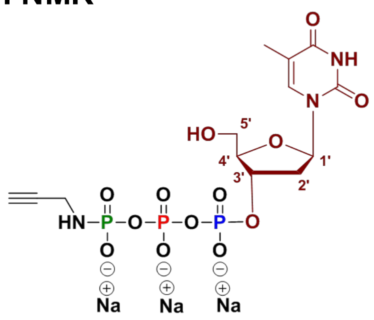
$^{31}P$-$^{1}H$ NMR



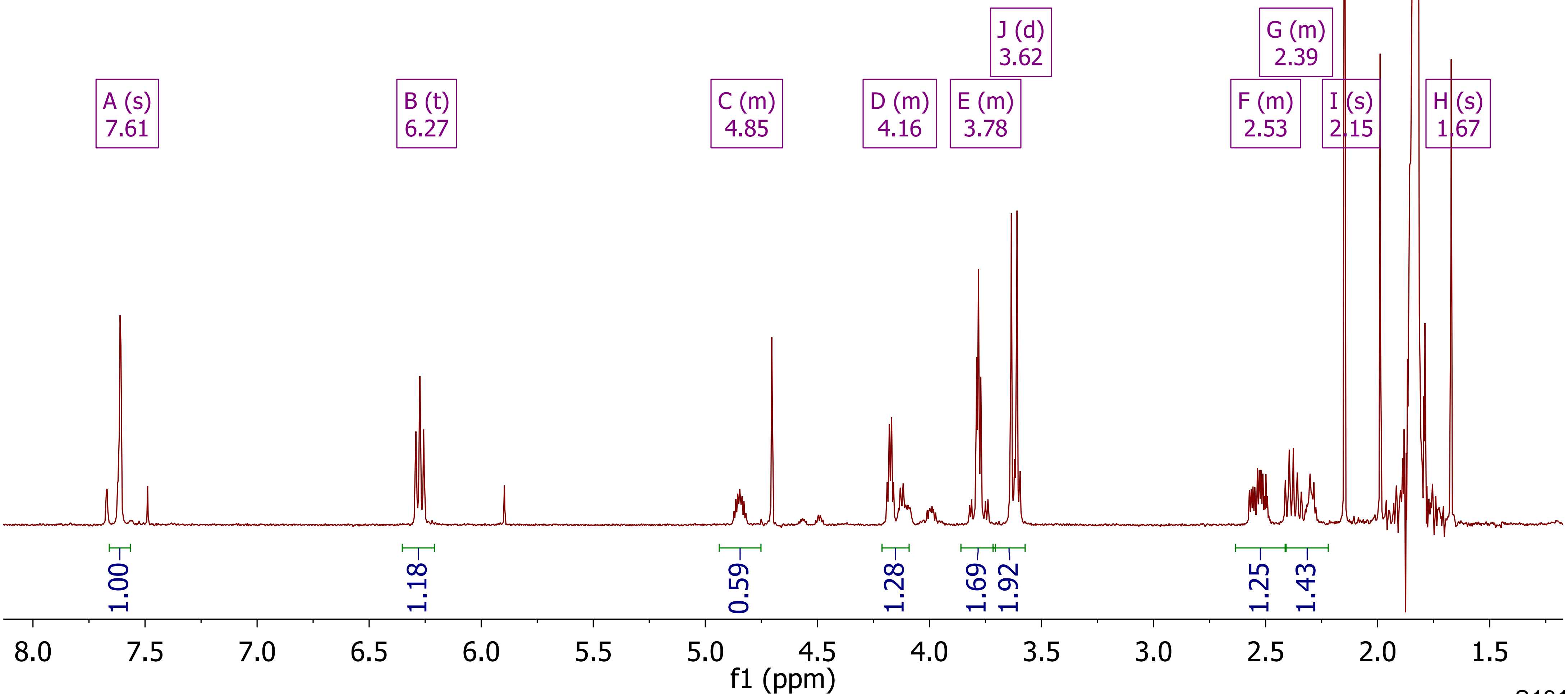
Product after purifucation by MPLC



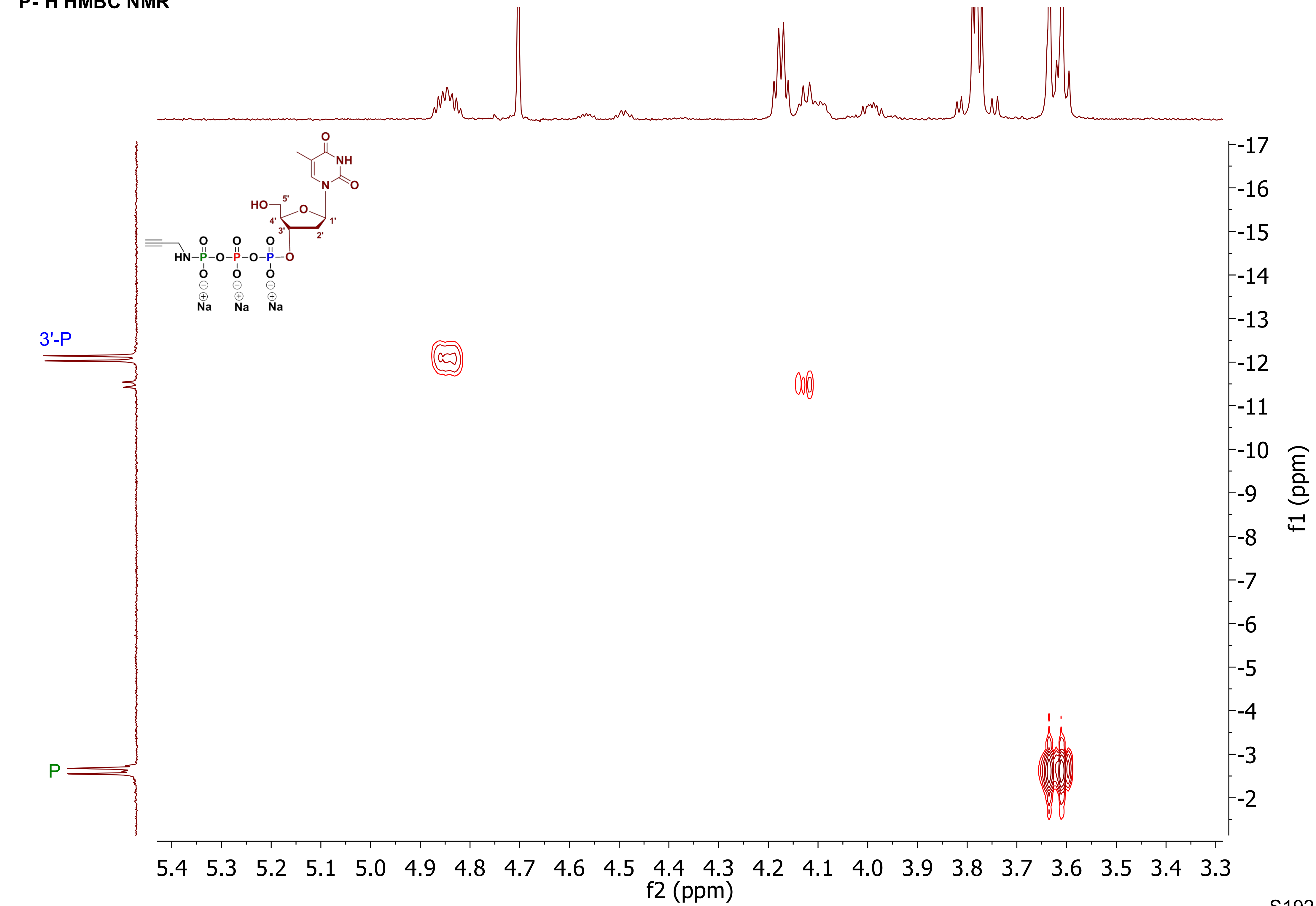
## $^1$H NMR



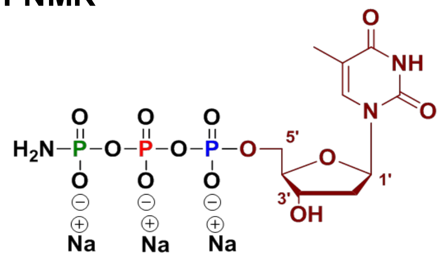
Product after purifucation by MPLC



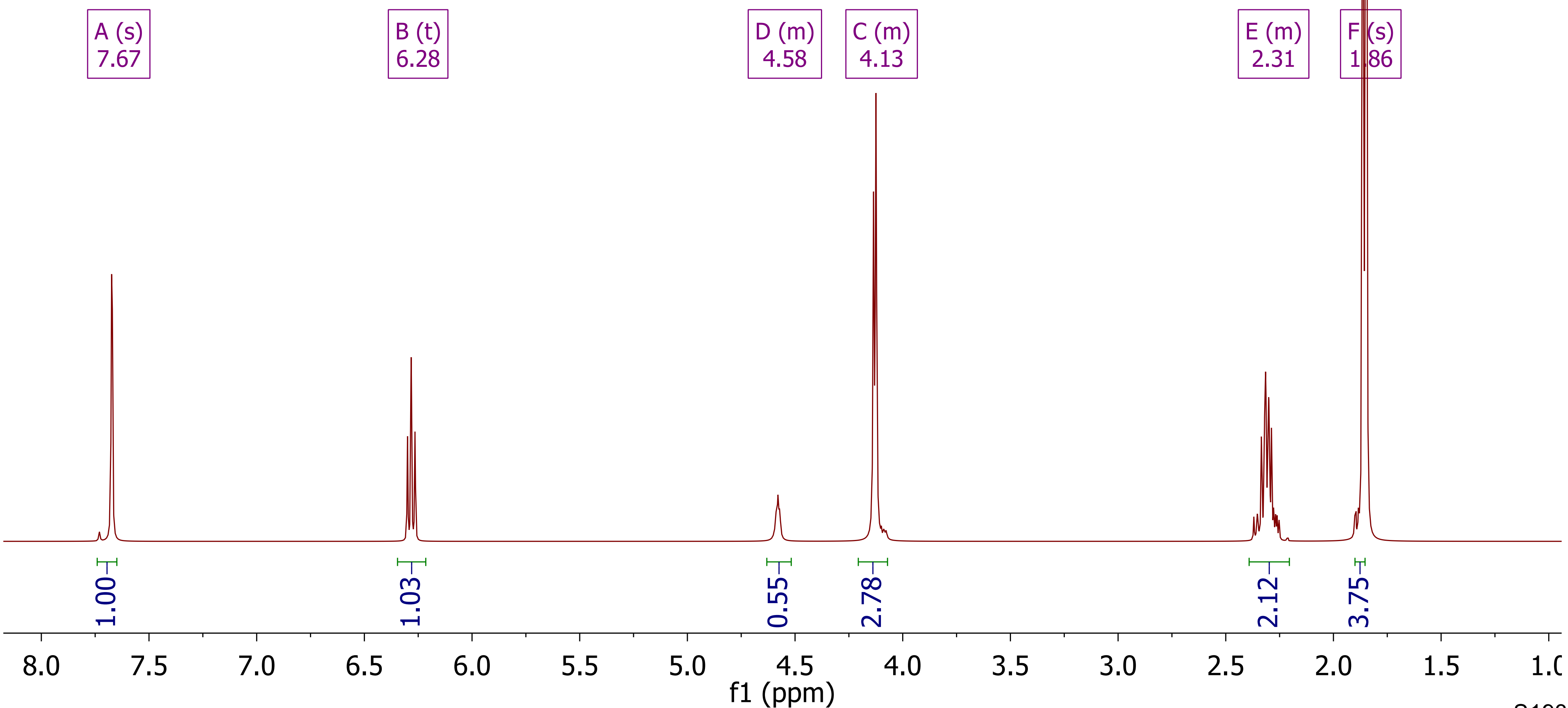
**$^{31}$P-$^{1}$H HMBC NMR**



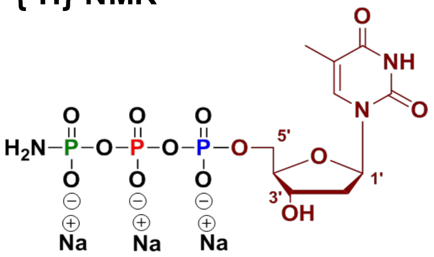
**$^1$H-NMR**



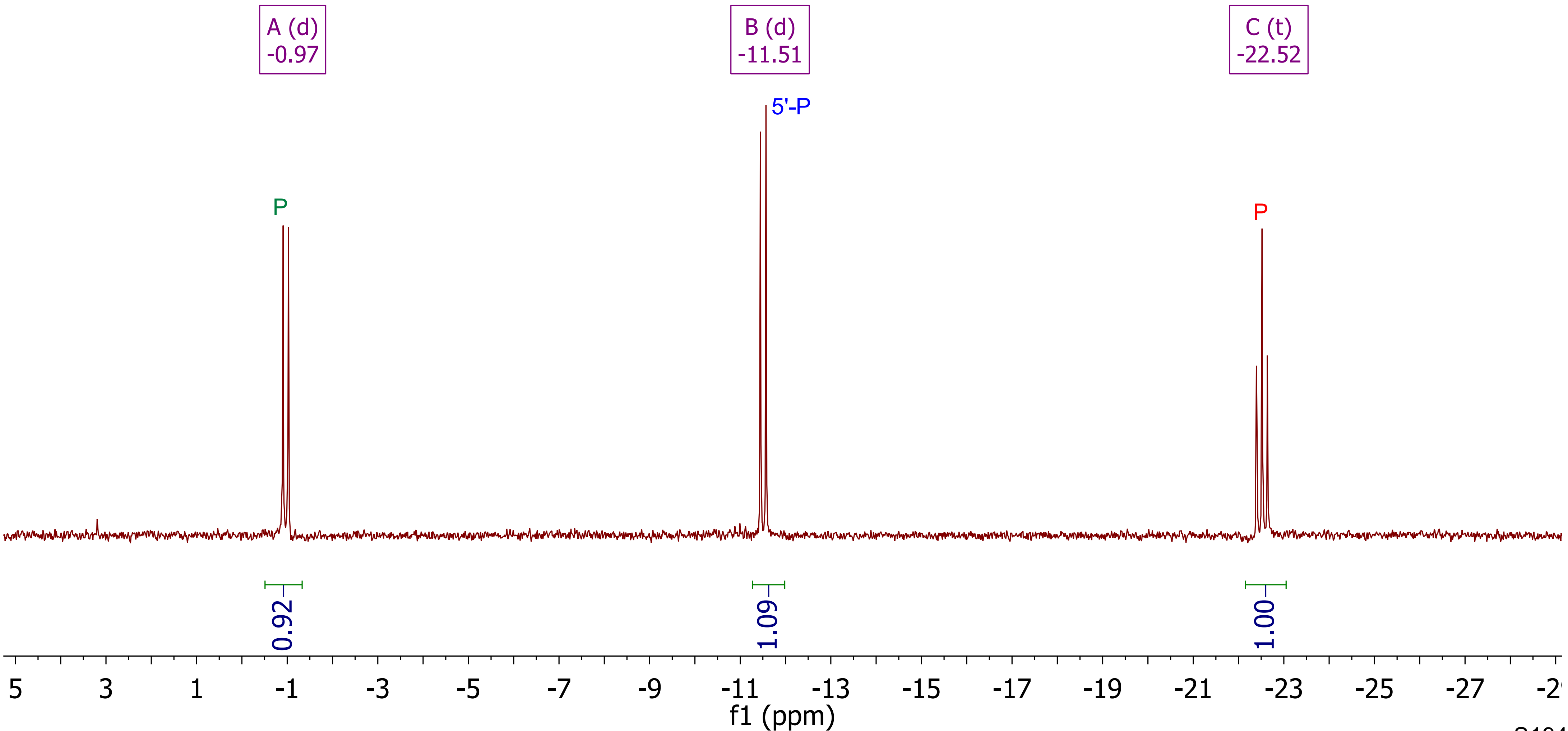

Product after purifucation by MPLC



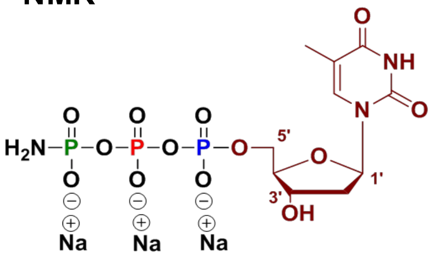
**$^{31}P\{^1H\}$-NMR**



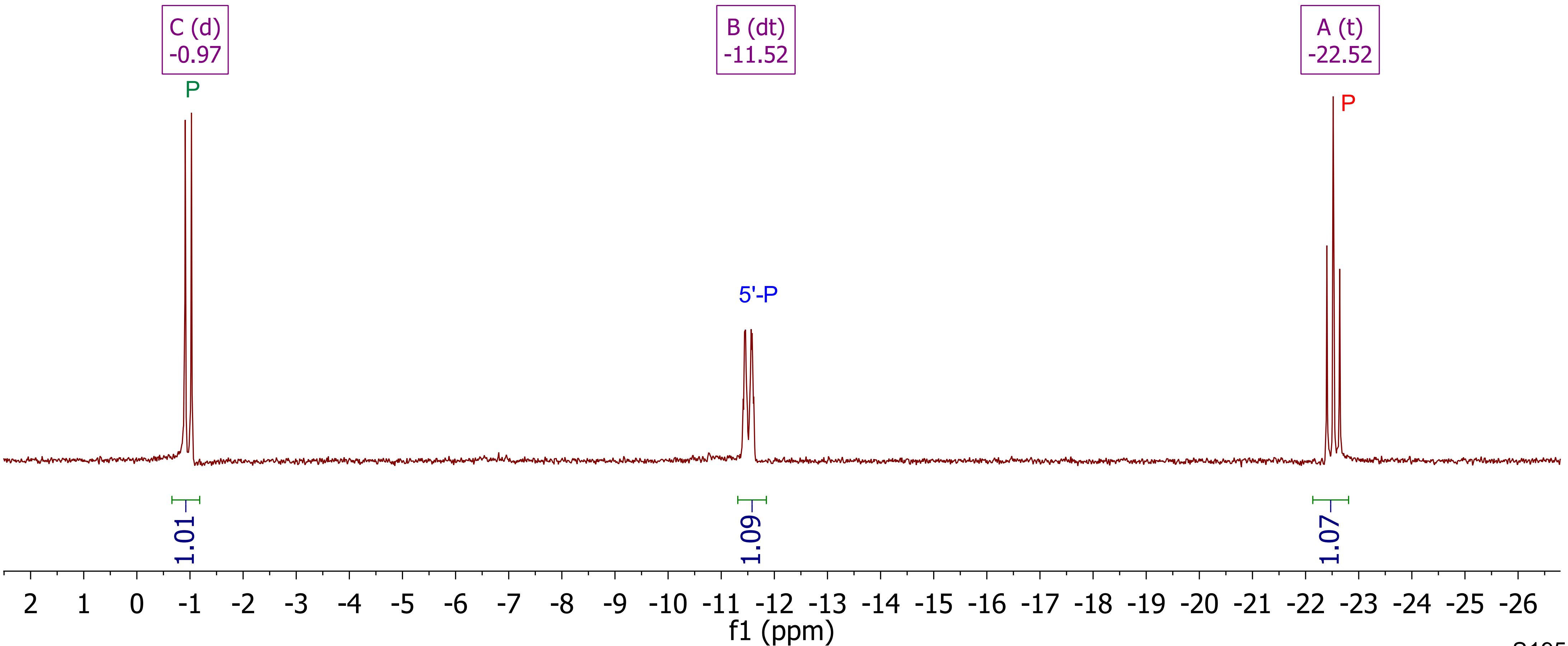

Product after purifucation by MPLC



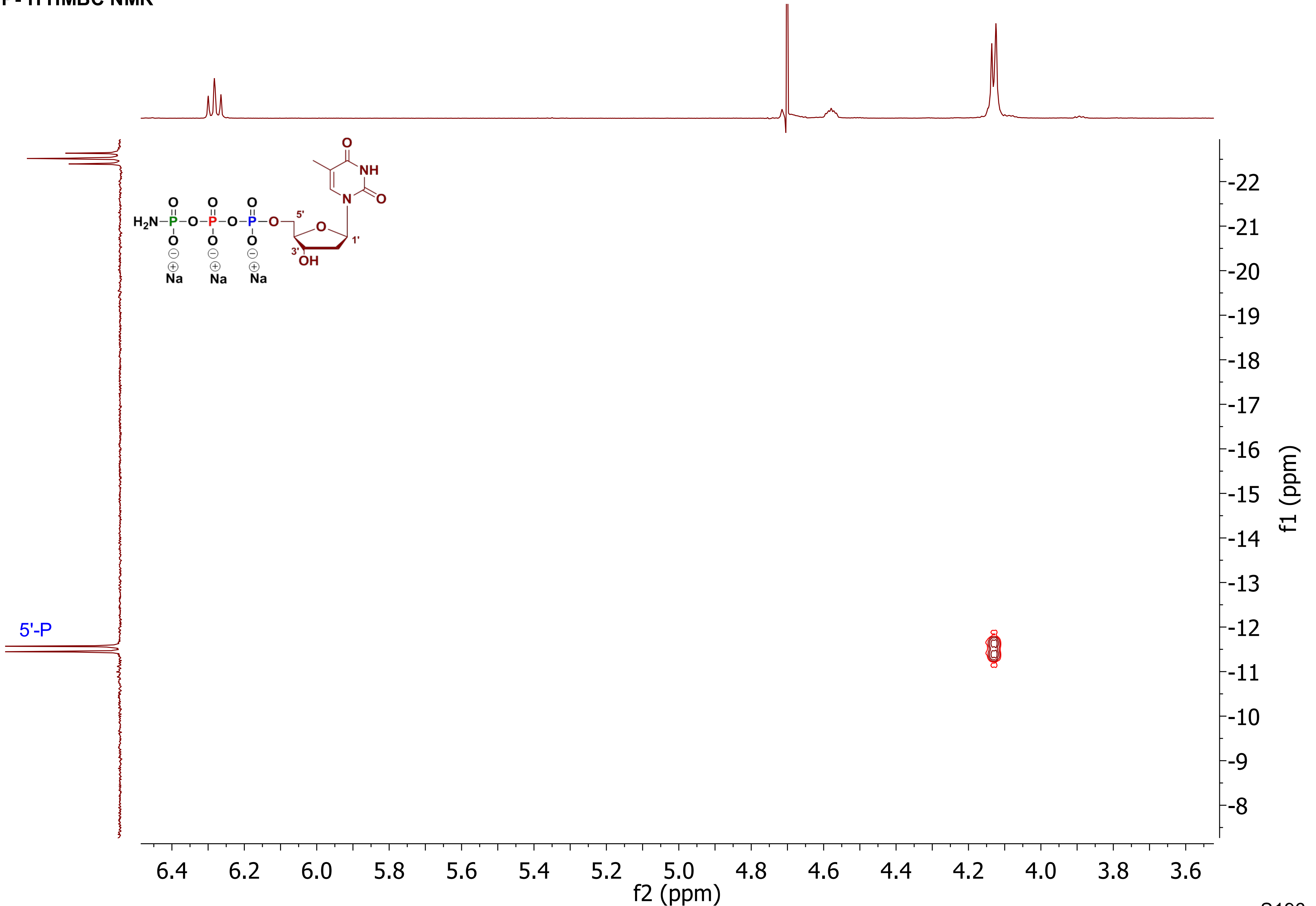
$^{31}$P NMR



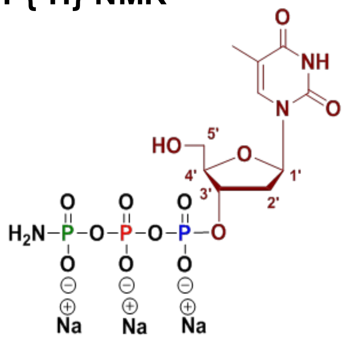

Product after purifucation by MPLC



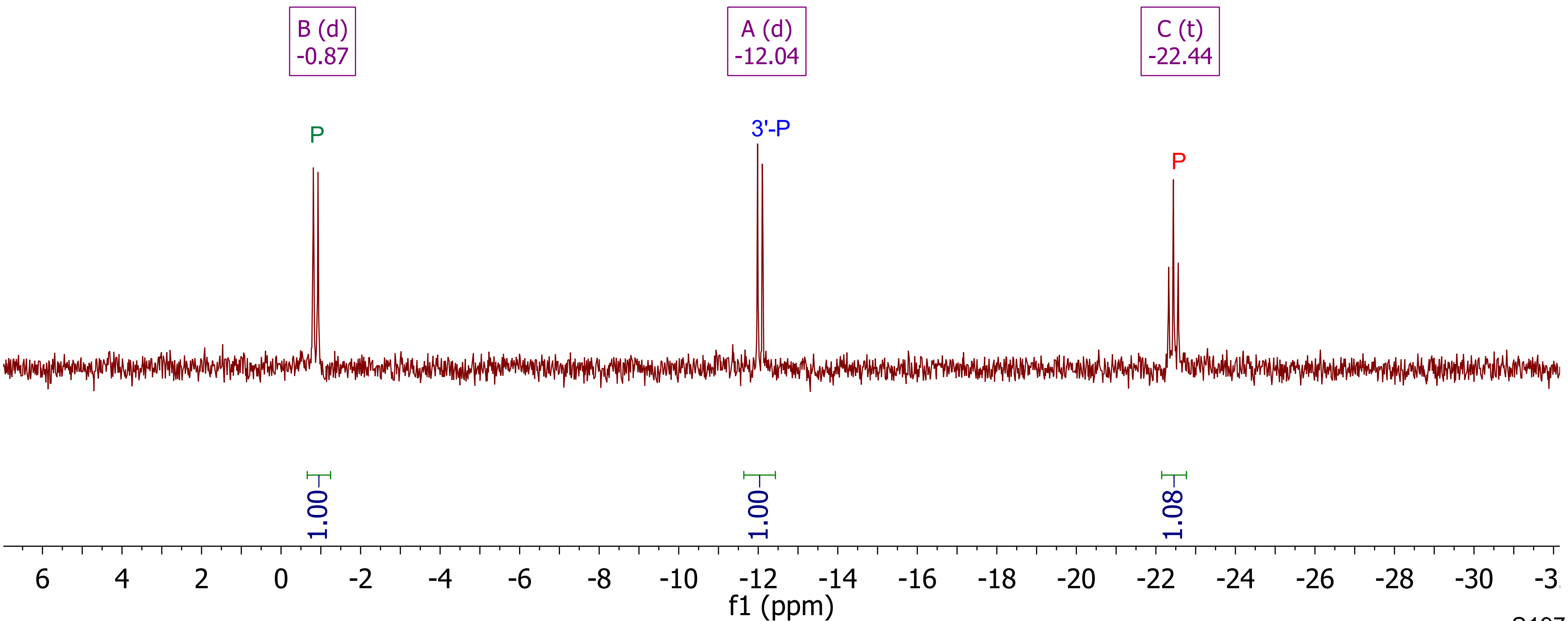
**$^{31}$P-$^{1}$H HMBC NMR**



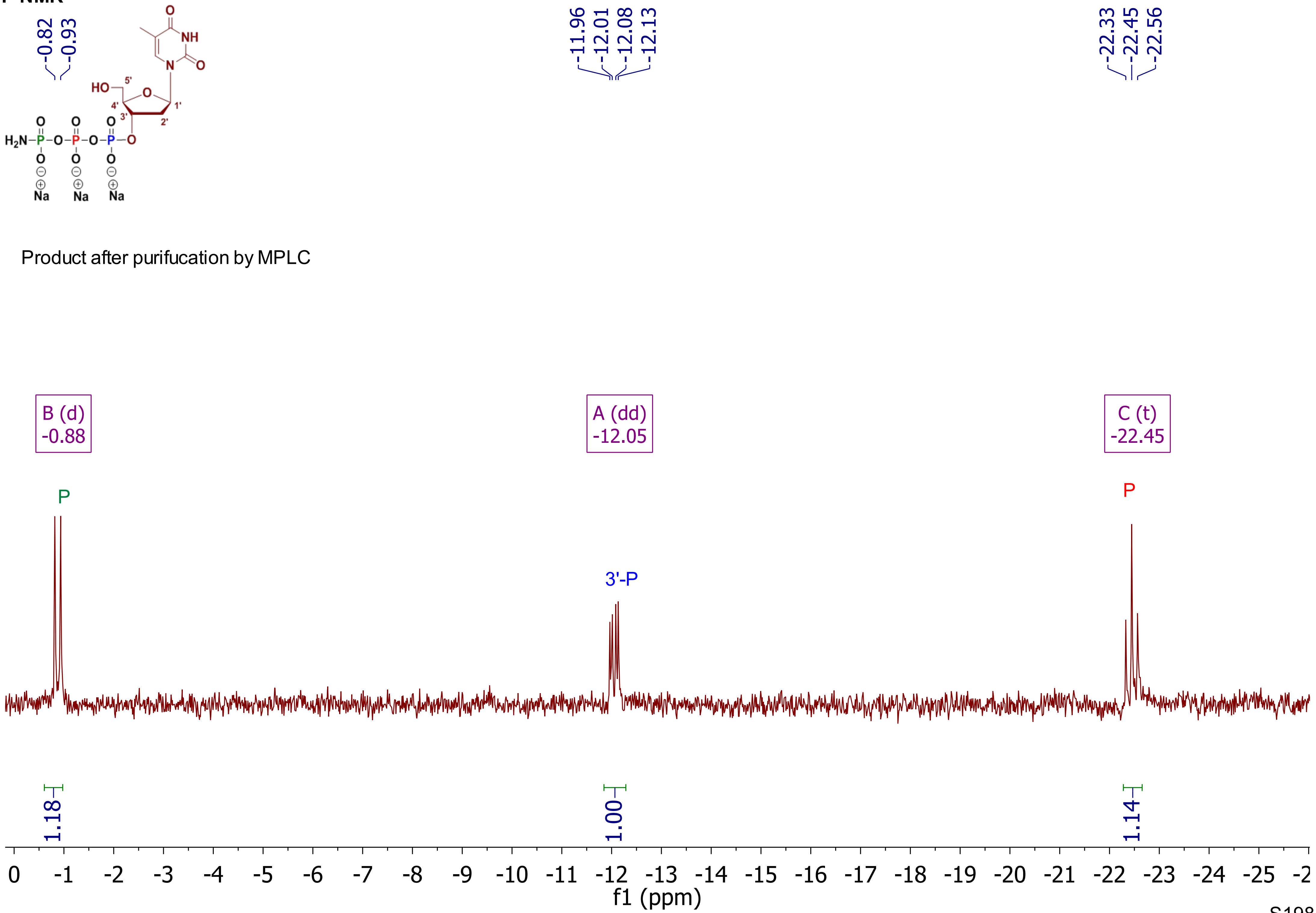
$^{31}P\{^1H\}$-NMR

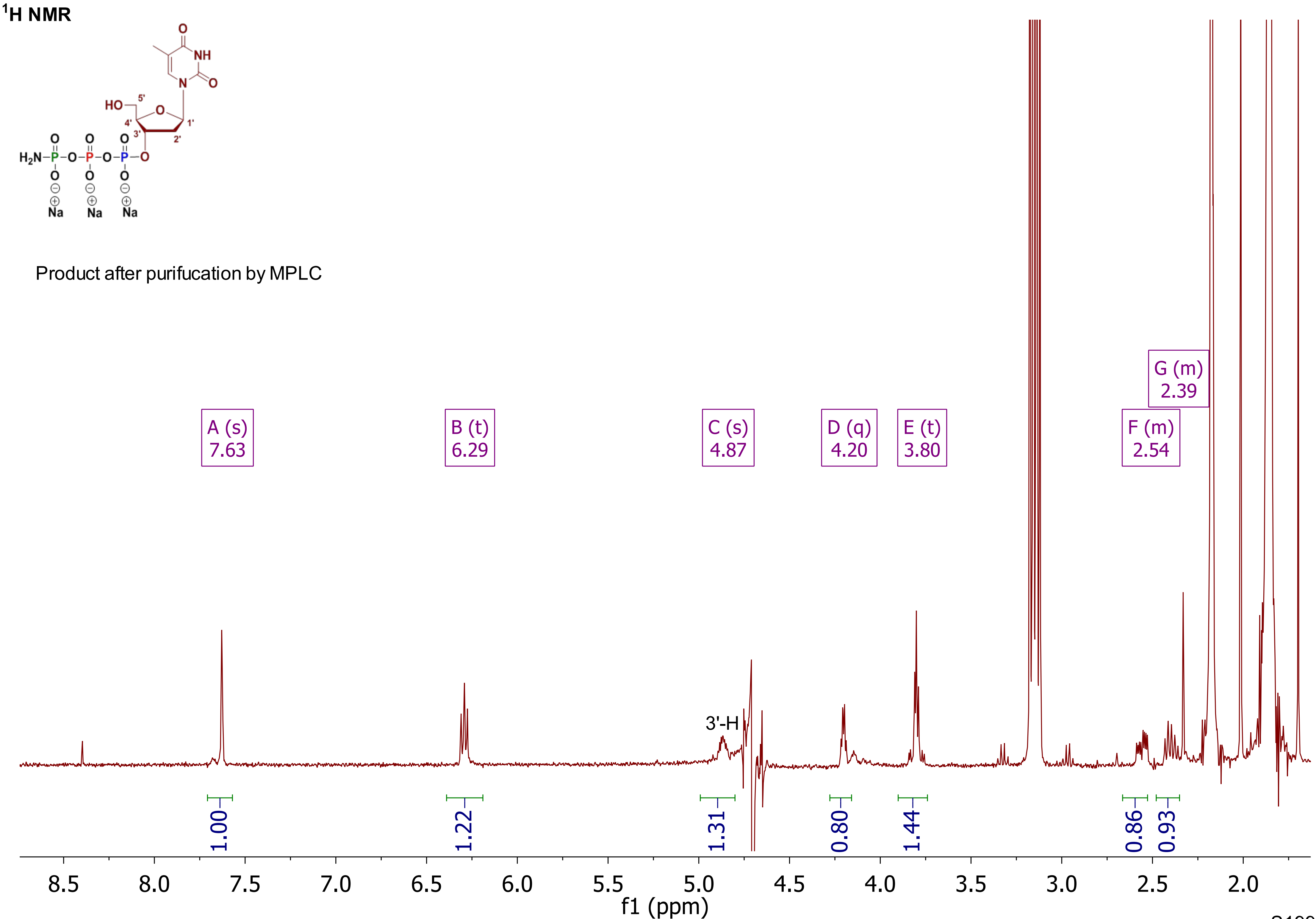


Product after purifucation by MPLC

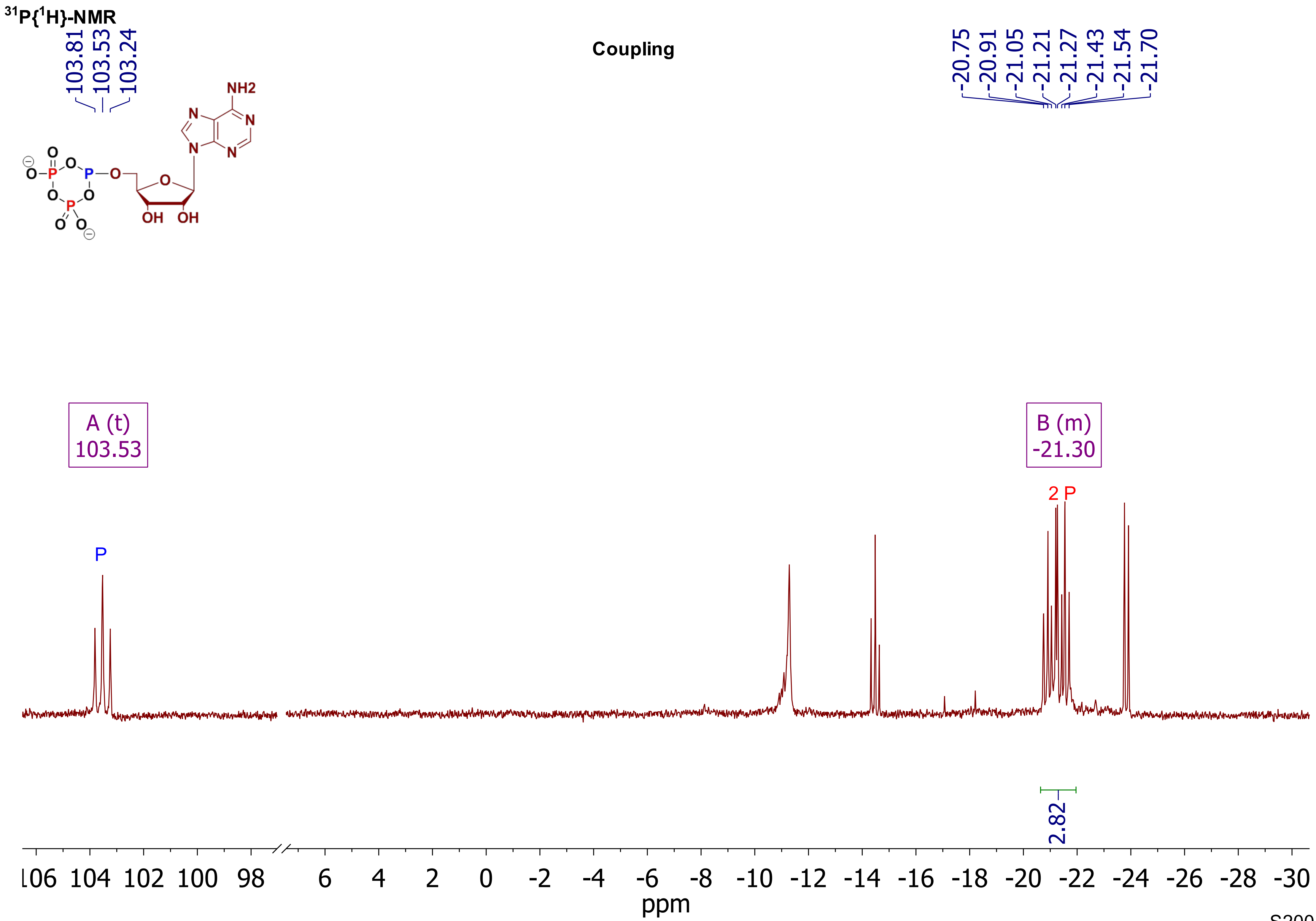


**$^{31}$P NMR**

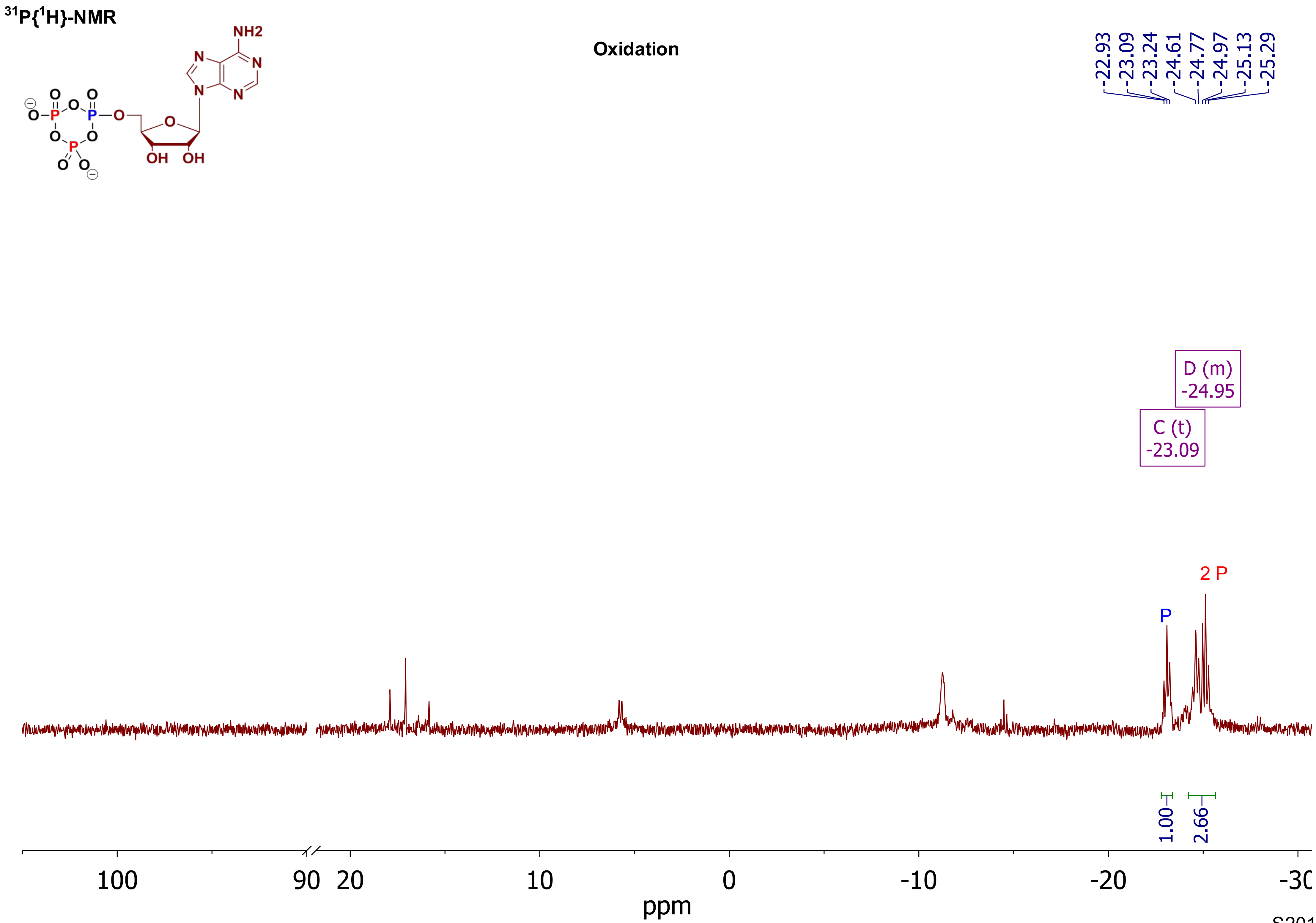

-0.82
-0.93
-11.96
-12.01
-12.08
-12.13
-22.33
-22.45
-22.56
O
NH
N
O
HO
5'
O
4'
3'
1'
2'
$H_2N$
P
O
Na
Product after purifucation by MPLC
B (d)
-0.88
A (dd)
-12.05
C (t)
-22.45
P
3'-P
P
1.18
1.00
1.14
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
-19
-20
-21
-22
-23
-24
-25
-2
f1 (ppm)



$^1$H NMR
Product after purifucation by MPLC
A (s) 7.63
B (t) 6.29
C (s) 4.87
D (q) 4.20
E (t) 3.80
F (m) 2.54
G (m) 2.39
3'-H
1.00
1.22
1.31
0.80
1.44
0.86
0.93
f1 (ppm)

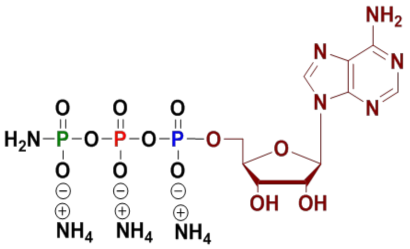


$^{31}P\{^1H\}$-NMR
Coupling
103.81
103.53
103.24
-20.75
-20.91
-21.05
-21.21
-21.27
-21.43
-21.54
-21.70
NH2
A (t)
103.53
B (m)
-21.30
P
2 P
2.82
106 104 102 100 98 6 4 2 0 -2 -4 -6 -8 -10 -12 -14 -16 -18 -20 -22 -24 -26 -28 -30
ppm

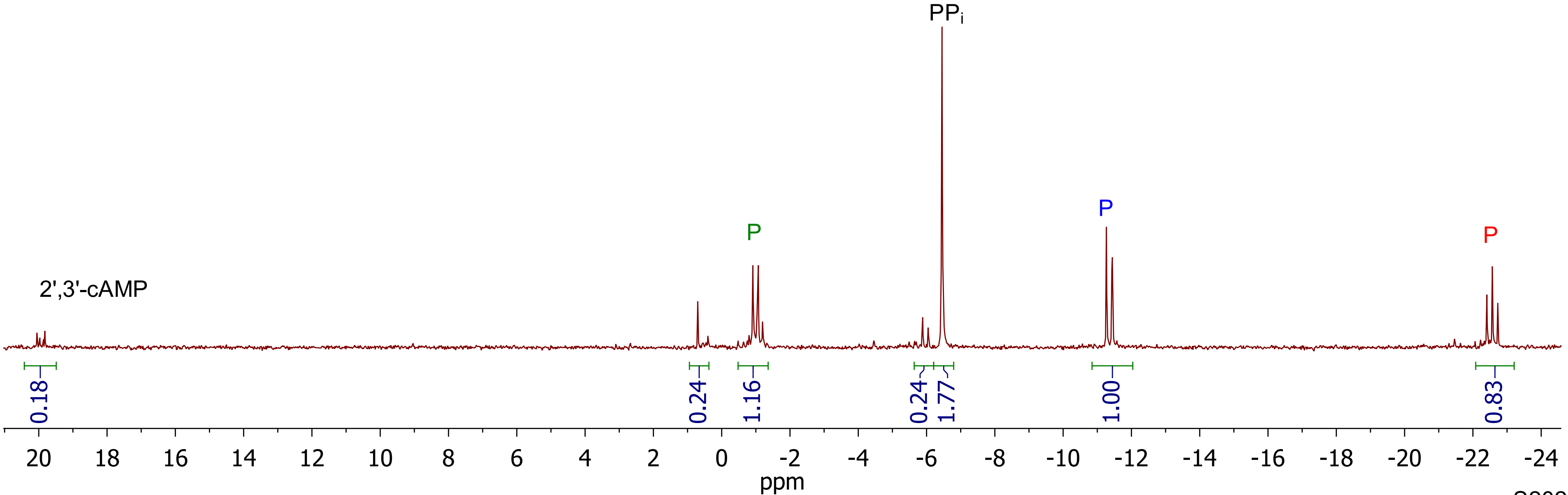


$^{31}P\{^{1}H\}$-NMR
Oxidation
NH2
-22.93
-23.09
-23.24
-24.61
-24.77
-24.97
-25.13
-25.29
D (m)
-24.95
C (t)
-23.09
2 P
P
1.00
2.66
100
90
20
10
0
-10
-20
-30
ppm

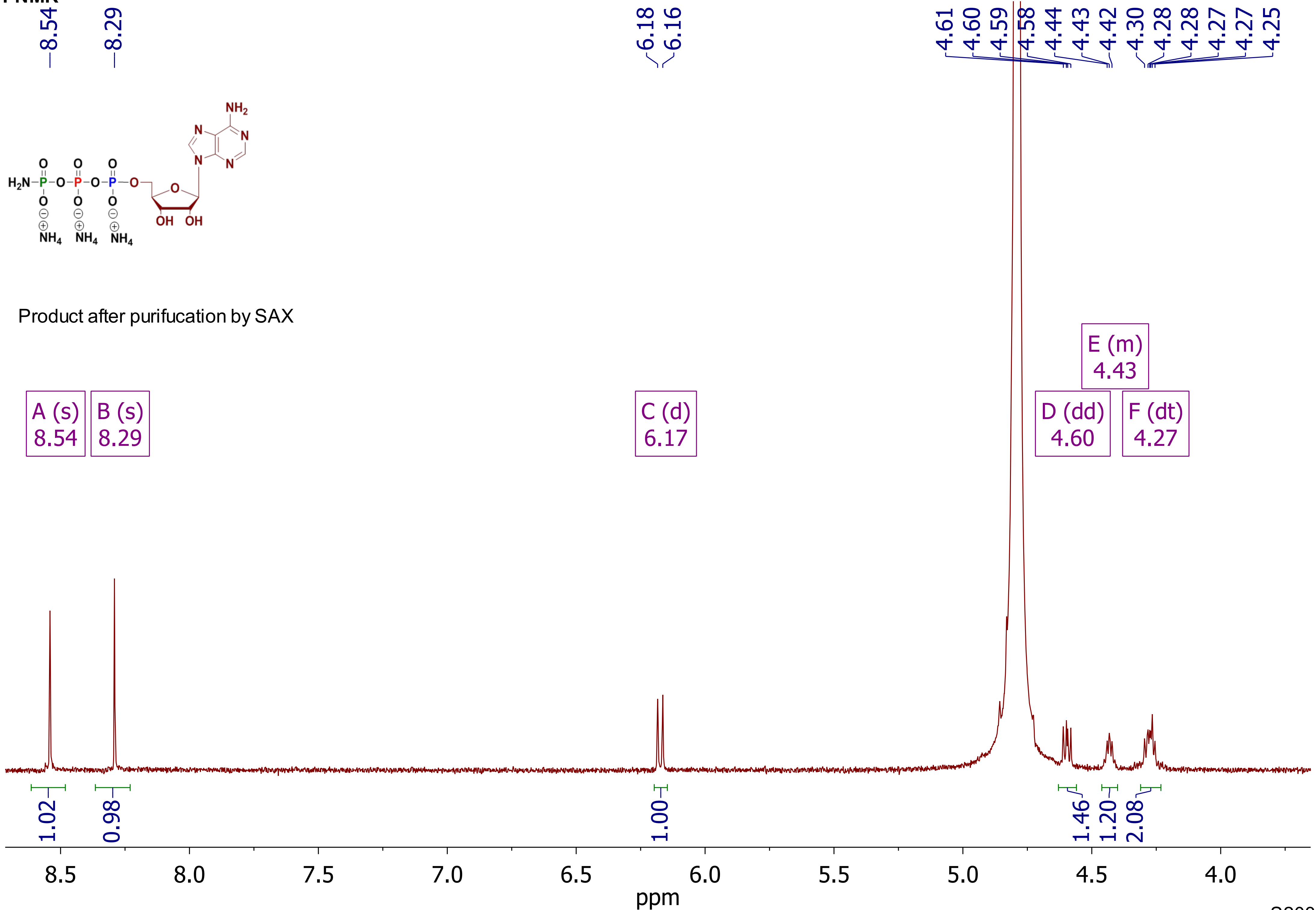
**$^{31}P\{^{1}H\}$-NMR**




Crude product after precipitation

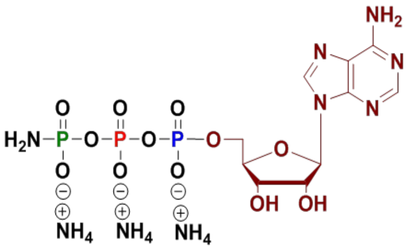


$^1$H-NMR

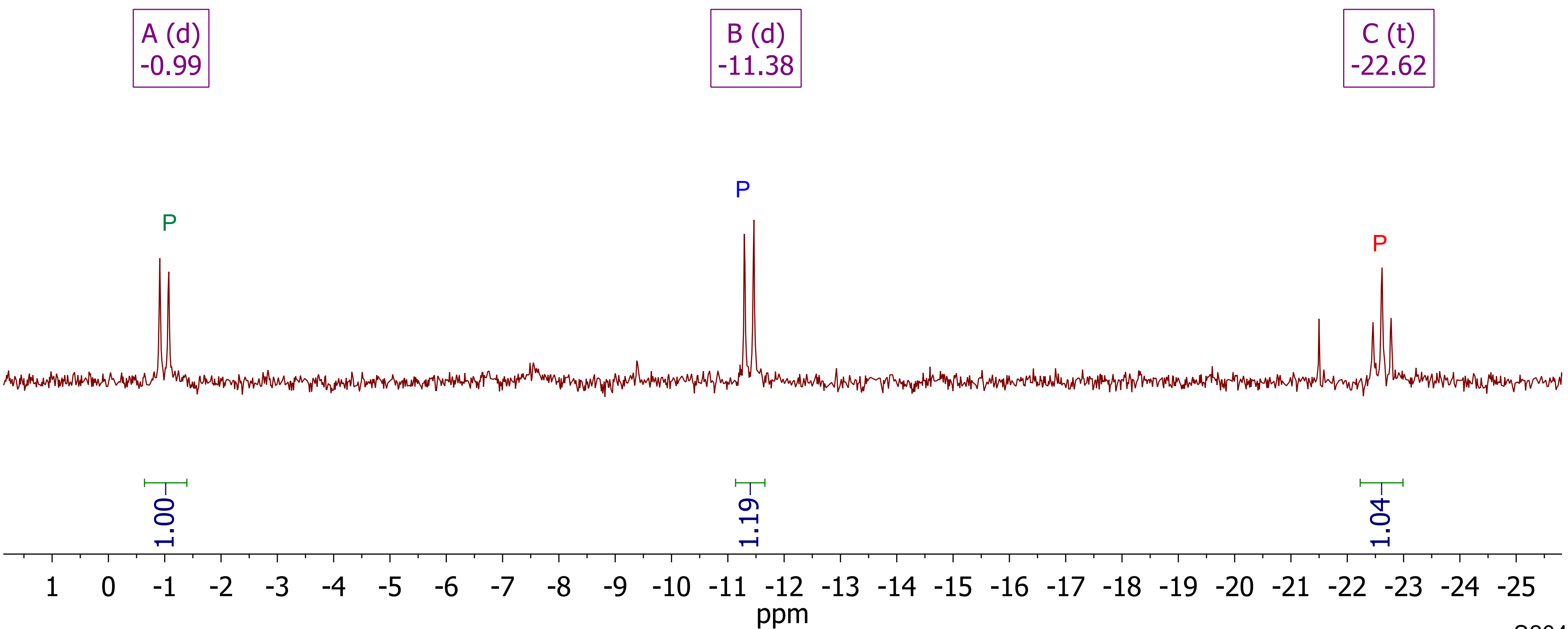


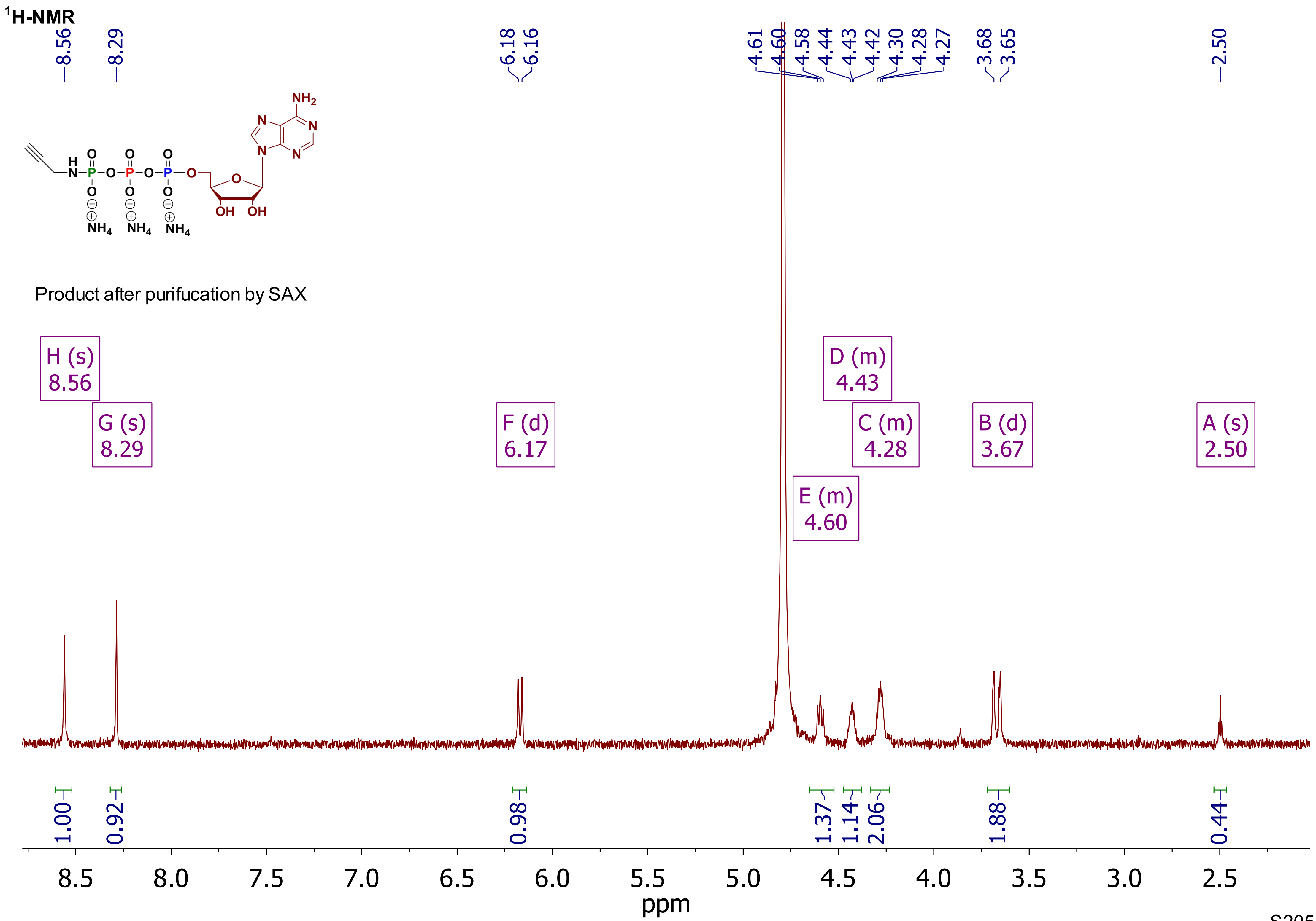
8.54
8.29
6.18
6.16
4.61
4.60
4.59
4.58
4.44
4.43
4.42
4.30
4.28
4.28
4.27
4.27
4.25
NH2
N
N
N
N
H2N
O
OH
OH
NH4
NH4
NH4
Product after purifucation by SAX
E (m)
4.43
A (s)
8.54
B (s)
8.29
C (d)
6.17
D (dd)
4.60
F (dt)
4.27
1.02
0.98
1.00
1.46
1.20
2.08
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm

**$^{31}P\{^1H\}$-NMR**

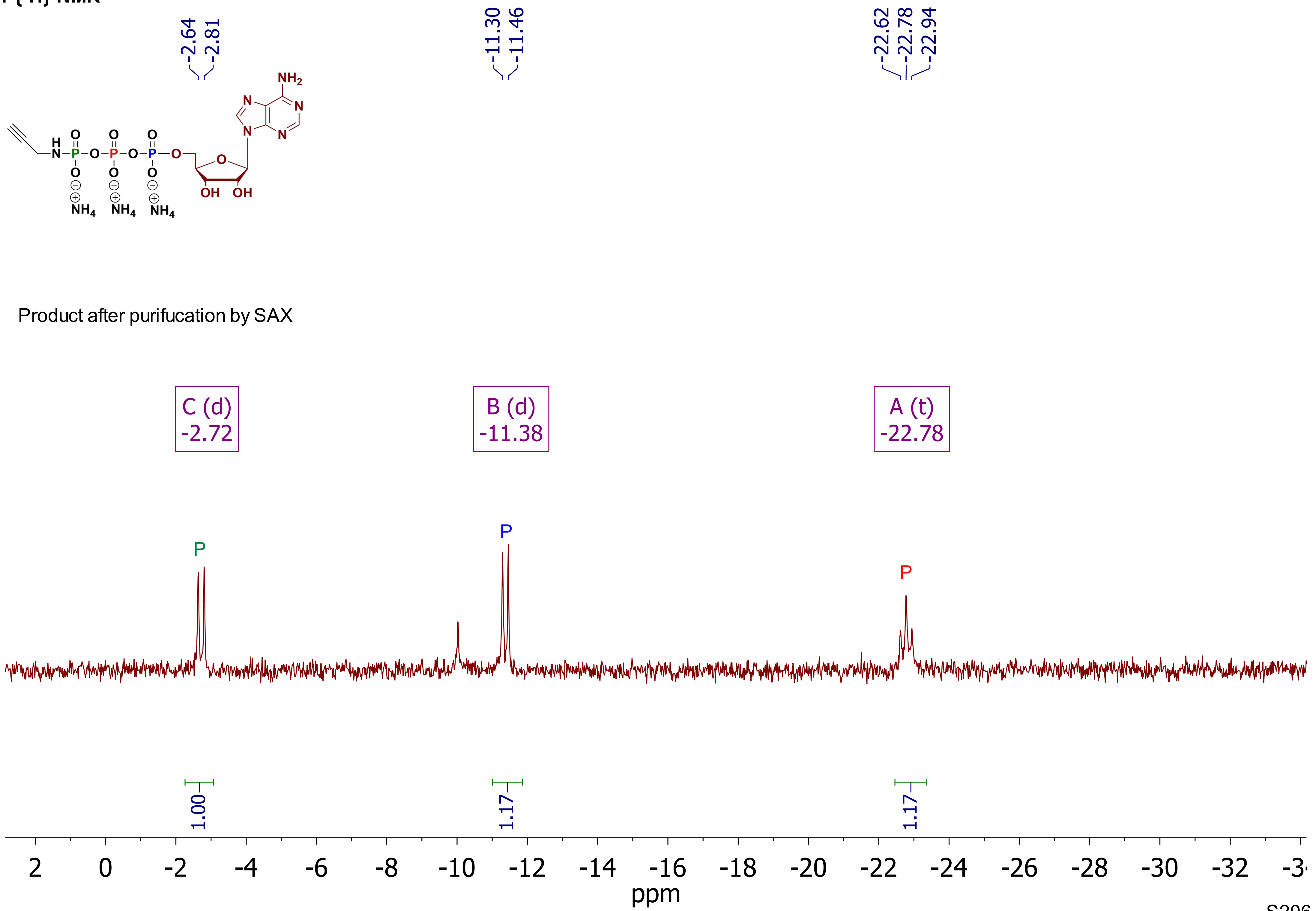


Product after purifucation by SAX

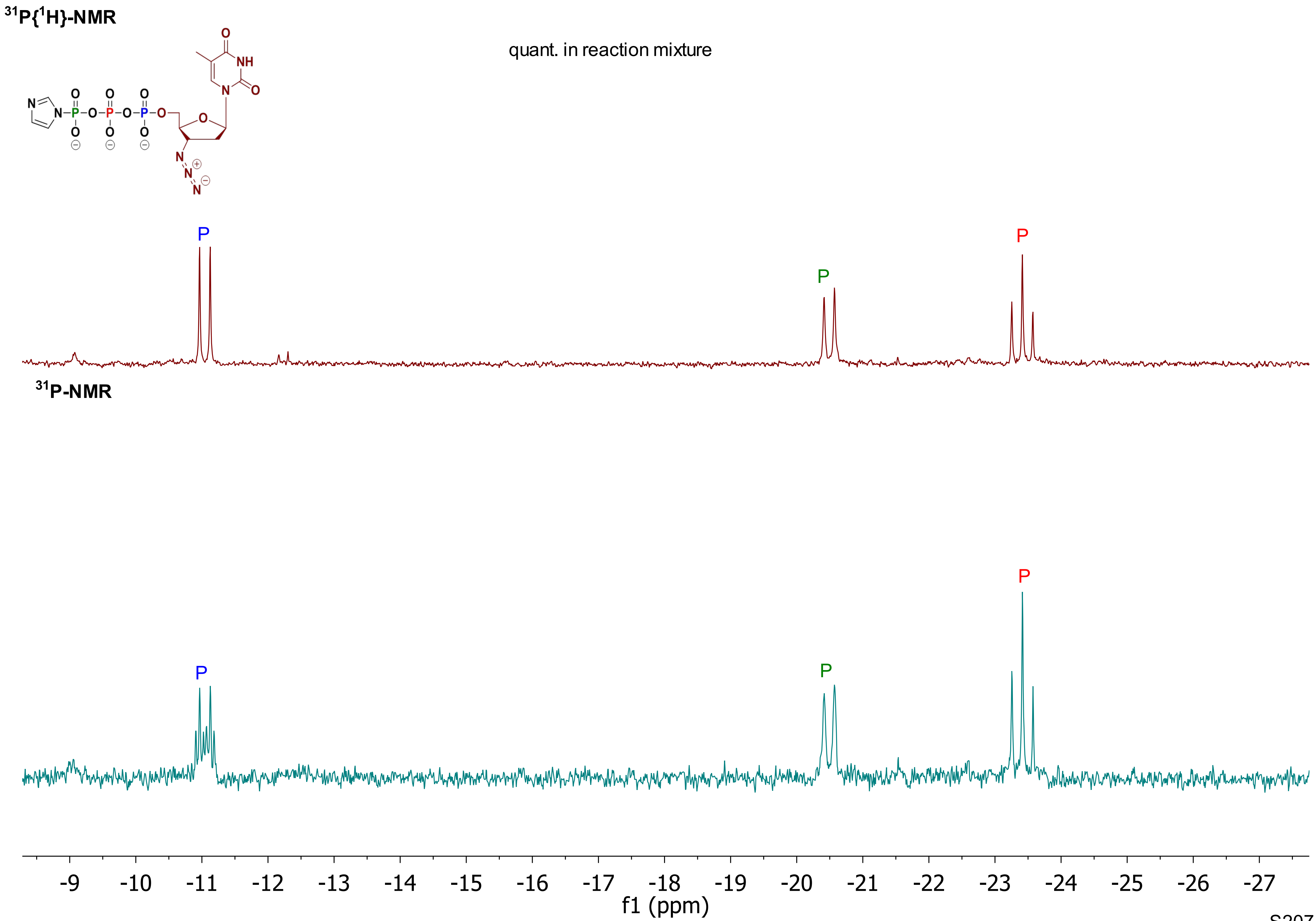



1H-NMR
8.56
8.29
6.18
6.16
4.61
4.60
4.58
4.44
4.43
4.42
4.30
4.28
4.27
3.68
3.65
2.50
NH2
N
N
N
N
H
N
P
O
O
O
O
P
O
O
O
P
O
O
O
O
OH
OH
NH4
NH4
NH4
Product after purifucation by SAX
H (s)
8.56
G (s)
8.29
F (d)
6.17
D (m)
4.43
C (m)
4.28
E (m)
4.60
B (d)
3.67
A (s)
2.50
1.00
0.92
0.98
1.37
1.14
2.06
1.88
0.44
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm

**$^{31}P\{^{1}H\}$-NMR**

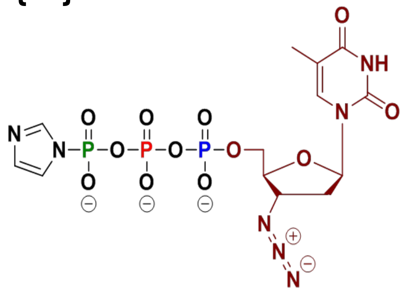


-2.64
-2.81
-11.30
-11.46
-22.62
-22.78
-22.94
Product after purifucation by SAX
C (d)
-2.72
B (d)
-11.38
A (t)
-22.78
P
P
P
1.00
1.17
1.17
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
ppm

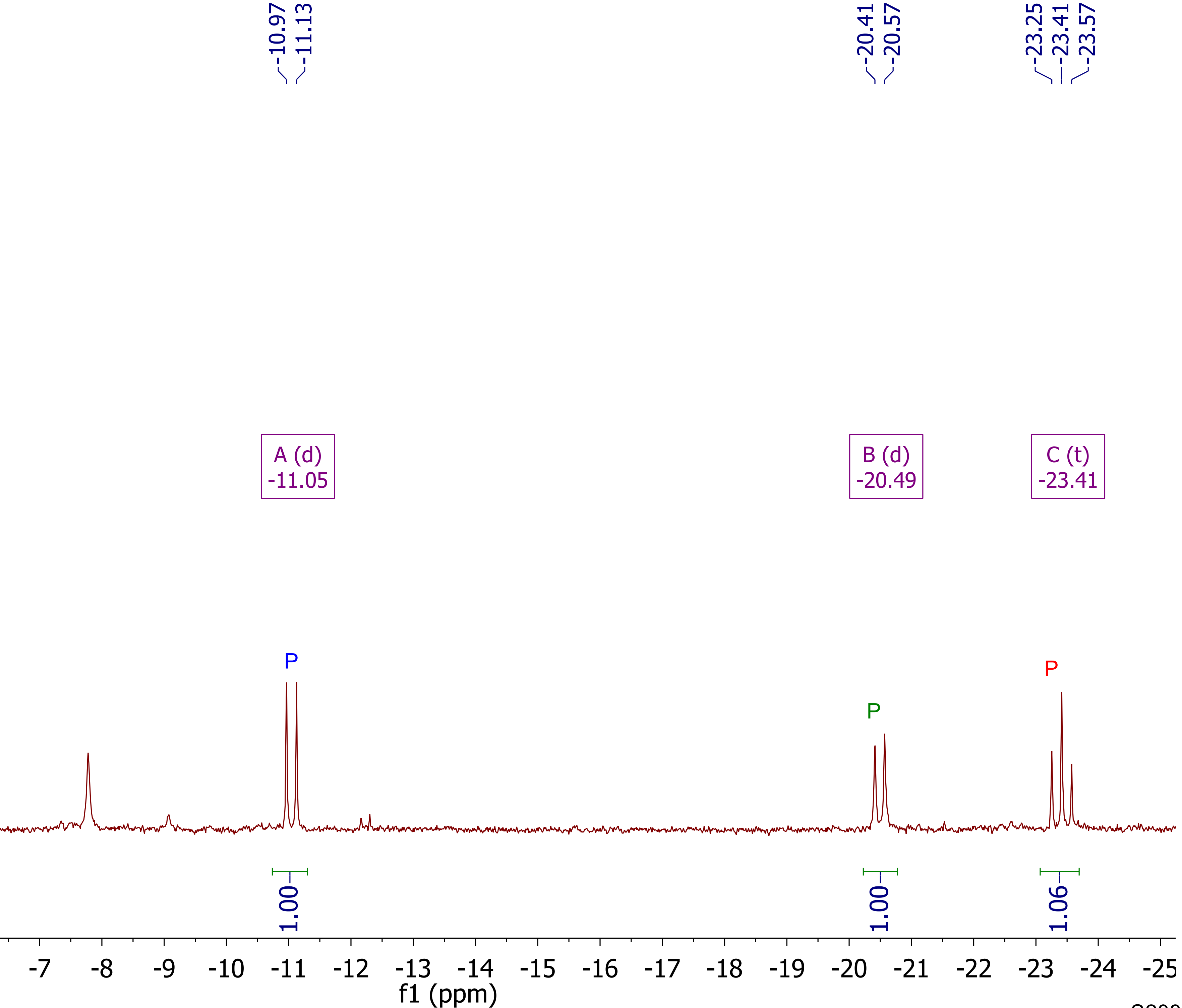


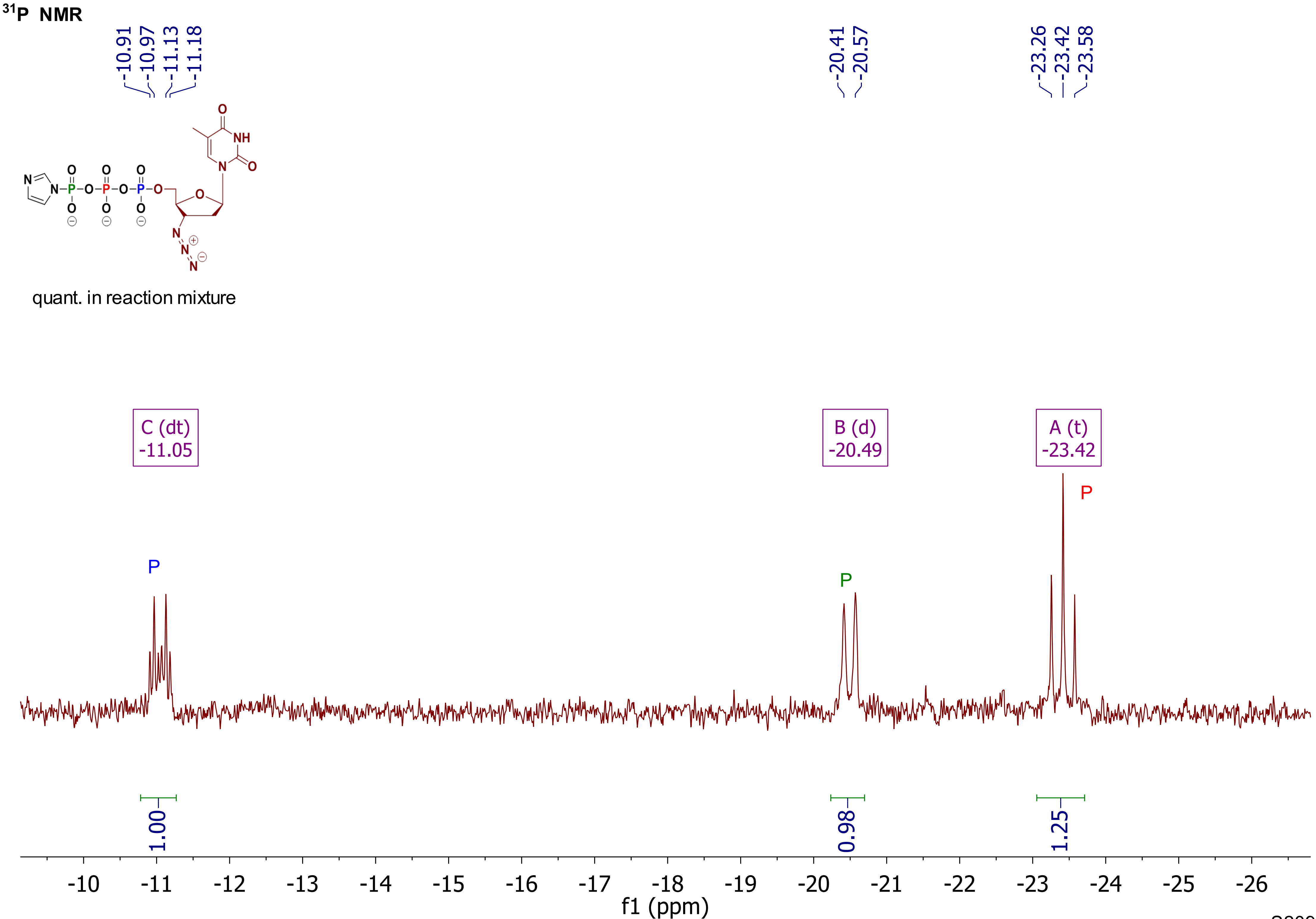
31P{1H}-NMR
quant. in reaction mixture
31P-NMR
P
P
P
P
P
P
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
-19
-20
-21
-22
-23
-24
-25
-26
-27
f1 (ppm)

$^{31}P\{^{1}H\}$-NMR



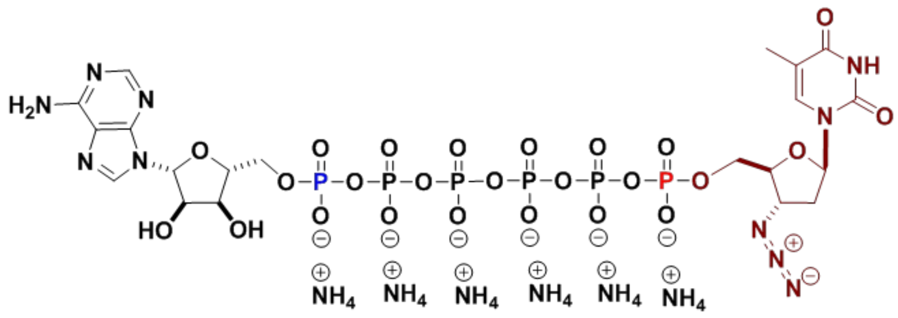

quant. in reaction mixture



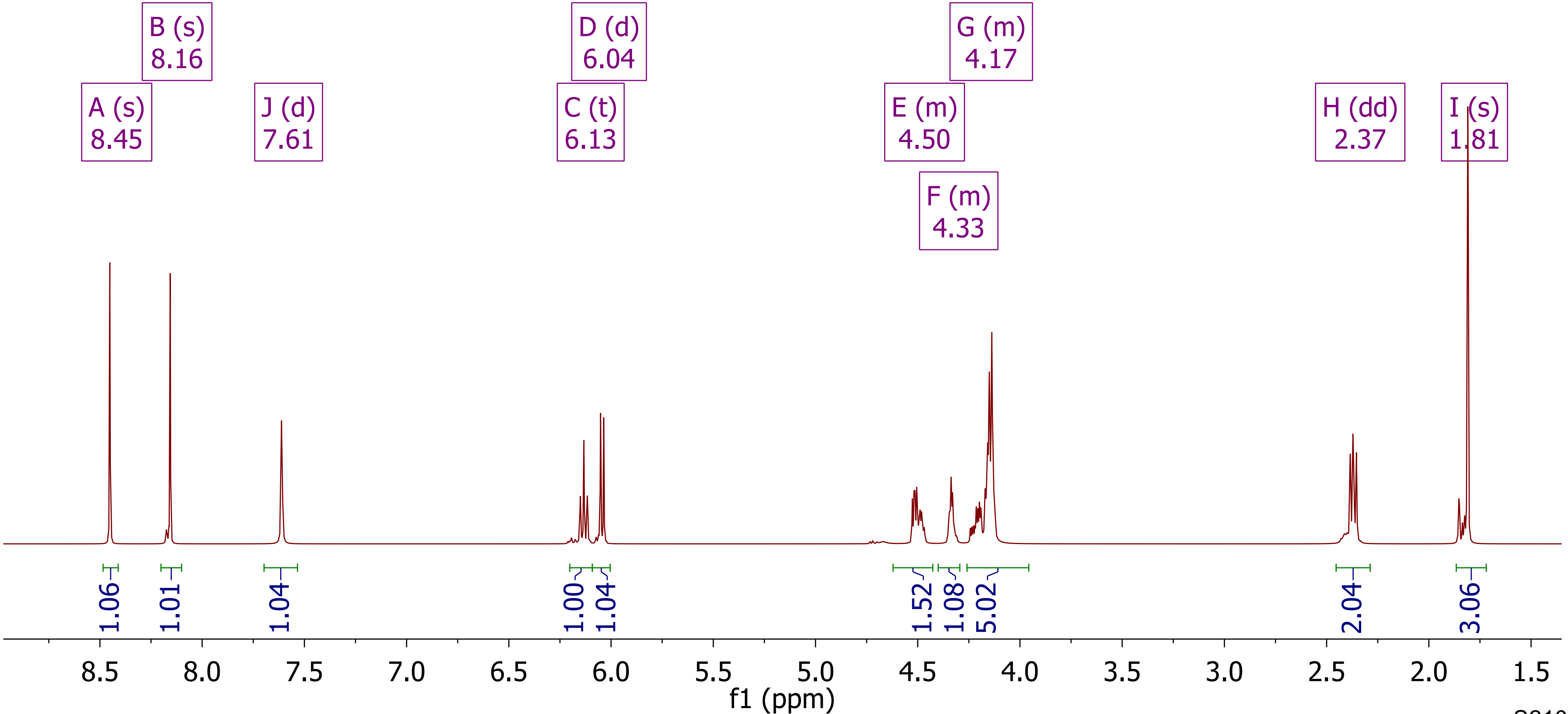


31P NMR
-10.91
-10.97
-11.13
-11.18
-20.41
-20.57
-23.26
-23.42
-23.58
O
NH
N
O
N
N
P
P
P
O
O
O
O
O
O
O
O
O
O
O
N
N
N
quant. in reaction mixture
C (dt)
-11.05
B (d)
-20.49
A (t)
-23.42
P
P
P
1.00
0.98
1.25
-10
-11
-12
-13
-14
-15
-16
-17
-18
-19
-20
-21
-22
-23
-24
-25
-26
f1 (ppm)

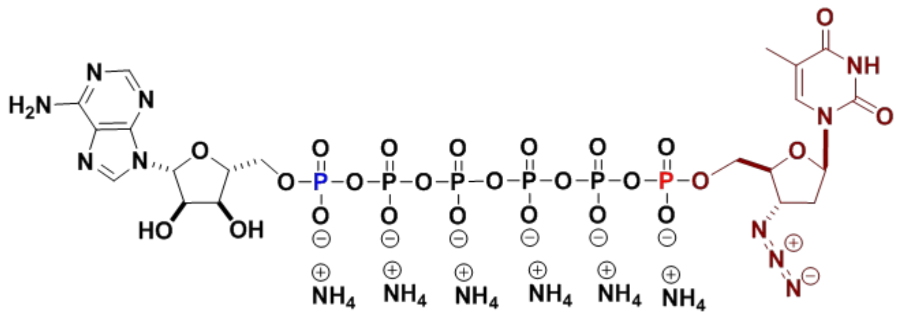
**$^1$H-NMR**



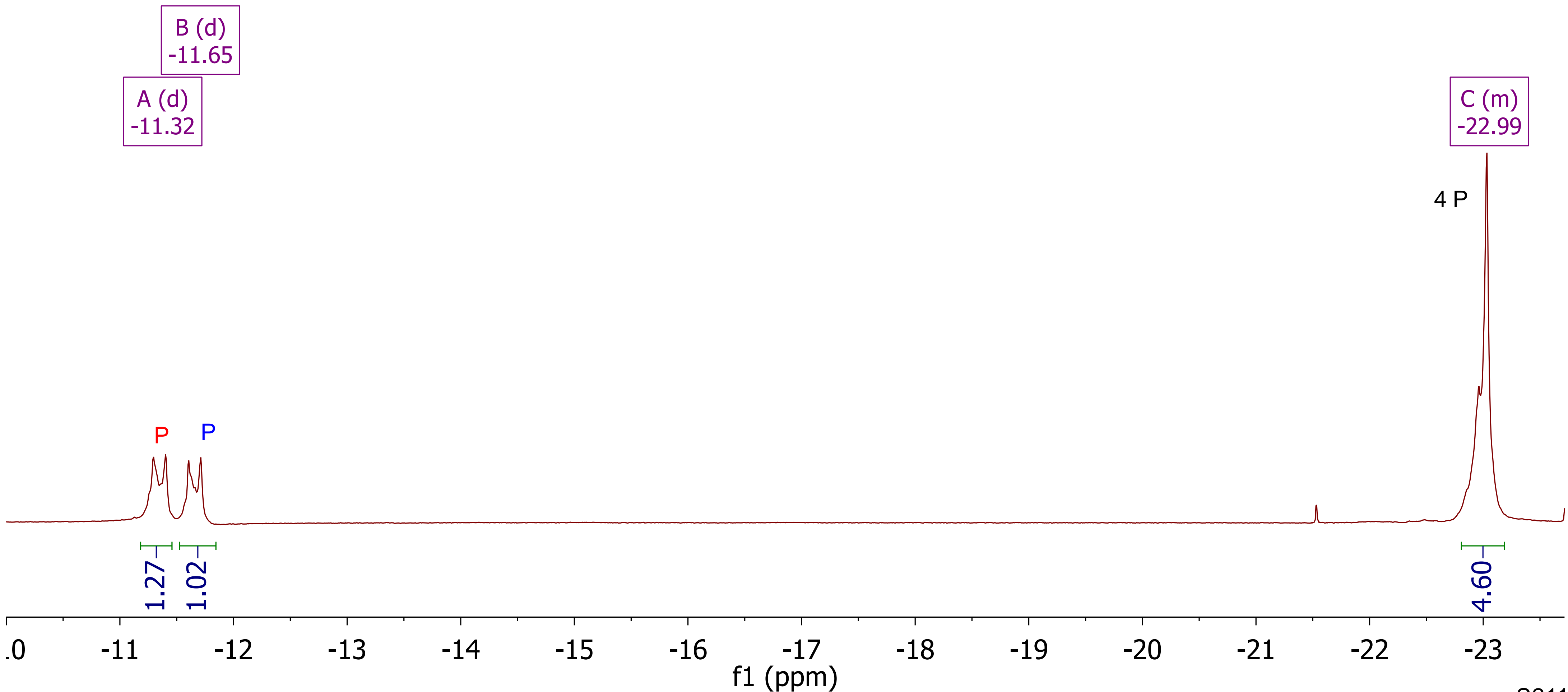
Product after purifucation by SAX



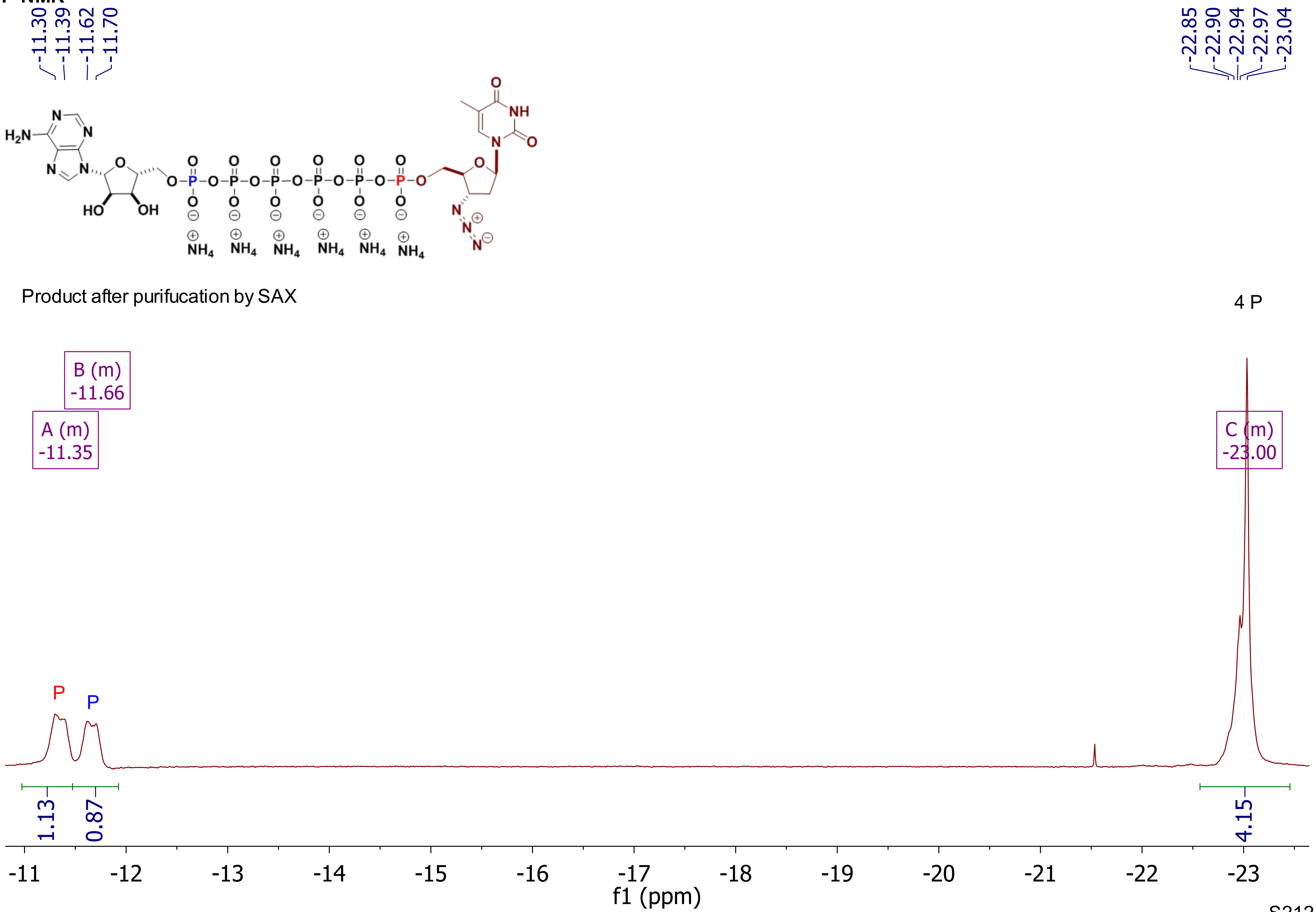
**$^{31}P\{^1H\}$-NMR**



Product after purifucation by SAX

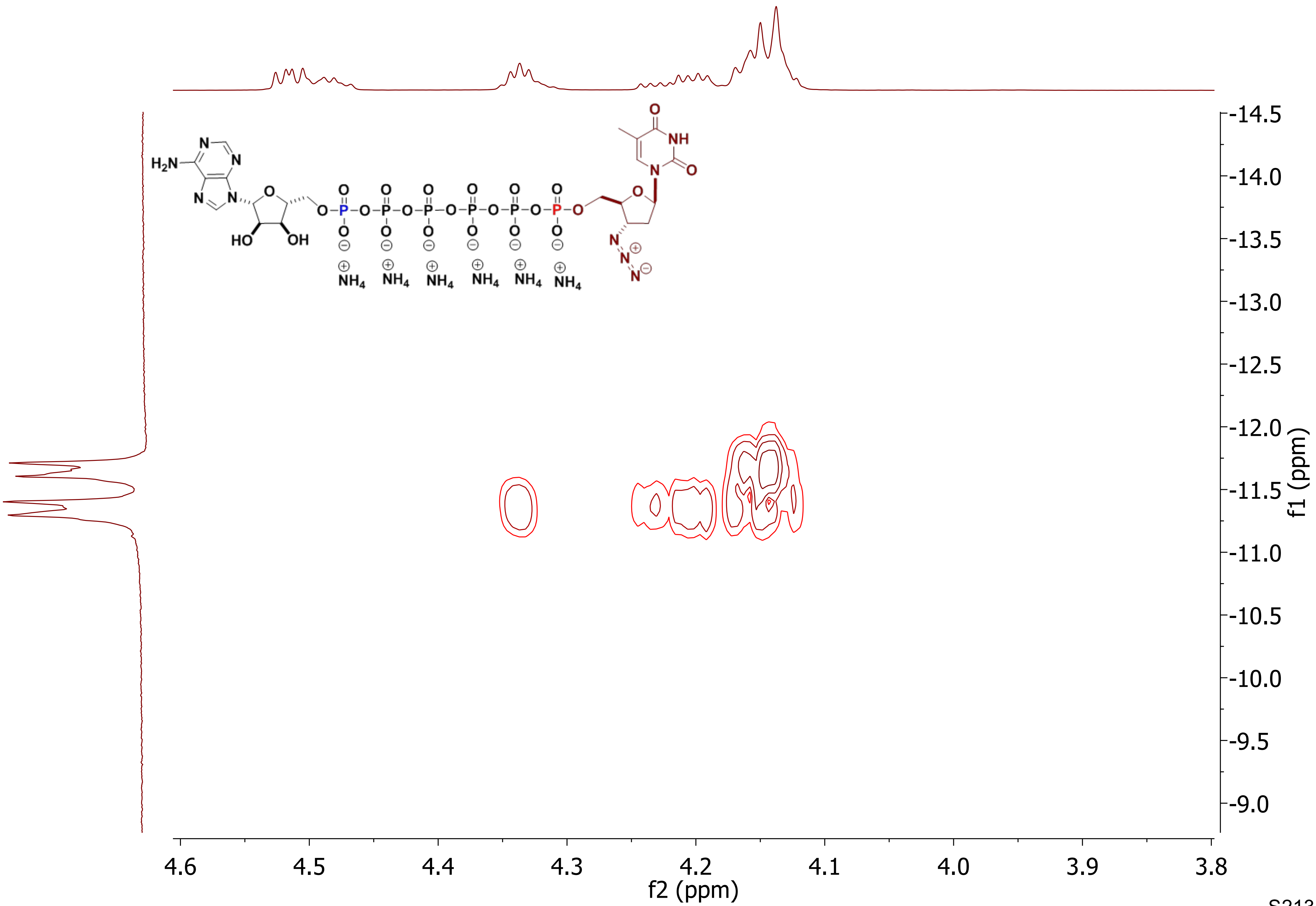


**$^{31}$P-NMR**

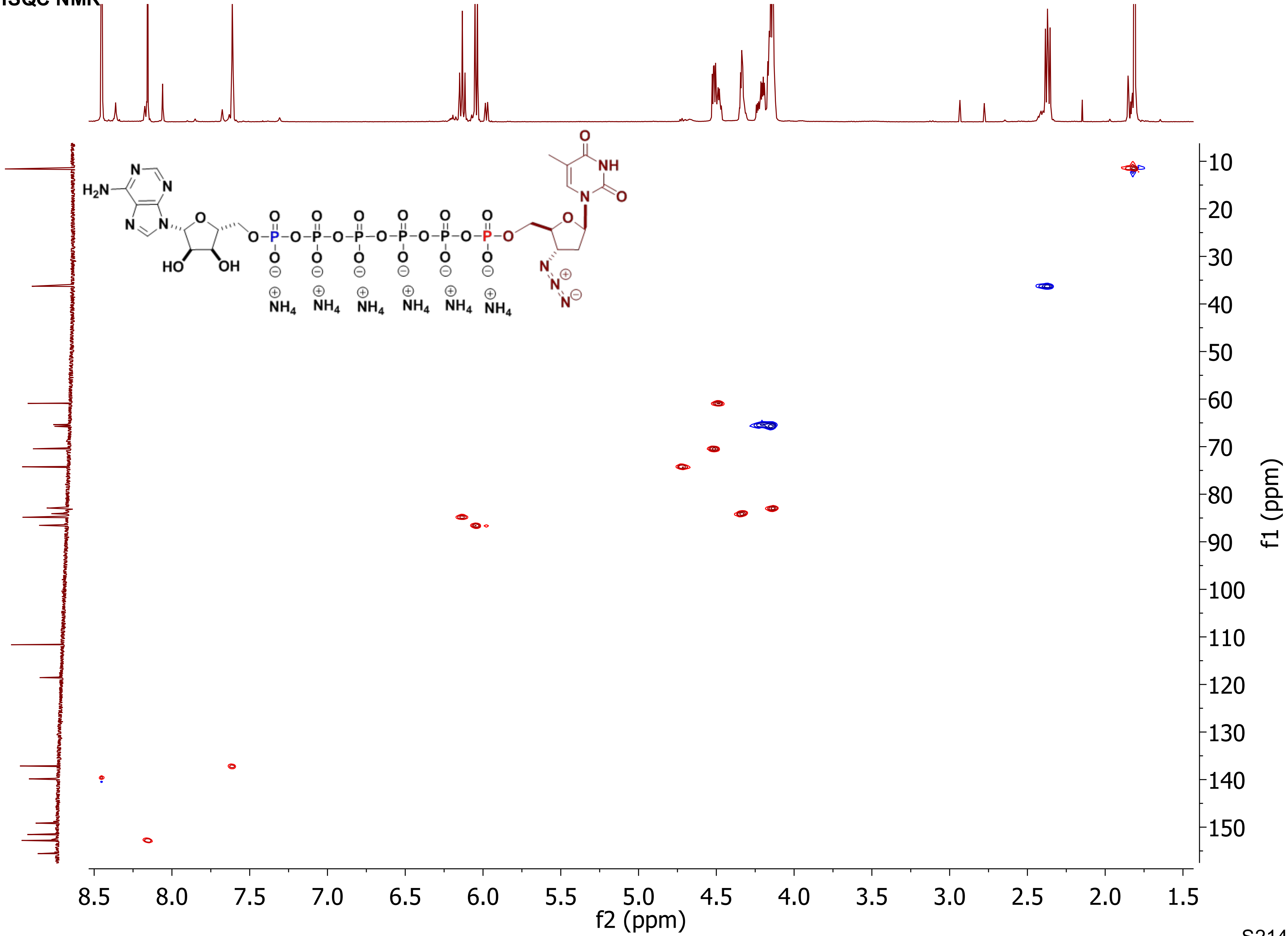


-11.30
-11.39
-11.62
-11.70
-22.85
-22.90
-22.94
-22.97
-23.04
Product after purifucation by SAX
4 P
B (m) -11.66
A (m) -11.35
C (m) -23.00
P
P
1.13
0.87
4.15
f1 (ppm)

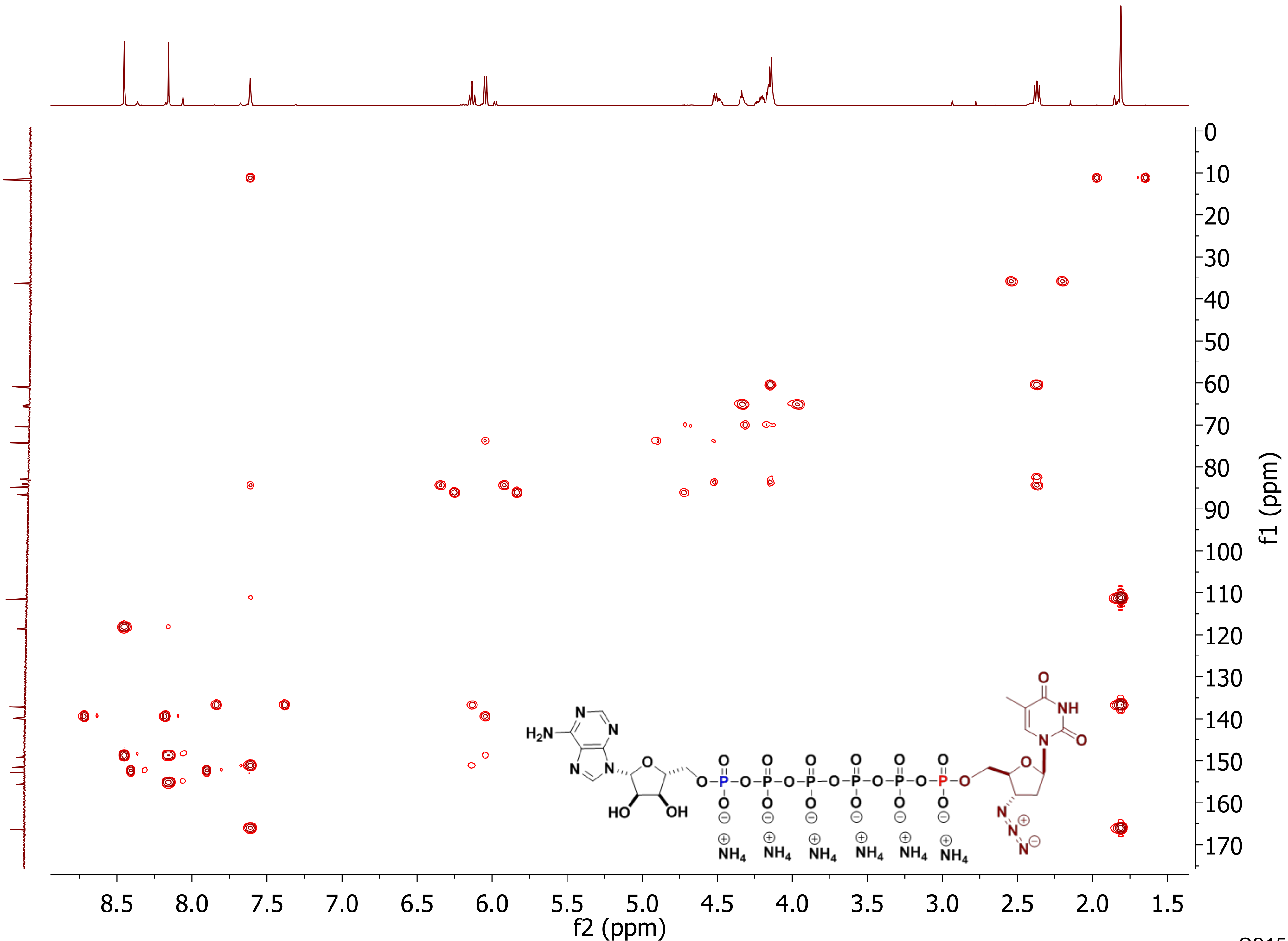
$^{31}P$-$^{1}H$ HMBC NMR



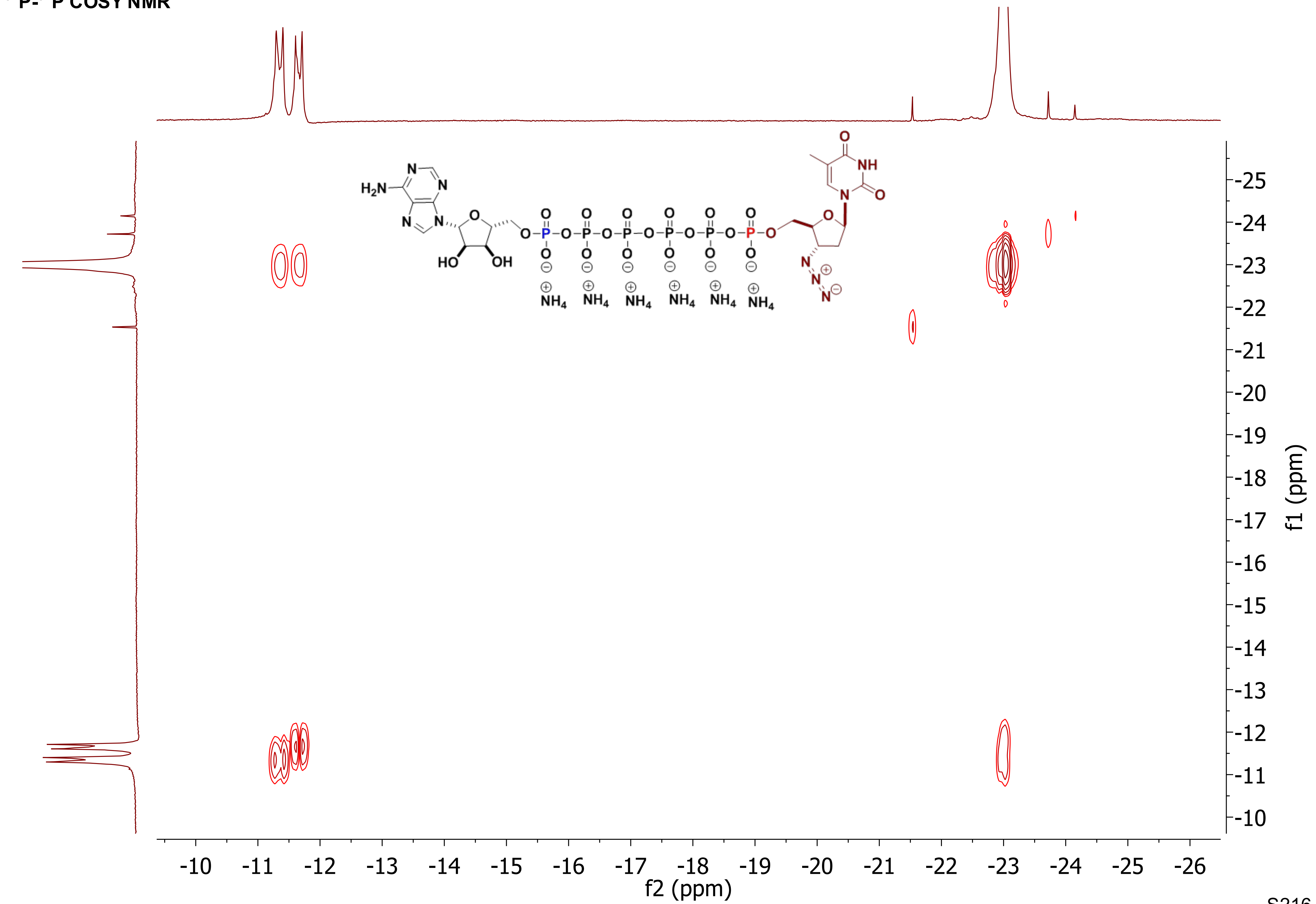
$^{13}$C-$^{1}$H HSQC NMR



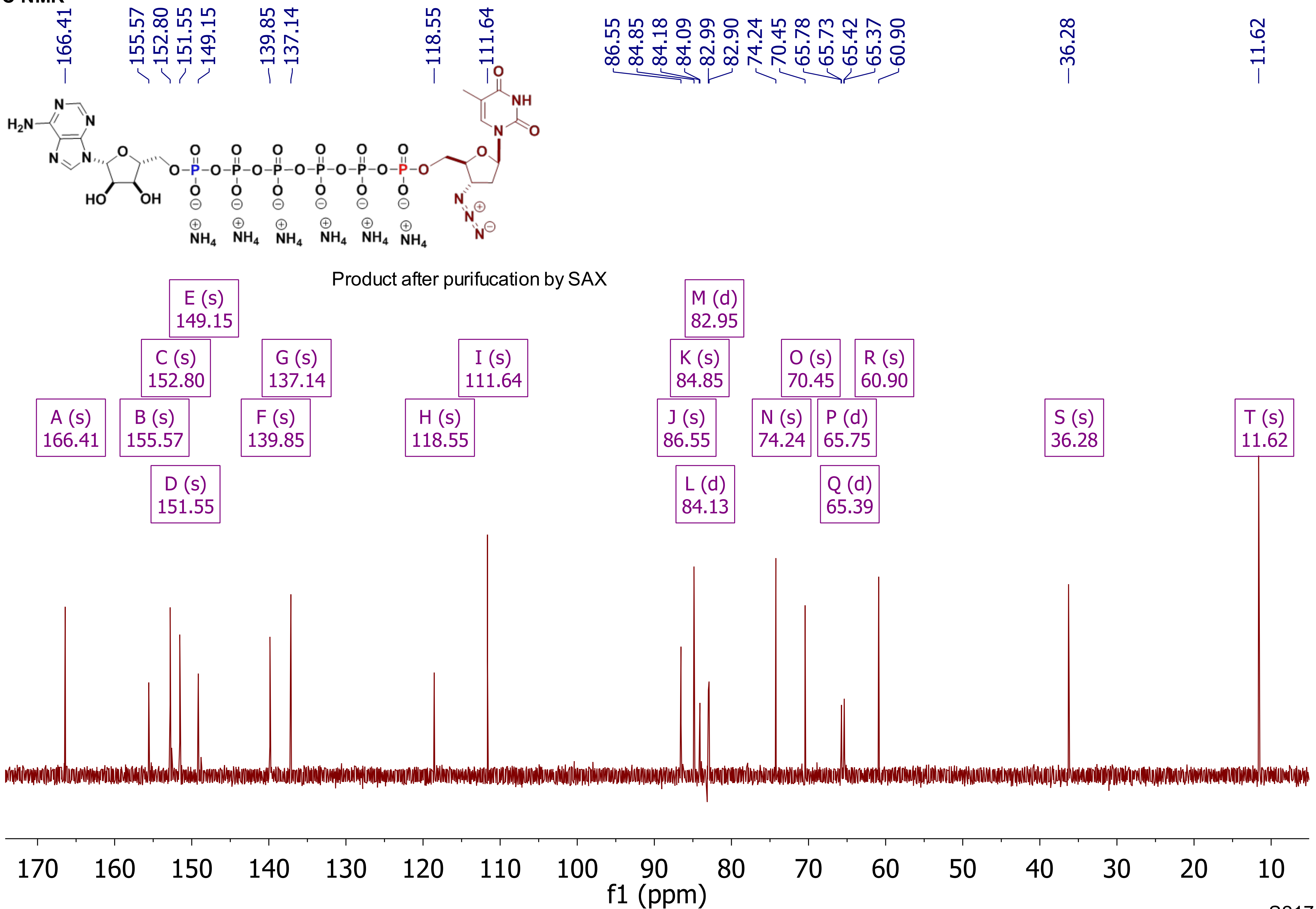
## $^{13}C$-$^{1}H$ HMBC NMR



$^{31}P$-$^{31}P$ COSY NMR

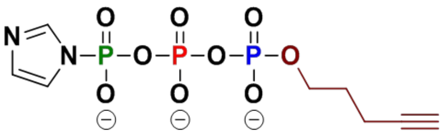


$^{13}C$ NMR

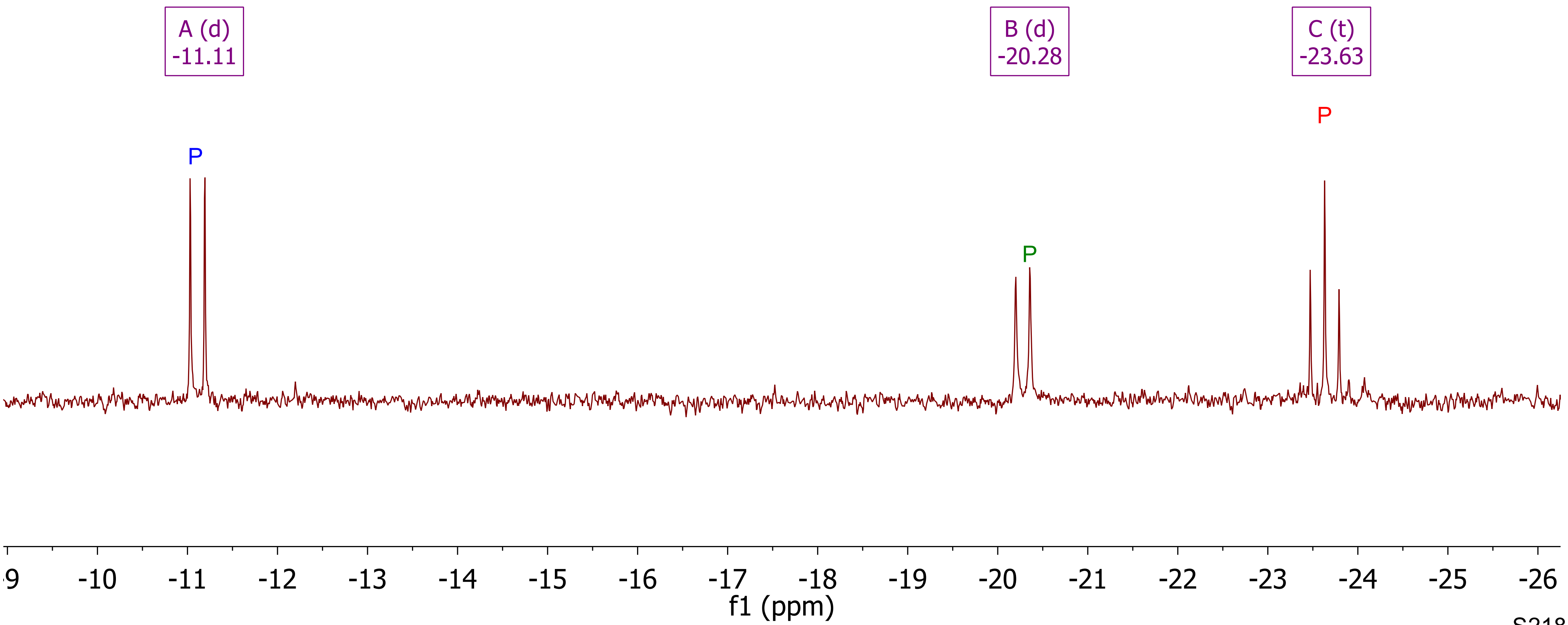


166.41
155.57
152.80
151.55
149.15
139.85
137.14
118.55
111.64
86.55
84.85
84.18
84.09
82.99
82.90
74.24
70.45
65.78
65.73
65.42
65.37
60.90
36.28
11.62
Product after purifucation by SAX
E (s) 149.15
M (d) 82.95
C (s) 152.80
G (s) 137.14
I (s) 111.64
K (s) 84.85
O (s) 70.45
R (s) 60.90
A (s) 166.41
B (s) 155.57
F (s) 139.85
H (s) 118.55
J (s) 86.55
N (s) 74.24
P (d) 65.75
S (s) 36.28
T (s) 11.62
D (s) 151.55
L (d) 84.13
Q (d) 65.39
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

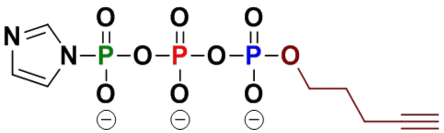
**$^{31}P\{^1H\}$-NMR**



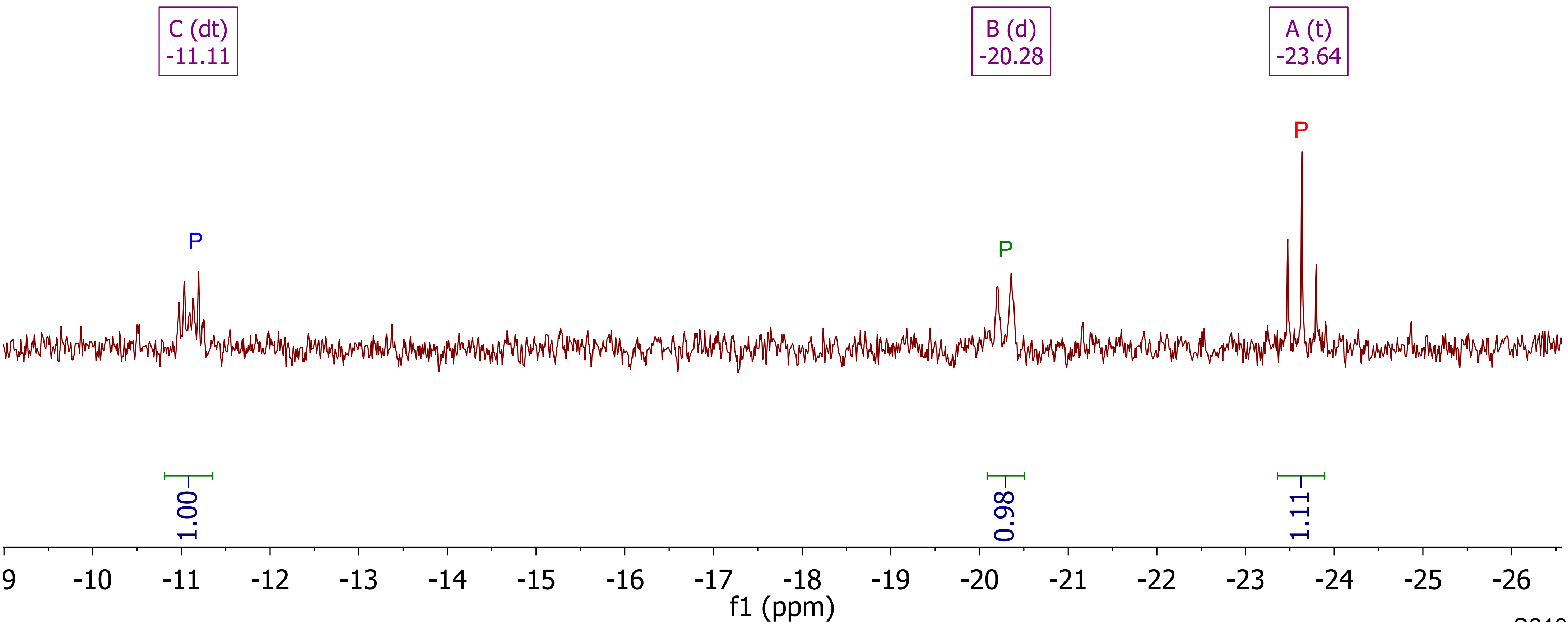
quant. in reaction mixture



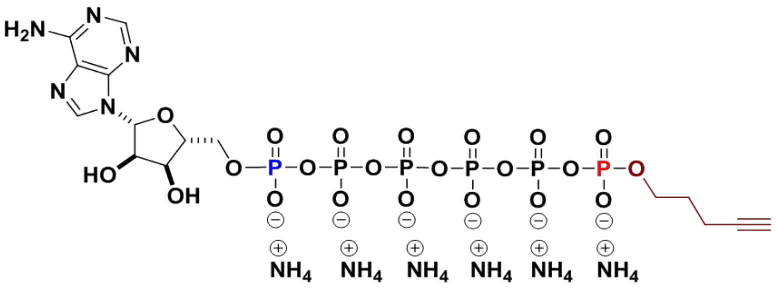
$^{31}$P-$^{1}$H NMR



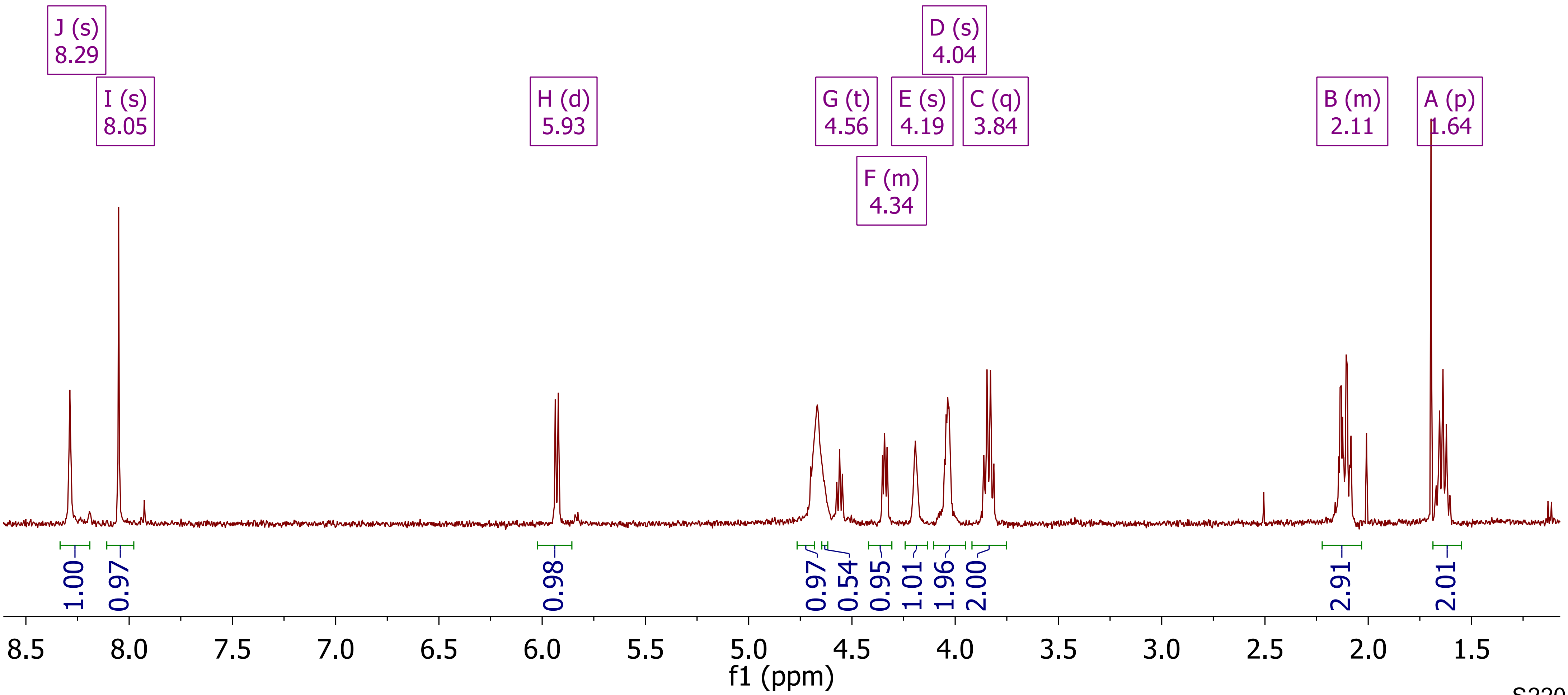
quant. in reaction mixture



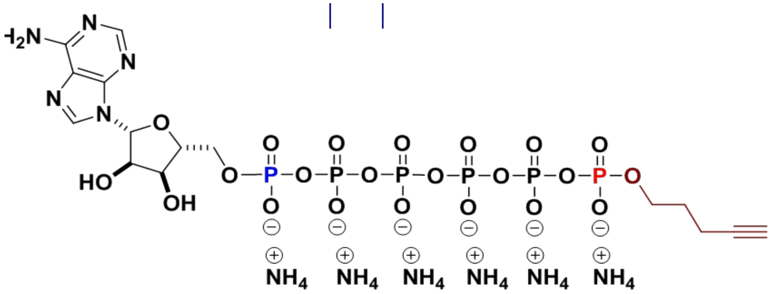
$^1$H-NMR



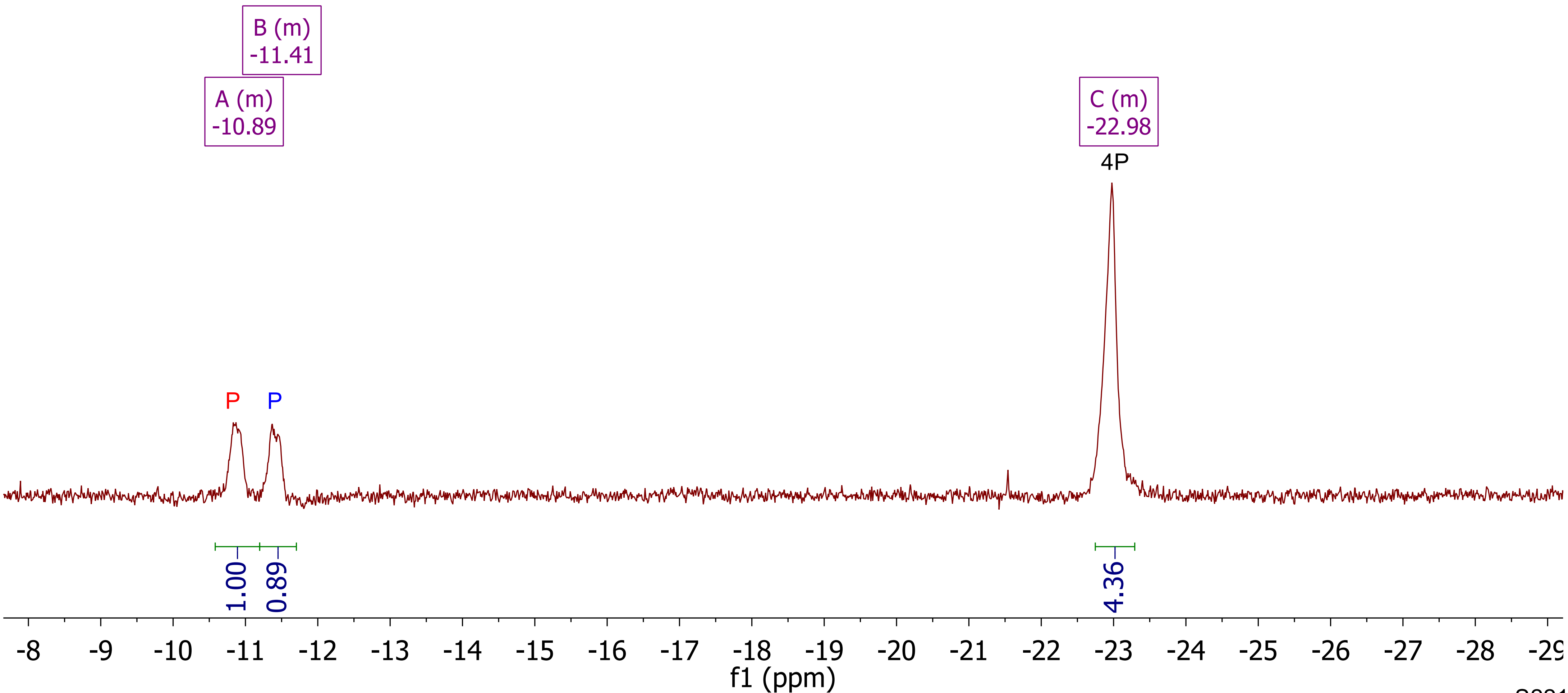
Product after purifucation by SAX



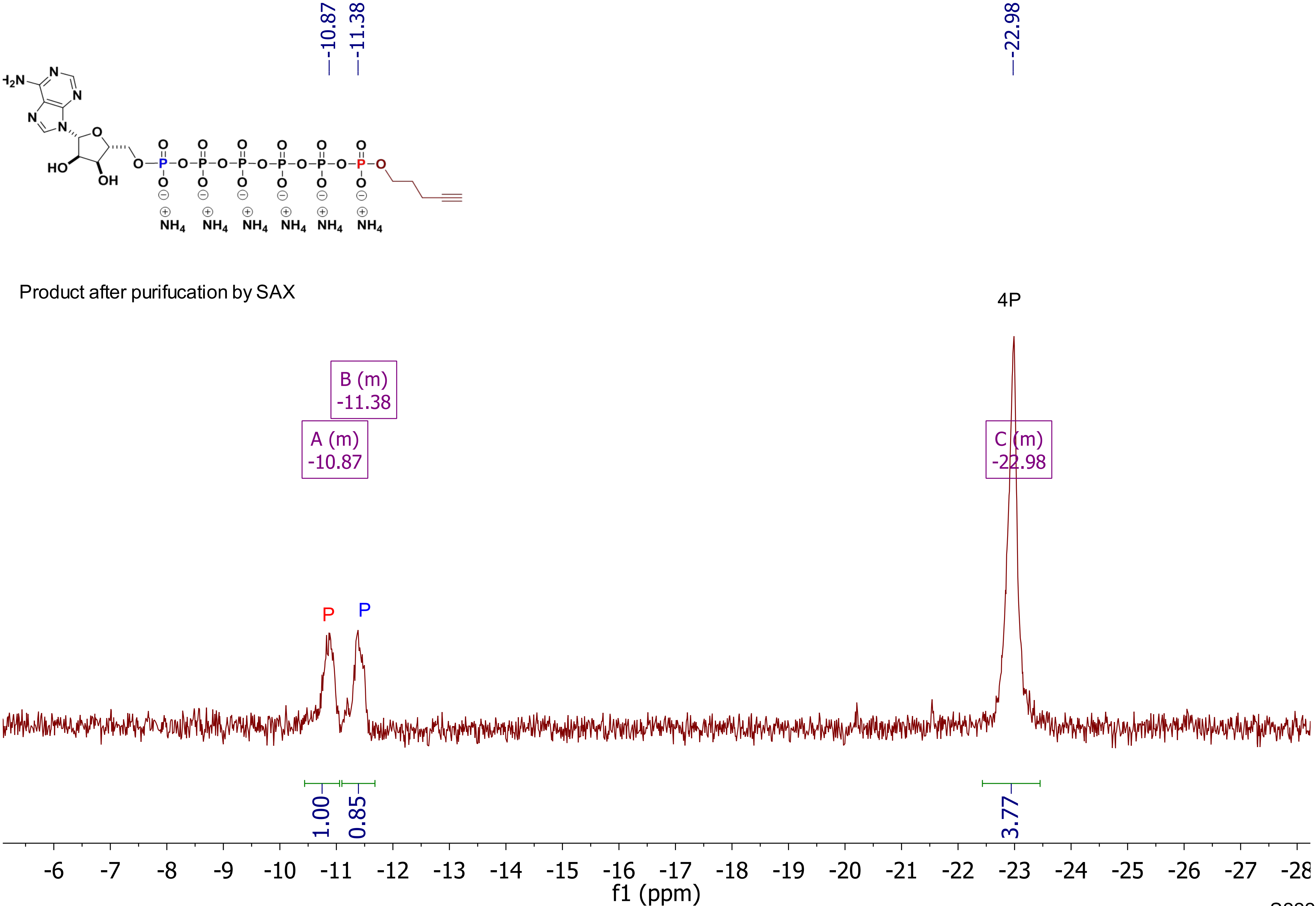
**$^{31}P\{^{1}H\}$-NMR**



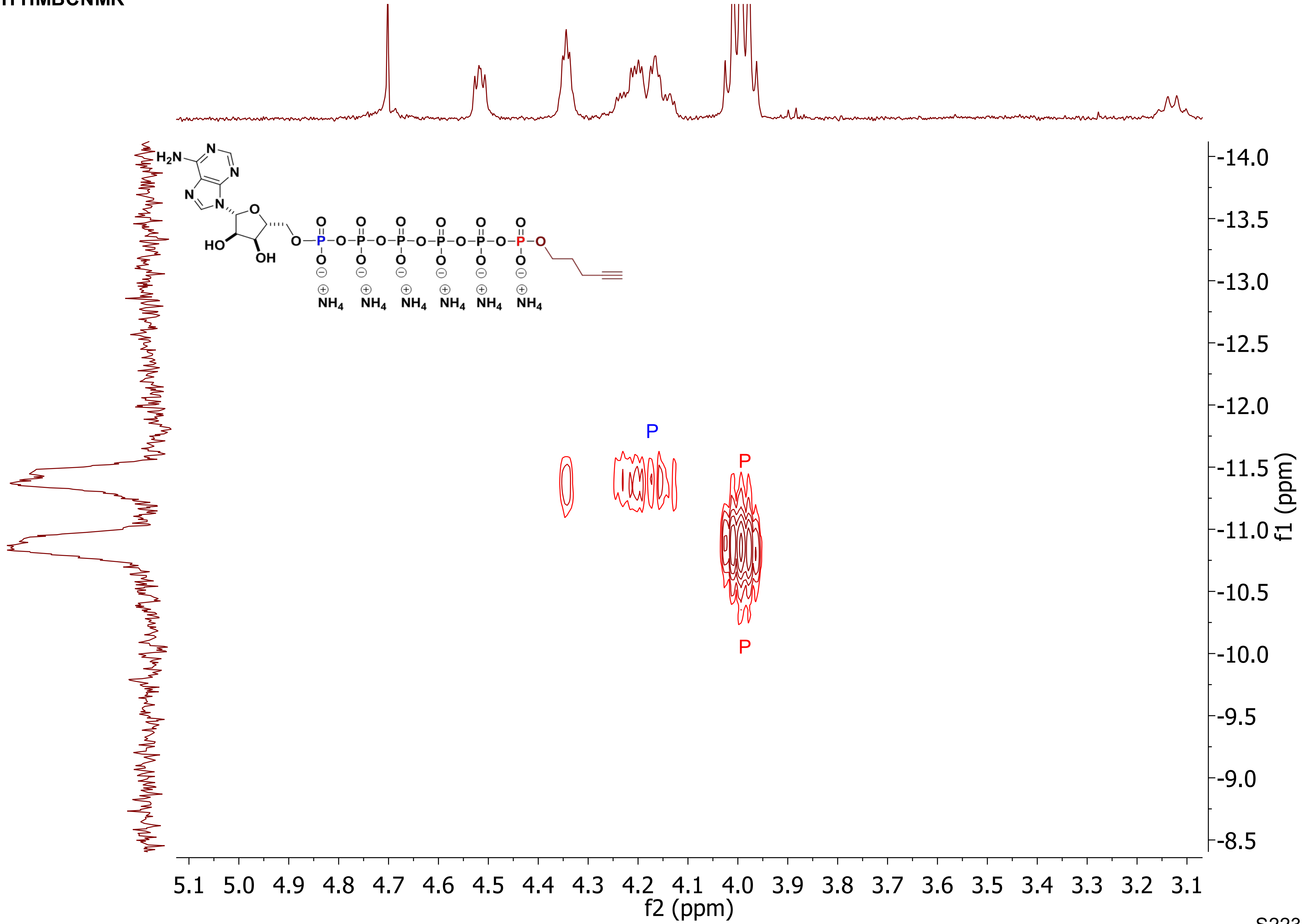

Product after purifucation by SAX



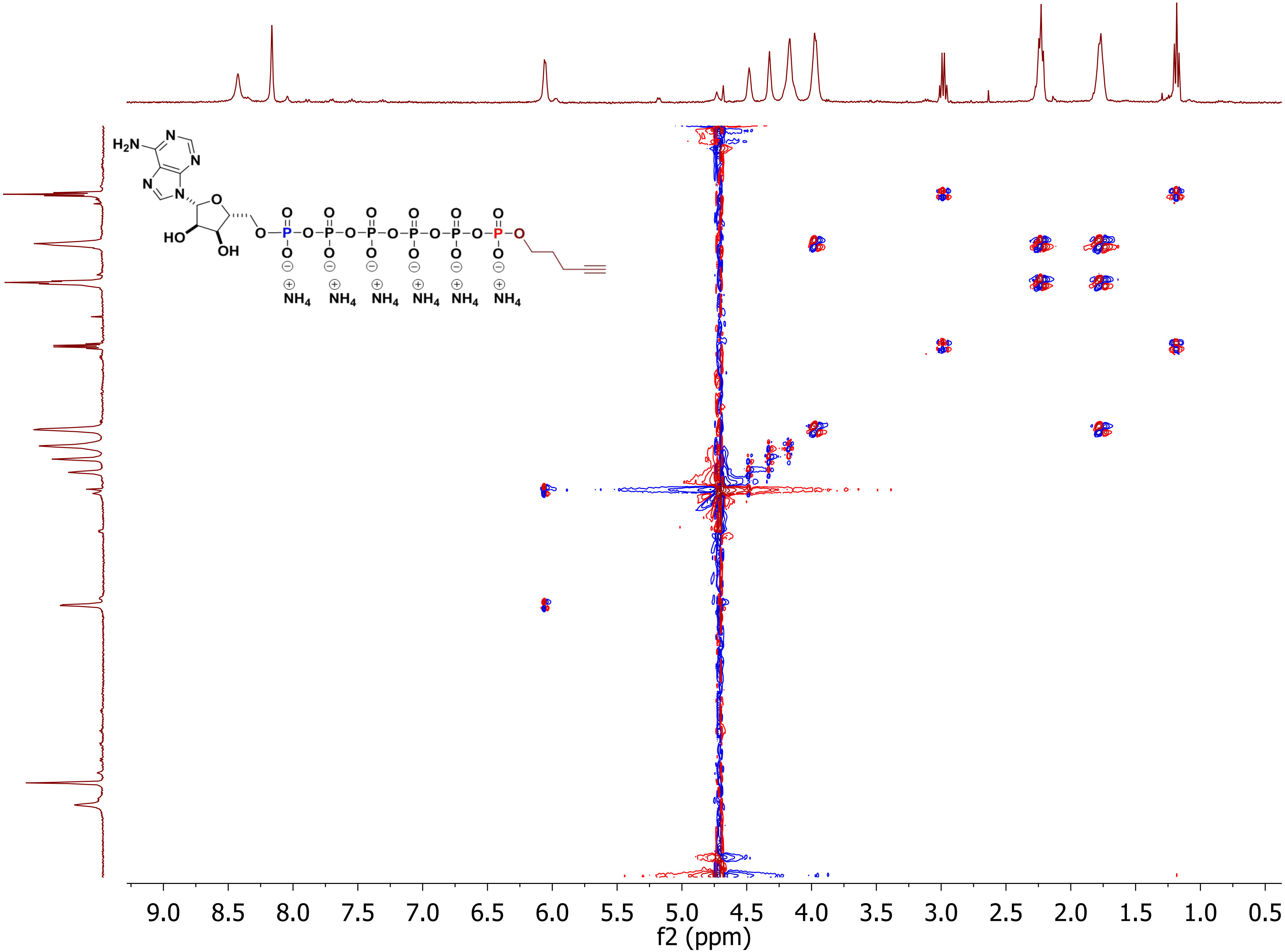
$^{31}$P-NMR


-10.87
-11.38
-22.98
$H_2N$
N
N
N
N
O
HO
OH
O
P
$NH_4$
Product after purifucation by SAX
4P
B (m)
-11.38
A (m)
-10.87
C (m)
-22.98
P
P
1.00
0.85
3.77
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
-18
-19
-20
-21
-22
-23
-24
-25
-26
-27
-28
f1 (ppm)

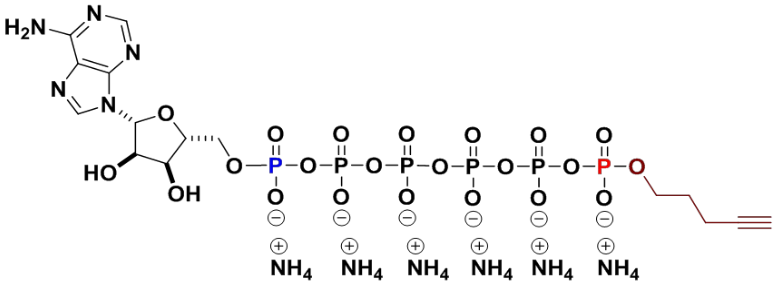
$^{31}P$-$^{1}H$ HMBCNMR

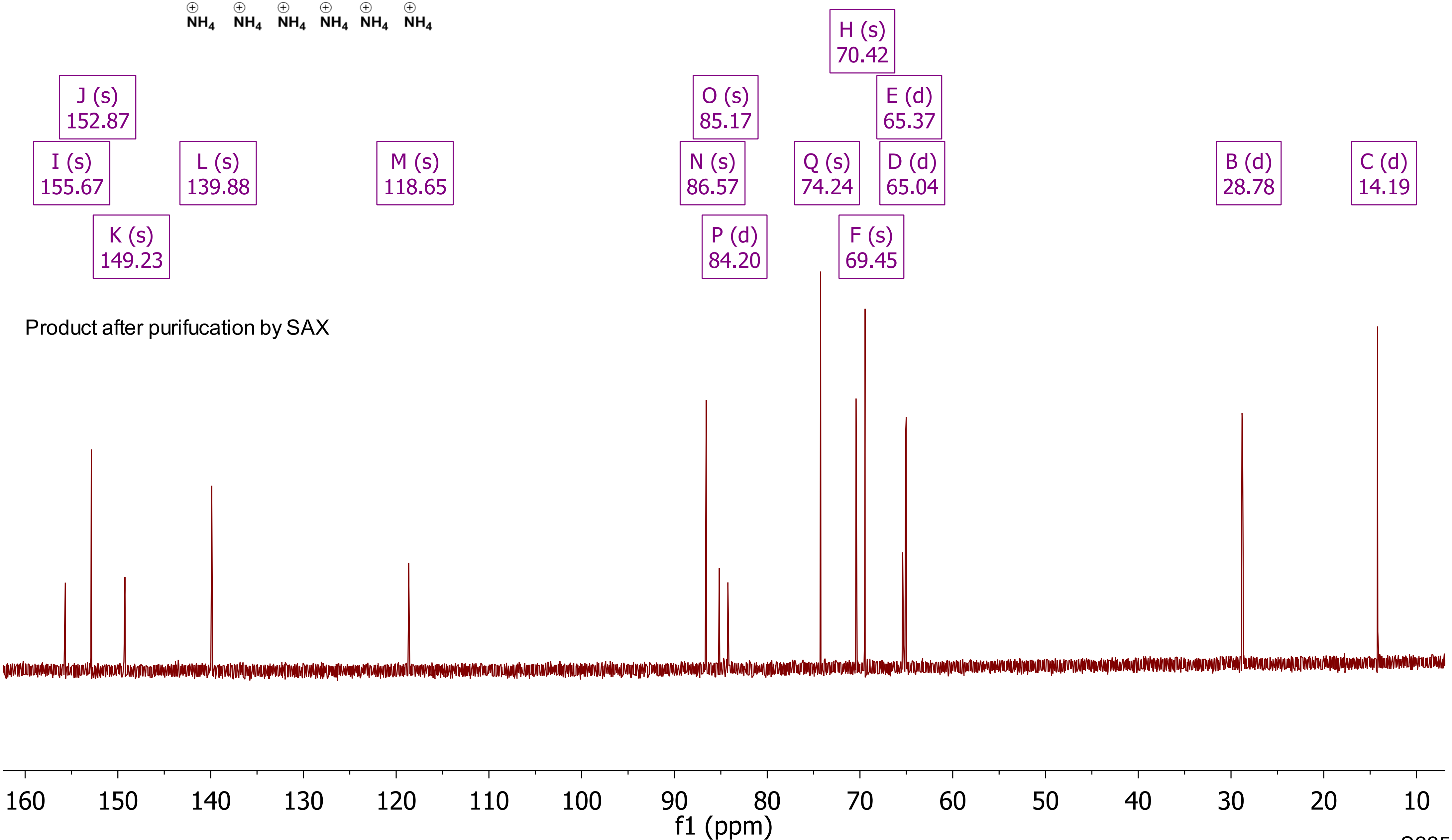


$^{1}H$-$^{1}H$ COSY NMR



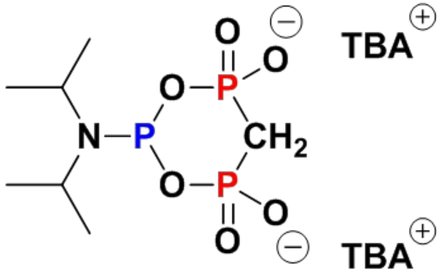
## $^{13}$C NMR

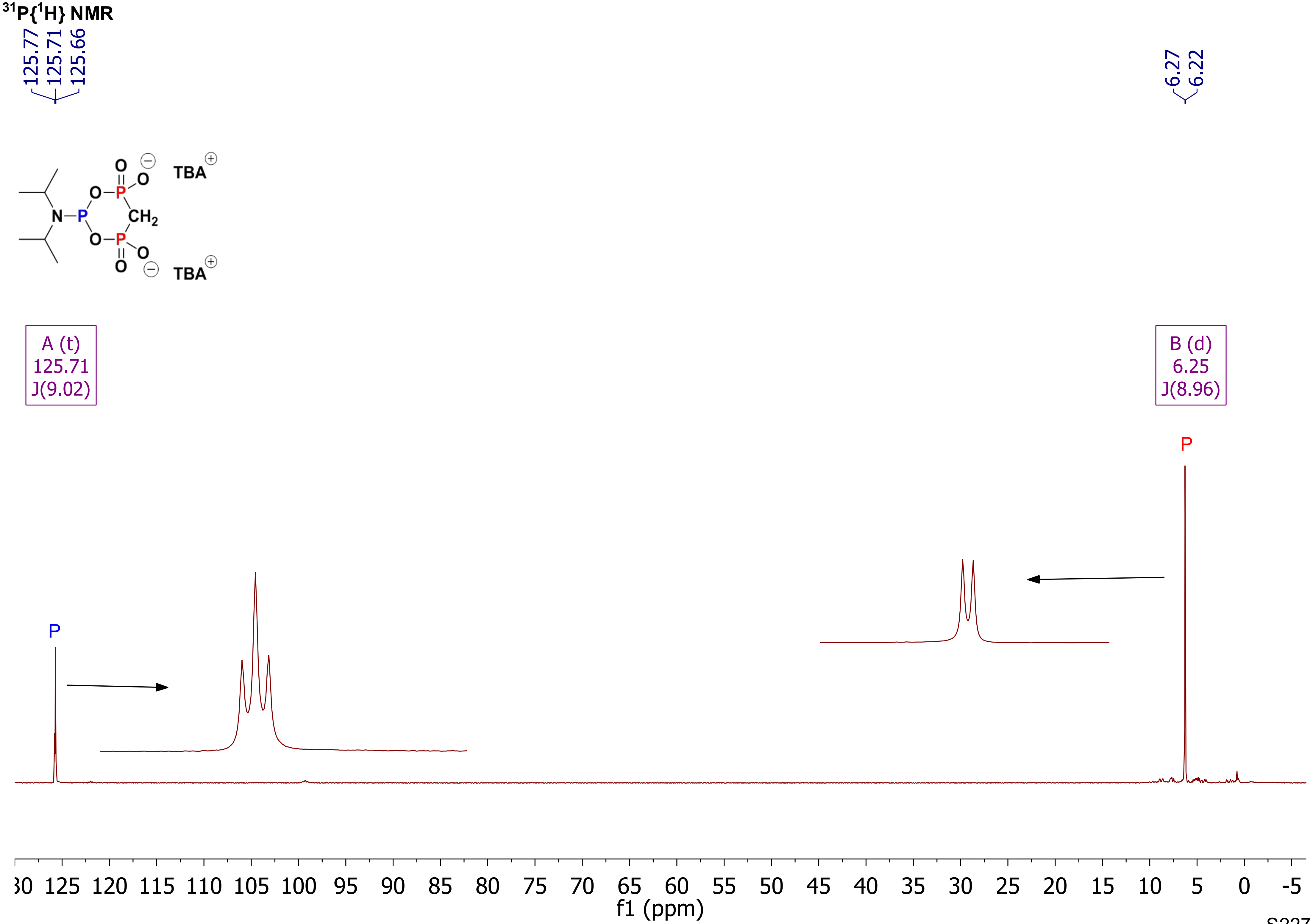




H (s)
70.42
J (s)
152.87
O (s)
85.17
E (d)
65.37
I (s)
155.67
L (s)
139.88
M (s)
118.65
N (s)
86.57
Q (s)
74.24
D (d)
65.04
B (d)
28.78
C (d)
14.19
K (s)
149.23
P (d)
84.20
F (s)
69.45
Product after purifucation by SAX
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

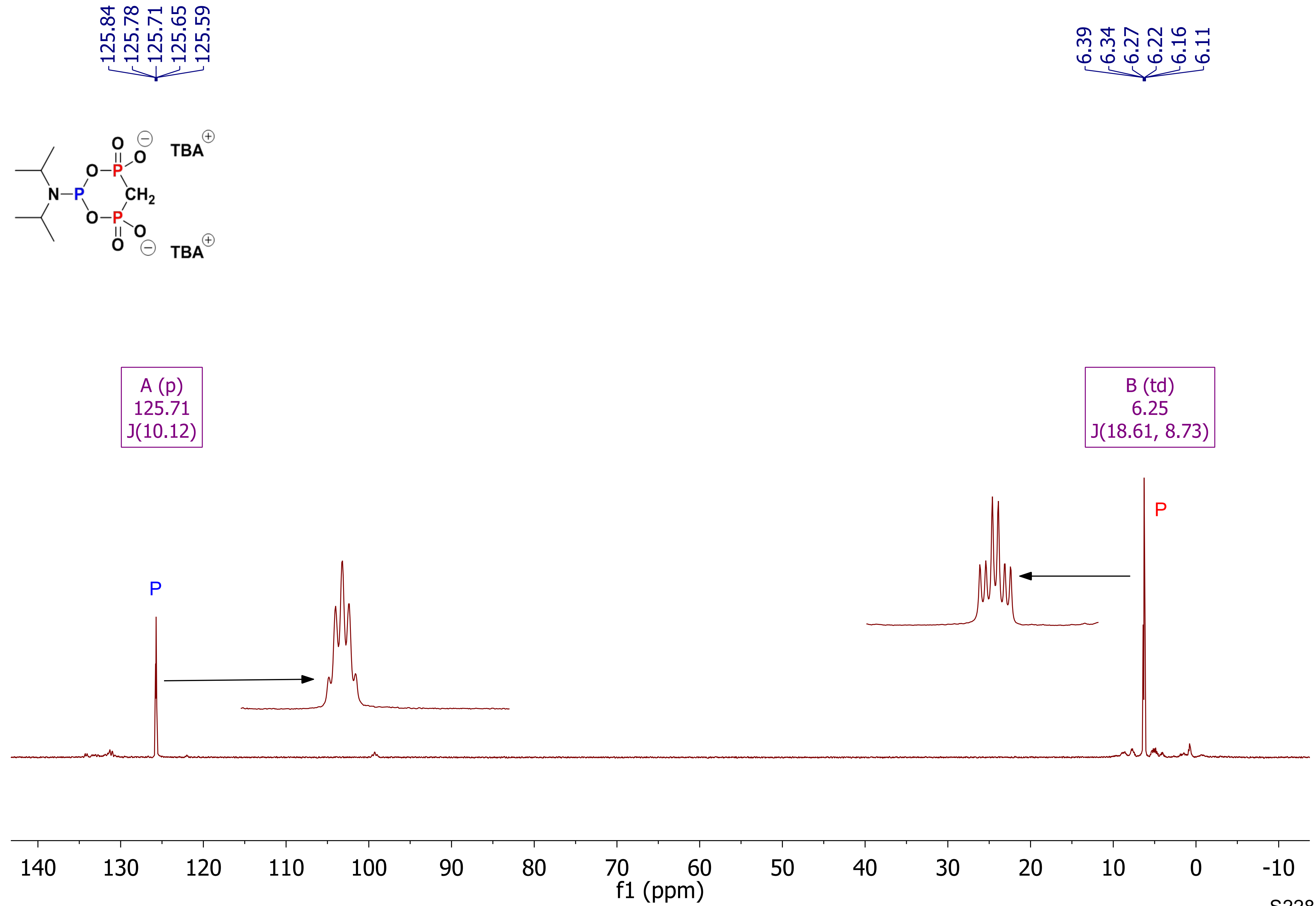
# Synthesis of triphosphates based on c-Py$_{CH2}$PA ($A_2$)



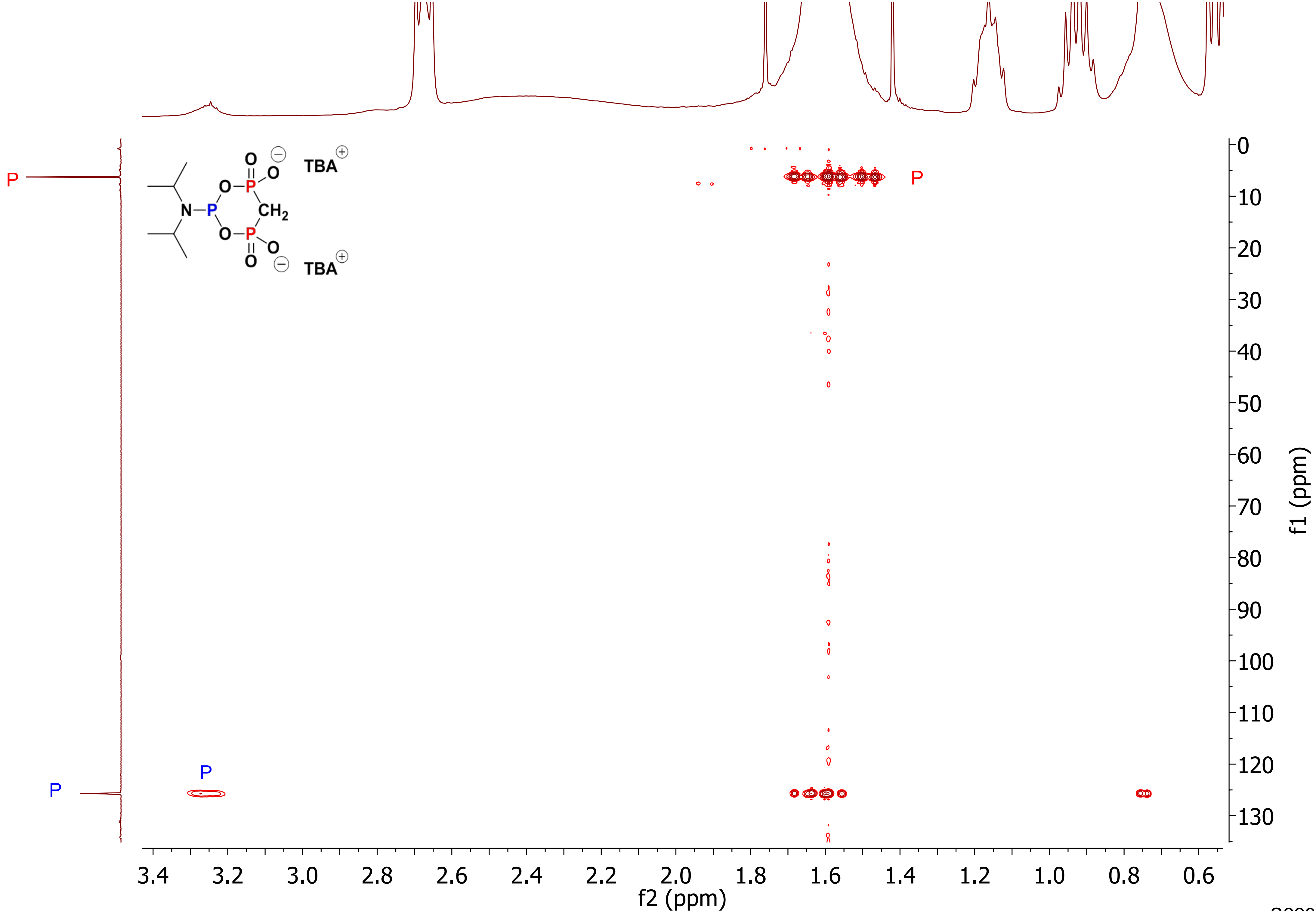


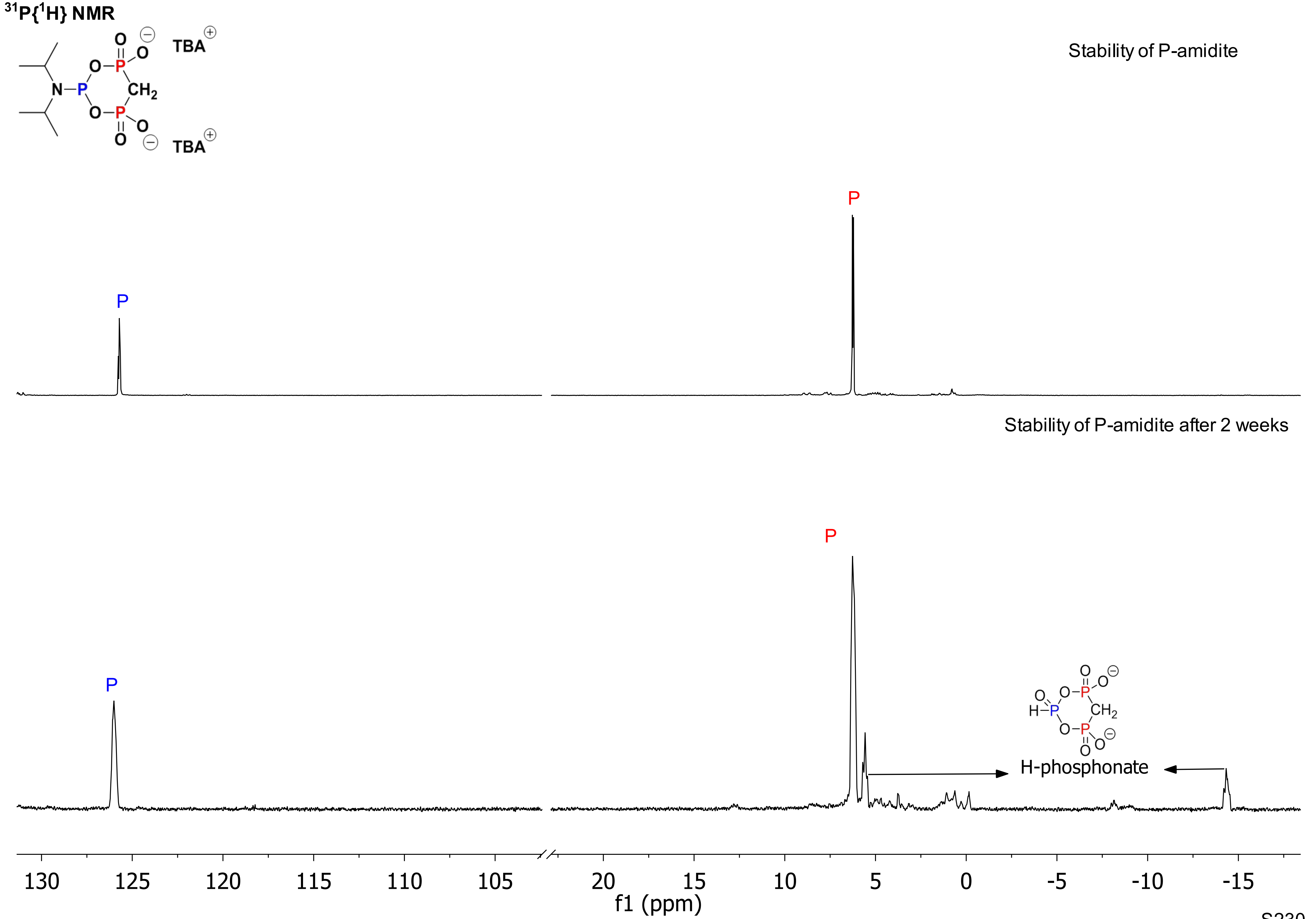
$^{31}P\{^1H\}$ NMR
125.77
125.71
125.66
6.27
6.22
TBA
TBA
A (t)
125.71
J(9.02)
B (d)
6.25
J(8.96)
P
P
f1 (ppm)

**$^{31}$P NMR**

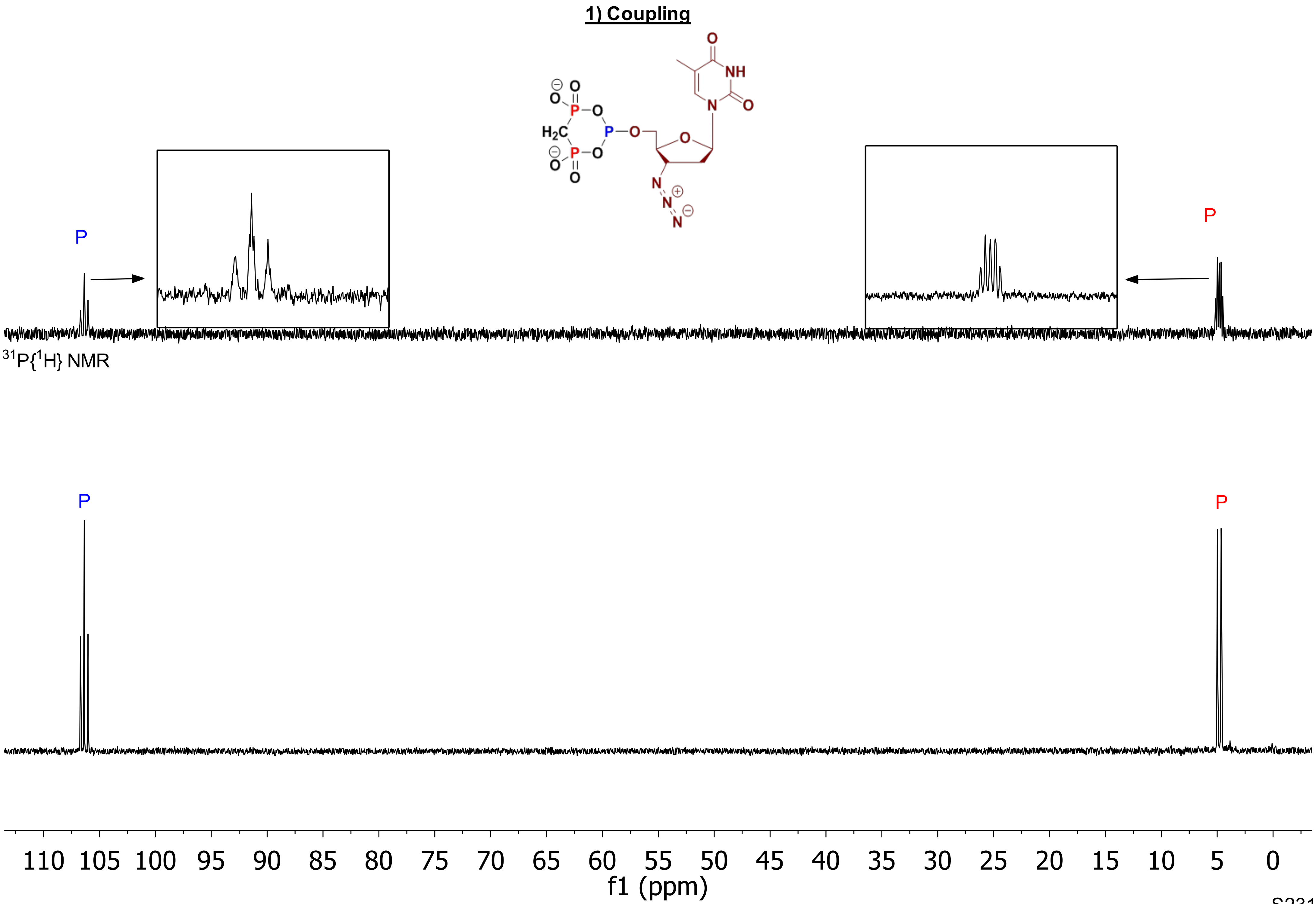

125.84
125.78
125.71
125.65
125.59
6.39
6.34
6.27
6.22
6.16
6.11
O
O
O
P
O
N
P
CH2
O
P
O
O
TBA
TBA
A (p)
125.71
J(10.12)
B (td)
6.25
J(18.61, 8.73)
P
P
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

$^{31}$P-$^{1}$H HMBC NMR

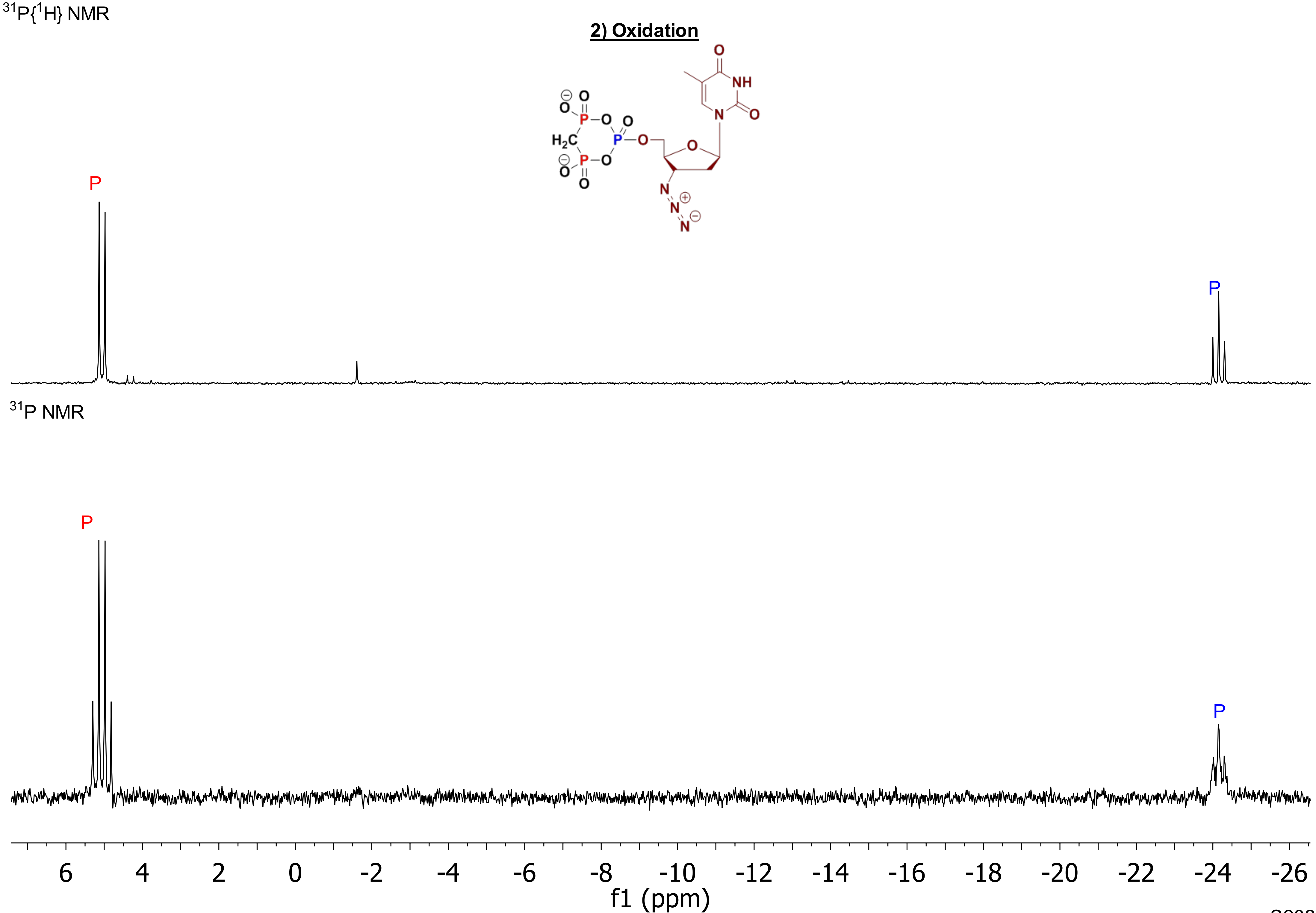



31P{1H} NMR
TBA
TBA
Stability of P-amidite
P
P
Stability of P-amidite after 2 weeks
P
P
H-phosphonate
130
125
120
115
110
105
20
15
10
5
0
-5
-10
-15
f1 (ppm)

$^{31}$PNMR

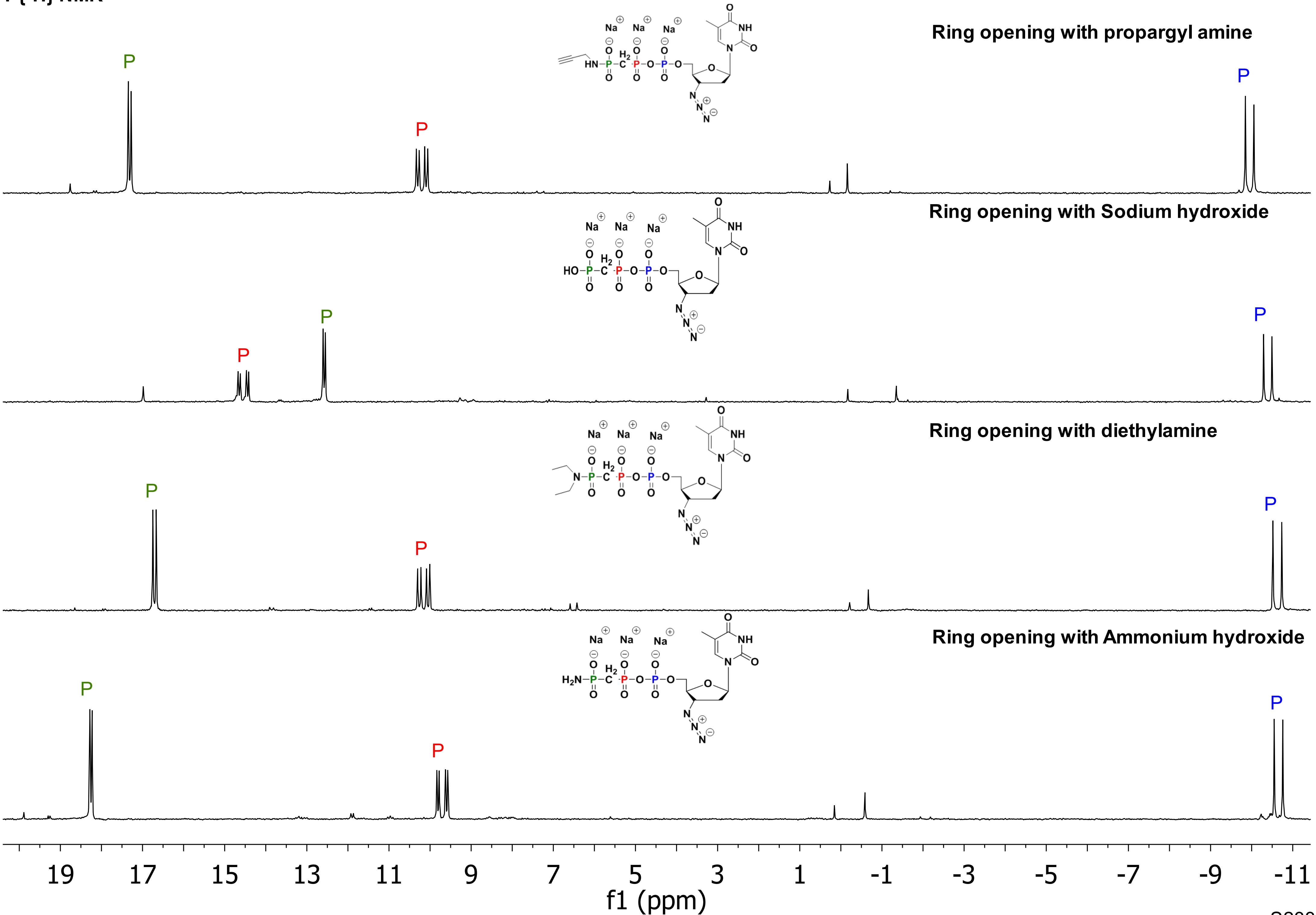


1) Coupling
P
P
$^{31}$P{$^1$H} NMR
P
P
110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0
f1 (ppm)

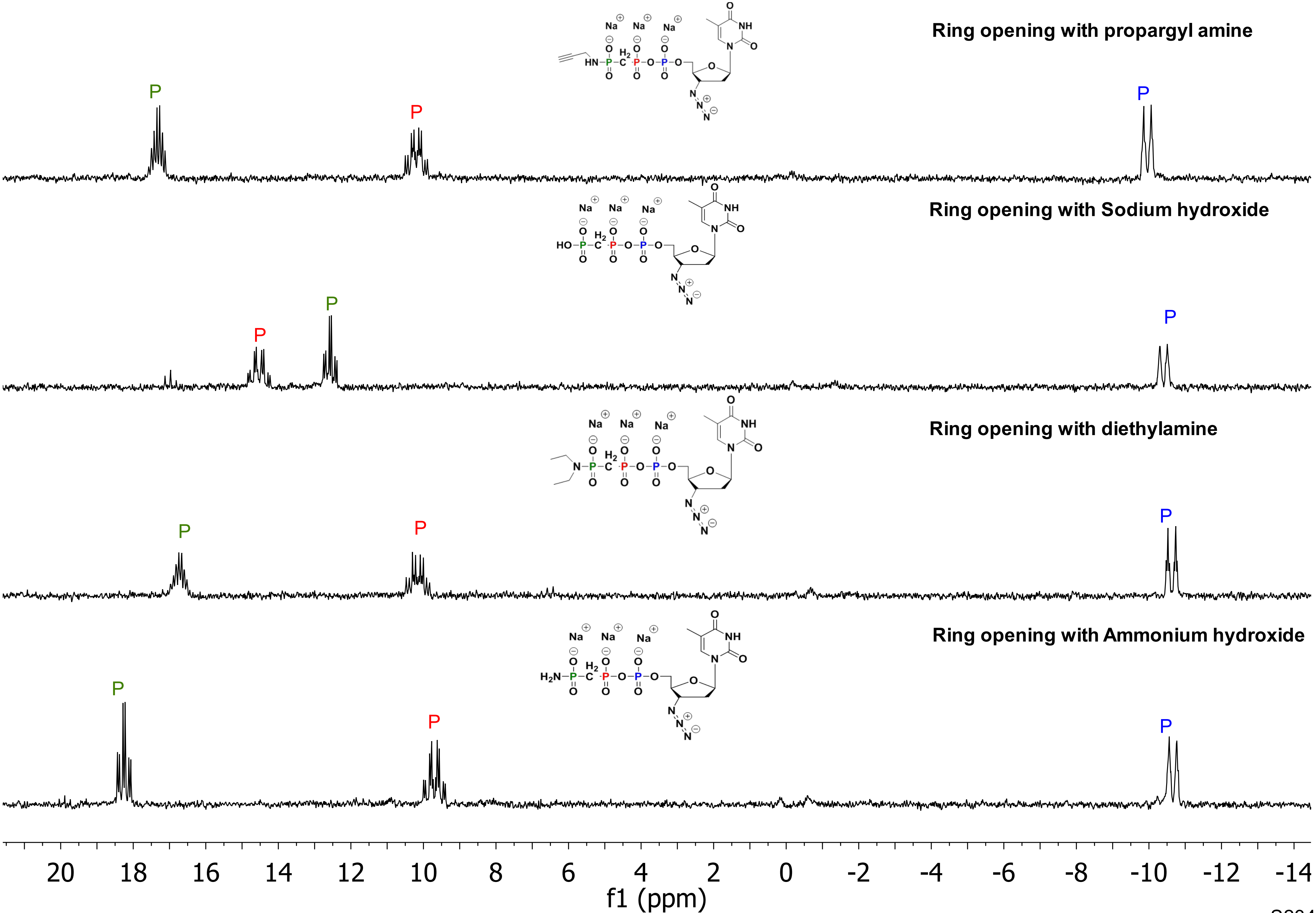


$^{31}P\{^1H\}$ NMR
2) Oxidation
P
P
$^{31}P$ NMR
P
P
6
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
-22
-24
-26
f1 (ppm)

$^{31}P\{^1H\}$ NMR


Ring opening with propargyl amine
Ring opening with Sodium hydroxide
Ring opening with diethylamine
Ring opening with Ammonium hydroxide
P
19
17
15
13
11
9
7
5
3
1
-1
-3
-5
-7
-9
-11
f1 (ppm)

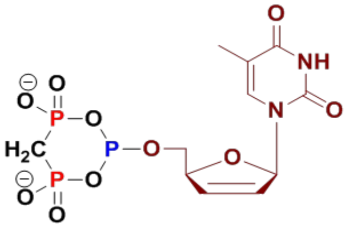
## $^{31}$P NMR



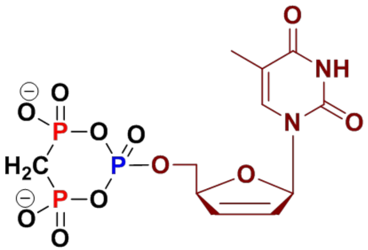
Ring opening with propargyl amine
Ring opening with Sodium hydroxide
Ring opening with diethylamine
Ring opening with Ammonium hydroxide
20 18 16 14 12 10 8 6 4 2 0 -2 -4 -6 -8 -10 -12 -14
f1 (ppm)

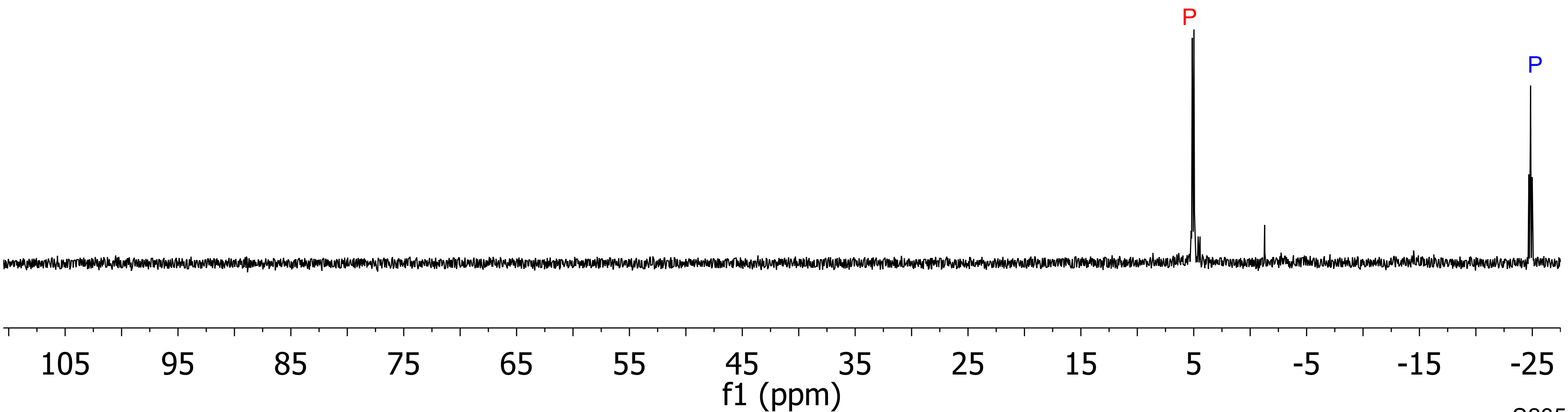
**$^{31}P\{^{1}H\}$ NMR**

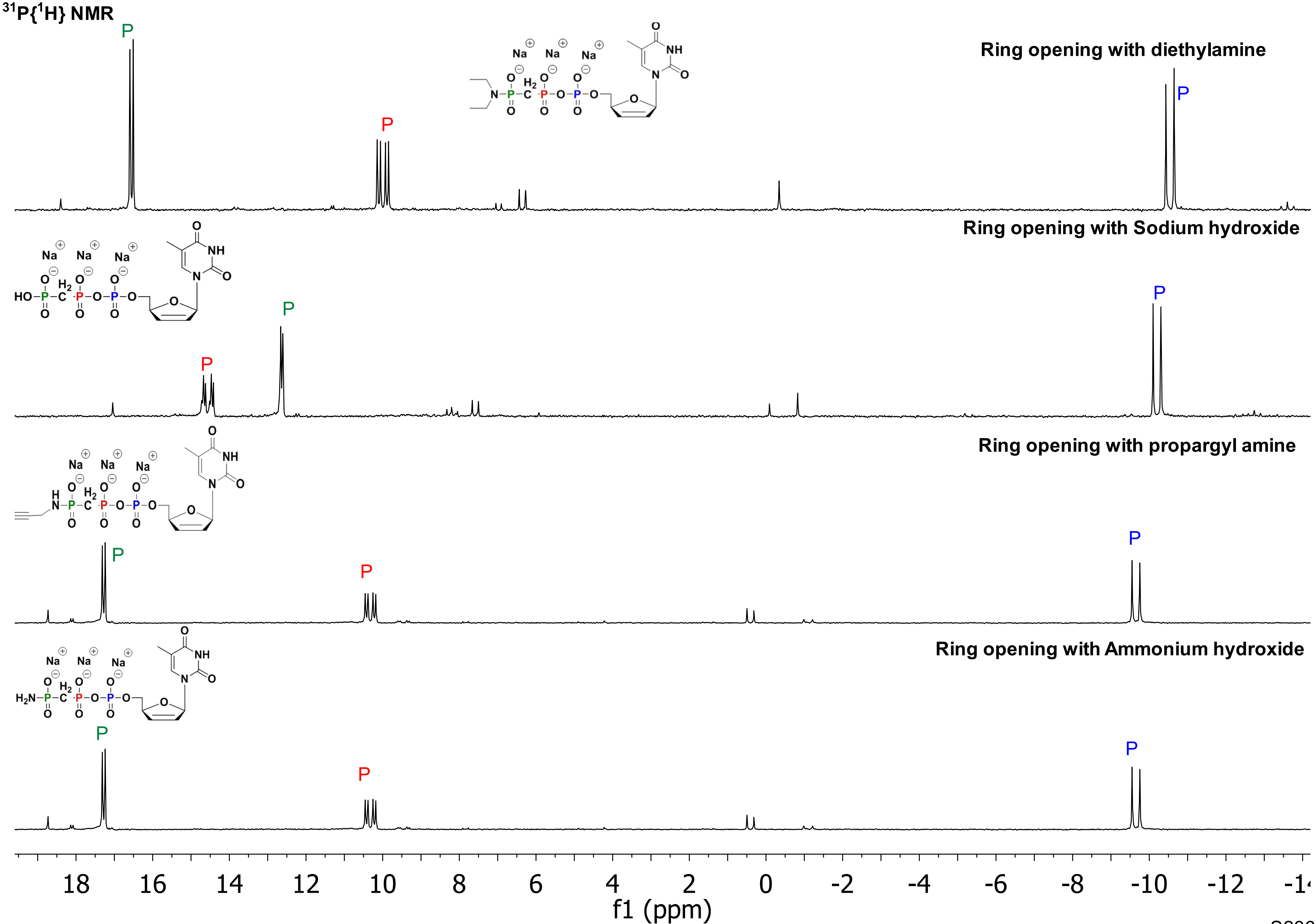
**1) Coupling**

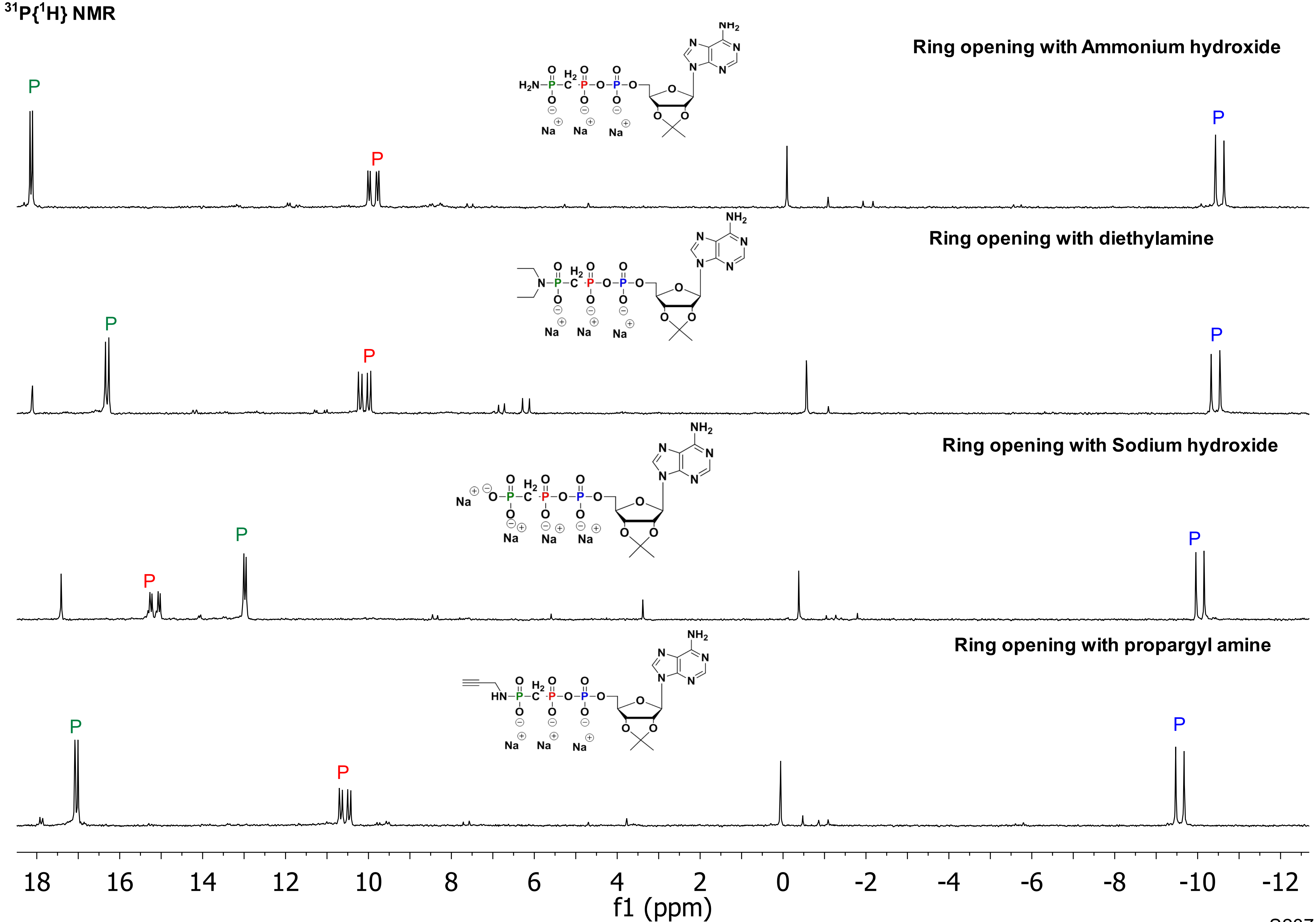



**2) Oxidation**

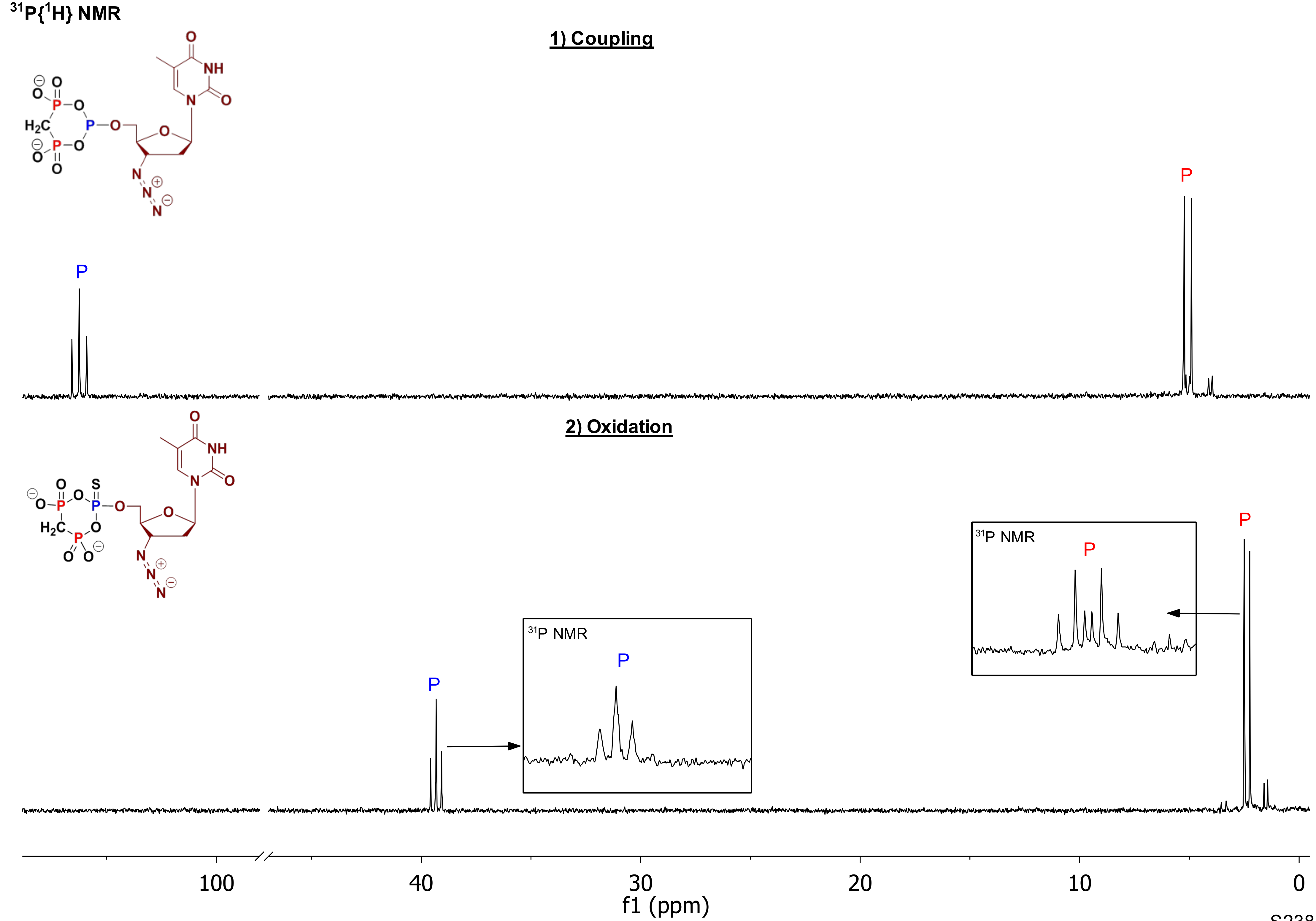





31P{1H} NMR
Ring opening with diethylamine
Ring opening with Sodium hydroxide
Ring opening with propargyl amine
Ring opening with Ammonium hydroxide
f1 (ppm)

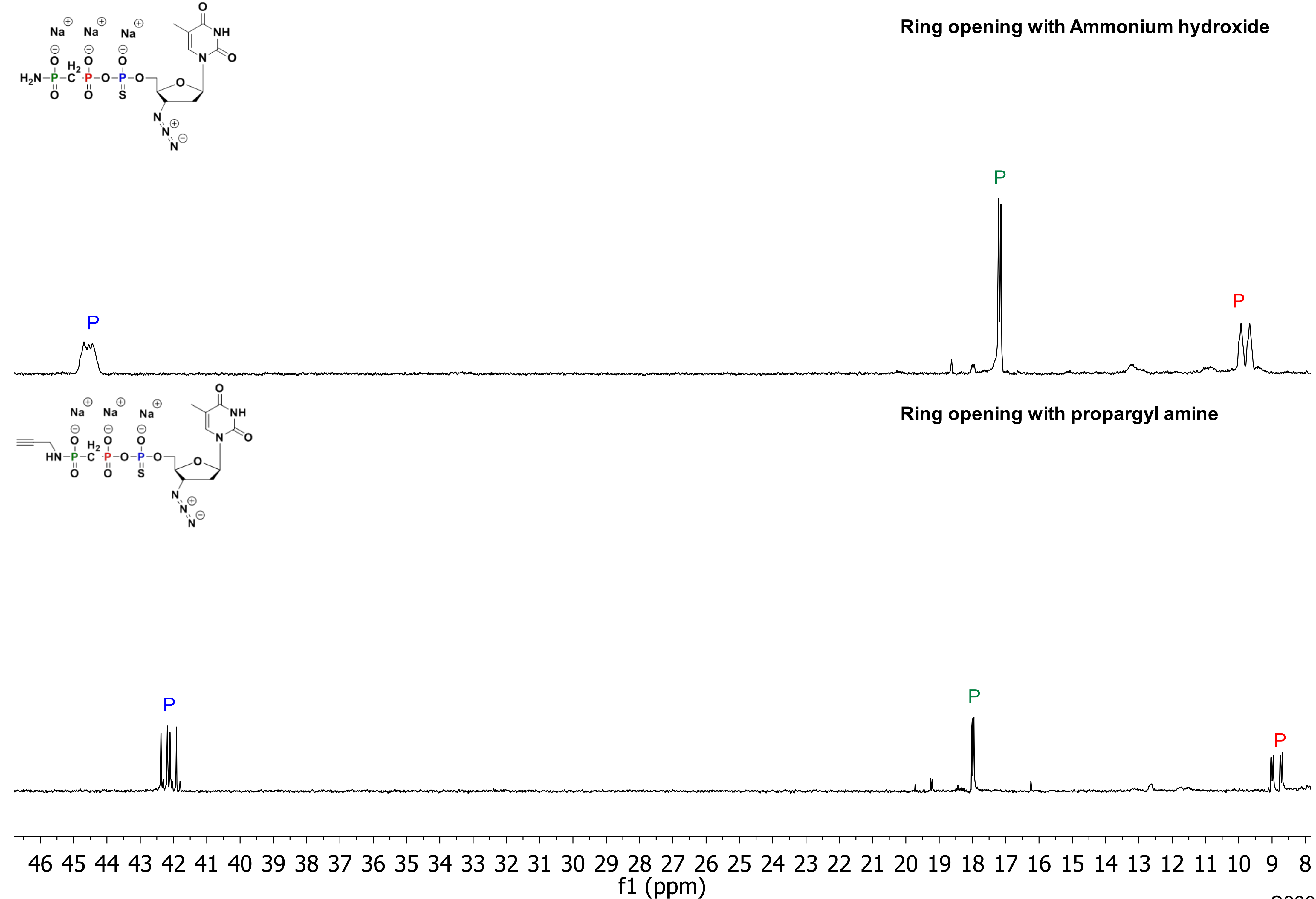


$^{31}P\{^1H\}$ NMR
Ring opening with Ammonium hydroxide
Ring opening with diethylamine
Ring opening with Sodium hydroxide
Ring opening with propargyl amine
18
16
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
-12
f1 (ppm)



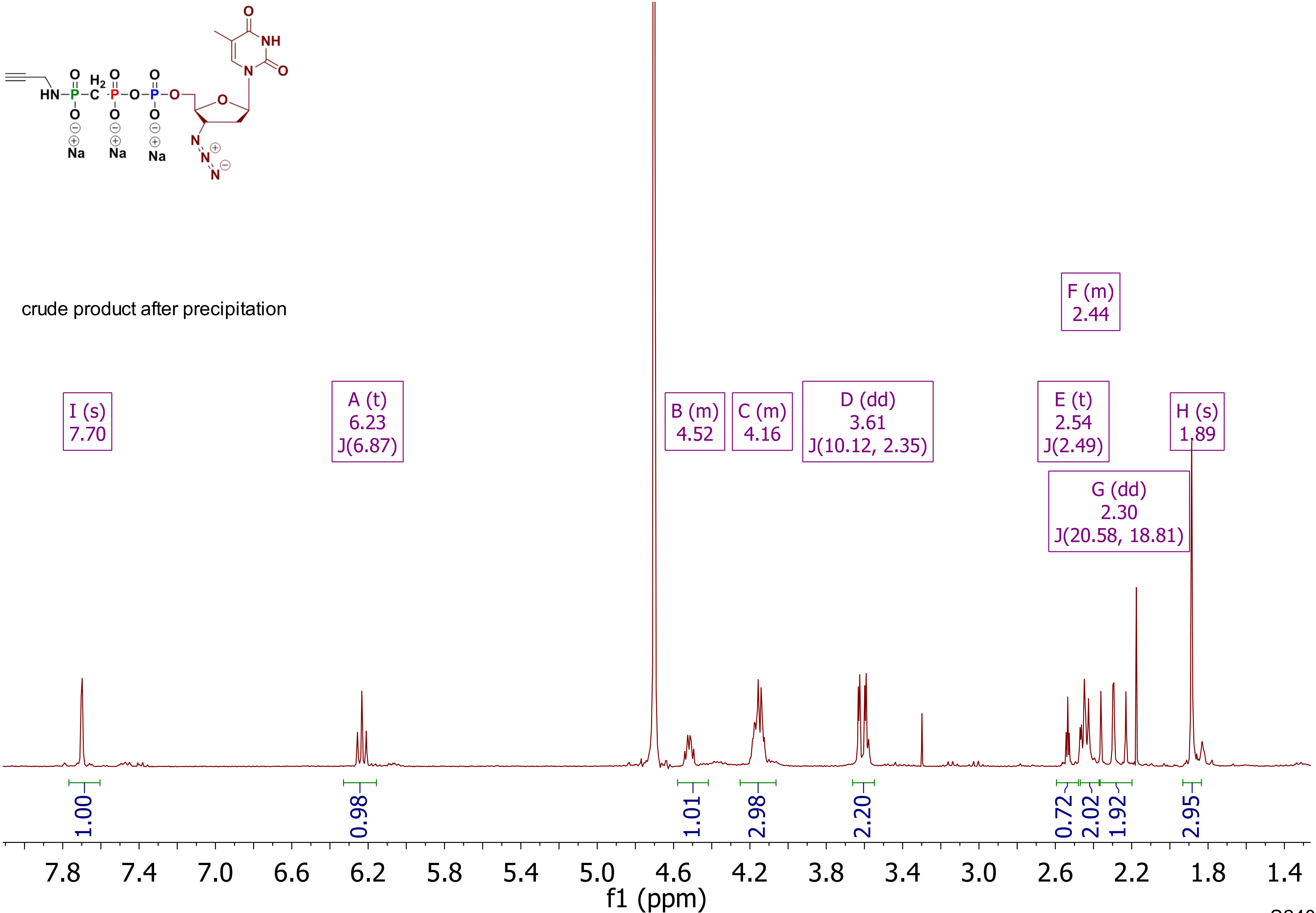
31P{1H} NMR
1) Coupling
P
P
2) Oxidation
31P NMR
P
31P NMR
P
P
P
100
40
30
20
10
0
f1 (ppm)

**$^{31}P\{^1H\}$ NMR**



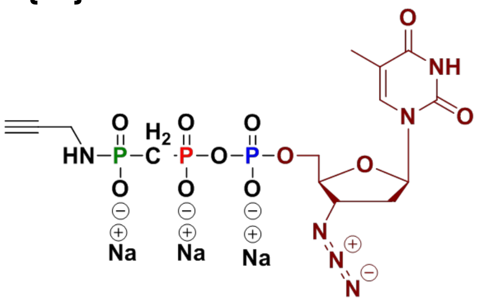
Ring opening with Ammonium hydroxide
Ring opening with propargyl amine
P
P
P
P
P
P
46 45 44 43 42 41 40 39 38 37 36 35 34 33 32 31 30 29 28 27 26 25 24 23 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8
f1 (ppm)

$^{1}$H NMR

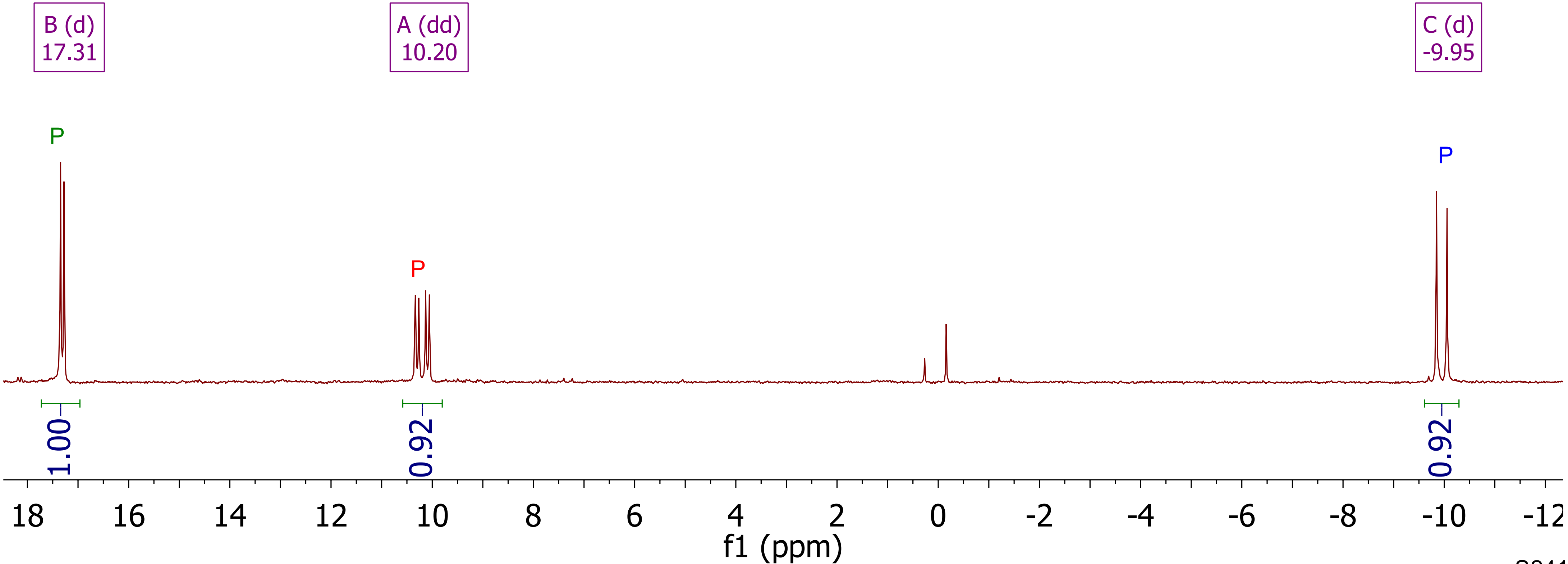


O
NH
N
O
O
HN
P
C
$H_2$
O
P
O
P
O
O
Na
Na
Na
N
N
N
crude product after precipitation
F (m)
2.44
I (s)
7.70
A (t)
6.23
J(6.87)
B (m)
4.52
C (m)
4.16
D (dd)
3.61
J(10.12, 2.35)
E (t)
2.54
J(2.49)
H (s)
1.89
G (dd)
2.30
J(20.58, 18.81)
1.00
0.98
1.01
2.98
2.20
0.72
2.02
1.92
2.95
7.8
7.4
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4
f1 (ppm)

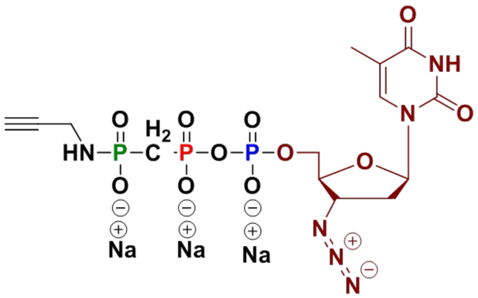
$^{31}P\{^{1}H\}$ NMR



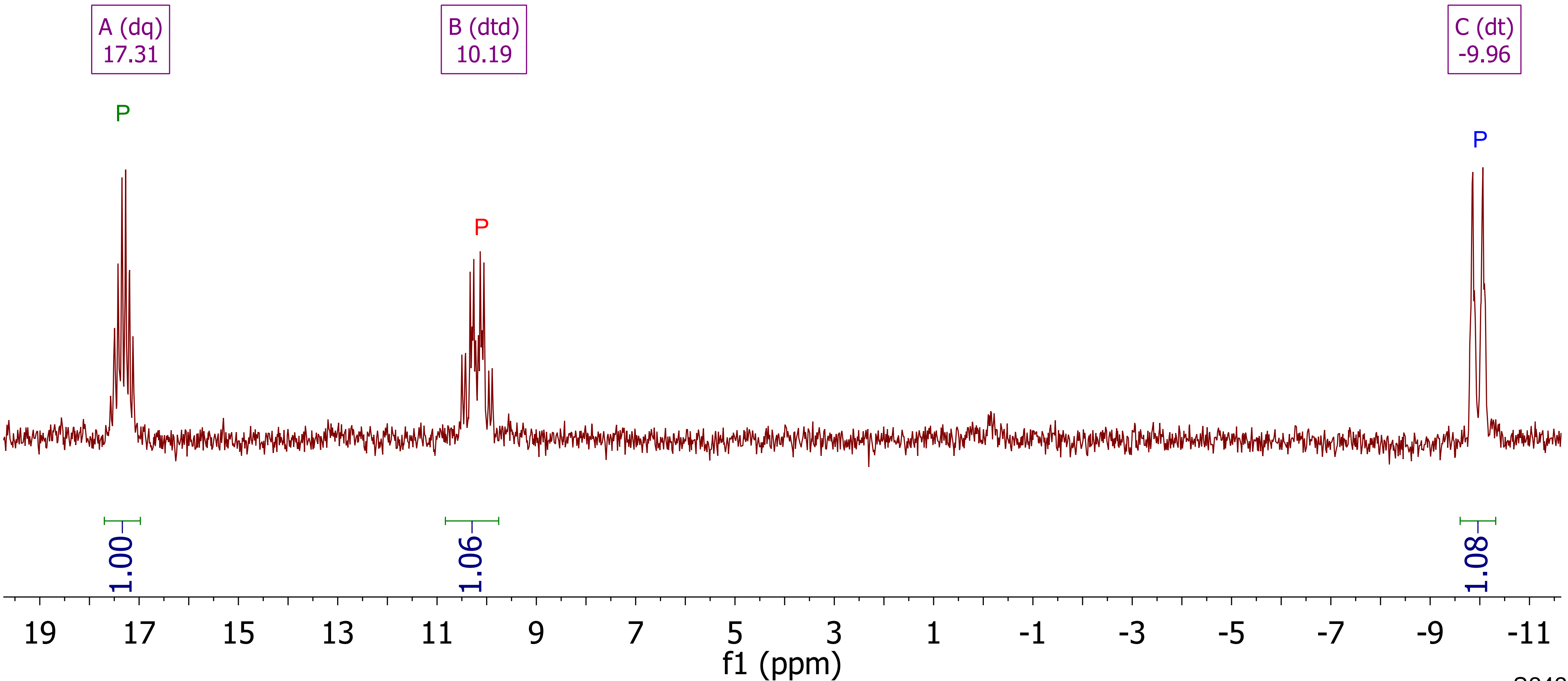
crude product after precipitation



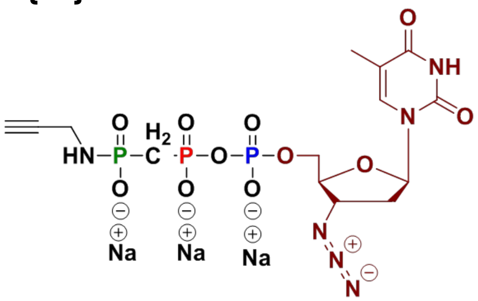
**$^{31}$P NMR**



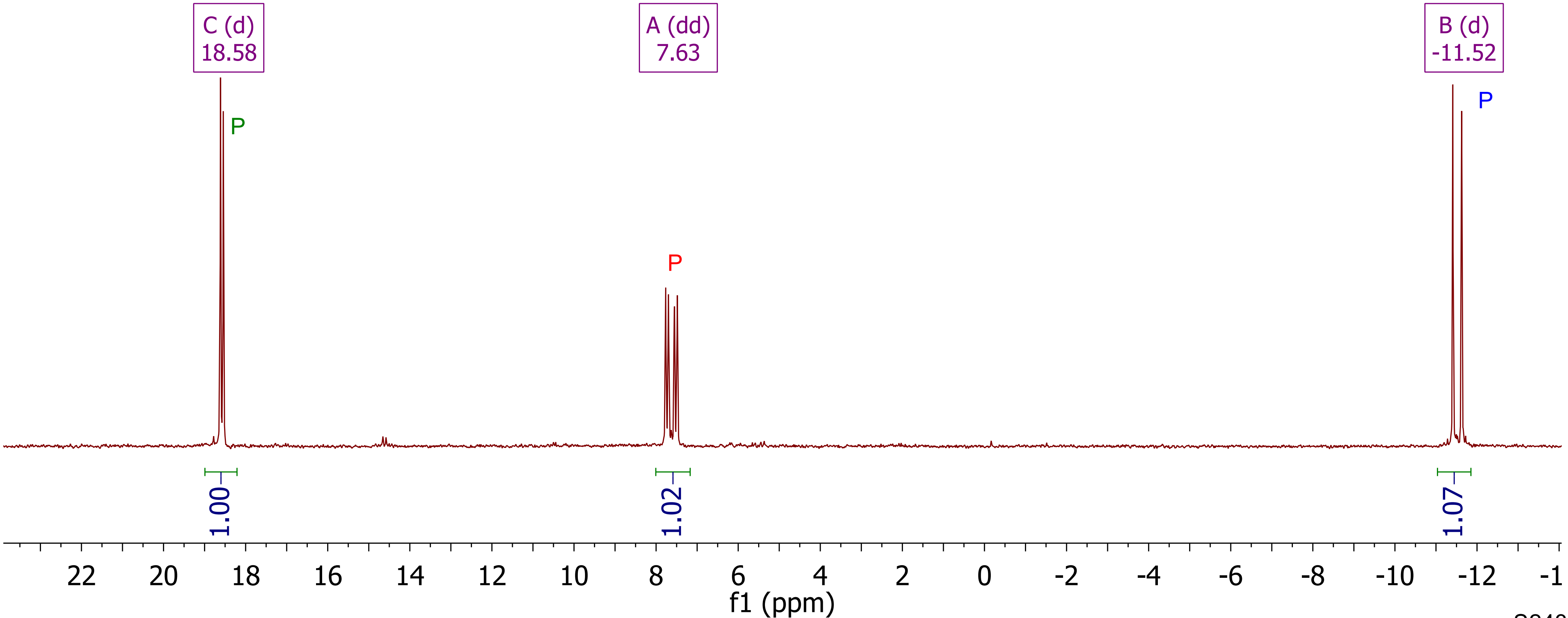
crude product after precipitation



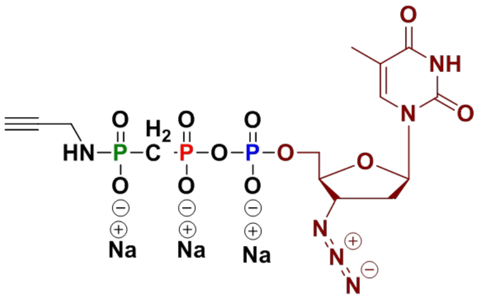
$^{31}P\{^{1}H\}$ NMR



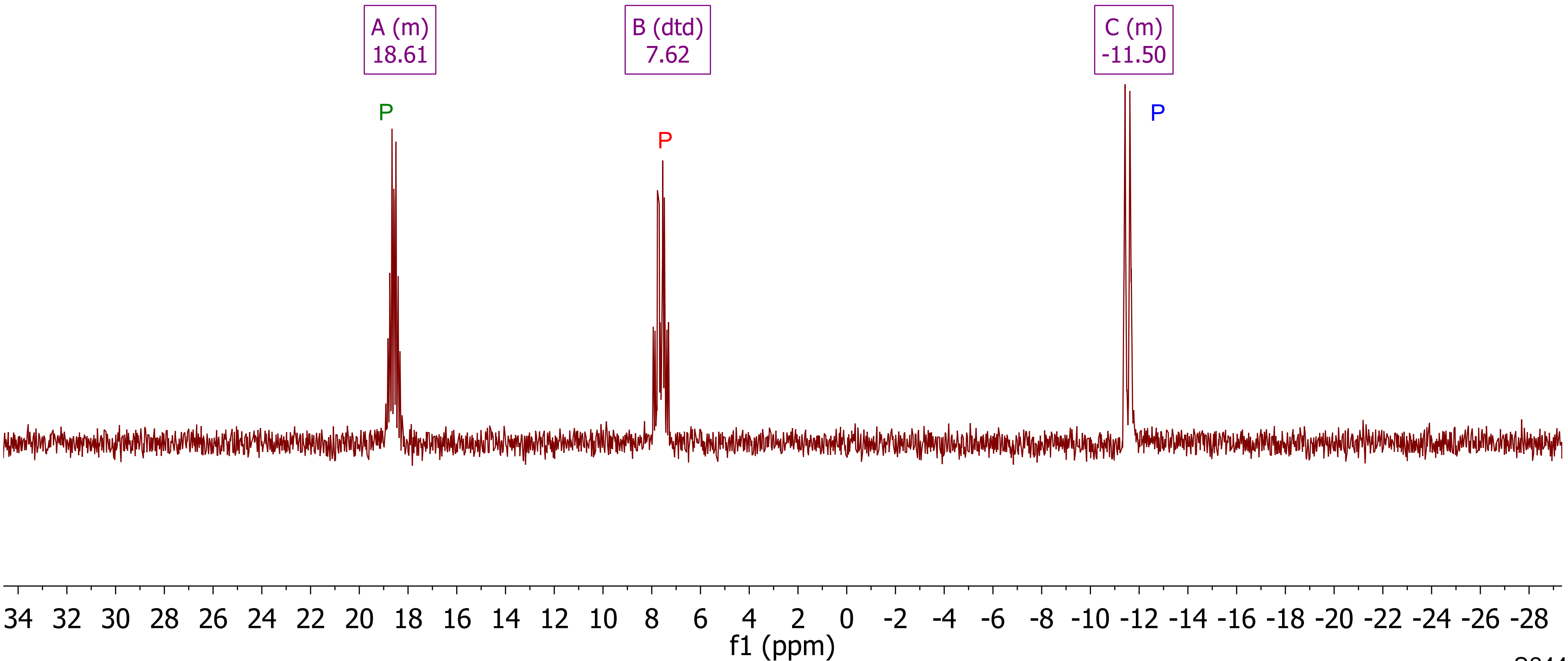
Product after precipitation with $NaClO_4$
reaction on 0.78 mmol scale



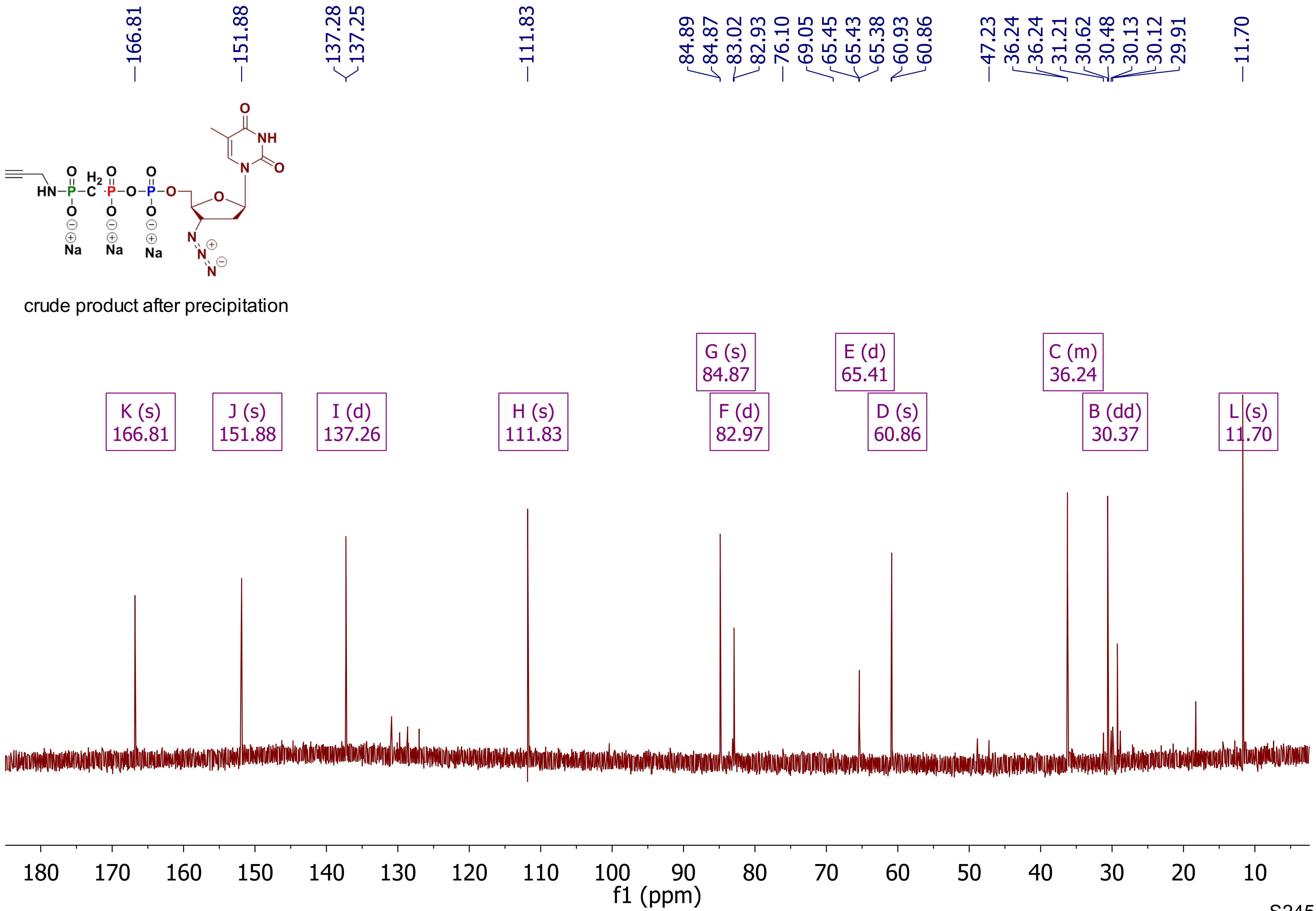
**$^{31}$P NMR**



Product after precipitation with $NaClO_4$
reaction on 0.78 mmol scale

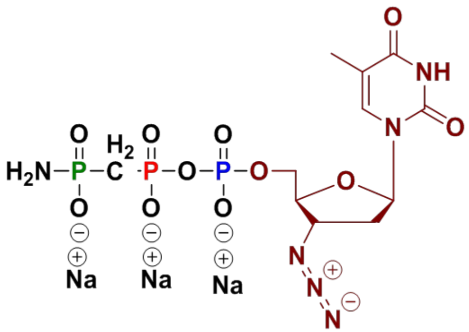


**$^{13}$C NMR**

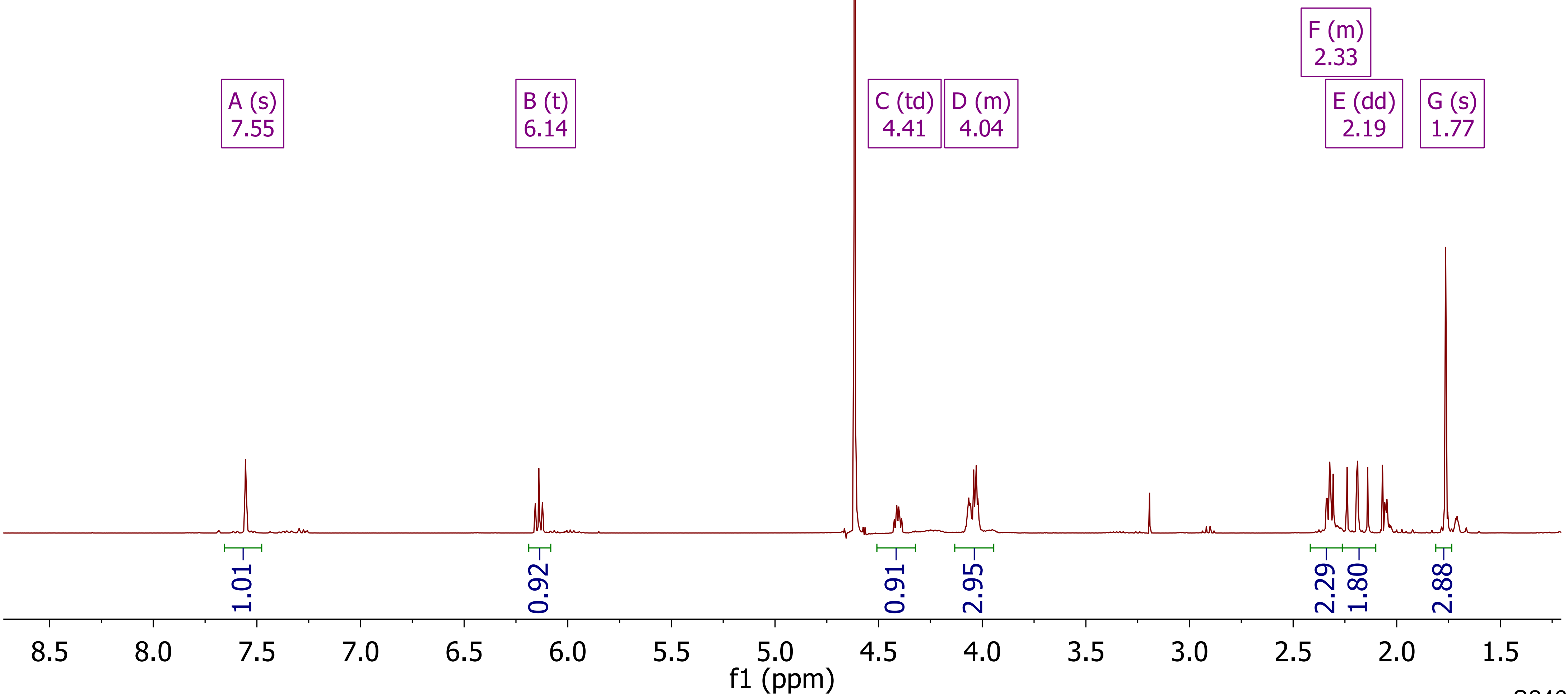


166.81
151.88
137.28
137.25
111.83
84.89
84.87
83.02
82.93
76.10
69.05
65.45
65.43
65.38
60.93
60.86
47.23
36.24
36.24
31.21
30.62
30.48
30.13
30.12
29.91
11.70
HN
P
C
$H_2$
O
Na
N
NH
crude product after precipitation
G (s) 84.87
E (d) 65.41
C (m) 36.24
K (s) 166.81
J (s) 151.88
I (d) 137.26
H (s) 111.83
F (d) 82.97
D (s) 60.86
B (dd) 30.37
L (s) 11.70
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

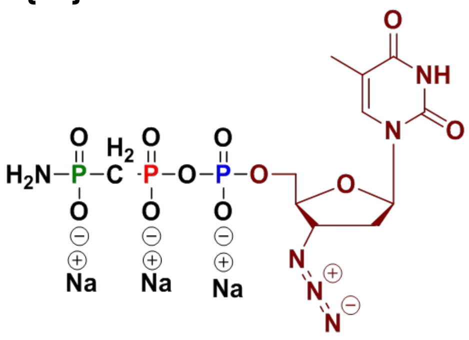
$^1$H NMR



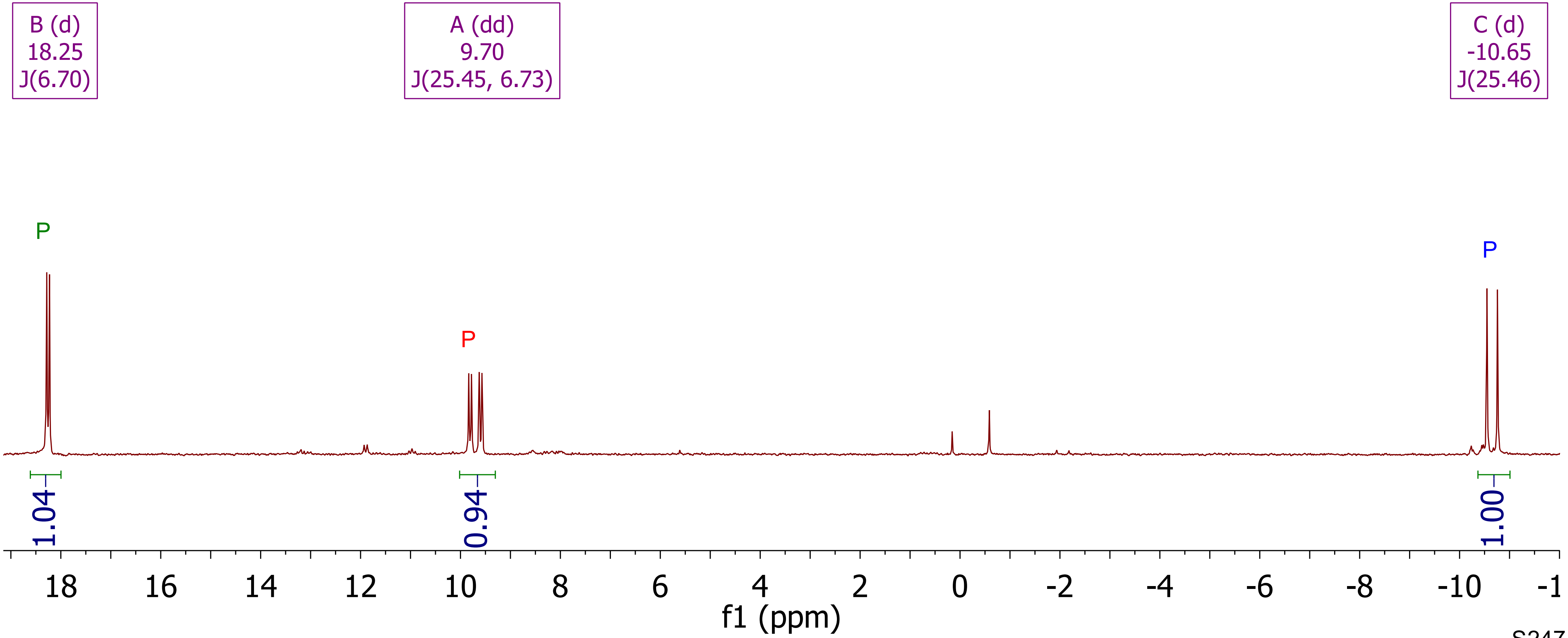
Crude product after precipitation



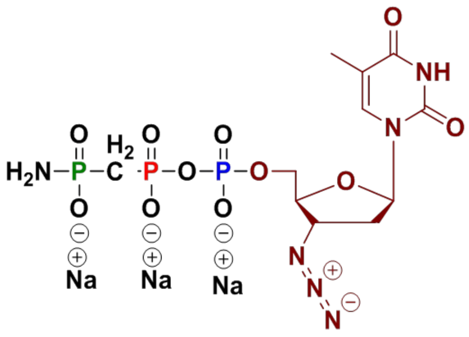
$^{31}P\{^{1}H\}$ NMR



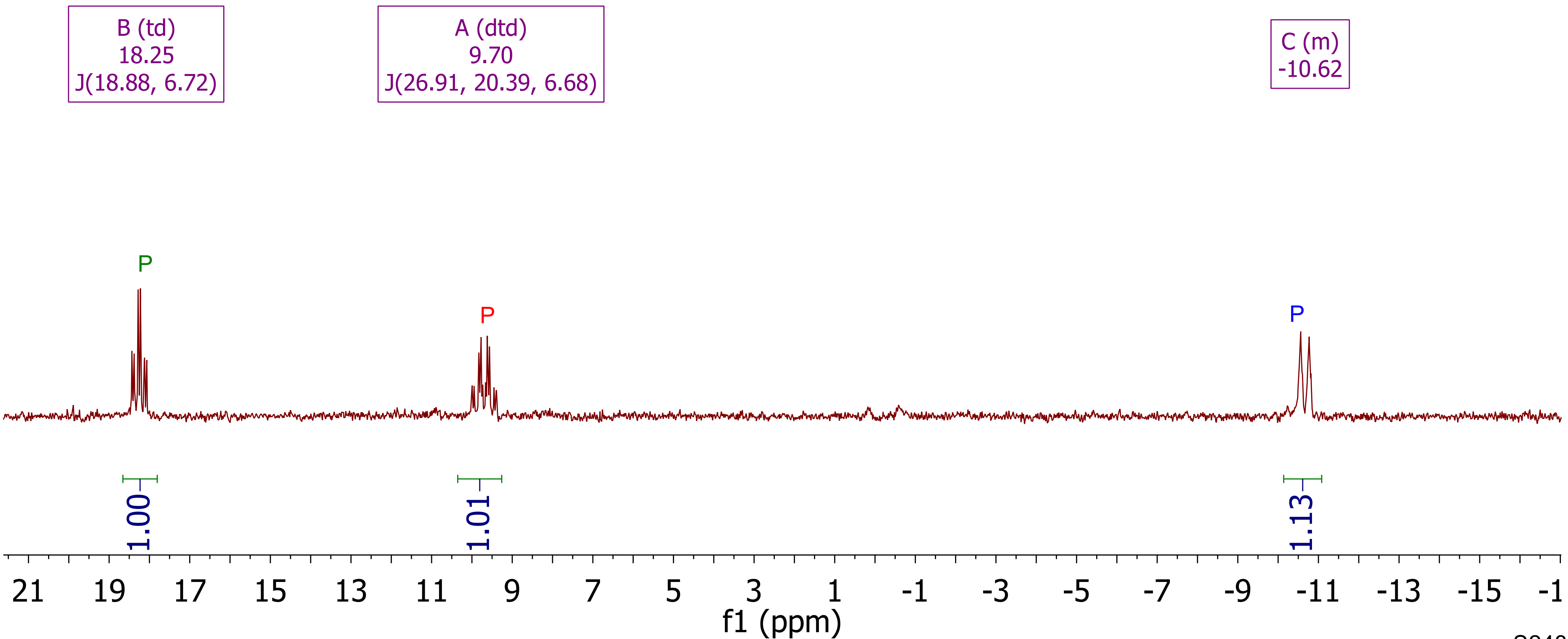
crude product after precipitation



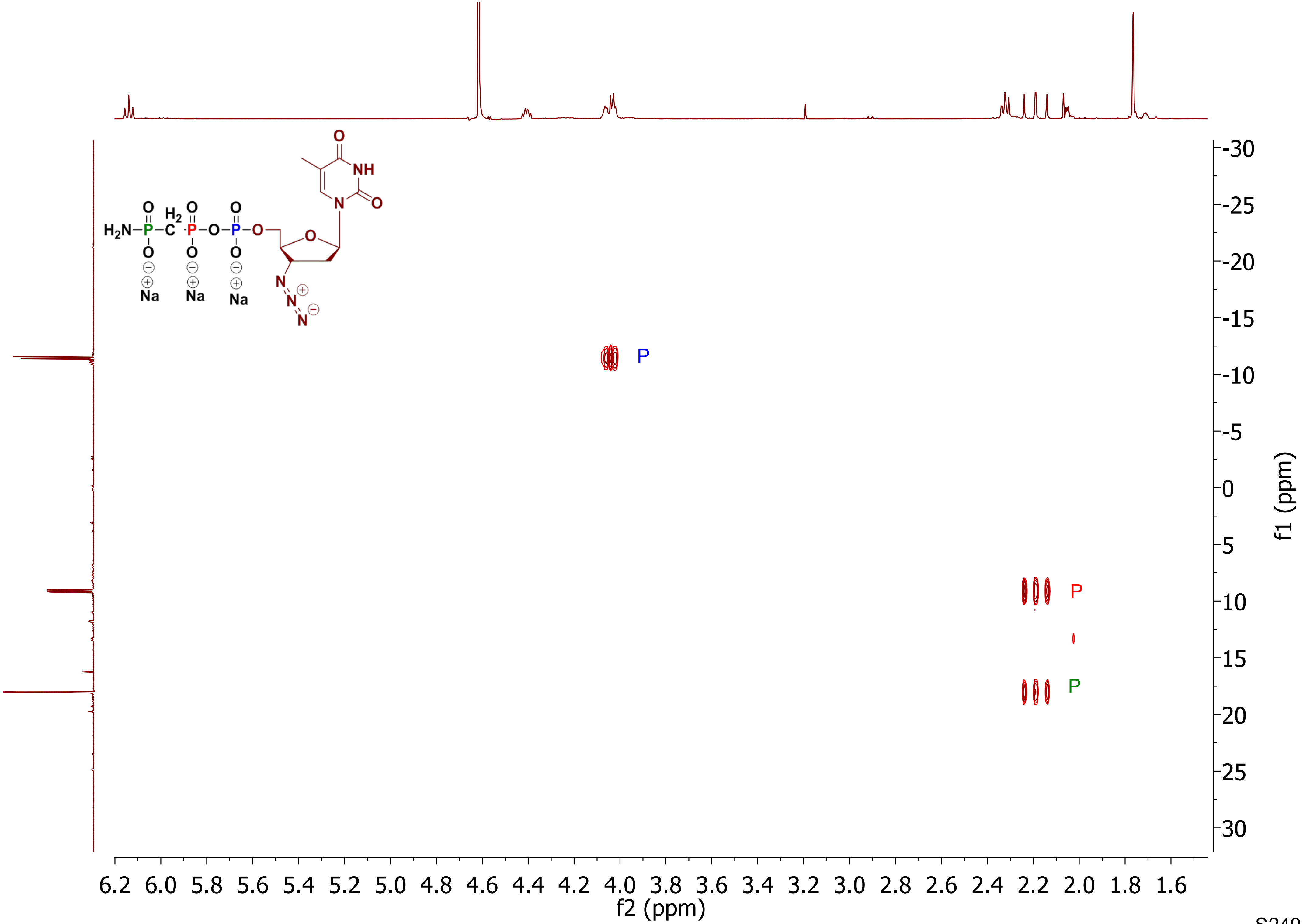
**$^{31}$P NMR**



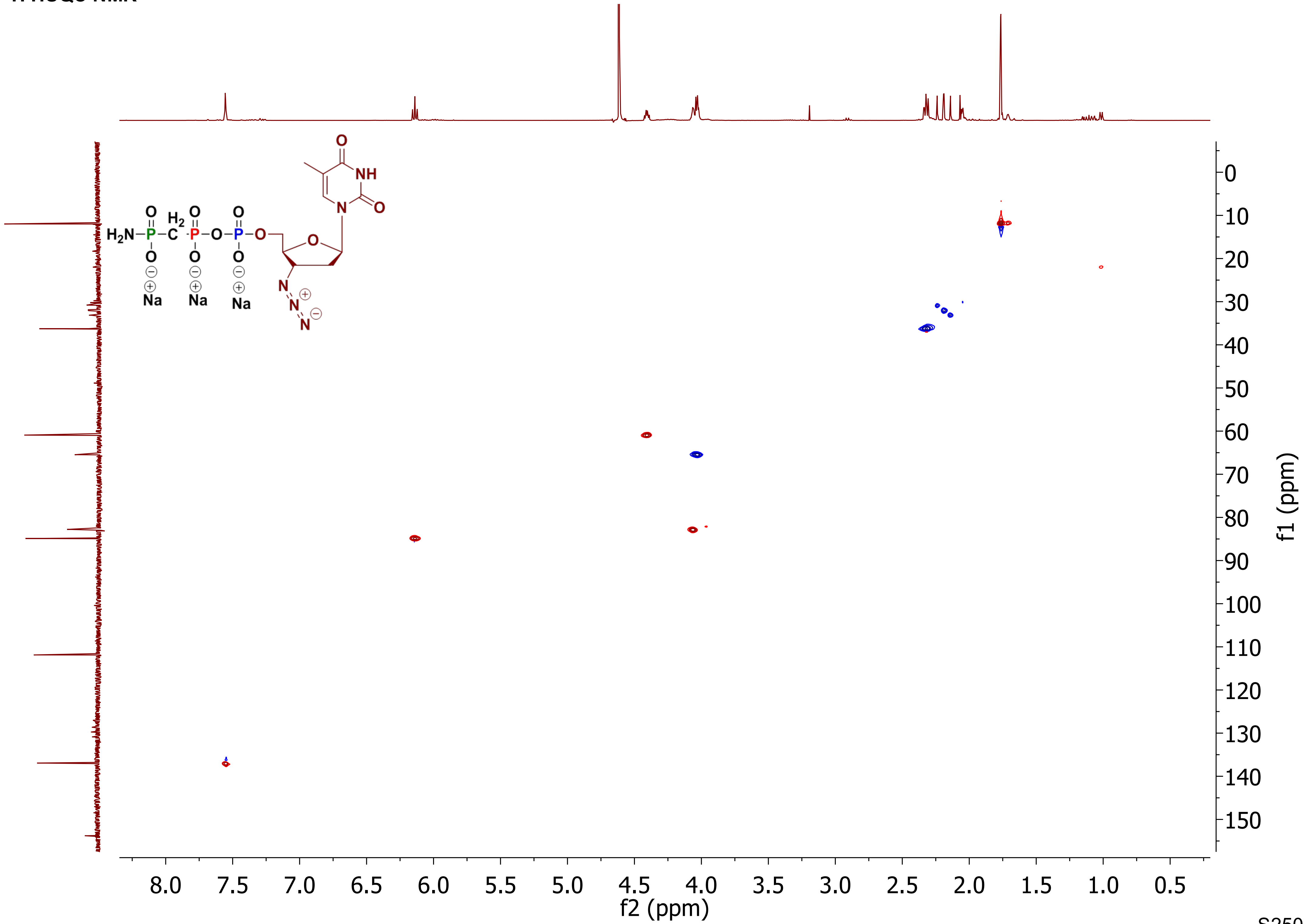
crude product after precipitation



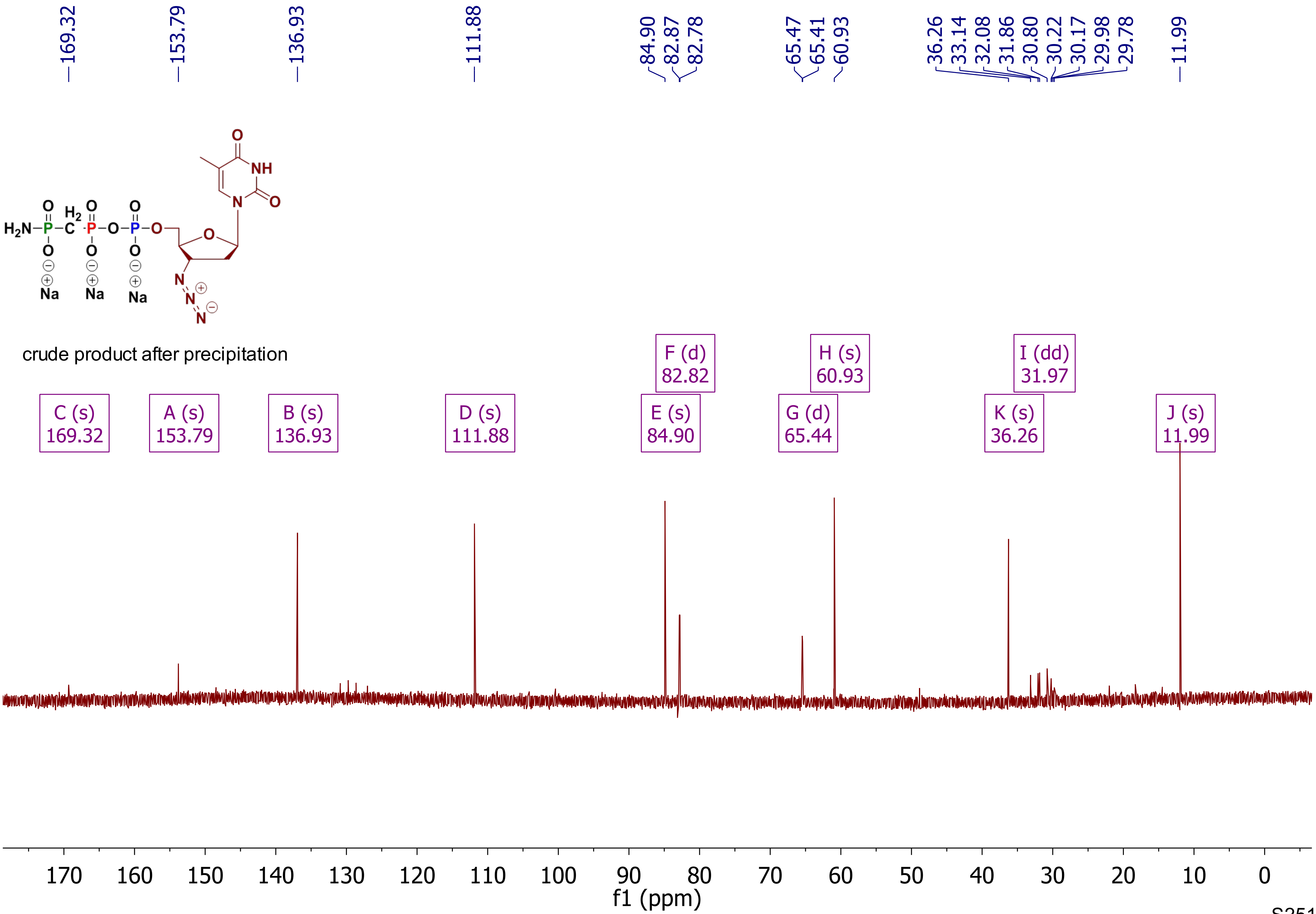
**$^{31}$P-$^{1}$H HMBC NMR**



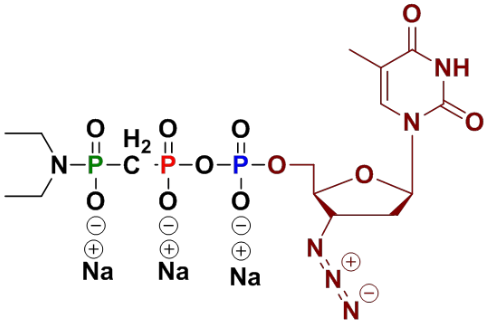
$^{13}C$-$^{1}H$ HSQC NMR



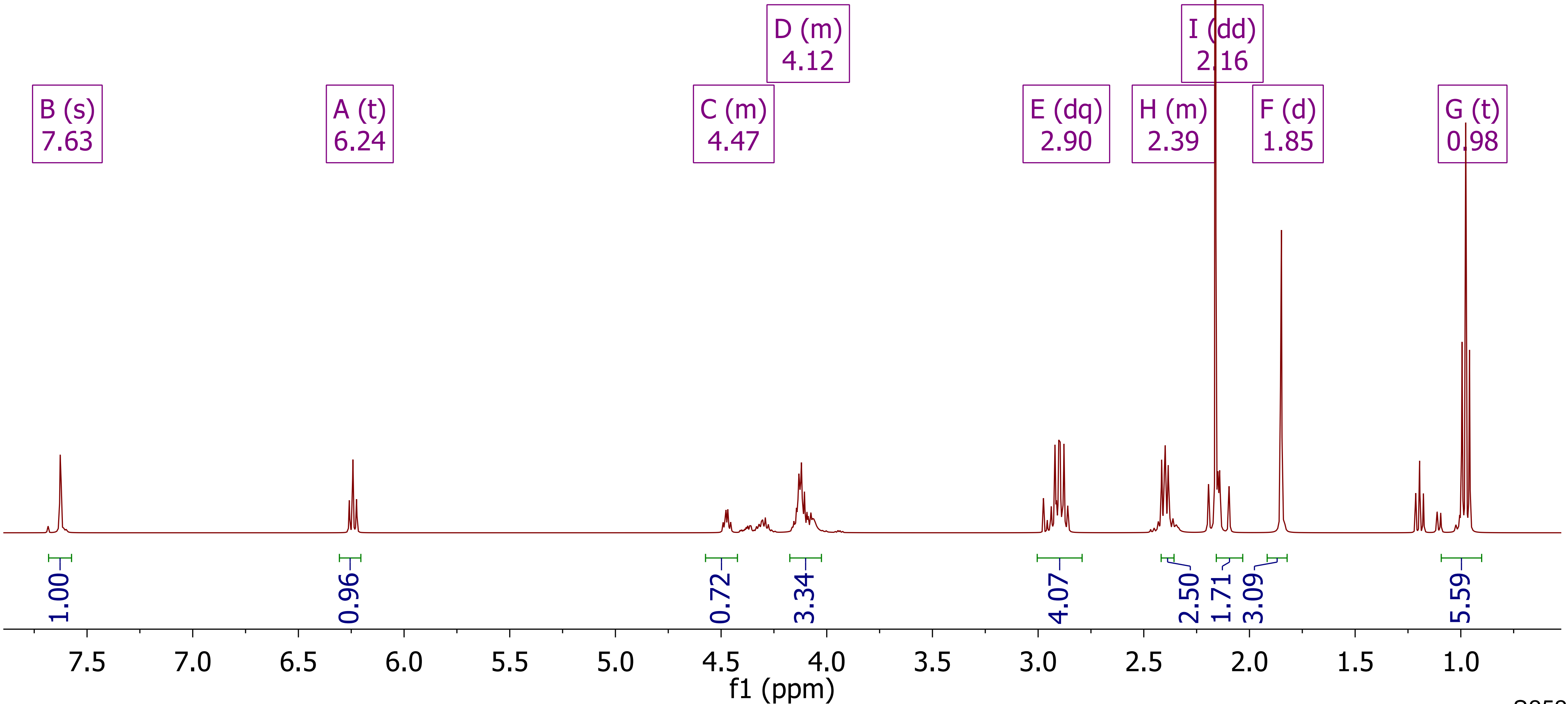
$^{13}C$ NMR


169.32
153.79
136.93
111.88
84.90
82.87
82.78
65.47
65.41
60.93
36.26
33.14
32.08
31.86
30.80
30.22
30.17
29.98
29.78
11.99
crude product after precipitation
C (s) 169.32
A (s) 153.79
B (s) 136.93
D (s) 111.88
E (s) 84.90
F (d) 82.82
G (d) 65.44
H (s) 60.93
I (dd) 31.97
K (s) 36.26
J (s) 11.99
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

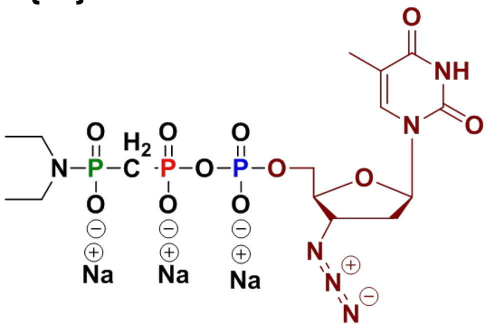
$^1H$ NMR



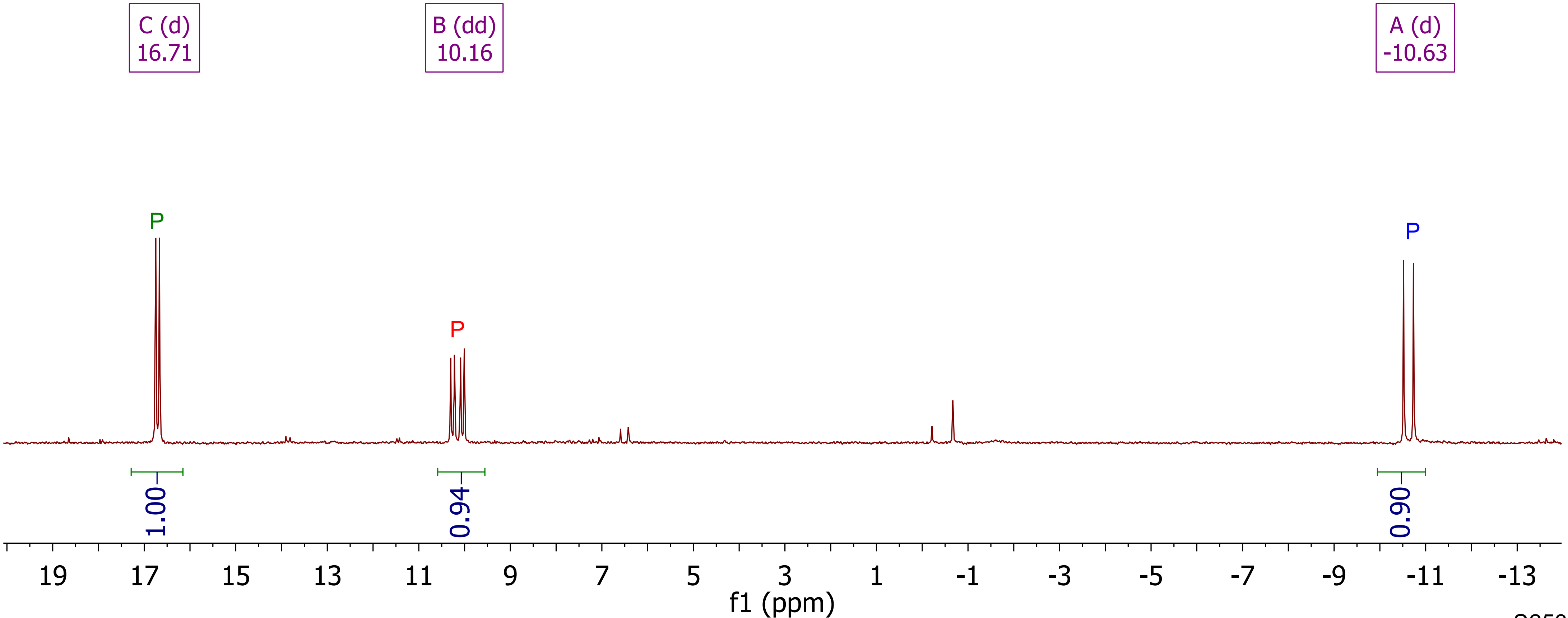
crude product after precipitation



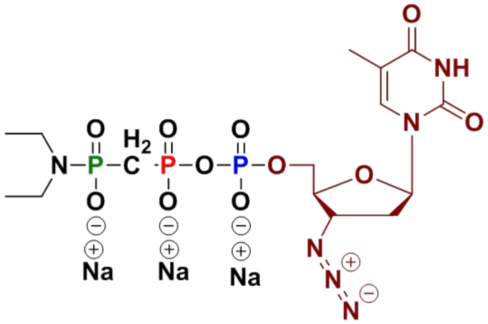
$^{31}P\{^{1}H\}$ NMR



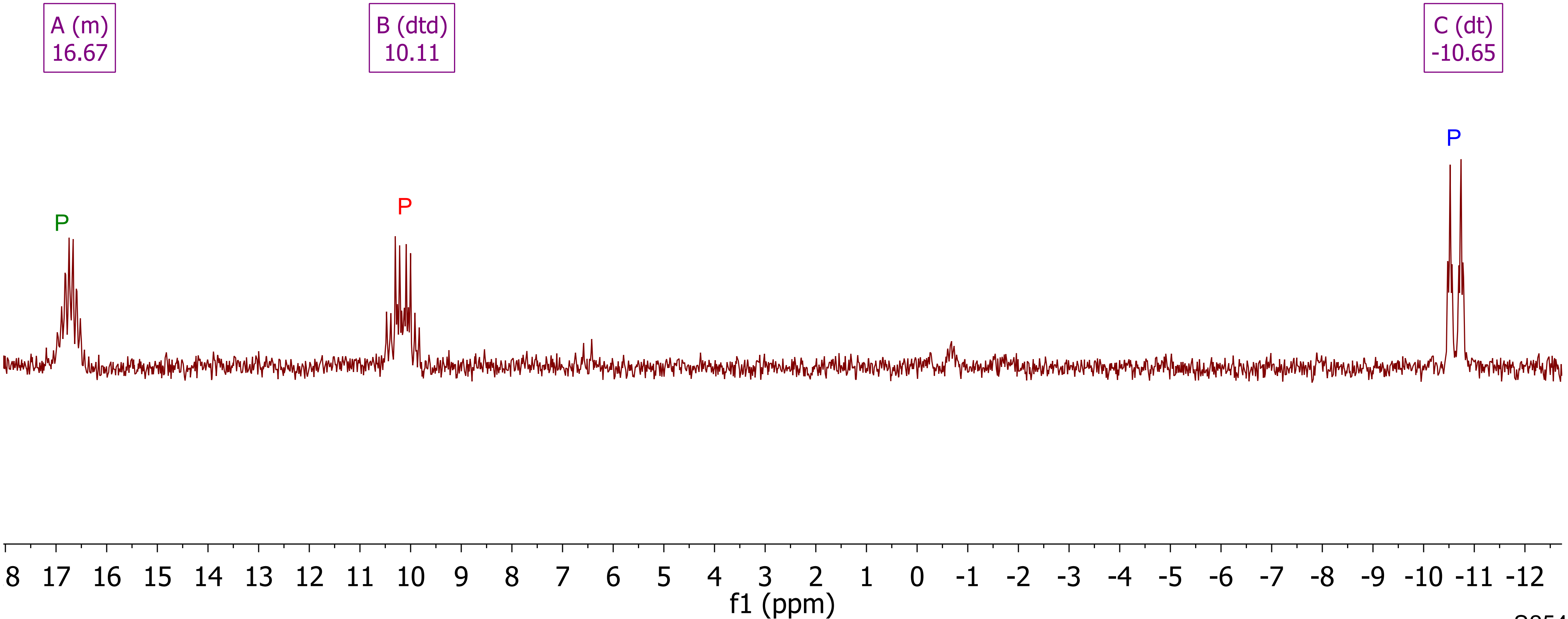
crude product after precipitation



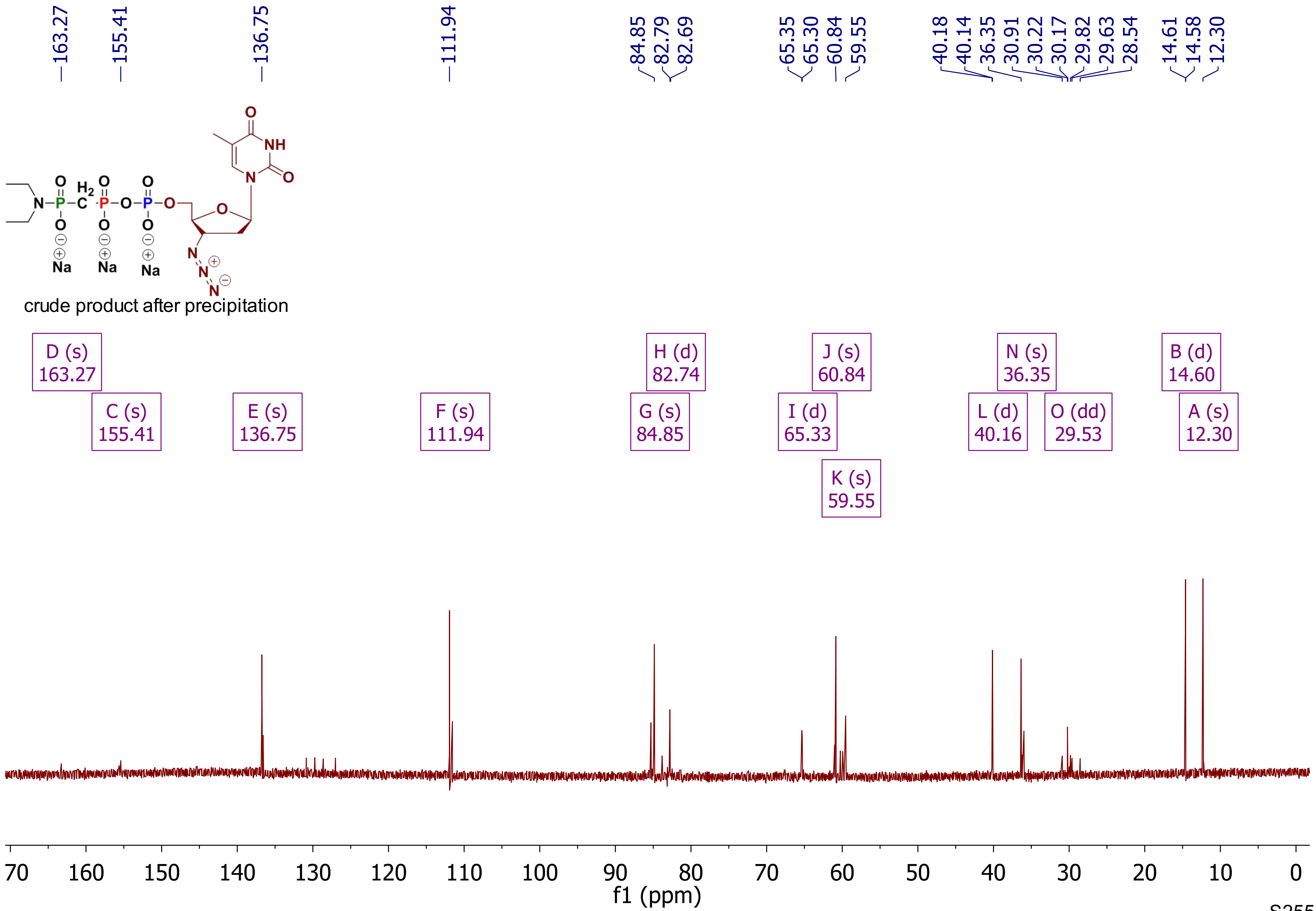
**$^{31}$P NMR**




crude product after precipitation

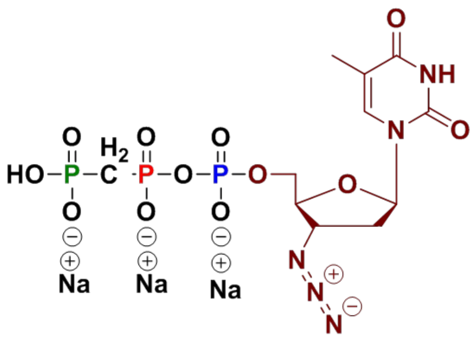


## $^{13}$C NMR

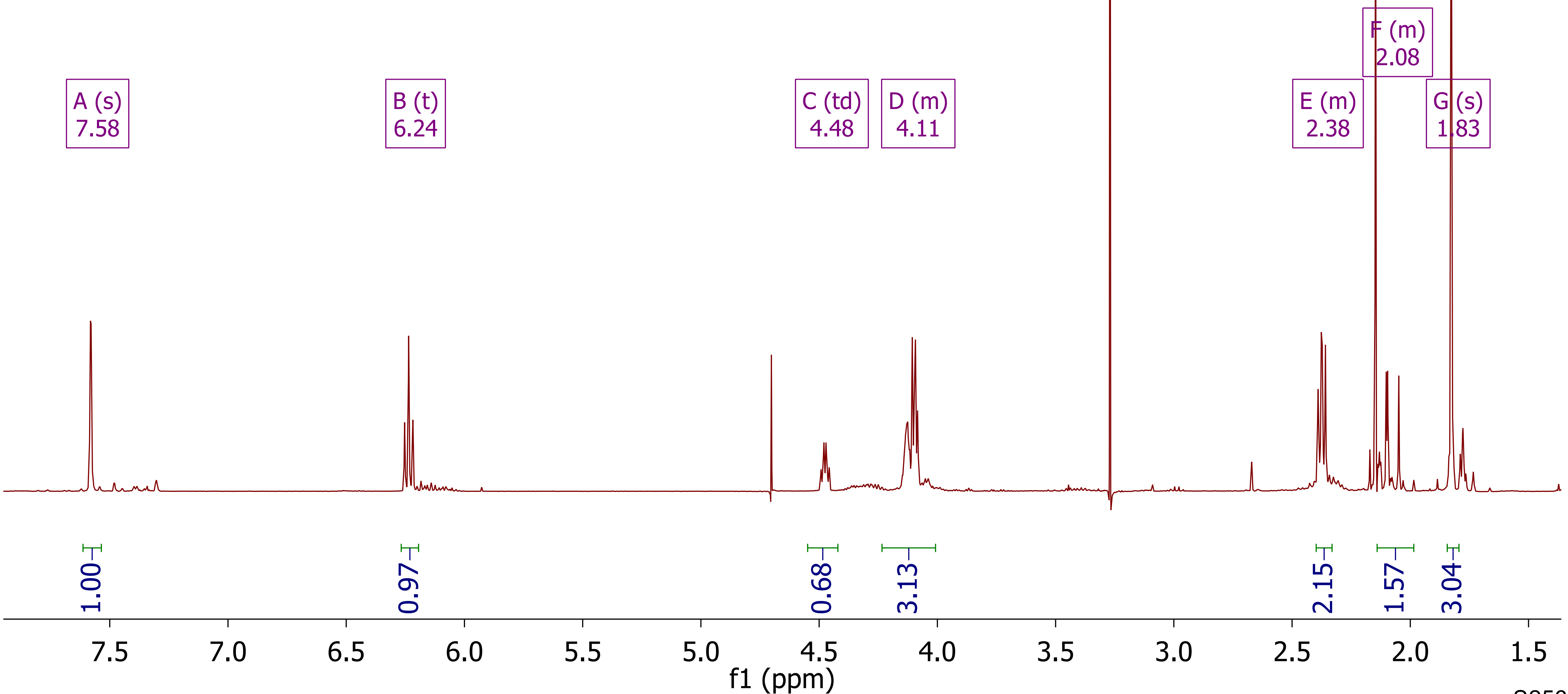


163.27
155.41
136.75
111.94
84.85
82.79
82.69
65.35
65.30
60.84
59.55
40.18
40.14
36.35
30.91
30.22
30.17
29.82
29.63
28.54
14.61
14.58
12.30
crude product after precipitation
D (s) 163.27
C (s) 155.41
E (s) 136.75
F (s) 111.94
H (d) 82.74
G (s) 84.85
J (s) 60.84
I (d) 65.33
K (s) 59.55
N (s) 36.35
L (d) 40.16
O (dd) 29.53
B (d) 14.60
A (s) 12.30
f1 (ppm)

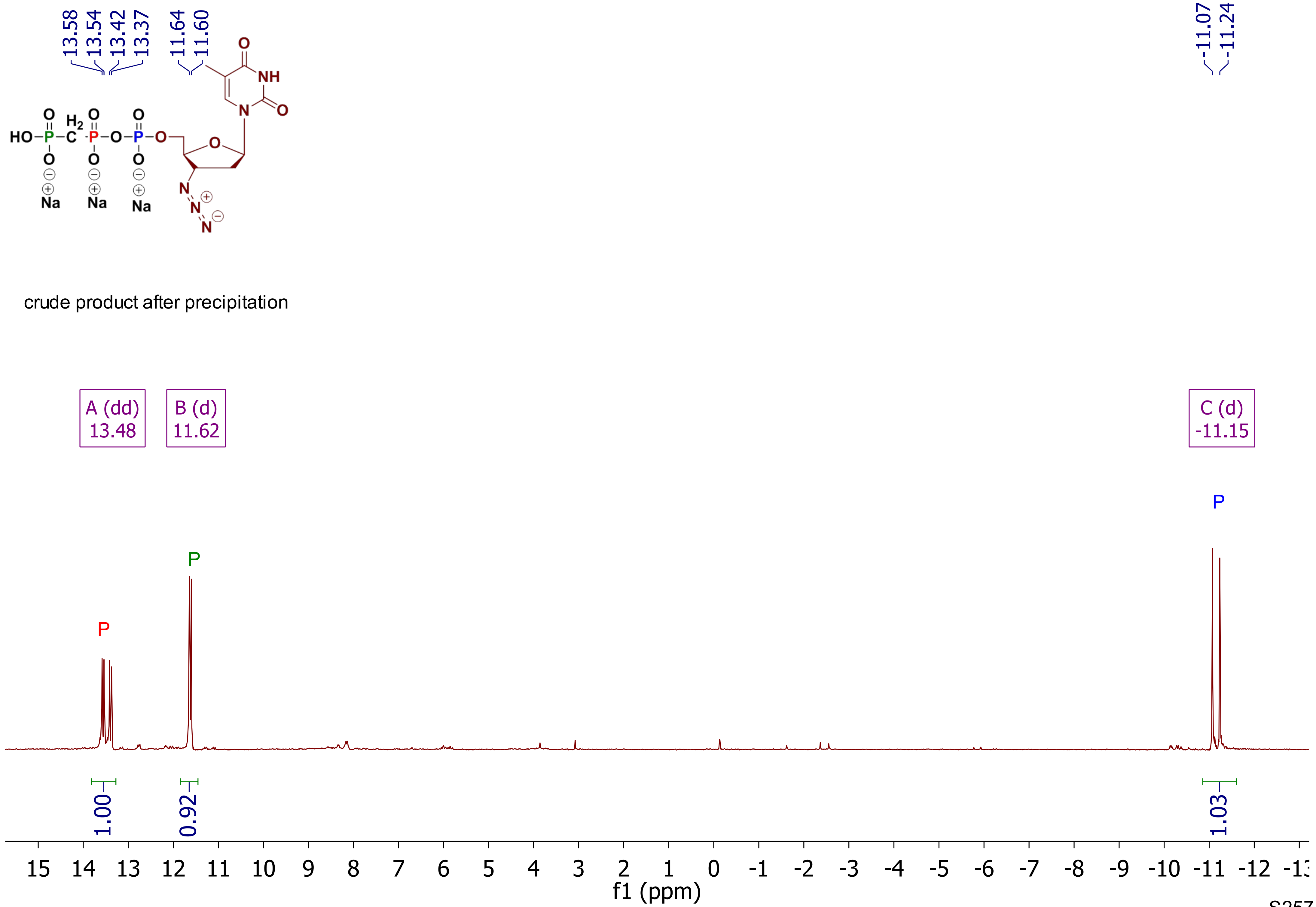
**$^1$H NMR**



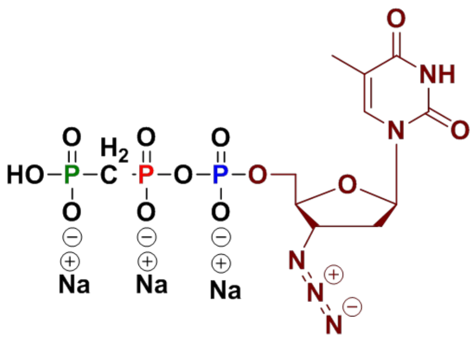

crude product after precipitation



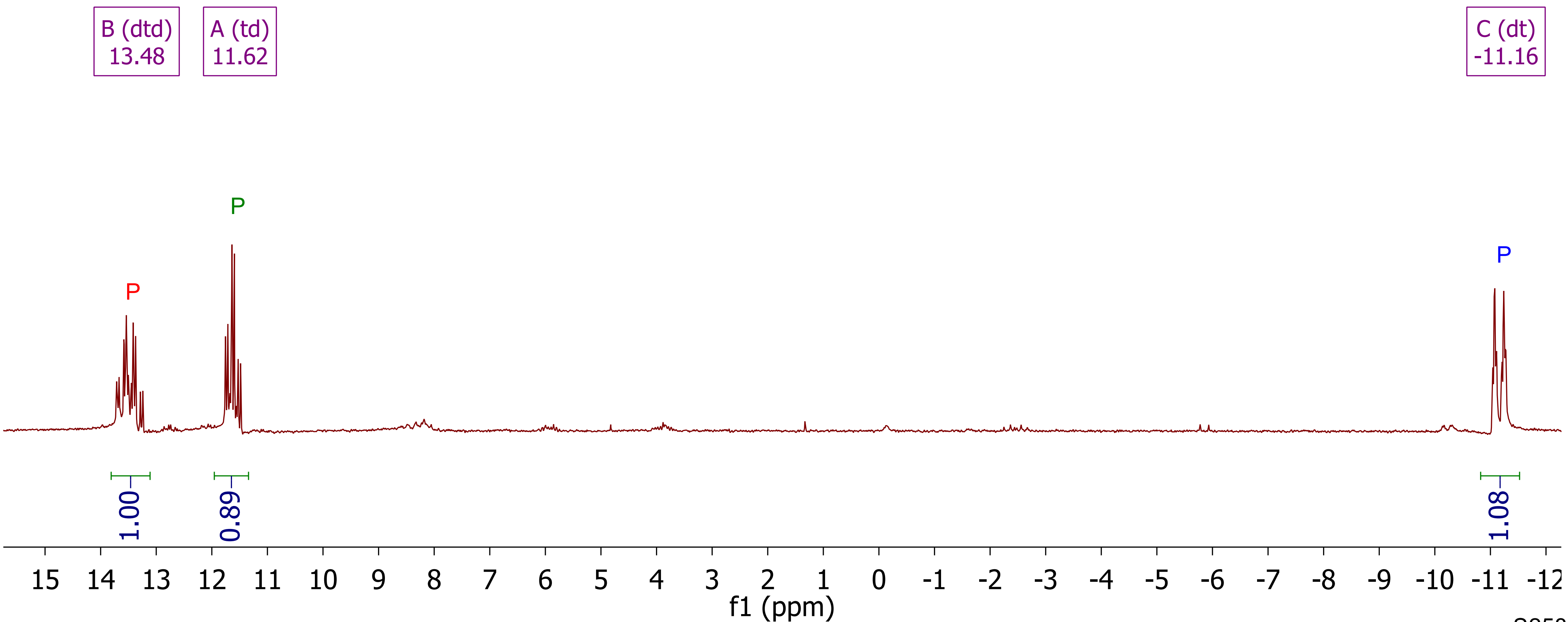
$^{31}P\{^{1}H\}$ NMR


13.58
13.54
13.42
13.37
11.64
11.60
-11.07
-11.24
O
NH
O
N
O
O
O
$H_2$
HO
P
C
P
O
P
O
O
O
O
O
Na
Na
Na
N
N
N
crude product after precipitation
A (dd)
13.48
B (d)
11.62
C (d)
-11.15
P
P
P
1.00
0.92
1.03
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
f1 (ppm)

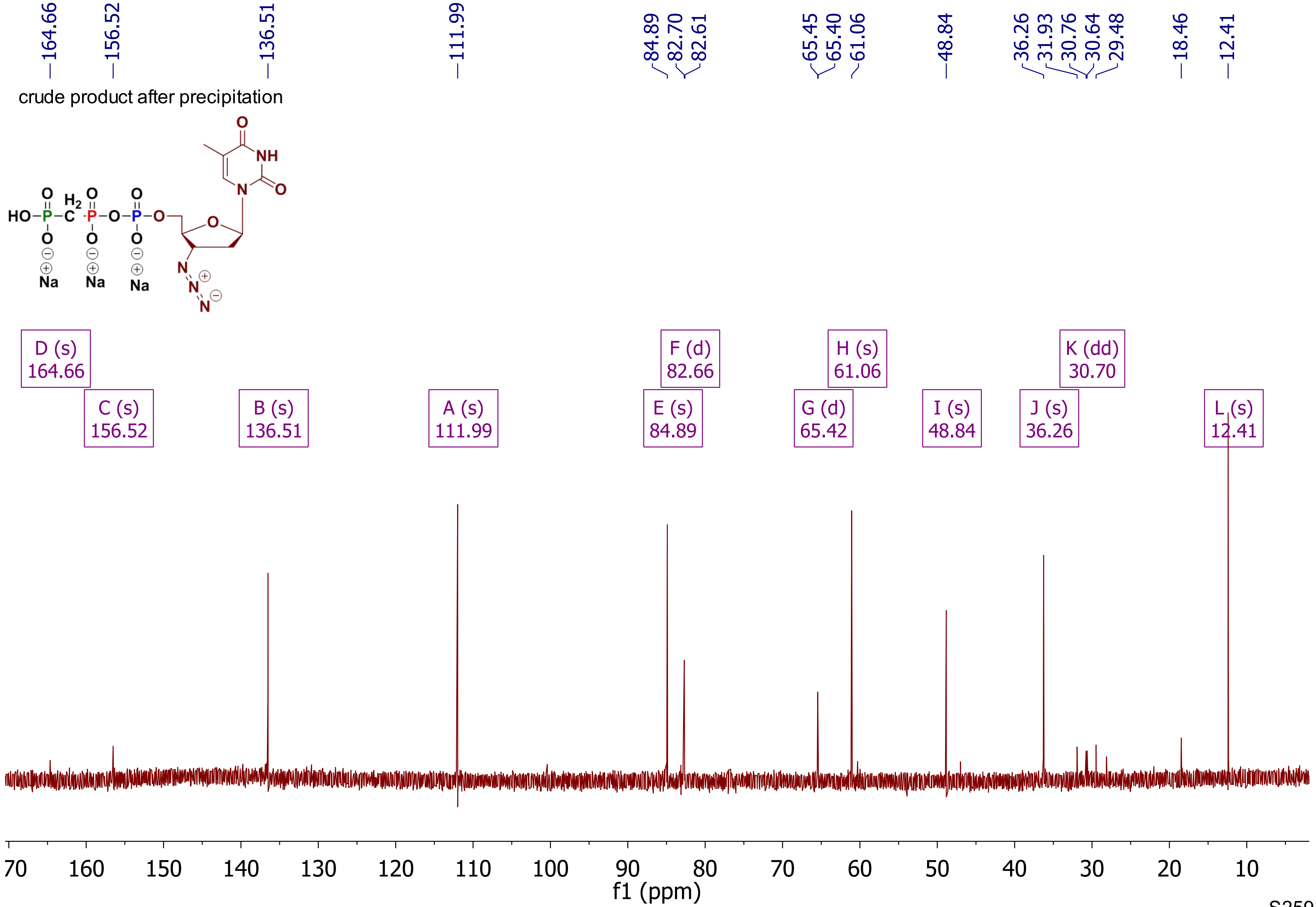
**$^{31}$P NMR**



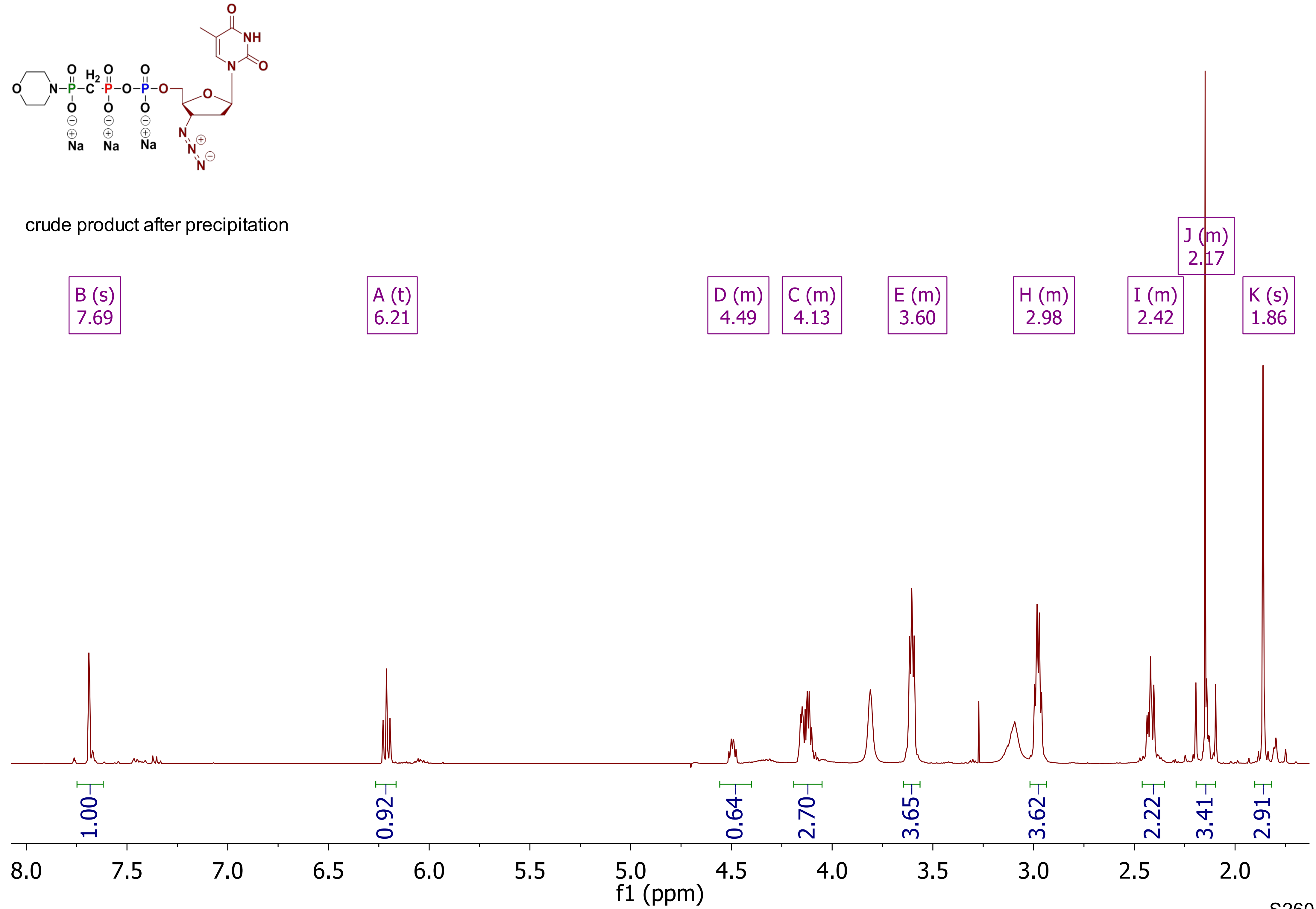
crude product after precipitation



**$^{13}$C NMR**

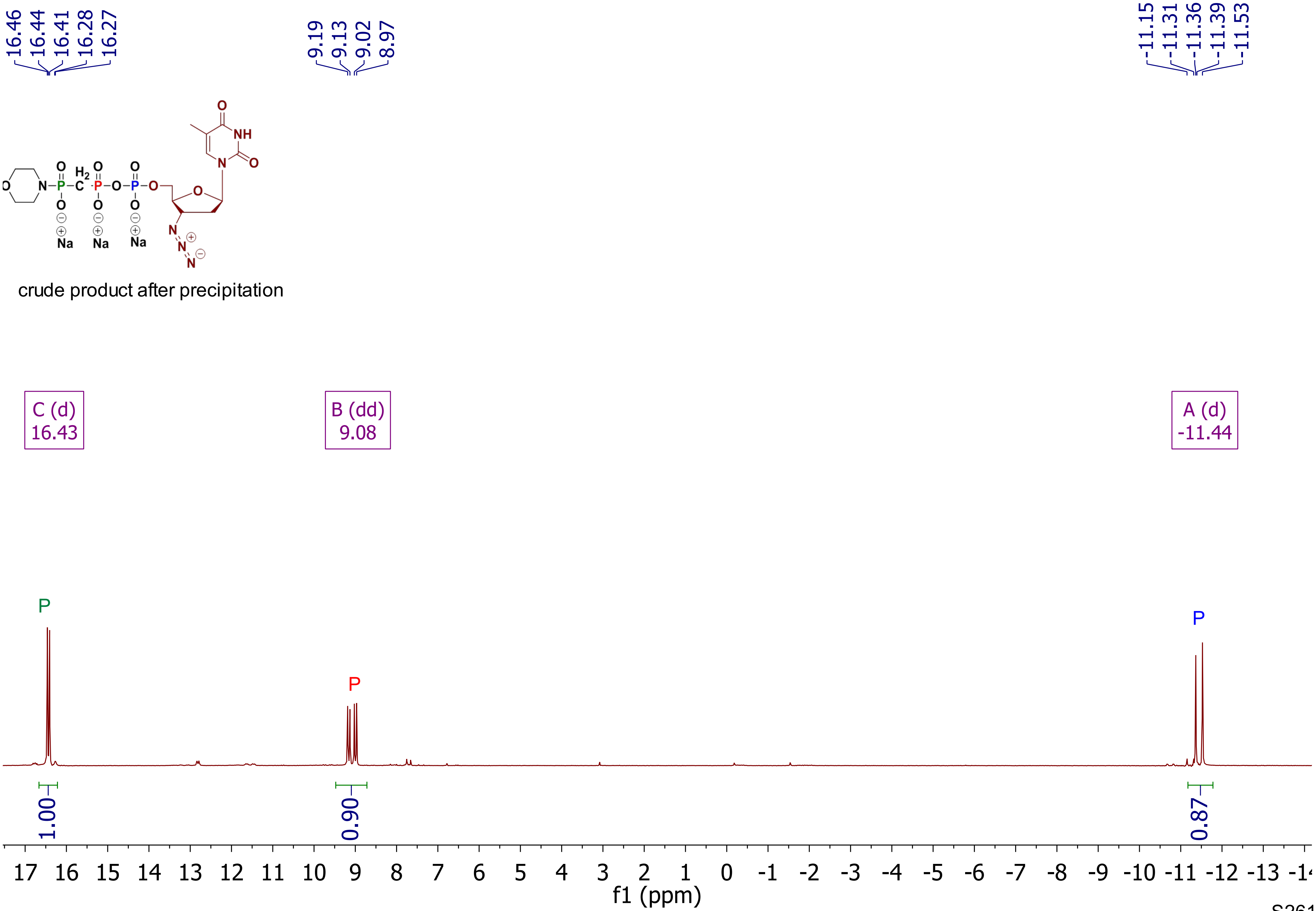

164.66
156.52
136.51
111.99
84.89
82.70
82.61
65.45
65.40
61.06
48.84
36.26
31.93
30.76
30.64
29.48
18.46
12.41
crude product after precipitation
D (s) 164.66
C (s) 156.52
B (s) 136.51
A (s) 111.99
F (d) 82.66
E (s) 84.89
H (s) 61.06
G (d) 65.42
I (s) 48.84
K (dd) 30.70
J (s) 36.26
L (s) 12.41
f1 (ppm)

**$^1$H NMR**

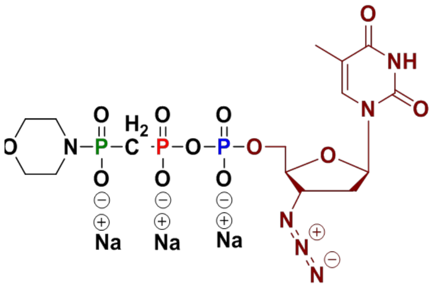


O
NH
N
O
O
N
P
C
$H_2$
P
O
P
O
O
Na
Na
Na
N
N
N
crude product after precipitation
B (s)
7.69
A (t)
6.21
D (m)
4.49
C (m)
4.13
E (m)
3.60
H (m)
2.98
I (m)
2.42
J (m)
2.17
K (s)
1.86
1.00
0.92
0.64
2.70
3.65
3.62
2.22
3.41
2.91
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

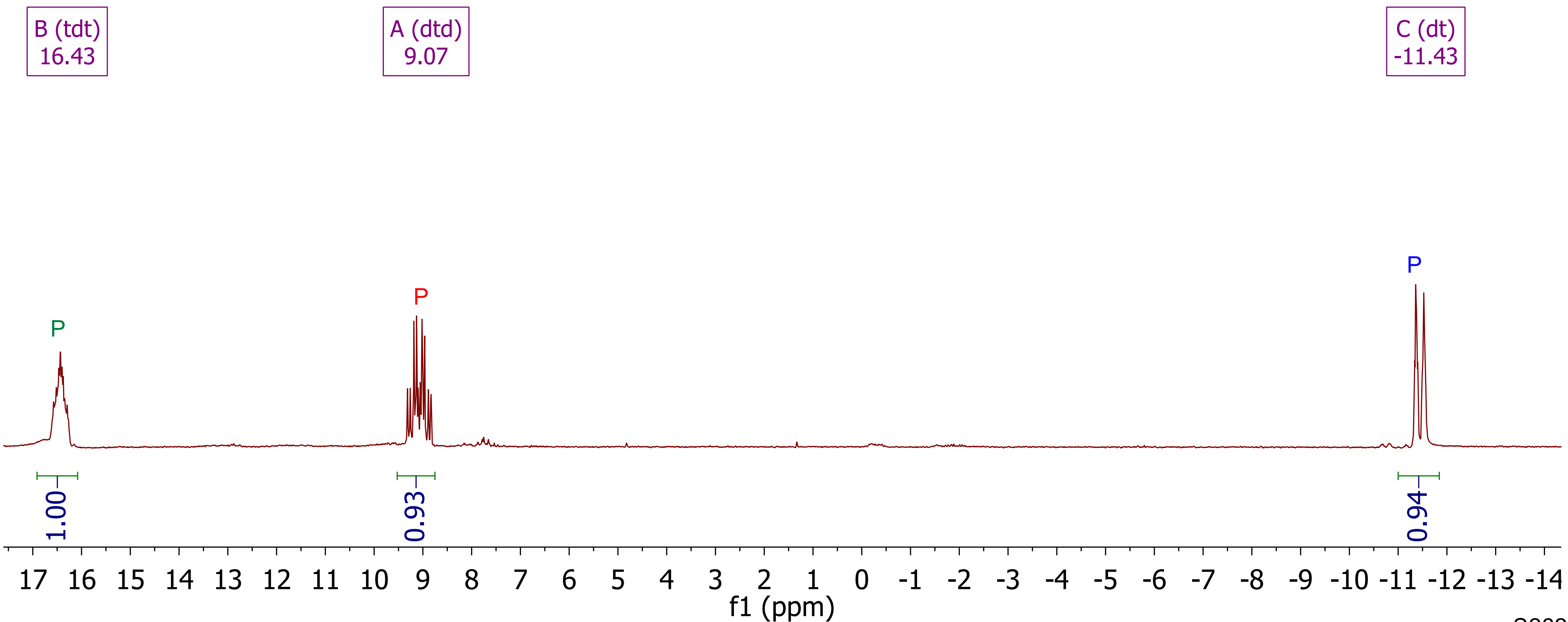
$^{31}P\{^{1}H\}$ NMR


16.46
16.44
16.41
16.28
16.27
9.19
9.13
9.02
8.97
-11.15
-11.31
-11.36
-11.39
-11.53
crude product after precipitation
C (d)
16.43
B (dd)
9.08
A (d)
-11.44
P
P
P
1.00
0.90
0.87
17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13
f1 (ppm)

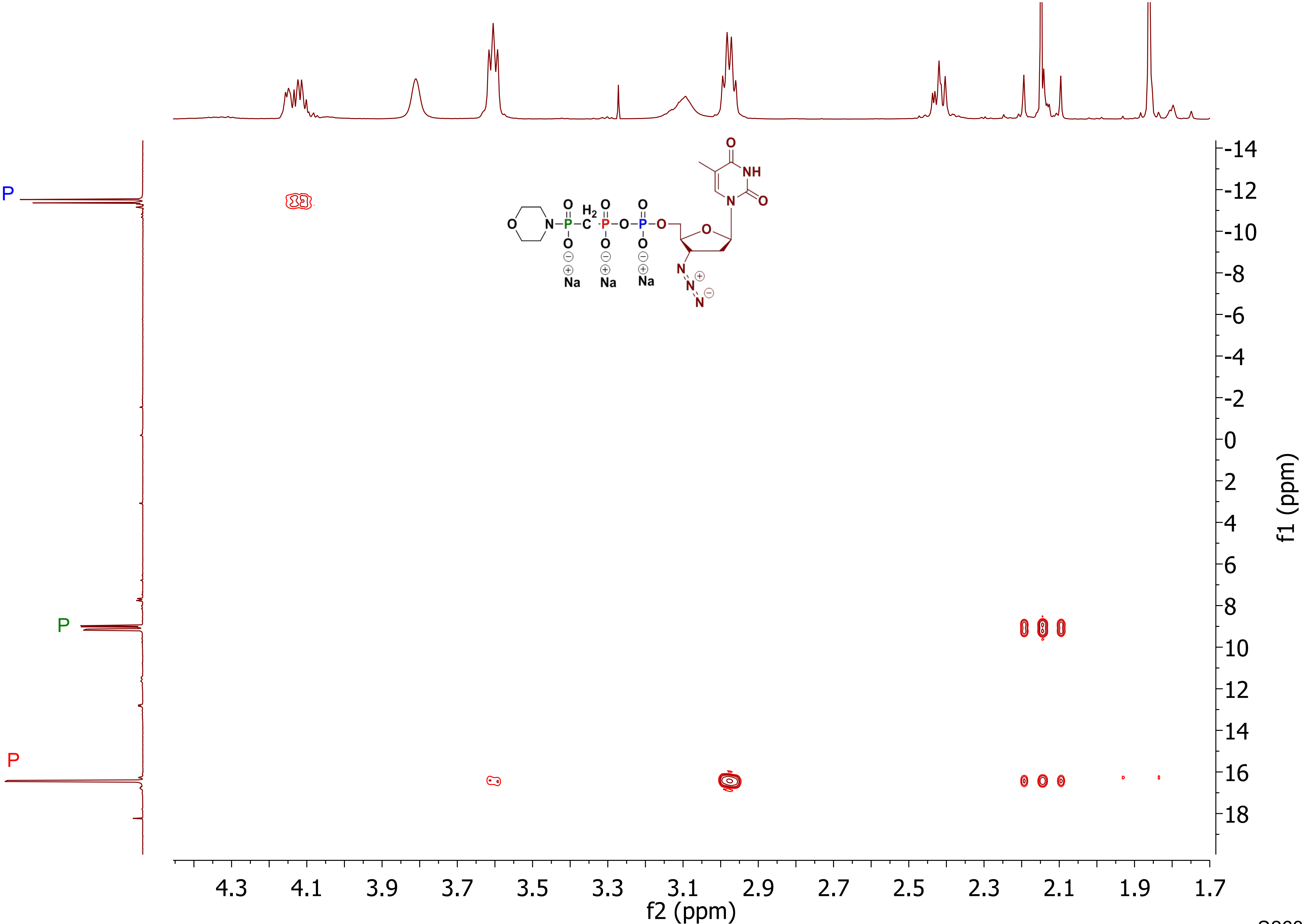
**$^{31}P$ NMR**



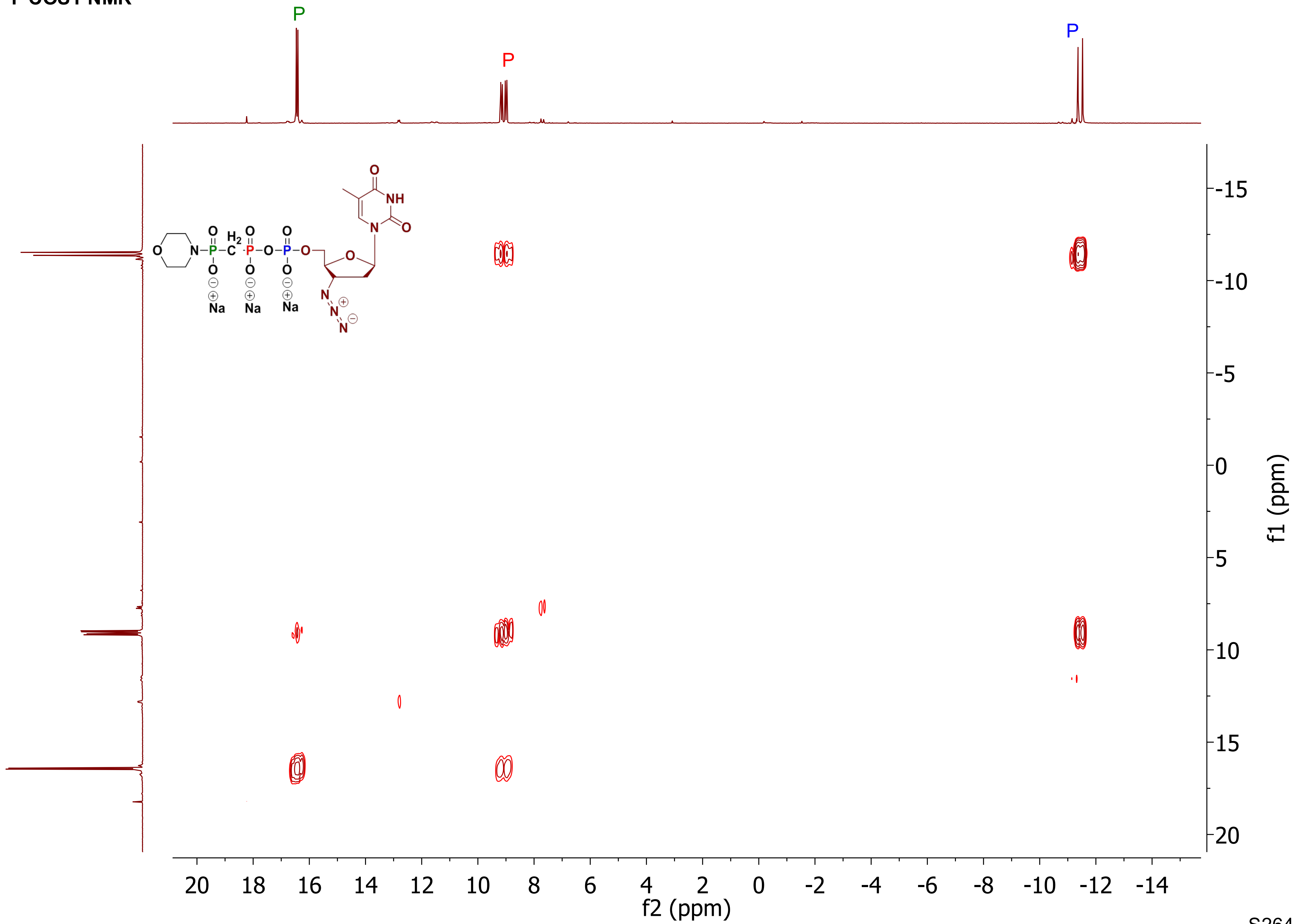
crude product after precipitation



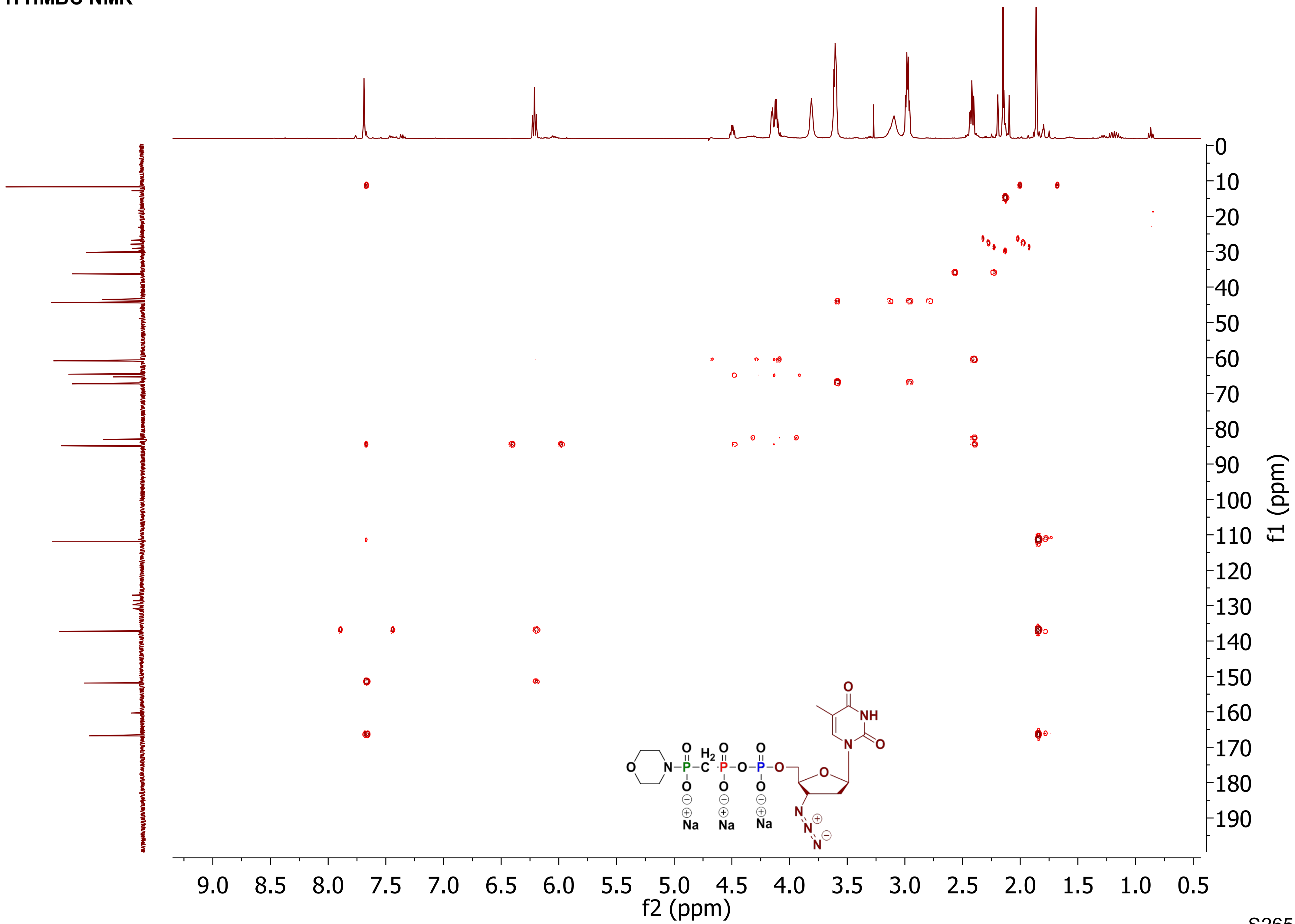
$^{31}$P-$^{1}$H HMBC NMR



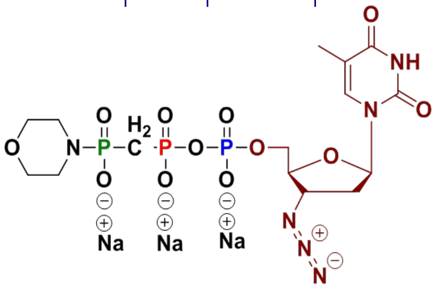
**$^{31}P$-$^{31}P$ COSY NMR**



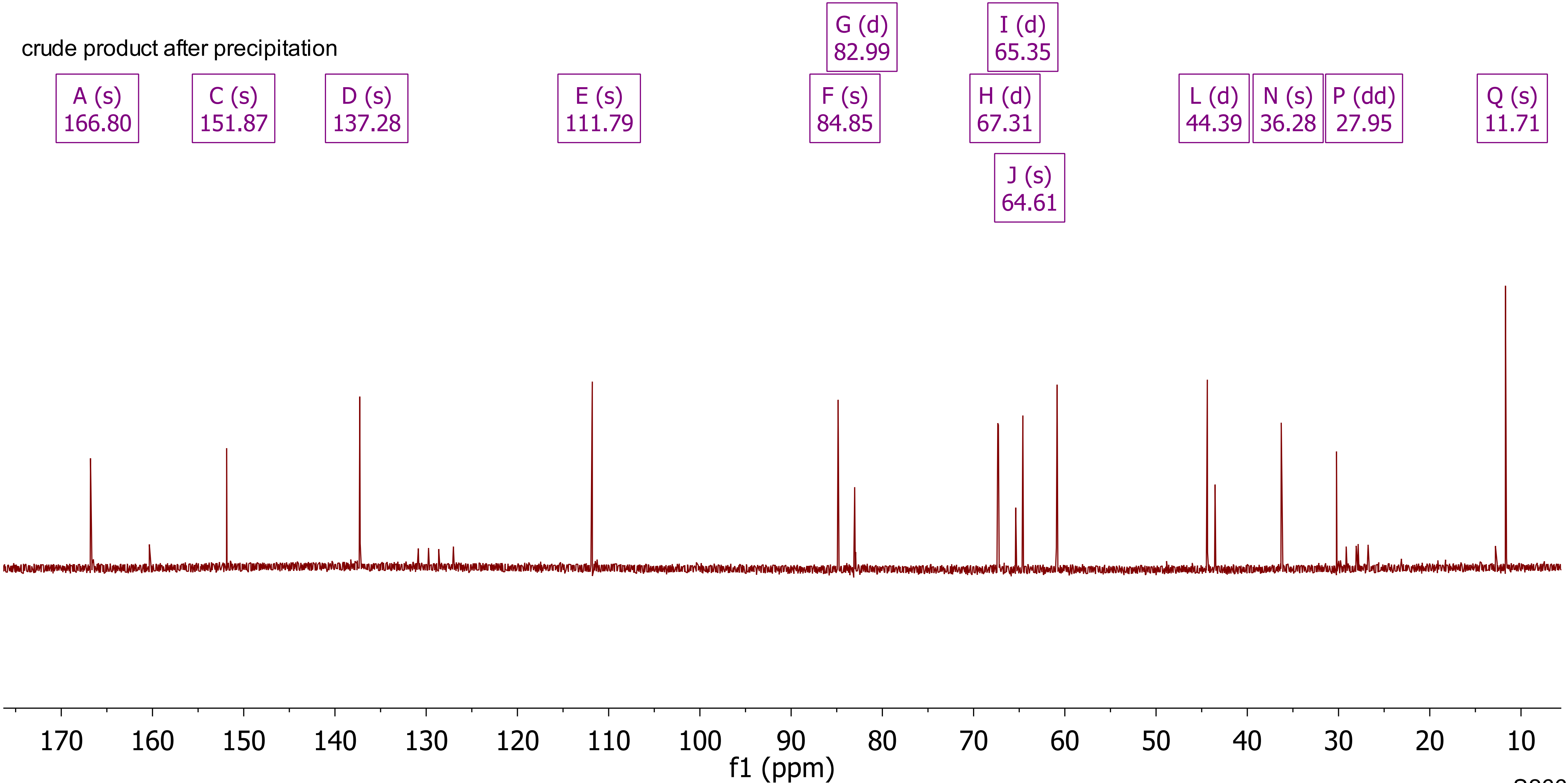
**$^{13}$C-$^{1}$H HMBC NMR**



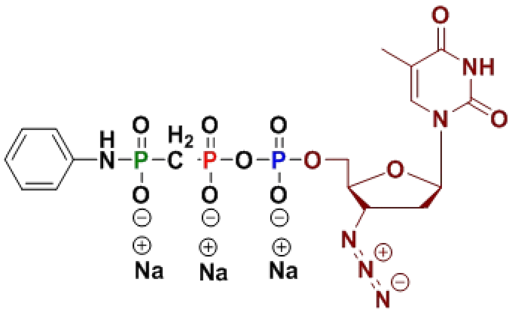
## $^{13}$C NMR



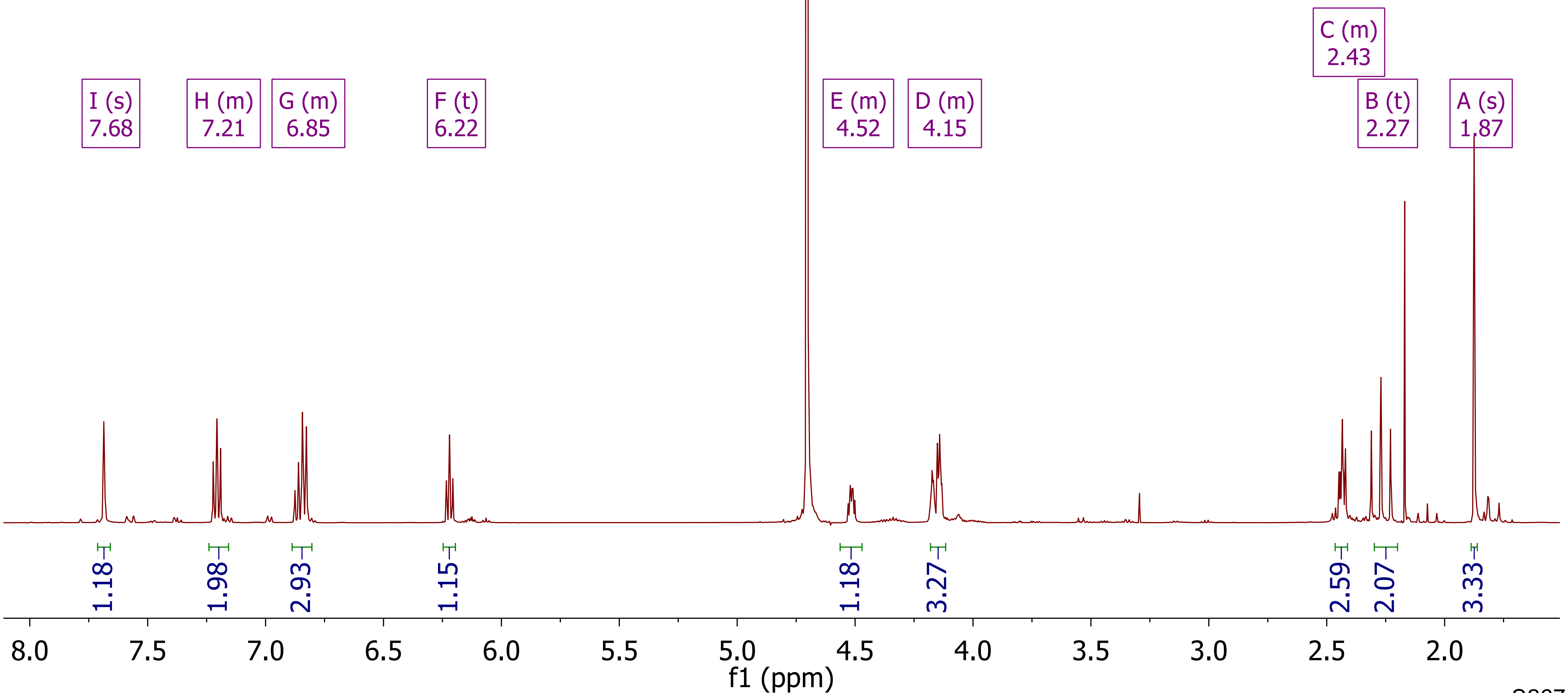
crude product after precipitation



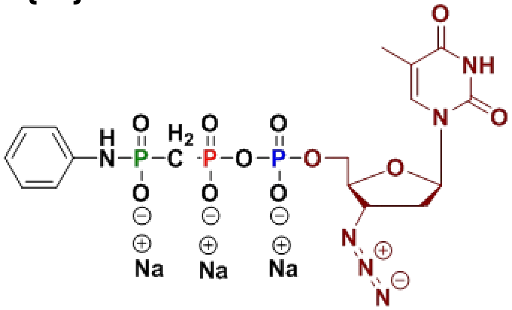
$^{1}$H NMR



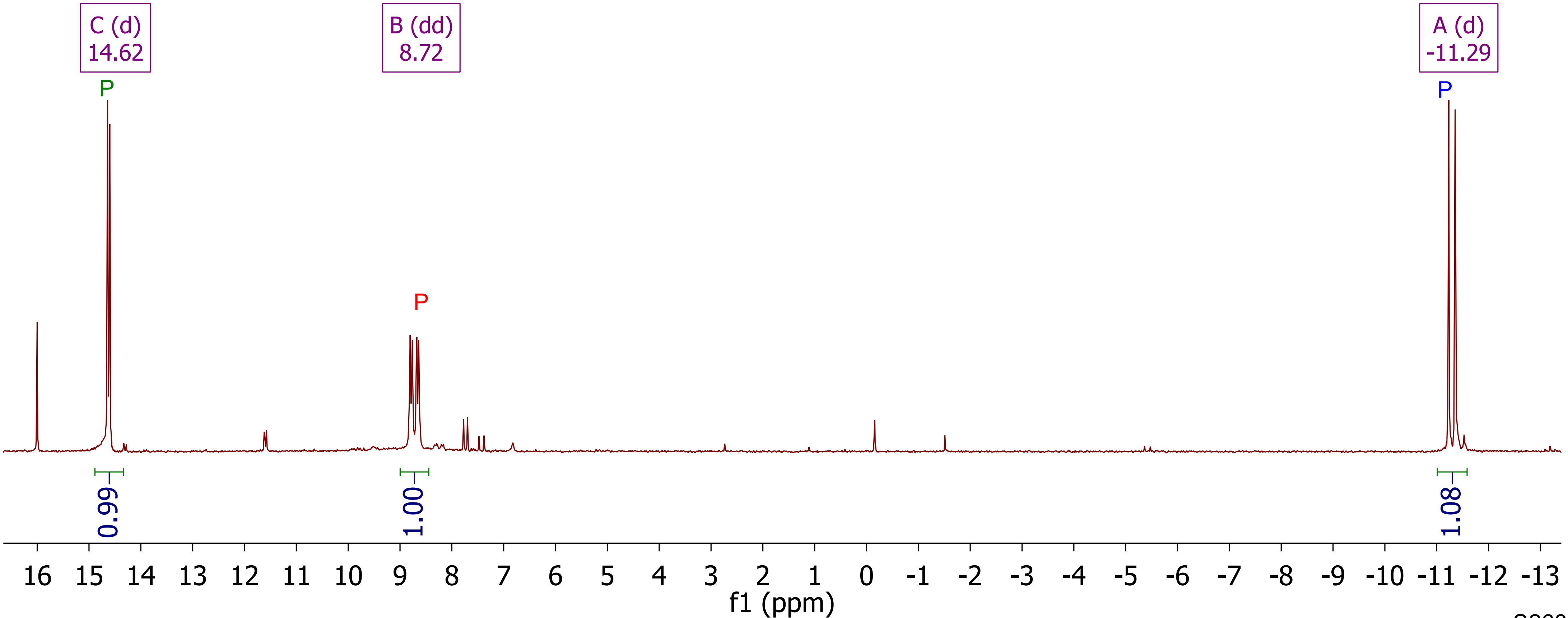
Crude after precipitation



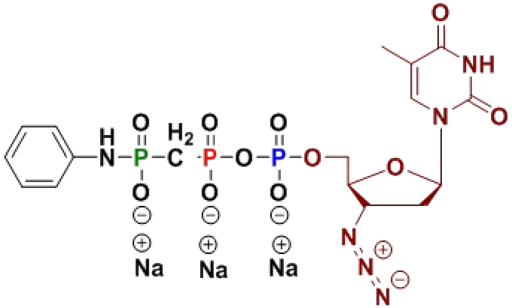
**$^{31}P\{^{1}H\}$ NMR**



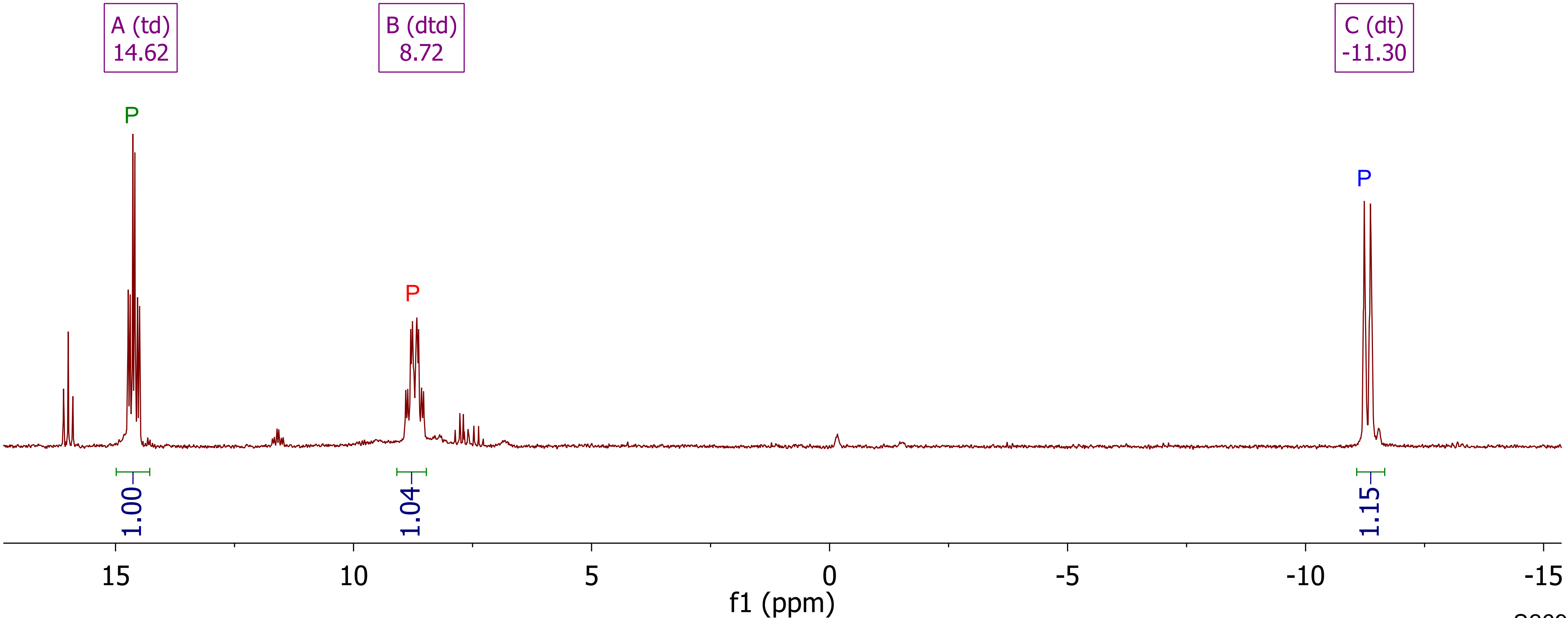
crude product after precipitation



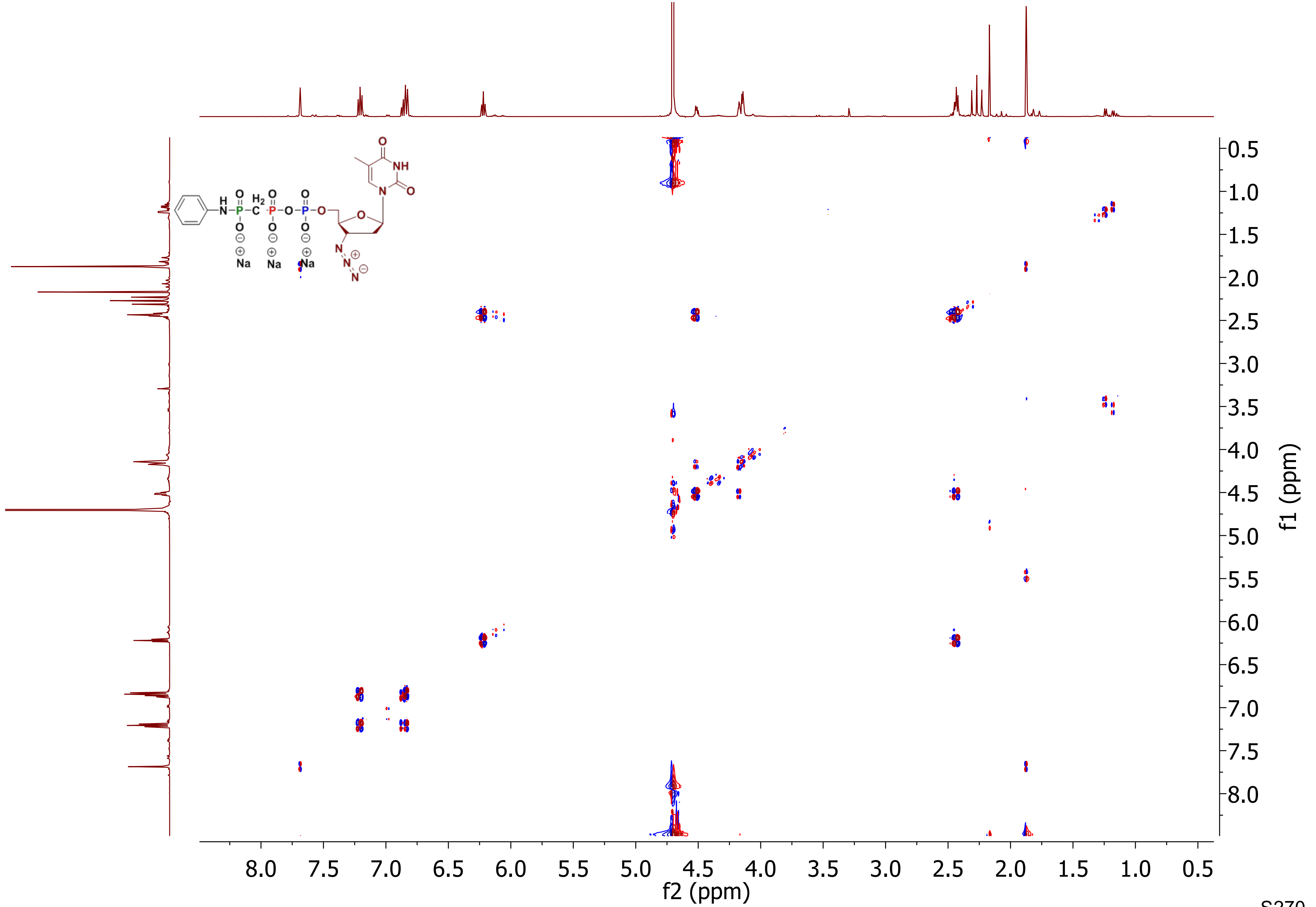
**$^{31}$P NMR**



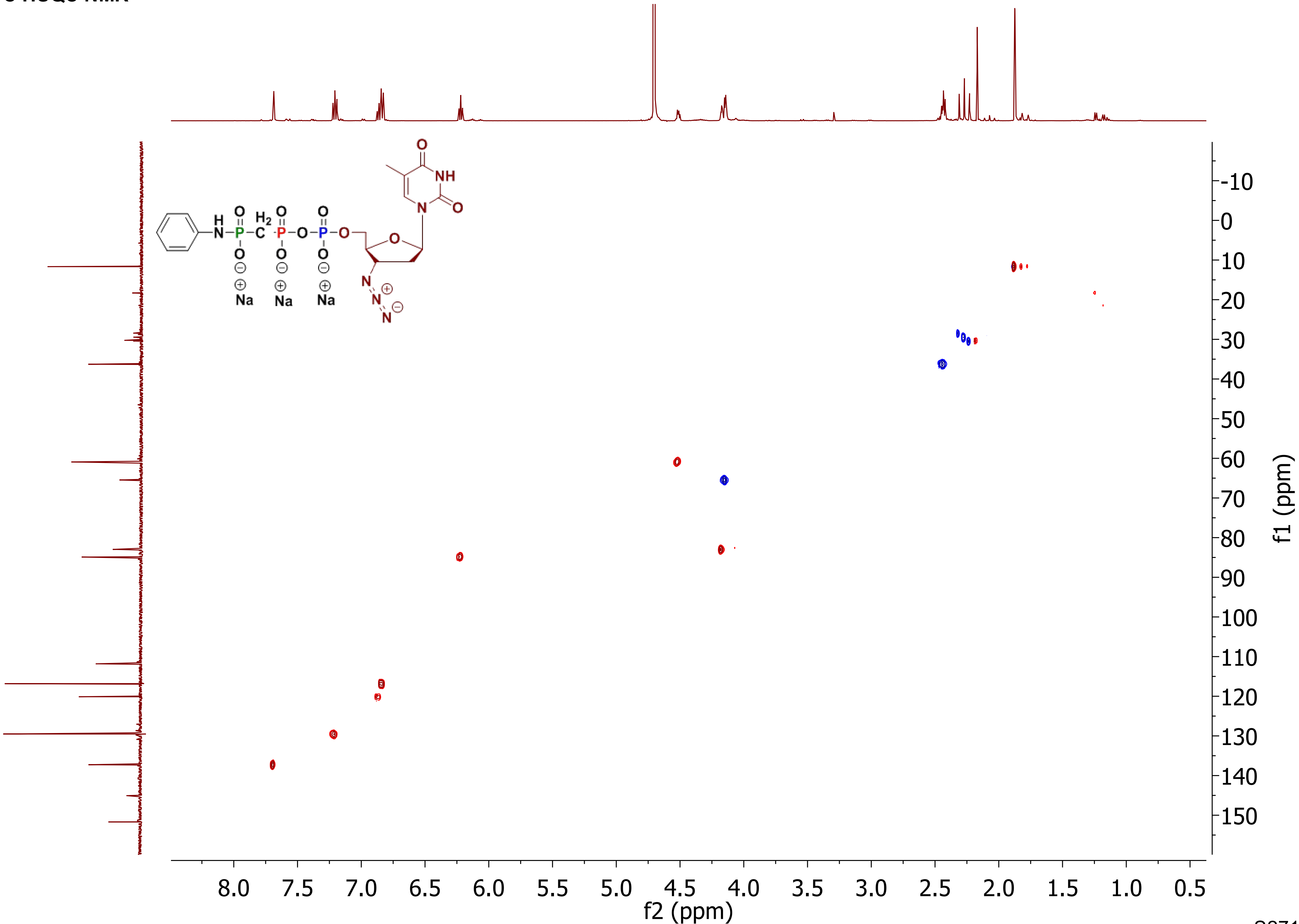
crude product after precipitation



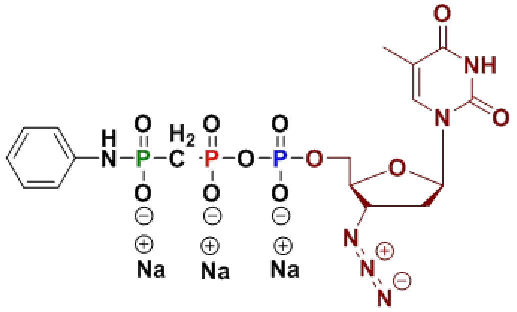
**$^1$H-$^1$H COSY NMR**



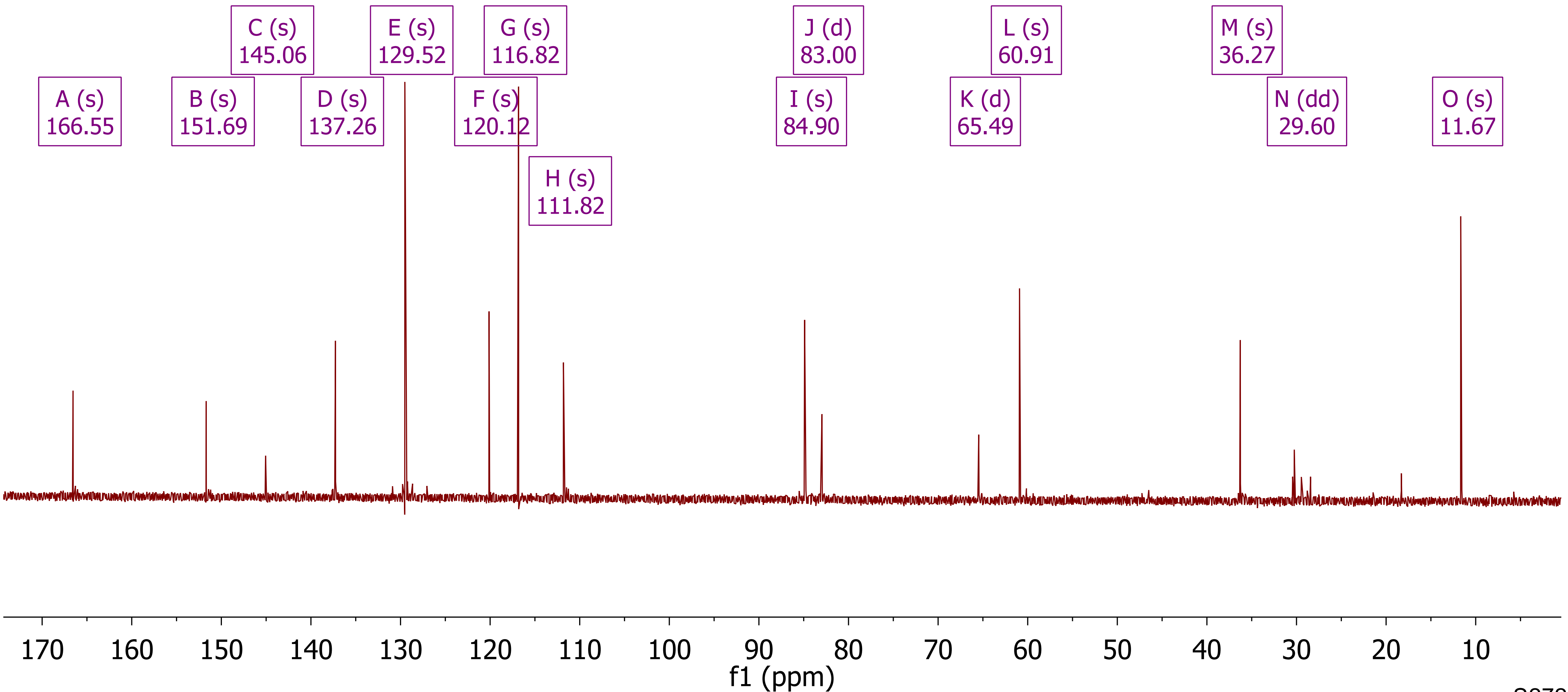
$^1H$-$^{13}C$ HSQC NMR



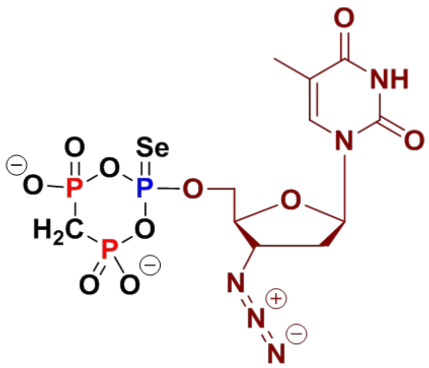
**$^{13}$C NMR**



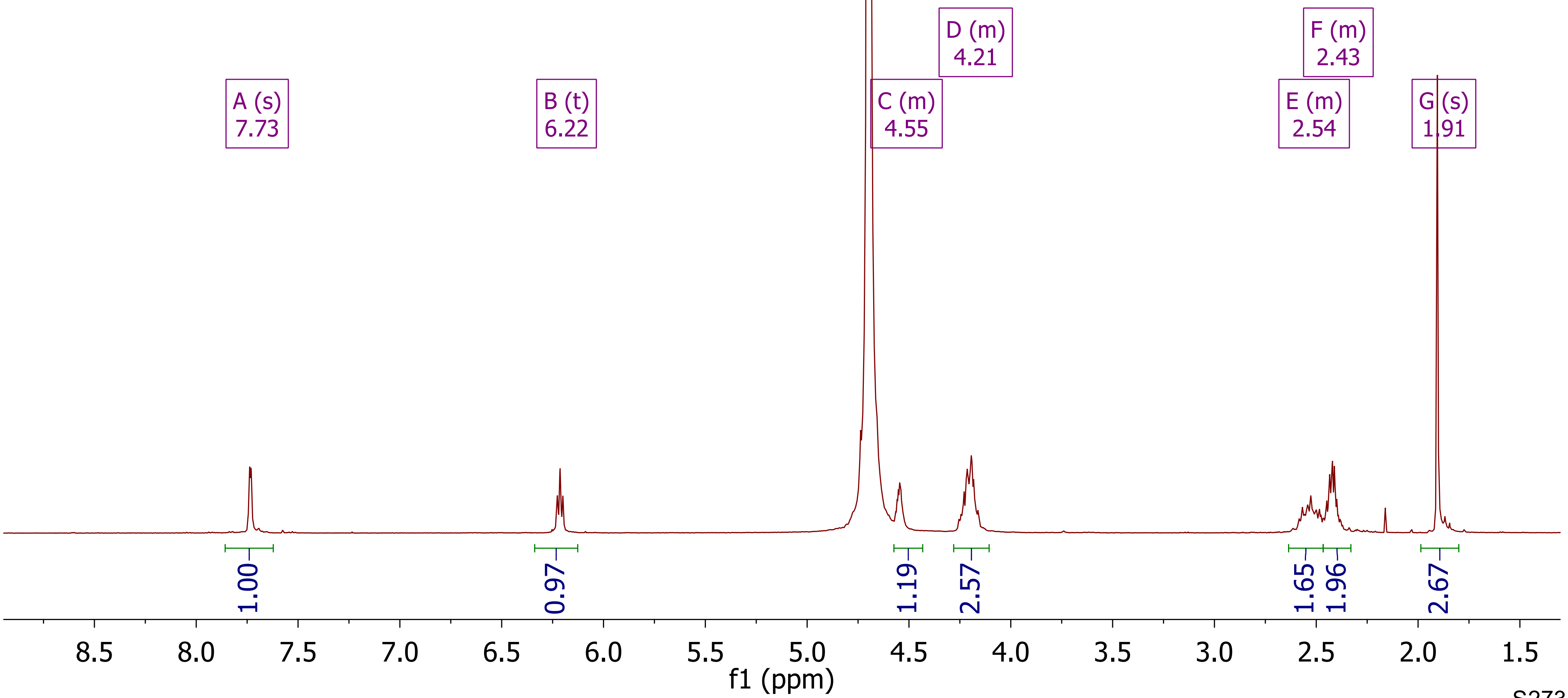
crude product after precipitation



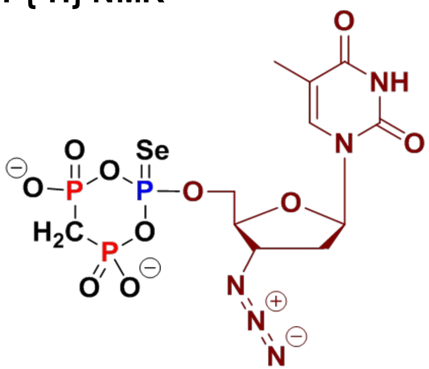
**$^1$H NMR**



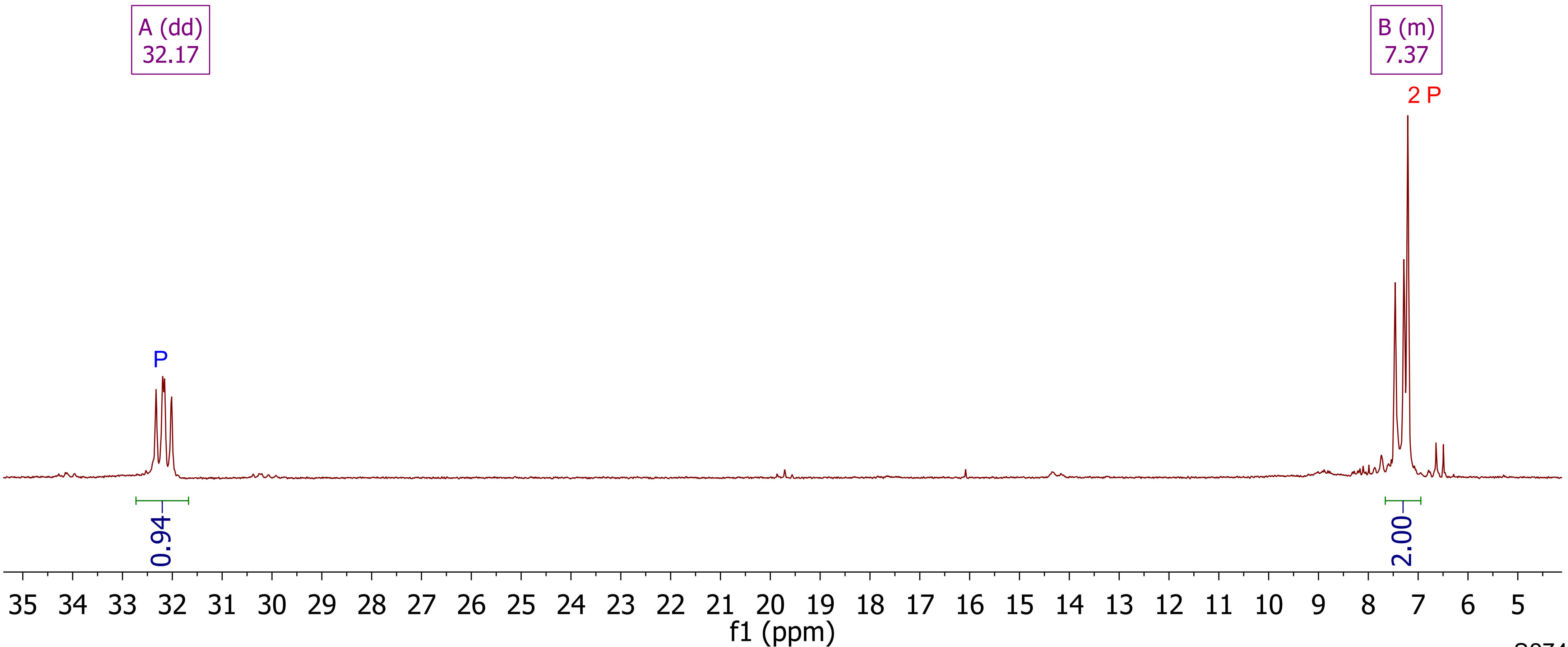
product after SAX Chromatography



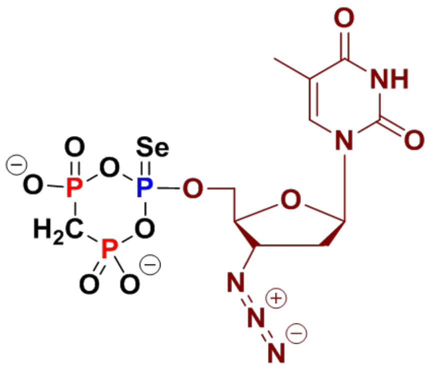
$^{31}P\{^1H\}$ NMR



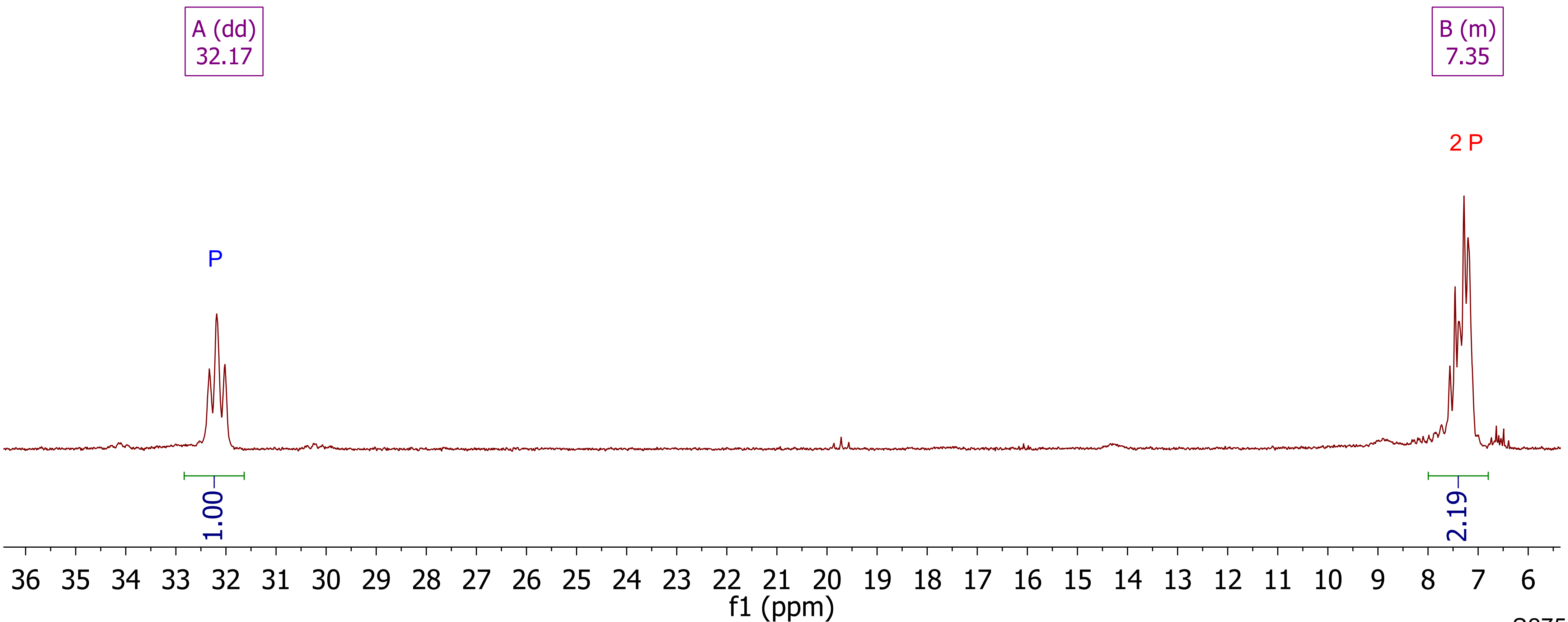
product after SAX Chromatography



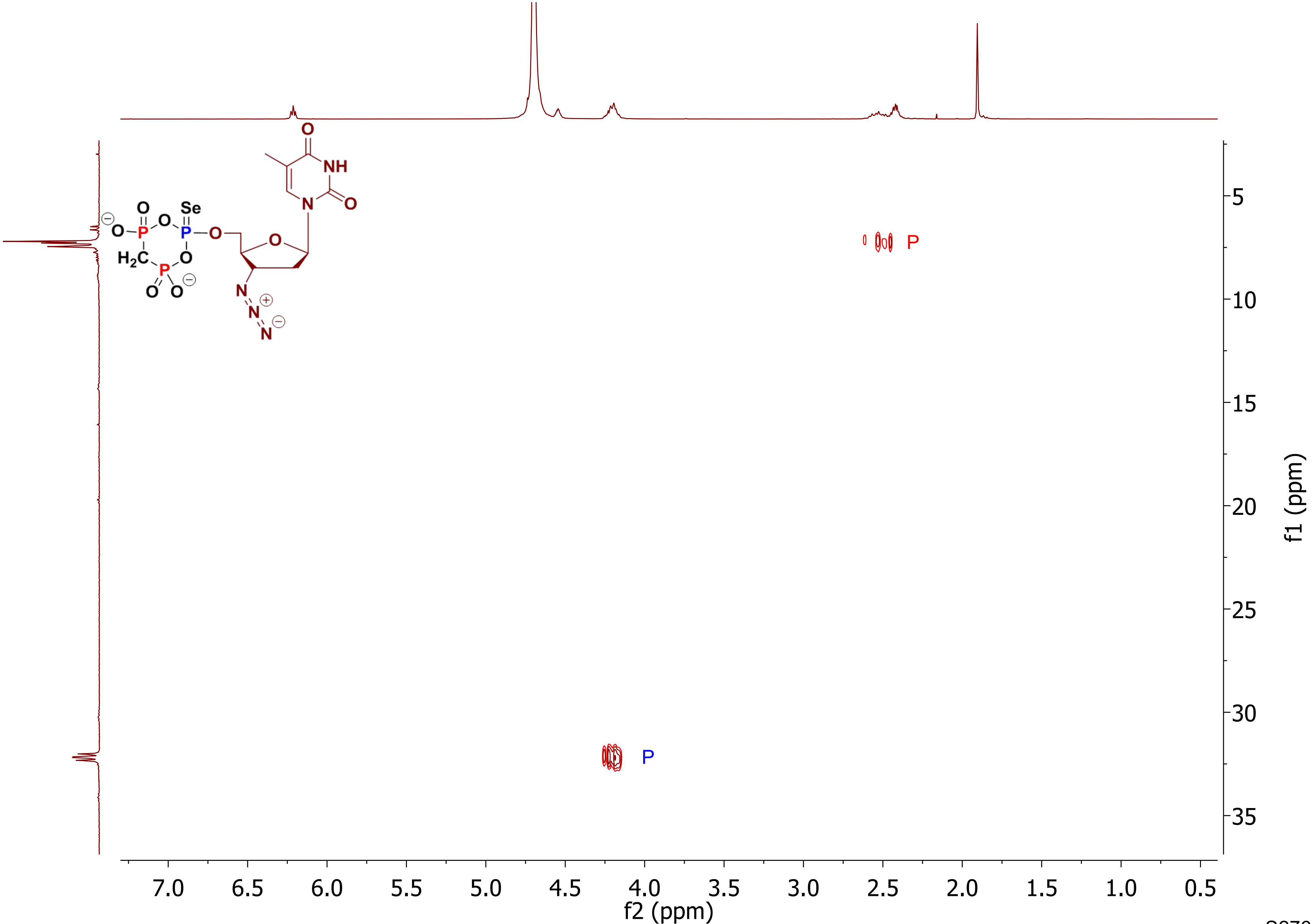
**$^{31}$P NMR**



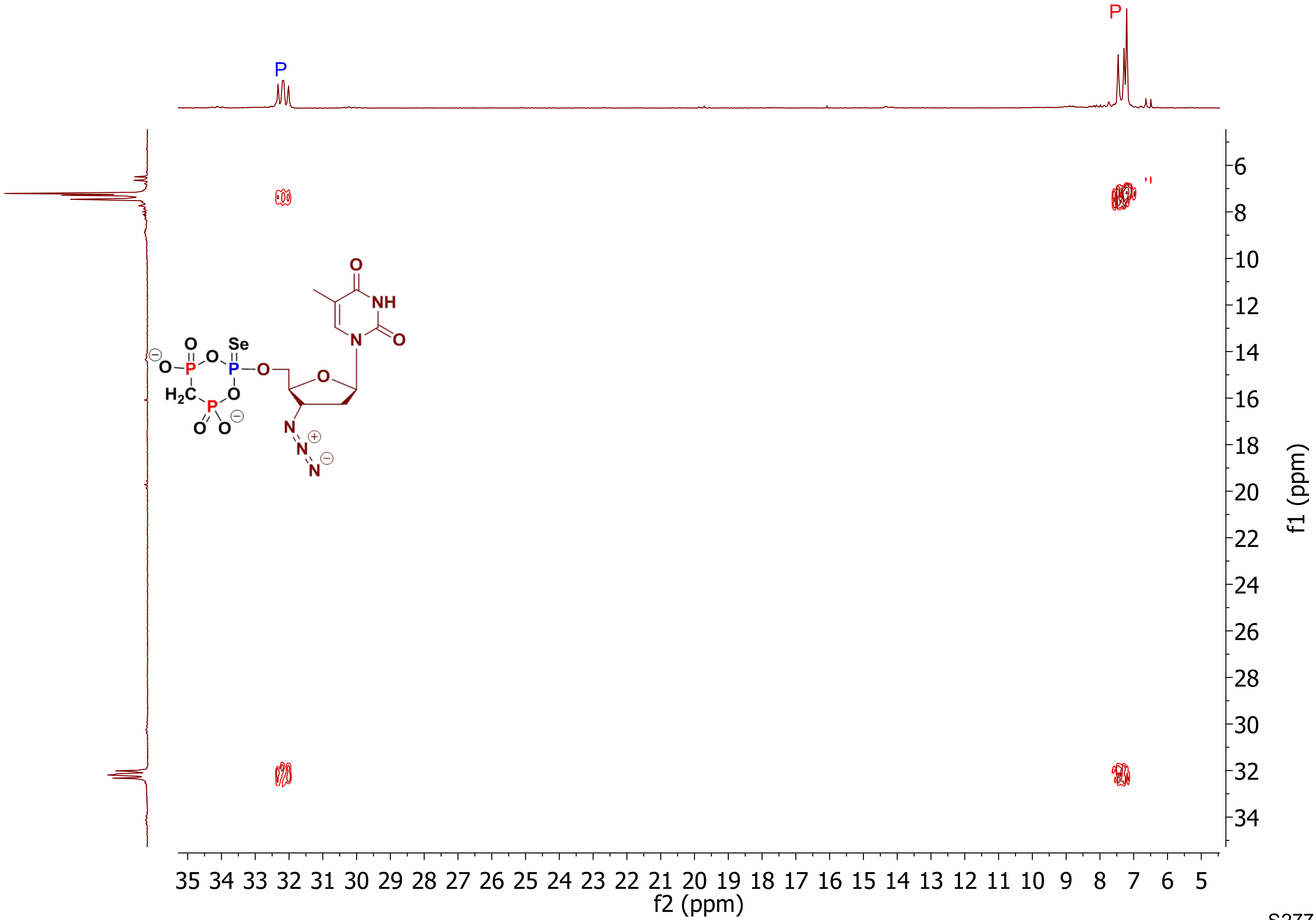
product after SAX Chromatography



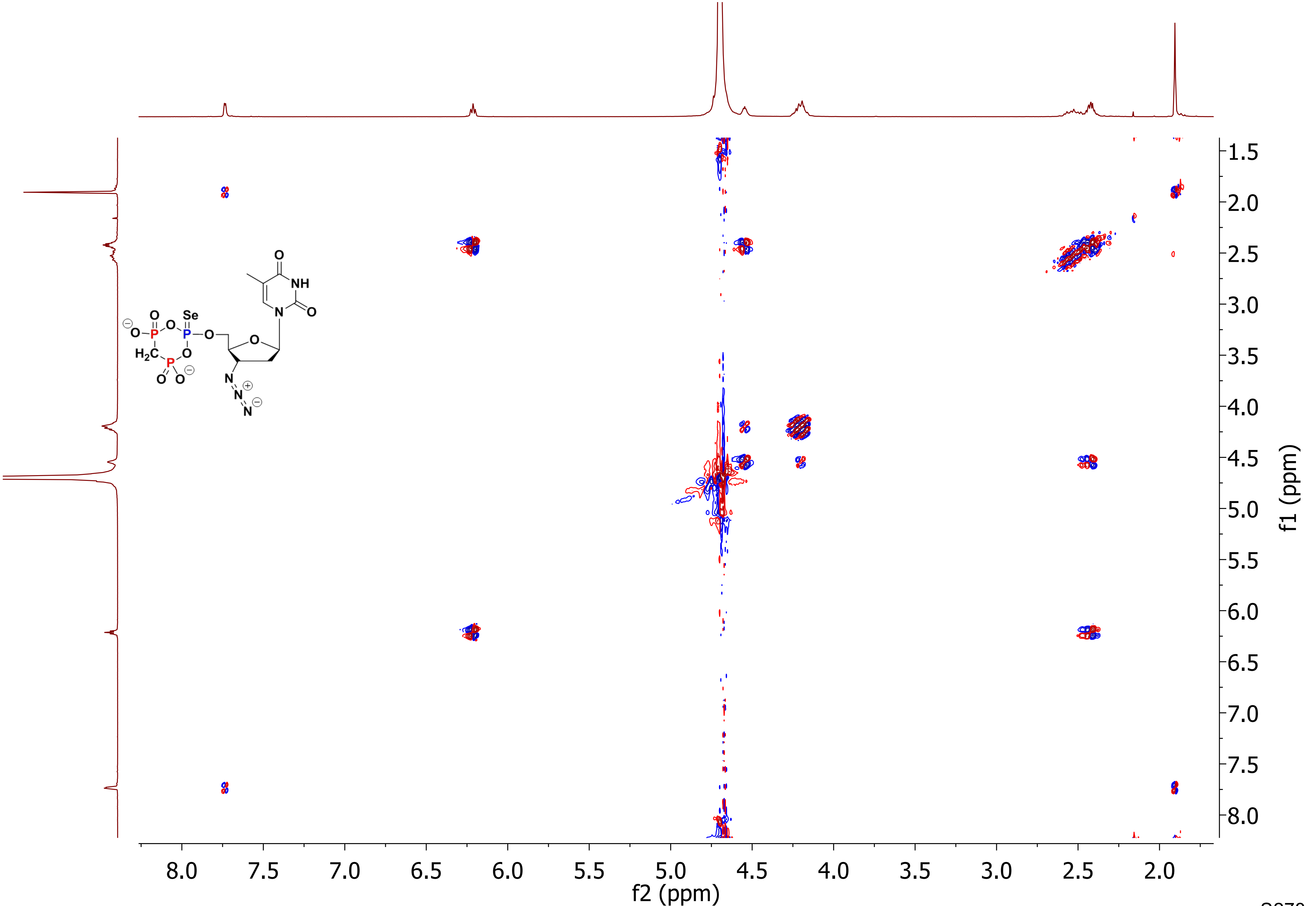
**$^{31}$P-$^{1}$H HMBC NMR**



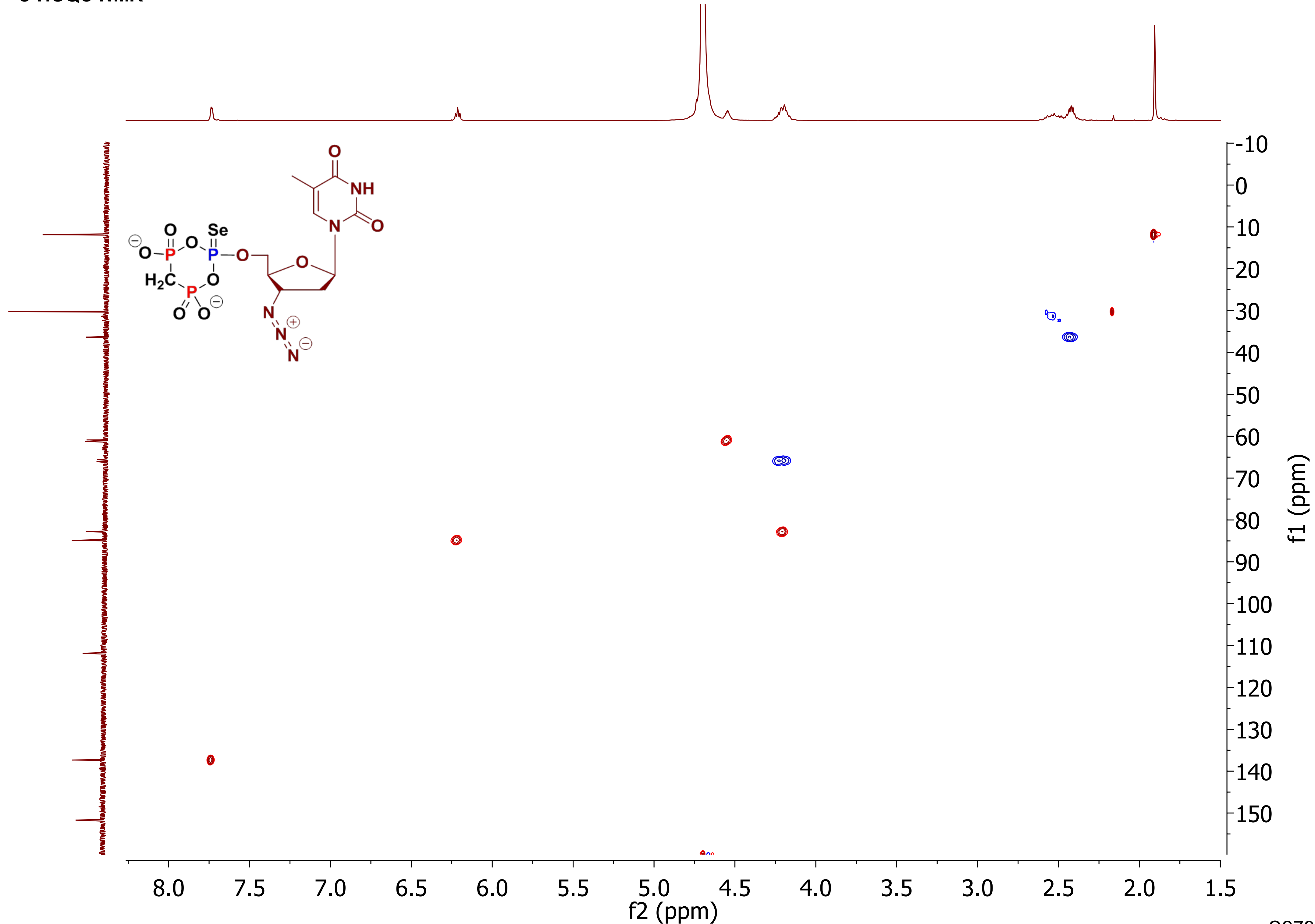
## $^{31}P$-$^{31}P$ COSY NMR



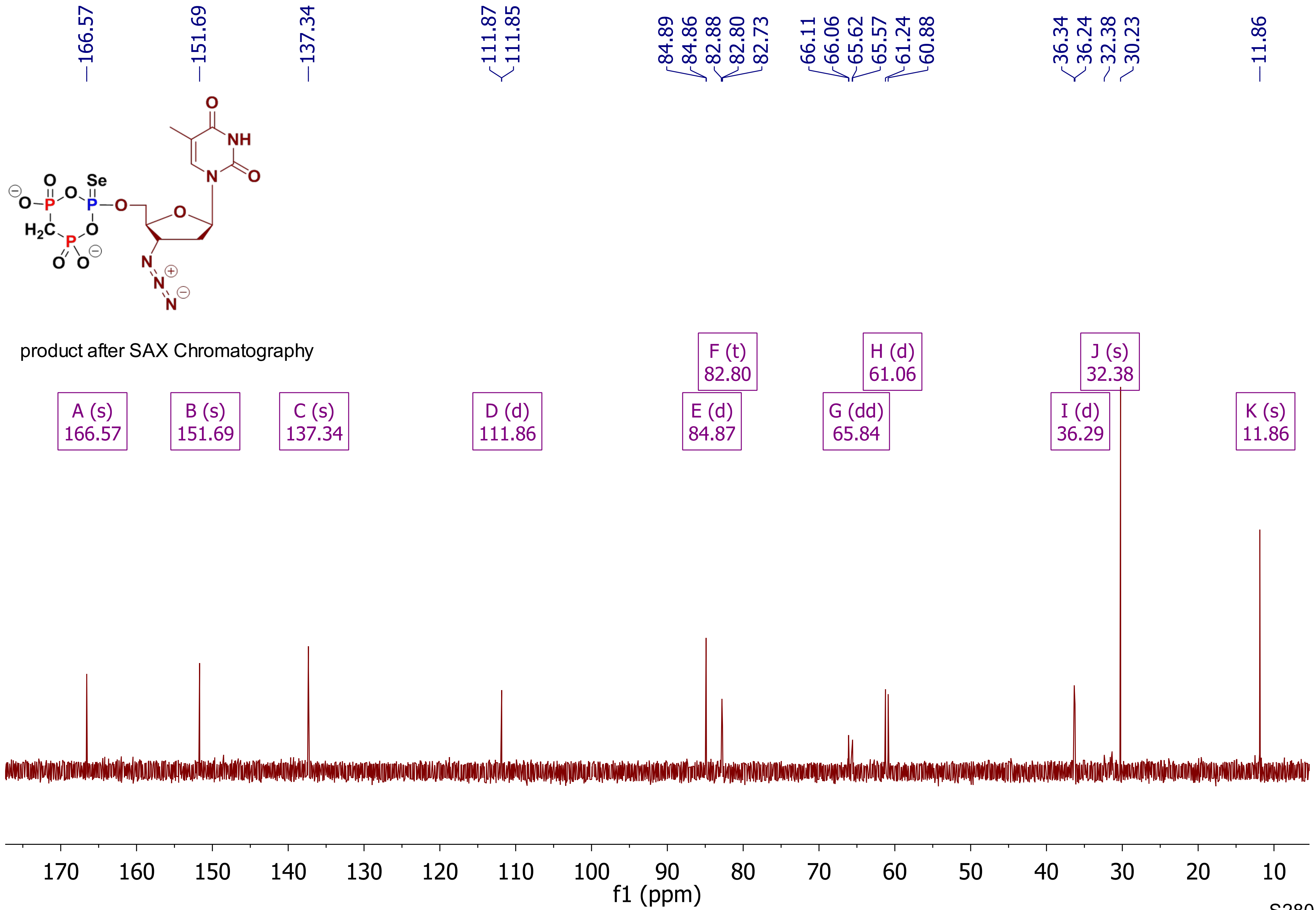
**$^1H$-$^1H$ HMBC NMR**



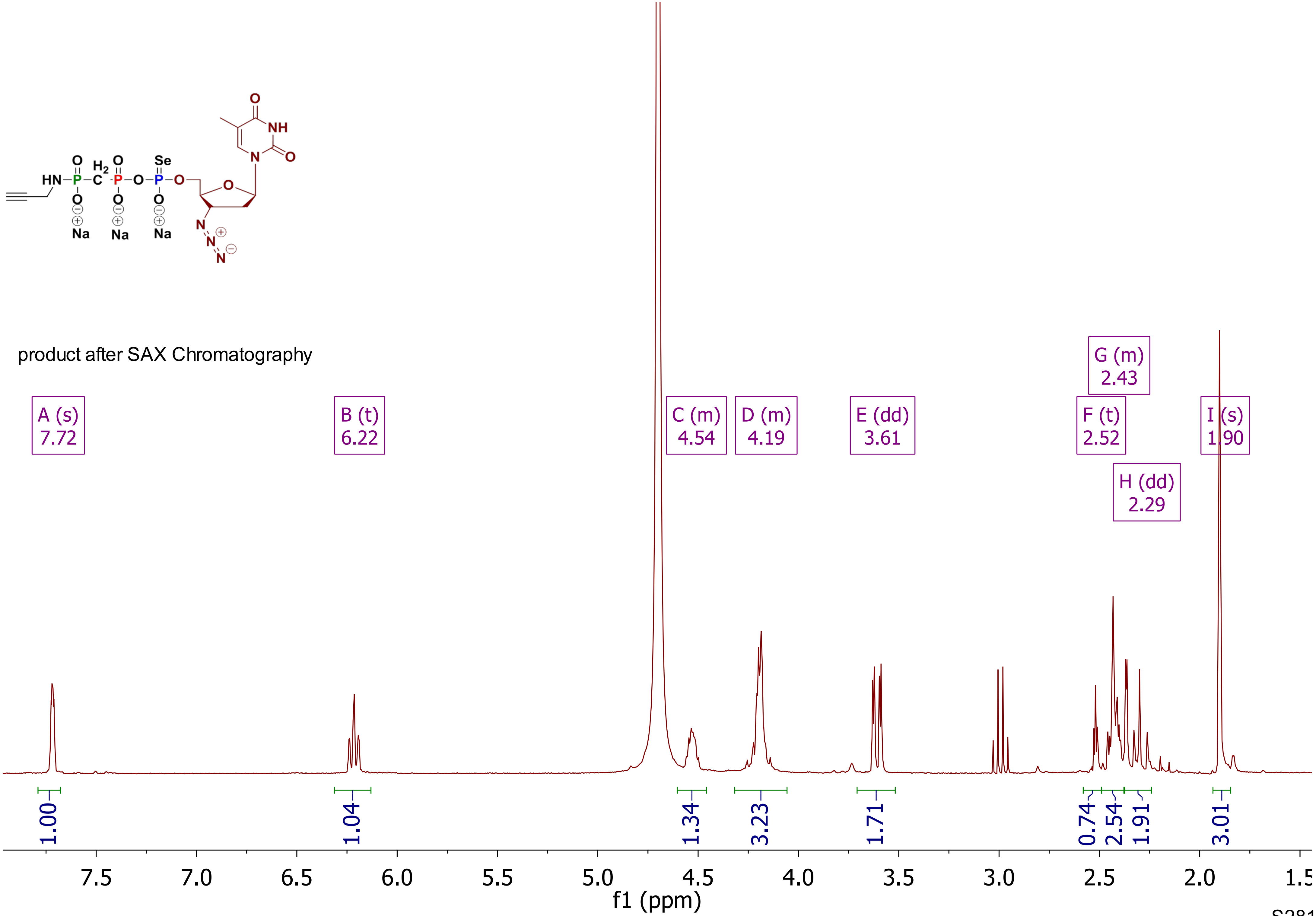
$^{1}H$-$^{13}C$ HSQC NMR



$^{13}C$ NMR

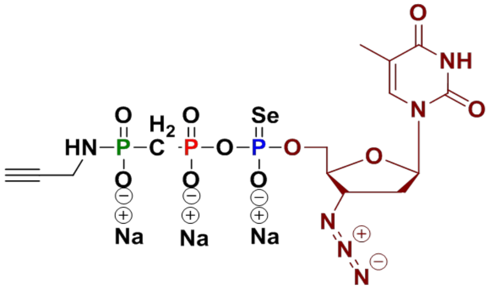

166.57
151.69
137.34
111.87
111.85
84.89
84.86
82.88
82.80
82.73
66.11
66.06
65.62
65.57
61.24
60.88
36.34
36.24
32.38
30.23
11.86
product after SAX Chromatography
F (t) 82.80
H (d) 61.06
J (s) 32.38
A (s) 166.57
B (s) 151.69
C (s) 137.34
D (d) 111.86
E (d) 84.87
G (dd) 65.84
I (d) 36.29
K (s) 11.86
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

$^1$H NMR

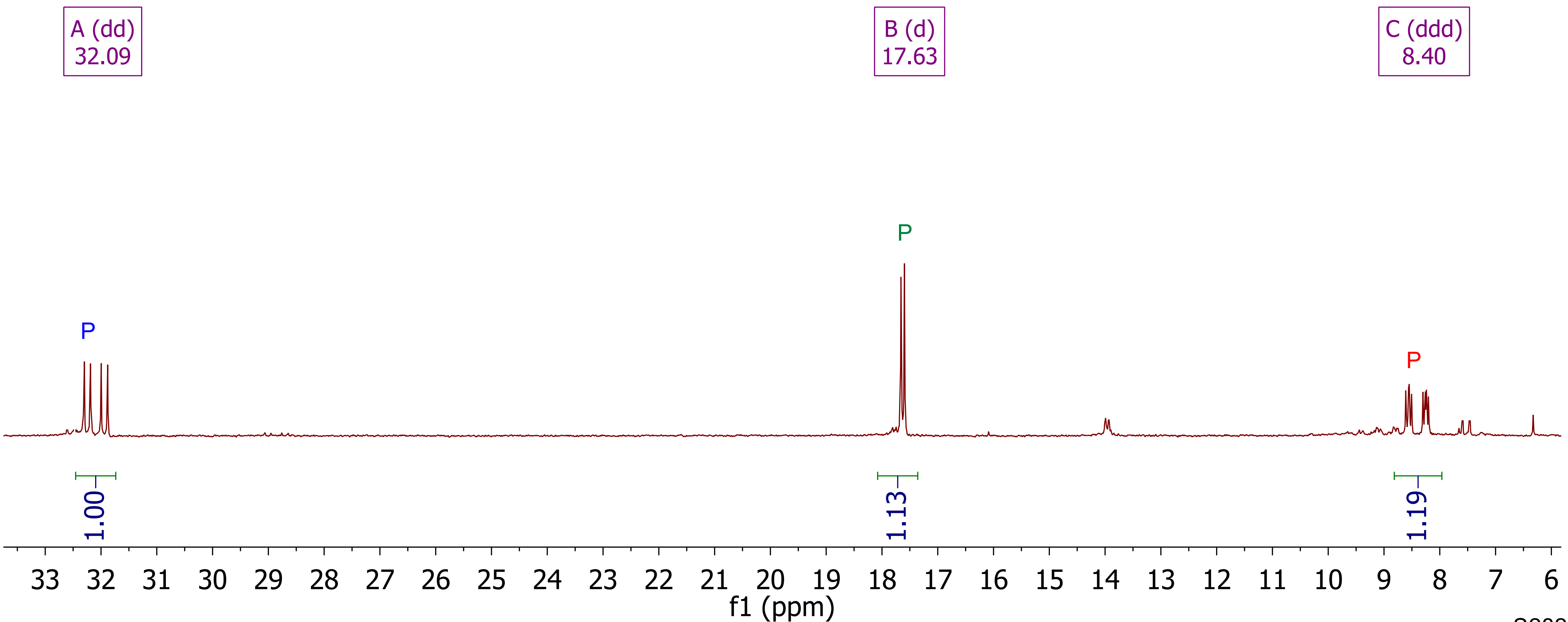


product after SAX Chromatography
A (s) 7.72
B (t) 6.22
C (m) 4.54
D (m) 4.19
E (dd) 3.61
F (t) 2.52
G (m) 2.43
H (dd) 2.29
I (s) 1.90
1.00
1.04
1.34
3.23
1.71
0.74
2.54
1.91
3.01
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

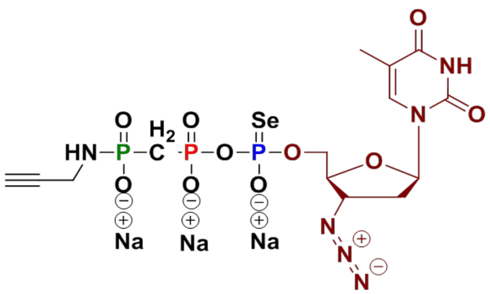
**$^{31}P\{^1H\}$ NMR**



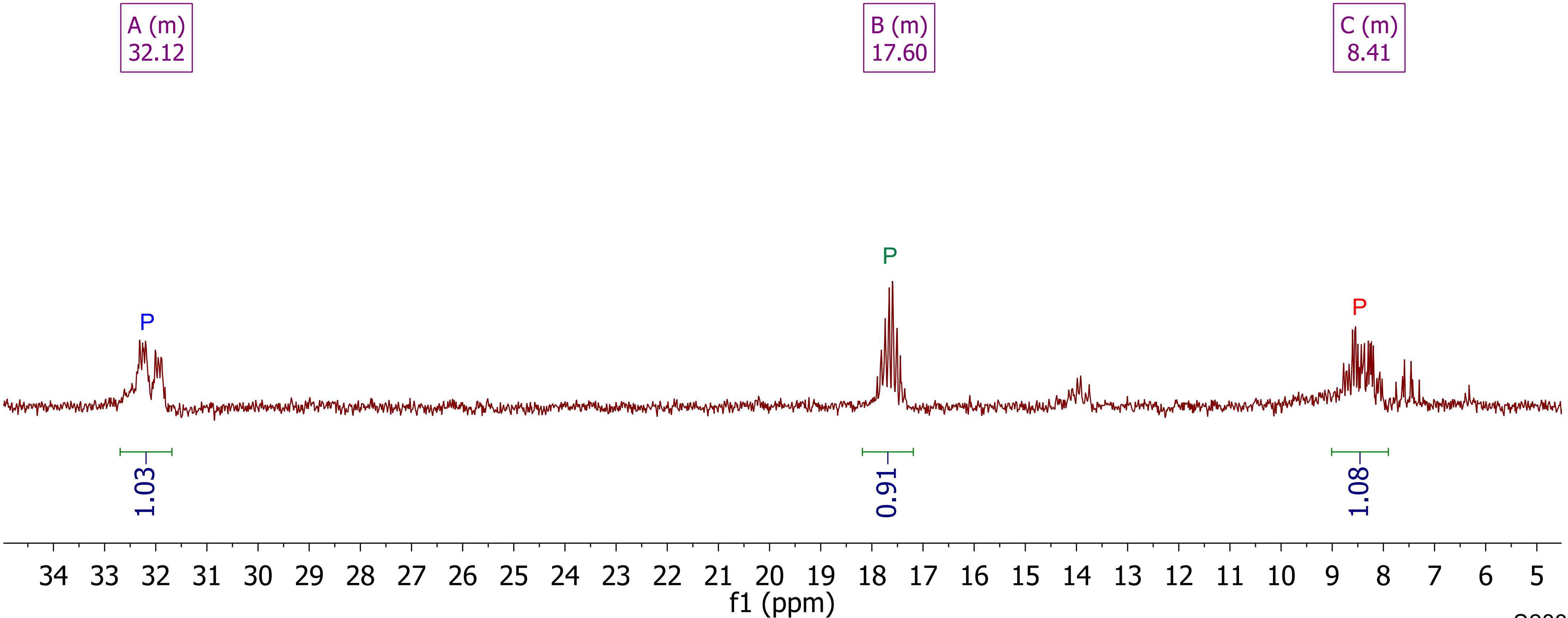

product after SAX Chromatography



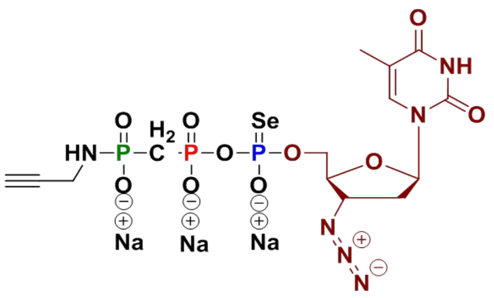
**$^{31}$P NMR**



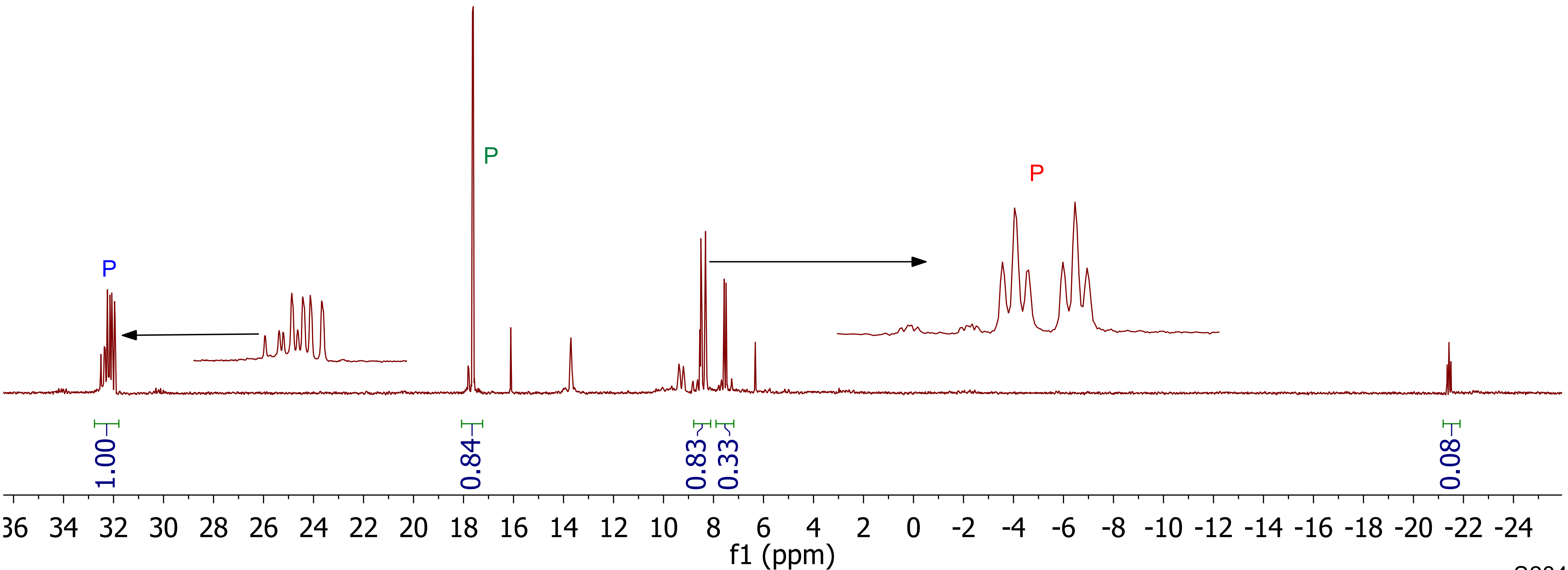

product after SAX Chromatography



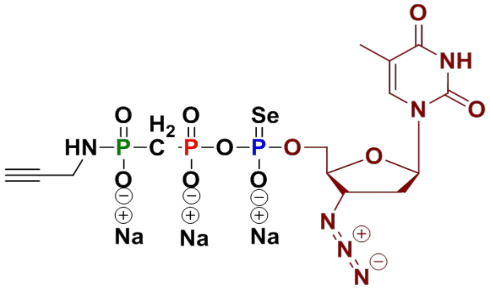
**$^{31}P\{^1H\}$ NMR**



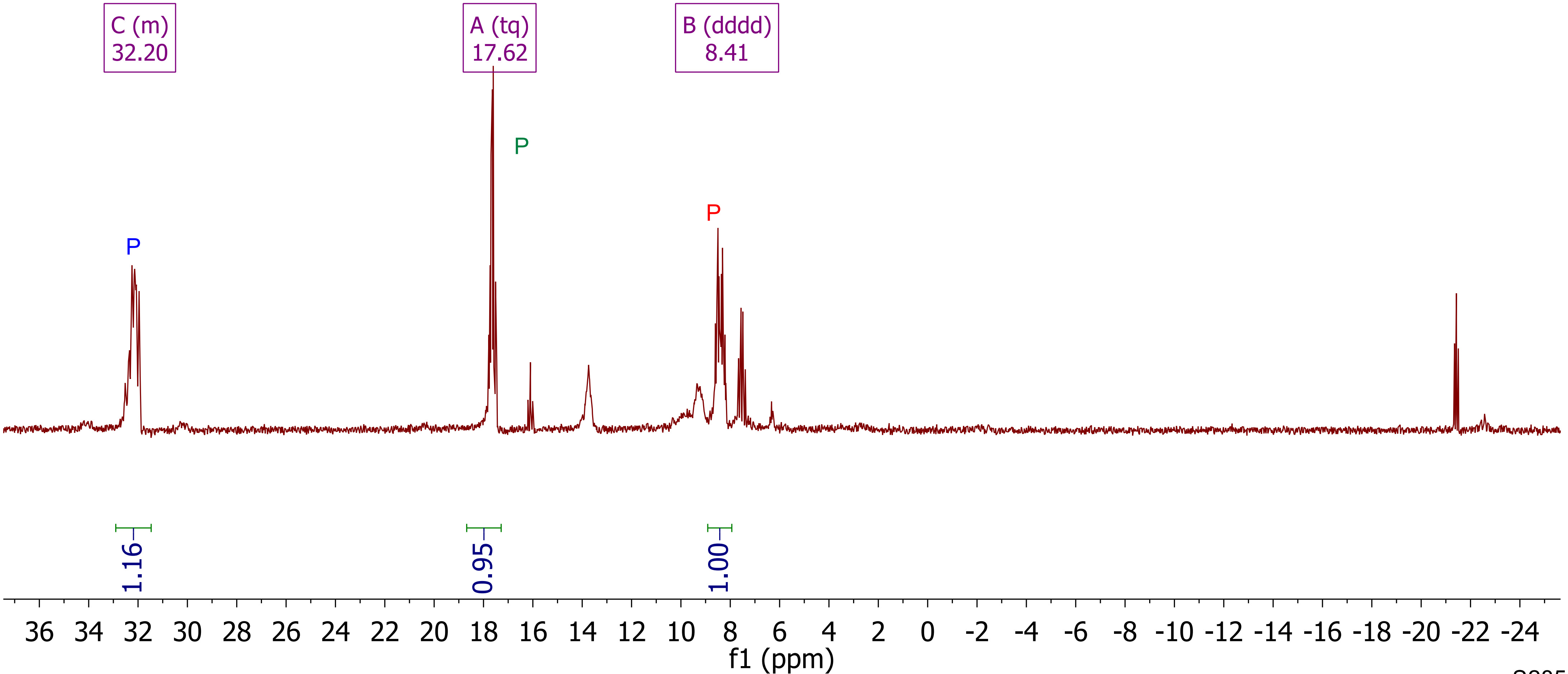
purified by SAX
after one day decomposition was observed



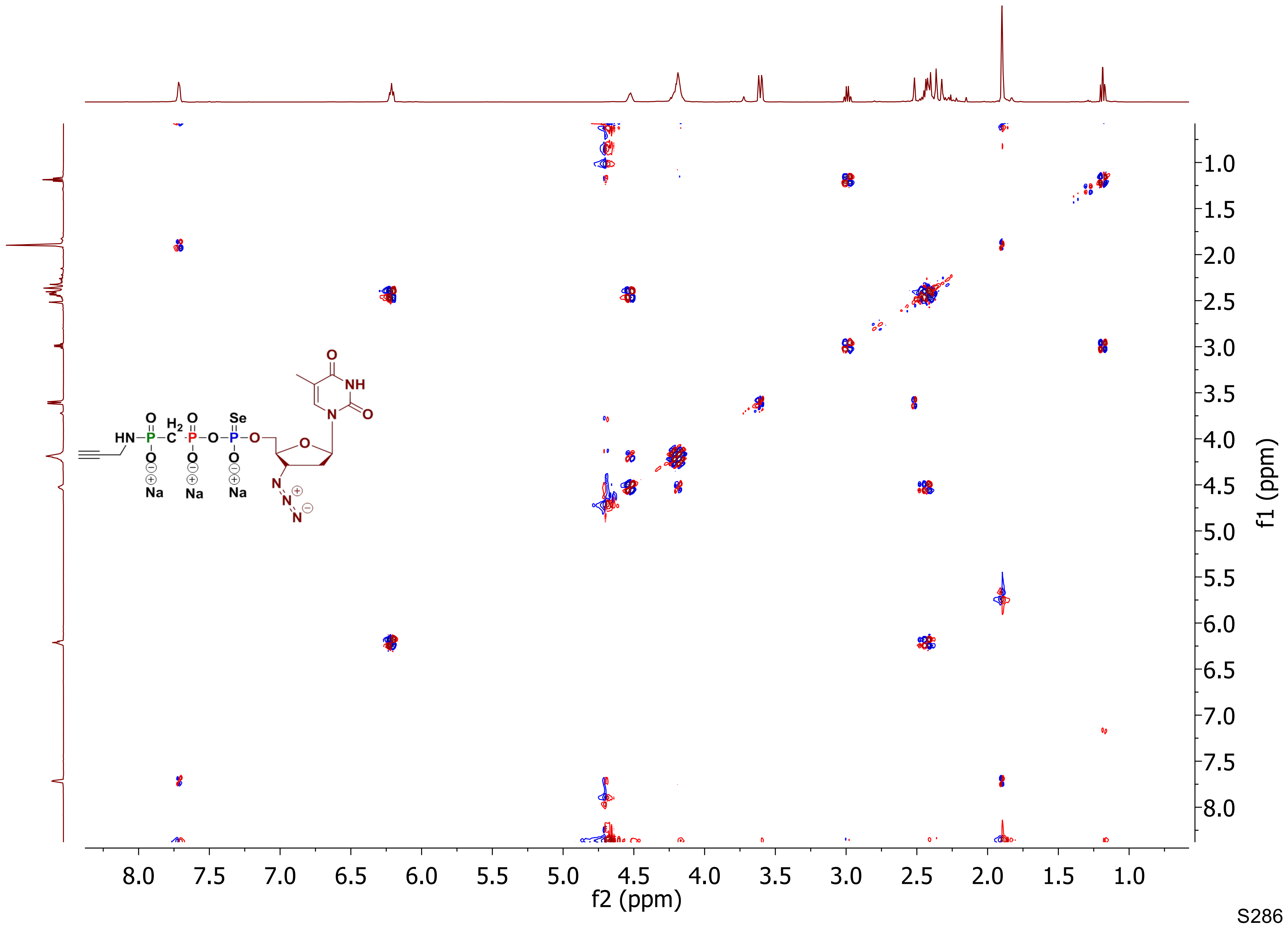
$^{31}$P NMR



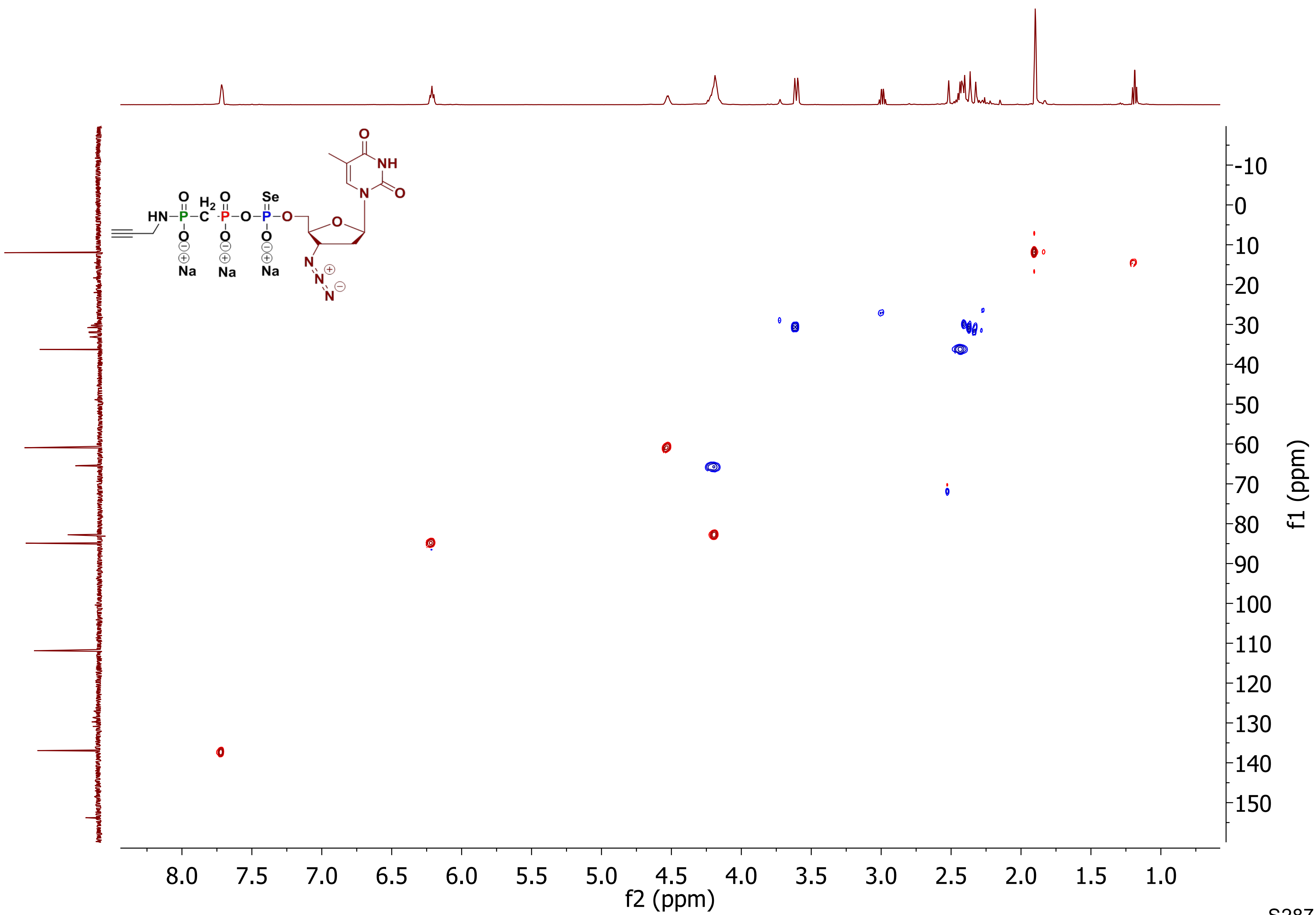
purified by SAX
after one day decomposition was observed



$^1H$-$^1H$ Cosy NMR

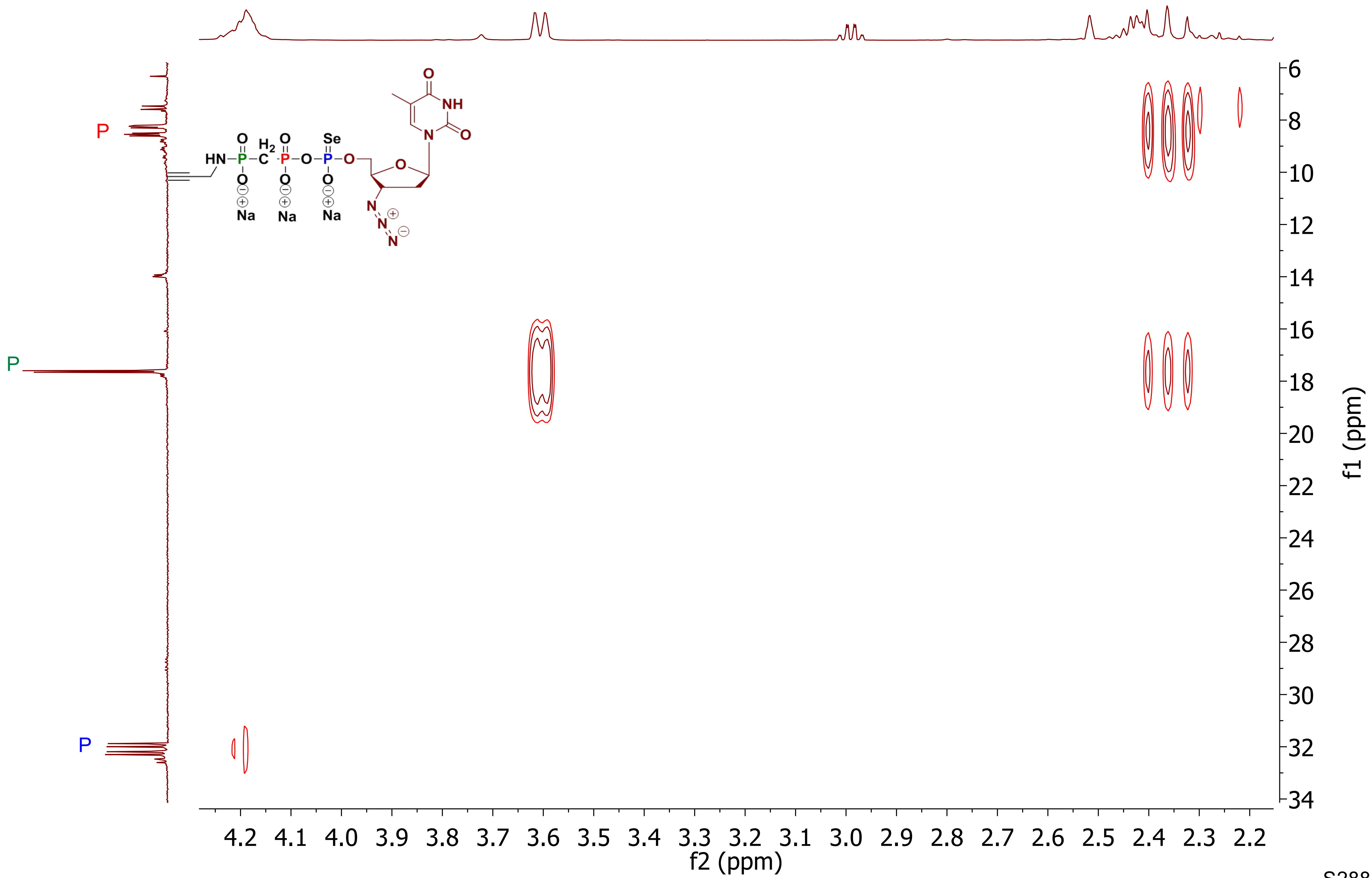


## $^1H$-$^{13}C$ HSQC NMR

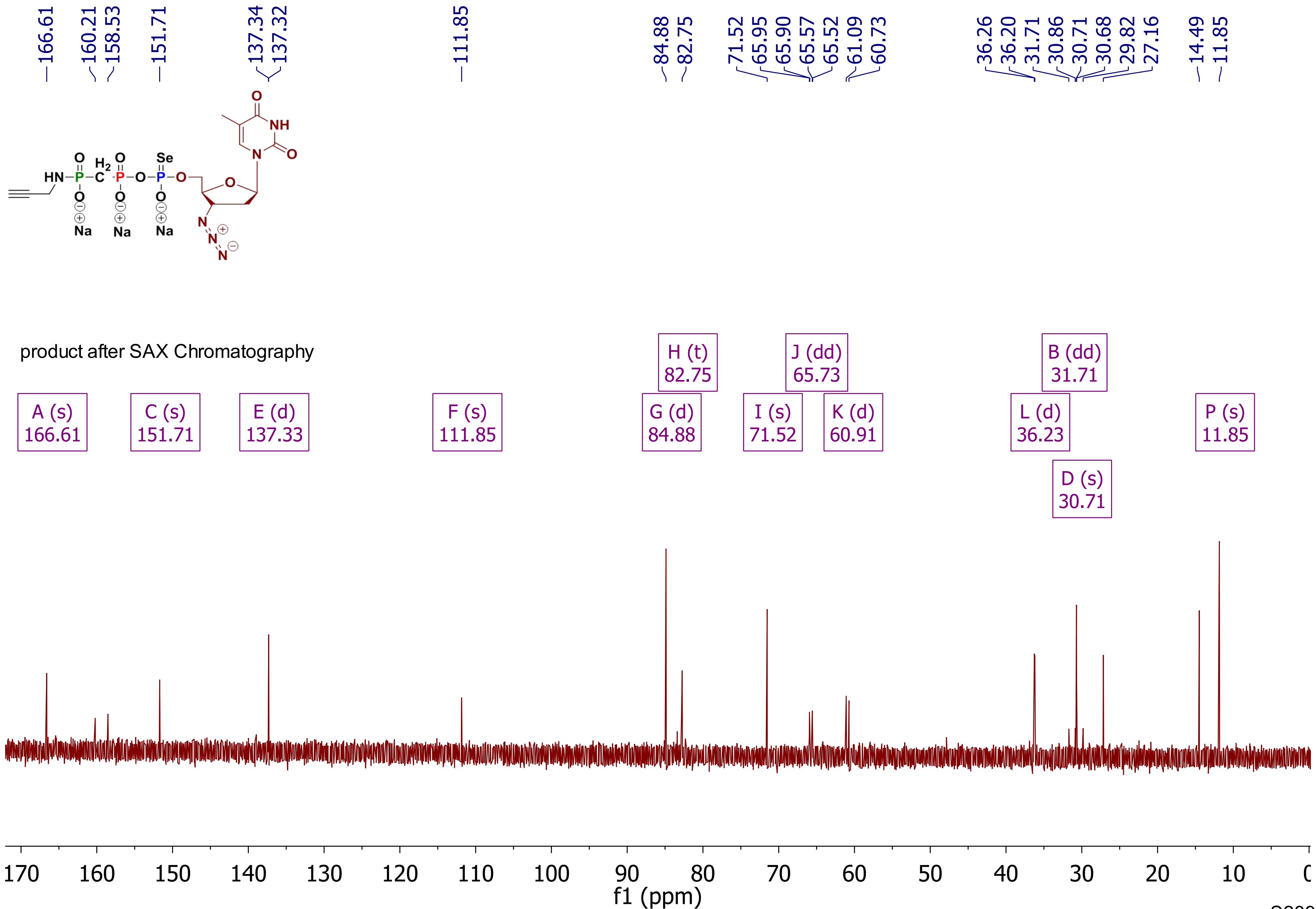


## $^{31}$P-$^{1}$H HMBC NMR

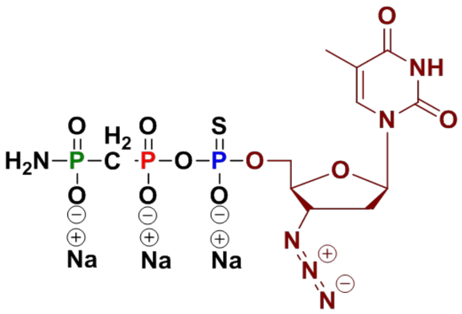
after SAX Chromatography



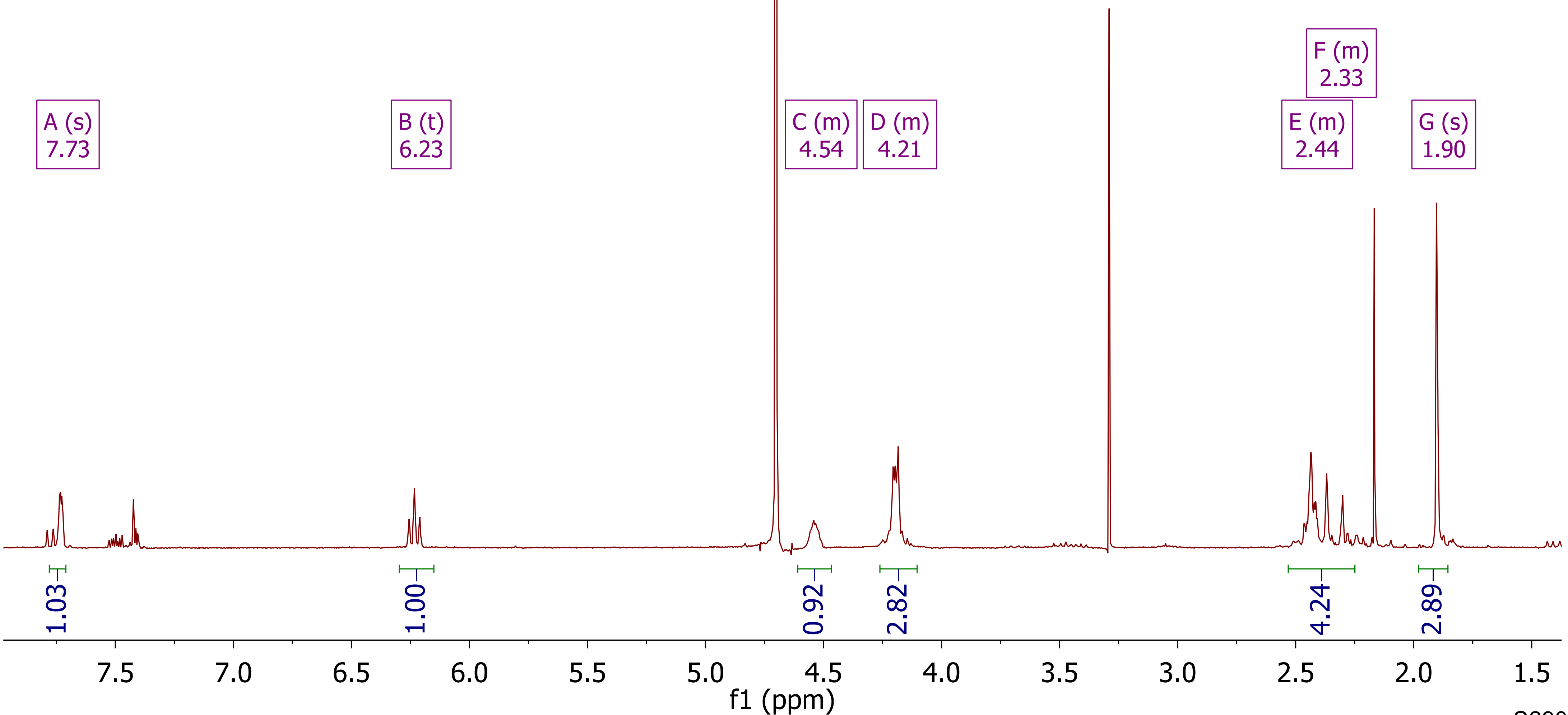
$^{13}C$ NMR


166.61
160.21
158.53
151.71
137.34
137.32
111.85
84.88
82.75
71.52
65.95
65.90
65.57
65.52
61.09
60.73
36.26
36.20
31.71
30.86
30.71
30.68
29.82
27.16
14.49
11.85
product after SAX Chromatography
H (t) 82.75
J (dd) 65.73
B (dd) 31.71
A (s) 166.61
C (s) 151.71
E (d) 137.33
F (s) 111.85
G (d) 84.88
I (s) 71.52
K (d) 60.91
L (d) 36.23
P (s) 11.85
D (s) 30.71
f1 (ppm)

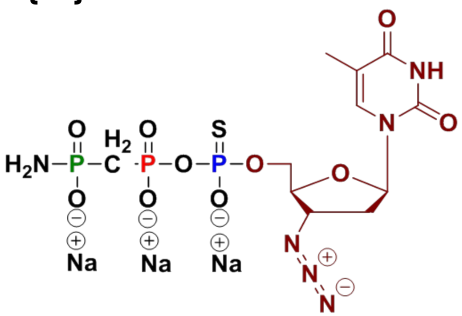
[1]H NMR



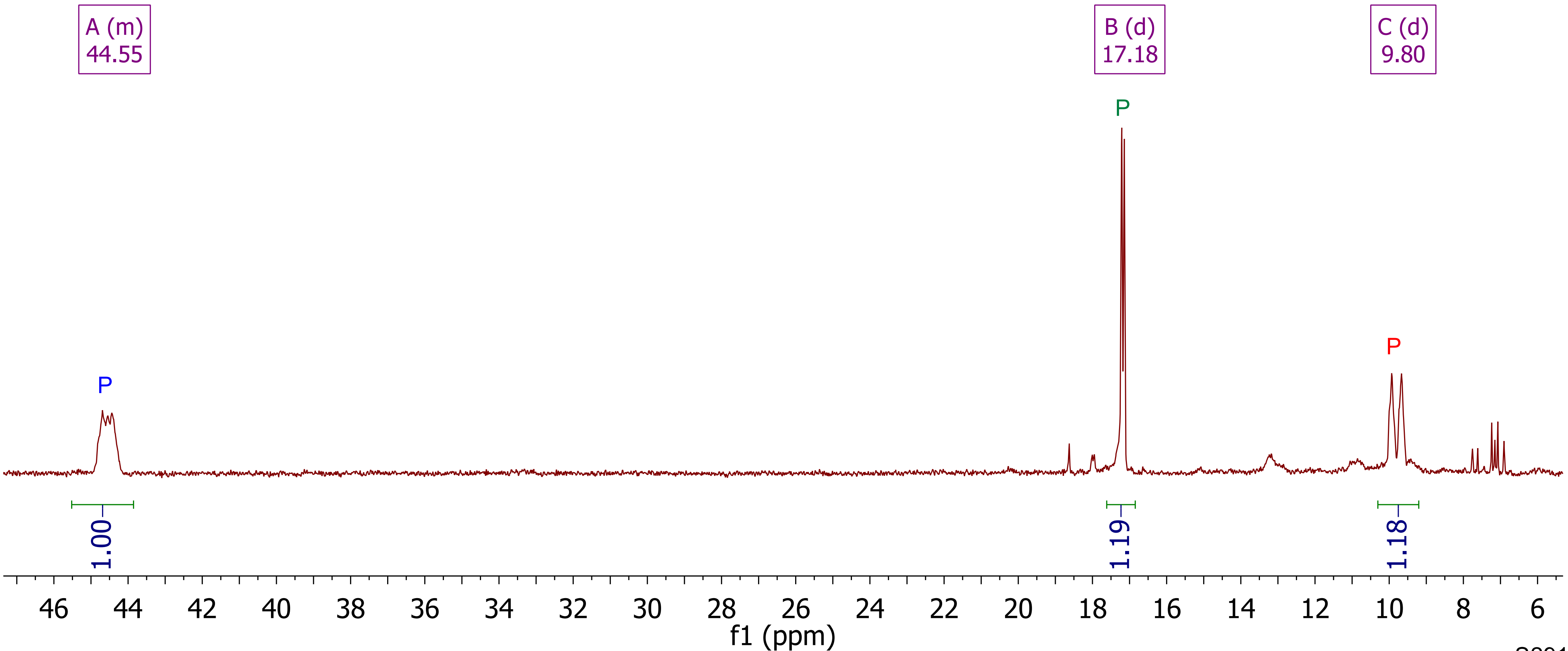

crude product after precipitation



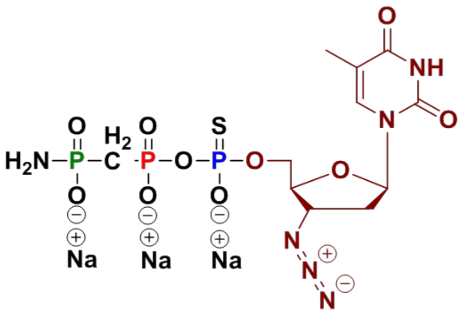
**$^{31}P\{^1H\}$ NMR**



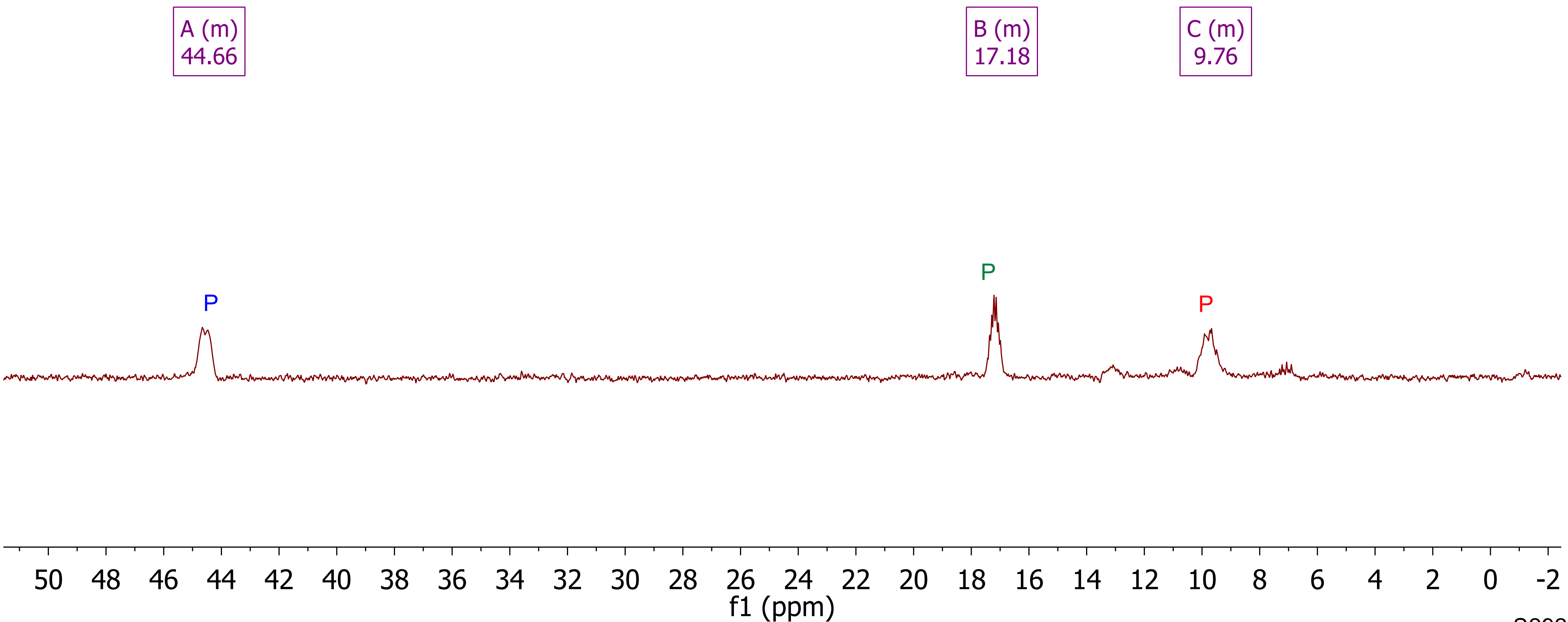
crude product after precipitation



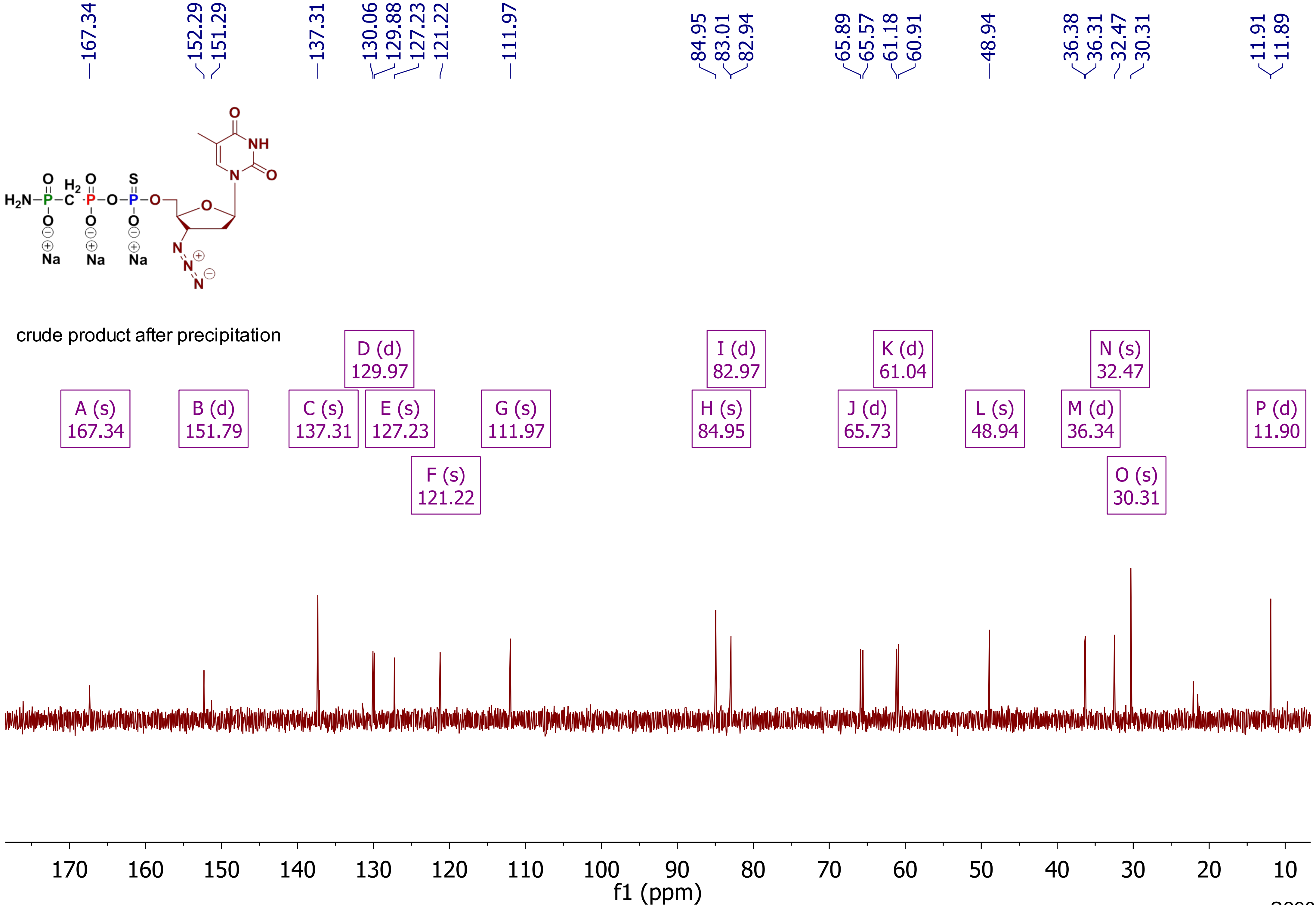
## $^{31}$P NMR

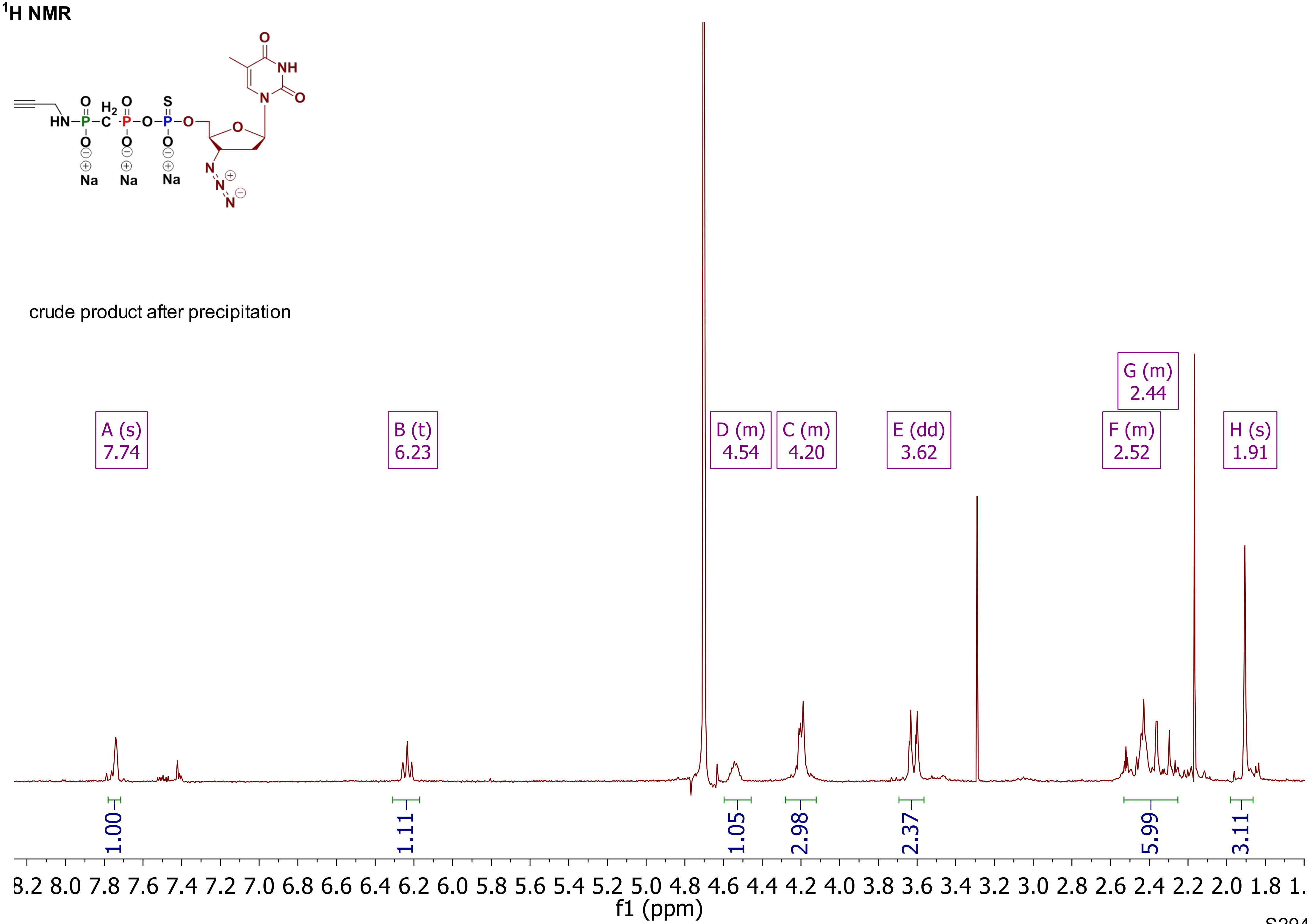


crude product after precipitation



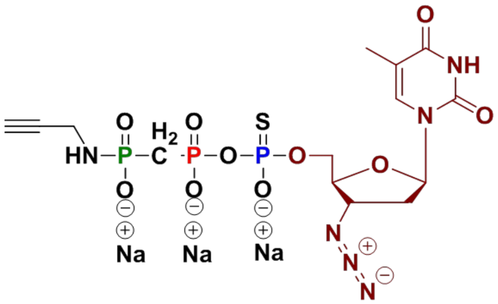
$^{13}$C NMR


167.34
152.29
151.29
137.31
130.06
129.88
127.23
121.22
111.97
84.95
83.01
82.94
65.89
65.57
61.18
60.91
48.94
36.38
36.31
32.47
30.31
11.91
11.89
crude product after precipitation
A (s) 167.34
B (d) 151.79
C (s) 137.31
D (d) 129.97
E (s) 127.23
F (s) 121.22
G (s) 111.97
H (s) 84.95
I (d) 82.97
J (d) 65.73
K (d) 61.04
L (s) 48.94
M (d) 36.34
N (s) 32.47
O (s) 30.31
P (d) 11.90
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

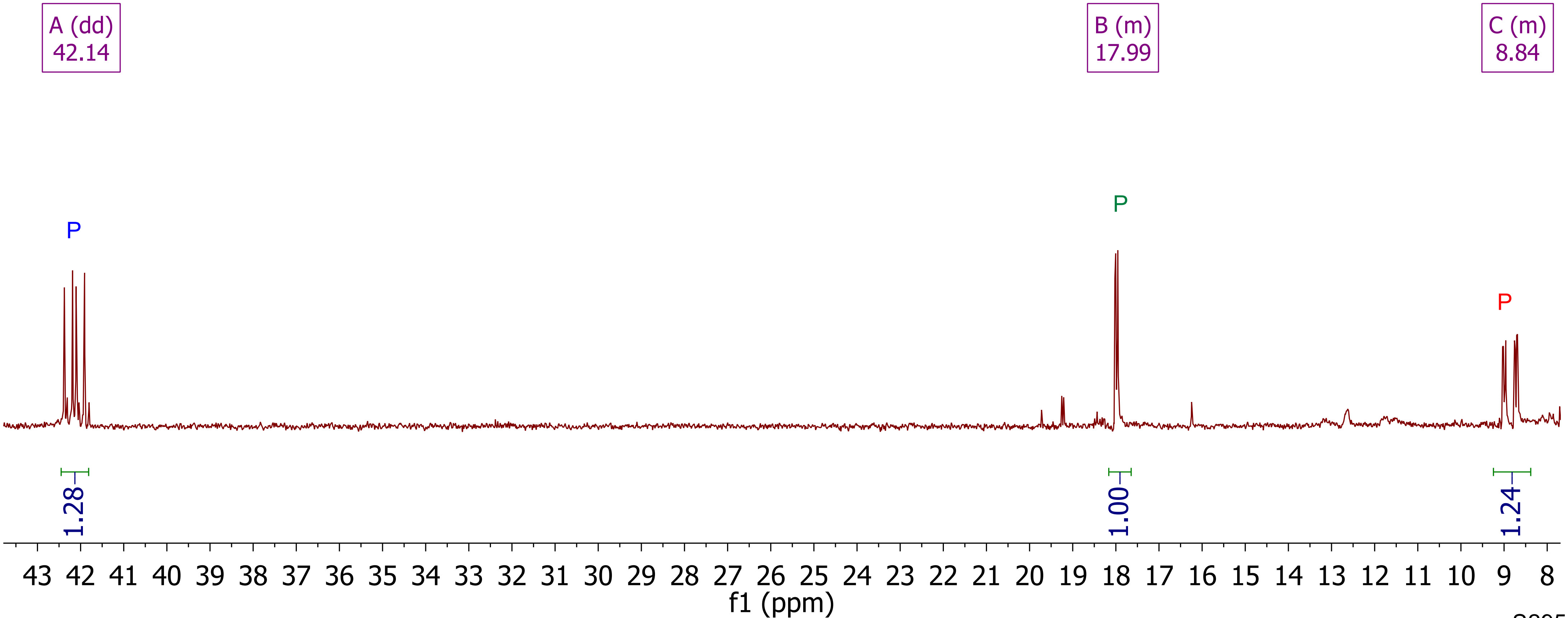


1H NMR
crude product after precipitation
A (s)
7.74
B (t)
6.23
D (m)
4.54
C (m)
4.20
E (dd)
3.62
G (m)
2.44
F (m)
2.52
H (s)
1.91
1.00
1.11
1.05
2.98
2.37
5.99
3.11
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.
f1 (ppm)

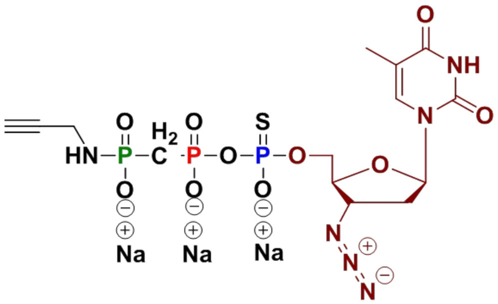
$^{31}P\{^1H\}$ NMR



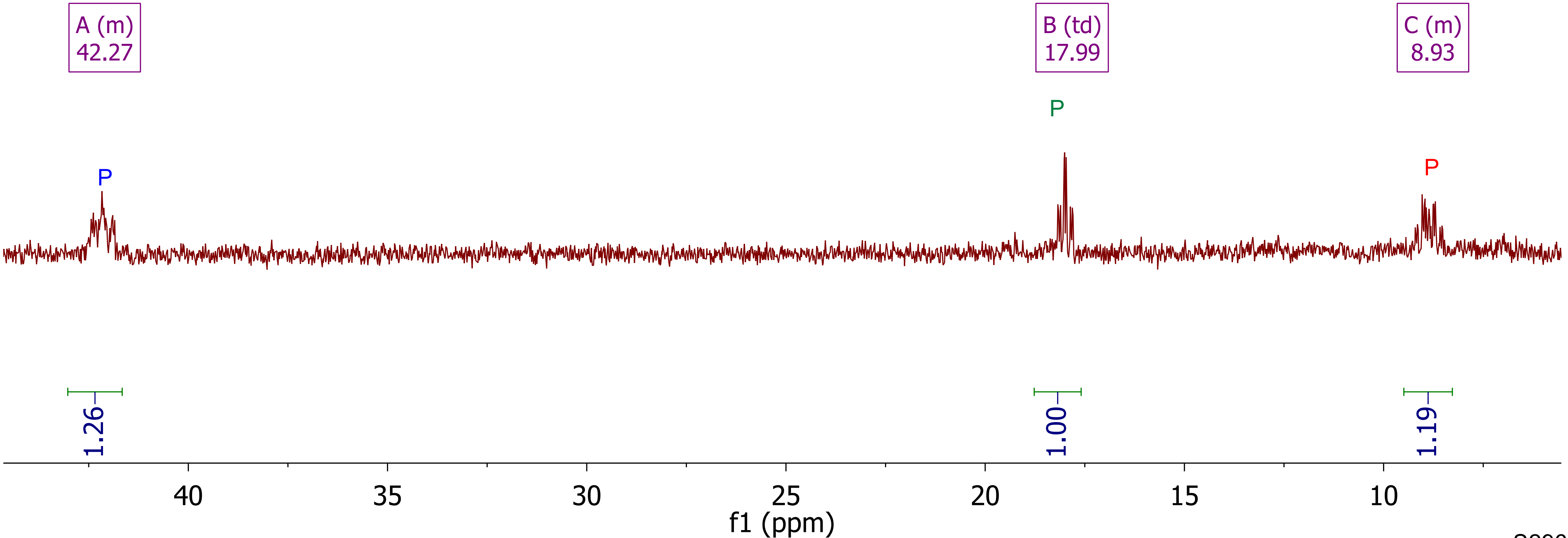

crude product after precipitation



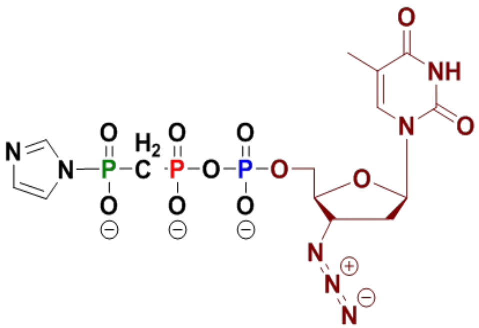
$^{31}$P NMR



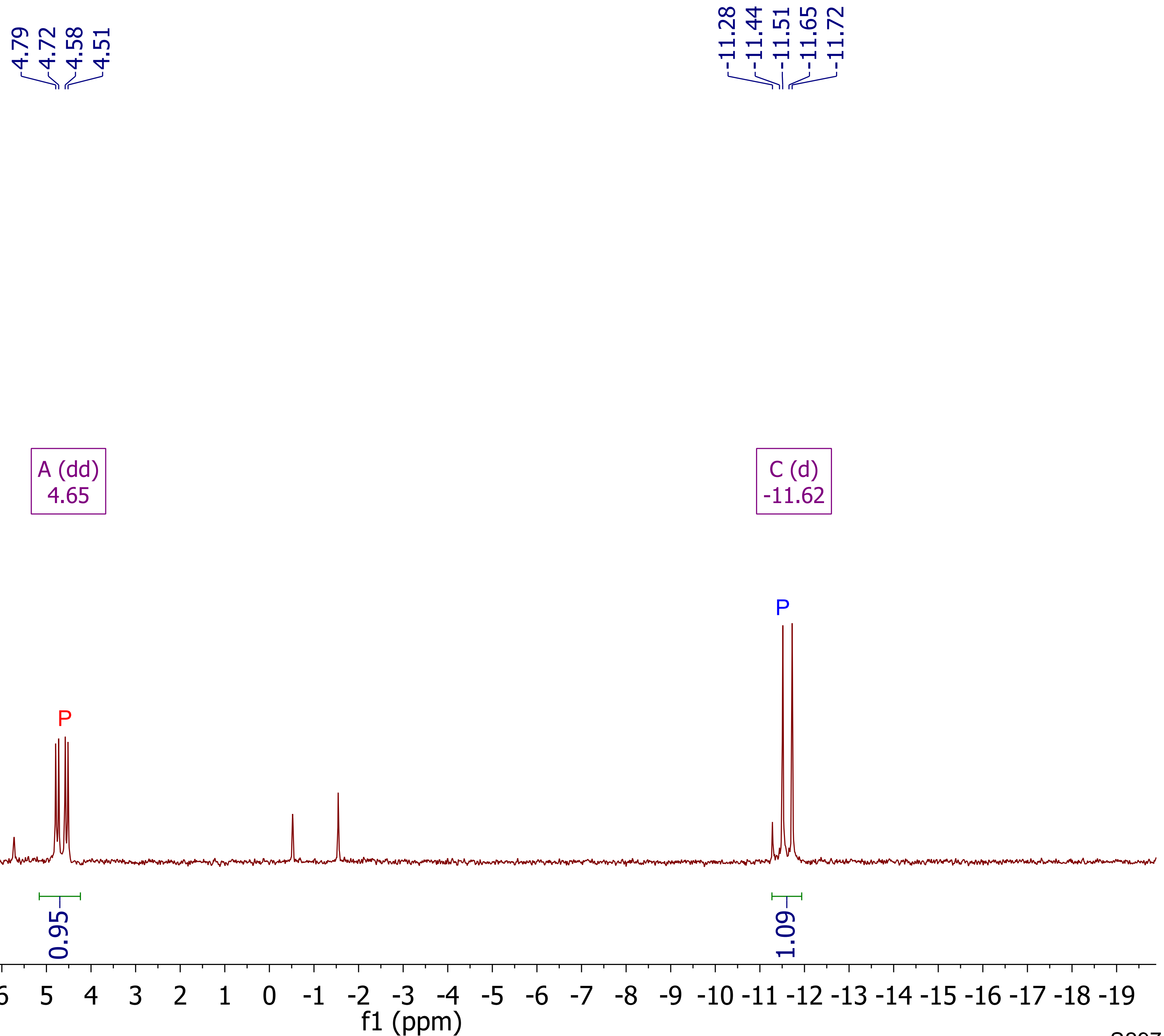
crude product after precipitation



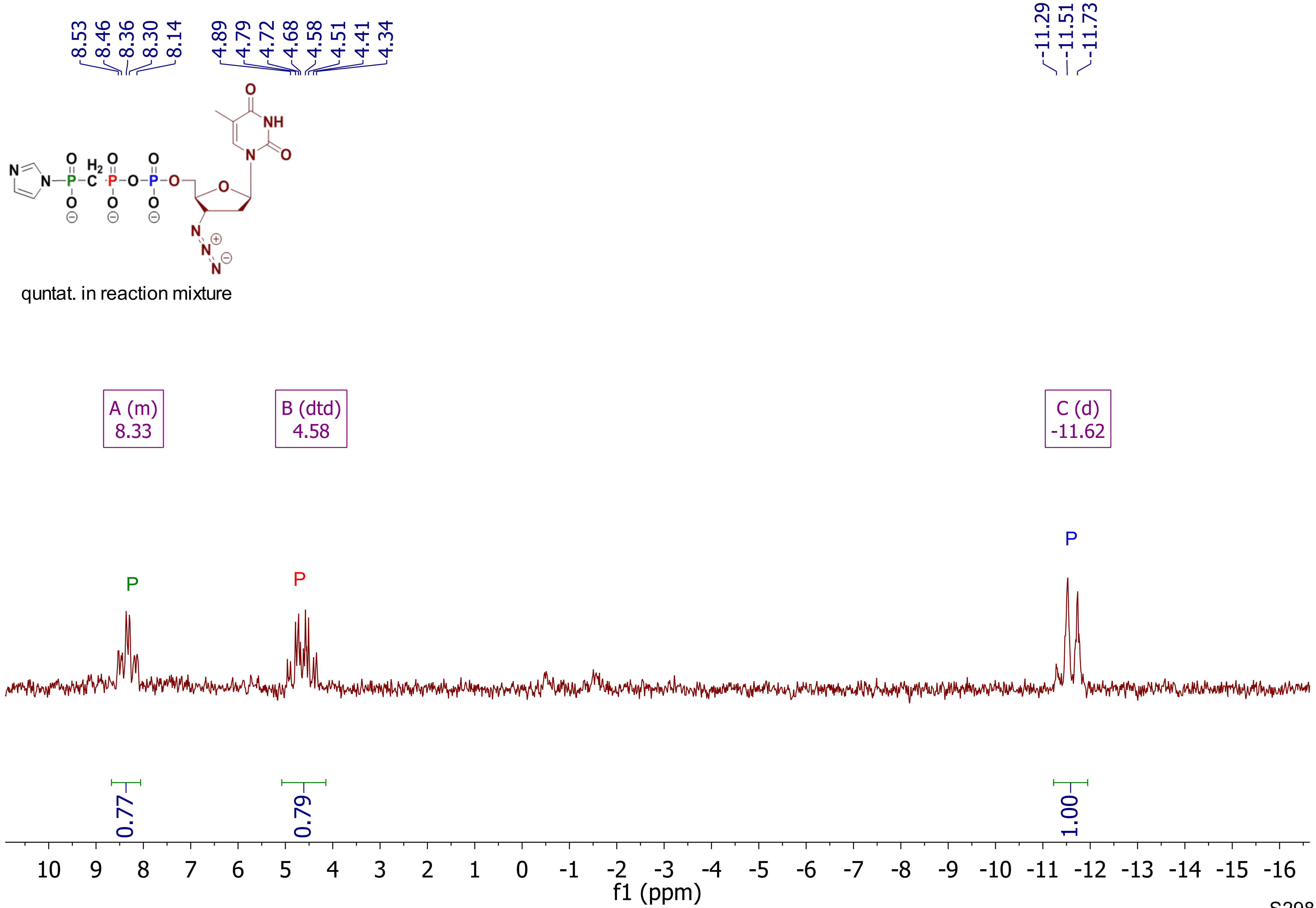
$^{31}P\{^1H\}$ NMR



qunatat. in reaction mixture

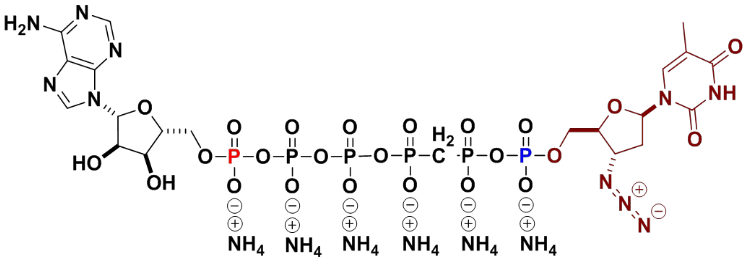


$^{31}$P NMR

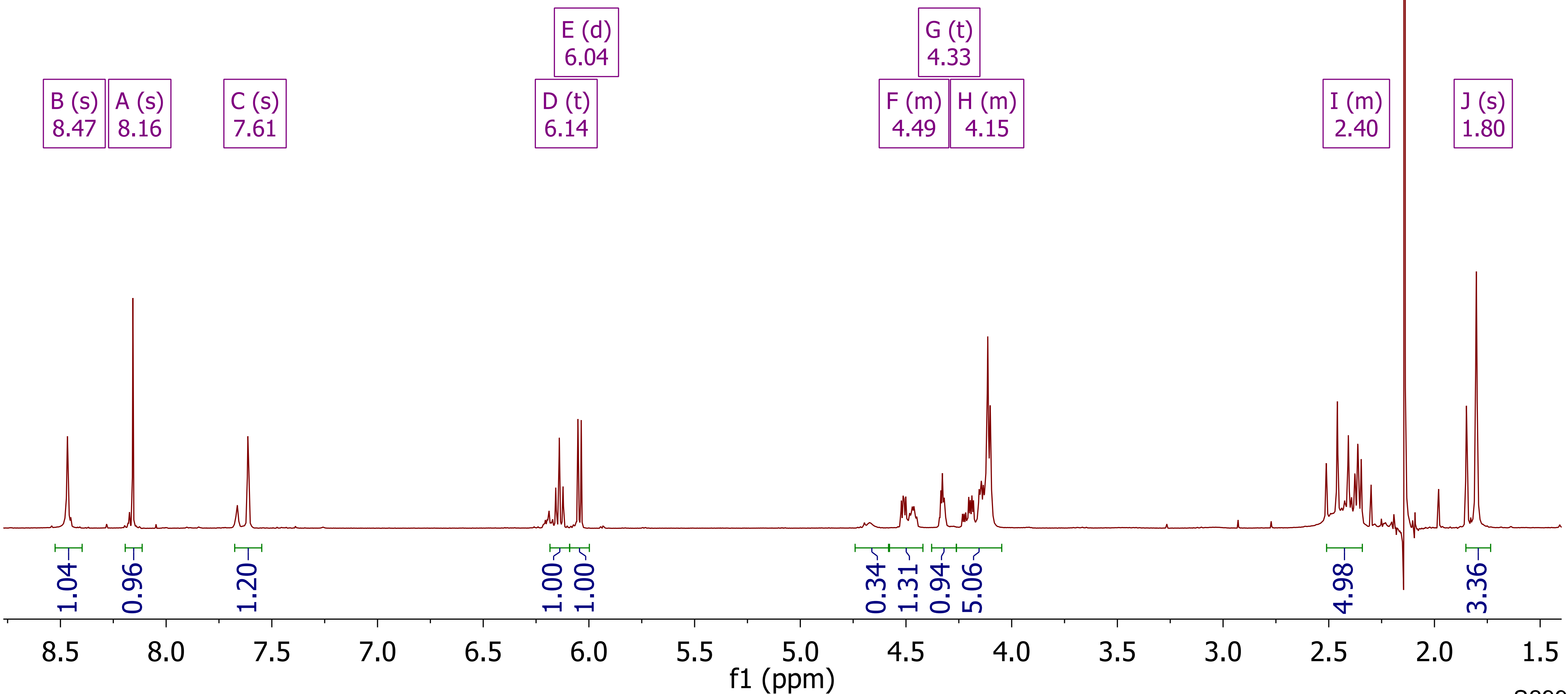


8.53
8.46
8.36
8.30
8.14
4.89
4.79
4.72
4.68
4.58
4.51
4.41
4.34
-11.29
-11.51
-11.73
O
NH
O
N
O
N
H2
P
C
O
O
O
N
N
N
qunttat. in reaction mixture
A (m)
8.33
B (dtd)
4.58
C (d)
-11.62
P
P
P
0.77
0.79
1.00
10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16
f1 (ppm)

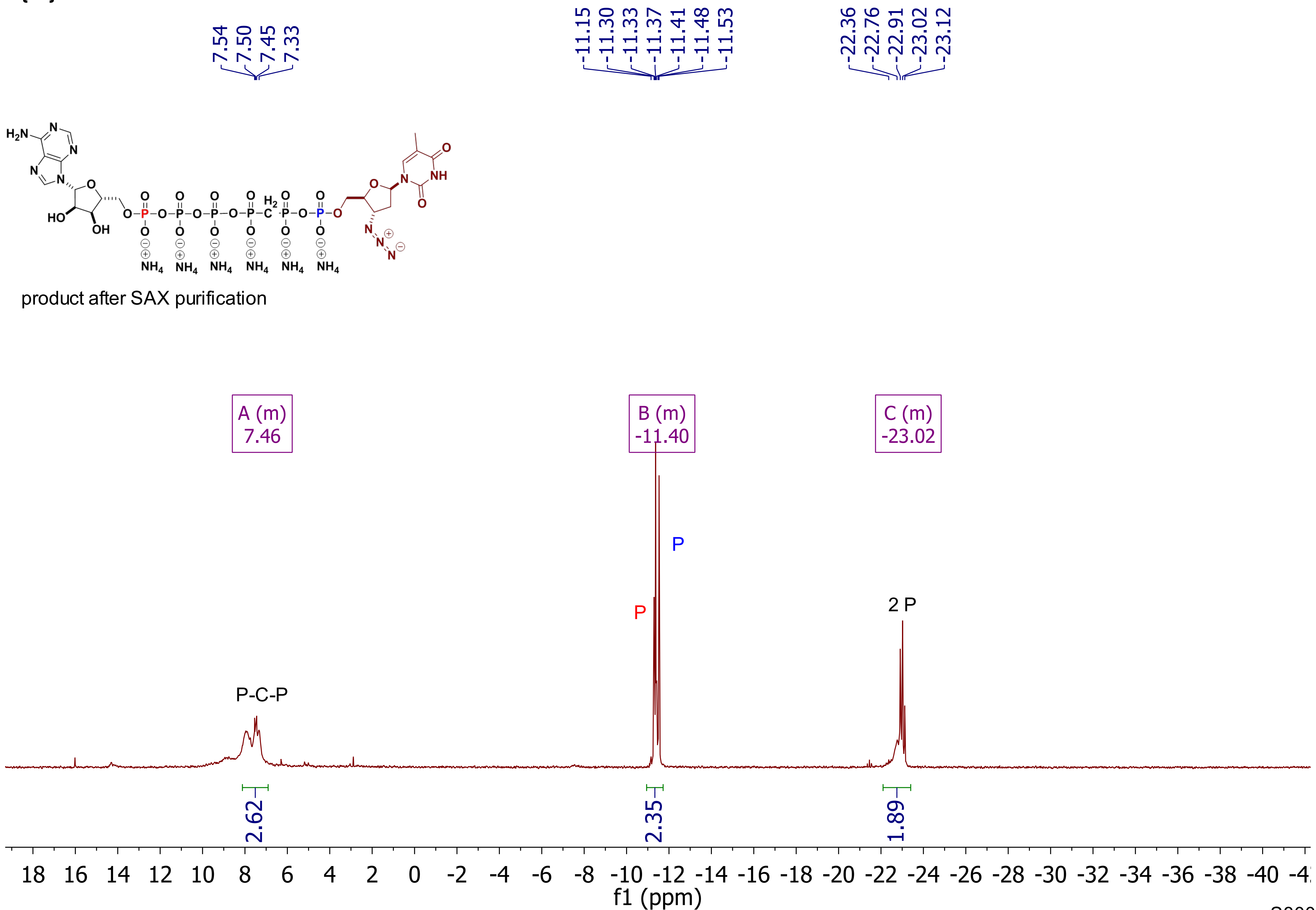
## $^1H$ NMR



product after SAX purification

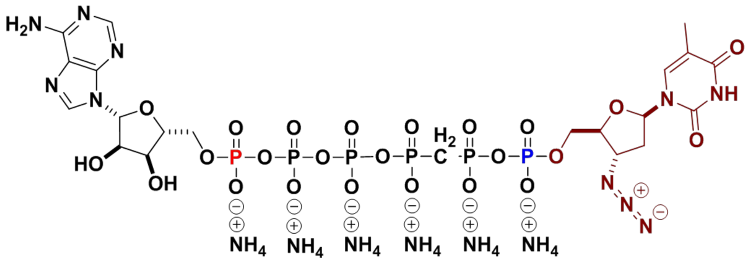


$^{31}P\{^1H\}$ NMR

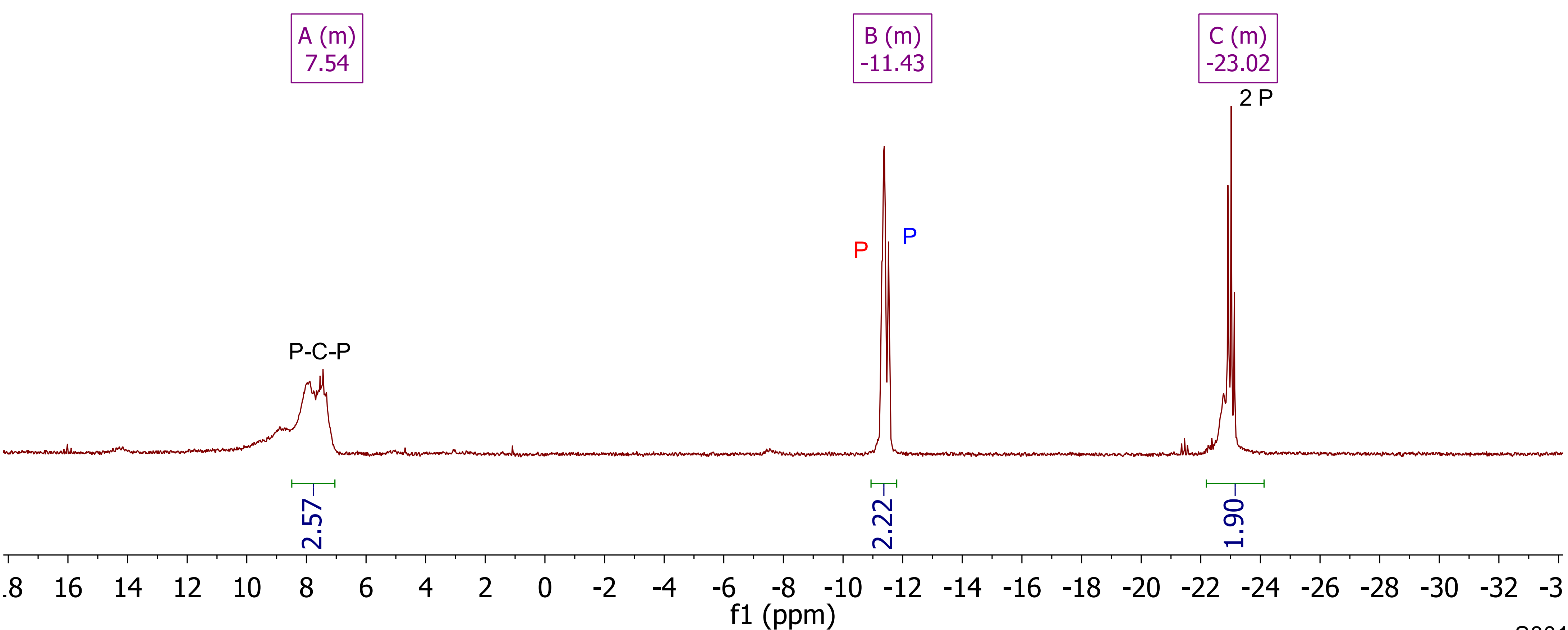


7.54
7.50
7.45
7.33
-11.15
-11.30
-11.33
-11.37
-11.41
-11.48
-11.53
-22.36
-22.76
-22.91
-23.02
-23.12
product after SAX purification
A (m) 7.46
B (m) -11.40
C (m) -23.02
P
P
2 P
P-C-P
2.62
2.35
1.89
18 16 14 12 10 8 6 4 2 0 -2 -4 -6 -8 -10 -12 -14 -16 -18 -20 -22 -24 -26 -28 -30 -32 -34 -36 -38 -40
f1 (ppm)

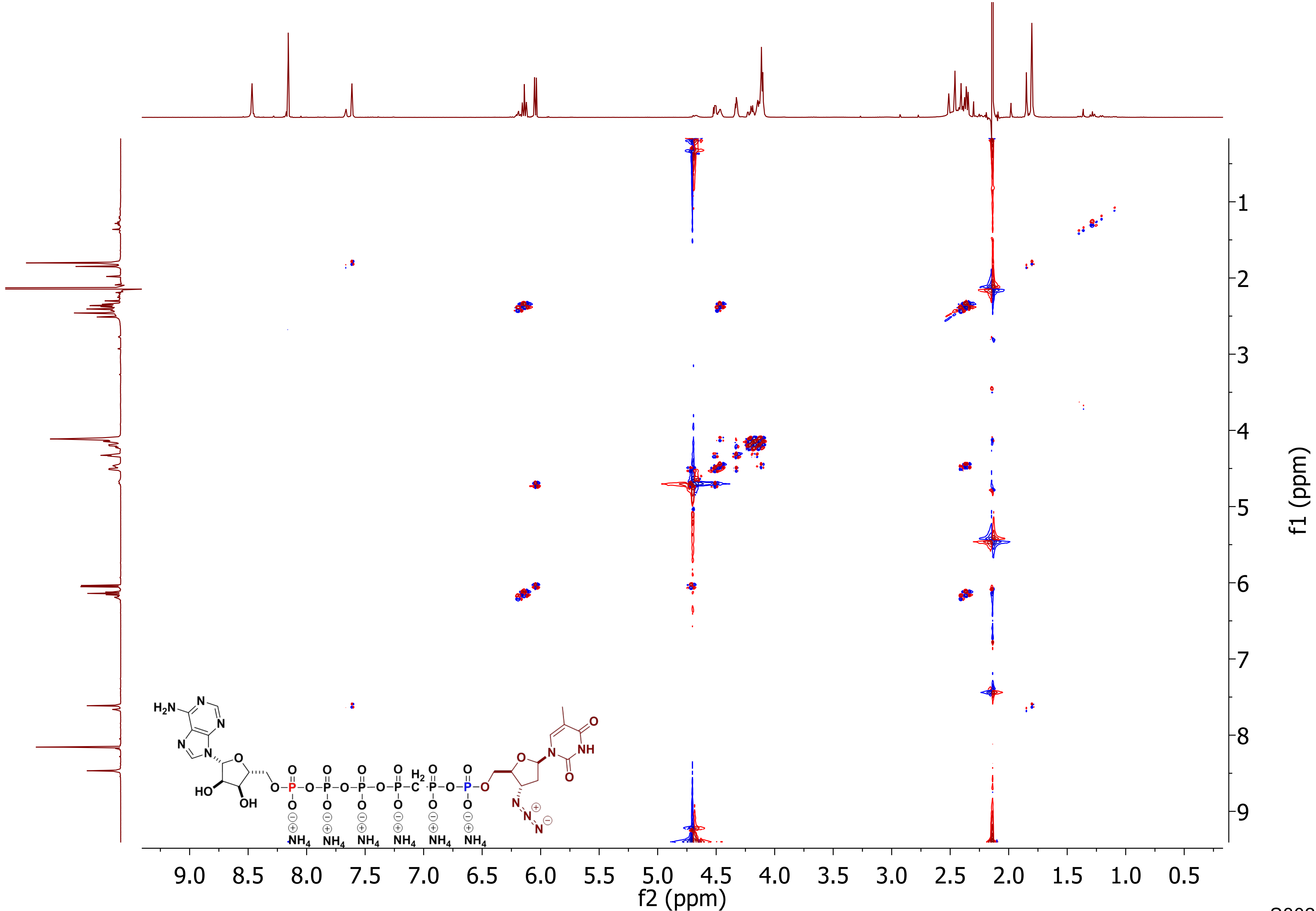
## $^{31}$P NMR



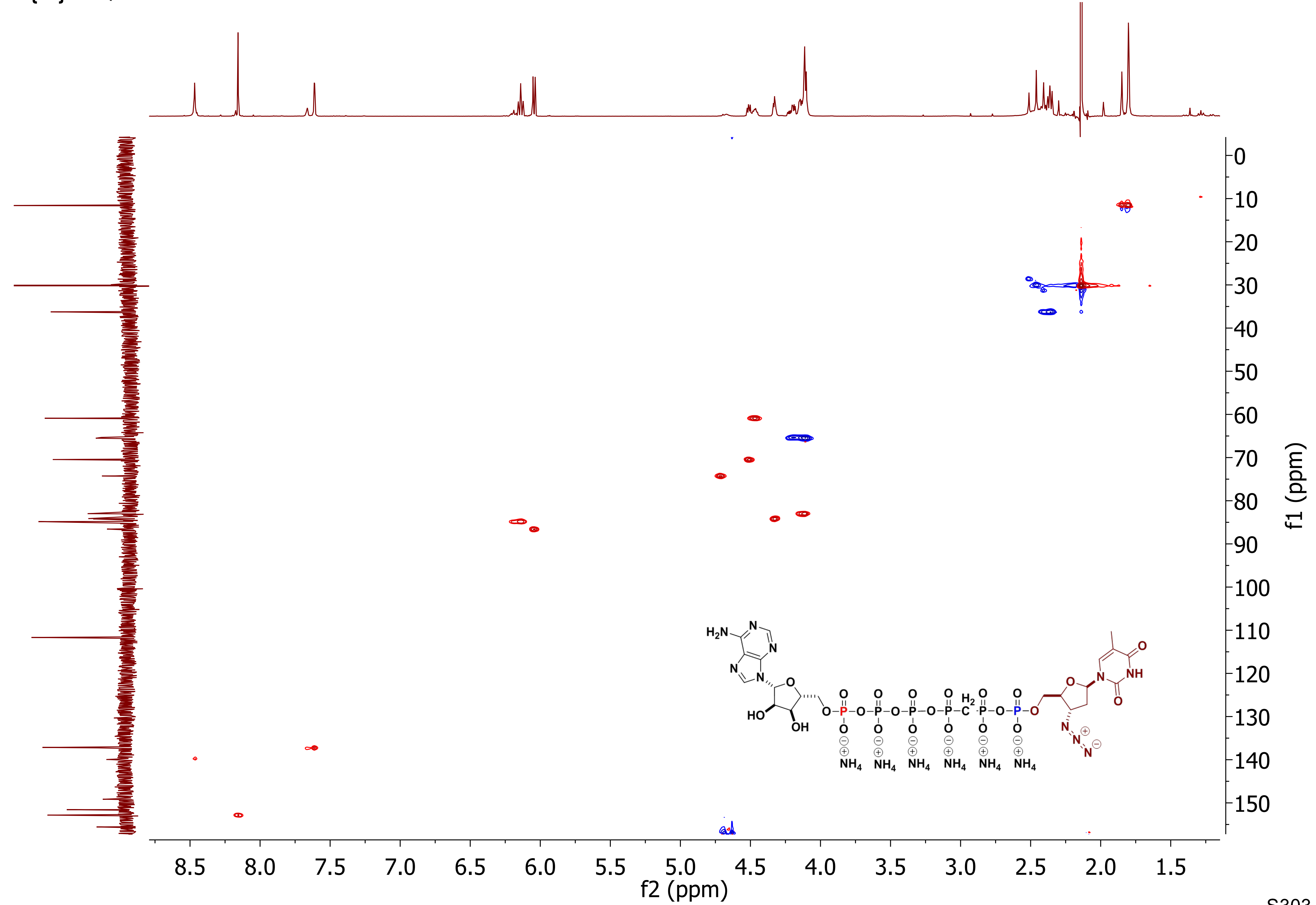
product after SAX purification



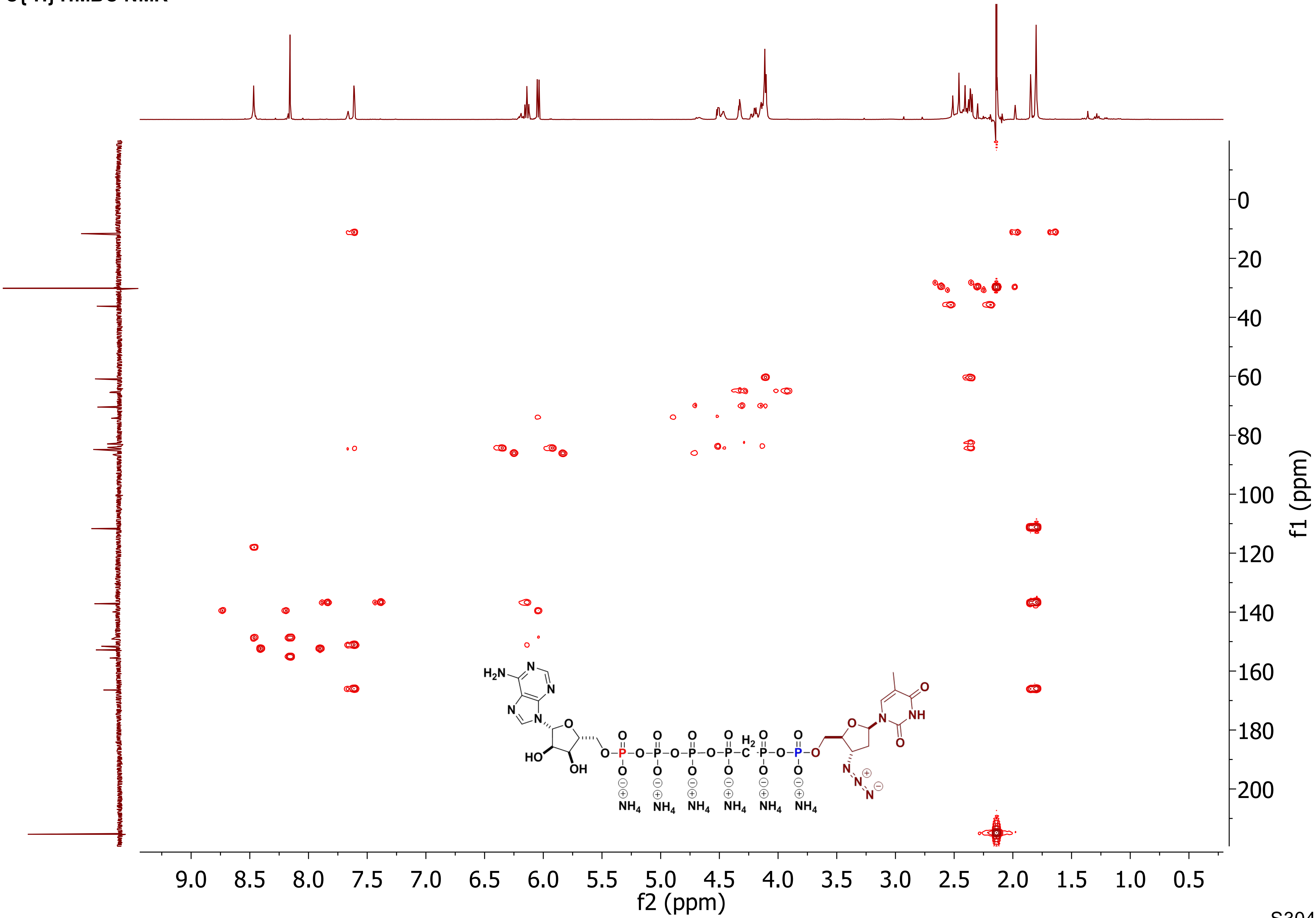
## $^1H$-$^1H$ COSY NMR



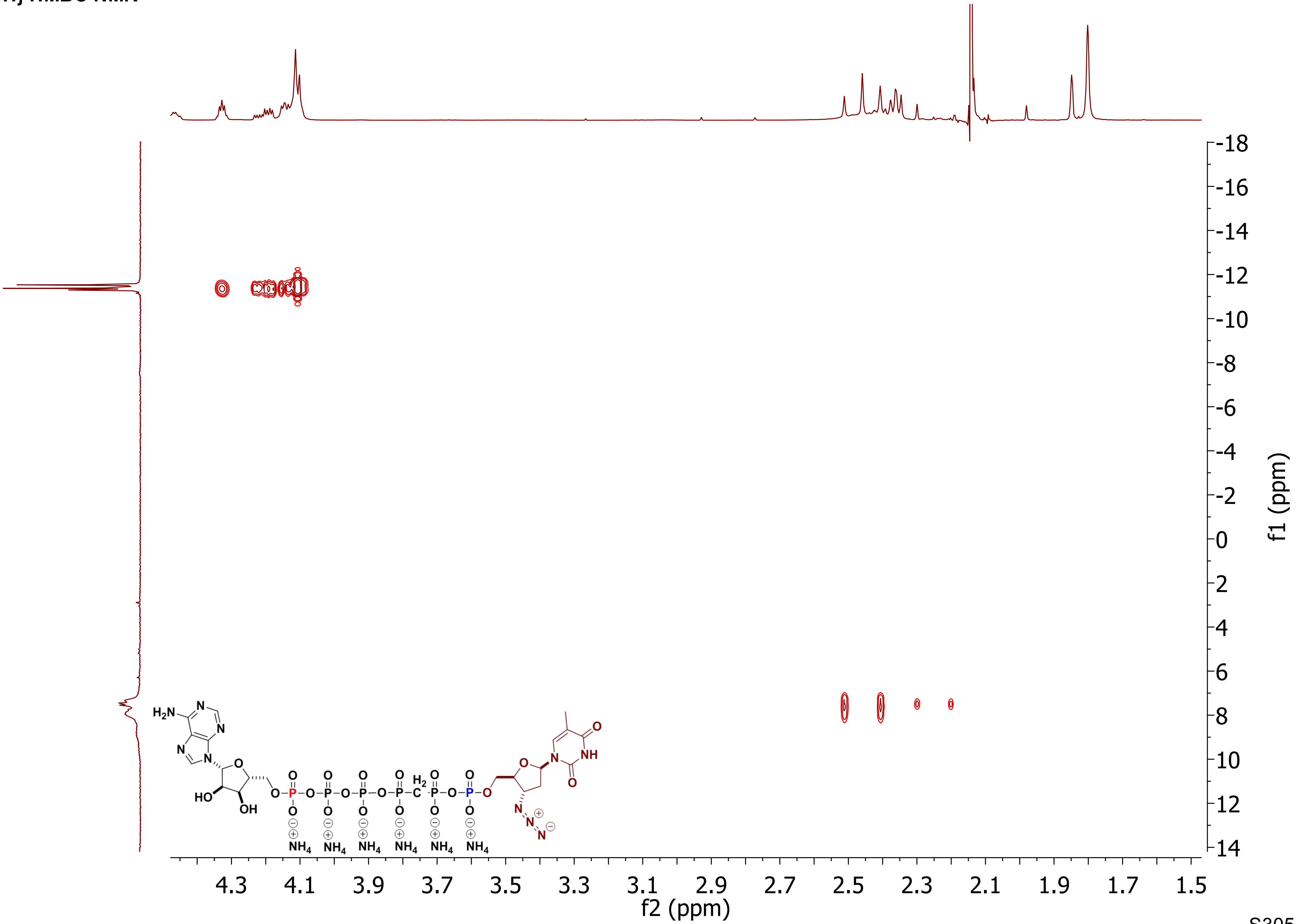
$^{13}C\{^1H\}$ HSQC NMR



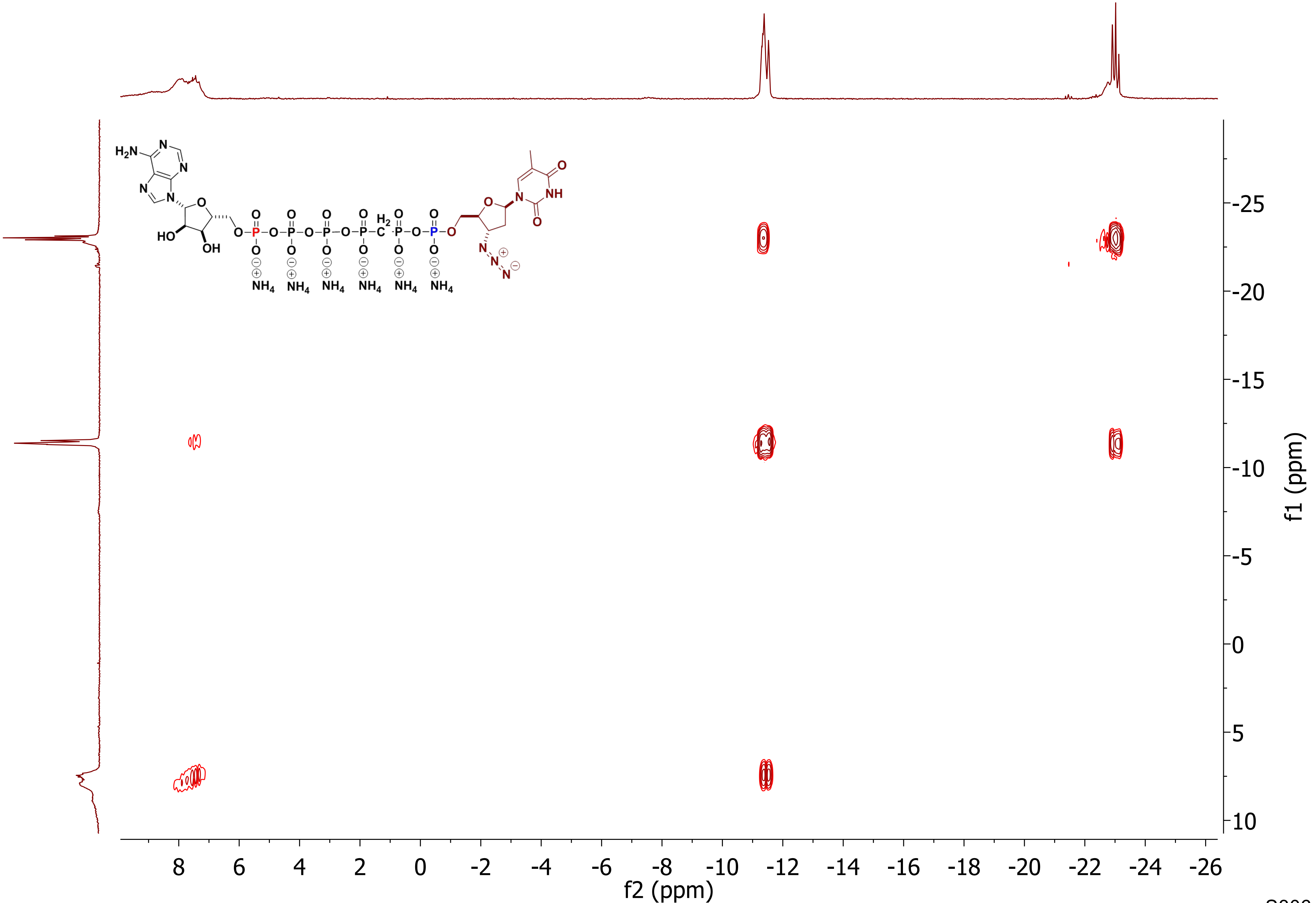
$^{13}C\{^1H\}$ HMBC NMR



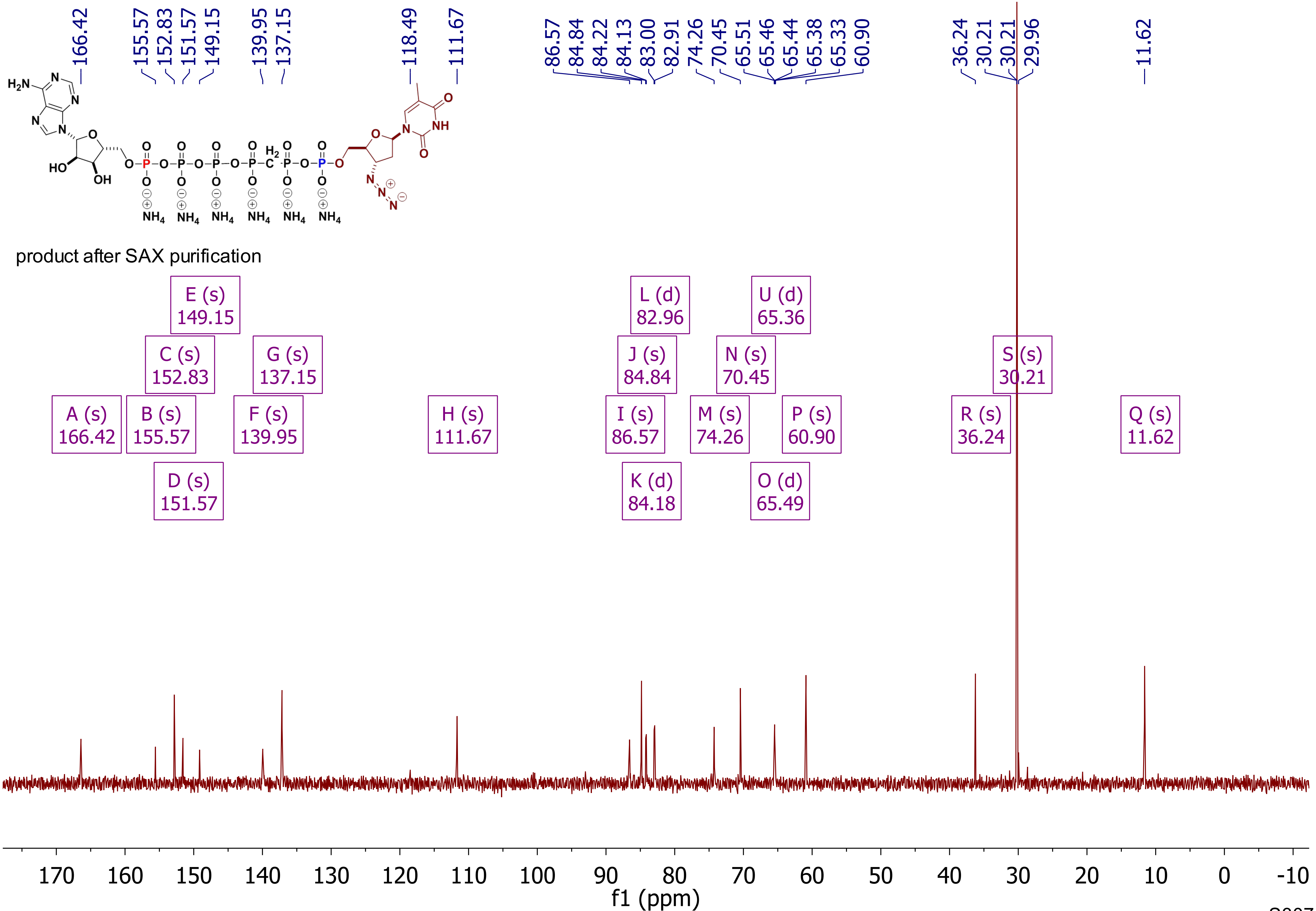
**$^{31}P\{^{1}H\}$ HMBC NMR**



## $^{31}P$ - $^{31}P$ COSY NMR

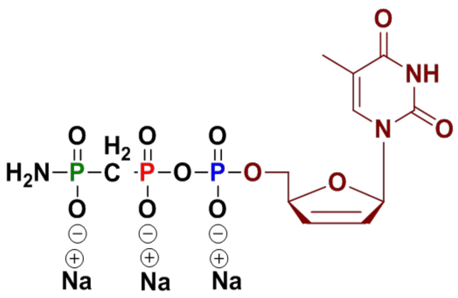


## $^{13}C$ NMR

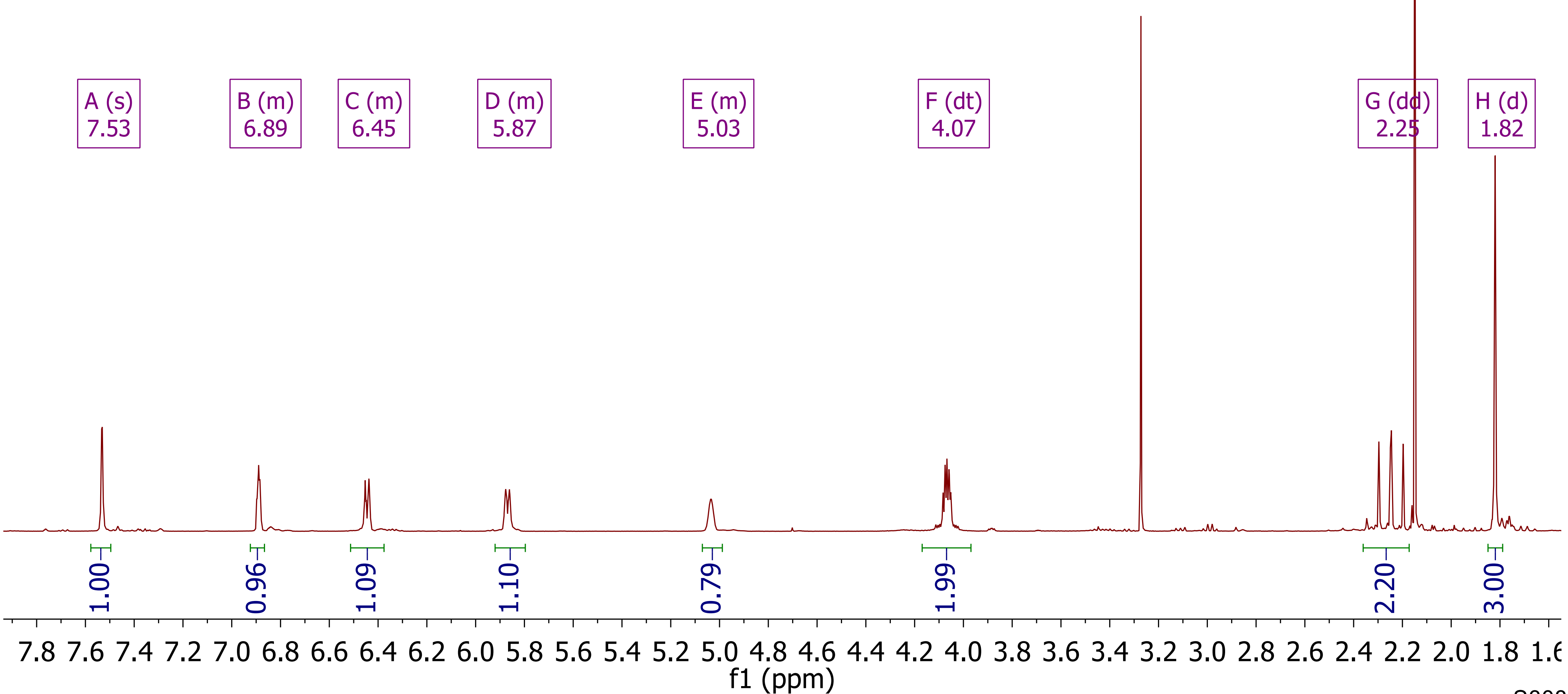


166.42
155.57
152.83
151.57
149.15
139.95
137.15
118.49
111.67
86.57
84.84
84.22
84.13
83.00
82.91
74.26
70.45
65.51
65.46
65.44
65.38
65.33
60.90
36.24
30.21
30.21
29.96
11.62
product after SAX purification
E (s) 149.15
C (s) 152.83
G (s) 137.15
A (s) 166.42
B (s) 155.57
F (s) 139.95
D (s) 151.57
H (s) 111.67
L (d) 82.96
J (s) 84.84
I (s) 86.57
K (d) 84.18
U (d) 65.36
N (s) 70.45
M (s) 74.26
P (s) 60.90
O (d) 65.49
S (s) 30.21
R (s) 36.24
Q (s) 11.62
f1 (ppm)

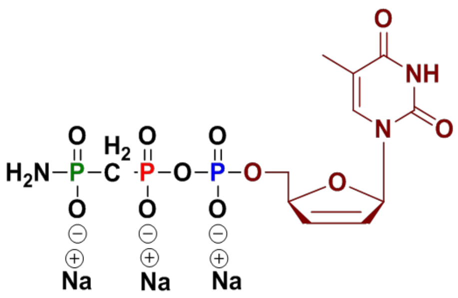
$^1$H NMR



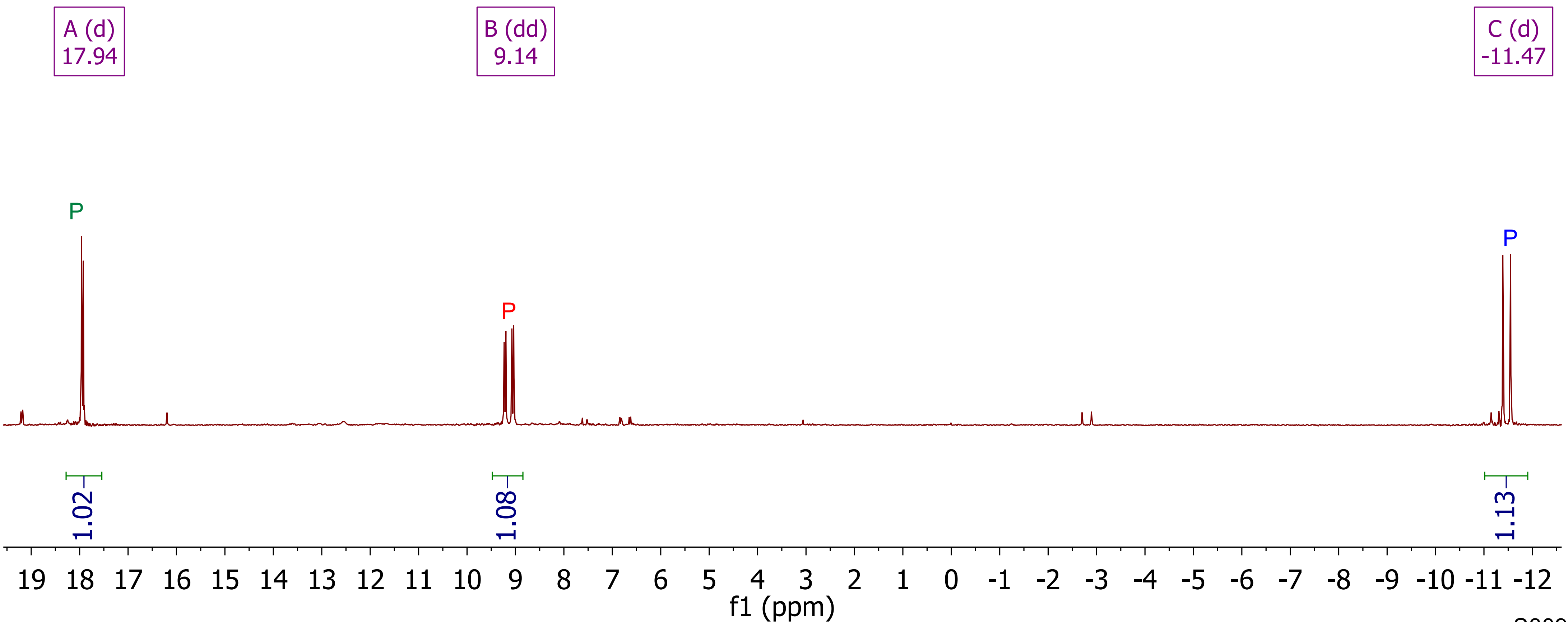
crude product after precipitation



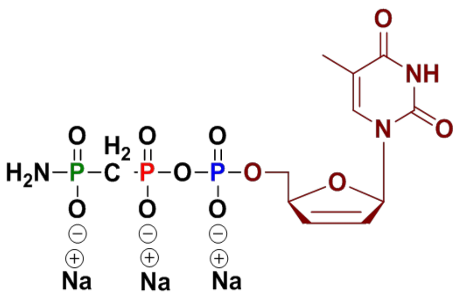
**$^{31}P\{^1H\}$ NMR**



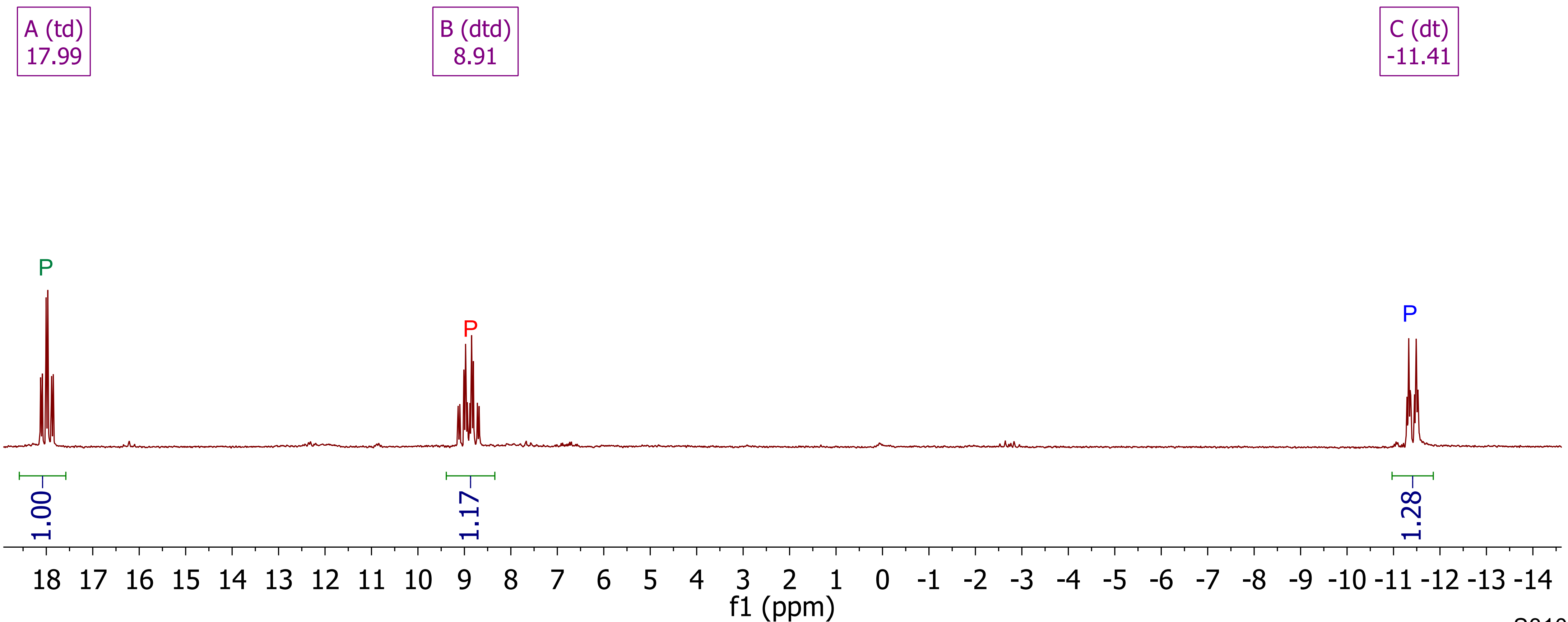
crude product after precipitation



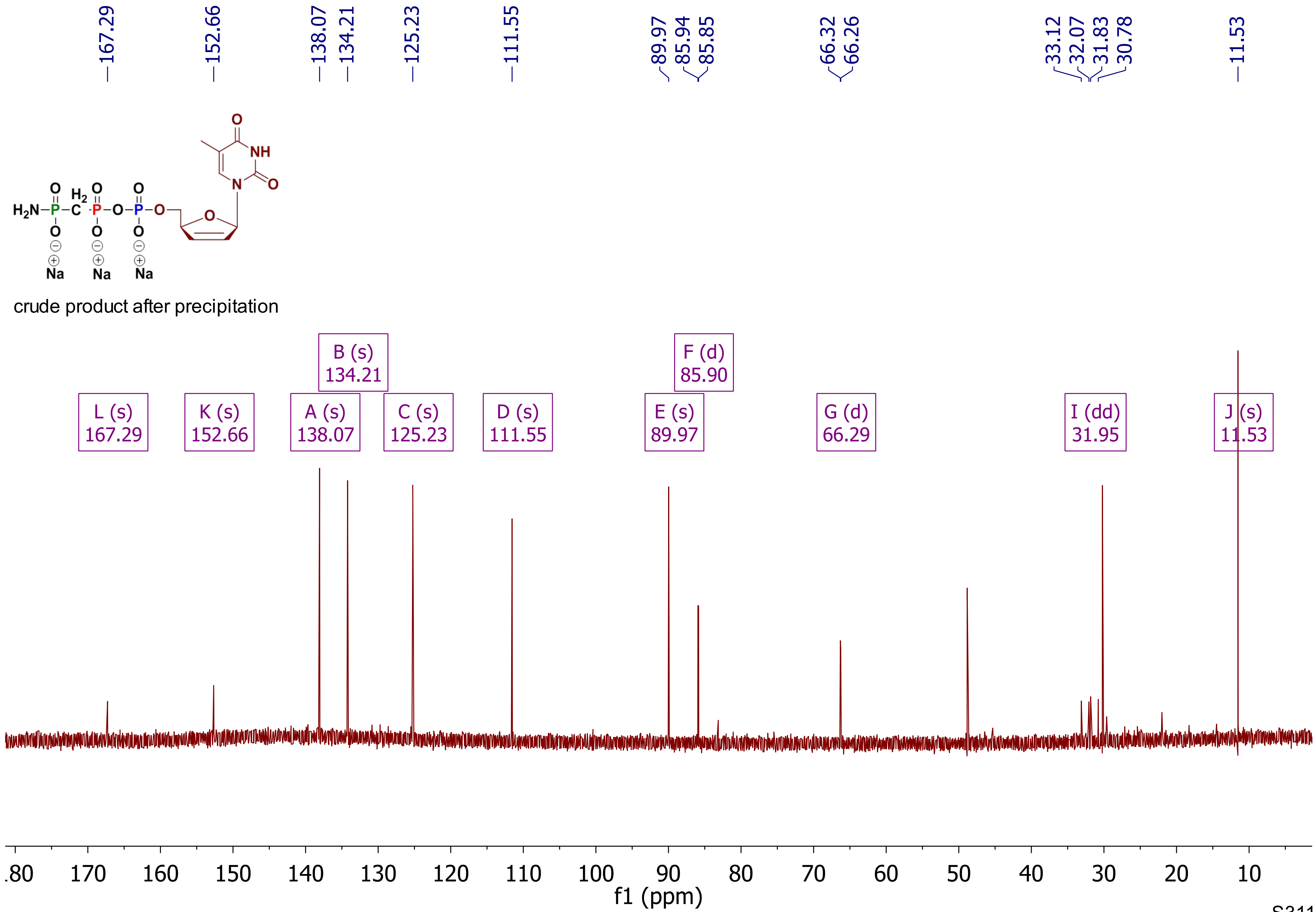
**$^{31}$P NMR**




crude product after precipitation

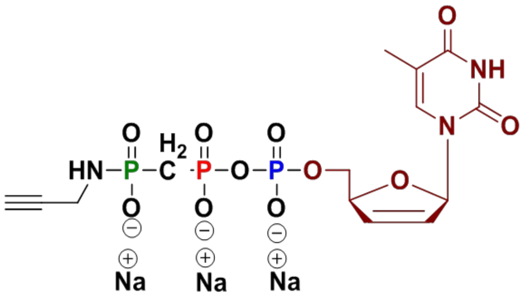


**$^{13}$C NMR**

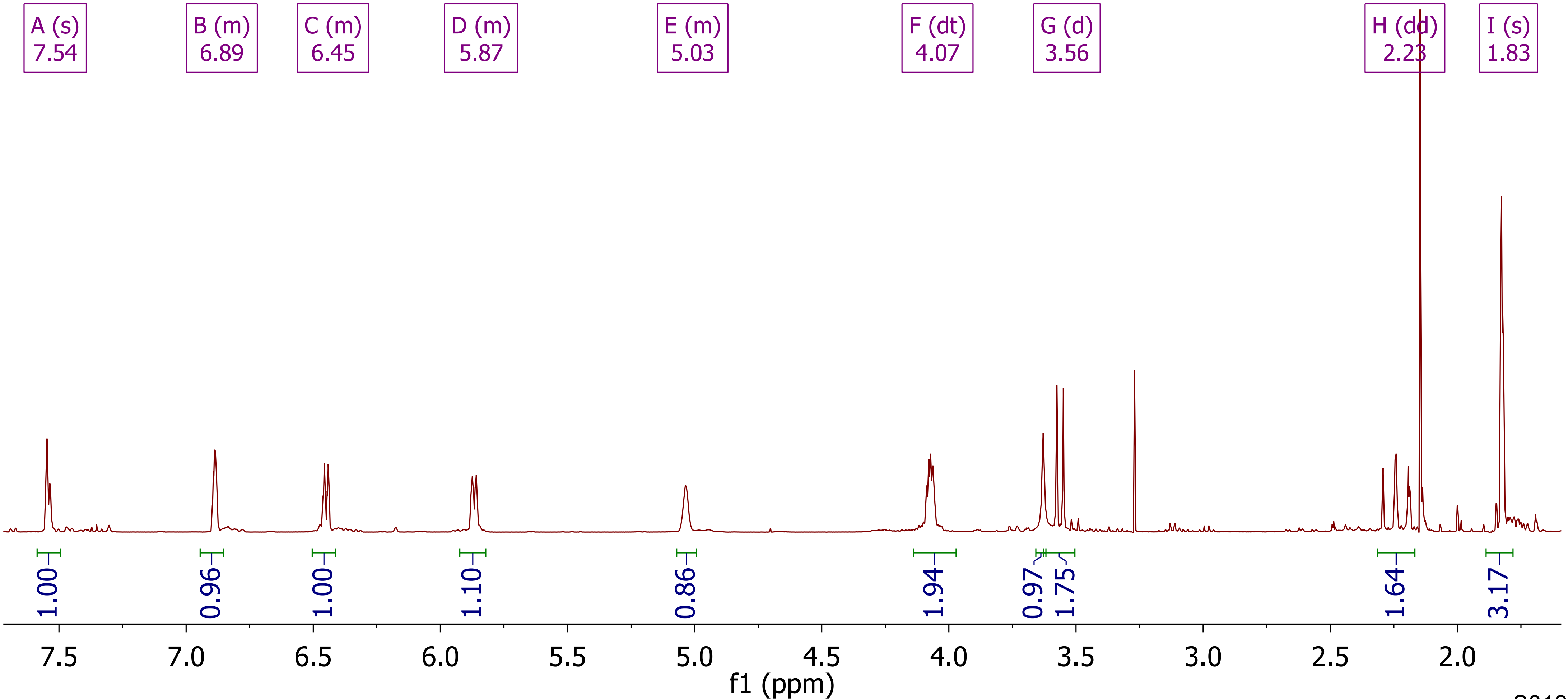


167.29
152.66
138.07
134.21
125.23
111.55
89.97
85.94
85.85
66.32
66.26
33.12
32.07
31.83
30.78
11.53
crude product after precipitation
B (s) 134.21
F (d) 85.90
L (s) 167.29
K (s) 152.66
A (s) 138.07
C (s) 125.23
D (s) 111.55
E (s) 89.97
G (d) 66.29
I (dd) 31.95
J (s) 11.53
f1 (ppm)

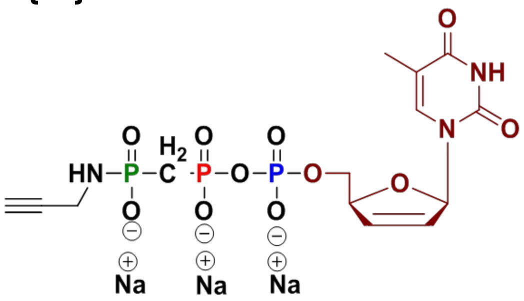
## $^1$H NMR



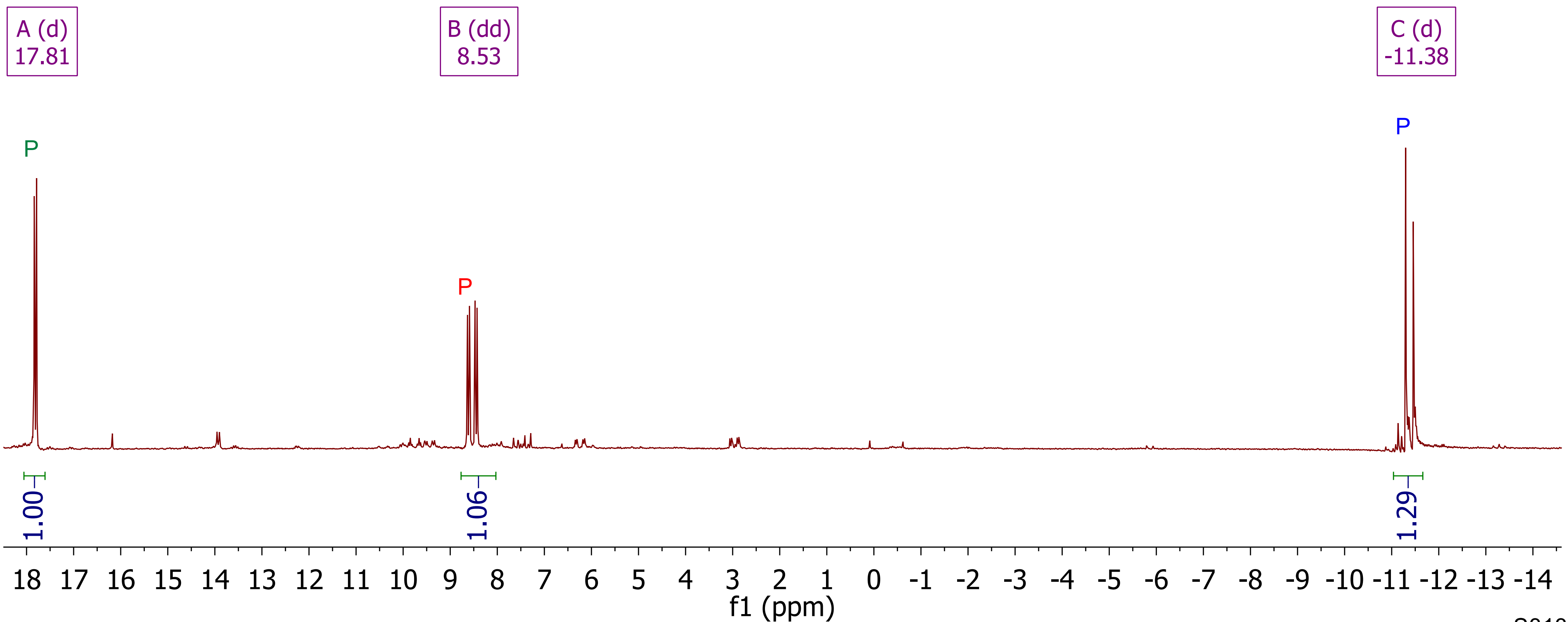
crude product after precipitation



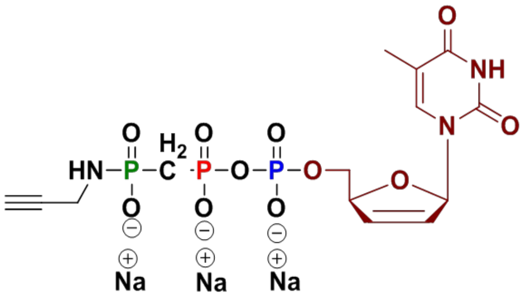
**$^{31}P\{^{1}H\}$ NMR**



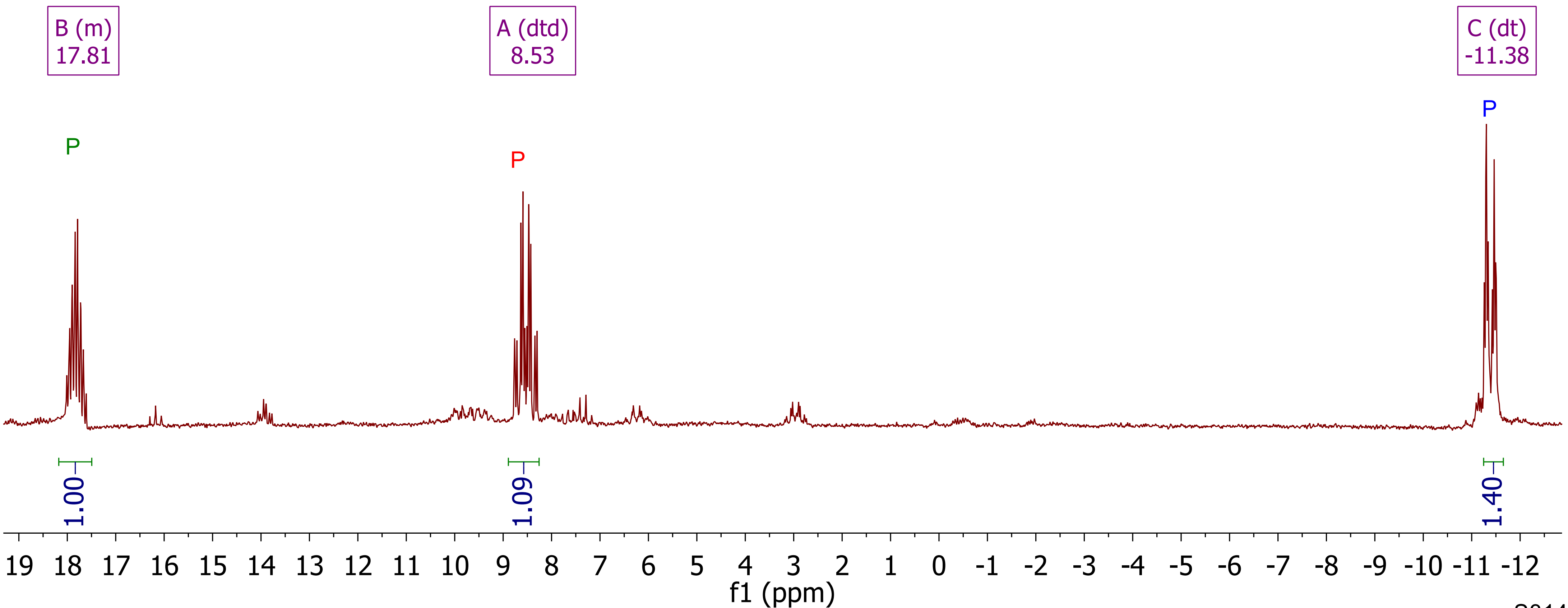
crude product after precipitation



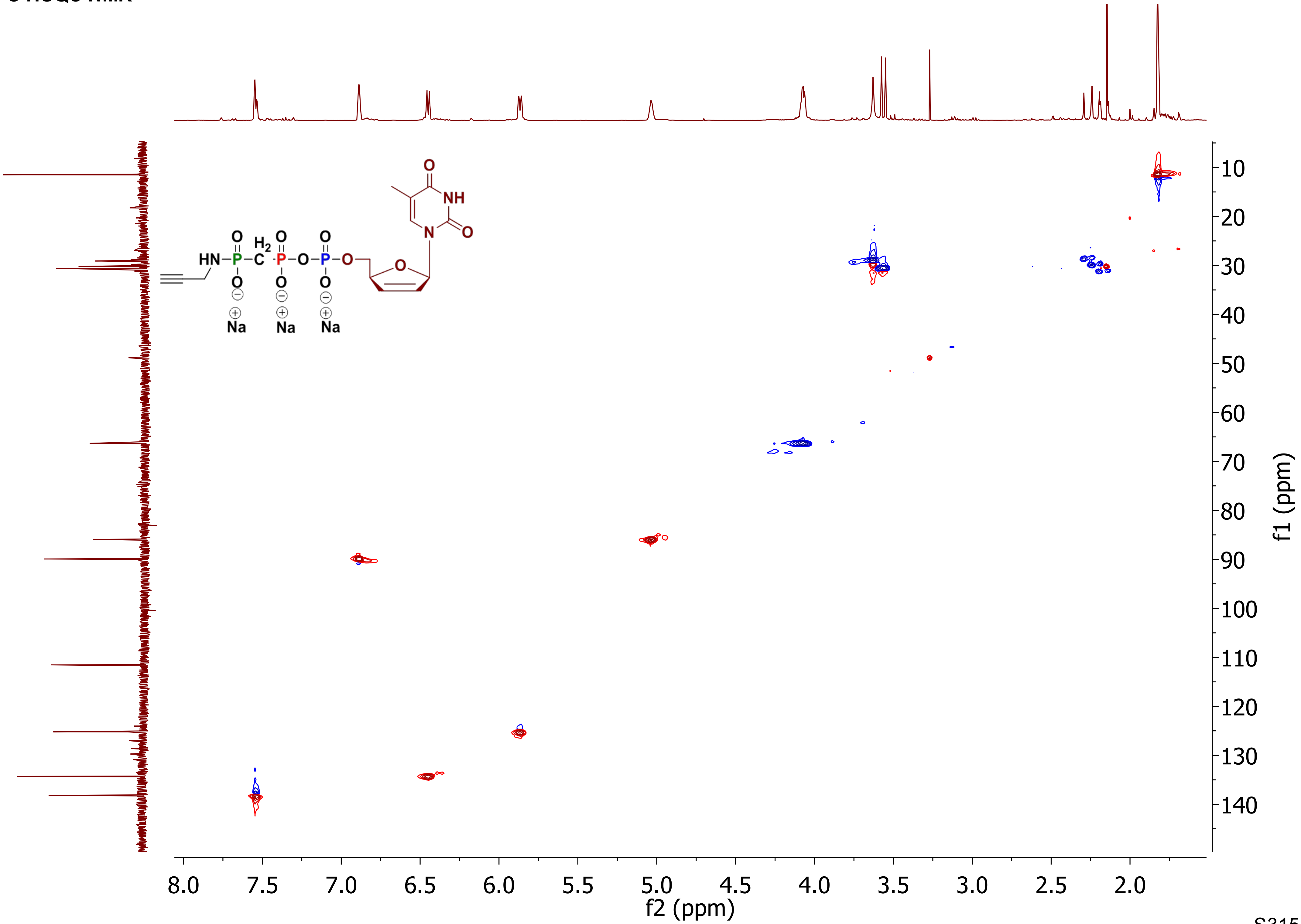
**$^{31}$P NMR**




crude product after precipitation

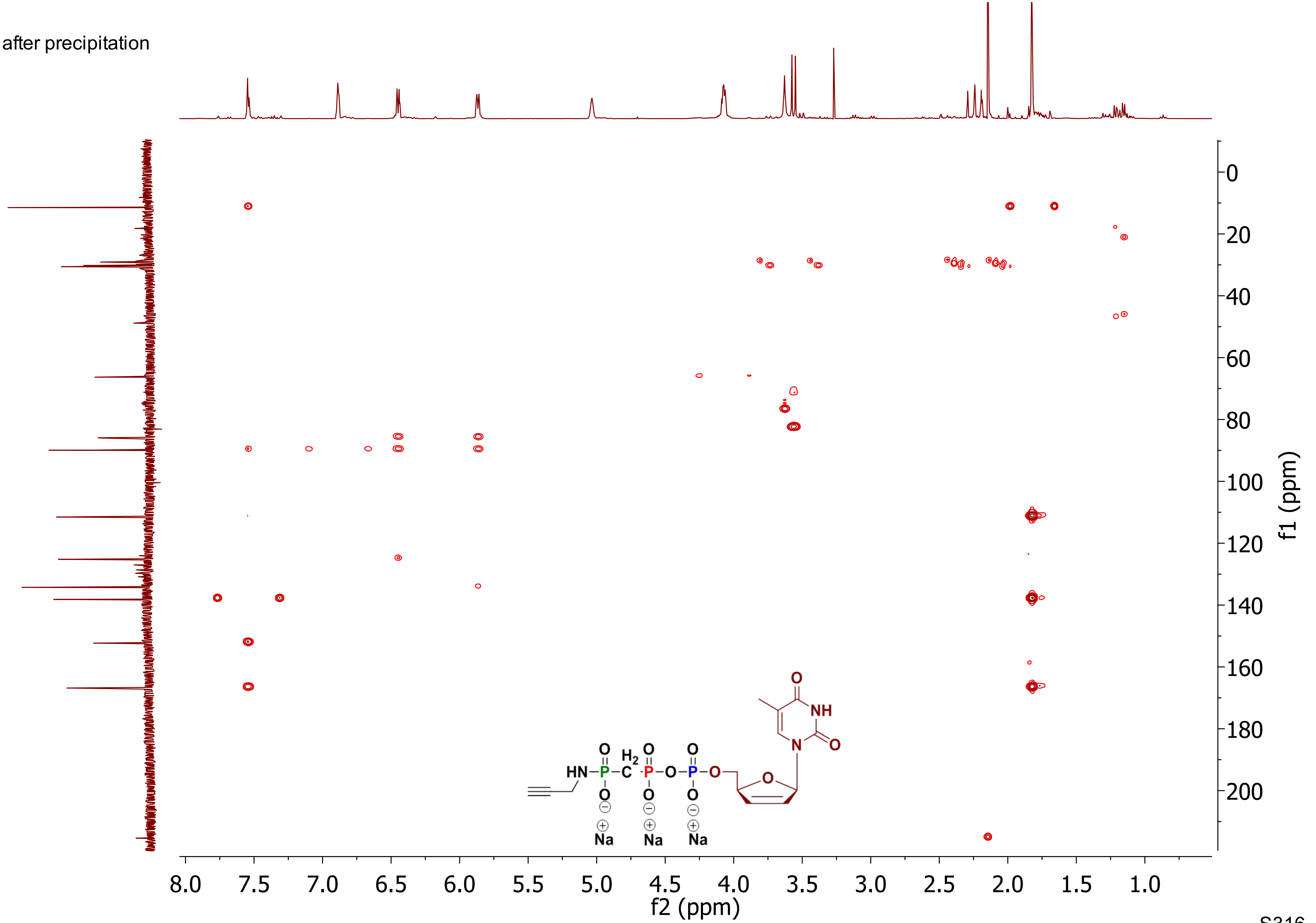


$^{1}H$-$^{13}C$ HSQC NMR

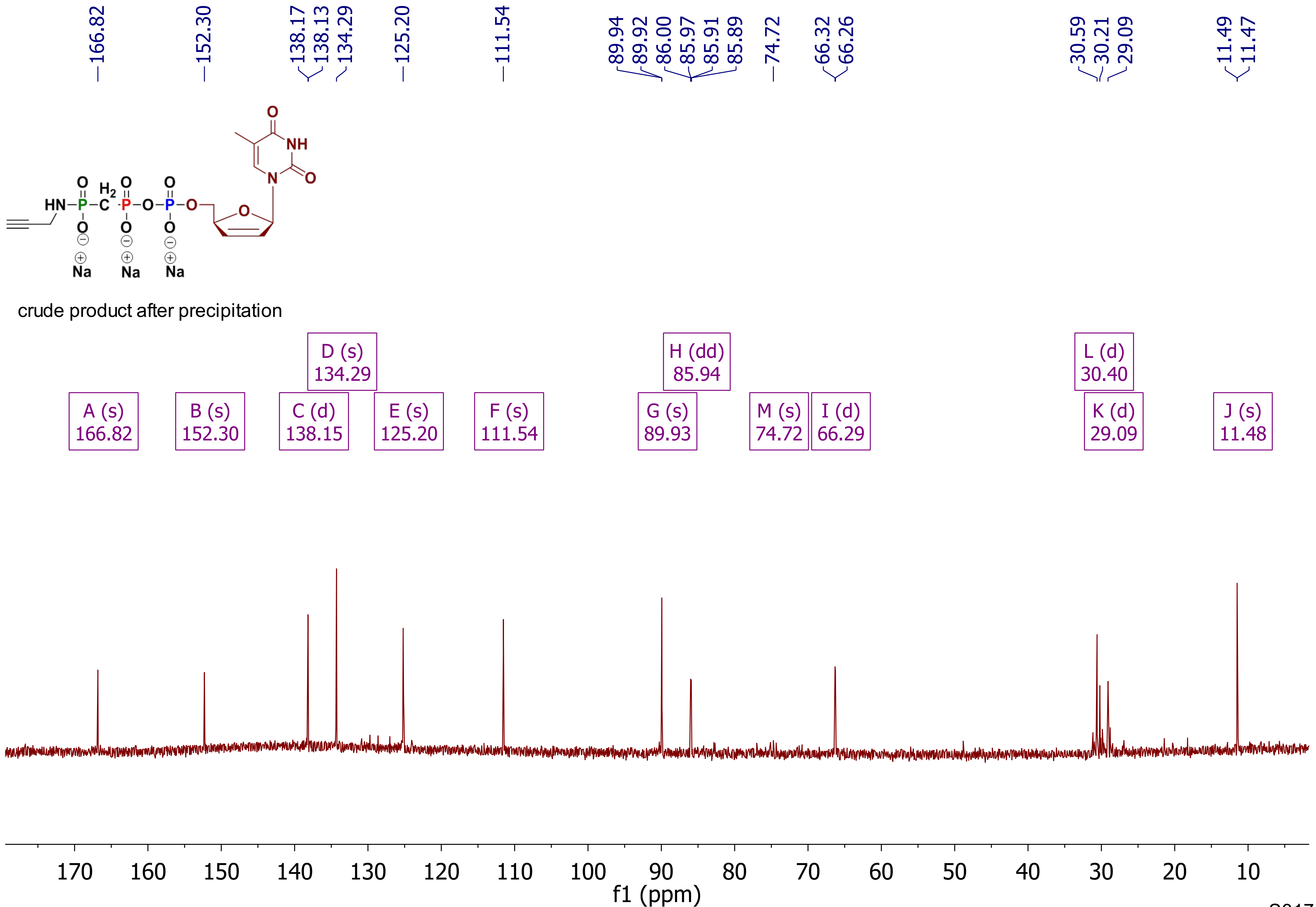


$^{1}H$-$^{13}C$ HMBC NMR

after precipitation

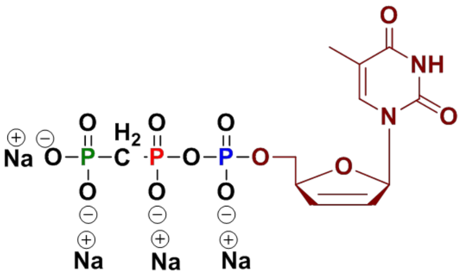


## $^{13}$C NMR

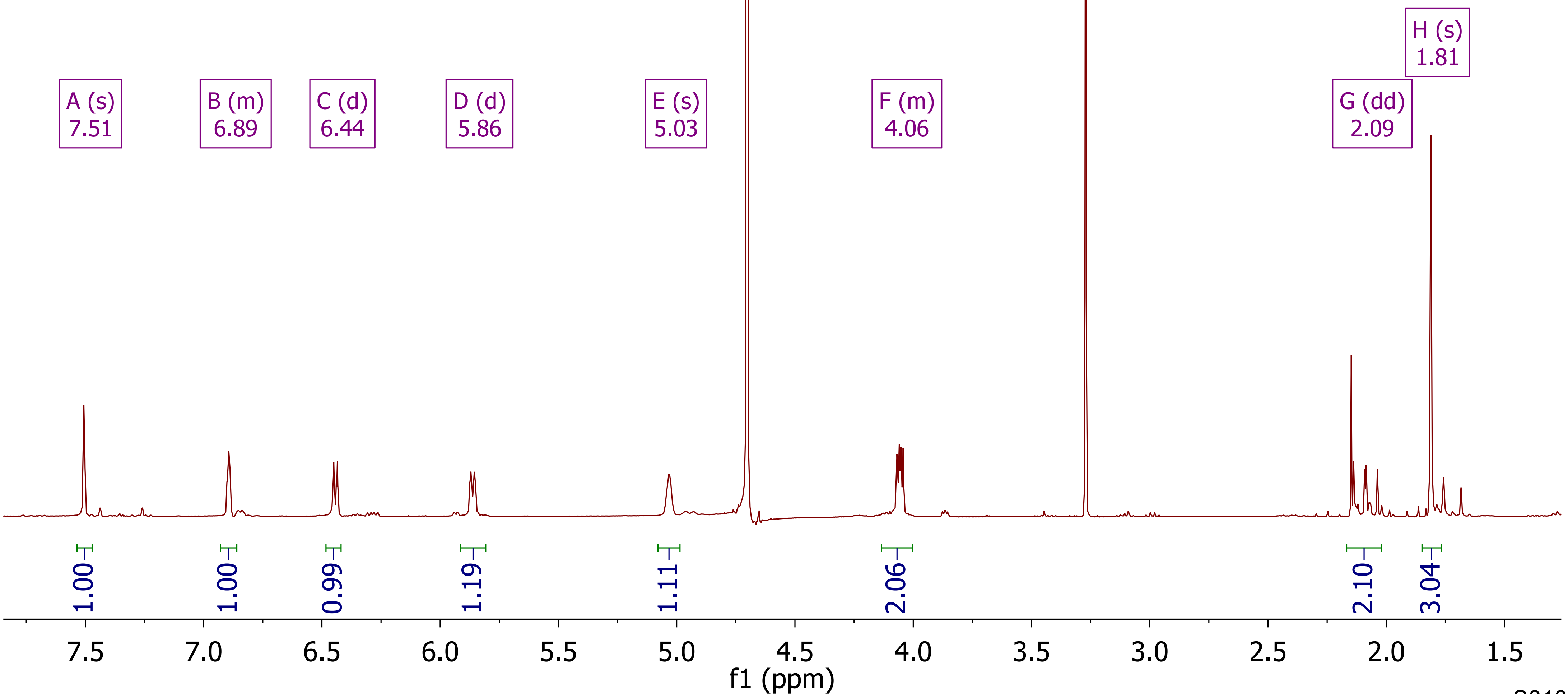


166.82
152.30
138.17
138.13
134.29
125.20
111.54
89.94
89.92
86.00
85.97
85.91
85.89
74.72
66.32
66.26
30.59
30.21
29.09
11.49
11.47
O
NH
O
N
O
H2
O
O
O
HN
P
C
P
O
P
O
O
O
O
Na
Na
Na
crude product after precipitation
D (s) 134.29
H (dd) 85.94
L (d) 30.40
A (s) 166.82
B (s) 152.30
C (d) 138.15
E (s) 125.20
F (s) 111.54
G (s) 89.93
M (s) 74.72
I (d) 66.29
K (d) 29.09
J (s) 11.48
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

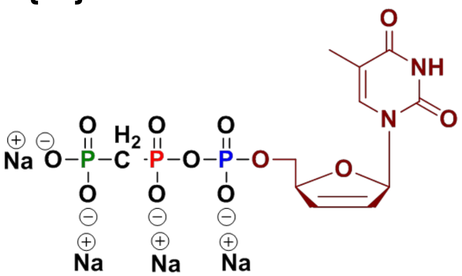
$^1$H - NMR



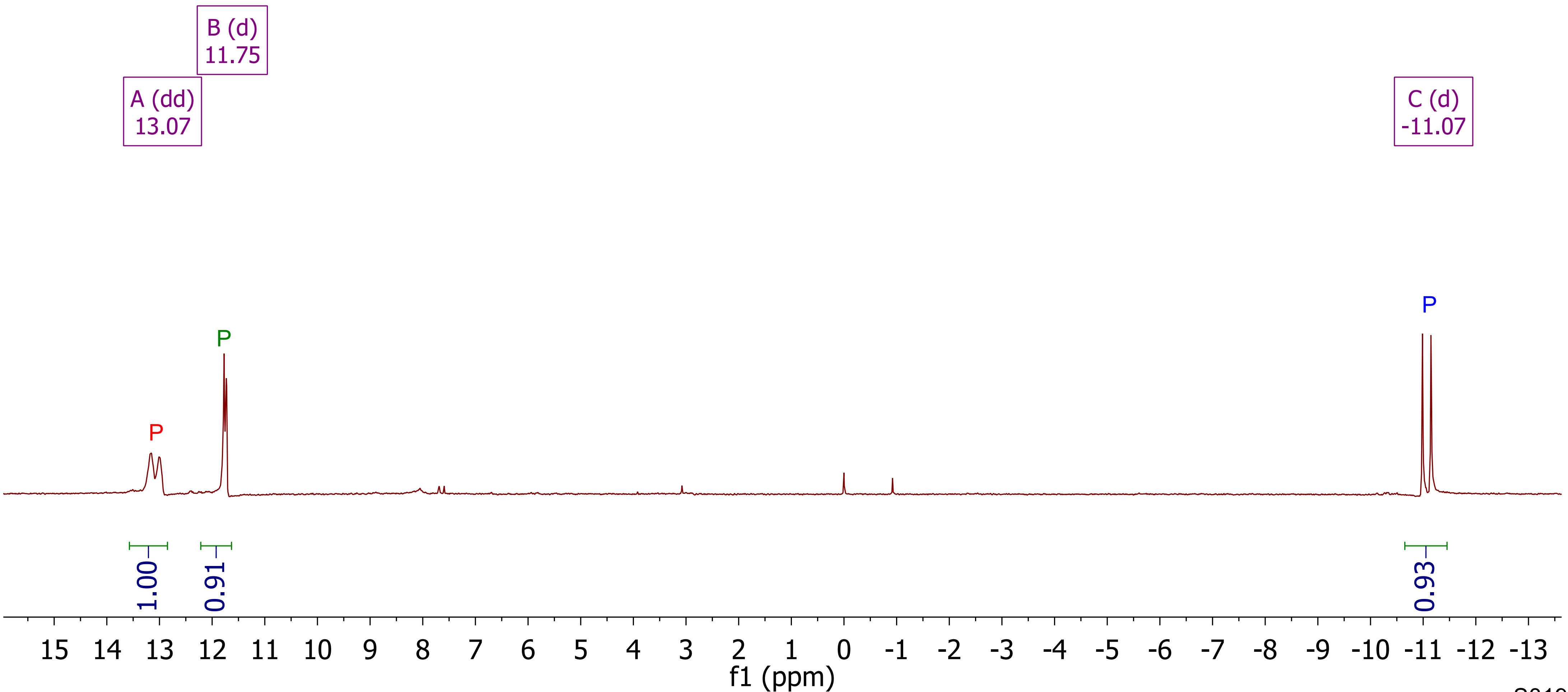
crude product after precipitation



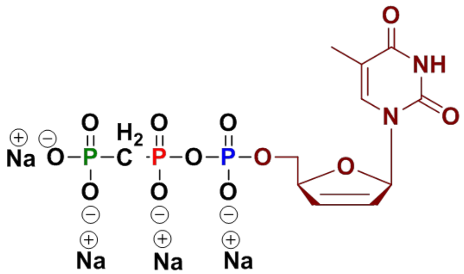
$^{31}P\{^{1}H\}$ NMR



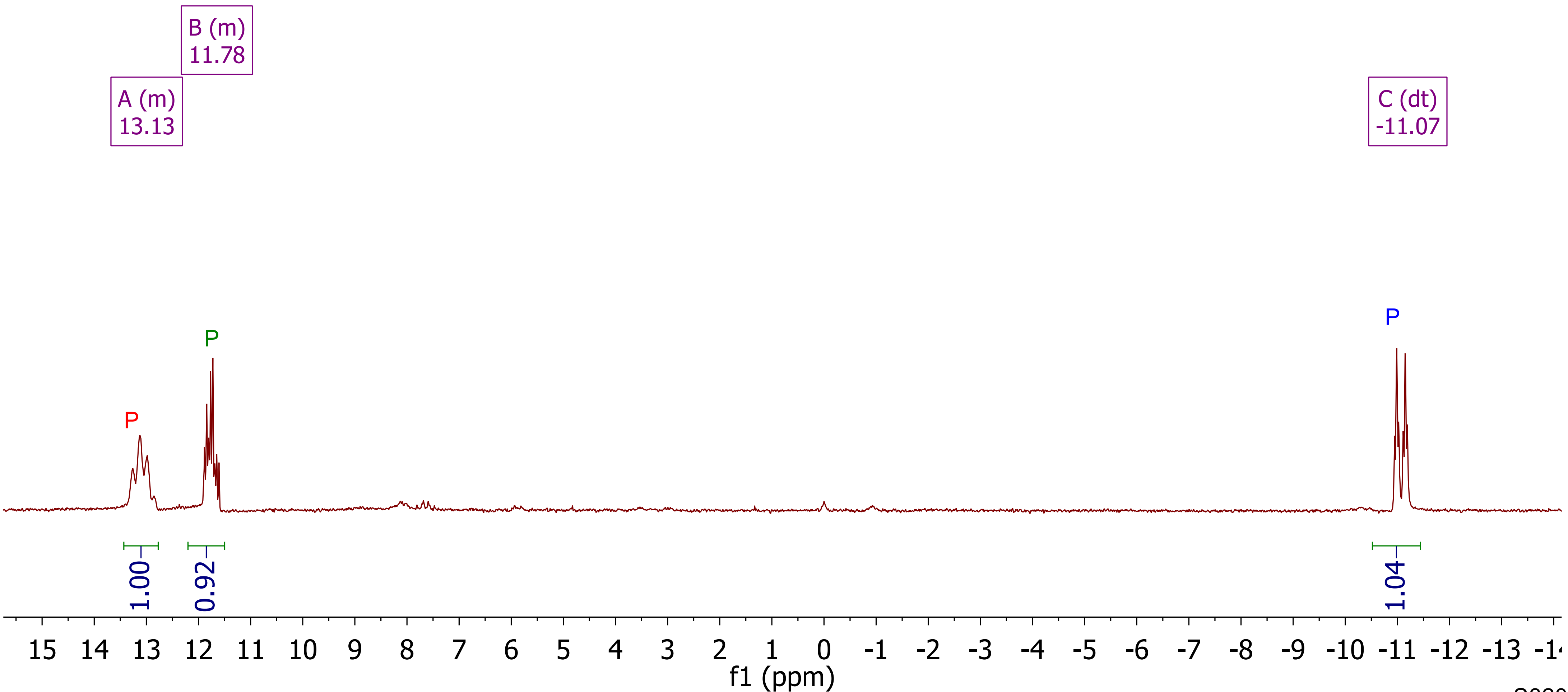
crude product after precipitation



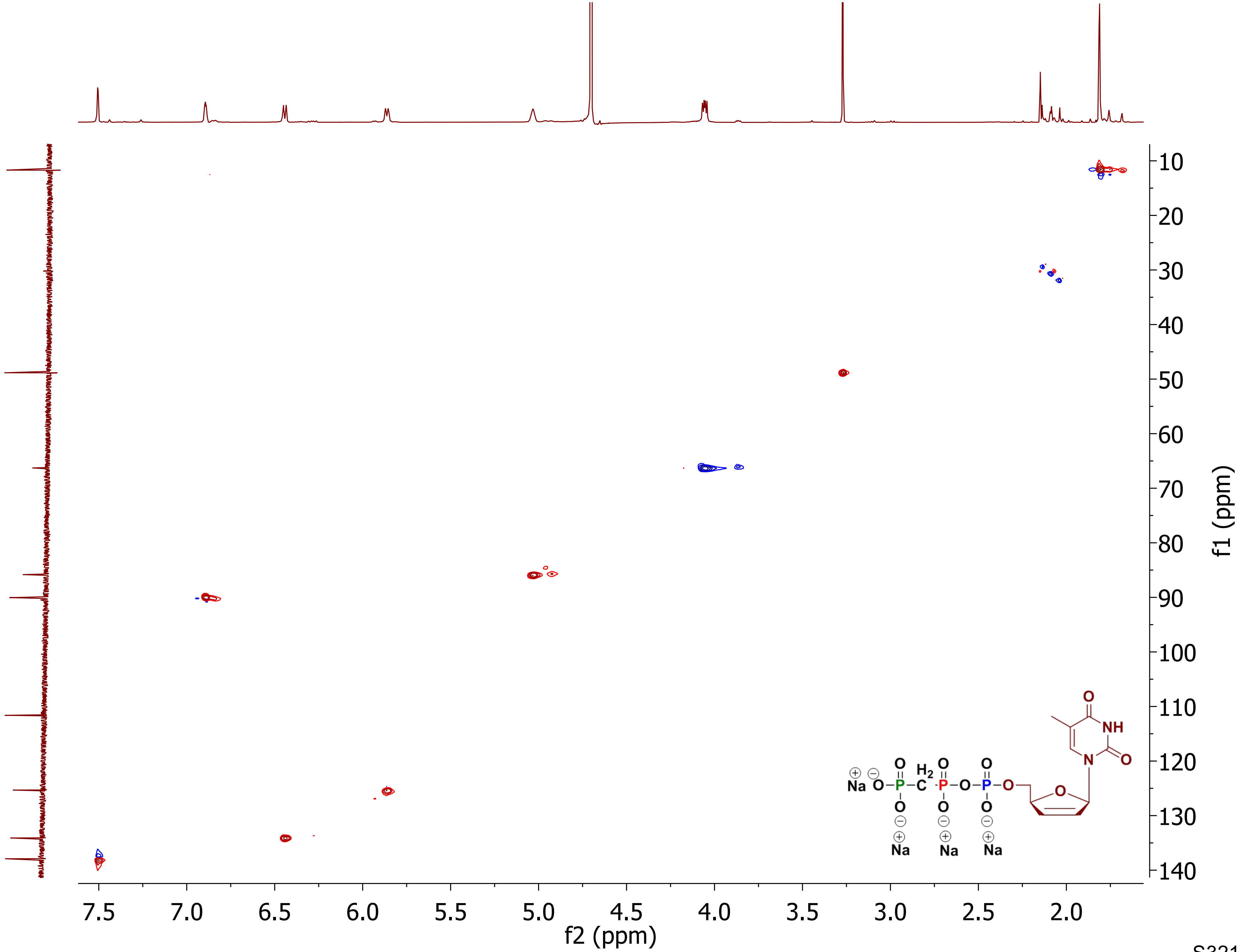
## $^{31}$P NMR



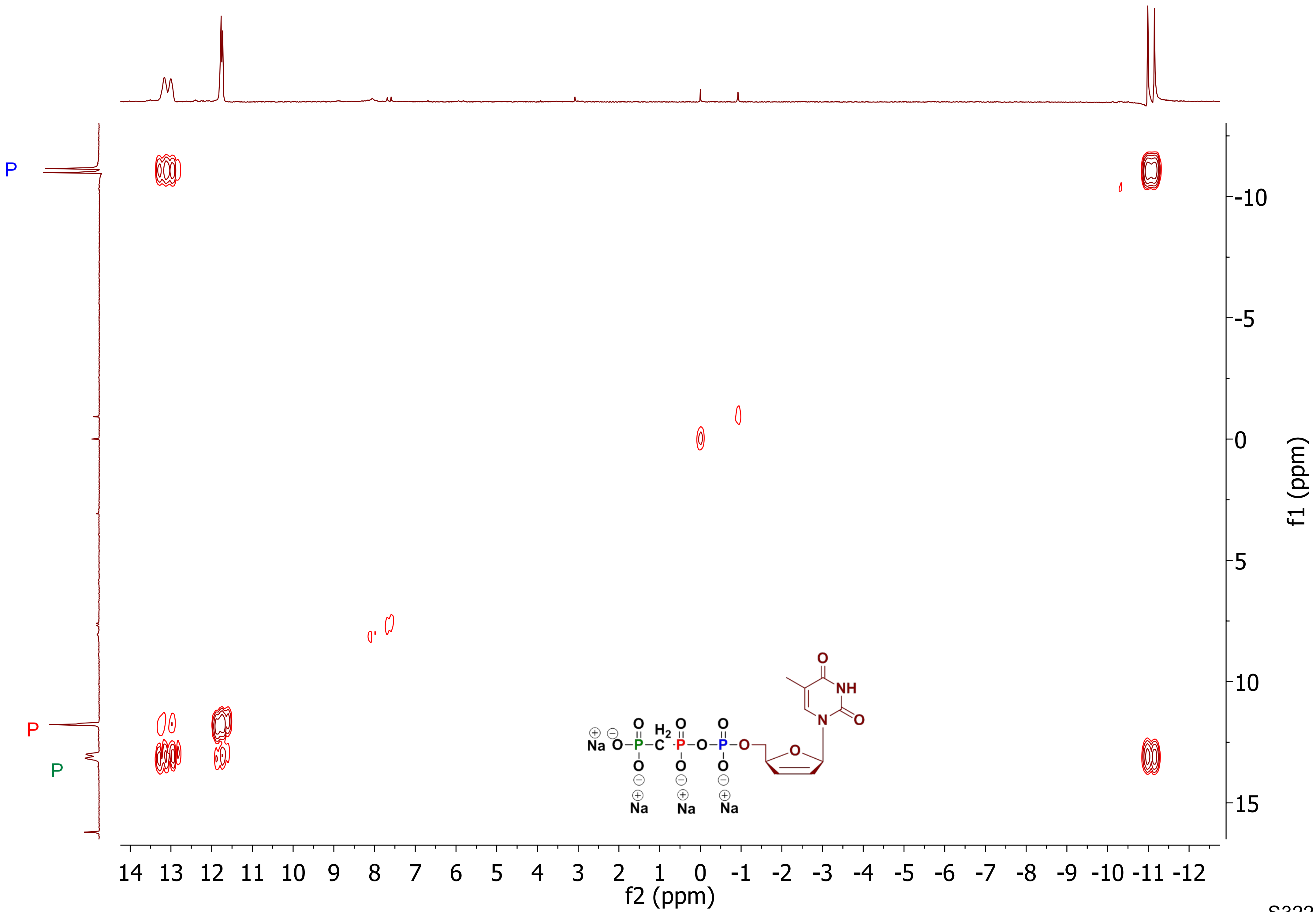
crude product after precipitation



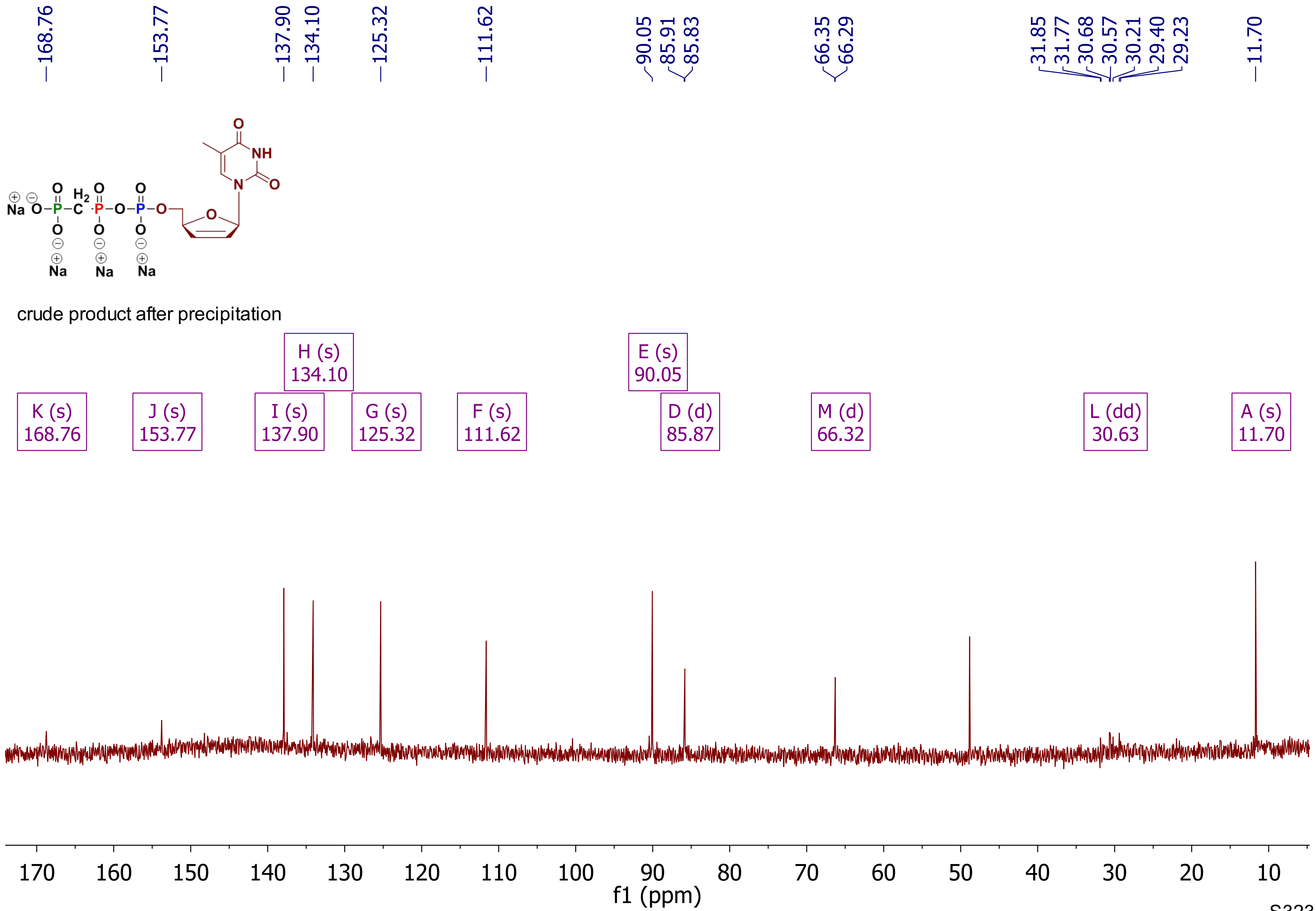
$^1H$-$^{13}C$ HSQC NMR



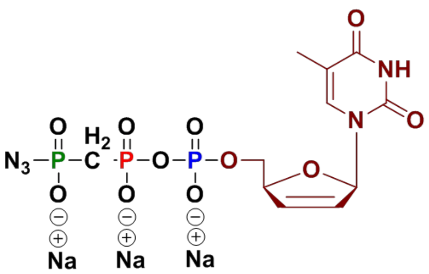
**$^{31}P$-$^{31}P$ COSY NMR**



$^{13}C$ NMR

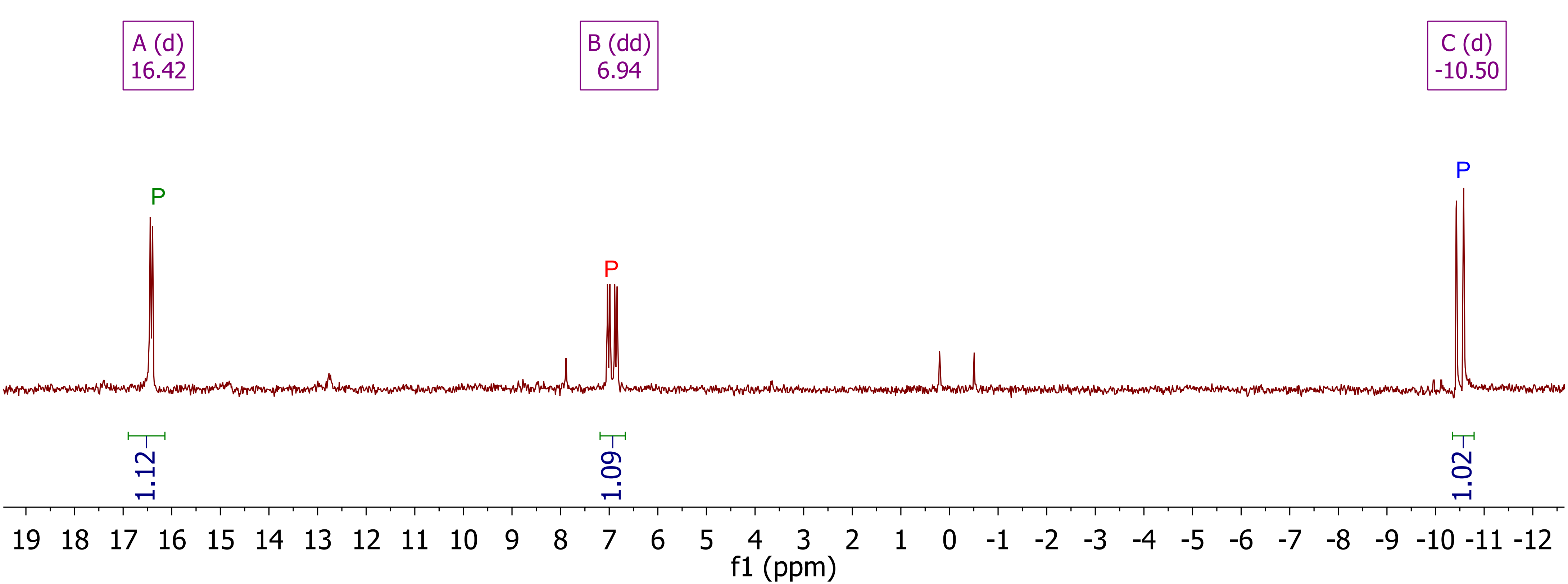

168.76
153.77
137.90
134.10
125.32
111.62
90.05
85.91
85.83
66.35
66.29
31.85
31.77
30.68
30.57
30.21
29.40
29.23
11.70
crude product after precipitation
H (s) 134.10
E (s) 90.05
K (s) 168.76
J (s) 153.77
I (s) 137.90
G (s) 125.32
F (s) 111.62
D (d) 85.87
M (d) 66.32
L (dd) 30.63
A (s) 11.70
f1 (ppm)

$^{31}P\{^1H\}$ NMR




crude product after precipitation

Ring opening with tetramethyl guanidinium azide

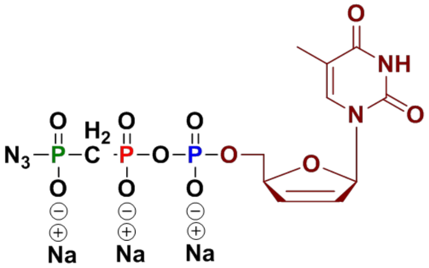


**$^{31}$P-$^{1}$H NMR**

16.55 16.51 16.43 16.38 16.31 16.26

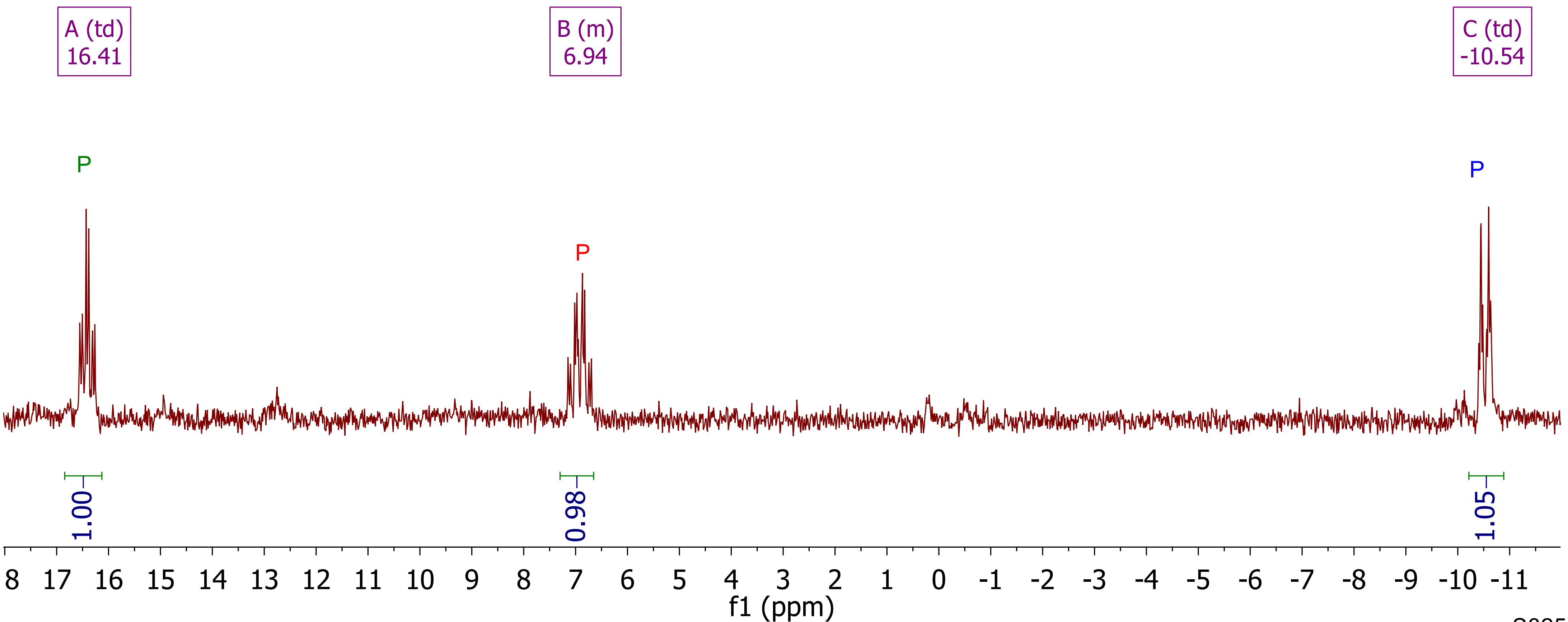
7.15 7.10 7.02 6.97 6.95 6.89 6.87 6.82 6.74 6.70

-10.44 -10.48 -10.59 -10.63

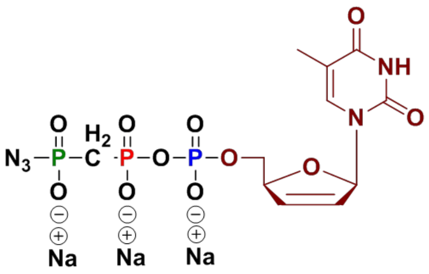



crude product after precipitation

Ring opening with tetramethyl guanidinium azide

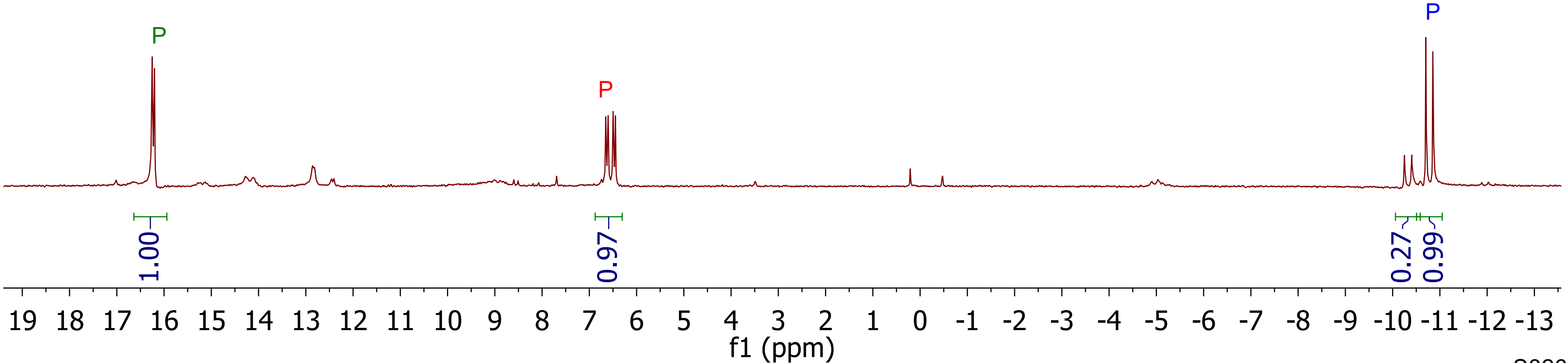


**$^{31}P\{^{1}H\}$ NMR**

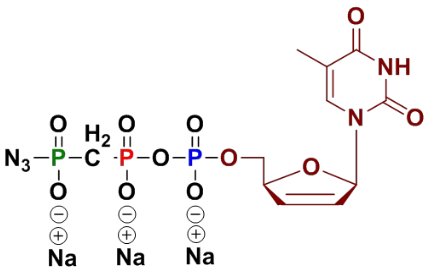



Ring opening with $LiN_3$

crude product after precipitation

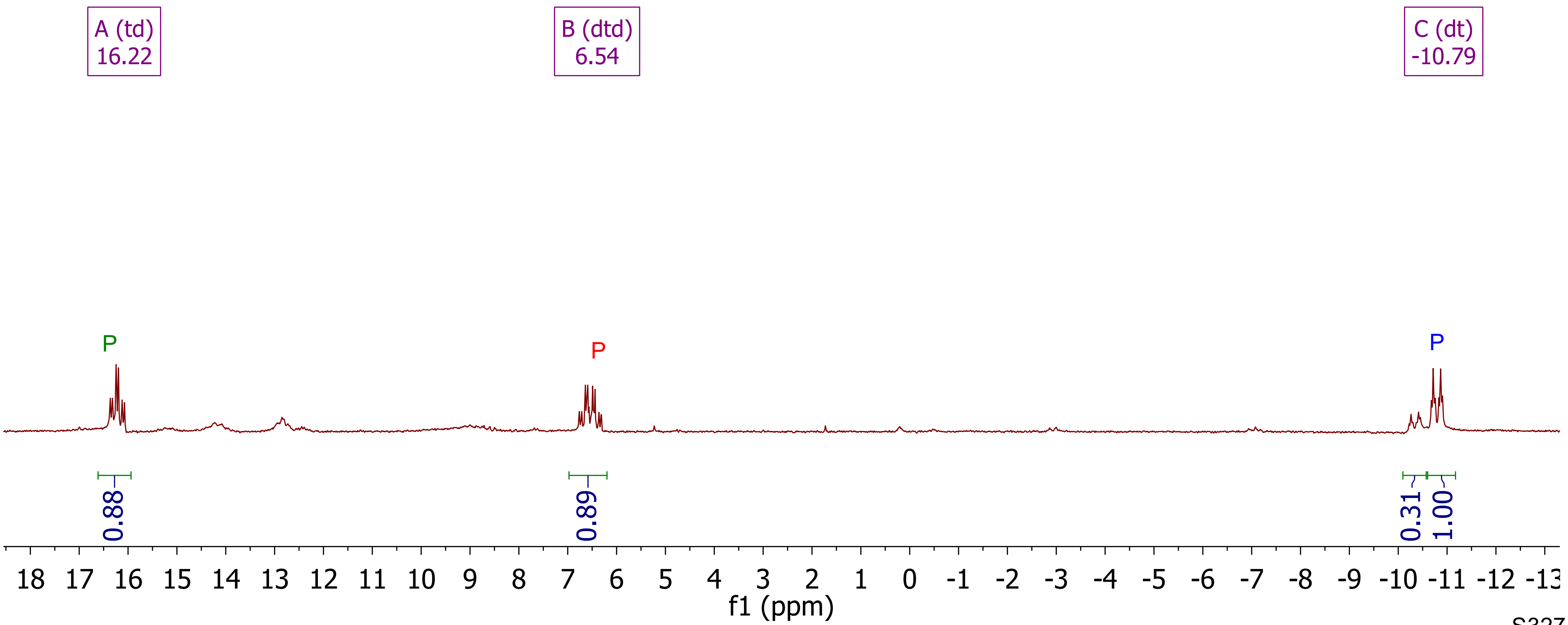


**$^{31}P$-$^{1}H$ NMR**

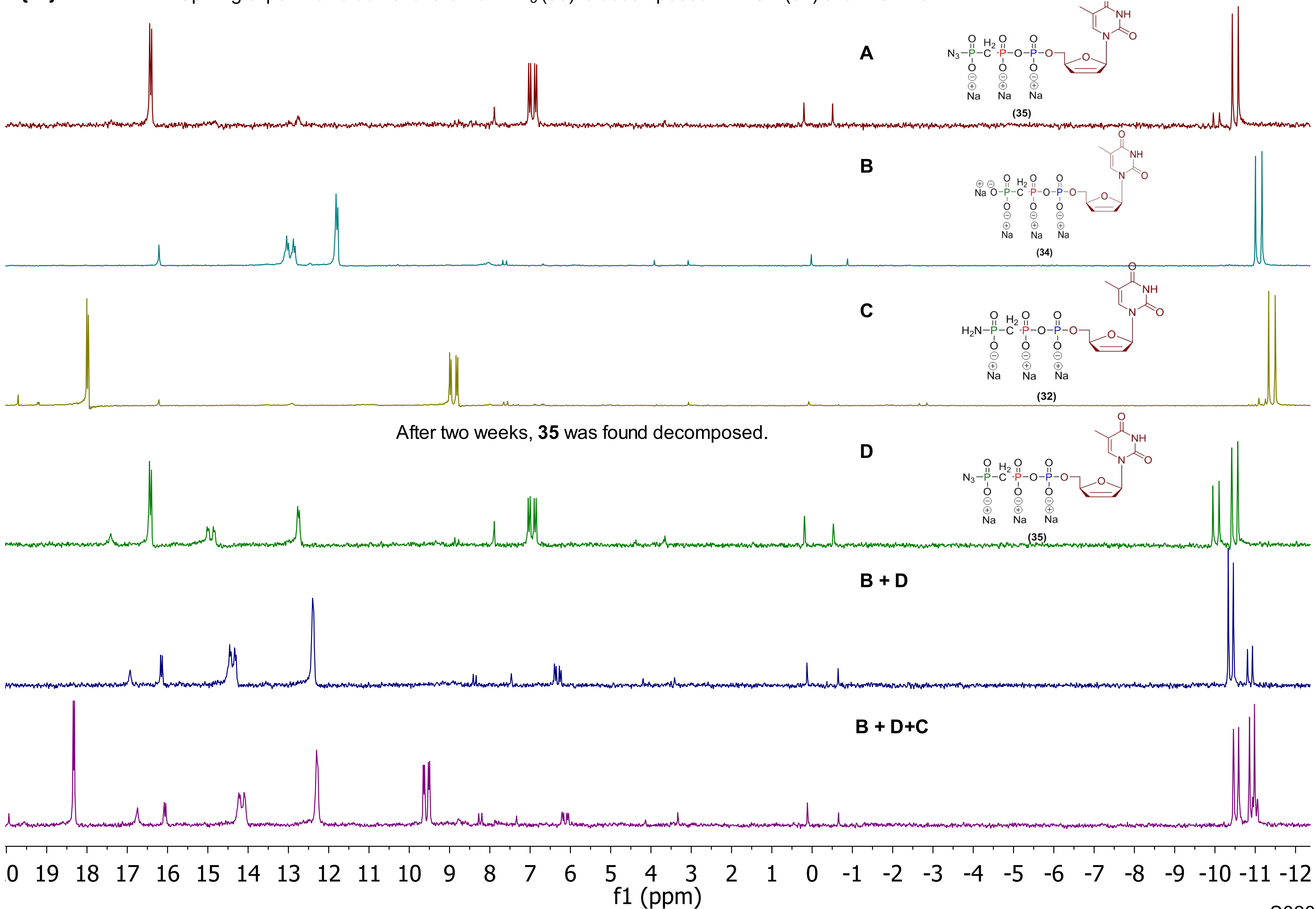


Ring opening with $LiN_3$

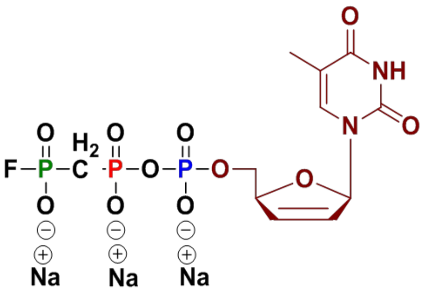
crude product after precipitation



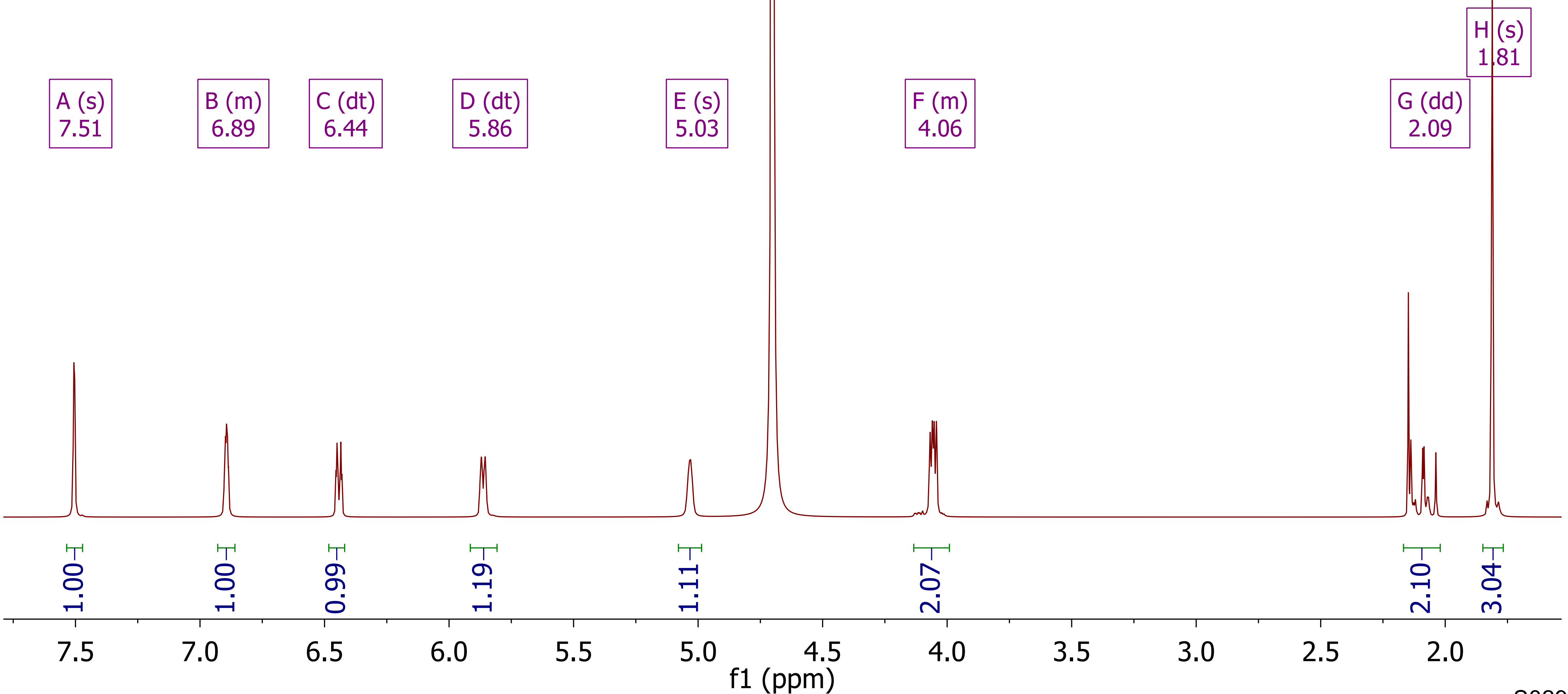
$^{31}P\{^1H\}$ NMR Spiking experiments demonstrate that P-$N_3$ (**35**) is decomposed in P-OH (**34**) over the time



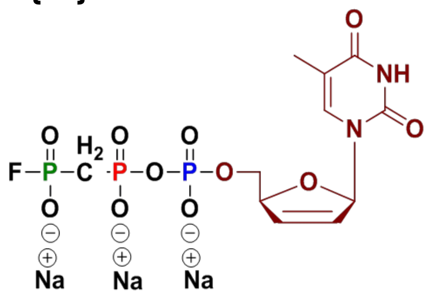
**$^1$H - NMR**



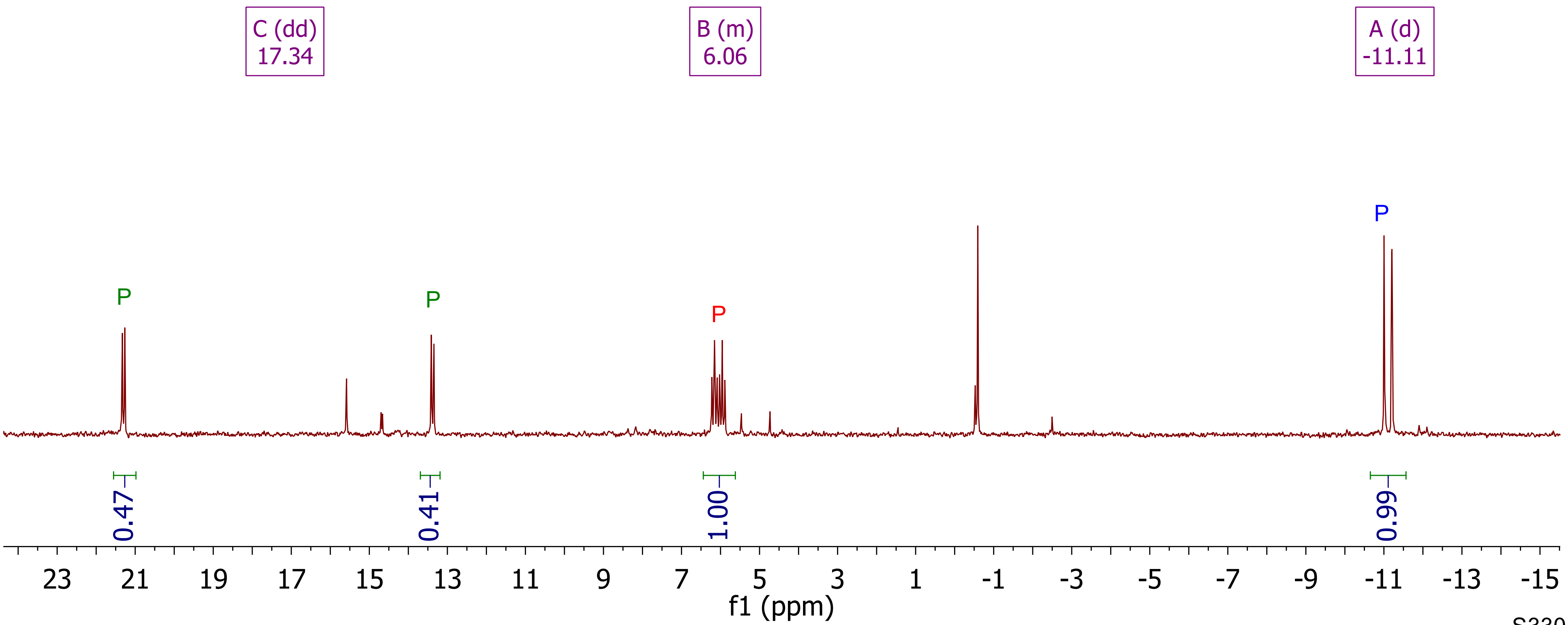

crude product after precipitation



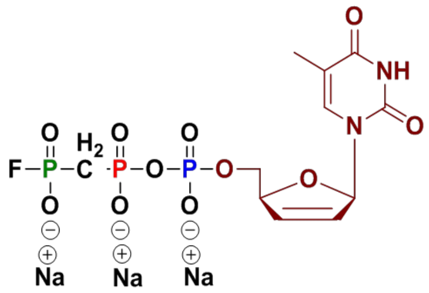
**$^{31}P\{^{1}H\}$ NMR**



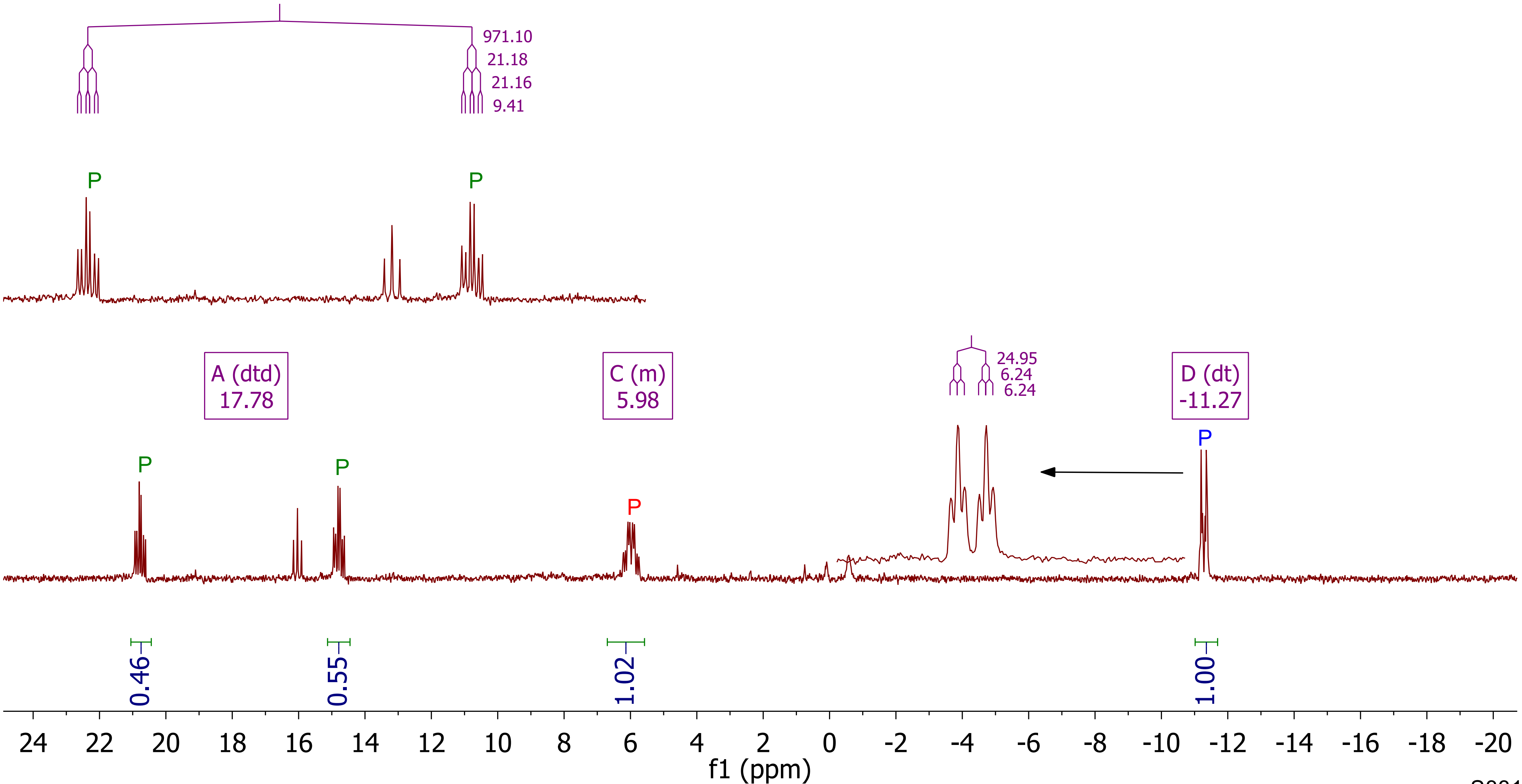

crude product after precipitation



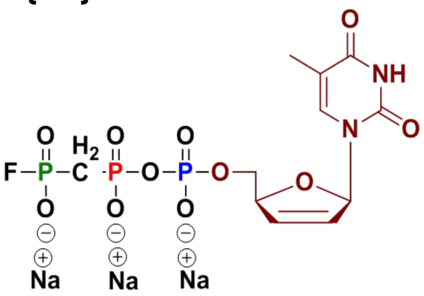
**$^{31}P$-$^{1}H$ NMR**



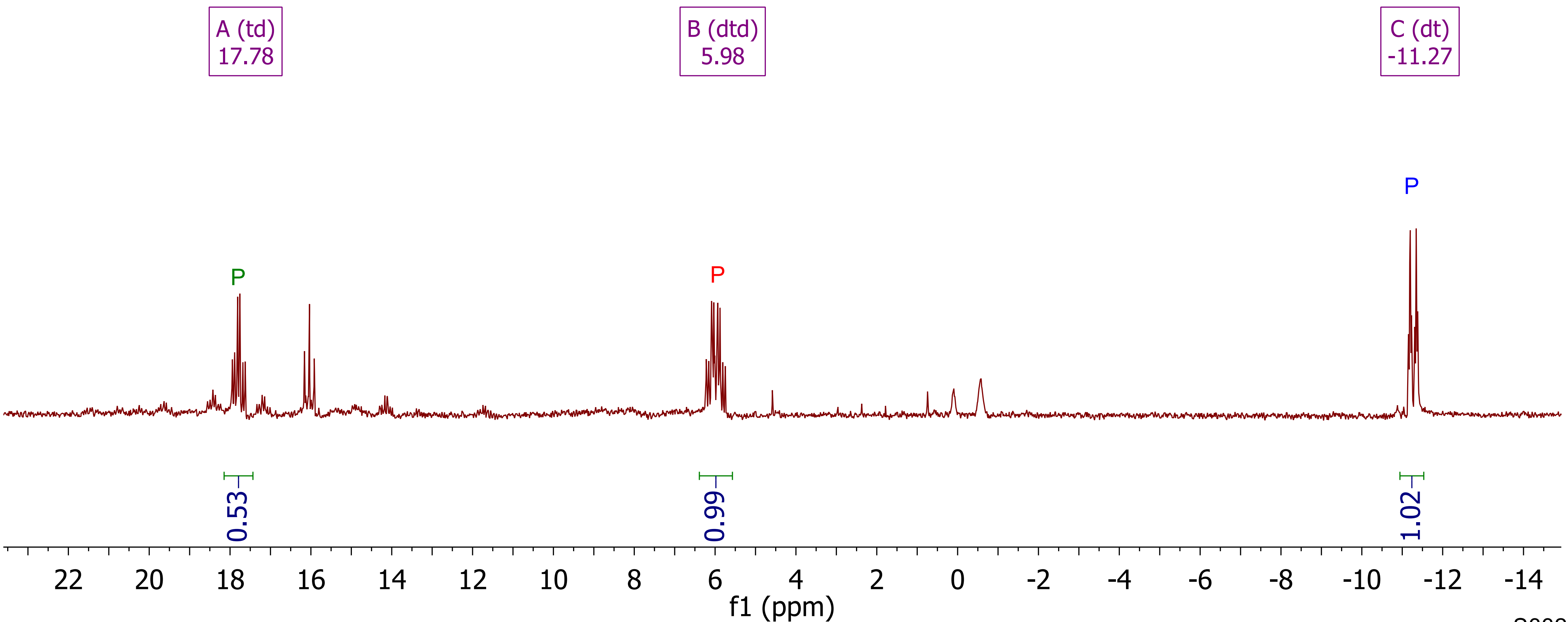

crude product after precipitation



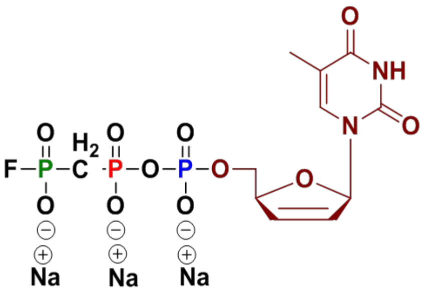
$^{31}P\{^{19}F\}$ NMR



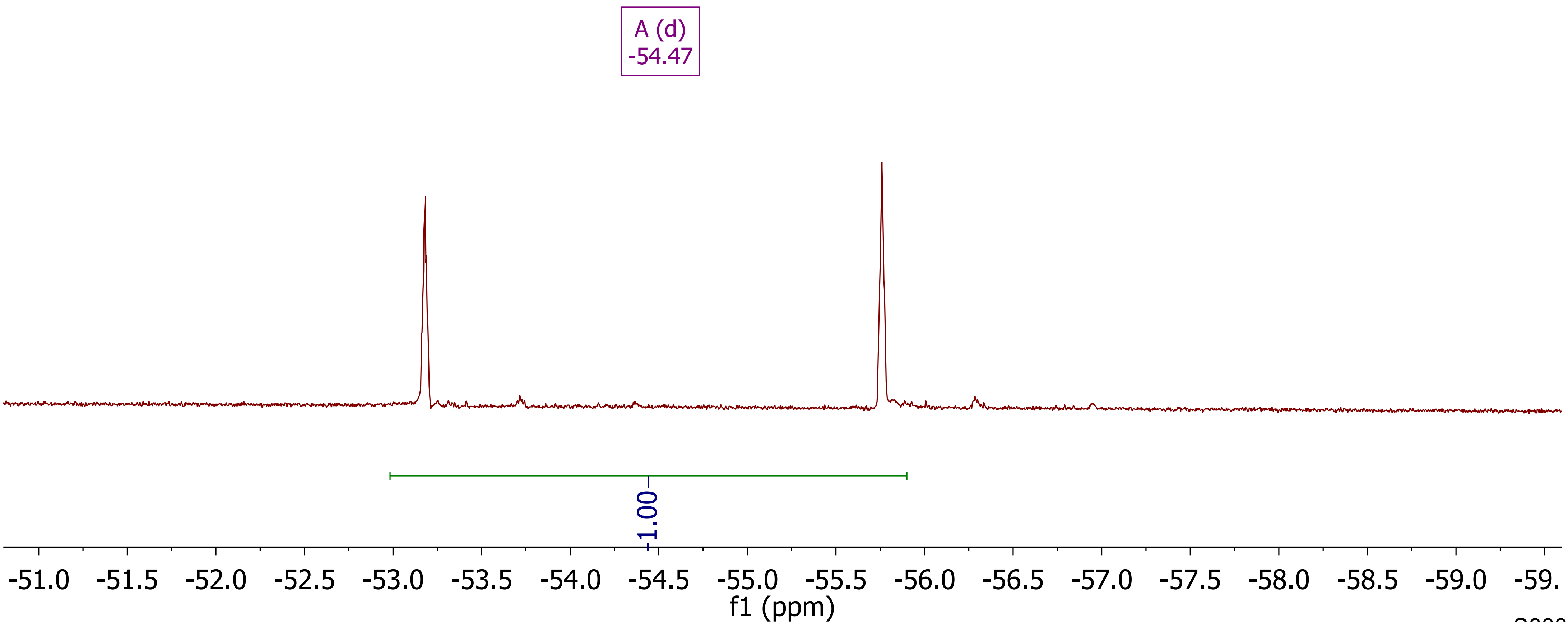
crude product after precipitation



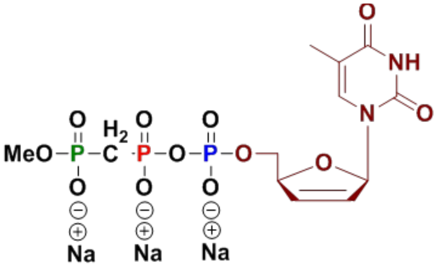
**$^{19}$F NMR**



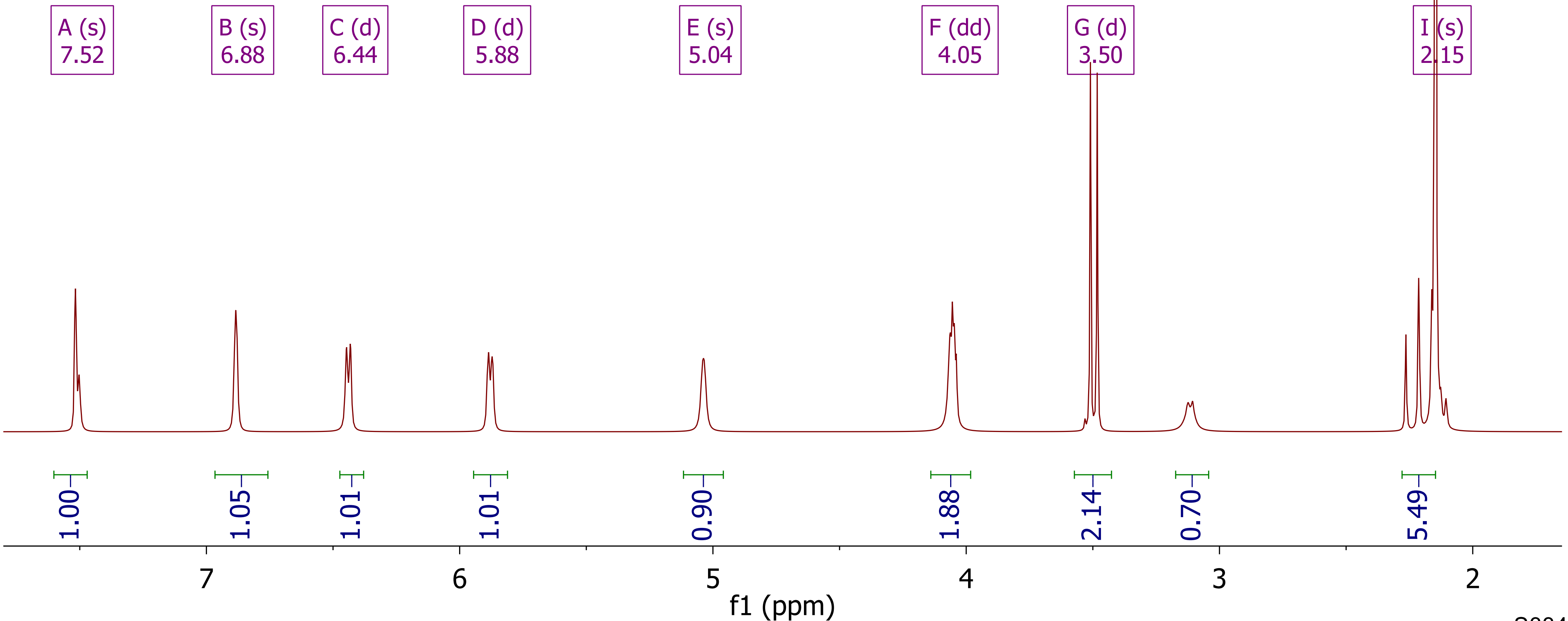
crude product after precipitation



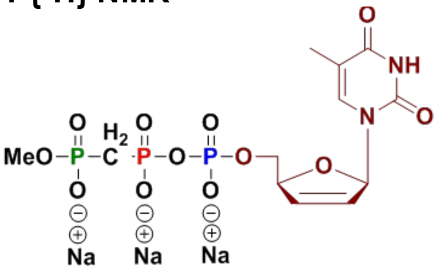
**$^1$H NMR**



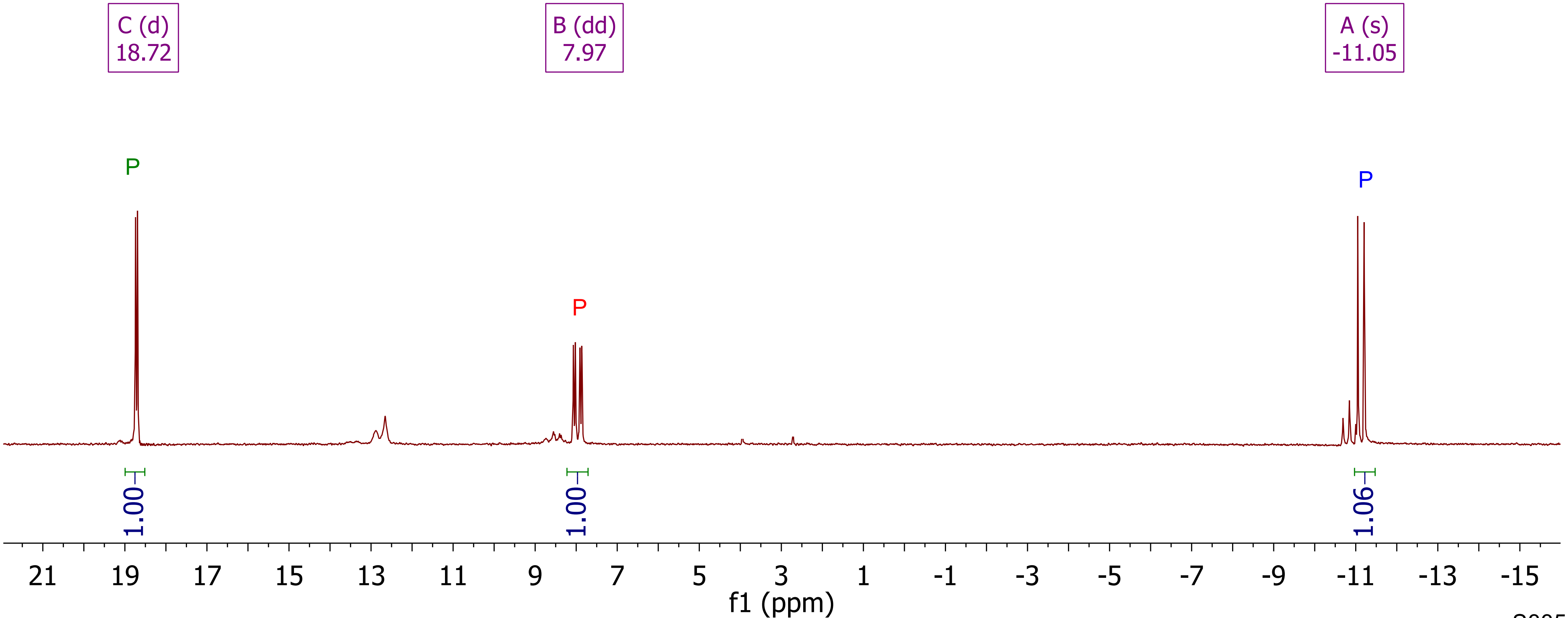
crude product after precipitation



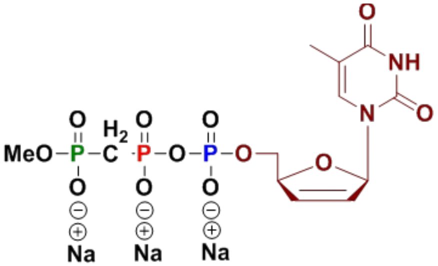
$^{31}P\{^1H\}$ NMR



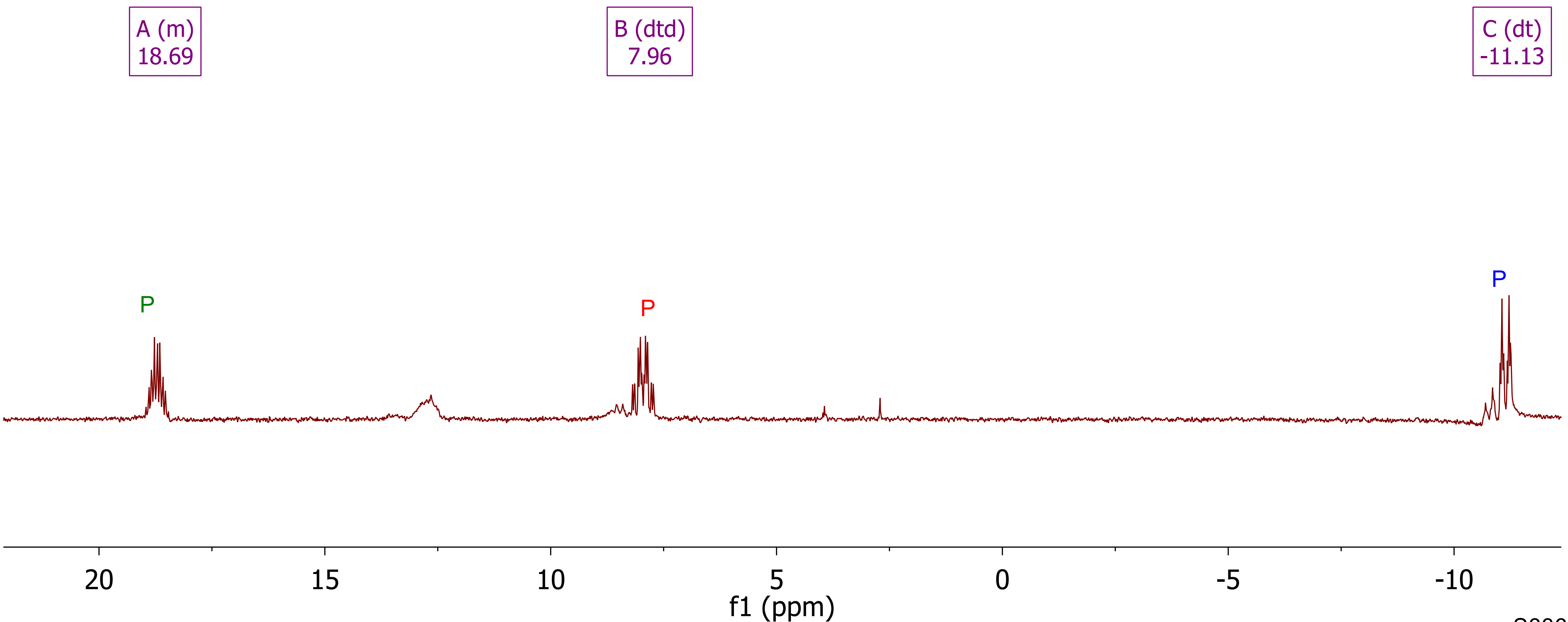

crude product after precipitation



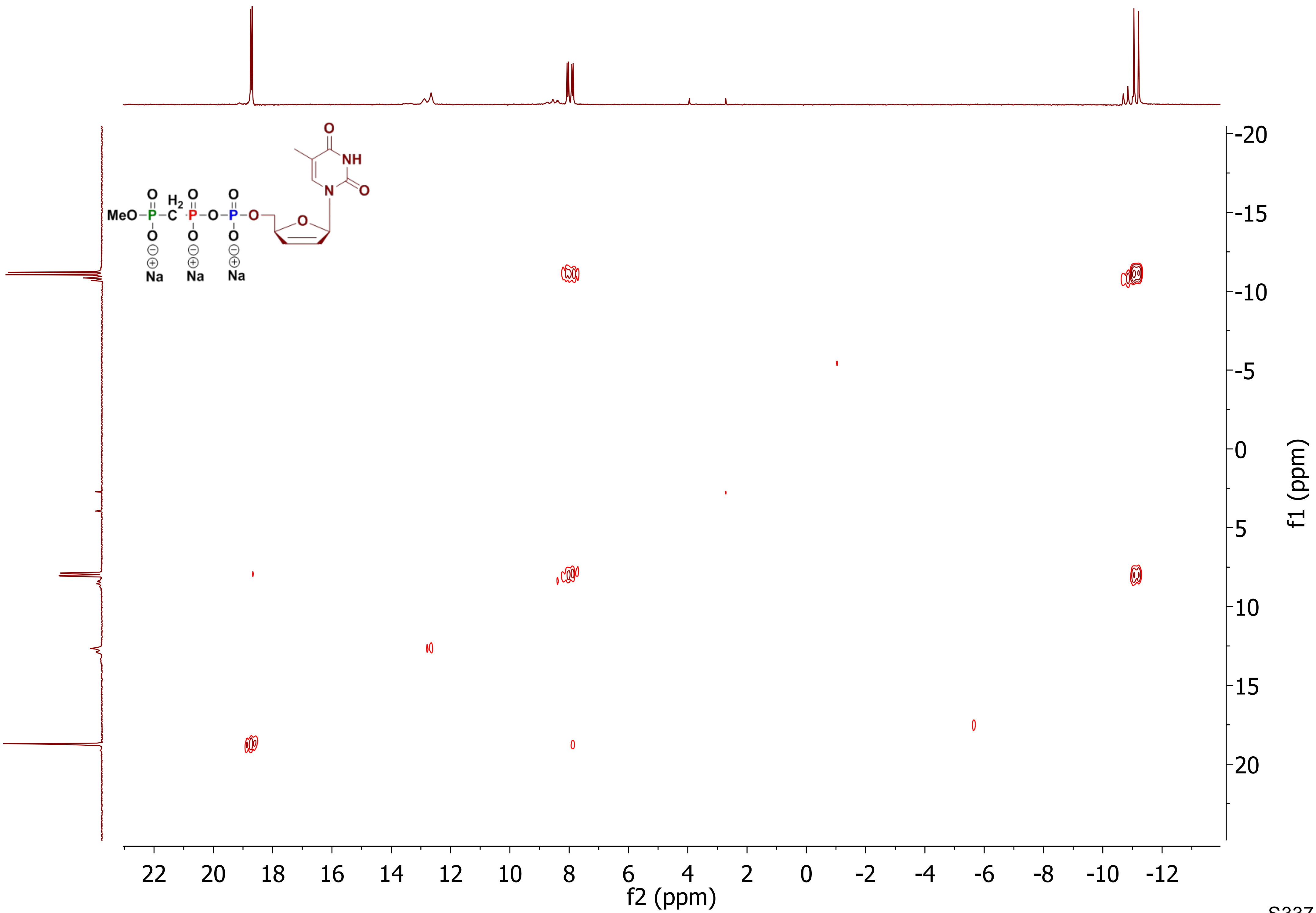
**$^{31}$P-$^{1}$H NMR**



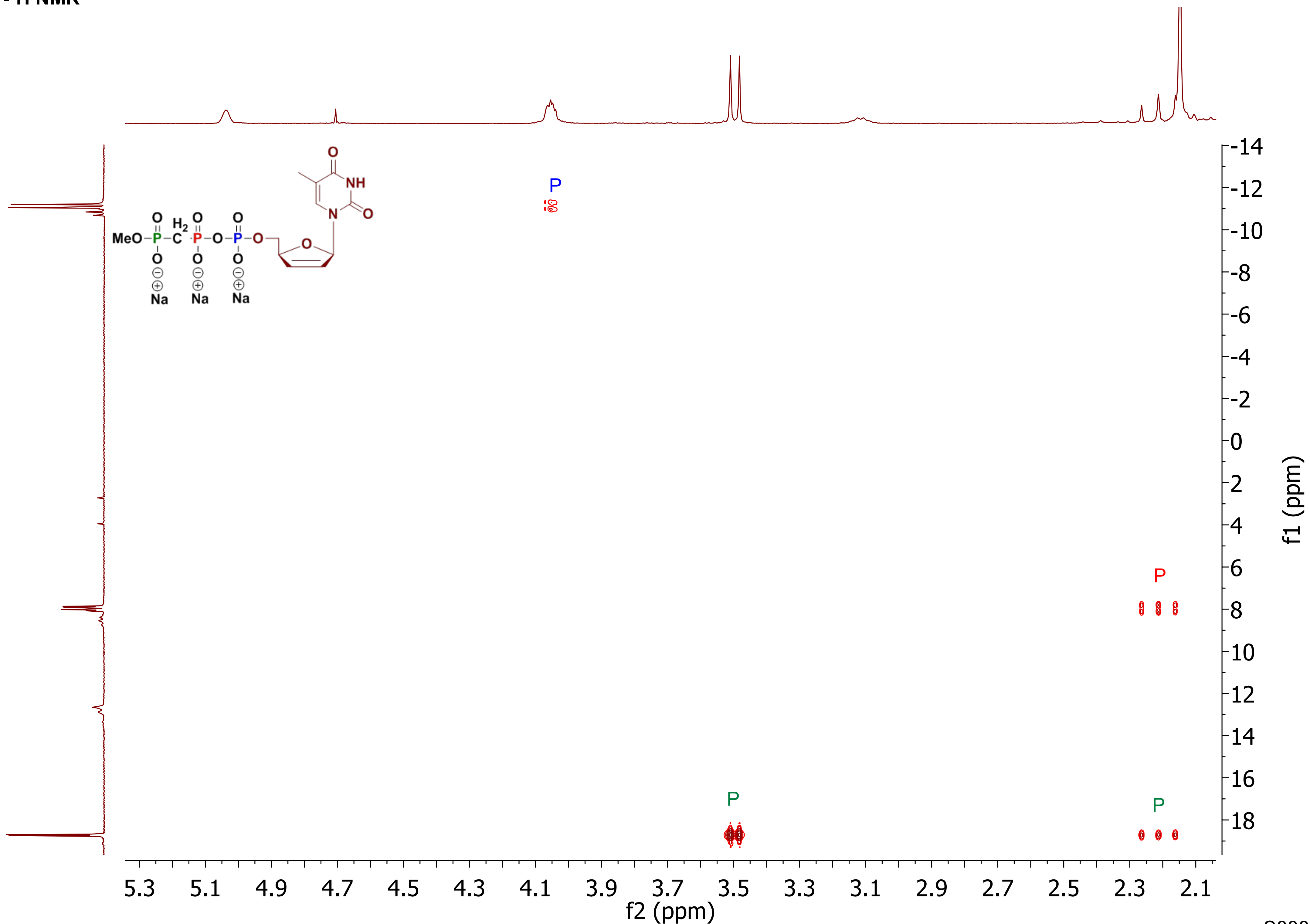
crude product after precipitation



$^{31}$P-$^{31}$P NMR

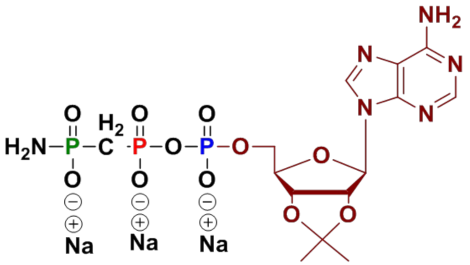

MeO
Na
NH
O
N
f2 (ppm)
f1 (ppm)
22 20 18 16 14 12 10 8 6 4 2 0 -2 -4 -6 -8 -10 -12
-20 -15 -10 -5 0 5 10 15 20

$^{31}$P-$^{1}$H NMR

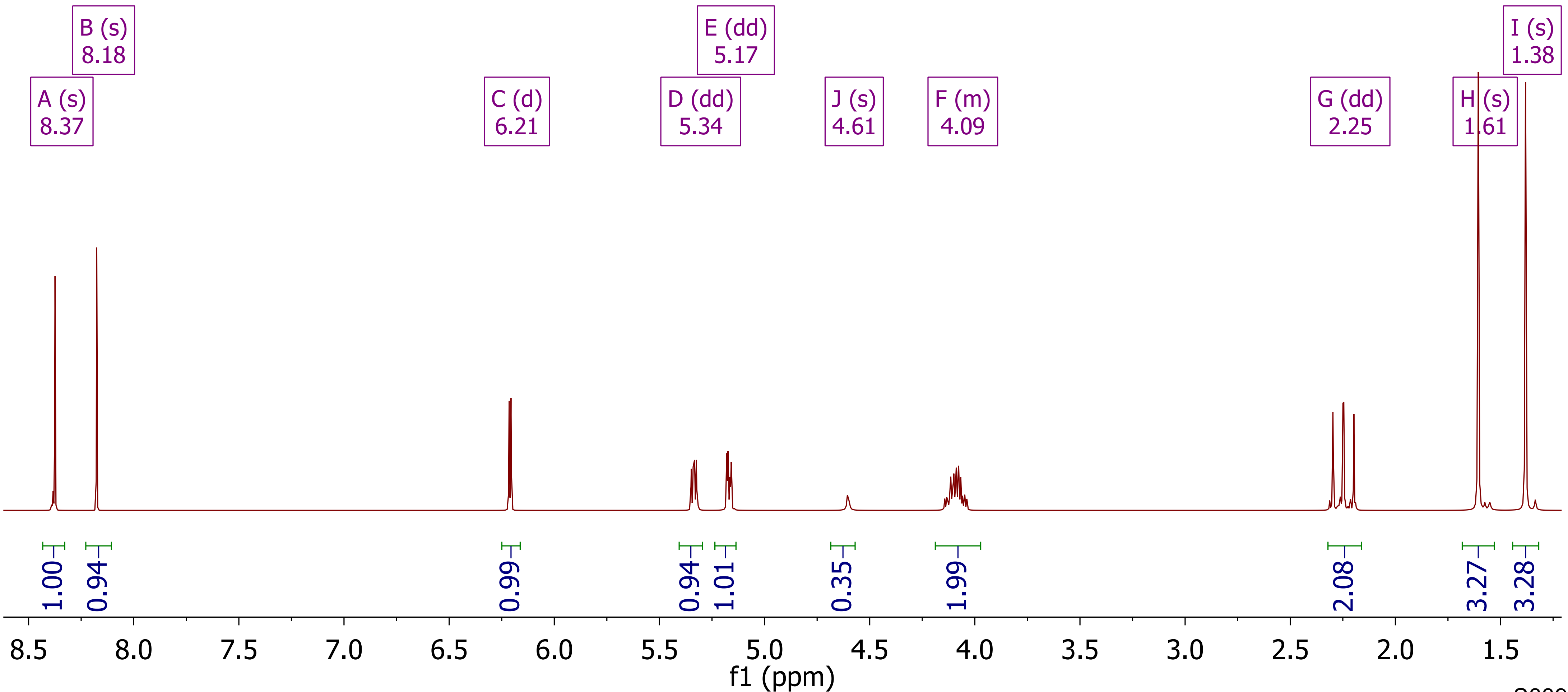


MeO
H2
Na
Na
Na
NH
P
P
P
P
5.3
5.1
4.9
4.7
4.5
4.3
4.1
3.9
3.7
3.5
3.3
3.1
2.9
2.7
2.5
2.3
2.1
f2 (ppm)
-14
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
f1 (ppm)

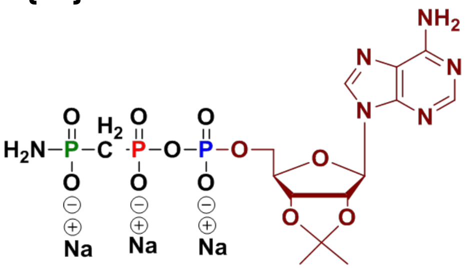
$^1$H NMR



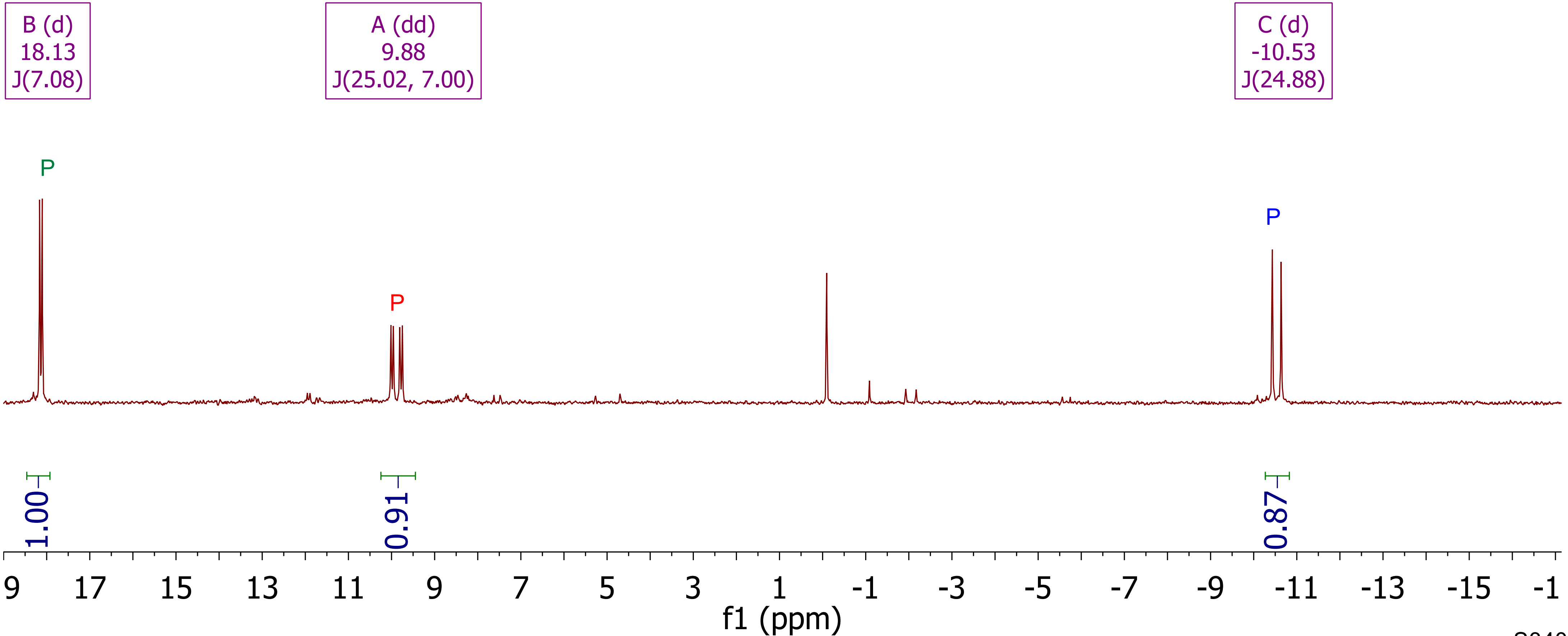
crude product after precipitation



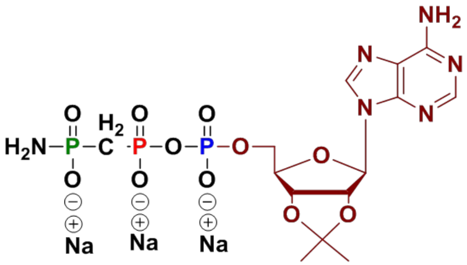
$^{31}P\{^{1}H\}$ NMR



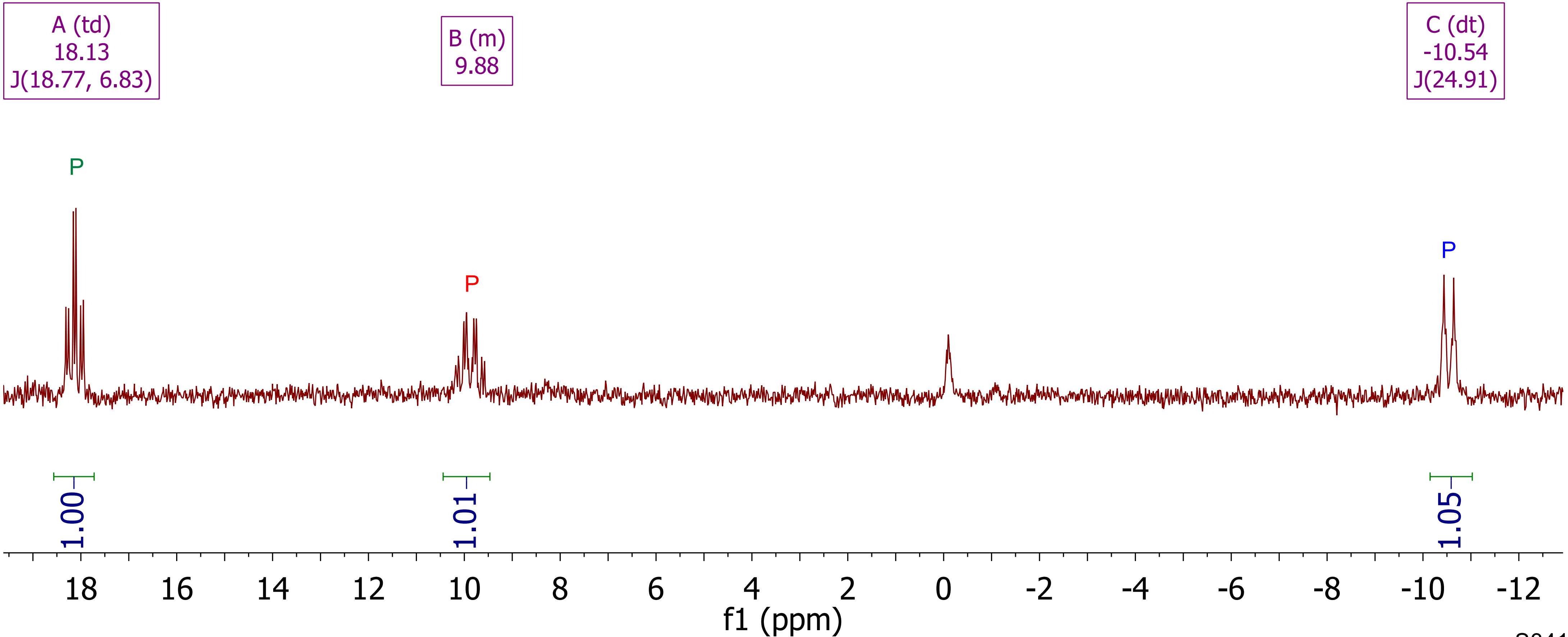
crude product after precipitation



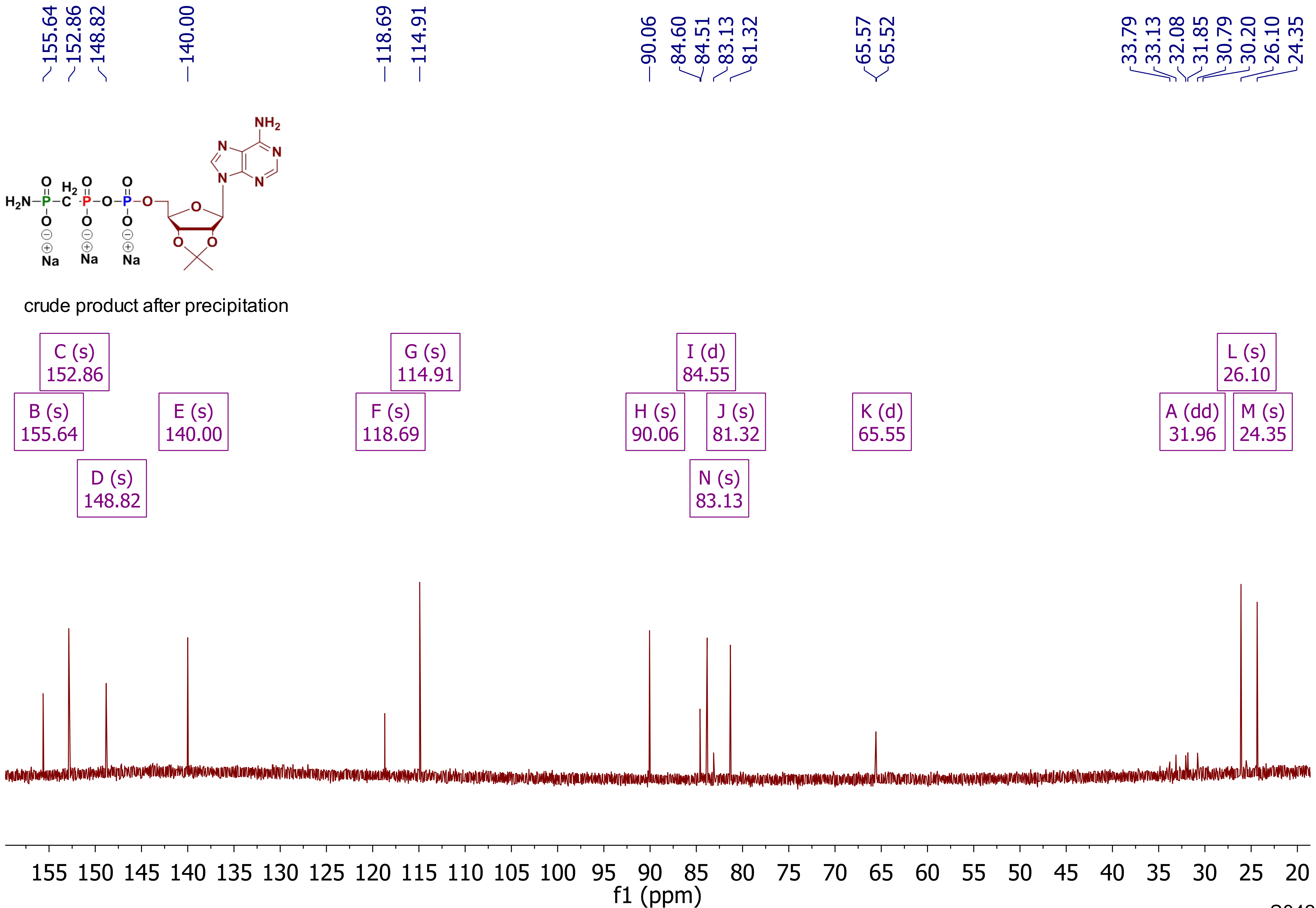
**$^{31}$P NMR**



crude product after precipitation

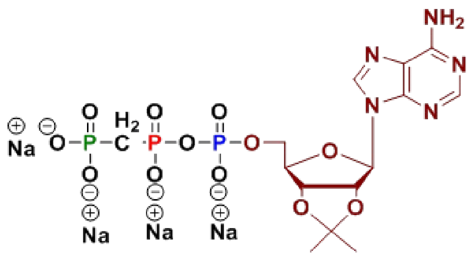


## $^{13}$C NMR

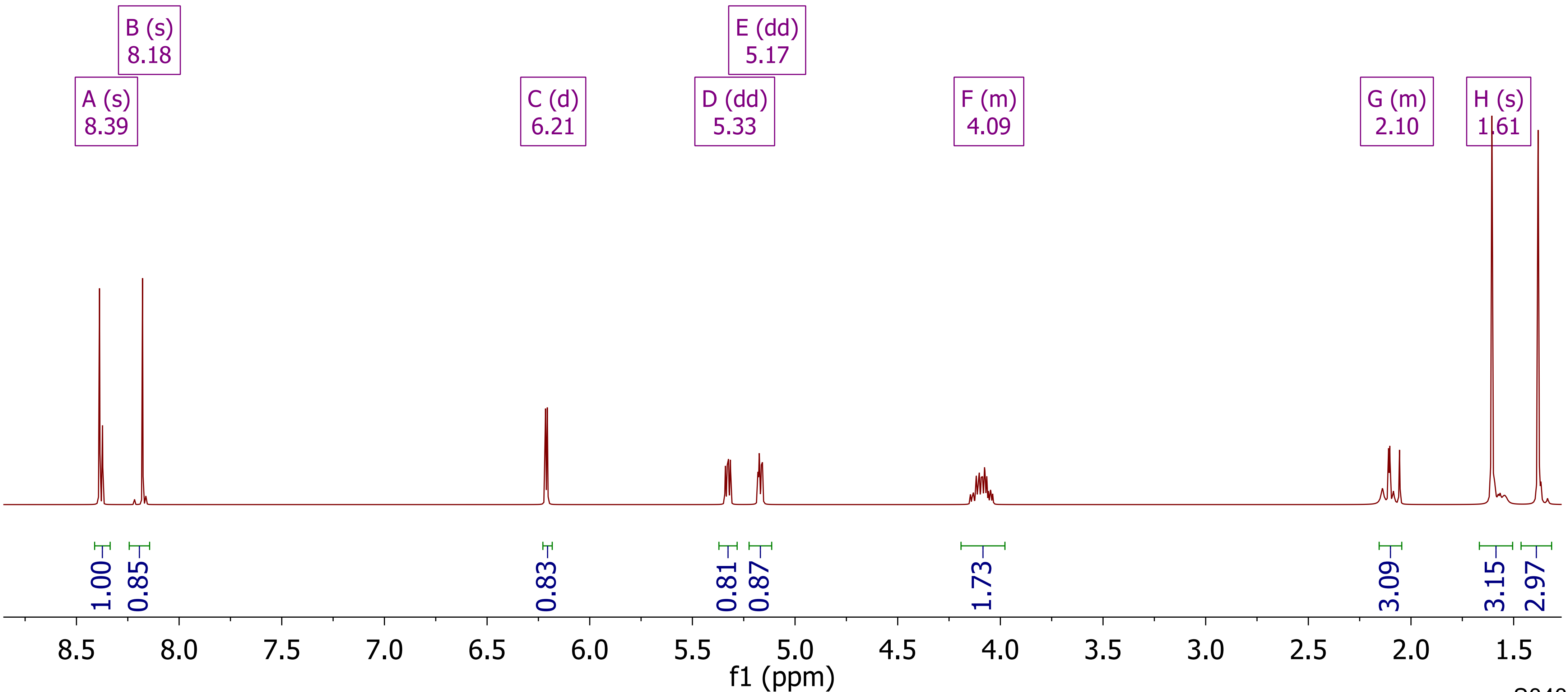



crude product after precipitation

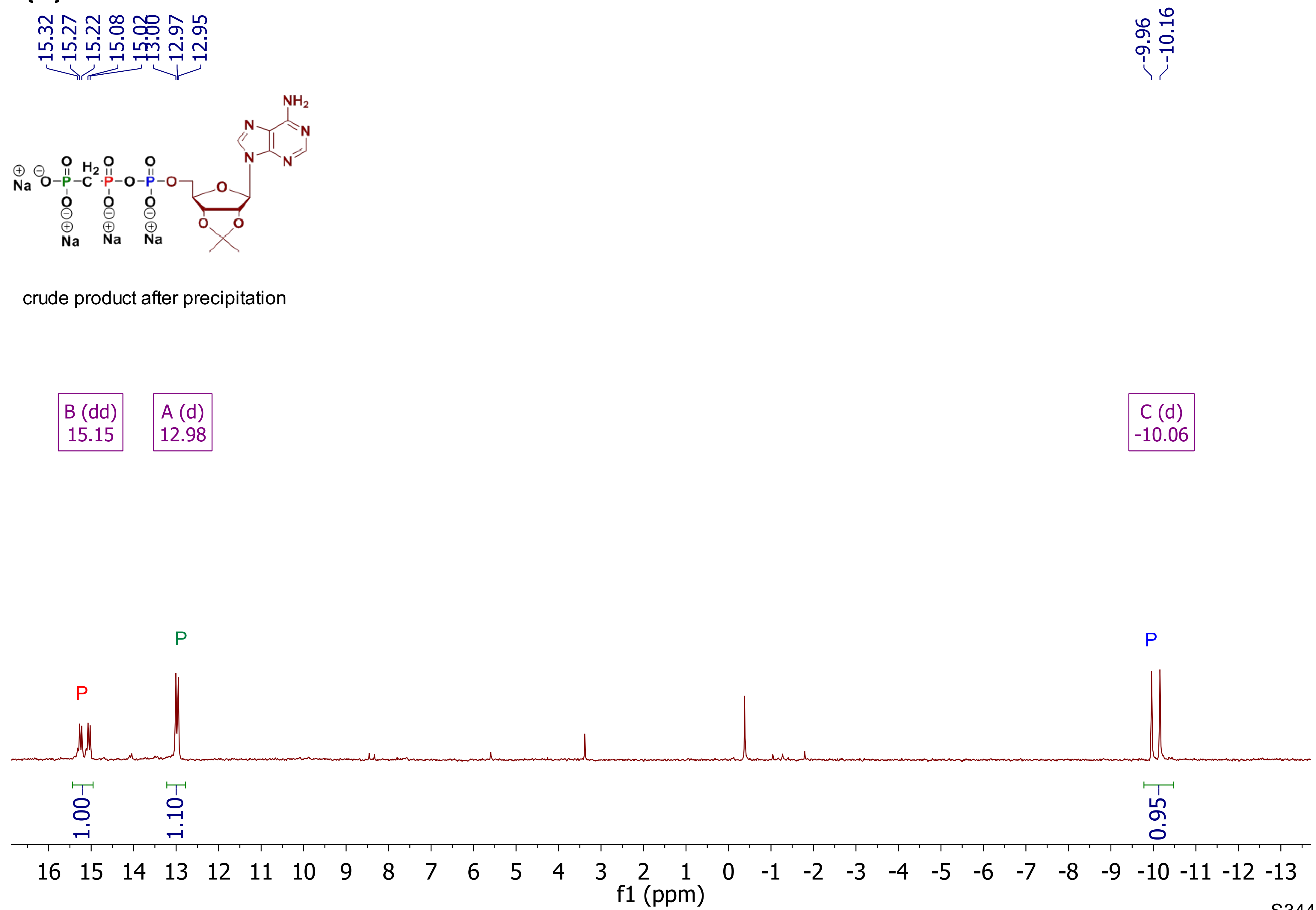
$^1$H NMR



crude product after precipitation

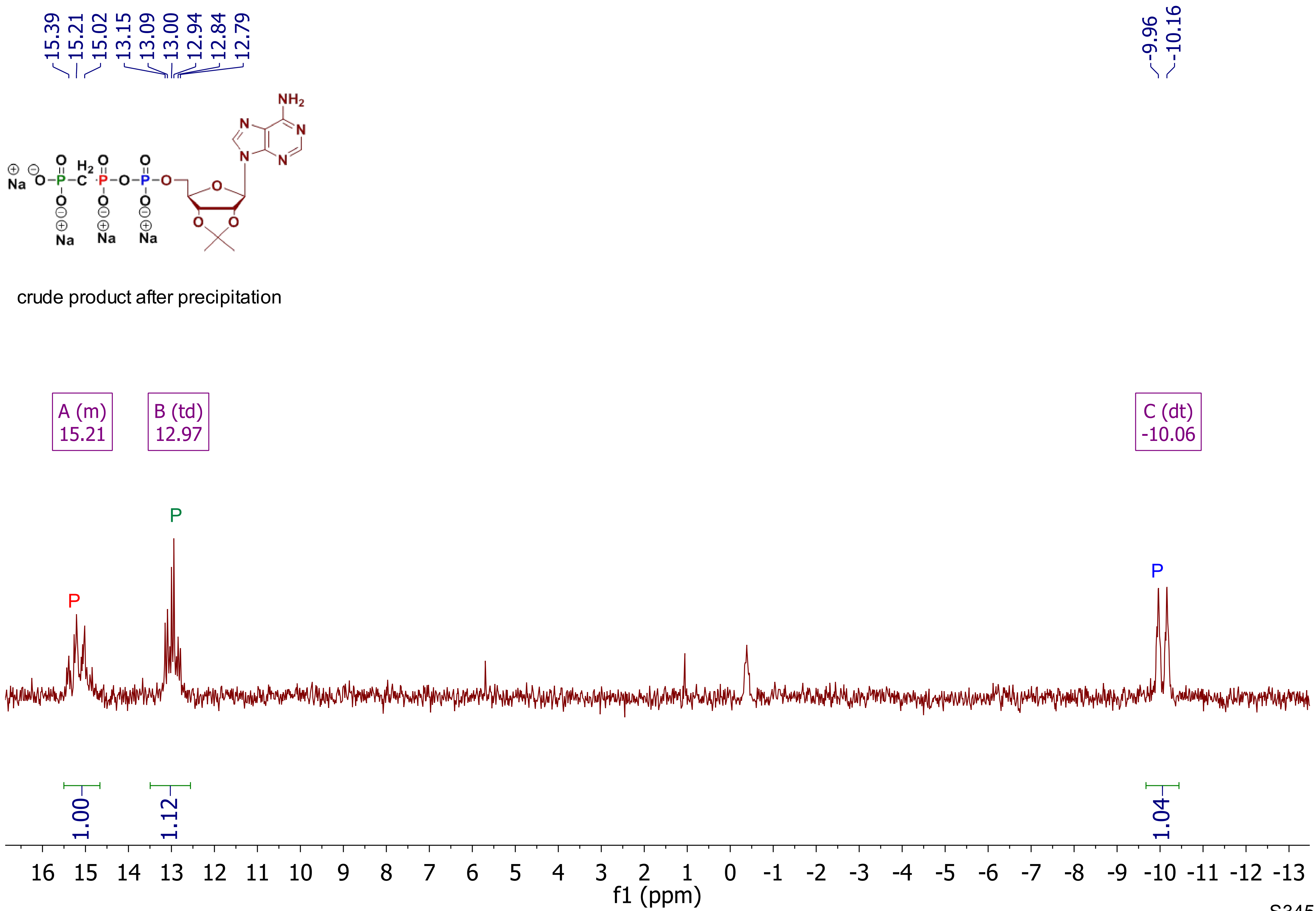


$^{31}P\{^1H\}$ NMR



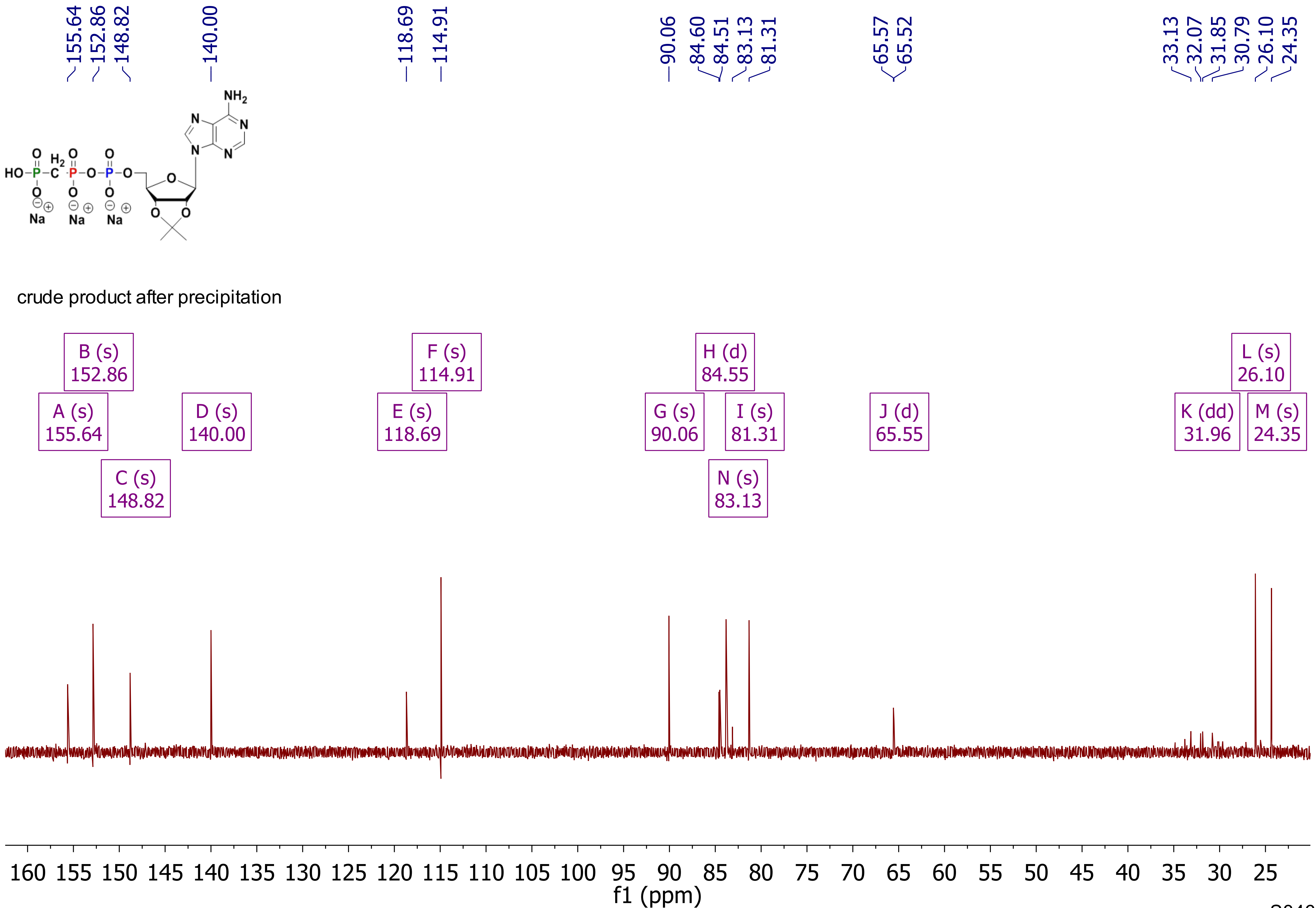
15.32
15.27
15.22
15.08
15.02
13.00
12.97
12.95
-9.96
-10.16
Na
Na
Na
Na
NH2
crude product after precipitation
B (dd)
15.15
A (d)
12.98
C (d)
-10.06
P
P
P
1.00
1.10
0.95
f1 (ppm)

$^{31}P$ NMR

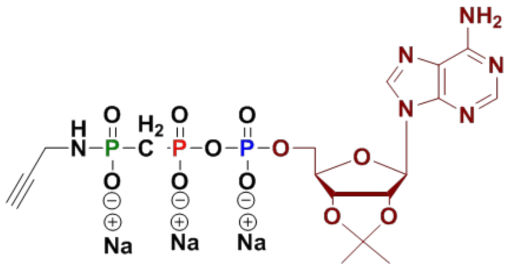


15.39
15.21
15.02
13.15
13.09
13.00
12.94
12.84
12.79
-9.96
-10.16
NH2
N
O
Na
P
H2
C
crude product after precipitation
A (m)
15.21
B (td)
12.97
C (dt)
-10.06
1.00
1.12
1.04
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13
f1 (ppm)

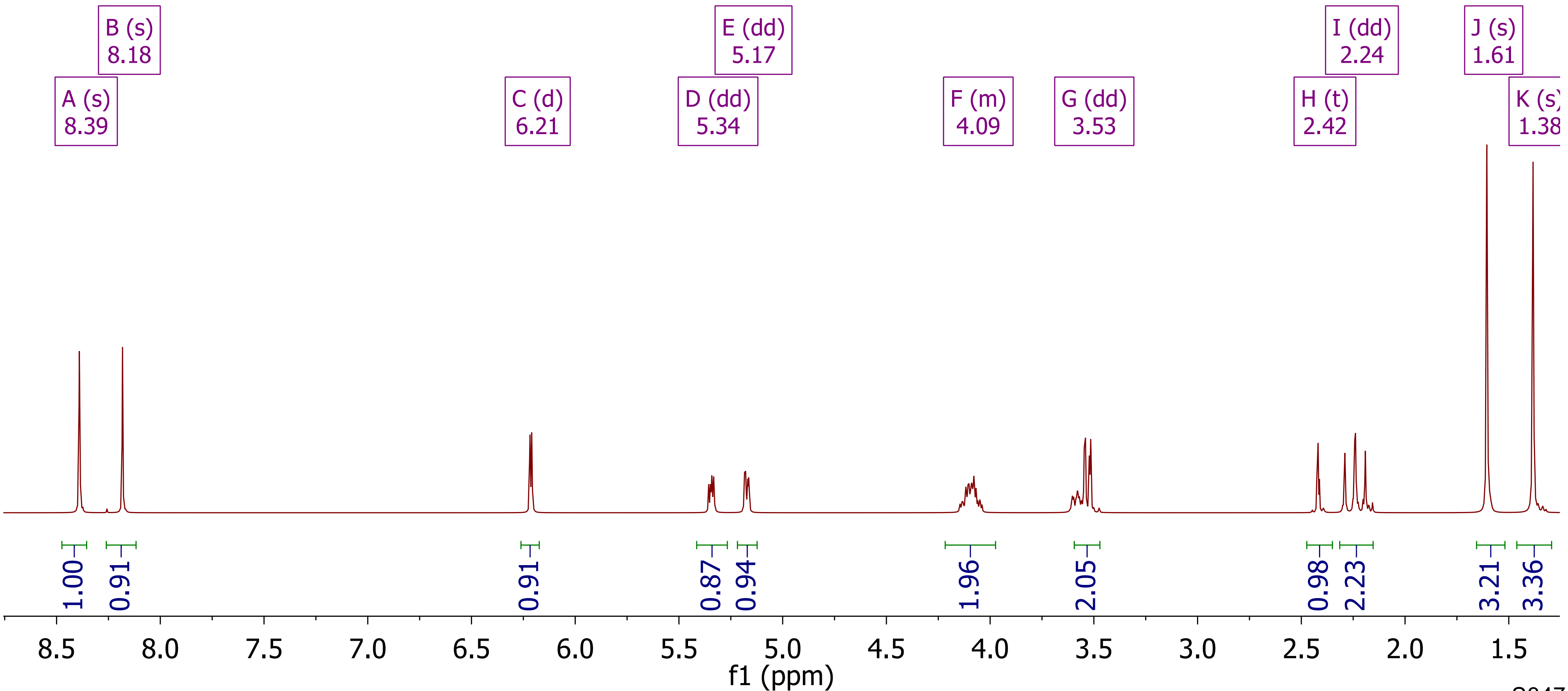
**$^{13}$C NMR**


155.64
152.86
148.82
140.00
118.69
114.91
90.06
84.60
84.51
83.13
81.31
65.57
65.52
33.13
32.07
31.85
30.79
26.10
24.35
crude product after precipitation
A (s) 155.64
B (s) 152.86
C (s) 148.82
D (s) 140.00
E (s) 118.69
F (s) 114.91
G (s) 90.06
H (d) 84.55
I (s) 81.31
N (s) 83.13
J (d) 65.55
K (dd) 31.96
L (s) 26.10
M (s) 24.35
f1 (ppm)

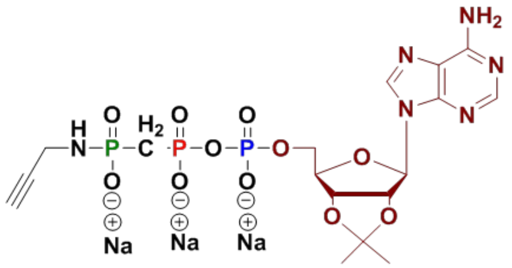
**$^1$H NMR**



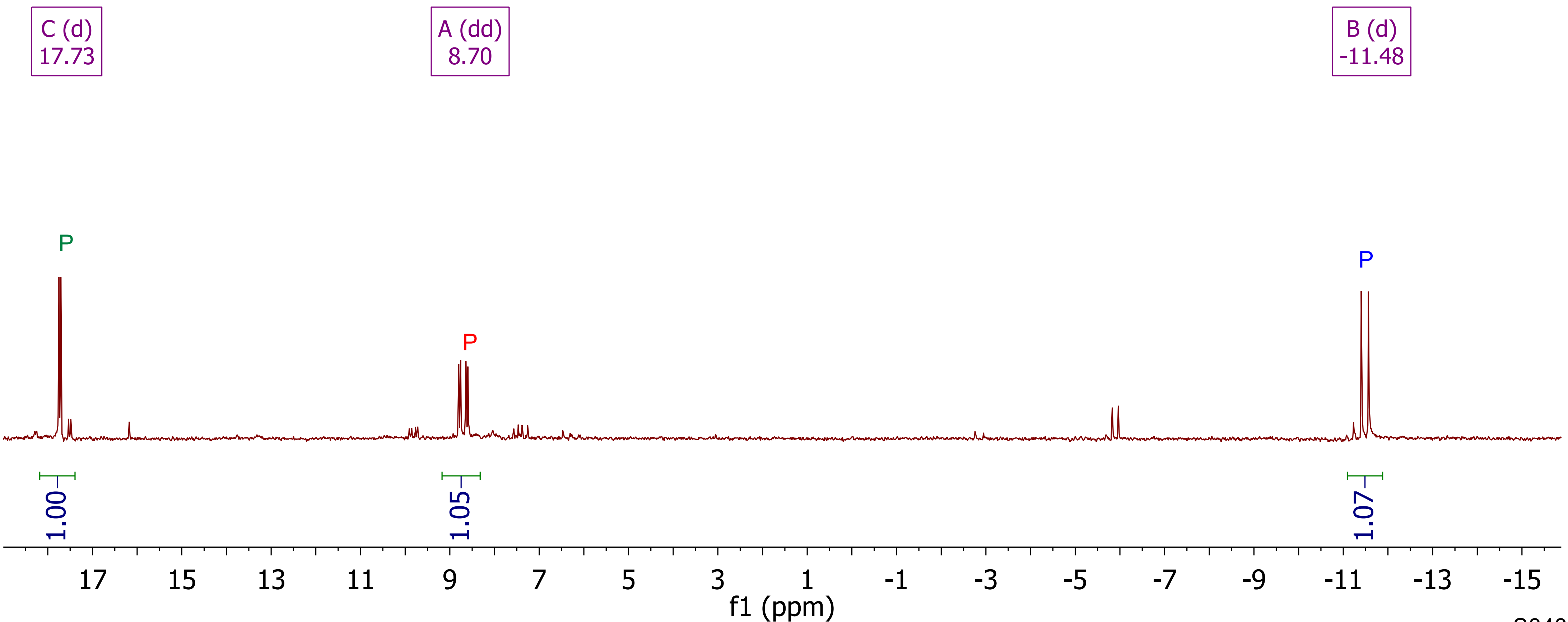
crude product after precipitation



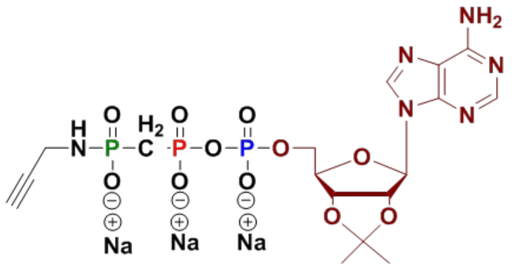
**$^{31}P\{^1H\}$ NMR**



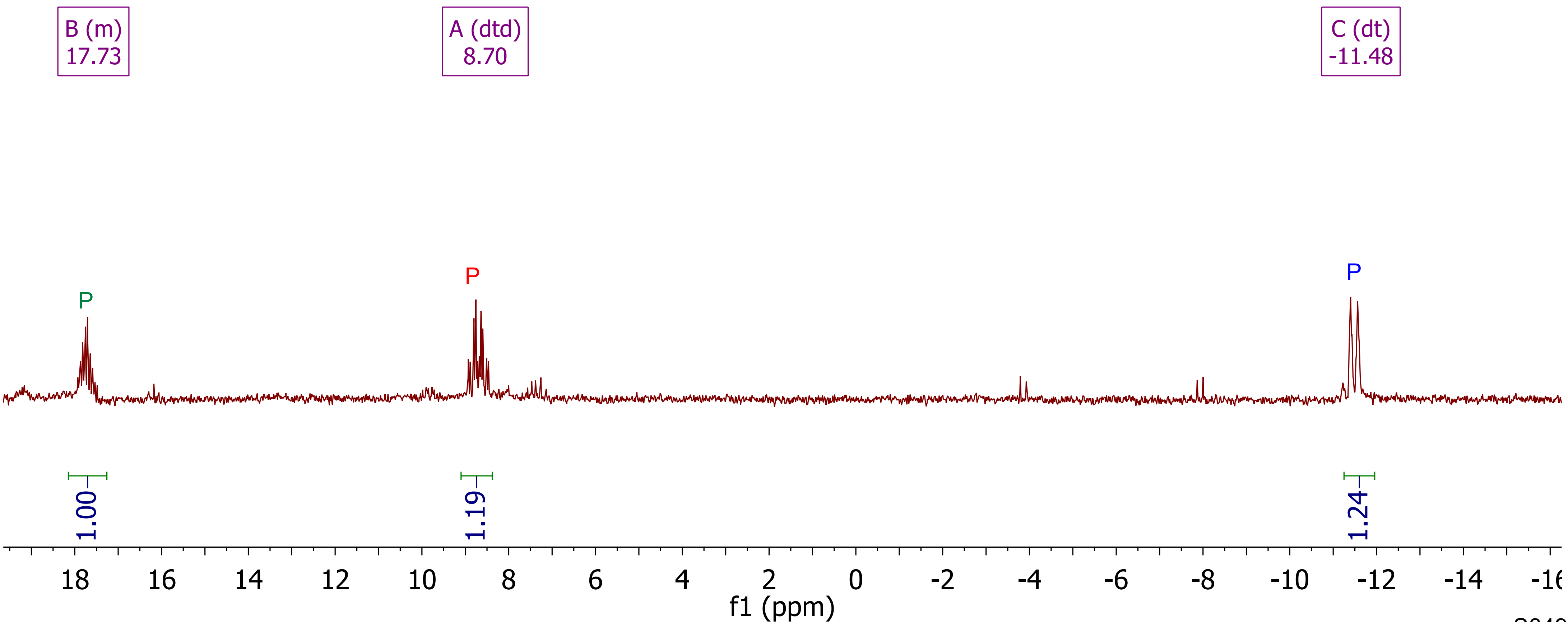
crude product after precipitation



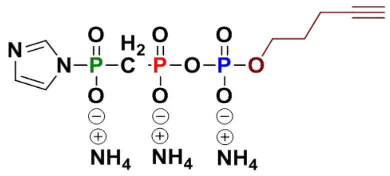
$^{31}$P NMR



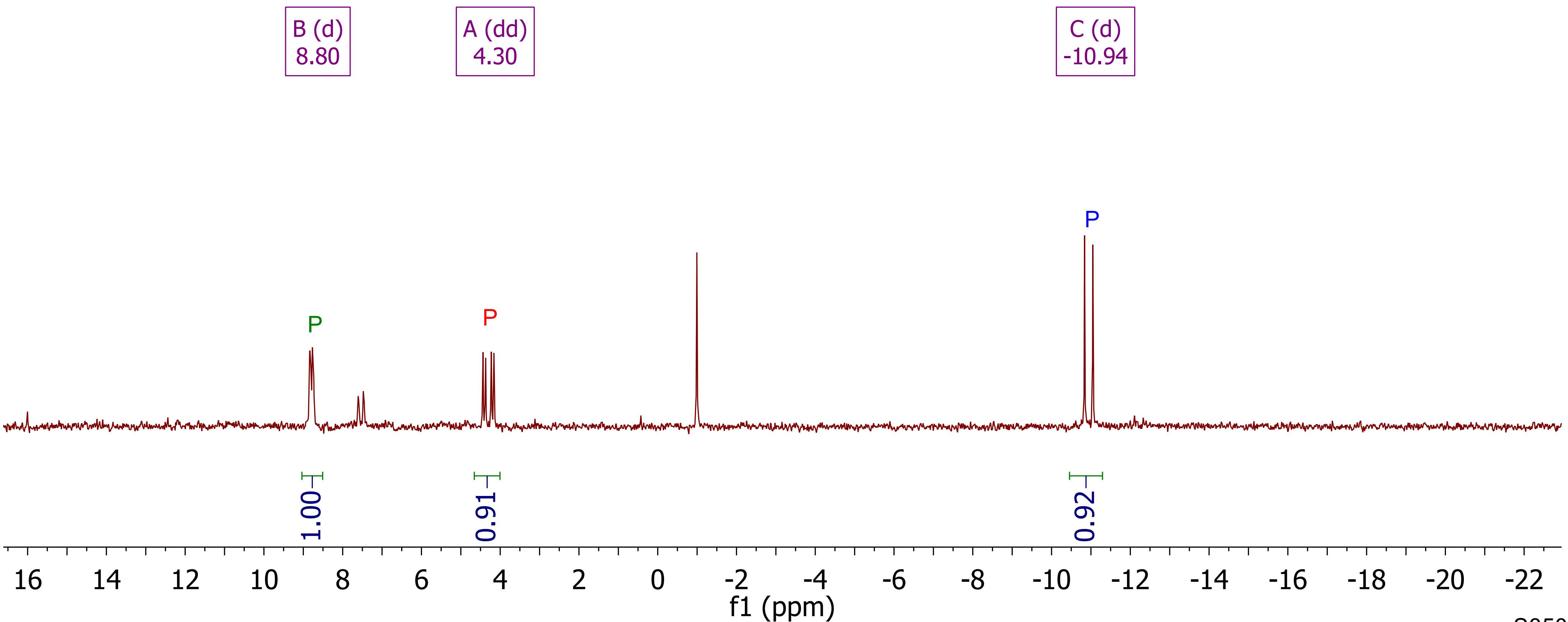
crude product after precipitation



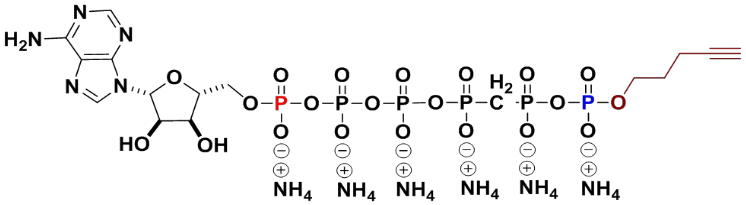
**$^{31}P\{^1H\}$ NMR**



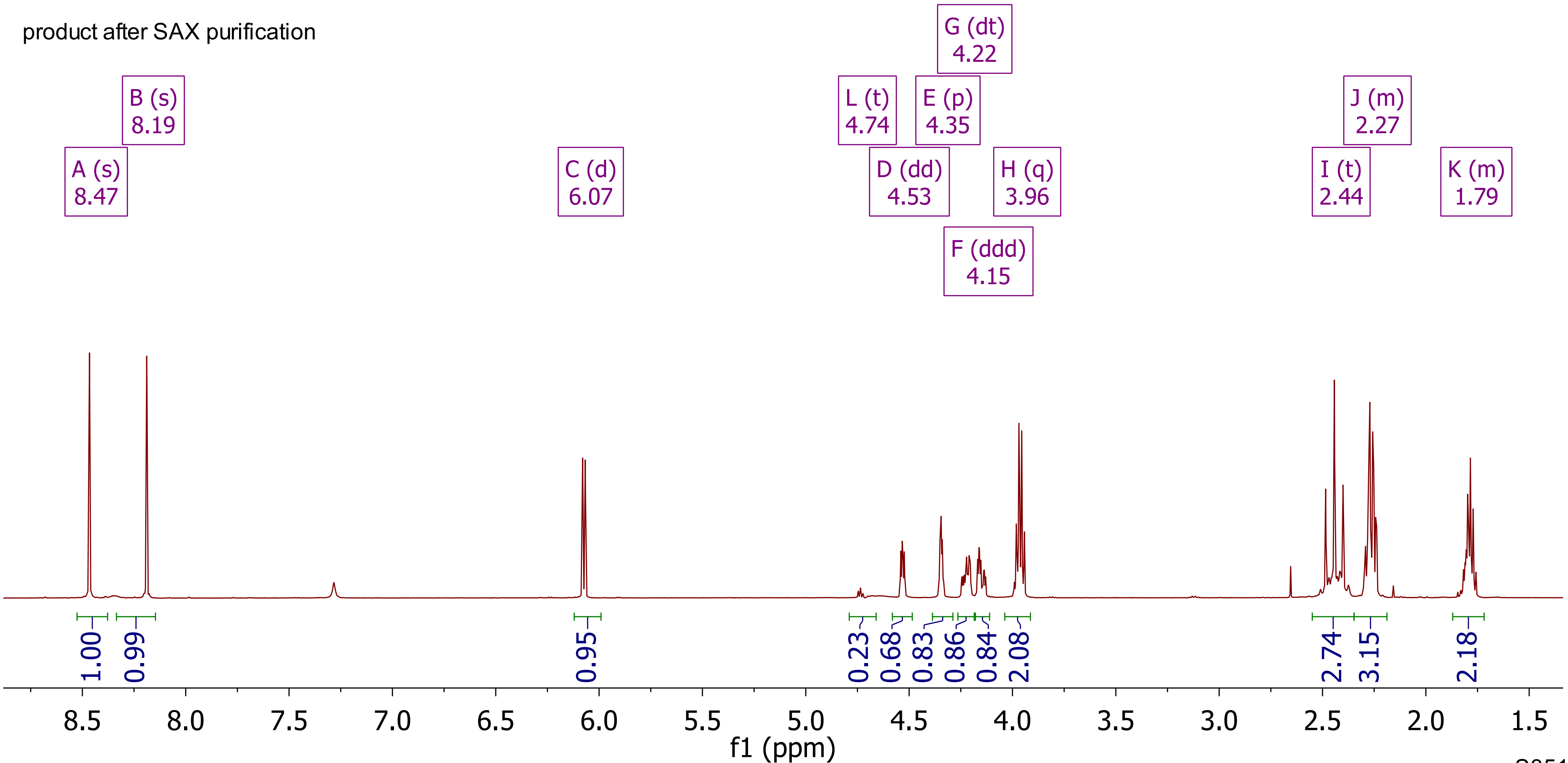

quantat. in reaction mixture



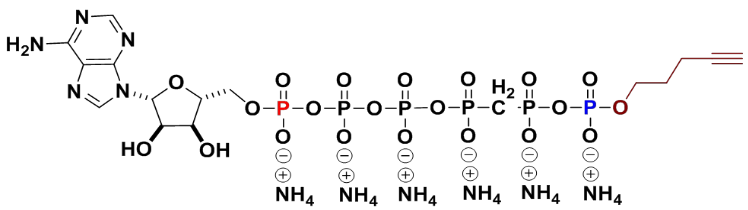
$^{1}$H NMR

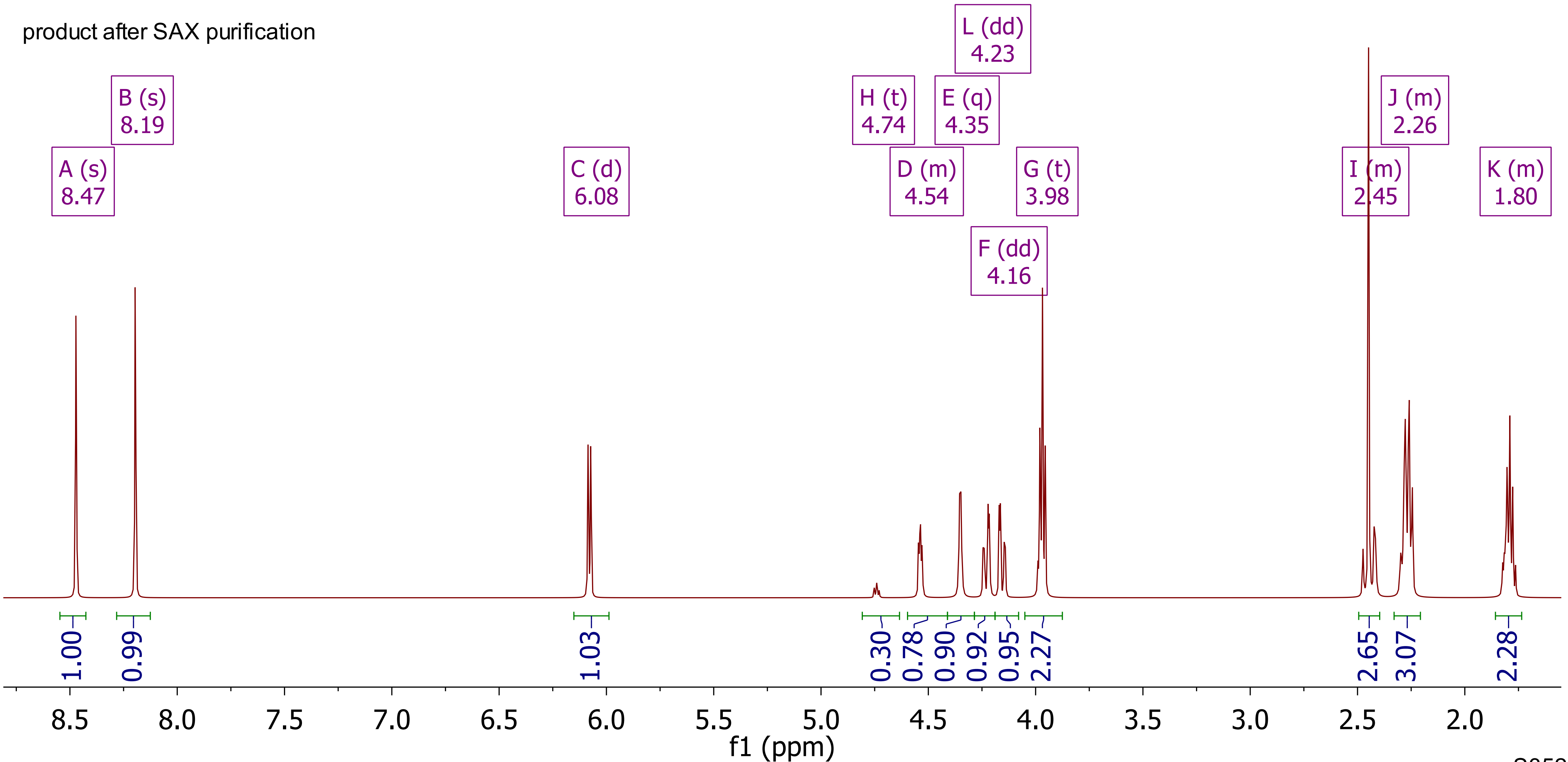


product after SAX purification



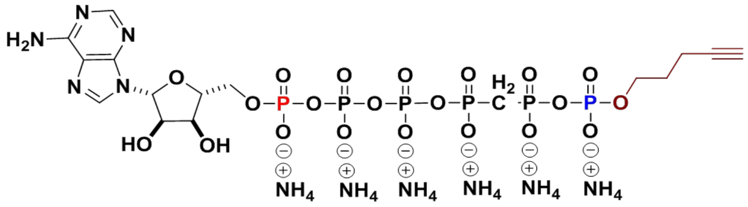
$^1H\{^{31}P\}$ NMR



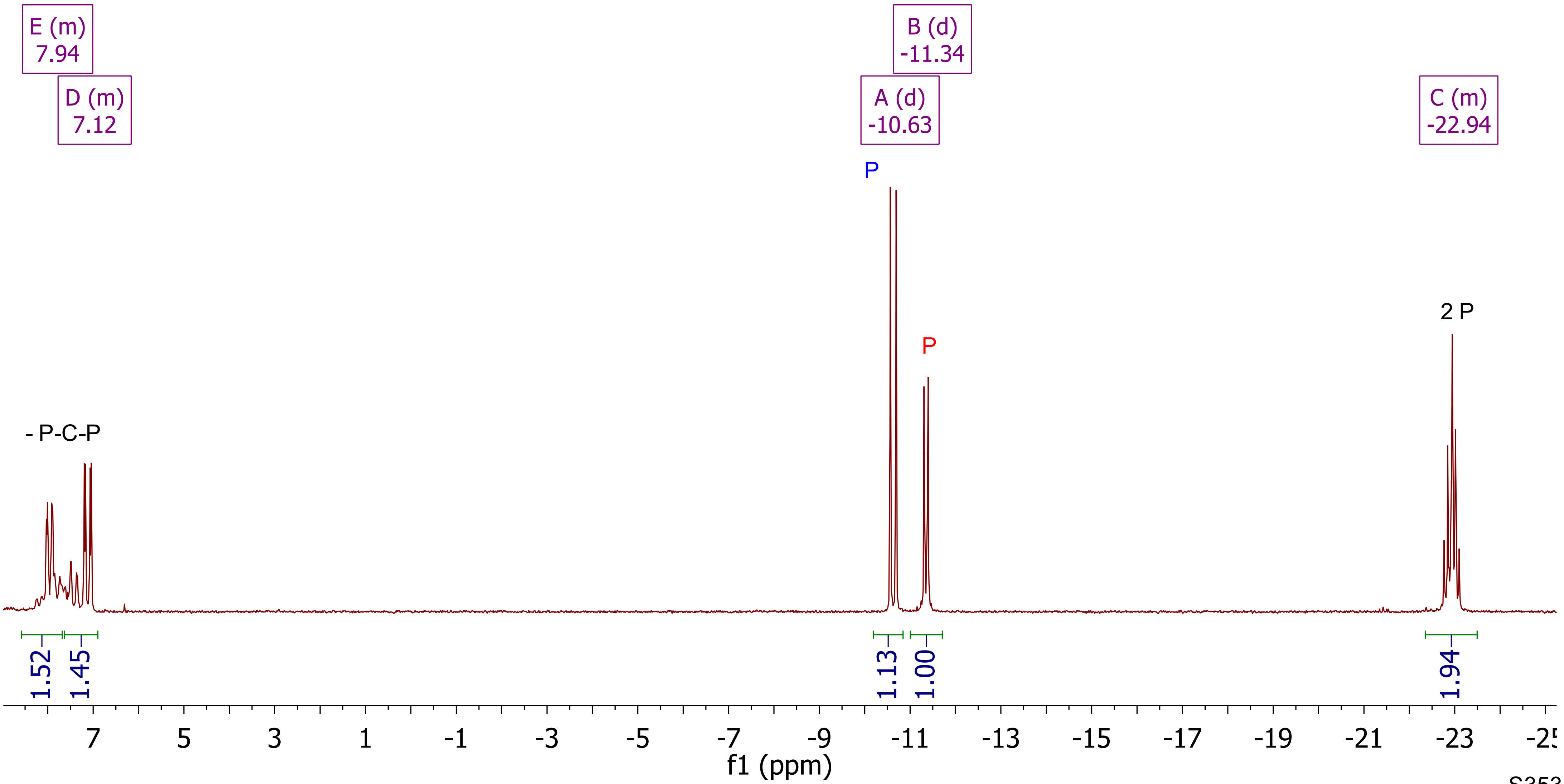


product after SAX purification
A (s) 8.47
B (s) 8.19
C (d) 6.08
H (t) 4.74
D (m) 4.54
E (q) 4.35
L (dd) 4.23
F (dd) 4.16
G (t) 3.98
I (m) 2.45
J (m) 2.26
K (m) 1.80
1.00
0.99
1.03
0.30
0.78
0.90
0.92
0.95
2.27
2.65
3.07
2.28
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

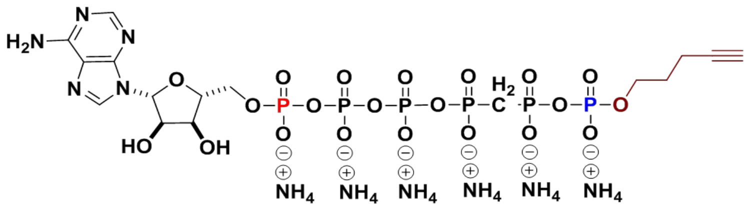
**$^{31}P\{^1H\}$ NMR**



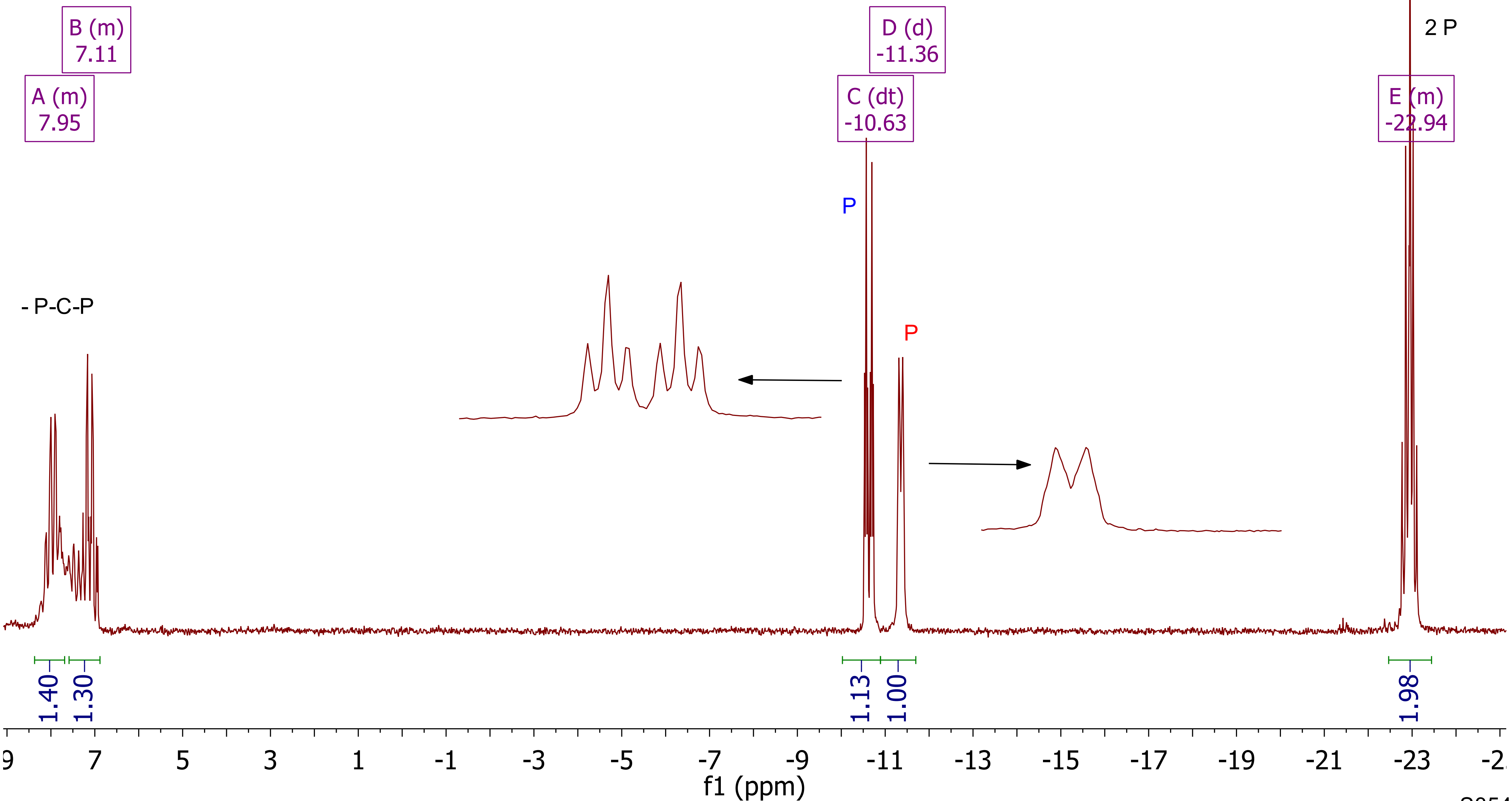
product after SAX purification



**$^{31}$P NMR**

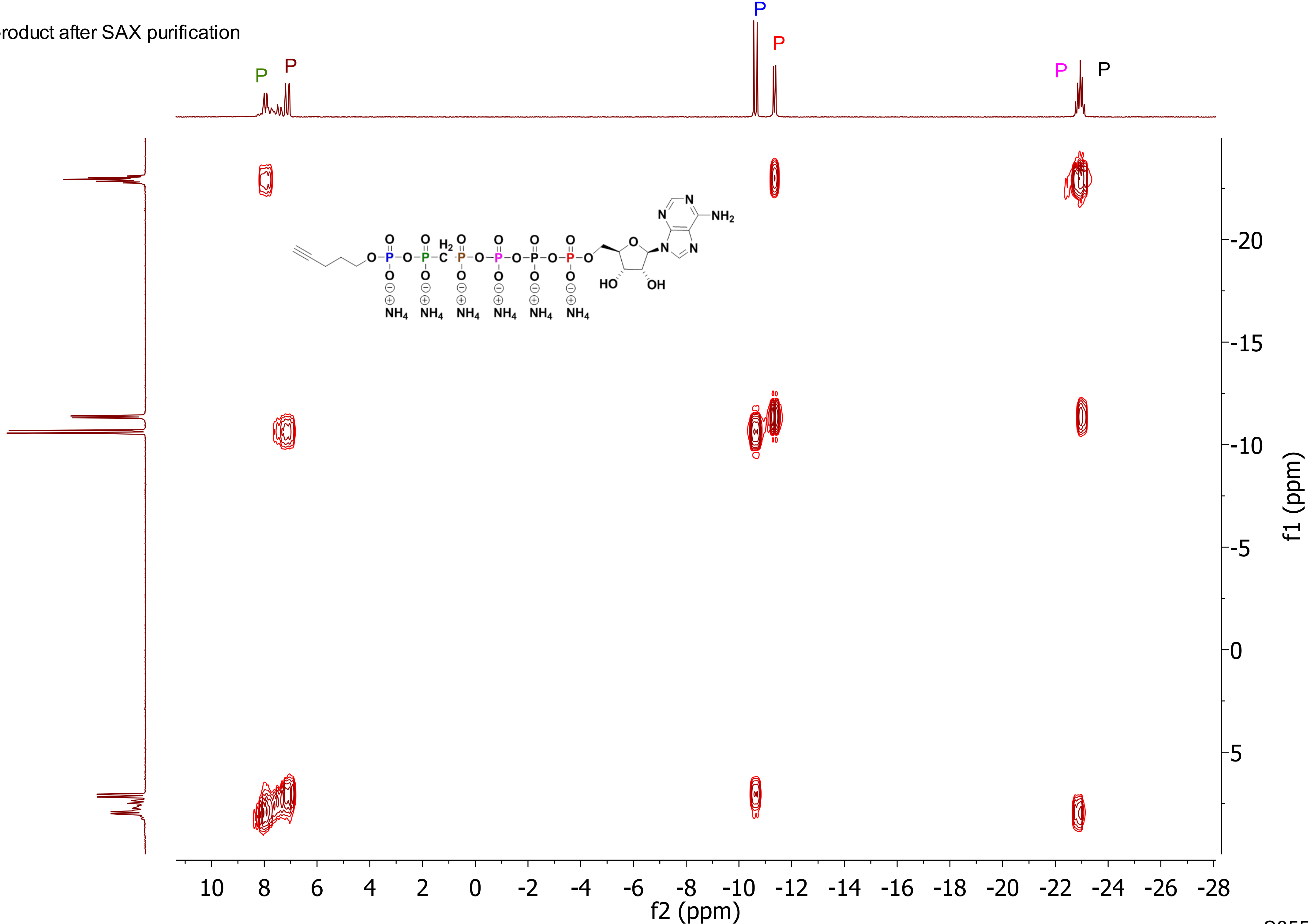


product after SAX purification



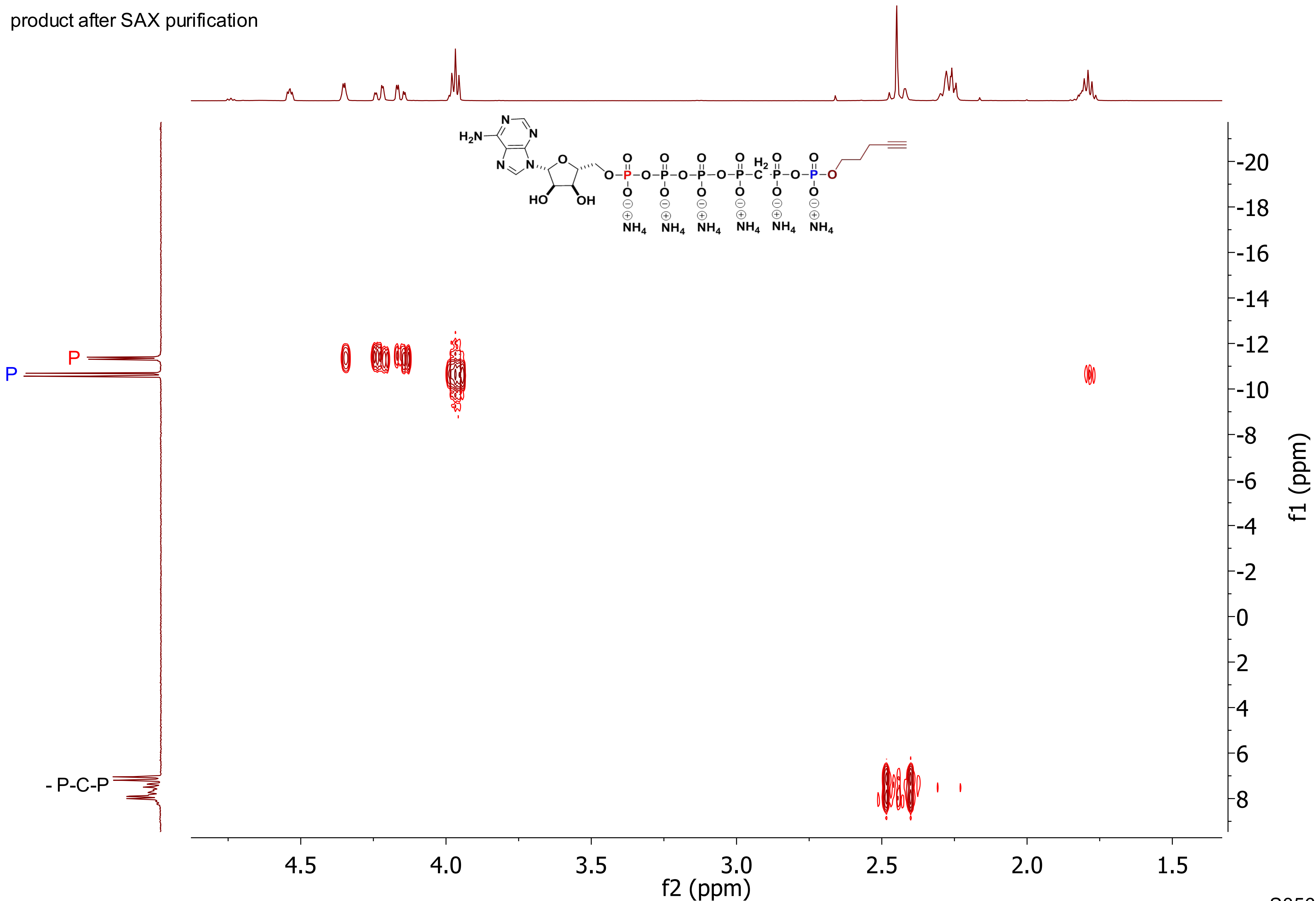
**$^{31}$P-$^{31}$P COSY NMR**

product after SAX purification

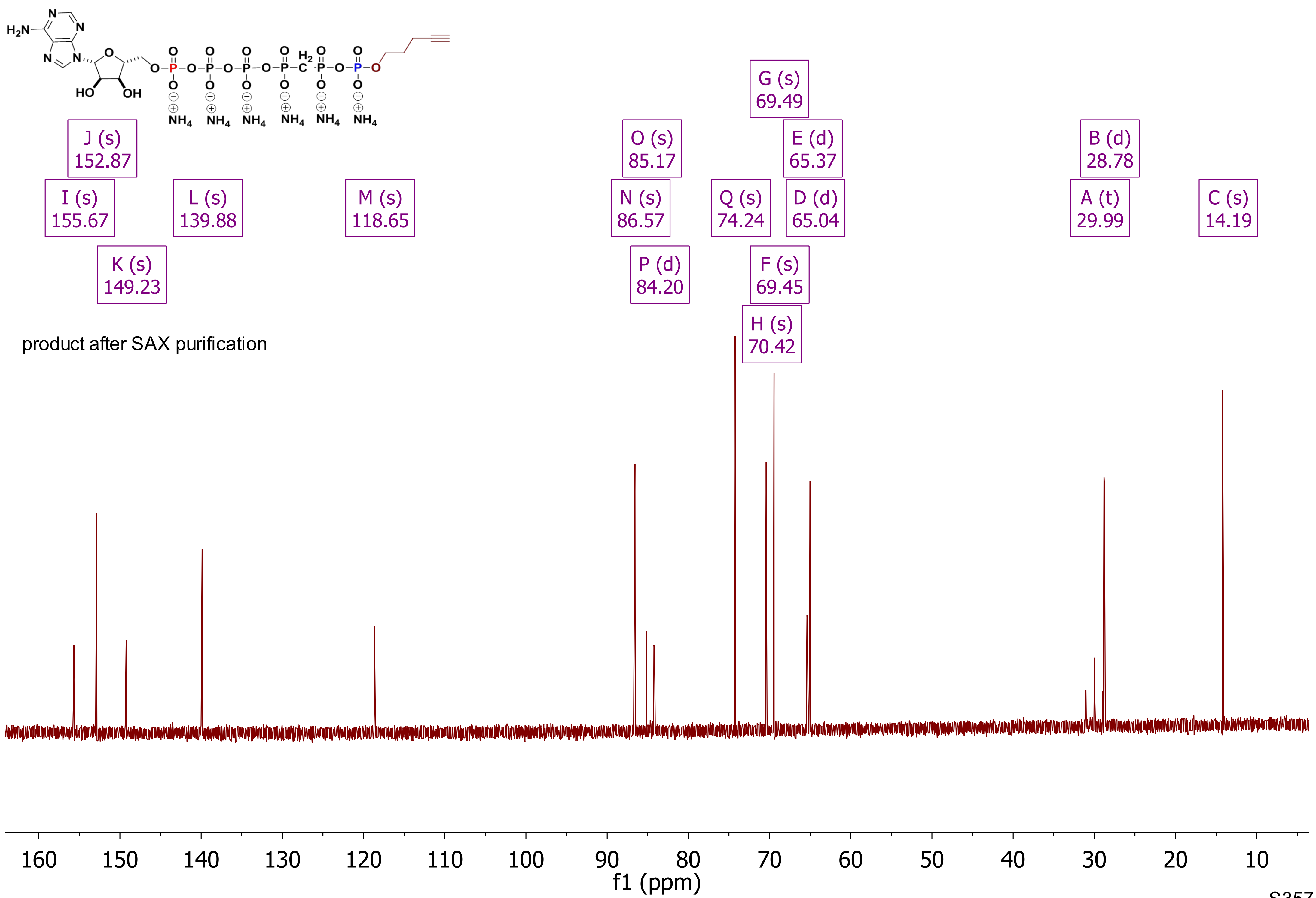


**$^{31}$P-$^{1}$H HMBC NMR**

product after SAX purification

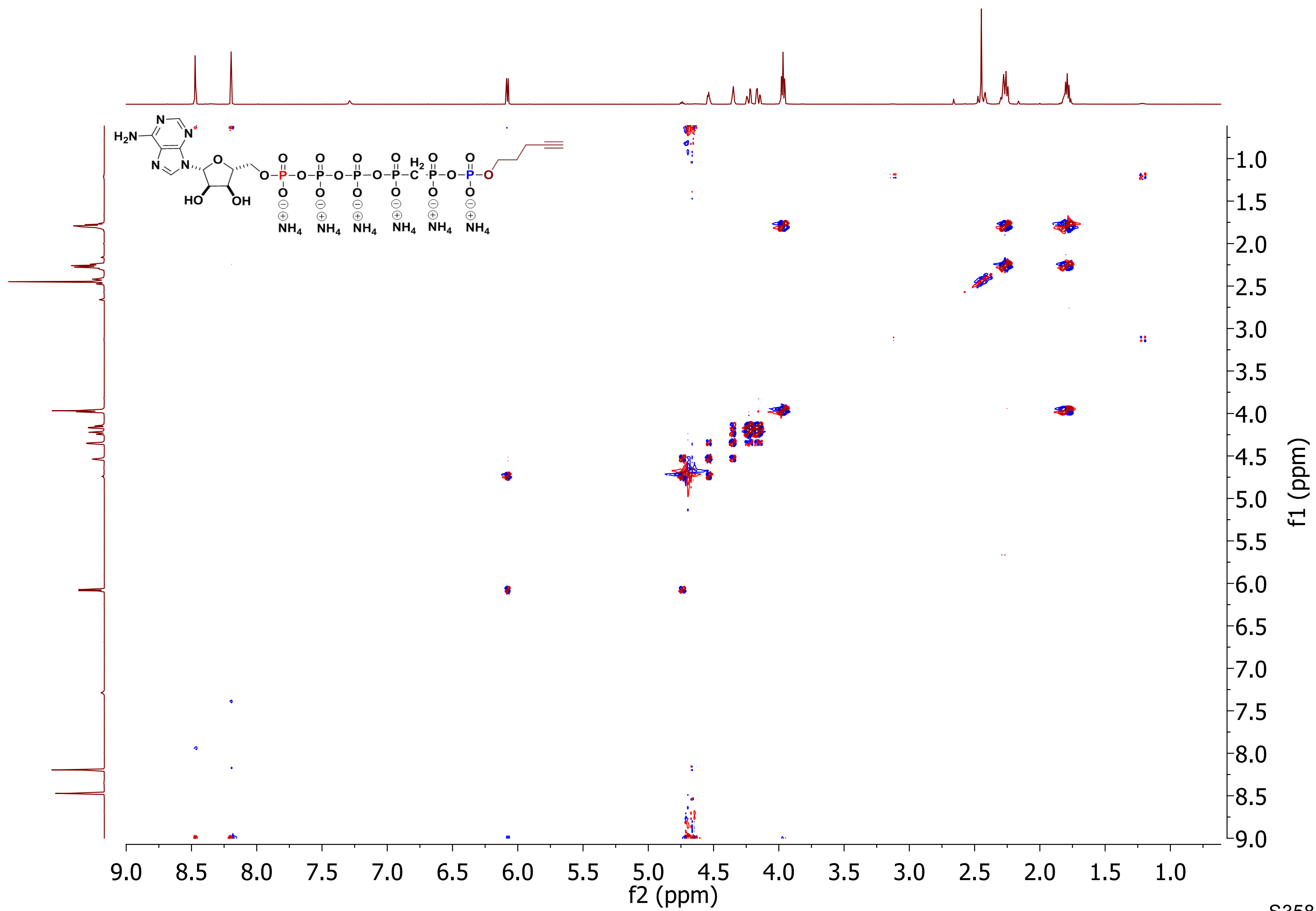


## $^{13}$C NMR

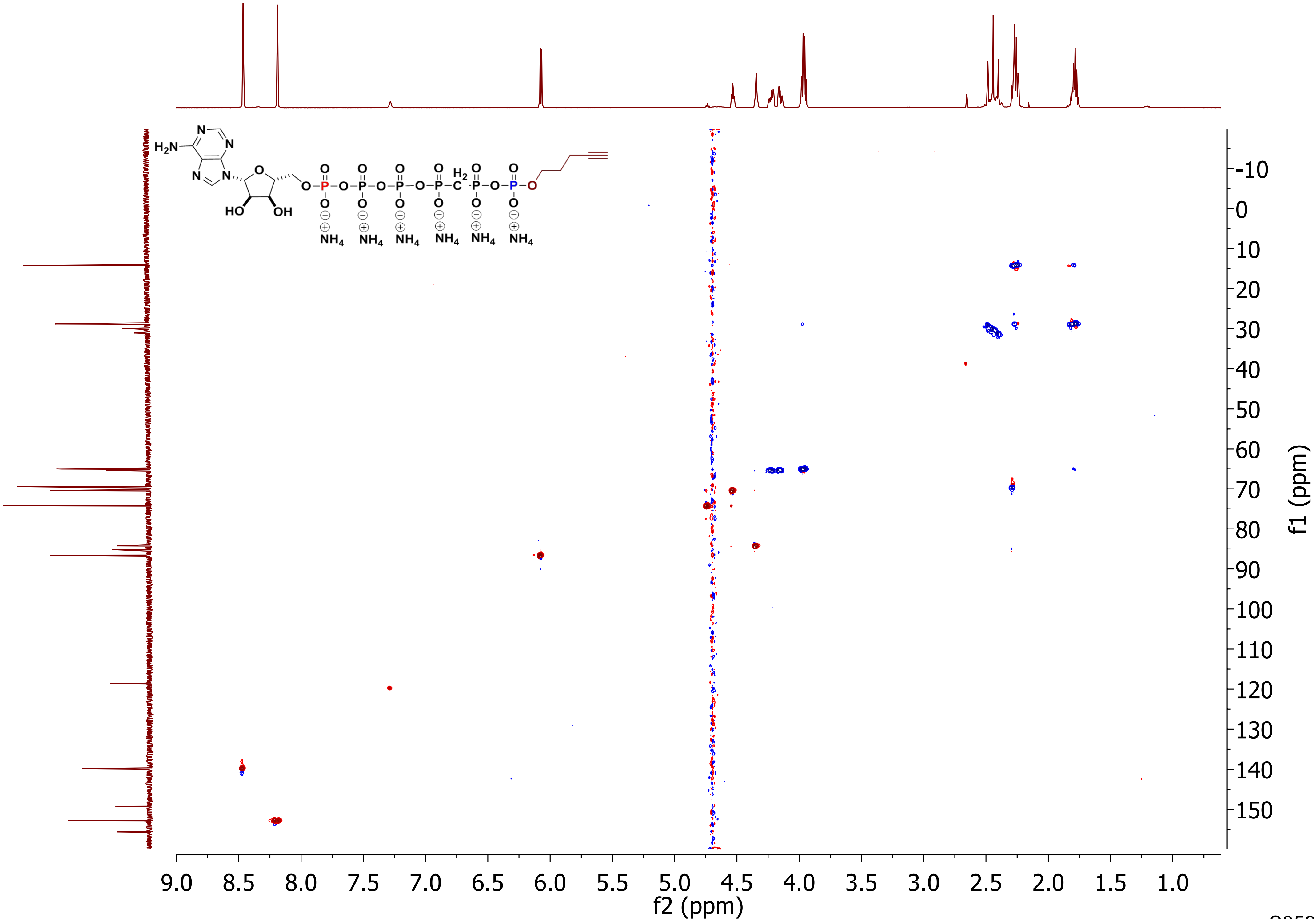


J (s) 152.87
I (s) 155.67
L (s) 139.88
M (s) 118.65
K (s) 149.23
O (s) 85.17
N (s) 86.57
P (d) 84.20
G (s) 69.49
Q (s) 74.24
E (d) 65.37
D (d) 65.04
F (s) 69.45
H (s) 70.42
B (d) 28.78
A (t) 29.99
C (s) 14.19
product after SAX purification
f1 (ppm)

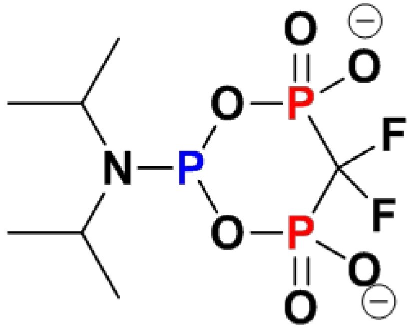
$^1$H-$^1$H COSY NMR

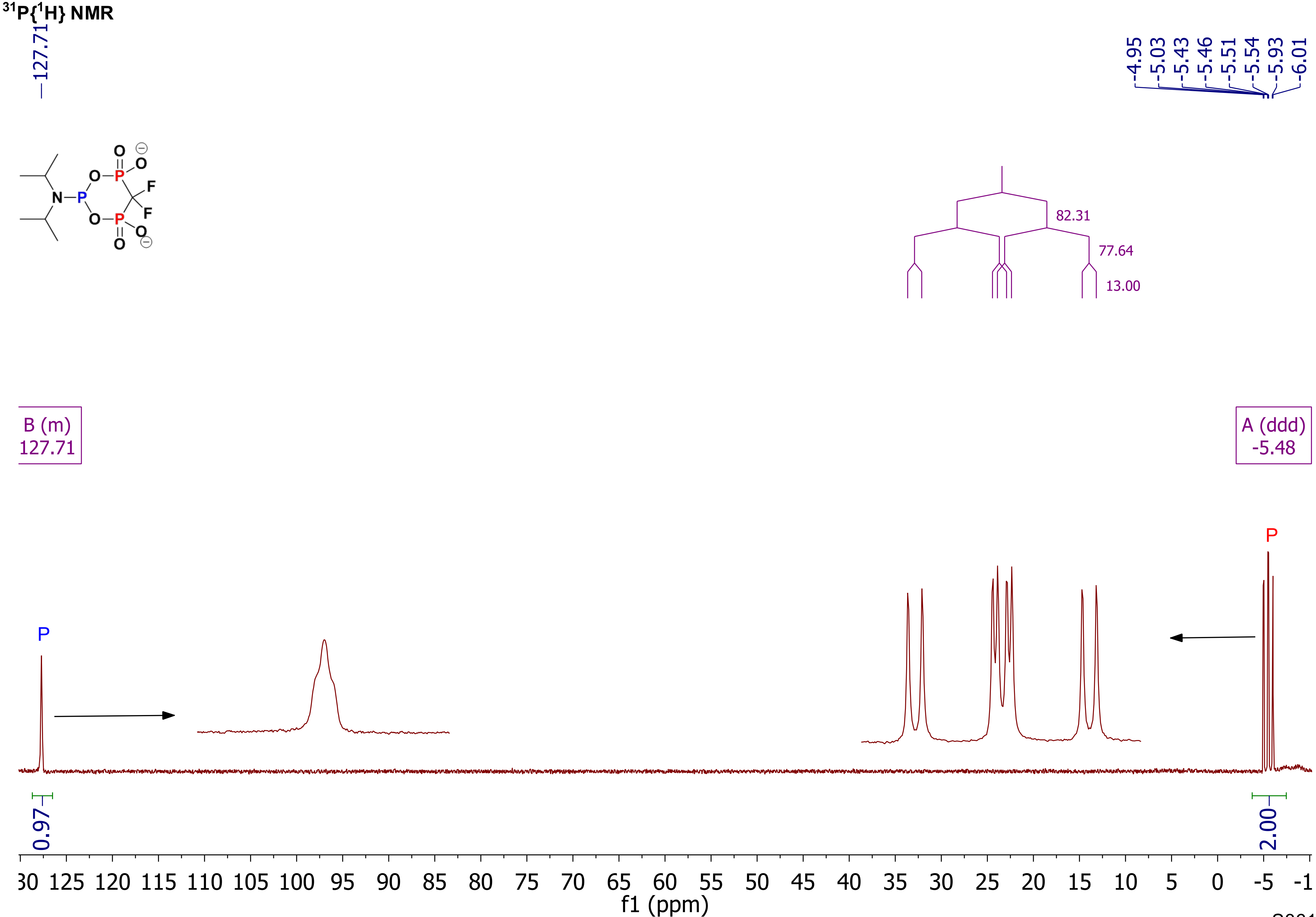


**$^{1}H$-$^{13}C$ HSQC NMR**

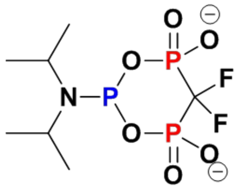


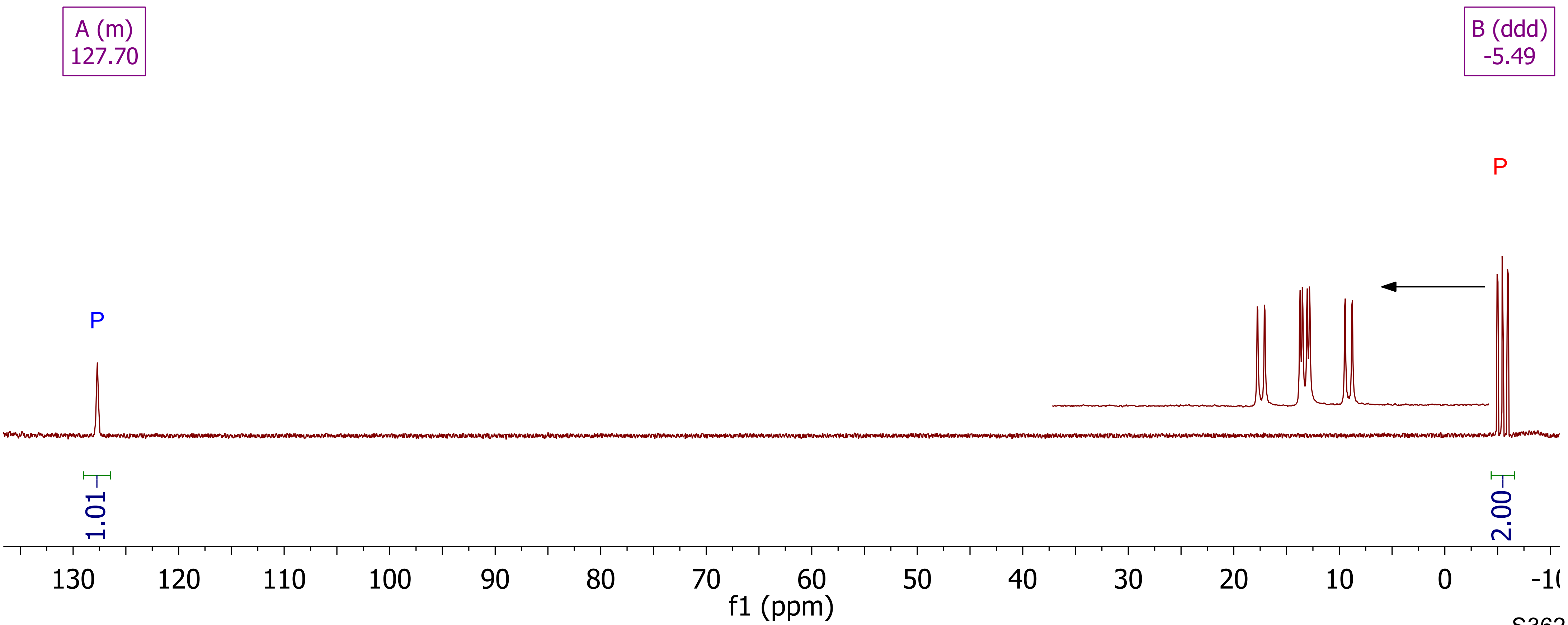
# Synthesis of triphosphates based on *c*-$Py_{CF2}$PA ($A_3$)



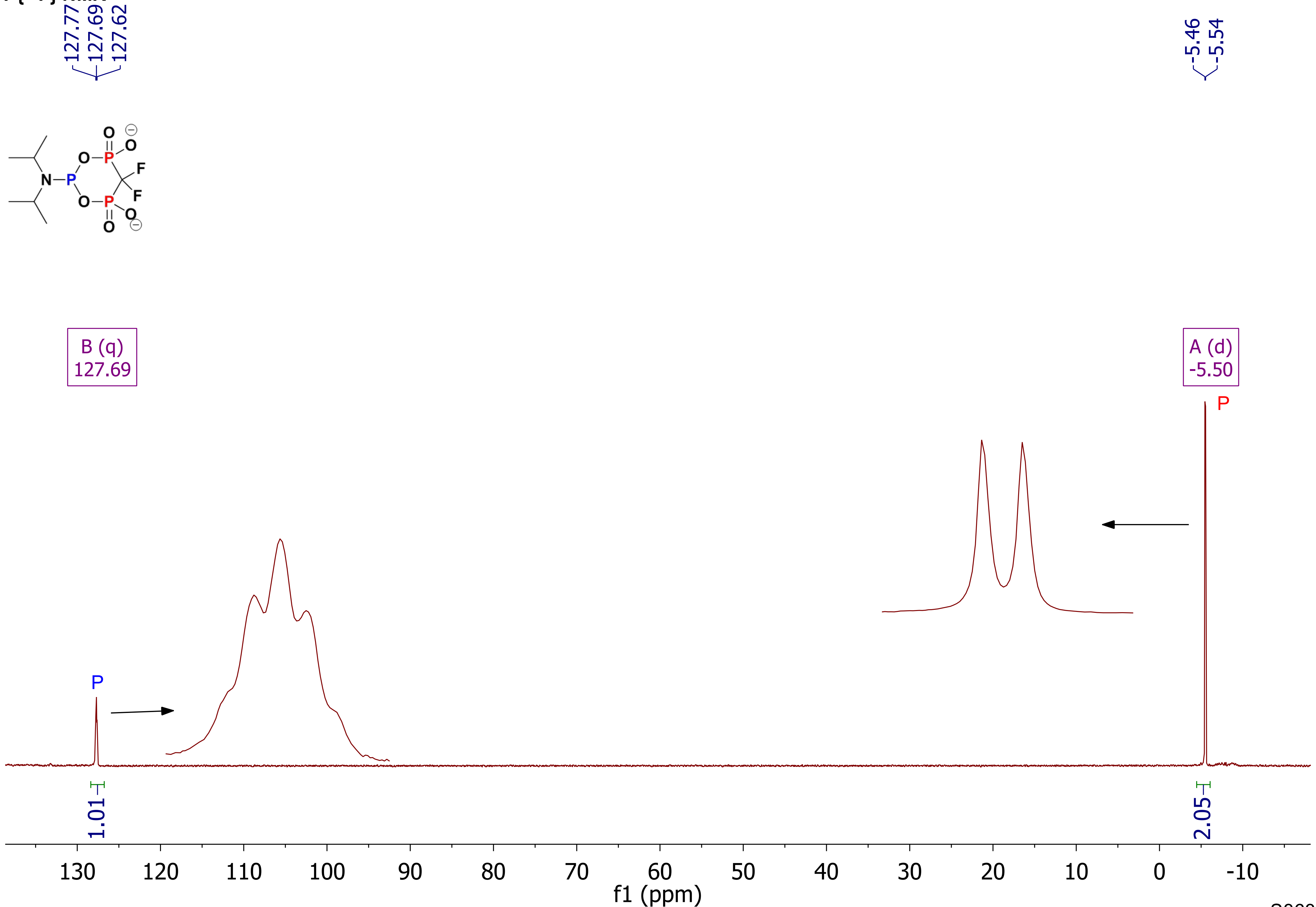

31P{1H} NMR
127.71
-4.95
-5.03
-5.43
-5.46
-5.51
-5.54
-5.93
-6.01
82.31
77.64
13.00
B (m)
127.71
A (ddd)
-5.48
P
P
0.97
2.00
130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 -5 -10
f1 (ppm)

**$^{31}$P NMR**



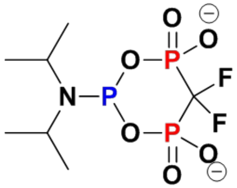

A (m)
127.70
B (ddd)
-5.49
P
P
1.01
2.00
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

**$^{31}P\{^{19}F\}$ NMR**

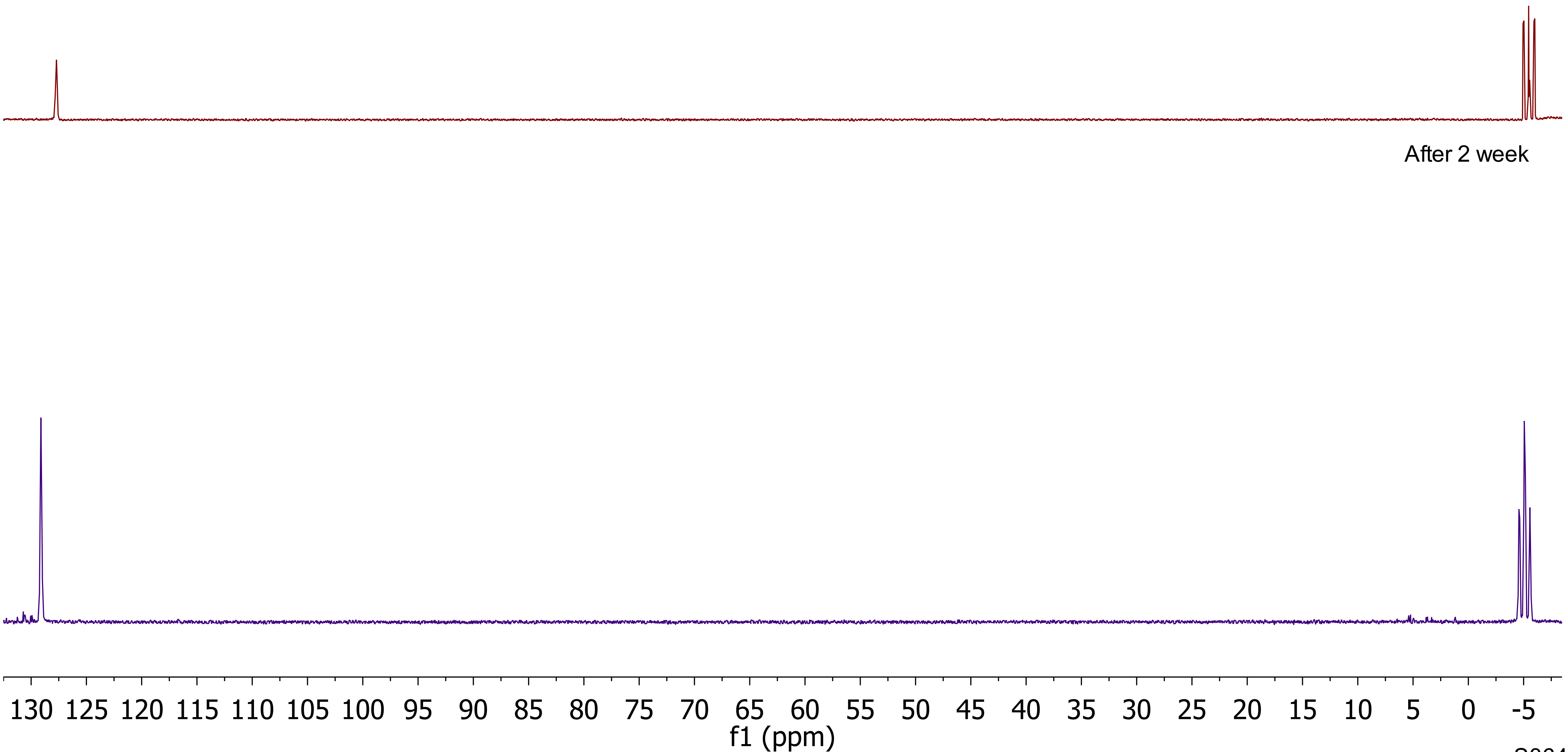


127.77
127.69
127.62
-5.46
-5.54
O
O
O
P
F
N
P
O
O
P
F
O
O
B (q)
127.69
A (d)
-5.50
P
P
1.01
2.05
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

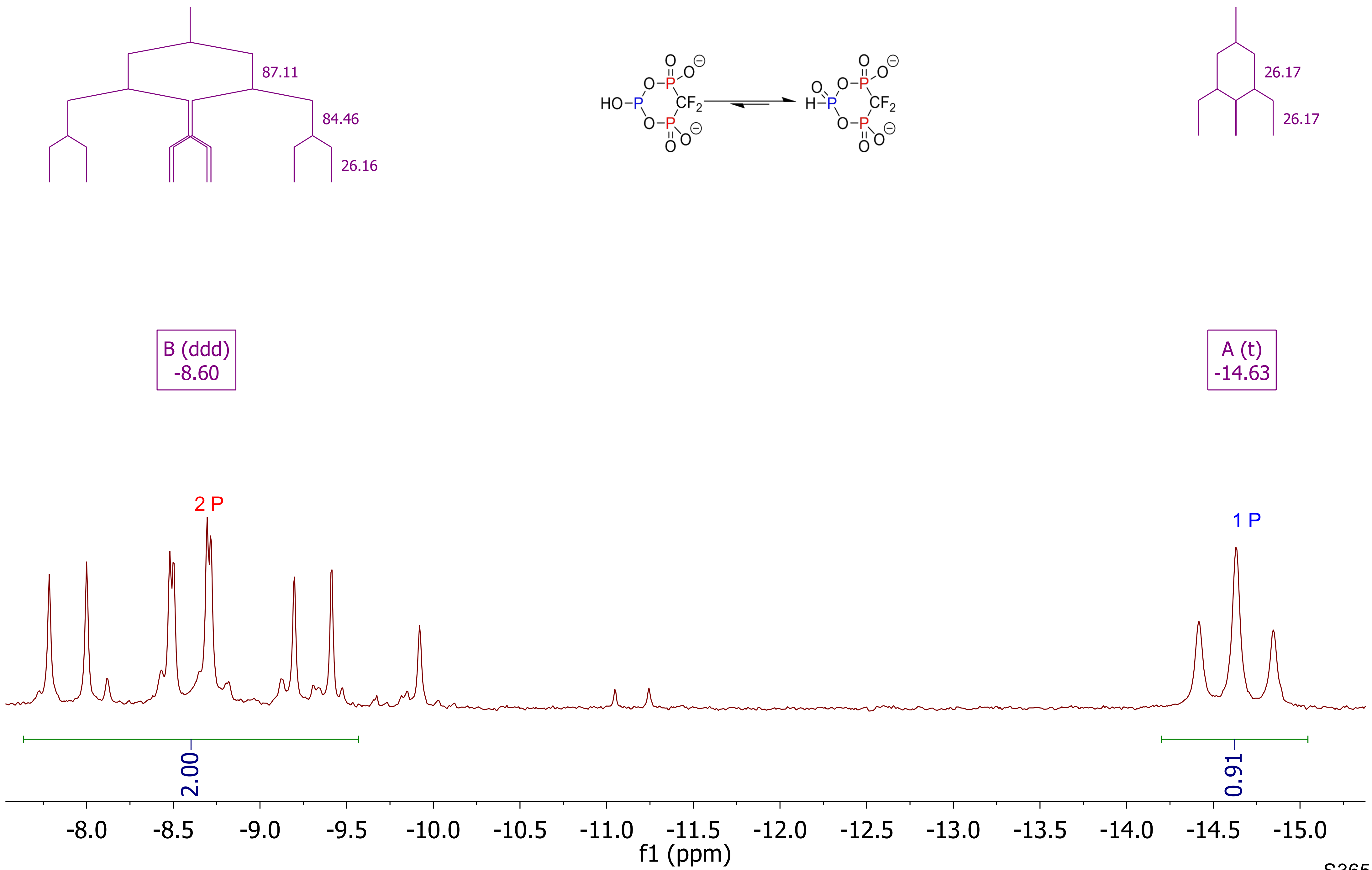
**$^{31}P\{^1H\}$ NMR**



Stability studies

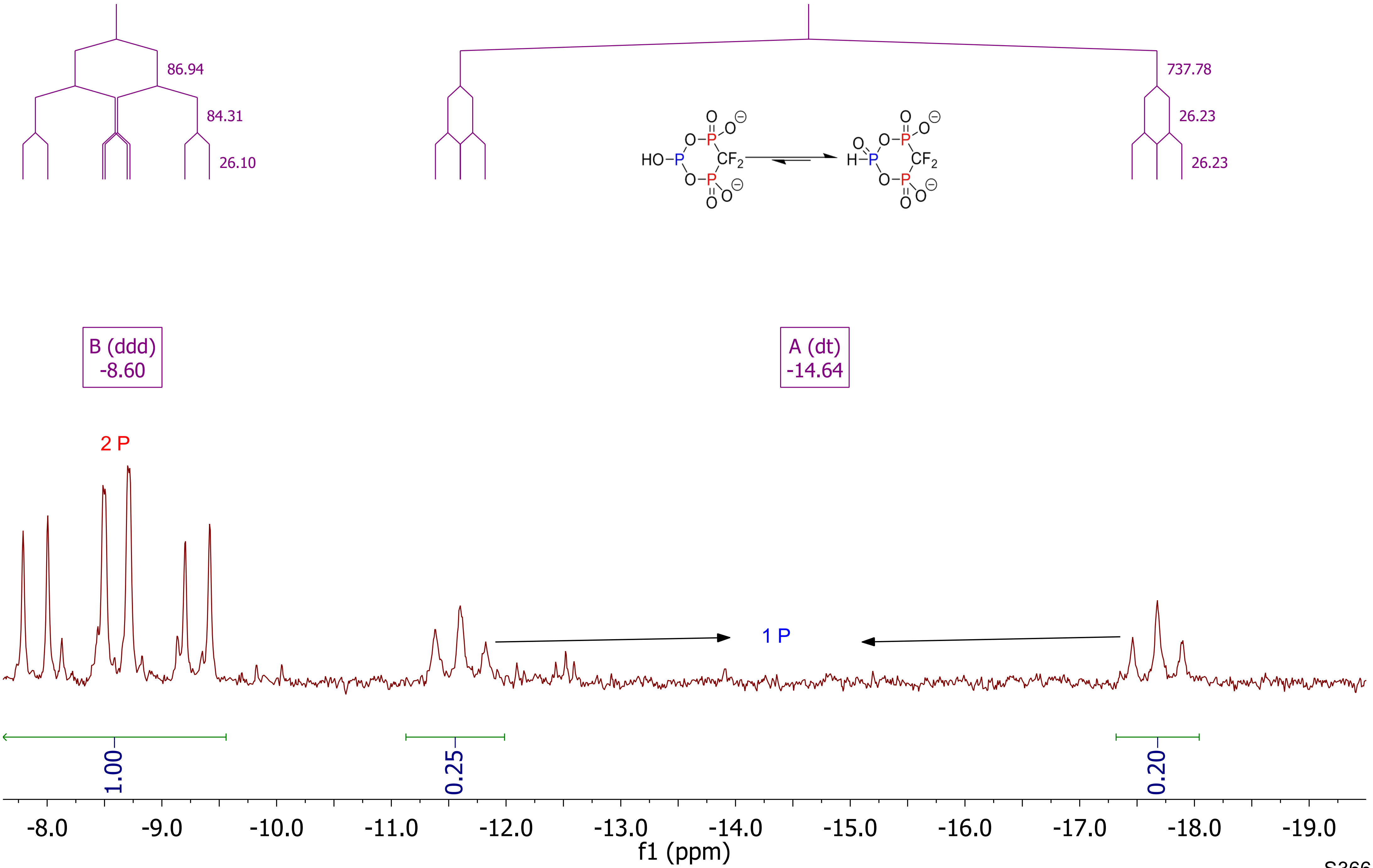


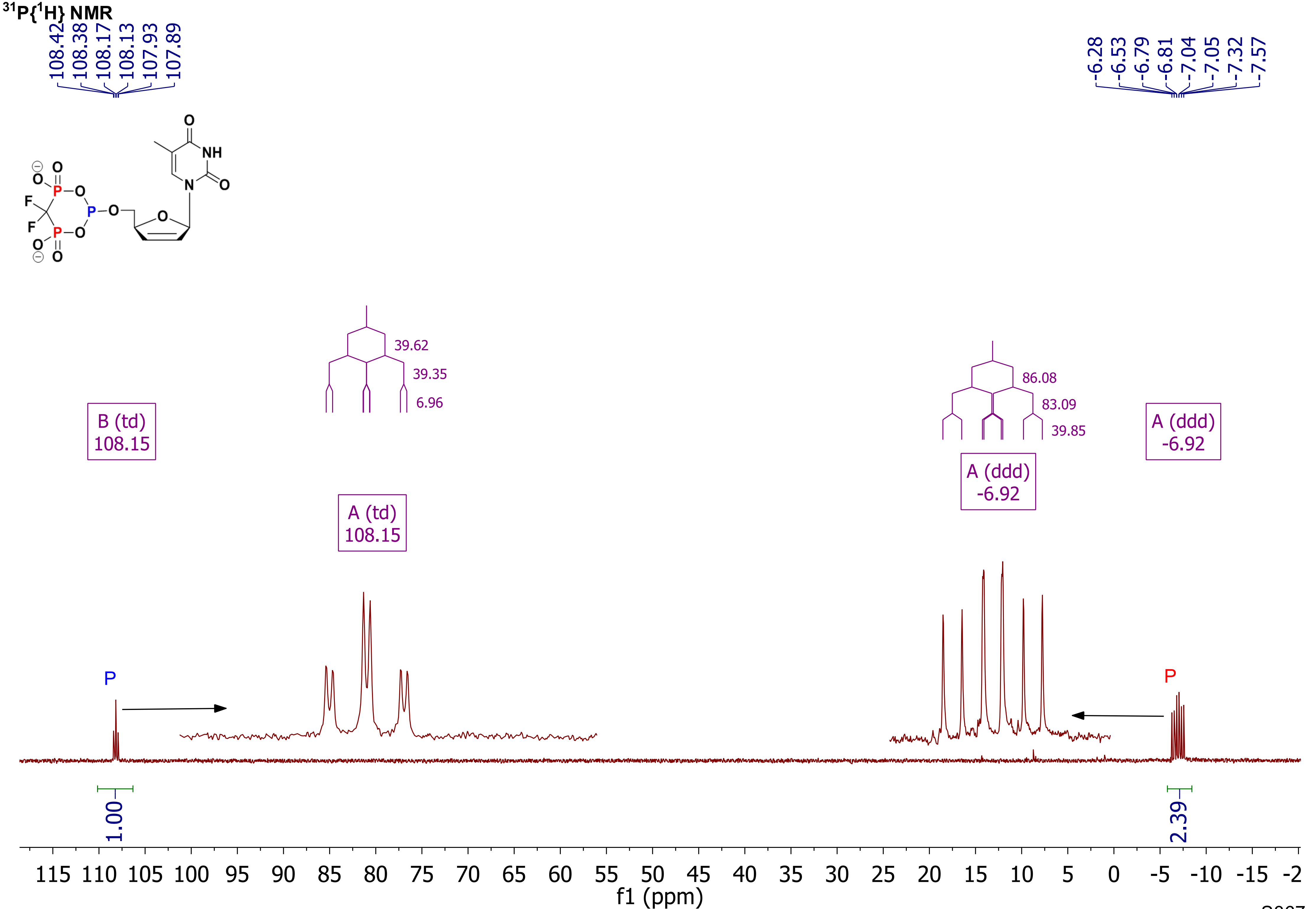
$^{31}P\{^1H\}$ NMR


87.11
84.46
26.16
26.17
26.17
HO–P
CF2
H–P
B (ddd)
-8.60
A (t)
-14.63
2 P
1 P
2.00
0.91
f1 (ppm)

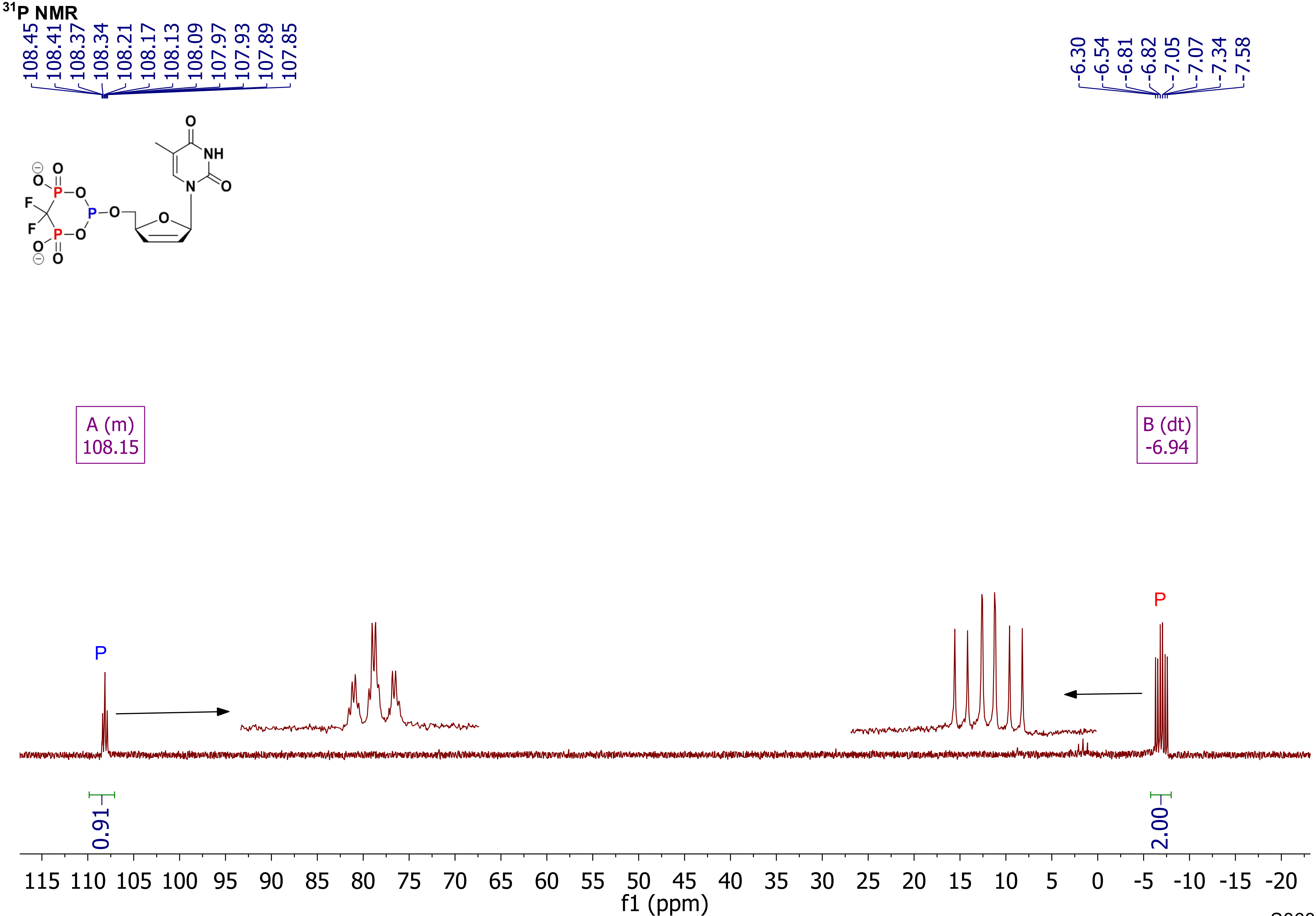
**$^{31}$P NMR**

After six weeks



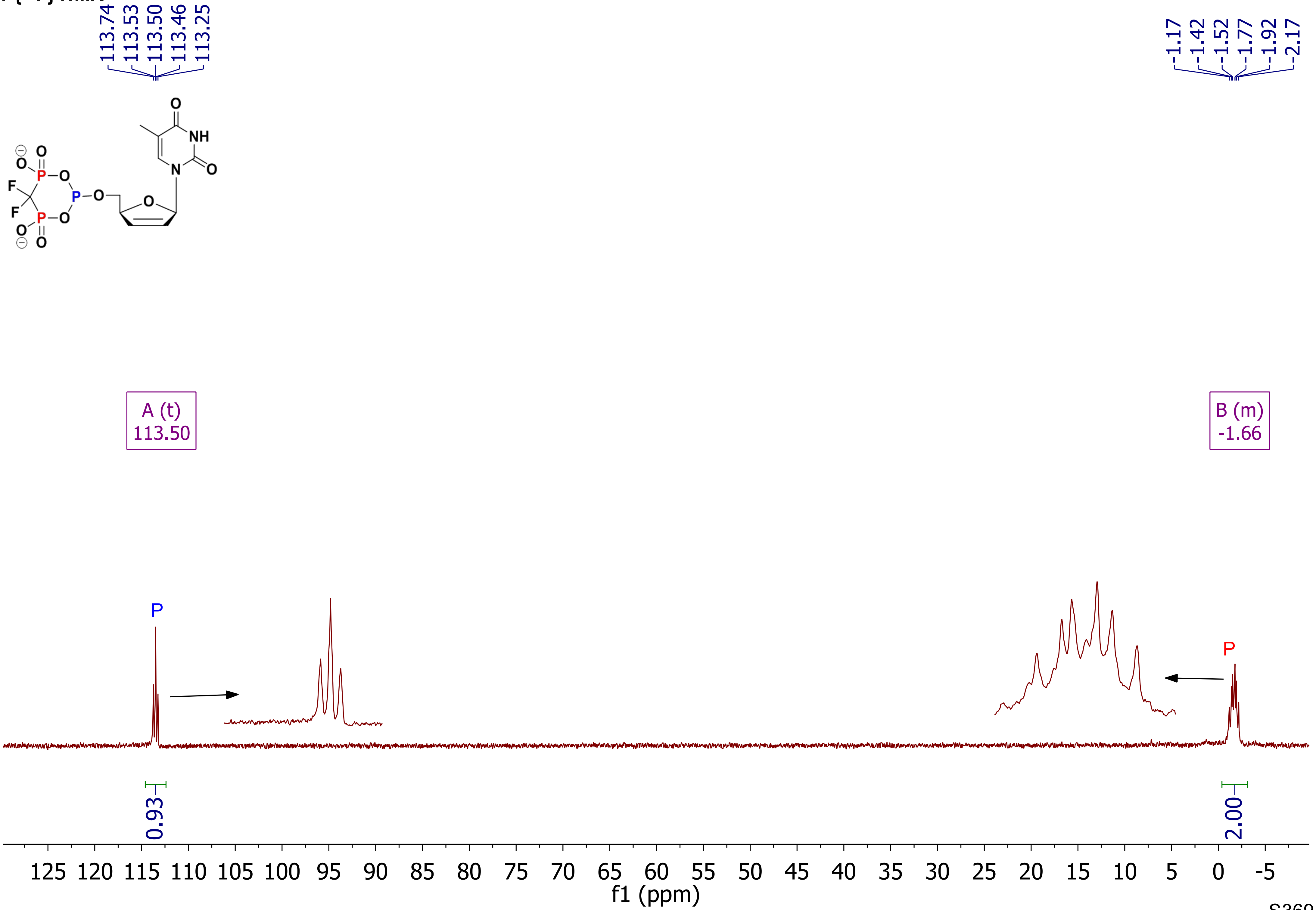


31P{1H} NMR
108.42
108.38
108.17
108.13
107.93
107.89
-6.28
-6.53
-6.79
-6.81
-7.04
-7.05
-7.32
-7.57
39.62
39.35
6.96
86.08
83.09
39.85
B (td)
108.15
A (td)
108.15
A (ddd)
-6.92
A (ddd)
-6.92
P
P
1.00
2.39
115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 -5 -10 -15 -2
f1 (ppm)

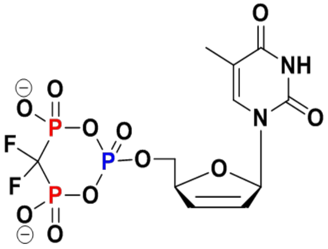


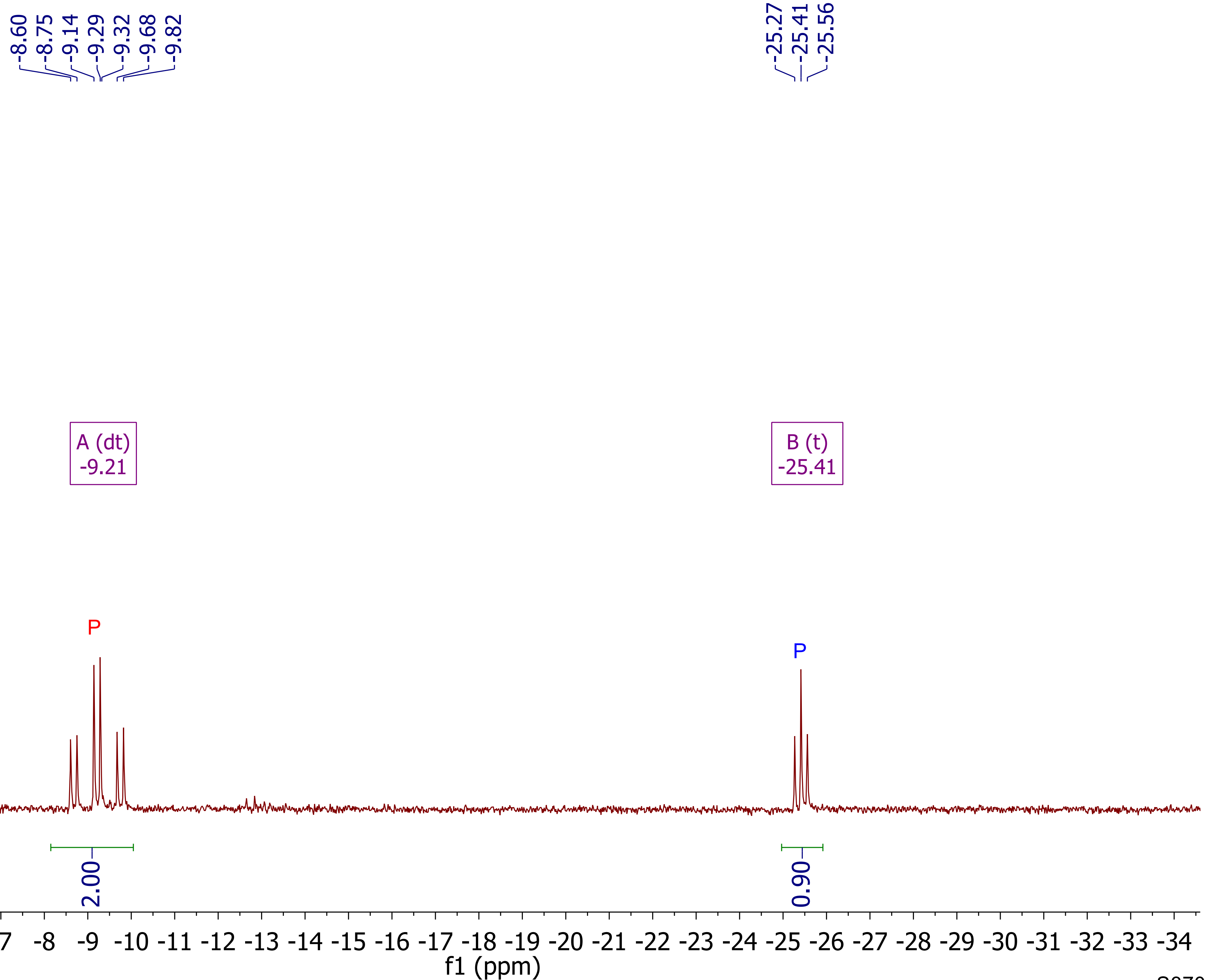
31P NMR
108.45
108.41
108.37
108.34
108.21
108.17
108.13
108.09
107.97
107.93
107.89
107.85
-6.30
-6.54
-6.81
-6.82
-7.05
-7.07
-7.34
-7.58
A (m)
108.15
B (dt)
-6.94
P
P
0.91
2.00
115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 -5 -10 -15 -20
f1 (ppm)

$^{31}P\{^{19}F\}$ NMR


113.74
113.53
113.50
113.46
113.25
-1.17
-1.42
-1.52
-1.77
-1.92
-2.17
A (t)
113.50
B (m)
-1.66
P
P
0.93
2.00
125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 -5
f1 (ppm)

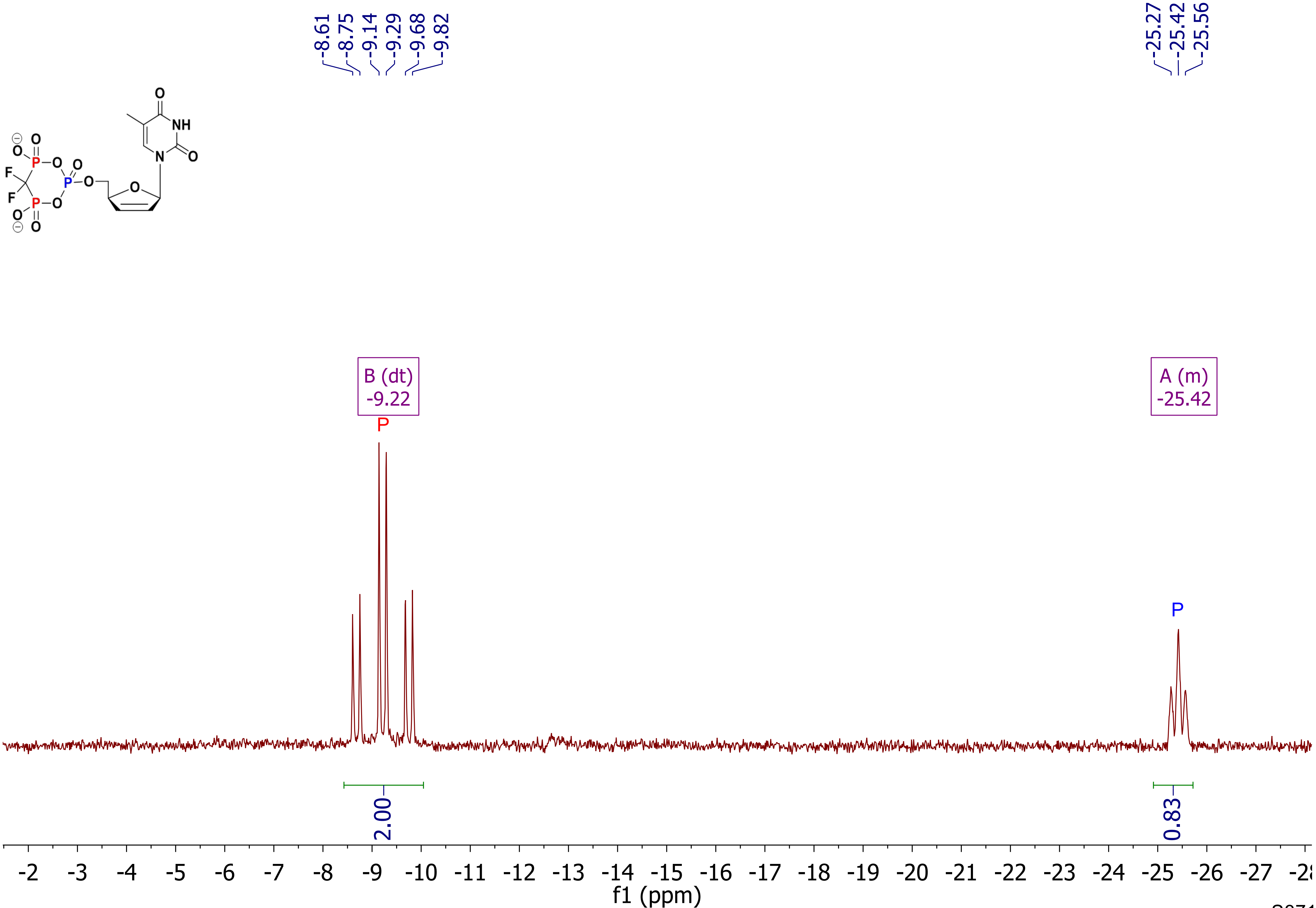
**$^{31}P\{^1H\}$ NMR**



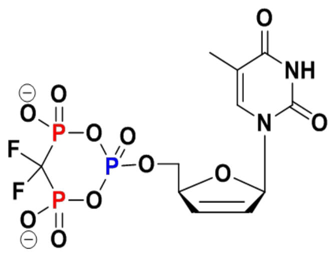


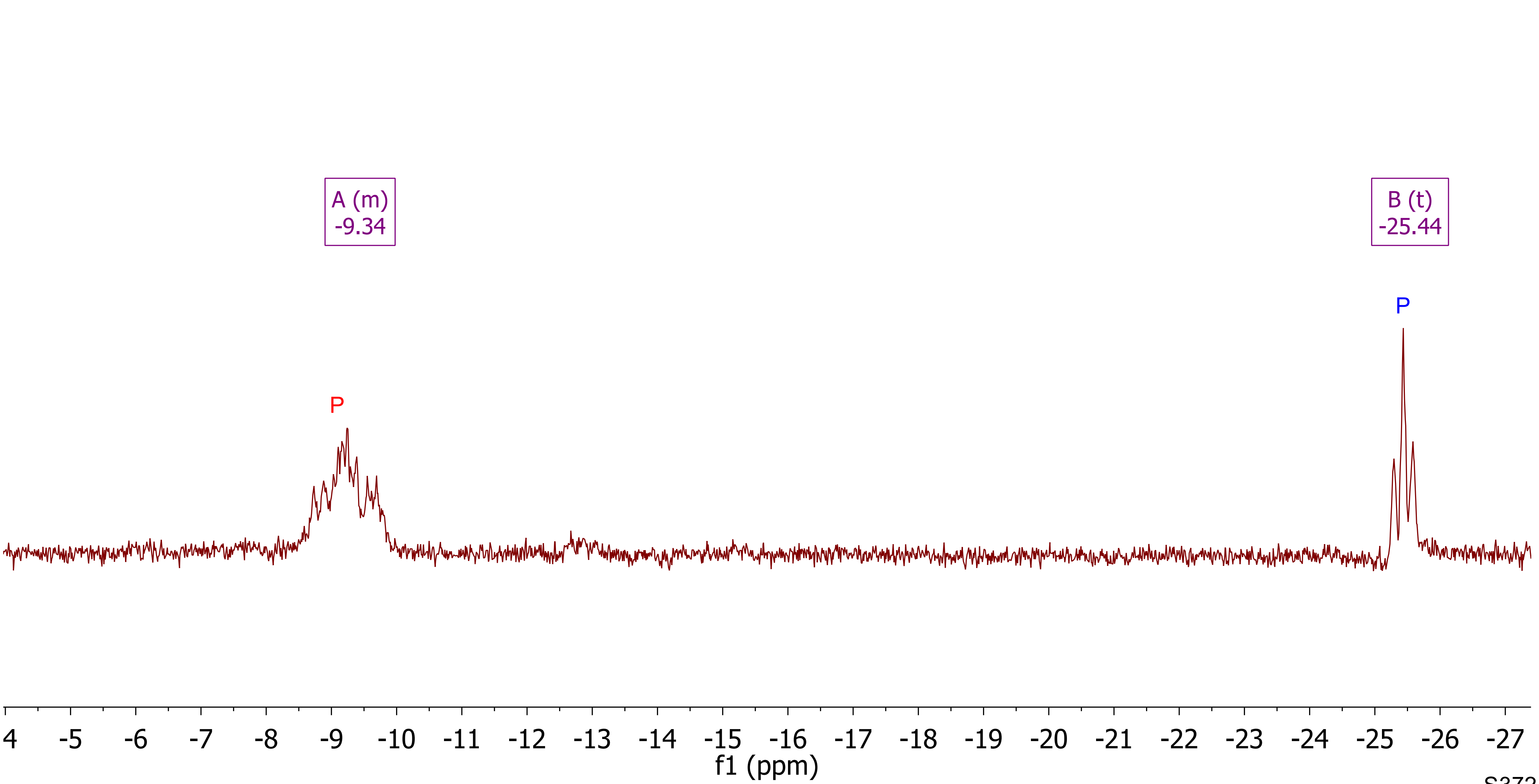
-8.60
-8.75
-9.14
-9.29
-9.32
-9.68
-9.82
-25.27
-25.41
-25.56
A (dt)
-9.21
B (t)
-25.41
P
P
2.00
0.90
-3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23 -24 -25 -26 -27 -28 -29 -30 -31 -32 -33 -34
f1 (ppm)

$^{31}$P NMR

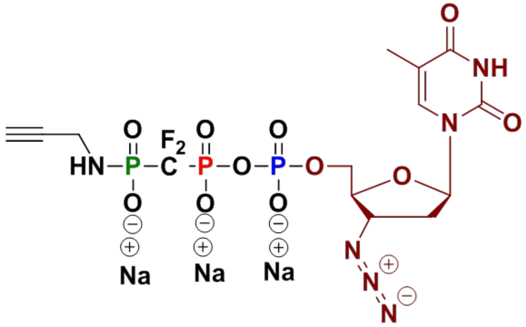

-8.61
-8.75
-9.14
-9.29
-9.68
-9.82
-25.27
-25.42
-25.56
B (dt)
-9.22
A (m)
-25.42
2.00
0.83
f1 (ppm)

**$^{31}P\{^{19}F\}$ NMR**

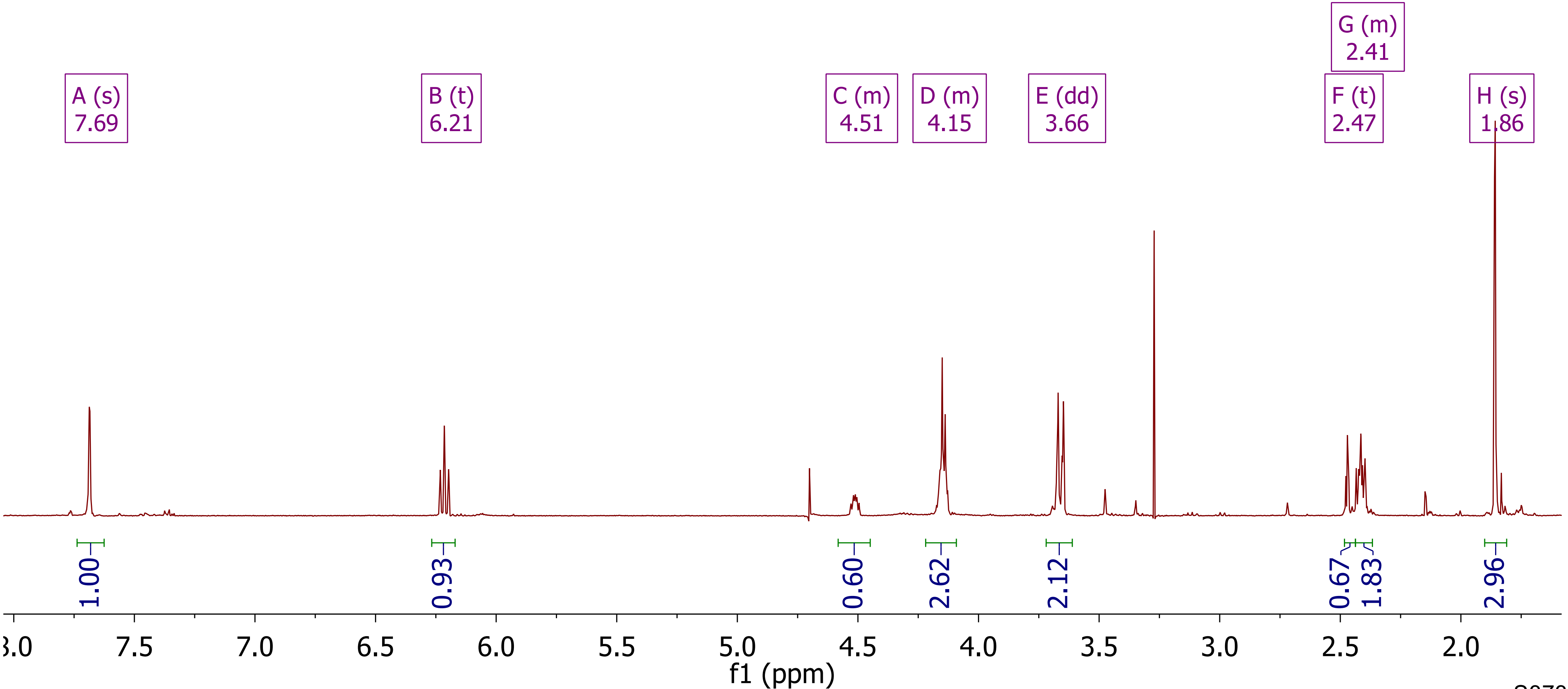



-8.73
-8.88
-9.18
-9.38
-9.55
-9.69
-25.29
-25.44
-25.58
A (m)
-9.34
B (t)
-25.44
P
P
4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23 -24 -25 -26 -27
f1 (ppm)

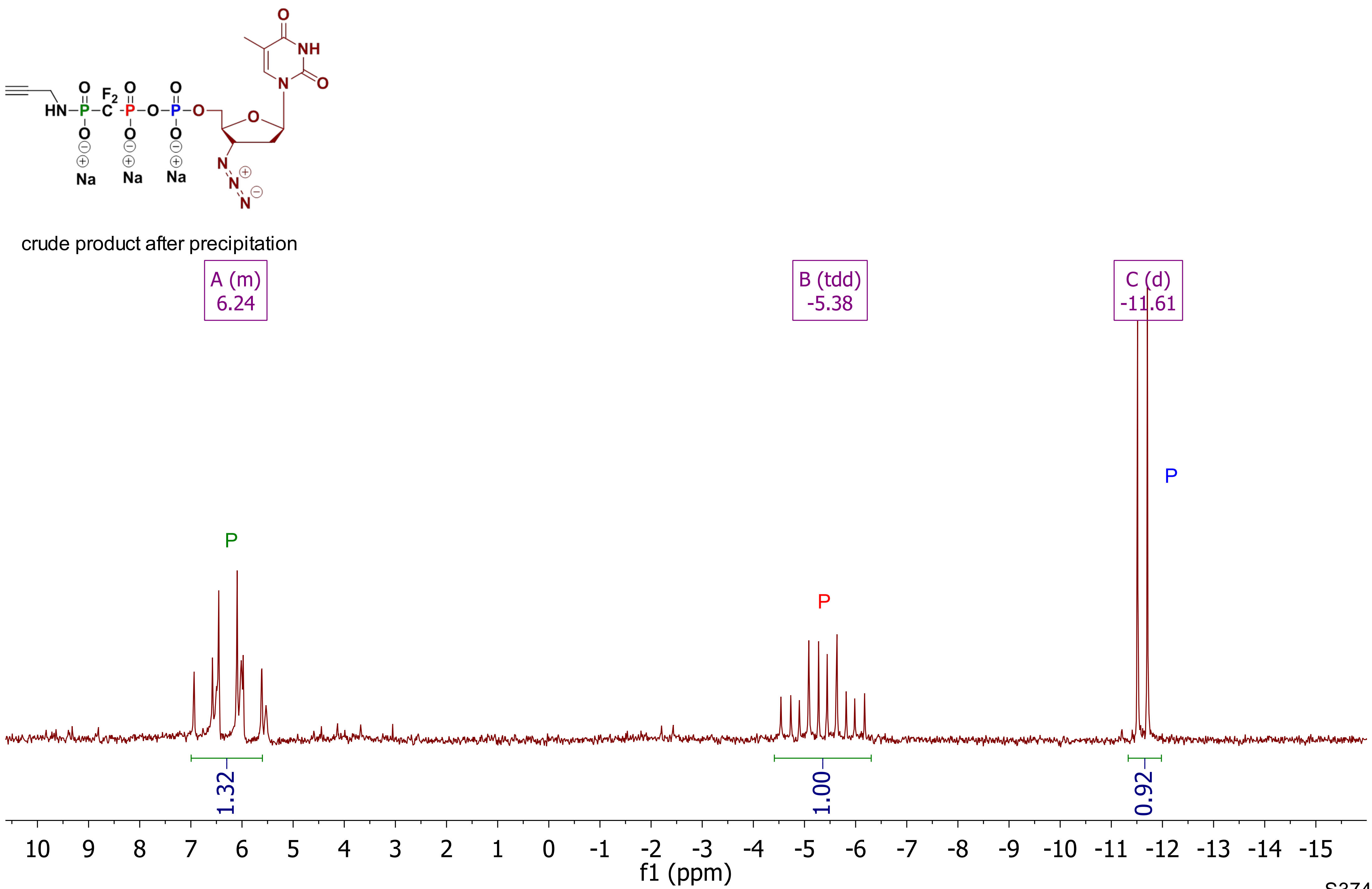
$^{1}$H NMR



crude product after precipitation

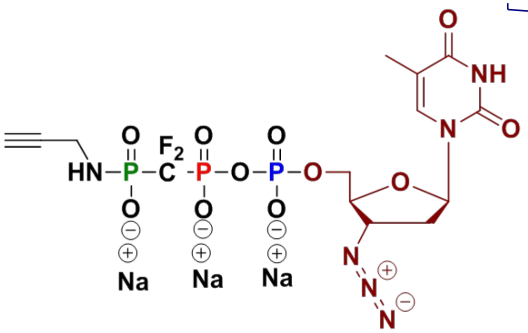


$^{31}P\{^1H\}$ NMR

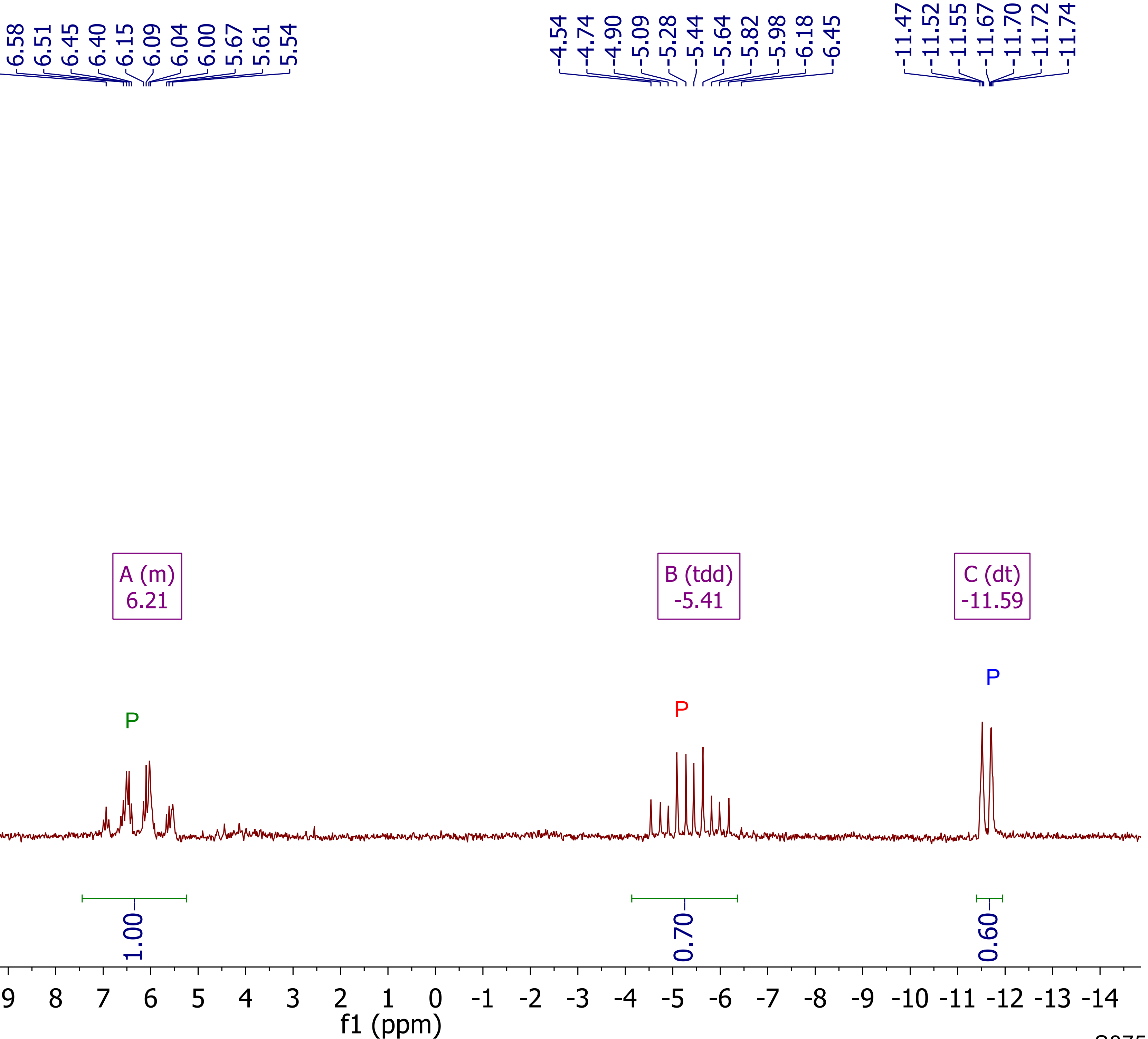


HN
F2
Na
Na
Na
NH
crude product after precipitation
A (m)
6.24
B (tdd)
-5.38
C (d)
-11.61
P
P
P
1.32
1.00
0.92
10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15
f1 (ppm)

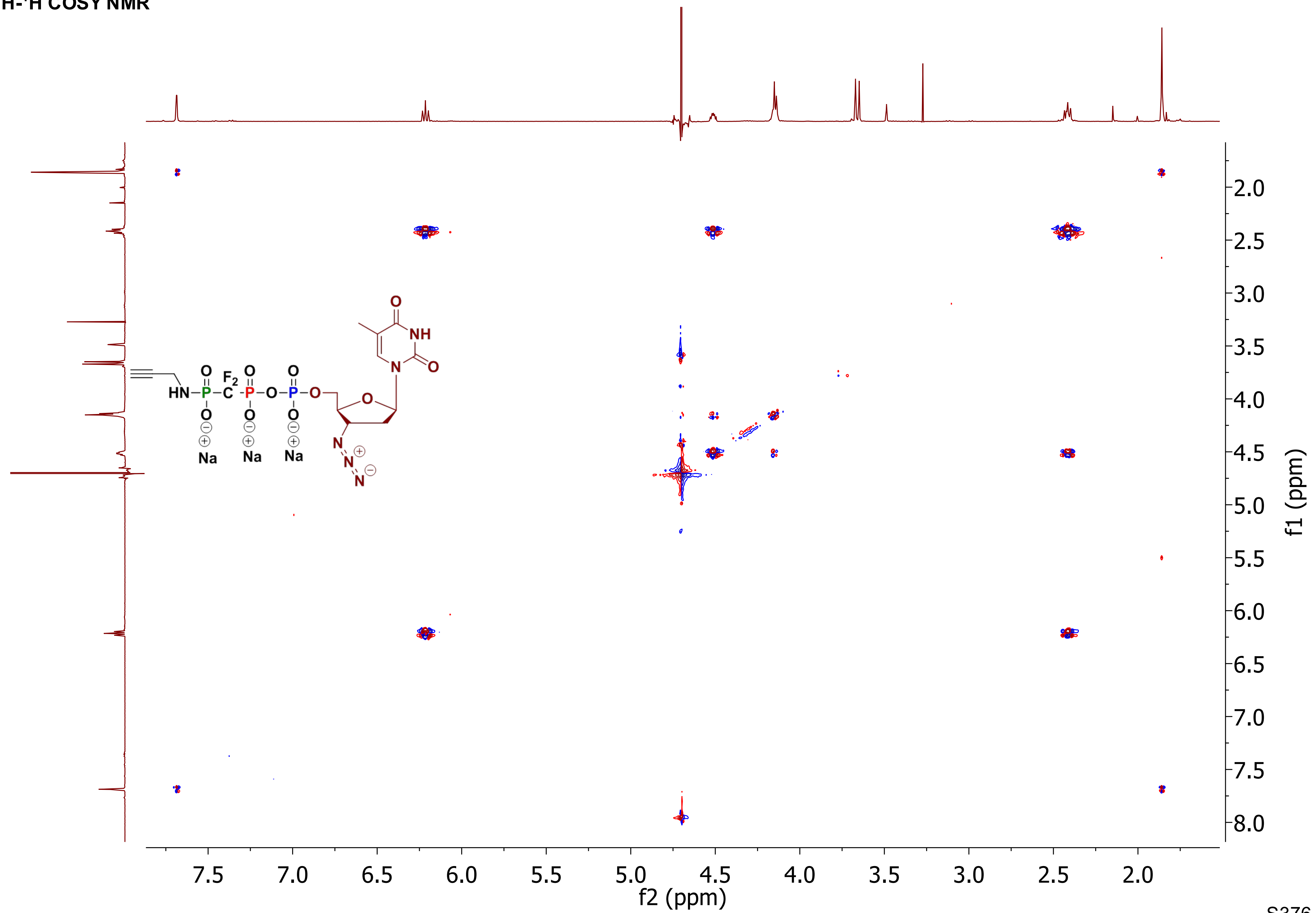
$^{31}$P NMR



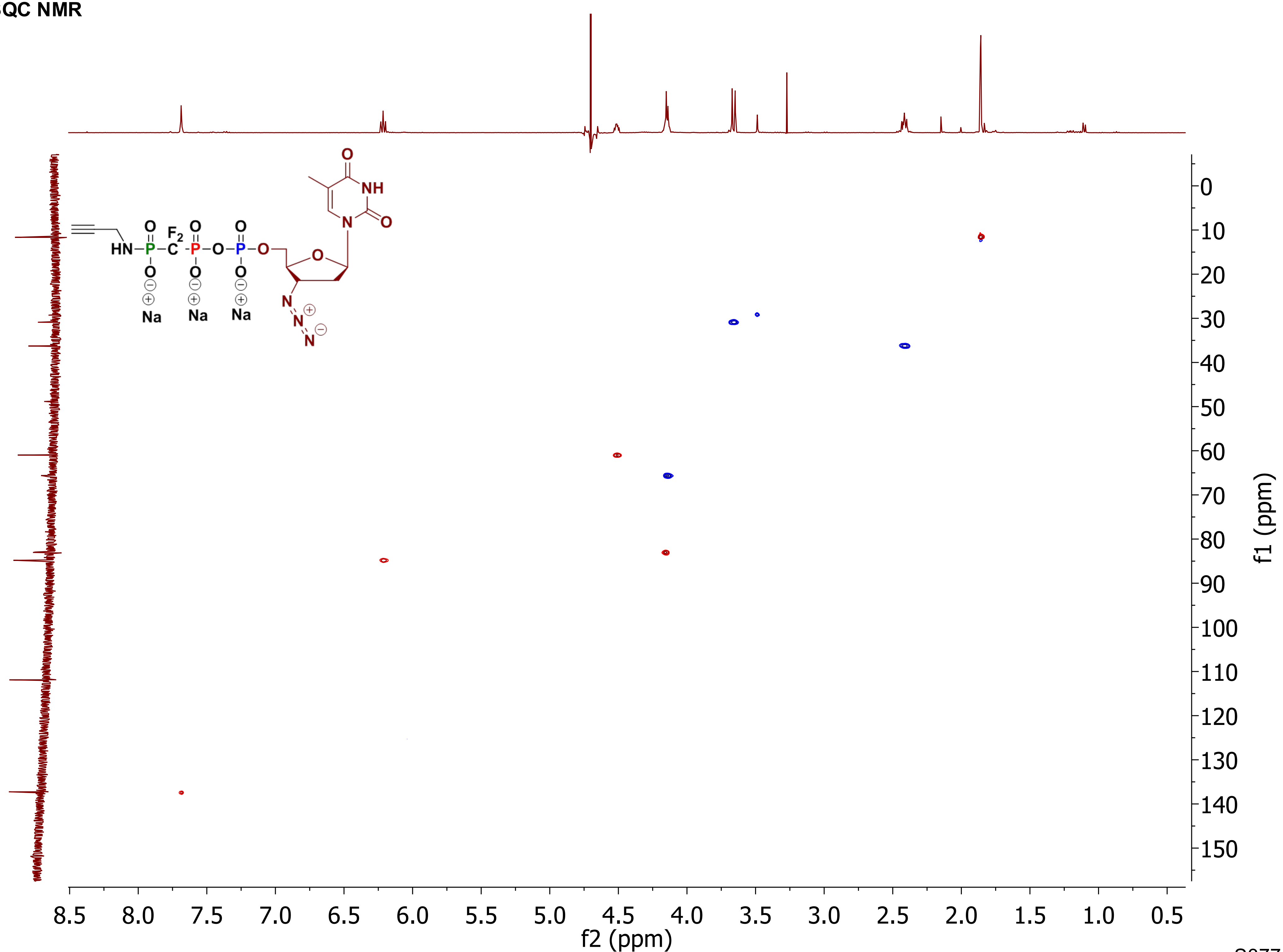
crude product after precipitation



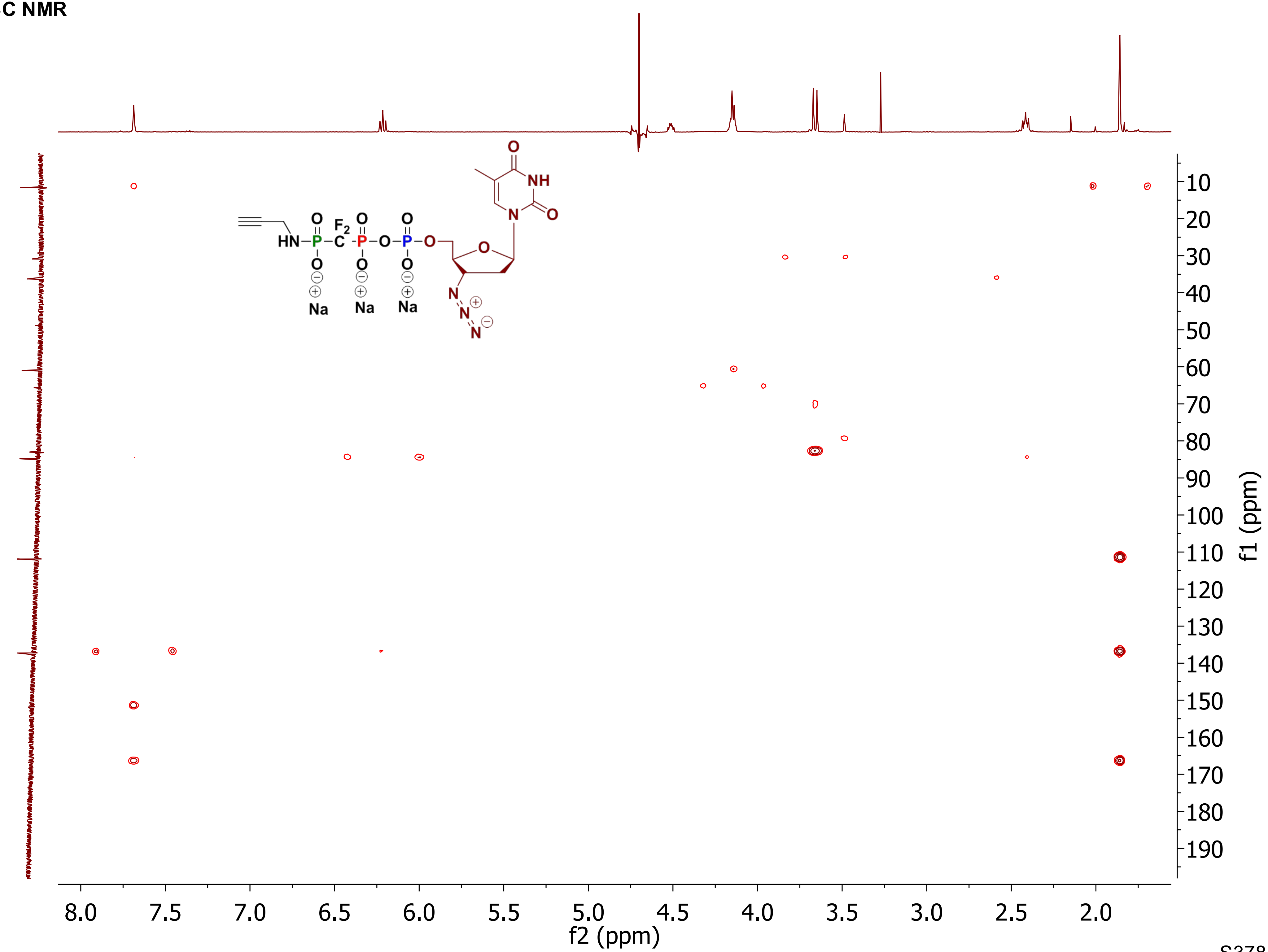
**$^{1}H$-$^{1}H$ COSY NMR**



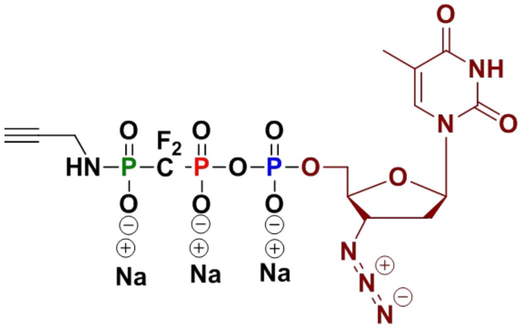
$^{1}H$-$^{13}C$ HSQC NMR



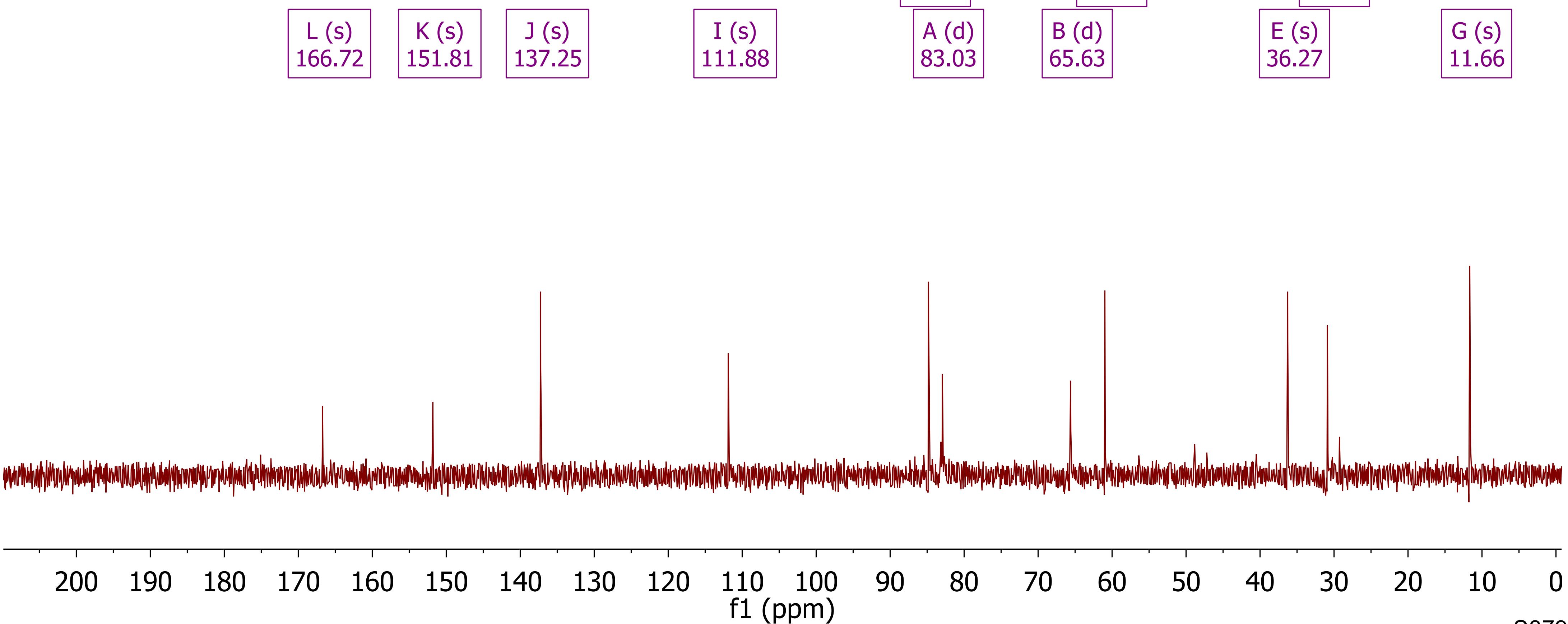
$^1$H-$^1$H HMBC NMR



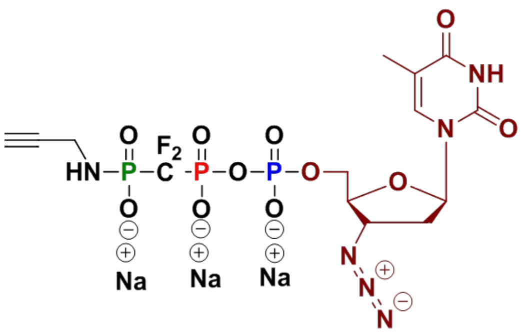
$^{13}C$ NMR



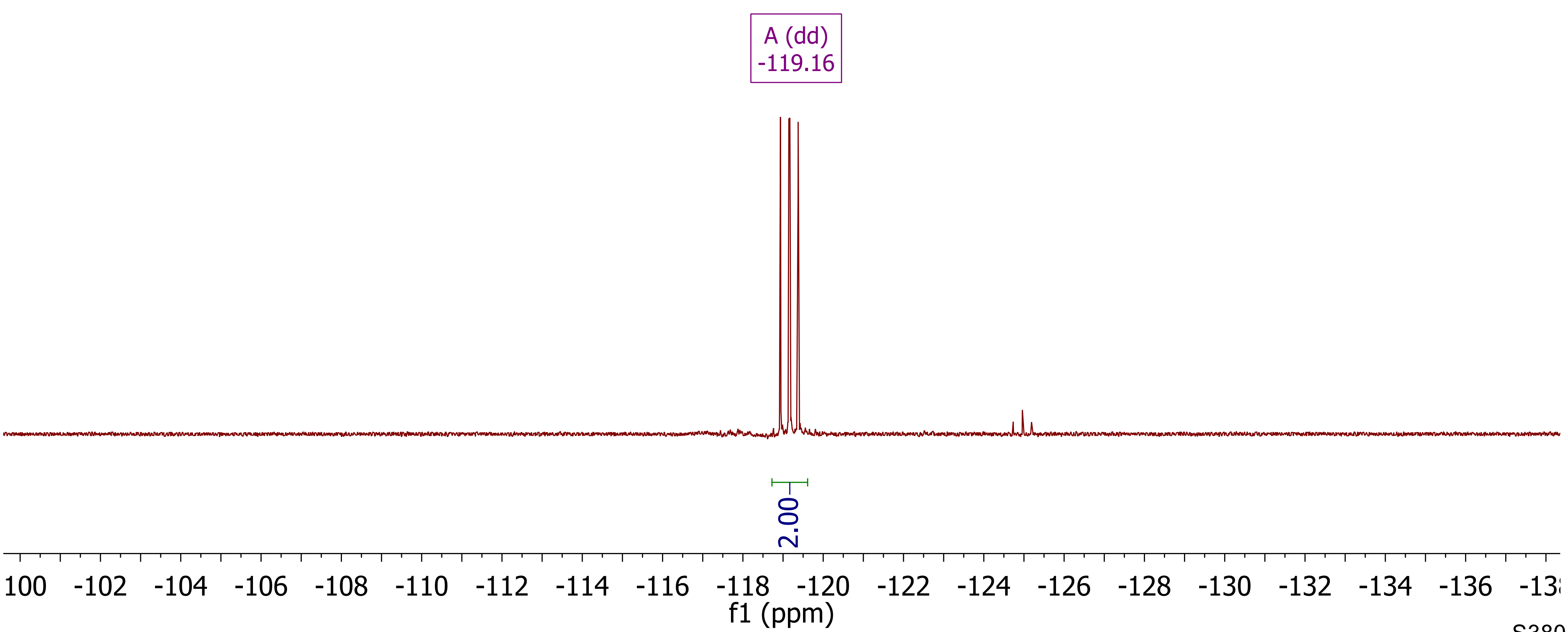
crude product after precipitation



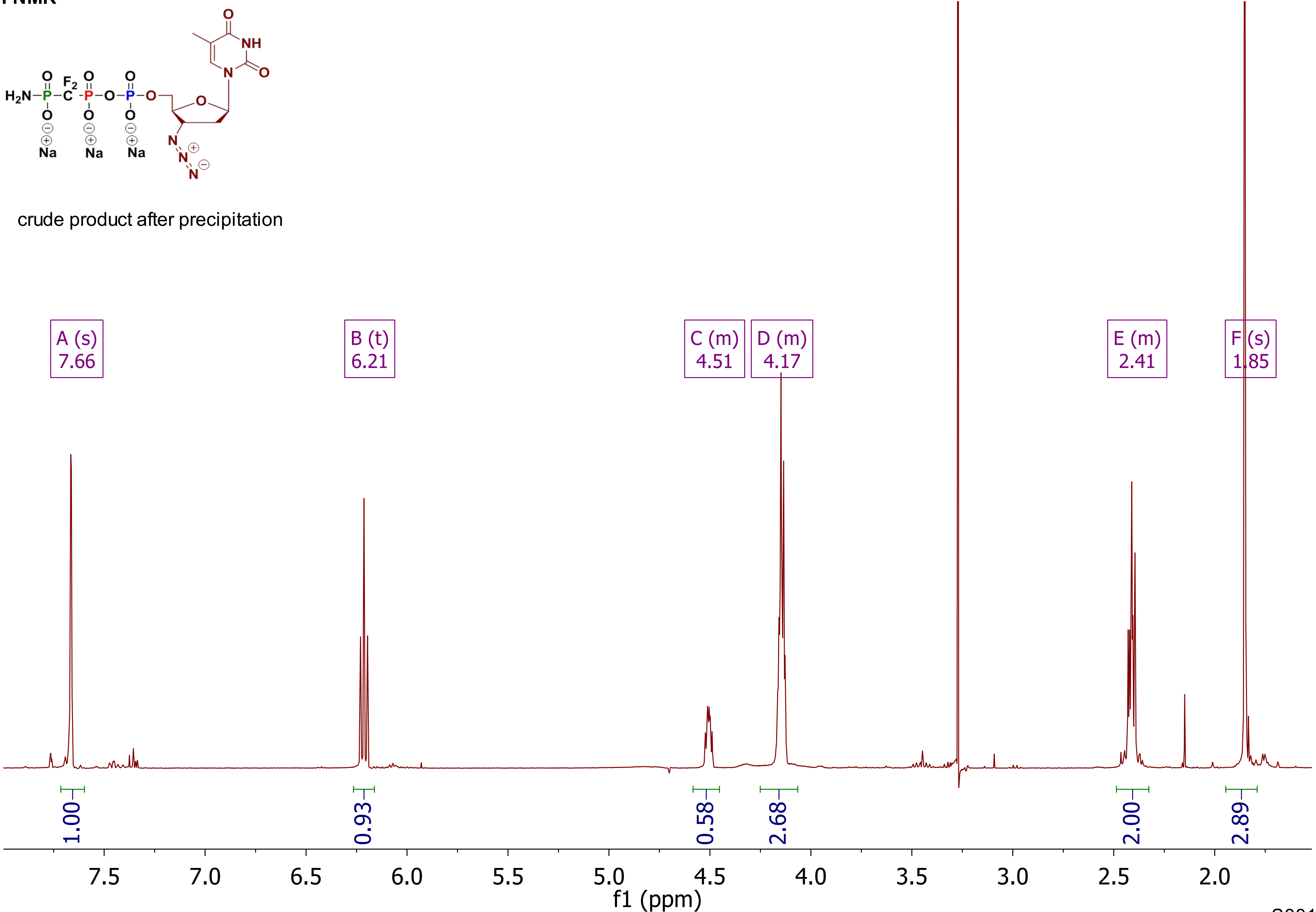
**$^{19}$F NMR**



crude product after precipitation

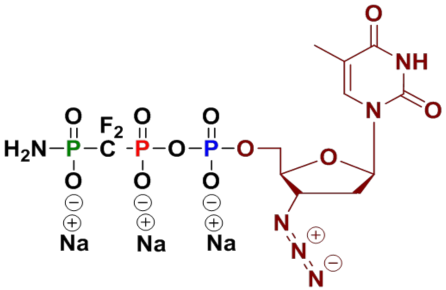


**$^1H$ NMR**

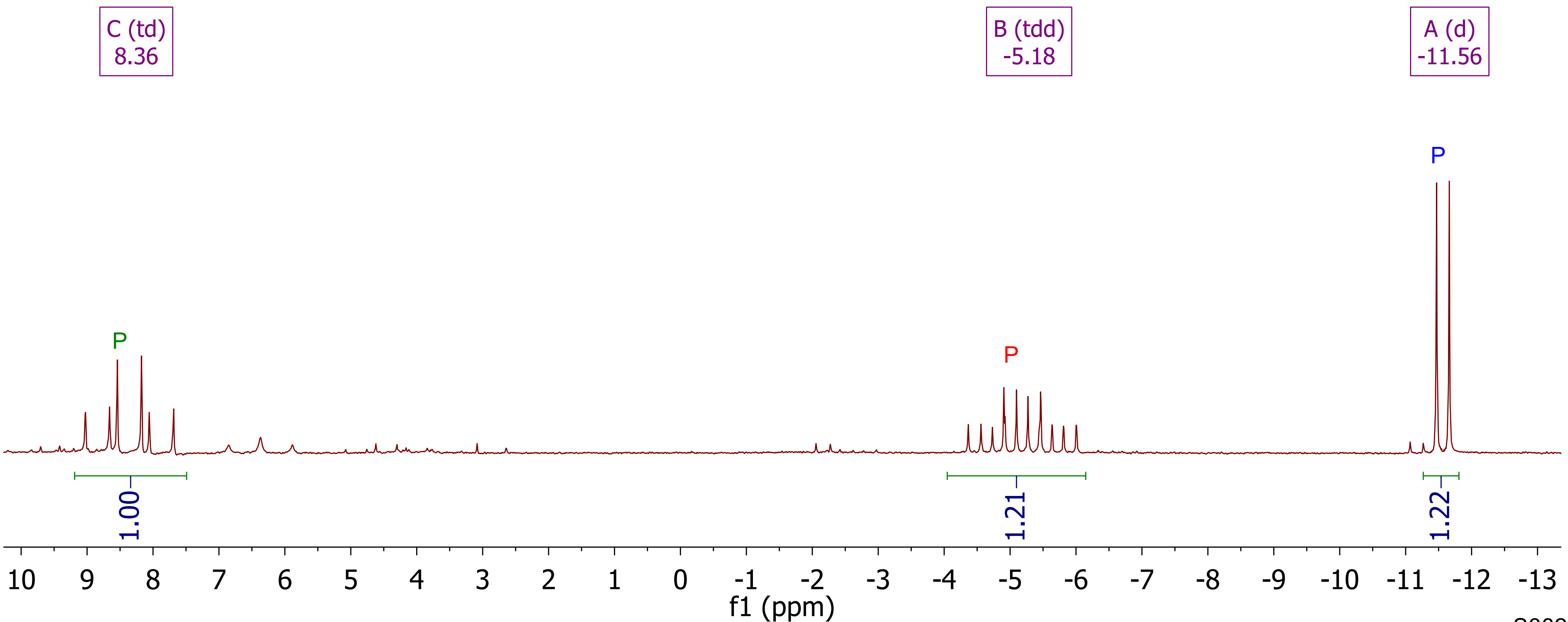


crude product after precipitation
A (s) 7.66
B (t) 6.21
C (m) 4.51
D (m) 4.17
E (m) 2.41
F (s) 1.85
1.00
0.93
0.58
2.68
2.00
2.89
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

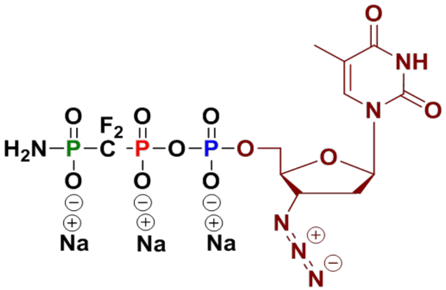
$^{31}P\{^{1}H\}$ NMR



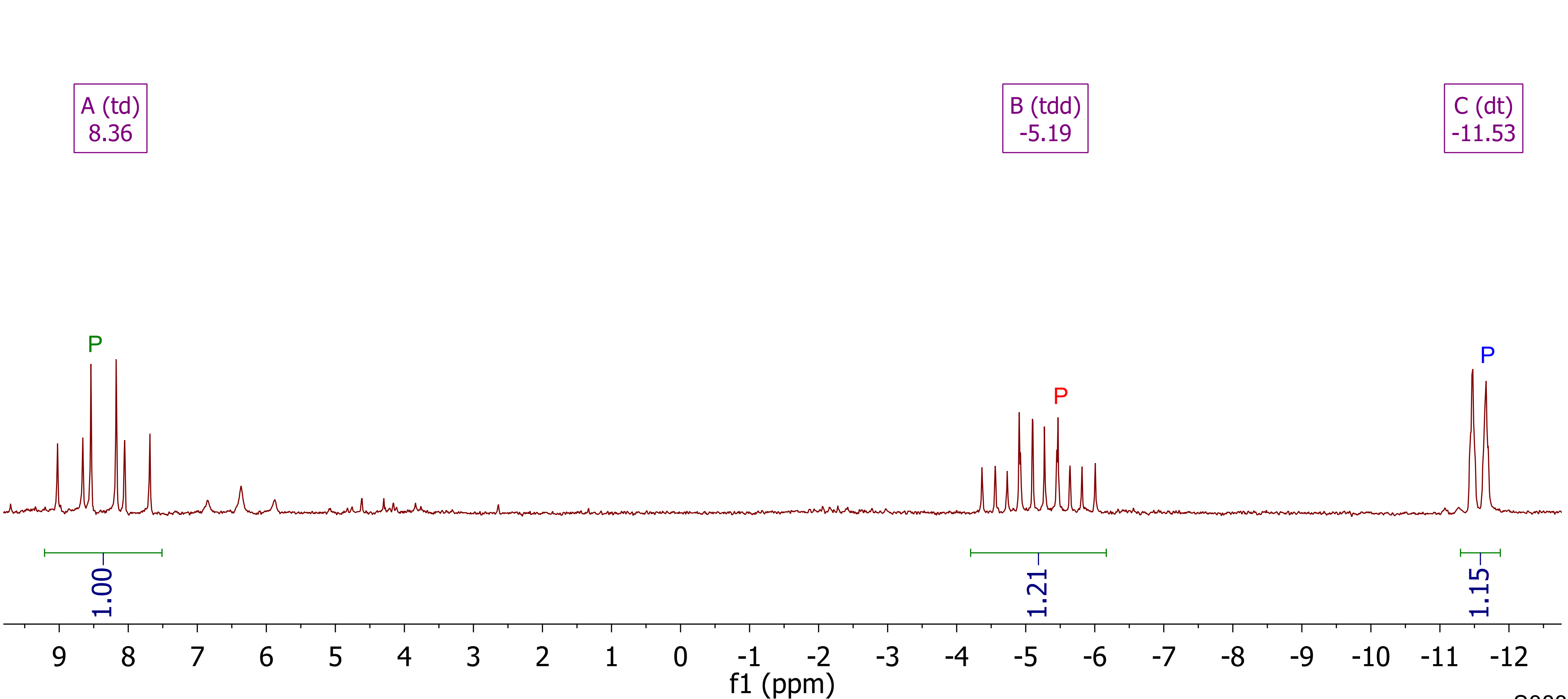
crude product after precipitation



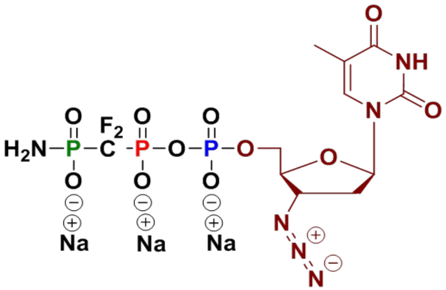
**$^{31}$P NMR**

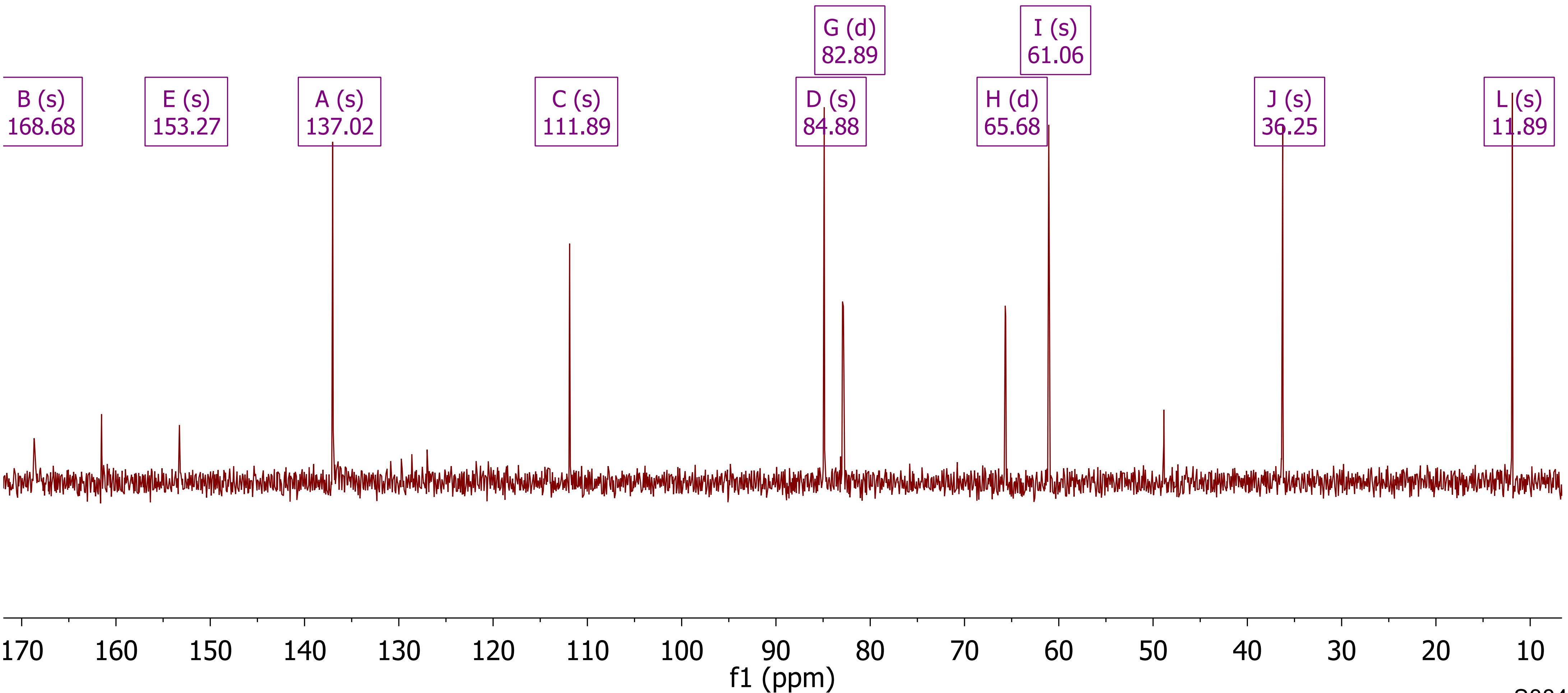


crude product after precipitation

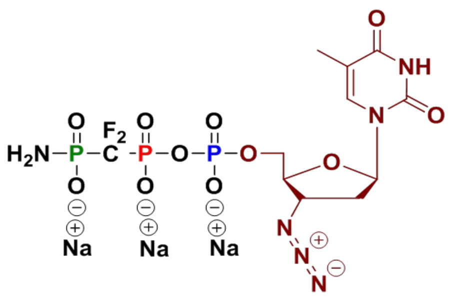


$^{13}C$ NMR

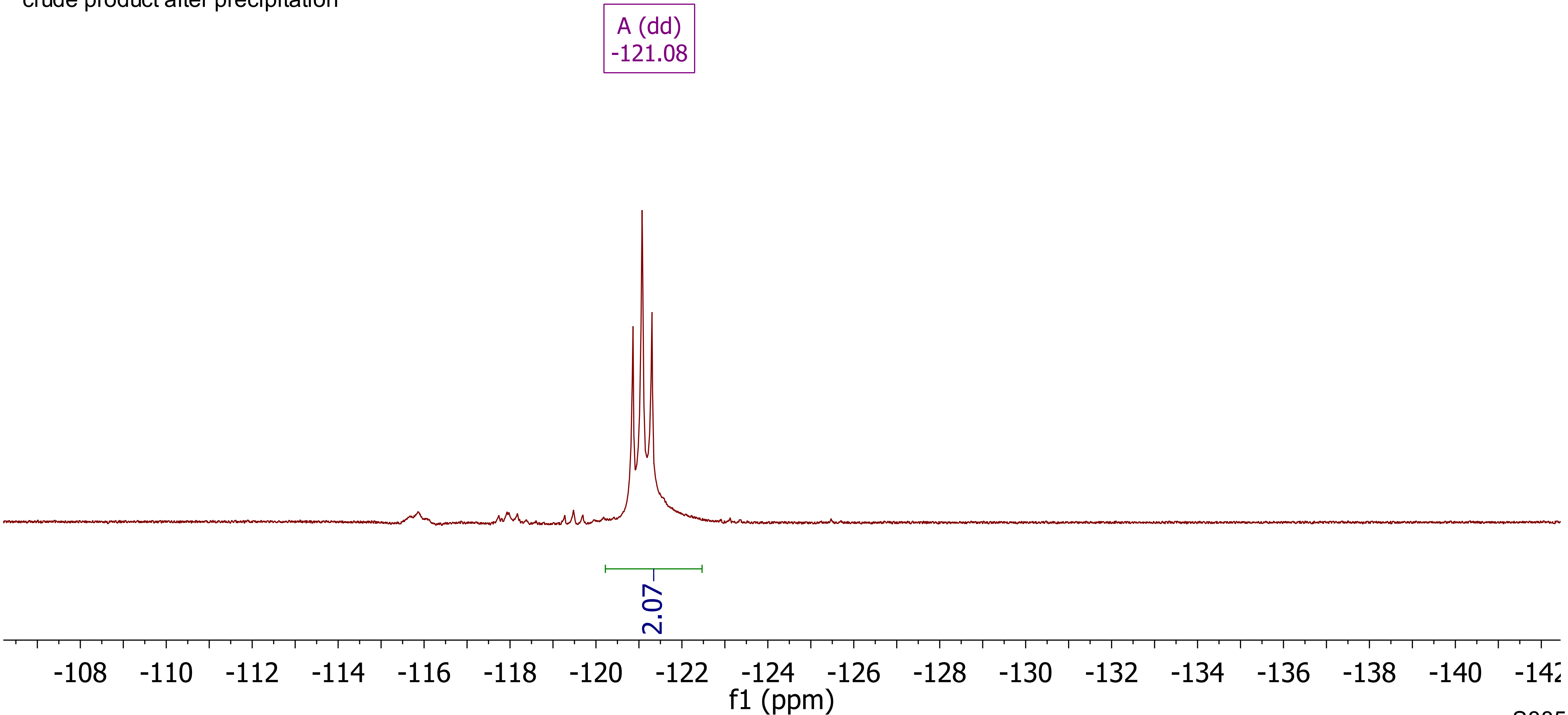


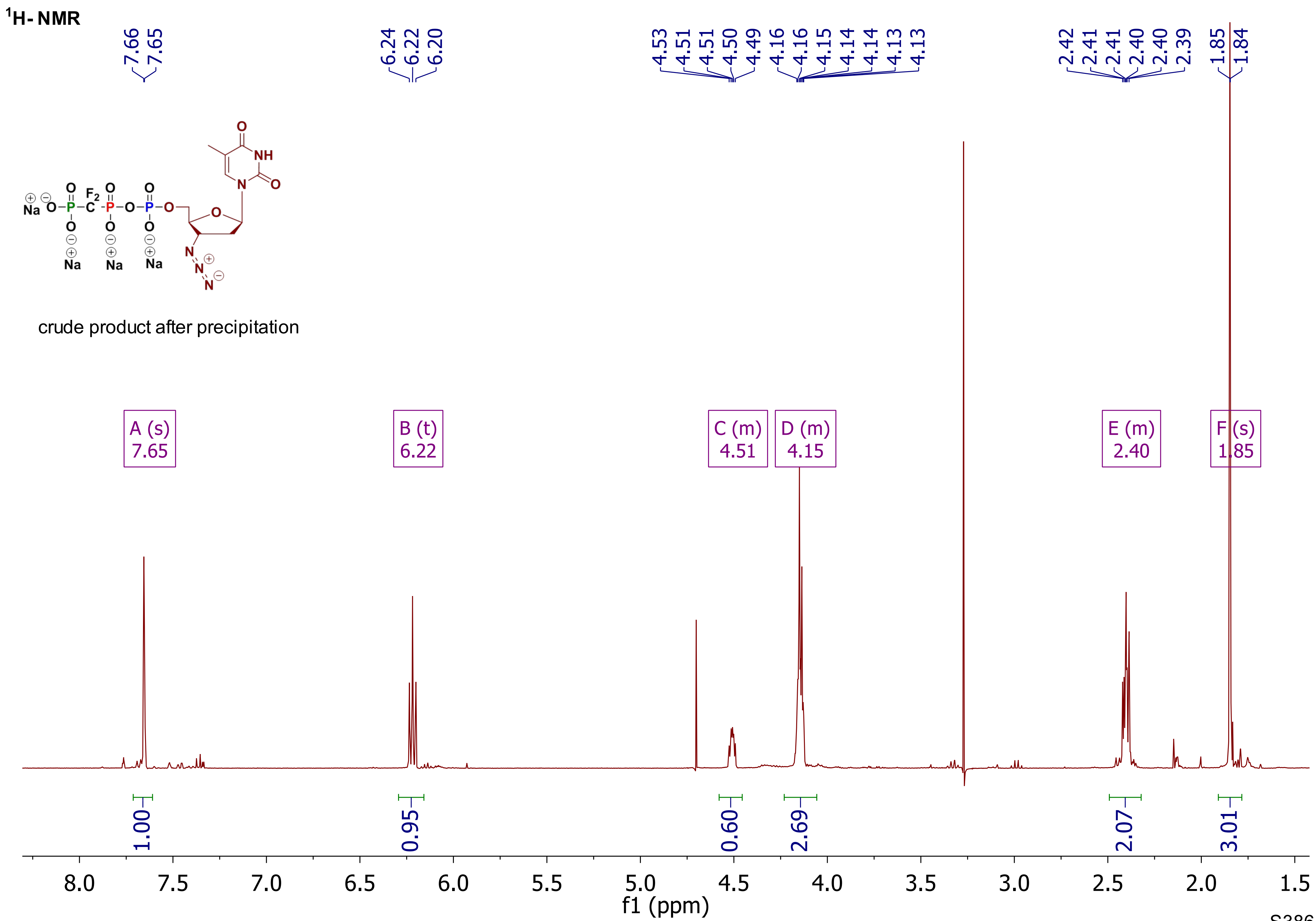

168.68
153.27
137.02
111.89
84.88
82.94
82.85
65.68
61.06
36.25
11.89
crude product after precipitation
B (s) 168.68
E (s) 153.27
A (s) 137.02
C (s) 111.89
D (s) 84.88
G (d) 82.89
H (d) 65.68
I (s) 61.06
J (s) 36.25
L (s) 11.89
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

$^{19}$F NMR



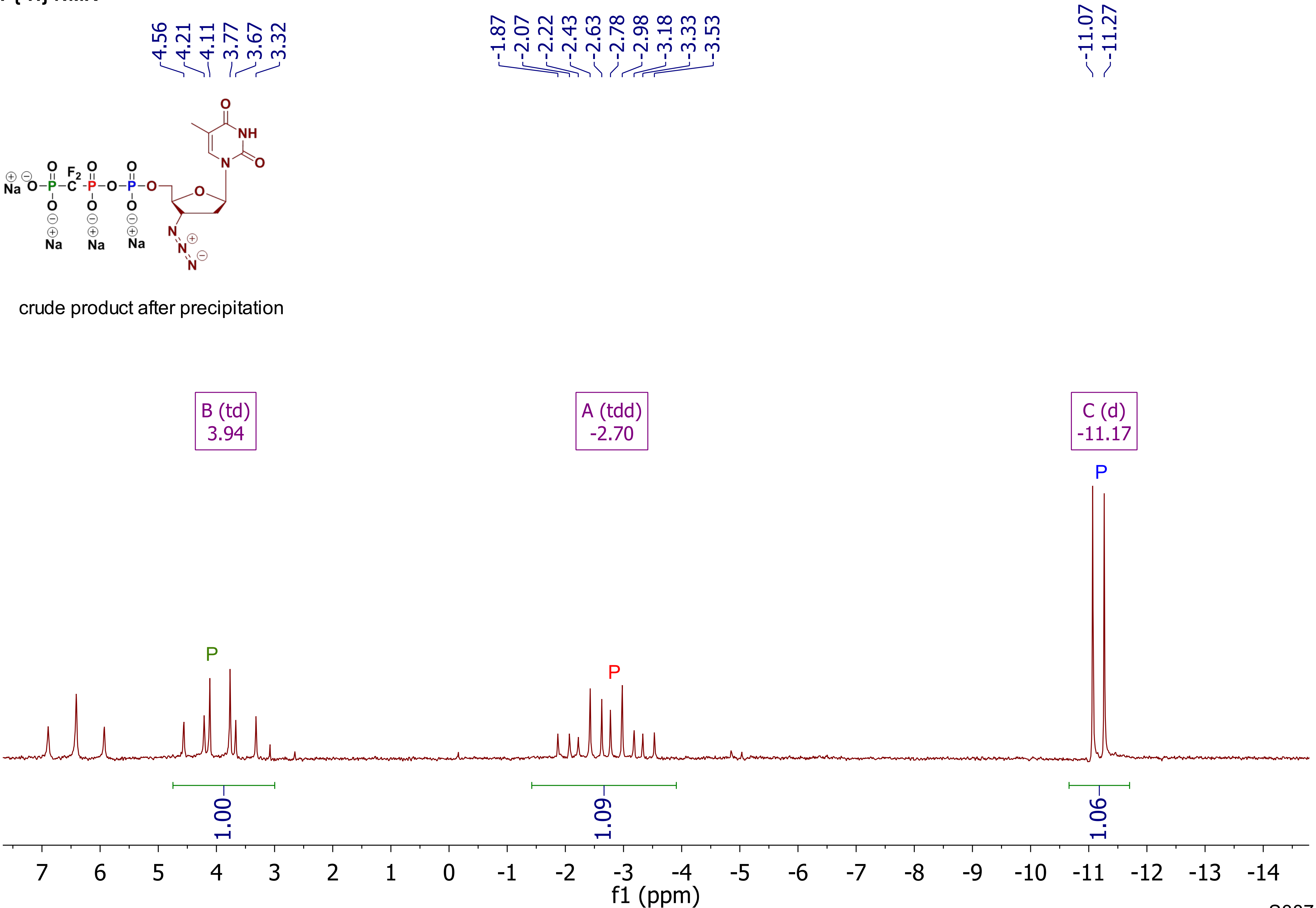
crude product after precipitation



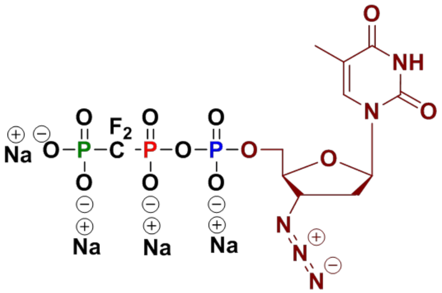


1H- NMR
crude product after precipitation
7.66 7.65
6.24 6.22 6.20
4.53 4.51 4.51 4.50 4.49 4.16 4.16 4.15 4.14 4.14 4.13 4.13
2.42 2.41 2.41 2.40 2.40 2.39 1.85 1.84
A (s) 7.65
B (t) 6.22
C (m) 4.51
D (m) 4.15
E (m) 2.40
F (s) 1.85
1.00
0.95
0.60
2.69
2.07
3.01
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f1 (ppm)

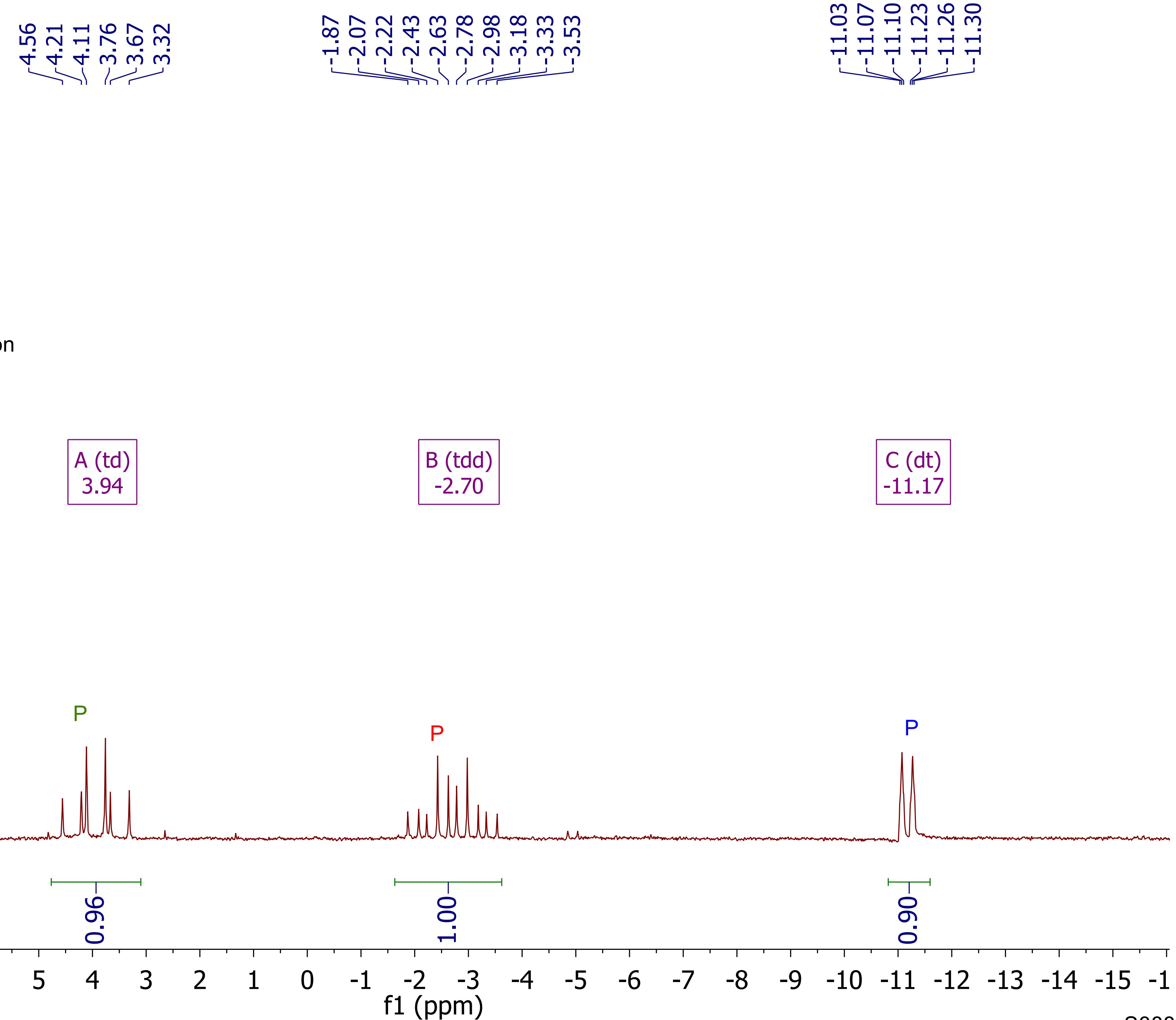
**$^{31}P\{^{1}H\}$ NMR**


4.56
4.21
4.11
3.77
3.67
3.32
-1.87
-2.07
-2.22
-2.43
-2.63
-2.78
-2.98
-3.18
-3.33
-3.53
-11.07
-11.27
crude product after precipitation
B (td)
3.94
A (tdd)
-2.70
C (d)
-11.17
P
P
P
1.00
1.09
1.06
f1 (ppm)

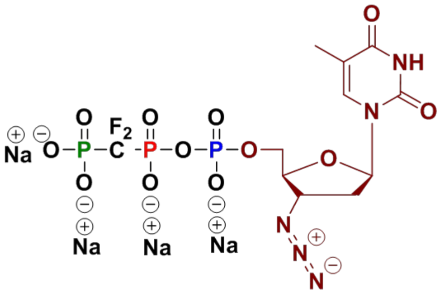
## $^{31}$P NMR



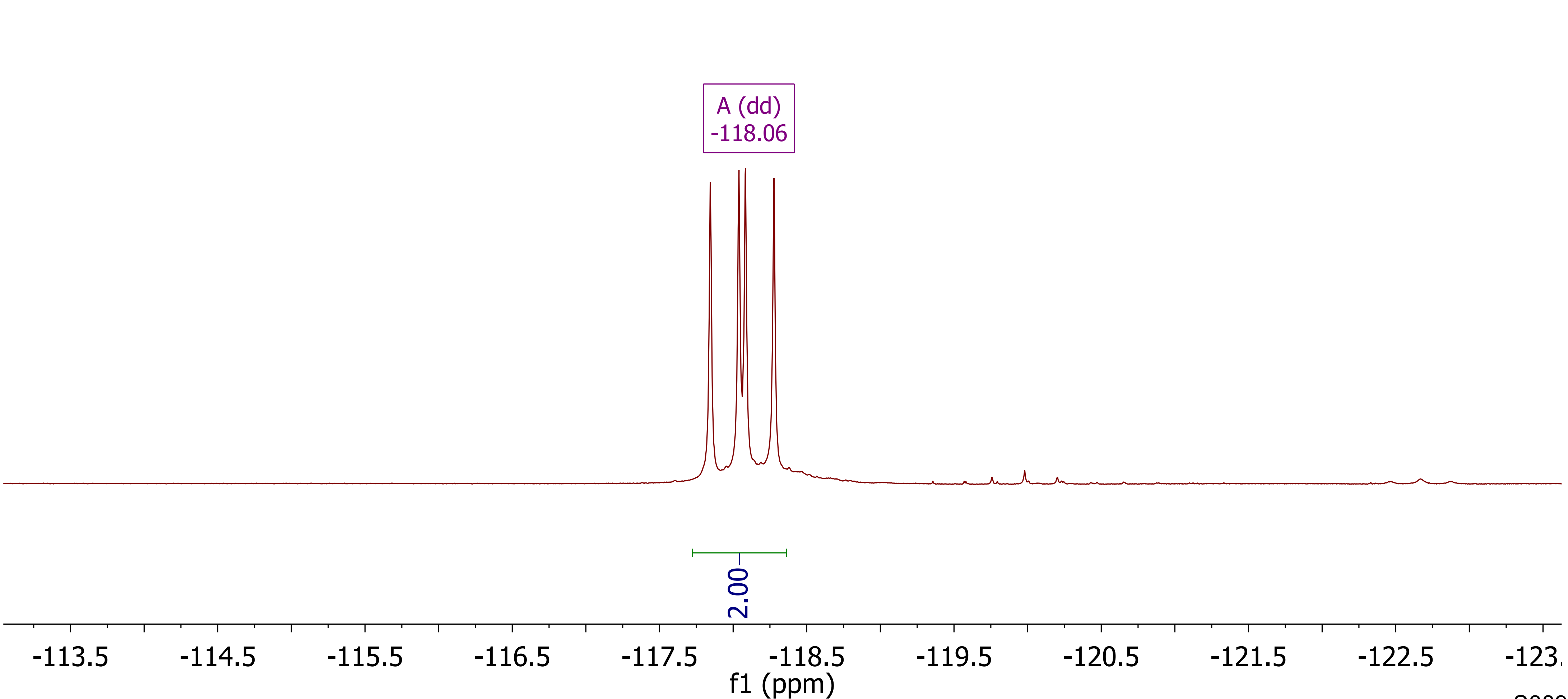

crude product after precipitation



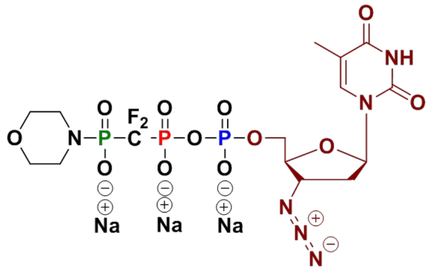
$^{31}P\{^{19}F\}$ NMR



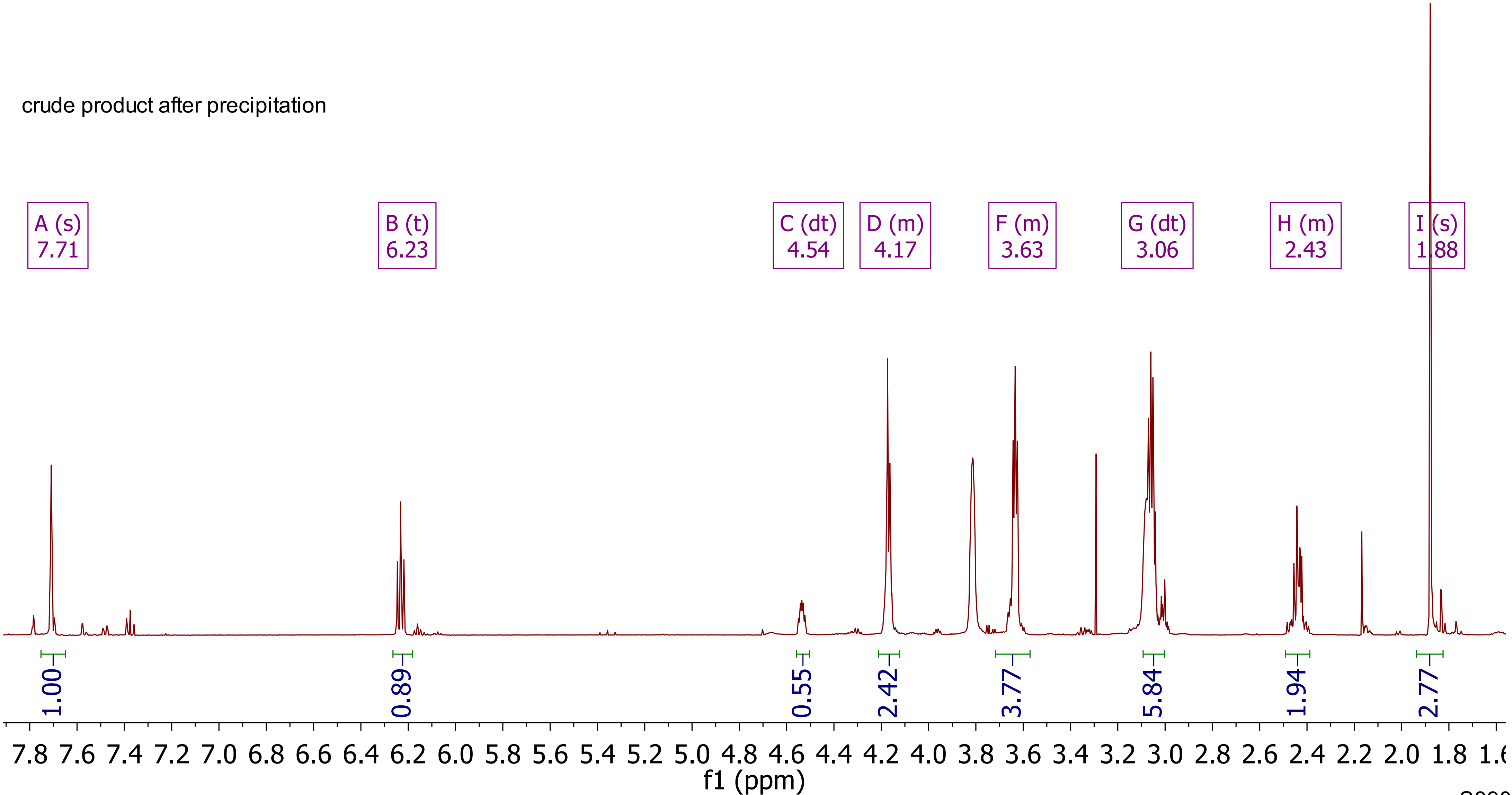
crude product after precipitation



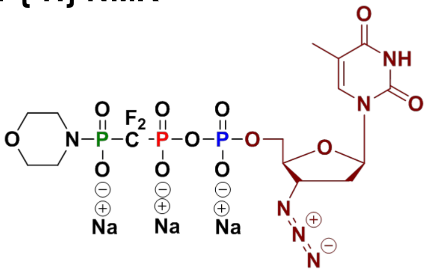
$^{1}$H NMR



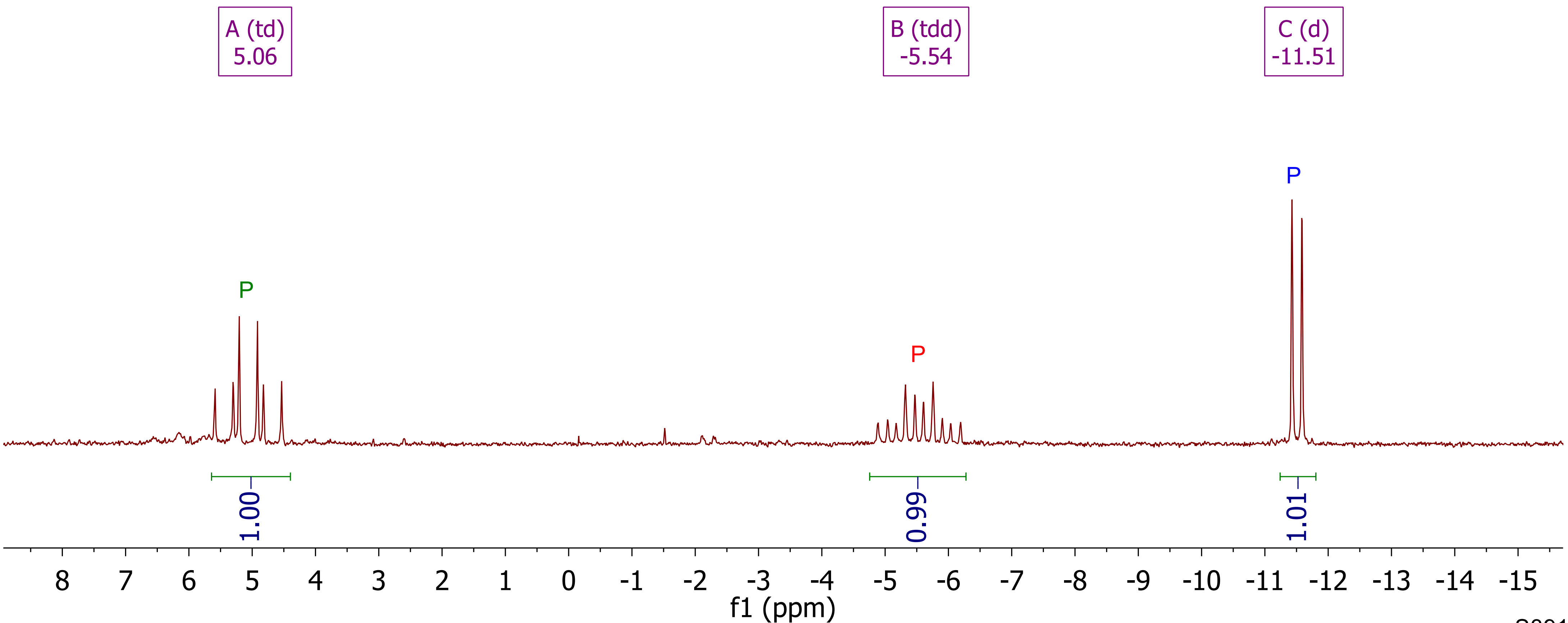
crude product after precipitation



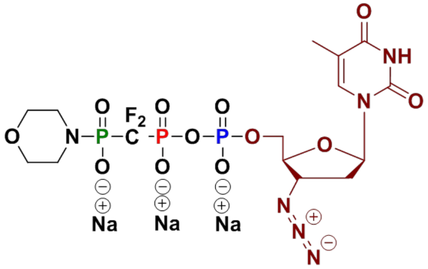
**$^{31}P\{^1H\}$ NMR**



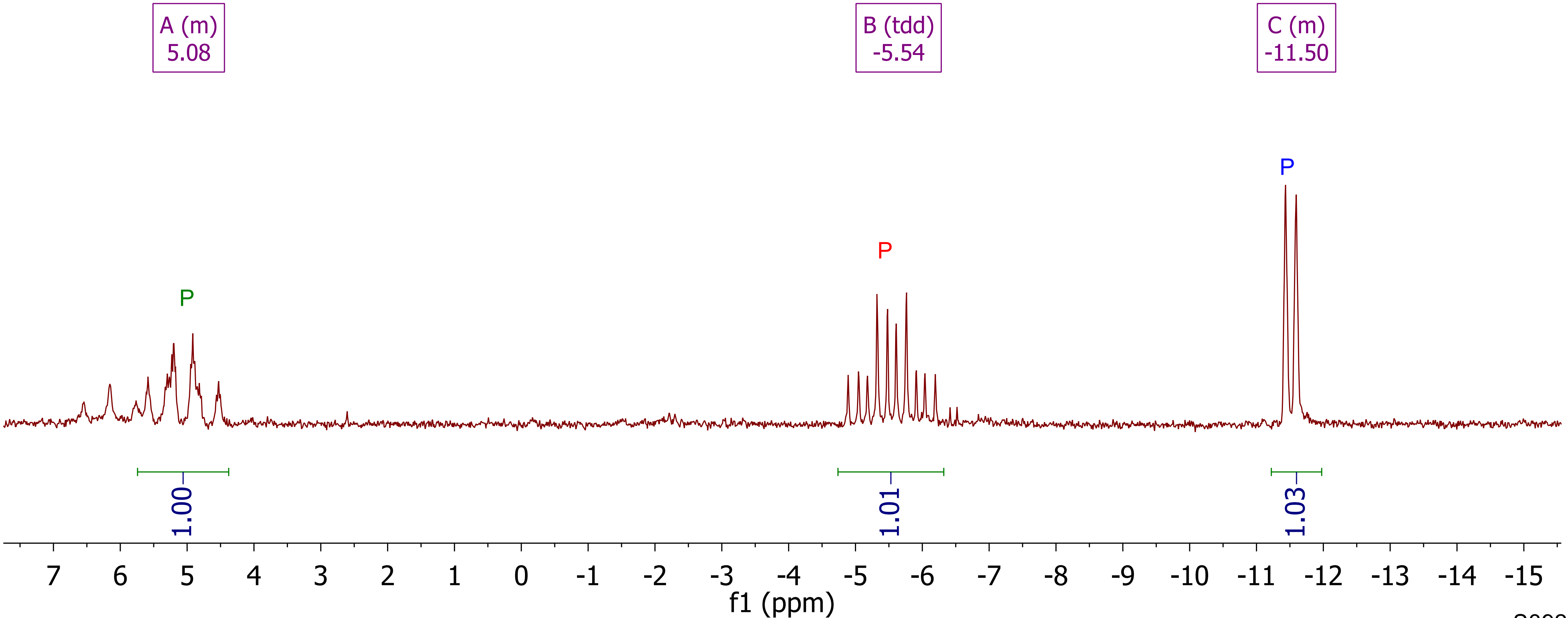
crude product after precipitation



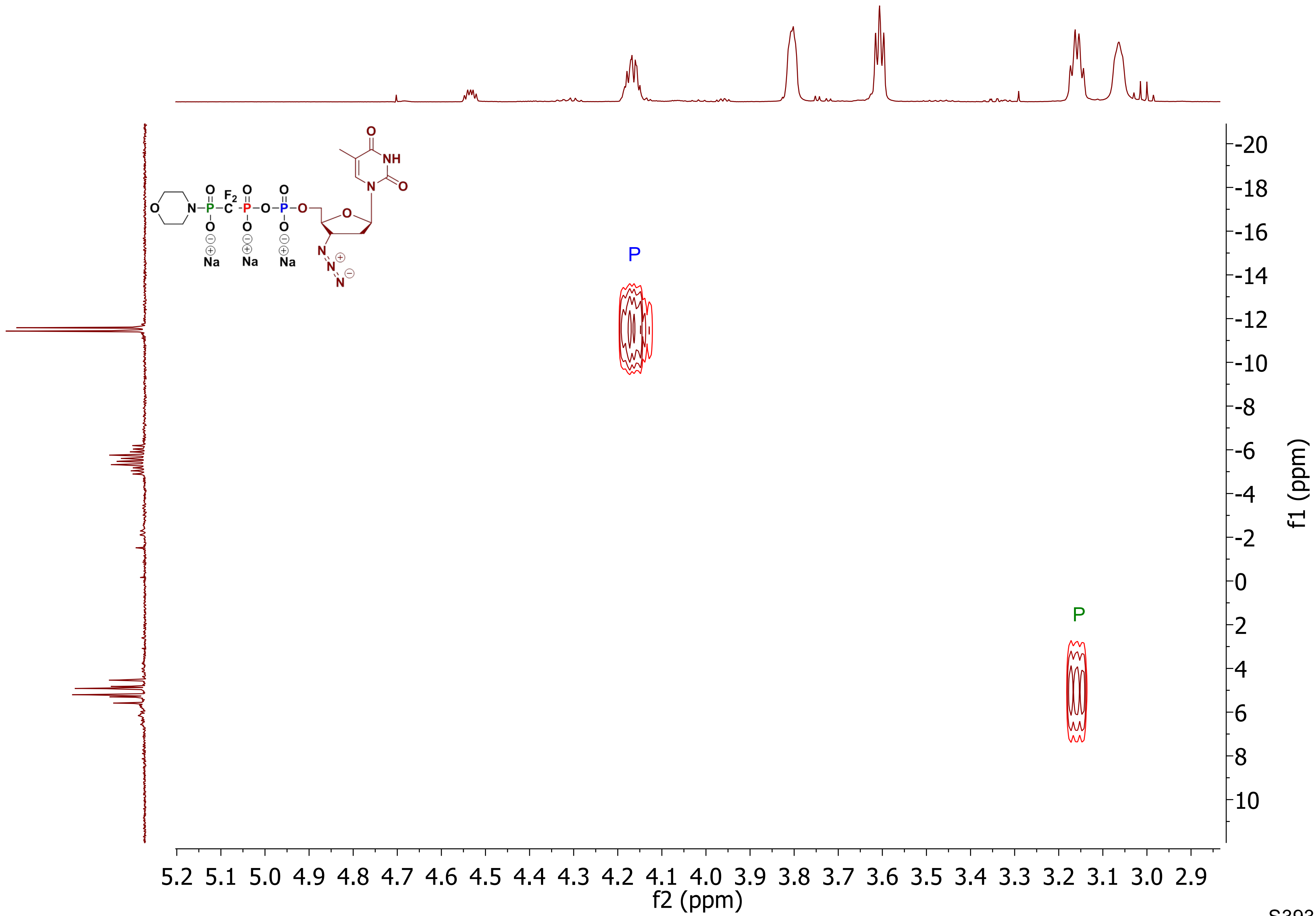
**$^{31}$P NMR**



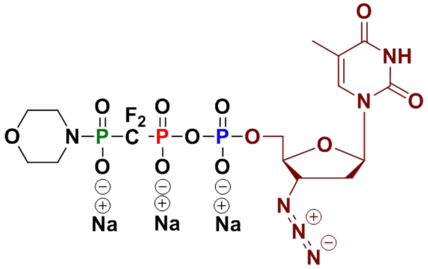

crude product after precipitation



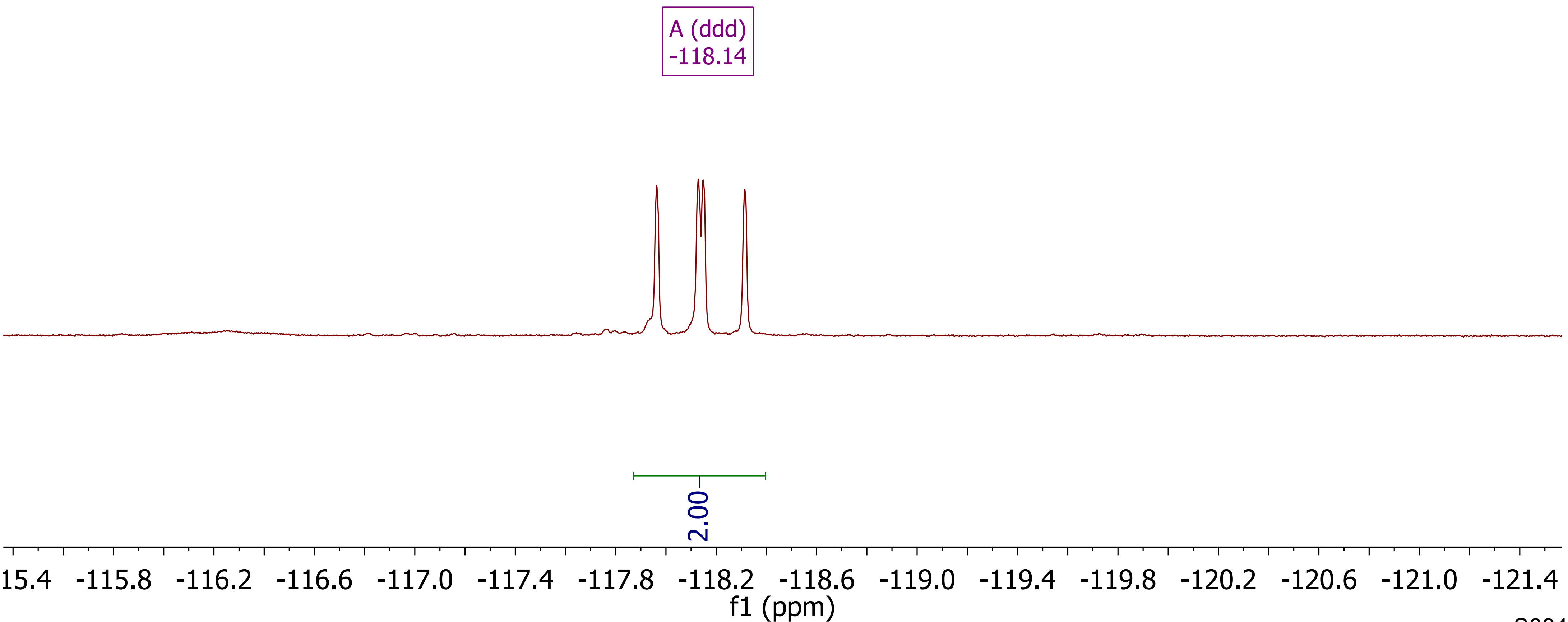
$^{31}P$-$^{1}H$ HMBC NMR



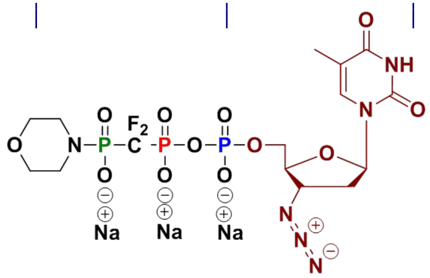
$^{19}$F NMR



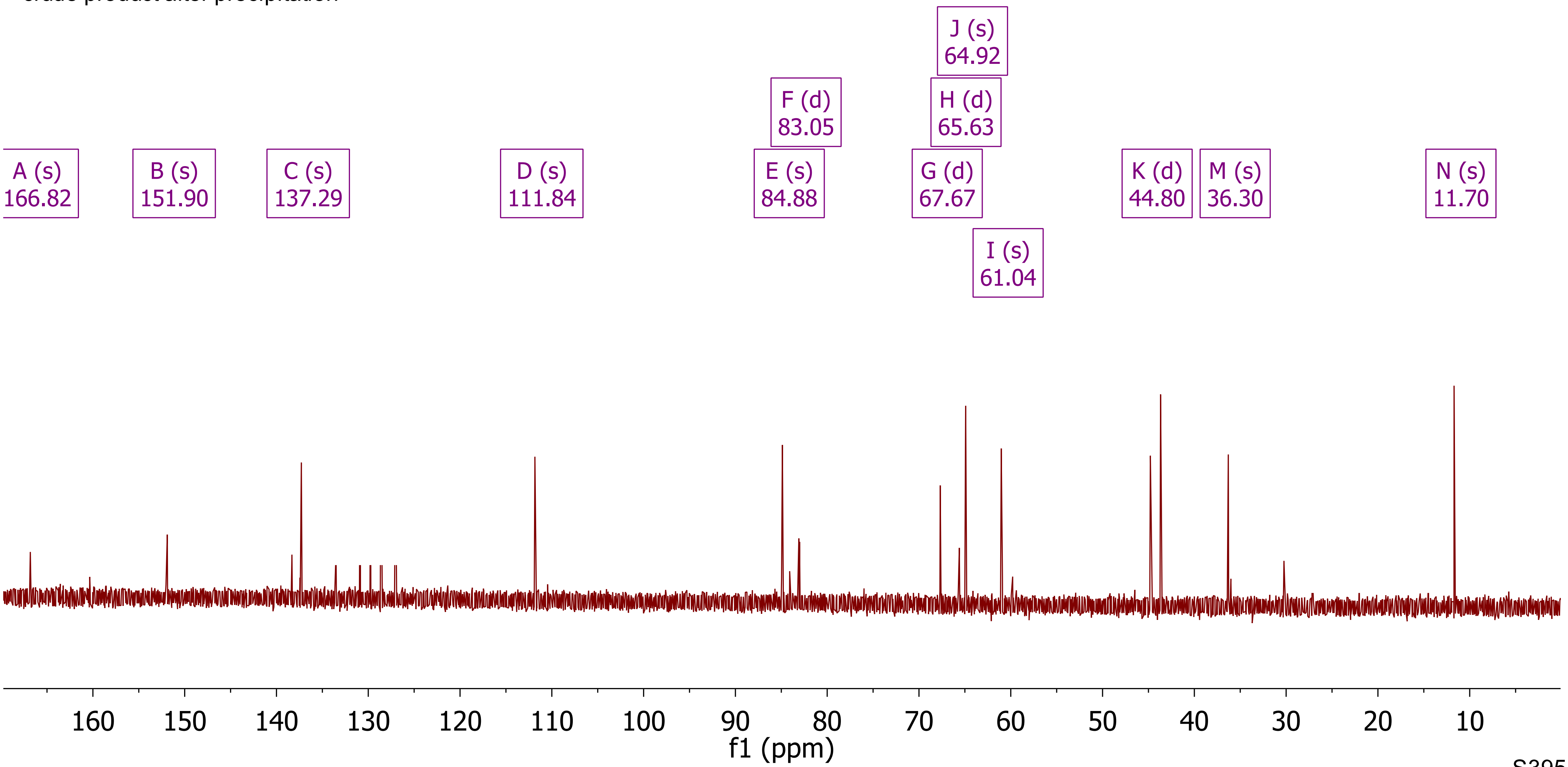
crude product after precipitation



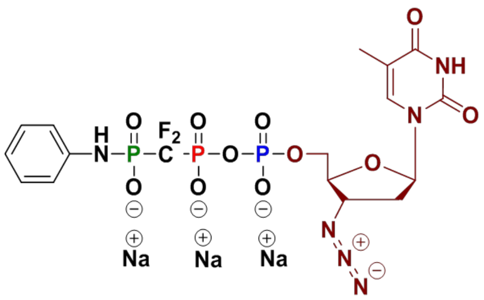
## $^{13}C$ NMR



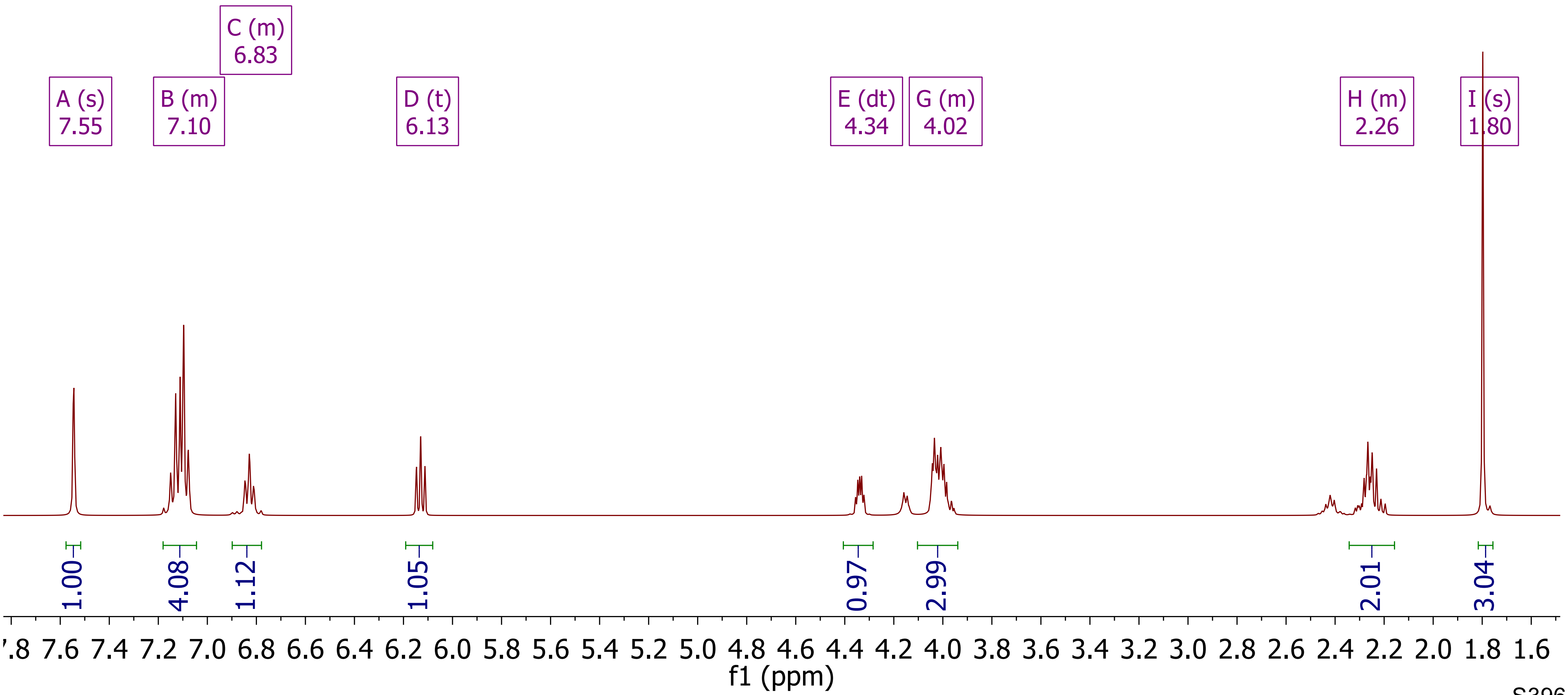
crude product after precipitation



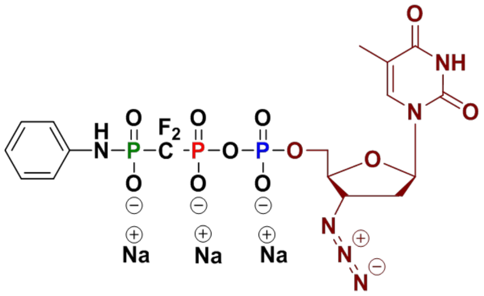
$^{1}$H NMR



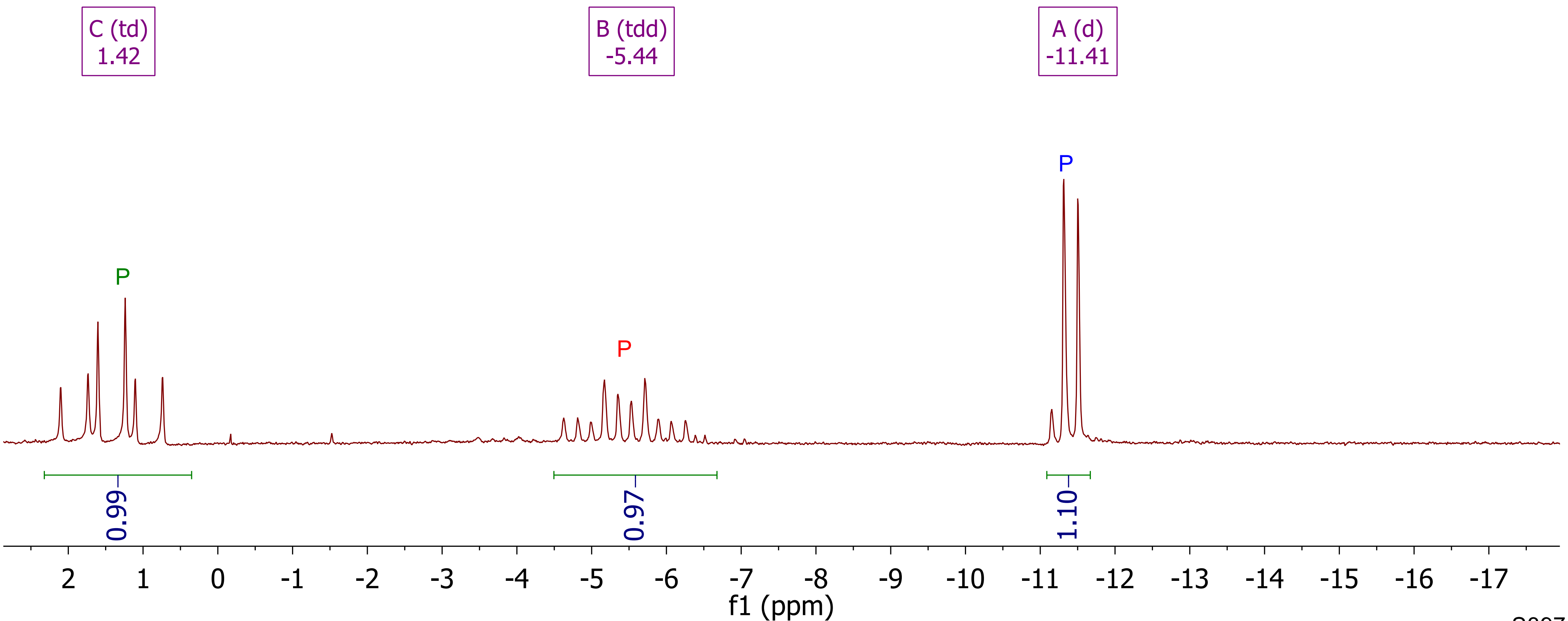
crude product after precipitation



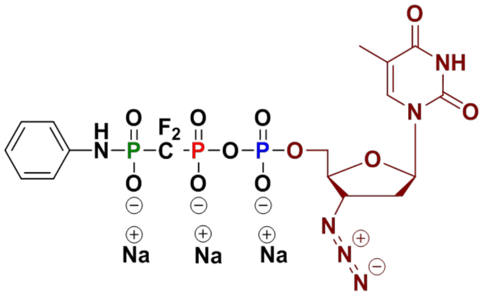
$^{31}P\{^1H\}$ NMR



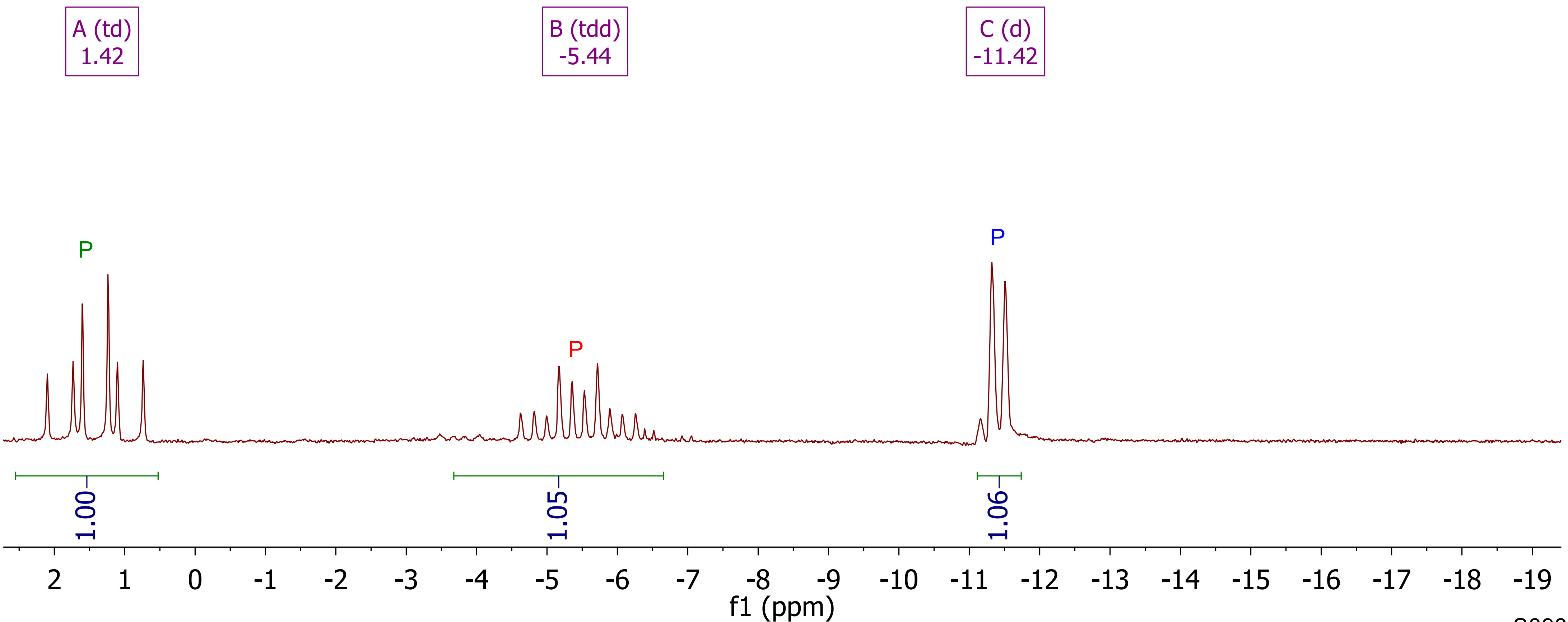
crude product after precipitation



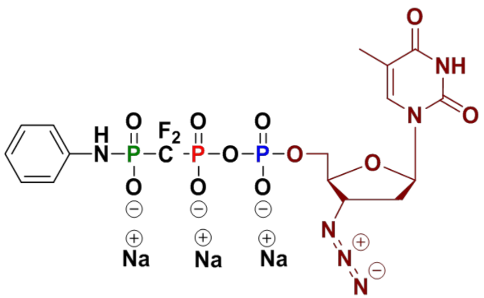
$^{31}$P NMR



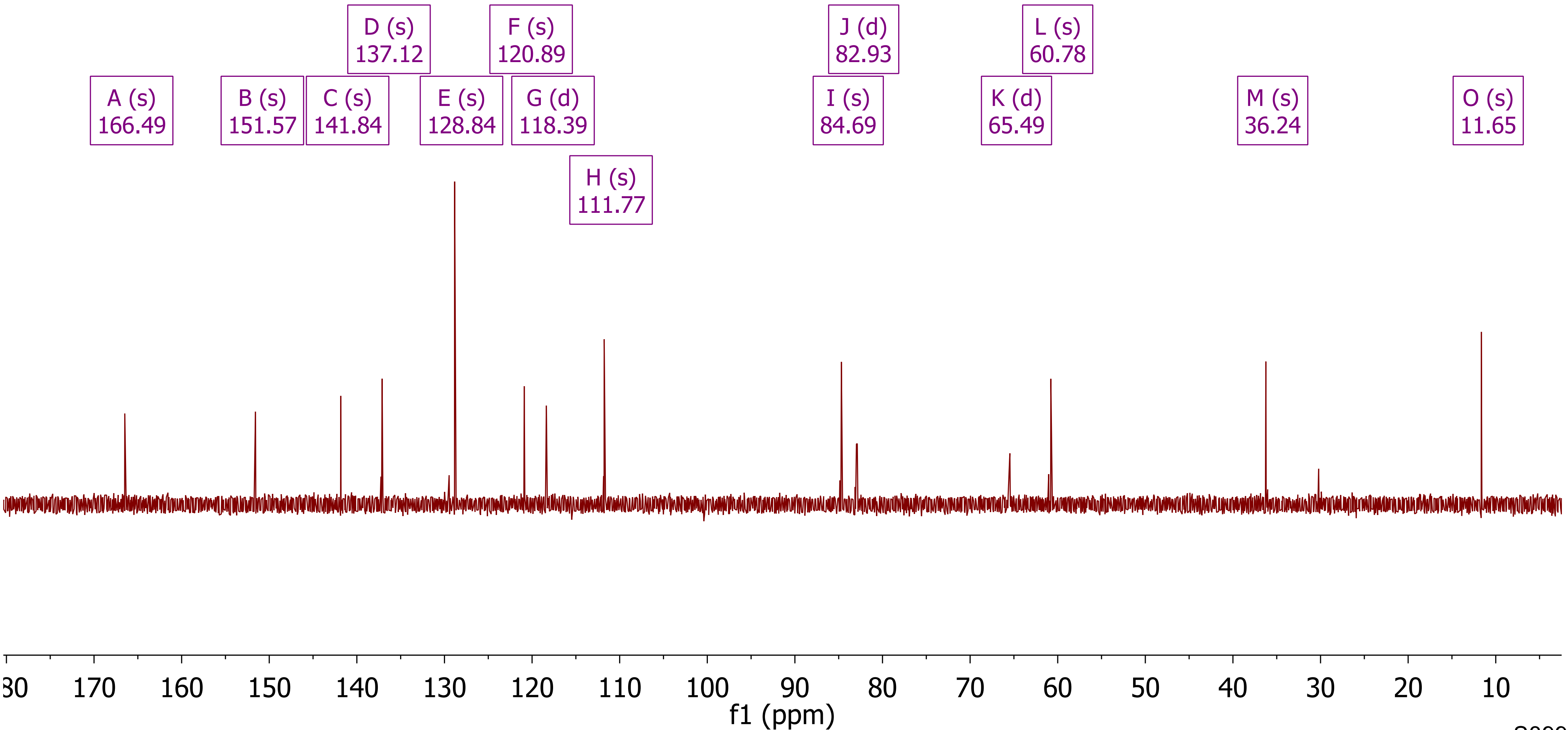
crude product after precipitation



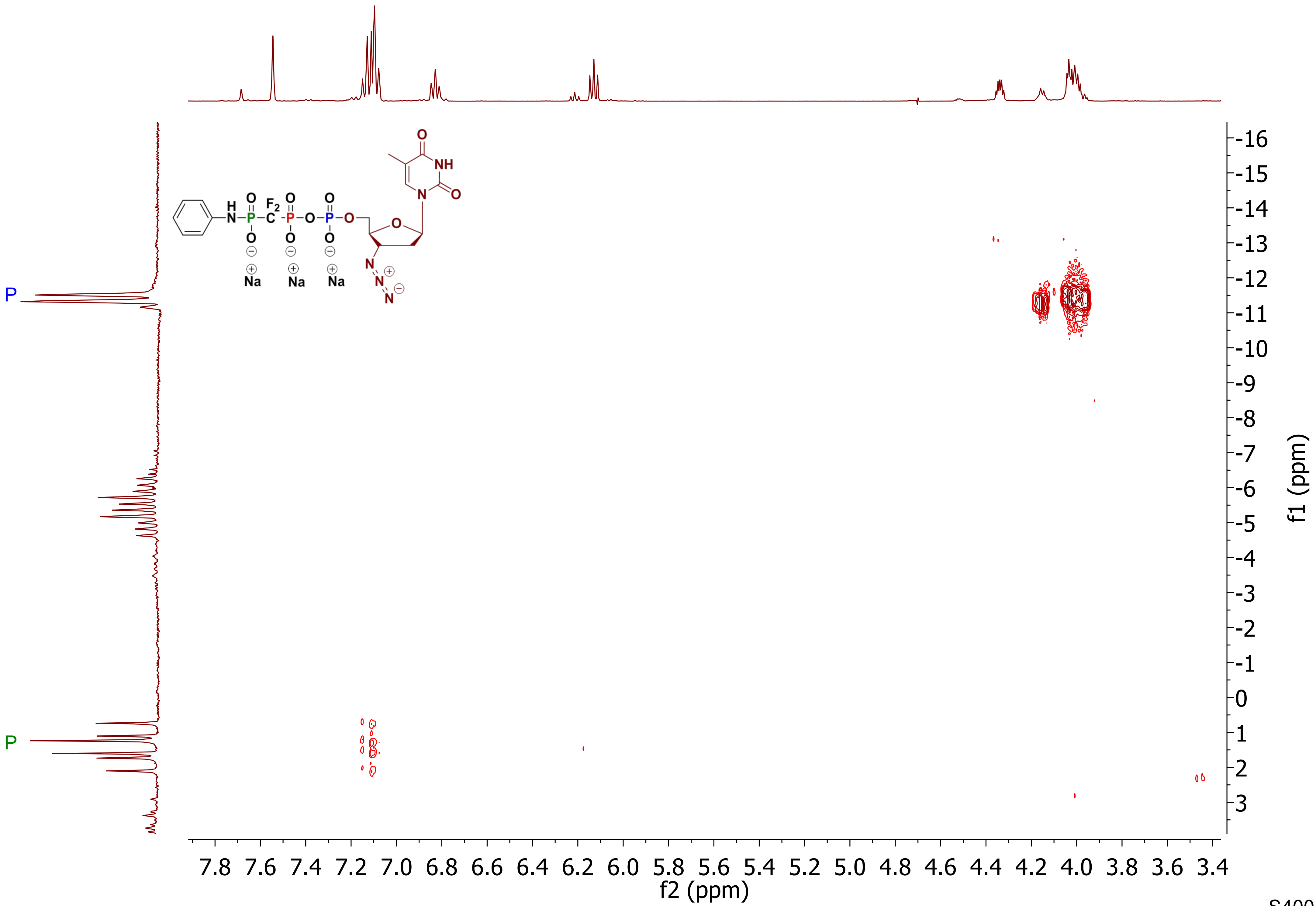
**$^{13}$C NMR**



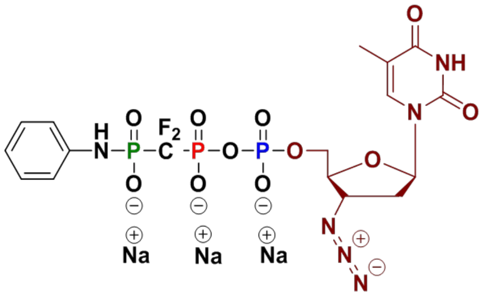
crude product after precipitation



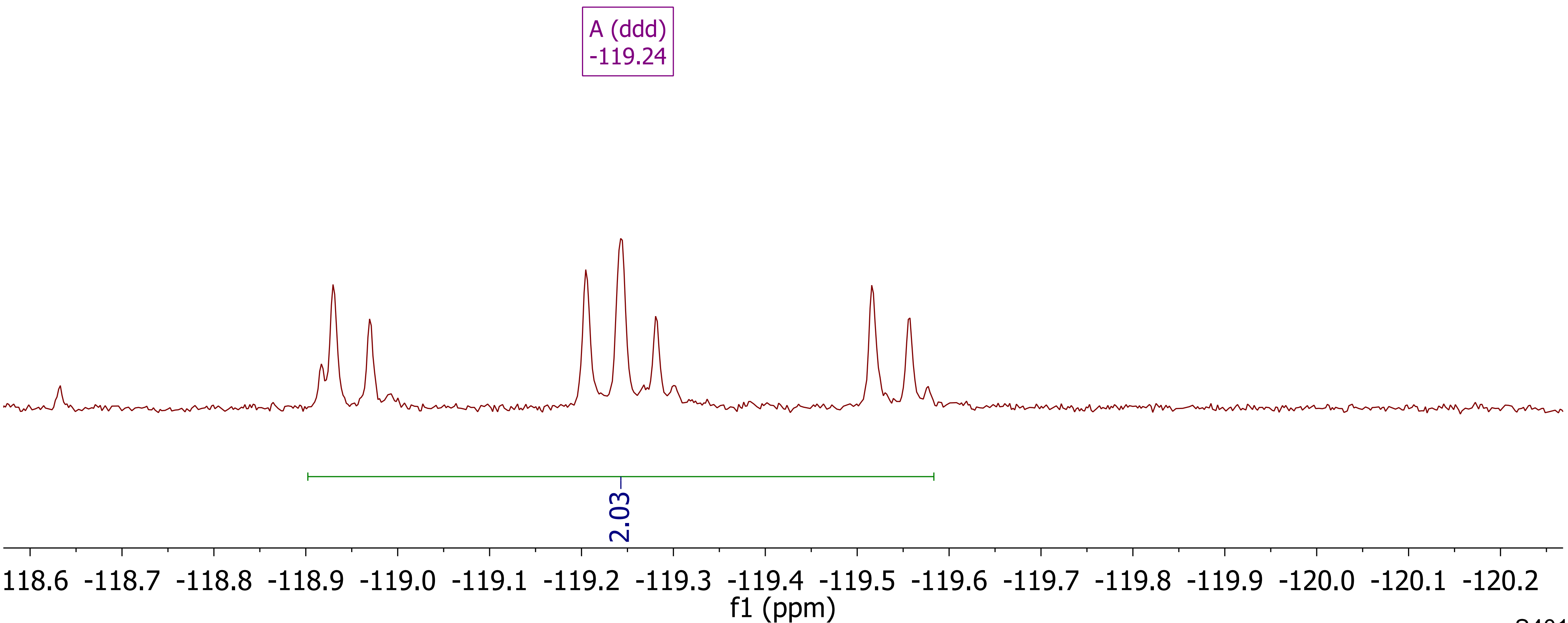
**$^{31}$P-$^{1}$H HMBC NMR**



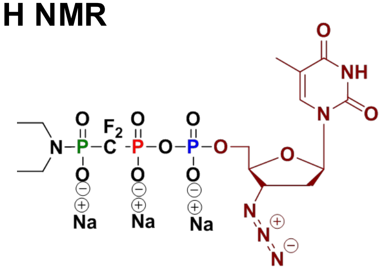
$^{19}$F NMR



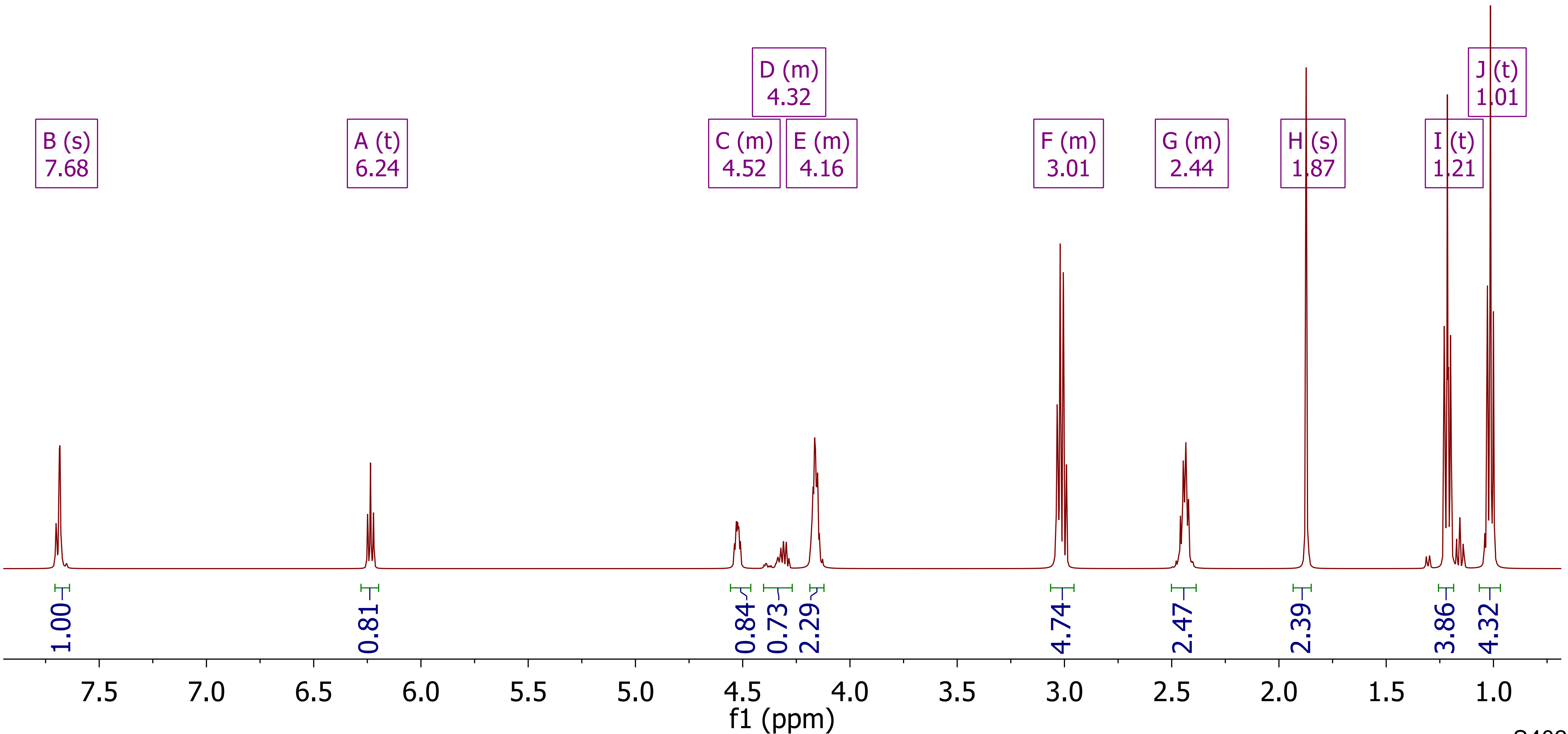
crude product after precipitation



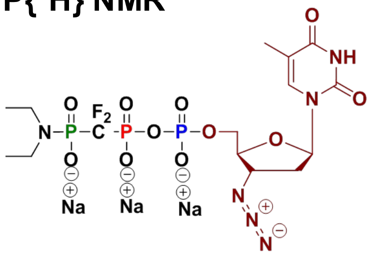
$^1$H NMR



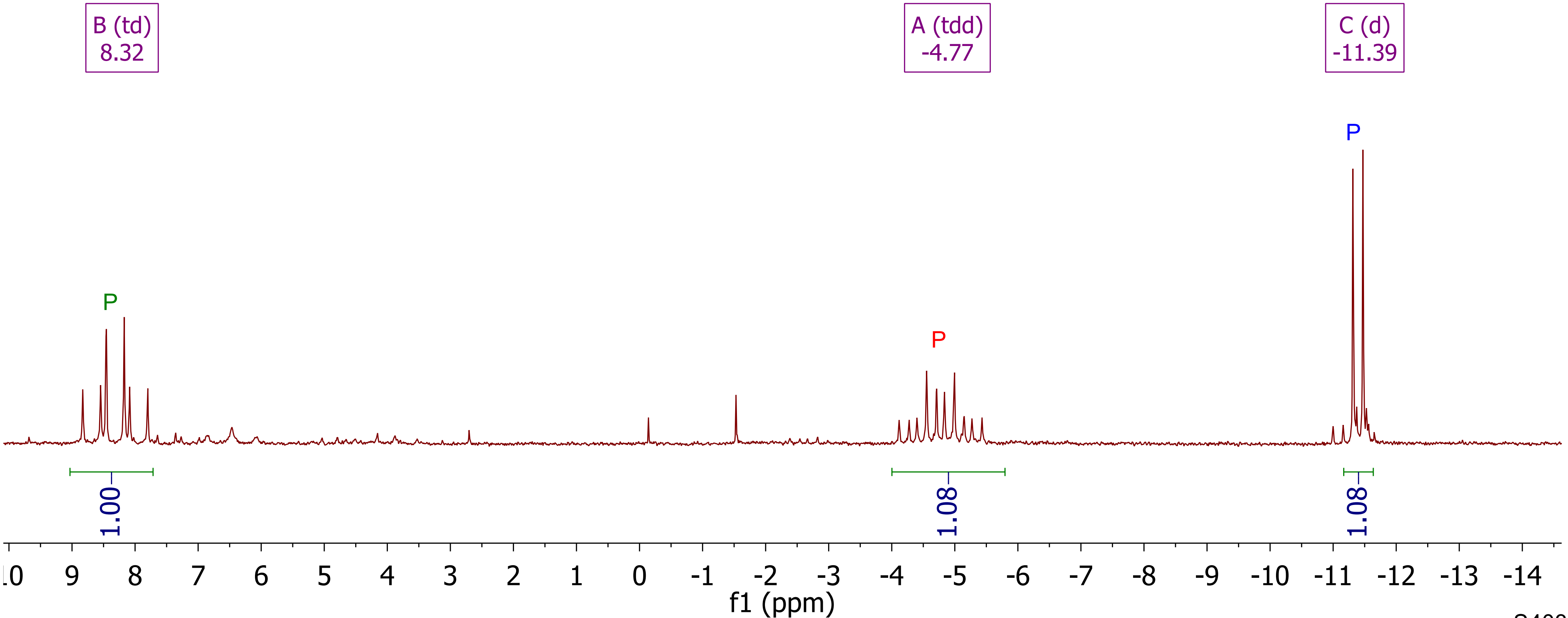
crude product after precipitation

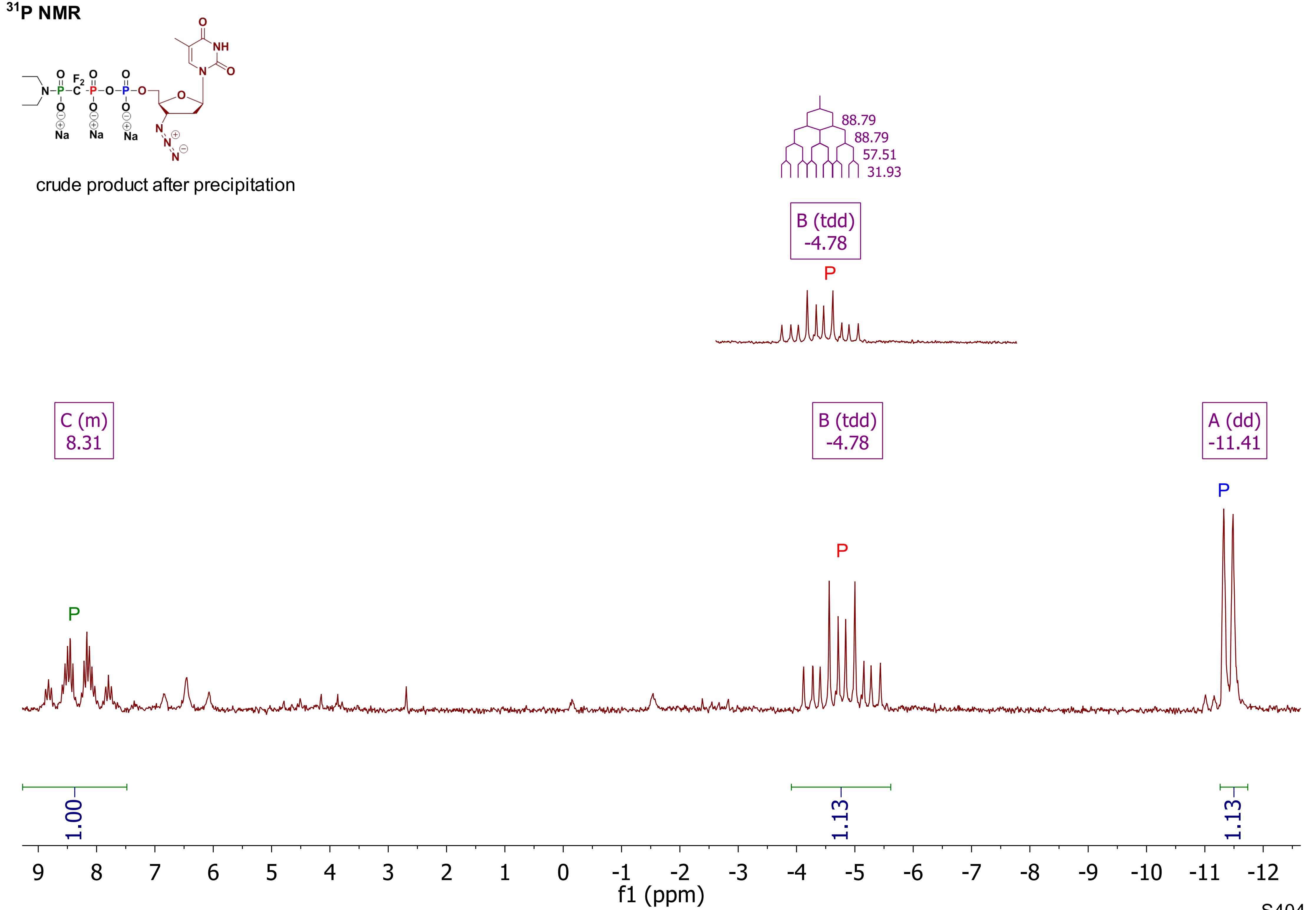


**$^{31}P\{^{1}H\}$ NMR**



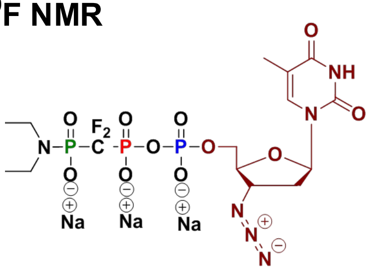
crude product after precipitation



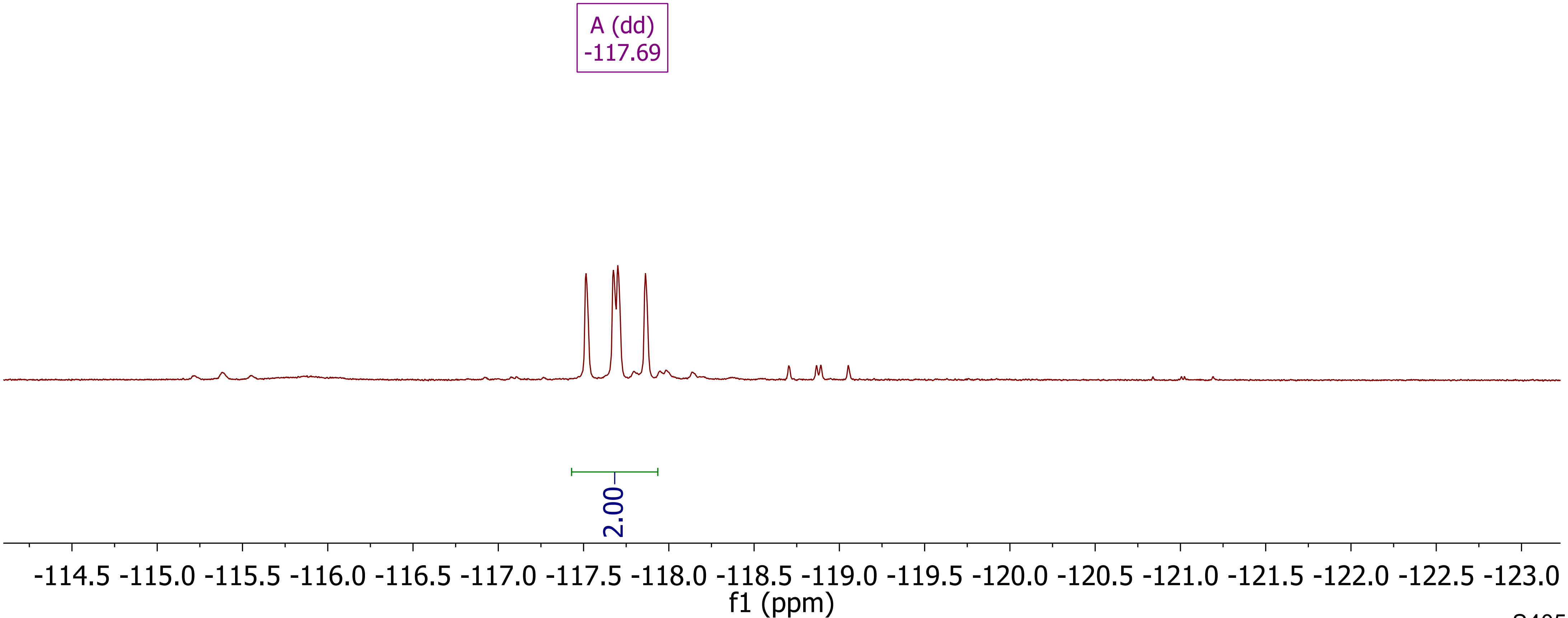


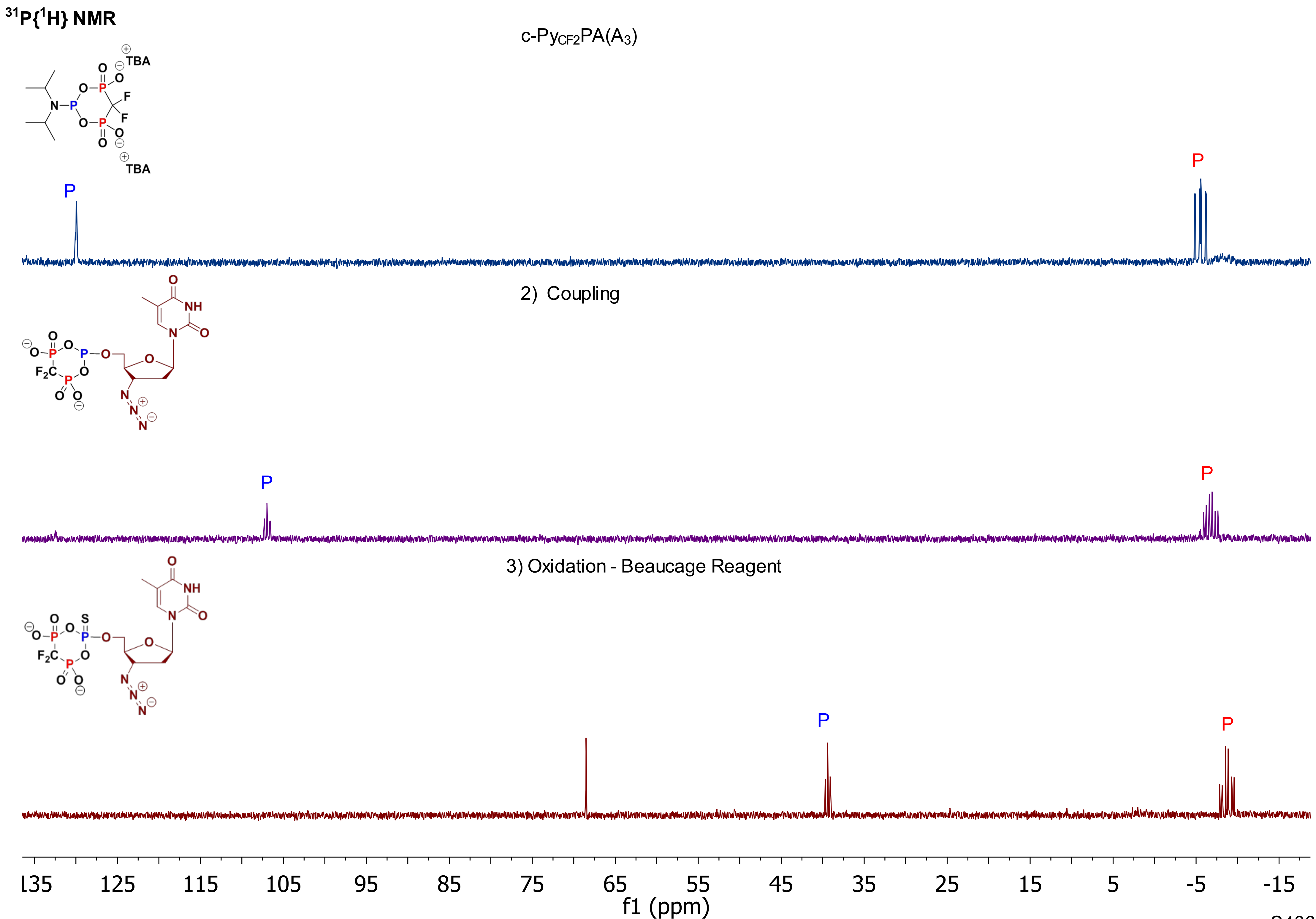
31P NMR
crude product after precipitation
88.79
88.79
57.51
31.93
B (tdd)
-4.78
P
C (m)
8.31
B (tdd)
-4.78
A (dd)
-11.41
P
P
P
1.00
1.13
1.13
9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12
f1 (ppm)

**$^{19}$F NMR**

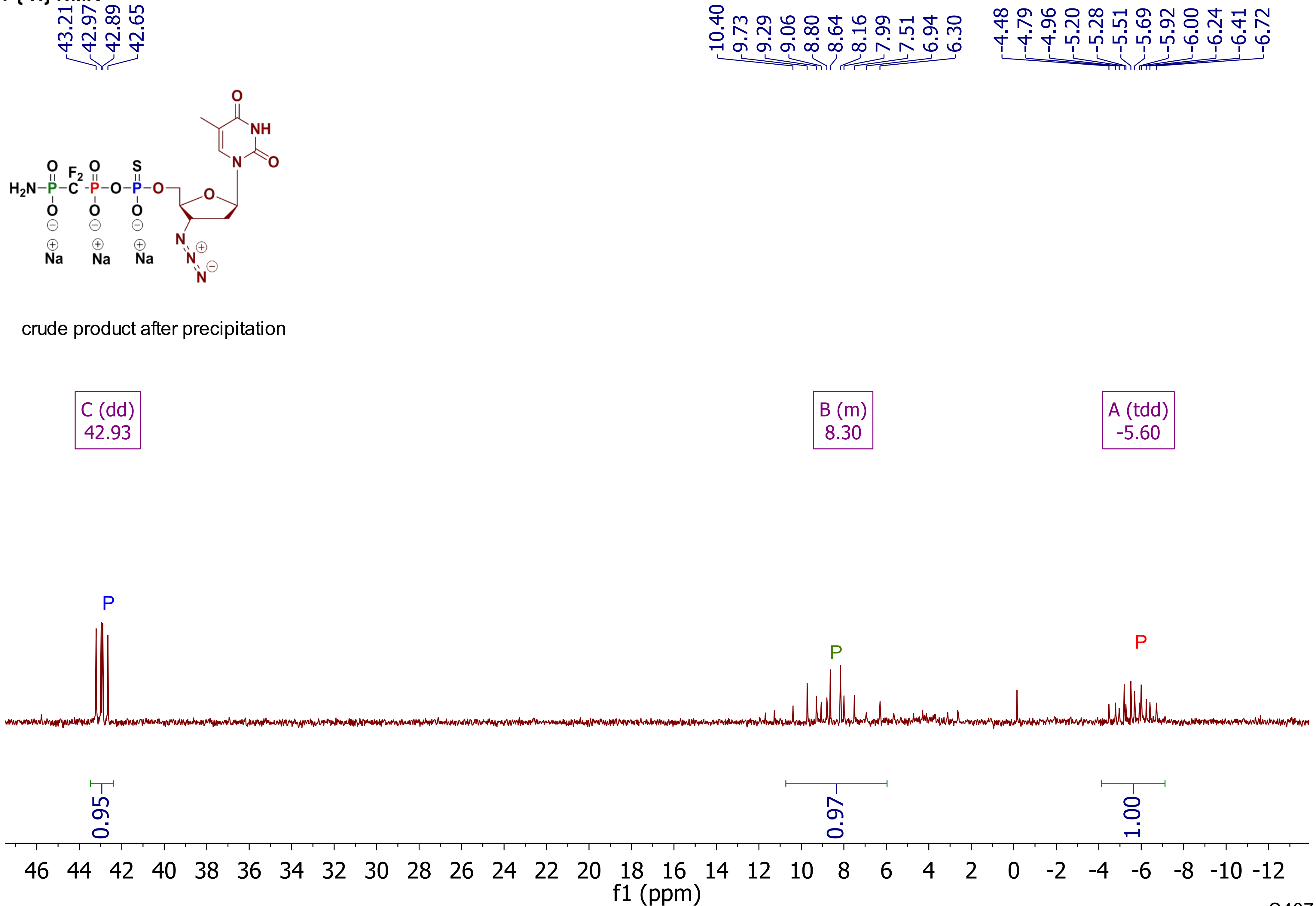



crude product after precipitation

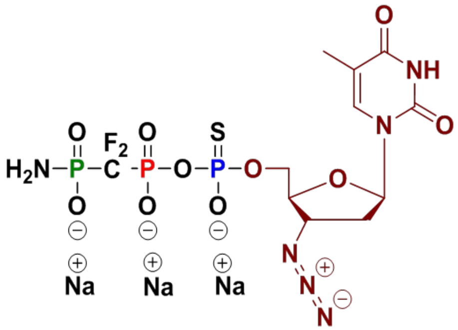



31P{1H} NMR
c-PyCF2PA(A3)
TBA
TBA
2) Coupling
3) Oxidation - Beaucage Reagent
135
125
115
105
95
85
75
65
55
45
35
25
15
5
-5
-15
f1 (ppm)

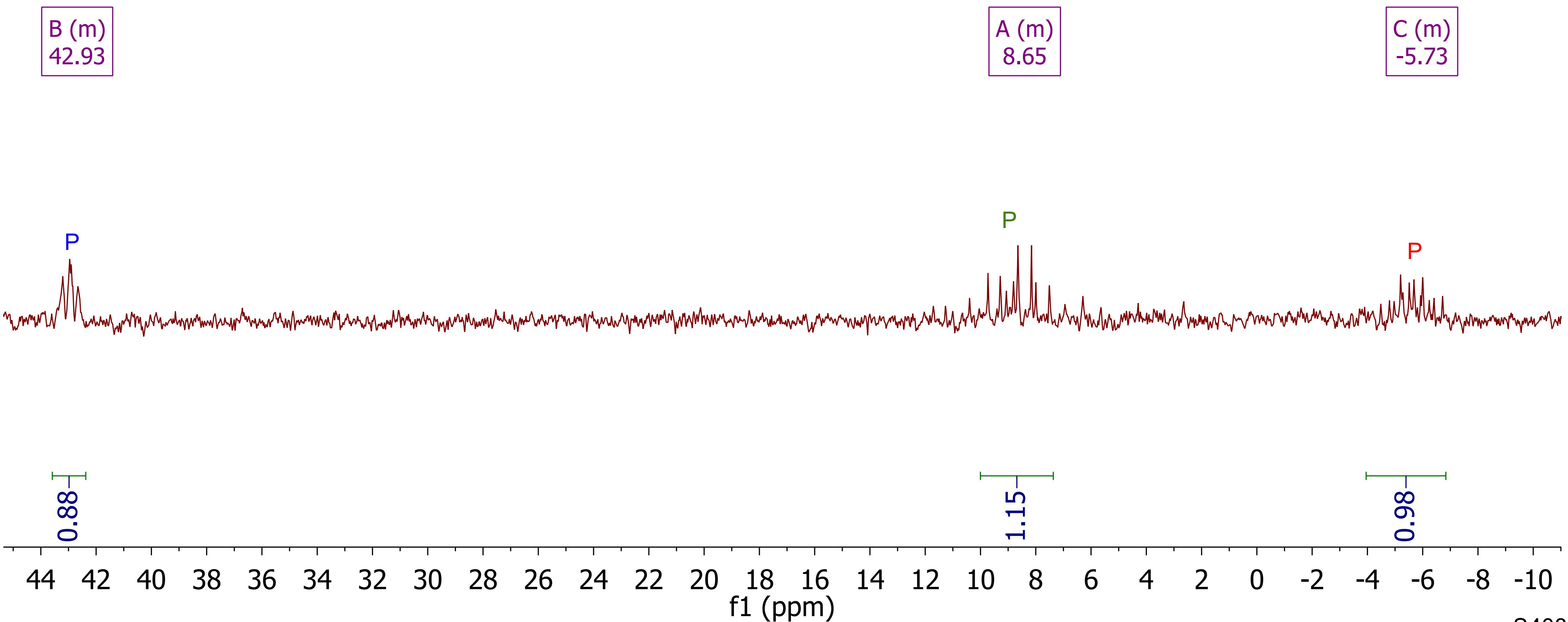
**$^{31}P\{^1H\}$ NMR**


43.21
42.97
42.89
42.65
10.40
9.73
9.29
9.06
8.80
8.64
8.16
7.99
7.51
6.94
6.30
-4.48
-4.79
-4.96
-5.20
-5.28
-5.51
-5.69
-5.92
-6.00
-6.24
-6.41
-6.72
crude product after precipitation
C (dd)
42.93
B (m)
8.30
A (tdd)
-5.60
P
P
P
0.95
0.97
1.00
46 44 42 40 38 36 34 32 30 28 26 24 22 20 18 16 14 12 10 8 6 4 2 0 -2 -4 -6 -8 -10 -12
f1 (ppm)

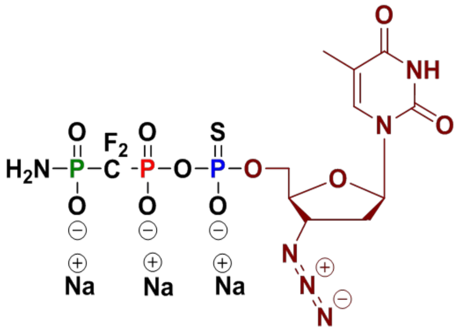
$^{31}$P NMR



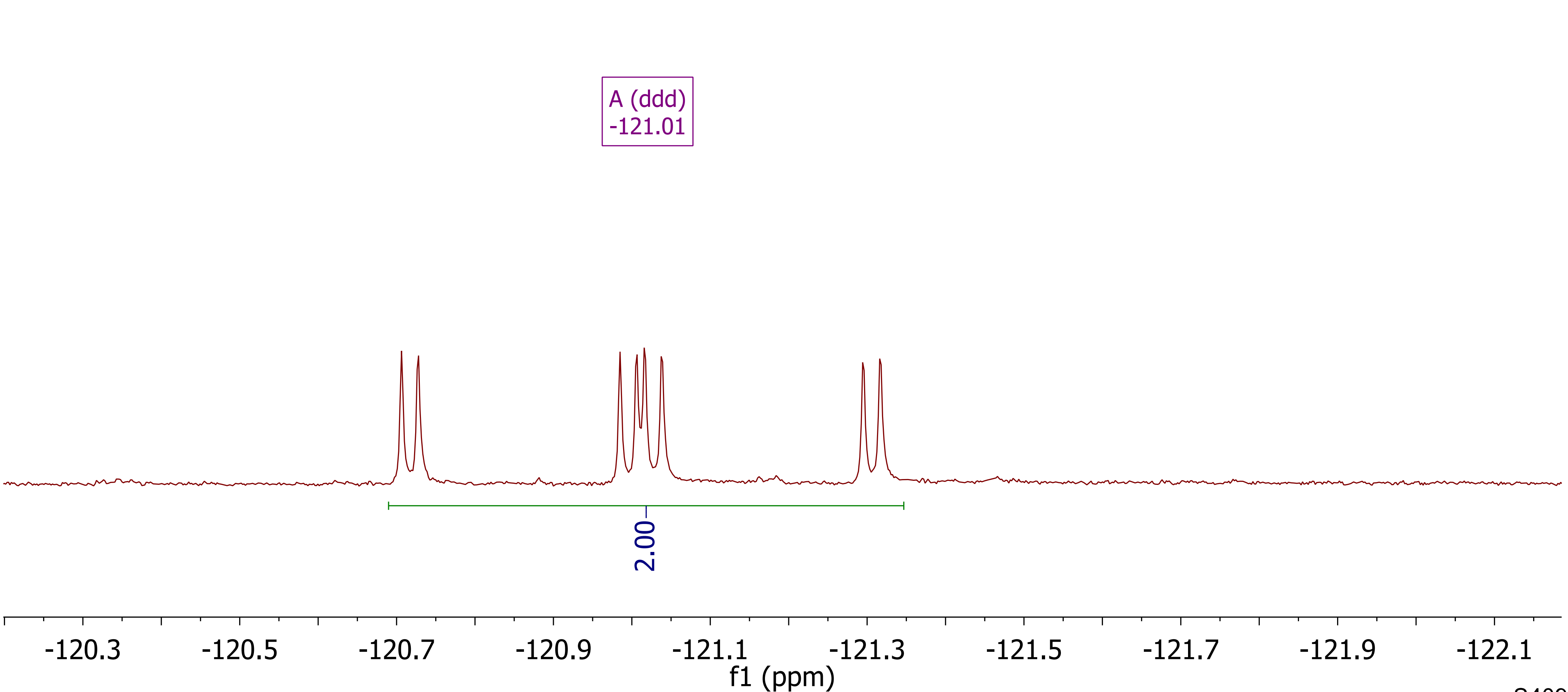
crude product after precipitation

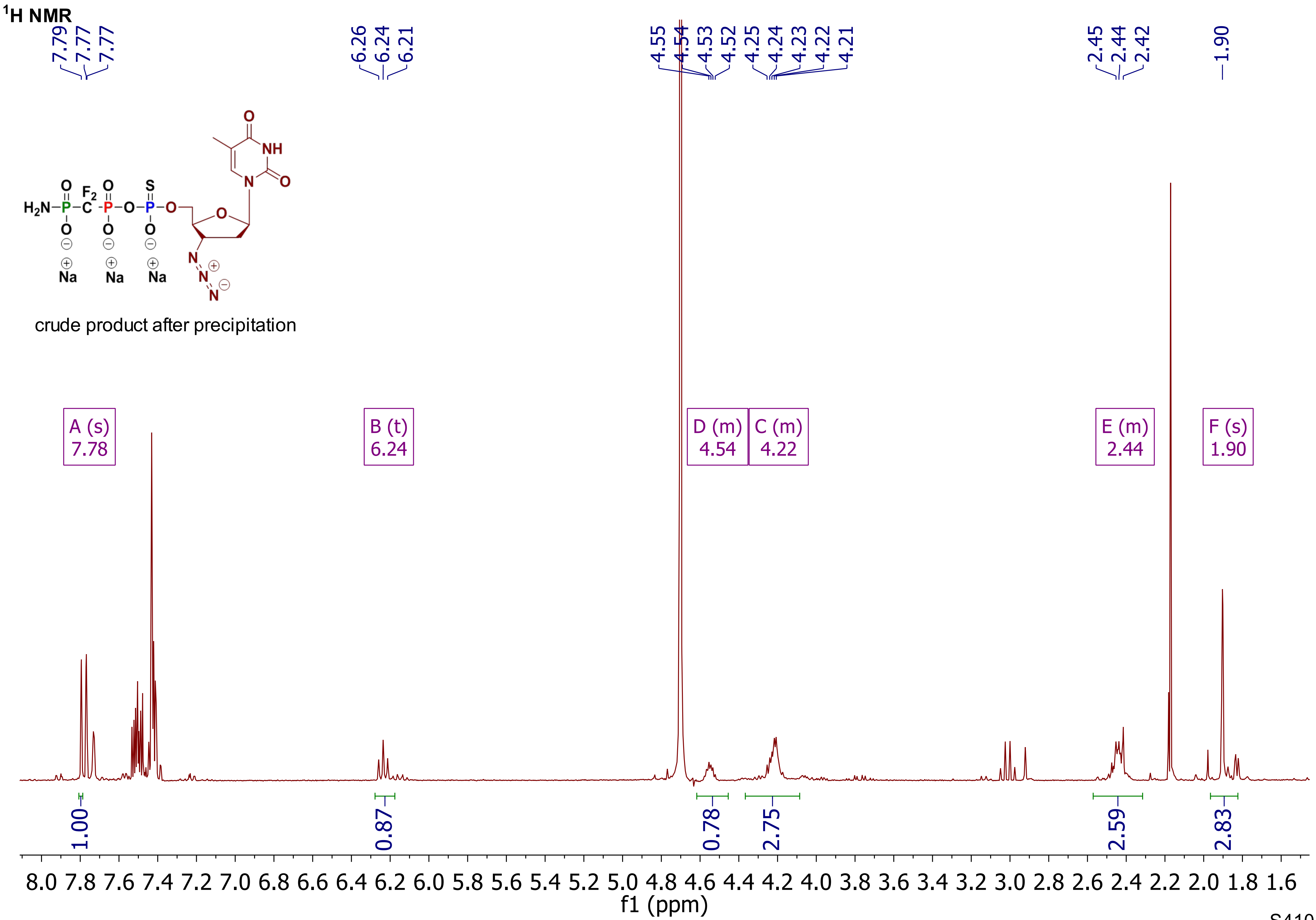


$^{19}$F NMR



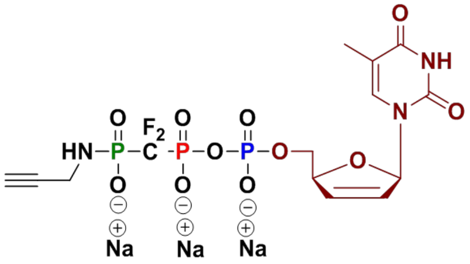
crude product after precipitation



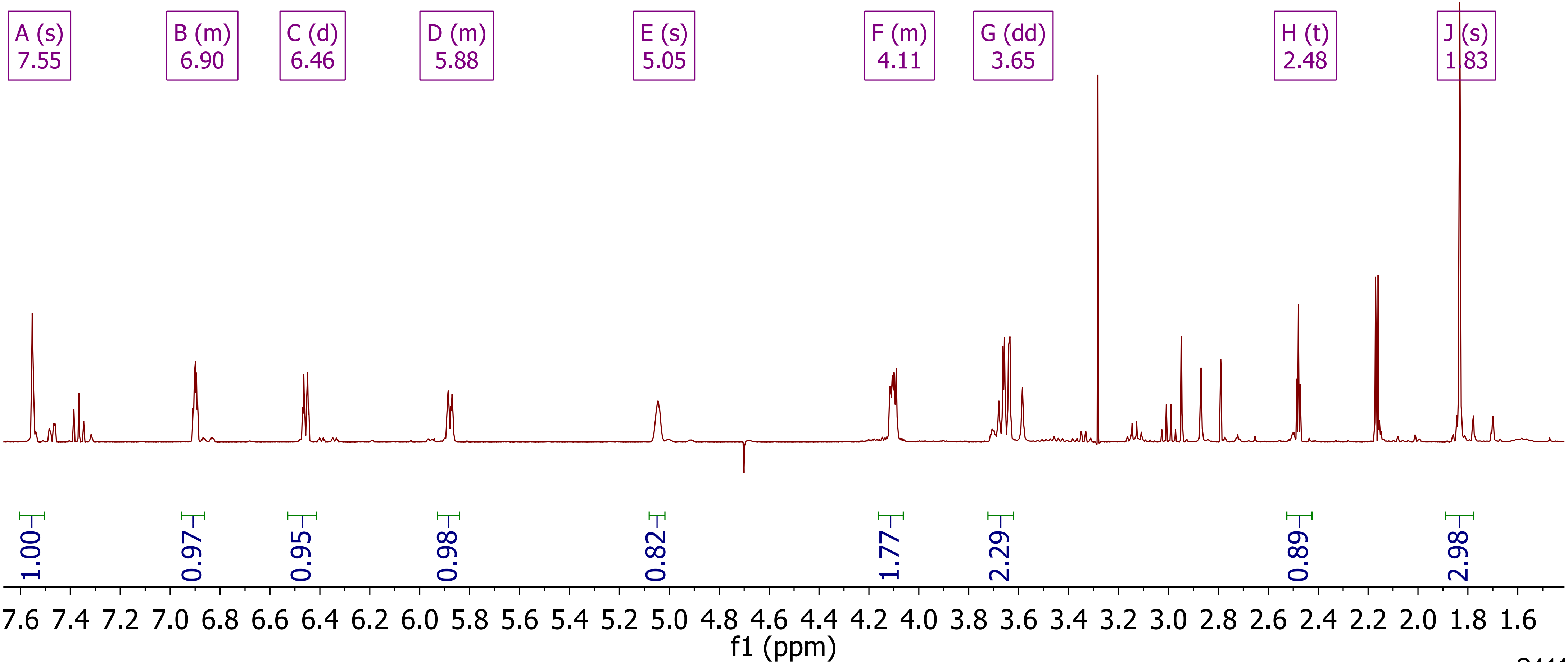


1H NMR
7.79
7.77
7.77
6.26
6.24
6.21
4.55
4.54
4.53
4.52
4.25
4.24
4.23
4.22
4.21
2.45
2.44
2.42
1.90
crude product after precipitation
A (s) 7.78
B (t) 6.24
D (m) 4.54
C (m) 4.22
E (m) 2.44
F (s) 1.90
1.00
0.87
0.78
2.75
2.59
2.83
8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6
f1 (ppm)

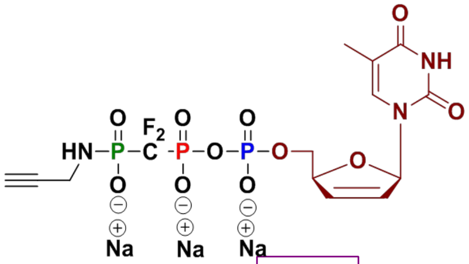
$^1$H NMR



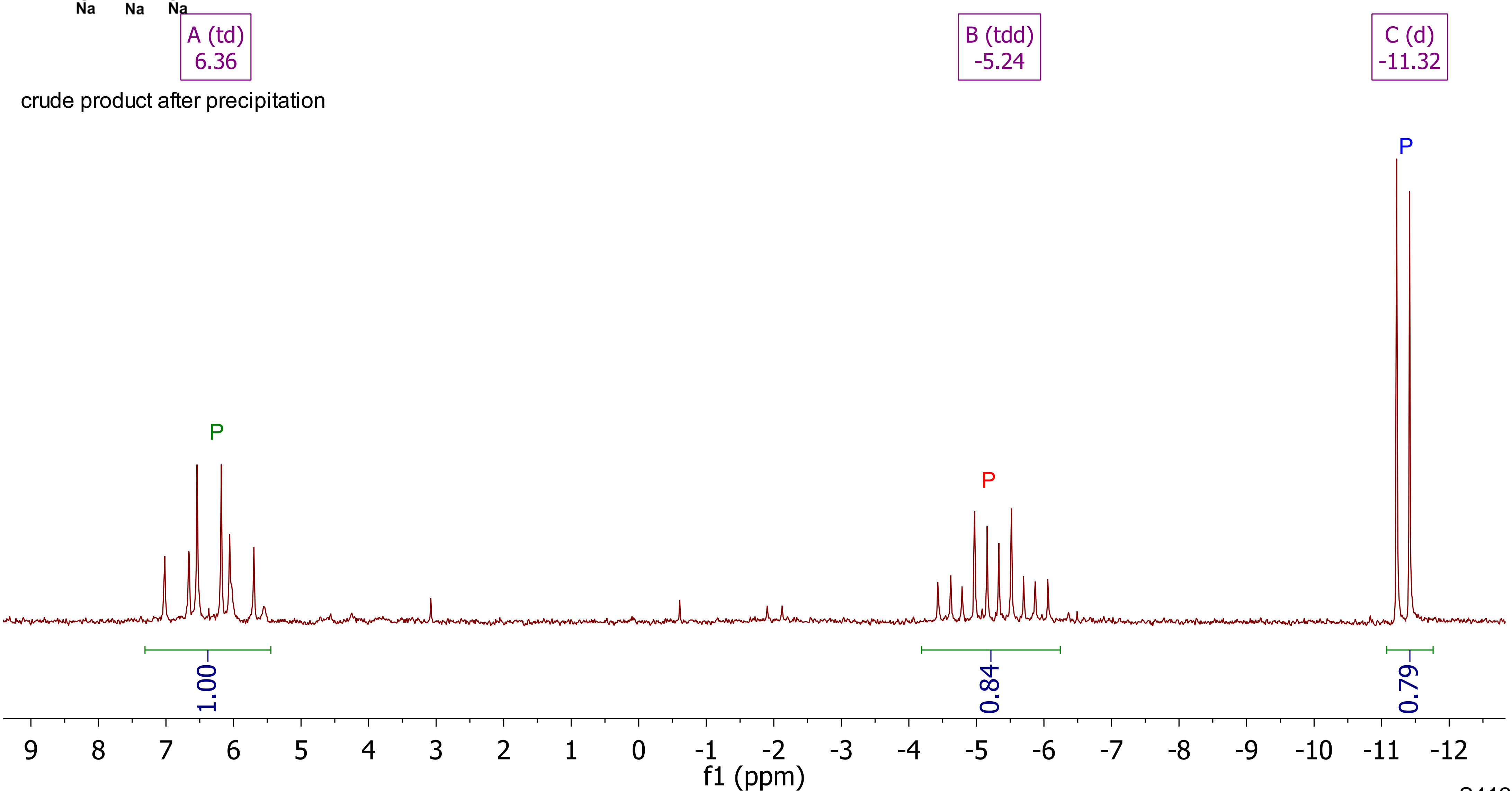
crude product after precipitation



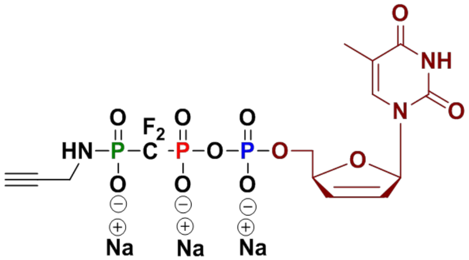
**$^{31}P\{^1H\}$ NMR**



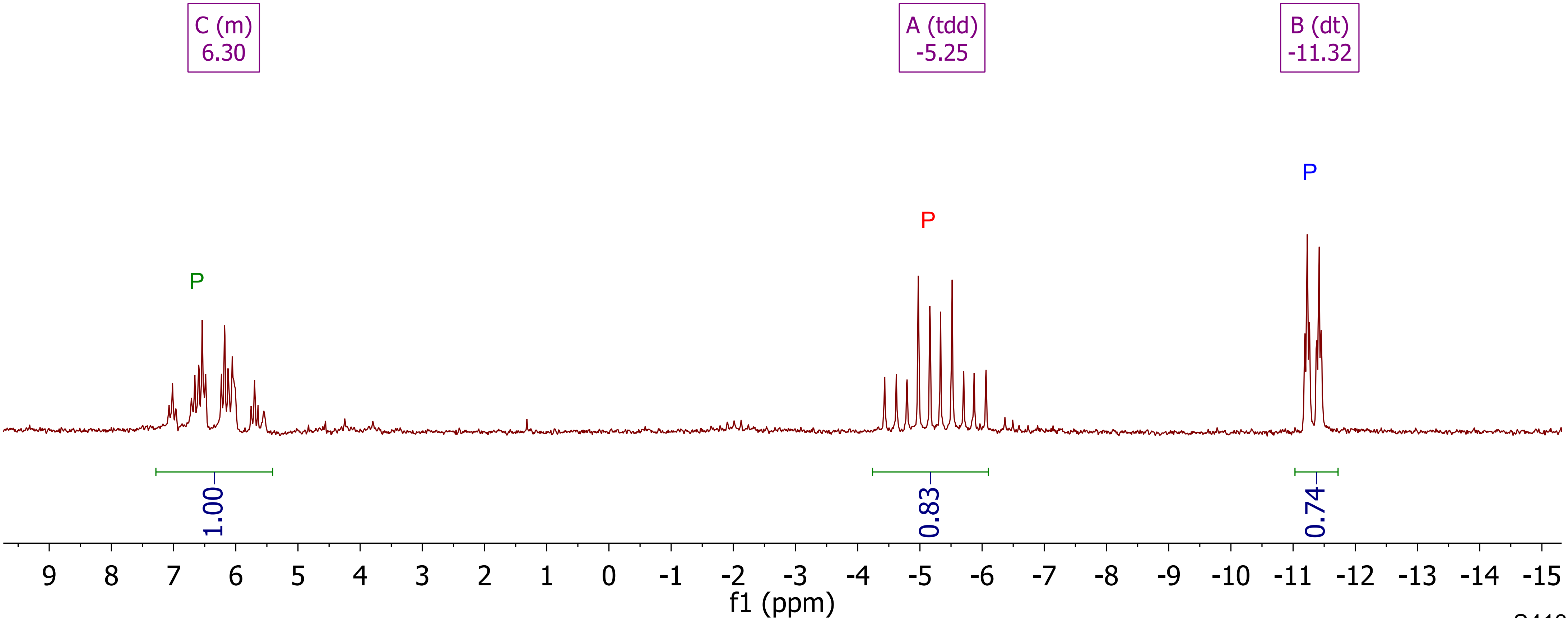
crude product after precipitation



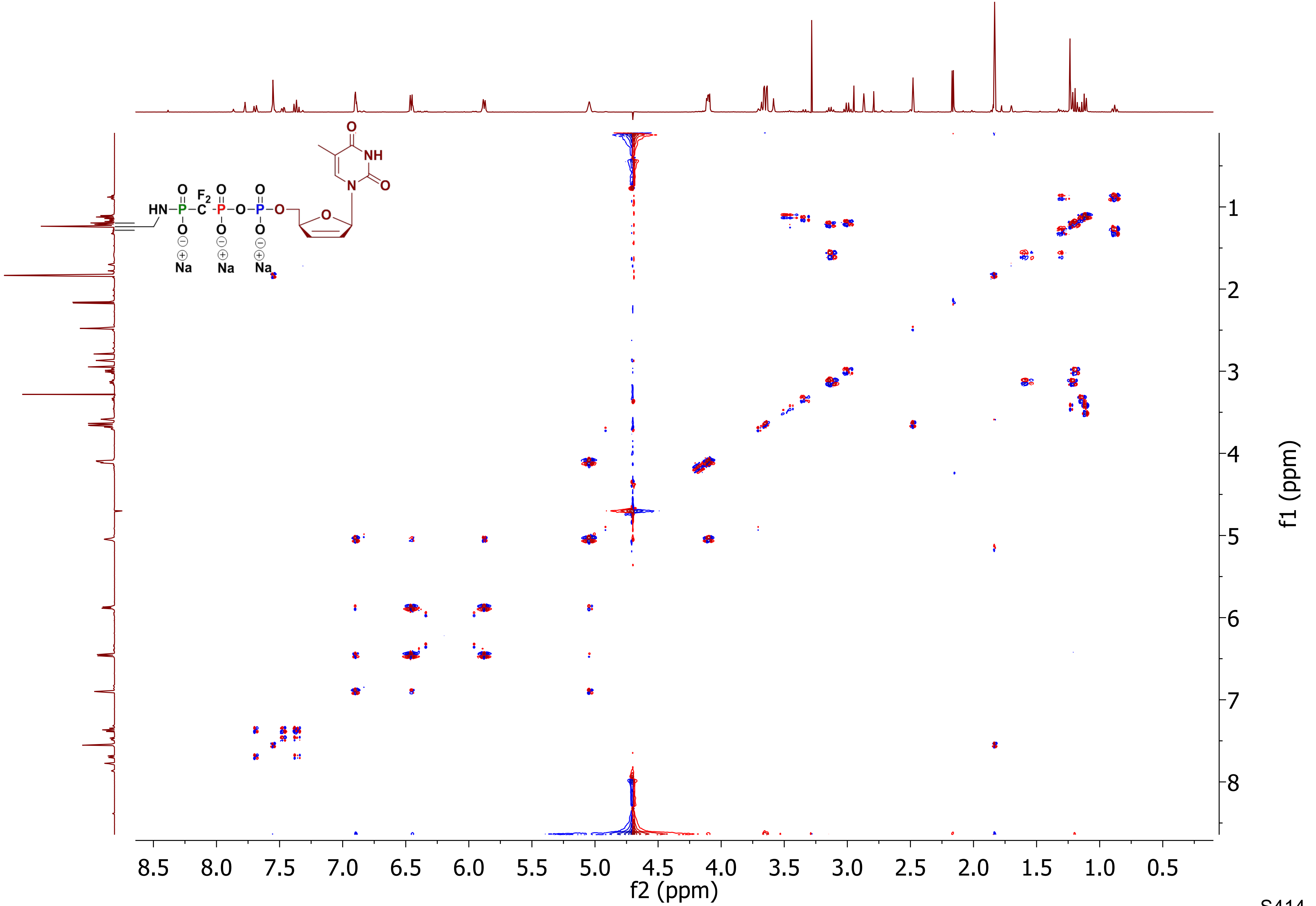
**$^{31}$P NMR**



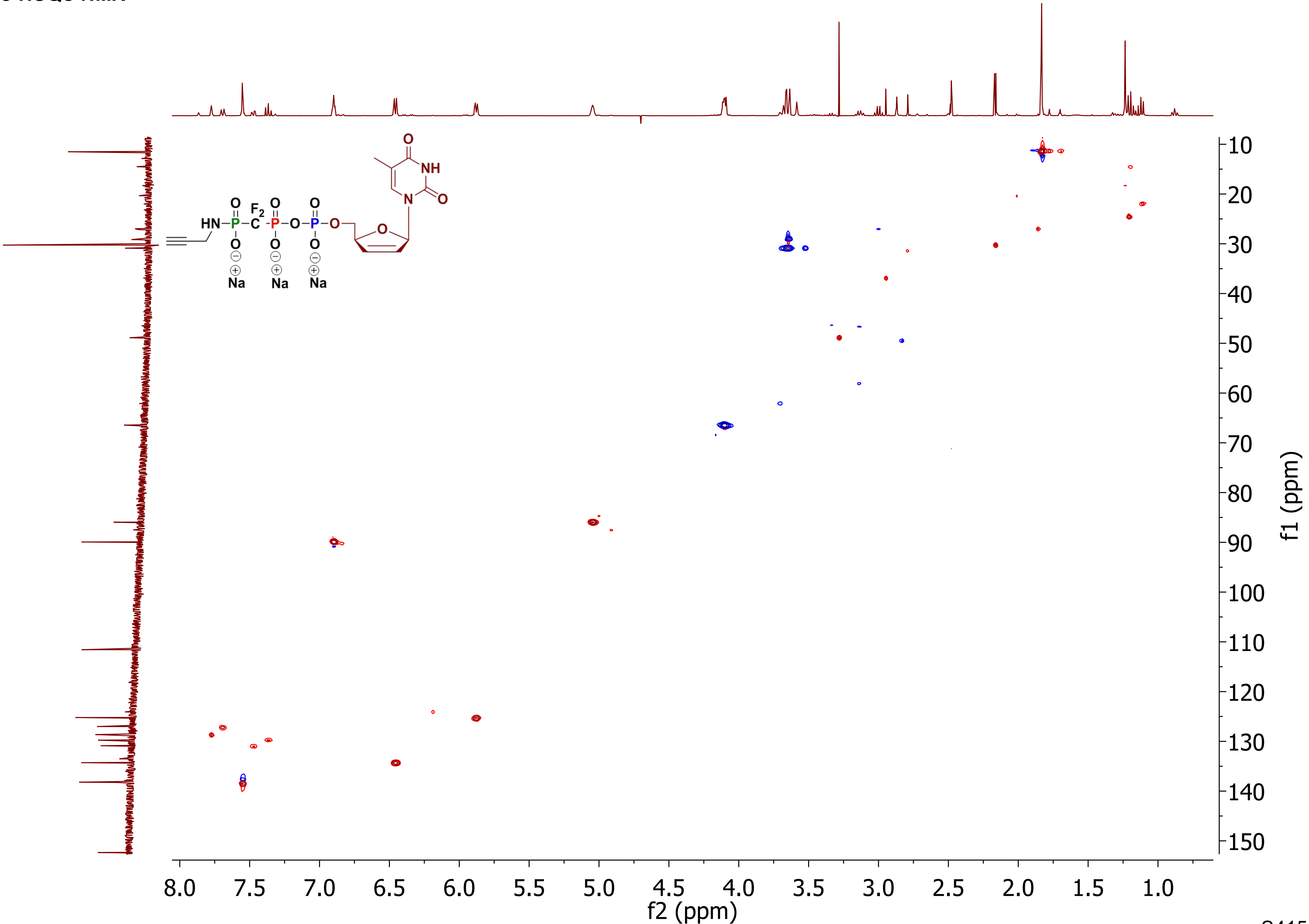
crude product after precipitation



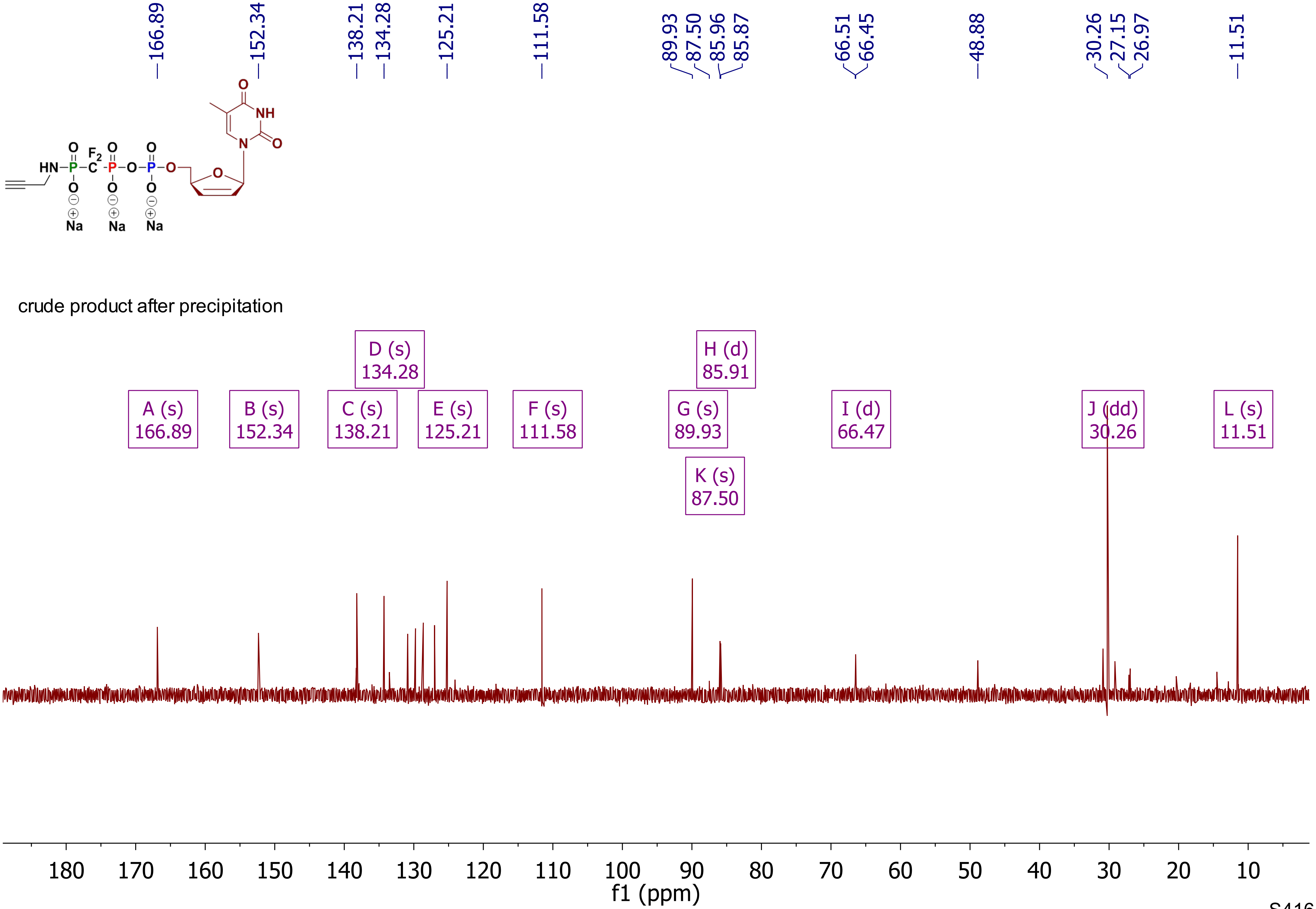
**$^1$H-$^1$H COSY NMR**



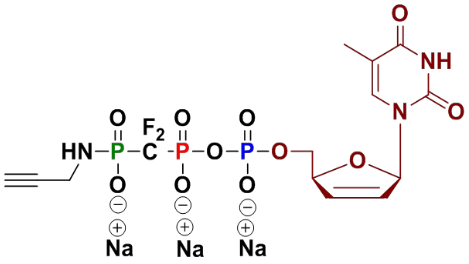
$^1H$-$^{13}C$ HSQC NMR



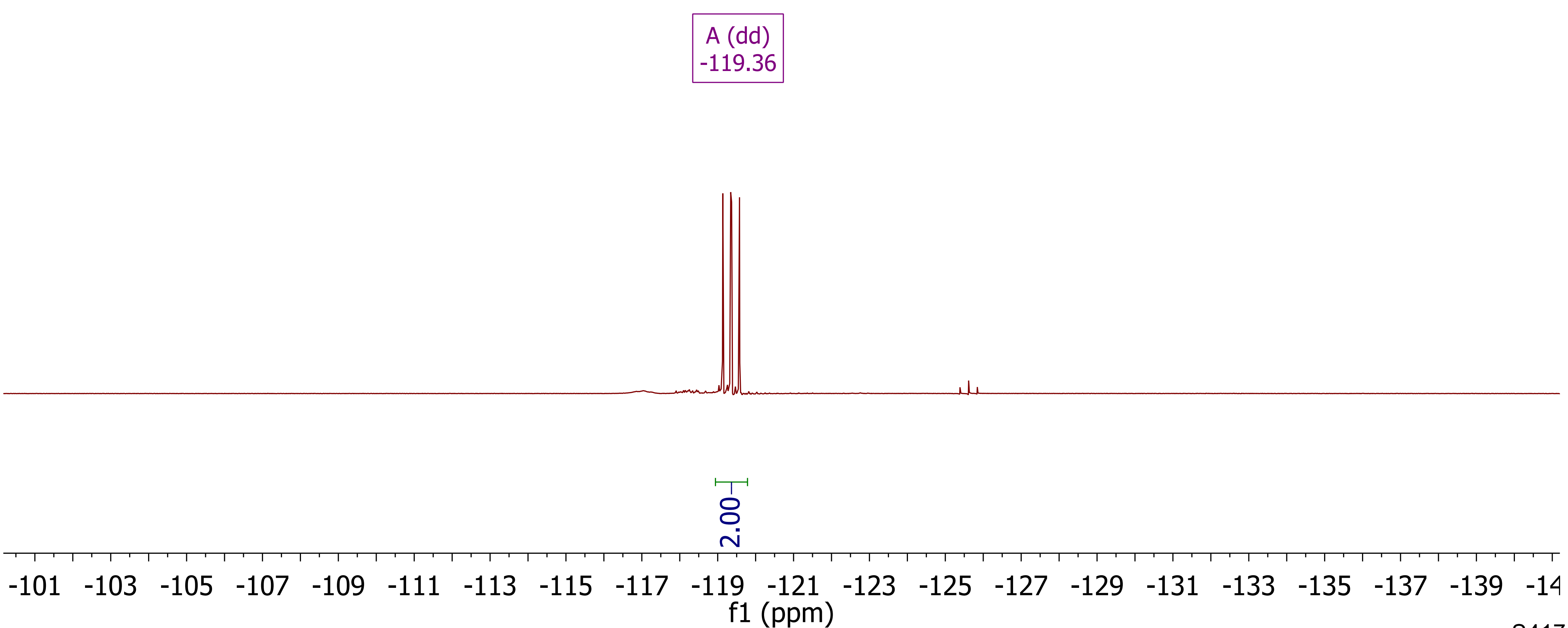
## $^{13}$C NMR


166.89
152.34
138.21
134.28
125.21
111.58
89.93
87.50
85.96
85.87
66.51
66.45
48.88
30.26
27.15
26.97
11.51
crude product after precipitation
A (s) 166.89
B (s) 152.34
C (s) 138.21
D (s) 134.28
E (s) 125.21
F (s) 111.58
G (s) 89.93
H (d) 85.91
K (s) 87.50
I (d) 66.47
J (dd) 30.26
L (s) 11.51
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

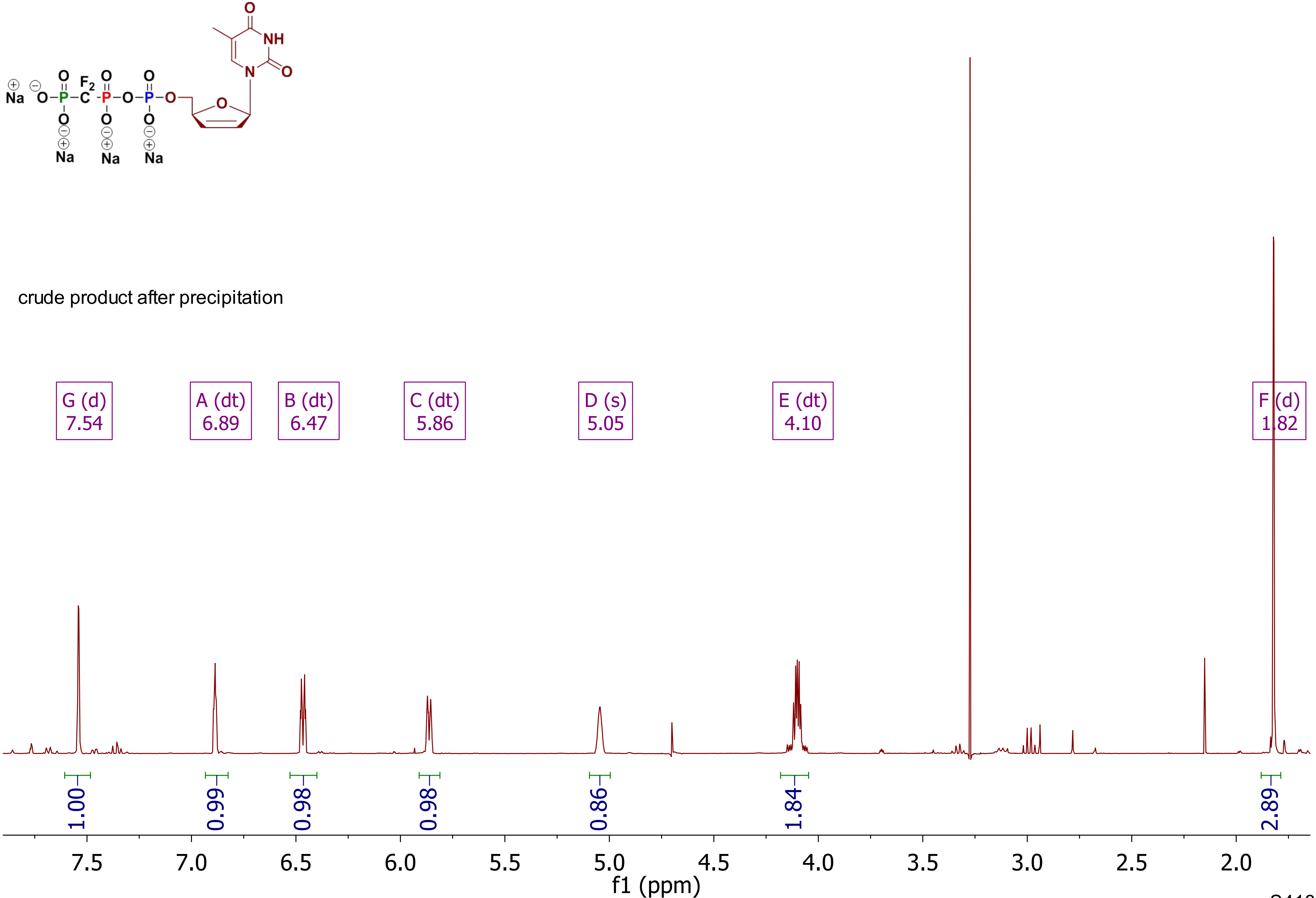
$^{19}F$ NMR




crude product after precipitation

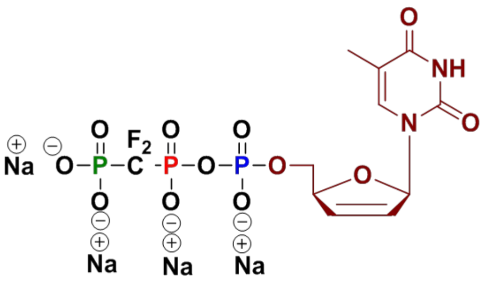


$^1$H NMR

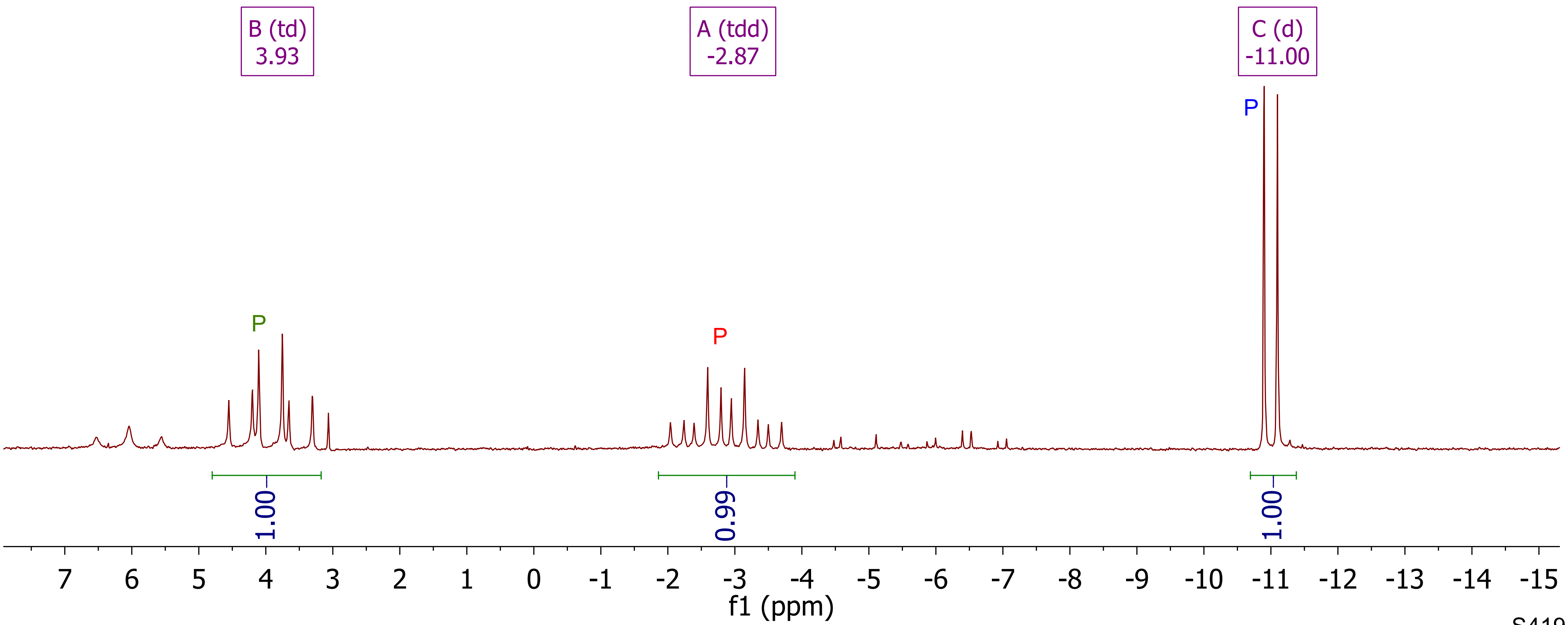


crude product after precipitation
G (d) 7.54
A (dt) 6.89
B (dt) 6.47
C (dt) 5.86
D (s) 5.05
E (dt) 4.10
F (d) 1.82
1.00
0.99
0.98
0.98
0.86
1.84
2.89
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

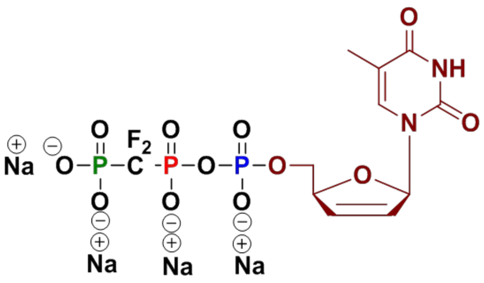
**$^{31}P\{^1H\}$ NMR**



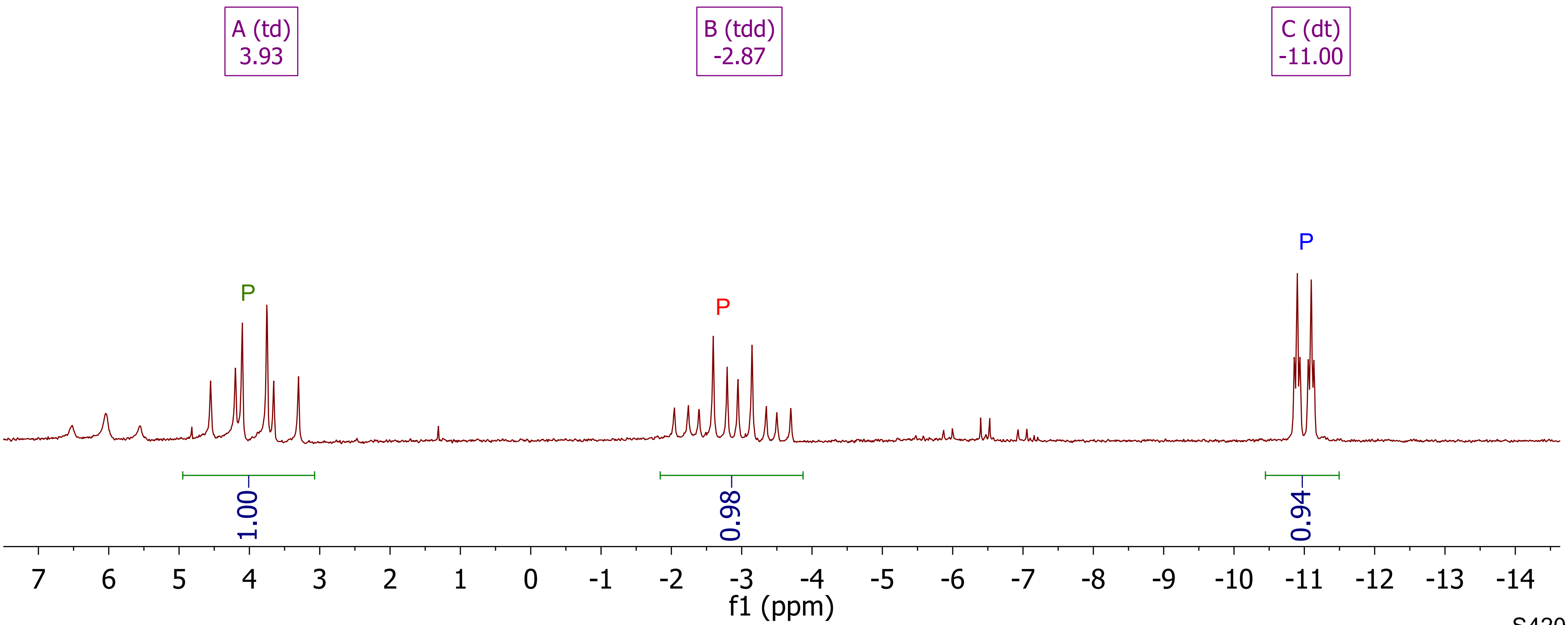
crude product after precipitation



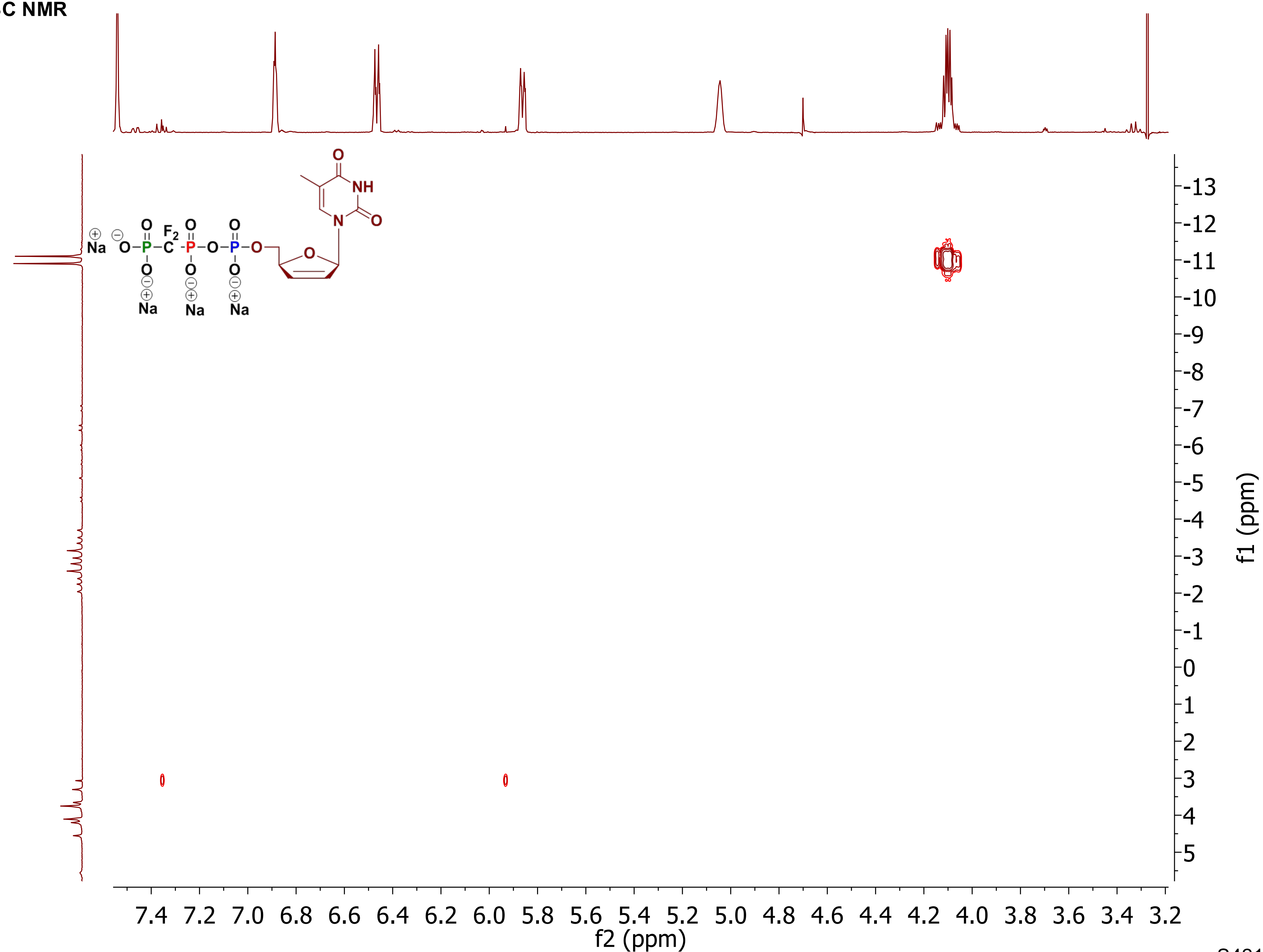
**$^{31}$P NMR**



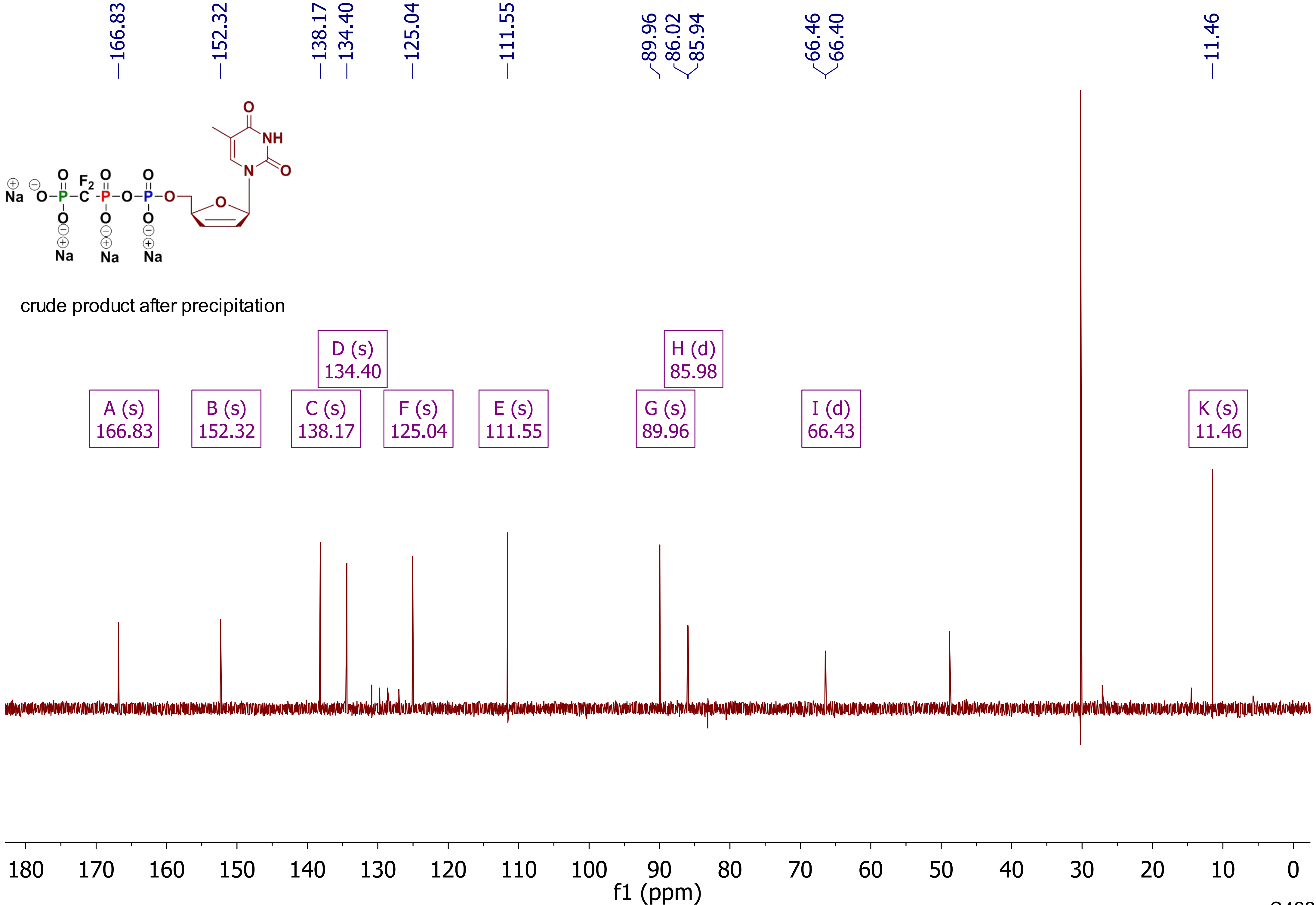
crude product after precipitation



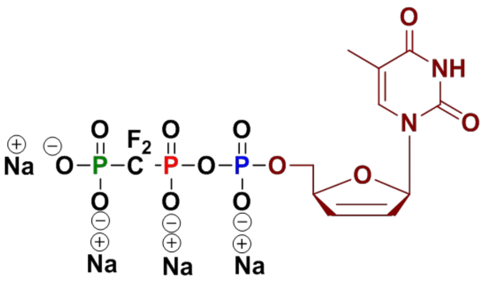
$^{31}P$-$^{1}H$ HMBC NMR



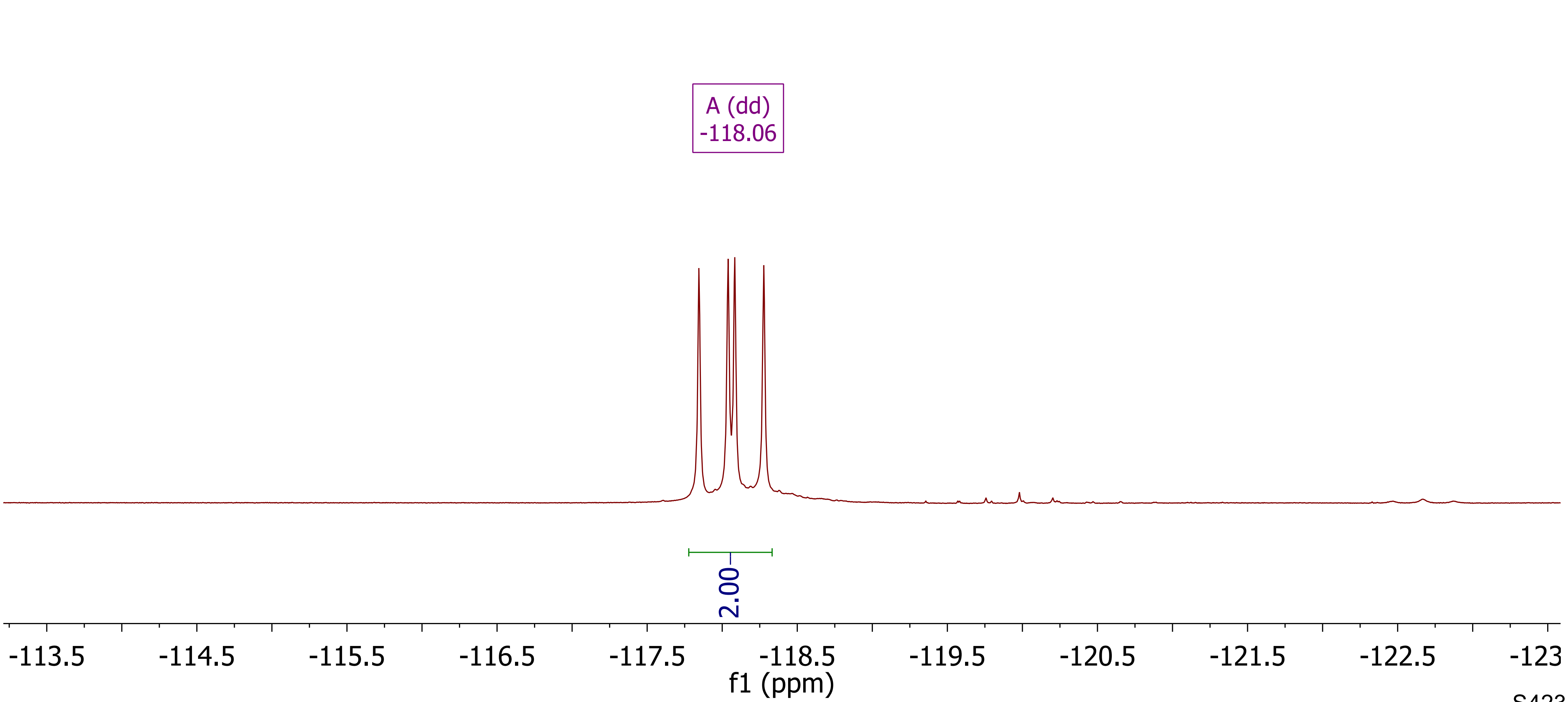
$^{13}C$ NMR


166.83
152.32
138.17
134.40
125.04
111.55
89.96
86.02
85.94
66.46
66.40
11.46
crude product after precipitation
A (s) 166.83
B (s) 152.32
C (s) 138.17
D (s) 134.40
F (s) 125.04
E (s) 111.55
G (s) 89.96
H (d) 85.98
I (d) 66.43
K (s) 11.46
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

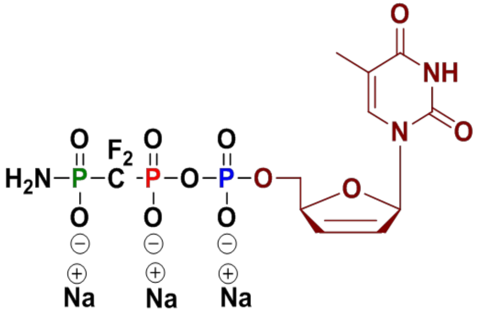
**$^{19}$F NMR**

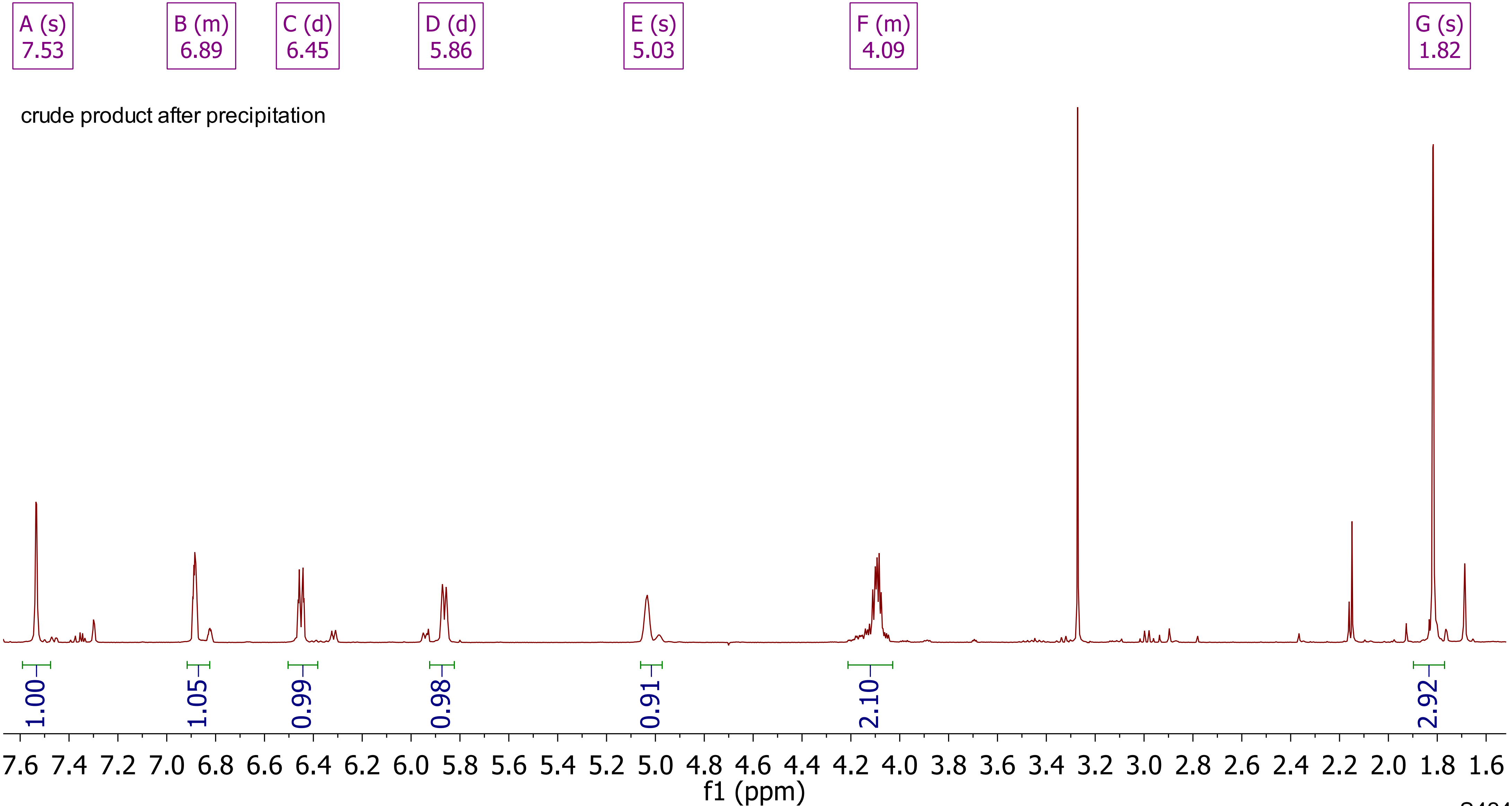


crude product after precipitation

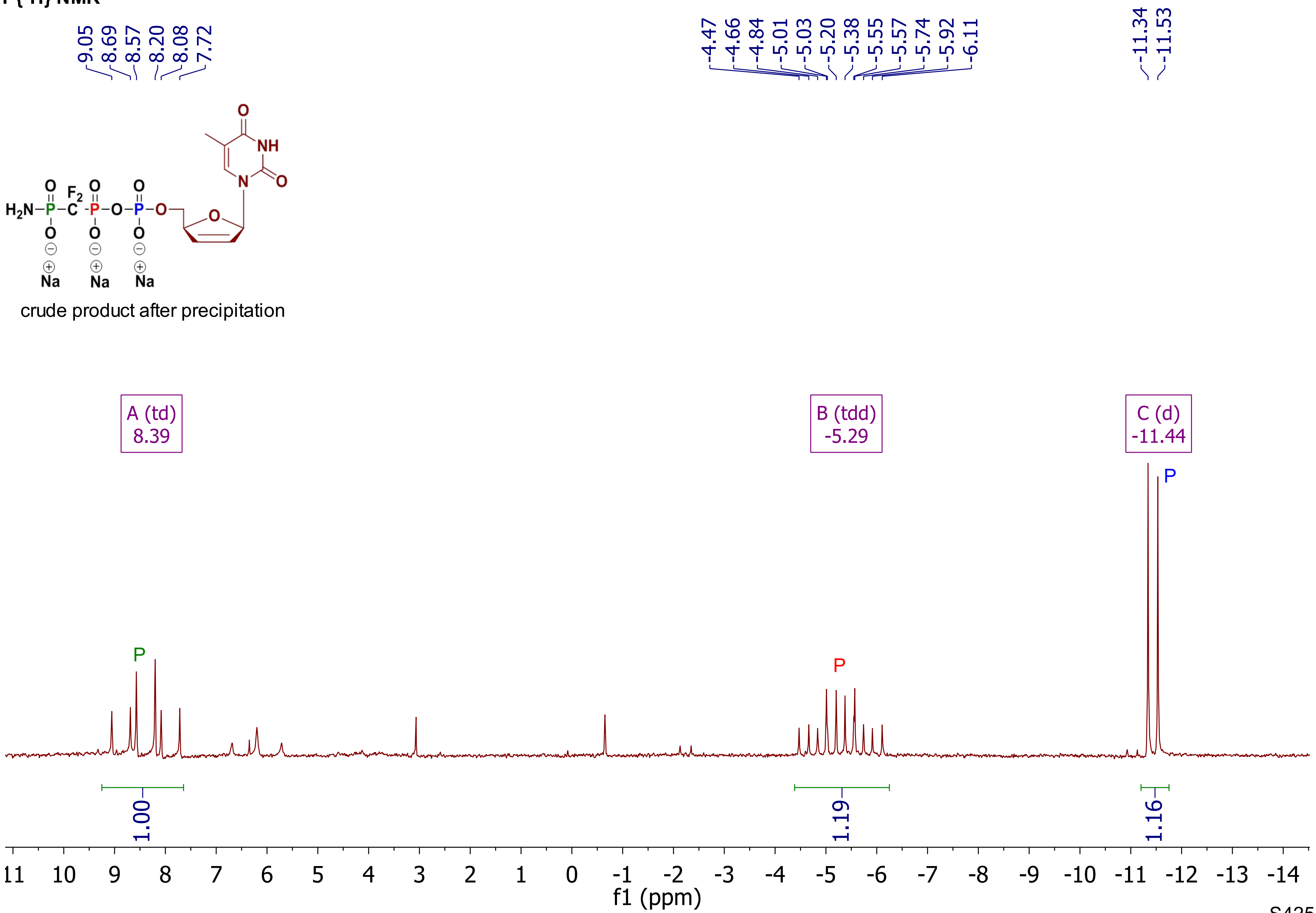


## $^1$H NMR



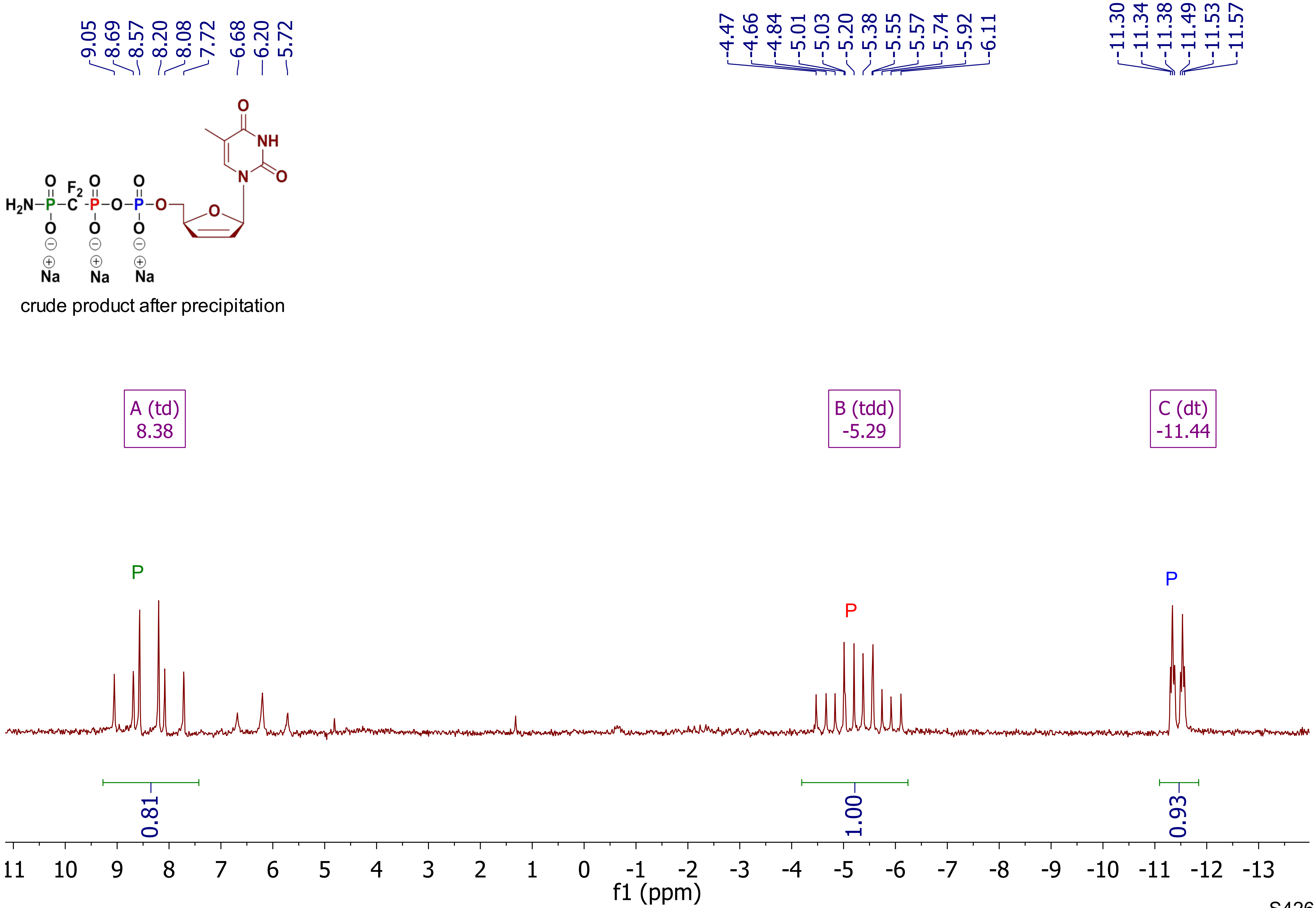

A (s)
7.53
B (m)
6.89
C (d)
6.45
D (d)
5.86
E (s)
5.03
F (m)
4.09
G (s)
1.82
crude product after precipitation
1.00
1.05
0.99
0.98
0.91
2.10
2.92
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6
f1 (ppm)

$^{31}P\{^1H\}$ NMR

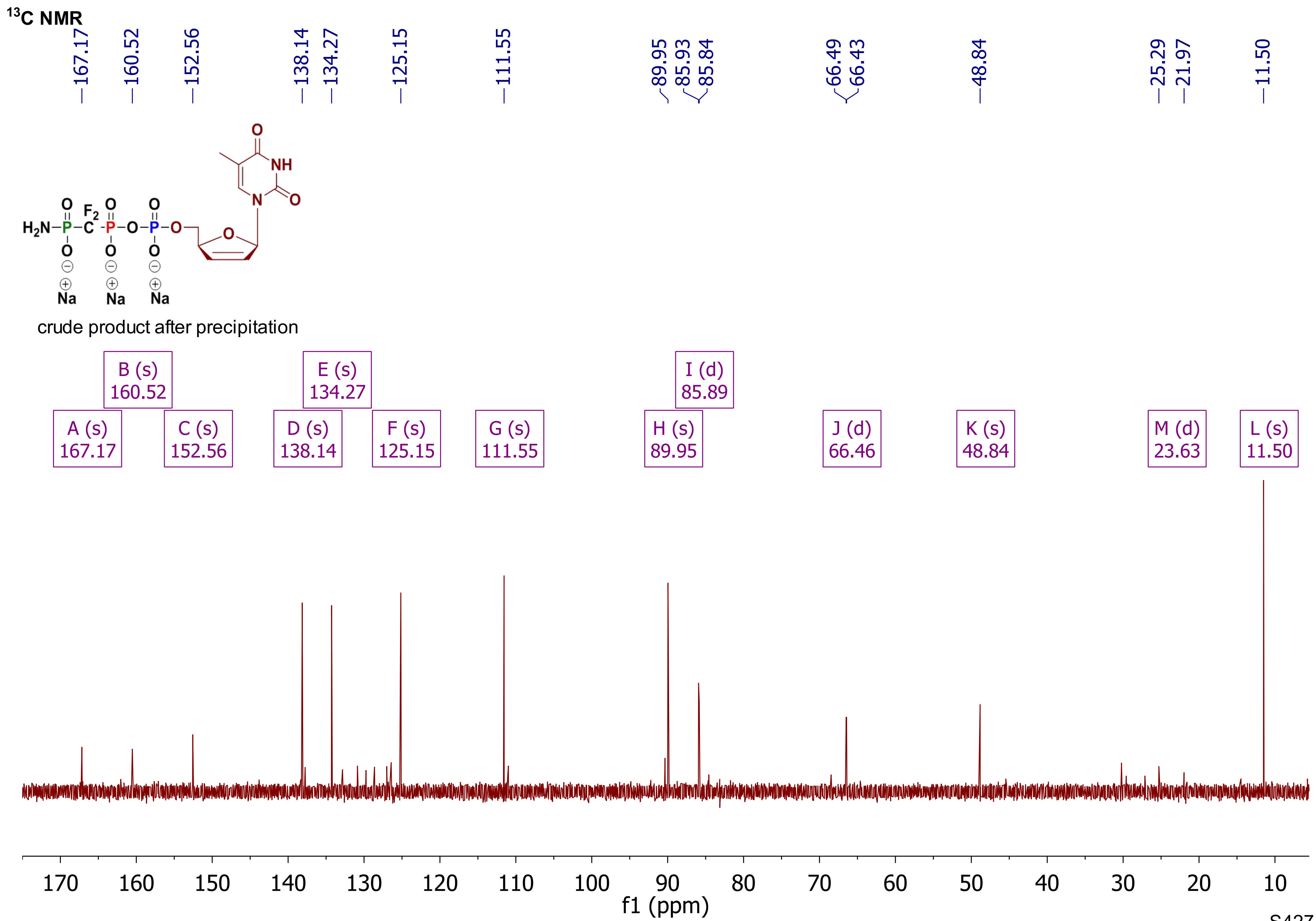


9.05
8.69
8.57
8.20
8.08
7.72
-4.47
-4.66
-4.84
-5.01
-5.03
-5.20
-5.38
-5.55
-5.57
-5.74
-5.92
-6.11
-11.34
-11.53
crude product after precipitation
A (td)
8.39
B (tdd)
-5.29
C (d)
-11.44
P
P
P
1.00
1.19
1.16
f1 (ppm)

**$^{31}$P NMR**

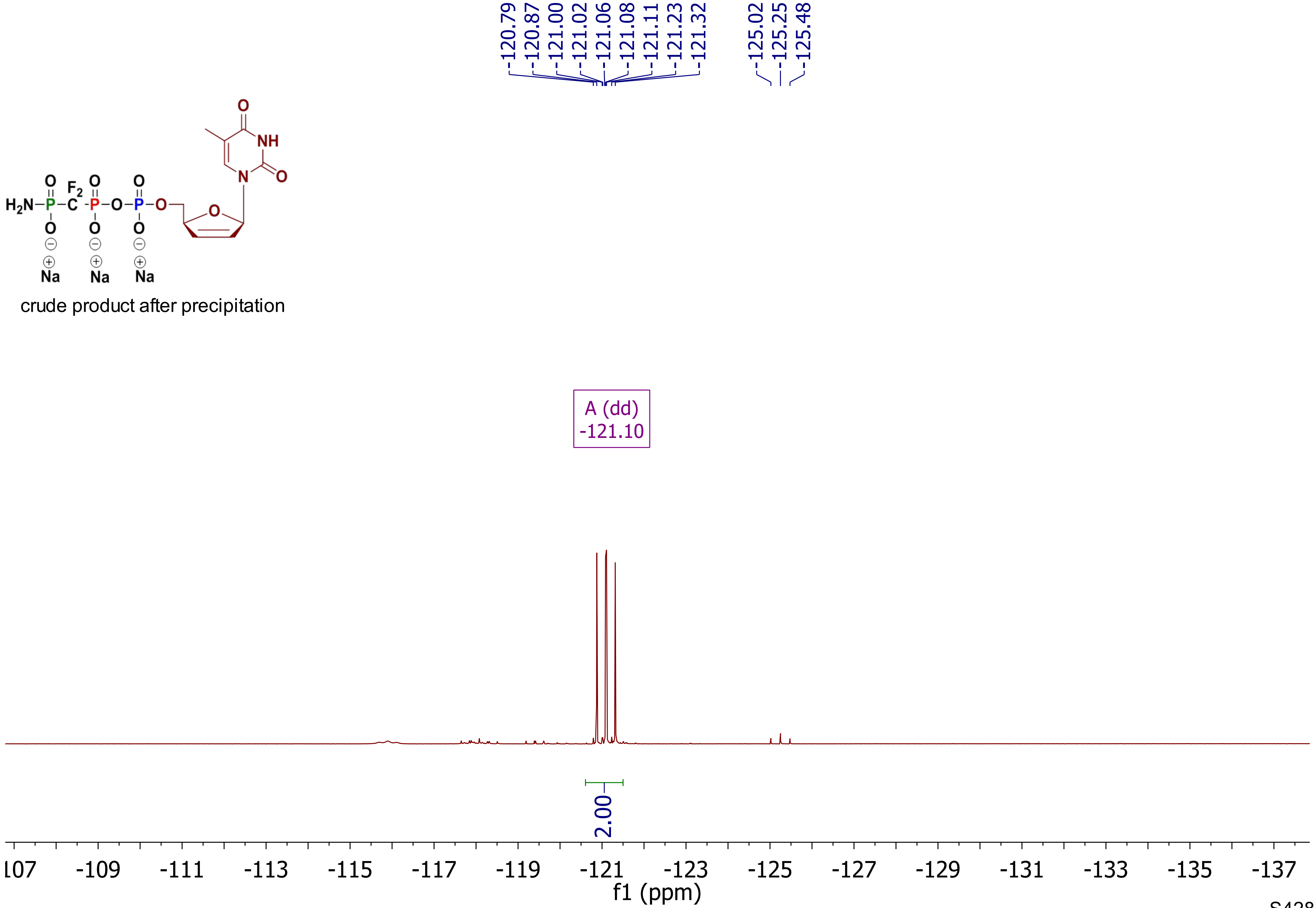


9.05
8.69
8.57
8.20
8.08
7.72
6.68
6.20
5.72
-4.47
-4.66
-4.84
-5.01
-5.03
-5.20
-5.38
-5.55
-5.57
-5.74
-5.92
-6.11
-11.30
-11.34
-11.38
-11.49
-11.53
-11.57
crude product after precipitation
A (td) 8.38
B (tdd) -5.29
C (dt) -11.44
P
P
P
0.81
1.00
0.93
11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13
f1 (ppm)



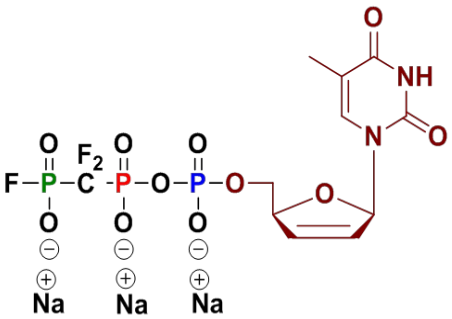
13C NMR
167.17
160.52
152.56
138.14
134.27
125.15
111.55
89.95
85.93
85.84
66.49
66.43
48.84
25.29
21.97
11.50
crude product after precipitation
A (s) 167.17
B (s) 160.52
C (s) 152.56
D (s) 138.14
E (s) 134.27
F (s) 125.15
G (s) 111.55
H (s) 89.95
I (d) 85.89
J (d) 66.46
K (s) 48.84
M (d) 23.63
L (s) 11.50
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

## $^{19}$F NMR

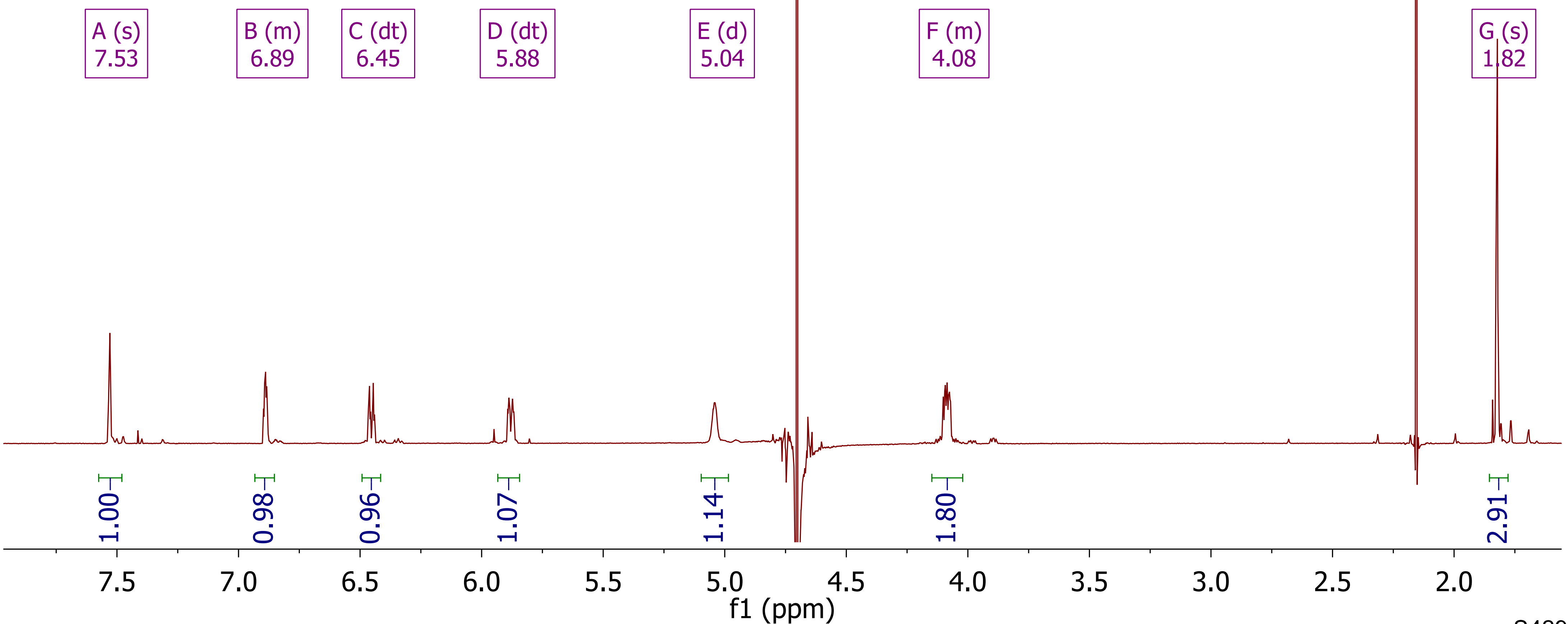



crude product after precipitation

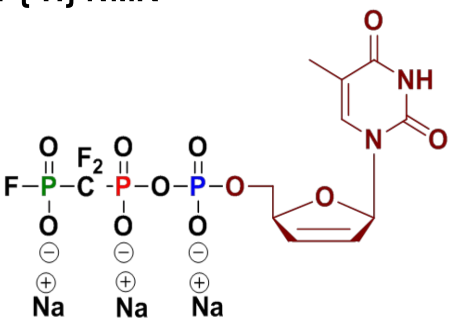
$^{1}$H NMR



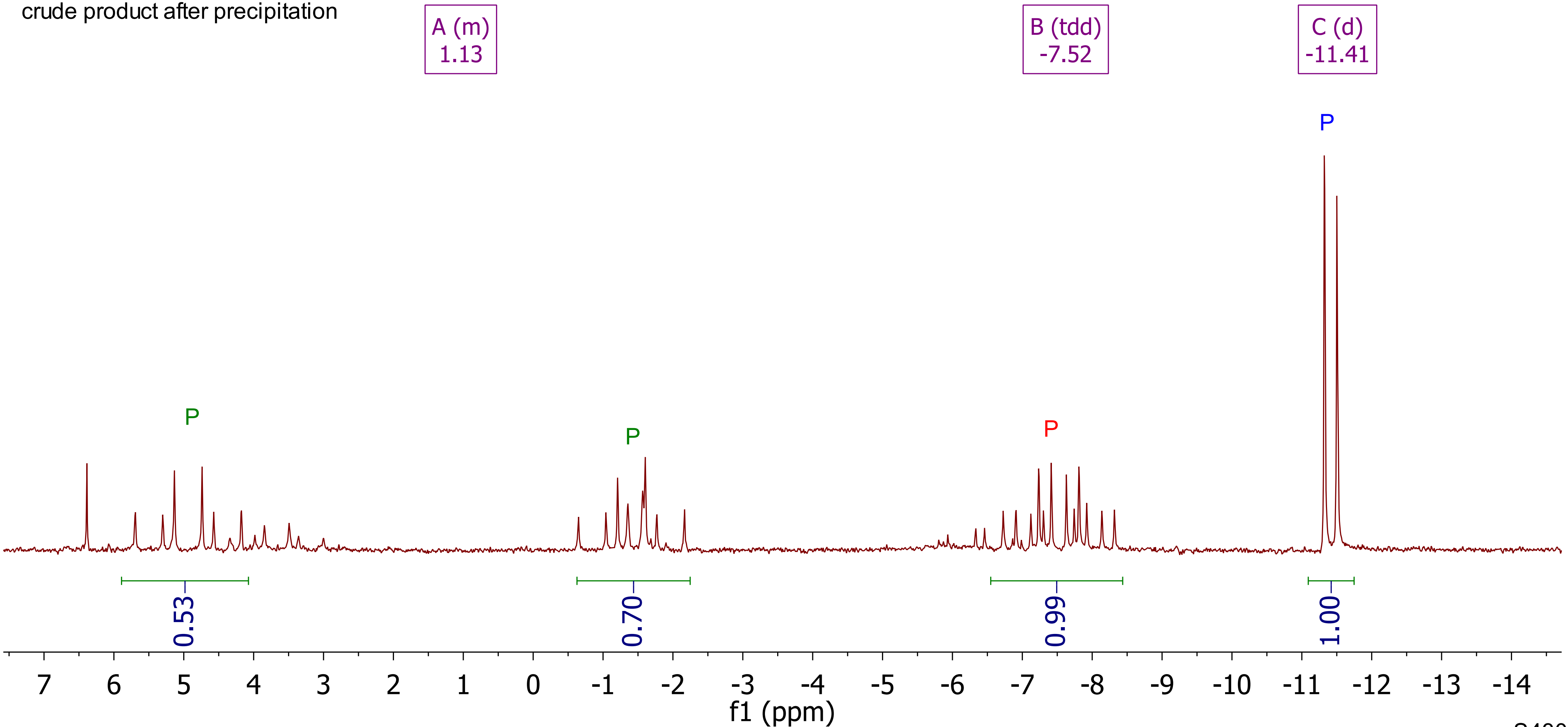

crude product after precipitation



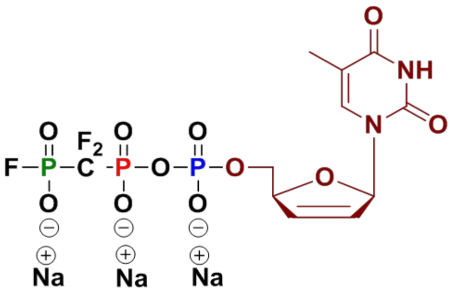
$^{31}P\{^{1}H\}$ NMR



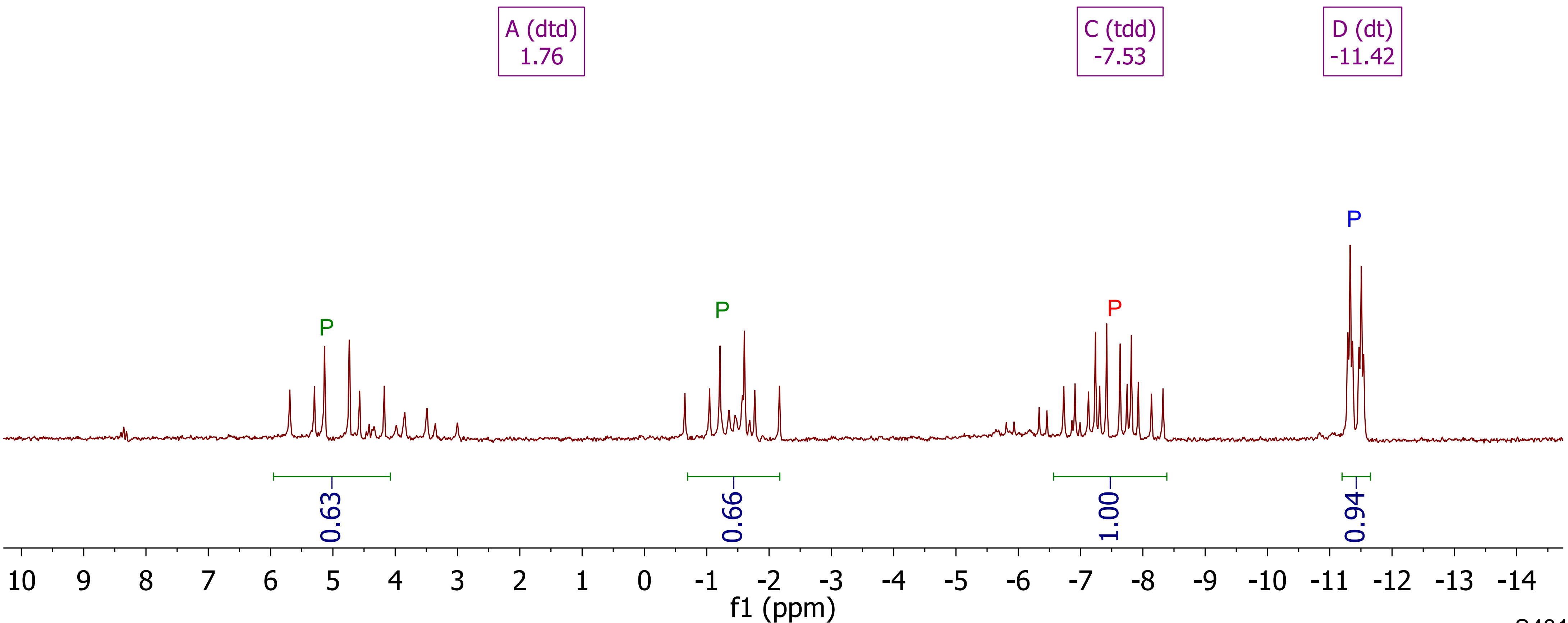
crude product after precipitation



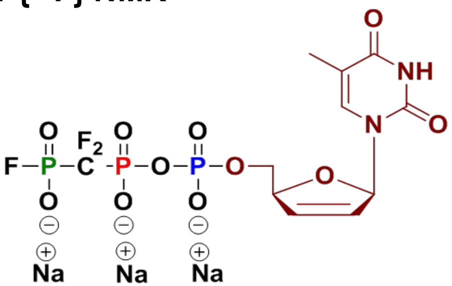
**$^{31}$P-$^{1}$H NMR**



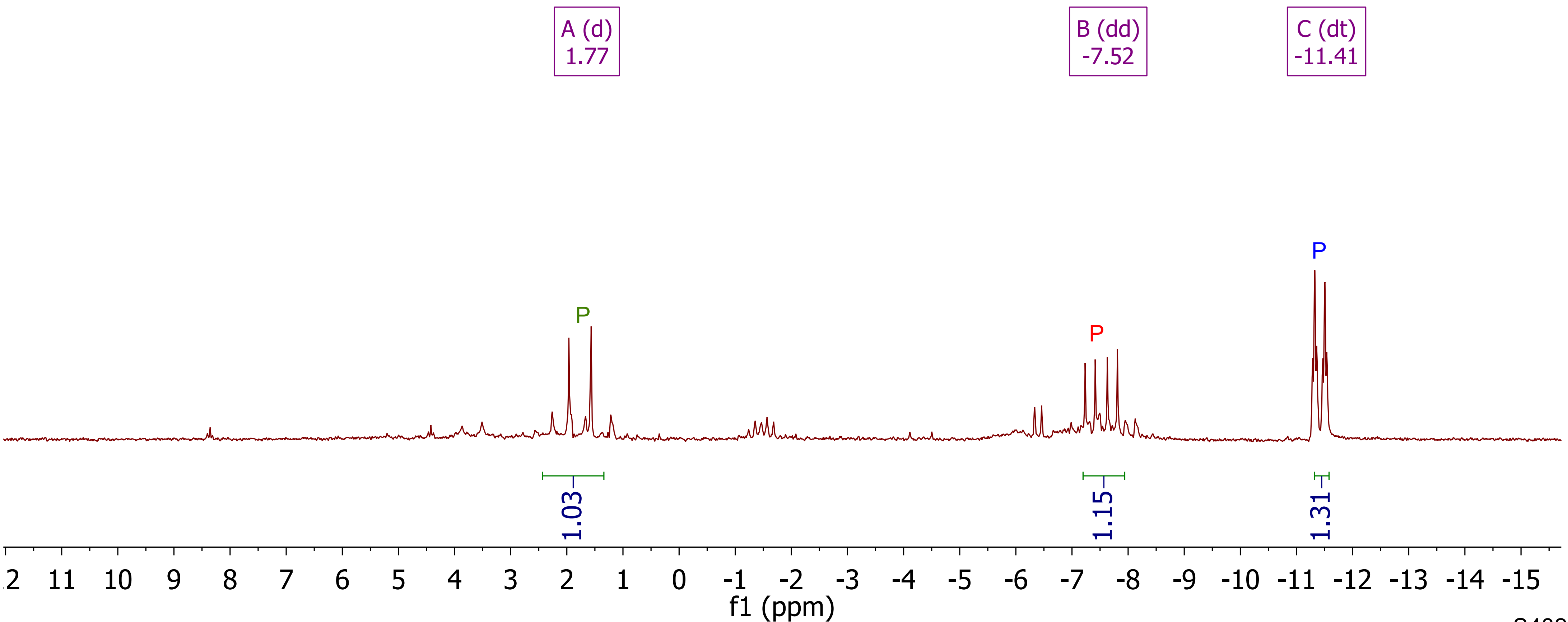

crude product after precipitation



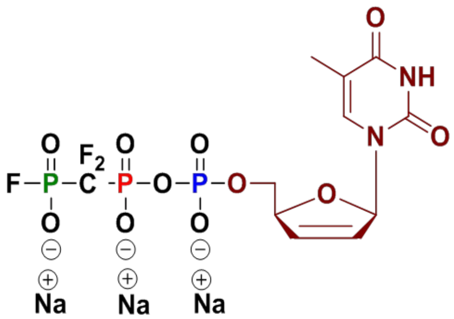
**$^{31}P\{^{19}F\}$ NMR**



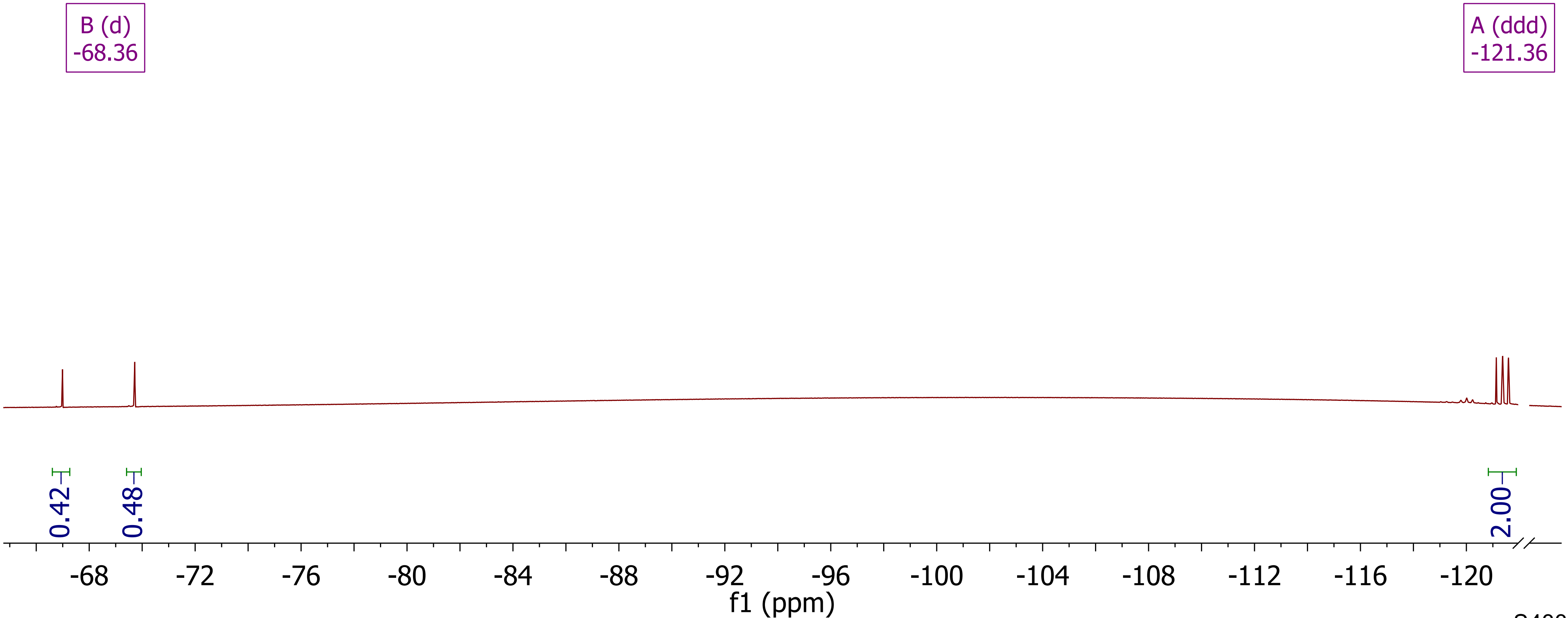

crude product after precipitation



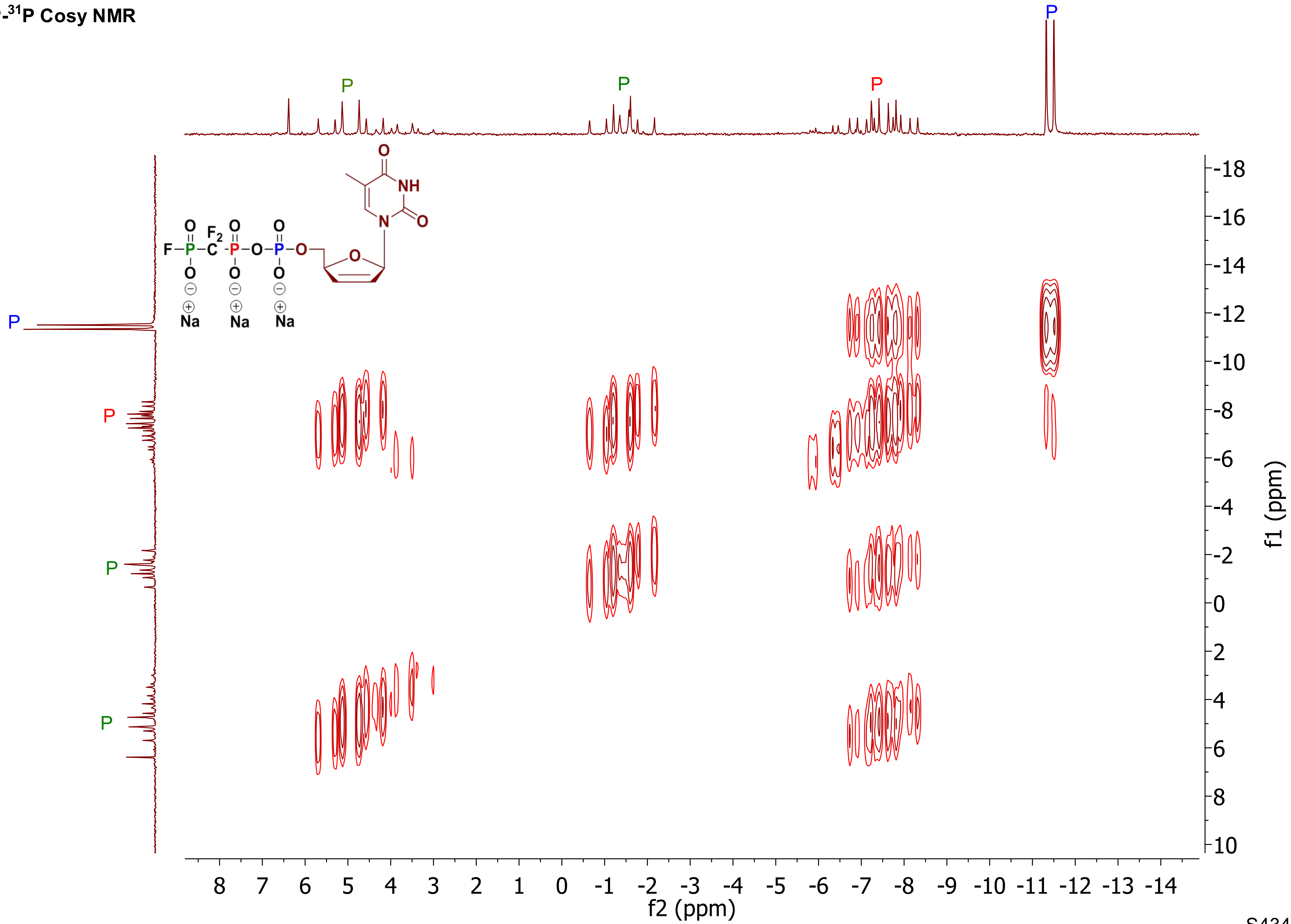
**$^{19}$F NMR**



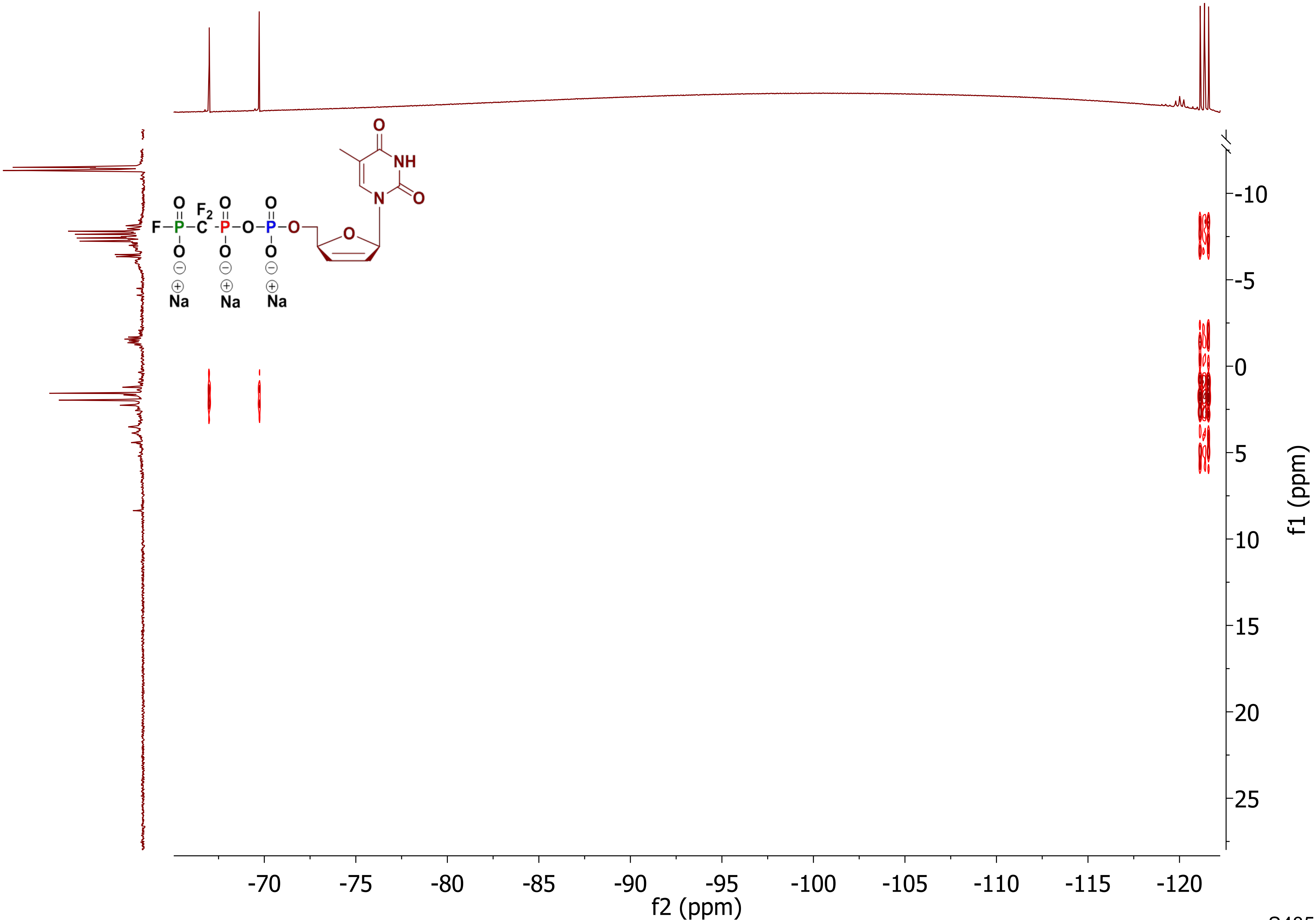

crude product after precipitation



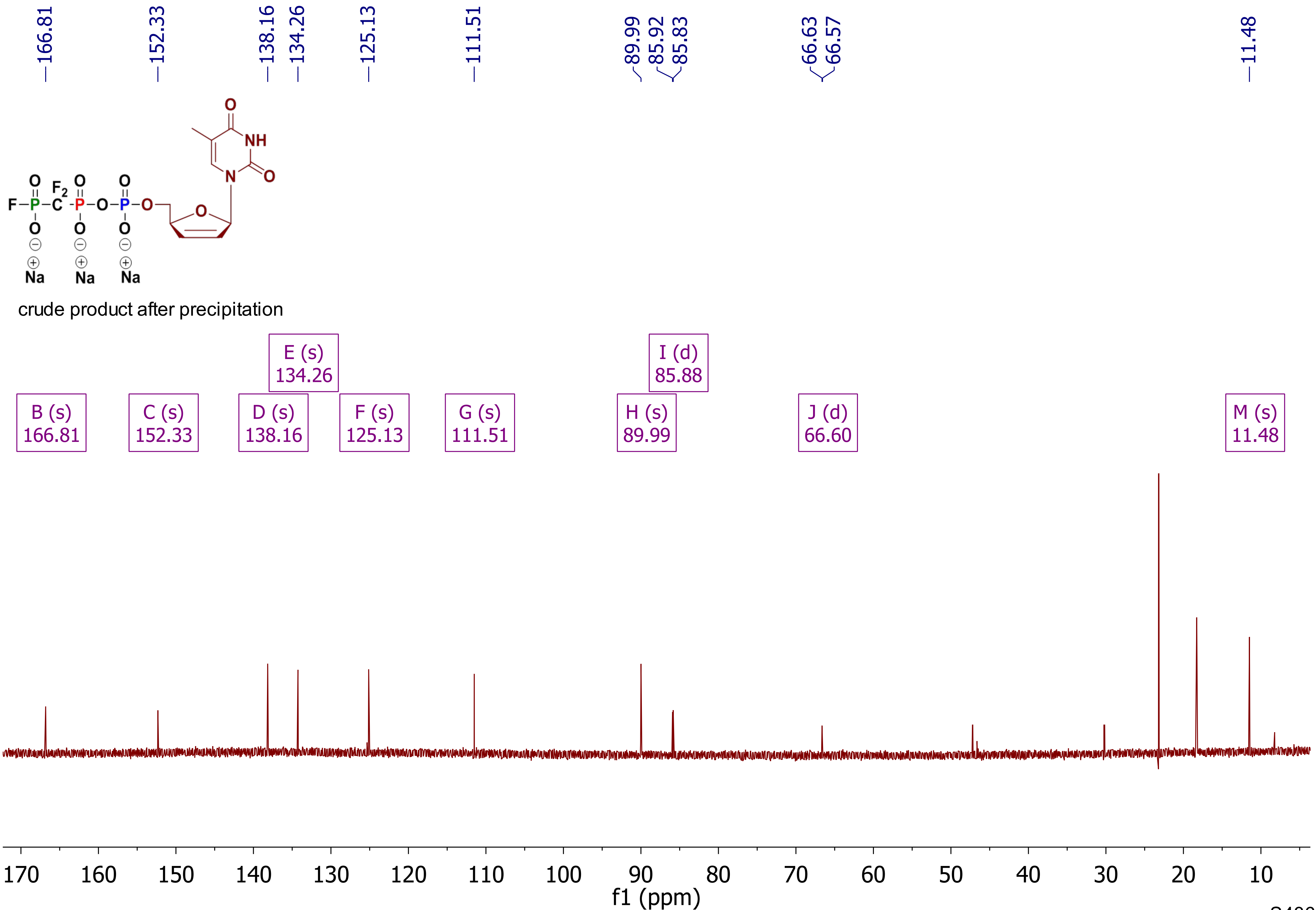
$^{31}P$-$^{31}P$ Cosy NMR



**$^{31}P$-$^{19}F$ HMBC NMR**

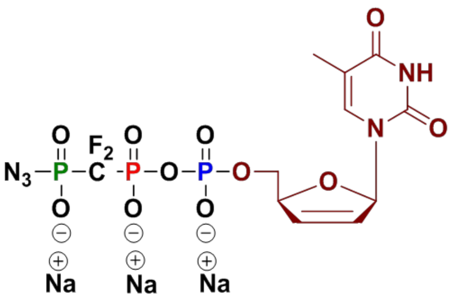


## $^{13}C$ NMR

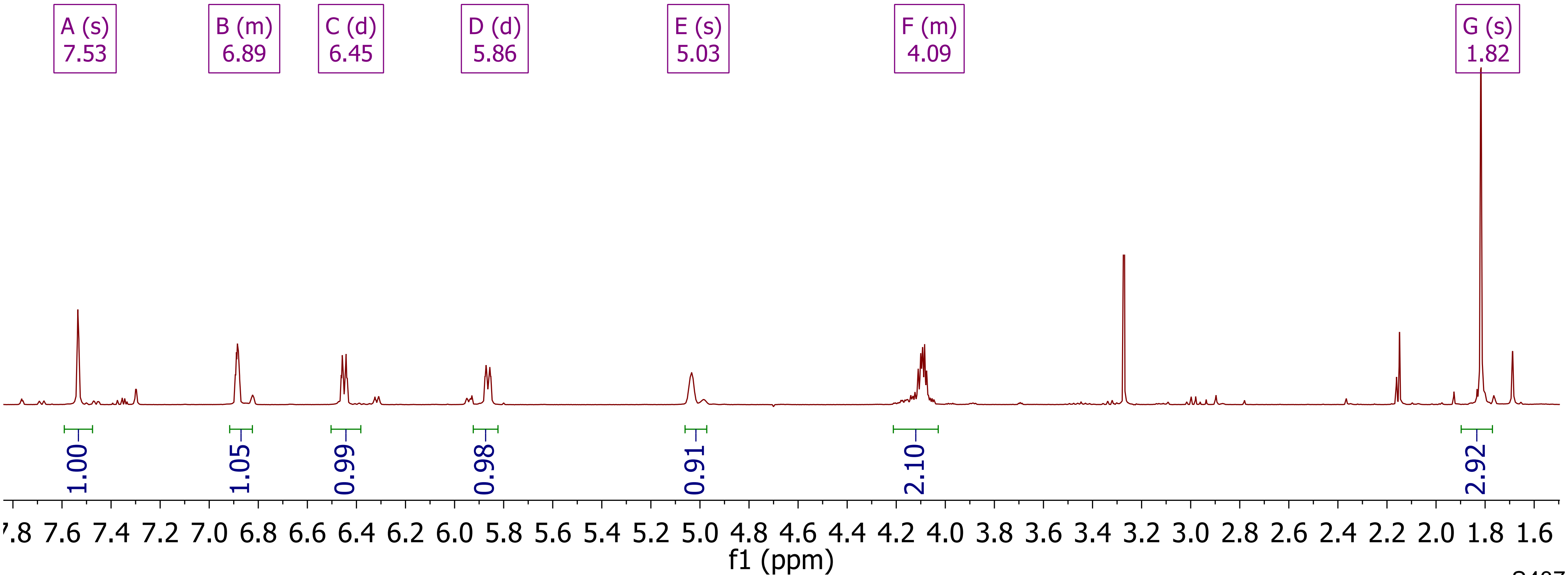



crude product after precipitation

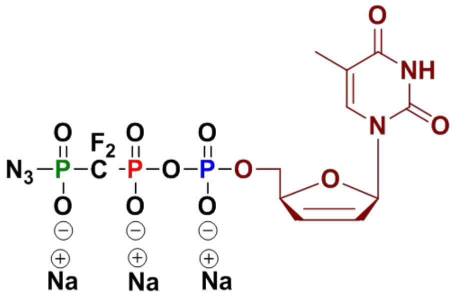
**$^1H$ NMR**



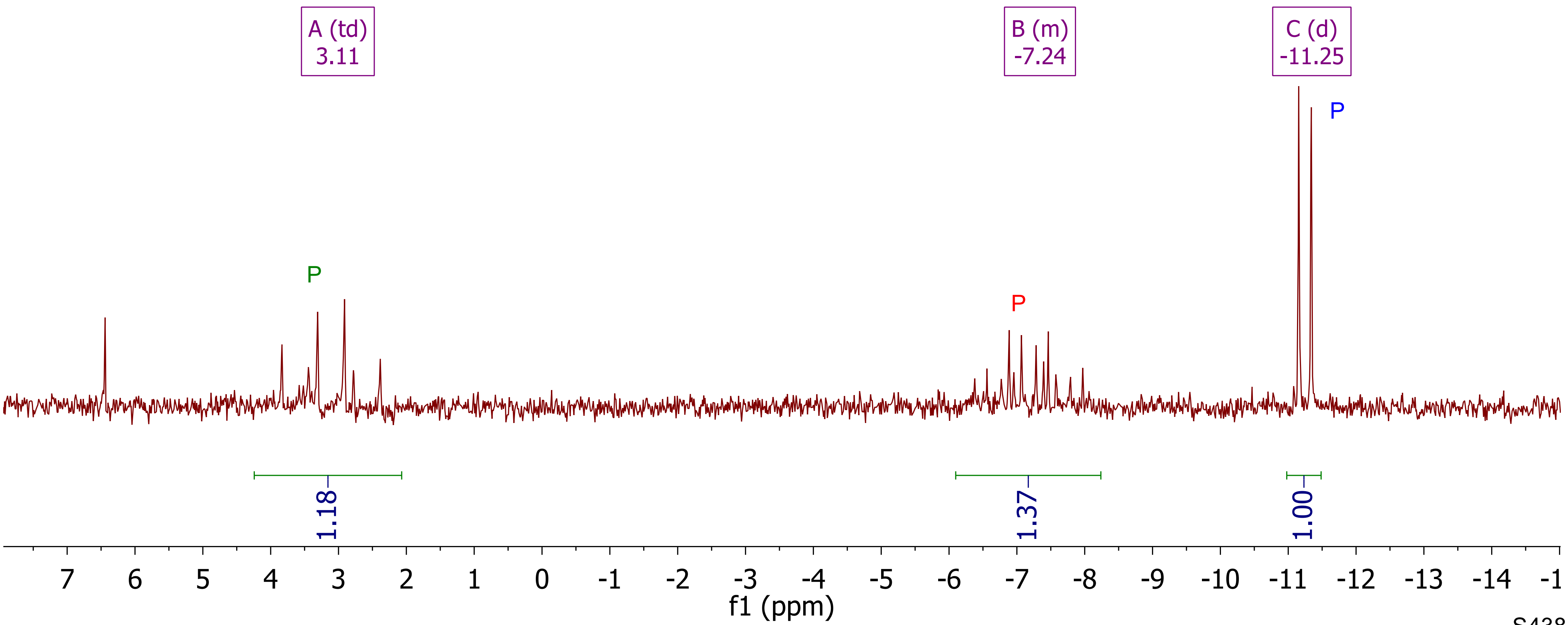
crude product after precipitation



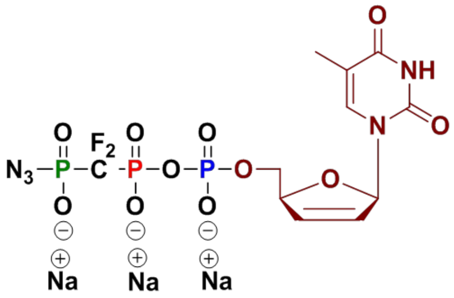
**$^{31}P\{^1H\}$ NMR**



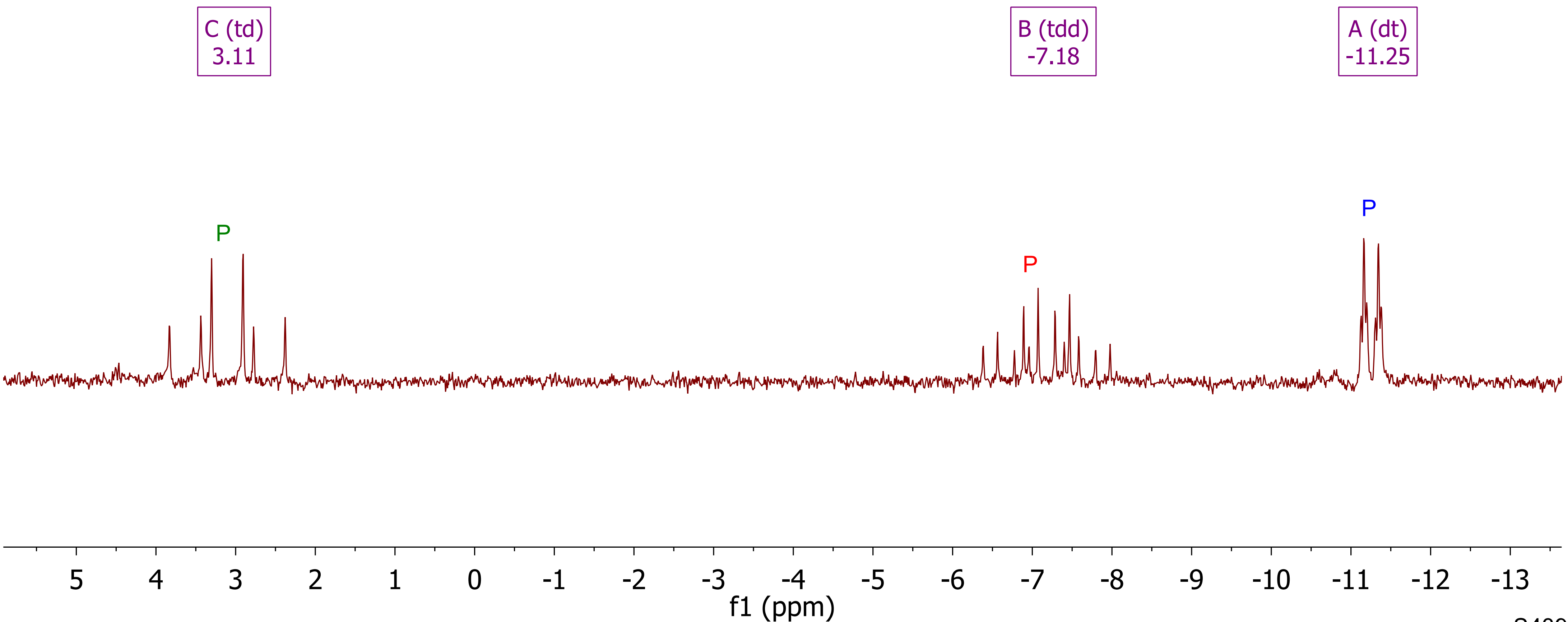
crude product after precipitation



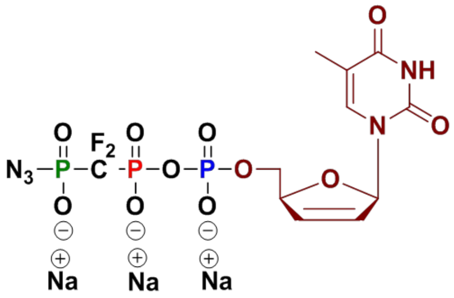
$^{31}$P-$^{1}$H NMR



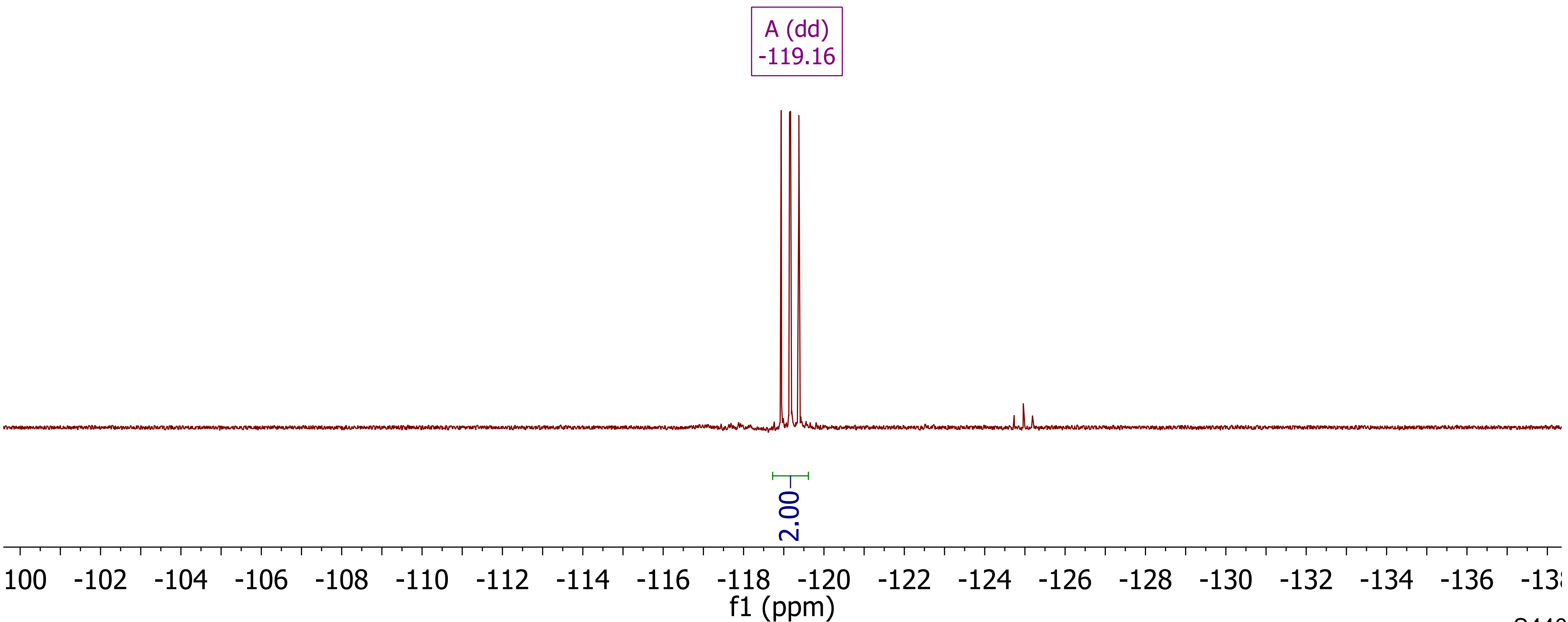
crude product after precipitation



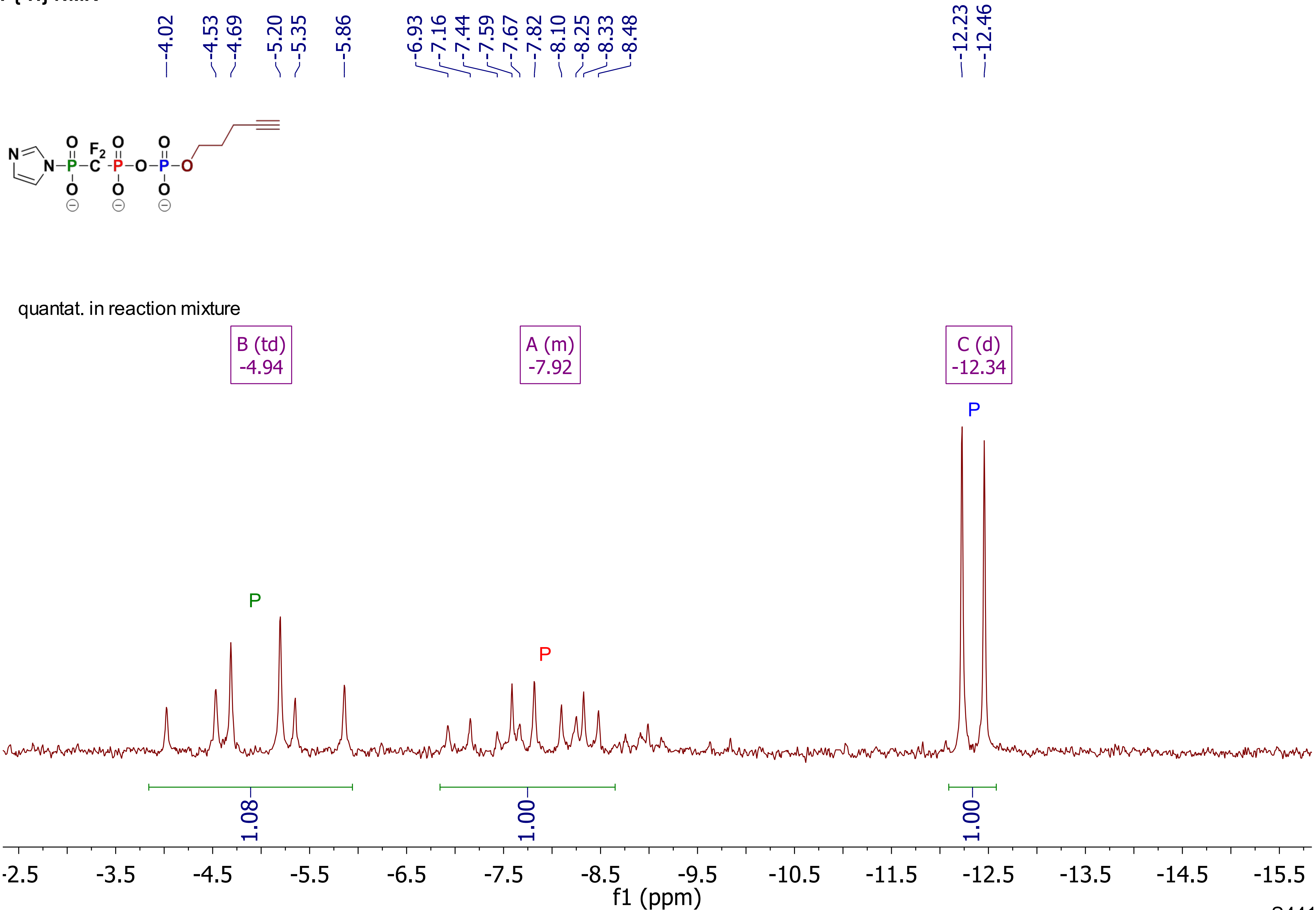
$^{19}$F NMR



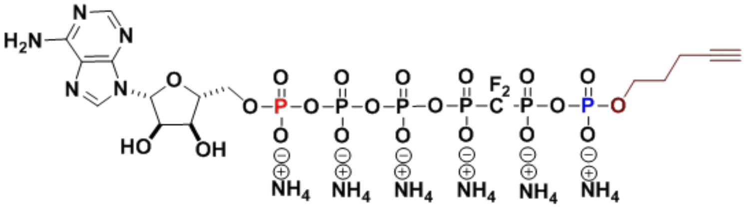

crude product after precipitation



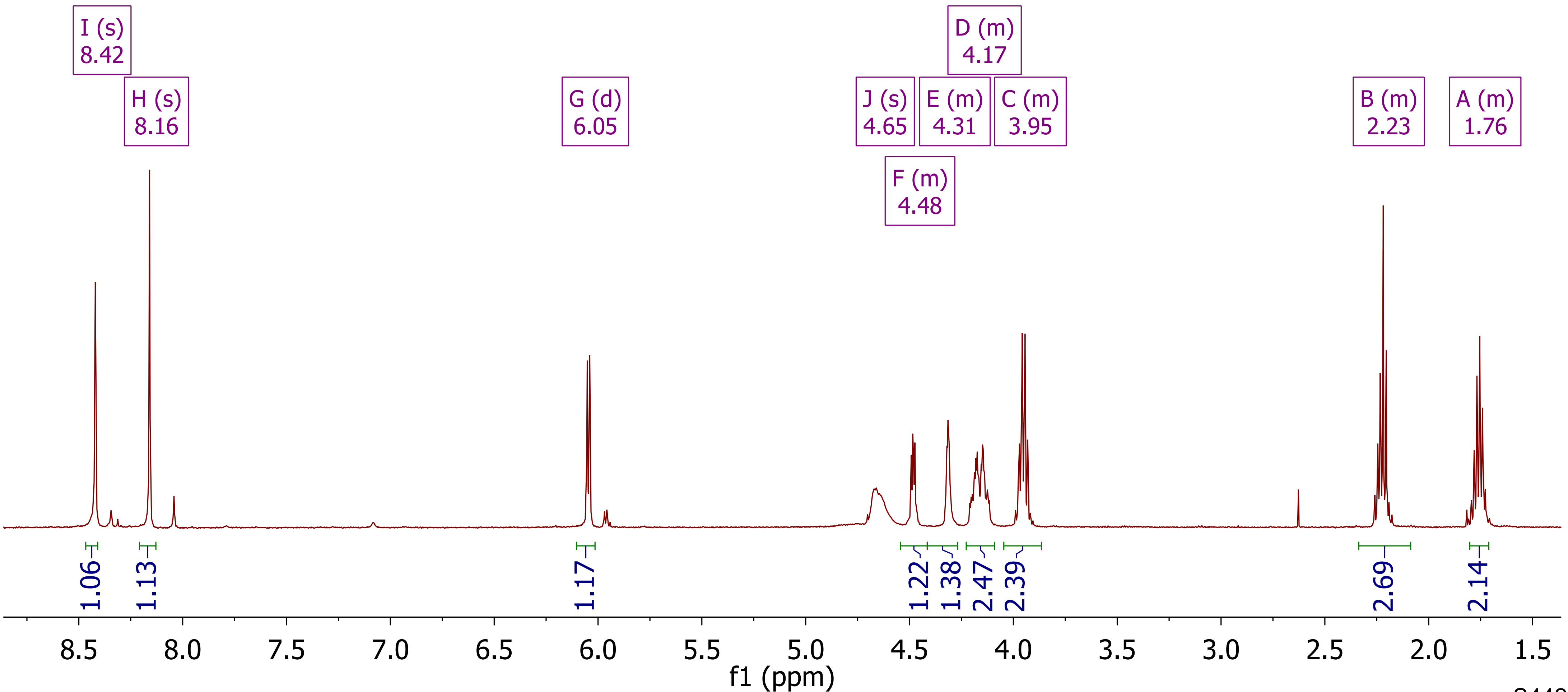
**$^{31}P\{^{1}H\}$ NMR**


-4.02
-4.53
-4.69
-5.20
-5.35
-5.86
-6.93
-7.16
-7.44
-7.59
-7.67
-7.82
-8.10
-8.25
-8.33
-8.48
-12.23
-12.46
quantat. in reaction mixture
B (td)
-4.94
A (m)
-7.92
C (d)
-12.34
P
P
P
1.08
1.00
1.00
-2.5
-3.5
-4.5
-5.5
-6.5
-7.5
-8.5
-9.5
-10.5
-11.5
-12.5
-13.5
-14.5
-15.5
f1 (ppm)

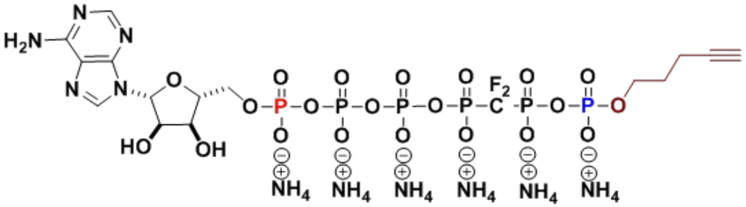
$^1$H NMR



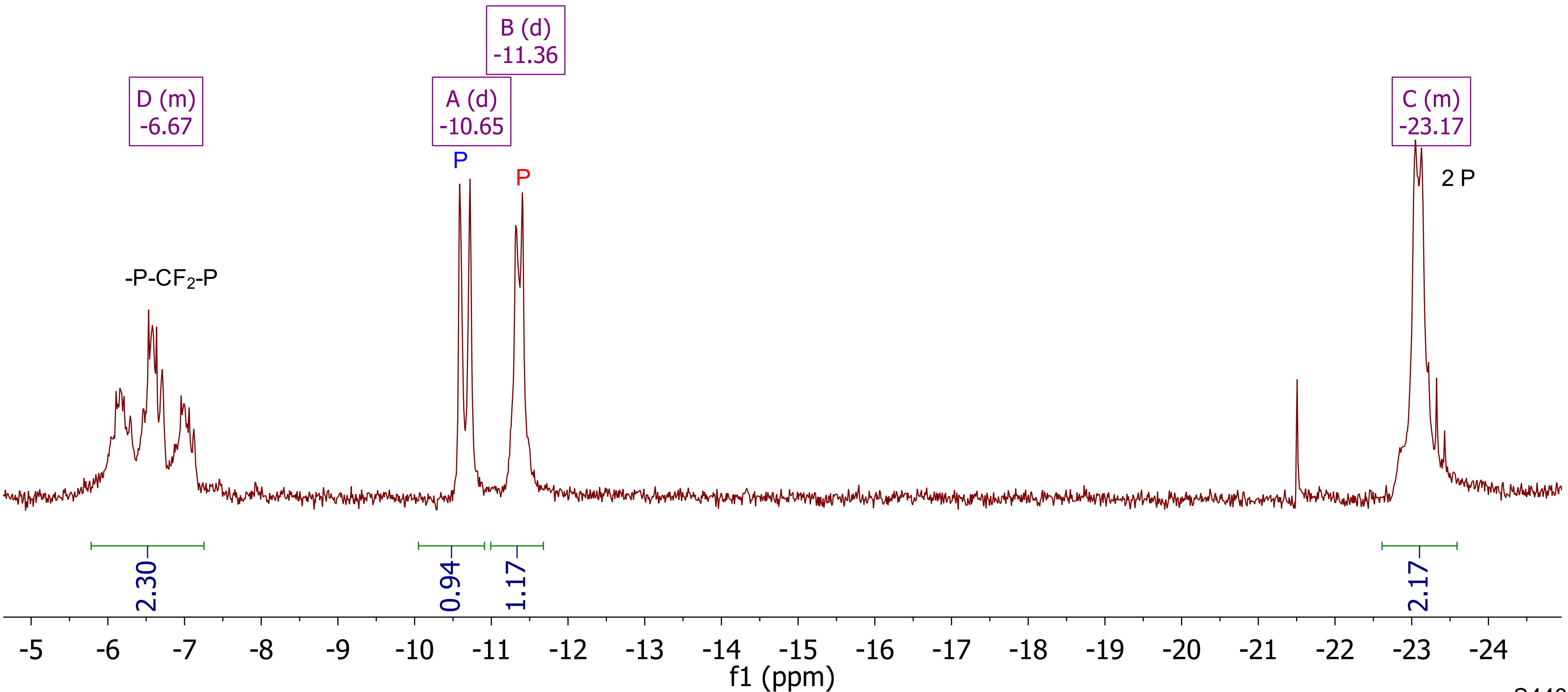
product purified by SAX



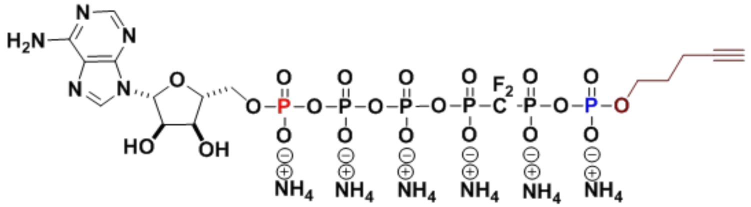
$^{31}P\{^1H\}$ NMR



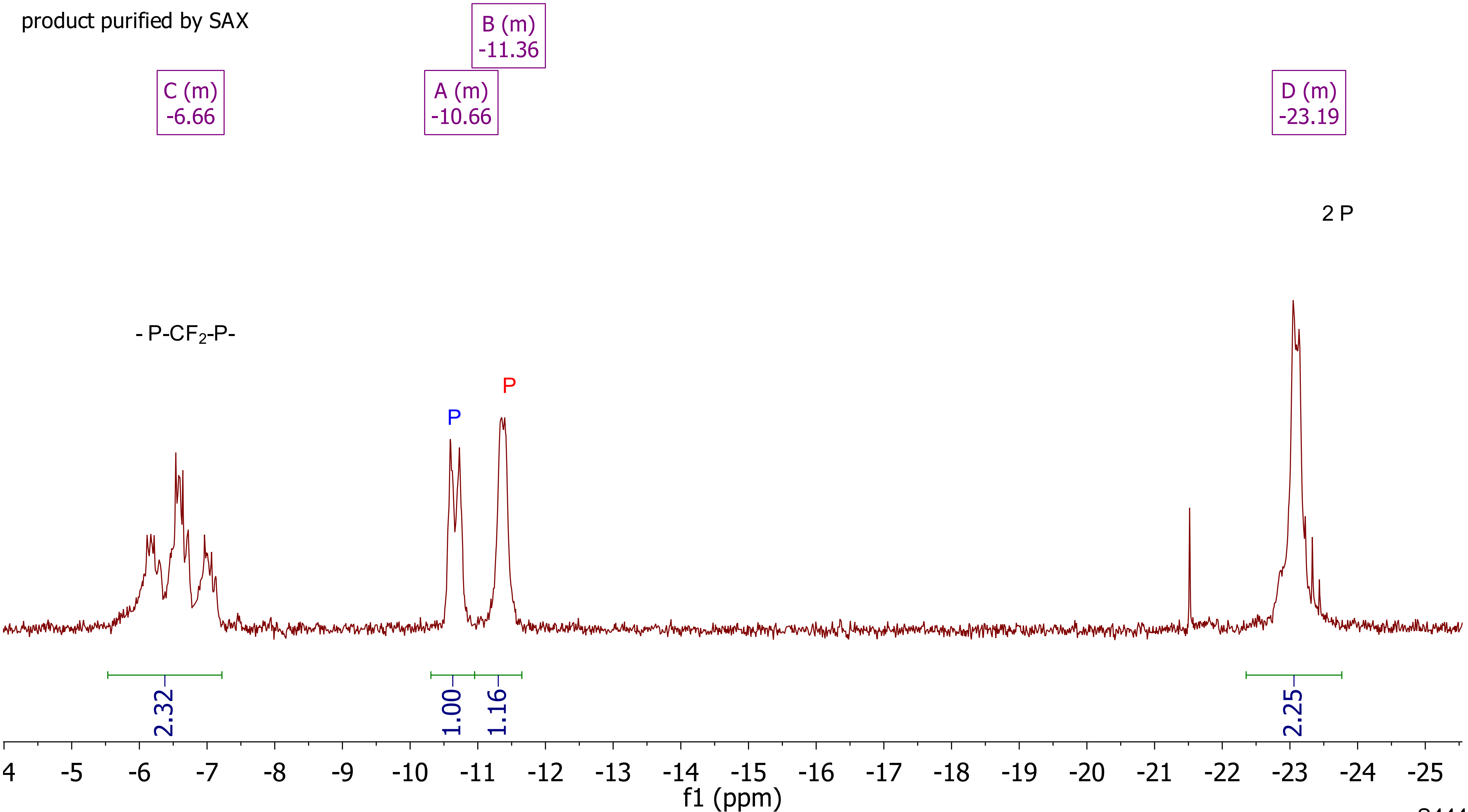

product purified by SAX



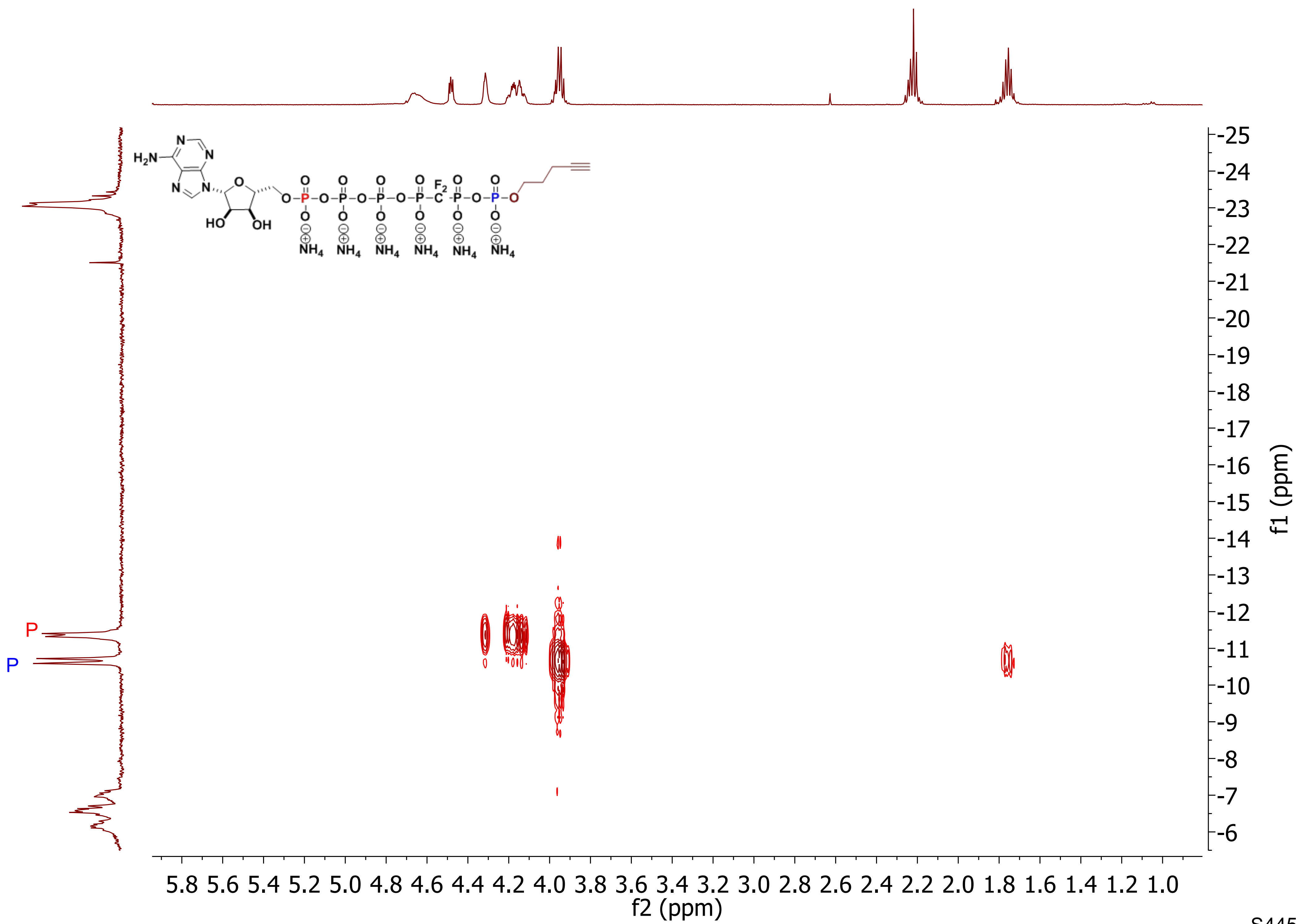
**$^{31}$P NMR**



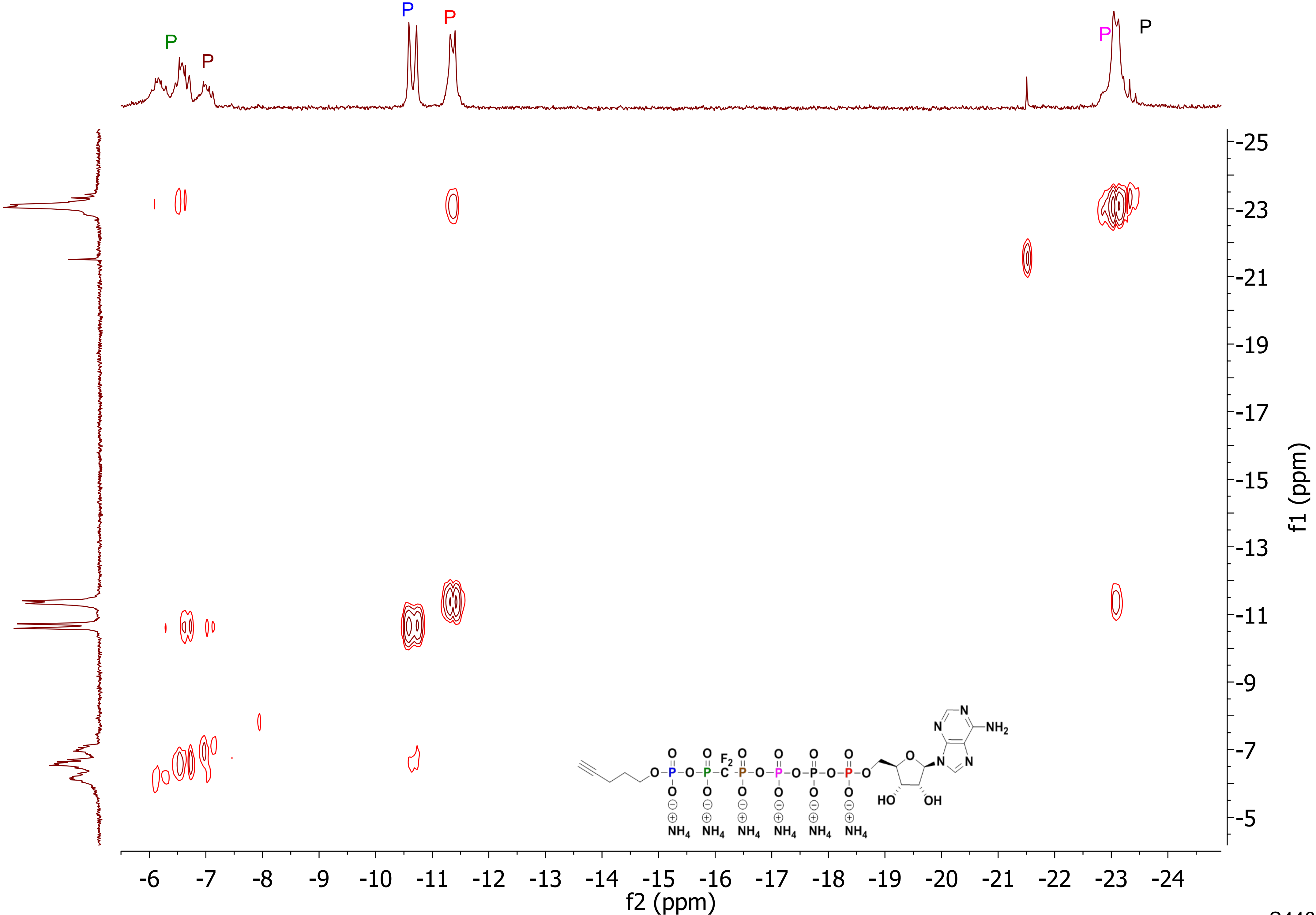

product purified by SAX



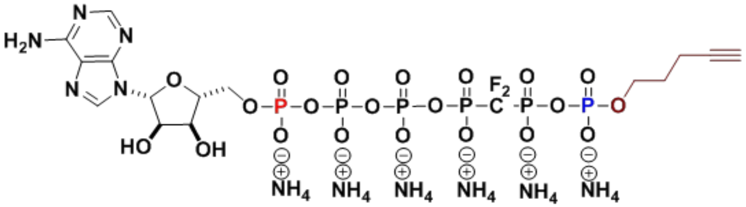
$^{31}P$-$^{1}H$ HMBC NMR



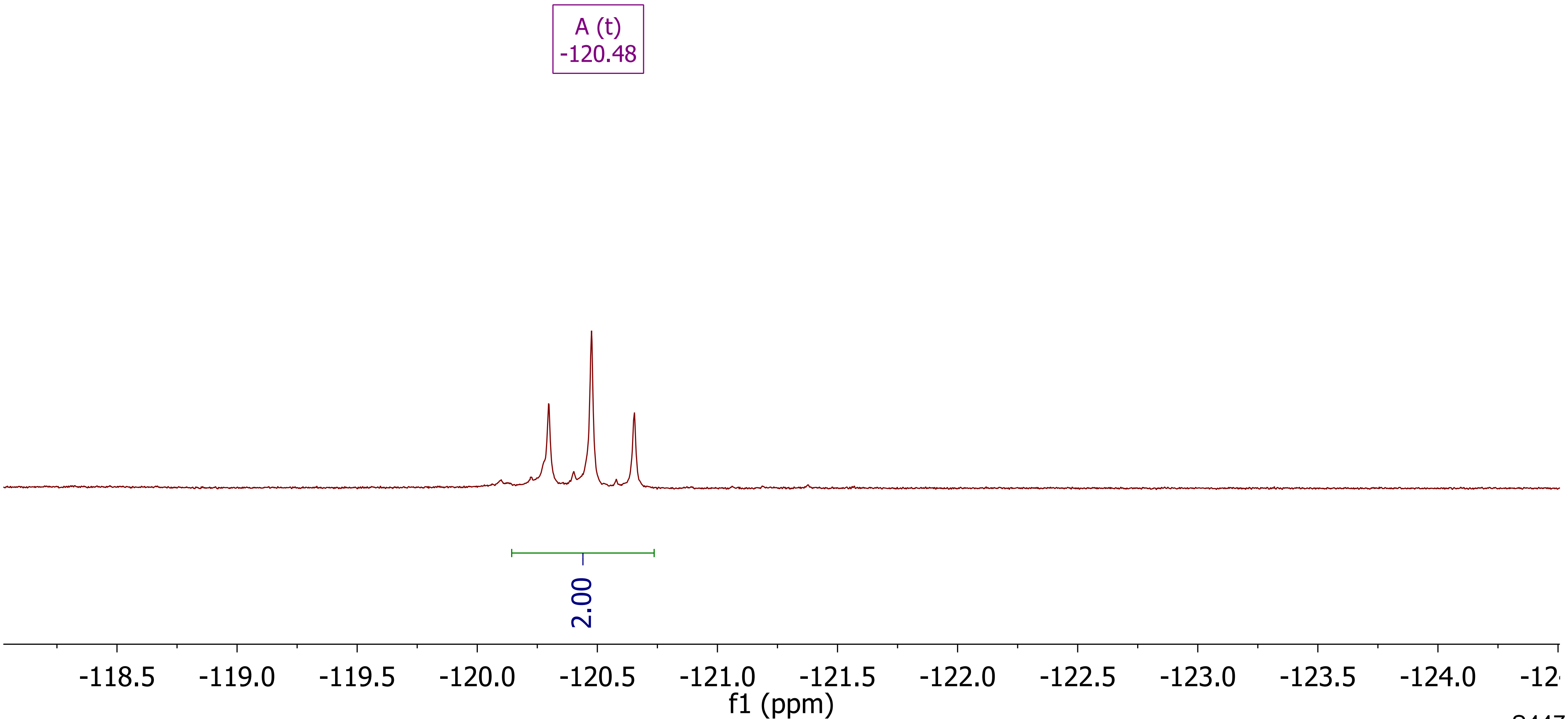
$^{31}P$-$^{31}P$ COSY NMR



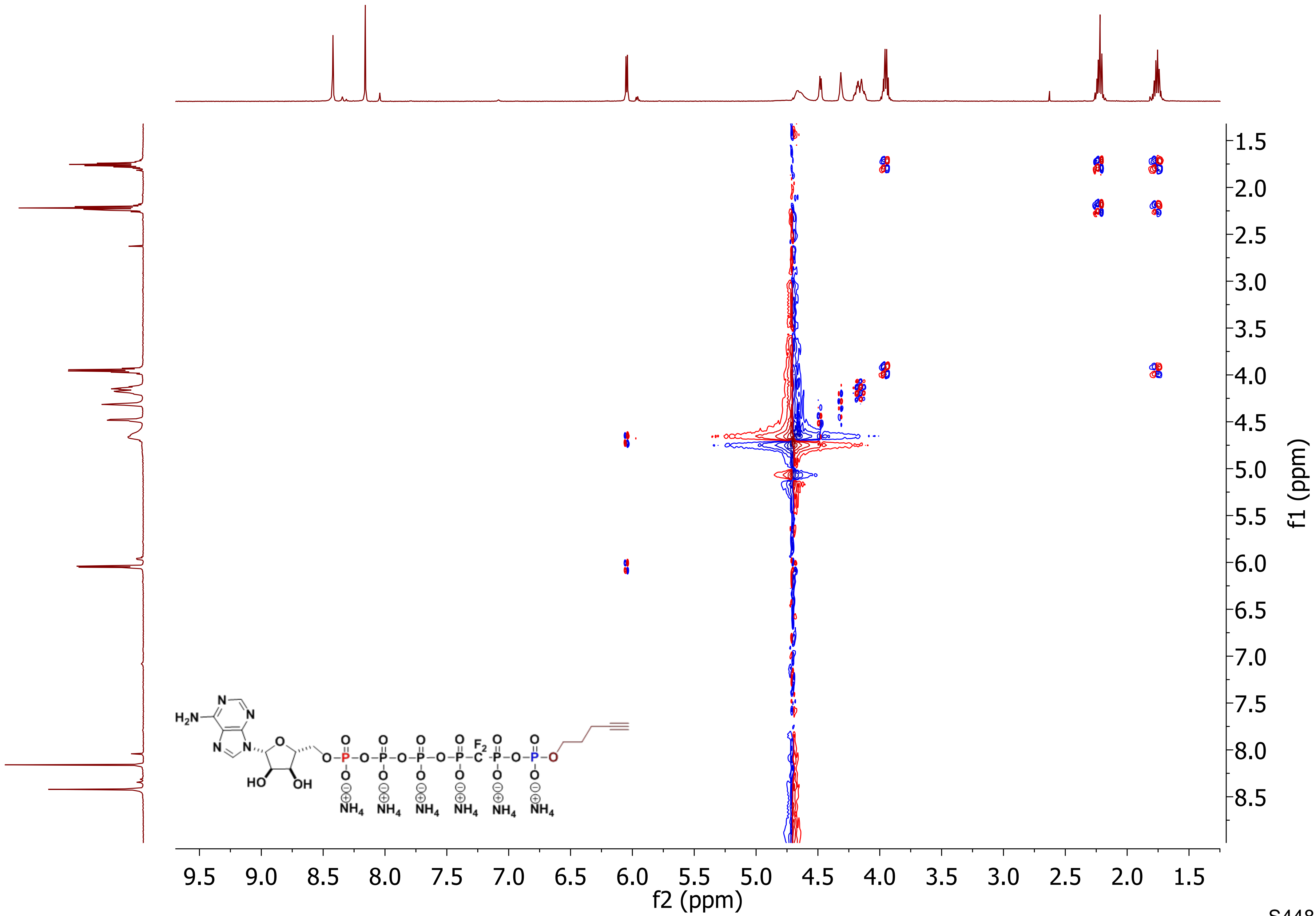
$^{19}$F NMR



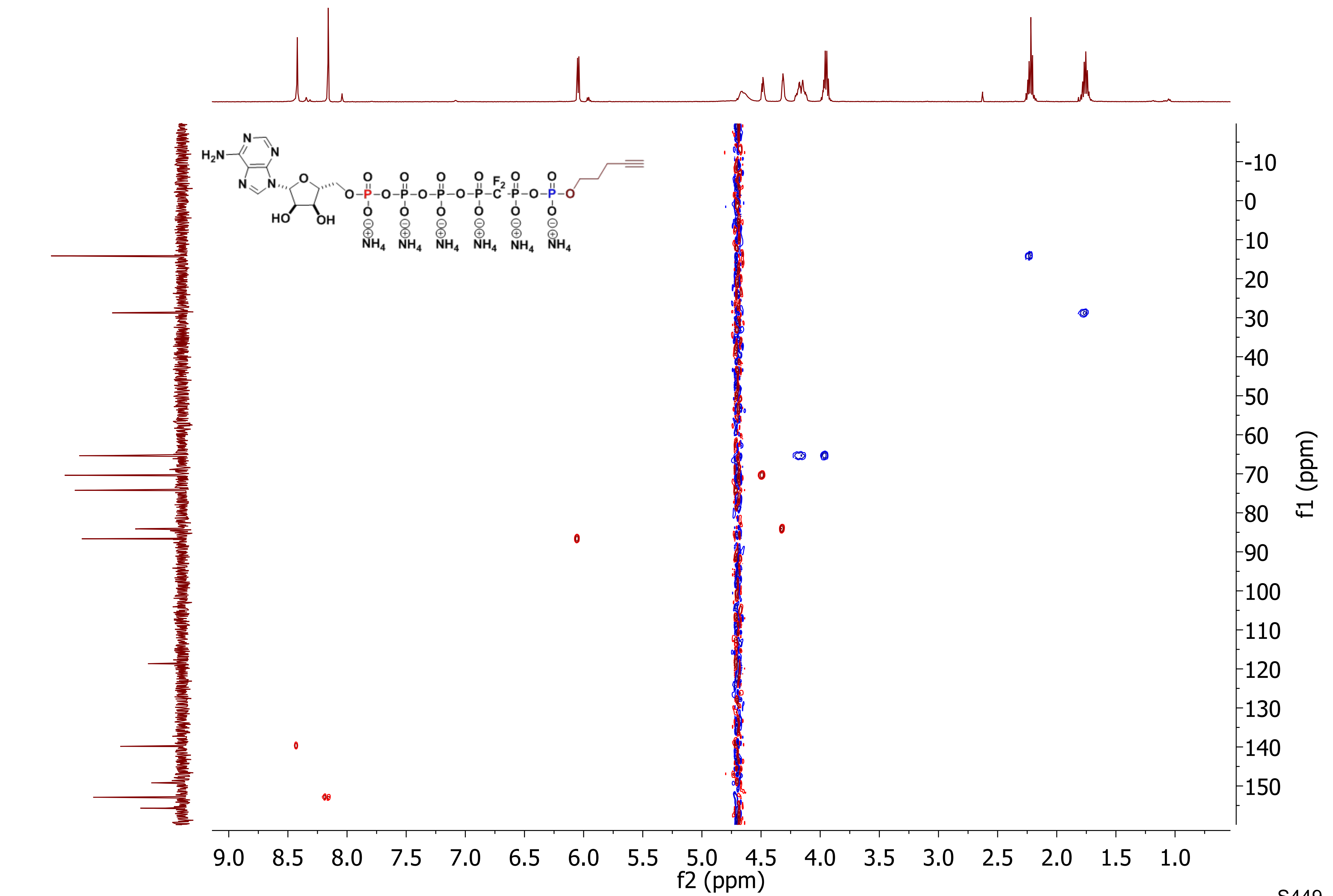
product purified by SAX



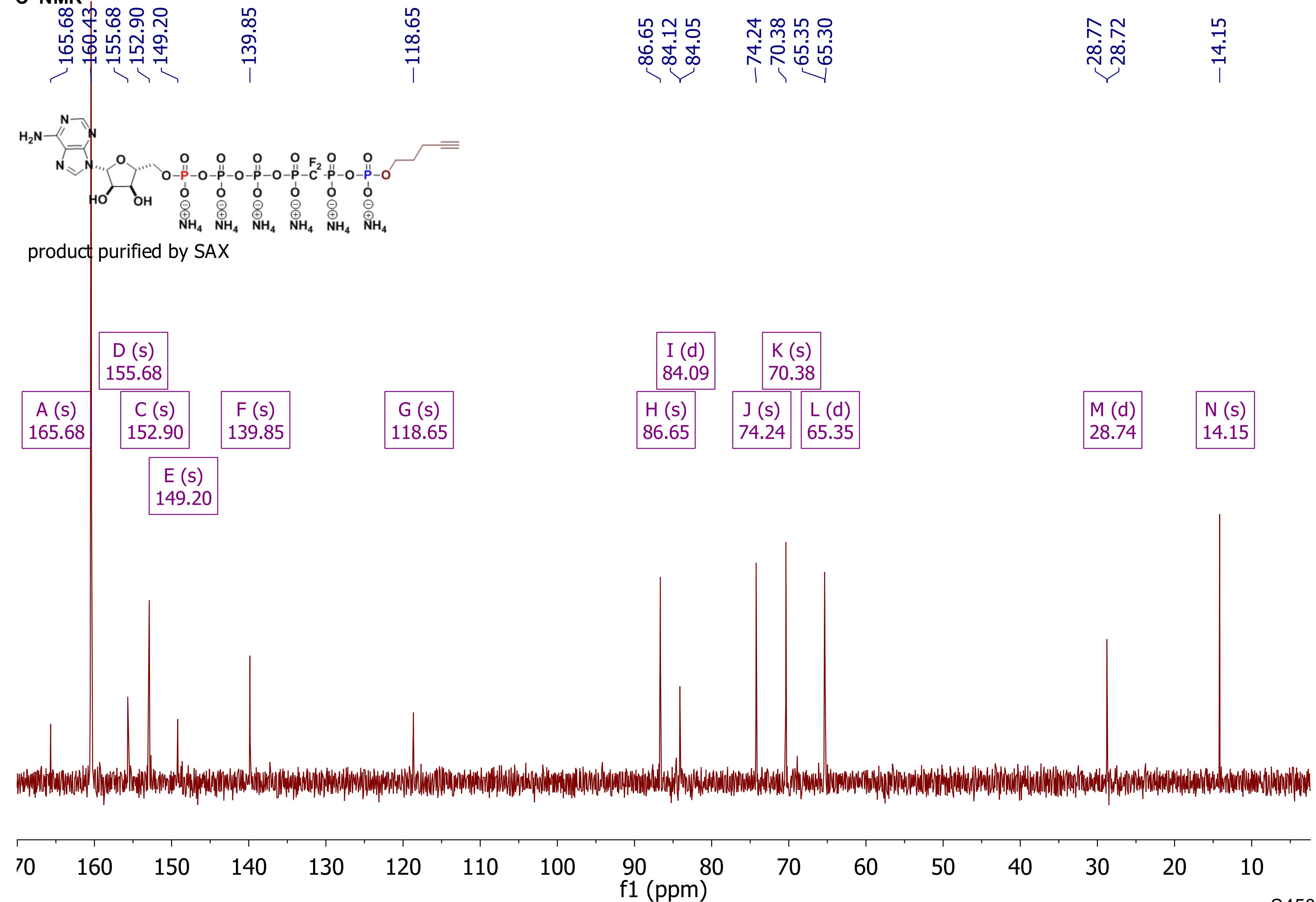
**$^1$H- $^1$H HSQC NMR**



$^{1}H$- $^{13}C$ HSQC NMR

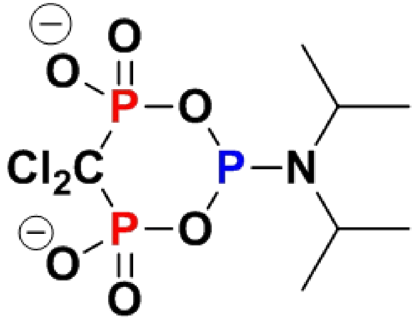


$^{13}$C NMR

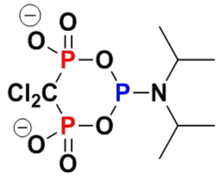


165.68
160.43
155.68
152.90
149.20
139.85
118.65
86.65
84.12
84.05
74.24
70.38
65.35
65.30
28.77
28.72
14.15
product purified by SAX
A (s) 165.68
D (s) 155.68
C (s) 152.90
E (s) 149.20
F (s) 139.85
G (s) 118.65
H (s) 86.65
I (d) 84.09
J (s) 74.24
K (s) 70.38
L (d) 65.35
M (d) 28.74
N (s) 14.15
f1 (ppm)

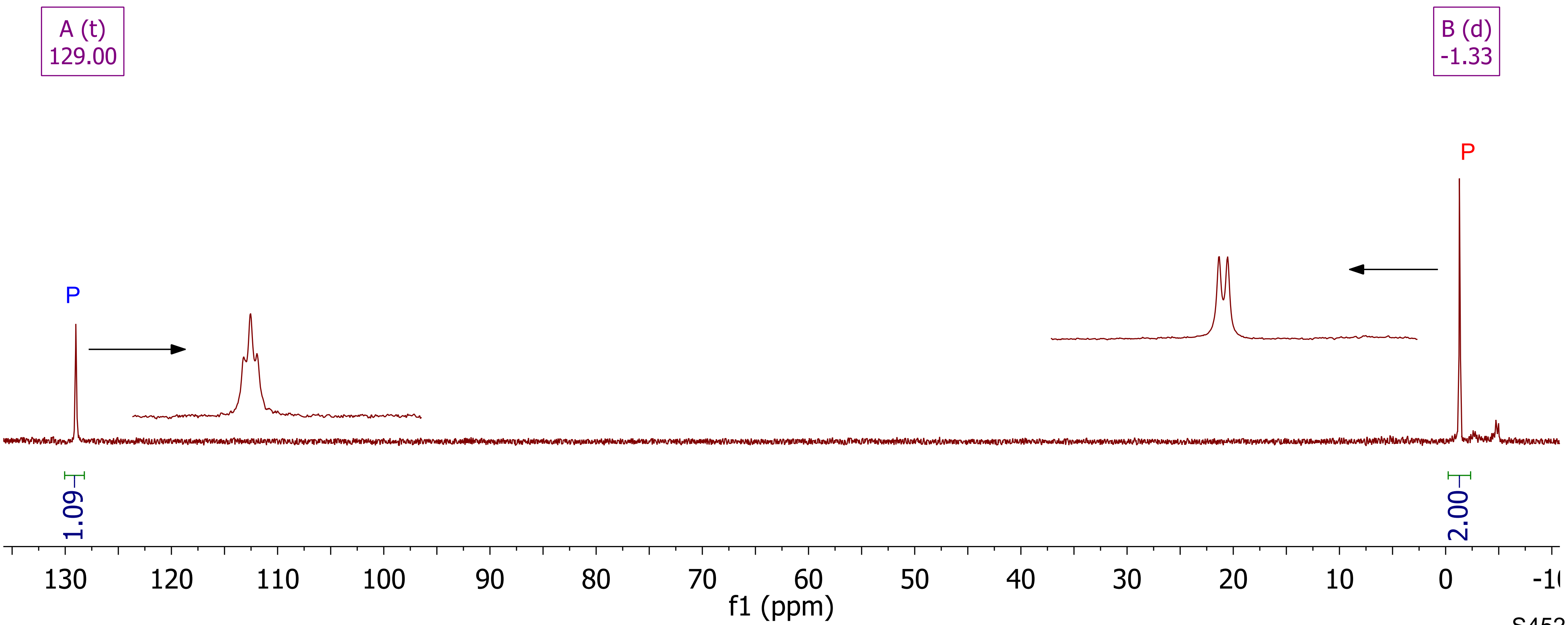
# Synthesis of triphosphates based on c-Py$_{CCl2}$PA ($A_4$)



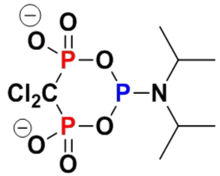
$^{31}P\{^1H\}$ NMR



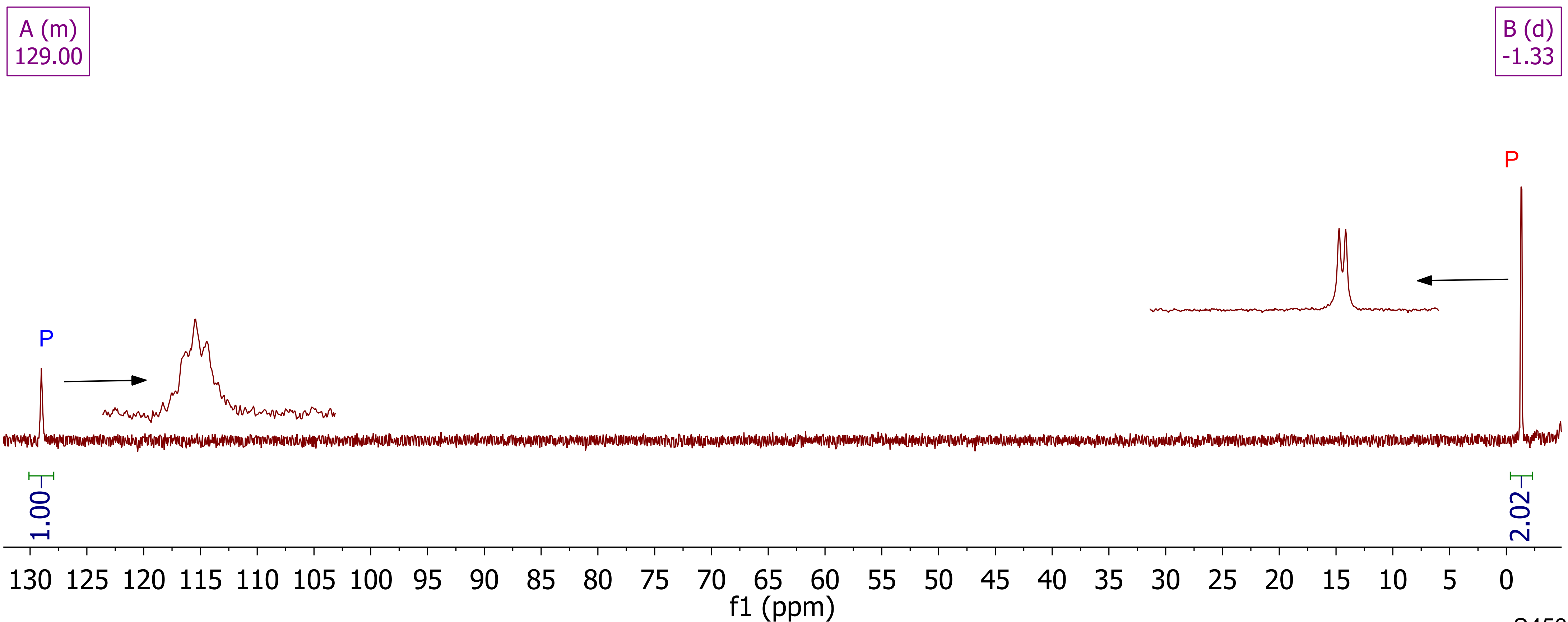
c-Py$_{CCl2}$PA(A$_4$)



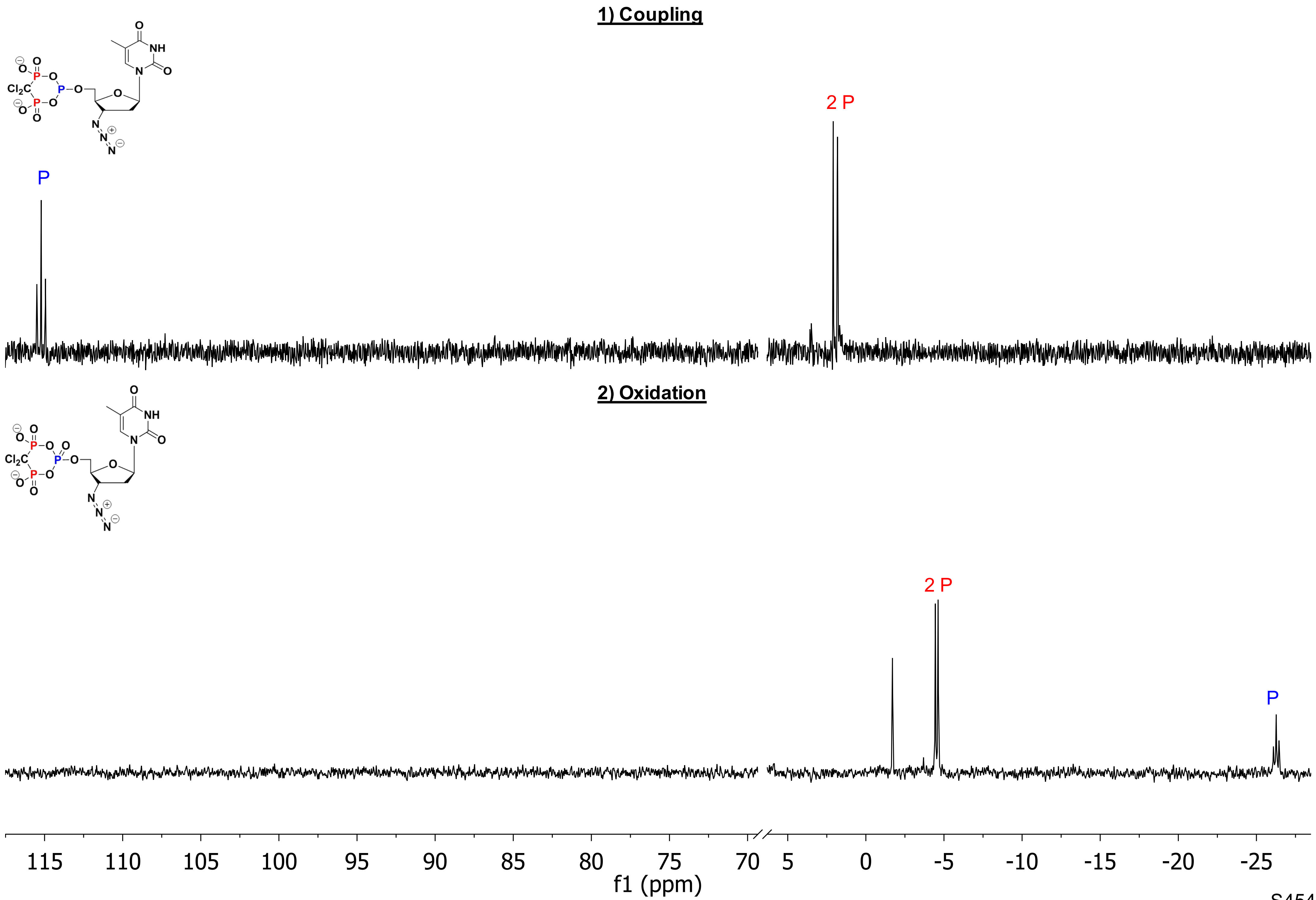
**$^{31}$P NMR**



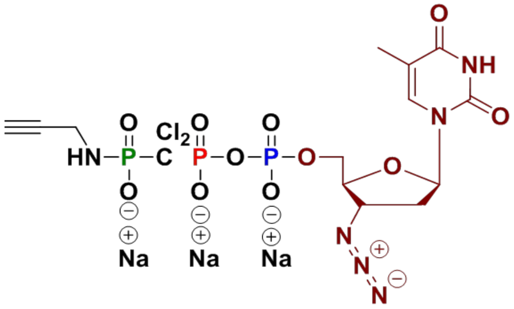
**c-Py$_{CCl2}$PA(A$_4$)**



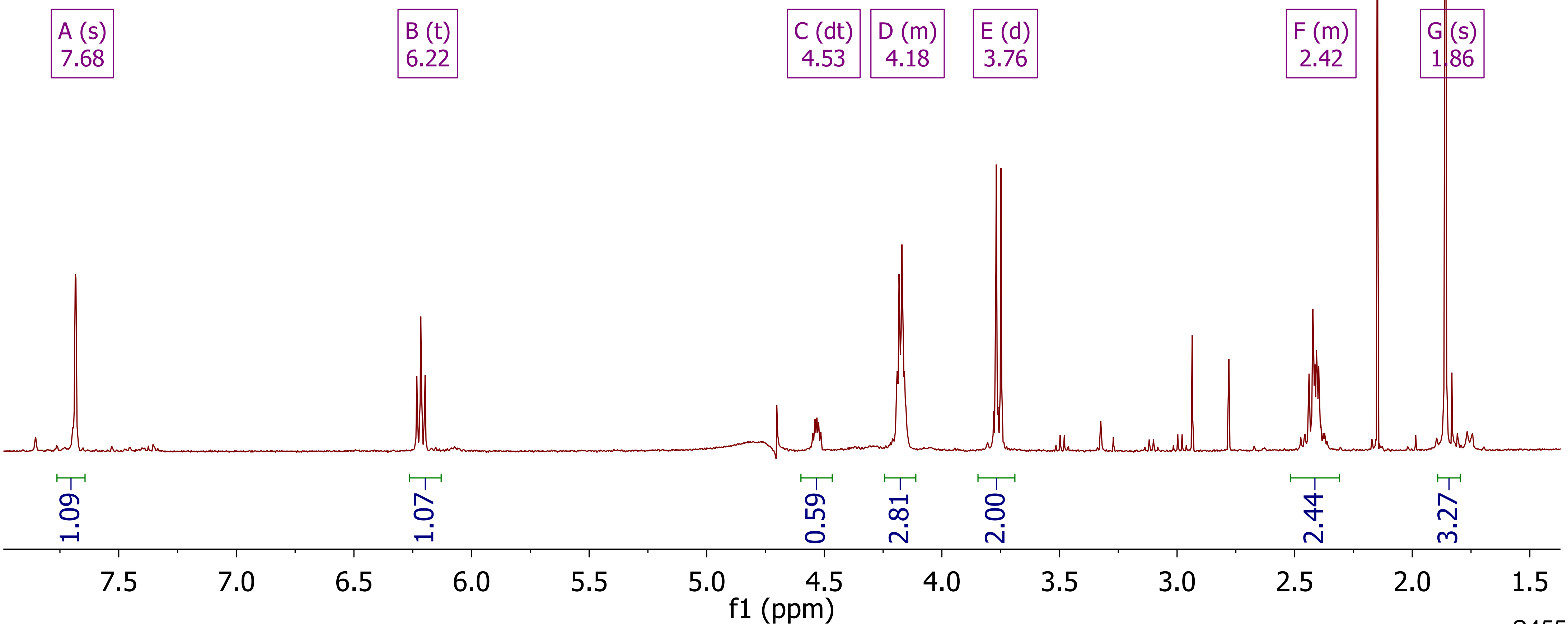
**$^{31}P\{^1H\}$ NMR**


1) Coupling
P
2 P
2) Oxidation
2 P
P
115 110 105 100 95 90 85 80 75 70 5 0 -5 -10 -15 -20 -25
f1 (ppm)

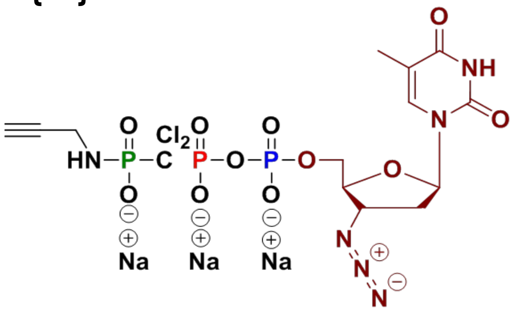
$^{1}$H NMR



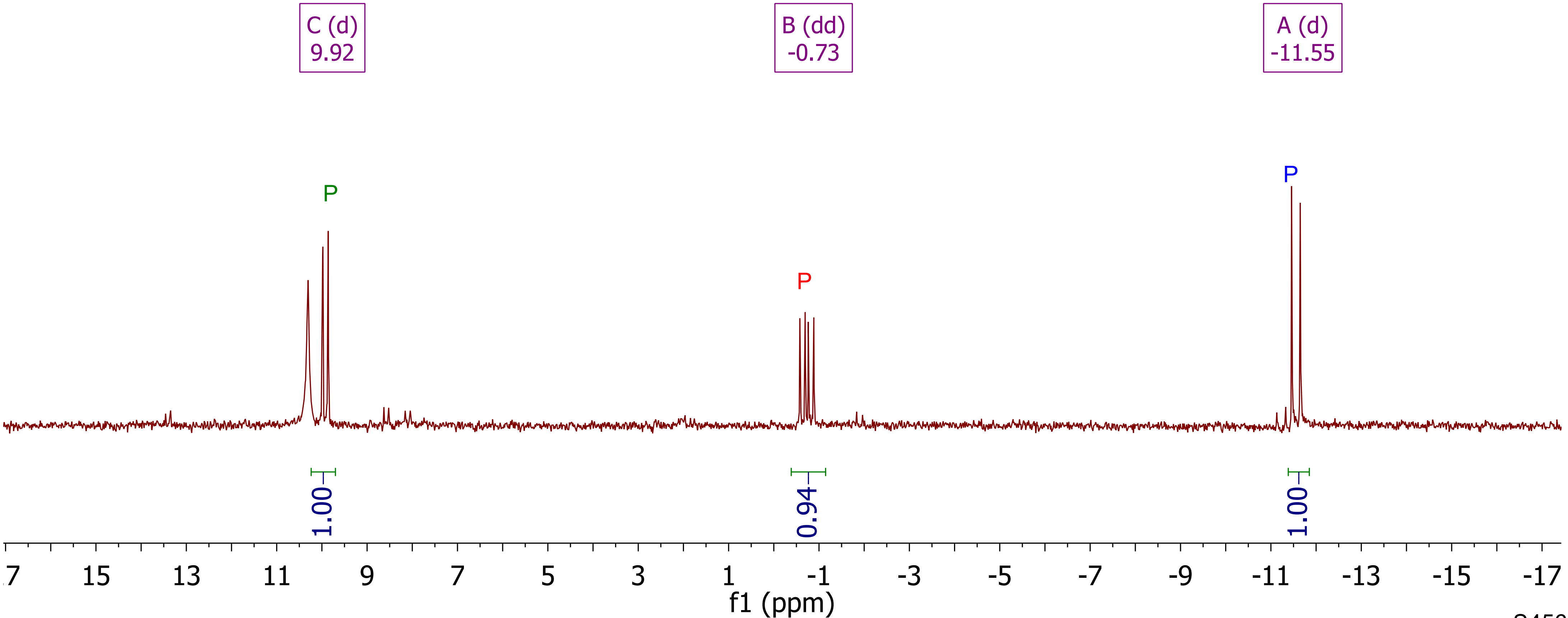
crude product after precipitation



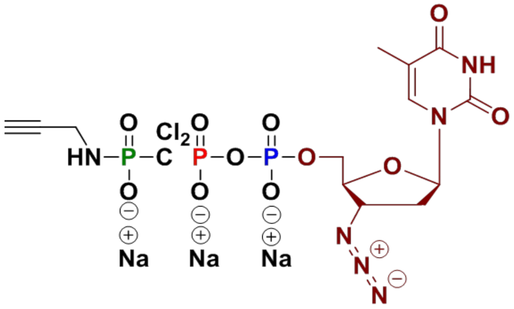
$^{31}P\{^1H\}$ NMR



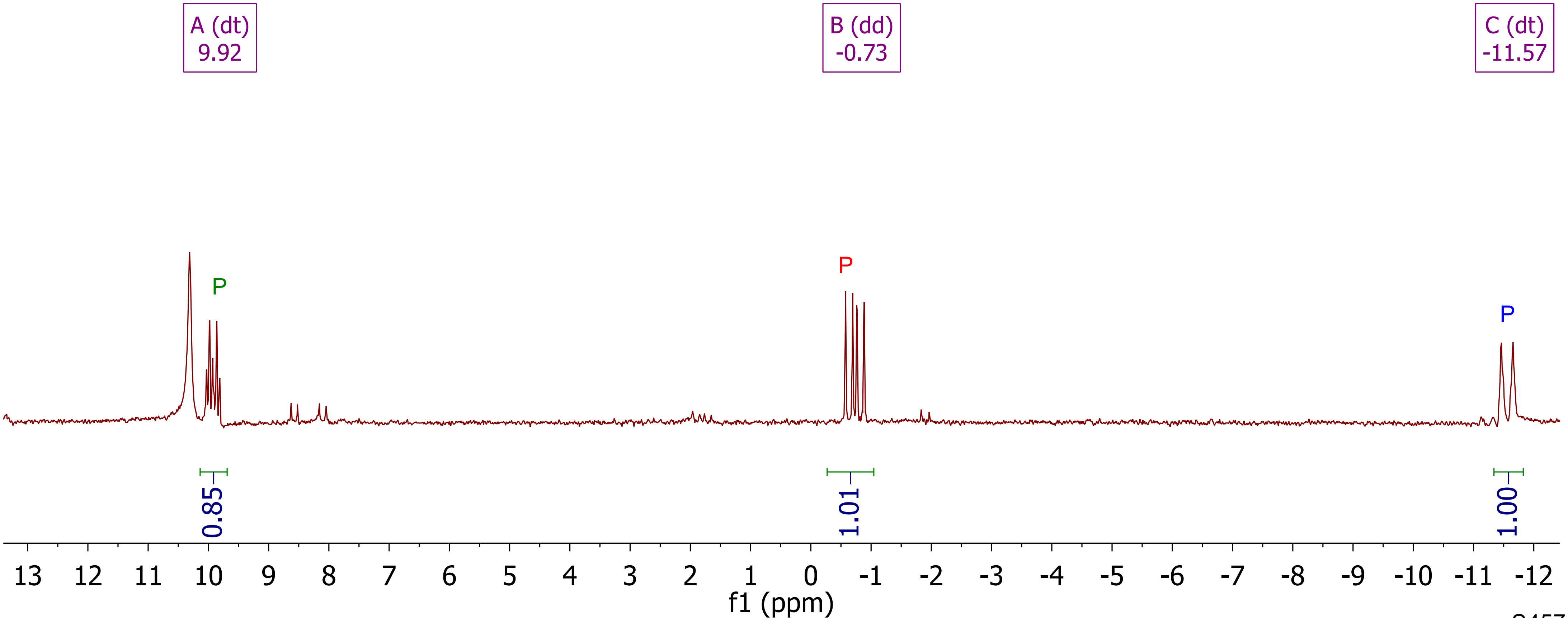
crude product after precipitation



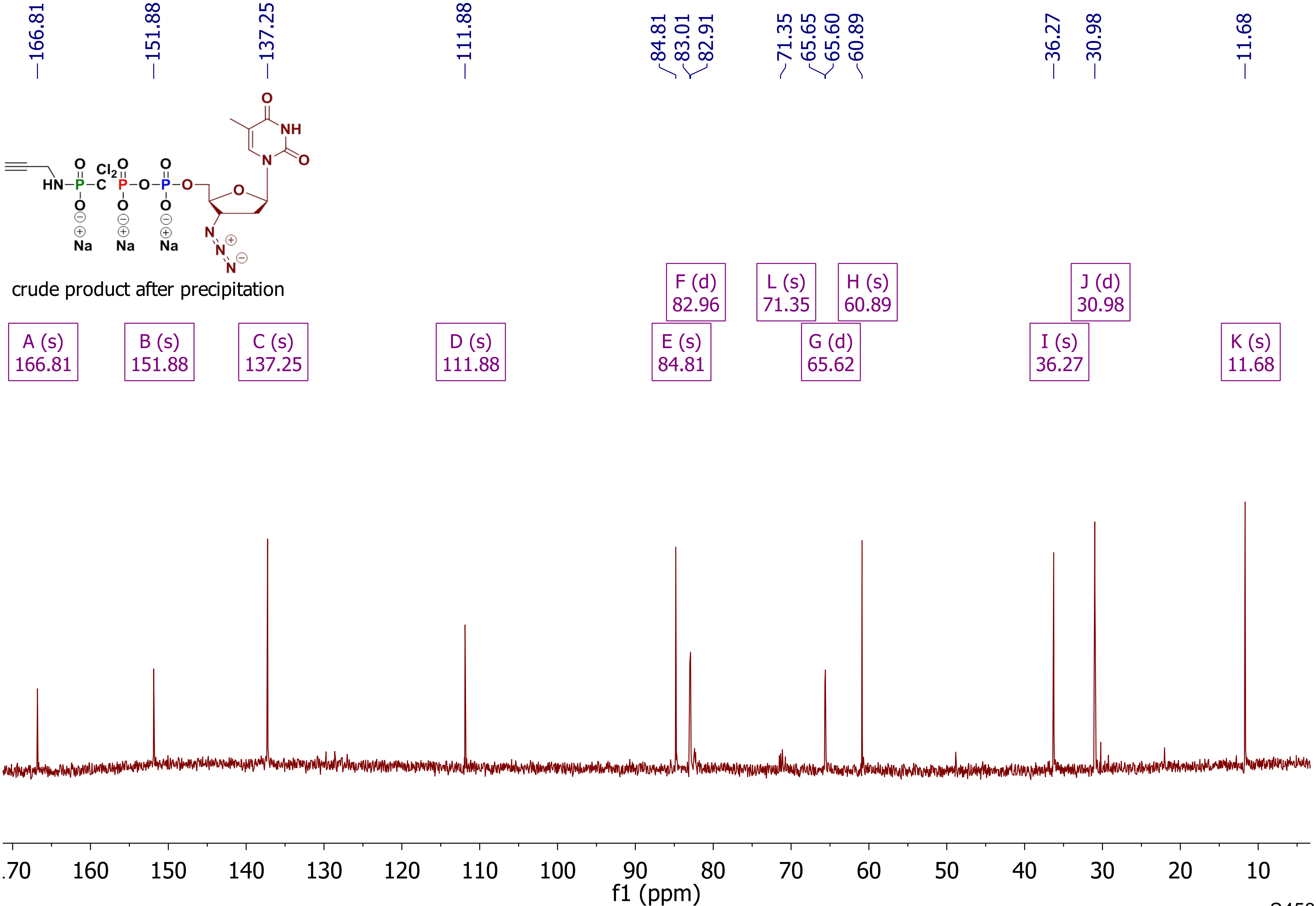
**$^{31}$P NMR**



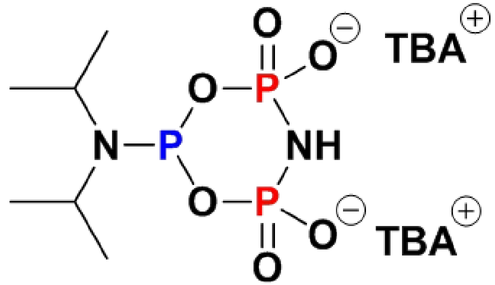

crude product after precipitation



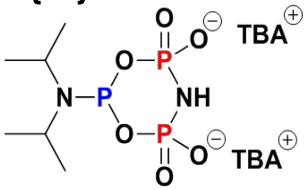
**$^{13}$C NMR**


166.81
151.88
137.25
111.88
84.81
83.01
82.91
71.35
65.65
65.60
60.89
36.27
30.98
11.68
crude product after precipitation
A (s) 166.81
B (s) 151.88
C (s) 137.25
D (s) 111.88
E (s) 84.81
F (d) 82.96
L (s) 71.35
G (d) 65.62
H (s) 60.89
I (s) 36.27
J (d) 30.98
K (s) 11.68
f1 (ppm)

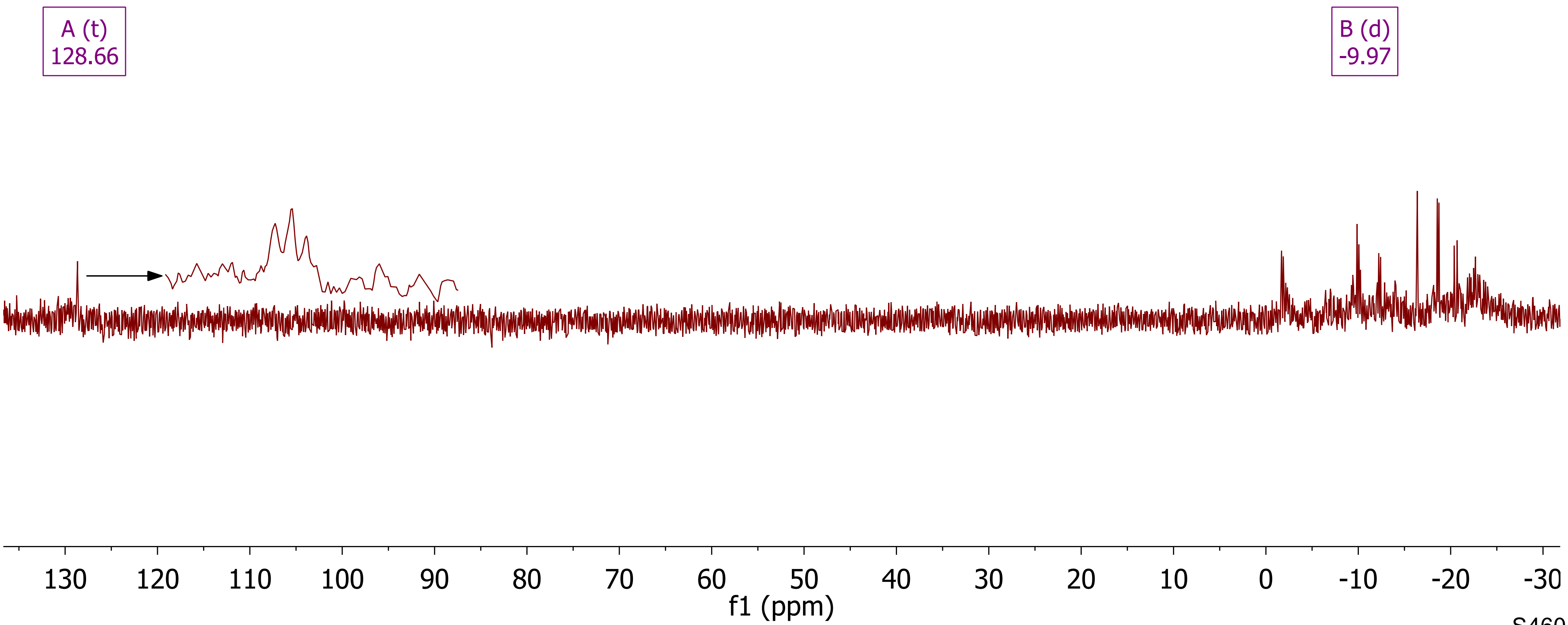
# Synthesis of triphosphates based on c-Py$_{NH}$PA (A$_5$)



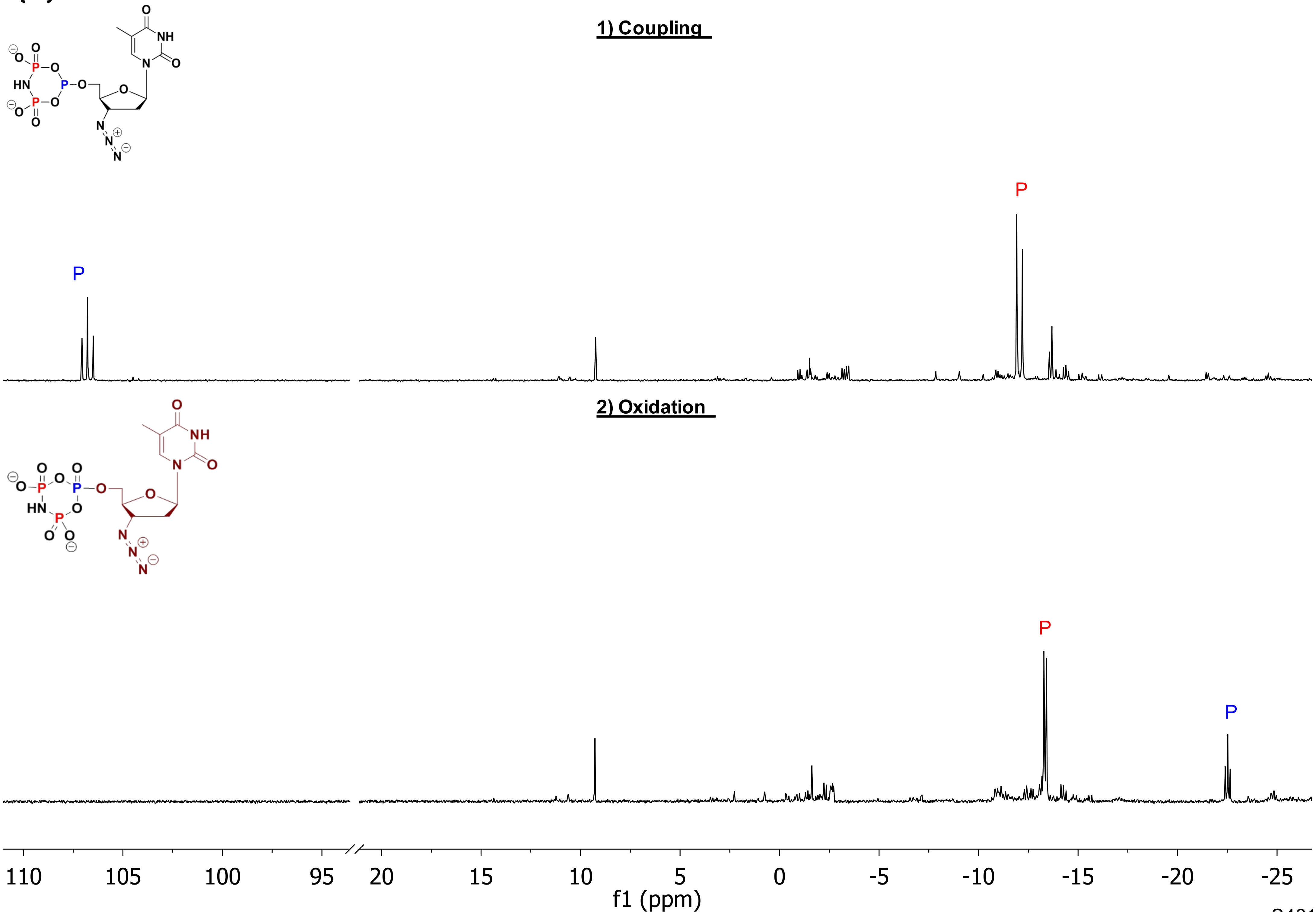
**$^{31}P\{^{1}H\}$ NMR**



The $cPy_{NH}PA$ was not stable inside the nmr tube

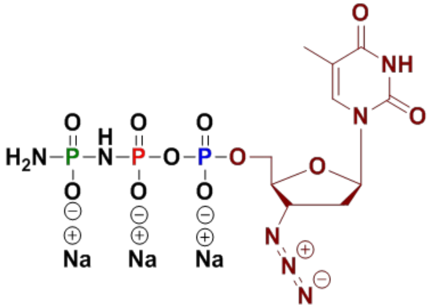


**$^{31}P\{^1H\}$ NMR**

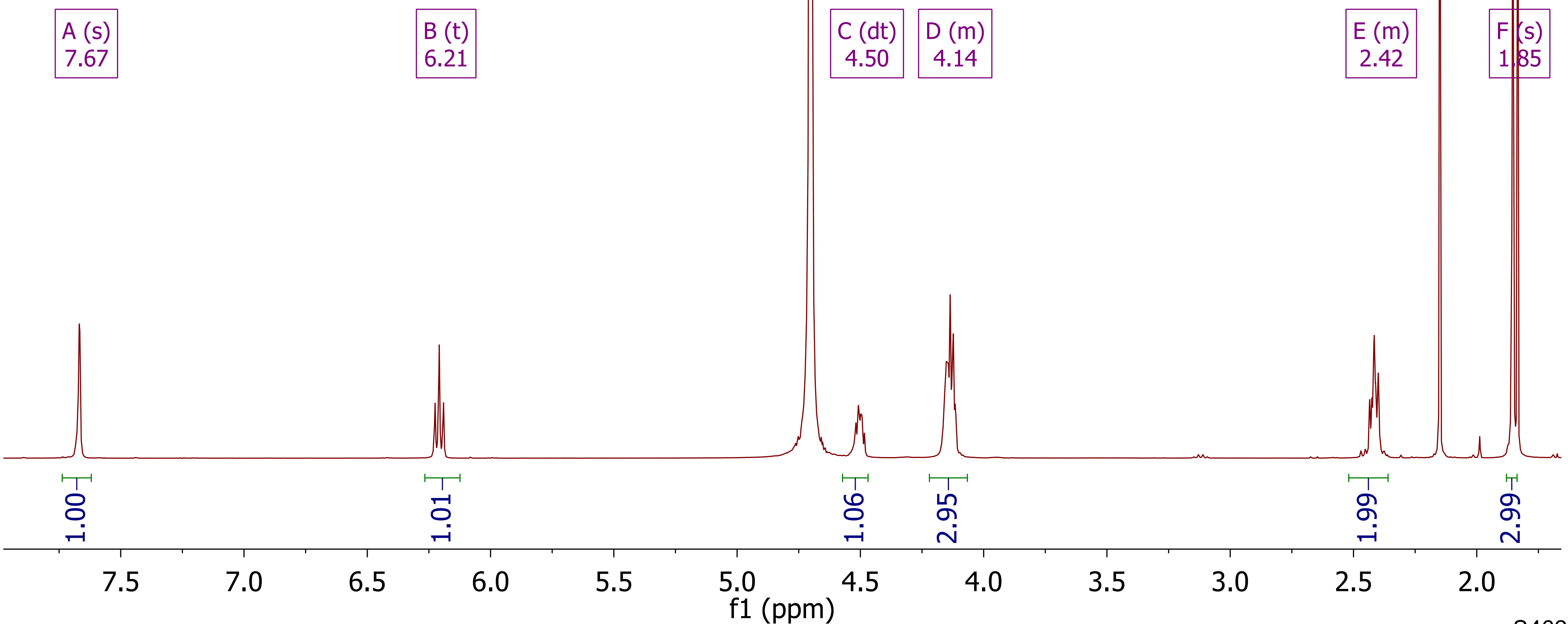


1) Coupling
P
P
2) Oxidation
P
P
110
105
100
95
20
15
10
5
0
-5
-10
-15
-20
-25
f1 (ppm)

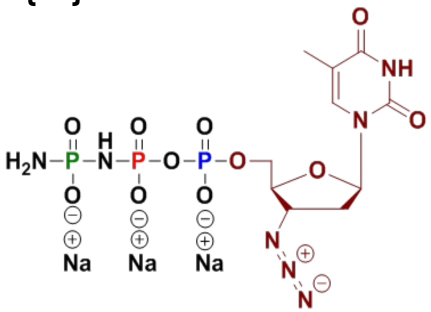
$^1$H NMR



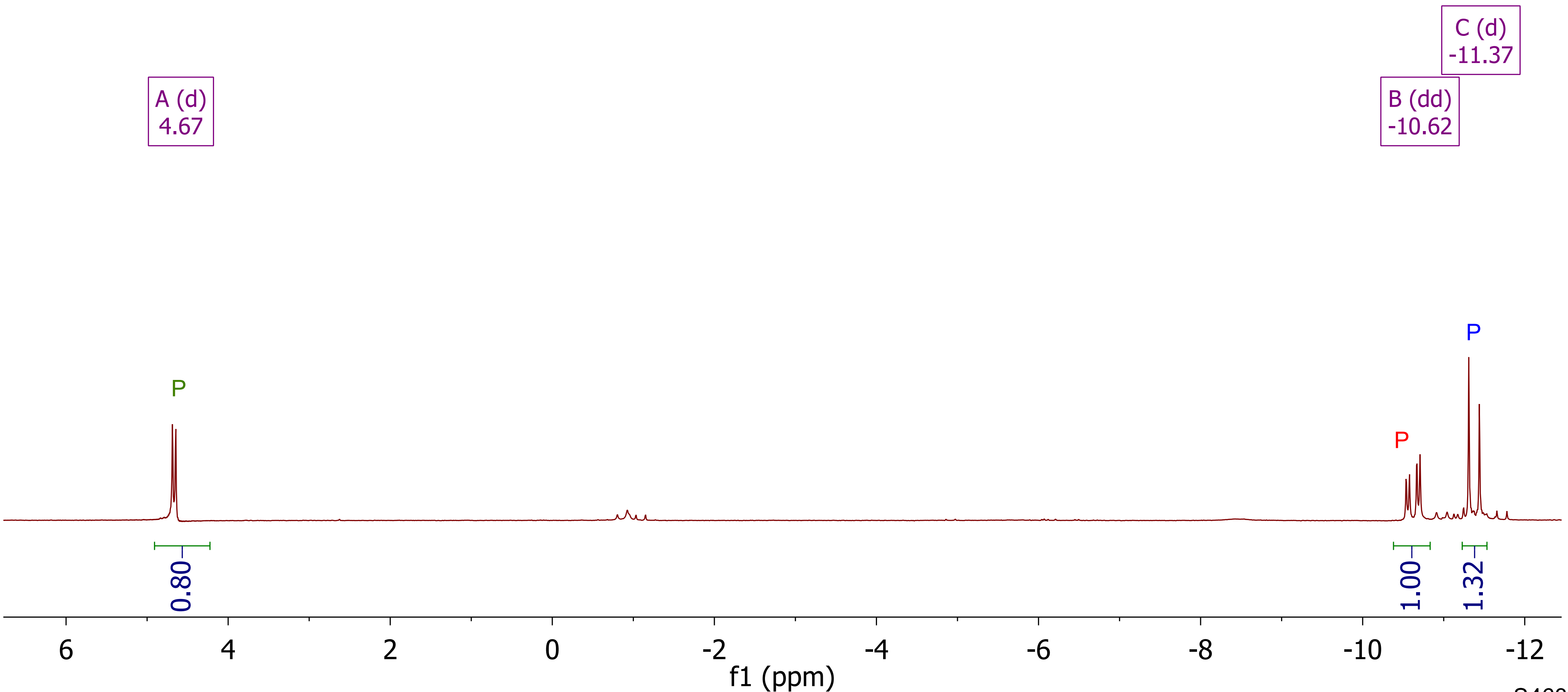
product after MPLC



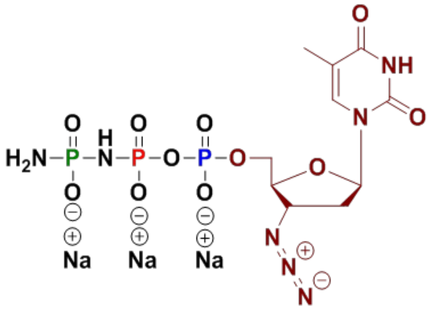
$^{31}P\{^{1}H\}$ NMR



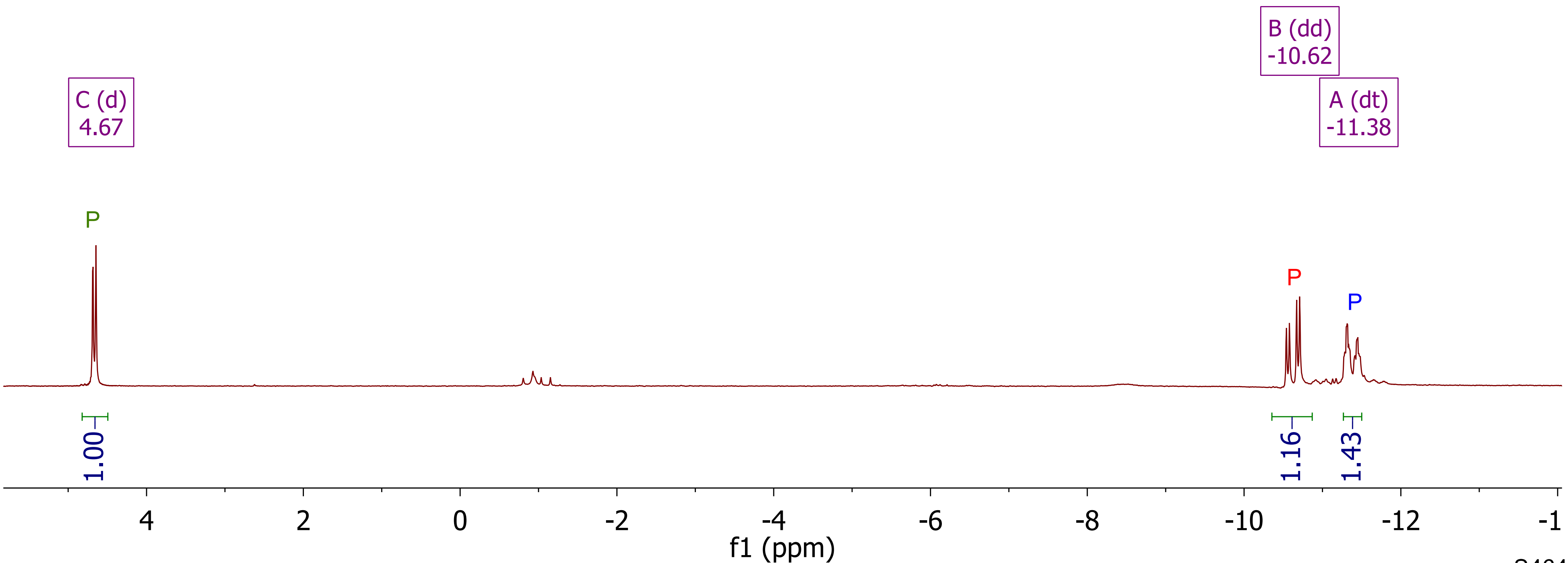

product after MPLC



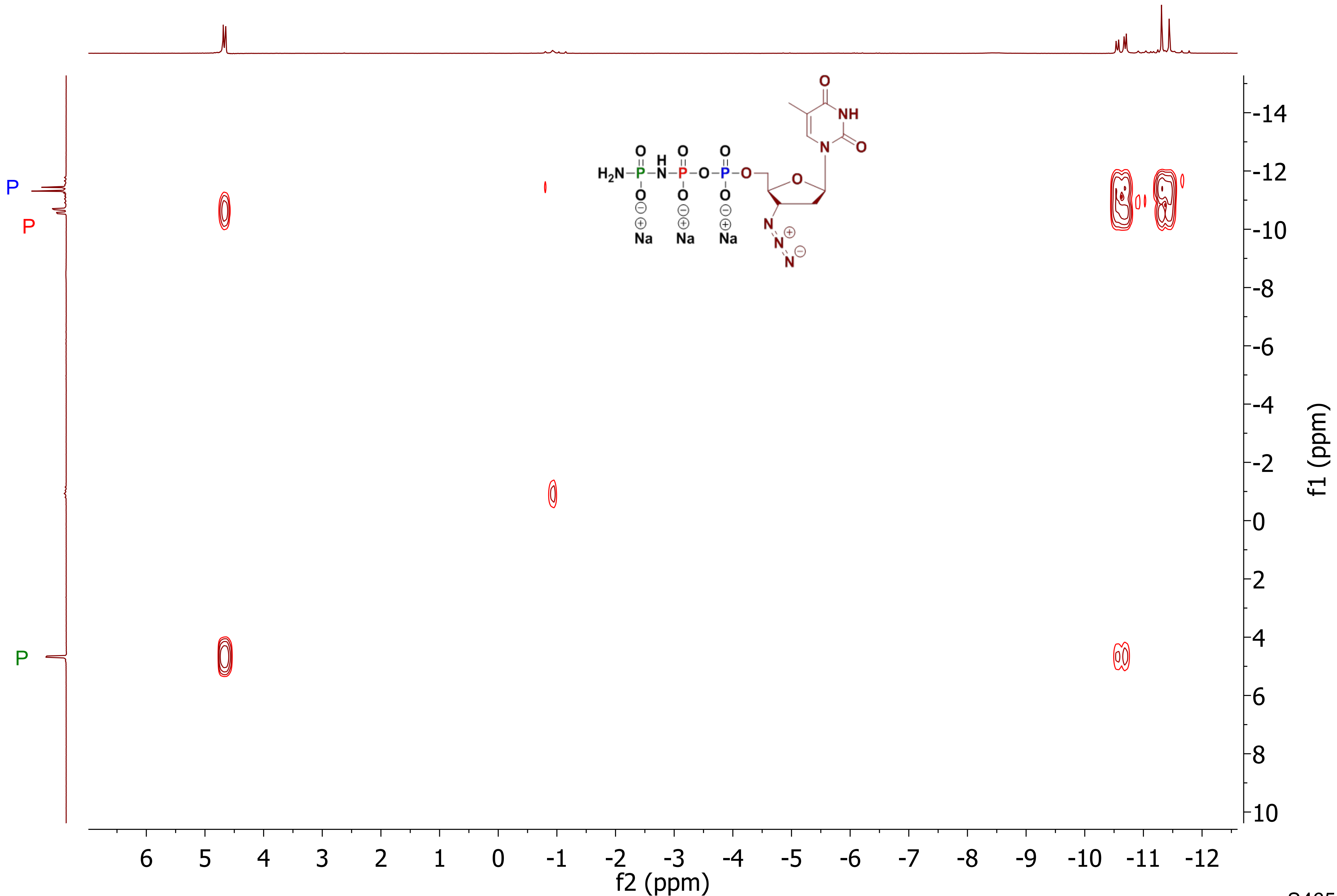
**$^{31}P$-$^{1}H$ NMR**



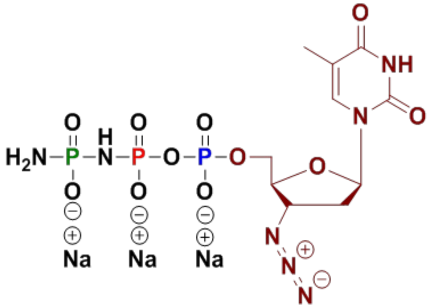
product after MPLC



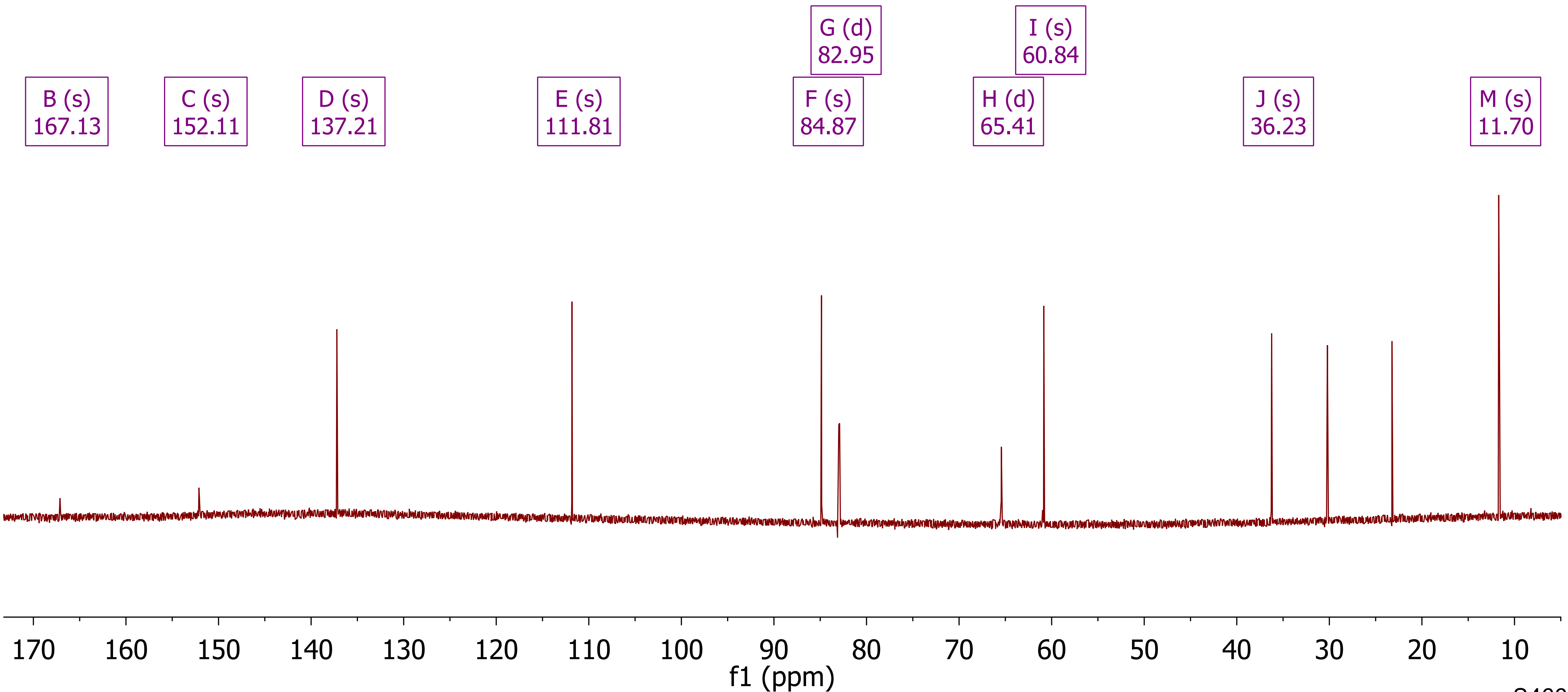
$^{31}P$-$^{31}P$ NMR Cosy



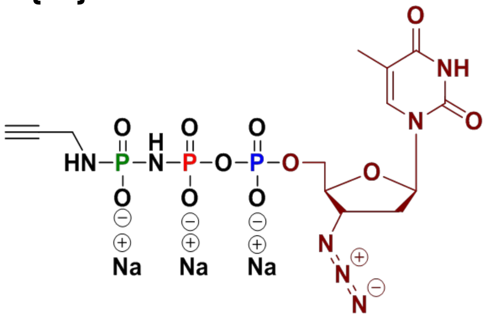
## $^{13}$C NMR



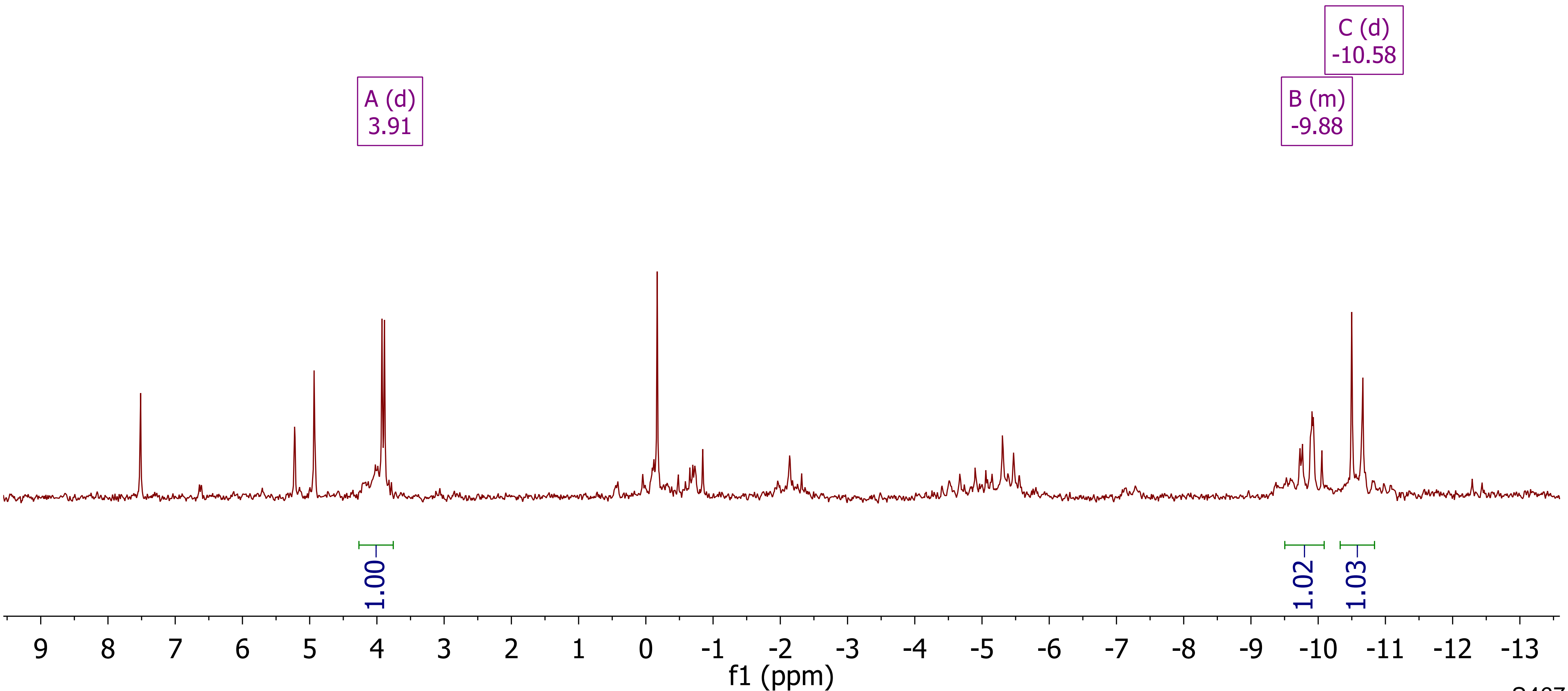
product after MPLC



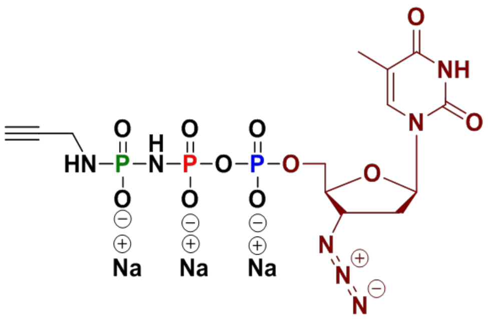
**$^{31}P\{^1H\}$ NMR**



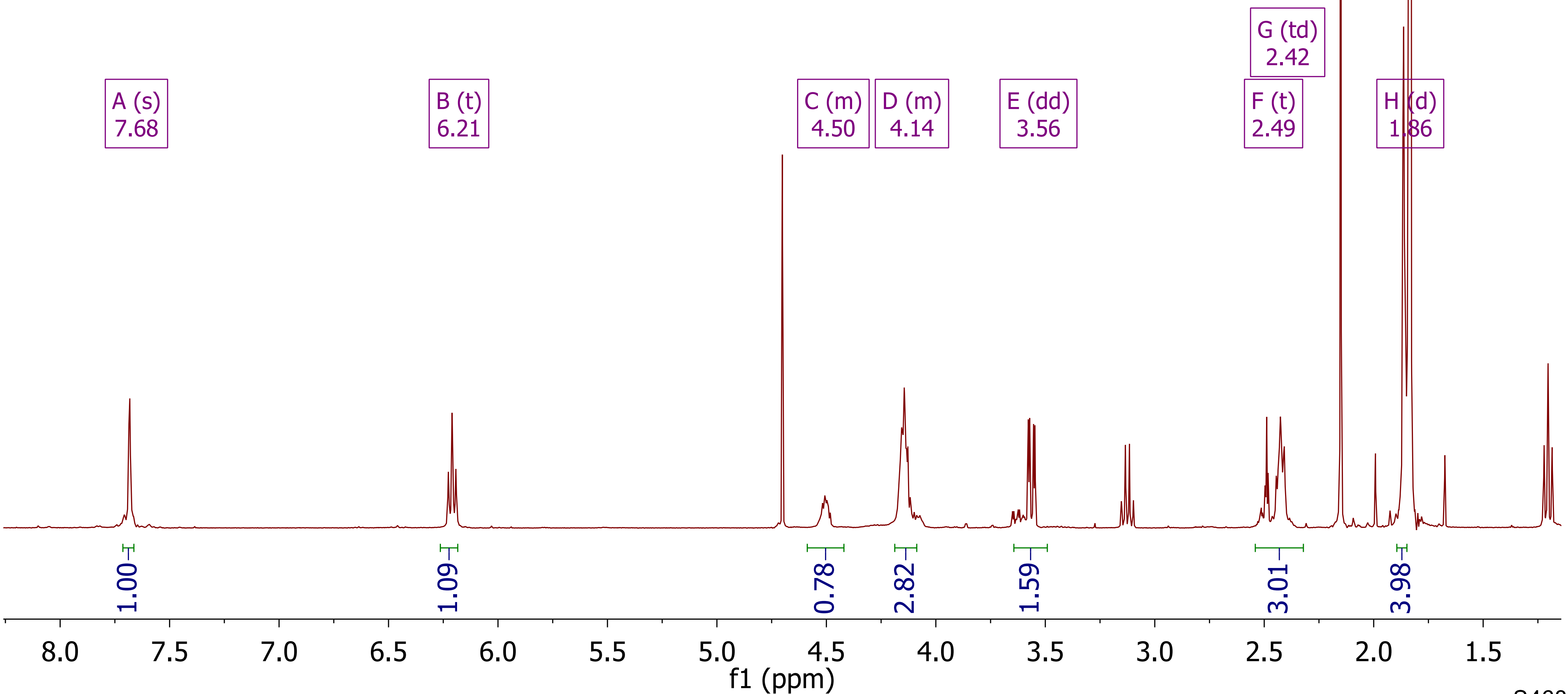

After precipitation crude



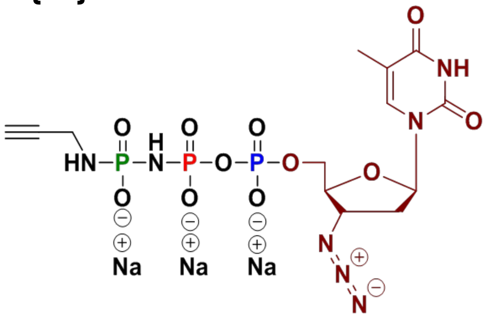
$^1$H NMR



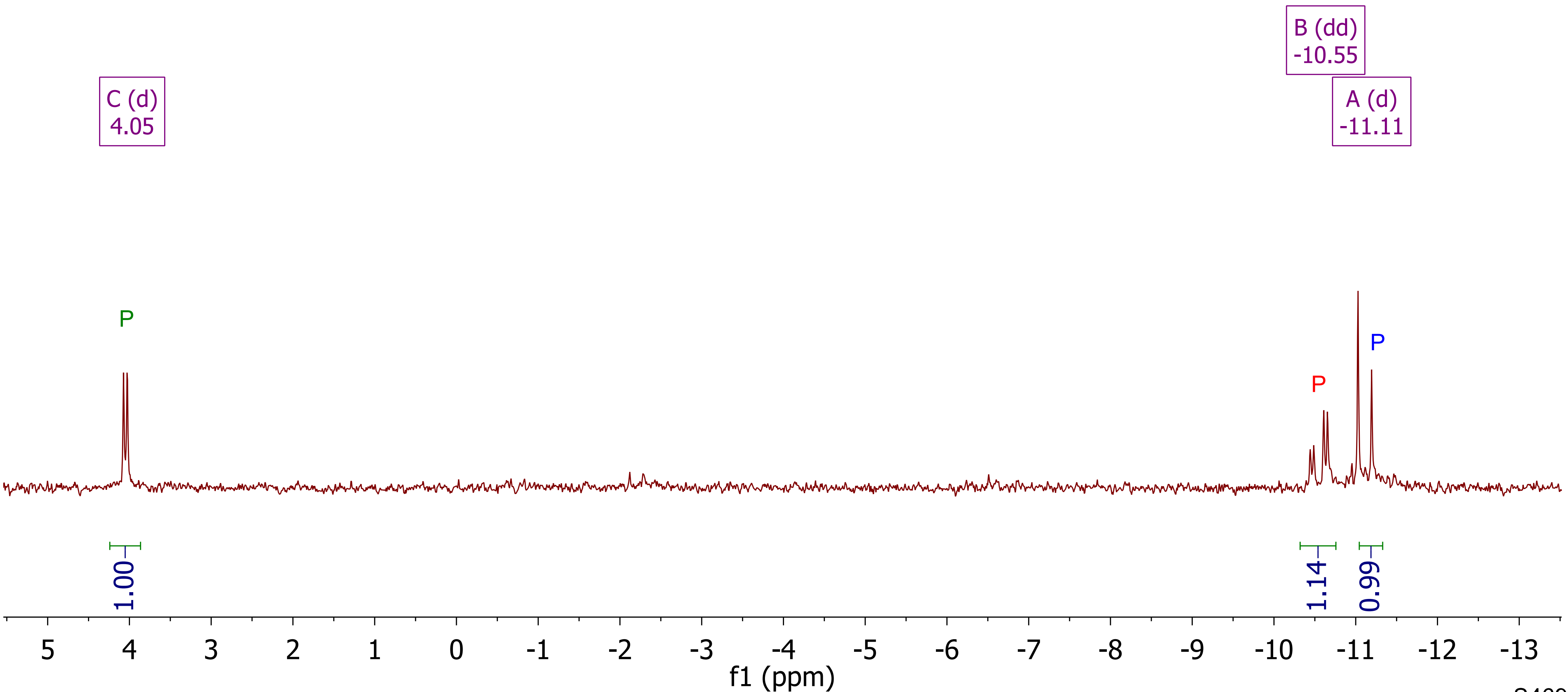
product after MPLC



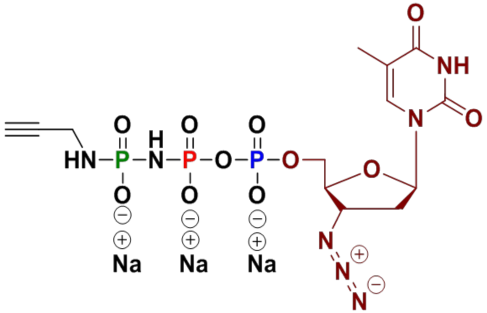
**$^{31}P\{^1H\}$ NMR**



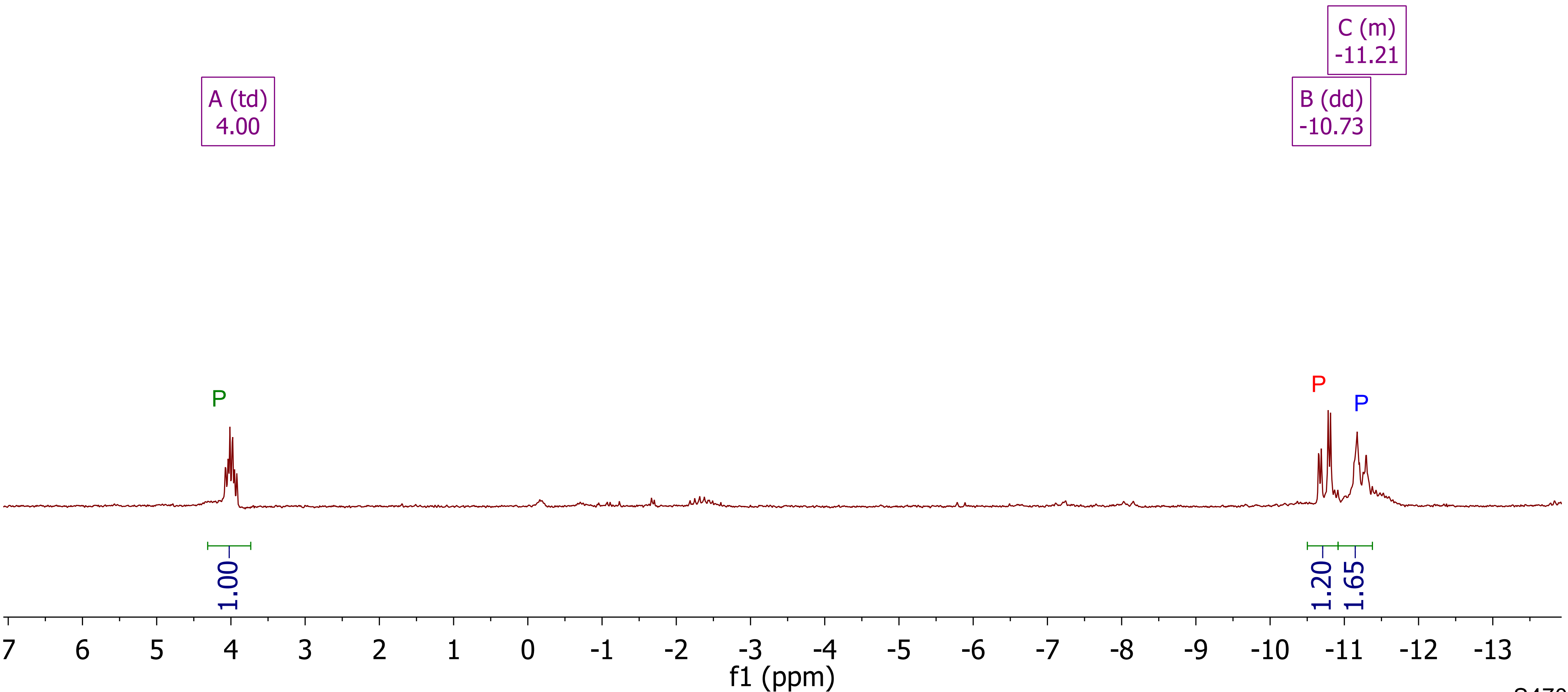
product after MPLC



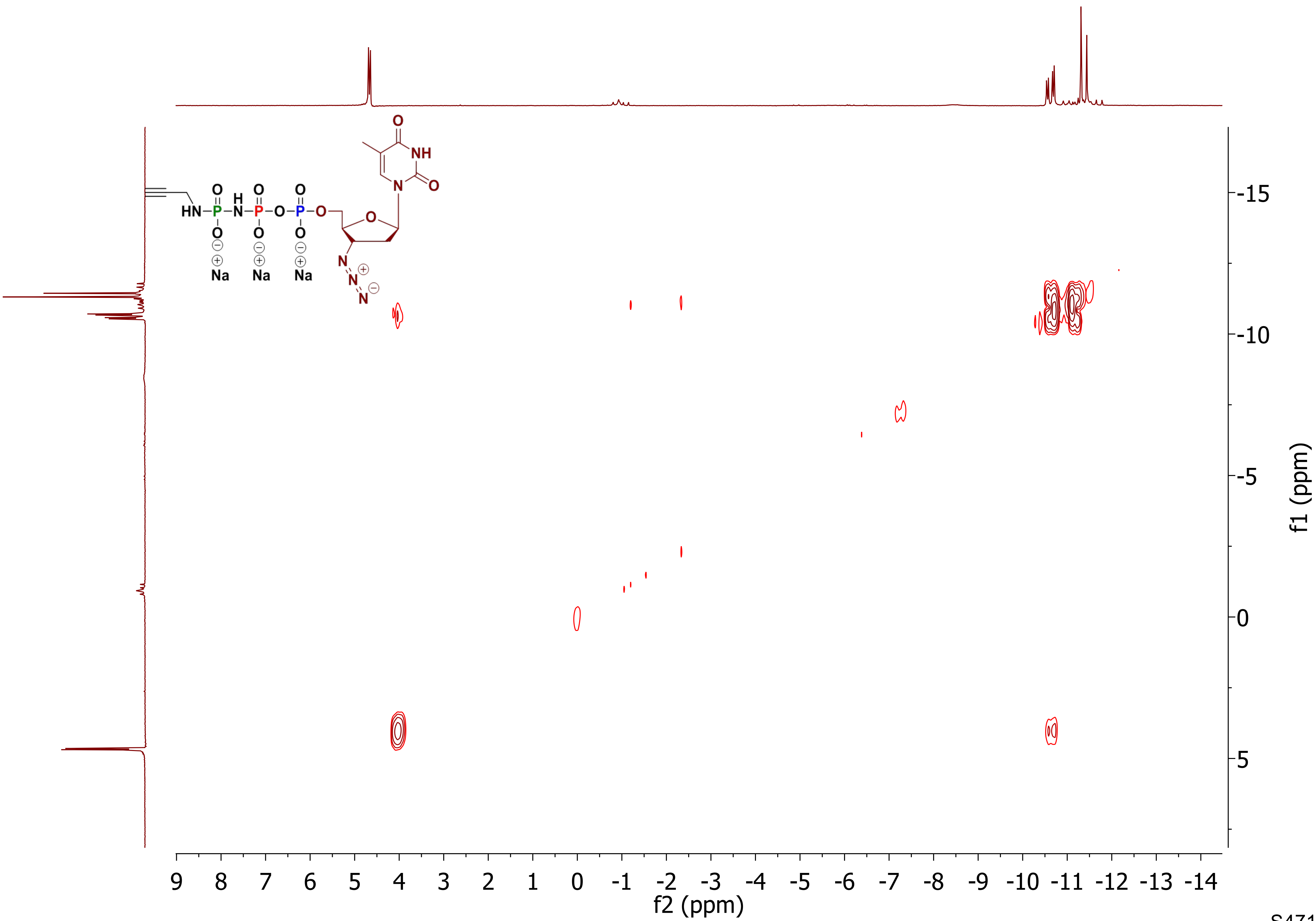
**$^{31}$P-$^{1}$H NMR**



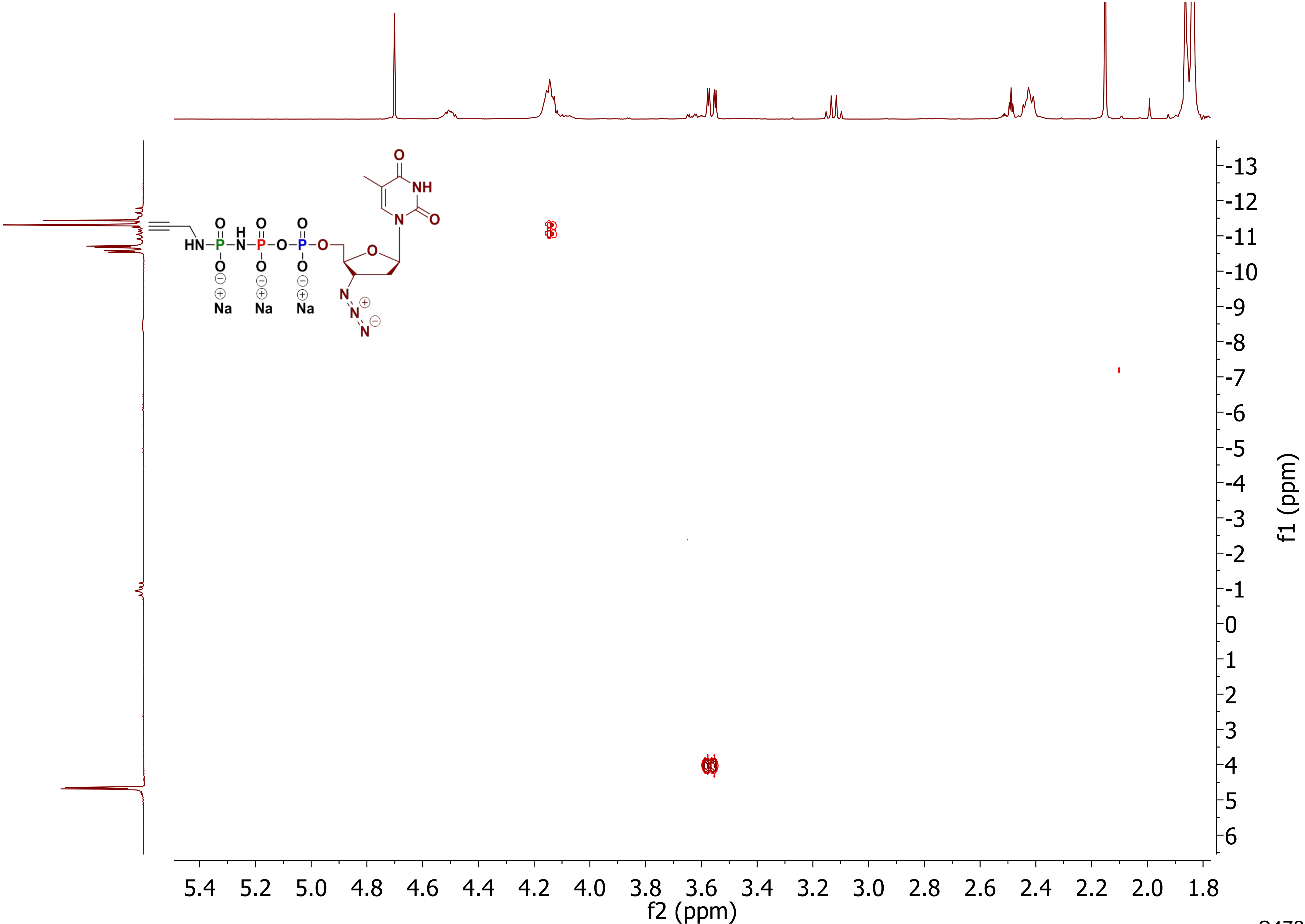
product after MPLC

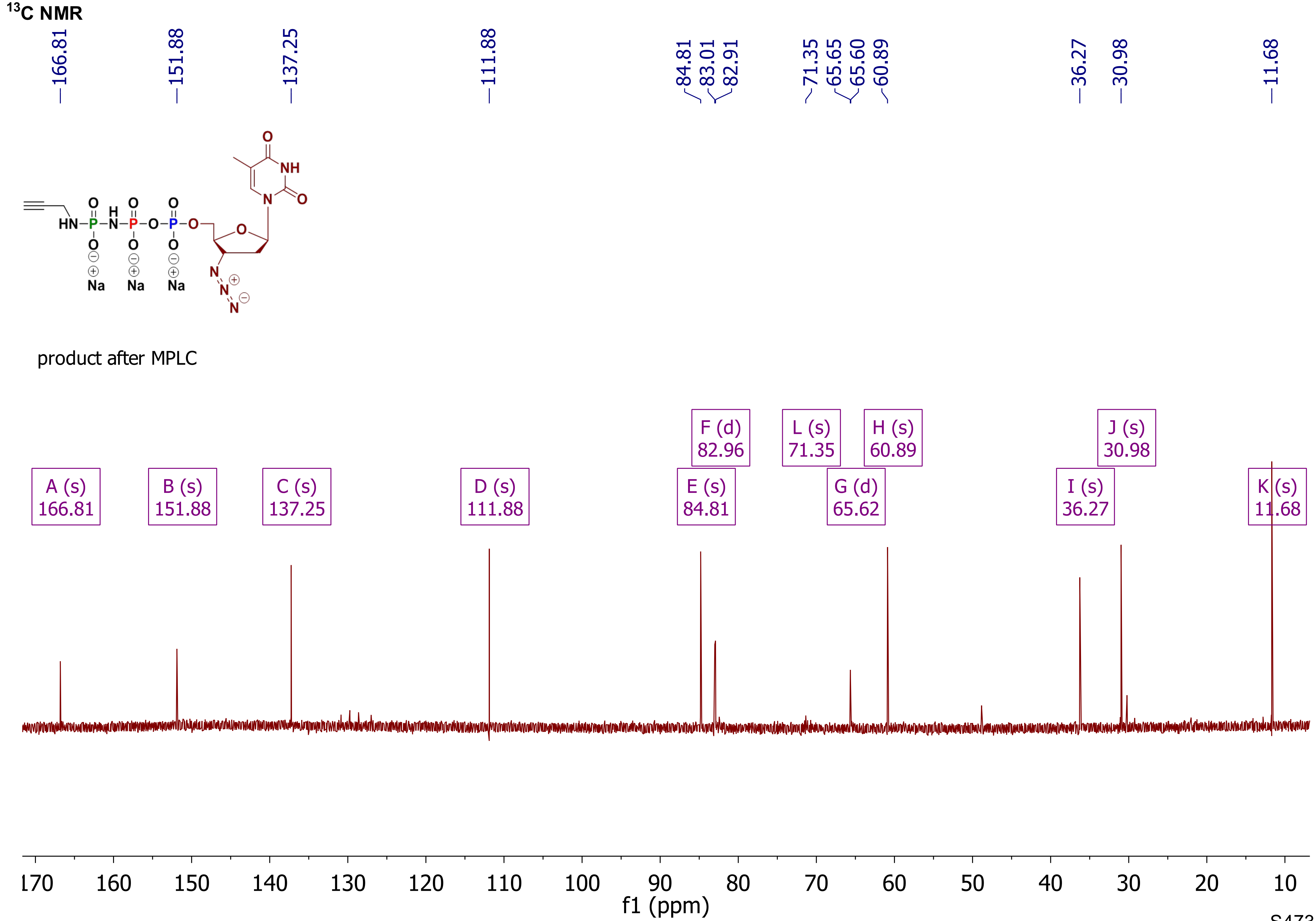


**$^{31}P$-$^{31}P$ COSY NMR**



**$^{31}$P-$^{1}$H HMBC NMR**




13C NMR
166.81
151.88
137.25
111.88
84.81
83.01
82.91
71.35
65.65
65.60
60.89
36.27
30.98
11.68
O
NH
N
O
HN
H
N
O
P
Na
Na
Na
N
product after MPLC
A (s) 166.81
B (s) 151.88
C (s) 137.25
D (s) 111.88
E (s) 84.81
F (d) 82.96
L (s) 71.35
G (d) 65.62
H (s) 60.89
I (s) 36.27
J (s) 30.98
K (s) 11.68
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)