Supporting Information

Synthesis of End-functionalized Phosphate and Phosphonate- polypeptides by Ring-Opening Polymerization of their Corresponding *N*-carboxyanhydride (NCA)

*Soumen Das, Mrityunjoy Kar and Sayam Sen Gupta**

Materials and method:

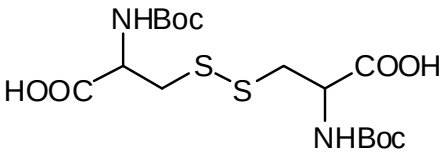
All chemicals were purchased from sigma-aldrich and used as received unless otherwise specified. All the solvents used were obtained from Merk India. Hexanes, DMF and acetonitrile were dried by conventional methods and stored in the glove box. THF was freshly distilled over sodium wire and ethyl acetate was freshly distilled from calcium hydride. FT-IR spectra were recorded on Perkin Elmer FT-IR spectrum GX instrument. ^{1}H NMR and ^{31}P NMR spectrum was obtained with Bruker spectrometer (200.13 MHz, 400.13 MHz). ^{31}P NMR shifts are reported in ppm relative to 85% H_3PO_4 at 0 ppm. ^{13}C NMR spectrum and DEPT were recorded on Bruker spectrometer (50.23 MHz) and reported relative signals according to deuterated solvent used. Size exclusion chromatography of the polymer was performed in VISKOTEK TDA 305-040 TRIPLE DETECTOR ARRAY refractive index (RI), viscometer (VISC), low angle light scattering (LALS), right angle light scattering (RALS) GPC/SEC MODULE. Separations were achieved by three columns (T6000M, GENERAL MIXED ORG 300X7.8 MM) and one guard column (TGAURD, ORG GUARD COL 10x4.6 MM), 0.025 M LiBr in DMF as the eluent at 60 °C. GPC/LS samples were prepared at concentrations of 5 mg/mL. A constant flow rate of 1 mL/min was maintained, and the instrument was calibrated using PMMA standards.

Circular Dichroism Measurements

Solutions of polymers were filtered through 0.22 μm syringe filters. CD (180−250 nm) spectra of the phospho-polypeptides (0.25 to 1.0 mg/mL in acetonitrile or in 10 mM phosphate buffer pH 7.2) were recorded (JASCO CD SPECTROPOLARIMETER, Model J-815) in a cuvette with a 1 mm path length. All the spectra were recorded for an average of three scans and the spectra were reported as a function of molar ellipticity [θ] versus wavelength. The molar ellipticity was calculated using the standard formula, [θ]=

(θ×100×Mw)/(C×l), where θ= experimental ellipticity in milli degrees, Mw = average molecular weight, C= concentration in mg/mL, and l = path length in cm. The % α helicity was calculated by using the formula % α helicity= $(-[\theta]_{222nm} + 3000)/ 39000$.

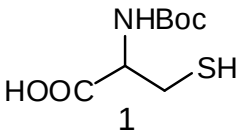
Synthesis of N-Boc-L-Cystine:



L-Cystine (10 g, 41.6 mmol) was dissolve in 9:1 water:tetrahydrofuran (100 mL). 6 M NaOH in water was added drop wise until pH 10 was reached. Then di-*tert*-butyl dicarbonate (24.5 g, 112.3 mmol) was added drop wise into that solution. The reaction mixture was stirred for 24 hrs. Then the reaction mixture acidified by drop wise addition of 2 (N) HCl with stirring until the solution reached pH 2. The solids were extracted with ethyl acetate (3x200 mL) and the combined organic layer washed with pH 2 water (2x100 mL) followed by brine solution and dried over sodium sulphate. Solution was filtered and concentrated under reduced pressure to get a white solid compound. The white solids were washed with hexane for several times to get completely pure N-Boc-L-Cystine (98% yield).

^{1}H NMR (200.13 MHz, DMSO-d_6): δ 1.37 (s, 18H), 2.80-2.92 (dd, *J*=13.39, 9.98, 2H), 3.07-3.16 (dd, *J*=13.52, 4.17, 2H), 4.08-4.22 (m, 2H); ^{13}C NMR (50.23 MHz, DMSO-d_6): δ 28.21 (6C), 39.84 (2C), 52.74 (2C), 78.33 (2C), 155.43 (2C), 172.5 (2C)

Synthesis of N-Boc-L-Cysteine:

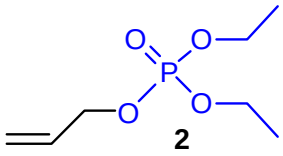


N-Boc-L-Cystine (10.0 g, 22.7 mmol) was dissolve in 200 mL of THF: H_2O (10:1) and triphenyl phosphine (6.54 g, 24.9mmol) was added. The reaction mixture stirred at room temperature for overnight. The THF was evaporated under reduced pressure, then water was added (100 mL) and the solution was made basic to pH 10 with 6 M NaOH. The aqueous phase was extracted with ethyl acetate (4x100 mL) to remove excess PPh_3 and PPh_3O. Then 2 (N) HCl was added drop wise with stirring until the solution reached pH 2, with the

formation of white precipitate. The white solids were extracted by ethyl acetate (3x100mL) and the combined organic phases were washed with pH 2 water (100 mL) followed by brine, and dried with Na_2SO_4. The solution was filtered and condensed to give colourless oily N-Boc-L-Cysteine **1** in 97% yields.

^{1}H NMR (200.13 MHz, DMSO-d_6): δ 1.43 (s, 9H), 2.46-2.54 (t, 1H), 2.66-2.95 (m, 2H), 4.01-4.15 (m, 1H); ^{13}C NMR (50.23 MHz, DMSO-d_6): δ 28.14 (3C), 39.30, 56.10, 78.27, 155.37, 172.09

Synthesis of allyl diethylphosphate (**2**):

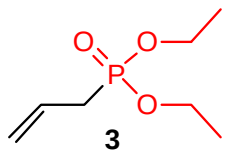


2

To a solution of diethyl chlorophosphate (2.0 g, 11.6 mmol) in dry THF (20 mL) was added a solution of allyl alcohol (0.66 g, 11.37 mmol) and triethyl amine (1.4 g, 13.92 mmol) in dry THF (20 ML) at 0 °C under nitrogen. The resulting mixture was stirred at room temperature for overnight. A precipitated white mass was filtered off and the filtrate was concentrated by evaporation of the solvent to obtained allyl diethylphosphate **2** as a transparent liquid (96% yield).

^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.19-1.26 (m, 6H), 3.99-4.08 (m, 4H), 4.38-4.46 (m, 2H), 5.11-5.30 (m, 2H), 5.74-5.93 (m, 1H); ^{13}C NMR (50.23 MHz, $CDCl_3$): δ 16.74 (2C), 64.41 (2C), 68.41, 118.61, 133.24

Synthesis of allyl diethylphosphonate (**3**):

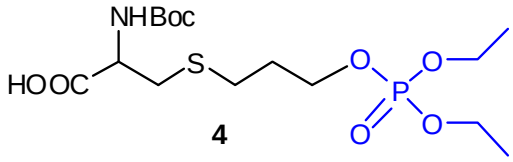


3

Triethyl phosphite (25 g, 150 mmol) and allyl bromide (27.22 g, 225 mmol) were taken into a 100 mL RB equipped with a condenser. The reaction mixture was heated for 20h at 150 °C. Then it was distilled under reduced pressure at temperature 85°-90 °C to obtained allyl diethylphosphonate **3** as a colourless liquid (90% yield).

^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.14-1.21 (m, 6H), 2.39-2.54 (m, 2H), 3.89-4.03 (m, 4H), 5.02-5.12 (m, 4H), 5.54-5.78 (m, 1H); ^{13}C NMR (50.23 MHz, $CDCl_3$): δ 16.26 (2C), 31.95, 61.38 (2C), 119.33, 127.05

Synthesis of N-Boc-L-cysteine-diethylphosphate (**4**):

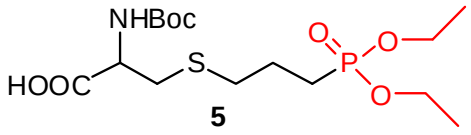


4

N-Boc-Cysteine **1** (2.84 g, 12.87 mmol) and allyl diethylphosphate **2** (1.0 g, 5.15 mmol) dissolved in dry DMF (25 mL) in a 50 mL test tube with joint. 2, 2-dimethoxy-2-phenyl acetophenone (0.396 g, 1.54 mmol) was added and reaction mixture was vacuum purged and backfilled with nitrogen for three times. Then the test tube irradiated to 365 nm light for 1h. The yellowish coloured DMF solution added into 300 mL of water and extracted with ethyl acetate (3x100 mL). The ethyl acetate layers were washed with water then brine, dried over Na_2SO_4, filtered and condensed to oil. The crude product was purified by silica gel column chromatography using ethyl acetate-pet ether with1% acetic acid as the mobile phase to afford the colourless oily N-Boc-L-cysteine-diethylphosphate **4** (94% yield).

^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.22-1.29 (t, *J*=6.32, 6H), 1.35 (s, 9H), 1.78-1.91 (m, 2H), 2.54-2.61 (t, *J*=6.82, 2H), 2.82-3.02 (m, 2H), 3.97-4.11 (m, 6H), 4.39-4.48 (m, 1H); ^{13}C NMR (50.23 MHz, $CDCl_3$): δ 15.93 (2C), 28.07 (3C), 28.28, 29.82, 52.99, 64.06 (2C), 65.9, 79.88, 155.22, 173.61; ^{31}P NMR (400.13 MHz, $CDCl_3$): δ -1.51

Synthesis of N-Boc-L-cysteine-diethylphosphonate (**5**):



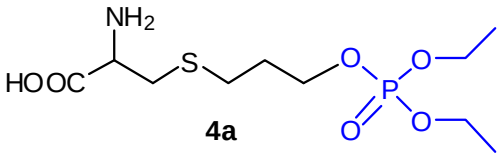
5

N-Boc-L-cysteine-diethylphosphonate **5** was prepared from N-Boc-L-Cysteine **1** and allyl diethylphosphonate **3** according to the procedure for **4** and was recovered as colourless oil (82% yields).

^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.23-1.30 (t, *J*= 7.07, 6H), 1.39 (s, 9H), 1.70-1.87 (m, 4H), 2.55-2.61 (t, *J*=6.69, 2H), 2.85-3.05 (m, 2H), 3.99-4.13 (m, 4H), 4.43-4.49 (m, 1H); ^{13}C

NMR (50.23 MHz, $CDCl_3$): δ 16.14 (2C), 21.98, 22.46, 25.27, 28.14 (3C), 34.06, 53.08, 62.01 (2C), 79.94, 155.25, 173.37; ^{31}P NMR (400.13 MHz, $CDCl_3$): δ 33.24

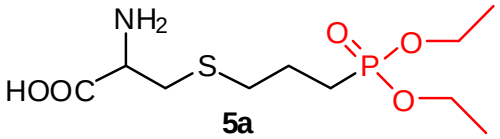
General procedure for the removal of Boc group:



4a

N-Boc-L-cysteine-diethylphosphate **4** (1.0 g, 2.4 mmol) was added into the HCl/THF mixture (4 M, 20 mL) and stirred for 4h at room temperature. The solvent was removed under reduced pressure to yield the product **4a** as oil. The oily product was washed with ethyl acetate for several times and then dried at room temperature under vacuum for 24h (97% yields).

^{1}H NMR (200.13 MHz, DMSO-d_6): δ 1.19-1.26 (m, 6H), 1.82-1.92 (m, 2H), 2.60-2.68 (t, *J*=7.2, 2H), 3.04-3.07 (d, *J*= 5.43, 2H), 3.93-4.08 (m, 6H), 4.12 (br, 1H), 8.64 (br, 3H); ^{13}C NMR (50.23 MHz, DMSO-d_6): δ 15.67 (2C), 24.74 , 27.27, 30.68, 51.44, 62.83 (2C), 66.98, 169.17

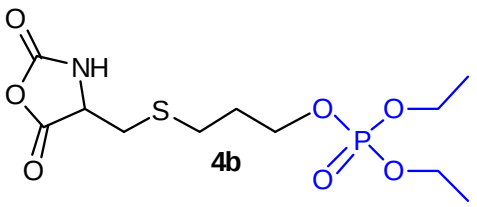


5a

5a was prepared from **5** according to the above procedure and recovered as oil (96% yield).

^{1}H NMR (200.13 MHz, DMSO-d_6): δ 1.18-1.25 (m, 6H), 1.82-1.90 (m, 2H), 2.61-2.68 (t, *J*=6.44, 2H), 3.02-3.05 (d, *J*= 5.43, 2H), 3.89-4.04 (m, 4H), 4.10 (br, 1H), 8.66 (br, 3H); ^{13}C NMR (50.23 MHz, DMSO-d_6): δ 16.38 (2C), 22.25 (2C), 25.16, 30.87, 51.93, 61.07 (2C), 169.49

Synthesis of diethylphosphate-L-Cysteine-N-Carboxyanhydride (**4b**):



4b

To H_2N-L-Cysteine-phosphate **4a** (0.5 g, 1.58 mmol) freshly distilled out THF was added under argon followed by a solution of triphosgene (0.234 g, 0.79 mmol) in dry THF (2 mL). Then the reaction mixture was stirred at 50 $^{\circ}$C for 1.5h under argon. The solution was evaporated to dryness under reduced pressure to get yellowish oil. The crude product was purified by silica gel column chromatography with a gradient of freshly distilled 95% ethyl acetate in dry hexanes. Collect 12 different fractions (10 mL) from column and analyzed by TLC. Fractions containing pure NCA were combined and removal solvent under reduced pressure to give diethylphosphate-L-Cysteine-NCA **4b** as colourless oil (68% yield). Diethylphosphate-L-Cysteine-NCA can be obtained as a solid by precipitation according to the following procedure. The oily product obtained from column was dissolved in 5 mL of dry $CHCl_3$ and precipitated into 100 mL of dry hexane to get a white solid product.

^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.31-1.38 (m, 6H), 1.90-2.02 (m, 2H), 2.63-3.14 (m, 4H), 4.03-4.22 (m, 6H), 4.53-4.58 (m, 1H), 8.12 (br, 1H); ^{13}C NMR (50.23 MHz, $CDCl_3$): δ 16.17 (2C), 29.04, 29.93, 33.53, 58.41, 64.51 (2C), 65.71, 152.00, 169.09; FT-IR ($CHCl_3$) 1785 cm^{-1} and 1860 cm^{-1} υ_{CO} (unsymmetrical stretching).

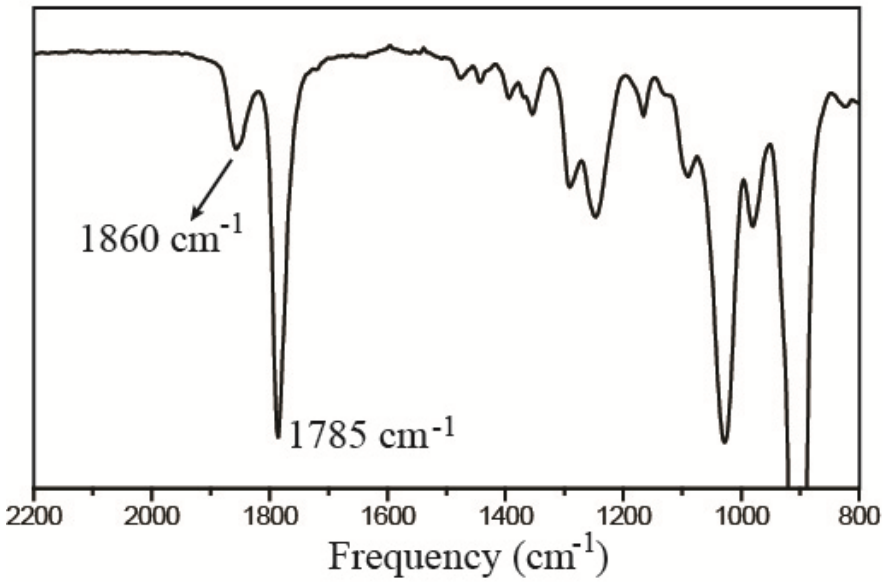
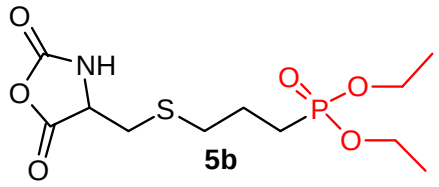


Figure 1. FT-IR Spectra of diethylphosphate-L-Cysteine-N-Carboxyanhydride in $CHCl_3$, shows two unsymmetrical infrared stretching at 1860 and 1785 cm^{-1}.

Synthesis of diethylphosphonate-L-Cysteine-N-Carboxyanhydride (**5b**):

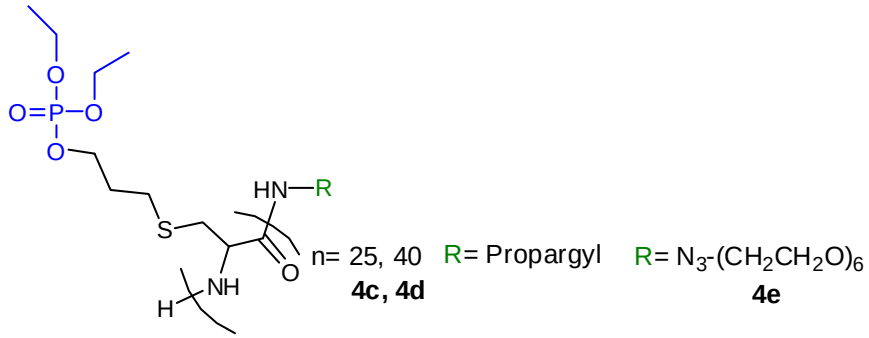


5b

Diethylphosphonate-L-Cysteine-N-Carboxyanhydride **5b** was prepared from **5a** according to the procedure for **4b** and recovered as colourless oil (65% yields).

^{1}H NMR (200.13 MHz, $CDCl_3$): δ 1.32-1.38 (m, 6H), 1.82-2.05 (m, 4H), 2.67-3.15 (m, 4H), 4.01-4.26 (m, 4H), 4.52-4.57 (m, 1H), 8.27 (br, 1H); ^{13}C NMR (50.23 MHz, $CDCl_3$): δ 16.47 (2C), 22.00, 24.81, 32.88 (2C), 58.68, 62.19 (2C), 151.98, 169.11; FT-IR ($CHCl_3$) 1785 cm^{-1} and 1860 cm^{-1} υ_{CO} (unsymmetrical stretching).

Synthesis of poly-diethylphosphate-L-Cysteine (**4c**, **4d** and **4e**):



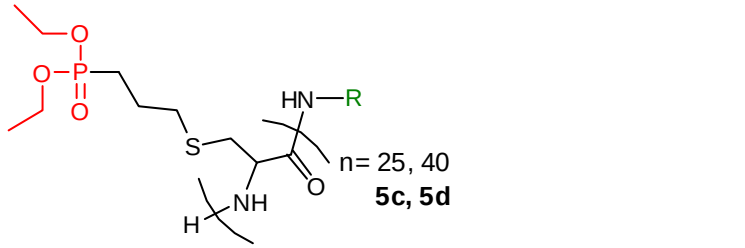
4c, 4d **4e**

To a solution of diethylphosphate-L-Cysteine NCA **4b** in dry dioxane or DMF (100 mg/mL) was added with propargyl amine or azido-PEG-NH_2 (0.5 M) as initiator inside the glove box. The reaction was stirred at room temperature and the reaction generally completed within 36 to 72 hrs. The progress of the reaction was monitored by FT-IR spectroscopy by comparing with the intensity of the initial NCA's anhydride stretching at 1785 cm^{-1} and 1860 cm^{-1}. Aliquots were removed after completion of the reaction for GPC analysis. Reaction were removed from the glove box and precipitated into diethyl ether. Solids were collected by centrifugation and washed with pH 2.0 water (HCl) followed by DI water. The polymers were lypholized to yield white solids (87-92% yield).

Polymer **4c**. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.31-1.35 (m, 6H), 1.94-1.95 (m, 2H), 2.25 (br, 1H for alkyne proton in initiator), 2.57-2.74 (m, 2H), 2.93-3.29 (m, 2H), 4.1-4.17 (m, 7H); ^{31}P NMR (400.13 MHz, $CDCl_3$): δ -0.52

Polymer **4d**. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.31-1.34 (m, 6H), 1.93-1.95 (m, 2H), 2.24 (br, 1H for alkyne proton in initiator), 2.57-3.29 (m, 4H), 4.08-4.15 (m, 7H); ^{31}P NMR (400.13 MHz, $CDCl_3$): δ -0.55

Synthesis of poly-diethylphosphonate-L-Cysteine (**5c**, **5d** and **5e**):



poly-diethylphosphonate-L-Cysteine (**5c**, **5d** and **5e**) were prepared by according the procedure for **4c** and recovered as white solid (87-92% yield)**.**

Polymer **5c.** ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.28-1.31 (m, 6H), 1.85 (br, 4H), 2.25 (br, 1H for alkyne proton in initiator), 2.51-3.27 (m, 4H), 4.05-4.12 (m, 5H); ^{31}P NMR (400.13 MHz, $CDCl_3$): δ 31.91

Polymer **5d.** ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.29-1.32 (m, 6H), 1.86 (br, 4H), 2.25 (br, 1H for alkyne proton in initiator), 2.52-3.30 (m, 4H), 4.06-4.13 (m, 5H); ^{31}P NMR (400.13 MHz, $CDCl_3$): δ 31.96

Polymer **5e.** ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.30-1.33 (m, 6H), 1.87 (br, 4H), 2.65-3.28 (m, 4H), 3.62-3.65 (m, for $-CH_2CH_2O$ unit in initiator), 4.08 (br, 5H)

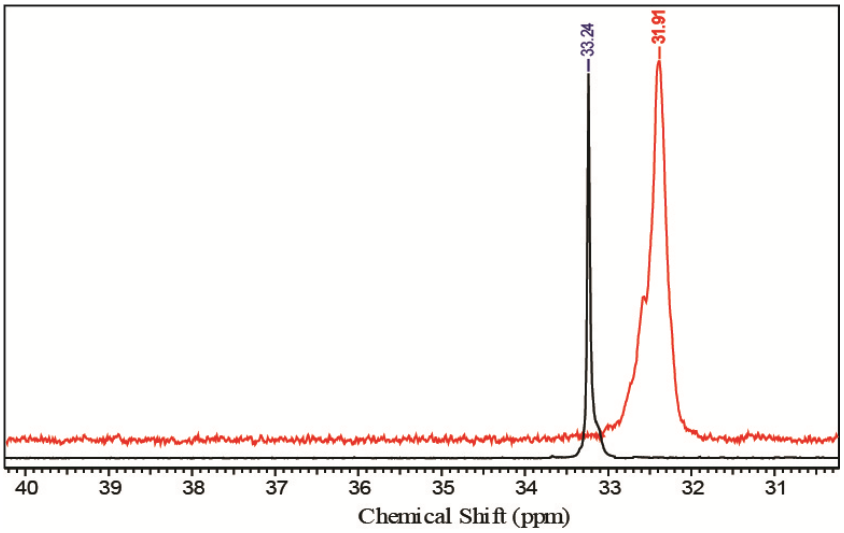
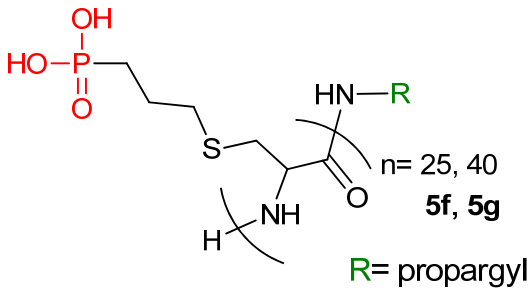


Figure 2. ^{31}P NMR shows that the broadening of phosphorous peak in phosphonate polypeptide. Black one represents ^{31}P NMR of N-Boc-L-cysteine-diethylphosphonate and red one for poly-diethylphosphonate-L-Cysteine.

Deprotection of poly-diethylphosphonate-L-Cysteine (**5c** and **5d**):

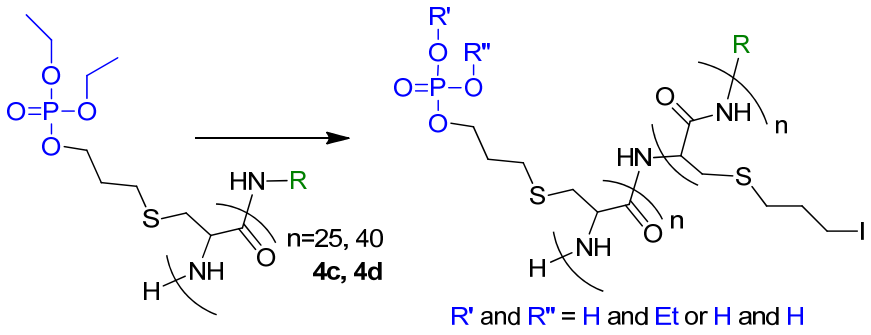


The deprotections were performing by *in situ* generation of iodotrimethylsilane. For example, polymer **5c** (100 mg, 0.0127 mmol) was dissolved in dry acetonitrile (5 mL) and sodium iodide (533.6 mg, 3.56 mmol) and trimethylsilyl chloride (386.7 mg, 3.56 mmol) were added sequentially. Then the reaction mixture was stirred at 45 $^{\circ}$C under N_2 atm for 24 hrs. The reaction mixture was evaporated to dryness to get a deep brown residue. The residue was re-dissolve in MeOH and dialyzed (using dialysis tubing MWCO of 2 KDa) against MeOH for 24 hrs to remove all the organic impurities. Then it dialyzed against DI water for another 48 hrs, with water changes at least 5 times. Dialyzed polymer was lyophilized to get **5f** as white solid (~85% yield).

Polymer **5f.** ^{1}H NMR (400.13 MHz, D_2O): δ 1.66-1.78 (br, 4H), 2.18 (br, 1H, for alkyne proton in initiator), 2.63 (br, 2H), 2.92-3.00 (br, 2H), 4.55 (br, 1H); ^{31}P NMR (400.13 MHz, D_2O): δ 25.30

Polymer **5g.** ^{1}H NMR (400.13 MHz, D_2O): δ 1.80-1.94 (br, 4H), 2.17 (br, 1H, for alkyne proton in initiator), 2.66 (br, 2H), 2.94-3.02 (br, 2H), 4.58 (br, 2H); ^{31}P NMR (400.13 MHz, D_2O): δ 25.37

Deprotection of poly-diethylphosphate-L-Cysteine (**4c** and **4d**):



Polymers **4c** and **4d** were deprotected by following the procedure of **5c** at 45 ^{0}C and at room temperature.

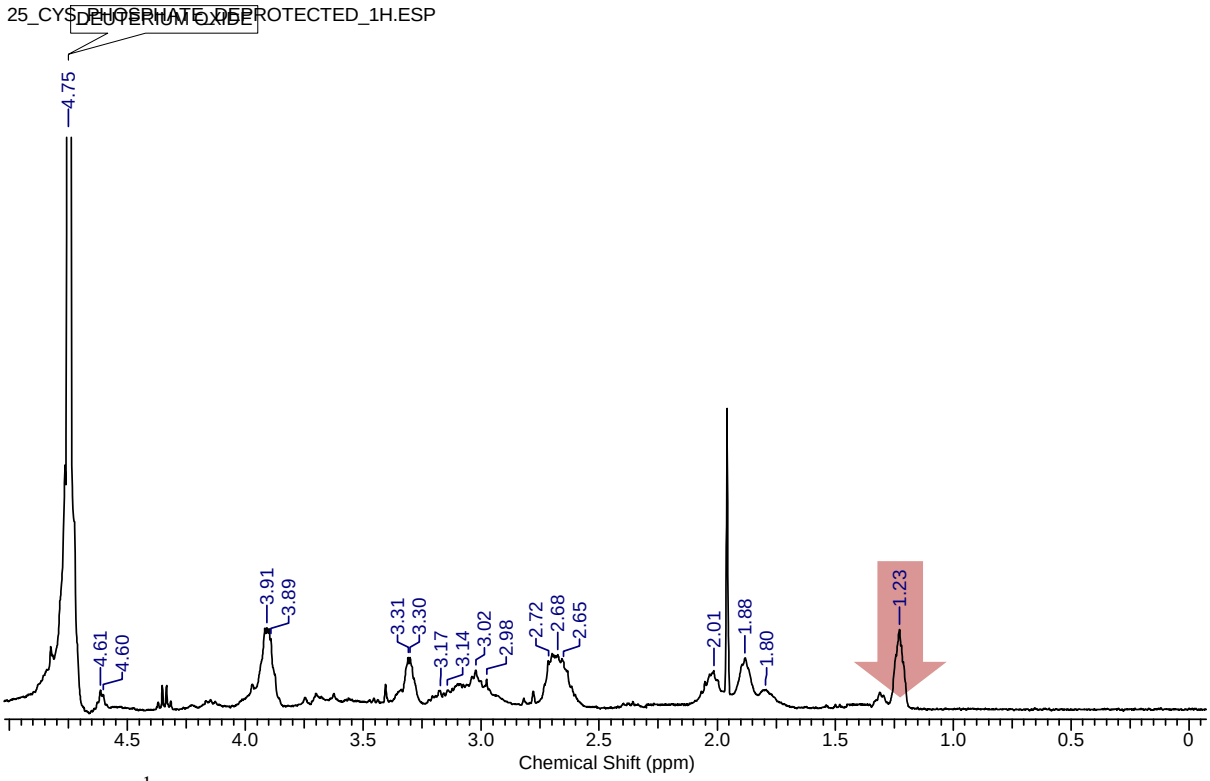


Figure 3. ^{1}H NMR spectra ($CDCl_3$) of deprotected 25-cysteine-Phosphate **4c**.

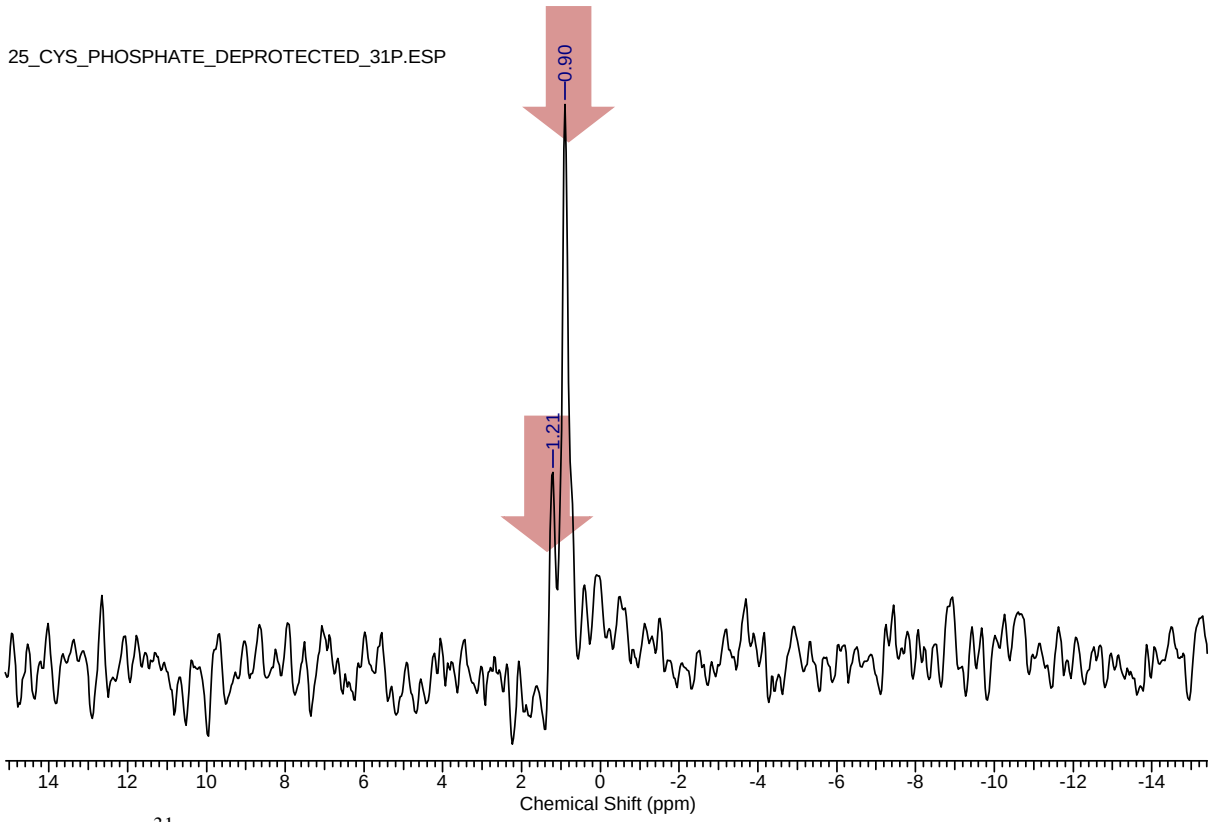
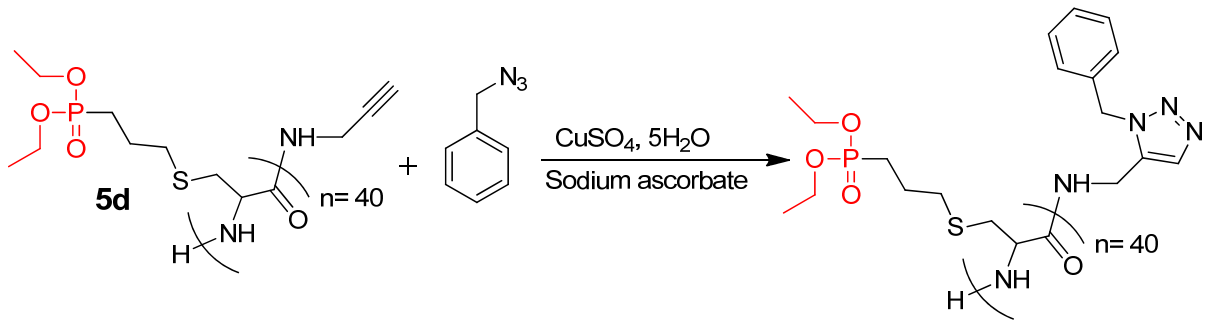


Figure 4. ^{31}P NMR spectra (D_2O) of deprotected 25-cysteine-Phosphate **4d** showing two phosphorus peaks.

Calculation of molecular weight by end group analysis

The alkyne terminated phosphopolypeptide **5d** was reacted with excess amount of benzyl azide (5 equivalent) in presence of $CuSO_4$, $5H_2O$ (5 equivalent) and sodium ascorbate (5 equivalent) in a solvent mixture THF: MeOH: H_2O (2: 2: 0.1). The reaction was left for 24 hr under argon atmosphere. Then solvent was removed under reduced pressure and residue was redissolved in dichloromethane. It was then washed multiple times using dilute aqueous ammonia solution to remove copper salt. The dichloromethane was removed and the residue was re-dissolved in MeOH. The resultant polymer re-precipitated for couple of times by addition of diethyl ether to the methanolic solution. The precipitated white polymer dried thoroughly and then went for NMR analysis (Figure 4).



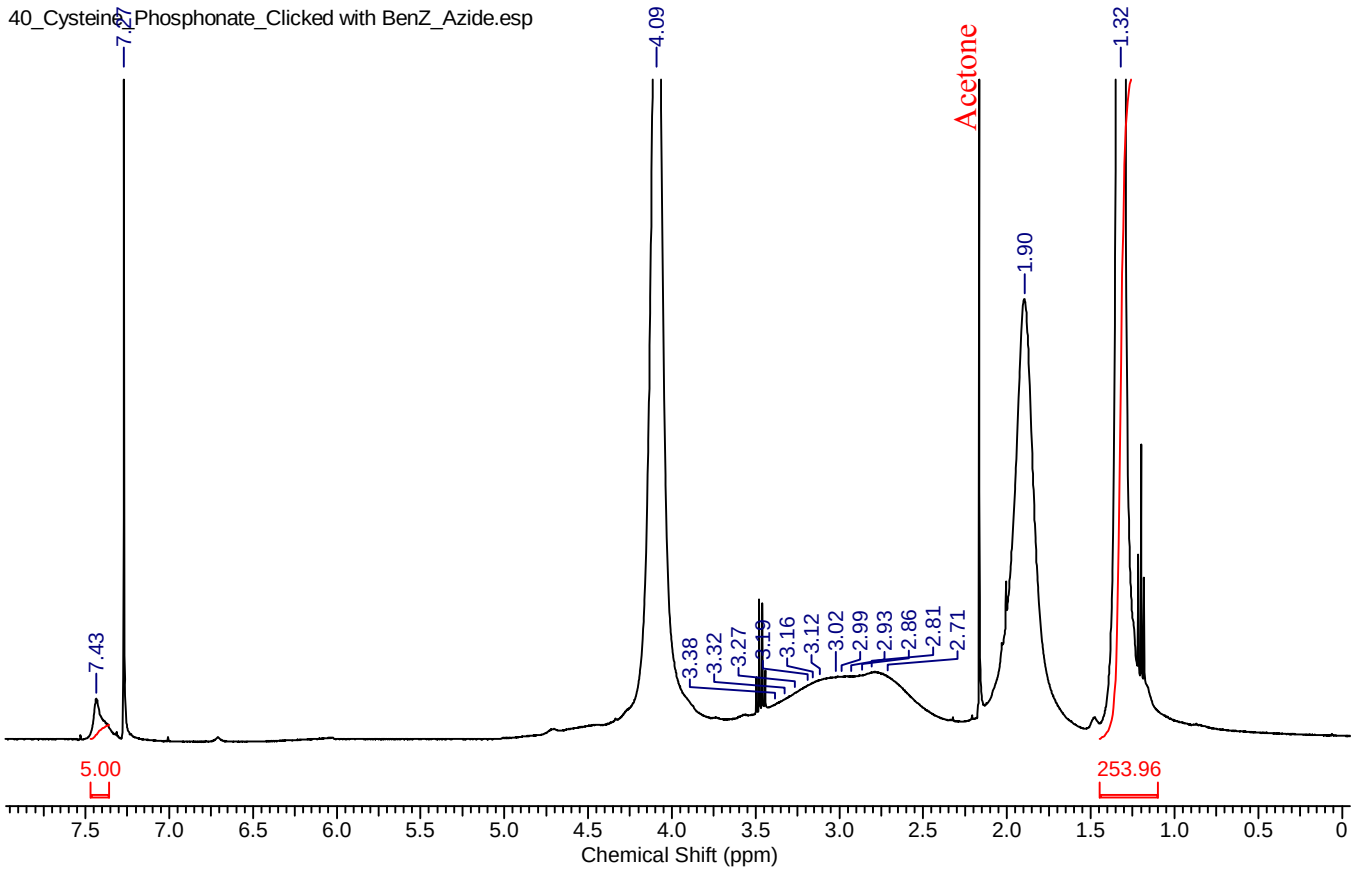


Figure 5: Calculation of polymer molecular weight (M_n) by using the characteristic proton peak of the aromatic moiety at 7.43 ppm in the *40*-diethylphosphonate-L-Cysteine after clicked.

Synthesis of fluorescein labelled Phosphonate-polypeptide:

The alkyne labelled fluorescein was prepared according to literature report (*Bioconjugate Chem*. **2005**, *16*, 1536). To a solution of 10 mg (0.0013 mmol) of **5e** in THF: MeOH: H_2O (2: 0.5: 0.25) was added alkyne fluorecein (2.0 mg, 3 eq), $CuSO_4$ (0.17 mg, 0.50 eq) and sodium ascorbate (1.0 eq) under nitrogen and the reaction mixture was stirred for 24 hrs. The completion of the reaction was monitored by the near dissaperance (more than 90-95%) of the azide stretching by FT-IR. Then, the solvent was removed under reduced pressure and the reaction mixture was dissolved in DCM. It was then washed multiple times using dilute aqueous ammonia solution to remove copper (I) salt and excess fluorecein alkyne. The dichloromethane was removed and the residue was re-dissolved in MeOH. The resultant polymer re-precipitated for couple of times by addition of diethyl ether to the methanolic

solution. Fluorecein labelled polymer was thoroughly dried (6 mg) and its absorption spectra taken in UV-vis spectrophotometer.

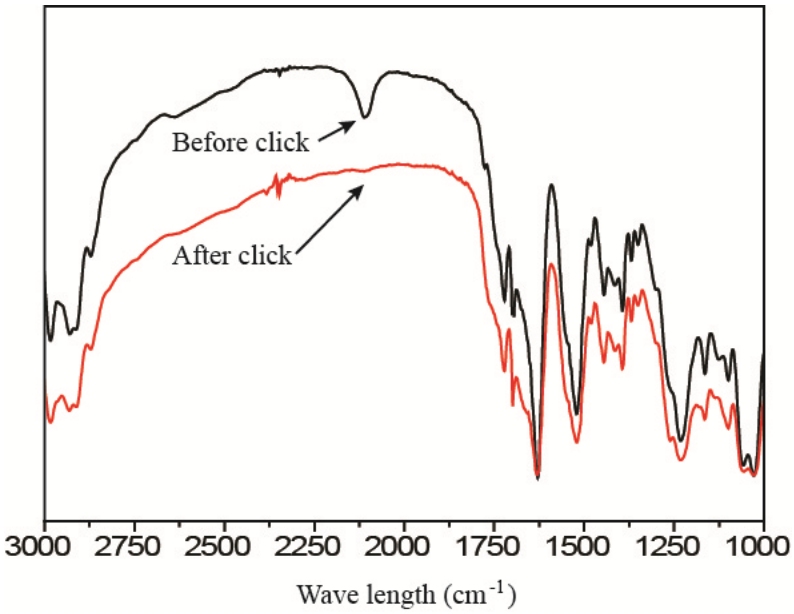


Figure 6. FT-IR spectra for azide functionalized polymer **5e** (black) and the crude reaction mixture upon completion of the click reaction (red).

*Method for estimation of azide concentration into **5e**:*

The fluorecein moiety was incorporated into **5e** using Cu (I) catalyzed azide–alkyne "click chemistry". The concentration of the fluorescein labelled polymer **5e** was calculated using the Mn value of 7,357 kDa that was obtained from NMR. Since only one fluorescein moiety will be conjugated to the polymer if all the polymer chains have one azide group attached to its end, the concentration of fluoresceinin solution would be equal to the concentration of the polymer. The concentration of fluorescein in solutions of **5e** was estimated from its absorption spectra (λ_{max}= 500 nm, ε= 90,000 $M^{-1}cm^{-1}$) in MeOH. The percentage of azide group incorporated was estimated from the ratio of the experimentally calculated concentration from absorption spectra of fluorescein to the theoretical concentration calculated from Mn values of **5e**.

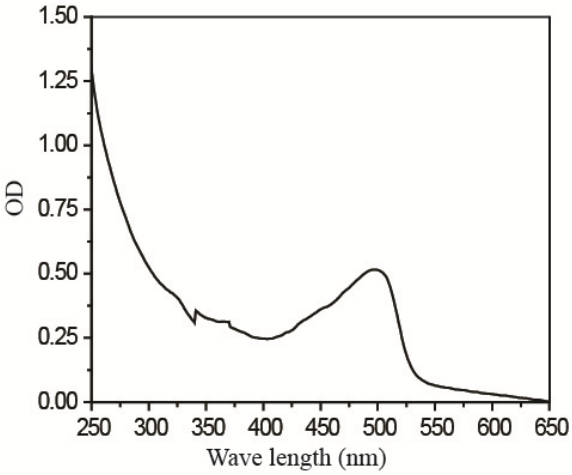


Figure 7. UV-VIS spectra of the fluorescein labelled polymer **5e** solution (6.6 μM) in MeOH.

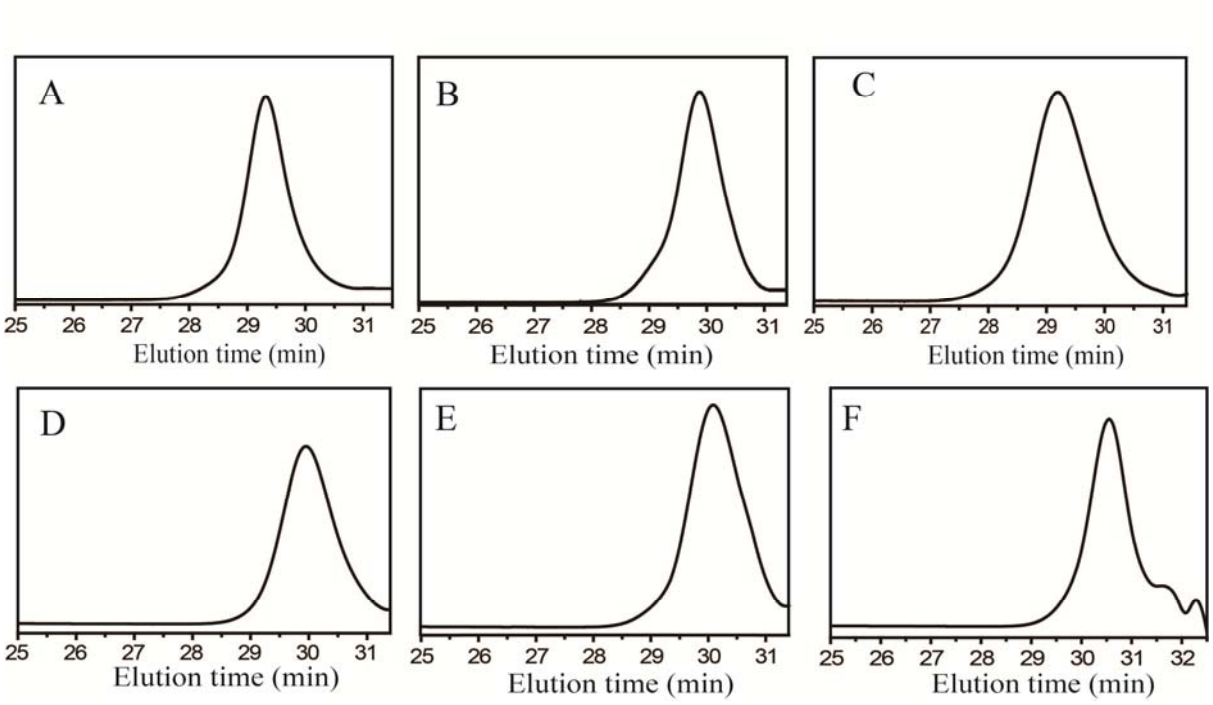


Figure 8. Size exclusion chromatogram of synthesized polymers (A) 40-diethylphosphate-L-Cysteine, (B) 25-diethylphosphate-L-Cysteine, (C) 40-diethylphosphonate-L-Cysteine, (D) 25-diethylphosphonate-L-Cysteine initiated by propargyl amine in DMF and (E) 25-diethylphosphonate-L-Cysteine (F) 15-diethylphosphonate-L-Cysteine initiated by azido-PEG-NH_2 in dioxane.

^{1}H, ^{13}C, DEPT and ^{31}P NMR Spectra of monomers and polymers

Figure 9: ^{1}H NMR of N-Boc-L-Cystine (DMSO-d6)

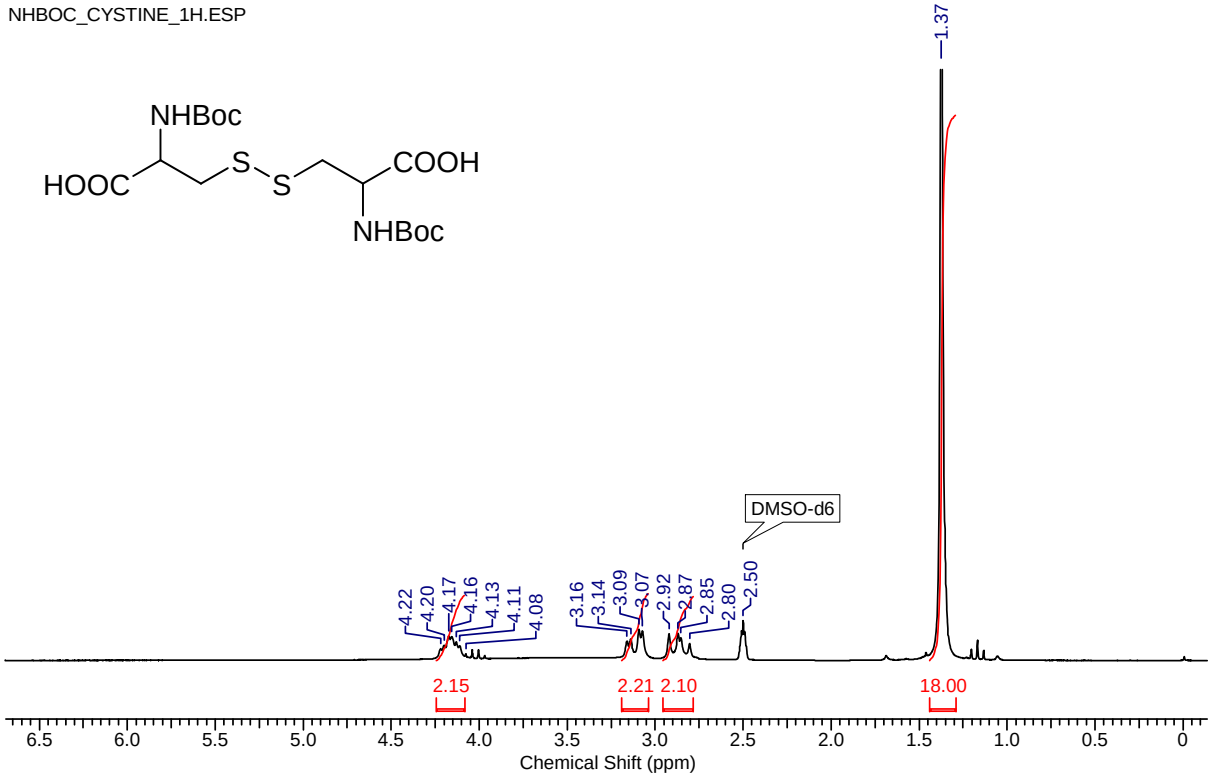


Figure 10: ^{13}C NMR of N-Boc-L-Cystine (DMSO-d6)

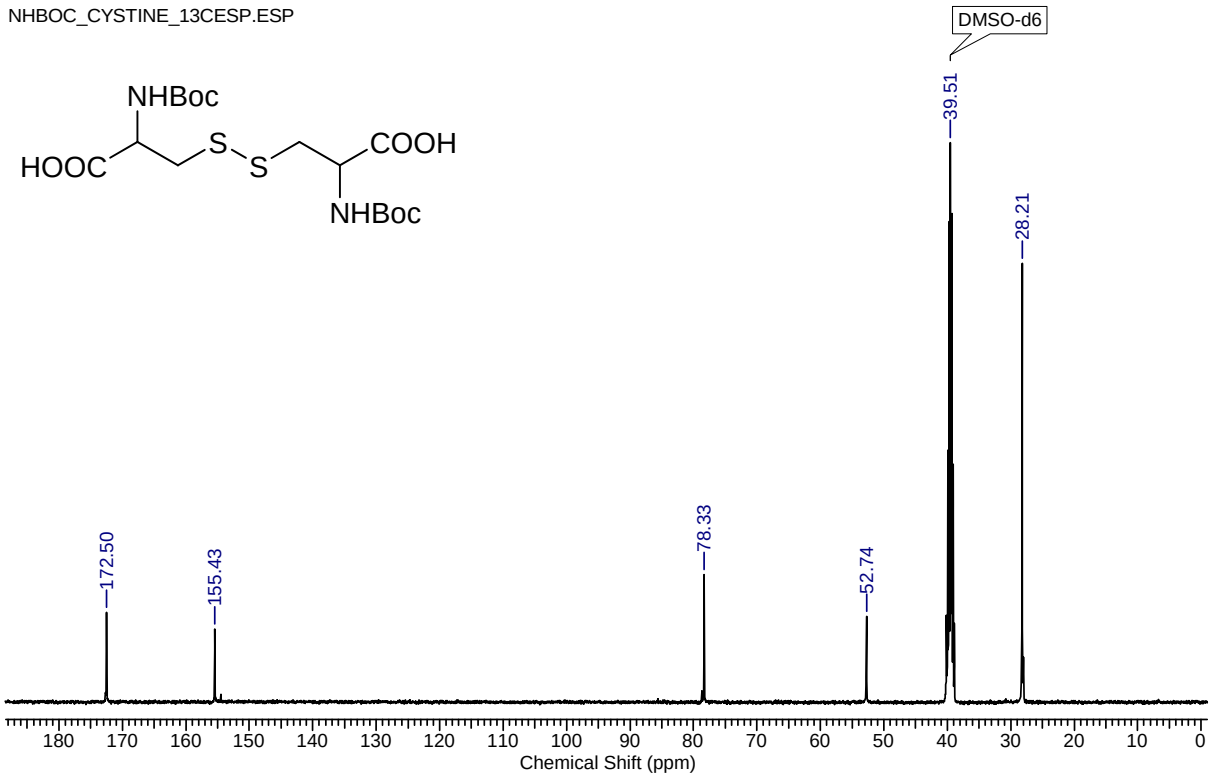


Figure 11: DEPT of N-Boc-L-Cystine (DMSO-d6)

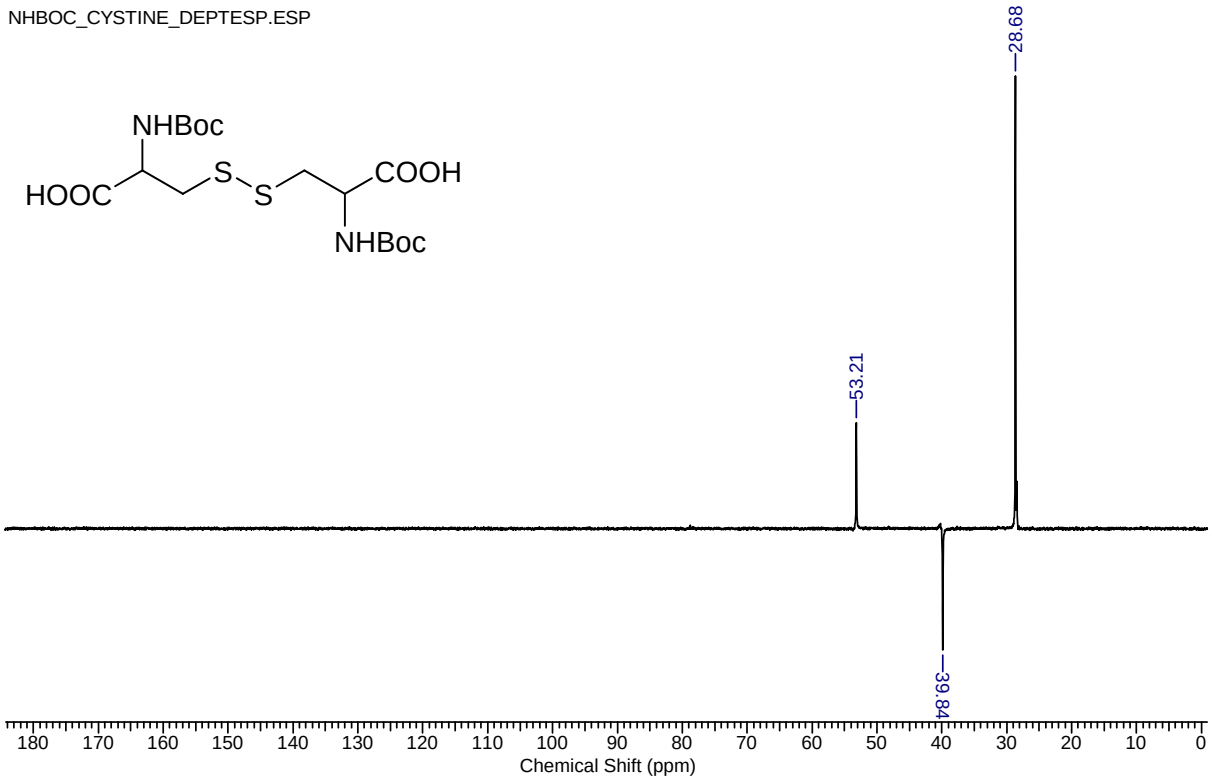


Figure 12: ^{13}C NMR of N-Boc-L-Cysteine (DMSO-d6)

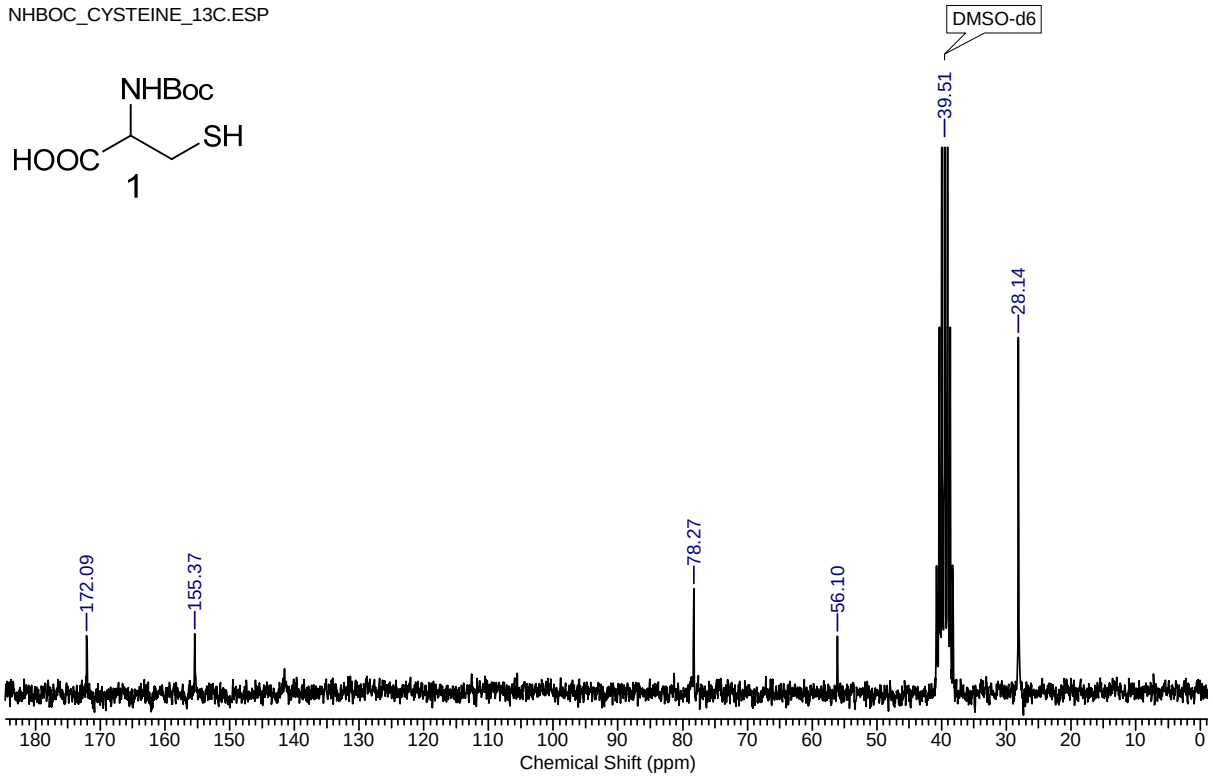


Figure 13: DEPT of N-Boc-L-Cysteine (DMSO-d6)

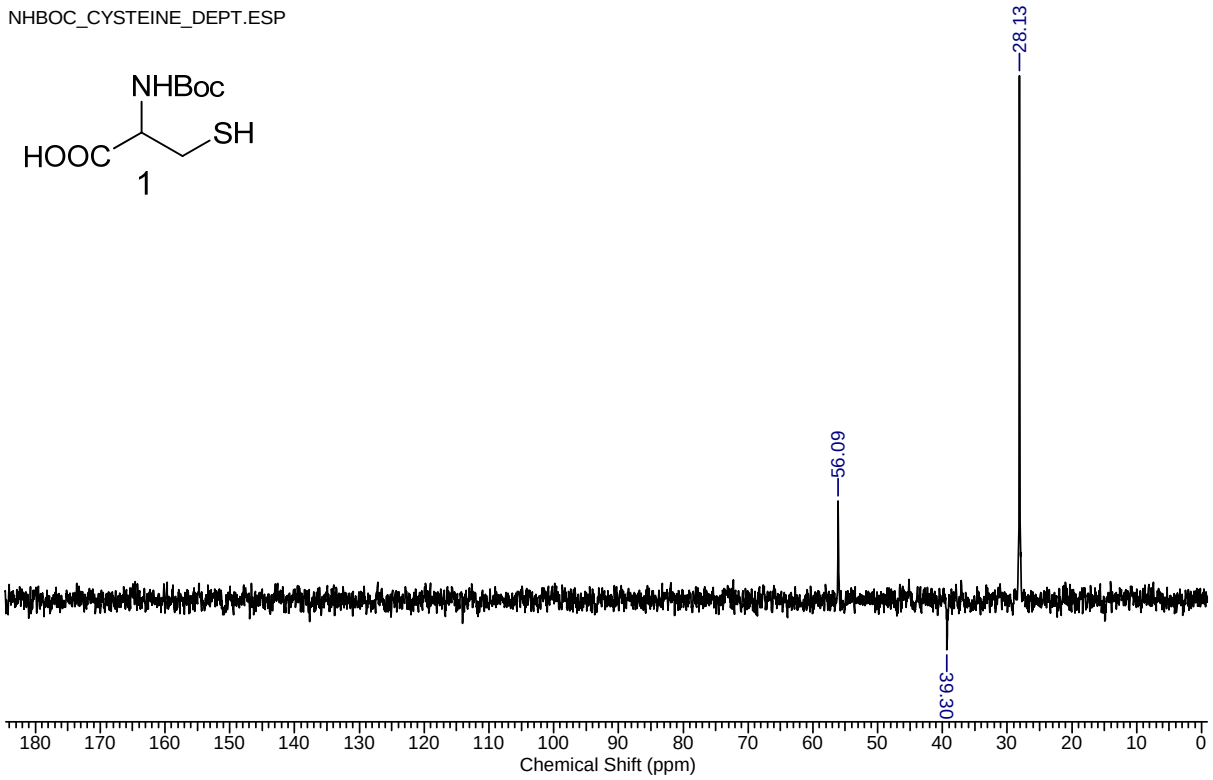


Figure 14: ^{1}H NMR of allyl diethylphosphate ($CDCl_3$)

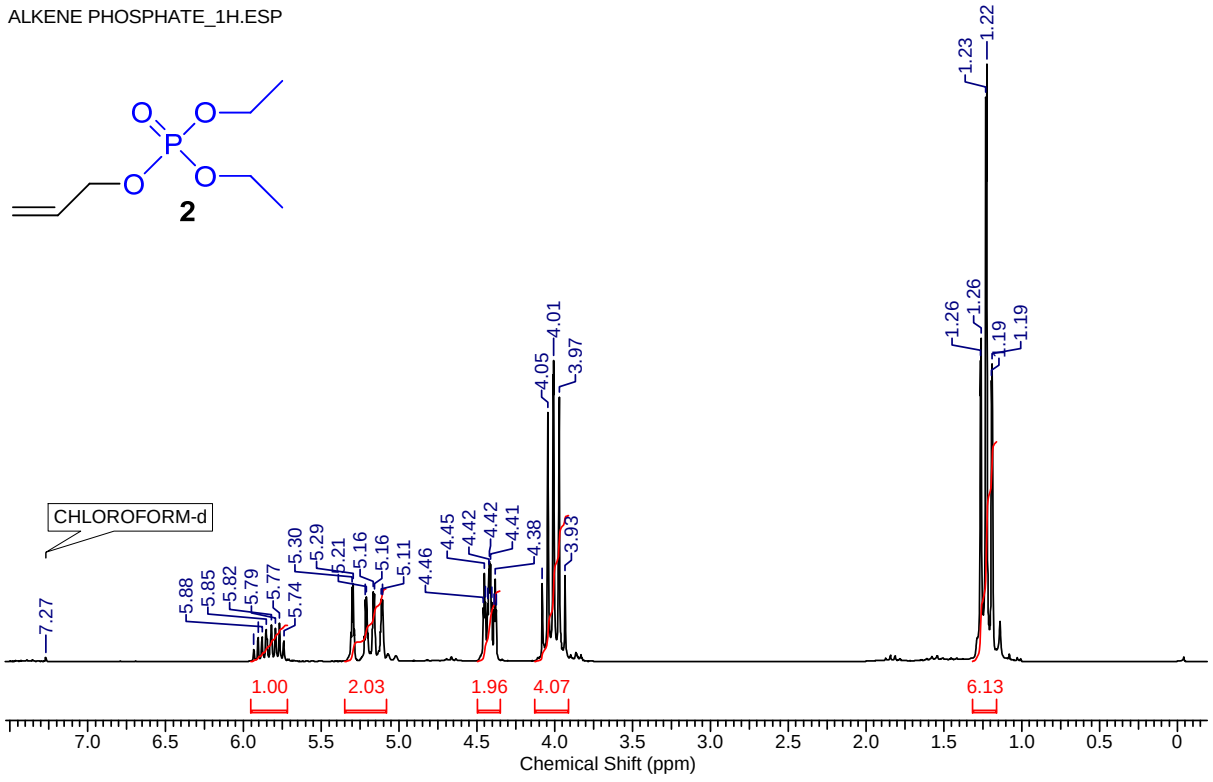


Figure 15: ^{13}C NMR of allyl diethylphosphate ($CDCl_3$)

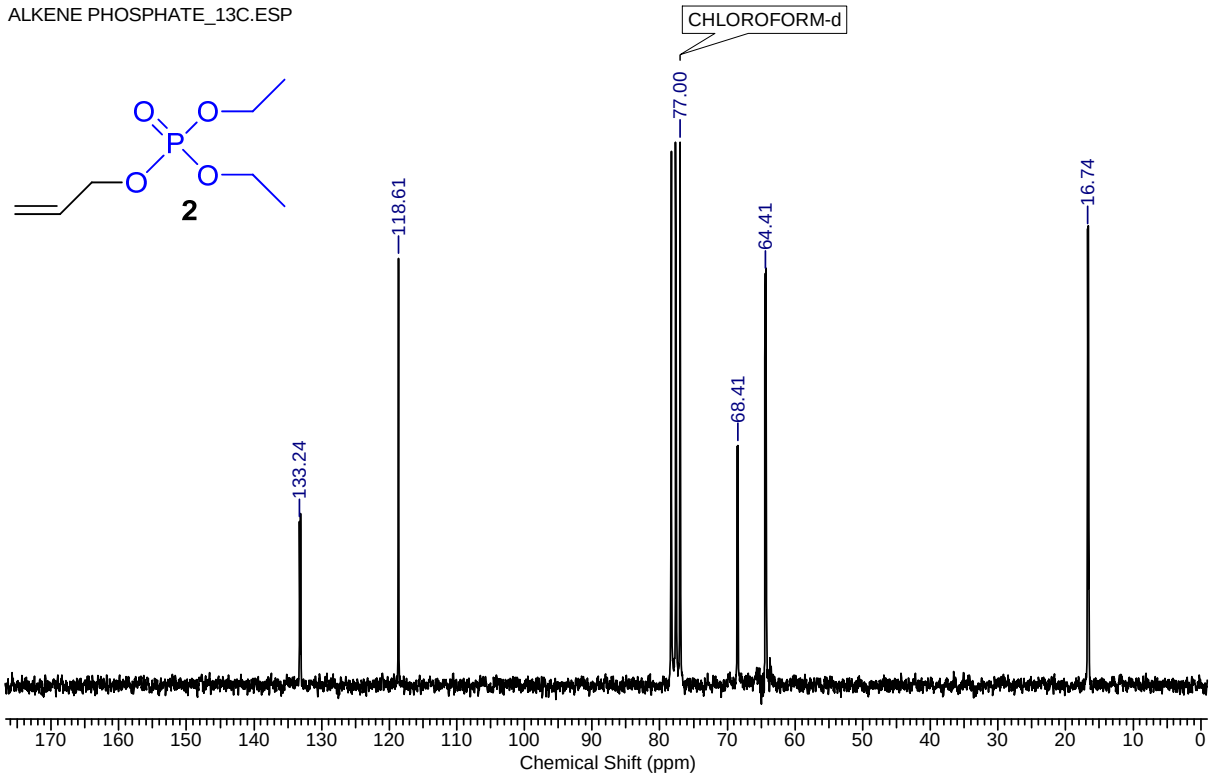


Figure 16: DEPT of allyl diethylphosphate ($CDCl_3$)

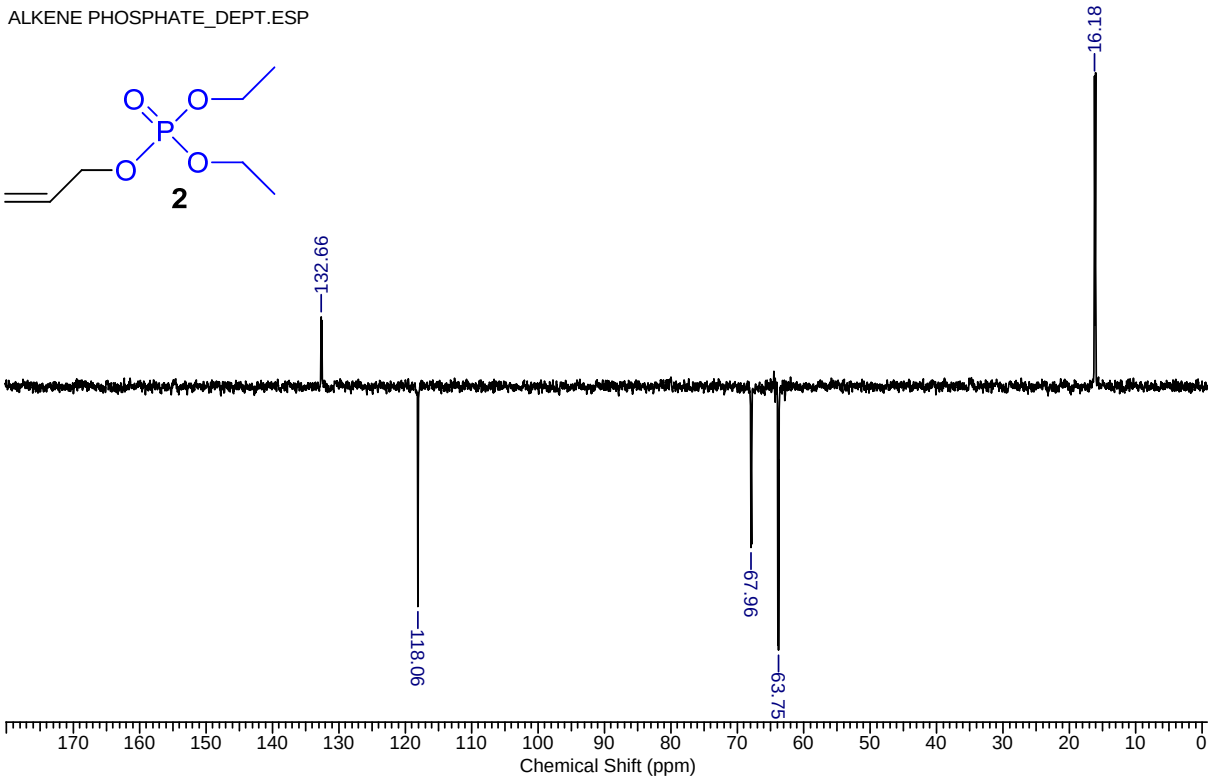


Figure 17: ^{1}H NMR of allyl diethylphosphonate ($CDCl_3$)

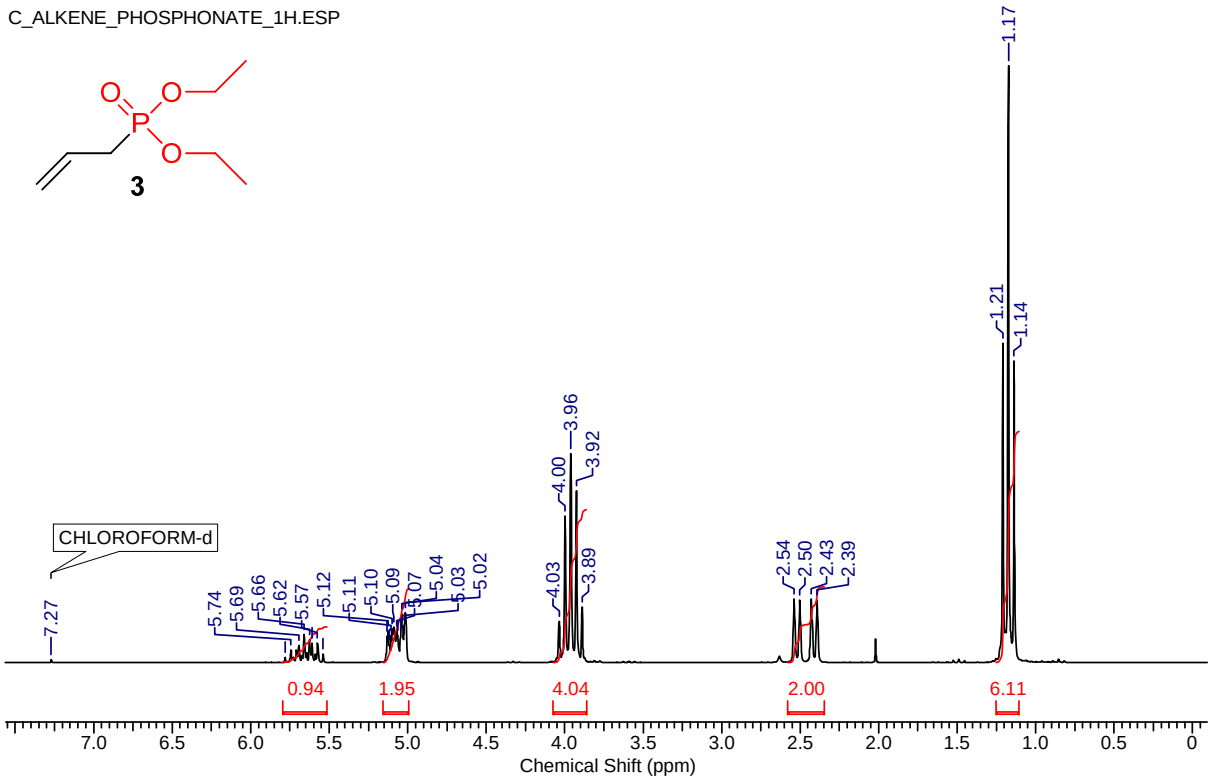


Figure 18: ^{13}C NMR of allyl diethylphosphonate ($CDCl_3$)

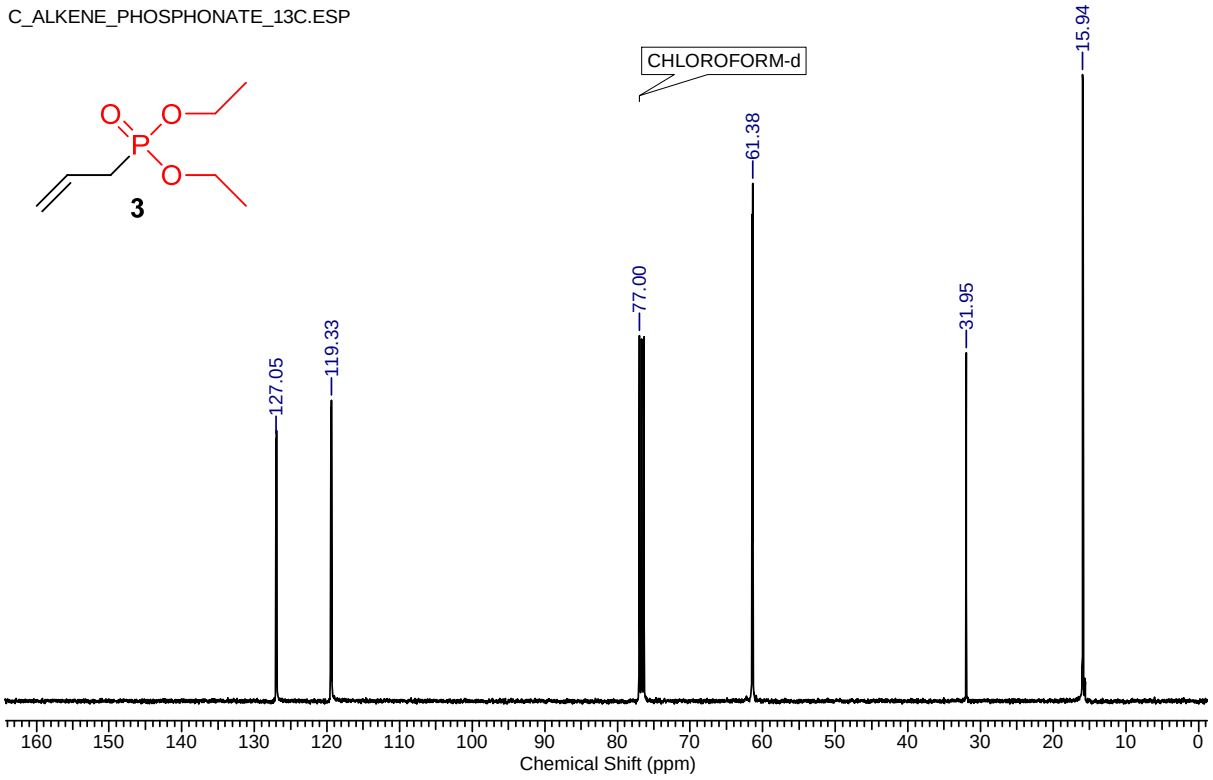


Figure 19: DEPT of allyl diethylphosphonate ($CDCl_3$)

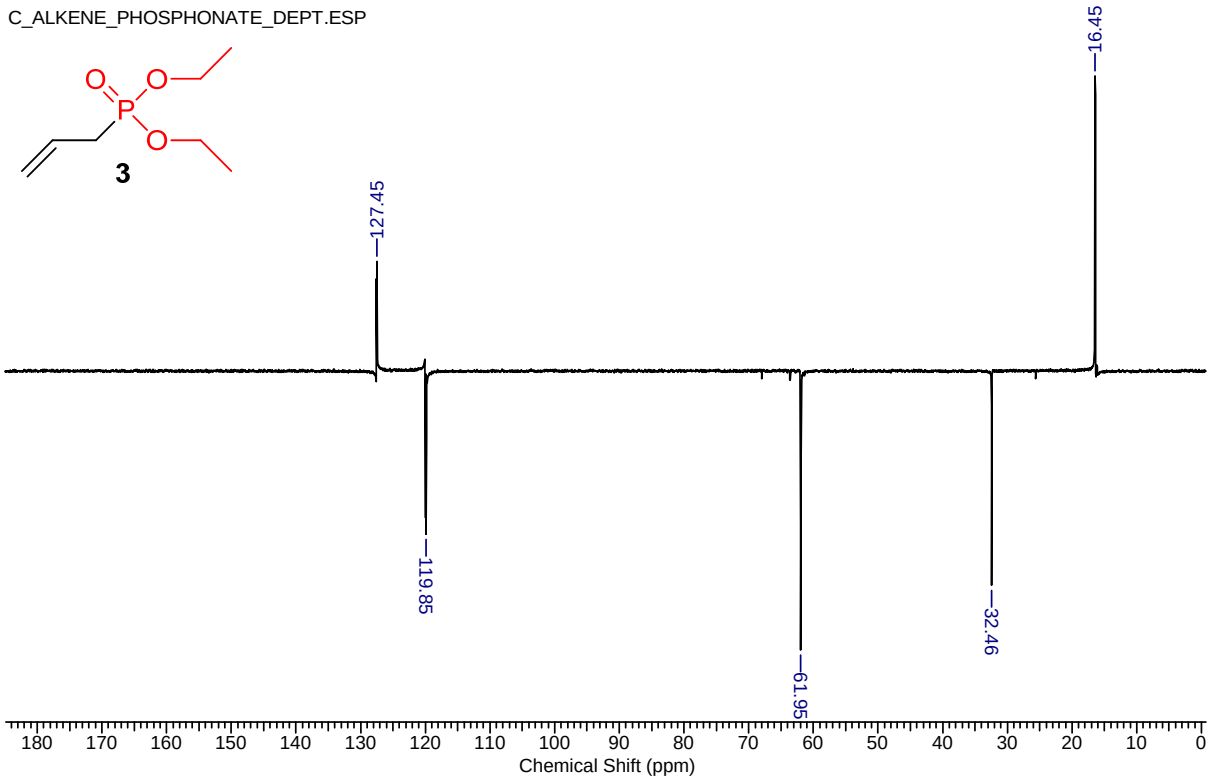


Figure 20: ^{1}H NMR of N-Boc-L-cysteine-diethylphosphate ($CDCl_3$)

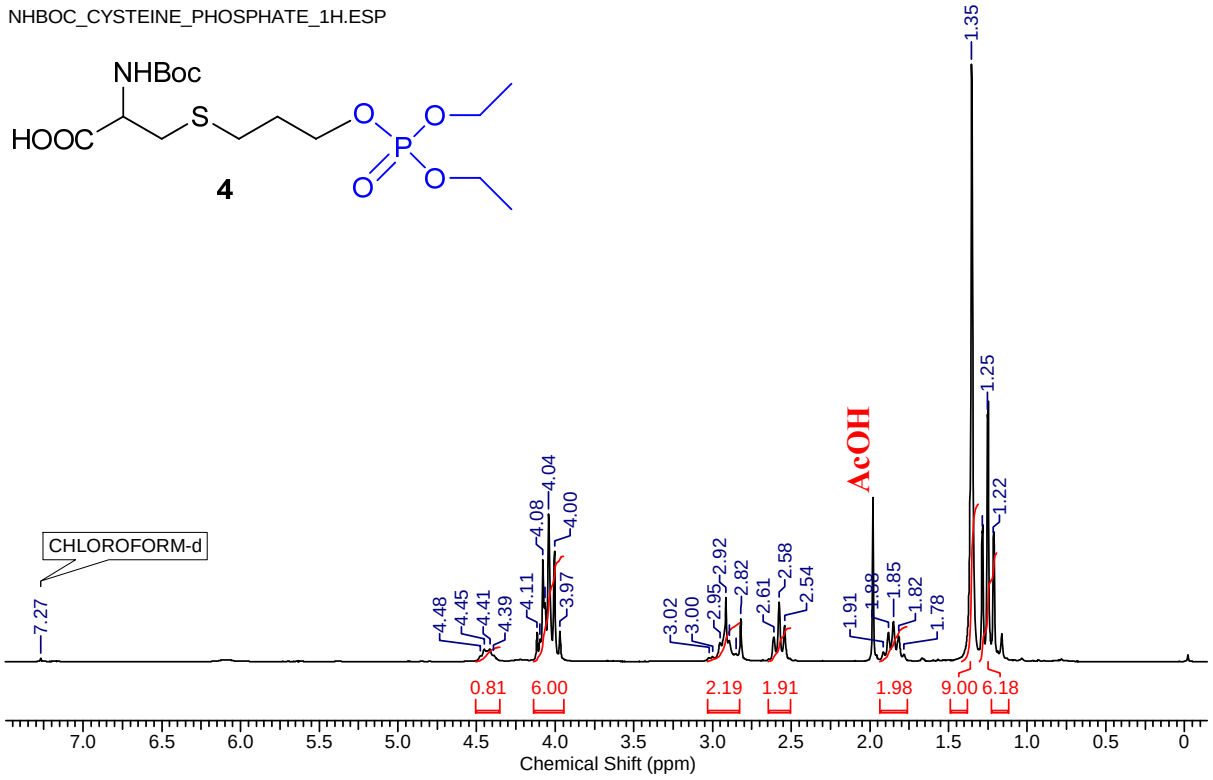


Figure 21: ^{13}C NMR of N-Boc-L-cysteine-diethylphosphate ($CDCl_3$)

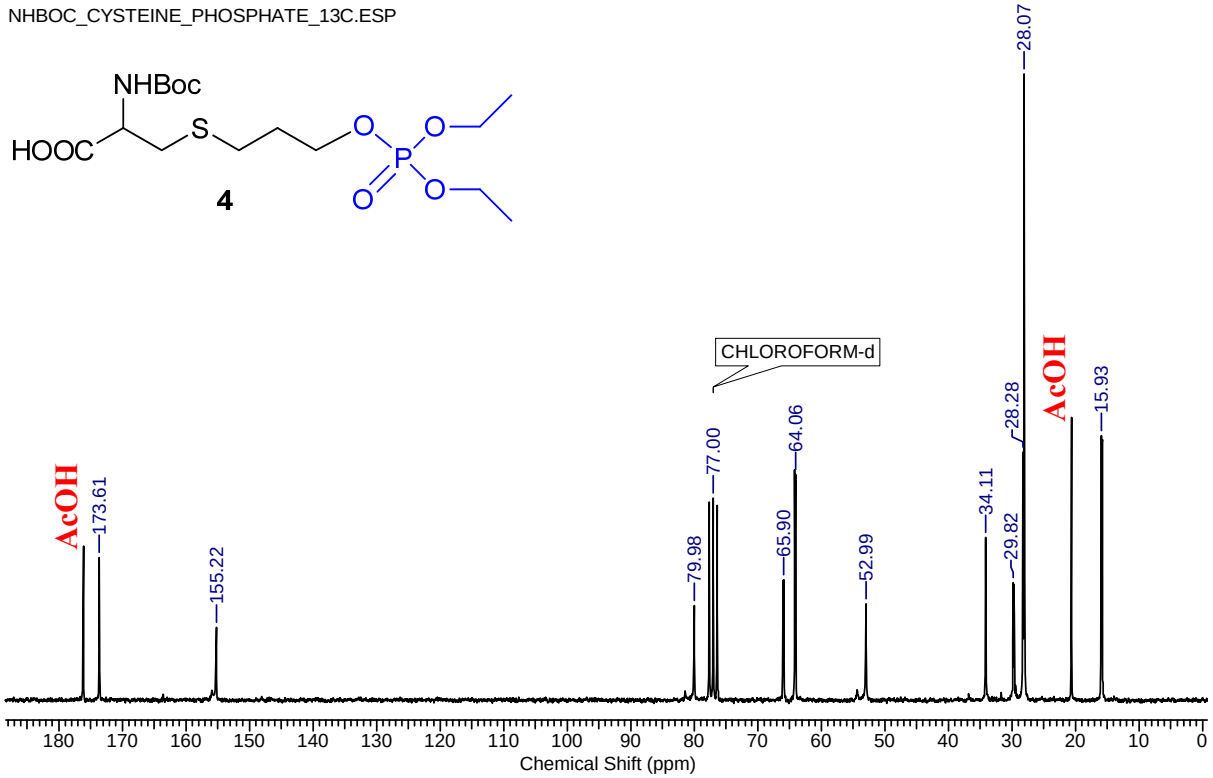


Figure 22: DEPT of N-Boc-L-cysteine-diethylphosphate ($CDCl_3$)

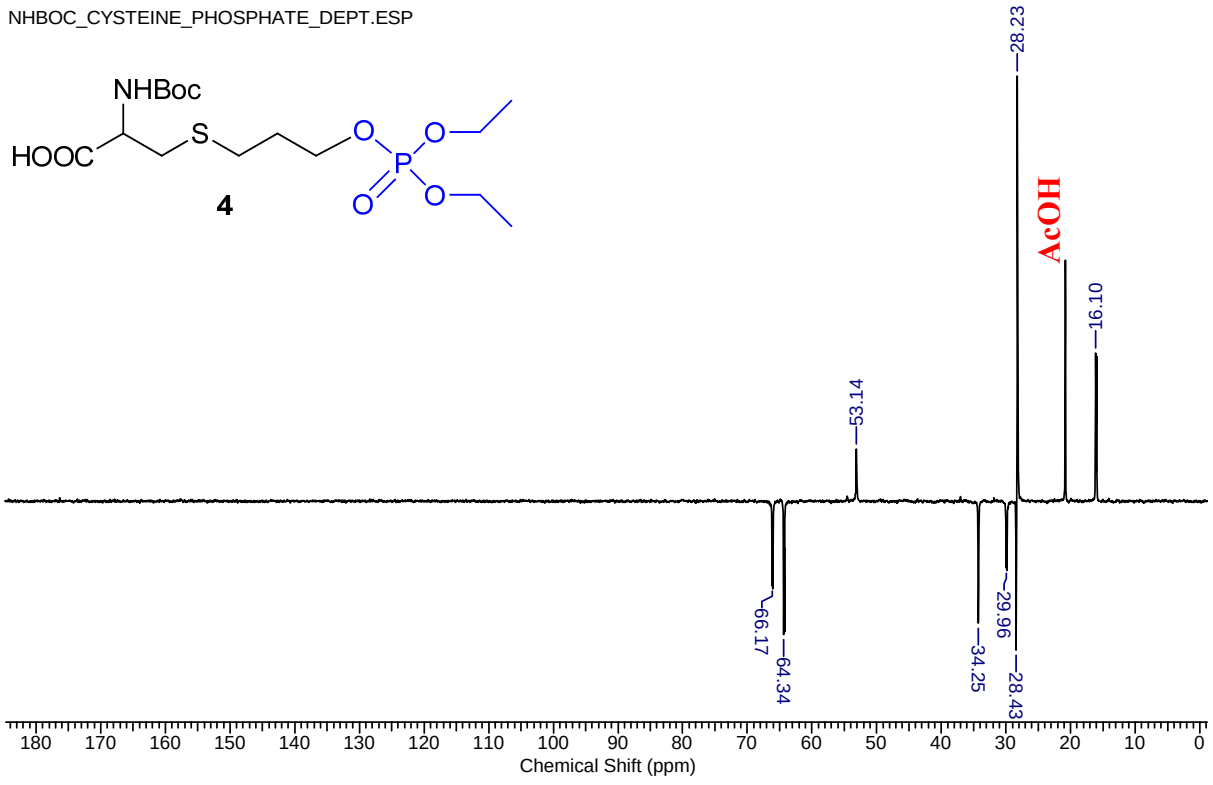


Figure 23: ^{31}P NMR of N-Boc-L-cysteine-diethylphosphate ($CDCl_3$)

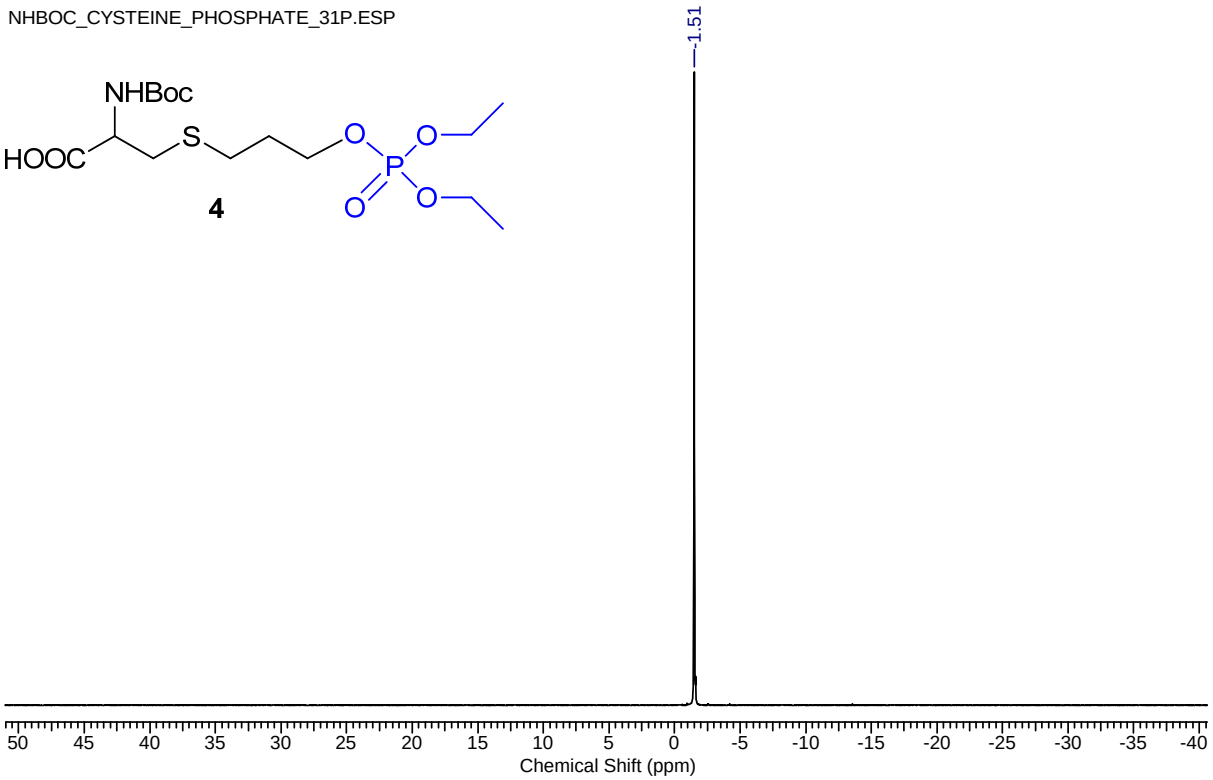


Figure 24: ^{1}H NMR of N-Boc-L-cysteine-diethylphosphonate ($CDCl_3$)

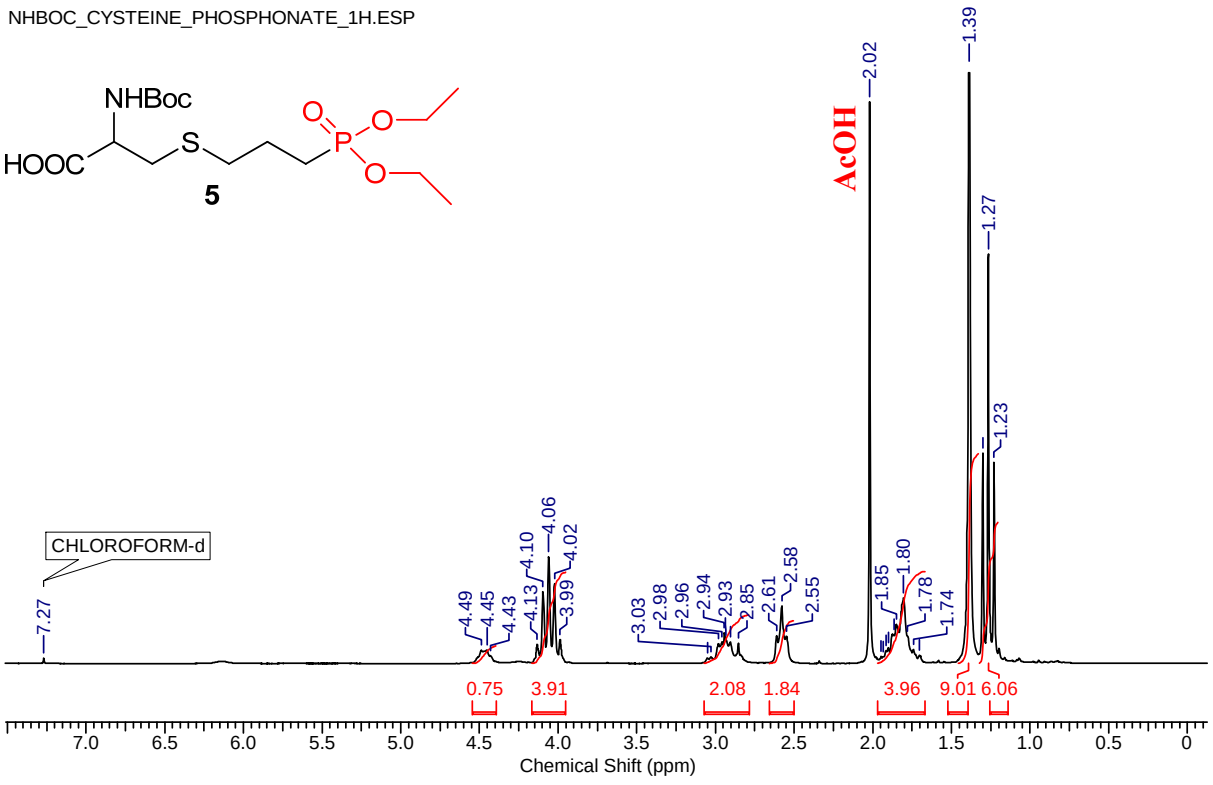


Figure 25: ^{13}C NMR of N-Boc-L-cysteine-diethylphosphonate ($CDCl_3$)

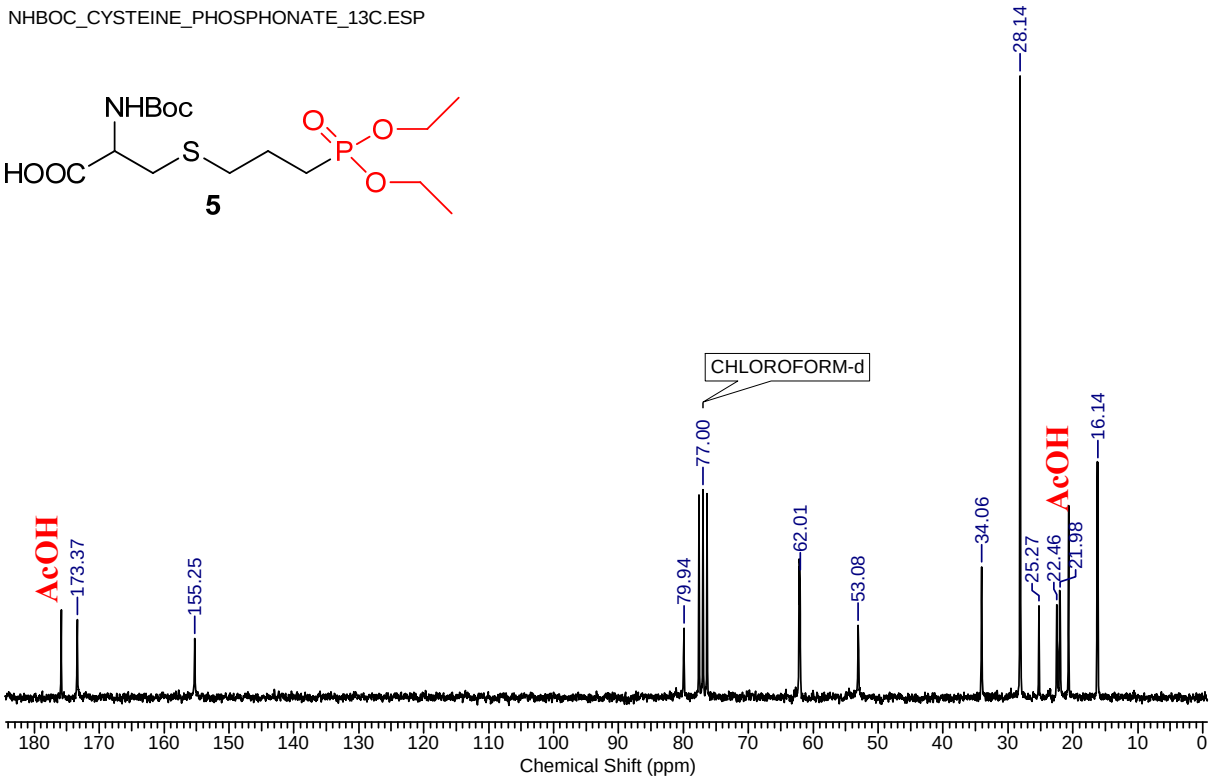


Figure 26: DEPT of N-Boc-L-cysteine-diethylphosphonate ($CDCl_3$)

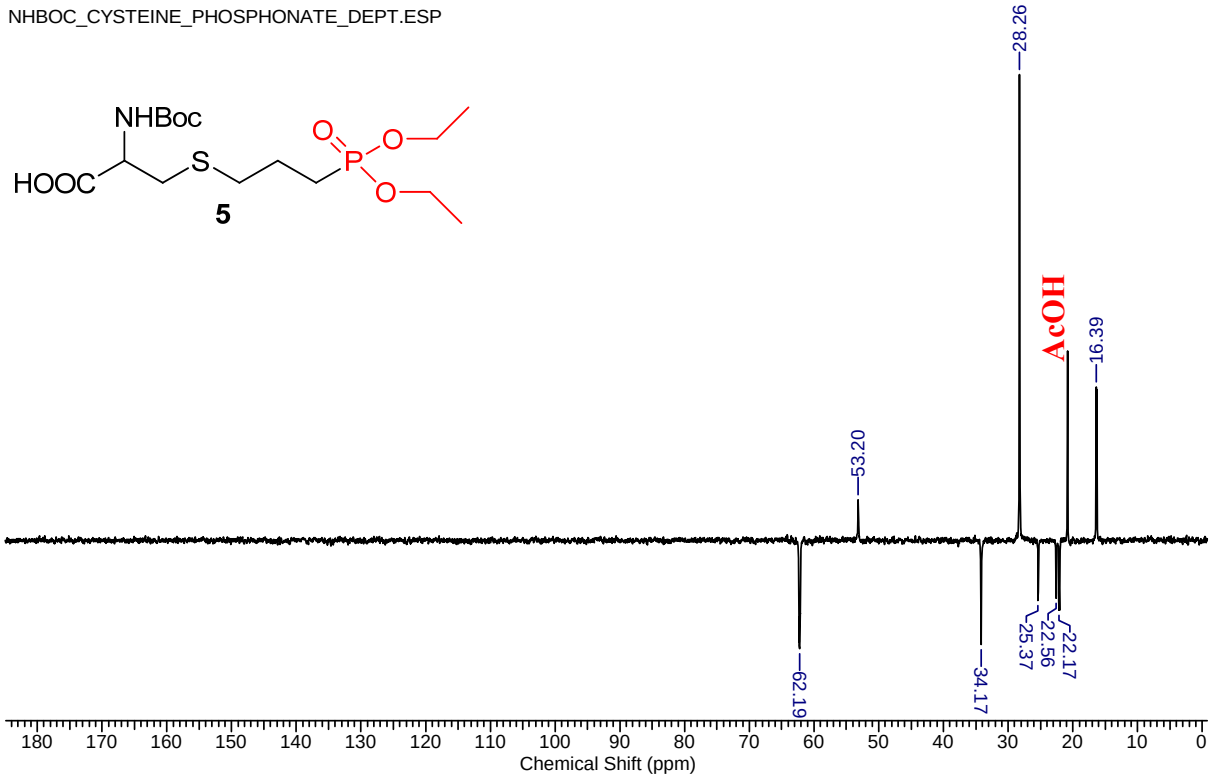


Figure 27: ^{31}P NMR of N-Boc-L-cysteine-diethylphosphonate ($CDCl_3$)

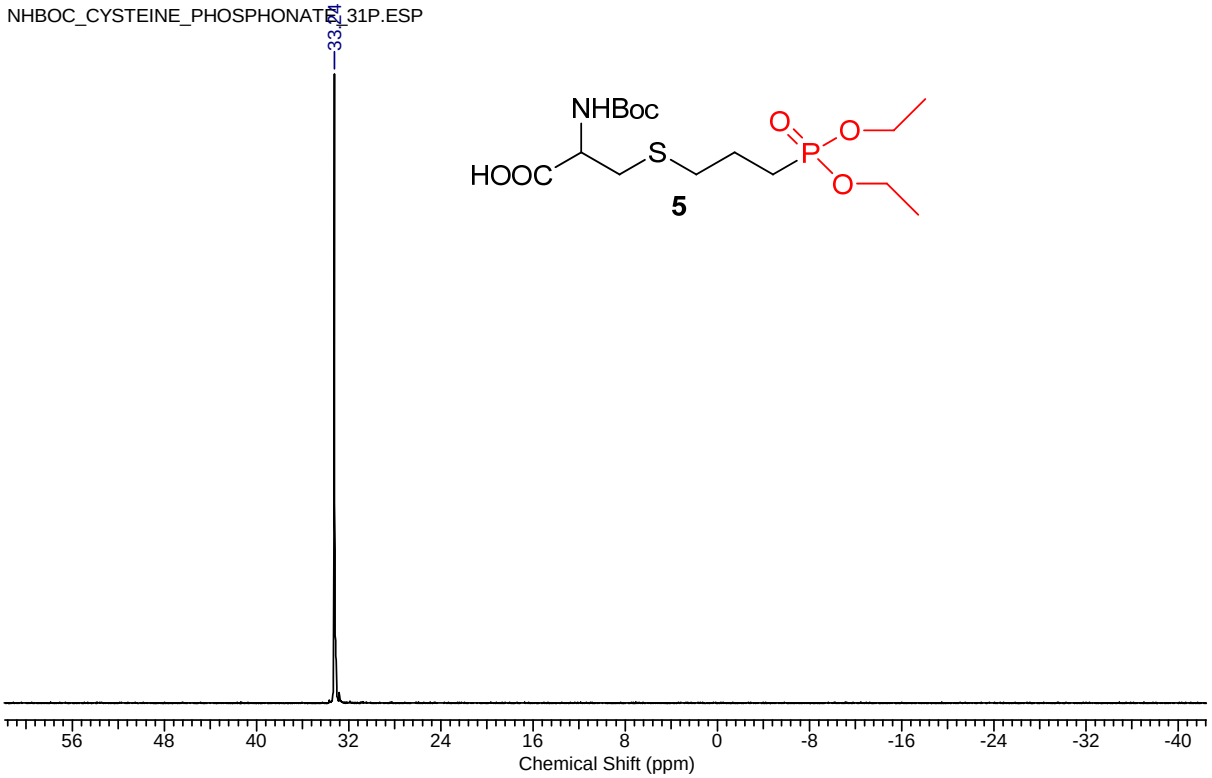


Figure 28: ^{1}H NMR of NH_2-L-cysteine-diethylphosphate (DMSO-d6)

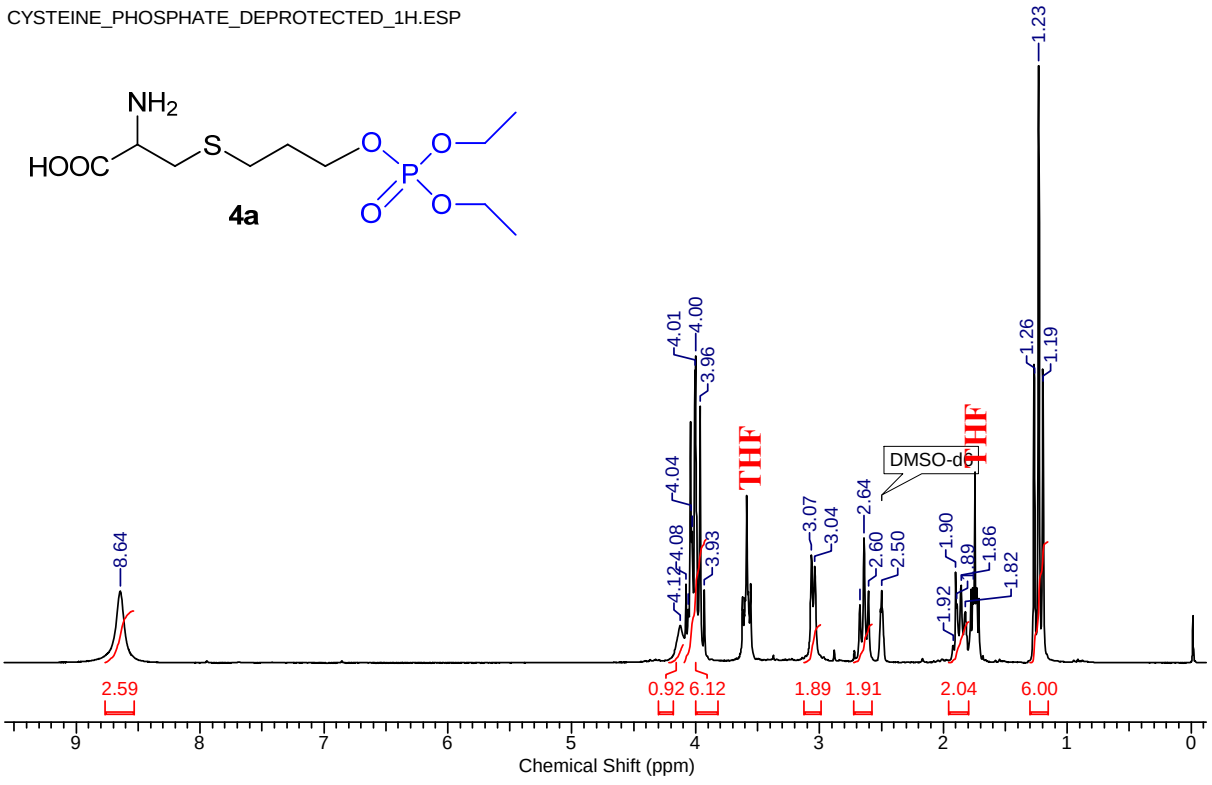


Figure 29: ^{13}C NMR of NH_2-L-cysteine-diethylphosphate (DMSO-d6)

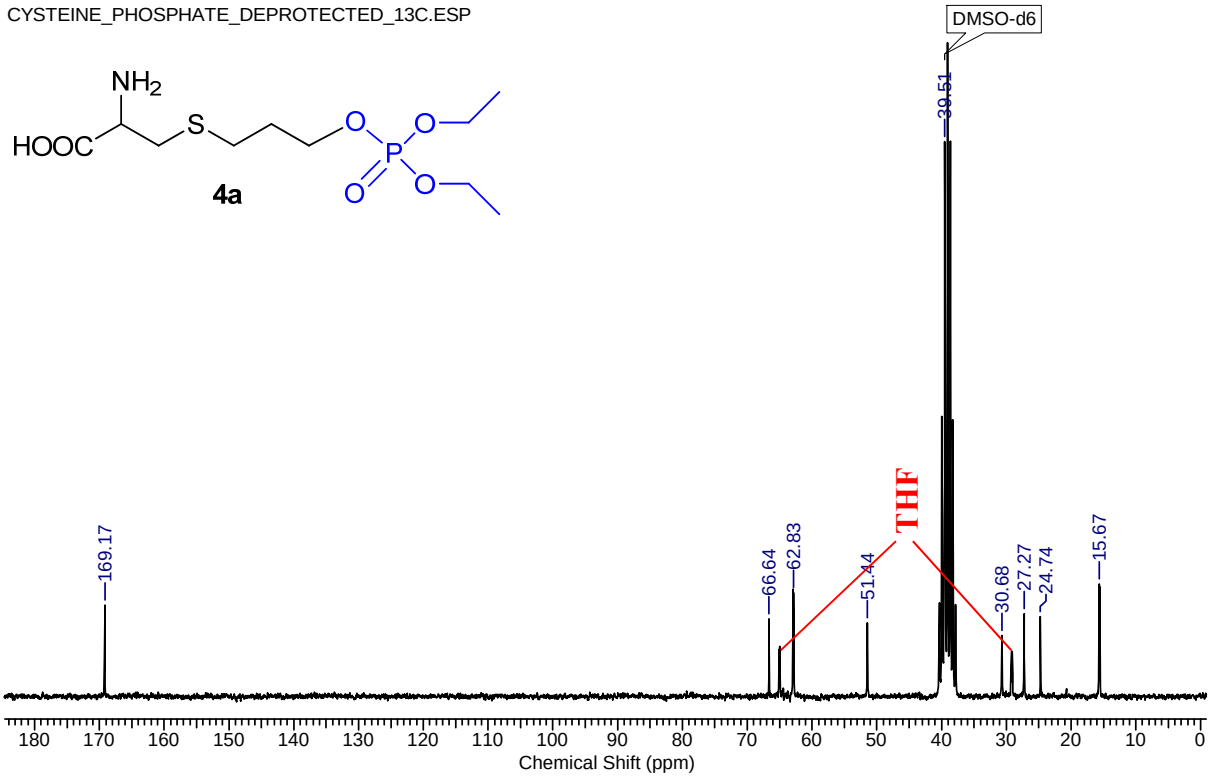


Figure 30: DEPT of NH_2-L-cysteine-diethylphosphate (DMSO-d6)

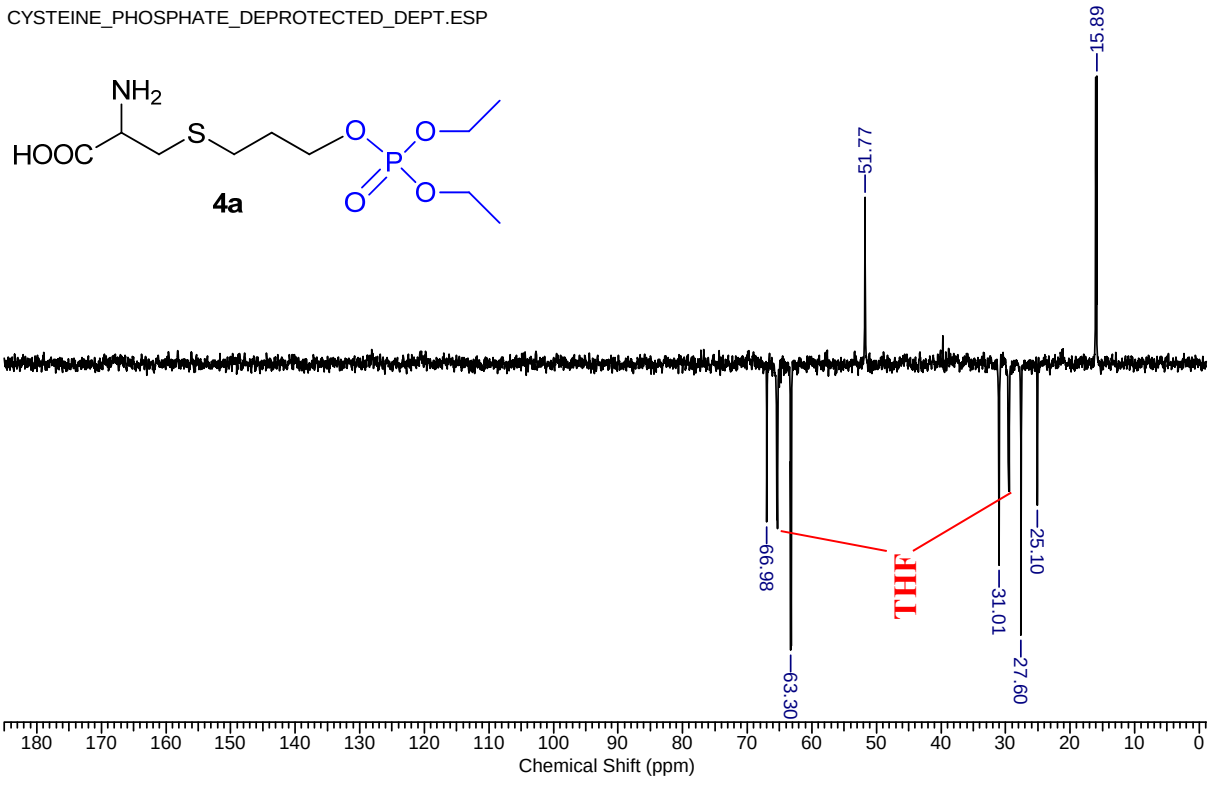


Figure 31: ^{1}H NMR of NH_2-L-cysteine-diethylphosphonate (DMSO-d6)

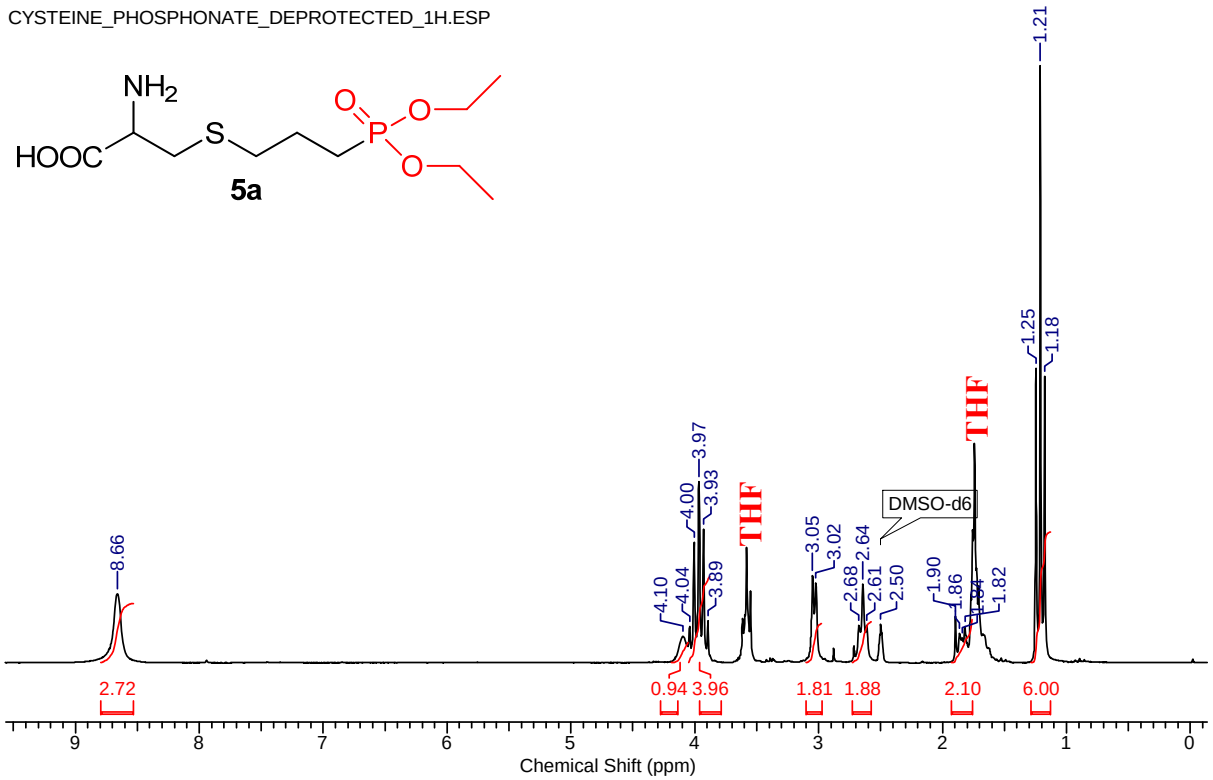


Figure 32: ^{13}C NMR of NH_2-L-cysteine-diethylphosphonate (DMSO-d6)

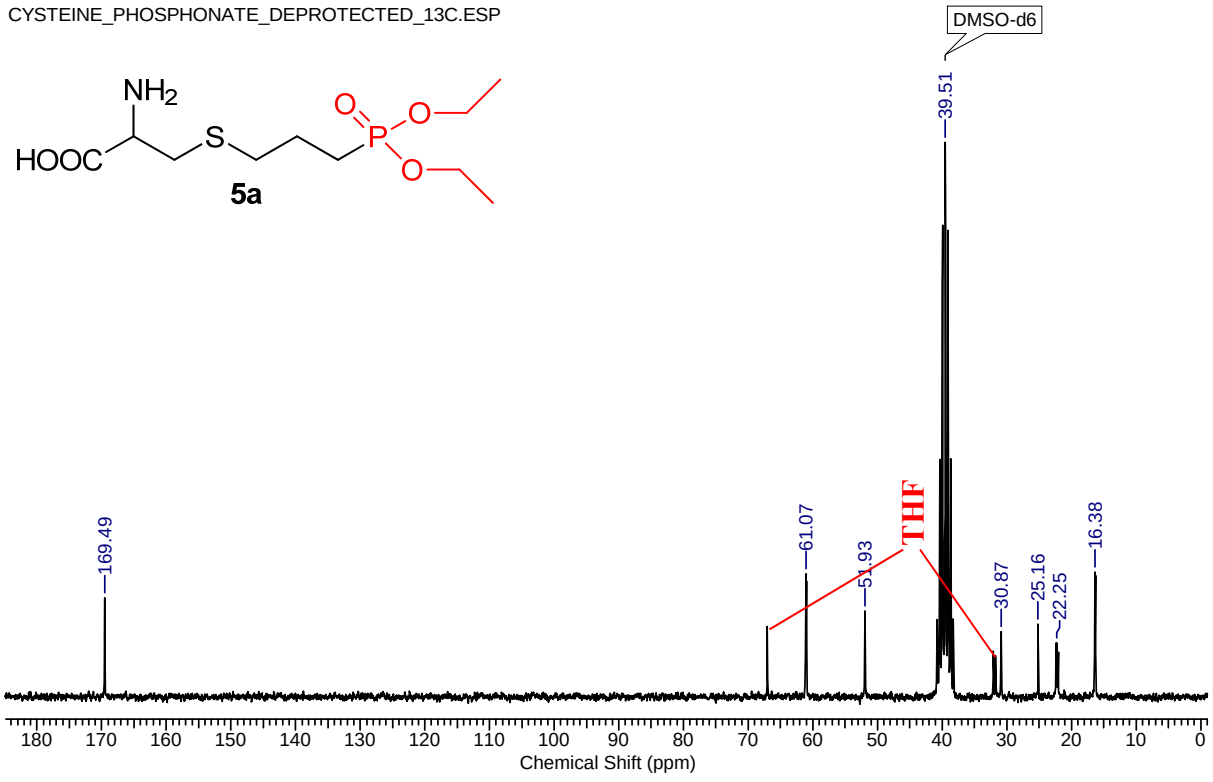


Figure 33: DEPT of NH_2-L-cysteine-diethylphosphonate (DMSO-d6)

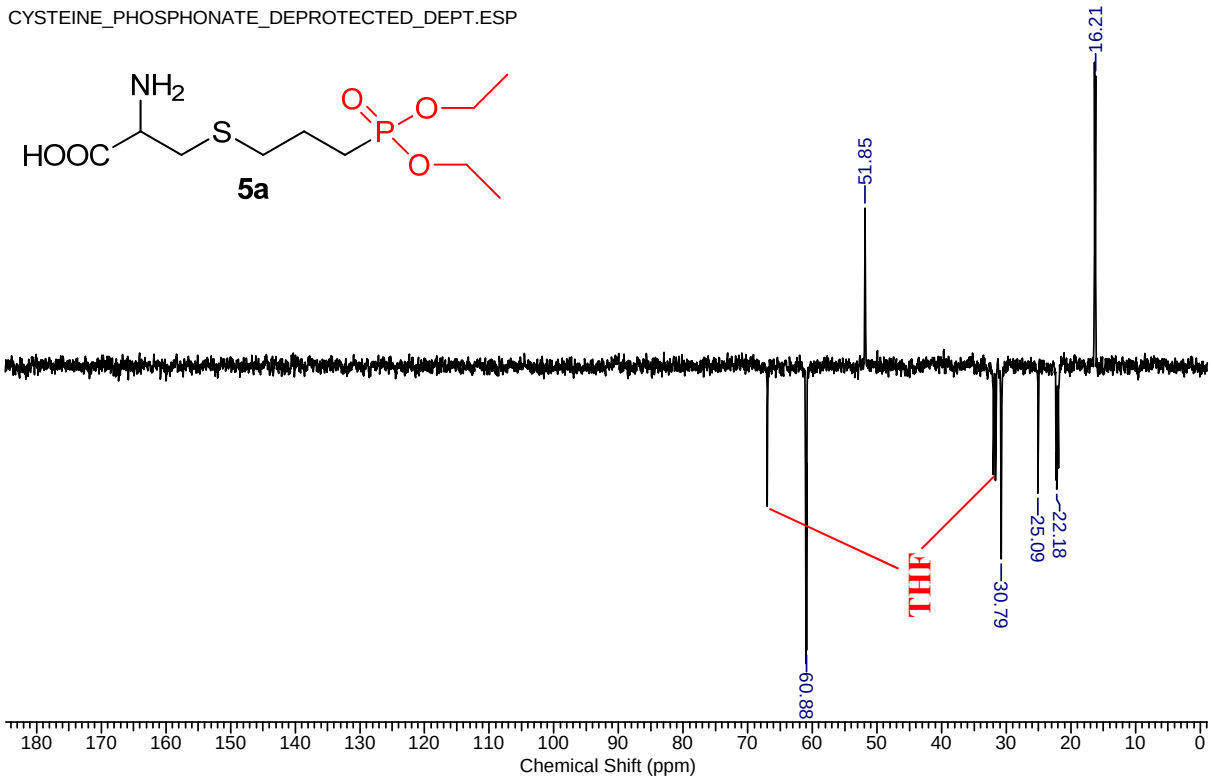


Figure 34: ^{1}H NMR of diethylphosphate-L-Cysteine-N-Carboxyanhydride ($CDCl_3$)

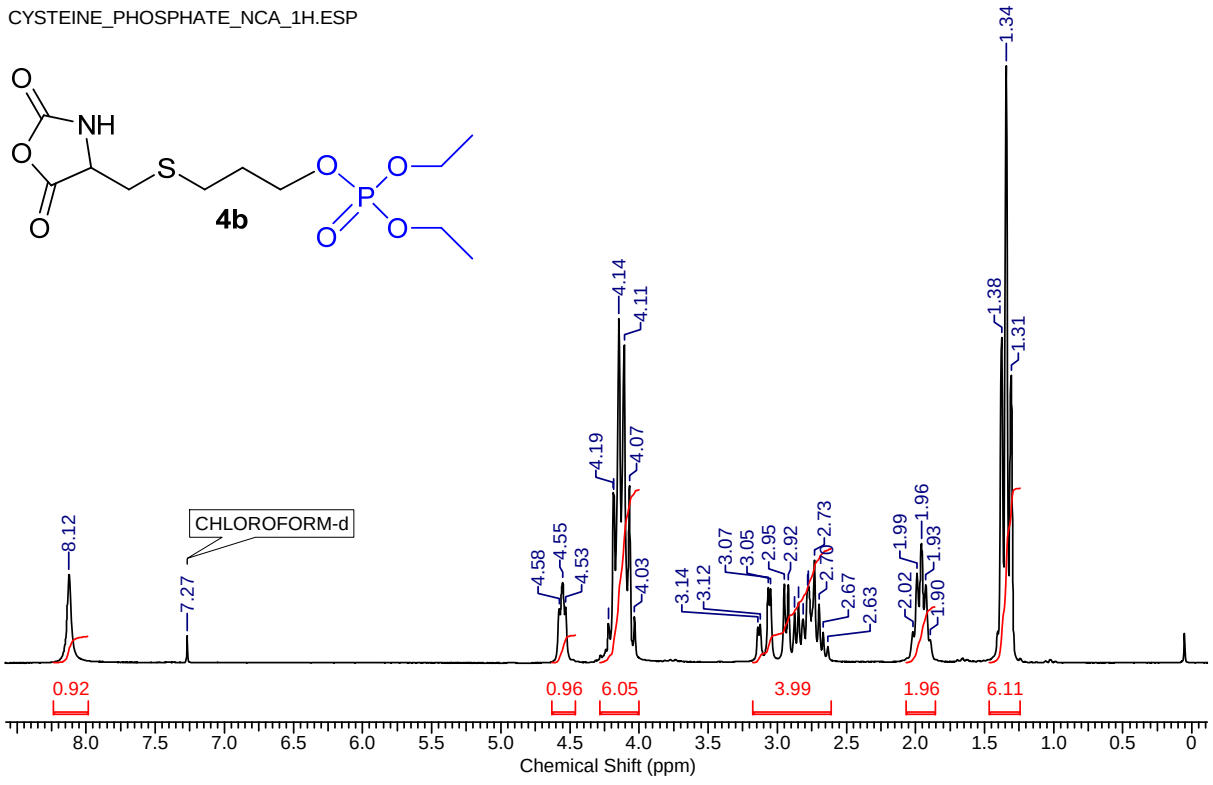


Figure 35: ^{13}C NMR of diethylphosphate-L-Cysteine-N-Carboxyanhydride ($CDCl_3$)

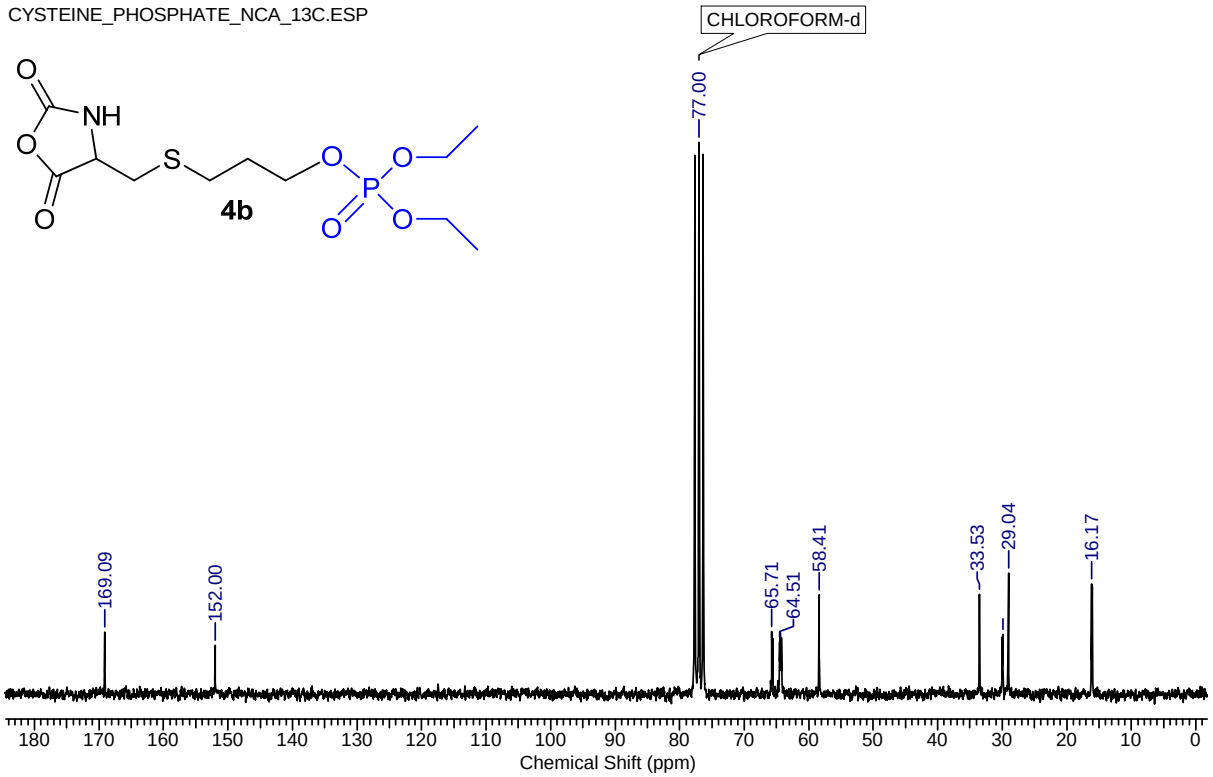


Figure 36: DEPT of diethylphosphate-L-Cysteine-N-Carboxyanhydride ($CDCl_3$)

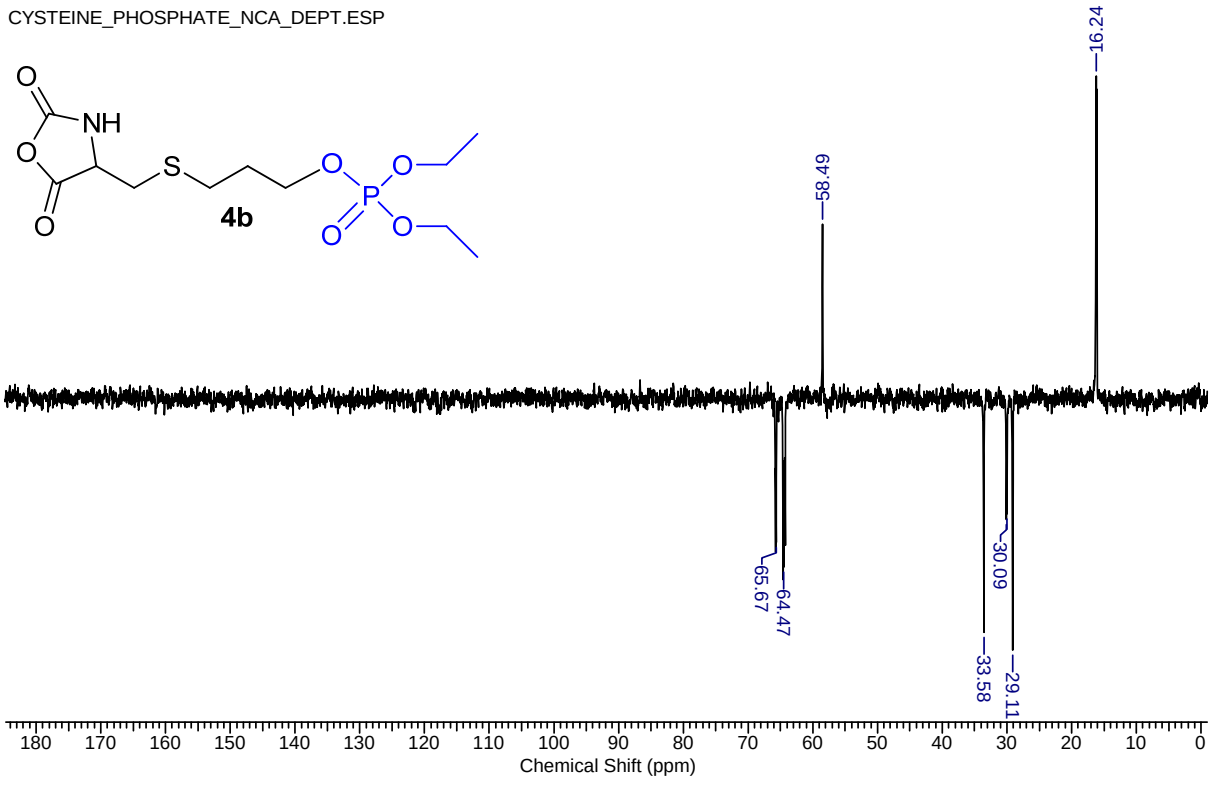


Figure 37: ^{1}H NMR of diethylphosphonate-L-Cysteine-N-Carboxyanhydride ($CDCl_3$)

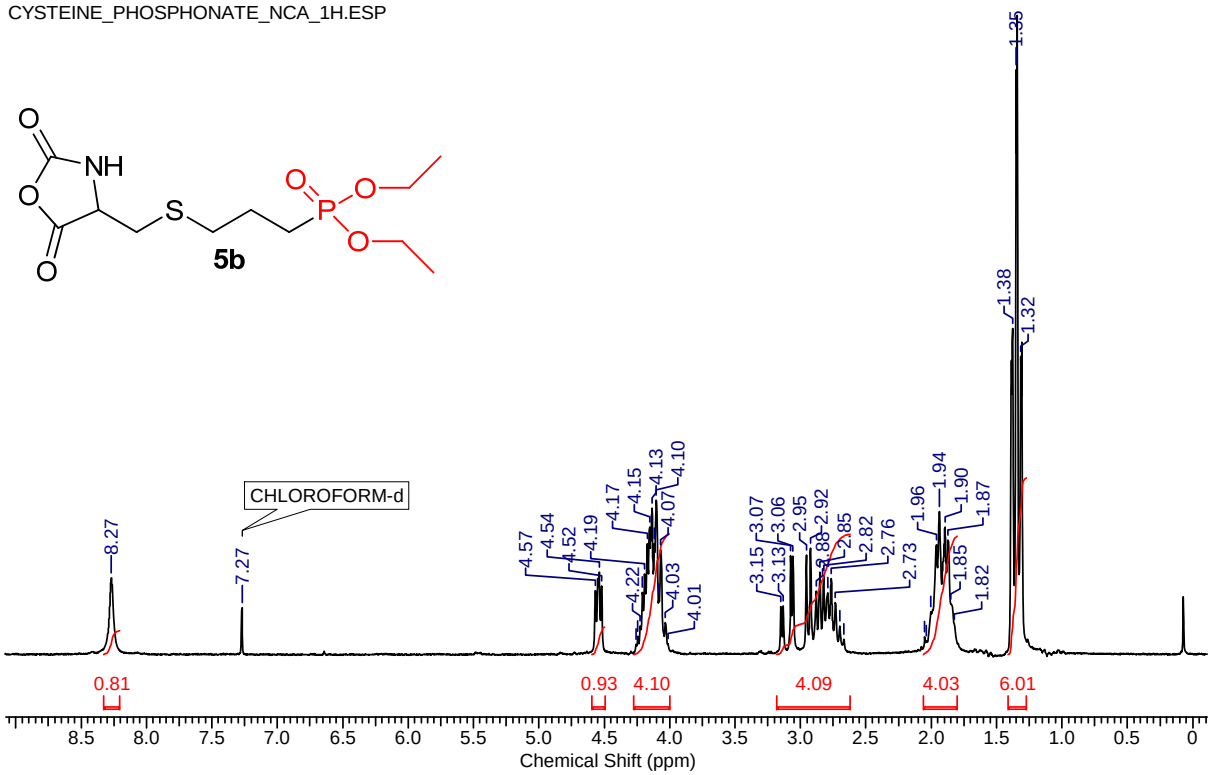


Figure 38: ^{13}C NMR of diethylphosphonate-L-Cysteine-N-Carboxyanhydride ($CDCl_3$)

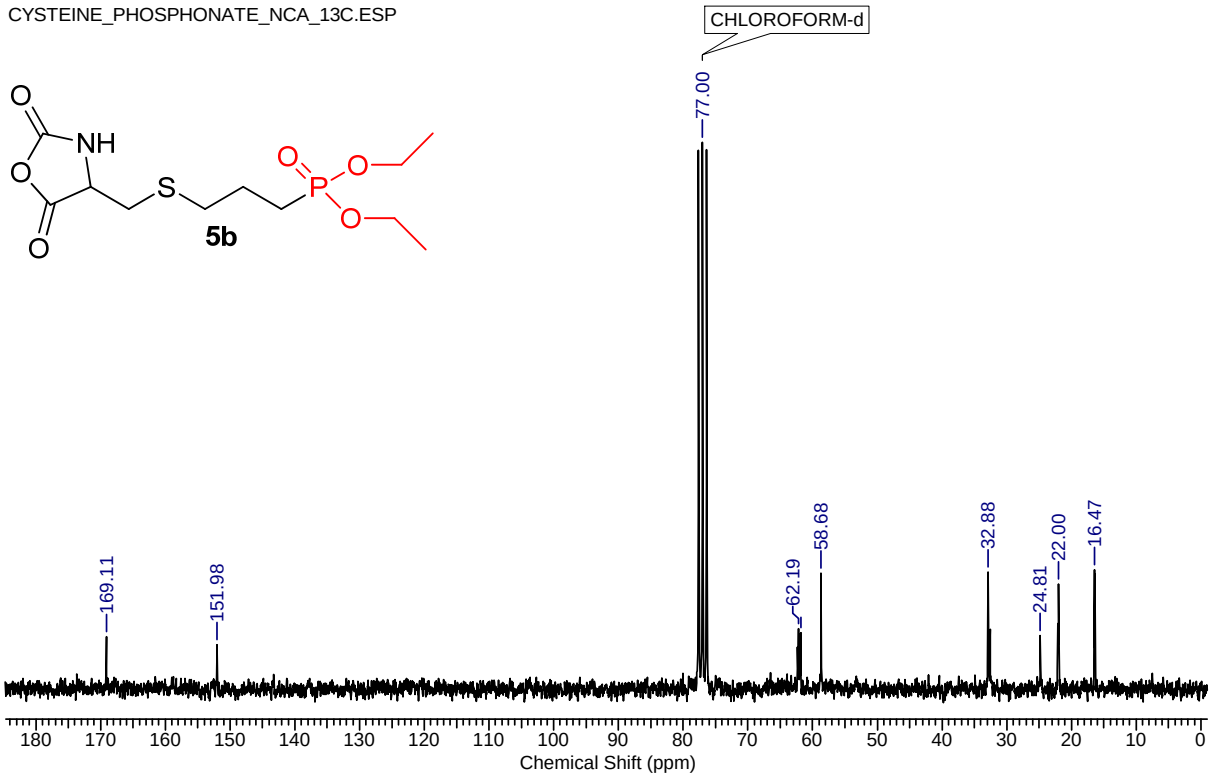


Figure 39: DEPT of diethylphosphonate-L-Cysteine-N-Carboxyanhydride ($CDCl_3$)

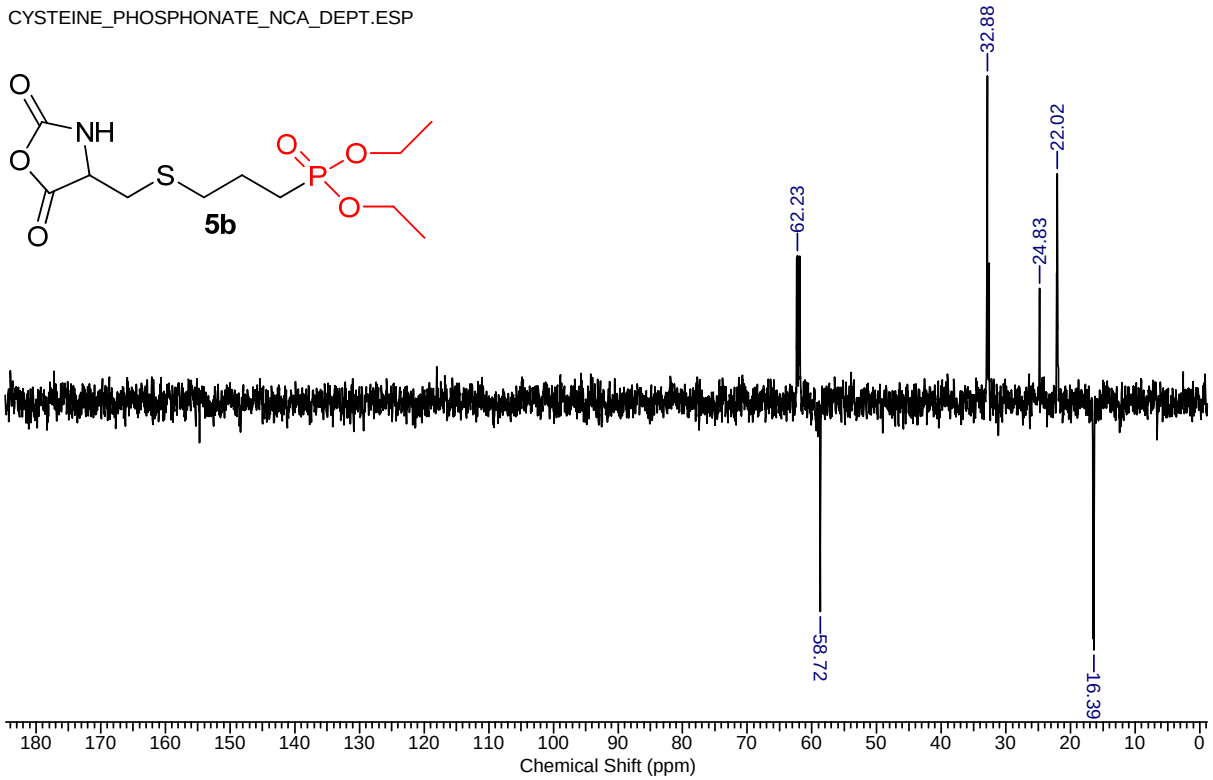


Figure 40: ^1H NMR of *25*-diethylphosphate-L-Cysteine ($CDCl_3$)

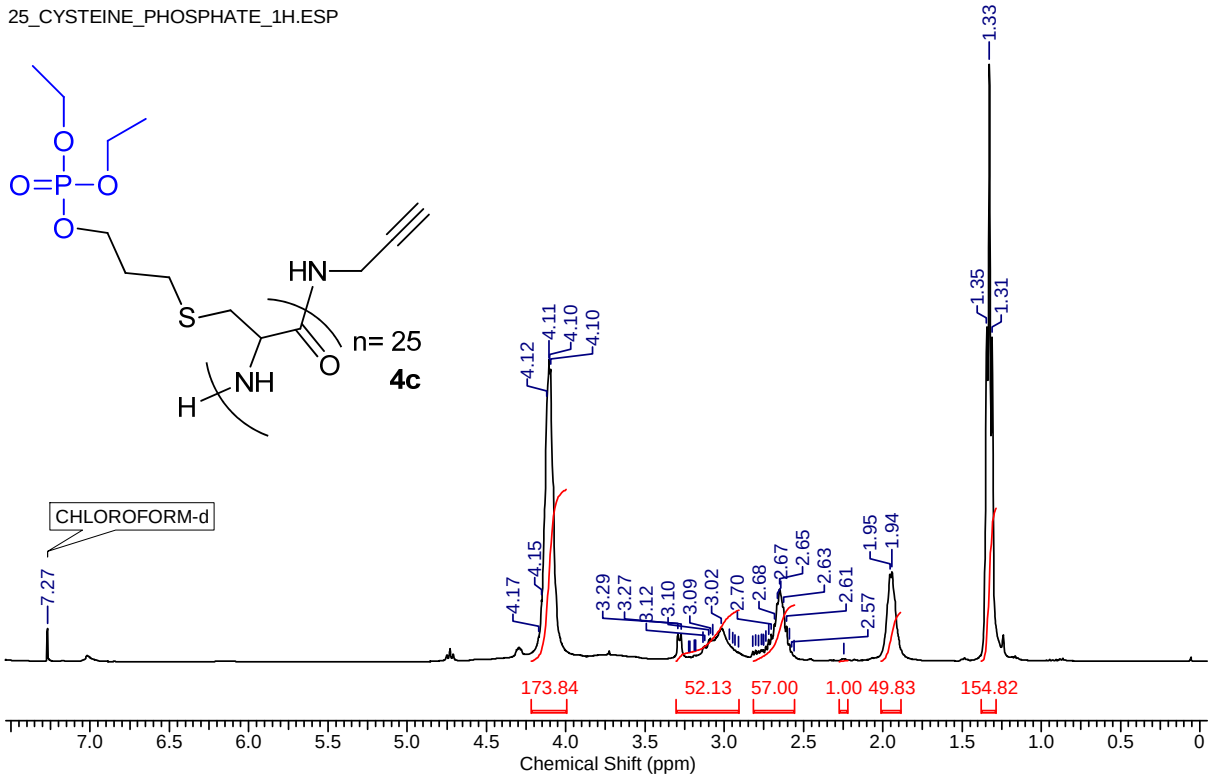


Figure 41: ^{31}P NMR of *25*-diethylphosphate-L-Cysteine ($CDCl_3$)

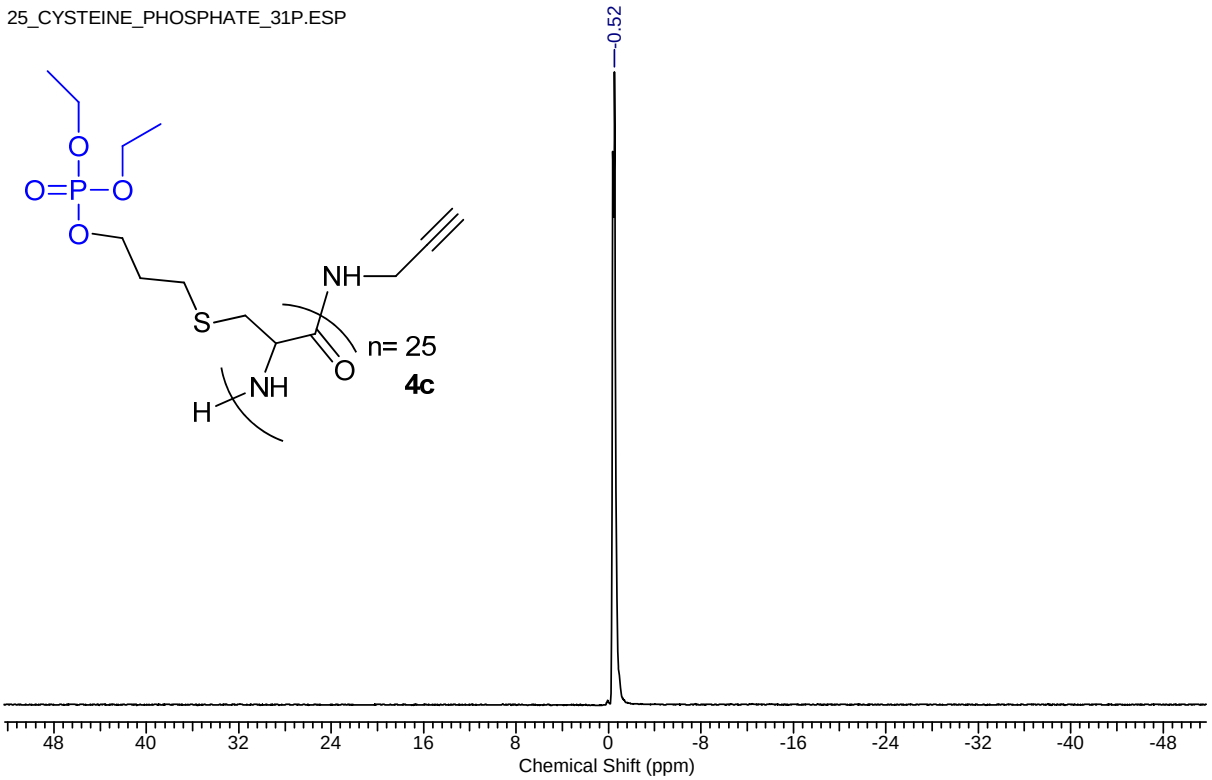


Figure 42: ^{1}H NMR of *40*-diethylphosphate-L-Cysteine ($CDCl_3$)

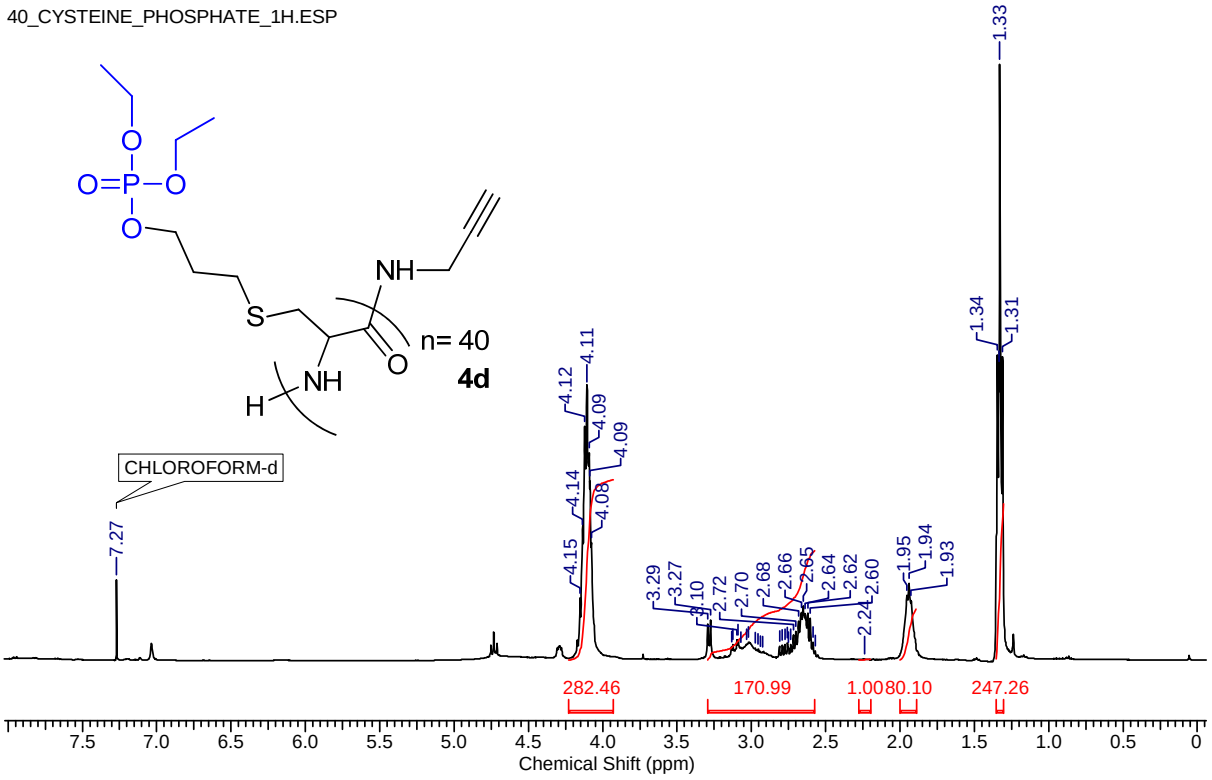


Figure 43: ^{31}P NMR of *40*-diethylphosphate-L-Cysteine ($CDCl_3$)

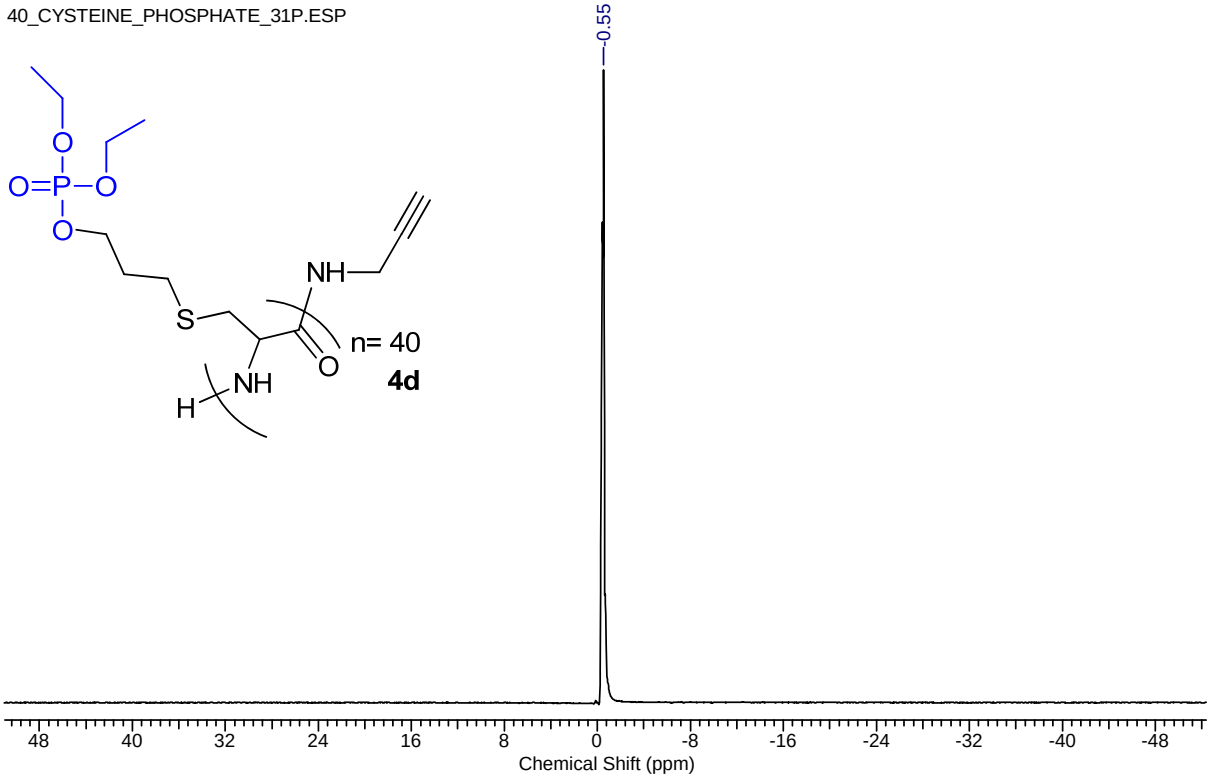


Figure 44: ^{1}H NMR of *25*-diethylphosphonate-L-Cysteine ($CDCl_3$)

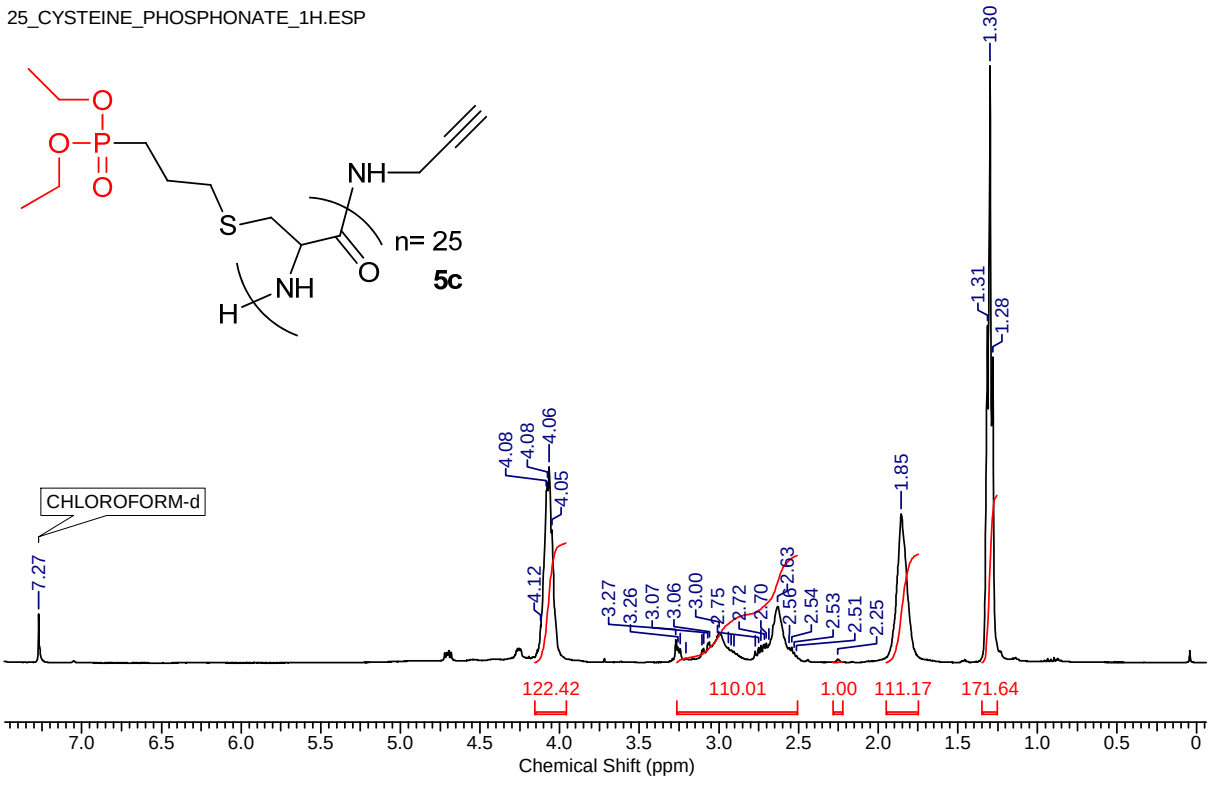


Figure 45: Calculation of polymer molecular weight (M_n) by using the characteristic proton peak of the initiator (alkyne C≡C-H) at 2.25 ppm in the *25*-diethylphosphonate-L-Cysteine.

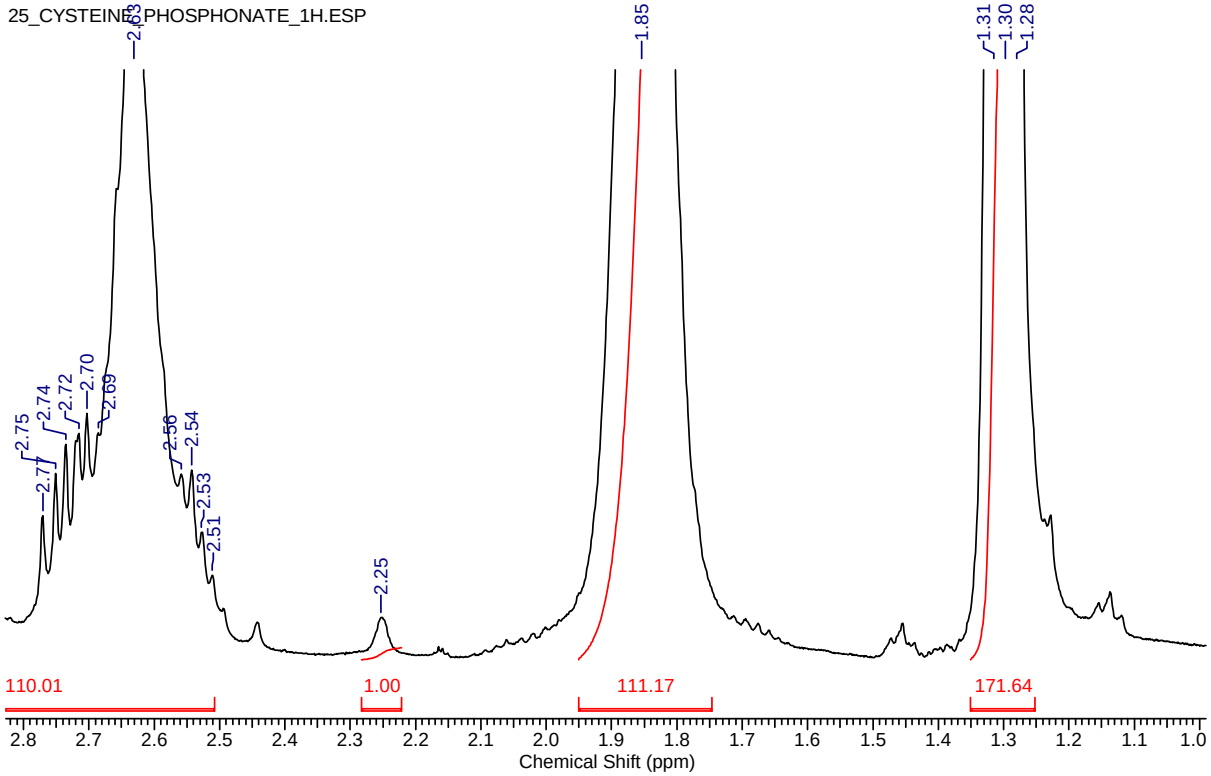


Figure 46: ^{31}P NMR of *25*-diethylphosphonate-L-Cysteine ($CDCl_3$)

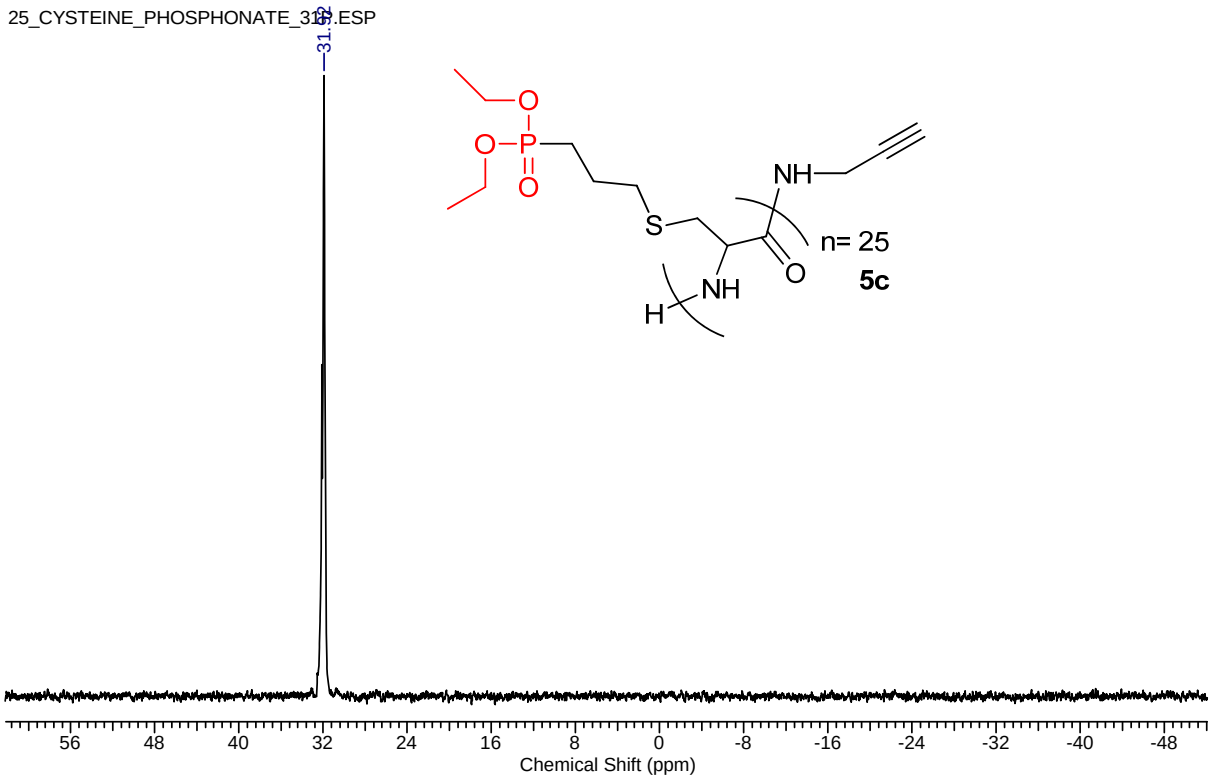


Figure 47: ^{1}H NMR of *40*-diethylphosphonate-L-Cysteine ($CDCl_3$)

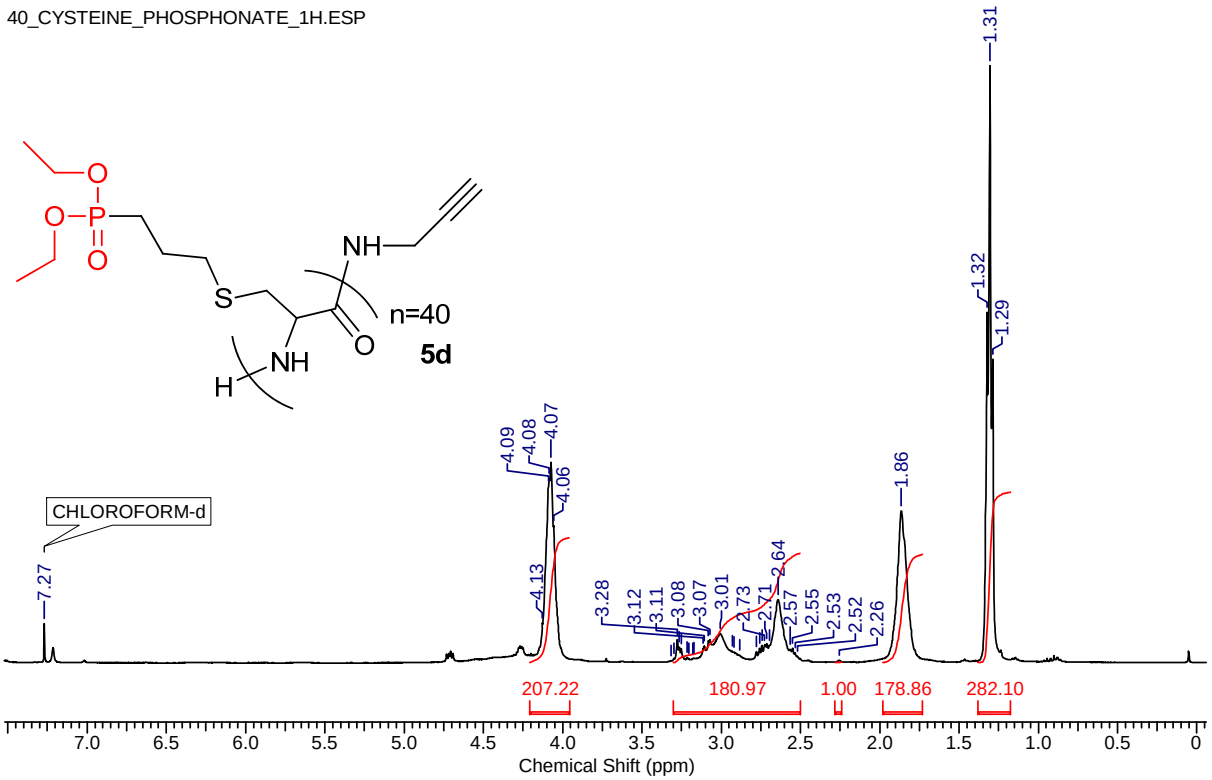


Figure 48: Calculation of polymer molecular weight (M_n) by using the characteristic proton peak of the initiator (alkyne C≡C-H) at 2.25 ppm in the *40*-diethylphosphonate-L-Cysteine.

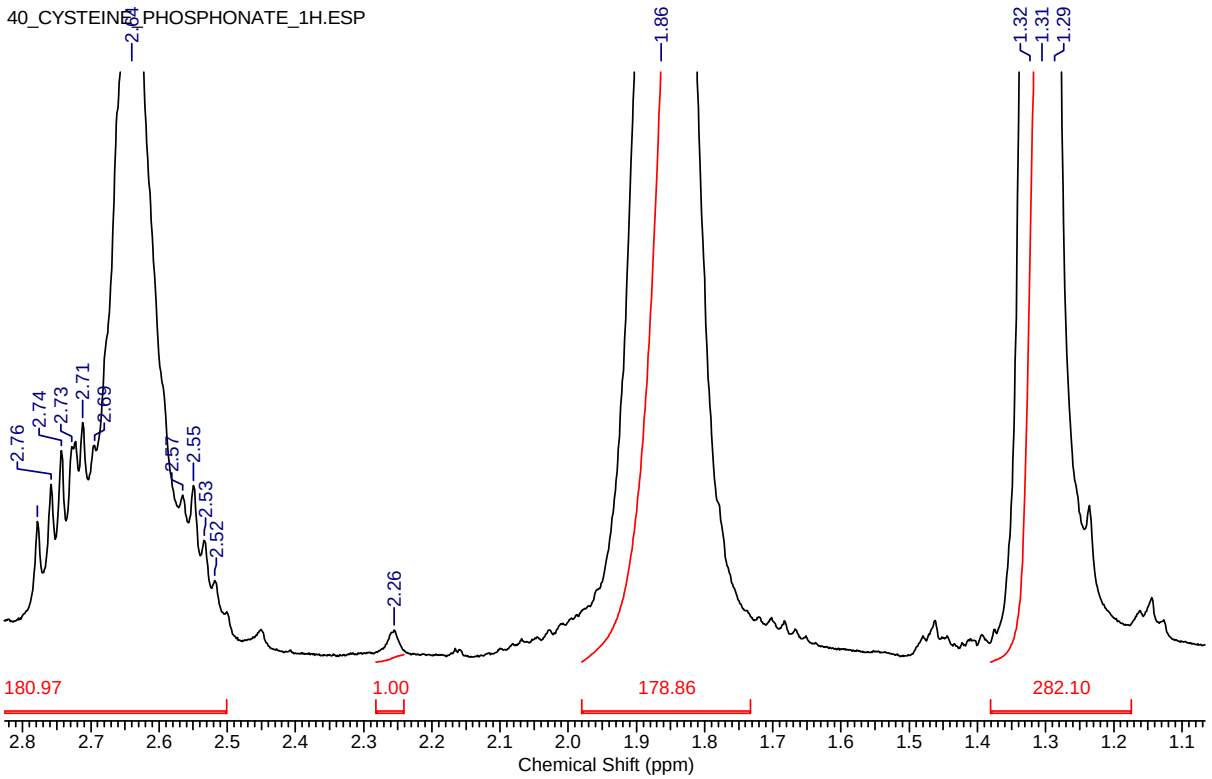


Figure 49: ^{31}P NMR of *40*-diethylphosphonate-L-Cysteine ($CDCl_3$)

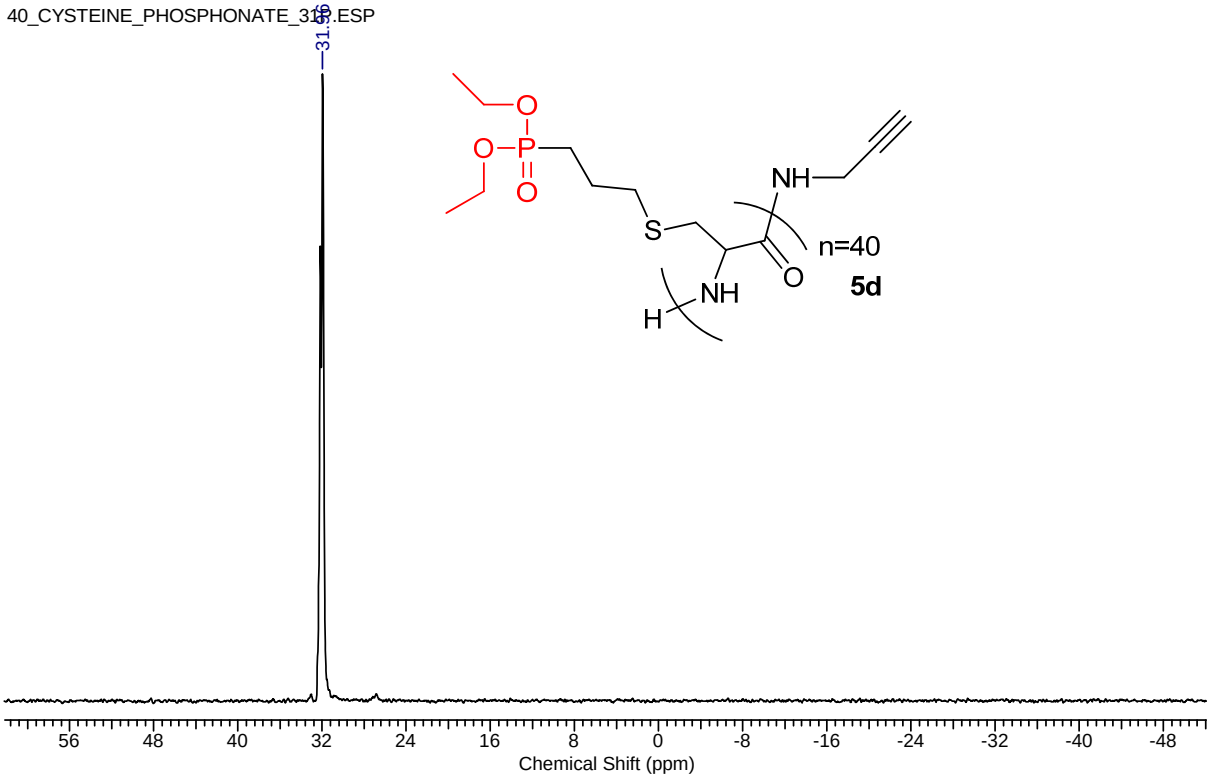


Figure 50: ^{1}H NMR of *25*-diethylphosphonate-L-Cysteine initiated by $N_3PEG_6NH_2$ ($CDCl_3$)

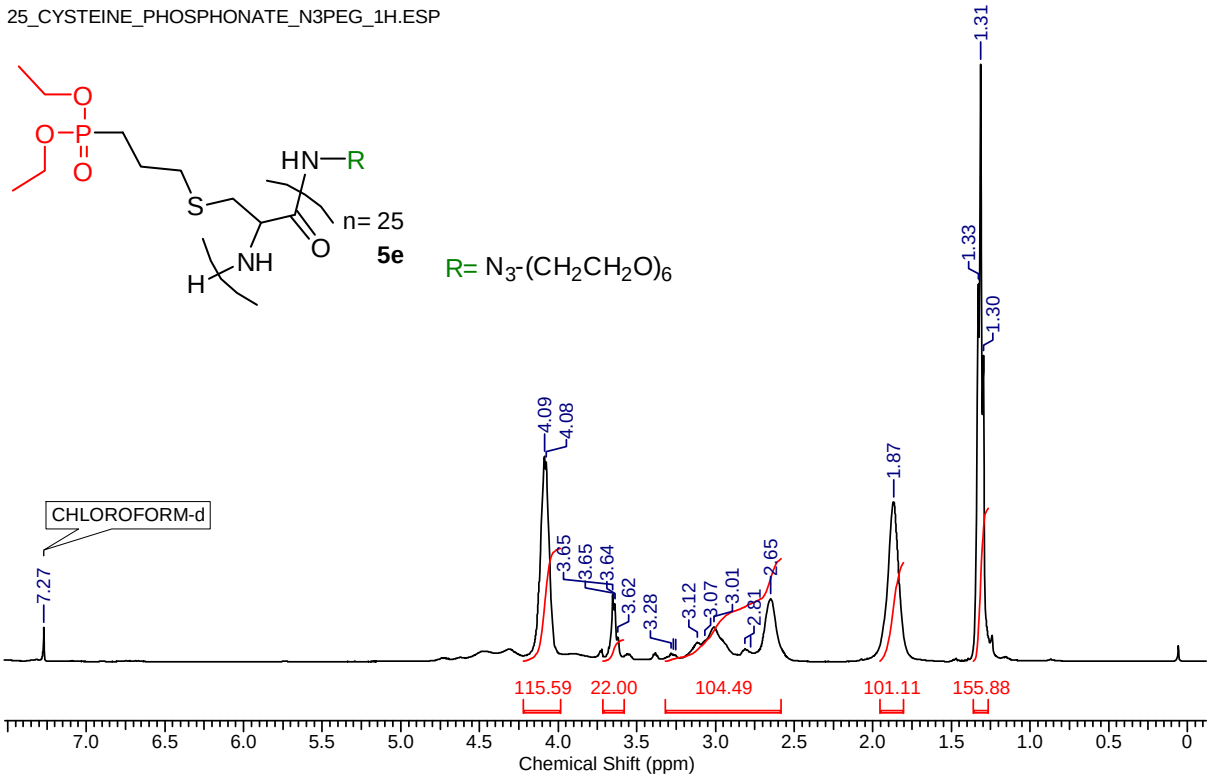


Figure 51: ^{1}H NMR of *25*-phosphonate-L-Cysteine (D_2O)

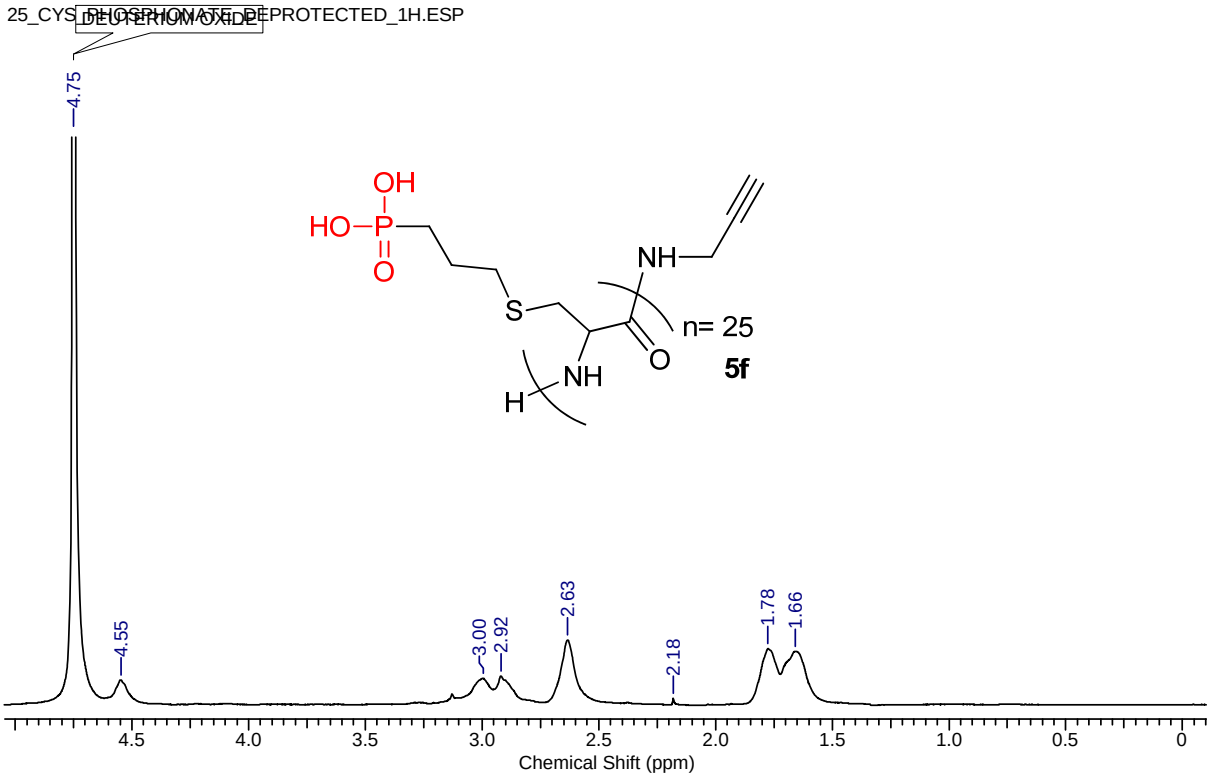


Figure 52: ^{31}P NMR of *25*-phosphonate-L-Cysteine (D_2O)

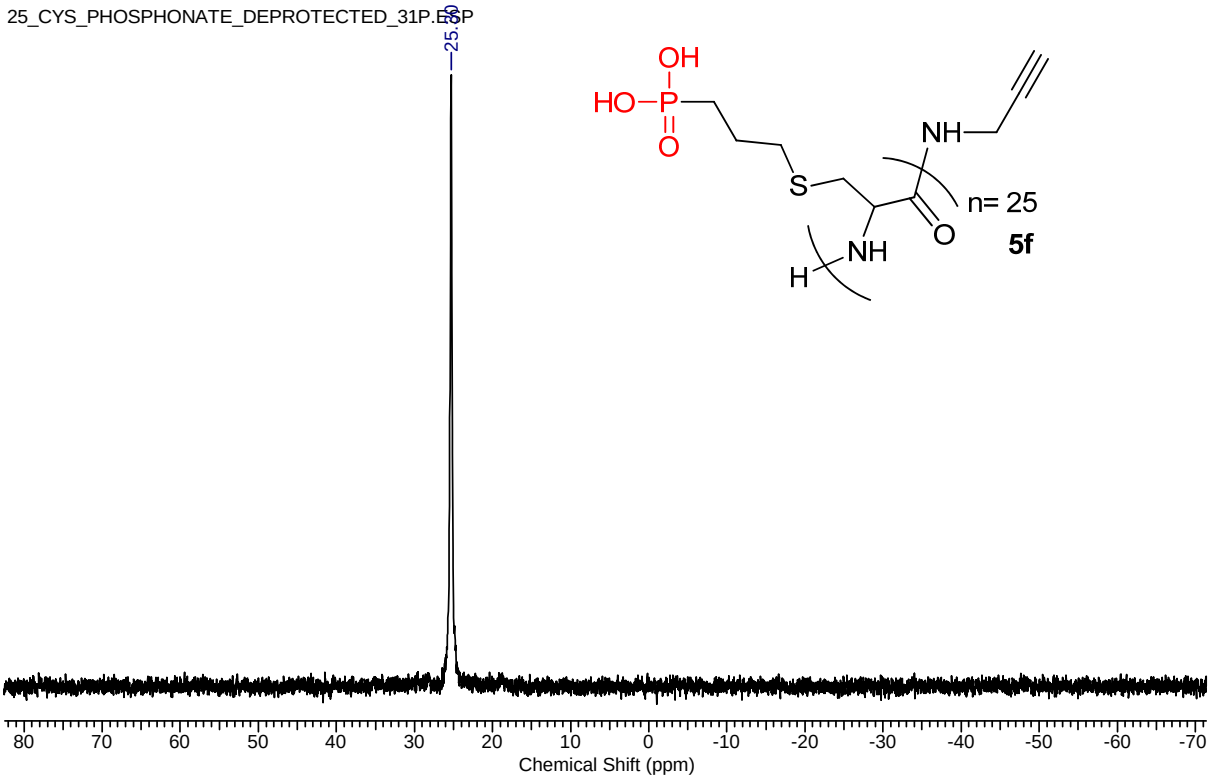


Figure 53: ^{31}P NMR of *40*-phosphonate-L-Cysteine (D_2O)