Electronic Supplementary Information (ESI)

Vitamin-Guanosine Monophosphate conjugates for *in vitro* transcription priming

Nikolaos Papastavrou[a], Omprakash Bande[a], Philippe Marlière[b*], Elisabetta Groaz[a*], and Piet Herdewijn[a*]

[a]Medicinal Chemistry, KU Leuven, Rega Institute for Medical Research, Herestraat 49−box 1041, 3000 Leuven, Belgium

[b]Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, 2 Rue Gaston Crémieux 91057 Evry, France

*piet.herdewijn@kuleuven.be

*elisabetta.groaz@kuleuven.be

* phmarliere@gmail.com

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General Methods and Materials

Materials and Instrumentation

All non-aqueous reactions were carried out in oven-dried glassware under an argon or nitrogen atmosphere using analytical grade solvents. Commercially available chemicals were of analytical or synthetic grade and used without further purification. Analytical grade solvents were used for HPLC purposes. Water was ultrapure (18 MΩcm) and prepared by a Millipore (MilliQ®) purification system. Reactions were monitored by TLC with UV-activated silica-coated aluminium plates acquired from Sigma-Aldrich (60778). Column chromatography was performed with silica gel 60 Å, 40-60 µM, Acros Organics (360050300). Preparative HPLC purification was carried out with a Phenomenex Gemini 110 Å column (C18, 10 µm, 21.2 mm × 250 mm) using a mobile phase of water/acetonitrile. High-resolution mass spectra (HRMS) were recorded in positive or negative ionization mode with a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). ^{1}H, ^{13}C, and ^{31}P spectra were recorded on a Bruker 300, 500, or 600 MHz spectrometer. Acrylamide-bisacrylamide stock solution 40% (29:1) was purchased by Carl-Roth (A121.1). Illustra NAP-25 columns were acquired from GE Healthcare (17085201). Oligonucleotides were purchased from IDT DNA Technologies (Leuven, Belgium), without prior purification. T7 RNA polymerase was obtained from Thermo Fisher Scientific (EP0112).

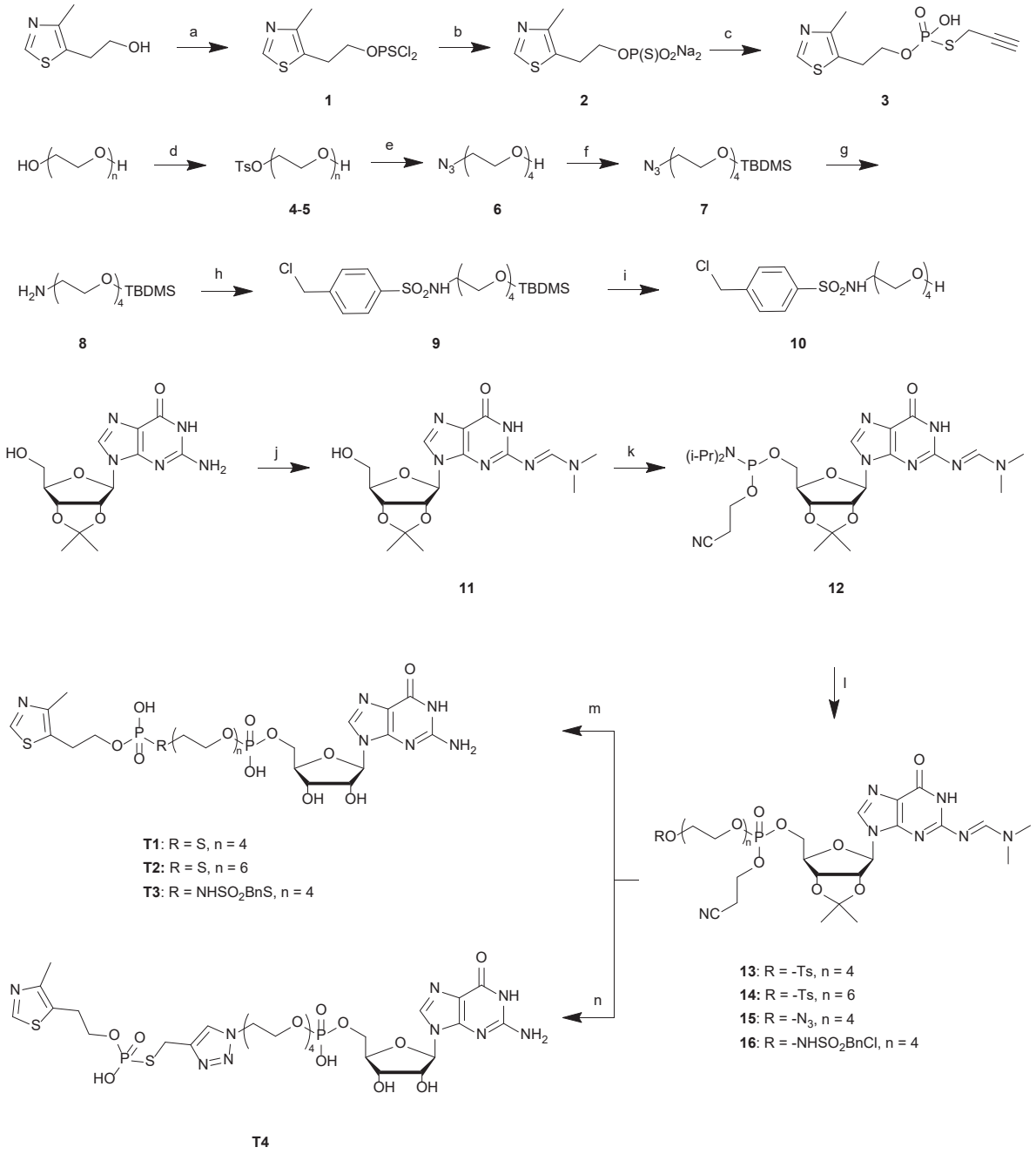
Methods

Oligonucleotide purification. Oligonucleotides were purified by 10% denaturing (7 M urea) polyacrylamide gel electrophoresis (PAGE) in a 1X Tris-Boric acid-EDTA buffer (TBE). The corresponding band was crushed and soaked in 0.3 M sodium acetate buffer (pH = 5.4) overnight at 37 °C, desalted on a NAP-25 column, ethanol precipitated, re-suspended in ultrapure water, and quantified with a ClarioStar microplate reader (LVis plate, BMG Labtech, Isogen Life Science).

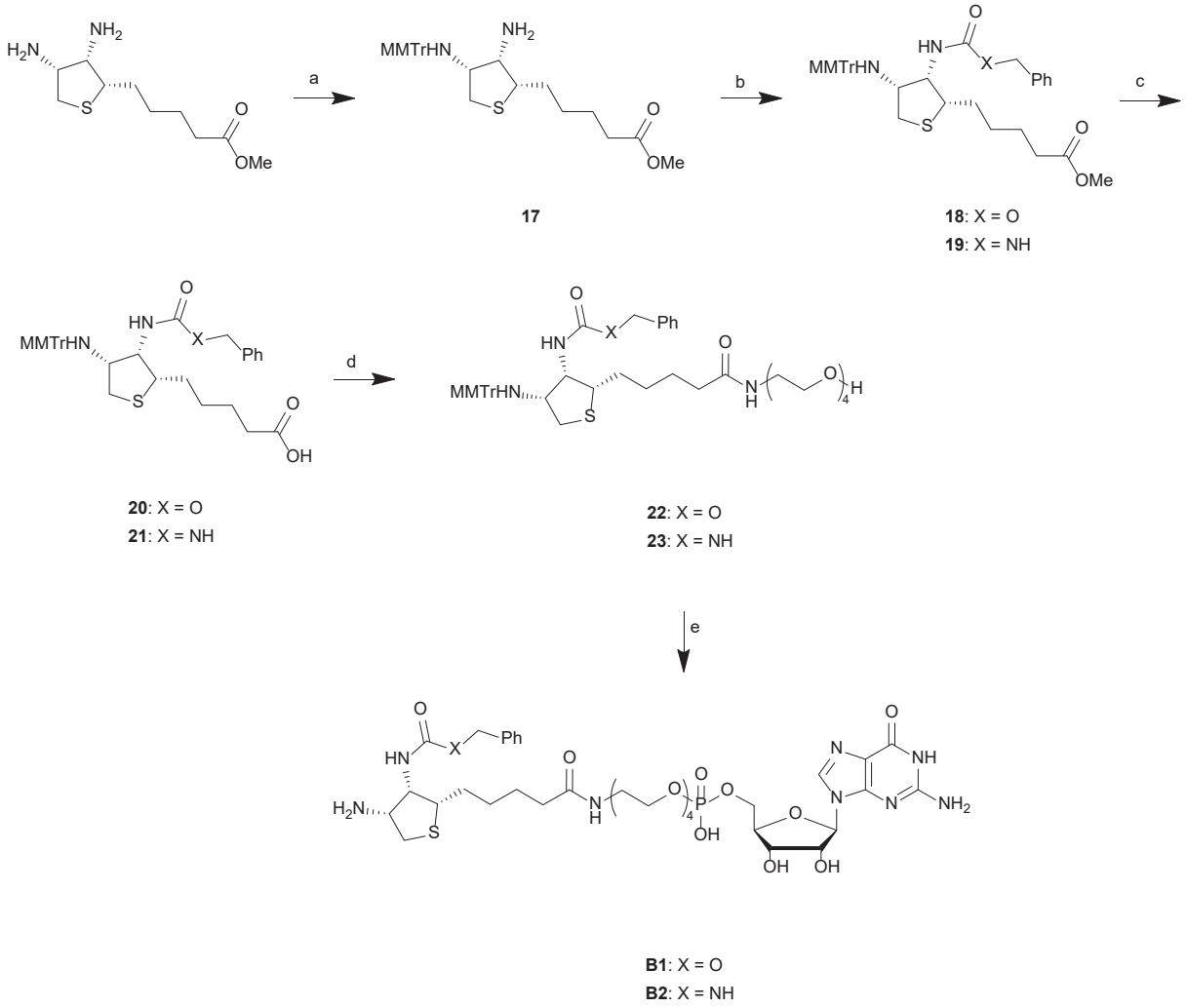
In vitro transcription. Equal amounts of the complementary strands (MT1 and MT1, Table S1) were mixed together in the presence of 10 mM Tris-HCl (pH = 7.0) and 80 mM NaCl to give a final concentration of 10 µM. The mixture was heated at 75 °C for 2 min, followed by slow cooling (0.1 °C/sec) to 16 °C to ensure complete hybridization. Transcription reactions were carried out at a final volume of 20 µL containing 1 µM double-stranded DNA template, 40 mM Tris-HCl (pH = 7.9), 6 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 10 mM NaCl, 2 mM spermidine, 0.6 U/µL of T7 RNA polymerase, 2 mM of the three NTPs (ATP, UTP, CTP), and varying concentrations of initiator nucleotide and GTP (as described in Figure 2). Transcription mixtures were incubated at 37 °C for 4 h.

In vitro transcription analysis. Transcription reactions were stopped by adding an equal volume of gel loading dye (90% v/v formamide, 10% TBE, 0.05% w/v xylene cyanol and bromophenol blue) and analysed by 12% denaturing PAGE. Glass plates (20 x 20 cm) were coated with 2% dichlorodimethylsilane in DCM prior to gel casting. Gels were run at a constant power of 30W, stained with 1X SYBR™ Safe (Thermo Fisher Scientific, S33102) in 1X TBE buffer, and scanned with a Typhoon FLA9500 imager (GE Healthcare). Gel band intensities were analysed using the ImageQuant™ TL 1D v8.1 software.

Synthetic Schemes (S1-S2)

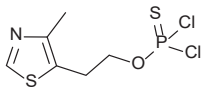


Scheme S1. a: $PSCl_3$, Et_3N, DCM, rt, 1 h, 59%; **b**: CH_3COONa, Et_3N, H_2O, 0-5 °C, 1 h, 62%; **c**: propargyl bromide, K_2CO_3, DMF, rt, o/n, 68%; **d**: TsCl, Et_3N, DCM, rt, 18 h, 82-89%; **e**: NaN_3, DMF 90 °C, 3 h, 92%; **f**: TBDPS-Cl, imidazole, DCM, rt, 20 h, 90%; **g**: H_2, Pd/C, MeOH, rt, 1 h, 98%; **h**: 4-(chloromethyl)benzene-1-sulfonyl chloride, Et_3N, DCM, rt, o/n, 75%; **i**: $Et_3N.3HF$, Et_3N, THF, rt, o/n, 99; **j**: DMFDMA, MeOH, rt, 3 h, 98%; **k**: 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, DIPEA, DCM, rt, 1 h, 86%; **l**: i) **4**-**6** or **10**, tetrazole, ACN, rt, 2 h. ii) tBuOOH/decane, rt, 1 h, 59-87% (over 2 steps); **m**: i) **2**, K_2CO_3, DMF, 50 °C, o/n. ii) HCOOH/ H_2O, rt, 3 d, 36-79% (over 2 steps); **n**: i) DBU, ACN, rt 1 h. ii) $[Cu(CH_3CN)_4]PF_6$, 45 °C, 2 d. iii) HCOOH/H_2O, rt, 3 d, 37% (over 3 steps).

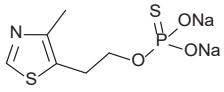


Scheme S2. a: MMTr-Cl, pyridine, rt, 12 h, 69%; **b**: benzyl chloroformate or benzyl isocyanate, DIPEA, rt, 30 min, 80-85%; **c**: 4N NaOH in MeOH/H_2O (4:1), THF, rt, 2 h, 74-77%; **d**: 2-(2-aminoethoxy)ethanol, DIPEA, HOBt, rt, 24 h, 84-85%; e: i) tetrazole, ACN, rt, 2 h. ii) t-BuOOH/decane, rt, 1 h. iii) DBU, DCM, rt, 2 h. iv) HCOOH/H_2O, rt, 3 d, 21-32% (over 4 steps).

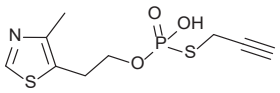
Chemical Synthesis



***O*-(2-(4-Methylthiazol-5-yl)ethyl) phosphorodichloridothioate (1).** 2-(4-Methylthiazol-5-yl)ethanol (1.0 eq, 3.00 g, 21 mmol) was dissolved in dry DCM (30 mL). The solution was cooled to 0 °C and Et_3N (2.5 eq, 7.3 mL, 52.4 mmol) was added. A solution of $PSCl_3$ (1.5 eq, 5.32 g, 31.4 mmol) in dry DCM (40 mL) was slowly added to the reaction mixture, which was then stirred overnight at room temperature. The mixture was concentrated under reduced pressure and the resulting residue was purified by silica gel column chromatography (Hexane/EtOAc, 6:4) to give the title compound (3.2 g, 59%) as a colourless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.63 (s, 1H), 3.66 (t, J = 7.1 Hz, 2H), 3.22 (t, J = 7.1 Hz, 2H), 2.42 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 150.0, 149.9, 127.2, 44.2, 29.6, 14.8; ^{31}P NMR (121 MHz, $CDCl_3$): δ 59.5.

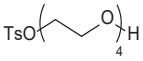


Sodium *O*-(2-(4-methylthiazol-5-yl)ethyl) phosphorothioate (2). Compound **1** (1.0 eq, 2.00 g, 7.3 mmol) was emulsified in a solution of CH_3COONa (4.4 eq, 2.64 g, 32.1 mmol) in H_2O (20 mL) and cooled to 0 °C. Then, Et_3N (4.4 eq, 4.48 mL, 32.1 mmol) was added dropwise and the mixture was left stirring below 5 °C for 1 h. The reaction mixture was allowed to warm to room temperature and neutralised with 1 N HCl. A solution of $BaCl_2$ (1.2 eq, 2.14 g, 8.8 mmol) in 10 mL of H_2O was added and a white precipitate was formed. The precipitate was collected by filtration, washed sequentially with H_2O (20 mL) and Et_2O (20 mL) and left to dry overnight over anhydrous $CaCl_2$. The dried powder was re-suspended in 10 mL of H_2O and 0.64 g of Na_2SO_4 was added. The precipitated $BaSO_4$ was filtered off and the remaining solution was freeze-dried to give the title compound (1.8 g, 62%) as a white solid. ^{1}H NMR (300 MHz, D_2O): δ 8.65 (br s, 1H), 3.88 (q, J = 7.0 Hz, 2H), 3.02 (t, J = 6.7 Hz, 2H), 2.29 (s, 3H); ^{13}C NMR (75 MHz, D_2O): δ 151.8, 148.6, 128.6, 64.2 (d, J = 4.9 Hz), 27.0 (d, J = 7.8 Hz), 13.5; ^{31}P NMR (121 MHz, D_2O): δ 42.6; HRMS (ESI-) calcd for $C_6H_9NO_3PS_2$ $[M\text{-}H]^-$ 237.9766, found 237.9764.

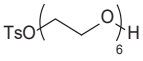


***O*-(2-(4-Methylthiazol-5-yl)ethyl) *S*-prop-2-yn-1-yl *O*-hydrogen phosphorothioate (3).** To a stirring solution of compound **2** (1.0 eq, 200 mg, 0.7 mmol) in 10 mL of DMF, K_2CO_3 (1.0 eq, 98 mg, 0.7 mmol) was added and the resulting suspension was stirred overnight at room temperature. The mixture was concentrated *in vacuo* and the residue was purified by HPLC (90-50% H_2O in ACN). Fractions containing the phosphorothiolate were collected and freeze dried to give the title compound (132 mg, 68%) as a white foam. ^{1}H NMR (600 MHz, D_2O) δ 8.75 (br s, 1H), 4.08 (q, J = 6.5 Hz, 2H), 3.14 (t, J = 6.0 Hz, 2H), 2.35

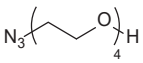
(s, 3H); ^{13}C NMR (151 MHz, D_2O) δ 153.7, 150.7 (d, J = 4.8 Hz), 129.8 (d, J = 12.4 Hz), 82.2 (d, J = 6.8 Hz), 73.2 (t, J = 3.8), 67.6 (d, J = 5.8 Hz), 28.2 (d, J = 8.5 Hz), 18.7 (d, J = 3.0 Hz), 15.3; ^{31}P NMR (121 MHz, D_2O): δ 19.1; HRMS (ESI-) calcd for $C_9H_{11}N_1O_3P_1S_2$ $[M-H]^-$ 275.9923, found 275.9925.



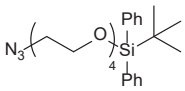
2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (4). Compound **4** was prepared according to a literature procedure[1]. Yield: 82%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 4.22–4.07 (m, 2H), 3.76–3.54 (m, 14H), 2.44 (s, 3H).



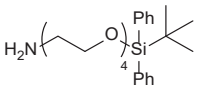
17-Hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (5). Compound **5** was prepared according to a literature procedure[1]. Yield: 89%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 4.19–4.14 (m, 2H), 3.80–3.46 (m, 22H), 2.45 (s, 3H).



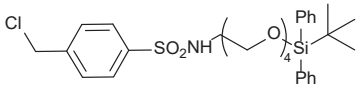
2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethanol (6). Compound **6** was prepared according to a literature procedure[1]. Yield: 92%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.76–3.55 (m, 14H), 3.43–3.32 (m, 2H).



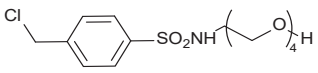
15-Azido-2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecane (7). A two-neck round-bottom flask was charged with compound **8** (1.0 eq, 4.00 g, 18 mmol) and imidazole (1.5 eq, 1.86 g, 27.2 mmol). Dry DCM (35 mL) was added and the solution was cooled to 0 °C. TBDPS-Cl (1.1 eq, 5.2 mL, 20 mmol) was slowly added and the mixture was allowed to warm to room temperature and stir for 20 h. The reaction mixture was washed with H_2O (50 mL x 1), brine (50 mL x 1), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (Hexane/EtOAc, 9:1 to 1:1) to give the title compound (7.5 g, 90%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (dd, J = 7.7, 1.7 Hz, 4H), 7.45–7.31 (m, 6H), 3.80 (t, J = 5.4 Hz, 2H), 3.69–3.57 (m, 12H), 3.36 (t, J = 5.2 Hz, 2H), 1.04 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 135.9, 134.0, 129.9, 127.9, 72.7, 71.1, 71.02, 71.00, 70.3, 63.7, 51.0, 27.1, 19.5. HRMS (ESI+) calcd for $C_{24}H_{39}N_4O_4Si_1$ $[M+NH_4]^+$ 475.2740, found 475.2739.



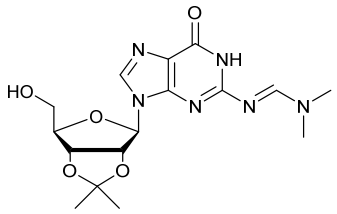
2,2-Dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-amine (8). Compound **9** (1.0 eq, 4.00 g, 8.4 mmol) was dissolved in 50 mL of dry MeOH and Pd/C (0.1 eq, 424 mg, 0.84 mmol) was added. The resulting suspension was placed under hydrogen gas and stirred at room temperature for 1 h. The reaction mixture was then filtered through a pad of Celite to remove the catalyst. Additional MeOH (50 mL x 2) was used to wash the Celite. After removal of all the volatiles under reduced pressure, the residue was purified by silica gel column chromatography (EtOAc/Et_3N, 95:5) to give the title compound (3.56 g, 98%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (dd, J = 7.7, 1.8 Hz, 4H), 7.44–7.34 (m, 6H), 3.81 (t, J = 5.3 Hz, 2H), 3.70–3.57 (m, 10H), 3.52 (t, J = 5.2 Hz, 2H), 2.86 (t, J = 5.2 Hz, 2H), 2.39 (br s, 2H), 1.05 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 135.9, 134.0, 129.9, 127.9, 73.0, 72.7, 71.04, 70.96, 70.9, 70.6, 63.8, 41.9, 27.1, 19.5; HRMS (ESI+) calcd for $C_{24}H_{38}N_1O_4Si_1$ $[M+H]^+$ 432.2564, found 432.2560.



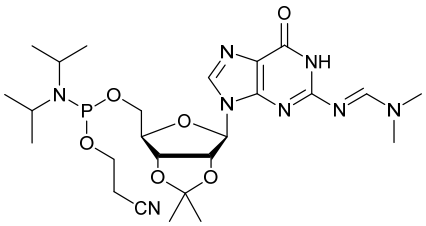
4-(Chloromethyl)-N-(2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)benzenesulfonamide (9). To a stirring solution of 4-(chloromethyl)benzenesulfonyl chloride (1.0 eq, 1.04 g, 4.6 mmol) and Et_3N (1.0 eq, 0.64 mL, 4.6 mmol) in 20 mL of dry DCM at 0 °C, a solution of compound **10** (1.0 eq, 2.00 g, 4.6 mmol) in 5 mL of dry DCM was slowly added and the reaction was allowed to stir at room temperature overnight. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (Hexane/EtOAc, 3:1 to 1:1) to give the title compound (2.3 g, 75%) as an off-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, J = 8.3 Hz, 2H), 7.68 (dd, J = 7.5, 1.5 Hz, 4H), 7.50 (d, J = 8.2 Hz, 2H), 7.46–7.32 (m, 6H), 5.37 (t, J = 5.8 Hz, 1H), 4.59 (s, 2H), 3.81 (t, J = 5.3 Hz, 2H), 3.70–3.46 (m, 7H), 3.12 (q, J = 5.4 Hz, 2H), 1.04 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 142.3, 140.6, 135.9, 134.0, 129.9, 129.3, 127.9, 127.8, 72.7, 71.03, 70.96, 70.8, 70.6, 69.5, 63.8, 45.2, 43.3, 27.1, 19.5; HRMS (ESI+) calcd for $C_{31}H_{42}Cl_1N_1O_6S_1Si_1Na$ $[M+Na]^+$ 642.2082, found 642.2085.



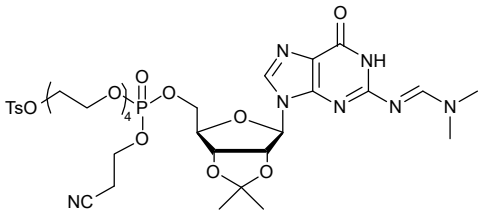
4-(Chloromethyl)-N-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)benzenesulfonamide (10). Compound **9** (1.0 eq, 1.00 g, 2.4 mmol) was dissolved in 15 mL of dry THF and placed under an inert atmosphere. Next, $Et_3N{\cdot}3HF$ (10.0 eq, 3.90 mL, 24 mmol) and Et_3N (2.0 eq, 0.65 mL, 4.7 mmol) were added to the mixture, which was allowed to stir at room temperature overnight. After removal of all the volatiles under reduced pressure, the resulting residue was purified by silica gel column chromatography (Hexane/EtOAc, 1:1 to 0:1) to give the title compound (0.99 g, 99%) as an off-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.5 Hz, 2H), 7.01 (t, J = 5.7 Hz, 1H), 4.62 (s, 1H), 3.79–3.59 (m, 10H), 3.54–3.47 (m, 4H), 3.13 (dd, J = 9.7, 5.8 Hz, 2H); ^{13}C NMR (75 MHz, CDCl3) δ 142.0, 141.0, 129.3, 127.8, 72.9, 71.0, 70.5, 70.2, 69.9, 61.8, 45.3, 43.3; HRMS (ESI+) calcd for $C_{15}H_{25}Cl_1N_1O_6S_1$ $[M+H]^+$ 382.1085, found 382.1089.



2-*N,N*-Dimethylaminomethylene-2',3'-*O,O*-isopropylidene Guanosine (11). Compound **11** was prepared according to a literature procedure[2]. Yield: 98%. ^{1}H NMR (300 MHz, MeOD): δ 8.68 (br s, 1H), 8.09 (s, 1H), 6.13 (d, J = 3.0 Hz, 1H), 5.32 (dd, J = 6.0, 3.1 Hz, 1H), 5.06 (dd, J = 6.1, 2.6 Hz, 1H), 4.35–4.29 (m, 1H), 3.81–3.68 (m, 2H), 3.24 (s, 3H), 3.15 (s, 3H) 1.63 (s, 3H), 1.42 (s, 3H).

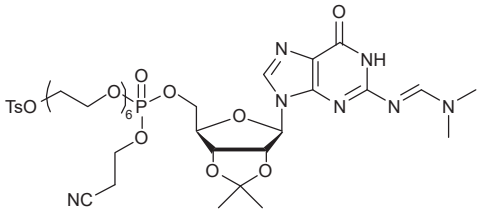


2-Cyanoethyl-*N,N*-diisopropylamino-5'-(2-*N*-dimethylaminomethylene-2',3'-*O,O*-isopropylideneguanosine) Phosphoramidite (12). Compound **12** was prepared according to a literature procedure[2]. Yield: 86%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.93 (br s, 1H), 8.63 (s, 1H), 7.90 (d, J = 11.4 Hz, 1H), 6.17–6.10 (m, 1H), 5.14 (dd, J = 6.1, 2.3 Hz, 1H), 5.02–4.94 (m, 1H), 4.45–4.42 (m, 1H), 3.95–3.69 (m, 4H), 3.64–3.50 (m, 2H), 3.19 (s, 3H), 3.10 (s, 3H), 2.78–2.61 (m, 2H), 1.63 (s, 3H), 1.40 (s, 3H), 1.20–1.13 (m, 12H).



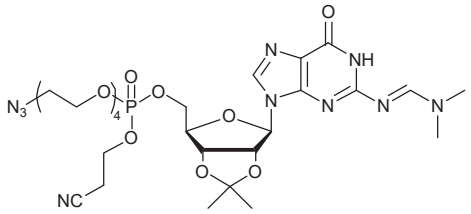
2-(2-(2-(2-(((2-Cyanoethoxy)(((3aR,4R,6R,6aR)-6-(2-((E)-((dimethylamino)methylene)amino)-6-oxo-1H-purin-9(6H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methoxy)phosphoryl)oxy)ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (13). To a stirring solution of phosphoramidite **5** (1.0 eq, 200 mg, 0.34 mmol) in dry ACN (10 mL) under an inert atmosphere, a solution of alcohol **6** (2.0 eq, 236 mg, 0.68 mmol) in 5 mL of dry ACN was added. The mixture was cooled to 0 °C in an ice-bath and a 0.45 M solution of tetrazole in acetonitrile (3.0 eq, 2.26 mL, 1.02 mmol) was slowly added. The ice-bath was removed and the reaction was allowed to warm to room temperature and stirred for an additional 2 h. The reaction progress was monitored by ^{31}P NMR. Upon completion, a 5.5 M

solution of *t*BuOOH in decane (1.1 eq, 0.075 mL, 0.37 mmol) was added to the reaction mixture. After 1 h, full oxidation of the phosphorus atom was confirmed by ^{31}P NMR and the mixture was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (DCM/MeOH, 1:0 to 9:1) to give the title compound (colourless oil, 244 mg, 87%) as an inseparable mixture of two isomers in a 93:7 ratio. ^{1}H NMR (600 MHz, MeOD-*d4*) (major): δ 8.64 (br s, 1H), 7.98 (d, *J* = 2.1 Hz, 2H), 7.77 (d, *J* = 7.8 Hz, 2H), 7.41 (d, *J* = 7.9 Hz, 2H), 6.15 (d, *J* = 2.5 Hz, 1H), 5.43 (dt, *J* = 6.0, 2.8 Hz, 1H), 5.11 (dd, *J* = 5.8, 3.4 Hz, 1H), 4.44 (dd, *J* = 8.4, 4.2 Hz, 1H), 4.35–4.26 (m, 2H), 4.24–4.09 (m, 6H), 3.68–3.48 (m, 14H), 3.21 (d, *J* = 1.0 Hz, 3H), 3.12 (d, *J* = 0.5 Hz, 3H), 2.84 (ddd, *J* = 12.3, 8.7, 5.3 Hz, 2H), 2.43 (s, 3H), 1.59 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (151 MHz, MeOD-*d4*) (major): δ 160.1, 159.8, 159.2, 151.6, 146.5, 139.3, 134.4, 131.1, 129.0, 118.6, 115.7, 91.3 (d, *J* = 4.2 Hz), 86.0 (d, *J* = 5.7 Hz), 85.3 (d, *J* = 4.1 Hz), 82.4 (d, *J* = 10.3 Hz), 71.54, 71.47, 71.4, 71.0, 70.8 (d, *J* = 6.1 Hz), 69.7, 68.5 (d, *J* = 4.5 Hz), 64.1 (t, *J* = 5.1 Hz), 41.8, 35.4, 27.5, 25.6, 21.6, 20.0 (d, *J* = 7.4 Hz). ^{1}H NMR (600 MHz, MeOD-*d4*) (minor): δ 8.64 (s, 1H), 7.98 (d, *J* = 2.1 Hz, 2H), 7.70 (d, *J* = 7.8 Hz, 2H), 7.22 (d, *J* = 7.9 Hz, 2H), 6.15 (d, *J* = 2.5 Hz, 1H), 5.43 (dt, *J* = 6.0, 2.8 Hz, 1H), 5.11 (dd, *J* = 5.8, 3.4 Hz, 1H), 4.44 (dd, *J* = 8.4, 4.2 Hz, 1H), 4.35–4.26 (m, 2H), 4.24–4.09 (m, 6H), 3.68–3.48 (m, 14H), 3.21 (d, *J* = 1.0 Hz, 3H), 3.12 (d, *J* = 0.5 Hz, 3H), 2.84 (ddd, *J* = 12.3, 8.7, 5.3 Hz, 2H), 2.36 (s, 3H), 1.59 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (151 MHz, MeOD-*d4*) (minor): δ 160.1, 159.8, 159.2, 151.6, 146.5, 139.3, 134.4, 129.8, 127.0, 118.6, 115.7, 91.3 (d, *J* = 4.2 Hz), 86.0 (d, *J* = 5.7 Hz), 85.3 (d, *J* = 4.1 Hz), 82.4 (d, *J* = 10.3 Hz), 71.54, 71.47, 71.4, 71.0, 70.8 (d, *J* = 6.1 Hz), 69.7, 68.5 (d, *J* = 4.5 Hz), 64.1 (t, *J* = 5.1 Hz), 41.8, 35.4, 27.5, 25.6, 21.3, 20.0 (d, *J* = 7.4 Hz). ^{31}P NMR (121 MHz, MeOD-*d4*) (major and minor): δ -2.08, -2.10. HRMS (ESI+) calcd for $C_{34}H_{49}N_7O_{14}P_1S_1$ $[M+H]^+$ 842.2790, found 842.2811.

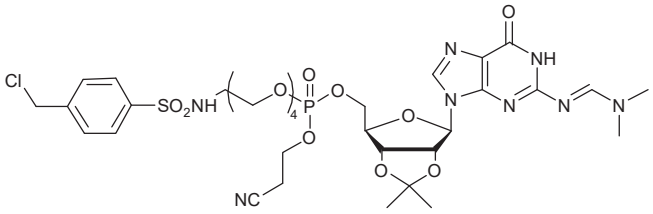


17-(((2-Cyanoethoxy)(((3aR,4R,6R,6aR)-6-(2-((E)-((dimethylamino)methylene)amino)-6-oxo-1H-purin-9(6H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methoxy)phosphoryl)oxy)-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (14). Compound **14** was obtained as an inseparable mixture of two isomers in a 9:1 ratio (colourless oil, 243 mg, 77%) starting from alcohol **7** (2.0 eq, 296 mg, 0.68 mmol) following the same procedure described for compound **13**. ^{1}H NMR (600 MHz, MeOD-*d4*) (major): δ 8.65 (br s, 1H), 7.98 (d, *J* = 2.7 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 7.9 Hz, 2H), 6.15 (d, *J* = 2.2 Hz, 1H), 5.44 (dt, *J* = 5.9, 2.8 Hz, 1H), 5.11 (ddd, *J* = 6.2, 3.4, 1.6 Hz, 1H), 4.44 (dd, *J* = 8.4, 4.1 Hz, 1H), 4.35–4.26 (m, 2H), 4.25–4.11 (m, 6H), 3.66–3.51 (m, 22H), 3.22 (s, 3H), 3.13 (s, 3H), 2.85 (ddd, *J* = 12.5, 8.9, 5.4 Hz, 2H), 2.44 (s, 3H), 1.60 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (151 MHz, MeOD-*d4*) (major): δ 160.1, 159.8, 159.2, 151.6, 146.5, 139.4 (d, *J* = 6.1 Hz), 134.4, 131.1, 129.1, 121.0, 118.6, 115.7, 91.3 (d, *J* = 4.3 Hz), 86.1 (d, *J* = 3.9 Hz), 85.3 (d, *J* = 5.0 Hz), 82.4 (d, *J* = 11.6 Hz), 71.6, 71.50, 71.46, 71.0, 70.8 (d, *J* = 6.1 Hz), 69.7, 69.0, 68.5, 64.2 (t, *J* = 5.1 Hz), 41.7, 35.4, 27.5, 25.6, 21.6, 20.0 (d, *J* = 7.4 Hz). ^{1}H NMR (600 MHz, MeOD-*d4*) (minor): δ 8.65 (s, 1H), 7.98 (d, *J* = 2.7 Hz, 1H), 7.70 (d, *J* = 8.2 Hz, 2H), 7.22 (d, *J* = 7.9 Hz, 2H), 6.15 (d, *J* = 2.2 Hz, 1H), 5.44 (dt, *J* = 5.9, 2.8 Hz, 1H), 5.11 (ddd, *J* = 6.2, 3.4, 1.6 Hz, 1H), 4.44 (dd, *J* = 8.4, 4.1 Hz, 1H), 4.35–4.26 (m, 2H), 4.25–4.11 (m, 6H), 3.66–3.51 (m, 22H), 3.22 (s, 3H), 3.13 (s, 3H), 2.85 (ddd, *J* = 12.5, 8.9, 5.4 Hz, 2H), 2.36 (s, 3H), 1.60 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (151 MHz, MeOD-*d4*) (minor): δ

160.1, 159.8, 159.2, 151.6, 146.5, 139.4 (d, *J* = 6.1 Hz), 134.4, 129.8, 127.0, 121.0, 118.6, 115.7, 91.3 (d, *J* = 4.3 Hz), 86.1 (d, *J* = 3.9 Hz), 85.3 (d, *J* = 5.0 Hz), 82.4 (d, *J* = 11.6 Hz), 71.6, 71.50, 71.46, 71.0, 70.8 (d, *J* = 6.1 Hz), 69.7, 69.0, 68.5, 64.2 (t, *J* = 5.1 Hz), 41.7, 35.4, 27.5, 25.6, 21.3, 20.0 (d, *J* = 7.4 Hz). ^{31}P NMR (121 MHz, MeOD-*d4*) (major and minor): *δ* -2.08, -2.11; HRMS (ESI+) calcd for $C_{38}H_{57}N_7O_{16}P_1S_1$ $[M+H]^+$ 930.3314, found 930.3322.

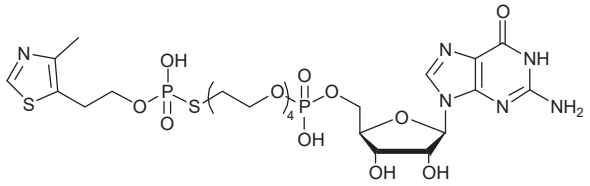


2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl (2-cyanoethyl) (((3aR,4R,6R,6aR)-6-(2-((E)-((dimethylamino)methylene)amino)-6-oxo-1H-purin-9(6H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl) phosphate (15). Compound **15** was obtained as a colourless oil (148 mg, 61%) from alcohol **8** (2.0 eq, 150 mg, 0.68 mmol) following the same procedure described for compound **13**. ^{1}H NMR (600 MHz, MeOD-*d4*): *δ* 8.65 (br s, 1H), 7.98 (d, *J* = 2.3 Hz, 1H), 6.16 (d, *J* = 2.4 Hz, 1H), 5.44 (dt, *J* = 5.9, 2.8 Hz, 1H), 5.11 (ddd, *J* = 6.2, 3.3, 1.7 Hz, 1H), 4.44 (dd, *J* = 8.5, 4.0 Hz, 1H), 4.35–4.26 (m, 2H), 4.24–4.11 (m, 4H), 3.66–3.58 (m, 12H), 3.37–3.34 (m, 2H), 3.22 (s, 3H), 3.13 (s, 3H), 2.85 (ddd, *J* = 13.4, 9.2, 5.4 Hz, 2H), 1.60 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (151 MHz, MeOD-*d4*): *δ* 160.1, 159.8, 159.2, 151.6, 139.3, 121.0, 118.5, 115.7, 91.4 (d, *J* = 5.7 Hz), 86.1, 85.3 (d, *J* = 4.8 Hz), 82.4 (d, *J* = 12.1 Hz), 71.6, 71.5, 71.0, 70.8 (d, *J* = 6.2 Hz), 69.0 (d, *J* = 2.6 Hz), 68.5 (d, *J* = 3.8 Hz), 64.1 (t, *J* = 5.5 Hz), 51.8, 41.7, 35.4, 27.4, 25.6, 20.0 (d, *J* = 7.4 Hz); ^{31}P NMR (121 MHz, MeOD-*d4*): *δ* -2.08, -2.09. HRMS (ESI+) calcd for $C_{27}H_{41}N_{10}O_{11}P_1Na$ $[M+Na]^+$ 735.2586, found 735.2576.

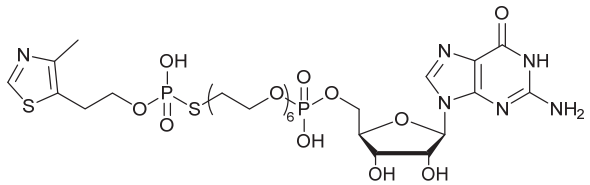


2-(2-(2-(2-(4-(Chloromethyl)phenylsulfonamido)ethoxy)ethoxy)ethoxy)ethyl (2-cyanoethyl) (((3aR,4R,6R,6aR)-6-(2-((E)-((dimethylamino)methylene)amino)-6-oxo-1H-purin-9(6H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl) phosphate (16). Compound **16** was obtained as a colourless oil (176 mg, 59%) starting from alcohol **12** (2.0 eq, 260 mg, 0.68 mmol) following the same procedure described for compound **13**. ^{1}H NMR (600 MHz, MeOD): *δ* 8.65 (br s, 1H), 7.98 (d, *J* = 1.9 Hz, 1H), 7.84 (d, *J* = 7.7 Hz, 2H), 7.59 (d, *J* = 8.1 Hz, 2H), 6.16 (d, *J* = 2.5 Hz, 1H), 5.44 (ddd, *J* = 6.5, 4.2, 2.5 Hz, 1H), 5.11 (ddd, *J* = 6.2, 3.3, 1.4 Hz, 1H), 4.44 (dd, *J* = 8.6, 4.4 Hz, 1H), 4.34–4.27 (m, 2H), 4.24–4.12 (m, 4H), 3.66–3.49 (m, 8H), 3.48–3.40 (m, 4H), 3.22 (s, 3H), 3.12 (s, 3H), 3.04 (td, *J* = 5.5, 1.9 Hz, 2H), 2.86–2.81 (m, 2H), 1.60 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (151 MHz, MeOD): *δ* 160.1, 159.8, 159.2, 151.6, 144.0, 141.9, 139.3, 130.7, 128.4, 121.0, 118.6, 115.7, 91.3 (d, *J* = 7.3 Hz), 86.1 (s), 85.3 (d, *J* = 7.1 Hz), 82.4 (d, *J* = 11.6 Hz),

71.5, 71.5, 71.2, 70.8 (d, *J* = 6.2 Hz), 70.6, 68.9, 68.5, 64.1 (t, *J* = 5.2 Hz), 45.2, 44.0, 41.7, 35.4, 27.46, 25.6, 20.0 (d, *J* = 7.4 Hz); ^{31}P NMR (121 MHz, MeOD): *δ* -2.06, -2.11. HRMS (ESI+) calcd for $C_{34}H_{49}Cl_1N_8O_{13}P_1S_1$ $[M+H]^+$ 875.2560, found 875.2557.

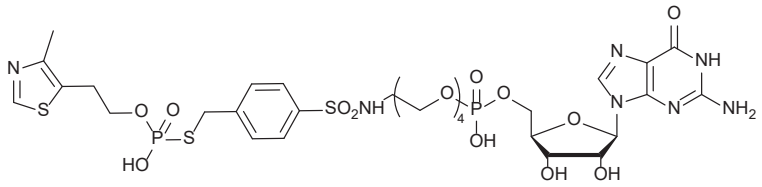


((2R,3S,4R,5R)-5-(2-Amino-6-oxo-1H-purin-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (2-(2-(2-(2-((hydroxy(2-(4-methylthiazol-5-yl)ethoxy)phosphoryl)thio)ethoxy)ethoxy)ethoxy)ethyl) hydrogen phosphate (T1). Compound **2** (1.0 eq, 50 mg, 0.18 mmol) and K_2CO_3 (1.0 eq, 25 mg, 0.18 mmol) were suspended in 10 mL of DMF. Compound **13** (1.0 eq, 151 mg, 0.18 mmol) was added and the mixture was heated at 50 °C overnight. The solvent was removed under reduced pressure and the residue was dissolved in 60% HCOOH in H_2O. The mixture was allowed to stir at room temperature for 3 days. The acid was removed under vacuum and the aqueous solution was lyophilised. The resulting residue was purified by HPLC (90-50% H_2O in ACN). The fractions containing the thiophosphate were collected and freeze dried to give the title compound (108 mg, 79%) as a white solid. ^{1}H NMR (600 MHz, D_2O): *δ* 8.84 (br s, 1H), 8.08 (br s, 1H), 5.91 (d, *J* = 5.7 Hz, 1H), 4.52–4.49 (m, 1H), 4.34–4.31 (m, 1H), 4.13–4.09 (m, 2H), 4.05 (q, *J* = 6.1 Hz, 2H), 3.93 (td, *J* = 6.1, 3.6 Hz, 2H), 3.64–3.59 (m, 8H), 3.56–3.53 (m, 2H), 3.51 (t, *J* = 6.5 Hz, 2H), 3.13 (t, *J* = 5.8 Hz, 2H), 2.66 (dt, *J* = 13.0, 6.4 Hz, 2H), 2.36 (s, 3H); ^{13}C NMR (151 MHz, D_2O): *δ* 160.1, 155.2, 153.6, 153.1, 149.6, 138.8, 130.3, 117.5, 88.3, 85.0 (d, *J* = 8.8 Hz), 74.9, 71.8, 71.34, 71.3, 70.9, 70.8 (d, *J* = 5.1 Hz), 70.6, 67.0 (d, *J* = 5.7 Hz), 66.2 (d, *J* = 4.8 Hz), 66.1 (d, *J* = 5.1 Hz), 30.2, 27.9 (d, *J* = 8.6 Hz), 14.7; ^{31}P NMR (202 MHz, D_2O): *δ* 20.8, 0.30; HRMS (ESI-) calcd for $C_{24}H_{37}N_6O_{14}P_2S_2$ $[M-H]^-$ 759.1289, found 759.1301.

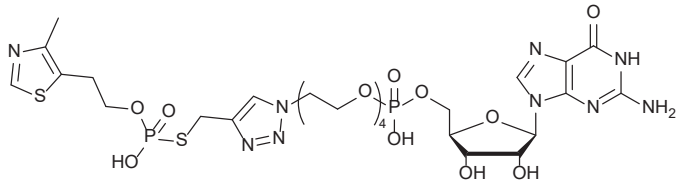


((2R,3S,4R,5R)-5-(2-Amino-6-oxo-1H-purin-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (17-((hydroxy(2-(4-methylthiazol-5-yl)ethoxy)phosphoryl)thio)-3,6,9,12,15-pentaoxaheptadecyl) hydrogen phosphate (T2). Compound **T2** was obtained as a white solid (94 mg, 62%) from tosylate **14** (1.0 eq, 167 mg, 0.18 mmol) following the same procedure described for compound **T1**. ^{1}H NMR (500 MHz, D_2O): *δ* 8.75 (br s, 1H), 8.08 (br s, 1H), 5.92 (d, *J* = 5.8 Hz, 1H), 4.51 (dd, *J* = 5.1, 3.8 Hz, 1H), 4.33 (dt, *J* = 6.5, 3.2 Hz, 1H), 4.11 (t, *J* = 3.6 Hz, 2H), 4.06 (dd, *J* = 12.5, 6.1 Hz, 2H), 3.92 (dt, *J* = 5.7, 3.6 Hz, 2H), 3.67–3.58 (m, 18H), 3.54 (t, *J* = 6.4 Hz, 2H), 3.13 (t, *J* = 5.9 Hz, 2H), 2.67 (dt, *J* = 13.0, 6.4 Hz, 2H), 2.36 (s, 3H); ^{13}C NMR (151 MHz, D_2O): *δ* 159.9, 158.5, 153.6, 151.6, 151.4, 148.7, 137.0, 127.9, 115.8, 86.5, 83.2 (d, *J* = 8.8 Hz), 73.1, 70.0, 69.6, 69.14 (d, *J* = 1.4 Hz), 69.06, 68.8, 65.4 (d, *J* = 5.6 Hz), 64.5 (d, *J* = 4.5 Hz), 64.2 (d, *J* = 5.2 Hz),

28.5, 26.2 (d, *J* = 8.6 Hz), 13.3; ^{31}P NMR (202 MHz, D_2O): *δ* 20.9, 0.29; HRMS (ESI-) calcd for $C_{28}H_{45}N_6O_{16}P_2S_2$ $[M-H]^-$ 847.1813, found 847.1842.

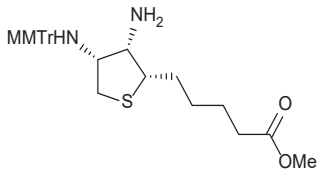


((2R,3S,4R,5R)-5-(2-Amino-6-oxo-1H-purin-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (2-(2-(2-(2-(4-(((hydroxy(2-(4-methylthiazol-5-yl)ethoxy)phosphoryl)thio)methyl)phenylsulfonamido)ethoxy)ethoxy)ethoxy)ethyl) hydrogen phosphate (T3). Compound **T3** was obtained as a white solid (60 mg, 36%) from chloride **16** (1.0 eq, 157 mg, 0.18 mmol) following the same procedure described for compound **T1**. ^{1}H NMR (600 MHz, D_2O): *δ* 9.62 (s, 1H), 8.84 (br s, 1H), 7.66 (d, *J* = 8.5 Hz, 2H), 7.54 (d, *J* = 8.5 Hz, 2H), 6.00 (d, *J* = 3.9 Hz, 1H), 4.69–4.67 (m, 1H), 4.47 (t, *J* = 5.2 Hz, 1H), 4.35 (td, *J* = 5.2, 2.7 Hz, 1H), 4.23 (ddd, *J* = 11.8, 4.4, 2.8 Hz, 1H), 4.12 (ddd, *J* = 11.8, 4.4, 2.8 Hz, 1H), 4.00–3.97 (m, 3H), 3.73–3.60 (m, 10H), 3.56–3.53 (m, 2H), 3.50–3.47 (m, 2H), 3.45–3.42 (m, 2H), 3.01 (t, *J* = 5.2 Hz, 2H), 2.86 (t, *J* = 5.7 Hz, 2H), 2.40 (s, 3H); ^{13}C NMR (151 MHz, D_2O): *δ* 155.0, 154.4, 153.6, 149.3, 144.6, 141.0, 136.4, 135.3, 132.4, 129.2, 126.1, 109.2, 88.6, 83.1 (d, *J* = 8.5 Hz), 73.6, 69.4 (d, *J* = 7.3 Hz), 69.0, 68.93, 68.89, 68.74, 68.68, 68.1, 64.2 (d, *J* = 5.2 Hz), 63.5 (d, *J* = 4.5 Hz), 63.4 (d, *J* = 5.2 Hz), 41.59, 33.0 (d, *J* = 2.7 Hz), 25.7 (d, *J* = 8.4 Hz), 10.47; ^{31}P NMR (202 MHz, D_2O): *δ* 19.5, 0.29. HRMS (ESI-) calcd for $C_{31}H_{44}N_7O_{16}P_2S_3$ $[M-H]^-$ 928.1487, found 928.1470.

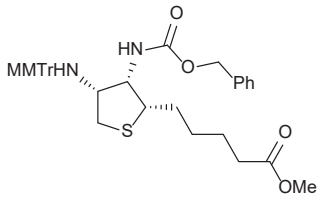


((2R,3S,4R,5R)-5-(2-Amino-6-oxo-1H-purin-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (2-(2-(2-(2-(4-(((hydroxy(2-(4-methylthiazol-5-yl)ethoxy)phosphoryl)thio)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl) hydrogen phosphate (T4). Compound **15** (1.0 eq, 100 mg, 0.12 mmol) and DBU (2.0 eq, 35 µL, 0.24 mmol) were dissolved in 10 mL of dry ACN and the solution was stirred at room temperature. After 1 h, compound **3** (1.2 eq, 33 mg, 0.14 mmol) and $[Cu(CH_3CN)_4]PF_6$ (0.1 eq, 5 mg, 0.01 mmol) were added to the mixture, which was allowed to stir at 45 °C for 2 d. The solvent was removed under reduced pressure and the residue was dissolved in 60% HCOOH in H_2O. The mixture was allowed to stir at room temperature for 3 d. The acid was removed under vacuum and the aqueous solution was lyophilised. The resulting residue was purified by HPLC (90-50% H_2O in ACN). The fractions containing the thiophosphate were collected and freeze-dried to give the title compound (37 mg, 37%) as a yellowish solid. ^{1}H NMR (600 MHz, D_2O): *δ* 8.65 (br s, 1H), 8.04 (br s, 1H), 7.82 (br s, 1H), 5.88 (d, *J* = 5.8 Hz, 1H), 4.49–4.47 (m, 3H), 4.29 (dt, *J* = 6.6, 3.4 Hz, 1H), 4.10–4.07 (m, 2H), 3.91–3.88 (m, 2H), 3.85–3.81 (m, 4H), 3.79 (d, *J* = 13.0 Hz, 2H), 3.59–3.57 (m, 2H), 3.54–3.46 (m, 8H), 2.90 (t, *J* = 6.2 Hz, 2H), 2.27 (s, 3H); ^{13}C NMR (151 MHz, D_2O): *δ* 160.1, 155.2, 153.3, 153.1, 150.3, 146.5, 138.7, 129.3, 125.6, 117.5, 88.2, 85.0 (d, *J* =

8.8 Hz), 74.9, 71.8, 71.3 (d, *J* = 7.5 Hz), 70.9 (d, *J* = 6.3 Hz), 70.8 (d, *J* = 9.3 Hz), 70.0, 66.9 (d, *J* = 5.5 Hz), 66.2 (d, *J* = 4.8 Hz), 66.1 (d, *J* = 5.3 Hz), 51.3, 27.8 (d, *J* = 8.1 Hz), 24.9, 15.0; ^{31}P NMR (202 MHz, D_2O): *δ* 19.5, 0.22; HRMS (ESI-) calcd for $C_{27}H_{40}N_9O_{14}P_2S_2$ $[M-H]^-$ 840.1616, found 840.1614.

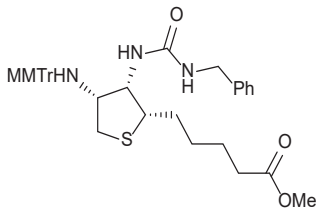


Methyl 5-((2S,3S,4R)-3-amino-4-(((4-methoxyphenyl)diphenylmethyl)amino)tetrahydrothiophen-2-yl)pentanoate (17). Methyl 5-((2S,3S,4R)-3,4-diaminotetrahydrothiophen-2-yl)pentanoate (1.0 eq, 3.0 g, 12.9 mmol) was co-evaporated twice with dry pyridine, dissolved in 55 mL of dry pyridine, and cooled to 0°C. 4-Methoxytriphenylmethyl chloride (1.2 eq, 4.78 g, 15.5 mmol) was added dropwise and the mixture was allowed to stir at room temperature for 12 h. The reaction was then quenched with MeOH and the organic solvents were evaporated under reduced pressure. The residue was dissolved in 50 mL of DCM and washed with water (50 mL x 1). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (Hexane/EtOAc/Et_3N 80:20:1) to give the title compound (4.5 g, 69%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$): *δ* 7.55–7.52 (m, 4H), 7.46–7.42 (m, 2H), 7.28–7.23 (m, 4H), 7.19–7.14 (m, 2H), 6.81–6.78 (m, 2H), 3.76 (s, 3H), 3.64 (s, 3H), 3.03 (br s, 1H), 2.97 (ddd, *J* = 8.0, 6.9, 3.6 Hz, 1H), 2.68–2.63 (m, 1H), 2.52 (t, *J* = 10.6 Hz, 1H), 2.26–2.21 (m, 2H), 1.68 (t, *J* = 3.7 Hz, 1H), 1.58–1.43 (m, 3H), 1.39–1.30 (m, 1H), 1.18–1.08 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): *δ* 173.9, 158.0, 147.1, 147.0, 138.9, 129.7, 128.4, 127.8, 126.3, 113.2, 70.6, 61.2, 55.2, 55.1, 51.4, 50.2, 33.7, 33.3, 30.8, 27.5, 24.7; HRMS (ESI+) calcd for $C_{30}H_{36}N_2O_3SNa$ $[M+Na]^+$ 527.2339, found 527.2338.

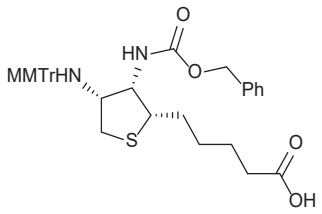


Methyl 5-((2S,3S,4R)-3-(((benzyloxy)carbonyl)amino)-4-(((4-methoxyphenyl)diphenylmethyl)amino)tetrahydrothiophen-2-yl)pentanoate (18). To a solution of compound **17** (1.0 eq, 1.4 g, 2.8 mmol) in 20 mL of dry DCM at 0 °C was first added DIPEA (2.0 eq, 0.96 mL, 5.6 mmol), followed by benzyl chloroformate (1.1 eq, 0.43 mL, 3.1 mmol). The reaction mixture was stirred for 30 min at room temperature, diluted with DCM (30 mL), and washed with H_2O (1 x 50 mL) and brine (1 x 50 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (Hexane/EtOAc/Et_3N 90:10:1) to give the title compound (1.55 g, 85%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.57–7.12 (m, 17H), 6.83–6.71 (m, 2H), 5.23 (d, *J* = 12.3 Hz, 1H), 5.18 (d, *J* = 12.3 Hz, 1H), 4.95 (d, *J* = 10.3 Hz, 1H), 4.07–4.0 (m, 1H), 3.77 (s, 3H), 3.64 (s, 3H), 3.25– 3.06 (m, 2H), 2.27–2.15 (m, 2H), 1.98–1.84 (m, 3H), 1.65–1.22 (m, 3H), 1.38–1.29 (m, 1H), 1.28–1.14 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): *δ* 173.8, 158.0, 156.7, 146.7, 146.6,

138.4, 136.4, 129.7, 128.6, 128.4, 128.2, 128.1, 126.4, 113.4, 70.6, 66.9, 60.3, 58.6, 55.2, 51.4, 47.5, 33.7, 33.1, 30.6, 27.6, 24.6; HRMS (ESI+) calcd for $C_{38}H_{42}N_2O_5SNa$ $[M+Na]^+$ 661.2706, found 661.2703.

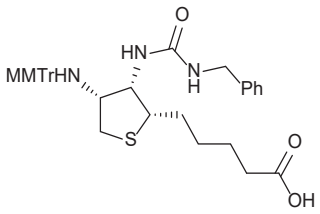


Methyl 5-((2S,3S,4R)-3-(3-benzylureido)-4-(((4-methoxyphenyl)diphenylmethyl)amino)tetrahydrothiophen-2-yl)pentanoate (19). To a solution of compound **17** (1.0 eq, 0.6 g, 1.2 mmol) in 6 mL of dry DCM at 0 °C was first added DIPEA (2.0 eq, 0.43 mL, 2.4 mmol), followed by benzyl isocyanate (1.1 eq, 0.16 mL, 1.3 mmol). The reaction mixture was stirred for 1 h at room temperature, diluted with DCM (30 mL), and washed with H_2O (1 x 50 mL) and brine (1 x 50 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (Hexane/EtOAc/Et_3N 90:10:1) to give the title compound (0.61 g, 80%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.55–7.46 (m, 3H), 7.40 (d, J = 8.0 Hz, 2H), 7.31–7.14 (m, 12H), 6.78 (d, J = 8.0 Hz, 2H), 5.70 (br s, 1H), 5.16 (br s, 1H), 4.47–4.36 (m, 1H), 4.34–4.22 (m, 1H), 3.75 (s, 3H), 3.63 (s, 3H), 3.22–3.08 (m, 2H), 2.59–2.49 (m, 1H), 2.20 (t, J = 7.2 Hz, 2H), 2.07 (br s, 1H), 1.95 –1.73 (m, 2H), 1.62–1.52 (m, 1H), 1.51–1.40 (m, 1H), 1.35–1.24 (m, 1H), 1.23–1.12 (m, 2H), 1.10–1.01 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 175.4, 160.4, 159.5, 148.2, 148.1, 140.8, 140.0, 131.2, 130.1, 129.9, 129.9, 129.5, 128.7, 128.6, 127.8, 114.8, 72.0, 62.1, 59.6, 56.6, 52.9, 49.2, 47.6, 45.9, 35.2, 34.5, 32.2, 29.2, 26.1; HRMS (ESI+) calcd for $C_{38}H_{43}N_3O_4SNa$ $[M+Na]^+$ 660.2866, found 660.2873.

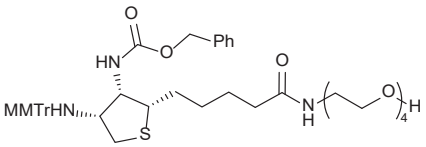


5-((2S,3S,4R)-3-(((Benzyloxy)carbonyl)amino)-4-(((4-methoxyphenyl)diphenylmethyl)amino)tetrahydrothiophen-2-yl)pentanoic acid (20). To a solution of compound **18** (400 mg, 0.6 mmol) in THF (3 mL) was added 1 mL of a 4 M solution of NaOH in MeOH/H_2O (4:1). The reaction mixture was stirred for 2 h at room temperature, diluted with EtOAc and neutralised with TEEA buffer. The organic layer was collected, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (DCM/MeOH/Et_3N 90:10:1) to give the title compound (290 mg, 74%) as a thick liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.17 (br s, 1H), 7.58–7.07 (m, 17H), 6.81–6.69 (m, 2H), 5.25 (d, J = 12.3 Hz, 1H), 5.25 (d, J = 12.3 Hz, 1H), 4.98 (d, J = 10.3 Hz, 1H), 4.08 (br s, 1H), 3.77 (s, 3H), 3.20–3.16 (m, 2H), 2.19–2.16 (m, 2H), 1.95–1.85 (m, 3H), 1.64–1.47 (m, 3H), 1.42–1.30 (m, 1H), 1.24–1.17 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.7, 158.0, 156.7, 146.8, 146.6,

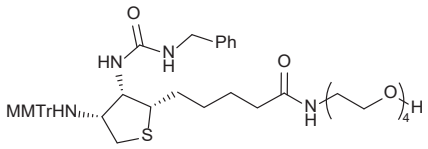
138.5, 136.5, 129.7, 128.6, 128.4, 128.1, 128.0, 126.4, 113.3, 70.5, 66.9, 60.4, 58.8, 55.2, 47.6, 36.0, 33.0, 30.8, 28.0, 25.6; HRMS (ESI-) calcd for $C_{37}H_{39}N_2O_5S$ $[M-H]^-$ 623.2584, found 623.2585.



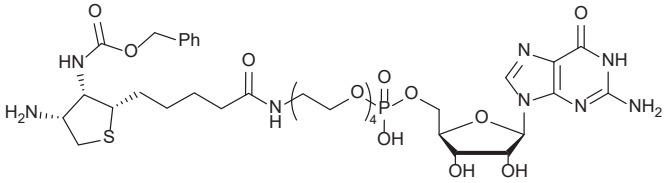
5-((2S,3S,4R)-3-(3-Benzylureido)-4-(((4-methoxyphenyl)diphenylmethyl)amino)tetrahydrothiophen-2-yl)pentanoic acid (21). Compound **21** was obtained as a thick liquid (380 mg, 77%) from ester **3** (500 mg, 0.6 mmol) following the same procedure described for compound **19**. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.50–7.44 (m, 4H), 7.37–7.16 (m, 12H), 7.21–7.16 (m, 2H), 6.85–6.82 (m, 2H), 6.58 (t, J = 6.0 Hz, 1H), 5.94 (d, J = 9.7 Hz, 1H), 4.38 (dd, J = 15.5, 6.0 Hz, 1H), 4.32 (dd, J = 15.5, 6.0 Hz, 1H), 4.23 (dt, J = 8.9, 4.0 Hz, 1H), 3.72 (s, 3H), 3.15–3.10 (m, 1H), 3.04–2.99 (m, 1H), 2.56 (d, J = 8.2 Hz, 1H), 2.13 (t, J = 7.5 Hz, 2H), 2.08 (t, J = 5.1 Hz, 1H), 1.60–1.51 (m, 1H), 1.52–1.45 (m, 1H), 1.44–1.39 (m, 2H),1.32–1.25 (m, 1H), 1.21–1.12 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 174.4, 158.9, 157.6, 147.2, 147.1, 141.1, 138.6, 129.6, 128.3, 128.3, 128.0, 126.9, 126.6, 126.3, 113.3, 70.1, 60.6, 56.8, 55.1, 47.6, 43.0, 33.6, 32.2, 30.9, 27.3, 24.6; HRMS (ESI+) calcd for $C_{37}H_{41}N_3O_4SNa$ $[M+Na]^+$ 646.2710, found 646.2728.



Benzyl ((2S,3S,4R)-2-(1-hydroxy-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-yl)-4-(((4-methoxyphenyl)diphenylmethyl)amino)tetrahydrothiophen-3-yl)carbamate (22). EDCI.HCl (1.2 eq, 0.37 g, 1.9 mmol) and DIPEA (2.5 eq, 0.72 mL, 4.0 mmol) were added to a solution of 2-(2-aminoethoxy)ethanol (1.2 eq, 0.35 mL, 1.9 mmol), compound **20** (1.0 eq, 1.00 g, 1.6 mmol), and HOBt (1.2 eq, 0.26 g, 1.9 mmol) in dry DMF (7 mL), and the mixture was stirred for 24 h at room temperature. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (DCM/MeOH/Et_3N 95:4:1) to give the title compound (1.1 g, 85%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.81 (t, J = 5.5 Hz, 1H), 7.45–7.17 (m, 18H), 6.87–6.79 (m, 2H), 5.23 (d, J = 12.7 Hz, 1H), 5.14 (d, J = 12.7 Hz, 1H), 4.60 (t, J = 5.5 Hz, 1H), 4.07 (quint, J = 4.2 Hz, 1H), 3.72 (s, 3H), 3.49–3.47 (m, 10H), 3.42–3.38 (m, 4H), 3.19–3.08 (m, 4H), 2.30 (t, J = 10.4 Hz, 1H), 1.99 (t, J = 7.4 Hz, 2H), 1.55–1.61 (m, 1H), 1.41–1.32 (m, 3H), 1.27–1.04 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.2, 157.7, 157.2, 147.2, 147.1, 138.6, 137.6, 129.4, 128.5, 128.1, 128.1, 127.8, 127.6, 126.3, 113.4, 72.4, 69.92, 69.86, 69.8, 69.6, 69.3, 65.4, 60.3, 60.0, 58.8, 55.1, 52.1, 47.9, 38.5, 35.3, 31.5, 31.0, 27.5, 25.4; HRMS (ESI+) calcd for $C_{45}H_{57}N_3O_8SNa$ $[M+Na]^+$ 822.3758, found 822.3751.

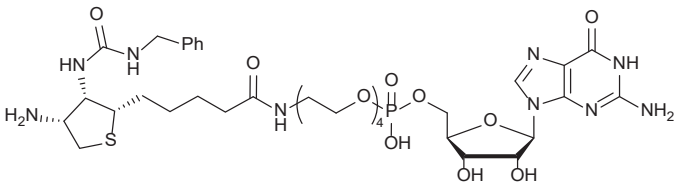


5-((2S,3S,4R)-3-(3-Benzylureido)-4-(((4-methoxyphenyl)diphenylmethyl)amino)tetrahydrothiophen-2-yl)-N-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)pentanamide (23). Compound **23** was obtained as a white solid (0.62 g, 84%) from carboxylic acid **5** following the same procedure described for compound **21**. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.80 (t, J = 5.6 Hz, 1H), 7.48–7.43 (m, 4H), 7.37–7.23 (m, 12H), 7.20–7.16 (m, 2H), 6.86–6.81 (m, 2H), 6.55 (t, J = 6.0 Hz, 1H), 5.91 (d, J = 9.7 Hz, 1H), 4.58 (t, J = 5.5 Hz, 1H), 4.35 (d, J = 15.5, 6.0 Hz, 1H), 4.35 (d, J = 15.5, 5.6 Hz, 1H), 4.23 (dt, J = 8.9, 4.0 Hz, 1H), 3.72 (s, 3H), 3.50–3.47 (m, 8H), 3.41–3.35 (m, 4H), 3.17 (q, J = 5.9 Hz, 2H), 3.14–3.10 (m, 1H), 3.03–2.97 (m, 1H), 2.55 (d, J = 8.1 Hz, 1H), 2.06 (t, J = 10.5 Hz, 1H), 2.00 (t, J = 7.5 Hz, 2H), 1.60–1.50 (m, 1H), 1.49–1.44 (m, 1H), 1.42–1.37 (m, 2H), 1.30–1.22 (m, 1H), 1.18–1.06 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 172.1, 158.8, 157.6, 147.2, 147.1, 141.1, 138.6, 129.6, 128.3, 128.3, 128.0, 126.9, 126.6, 126.3, 113.3, 72.4, 70.0, 69.9, 69.84, 69.81, 69.6, 69.2, 60.7, 60.3, 56.8, 55.1, 47.6, 43.0, 38.5, 35.2, 32.2, 30.9, 27.5, 25.4; HRMS (ESI+) calcd for $C_{45}H_{57}N_3O_8SNa$ $[M+Na]^+$ 821.3918, found 821.3937.



Benzyl ((2S,3S,4R)-4-amino-2-(1-((2R,3S,4R,5R)-5-(2-amino-6-oxo-1H-purin-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-16-oxo-2,3,6,9,12-pentaoxa-15-azaicosan-20-yl)tetrahydrothiophen-3-yl)carbamate (B1). To a stirring solution of phosphoramidite **5** (1.0 eq, 200 mg, 0.34 mmol) in dry ACN (10 mL), a solution of alcohol **22** (2.0 eq, 424 mg, 0.68 mmol) in 5 mL of dry ACN was added. The mixture was cooled to 0 °C in an ice-bath and a 0.45 M solution of tetrazole in acetonitrile (3.0 eq, 2.26 mL, 1.02 mmol) was slowly added. The ice-bath was removed and the reaction was allowed to warm to room temperature and stirred for an additional 2 h. The reaction progress was monitored by ^{31}P NMR. Upon completion, a 5.5 M solution of *t*BuOOH in decane (1.1 eq, 0.075 mL, 0.37 mmol) was added to the reaction mixture. After 1 h, full oxidation of the phosphorus atom was confirmed by ^{31}P NMR and the mixture was concentrated *in vacuo*. The crude compound was dissolved in 5 mL of DCM and treated with DBU (2.0 eq, 0.1 mL, 0.68 mmol) for 2 h at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in 60% HCOOH in H_2O. The mixture was allowed to stir at room temperature for 3 d. The acid was removed under vacuum and the aqueous solution was lyophilised. The resulting residue was purified by HPLC (90-50% H_2O in ACN). The fractions containing the thiophosphate were collected and freeze dried to give the title compound (95 mg, 32%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.40 (br s, 2H), 7.94 (s, 1H), 7.90 (t, J = 5.4 Hz, 1H), 7.39–7.33 (m, 5H), 7.32–7.28 (m, 1H), 7.25 (d, J = 9.9 Hz, 1H), 6.57 (br s, 2H), 5.70 (d, J = 6.1 Hz, 1H), 5.45 (br s, 1H), 5.33 (br s, 1H), 5.18 (d, J = 12.7 Hz, 1H), 4.98 (d, J = 12.7 Hz, 1H), 4.48 (br s, 1H), 4.45–4.41 (m, 1H), 4.14–4.11 (m, 1H), 4.00–3.95 (m, 1H), 3.90–3.85 (m, 1H), 3.83–3.75 (m, 1H), 3.76–3.72 (m, 2H), 3.55–3.52 (m, 1H), 3.53–3.40 (m, 12H), 3.41–3.39 (m, 2H), 3.20–3.14 (m, 2H), 3.08–3.05 (m, 1H), 2.93 (t, J = 10.7 Hz, 1H), 2.02 (t, J = 6.8 Hz, 2H), 1.64–1.56 (m, 1H),

1.48–1.28 (m, 3H), 1.22–1.13 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 172.1, 156.9, 156.8, 153.8, 151.6, 137.1, 135.7, 128.4, 127.9, 127.6, 116.6, 83.8 (d, J = 7.6 Hz), 73.7, 71.0, 70.4 (d, J = 7.3 Hz), 69.8, 69.7, 69.6, 69.2, 65.7, 64.7 (d, J = 4.0 Hz), 63.6 (d, J = 5.4 Hz), 56.5, 54.2, 48.8, 38.7, 35.3, 30.4, 29.6, 27.3, 25.4; ^{31}P NMR (121 MHz, DMSO-d_6): δ -1.27; HRMS (ESI-) calcd for $C_{35}H_{52}N_8O_{14}PS$ $[M-H]^-$ 871.3066, found 871.3064.



5-((2S,3S,4R)-4-Amino-3-(3-benzylureido)tetrahydrothiophen-2-yl)-N-(1-((2R,3S,4R,5R)-5-(2-amino-6-oxo-1H-purin-9(6H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-2,3,6,9,12-pentaoxatetradecan-14-yl)pentanamide (B2). Compound **B2** was obtained as a white solid (62 mg, 21%) from alcohol **23** (2.0 eq, 543 mg, 0.68 mmol) following the same procedure described for compound **B1**. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.34 (br s, 2H), 7.96 (s, 1H), 7.92 (t, J = 5.6 Hz, 1H), 7.31–7.24 (m, 5H), 7.23–7.17 (m, 1H), 7.10 (t, J = 5.9 Hz, 1H), 6.56 (br s, 2H), 6.37 (d, J = 9.9 Hz, 1H), 5.70 (d, J = 6.0 Hz, 1H), 5.44 (br s, 1H), 5.32 (br s, 1H), 4.56–4.51 (m, 1H), 4.46 (t, J = 5.3 Hz, 1H), 4.35 (dd, J = 15.5, 6.5 Hz, 1H), 4.17 (dd, J = 15.5, 5.4 Hz, 1H), 4.13–4.10 (m, 1H), 3.97 (q, J = 3.7 Hz, 1H), 3.92–3.86 (m, 1H), 3.82–3.74 (m, 3H), 3.71–3.65 (m, 1H), 3.55–3.45 (m, 12H), 3.44 –3.48 (m, 2H), 3.19–3.16 (m, 2H), 3.09–3.05 (m, 1H), 2.81 (t, J = 10.6 Hz, 1H), 2.09–1.99 (m, 2H), 1.66–1.62 (m, 1H), 1.53–1.34 (m, 3H), 1.26–1.12 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 172.3, 158.6, 157.1, 154.1, 151.8, 141.0, 135.9, 128.5, 127.2, 126.8, 116.8, 86.7, 84.0 (d, J = 7.4 Hz), 74.0, 71.1, 70.6 (d, J = 7.2 Hz), 70.1, 70.0, 69.8, 69.5, 64.9 (d, J = 4.3 Hz), 64.0 (d, J = 5.1 Hz), 55.3, 54.7, 49.7, 43.1, 38.9, 35.4, 30.6, 29.8, 27.6, 25.6; ^{31}P NMR (202 MHz, DMSO-d_6): δ -1.12; HRMS (ESI-) calcd for $C_{35}H_{53}N_9O_{13}S$ $[M-H]^-$ 870.3226, found 870.3248.

PAGE images (S3-S8)

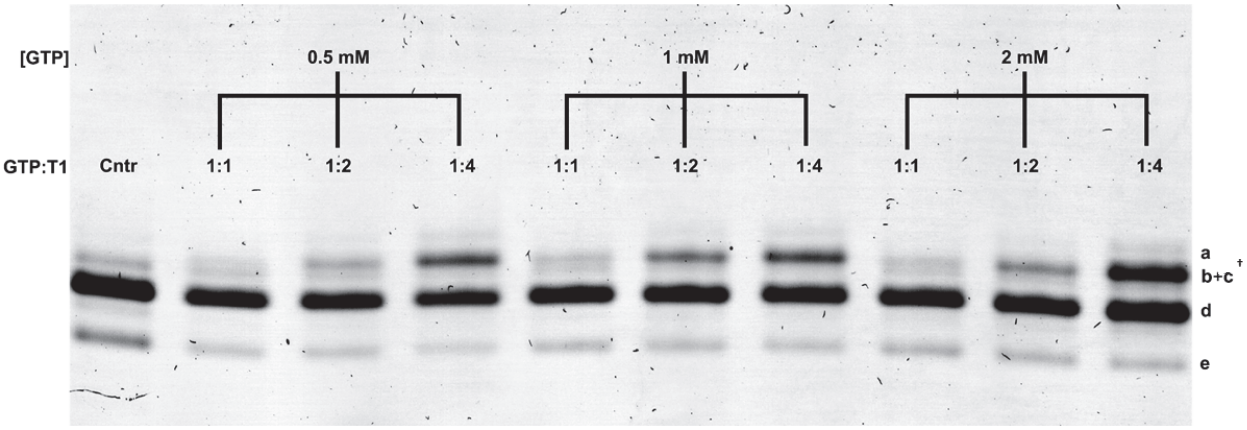


Figure S3. Denaturing PAGE analysis of transcription initiation with **T1**. **Cntr**, reference *in vitro* transcription (no **T1**, 2 mM GTP); **a**, 5′-modified 28-mer (n*+1); **b**, 5′-modified 27-mer (n*); **c**, 28-mer (n+1); **d**, 27-mer (n); **e**, 25-mer (early termination product). † The appearance of approximately 1% n+1 band was taken into consideration for calculating the relative RNA yields.

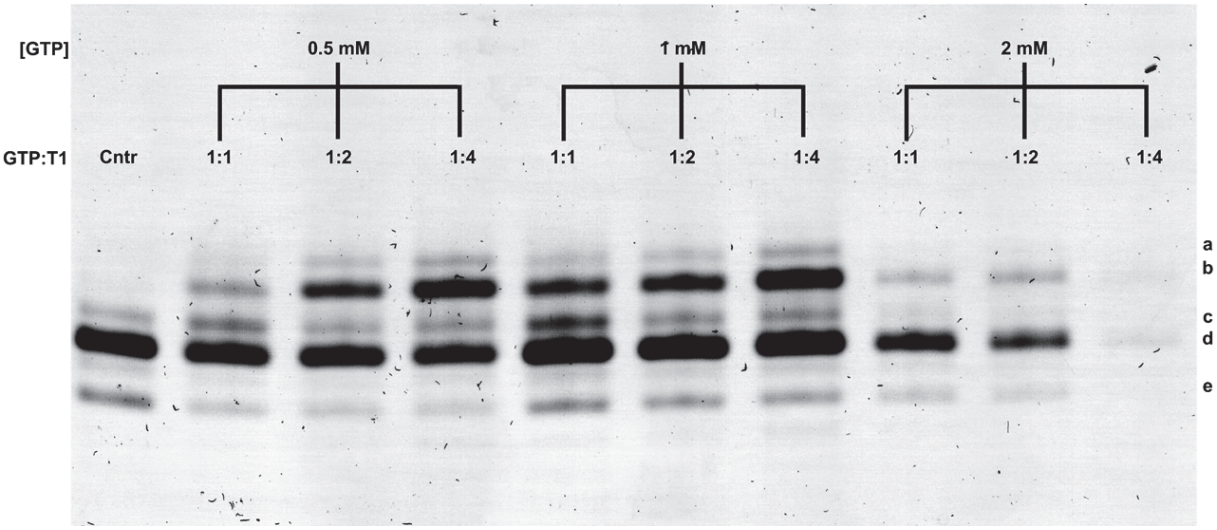


Figure S4. Denaturing PAGE analysis of transcription initiation with **T2**. **Cntr**, reference *in vitro* transcription (no **T2**, 2 mM GTP); **a**, 5′-modified 28-mer (n*+1); **b**, 5′-modified 27-mer (n*); **c**, 28-mer (n+1); **d**, 27-mer (n); **e**, 25-mer (early termination product).

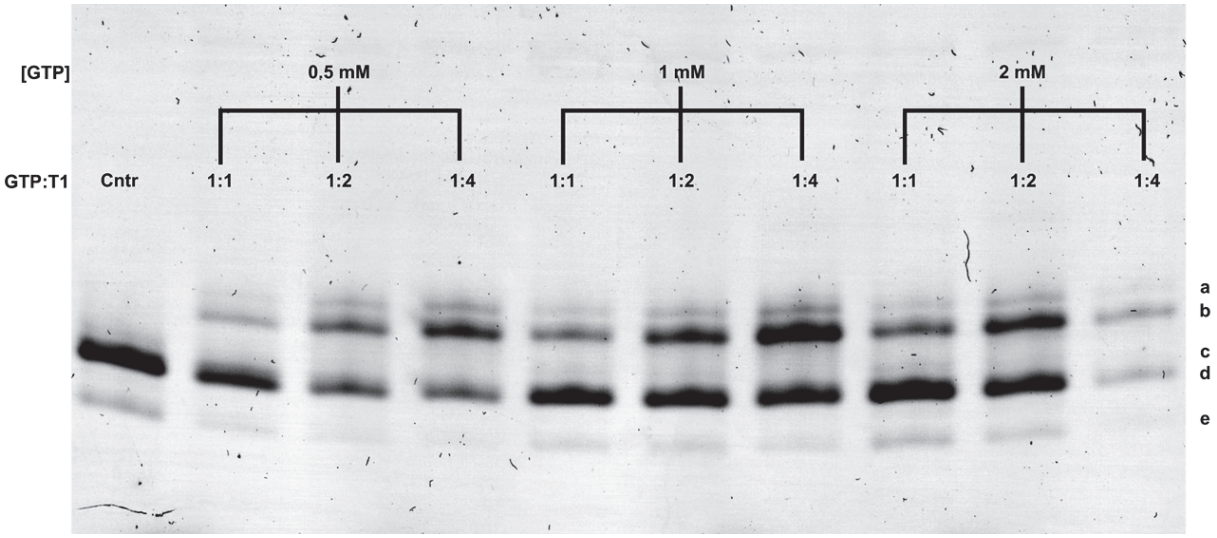


Figure S5. Denaturing PAGE analysis of transcription initiation with **T3**. **Cntr**, reference *in vitro* transcription (no **T3**, 2 mM GTP); **a**, 5'-modified 28-mer (n*+1); **b**, 5'-modified 27-mer (n*); **c**, 28-mer (n+1); **d**, 27-mer (n); **e**, 25-mer (early termination product).

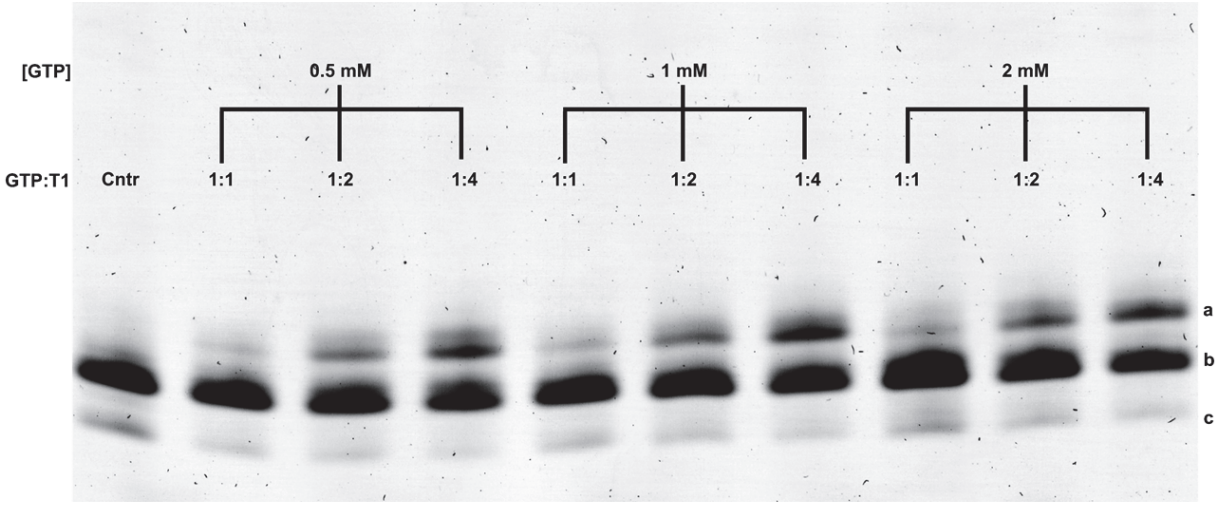


Figure S6. Denaturing PAGE analysis of transcription initiation with **T4**. **Cntr**, reference *in vitro* transcription (no **T4**, 2 mM GTP); **a**, 5'-modified 27-mer (n*); **b**. 27-mer (n); **c**, 25-mer (early termination product).

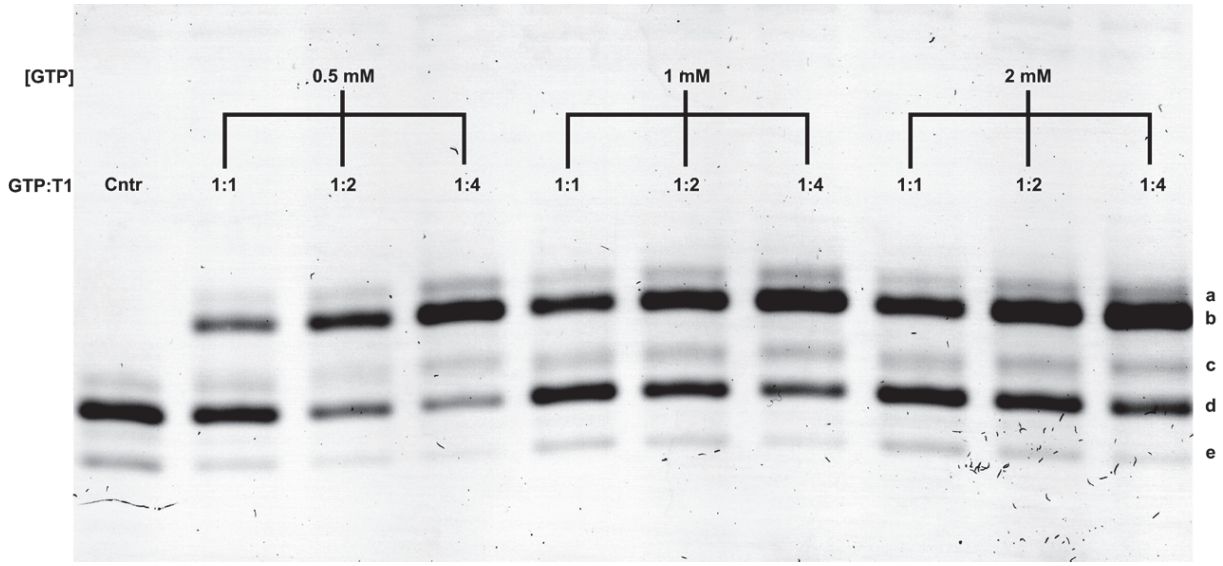


Figure S7. Denaturing PAGE analysis of transcription initiation with **B1**. **Cntr**, reference *in vitro* transcription (no **B1**, 2 mM GTP); **a**, 5′-modified 28-mer (n*+1); **b**, 5′-modified 27-mer (n*); **c**, 28-mer (n+1); **d**, 27-mer (n); **e**, 25-mer (early termination product).

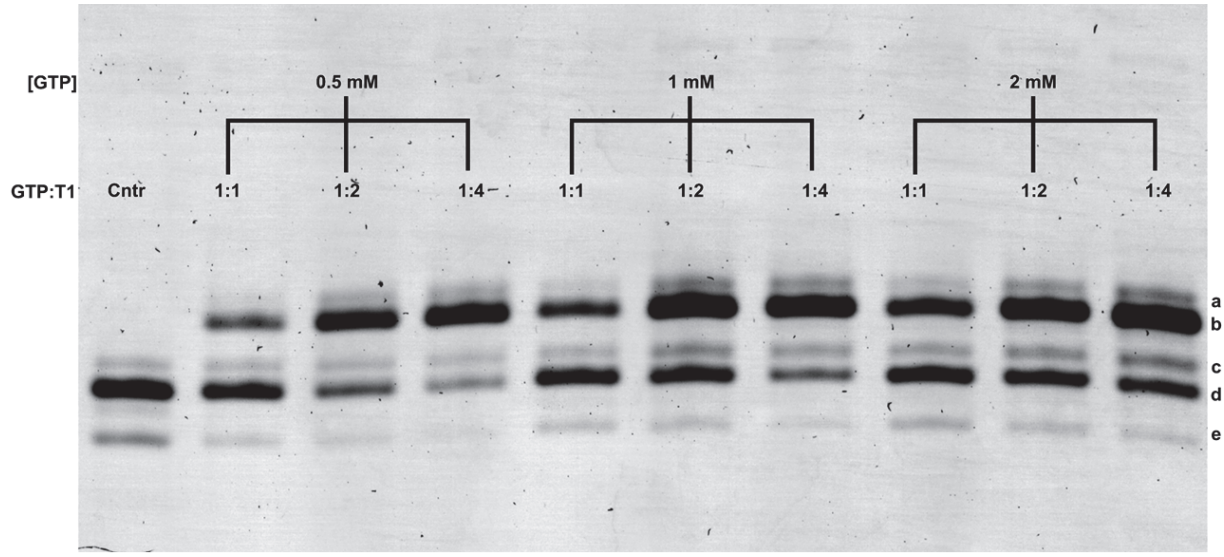


Figure S8. Denaturing PAGE analysis of transcription initiation with **B2**. **Cntr**, reference *in vitro* transcription (no **B2**, 2 mM GTP); **a**, 5′-modified 28-mer (n*+1); **b**, 5′-modified 27-mer (n*); **c**, 28-mer (n+1); **d**, 27-mer (n); **e**, 25-mer (early termination product).

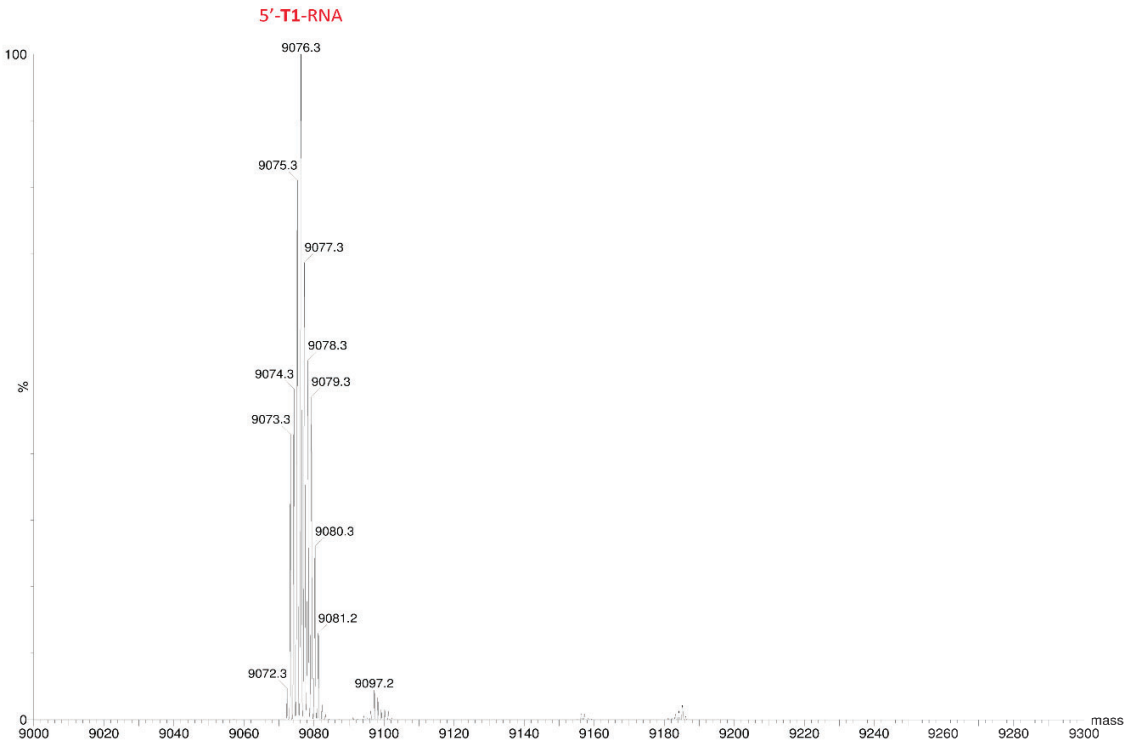
Table of Sequences (S9)

CODE	SEQUENCE	
MT1	5'-TAATACGACTCACTATAGGGAGCCTGAGCCTCCAGTCTCCAGTC-3'	sense
MT2	5'-**m**G**m**ACTGGAGACTGGAGGCTCAGGCTCCCTATAGTGAGTCGTATTA-3'	antisense

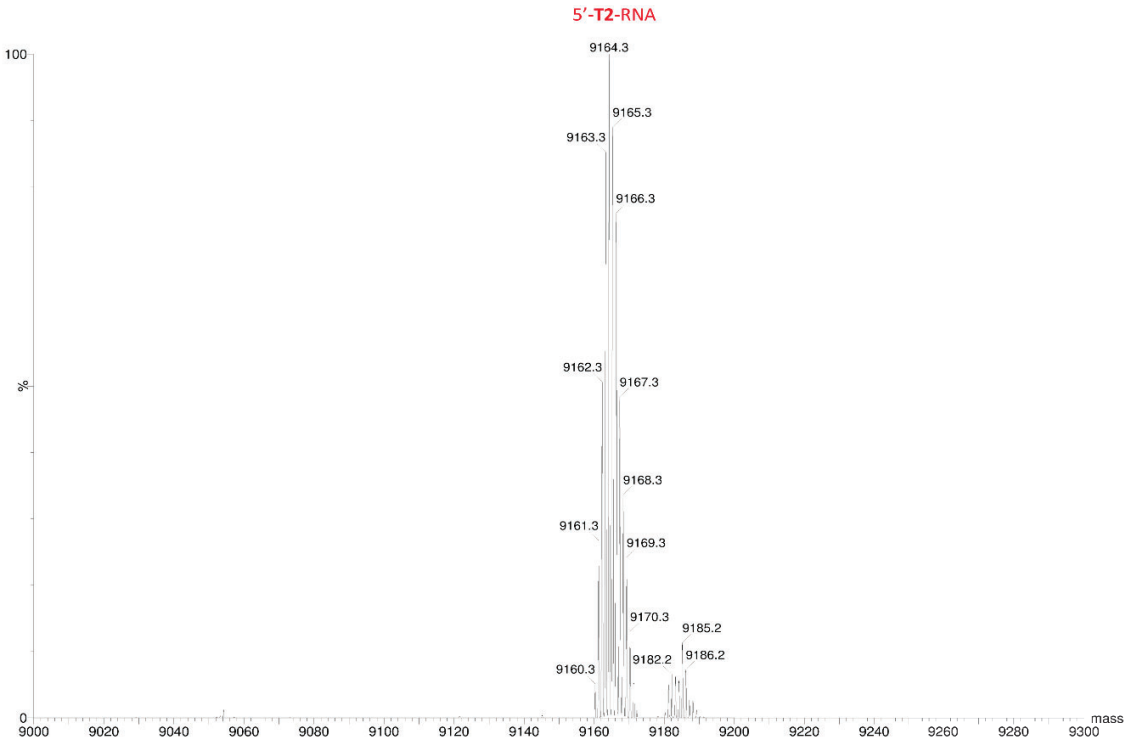
Table S9. Sequences of the DNA oligonucleotides used to prepare the DNA template for *in vitro* transcription. The consensus T7 promoter sequence is underlined. Methyl-modifications are shown in bold.

References

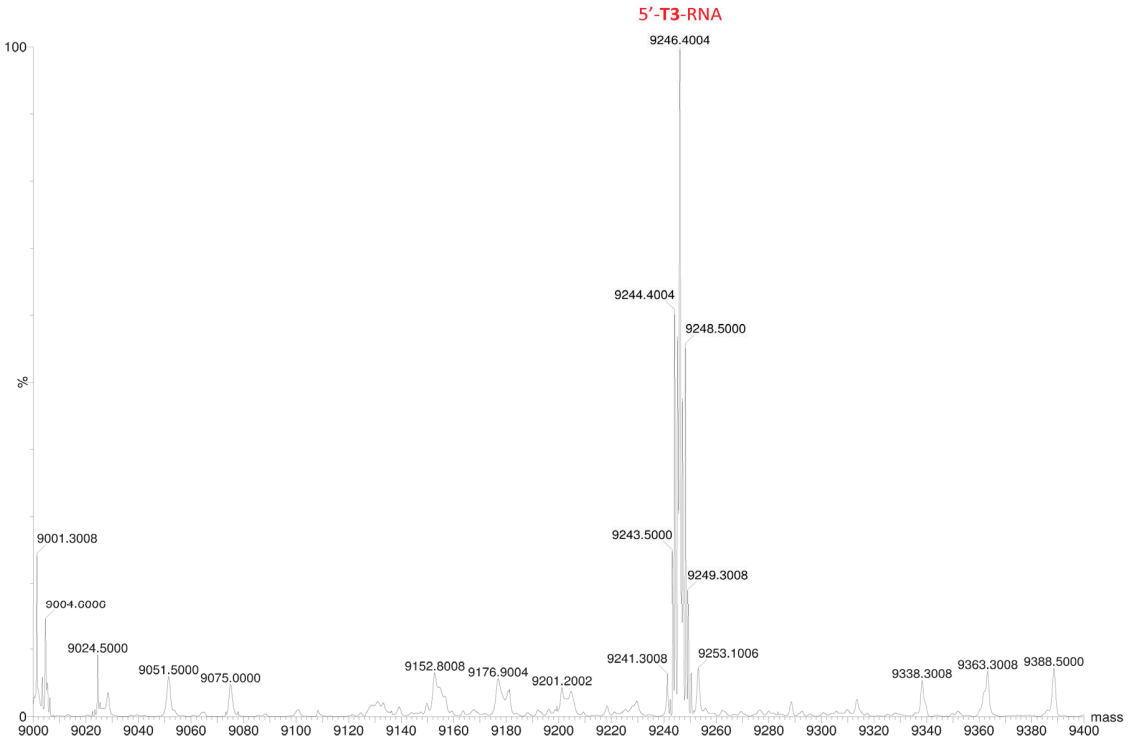
1. K. Heller, P. Ochtrop, M. F. Albers, F. B. Zauner, A. Itzen and C. Hedberg, *Angewandte Chemie International Edition*, 2015, **54**, 10327-10330.
2. L. Zhang, L. Sun, Z. Cui, R. L. Gottlieb and B. Zhang, *Bioconjug Chem*, 2001, **12**, 939-948.



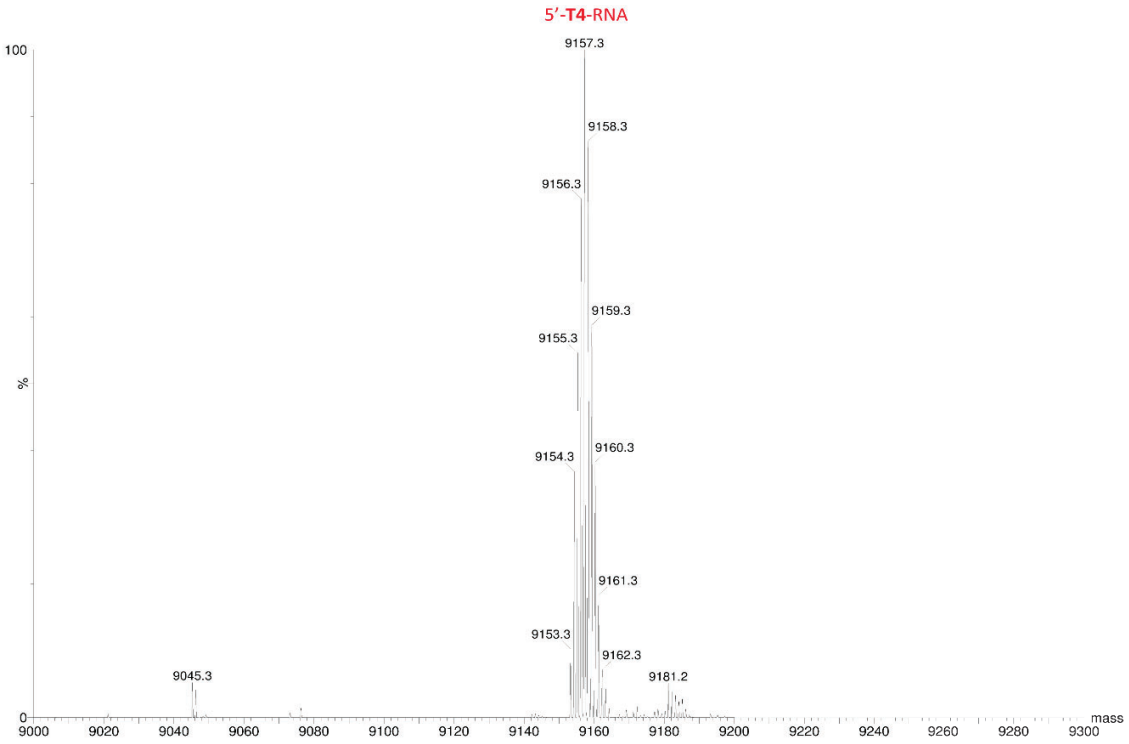
Mass spectrum of **T1**-modified RNA



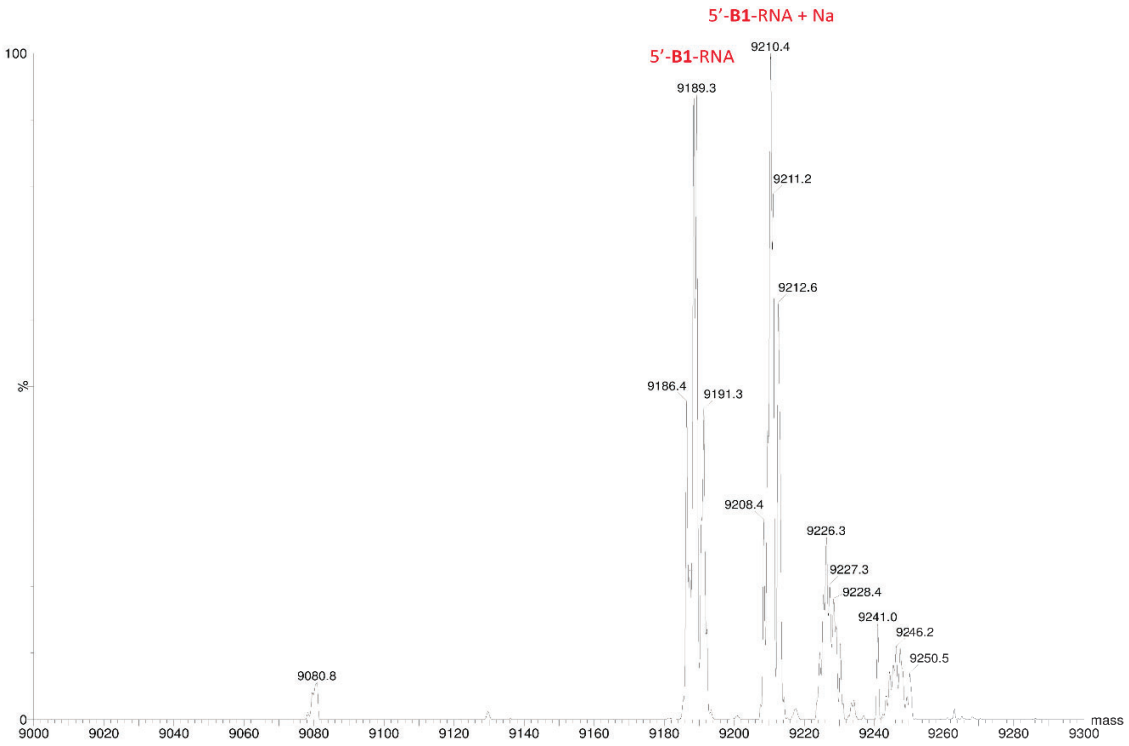
Mass spectrum of **T2**-modified RNA



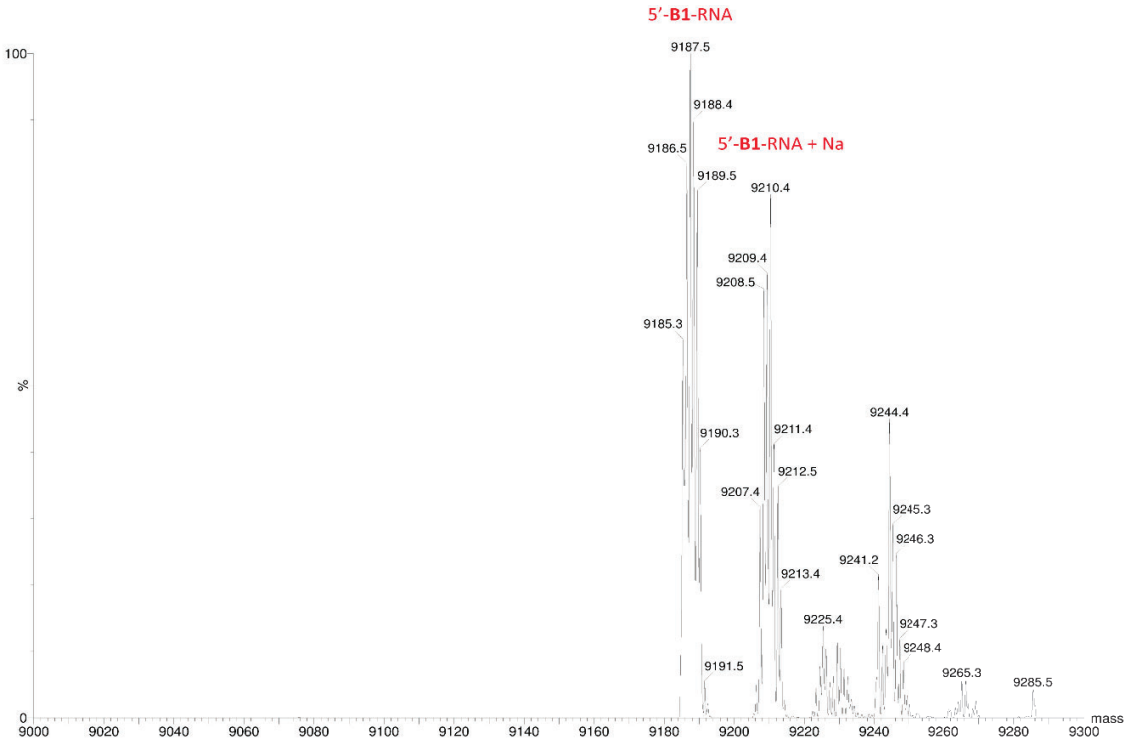
Mass spectrum of **T3**-modified RNA



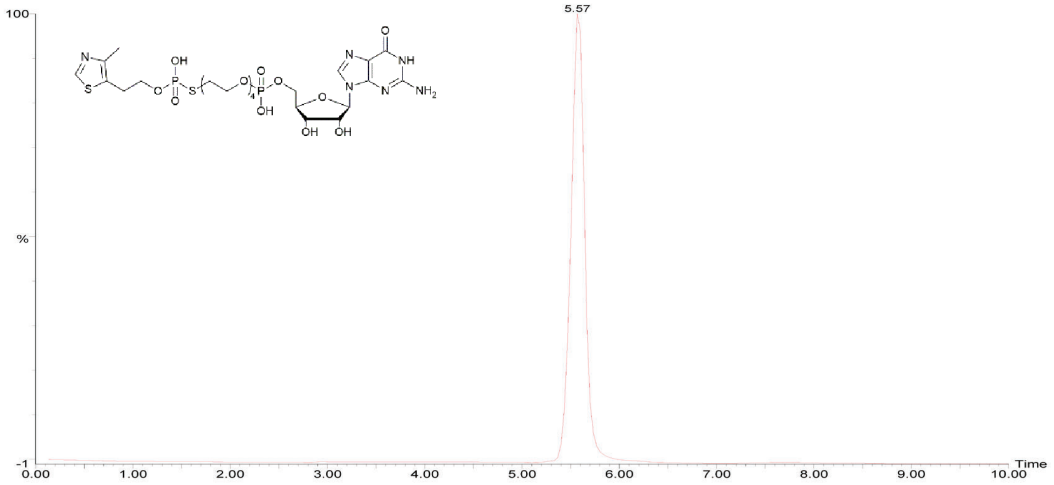
Mass spectrum of **T4**-modified RNA



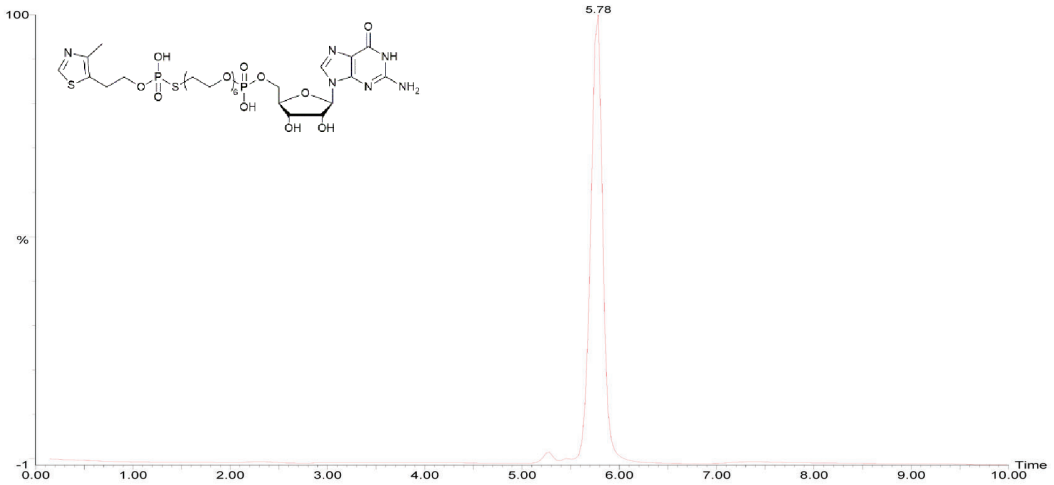
Mass spectrum of **B1**-modified RNA



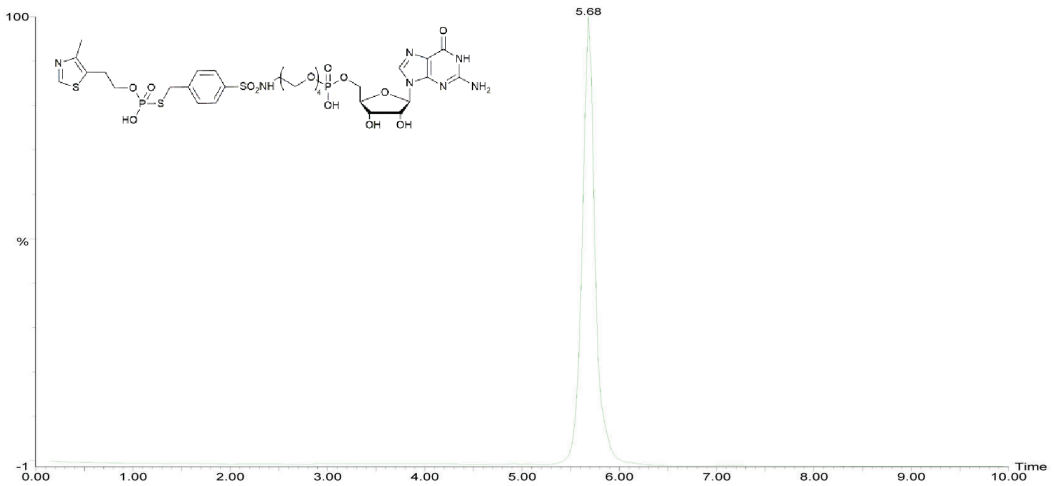
Mass spectrum of **B2**-modified RNA



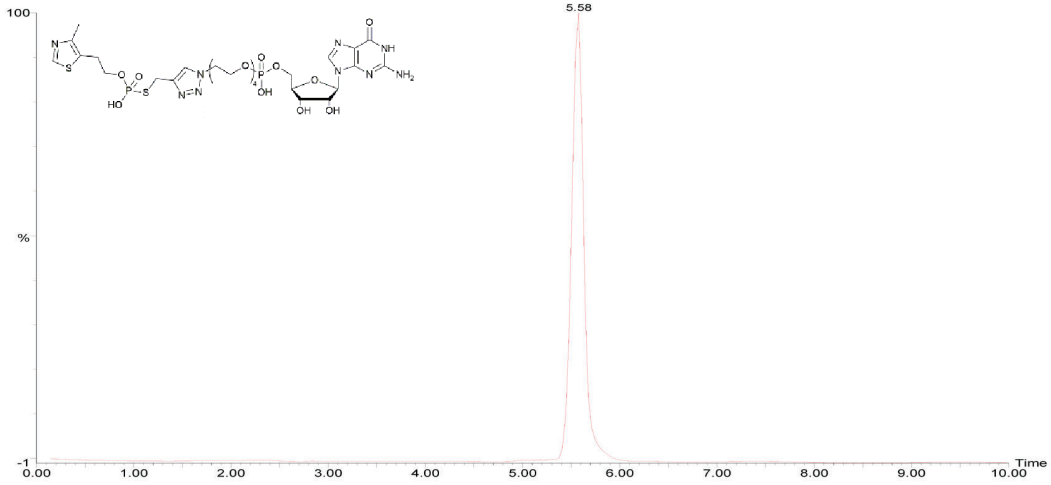
HPLC analysis of initiator **T1**



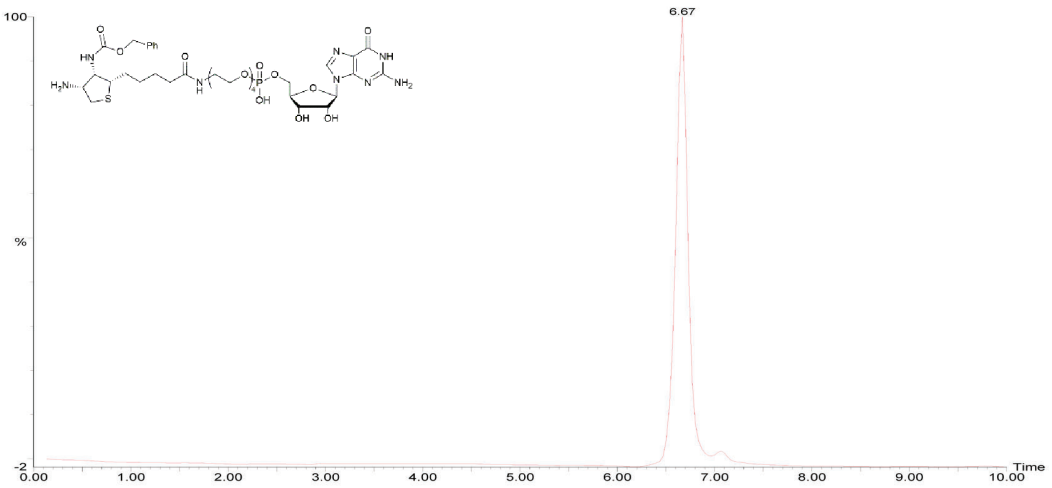
HPLC analysis of initiator **T2**



HPLC analysis of initiator **T3**



HPLC analysis of initiator **T4**



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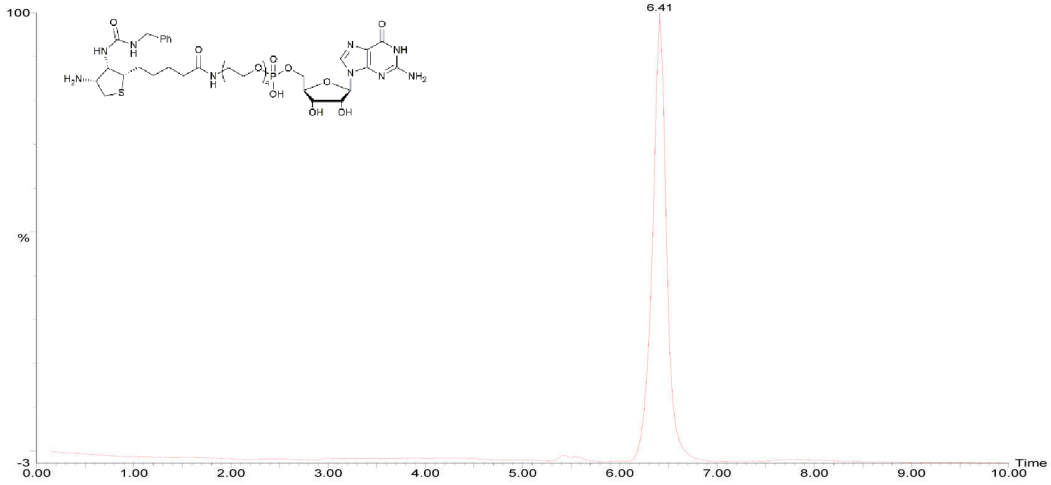

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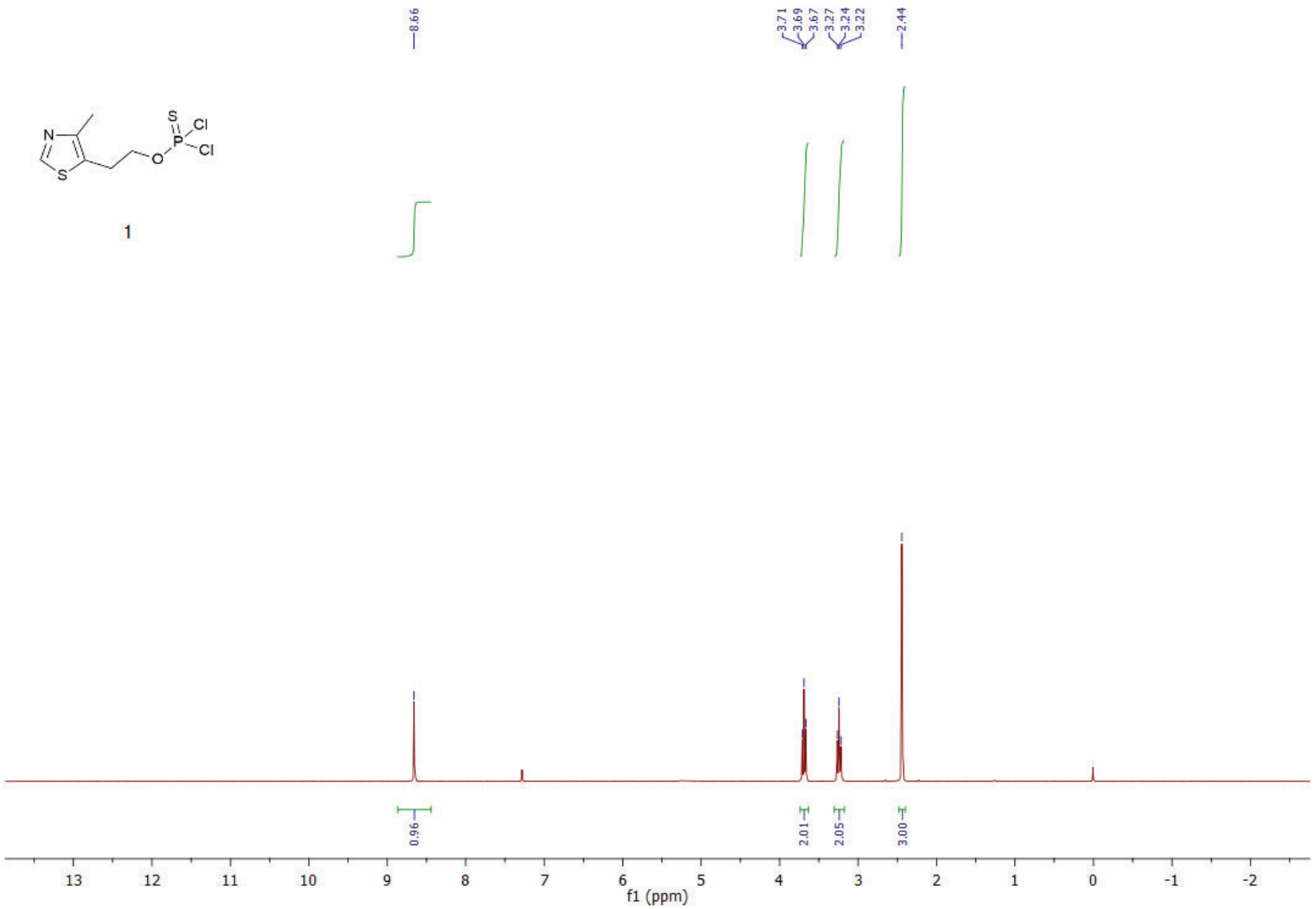
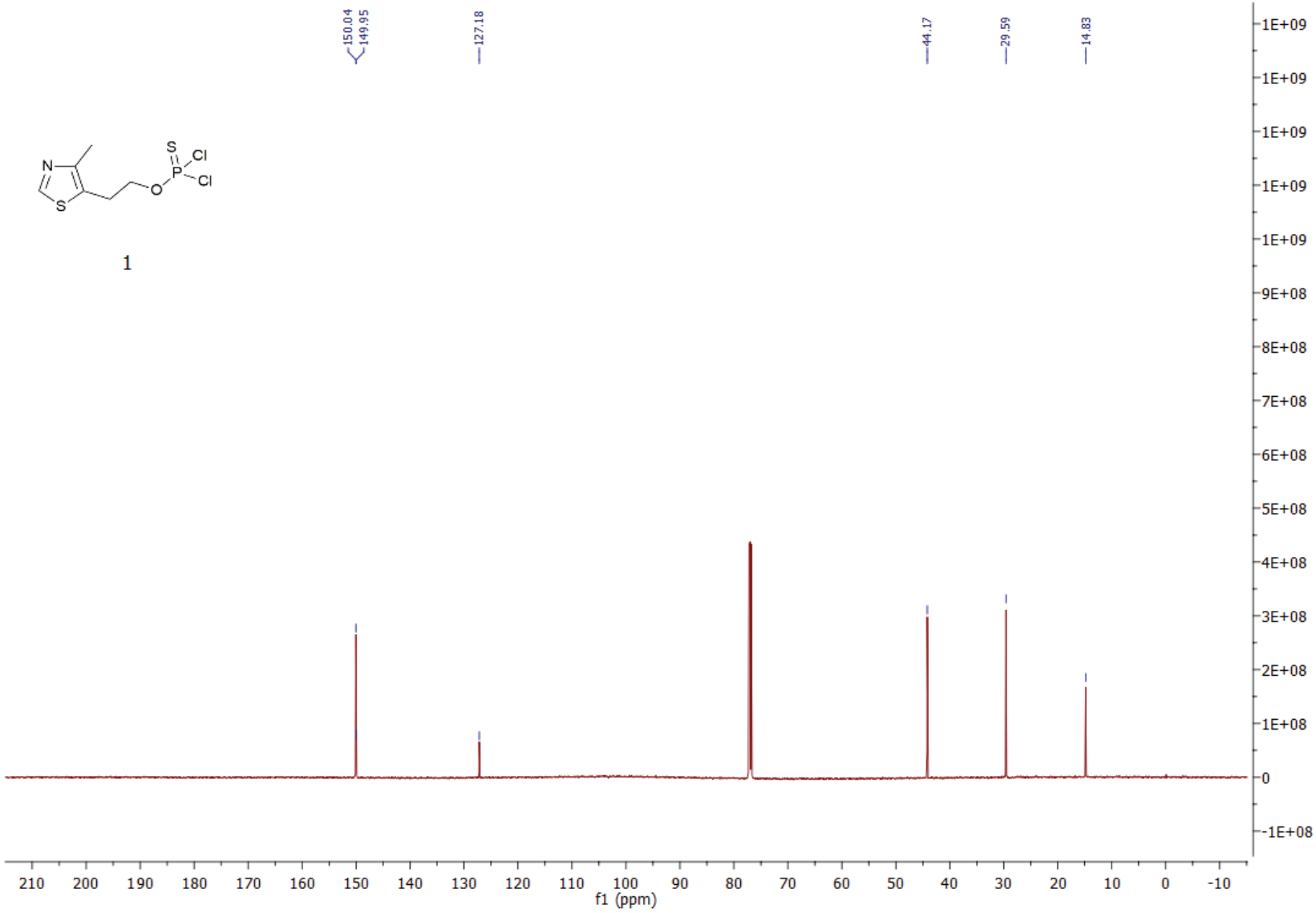


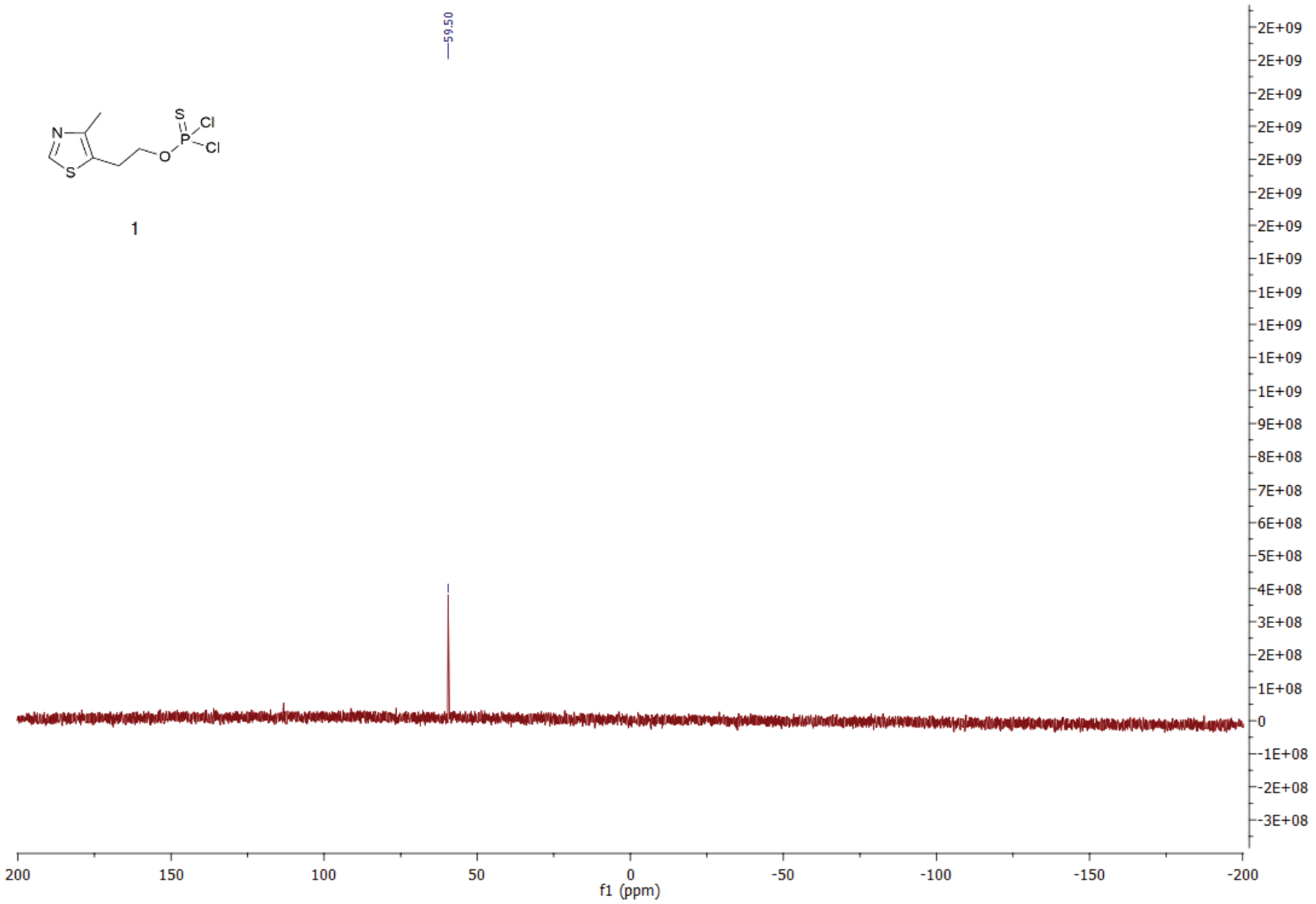
1

^{1}H NMR spectrum of compound **1** (600 MHz, $CDCl_3$)



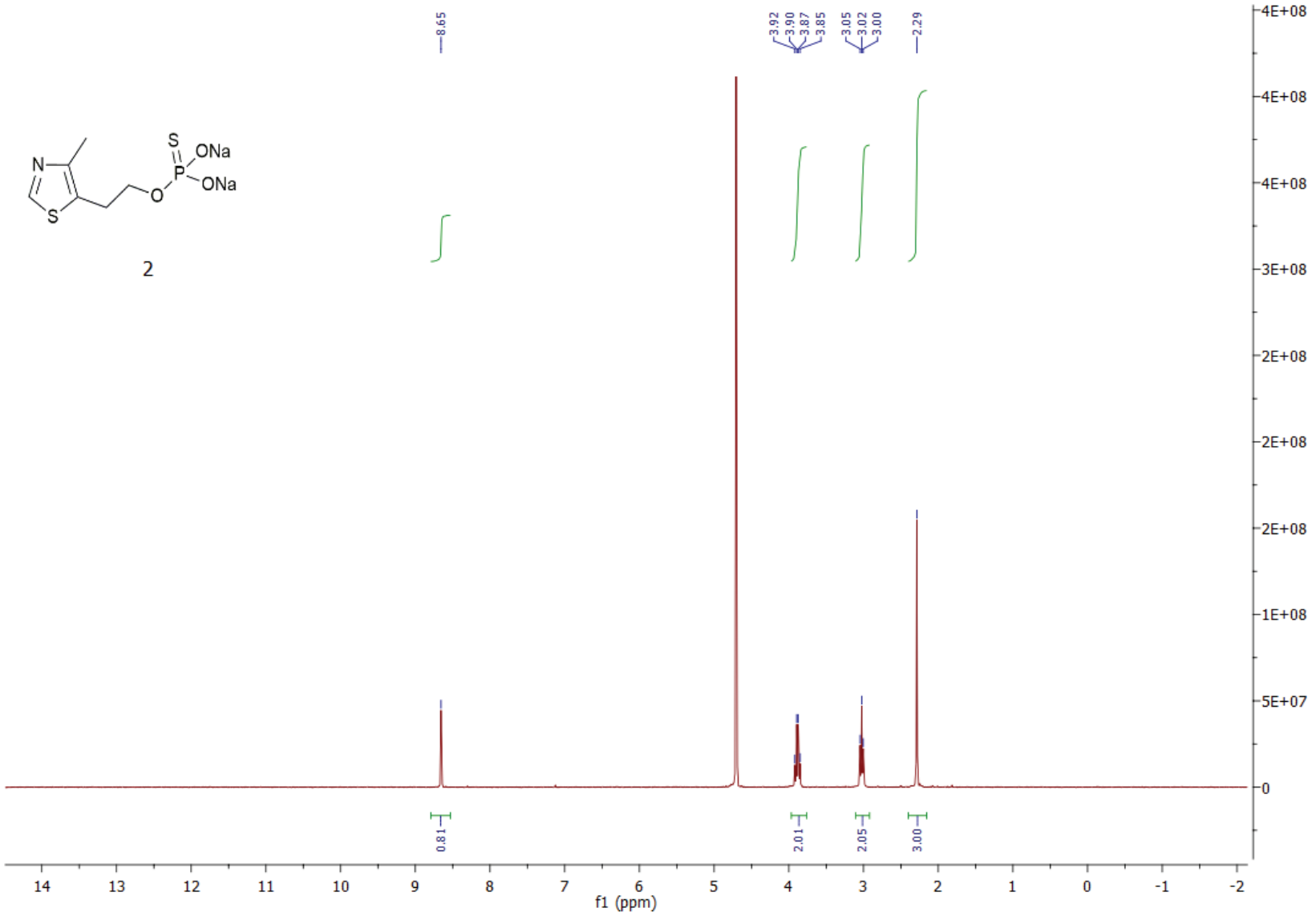
1

^{13}C NMR spectrum of compound **1** (150 MHz, $CDCl_3$)

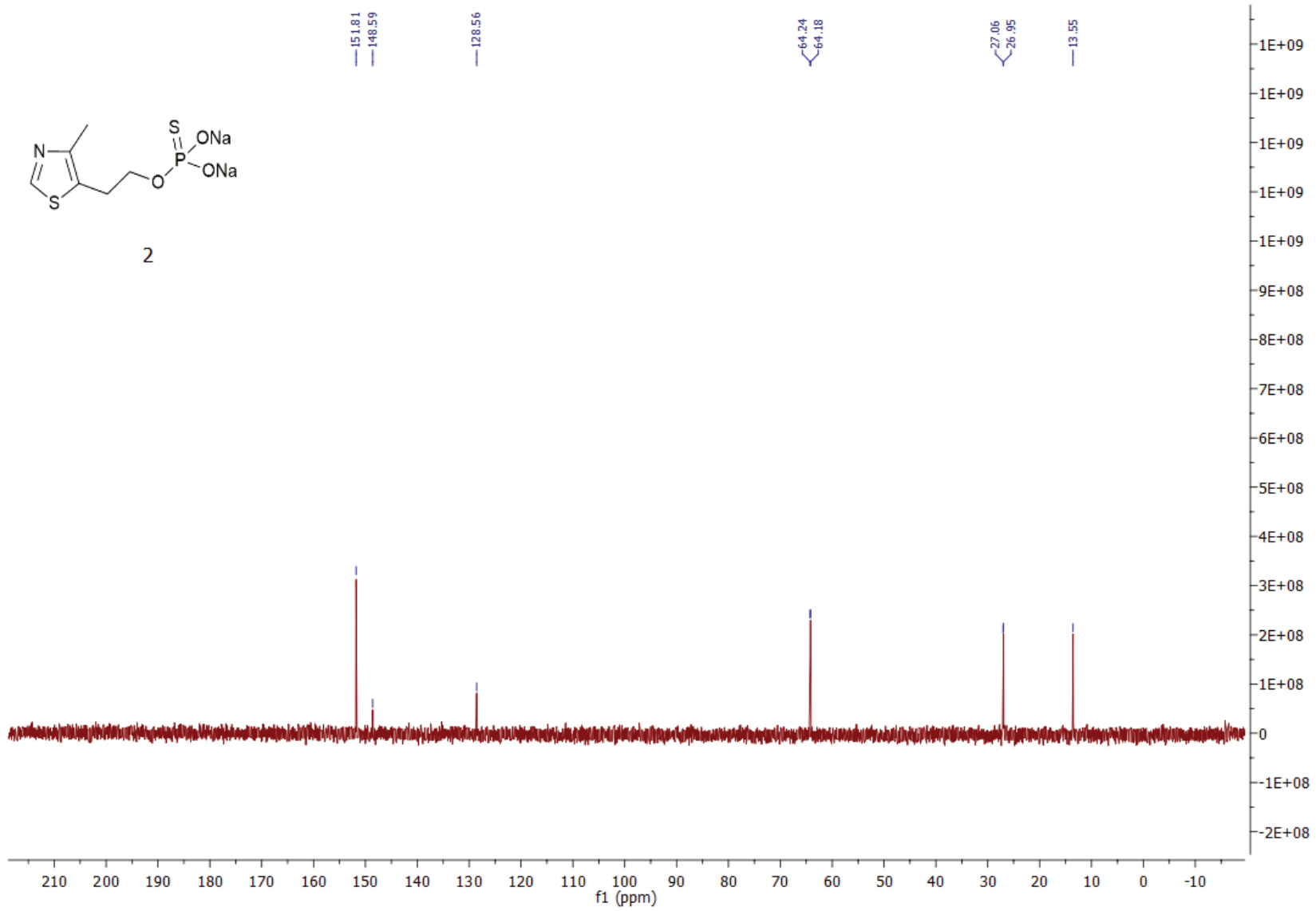


1

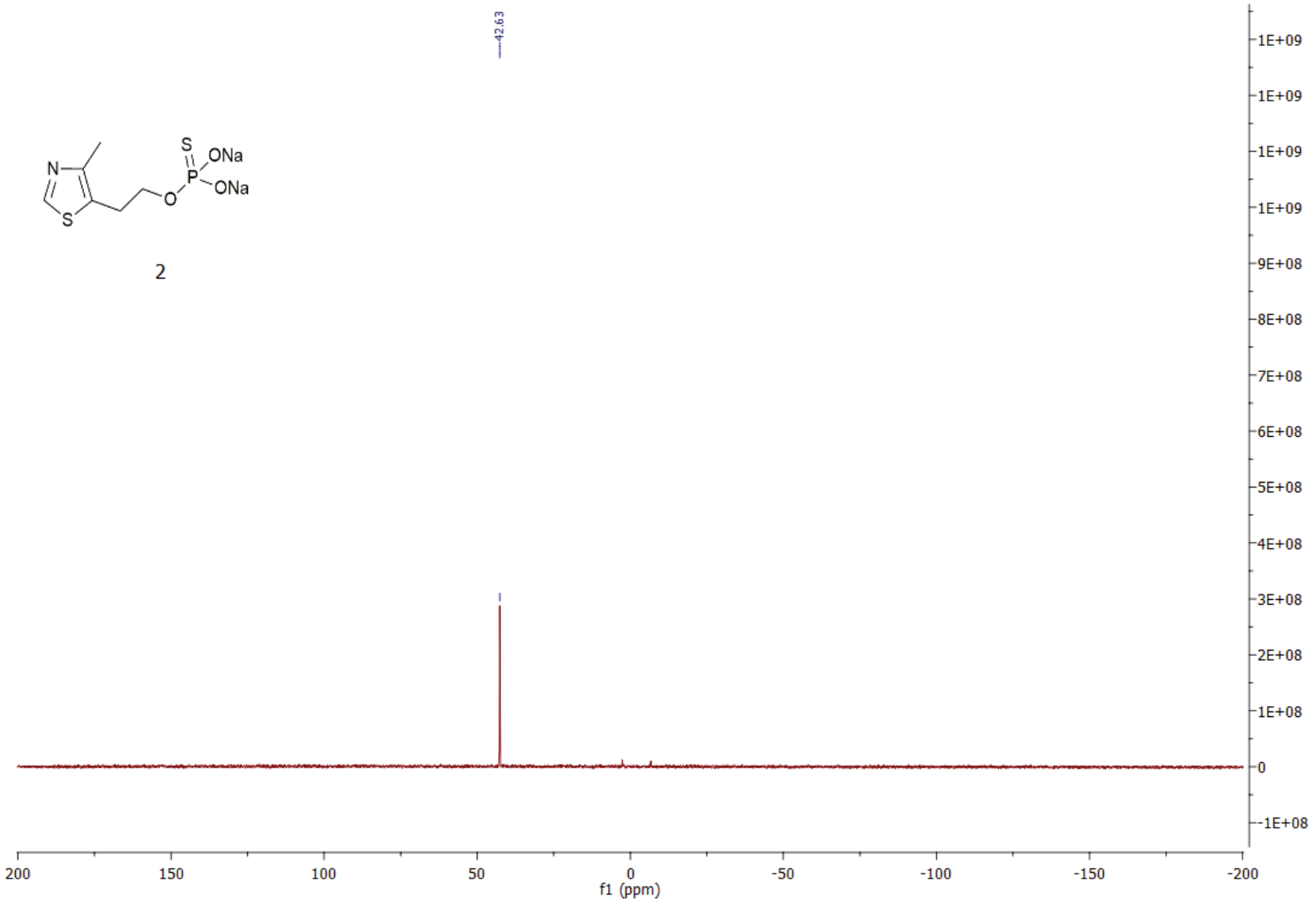
^{31}P NMR spectrum of compound **1** (121 MHz, $CDCl_3$)



^{1}H NMR spectrum of compound **2** (300 MHz, D_2O)

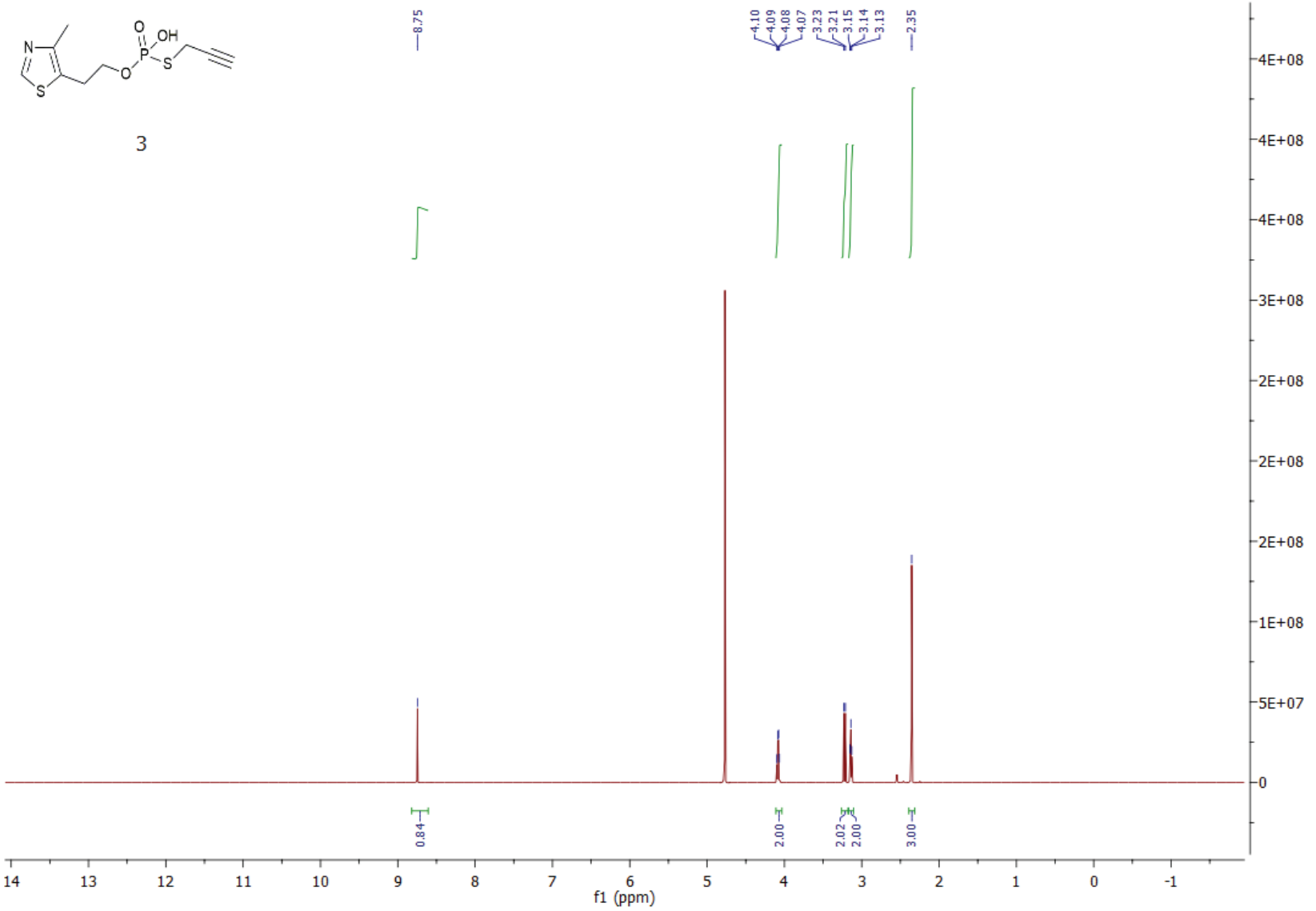


^{13}C NMR spectrum of compound **2** (75 MHz, D_2O)

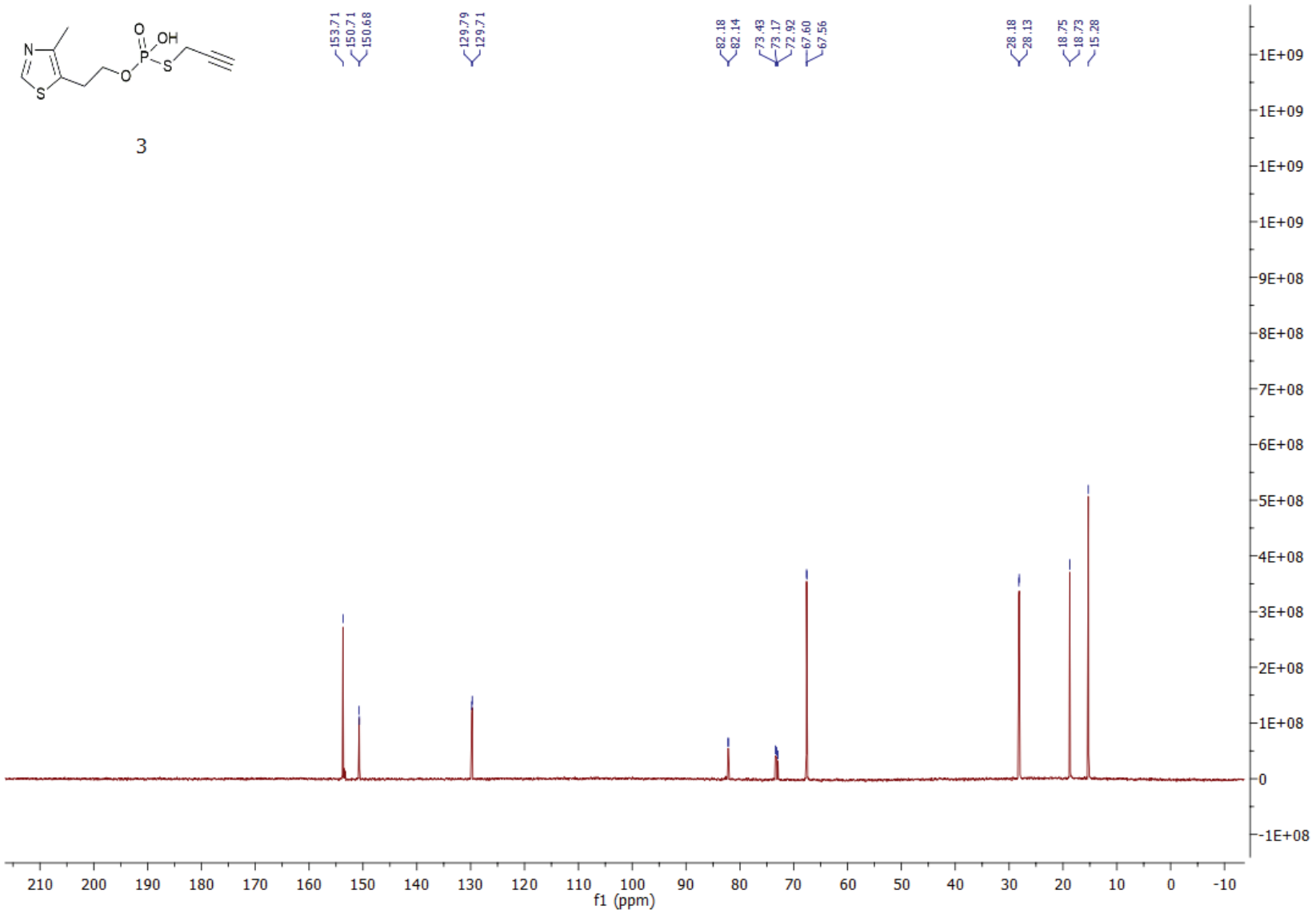


2

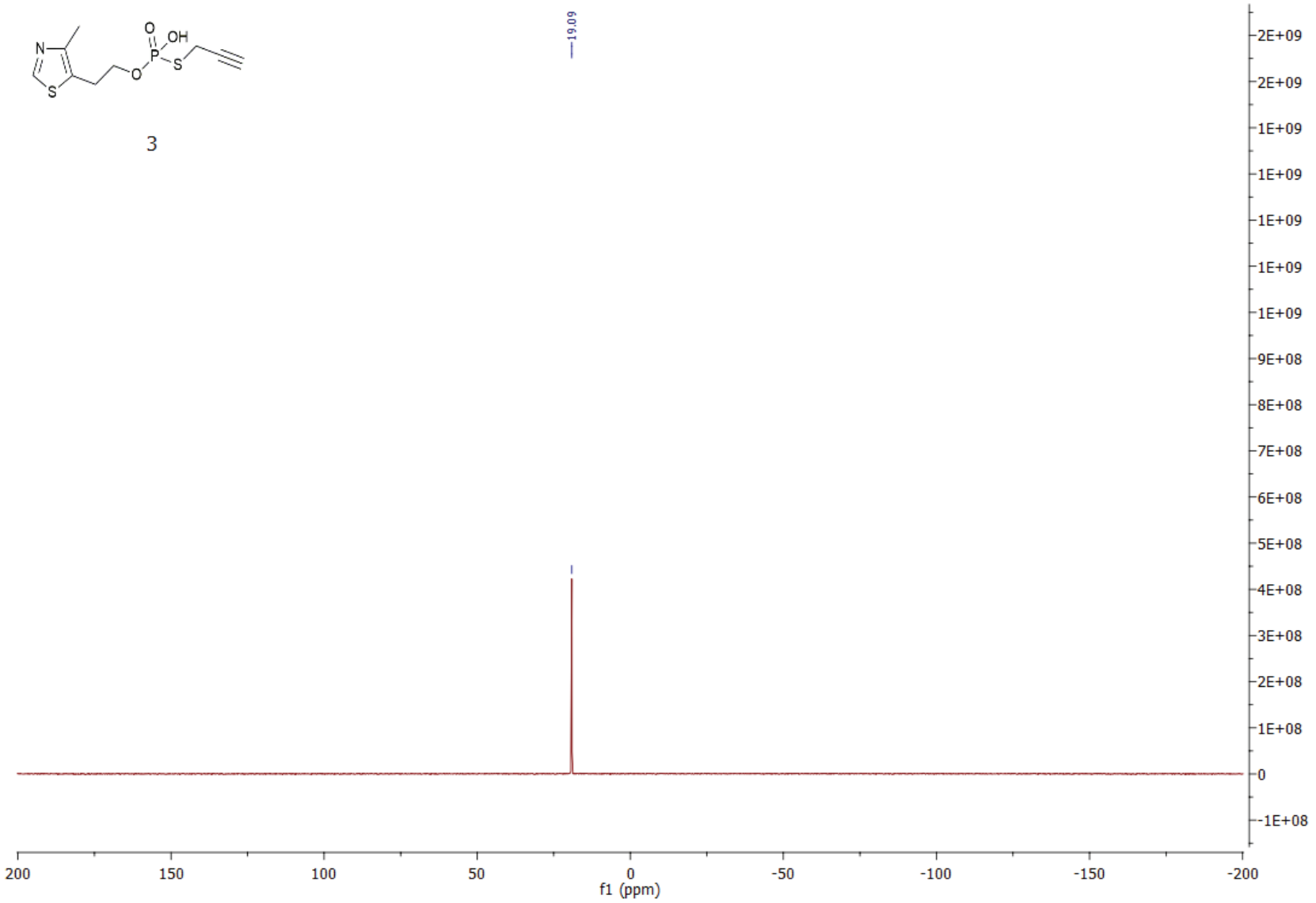
^{31}P NMR spectrum of compound **2** (121 MHz, D_2O)



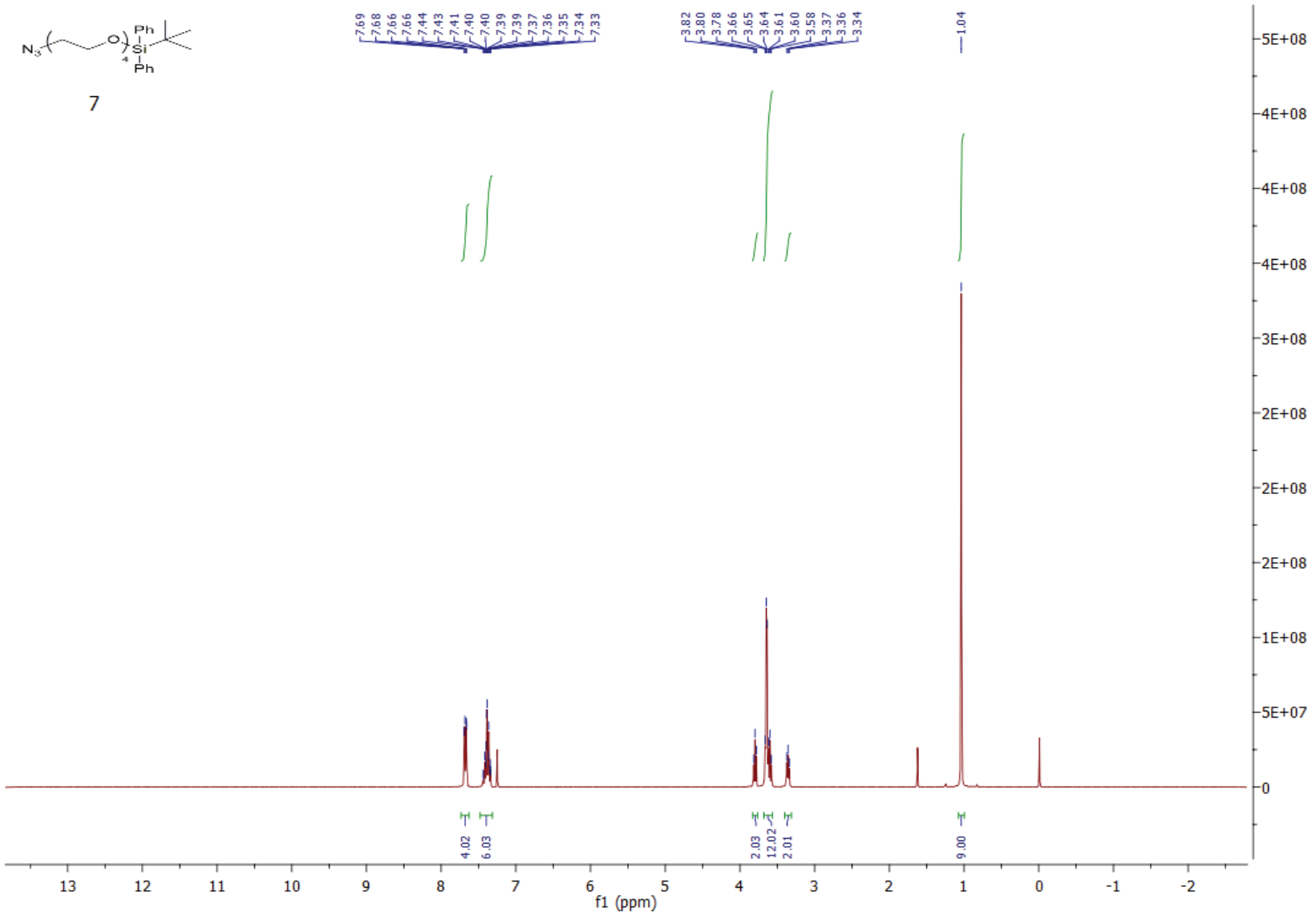
^{1}H NMR spectrum of compound **3** (600 MHz, D_2O)



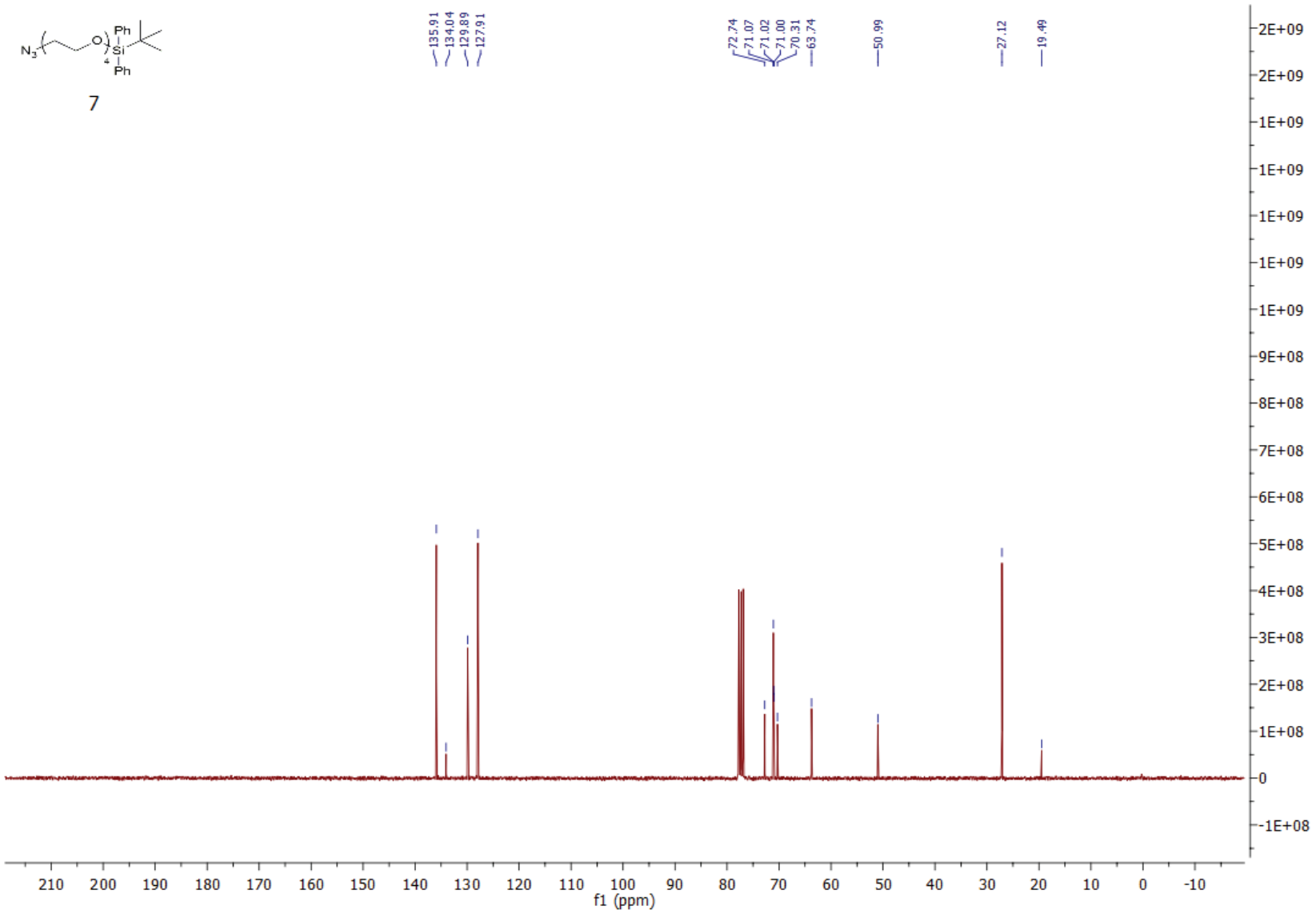
^{13}C NMR spectrum of compound **3** (151 MHz, D_2O)



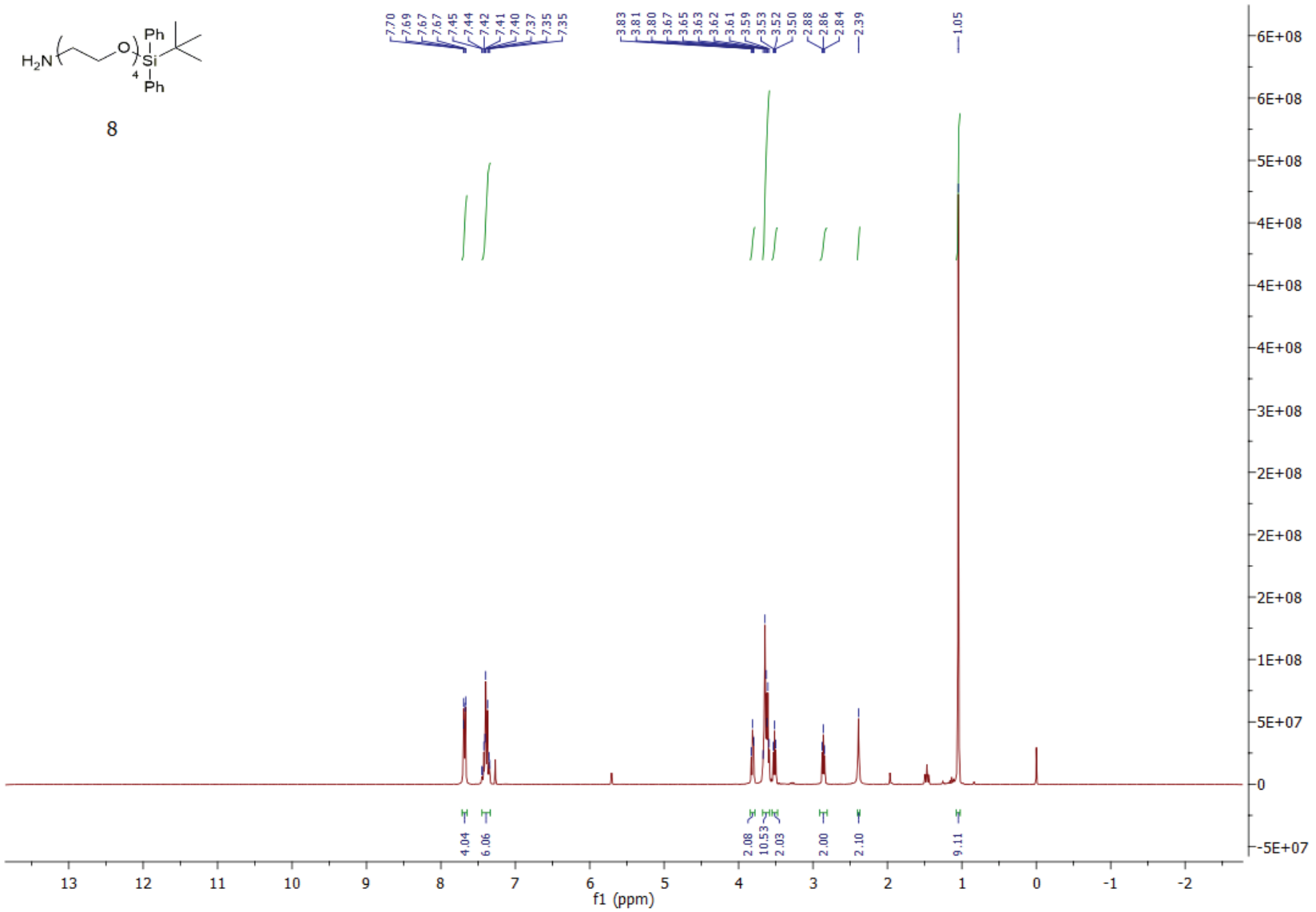
^{31}P NMR spectrum of compound **3** (121 MHz, D_2O)



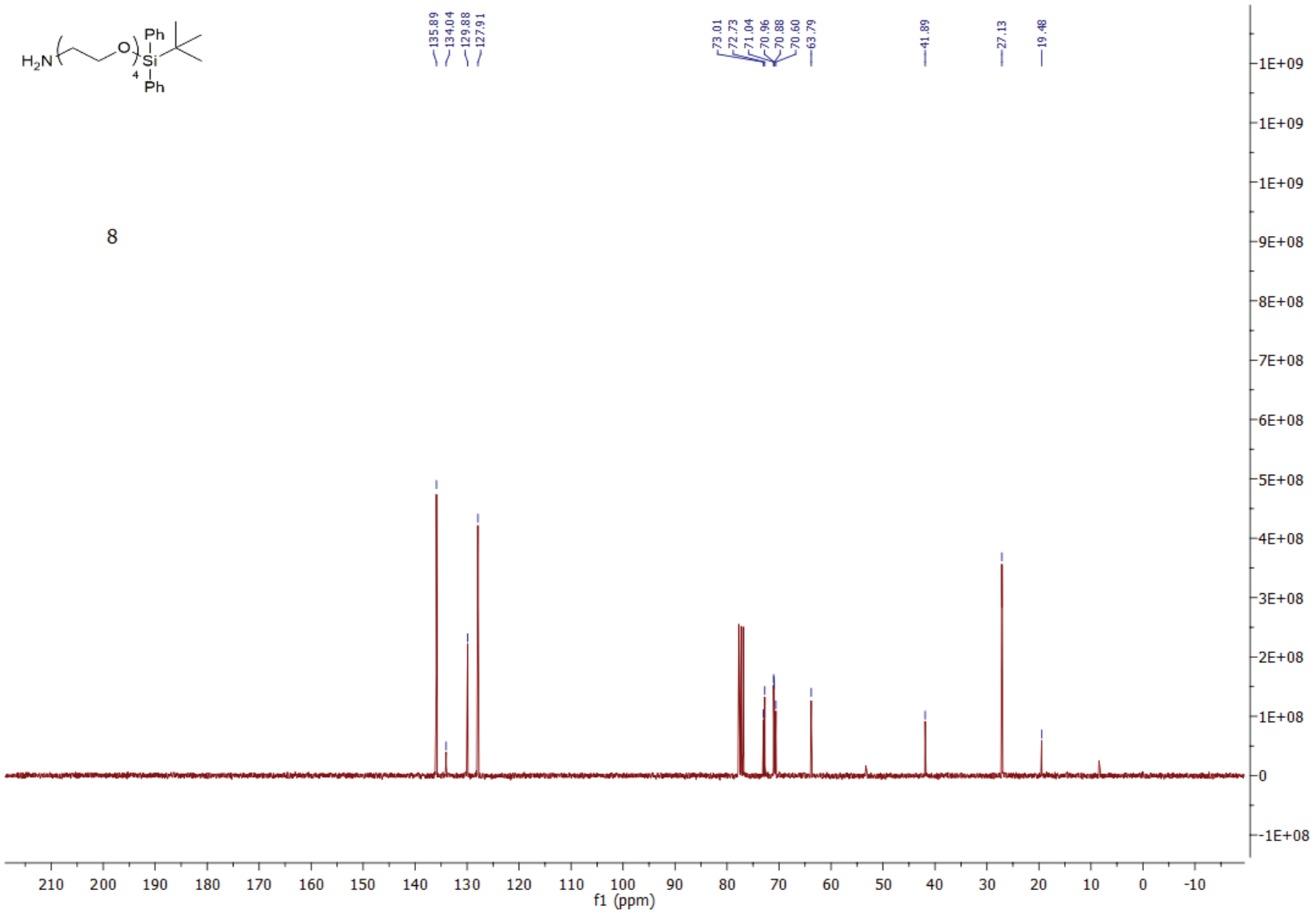
^{1}H NMR spectrum of compound **7** (300 MHz, $CDCl_3$)



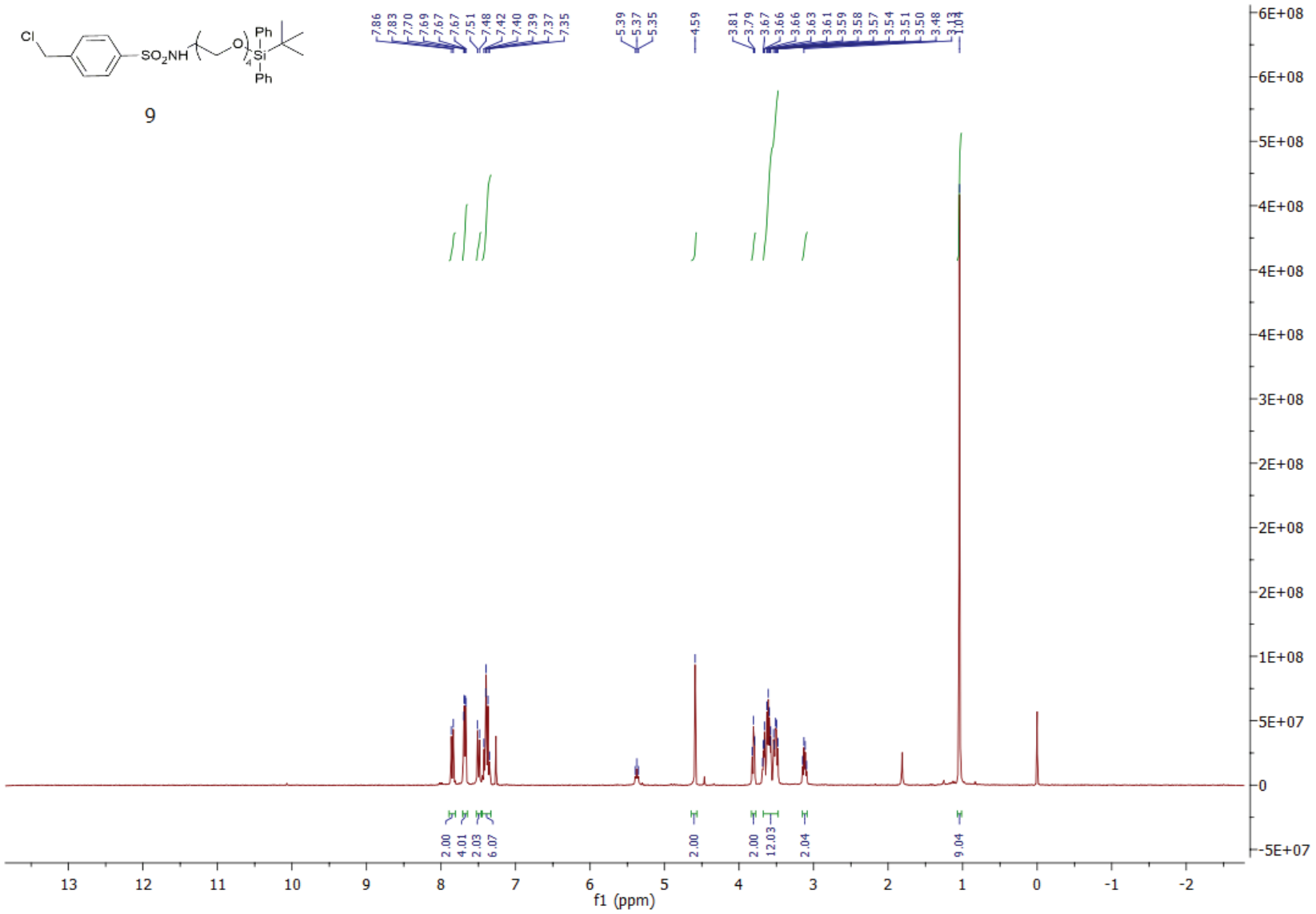
^{13}C NMR spectrum of compound **7** (75 MHz, $CDCl_3$)



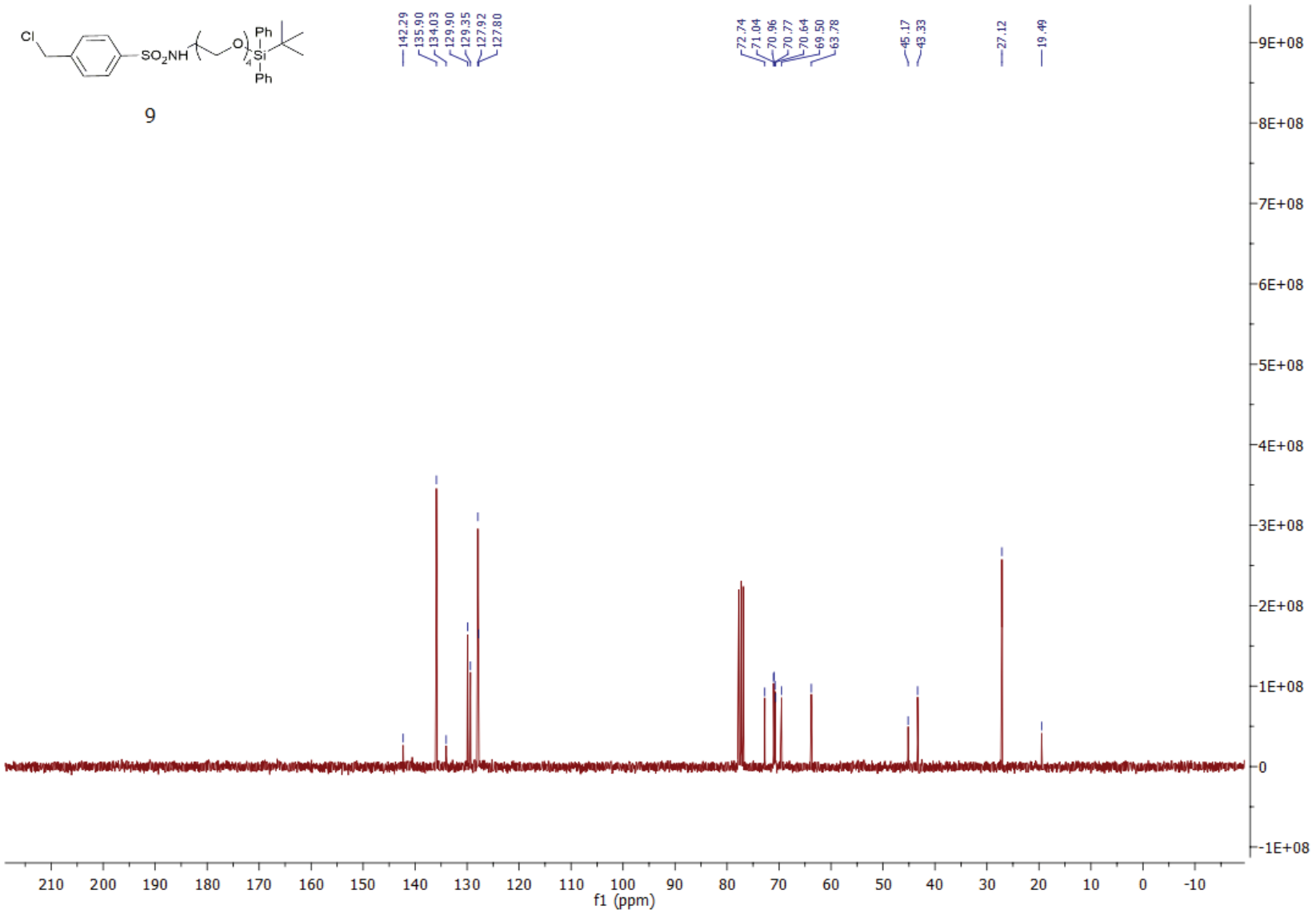
^{1}H NMR spectrum of compound **8** (300 MHz, $CDCl_3$)



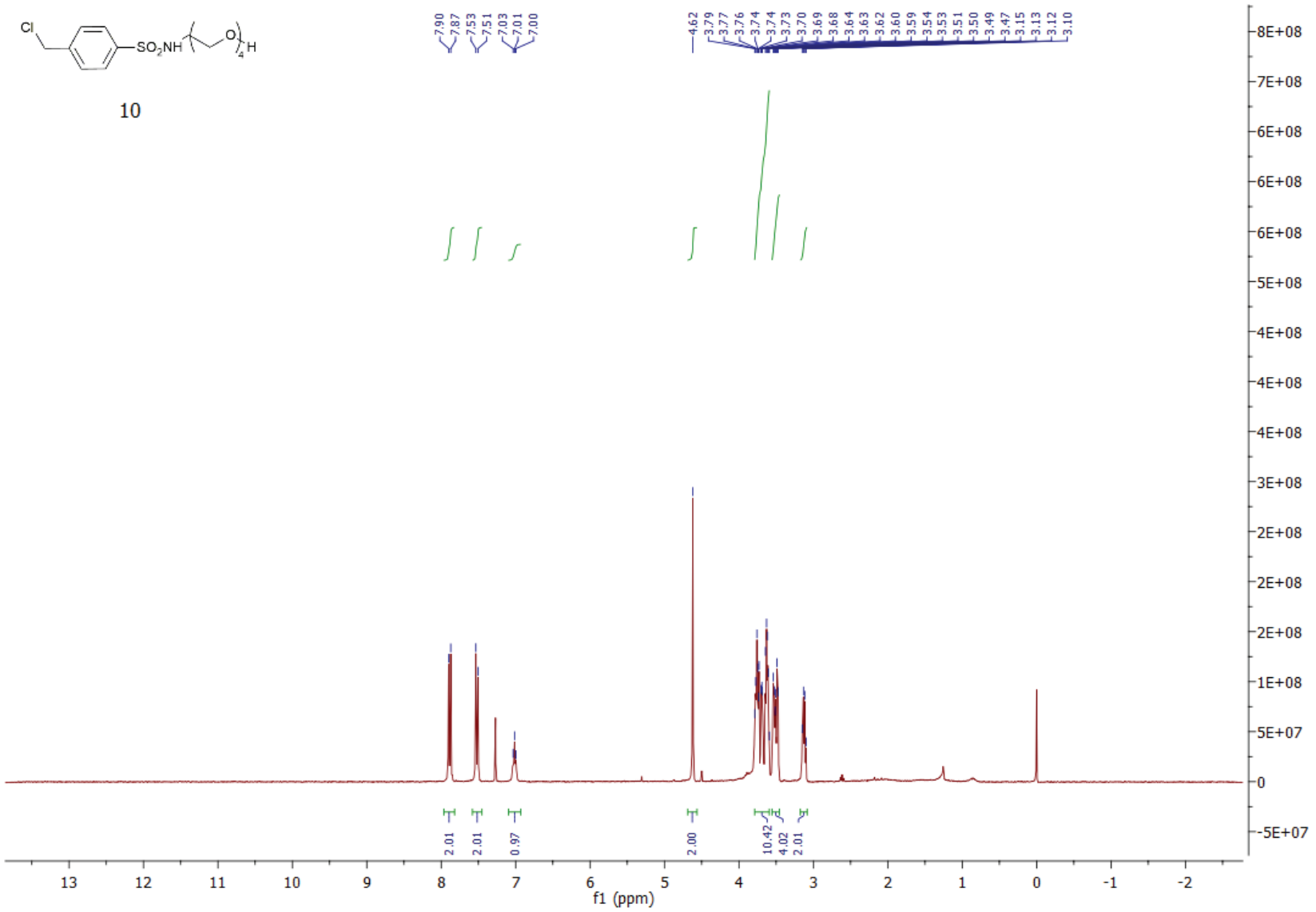
^{13}C NMR spectrum of compound **8** (75 MHz, $CDCl_3$)



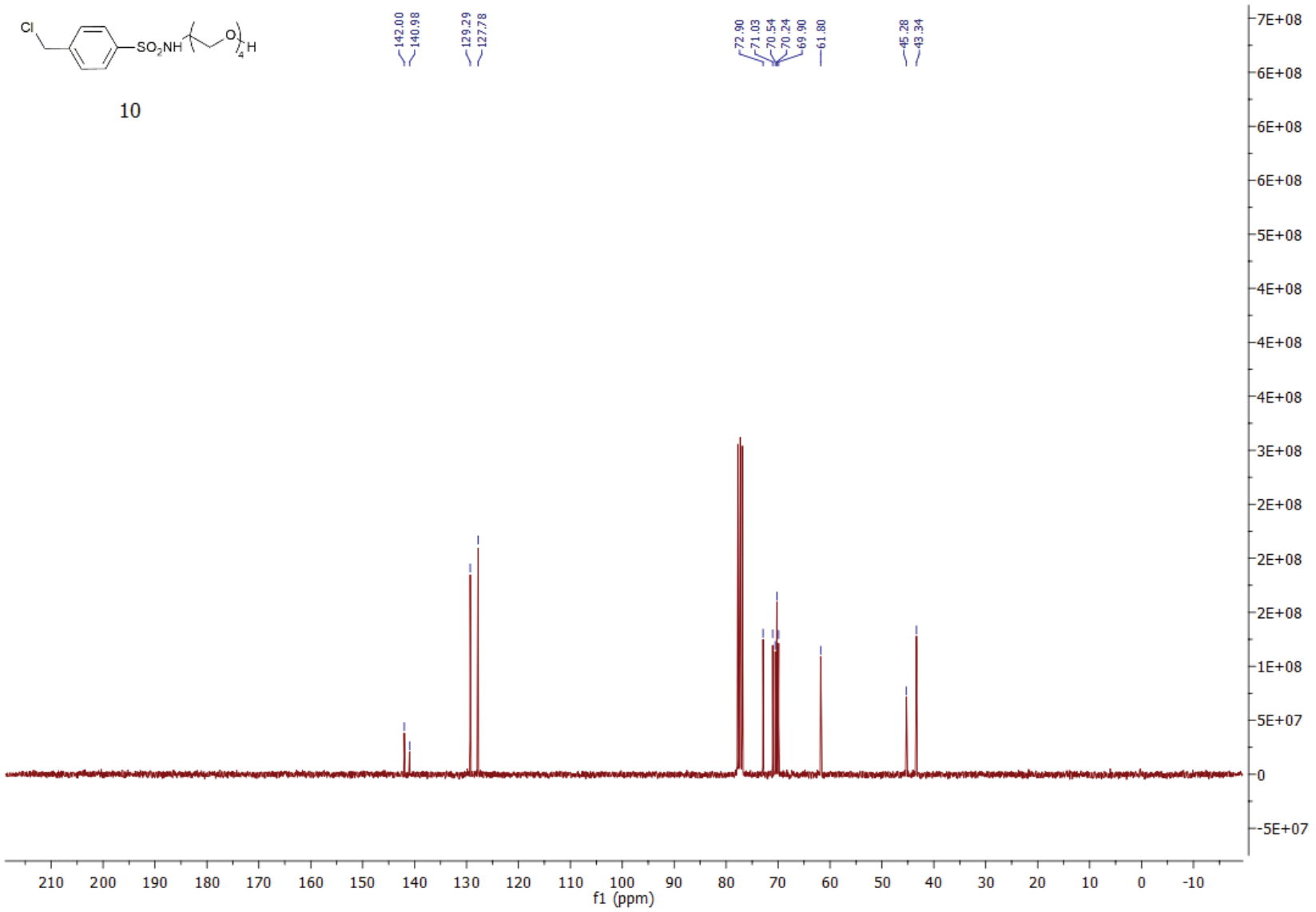
^{1}H NMR spectrum of compound **9** (300 MHz, $CDCl_3$)



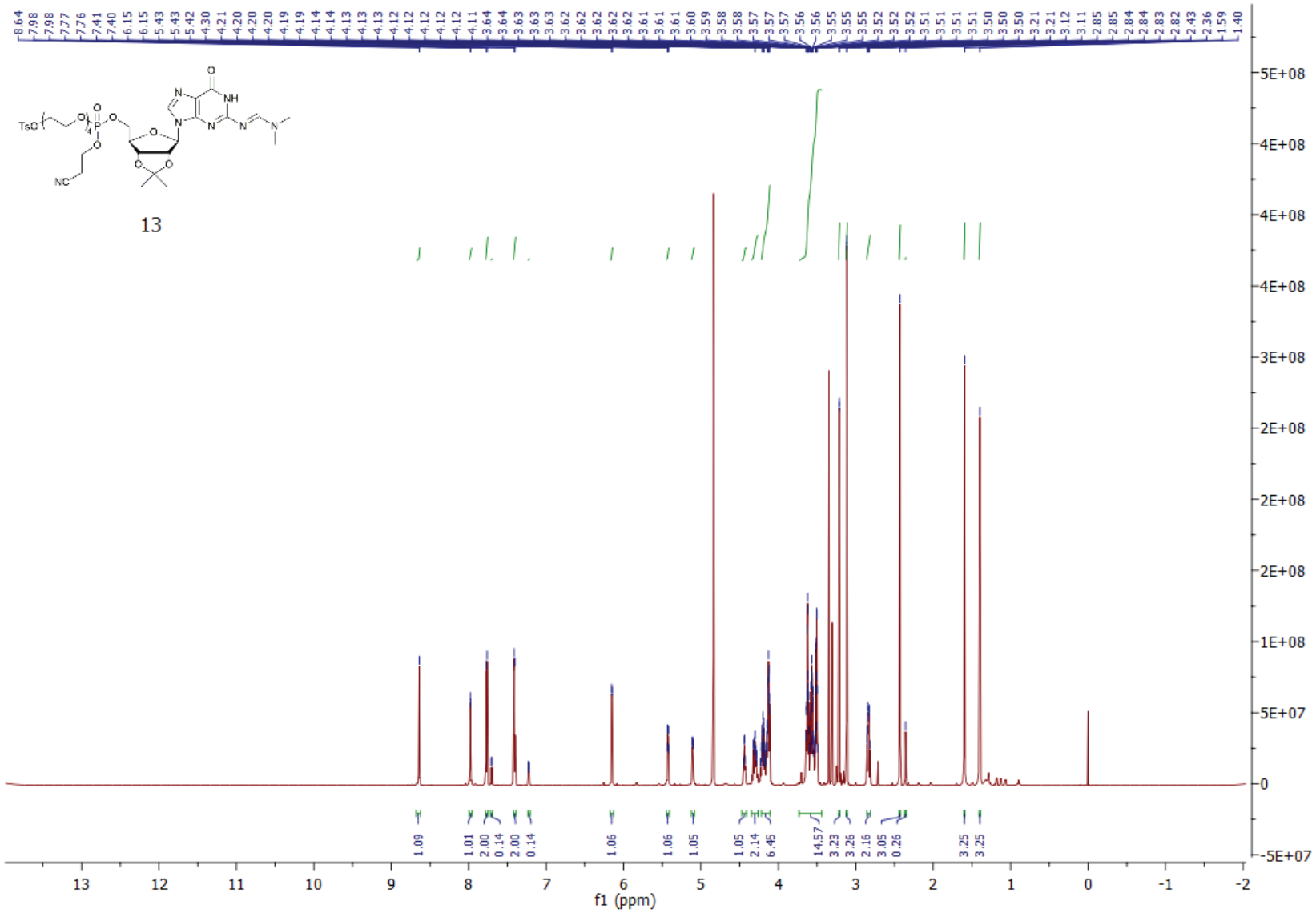
^{13}C NMR spectrum of compound **9** (150 MHz, $CDCl_3$)



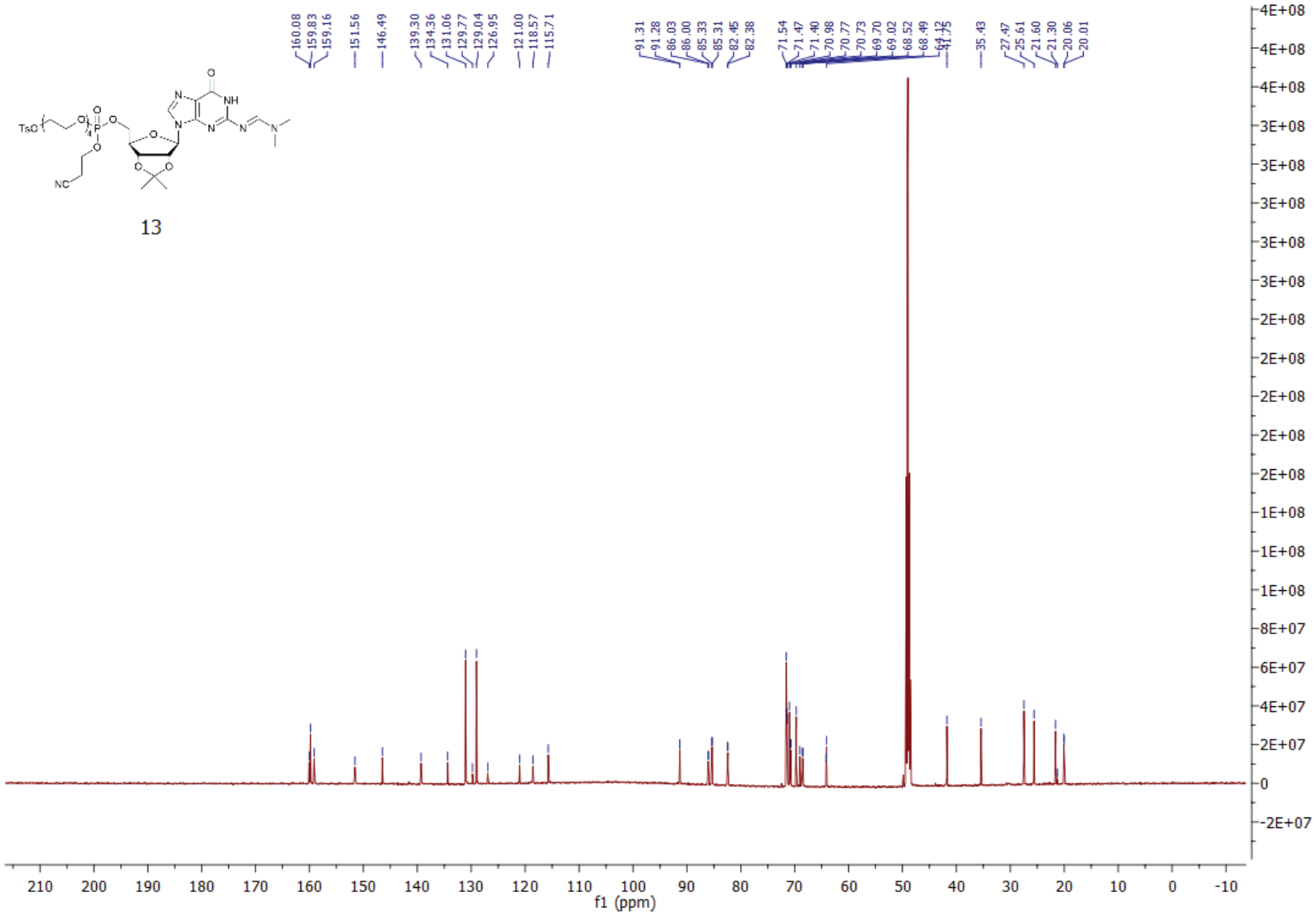
^{1}H NMR spectrum of compound **10** (300 MHz, $CDCl_3$)



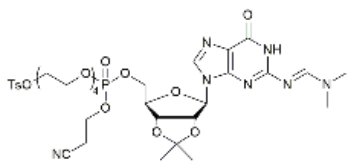
^{13}C NMR spectrum of compound **10** (75 MHz, $CDCl_3$)



^{1}H NMR spectrum of compound **13** (600 MHz, MeOD)

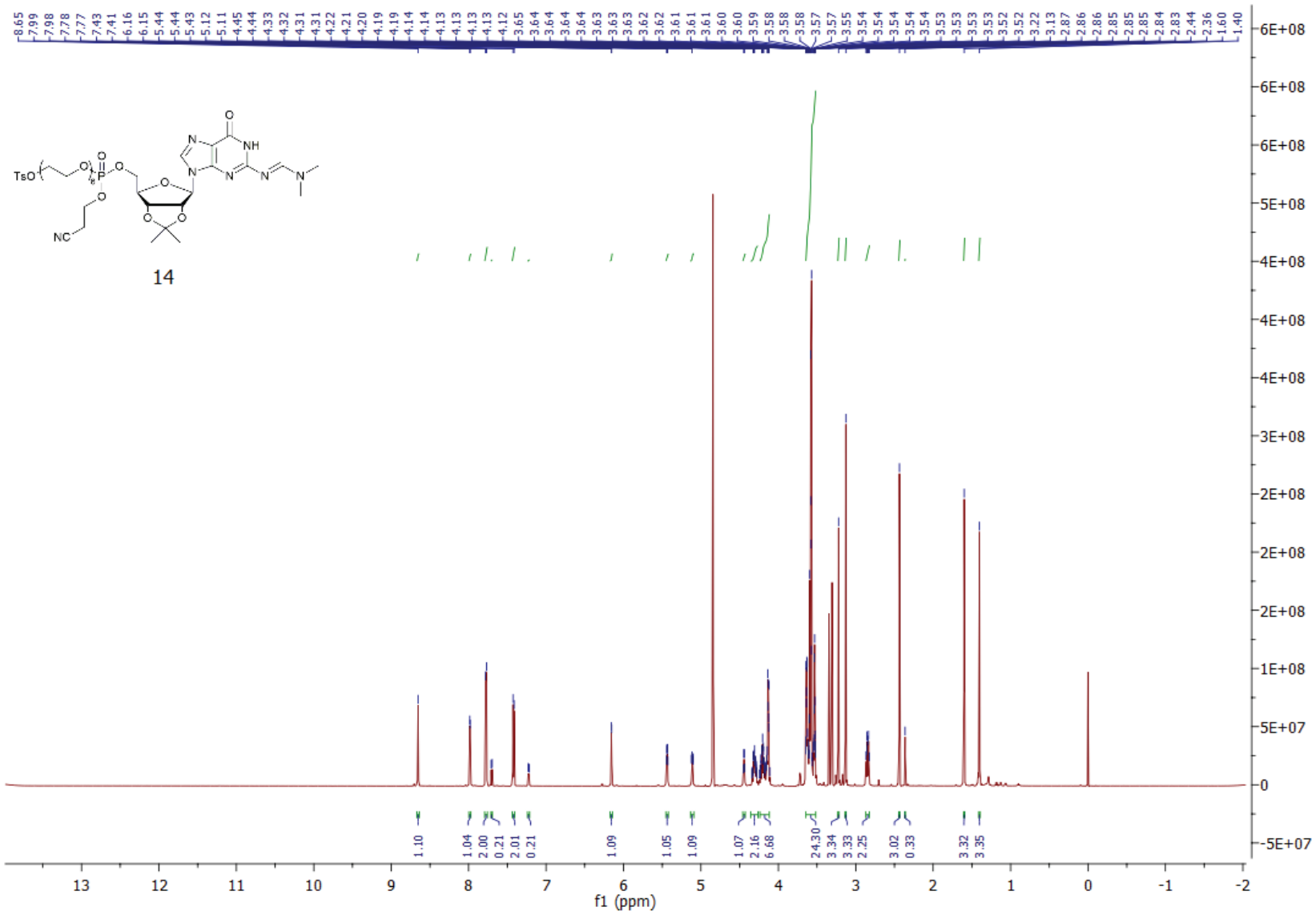


^{13}C NMR spectrum of compound **13** (151 MHz, MeOD)

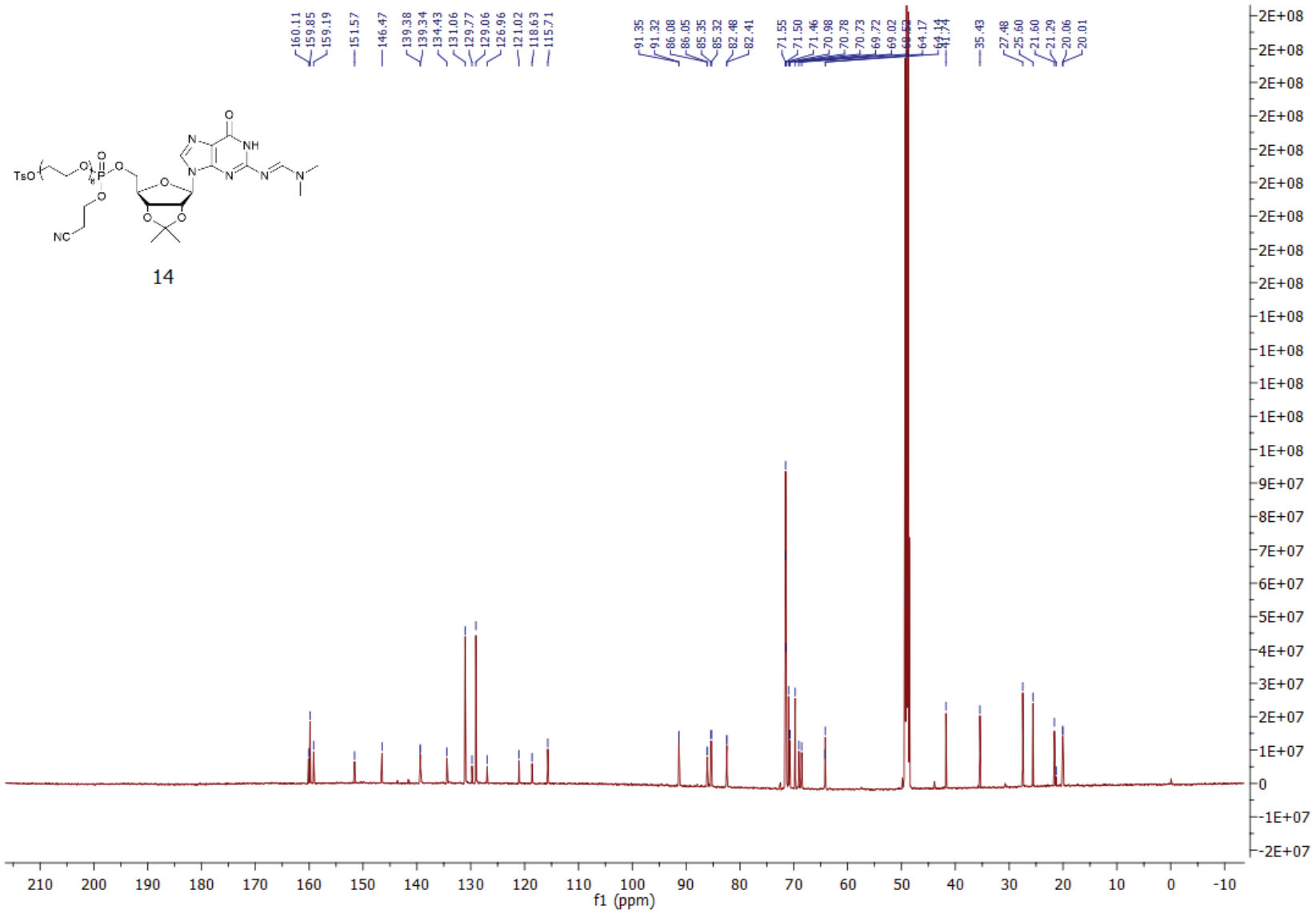


13

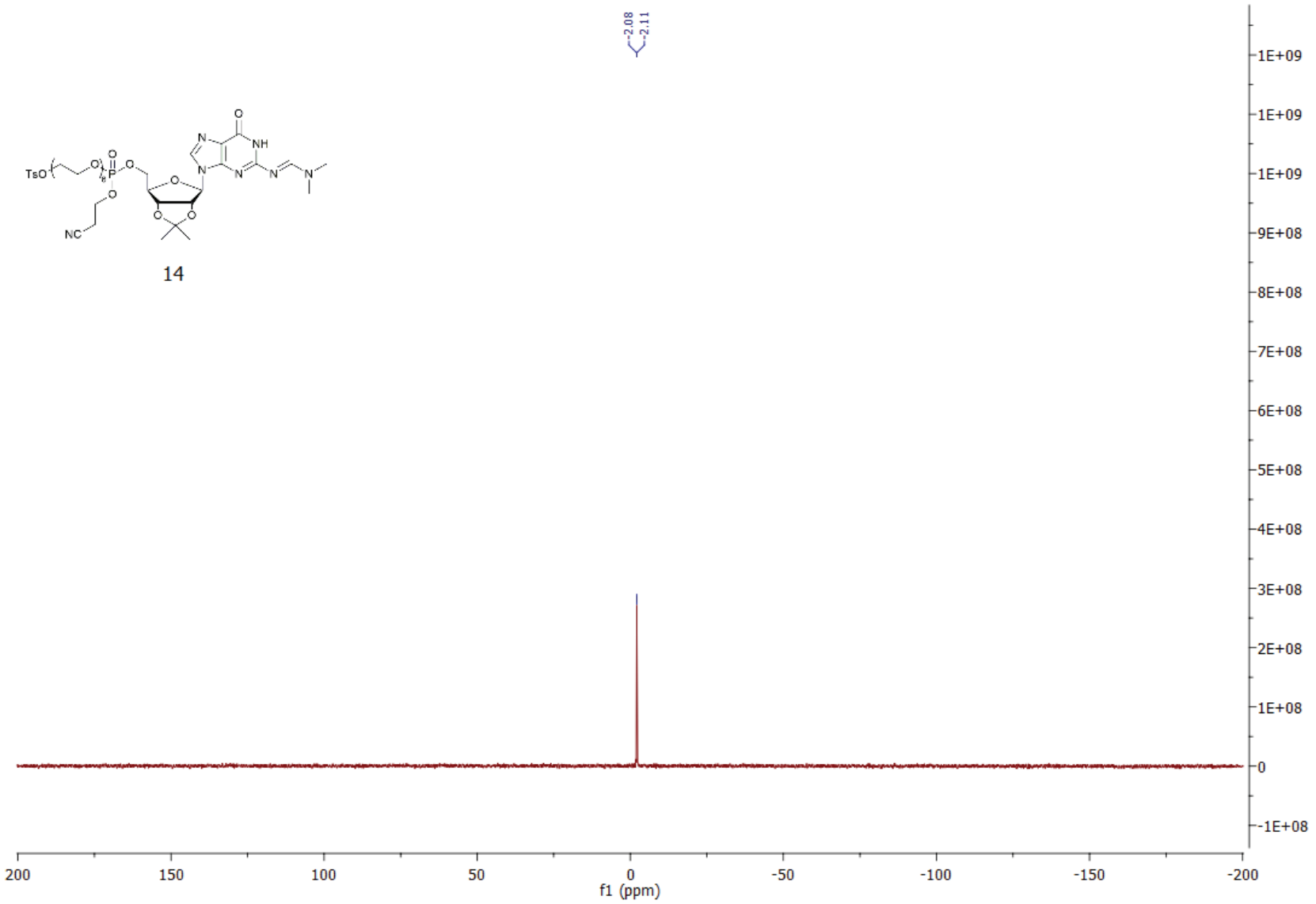
^{31}P NMR spectrum of compound **13** (121 MHz, MeOD)



^{1}H NMR spectrum of compound **14** (600 MHz, MeOD)

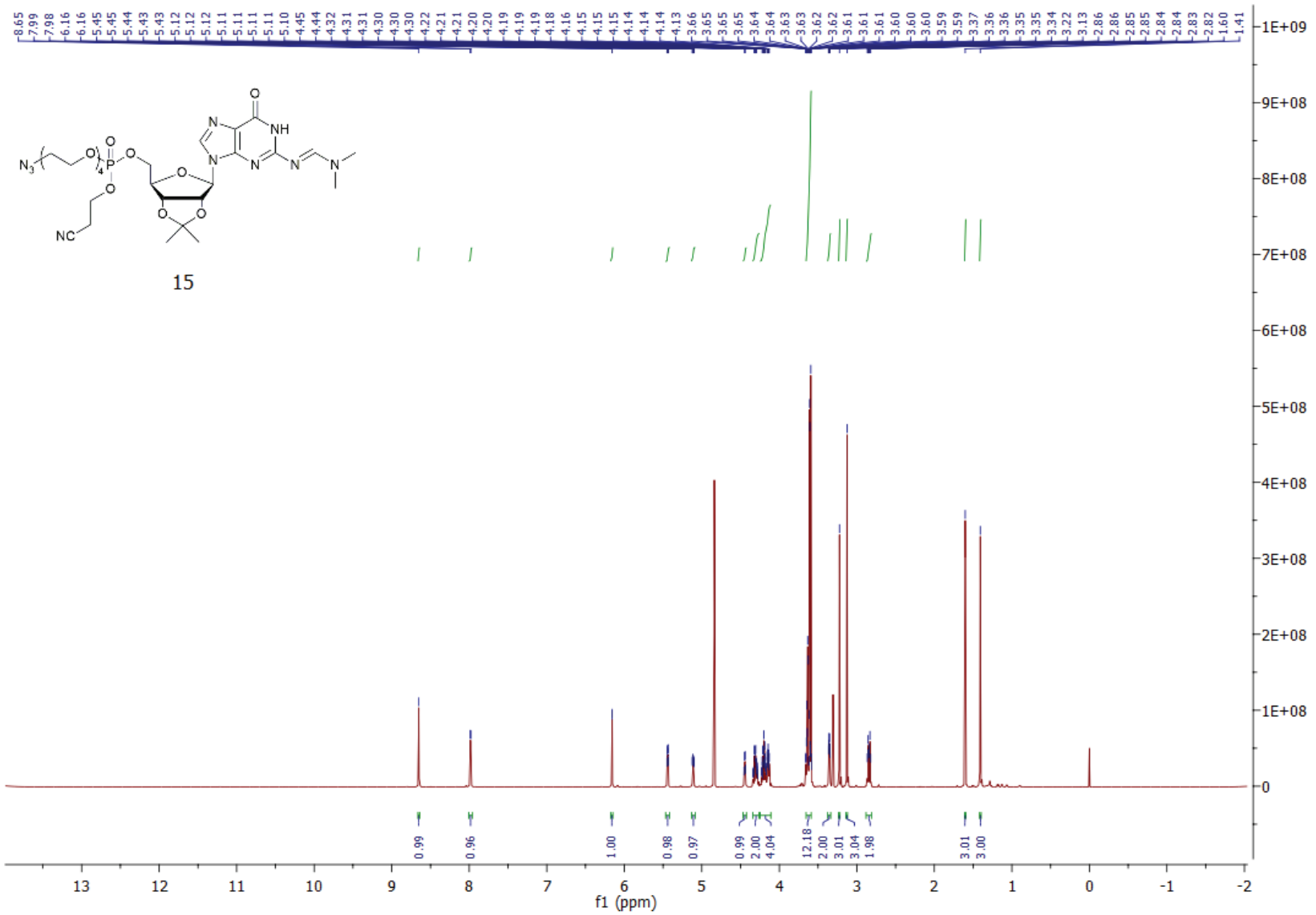


^{13}C NMR spectrum of compound **14** (151 MHz, MeOD)

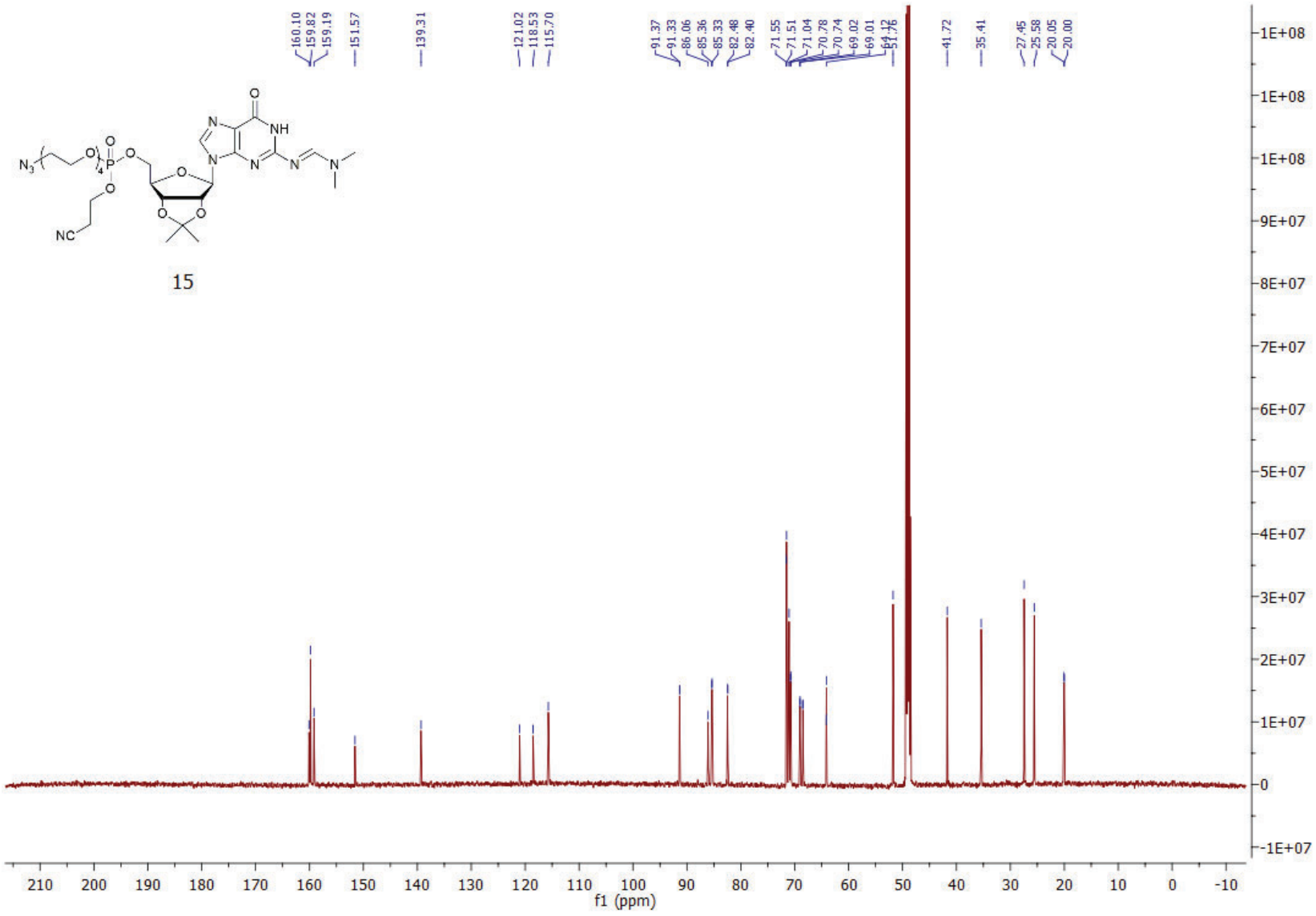


14

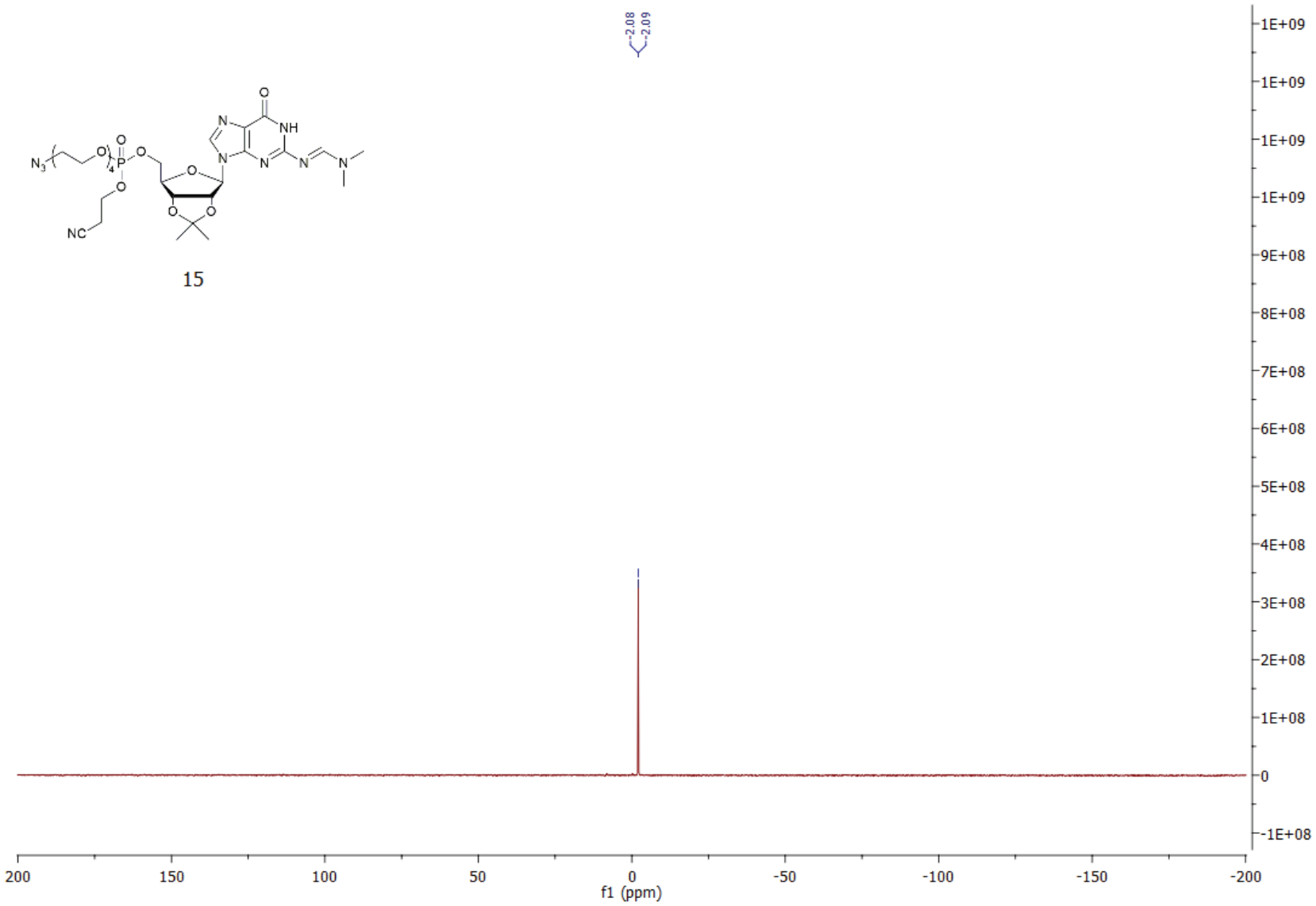
^{31}P NMR spectrum of compound **14** (121 MHz, MeOD)



^{1}H NMR spectrum of compound **15** (600 MHz, MeOD)

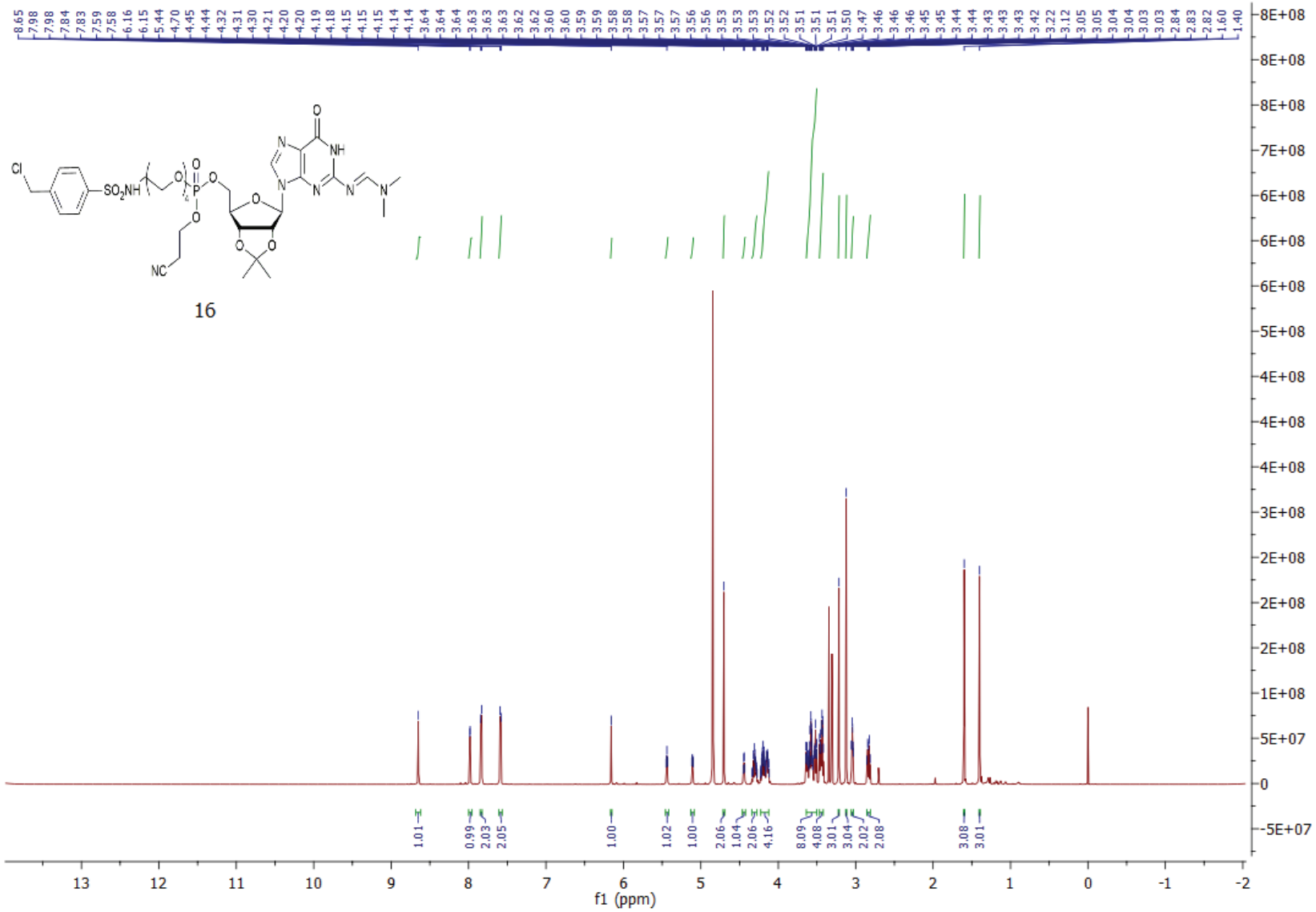


^{13}C NMR spectrum of compound **15** (151 MHz, MeOD)

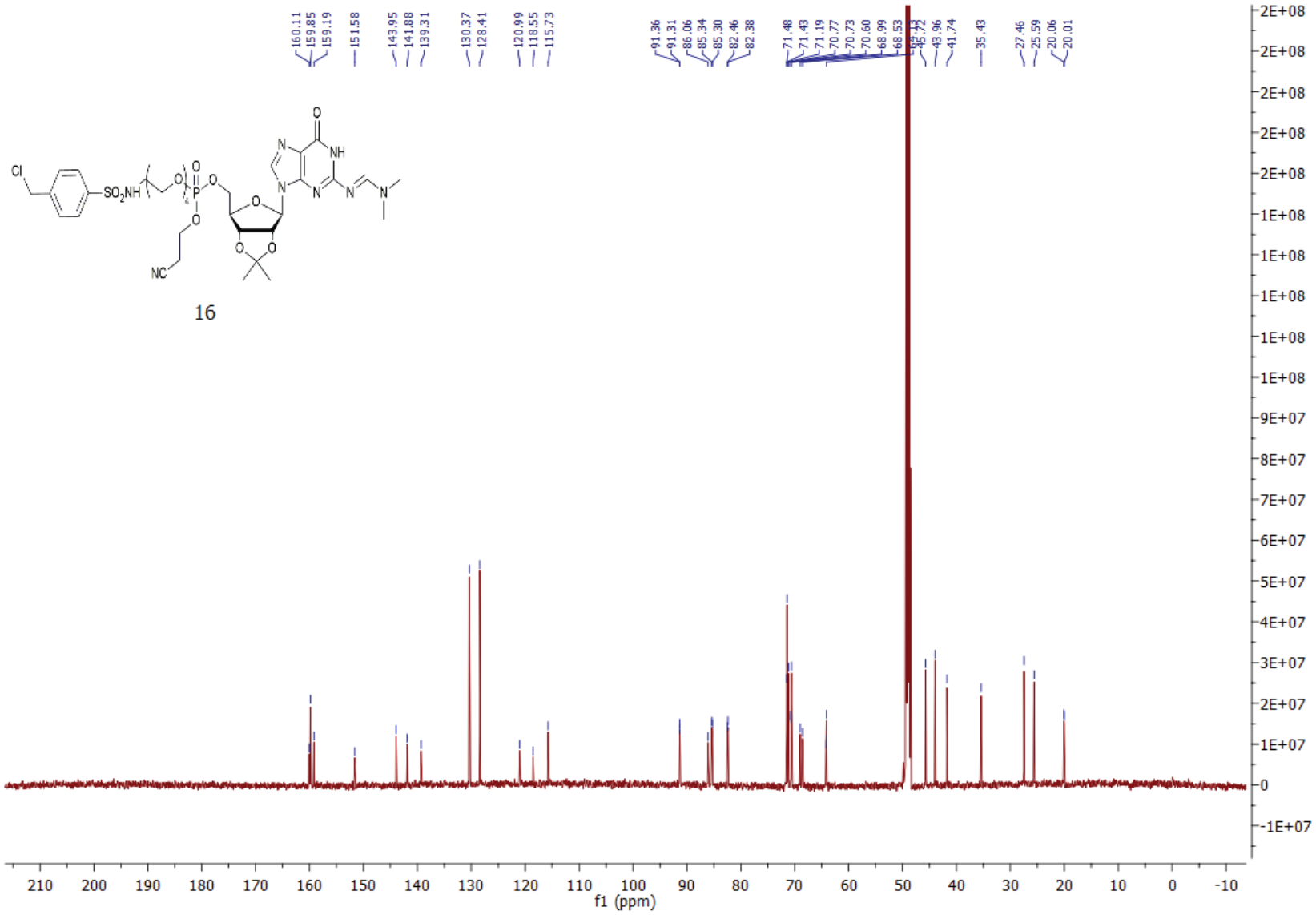


15

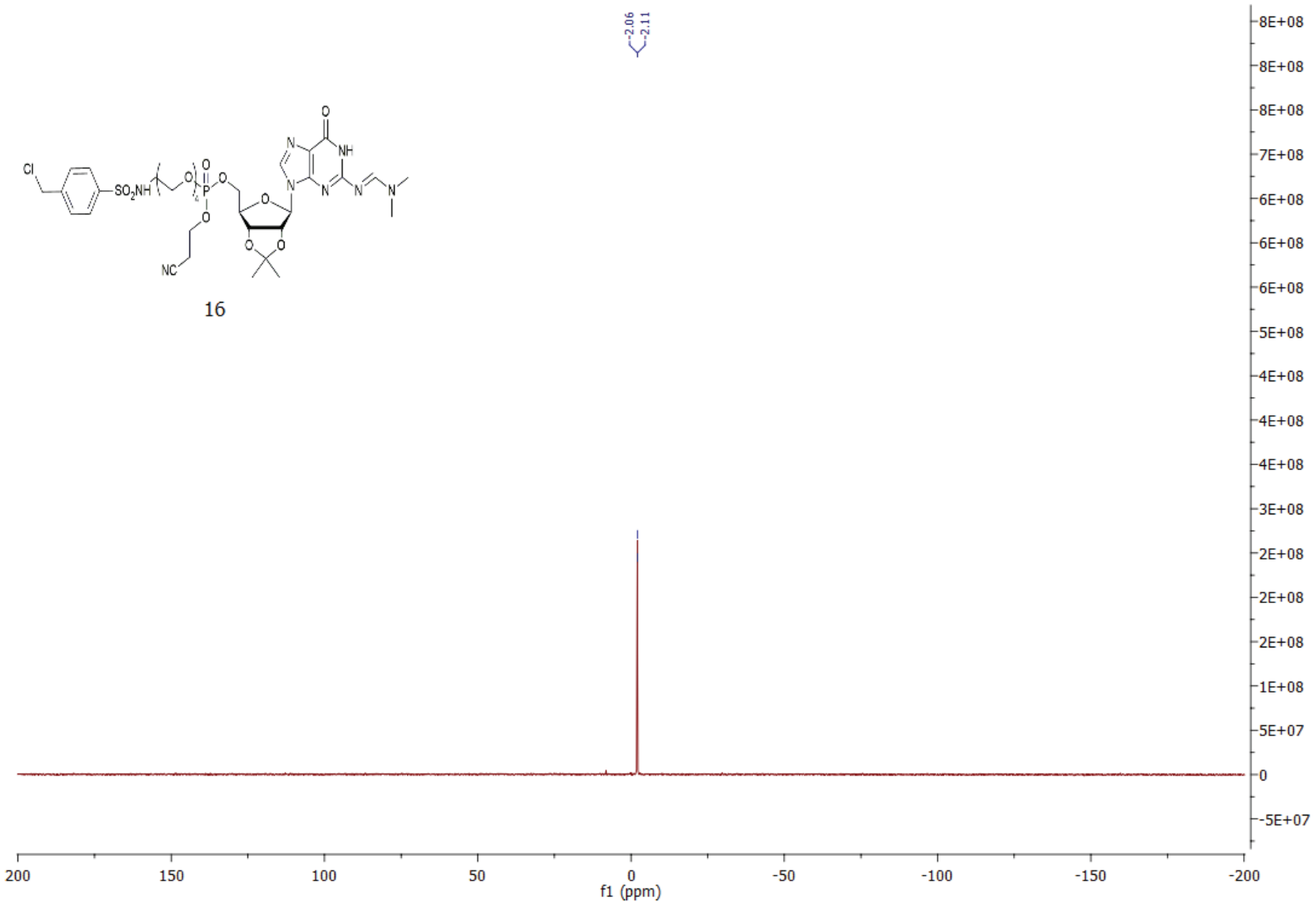
^{31}P NMR spectrum of compound **15** (121 MHz, MeOD)



^{1}H NMR spectrum of compound **16** (600 MHz, MeOD)

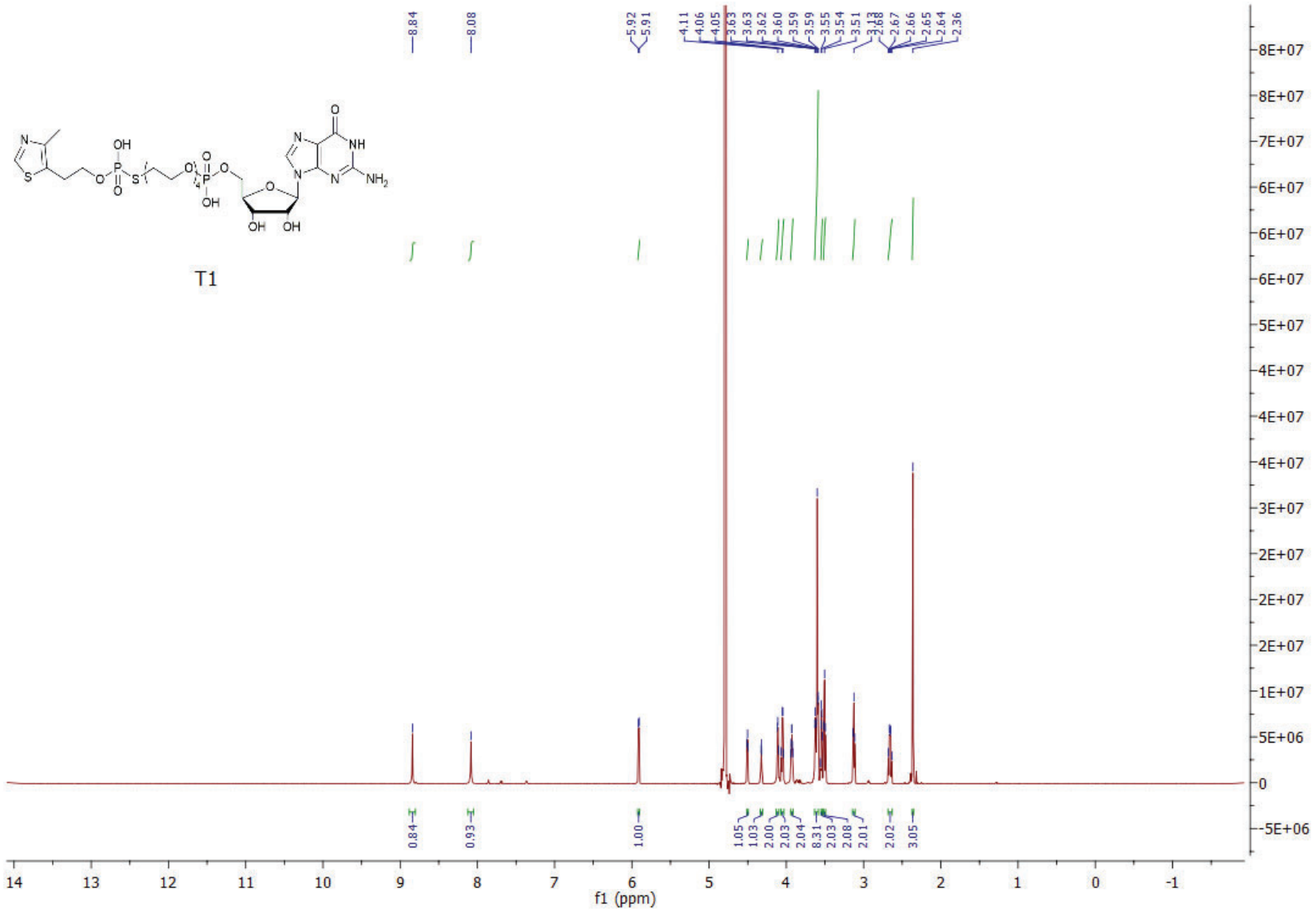


^{13}C NMR spectrum of compound **16** (151 MHz, MeOD)

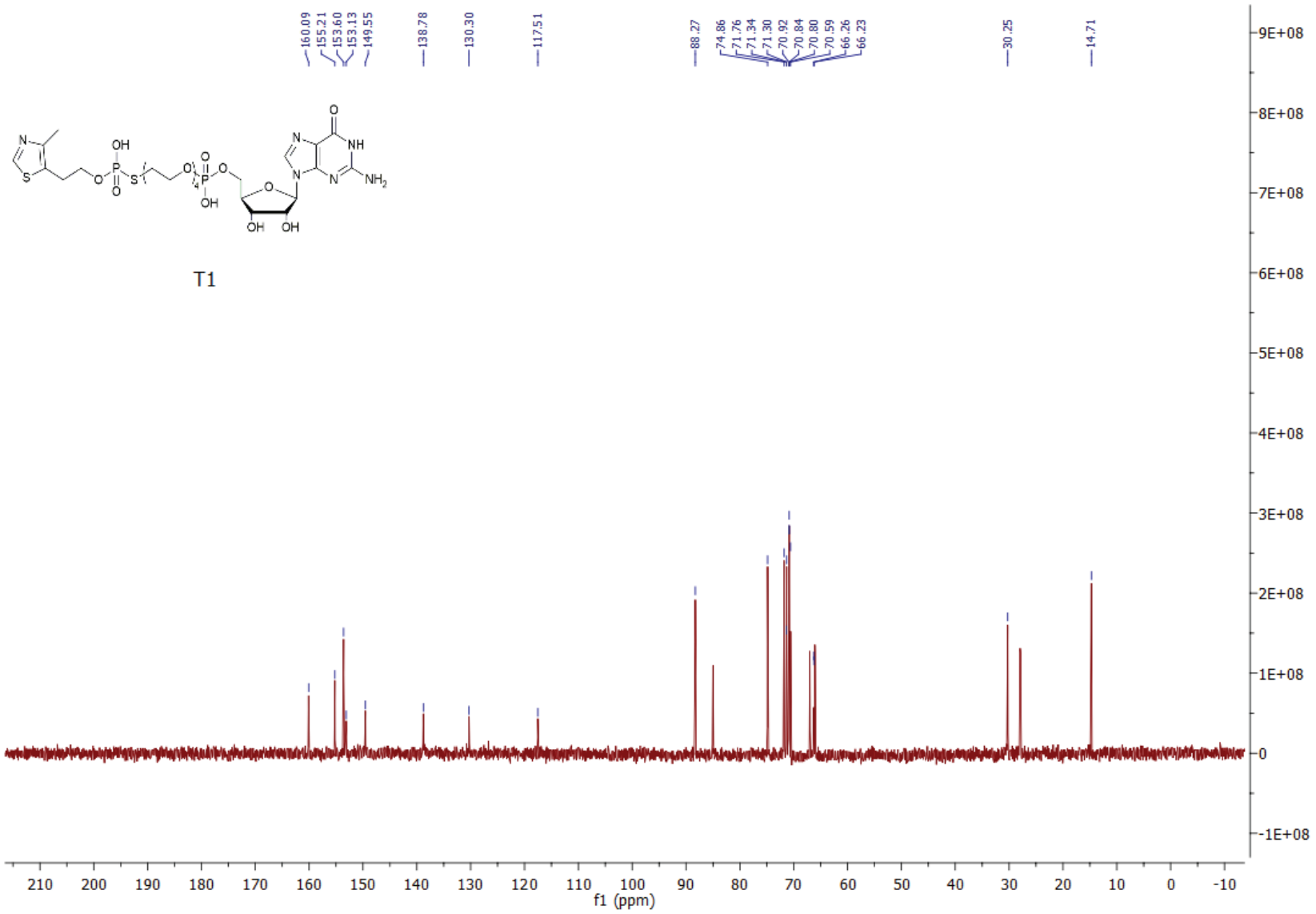


16

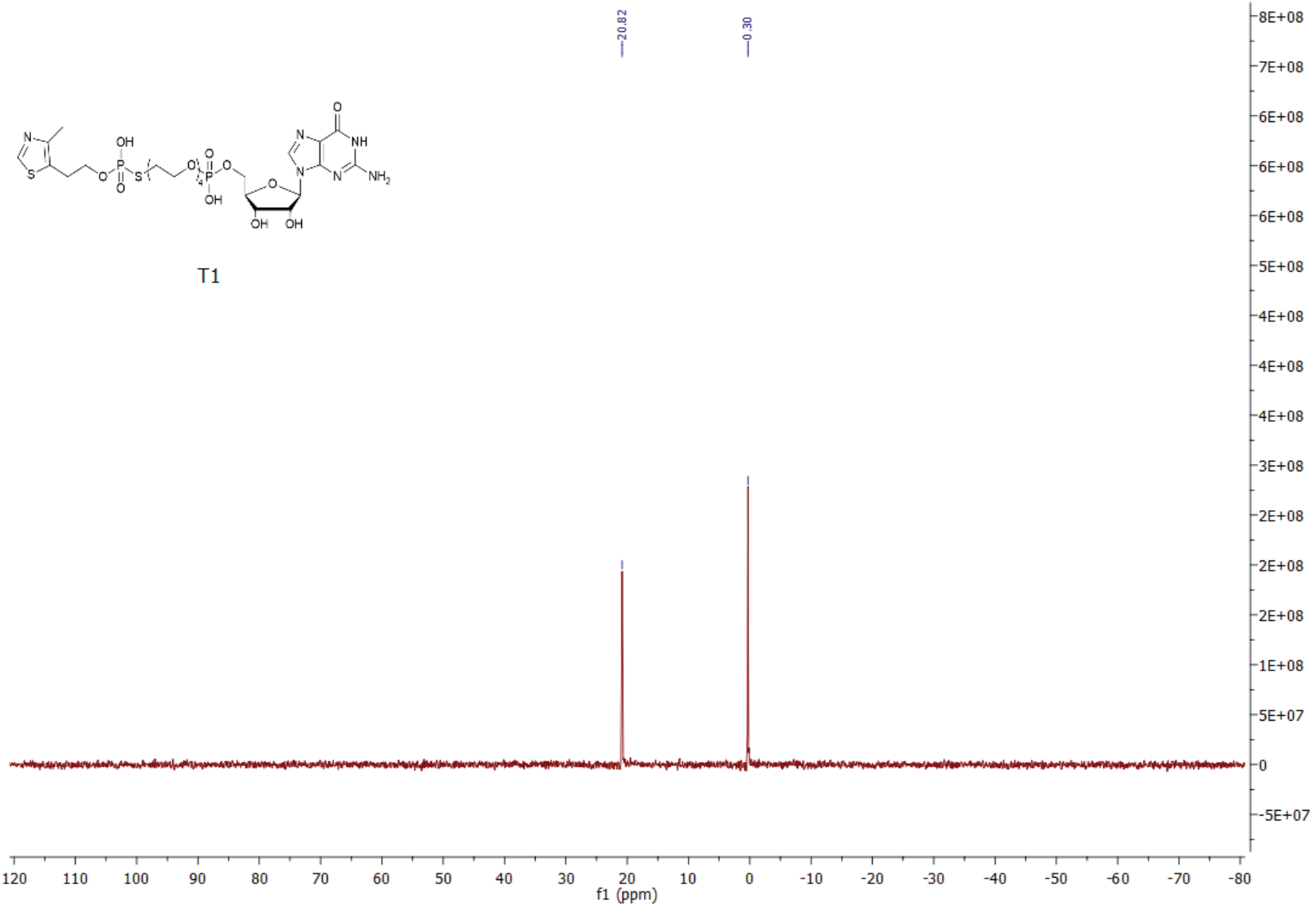
^{31}P NMR spectrum of compound **16** (121 MHz, MeOD)



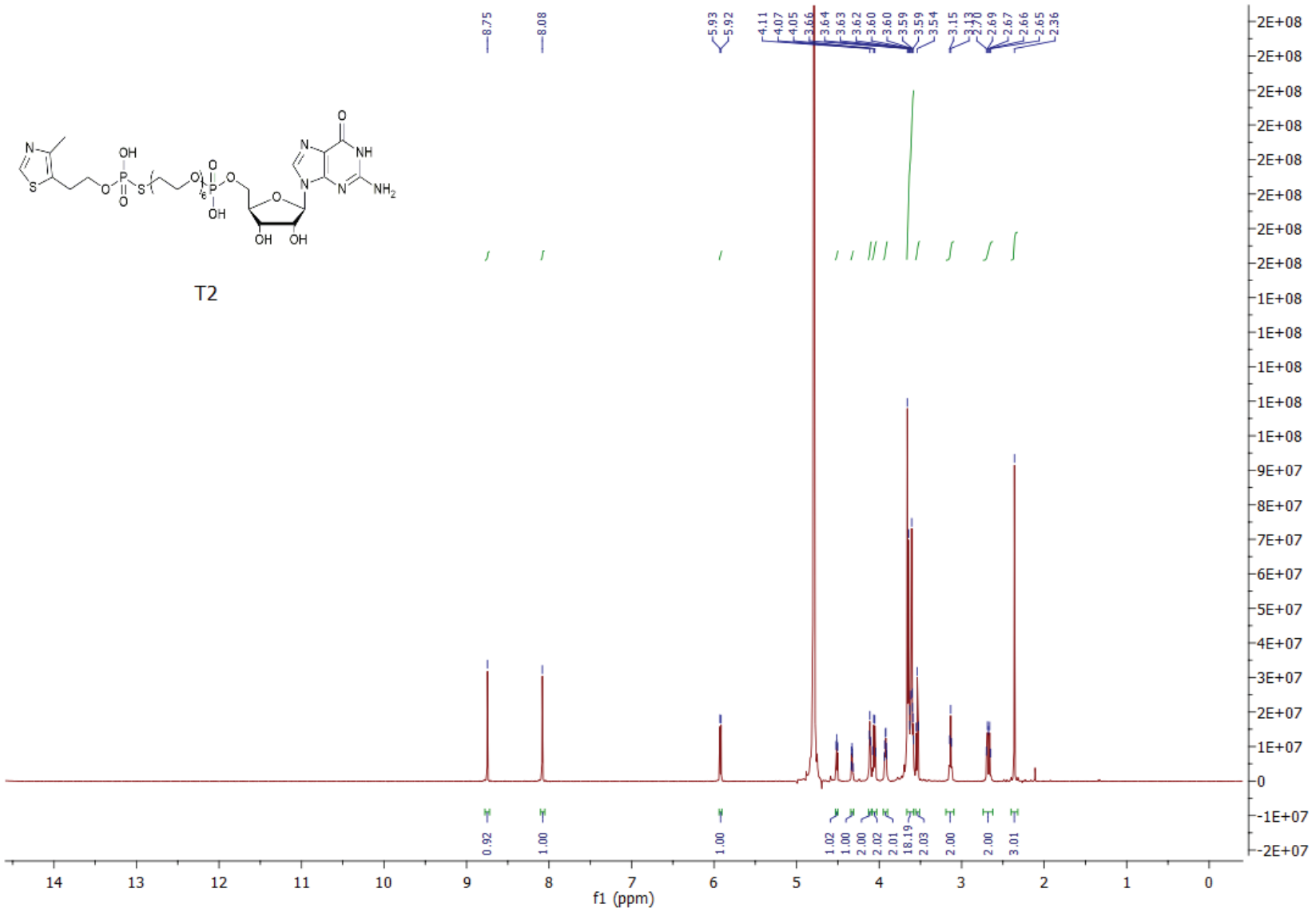
^{1}H NMR spectrum of compound **T1** (600 MHz, D_2O)



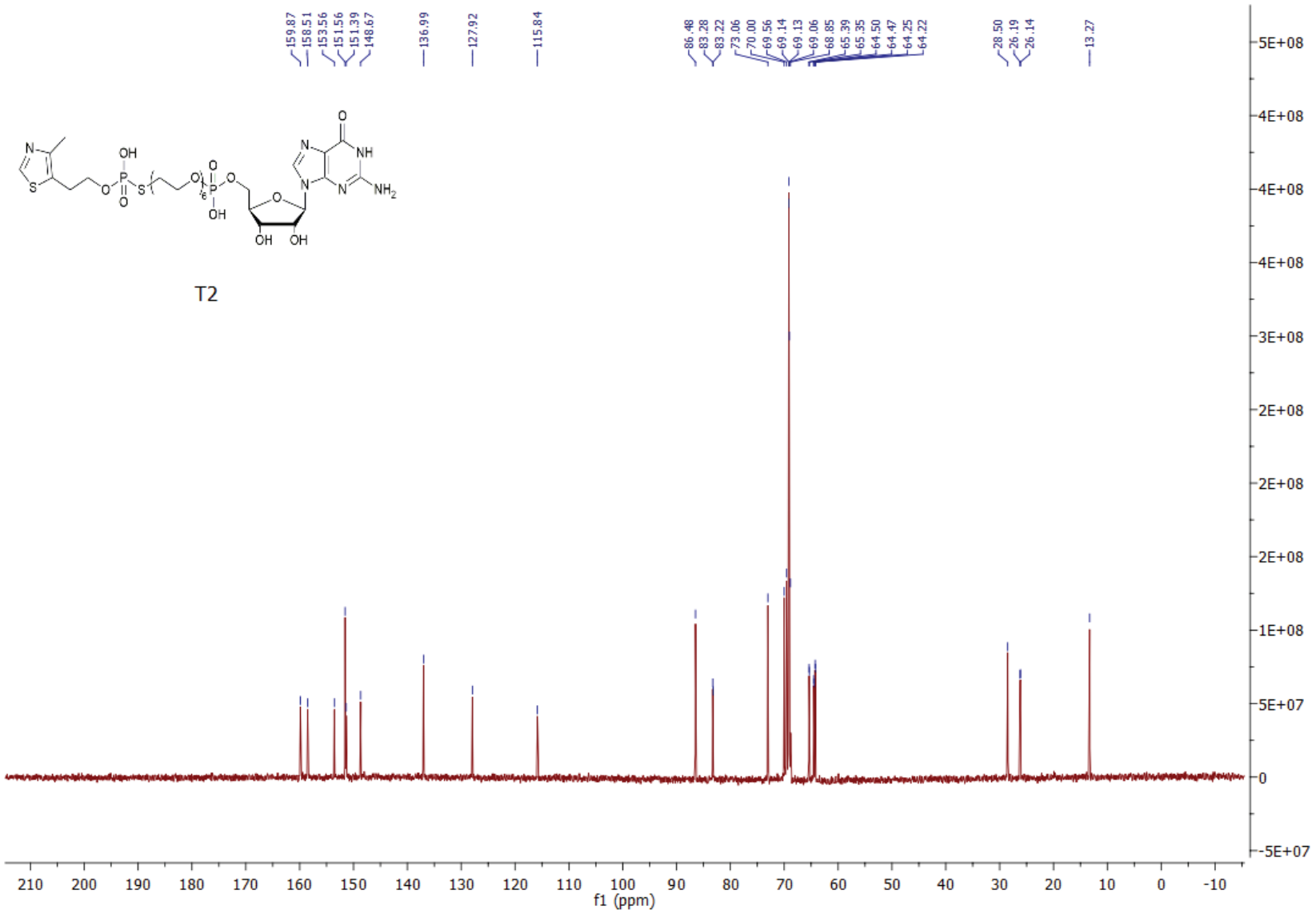
^{13}C NMR spectrum of compound **T1** (151 MHz, D_2O)



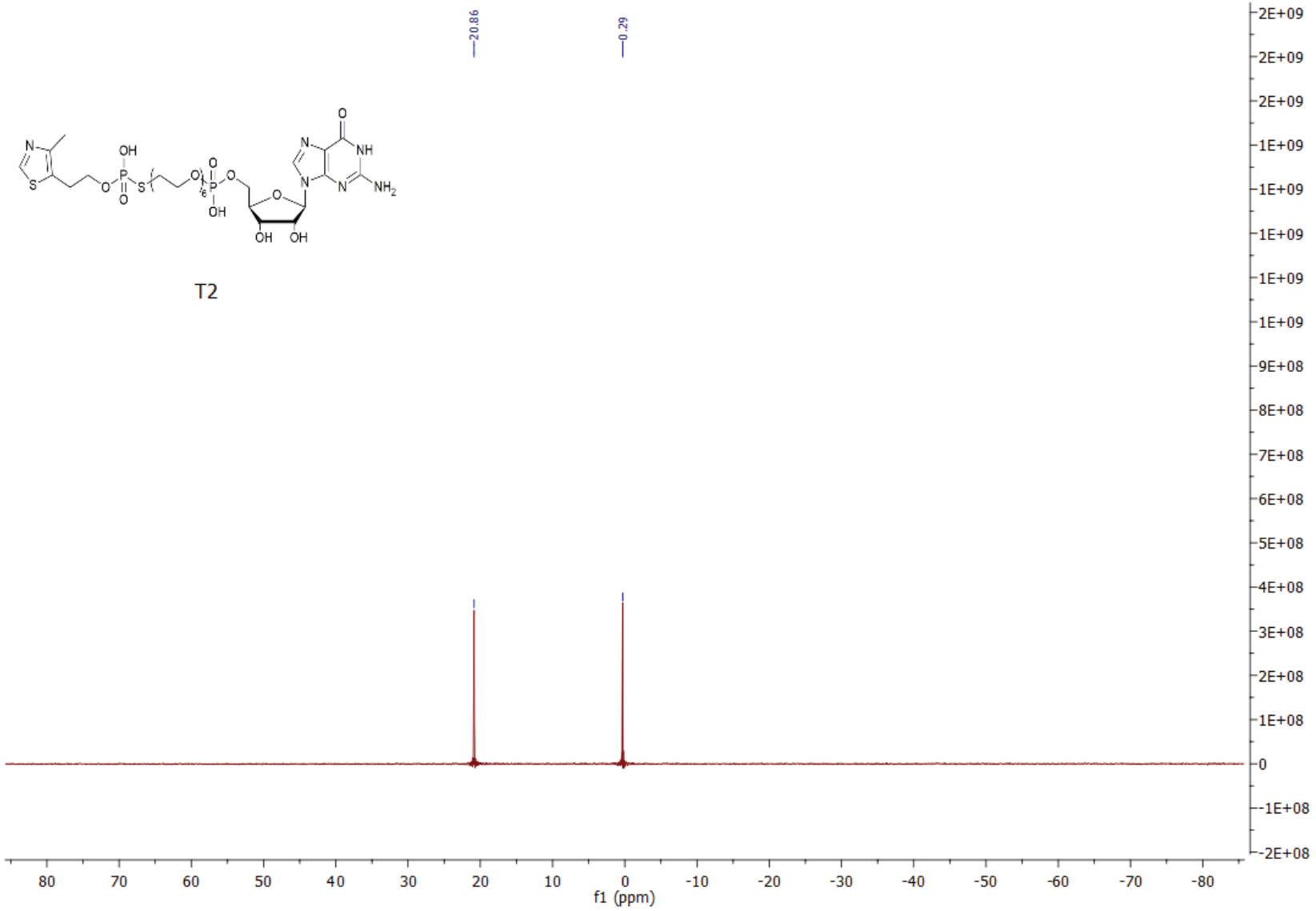
^{31}P NMR spectrum of compound **T1** (121 MHz, D_2O)



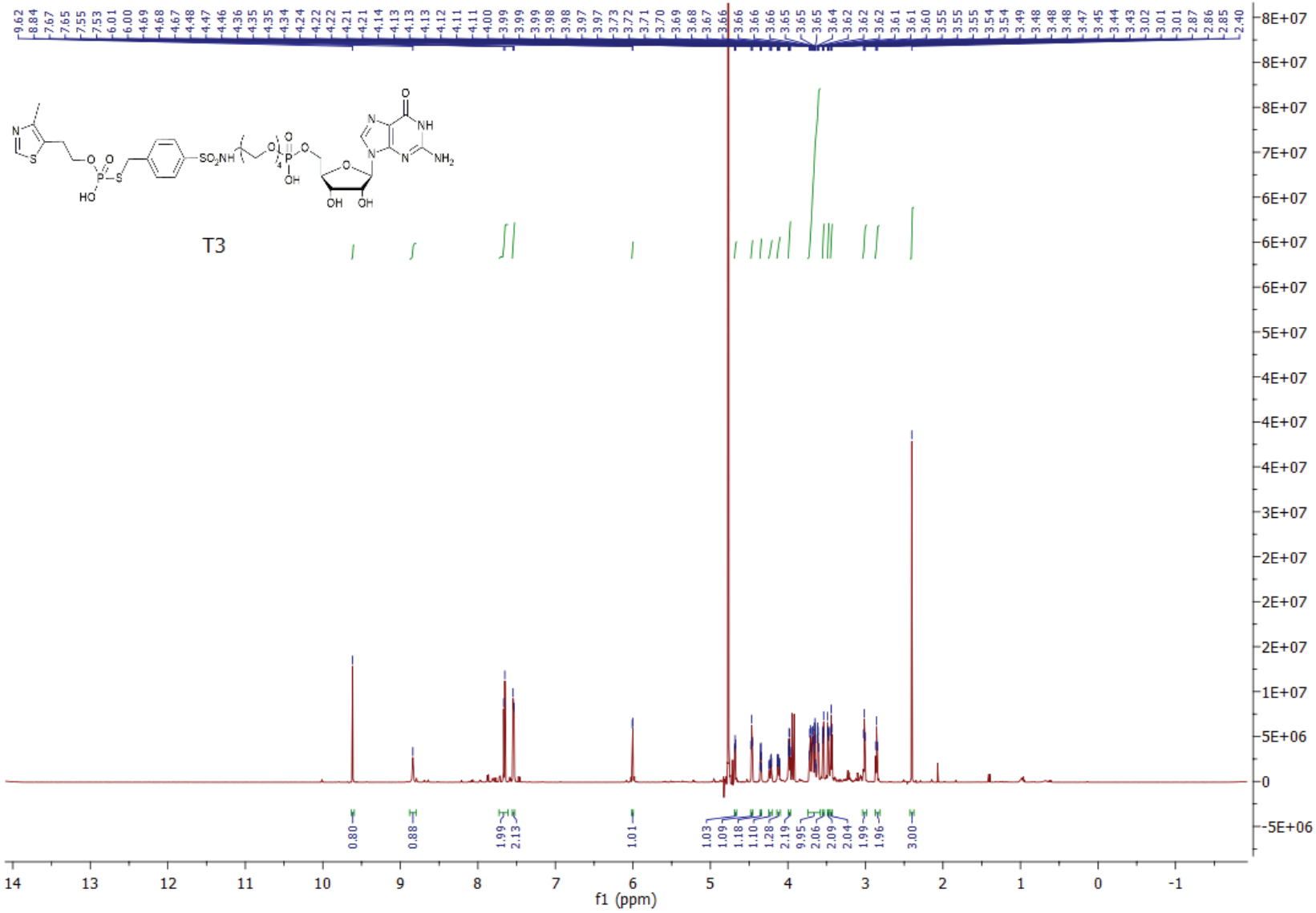
^{1}H NMR spectrum of compound **T2** (600 MHz, D_2O)



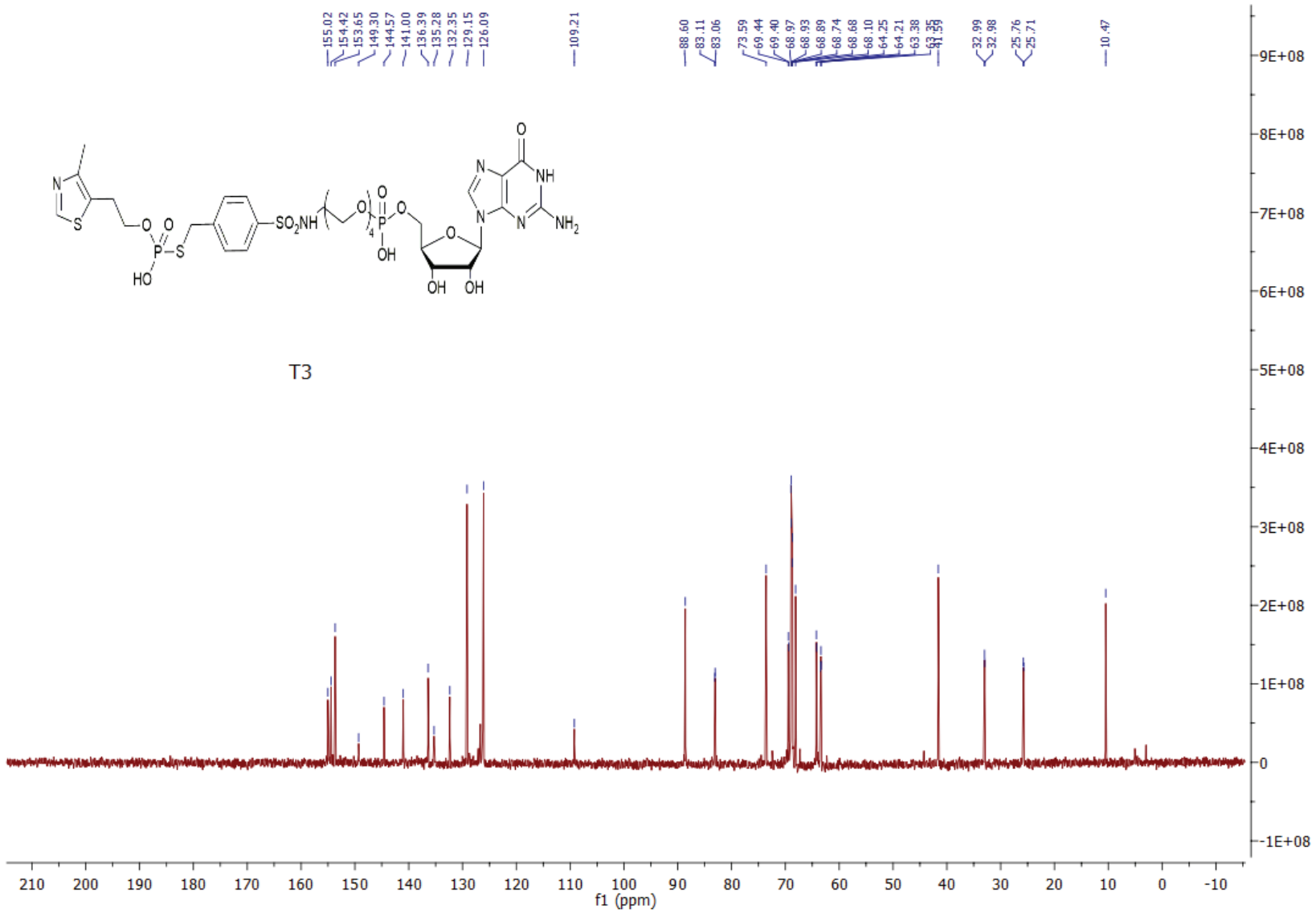
^{13}C NMR spectrum of compound **T2** (151 MHz, D_2O)



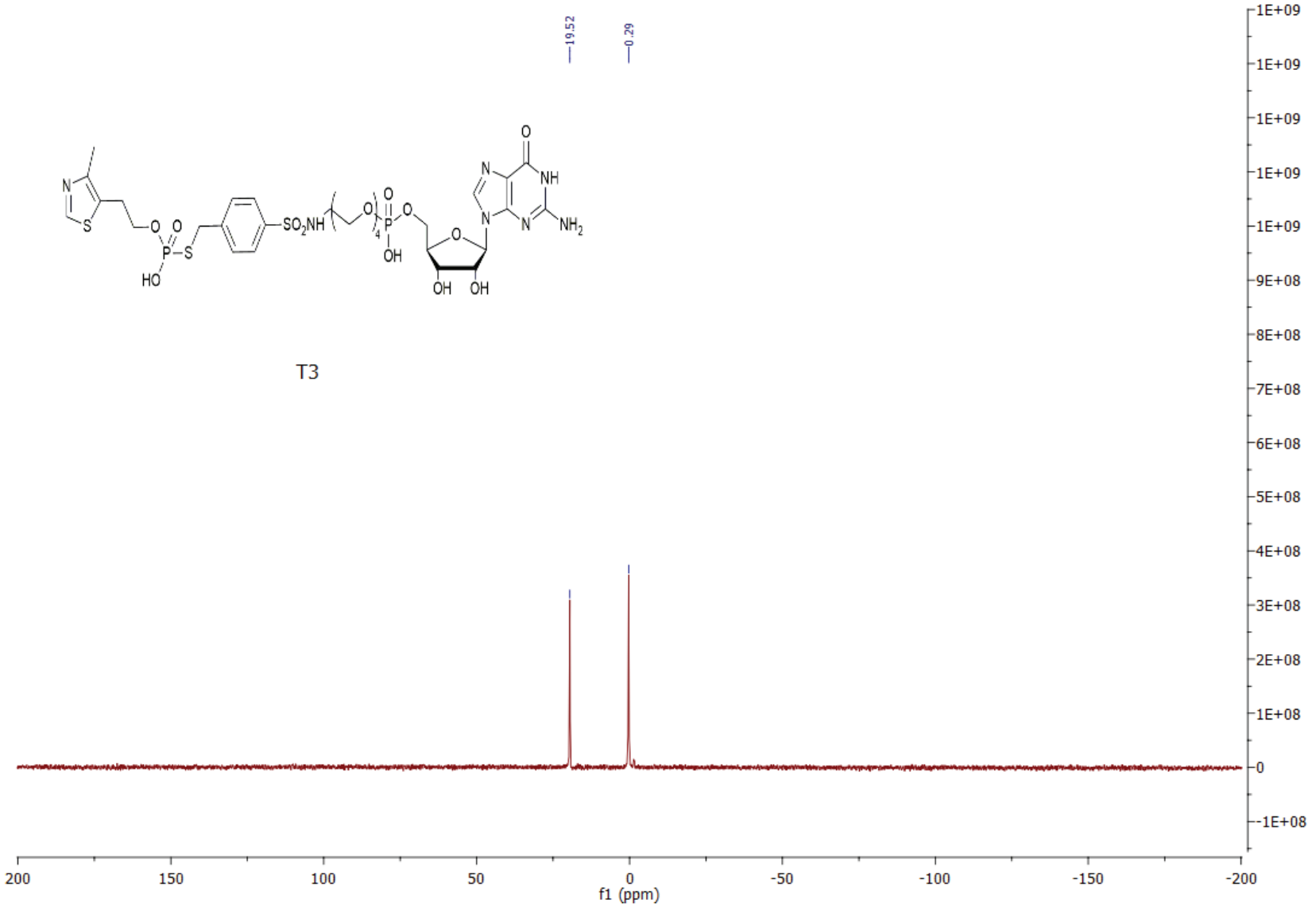
^{31}P NMR spectrum of compound **T2** (121 MHz, D_2O)



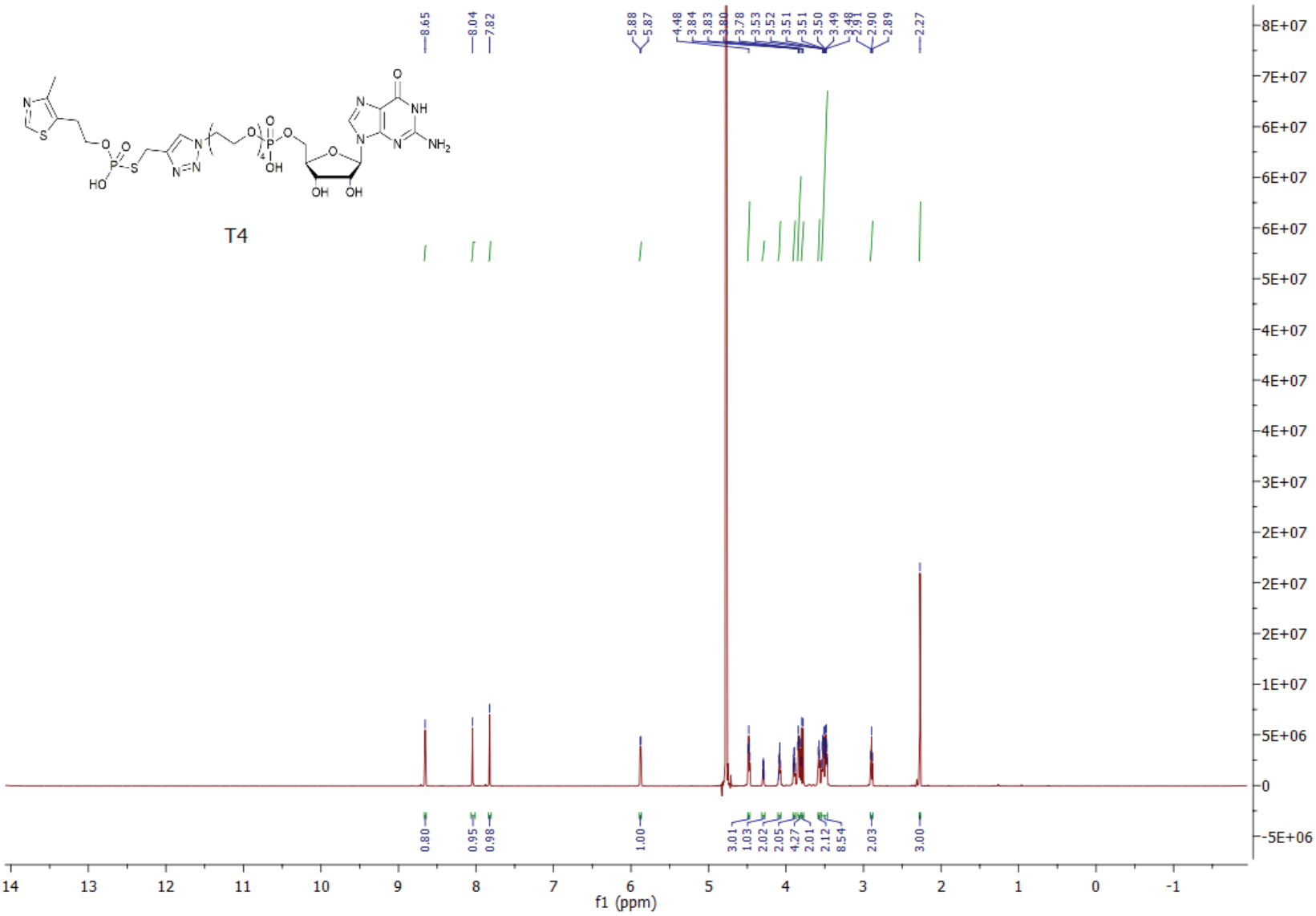
^{1}H NMR spectrum of compound **T3** (600 MHz, D_2O)



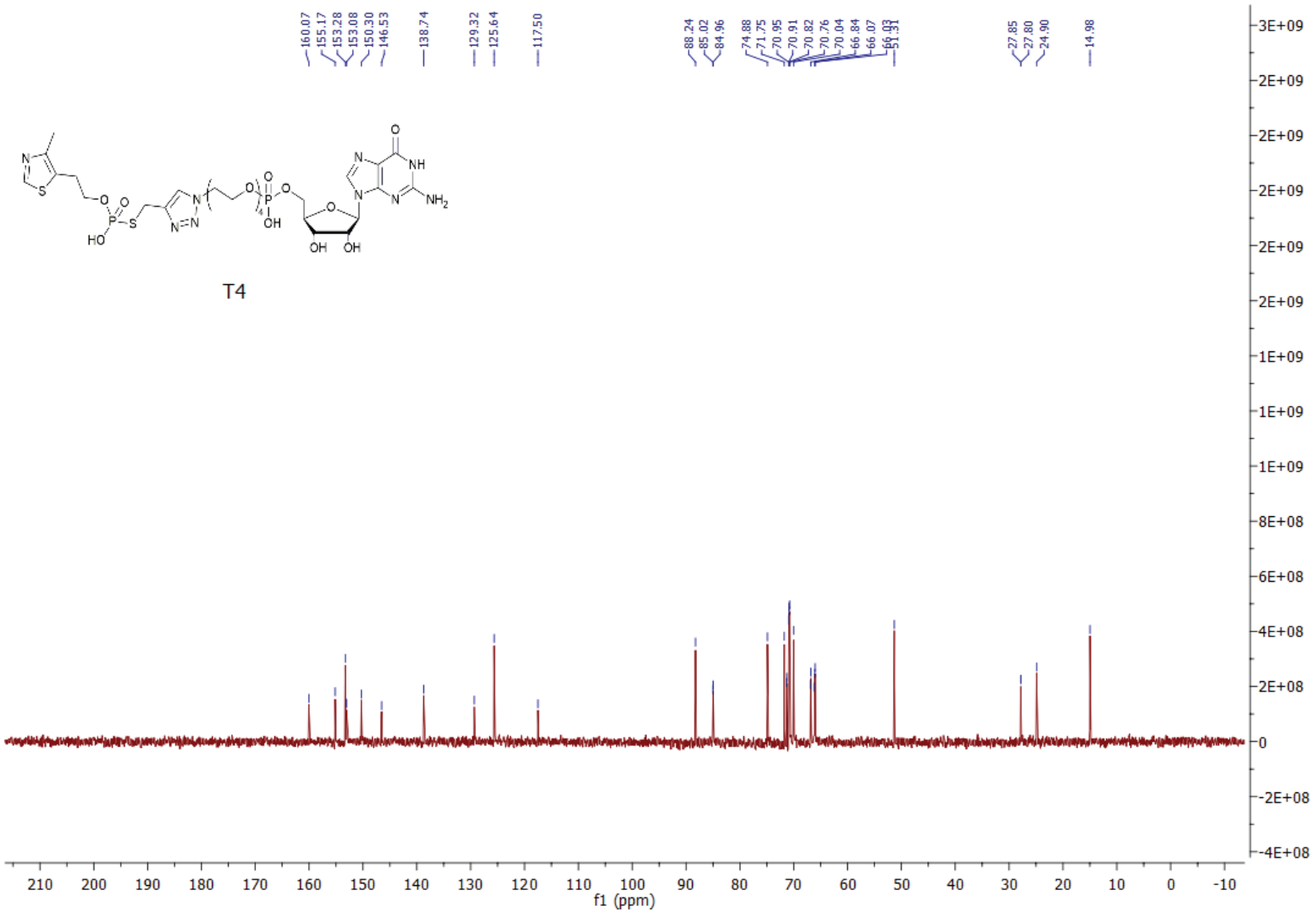
^{13}C NMR spectrum of compound **T3** (151 MHz, D_2O)



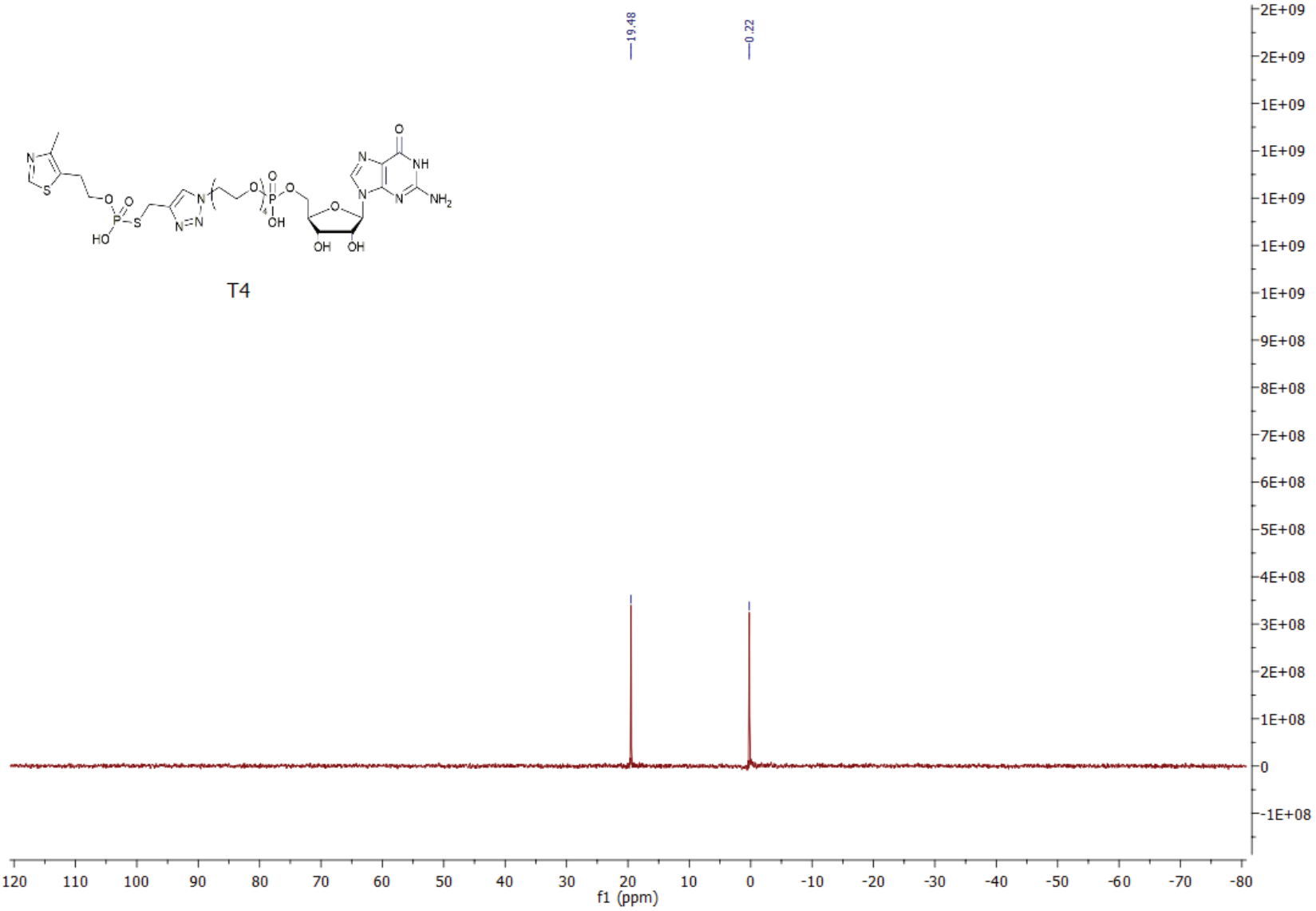
^{31}P NMR spectrum of compound **T3** (121 MHz, D_2O)



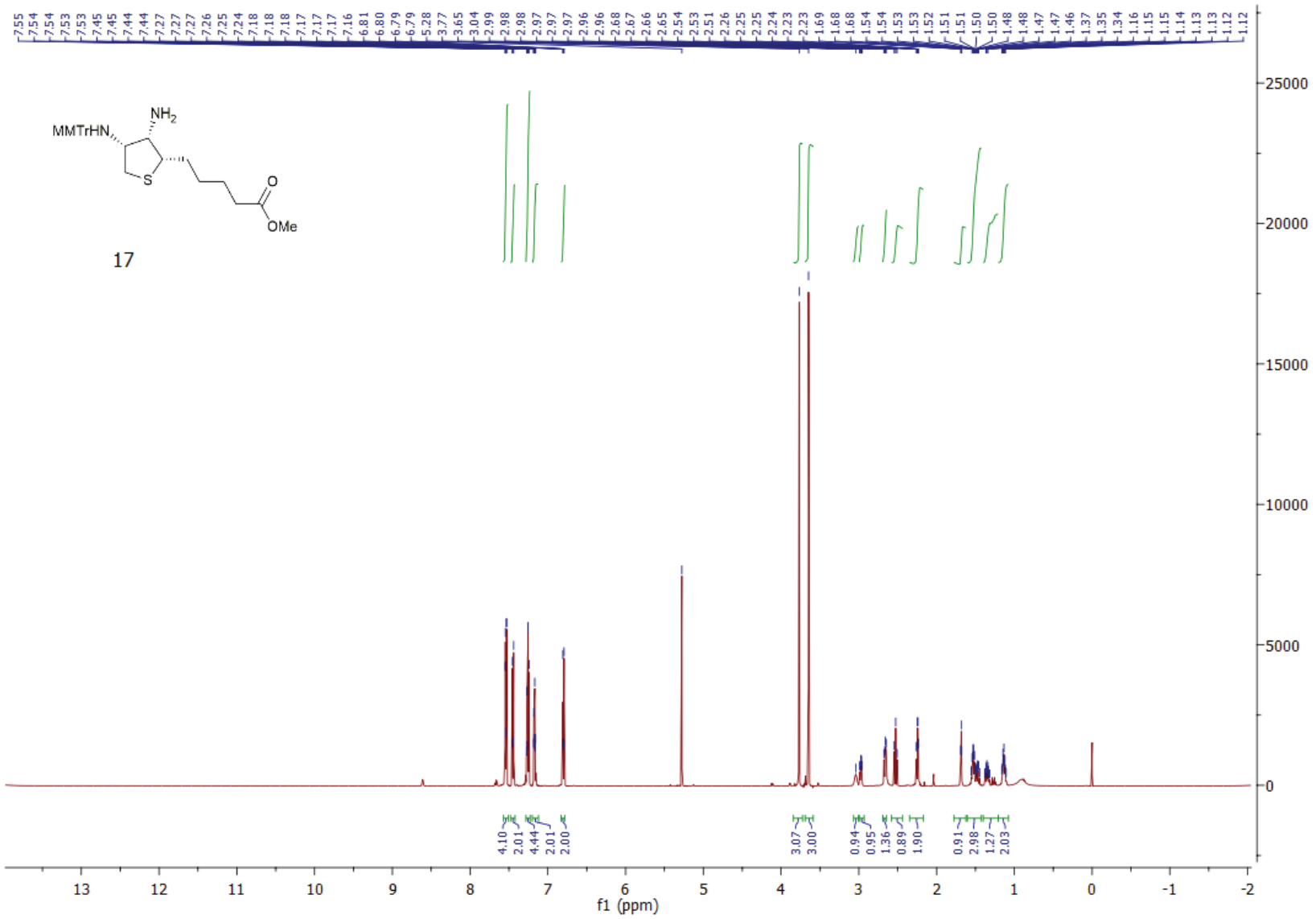
^{1}H NMR spectrum of compound **T4** (600 MHz, D_2O)



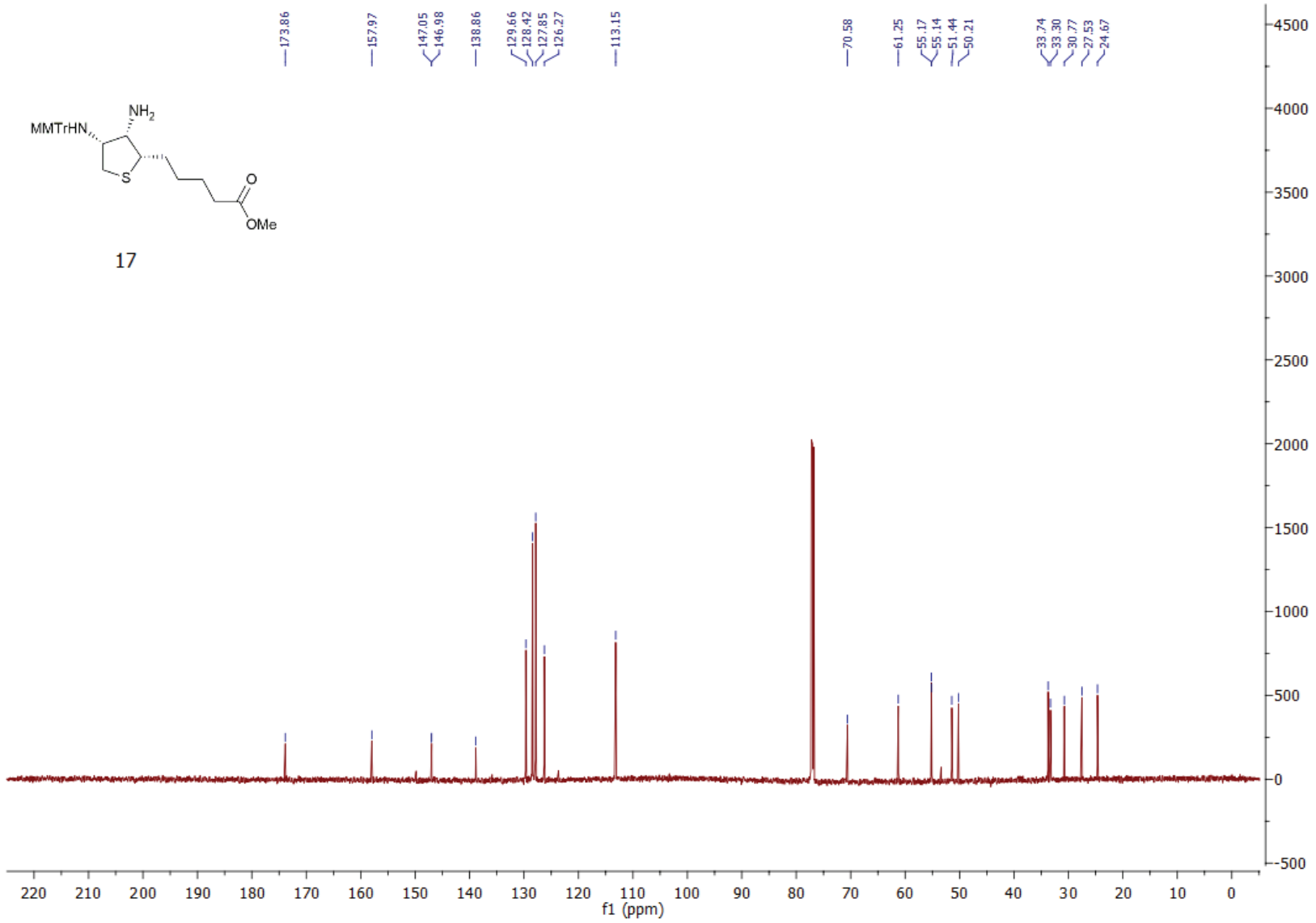
^{13}C NMR spectrum of compound **T4** (151 MHz, D_2O)



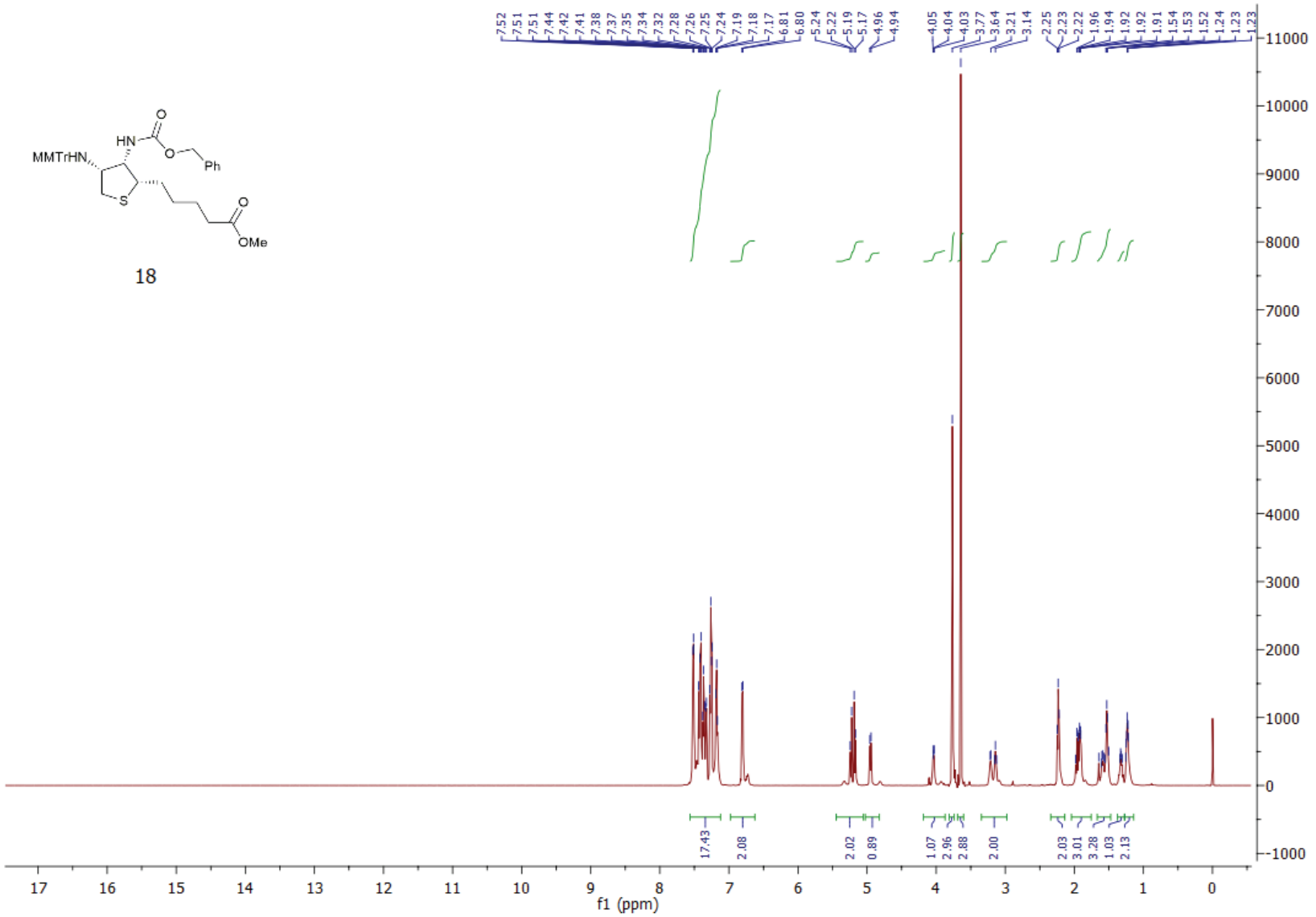
^{31}P NMR spectrum of compound **T4** (121 MHz, D_2O)



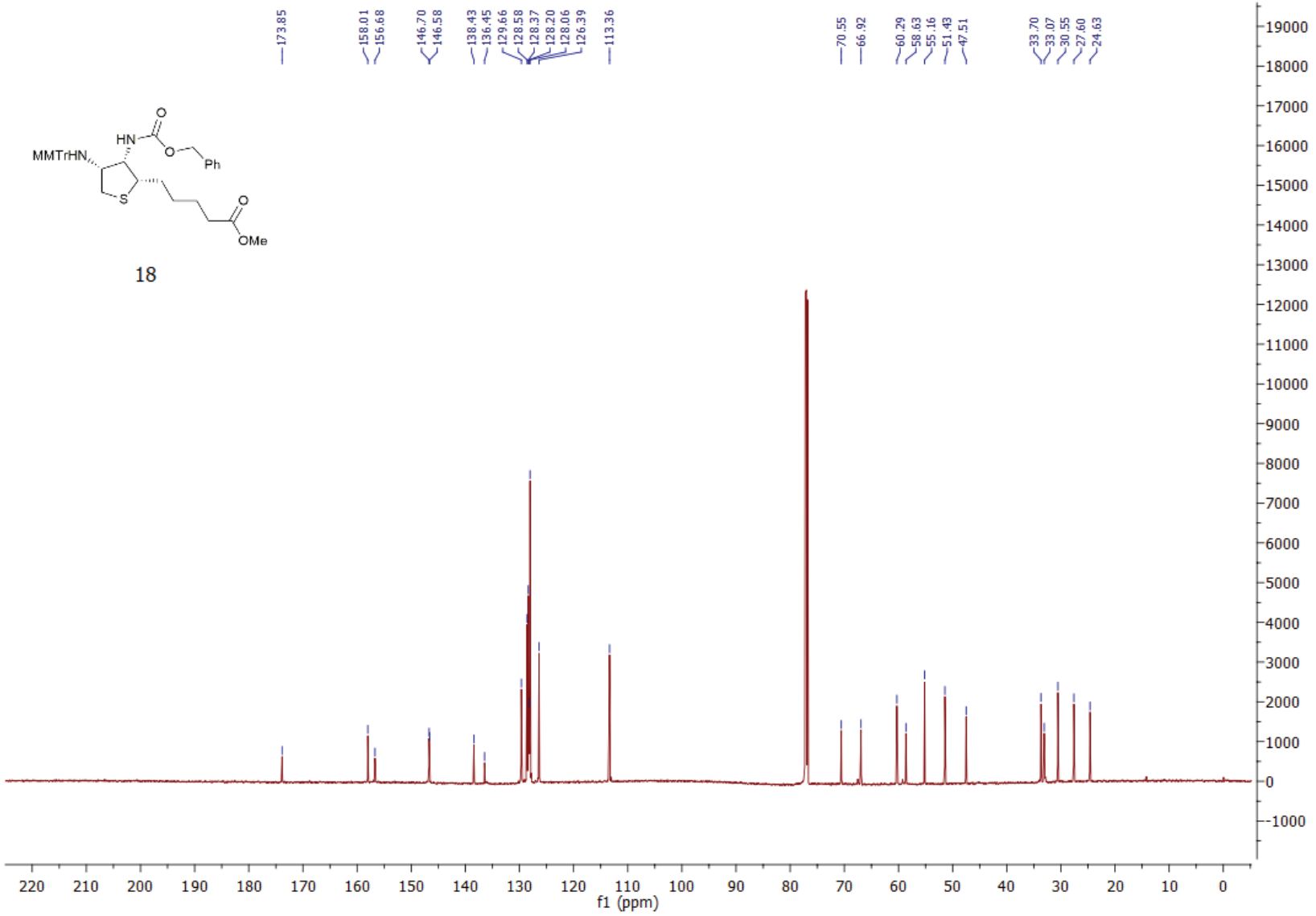
^{1}H NMR spectrum of compound **17** (600 MHz, $CDCl_3$)



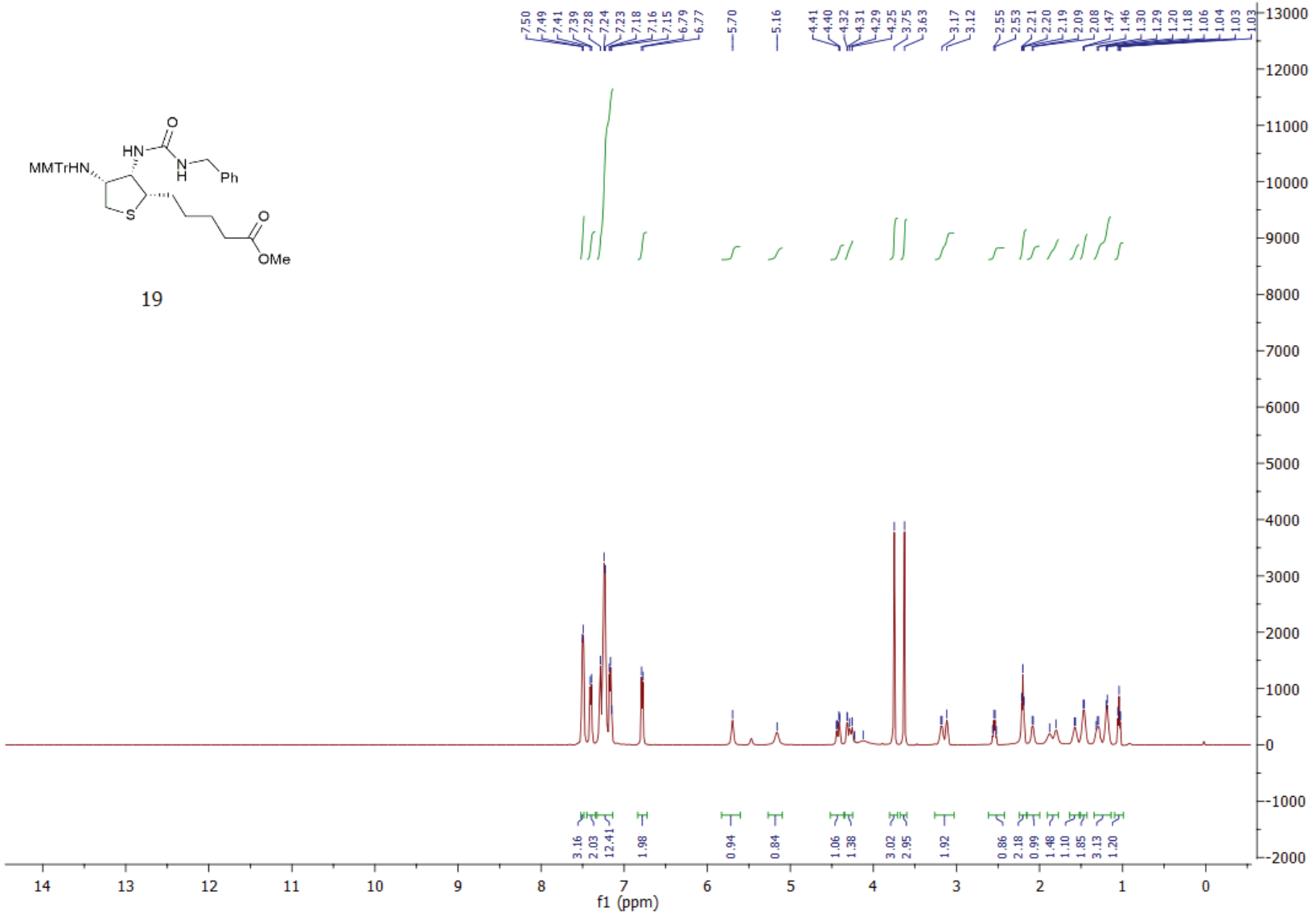
^{13}C NMR spectrum of compound **17** (151 MHz, $CDCl_3$)



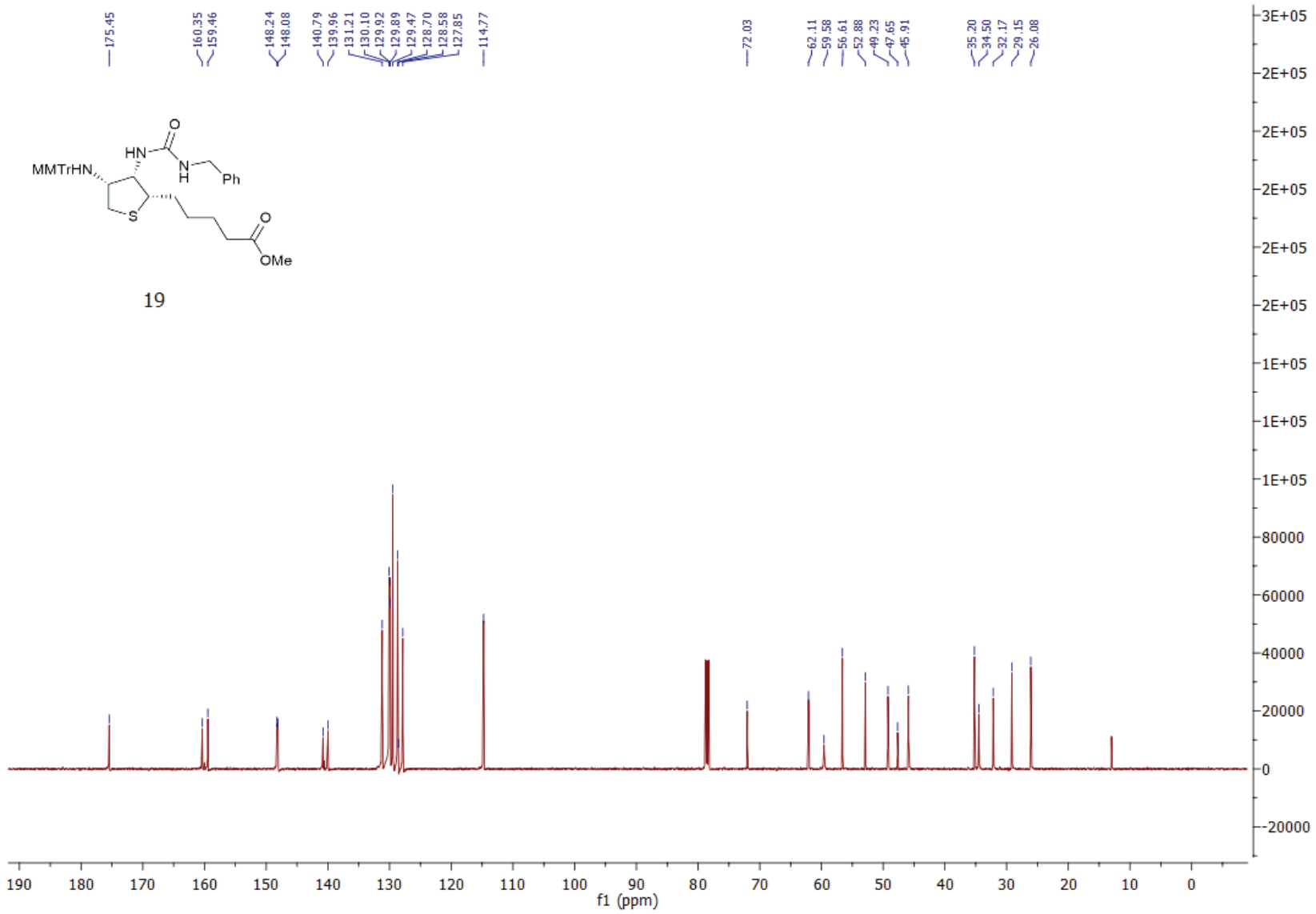
^{1}H NMR spectrum of compound **18** (300 MHz, $CDCl_3$)



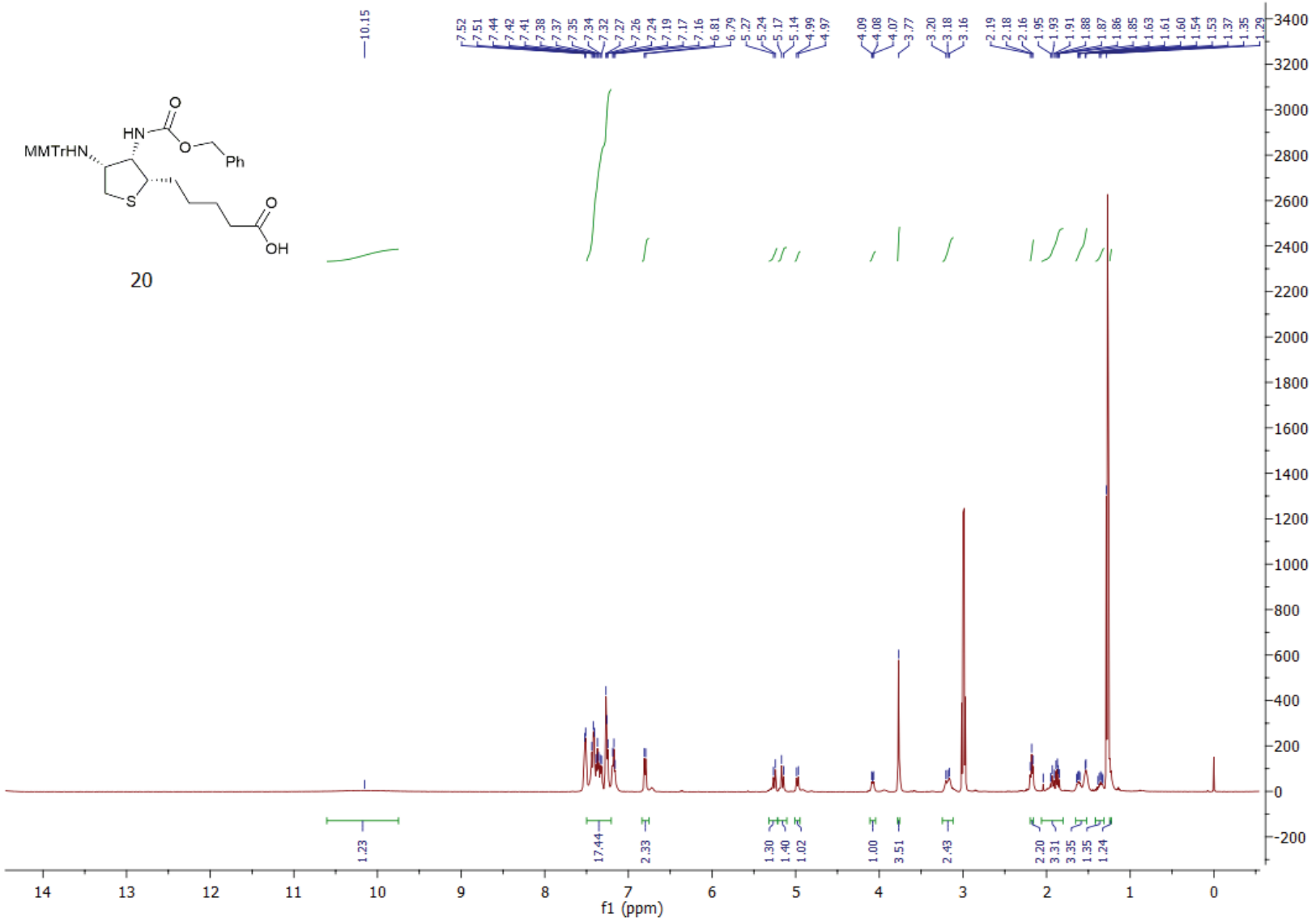
^{13}C NMR spectrum of compound **18** (151 MHz, $CDCl_3$)



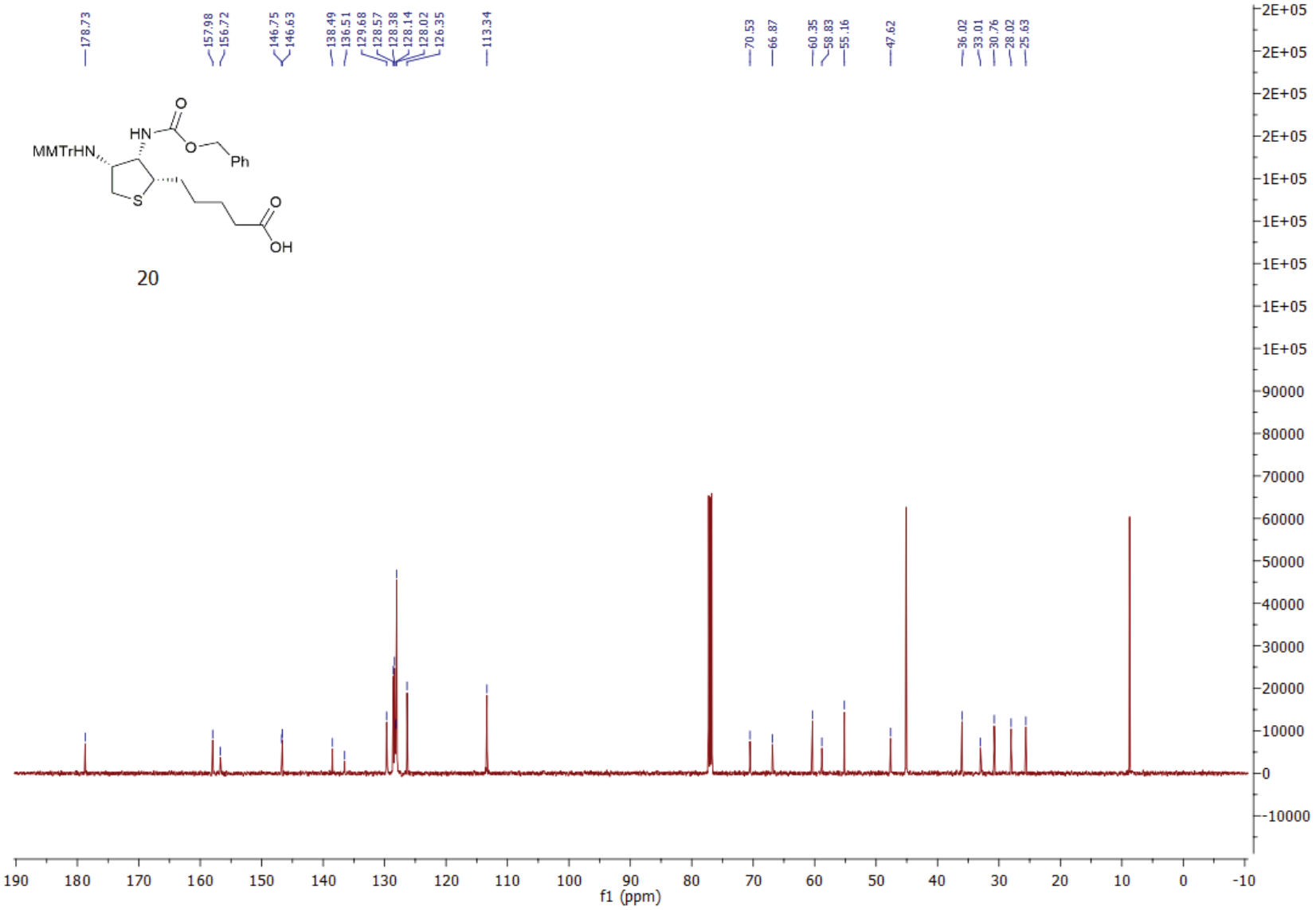
^{1}H NMR spectrum of compound **19** (500 MHz, $CDCl_3$)



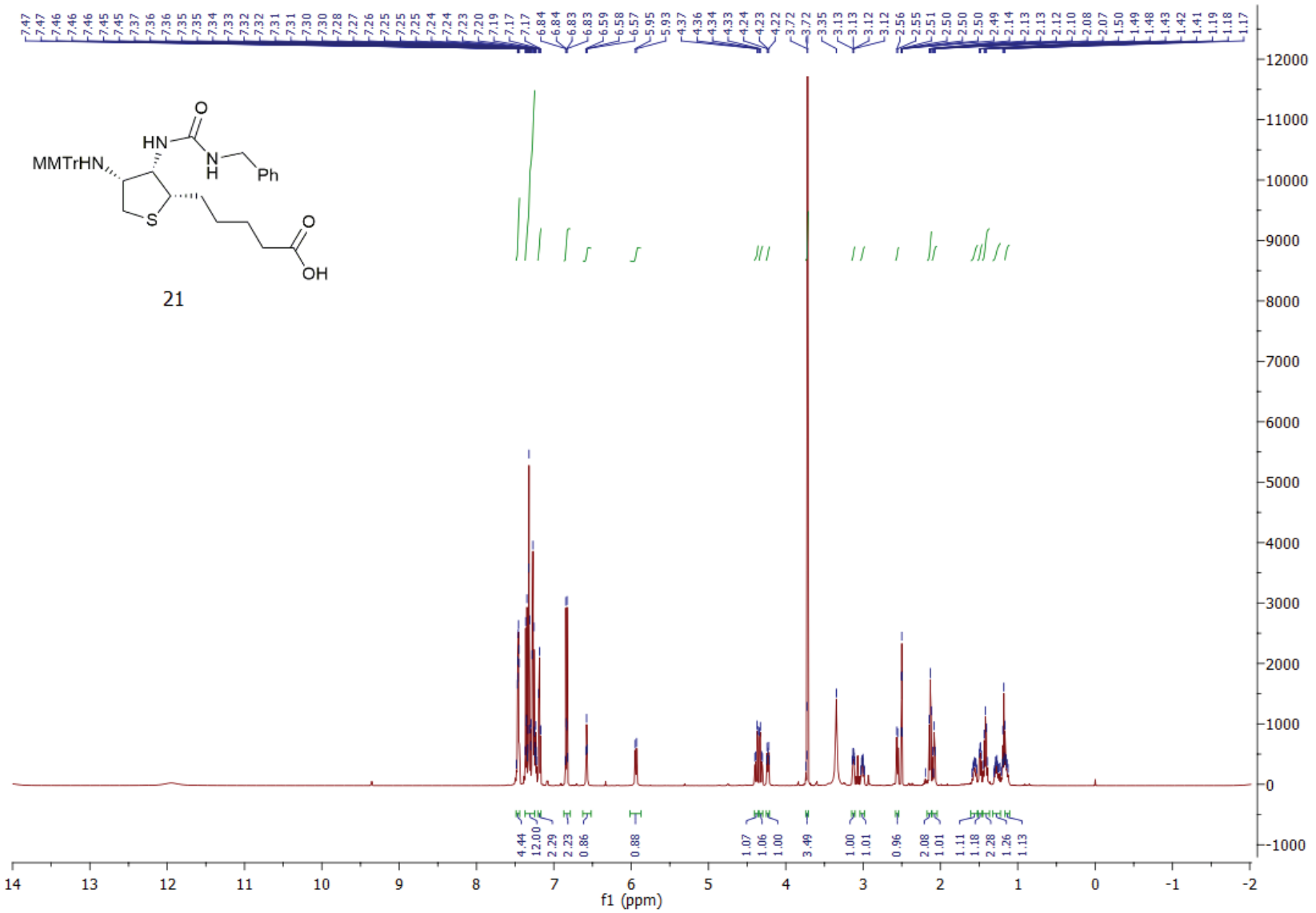
^{13}C NMR spectrum of compound **19** (126 MHz, $CDCl_3$)



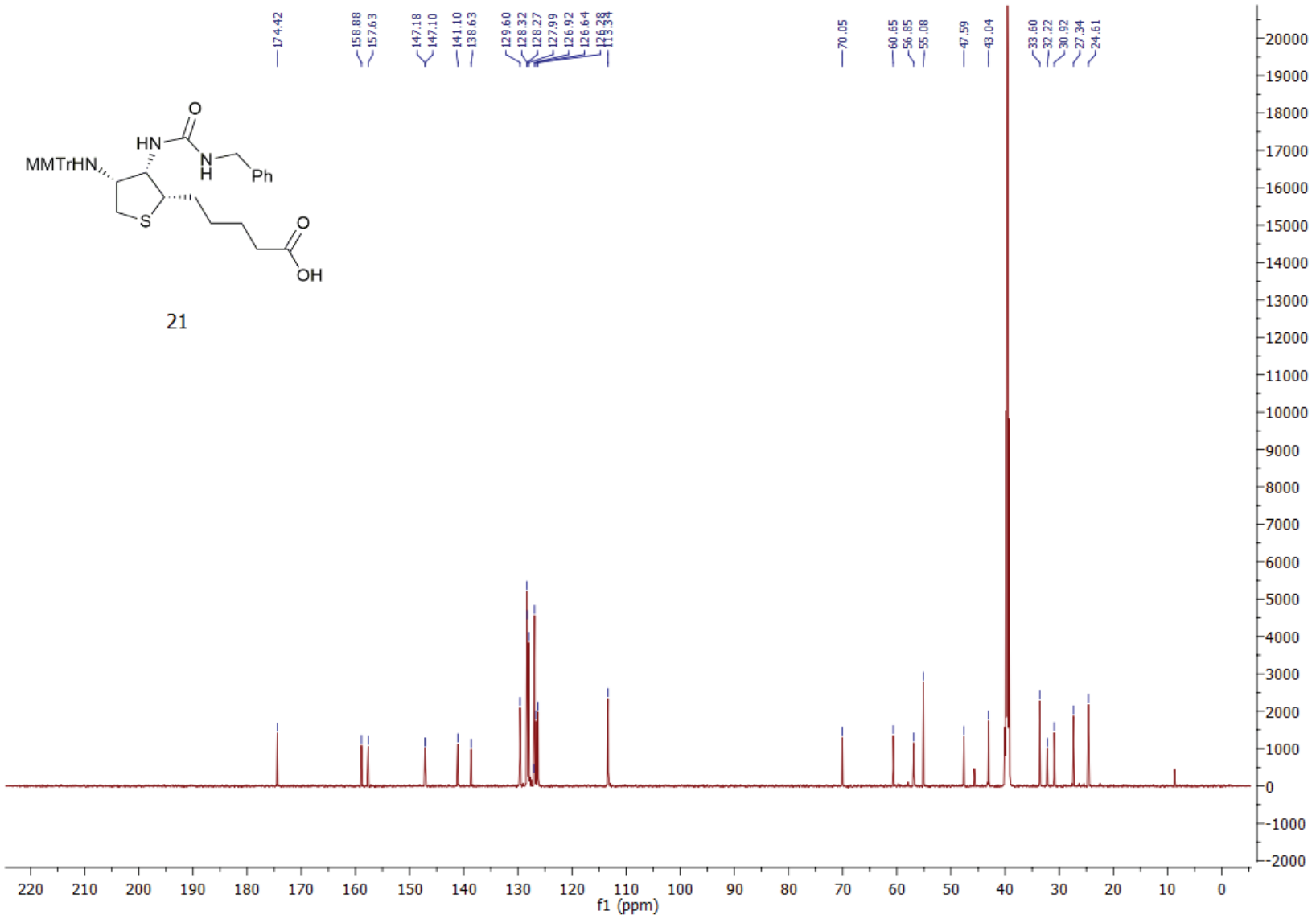
^{1}H NMR spectrum of compound **20** (500 MHz, $CDCl_3$)



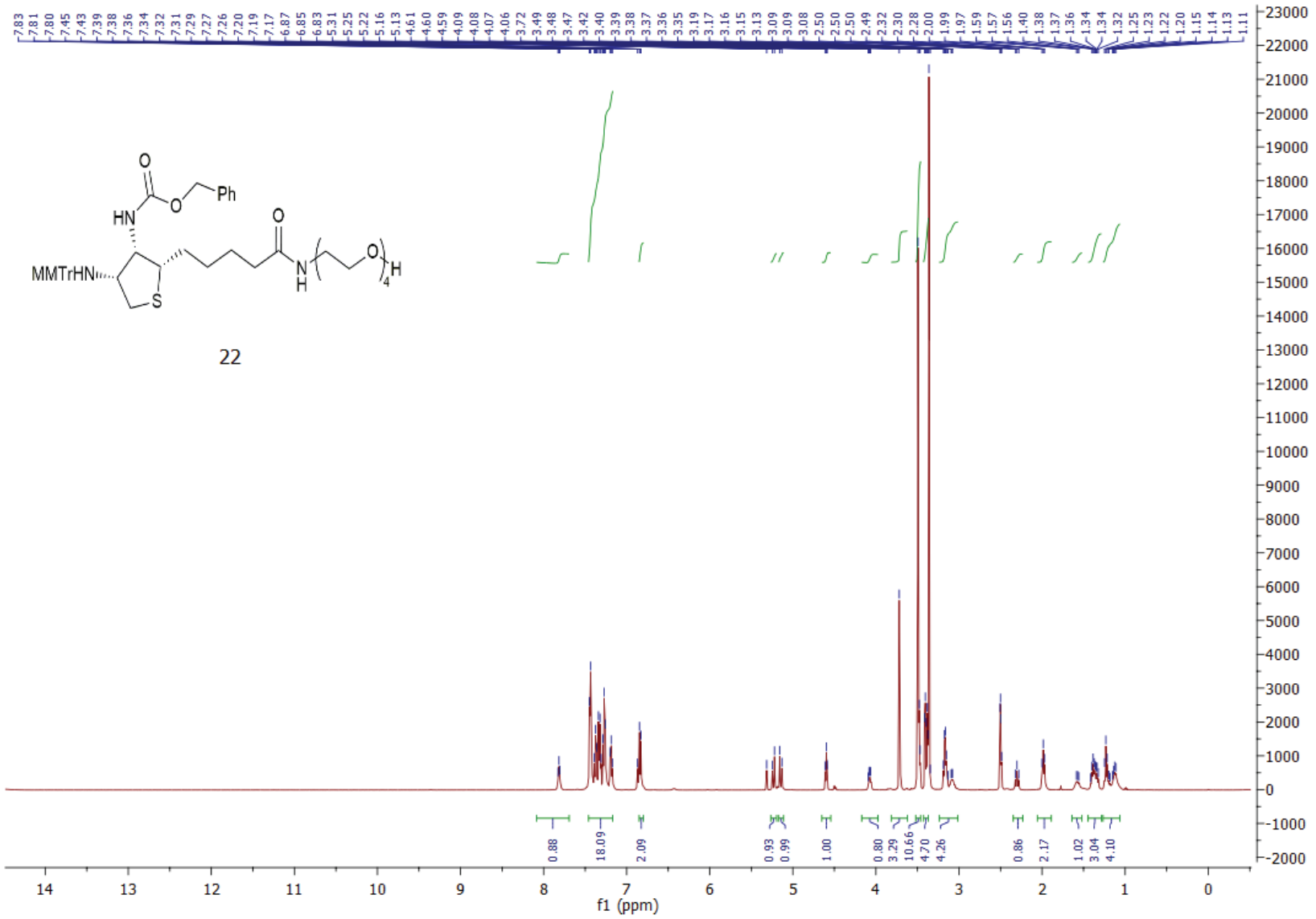
^{13}C NMR spectrum of compound **20** (126 MHz, $CDCl_3$)



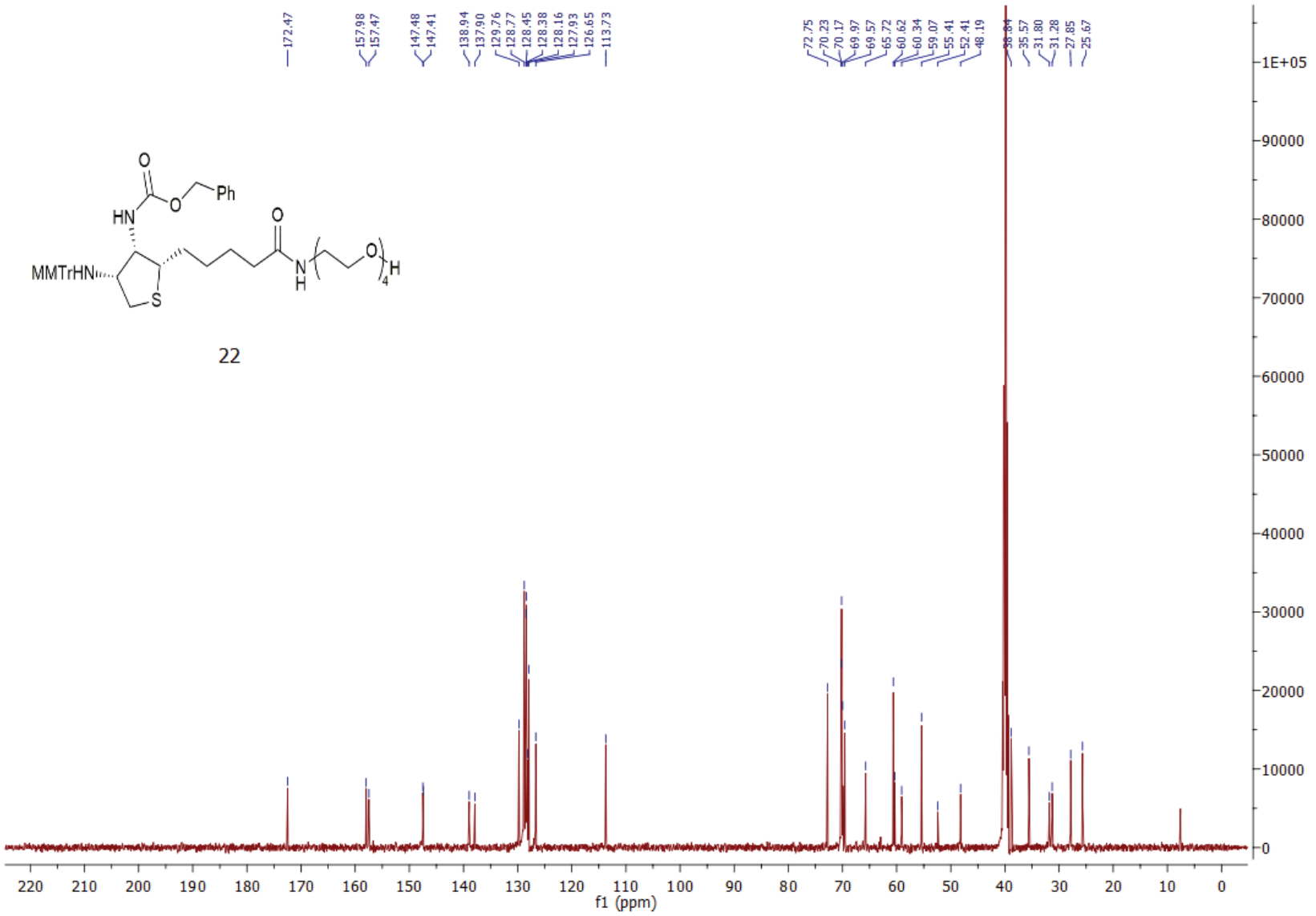
^{1}H NMR spectrum of compound **21** (600 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **21** (151 MHz, DMSO-d_6)

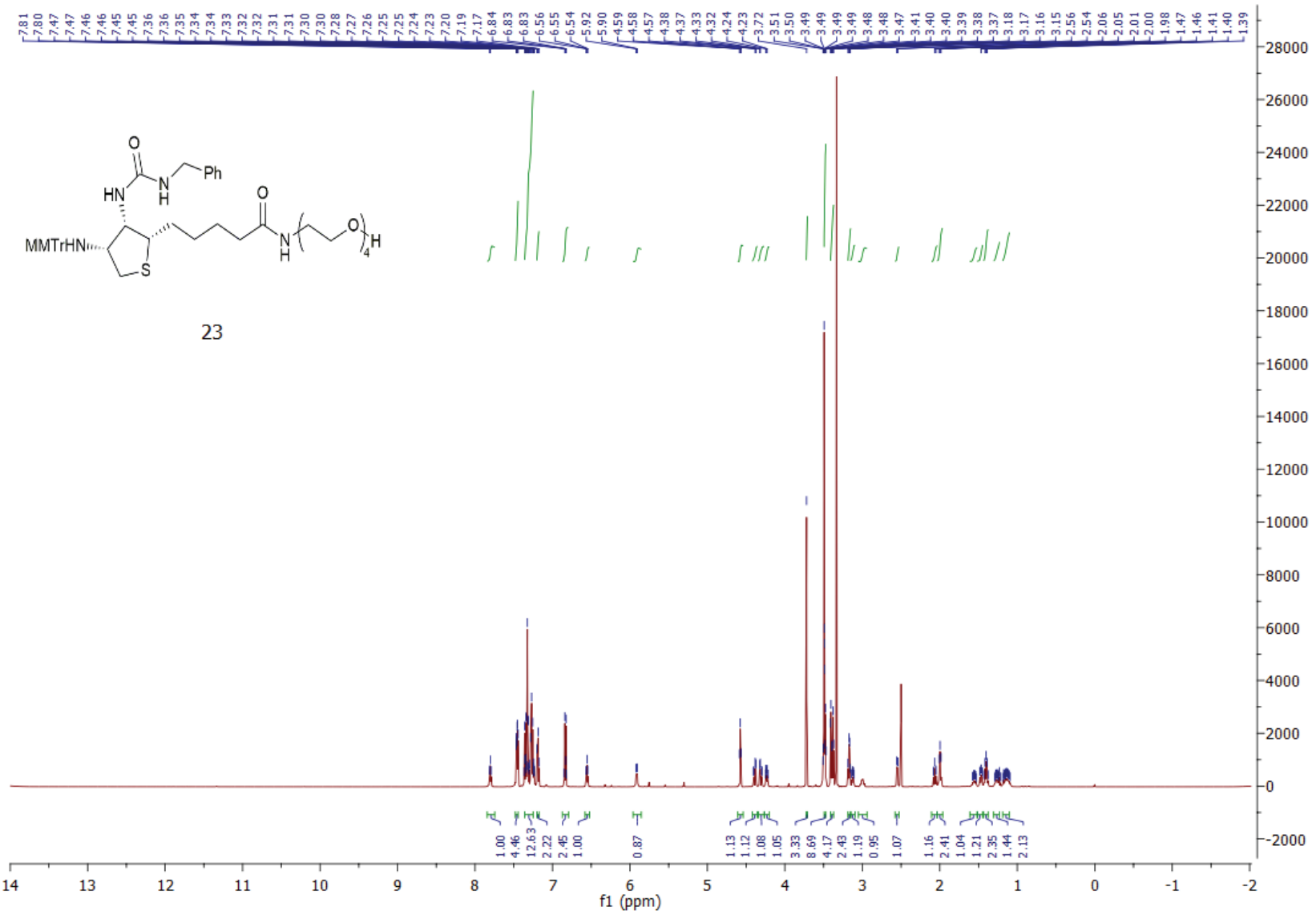


^{1}H NMR spectrum of compound **22** (500 MHz, DMSO-d_6)

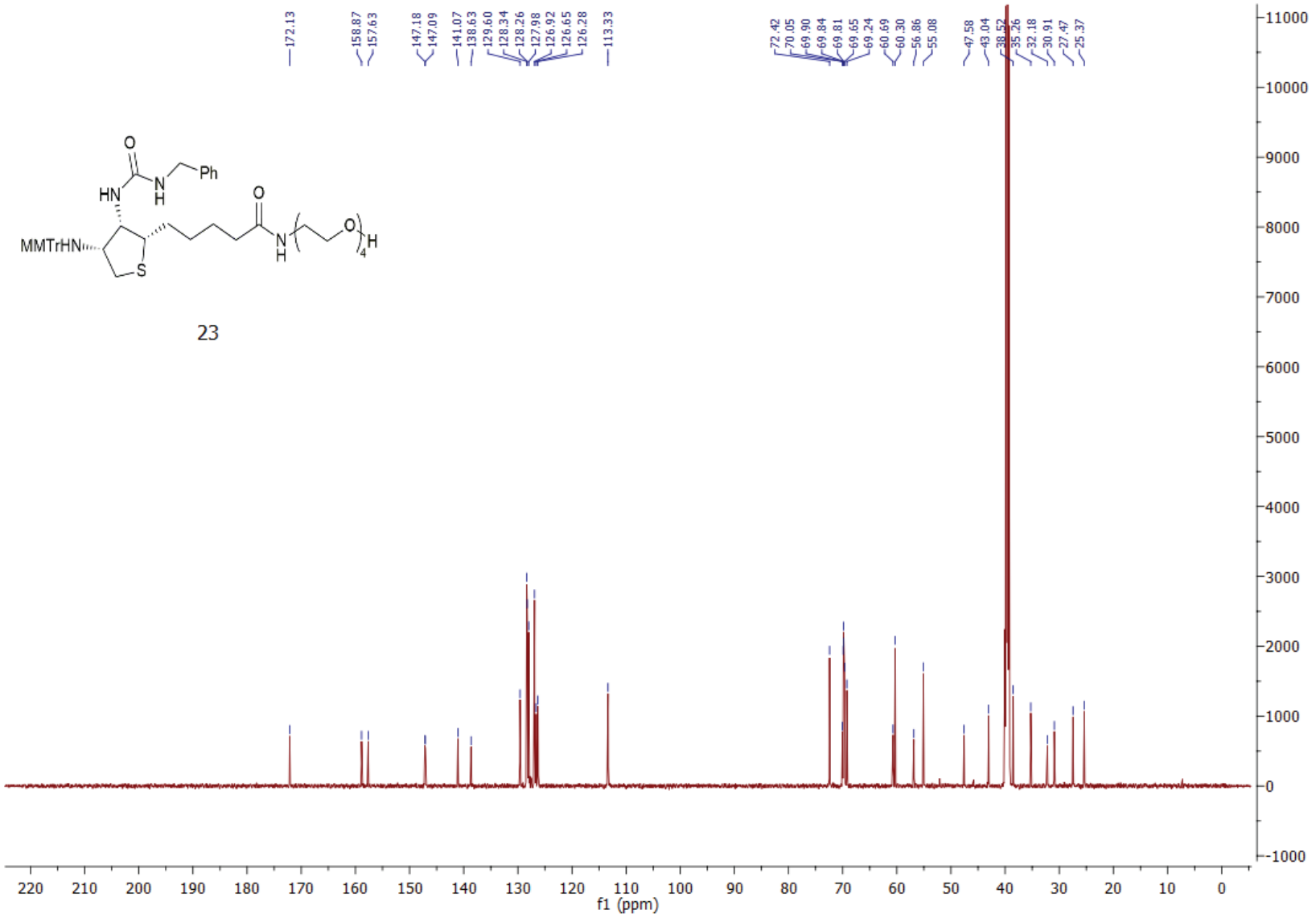


22

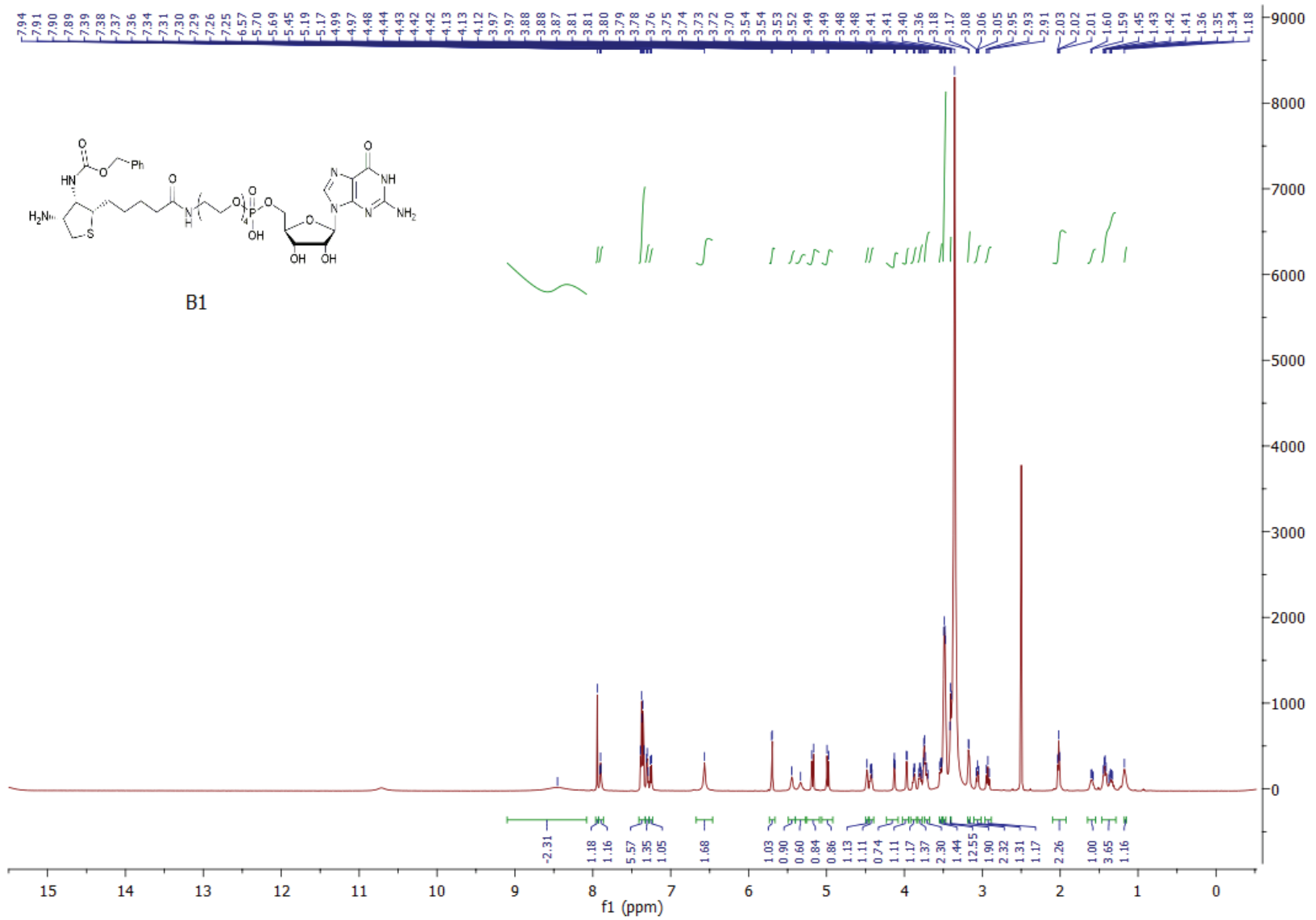
^{13}C NMR spectrum of compound **22** (126 MHz, DMSO-d_6)



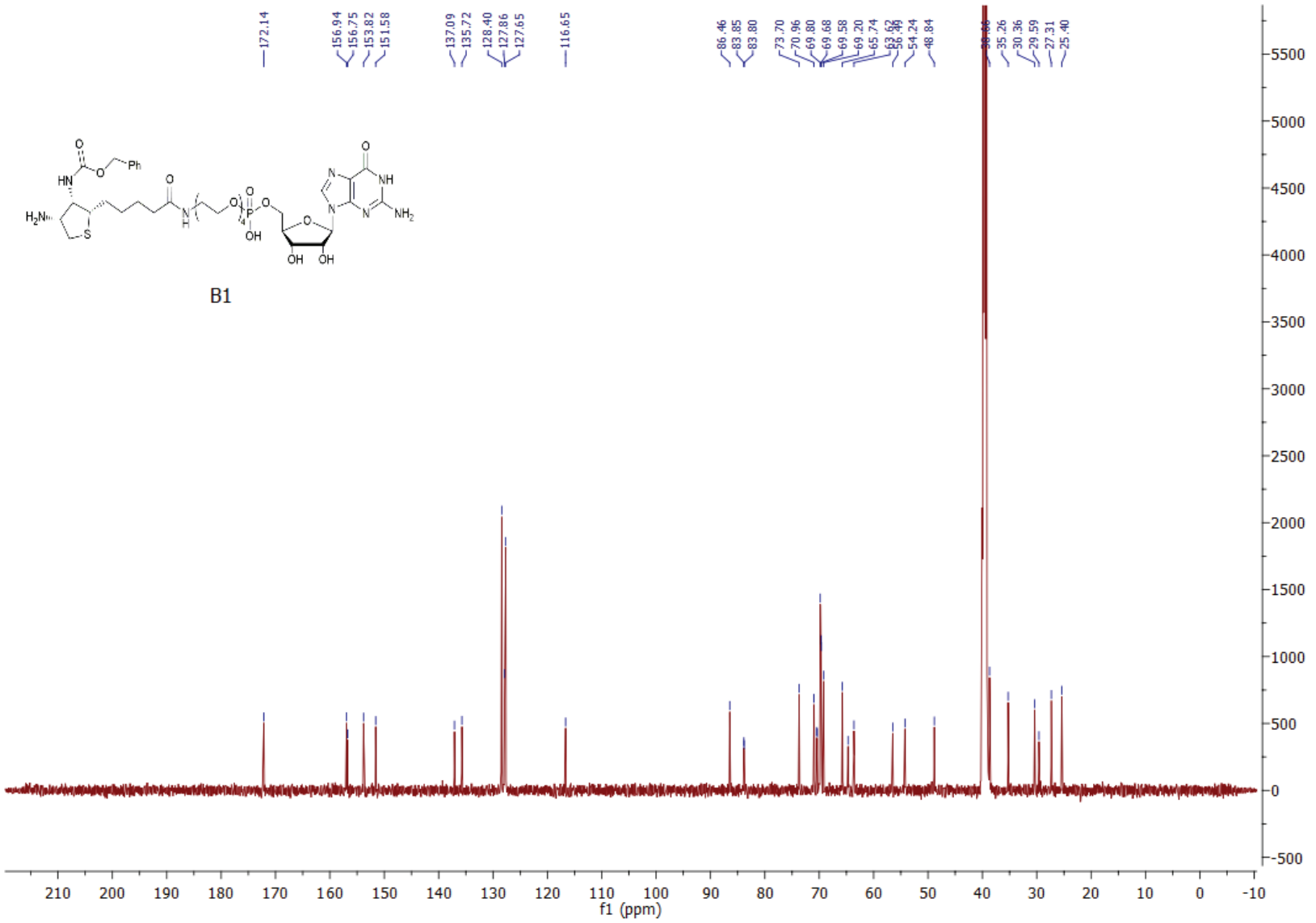
^{1}H NMR spectrum of compound **23** (600 MHz, DMSO-d_6)



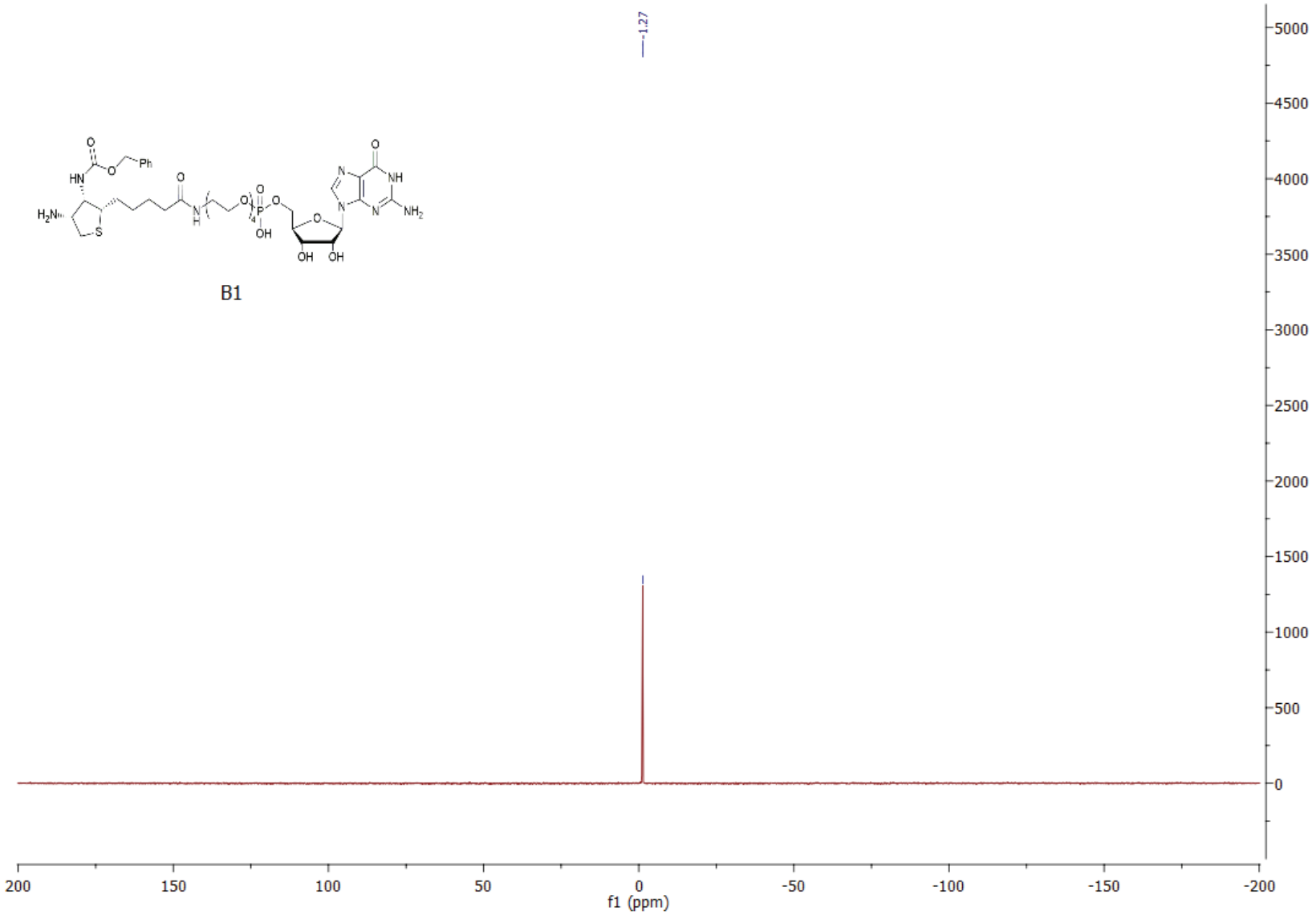
^{13}C NMR spectrum of compound **23** (151 MHz, DMSO-d_6)



^{1}H NMR spectrum of compound **B1** (600 MHz, DMSO-d_6)

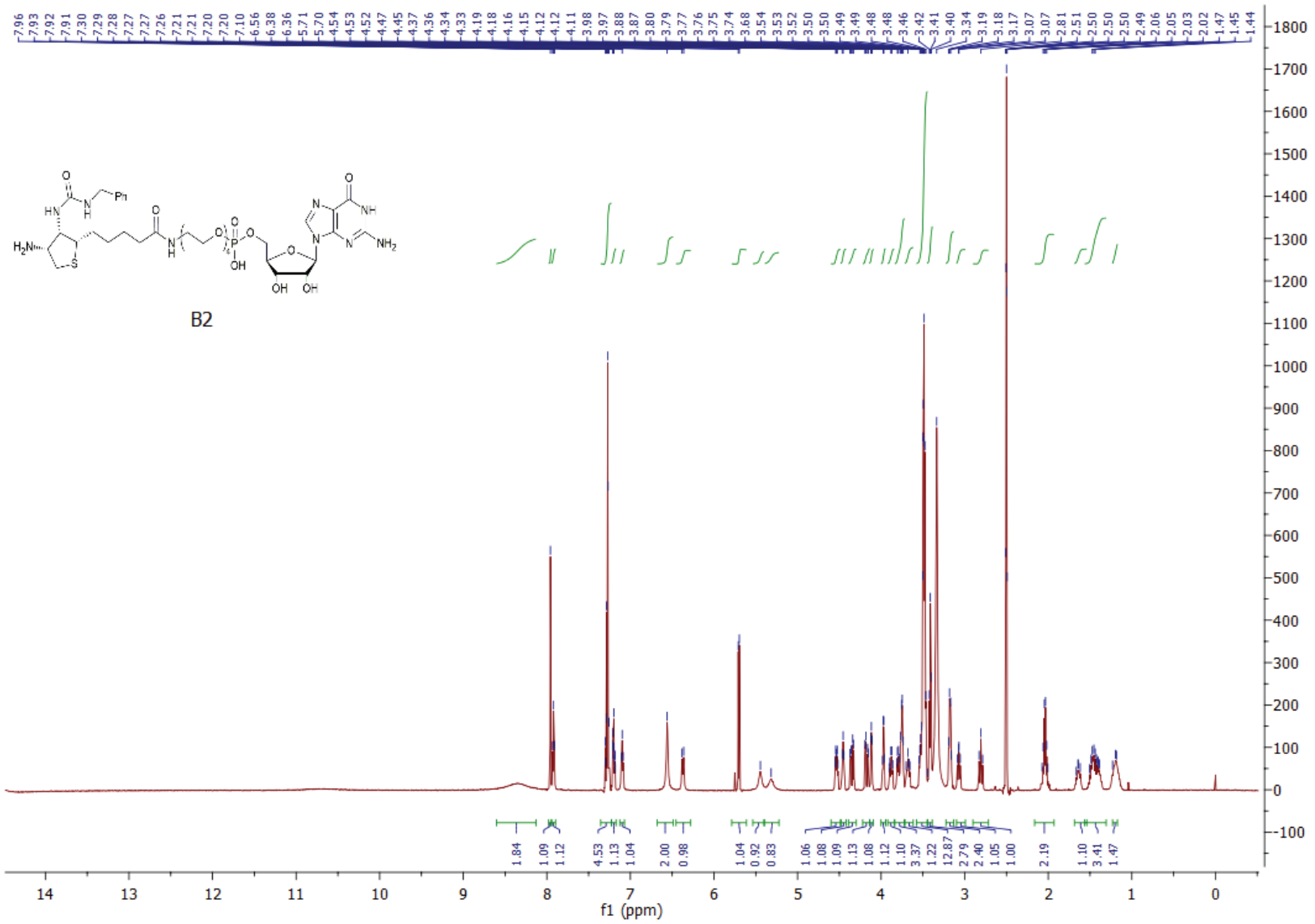


^{13}C NMR spectrum of compound **B1** (151 MHz, DMSO-d_6)

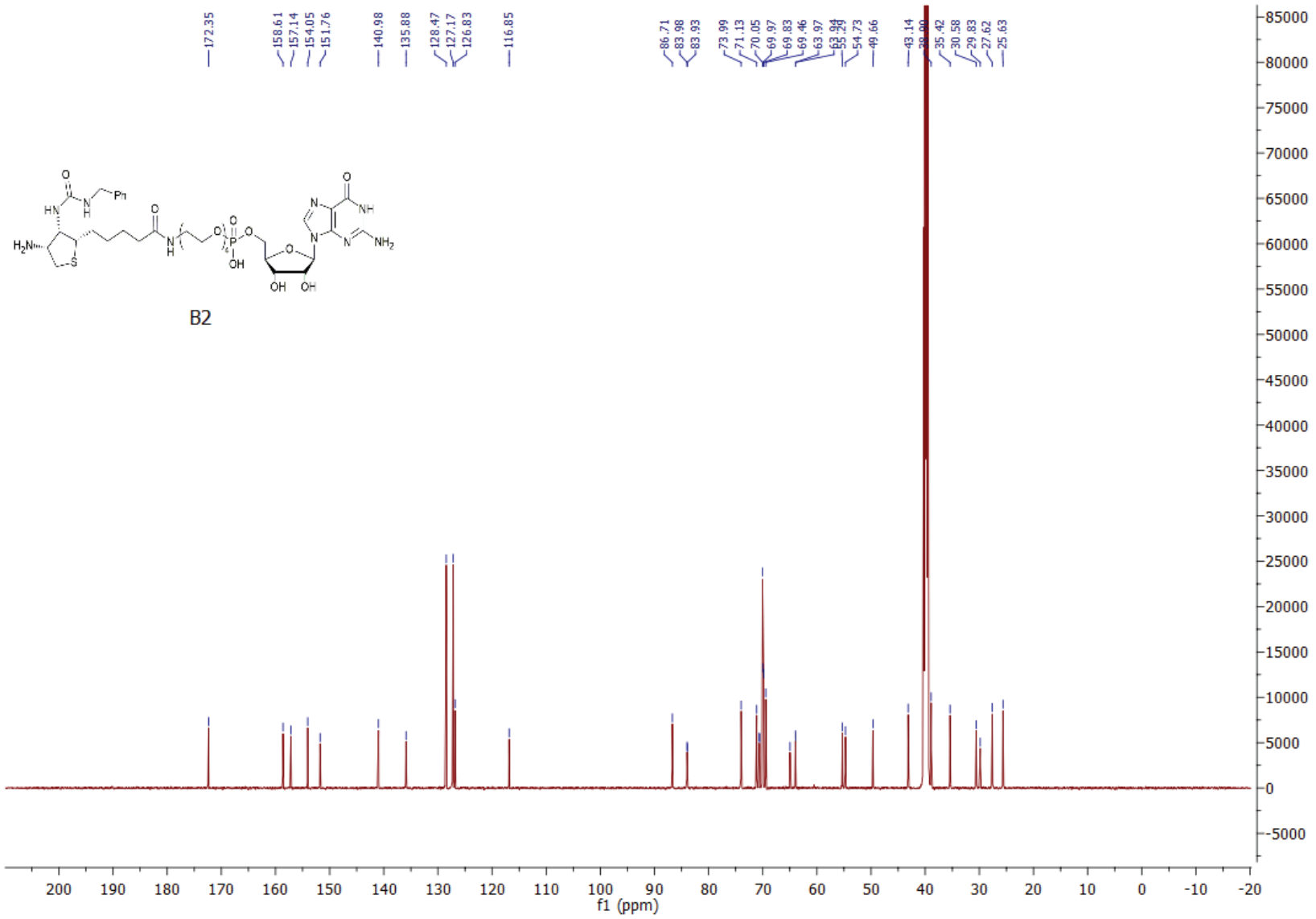


B1

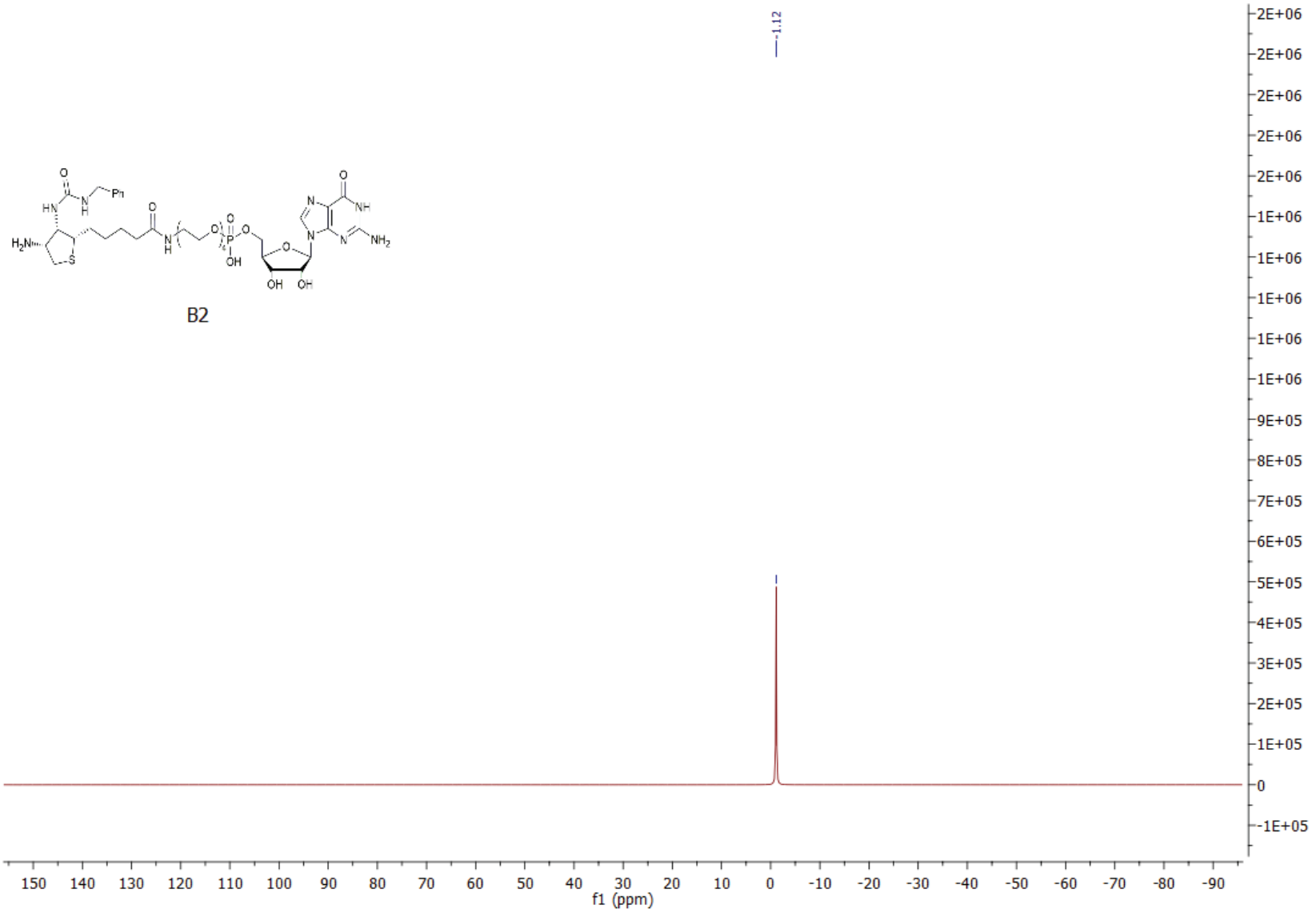
^{31}P NMR spectrum of compound B1 (121 MHz, DMSO-d_6)



^{1}H NMR spectrum of compound **B2** (500 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **B2** (151 MHz, DMSO-d_6)



B2

^{31}P NMR spectrum of compound **B2** (202 MHz, DMSO-d_6)