Supplemental Information

ω-Hydroxy isoprenoid bisphosphonates as linkable GGDPS inhibitors

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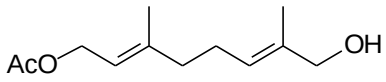
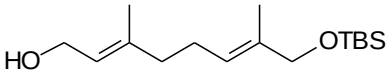
General experimental conditions. Tetrahydrofuran and diethyl ether were freshly distilled from sodium-benzophenone, while methylene chloride was distilled from calcium hydride prior to use. All other reagents and solvents were purchased from commercial sources and used without further purification. All reactions in nonaqueous solvents were conducted in flame-dried glassware under a positive pressure of argon and with magnetic stirring. All NMR spectra were obtained at 400 or 500 MHz for ^{1}H, and 100 MHz for ^{13}C, with internal standards of $(CH_3)_4Si$ (1H, 0.00) or $CHCl_3$ (^{1}H, 7.27; ^{13}C, 77.2 ppm) for non-aqueous samples or H_2O (^{1}H, 4.80) for aqueous samples. The ^{31}P chemical shifts were reported in ppm relative to 85% H_3PO_4 (external standard). High resolution mass spectra were obtained at the University of Iowa Mass Spectrometry Facility. Silica gel (60 Å, 0.040–0.063 mm) was used for flash chromatography.

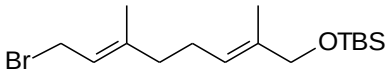


(2*E*,6*E*)-8-Hydroxy-3,7-dimethylocta-2,6-dien-1-yl Acetate (8). In a 250 mL round bottom flask, SeO_2 (188 mg, 1.7 mmol), salicylic acid (550 mg, 4 mmol), and t-BuOOH (20 mL of 70% solution in H_2O) were added sequentially to CH_2Cl_2 (60 mL) and the mixture was stirred for 30 min at 0 °C, then geranyl acetate (**7**, 8.6 mL, 40 mmol) was added. The biphasic mixture was stirred for an additional 22 h at 0 °C and then concentrated under reduced pressure at below 30 °C. After Et_2O (30 mL) was added, the organic layer was washed with 10% KOH (10 mL, 4 times), H_2O (8 mL, 3 times), and brine (10 mL), dried (Na_2SO_4), and finally concentrated in vacuo. The initial product was purified by gradient column chromatography (hexane:EtOAc = 7:3) to afford compound **8** (5.8 g, 63%) as a colorless oil. A second fraction contained the corresponding aldehyde, which was reduced to the alcohol by slow addition of $NaBH_4$ at 0 °C to give an additional

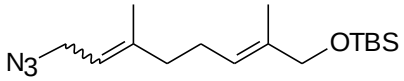
1.01 g of the compound **8**. Material from both fractions was combined to give a total yield of 74%, with spectral data in accord with the literature.[1]



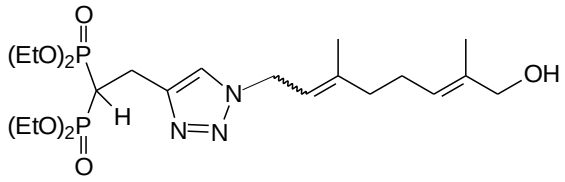
(2*E*,6*E*)-8-*tert*-Butyldimethylsilyloxy-3,7-dimethylocta-2,6-dien-1-ol[2] (10). A 250 mL round bottom flask was charged with the acetate **9**[2] (5.63 g, 18.0 mmol), K_2CO_3 (9.94 g, 72 mmol), and MeOH (100 mL). The mixture was stirred for 10 hours at rt. Methanol was removed under reduced pressure, and the residue was dissolved in a mixture of hexane (75 mL) and ether (75 mL). The solid residue was removed by filtration, and the filtrate was washed with H_2O (50 mL, 2 times), dried (Na_2SO_4), and concentrated using a rotary evaporator to afford the alcohol **10** as a yellow oil (5.06 g, 99%).



((2*E*,6*E*)-8-*tert*-Butyldimethylsilyloxy-3,7-dimethylocta-2,6-dien-1-ol[3] (11). A solution of the allylic alcohol **10** (4.77 g, 16.8 mmol) in THF (60 mL) was cooled to 0 °C followed by slow addition of pyridine (1.38 g, 17 mmol) and PBr_3 (4.60 g, 17 mmol). The reaction mixture was stirred for 40 minutes while maintaining the temperature at 0-5 °C, then diluted with hexane (100 mL) and quenched by addition of H_2O (50 mL). The organic layer was collected, washed with aq. $NaHCO_3$ (20 mL), H_2O (40 mL) and brine (50 mL), dried (Na_2SO_4), filtered through celite and basic alumina, and finally concentrated under reduced pressure to obtain **11** as a colorless oil (5.65 g, 97%) The ^1H NMR data matched that in the literature.[3]

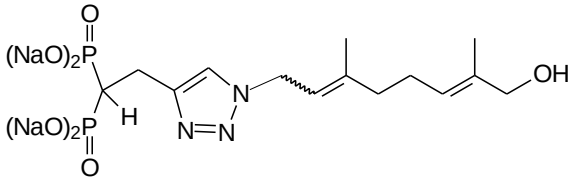


(2*E*,6*E*)-8- *tert*-Butyldimethylsilyloxy-3,7-dimethylocta-2,6-dien-1-azide (12). In an oven dried round bottom flask, compound **11** (5.48 g, 15.8 mmol) was dissolved in DMF (30 mL). After sodium azide (1.92 g, 30 mmol) was added, the mixture was stirred for 10 h at 45 °C. Upon completion of the reaction as monitored by TLC, the mixture was diluted with hexane (100 mL) and filtered to remove the solid residue. The hexane layer was collected, the filtrate was extracted with hexanes (50 mL, 2 times) and the extracts were combined, washed with brine (20 mL), dried (Na_2SO_4), and concentrated under reduced pressure to obtain the azide **12** as a colorless oil (4.39 g, 90%) which was carried immediately to the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40-5.34 (m, 2H), 4.03 (s, 2H) 3.80-3.77 (m, 2H), 2.21-2.16 (m, 4H), 1.83 (d, *J* = 1.1Hz, 1H), 1.73 (d, *J* = 1.1Hz, 2H), 1.63 (s, 3H), 0.93 (2s, 9H), 0.08 (2s, 6H).



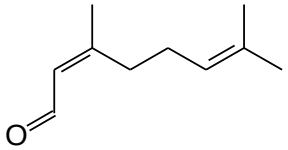
ω-Hydroxy bisphosphonate 14. A 100 mL flask was charged with acetylene **13** (391 mg, 1.2 mmol), sodium ascorbate (55 mg, 0.3 mmol), $CuSO_4 \cdot 5H_2O$ (37 mg, 0.15 mmol), ^{t}BuOH (7 mL) and H_2O (2 mL). A solution of azide **12** (515 mg, 1.7 mmol) in ^{t}BuOH (1 mL) was added to the mixture and it was stirred for 22 hours at rt. The reaction mixture was partitioned between EtOAc (80 mL) and brine (50 mL), and the organic layer was collected. The aqueous layer was extracted with EtOAc (50 mL, 2 times), the combined extracts were washed with H_2O (30 mL), dried (Na_2SO_4), and concentrated under reduced pressure to afford the triazole intermediate. This intermediate was then dissolved in AcOH (3 mL), H_2O (1 mL) was added to the reaction mixture and it was allowed to stir for 1 h at

rt. The reaction mixture was then concentrated under reduced pressure and partitioned between aq. $NaHCO_3$ (5 mL) and EtOAc (20 mL). The organic layer was collected, the aqueous layer was extracted with EtOAc (15 mL, 2 times), and then the extracts were combined, dried (Na_2SO_4), and concentrated at vacuo. The resulting material was subjected to SiO_2 column chromatography using hexanes:EtOH (4:1) to afford the desired triazole **14** (375 mg, 60% over 2 steps) as a mixture of *E* and *Z* isomers (~2:1) by integration of the triazole hydrogen resonance in the ^{1}H NMR spectrum: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 0.3 H), 7.47 (s, 0.6H), 5.36-5.29 (m, 2H), 4.87-4.85 (m, 2H), 4.12-4.00 (m, 8H), 3.92 (s, 2H), 3.30-3.21 (dt, $^2J_{HP}$ = 26.4 Hz, J = 6.5 Hz, 2H), 2.97-2.82 (m, 1H), 2.19-2.10 (m, 4H), 1.72-1.59 (4 s, 6H), 1.26-1.19 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.0, 144.9, 142.7, 141.6, 136.1, 135.7, 123.2, 122.6, 122.0, 121.8, 118.8, 117.3, 67.7, 67.5, 62.9-62.4 (4C), 47.8, 47.7, 38.9, 36.7 (t, $^2J_{CP}$ = 132.1 Hz), 31.5, 25.1, 25.0, 23.0, 22.1, 22.1, 22.0, 16.3-16.2 (4C), 13.7, 13.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.5, 22.5. HRMS (ES^+, *m*/*z*) calcd for $(M+H)^+$ $C_{22}H_{41}N_3P_2O_7$: 522.2498; found: 522.2493.

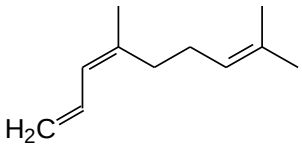


ω -Hydroxy bisphosphonate salt 15. A solution of the phosphonate esters **14** (260 mg, 0.5 mmol) in CH_2Cl_2 was cooled to 0 °C. Collidine (605 mg, 5 mmol) was then added slowly followed by the addition of TMSBr (918 mg, 6 mmol). The temperature was gradually raised to rt and the reaction was stirred for 13 hours under argon. After the solvent was removed under reduced pressure, toluene (10 mL) was added to the residue and concentrated in vacuo (repeated three times). A solution of 1N NaOH (2.5 mL) was

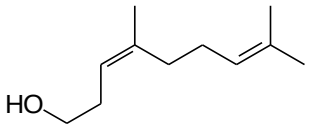
added to the reaction mixture and it was stirred for 20 minutes at rt. The reaction mixture then was transferred into a conical flask, and precipitation was encouraged by addition of anhydrous acetone. After the flask was stored in a freezer for 20 minutes, the product was collected by filtration. The solid precipitate was dissolved in water and dried on a lyophilizer to afford the desired tetra sodium salt **15** (180 mg, 75%): ^{1}H NMR (400 MHz, D_2O) δ 7.73-7.72 (2s, 1H), 5.45-5.35 (m, 1H), 5.31-5.26 (m, 1H), 4.88-4.85 (m, 2H), 3.80 (s, 2H), 3.11-3.02 (dt, $^{2}J_{HP}$ = 15.2 Hz, J = 6.8 Hz, 2H), 2.18-2.03 (m, 5H), 1.70 (s, 3H), 1.53-1.49 (2s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.3, 143.6, 143.3, 135.1, 134.7, 126.0, 125.8, 123.5, 121.8, 117.6, 117.2, 67.5, 67.4, 47.8, 47.7, 40.1 (t, $^{2}J_{CP}$ = 111.6 Hz), 38.2, 30.9, 28.2, 25.3, 25.0, 22.4, 22.3, 15.6, 12.9; ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.0. HRMS (ES^-, *m*/*z*) calcd for $(M\text{-}H)^-$ $C_{14}H_{24}N_3P_2O_7$: 408.1084; found: 408.1078.



(2*Z*)-3,7-Dimethyl-2,6-octadienal (17). Neral was prepared through a TEMPO oxidation as previously reported.[4] Alternatively, in an aluminum foil wrapped 500 mL round bottom flask, nerol (15.6 g, 102 mmol), MnO_2 (in activated charcoal, 32 g), and CH_2Cl_2 (100 mL) were added. The mixture was stirred for 5 days with addition of MnO_2 (5 g) every day. Once the reaction was complete as confirmed by TLC analysis, the solid was removed by filtration through a celite pad. The filtrate then was concentrated under reduced pressure to afford neral (**17,** 15.4 g, 100%) as a colorless oil.

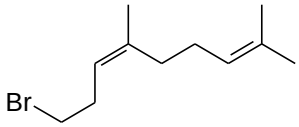


(3*Z*)-4,8-Dimethylnona-1,3,7-triene (**18).** To a flame-dried 500 mL round-bottomed flask was added triphenylphosphonium bromide (63.8 g, 180 mmol) and potassium *t*-butoxide (20.2 g, 180 mmol). The flask was charged with THF (150 mL) under argon. Neral (21 g, 150 mmol) then was added as a solution in THF (50 mL). The reaction mixture was allowed to stir at rt for 30 minutes, transferred to a conical flask and diluted with Et_2O (50 mL) and pentane (50 mL). Upon addition of $ZnBr_2$ (8 g) to the reaction mixture, a white precipitate of $Ph_3P(O)$ was formed as a (TPPO)-Zn complex. The solution was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The oil obtained was dissolved in pentane (100 mL) and the remaining TPPO was precipitated by further addition of $ZnBr_2$ (5 g). The resulting solid was removed by filtration and the filtrate was concentrated in vacuo. The initial product was purified by column chromatography on SiO_2 (100% hexanes) to afford the triene **18** as a clear, colorless oil (19.5 g, 88%). All spectral data were in accordance with the literature.[4]



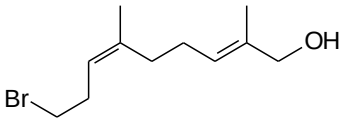
(**3*Z*)-4,8-Dimethylnona-3,7-dien-1-ol (19).** A procedure used to prepare homogeraniol[5] was adapted to prepare homonerol. Accordingly, a flame dried 500 mL flask was charged with triene (12 g, 80 mmol) and anhydrous THF (50 mL) under argon. The solution was cooled to 0 °C and 9-BBN (160 mL of a 0.5 M solution in THF) was added in portions

over 2.5 h while keeping the temperature below 0 °C. The reaction mixture was stirred for 6 hours at 0 °C, gradually allowed to warm to rt, and stirred 6 hours at rt. The solution was then cooled to -10 °C, NaOH (30 mL, 3M) and H_2O_2 (30 mL, 30% solution) were added slowly. The mixture was stirred for 2 h at 0 °C and 1 h at rt. The resulting two layers were separated to collect the organic layer, and the aqueous layer was extracted with Et_2O (100 mL, 2 times). The organic extracts were combined, dried (Na_2SO_4), and concentrated in vacuo to yield an oil. This product was purified by column chromatography on SiO_2 (EtOAc:hexanes = 1:4) to afford the desired homonerol (**19**) as a clear, isomerically pure, colorless oil (6.38 g, 48%). All spectral data were in accordance with the literature.[6]

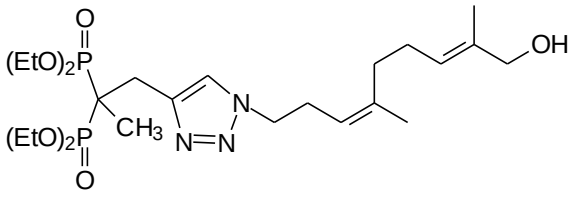


(6*Z*)-9-Bromo-2,6-dimethylnonan-2,6-diene (**20).** According to a previously published procedure, homonerol was converted to the corresponding bromide **20** through an intermediate mesylate.[6] To a flame-dried 100 mL round-bottomed flask was added homonerol (1.40 g, 8.3 mmol), CH_2Cl_2 (50 mL), and Et_3N (1.51 g, 15 mmol) at 0 °C under argon. The mixture was stirred for 30 minutes, then MsCl (1.1 mL, 13 mmol) was added slowly at 0 °C, and stirring was continued for 4 hours. Upon completion, the reaction was diluted with CH_2Cl_2 (50 mL), washed with 1N HCl (20 mL), sat. $NaHCO_3$ (20 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure to afford the mesylate (1.95 g) which was carried to the next step without further purification. The mesylate was dissolved in DMF (20 mL) followed by the addition of NaBr[7] (1.24 g, 12 mmol). The mixture was stirred for 16 hours at 40 °C while monitoring

the reaction progress by TLC. After consumption of the starting material, the mixture was extracted with hexane (50 mL, 4 times), the extracts were combined, washed with H_2O (30 mL, 2 times), and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was subjected to column chromatography (SiO_2, EtOAc:hexane = 1:9) to afford the desired homoneryl bromide **20**[8] (1.63 g, 85% over 2 steps) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.16-5.09 (m, 2H), 3.33 (t, *J* = 7.2Hz, 2H), 2.59-2.54 (m, 2H), 2.08-2.03 (m, 4H), 1.73 (s, 3H), 1.70 (s, 3H), 1.62 (s, 3H).

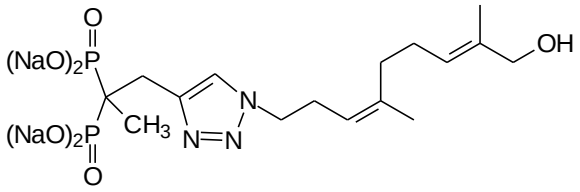


(6*Z*)-9-Bromo-2,6-dimethylnonan-2,6-dien-1-ol (**21**)**.** In a 50 mL round bottom flask, SeO_2 (166 mg, 1.5 mmol), salicylic acid (40 mg, 0.3 mmol), and *t*-BuOOH (2 mL, 5-6M solution in hexane) were added sequentially to CH_2Cl_2 (6 mL) and the reaction mixture was stirred for 30 mins at 0 °C, then homoneryl bromide (696 mg, 3 mmol) was added.[9] The mixture was stirred for an additional 14 hours at 0 °C, and then was concentrated under reduced pressure at 30 °C. After Et_2O (30 ml) was added, the organic layer was washed with 10% KOH (5 mL, 3 times), H_2O (8 mL, 2 times), and brine (8 mL), then dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, EtOAc:hexanes = 1:4) to afford alcohol **21** (186 mg, 25%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (m, 1H), 5.14 (t, *J* = 7.0 Hz, 1H), 4.00 (s, 2H), 3.33 (t, *J* = 7.2 Hz, 2H), 2.55 (m, 2H), 2.16-2.06 (m, 4H), 1.73 (s, 3H), 1.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4, 135.4, 125.5, 122.1, 68.9, 33.1, 31.8, 31.7, 26.1, 23.5, 13.8; HRMS (EI^+, *m*/*z*) calcd for (M^+) $C_{11}H_{19}BrO$: 246.0614 [M^+], 248.0594 [M^++2]; found: 246.0625 [M], 248.0603 [M+2].



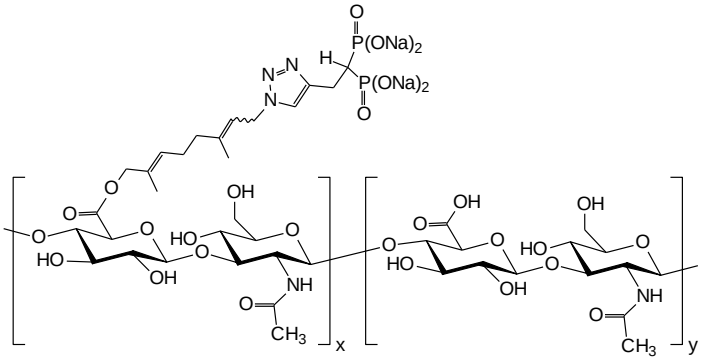
ω-Hydroxy bisphosphonate 24. In a 50 mL flask equipped with a magnetic stirrer, allylic bromide **21** (220 mg, 0.9 mmol) was dissolved in DMF (4.5 mL). Solid NaN_3 (87 mg, 1.35 mmol) was added and the reaction mixture was stirred at 60 °C while monitoring the reaction progress by TLC. After 16 hours, the solution was diluted with Et_2O (30 mL), the solid was removed through filtration, and the filtrate was concentrated under reduced pressure to afford homoneryl azide which was used without further purification. In a 50 mL flask, **23**[10] (325 mg, 1 mmol), $CuSO_4.5H_2O$ (3 drops of sat. solution), sodium ascorbate (39 mg, 0.2 mmol) and a mixture of *tert*-BuOH/H_2O (3/1 mL) were added sequentially. The homoneryl azide (in 2 mL of ^{t}BuOH) was then added to the reagent mixture and the reaction was stirred for 12 hours at rt. Upon completion, the reaction mixture was partitioned between CH_2Cl_2 (30 mL) and brine (20 mL). The organic layer was collected, the aqueous layer was further extracted with CH_2Cl_2 (30 mL, 3 times), and the combined extracts were dried (Na_2SO_4) and concentrated. The residue was then subjected to column chromatography (SiO_2, CH_2Cl_2:EtOH = 9:1) to afford the desired triazole **24** (370 mg, 75%) as a viscous oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 5.36 (t, J = 6.3 Hz, 1H), 5.07 (t, J = 7.1 Hz, 1H), 4.26 (t, J = 7.3 Hz, 2H), 4.19-4.07 (m, 8H), 3.97 (s, 2H), 3.30 (t, $^3J_{HP}$ = 14.2 Hz, 2H), 2.76 (s, 1H, OH), 2.58-2.52 (m, 2H), 2.09-2.03 (m, 4H), 1.68 (s, 3H), 1.62 (s, 3H), 1.45 (t, $^3J_{HP}$ = 16.6 Hz, 3H), 1.29-1.25 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.4, 138.8, 135.5, 124.2, 124.1, 119.8, 68.0, 63.0-62.6 (4C, m), 50.2, 41.5 (t, $^2J_{CP}$ =

133.0 Hz), 31.5, 28.9, 28.8, 25.5, 23.2, 16.4-16.3 (4C), 13.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 25.94; HRMS (ES+, *m*/*z*) calcd for $(M+H)^+$ $C_{24}H_{45}N_3P_2O_7$: 550.2800; found: 550.2812.



ω-Hydroxy bisphosphonate salt 25. A solution of the tetraethyl ester **24** (80 mg, 0.15 mmol) in CH_2Cl_2 (3 mL) was cooled to 0 °C. Collidine (121 mg, 1 mmol) was then added slowly to the solution followed by the addition of TMSBr (184 mg, 1.2 mmol). The reaction was allowed to warm to room temperature gradually and stirred under argon while the progress of the reaction was monitored periodically by ^{31}P NMR. After 12 hours, the solvent was removed under reduced pressure, and then 10 mL of toluene was added to the residue and the volatiles were removed in vacuo (repeated three times). A solution of 1N NaOH (1.0 mL) was added to the residue and it was stirred for 20 minutes at rt. The reaction mixture was transferred into a conical flask, the salt was precipitated by addition of anhydrous acetone, and after storage at 0 °C for 20 minutes the reaction was filtered. The solid precipitate was dissolved in water and the aqueous solution was dried in a lyophilizer. After analysis of the ^{1}H NMR spectrum showed the presence of collidine (0.3 mmol) the final cycle was repeated. Thus, treatment with NaOH (0.4 mL 1N solution), precipitation by addition of anhydrous acetone, dissolution in H_2O, and lyophilization overnight provided the desired tetra-sodium salt **25** (39 mg, 50%): ^{1}H NMR (400 MHz, D_2O) δ 7.78 (s, 1H), 5.25 (t, *J* = 6.1 Hz, 1H), 5.06 (t, *J* = 7.1 Hz, 1H), 4.26 (t, *J* = 6.4 Hz, 2H), 3.82 (s, 2H), 3.06 (t, $^3J_{HP}$ = 13.2 Hz, 2H), 2.47 (m, 2H), 1.88-1.81 (m, 4H), 1.56 (s, 3H), 1.51 (s, 1H), 1.17 (s, 1H, OH), 1.07 (t, $^3J_{HP}$ = 14.9 Hz, 3H); ^{13}C NMR (100 MHz,

D_2O) δ 145.1, 139.9, 134.6, 126.5, 125.8, 120.1, 67.6, 50.2, 40.0 (t, $^2J_{CP}$ = 113.0 Hz), 30.5, 28.3, 28.2, 25.3, 22.4, 18.3, 12.9; ^{31}P NMR (162 MHz, D_2O) δ 23.2; HRMS (ES^-, *m*/*z*) calcd for $(M\text{-}H)^-$ $C_{17}H_{29}N_3P_2O_7$: 436.1396; found: 436.1402.



Hyaluronic acid conjugate 27. Hyaluronic acid (**26**, 10 kDa) was dissolved in 20 ml nanopure water and stirred for 15 mins. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide (10x molar excess to bisphosphonate **15**) was added to the aqueous mixture and stirred for 30 min to allow activation of the carboxylic groups. Compound **15** (103 µl, 0.1 M) was added dropwise to achieve an overall 20 wt% composition in the reaction mixture. The reaction was allowed to stir for 24 h at rt. The reaction contents then were transferred to dialysis tubing (MWCO: 3500 Da) and dialyzed against nanopure water for 48 h. The final dialyzed product was transferred to a lyophilizer to obtain a white fluffy powder, which was stored at -20 °C. The reaction yield was 72% relative to compound **26** and drug conjugation was 2.7 wt%. The conjugate was characterized using ^{1}H and ^{31}P NMR.

Immunoblot analysis. RPMI-8226 or MM.1S (ATCC, Manassas, VA) cells were incubated (37 ºC and 5% CO_2) with test compounds for 48 hrs in RPMI-1640 media containing 10% fetal bovine serum and penicillin-streptomycin. Whole cell lysate was obtained using RIPA buffer (0.15 M NaCl, 1% sodium deoxycholate, 0.1% SDS, 1% Triton

(v/v) X-100, 0.05 M Tris HCl) containing protease and phosphatase inhibitors. Protein content was determined using the bicinchoninic acid (BCA) method (Pierce Chemical, Rockford, IL). Equivalent amounts of cell lysate were resolved by SDS-PAGE, transferred to polyvinylidene difluoride membrane, probed with the appropriate primary antibodies and detected using HRP-linked secondary antibodies and Millipore Immobilon ECL Ultra Substrate (for Rap1a) or Bio-Rad Clarity ECL substrate (for β-tubulin) western blotting reagents per manufacturer's protocols. The Rap1a antibody was purchased from Santa Cruz Biotechnology (sc-373968) and the β-tubulin antibody was purchased from Sigma (T5201).

Lambda light chain ELISA. A human lambda light chain kit (Bethyl Laboratories, Montgomery, TX) was used to quantify intracellular monoclonal protein levels in whole cell lysate. Lambda light chain levels were normalized to total protein levels (as determined by BCA).

FDPS and GGDPS enzyme assays. Recombinant FDPS was kindly provided by Dr. Raymond Hohl (Penn State Cancer Institute). Recombinant GGDPS was kindly provided by Dr. Edward Snell (Hauptman-Woodward Medical Research Institute). Enzyme assays were performed as previously described.[11] Compounds were tested in duplicate at multiple concentrations and three independent experiments were performed.

Statistics. Two-tailed *t*-testing was used to calculate statistical significance. An α of 0.05 was set as the level of significance. CompuSyn software (ComboSyn, Inc.,) was used to analyze the concentration response curves and determine the IC_{50} values. This software is based on the work of Chou and Talalay.[12,13]

References

(1) Yu, X.-J.; Zhang, H.; Xiong, F.-J.; Chen, X.-X.; Chen, F.-E. *Helv. Chim. Acta* **2008**, *91*, 1967.

(2) Reardon, M. B.; Xu, M.; Tan, Q.; Baumgartel, P. G.; Augur, D. J.; Huo, S.; Jakobsche, C. E. *J. Org. Chem.* **2016**, *81*, 10964.

(3) Lee, E. R.; Lakomy, I.; Bigler, P.; Scheffold, R. *Helv. Chim. Acta* **1991**, *74*, 146.

(4) Kliman, L. T.; Mlynarski, S. N.; Ferris, G. E.; Morken, J. P. *Angewandte Chemie-International Edition* **2012**, *51*, 521.

(5) Huang, H. J.; Yang, W. B. *Tetrahedron Lett.* **2007**, *48*, 1429.

(6) Matthiesen, R. A.; Wills, V. S.; Metzger, J. I.; Holstein, S. A.; Wiemer, D. F. *J. Org. Chem.* **2016**, *81*, 9438.

(7) Smitt, O.; Hogberg, H. E. *Tetrahedron* **2002**, *58*, 7691.

(8) Cabello, N.; Rodriguez, C.; Echavarren, A. M. *Synlett* **2007**, 1753.

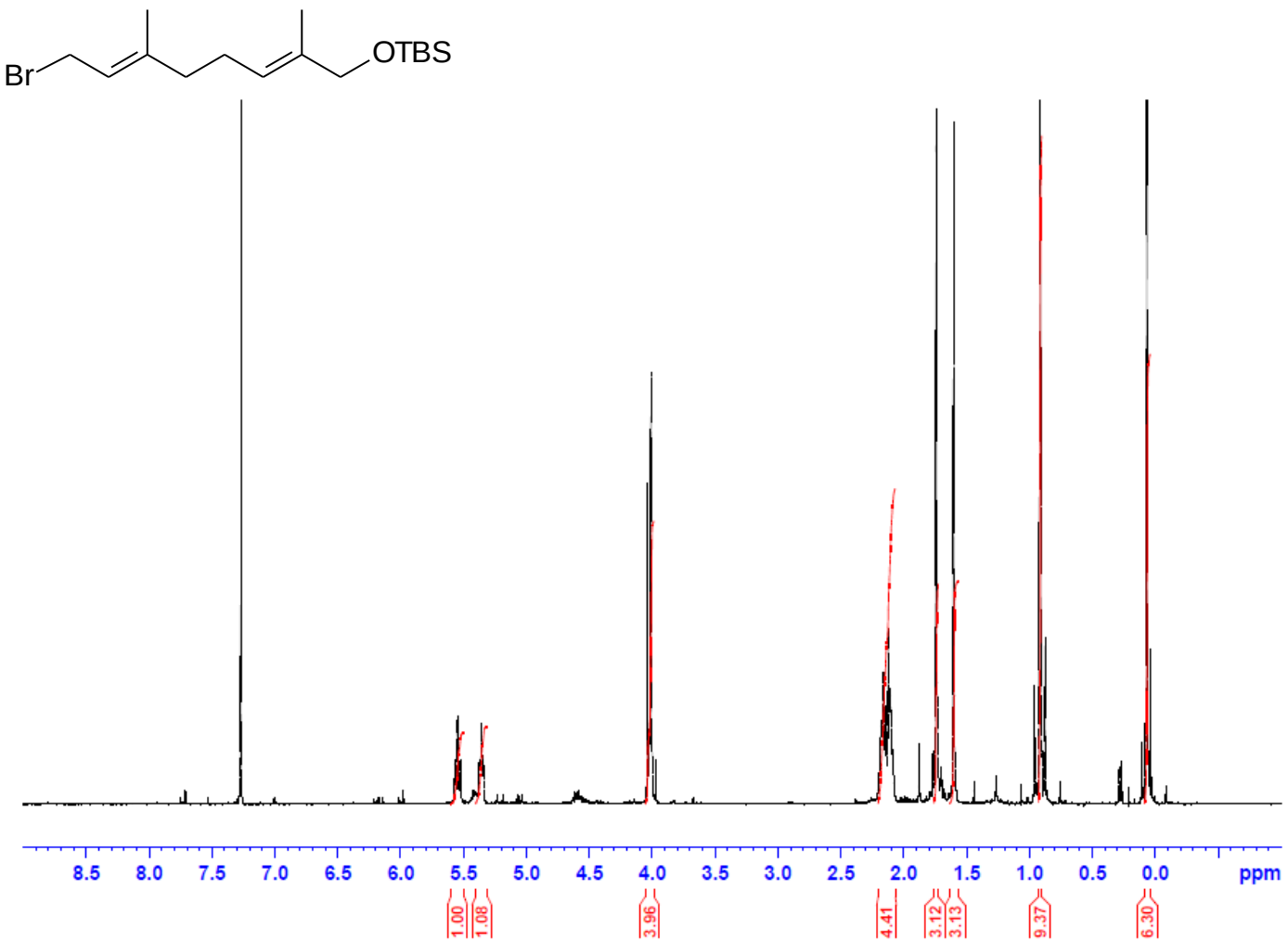
(9) Huang, Q. H.; Rawal, V. H. *Organic Letters* **2006**, *8*, 543.

(10) Matthiesen, R. A.; Varney, M. L.; Xu, P. C.; Rier, A. S.; Wiemer, D. F.; Holstein, S. A. *Bioorg. Med. Chem.* **2018**, *26*, 376.

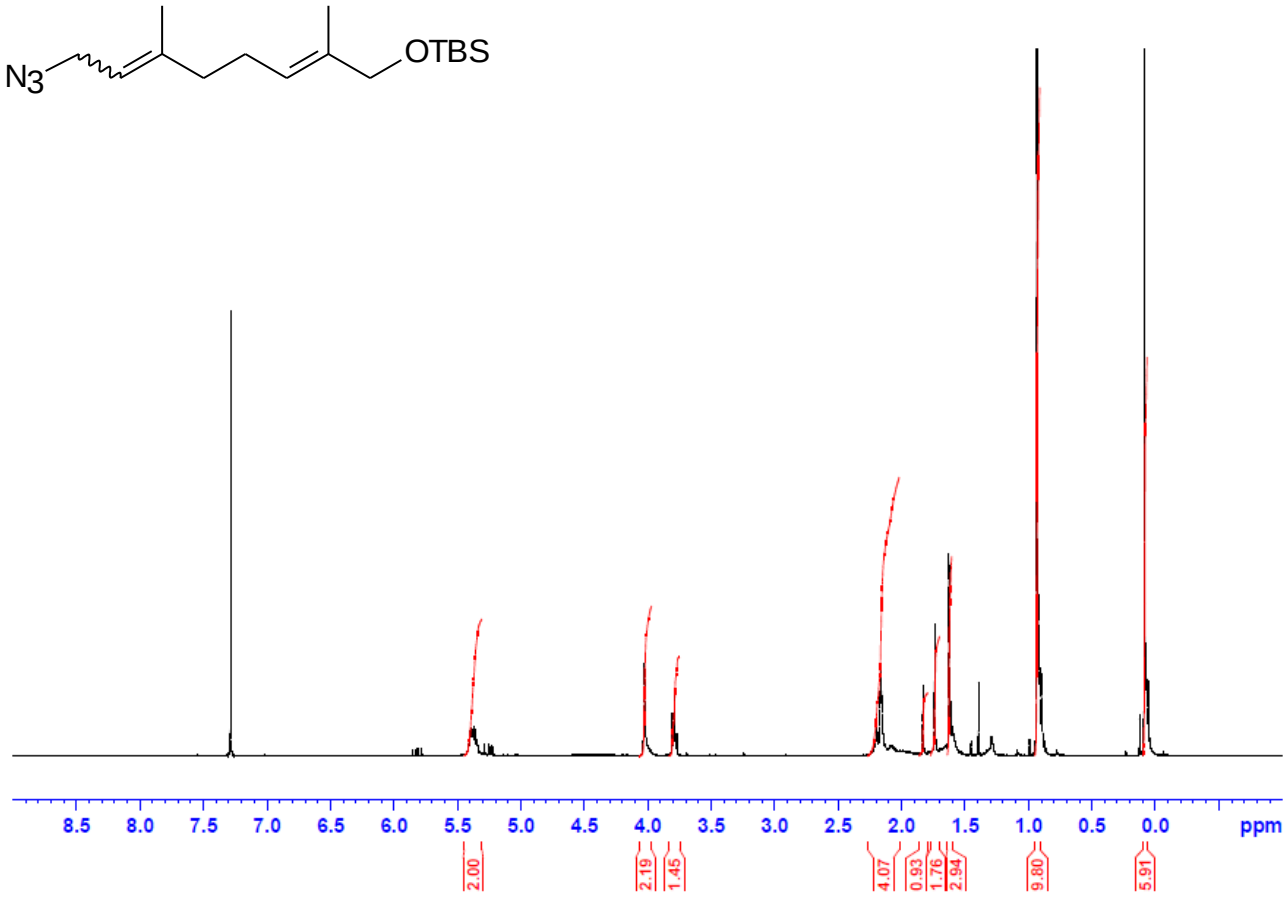
(11) Wills, V. S.; Allen, C.; Holstein, S. A.; Wiemer, D. F. *ACS Med Chem Lett* **2015**, *6*, 1195.

(12) Chou, T. C. *Pharmacological reviews* **2006**, *58*, 621.

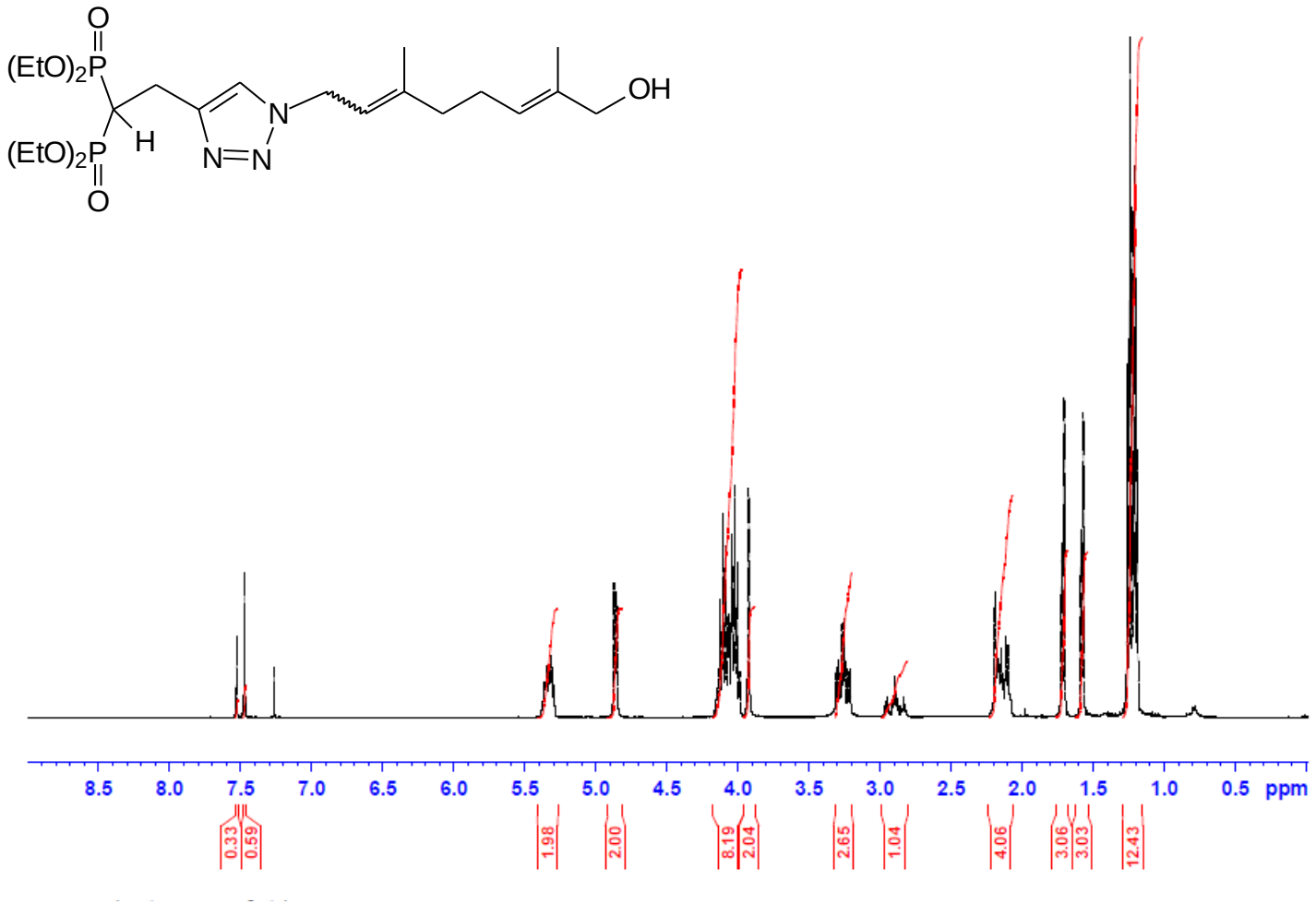
(13) Chou, T. C.; Talalay, P. *Adv. Enzyme Regul.* **1984**, *22*, 27.



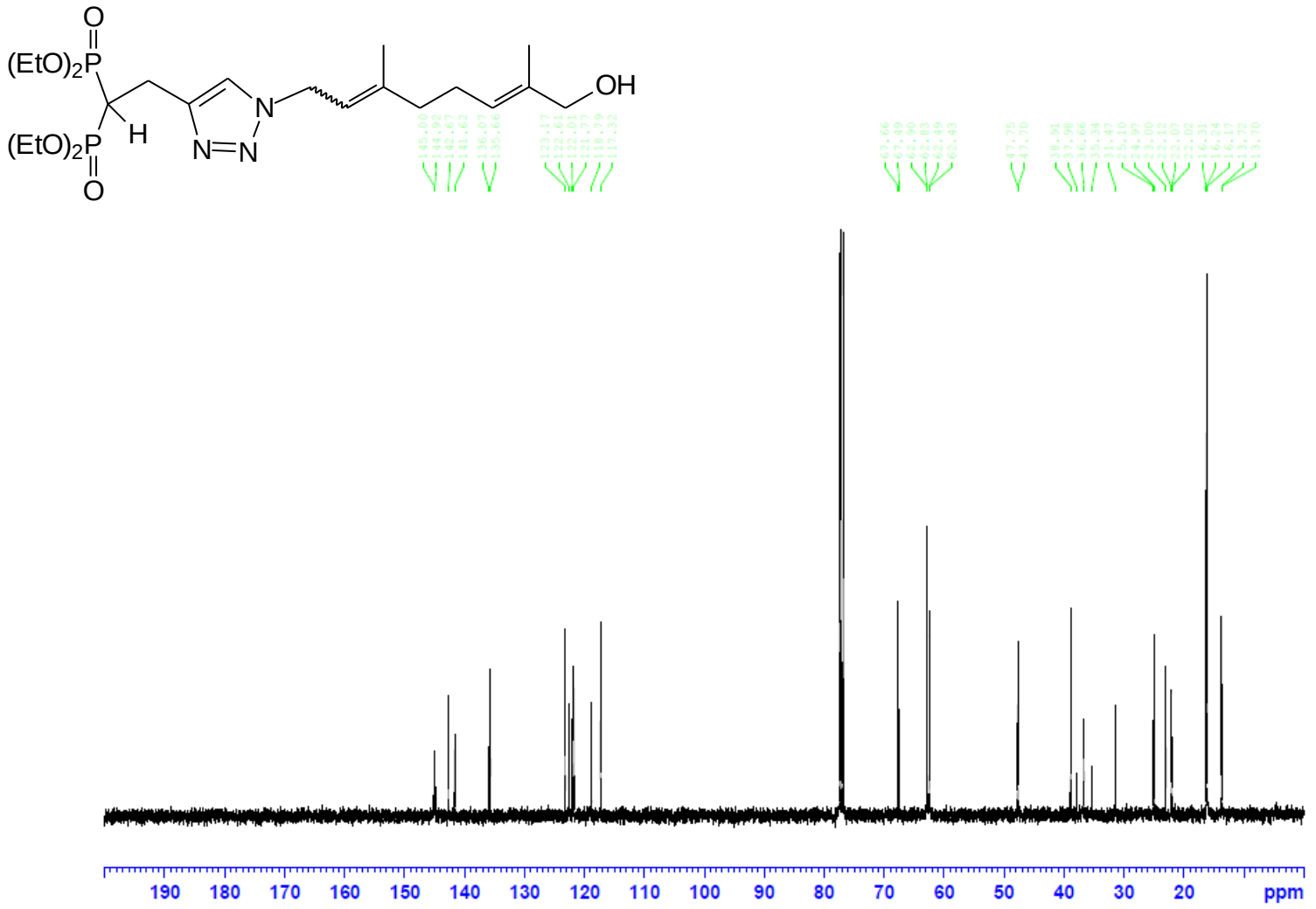
^{1}H NMR Spectrum of Compound **11**



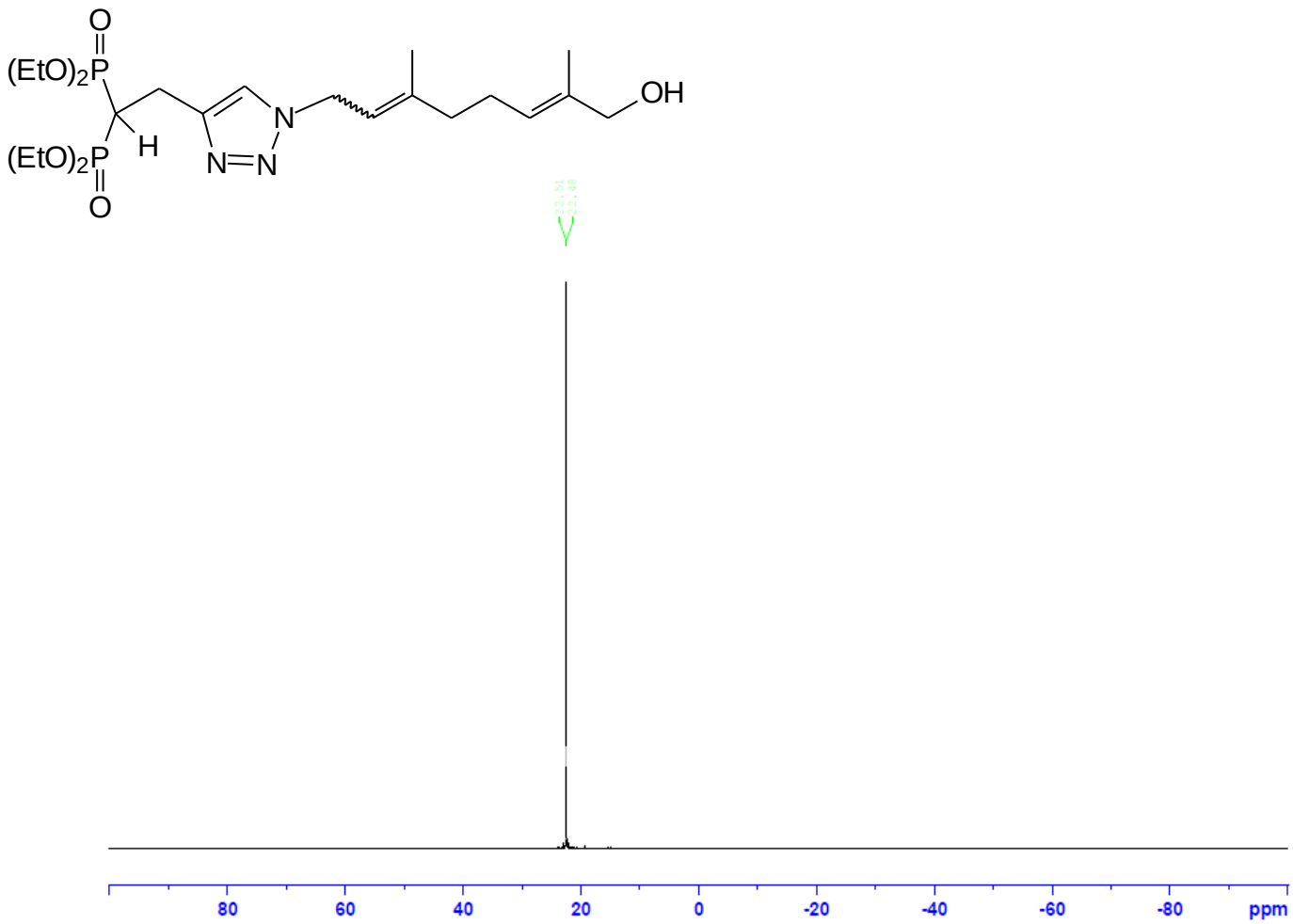
^{1}H NMR Spectrum of Compound **12**



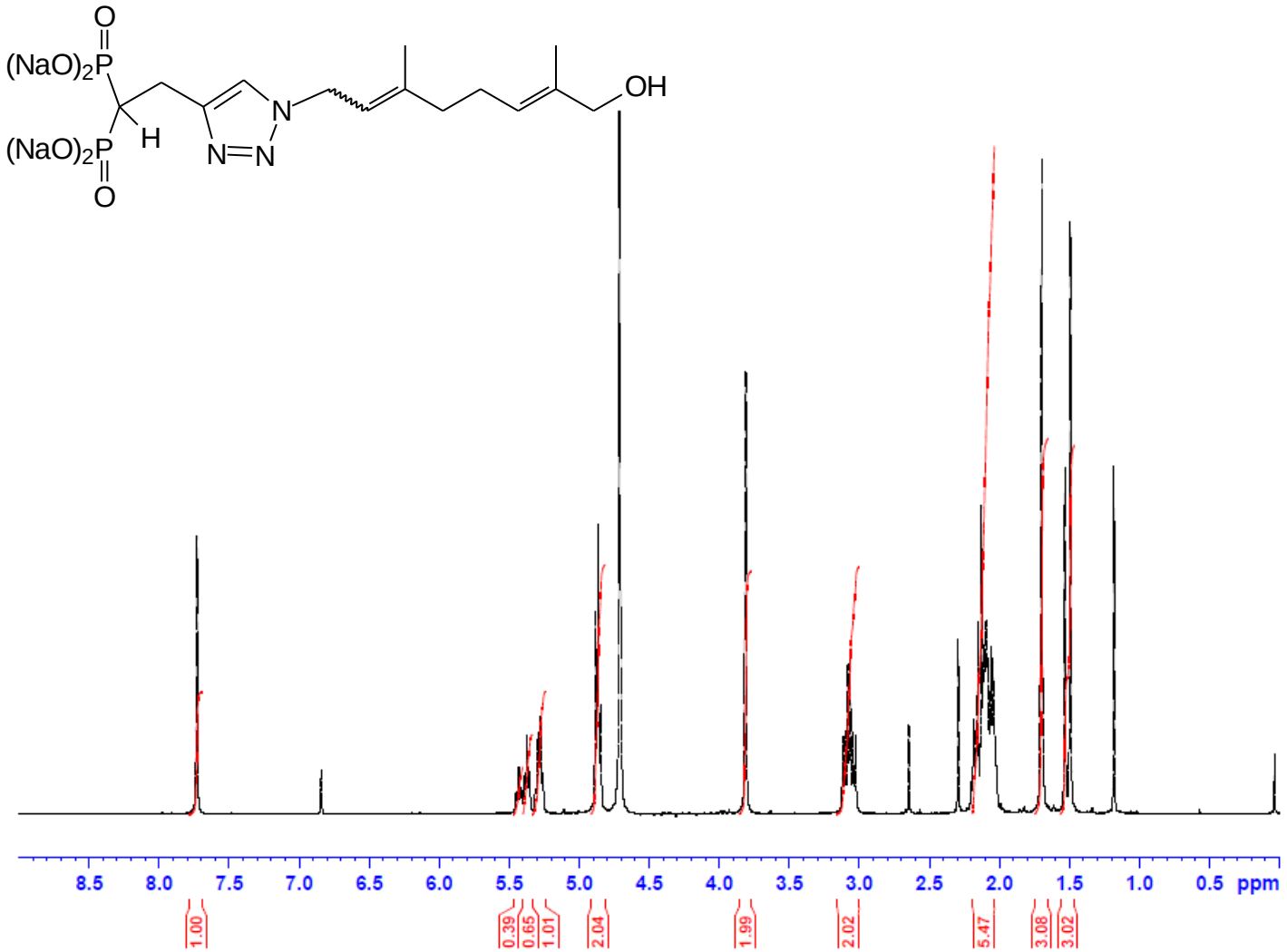
^{1}H NMR Spectrum of Compound **14**



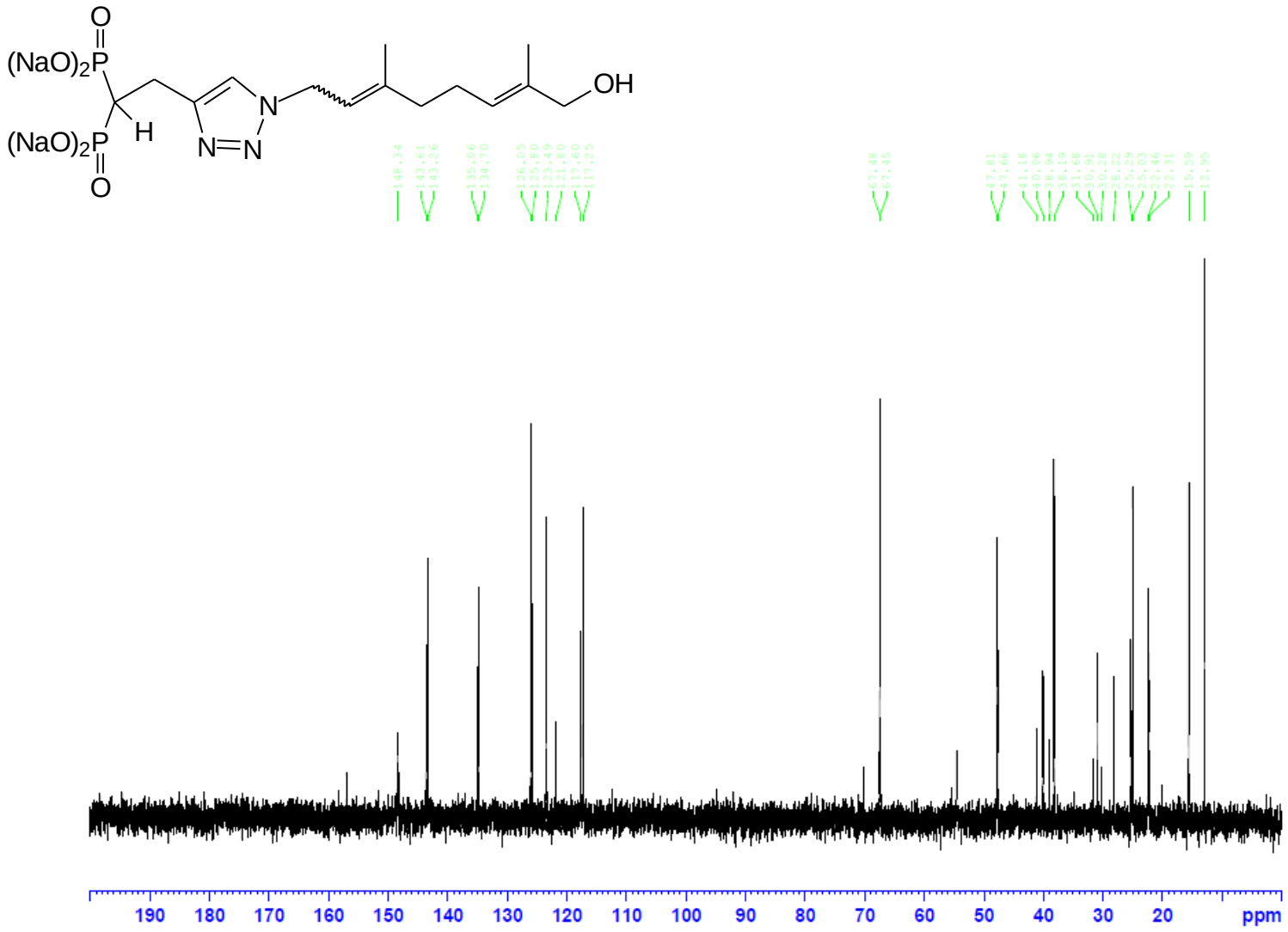
^{13}C NMR Spectrum of Compound **14**



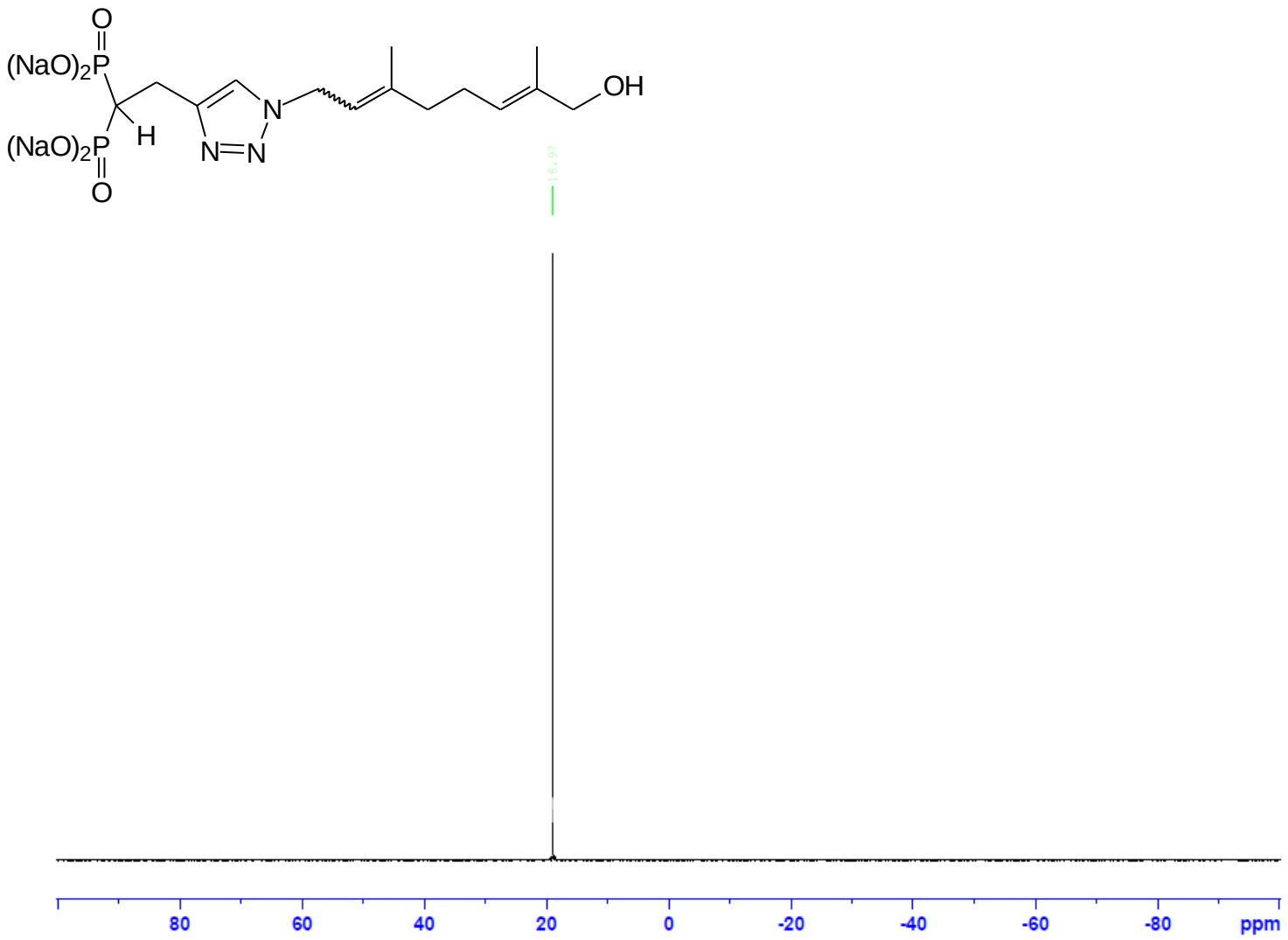
^{31}P NMR Spectrum of Compound **14**



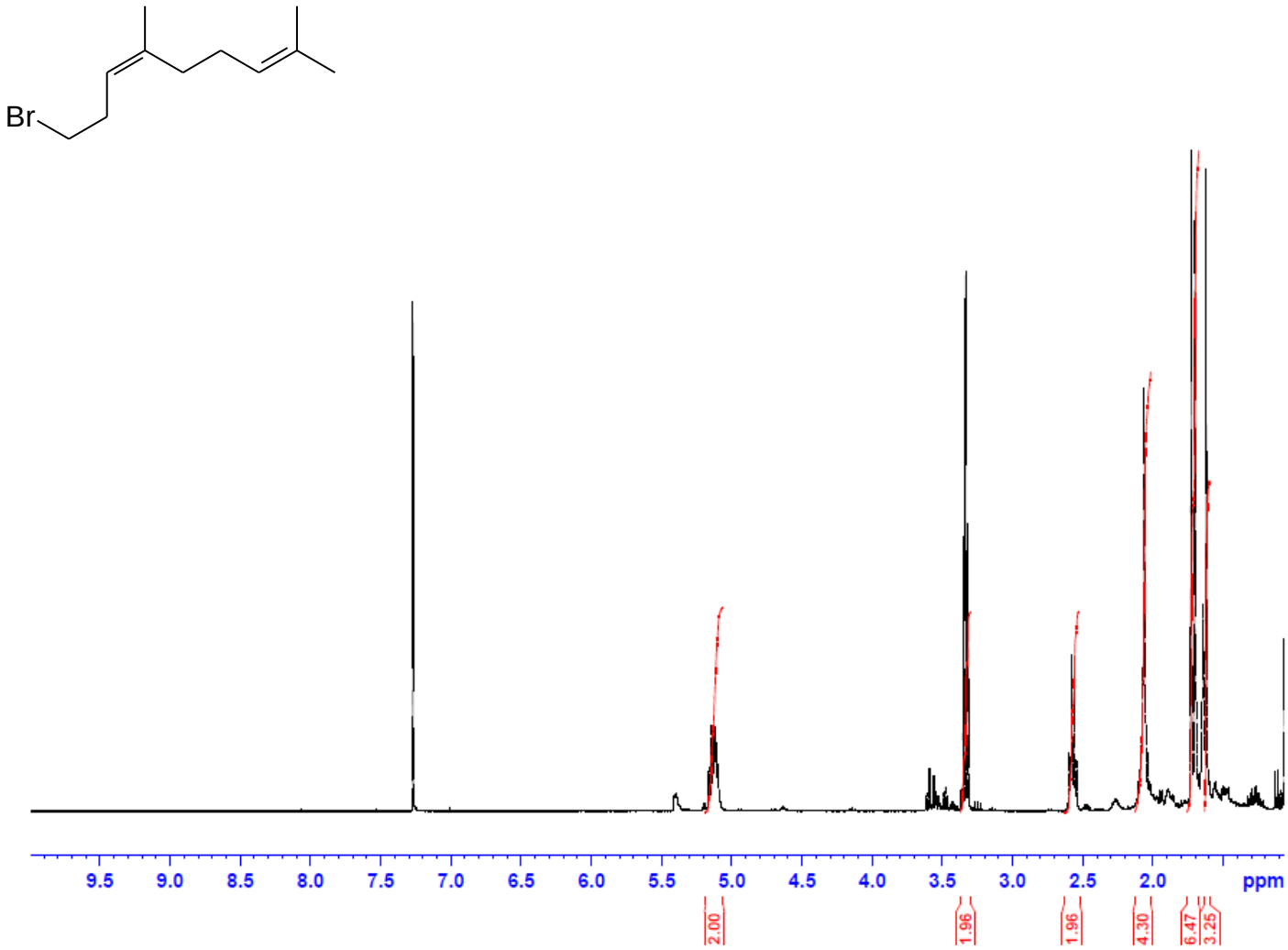
^{1}H NMR Spectrum of Compound **15**



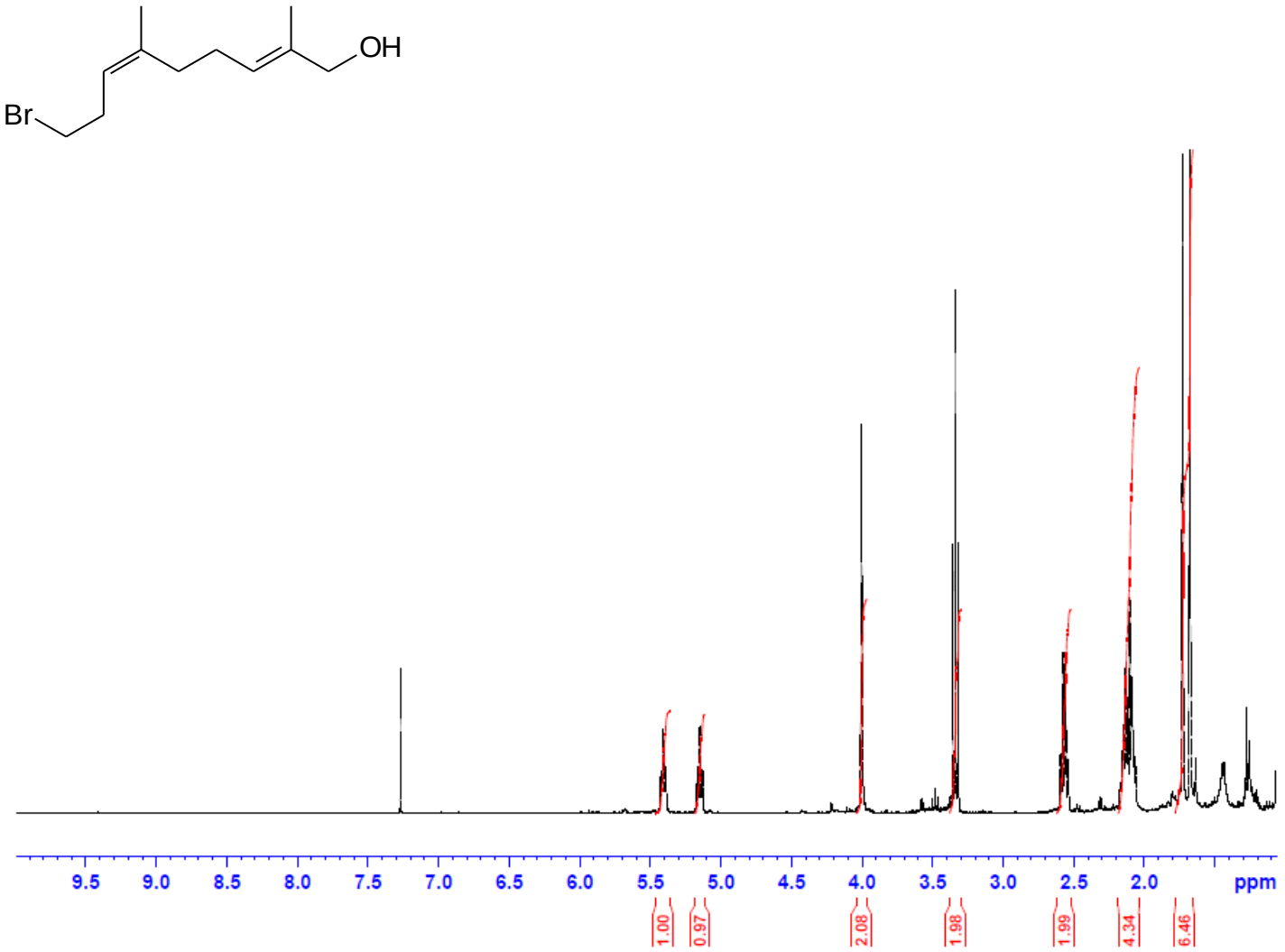
^{13}C NMR Spectrum of Compound **15**



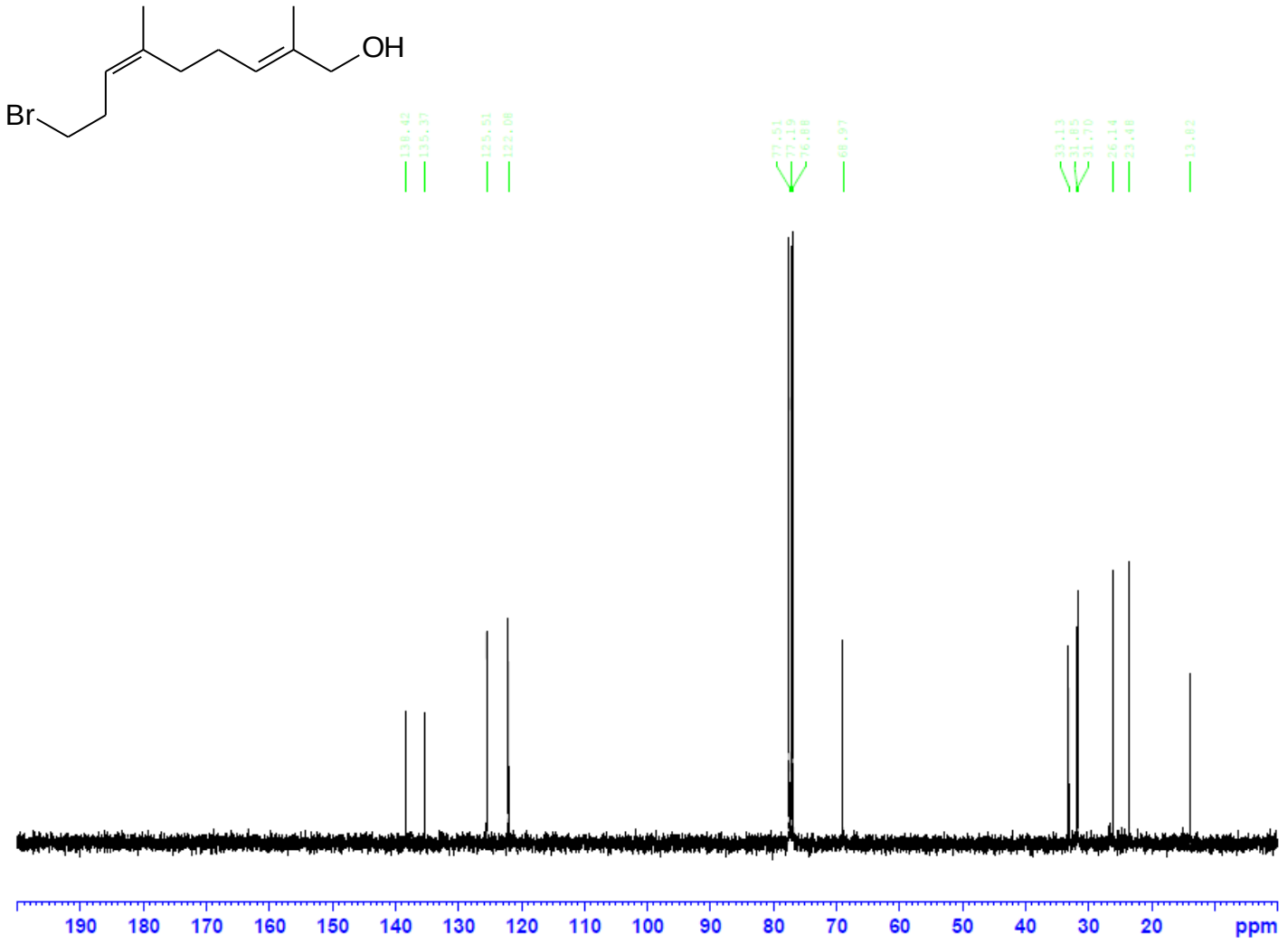
^{31}P NMR Spectrum of Compound **15**



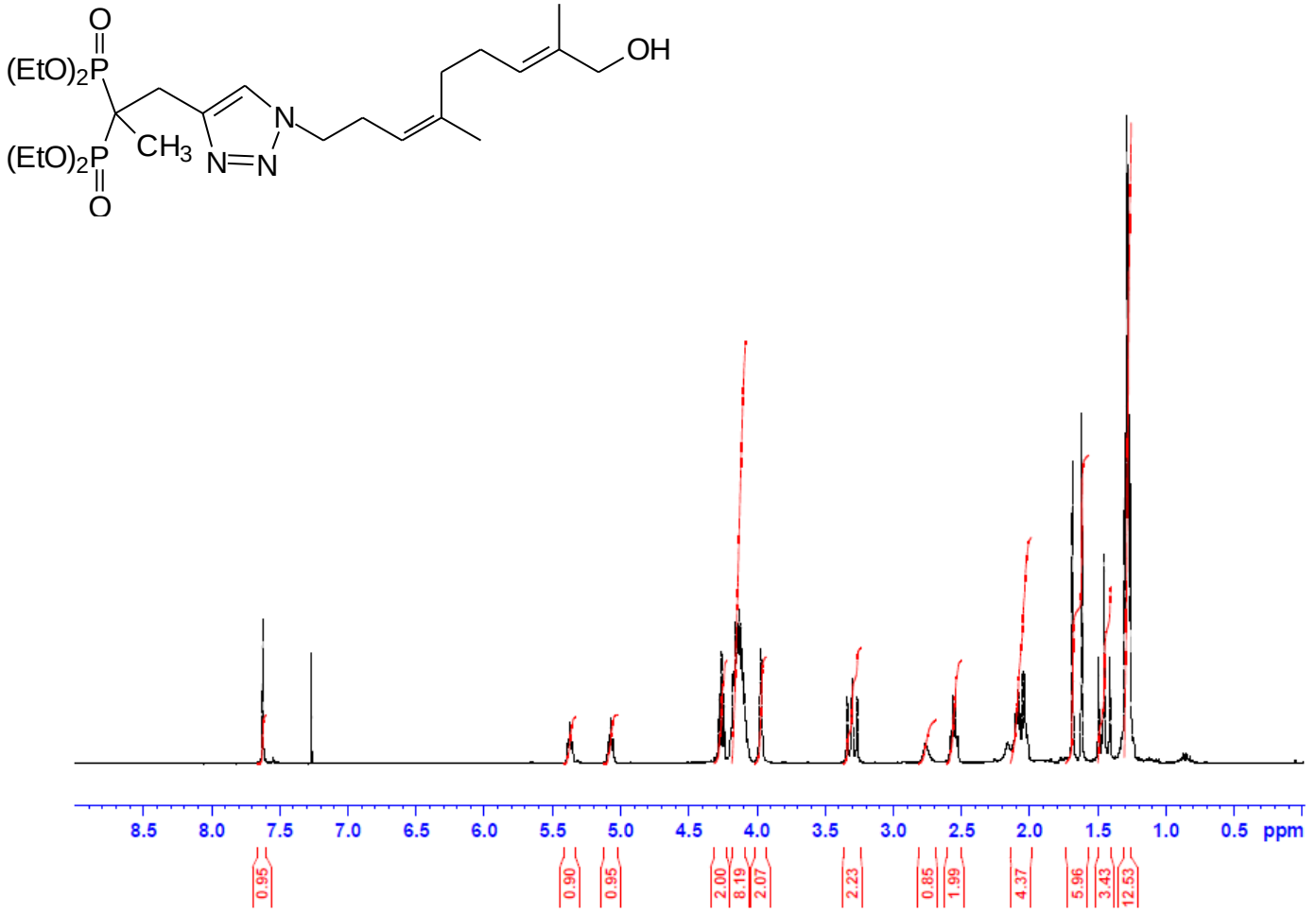
^{1}H NMR Spectrum of Compound **20**



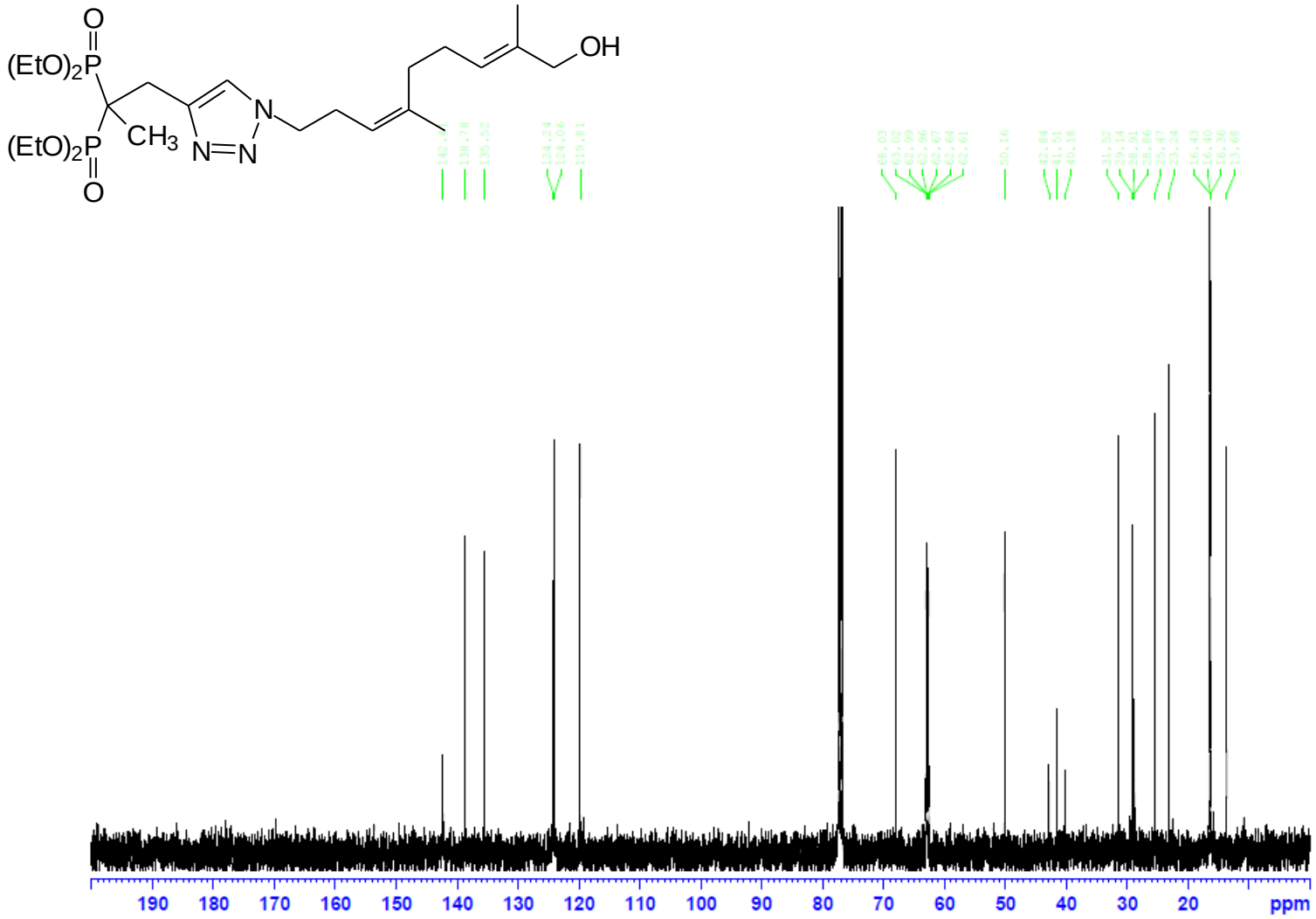
^{1}H NMR Spectrum of Compound **21**



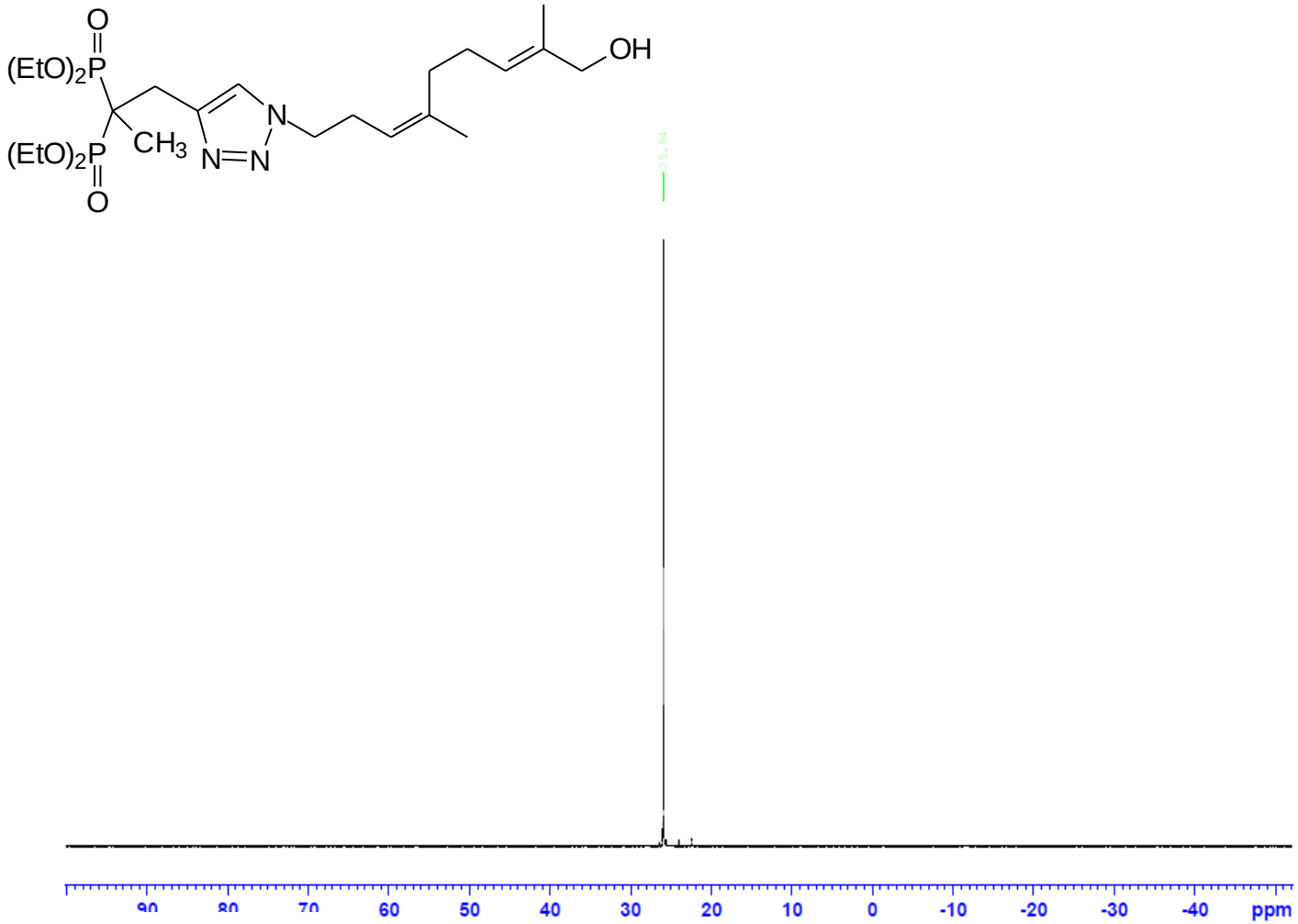
^{13}C NMR Spectrum of Compound **21**



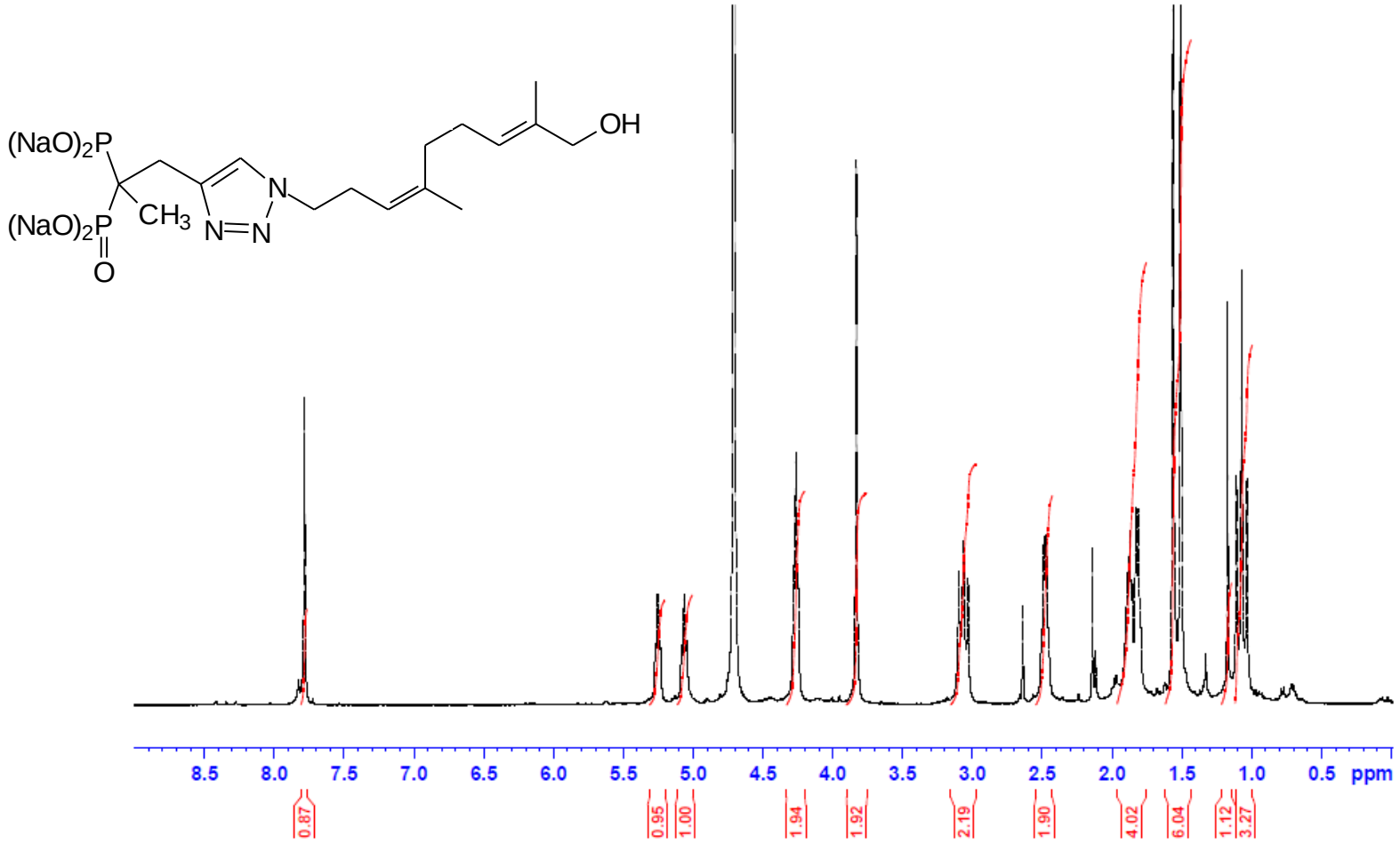
^{1}H NMR Spectrum of Compound **24**



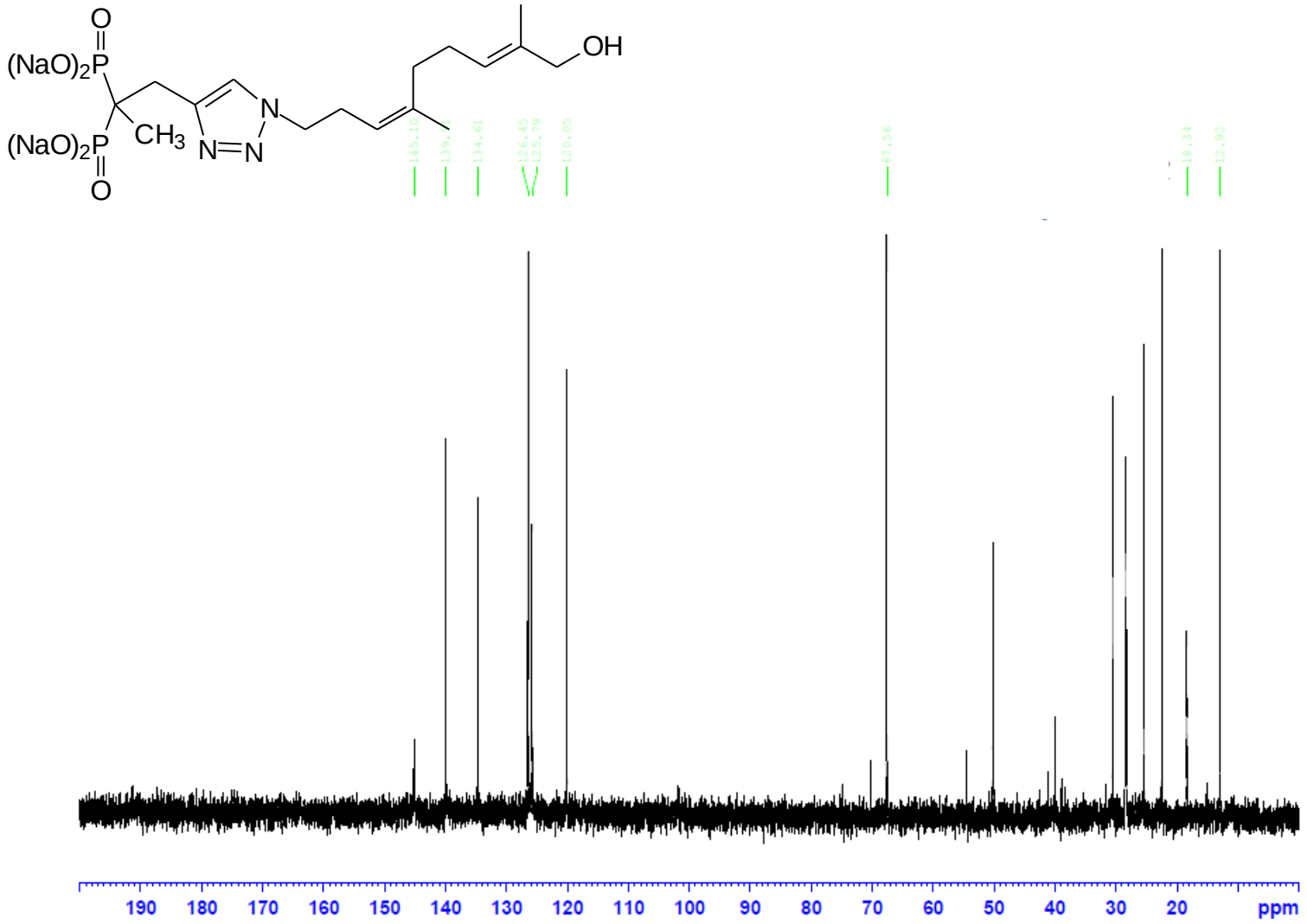
^{13}C NMR Spectrum of Compound **24**



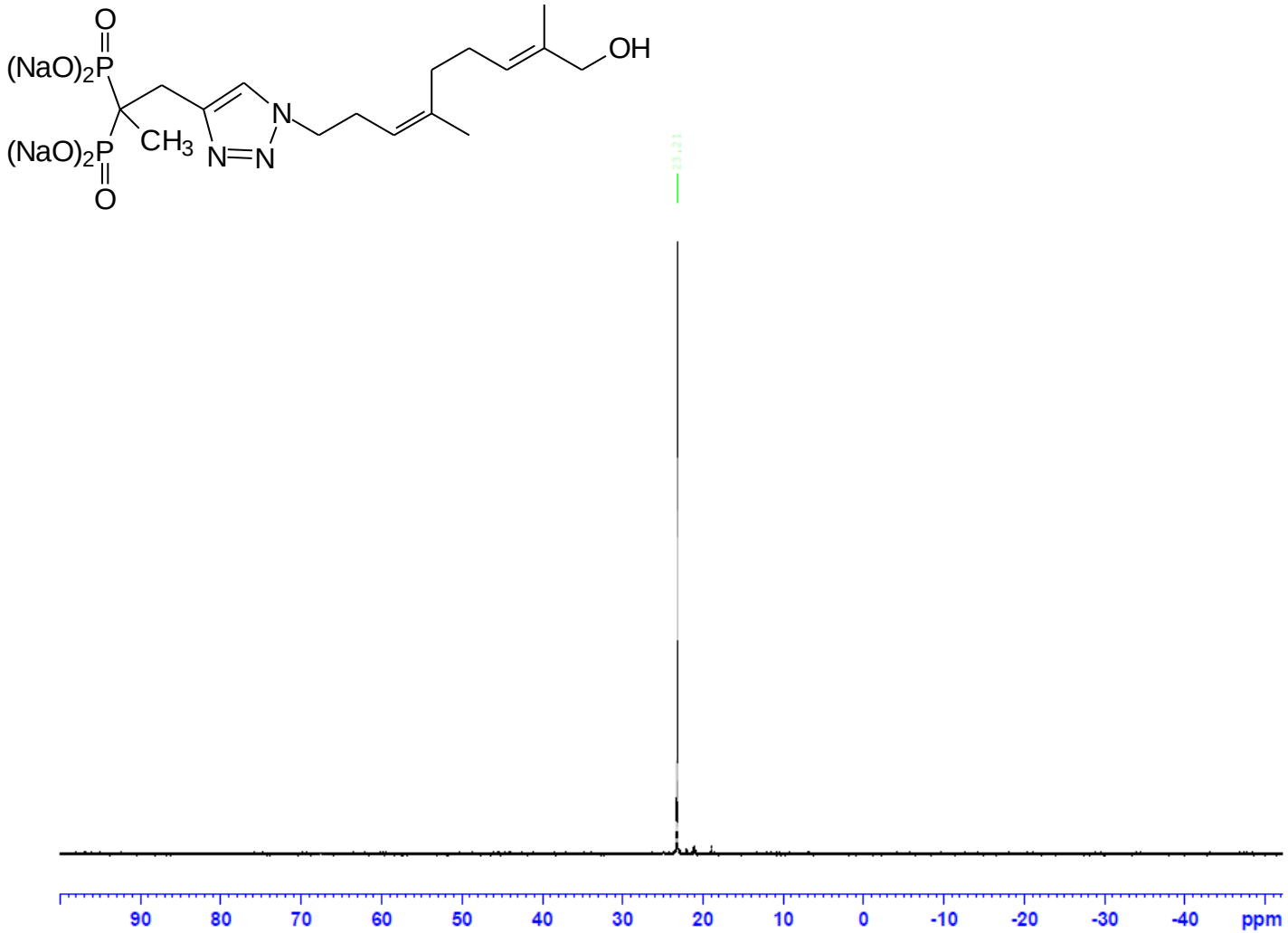
^{31}P NMR Spectrum of Compound **24**



^{1}H NMR Spectrum of Compound **25**



^{13}C NMR Spectrum of Compound **25**



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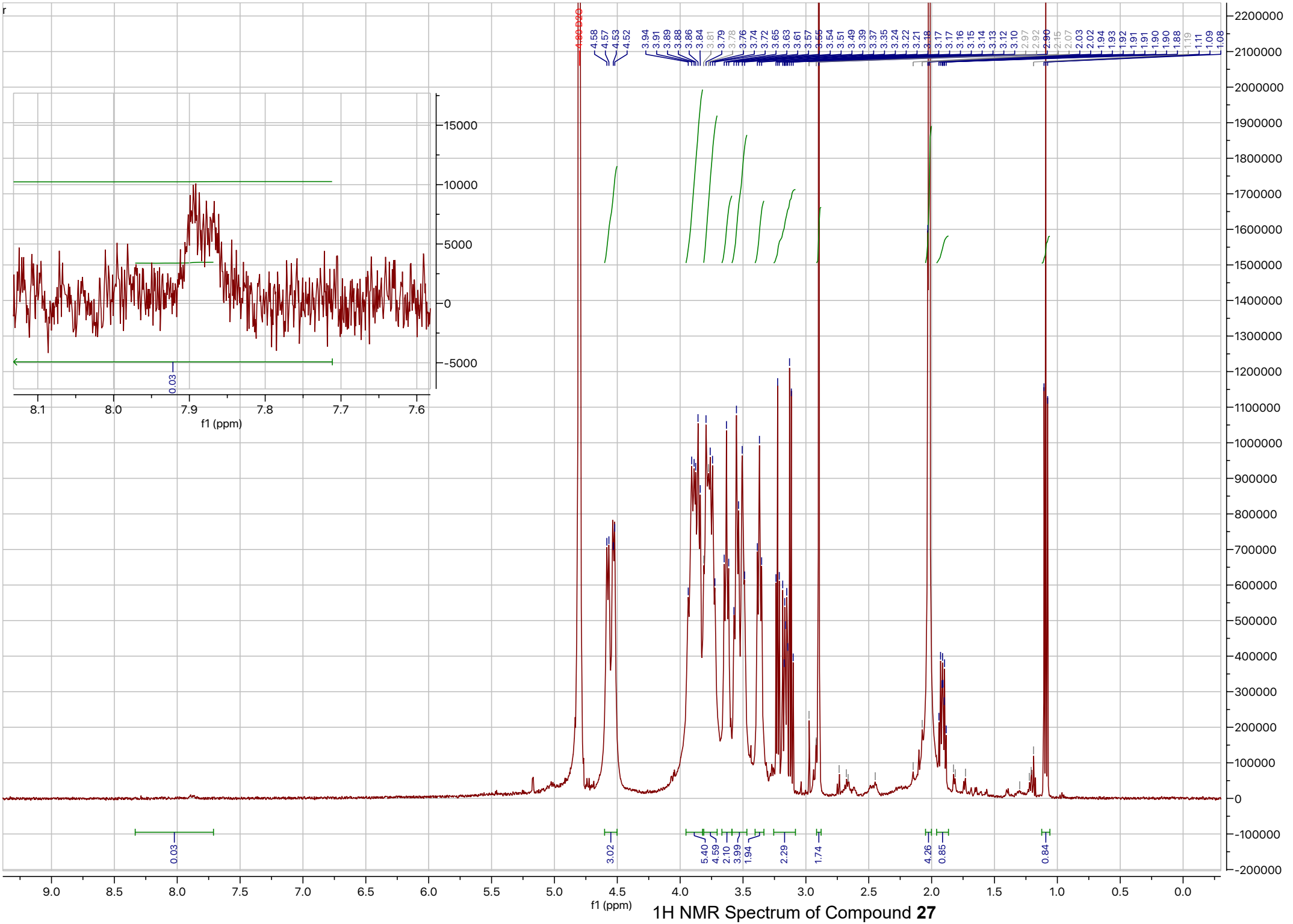
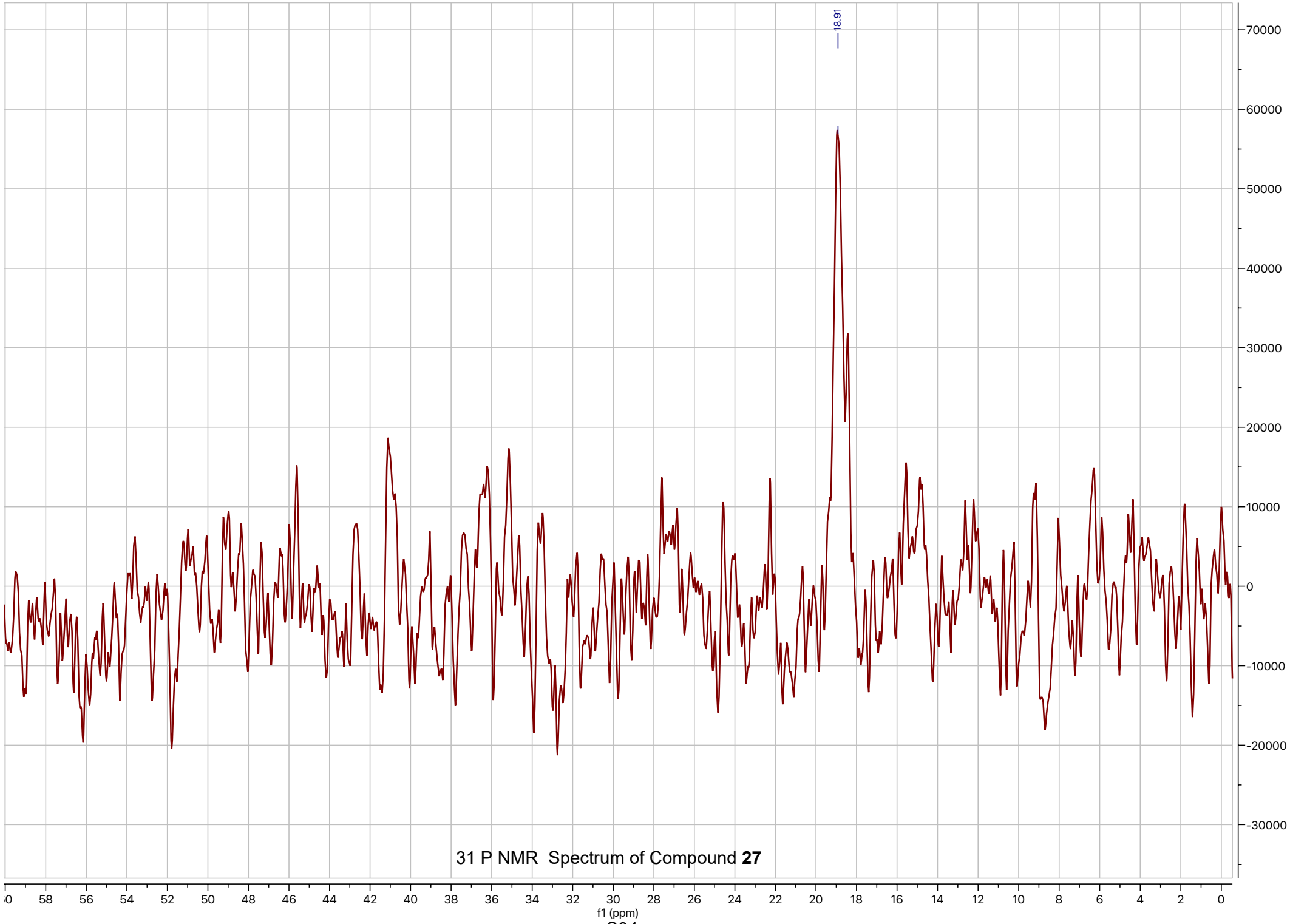


1H NMR Spectrum of Compound **27**



31 P NMR Spectrum of Compound **27**