"Enantioselective Inhibition of Squalene Synthase by Aziridine Analogues of Presqualene Diphosphate"

Ali Koohang, Jessica L. Bailey, and Robert M. Coates*

Department of Chemistry, University of Illinois, 600 South Mathews Avenue, Urbana, Illinois 61801

rm*coates@uiuc.edu*

Hans Erickson, David Owen, and C. Dale Poulter

Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

SUPPORTING INFORMATION

Table of Contents

A. General Experimental and Instrumentation……...S1

B. Preparative Procedures and Characterization Data for Aziridines…….S3

C. Preparative Procedures and Characterization Data for Diphosphates and Methanediphosphonates……..S13

D. Preparative Procedures and Characterization Data for Camphanate Esters……..S19

E. Experimental Procedures for Squalene Synthase Purification……...S20

F. ^{1}H, ^{13}C, and/or ^{31}P NMR Spectra of Selected Compounds ……....S22

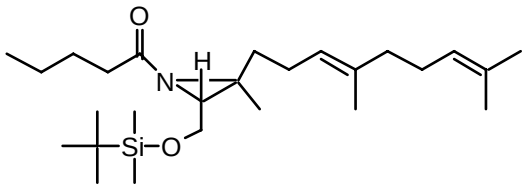
A. General Experimental and Instrumentation

All reactions except enzyme inhibition experiments were performed under N_2 using oven-dried glassware. Et_2O, THF, and benzene were distilled from sodium/benzophenone ketyl before use. CH_2Cl_2,

pentane, DMF, and toluene were distilled from calcium hydride before use. Et_3N was distilled from P_2O_5 and stored over KOH pellets. Methanesulfonyl chloride and CH_3CN were distilled from P_2O_5 and stored over molecular sieves. The Et_2AlCl was purchased as a 1.8 M solution in toluene. Solvents used for chromatography were distilled prior to use. All other reagents and solvents used were reagent grade. Column chromatography was performed according to Still's procedure[1] using 100-200 times excess 32-64 micron grade silica gel. TLC analysis was performed using TLC plates (0.25 mm 60 F-254 silica gel). Visualization of the developed plates was accomplished by staining with ethanolic phosphomolybdic acid, ceric ammonium molybdate, or *p*-anisaldehyde followed by heating on a hotplate (ca. 120 °C) or by staining with iodine vapor.

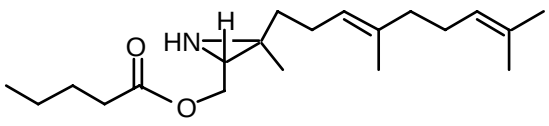
NMR spectra data were collected with either 400 or 500 MHz spectrometers. The following solvents and reference values (ppm) were used: $CDCl_3$ (^1H: 7.26, ^{13}C: 77.0), C_6D_6 (^1H: 7.15, ^{13}C: 128.0), CD_3OD (^1H: 3.34, ^{13}C: 63.08), and CD_3CN (^1H: 1.94, ^{13}C: 1.39). ^{31}P NMR spectra were referenced externally with 85% H_3PO_4. The abbreviation "app" (apparent) in ^1H NMR assignments refers to the appearance of the multiplet observed, and the multiplicity and coupling constants deduced in these cases were obtained by first-order coupling analysis. The purity of all products was determined to be >95% by NMR analysis unless specified otherwise. Samples for IR analysis were prepared as neat liquids on NaCl plates unless specified otherwise and data are reported as wave numbers (cm^{-1}). Optical rotations were taken in chloroform at ambient temperatures, which was typically 21-23°C.

B. Preparative Procedures and Characterization Data for Aziridines



(±)-*trans*-3-[(*tert*-Butyldimethylsilyl)oxy]methyl-2-((*E*)-4,8-dimethyl-3,7-nonadienyl) -2-methyl-1-(1-oxo-pentyl)aziridine (19)

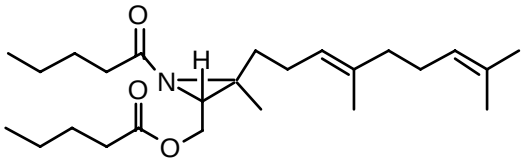
The following procedure is based on that reported by Meyers.[2] A solution of **9**-OTBDMS (118 mg, 0.33 mmol) and Et_3N (94 μL, 68 mg, 0.67 mmol) in 5 mL of ether was stirred and cooled at 0 °C under N_2 as valeryl chloride (48 μL, 48 mg, 0.4 mmol) was added dropwise. After 50 min, H_2O (5 mL) was added to the yellow suspension, and the aq layer was extracted with ether (3x10 mL). The combined organic layers were dried ($MgSO_4$), and concentrated by rotary evaporation to a yellow oil. Immediate purification by flash chromatography (10% EtOAc/hexanes as eluent) afforded **19** (109 mg, 75%) as a clear oil. Prolonged storage of this material even at -20°C resulted in decomposition. Data for **19**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 3H), 0.06 (s, 3H), 0.89 (s, 9H), 0.90 (t, J = 7.2 Hz, 3H), 0.93 (m, 1H), 1.29 (s, 3H), 1.32 (m, 3H), 1.57 (s, 6H), 1.60 (m, 1H), 1.66 (s, 3H), 1.99 (m, 6H), 2.16 (m, 1H), 2.21 and 2.34 (d of AB quartets, J_d = 7.6 Hz, J_{AB} = 15.2 Hz, 2H), 2.45 (dd, J = 7.2, 6.0 Hz, 1H), 3.53 (dd, J = 10.8, 6.8 Hz, 1H), 3.84 (dd, J = 11.2, 6.0 Hz, 1H), 5.07 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ -5.5, -5.3, 13.8, 15.9, 17.1, 17.6, 18.1, 22.4, 25.1, 25.6, 25.7, 26.5, 27.3, 37.2, 38.1, 39.6, 46.0, 46.5, 61.7, 122.8, 124.1, 131.3, 135.8, 183.6; IR (neat) ν_{max} 2957, 1692, 1462, 1383, 1256, 1089, 838, 777 cm^{-1}.



(±)-*trans*-2-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-2-methylaziridine-3-yl]methyl Pentanoate (20).

The following procedure is based on that reported by Corey.[3] A solution of **19** (96 mg, 0.22 mmol) in THF (1 mL) was stirred and cooled at 0 °C under N_2 as TBAF•$3H_2O$ (140 mg, 0.44 mmol) was added

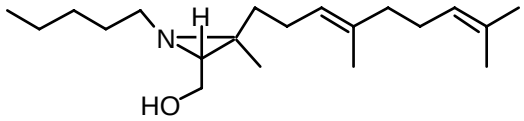
in small portions. The suspension was warmed to room temp, stirred for 2 h, and partitioned between satd NaCl (5 mL) and EtOAc (5 mL). The aq layer was washed with EtOAc (3x20 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated by rotary evaporation to a pale yellow oil. Purification by flash chromatography (50% MeOH/CH_2Cl_2) afforded 48 mg (68%) of **20** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.45 (broad s, 1H, NH, exchanges with D_2O), 0.89 (t, *J* = 7.2 Hz, 3H), 1.18 (s, 3H), 1.33 (m, 4H), 1.57 (s, 3H), 1.58 (m, 2H), 1.59 (s, 3H), 1.66 (d, *J* = 0.8 Hz, 3H), 1.96 (m, 2H), 2.09 (m, 5H), 2.31 (t, *J* = 7.6 Hz, 2H), 4.08 (d, *J* = 6.4 Hz, 2H), 5.06 (t of septets, *J* = 7.2, 1.6 Hz, 1H), 5.06 (t of sextets, *J* = 7.2, 1.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 13.7, 15.9, 17.4, 17.6, 22.2, 24.6, 25.6, 26.5, 26.9, 33.9, 38.7, 39.3, 39.6, 41.3, 64.6, 123.3, 124.1, 131.4, 135.7, 173.8; IR (neat) ν_{max} 1737 (C=O) cm^{-1}



(±)-*trans*-2-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-2-methyl-1-[(pentanoyl)aziridine-3-yl]methyl Pentanoate (22).

The following procedure is based on that reported by Meyers.[2] A solution of **9**-OH (246 mg, 1.03 mmol) and Et_3N (0.60 mL, 0.43 g, 4.32 mmol) in 10 mL of ether was stirred and cooled at 0 °C under N_2 as pentanoyl chloride (0.38 mL, 0.39 g, 3.24 mmol) was added dropwise. After 2 h, distilled H_2O (10 mL) was added to the yellow suspension, and the aq layer was extracted with ether (3x10 mL). The combined organic layers were dried ($MgSO_4$), and concentrated by rotary evaporation to a yellow oil. Immediate purification by flash chromatography (10% EtOAc/hexanes) afforded 334 mg (80%) of **22** as a clear oil. Prolonged storage of this material even at -20 °C resulted in decomposition products. Data for **22**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, *J* = 7.2 Hz, 6H), 0.93 (m, 1H), 1.33 (s, 3H), 1.35 (m, 4H), 1.59 (s, 6H), 1.63 (m, 4H), 1.67 (s, 3H), 2.00 (m, 6H), 2.24 (m, 2H), 2.35 (t, *J* = 7.2 Hz, 2H), 2.45 (t, *J* = 7.2 Hz, 1H), 2.57 (t, *J* = 6.8 Hz, 1H), 4.09 and 4.21 (d of AB quartets, J_d = 6.4 Hz, J_{AB} = 12.0 Hz, 2H), 5.06 (t of

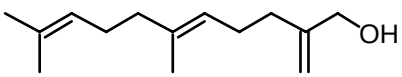
septets, J = 6.8, 1.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 15.9, 17.4, 17.6, 22.2, 22.4, 25.1, 25.6, 26.5, 26.9, 27.2, 33.8, 37.3, 37.7, 39.6, 42.6, 46.7, 62.7, 122.4, 124.0, 131.5, 136.2, 173.5, 183.5; IR (neat) ν_{max} 2959, 1737 (ester C=O), 1654 (amide C=O), 1455, 1381, 1110 cm^{-1}. Note: the ^{13}C NMR spectrum of **22** shows 23 signals instead of the expected 25. A likely explanation is that the missing peaks are superimposed on two other peaks.

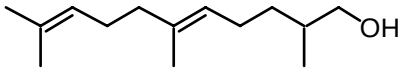


(±)-*trans*-2-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-3-hydroxymethyl-2-methyl-1-(pentyl) aziridine (21). Method A: By $LiAlH_4$ reduction of *N*-pentanoyl-*O*-silyl aziridine (21).

The following procedure is based on that reported by Huisgen et al.[4] A solution of $LiAlH_4$ (43 mg, 1.14 mmol) in ether (5 mL) was stirred and cooled at 0 °C under N_2 as a solution of **19** (99 mg, 0.23 mmol) in ether (2 mL) was added dropwise. The gray suspension was heated at reflux for 48 h, cooled to 0 °C, and stirred as H_2O (0.05 mL), 15% NaOH (0.05 mL), and H_2O (0.2 mL) were added dropwise in succession with occasional additions of ether (ca. 10 mL). The white solid was filtered and washed with EtOAc (20 mL). The filtrates were combined, dried ($MgSO_4$), and concentrated by rotary evaporation to a pale yellow oil. Purification by flash chromatography (10% MeOH/CH_2Cl_2) afforded 32 mg (46%) of **21** as a colorless oil and 15 mg (28%) of aziridino alcohol **9**-OH. Data for **21**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H), 1.13 (s, 3H), 1.30 (m, 5H), 1.43 (ddd, J = 13.2, 10.0, 6.4 Hz, 1H), 1.57 (m, 3H), 1.59 (s, 3H), 1.61 (s, 3H), 1.67 (d, J = 0.8 Hz, 3H), 1.97 (m, 2H), 2.05 (m, 2H), 2.17 (m, 2H), 2.31 (dt, J = 12.0, 7.2 Hz, 1H), 2.40 (s, 1H, OH, exch with D_2O), 2.68 (dt, J = 11.4, 8.0 Hz, 1H), 3.51 (dd, J = 11.2, 6.0 Hz, 1H), 3.70 (dd, J = 11.2, 5.6 Hz, 1H), 5.08 (t of septets, J = 7.2, 1.6 Hz, 1H), 5.11 (t of sextets, J = 7.0, 1.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.0, 15.9, 17.6, 18.9, 22.5, 25.1, 25.6, 26.5, 29.5, 30.1, 32.5, 39.6, 43.9, 50.5, 52.1, 60.0, 123.3, 124.1, 131.4, 135.6; IR (neat) ν_{max} 3375, 2958, 1455, 1381 cm^{-1}.

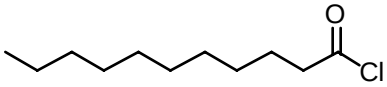
Method B: By $LiAlH_4$ reduction of *N,O*-diacyl aziridine 22. The *N*-pentyl aziridine **21** was prepared from the diacyl aziridine **22** (334 mg, 0.82 mmol) as described above for reduction of **19**. Purification by flash chromatography (10% MeOH/CH_2Cl_2 as eluent) afforded 170 mg (67%) of **21** and 25 mg of **9**-OH (13%). Spectral data were identical to those of the products obtained from reduction of **19**.





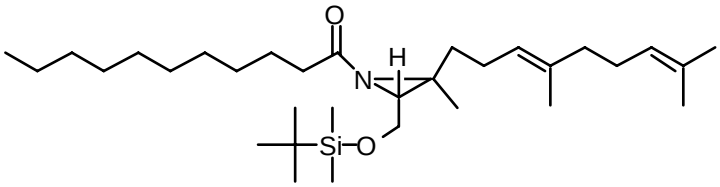
(*E*)-6,10-Dimethyl-2-methylene-5,9-undecadien-1-ol (16-OH and 17).

The following procedure is based on that reported by Marshall.[5] A suspension of 60% NaH-mineral oil dispersion (132 mg, 3.29 mmol) in 10 mL of diethyleneglycol monomethyl ether was stirred at room temp under N_2 as homogeranyl malonate **15** (851 mg, 2.74 mmol) was added dropwise. After heating at reflux for 12 h, the suspension was cooled to room temp, and $LiAlH_4$ (270 mg, 7.12 mmol) was added slowly. After heating at reflux for 3 h, the suspension was cooled to room temp, and the slurry was diluted with 15 mL of ether and hydrolyzed with H_2O (2 mL). The resulting white precipitate was filtered and washed with EtOAc (25 mL). The filtrates were combined, dried ($MgSO_4$), and concentrated by rotary evaporation to give a yellow oil. Purification by flash chromatography (50% EtOAc/hexanes) afforded 350 mg of a pale yellow oil that consisted of **16**-OH and dihydro-**16**-OH as a 3:1 mixture according to the ^1H NMR spectrum. Data for **16**-OH in the mixture: ^1H NMR (400 MHz, $CDCl_3$) δ 1.58 (s, 6H), 1.66 (d, *J* = 0.8 Hz, 3H), 1.88 (s, 1H, OH), 2.07 (m, 8H), 4.05 (s, 2H), 4.86 (d, *J* = 1.6 Hz, 1H), 5.01 (d, *J* = 1.6 Hz, 2H), 5.10 (m, 2H). Data for dihydro-**16**-OH in the mixture : ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (d, *J* = 6.4 Hz, 3H), 1.15 (m, 1H), 1.32 (s, 1H, OH, exch with D_2O), 1.44 (dddd, *J* = 13.6, 9.6, 6.8, 5.6 Hz, 1H), 1.59 (s, 6H, CH_3), 1.64 (m, 1H), 1.67 (d, *J* = 1.2 Hz, 3H), 2.02 (m, 6H), 3.42 (dd, *J* = 10.4, 6.8 Hz, 1H), 3.51 (dd, *J* = 10.4, 6.0 Hz, 1H), 5.09 (m, 2H).



Undecanoyl Chloride.

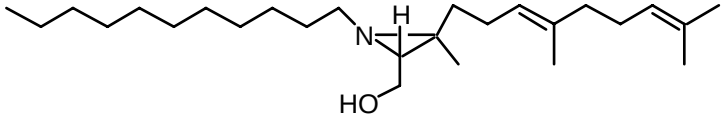
The following procedure is based on that reported by Ashton.[6] A solution of undecanoic acid (2.01 g, 9.51 mmol) in 5 mL of benzene was stirred at room temp under N_2 as oxalyl chloride (1.82 mL, 2.65 g, 20.9 mmol) was added dropwise over 10 min. After 0.5 h, gas evolution was ceased, and the clear solution was concentrated by rotary evaporation to a pale yellow oil. Purification by fractional distillation afforded undecanoyl chloride (1.91 g, 87%) as clear oil: bp 95-96 °C/mm Hg (lit.[6] bp 130 °C/15 mm Hg); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 6.8 Hz, 3H), 1.30 (m, 14 H), 1.7 (quintet, J = 7.4 Hz, 2H), 2.87 (t, J = 7.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.1, 22.6, 25.0, 28.4, 29.0, 29.2, 29.3, 29.4, 31.8, 47.1, 173.8. No spectral data could be found in the literature.



(±)-*trans*-3-[(*tert*-Butyldimethylsilyl)oxymethyl]-2-((*E*)-4,8-dimethyl-3,7-nonadienyl) -2-methyl-1-(1-oxo-undecyl)aziridine (*N*-Undecyl-7-*O*-TBDMS).

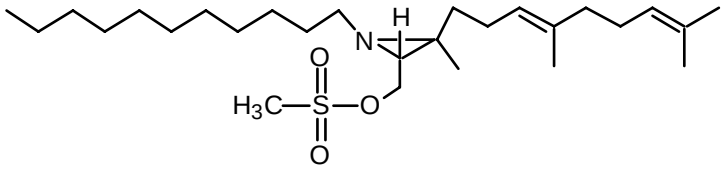
The *N*-acyl aziridine was prepared from **(±)-9**-OTBDMS (774 mg, 2.20 mmol) as described above for **19** using NEt_3 (0.63 mL, 456 mg, 4.49 mmol) and undecanoyl chloride (0.72 mL, 675 mg, 3.30 mmol) in ether (30 mL) at 0 °C for 50 min that upon workup afforded a yellow oil. Immediate purification by flash chromatography (10% EtOAc/hexanes) afforded 738 mg of *N*-undecyl-**7**-*O*-TBDMS ether (65%) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 3H), 0.06 (s, 3H), 0.86 (t, J = 6.8 Hz, 3H), 0.89 (s, 9H), 0.93 (m, 1H), 1.24 (m, 15H), 1.29 (s, 3H), 1.57 (s, 6H), 1.60 (m, 1H), 1.66 (d, J = 0.8 Hz, 3H), 1.99 (m, 6H), 2.17 (m, 1H), 2.21 and 2.34 (d of AB quartets, J_d = 7.6 Hz, J_{AB} = 15.2 Hz, 2H), 2.45 (dd, J = 7.2, 5.6 Hz, 1H), 3.53 (dd, J = 10.8, 6.8 Hz, 1H), 3.84 (dd, J = 11.2, 6.0 Hz, 1H), 5.07 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ -5.5, -5.3, 14.1, 15.9, 17.1, 17.6, 18.1, 22.6, 25.1, 25.2, 25.6, 25.7, 26.6, 29.3, 29.4,

29.5, 31.8, 37.5, 38.1, 39.6, 46.0, 46.5, 61.7, 122.8, 124.1, 131.3, 135.8, 183.6; IR (neat) ν_{max} 2928, 1691, 1461, 1372, 1254, 1091, 836, 776 cm^{-1}. Prolonged storage of this material even at -20°C resulted in decomposition products. The ^{13}C NMR spectrum shows 28 signals instead of the expected 30. Some methylene signals may be superimposed.



(±)-*trans*-2-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-3-hydroxymethyl-2-methyl-1-undecylaziridine (8-OH).

The aziridino alcohol was prepared from *N*-undecyl-**7**-*O*-TBDMS ether (738 mg, 1.42 mmol) as described above for **21** using $LiAlH_4$ (323 mg, 8.25 mmol) in ether (40 mL) at reflux for 48 h that after workup gave a pale yellow oil. Purification by flash chromatography (10% $MeOH/CH_2Cl_2$) afforded 320 mg (58%) of (±)-**8**-OH as a colorless oil and 130 mg (39%) of N-H aziridino alcohol (±)-**6**-OH. Data for (±)-**8**-OH: ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.8 Hz, 3H), 1.13 (s, 3H), 1.26 (m, 17H), 1.41 (ddd, *J* = 13.2, 10.0, 6.4 Hz, 1H), 1.55 (m, 3H), 1.58 (s, 3H), 1.59 (s, 3H), 1.66 (d, *J* = 0.8 Hz, 3H), 1.96 (m, 2H), 2.05 (q, *J* = 7.2 Hz, 2H), 2.15 (m, 2H), 2.32 (dt, *J* = 12.0, 7.6 Hz, 1H), 2.62 (dt, *J* = 12.0, 7.6 Hz, 1H), 3.04 (s, 1H, OH, exch with D_2O), 3.47 (dd, *J* = 11.2, 6.4 Hz, 1H), 3.67 (dd, *J* = 11.2, 5.2 Hz, 1H), 5.06 (t of septets, *J* = 7.2, 1.2 Hz, 1H), 5.09 (t of sextets, *J* = 6.4, 1.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.1, 15.9, 17.6, 19.0, 25.2, 25.6, 26.5, 27.4, 29.3, 29.5, 30.5, 31.8, 32.5, 39.6, 43.5, 50.4, 52.3, 60.1, 123.4, 124.1, 131.3, 131.3, 135.5; IR (neat) ν_{max} 3321, 2924, 1466, 1375, 1106, 1031 cm^{-1}. The ^{13}C NMR spectrum shows 23 signals instead of required 26. The increased intensity of the peak at 29.6 ppm indicates the likelihood that methylene carbon peaks are superimposed.

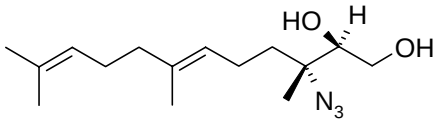


(±)-*trans*-2-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-2-methyl-3-(methanesulfonyloxy) methyl-1-undecylaziridine (8-OMs).

The following procedure is based on that reported by Crossland.[7] A solution of (±)-**8**-OH (114 mg, 0.29 mmol) and NEt_3 (77 μL, 41 mg, 0.41 mmol) in CH_2Cl_2 (3 mL) was stirred and cooled at -15 °C under N_2 as MsCl (24 μL, 55 mg, 0.45 mmol) was added dropwise. After 0.5 h, satd $NaHCO_3$ (10 mL) was added to the suspension, and the aq layer was extracted with EtOAc (3x10 mL). The combined extracts were washed with 30 mL of satd NaCl, dried (Na_2SO_4), and concentrated by rotary evaporation to a crude yellow oil. Purification by flash chromatography (50% EtOAc/hexanes) afforded 133 mg (97%) of (±)-**8**-OMs as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 6.8 Hz, 3H),1.17 (s, 3H), 1.25 (m, 17H), 1.44 (ddd, J = 13.6, 10.4, 6.4 Hz, 1H), 1.54 (m, 3H), 1.60 (s, 3H), 1.61 (s, 3H), 1.68 (d, J = 0.8 Hz, 3H), 1.98 (m, 2H), 2.06 (q, J = 7.1 Hz, 2H), 2.17 (m, 2H), 2.31 (dt, J = 11.2, 7.2 Hz, 1H), 2.63 (dt, J = 11.6, 7.6 Hz, 1H), 3.02 (s, 3H), 4.17 and 4.22 (d of AB quartet, J_d = 6.0 Hz, J_{AB}= 10.8 Hz, 2H), 5.08 (t of septets, J = 6.8, 1.2 Hz, 1H), 5.11 (t of sextets, J = 7.2, 1.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.1, 15.9, 17.6, 19.6, 22.6, 25.2, 25.6, 26.5, 27.3, 29.3, 29.55, 29.57, 29.63, 30.3, 31.8, 32.2, 37.4, 39.6, 46.7, 52.1, 70.6, 123.2, 124.1, 131.4, 135.7; IR (neat) ν_{max} 2927, 1453, 1357, 1176, 945, 813 cm^{-1}. Anal. calcd for $C_{27}H_{51}NO_3S$: C, 69.03; H, 10.94; N, 2.98; S, 6.82. Found: C, 68.56; H, 11.04; N, 2.62. The ^{13}C NMR spectrum shows 25 signals instead of required 27. The doubled intensity of the peaks at 29.55 and 29.57 ppm indicate the likelihood that two similar methylene peaks are superimposed on each other.

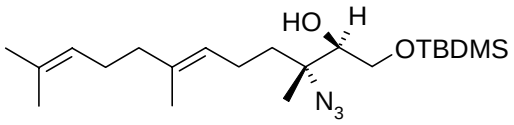
The following procedures for preparation and purification of the ent series (*ent*-**11**, *ent*-**11**-OTBDMS, *ent*-**12**, *ent*-**9**-OH, *ent*-**9**-OTBDMS, *ent*-**6**-OMs, and *ent*-**6**-OPP) were carried out as described

in the Experimental Section for their enantiomers. The physical properties of the ent compounds were identical to those reported in detail in the Experimental section, except for optical rotations.



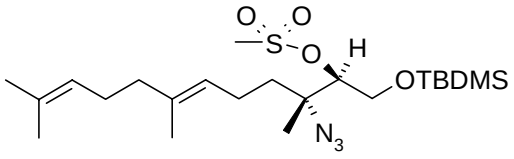
(2*R*, 3*R*)-3-Azido-3,7,11-trimethyl-(*E*)-6,10-dodecadien-1,2-diol (*ent*-11).

The (2*R*, 3*R*) azido diol was prepared from (-)-2, 3-epoxyfarnesol (**10**, 5.10 g, 21.4 mmol) as described for **11** using NaN_3 (3.08 g, 47.4 mmol), and Et_2AlCl (23.8 mL, 42.8 mmol, 1.8 M in toluene) in toluene (50 mL). The yield was 3.95 g (66%) of *ent*-**11** as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (101 MHz, $CDCl_3$), IR; $[\alpha]_D$ = -38.5 ± 0.2, (*c* = 1.12 in $CHCl_3$).



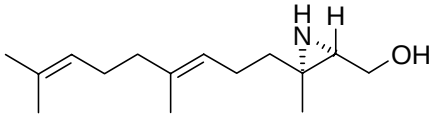
(2*R*,3*R*)-3-Azido-1-(*tert*-butyldimethylsilyloxy)-3,7,11-trimethyl-(*E*)-6,10-dodecadien-2-ol (*ent*-11-OTBDMS).

The (2*R*,3*R*) silyl ether was prepared from azido diol *ent*-**11** (3.85 g, 13.7 mmol) as described for **11**-OTBDMS using imidazole (5.56 g, 82.2 mmol), 4-DMAP (0.169 g, 1.37 mmol), and TBDMSCl (6.21 g, 41.1 mmol) in DMF (37 mL). Purification by flash chromatography (5% EtOAc/hexanes) afforded 4.64 g (85%) of *ent*-**11**-OTBDMS as a clear, colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (126 MHz, $CDCl_3$), IR.



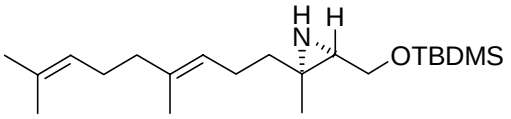
(2*R*,3*R*)-3-Azido-1-(*tert*-butyldimethylsilyloxy)-3,7,11-trimethyl-(*E*)-6,10-dodecadien-2-yl Methanesulfonate (*ent*-12).

The (2*S*,3*R*) azido mesylate was prepared from *ent*-**11**-OTBDMS (4.41 g, 13.0 mmol) as described for **12** using Et_3N (5.62g, 49.1 mmol), and methanesulfonyl chloride (5.12g, 50.6 mmol) in CH_2Cl_2 (50 mL) at 0 °C. Purification by flash chromatography (5% EtOAc/hexanes) afforded 4.22 g (81%) of *ent*-**12** as a pale, yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (101 MHz, $CDCl_3$), IR.



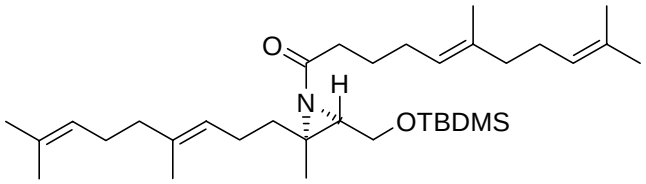
(2*S*,3*R*)-*trans*-3-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-3-methylaziridine-2-methanol (*ent*-9-OH).

The (2*S*,3*R*) aziridine was prepared from *ent*-**12** (2.45 g, 5.17 mmol) as described for **9**-OH using $LiAlH_4$ (0.495g, 12.9 mmol) in Et_2O (15mL) at room temp for 4 h. Workup and purification by flash chromatography (10% MeOH/CH_2Cl_2) gave 1.02 g (83%) of *ent*-**9**-OH as a pale, yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (126 MHz, $CDCl_3$), $[\alpha]_D = +19.3 \pm 0.2$, (*c* = 1.21 in $CHCl_3$).



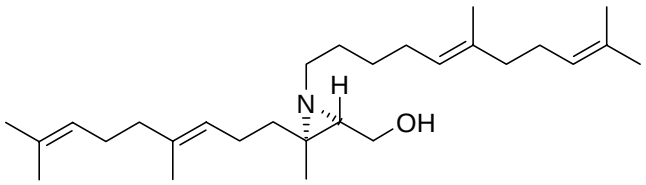
(2*S*,3*R*)-*trans*-3-[(*tert*-Butyldimethylsilyl)oxy]methyl-2-((*E*)-4,8-dimethyl-3,7-nonadienyl)-2-methylaziridine (*ent*-9-OTBDMS).

The aziridine was prepared from *ent*-**9**-OH (0.676 g, 2.86 mmol) as described for **9**-OTBDMS using imidazole (0.485 g, 7.15 mmol) and TBDMSCl (0.517g, 3.43 mmol) in DMF (1.5 mL). Purification by flash chromatography (10% MeOH/CH_2Cl_2) gave 0.753 g (75%) of *ent*-**9**-OTBDMS as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (101 MHz, $CDCl_3$).



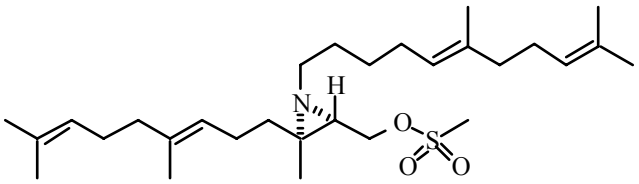
(2*S*, 3*R*)-*trans*-2-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-1-((*E*)-6,10-dimethyl-5,9-undecadienyl)-3-[(*tert*-butyldimethylsilyl)oxy]methyl-2-methylaziridine (*ent*-18).

The (2*S*,3*R*)-*N*-acyl aziridine was prepared from *ent*-**9**-OTBDMS (208 mg, 0.587 mmol) and Et_3N (245 μL, 178 mg, 1.76 mmol) in Et_2O (5mL) as described for its enantiomer **(18)** using bishomogeranic acid (**14b**, 123mg, 0.587 mmol), oxalyl chloride (56 μL, 82mg, 0.646 mmol), and pyridine (52 μL, 51mg, 0.646 mmol) in benzene (5 mL) that upon workup afforded 220 mg of *ent*-**18** as a yellow oil.



(2*S*, 3*R*)-*trans*-3-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-1-((*E*)-6,10-dimethyl-5,9-undecadienyl)-3-methylaziridine-2-methanol (*ent*-6-OH).

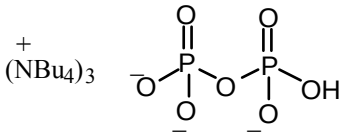
The (2*S*,3*R*)-*N*-alkyl aziridino alcohol was prepared from *N*-acyl aziridine (*ent*-**18**, 220 mg, 0.405 mmol) as described for **6**-OH using $LiAlH_4$ (80.3mg, 2.03 mmol) in ether (4.5 mL) at reflux for 48 h. Workup and purification by flash chromatography (50% EtOAc/hexanes) gave 63 mg (38%) of the *N*-alkyl aziridino alcohol *ent*-**6**-OH, 22 mg (25%) of NH aziridino alcohol *ent*-**9**-OH, and 20 mg (26%) of trishomogeraniol. Characterization of *ent*-**6**-OH: ^1H NMR (400 MHz, $CDCl_3$) ^{13}C NMR (101 MHz, $CDCl_3$), $[\alpha]_D = +4.93 \pm 0.4$, ($c$ = 1.09 in $CHCl_3$).



(2*S*, 3*R*)-*trans*-3-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-1-((*E*)-6,10-dimethyl-5,9-undecadienyl)- 3-methylaziridine-2-methanol Methanesulfonate (*ent*-6-OMs)

The following procedure is based on that reported by Koohang for (±)-**6**-OMs.[8] A solution of *ent*-**6**-OH (46 mg, 0.111 mmol) obtained from previous step and Et_3N (23 μL, 17 mg, 0.166 mmol) in CH_2Cl_2 (1.2 mL) was stirred at -20 °C under N_2 as methanesulfonyl chloride (10 μL, 15 mg, 0.133 mmol) was added dropwise via syringe. After 30 min, satd $NaHCO_3$ (1 mL) was added, and the solution was stirred vigorously for 5 min. The organic phase was removed, and the aq phase was extracted with EtOAc (3 x 4 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give 52 mg of a yellow oil. Purification by flash column chromatography (50% EtOAc/hexanes) gave 46 mg (86%) of the mesylate as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.18 (s, 3H), 1.43 (m, 7H), 1.59 (s, 3H), 1.60 (s, 6H), 1.61 (s, 3H), 1.67 (s, 3H), 1.97-2.22 (m, 12H), 2.31 (dt, J = 11.8, 7.6 Hz, 1H), 2.65 (dt, J = 11.6, 7.4 Hz, 1H), 3.02 (s, 3H), 4.21 (m, 2H), 5.10 (m, 4H).

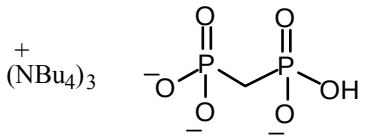
C. Preparative Procedures and Characterization Data for Diphosphates and Methylene Diphosphates



Tris(tetra-n-butylammonium) Hydrogen Diphosphate.[9a,b]

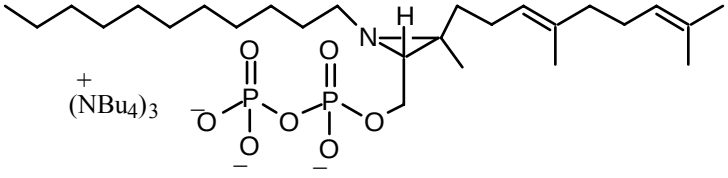
A solution of disodium dihydrogen pyrophosphate (3.13 g, 14 mmol) in deionized H_2O (25 mL) containing concd. NH_4OH (1 mL) was loaded onto a column (2 cm x 30 cm) of Dowex AG 50W-X8 cation exchange resin (100-200 mesh, H^+) and eluted with deionized H_2O. The first 150 mL of the eluent was stirred and titrated to pH of 7 immediately at room temp by slow addition of 44% aq NBu_4OH.

Lyophilization (0.005 mmHg) of this solution for 24 h afforded 10.1 g (80%) of a white powder that was used without further purification. The following ^{1}H, ^{13}C and ^{31}P NMR spectral data for a small sample of the product were similar to those in the literaturer:[9a,b] ^{1}H NMR (400 MHz, CD_3OD) δ 0.71 (t, J = 7.2 Hz, 36H), 1.10 (sextet, J = 7.2 Hz, 24H), 1.34 (m, 24H), 2.93 (m, 24H); ^{13}C NMR (101 MHz, CD_3OD) δ 13.9, 20.6, 24.7, 59.3; ^{31}P NMR (162 MHz, CD_3OD) δ -1.54 (s, 2P, PPi), 8.27 (s, 0.06, 1P, Pi).



Tris(tetra-*n*-butylammonium) Hydrogen Methanediphosphonate[9a,b]

A solution of methanediphosphonic acid, trisodium salt (1.00 g, 3.18 mmol) in deionized H_2O (10 mL) was loaded onto a column (2 cm x 26 cm) of Dowex AG 50W-X8 cation exchange resin (100-200 mesh, H^+) and eluted with deionized H_2O. The first 120 mL of the eluent was stirred and titrated slowly to pH of 10.0 at room temp with 44% aq NBu_4OH. Lyophilization (0.005 mmHg) of this light pink aq solution for 24 h afforded 2.80 g (98%) of a hygroscopic oily solid that was used without further purification. The following ^{1}H, ^{13}C and ^{31}P NMR spectral data for a small sample of the product are similar to those in the literature.[9a,b] ^{1}H NMR (400 MHz, CD_3OD) δ 1.01 (t, J = 7.6 Hz, 36H) , 1.43 (sextet, J = 6.8 Hz, 24H), 1.66 (m, 24H), 2.05 (t, J = 18.8 Hz, 2H), 3.27 (m, 24H), 5.62 (s, 6H, H_2O); ^{13}C NMR (101 MHz, CD_3OD) δ 14.2, 20.6, 24.7, 31.4 (t, J = 109.8 Hz), 59.2; ^{31}P NMR (162 MHz, CD_3OD) δ 20.3 (s).



(±)-*trans*-[2-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-2-methyl-1-undecylaziridin-3-yl] methyl Diphosphate, Tris(tetrabutylammonium) Salt ((±)-8-OPP).

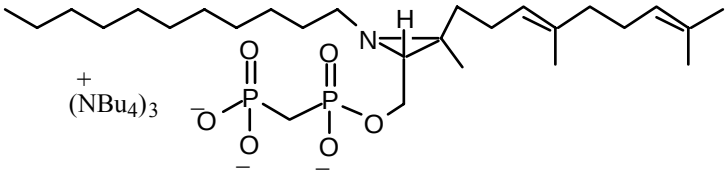
The aziridino diphosphate (±)-**8**-OPP was prepared from mesylate (±)-**8**-OMs (92 mg, 0.19 mmol) as described for enantiopure **6**-OPP using $(NBu_4)_3HP_2O_7$ (165 mg, 0.183 mmol) and activated 4Å powdered molecular sieves in anhyd CH_3CN (1 mL) at room temp for 48 h that upon workup afforded 350 mg of (±)-**8**-OPP (82%) as a 1:1 mixture of the NBu_4^+ salt and $(NBu_4)_3HP_2O_7$ (^{31}P NMR spectral analysis) as well as ~14 mg of recovered starting material. Data for (±)-**8**-OPP: ^{31}P NMR (162 MHz, CD_3OD) δ -5.79 (d, *J* = 19.6 Hz, 1P), -5.09 (d, *J* = 19.6 Hz, 1P), -3.10 (s, 2P, PPi).

Ion Exchange Chromatography: A solution of (±)-**8**-OPP in dry CH_3CN (1 mL) was loaded onto the ion exchange column (Dowex 50W-X8, NH_4^+ form) and eluted (flow rate = 5 mL/10 min) with the ion exchange buffer (25 mM aq NH_4CO_3) into 5 mL receiving tubes containing concd NH_4OH (0.5 mL each). The iodine active fractions (3-8, analyzed by silica gel TLC, isopropyl alcohol (IPA):concd NH_4OH:H_2O (8:1:1)) were combined and lyophilized. Inorganic diphosphate (40 mg) was completely removed by washing the solid with CH_3OH (2x5 mL). The organic extracts were combined and concentrated under reduced pressure at 35°C to afford 110 mg of (±)-**8**-OPP as the NH_4^+ form and the cyclic monophosphate byproduct **24** or **25** (2:1 by ^{31}P NMR analysis in favor of the aziridine). Data for **8**-OPP NH_4^+ form: ^{31}P NMR (162 MHz, CD_3OD) δ -7.19 (d, *J* = 20.72 Hz, 1P), -5.85 (d, *J* = 20.72 Hz, 1P), -1.64 (s). ^1H NMR analysis of this sample was indicative of complete ion exchange (absence of butyl groups).

Cellulose Chromatography: The (±)-**8**-OPP NH_4^+ form (110 mg) obtained from the previous step was dissolved in CH_3OH (1 mL) and loaded onto a cellulose column (2.5 x 32 cm; Whatman CF11 medium-grade-cellulose). The column was eluted (flow rate = 5 mL/10 min) with IPA:concd NH_4OH:H_2O (8:1:1). Each fraction was analyzed by silica gel TLC using IPA-$CHCl_3$-CH_3CN-25 mM aq NH_4CO_3 (5:1:2:2) as the developing solvent. Two sets of fractions (14-20 and 32-41) were collected and concentrated at 35 °C to ca 10 mL each, and the remaining aq solutions were lyophilized. The first set (fractions 14-20, 35 mg

total) was determined to be the rearranged cyclic monophosphate **24** or **25** by ^{1}H NMR and ^{31}P NMR (CD_3OD) analyses. Data for (±)-**24** or (±)-**25**: ^{1}H NMR (500 MHz, CD_3OD) δ 0.89 (t, *J* = 7.0 Hz, 3H), 1.29 (m, 16H), 1.45 (d, *J* = 1.5 Hz, 3H), 1.58 (s, 3H), 1.64 (s, 3H), 1.66 (s, 3H), 1.77 (m, 4H), 2.10 (m, 7H), 3.11 (td, *J* = 12.0, 5.3 Hz, 1H), 3.20 (td, *J* = 11.0, 5.3 Hz, 1H), 4.36 (ddd, *J* = 21.0, 13.5, 2.5 Hz, 1H), 4.36 (dd, *J* = 14.0, 4.5 Hz, 1H), 5.08 (t of septets, *J* = 7.0, 1.5 Hz, 1H), 5.16 (app t, J_{app} = 5.5 Hz, 1H); ^{31}P NMR (162 MHz, CD_3OD) δ -1.64 (s); MS (+) FAB m/z 472.4.

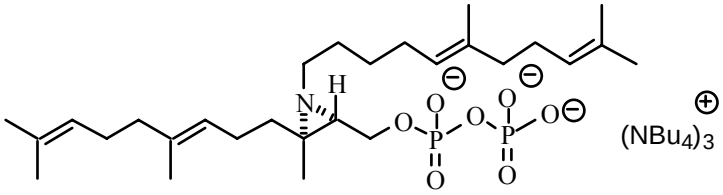
The material that was recovered from the second set of fractions (32-41, 26 mg total) was insoluble in aqueous and most organic solvents including CH_3OH. A small amount of this sample was dissolved in benzene-d_6 but no definitive assignments were made due to the observation of broad peaks both in ^{1}H NMR and ^{31}P NMR spectra.



(±)-*trans*-[2-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-2-methyl-1-undecylaziridin-3-yl] methyl Methanediphosphonate, Tris(tetrabutylammonium) Salt (8-OMDP).

A solution of mesylate (±)-**8**-OMs (85 mg, 0.18 mmol) in anhyd CH_3CN (1 mL) containing 4Å molecular sieves was stirred at room temp under N_2 as $(NBu_4)_3$HMDP (326 mg, 0.36 mmol) in anhyd CH_3CN (1 mL) was added dropwise. A light yellow color developed during the addition. After 60 h, the molecular sieves were filtered, and the filtrate was extracted with hexanes (5x5 mL). Concentration of the CH_3CN soluble fractions by rotary evaporation at 35 °C afforded 403 mg of (±)-**8**-OMDP (87%) as a 1:1.5 mixture of the NBu_4^+ salt and $(NBu_4)_3$HMDP according to ^{31}P NMR analysis. Data for (±)-**8**-OMDP: ^{31}P NMR (162 MHz, CD_3OD) δ 15.15 (d, *J* = 2.99 Hz, 2P), 19.13 (s, 6P, inorganic methanediphosphonate), 23.28 (d, *J* = 2.99 Hz, 1P). Ion exchange chromatography on (±)-**8**-OMDP (146 mg) followed by CH_3OH extraction (16 mg of $(NH_4)_3$HOMDP was separated) as described above for (±)-**8**-OPP afforded 37 mg of (±)-**8**-OMDP as a 1:1 mixture of the NH_4^+ form of (±)-**8**-OMDP and what are presumed to be cyclic methanediphosphonates similar to (±)-**24** and/or (±)-**25**. Cellulose chromatography

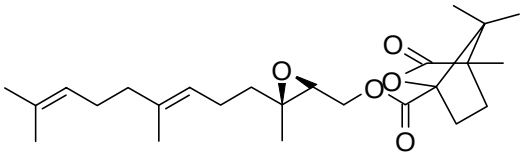
on this mixture resulted in further conversion of the aziridine to the cyclic methanephosphonates and 33 mg of the mixture was recovered after lyophilization (by ^{31}P NMR analysis). The major isomer in the cyclic mixture was partially separated according to solubility differences in benzene. While the minor isomer was soluble in benzene, the major isomer (10 mg) precipitated as an off-white powder. Data for (±)-**8**-OMDP in NBu_4^+ form: ^{31}P NMR (162 MHz, CD_3OD) δ 15.15 (d, *J*= 2.42 Hz, 1P), 23.28 (d, *J*= 3.56 Hz, 1P). Data for (±)-**8**-OMDP in NH_4^+ form: ^{31}P NMR (162 MHz, CD_3OD) δ 17.80 (d, *J*= 7.28 Hz, 1P), 21.77 (d, *J*= 5.99 Hz, 1P). Data for the major cyclized isomer: ^{1}H-NMR (500 MHz, CD_3OD) δ 0.89 (t, *J* = 7.0 Hz, 3H), 1.29 (m, 16H), 1.55 (d, *J* = 2.0 Hz, 3H), 1.59 (s, 3H), 1.64 (s, 3H), 1.65 (d, *J* = 0.5 Hz, 3H), 1.83 (m, 4H), 2.13 (m, 7H), 2.38 (ddd, *J* = 20.0, 18.0, 5.0 Hz, 2H), 2.98 (td, *J* = 12.0, 5.0 Hz, 1H), 3.23 (td, *J* = 12.0, 5.0 Hz, 1H), 4.58 (ddd, *J* = 21.5, 14.0, 1.5 Hz, 1H), 5.08 (t of septets, *J* = 7.0, 1.5 Hz, 1H), 5.16 (pseudo t, *J* = 7.0 Hz, 1H); ^{31}P NMR (162 MHz, CD_3OD) δ 12.81 (d, *J*= 15.86 Hz, 1P), 26.93 (d, *J*= 15.86 Hz, 1P); MS (+) FAB m/z 548.3. Data for the minor cyclized isomer: ^{31}P NMR (162 MHz, CD_3OD) δ 13.31 (d, *J* = 7.3 Hz, 1P), 26.72 (d, *J* = 9.9 Hz, 1P).



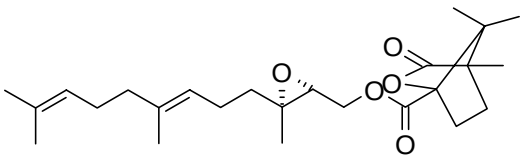
(2*S*, 3*R*)-*trans*-[2-((*E*)-4,8-Dimethyl-3,7-nonadienyl)-1-((*E*)-6,10-dimethyl-5,9-undecadienyl)-2-methyl-aziridin-3-yl]methyl Diphosphate, Tris(tetrabutylammonium) Salt (*ent*-6-OPP).

The (2*S*,3*R*) aziridino diphosphate *ent*-**6**-OPP was prepared from enantio pure mesylate *ent*-**6**-OMs (44 mg, 0.091 mmol) as described for its enantiomer using $(NBu_4)_3HP_2O_7$ (165 mg, 0.183 mmol) and activated 3Å powdered molecular sieves in anhyd CH_3CN (1 mL) at room temp for 60 h. Workup afforded 103 mg (73%) of *ent*-**6**-OPP and $(NBu_4)_3HP_2O_7$ (1:1 by ^{31}P NMR analysis) as a brown viscous oil as well as ~10 mg of recovered starting material *ent*-**6**-OMs. Data for *ent*-**6**-OPP: ^{31}P NMR (162 MHz, CD_3OD) δ –6.0 (d, J = 19.5 Hz, 1P), -5.2 (d, J = 19.5 Hz, 0.97 P), -3.9 (s, 1.78 P, PP_i), 6.2 (s, 0.14P, P_i). ^{1}H NMR data are given in the ms experimental section.

D. Preparative Procedures and Characterization Data for Camphanate Esters

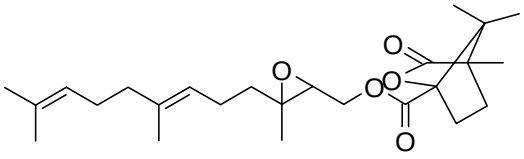


(2*R*, 3*S*)-*trans*-2,3-Epoxy-3,7,11-trimethyl-(*E*)-6,10-dodecadien-1-yl (*S*)-Camphanate. The following procedure is based on that reported by Gerlach.[10] A solution of (-)-2,3-epoxyfarnesol ((-)-**10**, 56.5 mg, 0.24 mmol) and 4-DMAP (4 mg, 0.033 mmol) in pyridine (2 mL) was stirred at room temp. under N_2 as (1*S*)-(-)-camphanic chloride (64.2 mg, 0.28 mmol) was added in portions. After 12 h, hexane (3 mL) and 1M HCl (3 mL) were added, and the mixture was vigorously stirred for 10 min. The aqueous layer was extracted with hexane (3 x 5 mL). The organic extracts were combined, washed with 1M HCl (2 x 5 mL), sat'd $NaHCO_3$ (2 x 5 mL), water (3 x 5 mL); dried ($MgSO_4$) and concentrated under reduced pressure to give 92 mg of a pale yellow oil. Purification by flash column chromatography (25% EtOAc/hexanes) afforded 87.6 mg (88%) of the camphanate ester as a clear, colorless oil: ^{1}H NMR (400 MHz, CD_3OD) δ 0.95 (s, 3H), 1.09 (s, 3H), 1.10 (s, 3H), 1.34 (s, 3H), 1.46 (dt, J = 8.4, 2.5 Hz, 1H), 1.59 (s, 3H), 1.62 (s, 3H), 1.64 (m, 2H), 1.66 (s, 3H), 2.02 (m, 8H), 2.46-2.54 (8 peak m, 1H), 3.05 (app dd, J_{app} = 7.1, 4.2 Hz, 1H), 4.25 (A<u>B</u>X, J_{AB} = 12.2 Hz, J_{BX} = 7.1 Hz, 1H), 4.50 (<u>A</u>BX, J_{AB} = 12.0 Hz, J_{AX} = 4.1 Hz, 1H) , 5.10 (m, 2H); some peaks of the diastereomer were also present (~6%): δ 4.27 (A<u>B</u>X, J_{AB} = 12.1 Hz, J_{BX} = 7.2 Hz, 0.029H), 4.44 (<u>A</u>BX, J_{AB} = 12.1 Hz, J_{AX} = 4.1 Hz, 0.030H). The diasteromeric ratio was 94:6 (±0.1).



(2*S*, 3*R*)-*trans*-2,3-Epoxy-3,7,11-trimethyl-(*E*)-6,10-dodecadien-1-yl (*S*)-Camphanate. The reaction of (+)-2,3-epoxyfarnesol (62 mg, 0.26 mmol), 4-DMAP (3.2 mg, 0.026 mmol) and (1*S*)-camphanic chloride (67 mg, 0.31 mmol) in pyridine was carried out as described above for (-)-2,3-epoxyfarnesol. The yield

was 65%. ^{1}H NMR (500 MHz, CD_3OD) δ 0.95 (s, 3H), 1.09 (s, 3H), 1.10 (s, 3H), 1.33 (s, 3H), 1.47 (m, 1H), 1.59 (s, 3H), 1.61 (s, 3H), 1.65 (m, 2H), 1.66 (s, 3H), 1.99 (m, 8H), 2.50 (8 peak m, 1H), 3.05 (app dd, J_{app} = 7, 4 Hz, 1H), 4.26 (app dd, J_{app} = 12, 7.5 Hz, 1H), 4.45 (app dd, J_{app} = 12.5, 4.5 Hz, 1H) , 5.10 (m, 2H).



(±)-(2*S, 3*R**)-*trans*-2,3-Epoxy-3,7,11-trimethyl-(*E*)-6,10-dodecadien-1-yl (*S*)-Camphanate** The reaction of (±)-2,3-epoxyfarnesol (53.5 mg, 0.23 mmol), 4-DMAP (4 mg, 0.033 mmol) and (1*S*)-camphanic chloride (60.3 mg, 0.27 mmol) in pyridine was carried out as described above for (-)-2,3-epoxyfarnesol. The yield was 71 mg (75%): ^{1}H NMR (400 MHz, CD_3OD) δ 0.95 (s, 3H), 1.09 (s, 3H), 1.10 (s, 3H), 1.33 (s, 3H), 1.44 (dt, J = 8.3, 2.6 Hz, 1H), 1.59 (s, 3H), 1.61 (s, 3H), 1.64 (m, 2H), 1.66 (s, 3H), 2.05 (m, 8H), 2.46-2.54 (12 peak m, 1H), 3.05 (app dd, J_{app} = 7.3, 4.2 Hz, 1H), 4.24 (app dd, J_{app} = 15.3, 7.0 Hz, 1H, diastereomer B), 4.27 (app dd, J_{app} = 15.3, 7.2 Hz, diastereomer A), 4.44 (app dd, J_{app} = 12.1, 4.0 Hz, 1H, diastereomer B), 4.49 (app dd, J_{app} = 12.1, 4.0 Hz, 1H, diastereomer A), 5.11 (m, 2H).

E. Experimental Procedures for Squalene Synthase Purification

Materials and methods: [1-^{1}H]FPP (15 Ci/mmol) was purchased from a commercial vendor, and was diluted to the desired specific activity with unlabelled FPP, synthesized according to the procedures of Davisson et al.[9] Concentrations of stock solutions of diphosphates were determined by phosphate analysis.[11] Tween 80, Cytoscint scintillation fluor, Gamma Bind Sepharose, MOPS, DTT, β-NADPH, squalene, leupeptin, IPTG, glycerol, ligroine (boiling range 90-110 °C), toluene, K_2HPO_4, NaCl, $MgCl_2$ and methanol were acquired from commercial suppliers. E. Coli XA90 (F'Lac1^{q1}) was provided by Professor Gregory Verdine (Harvard University).

Purification of squalene synthase mutants: The expression plasmid pDY14 contains the *S. cerevisiae ERG9* coding region and *rrB* terminator downstream of the synthetic *tac* promoter. This plasmid encodes a squalene synthase that is missing 25 C-terminal amino acids, and ends with the sequence 415QEEF419. The recombinant enzyme is soluble, fully active and easily purified by immunoaffinity chromatography.[12] The protein was purified from E.coli XA90/pDY14 cells according to the procedures of Zhang[13] except that the YL1/2 monoclonal antibody affinity resin was utilized after the DEAE-cellulose step.

A sample (1-2 g) of frozen cells was thawed on ice and suspended in 30 mL of extraction buffer (10 mM K_3PO_4, pH 7.4, 10 mM BME , 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, 10 ug/mL leupeptin and 10 ug/mL pepstatin A) at 4 °C. Cells were disrupted by sonication, and the suspension was clarified by centrifugation at 16000 rpm for 30 min. The clarified supernatant was loaded onto a 2.5 x 12 cm DE52 column. A step gradient of 70, 150 and 500 mM K_3PO_4, pH 7.4, 10 mM BME, separated about half of the unwanted protein as well as other non-protein cell components. The squalene synthase containing fraction eluted with the 150 mM phosphate wash. The protein solution was then loaded onto the antibody column (0.1 mL/min) and the unbound protein that eluted was monitored by UV spectroscopy (280 nm). After the addition, the column was washed with 50 mM phosphate buffer and the bound protein was eluted with 5 mM Asp-Phe buffer. The protein eluted as a single peak and was generally >95% pure by SDS-PAGE electrophoresis with 12% gels and coomassie blue staining using standard procedures. Protein concentrations were determined by the method of Bradford.[14] The enzyme was stored in 20% (v/v) glycerol, frozen on dry ice and stored at -70 °C until required.

Abbreviations used: BME, 2-mercaptoethanol; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl) ether; IPTG, isopropyl β-D-thiogalactoside; MOPS, 3-(*N*-morpholino)propanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

References

(1) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

(2) Meyers, A. I.; Temple, D. L.; Nolen, R. L.; Mihelich, E. D. *J. Org. Chem.* **1974**, *39*, 2778-2783.

(3) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *92*, 6190-6191.

(4) Huisgen, R.; Moebius, L.; Mueller, G.; Stangl, H.; Szeimies, G.; Vernon, J. M. *Chem. Ber.* **1965**, *98*, 3992-4013.

(5) Marshall, J. A.; Andersen, N. H.; Hochstetler, A. R.; *J. Org. Chem.* **1967**, *32*, 113-118.

(6) Ashton, R.; Robinson, R.; Smith, J. C. *J. Chem. Soc.* **1936**, 283-285.

(7) Crossland, R. K.; Servis, K. L. *J. Org. Chem.* **1970**, *35*, 3195-3196.

(8) Koohang, A.; Coates, R. M.; Owen, D.; Poulter, C. D. *J. Org. Chem.* **1999**, *64*, 6-7.

(9) (a) Davisson, V. J.; Woodside, A. B.; Neal, T. R.; Stremler, K. E.; Muehlbacher, M.; Poulter, C. D. *J. Org. Chem.* **1986**, *51*, 4768-4779. (b) Davisson, V. J.; Davis, D. R.; Dixit, V. M.; Poulter, C. D. *J. Org. Chem.* **1987**, *52*, 1794-1801.

(10) Gerlach, H.; Zagalak, B. *Chem. Commun.* **1973**, 274-275.

(11) Cassidy, P. B.; Dolence, J. M.; Poulter, C. D. *Methods Enzymol.* **1995**, *250*, 30-43.

(12) Mayer, M. P.; Prestwich, G. D.; Dolence, J. M.; Bond, P. D.; Wu, H-Y; Poulter, C. D. *Gene* **1993**, *132*, 41-47.

(13) Zhang, D.; Jennings, S. M.; Robinson, G. W.; Poulter, C. D. *Arch. Biochem. Biophys.* **1993**, *304*, 133-143.

(14) Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248-254.

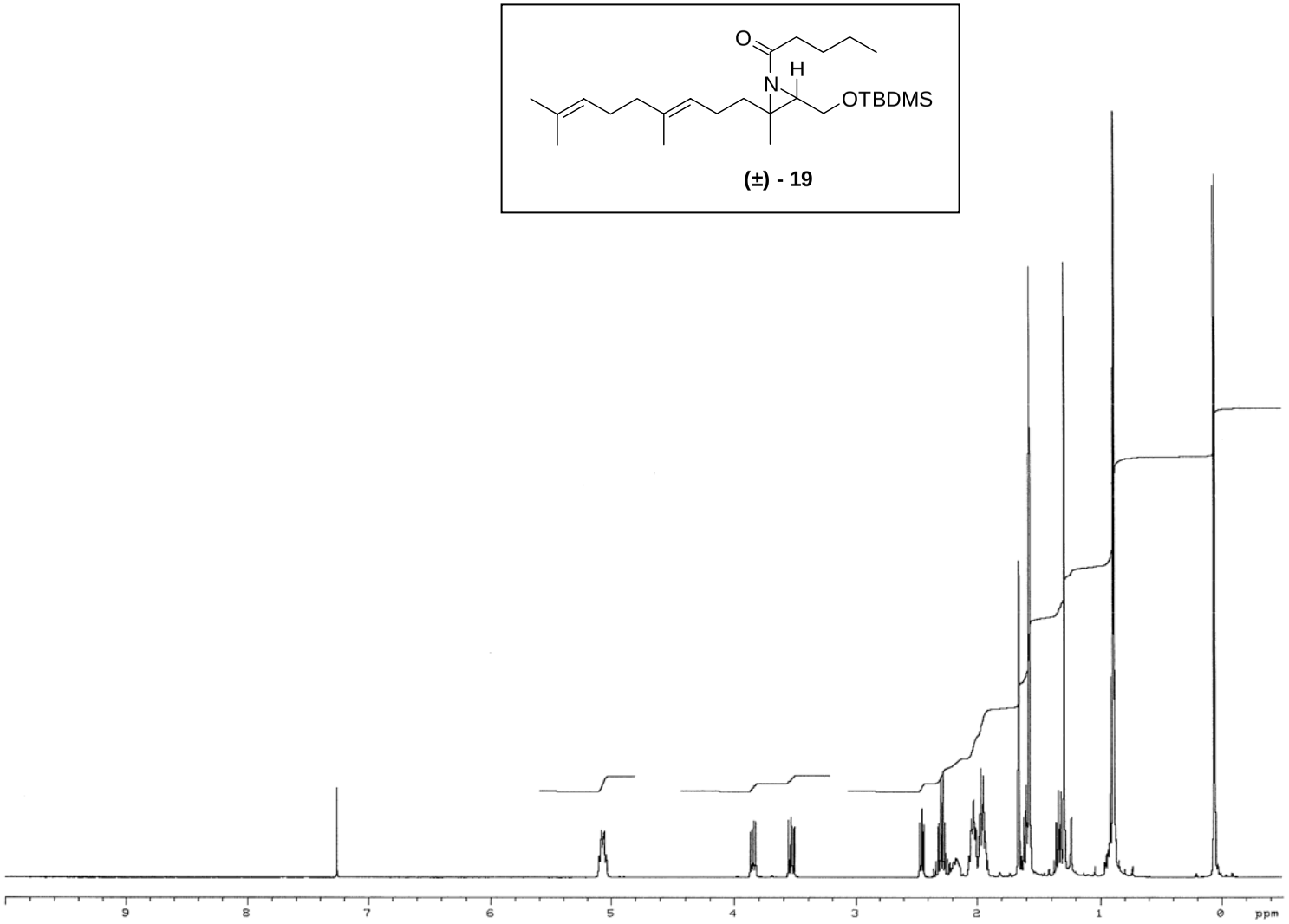


Figure 1: ^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of *N*-acyl, O-silyl aziridine **(±)-19.**

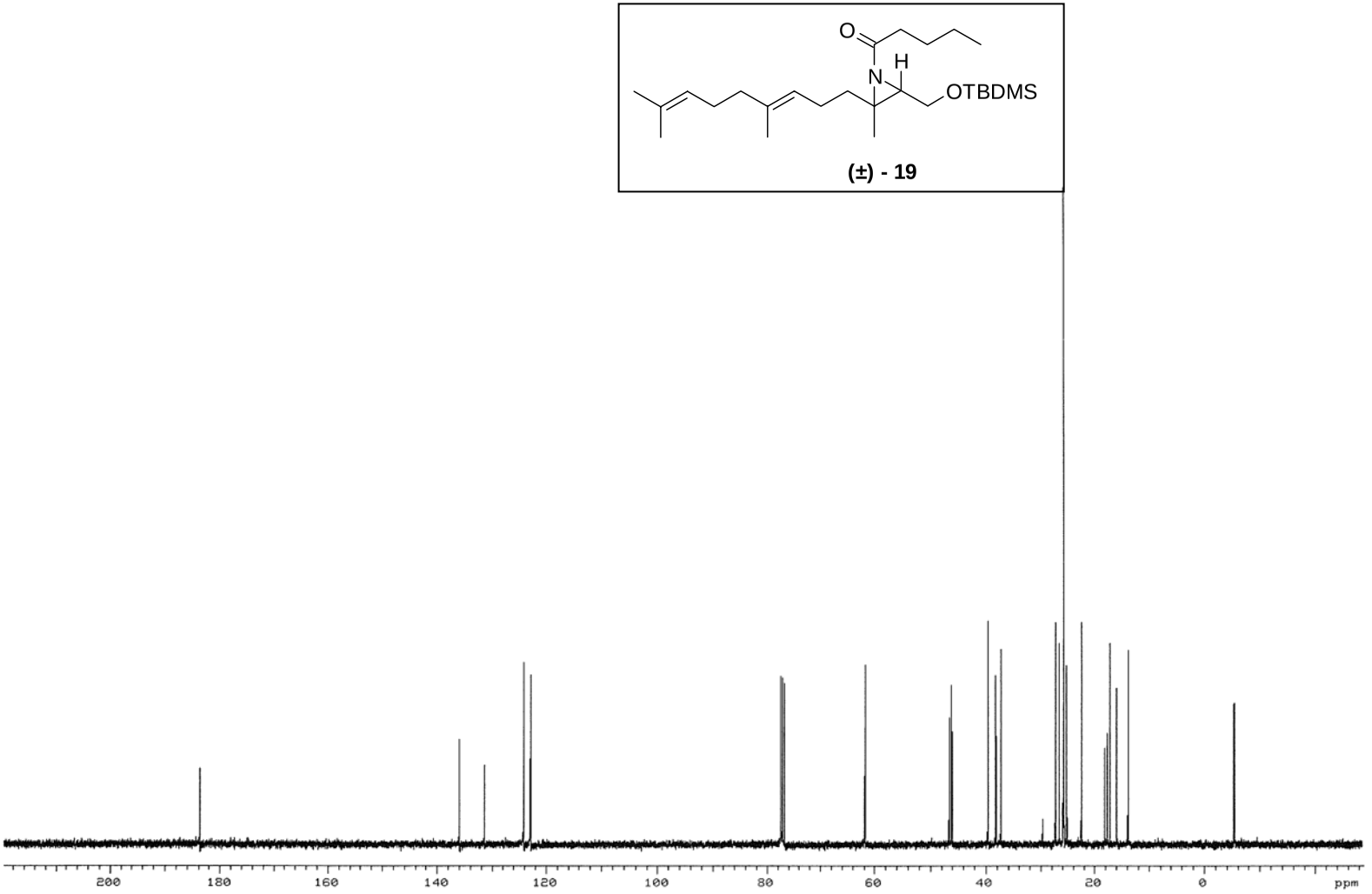


Figure 2: ^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of *N*-acyl, O-silyl aziridine **(±)-19.**

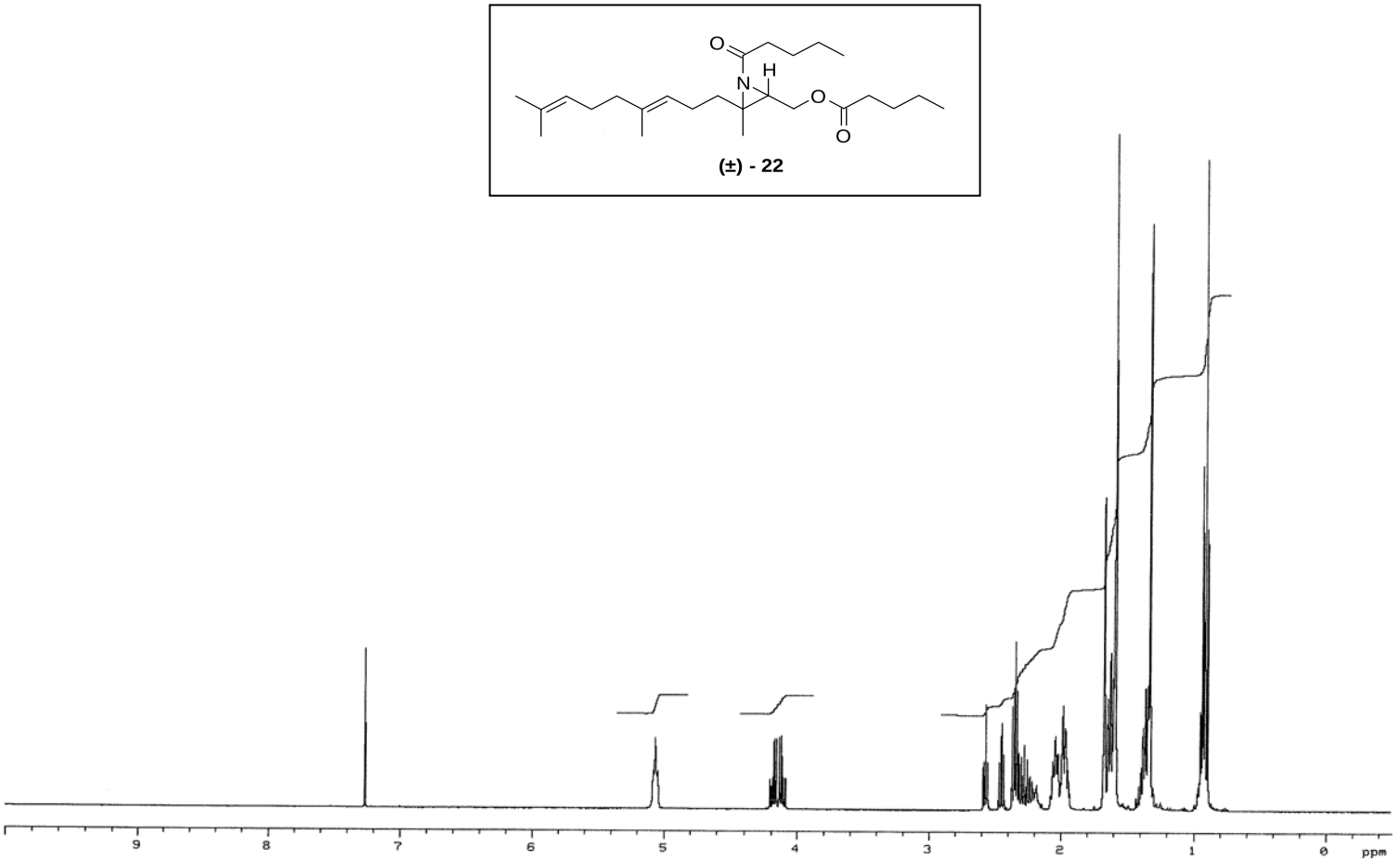
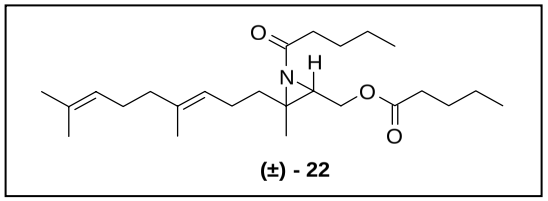


Figure 3: ^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of *N*-acyl, O-acyl aziridine **(±)-22.**



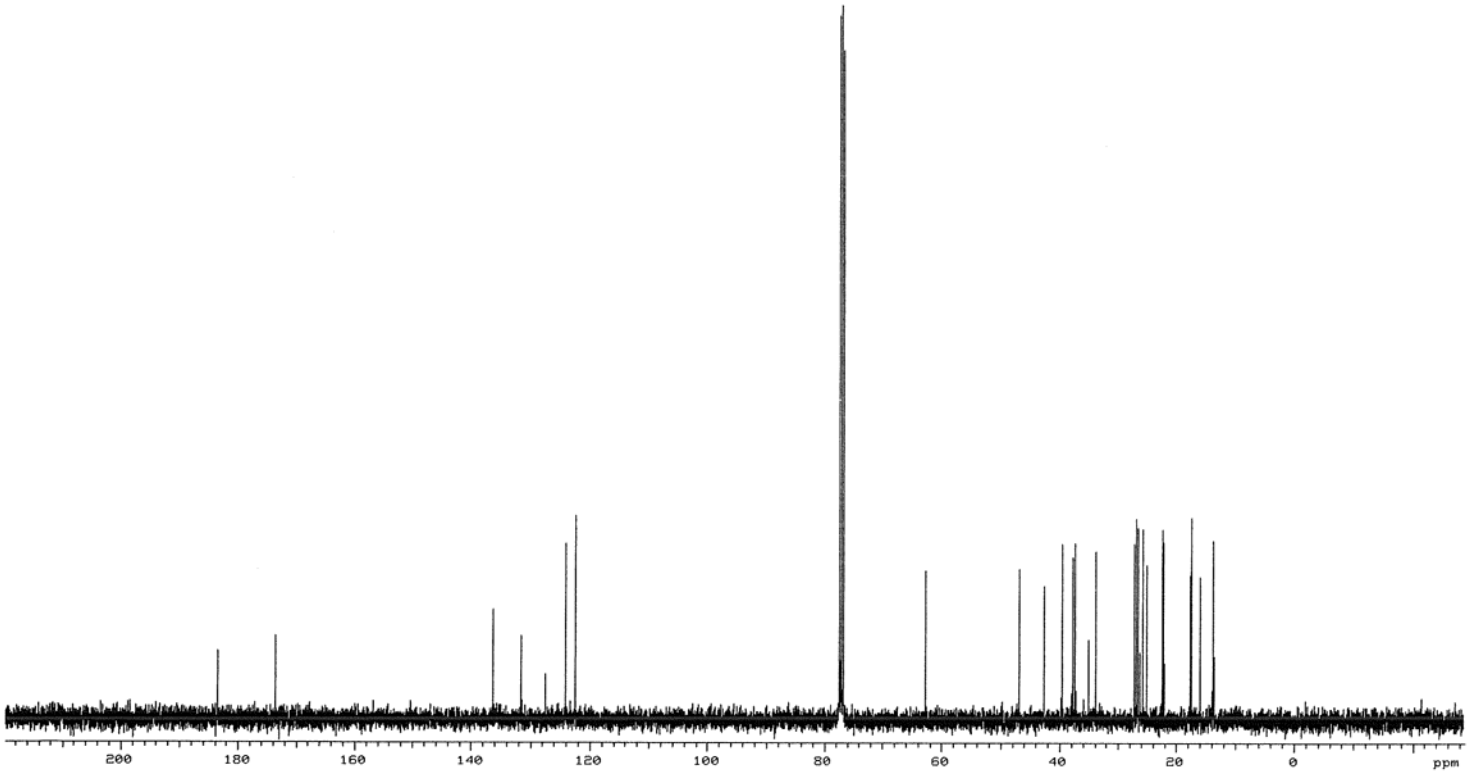


Figure 4: ^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of *N*-acyl, O-acyl aziridine **(±)-22.**

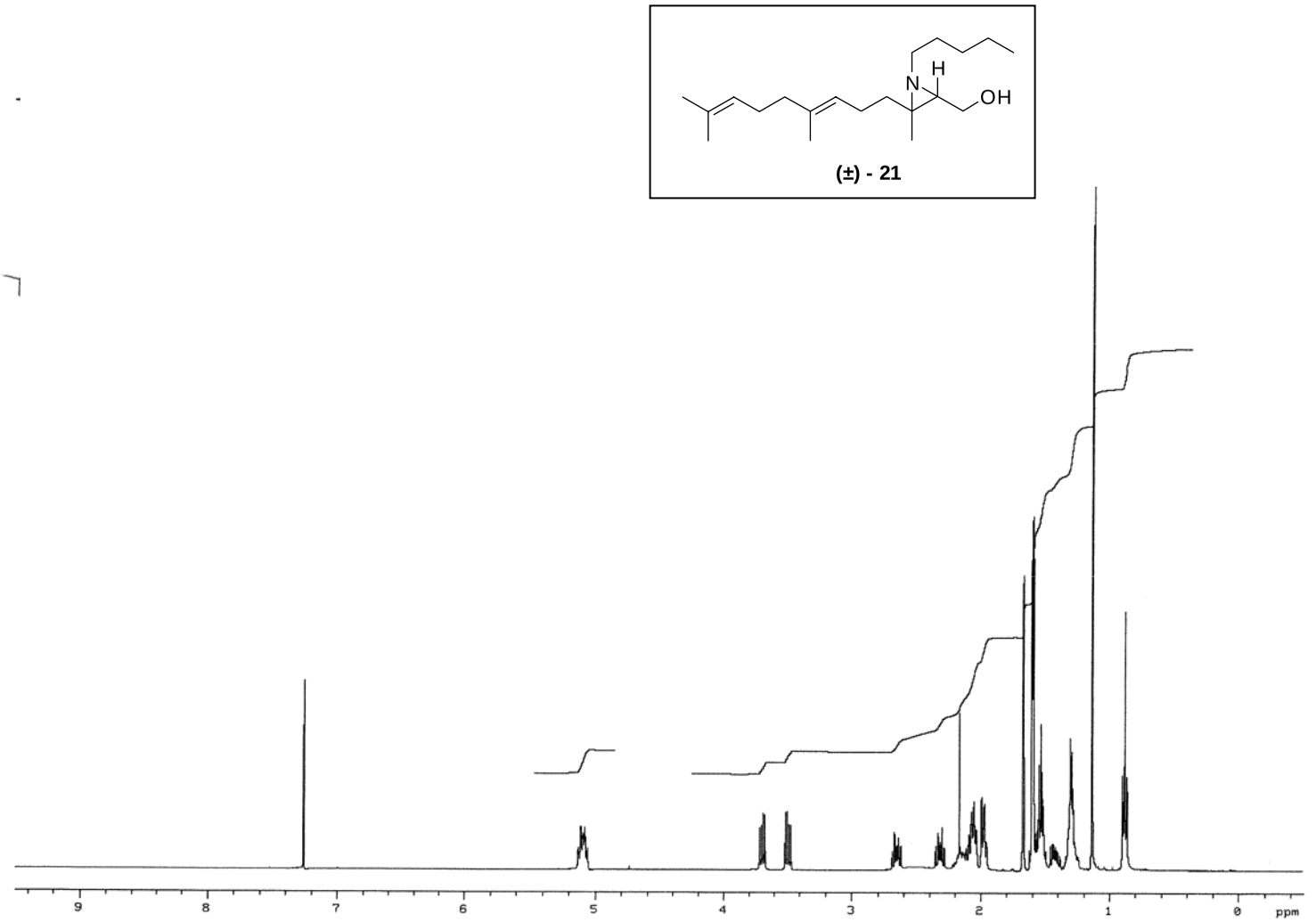


Figure 5: ^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of *N*-pentyl aziridino alcohol **(±)-21.**

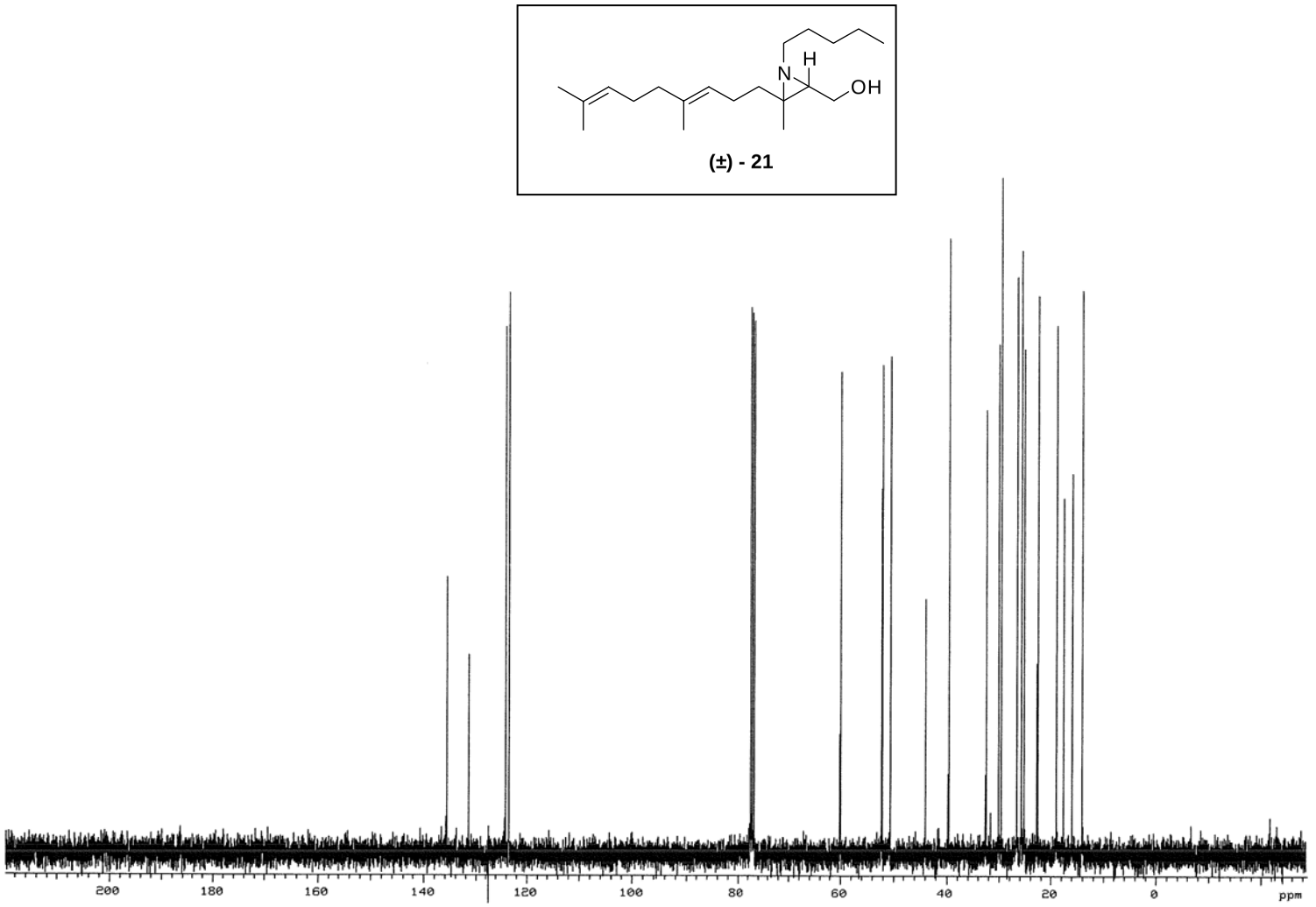


Figure 6: ^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of *N*-pentyl aziridino alcohol **(±)-21.**

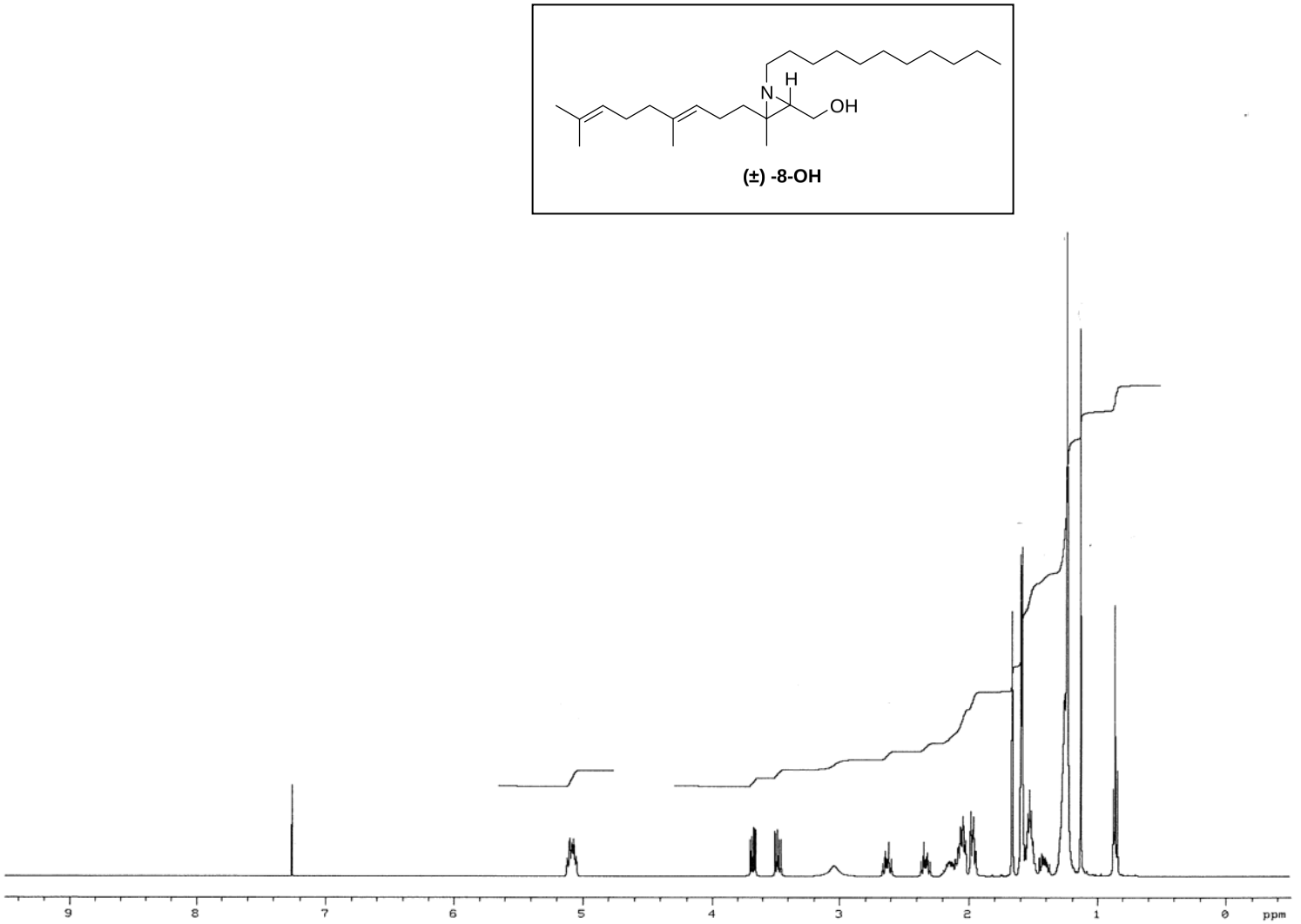


Figure 7: ^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of *N*-undecyl aziridino alcohol **(±)-8-OH.**

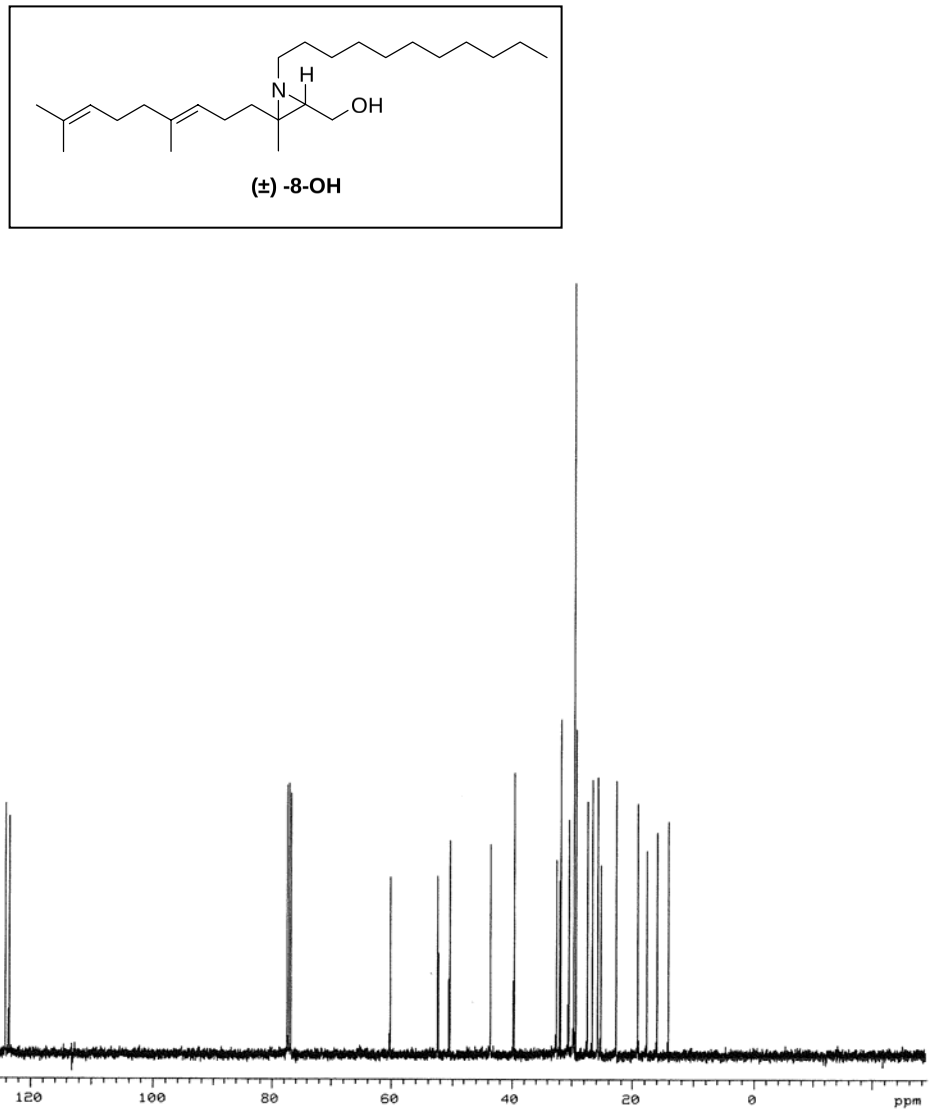
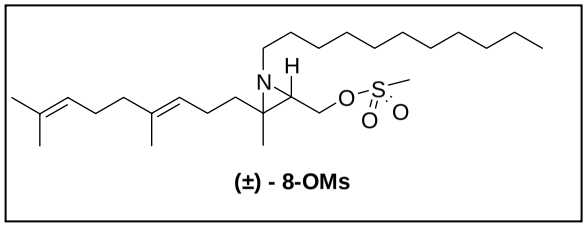


Figure 8: ^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of *N*-undecyl aziridino alcohol **(±)-8-OH.**



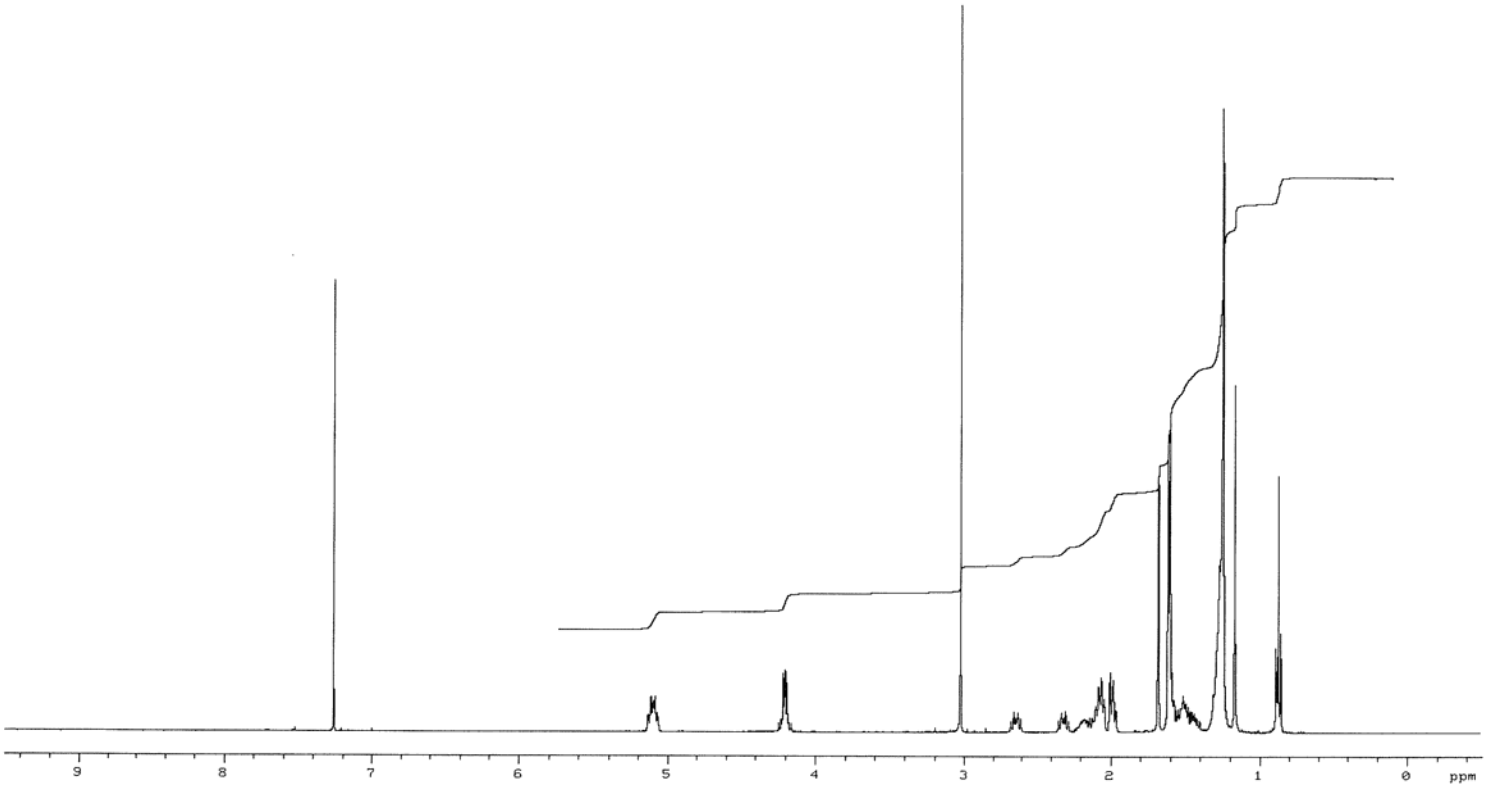


Figure 9: ^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of *N*-undecyl aziridino mesylate **(±)-8-OMs.**

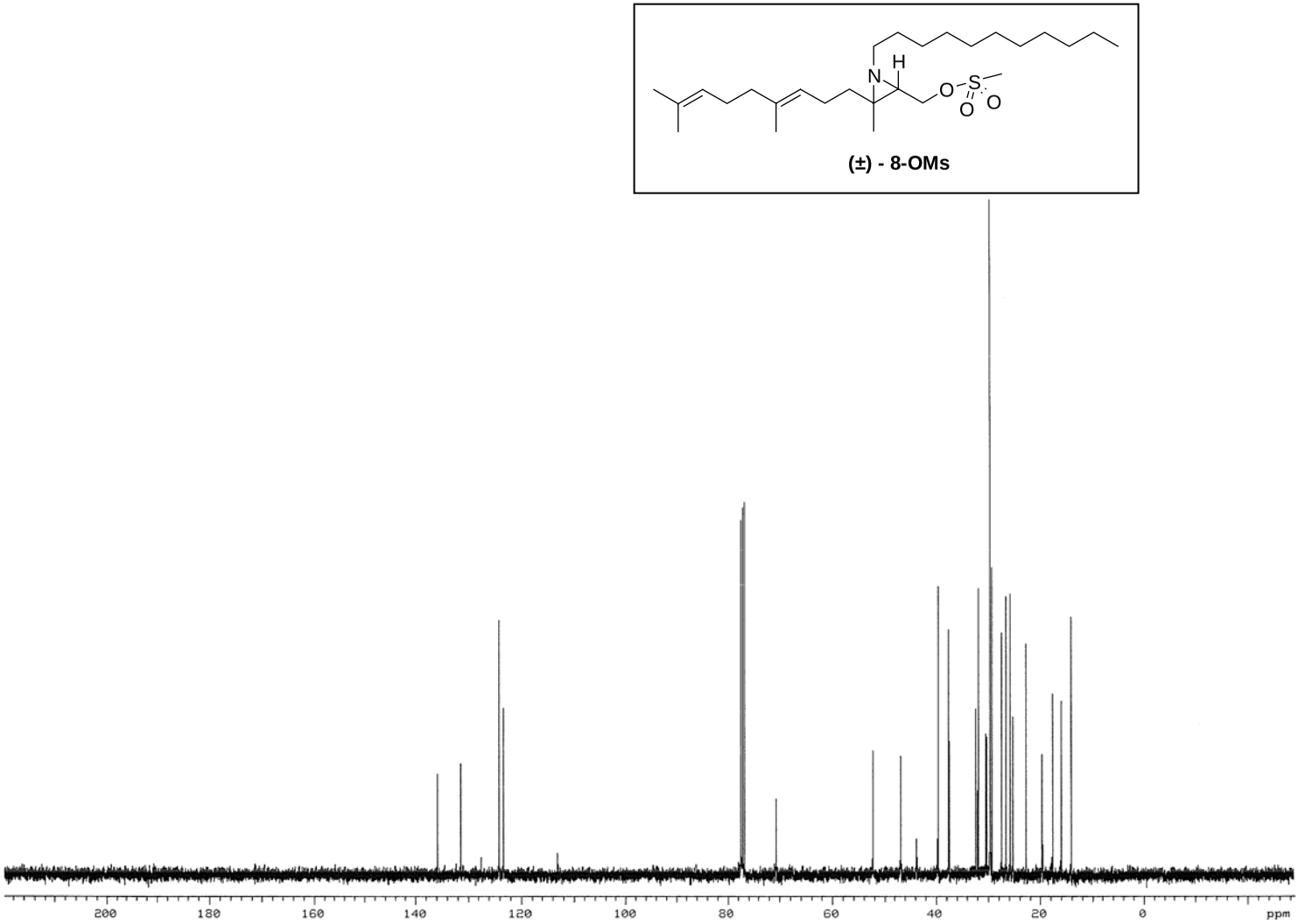


Figure 10: ^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of *N*-undecyl aziridino mesylate **(±)-8-OMs.**

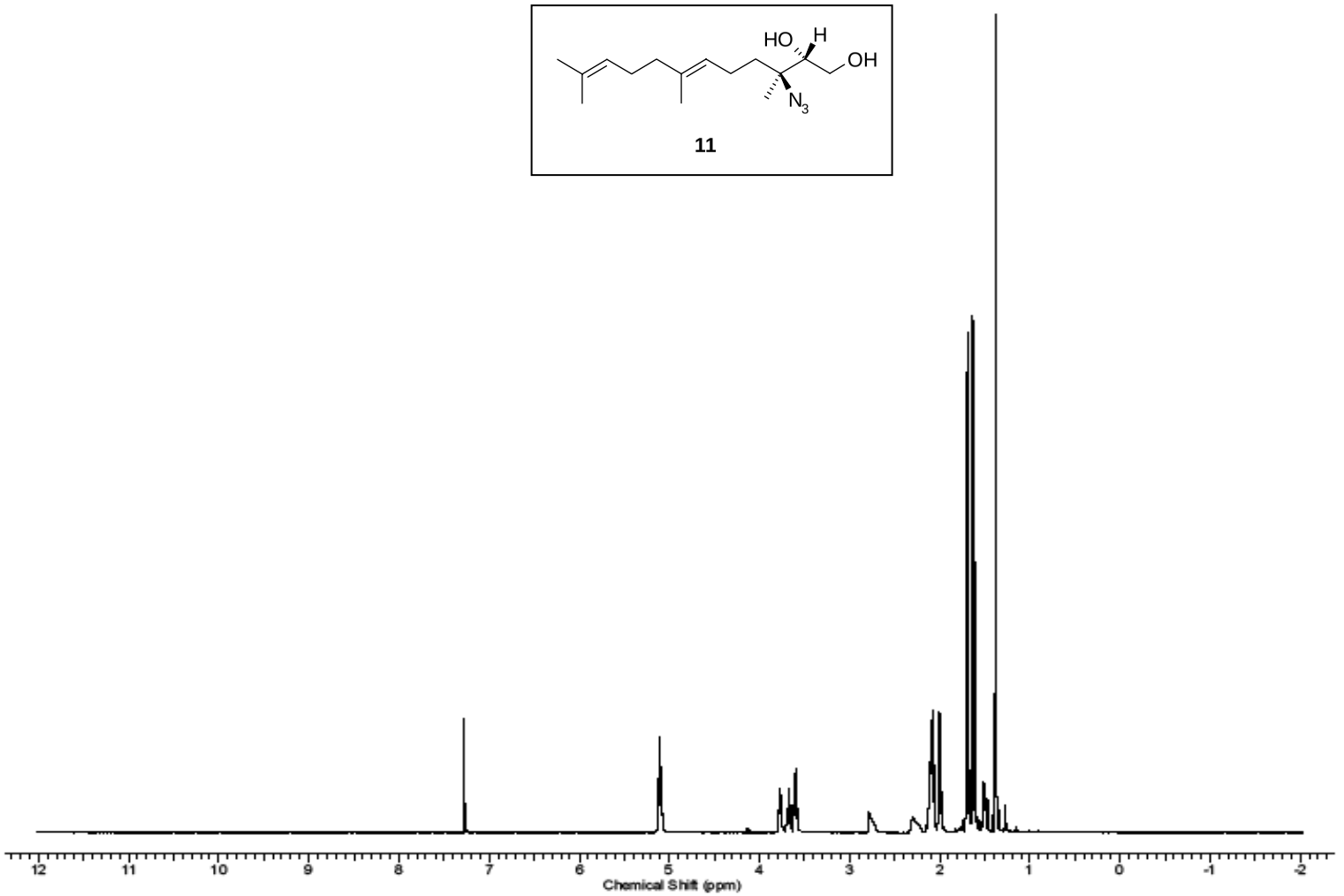


Figure 11: ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of (2*S*, 3*S*) azido diol **11.**

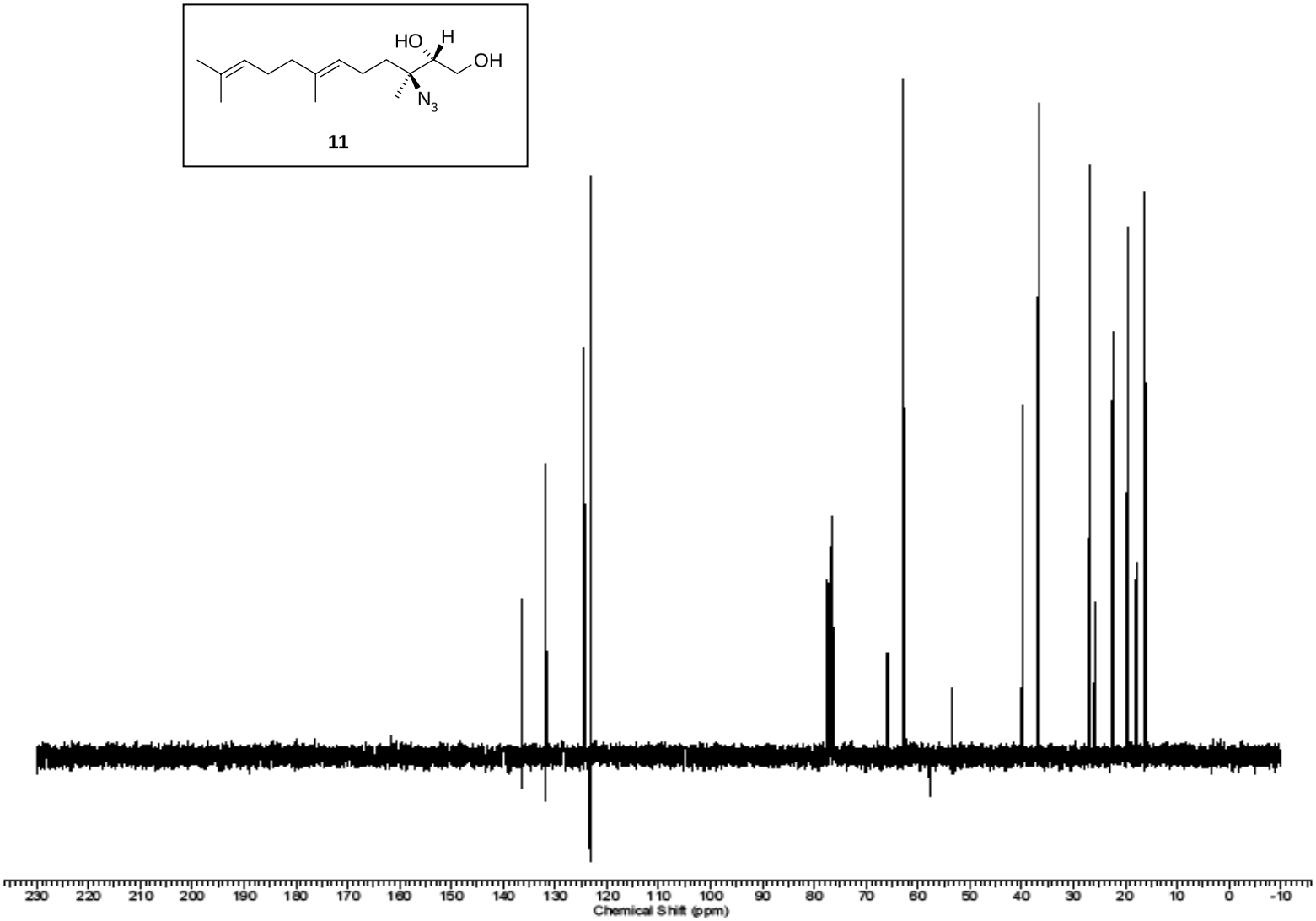


Figure 12: ^{13}C NMR Spectrum (126 MHz, $CDCl_3$) of (2*S*, 3*S*) azido diol **11.**

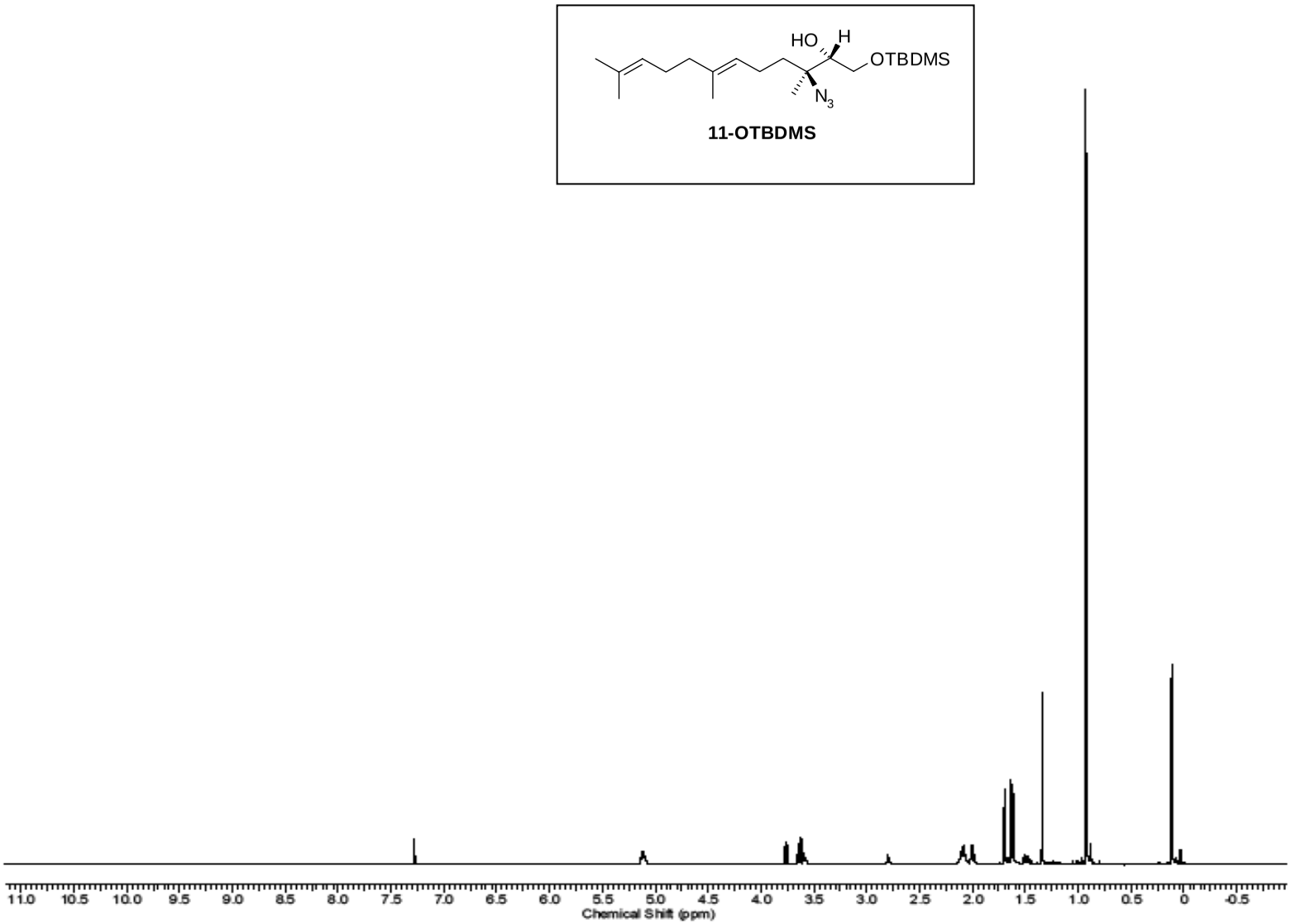


Figure 13: ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of (2*S*, 3*S*) silyl ether **11-OTBDMS.**

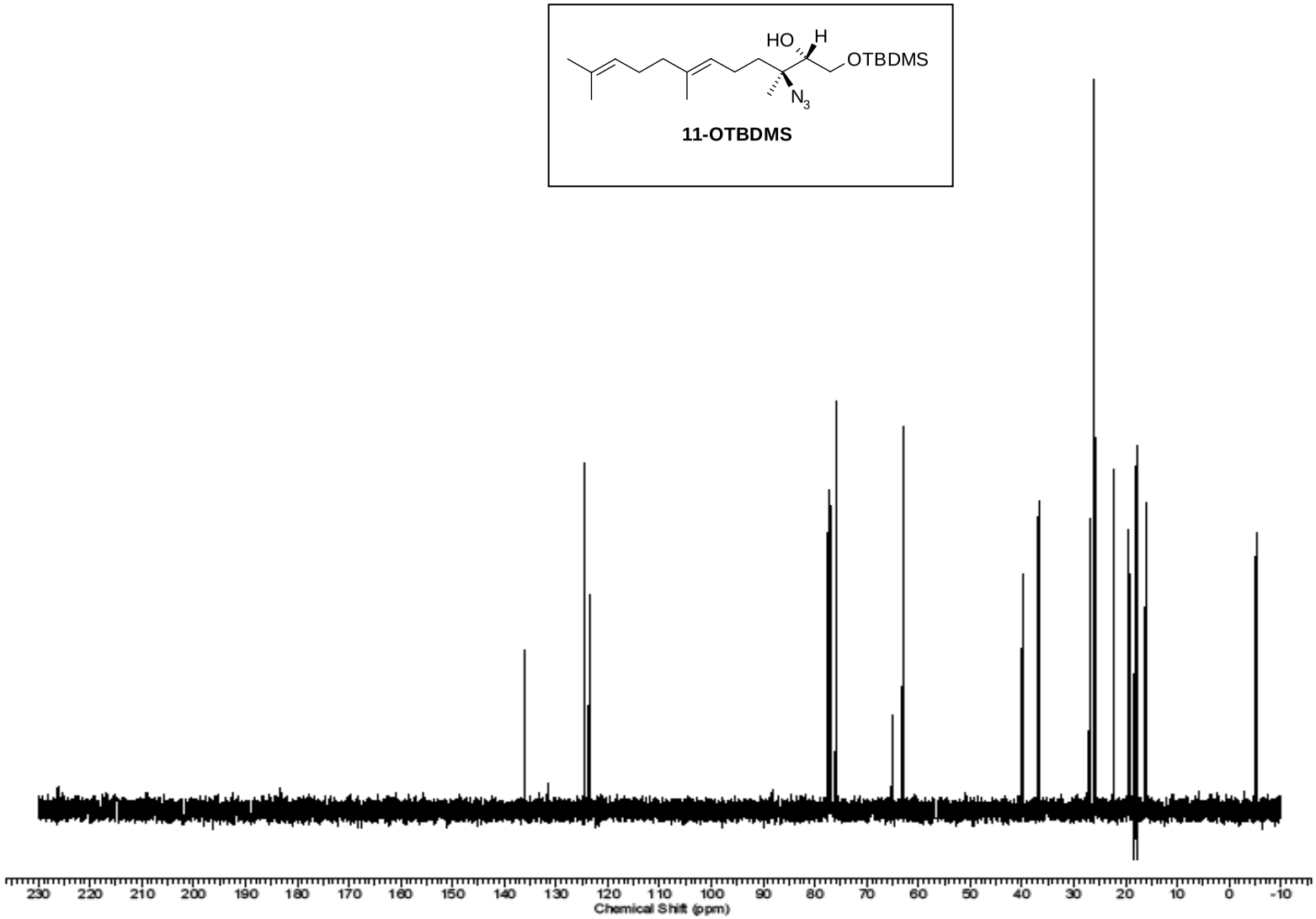


Figure 14: ^{13}C NMR Spectrum (126 MHz, $CDCl_3$) of (2*S*, 3*S*) silyl ether **11-OTBDMS.**

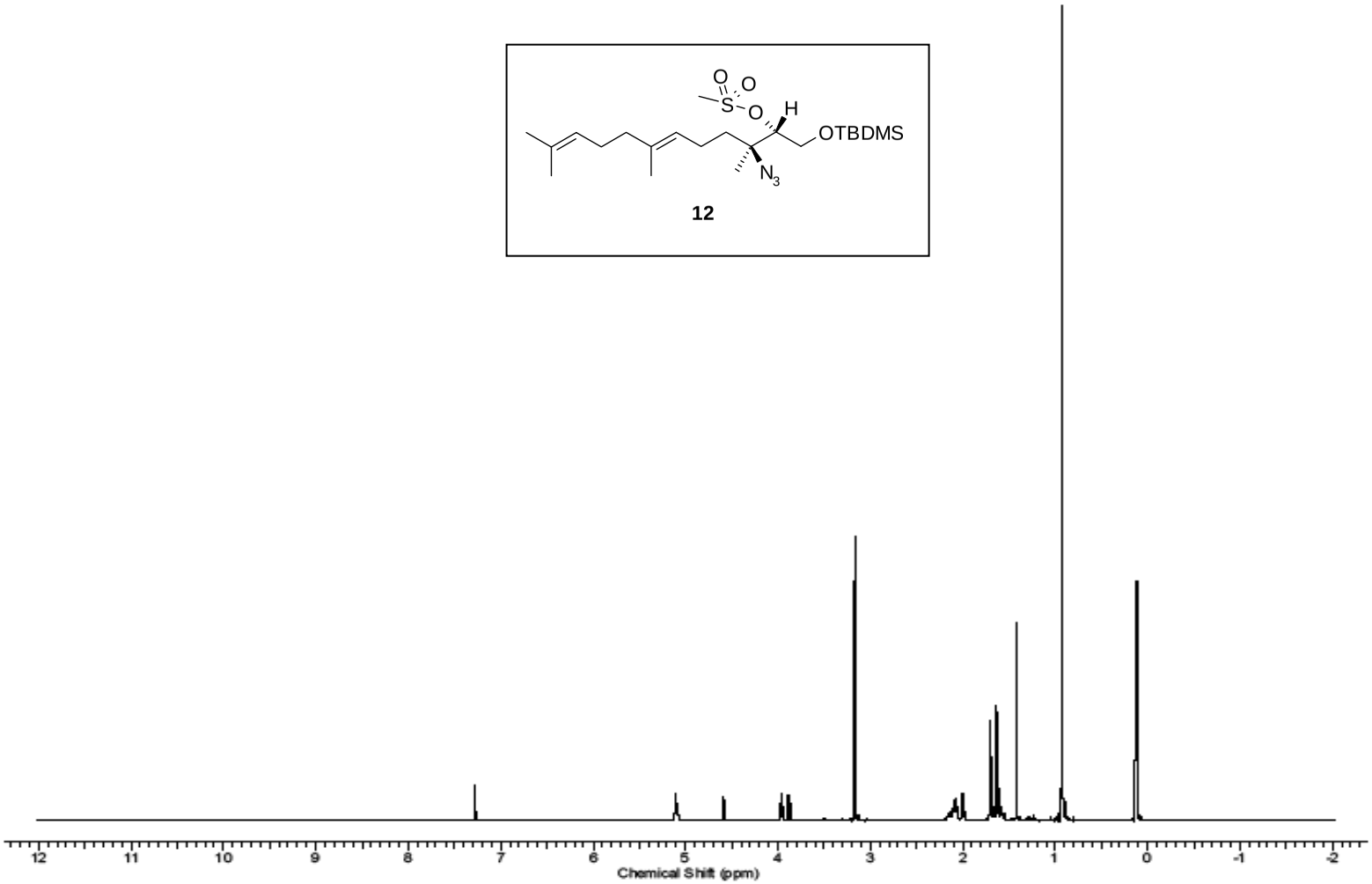


Figure 15: ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of (2*S*, 3*S*) azido mesylate **12.**

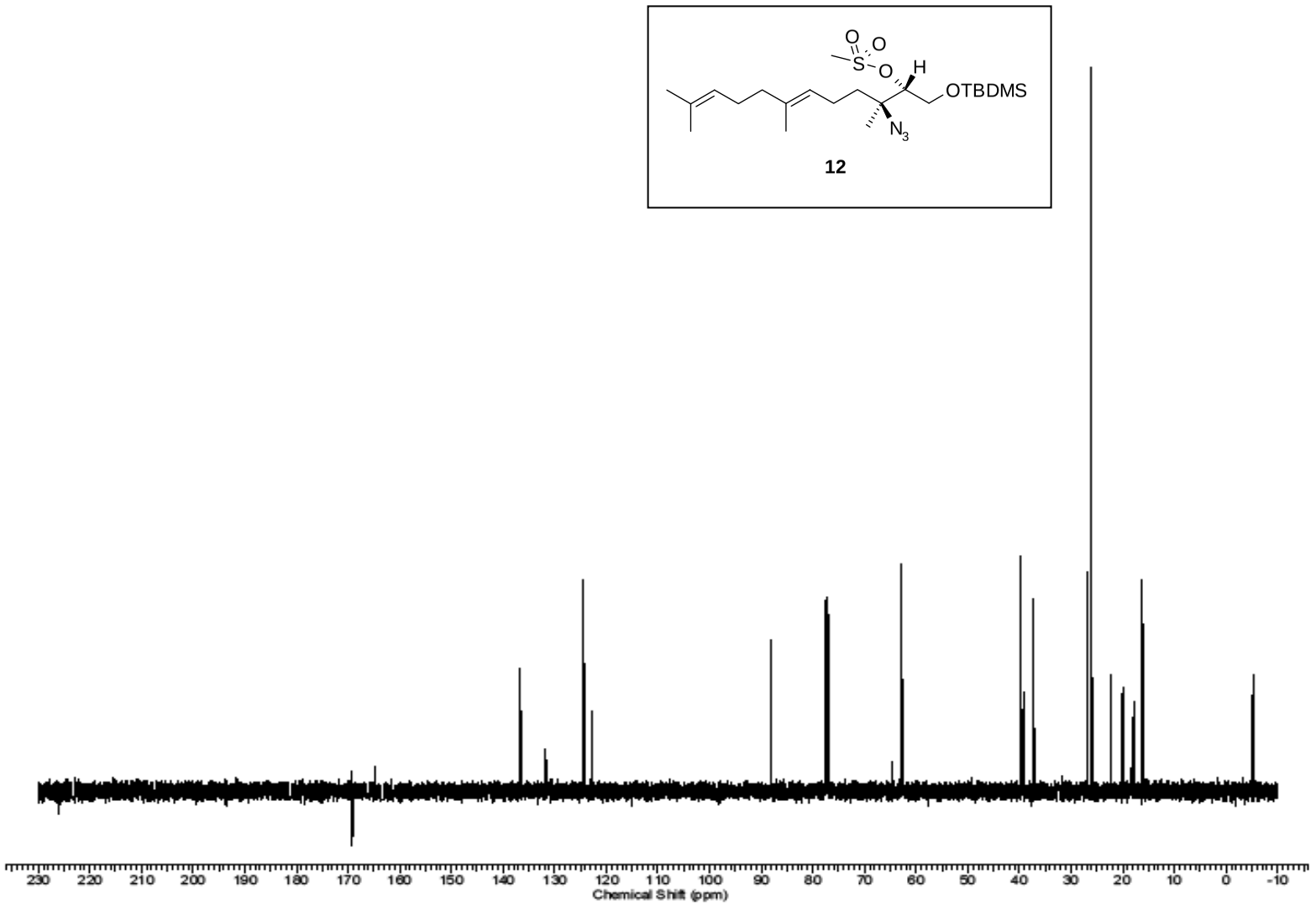


Figure 16: ^{13}C NMR Spectrum (126 MHz, $CDCl_3$) of (2*S*, 3*S*) azido mesylate **12.**

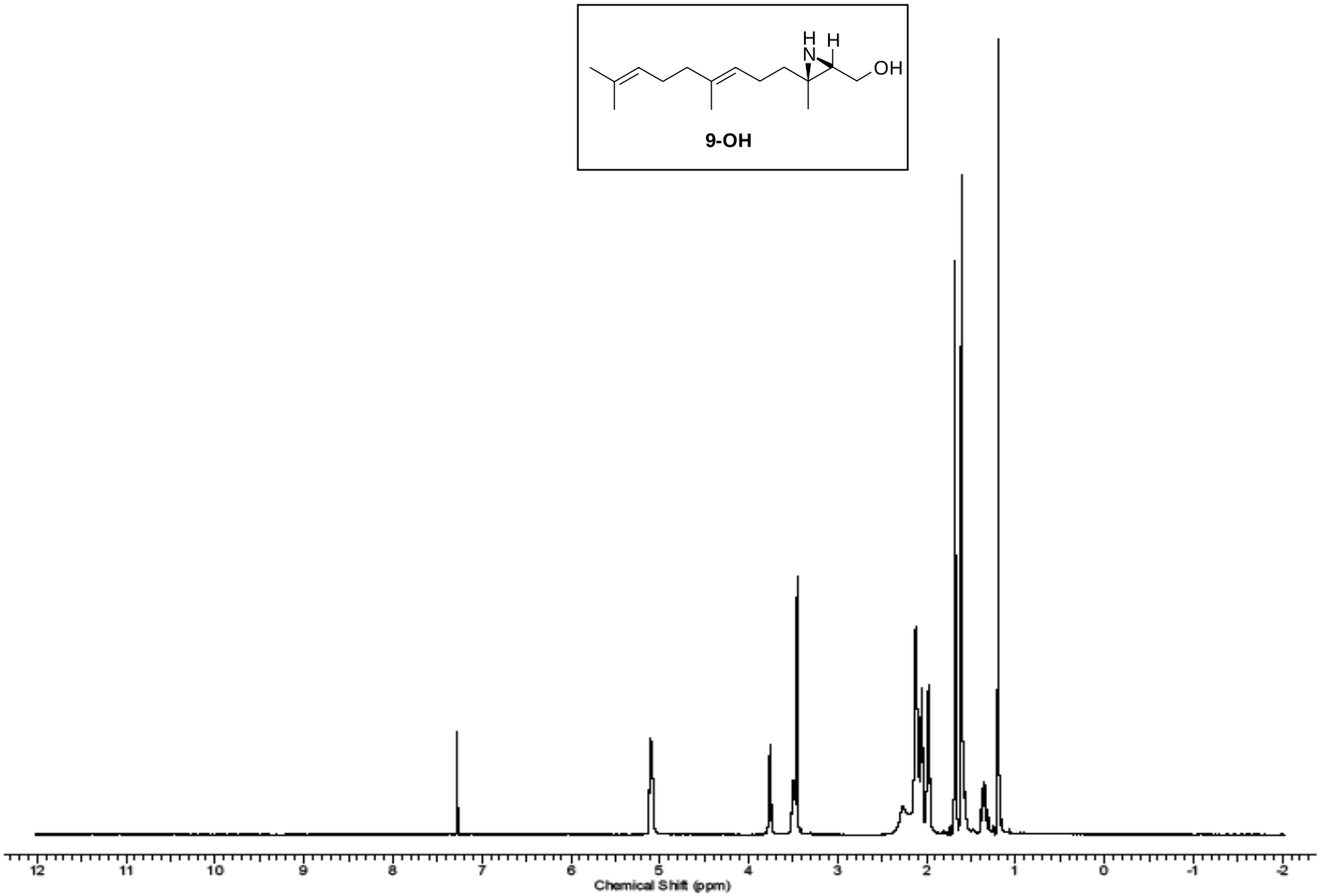


Figure 17: ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of (2*R*, 3*S*) aziridino alcohol **9-OH.**

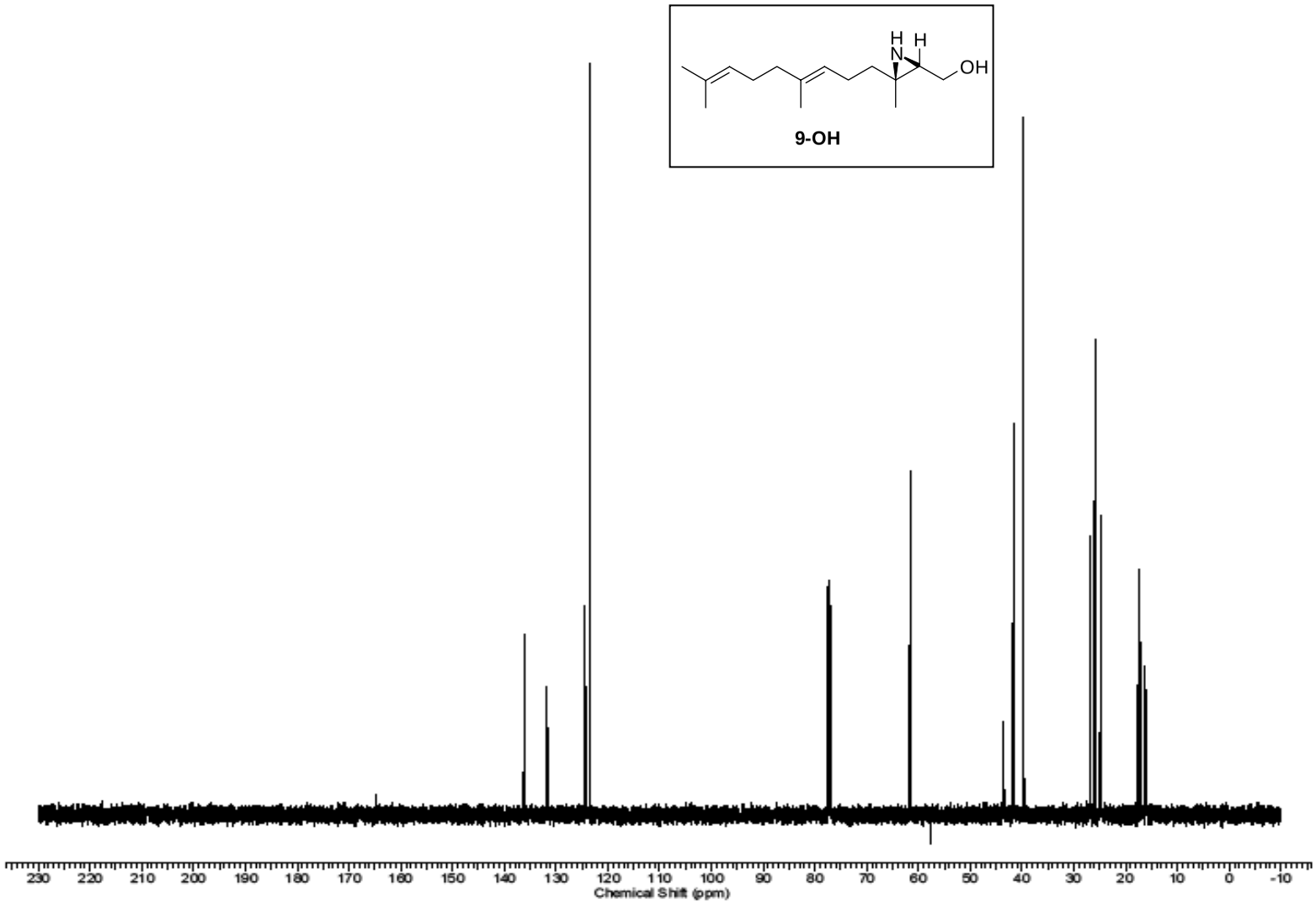


Figure 18: ^{13}C NMR Spectrum (126 MHz, $CDCl_3$) of (2*R*, 3*S*) aziridino alcohol **9-OH.**

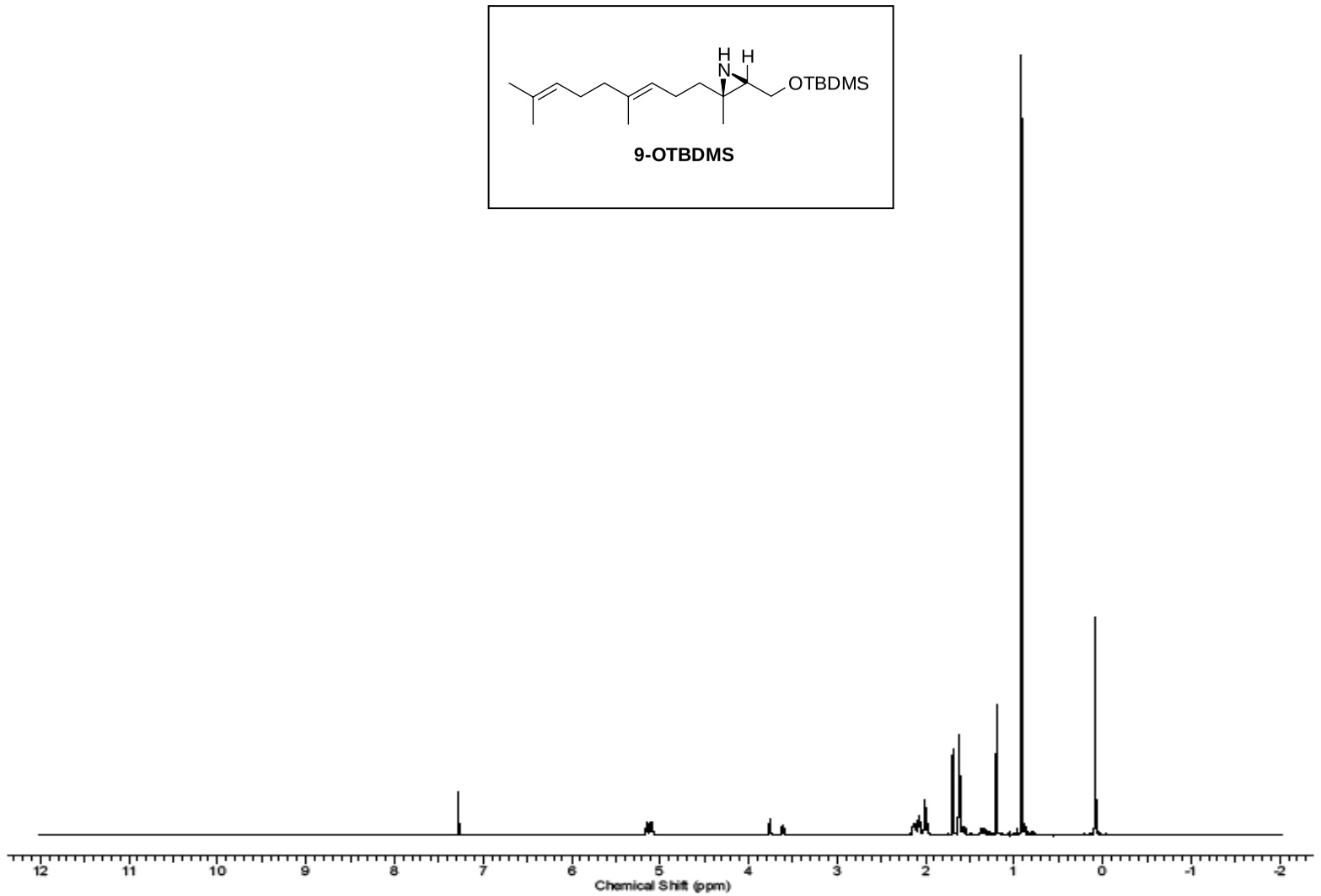


Figure 19: ^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of (2*R*, 3*S*) aziridine **9-OTBDMS.**

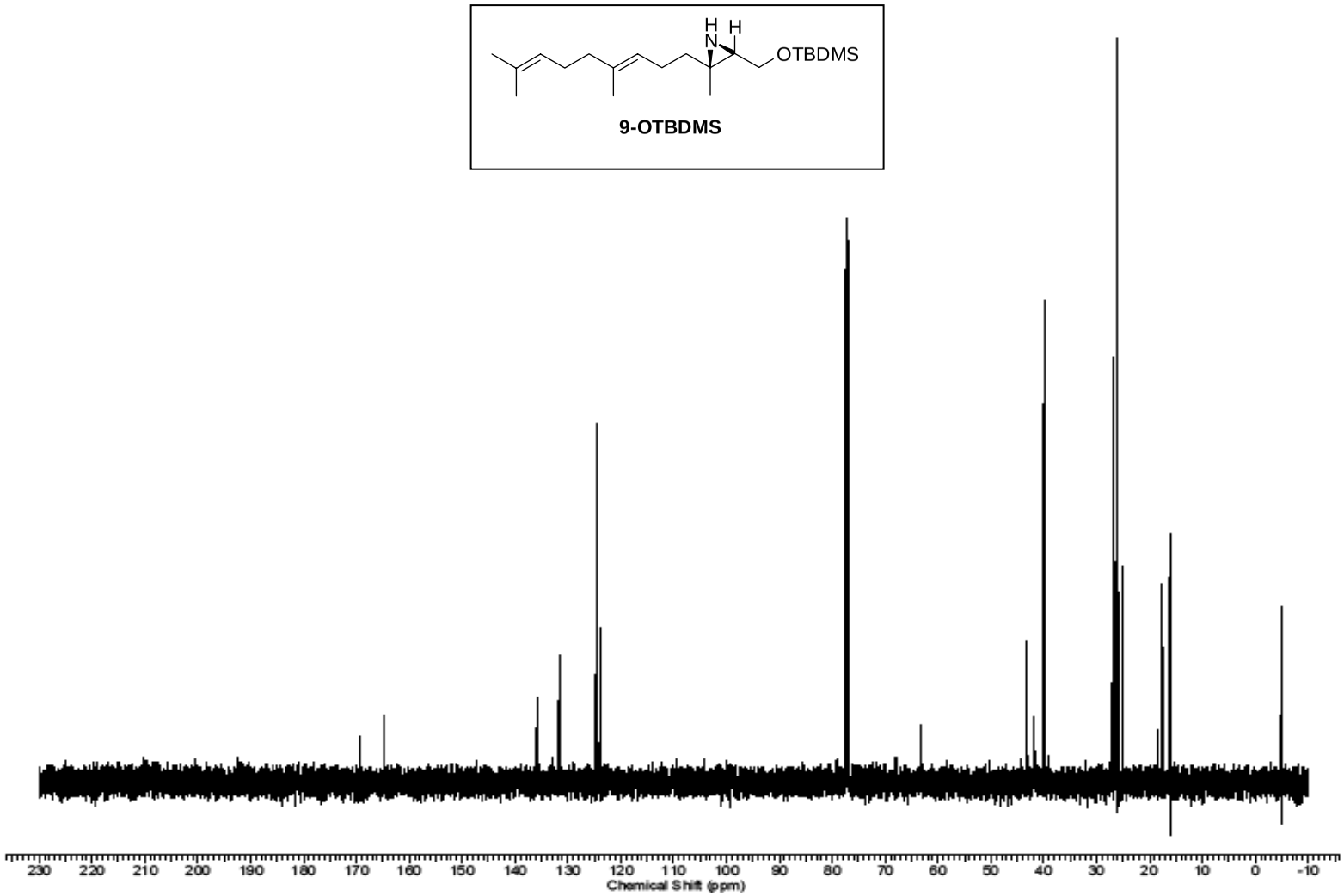


Figure 20: ^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of (2*R*, 3*S*) aziridine **9-OTBDMS.**

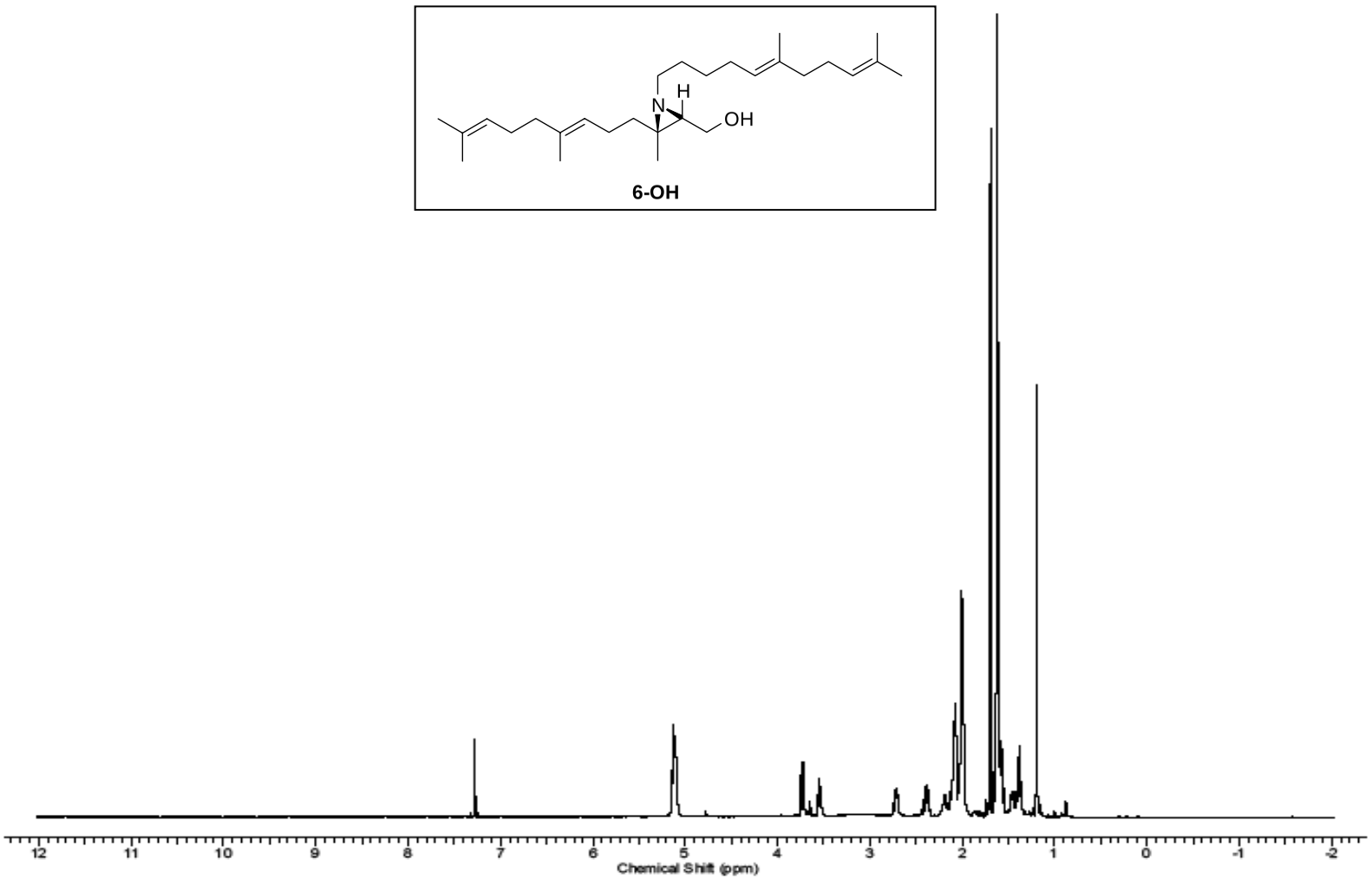


Figure 21: ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of (2*R*, 3*S*) *N*-alkyl aziridino alcohol **6-OH.**

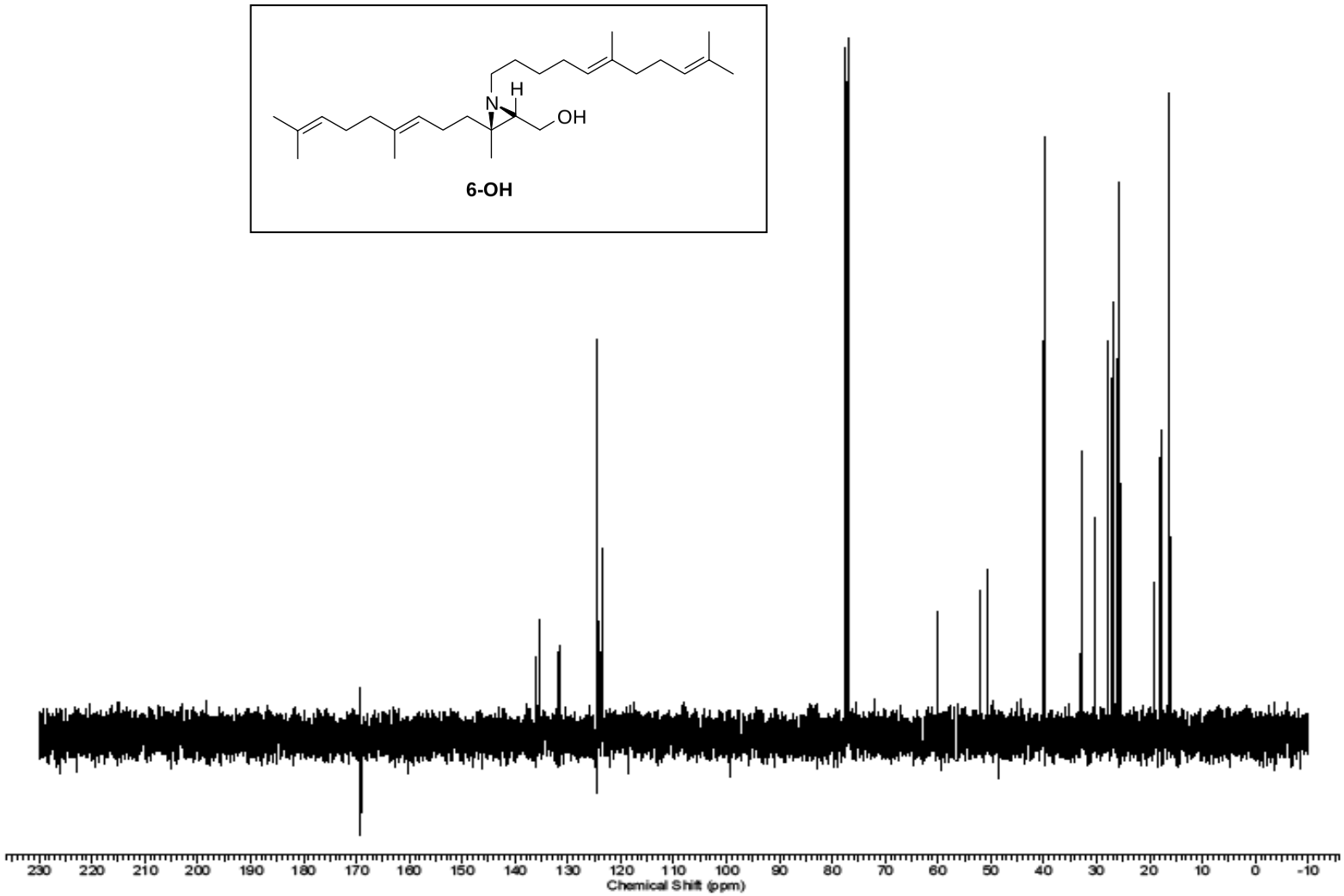


Figure 22: ^{13}C NMR Spectrum (126 MHz, $CDCl_3$) of (2*R*, 3*S*) *N*-alkyl aziridino alcohol **6-OH.**

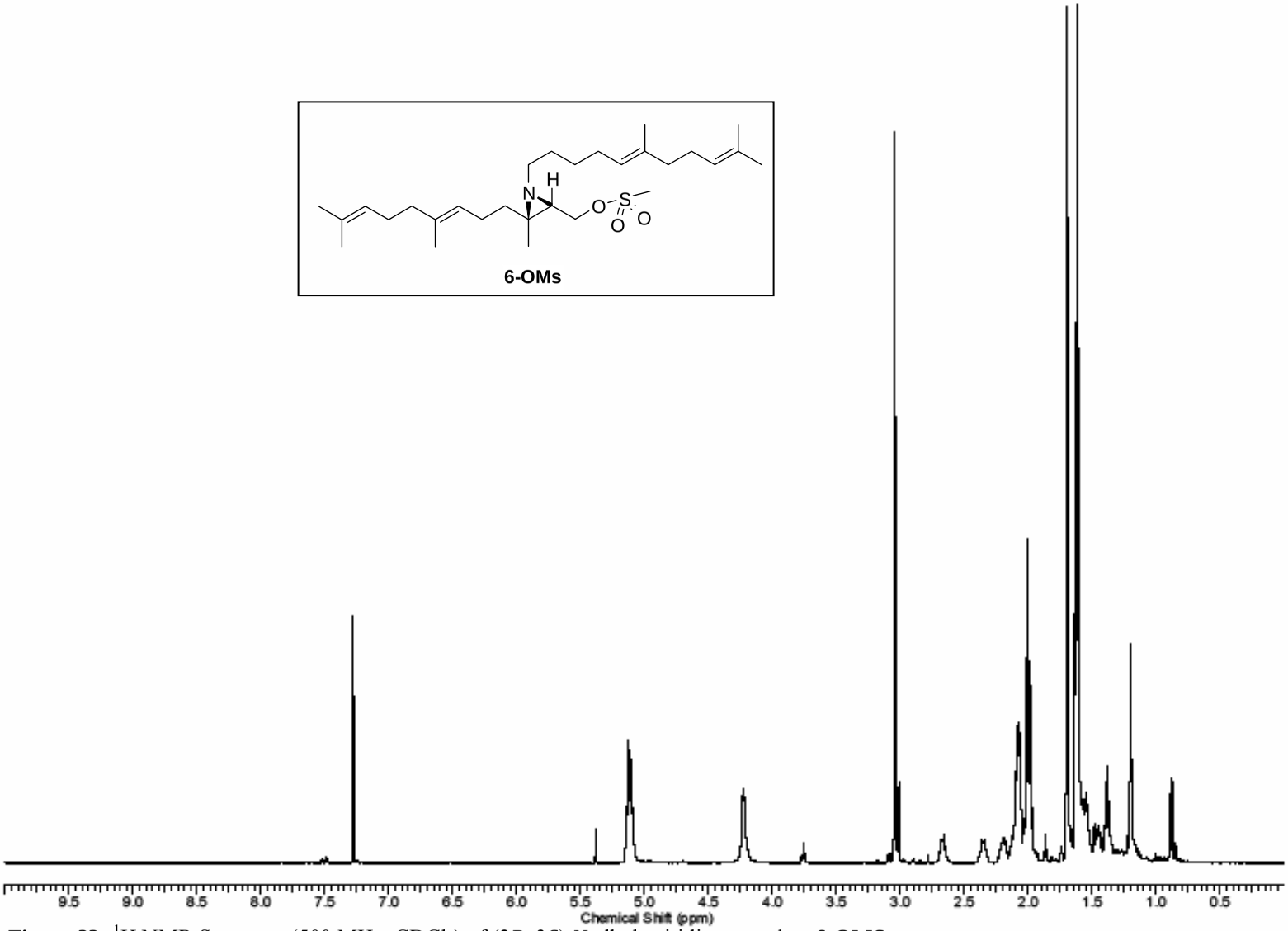


Figure 23: ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of (2*R*, 3*S*) *N*-alkyl aziridino mesylate **6-OMS.**

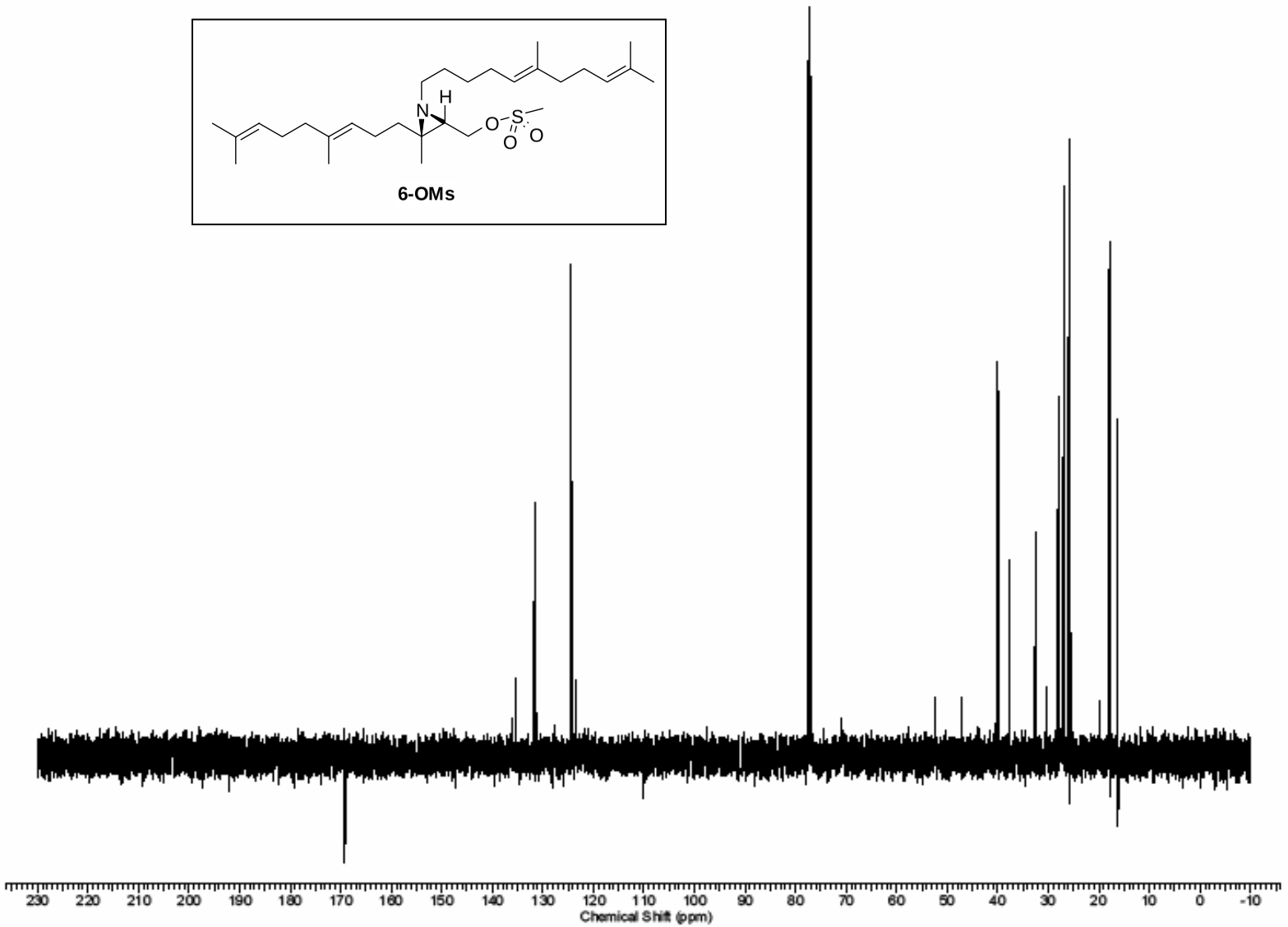


Figure 24: ^{13}C NMR Spectrum (126 MHz, $CDCl_3$) of (2*R*, 3*S*) *N*-alkyl aziridino mesylate **6-OMS.**

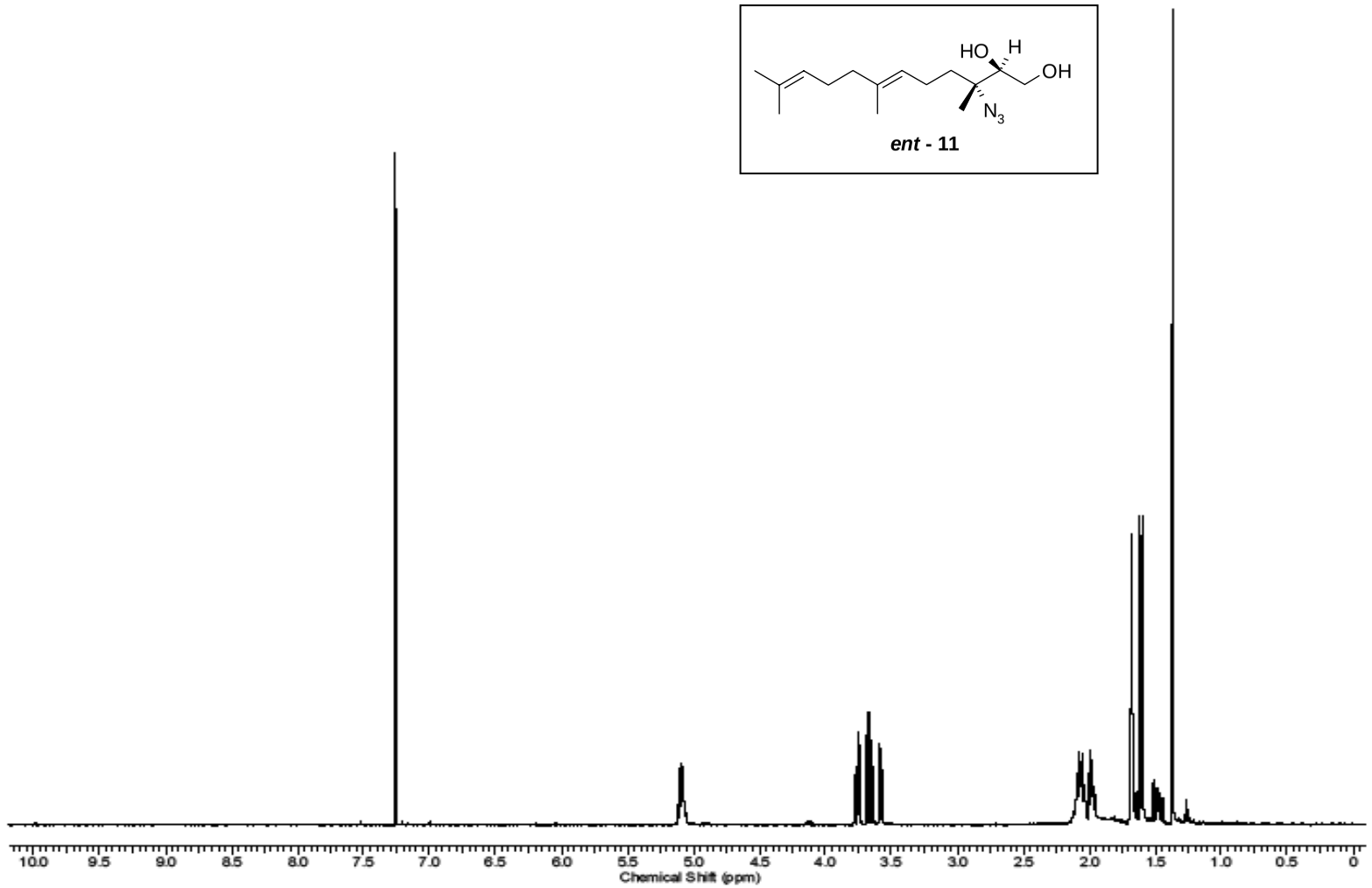


Figure 25: ^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of (2*R*, 3*R*) azido diol ***ent*-11.**

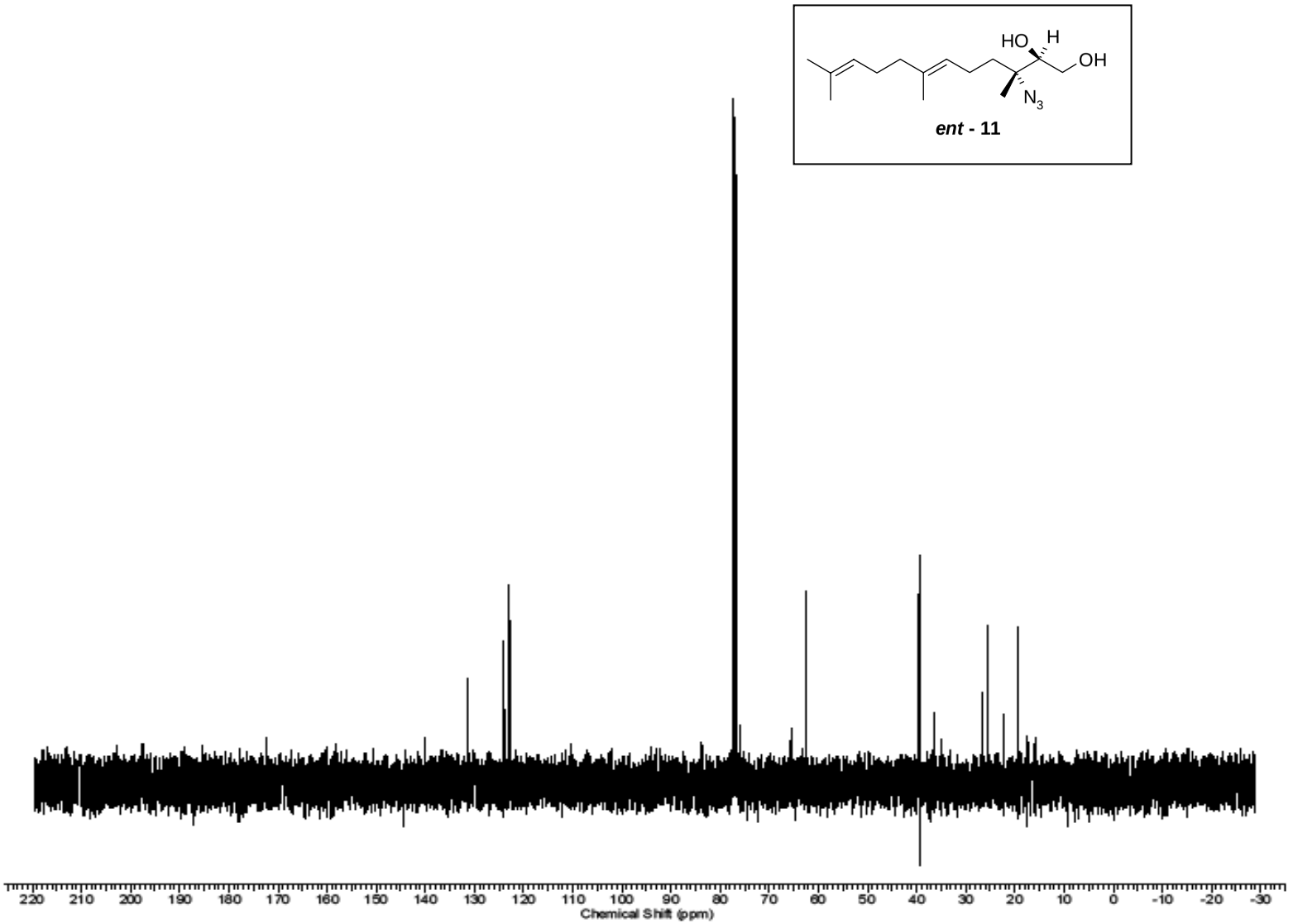


Figure 26: ^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of (2*R*, 3*R*) azido diol ***ent*-11.**

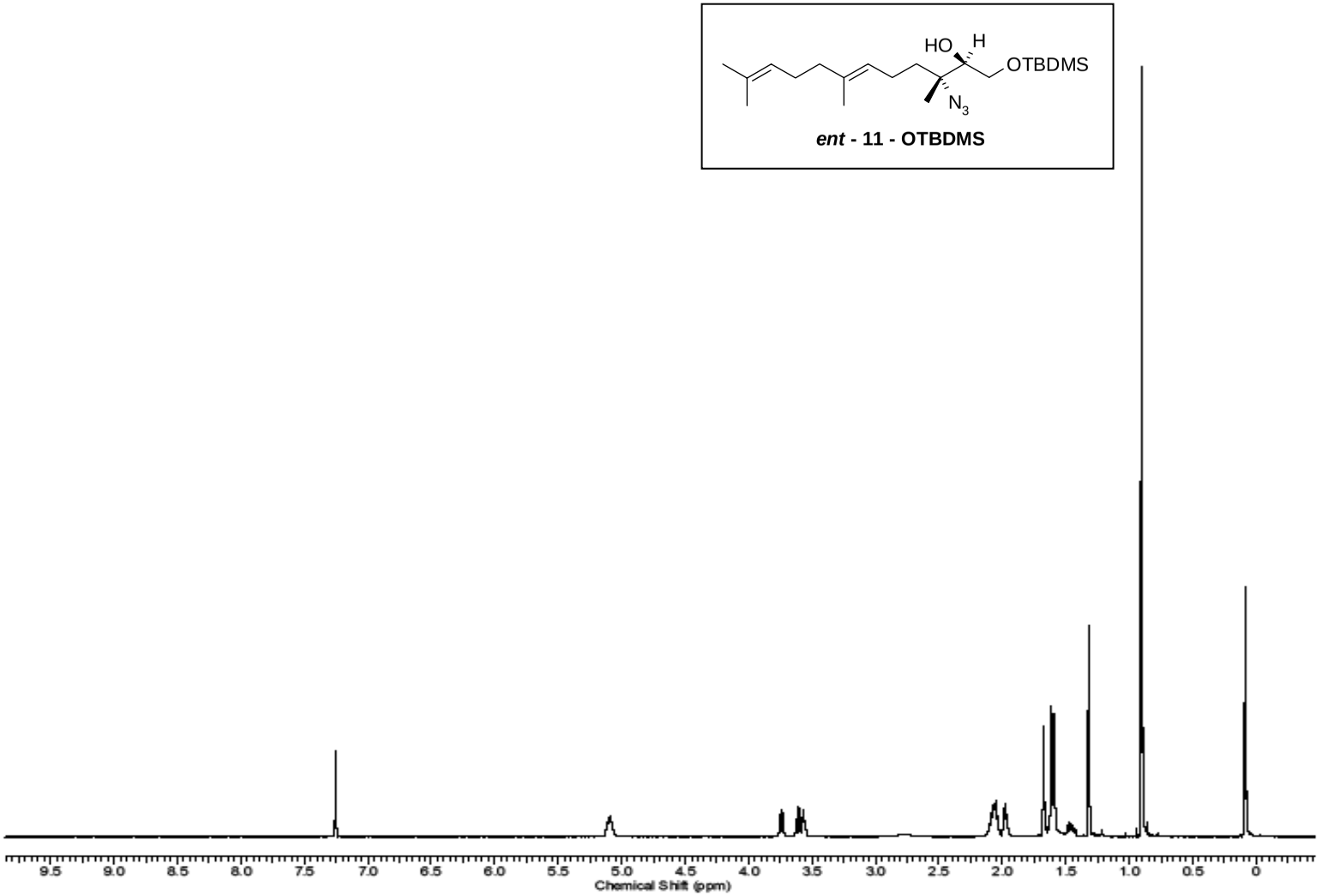


Figure 27: ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of (2*R*, 3*R*) silyl ether ***ent*-11-OTBDMS.**

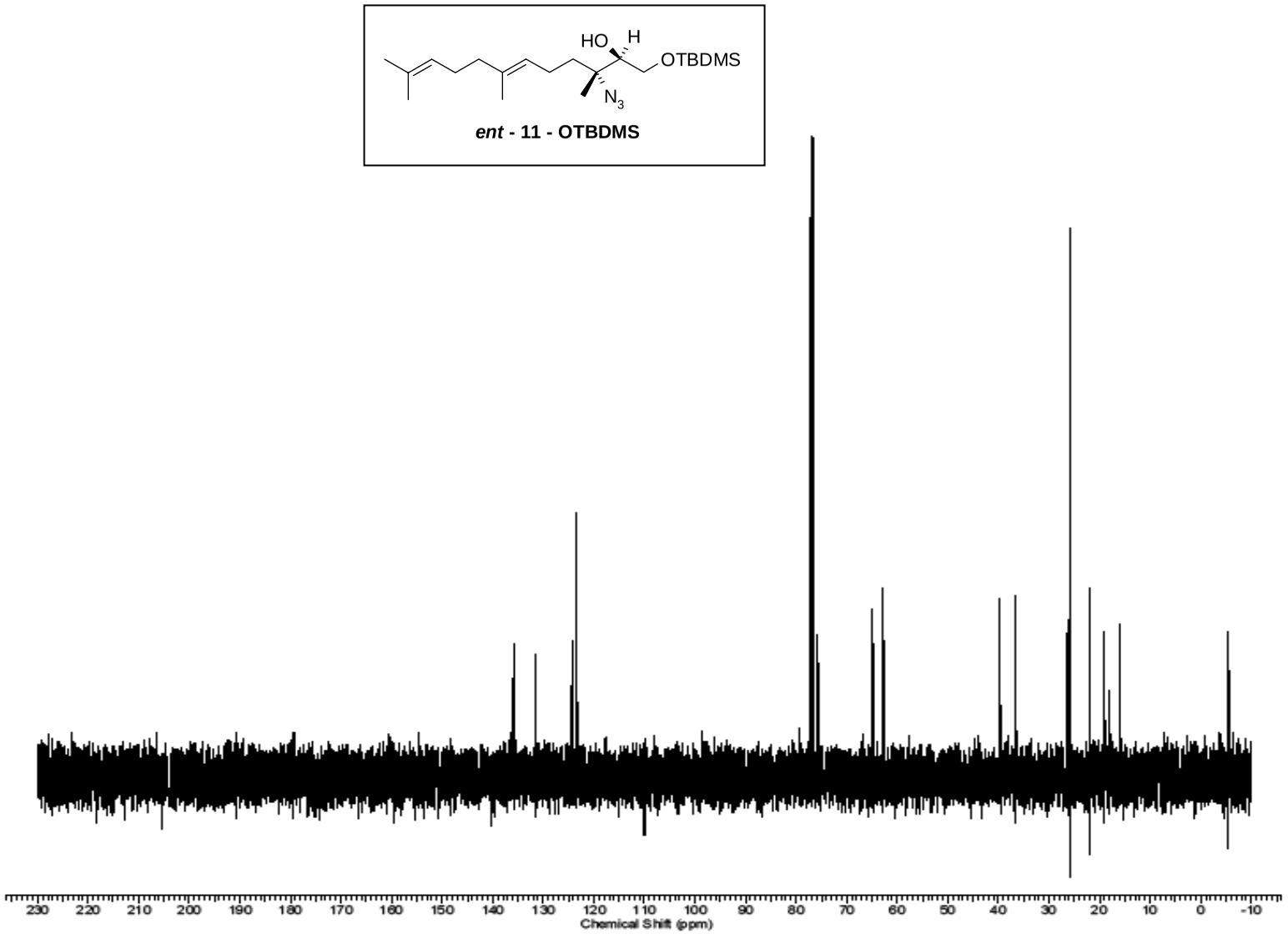


Figure 28: ^{13}C NMR Spectrum (126 MHz, $CDCl_3$) of (2*R*, 3*R*) silyl ether ***ent*-11-OTBDMS.**

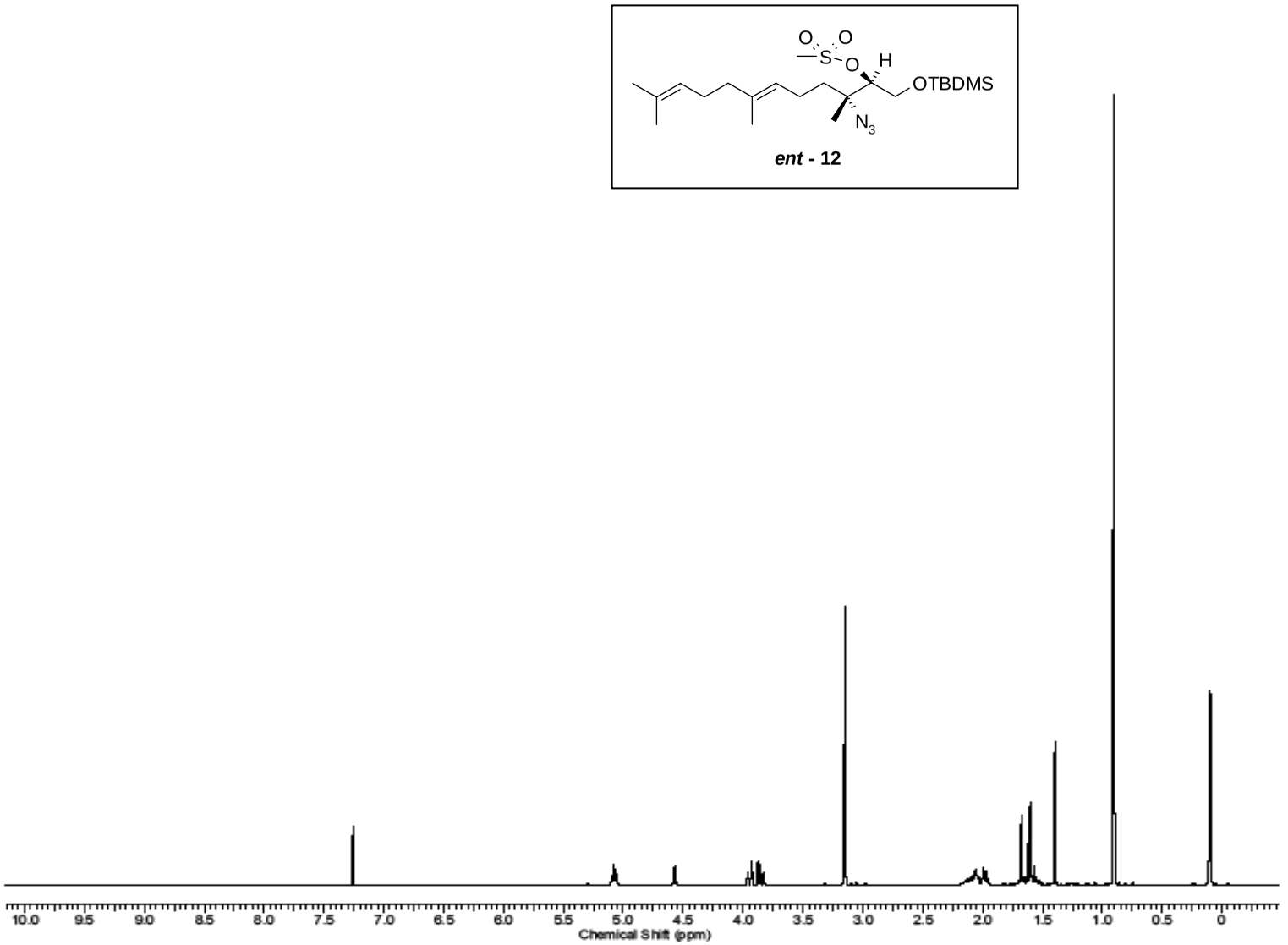


Figure 29: ^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of (2*R*, 3*R*) azido mesylate ***ent*-12.**

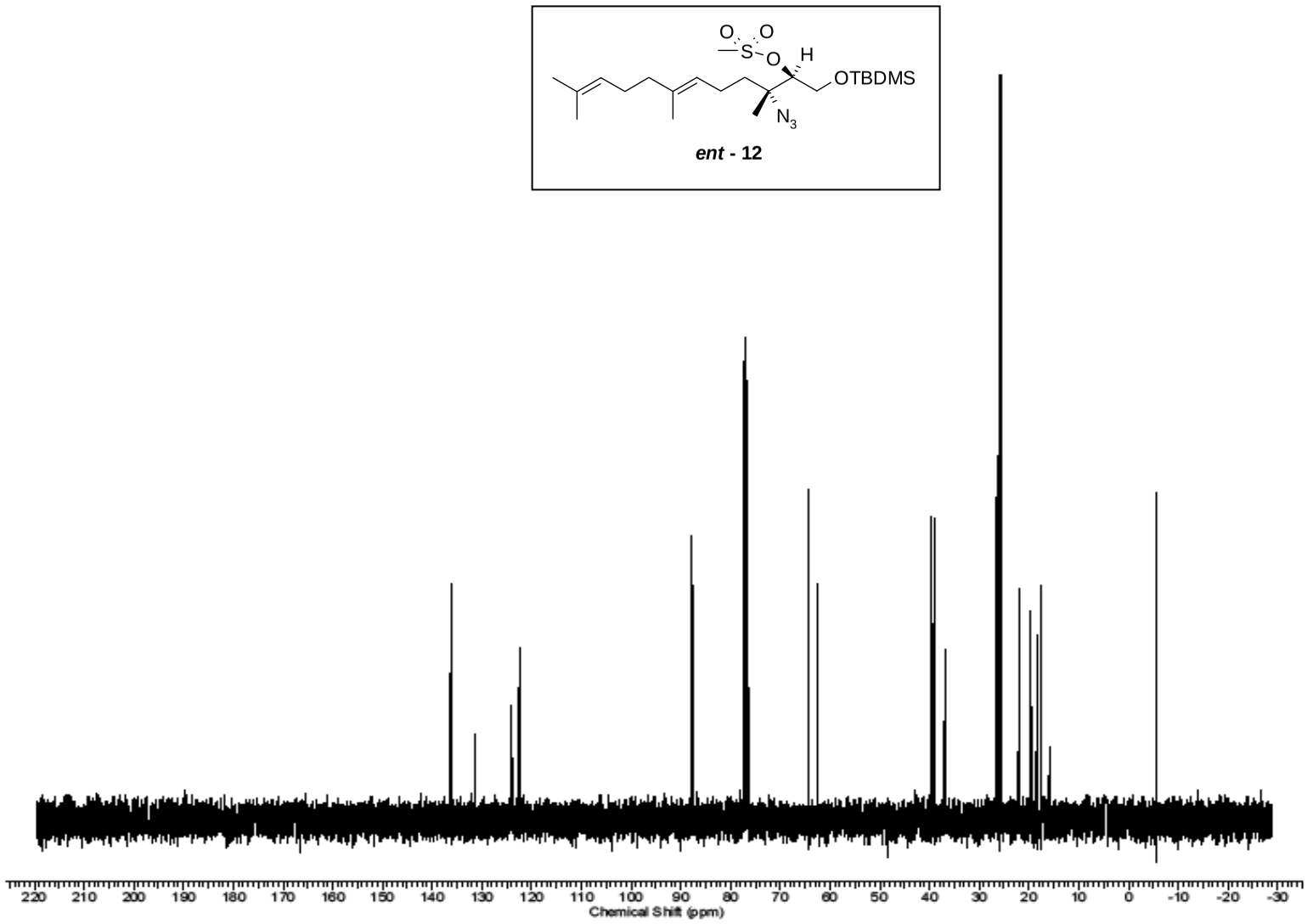


Figure 30: ^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of (2*R*, 3*R*) azido mesylate ***ent*-12.**

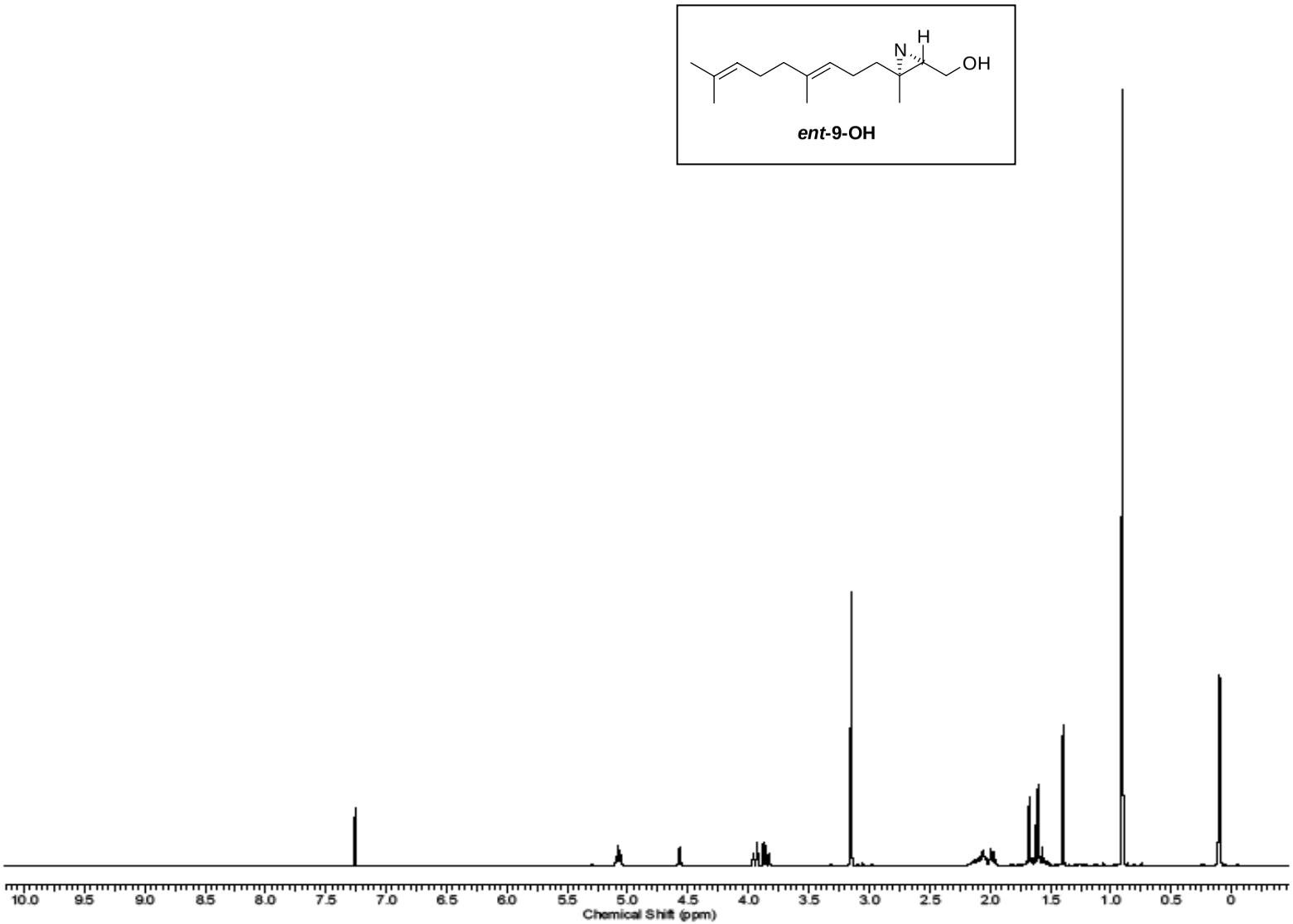


Figure 31: ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of (2*S*, 3*R*) aziridino alcohol ***ent*-9-OH.**

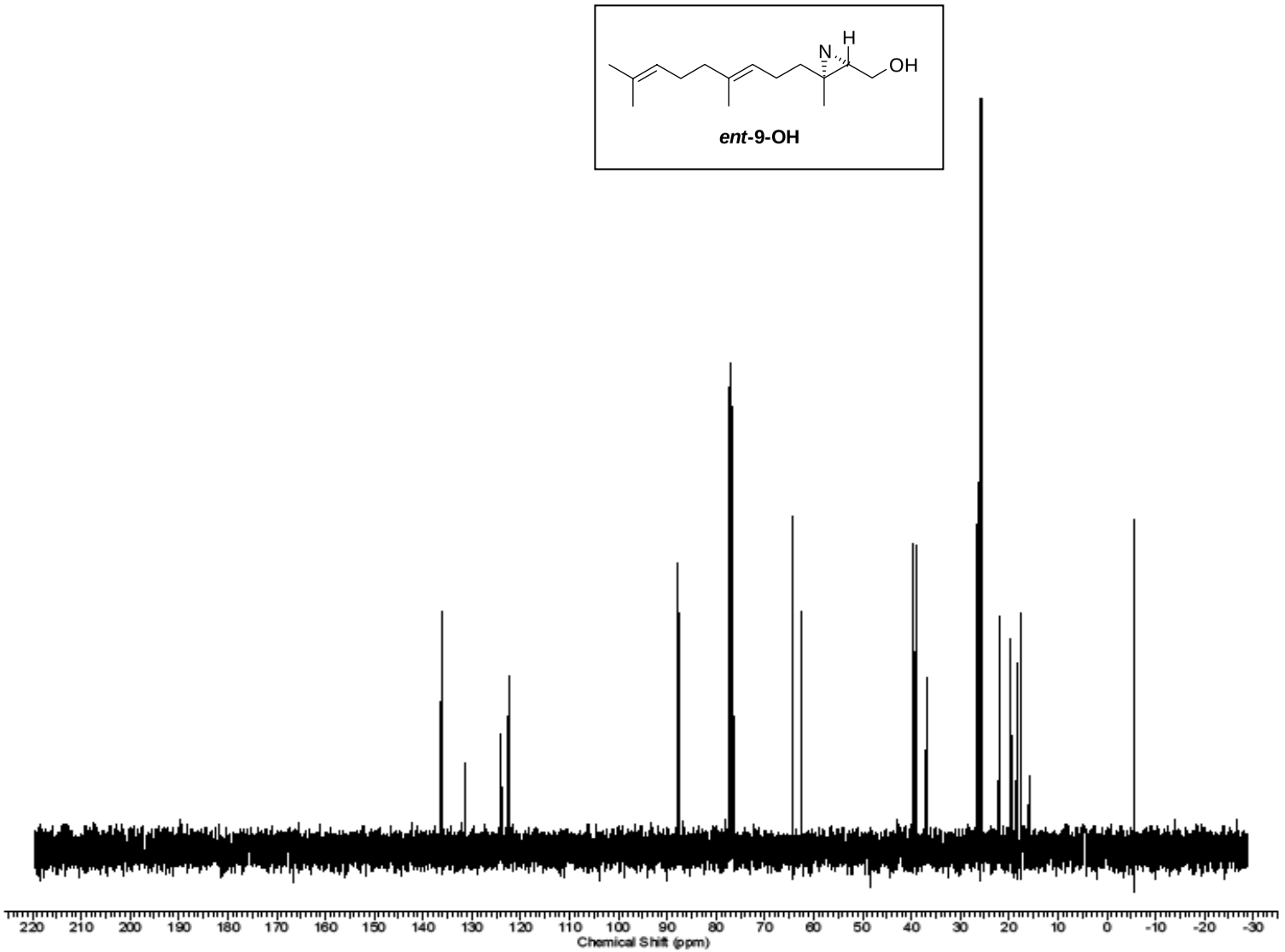


Figure 32: ^{13}C NMR Spectrum (126 MHz, $CDCl_3$) of (2*S*, 3*R*) aziridino alcohol ***ent*-9-OH.**

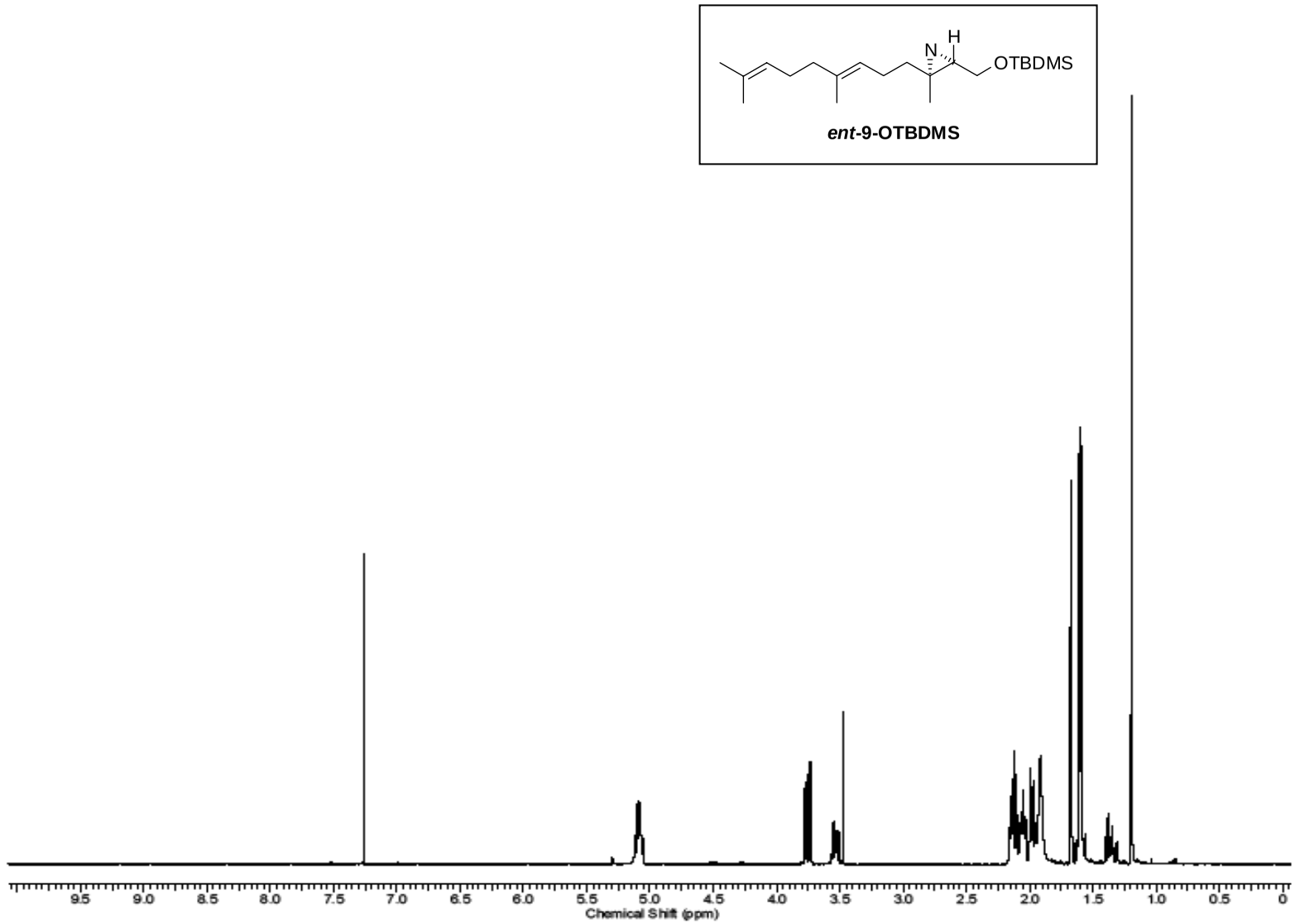


Figure 33: ^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of (2*S*, 3*R*) aziridine ***ent*-9-OTBDMS.**

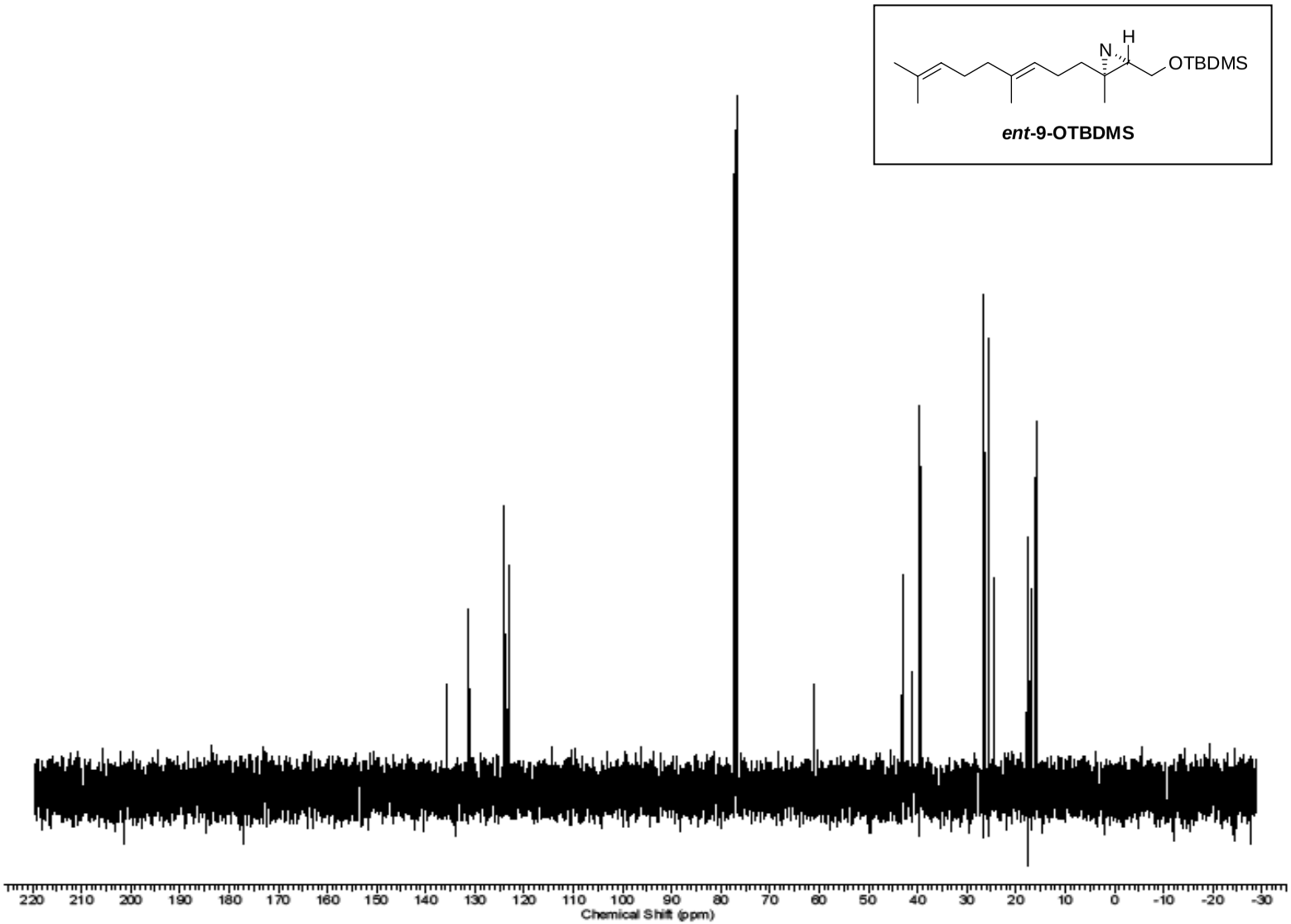


Figure 34: ^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of (2*S*, 3*R*) aziridine ***ent*-9-OTBDMS.**

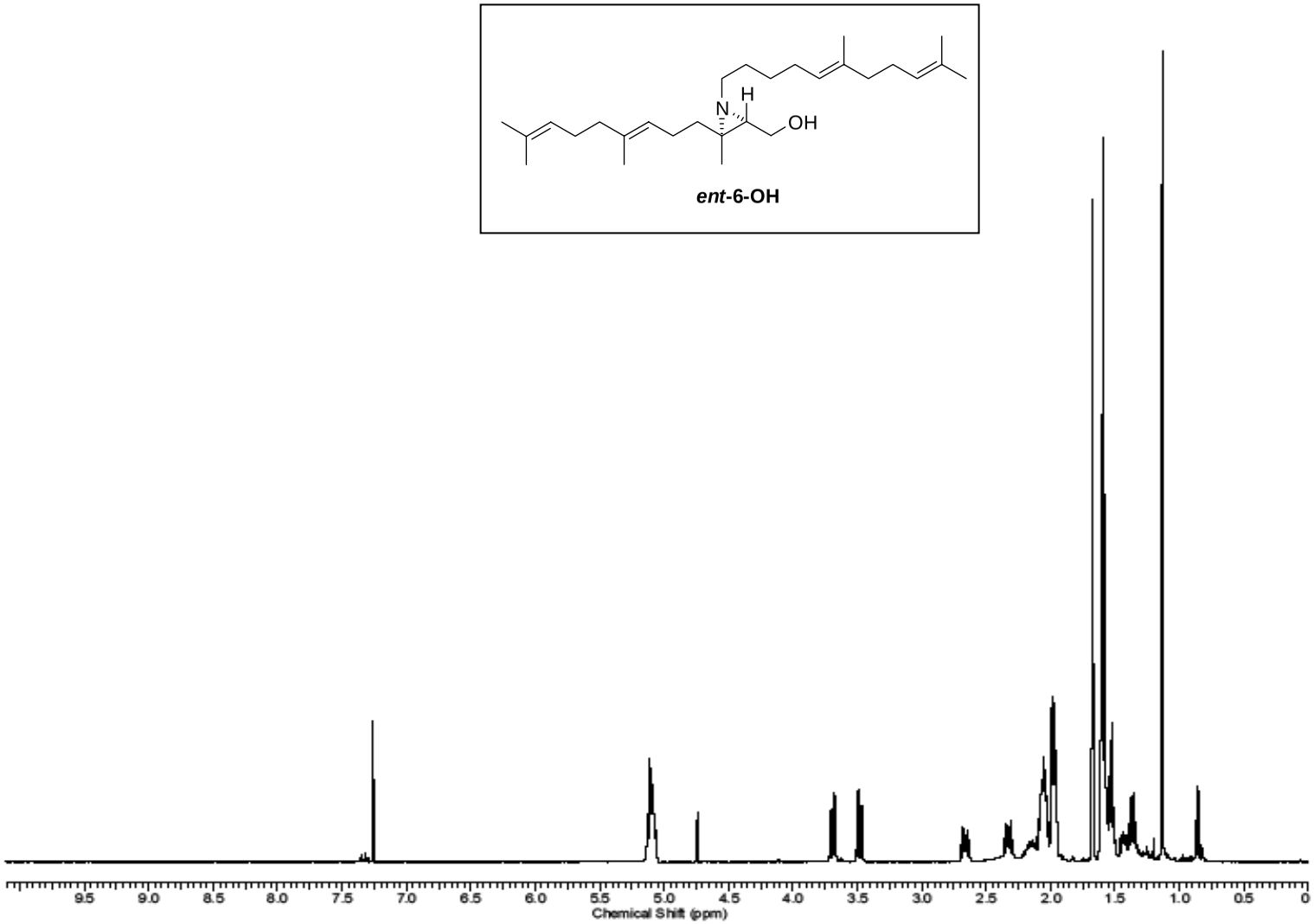


Figure 35: ^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of (2*S*, 3*R*) *N*-alkyl aziridino alcohol ***ent*-6-OH.**

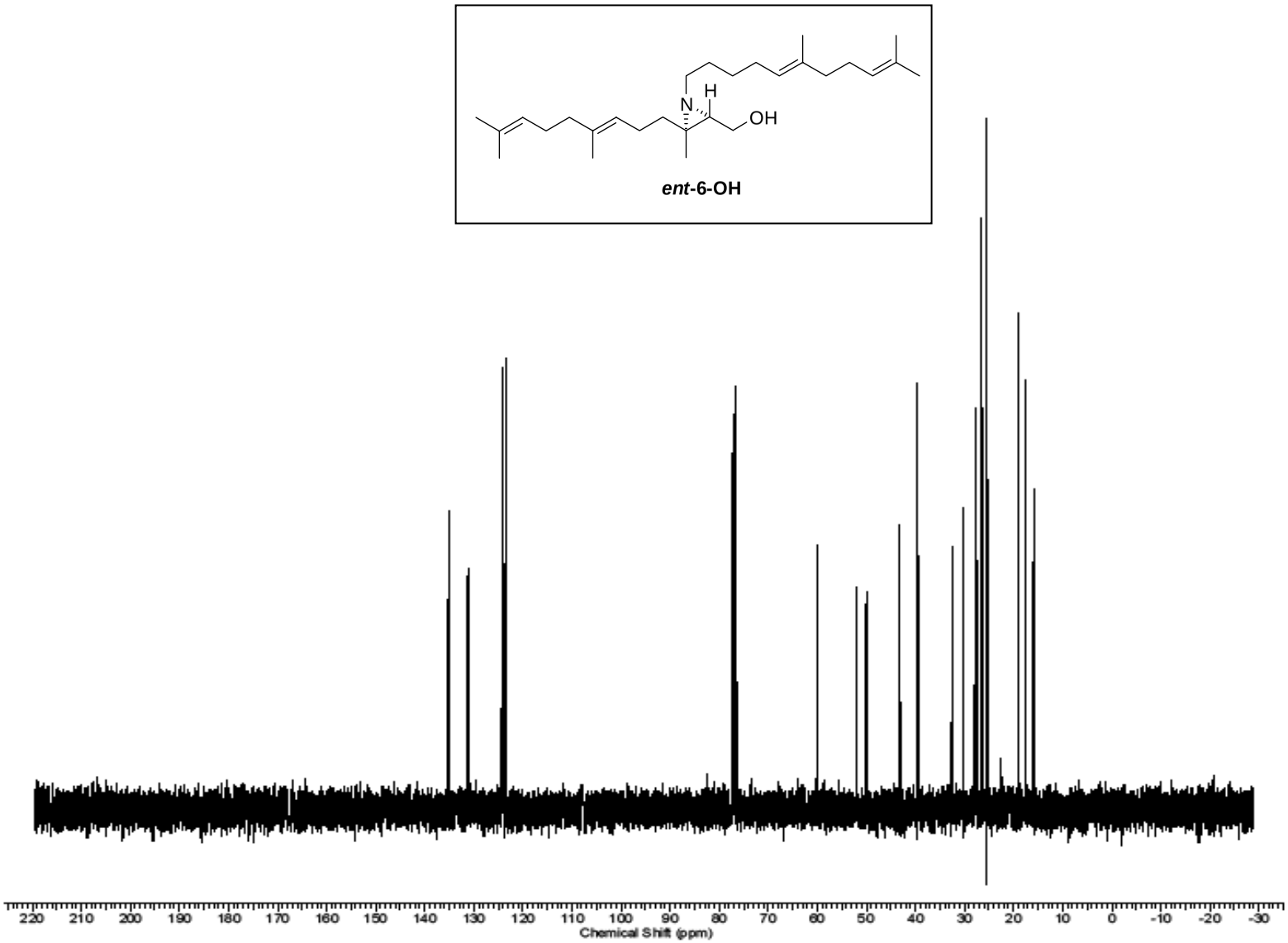


Figure 36: ^{13}C NMR Spectrum (101 MHz, $CDCl_3$) of (2*S*, 3*R*) *N*-alkyl aziridino alcohol ***ent*-6-OH.**

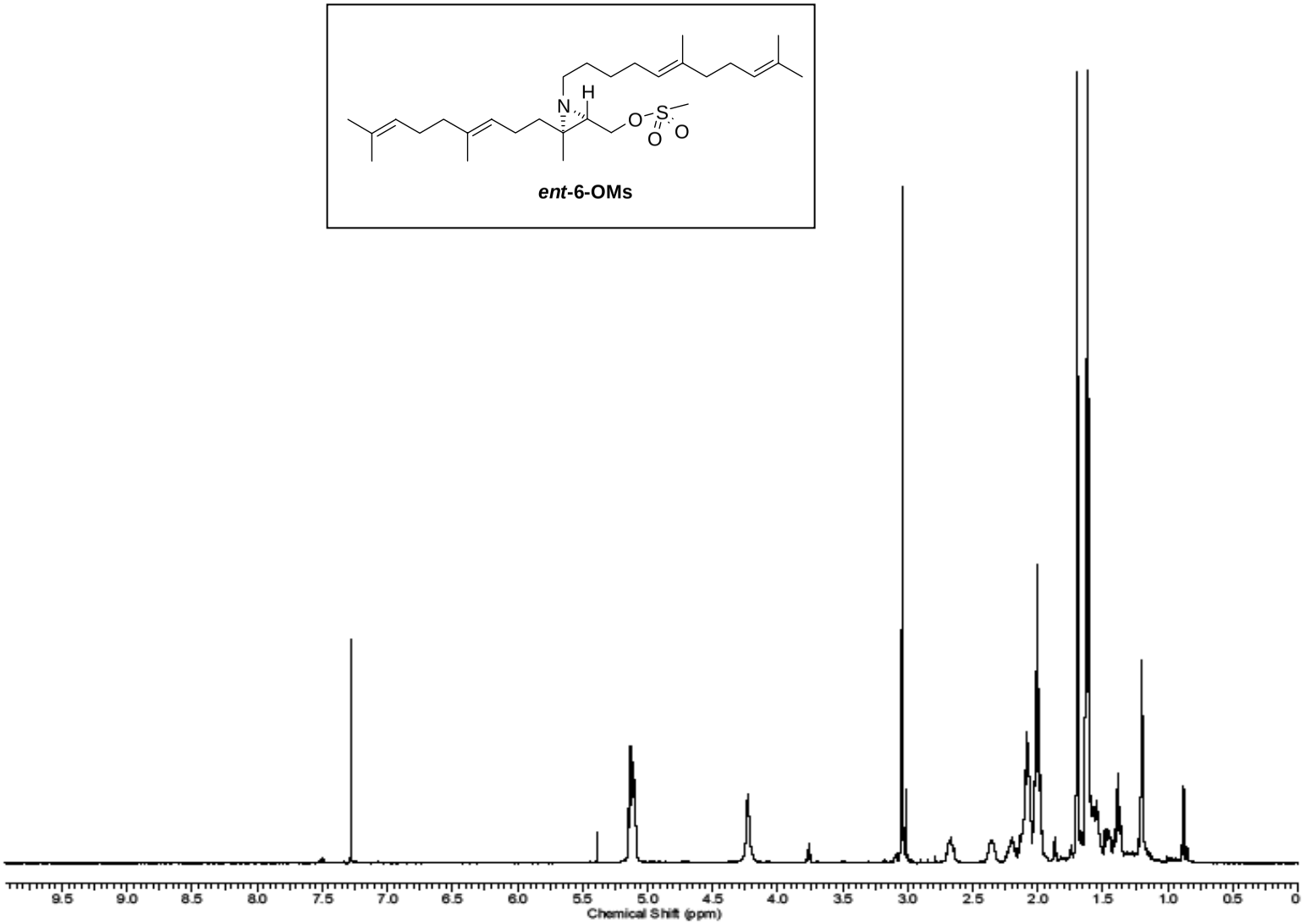


Figure 37: ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of (2*S*, 3*R*) *N*-alkyl aziridino mesylate ***ent*-6-OMs.**

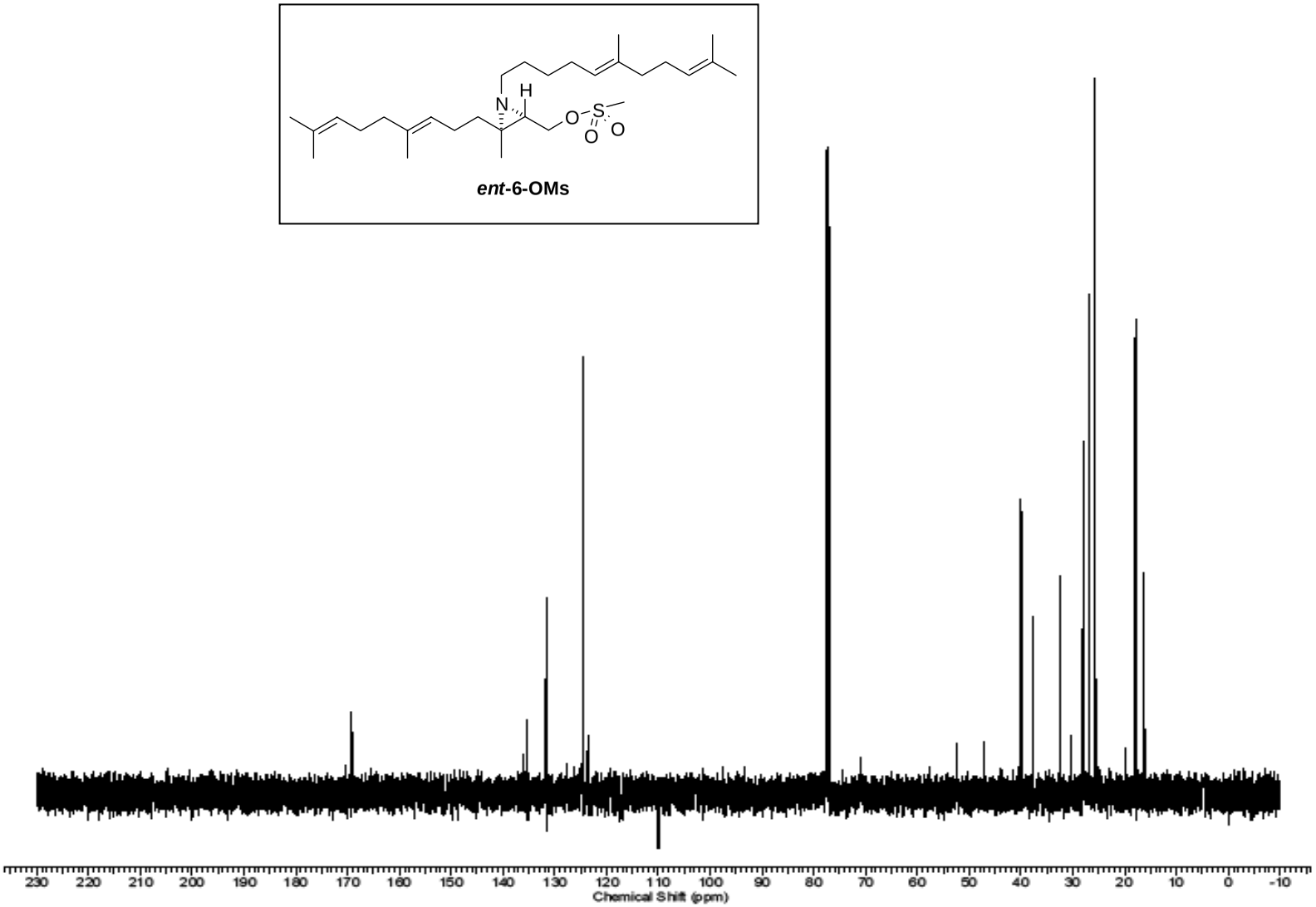
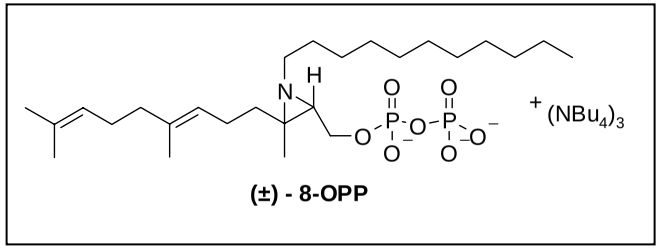


Figure 38: ^{13}C NMR Spectrum (126 MHz, $CDCl_3$) of (2*S*, 3*R*) *N*-alkyl aziridino mesylate ***ent*-6-OMs.**



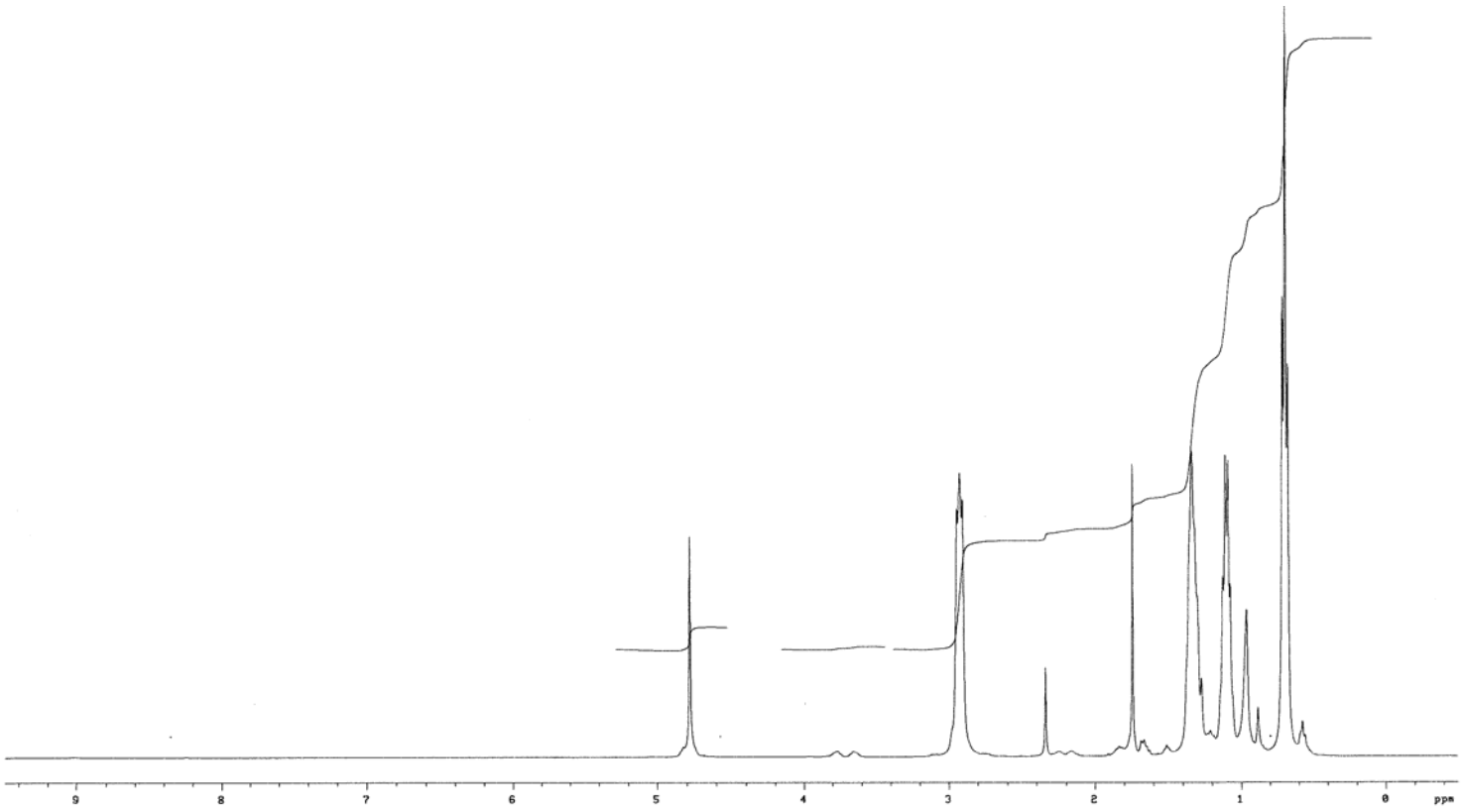


Figure 39: ^{1}H NMR Spectum **(**400 MHz, CD_3OD) of *N*-undecyl aziridino diphosphate **(±)-8-OPP.**

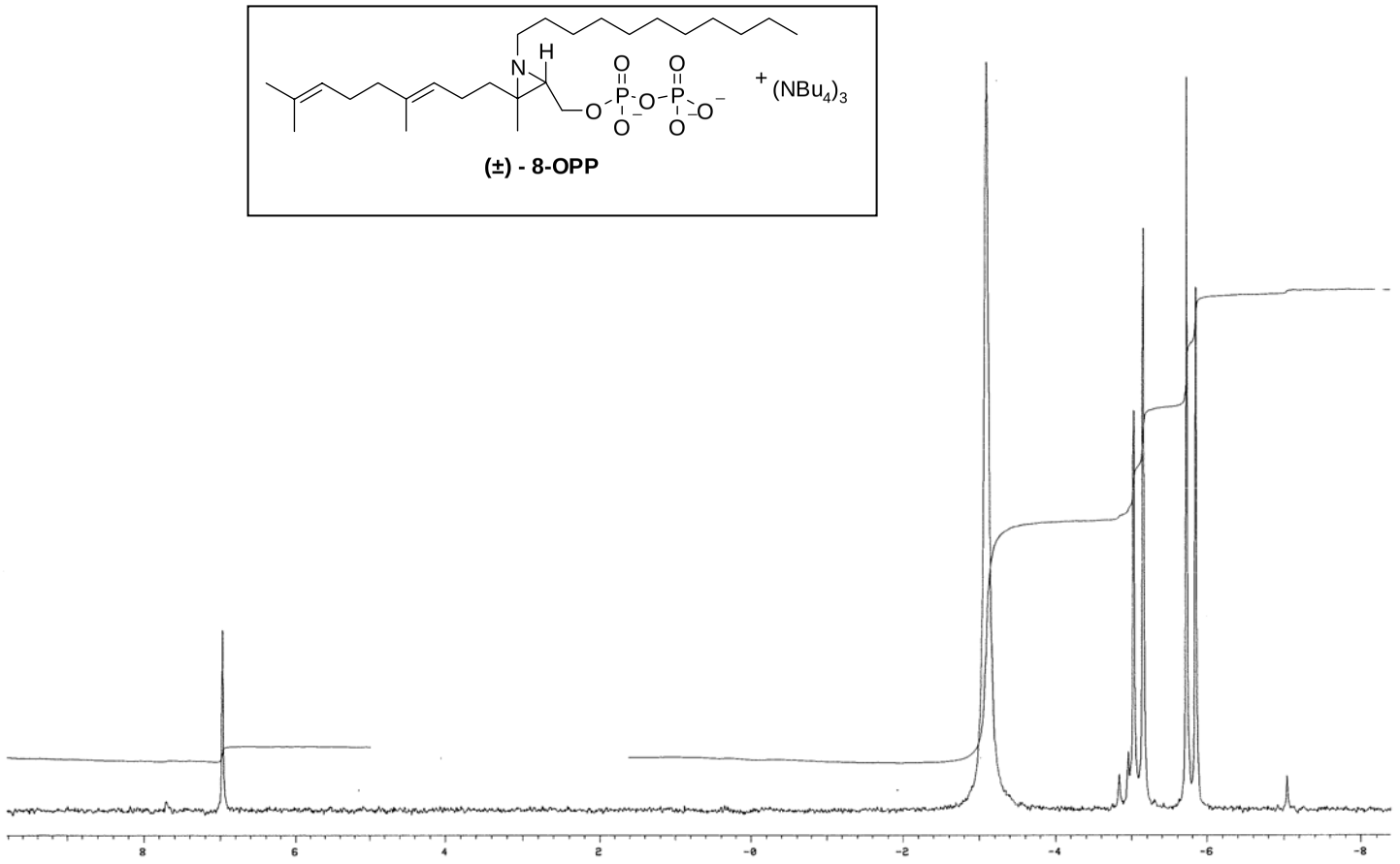


Figure 40: ^{31}P NMR Spectrum (162 MHz, CD_3OD) of *N*-undecyl aziridino diphosphate **(±)-8-OPP.**

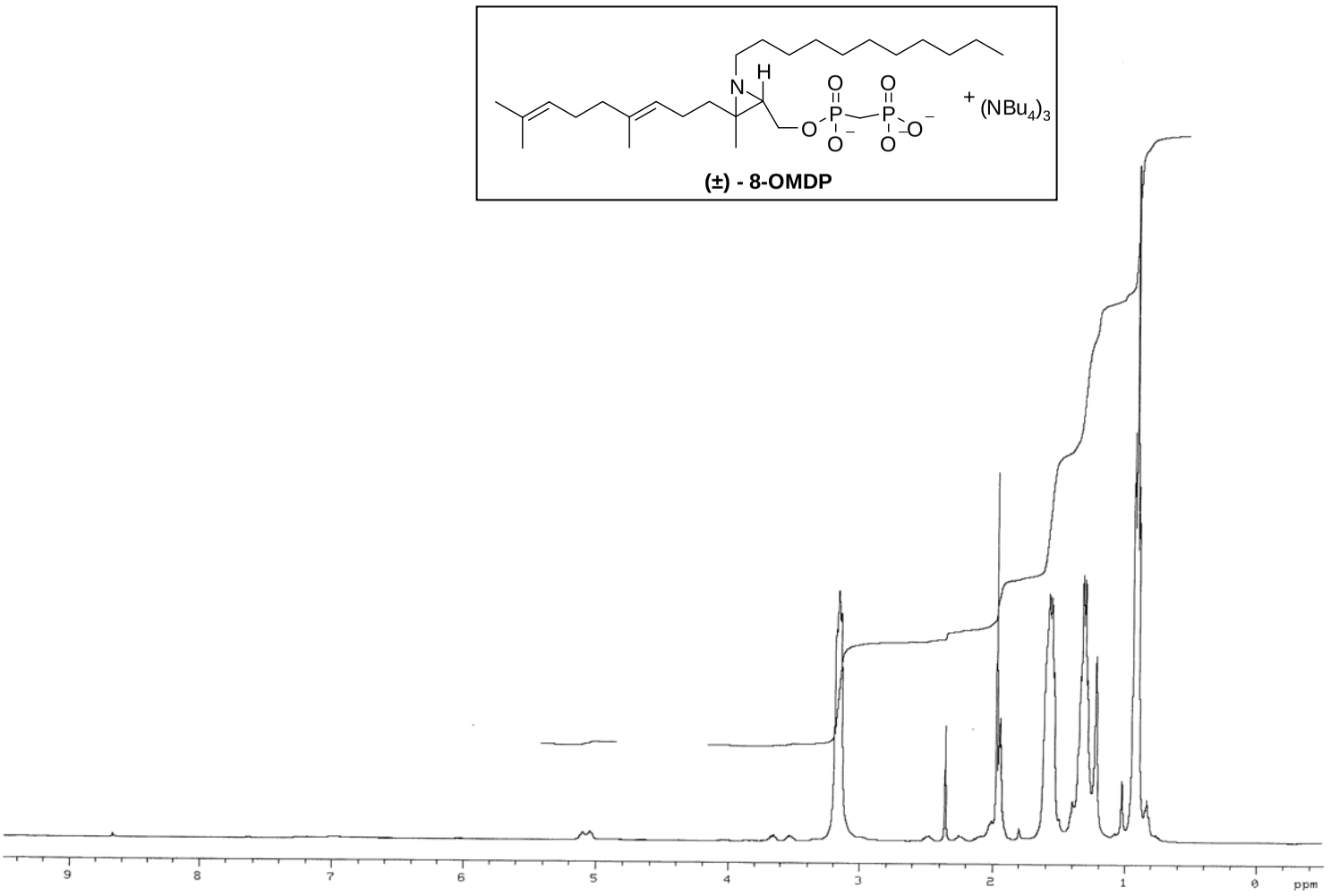


Figure 41: ^{1}H NMR Spectum **(**400 MHz, CD_3OD) of *N*-undecyl aziridino methanediphosphonate **(±)-8-OMDP.**

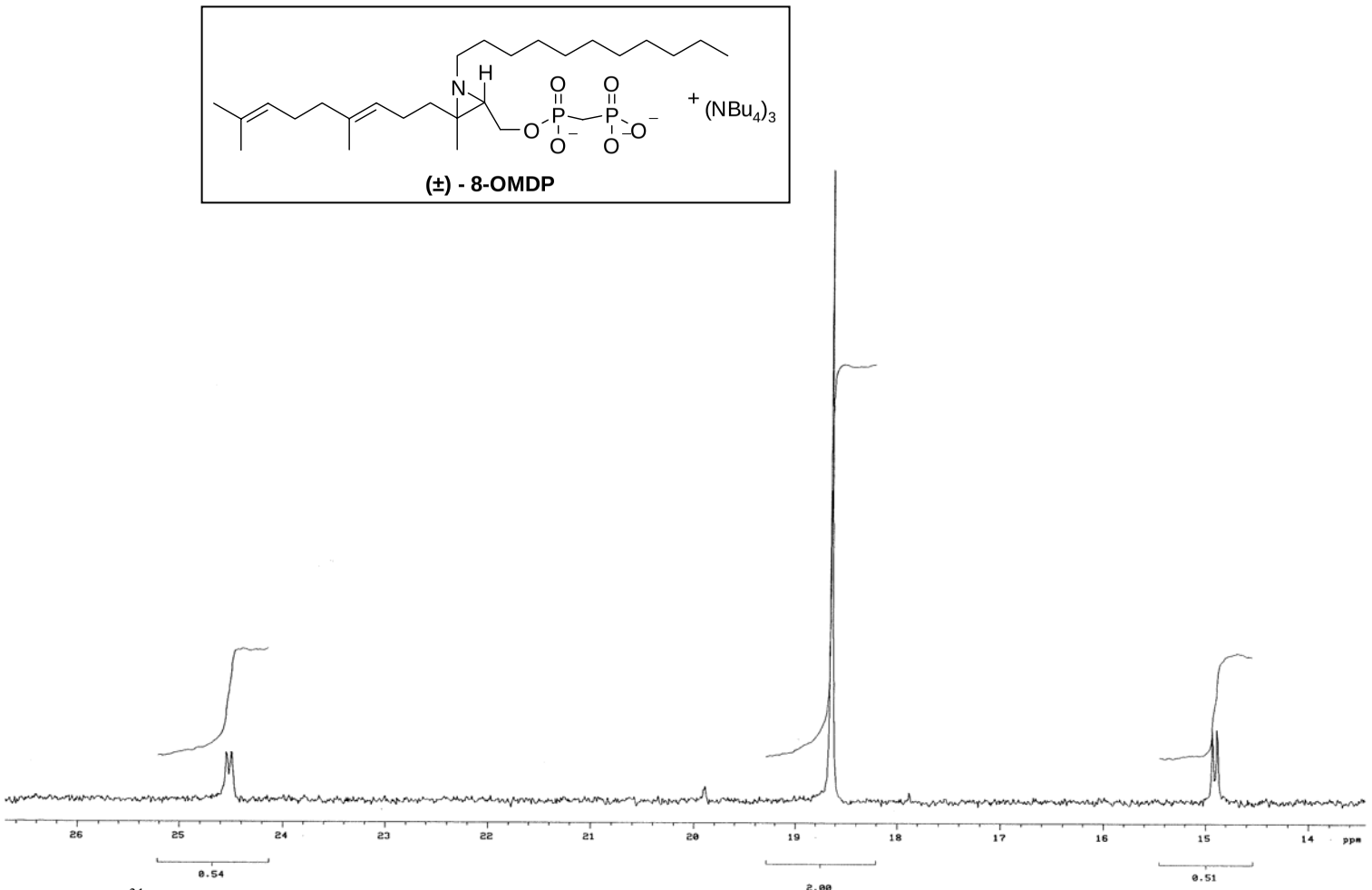


Figure 42: ^{31}P NMR Spectrum (162 MHz, CD_3OD) of *N*-undecyl aziridino methanediphosphonate **(±)-8-OMDP.**

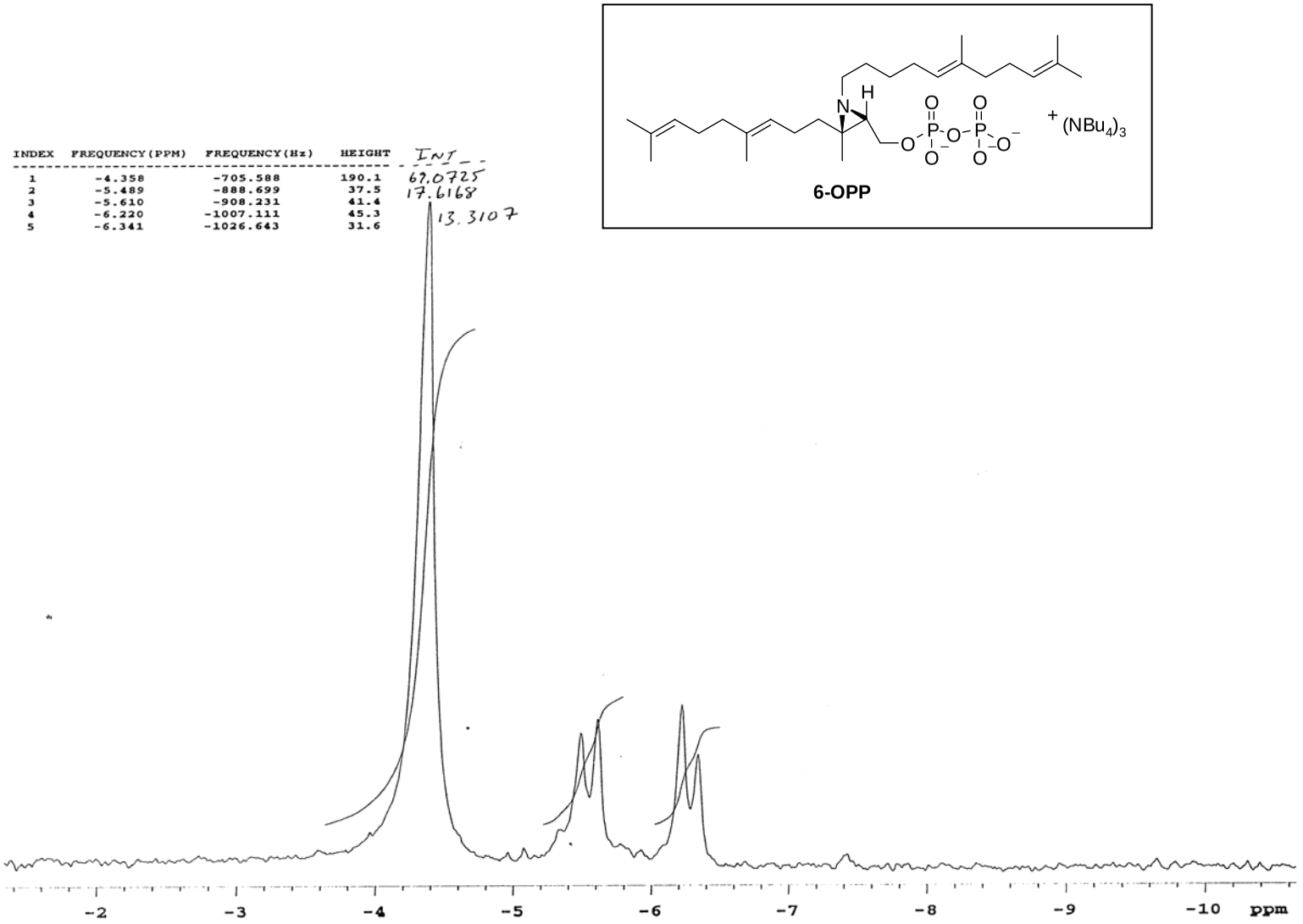


Figure 43: ^{31}P NMR Spectrum (162 MHz, CD_3OD) of (2*R*, 3*S*) *N*-alkyl aziridino diphosphate **6-OPP.**

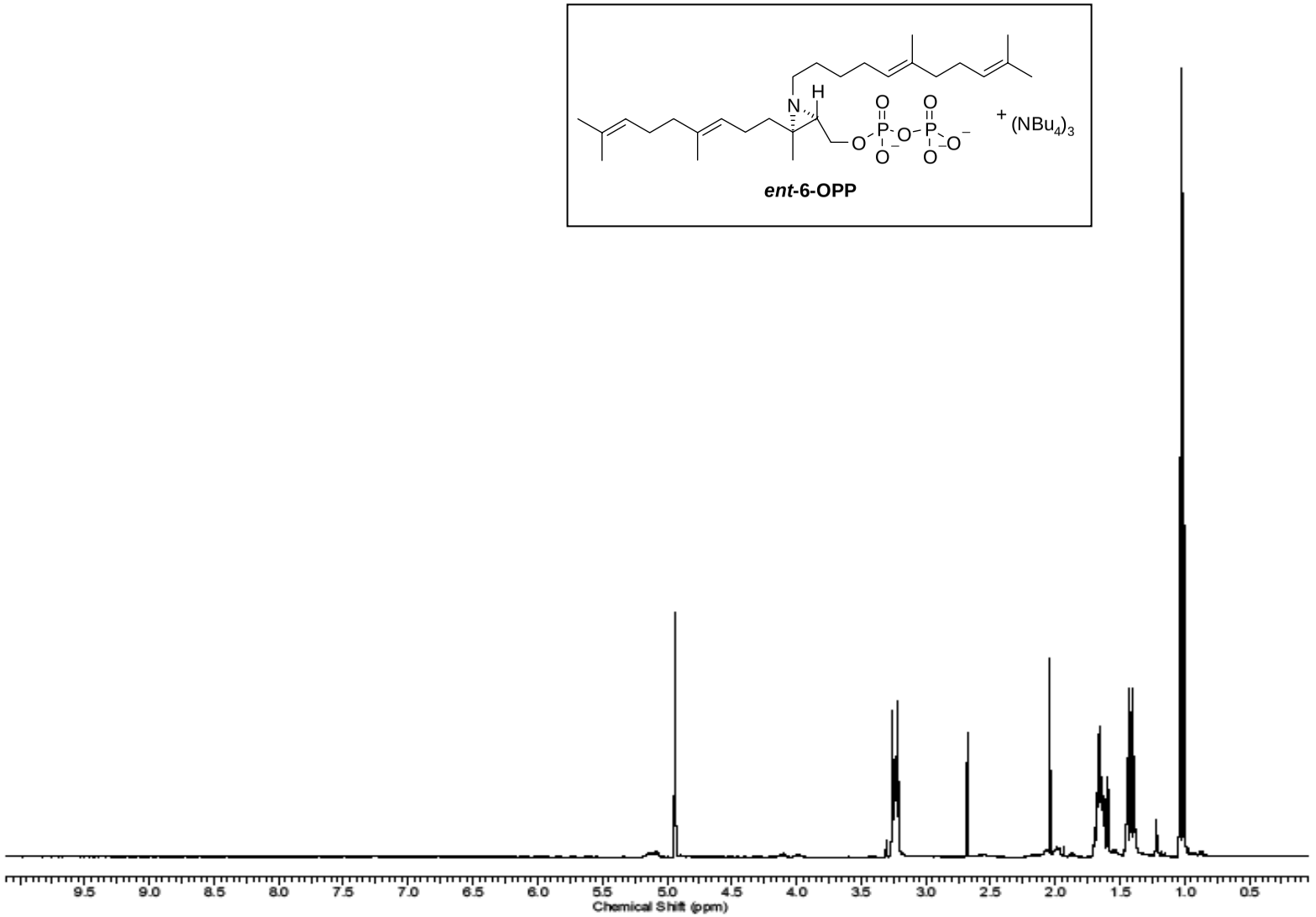


Figure 44: ^{1}H NMR Spectum **(**400 MHz, CD_3OD) of (2*S*, 3*R*) *N*-alkyl aziridino diphosphate ***ent*-6-OPP.**

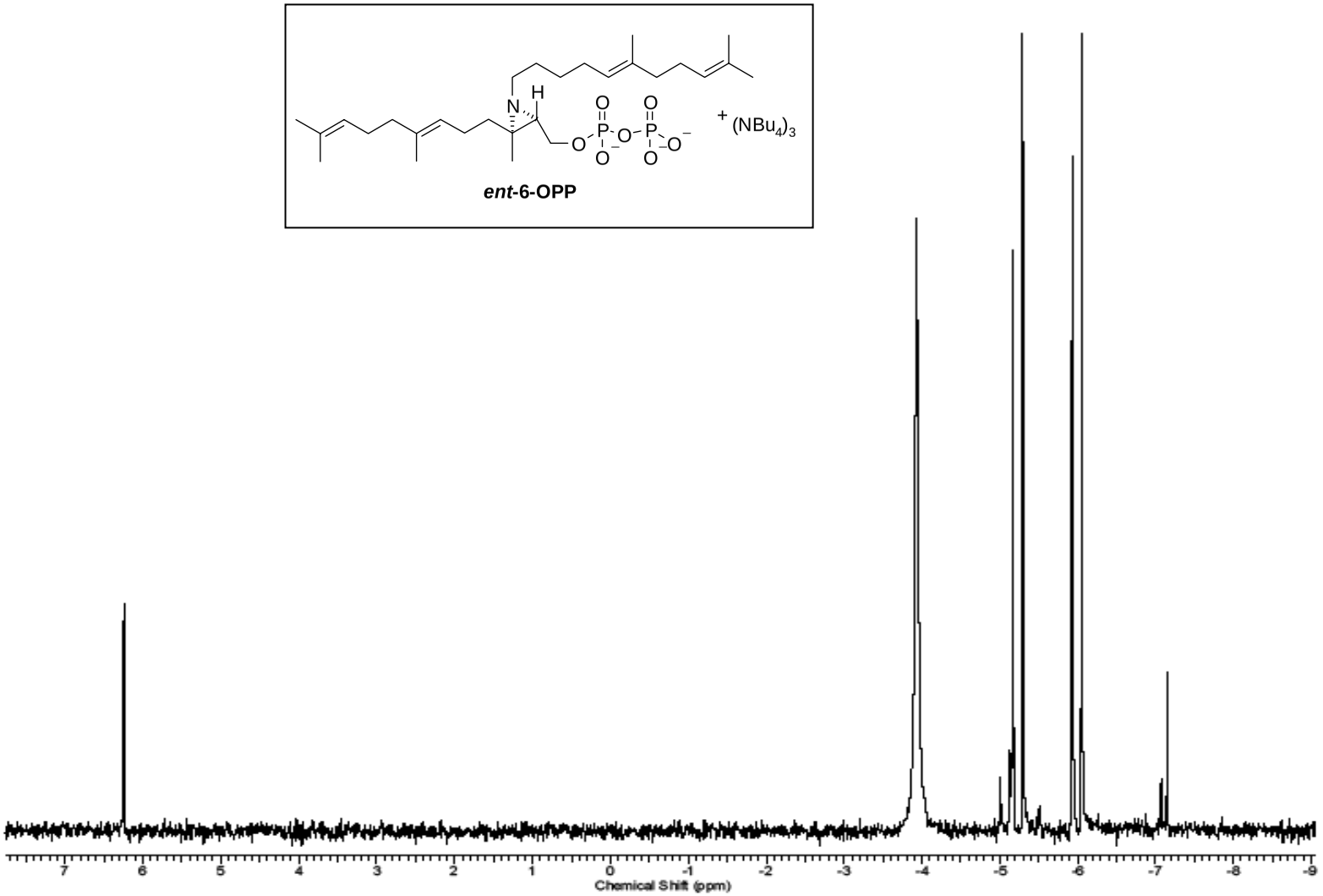


Figure 45: ^{31}P NMR Spectrum (162 MHz, CD_3OD) of (2*S*, 3*R*) *N*-alkyl aziridino diphosphate ***ent*-6-OPP.**

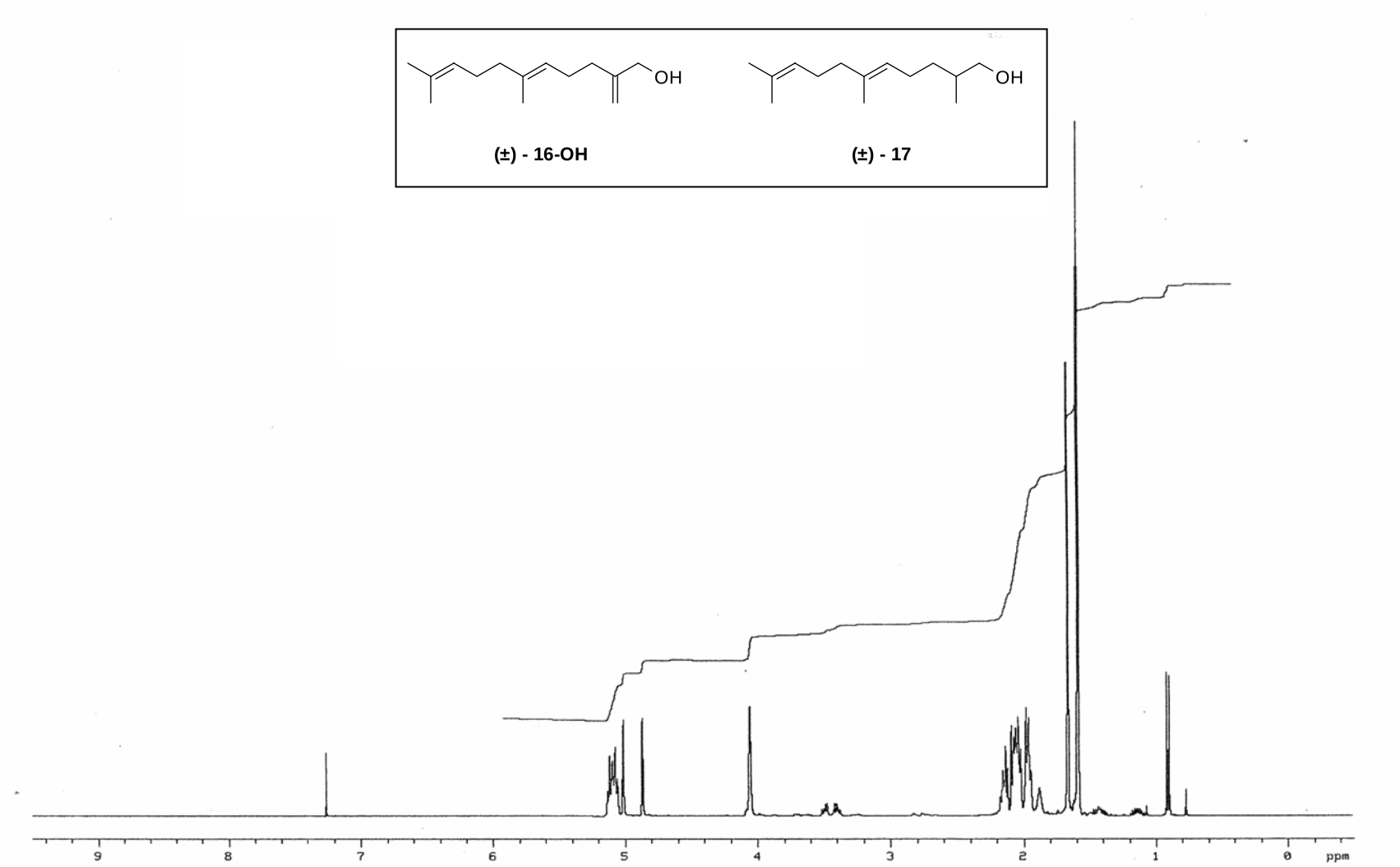


Figure 46: ^{1}H NMR Spectrum (400 MHz, $CDCl_3$) of alcohols **(±)-16-OH** and **(±)-17.**

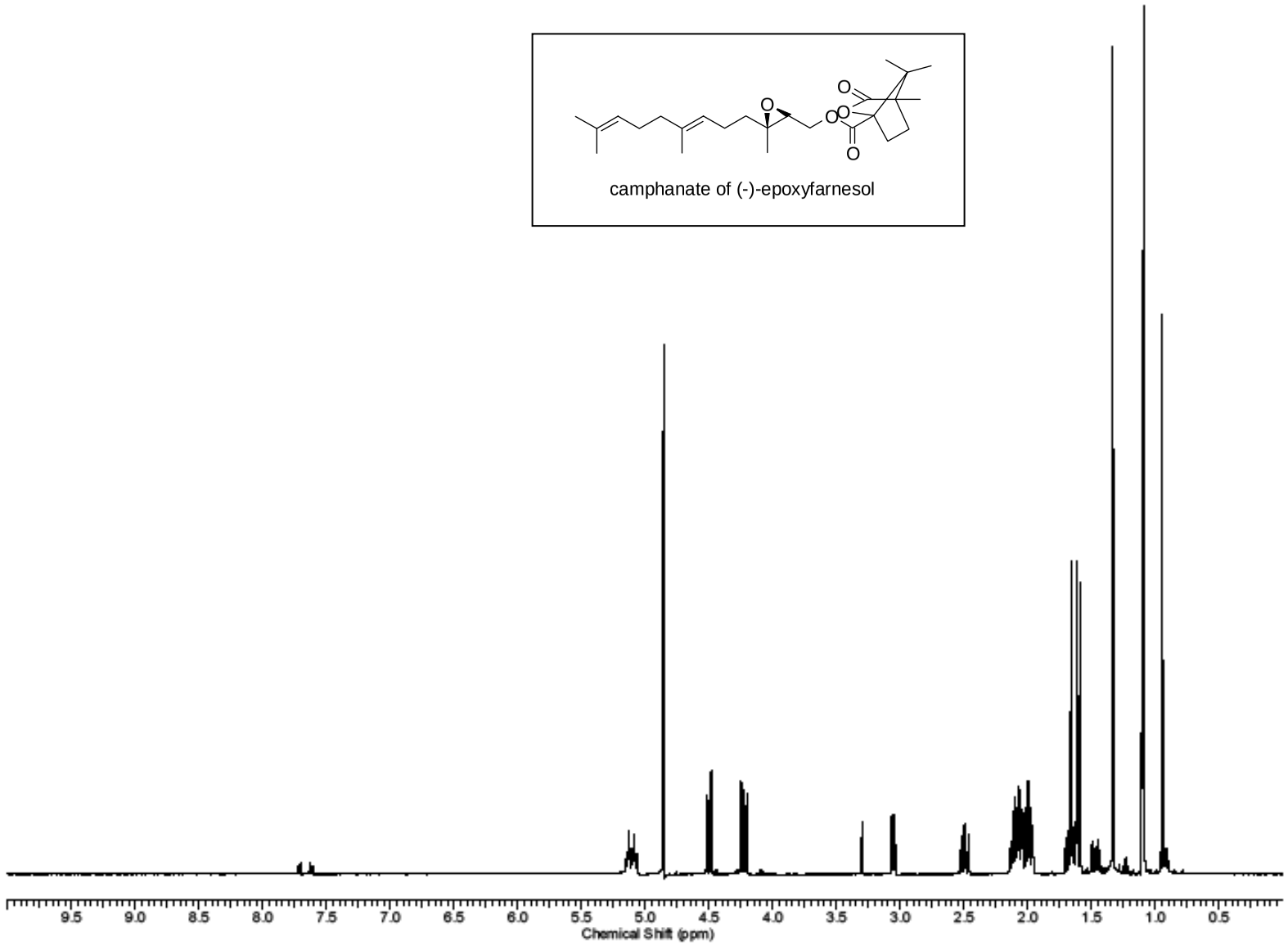


Figure 47: ^{1}H NMR Spectum **(**500 MHz, CD_3OD) the camphanate of **(-)-expoxyfarnesol.**

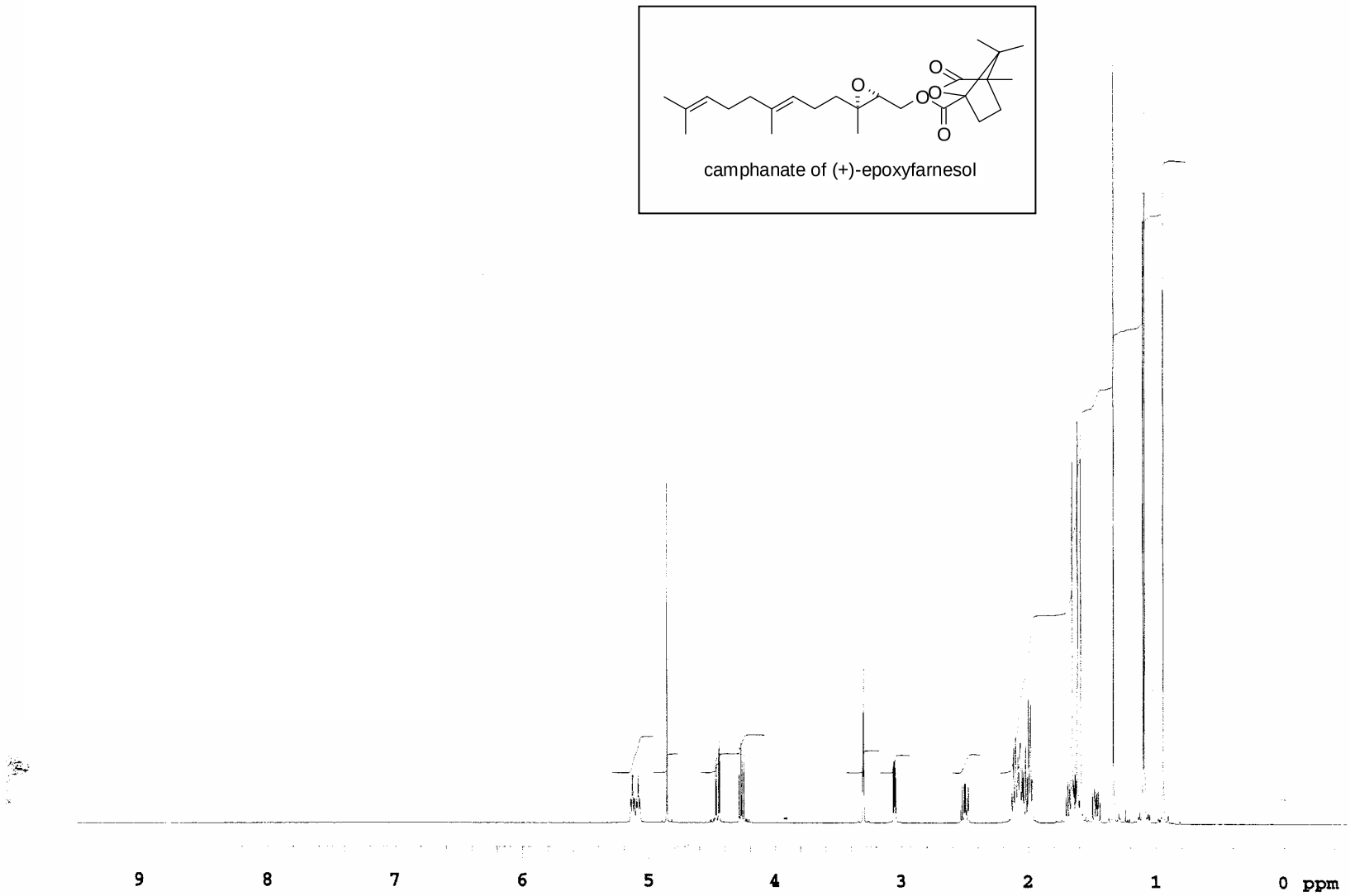


Figure 48: ^{1}H NMR Spectum **(**400 MHz, CD_3OD) the camphanate of **(+)-epoxyfarnesol.**

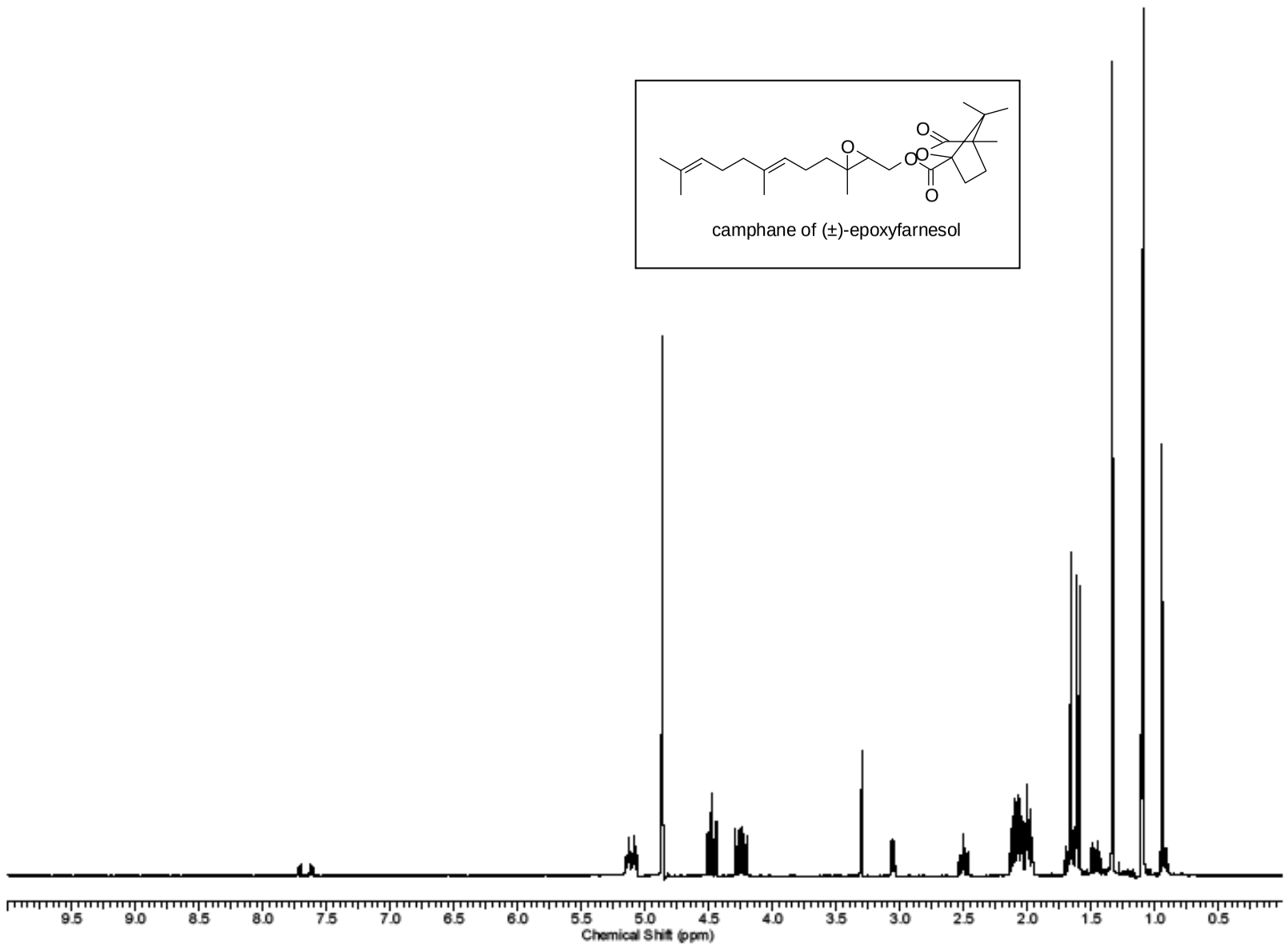


Figure 49: ^{1}H NMR Spectum **(**400 MHz, CD_3OD) the camphanate of **(±)-epoxyfarnesol.**