Supporting Information for:

A Practical Entry to β–Aryl-β-Alkyl Aminoalcohols: Application to the Synthesis of a Potent BACE1 Inhibitor

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Content

1. General Procedures

All reactions involving moisture-sensitive chemicals were carried out in flame-dried glassware with magnetic stirring under nitrogen. Column chromatography refers to flash chromatography and was performed on silica gel 60, 230-400 mesh. Flash chromatography was also performed on silica gel using automated purification system with the indicated eluent. High resolution mass spectra were recorded on a mass spectrometer configured with an electrospray ionization source, maintained at 140 ºC, using nitrogen as the nebulizer gas and Lockmass device for mass calibration using Leucine-Enkephaline as standard substance. Spectra were acquired in positive ionization mode by scanning from 100 to 750 in 0.5 s using a dwell time of 0.1 s. The capillary needle voltage was 2.5 kV and the cone voltage was 20 V.

^{1}H and ^{13}C NMR-spectra were recorded at 303 K either on a 300 MHz, 400 MHz or 500 MHz spectrometer with standard pulse sequences. The chemical shifts are reported relative to trimethylsilane (TMS), which was used as internal standard, or to residual $CHCl_3$ (δ 7.26). ^{13}C spectra were referred to the central component of the $CDCl_3$ triplet at δ 77.0. Carbon substitution degrees were established by J-MOD and DEPT pulse sequences. A combination of COSY, HMQC, and NOE experiments was utilized when necessary for the assignment of ^{1}H and ^{13}C chemical shifts. All operations involving air-sensitive reagents were performed under an inert atmosphere of dry nitrogen or argon using syringes, oven-dried glassware, and freshly distilled and dried solvents.

Melting points were determined in open capillary tubes with a temperature gradient of 10 ºC/minute. The maximum temperature was 300 ºC. The melting points were read from a digital display and are uncorrected.

2. BACE1 assays

Enzymatic assay: BACE1 was assayed fluorimetrically on Monaco Safas spektrofluorometer flx. The wavelength for excitation was 328 nm and for emission 400 nm. The reactions were followed at 25 °C over 20 min. A human recombinant BACE1 (Enzo Life Sciences, Lörrach, Germany) stock solution of 500 µg/mL was diluted 1:10 with assay buffer (20 mM sodium acetate pH 4.5, 0.1% CHAPS, 0.1% Top Block). Inhibitor stock solutions were prepared with DMSO. A 1 mM stock solution of the substrate Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(Dnp)-OH (Bachem, Bubendorf, Switzerland) was diluted 1:10 with 50% DMSO and 1:3 with ammonium acetate buffer (10 mM, pH 7.4). The final concentration of DMSO was 2.4%, and the final concentration of the substrate was 2.5 µM. Assays were performed with a final concentration of 0.5 µg/mL of BACE1. To a cuvette 905 µL assay buffer, inhibitor solution and DMSO in a total volume of 10 µL and 10 µL BACE1 solution were added, thoroughly mixed and incubated for 1 h at 25 °C. The reaction was initiated by adding 75 µL of the substrate. Experiments were performed in duplicate with five different inhibitor concentrations and IC_{50} values obtained.

Cellular Assay: In an αlisa assays the levels of $A\beta_{TOT}$ produced and secreted into the medium of human neuroblastoma SKNBE2 cells were quantified. The assay is based on the human neuroblastoma SKNBE2 expressing the wild type Amyloid Precursor Protein (hAPP695). The compounds were diluted and added to the cells, incubated for 18 h and then AβTOT was measured by sandwich αlisa. αlisa is a sandwich assay using biotinylated antibody AbN/25 attached to streptavidin coated beads and antibody Ab4G8 for the detection of $A\beta_{TOT}$. In the presence of $A\beta_{TOT}$, the beads come into close proximity. The excitation of the donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the acceptor beads, resulting in light emission. Light emission is measured after 1 hour incubation (excitation at 650 nm and emission at 615 nm). A best-fit curve was fitted to the plot of % Controlmin versus compound concentration and an IC_{50} value was obtained.

3. Stereochemical determination of 7 (SFC analysis and VCD)

A sample of racemic aminoalcohol *rac*-**7** was separated into the corresponding enantiomers by preparative SFC on (Chiralpak Daicel AD x 250 mm, Mobile phase (CO_2, MeOH with 0.2% $iPrNH_2$)). Using this technique, the enantiomeric excess of aminoalcohol **7** was determined to be 96.80%.

Report SFC-MS

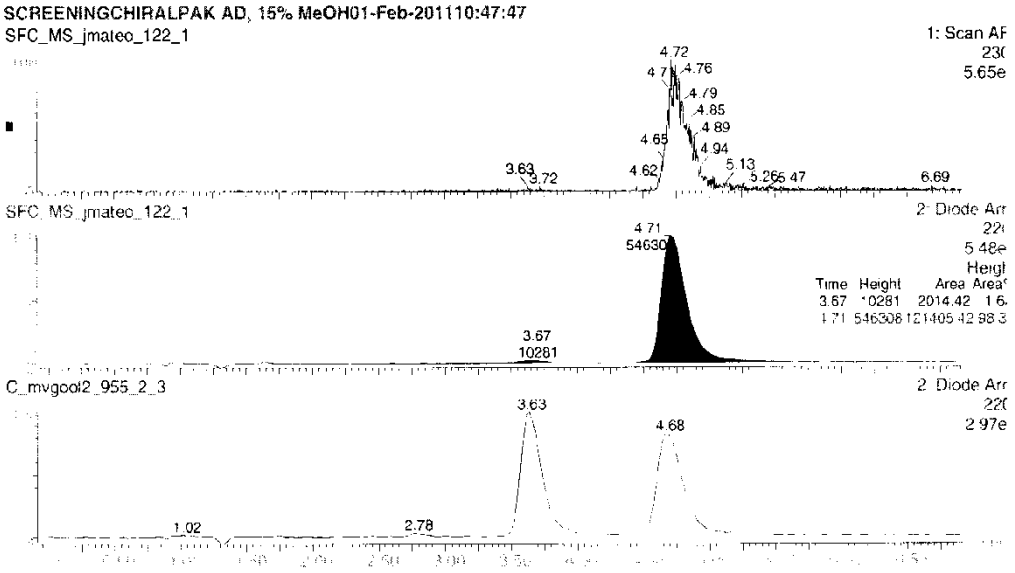
NotebookID: jimateo_122_1
Analyst: sthomas0
Method: SFC_AD_MEOH_15_3ml
Conclusion: N/A
Date: 01-february-2011
Remark: Check EE purity, (1.60 % isomer 1), (98.40 % isomer 2).

Method description:

Columns: Chiralpak AD 250 mm x 4.6 mm
Flow: 3 ml/min
Mobile phase: 15% MeOH (+0.3% iPrNH2) hold 7 min.
Temperature: 35 °C

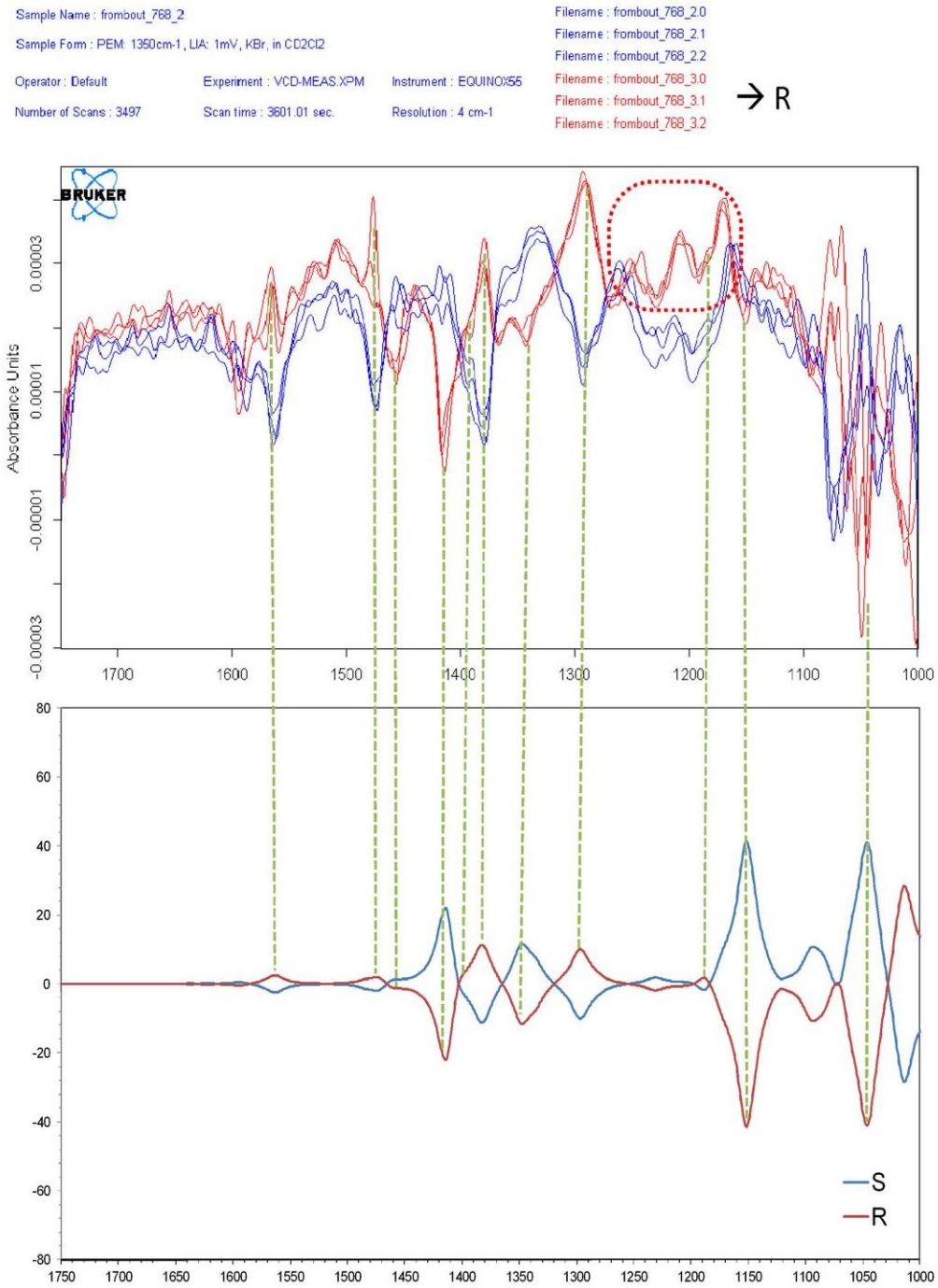
Conclusion/Chromatogram/spectra

Rt (4.68 min)	UV: 98.40 %	isomer 2
Rt (3.63 min)	UV: 1.60 %	isomer 1



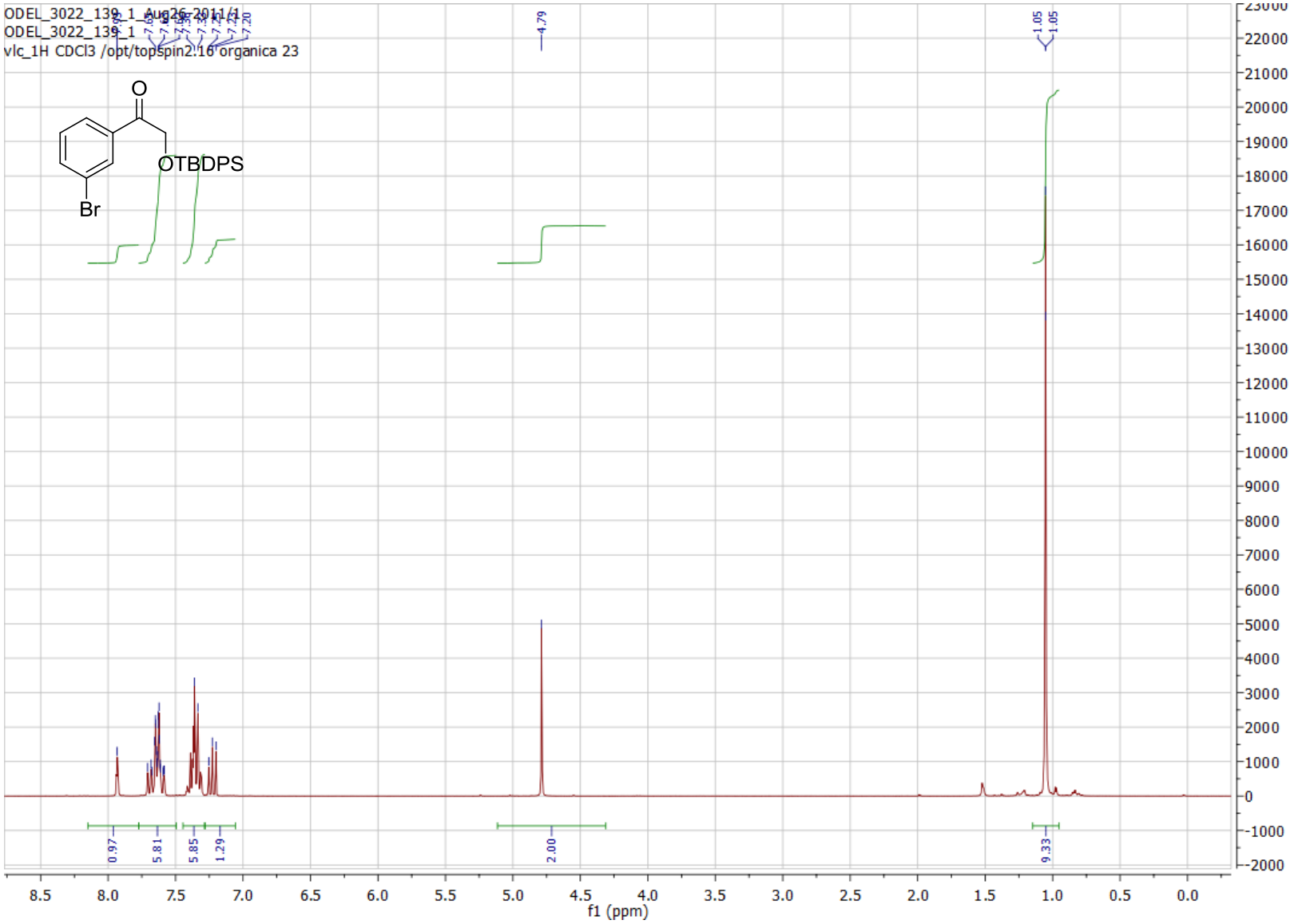
Vibrational circular dichroism (VCD) spectrum of (*R*)-7

For key intermediates the absolute configuration of the chiral center was established *via* comparison with samples of known configuration or, for coupound **7**, the use of vibrational circular dichroism (VCD). VCD spectra were measured at a resolution of 4 cm^{-1} under an ambient temperature. Samples were dissolved in CD_2Cl_2 and then placed in a KBr cell. The VCD spectra were corrected by a solvent spectrum obtained at the same experimental conditions.

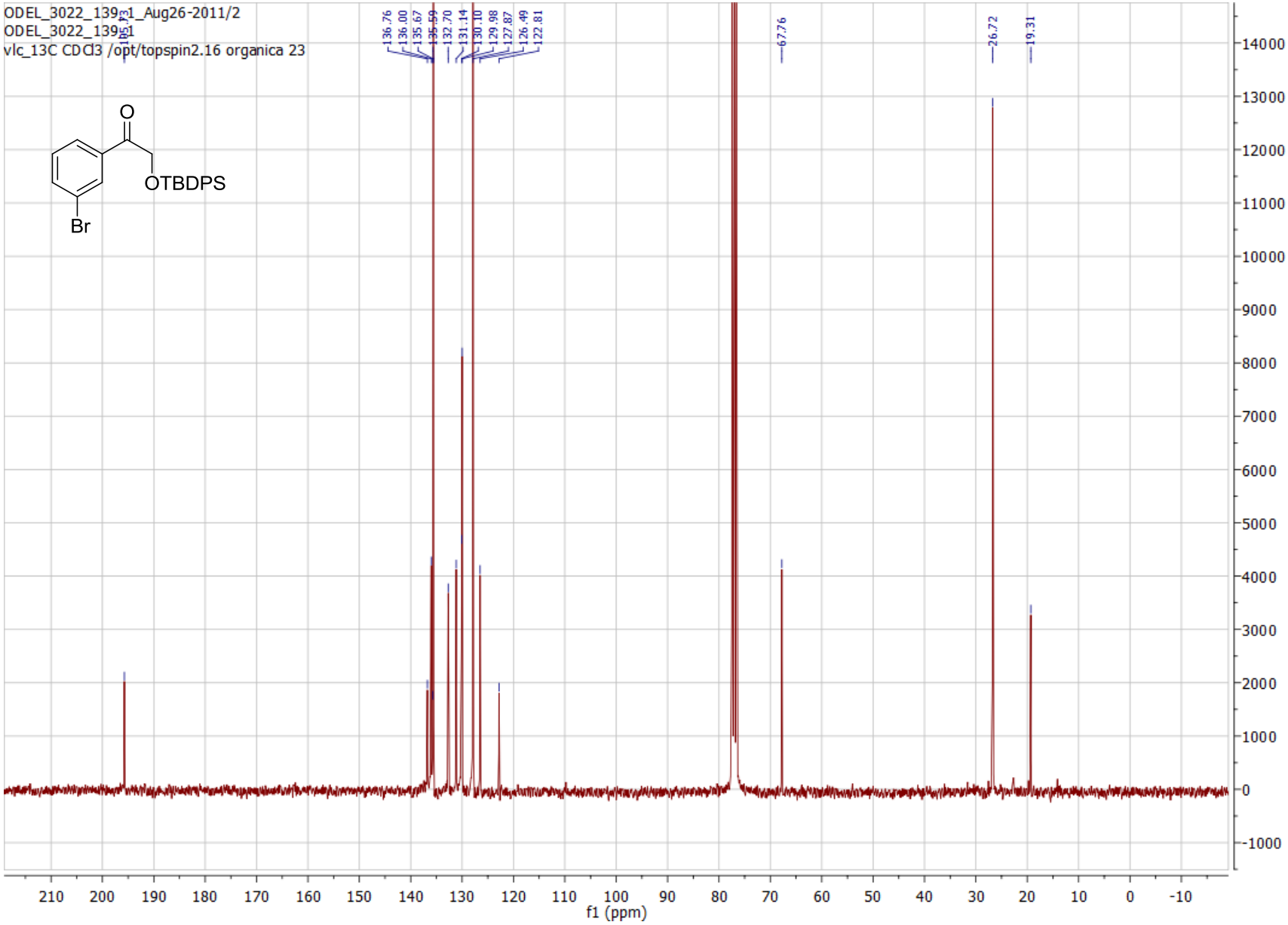


4. ^{1}H and ^{13}C Spectra

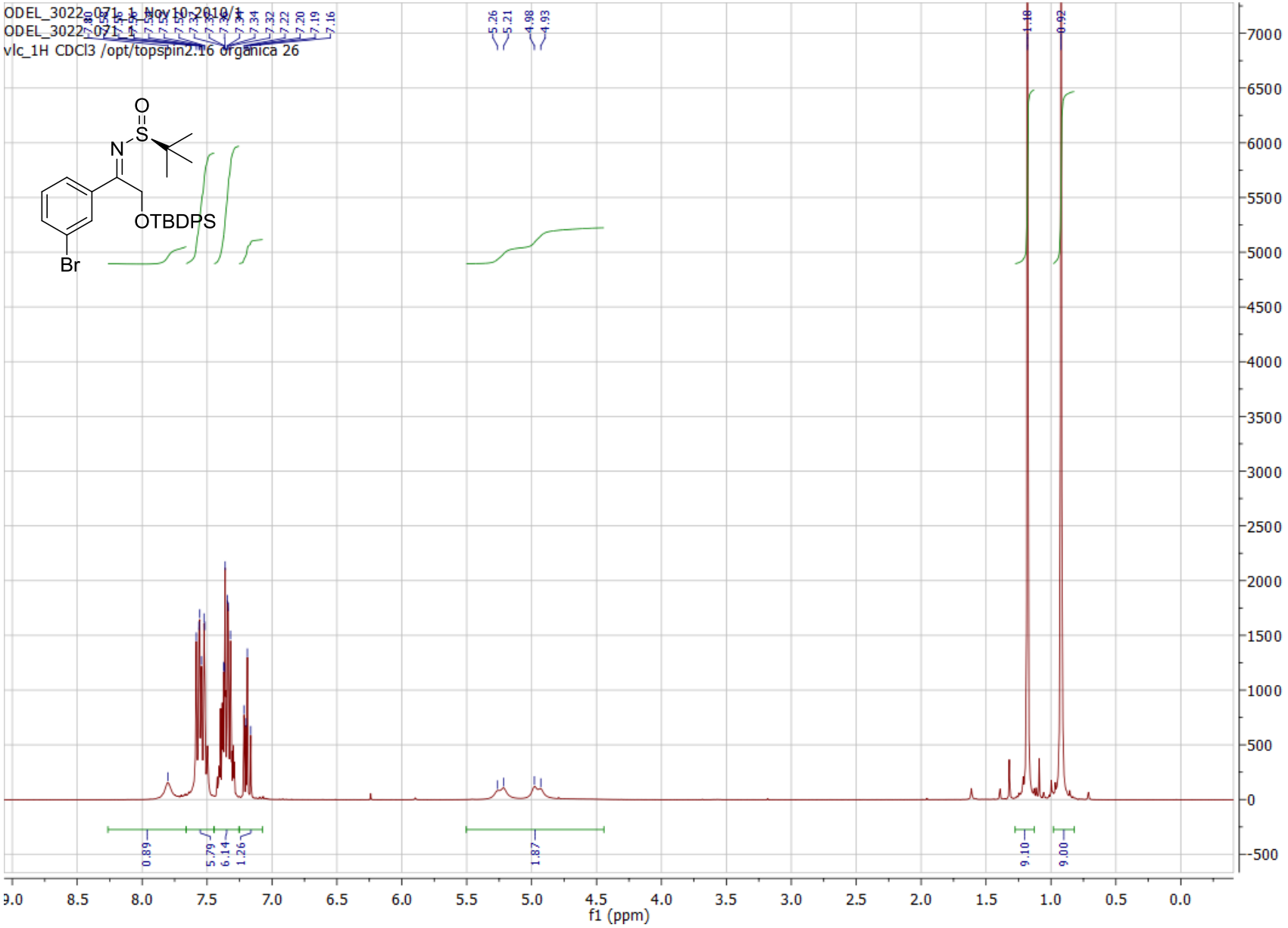
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of 1-(3-Bromophenyl)-2-(*tert*-butyldiphenylsilyloxy)ethanone (**2b**)



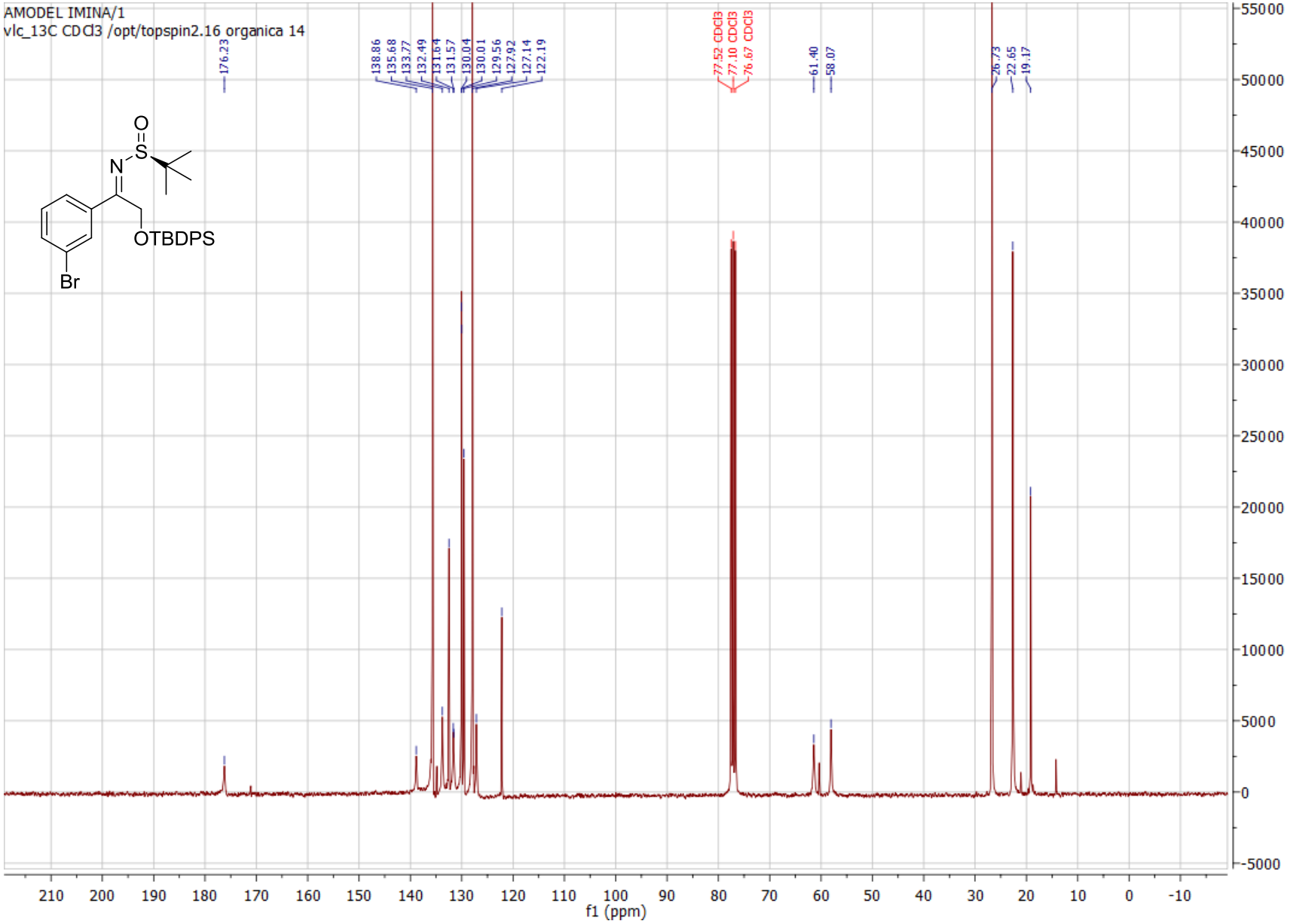
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of 1-(3-Bromophenyl)-2-(*tert*-butyldiphenylsilyloxy)ethanone (**2b**)



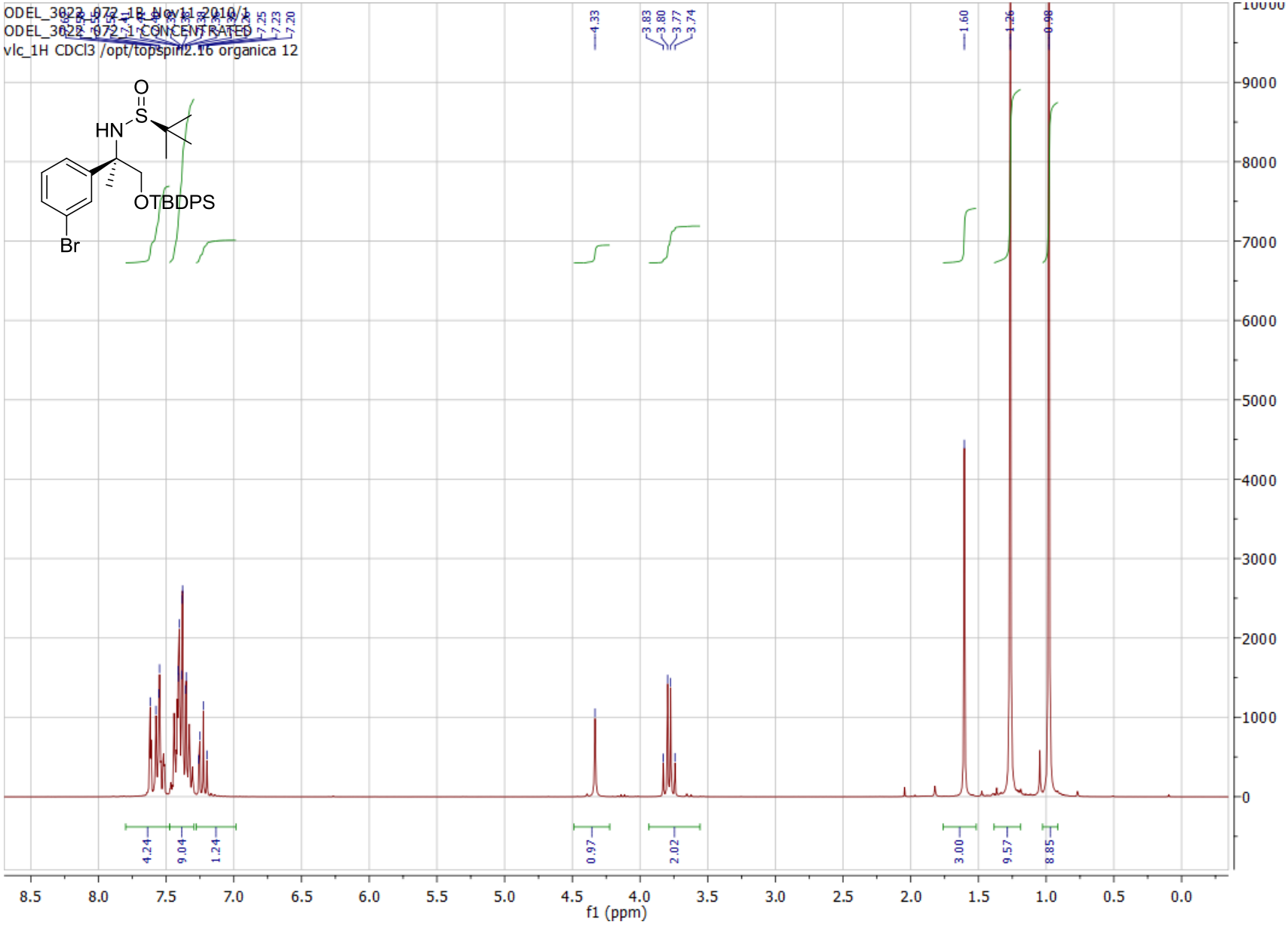
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*S*,*Z*)-*N*-(1-(3-Bromophenyl)-2-(*tert*-butyldiphenylsilyloxy)ethylidene)-2-methylpropane-2-sulfinamide (**3b**)



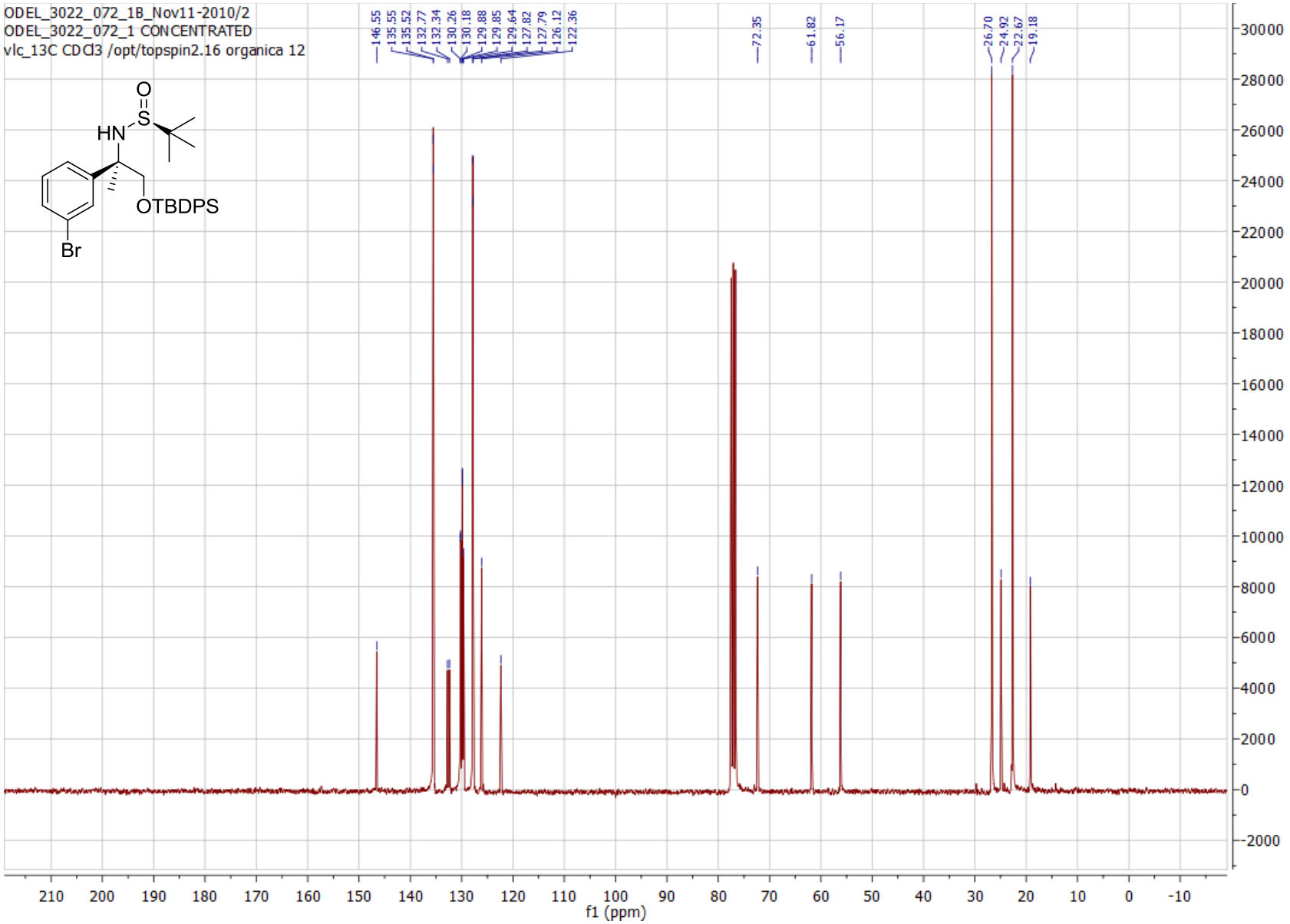
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*S*,*Z*)-*N*-(1-(3-Bromophenyl)-2-(*tert*-butyldiphenylsilyloxy)ethylidene)-2-methylpropane-2-sulfinamide (**3b**)



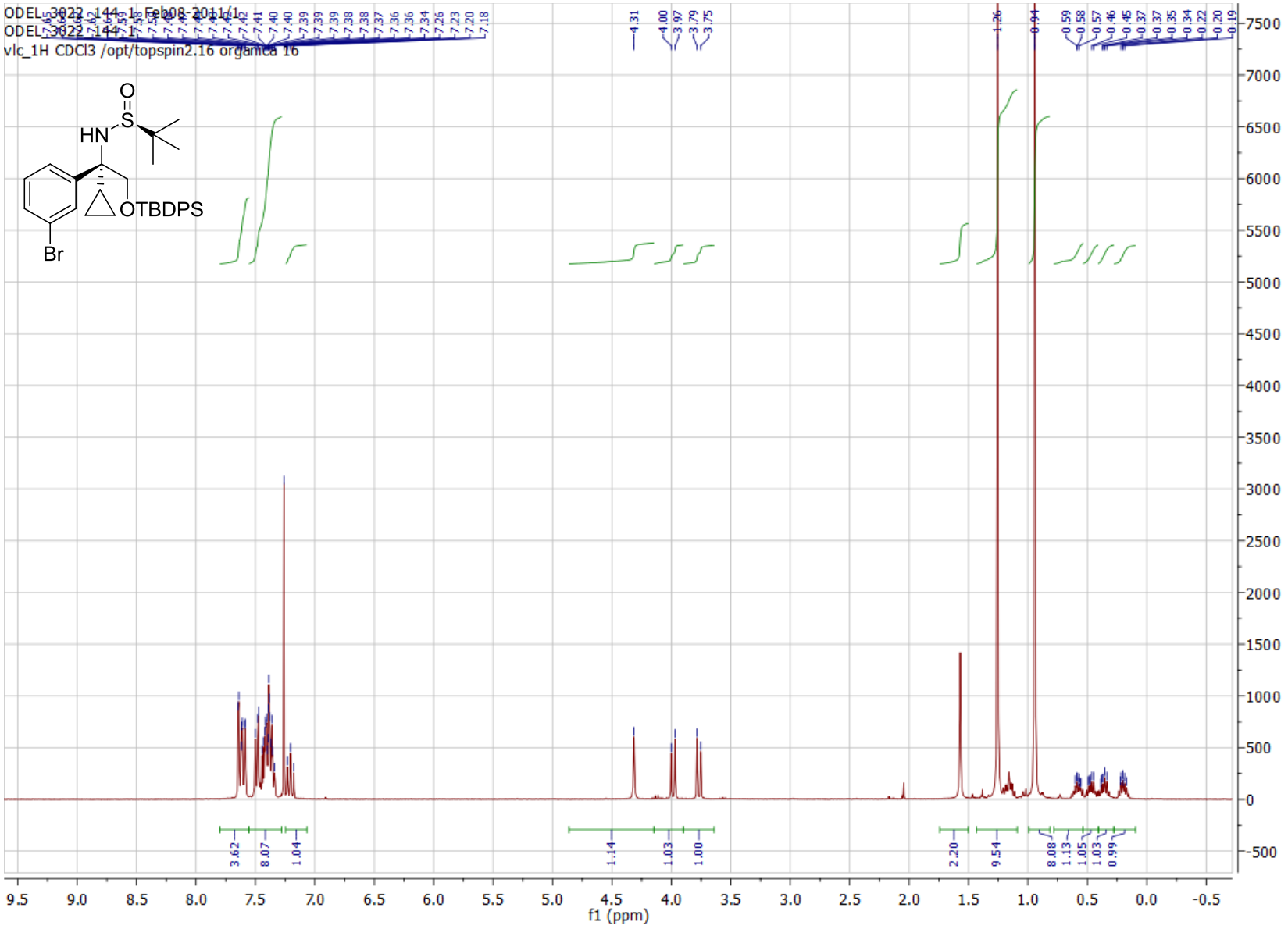
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*S*)-*N*-((*R*)-2-(3-Bromophenyl)-1-(*tert*-butyldiphenylsilyloxy)propan-2-yl)-2-methylpropane-2-sulfinamide (**4b**)



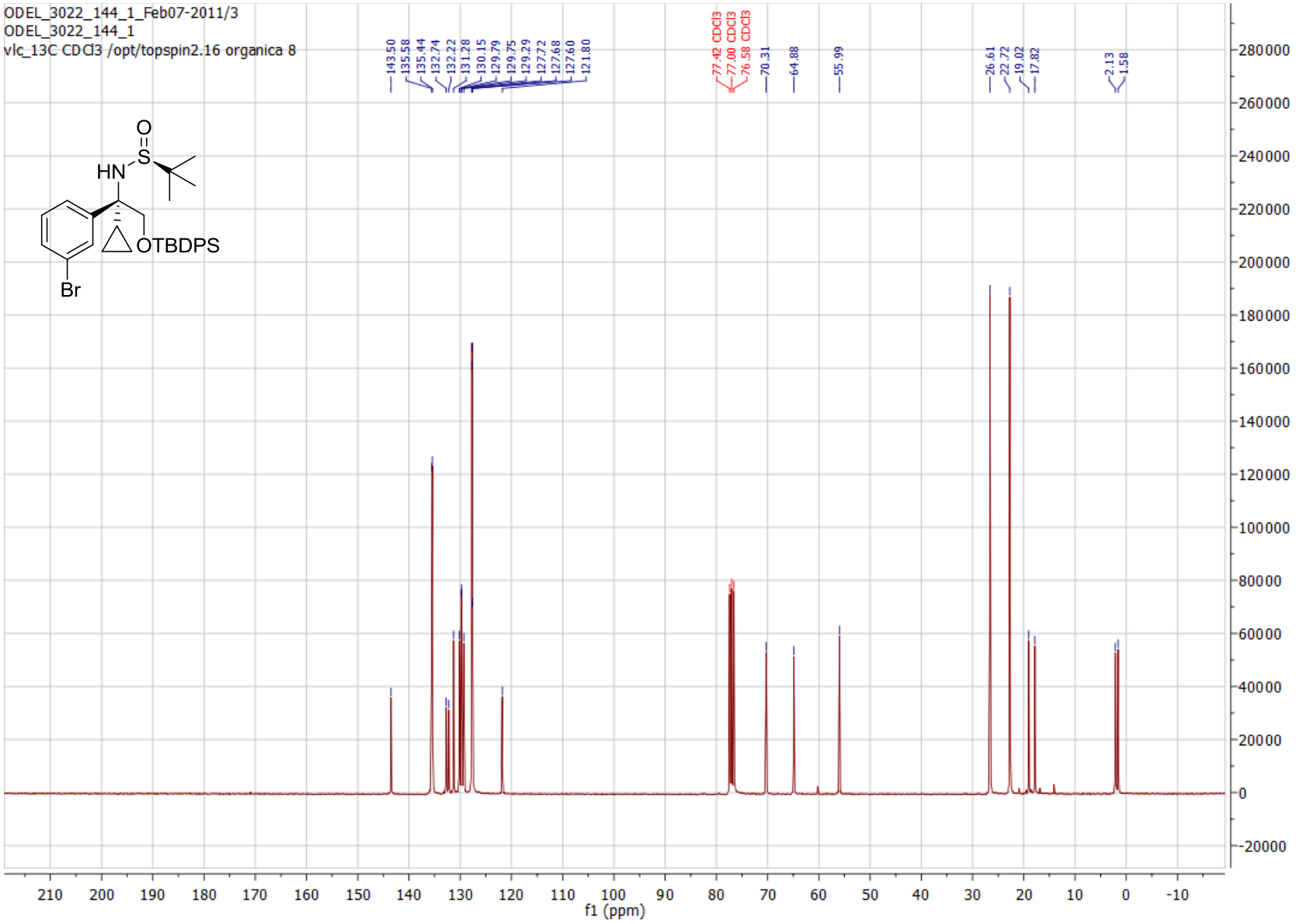
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*S*)-*N*-((*R*)-2-(3-Bromophenyl)-1-(*tert*-butyldiphenylsilyloxy)propan-2-yl)-2-methylpropane-2-sulfinamide (**4b**)



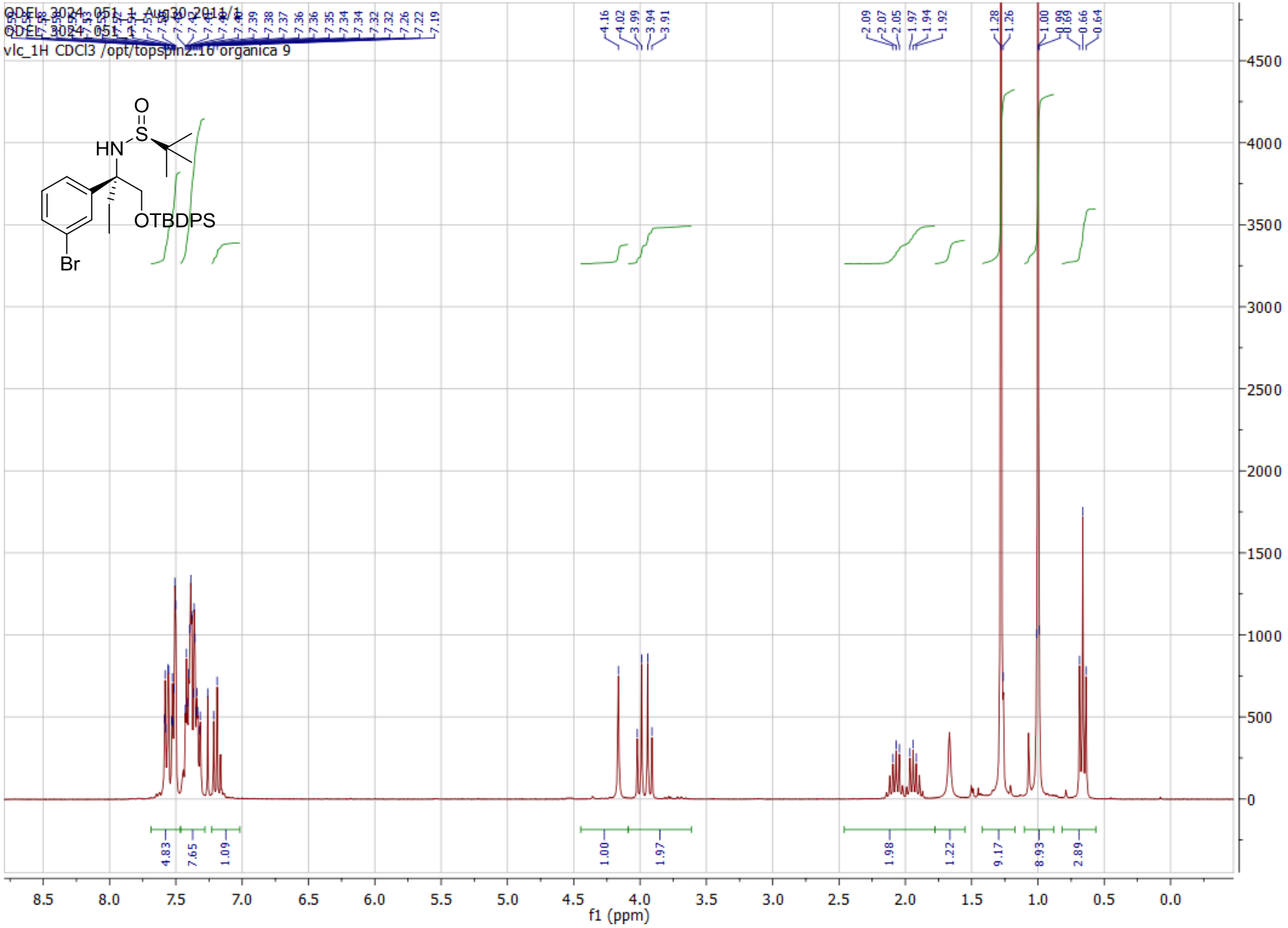
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*S*)-*N*-((*R*)-1-(3-Bromophenyl)-2-(*tert*-butyldiphenylsilyloxy)-1-cyclopropylethyl)-2-methylpropane-2-sulfinamide (**4c**)



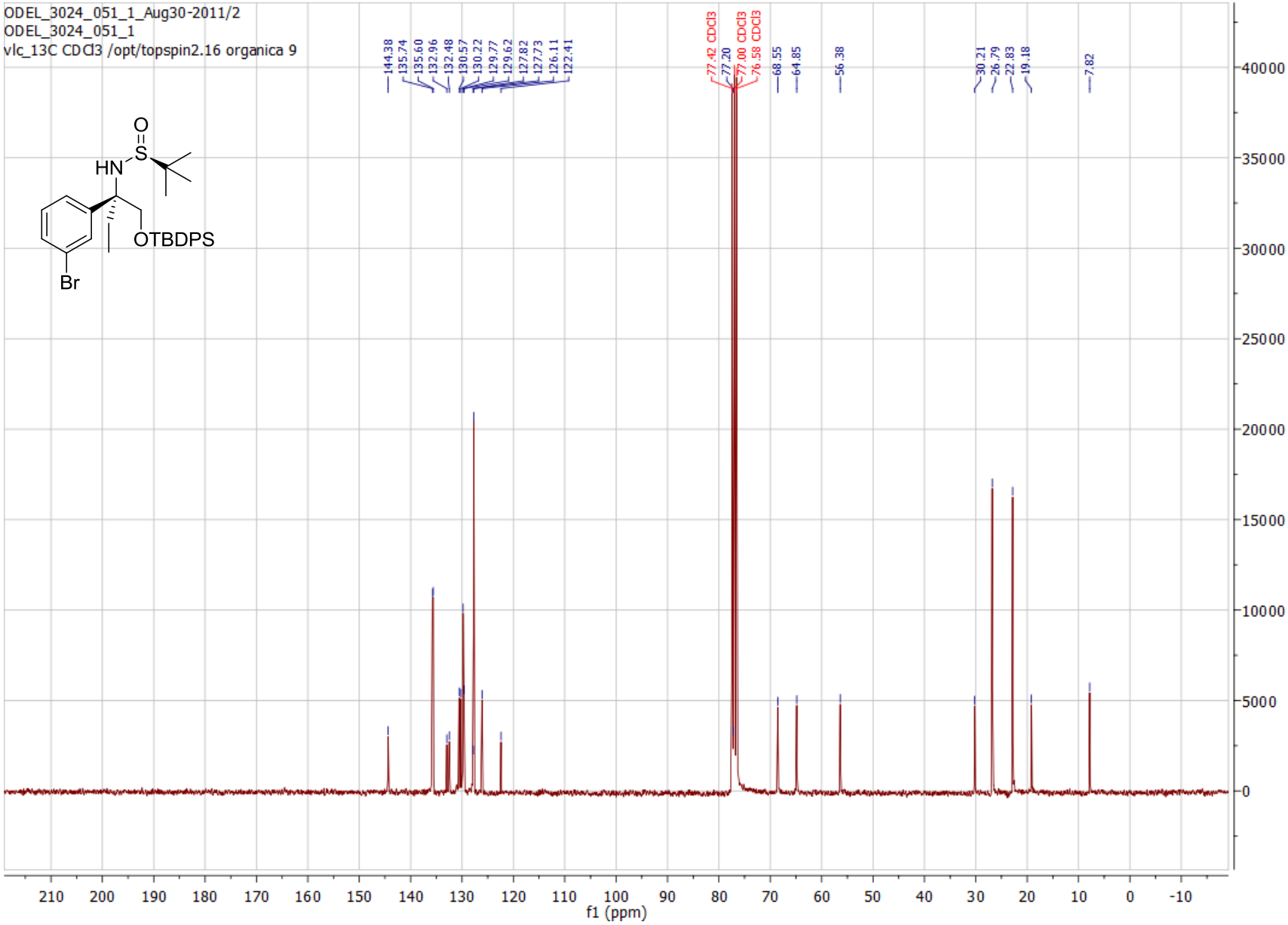
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*S*)-*N*-((*R*)-1-(3-Bromophenyl)-2-(*tert*-butyldiphenylsilyloxy)-1-cyclopropylethyl)-2-methylpropane-2-sulfinamide (**4c**)



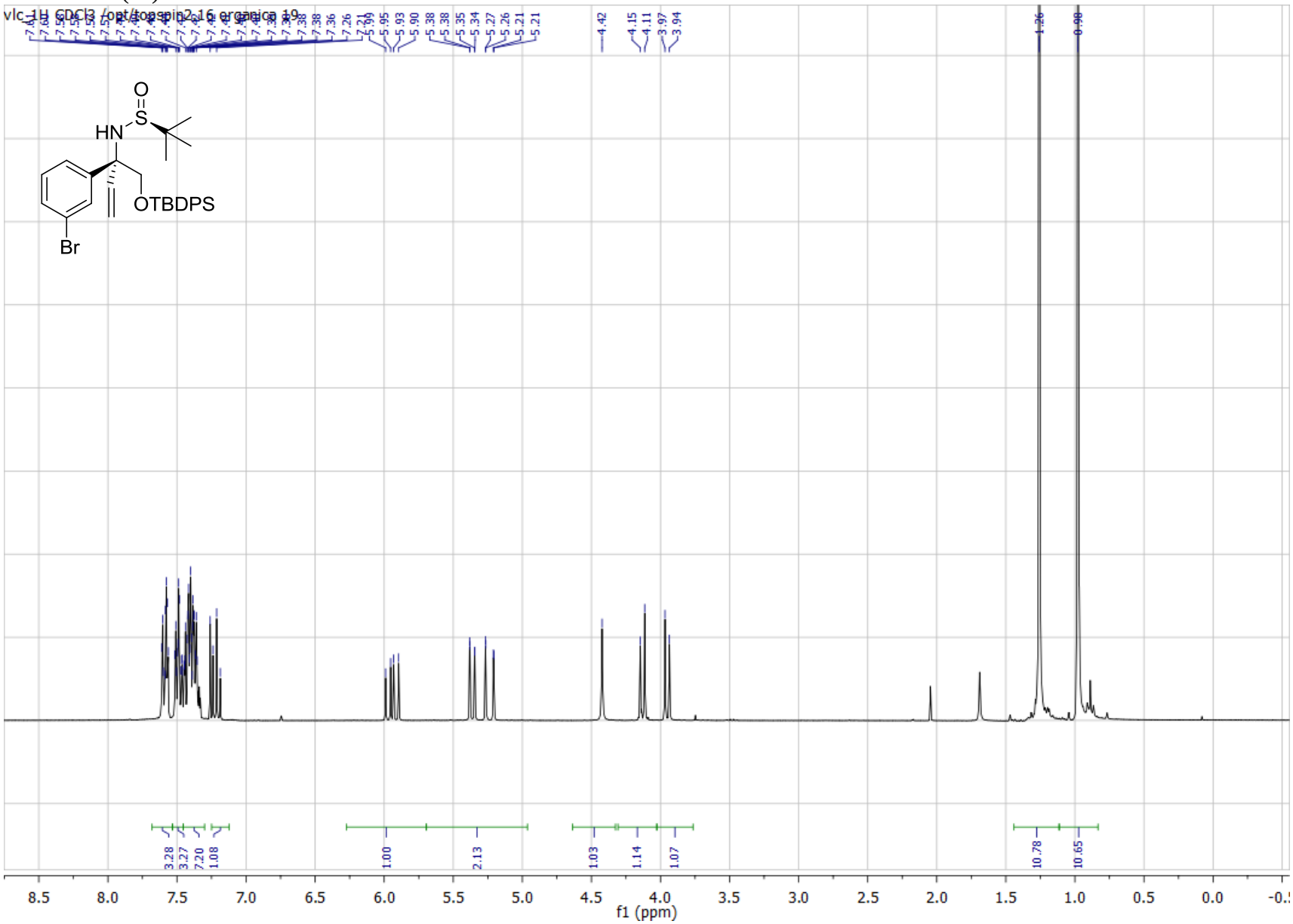
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*S*)-*N*-((*R*)-2-(3-Bromophenyl)-1-(*tert*-butyldiphenylsilyloxy)butan-2-yl)-2-methylpropane-2-sulfinamide (**4d**)



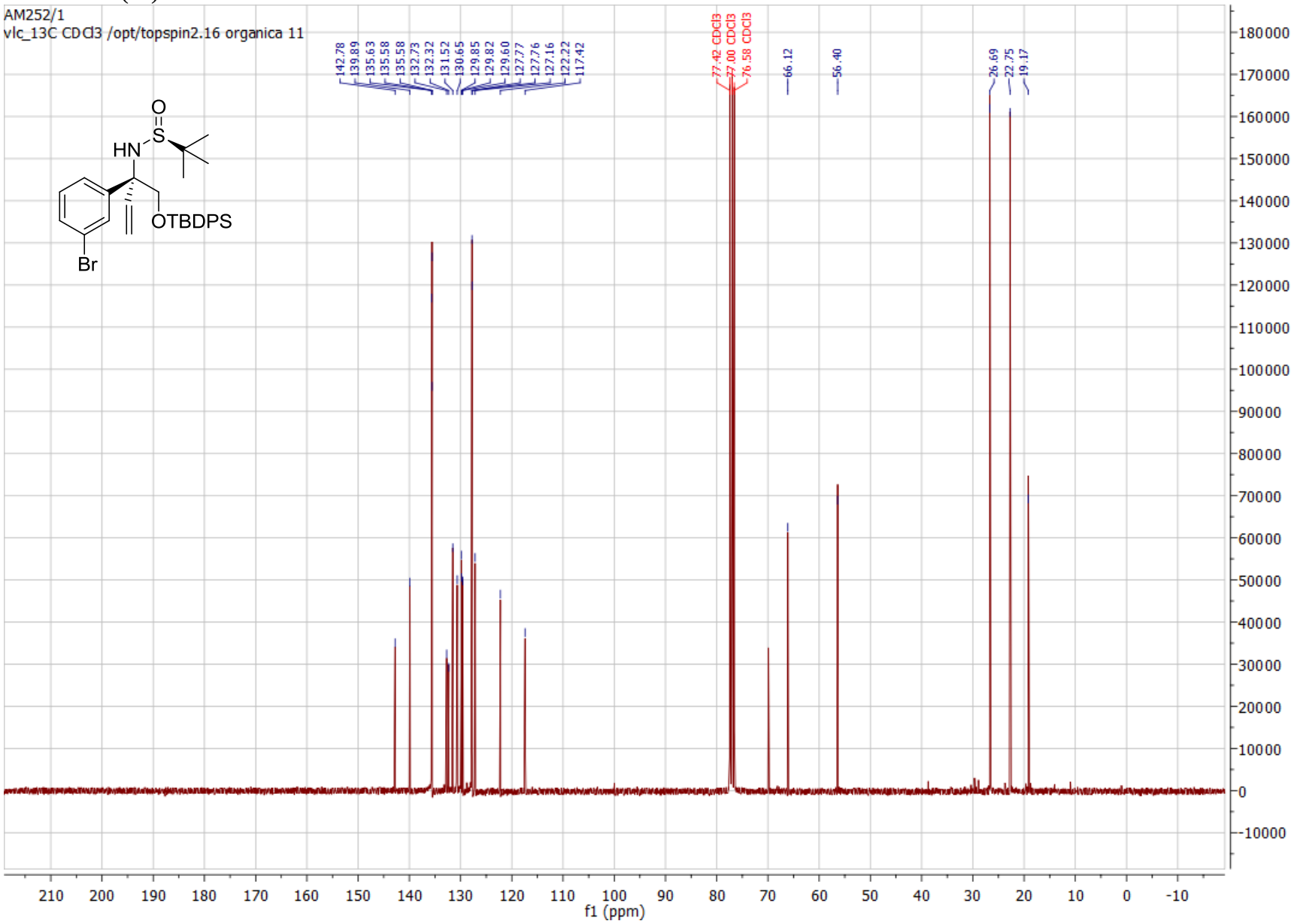
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*S*)-*N*-((*R*)-2-(3-Bromophenyl)-1-(*tert*-butyldiphenylsilyloxy)butan-2-yl)-2-methylpropane-2-sulfinamide (**4d**)



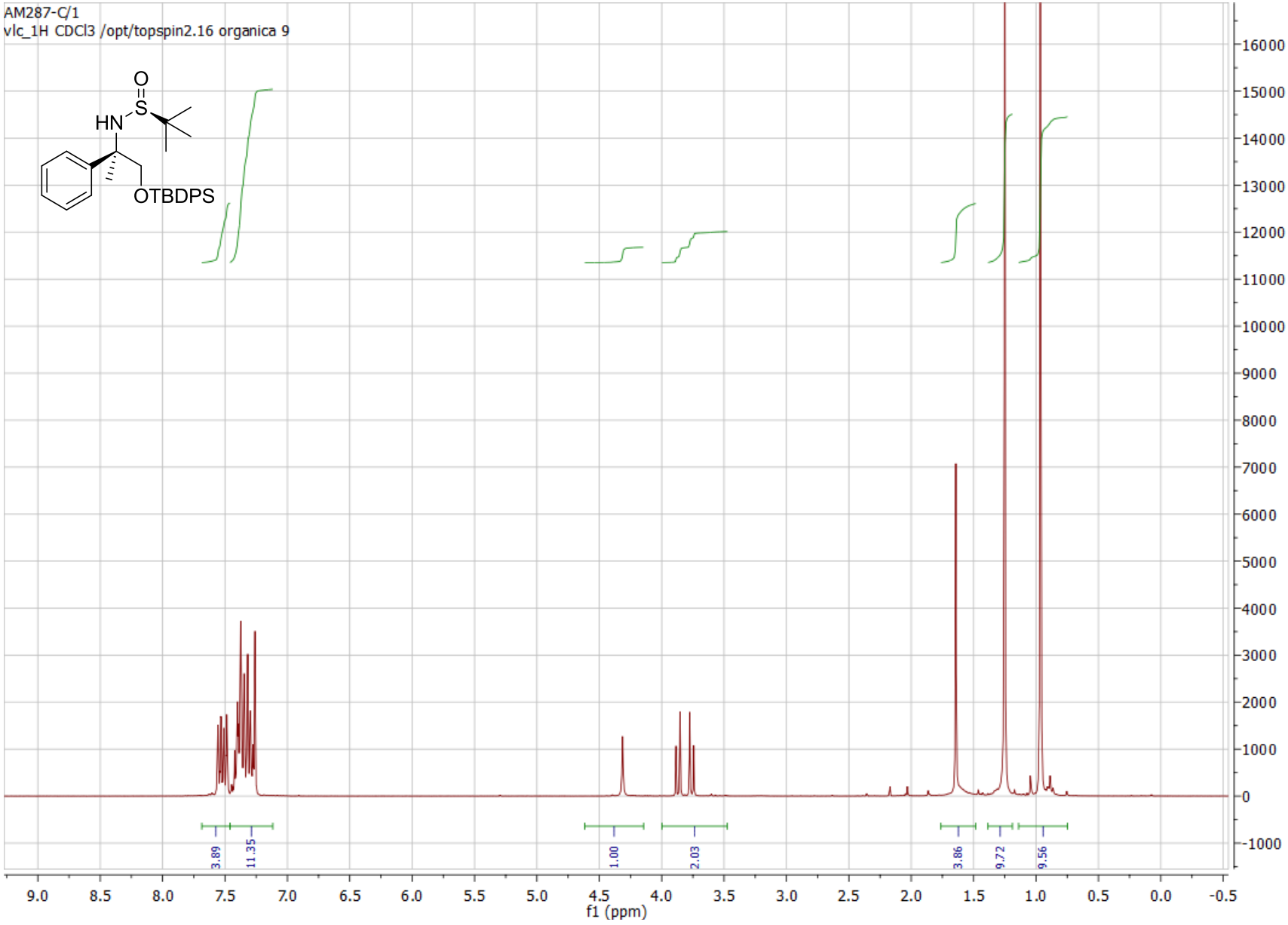
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*S*)-*N*-((*R*)-2-(3-Bromophenyl)-1-(*tert*-butyldiphenylsilyloxy)but-3-en-2-yl)-2-methylpropane-2-sulfinamide (**4e**)



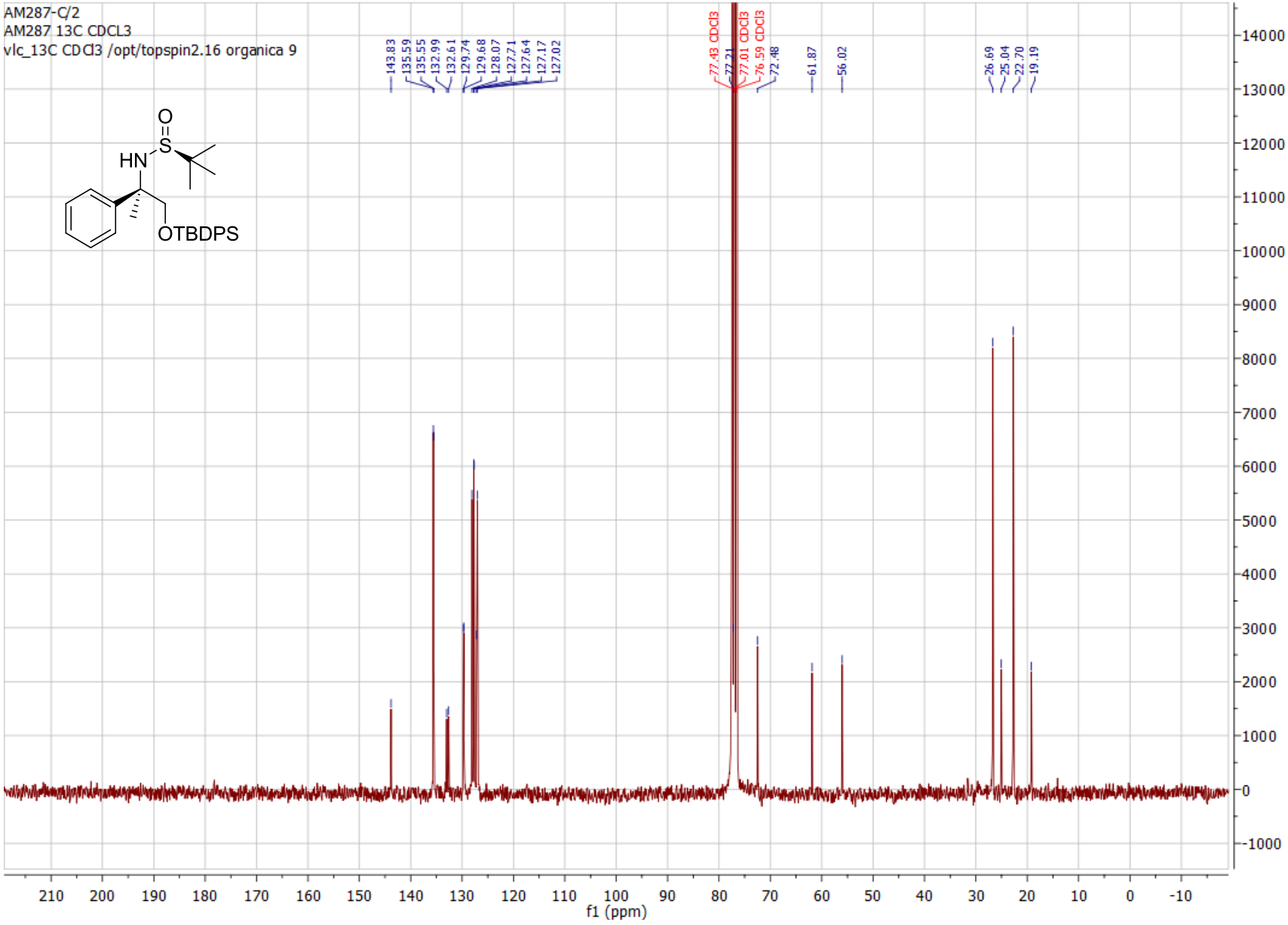
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*S*)-*N*-((*R*)-2-(3-Bromophenyl)-1-(*tert*-butyldiphenylsilyloxy)but-3-en-2-yl)-2-methylpropane-2-sulfinamide (**4e**)



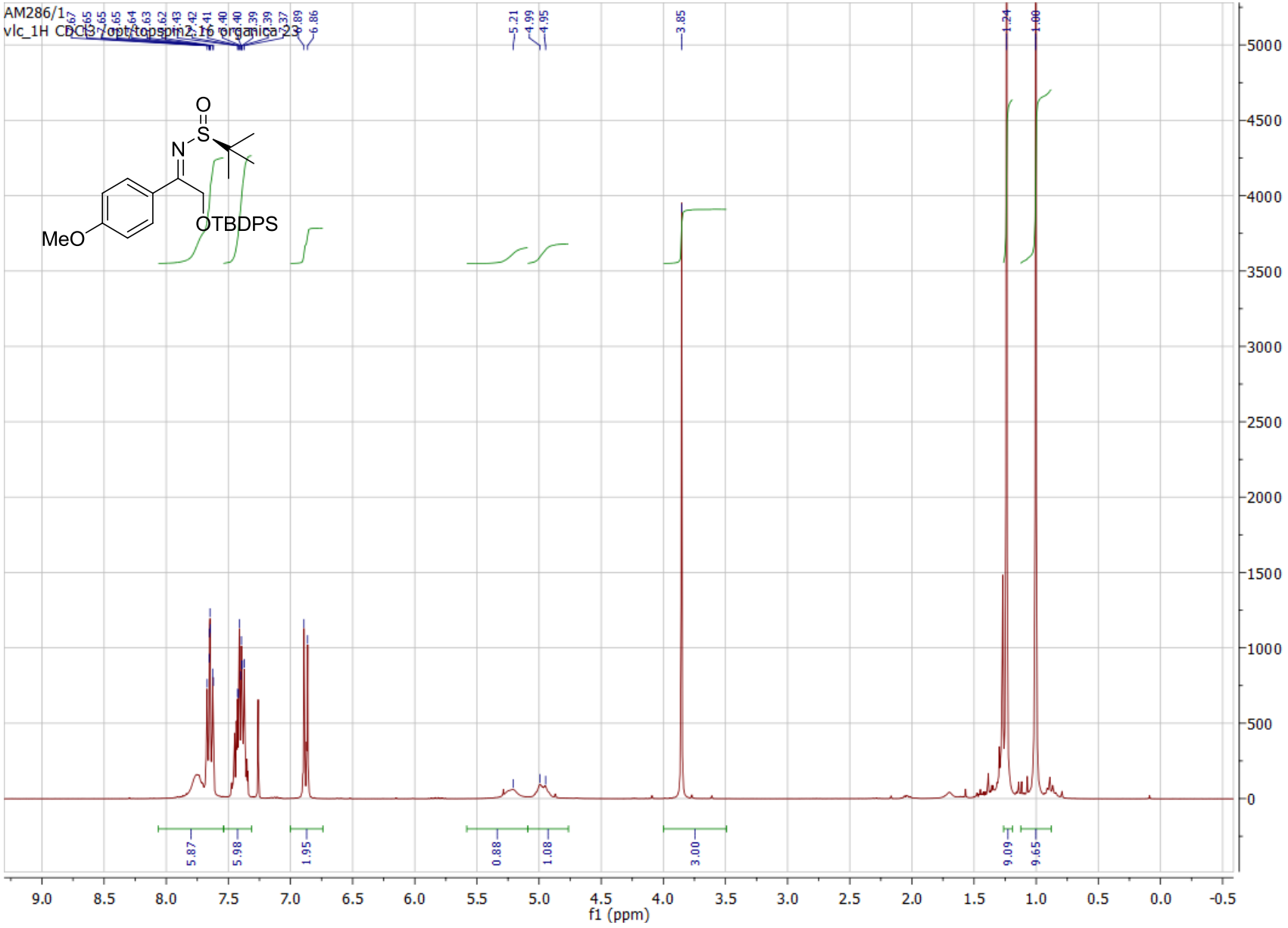
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*S*)-*N*-((*R*)-1-(*tert*-butyldiphenylsilyloxy)-2-phenylpropan-2-yl)-2-methylpropane-2-sulfinamide (**4g**)



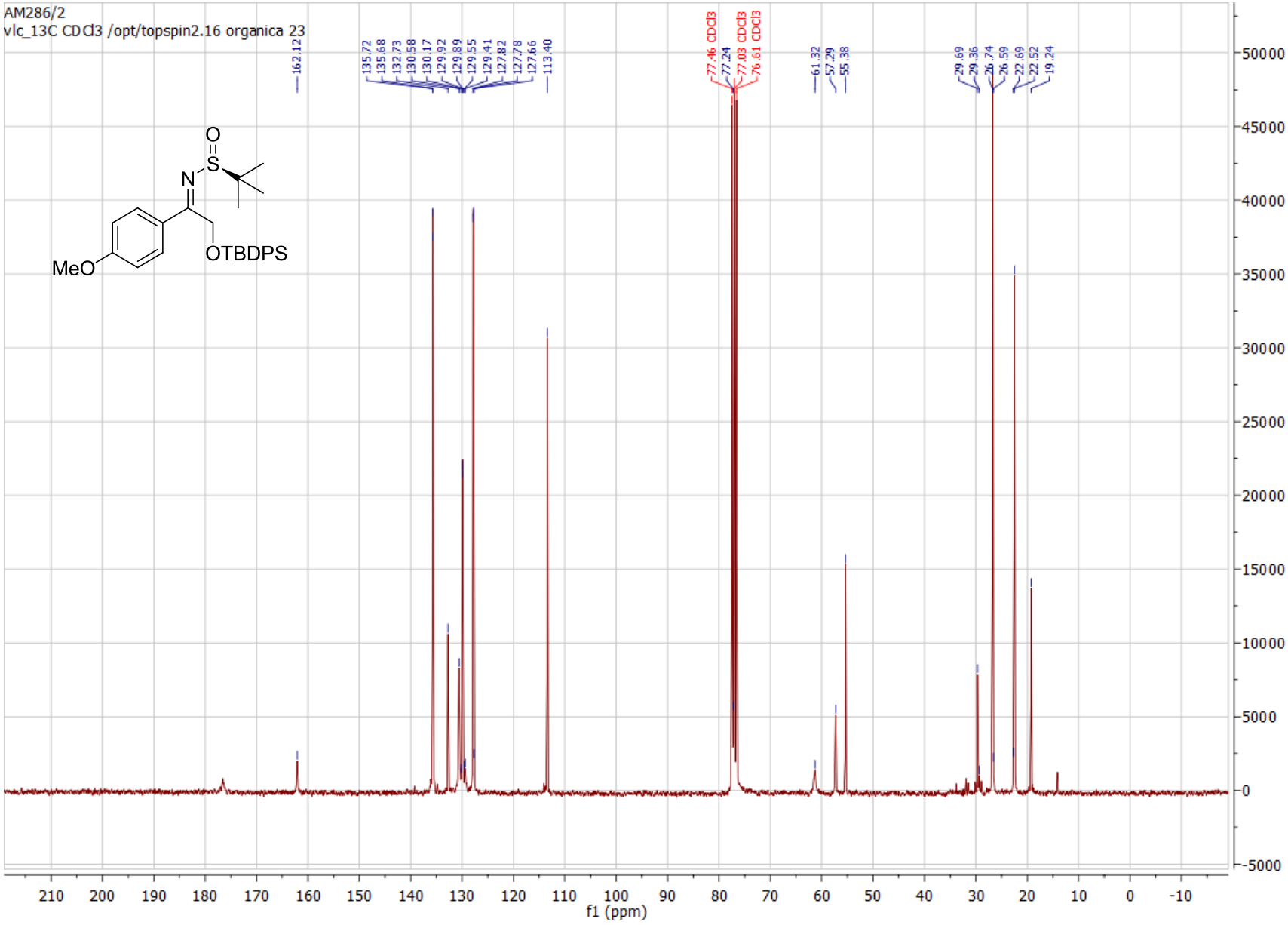
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*S*)-*N*-((*R*)-1-(*tert*-butyldiphenylsilyloxy)-2-phenylpropan-2-yl)-2-methylpropane-2-sulfinamide (**4g**)



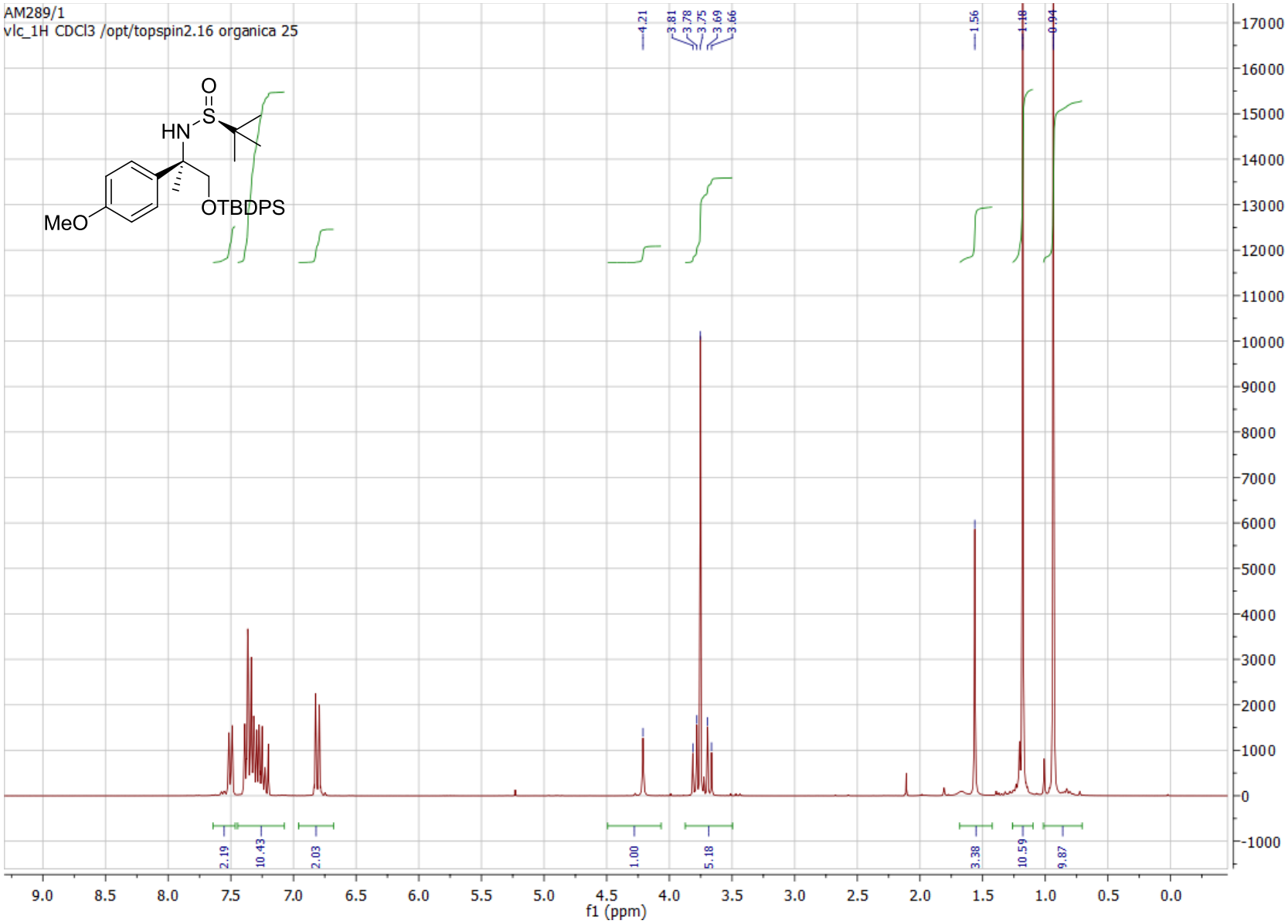
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*S*,*Z*)-*N*-(2-(*tert*-butyldiphenylsilyloxy)-1-(4-methoxyphenyl)ethylidene)-2-methylpropane-2-sulfinamide (**3d**)



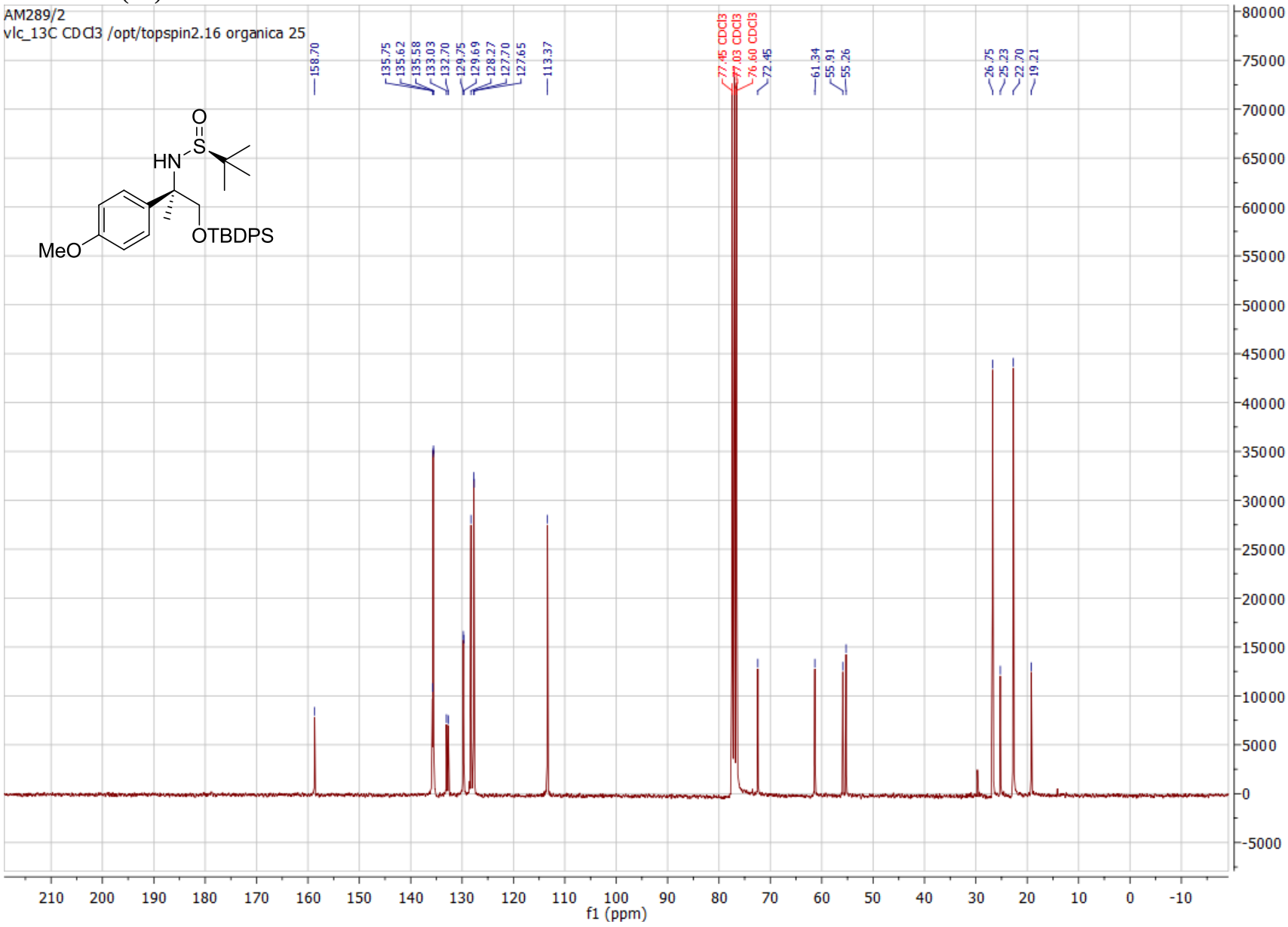
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*S*,*Z*)-*N*-(2-(*tert*-butyldiphenylsilyloxy)-1-(4-methoxyphenyl)ethylidene)-2-methylpropane-2-sulfinamide (**4h**)



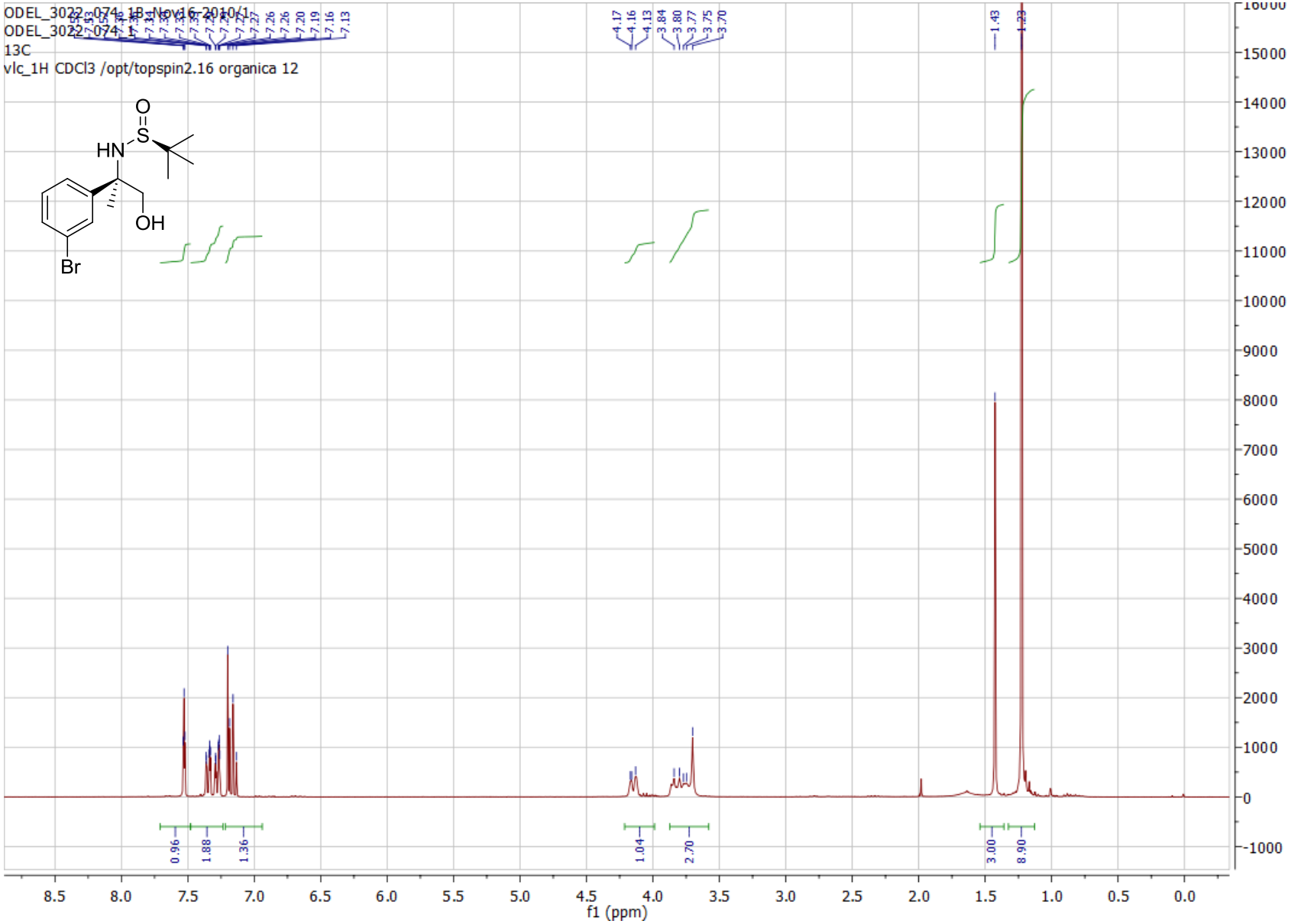
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*S*)-*N*-((*R*)-1-(*tert*-butyldiphenylsilyloxy)-2-(4-methoxyphenyl)propan-2-yl)-2-methylpropane-2-sulfinamide (**4h**)



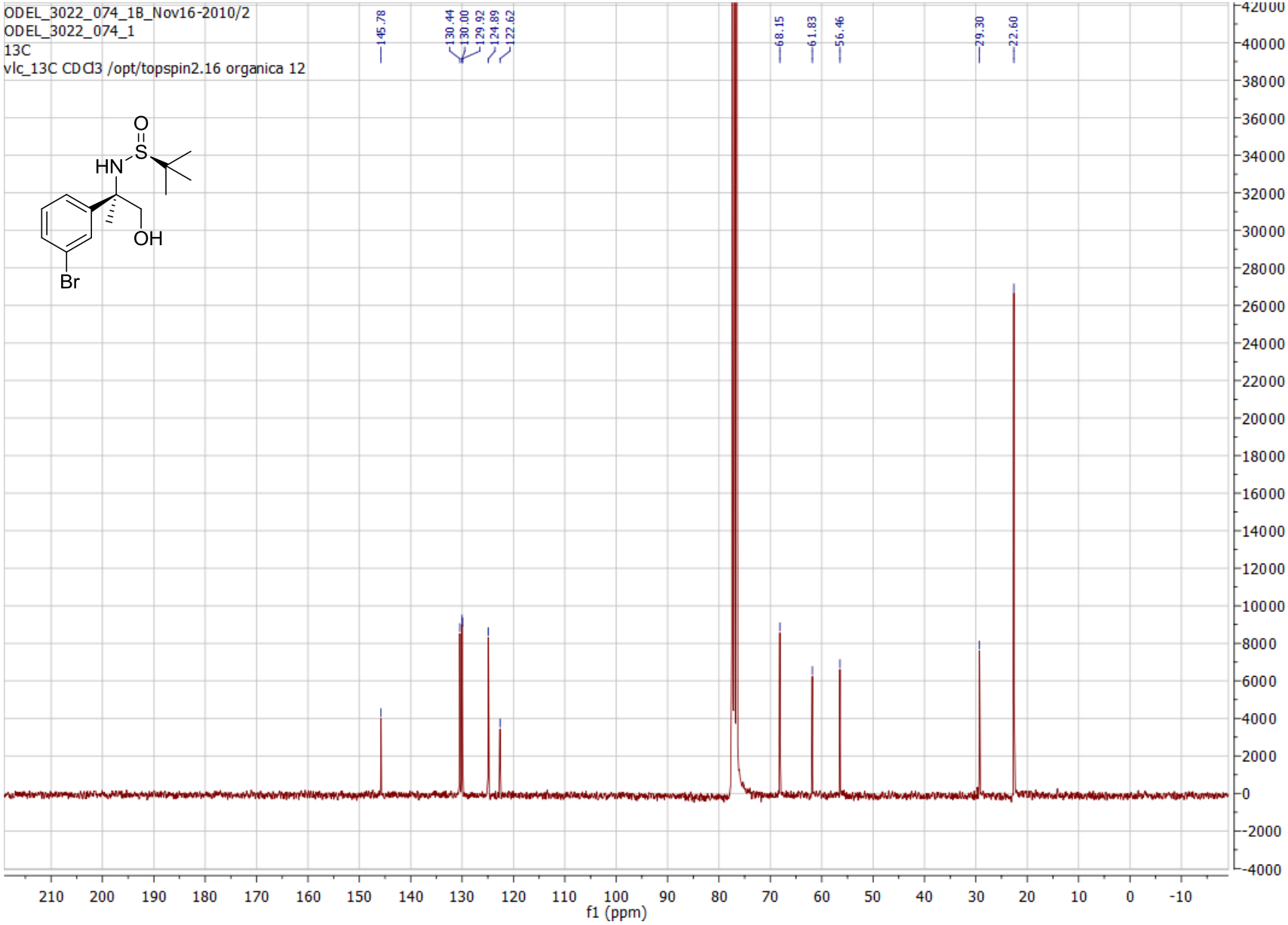
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*S*)-*N*-((*R*)-1-(*tert*-butyldiphenylsilyloxy)-2-(4-methoxyphenyl)propan-2-yl)-2-methylpropane-2-sulfinamide (**4h**)



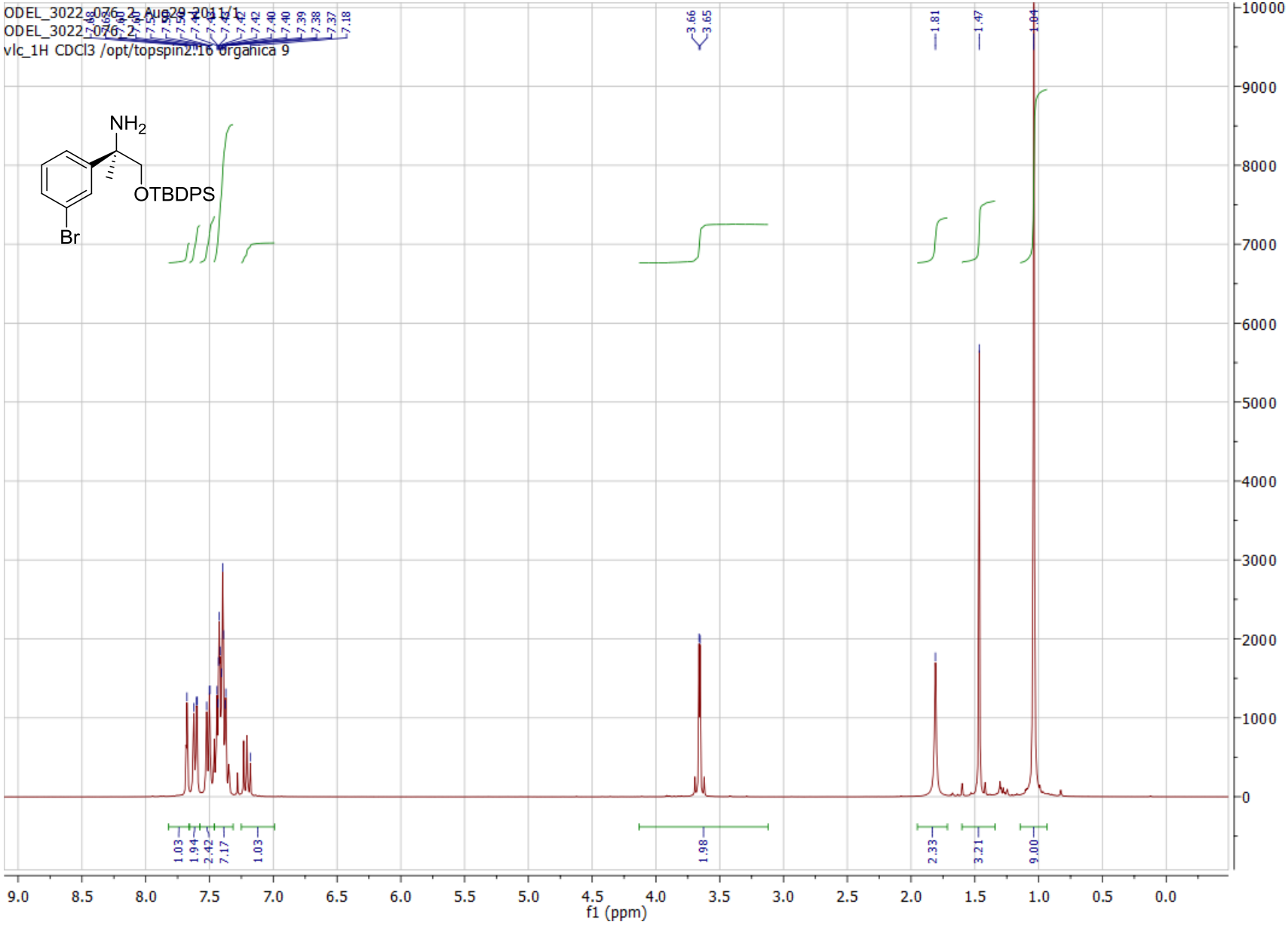
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*S*)-*N*-((*R*)-2-(3-bromophenyl)-1-hydroxypropan-2-yl)-2-methylpropane-2-sulfinamide (**5**)



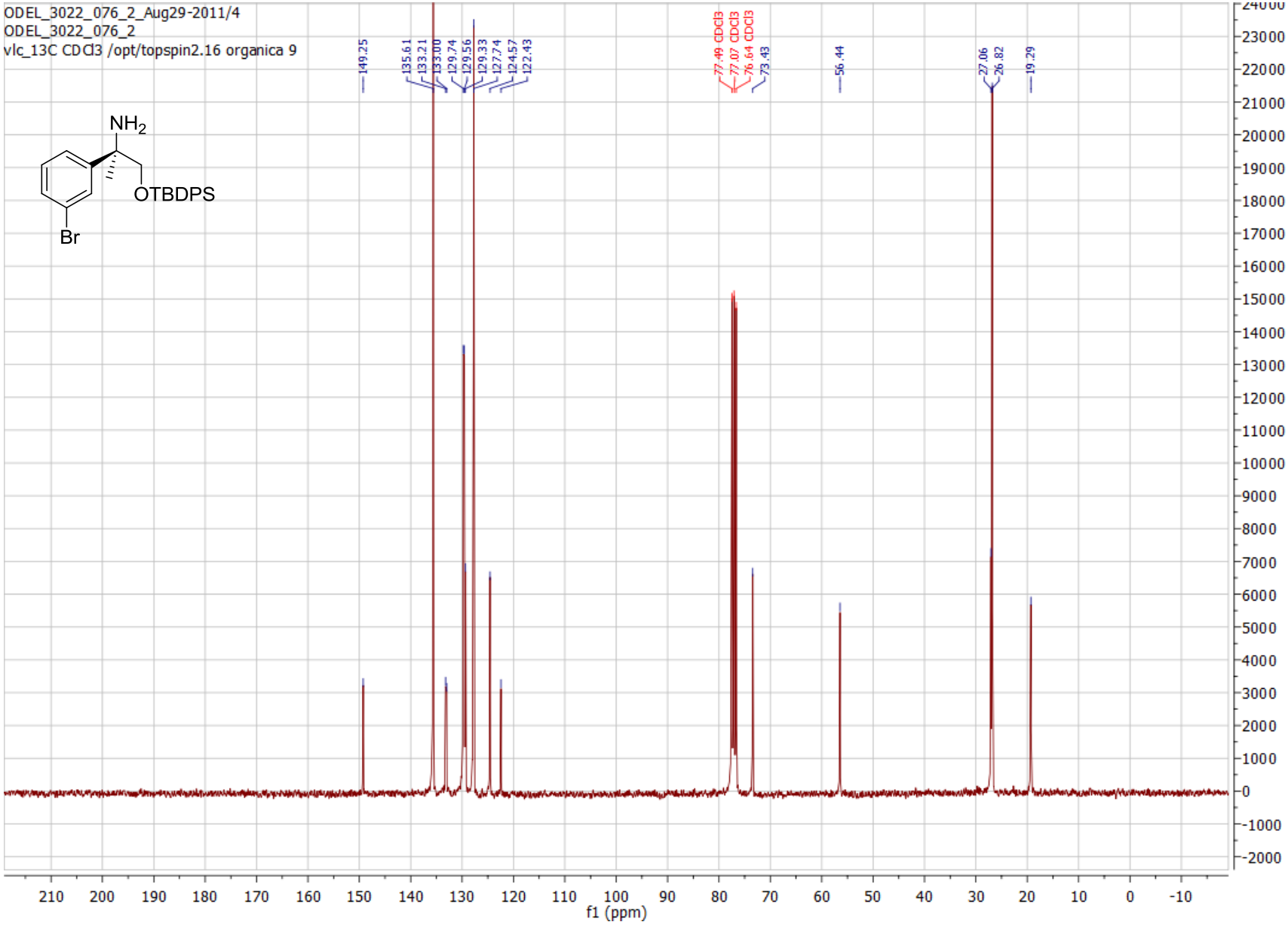
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*S*)-*N*-((*R*)-2-(3-bromophenyl)-1-hydroxypropan-2-yl)-2-methylpropane-2-sulfinamide (**5**)



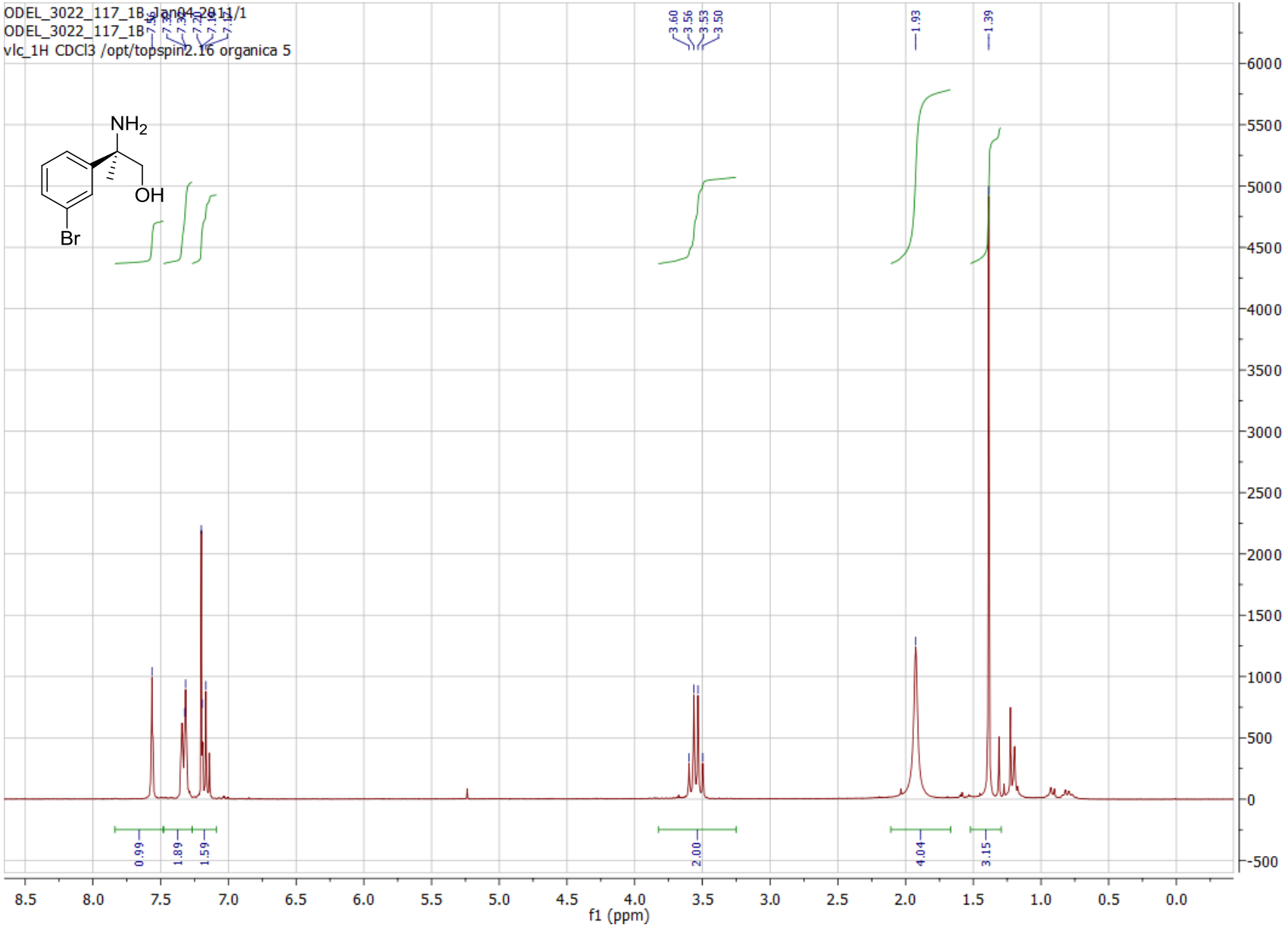
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*R*)-2-(3-Bromophenyl)-1-(*tert*-butyldiphenylsilyloxy)propan-2-amine (**6**)



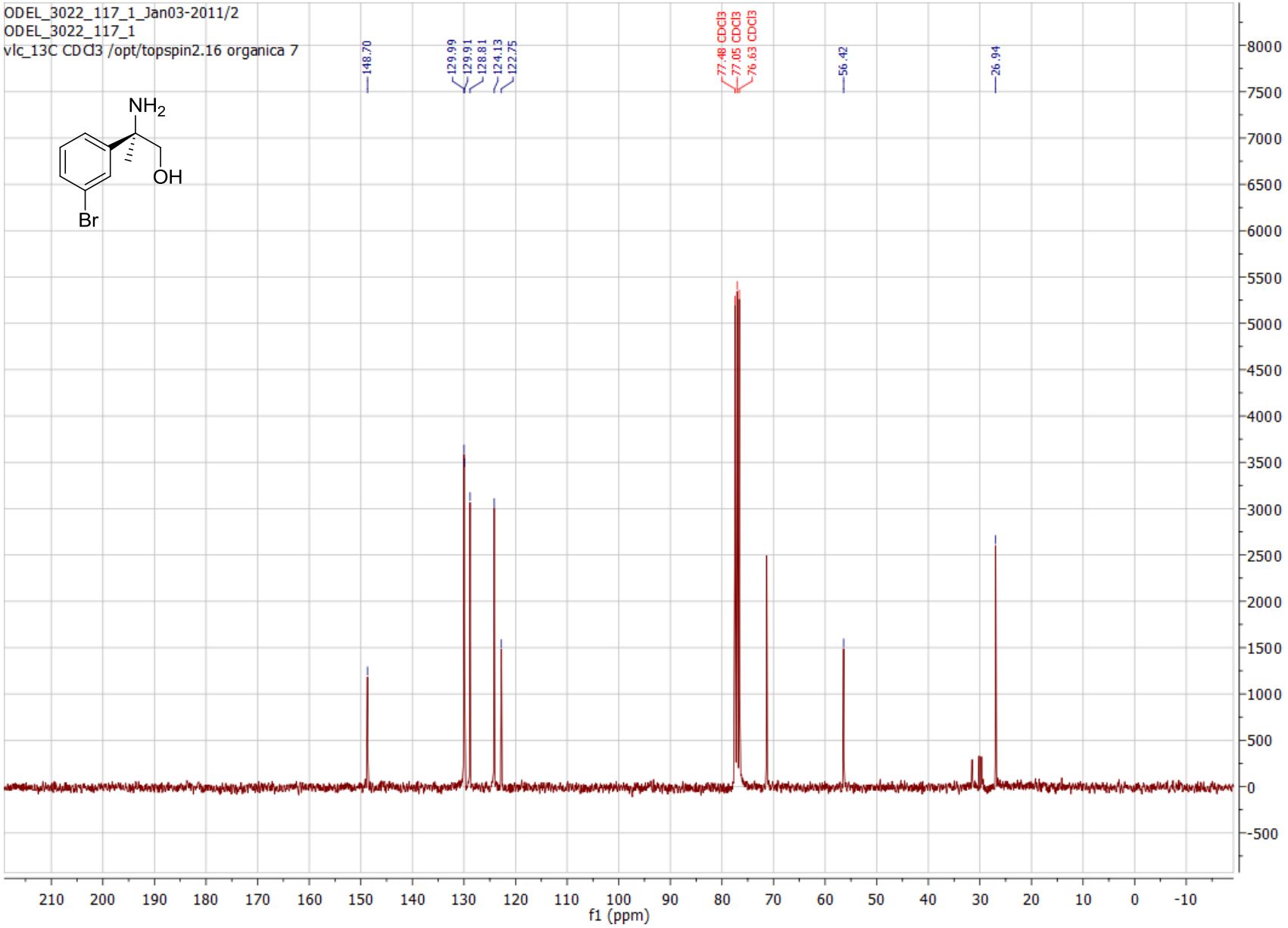
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*R*)-2-(3-Bromophenyl)-1-(*tert*-butyldiphenylsilyloxy)propan-2-amine (**6**)



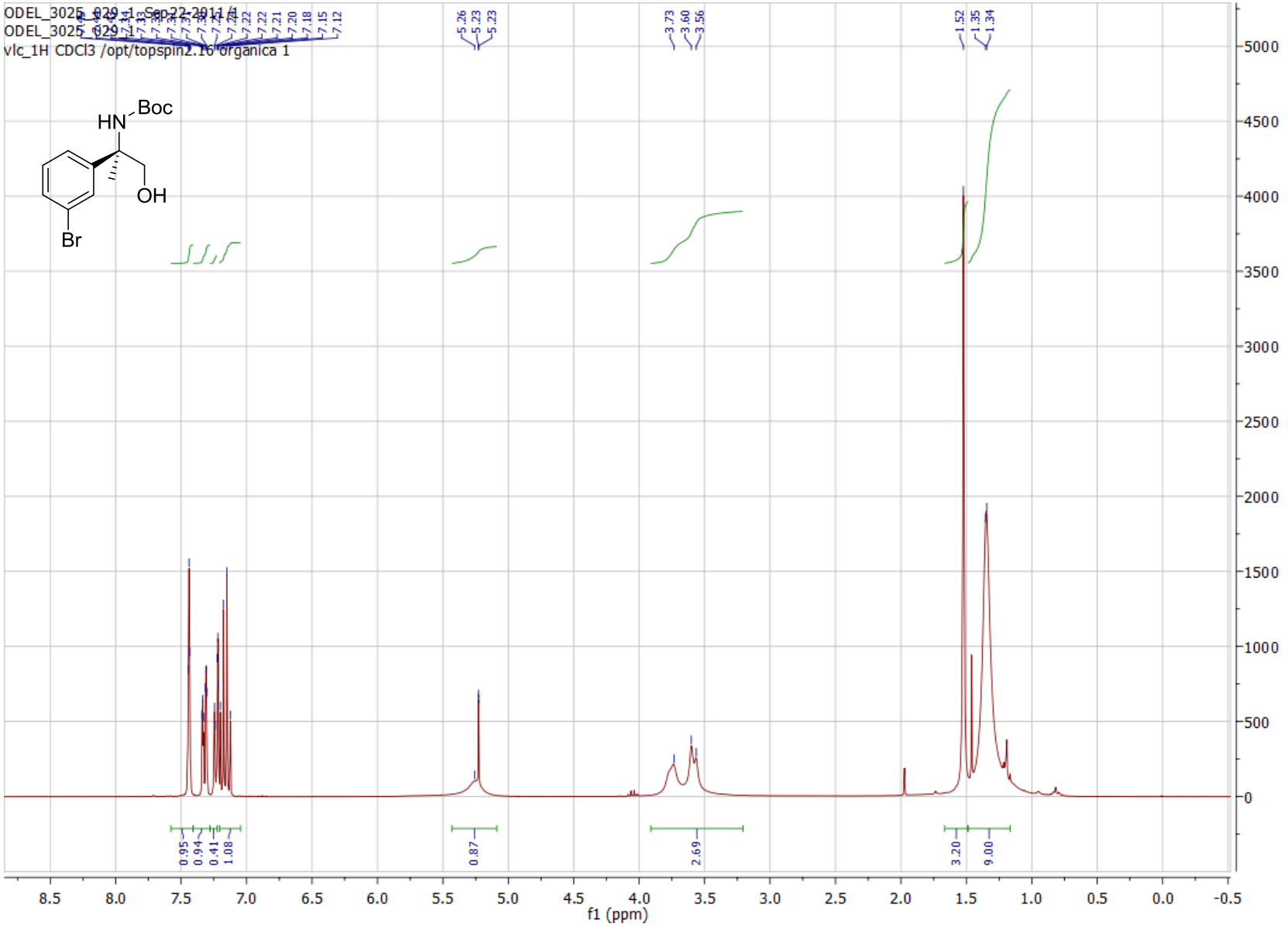
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*R*)-2-amino-2-(3-bromophenyl)propan-1-ol (**7**)



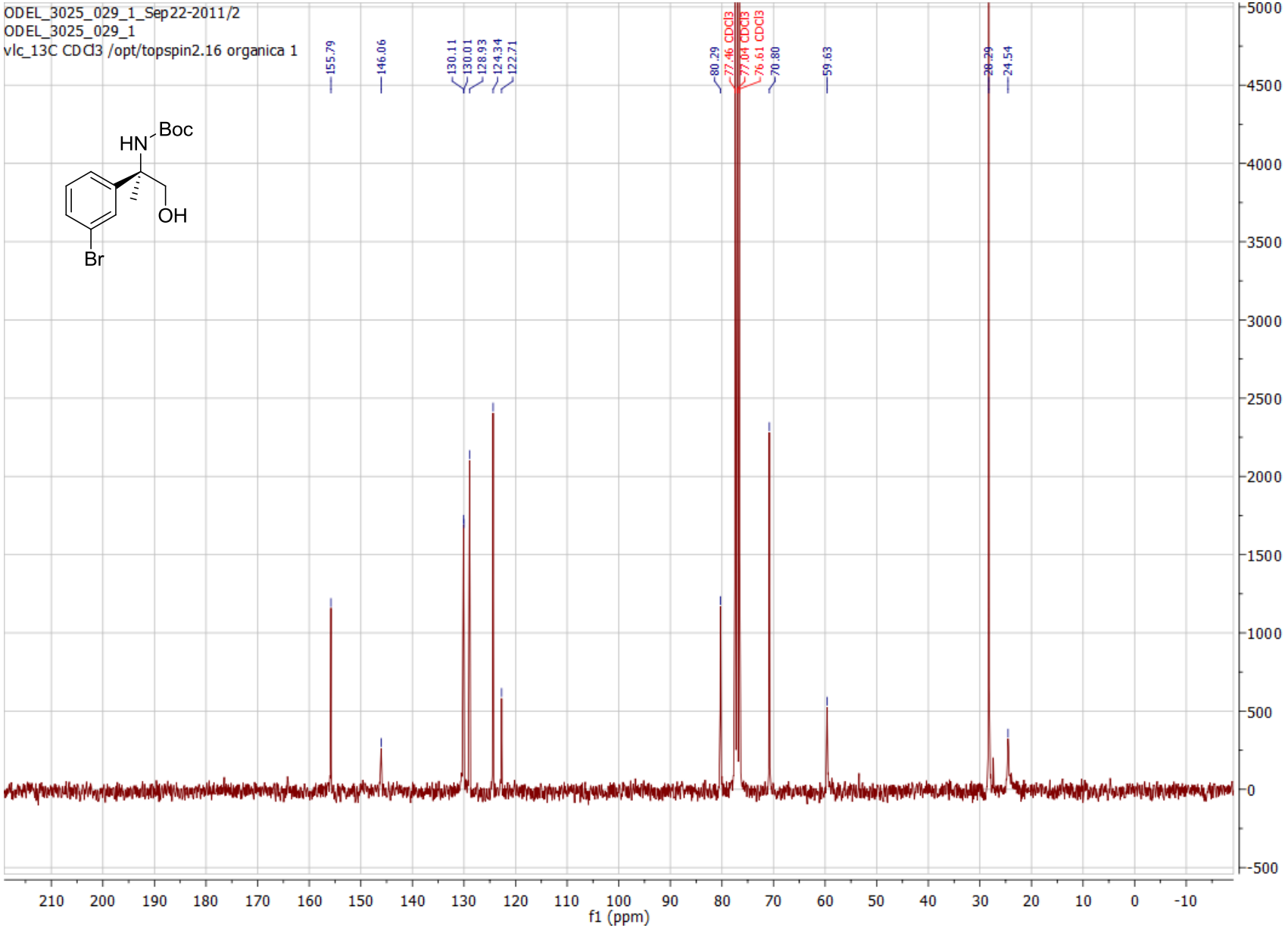
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*R*)-2-amino-2-(3-bromophenyl)propan-1-ol (**7**)



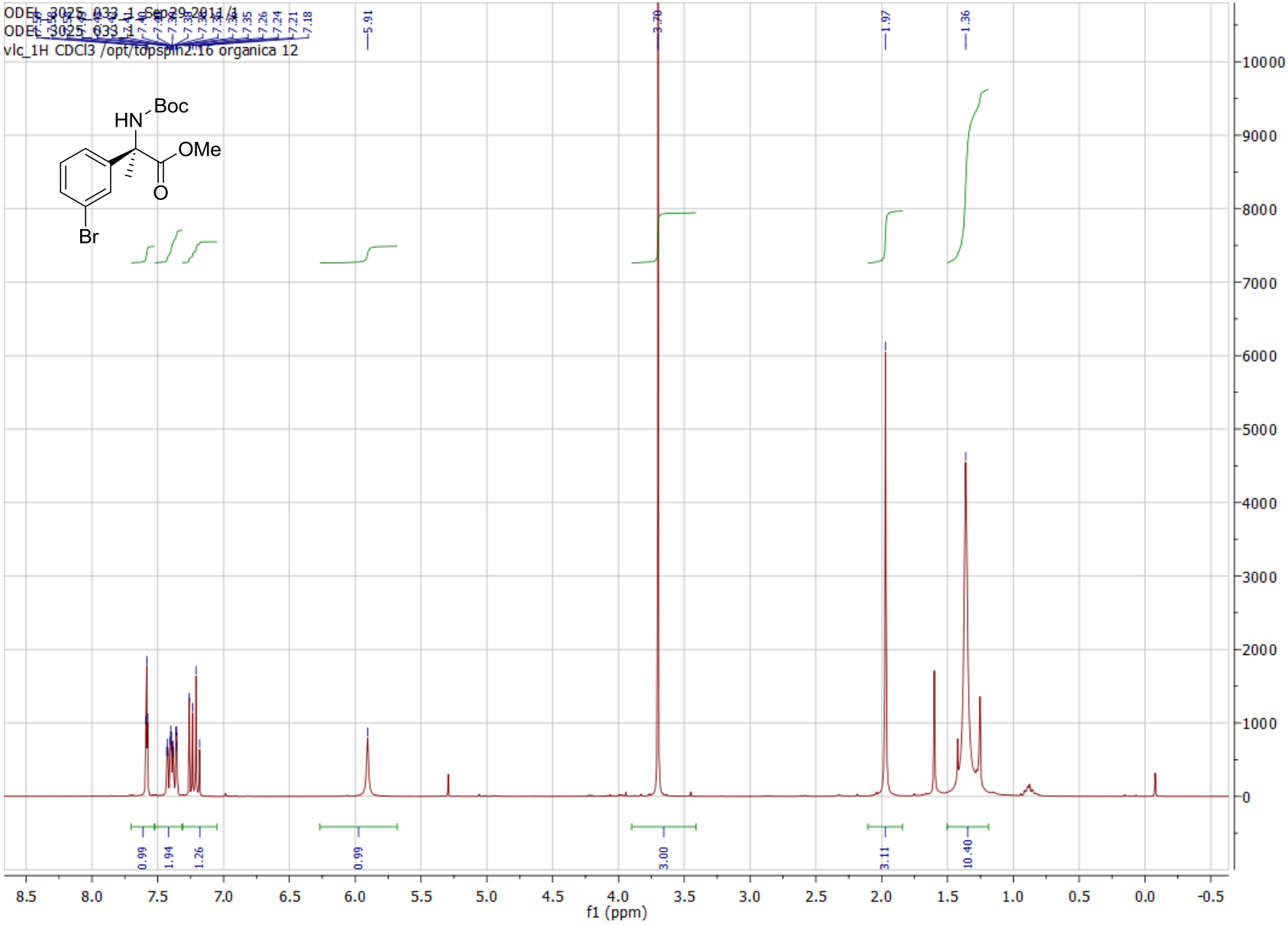
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*R*)-*tert*-Butyl 2-(3-bromophenyl)-1-hydroxypropan-2-ylcarbamate (**8**)



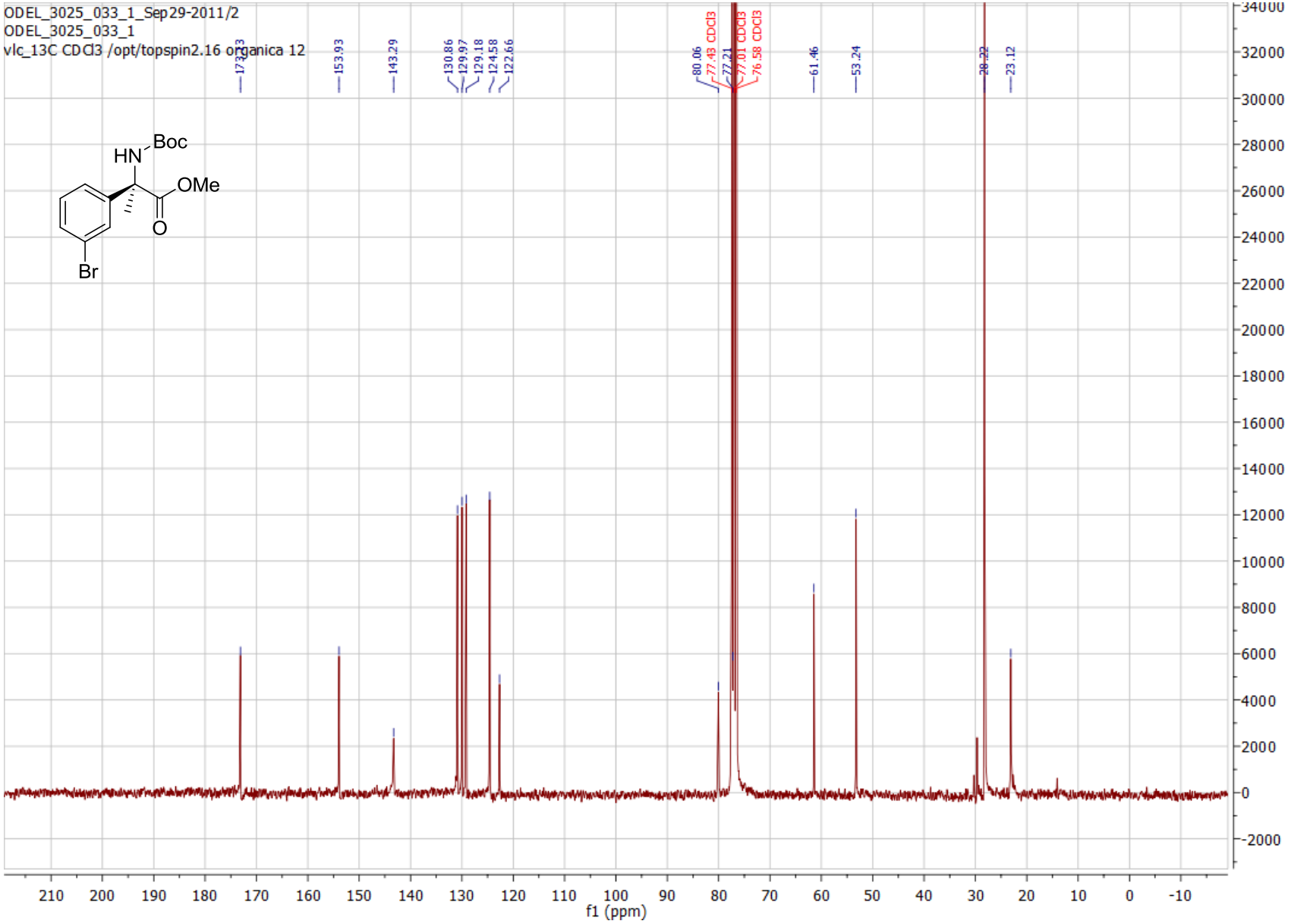
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*R*)-*tert*-Butyl 2-(3-bromophenyl)-1-hydroxypropan-2-ylcarbamate (**9**)



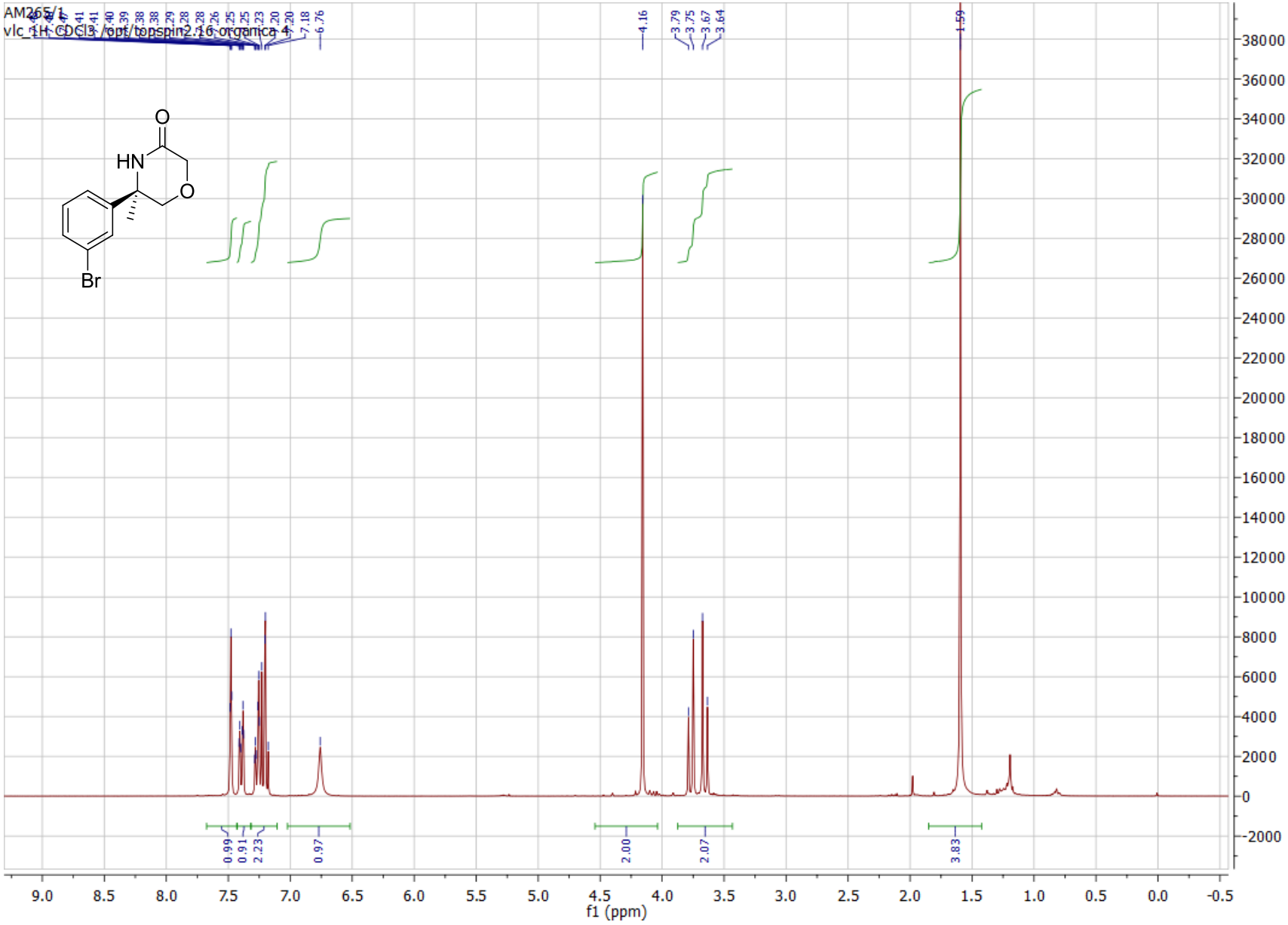
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*R*)-Methyl 2-(3-bromophenyl)-2-(*tert*-butoxycarbonylamino)propanoate (**9**)



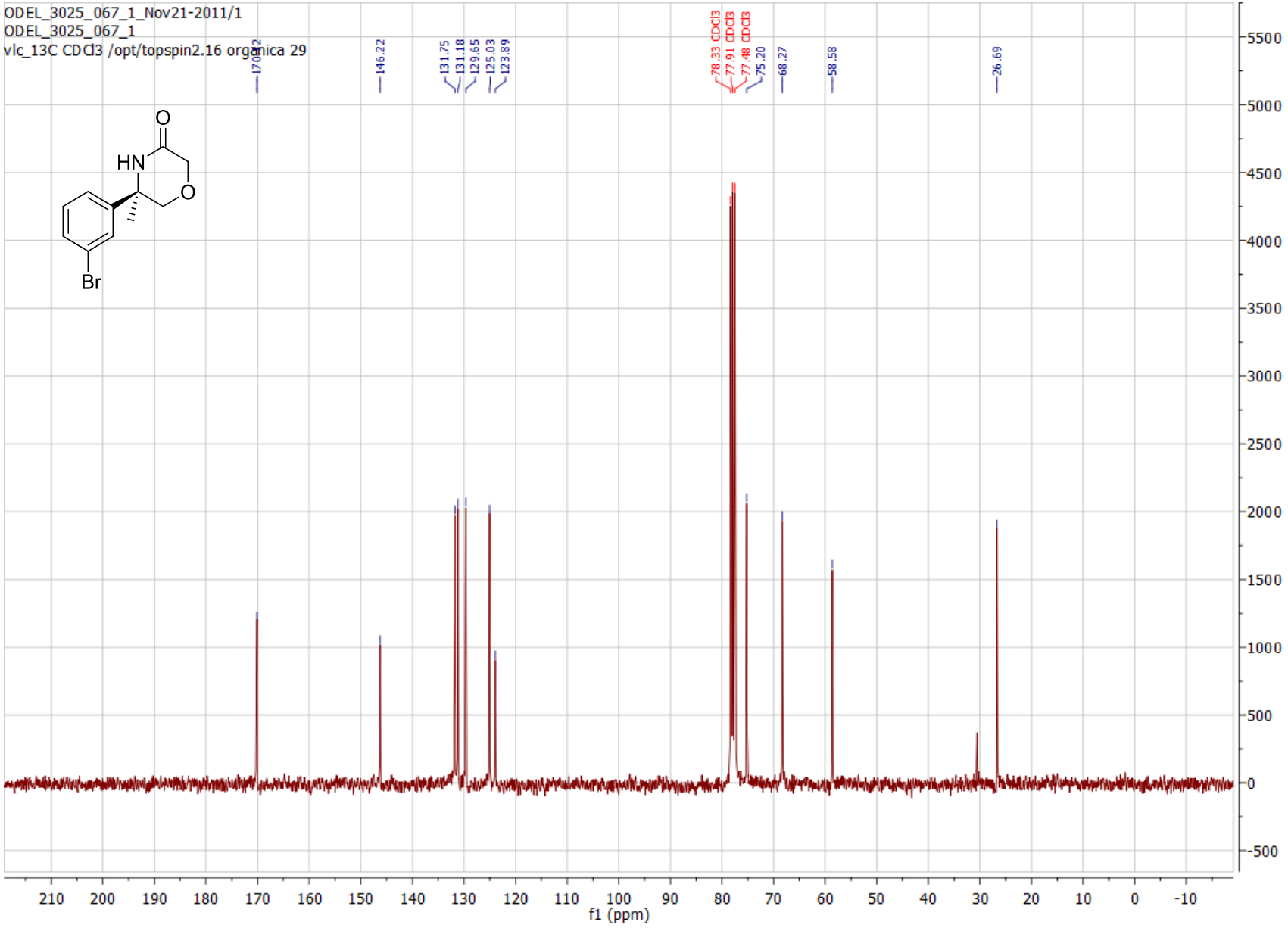
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*R*)-Methyl 2-(3-bromophenyl)-2-(*tert*-butoxycarbonylamino)propanoate (**9**)



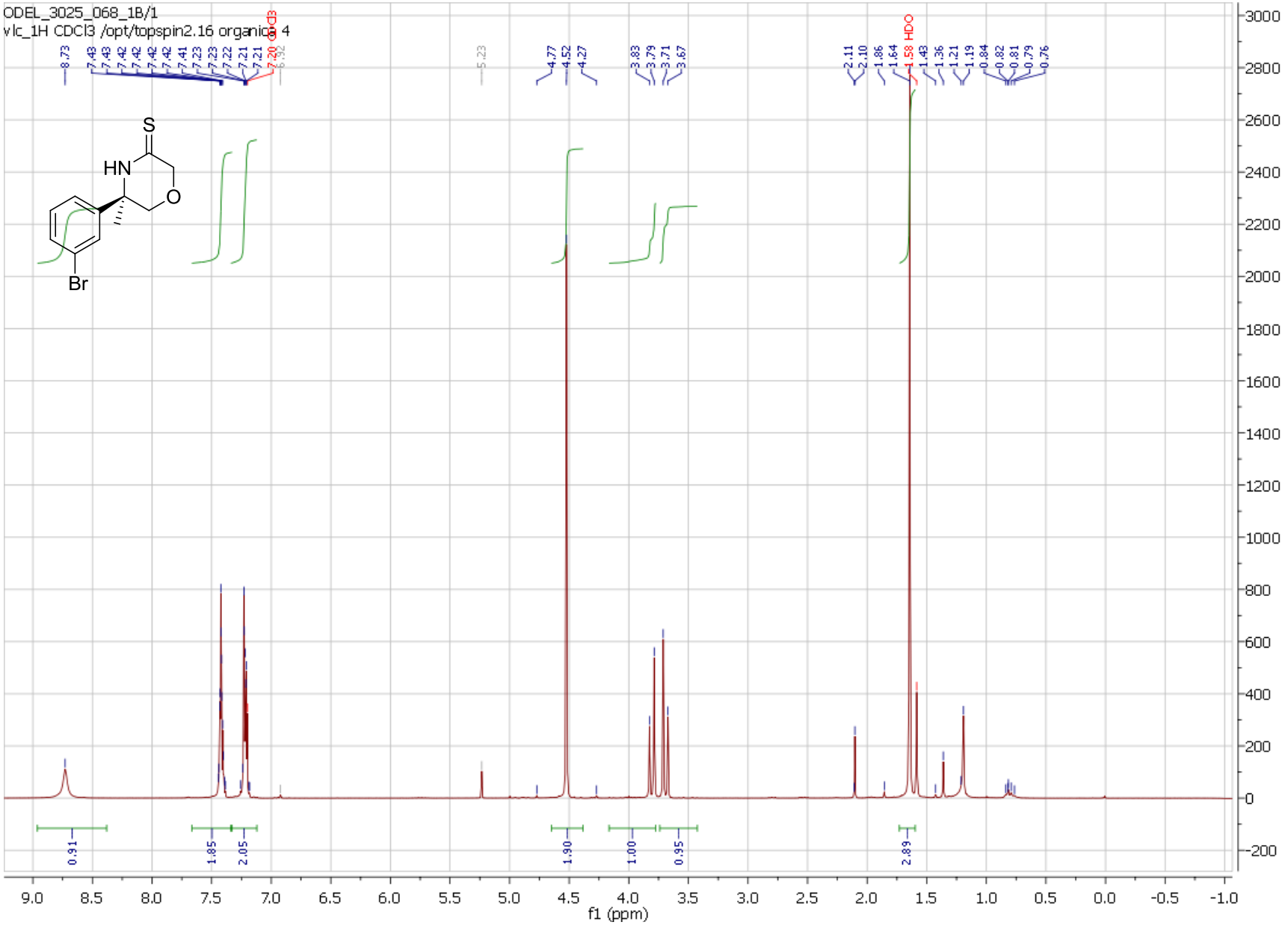
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*R*)-5-(3-Bromophenyl)-5-methylmorpholin-3-one (**10**)



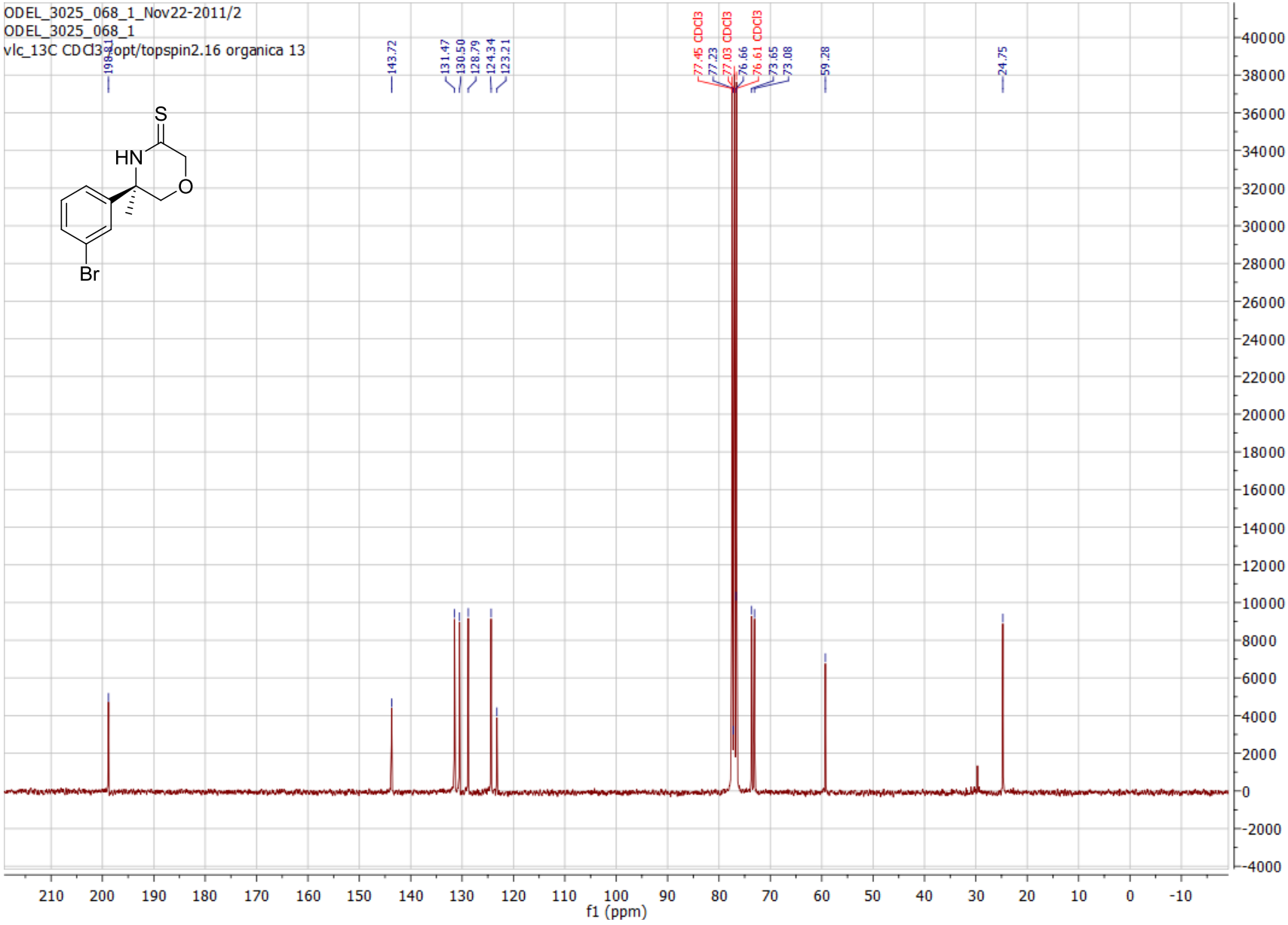
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*R*)-5-(3-Bromophenyl)-5-methylmorpholin-3-one (**10**)



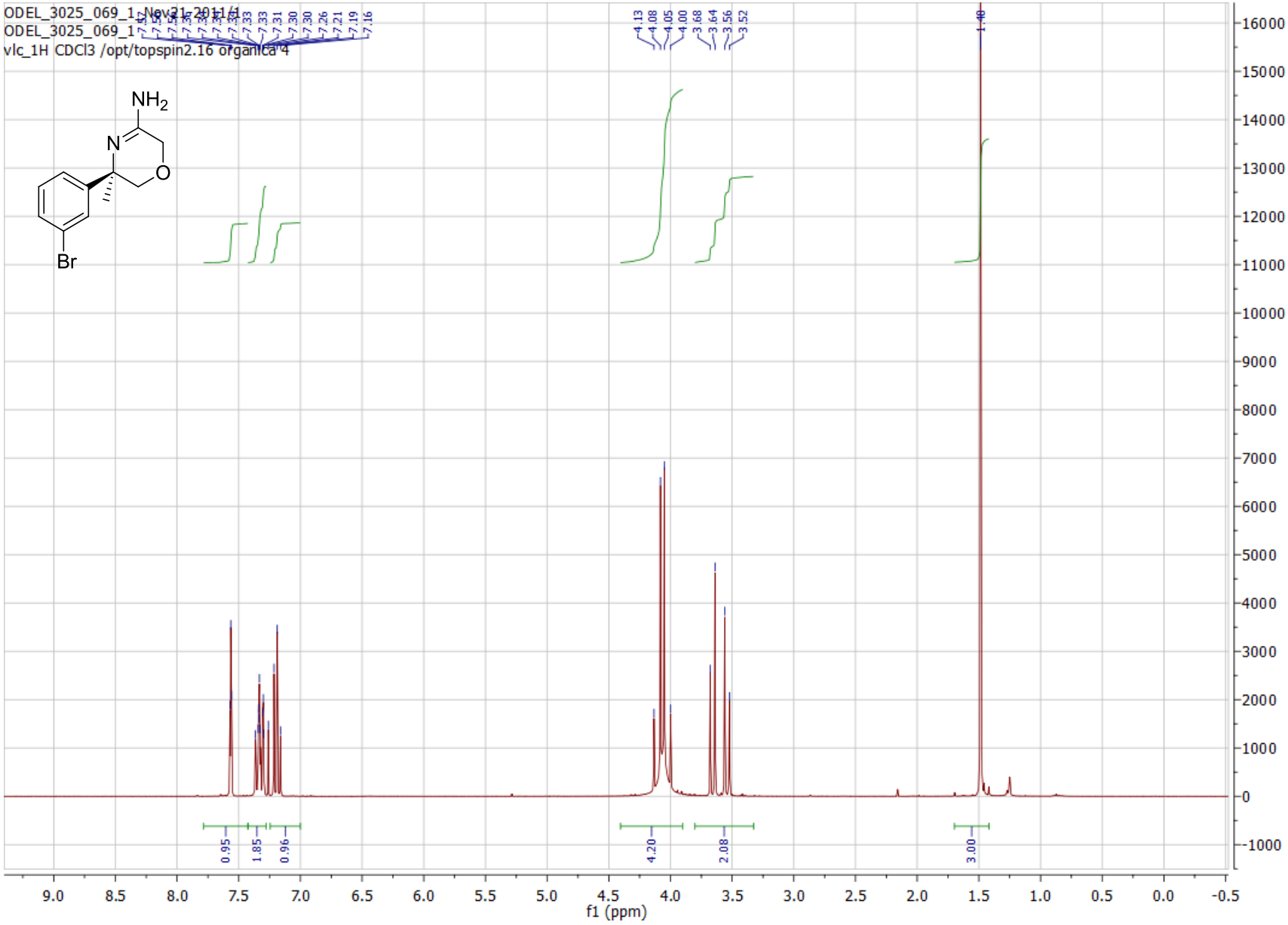
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*R*)-5-(3-Bromophenyl)-5-methylmorpholine-3-thione (**11**)



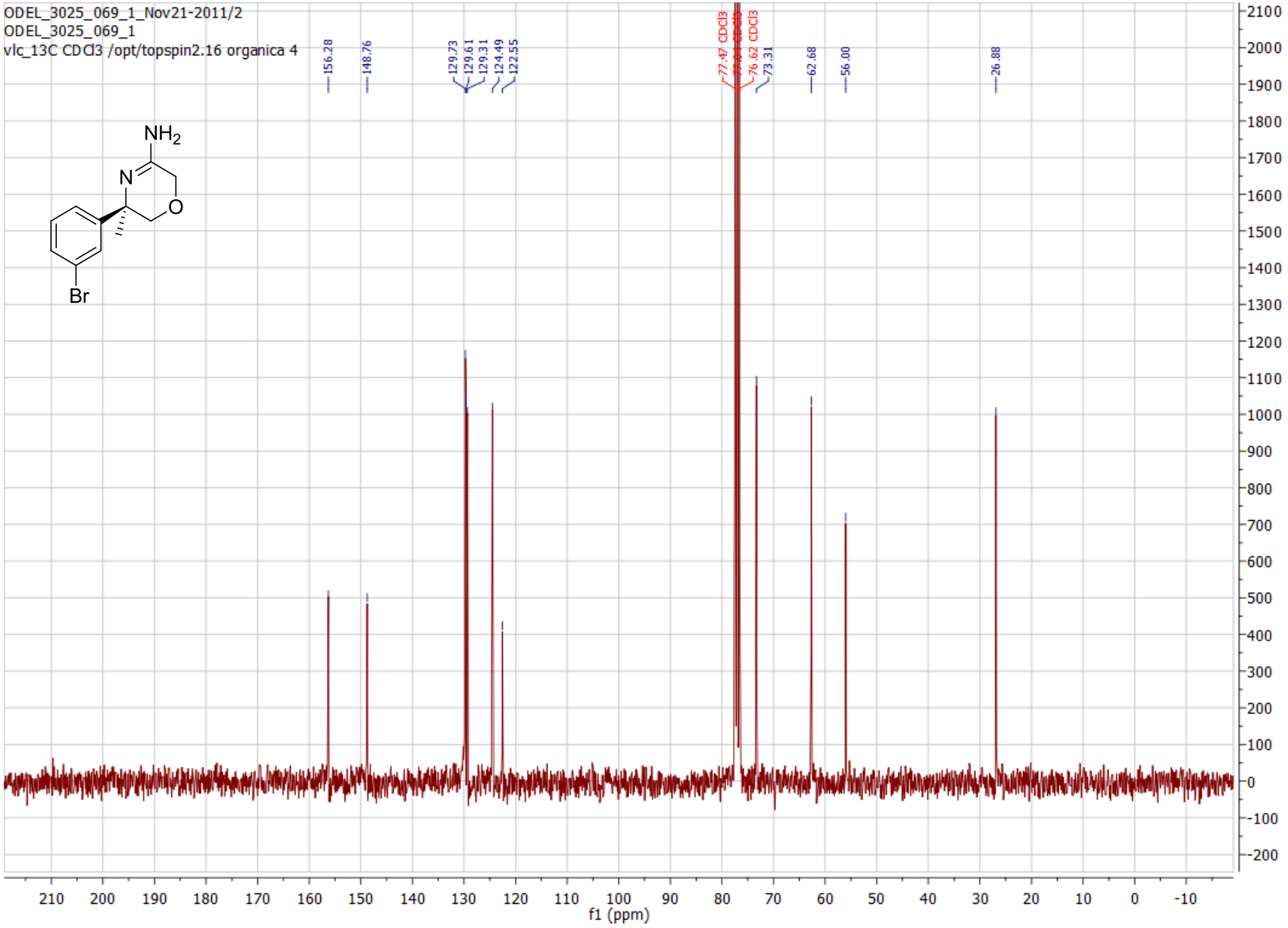
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*R*)-5-(3-Bromophenyl)-5-methylmorpholine-3-thione (**11**)



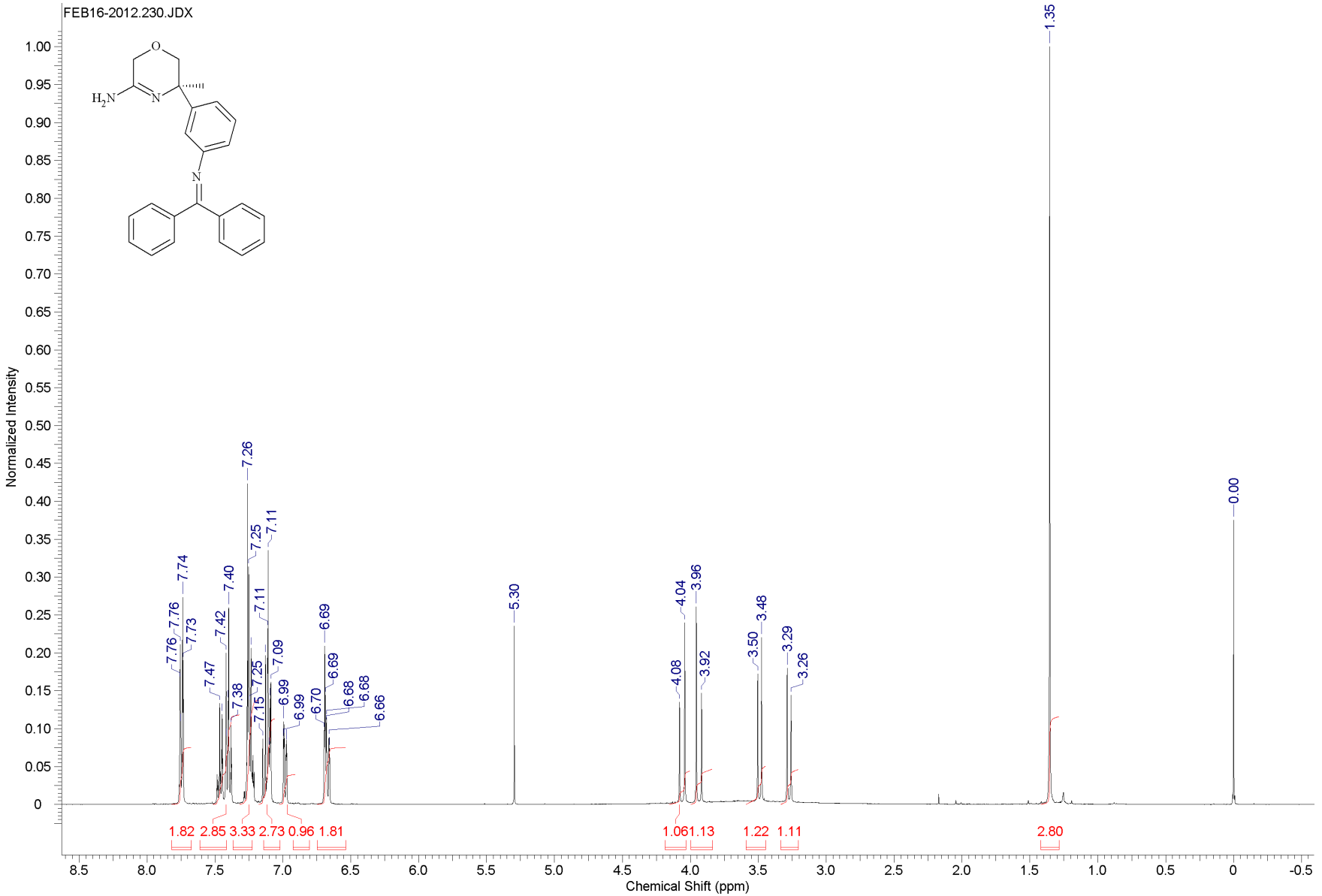
^{1}H-NMR ($CDCl_3$, 300 MHz) Spectrum of (*R*)-5-(3-bromophenyl)-5-methyl-5,6-dihydro-2H-1,4-oxazin-3-amine (**12**)



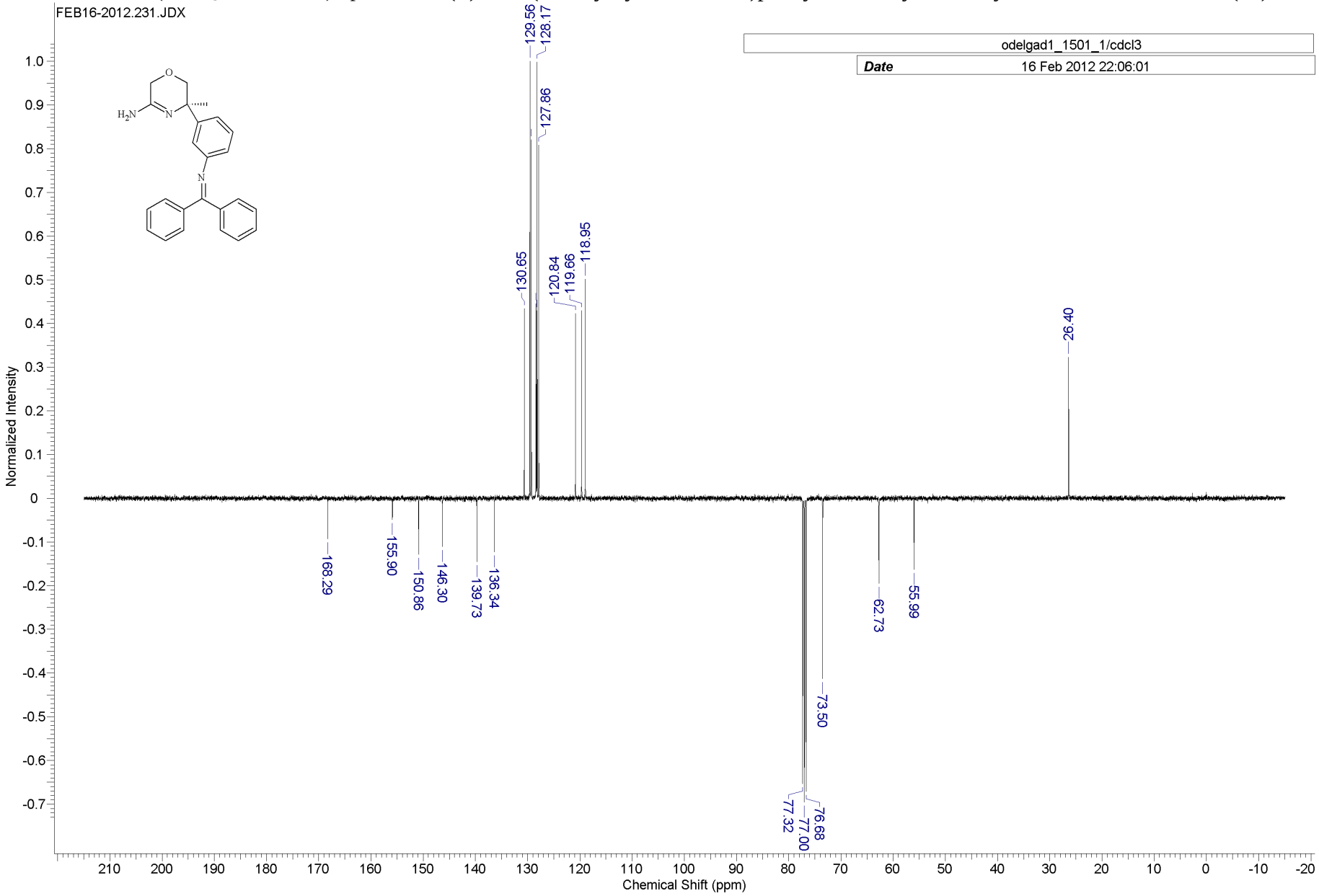
^{13}C-NMR ($CDCl_3$, 75.5 MHz) Spectrum of (*R*)-5-(3-bromophenyl)-5-methyl-5,6-dihydro-2H-1,4-oxazin-3-amine (**12**)



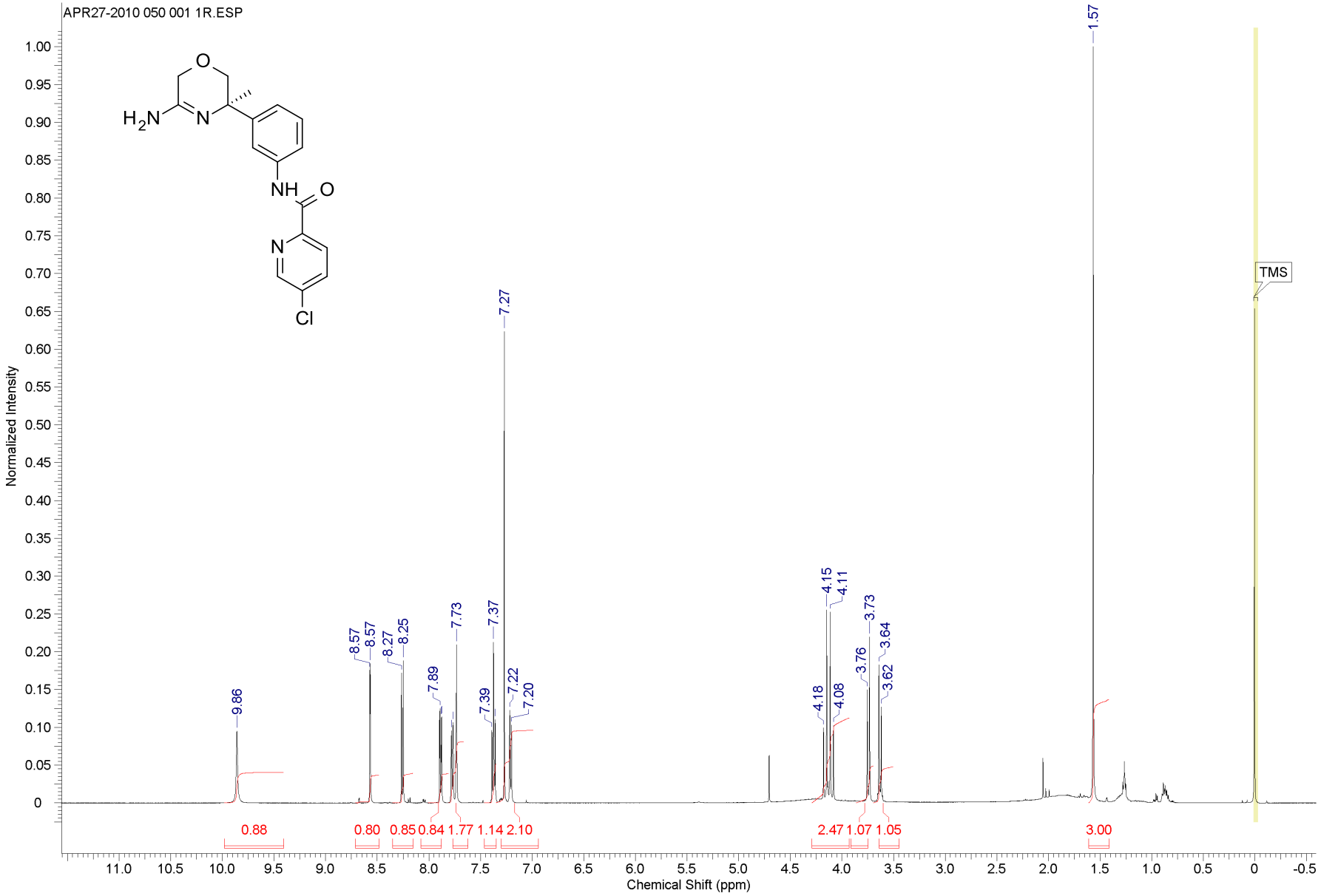
^{1}H-NMR ($CDCl_3$, 400 MHz) Spectrum of (*R*)-5-[3-(benzhydrylideneamino)phenyl]-5-methyl-2,6-dihydro-1,4-oxazin-3-amine (**13**)



^{13}C J-MOD NMR ($CDCl_3$, 100.6 MHz) Spectrum of (*R*)-5-[3-(benzhydrylideneamino)phenyl]-5-methyl-2,6-dihydro-1,4-oxazin-3-amine (**13**)



^{1}H-NMR ($CDCl_3$, 500 MHz) Spectrum of (*R*)-*N*-(3-(5-Amino-3-methyl-3,6-dihydro-2H-1,4-oxazin-3-yl)phenyl)-3-chloropicolinamide (**15**)



^{13}C-JMOD NMR ($CDCl_3$, 500 MHz) Spectrum of (*R*)-*N*-(3-(5-Amino-3-methyl-3,6-dihydro-2H-1,4-oxazin-3-yl)phenyl)-3-chloropicolinamide (**15**)