Supporting information

SYNTHESIS OF CYCLOOXYGENASE METABOLITES OF 8,9-EPOXYEICOSATRIENOIC ACID (EET): 11- AND 15-HYDROXY 8,9-EETS

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Supporting Figures (UHPLC-MS data)

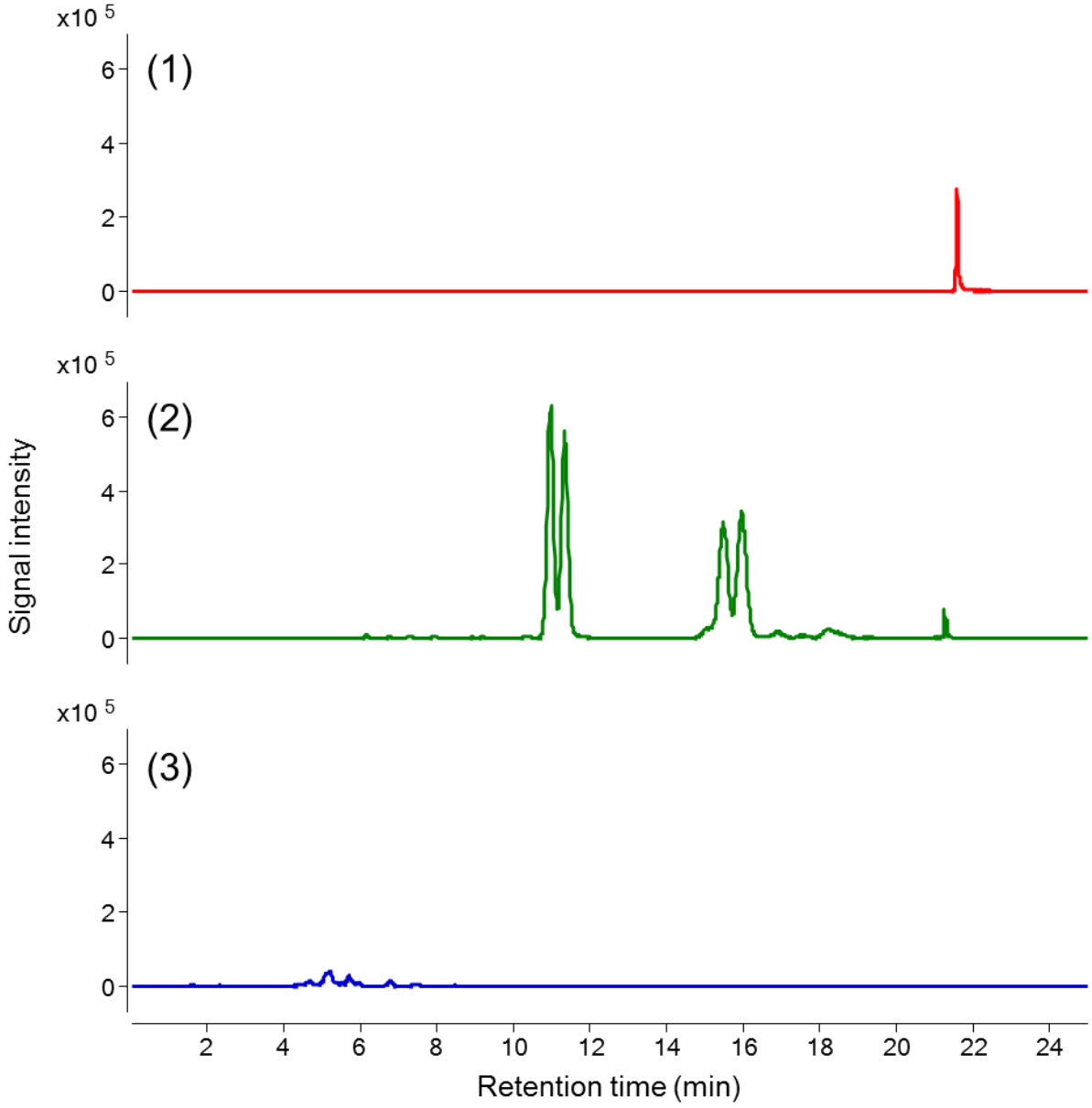


Figure S1. Extracted ion chromatograms for products formed in autoxidation reaction (see Scheme 1 of the paper). 1) 8,9-EET (*m/z* 319.2273); 2) EHETs (*m/z* 335.2222); 3) hydroperoxy 8,9-EETs (*m/z* 351.2172).

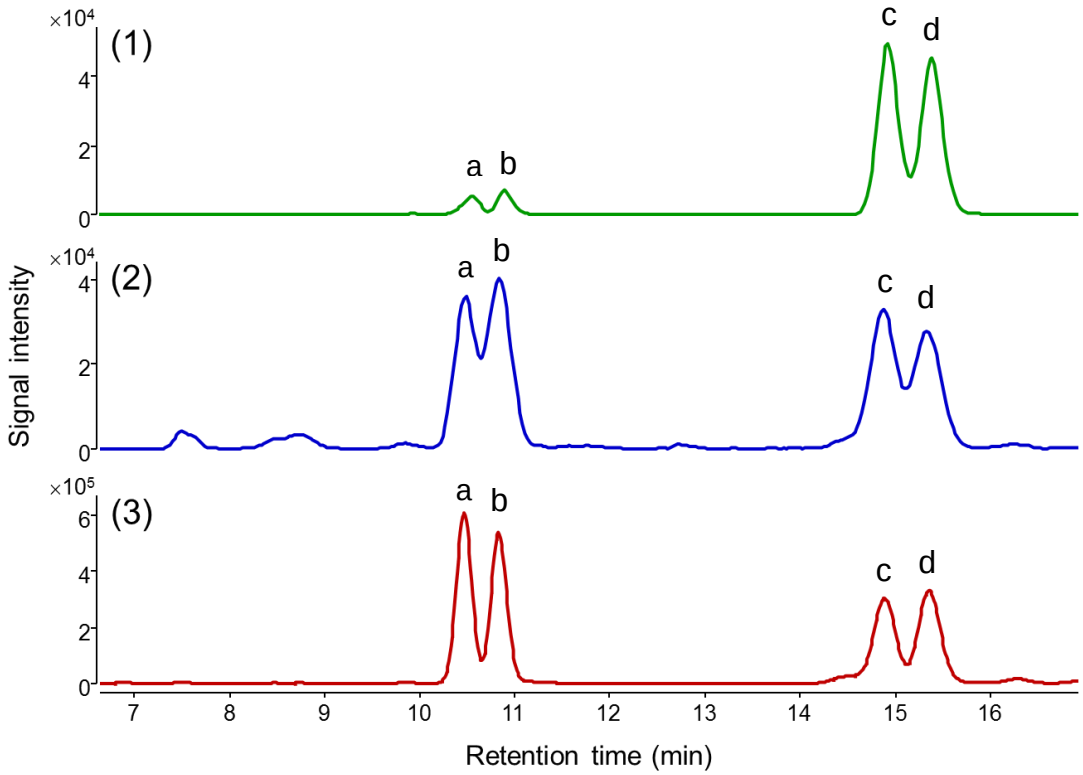


Figure S2. Extracted ion chromatograms (*m/z* 335.2222) for products obtained from 1) COX-1 enzymatic hydroxylation of (±)-8,9-EET; 2) COX-2 enzymatic hydroxylation of (±)-8,9-EET; 3) autoxidation of (±)-8,9-EET. The relative stereochemistry for EHETs corresponding to each chromatographic peak is assigned as follows: a) 8*S*,9*R*,15*R*-EHET; b) 8*R*,9*S*,15*R*-EHET; c) 8*R*,9*S*,11*R*-EHET; d) 8*S*,9*R*,11*R*-EHET. MS/MS spectra for peaks a-d from both enzymatic and autoxidation reactions are presented below in figures S6-S13.

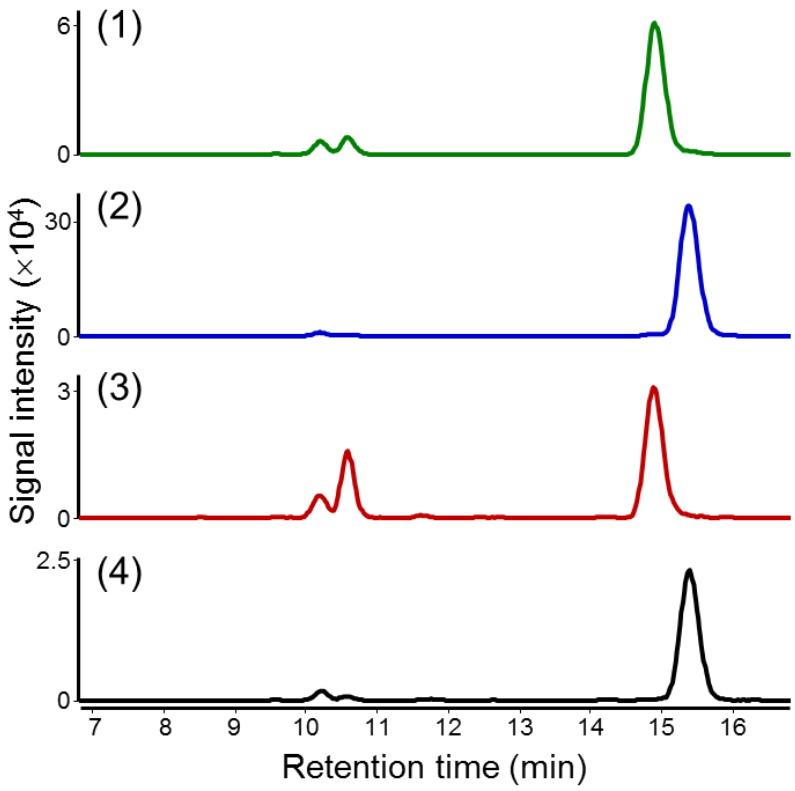


Figure S3. Extracted ion chromatograms (*m/z* 335.2222) for products obtained from 1) COX-1 enzymatic hydroxylation of 8*R*,9*S*-EET; 2) COX-1 enzymatic hydroxylation of 8*S*,9*R*-EET; 3) COX-2 enzymatic hydroxylation of 8*R*,9*S*-EET; 4) COX-2 enzymatic hydroxylation of 8*S*,9*R*-EET.

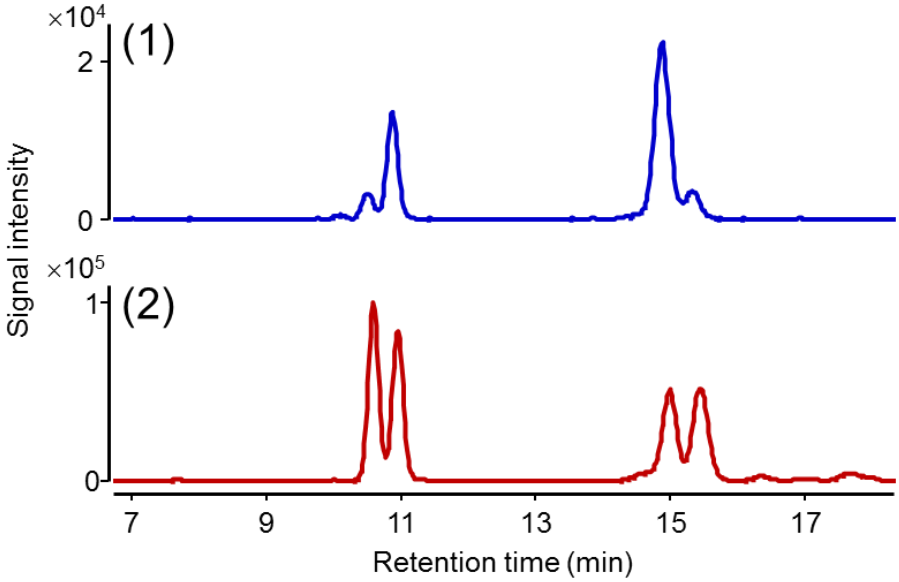


Figure S4. Extracted ion chromatograms (*m/z* 335.2222) for 1) synthetic 8*R*,9*S*,11*R*-EHET and 8*R*,9*S*,15*R*-EHET co-injected with products formed from the enzymatic reaction between COX-2 and (±)-8,9-EET; 2) products formed from free radical autoxidation of (±)-8,9-EET.

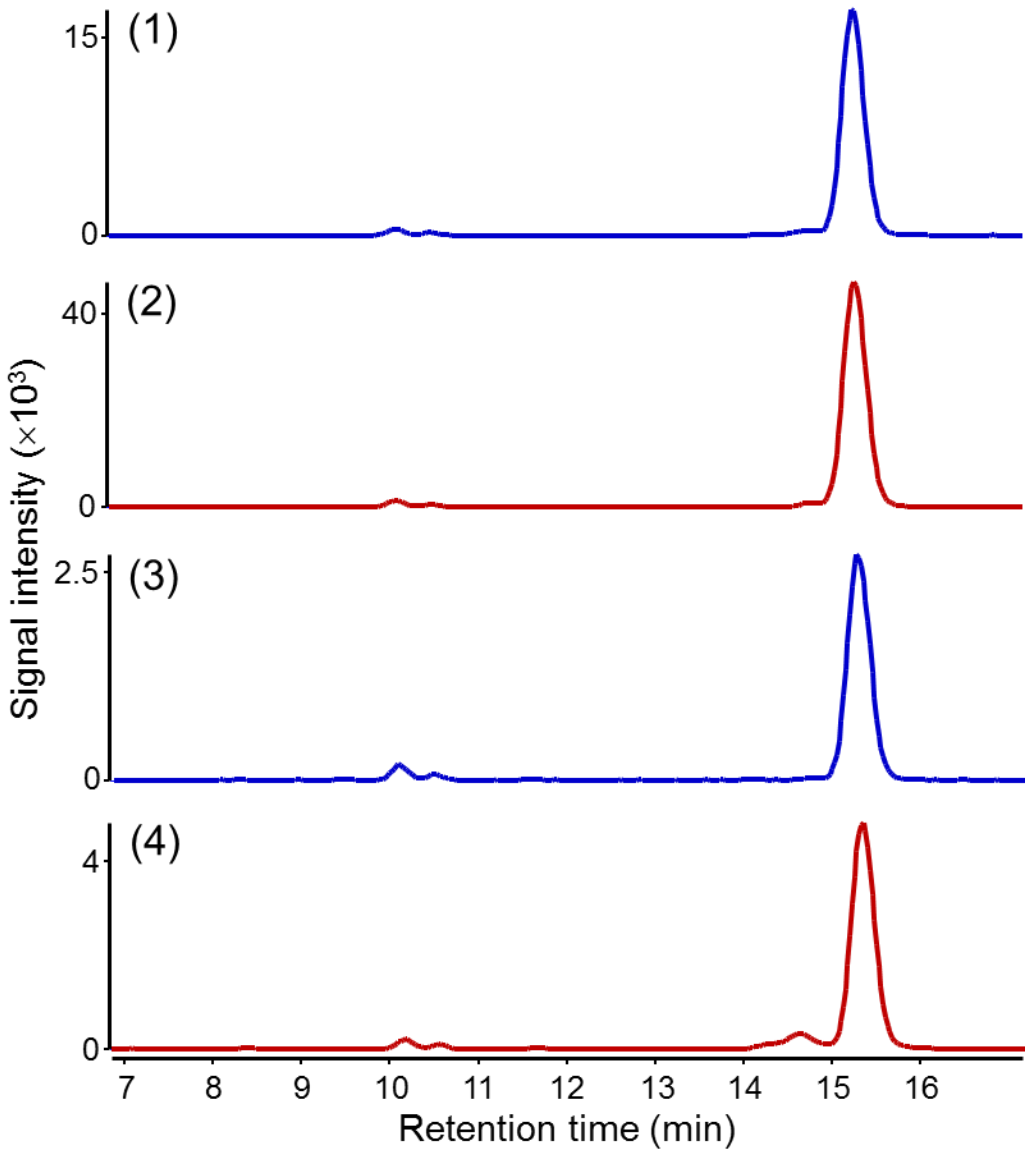


Figure S5. Extracted ion chromatograms (*m/z* 335.2222) for 1) COX-1 enzymatic hydroxylation of 8*S*,9*R*-EET; 2) synthetic 8*S*,9*R*,11*R*-EET co-injected with products formed from the enzymatic reaction between COX-1 and 8*S*,9*R*-EET; 3) COX-2 enzymatic hydroxylation of 8*S*,9*R*-EET; 4) synthetic 8*S*,9*R*,11*R*-EET co-injected with products formed from the enzymatic reaction between COX-2 and 8*S*,9*R*-EET.

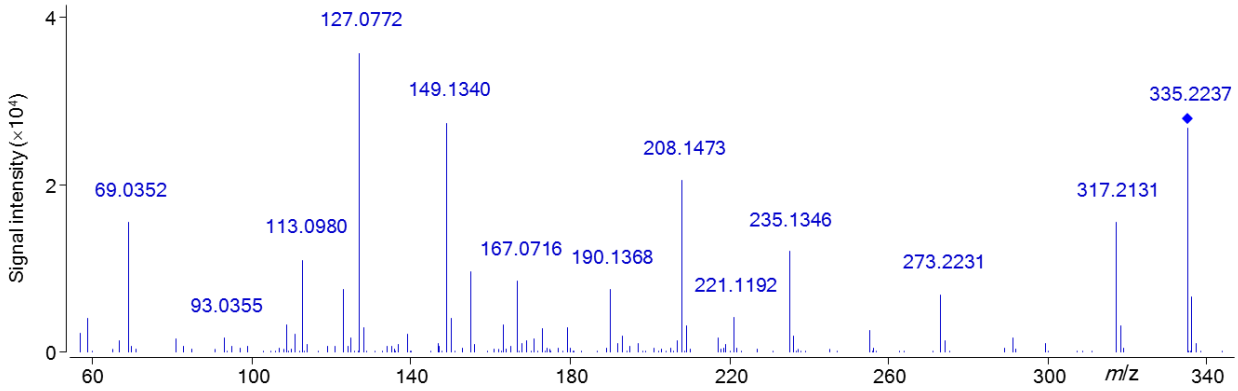


Figure S6. MS/MS spectrum for peak a) from autoxidation reaction of the (±)-8,9-EET.

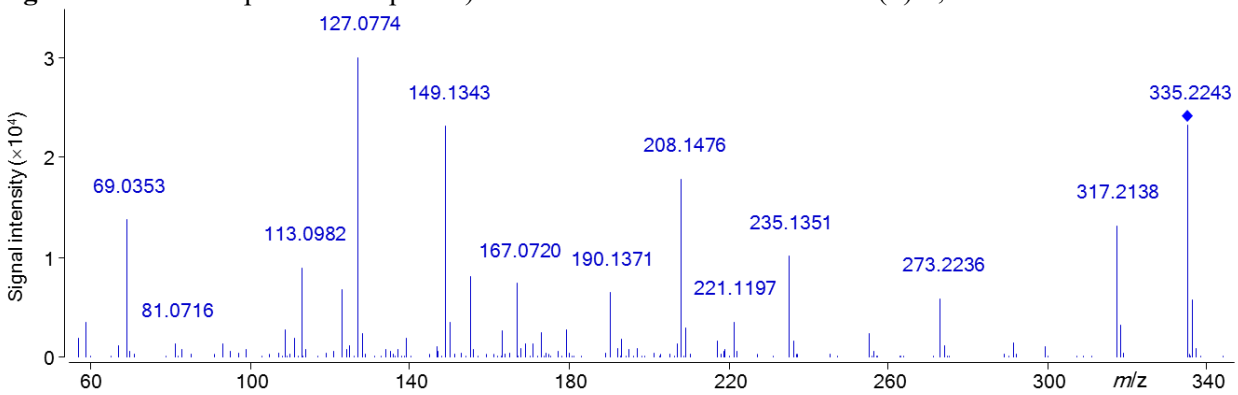


Figure S7. MS/MS spectrum for peak b) from autoxidation reaction of the (±)-8,9-EET.

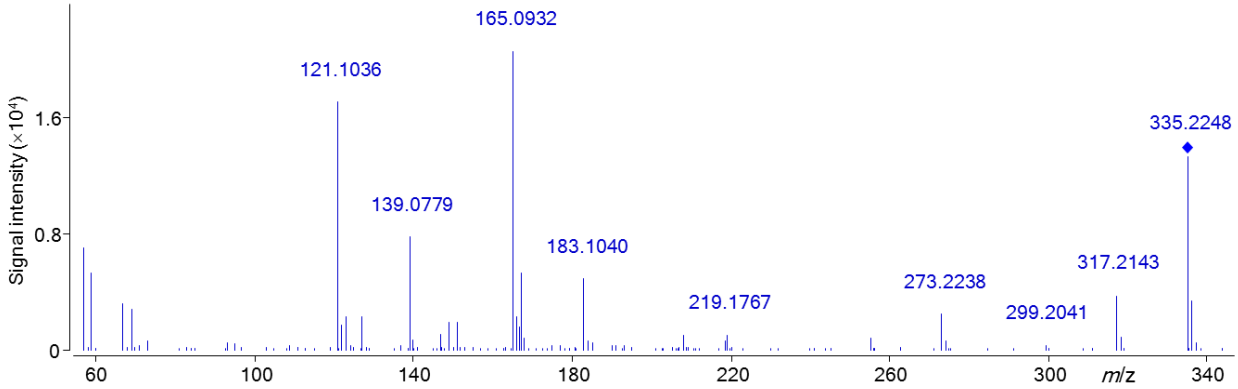


Figure S8. MS/MS spectrum for peak c) from autoxidation reaction of the (±)-8,9-EET.

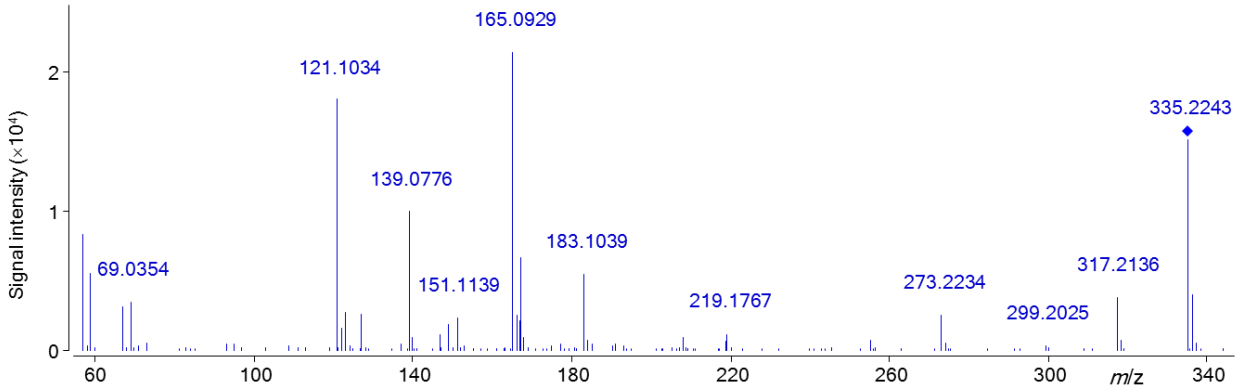


Figure S9. MS/MS spectrum for peak d) from autoxidation reaction of the (±)-8,9-EET.

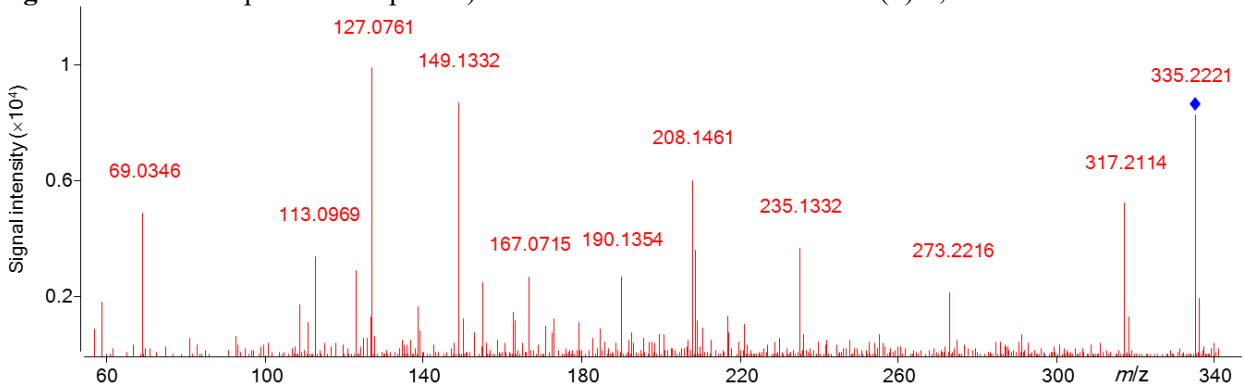


Figure S10. MS/MS spectrum for peak a) from COX-2 enzymatic hydroxylation of the (±)-8,9-EET

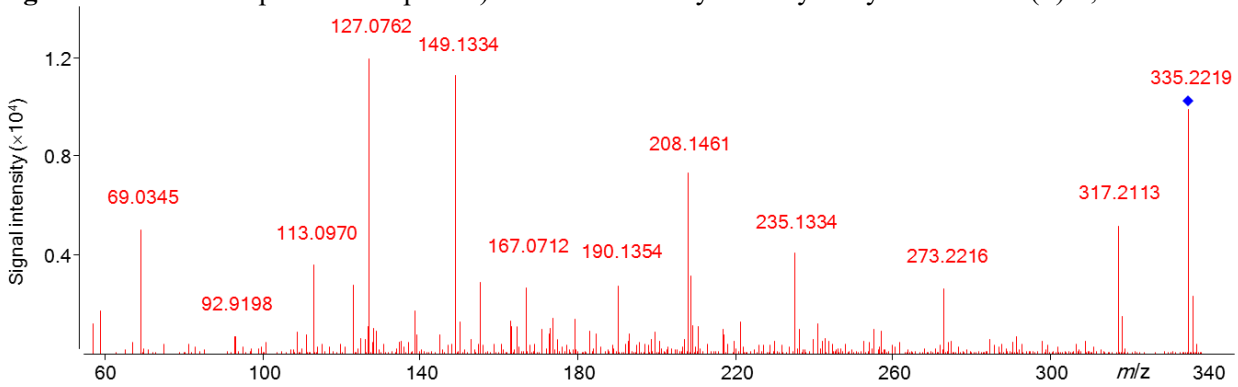


Figure S11. MS/MS spectrum for peak b) from COX-2 enzymatic hydroxylation of the (±)-8,9-EET

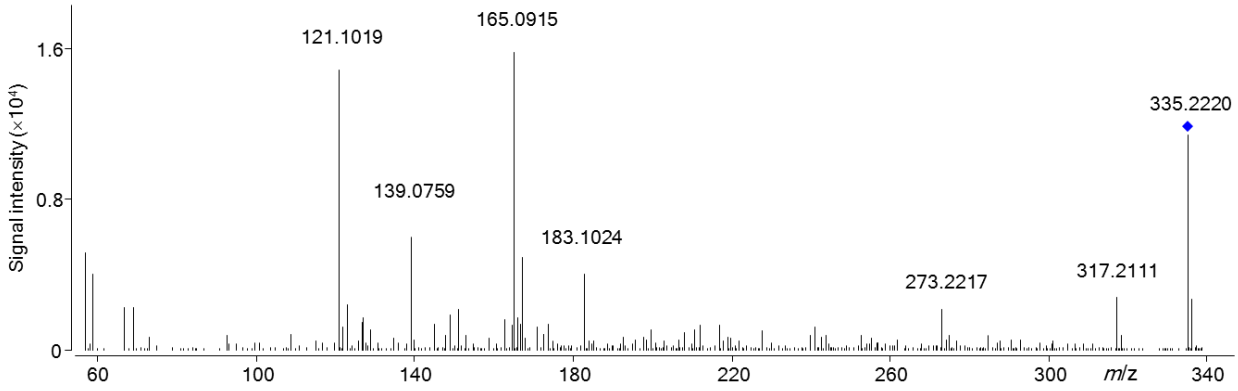


Figure S12. MS/MS spectrum for peak c) from COX-2 enzymatic hydroxylation of the (±)-8,9-EET

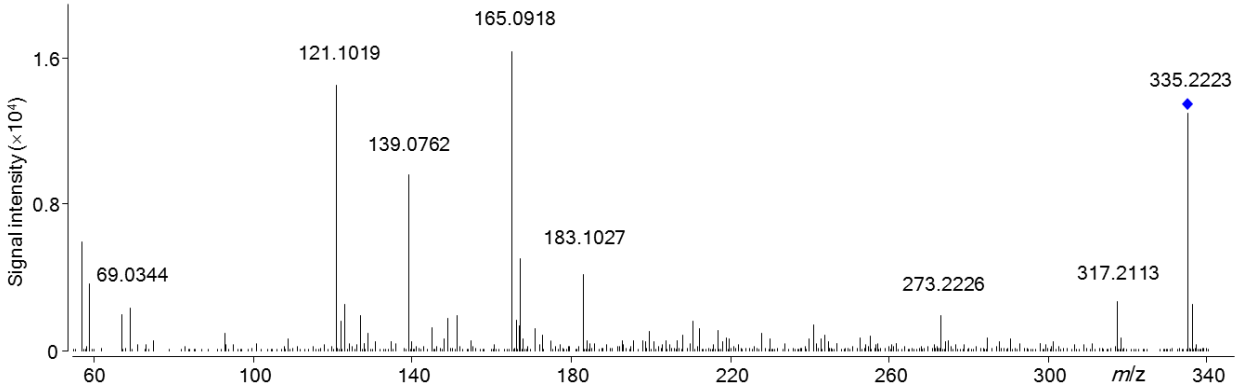


Figure S13. MS/MS spectrum for peak d) from COX-2 enzymatic hydroxylation of the (±)-8,9-EET

General Information

Materials. 8,9-EET was chemically synthesized in our lab[1] and stored at −80 °C. Arachidonic acid, ovine COX-1, and human recombinant COX-2 were purchased from Cayman Chemical Company (Ann Arbor, MI). Optima™ grade methanol and water were purchased from Fisher Scientific (Waltham, MA). The sEH inhibitor 1471 was synthesized in house. Radical initiator V-70 was purchased from Wako Chemicals (Richmond, VA). All other chemical reagents were purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI) or Fisher Scientific (Houston, TX).

All reactions were carried out under an atmosphere of dry nitrogen. All chemicals purchased from commercial sources were used as received without further purification unless indicated. Analytical thin-layer chromatography (TLC) was performed on Merck TLC silica gel 60 F_{254} plates. Flash chromatography was performed on silica gel (230–400 mesh) from Macherey Nagel. NMR spectra were recorded on Varian VNMRS 600, Inova 400, Mercury 300 or Bruker Avance III 800 MHz instruments. Multiplicity is described by the abbreviations b = broad, s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet. Chemical shifts are given in ppm. ^{1}H NMR spectra were referenced to the residual solvent peak at δ = 7.26 ($CDCl_3$). ^{13}C NMR spectra were referenced to the solvent peak at δ = 77.16 ($CDCl_3$). FT-IR spectra were recorded on a Thermo Scientific NICOLET IR100 FT-IR spectrometer and are reported in wave numbers (cm^{-1}).

8,9-EET metabolism by COX-1 and COX-2. EHET products were formed from 8,9-EET incubations with COX-1 and COX-2. These enzymatic reactions were carried out using 2 mL plastic Eppendorf vials in Tris-HCl buffer (100 mM, pH 8.0, with 5 mM EDTA) containing bovine hematin (2 μM) and phenol (1 mM). This solution was warmed to 37 ºC before adding COX-1 or COX-2 ($C_{final} \approx 5$ μg/mL). 8,9-EET (C_{final} = 40 μM) in 1:1 ethanol/Tris-HCl buffer was then added to initiate the reaction. The amount of ethanol was 0.8% of the total incubation volume (300 μL). Reactions were stopped after 5 min with ice-cold methanol (600 μL), centrifuged at 13,000g for 10 min, and filtered. Products from these reactions were analyzed using LC-QToF-MS or LC-MS/MS.

LC–MS Analysis. The system consisted of an Agilent 1290 Infinity LC system (Agilent Technologies) with a pump (G4220A), a column oven (G1316C), an autosampler (G4226A), and an Agilent 6550 iFunnel QTOFMS. Samples were separated on an Acquity UPLC CSH C18 column (100 × 2.1 mm; 1.7 μm) (Waters). The column was maintained at 40 °C at a flow-rate of 0.4 mL/min. The mobile phases consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The separation was conducted with the following gradient: 0–20 min 40% (B); 20–20.5 min 100% (B); 20.5–25.0 min 100% (B); 25.0–25.1 min 40% (B); 25.1–30 min 40% (B). A sample volume of 3 μL was used for the injection. Sample temperature was maintained at 4 °C. The QTOF instrument was operated in electrospray ionization (ESI) in negative mode with following parameters: MS[1] mass range: *m/z* 50–1700; MS/MS mass range: *m/z* 50–1700; collision energy: −15 eV; capillary voltage: −3.5 kV; nozzle voltage: −1 kV; gas temperature, 200 °C; drying gas (nitrogen), 14 L/min; nebulizer gas (nitrogen), 35 psi; sheath gas temperature, 350 °C; sheath gas flow (nitrogen), 11 L/min; acquisition rate MS[1], 5 spectra/s (200 ms); acquisition rate MS/MS, 1 spectrum/s (1000 ms); precursor ion, *m/z* 335.2222; isolation window, 1.3 *m/z*. The instrument was tuned using an Agilent tune mix (mass resolving power ~20,000 FWHM). A reference solution (*m/z* 119.0363, *m/z* 966.0007) was used to correct small mass drifts during the acquisition. For the data processing the MassHunter Qualitative (B.05.00) Analysis (Agilent) software program was used.

[1] Morisseau, C.; Inceoglu, B.; Schmelzer, K.; Tsai, H.-J.; Jinks, S. L.; Hegedus, C. M.; Hammock, B. D. *J. Lipid Res.* **2010**, *51* (12), 3481.

GC–MS Analysis. The system consisted of an Agilent GC–MS system (Agilent Technologies); a 7693 Series autosampler, a split/splitless injector, a 7890A GC system, and an accurate-mass quadrupole/time-of-flight mass spectrometer 7200 operated in positive chemical ionization (PCI). Injection parameters were as follows: injection volume, 1 µL; injector temperature, 250 °C; helium carrier gas flow, 1 mL/min; splitless period, 1 min. For GC separation a 30 m × 0.25 mm, 0.25 µm Rtx-5Sil MS (Restek) capillary column with a 10 m × 0.25 mm integrated Guard column was used with oven temperature program: 60 °C (1 min), 10 °C/min to 325 °C (hold 9.5 min). CI gas: methane; CI gas flow: 20 %. MS detection parameters were as follows: acquisition rate, 5 spectra/s; mass range, *m/z* 85–1000; MS ion source temperature, 300 °C; MS quadrupole temperature, 150 °C; electron multiplier voltage, 750 V. For the data processing the MassHunter Qualitative (B.05.00) Analysis (Agilent) software program was used.

Chiral separation of 8*R*,9*S*-EET and 8*S*,9*R*-EET.

Enantiomers of 8,9-EET were separated on Agilent 1200 Series HPLC system with Lux Cellulose-3 column (250 × 4.6 mm, 5.0 µm) and hexane/*i*PrOH/AcOH = 99.4:0.5:0.1 as mobile phase. A sample volume of 10 µL was used for the injection. Previous research shows that mouse liver soluble epoxide hydrolase (sEH) hydrolyzes 8*S*,9*R*-EET more than 10 times faster than 8*R*,9*S*-EET enantiomer.[2] This difference in hydrolysis kinetics was used for elucidation of the absolute stereochemistry of 8,9-EETs corresponding to each chromatographic peak. (±)-8,9-EET was reacted with rat sEH as described below and the residual 8,9-EET was analyzed by chiral HPLC. As can be seen from the scheme S12 the intensity of the second peak (RT~23.9 min) is significantly lower compared to the first peak (RT~22.3 min) suggesting it to be 8*S*,9*R*-EET.

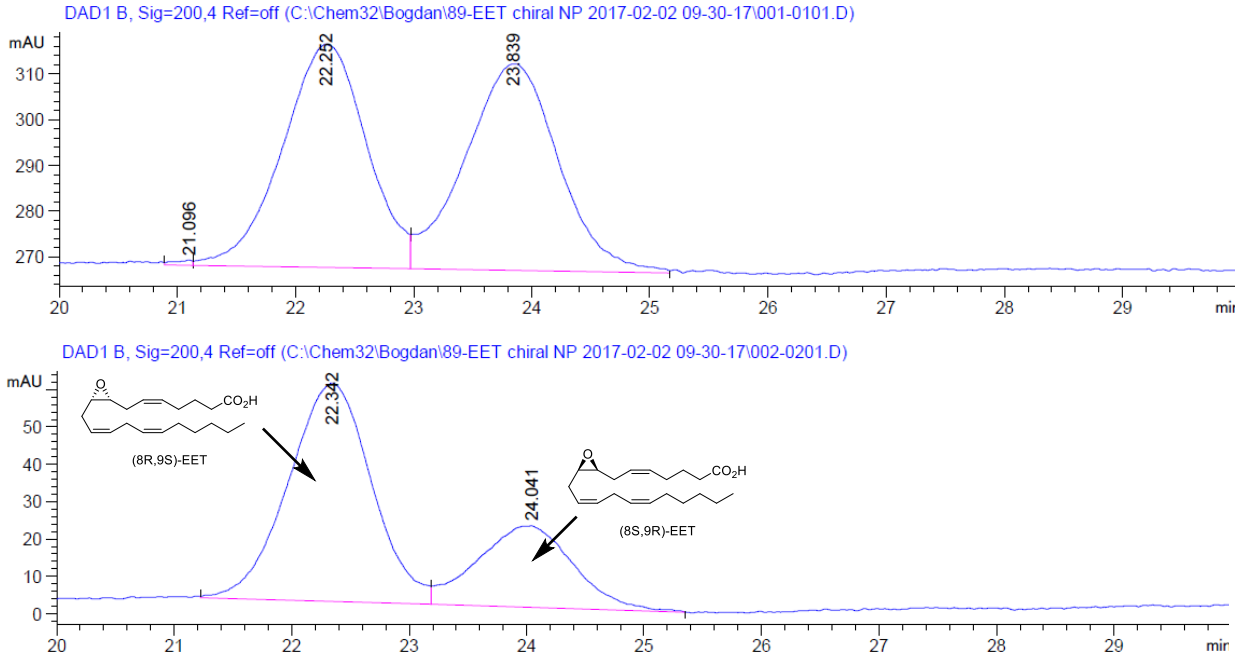


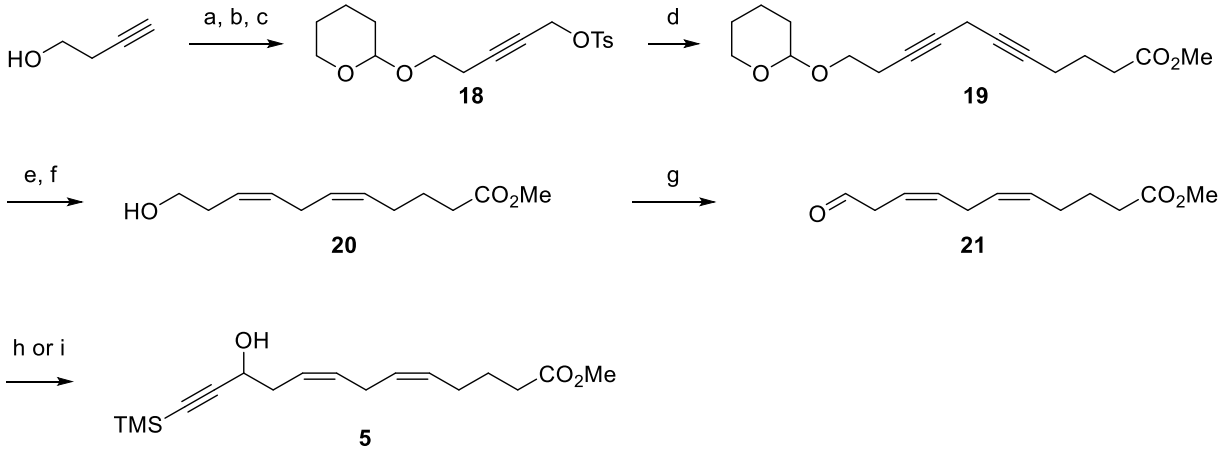
Figure S14. HPLC-UV chromatogram for residual 8,9-EET after incubation with soluble epoxide hydrolase (bottom) and blank reaction (same conditions without enzyme, top).

[2] D. C. Zeldin, J. Kobayashi, J. R. Falck, B. S. Winder, B. D. Hammock, J. R. Snapper and J. H. Capdevila, *J. Biol. Chem.*, 1993, **268**, 6402–7.

Hydrolysis of 8,9-EET by sEH. 2 µL of 8,9-EET (45 mM in EtOH) was added to the solution of pure sEH (5 µg/mL, from mouse liver cytosol) and BSA (0.1 g/mL) in PBS buffer (600 µL, 10 mM). The resulting mixture was incubated at 37 °C for 30 min, the reaction was stopped by the addition of MeOH (100 µL) and the residual 8,9-EET was extracted with Et_2O (3 × 100 µL). Combined ethereal solutions were dried over $MgSO_4$, filtered, evaporated under the stream of nitrogen and the residue was reconstituted in hexane (100 µL).

Alternative Synthetic Approach Toward Racemic Intermediate 5

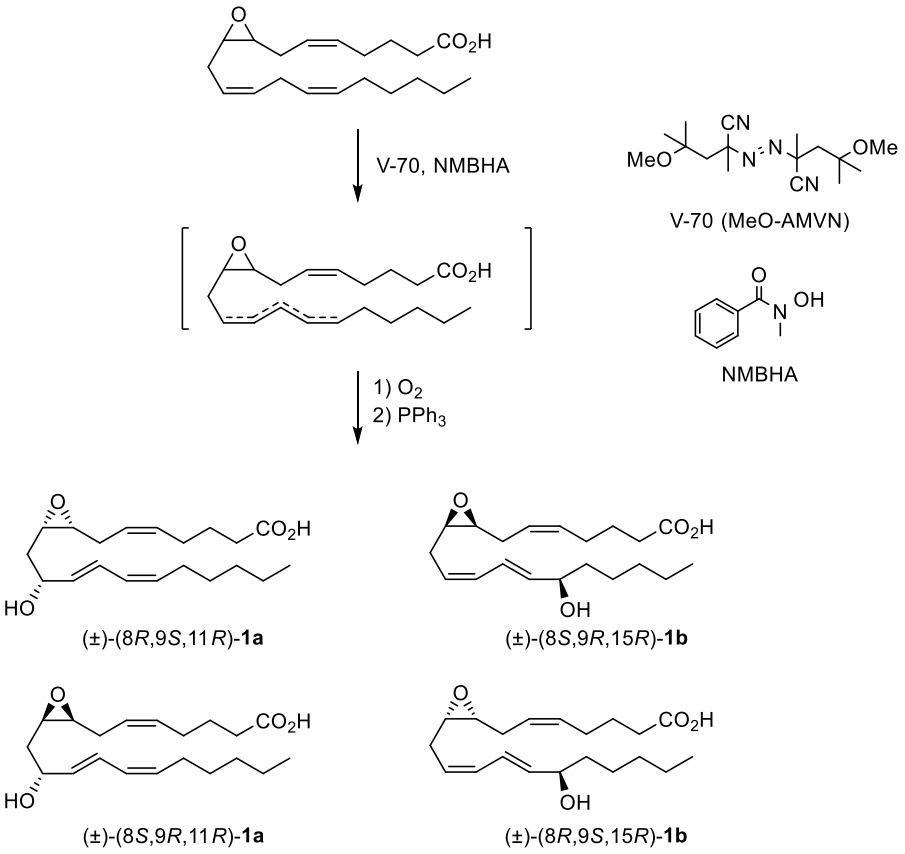
An alternative synthetic approach toward intermediate 5 commenced by a transformation of 3-butyne-1-ol into precursor **18** according to the literature procedure.[3] Copper catalyzed coupling between **18** and methyl hex-5-ynoate gave intermediate **19** which was subsequently hydrogenated in the presence of Lindlar catalyst and deprotected to give dienol **20**. The latter was oxidized with Dess-Martin periodinane into the corresponding aldehyde in almost quantitative yield. Treatment of aldehyde **21** with ((trimethylsilyl)ethynyl)lithium give propargylic alcohol **5** in only 35% yield, probably because of extensive side reactions such as enolysation, reduction and condensation which are well documented in the literature for this type of transformation. An alternative procedure where an alkynylcerium reagent[4] was used instead of alkynyllithiym did not provide any advantage in terms of product yield.



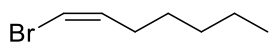
Scheme S1. a) DHP, CSA cat., CH_2Cl_2, 87%; b) BuLi, $(CH_2O)_n$, THF, −78 °C – rt, 68%; c) TsCl, KOH, Et_2O, 90%; d) methyl hex-5-ynoate, Cs_2CO_3, NaI, CuI, DMF, rt, 79%; e) Pd/$CaCO_3$, hexanes/EtOAc, 64%; f) pTSA, MeOH, 97%; g) DMP, CH_2Cl_2, 95%; h) TMSCCH, BuLi, THF, −78 °C, 35% i) TMSCCH, BuLi, $CeCl_3$, THF, −78 °C, 39%.

[3] Garcia, P.; Moulin, S.; Miclo, Y.; Leboeuf, D.; Gandon, V.; Aubert, C.; Malacria, M. *Chemistry* **2009**, *15* (9), 2129.
[4] Dimitrov, V.; Kostova, K.; Genov, M. *Tetrahedron Lett.* **1996**, *37* (37), 6787.

Experimental Procedures



Free-radical autoxidation of (±)-8,9-EET. To a vial containing (±)-8,9-EET (~0.18 mg, 0.55 μmol) were added solutions of NMBHA (40 mM, 25 μL) and V-70 (40 mM, 2.5 μL) in dry acetonitrile. The vial was closed, vortexed and left stay at room temperature for 16 h. PPh_3 (0.3 mg, 1.1 μmol) was added, the reaction mixture was vortexed and left stay at room temperature for 2 h. The reaction mixture was stored at −20 °C till analysis.

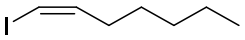


(dr = 98:2)

(*Z*)-1-Bromohept-1-ene.[5] A mixture of catecholborane (6 g, 50 mmol, 1 equiv) and 1-octyne (4.8 g, 50 mmol, 1 equiv) was stirred for 2 h under nitrogen at 70 °C. The reaction mixture was cooled to rt, CH_2Cl_2 (14 mL) was added and the resulting solution was cooled to −20 °C. A solution of Br_2 (17.6 g, 110 mmol, 2.2 equiv) in CH_2Cl_2 (22 mL) was added dropwise and the reaction mixture was cooled to −78 °C. NaOH aqueous solution (2 M, 113 mmol, 2.26 equiv) was added dropwise and the resulting mixture was warmed

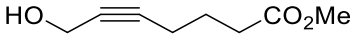
[5] Brown, H. C.; Subrahmanyam, C.; Hamaoka, T.; Ravindran, N.; Bowman, D. H.; Misumi, S.; Unni, M. K.; Somayaji, V.; Bhat, N. G. *J. Org. Chem.* **1989**, *54* (26), 6068.

to rt and stirred for 1 h. The reaction mixture was extracted with CH_2Cl_2 (3×30 mL). Combined organics were washed with brine, dried over $MgSO_4$, filtered and evaporated. Distillation of the residue (bp = 53–58 °C/10 mmHg) provide pure product as a colorless liquid (7.236 g, 82%).
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.10 (m, 2H), 2.19 (m, 2H), 1.48 – 1.29 (m, 6H), 0.90 (m, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 135.2, 107.7, 31.5, 29.8, 28.0, 22.6, 14.1.

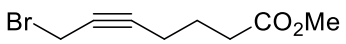


2 (dr = 98:2)

(*Z*)-1-Iodohept-1-ene 2.[5] A mixture of catecholborane (6 g, 50 mmol, 1 equiv) and 1-octyne (4.8 g, 50 mmol, 1 equiv) was stirred for 2 h under nitrogen at 70 °C. The reaction mixture was cooled to rt, 50 mL of water was added and the stirring was continued for 3 h at rt. The resulting white solid was filtered and washed with cold water. The resulting boronic acid was dissolved in diethyl ether/THF mixture (1:1, 50 mL), cooled to 0 °C and I_2 (34.3 g, 135 mmol, 2.7 equiv) was added. The resulting mixture was stirred for 6 h at 0 °C, quenched with aq. sol. of $Na_2S_2O_3$ (until the iodine color disappeared) and extracted with diethyl ether (3×200 mL). Combined organics were dried over $MgSO_4$, filtered and evaporated. Distillation of the residue (bp = 45–50 °C/~0.1 mmHg) give product slightly contaminated with *trans*-isomer (< 5%) as a pale-yellow liquid (5.484 g, 49%).
^{1}H NMR (600 MHz, $CDCl_3$) δ 6.17 (m, 2H), 2.13 (dt, J = 7.1, 7.1 Hz, 2H), 1.43 (m, 2H), 1.33 (m, 4H), 0.90 (t, J = 6.8 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 141.6, 82.3, 34.8, 31.4, 27.8, 22.7, 14.2.



Methyl 7-hydroxyhept-5-ynoate. BuLi (2.44M, 50.4 mL, 123 mmol, 2.05 equiv) was added dropwise to a solution cooled to −78 °C of hex-5-ynoic acid (6.72 g, 60 mmol, 1 equiv) in dry THF (180 mL). The reaction mixture was stirred at this temperature for 30 min, warmed to 0 °C and stirred for another 30 min. Polyformaldehyde (2.16 g, 72 mmol, 1.2 equiv) was added in one portion and the resulting mixture was stirred at rt overnight, quenched with brine and 3 M HCl (to pH ~1–3) and extracted with diethyl ether (3×50 mL). Combined organics were dried over $MgSO_4$, filtered and evaporated. The residue was dissolved in MeOH (100 mL), TMSCl (0.15 mL) was added dropwise and the resulting mixture was stirred overnight at rt. The reaction mixture was then quenched with brine and sat. aq. sol. of $NaHCO_3$ (to pH ~7) and extracted with Et_2O (4×70 mL). Combined organics were evaporated under reduced pressure, and the residue was chromatographed (EtOAc/Hexanes = 20:80→25:75→30:70) to give pure product as clear oil (5.288 g, 56.5%).
^{1}H NMR (600 MHz, $CDCl_3$) δ 4.16 (d, J = 1.8 Hz, 2H), 3.62 (s, 3H), 2.78 – 2.59 (br m, 1H), 2.38 (t, J = 7.4 Hz, 2H), 2.22 (m, 2H), 1.77 (m, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 173.8, 84.7, 79.5, 51.6, 51.0, 32.8, 23.7, 18.2.



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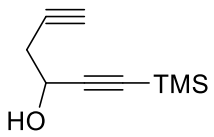
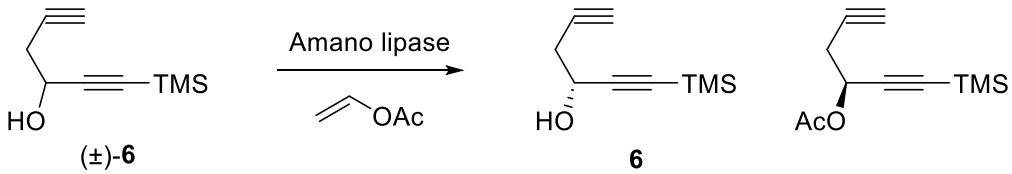
Methyl 7-bromohept-5-ynoate 5. PPh_3 (3.97 g, 15.2 mmol, 1.1 equiv) and CBr_4 (5.03 g, 15.2 mmol, 1.1 equiv) were added in one portion to a cooled to 0 °C solution of methyl 7-hydroxyhept-5-ynoate (2.15 g, 13.8 mmol, 1 equiv) in CH_2Cl_2 (120 mL). The reaction mixture was stirred at rt for 1 hour, concentrated to ~40 mL, diluted with hexane (50 mL) and chromatographed (EtOAc/Hexanes = 1:9) to give pure product as pale-yellow oil (2.848 g, 94%).
^{1}H NMR (600 MHz, $CDCl_3$) δ 3.88 (s, 2H), 3.64 (s, 3H), 2.39 (t, J = 7.4 Hz, 2H), 2.28 (m, 2H), 1.79 (m, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 173.3, 86.7, 76.2, 51.5, 32.7, 23.5, 18.3, 15.3.



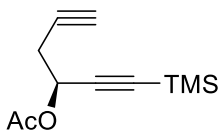
(±)-**6**

1-(Trimethylsilyl)hexa-1,5-diyn-3-ol (±)-6. To a suspension of Zn dust (1.04 g, 16 mmol, 1.6 equiv) in THF (14 mL) was added dibromoethane (0.1 mL) and the resulting mixture was heated to reflux for 5 min. It was then cooled to rt and TMSCl (0.19 mL) was added. The resulting mixture was stirred for 20 min at rt and cooled to −10 °C. A solution of propargyl bromide (80% in toluene, 1.67 mL, 2.23 g, 15 mmol, 1.5 equiv) in THF (1.5 mL) was slowly added. The reaction mixture was stirred at −10 °C for 1 h, cooled to −78 °C and a solution of 3-(trimethylsilyl)propiolaldehyde (1.26 g, 10 mmol, 1 equiv) in THF (6 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h, quenched with sat. aq. sol. of NH_4Cl (20 mL) and extracted with diethyl ether (3×20 mL). Combined organics were dried over $MgSO_4$, filtered and evaporated. Purification of the residue by flash column chromatography (EtOAc/hexanes = 15:85) provided pure product as a colorless oil (1.317 g, 79%).
^{1}H NMR (600 MHz, $CDCl_3$) δ 4.49 (s, 1H), 2.63 – 2.55 (m, 2H), 2.51 (br s, 1H), 2.09 (t, J = 2.6 Hz, 1H), 0.15 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 104.7, 90.4, 79.5, 71.4, 61.2, 28.5, -0.1.
IR (neat/cm^{-1}) 3294, 2960, 2900, 2177.
HRMS (CI), calculated for $C_{12}H_{23}OSi_2$ (MTMS$^+$) *m/z* 239.1282, found *m/z* 239.1287.

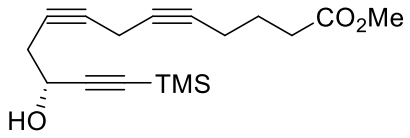


(±)-**6** **6**

(*R*)-1-(trimethylsilyl)hexa-1,5-diyn-3-ol 6. A mixture of 1-(Trimethylsilyl)hexa-1,5-diyn-3-ol (±)-**6** (3.26 g, 19.64 mmol, 1 equiv), Amano Lipase from *Pseudomonas fluorescens* (0.33 g) and vinyl acetate (32 mL) was stirred at room temperature for 2 days, filtered through a short pad of silicagel and evaporated. Purification of the residue by flash column chromatography (EtOAc/hexanes = 10:90→25:75) provided pure **6** as a colorless oil (1.460 g, 45%, ee > 96% as determined by chiral HPLC and NMR of MTPA derivatives). Acetate was obtained as a colorless liquid (1.525 g, 37%).
^{1}H NMR (600 MHz, $CDCl_3$) δ 4.49 (q, *J* = 5.5 Hz, 1H), 2.67 – 2.54 (m, 2H), 2.45 (d, *J* = 5.1 Hz, 1H), 2.09 (t, *J* = 2.6 Hz, 1H), 0.16 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 104.8, 90.5, 79.5, 71.4, 61.3, 28.6, -0.1.

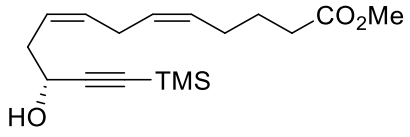


(*S*)-1-(trimethylsilyl)hexa-1,5-diyn-3-yl acetate.
^{1}H NMR (600 MHz, $CDCl_3$) δ 5.46 (t, *J* = 6.4 Hz, 1H), 2.63 (dd, *J* = 6.4, 2.6 Hz, 2H), 2.06 (s, 3H), 2.02 (t, *J* = 2.6 Hz, 1H), 0.13 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 169.5, 101.0, 91.3, 78.5, 71.0, 62.2, 25.8, 20.9, -0.3.



4

Methyl (*R*)-11-hydroxy-13-(trimethylsilyl)trideca-5,8,12-triynoate 4. A solution of 1-(trimethylsilyl)hexa-1,5-diyn-3-ol **6** (1.25 g, 7.53 mmol, 1 equiv) in DMF (5 mL) was added dropwise to a solution cooled to 0 °C of methyl 7-bromohept-5-ynoate **5** (1.65 g, 7.53 mmol, 1 equiv), Cs_2CO_3 (2.46 g, 7.53 mmol, 1 equiv), NaI (1.13 g, 7.53 mmol, 1 equiv) and CuI (1.43 g, 7.53 mmol, 1 equiv) in DMF (40 mL). The reaction mixture was stirred at rt overnight, diluted with Et_2O (50 mL) and filtered through a short pad of Celite. The Celite pad was washed with Et_2O. Combined organics were washed with sat. aq. sol. of NH_4Cl (40 mL), water (2×40 mL), dried over $MgSO_4$, filtered and evaporated. Purification of the residue by flash column chromatography (EtOAc/hexanes = 15:85→20:80) provided partially purified product as a yellow oil (1.443g, 63%). The reported NMR were taken on the partially purified product. Since this product decomposes rapidly it was moved to the next step immediately after taking the NMR.
^{1}H NMR (600 MHz, $CDCl_3$) δ 4.40 (dd, J = 6.6, 5.7 Hz, 1H), 3.61 (s, 3H), 3.08 (p, J = 2.3 Hz, 2H), 2.79 (br s, 1H), 2.51 (m, 2H), 2.36 (t, J = 7.4 Hz, 2H), 2.16 (tt, J = 6.9, 2.4 Hz, 2H), 1.74 (p, J = 7.1 Hz, 2H), 0.10 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 173.7, 105.3, 89.7, 79.3, 77.5, 75.6, 75.1, 61.3, 51.6, 32.9, 28.8, 23.8, 18.2, 9.8, -0.2.
IR (neat/cm^{-1}) 3467, 2956, 2175, 1736.
HRMS (CI), calculated for $C_{20}H_{33}O_3Si_2$ ($MTMS^+$) *m/z* 377.1963, found *m/z* 377.1968.



8

Methyl (*R*,5*Z*,8*Z*)-11-hydroxy-13-(trimethylsilyl)trideca-5,8-dien-12-ynoate 8. $NaBH_4$ (210 mg) was added to a mixture of EtOH (5 mL) and 2N aq. NaOH (0.25 mL) at rt. The resulting mixture was stirred for 10–15 min and filtered through a short pad of celite. The filtrate (1.18 mL) was added dropwise to a solution of $Ni(OAc)_2{\cdot}4H_2O$ (0.249 g, 1 mmol, 1 equiv) in EtOH (16 mL) with vigorous stirring under atmosphere of hydrogen. Ethylenediamine (0.167 mL, 0.15 g, 2.5 mmol, 2.5 equiv) and a solution of **4** (0.304 g, 1 mmol, 1 equiv) in EtOH (3 mL) were added and the reaction mixture was stirred at rt for 2–3

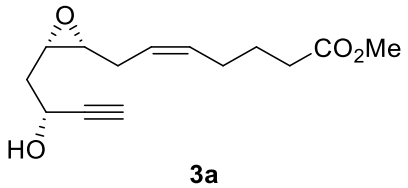
h. The reaction mixture was diluted with hexanes (20 mL), filtered through a short pad of silicagel and evaporated under reduced pressure. Purification of the residue by flash column chromatography (EtOAc/hexanes = 10:90→15:85) provided partially purified product as a pail-yellow oil (0.272 g, 88%, may contain traces of corresponding triene).

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.48 (m, 2H), 5.33 (m, 2H), 4.34 (t, J = 6.3 Hz, 1H), 3.63 (s, 3H), 2.77 (m, 2H), 2.45 (t, J = 6.8 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 2.06 (m, 2H), 1.66 (m, 2H), 0.12 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 174.2, 131.6, 129.1, 128.7, 124.1, 106.5, 89.3, 62.3, 51.6, 35.7, 33.4, 26.6, 25.9, 24.8, -0.1.

IR (neat/cm^{-1}) 3420, 3010, 2955, 2902, 2861, 1739.

HRMS (CI), calculated for $C_{20}H_{37}O_3Si_2$ (MTMS$^+$) *m/z* 381.2276, found *m/z* 381.2281.



3a

Methyl (*Z*)-7-((2*R*,3*S*)-3-((*R*)-2-hydroxybut-3-yn-1-yl)oxiran-2-yl)hept-5-enoate 3a. To a cooled to 0 ºC solution of **8** (5.74 g, 18.6 mmol, 1 equiv) and $VO(acac)_2$ (0.2 g, 0.75 mmol, 0.04 equiv) in CH_2Cl_2 (180 mL) was added tert-butyl hydroperoxide (5-6 M in decane, 5.42 mL, 29.8 mmol, 1.6 equiv) dropwise. The reaction was stirred at 0 °C for 3 h and then gradually warmed up to rt overnight. After 24 h, Me_2S (1.5 mL) was added, the reaction mixture was concentrated to ~60 mL and chromatographed (EtOAc/hexanes = 30:70) to give product as a pale-yellow oil (4.29 g, 71%, as 70:30 mixture of diastereomers).

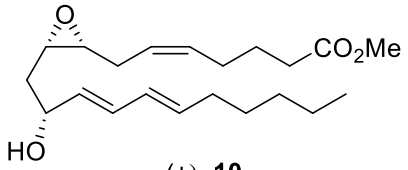
To a solution of this product (0.58 g, 1.8 mmol, 1 equiv) in MeOH (10 mL) was added K_2CO_3 (0.124 g, 0.9 mmol, 0.5 equiv) in one portion. The reaction mixture was stirred at rt overnight, concentrated to ~1–2 mL and chromatographed (EtOAc/hexanes = 30:70) to give the product as a pale-yellow oil (0.429 g, 95%, as ~70:30 mixture of diastereomers). Major diastereoisomer was partially separated by flash column chromatography (EtOAc/hexanes = 30:70) for analytical purposes.

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.47 (m, 2H), 4.59 (t, J = 6.2 Hz, 1H), 3.64 (s, 3H), 3.16 (dt, J = 7.7, 4.5 Hz, 1H), 2.95 (td, J = 6.4, 4.3 Hz, 1H), 2.93 (br s, 1H), 2.51 (d, J = 2.1 Hz, 1H), 2.32 (m, 1H), 2.30 (t, J = 7.4 Hz, 2H), 2.22 (m, 1H), 2.07 (q, J = 6.7 Hz, 2H), 2.01 (m, 1H), 1.91 (ddd, J = 14.0, 7.5, 6.5 Hz, 1H), 1.68 (p, J = 7.4 Hz, 2H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 174.2, 131.6, 124.8, 84.1, 73.7, 60.5, 56.0, 54.0, 51.6, 36.0, 33.4, 26.8, 26.5, 24.7.

IR (neat/cm^{-1}) 3410, 3283, 2952, 2094, 1731, 1652.

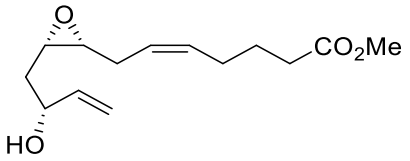
HRMS (CI), calculated for $C_{17}H_{29}O_4Si$ (MH$^+$) *m/z* 325.1830, found *m/z* 325.1835.



(±)–**10**

(±)-Methyl (*Z*)-7-((2*R*,3*S*)-3-((*R*,3*E*,5*E*)-2-hydroxyundeca-3,5-dien-1-yl)oxiran-2-yl)hept-5-enoate 10. Bu_3SnH (0.481 g, 1.65 mmol, 1.1 equiv) was added dropwise to a cooled to 0 °C solution of **3a** (0.379

g, 1.5 mmol, 1 equiv), **2** (0.319 g, 1.8 mmol, 1.2 equiv) and $Pd(PPh_3)_3Cl_2$ (5.3 mg, 7.5 μmol, 0.005 equiv) in THF (2 mL). The reaction mixture was stirred at rt for 3 days, filtered through a short pad of silicagel and evaporated. Purification of the residue by flash column chromatography (EtOAc/hexanes = 20:80→30:70) give partially purified product as a colorless oil (mixture of isomers, 0.105 g, 20%). The major product was isolated by HPLC purification (<5% yield).
^{1}H NMR (800 MHz, $CDCl_3$) δ 6.25 (dd, *J* = 15.3, 10.4 Hz, 1H), 6.03 (dd, *J* = 15.1, 10.5 Hz, 1H), 5.73 (dt, *J* = 14.7, 7.0 Hz, 1H), 5.62 (dd, *J* = 15.3, 7.0 Hz, 1H), 5.49 (m, 2H), 4.43 (m, 1H), 3.67 (s, 3H), 3.09 (dt, *J* = 8.4, 4.3 Hz, 1H), 2.95 (td, *J* = 6.4, 4.3 Hz, 1H), 2.37 (m, 1H), 2.32 (t, *J* = 7.4 Hz, 2H), 2.21 (m, 1H), 2.08 (m, 4H), 1.87 (ddd, *J* = 14.2, 5.4, 4.4 Hz, 1H), 1.71 (m, 3H), 1.38 (m, 2H), 1.29 (m, 4H), 0.88 (t, *J* = 7.1 Hz, 3H).
^{13}C NMR (201 MHz, $CDCl_3$) δ 174.2, 136.5, 132.2, 131.8, 131.6, 129.3, 124.9, 71.6, 55.9, 54.8, 51.7, 35.4, 33.5, 32.8, 31.5, 29.0, 26.9, 26.6, 24.8, 22.7, 14.2.
IR (neat/cm^{-1}) 3513, 3028, 2954, 2927, 2855, 1738, 1658.
HRMS (ESI), calculated for $C_{20}H_{31}O_4$ ([M–Me]$^-$) *m/z* 335.2228, found *m/z* 335.2232.



Methyl (*Z*)-7-((2*R*,3*S*)-3-((*R*)-2-hydroxybut-3-en-1-yl)oxiran-2-yl)hept-5-enoate. $NaBH_4$ (210 mg) was added to a mixture of EtOH (5 mL) and 2 N aq. NaOH (0.25 mL) at rt. The resulting mixture was stirred for 10–15 min and filtered through a short pad of celite. The filtrate (0.33 mL) was added dropwise to a solution of $Ni(OAc)_2{\cdot}4H_2O$ (69 mg, 0.28 mmol, 1 equiv) in EtOH (4.8 mL) with vigorous stirring under atmosphere of hydrogen. Ethylenediamine (46 μL, 42 mg, 0.7 mmol, 2.5 equiv) and a solution of **3a** (70 mg, 0.28 mmol, 1 equiv) in EtOH (1–2 mL) were added and the reaction mixture was stirred at rt for 3–5 min. The reaction mixture was diluted with hexanes (10 mL), filtered through a short pad of silicagel and evaporated under reduced pressure. The crud product containing up to 30% of overreduced product was used in the next step without further purification.

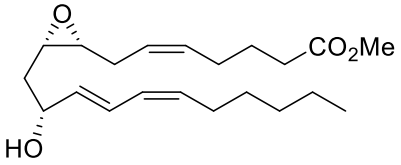
Synthesis of the 11-hydroxy EET methyl esters 9. *Procedure A.* Triethylamine (10 μL, 7.2 mg, 71 μmol, 1 equiv) was added to a degassed solution of methyl (*Z*)-7-((2*R*,3*S*)-3-((*R*)-2-hydroxybut-3-en-1-yl)oxiran-2-yl)hept-5-enoate (18 mg, 71 μmol, 1 equiv), **2** (16 mg, 71 μmol, 1 equiv), $Pd(OAc)_2$ (16 mg, 71 μmol, 1 equiv), Cs_2CO_3 (25 mg, 71 μmol, 1 equiv) and TBAB (23 mg, 71 μmol, 1 equiv) in DMF (0.5 mL). The reaction mixture was stirred at rt for 24 h and chromatographed (EtOAc/hexanes = 20:80→30:70) to give product that contained some impurities. Analytically pure product was obtained after HPLC purification as a colorless oil (7.5 mg, 30%).

Procedure B. Procedure developed by Darwish *et al*.[6] for regioselective hydrostannylation was used for the first step. $Pd_2(dba)_3$ (4mg, 4.4 μmol, 0.01 equiv), Cy_3PHBF_4 (3.24 mg, 8.8 μmol, 0.02 equiv) and *i*Pr_2NEt (114 mg, 0.88 mmol, 2 equiv) were dissolved in CH_2Cl_2 (2.5 mL) and the resulting mixture was stirred at rt for 10 min. A solution of **3** (mixture of diastereomers, 111 mg, 0.44 mmol, 1 equiv) in CH_2Cl_2 (1 mL) was added and the resulting mixture was cooled to 0 °C. A solution of Bu_3SnH (180 mg, 0.62 mmol, 1.4 equiv) in CH_2Cl_2 (0.8 mL) was added dropwise over 10 min; the reaction mixture was stirred at 0 °C for 3 h and chromatographed (EtOAc/hexanes = 50:50) to give partially purified methyl (*Z*)-7-

[6] A. Darwish, A. Lang, T. Kim and J. M. Chong, *Org. Lett.*, 2008, **10**, 861–864.

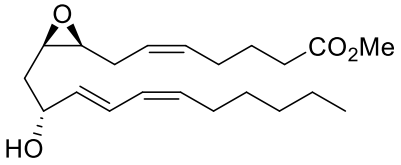
((2*S*,3*R*)-3-((*R*,*E*)-2-hydroxy-4-(tributylstannyl)but-3-en-1-yl)oxiran-2-yl)hept-5-enoate as a colorless oil (171 mg).

CuTC (91 mg, 0.48 mmol, 1.5 equiv) was added in one portion to a cooled to 0 °C solution of methyl (*Z*)-7-((2*S*,3*R*)-3-((*R*,*E*)-2-hydroxy-4-(tributylstannyl)but-3-en-1-yl)oxiran-2-yl)hept-5-enoate (171 mg, 0.315 mmol, 1 equiv) and **2** (84 mg, 0.375 mmol, 1.2 equiv) in DMF (3 mL). The resulting mixture was stirred at 0 °C for 3 h, quenched with water (15 mL) and extracted with Et_2O (4×6 mL). Combined organics were washed with water, dried over $MgSO_4$, filtered and evaporated. Purification of the residue by flash column chromatography (EtOAc/hexanes = 33:67→50:50) give pure product as a mixture of diastereomers **9a** and **9b** (colorless oil, 72.4 mg, 47% for two steps). Diastereoisomers **9a** and **9b** were separated by a reverse phase HPLC.



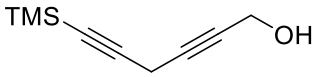
9a

Methyl (*Z*)-7-((2*R*,3*S*)-3-((*R*,3*E*,5*Z*)-2-hydroxyundeca-3,5-dien-1-yl)oxiran-2-yl)hept-5-enoate 9a.
^{1}H NMR (800 MHz, $CDCl_3$) δ 6.57 (dd, J = 15.2, 11.1 Hz, 1H), 5.98 (dd, J = 11.0, 11.0 Hz, 1H), 5.70 (dd, J = 15.2, 6.8 Hz, 1H), 5.54 – 5.44 (m, 3H), 4.47 (q, J = 6.4 Hz, 1H), 3.67 (s, 3H), 3.10 (dt, J = 8.4, 4.3 Hz, 1H), 2.95 (td, J = 6.4, 4.3 Hz, 1H), 2.39 – 2.34 (m, 1H), 2.32 (t, J = 7.4 Hz, 2H), 2.24 – 2.15 (m, 4H), 2.10 (q, J = 7.2 Hz, 2H), 1.89 (dt, J = 14.2, 4.8 Hz, 1H), 1.76 – 1.68 (m, 3H), 1.38 (m, 2H), 1.30 (m, 4H), 0.88 (t, J = 7.0 Hz, 3H).
^{13}C NMR (201 MHz, $CDCl_3$) δ 174.1, 134.4, 133.9, 131.6, 127.6, 126.7, 124.9, 71.6, 55.9, 54.8, 51.7, 35.4, 33.5, 31.6, 29.4, 27.9, 26.9, 26.5, 24.8, 22.7, 14.2.
IR (neat/cm^{-1}) 3445, 3007, 2957, 2926, 2858, 1736.
HRMS (ESI), calculated for $C_{20}H_{31}O_4$ ([M–Me]$^-$) *m/z* 335.2228, found *m/z* 335.2228.

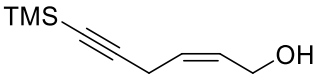


9b

Methyl (*Z*)-7-((2*S*,3*R*)-3-((*R*,3*E*,5*Z*)-2-hydroxyundeca-3,5-dien-1-yl)oxiran-2-yl)hept-5-enoate 9b.
^{1}H NMR (800 MHz, $CDCl_3$) δ 6.57 (dd, *J* = 15.2, 11.1 Hz, 1H), 5.97 (dd, *J* = 11.0, 11.0 Hz, 1H), 5.70 (dd, *J* = 15.2, 6.8 Hz, 1H), 5.53 – 5.44 (m, 3H), 4.47 (q, *J* = 6.9 Hz, 1H), 3.66 (s, 3H), 3.10 (dt, *J* = 8.4, 4.3 Hz, 1H), 2.95 (td, *J* = 6.4, 4.2 Hz, 1H), 2.36 (m, 1H), 2.32 (t, *J* = 7.4 Hz, 2H), 2.24 – 2.15 (m, 4H), 2.09 (q, *J* = 7.3 Hz, 2H), 1.89 (ddd, *J* = 14.3, 5.4, 4.3 Hz, 1H), 1.75 – 1.68 (m, 3H), 1.37 (m, 2H), 1.29 (m, 4H), 0.88 (t, *J* = 6.9 Hz, 3H).
^{13}C NMR (201 MHz, $CDCl_3$) δ 174.2, 134.4, 133.8, 131.6, 127.5, 126.6, 124.9, 71.6, 55.9, 54.8, 51.7, 35.4, 33.5, 31.6, 29.4, 27.9, 26.9, 26.5, 24.8, 22.7, 14.2.
HRMS (ESI), calculated for $C_{20}H_{31}O_4$ ([M–Me]$^-$) *m/z* 335.2228, found *m/z* 335.2228.

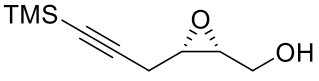


6-(Trimethylsilyl)hexa-2,5-diyn-1-ol. Propargyl alcohol (2.8 g, 50 mmol, 1 equiv) was added dropwise to a cooled to 0 °C mixture of Cs_2CO_3 (16.3 g, 50 mmol, 1 equiv), NaI (7.5 g, 50 mmol, 1 equiv) and CuI (9.5 g, 50 mmol, 1 equiv) in DMF (300 mL). The resulting mixture was stirred for 10 min and (3-bromoprop-1-yn-1-yl)trimethylsilane (9.55 g, 50 mmol, 1 equiv) was added dropwise. After stirring for 20 h at room temperature diethyl ether (500 mL) was added and the resulting mixture was filtered through a short pad of celite. The filtrate was washed with sat. aq. sol. of NH_4Cl (200 mL), water (400 mL), dried over $MgSO_4$, filtered and evaporated. Purification of the residue by flash column chromatography (EtOAc/hexanes = 16:84→20:80) provided partially purified product as an orange oil (5.193 g, 62%). The reported NMR were taken on the partially purified product. Since this product decomposes rapidly it was moved to the next step immediately after taking the NMR.
^{1}H NMR (600 MHz, $CDCl_3$) δ 4.24 (t, J = 2.2 Hz, 2H), 3.24 (t, J = 2.2 Hz, 2H), 2.11 (s, 1H), 0.14 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 99.5, 85.6, 79.7, 79.0, 51.3, 11.1, 0.0.



15

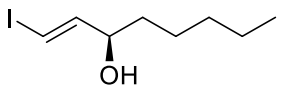
(*Z*)-6-(Trimethylsilyl)hex-2-en-5-yn-1-ol 15. $NaBH_4$ (630 mg) was added to a mixture of EtOH (15 mL) and 2N aq. NaOH (0.75 mL) at rt. The resulting mixture was stirred for 10–15 min and filtered through a short pad of celite. The filtrate (12.2 mL) was added dropwise to a solution of $Ni(OAc)_2{\cdot}4H_2O$ (2.565 g, 10.3 mmol, 0.4 equiv) in EtOH (170 mL) with vigorous stirring under an atmosphere of hydrogen. Ethylenediamine (1.72 mL, 1.55 g, 25.8 mmol, 1 equiv) and a solution of 6-(trimethylsilyl)hexa-2,5-diyn-1-ol (4.3 g, 25.8 mmol, 1 equiv) in EtOH (20 mL) were added and the reaction mixture was stirred at rt for 2–3 h. The reaction mixture was diluted with hexanes (200 mL), filtered through a short pad of silicagel and evaporated under reduced pressure. Purification of the residue by flash column chromatography (EtOAc/hexanes = 15:85→20:80) provided partially purified product (contaminated with < 10% of diene, detrimethylsilylated product and overreduced products) as a yellow oil (3.773 g, 87%).
^{1}H NMR (600 MHz, $CDCl_3$) δ 5.68 (m, 1H), 5.57 (m, 1H), 4.20 (d, J = 6.6 Hz, 2H), 3.02 (d, J = 7.0 Hz, 2H), 0.13 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 130.5, 126.8, 104.6, 85.1, 58.4, 18.7, 0.1.
IR (neat/cm^{-1}) 3332, 3028, 2959, 2898, 2176, 1709.
HRMS (CI), calculated for $C_{12}H_{25}OSi_2$ ($MTMS^+$) *m/z* 241.1438, found *m/z* 241.1444.



13

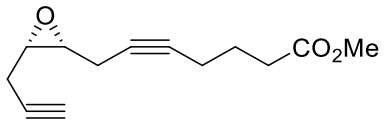
((2*R*,3*S*)-3-(3-(trimethylsilyl)prop-2-yn-1-yl)oxiran-2-yl)methanol 13. To a suspension of dry MS 4 Å (6.3 g) in dry CH_2Cl_2 (100 mL) was added $Ti(OiPr)_4$ (1.546 g, 5.44 mmol, 0.2 equiv) followed by the addition of (–)-DET (1.29 g, 6.26 mmol, 0.23 equiv) at −20 °C. After stirring for 15 min, tBuOOH (5–6 M in decane, 9.9 mL, 54.4 mmol, 2 equiv) was slowly added and the resulting solution was stirred for another 15 min at −20 °C. A solution of **15** (4.6 g, 27.2 mmol, 1 equiv) in CH_2Cl_2 (25 mL) was then added. The reaction mixture was stirred at −20 °C for 1.5 h and then was kept in the freezer (−20 °C)

without stirring overnight. The reaction mixture was quenched with 10% aq. sol. of tartric acid (21 mL) and the resulting mixture was stirred at rt for 1 h. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3×50 mL). Combined organics were dried over $MgSO_4$, filtered and evaporated. Purification of the residue by flash column chromatography (EtOAc/hexanes = 20:80→25:75→30:70) provided partially purified product (< 5% of contaminants) as a pale-yellow oil (3.323 g, 66%, 64% ee).
^{1}H NMR (600 MHz, $CDCl_3$) δ 3.82 (dd, J = 12.4, 4.9 Hz, 1H), 3.73 (dd, J = 12.5, 6.2 Hz, 1H), 3.24 (ddd, J = 7.0, 5.8, 4.2 Hz, 1H), 3.17 (ddd, J = 6.2, 4.8, 4.2 Hz, 1H), 2.72 (dd, J = 17.5, 5.8 Hz, 1H), 2.35 (dd, J = 17.5, 7.0 Hz, 1H), 2.29 (br s, 1H), 0.13 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 101.3, 87.8, 60.6, 56.3, 54.9, 20.2, 0.0.
IR (neat/cm^{-1}) 3452, 2959, 2900, 2178, 1741.
HRMS (CI), calculated for $C_{12}H_{25}O_2Si_2$ ($MTMS^+$) *m/z* 257.1388, found *m/z* 257.1393.



11

(*R*,*E*)-1-iodooct-1-en-3-ol 11 was synthesized from commercial (*R*)-oct-1-yn-3-ol according to the previously reported procedure.[7]
^{1}H NMR (600 MHz, $CDCl_3$) δ 6.51 (dd, *J* = 14.4, 6.0 Hz, 1H), 6.19 (dd, *J* = 14.4, 1.3 Hz, 1H), 4.07 (qd, *J* = 6.1, 1.2 Hz, 1H), 1.46 (m, 2H), 1.28 (m, 6H), 0.89 (s, 9H), 0.88 (t, *J* = 6.9 Hz, 3H), 0.04 (s, 3H), 0.03 (s, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 149.6, 75.5, 75.4, 37.7, 31.9, 26.0, 24.7, 22.7, 18.4, 14.1, -4.3, -4.7.

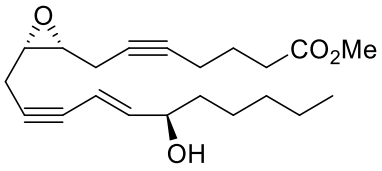


12

Methyl 7-((2*R*,3*S*)-3-(prop-2-yn-1-yl)oxiran-2-yl)hept-5-ynoate 12.
Triflate of **13** was prepared as follows: Triethylamine (164 mg, 1.6 mmol, 1.5 equiv) and Tf_2O (366 mg, 1.3 mmol, 1.2 equiv) were added dropwise to a cooled to −78 °C solution of **13** (200 mg, 1.1 mmol, 1 equiv) in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 30 min, quenched with sat. aq. sol. of NH_4Cl (5 mL) and extracted with CH_2Cl_2 (3×5 mL). Combined organics were dried over $MgSO_4$, filtered through a short pad of silicagel and evaporated. Crud product (308 mg) was used in the next step without further purification.
BuLi (2.5 M, 1.6 mL, 4 mmol, 4 equiv) was added dropwise to a cooled to −78 °C solution of hex-5-ynoic acid (224 mg, 2 mmol, 2 equiv) in THF (8 mL). The reaction mixture was stirred for 30 min, HMPA (2 mL) was added and the stirring was continued for another 15 min. A crude triflate of **13** was dissolved in THF (2 mL) at 0 °C and added via cannula. The reaction mixture was stirred at −78 °C for 20–30 min, quenched with 0.1 M HCl (to pH ~2–3) and extracted with Et_2O (3×15 mL). Combined organics were dried over $MgSO_4$, filtered and concentrated to ~5 mL. Methanol (1 mL) was added followed by the addition of the trimethylsilyldiazomethane solution in hexane (2 M, 4 mL, monitored by TLC). The residue was partially purified by flash column chromatography (EtOAc/hexanes = 10:90→15:85; three compounds with close Rf are combined together).

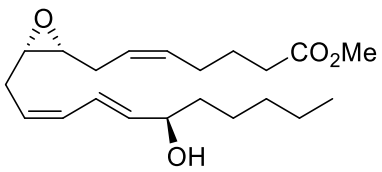
[7] Bischop, M.; Doum, V.; Nordschild née Rieche, A.; Pietruszka, J.; Sandkuhl, D. *Synthesis (Stuttg).* **2010**, *2010* (03), 527.

Partially purified product was dissolved in MeOH (10 mL), a catalytic amount of K_2CO_3 (~10 mg) was added and the resulting mixture was stirred at rt for 4–5 h. The reaction mixture was quenched with brine (10 mL) and extracted with Et_2O (3×15 mL). Combined organics were dried over $MgSO_4$, filtered and evaporated. Purification of the residue by flash column chromatography (EtOAc/hexanes = 10:90→15:85) provided pure product as a pale-yellow oil (100 mg, 42% for 3 steps).
^{1}H NMR (600 MHz, $CDCl_3$) δ 3.61 (s, 3H), 3.10 (m, 2H), 2.50 (m, 2H), 2.37 (t, J = 7.5 Hz, 2H), 2.31 (ddd, J = 17.4, 6.6, 2.8 Hz, 1H), 2.26 (ddt, J = 17.2, 6.6, 2.5 Hz, 1H), 2.17 (tt, J = 6.9, 2.4 Hz, 2H), 2.04 (t, J = 2.7 Hz, 2H), 1.74 (p, J = 7.2 Hz, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 173.5, 81.5, 79.1, 75.4, 70.6, 55.0, 54.5, 51.5, 32.8, 23.9, 18.6, 18.3, 18.2.
IR (neat/cm^{-1}) 3290, 2995, 2913, 2846, 1734.
HRMS (CI), calculated for $C_{13}H_{17}O_3$ ($[M+H]^+$) *m/z* 221.1172, found *m/z* 221.1178.



16

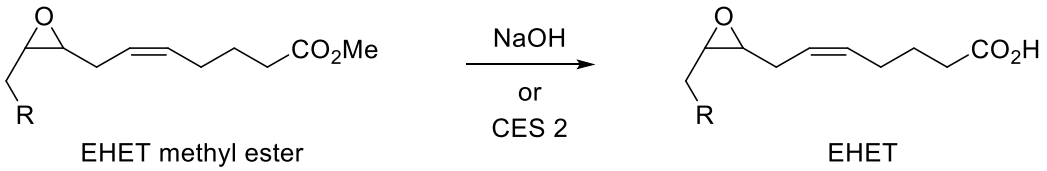
Methyl 7-((2*R*,3*S*)-3-((*R*,*E*)-6-hydroxyundec-4-en-2-yn-1-yl)oxiran-2-yl)hept-5-ynoate 16.
To a solution of (*R*,*E*)-1-iodooct-1-en-3-ol **11** (165 mg, 0.65 mmol, 1.5 equiv), $Pd(PPh_3)_2Cl_2$ (30 mg, 43 μmol, 0.1 equiv) and CuI (25 mg, 0.13 mmol, 0.3 equiv) in triethylamine (1 mL) stirred at rt for 10 min, was added dropwise a solution of **12** (95 mg, 0.43 mmol, 1 equiv) in triethylamine (3 mL). The reaction mixture protected from light with aluminum foil was stirred for 4 h at rt and evaporated under reduced pressure. Purification of the residue by flash column chromatography (Et_2O/hexanes = 50:50 + 1% Et_3N) provided pure product as a colorless oil (128 mg, 86%).
^{1}H NMR (600 MHz, $CDCl_3$) δ 6.04 (dd, J = 15.9, 6.2 Hz, 1H), 5.61 (d, J = 15.9 Hz, 1H), 4.05 (q, J = 6.5 Hz, 1H), 3.61 (s, 3H), 3.11 (m, 2H), 2.65 (ddd, J = 17.6, 5.7, 2.0 Hz, 1H), 2.51 (ddt, J = 17.0, 5.4, 2.4 Hz, 1H), 2.41 (ddd, J = 17.6, 6.7, 2.2 Hz, 1H), 2.37 (t, J = 7.5 Hz, 2H), 2.27 (ddt, J = 17.1, 6.5, 2.2 Hz, 2H), 2.18 (m, 2H), 1.75 (p, J = 7.2 Hz, 2H), 1.45 (m, 2H), 1.37 – 1.18 (m, 6H), 0.83 (t, J = 6.8 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 173.7, 145.7, 109.5, 84.9, 81.4, 80.6, 75.4, 72.1, 55.1, 54.8, 51.8, 36.9, 32.9, 31.7, 25.0, 24.0, 22.6, 19.3, 18.6, 18.2, 14.0.
IR (neat/cm^{-1}) 3469, 2953, 2932, 2858, 1736.
HRMS (CI), calculated for $C_{24}H_{39}O_4Si$ (MTMS$^+$) *m/z* 419.2612, found *m/z* 419.2618.



17

Methyl (*Z*)-7-((2*R*,3*S*)-3-((*R*,2*Z*,4*E*)-6-hydroxyundeca-2,4-dien-1-yl)oxiran-2-yl)hept-5-enoate 17.
$NaBH_4$ (210 mg) was added to a mixture of EtOH (5 mL) and 2N aq. NaOH (0.25 mL) at rt. The resulting mixture was stirred for 10–15 min and filtered through a short pad of celite. The filtrate (0.41 mL) was added dropwise to a solution of $Ni(OAc)_2{\cdot}4H_2O$ (86 mg, 0.35 mmol, 1 equiv) in EtOH (6 mL) with

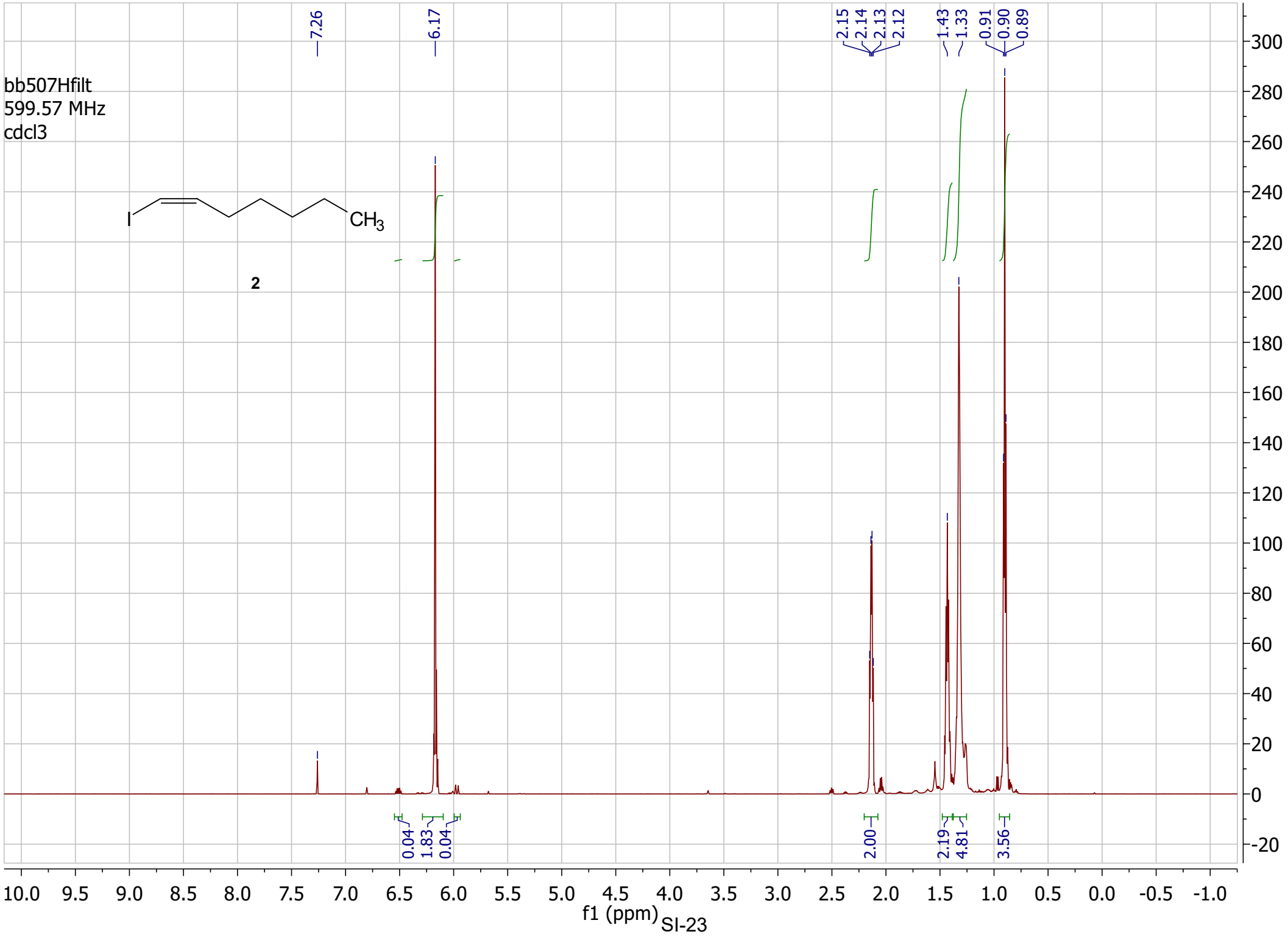
vigorous stirring under atmosphere of hydrogen. Ethylenediamine (58 µL, 52 mg, 0.87 mmol, 2.5 equiv) and a solution of **16** (120 mg, 0.35 mmol, 1 equiv) in EtOH (1–2 mL) were added and the reaction mixture was stirred at rt for ~3 h. The reaction mixture was diluted with hexanes (10 mL), filtered through a short pad of silicagel and evaporated under reduced pressure to give product containing small amount of impurities (118 mg, 97%). Analytically pure product was obtained after HPLC purification.
^{1}H NMR (600 MHz, $CDCl_3$) δ 6.47 (dd, J = 15.1, 11.1 Hz, 1H), 6.12 (dd, J = 10.9, 10.9 Hz, 1H), 5.74 (dd, J = 15.1, 6.6 Hz, 1H), 5.49 (m, 3H), 4.17 (q, J = 6.5, 6.0 Hz, 1H), 3.66 (s, 3H), 2.97 (m, 2H), 2.53 (m, 1H), 2.39 (m, 2H), 2.32 (t, J = 7.4 Hz, 2H), 2.23 (m, 1H), 2.10 (q, J = 6.8 Hz, 2H), 1.71 (p, J = 7.4 Hz, 2H), 1.59 – 1.48 (m, 2H), 1.44 – 1.26 (m, 6H), 0.89 (t, J = 6.7 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 174.1, 137.8, 131.5, 130.4, 126.1, 125.2, 125.0, 72.7, 56.5, 56.4, 51.7, 37.5, 33.5, 31.9, 30.5, 26.9, 26.4, 25.2, 24.8, 22.7, 14.2.
IR (neat/cm^{-1}) 3432, 3011, 2952, 2930, 2858, 1736.
HRMS (ESI), calculated for $C_{20}H_{31}O_4$ ($[M–Me]^-$) *m/z* 335.2228, found *m/z* 335.2237.



EHET methyl ester EHET

Hydrolysis of EHET methyl esters to free EHETs. An aqueous solution of NaOH (1 M, 2 µL) was added to the vial containing solution of corresponding EHET methyl ester (10 mM, 10 µL) in MeOH. The vial was flushed with nitrogen, closed, vortexed and allowed to stay overnight at 4 °C.

Alternatively, the EHETs were converted from their methyl esters to their carboxlic acids using recombinant human carboxylesterase 2 (CES 2). The CES 2 was produced in a baculovirus expression system, dislodged from membrane by weak detergent and partially purified by anion exchange chromatography. The purity was estimated by Coomassie staining on an SDS page gel and densitometry and the amount added is expressed as pure CES 2. In glass tubes (10 x 75 mm) containing CES 2 (27 µg/mL in Na_3PO_4 buffer (90 µL, 0.1 M pH 7.4 containing 0.1 mg/mL BSA)), 1 µL 10 mM EHET in DMSO was added and the mixture was incubated for 30 min at 37 °C. The enzymatic and chemical methods yielded products that were chromatographically and structurally indistinguishable.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2.13
2.12
1.43
1.33
0.91
0.90
0.89
I
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2
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0.04
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2.19
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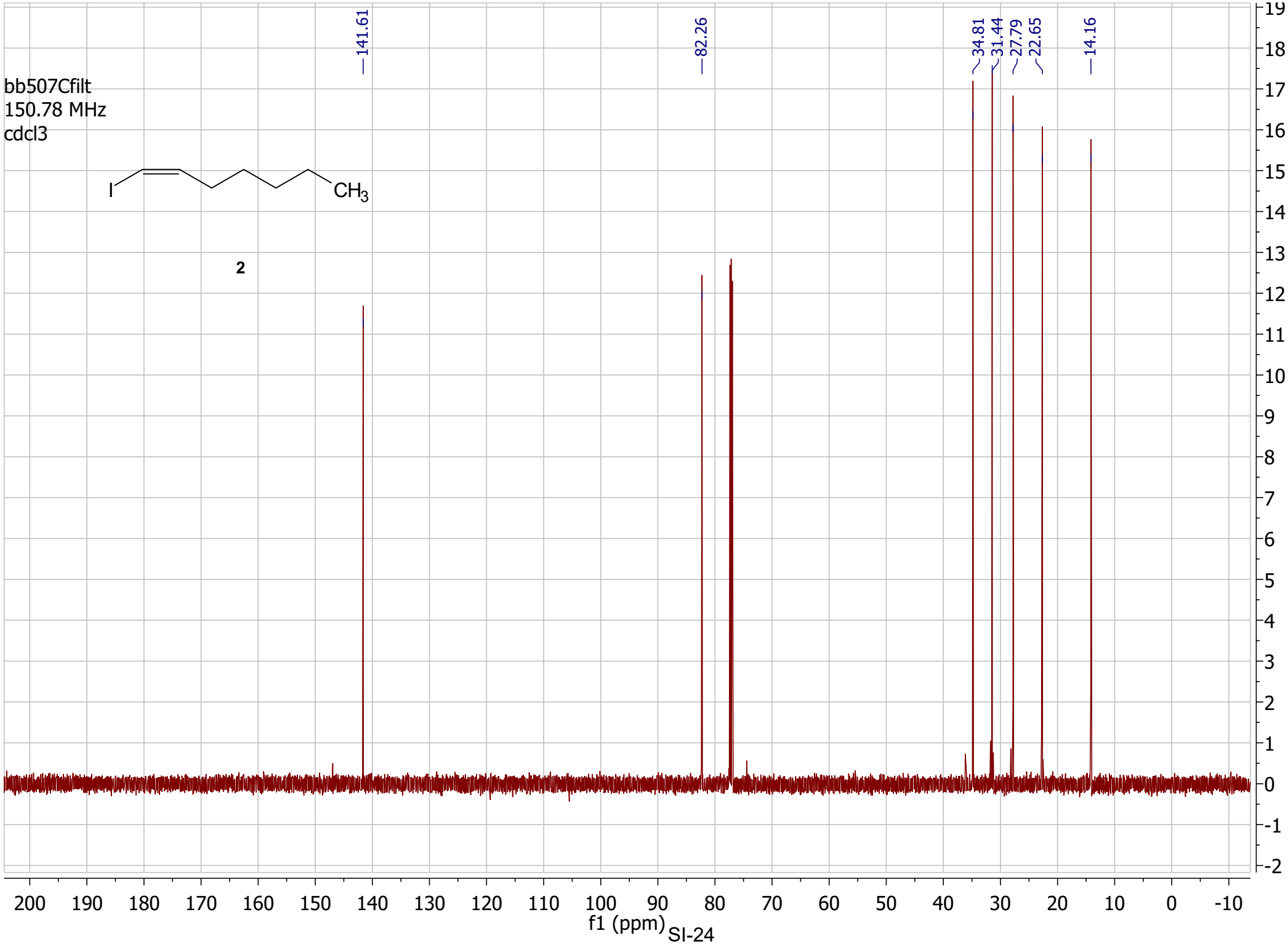
f1 (ppm)
SI-23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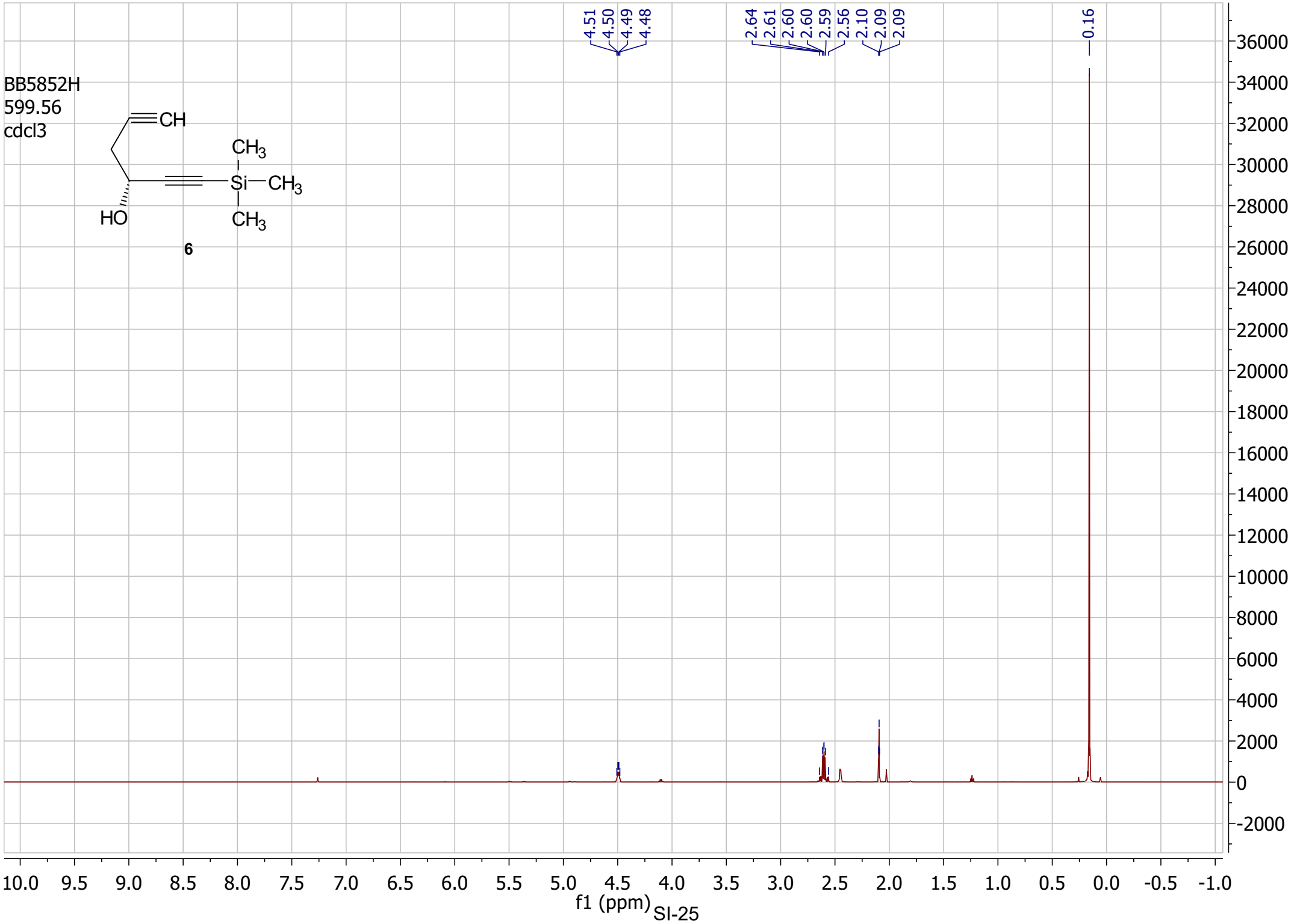
f1 (ppm)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2.60
2.60
2.59
2.56
2.10
2.09
2.09
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34000
32000
30000
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
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9.0
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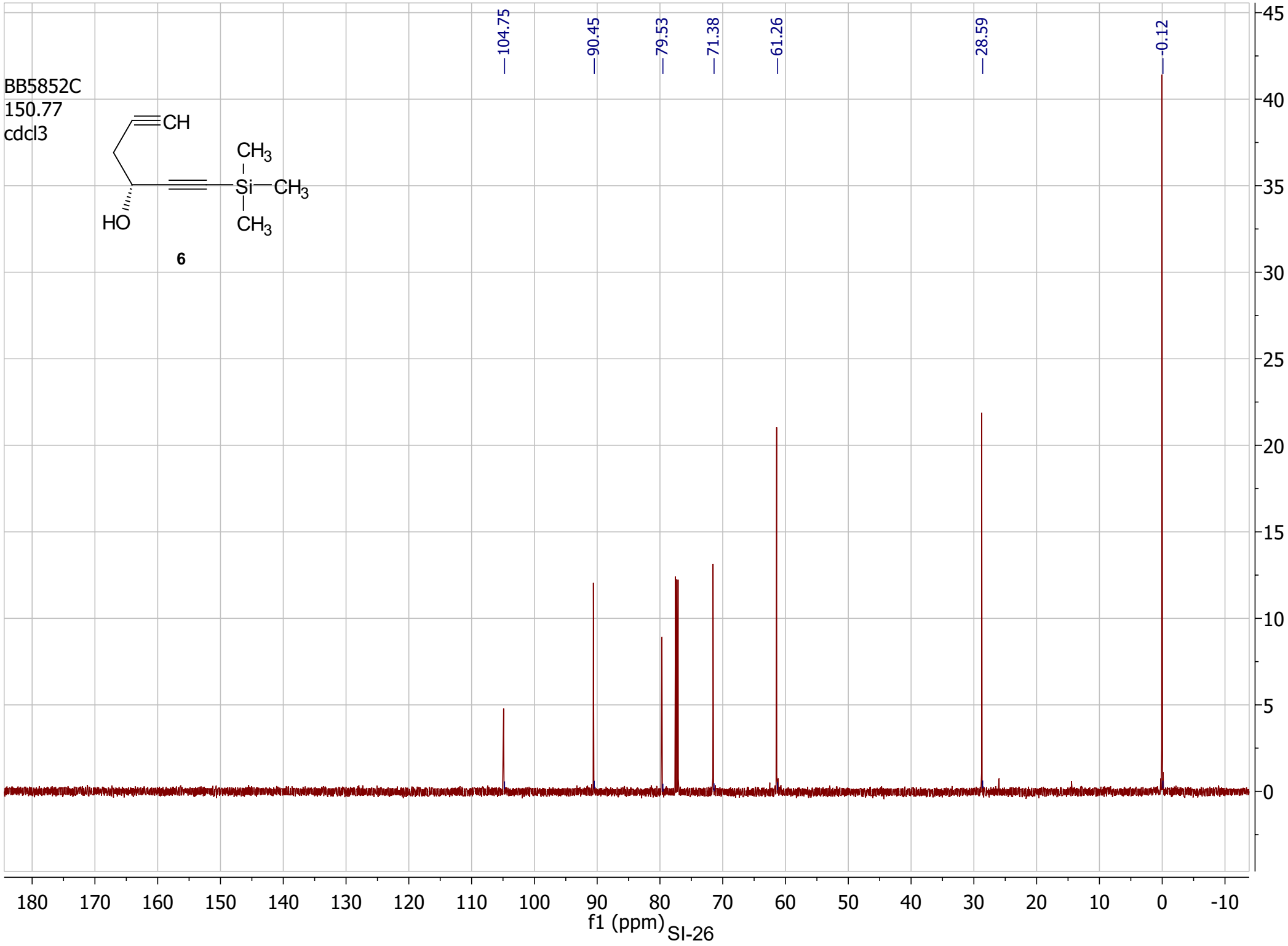
SI-25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45
40
35
30
25
20
15
10
5
0
180
170
160
150
140
130
120
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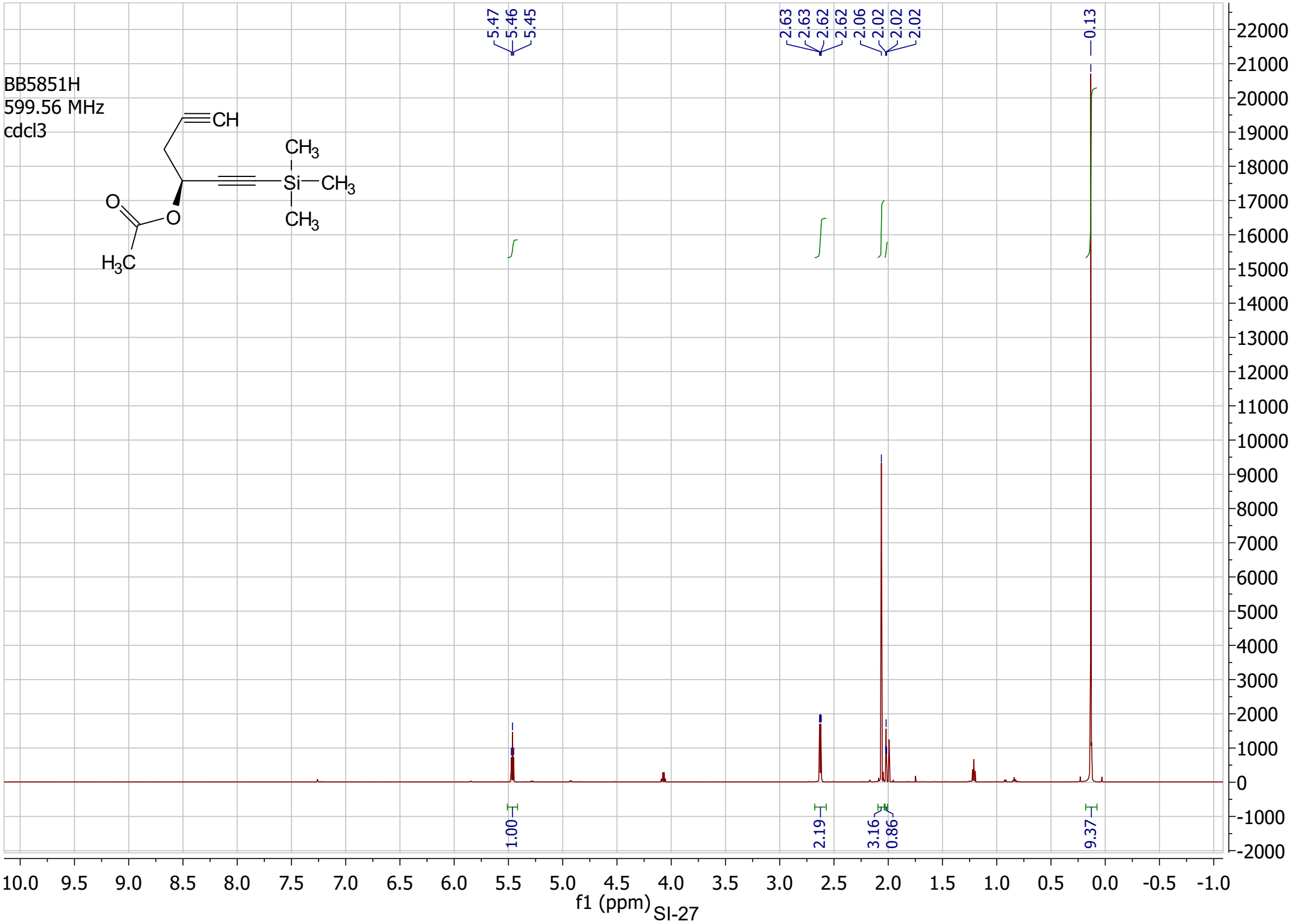
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)
SI-26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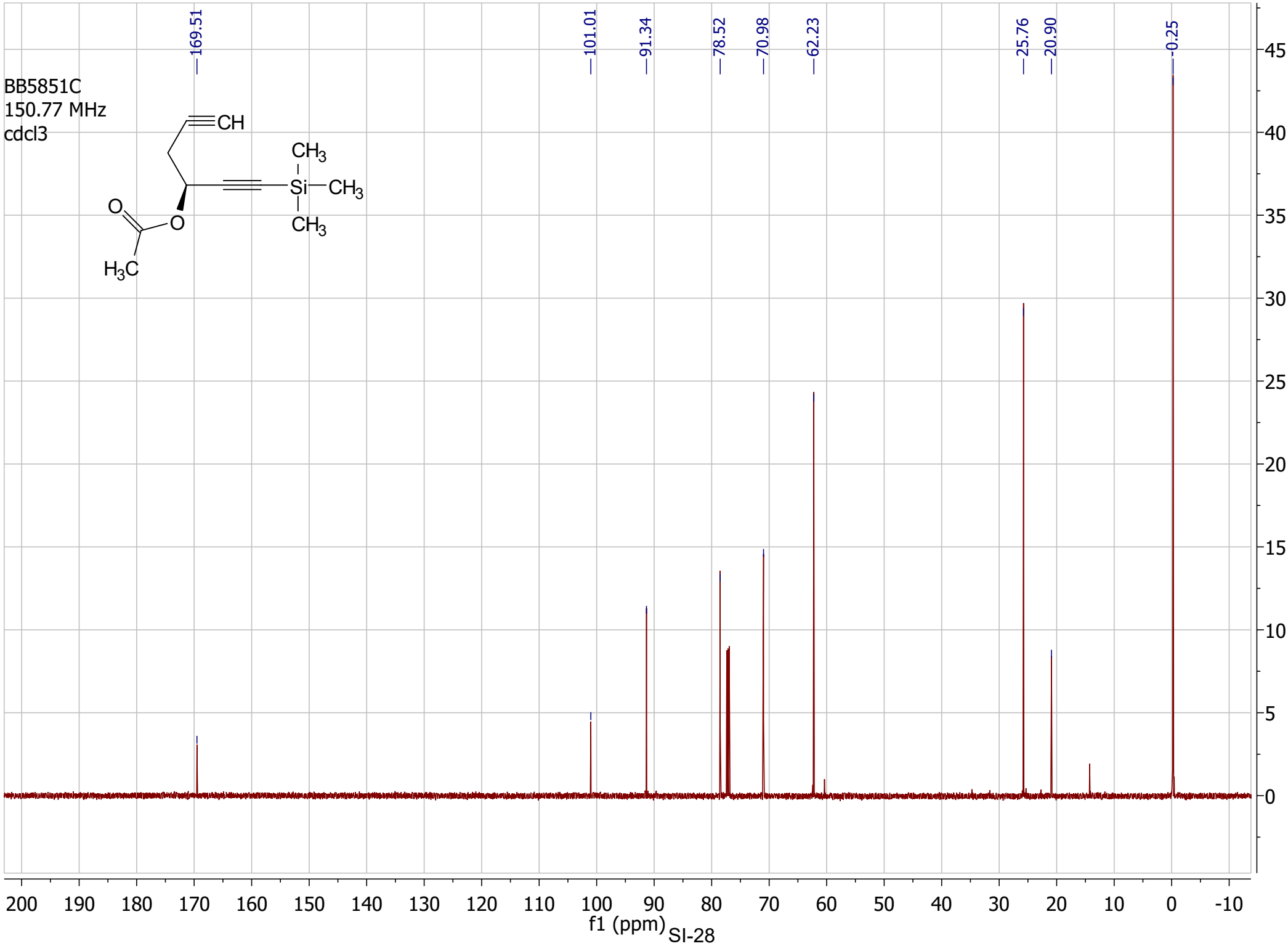
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CH3
Si
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CH3
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O
H3C
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f1 (ppm)
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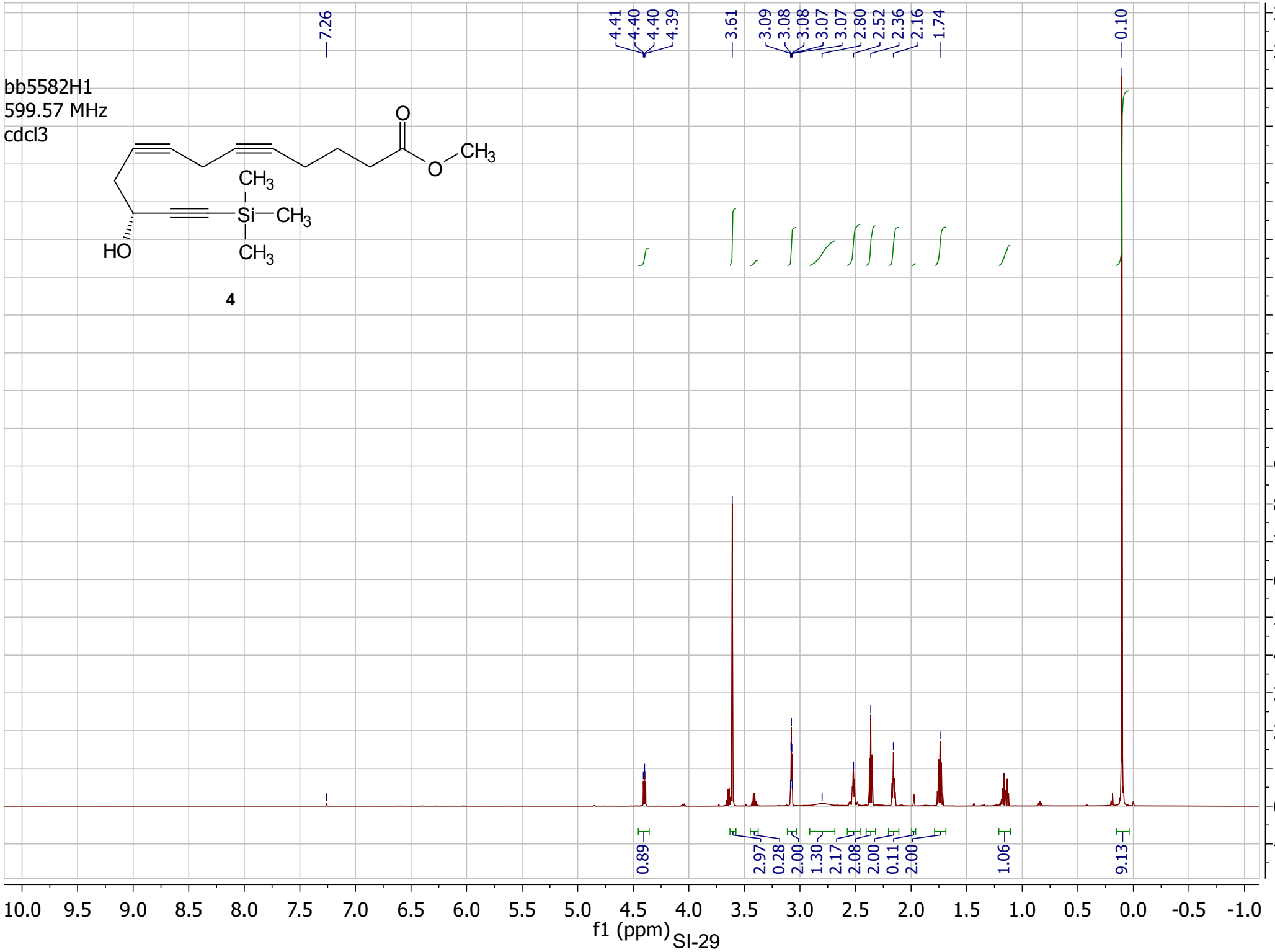
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3.09
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3.07
3.07
2.80
2.52
2.36
2.16
1.74
0.10
O
O
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CH3
Si
CH3
CH3
HO
4
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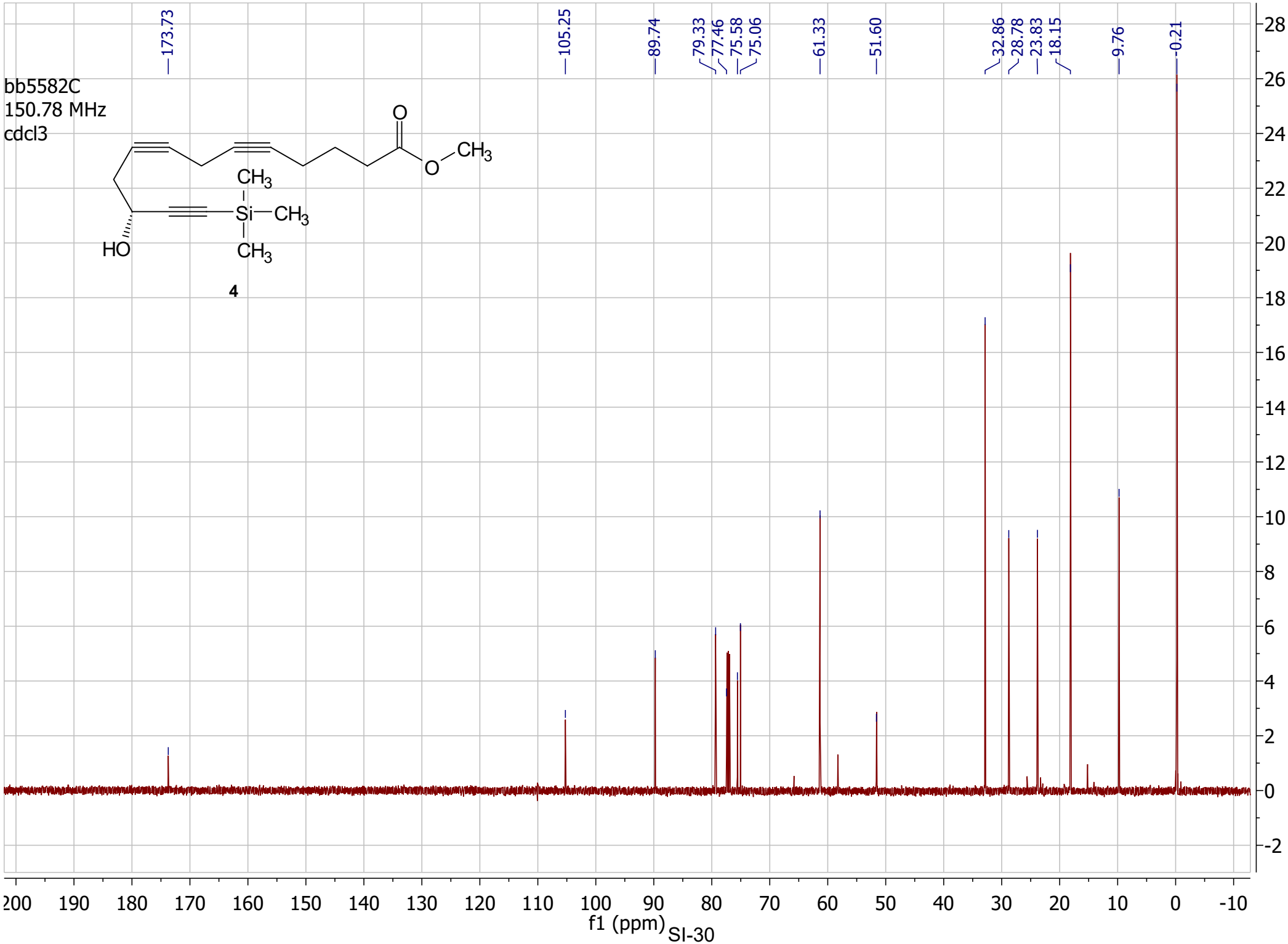
2.97
0.28
2.00
1.30
2.17
2.08
2.00
0.11
2.00
1.06
9.13
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)
SI-29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75.06
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32.86
28.78
23.83
18.15
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O
CH3
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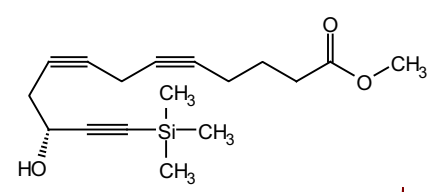
Si
CH3
CH3
HO
4
f1 (ppm)
SI-30



4

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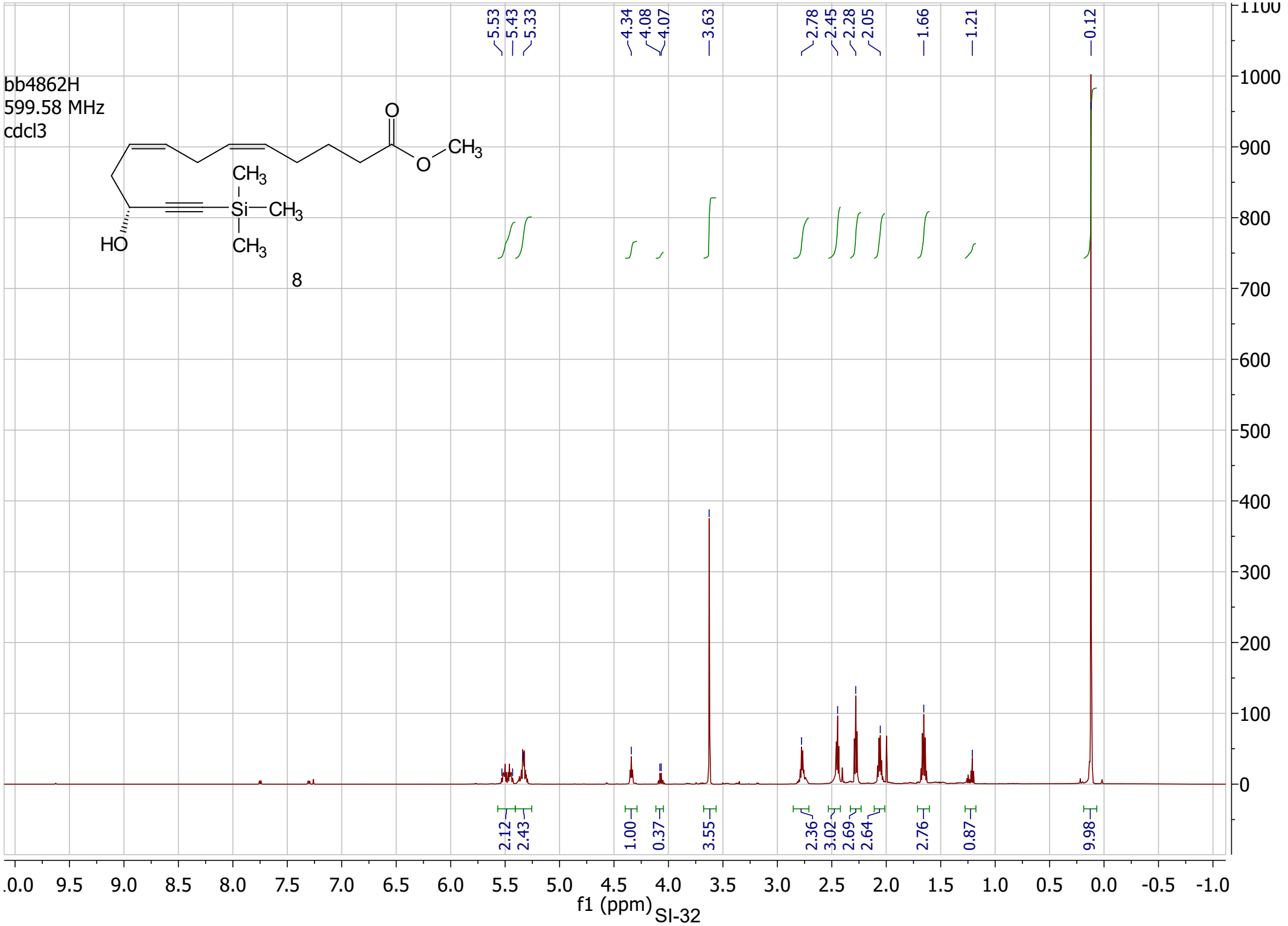
f2 (ppm)

f1 (ppm)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Si
CH3
O
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4.07
3.63
2.78
2.45
2.28
2.05
1.66
1.21
0.12
2.12
2.43
1.00
0.37
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2.36
3.02
2.69
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0.87
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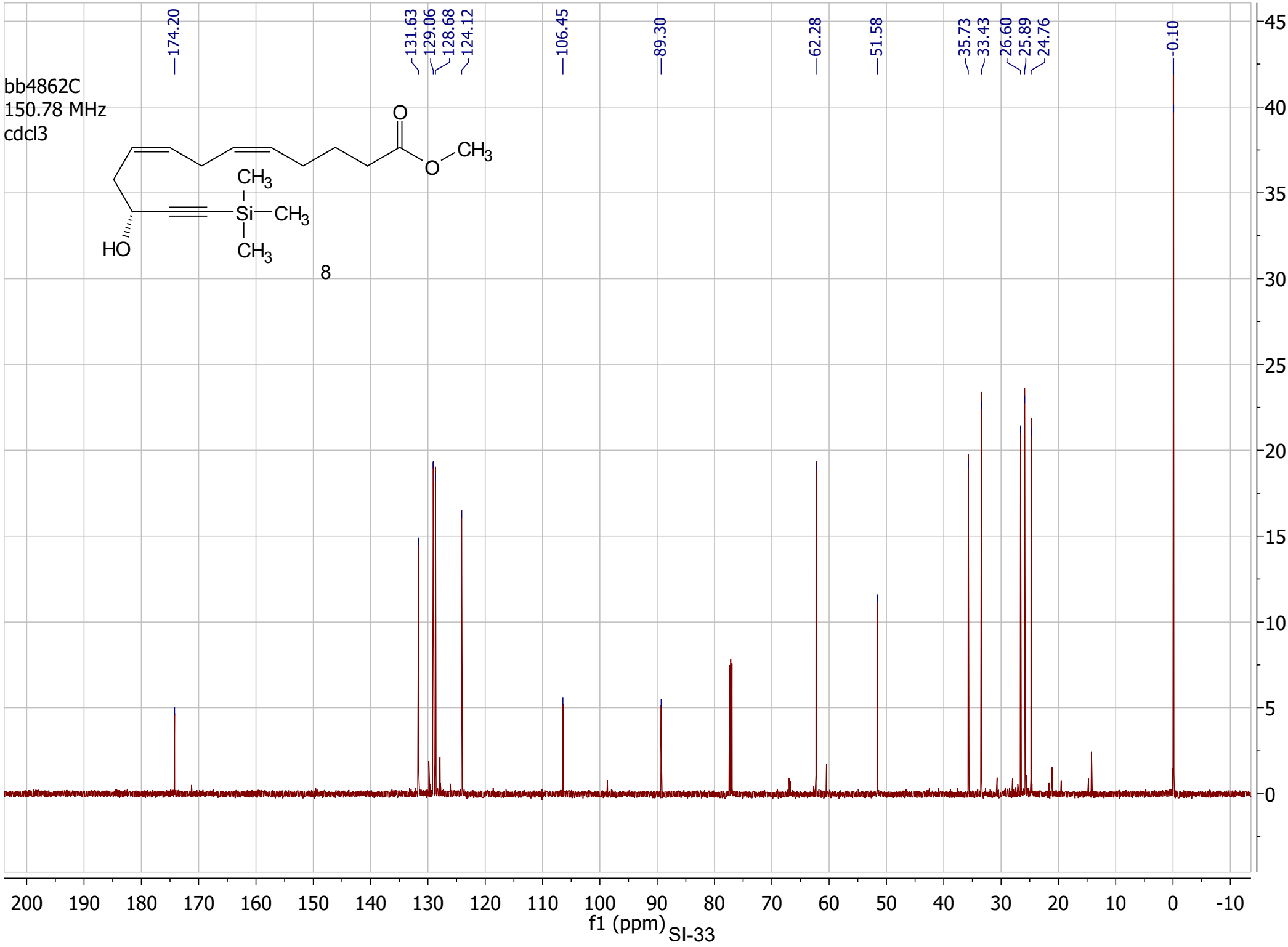
f1 (ppm)
SI-32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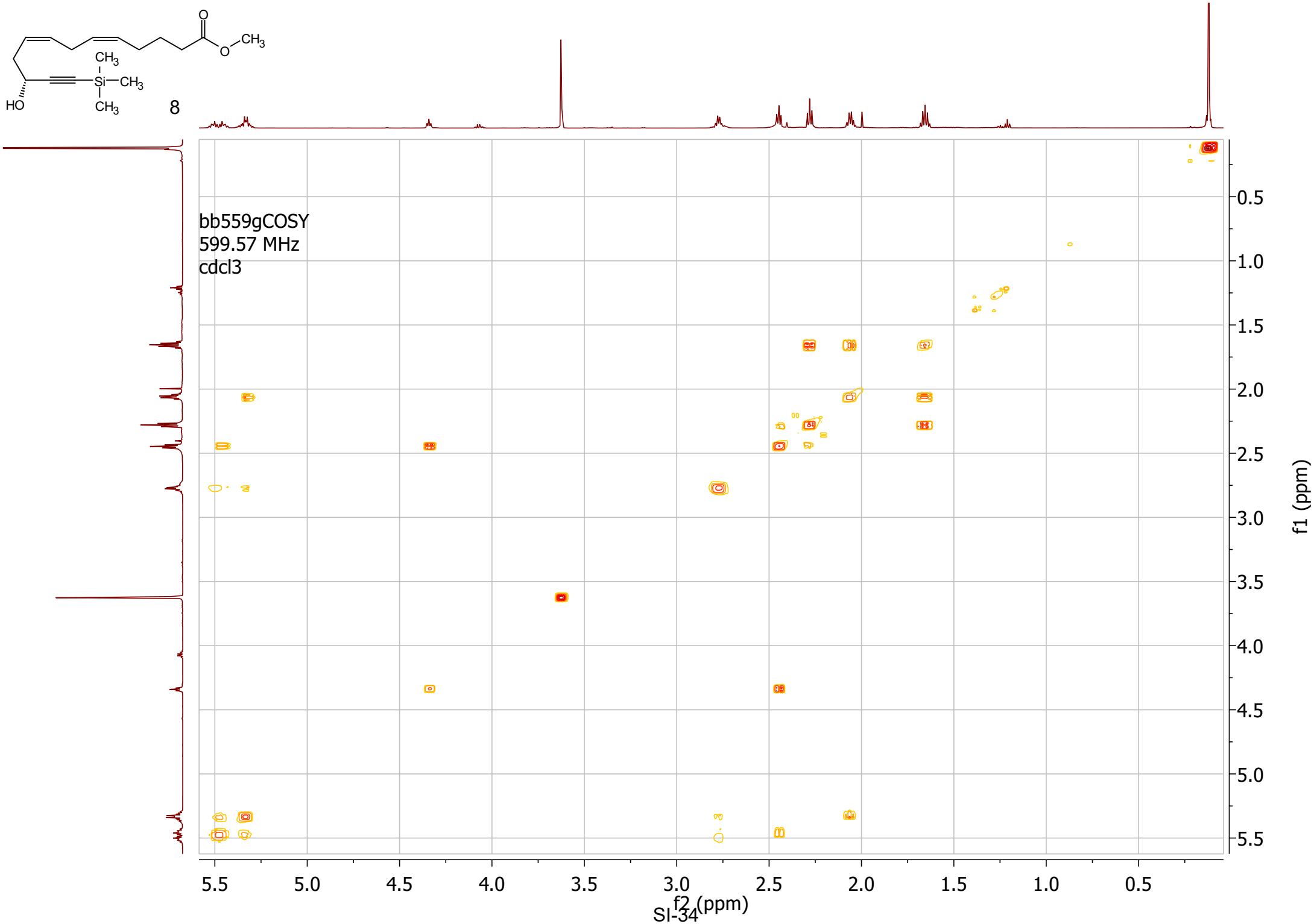
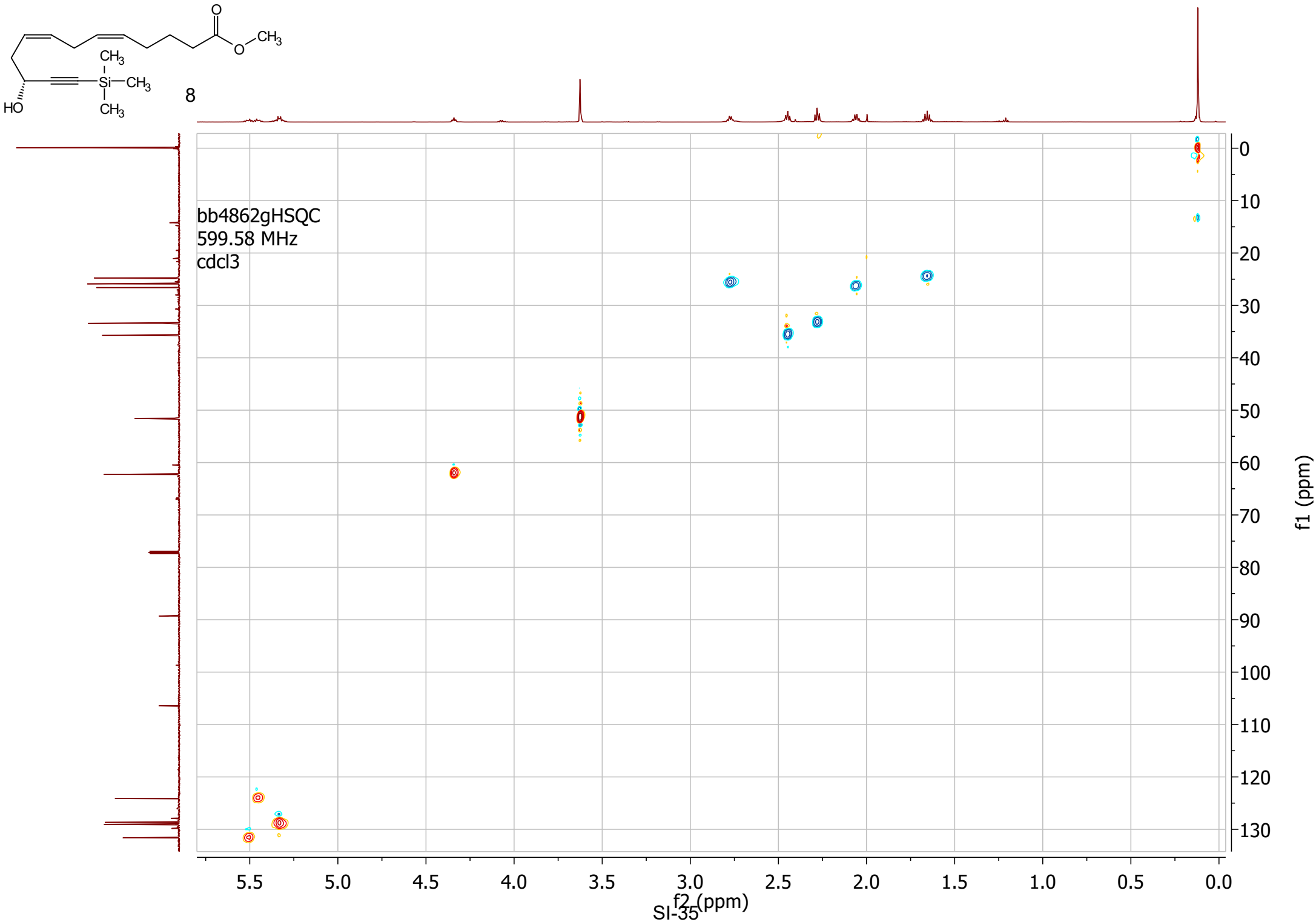
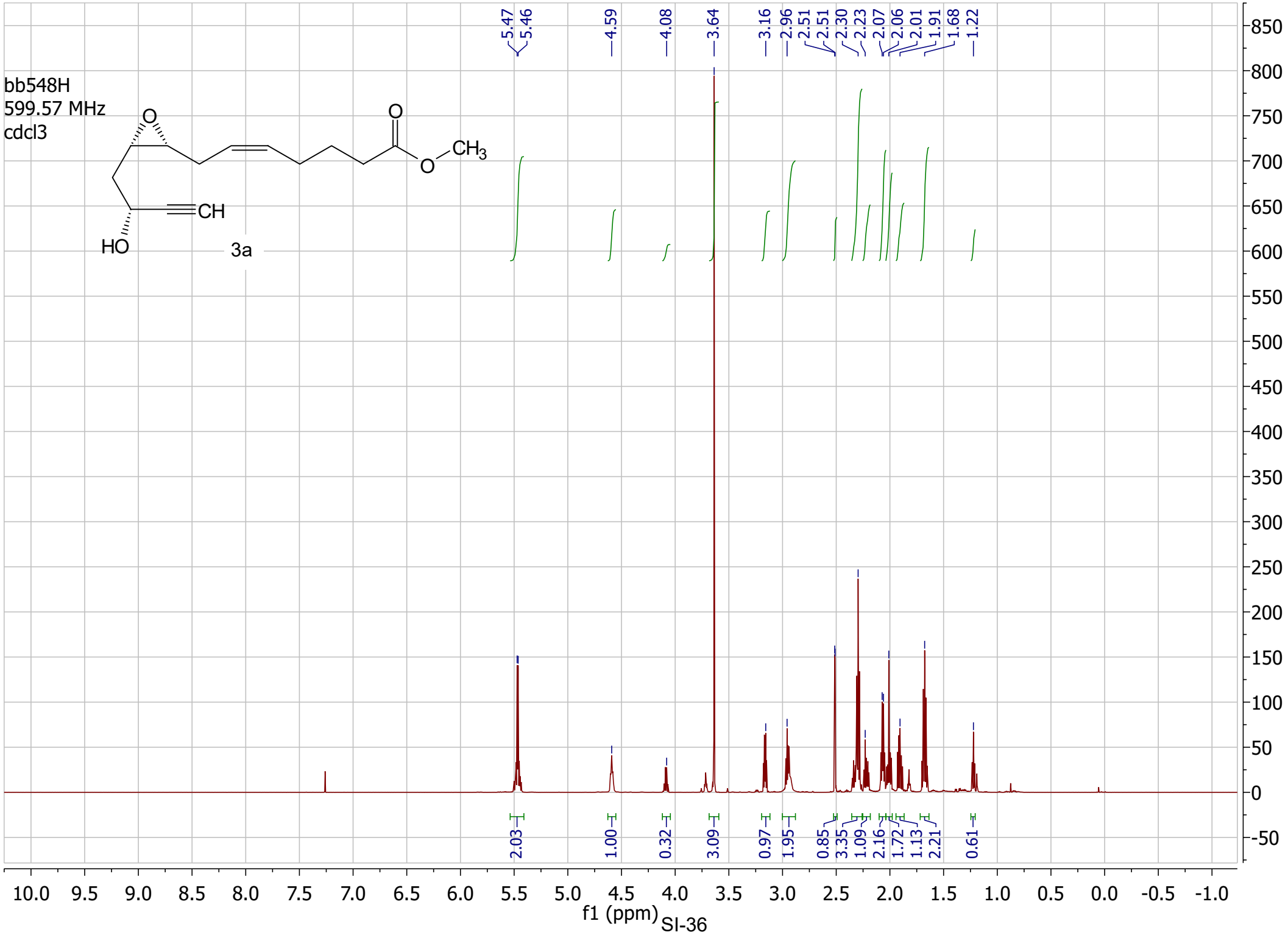
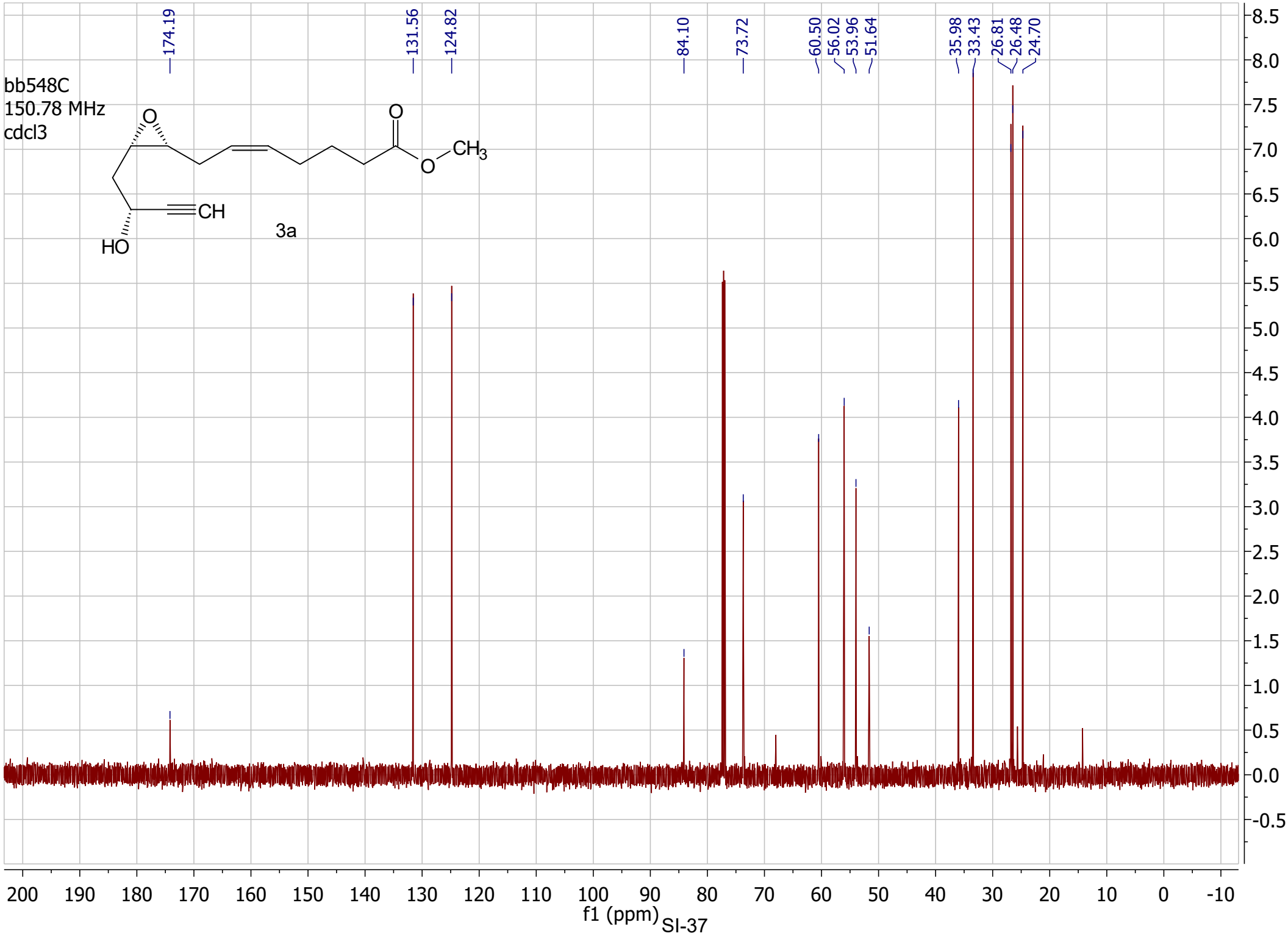
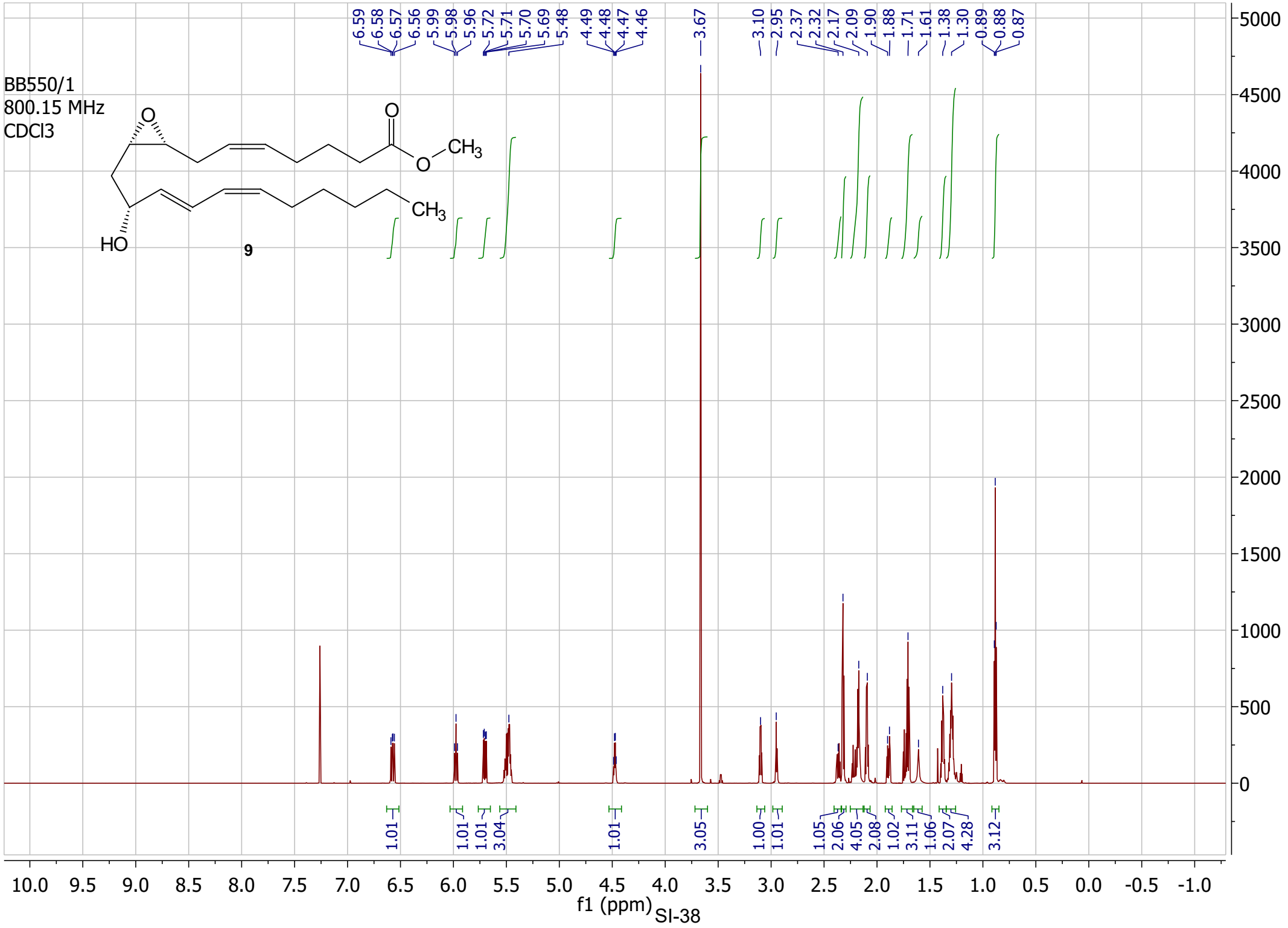
33.43
26.60
25.89
24.76
0.10
bb4862C
150.78 MHz
cdcl3
O
O
CH3
CH3
Si
CH3
CH3
HO
8
f1 (ppm)
SI-33

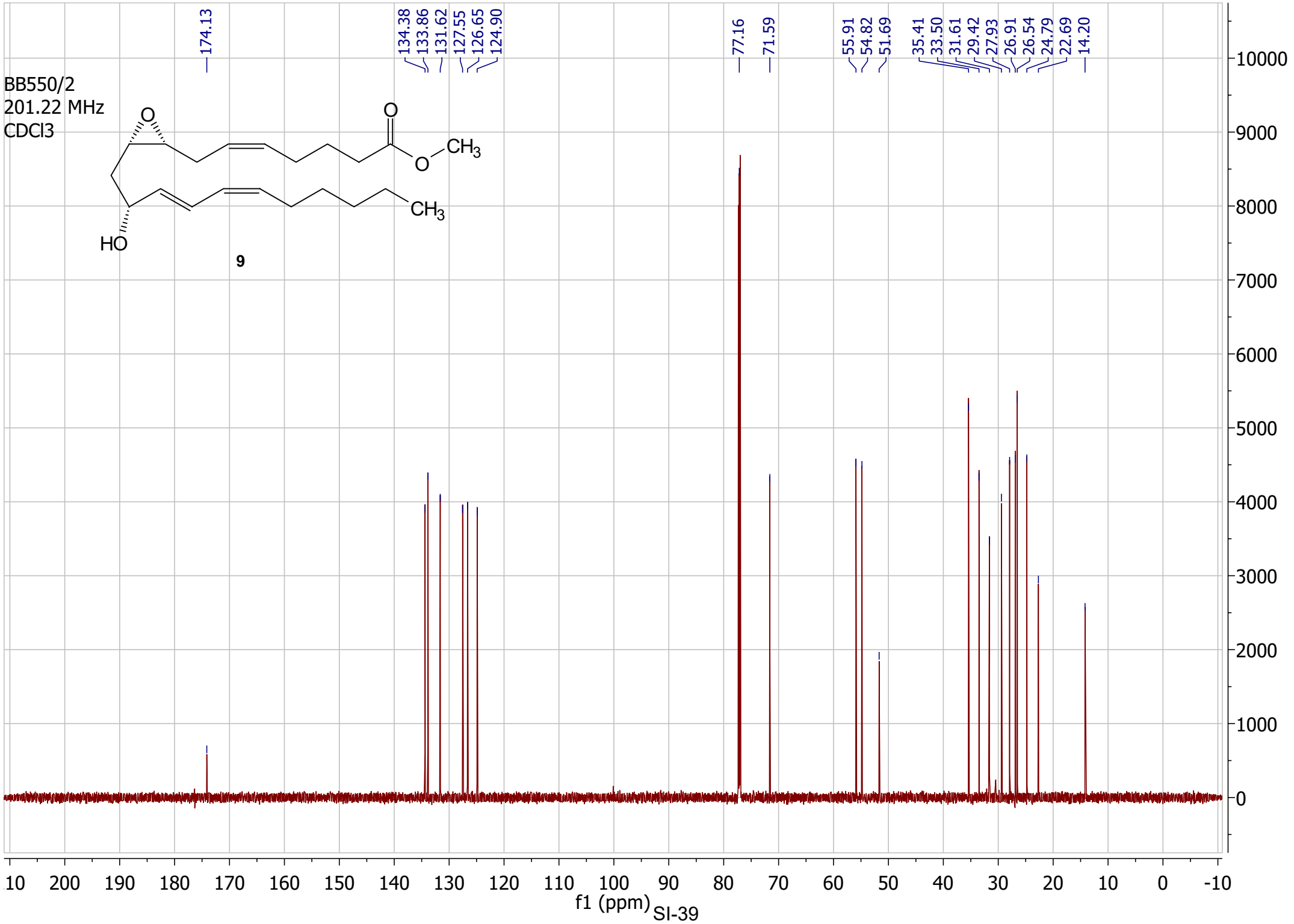

8
bb559gCOSY
599.57 MHz
cdcl3
f2 (ppm)
f1 (ppm)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

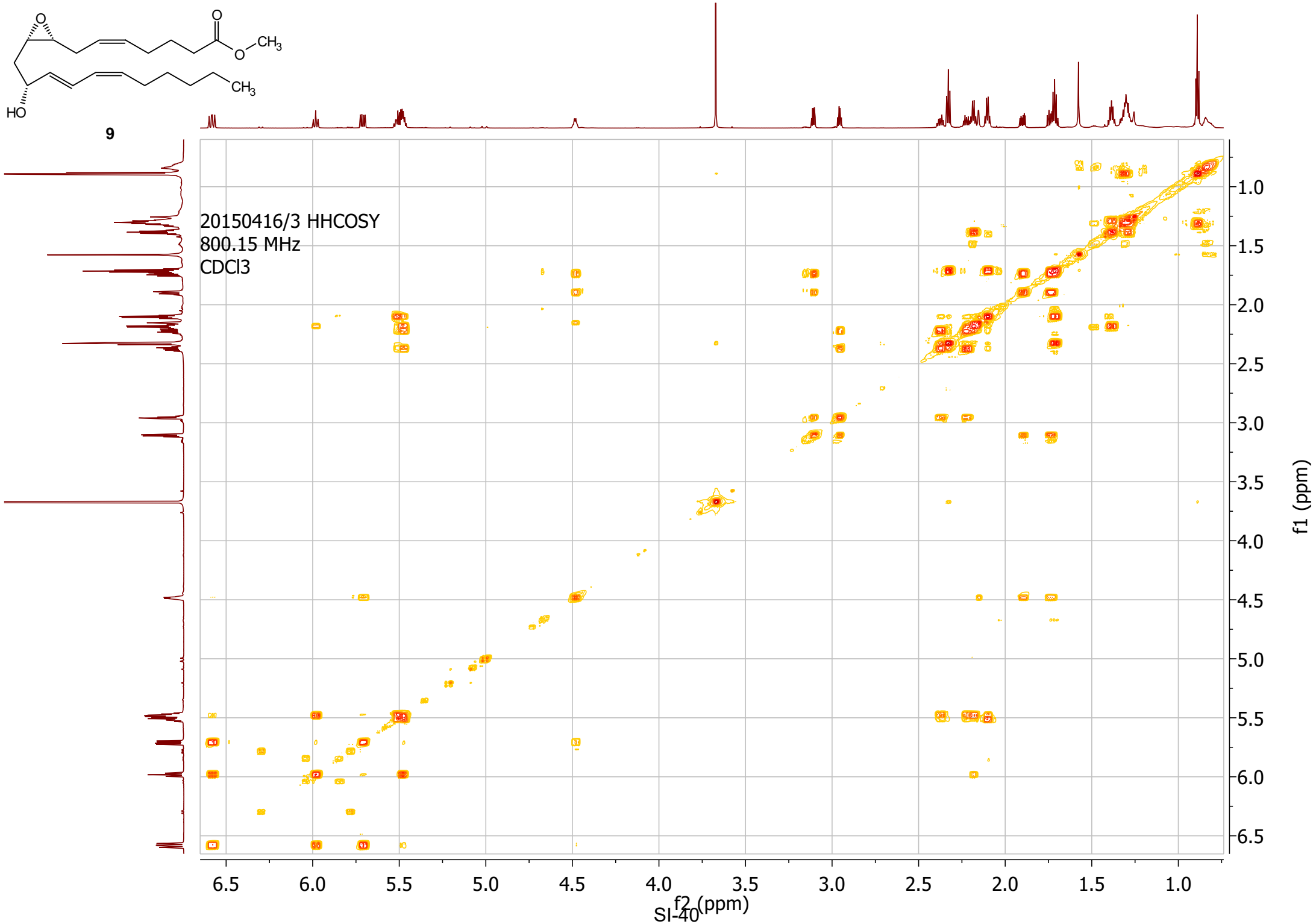

8
bb4862gHSQC
599.58 MHz
cdcl3
f2 (ppm)
f1 (ppm)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
80
90
100
110
120
130

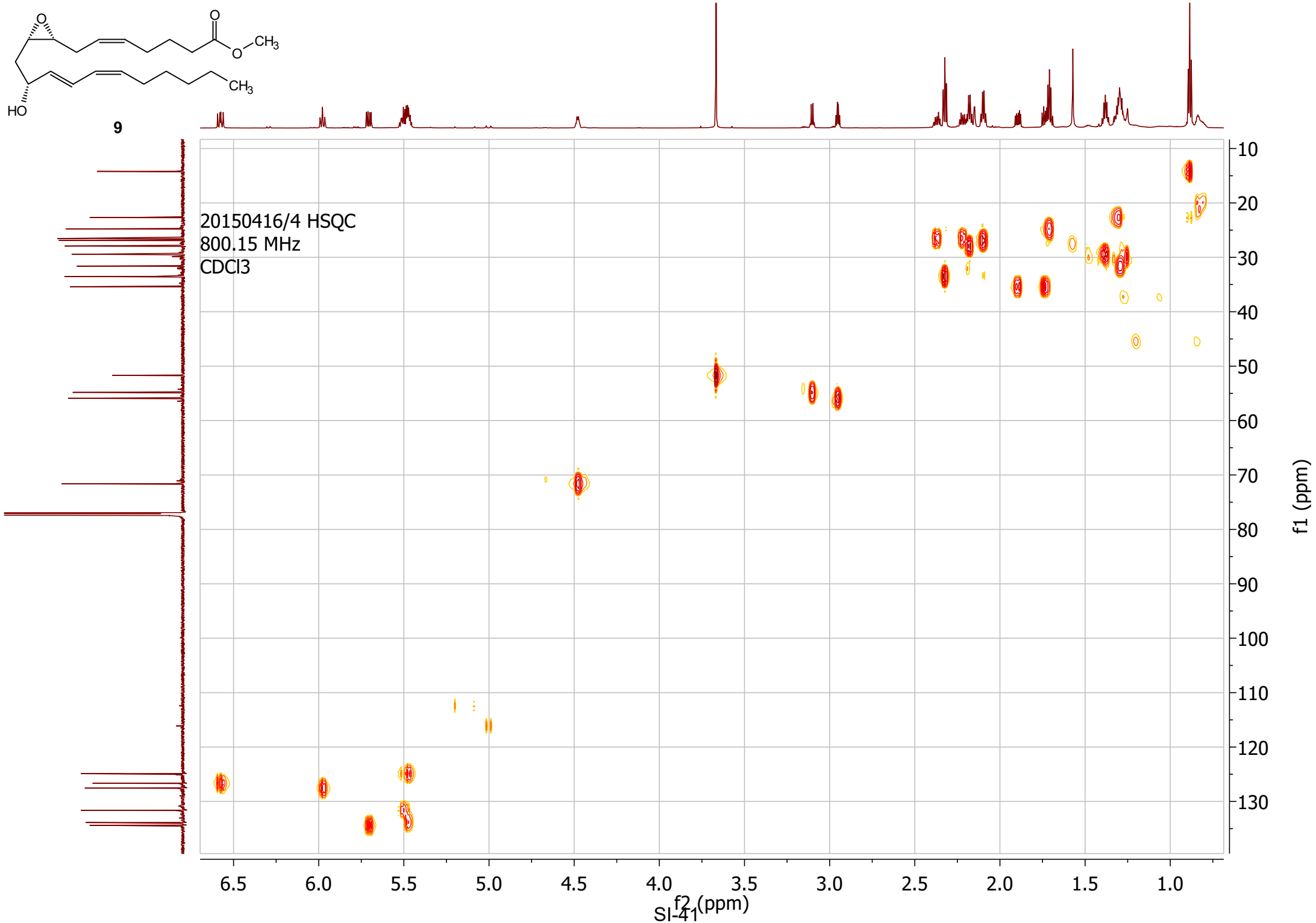

bb548H
599.57 MHz
cdcl3
O
O
O
CH3
CH
HO
3a
5.47
5.46
4.59
4.08
3.64
3.16
2.96
2.51
2.51
2.30
2.23
2.07
2.06
2.01
1.91
1.68
1.22
2.03
1.00
0.32
3.09
0.97
1.95
0.85
3.35
1.09
2.16
1.72
1.13
2.21
0.61
f1 (ppm)
SI-36

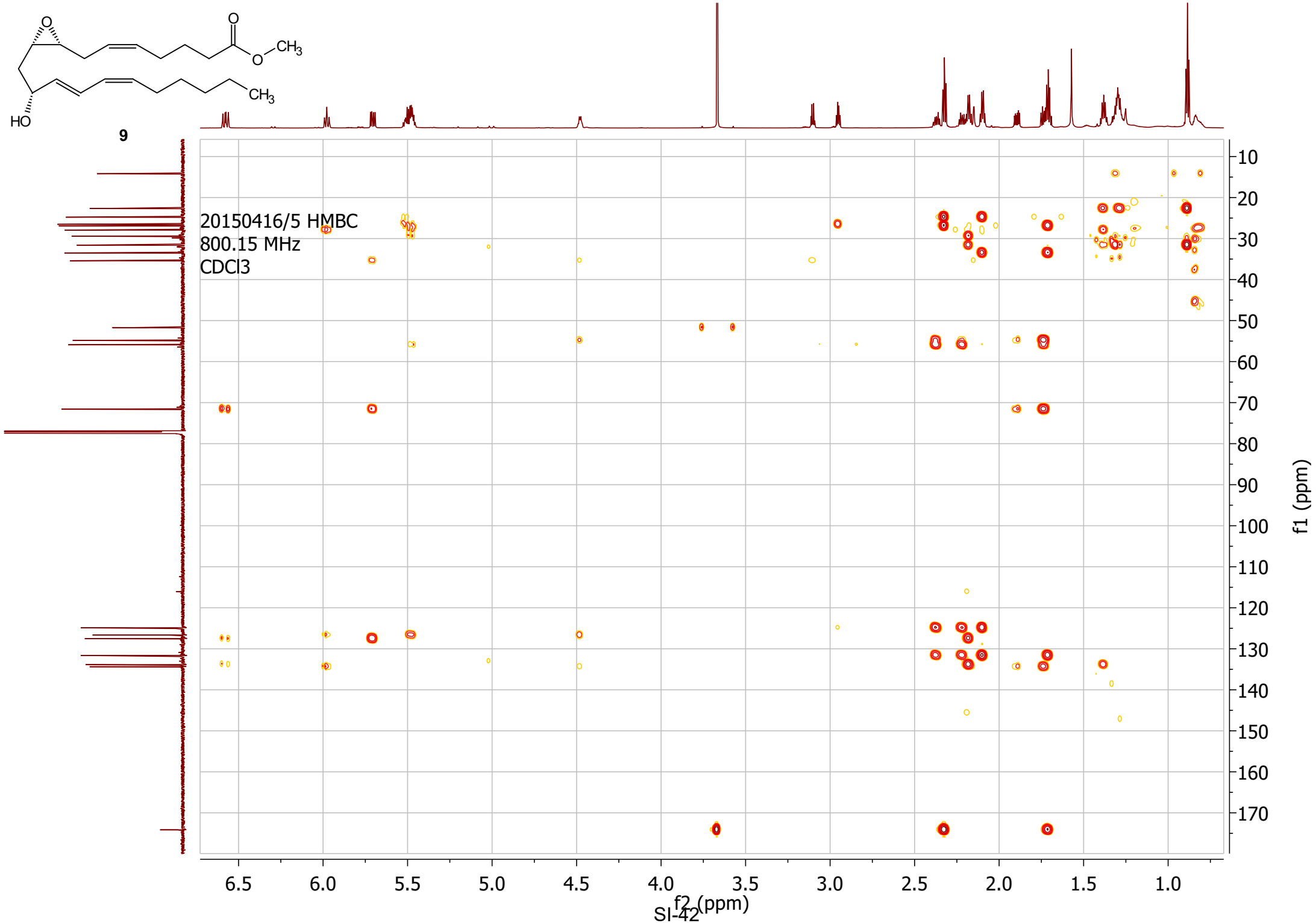

bb548C
150.78 MHz
cdcl3
174.19
131.56
124.82
84.10
73.72
60.50
56.02
53.96
51.64
35.98
33.43
26.81
26.48
24.70
O
O
O
CH3
CH
HO
3a
f1 (ppm)

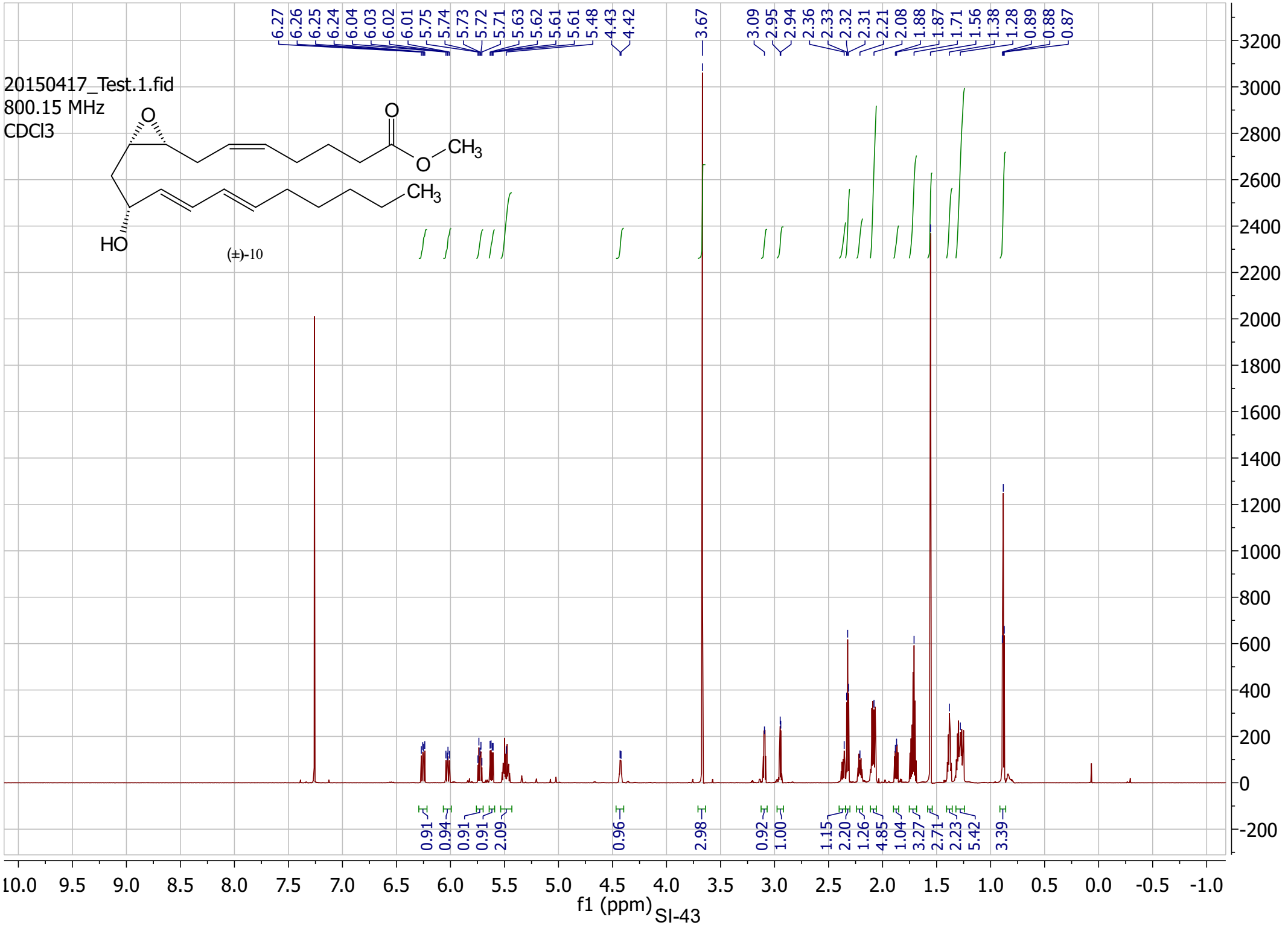

BB550/1
800.15 MHz
CDCl3
O
O
O
CH3
CH3
HO
9
6.59
6.58
6.57
6.56
5.99
5.98
5.96
5.72
5.71
5.70
5.69
5.48
4.49
4.48
4.47
4.46
3.67
3.10
2.95
2.37
2.32
2.17
2.09
1.90
1.88
1.71
1.61
1.38
1.30
0.89
0.88
0.87
1.01
1.01
1.01
3.04
1.01
3.05
1.00
1.01
1.05
2.06
4.05
2.08
1.02
3.11
1.06
2.07
4.28
3.12
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)
SI-38

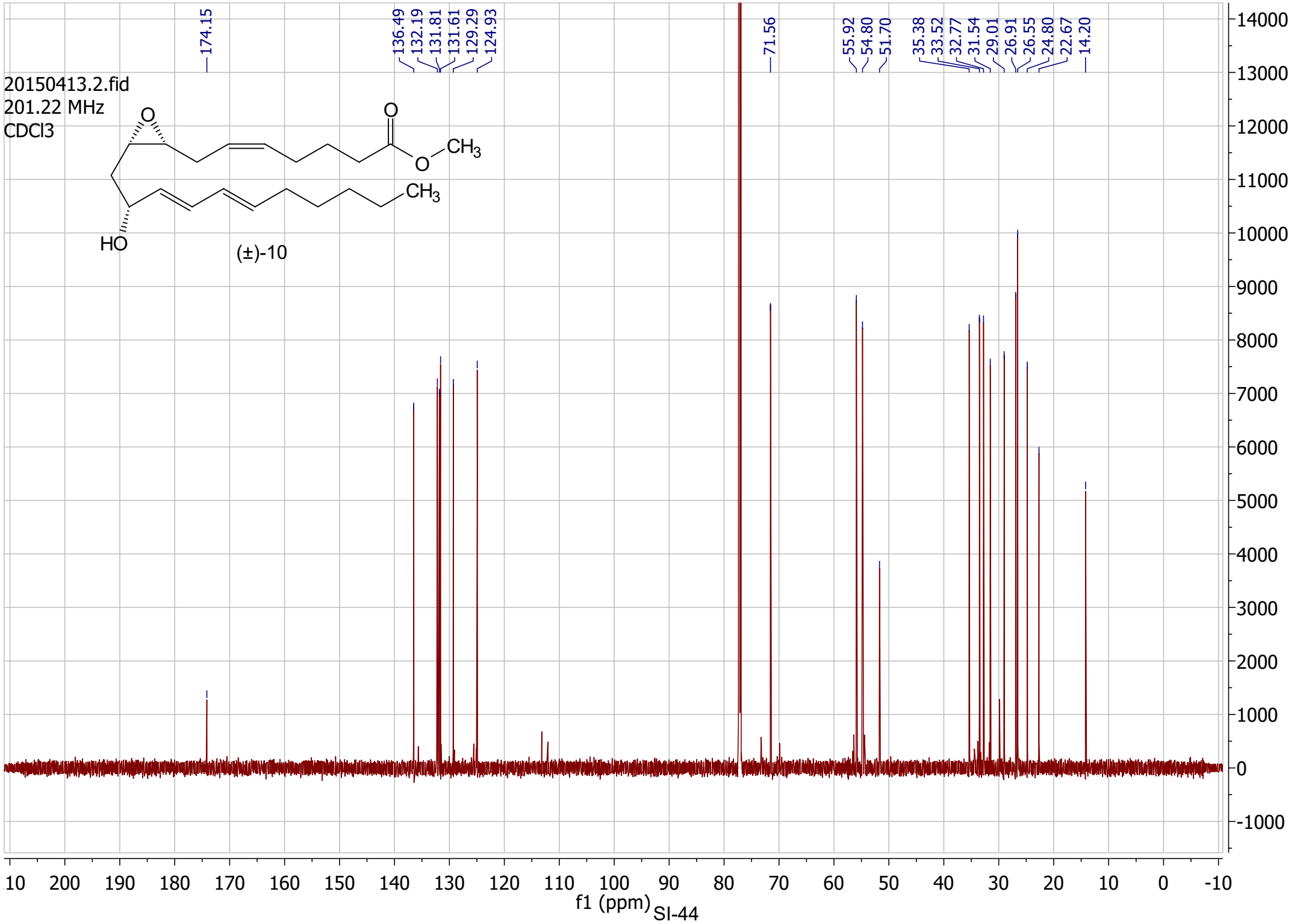

BB550/2
201.22 MHz
CDCl3
174.13
134.38
133.86
131.62
127.55
126.65
124.90
77.16
71.59
55.91
54.82
51.69
35.41
33.50
31.61
29.42
27.93
26.91
26.54
24.79
22.69
14.20
O
O
CH3
O
CH3
HO
9
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

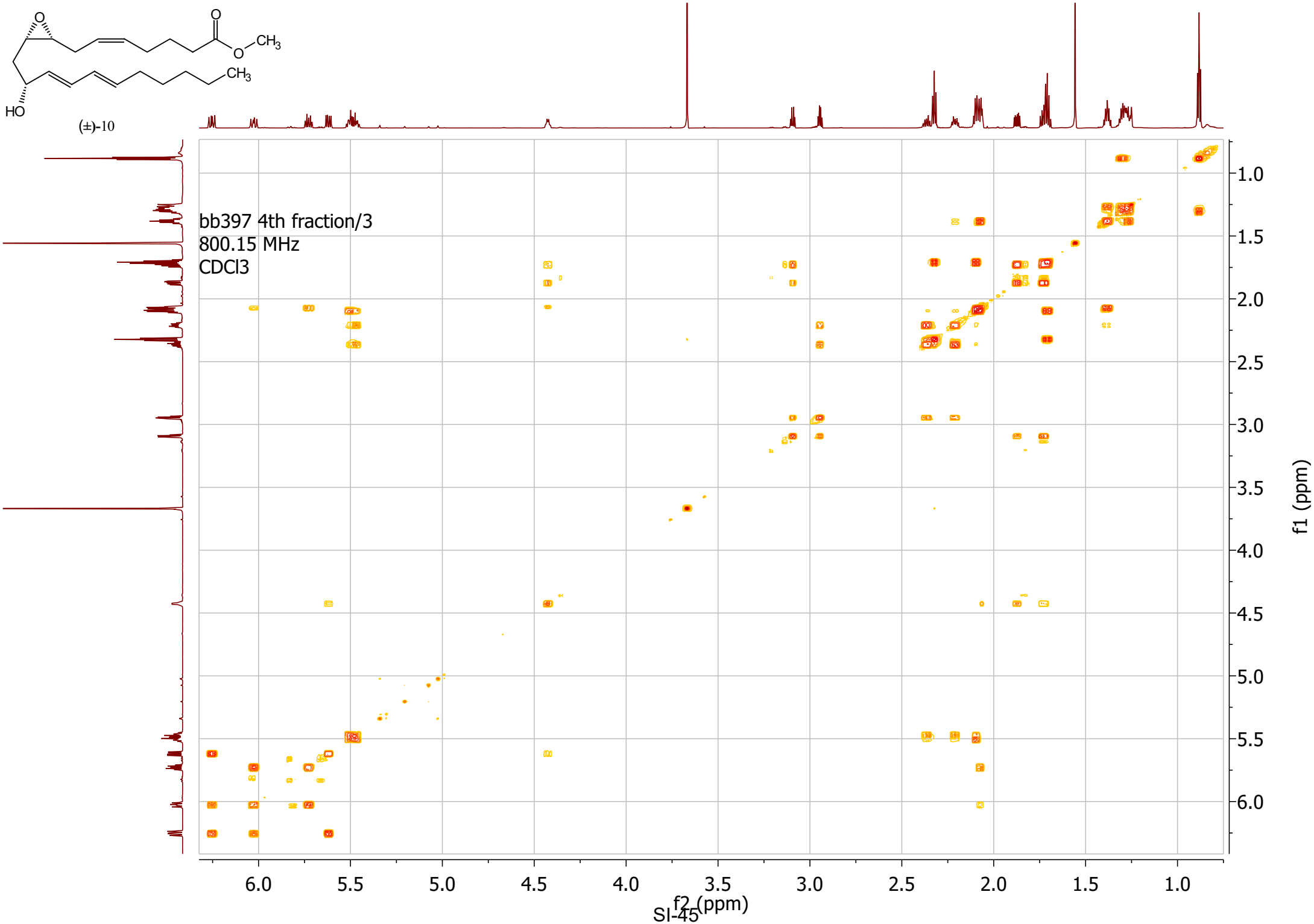

9
20150416/3 HHCOSY
800.15 MHz
CDCl3
f2 (ppm)
f1 (ppm)
SI-40

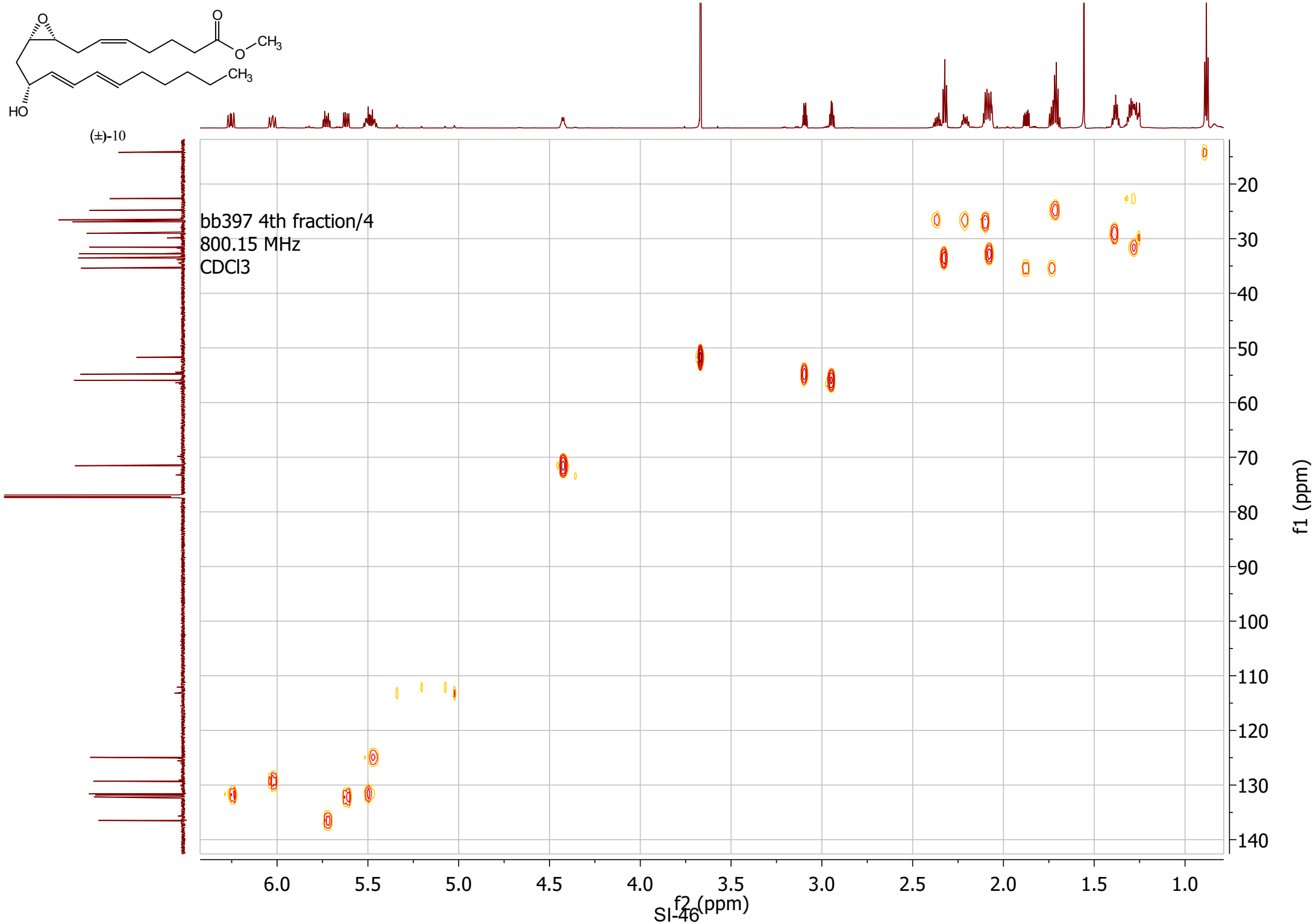

9
20150416/4 HSQC
800.15 MHz
CDCl3
f2 (ppm)
f1 (ppm)
SI-41

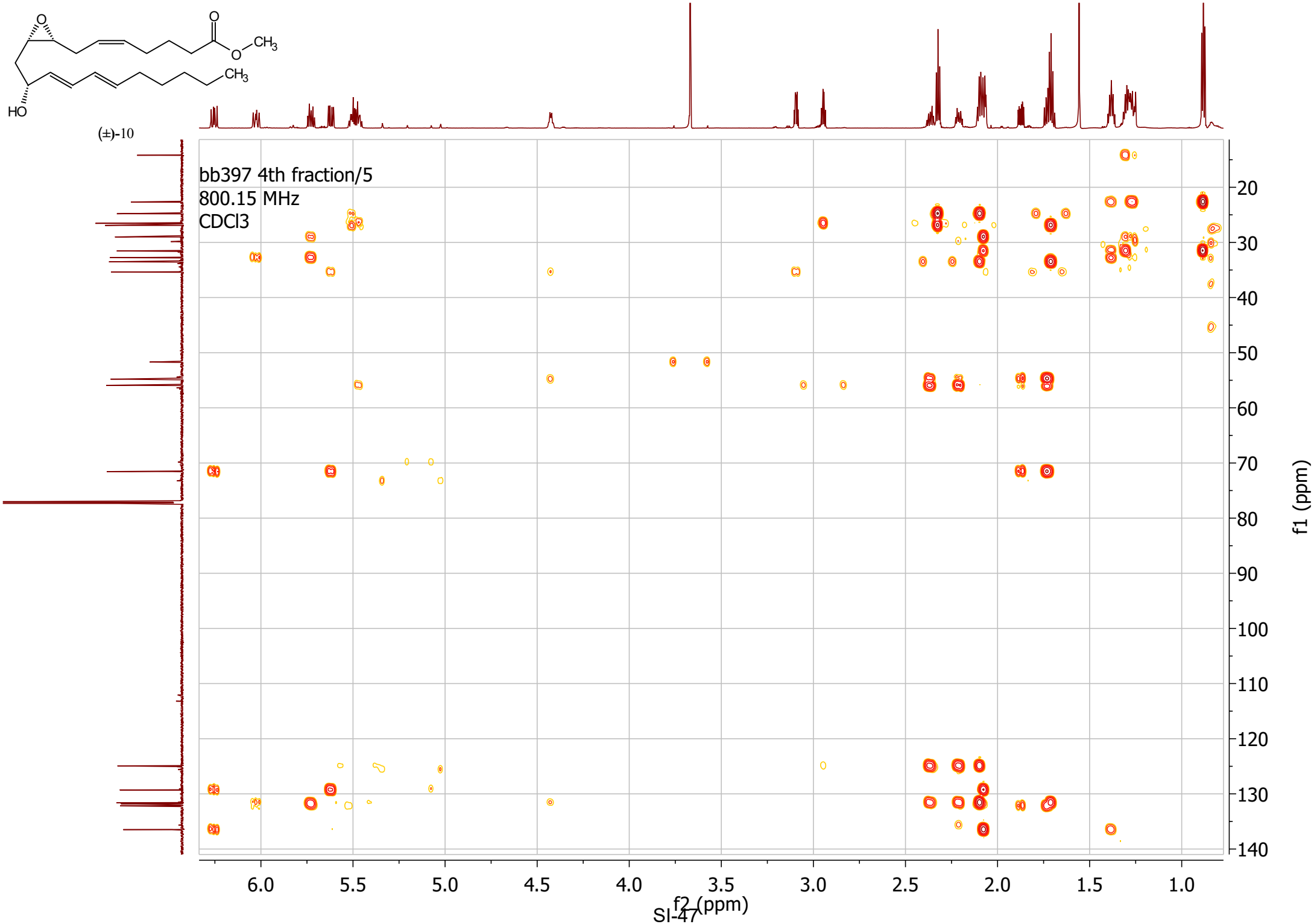

9
20150416/5 HMBC
800.15 MHz
CDCl3
f2 (ppm)
f1 (ppm)
SI-42

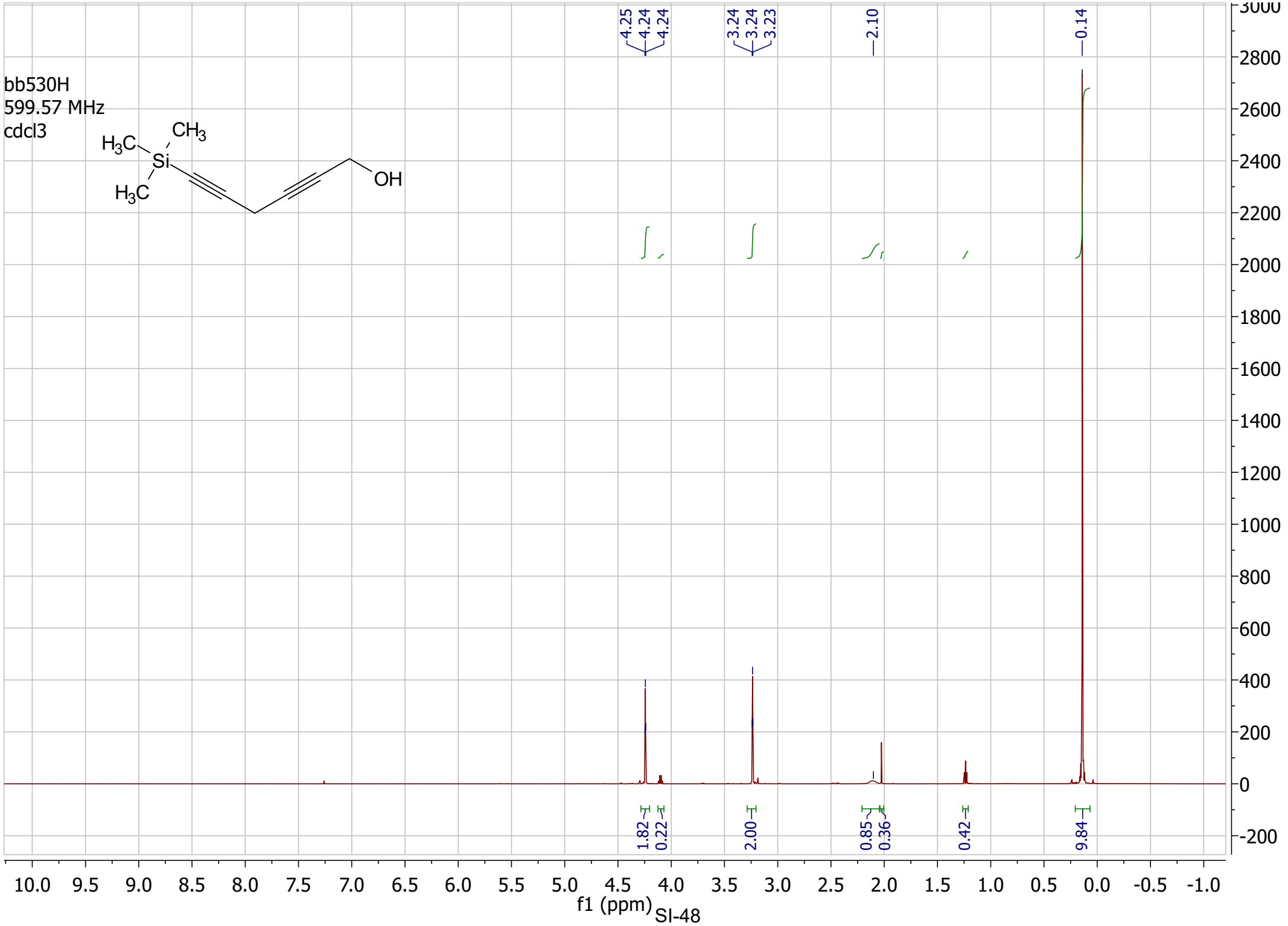

20150417_Test.1.fid
800.15 MHz
CDCl3
6.27 6.26 6.25 6.24 6.04 6.03 6.02 6.01 5.75 5.74 5.73 5.72 5.71 5.63 5.62 5.61 5.61 5.48 4.43 4.42
3.67
3.09 2.95 2.94 2.36 2.33 2.32 2.31 2.21 2.08 1.88 1.87 1.71 1.56 1.38 1.28 0.89 0.88 0.87
O
O
O
CH3
CH3
HO
(±)-10
0.91 0.94 0.91 0.91 2.09 0.96 2.98 0.92 1.00 1.15 2.20 1.26 4.85 1.04 3.27 2.71 2.23 5.42 3.39
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)
SI-43

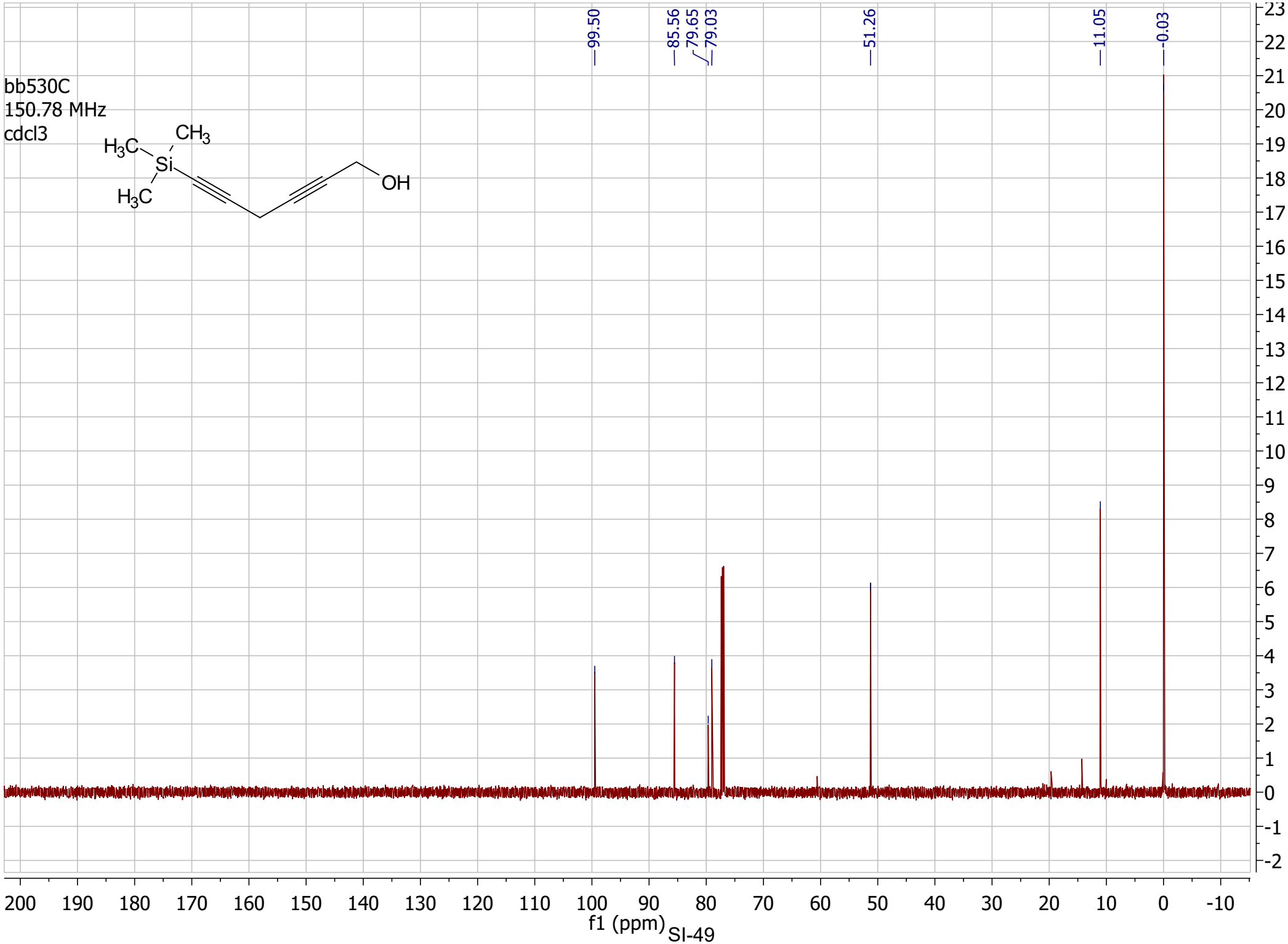

174.15
136.49
132.19
131.81
131.61
129.29
124.93
71.56
55.92
54.80
51.70
35.38
33.52
32.77
31.54
29.01
26.91
26.55
24.80
22.67
14.20
20150413.2.fid
201.22 MHz
CDCl3
O
O
O
CH3
CH3
HO
(±)-10
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
10 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

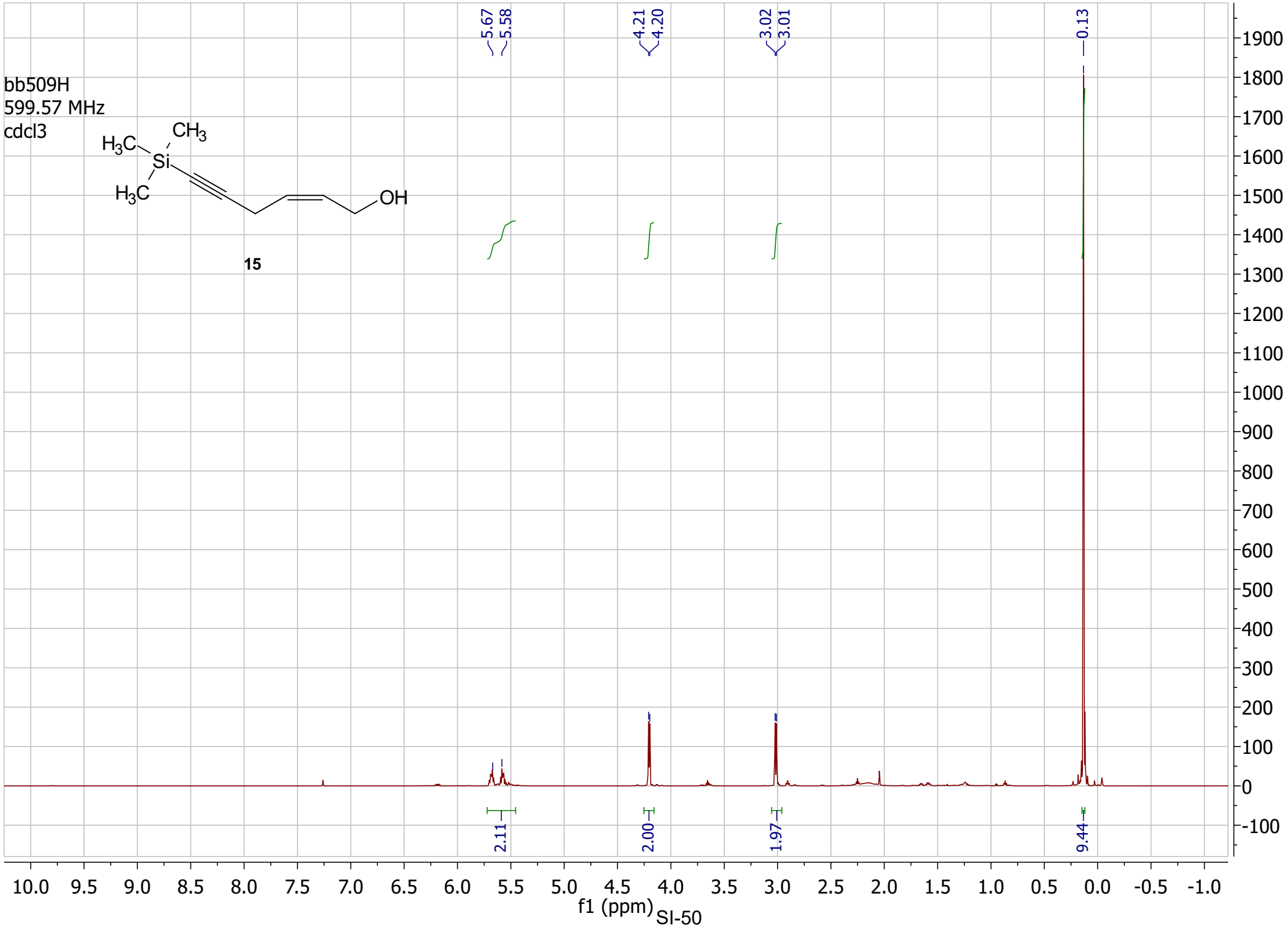

(±)-10
bb397 4th fraction/3
800.15 MHz
CDCl3
f2 (ppm)
f1 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

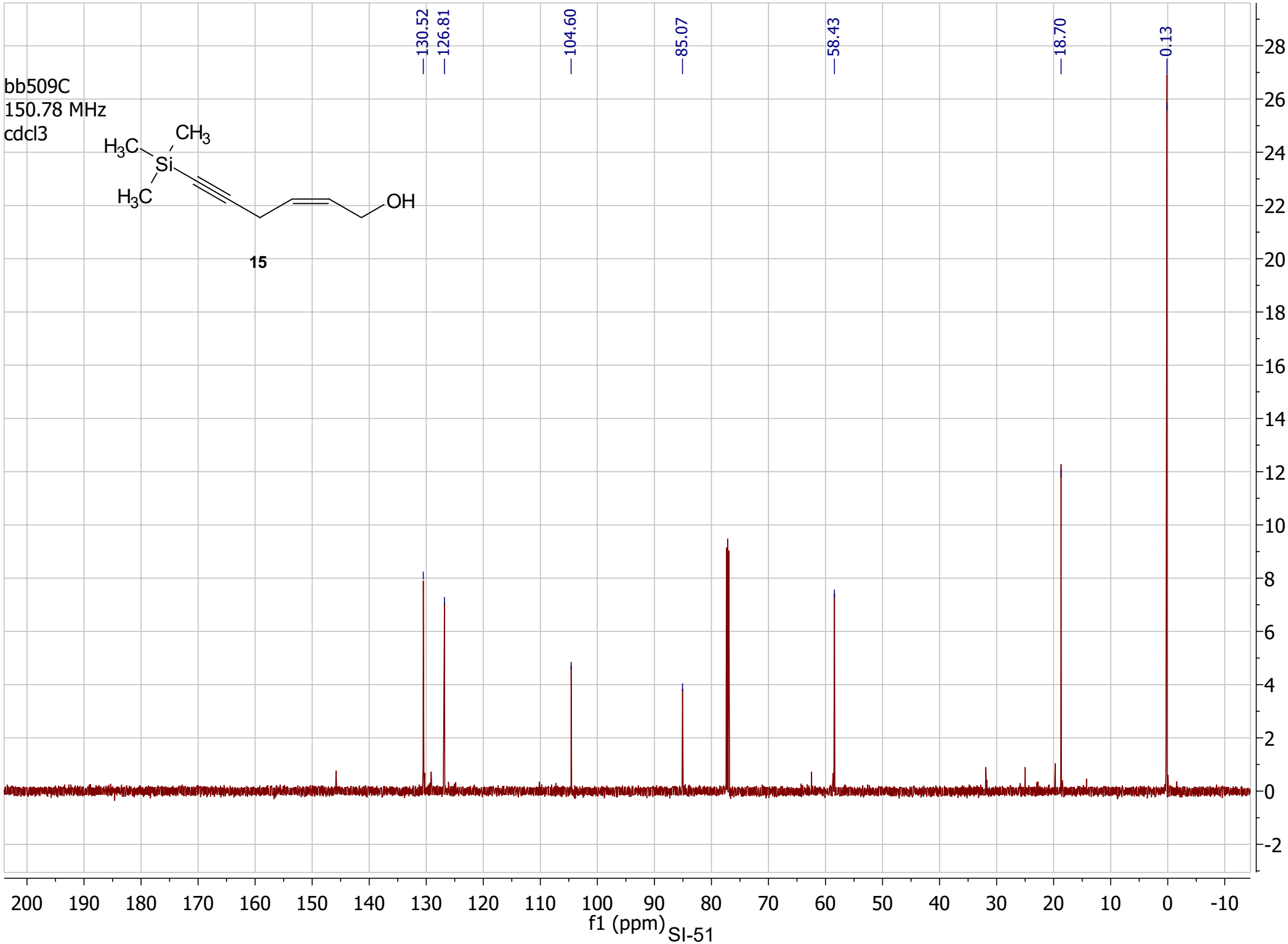

(±)-10
bb397 4th fraction/4
800.15 MHz
CDCl3
f2 (ppm)
f1 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
20
30
40
50
60
70
80
90
100
110
120
130
140

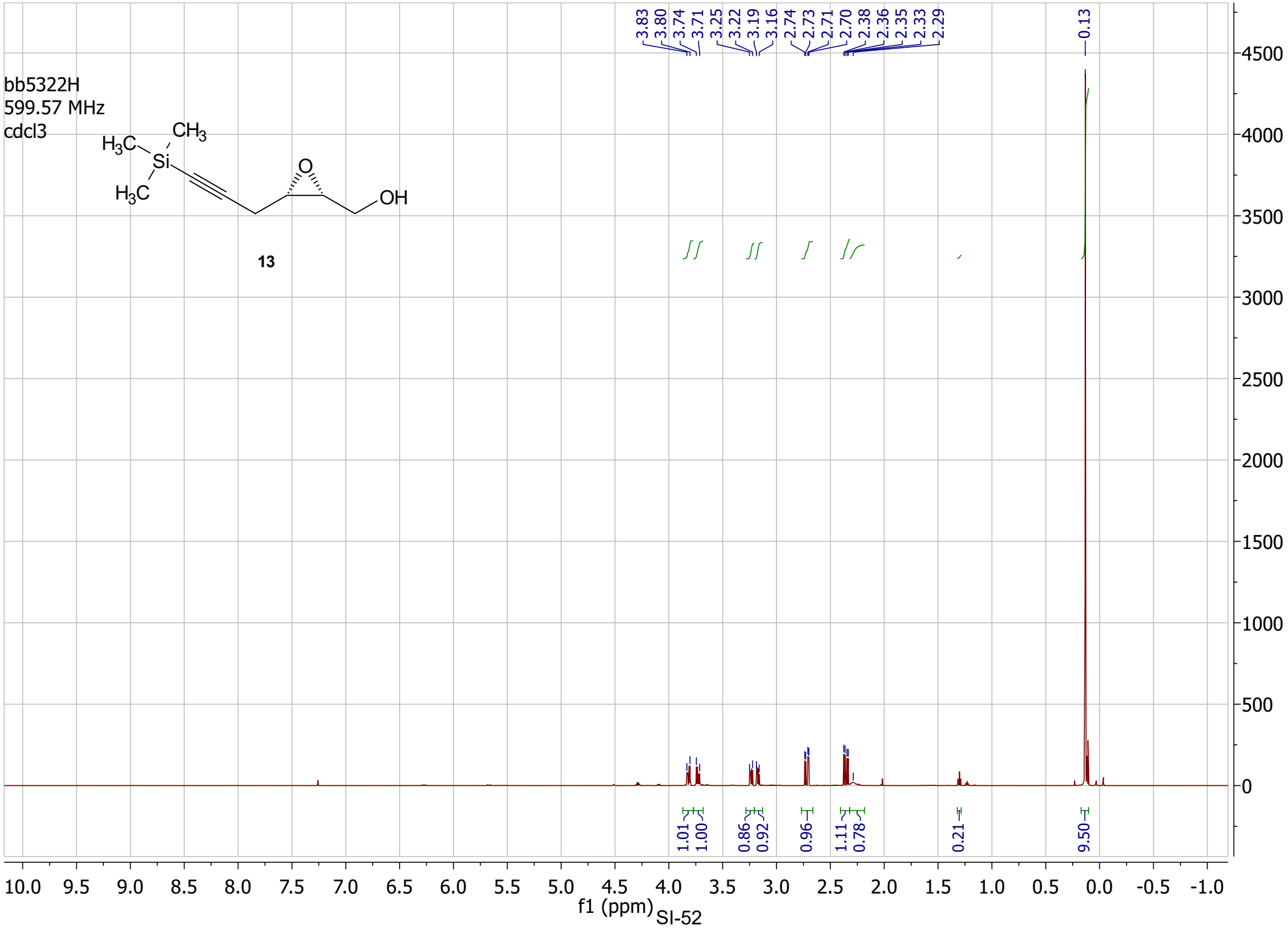

(±)-10
bb397 4th fraction/5
800.15 MHz
CDCl3
f2 (ppm)
f1 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
20
30
40
50
60
70
80
90
100
110
120
130
140

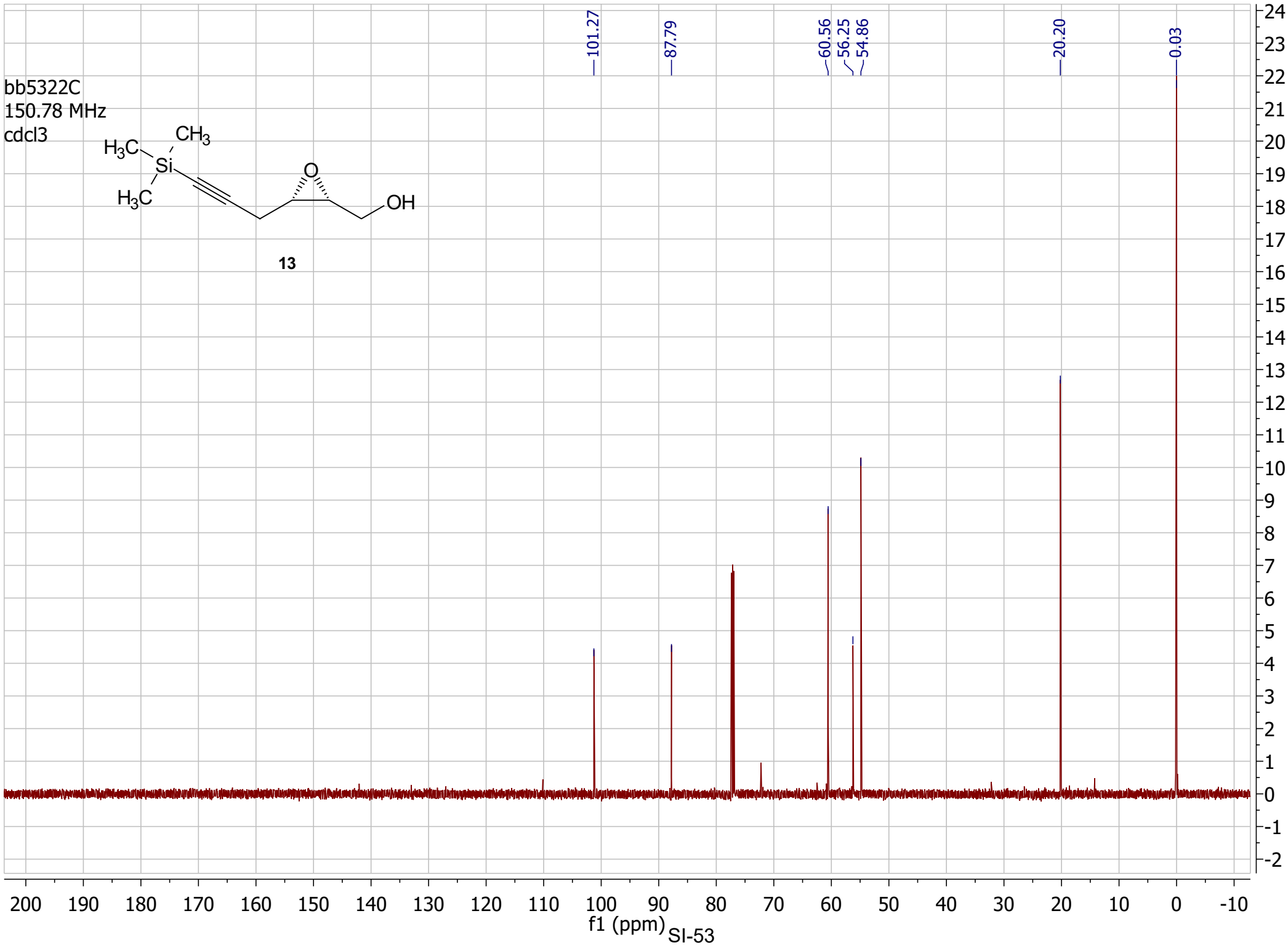

bb530H
599.57 MHz
cdcl3
H3C
CH3
Si
H3C
OH
4.25
4.24
4.24
3.24
3.24
3.23
2.10
0.14
1.82
0.22
2.00
0.85
0.36
0.42
9.84
f1 (ppm)
SI-48

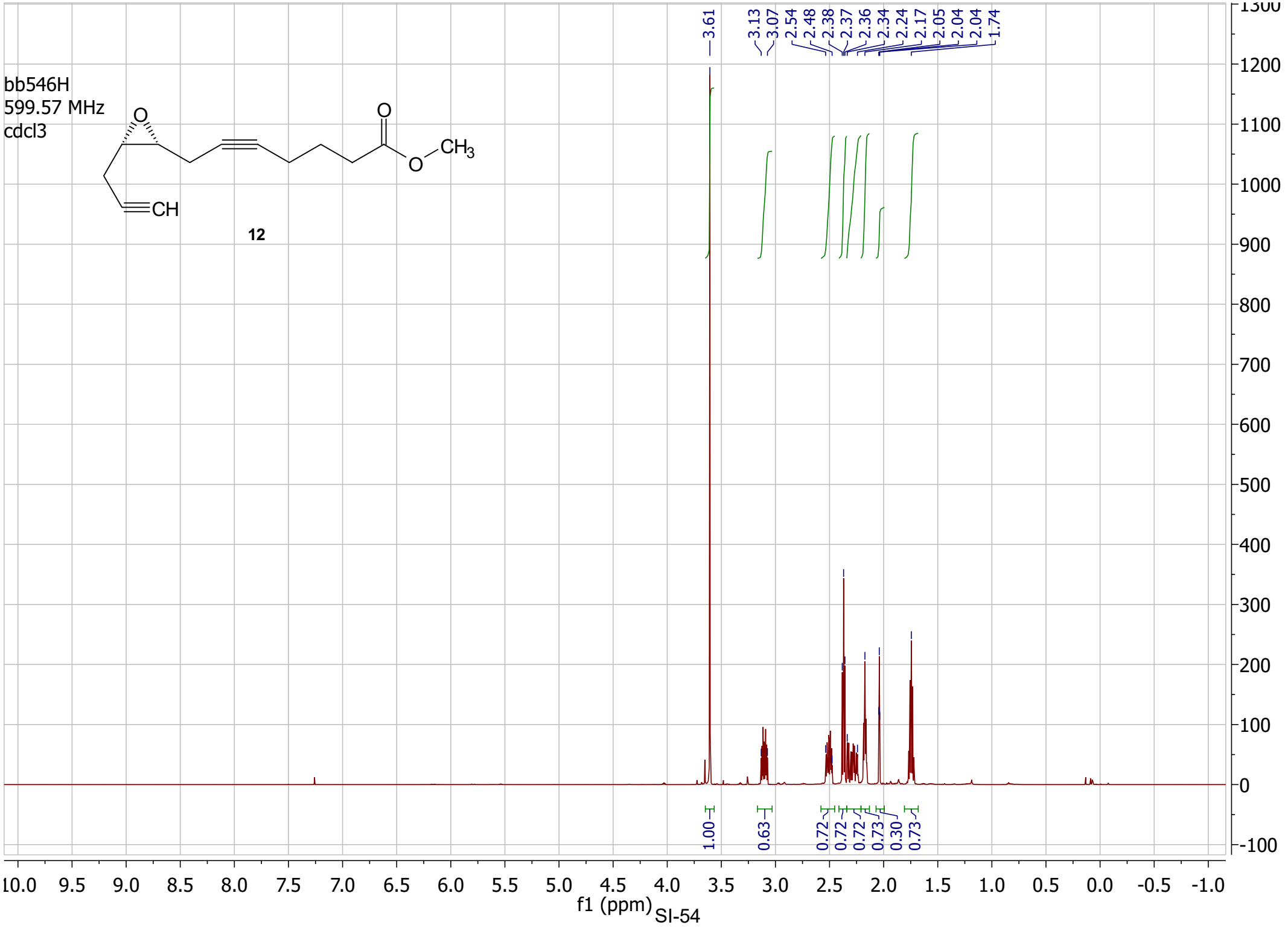

bb530C
150.78 MHz
cdcl3
H3C
CH3
Si
H3C
OH
99.50
85.56
79.65
79.03
51.26
11.05
0.03
f1 (ppm)
SI-49

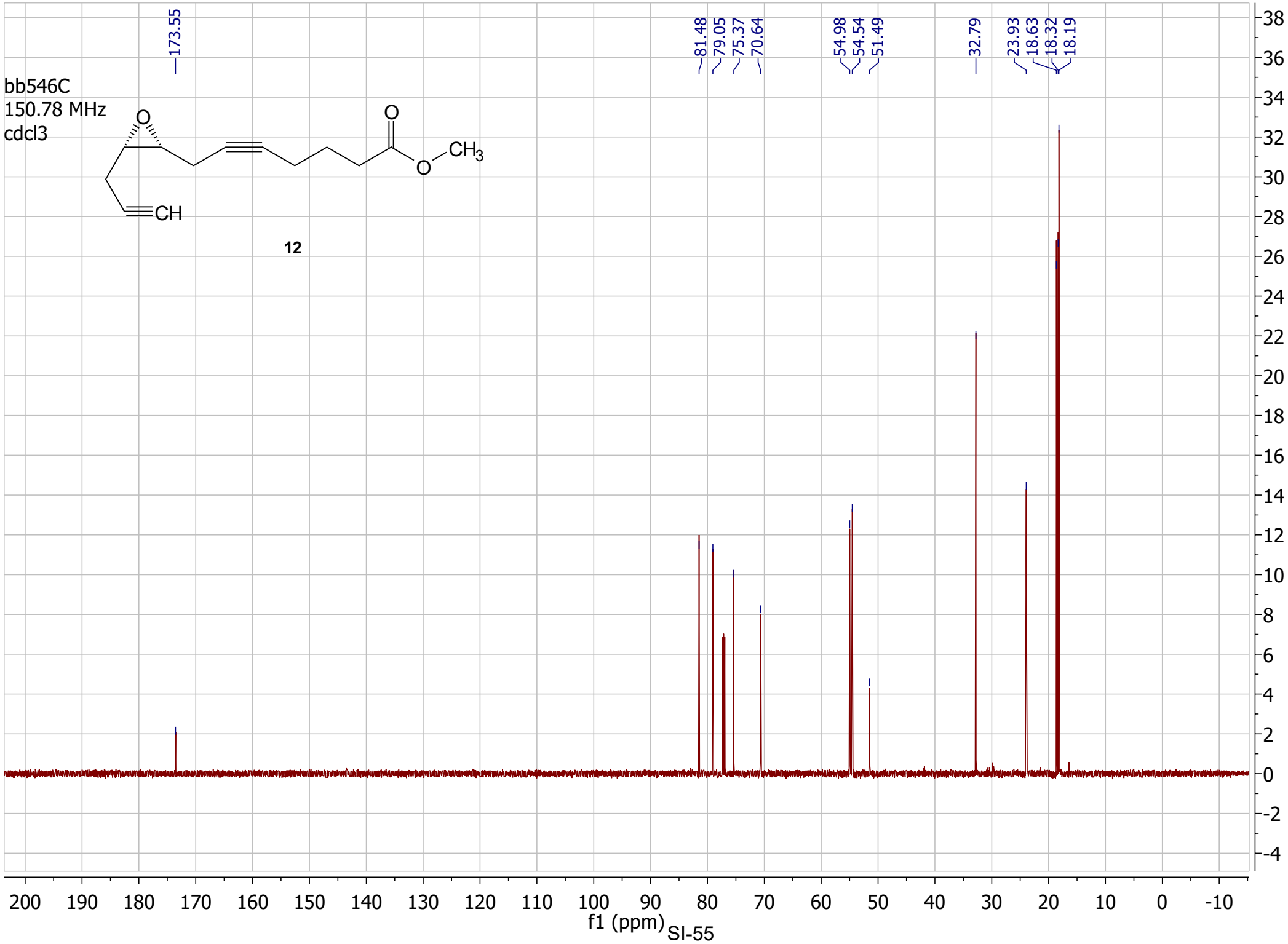

bb509H
599.57 MHz
cdcl3
H3C
CH3
Si
H3C
OH
15
5.67
5.58
4.21
4.20
3.02
3.01
0.13
2.11
2.00
1.97
9.44
f1 (ppm)
SI-50

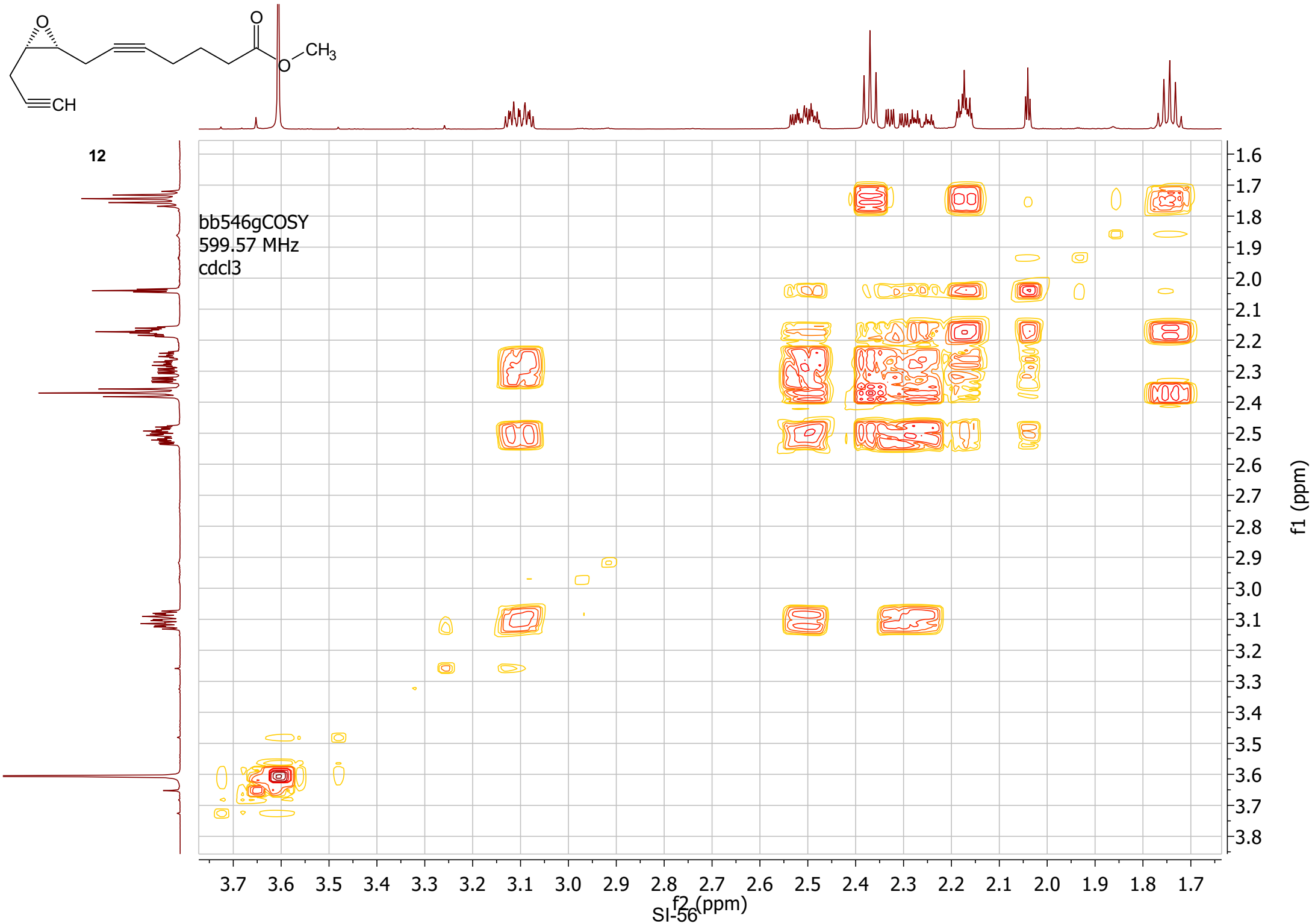

130.52
126.81
104.60
85.07
58.43
18.70
0.13
bb509C
150.78 MHz
cdcl3
H3C
CH3
Si
H3C
OH
15
f1 (ppm)
SI-51


bb5322H
599.57 MHz
cdcl3
13
3.83
3.80
3.74
3.71
3.25
3.22
3.19
3.16
2.74
2.73
2.71
2.70
2.38
2.36
2.35
2.33
2.29
0.13
1.01
1.00
0.86
0.92
0.96
1.11
0.78
0.21
9.50
f1 (ppm)
SI-52

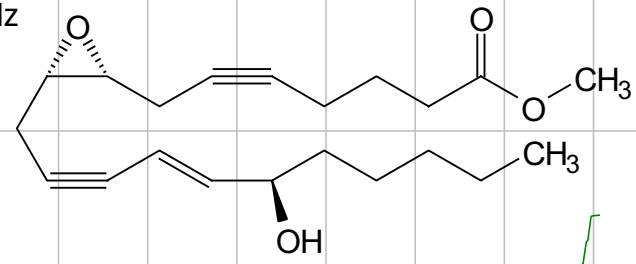

bb5322C
150.78 MHz
cdcl3
H3C
CH3
Si
H3C
O
OH
13
101.27
87.79
60.56
56.25
54.86
20.20
0.03
f1 (ppm)
SI-53


bb546H
599.57 MHz
cdcl3
O
O
O
CH3
CH
12
3.61
3.13
3.07
2.54
2.48
2.38
2.37
2.36
2.34
2.24
2.17
2.05
2.04
2.04
1.74
1.00
0.63
0.72
0.72
0.72
0.73
0.30
0.73
f1 (ppm)
SI-54

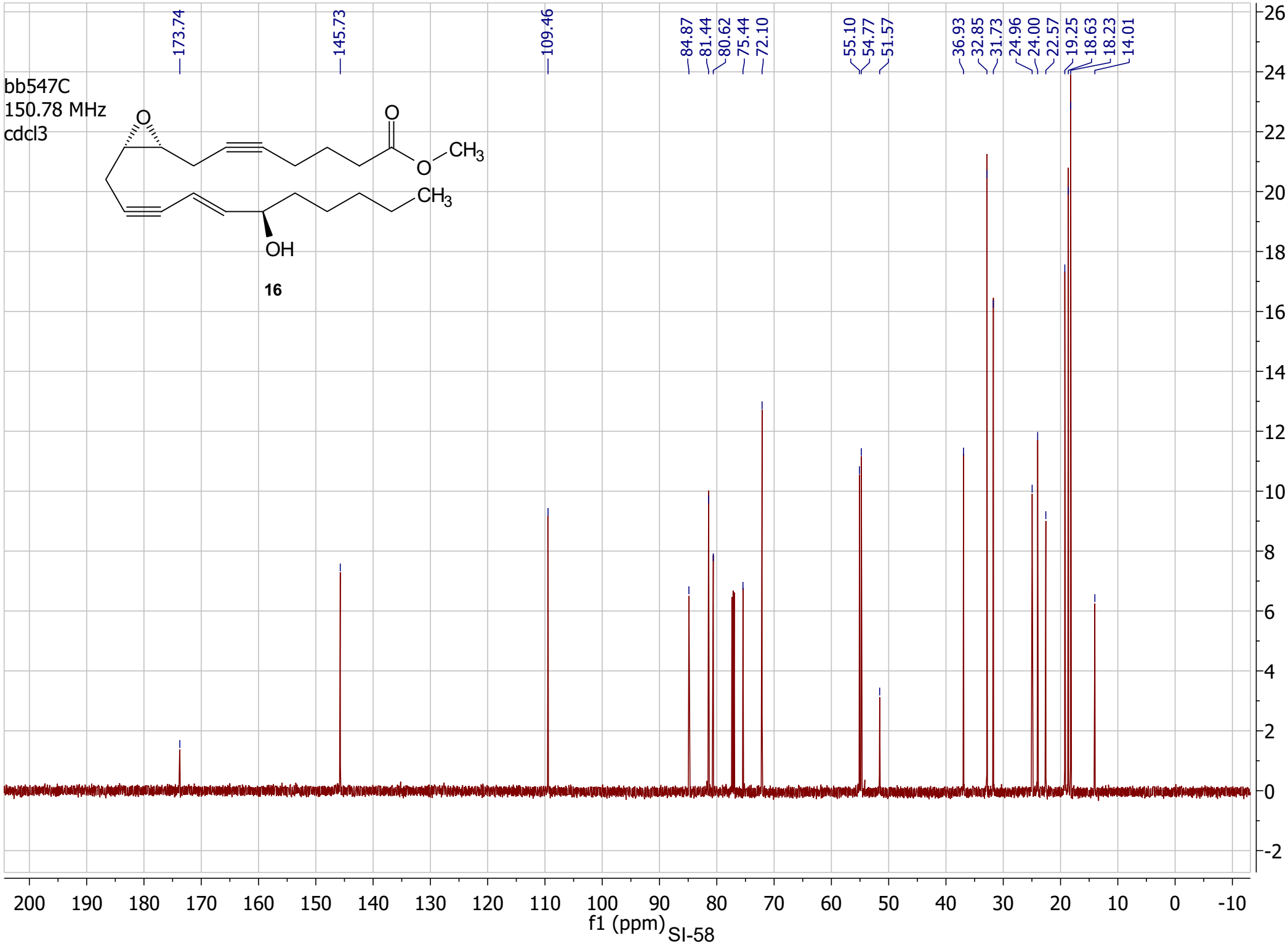

bb546C
150.78 MHz
cdcl3
173.55
81.48
79.05
75.37
70.64
54.98
54.54
51.49
32.79
23.93
18.63
18.32
18.19
O
O
O
CH3
CH
12
f1 (ppm)

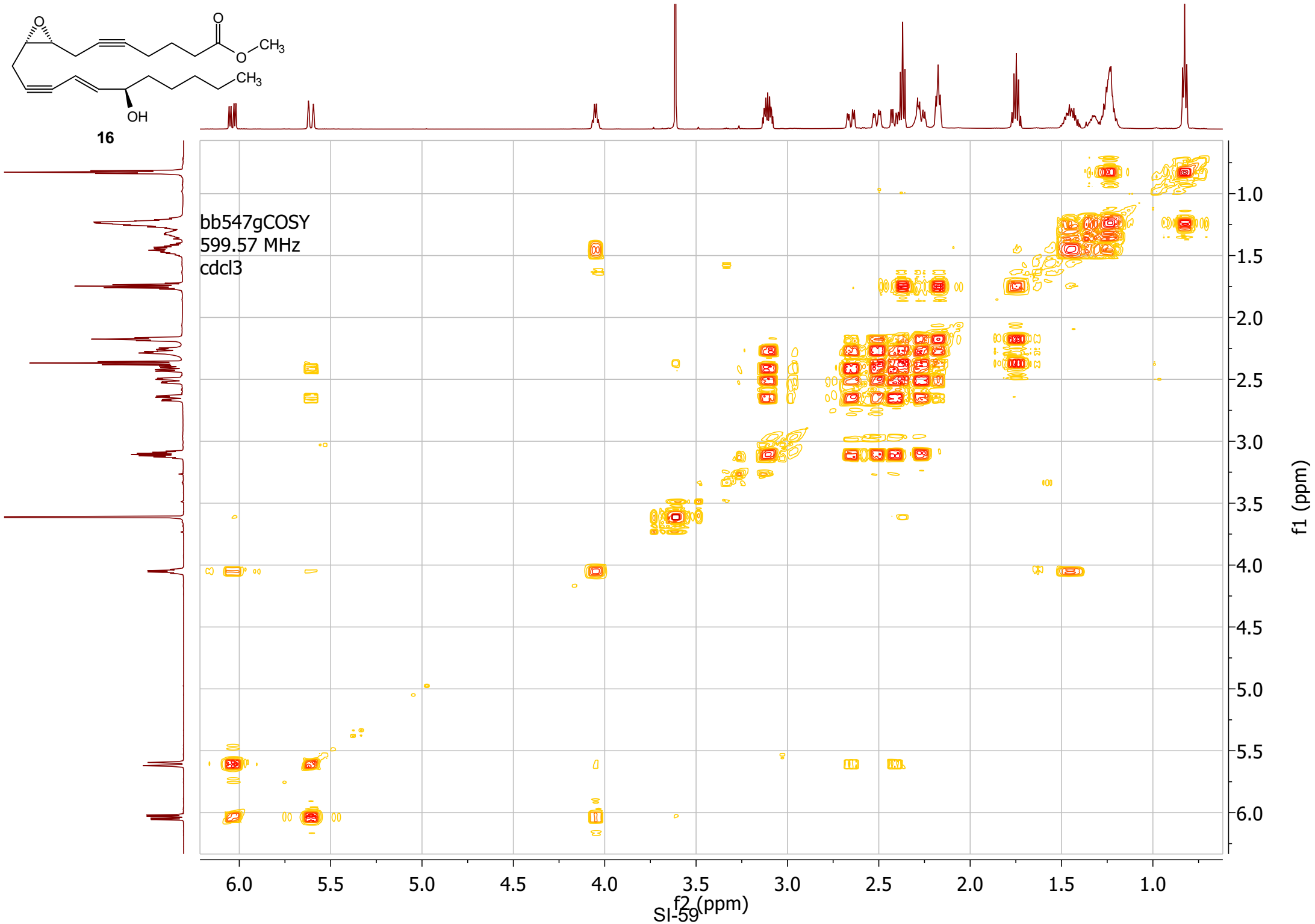

12
bb546gCOSY
599.57 MHz
cdcl3
f2 (ppm)
f1 (ppm)

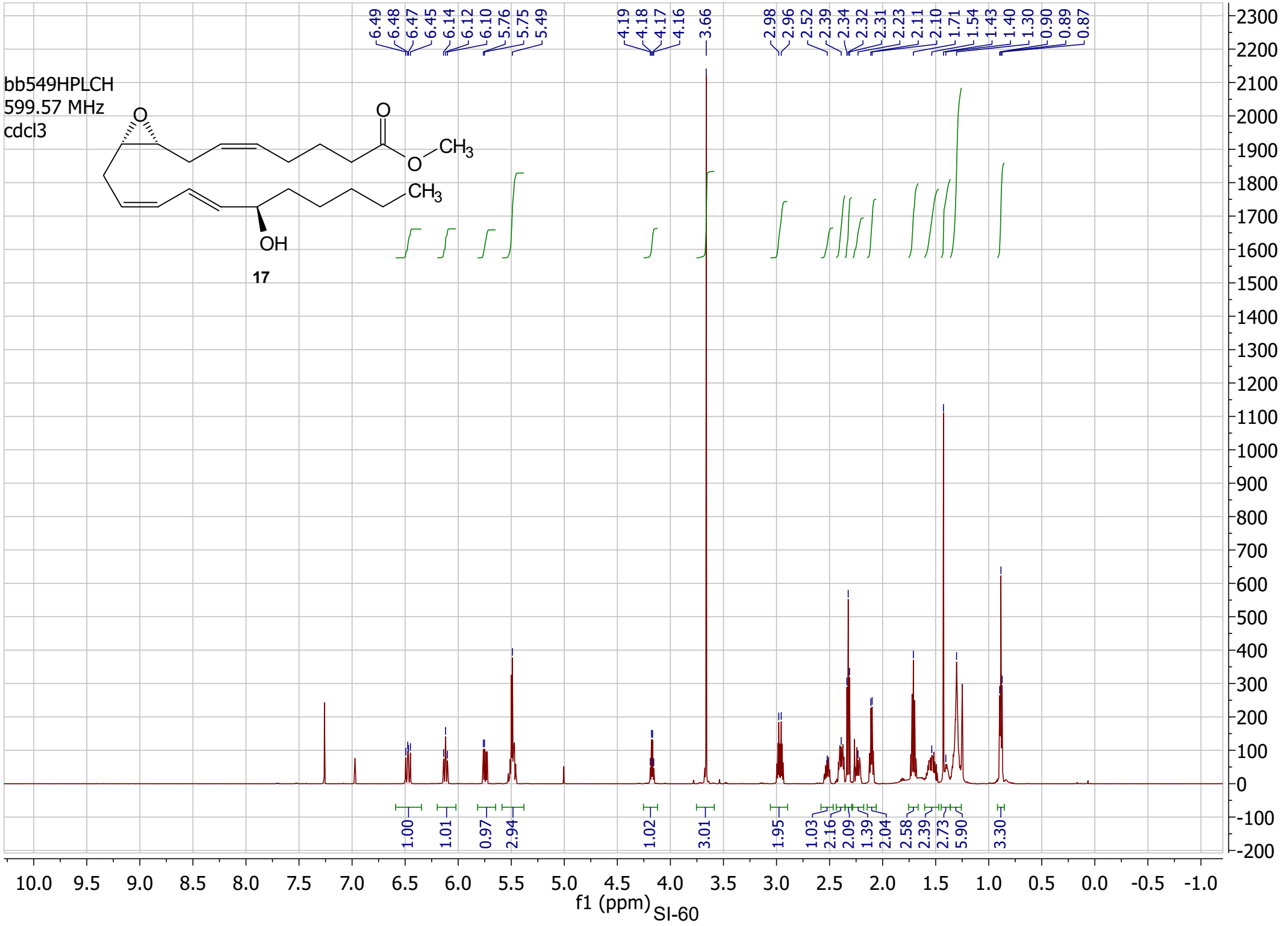
bb547H
599.57 MHz
cdcl3

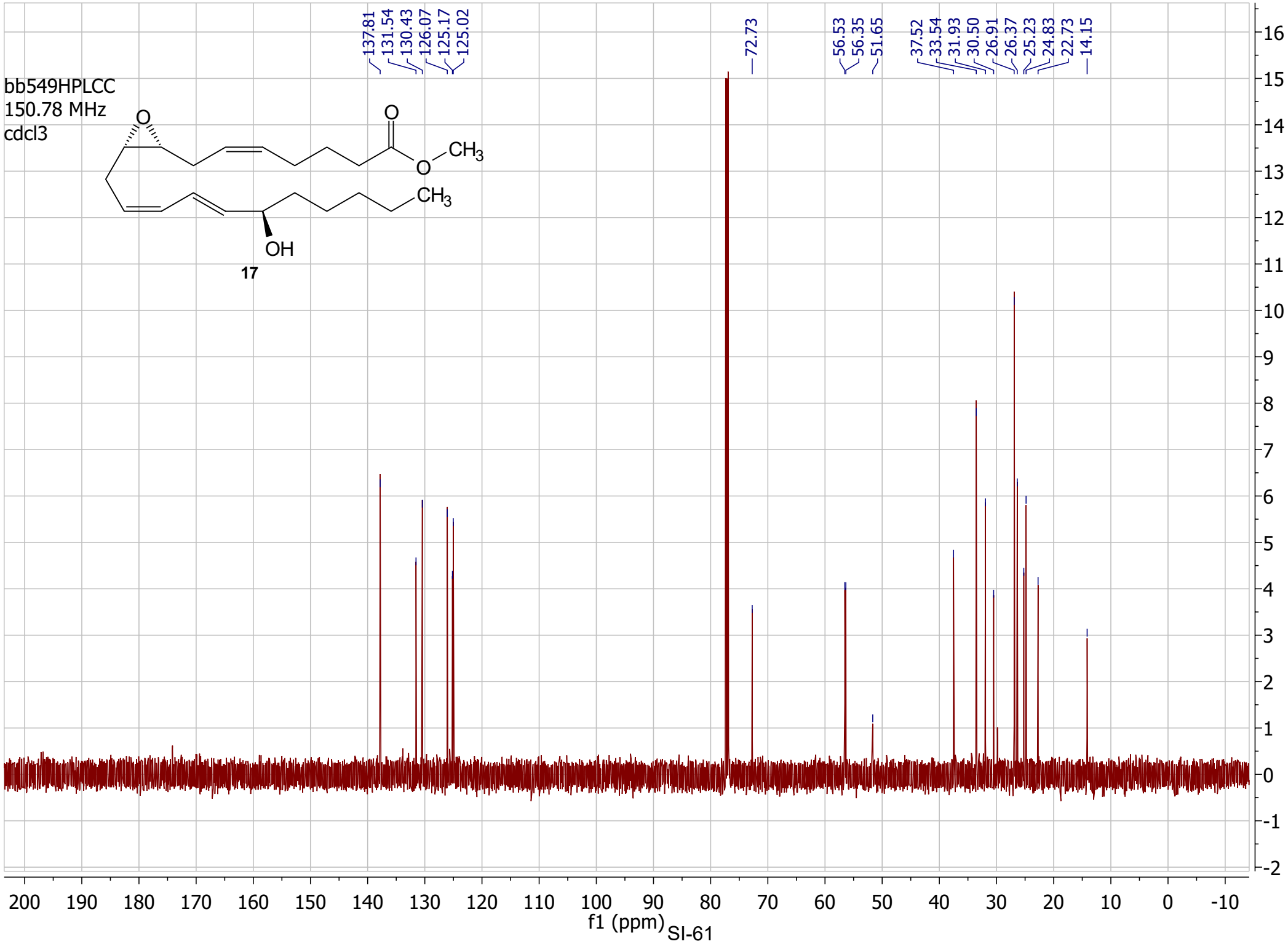


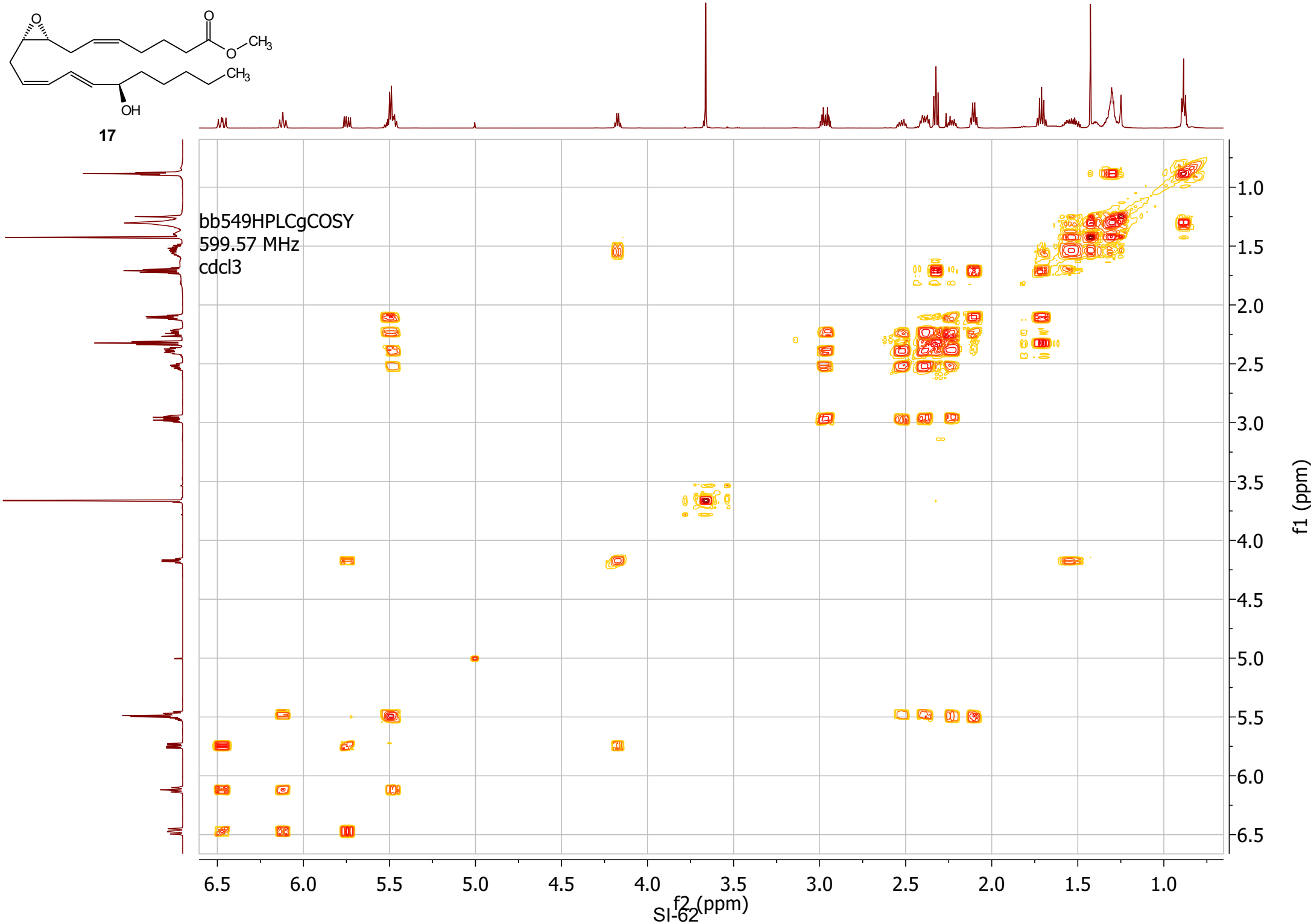
16

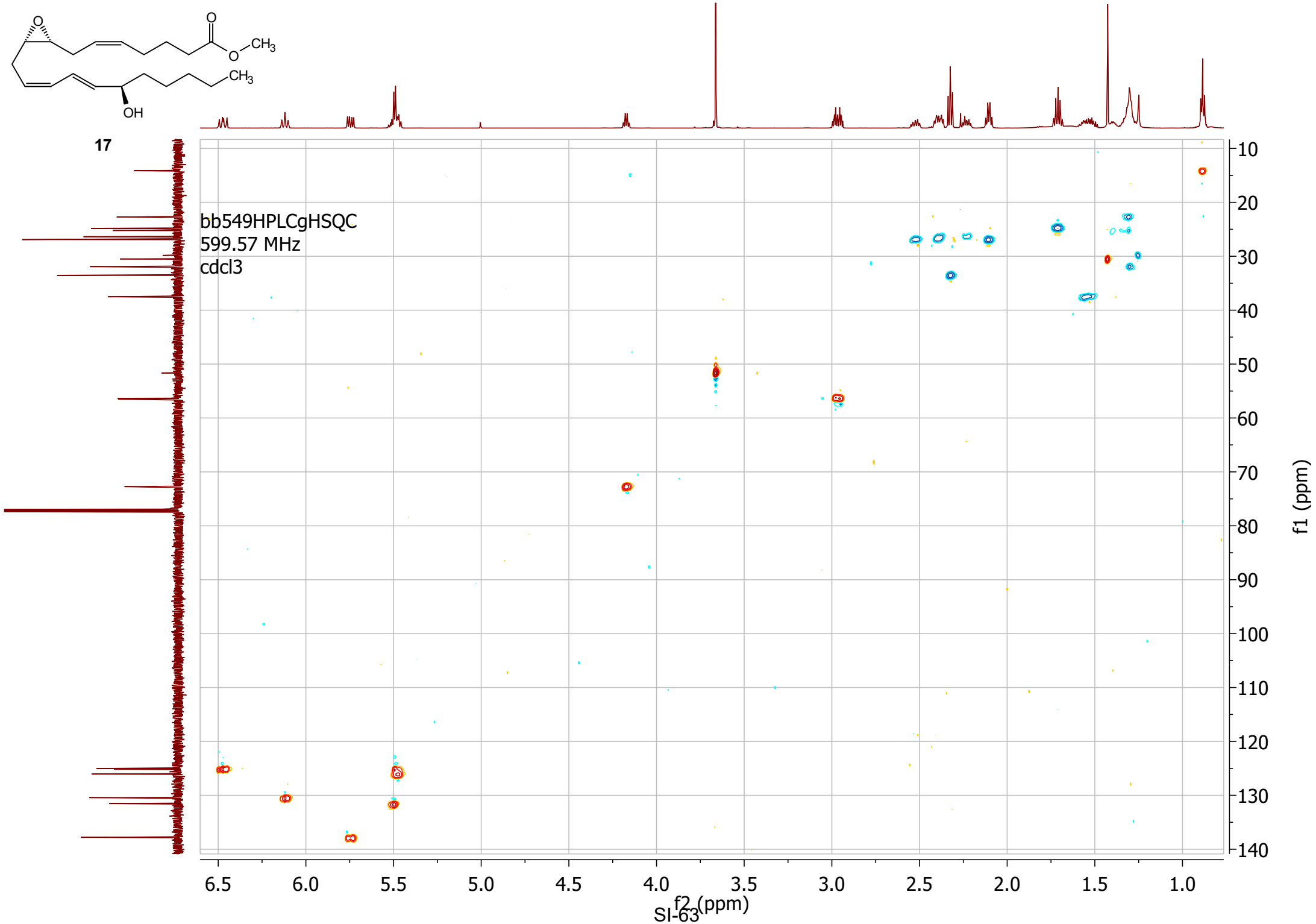

bb547C
150.78 MHz
cdcl3
173.74
145.73
109.46
84.87
81.44
80.62
75.44
72.10
55.10
54.77
51.57
36.93
32.85
31.73
24.96
24.00
22.57
19.25
18.63
18.23
14.01
O
O
O
CH3
CH3
OH
16
f1 (ppm)


16
bb547gCOSY
599.57 MHz
cdcl3
f2 (ppm)
f1 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0


bb549HPLCH
599.57 MHz
cdcl3
O
O
O
CH3
CH3
OH
17
6.49
6.48
6.47
6.45
6.14
6.12
6.10
5.76
5.75
5.49
4.19
4.18
4.17
4.16
3.66
2.98
2.96
2.52
2.39
2.34
2.32
2.31
2.23
2.11
2.10
1.71
1.54
1.43
1.40
1.30
0.90
0.89
0.87
1.00
1.01
0.97
2.94
1.02
3.01
1.95
1.03
2.16
2.09
1.39
2.04
2.58
2.39
2.73
5.90
3.30
f1 (ppm)
SI-60


bb549HPLCC
150.78 MHz
cdcl3
137.81
131.54
130.43
126.07
125.17
125.02
72.73
56.53
56.35
51.65
37.52
33.54
31.93
30.50
26.91
26.37
25.23
24.83
22.73
14.15
O
O
O
CH3
CH3
OH
17
f1 (ppm)
SI-61


17
bb549HPLCgCOSY
599.57 MHz
cdcl3
f2 (ppm)
f1 (ppm)
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0


17
bb549HPLCgHSQC
599.57 MHz
cdcl3
f2 (ppm)
f1 (ppm)
6.5
6.0
5.5
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