Supporting Information

Expanding the pleuromutilin class of antibiotics by *de novo* chemical synthesis

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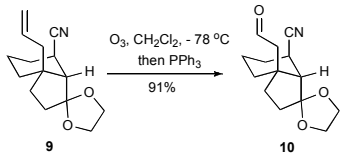
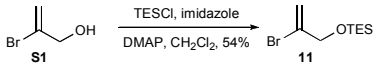
General Methods. All reactions were carried out under an atmosphere of argon with magnetic stirring unless otherwise indicated. Palladium (II) acetate was purchased from Gelest. In other cases, commercial reagents of high purity were purchased from either Aldrich or Acros and used without further purification. Tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), toluene, benzene, ether (Et_2O), acetonitrile (CH_3CN), triethylamine (NEt_3), and pyridine were dried by passing through activated alumina columns. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm Whatman silica gel plates Partisil K6F (60 Å) using UV light as a visualizing agent and aqueous potassium permanganate or ethanolic *p*-anisaldehyde solution and heat as developing agents. Silica gel from SiliCycle silicaFlash P60 40-63 μm (230-400 mecsh) or from Dynamic Adsorbent Inc 32-63 μm was used for flash column chromatography (FCC).

Instrumentation. FT-IR spectra were obtained on a Perkin-Elmer Paragon 500 or a NICOLET 6700. Nuclear magnetic resonance (NMR) spectra were obtained on a 500 MHz Bruker AVANCE spectrometer and calibrated to the residual solvent peak. Coupling constant values were extracted assuming first-order coupling and are given in Hz. The multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad signal. High resolution mass spectra were obtained on a Kratos MS 50 using electrospray ionization (ESI).

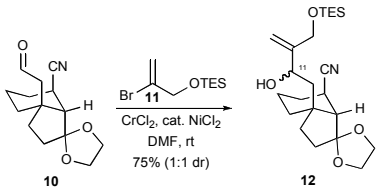


(3a'*S*,7'*S*,7a'*S*)-3a'-(2-oxoethyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbonitrile (**10**): a stream of ozone was bubbled into a solution of **9**[1] (871 mg, 3.53 mmol) in dichloromethane (30 ml) at -78 °C. Upon consumption of **9** by TLC analysis, a stream of O_2 was bubbled into the reaction for 5 minutes at -78 °C before adding triphenylphosphine (1.39 g, 5.31 mmol). The reaction was allowed to warm to rt and stirred for 3 hours. Evaporation of the dichloromethane and FCC using 40% ethyl acetate in hexanes with 1% triethylamine gave **10** as a colorless oil (791 mg, 91%). ^{1}H-NMR

(500 MHz, $CDCl_3$): δ 9.74 (1H, t, *J* = 2.1 Hz), 3.95 – 3.75 (4H, m), 2.86 (1H, dd, *J* = 16.5, 2.3 Hz), 2.55 (2H, ddd, *J* = 18.5, 12.0, 3.3 Hz), 2.08 (1H, d, *J* = 7.2 Hz), 1.98 (3H, dt, *J* = 16.1, 6.1 Hz), 1.75 (1H, dt, *J* = 13.4, 8.3 Hz), 1.67 – 1.41 (6H, m). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 200.98, 122.26, 116.54, 64.01, 63.32, 51.31, 50.94, 40.80, 33.30, 30.82, 30.21, 26.12, 24.65, 17.83. IR (neat): ν 2943, 2874, 2236, 1720 cm^{-1}. HRMS (ESI+): calculated for $C_{14}H_{19}NNaO_3$ ($[M+Na]^+$) 272.1263, found 272.1271.

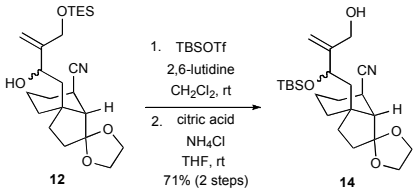


(2-bromoallyloxy)triethylsilane (**11**): to a solution of 2-bromoallyl alcohol (1.10 g, 8.09 mmol) in dichloromethane (25 ml) was added triethylsilylchloride (TESCl, 1.51 g, 10.11 mmol), imidazole (1.09 g, 16.18 mmol), and 4-dimethylaminopyridine (DMAP, 200 mg, 1.62 mmol) at 0 °C. The reaction stirred at rt and was monitored by TLC. Upon consumption of the 2-bromoallyl alcohol, water (10 ml) was added and the organic layer was separated, dried using Na_2SO_4 and evaporated. FCC using pentane gave **11** as a colorless oil (1.10 g, 54%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.91 (1H, d, *J* = 1.7 Hz), 5.47 (1H, d, *J* = 1.6 Hz), 4.15 (2H, t, *J* = 1.6 Hz), 0.90 (9H, t, *J* = 8.0 Hz), 0.57 (6H, q, *J* = 8.0 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 131.71, 114.70, 67.14, 6.73, 4.38. IR (neat): ν 2921, 2878, 1642, 1461 cm^{-1}.



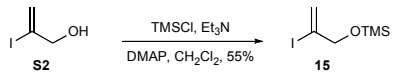
(3a'*S*,7'*S*,7a'*S*)-3a'-(2-hydroxy-3-((triethylsilyloxy)methyl)but-3-enyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbonitrile (**12**): to a solution of chromium (II) chloride (1.22 g, 10.03 mmol) and nickel (II) chloride (52 mg, 0.40 mmol) in degassed DMF (40) was added a solution of **10** (500 mg, 2.01 mmol) and **11** (1.10 g, 4.40 mmol) in degassed DMF (10 ml) at rt. The reaction stirred at rt and was monitored by TLC. Upon consumption of **10**, a 1.0 M solution of sodium serinate (50 ml) and ethyl acetate (50 ml) were added at 0 °C and the reaction stirred at rt for 1 hour. The aqueous layer was separated and extracted with ethyl acetate (3 x 25 ml). The organic layers were

combined, washed with water (2 x 25 ml), dried using $MgSO_4$ and evaporated. FCC using 15% ethyl acetate in hexane gave a partially separable mixture of C11 epimers (**12**) as a colorless oil (632 mg, 1:1 dr, 75%). Less polar C-11 epimer: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.02 (1H, s), 4.99 (1H, d, *J* = 1.3 Hz), 4.31 – 4.21 (2H, m), 4.15 (1H, d, *J* = 13.2 Hz), 3.97 – 3.79 (4H, m), 2.91 (1H, s), 2.51 (1H, td, *J* = 8.8, 4.4 Hz), 2.12 (1H, d, *J* = 8.1 Hz), 2.07 – 1.88 (4H, m), 1.75 – 1.48 (5H, m), 1.46 – 1.30 (3H, m), 0.91 (9H, t, *J* = 7.9 Hz), 0.57 (6H, q, *J* = 8.0 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 150.96, 123.61, 110.37, 71.22, 65.16, 64.37, 64.04, 51.88, 46.11, 43.05, 34.67, 32.01, 31.24, 27.60, 26.04, 19.12, 6.84, 4.35. IR (neat): ν 3503, 2923, 2853, 1457 cm^{-1}. HRMS (ESI+) calculated for $C_{23}H_{40}NO_4Si$ ($[M+H]^+$): 422.2727, found 422.2725.

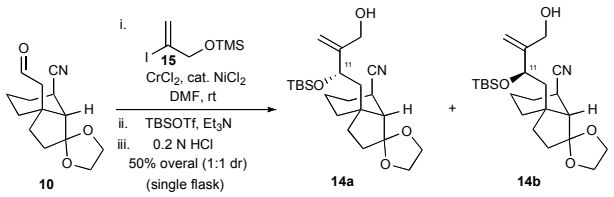


(3a'*S*,7'*S*,7a'*S*)-3a'-(2-(*tert*-butyldimethylsilyloxy)-3-(hydroxymethyl)but-3-enyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbonitrile (**14**): to a solution of **12** (less polar C-11 epimer) (235 mg, 0.56 mmol) in dichloromethane (10 ml) was added t-butyldimethylsilyl trifluoromethanesulfonate (0.14 ml, 0.61 mmol), 2,6-lutidine (0.10 ml, 0.84 mmol) at 0 °C. The reaction stirred at rt and was monitored by TLC. Upon consumption of **12**, saturated aqueous $NaHCO_3$ (5 ml) was added and the organic layer was separated, dried using Na_2SO_4 and evaporated. The crude product was dissolved in THF (8 ml) and a 1.0 M solution of citric acid (1.8 ml) and saturated aqueous NH_4Cl (1.8 ml) were added at 0 °C. The reaction stirred at 0 °C for 6 hours and was warmed to rt at which point stirring continued for an additional 3 hours. A saturated aqueous solution of $NaHCO_3$ (5 ml) and ether (10 ml) were added at 0 °C. The aqueous layer was separated and extracted with ether (2 x 5 ml). The organic layers were combined, dried using $MgSO_4$, and evaporated. FCC using 30% ethyl acetate in hexane gave **14** as a colorless oil (167 mg, 71% from **12**). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.04 (2H, s), 4.40 (1H, t, *J* = 5.5 Hz), 4.28 (1H, dd, *J* = 14.2, 4.4 Hz), 4.17 (1H, dd, *J* = 14.1, 6.6 Hz), 4.00 – 3.61 (4H, m), 2.67 (1H, d, *J* = 4.9 Hz), 2.11 (2H, dd, *J* = 14.1, 7.5 Hz), 2.07 – 1.78 (4H, m), 1.77 – 1.33 (8H, m), 0.85 (9H, s), 0.07 (3H, s), 0.00 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ

151.67, 123.72, 117.76, 111.60, 73.64, 65.04, 63.97, 62.95, 52.49, 45.39, 42.14, 34.66, 32.98, 30.25, 26.42, 25.86, 24.80, 18.39, 17.97, -4.13, -4.81. IR (neat): ν 3459, 2929, 2854, 2240, 1461, 1254 cm^{-1}. HRMS (ESI+) calculated for $C_{23}H_{40}NO_4Si$ ($[M+H]^+$): 422.2648, found 422.2722.

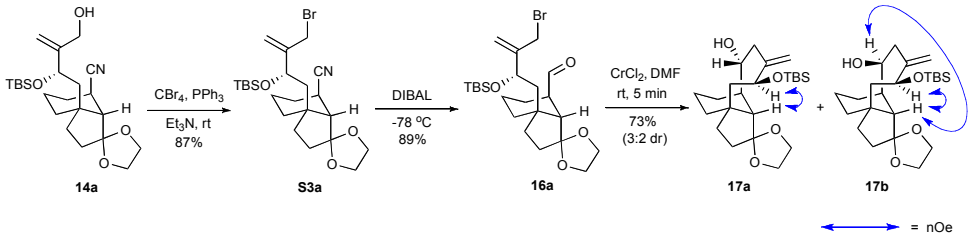


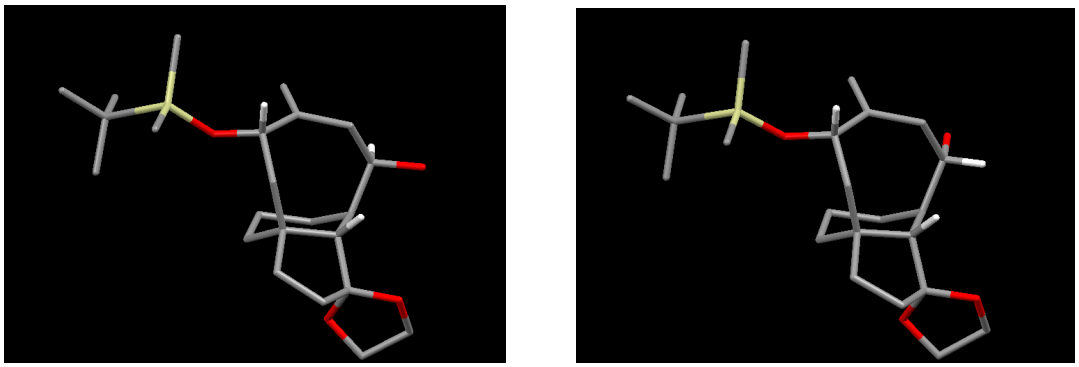
(2-iodoallyloxy)trimethylsilane (**15**): to a solution of 2-iodoallyl alcohol[2] (10.00 g, 54.34 mmol) in dichloromethane (100 ml) was added trimethylsilylchloride (13.03 g, 107.98 mmol), triethylamine (23.00 ml, 165.32 mmol), and 4-dimethylaminopyridine (1.32 g, 10.80 mmol) at 0 °C. The reaction stirred at rt and was monitored by TLC. Upon consumption of the 2-iodoallyl alcohol, water (50 ml) was added and the organic layer was separated, dried using $MgSO_4$ and evaporated. Distillation (70 °C @ 10 torr) provided **15** as a pale orange oil (7.70 g, 55%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.25 (1H, d, *J* = 1.6 Hz), 5.66 (1H, d, *J* = 1.6 Hz), 4.00 (2H, s), 0.00 (9H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 123.77, 110.03, 71.14, 0.00. IR (neat): ν 2957, 2850, 1626, 1452 cm^{-1}.



(3a'*S*,7'*S*,7a'*S*)-3a'-((*S*)-2-(*tert*-butyldimethylsilyloxy)-3-(hydroxymethyl)but-3-enyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbonitrile (**14a**) & (3a'*S*,7'*S*,7a'*S*)-3a'-((*R*)-2-(*tert*-butyldimethylsilyloxy)-3-(hydroxymethyl)but-3-enyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbonitrile (**14b**): to a solution of chromium (II) chloride (3.53 g, 28.92 mmol) and nickel (II) chloride (4 mg, 0.03 mmol) in degassed DMF (40) was added a solution of **10** (720 mg, 2.89 mmol) and **15** (2.96 g, 11.56 mmol) in degassed DMF (10 ml) at rt. The reaction stirred at rt and was monitored by TLC. Upon consumption of **10**, triethylamine (17.00 ml, 12.34 mmol) and t-butyldimethylsilyl trifluoromethanesulfonate (16.0 ml, 14.20 mmol) were added successively at rt. After stirring for 30 minutes at rt, the silylation was deemed complete by TLC. A 0.2 M solution of HCl (100 ml) and ethyl acetate (100 ml) were then added at

rt and the reaction stirred for 15 minutes. The aqueous layer was separated and extracted with ethyl acetate (3 x 50 ml). The organic layers were combined, dried using Na_2SO_4 and evaporated. FCC using 25% ethyl acetate in hexanes gave a partially separable mixture of **14a** and **14b** (609 mg, 1:1 dr, 50% from **10**). **14a**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.04 (2H, s), 4.40 (1H, t, *J* = 5.5 Hz), 4.28 (1H, dd, *J* = 14.2, 4.4 Hz), 4.17 (1H, dd, *J* = 14.1, 6.6 Hz), 4.00 – 3.61 (4H, m), 2.67 (1H, d, *J* = 4.9 Hz), 2.11 (2H, dd, *J* = 14.1, 7.5 Hz), 2.07 – 1.78 (4H, m), 1.77 – 1.33 (8H, m), 0.85 (9H, s), 0.07 (3H, s), 0.00 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 151.67, 123.72, 117.76, 111.60, 73.64, 65.04, 63.97, 62.95, 52.49, 45.39, 42.14, 34.66, 32.98, 30.25, 26.42, 25.86, 24.80, 18.39, 17.97, -4.13, -4.81. **14b**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.98 (2H, s), 4.31 (1H, dd, *J* = 8.1, 3.5 Hz), 4.20 (2H, s), 3.93 – 3.73 (4H, m), 2.00 – 1.78 (6H, m), 1.71 (1H, dd, *J* = 14.8, 8.2 Hz), 1.63 – 1.45 (6H, m), 1.37 (2H, dd, *J* = 9.3, 3.9 Hz), 0.79 (9H, s), 0.00 (3H, s), -0.07 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 152.01, 123.72, 117.82, 111.19, 73.02, 65.02, 64.26, 63.18, 53.07, 46.32, 42.48, 34.74, 32.67, 30.12, 27.23, 25.92, 25.51, 18.80, 17.98, -3.99, -4.59. IR (neat): ν 3459, 2929, 2854, 2240, 1461, 1254 cm^{-1}. HRMS (ESI+) calculated for $C_{23}H_{40}NO_4Si$ ($[M+H]^+$): 422.2727, found 422.2722.





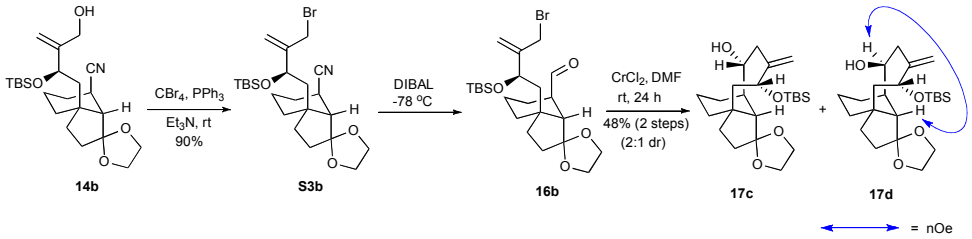
The three-dimensional representations of **17a** (left) & **17b** (right) were created by *Spartan*, and displayed by *Mercury*. Other irrelevant hydrogen atoms were omitted for clarity. (gray - carbon; red – oxygen; yellow – silicon; white – hydrogen.)

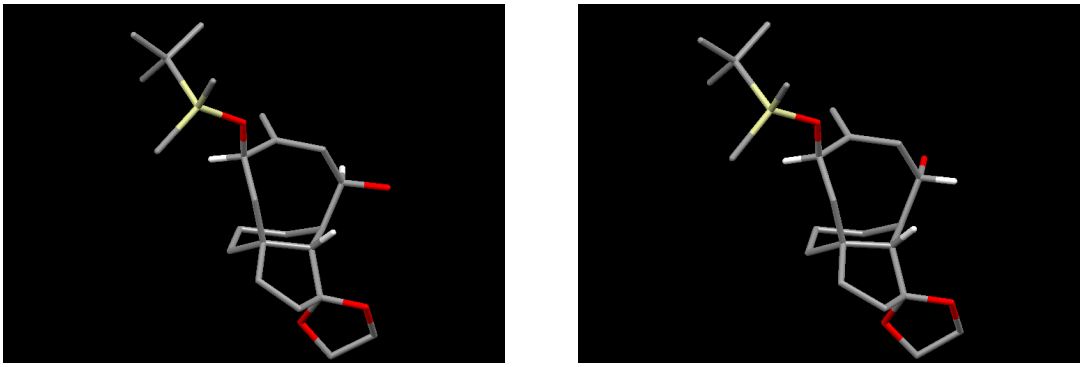
(3a'*S*,7'*R*,7a'*S*)-3a'-((*S*)-3-(bromomethyl)-2-(*tert*-butyldimethylsilyloxy)but-3-enyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbonitrile (**S3a**): to a solution of **14a** (19 mg, 0.045 mmol) in dichloromethane (5 ml) was added triethylamine (0.06 ml, 0.45 mmol), triphenylphosphine (60 mg, 0.23 mmol) and carbon tetrabromide (76 mg, 0.23 mmol) successively at rt. The reaction stirred at rt and was monitored by TLC. Upon consumption of **14a**, the dichloromethane was evaporated and the crude material was purified by FCC using 10% ethyl acetate in hexanes to give **S3a** as a colorless oil (19 mg, 87%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.31 (1H, s), 5.27 (1H, s), 4.46 (1H, d, J = 8.3 Hz), 4.03 (2H, s), 3.98 – 3.82 (4H, m), 2.77 – 2.65 (1H, m), 2.17 (1H, ddd, J = 14.9, 8.3, 2.4 Hz), 2.03 – 1.93 (2H, m), 1.88 (2H, dd, J = 11.3, 5.2 Hz), 1.82 – 1.37 (8H, m), 0.89 (9H, s), 0.09 (3H, s), 0.00 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 148.69, 123.65, 117.77, 115.97, 71.50, 65.09, 63.90, 53.48, 46.38, 42.45, 34.75, 33.10, 32.67, 29.82, 26.32, 25.91, 24.76, 18.32, 17.96, -3.88, -4.81. IR (neat): ν 2931, 2859, 2237, 1462, 1257 cm^{-1}. HRMS (ESI+) calculated for $C_{23}H_{39}BrNO_3Si$ ($[M+H]^+$): 484.1883, found 484.1877.

(3a'*S*,7'*R*,7a'*S*)-3a'-((*S*)-3-(bromomethyl)-2-(*tert*-butyldimethylsilyloxy)but-3-enyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbaldehyde (**16a**): to a solution of **S3a** (19 mg, 0.04 mmol) in toluene (6 ml) was added a 1.0 M solution of diisobutylaluminum hydride in toluene (0.05 ml, 0.05 mmol) dropwise at -78 ^{o}C. The reaction stirred at -78 ^{o}C and was monitored by TLC. Upon consumption of **S3a**, MeOH (0.5 ml) was added at -78 ^{o}C followed and the cold bath was removed. Upon warming to rt, a 1.0 M solution of aqueous citric acid (3 ml) and ether (5 ml) were added. The reaction stirred for an additional 30 minutes at rt and the aqueous layer was separated and extracted with ether (2 x 5 ml). The organic layers were combined, dried using $MgSO_4$ and evaporated. FCC using 10% ethyl acetate in hexane with 1% triethylamine gave **16a** as a colorless oil (17 mg, 89%). This aldehyde was sensitive to air oxidation upon standing and was therefore used immediately for the next step. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.60 (1H, d, J = 0.8 Hz), 5.20 (1H, s), 5.17 (1H, s), 4.35 (1H, dd, J = 7.6, 3.0 Hz), 3.93 (2H, q, J = 11.2 Hz), 3.81 – 3.61 (4H, m), 2.35 (1H, dd, J = 12.3, 6.2 Hz), 2.11 (1H, d, J = 6.5 Hz), 1.91 – 1.81 (2H, m), 1.72 – 1.32 (10H, m), 0.80 (9H, s), 0.00 (3H, s), -0.09 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 204.59, 148.84, 118.64, 115.77, 71.55,

64.69, 64.02, 52.02, 46.69, 46.47, 42.34, 35.02, 32.57, 32.37, 30.48, 25.90, 22.97, 19.19, 17.96, -3.97, -4.81. IR (neat): ν 2930, 2858, 2714, 1722, 1471, 1257, 1090 cm^{-1}. HRMS (ESI+) calculated for $C_{23}H_{39}BrNaO_4Si$ ($[M+Na]^+$): 509.1699, found 509.1695.

tricyclic pleuromutilin derivatives **17a** & **17b**: to a solution of chromium (II) chloride (70 mg, 0.57 mmol) in degassed dimethylformamide (5 ml) was added a solution of **16a** (9 mg, 0.019 mmol) in degassed dimethylformamide (1 ml) at rt. The reaction stirred at rt and was monitored by TLC. Upon completion of the reaction (~ 5 min), a 1.0 M solution of sodium serinate (5 ml) and ethyl acetate (5 ml) were added at 0 ^oC. The reaction stirred for 15 minutes at rt and the aqueous layer was separated and extracted with ethyl acetate (3 x 2 ml). The organic layers were combined, dried using Na_2SO_4 and evaporated. FCC using 20% ethyl acetate in hexanes gave a partially separable mixture of epimers **17a** and **17b** (5.5 mg, 3:2 dr, 73%). **17a**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.21 (1H, s), 4.98 (1H, s), 4.11 (1H, d, *J* = 8.7 Hz), 3.95 – 3.84 (3H, m), 3.79 – 3.65 (2H, m), 2.59 (1H, d, *J* = 14.2 Hz), 2.45 (1H, dd, *J* = 14.2, 8.3 Hz), 2.06 (1H, s), 1.97 – 1.15 (14H, m), 0.87 (9H, s), 0.03 (3H, s), 0.00 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 150.39, 119.70, 114.26, 74.68, 72.02, 65.06, 63.57, 46.72, 46.52, 39.94, 38.98, 38.49, 36.15, 35.25, 31.84, 25.88, 24.24, 18.13, 17.57, -4.55, -4.74. **17b**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.07 (1H, s), 5.04 (1H, s), 4.42 (1H, s), 3.98 – 3.88 (3H, m), 3.87 – 3.76 (2H, m), 2.79 (1H, t, *J* = 12.4 Hz), 2.16 (2H, dd, *J* = 16.6, 9.2 Hz), 2.02 – 1.88 (2H, m), 1.86 – 1.28 (12H, m), 0.88 (9H, s), 0.04 (3H, s), 0.00 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 149.68, 119.66, 115.99, 80.91, 75.52, 64.97, 64.07, 50.52, 46.47, 39.57, 39.49, 34.69, 33.96, 33.78, 33.66, 25.90, 25.80, 19.55, 18.80, 18.11, -4.81, -5.01. IR (neat): ν 3401, 2929, 2870, 1462, 1246, 1038 cm^{-1}. HRMS (ESI+) calculated for $C_{23}H_{41}O_4Si$ ($[M+H]^+$): 409.2774, found 409.2772.





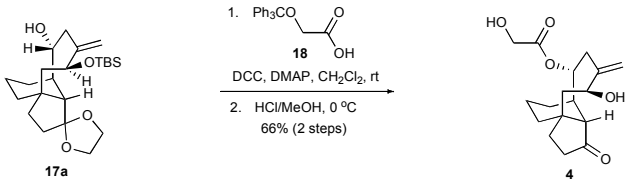
The three-dimensional representations of **17c** (left) & **17d** (right) were created by *Spartan*, and displayed by *Mercury*. Other irrelevant hydrogen atoms were omitted for clarity. (gray - carbon; red – oxygen; yellow – silicon; white – hydrogen.)

(3a'*S*,7'*R*,7a'*S*)-3a'-((*R*)-3-(bromomethyl)-2-(*tert*-butyldimethylsilyloxy)but-3-enyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbonitrile (**S3b**): to a solution of **14b** (150 mg, 0.356 mmol) in dichloromethane (10 ml) was added triethylamine (0.50 ml, 3.56 mmol), triphenylphosphine (466 mg, 1.78 mmol) and carbon tetrabromide (590 mg, 1.78 mmol) successively at rt. The reaction stirred at rt and was monitored by TLC. Upon consumption of **14b**, the dichloromethane was evaporated and the crude material was purified by FCC using 10% ethyl acetate in hexanes to give **S3b** as a colorless oil (156 mg, 90%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.25 (1H, s), 5.19 (1H, s), 4.42 (1H, d, J = 8.8 Hz), 4.03 (1H, d, J = 10.9 Hz), 3.99 – 3.65 (5H, m), 2.67 – 2.42 (1H, m), 2.15 – 1.80 (5H, m), 1.80 – 1.50 (6H, m), 1.40 (2H, d, J = 3.2 Hz), 0.84 (9H, s), 0.05 (3H, s), 0.04 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 149.13, 123.75, 117.89, 115.57, 70.37, 65.02, 64.58, 52.75, 47.07, 42.75, 34.64, 33.35, 32.63, 30.35, 27.58, 26.00, 25.88, 19.03, 17.97, -3.80, -4.48.

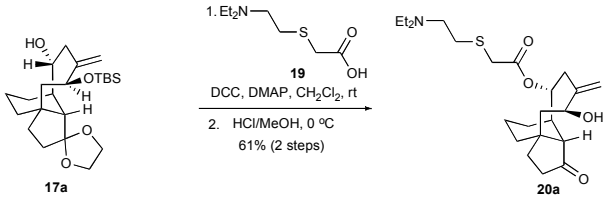
(3a'*S*,7'*R*,7a'*S*)-3a'-((*R*)-3-(bromomethyl)-2-(*tert*-butyldimethylsilyloxy)but-3-enyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbaldehyde (**16b**): to a solution of **S3b** (122 mg, 0.253 mmol) in toluene (10 ml) was added a 1.0 M solution of diisobutylaluminum hydride in toluene (0.304 ml, 0.304 mmol) dropwise at -78 °C. The

reaction stirred at -78 °C and was monitored by TLC. Upon consumption of **S3b**, MeOH (0.5 ml) was added at -78 °C followed and the cold bath was removed. Upon warming to rt, a 1.0 M solution of aqueous citric acid (5 ml) and ether (5 ml) were added. The reaction stirred for an additional 30 minutes at rt and the aqueous layer was separated and extracted with ether (2 x 5 ml). The organic layers were combined, dried using $MgSO_4$ and evaporated to afford the crude aldehyde **16b**. This aldehyde was used for the next step without further purification.

tricyclic pleuromutilin derivatives **17c** & **17d**: in a separate flask, a solution of the crude aldehyde **16b** in degassed dimethylformamide (5 ml) was added to a solution of chromium (II) chloride (70 mg, 0.57 mmol) in degassed dimethylformamide (50 ml) at rt. The reaction stirred at rt and was monitored by TLC. After stirring for 24 hours, a 1.0 M solution of sodium serinate (50 ml) and ethyl acetate (50 ml) were added at 0 °C. The reaction stirred for 15 minutes at rt and the aqueous layer was separated and extracted with ethyl acetate (3 x 25 ml). The organic layers were combined, dried using Na_2SO_4 and evaporated. FCC using 15% ethyl acetate in hexanes gave a partially separable mixture of epimers **17c** and **17d** (49 mg, 2:1 dr, 48% from **S3b**). **17c:** ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.36 (1H, s), 5.10 (1H, s), 4.28 (1H, dd, *J* = 10.2, 3.1 Hz), 3.98 – 3.87 (3H, m), 3.84 – 3.76 (1H, m), 3.68 (1H, d, *J* = 3.6 Hz), 2.63 (1H, dd, *J* = 14.2, 6.2 Hz), 2.32 (1H, d, *J* = 14.2 Hz), 2.25 – 2.11 (2H, m), 1.99 (1H, dt, *J* = 8.8, 4.3 Hz), 1.88 (1H, dd, *J* = 13.4, 3.3 Hz), 1.85 – 1.37 (9H, m), 1.24 – 1.13 (2H, m), 0.88 (9H, s), 0.02 (3H, s), 0.00 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 149.27, 119.74, 116.25, 74.04, 73.54, 64.93, 64.23, 42.98, 40.78, 38.49, 38.12, 36.21, 35.43, 33.24, 29.72, 25.89, 25.04, 19.56, 18.35, -4.66, -5.12. **17d**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.39 (1H, s), 5.08 (1H, t, *J* = 1.5 Hz), 4.26 (1H, dd, *J* = 10.6, 3.9 Hz), 3.99 – 3.88 (3H, m), 3.86 – 3.75 (2H, m), 2.52 (1H, dd, *J* = 13.1, 1.9 Hz), 2.17 (2H, ddd, *J* = 19.2, 12.9, 3.4 Hz), 1.95 (1H, dd, *J* = 13.0, 3.9 Hz), 1.90 (1H, s), 1.73 – 1.39 (12H, m), 0.87 (9H, s), 0.02 (3H, s), 0.00 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 150.77, 119.75, 115.13, 80.46, 73.65, 64.89, 64.30, 57.94, 46.63, 41.04, 39.80, 37.95, 35.49, 34.47, 32.94, 29.72, 25.89, 25.80, 20.20, 19.91, 18.40, -4.66, -5.19. IR (neat): ν 3401, 2929, 2870, 1462, 1246, 1038 cm^{-1}. HRMS (ESI+) calculated for $C_{23}H_{41}O_4Si$ ($[M+H]^+$): 409.2774, found 409.2772.

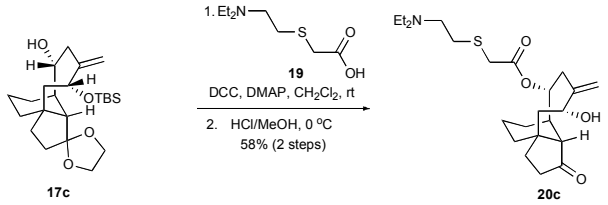


tricyclic pleuromutilin derivative **4**: To a solution of **17a** (5 mg, 0.022 mmol) and **18** (28 mg, 0.088 mmol) in dichloromethane (2 ml) was added dicyclohexylcarbodiimide (18 mg, 0.088 mmol) and 4-dimethylaminopyridine (1 mg, 0.008 mmol) at rt. The reaction stirred for 1 hour at which point **17a** was completely consumed. Water (1 ml) was added to the reaction and the aqueous layer was separated and extracted with dichloromethane (2 ml). The organic layers were combined, dried using Na_2SO_4 and evaporated. FCC using 5% ethyl acetate in hexane gave the desired acylation product with some impurities that were inseparable by FCC. This mixture was taken on directly to the next step without further purification. A 3% solution of HCl in MeOH (3 ml) was added to the mixture at 0 ^oC. The reaction stirred at 0 ^oC and was monitored by TLC. Upon completion of the reaction (~4 hours), solid $NaHCO_3$ (400 mg) was added and the reaction stirred for 5 minutes before filtering through a small plug of celite. Evaporation and FCC using 60% ethyl acetate in hexane gave **4** as a clear colorless oil (2.5 mg, 66%, 2 steps). ^{1}H-NMR (500 MHz, MeOD): δ 5.22 (1H, d, J = 1.2 Hz), 5.00 (1H, dt, J = 8.7, 5.3 Hz), 4.92 (1H, s), 4.08 (1H, d, J = 9.6 Hz), 4.03 (2H, d, J = 1.7 Hz), 2.51 – 2.37 (3H, m), 2.31 – 2.13 (3H, m), 1.90 (2H, dt, J = 14.5, 6.0 Hz), 1.66 (1H, d, J = 14.9 Hz), 1.61 – 1.42 (3H, m), 1.34 – 1.02 (6H, m). ^{13}C-NMR (125 MHz, MeOD): δ 219.79, 174.14, 151.81, 113.72, 77.17, 70.72, 61.25, 54.02, 45.41, 40.54, 39.63, 36.66, 35.08, 33.21, 32.35, 24.34, 17.66. IR (neat): ν 3401, 2926, 1734, 1724, 1428, 1117 cm^{-1}. HRMS (ESI+) calculated for $C_{17}H_{24}NaO_5$ ([M+Na]$^+$): 331.1521, found 331.1518.



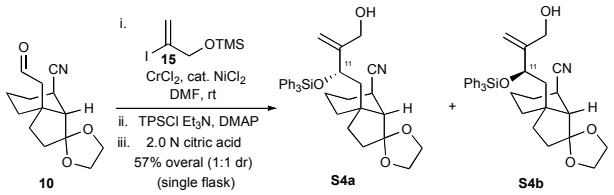
tricyclic pleuromutilin derivative **20a**: to a solution of **17a** (40 mg, 0.098 mmol) and **19** (28 mg, 0.147 mmol) in dichloromethane (5 ml) was added dicyclohexylcarbodiimide (30

mg, 0.147 mmol) and 4-dimethylaminopyridine (1 mg, 0.008 mmol) at rt. The reaction stirred for 12 hours at which point **17a** was completely consumed. Water (3 ml) was added to the reaction and the aqueous layer was separated and extracted with dichloromethane (2 ml). The organic layers were combined, dried using Na_2SO_4 and evaporated. To the crude product was added a 3% solution of HCl in MeOH (4 ml) at 0 ^oC. The reaction stirred at 0 ^oC and was monitored by TLC. Upon completion of the reaction (~2 hours), solid $NaHCO_3$ (400 mg) was added and the reaction stirred for 5 minutes before filtering through a small plug of celite. Evaporation and FCC using 10% MeOH in dichloromethane gave **20a** as a clear colorless oil (25 mg, 61% from **17a**). ^{1}H-NMR (500 MHz, D_2O): δ 5.15 (1H, s), 4.98 (1H, s), 4.94 (1H, dt, *J* = 8.5, 5.2 Hz), 4.20 (1H, d, *J* = 9.8 Hz), 3.36 (2H, dd, *J* = 8.7, 5.1 Hz), 3.20 – 3.10 (2H, m), 3.00 (4H, q, *J* = 7.3 Hz), 2.90 – 2.78 (2H, m), 2.55 (1H, s), 2.47 (2H, d, *J* = 4.9 Hz), 2.31 (2H, dd, *J* = 9.8, 5.5 Hz), 2.23 (1H, s), 1.98 – 1.82 (2H, m), 1.68 (1H, d, *J* = 14.8 Hz), 1.60 – 1.40 (2H, m), 1.24-1.05 (6H, m), 1.14 (6H, t, *J* = 7.3 Hz). ^{13}C-NMR (125 MHz, D_2O): δ 224.44, 172.53, 149.02, 113.84, 77.43, 69.80, 52.82, 50.40, 47.19, 42.72, 38.24, 37.75, 35.01, 34.29, 33.30, 31.47, 30.70, 26.32, 22.98, 16.06, 8.50. IR (neat): ν 3391, 2926, 2854, 1732, 1715, 1652, 1456, 1275 cm^{-1}. HRMS (ESI+) calculated for $C_{23}H_{38}NO_4S$ ($[M+H]^+$): 424.2522, found 424.2517.



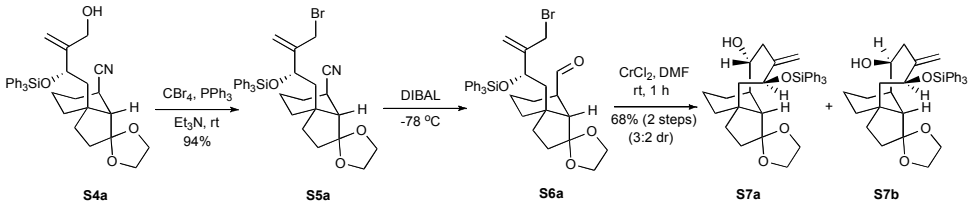
tricyclic pleuromutilin derivative **20c**: To a solution of **17c** (16 mg, 0.039 mmol) and **19** (9 mg, 0.047 mmol) in dichloromethane (5 mL) was added dicyclohexylcarbodiimide (12 mg, 0.058 mmol) and 4-dimethylaminopyridine (1 mg, 0.008 mmol) at rt. The reaction stirred overnight, and additional portions of **19** (9 mg, 0.047 mmol) and dicyclohexylcarbodiimide (12 mg, 0.058 mmol) were added during this period. After **17c** was nearly consumed, water (5 mL) was added to the reaction and the aqueous layer was separated and extracted twice with dichloromethane (5 mL). The organic layers were combined, dried using $MgSO_4$, and evaporated. FCC using 5% MeOH in

dichloromethane gave 22 mg coupling product, which was taken on directly to the next step. A 3% solution of HCl in MeOH (8.25 mL) was added to the mixture at 0 °C and allowed to stir at 0 °C for 1 hr. The reaction was then allowed to warm to room temperature and was complete by TLC after 1.5 hrs. Solid $NaHCO_3$ was added (100 mg) and the reaction was allowed to stir for 5 minutes before filtering through a small plug of Celite. Evaporation and FCC using 7-10% MeOH in dichloromethane gave **20c** as a colorless oil (9.5 mg, 58%, 2 steps). ^{1}H-NMR (500 MHz, D_2O): δ 5.31 (1H, s), 5.12 (1H, s), 5.05 (1H, t, *J* = 5.7), 4.59 (1H, d, *J* = 8.3), 3.47 (1H, d, *J* = 3.3), 3.38 (2H, t, *J* = 7.4), 3.25 (4H, q, *J* = 7.1), 3.01 - 2.93 (2H, m), 2.74 (1H, dd, *J* = 14.9, 7.1), 2.65 (1H, s), 2.49 (2H, d, *J* = 13.3), 2.36 - 2.30 (2H, m), 2.07 (1H, d, *J* = 12.3), 1.96 - 1.83 (2H, m), 1.79 - 1.72 (1H, m), 1.56 (2H, dd, *J* = 33.0, 14.1), 1.44 - 1.17 (11H, m). ^{13}C-NMR (125 MHz, D_2O): δ 227.15, 171.96, 147.74, 117.30, 77.91, 72.95, 51.29, 50.29, 47.41, 38.82, 36.27, 35.32, 34.46, 33.31, 32.50, 25.93, 23.31, 18.68, 8.00. HRMS (ESI+) calculated for $C_{23}H_{38}NO_4S$ ($[M+H]^+$): 424.2522, found 424.2518.



(3a'*S*,7'*S*,7a'*S*)-3a'-(3-(hydroxymethyl)-2-(triphenylsilyloxy)but-3-enyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbonitrile (**S4**): To a solution of chromium (II) chloride (3.48 g, 14.26 mmol) and nickel (II) chloride (3.7 mg, 0.028 mmol) in degassed DMF (30 ml) was added a solution of **10** (710 mg, 2.83 mmol) and **15** (2.90 g, 11.32 mmol) in degassed DMF (15 ml) at rt. The reaction stirred at rt and was monitored by TLC. Upon consumption of **10** (~3 hours), triethylamine (11.84 ml, 84.93 mmol), 4-dimethylaminopyridine (345 mg, 2.83 mmol) and triphenylsilylchloride (16.69 g, 56.62 mmol) in dimethylformamide (30 ml) were added successively at rt. After stirring for 3 hours at rt, the silylation was deemed complete by TLC. A 1.0 N solution of sodium serinate (300 ml) and ethyl acetate (200 ml) were then added at rt and the reaction stirred for 30 minutes. The aqueous layer was separated and extracted with ethyl acetate (2 x 150 ml). The organic layers were combined and a solution of 2.0 N aqueous citric

acid was added at rt. The reaction stirred for ~1 hour and the organic layer was separated, washed with sat. $NaHCO_3$, dried using Na_2SO_4 and evaporated. FCC using ethyl acetate in hexanes (15% → 40%) gave **S4** as a colorless oil (908 mg, 1:1 dr, 57% from **10**). Desired C-11 epimer **S4a**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.57 (6H, d, *J* = 6.8 Hz), 7.37 (3H, t, *J* = 7.3 Hz), 7.31 (6H, t, *J* = 7.2 Hz), 4.95 (1H, s), 4.83 (1H, s), 4.52 (1H, t, *J* = 6.1 Hz), 4.22 (1H, dd, *J* = 14.3, 4.2 Hz), 4.06 (1H, dd, *J* = 14.3, 7.8 Hz), 4.01 – 3.71 (4H, m), 2.54 (1H, dd, *J* = 11.4, 5.9 Hz), 2.13 (1H, dd, *J* = 14.7, 5.7 Hz), 1.81 (3H, ddd, *J* = 13.3, 11.1, 5.8 Hz), 1.69 (2H, t, *J* = 7.9 Hz), 1.48 – 1.12 (8H, m). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 149.36, 134.65, 132.94, 129.07, 126.79, 122.59, 116.43, 112.03, 73.61, 63.94, 62.92, 61.24, 52.09, 43.97, 40.96, 33.59, 31.34, 28.76, 25.41, 23.77, 17.19. IR (neat): ν 3468, 3069, 2929, 2237, 1429, 1116, 1024 cm^{-1}. HRMS (ESI+) calculated for $C_{35}H_{40}NO_4Si$ ([M+H]$^+$): 566.2727, found 566.2715.

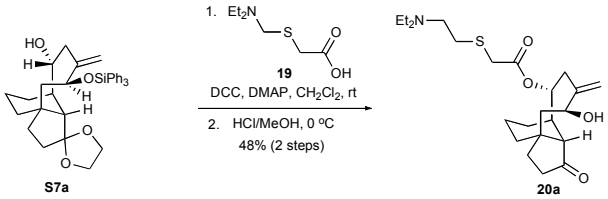


(3a'*S*,7'*R*,7a'*S*)-3a'-((*S*)-3-(bromomethyl)-2-(triphenylsilyloxy)but-3-enyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbonitrile (**S5a**): to a solution of **S4a** (140 mg, 0.247 mmol) in dichloromethane (15 ml) was added triethylamine (0.34 ml, 2.47 mmol), triphenylphosphine (324 mg, 1.24 mmol) and carbon tetrabromide (409 mg, 1.24 mmol) successively at rt. The reaction stirred at rt and was monitored by TLC. Upon consumption of **S4a**, the dichloromethane was evaporated and the crude material was purified by FCC using 20% ethyl acetate in hexanes to give **S5a** as a colorless oil (145 mg, 94%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.56 (6H, d, *J* = 6.8 Hz), 7.33 (9H, dt, *J* = 29.0, 7.2 Hz), 5.12 (1H, s), 5.07 (1H, s), 4.53 (1H, t, *J* = 5.6 Hz), 3.91 (1H, d, *J* = 11.7 Hz), 3.88 – 3.69 (5H, m), 2.61 – 2.49 (1H, m), 2.29 (1H, dd, *J* = 14.8, 6.5 Hz), 1.87 – 1.61 (5H, m), 1.54 – 1.06 (7H, m). ^{13}C-NMR (125 MHz, $CDCl_3$): δ146.06, 134.68, 132.89, 129.02, 126.76, 122.55, 116.80, 116.48, 72.50, 63.95, 62.88, 52.75, 44.59, 41.24, 33.62, 31.38, 30.64, 28.35, 25.38, 23.80, 17.24. IR (neat): ṇ2924, 2860, 2236, 1429,

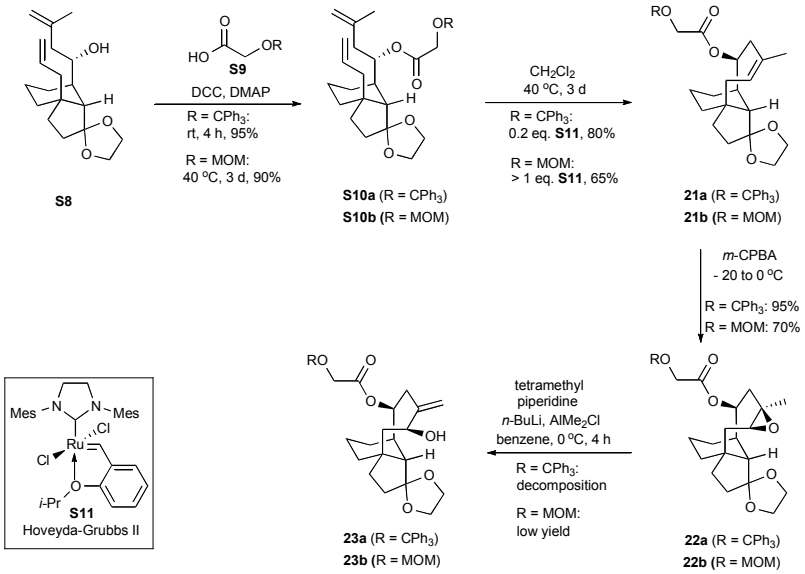
1116, 1024 cm^{-1}. HRMS (ESI+) calculated for $C_{35}H_{39}BrNO_3Si$ ($[M+H]^+$): 628.1883, found 628.1793.

(3a'*S*,7'*R*,7a'*S*)-3a'-((*S*)-3-(bromomethyl)-2-(triphenylsilyloxy)but-3-enyl)octahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-carbaldehyde (**S6a**): to a solution of **S5a** (132 mg, 0.210 mmol) in toluene (10 ml) was added a 1.0 M solution of diisobutylaluminum hydride in toluene (0.253 ml, 0.253 mmol) dropwise at -78 ^oC. The reaction stirred at -78 ^oC and was monitored by TLC. Upon consumption of **S5a**, MeOH (0.5 ml) was added at -78 ^oC followed and the cold bath was removed. Upon warming to rt, a 1.0 M solution of aqueous citric acid (5 ml) and ether (5 ml) were added. The reaction stirred for an additional 30 minutes at rt and the aqueous layer was separated and extracted with ether (2 x 5 ml). The organic layers were combined, dried using $MgSO_4$ and evaporated to afford the crude aldehyde. This aldehyde **S6a** was used for the next step without further purification.

tricyclic pleuromutilin derivatives **S7a** & **S7b**: to a solution of chromium (II) chloride (385 mg, 3.16 mmol) in degassed dimethylformamide (70 ml) was added a solution of crude aldehyde **S6a** in degassed dimethylformamide (10 ml) at rt. The reaction stirred at rt and was monitored by TLC. Upon completion of the reaction (~ 1 h), a 1.0 M solution of sodium serinate (50 ml) and ethyl acetate (50 ml) were added at 0 ^oC. The reaction stirred for 30 minutes at rt and the aqueous layer was separated and extracted with ethyl acetate (2 x 50 ml). The organic layers were combined, dried using Na_2SO_4 and evaporated. FCC using 30% ethyl acetate in hexanes gave a partially separable mixture of epimers **S7a** and **S7b** (76 mg, 3:2 dr, 68%). **S7a**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.60 – 7.49 (6H, m), 7.32 (9H, dt, *J* = 28.6, 7.2 Hz), 5.15 (1H, s), 4.98 (1H, s), 4.28 (1H, d, *J* = 9.3 Hz), 3.85 – 3.72 (3H, m), 3.71 – 3.54 (2H, m), 2.57 (1H, d, *J* = 14.2 Hz), 2.34 (1H, dd, *J* = 14.2, 7.7 Hz), 2.05 – 1.95 (1H, m), 1.91 – 0.76 (14H, m). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 147.60, 134.53, 133.52, 128.92, 126.74, 118.42, 115.88, 73.29, 72.05, 63.92, 62.43, 44.66, 43.92, 37.46, 37.19, 37.02, 34.14, 34.01, 30.78, 23.36, 16.81. IR (neat): ṇ3396, 2925, 2855, 1429, 1261, 1116, 1024. HRMS (ESI+) calculated for $C_{35}H_{41}O_4Si$ ($[M+H]^+$): 553.2774, found 553.2767.



tricyclic pleuromutilin derivative **20a**: to a solution of **S7a** (33 mg, 0.060 mmol) and **19** (24 mg, 0.126 mmol) in dichloromethane (5 ml) was added dicyclohexylcarbodiimide (30 mg, 0.147 mmol) and 4-dimethylaminopyridine (1 mg, 0.008 mmol) at rt. The reaction stirred for 12 hours at which point **S7a** was completely consumed. Water (3 ml) was added to the reaction and the aqueous layer was separated and extracted with dichloromethane (2 ml). The organic layers were combined, dried using Na_2SO_4 and evaporated. To the crude product was added a 3% solution of HCl in MeOH (4 ml) at 0 ^oC. The reaction stirred at 0 ^oC and was monitored by TLC. Upon completion of the reaction (~2 hours), solid $NaHCO_3$ (500 mg) was added and the reaction stirred for 5 minutes before filtering through a small plug of celite. Evaporation and FCC using 10% MeOH in dichloromethane gave **20a** as a clear colorless oil (12 mg, 48% from **S7a**).



For general experimental procedures, see Ref. 1. (Characterization of **S10a**, **21a**, **22a** included).

(*S*)-1-((3a'*S*,7'*R*,7a'*S*)-3a'-allyloctahydrospiro[[1,3]dioxolane-2,1'-indene]-7'-yl)-3-methylbut-3-enyl 2-(methoxymethoxy)acetate (**S10b**): ^{1}H-NMR (500 MHz, $CDCl_3$): δ

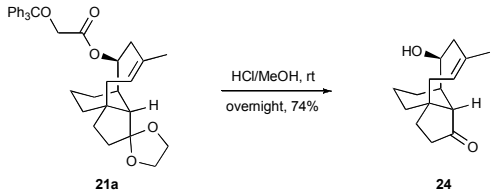
5.86 - 5.73 (1H, m), 5.50 - 5.46 (1H, m), 5.07 (1H, s), 5.05 (1H, d, J = 6.1), 4.76 - 4.65 (4H, m), 4.16 - 4.09 (3H, m), 4.05 (1H, dd, J = 13.4, 7.4), 3.92 (1H, dd, J = 12.9, 7.0), 3.85 (1H, dd, J = 13.6, 6.8), 3.38 (3H, s), 2.31 - 2.01 (5H, m), 1.78 - 1.18 (14H, m). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 169.71, 142.65, 135.62, 118.64, 117.74, 113.18, 96.30, 73.42, 64.75, 64.14, 63.96, 55.83, 49.90, 45.32, 41.28, 38.11, 35.50, 34.66, 33.79, 30.88, 22.11, 20.14, 17.55. IR (neat): ν 2939, 1750, 1441, 1194, 1151, 1120, 1060 cm^{-1}. HRMS (ESI+): calculated for $C_{23}H_{37}O_6$ ($[M+H]^+$) 409.2590, found 409.2583.

tricyclic pleuromutilin derivative **21b**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.44 (1H, t, *J* = 8.0), 4.76 (1H, dd, *J* = 10.4, 5.5), 4.73 (2H, s), 4.16 (2H, s), 3.93 - 3.84 (3H, m), 3.74 - 3.69 (1H, m), 3.41 (3H, s), 2.93 (1H, t, *J* = 12.3), 2.49 (1H, dd, *J* = 14.1, 8.5), 2.14 (1H, t, *J* = 6.3), 1.92 (1H, s), 1.87 (3H, s), 1.85 - 1.48 (10H, m), 1.36 (1H, s), 1.19 (1H, d, *J* = 13.0). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 169.52, 137.69, 123.66, 119.41, 96.48, 75.76, 65.17, 64.51, 63.46, 55.98, 47.42, 42.94, 36.31, 35.54, 35.30, 34.29, 33.40, 31.54, 24.10, 20.08, 19.68. IR (neat): ν 2933, 1750, 1202, 1151, 1123, 1062, 1029 cm^{-1}. HRMS (ESI+): calculated for $C_{21}H_{33}O_6$ ($[M+H]^+$) 381.2277, found 381.2276.

tricyclic pleuromutilin derivative **22b**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.01 (1H, ddd, *J* = 11.3, 5.7, 2.6), 4.73 (2H, s), 4.18 (2H, s), 3.96 - 3.90 (2H, m), 3.89 (1H, dd, *J* = 11.0, 4.9), 3.78 - 3.71 (1H, m), 3.41 (3H, s), 2.81 (1H, dd, *J* = 10.2, 5.0), 2.21 (1H, t, *J* = 5.4), 2.00 - 1.68 (8H, m), 1.61 - 1.39 (9H, m), 1.11 (1H, d, *J* = 12.8). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 169.46, 119.27, 96.47, 74.97, 65.33, 64.42, 63.58, 60.55, 58.74, 55.98, 48.17, 40.06, 36.83, 36.63, 36.09, 35.10, 32.25, 31.53, 22.40, 19.51, 19.27. IR (neat): ν 2936, 1752, 1202, 1151, 1122, 1062, 1030 cm^{-1}. HRMS (ESI+): calculated for $C_{21}H_{33}O_7$ ($[M+H]^+$) 397.2226, found 397.2227.

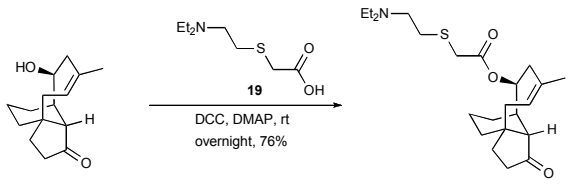
tricyclic pleuromutilin derivative **23b**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.37 (1H, s), 5.34 (1H, s), 4.90 (1H, ddd, *J* = 11.7, 6.1, 3.0), 4.73 (2H, s), 4.32 (1H, d, *J* = 10.3), 4.19 (2H, s), 3.93 - 3.83 (3H, m), 3.75 - 3.66 (1H, m), 3.41 (3H, s), 2.68 (1H, t, *J* = 12.4), 2.36 (1H, d, *J* = 11.4), 2.21 (1H, s), 2.10 (1H, dd, *J* = 14.6, 11.2), 1.92 (1H, s), 1.87 - 1.62 (6H, m), 1.59 - 1.37 (5H, m), 1.24 (1H, d, *J* = 12.2). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 169.54,

150.58, 120.40, 119.44, 96.46, 82.27, 71.58, 65.23, 64.45, 63.64, 55.96, 45.99, 41.35, 38.51, 36.46, 34.82, 32.34, 32.15, 31.83, 19.43, 19.24. IR (neat): ν 3470, 2937, 1749, 1204, 1151, 1122, 1061 cm^{-1}. HRMS (ESI+): calculated for $C_{21}H_{33}O_7$ ($[M+H]^+$) 397.2226, found 397.2221.



21a **24**

tricyclic pleuromutilin derivative **24**: concentrated HCl (0.7 mL) was dissolved in a mixture of 5 mL methanol and 1 ml dichloromethane. To a vial with the substrate **21a**[1] (50 mg, 0.087 mg) was added this solution. The mixture was stirred overnight and monitored by TLC. Upon complete consumption of **21a**, the solvent was removed under reduced pressure, and then ether and water was added. The aqueous layer was separated and extracted with ether and then dichloromethane. The organic layers were combined, dried using Na_2SO_4 and evaporated. The residue was dissolved with a small amount of solvent mixture (ethyl acetate/hexane/dichloromethan) before loaded to the FCC column; FCC using ethyl acetate in hexane (9% → 25%) gave **24** (15 mg, 74%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.48 (1H, t, *J* = 8.0), 3.66 (1H, dd, *J* = 10.5, 5.7), 2.88 (1H, t, *J* = 12.3), 2.67 (1H, s), 2.54 (1H, dd, *J* = 14.1, 8.6), 2.22 (2H, dt, *J* = 17.2, 8.4), 1.97 (1H, s), 1.94 - 1.62 (9H, m), 1.59 - 1.52 (1H, m), 1.43 - 1.34 (2H, m), 1.33 - 1.23 (1H, m), 1.16 - 1.05 (1H, m). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 218.74, 139.12, 122.93, 71.39, 53.82, 43.28, 38.34, 35.75, 35.15, 34.34, 33.05, 32.96, 24.41, 19.79, 18.66. IR (neat): ν 3413, 2924, 2855, 1734, 1474, 1454, 1232, 1171, 1106, 1078, 1060, 1048, 1035, 1018 cm^{-1}. HRMS (ESI+): calculated for $C_{15}H_{23}O_2$ ($[M+H]^+$) 235.1698, found 235.1696.



24 **25**

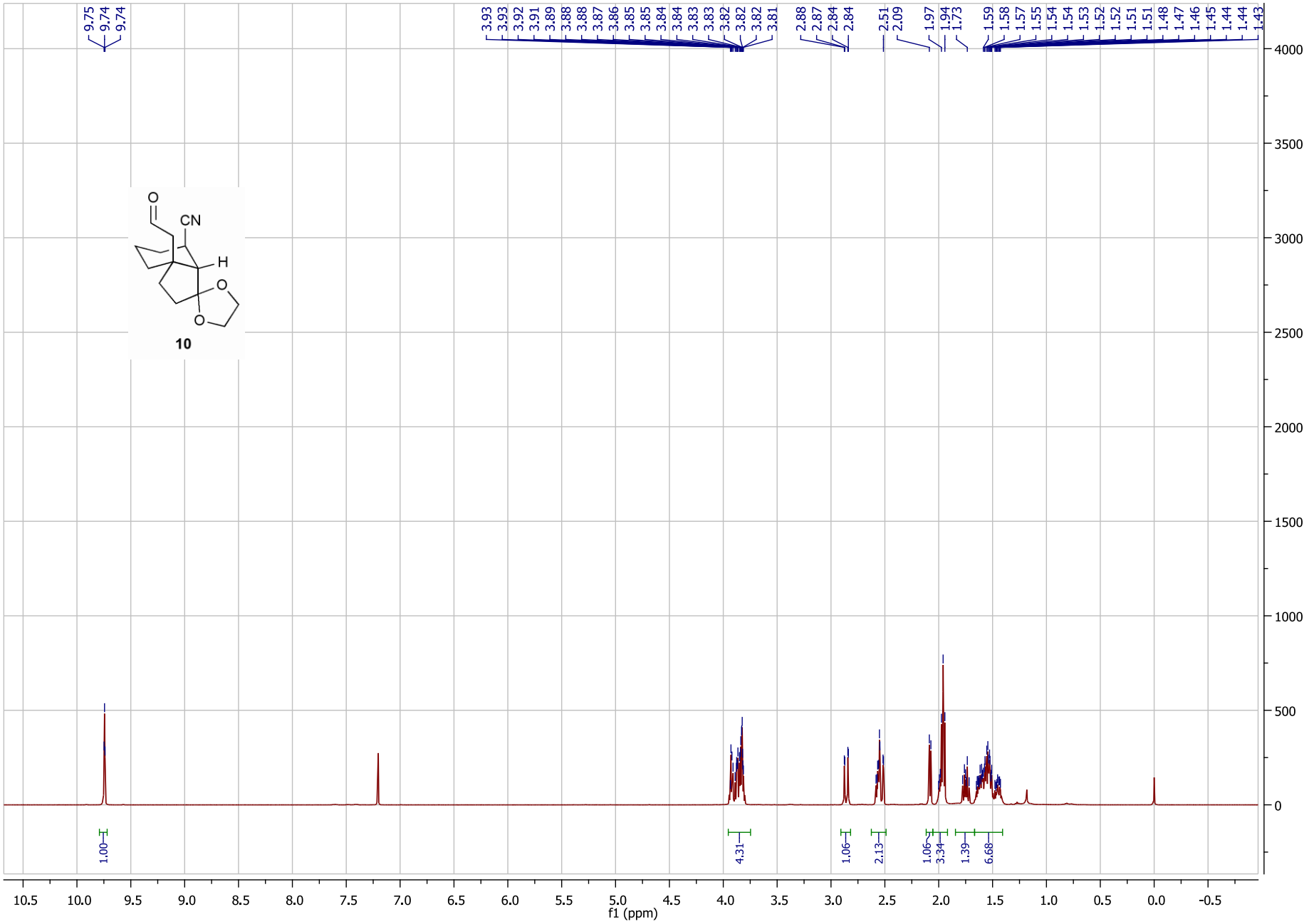
tricyclic pleuromutilin derivative **25**: alcohol **24** (6 mg, 0.026 mmol) and acid **19** (15 mg, 0.077 mmol) were dissolved in 3 mL dichloromethane. To this mixture was added N,N'-

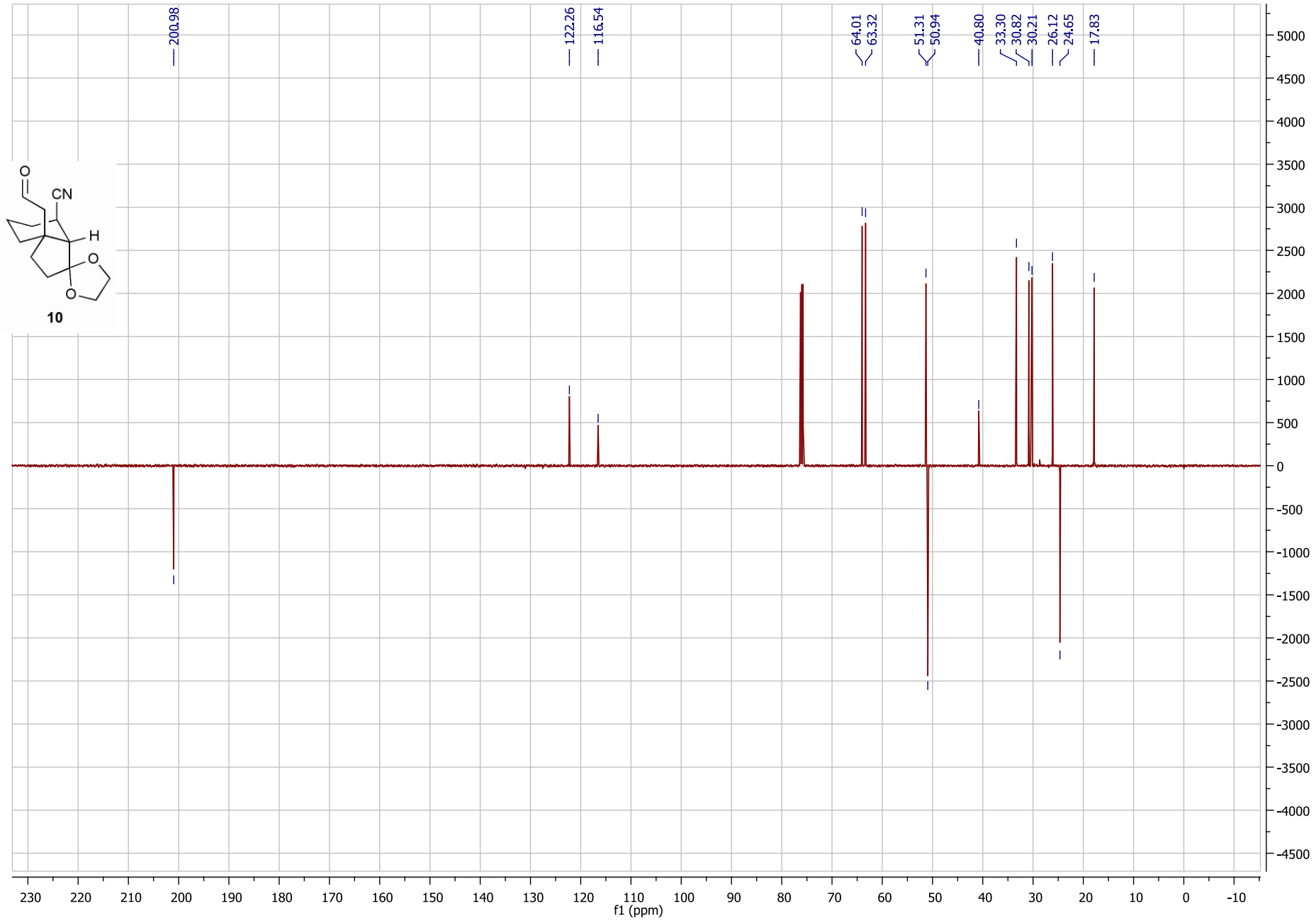
Dicyclohexylcarbodiimide (DCC, 16 mg, 0.077 mmol) and 4-Dimethylaminopyridine (DMAP, 0.3 mg, 0.0026 mmol) at room temperature and then stirred overnight. The mixture was filtered through Celite to remove insoluble impurities. FCC using 6% methanol in dichloromethane multiple times gave pure **25** (7.9 mg, 76%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.53 (1H, t, *J* = 8.0), 4.73 (1H, dd, *J* = 11.8, 5.8), 3.26 (2H, s), 2.96 (1H, t, *J* = 12.4), 2.75 (5H, bs), 2.57 (5H, dt, *J* = 14.4, 7.7), 2.31 - 2.17 (2H, m), 2.00 (1H, s), 1.95 - 1.76 (7H, m), 1.70 (1H, dd, *J* = 23.0, 10.4), 1.58 (1H, dd, *J* = 11.5, 9.4), 1.49 - 1.36 (2H, m), 1.32 - 1.23 (1H, m), 1.21 - 1.09 (1H, m), 1.05 (6H, t, *J* = 7.1). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 217.39, 169.65, 138.45, 123.53, 74.14, 53.62, 52.26, 47.04, 43.17, 35.55, 34.99, 34.33, 34.19, 32.97, 32.85, 32.84, 30.02, 23.95, 19.82, 19.66, 11.77. IR (neat): ν 2964, 2925, 2850, 1736, 1455, 1382, 1267, 1105 cm^{-1}. HRMS (ESI+): calculated for $C_{23}H_{38}NO_3S$ ($[M+H]^+$) 408.2572, found 408.2578.

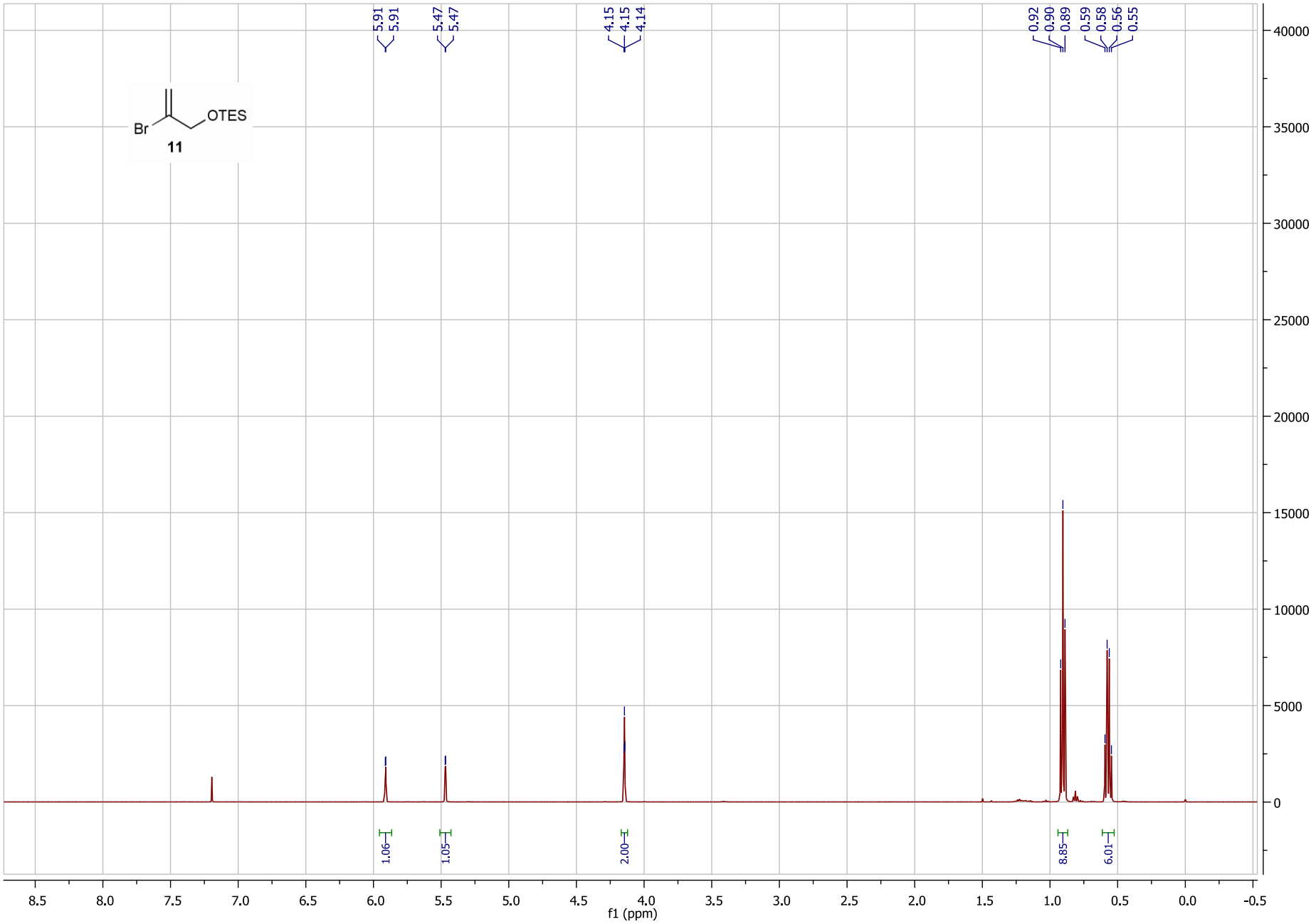
Determination of MIC values for pleuromutilin family members versus *M. tuberculosis* mc^{2}7000. A drug susceptibility assay in 96-well plate format by Alamar Blue (resazurin) viability assay was modified from Franzblau and co-workers.[3] The bacteria were grown to mid-log phase in ADC supplemented 7H9 media (Middlebrook) (OD_{600} = 0.5) and diluted to OD_{600} = 0.003 into 7H9 media containing 0.05% tyloxapol, 0.2% dextrose, and 25 ng/mL pantothenate. 196 μL of diluted culture was dispensed into each well of a sterile 96-well plate. Compounds were dissolved in DMSO and subsequent ½ serial dilutions were performed in DMSO. 4 μL of serial diluted drug solutions were added to testing wells, while the outer perimeter wells were injected with the same volume of DMSO for a negative control. The plates were sealed with film and were incubated at 37 °C for 5 days with shaking. Resazurin stock solution was added to every well to a final concentration of 5 μg/mL. The plates were reincubated at 37 °C for 24 h, and the colors of all wells were recorded. A blue color in the well was interpreted as no respiration, and a pink color was scored as viable cells, reducing resazurin to resorufin coupled to respiration. Wells appearing as violet after 24 h of incubation would invariably change to pink after longer incubation and thus were scored as growth (while the adjacent blue wells remained blue). The MIC was recorded as the lowest drug concentration that prevented a color change from blue to pink.

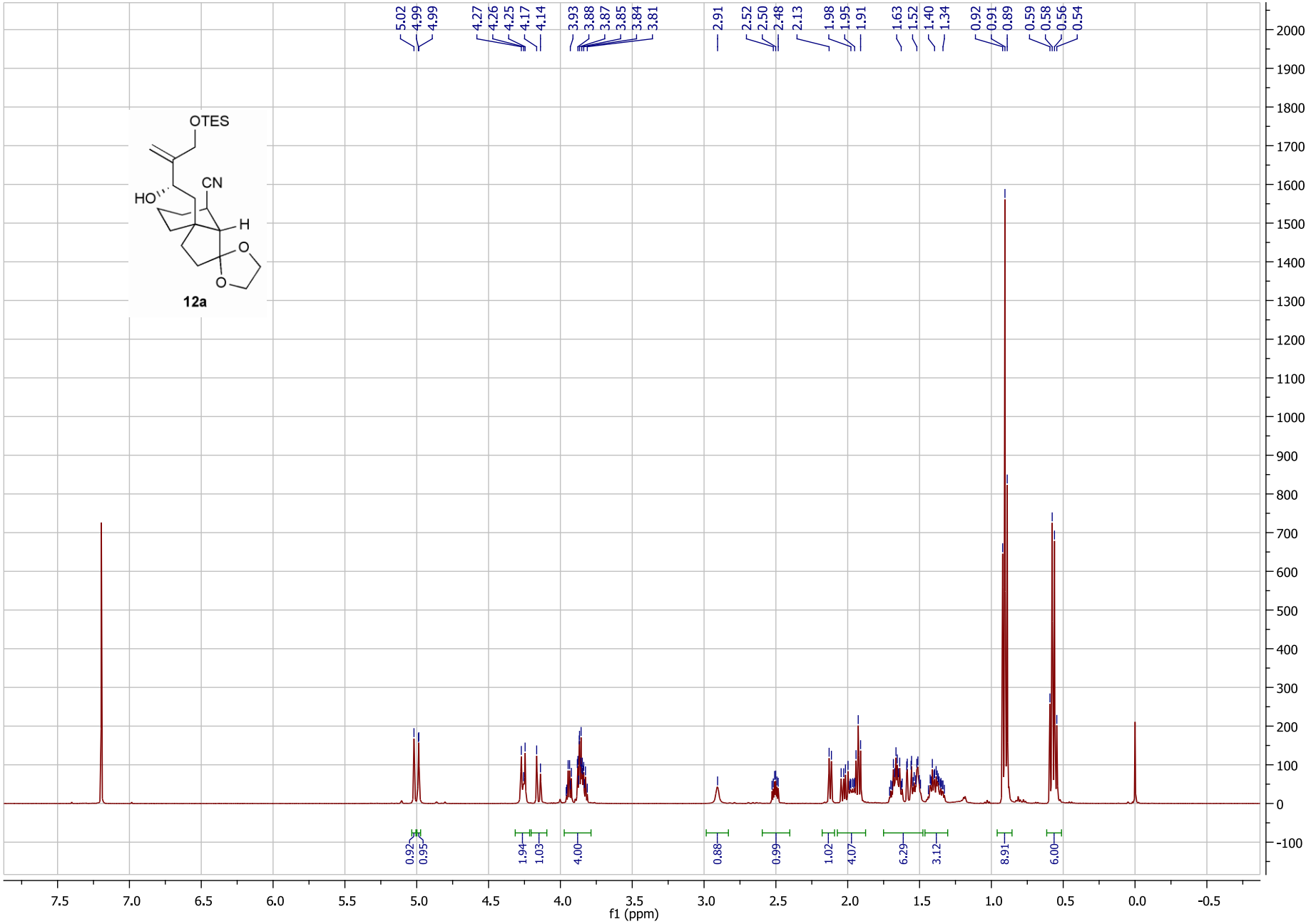
References:

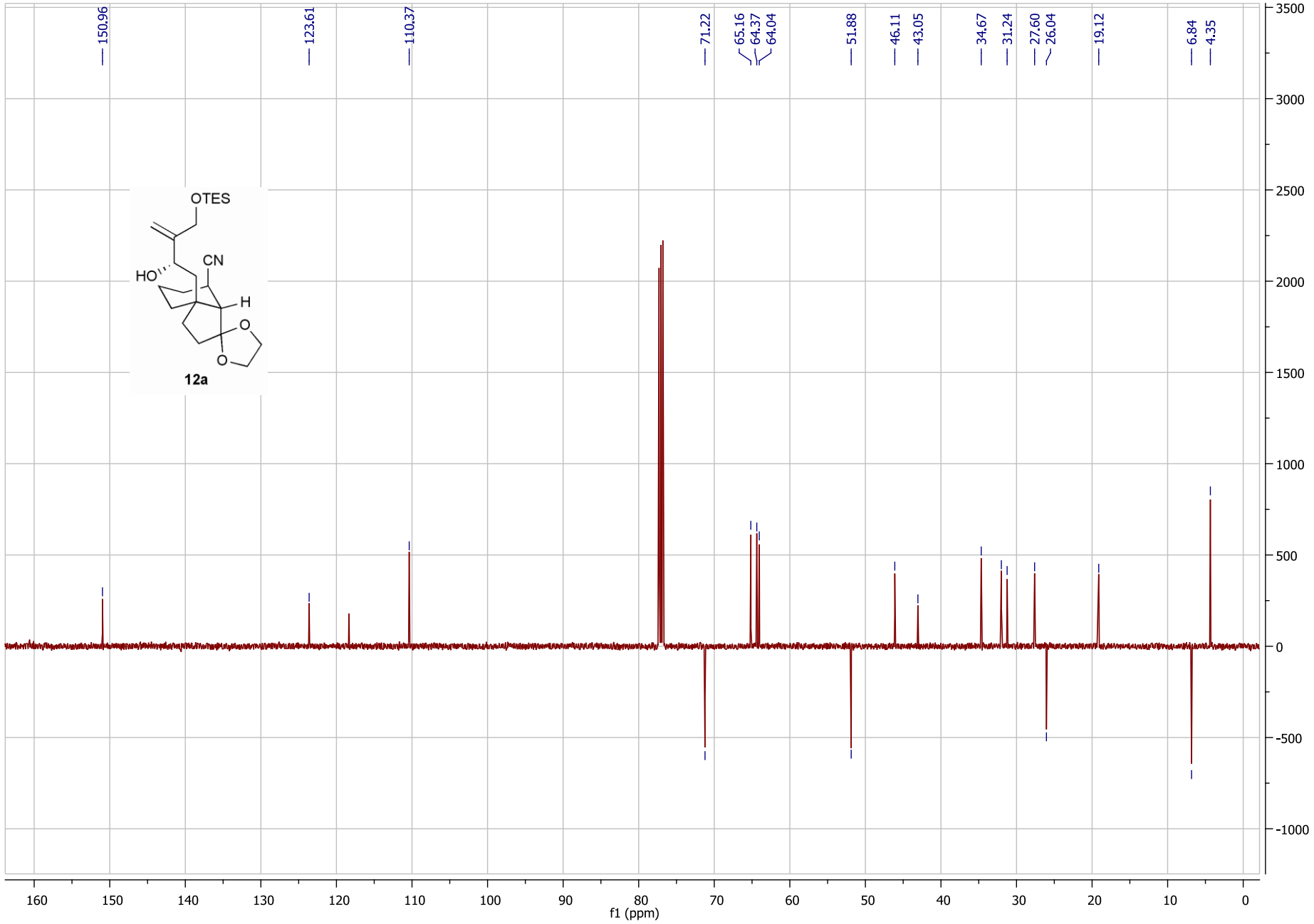
1. J. Liu, S. D. Lotesta and E. J. Sorensen, *Chem. Commun.*, 2011, **47**, 1500.
2. M. Kurosu, M. Lin, Y. Kishi, *J. Am. Chem. Soc.,* 2004, **126**, 12248.
3. L. A. Collins and S. Franzblau, *Antimicrob. Agents Chemother.*, 1997, **41**, 1004.

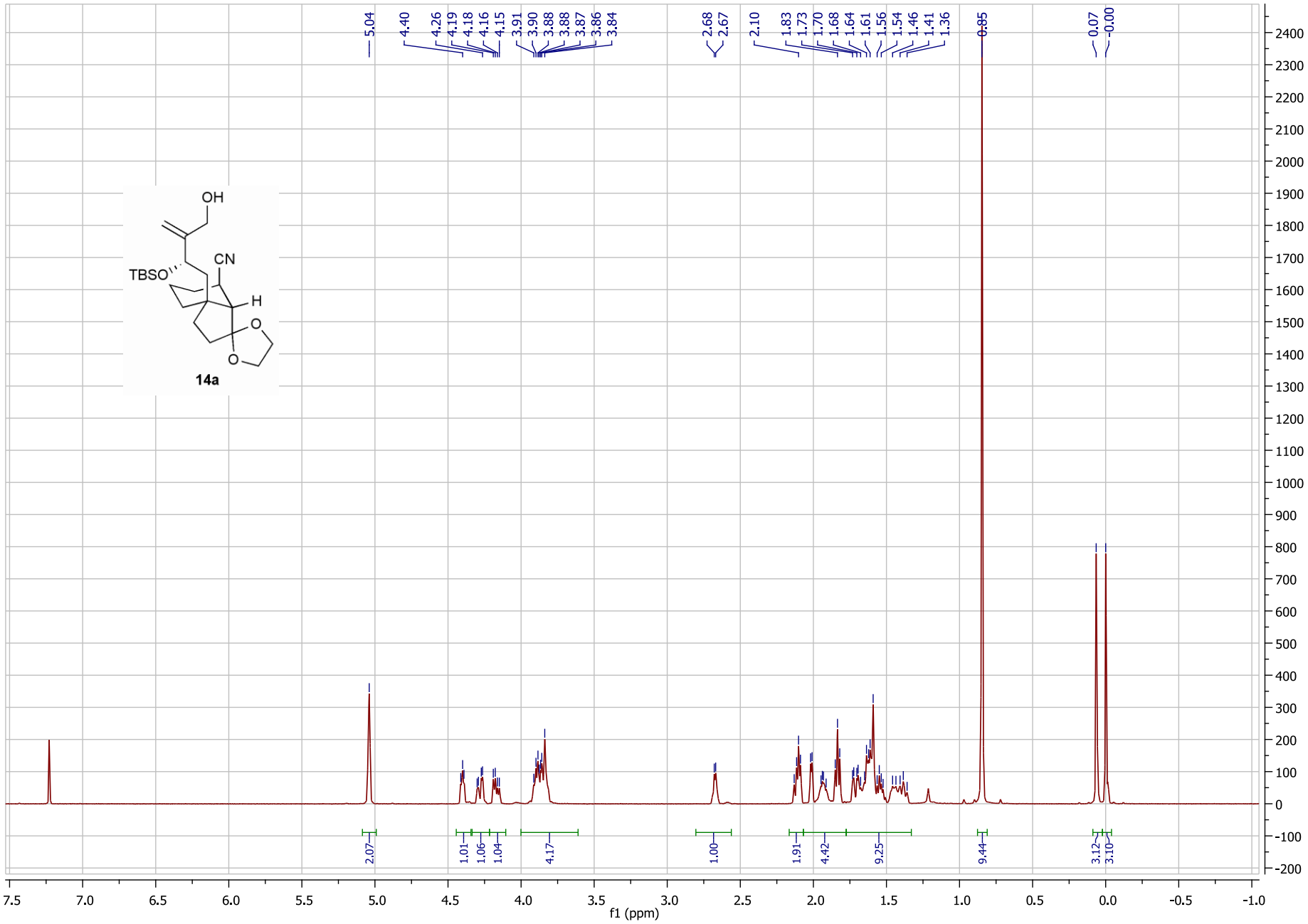


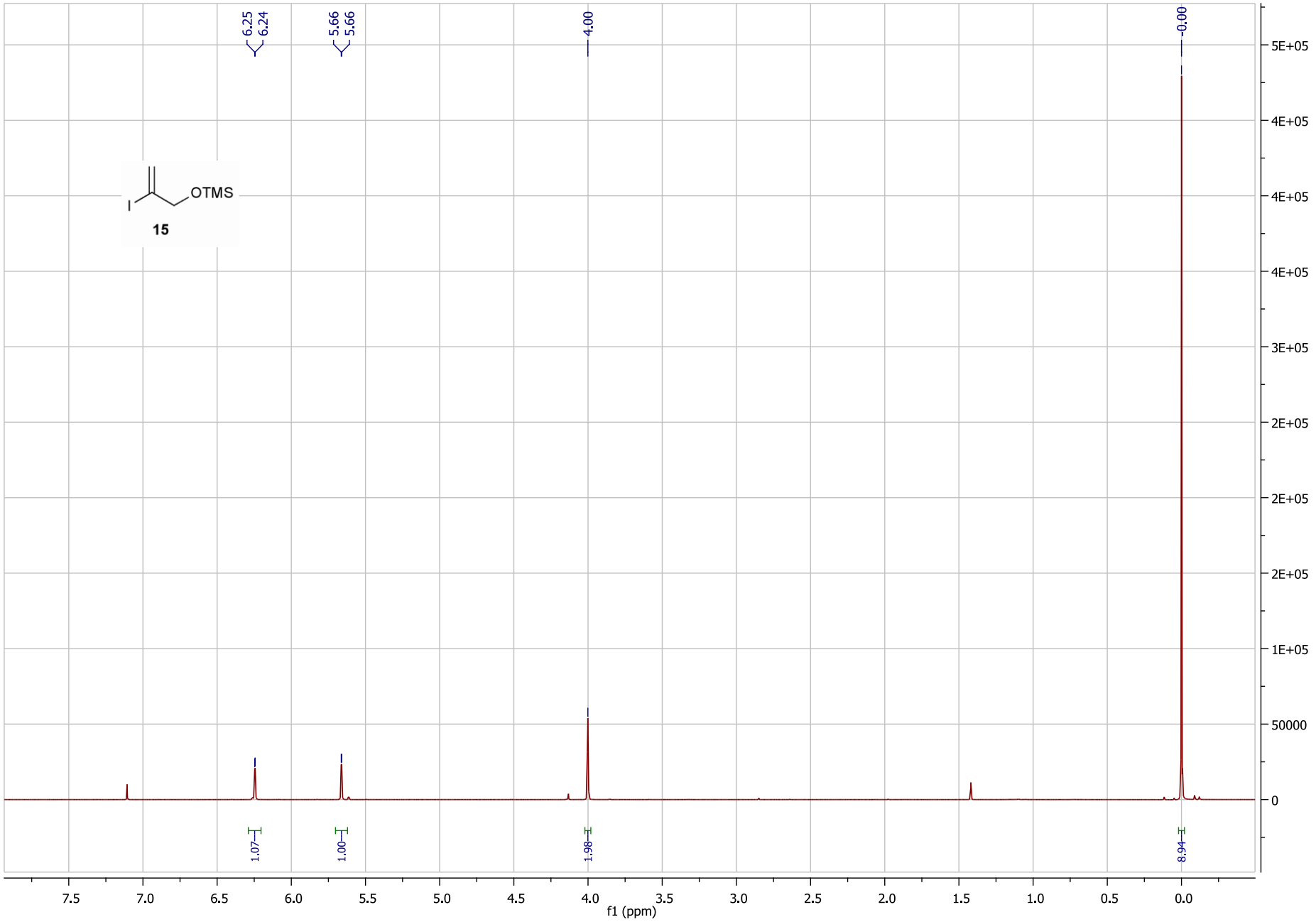


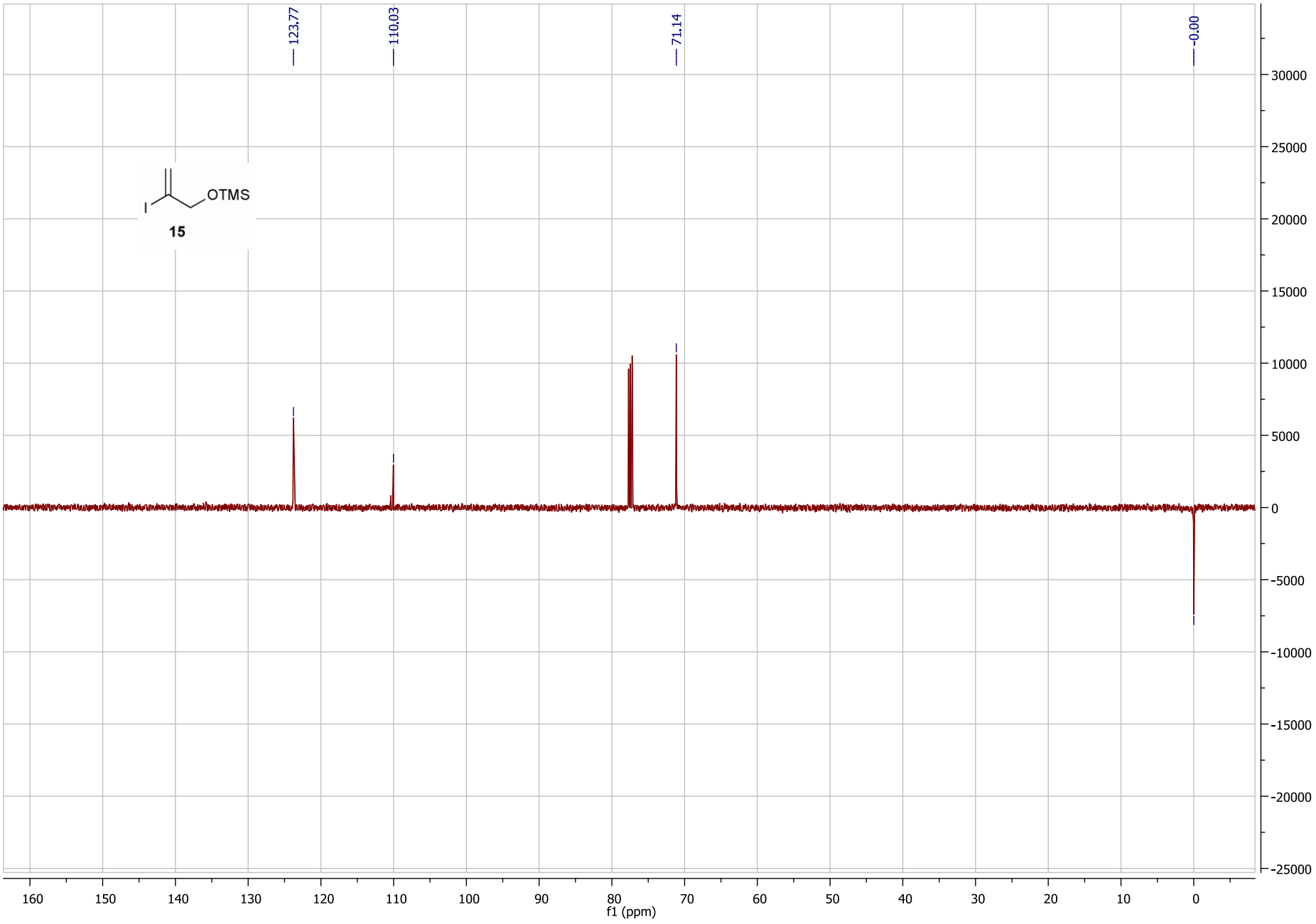


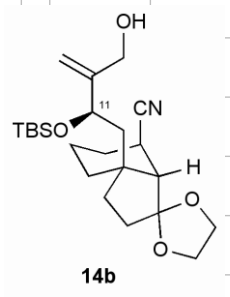




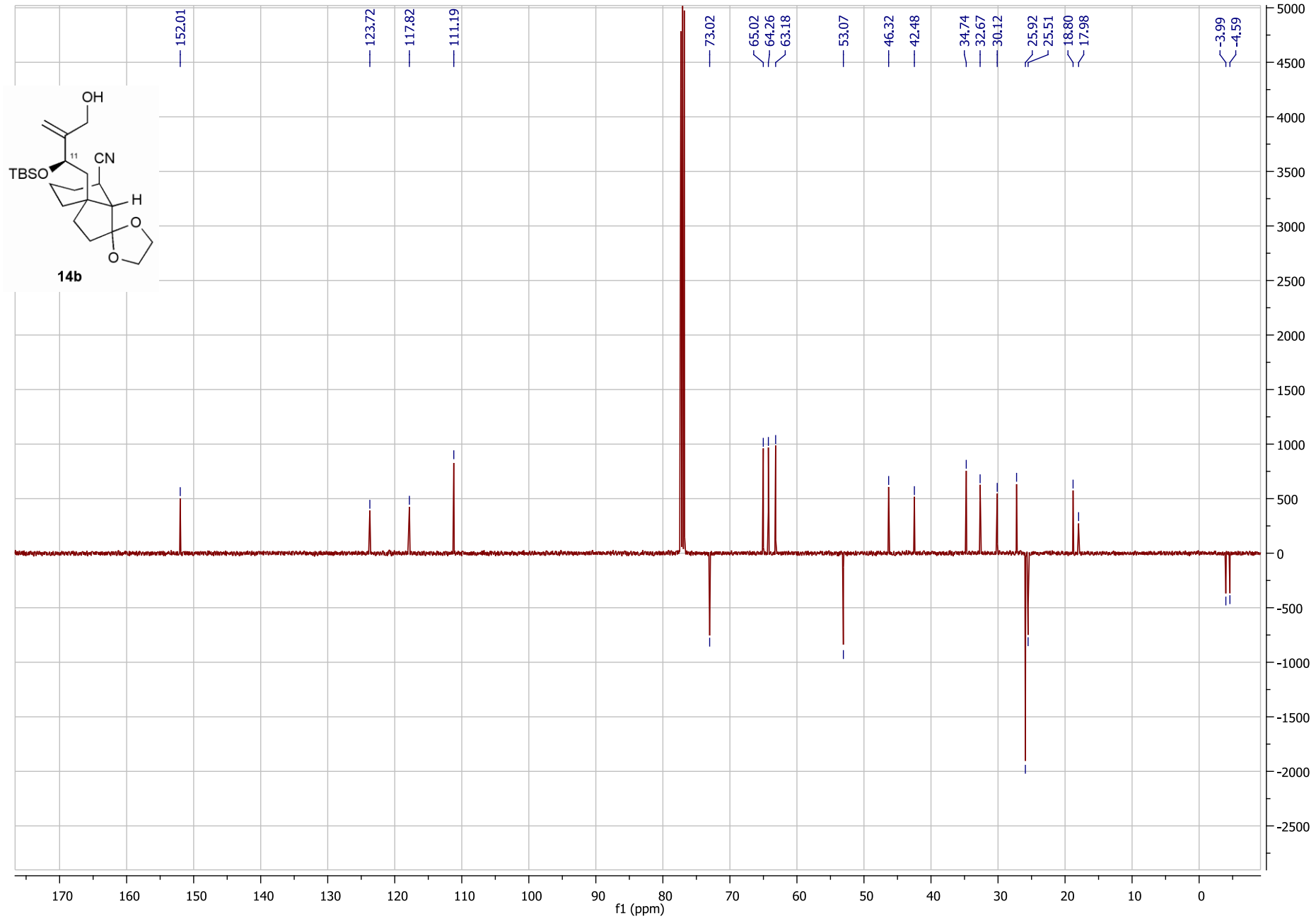


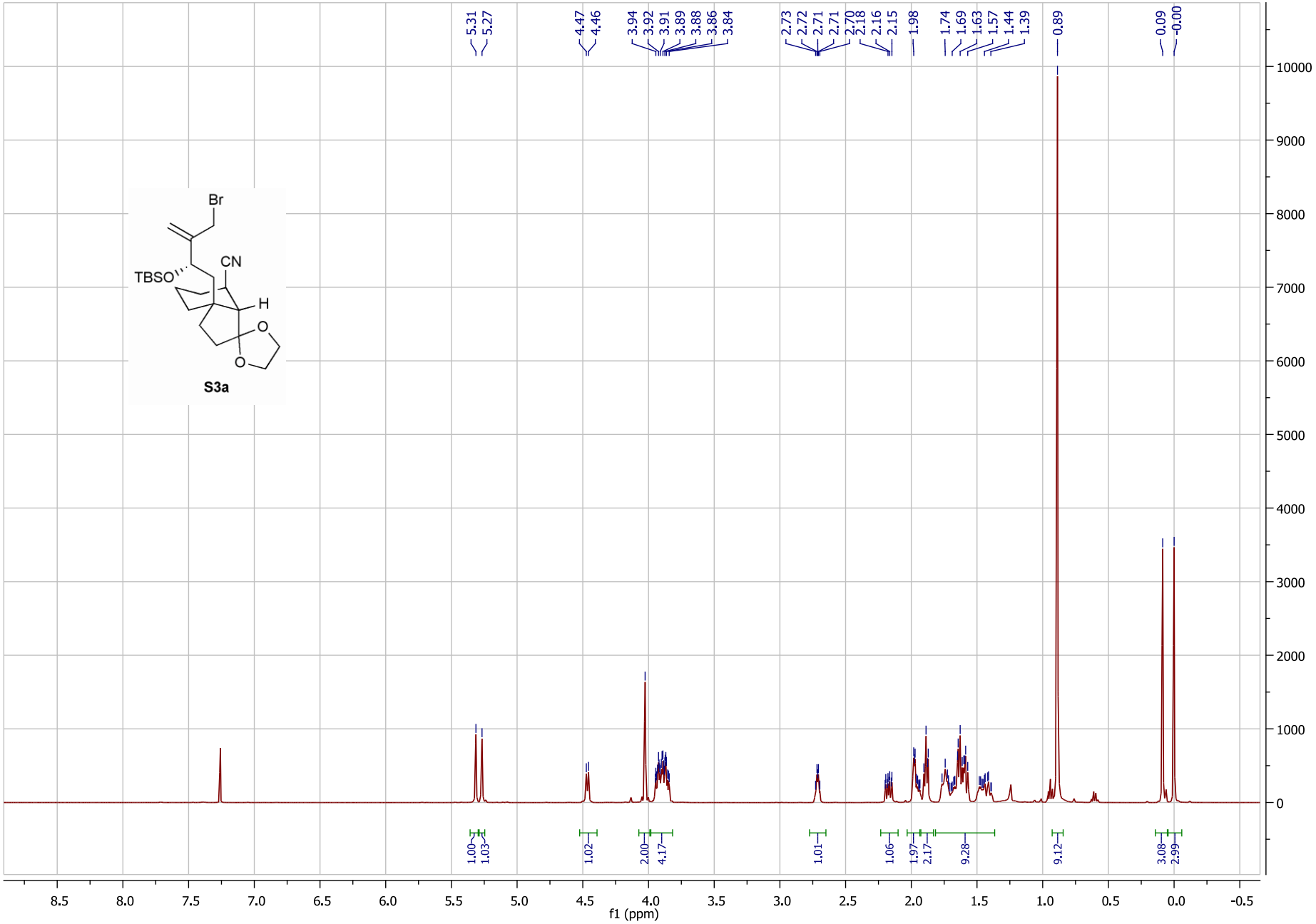


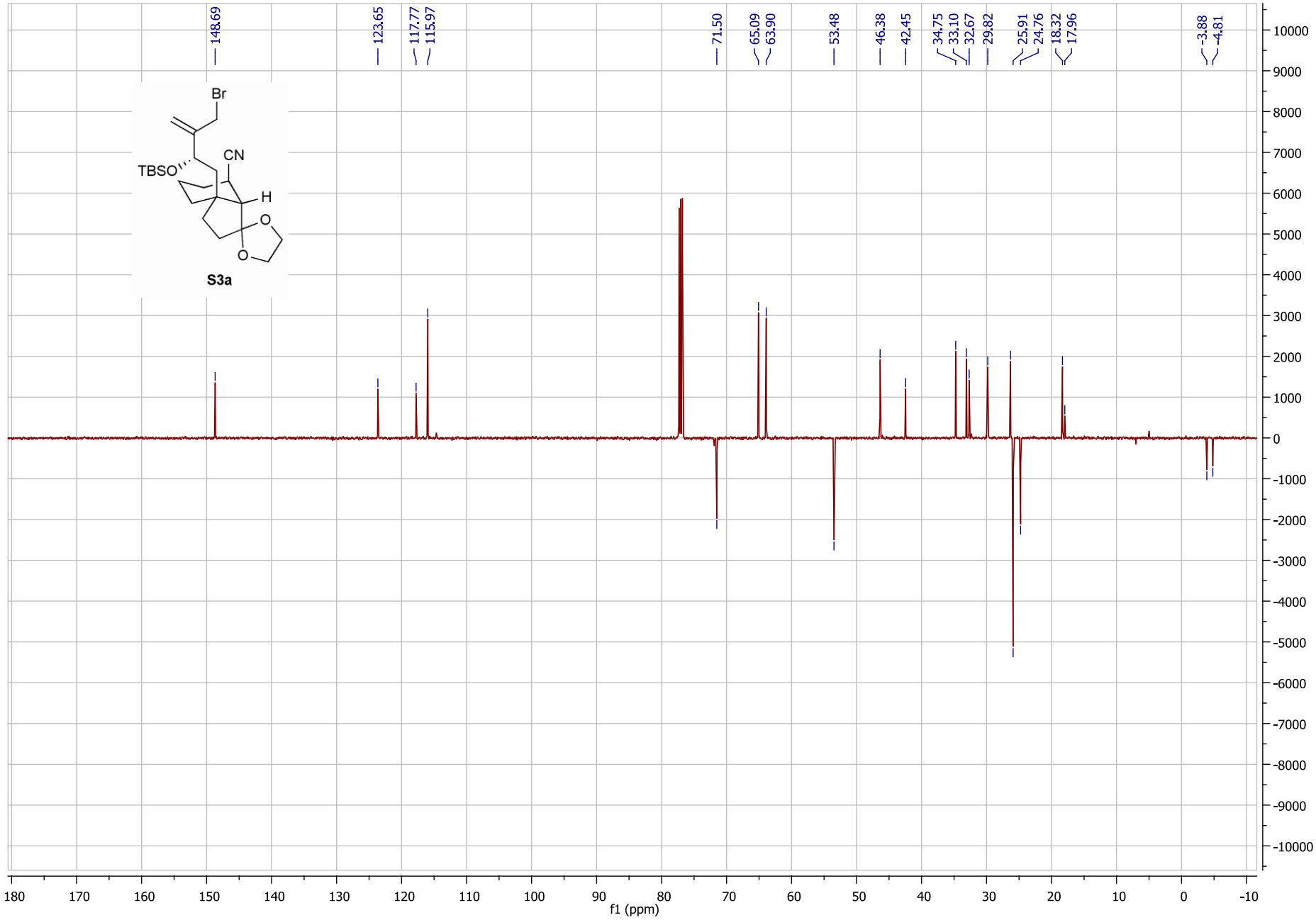


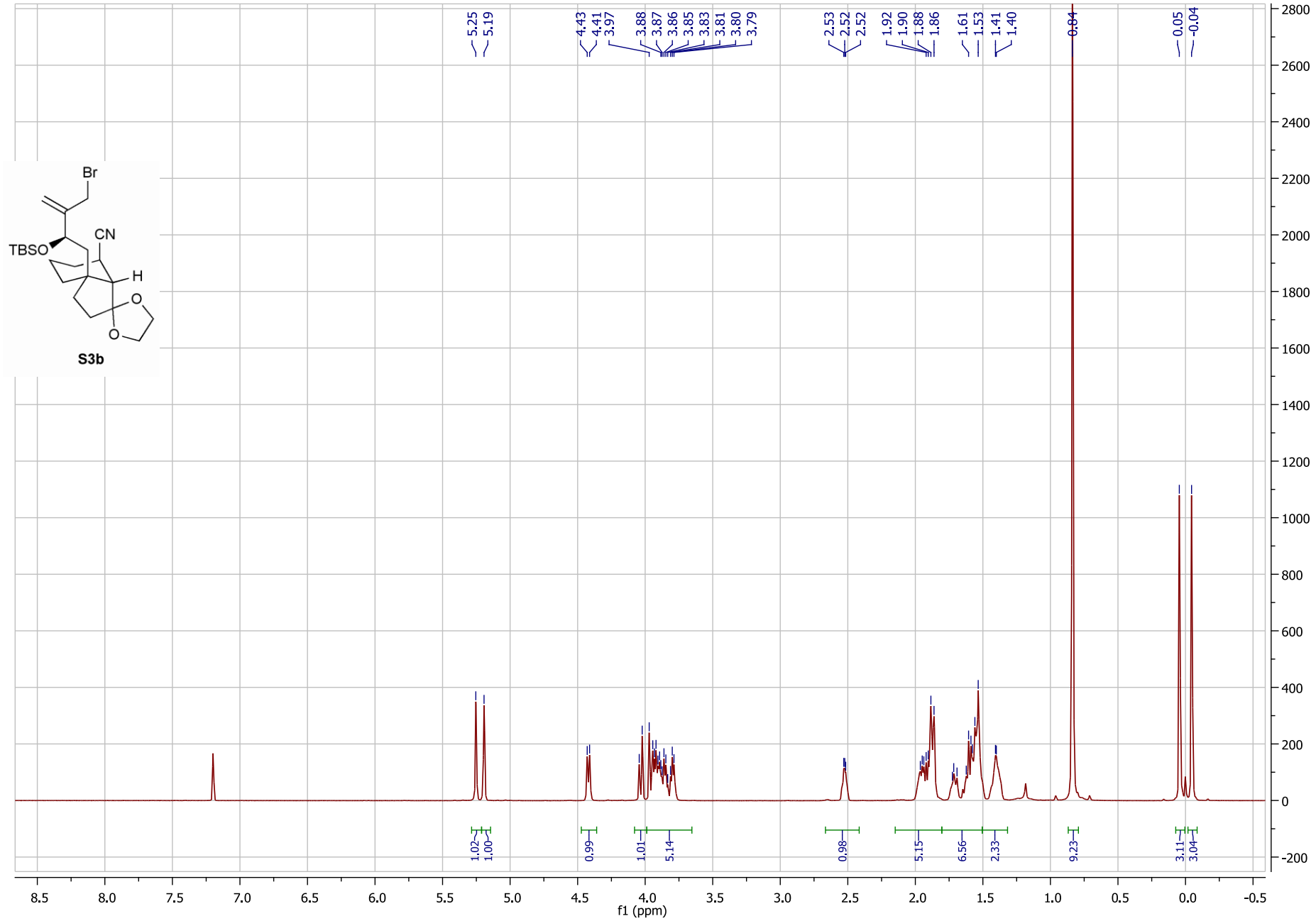


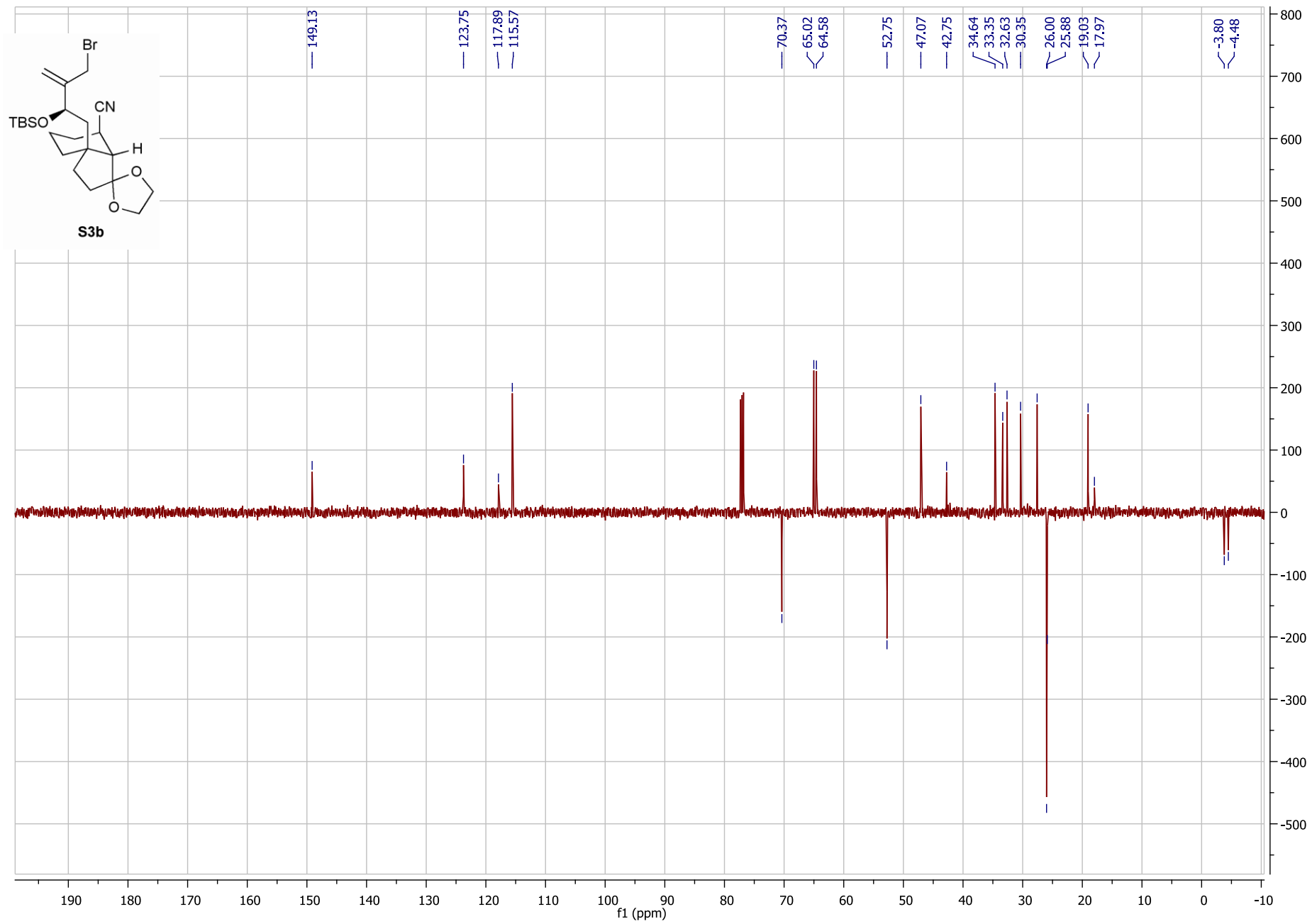
14b

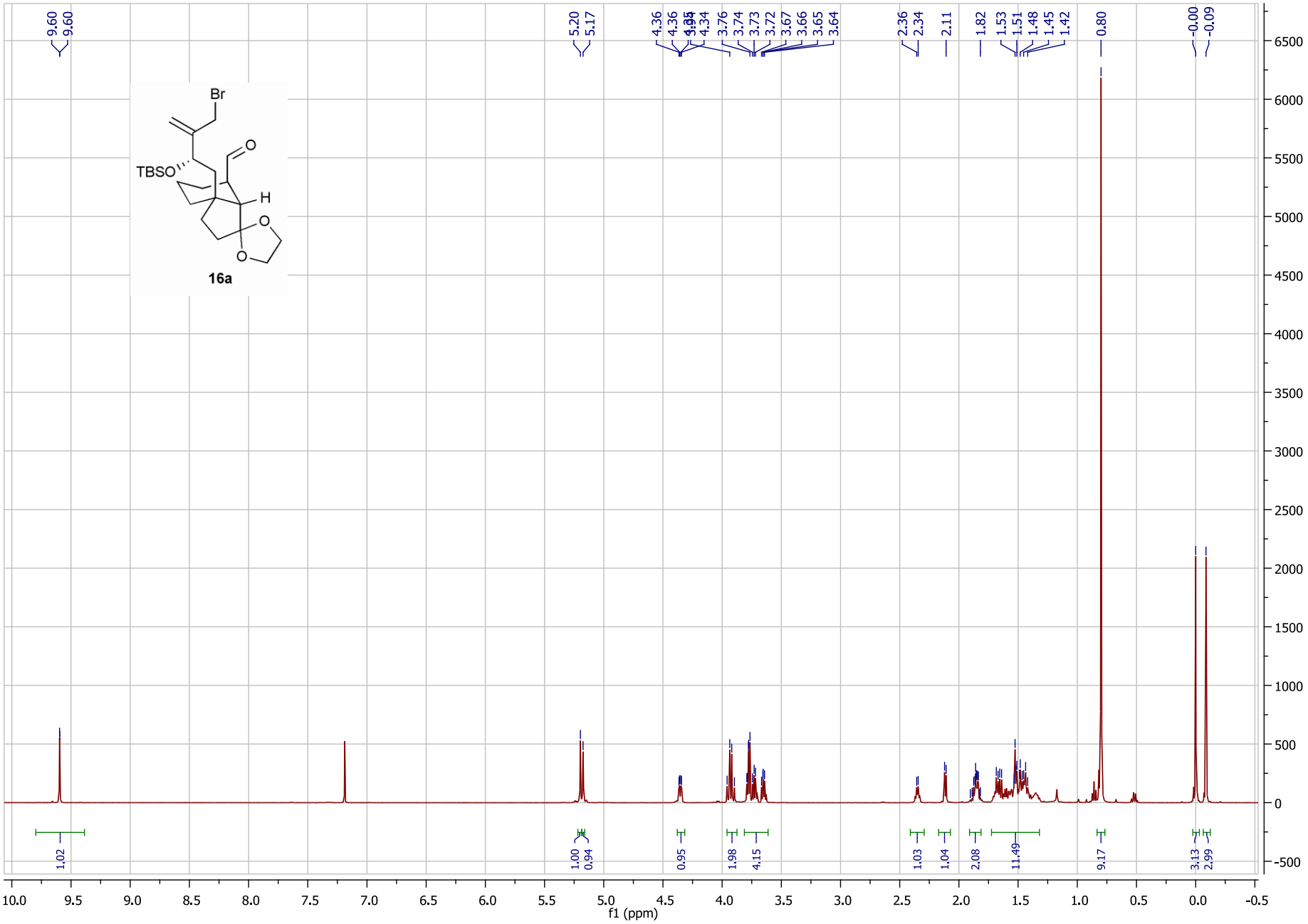


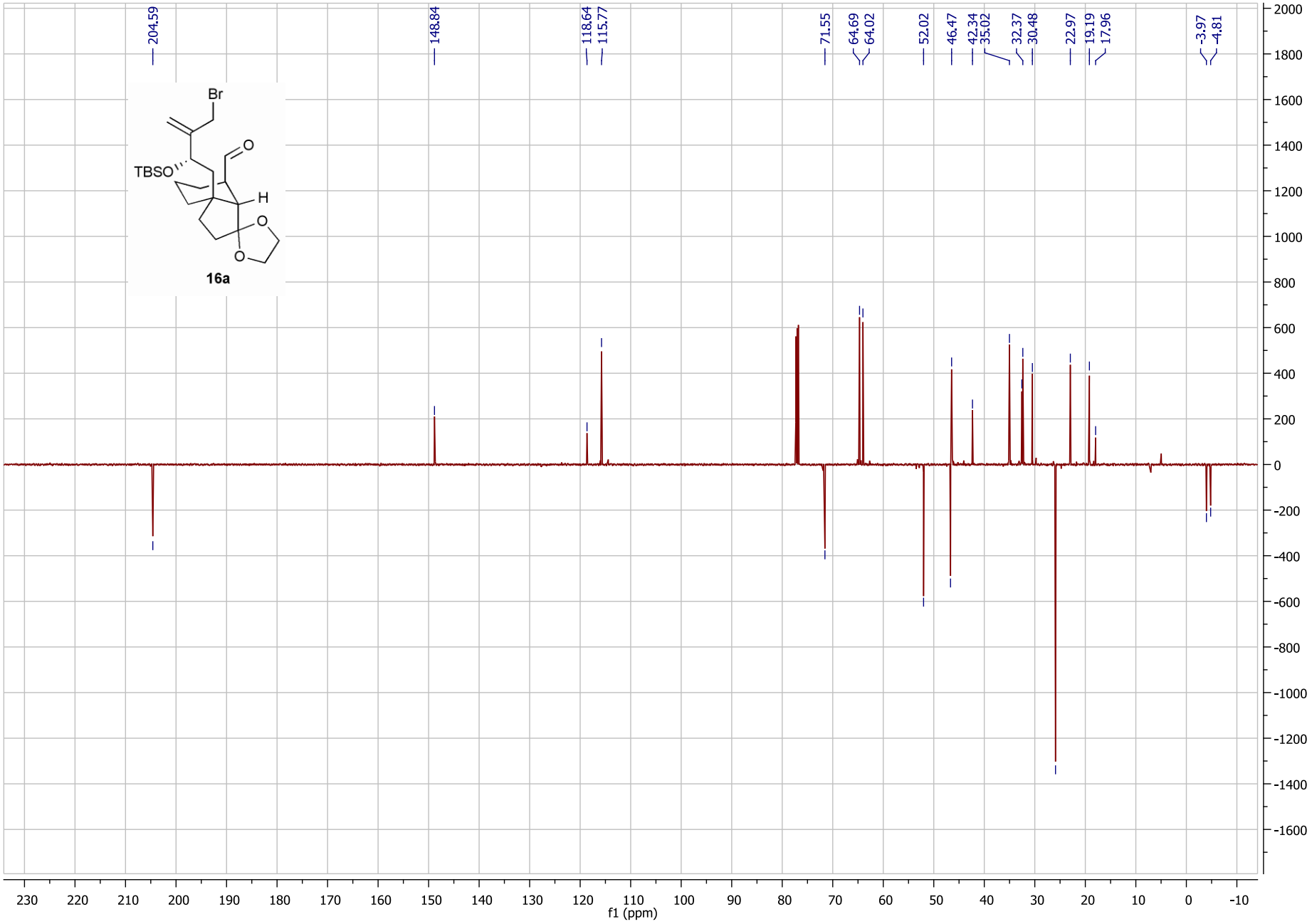


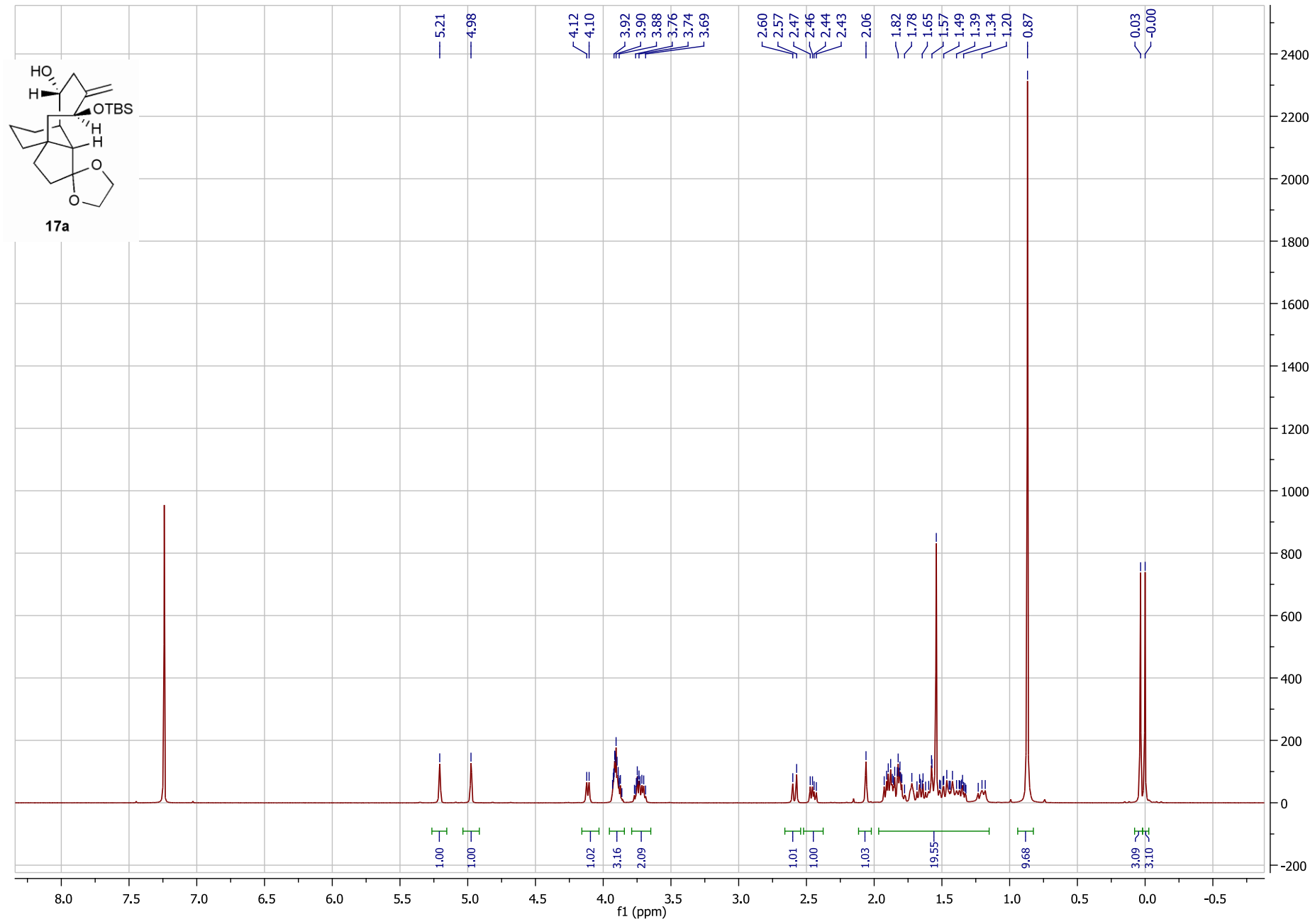


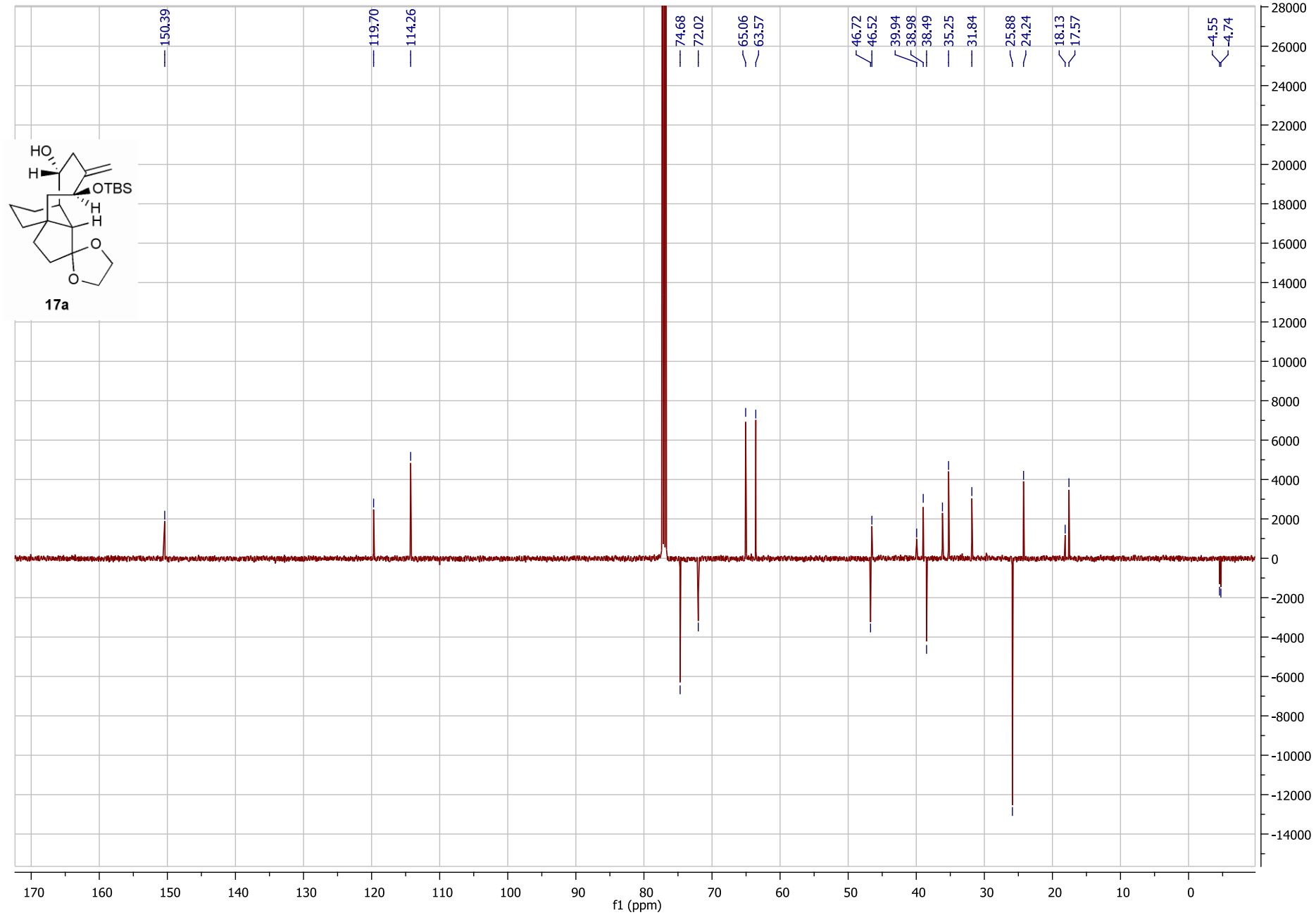


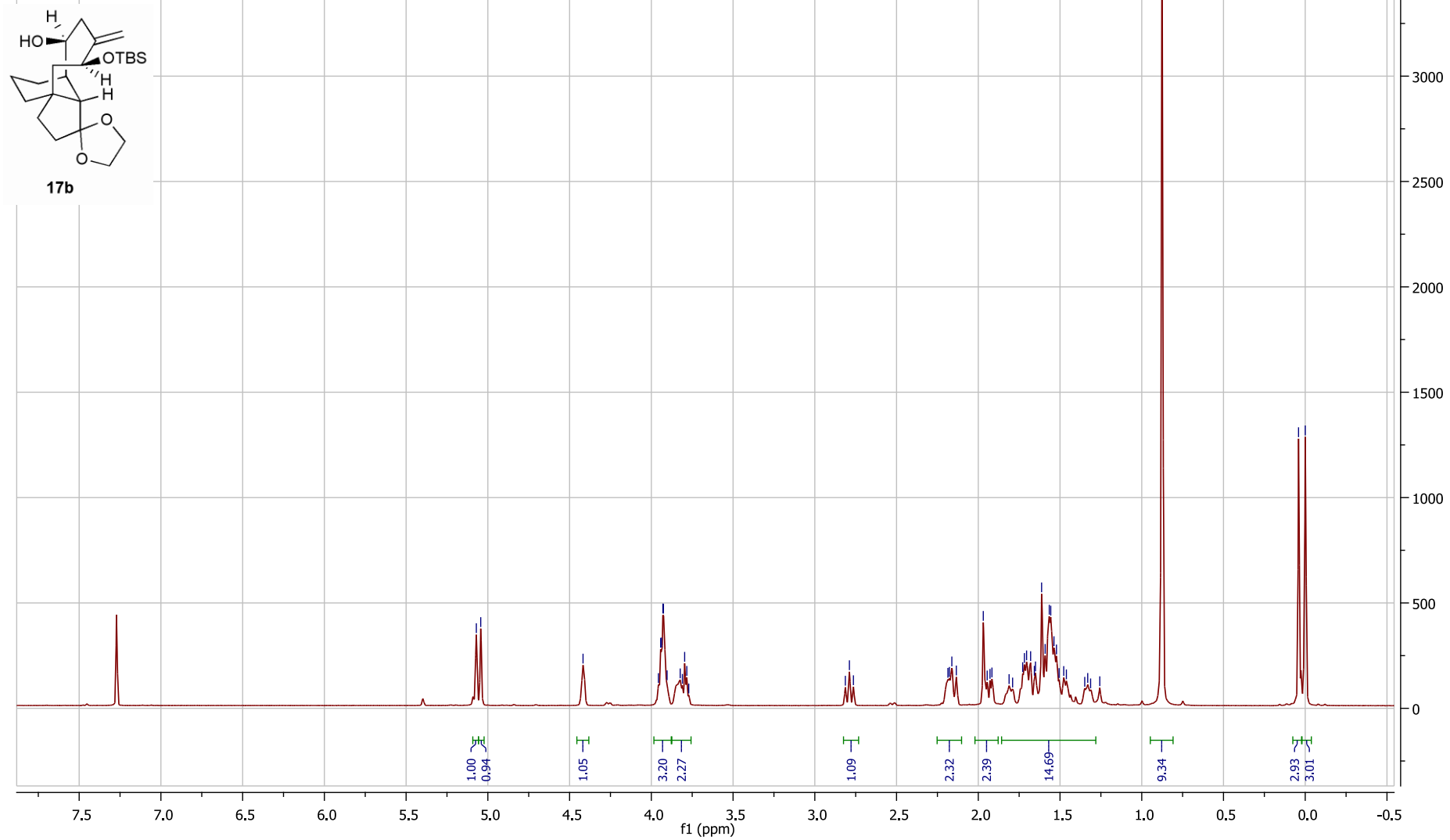




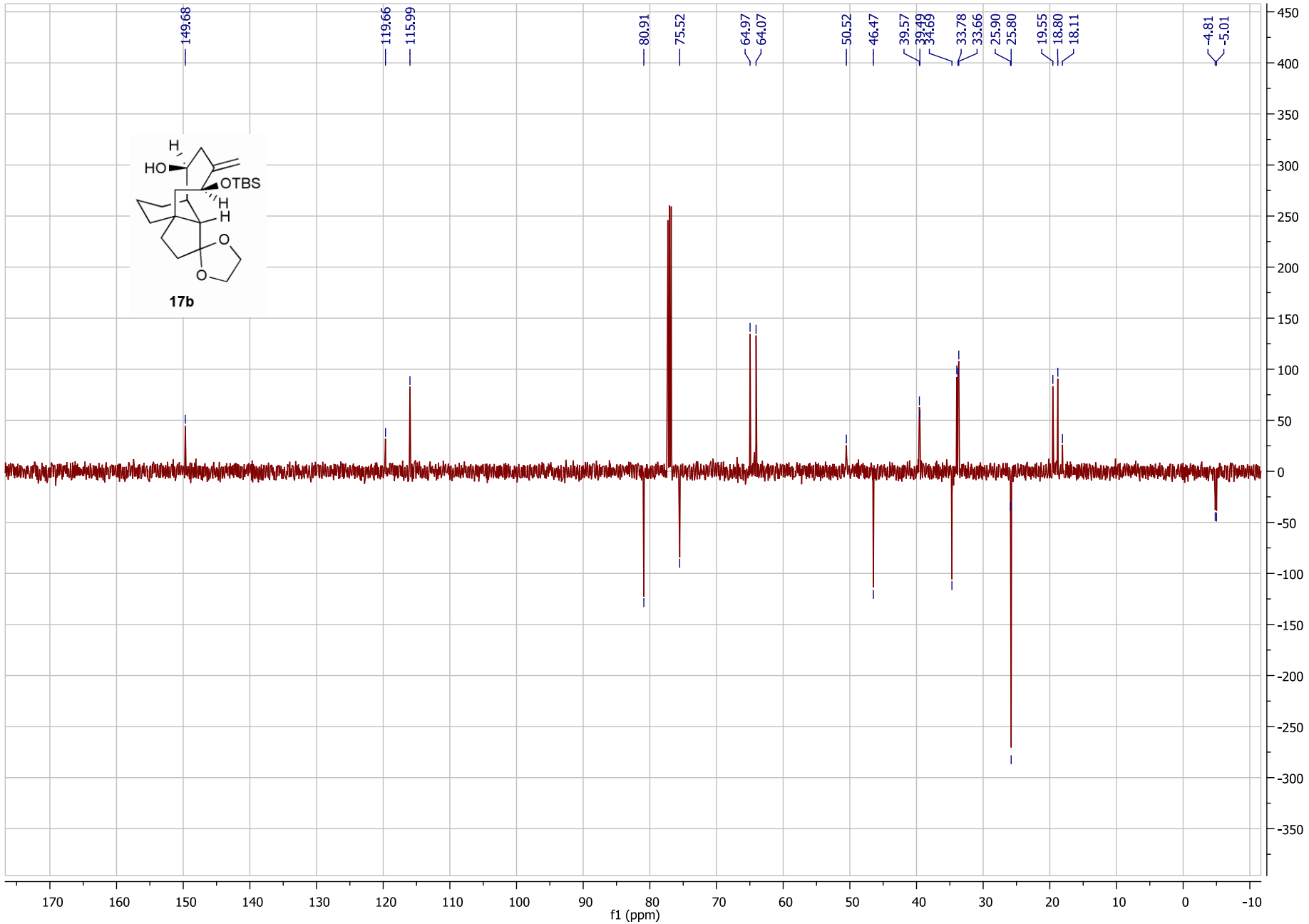




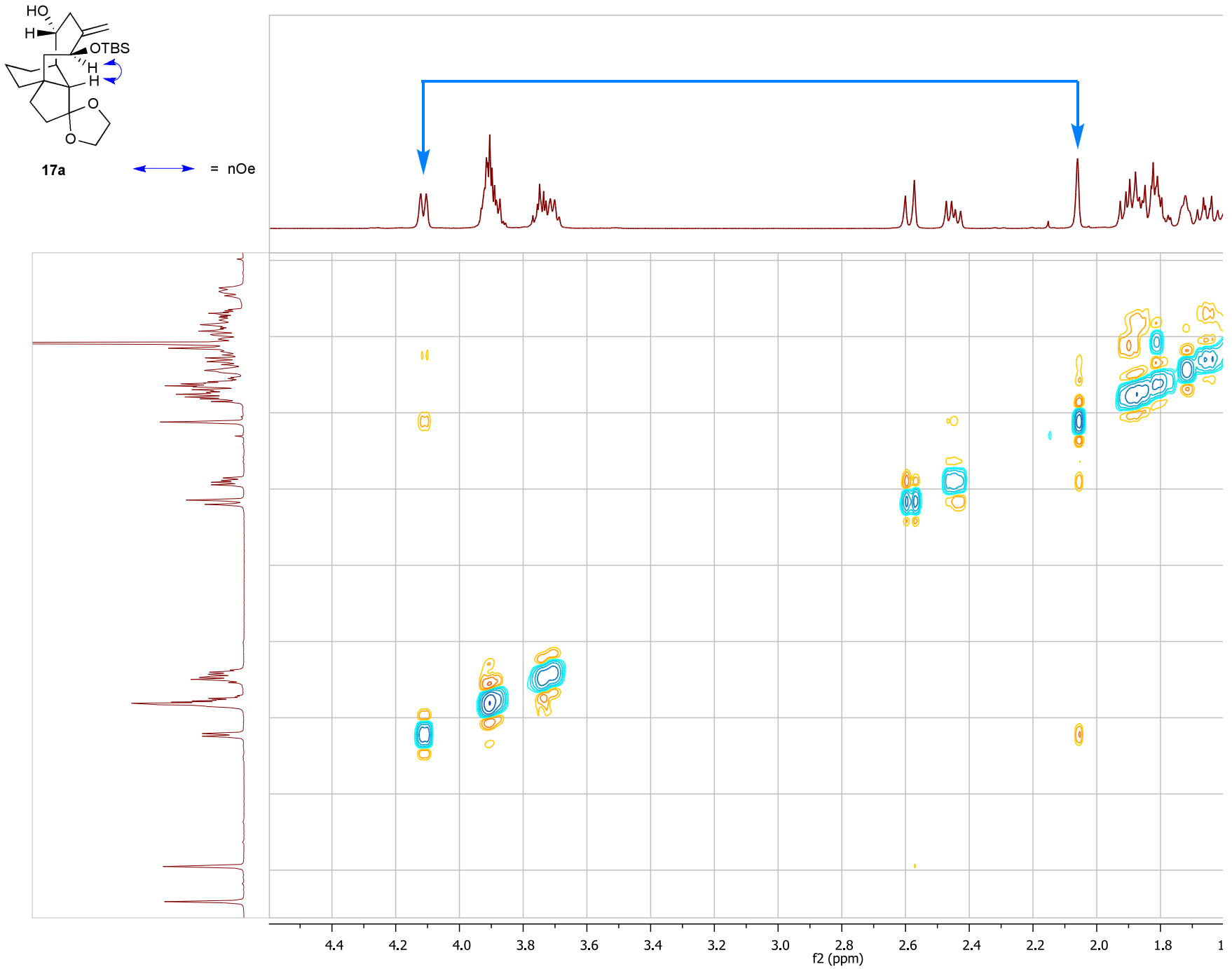




17b



NOESY analysis of 17a



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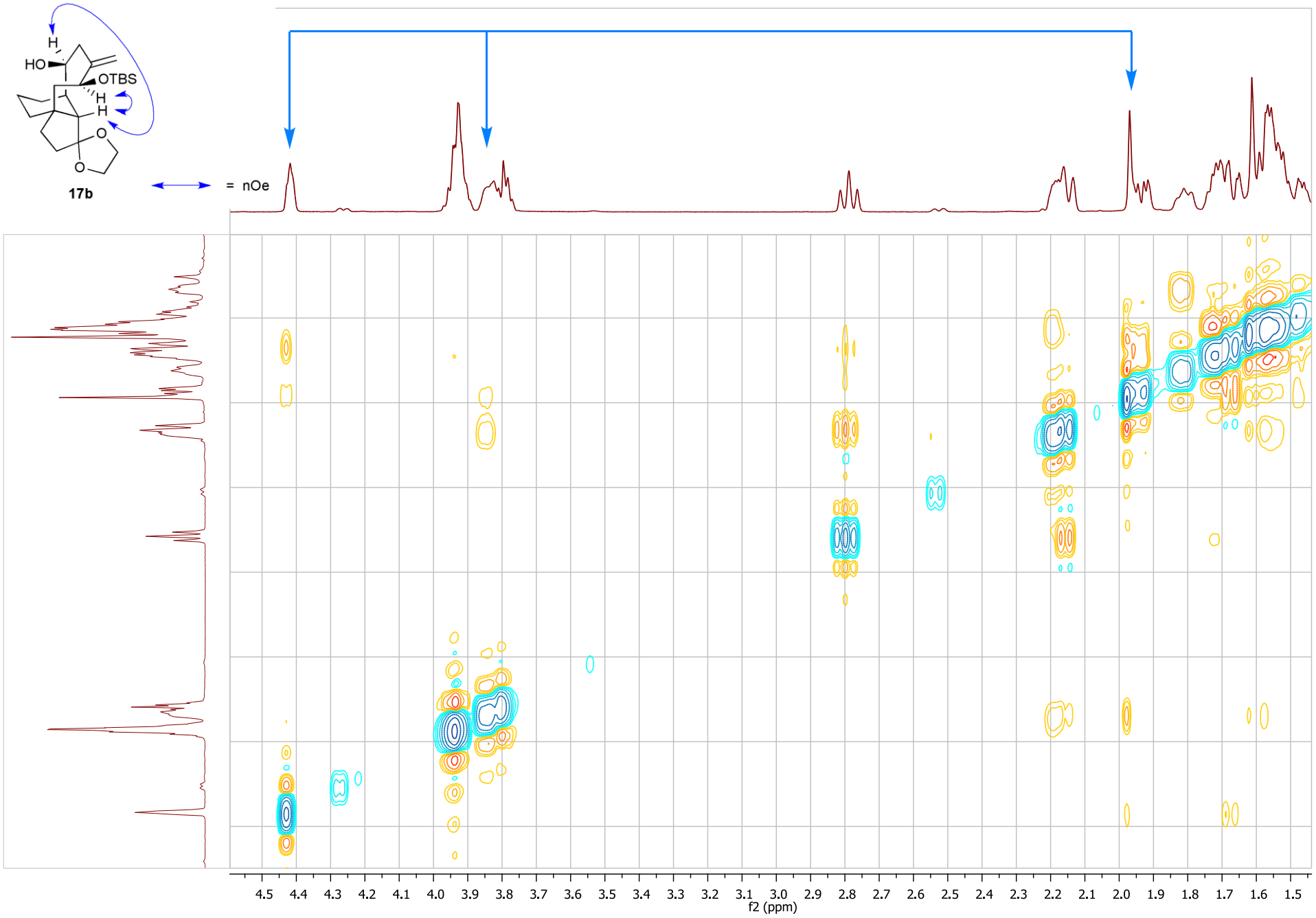

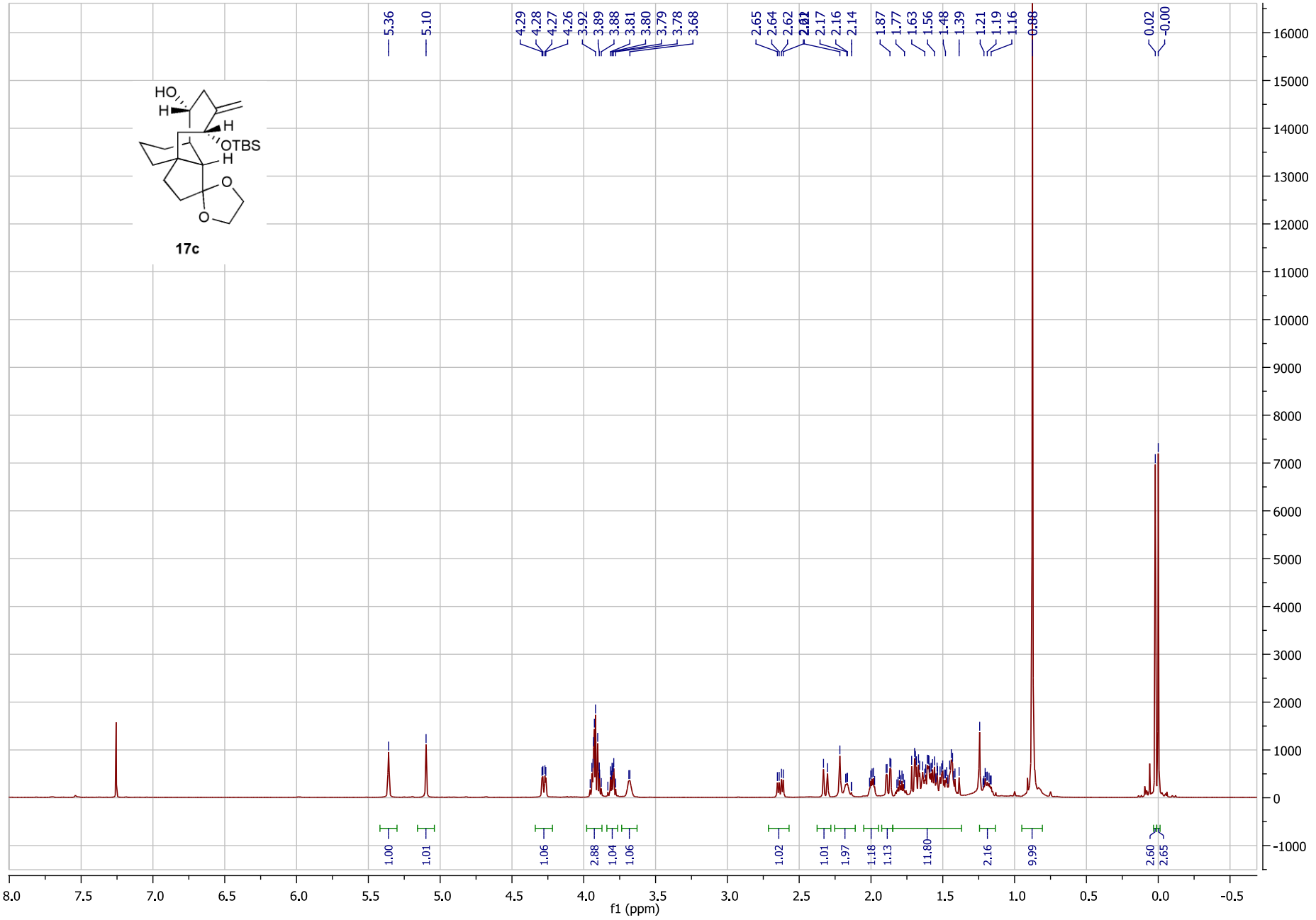

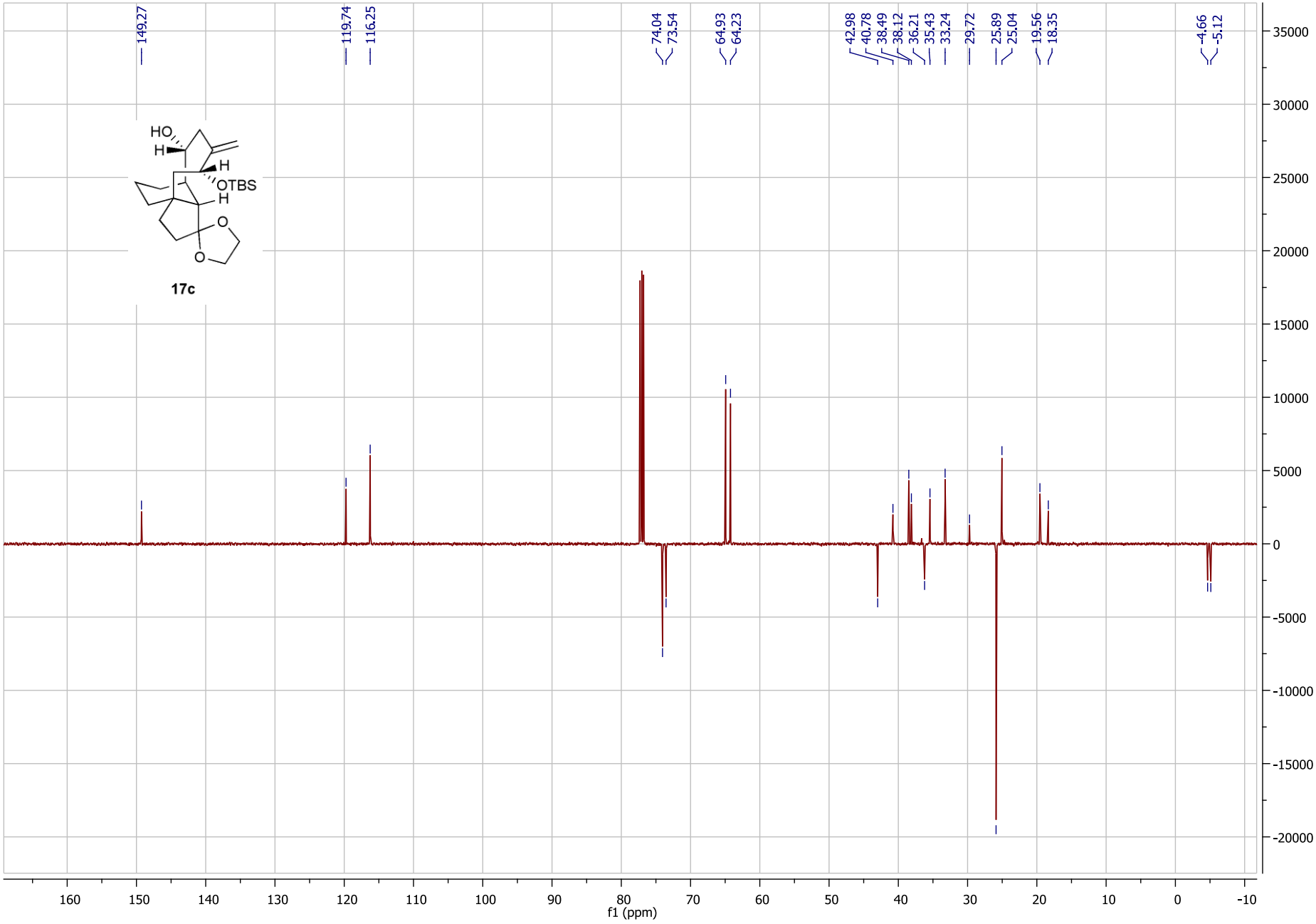

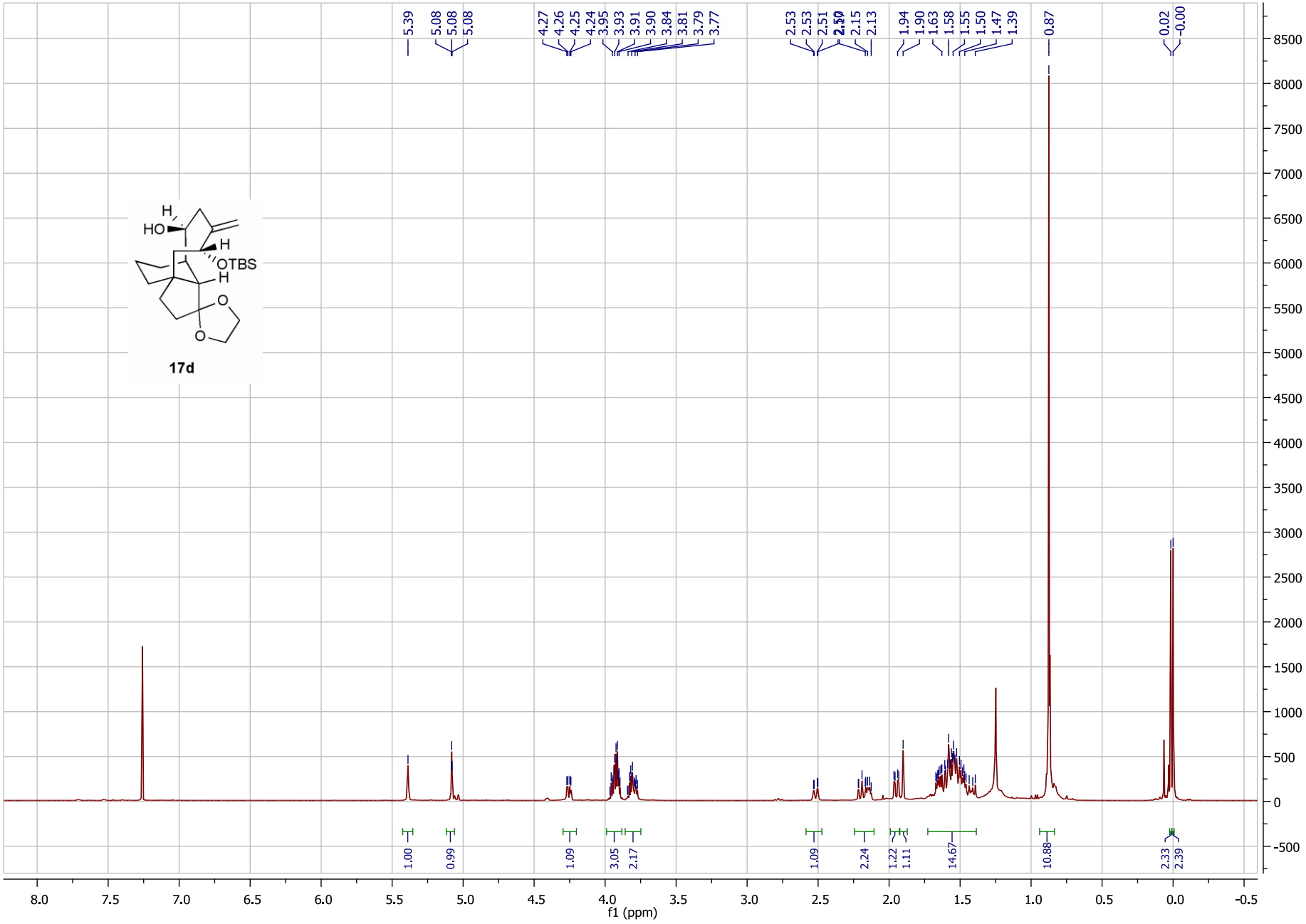

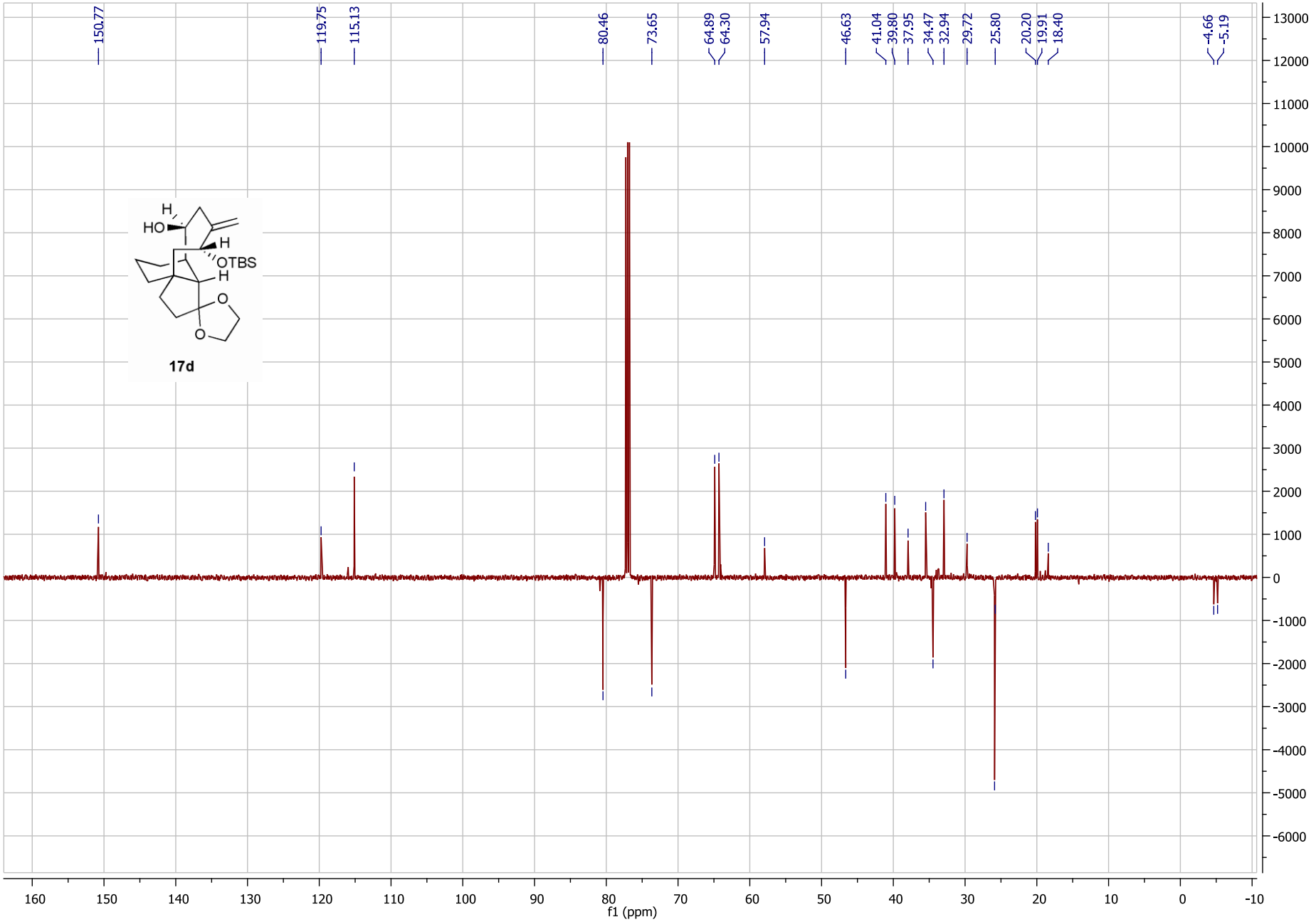

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