SUPPORTING INFORMATION

Chemoenzymatic Synthesis of Spinosyn A

Hak Joong Kim,[†‡] Sei-hyun Choi,[†] Byung-sun Jeon,[†] Namho Kim,[†] Rongson Pongdee,[†] Qingquan Wu,[†] and Hung-wen Liu[†]*
[†] Division of Medicinal Chemistry, College of Pharmacy, and Department of Chemistry, The University of Texas at Austin, Austin, Texas 78712, USA
[‡] Department of Chemistry, Korea University, Seoul 136-701, Republic of Korea

I. General Experimental Procedures

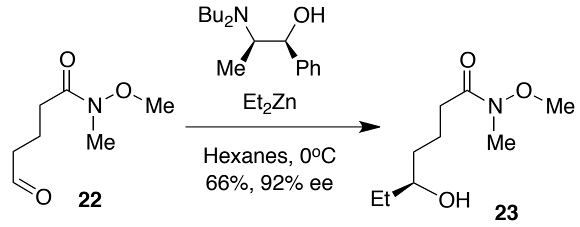
Analytical thin layer chromatography (TLC) was carried out on pre-coated TLC glass plates (Silica gel, grade 60, F_{254}, 0.25 mm layer thickness, EMD Chemicals). Flash column chromatography was performed on silica gel (230-400 mesh, grade 60, Sorbent Technologies). High-performance liquid chromatography (HPLC) applications were performed on a Beckman Coulter HPLC instrument equipped with a UV detector. The NMR spectra were acquired on a Varian Unity 500 or 300 spectrometer housed in the NMR Facility of the Department of Chemistry, University of Texas at Austin. The mass spectroscopic analysis was done at the Mass Spectrometry and Proteomics Facility in the Department of Chemistry and the Institute for Cellular and Molecular Biology, University of Texas at Austin. All chemicals were purchased from Sigma-Aldrich, Fisher Scientific or Acros Chemicals, and were used without further purification unless specified.

II. Enzyme Preparation

All enzymes used in this study including SpnJ,[1] SpnM,[2] SpnF,[2] SpnL,[2] SpnG,[3] SpnH,[4] SpnI,[4] and SpnK,[4] were prepared following previously reported procedures.

III. Chemical Synthesis of the Presumed Post-PKS Precursor (11)

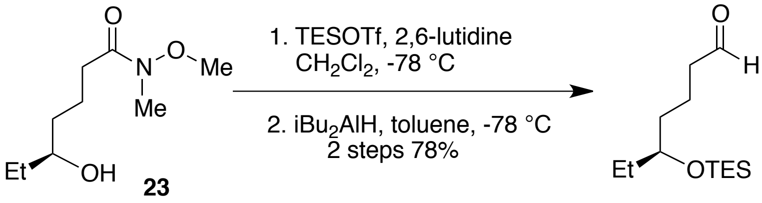
III.1. Synthesis of Fragment A (19)



(*S*)-5-Hydroxy-*N*-methoxy-*N*-methylheptanamide (23). To a mixture of aldehyde **22**[5] (2.61 g, 16.4 mmol) in 50 mL anhydrous hexanes at room temperature was added (-)-(*1S,2R*)-*N,N*-dibutylnorephedrine (DBNP, 0.273 mL, 0.984 mmol) and the reaction was stirred at room temperature for 30 min. Then, the reaction was cooled to 0 ºC and diethyl zinc (1.1 M in toluene, 37 mL, 41 mmol) was added. After stirring for 24 hr, the reaction was quenched by the addition of a saturated ammonium chloride solution (30 mL). The mixture was extracted with dichloromethane (30 mL × 5), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 1/3) to afford alcohol **23** (2.05 g, 10.8 mmol, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.65 (s, 3H, OMe), 3.48 (ddd, *J* = 12, 7.9, 4.5 Hz, 1H, 5-H), 3.15 (s, 3H, NMe), 2.43 (*br* t, *J* = 5.5 Hz, 2H, 2-H), 1.77-1.67 (m, 2H, 3-H), 1.53-1.37 (m, 4H, 4-H, 6-H), 0.91 (t, *J* = 7.5 Hz, 3H, 7-H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.7. 72.6, 61.2, 36.5, 32.2, 31.6, 30.1, 20.3, 9.90; HRMS (CI+) *m/z* for $C_9H_{10}NO_3$ $[M+H]^+$, calc. 190.1443, found 190.1447.

To determine the absolute stereochemistry as well as the enantiomeric purity of the product, the Mosher method[6] based on ^{19}F-NMR analysis of the diastereomeric MTPA ester derivatives of alcohol **23** was applied: **(*S*)-MTPA-ester of 23:** To a clear solution of alcohol **23** (0.05 g, 0.26 mmol) in 4 mL anhydrous dichloromethane at room temperature was added dry pyridine (0.065 mL, 0.81 mmol) followed by (*R*)-(–)-*α*-methoxy-*α*-trifluoromethylphenylacetic acid chloride ((*R*)-(–)-MTPA-Cl, 0.095 mL, 0.49 mmol). After stirring for 2 hr, the reaction mixture was quenched by the addition of water (1 mL). The aqueous layer was extracted with dichloromethane (3 mL x 3), and the combined organic layers were dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was subjected to flash column chromatography (hexanes/ethyl acetate = 1/1) to afford (*S*)-MTPA-ester (0.086 g, 0.22 mmol). In an entirely analogous fashion, the (*R*)-MTPA-ester was prepared using (*S*)-(+)-MTPA-Cl. **(*R*)-MTPA-ester of 23:** ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.54-7.52 (m, 2H, Ph), 7.39-7.36 (m, 3H, Ph), 5.07-5.03 (m, 1H, 5-H), 3.63 (s, 3H, OMe), 3.54-3.53 (m, 3H, OMe from Mosher), 3.14 (s, 3H, NMe), 2.44-2.30 (m, 2H, 2-H), 1.71-1.51 (m, 6H, 3-H, 4-H, 6-H), 0.91 (t, *J* = 7.5 Hz, 3H, 7-H); ^{19}F NMR ($CDCl_3$, 470 MHz) δ -71.72 ppm (integration value = 907.52), -71.76 (integration value = 77.68). **(*S*)-MTPA-ester of 23:** ^{1}H NMR ($CDCl_3$, 500

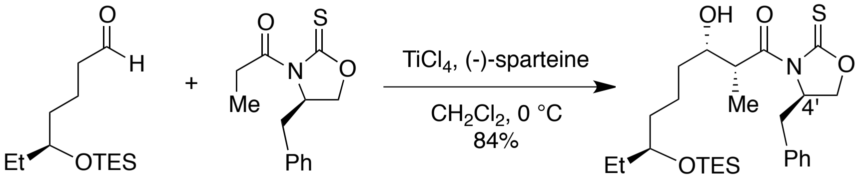
MHz) δ 7.54-7.52 (m, 2H, Ph), 7.39-7.36 (m, 3H, Ph), 5.07-5.02 (m, 1H, 5-H), 3.64 (s, 3H, OMe), 3.54-3.53 (m, 3H, OMe from Mosher), 3.15 (s, 3H, NMe), 2.48-2.34 (m, 2H, 2-H), 1.72-1.57 (m, 6H, 3-H, 4-H, 6-H), 0.79 (t, *J* = 7.5 Hz, 3H, 7-H); ^{19}F NMR (CDCl$_3$, 470 MHz) δ -71.72 ppm (integration value = 84.77), -71.76 (integration value = 941.45).



23

24

(*S*)-5-(Triethylsilyloxy)heptanal (24). To a clear solution of alcohol **23** (0.238 g, 1.25 mmol) in 5 mL anhydrous dichloromethane at -78 ºC was added 2,6-lutidine (0.290 mL, 2.50 mmol) followed by triethylsilyl trifluromethanesulfonate (TESOTf, 0.339 mL, 1.50 mmol). After stirred for 3 hr during which the temperature was maintained at -78 ºC, the reaction mixture was poured into a saturated ammonium chloride solution (20 mL). The mixture was extracted with dichloromethane (20 mL × 3), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was subjected to flash column chromatography (hexanes/ethyl acetate = 1/1) to afford a silyl ether (0.310 g, 1.03 mmol). To a solution of the silyl ether in toluene (4 mL) at -78 °C was added DIBAL-H (1 M in hexanes, 2.06 mL, 2.06 mmol). After 2 hr stirring at -78 ºC, the reaction was quenched by adding methanol (2 mL) followed by a saturated Rochelle's salt solution (3 mL). The resulting mixture was stirred at room temperature for an additional 30 min. The reaction mixture was extracted with ethyl acetate (20 mL × 3), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 19/1) to afford aldehyde **24** (0.237 g, 0.970 mmol, 2 steps 78%). ^{1}H NMR (CDCl$_3$, 500 MHz) δ 9.74 (t, *J* = 1.8 Hz, 1H, 1-H), 3.59 (quint, *J* = 5.8 Hz, 1H, 5-H), 2.40 (dt, *J* = 7.4, 1.8 Hz, 2H, 2-H), 1.73-1.56 (m, 2H, 3-H), 1.48-1.36 (m, 4H, 4-H, 6-H), 0.937 (t, *J* = 7.8 Hz, 9H, TES), 0.85 (t, *J* = 7.4 Hz, 3H, 7-H), 0.58 (q, *J* = 7.8 Hz, 6H, TES); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 202.6, 73.1, 44.0, 35.9, 29.8, 18.0, 9.55, 6.92, 5.08; HRMS (CI+) *m/z* for $C_{13}H_{29}O_2Si$ [M+H]$^+$, calc. 245.1937, found 245.1925.

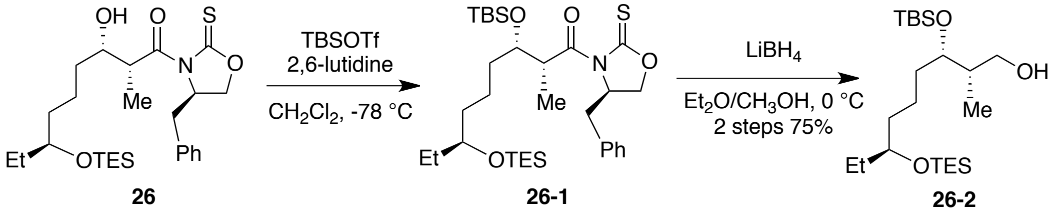


24

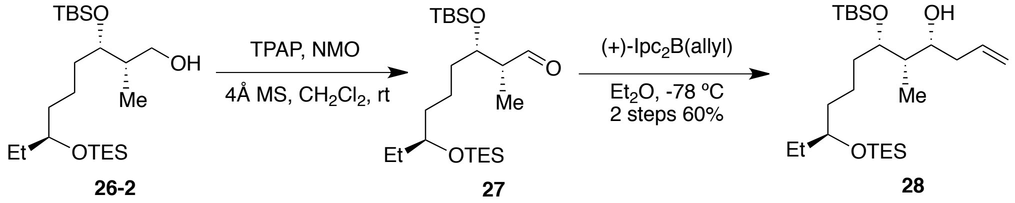
25

26

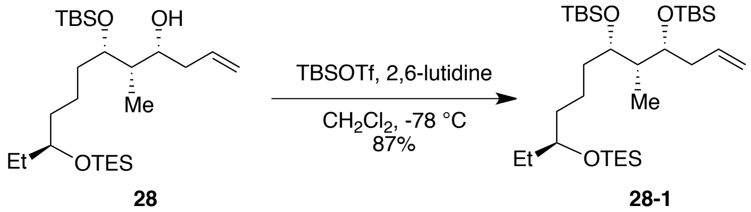
(2*R*,3*S*,7*S*)-1-((*R*)-4′-Benzyl-2′-thioxooxazolidin-3′-yl)-3-hydroxy-2-methyl-7-(triethylsilyloxy)nonan-1-one (26). To a clear solution of oxazolidino-2-thione **25** (0.528 g, 2.12 mmol) in 14 mL anhydrous dichloromethane at 0 ºC was added titanium(IV) chloride (0.243 mL, 2.22 mmol) resulting in a yellow suspension. After 5 min of stirring, (-)-sparteine (1.20 mL, 5.30 mmol) was added to the suspension to produce a dark red solution. The reaction continued at 0 ºC for 20 min for complete enolization, and then a precooled solution of aldehyde **24** (0.780 g, 3.19 mmol) in 3 mL anhydrous dichloromethane was slowly transferred to the reaction mixture at 0 ºC *via* cannula, turning the reaction to a lighter shade of red. After 1 hr, the mixture was poured into a brine solution (100 mL), extracted with dichloromethane (25 mL × 5), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 4/1) to afford *syn*-aldol adduct **26** (0.848 g, 1.72 mmol) in 84% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33-7.29 (m, 2H, Ph), 7.28-7.24 (m, 1H, Ph), 7.20-7.18 (m, 2H, Ph), 4.96-4.91 (m, 1H, 4'-H), 4.67 (dq, *J* = 6.9, 2.7 Hz, 1H, 2-H), 4.32-4.26 (m, 2H, 5'-H), 3.98-3.96 (m, 1H, 3-H), 3.57 (quint, *J* = 5.7 Hz, 1H, 7-H), 3.22 (dd, *J* = 13.3, 3.5 Hz, 1H, $PhCH_2$), 2.74 (dd, *J* = 13.3, 10 Hz, 1H, $PhCH_2$), 1.58-1.28 (m, 8H, 4-H, 5-H, 6-H, 8-H), 1.27 (d, *J* = 6.9 Hz, 3H, 2-Me), 0.937 (t, *J* = 8 Hz, 9H, TES), 0.849 (t, *J* = 7.5 Hz, 3H, 9-H), 0.574 (q, *J* = 8 Hz, 6H, TES); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.0, 178.4, 135.1, 129.4, 129.0, 127.5, 73.4, 71.6, 70.2, 59.9, 42.0, 37.6, 36.5, 34.3, 29.8, 21.7, 10.3, 9.62, 6.96, 5.12; HRMS (CI+) *m/z* for $C_{26}H_{44}N_1O_4Si_1S_1$ $[M+H]^+$, calc. 494.2760, found 494.2784.



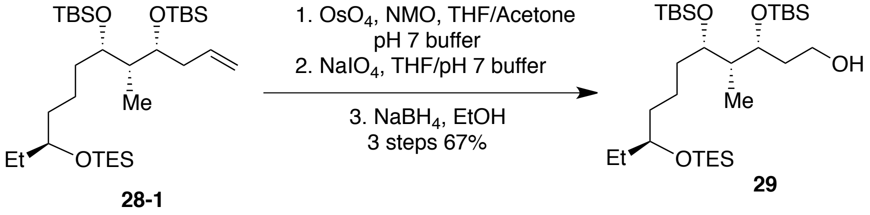
(2*S*,3*S*,7*S*)-3-(*tert*-Butyldimethylsilyloxy)-2-methyl-7-(triethylsilyloxy)nonan-1-ol (26-2). To a clear solution of *syn*-aldol adduct **26** (3.51 g, 7.12 mmol) in 50 mL anhydrous dichloromethane was added 2,6-lutidine (1.70 mL, 14.2 mmol) followed by *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf, 1.96 mL, 8.54 mmol) at -78 ºC and the reaction continued with the temperature maintained at -78 °C for 2 hr. Then, the reaction mixture was poured into a saturated sodium bicarbonate solution (50 mL), extracted with dichloromethane (100 mL × 3), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was subjected to flash column chromatography (hexanes/ethyl acetate = 19/1) to afford silyl ether **26-1** (3.99 g, 6.58 mmol, 92%). To a solution of **26-1** in anhydrous diethyl ether (40 mL) and methanol (0.340 mL) at 0 ºC was added lithium borohydride (2 M in tetrahydrofuran, 4.23 mL, 8.47 mmol). The immediate evolution of a gas, presumably molecular hydrogen, was noted. After 15 min stirring at 0 ºC, the reaction mixture was warmed to room temperature over 1 hr, at which time 15% sodium hydroxide (40 mL) was added, and the reaction mixture was stirred for additional 30 min. The mixture was then extracted with ethyl acetate (40 mL × 3), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 19/1) to afford the primary alcohol **26-2** (2.24 g, 5.28 mmol, 81%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.76-3.71 (m, 1H, 3-H or 7-H), 3.68-3.64 (m, 1H, 1-H), 3.59-3.55 (m, 1H, 3-H or 7-H), 3.49 (dd, *J* = 10.6, 5.2 Hz, 1H, 1-H), 1.95-1.91 (m, 1H, 2-H), 1.57-1.36 (m, 7H, 4-H, 5-H, 6-H, 8-H), 1.26-1.18 (m, 1H, 5-H), 0.94 (t, *J* = 8 Hz, 6H, TES), 0.88 (s, 9H, TBS), 0.85 (t, *J* = 7.4 Hz, 3H, 9-H), 0.79 (d, *J* = 6.9 Hz, 3H, 2-Me), 0.57 (q, *J* = 8 Hz, 6H, TES), 0.07 (s, 3H, TBS), 0.05 (s, 3H, TBS); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 75.9, 73.3, 66.1, 39.5, 36.8, 32.6, 29.8, 25.8, 22.0, 18.0, 11.9, 9.66, 6.95, 6.57, 5.79, 5.12, -4.40, -4.49; HRMS (CI+) *m/z* for $C_{22}H_{51}O_3Si_2$ $[M+H]^+$, calc. 419.3377, found 419.3374.



(4*R*,5*S*,6*S*,10*S*)-6-(*tert*-Butyldimethylsilyloxy)-5-methyl-10-(triethylsilyloxy)dodec-1-en-4-ol (28). To a solution of primary alcohol **26-2** (0.870 g, 2.05 mmol) in anhydrous dichloromethane (20 mL) at room temperature was added activated 4 Å molecular sieve (MS) and the resulting suspension was stirred at ambient temperature for 10 min to get rid of moisture. To the suspension, *N*-methylmorpholine oxide (NMO) (0.480 g, 4.10 mmol) and tetrapropylammonium perruthenate (TPAP) (0.036 g, 0.103 mmol) were added in sequence. After 30 min stirring at room temperature, 10% sodium thiosulfate ($Na_2S_2O_3$) solution (20 mL) was added to quench the excess oxidants. The reaction mixture was then extracted with dichloromethane (20 mL × 3), washed with a brine solution (20 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 50/1) to afford aldehyde **27** (0.742 mg, 1.76 mmol, 86%). For application of Brown's asymmetric allylation, the reagent of (+)-*B*-allyldiisopinocampheylborane [(+)-Ipc_2B(allyl)] was prepared as follows. To a solution of (+)-*B*-methoxydiisopinocampheylborane [(+)-Ipc_2BOMe] (0.164 g, 0.519 mmol) in anhydrous ethyl ether (2 mL) at room temperature was added allylmagnesium bromide (1 M in ethyl ether, 0.472 mL, 0.472 mmol) and the resulting white suspension was stirred for 1 hr. After the mixture was cooled to -78 ºC, a solution of aldehyde **27** (0.100 g, 0.236 mmol) in anhydrous ethyl ether (1 mL) was added to the solution containing (+)-Ipc_2B(allyl) and the mixture was stirred at -78 °C for an additional 30 min. Methanol (1 mL) was added to quench the reaction, followed by the addition of 1 N sodium hydroxide (1 mL) and 30% hydrogen peroxide solution (0.340 mL, 0.944 mmol). After overnight stirring at room temperature, the reaction mixture was poured into a saturated sodium bicarbonate solution (10 mL), extracted with ethyl acetate (10 mL × 3), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 50/1) to afford homoallylic alcohol **28** (0.070 g, 1.53 mmol, 70%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.83-5.75 (m, 1H, 2-H), 5.11-5.03 (m, 2H, 1-H), 3.82-3.76 (m, 2H, 6-H, 10-H), 3.55 (*app* quint, *J* = 6 Hz, 1H, 4-H), 2.72 (*br* s, 1H, 4-O*H*), 2.30-2.24 (m, 1H, 3-H), 2.22-2.16 (m, 1H, 3-H), 1.61-1.17 (m, 9H, 5-H, 7-H, 8-H, 9-H, 11-H), 0.94 (t, *J* = 8 Hz, 9H, TES), 0.87 (*app* s, 12H, TBS, 2-Me), 0.85 (t, *J* = 7.4 Hz, 3H, 12-H), 0.57 (q, *J* = 8 Hz, 6H, TES), 0.074 (s, 3H, TBS), 0.069 (s, 3H, TBS); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 135.4, 117.1, 77.5, 74.3, 73.4, 39.8, 39.0, 36.7, 35.0, 29.9, 25.9, 21.3, 18.0, 9.7, 6.9, 5.7, 5.1, -3.6, -4.5; HRMS (CI+) *m/z* for $C_{25}H_{53}O_3Si_2$ $[M+H]^+$, calc. 457.3533, found 457.3531. To confirm the relative stereochemistry between C-4 and C-5 (C-15 and C-17 in spinosyn A), compound **28** was derivatized to the corresponding acetonide as follows.[7] **(*S*)-6-((4′*S*,5′*R*,6′*R*)-6′-Allyl-2′,2′,5′-trimethyl-1′,3′-dioxan-4′-yl)hexan-3-ol.** To a solution of compound **28** (79 mg, 0.17 mmol) in tetrahydrofuran (2 mL) at 0 ºC was added tetrabutylammonium fluoride (1 M solution in tetrahydrofuran, 0.378 mL, 0.378 mmol). After 30 min stirring, the reaction mixture was warmed to room temperature and stirred for an additional 1 hr. The mixture was then poured into a saturated ammonium chloride solution (10 mL) and extracted with dichloromethane (10 mL × 3). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product, a triol, was used for the next step without further purification. To a solution of the triol obtained in the previous step (~40 mg, 0.17 mmol) in a mixture of 2,2-dimethoxypropane (1 mL) and dichloromethane (1 mL) were added several crystals of pyridinium *p*-toluenesulfonate (PPTS). The reaction mixture was stirred for 1.5 hr, then poured into a saturated sodium bicarbonate solution (10 mL), and extracted with dichloromethane (10 mL × 3). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 4/1) to afford the desired acetonide (33 mg, 0.122, 72% for 2 steps). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.78-5.69 (m, 1H, allyl 2″-H), 5.10-5.06 (m, 1H, allyl 1″-H), 5.03-5.00 (m, 1H, allyl 1″-H), 3.88 (td, *J* = 7.1, 2.3 Hz, 1H, 6′-H), 3.84-3.81 (m, 1H, 4′-H), 3.53-3.48 (m, 1H, 3-H), 2.30-2.24 (m, 1H, allyl 3″-H), 2.14-2.08 (m, 1H, allyl 3″-H), 1.53-1.26 (m, 9H, 2-H, 4-H, 5-H, 6-H, 5′-H), 1.39 (s, 3H, 2′-Me), 1.36 (s, 3H, 2′-Me), 0.91 (t, *J* = 7.5 Hz, 3H, 1-H), 0.82 (d, *J* = 6.8 Hz, 3H, 5′-Me); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 134.5, 116.8, 98.8, 73.3, 73.1, 73.0, 37.2, 36.7, 34.1, 32.7, 30.1, 30.0 (2′-Me), 21.5, 19.6 (2′-Me), 9.8, 4.5.

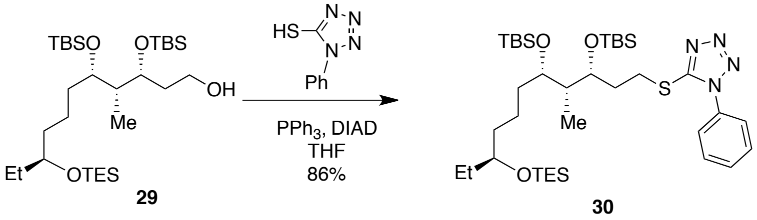


(4*R*,5*S*,6*S*,10*S*)-4,6-Bis(*tert*-Butyldimethylsilyloxy)-5-methyl-10-(triethylsilyloxy)dodec-1-ene (28-1). To a solution of alcohol **28** (3.78 g, 8.24 mmol) in anhydrous dichloromethane (50 mL) at -78 ºC was added 2,6-lutidine (2.87 mL, 24.7 mmol) followed by *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf, 2.82 mL, 12.3 mmol). After 2 hr of stirring at -78 ºC, the reaction mixture was warmed to room temperature over 2 hr and then quenched by the addition of a saturated sodium bicarbonate solution (50 mL). The reaction mixture was extracted with dichloromethane (50 mL × 3), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 50/1) to afford silyl ether **28-1** (4.11 g, 7.17 mmol, 87%). ^{1}H NMR (CDCl$_3$, 500 MHz) δ 5.81-5.73 (m, 1H, 2-H), 5.03-4.99 (m, 2H, 1-H), 3.75 (q, *J* = 5.6 Hz, 1H, 6-H or 10-H), 3.67 (q, *J* = 5.6 Hz, 1H, 6-H or 10-H), 3.54 (quint, *J* = 5.8 Hz, 1H, 4-H), 2.28-2.26 (m, 2H, 3-H), 1.62-1.18 (m, 9H, 5-H, 7-H, 8-H, 9-H, 11-H), 0.94 (t, 9H, *J* = 8 Hz, TES), 0.87 (d, *J* = 4 Hz, 18H, TBS), 0.85 (t, *J* = 7.4 Hz, 3H, 12-H), 0.84 (d, *J* = 7.5 Hz, 3H, 5-Me), 0.57 (q, *J* = 8 Hz, 6H, TES), 0.01-0.03 (m, 12H, TBS); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 135.0, 116.7, 73.5, 72.7, 72.2, 40.5, 39.7, 37.2, 35.1, 29.8, 26.0, 20.7, 18.2, 9.6, 9.3, 6.9, 5.1, -3.9, -4.47, -4.52; HRMS (CI+) *m/z* for $C_{31}H_{69}O_3Si_3$ [M+H]$^+$, calc. 573.4555, found 573.4557.

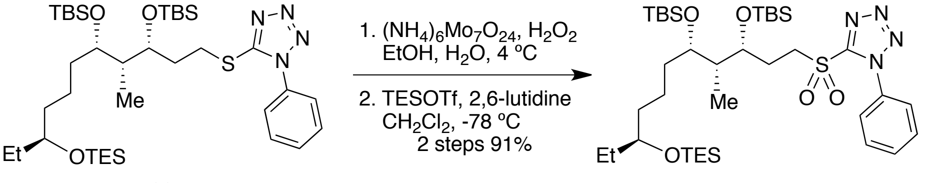


(3*R*,4*R*,5*S*,9*S*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-4-methyl-9-(triethylsilyloxy)undecan-1-ol (29). To a solution of olefin **28-1** (4.11 g, 7.17 mmol) in tetrahydrofuran (13 mL), acetone (13 mL), and pH 7 buffer (13 mL, potassium phosphate, Fisher Scientific, cat # SB109) at room temperature was added *N*-methylmorpholine oxide (NMO, 1.25 g, 10.7 mmol) followed by osmium tetroxide (0.090 g, 0.358 mmol). After stirred overnight, the reaction mixture was poured into 10% sodium thiosulfate ($Na_2S_2O_3$) solution (50 mL) and stirred for an additional 1 hr to reduce residual oxidants. Then, the mixture was extracted with ethyl acetate (50 mL × 5), washed with a brine solution (50 mL), dried over anhydrous sodium sulfate, concentrated under reduced pressure, and used without further purification. To a clear solution of the crude diol (4.6

g) in tetrahydrofuran (120 mL) and pH 7 buffer (40 mL, potassium phosphate, Fisher Scientific, cat # SB109) at room temperature was added sodium periodate (4.8 g, 22.7 mmol) in three portions at 10 min intervals. After 3 hr stirring, the reaction mixture was poured into a saturated sodium bicarbonate solution (50 mL), extracted with ethyl acetate (50 mL × 3), dried over anhydrous sodium sulfate, concentrated under reduced pressure, and the resulting crude product was used without further purification. To a solution of the crude aldehyde (4.35 g) in ethyl alcohol (70 mL) at room temperature was added sodium borohydride (0.427 g, 11.3 mmol). After 1 hr stirring, the reaction mixture was poured into a saturated ammonium chloride solution (50 mL), extracted with ethyl acetate (50 mL × 3), washed with a brine solution (50 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 19/1) to afford alcohol **29** (2.77 g, 4.80 mmol, 3 steps 67%). ^{1}H NMR (CDCl$_3$, 500 MHz) δ 3.88-3.85 (m, 1H, 5-H or 9-H), 3.83-3.78 (m, 1H, 5-H or 9-H), 3.69-3.64 (m, 2H, 1-H), 3.55 (quint, *J* = 5.8 Hz, 1H, 3-H), 1.89-1.19 (m, 11H, 2-H, 4-H, 6-H, 7-H, 8-H, 10-H), 0.94 (t, *J* = 8 Hz, 9H, TES), 0.88 (s, 9H, TBS), 0.86 (s, 9H, TBS), 0.89-0.85 (*app* m, 6H, 4-Me, 11-H), 0.58 (q, *J* = 8 Hz, 6H, TES), 0.08-0.01 (m, 12H, TBS); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 73.4, 72.8, 72.4, 59.8, 40.4, 37.0, 35.6, 35.5, 29.8, 25.9, 25.8, 21.1, 18.1, 18.0, 9.8, 9.6, 6.9, 5.2, -3.7, -4.1, -4.5; HRMS (CI+) *m/z* for $C_{30}H_{69}O_4Si_3$ [M+H]$^+$, calc. 577.4504, found 577.4483.



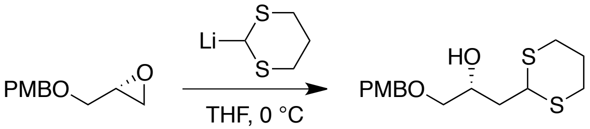
5-((3*R*,4*R*,5*S*,9*S*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-4-methyl-9-(triethylsilyloxy)undecylthio)-1-phenyl-1*H*-tetrazole (30). To a solution of alcohol **29** (0.184 g, 0.318 mmol) in anhydrous tetrahydrofuran (6 mL) at 0 °C was added 1-phenyl-1*H*-tetrazole-5-thiol (0.085 g, 0.477 mmol) followed by triphenylphosphine (PPh$_3$, 0.125 g, 0.477 mmol) and diisopropyl azodicarboxylate (DIAD, 0.094 mL, 0.477 mmol), and the resulting yellow suspension was stirred at 0 °C for 1 hr. The reaction mixture was warmed to room temperature over 1 hr, concentrated under reduced pressure, and directly subjected to flash column chromatography (hexanes/ethyl acetate = 50/1) to afford thioether **30** (0.202 g, 0.274 mmol, 86%). ^{1}H NMR (CDCl$_3$, 500 MHz) δ 7.54-7.49 (m, 5H, Ph), 3.84-3.78 (m, 2H, 9-H, 3-H), 3.56 (*app* quint, *J* = 5.8 Hz, 1H, 5-H), 3.41-3.30 (m, 2H, 1-H), 2.18-2.11 (m, 1H, 2-H), 2.00-1.93 (m, 1H, 2-H), 1.73-1.20 (m, 9H, 4-H, 6-H, 7-H, 8-H, 10-H), 0.92 (t, *J* = 8 Hz, 9H, TES), 0.88-0.83 (*app* m, 6 H, 4-Me, 11-H), 0.87 (s, 9H, TBS), 0.85 (s, 9H, TBS), 0.56 (q, *J* = 8 Hz, 6H, TES), 0.04-0.002 (m, 12H, TBS); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 154.3, 133.8, 130.0, 129.7, 123.8, 73.4, 72.5, 72.1, 40.8, 37.0, 35.5, 33.9, 29.7, 29.0, 26.0, 25.9, 21.1, 18.1, 18.0, 9.77, 9.62, 6.95, 6.56, 5.80, 5.12, -3.7, -4.1, -4.37, -4.40, -4.44; HRMS (CI+) *m/z* for $C_{37}H_{73}N_4O_3Si_3S_1$ $[M+H]^+$, calc. 737.4711, found 737.4689.



30 Fragment A (**19**)

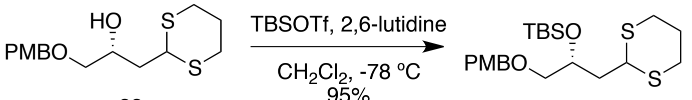
(3*S*,7*S*,8*R*,9*R*)-7,9-Bis(*tert*-butyldimethylsilyloxy)-8-methyl-11-(1-phenyl-1*H*-tetrazol-5-ylsulfonyl)undecan-3-ol (19, fragment A). To a solution of thioether **30** (2.43 g, 3.30 mmol) in ethyl alcohol (10 mL) at 0 °C was added a premixed oxidant (2 mL, ammonium molybdate/30% H_2O_2 = 2.4 g/10 mL), and the reaction was stirred at 0°C for 18 hr. Then, the reaction mixture was poured into water (30 mL), extracted with ethyl acetate (30 mL × 3), washed with a brine solution (30 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 19/1) to afford a sulfone with its triethylsilyl protecting group cleaved (2.04 g, 3.11 mmol, 95%). To a solution of the resulting alcohol (0.056 g, 0.0853 mmol) in dichloromethane (3 mL) at -78 °C was added 2,6-lutidine (0.015 mL, 0.128 mmol) followed by the addition of triethylsilyl trifluromethanesulfonate (TESOTf, 0.023 mL, 0.128 mmol). After 1 hr stirring at -78 °C, the reaction was quenched by the addition of a saturated ammonium chloride solution (1 mL). The mixture was extracted with dichloromethane (5 mL × 3), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexane/ethyl acetate = 49/1) to afford fragment A (**19**) (62.7 mg, 0.0815 mmol, 96%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70-7.67 (m, 2H, Ph), 7.60-7.56 (m, 3H, Ph), 3.88-3.85 (m, 1H, 3-H), 3.82-3.79 (m, 1H, 9-H), 3.77-3.73 (m, 2H, 1-H), 3.57 (*app* quint, *J* = 5.8 Hz, 1H, 5-H), 2.31-2.24 (m, 1H, 2-H), 2.17-2.10 (m, 1H, 2-H), 1.61-1.17 (m, 9H, 4-H, 6-H, 7-H, 8-H, 10-H), 0.93 (t, *J* = 8 Hz, 9H, TES), 0.91-0.83 (*app* m, 6H, 11-H, 4-Me), 0.89 (s, 9H, TBS), 0.83 (s, 9H, TBS), 0.57 (q, *J* = 8 Hz, 6H, TES), 0.07-0.001 (m, 12H, TBS); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 153.5, 133.1, 131.4, 129.7, 125.0, 73.3, 71.7, 71.6, 52.2, 40.7, 36.8, 35.6, 29.8, 26.4, 25.9, 25.9, 21.2, 18.1, 18.05, 9.62, 6.94, 5.12, -3.6, -4.3, -4.48, -4.51; HRMS (CI+) *m/z* for $C_{37}H_{73}N_4O_5Si_3S_1$ $[M+H]^+$, calc. 769.4610, found 769.4613.

III.2. Synthesis of Fragment B (20)



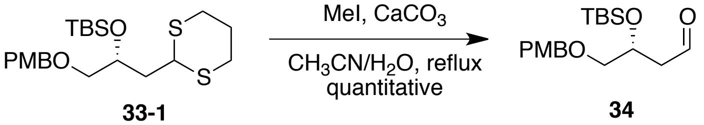
32 **33**

(*R*)-1-(1′,3′-Dithian-2-yl)-3-(4′′-methoxybenzyloxy)propan-2-ol (33). To a solution of 1,3-dithiane (1.44 g, 11.9 mmol) in tetrahydrofuran (10 mL) at -30 ºC was added *n*-butyl lithium (2.5 M solution in hexanes, 4.95 mL, 12.4 mmol), and the mixture was stirred for 2 hr while the temperature was maintained lower than -10 ºC. To this solution, after warmed to 0 ºC, was added epoxide **32** (1.66 g, 8.53 mmol). After 2 h of stirring, the reaction was quenched by adding a half-saturated ammonium chloride solution (20 mL) at room temperature, and the resulting mixture was extracted with ethyl acetate (30 mL × 3). The combined organic extracts were washed with a brine solution (50 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 5/2) to afford the secondary alcohol **33** (2.51 g, 7.98 mmol, 94%). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.27-7.23 (m, 2H, PMB), 6.90-6.86 (m, 2H, PMB), 4.48 (d, *J* = 1.7 Hz, 2H, PMB), 4.25 (dd, *J* = 9.6, 4.9 Hz, 1H, 2-H), 4.13-4.09 (m, 1H, 2′-H), 3.80 (s, 3H, PMB), 3.48 (dd, *J* = 9.6, 3.4 Hz, 1H, 3-H), 3.34 (dd, *J* = 9.6, 6.9 Hz, 1H, 3-H), 2.94-2.79 (m, 4H, 3′-H, 5′-H), 2.50 (d, *J* = 4.1 Hz, 1H, OH), 2.14-1.78 (m, 4H, 1-H, 4′-H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 159.3, 129.8, 129.3, 113.8, 73.6, 73.0, 67.1, 55.2, 43.7, 38.9, 30.3, 30.0, 25.9; HRMS (CI+) *m/z* for $C_{15}H_{22}NaO_3S_2$ [M+Na]$^+$, calc. 337.0904, found 337.0903.

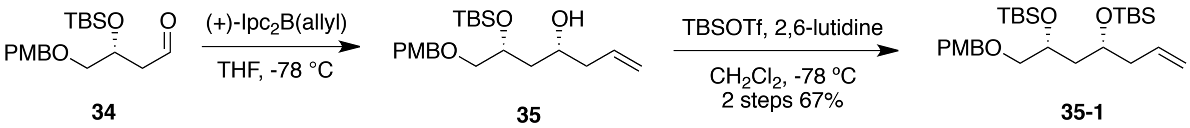


33 **33-1**

(*R*)-(1-(1′,3′-Dithian-2′-yl)-3-(4′′-methoxybenzyloxy)propan-2-yloxy)(*tert*-butyl)dimethylsilane (33-1). To a solution of the secondary alcohol **33** (75.4 mg, 0.239 mmol) and 2,6-lutidine (39 *μ*L, 0.334 mmol) at -78 ºC was added *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf, 66 *μ*L, 0.287 mmol). After 5 hr of stirring, the mixture was poured into a 1 N hydrochloric acid solution and extracted with dichloromethane (5 mL × 3). The combined organic extracts were washed with a brine solution (20 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 19/1) to afford silyl ether **33-1** (96.9 mg, 0.226 mmol, 95%). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.30-7.26 (m, 2H, PMB), 6.92-6.88 (m, 2H, PMB), 4.47 (s, 2H, PMB), 4.17-4.09 (m, 2H, 2-H, 2′-H), 3.83 (s, 3H, PMB), 3.34-3.31 (m, 2H, 3-H), 2.89-2.75 (m, 4H, 4′′-H, 6′′–H), 2.15-1.62 (m, 4H, 1-H, 5′′-H), 0.91 (s, 9H, TBS), 0.13 (s, 3H, TBS), 0.09 (s, 3H, TBS); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 159.1, 130.32, 129.2, 113.7, 74.3, 72.9, 67.9, 55.3, 43.6, 40.3, 30.5, 29.9, 26.0, 25.9, 18.1, -4.4, -4.8; HRMS (CI+) *m/z* for $C_{21}H_{37}O_3S_2Si$ [M+H]$^+$, calc. 429.1955, found 429.1948.

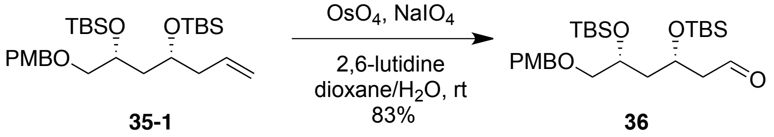


(*R*)-3-(*tert*-Butyldimethylsilyloxy)-4-(4′-methoxybenzyloxy)butanal (34). To a solution of dithiane **33-1** (41.4 mg, 96.5 μmol) in a mixture of acetonitrile (4 mL) and water (1 mL) at room temperature was added iodomethane (0.1 mL, excess) and calcium carbonate (35 mg, excess), and the reaction mixture was heated under reflux for 6 hr. Then, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (5 mL × 3). The combined organic extracts were washed with a brine solution (10 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 9/1) to afford aldehyde **34** (30 mg, 97 μmol, quantitative). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.72 (t, J = 2.7 Hz, 1H, 1-H), 7.20-7.16 (m, 2H, PMB), 6.83-6.81 (m, 2H, PMB), 4.39 (s, 2H, PMB), 4.28 (tt, J = 6.5, 5.1 Hz, 1H, 3-H), 3.75 (s, 3H, PMB), 3.41 (dd, J = 9.6, 5.1 Hz, 1H, 4-H), 3.30 (dd, J = 9.6, 6.5 Hz, 1H, 4-H), 2.58 (ddd, J = 15.9, 5.1, 2.0 Hz, 1H, 2-H), 2.50 (ddd, J = 15.9, 6.7, 2.7 Hz, 1H, 2-H), 0.8 (s, 9H, TBS), 0.0 (s, 6H, TBS); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.5, 159.2, 123.0, 129.3, 113.8, 73.7, 73.0, 67.3, 55.3, 49.0, 25.7, 18.0, -4.5, -5.0; HRMS (ESI+) *m/z* for $C_{18}H_{30}O_4S_i$ $[M+Na]^+$, calc. 361.1806, found 361.1794.

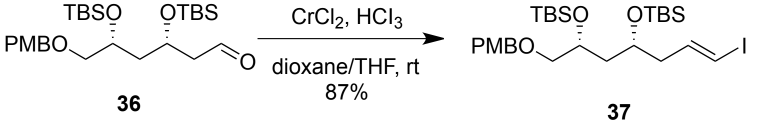


(2*R*,4*R*)-1-(4-Methoxybenzyloxy)-2,4-bis(*tert*-butyldimethylsilyloxy)hept-6-ene (35-1). To a stirred solution of (+)-Ipc_2BCl (1.74 g, 5.43 mmol) in anhydrous tetrahydrofuran (20 mL) was added 1.0 M allyl magnesium bromide (5.43 mL, 5.43 mmol) at -78 ºC. After 30 min, the resulting white suspension was warmed to room temperature and stirred for an additional 4 hr. The solution was then cooled to -78 ºC and treated dropwise with a solution of aldehyde **34** (1.42 g, 4.19 mmol) in anhydrous tetrahydrofuran (5 mL). After stirring at -78 ºC for 2 hr, the reaction was quenched by adding methanol (5 mL) followed by an alkaline hydrogen peroxide solution and stirred at room temperature for an additional 2 hr. The solution was diluted with ethyl ether (50 mL), washed with a brine solution (40 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 19/1) to afford the corresponding homoallylic alcohol **35** (1.39 g, 3.65 mmol, 87%). To a solution of the resulting alcohol **35** (1.39 g, 3.65 mmol) and 2,6-lutidine (0.55 mL, 4.75 mmol) in dichloromethane (25 mL) at -78 ºC was added *tert*-butyldimethylsilyl trifluoromethanesulfonate (1.00 mL, 4.38 mmol). After 1 hr, the reaction mixture was poured into a saturated sodium bicarbonate solution (40 mL) and extracted with dichloromethane (25 mL × 3). The combined organic extracts were washed with a brine solution (30 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexnase/ethyl acetate = 29/1) to afford compound **35-1** (1.39 g, 2.81 mmol, 77%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.26 (d, *J* = 8.7 Hz, 2H, PMB-aromatic *H*), 6.89 (d, *J* = 8.5Hz, 2H, PMB-aromatic *H*), 5.91-5.77 (m, 1H, 6-H), 5.05 (*app* d, *J* = 12 Hz, 2H, 7-H), 4.46 (s, 2H, PMB-CH_2), 3.97-3.83 (m, 2H, 2-H, 4-H), 3.80 (s, 3H, PMB-Me), 3.38 (d, *J* = 4.9 Hz, 2H, 1-H), 2.34-2.12 (m, 2H, 5-H), 1.78-1.58 (m, 2H, 3-H), 0.91 (s, 9H, TBS), 0.90 (s, 9H, TBS), 0.08 (s, 3H, TBS), 0.07 (s, 3H, TBS), 0.05 (s, 3H, TBS), 0.04 (s, 3H, TBS); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.0, 135.1, 130.5, 129.2, 116.9, 113.6, 74.6, 72.8, 69.1, 68.8, 55.2, 42.1, 41.6, 25.9, 25.9, 18.1, 18.0, -4.23, -4.41, -4.54, -4.82; HRMS (CI+) *m/z* for $C_{27}H_{49}O_4Si_2$ $[M-H]^+$, calc. 493.3169, found 493.3149.

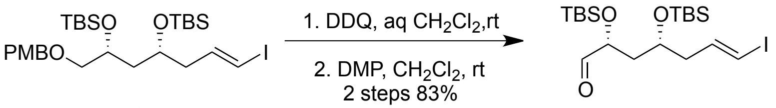
To confirm the relative stereochemistry between C-2 ad C-4 (C-9 and C-11 in spinosyn A), compound **35-1** was derivatized to give the corresponding acetonide as follows.[7] **(4*R*,6*R*)-4-Allyl-6-((*p*-methoxybenzyloxy)methyl)-2,2-dimethyl-1,3-dioxane.** To a solution of **35-1**, (79 mg, 0.160 mmol) in tetrahydrofuran (2 mL) at 0 ºC was added tetrabutylammonium fluoride (1 M solution in tetrahydrofuran, 0.320 mL, 0.320 mmol). After 30 min stirring, the reaction mixture was warmed to room temperature and stirred for an additional 1 hr. The mixture was then poured into a saturated ammonium chloride solution (10 mL) and extracted with dichloromethane (10 mL × 3). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure. To a solution of the resulting crude diol (25.8 mg, 96.9 μmol) in dichloromethane (5 mL) was added 2,2-dimethoxypropane (60 μL, 485 μmol) and a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) at room temperature. After 2 hr, the reaction mixture was concentrated under reduced pressure and subjected to flash column chromatography (hexanes/ethyl acetate = 9/1) to afford the desired acetonide product. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.24 (*app* d, *J* = 8.7 Hz, 2H, PMB), 6.85 (*app* d, *J* = 8.7 Hz, 2H, PMB), 5.84-5.70 (m, 1H, 2″-H), 5.10-5.01 (m, 2H, 1″-H), 4.48 (q, *J* = 11.8 Hz, 2H, PMB), 4.08-4.00 (m, 1H, 4-H, or 6-H), 3.92-3.83 (m, 1H, 4-H or 6-H), 3.78 (s, 3H, OMe of PMB), 3.45 (dd, *J* = 10, 5.9 Hz, 1H, 1′-H), 3.31 (dd, *J* = 10, 4.9 Hz, 1H, 1′-H), 2.33-2.24 (m, 1H, 3″-H), 2.14-2.08 (m, 1H, 3″-H), 1.52 (dt, *J* = 12.8, 2.6 Hz, 1H, 5-H), 1.43 (s, 3H, 2-Me), 1.39 (s, 3H, 2-Me), 1.16 (*app* q, *J* = 12 Hz, 1H, 5-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.2, 134.1, 130.3, 129.4, 117.1, 113.7, 98.6, 73.3, 73.1, 68.5, 68.3, 55.3, 40.8, 33.3, 30.1 (2-Me), 19.8 (2-Me).



(3*S*,5*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-6-(4-methoxybenzyloxy)hexanal (36). To a solution of olefin **35-1** (2.45 g, 6.63 mmol) in dioxane/H_2O = 3/1 (60 mL) was added 2,6-lutidine (1.17 mL, 10.0 mmol), osmium tetroxide (40 mg, 0.16 mmol), and sodium periodate (4.28 g, 20.0 mmol) in sequence at room temperature. After 5 hr of stirring, the solution was mixed with H_2O (50 mL) and dichloromethane (100 mL). The separated aqueous layer was extracted with dichloromethane (50 mL × 3) and the combined organic extracts were washed with a brine solution (100 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 19/1) to afford aldehyde **36** (2.73 g, 5.50 mmol, 83%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.77 (dd, *J* = 3.2, 2.1 Hz, 1H, 1-H), 7.22 (d, *J* = 8.8 Hz, 2H, PMB-aromatic *H*), 6.85 (d, *J* = 8.6 Hz, 2H, PMB-aromatic *H*), 4.42 (s, 2H, PMB-C*H*$_2$), 4.37-4.32 (m, 1H, 3-H), 3.87-3.82 (m, 1H, 5-H), 3.79 (s, 3H, PMB-Me), 3.37-3.30 (m, 2H, 6-H), 2.58-2.43 (m, 2H, 2-H), 1.75 (t, *J* = 6.8 Hz, 2H, 4-H), 0.85 (s, 9H, TBS), 0.84 (s, 9H, TBS), 0.03-0.02 (m, 12H, TBS); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.2, 159.1, 130.25, 129.2, 113.7, 74.3, 72.9, 68.7, 65.5, 55.2, 50.4, 42.7, 25.8, 25.7, 18.1, 17.9, -4.23, -4.41, -4.82, -4.87; HRMS (CI+) *m/z* for $C_{26}H_{47}O_5Si_2$ [M-H]$^+$, calc. 495.2962, found 495.2970.



(2*R*,4*R*,*E*)-1-((4-Methoxybenzyloxy)methyl)-2,4-bis(*tert*-butyldimethylsilyloxy)-7-iodohept-6-ene (37). To a stirred suspension of chromium(II) chloride (2.89 g, 23.6 mmol) in anhydrous dioxane/tetrahydrofuran = 6/1 (60 mL) was added a solution of aldehyde **36** (2.34 g, 4.71 mmol) and iodoform (3.70 g, 9.42 mmol) in anhydrous dioxane/tetrahydrofuran = 6/1 (5 mL) at 0 °C. The solution was protected from light and allowed to warm to room temperature over 20 hr. The resulting green solution was quenched with H_2O (50 mL), and the separated aqueous layer was extracted with ethyl acetate (50 mL × 3). The combined organic extracts were washed with a saturated sodium thiosulfate solution (100 mL) and a brine solution (100 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 97/3) to afford vinyl iodide **37** (2.53 g, 4.08 mmol, 87%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.23 (d, *J* = 8.7 Hz, 2H, PMB-aromatic *H*), 6.86 (d, *J* = 8.7 Hz, 2H, PMB-aromatic *H*), 6.53-6.43 (m, 1H, 6-H), 5.99 (d, *J* = 14 Hz, 1H, 7-H), 4.42 (s, 2H, PMB-C*H*$_2$), 3.87-3.81 (m, 2H, 2-H, 4-H), 3.79 (s, 3H, PMB-Me), 3.37-3.27 (m, 2H, 1-H), 2.28-2.20 (m, 1H, 5-H), 2.14-2.04 (m, 1H, 5-H), 1.72-1.54 (m, 2H, 3-H), 0.86 (s, 9H, TBS), 0.85 (s, 9H, TBS), 0.03-0.00 (m, 12H, TBS); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.0, 143.2, 130.4, 129.2, 113.7, 74.5, 72.9, 68.9, 68.0, 55.3, 43.4, 42.3, 25.9, 25.8, 18.1, 18.0, -4.20, -4.48, -4.56, -4.79; HRMS (CI+) *m/z* for $C_{27}H_{48}IO_4Si_2$ [M-H]$^+$, calc. 619.2136, found 619.2136.



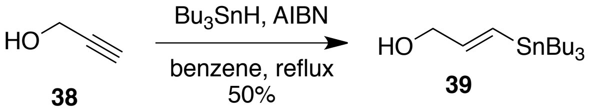
37

Fragment B (**20**)

(2*R*,4*R*,*E*)-2,4-Bis(*tert*-butyldimethylsilyloxy)-7-iodohept-6-enal (20, fragment B**).** To a solution of PMB ether **37** (3.10 g, 4.99 mmol) in CH_2Cl_2/H_2O = 10/1 (150 mL) was added 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 1.36 g, 5.99 mmol) at room temperature. After 1 hr of stirring, the solution was treated with a saturated sodium bicarbonate solution (100 mL). The separated aqueous layer was extracted with dichloromethane (50 mL × 3) and the combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 19/1) to afford a primary alcohol (2.35 g, 4.69 mmol, 94%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.46 (dt, *J* = 14.4, 7.5 Hz, 1H, 6-H), 6.03 (dt, *J* = 14.4, 1.2 Hz, 1H, 7-H), 3.89-3.76 (m, 2H, 2-H, 4-H), 3.58-3.51 (m, 1H, 1-H), 3.48-3.40 (m, 1H, 1-H), 2.30-2.12 (m, 2H, 3-H), 1.73-1.58 (m, 2H, 5-H), 0.87 (s, 9H, TBS), 0.86 (s, 9H, TBS), 0.06-0.03 (m, 12H, TBS); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 142.6, 77.0, 69.8, 68.2, 66.1, 43.7, 41.3, 25.79, 25.77, 18.0, 17.9, -4.36, -4.55, -4.64; HRMS (CI+) *m/z* for $C_{19}H_{42}IO_3Si_2$ $[M+H]^+$, calc. 501.1717, found 501.1713.

To a solution of the resulting alcohol (1.00 g, 2.00 mmol) in dichloromethane (25 mL) was added Dess-Martin periodinane (890 mg, 2.10 mmol) at 0 ºC. After stirring at room temperature for 1 hr, the solution was diluted with ethyl ether (50 mL). The resulting mixture was washed with a saturated sodium bicarbonate solution (50 mL), a saturated sodium thisulfate solution (50 mL), and a brine solution (50 mL). The dichloromethane solution was then dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes only to remove iodoform → hexanes/ethyl acetate = 29/1) to afford fragment B (**20**) (891 mg, 1.79 mmol, 89%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.57 (d, *J* = 1.4 Hz, 1H, 1-H), 6.46 (dt, *J* = 14.4, 7.8 Hz, 1H, 6-H), 6.05 (dt, *J* = 14.4, 1.3 Hz, 1H, 7-H), 4.05 (td, *J* = 6.1, 1.4 Hz, 1H, 2-H), 3.98-3.93 (m, 1H, 4-H), 2.29-2.16 (m, 2H, 5-H), 1.83-1.75 (m, 2H, 3-H), 0.90 (s, 9H, TBS), 0.85 (s, 9H, TBS), 0.07-0.04 (m, 12H, TBS); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 203.4, 142.3, 77.1, 74.7, 66.8, 43.5, 40.2, 25.8, 25.7, 18.1, 17.9, -4.36, -4.51, -4.64, -4.95; HRMS (CI+) *m/z* for $C_{19}H_{40}IO_3Si_2$ $[M+H]^+$, calc. 499.1561, found 499.1572.

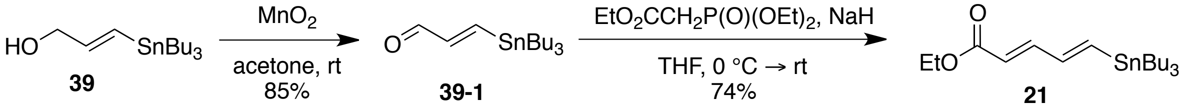
III.3. Synthesis of Fragment C (21)



38

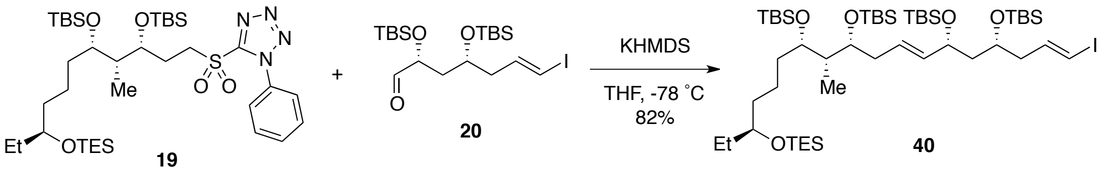
39

(*E*)-3-(Tributylstannyl)prop-2-en-1-ol (39). The stereoselective tin addition to propargylic alcohol was conducted following a known procedure.[8] Propargylic alcohol (**38**, 1.50 g, 26.7 mmol) was mixed with tributyltin hydride (9.21 mL, 34.7 mmol), to which was added 2,2′-azobis(2-methylpropionitrile) (AIBN, 43.8 mg, 0.267 mmol) at room temperature. The reaction was gradually heated to 80 ºC over 1 hr and was allowed to continue overnight under reflux. After completion of the reaction was confirmed by TLC analysis, the crude product was directly subjected to flash column chromatography (hexanes) to afford vinyl tin **39** (4.55 g, 13.1 mmol, 50%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.24-6.11 (m, 2H, 1-H, 2-H), 4.15 (*br* d, *J* = 3.1 Hz, 2H, 3-H), 1.58-1.43 (m, 6H, *Bu₃*Sn), 1.40-1.1.26 (m, 6H, *Bu₃*Sn), 0.98-0.80 (m, 15H, *Bu₃*Sn); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.0, 128.3, 66.4, 29.1, 27.3, 13.7, 9.4.

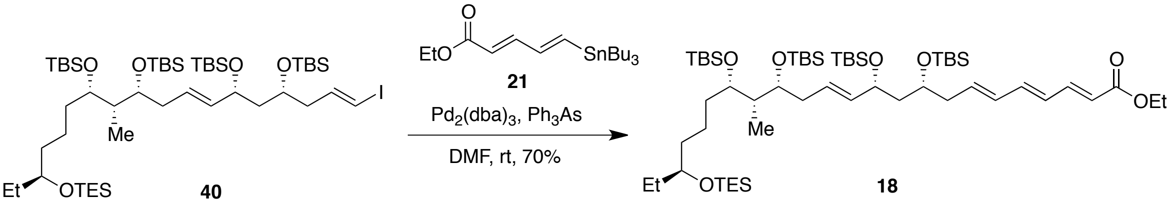


Ethyl (2*E*,4*Z*)-5-(tributylstannyl)penta-2,4-dienoate (21). To a solution of alcohol **39** (1.54 g, 4.43 mmol) in acetone (50 mL) at room temperature was added activated manganese oxide (3.85 g, 44.3 mmol). After stirring overnight, the reaction mixture was filtered over a pad of Celite to remove manganese oxide. The filtrate was concentrated under reduced pressure. The crude residue was briefly purified by flash column chromatography (hexanes only) to afford aldehyde **39-1** (1.29 g, 3.75 mmol, 85%). The resulting aldehyde was immediately used for the next step, a Horner-Wadsworth-Emmons reaction. To a solution of triethyl phosphonoacetate (1.08 mL, 5.45 mmol) in tetrahydrofuran (15 mL) at 0 ºC was slowly added sodium hydride (60% in mineral oil, 327 mg, 5.45 mmol). To the resulting suspension was added aldehyde **39-1** (1.26 g, 3.64 mmol). After stirring at 0 ºC for 4 hr, the reaction was quenched by the addition of a saturated ammonium chloride solution (15 mL), and the mixture was extracted with ethyl acetate (20 mL × 3). The combined organic extracts were washed with a brine solution (30 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 39/1) to afford fragment C for the Stille coupling (**21**, 1.10 g, 2.65 mmol, 74%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.18 (dd, *J* = 15.4, 10.2 Hz, 1H, 3-H), 6.81 (d, *J* = 18.8 Hz, 1H, 1-H), 6.64 (dd, *J* = 18.8, 10.2 Hz, 1H, 2-H), 5.79 (d, *J* = 15.4 Hz, 1H, 4-H), 4.20 (q, *J* = 7.1 Hz, 1H, CH_3CH_2OCO), 1.56-1.41 (m, 6H, *Bu₃*Sn), 1.30-1.26 (m, 9H, *Bu₃*Sn), 0.95-0.78 (m, 15H, *Bu₃*Sn, CH_3CH_2OCO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.4, 147.2, 146.3, 144.2, 119.9, 60.2, 29.0, 27.2, 13.7, 9.6; HRMS (CI+) *m/z* for $C_{19}H_{37}O_2Sn$ $[M+H]^+$, calc. 417.1816, found 417.1821.

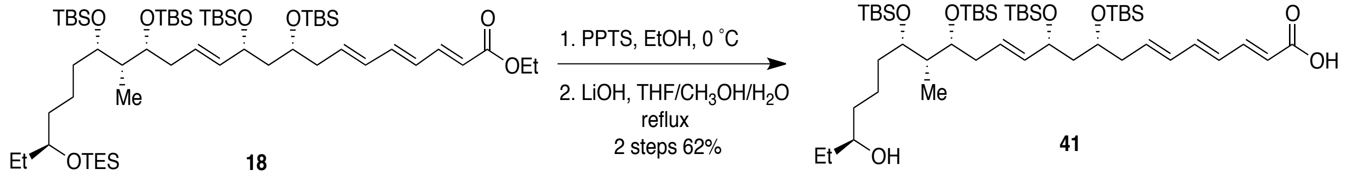
III.4. Completion of the Synthesis of the Presumed Post-PKS Precursor (11)



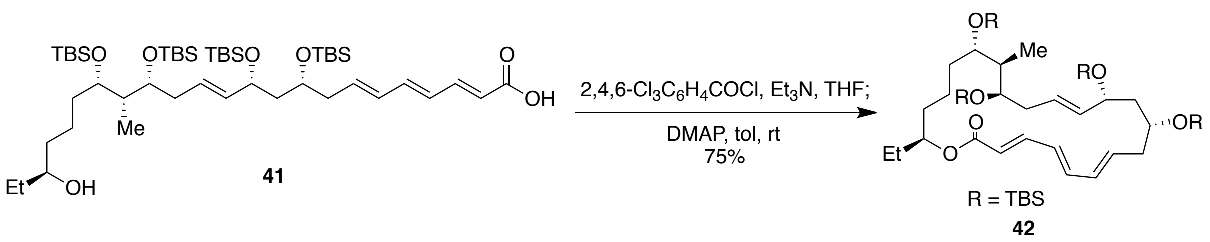
(1*E*,4*R*,6*R*,7*E*,10*R*,11*R*,12*S*,16*S*)-4,6,10,12-Tetrakis(*tert*-butyldimethylsilyloxy)-11-methyl-16-(triethylsilyloxy)-1-iodooctadeca-1,7-diene (40). To a solution of fragment A (**19**) (0.594 g, 0.772 mmol) in tetrahydrofuran (5 mL) at -78 °C was added potassium hexamethyldisilazide (KHMDS, 0.5 M in toluene, 1.85 mL, 0.927 mmol) dropwise over 10 min, and the mixture was stirred at -78 °C for 1 hr, at which time fragment B (**20**) (0.413 g, 0.849 mmol) was added to the resulting yellow solution at -78 °C. After stirring for 4 hr, the temperature was slowly raised to room temperature over 1 hr, and the reaction mixture was poured into a saturated sodium bicarbonate solution (10 mL). The resulting mixture was extracted with ethyl acetate (20 mL × 3), washed with a brine solution (20 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was subjected to short column chromatography (hexanes only) to afford the vinyl iodide **40** (663 mg, 0.636 mmol, 82%). ^{1}H NMR (CDCl$_3$, 500 MHz) δ 6.49 (ddd, *J* = 14.4, 8, 7 Hz, 1H, 2-H), 5.99 (d, *J* = 14.4 Hz, 1H, 1-H), 5.51 (dt, *J* = 15.5, 6.6 Hz, 1H, 8-H), 5.38 (dd, *J* = 15.5, 6.9 Hz, 1H, 7-H), 4.08 (*br* q, *J* = 12.6, 6.9 Hz, 1H, 6-H), 3.80 (*br* quint, *J* = 17.0, 11.8, 6.6 Hz, 1H, 4-H), 3.74 (q, *J* = 5.5 Hz, 1H, 10-H), 3.66 (br q, *J* = 10.5, 5.5 Hz, 1H, 11-H), 3.56 (quint, *J* = 5.8 Hz, 1H, 16-H), 2.30-2.22 (m, 3H, 3-H, 9-H), 2.08-2.14 (m, 1H, 3-H), 1.70 (ddd, *J* = 13.5, 7.7, 5.5 Hz, 1H, 5-H), 1.59-1.20 (m, 10H, 5-H, 11-H, 13-H, 14-H, 15-H, 17-H), 0.94 (t, *J* = 7.8 Hz, 9H, TES), 0.87-0.83 (m, 42H, TBS, 11-Me, 18-H), 0.57 (q, *J* = 7.8 Hz, 6H, TES), 0.04-(-0.01) (m, 24H, TBS); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 143.3, 135.5, 126.8, 76.4, 73.3, 73.0, 72.2, 70.8, 68.2, 46.0, 43.5, 41.3, 38.0, 37.3, 35.5, 29.5, 26.0, 25.9, 25.8, 21.0, 18.2, 18.14, 18.11, 18.0, 9.7, 9.5, 7.0, 5.1, -3.7, -3.8, -3.9, -4.3, -4.35, -4.4, -4.5, -4.7; HRMS (CI+) *m/z* for $C_{49}H_{105}O_5Si_5I$ [M+1]$^+$, calc. 1041.5926, found 1041.5837.



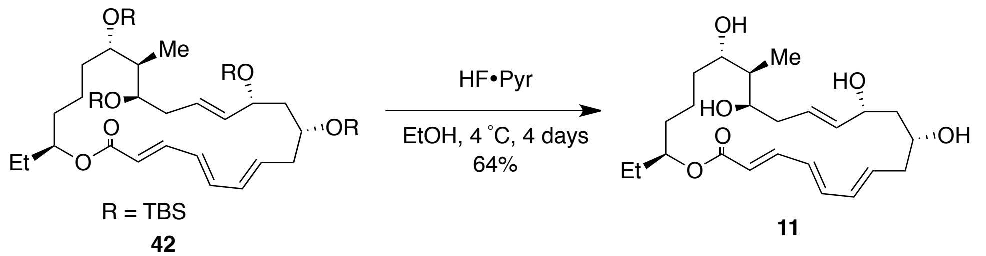
Ethyl (2*E*,4*E*,6*E*,9*R*,11*R*,12*E*,15*R*,16*R*,17*S*,21*S*)-9,11,15,17-tetrakis(*tert*-butyldimethylsilyloxy)-16-methyl-21-(triethylsilyloxy)tricosa-2,4,6,12-tetraenoate (18). To a solution of vinyl iodide **40** (485 mg, 0.466 mmol) and vinyl stannane **21** (165 mg, 1.31 mmol) in dimethylformamide (5 mL) at room temperature was added tris(dibenzylideneacetone)dipalladium(0) (21 mg, 0.023 mmol) and triphenylarsine (19 mg, 0.060 mmol). After 3 hr of stirring, the reaction mixture was concentrated under reduced pressure and diluted with water (10 mL). The suspension mixture was extracted with ethyl acetate (10 mL × 3). Then, the combined organic extracts were washed with a brine solution (20 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 49/1) to afford *(E)*-triene **18** (339 mg, 0.326 mmol, 70%) as a single product. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 7.27 (dd, *J* = 15.1, 11.3 Hz, 1H, 3-H), 6.50 (dd, *J* = 14.8, 10.5 Hz, 1H, 5-H), 6.19 (dd, *J* = 14.8, 11.3 Hz, 1H, 4-H), 6.12 (dd, *J* = 15.3, 10.5 Hz, 1H, 6-H), 5.88 (dt, *J* = 15.3, 7.5 Hz, 1H, 7-H), 5.82 (d, *J* = 15.1 Hz, 1H, 2-H), 5.51 (dt, *J* = 15.5, 7.2 Hz, 1H, 13-H), 5.38 (dd, *J* = 15.5, 6.8 Hz, 1H, 12-H), 4.18 (q, *J* = 7.2 Hz, 2H, $\mathrm{CH_3C}H_2\mathrm{OC(O)}$), 4.11 (*br* q, *J* = 12.9, 6.8 Hz, 1H, 11-H), 3.80 (quint, *J* = 5.9 Hz, 1H, 9-H), 3.74 (q, *J* = 5.5 Hz, 1H, 15-H), 3.66 (*br* q, *J* = 10.5, 5.5 Hz, 1H, 17-H), 3.56 (quint, *J* = 5.8 Hz, 1H, 21-H), 2.39-2.34 (m, 1H, 8-H), 2.24-2.18 (m, 3H, 14-H, 8-H), 1.70 (ddd, *J* = 13.7, 7.5, 5.9 Hz, 1H, 10-H), 1.61-1.35 (m, 10H, 10-H, 16-H, 18-H, 19-H, 20-H, 22-H), 1.27 (t, *J* = 7.2 Hz, 3H, $\mathrm{C}H_3\mathrm{CH_2OC(O)}$), 0.94 (t, *J* = 7.9 Hz, 9H, TES), 0.87-0.83 (m, 42H, TBS, 16-Me, 23-H), 0.57 (q, *J* = 7.9 Hz, 6H, TES), -0.01-0.04 (m, 24H, TBS); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 167.2, 144.7, 140.9, 136.6, 135.6, 132.0, 128.1, 126.8, 120.3, 73.3, 73.0, 72.3, 70.8, 69.0, 60.2, 46.2, 41.3, 40.8, 38.0, 37.3, 35.5, 29.5, 26.0, 25.9, 25.8, 21.0, 18.2, 18.1, 18.0, 14.3, 9.65, 9.51, 6.97, 5.13, -3.74, -3.80, -3.93, -4.27, -4.32, -4.37, -4.53, -4.73; HRMS (CI−) *m/z* for $C_{56}H_{114}O_7Si_5$ [M]$^-$, calc. 1038.7411, found 1038.7416.



(2*E*,4*E*,6*E*,9*R*,11*R*,12*E*,15*R*,16*R*,17*S*,21*S*)-9,11,15,17-Tetrakis(*tert*-butyldimethylsilyloxy)-21-hydroxy-16-methyl-tricosa-2,4,6,12-tetraenoic acid (41). To a solution of triene **18** (101 mg, 0.0971 mmol) in ethanol (2 mL) at 0 ºC was added pyridinium *p*-toluenesulfonate (PPTS, 2.4 mg, 0.0097 mmol). After 2 hr stirring at 0 ºC, the reaction mixture was poured into a brine solution (5 mL), extracted with ethyl acetate (10 mL × 3), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 19/1) to afford the corresponding secondary alcohol (70.2 mg, 0.0758 mmol, 78%). ^{1}H NMR (CDCl$_3$, 500 MHz) δ 7.28 (dd, *J* = 15.2, 11.4 Hz, 1H, 3-H), 6.51 (dd, *J* = 14.8, 10.7 Hz, 1H, 5-H), 6.19 (dd, *J* = 14.8, 11.4 Hz, 1H, 4-H), 6.12 (dd, *J* = 15.2, 10.7 Hz, 1H, 6-H), 5.89 (dt, *J* = 15.2, 7.6 Hz, 1H, 7-H), 5.82 (d, *J* = 15.2 Hz, 1H, 2-H), 5.54 (dt, *J* = 15.5, 7.1 Hz, 1H, 13-H), 5.40 (dd, *J* = 15.5, 6.9 Hz, 1H, 12-H), 4.18 (q, *J* = 7.2 Hz, 2H, $\mathrm{CH_3C}H_2\mathrm{OC(O)}$), 4.12 (*br* q, *J* = 13.1, 6.5 Hz, 1H, 11-H), 3.80 (*br* quint, *J* = 17.5, 11.8, 6.2 Hz, 1H, 9-H), 3.73 (*app* q, *J* = 5.5 Hz, 1H, 15-H), 3.67 (*app* q, *J* = 5.4 Hz, 1H, 17-H), 3.48 (m, 1H, 21-H), 2.39-2.34 (m, 1H, 8-H), 2.24-2.18 (m, 3H, 8-H, 14-H), 1.70 (ddd, *J* = 13.5, 7.2, 6.2 Hz, 1H, 10-H), 1.61-1.35 (m, 11H, 10-H, 16-H, 18-H, 19-H, 20-H, 22-H), 1.27 (t, *J* = 7.2 Hz, 3H, $\mathrm{C}\mathit{H}_3\mathrm{CH}_2\mathrm{OC(O)}$), 0.94 (t, *J* = 7.5 Hz, 3H, 23-H), 0.83-0.87 (m, 39H, TBS, 16-Me), 0.04-(-0.01) (m, 24H, TBS); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.2, 144.7, 140.9, 136.4, 135.7, 132.1, 128.2, 126.8, 120.3, 73.0, 72.6, 72.2, 70.8, 69.0, 60.2, 46.2, 40.8, 40.7, 37.7, 37.4, 35.1, 30.1, 29.7, 25.9, 25.7, 21.3, 18.2, 18.16, 18.13, 18.0, 14.3, 9.9, 9.3, -3.77, -3.82, -3.99, -4.28, -4.38, -4.59, -4.71; HRMS (CI–) *m/z* for $C_{50}H_{100}O_7Si_4$ $[M]^-$, calc. 924.6546, found 924.6544. To a solution of the alcohol obtained from the previous step (176 mg, 0.190 mmol) in tetrahydrofuran (4 mL) and methanol (4 mL) at room temperature was added 0.5 N lithium hydroxide solution (4 mL), and the mixture was stirred under reflux for 3 hr. The volatile solvents were then evaporated under reduced pressure and the pH of the aqueous solution was adjusted to around 6. The mixture was extracted with dichloromethane (10 mL × 3), washed with a brine solution (10 mL), dried over magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 4/1 to 2/1) to afford *seco*-acid **41** (135 mg, 0.150 mmol, 79%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36 (dd, *J* = 15.1, 11.3 Hz, 1H, 3-H), 6.55 (dd, *J* = 14.8, 10.5 Hz, 1H, 5-H), 6.22 (dd, *J* = 14.8, 11.3 Hz, 1H, 4-H), 6.14 (dd, *J* = 15.1, 10.5 Hz, 1H, 6-H), 5.94 (dt, *J* = 15.1, 7.4 Hz, 1H, 7-H), 5.82 (d, *J* = 15.1 Hz, 1H, 2-H), 5.54 (dt, *J* = 15.5, 7.2 Hz, 1H, 13-H), 5.39 (dd, *J* = 15.5, 6.8 Hz, 1H, 12-H), 4.12 (*br* q, *J* = 13.4, 6.4 Hz, 1H, 11-H), 3.81 (*br* quint, *J* = 17.5, 11.7, 6.1 Hz, 1H, 9-H), 3.73 (q, *J* = 5.5 Hz, 1H, 15-H), 3.67 (*br* q, *J* = 10.5, 5.5 Hz, 1H, 17-H), 3.51-3.45 (m, 1H, 21-H), 2.39-2.34 (m, 1H, 8-H), 2.24-2.18 (m, 3H, 8-H, 14-H), 1.70 (ddd, *J* = 13.5, 7.2, 6.4 Hz, 1H, 10-H), 1.61-1.18 (m, 10H, 10-H, 16-H, 18-H, 19-H, 20-H, 22-H), 0.92 (t, *J* = 7.5 Hz, 3H, 23-H), 0.87-0.83 (m, 36H, TBS), 0.83 (d, *J* = 6.9 Hz, 3H, 16-Me) 0.04-(-0.01) (m, 24H, TBS); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.3, 147.0, 142.0, 137.4, 135.6, 132.0, 127.9, 126.9, 119.1, 73.1, 72.6, 72.2, 70.9, 69.0, 46.1, 40.8, 40.6, 37.7, 37.4, 35.1, 30.1, 25.95, 25.93, 25.87, 21.3, 18.2, 18.16, 18.13, 18.0, 9.9, 9.3, -3.77, -3.82, -3.99, -4.28, -4.370, -4.378 -4.59, -4.71; HRMS (CI–) *m/z* for $C_{48}H_{96}O_7Si_4$ $[M]^-$, calc. 896.6233, found 896.6230.



9,11,15,17-Tetrakis(*tert*-butyldimethylsilyloxy)-macrolactone (42). A solution of triethylamine (0.04 M in tetrahydrofuran, 7.5 mL, 0.30 mmol) was mixed with *seco*-acid **41** (135 mg, 0.150 mmol). To that mixture at room temperature was added 2,4,6-trichlorobenzoyl chloride solution (0.04 M in tetrahydrofuran, 4.1 mL, 0.165 mmol). The reaction continued with stirring at room temperature for 1.5 hr, at which time the mixture was filtered over a pad of Celite, and the filtrate was concentrated under reduced pressure to afford a crude mixed anhydride. To a solution of *N*,*N*-dimethylaminopyridine (DMAP, 36.7 mg, 0.300 mmol) was added a solution of the obtained mixed anhydride in toluene (0.01 M, 15 mL) using a syringe pump over 2 hr, after which the syringe was rinsed with an additional 1 mL of toluene. After stirring for an additional 1 hr, the reaction mixture was poured into a saturated sodium bicarbonate solution (15 mL), extracted with ethyl acetate (15 mL × 3), washed with a brine solution (15 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude residue was subjected to flash column chromatography (hexanes/ethyl acetate = 49/1) to afford macrolactone **42** (99.2 mg, 0.113 mmol) in 75% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.24 (dd, *J* = 15.3, 11.3 Hz, 1H, 3-H), 6.47 (dd, *J* = 14.8, 10.7 Hz, 1H, 5-H), 6.21 (dd, *J* = 14.8, 11.3 Hz, 1H, 4-H), 6.11 (dd, *J* = 15.3, 10.7 Hz, 1H, 6-H), 5.79 (d, *J* = 15.3 Hz, 1H, 2-H), 5.80-5.75 (m, 1H, 7-H), 5.38 (dt, *J* = 15.5, 7.2 Hz, 1H, 13-H), 5.27 (dd, *J* = 15.5, 6.8 Hz, 1H, 12-H), 4.88-4.83 (m, 1H, 21-H), 4.01 (*br* q, *J* = 13.5, 6.8 Hz, 1H, 11-H), 3.75 (*br* ddd, *J* = 14.4, 8.9, 5.1 Hz, 1H, 9-H), 3.67 (*br* q, *J* = 9.0, 5.5 Hz, 1H, 15-H), 3.57 (*br* q, *J* = 10.2, 5.5 Hz, 1H, 17-H), 2.46-2.42 (m, 1H, 8-H), 2.24-2.18 (m, 2H, 14-H, 8-H), 2.12-2.08 (m, 1H, 14-H), 1.69-1.17 (m, 11H, 10-H, 16-H, 18-H, 19-H, 20-H, 22-H), 0.91 (t, *J* = 7.5 Hz, 3H, 23-H), 0.874 (s, 9H, TBS), 0.867 (s, 9H, TBS), 0.85 (s, 9H, TBS), 0.83 (s, 9H, TBS), 0.74 (d, 3H, *J* = 6.9 Hz, 16-Me) 0.04-(-0.01) (m, 24H, TBS); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.0, 144.8, 140.9, 136.0, 135.2, 132.0, 128.0, 127.1, 120.8, 75.2, 73.2, 72.1, 71.1, 69.1, 46.6, 42.4, 42.1, 38.4, 34.4, 33.4, 29.7, 27.8, 26.04, 25.99, 25.91, 25.8, 21.1, 18.18, 18.14, 18.08, 18.02, 10.2, 9.9, -3.47, -3.87, -3.97, -4.33, -4.41, -4.53 -4.62; HRMS (CI−) *m/z* for $C_{48}H_{94}O_6Si_4$ $[M]^-$, calc. 878.6128, found 878.6128.



Monomacrolactone (11). To a solution of protected macrolactone **42** (31.2 mg, 35.4 μmol) in ethanol (3 mL) was added hydrogen fluoride pyridine complex (0.3 mL) at 0 ºC. The reaction was stirred for 4 days while the temperature was maintained at 4 ºC. After completion of the reaction was confirmed by TLC analysis, the reaction was quenched by carefully addition of a saturated sodium bicarbonate solution at 0 °C. The mixture was then extracted with chloroform (20 mL × 3), and the combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure.

The crude residue was subjected to flash column chromatography ($CH_2Cl_2/CH_3OH = 93/7$) to afford macrolactone **11** (9.6 mg, 22 μmol) in 64% yield. ^{1}H NMR (DMSO-d$_6$, 500 MHz) δ 7.25 (dd, *J* = 15.2, 11.3 Hz, 1H, 3-H), 6.70 (dd, *J* = 14.9, 10.9 Hz, 1H, 5-H), 6.35 (dd, *J* = 14.9, 11.3 Hz, 1H, 4-H), 6.18 (dd, *J* = 15.2, 10.9 Hz, 1H, 6-H), 5.89 (ddd, *J* = 15.2, 10.3, 5.4 Hz, 1H, 7-H), 5.85 (d, *J* = 15.2 Hz, 1H, 2-H), 5.29 (*app* dd, *J* = 15.4, 7.2 Hz, 1H, 12-H), 5.18 (ddd, *J* = 15.4, 7.6, 5.9 Hz, 1H, 13-H), 4.75 (quint, *J* = 6.2 Hz, 1H, 21-H), 4.64 (*br* s, 1H, O*H*), 4.51 (*br* s, 1H, O*H*), 4.38 (*br* m, 2H, O*H*), 3.81-3.74 (m, 1H, 11-H), 3.72-3.66 (m, 1H, 9-H), 3.52-3.48 (m, 1H, 15-H), 3.47-3.43 (m, 1H, 17-H), 2.52-2.50 (m, 1H, 8-H), 2.08-1.86 (m, 3H, 8-H, 14-H), 1.60-1.45 (m, 5H, 10-H, 19-H, 22-H), 1.40-1.21 (m, 5H, 10-H, 18-H, 20-H), 1.19-1.54 (m, 1H, 16-H), 0.84 (t, *J* = 7.3 Hz, 3H, 23-H), 0.67 (d, *J* = 7.1 Hz, 3H, 16-Me); ^{13}C NMR (DMSO-d$_6$, 125 MHz) δ 165.9, 144.7, 141.5, 137.0, 136.2, 131.7, 127.8, 126.0, 120.2, 74.7, 74.4, 73.3, 69.4, 66.9, 54.9, 45.8, 42.8, 38.4, 33.8, 32.8, 27.3, 21.3, 9.72, 6.06, ; HRMS (CI−) *m/z* for $C_{24}H_{38}O_6$ [M]$^-$, calc. 422.2668, found 422.2664.

IV. Evaluation of the Effect of SpnF on the Transformation From Compound 12 to Compound 16

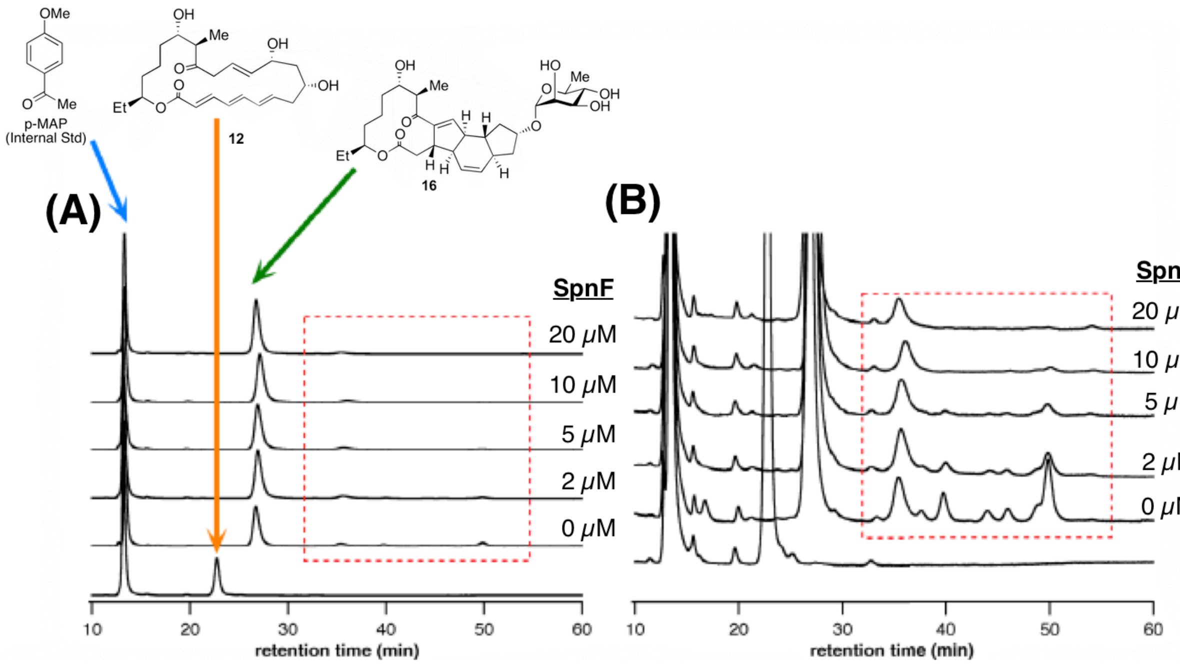


Figure S1. Evaluation of the physiological role of SpnF. (A) HPLC traces of a series of enzymatic reactions converting **12** and TDP-L-rhamnose (**5**) to **16** catalyzed by SpnM, SpnF, SpnL, and SpnG in which concentrations of SpnF were varied from 0 μM to 20 μM (*p*-MAP was used as the internal standard). (B) The amplitude of the HPLC traces was enlarged for analysis of the by-product profile of each incubation.

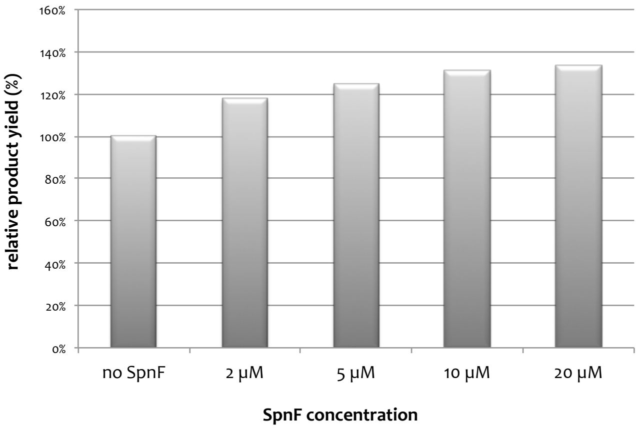


Figure S2. Effect of the SpnF concentration on the product yield of a series of reactions converting **12** to **16** catalyzed by SpnM, SpnF, SpnL, and SpnG. Relative product yields were calculated in reference to that of the incubation without SpnF. The yield of the latter case was set to be 100%.

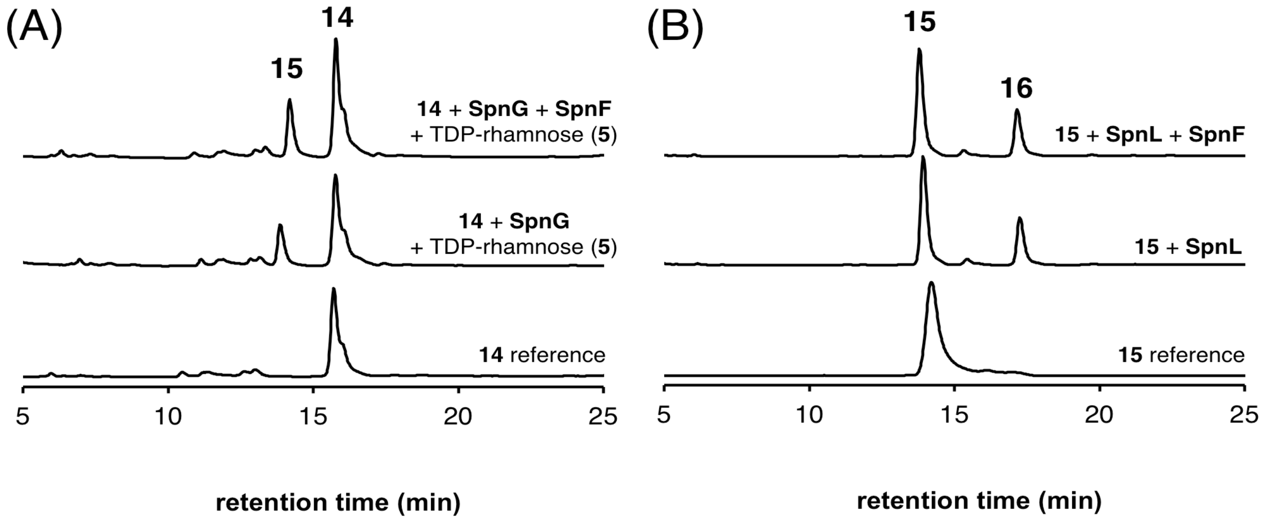


Figure S3. HPLC traces of the experiments to investigate the effect of SpnF on (A) the SpnG and (B) the SpnL reactions.

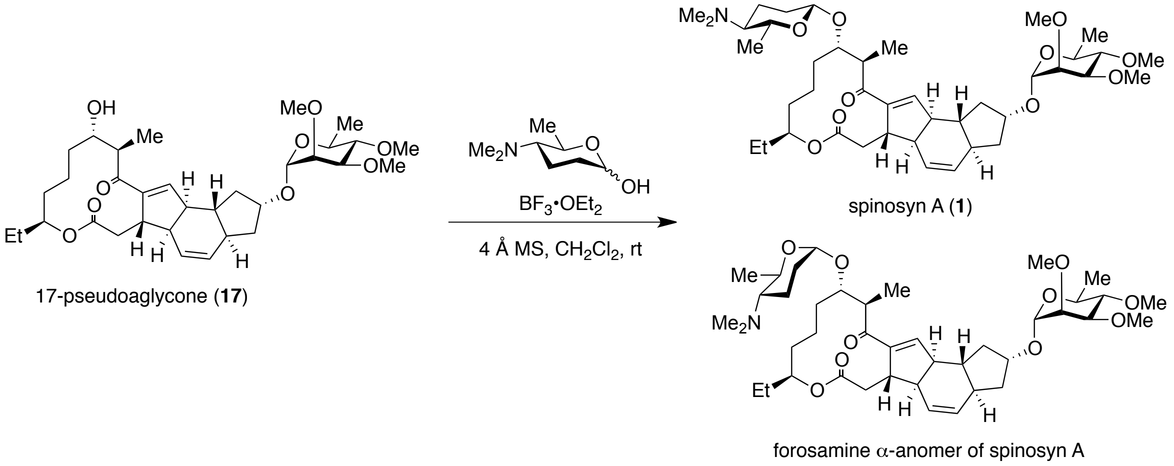
To investigate the effect of SpnF on the cycloaddition reaction, the following reactions were conducted. To a solution containing compound **12** (500 μM), TDP-L-rhamnose (chemoenzymatically prepared, 1 mM),[4] *p*-methoxyacetophenone (*p*-MAP as the internal standard, 500 μM), SpnF (concentration varied), SpnL (20 μM), and SpnG (15 μM) in 50 μL of 50 mM Tris•HCl (pH 8) buffer at 30 ºC, SpnM (0.5 μM) was added to initiate the enzymatic reaction. After 4 hr of incubation, at which point the substrate was completely consumed, the reaction was quenched by adding 200 μL of ethanol and the denatured enzymes were pelleted by centrifugation. The supernatant was then subjected to HPLC analysis to check the product profile (Varian Microsorb-MV 100-5 C18 250 × 4.6 mm, flow rate = 1 mL/min, UV detection at 280 nm). The products were eluted with water (A) and acetonitrile (B) using the linear gradient from 31% B to 36% B over 70 min. In this set of experiments, the concentrations of SpnF were varied from 0 μM to 20 μM to evaluate its effect on the product profile. As shown in Figure S1, when the concentration of SpnF increased, the reaction became "cleaner" since formation of byproducts eluted between 35 min and 50 min was significantly suppressed. It is important to note that the retention times of these peaks do not match with any of the characterized "on-path" intermediates. In addition, the product yield increased by nearly 30% when 20 μM SpnF was used as compared to its absence (Figure S2).

To test the possibility that the observed yield increase by SpnF is due to its positive influence on the rates of other participating enzymes through protein-protein interactions, the extents of substrate consumption in the absence and presence of SpnF were compared at a common time point. Specifically, SpnG (2.5 μM) or SpnL (2.5 μM) was added to a solution containing the corresponding substrate (100 μM of **14** and 200 μM of **5** for SpnG, 500 μM of **15** for SpnL) in 50 mM Tris•HCl buffer (pH 8), and the mixture was incubated at 30 ºC for 40 min or 90 min, respectively, before analyzed by HPLC (Varian Microsorb-MV 100-5 C18 250 × 4.6 mm, flow rate = 1 mL/min, UV detection at 254 nm, a linear gradient from 30% B to 45% B over 20 min where A: H_2O and B: acetonitrile). Another set of reactions under the same conditions except for inclusion of SpnF (10 μM) was also performed in parallel to investigate the effect of SpnF on these two respective reactions. As shown in Figure S3, supplementation of SpnF appeared to have no apparent effect on the extents of both SpnL- and SpnG-catalyzed reactions. In addition, it was demonstrated in our previous communication[2] that catalysis by SpnM is independent of SpnF. Thus, suppression of the off-path intermediates by SpnF shown in Figure S1 and S2 is unlikely due to its interactions with other enzymes. Based on these observations, we propose that SpnF suppresses formation of the off-path byproducts such as diastereomeric isomers and/or nucleophilic-addition adducts by accelerating the cycloaddition step, thus ensuring rapid enzymatic turnover of the chemically reactive intermediate **13** to **14**.

V. Chemoenzymatic Reconstitution of the Post-PKS Modifications to Produce Spinosyn A (1)

The enzymatic production of 17-pseudoaglycone (**17**) from the PKS product (**11**) was achieved under the following reaction conditions. Initially, the "multi-step, one-pot" tandem enzymatic conversion of **11** to **17** was tested under the condition where the concentrations of enzymes from operon III, SpnG and SpnH, were set at 1 μM based on our previous results from the investigation of the rhamnose methylation events.[4] However, under this condition, the conversion was slow and incomplete. Analysis of the reaction mixture revealed that the SpnH-catalyzed reaction, methylation at the *O*4-position of the rhamnose moiety, was the bottleneck. Thus, we decided to increase the concentrations of enzymes from operon III from 1 μM to 3 μM. **One-pot enzymatic conversion of the macrolactone intermediate 11 to 17-pseudoaglycone (17):** To a solution containing compound **11** (1 mM), TDP-L-rhamnose (1.7 mM), $MgCl_2$ (2 mM), SAM (15 mM), SpnM (5 μM), SpnF (20 μM), SpnG (3 μM), SpnL (5 μM), SpnI (10 μM), SpnK (5 μM), and SpnH (3 μM) in 250 μL of 50 mM Tris•HCl (pH 8) buffer at 30 ºC, SpnJ (5 μM) was added to initiate the reaction. After 4 days of incubation, at which point the substrate was completely consumed, the reaction was quenched by the addition of 250 μL of a cold 1:1 mixture of DMSO/CH_3CN and the denatured enzymes were pelleted by centrifugation. The supernatant (20 μL) was then subjected to reverse phase HPLC analysis (Alltech 4 × 250 mm Econosil C18 column, flow rate = 1mL/min, UV detection at 254 nm). The products were eluted with water (A) and acetonitrile (B) using the linear gradient from 30% B to 60% B over 60 min (see Figure 1B). The product yield was calculated to be 19.6% (average yield per step = 81.6%) by interpolating the integrated peak area with respect to the standard calibration curve made with standard samples of PSA-17 (**17**) whose concentrations was varied from 0.1 mM to 0.8 mM.

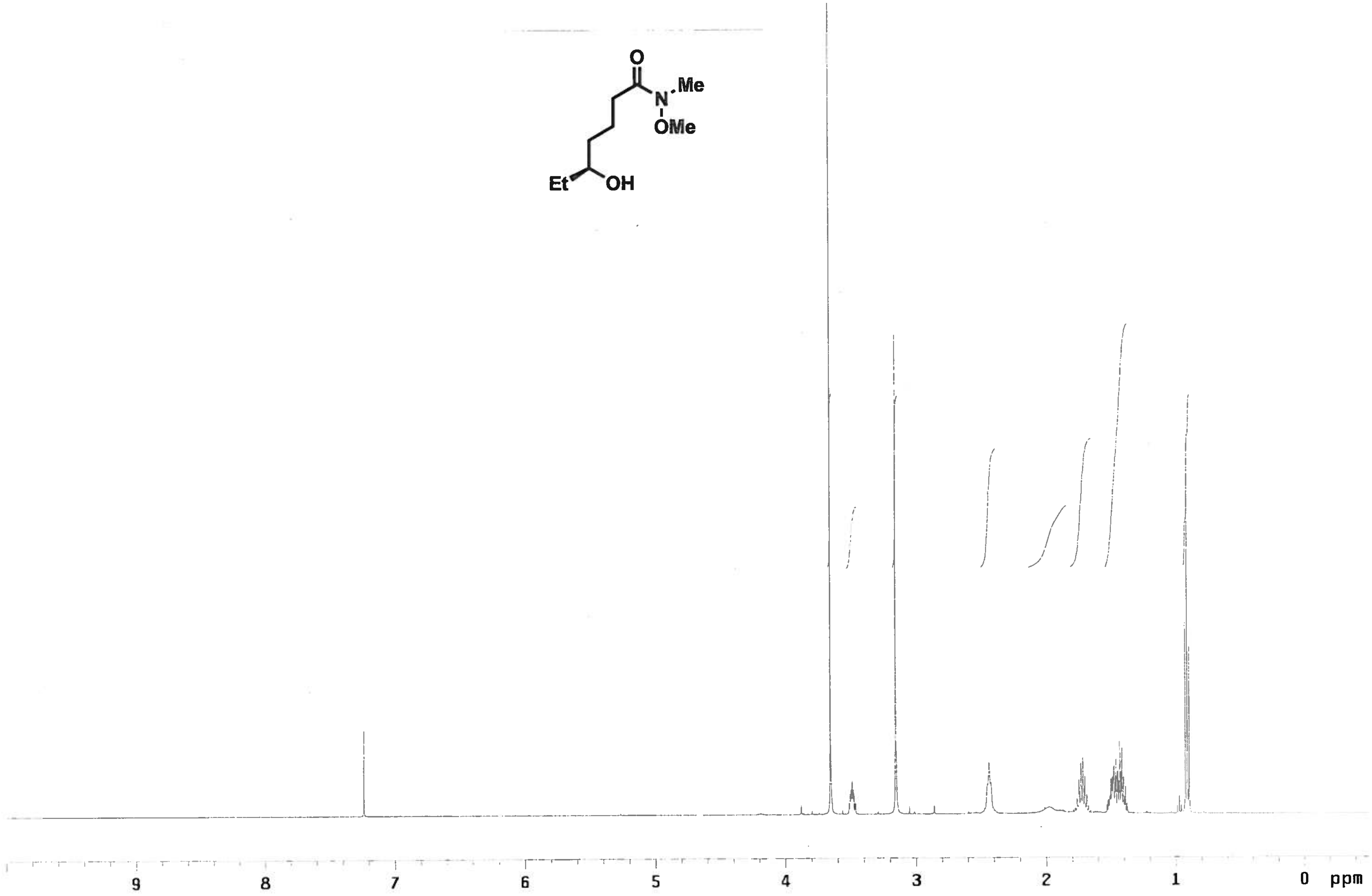
It should be noted that the curves shown the HPLC trace shown in Figure 1 in the main text is not a reliable indicator with regards to quantitating side reactions and/or decomposition products due to variability in their extinction coefficients at 254 nm. In other words, the minor peaks in some cases (e.g., those in Figure 1B (b)) are likely deceptively small, and some possibly significant decomposition products may not be observable by HPLC at all. While it was challenging to purify and characterize these minor impurities, we managed to isolate the major byproduct(s) from the SpnM reaction, which appeared to be the *cis*-isomer(s) of **13**. However, full characterization of this compound was not possible due to its scarcity.

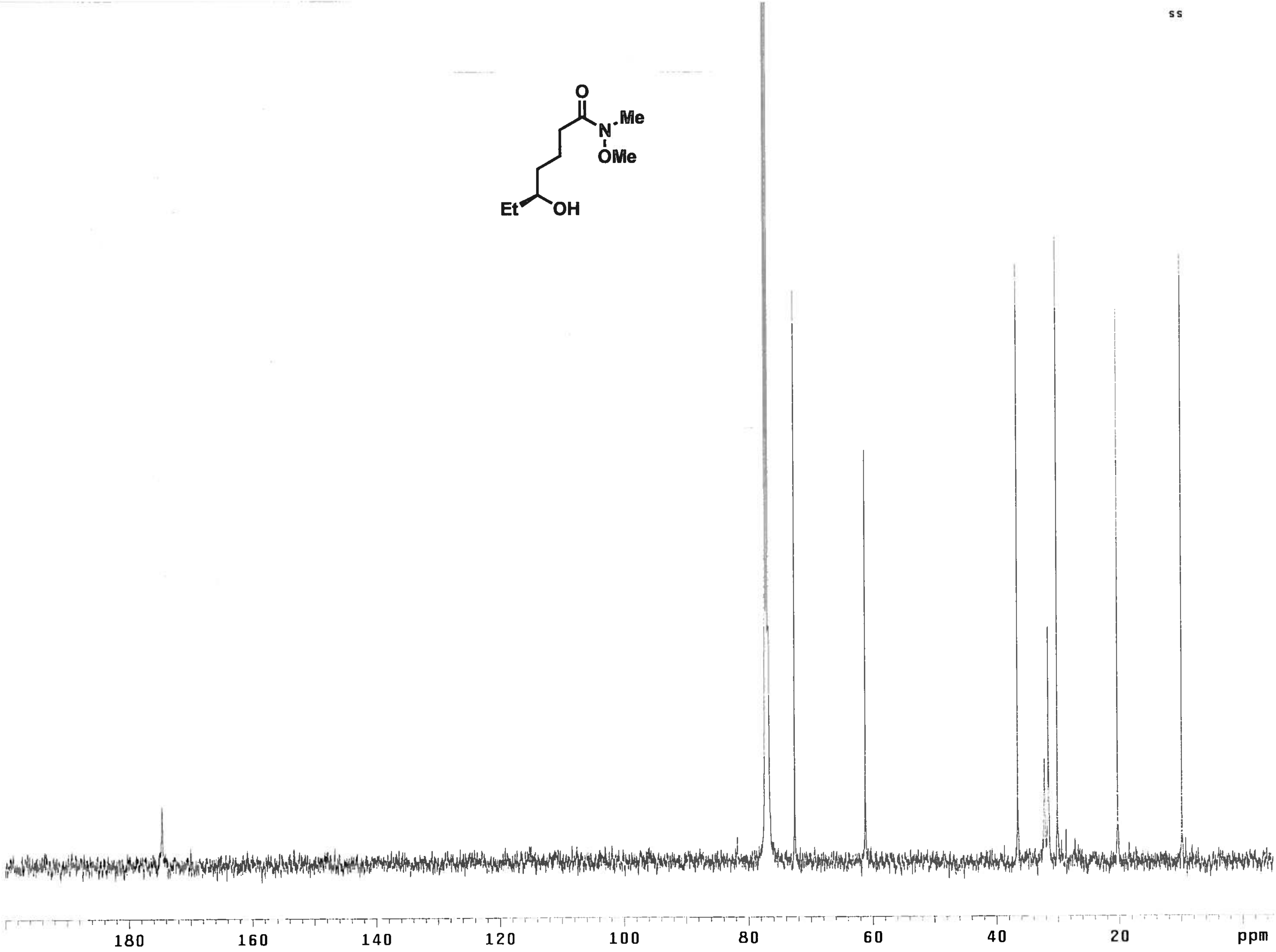


Lewis acid promoted attachment of D-forosamine to 17-pseudoaglycone (17) to afford spinosyn A (1): To a solution of D-forosamine[9] (126 mg, 0.79 mmol), 17-pseudoaglycone (**17**, 46.7 mg, 0.0791 mmol), and activated 4 Å molecular sieve in dichloromethane (5 mL) was added trifluoroboron etherate (BF_3•Et_2O, 0.1 mL, 0.08 mmol) over 3 min at 0 ºC. The reaction was stirred at 0 ºC for 15 min. Then, the ice bath was removed and the reaction mixture was slowly warmed to room temperature. After 6 hr, the reaction was quenched with 2~3 drops of saturated aqueous sodium bicarbonate. Then, the aqueous phase was extracted with dichloromethane (3 mL × 3). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was then subjected to flash column chromatography (SiO_2, MeOH/dichlromethane = 3/97) to afford 2:3 mixture of **1** and its *α*-anomer (16.8 mg combined, 29.0%, adjusted 62.5% based on the recovered **17**) with the recovery of 25 mg of 17-pseudoaglycone (**17**, 53.5%). The ^{1}H-NMR spectra of both compounds were consistent with the previously reported data.[10,11] Spinosyn A (**1**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.75 (s, 1H, 13-H), 5.86 (d, *J* = 9.6 Hz, 1H, 6-H), 5.78 (dt, *J* = 9.6, 2.8 Hz, 1H, 5-H), 4.86 (d, *J* = 1.6 Hz, 1H, 1′-H), 4.68-4.62 (m, 1H, 21-H), 4.41 (d, *J* = 7.0 Hz, 1H, 1″-H), 4.31-4.26 (m, 1H, 9-H), 3.66-3.58 (m, 1H, 17-H), 3.54-3.52 (m, 1H, 5″-H), 3.54 (s, 3H, OMe), 3.49 (s, 3H, OMe), 3.48 (s, 3H, OMe), 3.48-3.42 (m, 4H, 4-H, 2′-H, 3′-H, 5′-H), 3.31-3.25 (m, 1H, 16-H), 3.12-3.07 (m, 2H, 2-H, 3-H, 4′-H), 3.01-2.96 (m, 1H, 3-H), 2.89-2.82 (m, 1H, 12-H), 2.44-2.10 (m, 9H, 2-H, 7-H, 10-H, NCH_3), 2.04-1.86 (m, 4H, 8-H, 2″-H, 3″-H, 4″-H), 1.84-1.74 (m,1H, 19-H), 1.66-1.10 (m, 18H, 8-H, 10-H, 16-CH_3, 18-H, 19-H, 20-H, 22-H, 6′-H, 2″-H, 3″-H, 6″-H), 0.94-0.78 (m, 4H, 11-H, 23-H). The *α*-anomer of **1**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.78 (s, 1H, 13-H), 5.86 (d, *J* = 10 Hz, 1H, 6-H), 5.76 (dt, *J* = 9.6, 2.8 Hz, 1H, 5-H), 4.84 (d, *J* = 1.8 Hz 1H. 1′-H), 4.68-4.62 (m, 1H, 21-H), 4.39-4.26 (m, 2H, 1″-H, 9-H), 3.86-3.80 (m, 1H, 17-H), 3.55 (s, 3H, OMe), 3.54-3.52 (m, 1H. 5″-H), 3.49 (s, 3H, OMe), 3.48 (s, 3H, OMe), 3.48-3.42 (m, 4H, 4-H, 2′-H, 3′-H, 5′-H), 3.36-3.30 (m, 1H, 16-H), 3.13-2.98 (m, 3H, 2-H, 3-H, 4′-H), 2.91-2.84 (m, 1H, 12-H), 2.44-2.10 (m, 9H, 2-H, 7-H, 10-H, NCH_3), 2.04-1.86 (m, 4H, 8-H, 2″-H, 3″-H, 4″-H), 1.84-1.74 (m,1H, 19-H), 1.66-1.10 (m, 18H, 8-H, 10-H, 16-CH_3, 18-H, 19-H, 20-H, 22-H, 6′-H, 2″-H, 3″-H, 6″-H), 0.94-0.78 (m, 4H, 11-H, 23-H).

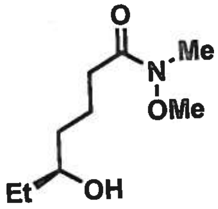
VI. References

(1) Kim, H. J.; Pongdee, R.; Wu, Q.; Hong, L.; Liu, H.-W. *J. Am. Chem. Soc.* **2007**, *129*, 14582.
(2) Kim, H. J.; Ruszczycky, M. W.; Choi, S.-H.; Liu, Y.-N.; Liu, H.-W. *Nature* **2011**, *473*, 109.
(3) Isiorho, E. A.; Liu, H.-W.; Keatinge-Clay, A. T. *Biochemistry* **2012**, *51*, 1213.
(4) Kim, H. J.; White-Phillip, J. A.; Ogasawara, Y.; Shin, N.; Isiorho, E. A.; Liu, H.-W. *J. Am. Chem. Soc.* **2010**, *132*, 2901.
(5) Molander, G. A.; McWilliams, J. C.; Noll, B. C. *J. Am. Chem. Soc.* **1997**, *119*, 1265.
(6) Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143.
(7) Rychnovsky, S. D.; Rogers, B. N.; Richardson, T. I. *Acc. Chem. Res.* **1998**, *31*, 9.
(8) Jung, M. E.; Light, L. A. *Tet. Lett.* **1982**, *23*, 3851.
(9) Creemer, L. C.; Kirst, H. A.; Paschal, J. W. *J. Antiobiot.* **1998**, *51*, 795.
(10) Evans, D. A.; Black, W. C. *J. Am. Chem. Soc.* **1993**, *115*, 4497.
(11) Paquette, L. A.; Collado, I.; Purdie, M. *J. Am. Chem. Soc.* **1998**, *120*, 2553.


O
N
Me
OMe
Et
OH
9
8
7
6
5
4
3
2
1
0
ppm

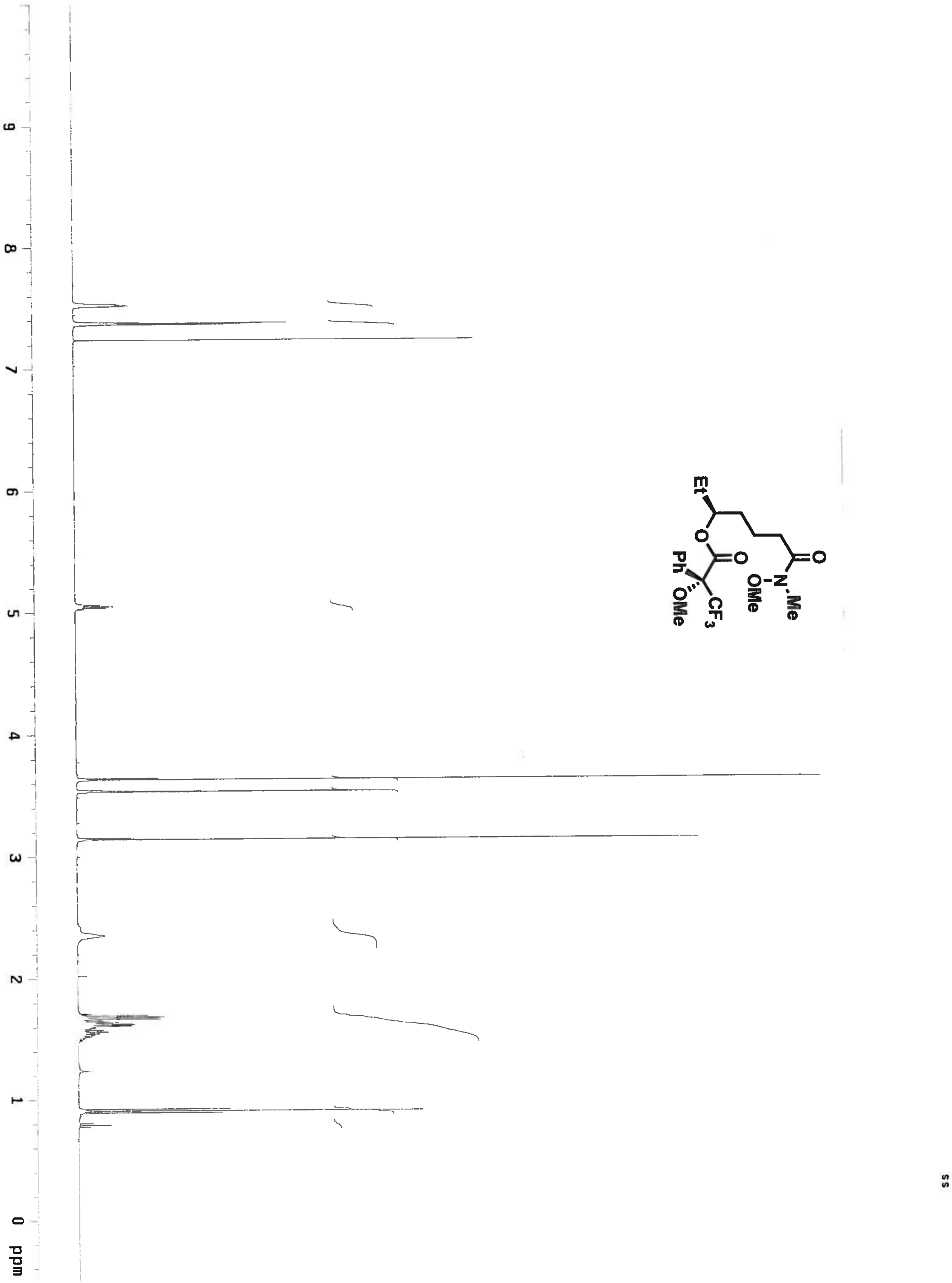

ss
O
N
Me
OMe
Et
OH
180
160
140
120
100
80
60
40
20
ppm

```
                           Elemental Composition                   Date : 23-JAN-2004

File:LIU8932 Ident:7_9 SMO(1,5) BSUB(128,15,-3.0)
ZAB-E4F CI+ Voltage BpI:2349312 TIC:43217808 Flags:NORM DEL ERR
File Text:I-RP-31
  Heteroatom Max:   50     Ion:  Both Even and Odd
  Limits:
                                                    -0.5     0     0      1     0
  190.144713                 5.5                    50.0   200   400      1     6

     Mass              mDa    PPM   Calc. Mass      DBE     C      H      N     O

  190.144713          -0.4   -2.1   190.144319      0.5     9     20      1     3
```

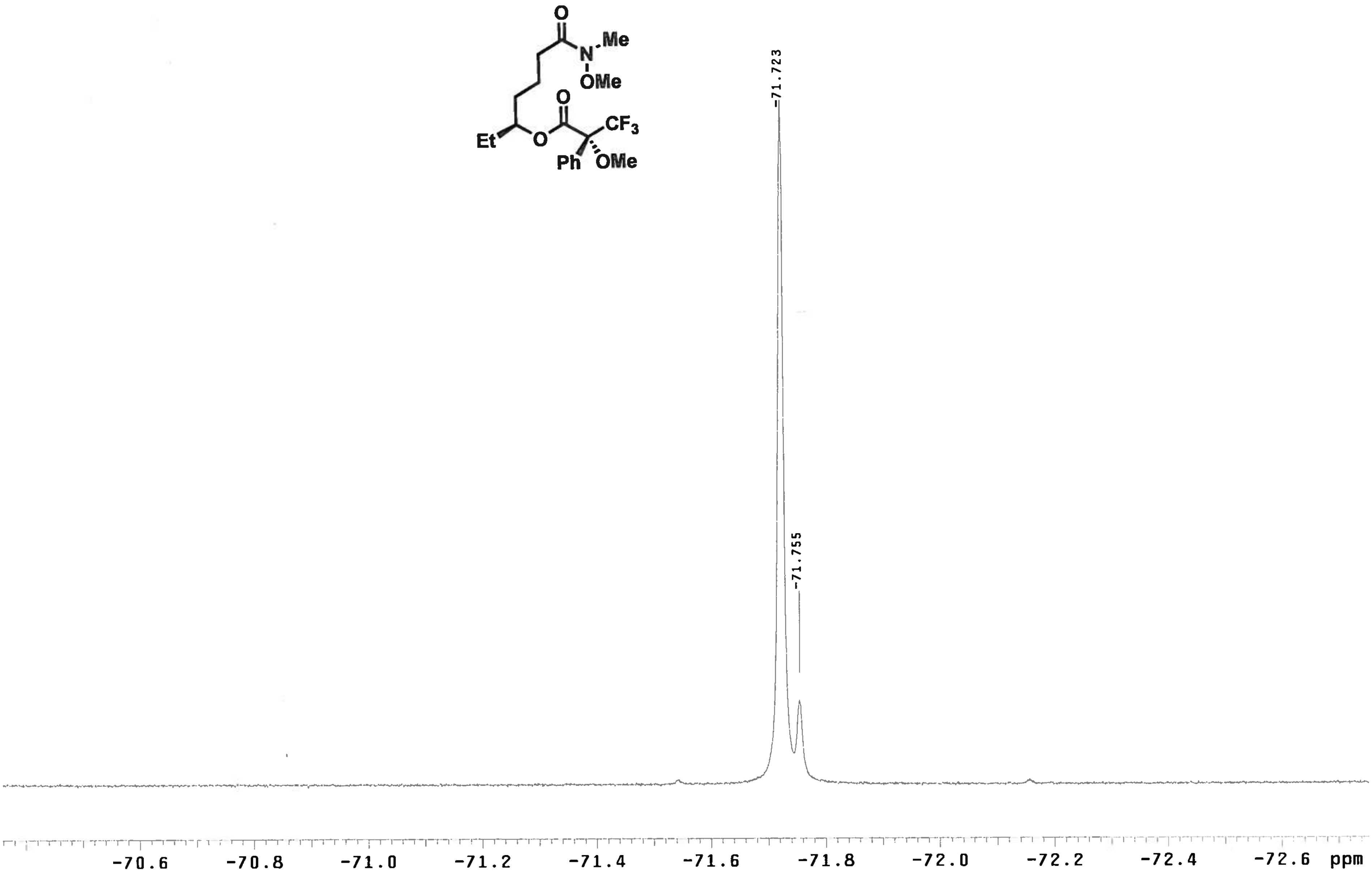


$C_9H_{19}NO_3$

189.1[illegible]5

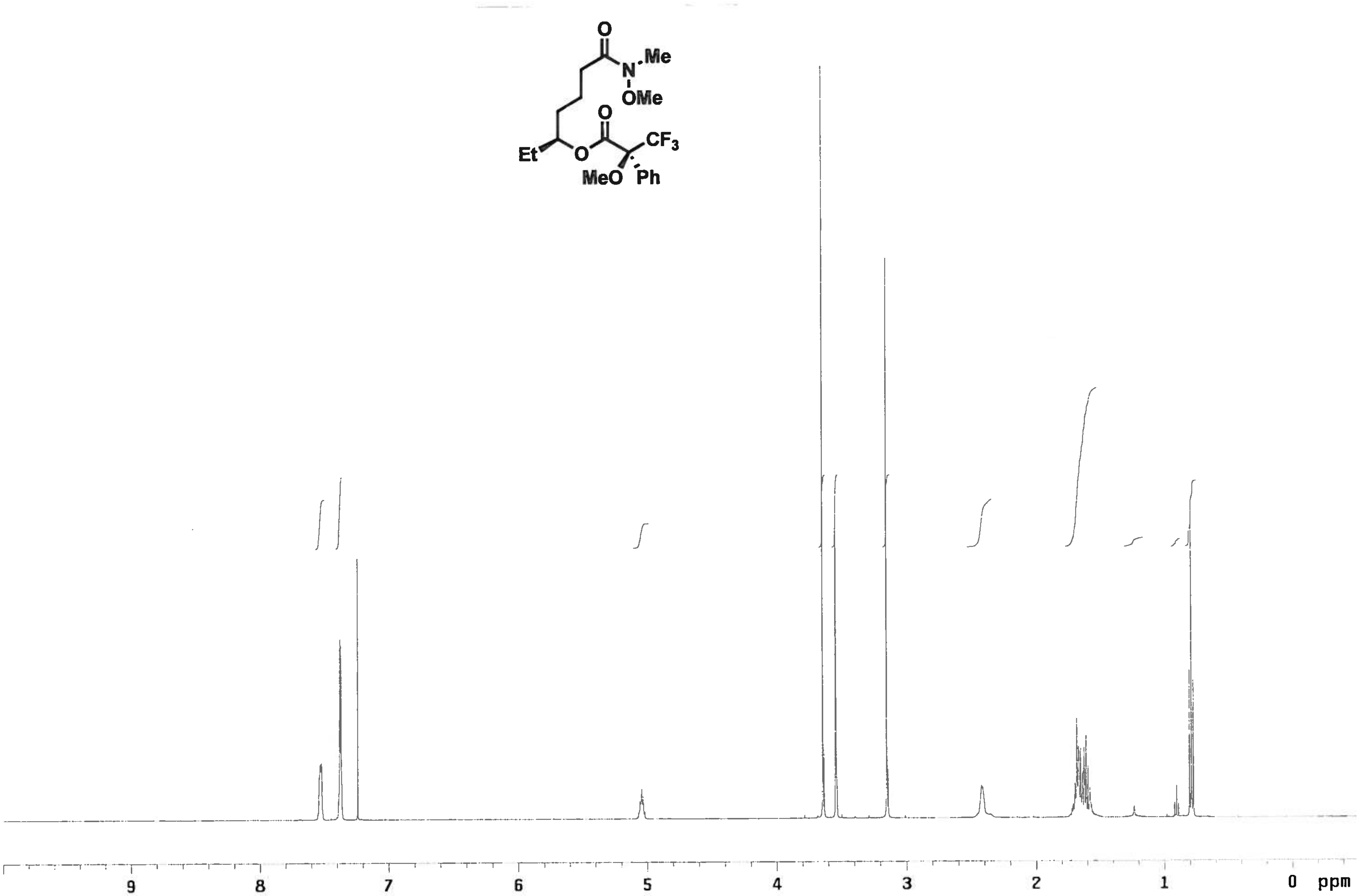

9
8
7
6
5
4
3
2
1
0
ppm
Et
O
O
Ph
OMe
CF3
O
N
Me
OMe
ss

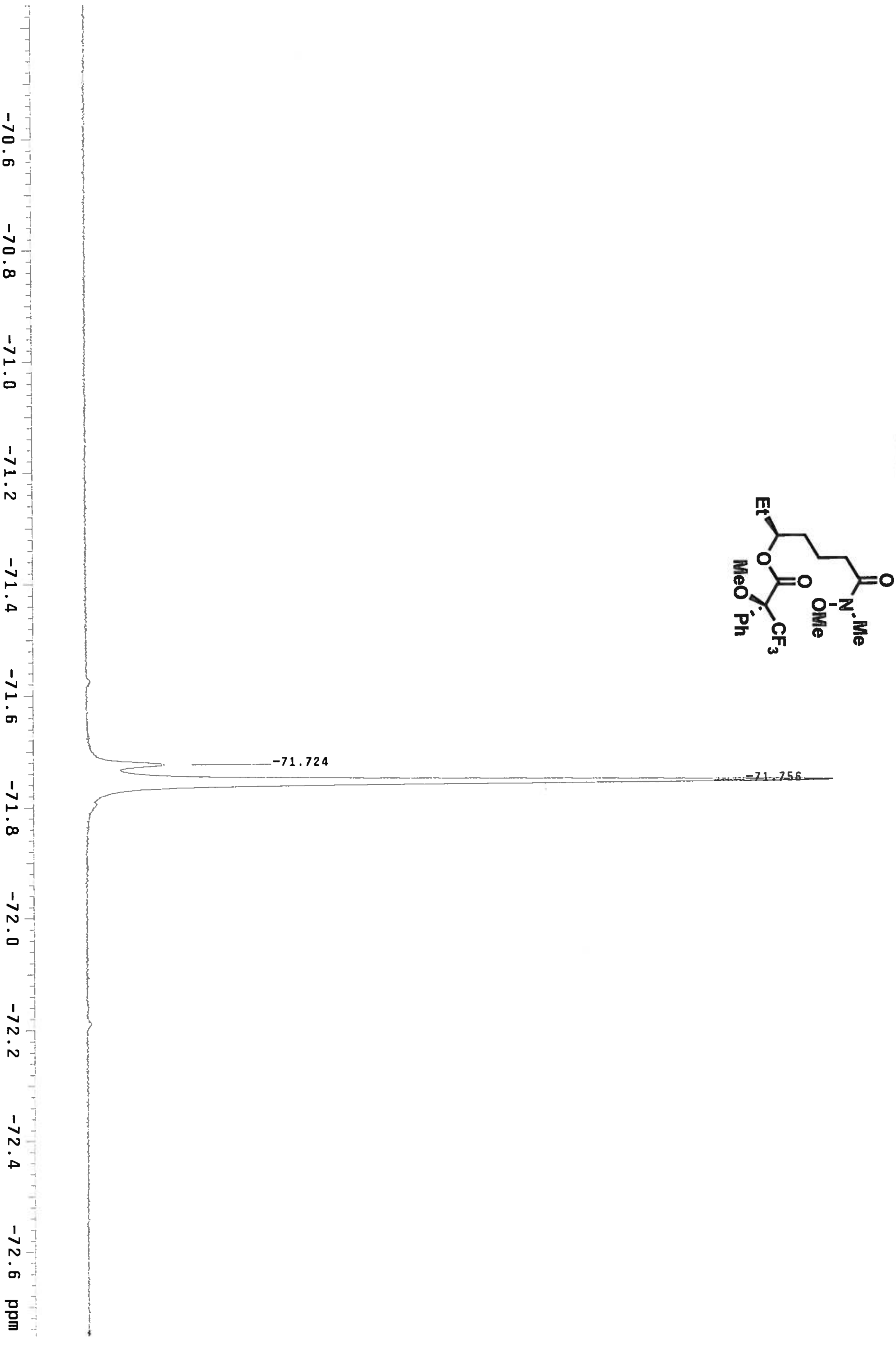
ss



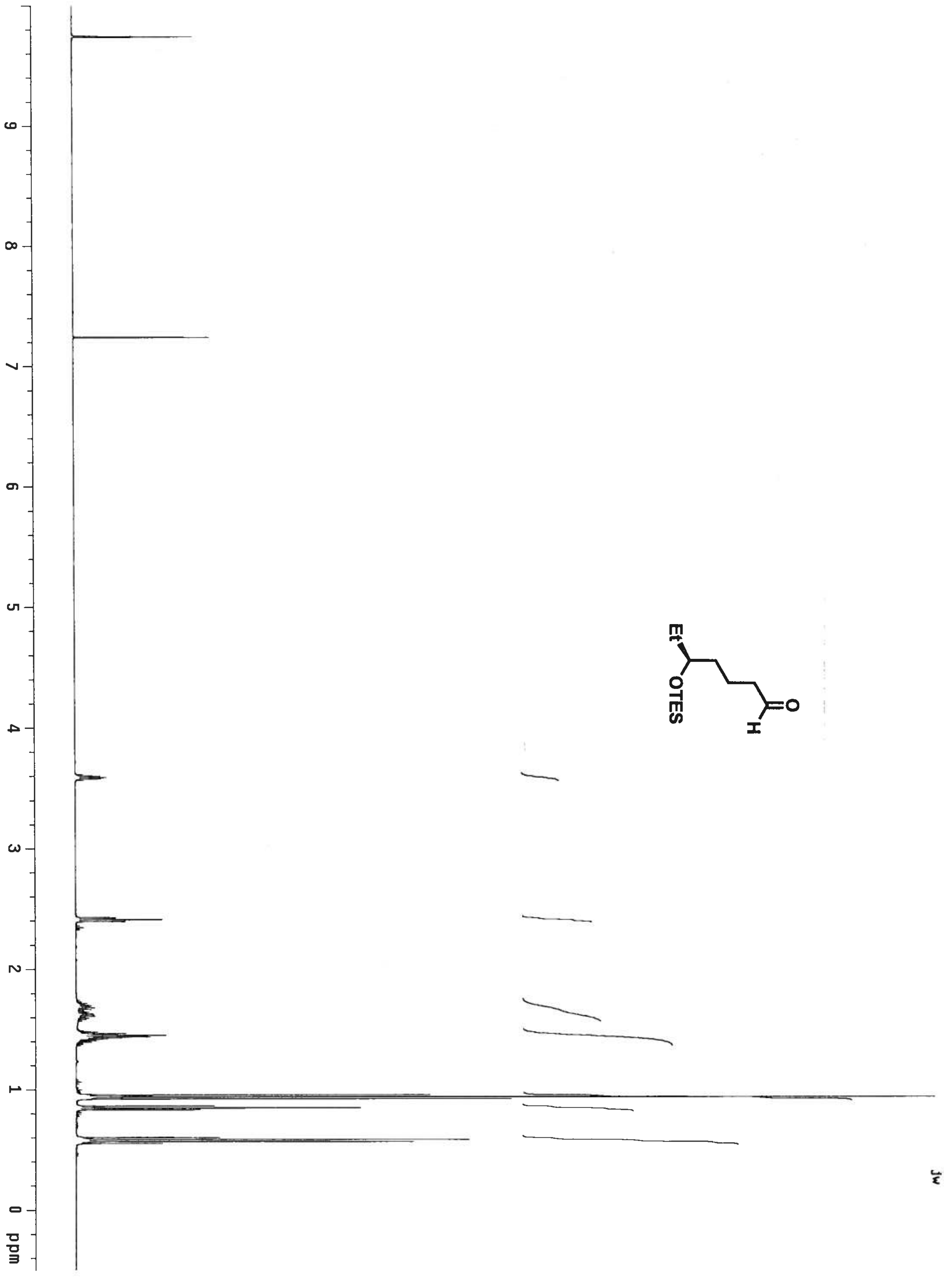
O
N
Me
OMe
O
CF3
Et
O
Ph
OMe
-71.723
-71.755
-70.6
-70.8
-71.0
-71.2
-71.4
-71.6
-71.8
-72.0
-72.2
-72.4
-72.6
ppm

ss

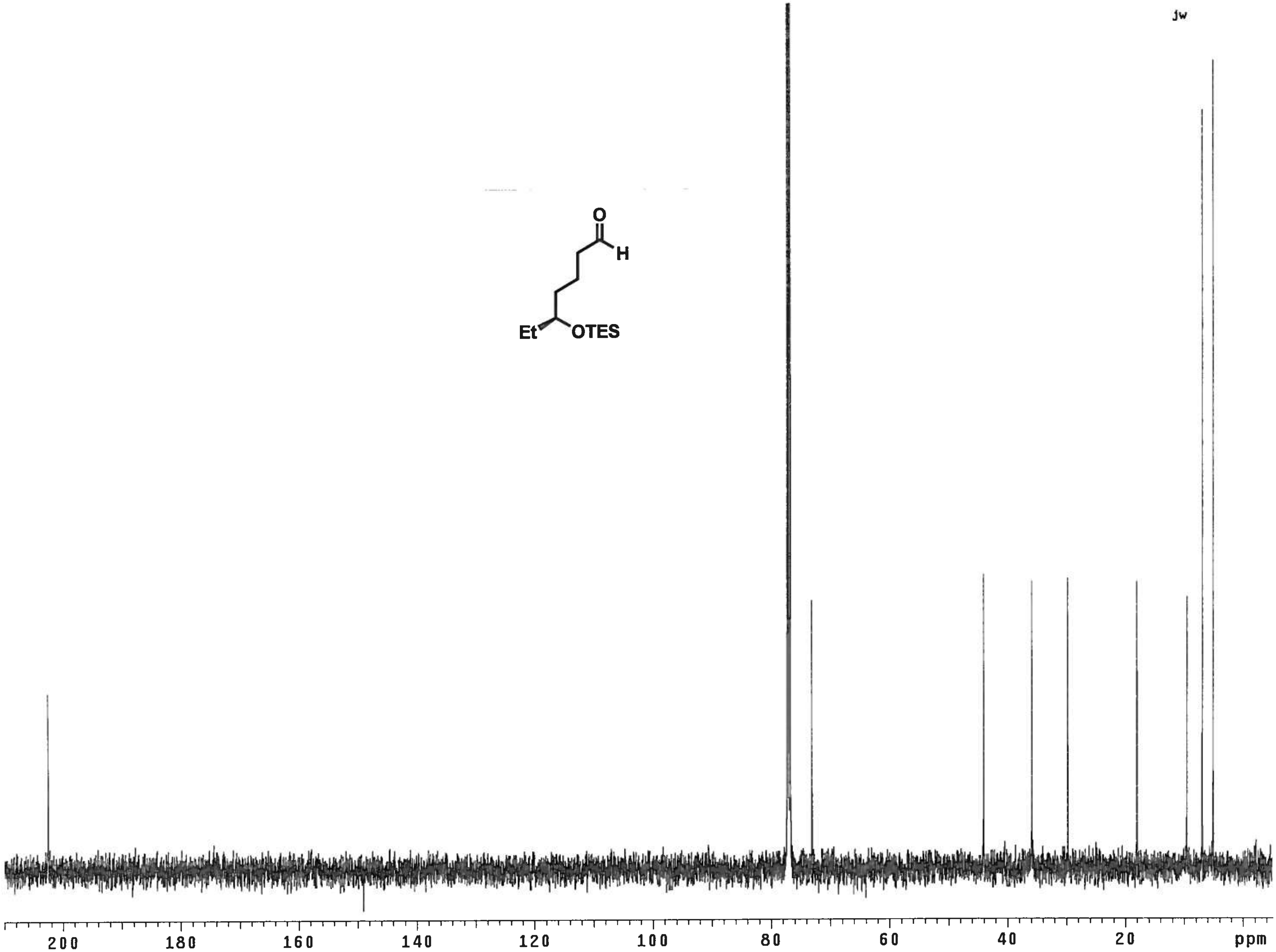



-70.6
-70.8
-71.0
-71.2
-71.4
-71.6
-71.8
-72.0
-72.2
-72.4
-72.6
ppm
-71.724
-71.756
Et
O
O
MeO
Ph
CF3
O
N
Me
OMe

ss

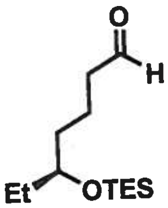


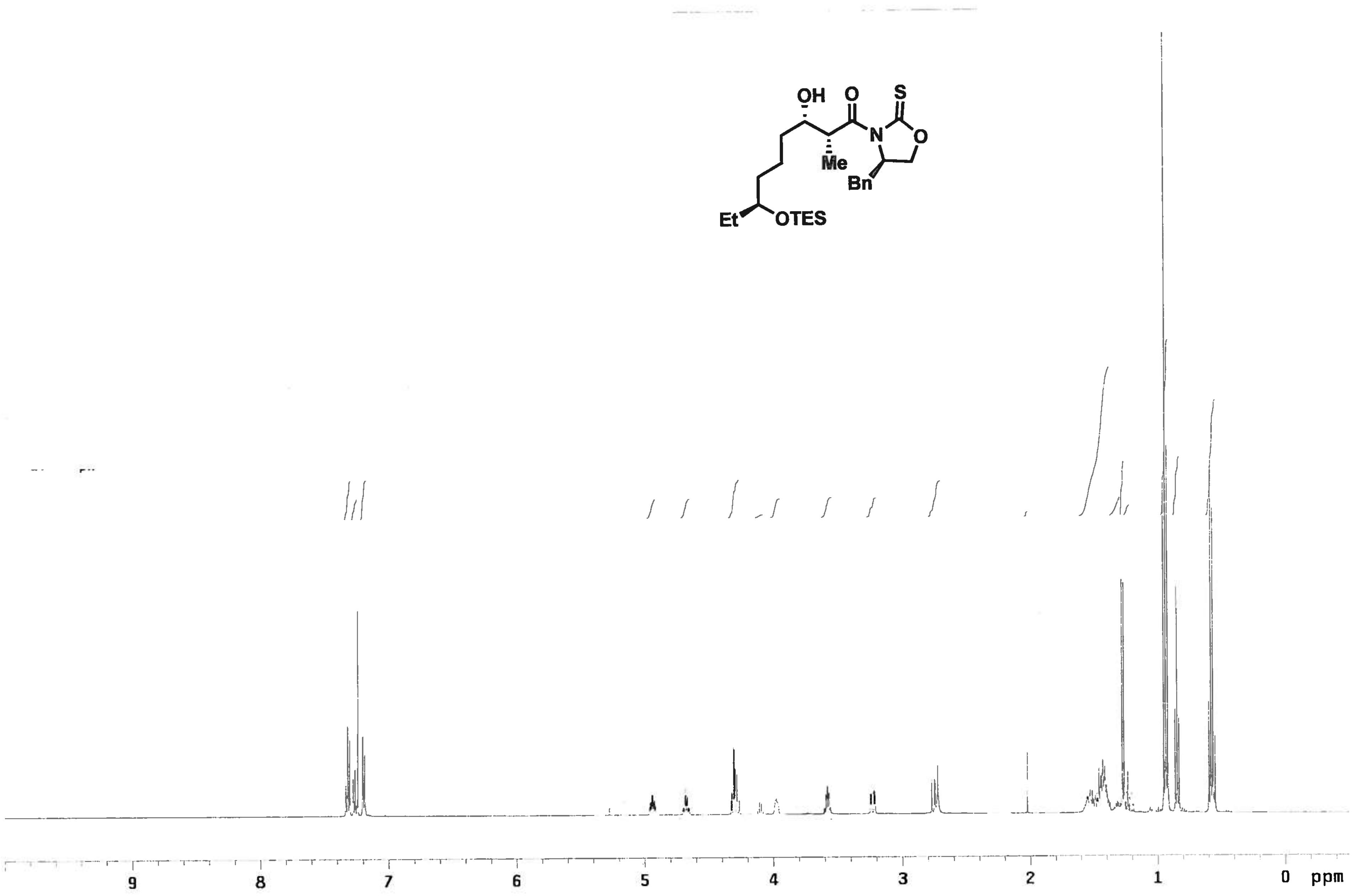
9
8
7
6
5
4
3
2
1
0
ppm
O
H
Et
OTES
Jw

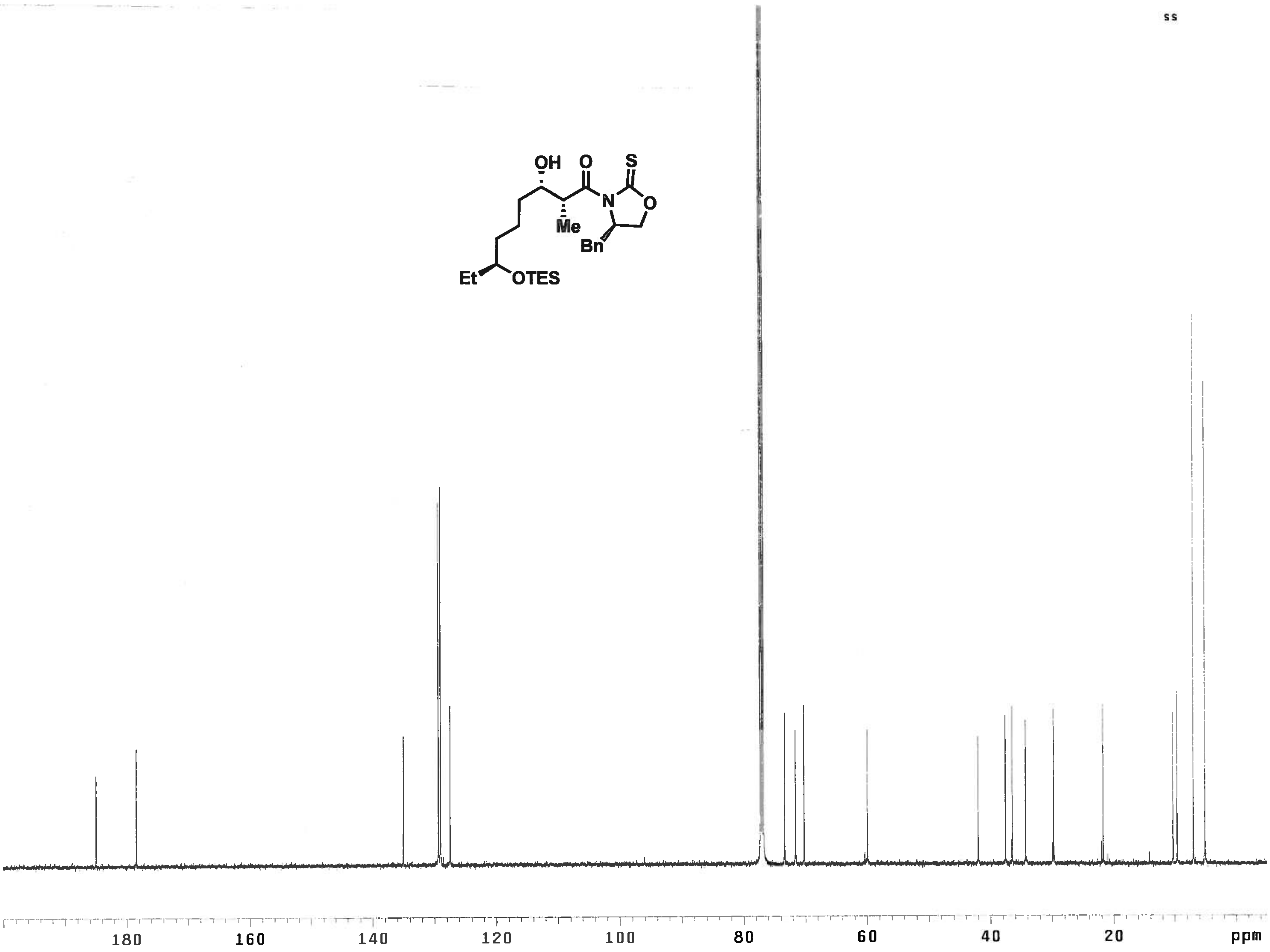


jw
O
H
Et
OTES
200
180
160
140
120
100
80
60
40
20
ppm

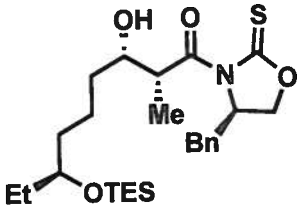
```
                        Elemental Composition                  Date : 23-JAN-2004

File:LIU8930 Ident:9_10 SMO(1,5) BSUB(128,15,-3.0)
ZAB-E4F CI+ Voltage BpI:343872 TIC:10107969 Flags:NORM DEL ERR
File Text:I-RP-42
  Heteroatom Max:    50    Ion:  Both Even and Odd
  Limits:
                                                  -0.5     0     0      0     1
  245.192532              5.5                     50.0   200   400      6     1

     Mass          mDa    PPM    Calc. Mass        DBE     C     H      O    Si

  245.192532       1.2    4.7    245.193684        0.5    13    29      2     1
```

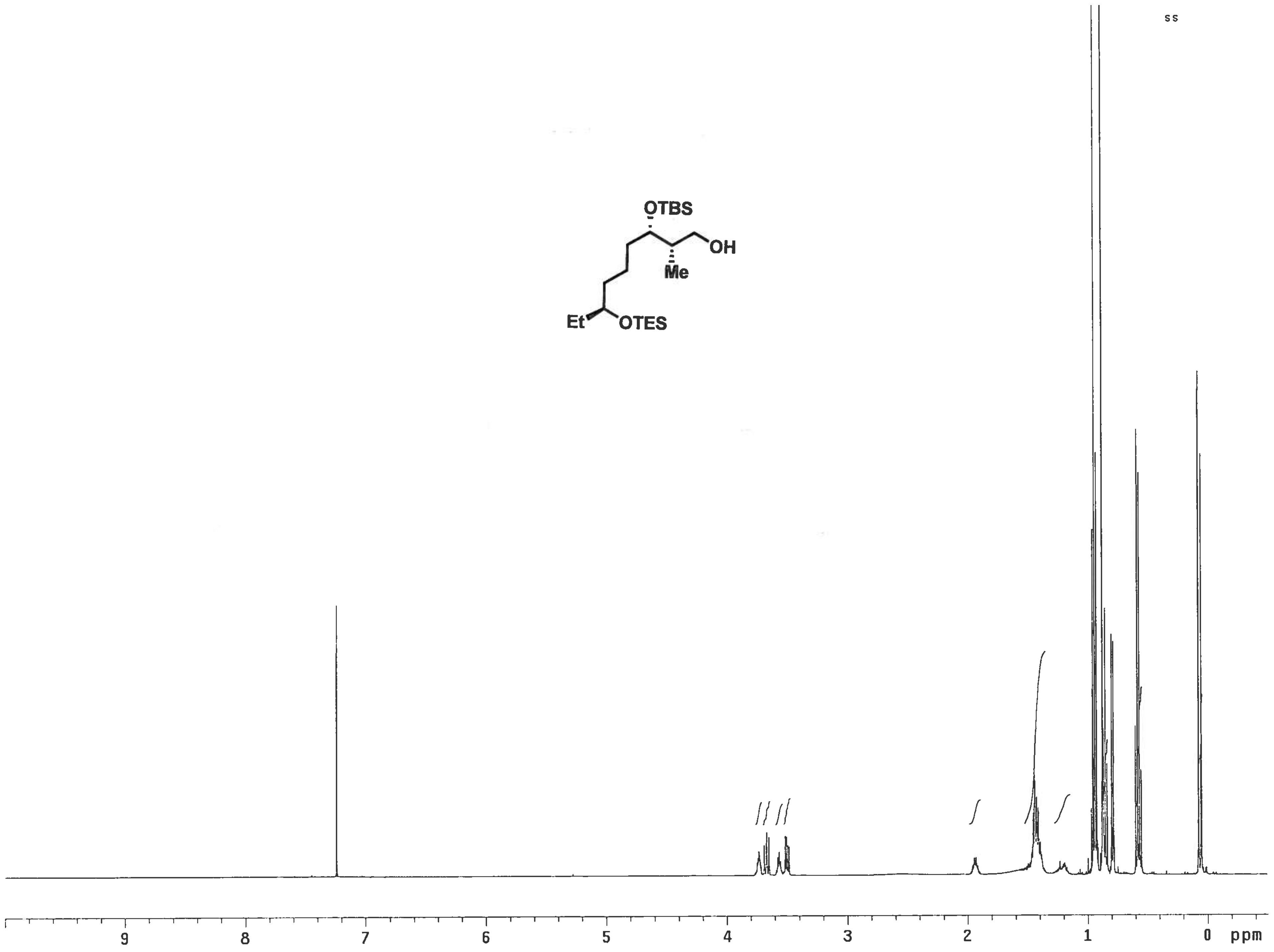


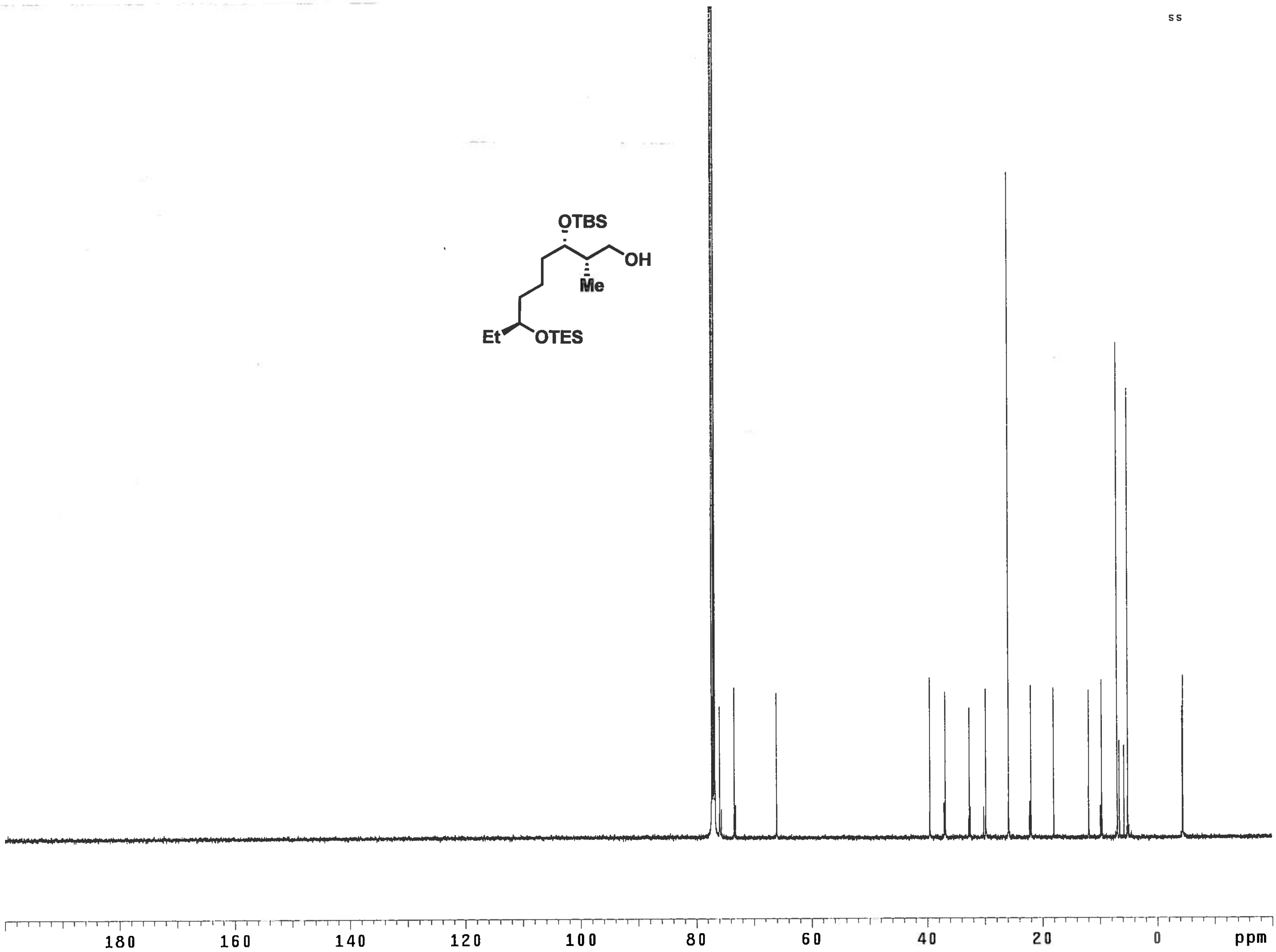

OH
O
S
N
O
Me
Bn
Et
OTES
9
8
7
6
5
4
3
2
1
0
ppm


ss
OH
O
S
N
O
Me
Bn
Et
OTES
180
160
140
120
100
80
60
40
20
ppm

```
                    Elemental Composition                  Date : 11-FEB-2004

File:LIU9899 Ident:59_62 SMO(1,5) BSUB(128,15,-3.0)
ZAB-E4F CI+ Voltage BpI:771136 TIC:20242570 Flags:NORM
File Text:I-RP-62
  Heteroatom Max:   20    Ion:  Both Even and Odd
  Limits:

                                                 -0.5    0     0     1    0    1    1
  494.278426           5.0                       20.0  200   400     1    8    1    1

     Mass             mDa     PPM   Calc. Mass    DBE    C     H     N    O   Si    S

  494.278426         -2.4    -4.8   494.276035    6.5   26    44     1    4    1    1
```




ss
OTBS
OH
Me
Et
OTES
9
8
7
6
5
4
3
2
1
0
ppm


ss
OTBS
OH
Me
Et
OTES
180
160
140
120
100
80
60
40
20
0
ppm

Elemental Composition Report

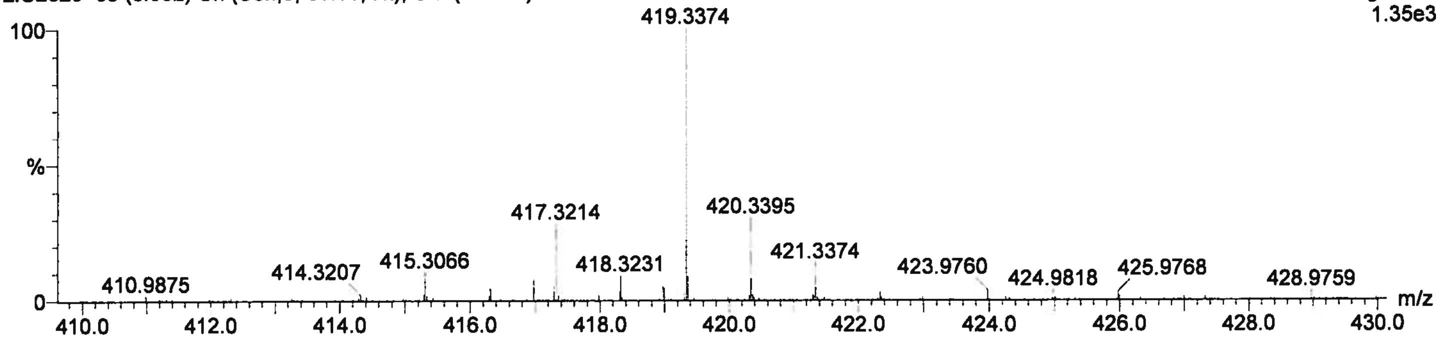
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -50.0, max = 100.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

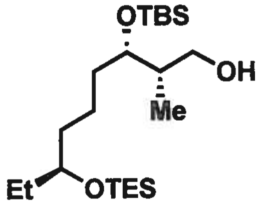
Monoisotopic Mass, Odd and Even Electron Ions
36 formula(e) evaluated with 1 results within limits (up to 10 closest results for each mass)

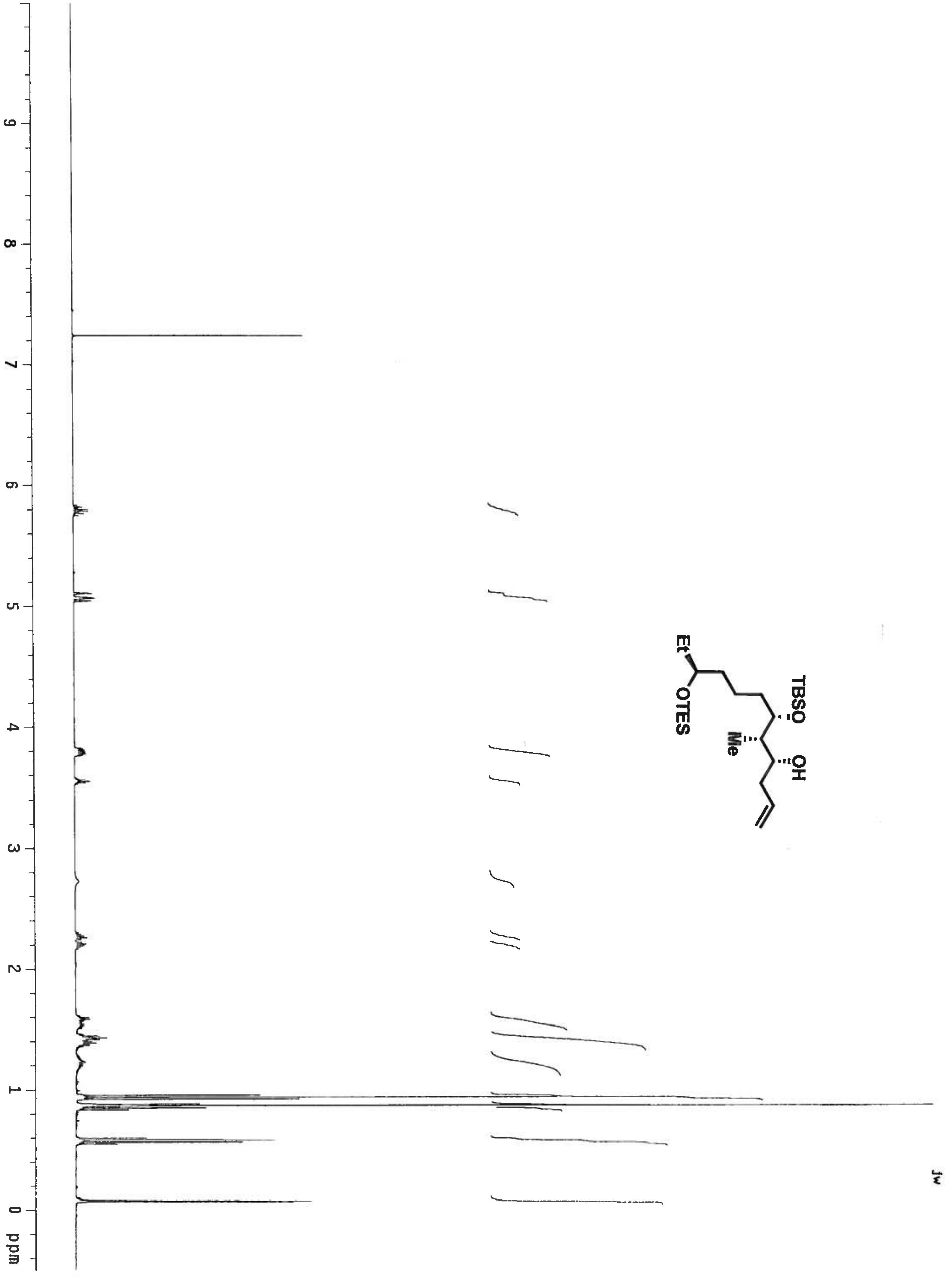
HJ03-174SM
LIU2029 93 (0.932) Cn (Cen,5, 80.00, Ht); Cm (91:119)

15-Nov-200508:42:24
Voltage CI+
1.35e3

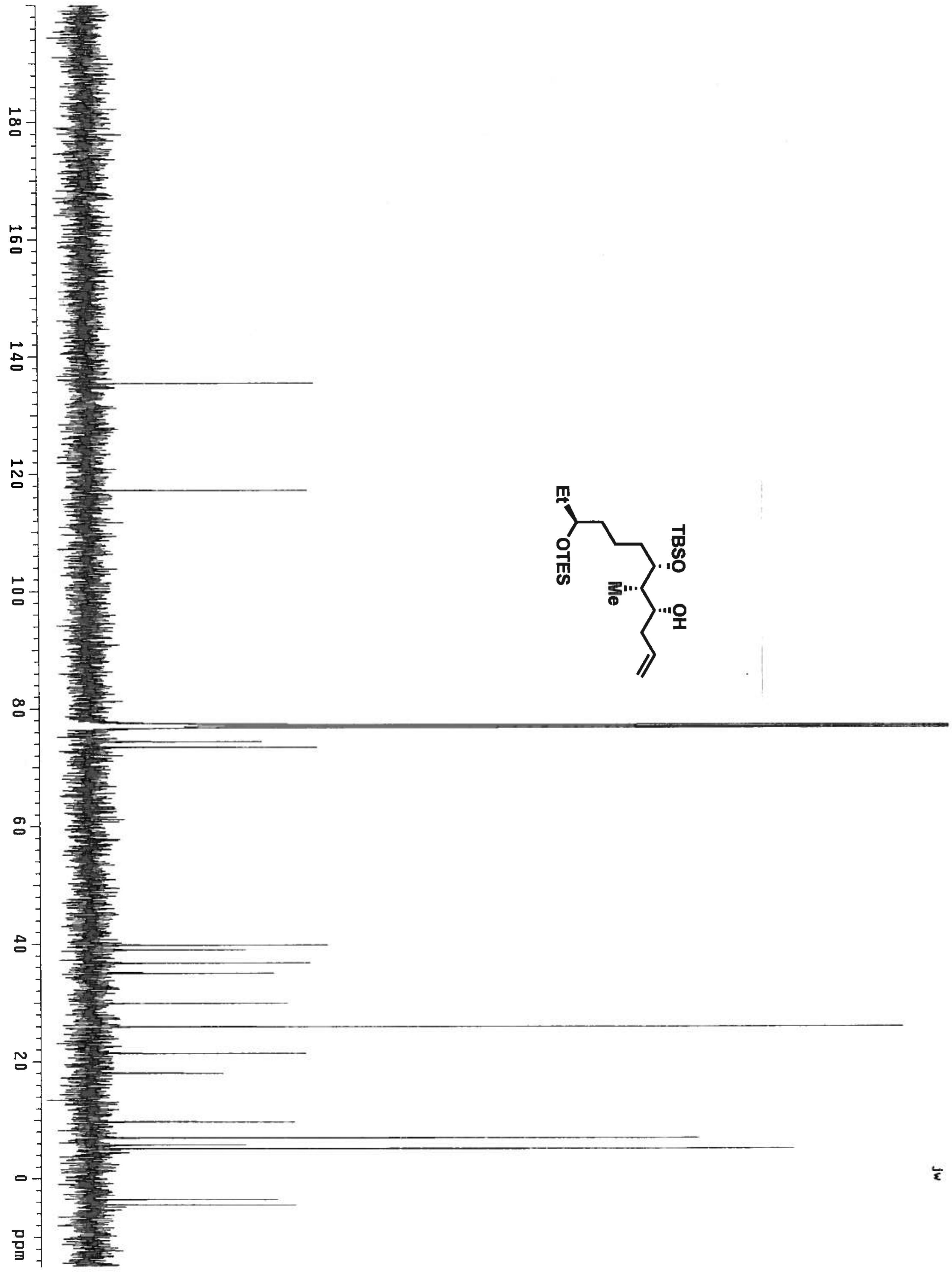


```
Minimum:                                      -50.0
Maximum:                     500.0    5.0     100.0

Mass        Calc. Mass       mDa      PPM     DBE      Score      Formula

419.3374    419.3377         -0.3     -0.7    -0.5     1          C22  H51  O3  Si2
```



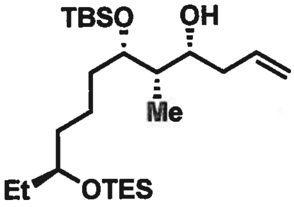


jw

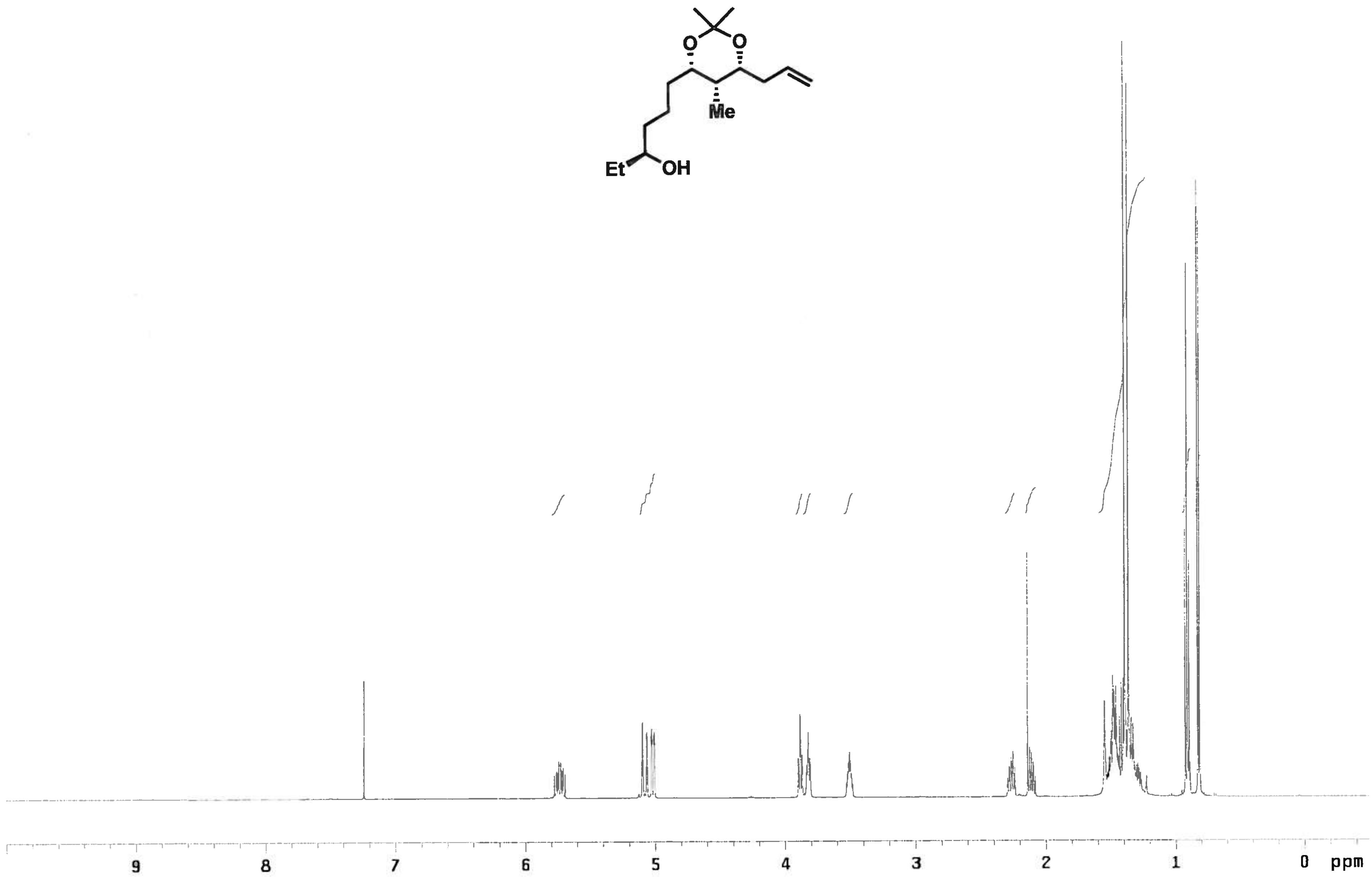


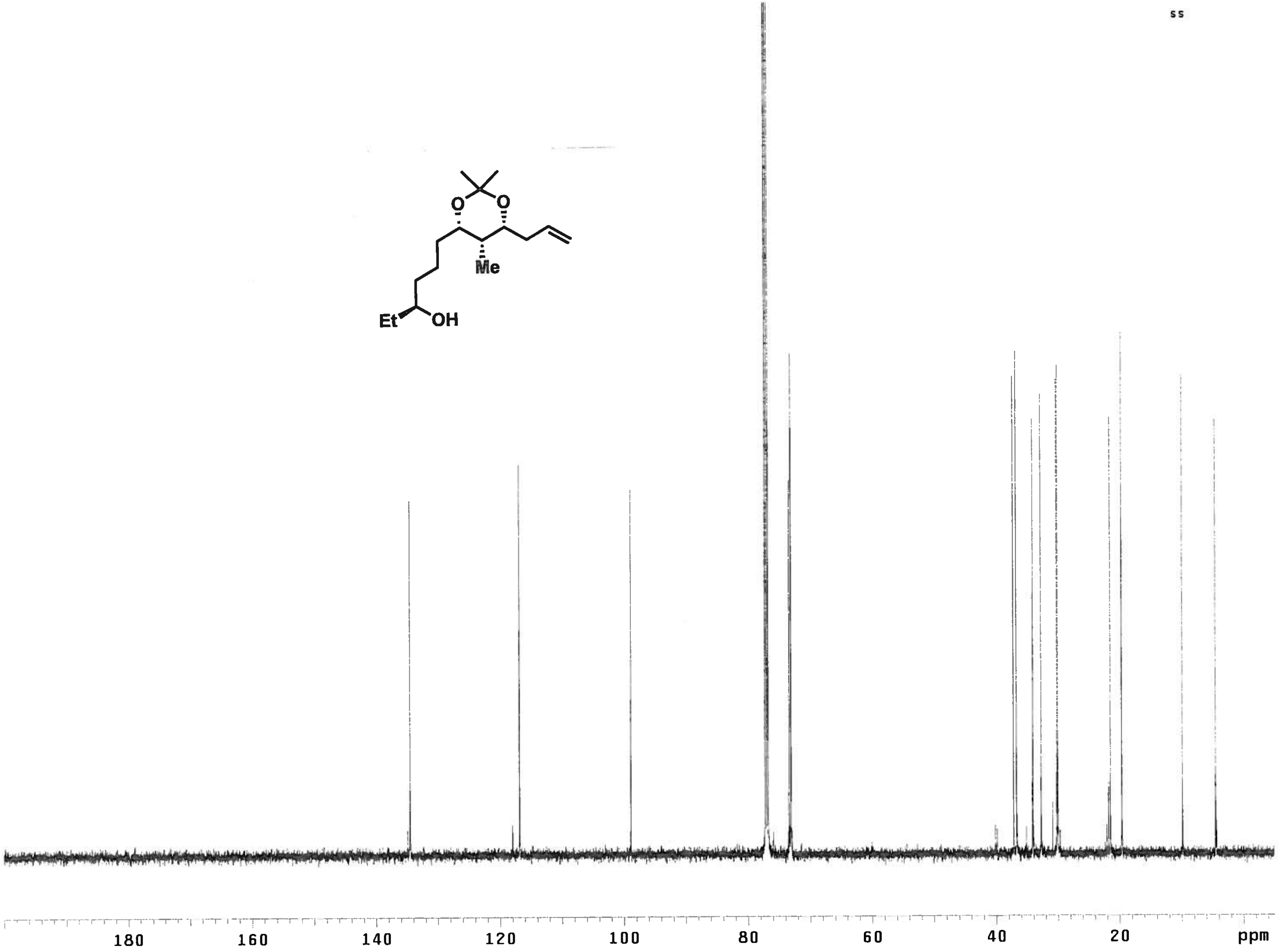
jw

```
                    Elemental Composition                Date : 25-JUN-2004

File:J25LN8912 Ident:26 SMO(1,7) PKD(5,3,5,0.01%,5.0,0.00%,F,F)
ZAB-E4F CI+ Voltage BpI:2159 TIC:1167147 Flags:NORM DEL ERR
File Text:I-RP-140B / RONGSUN
  Heteroatom Max:   20    Ion:  Both Even and Odd
  Limits:
                                                 -0.5     0     0     0     0
  457.353052            5.0                      16.0   200   400     3     2

     Mass          mDa     PPM    Calc. Mass      DBE     C     H     O    Si

  457.353052       0.3     0.6    457.353328      1.5    25    53     3     2
```

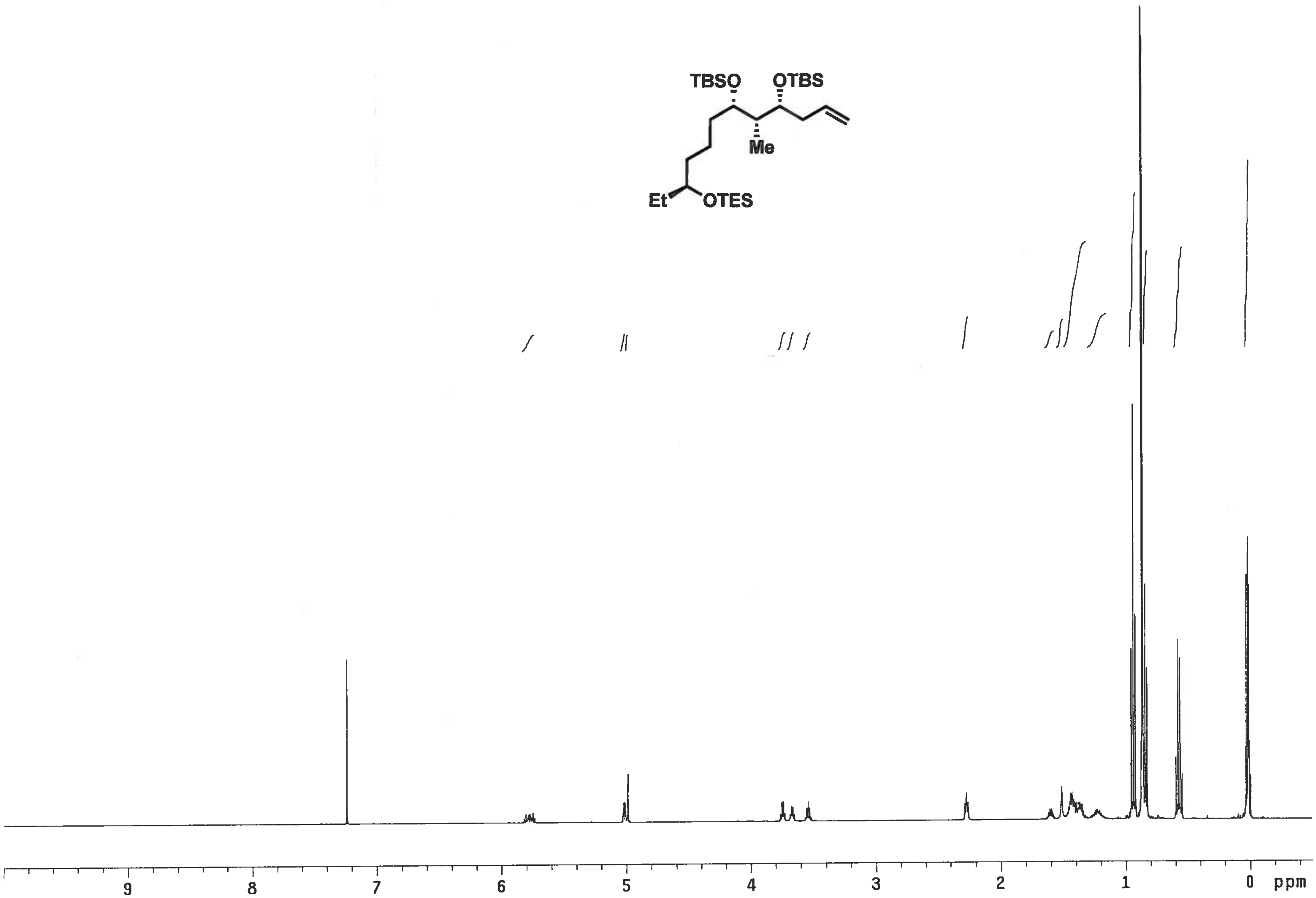


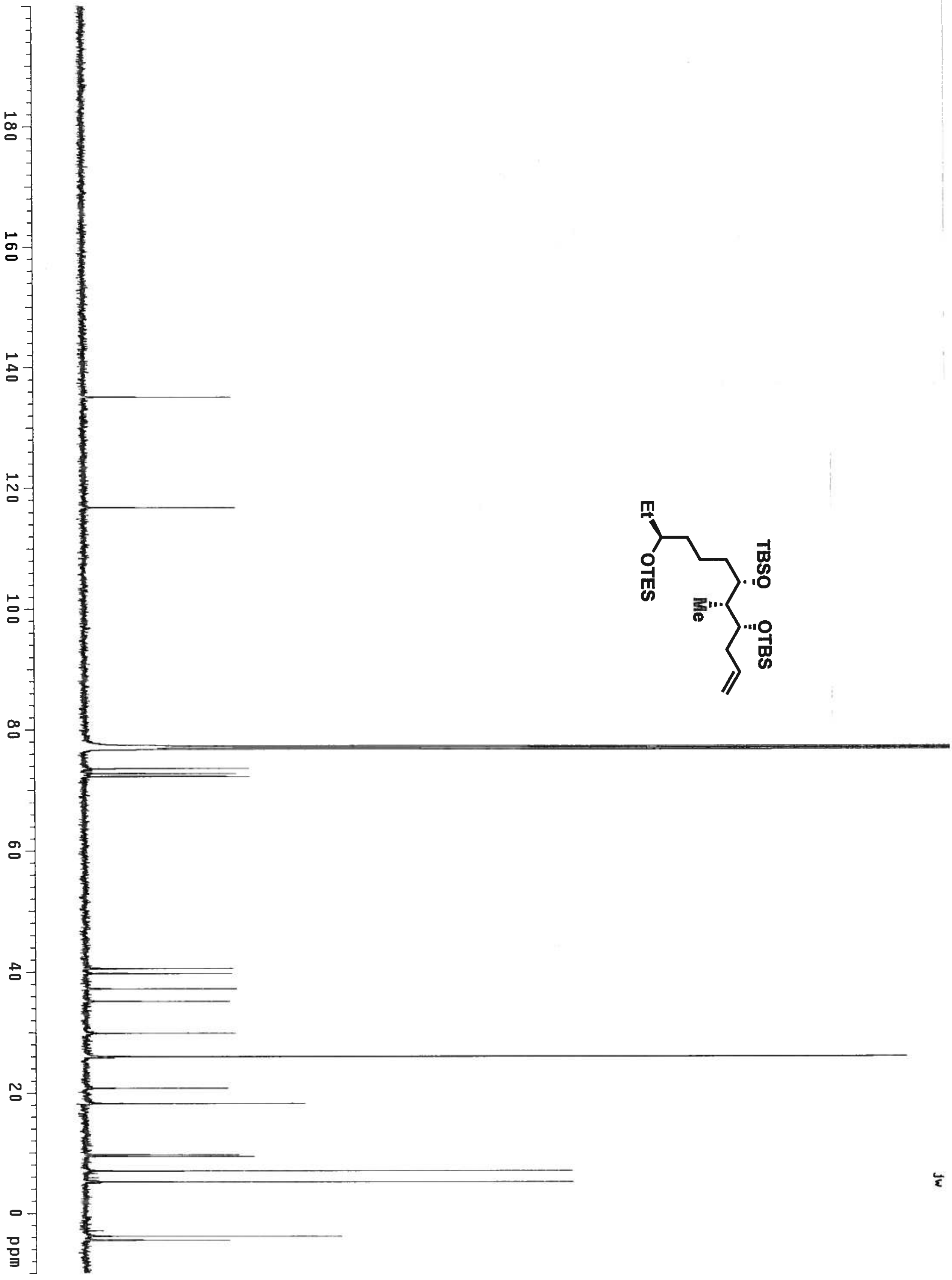
ss




ss
Et
OH
O
O
Me
180
160
140
120
100
80
60
40
20
ppm

jw





jw

Elemental Composition Report

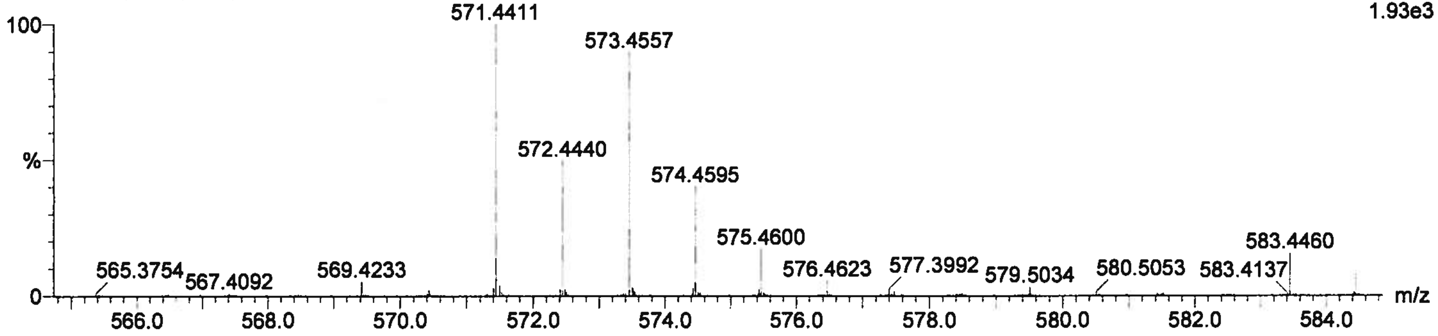
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -50.0, max = 100.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

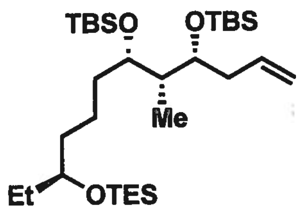
Monoisotopic Mass, Odd and Even Electron Ions
108 formula(e) evaluated with 1 results within limits (up to 10 closest results for each mass)

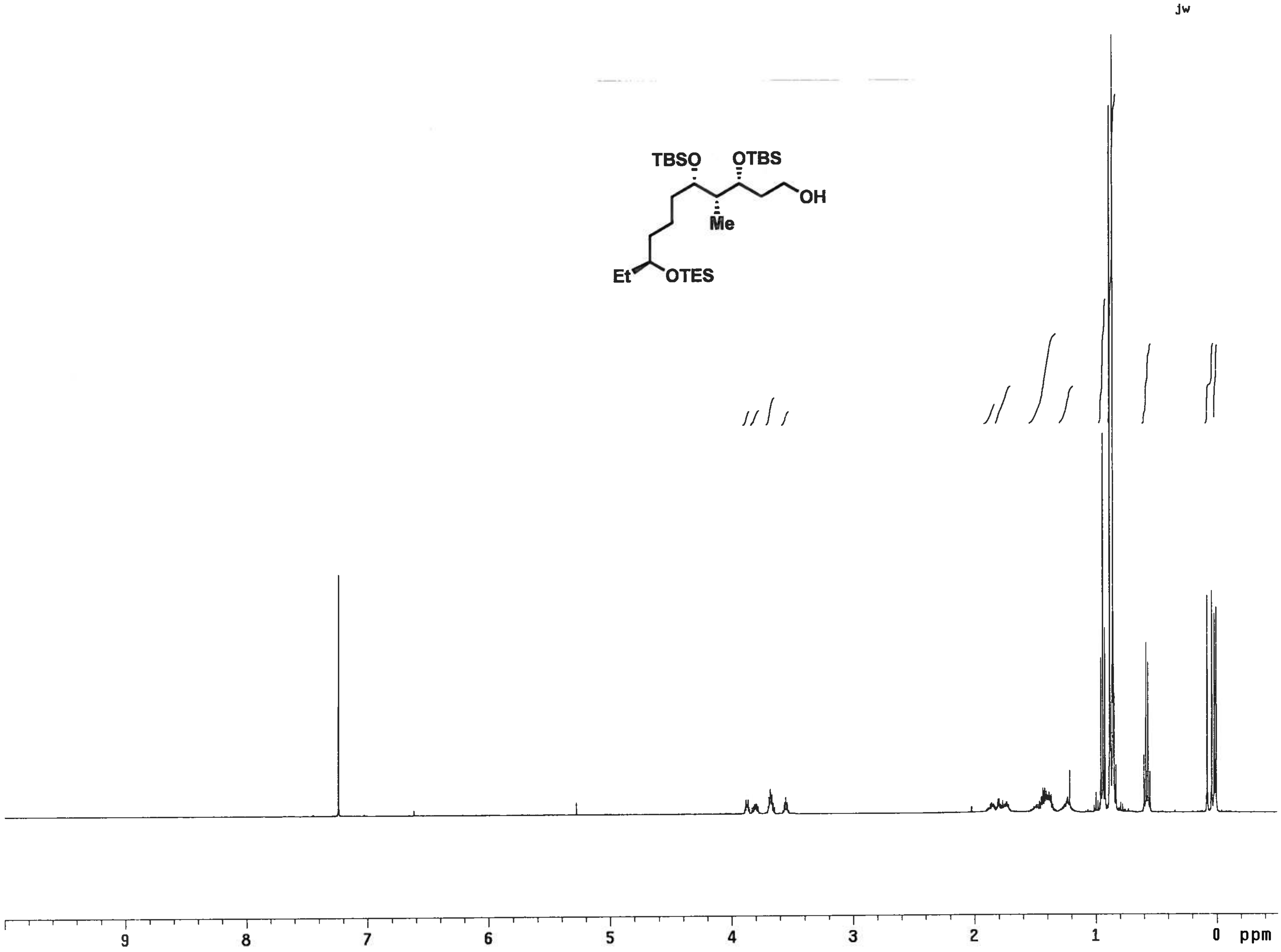
HJ04-010
LIU2032 32 (0.321) Cn (Cen,3, 80.00, Ht); Cm (25:50)

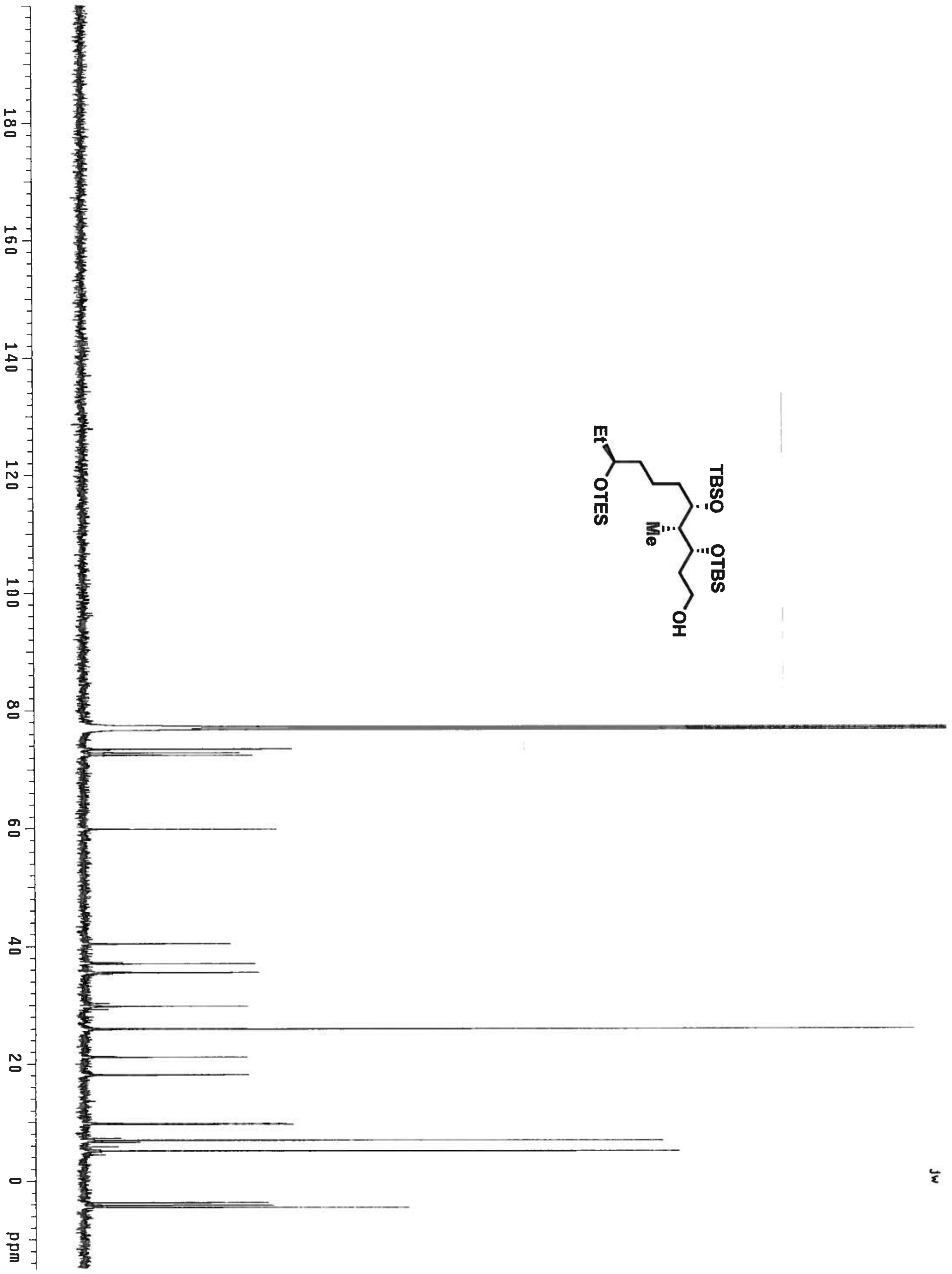
14-Nov-200517:20:42
Voltage CI+
1.93e3



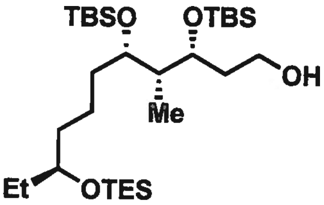
```
Minimum:                                      -50.0
Maximum:                  500.0      5.0      100.0

Mass       Calc. Mass     mDa        PPM      DBE        Score        Formula

573.4557   573.4555       0.2        0.4      0.5        1            C31   H69   O3   Si3
```



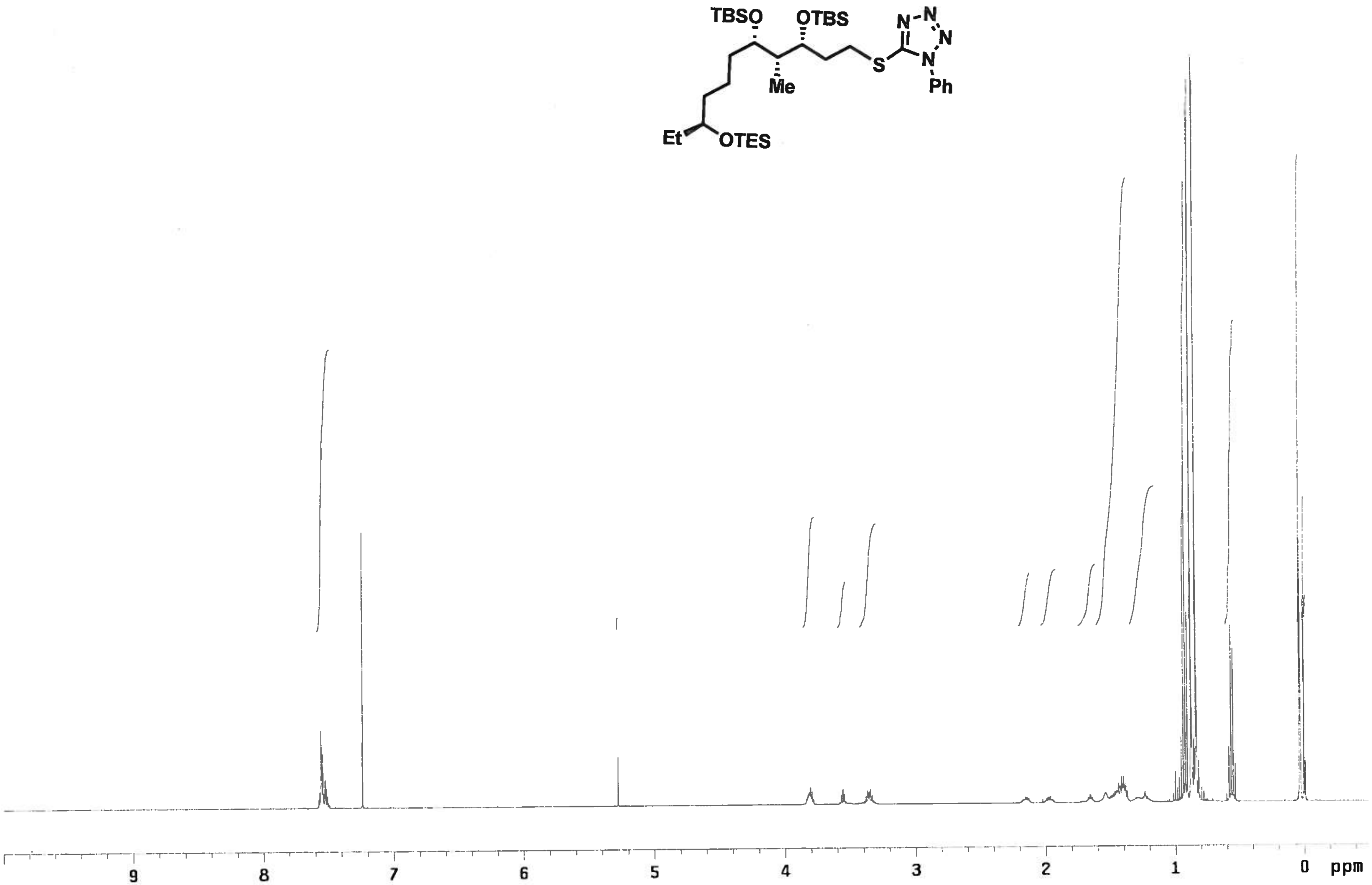

jw
TBSO
OTBS
OH
Me
Et
OTES
9
8
7
6
5
4
3
2
1
0
ppm

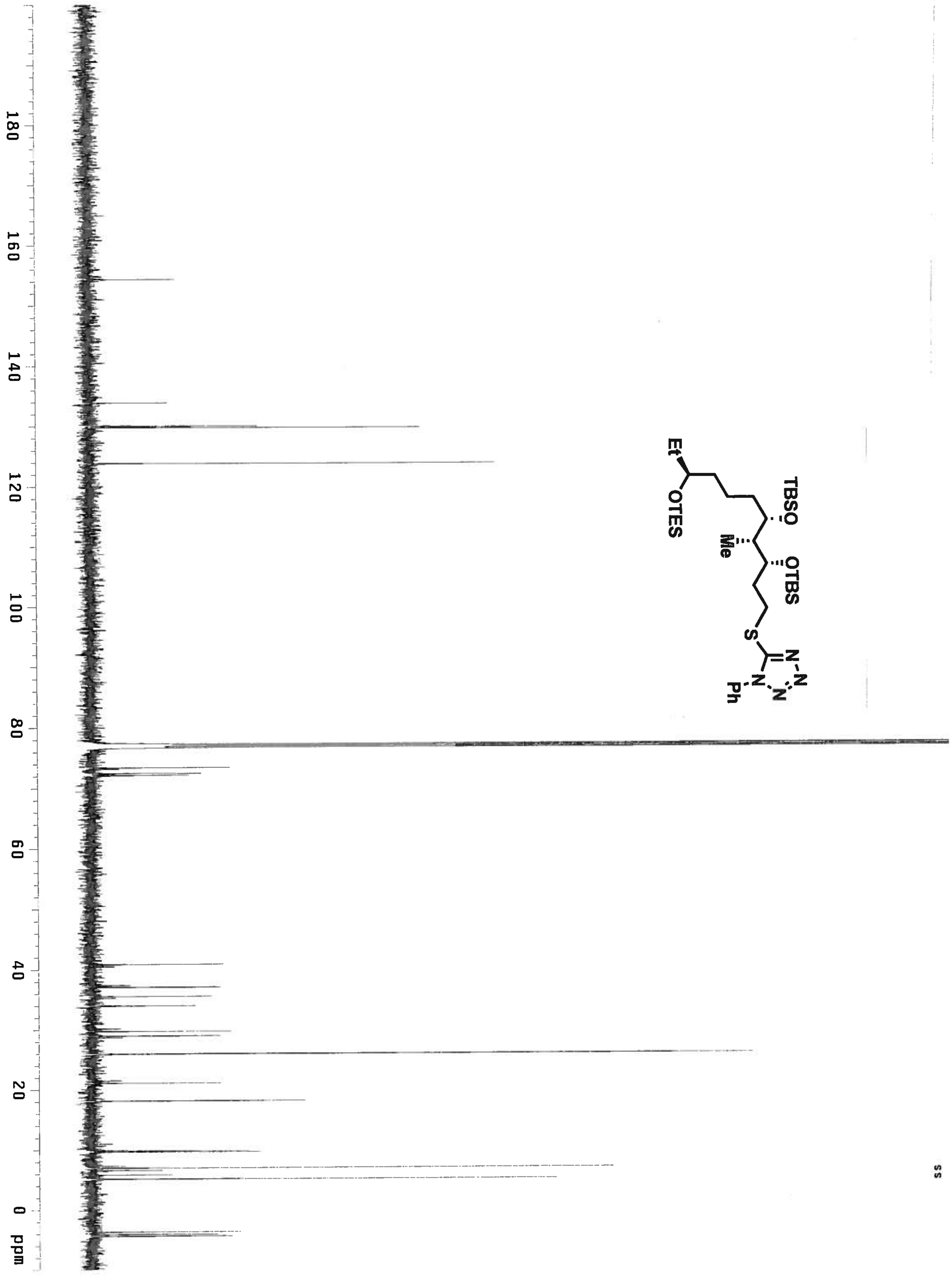

180
160
140
120
100
80
60
40
20
0
ppm
TBSO
OTBS
Me
OH
Et
OTES
jw

```
                         Elemental Composition                  Date : 27-APR-2004

File:LIU8908 Ident:14_18 SMO(1,5) BSUB(128,15,-3.0)
ZAB-E4F CI+ Voltage BpI:398384 TIC:19857072 Flags:NORM
File Text:FG 69
  Heteroatom Max:    50     Ion:  Both Even and Odd
  Limits:
                                                        -0.5    0     0     0    3
  577.448257              5.0                           60.0  200   400     9    3

     Mass           mDa    PPM    Calc. Mass     DBE     C     H     O   Si

  577.448257        2.1    3.7   577.450372     -0.5    30    69     4    3
```



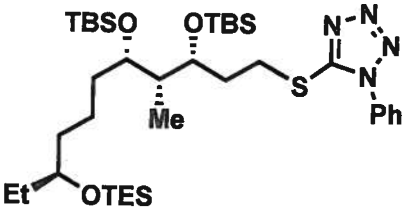
ss



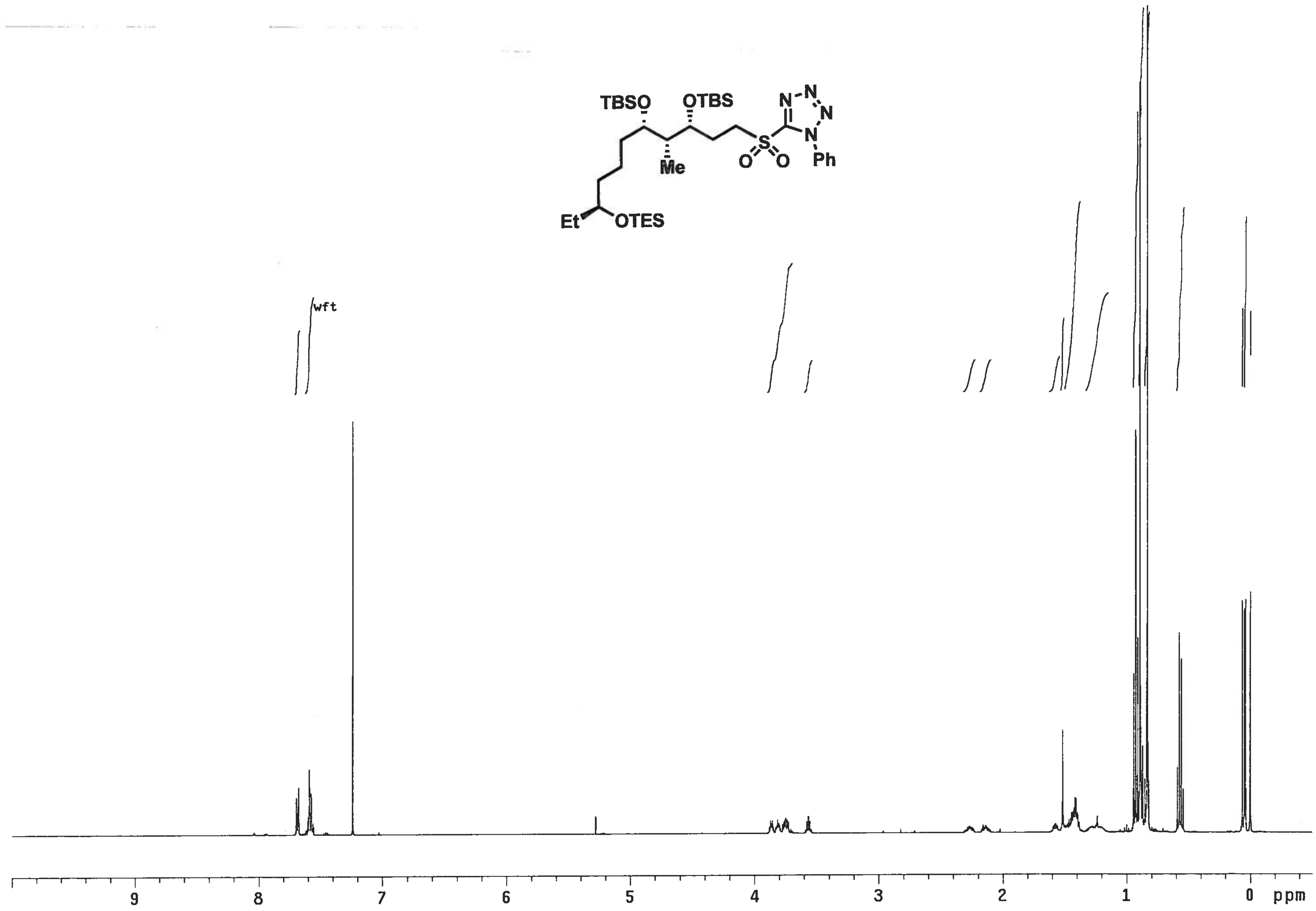


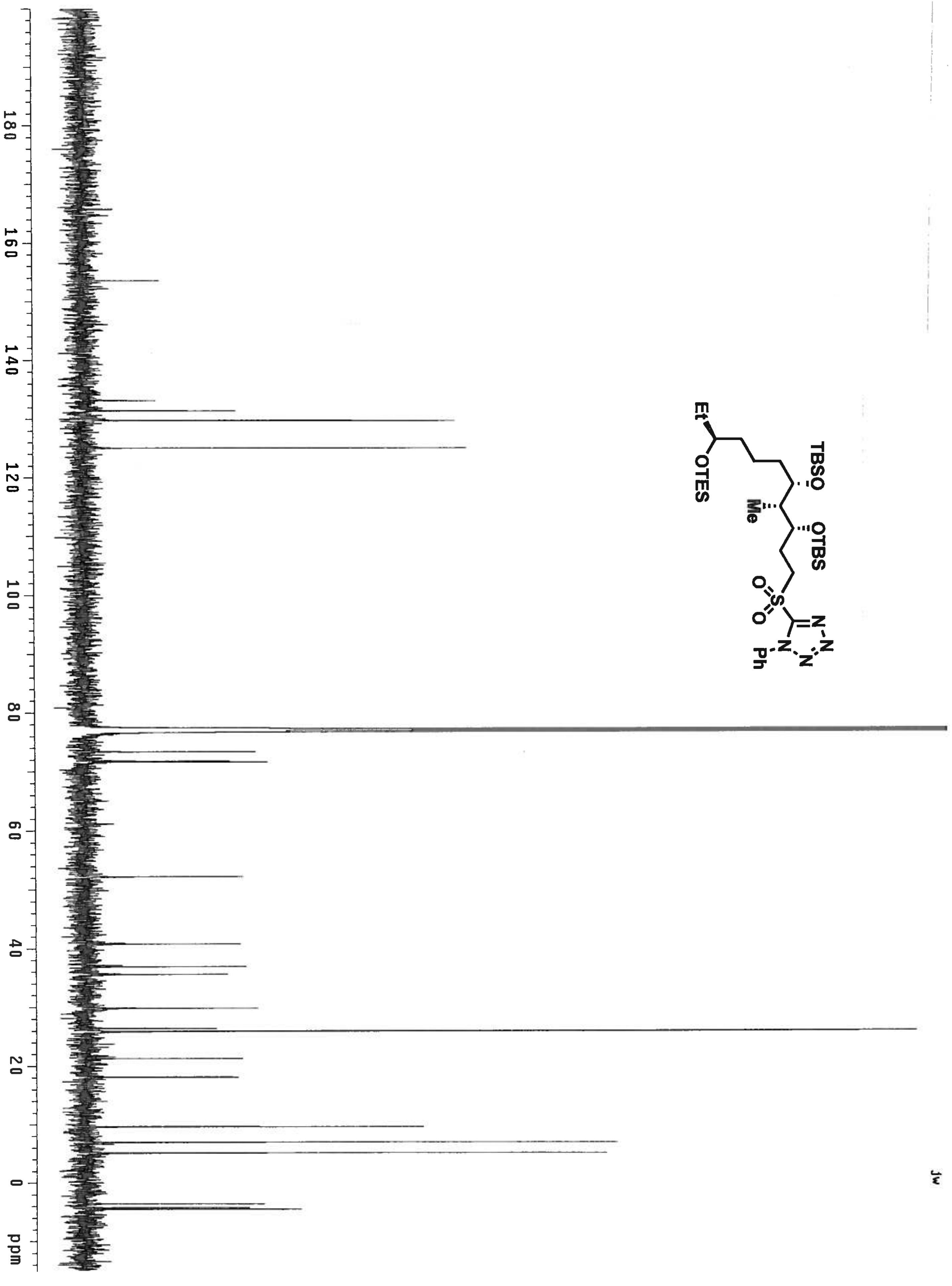
180
160
140
120
100
80
60
40
20
0
ppm
TBSO
OTBS
Me
Et
OTES
S
N
N
N
N
Ph
ss

```
                    Elemental Composition                Date : 27-APR-2004
File:LIU8907 Ident:18 SMO(1,5) BSUB(128,15,-3.0)
ZAB-E4F CI+ Voltage BpI:36256 TIC:2326358 Flags:NORM
File Text:FG 69
  Heteroatom Max:    50    Ion:  Both Even and Odd
  Limits:
                                                -0.5     0     0    4    0    3    1
  737.468879             5.0                    60.0   200   400    4    9    3    1

     Mass          mDa   PPM   Calc. Mass       DBE     C     H    N    O   Si    S

  737.468879       2.2   3.0   737.471125       6.5    37    73    4    3    3    1
```



jw

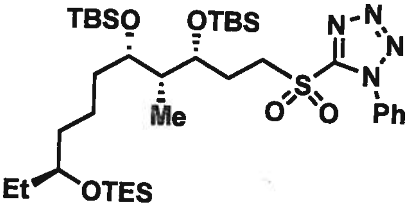


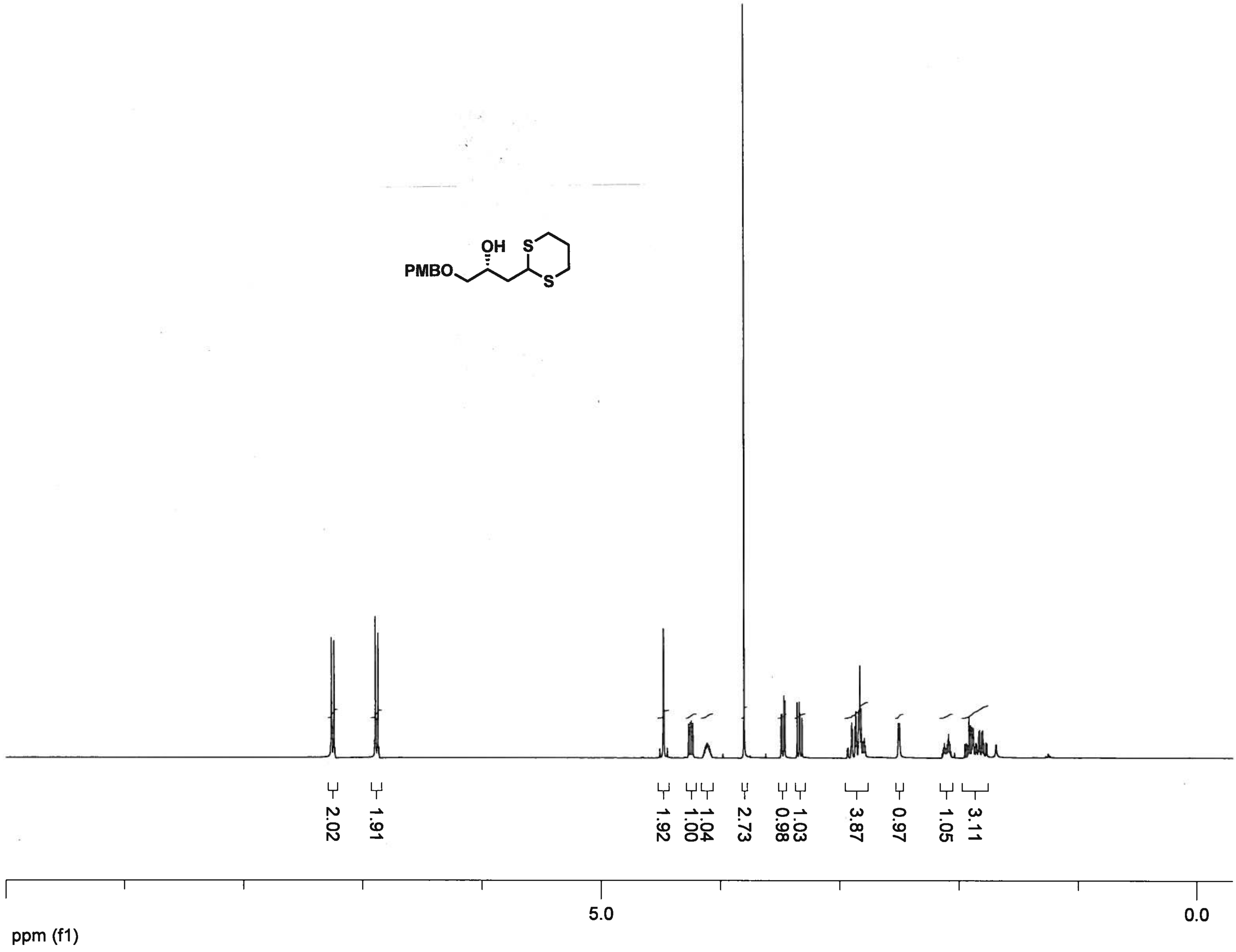


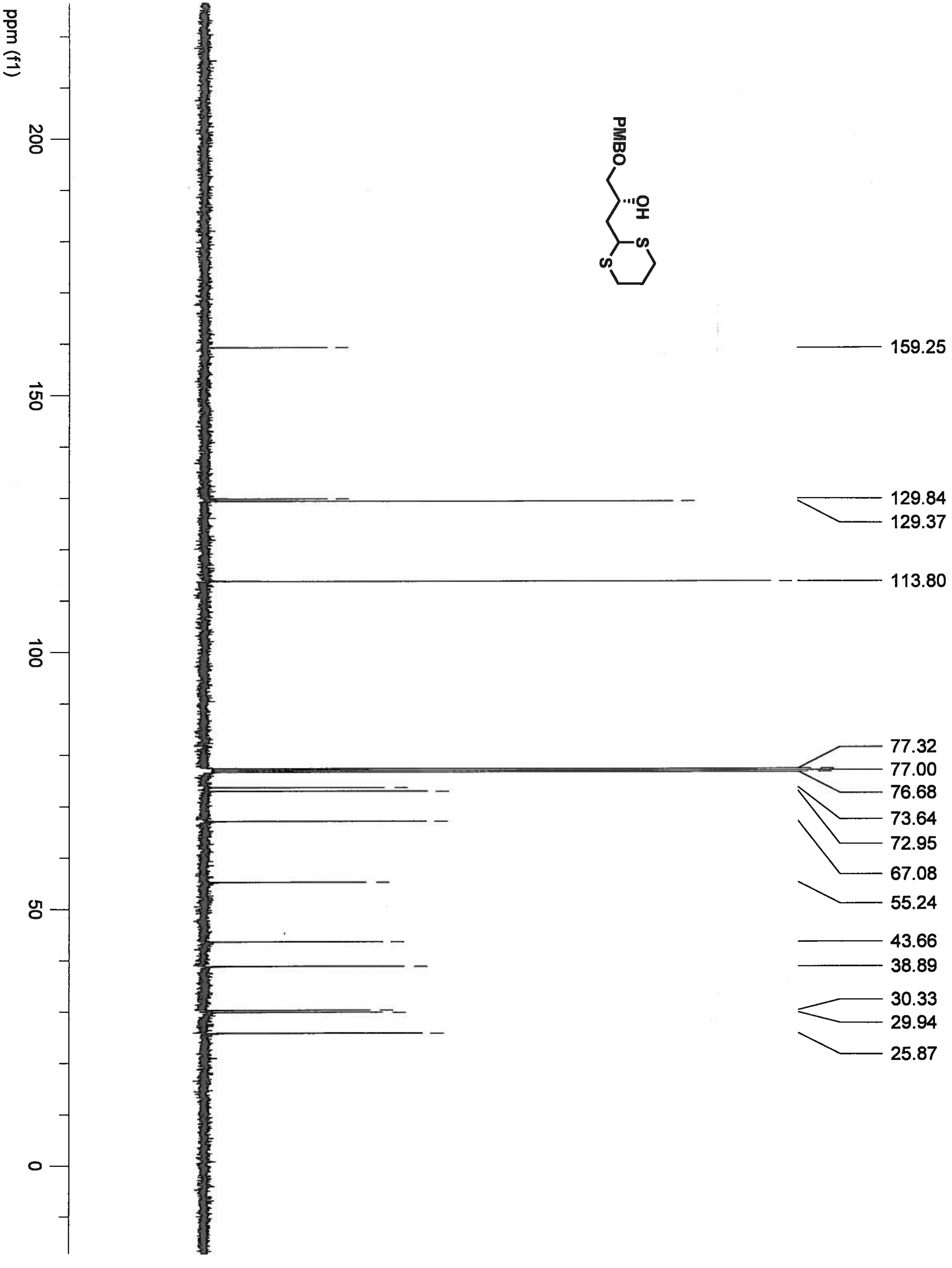
180
160
140
120
100
80
60
40
20
0
ppm
TBSO
OTBS
Me
Et
OTES
O
S
O
N
N
N
N
Ph

Jw

```
                         Elemental Composition                Date : 21-MAY-2004

File:LIU8909 Ident:46_49 SMO(1,5) BSUB(128,15,-3.0)
ZAB-E4F CI+ Voltage BpI:49478 TIC:4286041 Flags:NORM
File Text:I-RP-129
  Heteroatom Max:    20     Ion:  Both Even and Odd
  Limits:
                                                 -0.5     0     0    4    0    3    1
  769.461965            5.0                      20.0   200   400    4    8    3    1

     Mass          mDa     PPM    Calc. Mass      DBE     C     H    N    O   Si    S

  769.461965      -1.0    -1.3    769.460954      6.5    37    73    4    5    3    1
```




PMBO
OH
S
S
2.02
1.91
1.92
1.00
1.04
2.73
0.98
1.03
3.87
0.97
1.05
3.11
5.0
0.0
ppm (f1)


ppm (f1)
200
150
100
50
0
PMBO
OH
S
S
159.25
129.84
129.37
113.80
77.32
77.00
76.68
73.64
72.95
67.08
55.24
43.66
38.89
30.33
29.94
25.87

Elemental Composition Search Report:

Target Mass:

Target m/z = 337.0904 ± 0.002
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
Na	22.989770	1	1
O	15.994915	3	3
S	31.972071	2	2

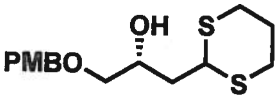
Additional Search Restrictions:

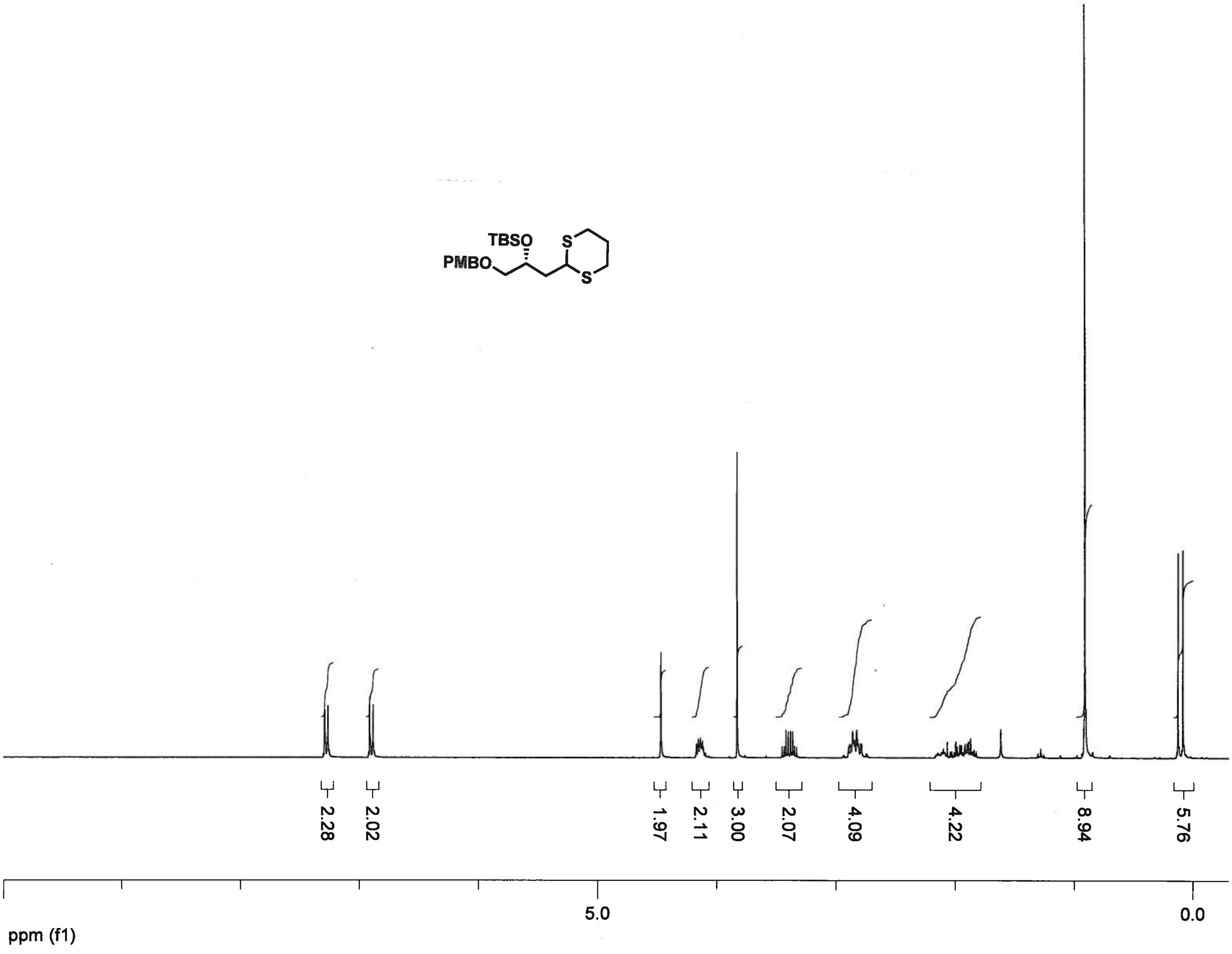
None

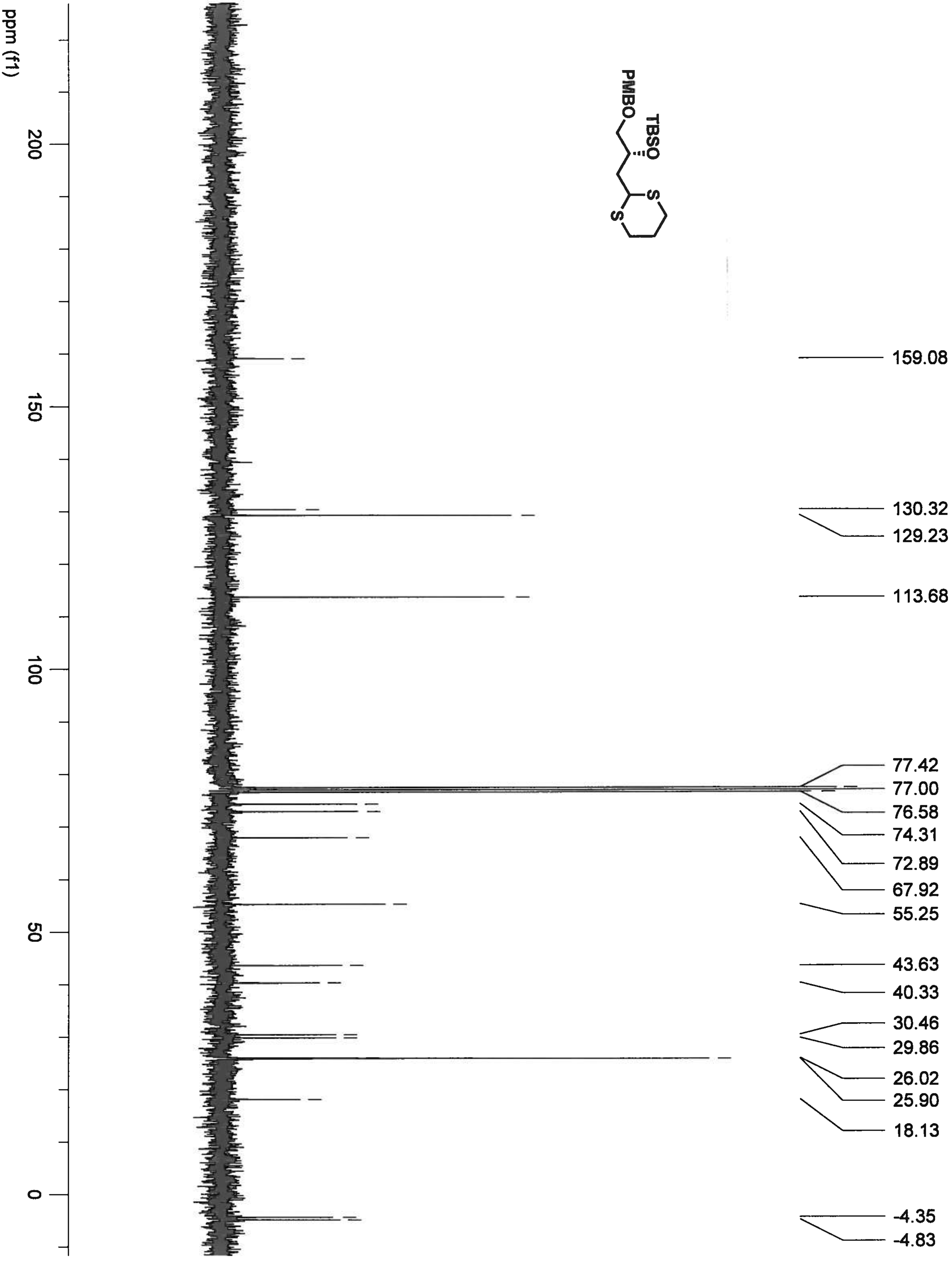
Search Results:

Number of Hits = 1

m/z	Delta m/z	DBE	Formula
337.09026	0.00014	5.0	$C_{15}H_{22}NaO_3S_2^{+1}$




TBSO
PMBO
S
S
2.28
2.02
1.97
2.11
3.00
2.07
4.09
4.22
8.94
5.76
5.0
0.0
ppm (f1)


ppm (f1)
200
150
100
50
0
PMBO
TBSO
S
S
159.08
130.32
129.23
113.68
77.42
77.00
76.58
74.31
72.89
67.92
55.25
43.63
40.33
30.46
29.86
26.02
25.90
18.13
-4.35
-4.83

Elemental Composition Search Report:

Target Mass:

Target m/z = 429.1955 ± 0.002
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	3	3
S	31.972071	2	2
Si	27.976927	1	1

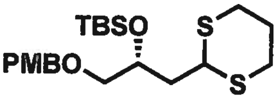
Additional Search Restrictions:

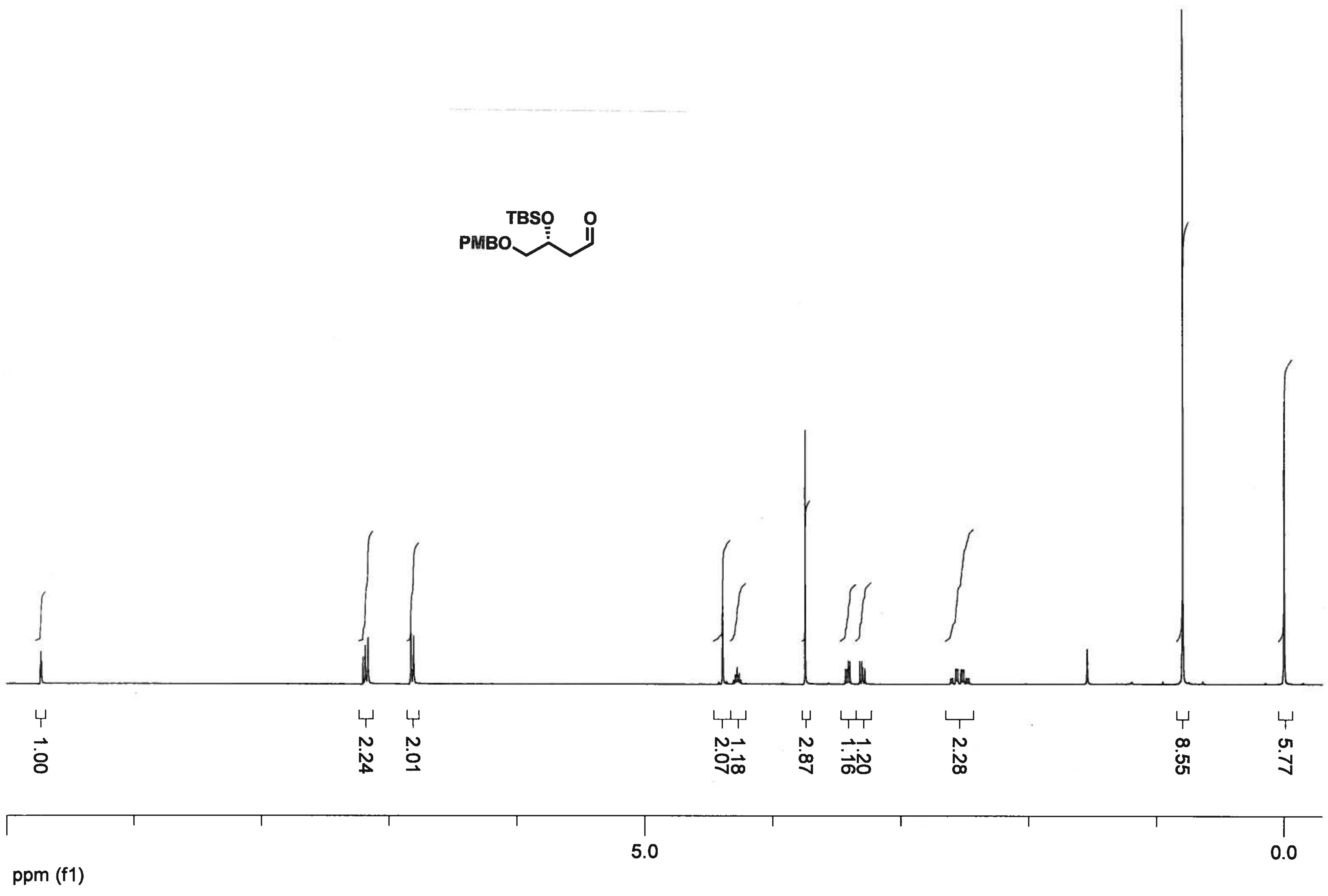
None

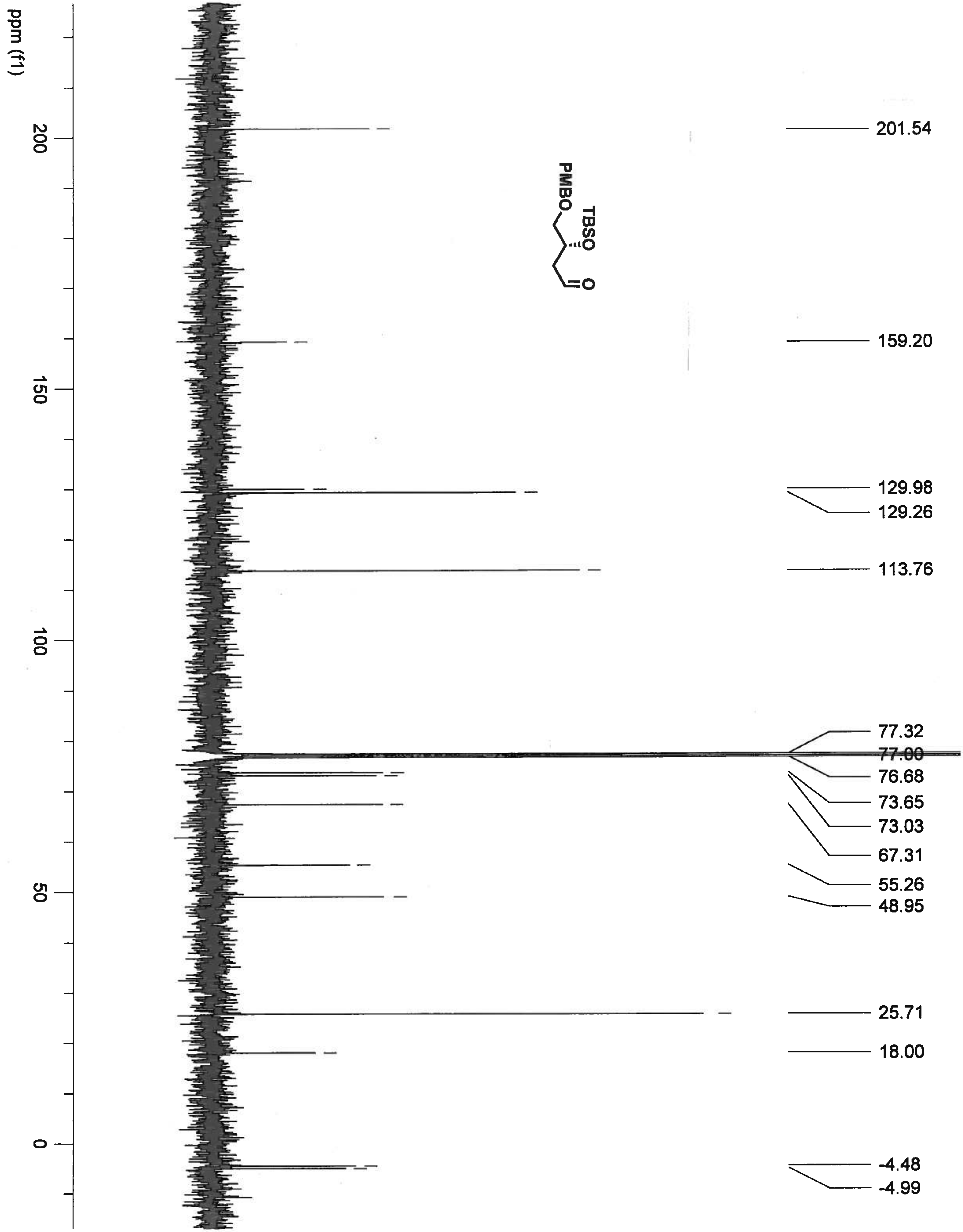
Search Results:

Number of Hits = 1

m/z	Delta m/z	DBE	Formula
429.19479	0.00071	4.5	$C_{21}H_{37}O_3S_2Si^{+1}$



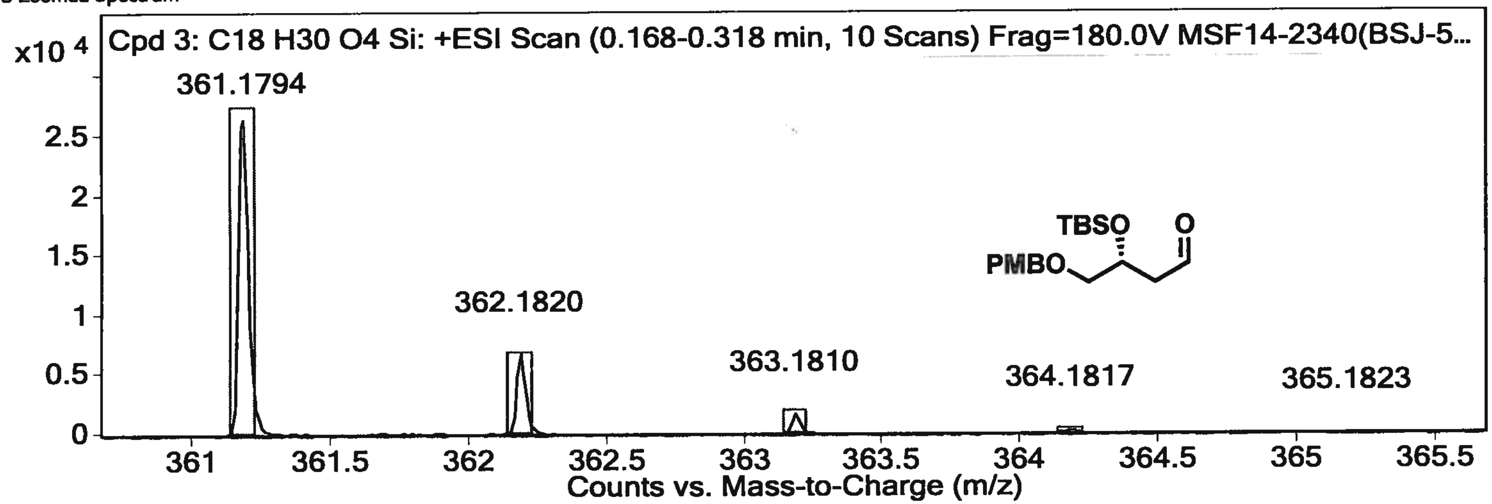

TBSO
O
PMBO
1.00
2.24
2.01
2.07
1.18
2.87
1.16
1.20
2.28
8.55
5.77
5.0
0.0
ppm (f1)


ppm (f1)
200
150
100
50
0
PMBO
TBSO
O
201.54
159.20
129.98
129.26
113.76
77.32
77.00
76.68
73.65
73.03
67.31
55.26
48.95
25.71
18.00
-4.48
-4.99

Target Compound Screening Report

Data File	MSF14-2340(BSJ-5-047)_hrESIpos5.d	**Sample Name**	2340(BSJ-5-047)	**Comment**	2340(BSJ-5-047)
Position	P1-A3	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	pos.m	**Acquired Time**	7/21/2014 4:47:02 PM	**DA Method**	Ian.m

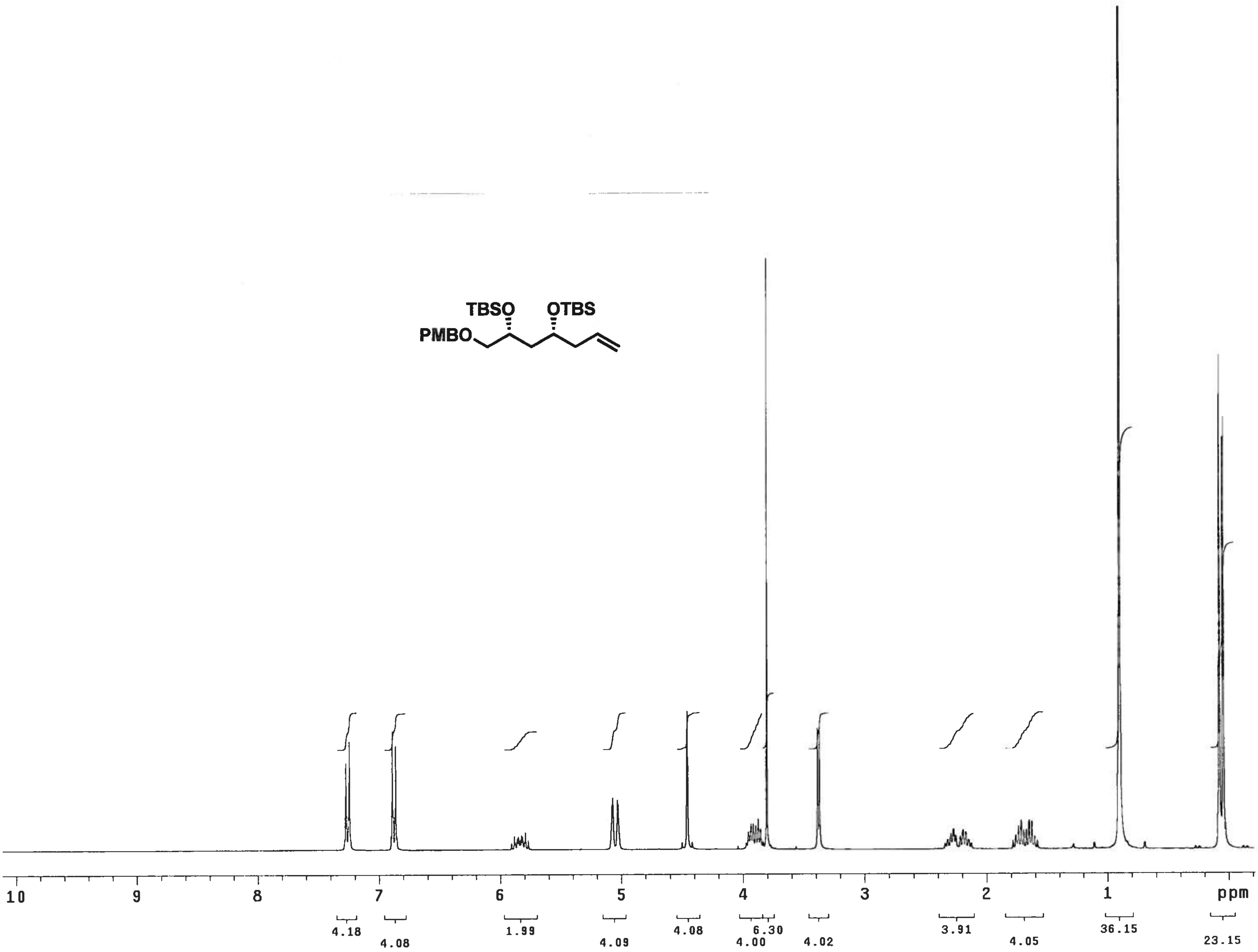
MS Zoomed Spectrum

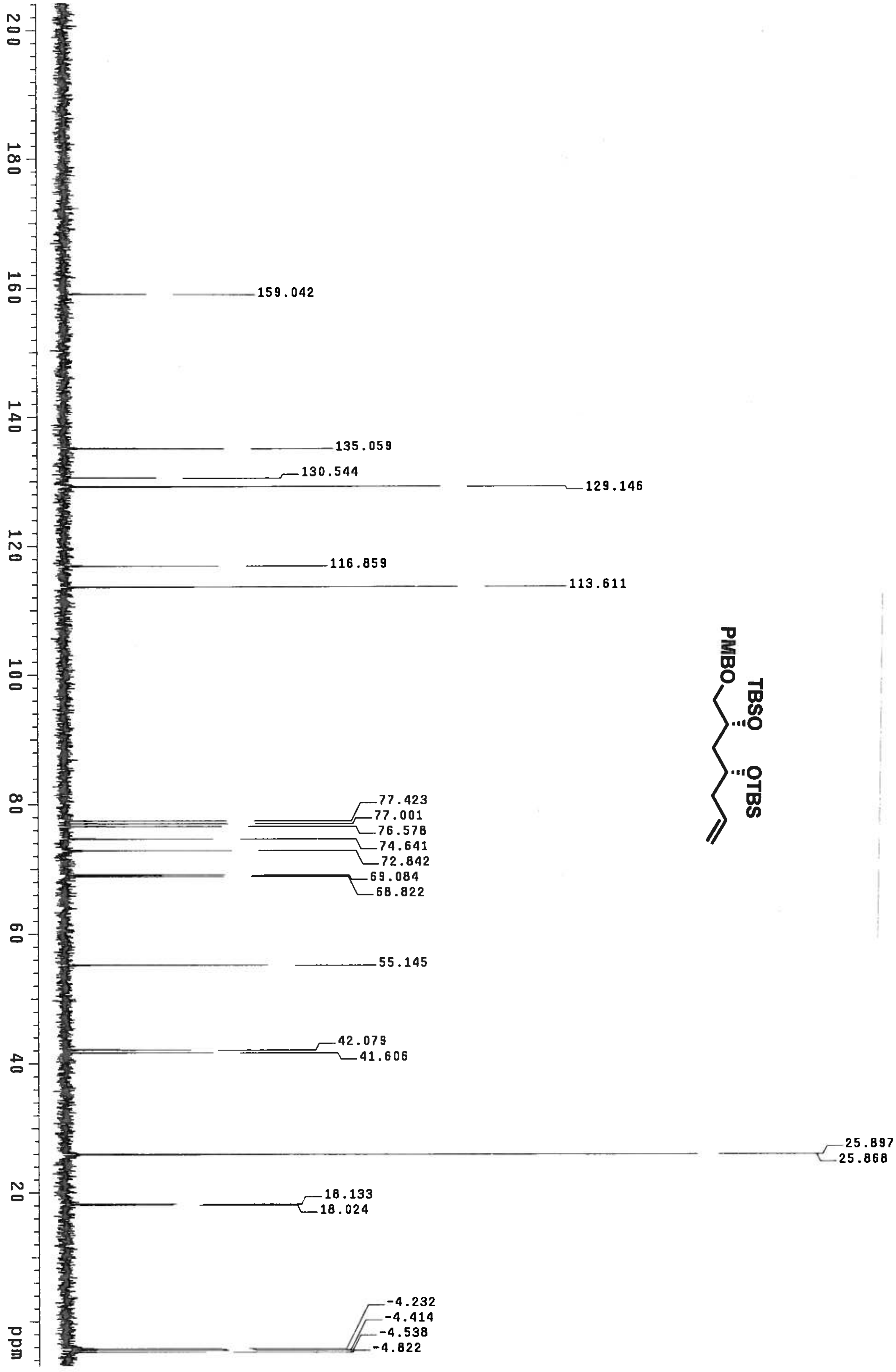


MS Spectrum Peak List

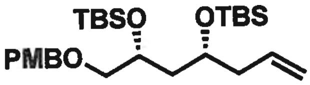
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
361.17940	361.18060	1	27353.18	C18H30O4Si	(M+Na)+	3.18
362.18200	362.18320	1	6789.95	C18H30O4Si	(M+Na)+	3.18
363.18100	363.18180	1	1710.78	C18H30O4Si	(M+Na)+	2.21
364.18170	364.18370	1	353.58	C18H30O4Si	(M+Na)+	5.48
365.18230	365.18550	1	41.43	C18H30O4Si	(M+Na)+	8.81
393.20600			227713.97			

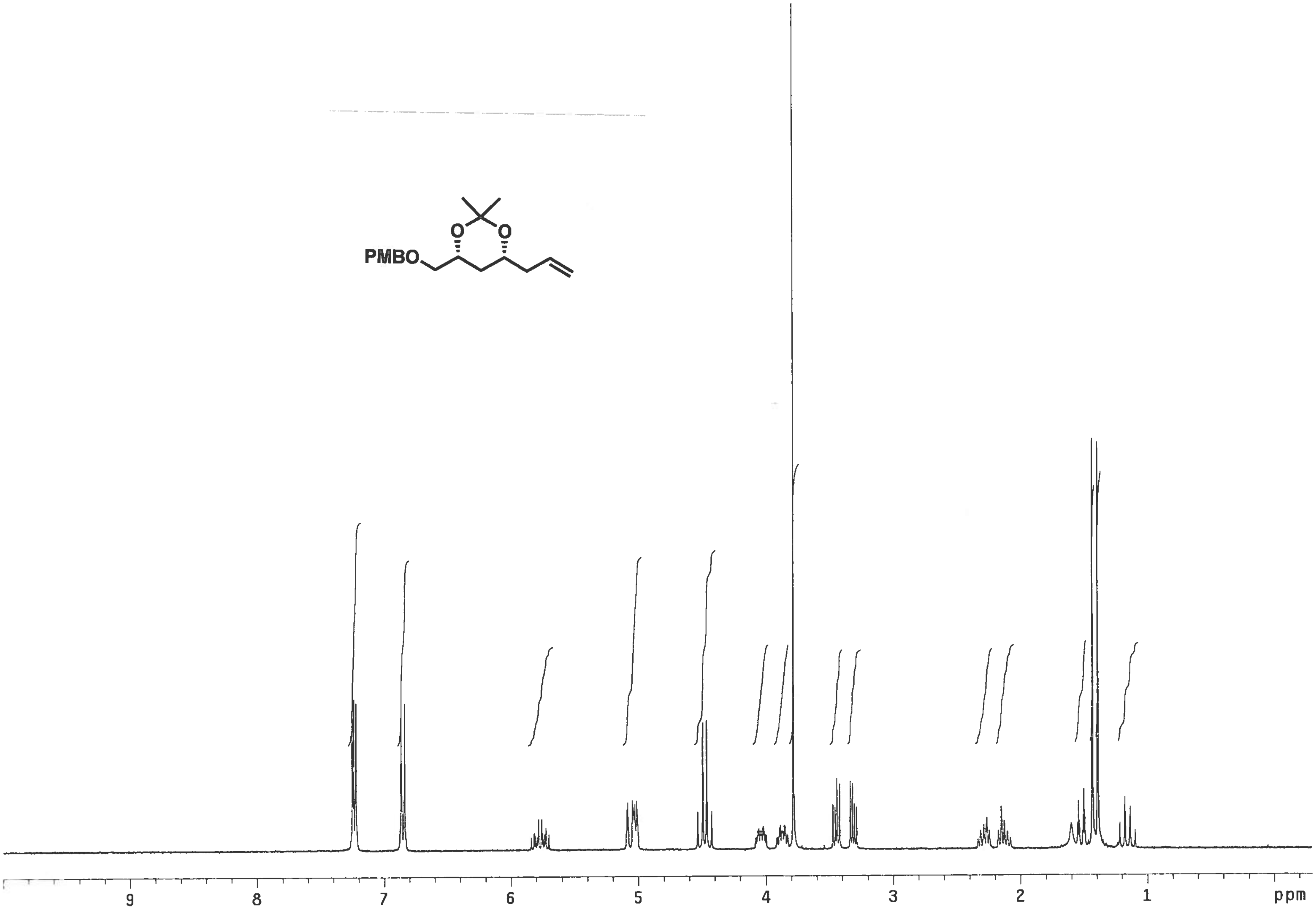
--- End Of Report ---

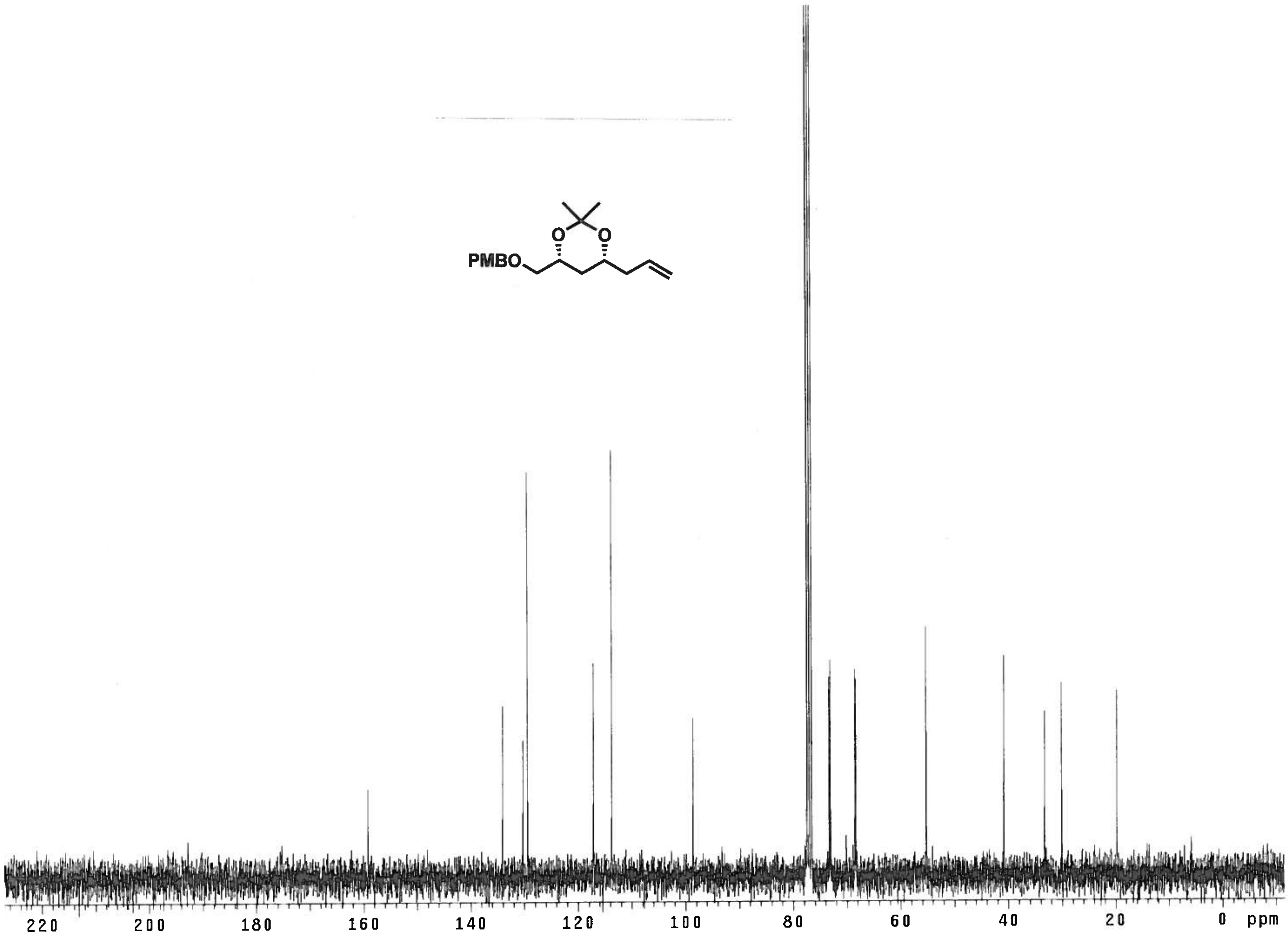

TBSO
OTBS
PMBO
10
9
8
7
6
5
4
3
2
1
ppm
4.18
4.08
1.99
4.09
4.08
4.00
6.30
4.02
3.91
4.05
36.15
23.15

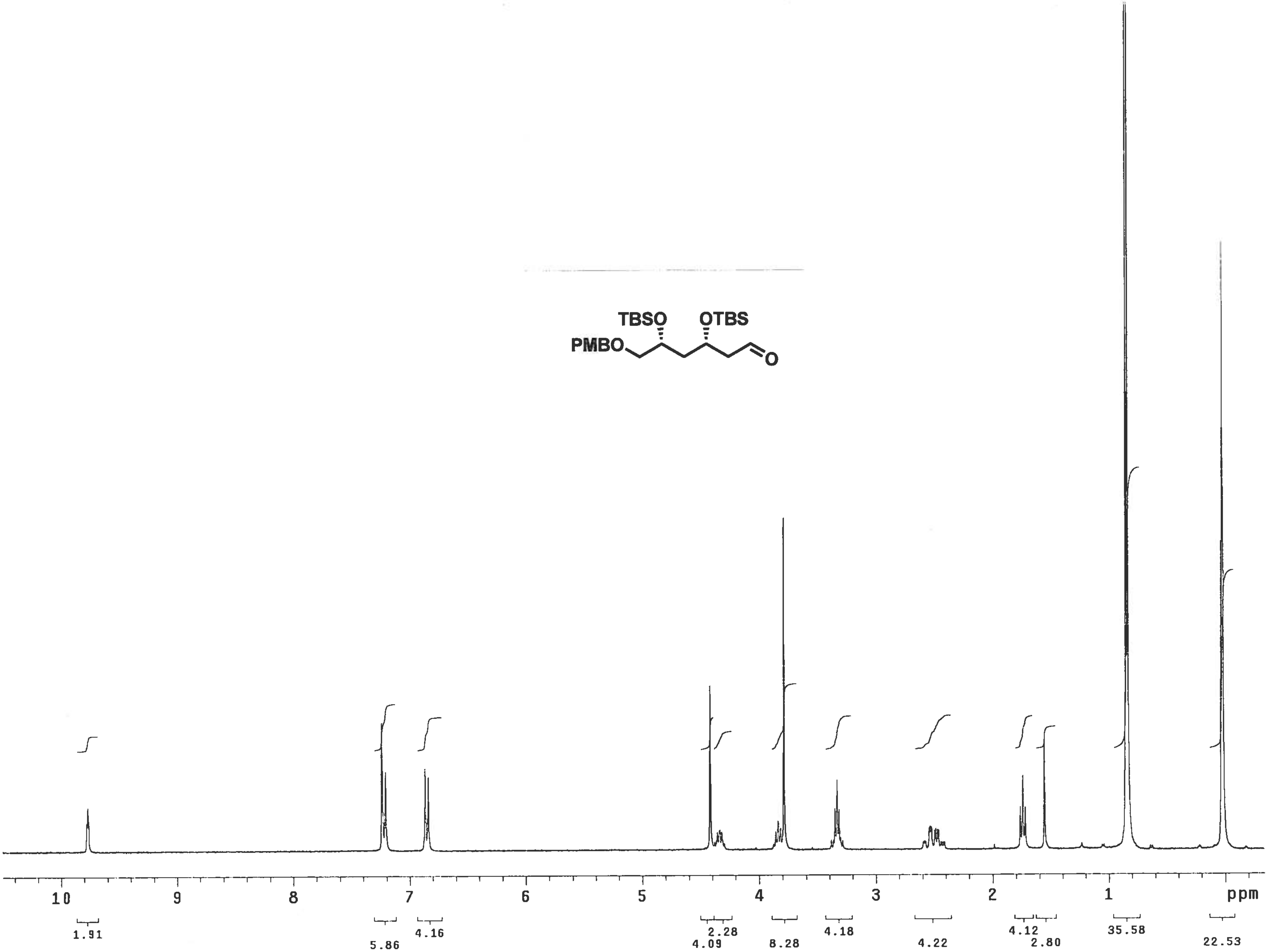

200
180
160
140
120
100
80
60
40
20
ppm
159.042
135.059
130.544
129.146
116.859
113.611
77.423
77.001
76.578
74.641
72.842
69.084
68.822
55.145
42.079
41.606
25.897
25.868
18.133
18.024
-4.232
-4.414
-4.538
-4.822
TBSO
OTBS
PMBO

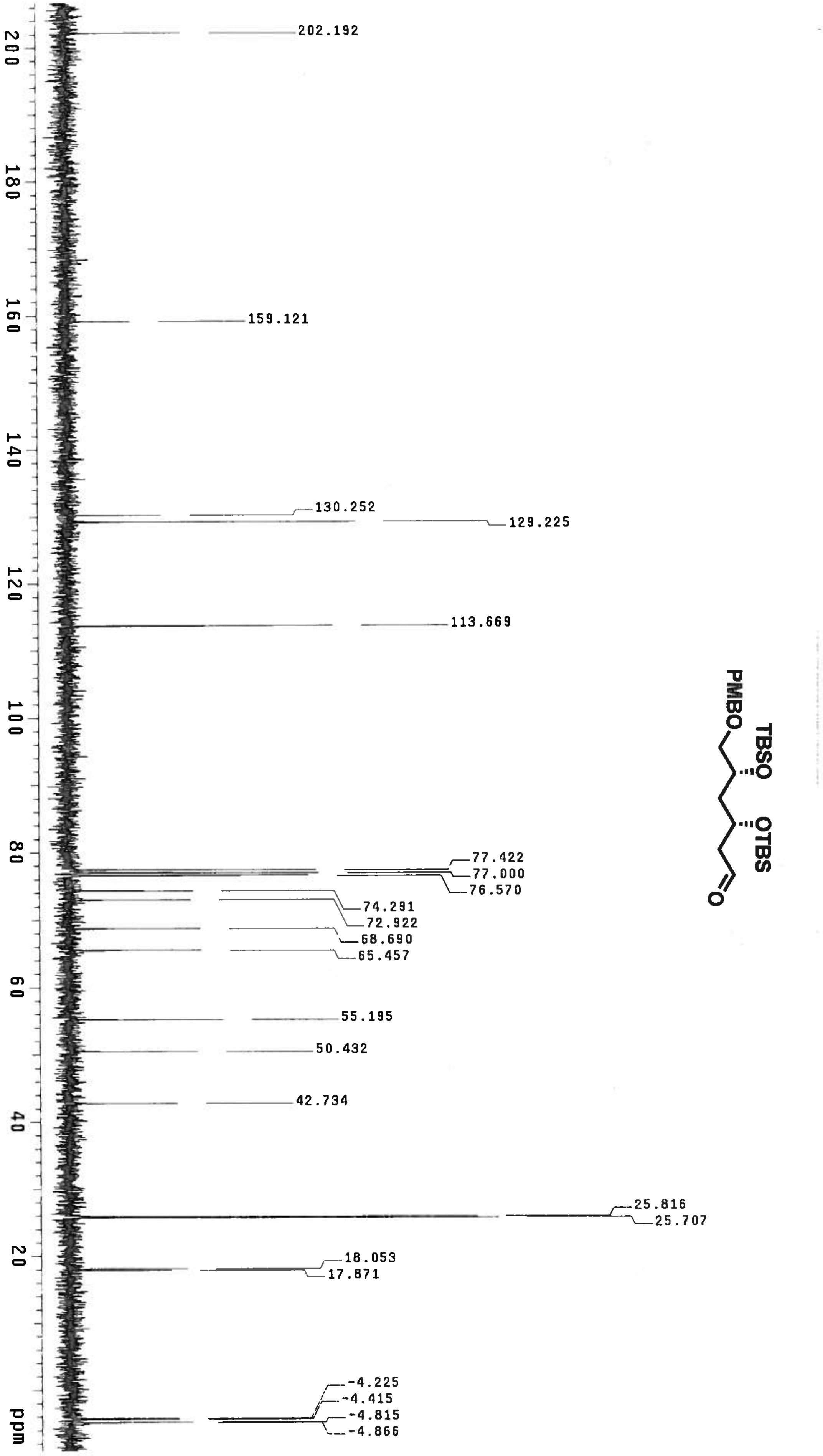
```
                    Elemental Composition                  Date : 12-OCT-2004

File:O12LU3699 Ident:1_5 SMO(1,7) PKD(7,3,7,0.50%,80.0,80.00%,F,F)
ZAB-E4F CI+ Voltage BpI:936128 TIC:35355800 Flags:NORM DEL ERR
File Text:QINGQUAN  WU   /  WQQ-03699
  Heteroatom Max:    20     Ion:  Both Even and Odd
  Limits:
                                               -0.5    0    0     0     0
  493.314893            5.0                    10.0  200  400     4     2

    Mass          mDa     PPM    Calc. Mass     DBE     C    H     O    Si

  493.314893      2.0     4.2    493.316942     5.5    27   49     4     2
```



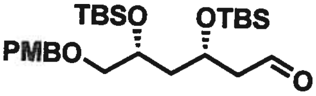

PMBO
O
O
9
8
7
6
5
4
3
2
1
ppm

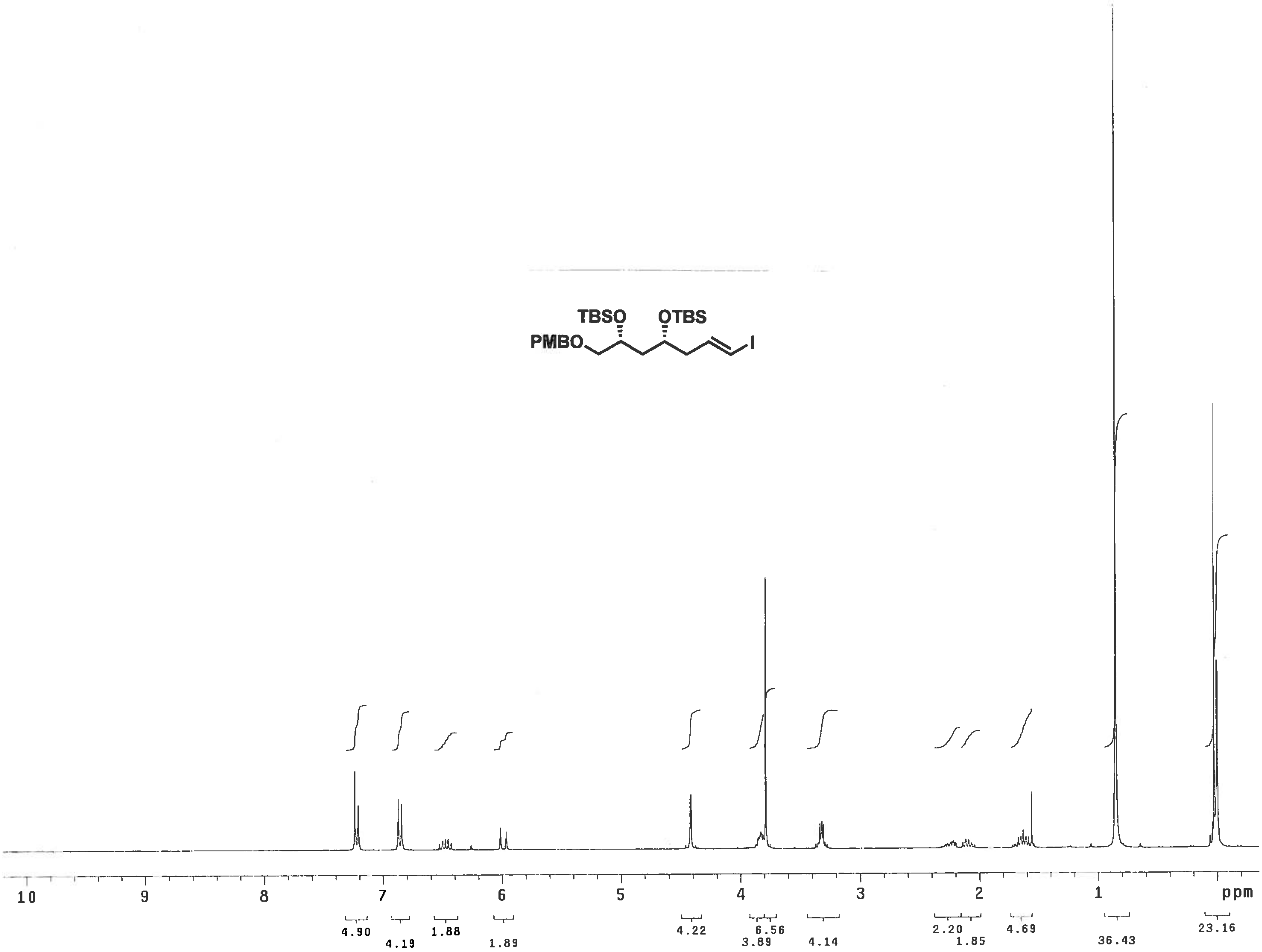

PMBO
O
O
220
200
180
160
140
120
100
80
60
40
20
0
ppm

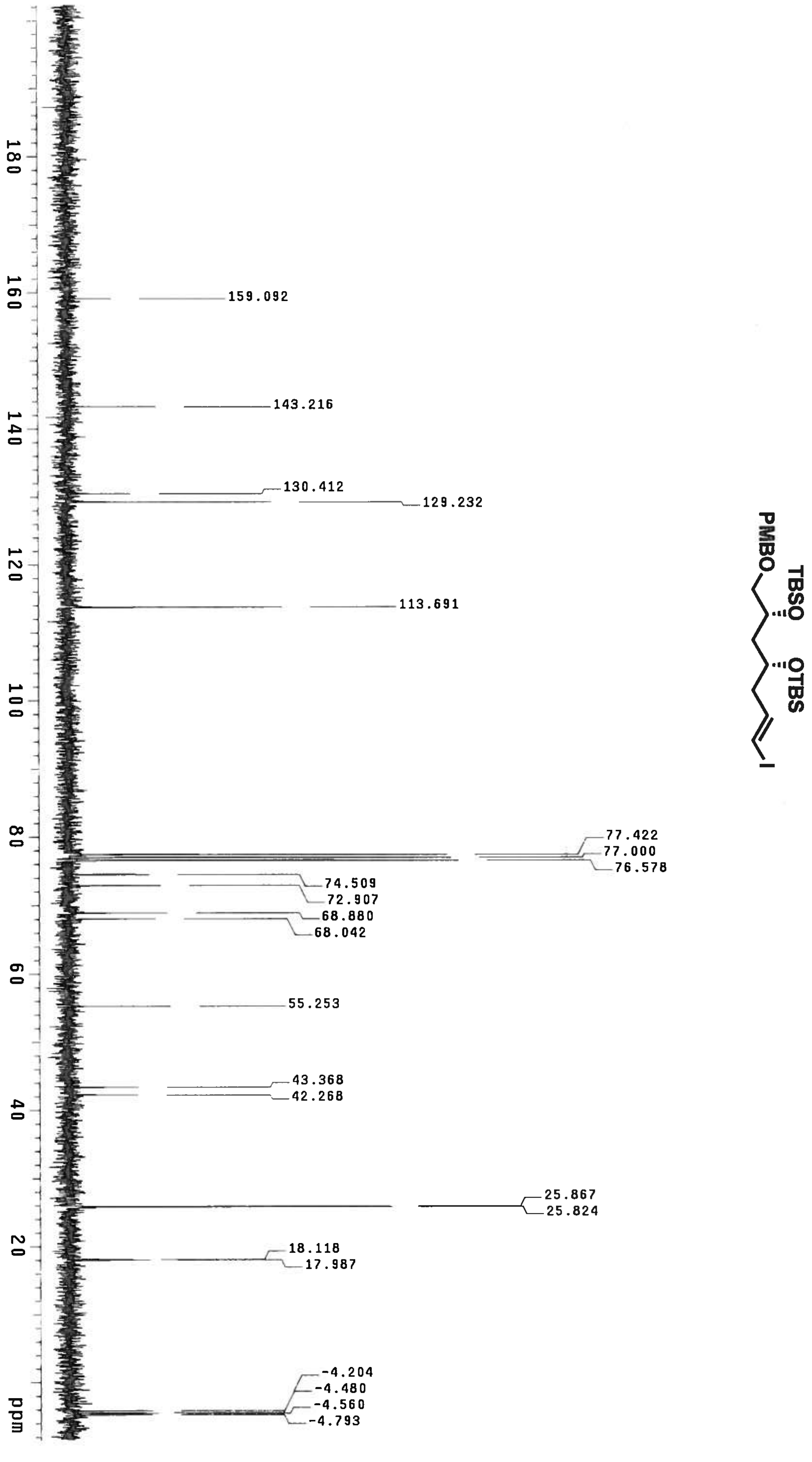

TBSO
OTBS
PMBO
O
10
9
8
7
6
5
4
3
2
1
ppm
1.91
5.86
4.16
2.28
4.09
8.28
4.18
4.22
4.12
2.80
35.58
22.53


202.192
159.121
130.252
129.225
113.669
77.422
77.000
76.570
74.291
72.922
68.690
65.457
55.195
50.432
42.734
25.816
25.707
18.053
17.871
-4.225
-4.415
-4.815
-4.866
200
180
160
140
120
100
80
60
40
20
ppm
PMBO
TBSO
OTBS
O

```
                         Elemental Composition                   Date : 12-OCT-2004

File:O12LU3700 Ident:3_6 SMO(1,7) PKD(7,3,7,0.50%,80.0,80.00%,F,F)
ZAB-E4F CI+ Voltage BpI:758560 TIC:29280920 Flags:NORM DEL ERR
File Text:QINGQUAN  WU  /  WQQ-03700
  Heteroatom Max:   20    Ion:  Both Even and Odd
  Limits:
                                                 -0.5     0     0     0     0
  495.297001             5.0                     10.0   200   400     5     2

     Mass            mDa     PPM   Calc. Mass     DBE     C     H     O    Si

  495.297001        -0.8    -1.6   495.296207     5.5    26    47     5     2
```

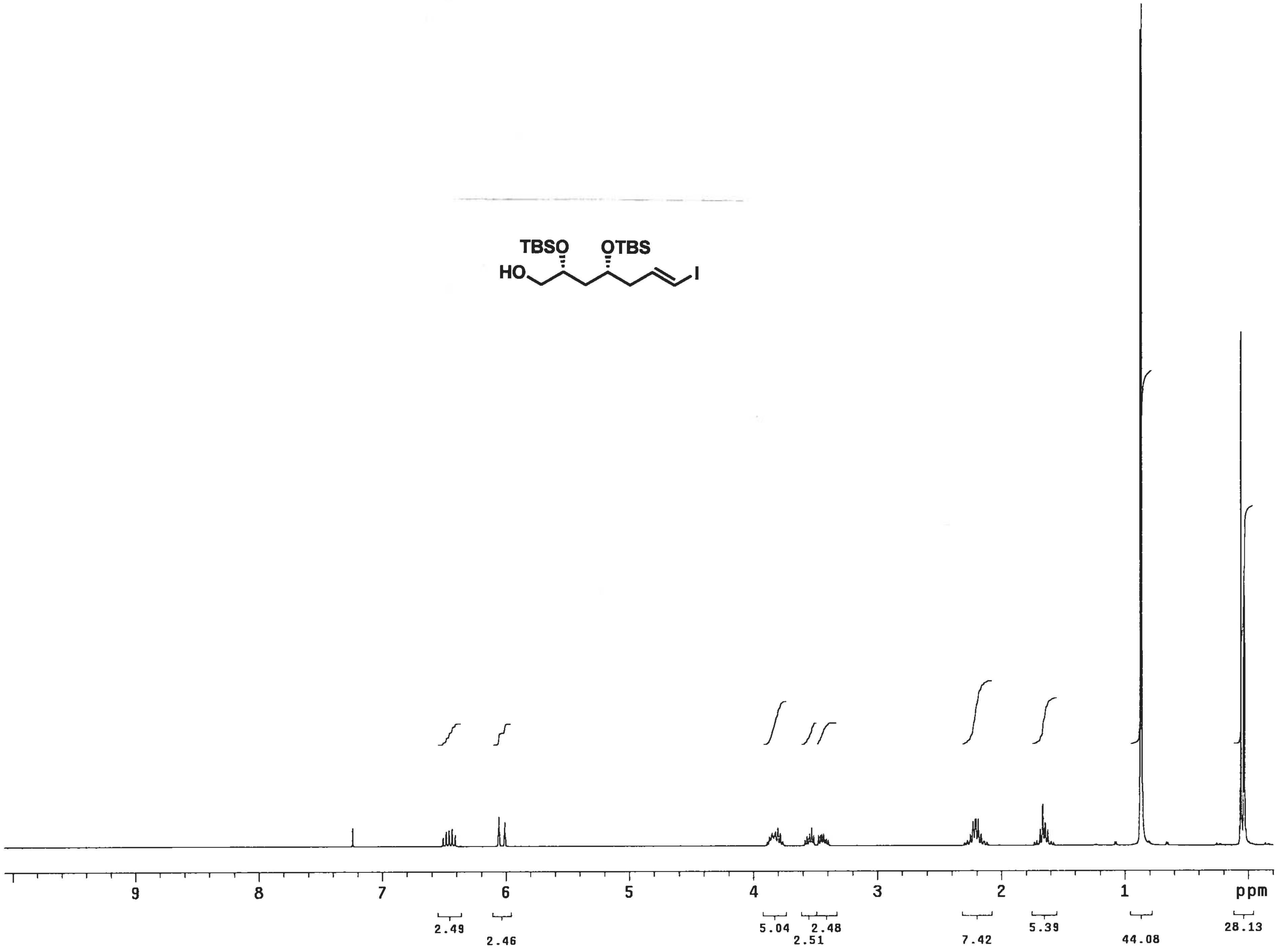


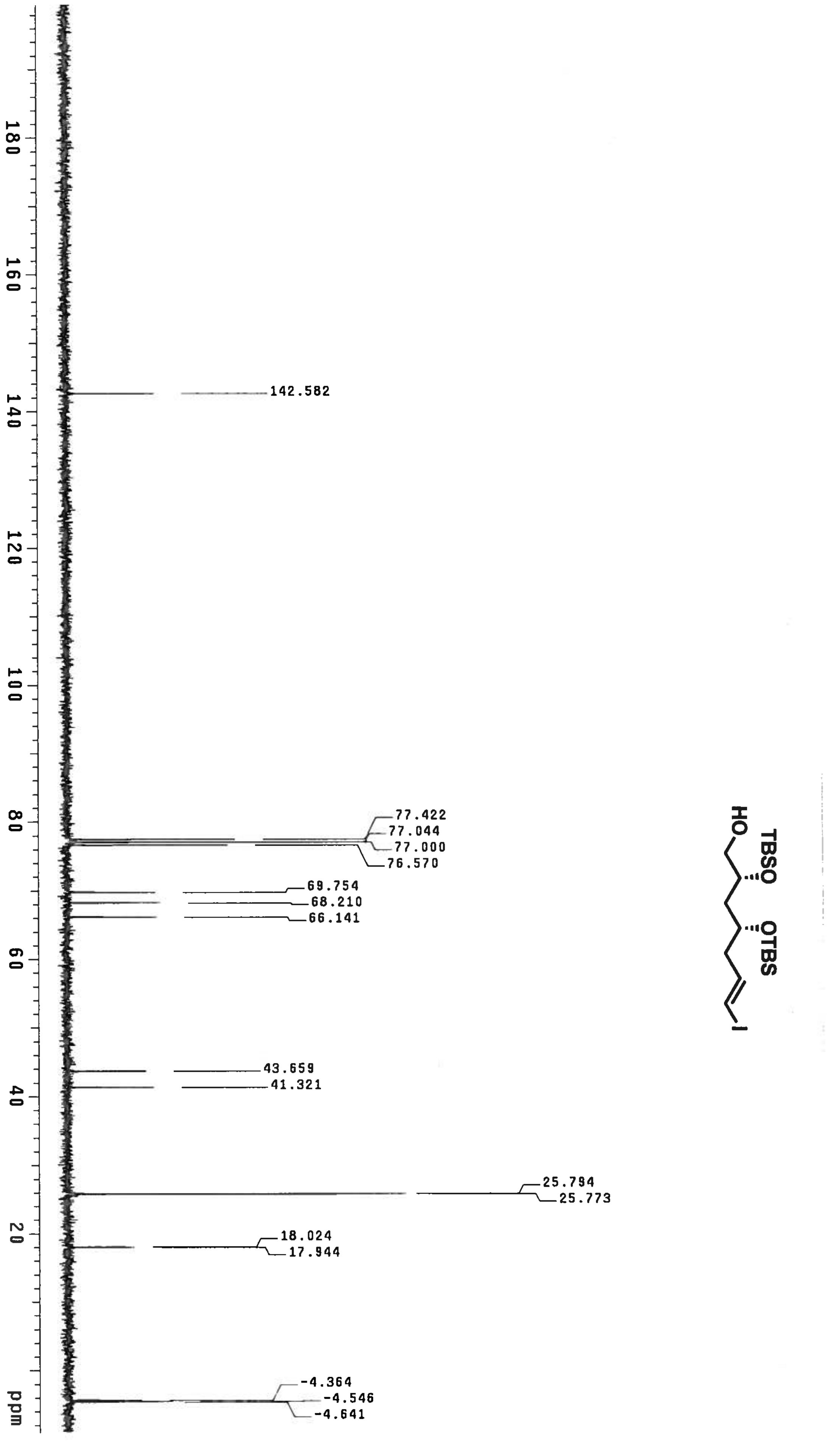

TBSO
OTBS
PMBO
I
10
9
8
7
6
5
4
3
2
1
ppm
4.90
4.19
1.88
1.89
4.22
6.56
3.89
4.14
2.20
1.85
4.69
36.43
23.16



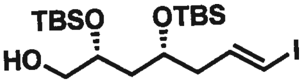
PMBO
TBSO
OTBS
I
159.092
143.216
130.412
129.232
113.691
77.422
77.000
76.578
74.509
72.907
68.880
68.042
55.253
43.368
42.268
25.867
25.824
18.118
17.987
-4.204
-4.480
-4.560
-4.793
180
160
140
120
100
80
60
40
20
ppm

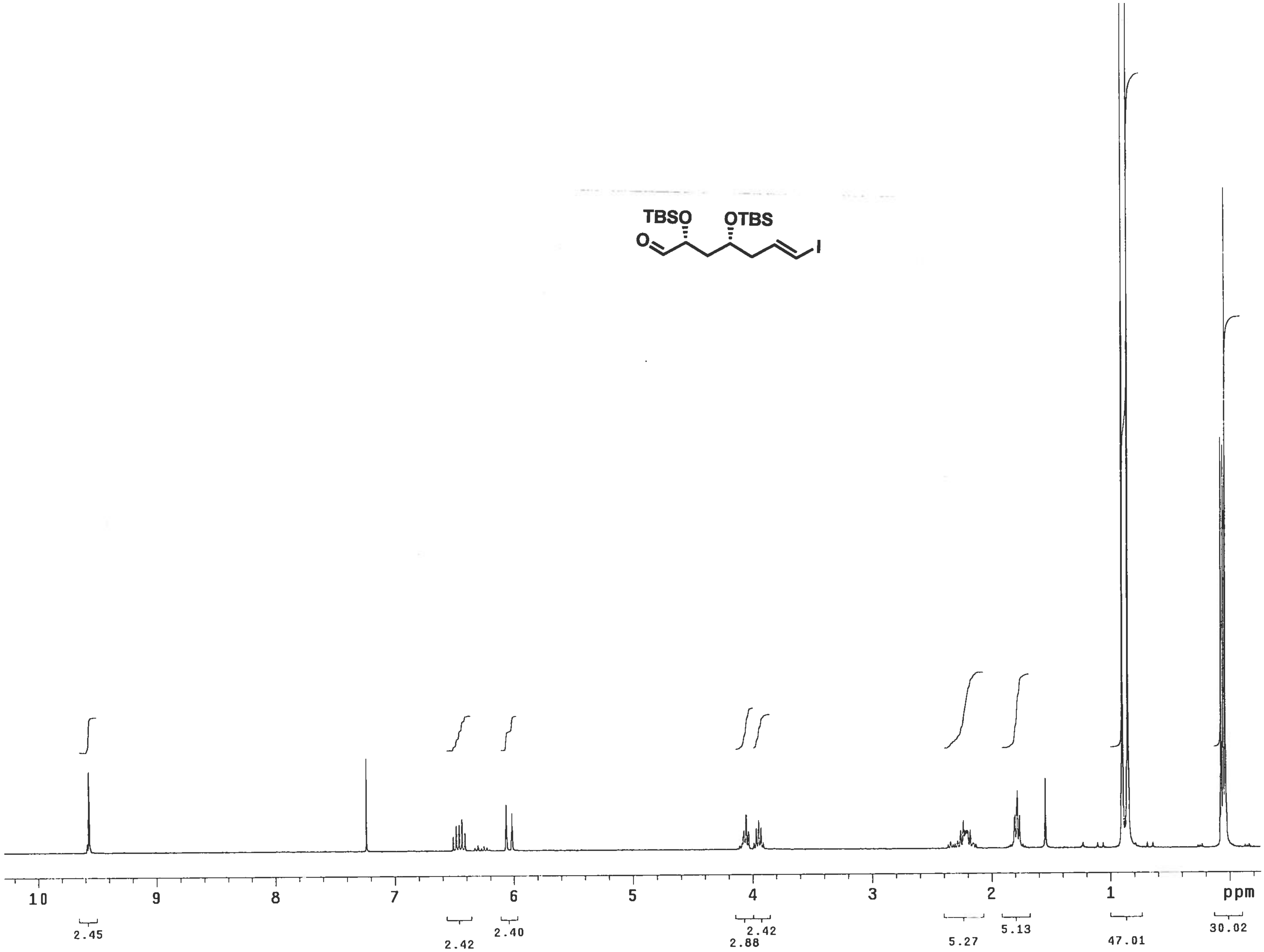
```
                         Elemental Composition                    Date : 14-OCT-2004

File:O14LU3761 Ident:5_6 SMO(1,5) PKD(7,3,7,0.50%,80.0,80.00%,F,F)
ZAB-E4F CI+ Voltage BpI:146832 TIC:8522244 Flags:NORM DEL ERR
File Text:WQQ-03761  /  QINGQUAN  WU            OPERATOR AND ANALYZER:  ALBERT
 Heteroatom Max:   30    Ion:  Both Even and Odd
 Limits:

                                                  -0.5    0    0    0    0    0
 619.213630            5.0                        20.0  200  400    4    2    1

    Mass           mDa   PPM    Calc. Mass    DBE    C    H    O   Si    I

 619.213630        0.0   -0.1   619.213594    5.5   27   48    4    2    1
```

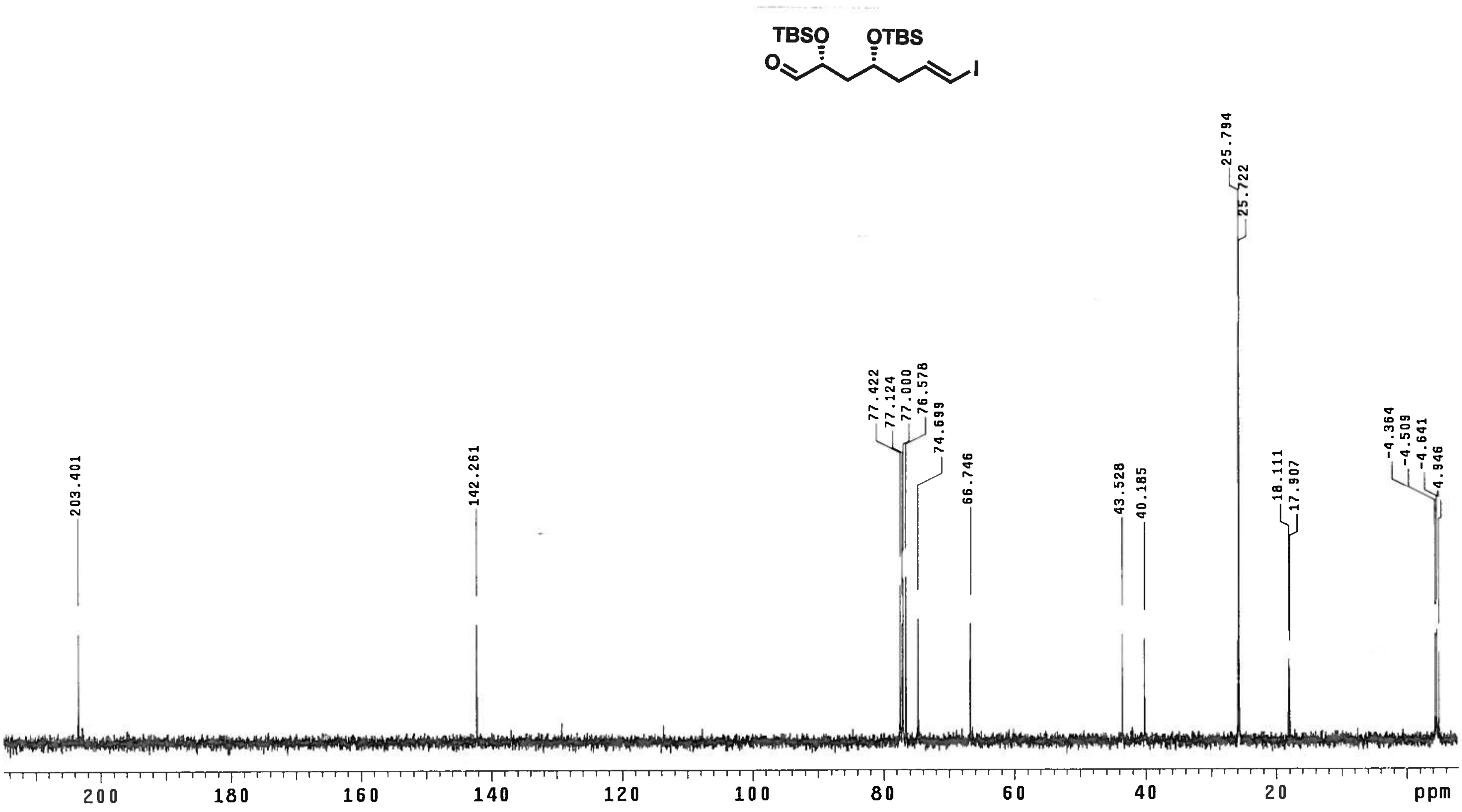

TBSO
OTBS
HO
I
9
8
7
6
5
4
3
2
1
ppm
2.49
2.46
5.04
2.51
2.48
7.42
5.39
44.08
28.13


142.582
77.422
77.044
77.000
76.570
69.754
68.210
66.141
43.659
41.321
25.794
25.773
18.024
17.944
-4.364
-4.546
-4.641
180
160
140
120
100
80
60
40
20
ppm
HO
TBSO
OTBS
I

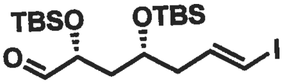
```
                     Elemental Composition                  Date : 14-OCT-2004

File:O14LU3762 Ident:14 SMO(1,5) PKD(7,3,7,0.50%,80.0,80.00%,F,F)
ZAB-E4F CI+ Voltage BpI:400640 TIC:10381599 Flags:NORM DEL ERR
File Text:WQQ-03762  /  QINGQUAN  WU
 Heteroatom Max:   30    Ion:  Both Even and Odd
 Limits:
                                               -0.5    0    0    0    0    0
 501.171312            5.0                     10.0  200  400    3    2    1

    Mass          mDa    PPM   Calc. Mass      DBE     C    H    O   Si    I

 501.171312       0.4    0.8   501.171729      0.5    19   42    3    2    1
```

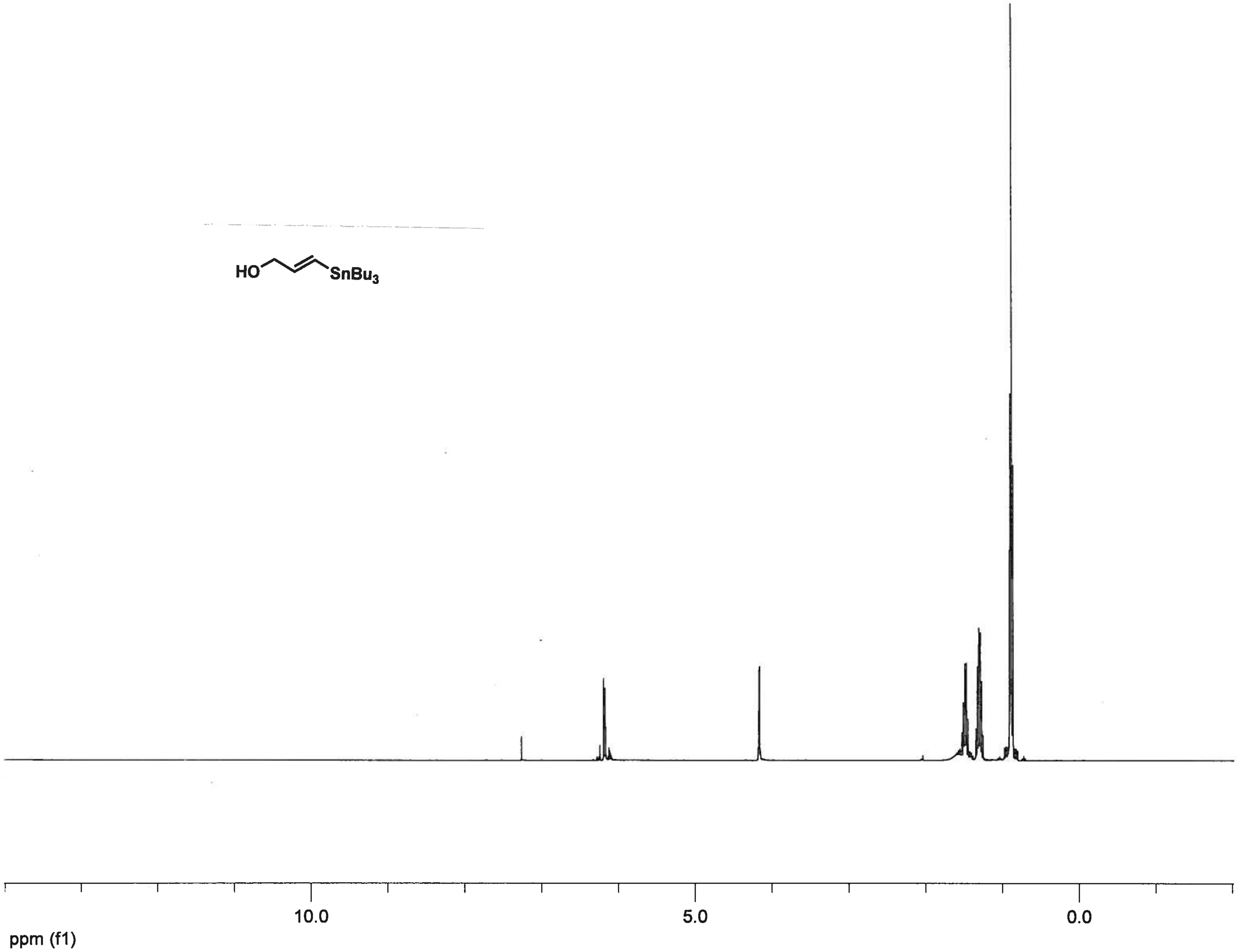


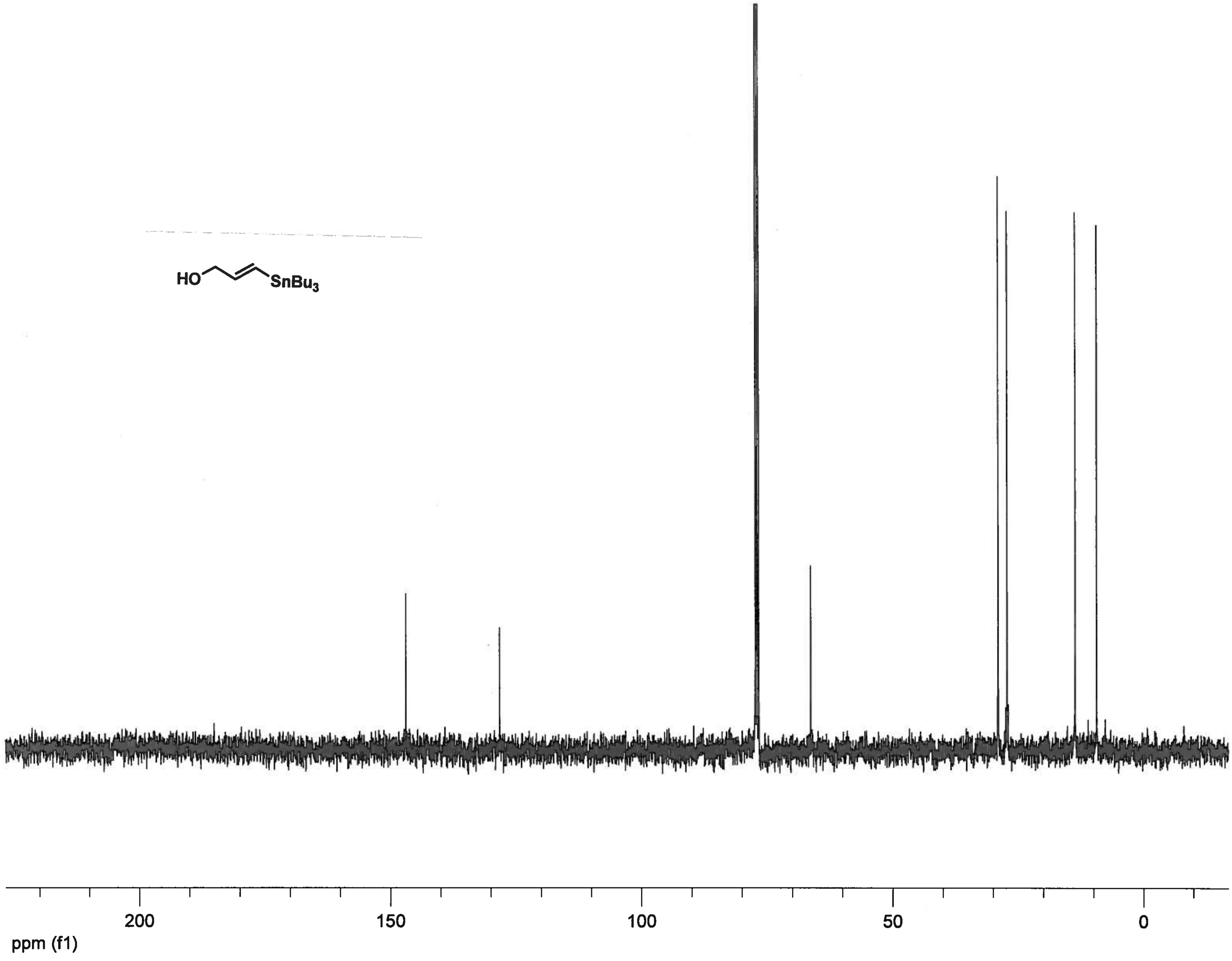

TBSO
OTBS
O
I
10
9
8
7
6
5
4
3
2
1
ppm
2.45
2.42
2.40
2.88
2.42
5.27
5.13
47.01
30.02


TBSO
OTBS
O
I
203.401
142.261
77.422
77.124
77.000
76.578
74.699
66.746
43.528
40.185
25.794
25.722
18.111
17.907
-4.364
-4.509
-4.641
4.946
200
180
160
140
120
100
80
60
40
20
ppm

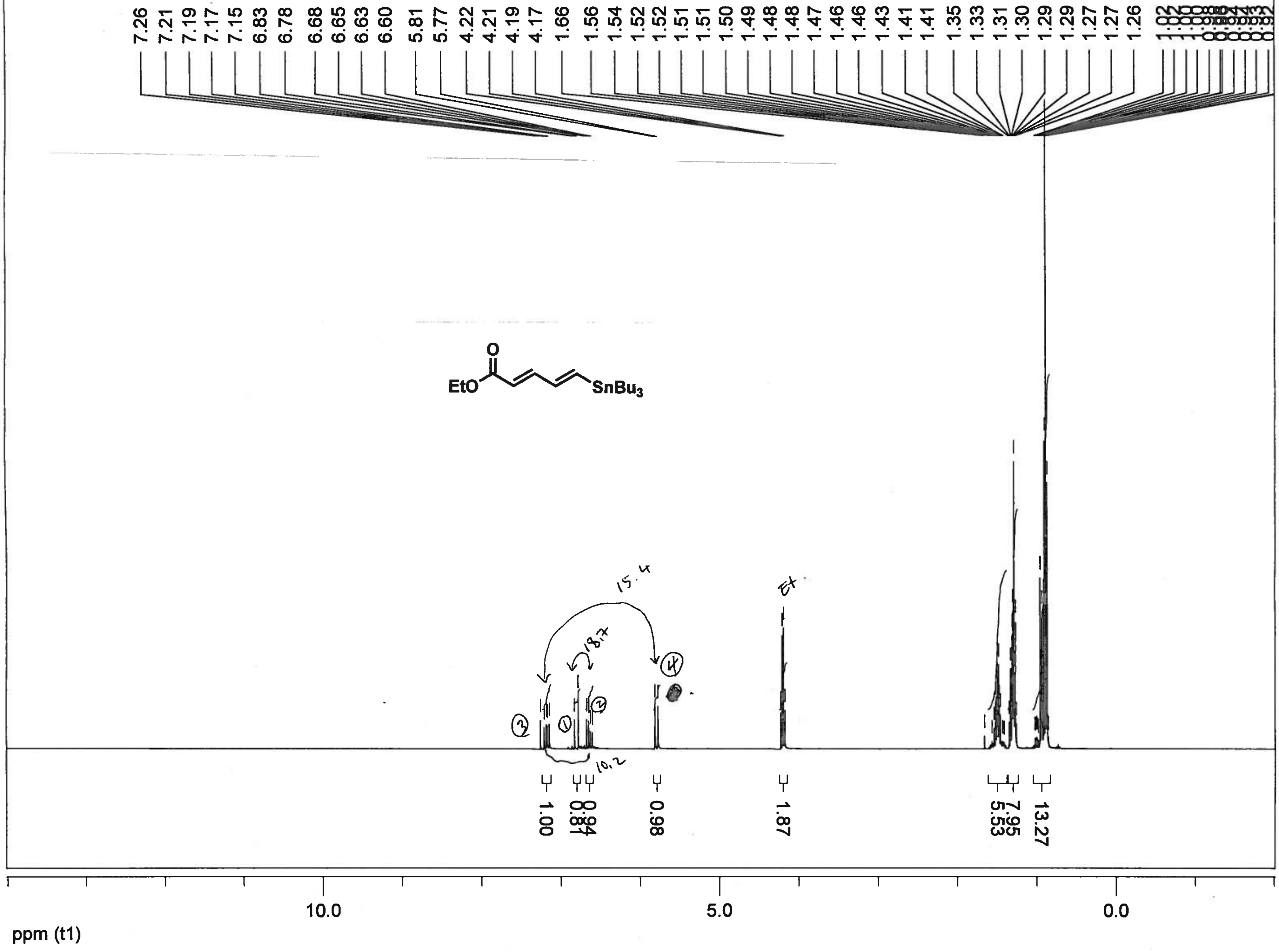
```
                         Elemental Composition                  Date : 13-OCT-2004
File:O13LU3760 Ident:1 SMO(1,5) PKD(7,3,7,0.50%,80.0,80.00%,F,F)
ZAB-E4F CI+ Voltage BpI:34432 TIC:2420439 Flags:NORM DEL ERR
File Text:WQQ-03760  /  QINGQUAN  WU
  Heteroatom Max:   20    Ion:  Both Even and Odd
  Limits:

                                                    -0.5    0    0     0     0     0
  499.157233               5.0                      10.0  200  400     3     2     1

    Mass           mDa     PPM    Calc. Mass      DBE     C    H     O    Si     I

  499.157233      -1.2    -2.3    499.156079      1.5    19   40     3     2     1
```

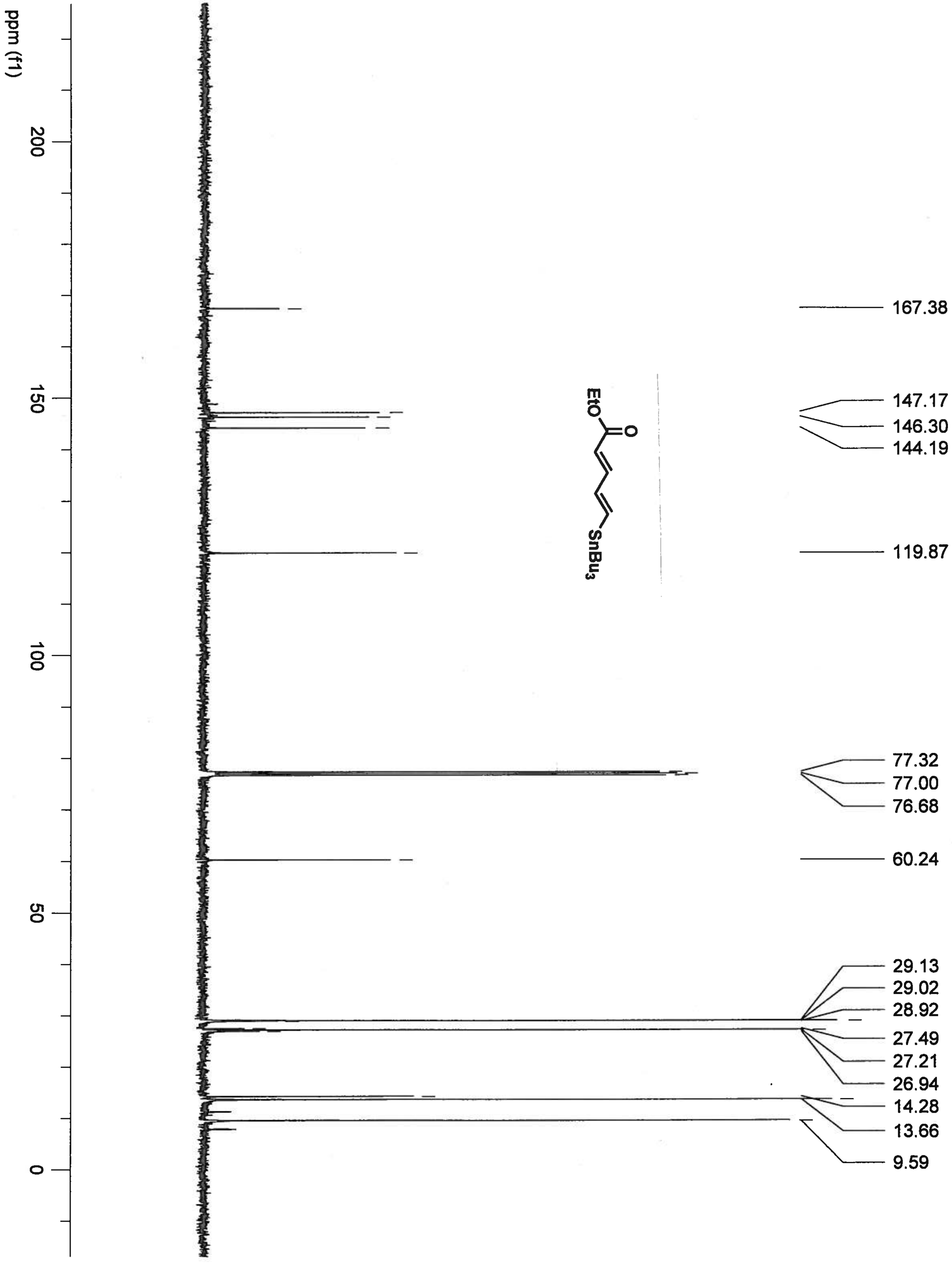



HO
SnBu3
10.0
5.0
0.0
ppm (f1)


HO
SnBu3
200
150
100
50
0
ppm (f1)

HJ10-182, HCDCl3, 400 MHz, Fragment C




ppm (f1)
200
150
100
50
0
EtO
O
SnBu$_3$
167.38
147.17
146.30
144.19
119.87
77.32
77.00
76.68
60.24
29.13
29.02
28.92
27.49
27.21
26.94
14.28
13.66
9.59

Elemental Composition Report

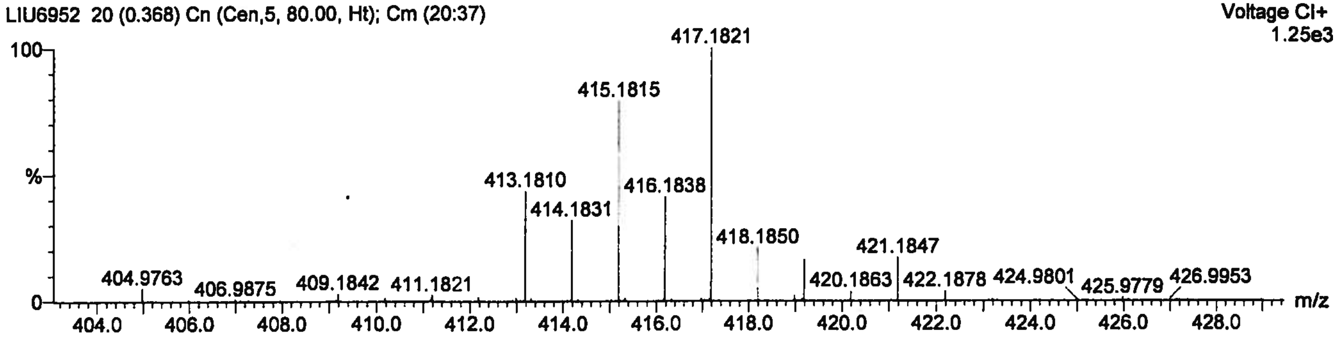
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1000.0, max = 1000.0
Selected filters: None

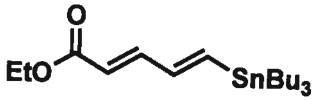
Monoisotopic Mass, Odd and Even Electron Ions
136 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 2-250 H: 1-100 O: 0-8 Sn: 1-2

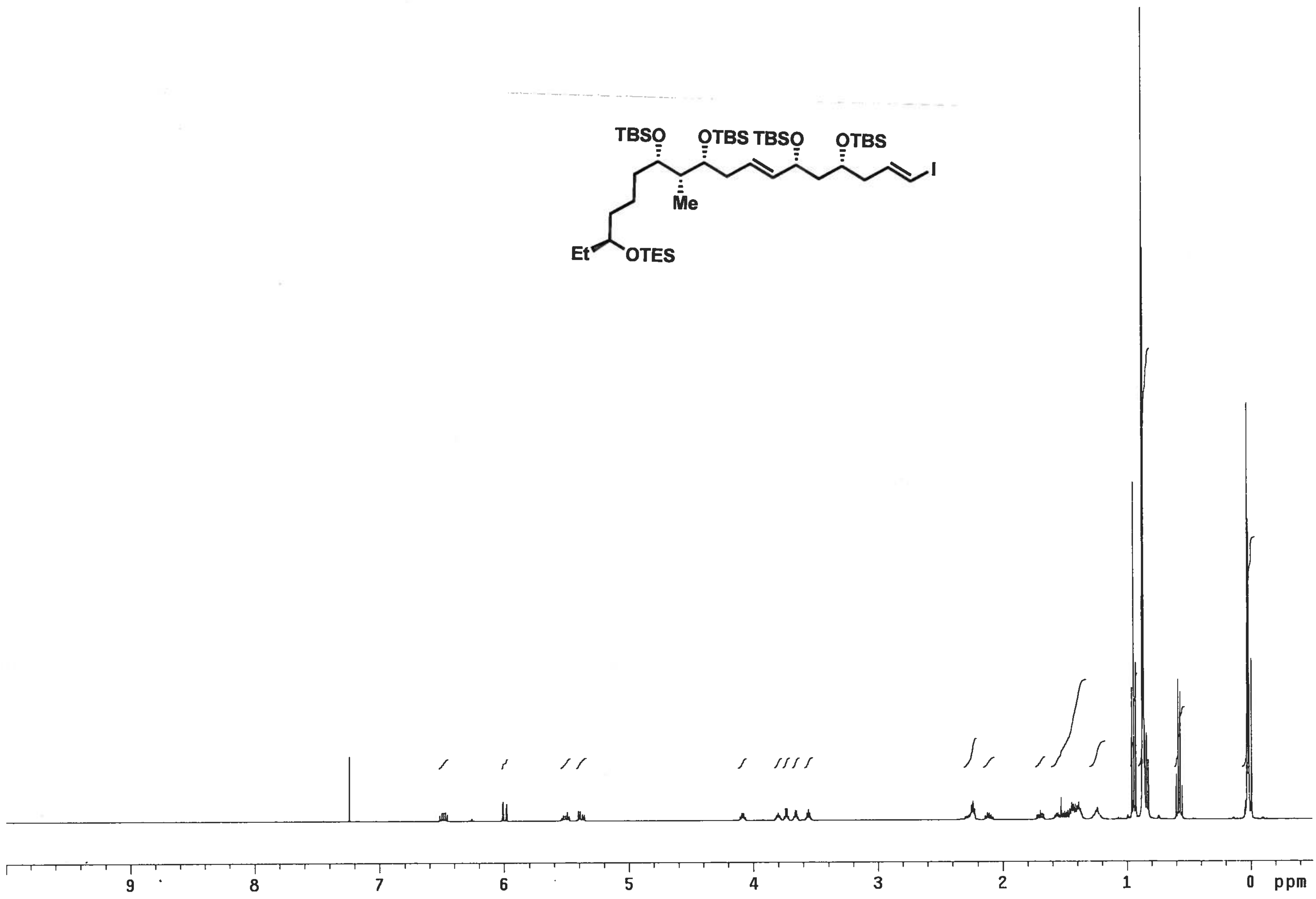
HJ09-062
LIU6952 20 (0.368) Cn (Cen,5, 80.00, Ht); Cm (20:37)

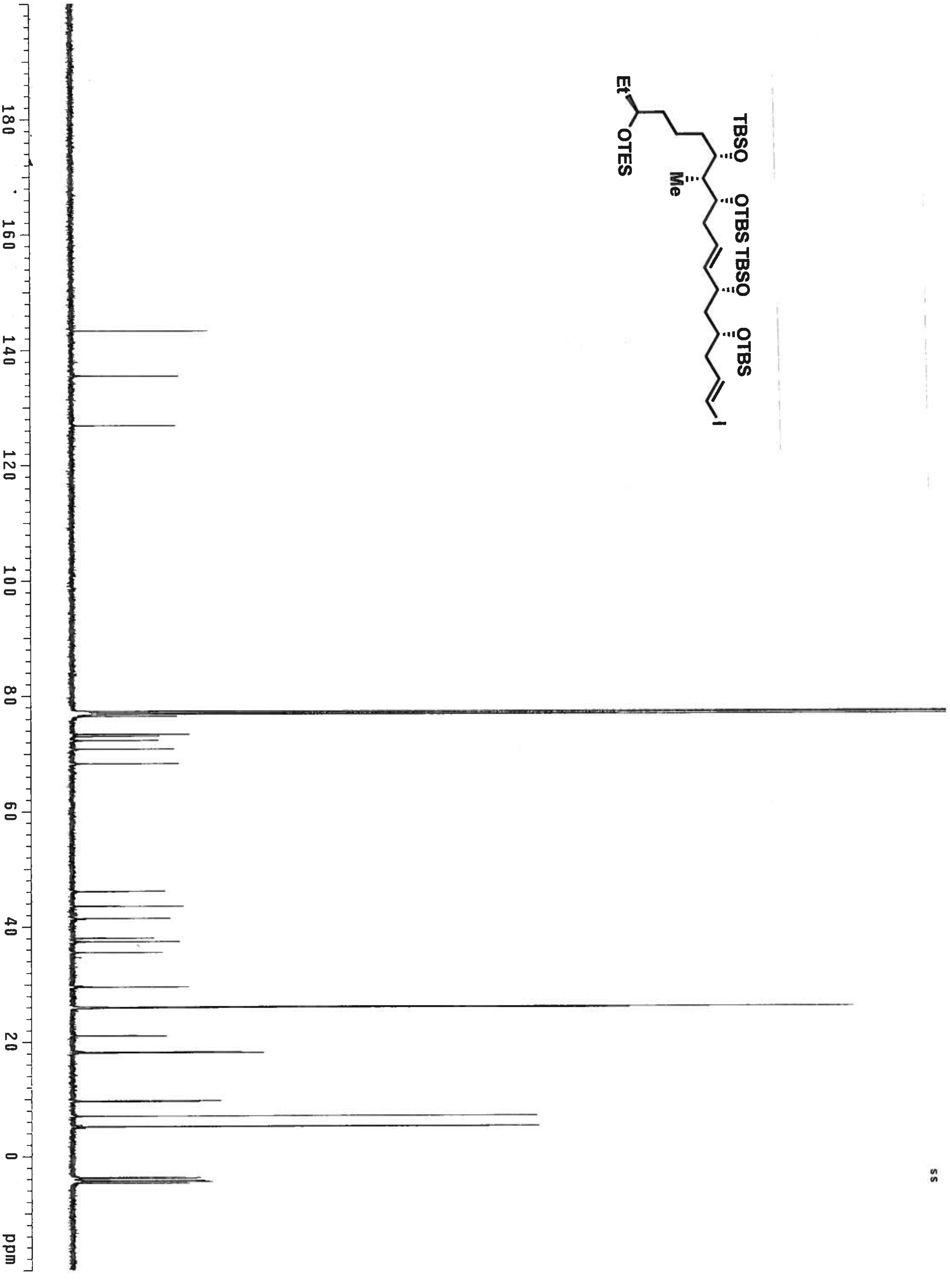
08-Dec-2008
Voltage CI+
1.25e3



```
Minimum:                              -1000.0
Maximum:                 20.0    5.0  1000.0

Mass       Calc. Mass    mDa     PPM   DBE     i-FIT    Formula

417.1821   417.1816      0.5     1.2   2.5     0.1      C19  H37  O2  Sn
```




TBSO
OTBS TBSO
OTBS
I
Me
Et
OTES
9
8
7
6
5
4
3
2
1
0
ppm



TBSO
OTBS
TBSO
OTBS
Me
Et
OTES
I
180
160
140
120
100
80
60
40
20
0
ppm
ss

Elemental Composition Report

Single Mass Analysis

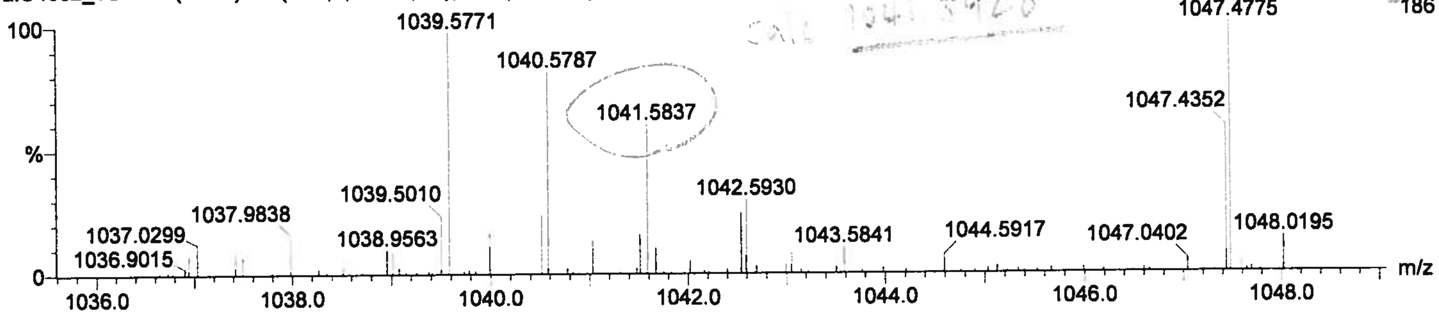
Tolerance = 5.0 PPM / DBE: min = -150.0, max = 150.0
Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions
21 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-250 H: 0-250 O: 5-5 Si: 5-5 I: 1-1

HJ05-108

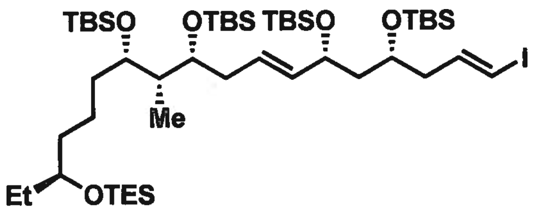
25-Apr-2006
100 Deg C
Voltage CI+

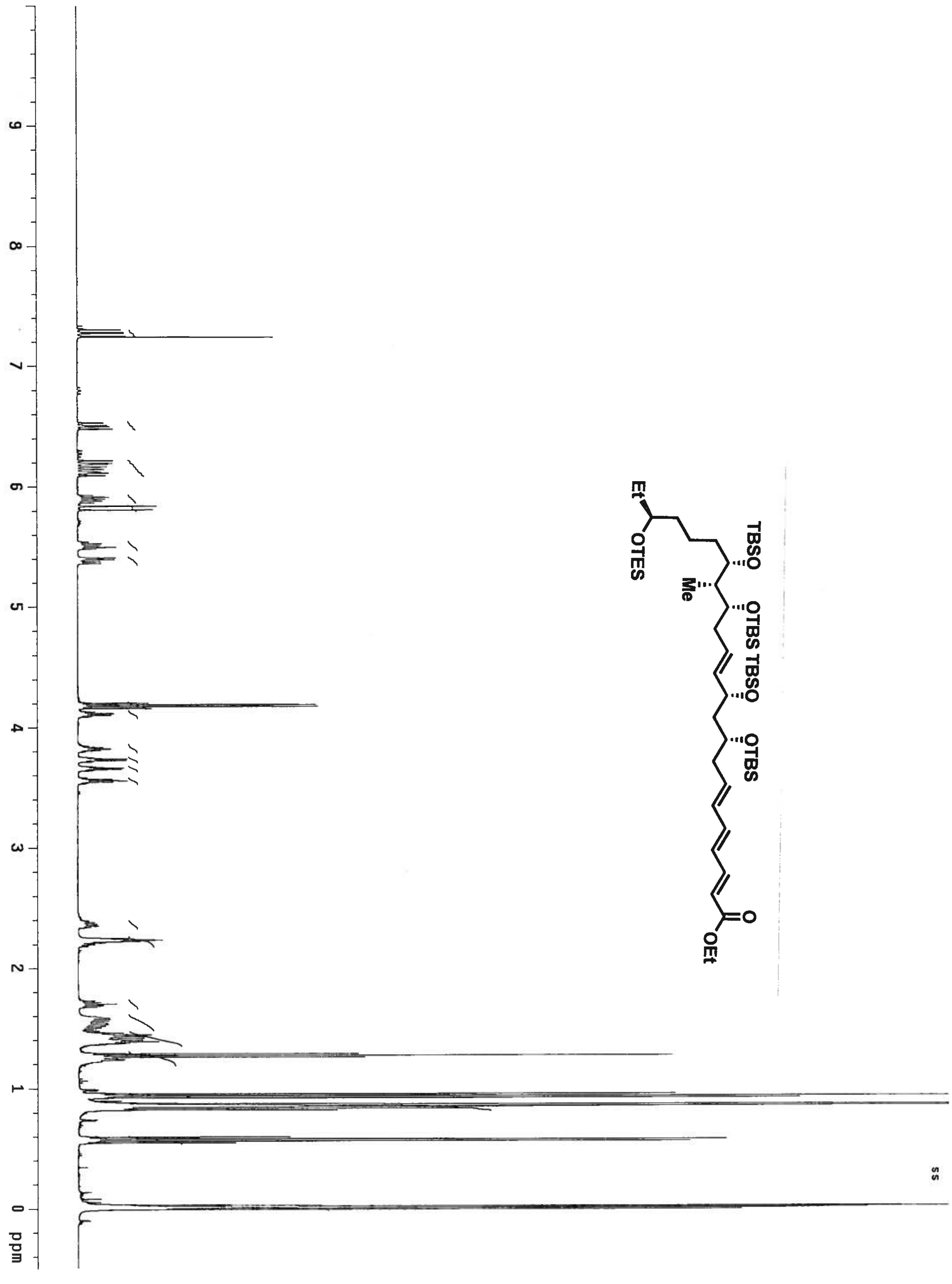
LIU4652_1U 253 (6.354) Cn (Cen,5, 80.00, Ht); Cm (253:259)

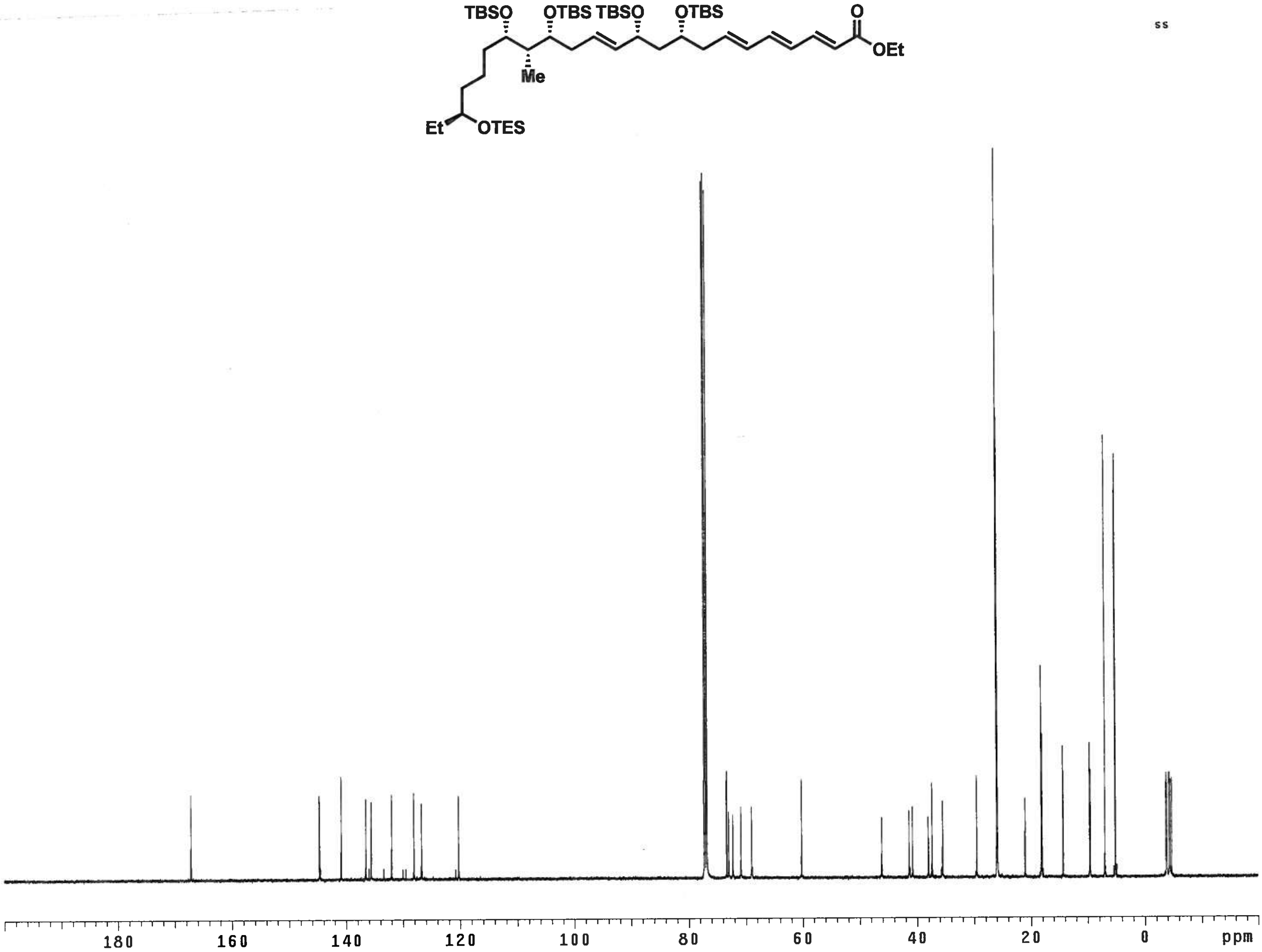


calc 1041.5926

```
Minimum:                                    -150.0
Maximum:                    500.0     5.0    150.0

Mass       Calc. Mass       mDa       PPM    DBE       i-FIT      Formula

1039.5771  1039.5775        -0.4      -0.4   2.5       1.5        C49  H104  O5  Si5  I
```




9
8
7
6
5
4
3
2
1
0
ppm
TBSO
OTBS TBSO
OTBS
Me
Et
OTES
O
OEt
ss


TBSO
OTBS
TBSO
OTBS
O
OEt
Me
Et
OTES
ss
180
160
140
120
100
80
60
40
20
0
ppm

Elemental Composition Report

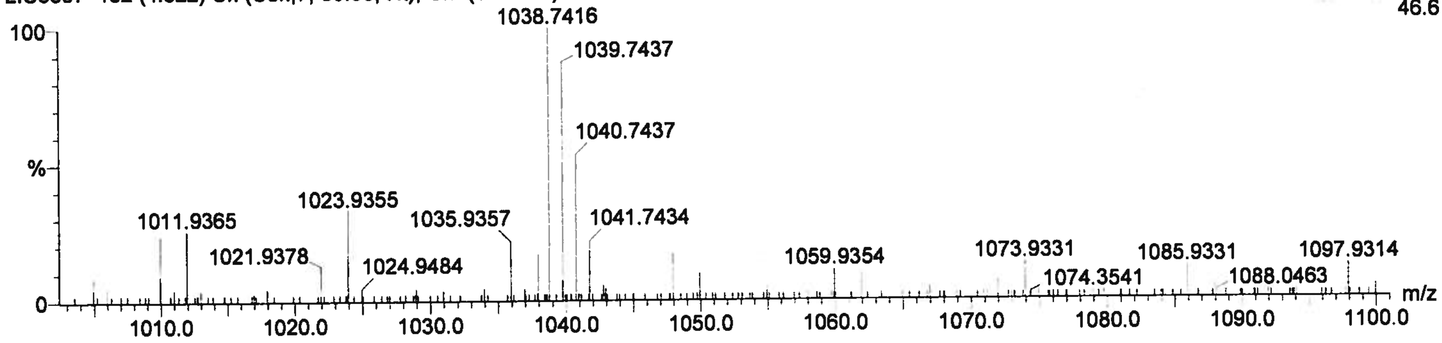
Single Mass Analysis

Tolerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

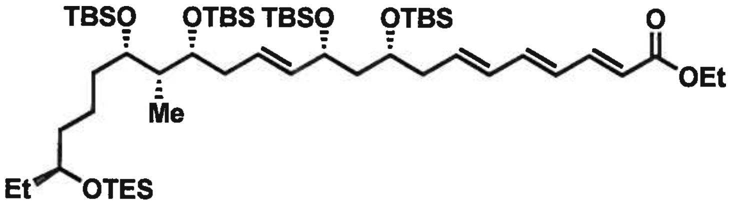
Monoisotopic Mass, Odd and Even Electron Ions
11 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-250 O: 7-7 Si: 5-5

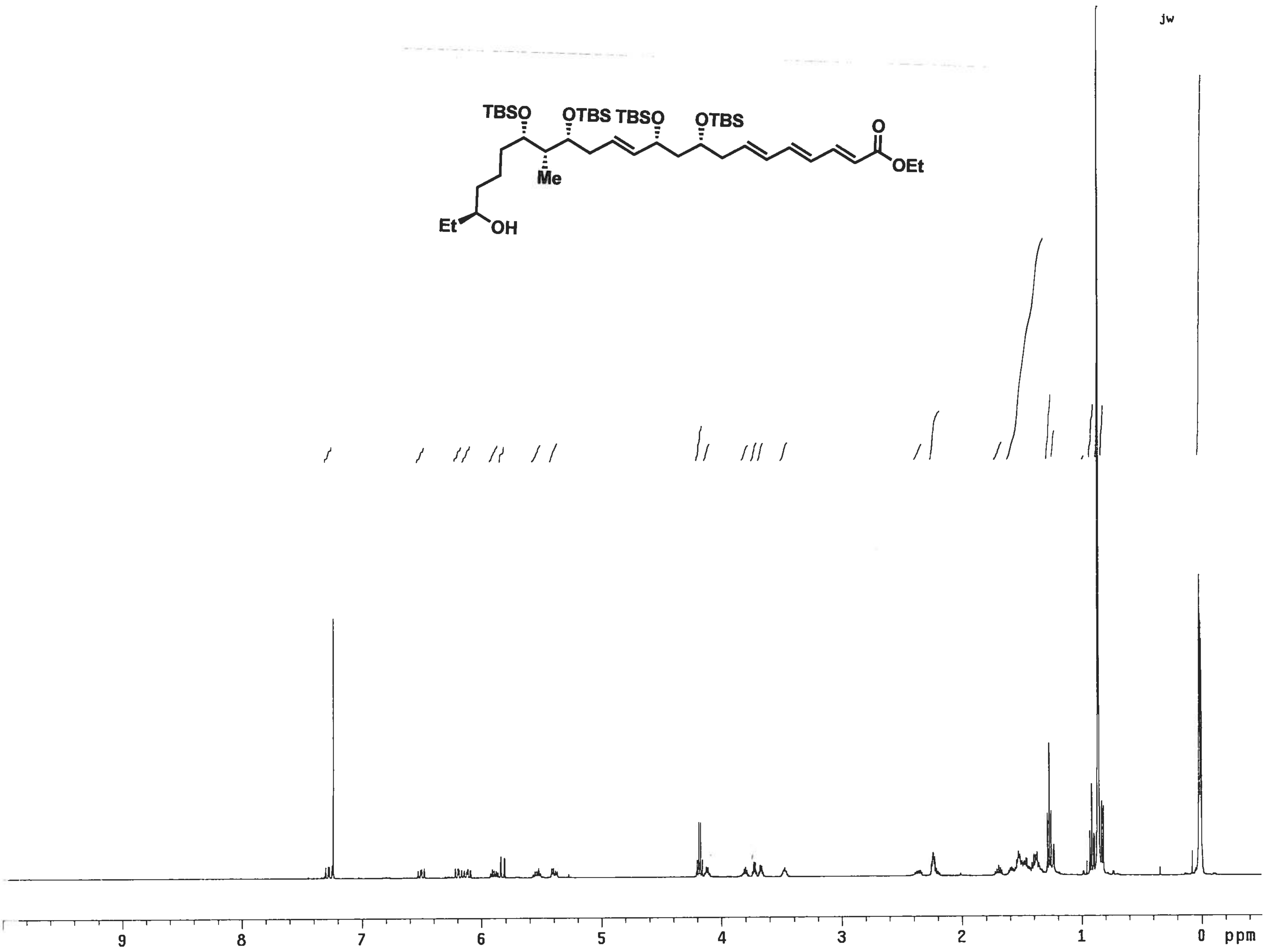
HJ05-050-2
LIU3897 152 (4.322) Cn (Cen,7, 80.00, Ht); Cm (150:302)

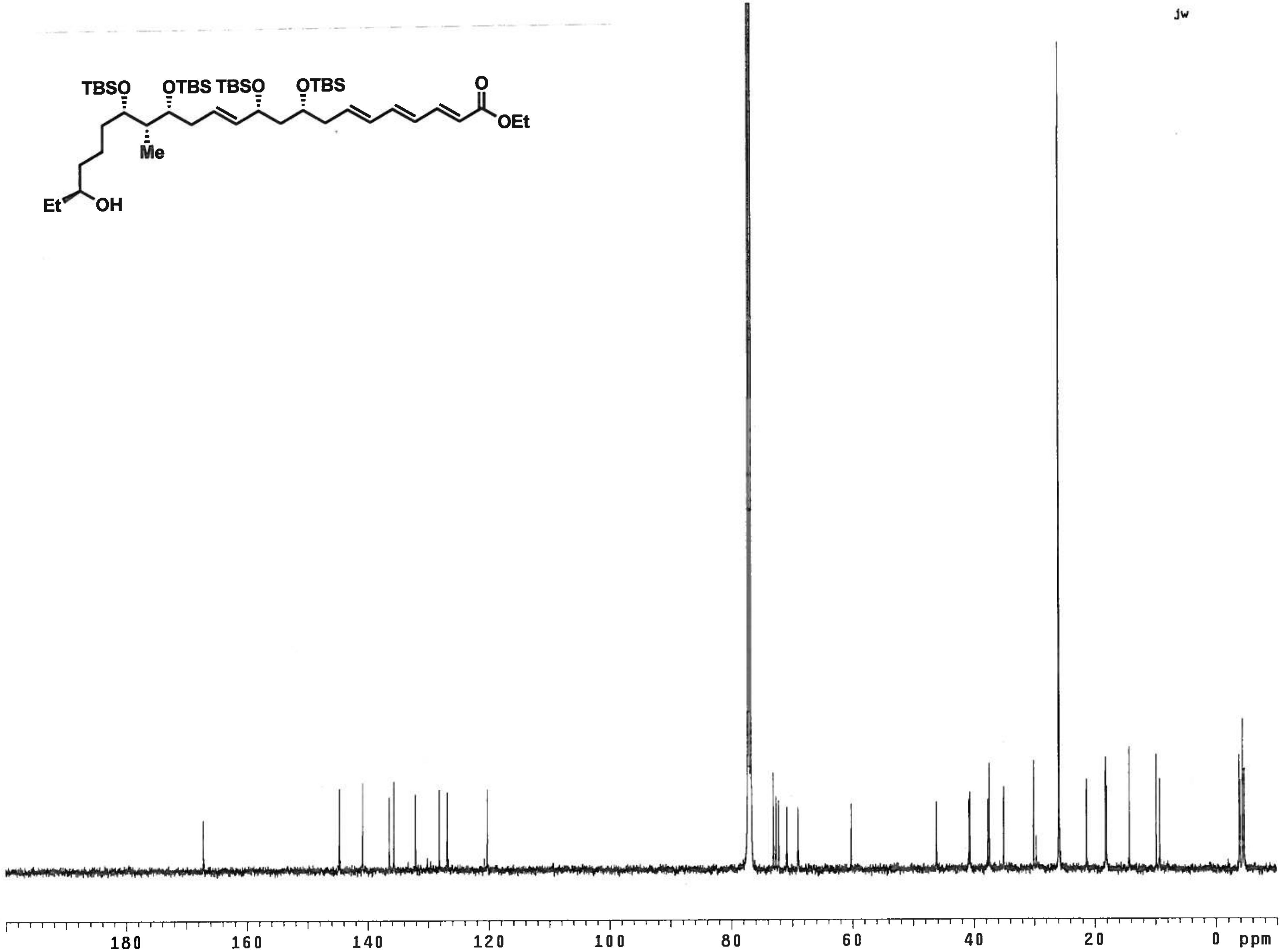
15-Mar-2006
Voltage CI-
46.6



```
Minimum:                                        -1.5
Maximum:                  5.0        50.0        50.0

Mass       Calc. Mass     mDa        PPM         DBE       i-FIT     Formula

1038.7416  1038.7411      0.5        0.5         5.0       0.0       C56  H114  O7  Si5
```




jw
TBSO
OTBS
TBSO
OTBS
O
OEt
Me
Et
OH
9
8
7
6
5
4
3
2
1
0
ppm


jw
TBSO
OTBS
TBSO
OTBS
O
OEt
Me
Et
OH
180
160
140
120
100
80
60
40
20
0
ppm

Elemental Composition Report

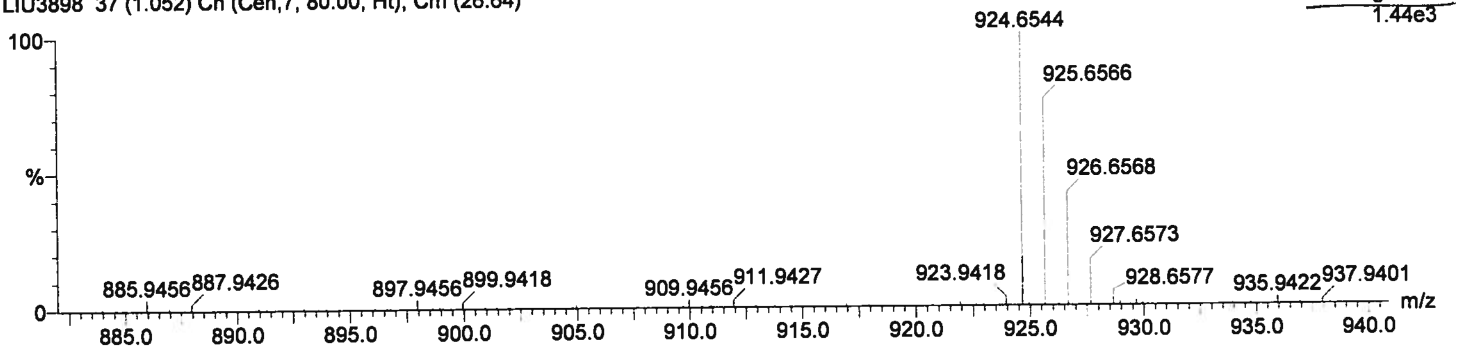
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

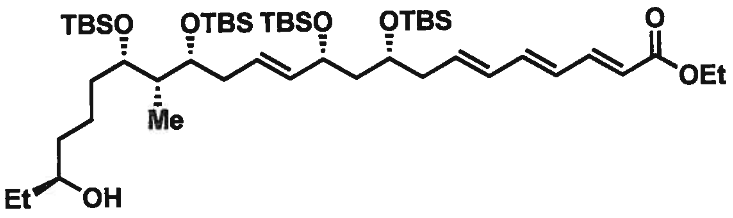
Monoisotopic Mass, Odd and Even Electron Ions
10 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-250 O: 7-7 Si: 4-4

HJ05-058
LIU3898 37 (1.052) Cn (Cen,7, 80.00, Ht); Cm (26:64)

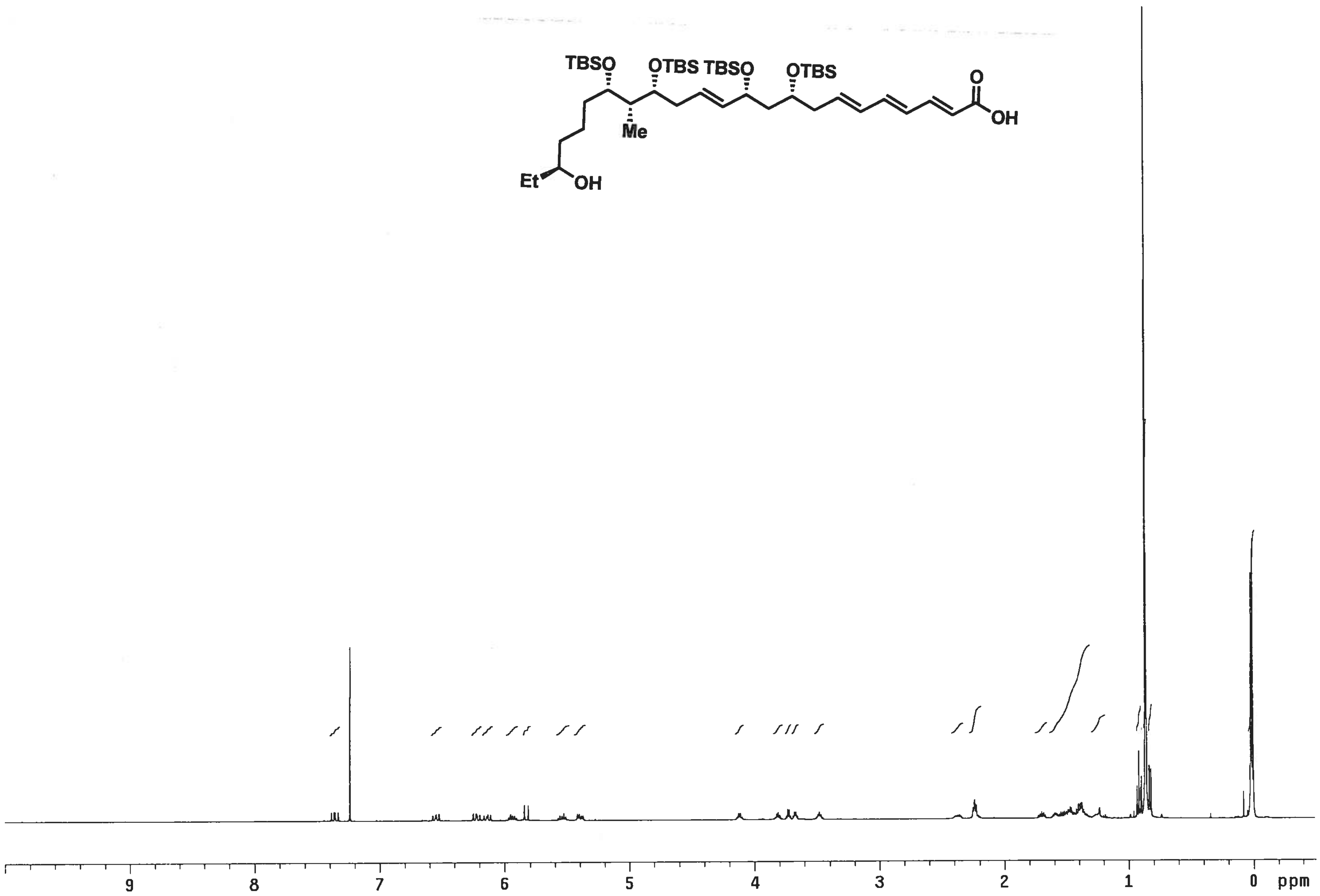
15-Mar-2006
Voltage CI-
1.44e3

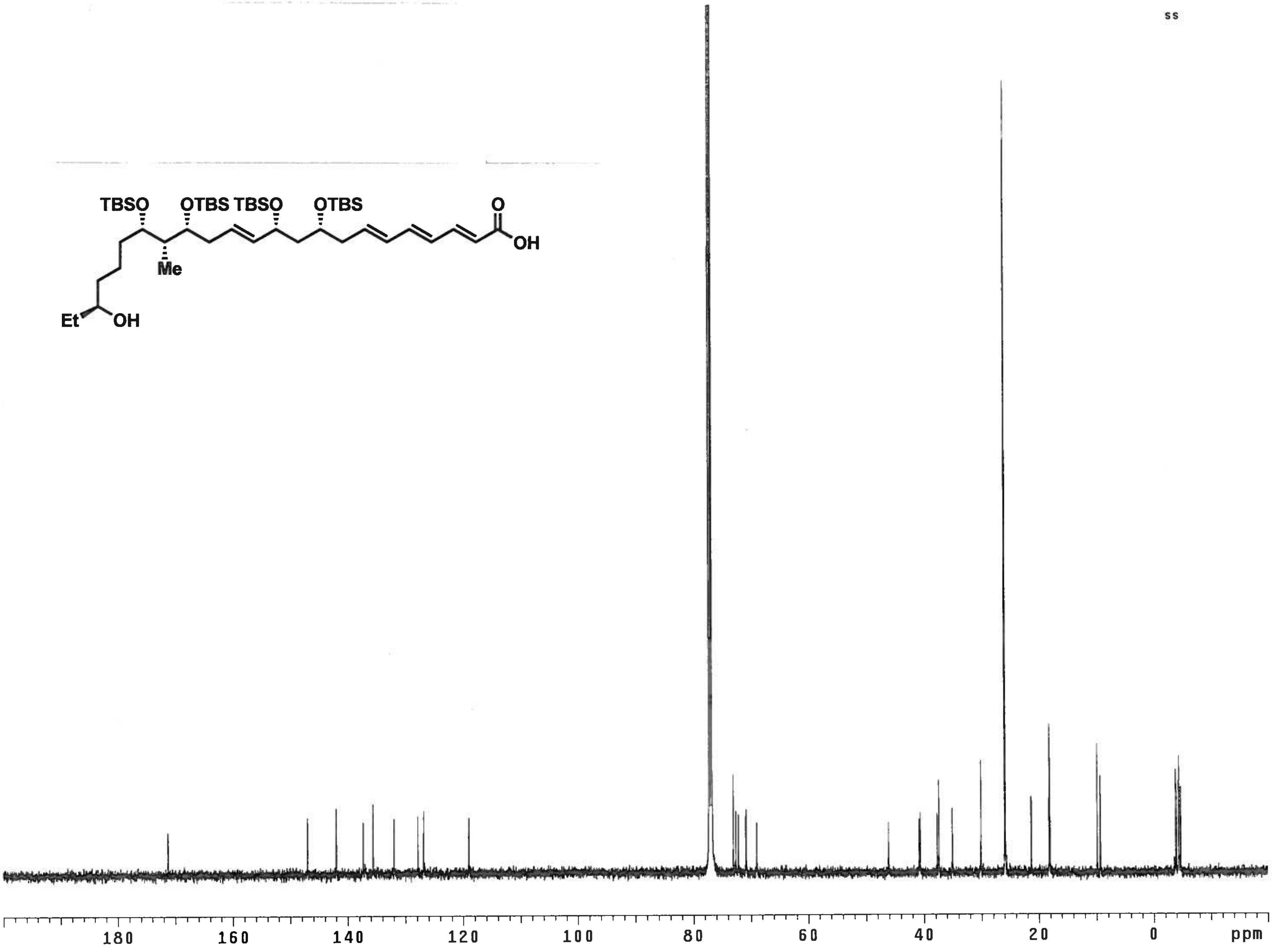


Minimum:				-1.5		
Maximum:		5.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
924.6544	924.6546	-0.2	-0.2	5.0	1.0	C50 H100 O7 Si4



ss




ss
TBSO
OTBS
TBSO
OTBS
O
OH
Me
Et
OH
180
160
140
120
100
80
60
40
20
0
ppm

Elemental Composition Report

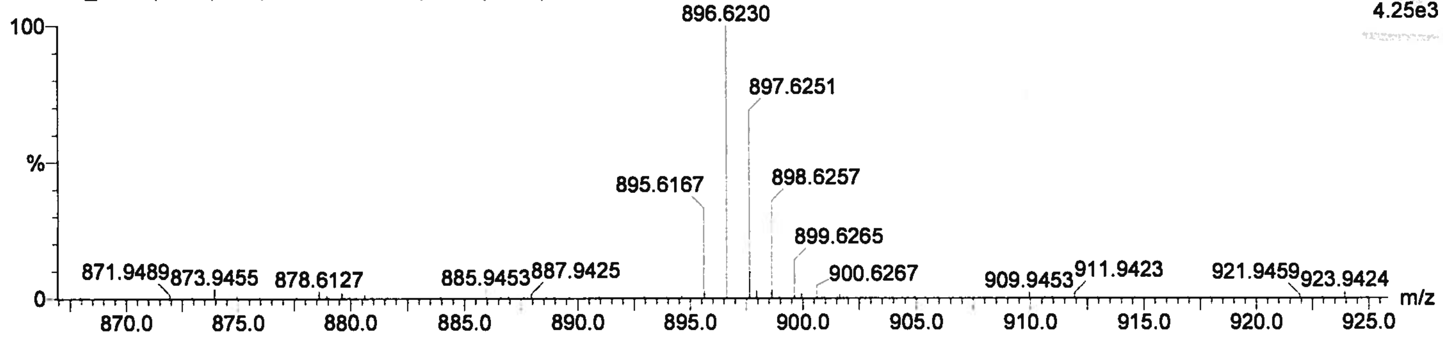
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

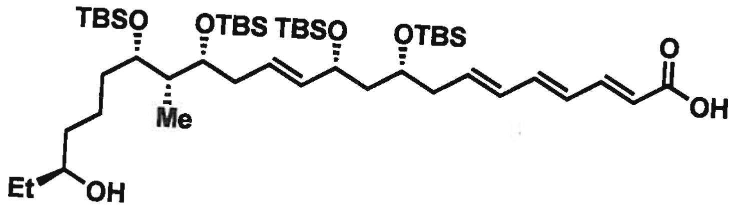
Monoisotopic Mass, Odd and Even Electron Ions
10 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-250 O: 7-7 Si: 4-4

HJ05-054
LIU3899_1 66 (1.877) Cn (Cen,7, 80.00, Ht); Cm (66:89)

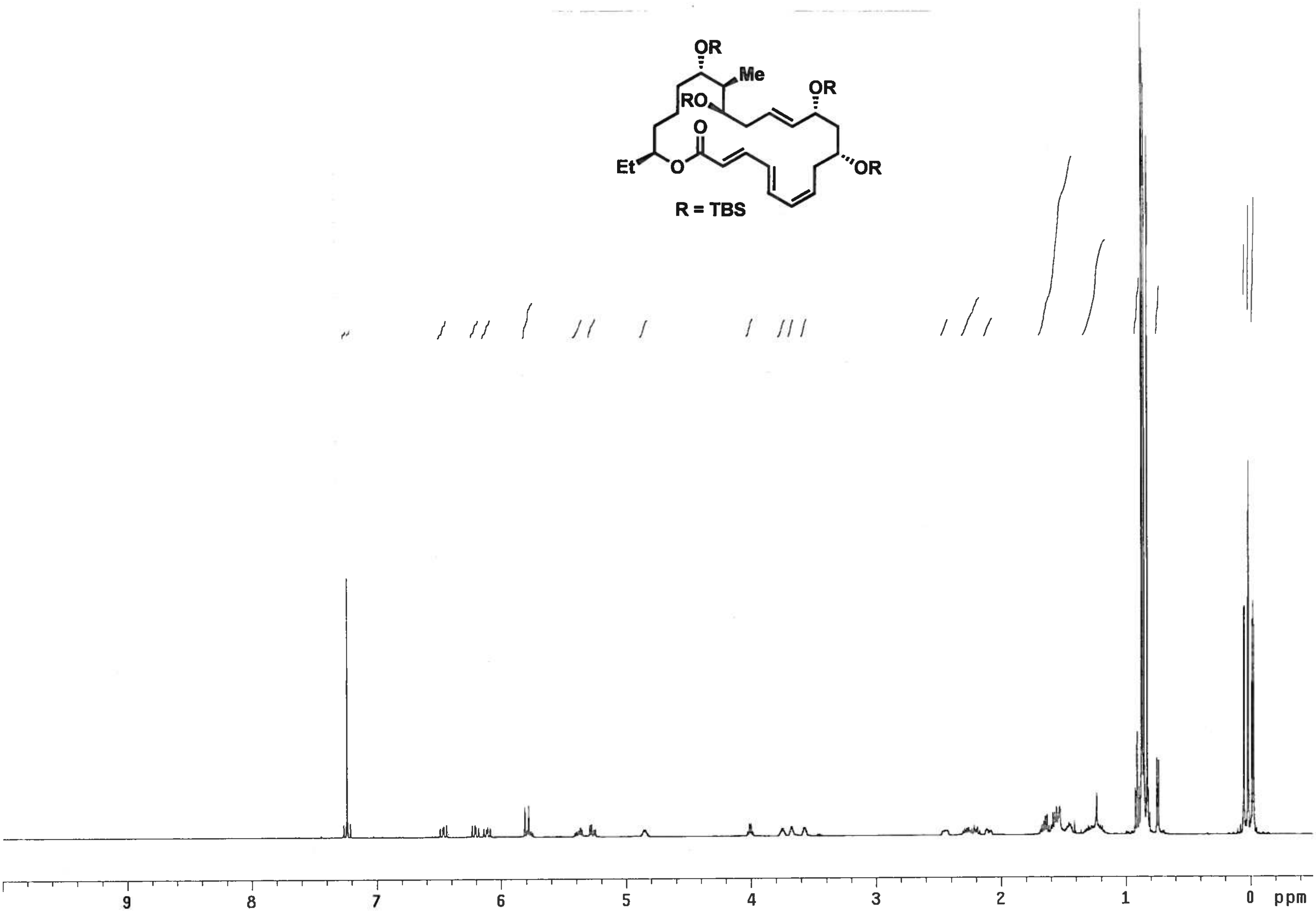
15-Mar-2006
Voltage CI-
4.25e3

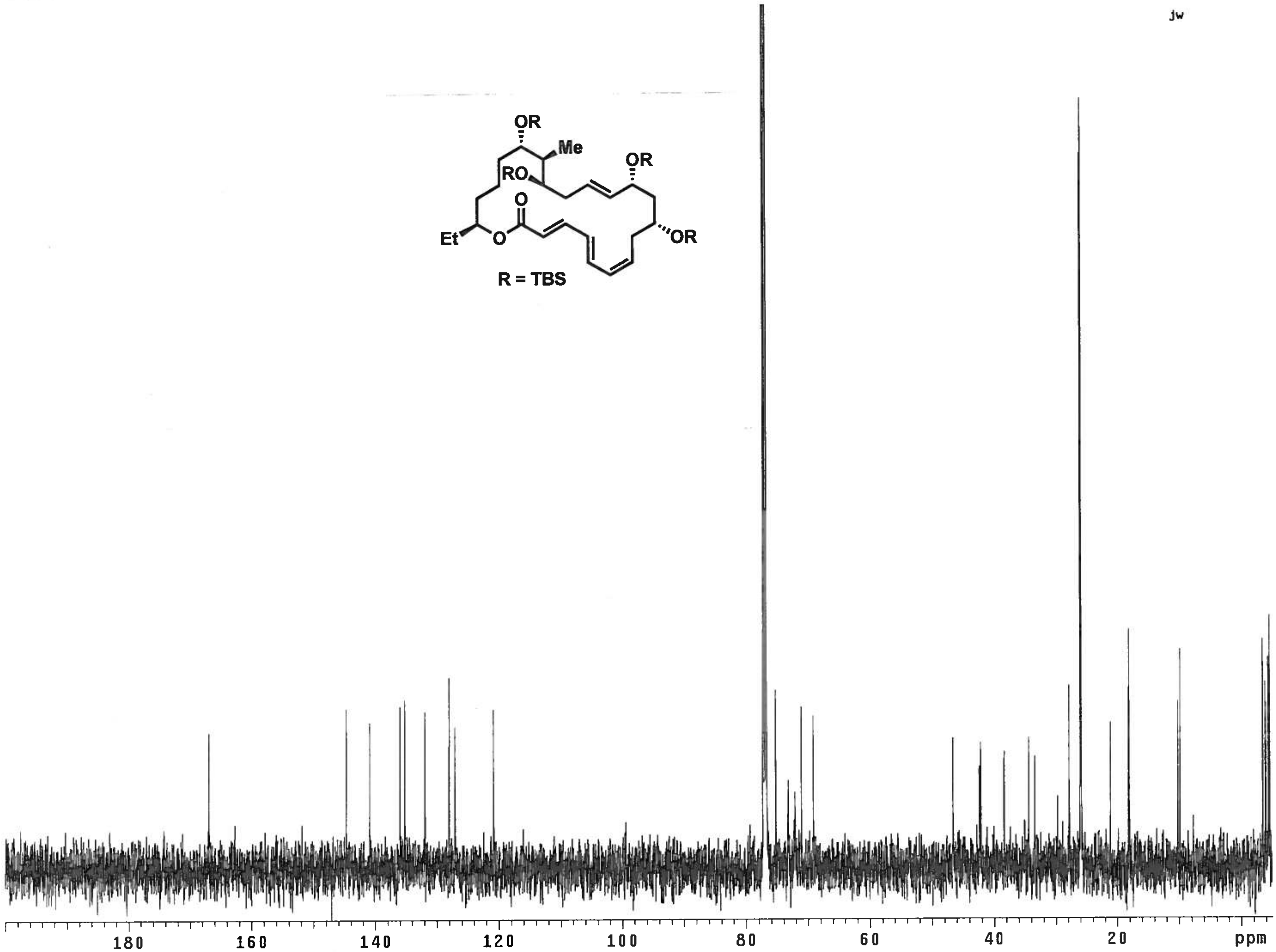


```
Minimum:                              -1.5
Maximum:              5.0      5.0    50.0

Mass       Calc. Mass  mDa     PPM    DBE      i-FIT     Formula

896.6230   896.6233    -0.3    -0.3   5.0      18.8      C48  H96  O7  Si4
```



jw




jw
OR
Me
RO
OR
O
Et
O
OR
R = TBS
180
160
140
120
100
80
60
40
20
ppm

Elemental Composition Report

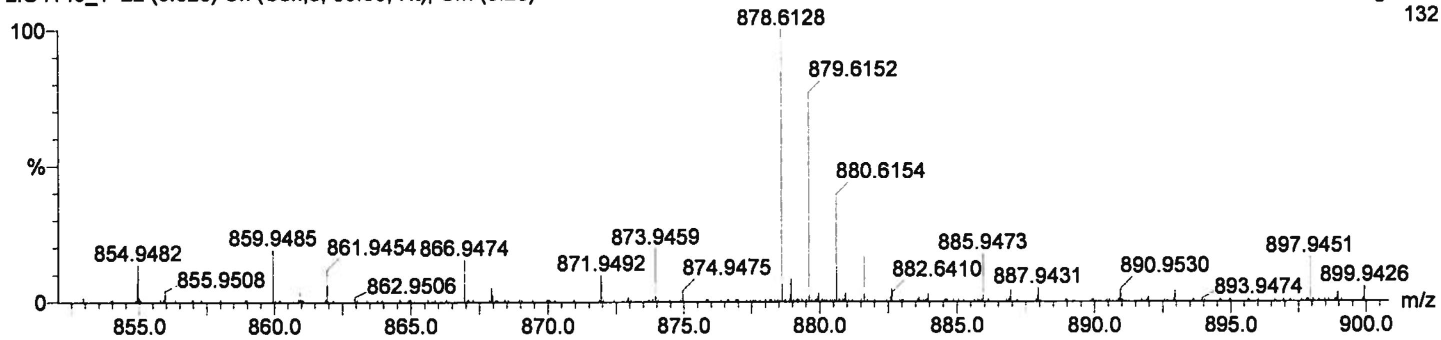
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -50.0, max = 100.0
Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions
17 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-250 H: 0-250 O: 6-6 Si: 4-4

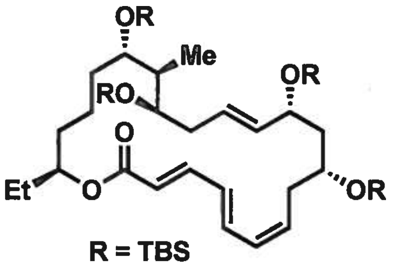
HJ05-074
LIU4149_1 22 (0.626) Cn (Cen,3, 80.00, Ht); Cm (3:23)

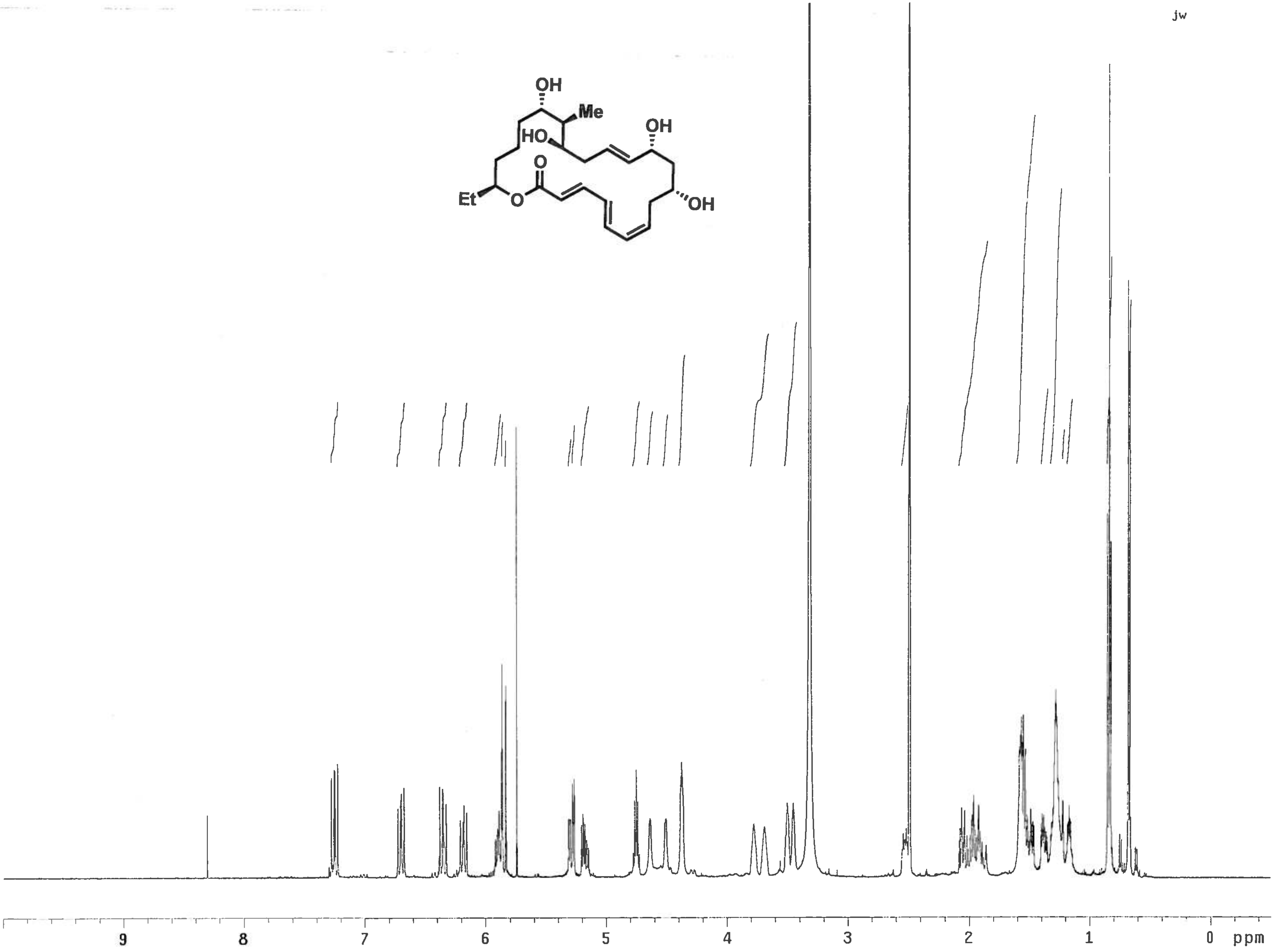
23-Mar-2006
Voltage CI-
132

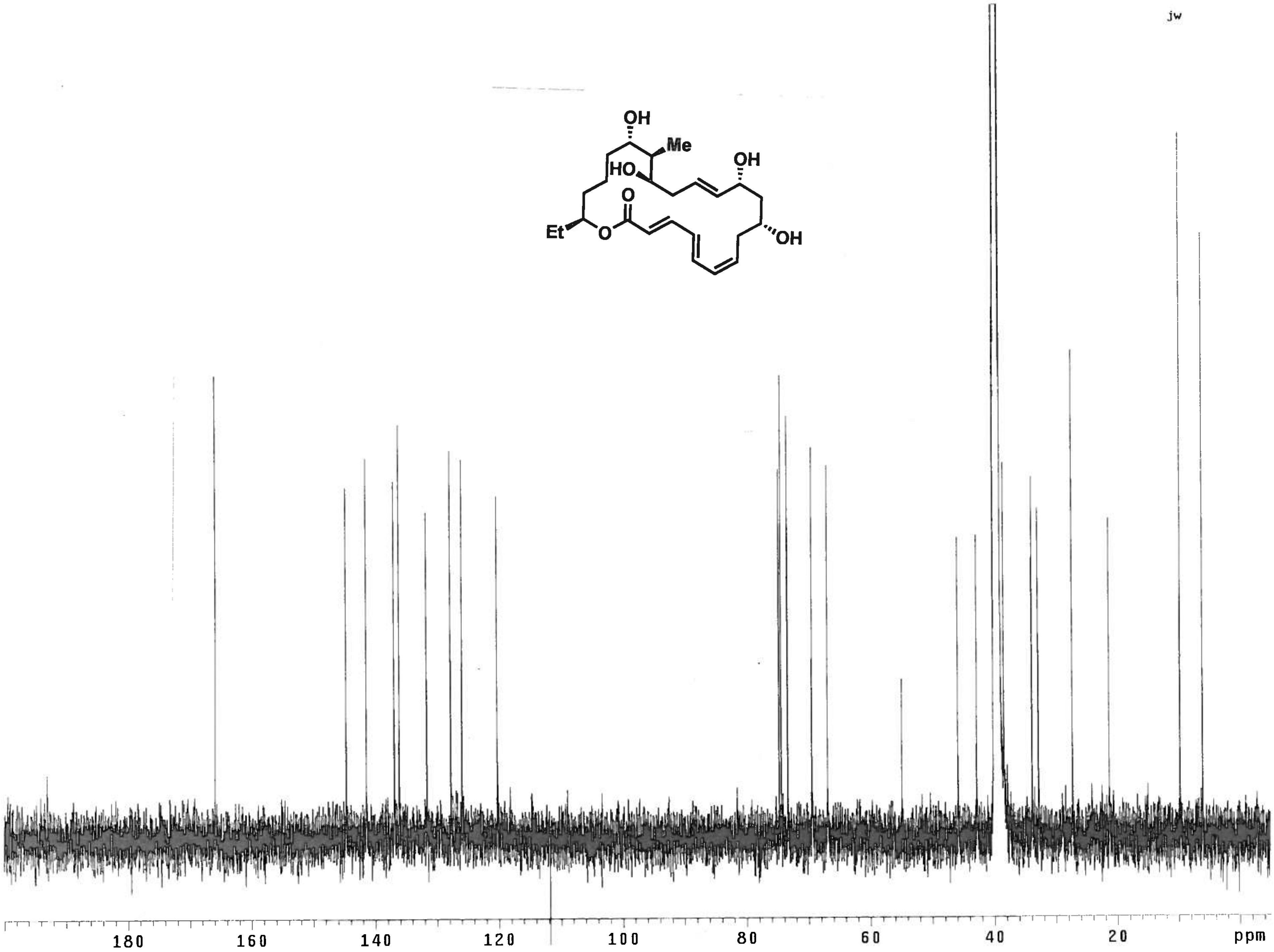


```
Minimum:                                       -50.0
Maximum:                   500.0     5.0       100.0

Mass        Calc. Mass     mDa       PPM       DBE        i-FIT       Formula

878.6128    878.6128       0.0       0.0       6.0        0.2         C48  H94  O6  Si4
```

M^+




OH
Me
HO
OH
O
Et
O
OH
jw
9
8
7
6
5
4
3
2
1
0 ppm


jw
OH
Me
HO
OH
O
Et
O
OH
180
160
140
120
100
80
60
40
20
ppm

Elemental Composition Report

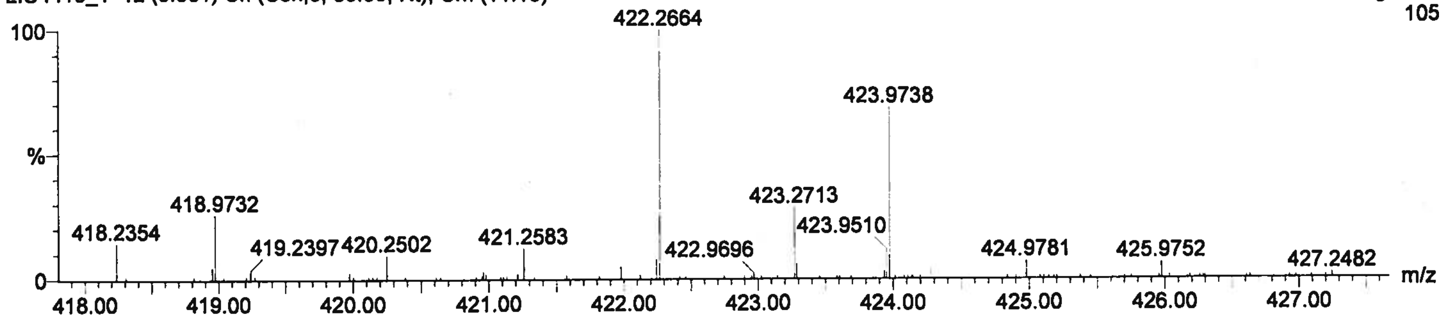
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -150.0, max = 150.0
Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions
21 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 1-250 H: 0-250 O: 6-6
HJ05-128

04-May-2006
100 Deg C
Voltage CI-
105

LIU4415_1 12 (0.301) Cn (Cen,5, 80.00, Ht); Cm (11:15)



```
Minimum:                                    -150.0
Maximum:                500.0      5.0      150.0

Mass       Calc. Mass   mDa        PPM      DBE       i-FIT     Formula

422.2664   422.2668     -0.4       -0.9     6.0       0.4       C24  H38  O6
```