Synthesis of a Bicyclobutane Fatty Acid Identified from the Cyanobacterium *Anabaena* PCC 7120

Sean DeGuire[a], Shutao Ma[b] and Gary A. Sulikowski[a,*]

[a]Departments of Chemistry and Biochemistry, Institute of Chemical Biology, Vanderbilt University, Nashville, TN 37235-1822, U.S.A.

[b]Department of Medicinal chemistry, School of Pharmaceutical Sciences, Shandong University, 44 West Culture Road, Jinan, 250012, P.R. China

Supporting Information

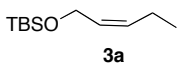
General Procedures: All non-aqueous reactions were performed in flame-dried or oven dried round-bottomed flasks under an atmosphere of argon. Where necessary (so noted) solutions were deoxygenated by alternate freeze (liquid nitrogen)/evacuation/argon-flush/thaw cycles (three iterations) or degassed by purging with argon for several minutes. Stainless steel syringes or cannulae were used to transfer air- and moisture-sensitive liquids. Reaction temperatures were controlled using a thermocouple thermometer and analog hotplate stirrer. Reactions were conducted at room temperature (rt, approximately 23 °C) unless otherwise noted. Flash column chromatography was conducted as described Still et. al. using silica gel 230-400 mesh.[1] Where necessary, silica gel was neutralized by treatment of the silica gel prior to chromatography with the eluent containing 1% triethylamine. Analytical thin-layer chromatography (TLC) was performed on E. Merck silica gel 60 F254 plates and visualized using UV, ceric ammonium molybdate, potassium permanganate, and anisaldehyde stains. Yields were reported as isolated, spectroscopically pure compounds.

[1] W. C. Still, M. Kahn, A. Mitra, *J Org Chem* **1978**, *43*, 2923.

Materials. Solvents were obtained from either an MBraun MB-SPS solvent system or freshly distilled (tetrahydrofuran was distilled from sodium-benzophenone; toluene was distilled from calcium hydride; dimethyl sulfoxide was distilled from calcium hydride and stored over 4Å molecular sieves). Commercial reagents were used as received with the following exceptions. The molarity of *n*-butyllithium and *tert*-butyllithium solutions were determined by titration using diphenylacetic acid as an indicator (average of three determinations).

Instrumentation. Reverse phase HPLC was conducted on a Varian ProStar HPLC system using a Phenomenex Luna 5u C18(2) 100A Axia 50 x 30.00 mm column. Infrared spectra were obtained as thin films on NaCl plates using a Thermo Electron IR100 series instrument and are reported in terms of frequency of absorption (cm^{-1}). ^{1}H NMR spectra were recorded on Bruker 300, 400, 500, or 600 MHz spectrometers and are reported relative to deuterated solvent signals. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad, app = apparent), coupling constants (Hz), and integration. ^{13}C NMR spectra were recorded on Bruker 75, 100, 125, or 150 MHz spectrometers and are reported relative to deuterated solvent signals. LC/MS was conducted and recorded on an Agilent Technologies 6130 Quadrupole instrument. High-resolution mass spectra were obtained from the Department of Chemistry and Biochemistry, University of Notre Dame using either a JEOL AX505HA or JEOL LMS-GCmate mass spectrometer.

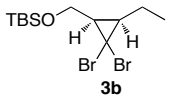
Compound Preparation



3a

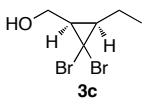
Silyl ether 3a To a solution of cis-2-penten-1-ol (**3**) (1.0 mL, 9.75 mmol) in dichloromethane (19 mL) at 0 °C was added triethylamine (2.04 mL, 14.63 mmol) and the resulting solution was maintained at 0 °C for 10 min, tert-butyldimethylchlorosilane (TBSCl) (1.76 g, 11.70 mmol) in DMF (4.0 mL) was added. The resulting solution was warmed to rt and maintained at that temperature for 4 h. Water (20 mL) was added to the reaction mixture and the aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The resulting residue was purified by flash chromatography (20:1 hexane-EtOAc) to afford 1.84 g

(94%) of **3a** as a colorless oil: R_f= 0.63 (10:1 hexane-EtOAc)); IR (neat) 2958, 1463 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.41-5.49 (m, 2H), 4.23 (d, J= 6.0 Hz, 2H), 2.03-2.06 (m, 2H), 0.97 (t, J= 7.5 Hz, 3H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ ; 132.7, 129.1, 59.6, 26.3, 21.1, 18.6, 14.4, -4.9; MS (ESI) m/z 199.1512 (M+H) [199.1513 calcd for $C_{11}H_{23}OSi$].



3b

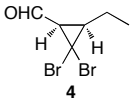
Dibromide 3b To a solution of silyl ether **3a** (1.0 g, 5.0 mmol) and benzyltriethylammonium chloride ($BnNEt_3Cl$) (20.0 mg, 0.1 mmol) in bromoform (2.85 mL, 17.5 mmol) was added a solution of NaOH (1.42 g, 35.4 mmol) in water (1.42 mL) over 1 h. The reaction mixture was stirred for 16 h. The mixture was diluted with water (50 mL) and filtered through a plug of Celite, washed with dichloromethane (150 mL). The aqueous layer was extracted with dichloromethane (3 x 30 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The resulting residue was purified by flash chromatography (hexane) to afford 1.49 g (80%) of **3b** as a colorless oil: R_f= 0.48 (20:1 hexane-EtOAc); IR (neat) 2957, 1463, 1255 cm^{-1}; ^1H NMR (500 MHz $CDCl_3$) δ 3.80 (dd, J= 11.2, 6.6 Hz, 1H), 3.58 (dd, J= 11.2, 7.2 Hz, 1H), 1.88 (dt, J= 11.0, 6.9 Hz, 1H), 1.63 (dt, J= 11.0, 7.3 Hz, 1H), 1.40-1.52 (m, 2H), 1.08 (t, J= 7.4 Hz, 3H), 0.91 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ ; 61.4, 35.6, 35.0, 34.3, 26.1, 20.8, 18.5, 13.1, -5.0, -5.1; MS (ESI) m/z 392.9858 (M+Na) [392.9855 calcd for $C_{12}H_{24}Br_2NaOSi$].



3c

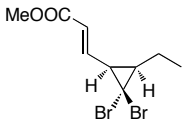
Alcohol 3c To a solution of dibromide **3b** (2.18 g, 5.8 mmol) in THF (58.0 mL) was added a solution of tetrabutylammonium (TBAF) (8.2 mL, 1.0 M in THF, 8.2 mmol). After 30 min, the solution was diluted with ethyl acetate (30 mL) and washed with 1 M aqueous HCl (50 mL). The aqueous layer was extracted with ethyl acetate (3 × 30 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The resulting residue was purified by flash chromatography (8:1 hexane-EtOAc) to afford 1.42 g (95%) of **3c** as a light yellow oil: R_f= 0.27 (4:1 hexane-EtOAc); IR (neat) 3351, 2967, 2931, 1460, cm^{-1}; ^1H NMR (500 MHz $CDCl_3$) δ 3.66-3.75 (m, 2H), 1.91-1.96 (m, 2H), 1.64-1.69 (m, 1H), 1.40-1.56 (m, 2H), 1.06 (t, J= 7.4 Hz, 3H); ^{13}C NMR (125

MHz, $CDCl_3$) δ 61.2, 36.0, 34.9, 34.2, 20.9, 13.1; MS (EI) m/z 255.9080 (M+H) [255.9098 calcd for $C_6H_{10}Br_2O$].



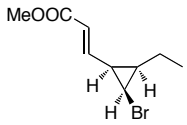
4

Aldehyde 4 To a solution of alcohol **3c** (2.48 g, 9.63 mmol) in dichloromethane (40 mL) and DMSO (10 mL) at -15 °C was added N,N,-diisopropylethylamine (5.02 mL, 28.84 mmol). The resulting solution was maintained for 10 min at -15 °C and SO_3·Pyr (4.59 g, 28.84 mmol) in DMSO (10 mL) was added dropwise. The resulting solution was maintained at -15 °C for 1 h and warmed to rt. After dilution with Et_2O (75 mL) the solution was washed sequentially with water (2 x 30 mL), brine (1 x 30 mL), 15% $NaHCO_3$ (1 x 30 mL) and saturated aqueous $CuSO_4$ (2 x 30 mL). The organic layer was then dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (8:1 hexane-EtOAc) to afford 1.94 g (79%) of **4** as a light yellow oil: R_f= 0.58 (4:1 hexane-EtOAc); IR (neat) 2969, 2874, 1707 cm^{-1}; ^1H NMR (500 MHz $CDCl_3$) δ 9.31 (d, J= 5.6 Hz, 1H), 2.40 (dd, J= 10.9, 5.6 Hz, 1H), 2.08-2.14 (m, 1H), 1.89-1.97 (m, 1H), 1.71-1.80 (m, 1H), 1.12 (t, J= 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 198.3, 41.6, 40.2, 29.1, 21.5, 13.0; MS (ESI) m/z 255.0, (M+H) [254.90 calcd for $C_6H_9Br_2O$].



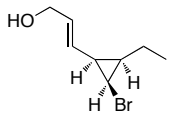
4a

Enoate 4a To a solution of methyl 2-(diethoxyphosphoryl)acetate (3.18 g, 18.17 mmol) in THF (90 mL) at 0 °C was added NaH (392 mg, 16.35 mmol) in 5 portions over 2 min. The resulting solution was maintained at 0 °C for 10 min then warmed to rt. Neat aldehyde **4** was added to the solution dropwise. The resulting reaction mixture was stored for 4 h then diluted with Et_2O (100 mL). The ethereal solution was then washed with 1 M NaOH (50 mL). The organic layer was separated and the aqueous layer was extracted with Et_2O (3 x 75 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (10:1 hexane-EtOAc) to afford 2.45 g (87%) of **4a** as a light yellow oil: R_f= 0.58 (4:1 hexane-EtOAc); IR (neat) 2968, 2933, 2875, 1708, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.60 (dd, J= 15.6, 10.0 Hz, 1H), 6.13 (d, J= 15.6 Hz, 1H), 3.75 (s, 3H), 2.42 (t, J= 10.4 Hz, 1H), 1.93 (ddd, J= 10.7, 7.5, 7.5 Hz, 1H), 1.63-1.72 (m, 1H), 1.47-1.54 (m, 1H), 1.06 (t, J= 7.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.0, 143.3, 125.1, 51.7, 39.9, 35.7, 35.1, 21.7, 12.5; MS (ESI) m/z 332.9114 (M+Na) [332.9096 calcd for $C_9H_{12}Br_2NaO_2$].



5

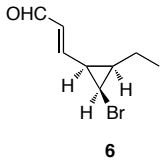
Cyclopropylbromide 5 To a solution of enoate **4a** (6.45 g, 19.73 mmol) in toluene (79 mL) at -78 °C was added triphenylstannane (6.93 mL, 19.73 mmol) and a solution of triethylborane (5.92 mL, 1 M in hexanes, 5.92 mmol). The reaction flask was fitted with a drying tube and the solution was maintained at -78 °C for 4 h. The reaction mixture was warmed to room temperature and poured into a saturated solution of potassium fluoride (100 mL) and the resulting mixture was stirred for an additional 3 h. The solution was then passed through a plug of Celite and washed with Et_2O (150 mL). The resulting solution was extracted with Et_2O (3 x 75 mL) The combined organics were dried ($MgSO_4$) and concentrated to ~80 mL in vacuo (toluene not removed). The resulting solution was filtered through a long plug of silica gel first with hexanes (500 mL) then with Et_2O (500 mL). The ethereal fractions were combined and concentrated in vacuo. The residue was purified by flash chromatography (10:1 hexane-Et_2O) to afford 4.37 g (95%) of **5** as a light yellow oil: R_f= 0.50 (4:1 hexane-EtOAc); IR (neat) 2968, 1732, 1645, 1435 cm^{-1}; ^1H NMR (400 MHz $CDCl_3$) δ 6.82 (dd, J= 15.6, 10.4 Hz, 1H), 6.05 (d, J= 15.6 Hz, 1H), 3.74 (s, 3H), 3.47 (t, J= 7.6 Hz, 1H), 1.86 (ddd, J= 9.9, 9.9, 7.3 Hz), 1.57-1.66 (m, 2H), 1.24-1.36 (m, 1H) 1.02 (t, J= 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.3, 146.2, 123.2, 51.5, 30.9, 26.5, 22.6, 19.8, 13.1; MS (ESI) m/z 254.9991 (M+Na) [254.9991 calcd for $C_9H_{13}BrNaO_2$].



5a

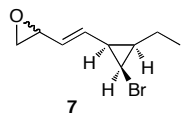
Allylic alcohol 5a To a solution of cyclopropylbromide **5** (4.96 g, 17.84 mmol) in dichloromethane (200 mL) at -78 °C was added neat diisobutylaluminum hydride (DIBAL-H) (7.57 mL, 42.46 mmol, 2.0 equiv) dropwise. The resulting solution was maintained at -78 °C for 45 min. The reaction mixture was warmed to rt, quenched with a saturated solution of Rochelle's salt (150 mL) and stirred for 2 h. The aqueous layer was extracted with Et_2O (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (4:1 hexane-EtOAc) to afford 3.85 g (95%) of **5a** as a yellow oil: R_f= 0.30 (hexanes-ethyl acetate, 4:1); IR (neat) 3309, 2964 cm^{-1}; ^1H NMR (500 MHz $CDCl_3$) δ 5.88 (dt, J= 15.4, 6.0 Hz, 1H), 5.51 (dd, J= 15.4, 9.3 Hz, 1H), 4.12 (d, J= 6.0 Hz, 2H), 3.34 (t, J= 7.5 Hz, 1H), 1.70-1.76 (m, 1H), 1.47-1.51 (m, 2H), 1.07-1.13 (m, 1H), 0.99 (t, J= 7.4 Hz, 3H); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 132.7, 128.4, 63.8, 31.1, 23.9, 22.0, 19.7, 13.2; MS (ESI) m/z 187.0088 (M+H–H_2O) [187.01 calcd for $C_8H_{12}Br$].



6

Enal 6 To a solution of allylic alcohol **5a** (1.0 g, 4.88 mmol) in dichloromethane (50 mL) was added manganese(IV) oxide (4.24 g, 48.76 mmol). The reaction mixture was stirred for 16 h and filtered through a plug of Celite. The filtrate was concentrated in vacuo. The residue was purified by flash chromatography (8:1 hexane-Et_2O) to afford 0.983 g (99%) of **6** as a colorless oil: R_f= 0.38 (4:1 hexane-EtOAc); IR (neat) 2965, 2862, 1683 cm^{-1}; ^1H NMR (500 MHz $CDCl_3$) δ 9.49 (d, J= 8.0 Hz, 1H), 6.67 (dd, J= 15.5, 10.2 Hz, 1H), 6.34 (dd, J= 15.5, 8.0 Hz, 1H), 3.56 (t, J= 7.3 Hz, 1H), 1.98 (dt, J= 9.9, 7.2 Hz, 1H), 1.58-1.65 (m, 2H), 1.42-1.47 (m, 1H), 1.01 (t, J= 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.0, 155.8, 135.2, 31.6, 27.7, 23.3, 20.0, 13.1; MS (ESI) m/z 224.9879 (M+Na) [224.9885 calcd for $C_8H_{11}BrNaO$].

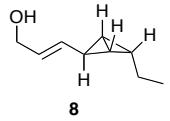


7

Epoxide 7 To a solution of aldehyde **6** (500 mg, 2.446 mmol) and ICH_2Cl (0.179 mL, 2.46 mmol) in THF (5 mL) at -78 °C was added a solution of *n*-BuLi (1.16 mL, 2.13 M in hexane, 2.46 mmol) dropwise. The resulting solution was maintained for 30 min at -78 °C and quenched with saturated NH_4Cl (10 mL). The reaction mixture was warmed to rt, diluted with water (3 mL) and extracted with Et_2O (3 x 10 mL). Combined ethereal extracts were dried ($MgSO_4$) and concentrated. Flash column chromatography of the resulting residue (4:1 hexane-Et_2O 4:1) yielded 318 mg (51%) of a mixture of chlorohydrins (**6a**) as a light yellow oil: ^1H NMR (400 MHz, $CDCl_3$): δ 5.77 (dd, J= 15.6, 6.4 Hz, 1H), 5.65 (dd, J= 15.2, 8.8 Hz, 1H), 4.36 (m, 1H), 3.60 (dd, J= 11.2, 4.0 Hz, 1H), 3.52 (dd, J= 10.4, 6.8 Hz, 1H), 3.62 (dd, J= 7.6, 7.6 Hz, 1H), 1.73 (ddd, J= 16.8, 9.2, 7.6 Hz, 1H), 1.46-1.53 (m, 2H), 1.11-1.15 (m, 1H), 0.99 (t, J= 7.6 Hz, 3H).

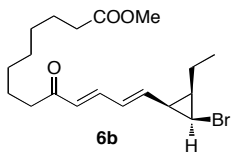
To a solution of chlorohydrins **6a** (50 mg, 0.20 mmol) in THF (2 mL) was added NaH (9.5 mg, 0.39 mmol). The resulting mixture was stirred for 1 h and quenched with 10% $NaHCO_3$ (3 mL) and water (3 mL) and separated. The aqueous was extracted with Et_2O (5 x 5 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated. The resulting residue was purified by flash column chromatography (10:1 hexane-Et_2O-1% Et_3N) to yield 36.3 mg (84%)

of epoxide **7** as a light yellow oil and as a mixture of diastereomers: IR (neat) 2965, 2929, 2873, 1248 cm^{-1}; ^1H NMR (400 MHz, C_6D_6): δ 5.75 (ddd, J= 15.2, 9.6, 9.2, 1H), 5.22 (dd, 15.2, 8.0 Hz, 1H), 3.01-3.05 (m, 1H), 2.88 (dd, J= 7.6, 7.6 Hz, 1H), 2.46-2.50 (m, 1H), 2.26 (dd, J= 5.6, 2.4 Hz, 1H), 1.32-1.42 (m, 3H), 1.17-1.23 (m, 1H), 0.87 (t, J=7.6 Hz, 3H). ^{13}C NMR (150 MHz, C_6D_6) (major isomer only): δ 132.1, 131.1, 51.9, 48.5, 31.0, 24.2, 22.3, 19.9, 13.1; MS (EI) m/z 216.0167 (M+H) [216.0150 calcd for $C_9H_{14}BrO$].



8

Bicyclobutane 8 To a solution of epoxide **7** (50 mg, 0.23 mmol) in THF (2.3 mL) at -78 °C was added 0.325 mL *n*-BuLi (2.13 M in hexanes, 0.325 mL, 0.69 mmol) slowly. The solution was maintained at -78 °C for 30 min. In a separate flask CuI (88 mg, 0.46 mmol) and LiCl (39 mg, 0.92 mmol) were weighed and the flask flame dried under vacuum, purged with argon and cooled to rt. The salts were dissolved in THF (1.15 mL) and stirred for 10 min. The resulting yellow solution was cooled to -78 °C. The prepared solution of cylopropyllithium was added to the yellow solution of CuI-LiCl dropwise via cannula. The resulting solution was stirred for 2 h, warmed to -15 °C and quenched with a 9:1 aqueous solution of saturated NH_4Cl and NH_4OH (3 mL). After warming to room temperature, the aqueous layer was extracted with Et_2O (4 x 7 mL). Organic extracts were dried ($MgSO_4$) and concentrated to yield bicyclobutane **8** (ca. 54 mg, crude) as a light yellow oil: ^1H NMR (400 MHz, C_6D_6): δ 5.67 (ddd, J= 15.2, 5.6, 5.2 Hz, 1H), 5.27 (dd, J= 15.6, 9.2 Hz, 1H), 3.89 (d, J=3.6 Hz, 2H), 2.44 (d, J= 8.8 Hz, 1H), 2.04 (m, 1H), 1.35-1.45 (m, 2H), 1.23-1.29 (m, 2H), 0.97 (t, J= 7.2 Hz, 3H). ^{13}C NMR (100 MHz, C_6D_6): δ 129.4 (CH), 129.3 (CH), 63.1(CH_2), 45.9 (CH), 44.8 (CH), 17.2 (CH_2), 13.6 (CH_3), 9.18 (CH).

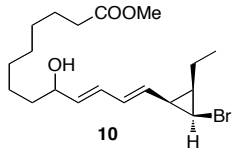


6b

Dienone 6b To a solution of β-keto phosphonate ester **9**[2] in THF at -78 °C was added KHMDS. The resulting solution was allowed to stir at -78 °C for 30 min and warmed to rt. Aldehyde **6** was added neat dropwise and the resulting solution was stirred 16 h. The reaction mixture was quenched with water (10 mL) and extracted with Et_2O (3 x 30 mL). Combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Flash column chromatography of the residue (8:1 hexane-EtOAc) yielded 762.2 mg (92%) of dienone **6b** as a

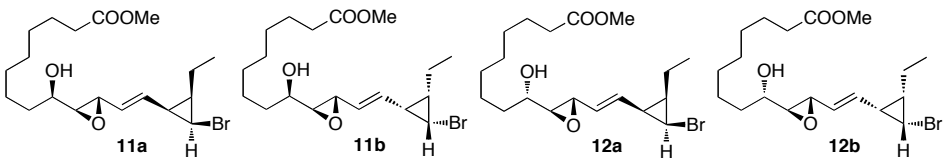
[2] I. Delamarche, P. Mossett, *J Org Chem* **1994**, *59*, 5453-5457.

pale yellow oil: IR (neat) 2932, 2858, 1737, 1685, 1660, 1628, 1595cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.17 (dd, J=15.5, 10.9 Hz, 1H), 6.42 (dd, 15.1, 10.9, 1H), 6.09 (d, J=15.5 Hz, 1H), 6.02 (dd, J=15.1, 9.8 Hz), 3.66 (s, 3H), 3.45 (dd, J=7.4, 7.4 Hz, 1H), 2.55 (t, J=7.5 Hz, 2H), 2.30 (t, J=7.5 Hz, 2H), 1.85 (ddd, J=9.7, 7.4, 7.4 Hz, 1H), 1.50-1.65 (m, 6H), 1.32 (br s, 6H), 1.23-1.31 (m, 1H), 1.01 (t, J=7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 201.1, 174.3, 142.2, 141.1, 131.6, 128.0, 51.5, 40.4, 34.1, 31.6, 29.2, 29.1, 29.0, 26.1, 25.0, 24.4, 23.4, 19.8, 13.1; MS (ESI) m/z 385.1379 (M+H) [385.1373 calcd for $C_{19}H_{30}BrO_3$].



10

Alcohol 10 To a stirred solution of dieneone **6b** (762 mg, 1.98 mmol) in methanol (15 mL) was added $CeCl_3{\cdot}7H_2O$ (2.2 g, 6.0 mmol). The resulting mixture was stirred for 30 min and $NaBH_4$ (150 mg, 3.95 mmol) was then added in 3 portions. The resulting solution was stirred for 10 min, quenched with water (10 mL), 2 M HCl (10 drops) and extracted with EtOAc (4 x 30 mL). Combined organic extracts were dried ($MgSO_4$) and concentrated. Flash column chromatography of the residue (4:1 hexane-EtOAc) yielded 754 mg (98%) of alcohol **16** as a colorless oil and as a mixture of diastereomers : IR (neat) 3455 (br), 2931, 2856, 1737 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.33–6.23 (m, 2H), 5.65–5.51 (m, 2H), 3.66 (s, 3H), 3.38 (dd,t, J=7.49, 7.49 Hz, 1H), 2.29 (t, J= 7.5 Hz, 2H), 1.75 (ddd, J= 9.6, 7.5, 7.5 Hz, 1H), 1.66–1.45 (m, 9H), 1.31 (s (br), 6H), 1.15 (dddd, J=9.66, 7.36, 7.36, 7.28 Hz, 1H), 1.00 (t, J= 7.5 Hz, 3H) ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.4, 134.4, 134.3 (isomer), 132.7, 130.5, 130.4 (isomer), 130.1, 72.7, 72.5 (isomer), 51.5, 37.3(2), 37.3(0) (isomer), 34.1, 31.5, 29.4, 29.2, 29.1, 25.4, 25.0, 24.5(3), 24.5(1) (isomer), 22.6, 19.7, 13.1; MS (ESI) m/z 369.1414, (M+H–H_2O) [369.1424 calcd for $C_{19}H_{30}BrO_2$].



11a **11b** **12a** **12b**

Epoxy alcohols syn (11a/11b) and anti (12a/12b) To a stirred solution of alcohol **10** (150 mg, 0.39 mmol) at 0 ºC in dichloromethane (15 mL) was added dimethyl dioxirane as a 0.05 M solution in acetone (7.75 mL, 0.39 mmol). The resulting solution was stirred for 10 min at 0 ºC and concentrated to yielded a colorless oil. Flash column chromatography (3:1-1% Et_3N hexane-

EtOAc) yielded a mixture of syn (**11**) and anti (**12**) epoxy alcohols. The syn isomers **11a**/**11b** (Rf= 0.22; 99.5 mg, 64%) proved to be the slower eluting isomers and the anti isomers **12a**/**12b** faster eluting isomers (Rf= 0.31; 49.0 mg, 31%). After flash chromatography syn and anti diastereomers were separated by HPLC for the purpose of characterization using a Varian Dynamax 8 μm Si column (2.14 x 25 cm) eluted at a flow rate of 10 mL/min with Hexane/*i*-propanol gradient (1 to 3% i-propanol) with UV light detection at 205 nm using a Varian 230 detector. Diastereomers **11a** and **11b** (and **12a** and **12b)** were not assigned.

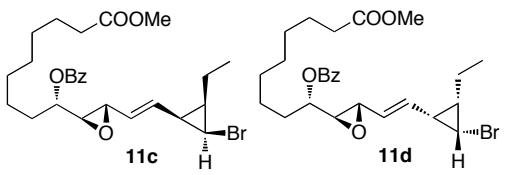
11a or **11b:** Retention time 33.2 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.79 (dd, J=15.5, 9.4 Hz, 1H), 5.45 (dd, J=15.5, 8.4 Hz, 1H), 3.84 (m, 1H), 3.66 (s, 3H), 3.49 (dd, J= 8.36, 2.28, 1H), 3.37 (dd, J=7.4, 7.4 Hz, 1H), 2.96 (dd, J= 2.7, 2.7 Hz, 1H), 2.30 (t, J= 7.5 Hz, 2H), 1.77 (ddd, J= 9.6, 7.6, 7.6 Hz, 1H), 1.46–1.65 (m, 8H), 1.32 (s(br), 6H), 1.16 (dddd, J=9.8, 9.6, 7.4, 7.4 Hz, 1H), 1.02 (t, J=7.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): 174.5, 133.0, 130.0, 68.6, 62.8, 55.1, 51.6, 34.2, 33.4, 30.9, 29.6, 29.3, 29.2, 25.3, 25.0, 24.4, 22.3, 19.7, 13.2.

11a or **11b:** Retention time 34.1 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.78 (dd, J= 15.4, 9.4 Hz, 1H), 5.47 (dd, J= 15.4, 8.1 Hz, 1H), 3.85 (m, 1H), 3.66 (s, 3H), 3.48 (dd, J= 8.2, 2.1 Hz, 1H), 3.37 (dd, J= 7.42, 7.42 Hz, 1H), 2.95 (dd, J= 2.64, 2.64 Hz, 1H), 2.30 (t, J= 7.5 Hz, 2H), 1.77 (ddd, J= 9.2, 7.6. 7.6 Hz, 1H), 1.45–1.65 (m 8H), 1.32 (s(br), 6H), 1.53 (m, 1H), 1.01 (t, J=7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): 174.5, 132.8, 129.9, 68.5, 62.9, 55.0, 51.6, 34.2, 33.4, 30.9, 29.6, 29.3, 29.2, 25.3, 25.0, 24.4, 22.3, 19.8, 13.1.

12a or **12b:** Retention time 41.0 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.77 (dd, J= 15.4, 9.5 Hz, 1H), 5.43 (dd, J= 15.5, 8.3 Hz, 1H) 3.66 (s, 3H), 3.52 (m, 1H), 3.39 (dd, J= 8.4, 2.2 Hz, 1H0, 3.36 (dd, J= 7.5, 7.5 Hz, 1H), 2.91 (dd, J=4.8, 2.3 Hz, 1H), 2.30 (t, J=7.5 Hz, 2H), 1.77 (ddd, J= 9.6, 7.4, 7.4 Hz, 1H), 1.51–1.65 (m, 8H), 1.31 (s(br), 6H), 1.12–1.21 (m, 1H), 1.02 (t, J=7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 174.4, 132.9, 129.9, 71.0, 63.4, 56.9, 51.6, 34.5, 34.2, 30.9, 29.5, 29.3, 29.2, 25.3, 25.0, 24.4, 22.2, 19.7, 13.2.

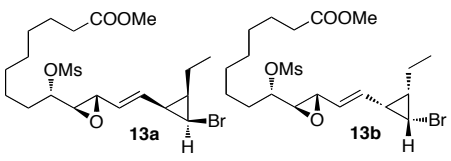
12a or **12b:** Retention Time 41.9 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.78 (dd, J= 15.5, 9.4 Hz, 1H), 5.46 (dd, J= 15.5, 8.1 Hz, 1H), 3.66 (s, 3H), 3.53 (m, 1H), 3.39 (dd, J=8.1, 2.3 Hz, 1H), 3.37 (dd, J= 7.4, 7.4 Hz, 1H), 2.91 (dd, J= 4.7, 2.3 Hz, 1H), 2.30 (t, J= 7.5 Hz, 2H), 1.76 (ddd, J= 9.4, 7.4, 7.4 Hz, 1H), 1.48–1.65 (m, 8H), 1.31 (s(br), 6H), 1.10–1.19 (m, 1H), 1.00 (t, J= 7.4 Hz,

1H). ^{13}C NMR (125 MHz, $CDCl_3$): 174.4, 132.8, 129.8, 71.0, 63.5, 56.8, 51.6, 34.6, 34.2, 30.9, 29.5, 29.3, 29.2, 25.4, 25.0, 24.4, 22.2, 19.7, 13.1.



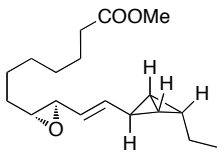
***p*-Nitro benzoate 11c/11d** To a solution of syn-epoxy alcohols **11a/11b** (182 mg, 0.45 mmol) in THF (4.5 mL) was added PPh_3 (237 mg, 0.90 mmol) and *p*-nitrobenzoic acid (151 mg, 0.90 mmol). The solution was maintained for 10 min and diisopropylazadicarboxylate (DIAD) was added dropwise. The resulting solution was maintained for 16 h, concentrated in vacuo and the residue purified by flash column chromatography (5:1-1% Et_3N hexane-EtOAc) to yield 215 mg (86%) of **11c/11d** as a colorless oil and as a mixture of diastereomers: IR (neat) 2933, 2860, 1729, 1529, 1271 cm^{-1}; ^1H NMR (400 MHz $CDCl_3$) δ 8.27 (d, J= 8.8 Hz, 2H), 8.18 (d, J= 8.8 Hz, 2H), 5.71-5.80 (m, 1H), 5.35-5.46 (m, 1H) 5.06-5.13 (m, 1H), 3.63 (s, 3H), 3.42-3.46 (m, 1H), 3.33 (t, J= 7.6 Hz, 1H), 3.01-3.05 (m, 1H), 2.26 (t, 7.6 Hz, 2H), 1.76-1.83 (m, 2H), 1.68-1.75 (m, 1H), 1.57-1.63 (m, 2H), 1.33-1.56 (m, 4H), 1.20-1.30 (m, 6H), 1.04-1.18 (m, 1H), 0.92-1.00 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 164.1, 150.7, 135.5, 133.1, 132.8 (isomer), 130.9, 130.8 (isomer), 129.5, 129.3 (isomer), 123.7, 74.0, 73.9 (isomer), 60.3, 60.2 (isomer), 56.7, 51.5, 34.1, 31.1(4), 31.1(0) (isomer), 30.8, 29.3, 29.1, 29.0, 25.0, 24.9, 24.4, 24.3 (isomer), 22.2, 19.7, 13.1, 13.0 (isomer); MS (ESI) 574.1368 m/z (M+Na) calcd for $C_{26}H_{34}BrNNaO_7$, 574.14].

Methanolysis of 11c/11d To a solution of *p*-nitrobenzoate ester **11c/11d** (44 mg, 0.08 mmol) in MeOH (0.7 mL) was added K_2CO_3 (11 mg, 0.08 mmol). The resulting solution was stirred for 30 minutes, quenched with saturated ammonium chloride (1 mL) and the aqueous layer extracted with dichloromethane (3 x 5 mL). Combined organic extracts were dried over Na_2SO_4, filtered and concentrated in vacuo. Flash column chromatography (4:1-1% Et_3N hexane:EtOAc) yielded 28 mg (86%) of anti-epoxy alcohols **12a/12b**.



Mesylate 13 To a solution of anti-epoxy alcohol **12** (248 mg, 0.61 mmol) in dichloromethane (20 mL) over activated 4 Å molecular sieves at 0 °C was added MsCl (0.142 mL, 1.84 mmol). The resulting solution was stirred at that temperature for 5 min and Et_3N (0.257 mL, 1.84 mmol) was added dropwise. One

crystal of 4,4-dimethylaminopyridine was added and the solution was warmed to room temperature and stirred for 16 h. The resulting solution was diluted with EtOAc (30 mL), washed with 15% $NaHCO_3$ (15 mL) and extracted EtOAc (3 x 30 mL). Combined organic extracts were dried over Na_2SO_4, filtered and concentrated in vacuo. Flash column chromatography (3:1-1% Et_3N hexane-EtOAc) to yield 165.1 mg (56%) of **13** as a light yellow oil and as a mixture of diastereomers: IR (neat) 2934, 1736, 1359, 1175, 925 cm^{-1}; ^1H NMR (400 MHz $CDCl_3$) δ 5.80 (dd, J= 15.2, 9.2 Hz, 1H), 5.40 (dd, J= 15.2, 8.4 Hz, 1H), 4.57-4.63 (m, 1H), 3.65 (s, 3H), 3.45-3.60 (m, 1H), 3.33-3.38 (m, 1H), 3.00-3.10 (m, 3H), 2.96-2.99 (m, 1H), 2.28 (t, J=7.6 Hz, 2H), 1.71-1.78 (m, 3H), 1.39-1.63 (m, 6H) 1.21-1.35 (m 6H), 1.10-1.20(m, 1H), 0.96-1.03 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 133.8, 133.6 (isomer), 129.0, 128.8 (isomer), 80.3, 80.2 (isomer), 60.0, 59.9 (isomer), 56.6, 56.5 (isomer) 51.6, 38.9, 38.8 (isomer), 34.1, 32.2, 30.8, 29.1, 29.0(5) (isomer), 24.9, 24.7, 24.5, 24.4, 22.2, 19.7, 13.1, 13.0 (isomer); MS (ESI) 521.1176 m/z, (M+Na+H_2O) [521.12 calcd for $C_{20}H_{35}BrNaO_7S$,].



2

Bicyclobutane 2 To a solution of mesylate **13** (53.7 mg, 0.11 mmol) in THF (2.0 mL) and water (2.79 mL) was added LiOH (117 mg, 2.79 mmol) at room temperature. The resulting solution was warmed to 60 °C, stirred for 2.5 h, cooled to room temperature, diluted with EtOAc (20 mL), and washed with saturated NH_4Cl (5 mL). The aqueous layer was extracted with EtOAc (4x 20 mL). Combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. Resulting oil was concentrated from benzene (3 x 5 mL) in vacuo, the residue dissolved in THF (14 mL) and the cooled to -78 °C. A solution of *t*-BuLi (1.50 M in pentane, 0.297 mL, 0.45 mmol) was added dropwise to the solution of crude carboxylic acid (**14**). The reaction mixture was stirred at -78 °C for 2 h, warmed to -20 °C and stirred an additional 30 min. The mixture was warmed to 0 °C and quenched with saturated NH_4Cl (0.2 mL). An ethereal solution of diazomethane (7 mL, ~0.25 M) was added and the mixture was stirred an additional 5 min, diluted with H_2O (10 mL) and extracted with EtOAc (4 x 20 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to yield a faint yellow oil (38 mg crude yield). The crude material was purified on RP-HPLC using a Varian microsorb-mv 4.6 x 250 mm column eluting with 85:15 (MeOH:H_2O) + 20 mM Et_3N at pH=8 at 0.5 mL/min with a UV detector at 205 nm. Aqueous fractions containing product were extracted with freshly distilled n-pentane (5 x 5 mL) and concentrated to yield 6.87 mg (20.3%) of **2** as a colorless oil: Retention

time 27 min; ^{1}H NMR (400 MHz, C_6D_6) δ 5.41 (dd, J=8.0, 15.6 Hz, 1H), 5.34 (dd, 7.2, 15.6 Hz, 1H), 3.37 (s, 3H), 2.93 (dd, J= 2.0, 6.8 Hz, 1H), 2.64 (dt, J= 2, 5.6 Hz, 1H), 2.34 (d, J= 8.0 Hz, 1H), 2.11 (t, J= 7.6 Hz, 2H), 1.92 (m, 1H), 1.49-1.58 (m, 2H), 1.23-1.42 (m, 6H), 1.06-1.19 (m, 8H), 0.87(t, J= 7.6 Hz, 3H); ^{13}C NMR (150 MHz, C_6D_6) δ 173.4, 132.8, 128.6, 60.4, 58.0, 51.0, 45.7, 44.6, 34.1, 32.4, 29.5(3), 29.4(8), 26.2, 25.2, 17.1, 13.6, 9.4, 9.3.

NMR comparison of Synthetic and Natural Bicyclobutane (2)

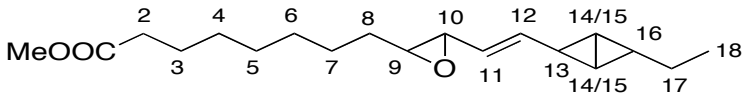
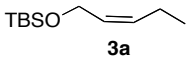


Table S1. Comparison of ^{13}C and ^{1}H NMR of natural and synthetic bicyclobutane **2**.

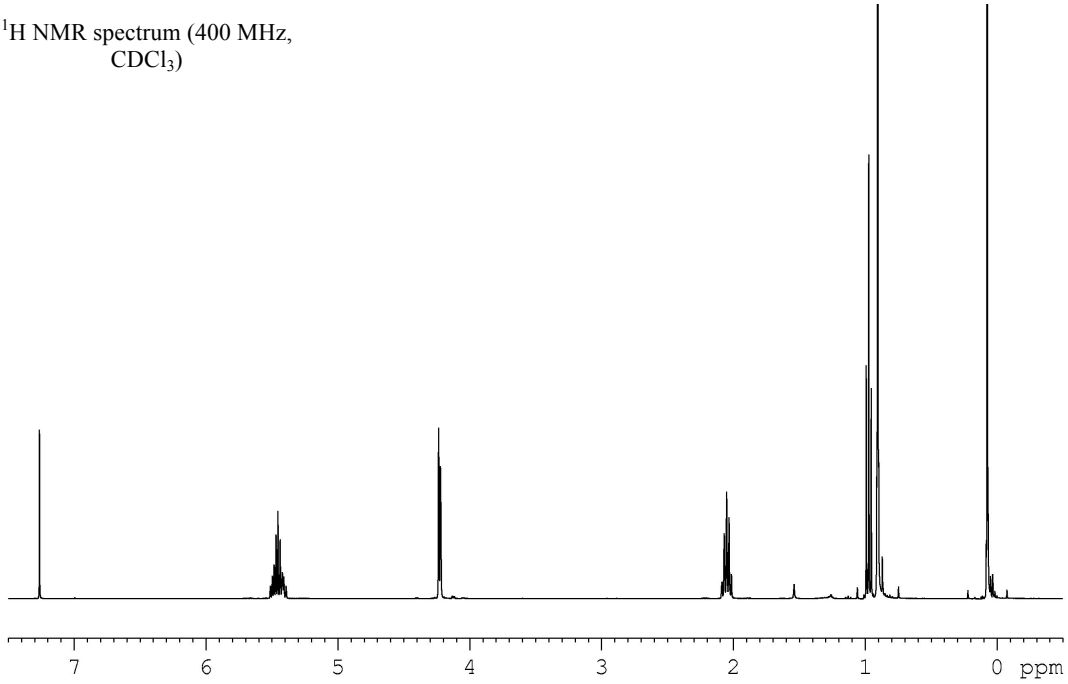
Carbon Number	^{13}C NMR (Literature)[3]	^{13}C NMR (Synthetic)	^{1}H NMR (Literature) [3]	^{1}H NMR (Synthetic)
1	~173	173.4	—	—
2	34.1	34.1	2.10 (t, *J*= 7.4 Hz)	2.11 (*J* = 7.6 Hz)
3	25.2	25.2	1.53 (q, *J*=7.4 Hz)	1.49-1.58 (m)
4, 5, 6	29.6, 29.5, 29.3	29.5, 29.5, 29.5	Not assigned	Not assigned
7	26.3	26.2	Not assigned	Not assigned
8	32.5	32.4	1.37 (m)	Not assigned
9	60.4	60.4	2.64 (dt, *J*= 5.6, 2 Hz)	2.64 (dt, *J*= 5.6, 2.0 Hz)
10	58.1	58.0	2.94 (dd, *J*= 7.1, 2.0 Hz)	2.93 (*J*= 6.8, 2.0 Hz)
11	~128	128.3	5.34 (dd, *J*= 15.4, 8.1 Hz)	5.34 (dd, *J*= 15.6, 7.2 Hz)
1	~133	132.7	5.39 (dd, *J*= 15.5, 8.2 Hz)	5.41 (dd, *J*= 15.5, 8.2 Hz)
13	44.7	44.6	2.36 (d, *J*= 8.2 Hz)	2.34 (d, *J*= 8.0 Hz)
14, 15	9.3, 9.2	9.3, 9.3	1.31, 1.34 (m)	Not assigned
16	45.8	45.7	1.91 (m)	1.92 (m)
17	17.1	17.1	1.16 (q, *J* = 7.3 Hz)	Not assigned
18	13.7	13.6	0.87 (t, *J*= 7.4 Hz)	0.87 (t, *J*= 7.6 Hz)
OMe	51.0	51.0	3.36 (s)	3.37 (s)

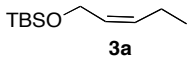
[3] C. Schneider, K. Niisuke, W. E. Boeglin, M. Voehler, D. F. Stec, N. A. Porter, A. R. Brash, *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 18941-18945.



3a

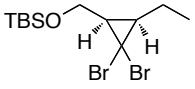
^{1}H NMR spectrum (400 MHz, $CDCl_3$)





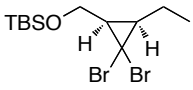
3a

^{13}C NMR spectrum (125 MHz, $CDCl_3$)



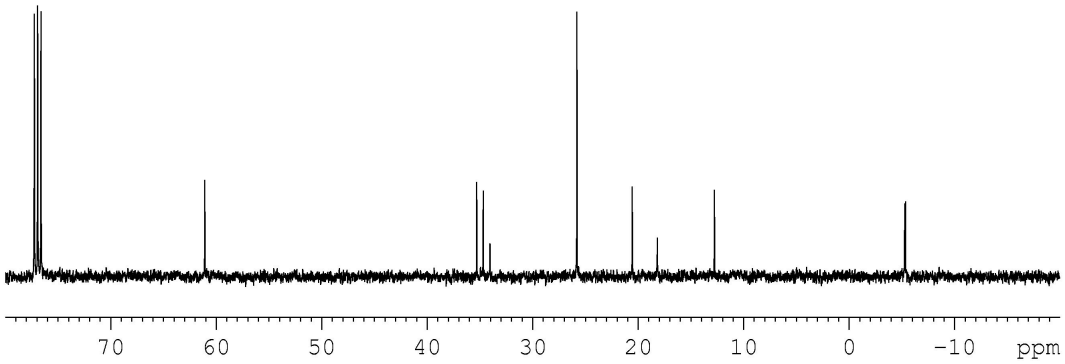
3b

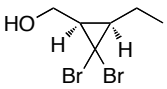
^{1}H NMR spectrum (400 MHz, $CDCl_3$)



3b

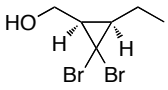
^{13}C NMR spectrum (125 MHz, $CDCl_3$)





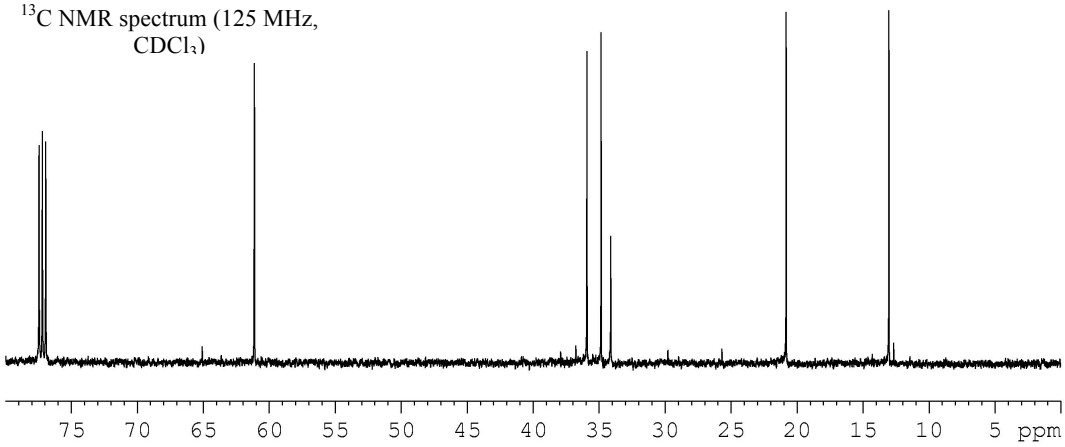
3c

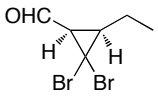
^{1}H NMR spectrum (400 MHz, $CDCl_3$)



3c

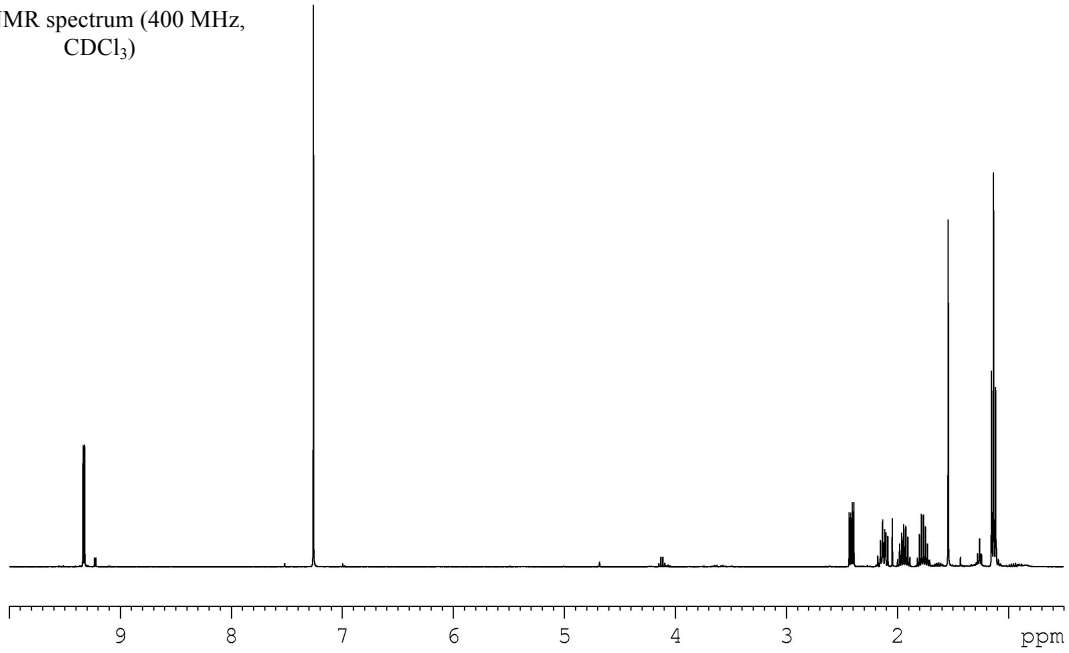
^{13}C NMR spectrum (125 MHz, $CDCl_3$)

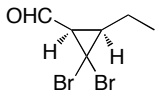




4

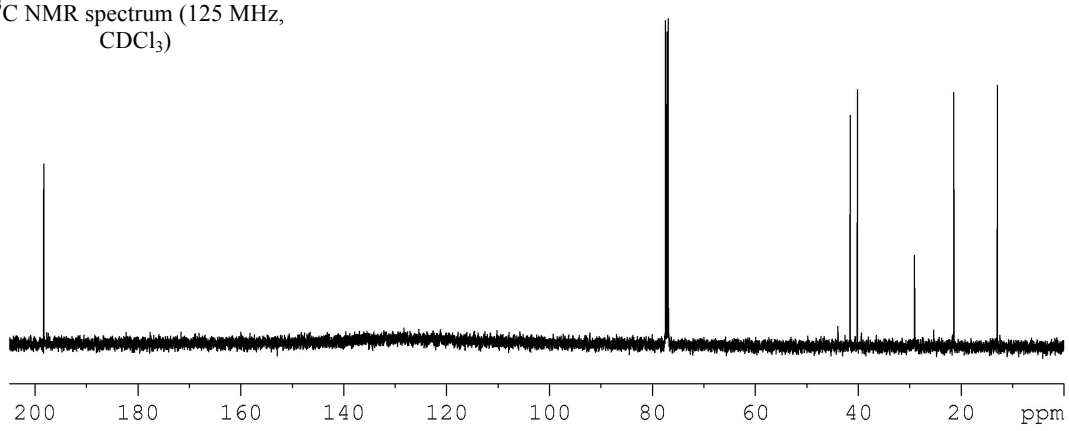
^{1}H NMR spectrum (400 MHz, $CDCl_3$)

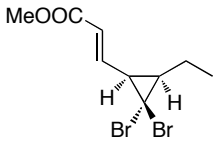




4

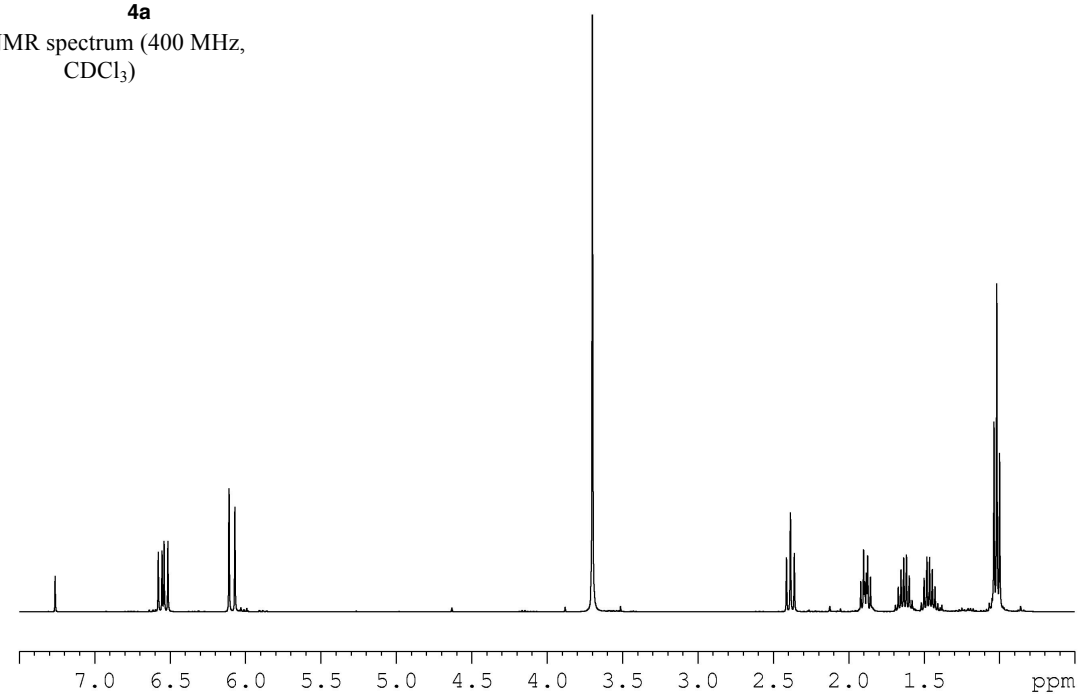
^{13}C NMR spectrum (125 MHz, $CDCl_3$)

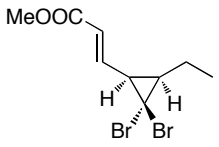




4a

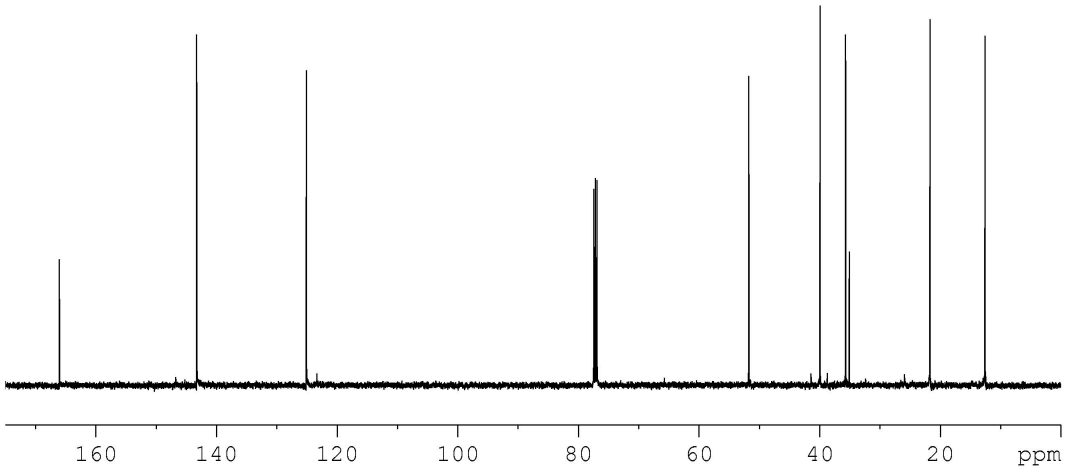
^{1}H NMR spectrum (400 MHz, $CDCl_3$)

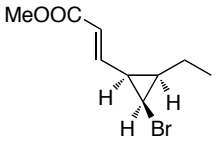




4a

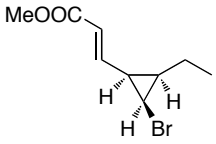
^{13}C NMR spectrum (125 MHz, $CDCl_3$)





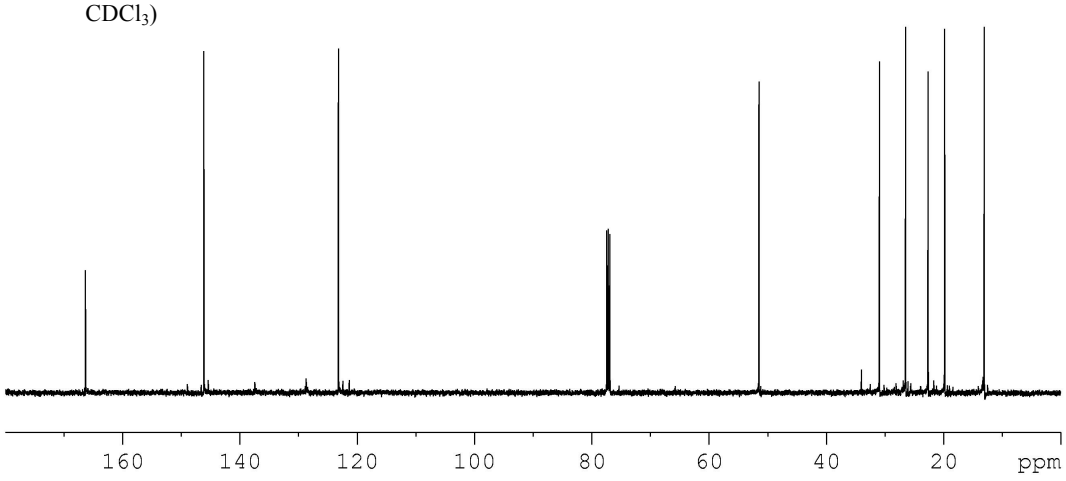
5

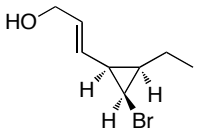
^{1}H NMR spectrum (400 MHz, $CDCl_3$)



5

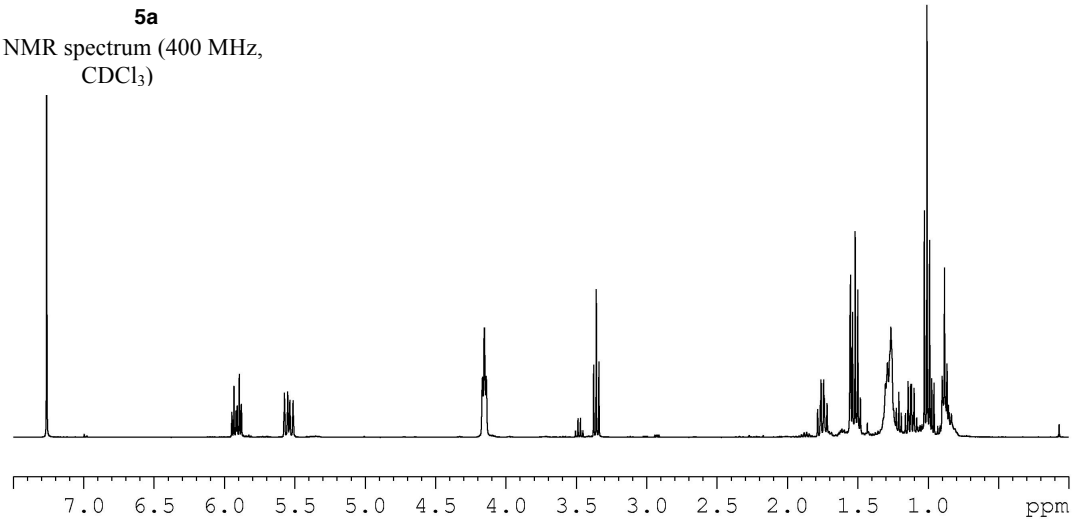
^{13}C NMR spectrum (125 MHz, $CDCl_3$)

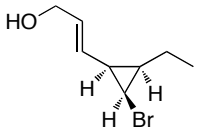




5a

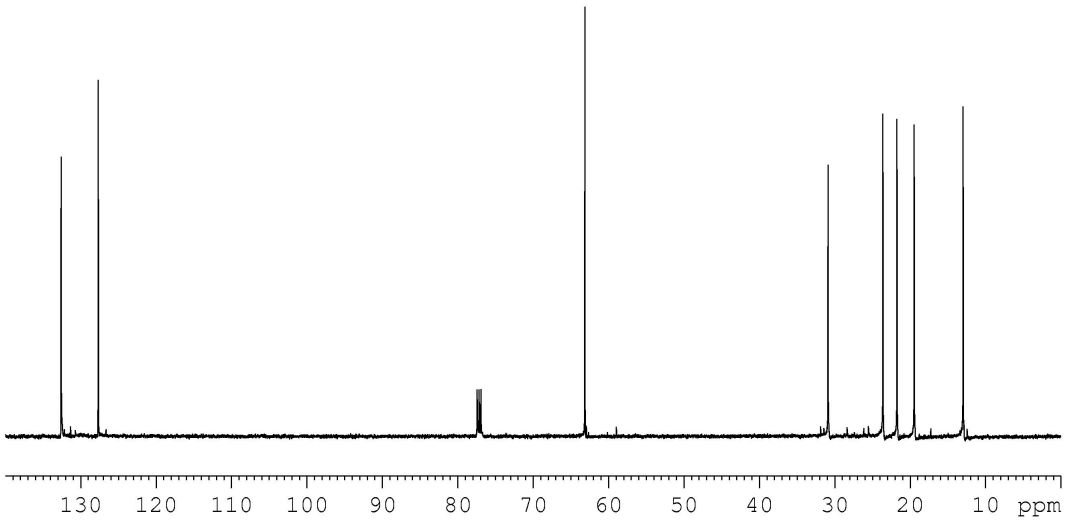
^{1}H NMR spectrum (400 MHz, $CDCl_3$)

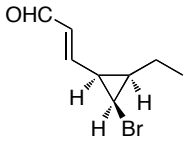




5a

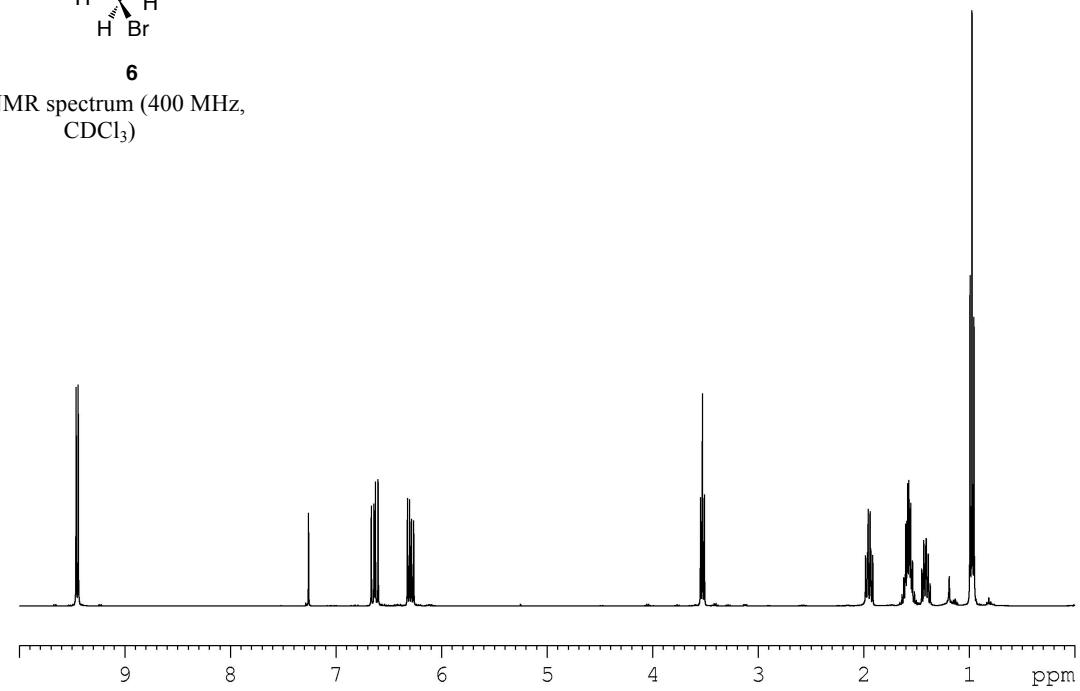
^{13}C NMR spectrum (125 MHz, $CDCl_3$)

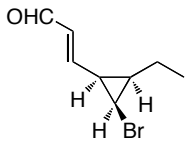




6

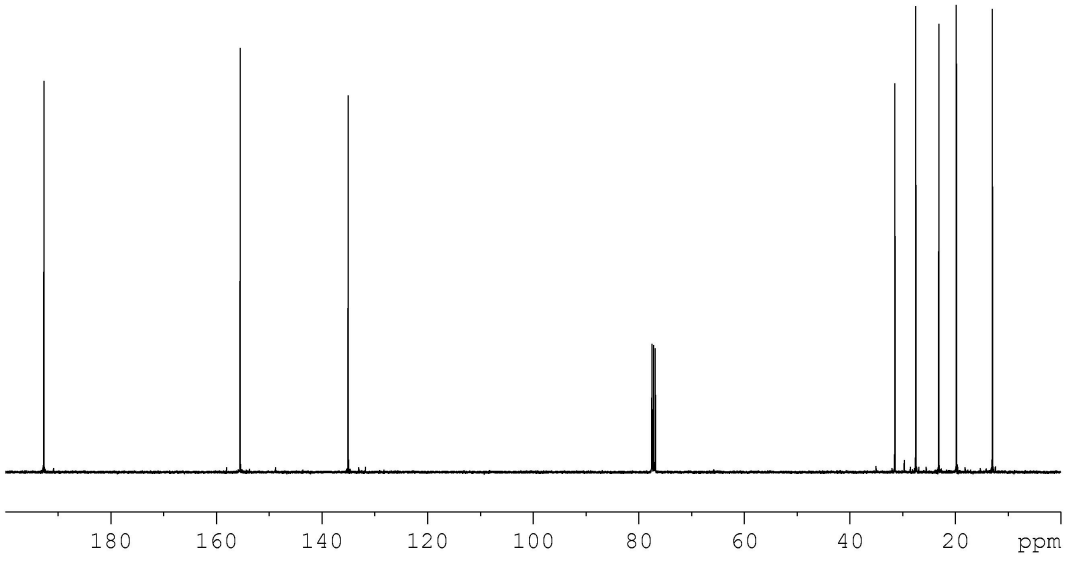
^{1}H NMR spectrum (400 MHz, $CDCl_3$)

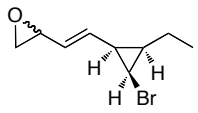




6

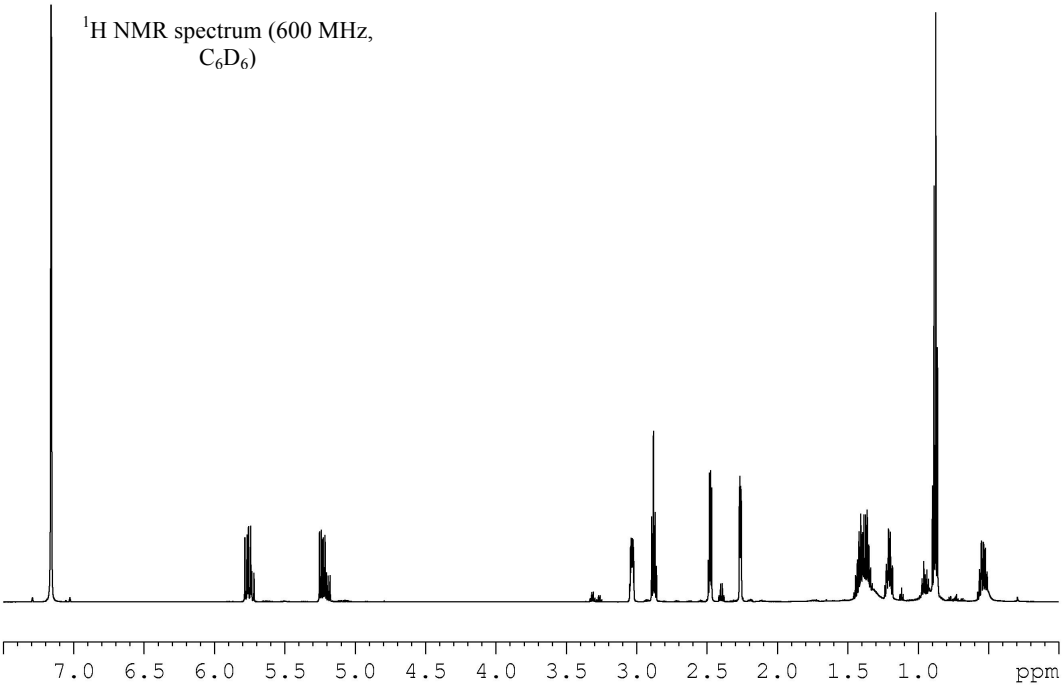
^{13}C NMR spectrum (125 MHz, $CDCl_3$)

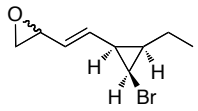




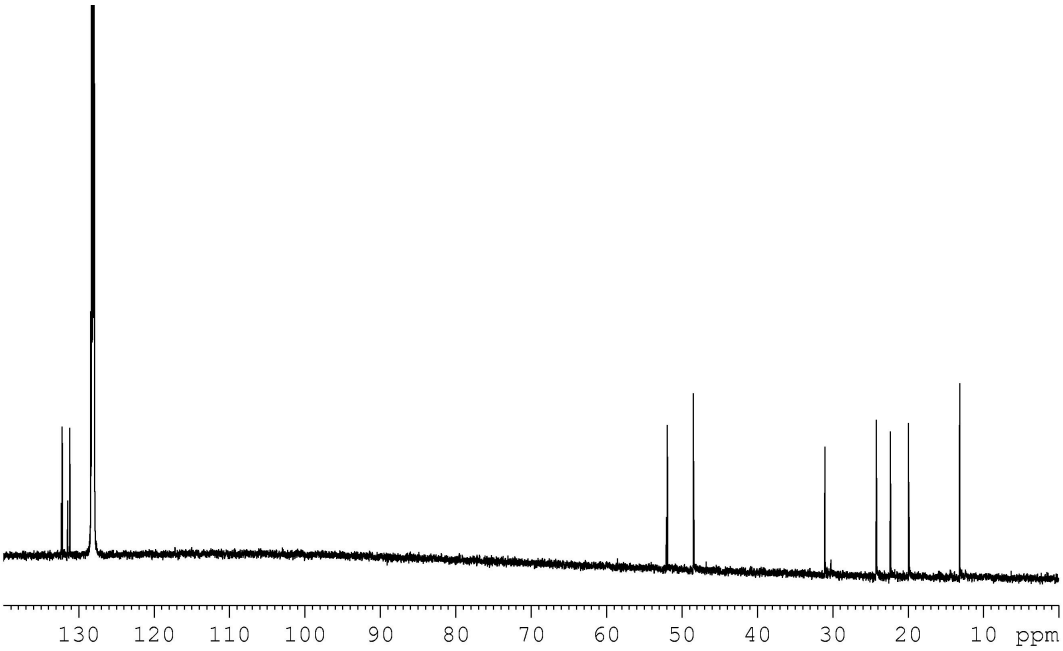
12

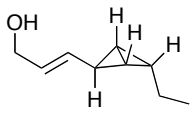
^{1}H NMR spectrum (600 MHz, C_6D_6)





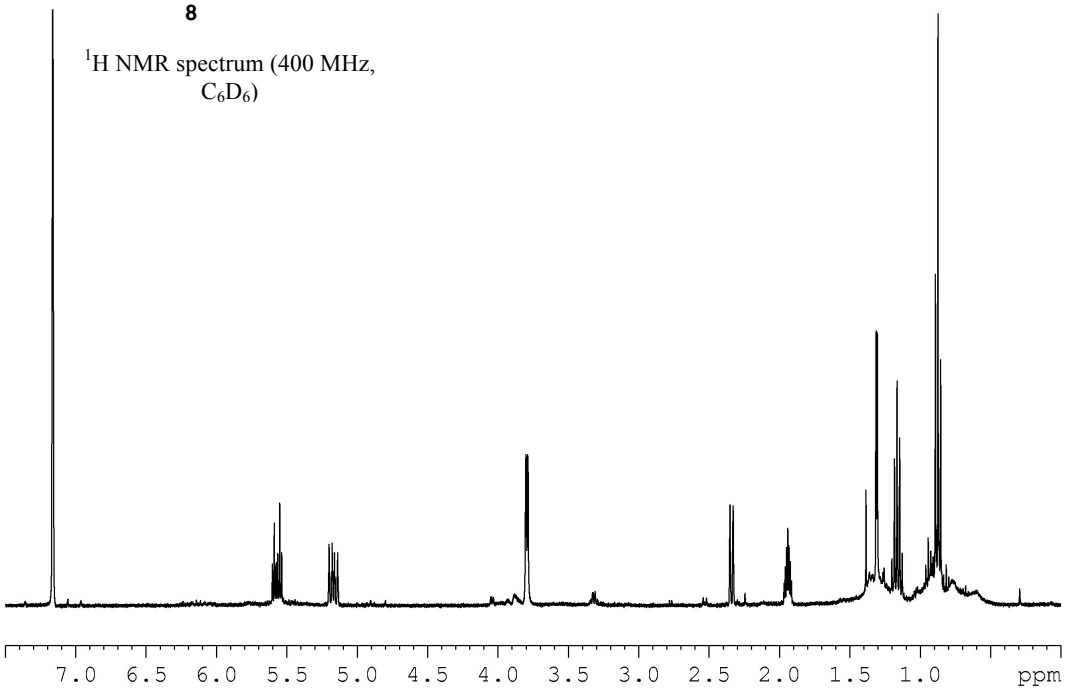
^{13}C NMR spectrum (150 MHz, C_6D_6)

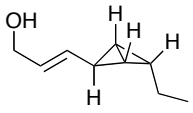




8

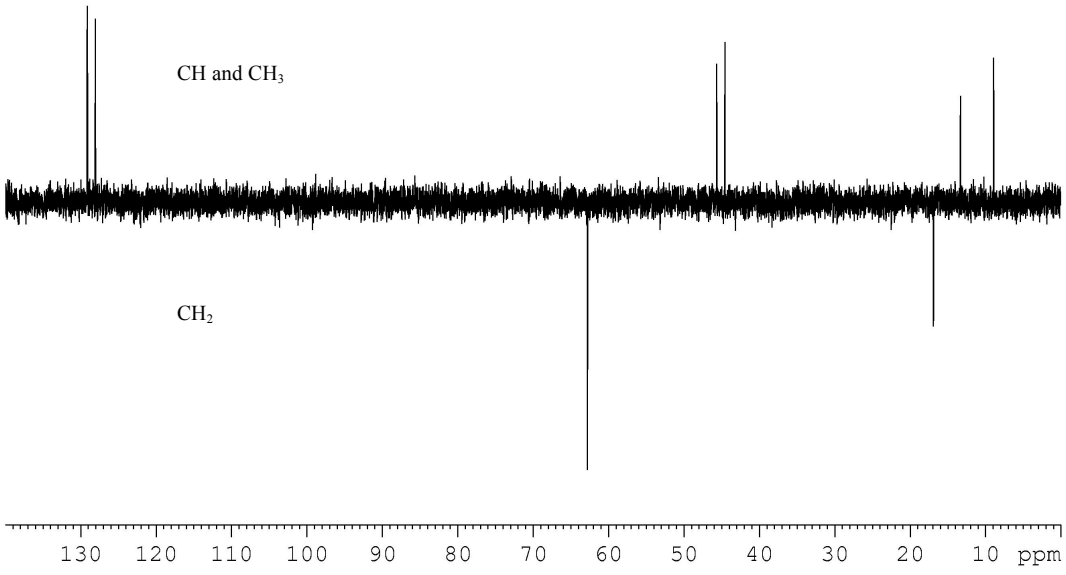
^{1}H NMR spectrum (400 MHz, C_6D_6)

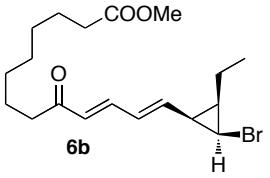




8

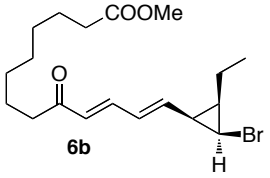
^{13}C DEPT135 NMR spectrum (100 MHz, C_6D_6)





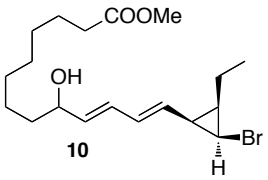
6b

^{1}H NMR Spectrum (400 MHz, $CDCl_3$)



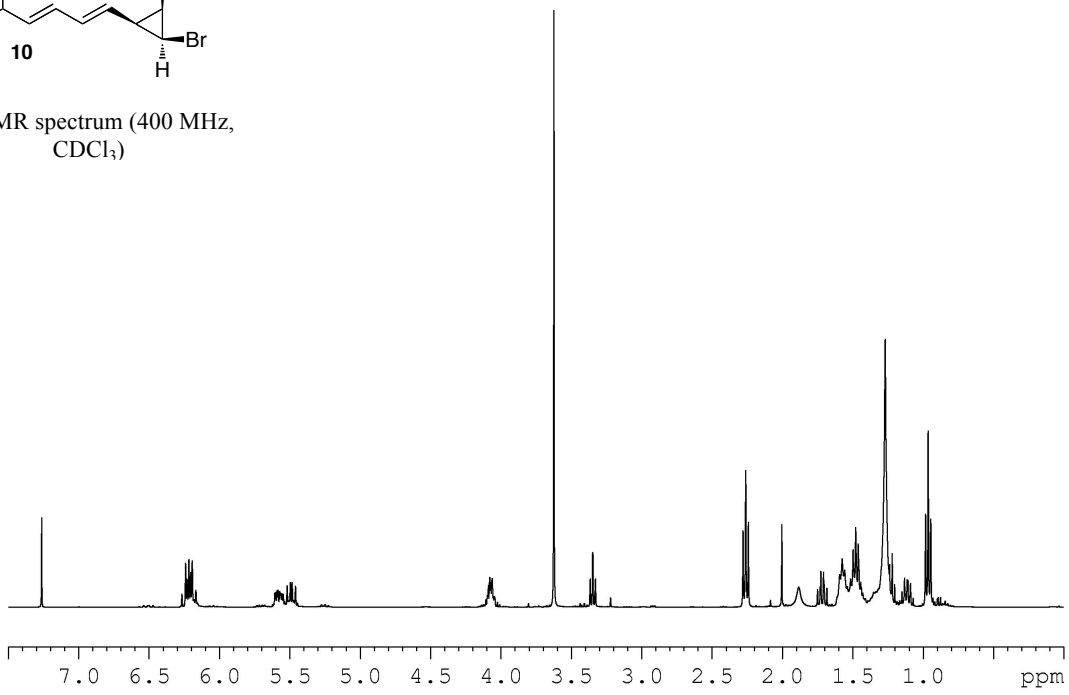
6b

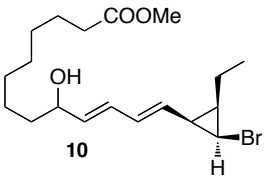
^{13}C NMR Spectrum (125 MHz, $CDCl_3$)



10

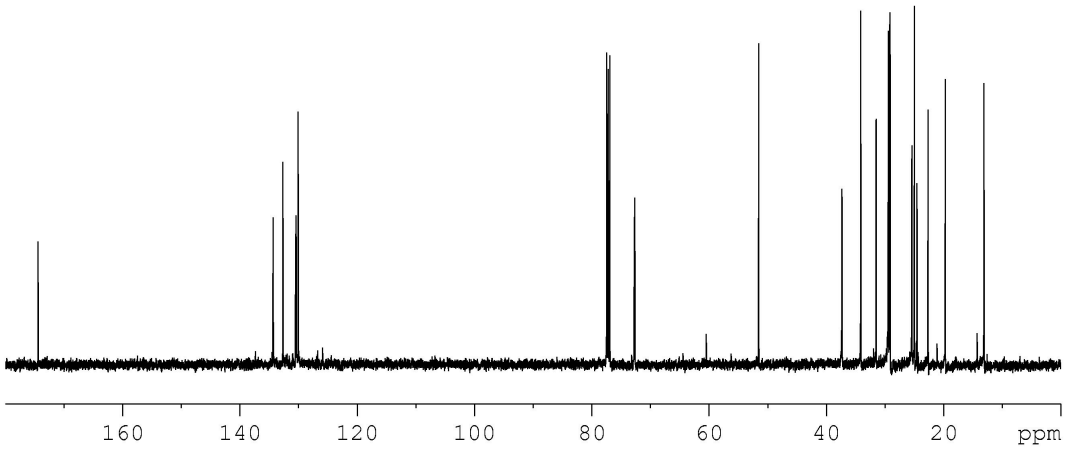
^{1}H NMR spectrum (400 MHz, $CDCl_3$)

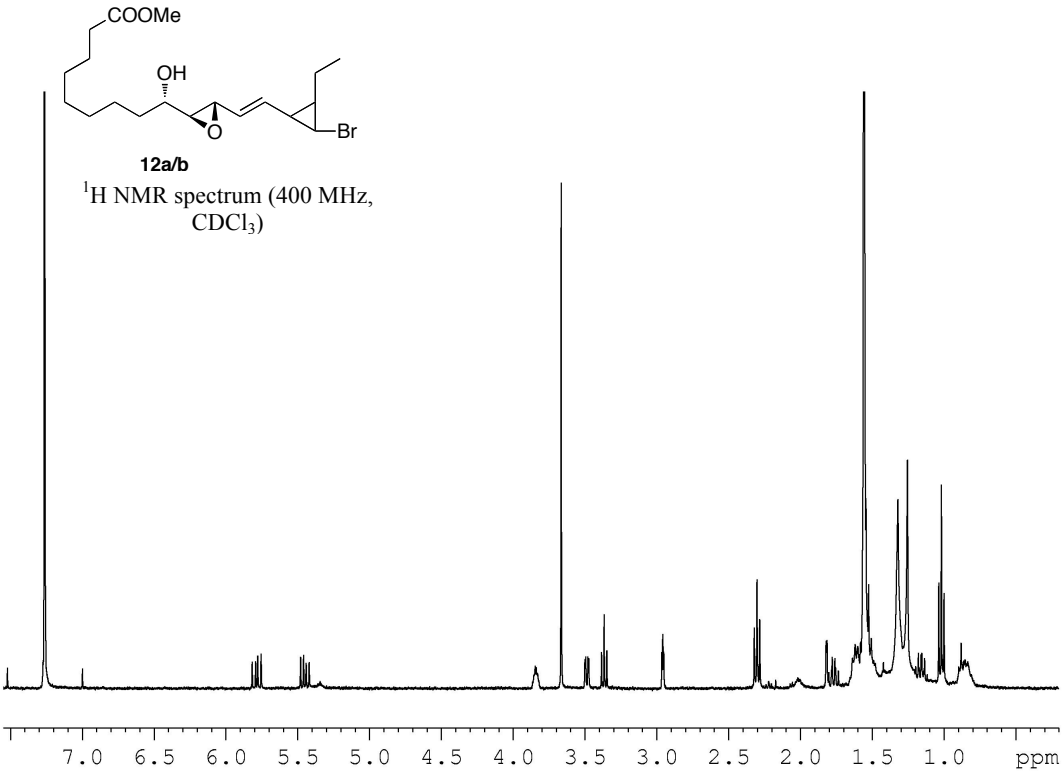




10

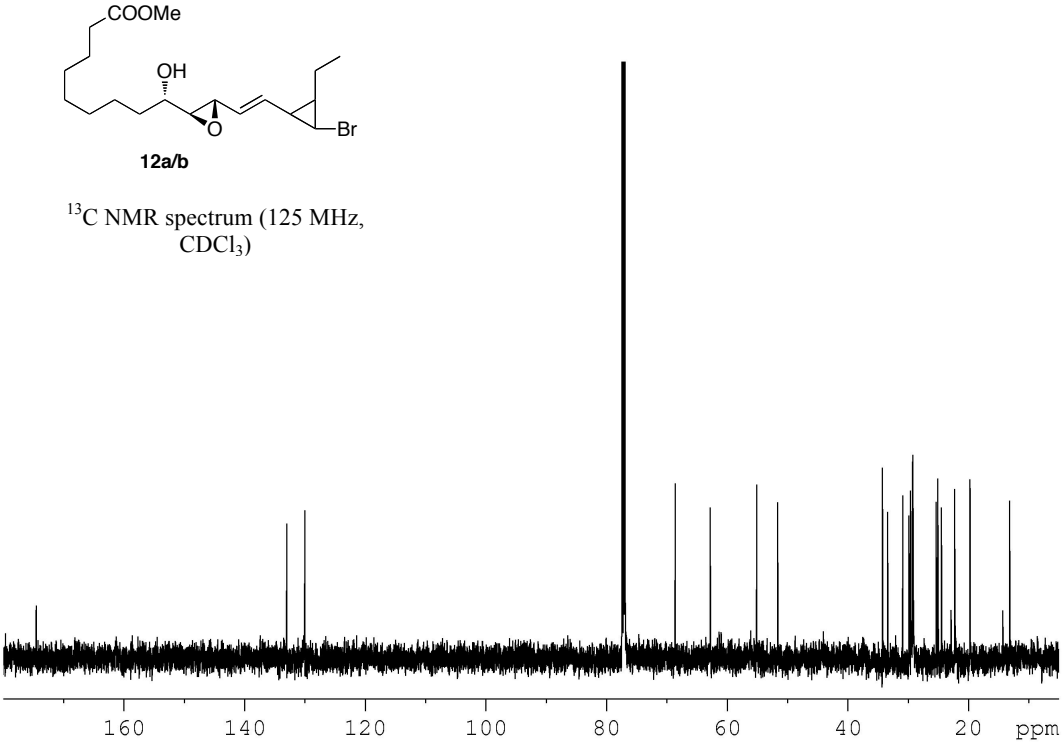
^{13}C NMR spectrum (125 MHz, $CDCl_3$)





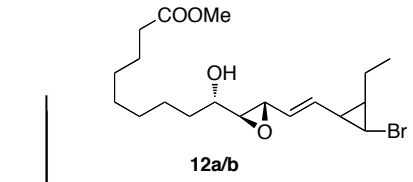
12a/b

^{1}H NMR spectrum (400 MHz, $CDCl_3$)



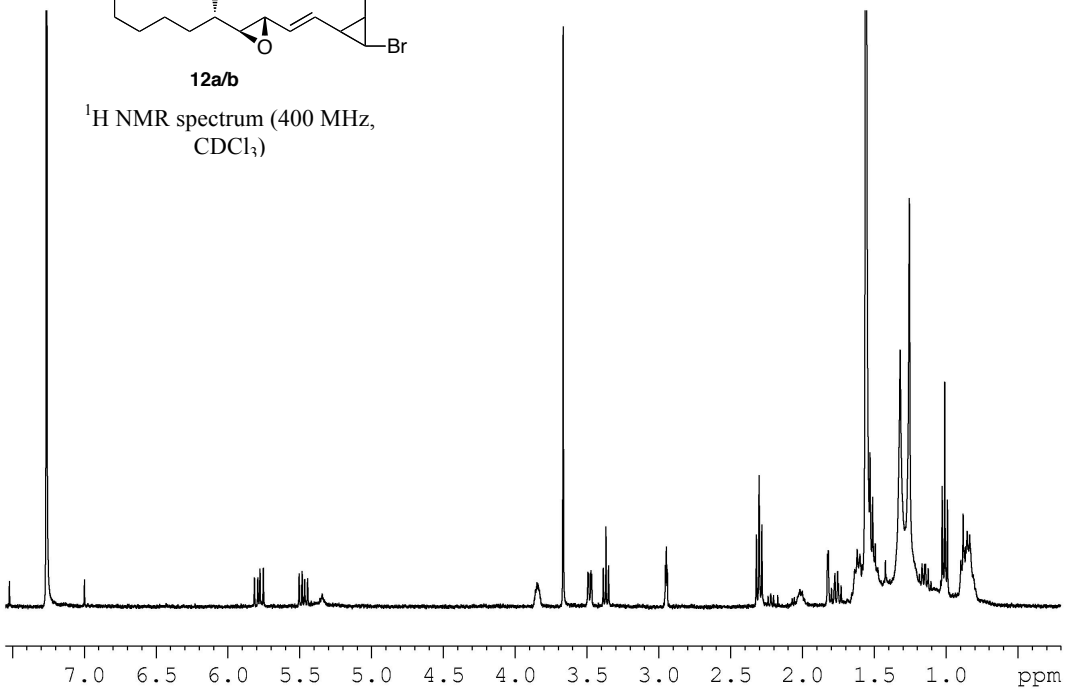
12a/b

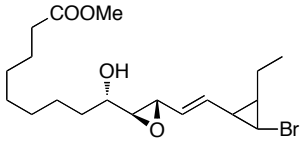
^{13}C NMR spectrum (125 MHz, $CDCl_3$)



12a/b

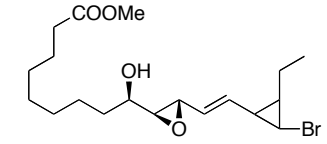
^{1}H NMR spectrum (400 MHz, $CDCl_3$)





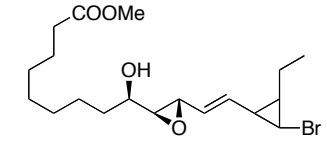
12a/b

^{13}C NMR spectrum (125 MHz, $CDCl_3$)



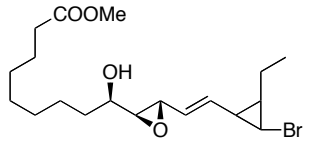
11a/b

^{1}H NMR spectrum (500 MHz, $CDCl_3$)



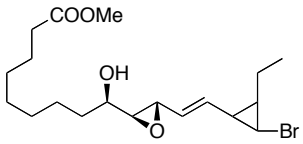
11a/b

^{13}C NMR spectrum (125 MHz, $CDCl_3$)



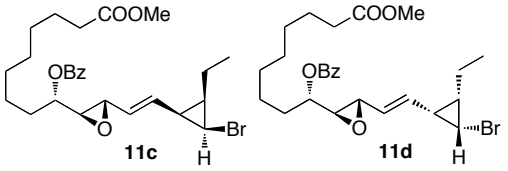
11a/b

^{1}H NMR spectrum (500 MHz, $CDCl_3$)

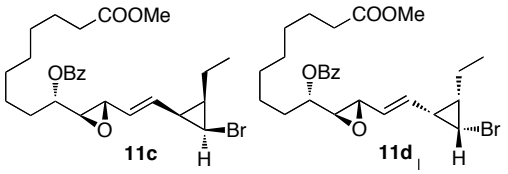


11a/b

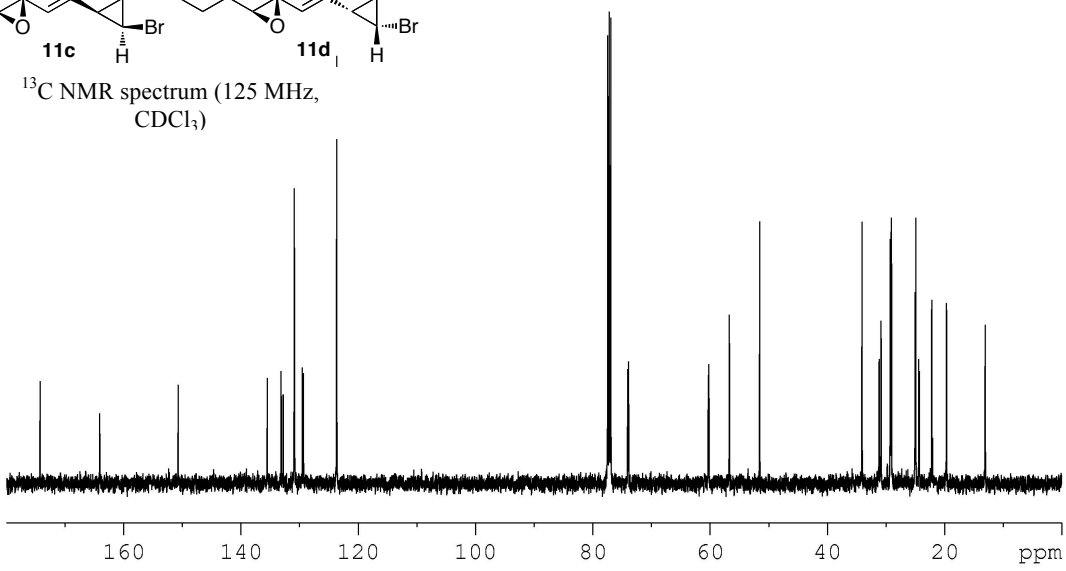
^{13}C NMR spectrum (125 MHz, $CDCl_3$)

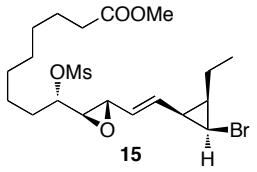


^{1}H NMR spectrum (400 MHz, $CDCl_3$)



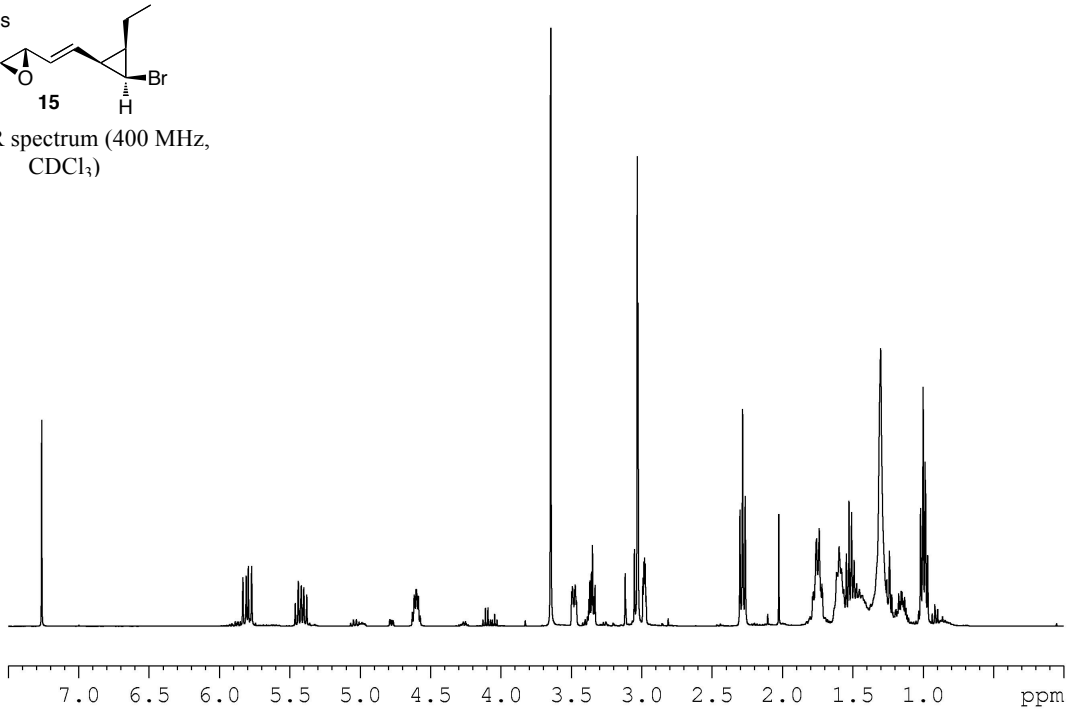
^{13}C NMR spectrum (125 MHz, $CDCl_3$)

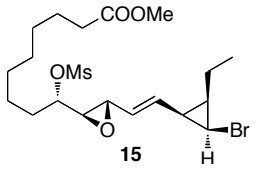




15

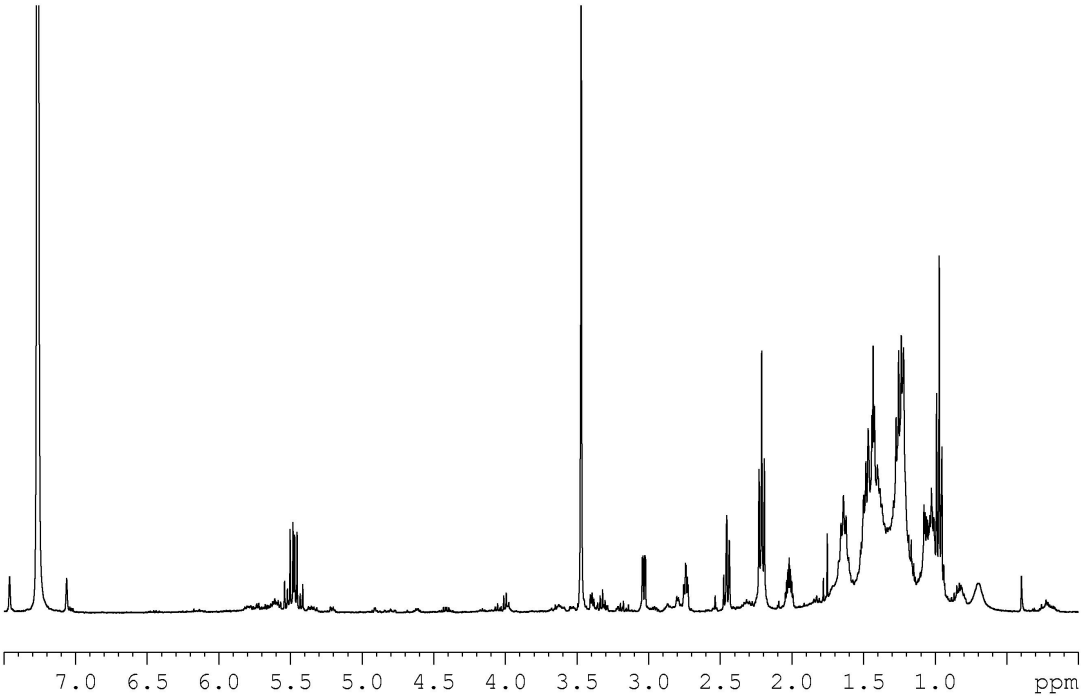
^{1}H NMR spectrum (400 MHz, $CDCl_3$)

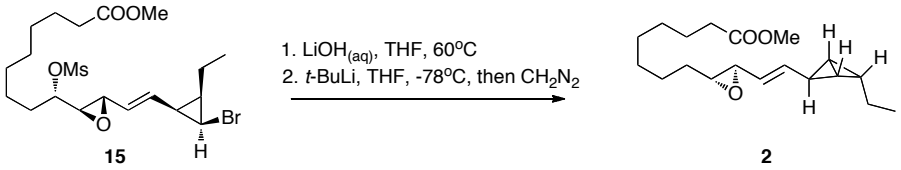




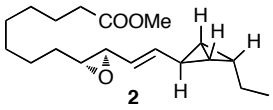
15

^{13}C NMR spectrum (125 MHz, $CDCl_3$)



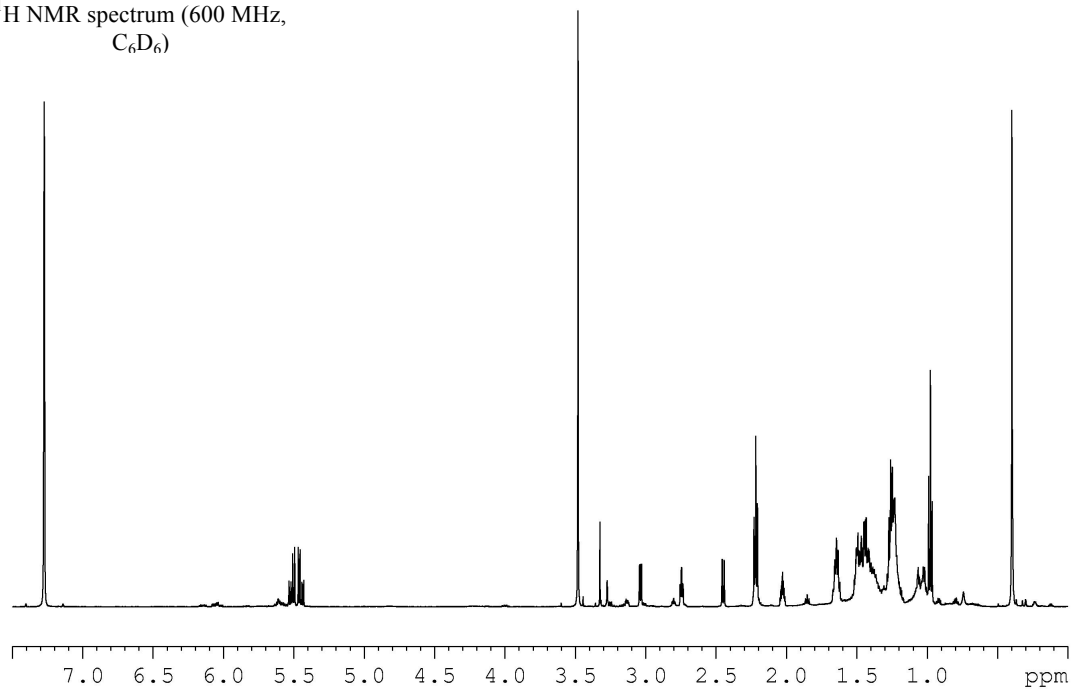


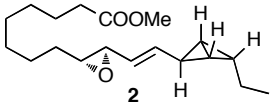
Crude ^{1}H NMR spectrum (400 MHz, C_6D_6)



2

^{1}H NMR spectrum (600 MHz, C_6D_6)





2

^{13}C NMR spectrum (150 MHz, C_6D_6)