SUPPORTING INFORMATION

Development of Allosteric NIK Ligands from Fragment-Based NMR Screening

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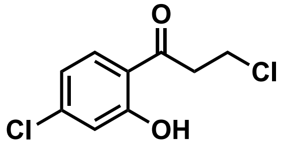
Chemistry – General. All air or moisture sensitive reactions were conducted in oven-dried glassware under inert argon atmosphere. Reactions were stirred with Teflon-coated stir bars. Solvents THF and CH_2Cl_2 were dried using an MBraun solvent purification system. Other solvents were purchased as ACS grade and were used as received. All reagents were purchased from commercial suppliers and were used without further purification. Water for reverse phase purification and SPR assays was purified using a MilliQ system (MilliporeSigma). Silica gel chromatography was performed using RediSep® R_f high performance silica gel columns (Teledyne-Isco) on a Combiflash NextGen 300+ instrument (Teledyne-Isco). C18 chromatography was performed using RediSep® high performance C18 columns (Teledyne-Isco) on a Combiflash NextGen 300+ instrument (Teledyne-Isco). ^1H and ^{13}C NMR spectra were collected on a Bruker Advance 500 MHz spectrometer at room temperature. Chemical shifts (δ) are reported in parts per million (ppm) and the residual solvent peak is used as a reference. Coupling constants (*J*) are reported in Hertz (Hz). High resolution mass spectrometry was performed on an Orbitrap HRMS LC-MS instrument at the Analytical Biochemistry Core Facility at the University of Minnesota Masonic Cancer Center. All assayed compounds were determined to be ≥ 95% purity at 215 nm and 254 nm by analytical reverse-phase HPLC analysis on an Agilent 1200-series instrument equipped with a diode array detector and a Luna C18 column (5 μm, 100 Å, 4.6 × 150 mm, Phenomenex). The analysis method (1.0 mL/min flow rate) involved isocratic H_2O (90% H_2O: 10% CH_3CN; 0-2 min) followed by a linear gradient to 85% CH_3CN (2-24 min) and finally a linear gradient to 95% CH_3CN (24-26 min). Safety statement: No unexpected or abnormally high safety hazards were encountered.

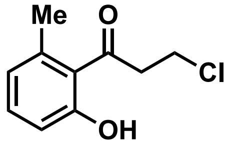
Chemistry – Procedures.

3-chloro-1-(4-chloro-2-hydroxyphenyl)propan-1-one (3a)



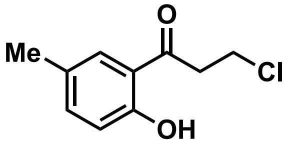
3a was prepared according to modification of prior report.[1] A mixture of m-chlorophenol (2.00 g, 15.6 mmol) and 3-chloropropionyl chloride (1.98 g, 15.6 mmol) were stirred at 110 °C for 45 minutes. The reaction mixture was cooled to room temperature and aluminum chloride (4.15g, 31.1 mmol) was added in portions. The reaction mixture was stirred at 90 °C for 2 hours. The reaction mixture was cooled to room temperature and quenched with crushed ice. The solution was partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL), the organic phase separated, washed with brine (75 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **3a** as a pale yellow solid (1.63 g, 48%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.15 (s, 1H), 7.65 (d, J = 5.0 Hz, 1H), 7.02 (d, J = 2.5 Hz, 1 H), 6.91 (dd, J = 8.5, 2.0 Hz, 2 H), 3.91 (t, J = 6.5 Hz, 1 H), 3.46 (t, J = 6.7 Hz, 2 H).

3-chloro-1-(2-hydroxy-6-methylphenyl)propan-1-one (3b)



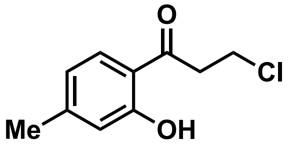
3b was prepared according to modification of prior report.[1] A mixture of m-cresol (1.00 g, 9.25 mmol) and 3-chloropropionyl chloride (1.17 g, 9.25 mmol) were stirred at 110 °C for 45 minutes. The reaction mixture was cooled to room temperature and aluminum chloride (3.70g, 27.7 mmol) was added in portions. The reaction mixture was stirred at 90 °C for 2 hours. The reaction mixture was cooled to room temperature and quenched with crushed ice. The solution was partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL), the organic phase separated, washed with brine (75 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **3b** as a pale yellow solid (781 mg, 43%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.44 (s, 1H), 7.29 (t, J = 7.5 Hz, 1 H), 6.84 (d, J = 8.3 Hz, 1 H), 6.74 (d, J = 7.5 Hz, 1 H), 3.92 (t, J = 6.7 Hz, 2 H), 3.44 (t, J = 6.7 Hz, 2 H), 2.59 (s, 3 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 204.6, 162.0, 138.8, 134.7, 123.4, 121.9, 116.5, 47.0, 38.8, 24.2. HRMS (ESI$^-$) *m/z* calcd for $C_{10}H_{11}O_2Cl$ (M-H$^+$): 197.0375, found: 197.0373.

3-chloro-1-(2-hydroxy-5-methylphenyl)propan-1-one (3c)



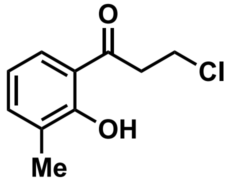
3c was prepared according to modification of prior report.[1] A mixture of p-cresol (2.00 g, 18.5 mmol) and 3-chloropropionyl chloride (2.35 g, 18.5 mmol) were stirred at 80 °C for 45 minutes. The reaction mixture was cooled to room temperature and aluminum chloride (4.93 g, 37.0 mmol) was added in portions. The reaction mixture was stirred at 120 °C for 24 hours. The reaction mixture was cooled to room temperature and quenched with crushed ice. The solution was partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL), the organic phase separated, washed with brine (75 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **3c** as a pale yellow solid (2.63 g, 72%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.85 (s, 1H), 7.50 (s, 1H), 7.31 (dd, J = 8.6, 2.3 Hz, 1H), 6.91 (d, J = 8.6 Hz, 1H), 3.92 (t, J = 6.7 Hz, 2H), 3.49 (t, J = 6.7 Hz, 2H), 2.32 (s, 3H).

3-chloro-1-(2-hydroxy-4-methylphenyl)propan-1-one (3d)



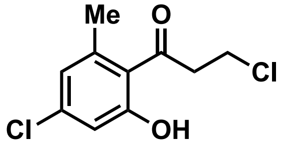
3d was prepared according to modification of prior report.[1] A mixture of m-cresol (1.00 g, 9.25 mmol) and 3-chloropropionyl chloride (1.17 g, 9.25 mmol) were stirred at 110 °C for 45 minutes. The reaction mixture was cooled to room temperature and aluminum chloride (3.70 g, 27.7 mmol) was added in portions. The reaction mixture was stirred at 90 °C for 2 hours. The reaction mixture was cooled to room temperature and quenched with crushed ice. The solution was partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL), the organic phase separated, washed with brine (75 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **3d** as a pale yellow solid (342 mg, 19%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.05 (s, 1H), 7.60 (d, J = 8.2 Hz, 1H), 6.80 (s, 1H), 6.73 (d, J = 8.1 Hz, 1H), 3.91 (t, J = 6.9 Hz, 2H), 3.45 (t, J = 6.7 Hz, 2H), 2.36 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 201.9, 162.8, 148.7, 129.7, 120.6, 118.8, 117.1, 40.8, 38.4, 22.1. HRMS (ESI$^-$) *m/z* calcd for $C_{10}H_{11}O_2Cl$ (M-H$^+$): 197.0375, found: 197.0374.

3-chloro-1-(2-hydroxy-3-methylphenyl)propan-1-one (3e)



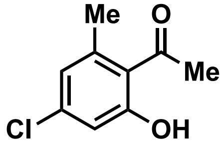
3e was prepared according to modification of prior report.[1] A mixture of o-cresol (2.00 g, 18.5 mmol) and 3-chloropropionyl chloride (2.35 g, 18.5 mmol) were stirred at 80 °C for 45 minutes. The reaction mixture was cooled to room temperature and aluminum chloride (4.93 g, 37.0 mmol) was added in portions. The reaction mixture was stirred at 120 °C for 24 hours. The reaction mixture was cooled to room temperature and quenched with crushed ice. The solution was partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL), the organic phase separated, washed with brine (75 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **3e** as a pale yellow solid (912 mg, 25%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.33 (s, 1H), 7.58 (d, J = 8.3 Hz, 1H), 7.37 (d, J = 7.2 Hz, 1H), 6.83 (t, J = 7.6 Hz, 1H), 3.92 (t, J = 6.8 Hz, 2H), 3.50 (t, J = 6.7 Hz, 2H), 2.27 (s, 3H).

3-chloro-1-(4-chloro-2-hydroxy-6-methylphenyl)propan-1-one (3f)



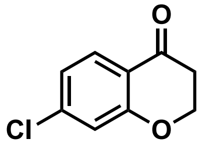
3f was prepared according to modification of prior report.[1] A mixture of 5-chloro-3-cresol (1.51 g, 10.6 mmol) and 3-chloropropionyl chloride (1.34 g, 10.6 mmol) were stirred at 110 °C for 45 minutes. The reaction mixture was cooled to room temperature and aluminum chloride (4.24 g, 31.8 mmol) was added in portions. The reaction mixture was stirred at 90 °C for 2 hours. The reaction mixture was cooled to room temperature and quenched with crushed ice. The solution was partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL), the organic phase separated, washed with brine (75 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **3f** as a pale yellow solid (834 mg, 34%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.92 (s, 1H), 6.88 (s, 1H), 6.74 (s, 1H), 3.91 (t, J = 6.9 Hz, 2H), 3.42 (t, J = 6.7 Hz, 2H), 2.58 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 204.0, 163.4, 140.7, 140.6, 123.8, 120.2, 116.8, 47.1, 38.7, 24.5. HRMS (ESI$^-$) m/z calcd for $C_{10}H_{10}O_2Cl_2$ (M-H$^+$): 230.9985, found: 230.9982.

1-(4-chloro-2-hydroxy-6-methylphenyl)ethan-1-one (3g)



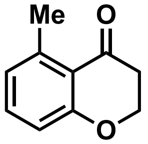
3g was prepared according to modification of prior report.[1] A mixture of 3-chloro-5-methylphenol (1.82 g, 12.8 mmol) and acetyl chloride (1.00 g, 12.8 mmol) were stirred at 110 °C for 45 minutes. The reaction mixture was cooled to room temperature and aluminum chloride (5.11 g, 38.3 mmol) was added in portions. The reaction mixture was stirred at 90 °C for 2 hours. The reaction mixture was cooled to room temperature and quenched with crushed ice. The solution was partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL), the organic phase separated, washed with brine (75 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **3g** as a pale yellow solid (984 mg, 42%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.64 (s, 1H), 6.86 (s, 1H), 6.72 (s, 1H), 2.66 (s, 3H), 2.58 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 205.5, 164.1, 141.3, 140.6, 123.5, 120.1, 116.8, 33.5, 24.6. HRMS (ESI$^-$) m/z calcd for $C_9H_9O_2Cl$ (M-H$^+$): 183.0218, found: 183.0212.

7-chlorochroman-4-one (4a)



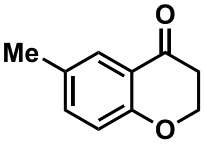
4a was prepared according to modification of prior report.[2] A mixture of **3a** (1.35 g, 6.16 mmol) and K_2CO_3 (1.70 g, 12.3 mmol) in anhydrous EtOH (40 mL) was stirred at room temperature under argon for 24 hours. The suspension was filtered and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **4a** as a white solid (688 mg, 61%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, J = 9.0 Hz, 1H), 7.01-6.98 (m, 2H), 4.55 (t, J = 6.3 Hz, 2H), 2.81 (t, J = 6.7 Hz, 2H).

5-methylchroman-4-one (4b)



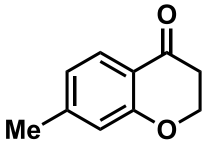
4b was prepared according to modification of prior report.[2] A mixture of **3b** (198 mg, 997 µmol) and K_2CO_3 (276 mg, 1.99 mmol) in anhydrous EtOH (10 mL) was stirred at room temperature under argon for 24 hours. The suspension was filtered and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **4b** as a white solid (45.8 mg, 28%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (t, J = 7.5 Hz, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.79 (d, J = 7.5 Hz, 1H), 4.48 (t, J = 6.3 Hz, 2H), 2.80 (t, J = 6.4 Hz, 2H), 2.64 (s, 3H).

6-methylchroman-4-one (4c)



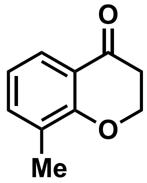
4c was prepared according to modification of prior report.[2] A mixture of **3c** (1.12 g, 5.64 mmol) and K_2CO_3 (1.56 g, 11.3 mmol) in anhydrous EtOH (50 mL) was stirred at room temperature under argon for 24 hours. The suspension was filtered and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **4c** as a white solid (672 mg, 73%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.28 (dd, J = 8.5, 2.4 Hz, 1H), 6.87 (d, J = 8.6 Hz, 1H), 4.51 (t, J = 6.1 Hz, 2H), 2.79 (t, J = 6.6 Hz, 2H), 2.31 (s, 3H).

7-methylchroman-4-one (4d)



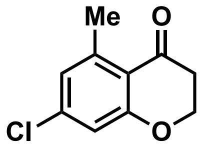
4d was prepared according to modification of prior report.[2] A mixture of **3d** (780 mg, 3.93 mmol) and K_2CO_3 (1.09 g, 7.85 mmol) in anhydrous EtOH (25 mL) was stirred at room temperature under argon for 24 hours. The suspension was filtered and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **4d** as a white solid (368 mg, 58%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, J = 7.9 Hz, 1H), 6.83 (d, J = 8.1 Hz, 1H), 6.77 (s, 1H), 4.51 (t, J = 6.3 Hz, 2H), 2.78 (t, J = 6.6 Hz, 2H), 2.35 (s, 3H).

8-methylchroman-4-one (4e)



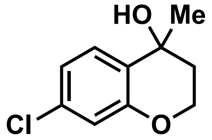
4e was prepared according to modification of prior report.[2] A mixture of **3e** (785 mg, 3.95 mmol) and K_2CO_3 (1.09 g, 7.90 mmol) in anhydrous EtOH (25 mL) was stirred at room temperature under argon for 24 hours. The suspension was filtered and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **4e** as a white solid (328 mg, 51%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 7.3 Hz, 1H), 6.91 (t, J = 7.7 Hz, 1H), 4.56 (t, J = 6.1 Hz, 2H), 2.80 (t, J = 6.7 Hz, 2H), 2.24 (s, 3H).

7-chloro-5-methylchroman-4-one (4f)



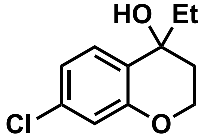
4f was prepared according to modification of prior report.[2] A mixture of **3f** (558 mg, 2.39 mmol) and K_2CO_3 (662 mg, 4.79 mmol) in anhydrous EtOH (16 mL) was stirred at room temperature under argon for 24 hours. The suspension was filtered and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **4f** as a white solid (263 mg, 56%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.86 (s, 1H), 6.80 (s, 1H), 4.48 (t, J = 6.3 Hz, 2H), 2.78 (t, J = 6.6 Hz, 2H), 2.62 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 192.5, 163.4, 144.1, 140.2, 125.0, 118.6, 116.1, 66.9, 39.1, 23.0. HRMS (ESI$^-$) *m/z* calcd for $C_{10}H_9O_2Cl$ (M-H$^+$): 195.0218, found: 195.0219.

7-chloro-4-methylchroman-4-ol (5)



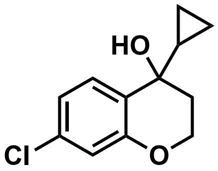
To a solution of **4a** (100 mg, 548 μmol) in anhydrous THF (2.74 mL, 0.20 M) was added MeMgBr (979 μL of 1.4 M solution in (1:3) THF:Toluene) at 0 °C under argon gas. The reaction was allowed to warm to room temperature as it stirred for 24 hours. The reaction was cooled to 0 °C and quenched with sat. aq. NH_4Cl solution (5 mL). The mixture was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **5** as a white solid (86.7 mg, 79%): ^{1}H NMR (500 MHz, DMSO-*d$_6$*) *δ* 7.46 (d, *J* = 8.4 Hz, 1H), 6.92 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.79 (d, *J* = 2.2 Hz, 1H), 5.20 (s, 1H), 4.26-4.15 (m, 2H), 1.98-1.87 (m, 2H), 1.45 (s, 3H). ^{13}C NMR (500 MHz, DMSO-*d$_6$*) *δ* 154.3, 131.9, 129.3, 128.8, 120.1, 115.8, 64.4, 63.6, 37.2, 29.7. HRMS (ESI$^-$) *m/z* calcd for $C_{10}H_{11}O_2Cl$ (M-H$^+$): 197.0375, found: 197.0375.

7-chloro-4-ethylchroman-4-ol (6)



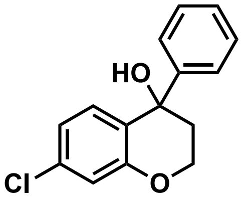
To a solution of **4a** (100 mg, 548 μmol) in anhydrous THF (2.74 mL, 0.20 M) was added EtMgBr (1.37 mL of 1.0 M solution in THF) at 0 °C under argon gas. The reaction was allowed to warm to room temperature as it stirred for 24 hours. The reaction was cooled to 0 °C and quenched with sat. aq. NH_4Cl solution (5 mL). The mixture was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **6** as a white solid (49.3 mg, 42%): ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.33 (d, *J* = 8.4 Hz, 1H), 6.90 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.84 (d, *J* = 2.1 Hz, 1H), 4.26-4.24 (m, 2H), 2.15-1.86 (m, 4H), 1.74 (s, 1H), 0.90 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) *δ* 155.3, 134.2, 127.6, 126.1, 121.0, 117.3, 68.5, 63.4, 34.0, 33.9, 8.38. HRMS (ESI$^-$) *m/z* calcd for $C_{11}H_{13}O_2Cl$ (M-H$^+$): 211.0531, found: 211.0528.

7-chloro-4-cyclopropylchroman-4-ol (7)



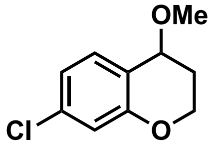
To a solution of **4a** (105 mg, 575 μmol) in anhydrous THF (2.88 mL, 0.20 M) was added *cyclo*PrMgBr (1.15 mL of 1.0 M solution in 2-methyl THF) at 0 °C under argon gas. The reaction was transferred to an oil bath and was refluxed under argon gas for 24 hours. The reaction was cooled to 0 °C and quenched with sat. aq. NH_4Cl solution (5 mL). The mixture was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by reverse phase C18 chromatography on a gradient of MeCN:H_2O (1:9) to (9:1) to give **7** as a white solid (78.0 mg, 60%): ^{1}H NMR (500 MHz, DMSO-*d₆*) *δ* 7.53 (d, *J* = 8.4 Hz, 1H), 6.92 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.81 (d, *J* = 2.3 Hz, 1H), 4.90 (s, 1H), 4.32-4.24 (m, 2H), 1.97-1.86 (m, 2H), 1.05-1.00 (tt, *J* = 8.3, 5.4 Hz, 1H), 0.70-0.65 (dtd, *J* = 8.9, 5.7, 4.0 Hz, 1H), 0.52-0.46 (tdd, *J* = 8.7, 5.8, 3.9 Hz, 1H), 0.37-0.32 (dtd, *J* = 8.8, 5.8, 4.0 Hz, 1H), 0.29-0.23 (tdd, *J* = 8.7, 5.8, 4.0 Hz, 1H). ^{13}C NMR (500 MHz, DMSO-d_6) *δ* 154.5, 132.2, 129.3, 128.4, 119.7, 115.7, 64.1, 63.3, 36.0, 20.9, -1.42, -0.97. HRMS (ESI$^-$) *m/z* calcd for $C_{12}H_{13}O_2Cl$ (M-H$^+$): 223.0531, found: 223.0531.

7-chloro-4-phenylchroman-4-ol (8)



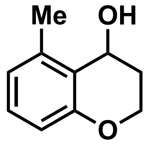
To a solution of **4a** (100 mg, 548 μmol) in anhydrous THF (2.74 mL, 0.20 M) was added PhMgBr (602 μL of 1.0 M solution in THF) at 0 °C under argon gas. The reaction was allowed to warm to room temperature as it stirred for 24 hours. The reaction was cooled to 0 °C and quenched with sat. aq. NH_4Cl solution (5 mL). The mixture was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by reverse phase C18 chromatography on a gradient of MeCN:H_2O (1:9) to (9:1) to give **8** as a white solid (36.2 mg, 25%): ^{1}H NMR (500 MHz, DMSO-d_6) *δ* 7.34-7.22 (m, 5H), 6.92 (s, 1H), 6.87 (s, 2H), 6.00 (s, 1H), 4.36-4.31 (ddd, *J* = 11.1, 9.8, 2.8 Hz, 1H), 4.14-4.10 (ddd, *J* = 11.1, 5.7, 3.5 Hz, 1H), 2.25-2.19 (ddd, *J* = 14.0, 9.8, 3.7 Hz, 1H), 2.10-2.05 (ddd, *J* = 14.1, 5.6, 2.9 Hz, 1H). ^{13}C NMR (500 MHz, DMSO-d_6) δ 155.2, 147.7, 132.4, 131.2, 128.1, 127.8, 126.7, 126.3, 120.1, 116.0, 69.5, 63.1, 38.8. HRMS (ESI$^-$) *m/z* calcd for $C_{15}H_{13}O_2Cl$ (M-H$^+$): 259.0531, found: 259.0529.

7-chloro-4-methoxychromane (9)



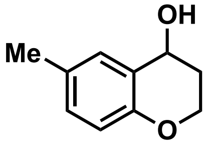
1 (223 mg, 1.21 mmol) was dissolved in anhydrous THF (5 mL) and was slowly added to a suspension of NaH (72.5 mg, 1.81 mmol, 60% dispersion in mineral oil) in anhydrous THF (5 mL) at room temperature under argon gas. The reaction was stirred for 30 minutes. Methyl iodide (171 mg, 1.21 mmol) was slowly added at room temperature under argon gas and the reaction was stirred for 3 hours. The reaction was quenched with crushed ice, the mixture was partitioned between EtOAc (25 mL) and sat. aq. $NaHCO_3$ solution (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **9** as a clear liquid (164 mg, 68%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.16 (d, *J* = 8.1 Hz, 1H), 6.88-6.85 (m, 2H), 4.29-4.21 (m, 3H), 3.42 (s, 3H), 2.16-1.98 (m, 2H). ^{13}C NMR (500 MHz, DMSO-d_6) δ 155.6, 134.9, 131.6, 120.4, 120.3, 117.3, 71.4, 62.4, 56.0, 27.1. HRMS (ESI$^-$) *m/z* calcd for $C_{10}H_{11}O_2Cl$ (M-H$^+$): 197.0375, found: 197.0374.

5-methylchroman-4-ol (10)



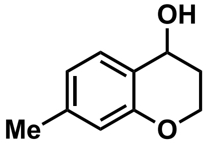
10 was prepared according to modification of prior report.[3] To a solution of **4b** (36.3 mg, 224 μmol) in anhydrous MeOH (678 μL, 0.33 M) was added $NaBH_4$ (9.31 mg, 246 μmol) at 0 °C. The reaction was stirred at 0 °C for 2 hours. The reaction was quenched by addition of H_2O (5 mL), the mixture was transferred to a separatory funnel and extracted with CH_2Cl_2 (10 mL x 3), then the combined organic phases were washed with 25 mL of brine. The organic phase was separated, dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by reverse phase C18 chromatography on a gradient of MeCN:H_2O (1:9) to (9:1) to give **10** as a white solid (22.8 mg, 62%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.03(t, *J* = 8.1 Hz, 1H), 6.70 (d, *J* = 7.4 Hz, 1H), 6.59 (d, *J* = 8.2 Hz, 1H), 5.08 (d, *J* = 5.4 Hz, 1H), 4.64 (m, 1H), 4.19-4.07 (m, 2H), 2.33 (s, 3H), 1.92-1.83 (m, 2H). MS (ESI$^-$) *m/z* calcd for $C_{10}H_{12}O_2$ (M-H$^+$): 163.1, found: 163.1.

6-methylchroman-4-ol (11)



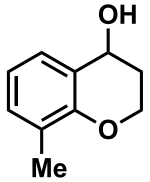
11 was prepared according to prior report.[3] To a solution of **4c** (624 mg, 3.85 mmol) in anhydrous MeOH (11.7 mL, 0.33 M) was added $NaBH_4$ (160 mg, 4.23 mmol) at 0 °C. The reaction was stirred at 0 °C for 2 hours. The reaction was quenched by addition of H_2O (10 mL), the mixture was transferred to a separatory funnel and extracted with CH_2Cl_2 (10 mL x 3), then the combined organic phases were washed with 25 mL of brine. The organic phase was separated, dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by reverse phase C18 chromatography on a gradient of MeCN:H_2O (1:9) to (9:1) to give **11** as a white solid (418 mg, 66%): ^1H NMR (500 MHz, DMSO-*d*$_6$) δ 7.09 (s, 1H), 6.92 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.62 (d, *J* = 8.3 Hz, 1H), 5.27 (d, *J* = 5.4 Hz, 1H), 4.56 (q, *J* = 5.0 Hz, 1H), 4.16-4.09 (m, 2H), 2.20 (s, 3H), 1.99-1.80 (m, 2H). MS (ESI$^-$) *m/z* calcd for $C_{10}H_{12}O_2$ (M-H$^+$): 163.1, found: 163.1.

7-methylchroman-4-ol (12)



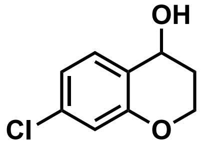
12 was prepared according to modification of prior report.[3] To a solution of **4d** (345 mg, 2.13 mmol) in anhydrous MeOH (6.45 mL, 0.33 M) was added $NaBH_4$ (88.5 mg, 2.34 mmol) at 0 °C. The reaction was stirred at 0 °C for 2 hours. The reaction was quenched by addition of H_2O (10 mL), the mixture was transferred to a separatory funnel and extracted with CH_2Cl_2 (10 mL x 3), then the combined organic phases were washed with 25 mL of brine. The organic phase was separated, dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by reverse phase C18 chromatography on a gradient of MeCN:H_2O (1:9) to (9:1) to give **12** as a white solid (212 mg, 61%): ^1H NMR (500 MHz, DMSO-*d*$_6$) δ 7.15 (d, *J* = 7.8 Hz, 1H), 6.67 (d, *J* = 7.8 Hz, 1H), 6.55 (s, 1H), 5.23 (d, *J* = 5.4 Hz, 1H), 4.56 (q, *J* = 4.9 Hz, 1H), 4.17-4.11 (m, 2H), 2.21 (s, 3H), 1.98-1.80 (m, 2H). MS (ESI$^-$) *m/z* calcd for $C_{10}H_{12}O_2$ (M-H$^+$): 163.1, found: 163.1.

8-methylchroman-4-ol (13)



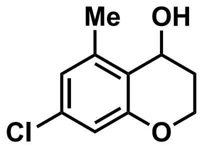
13 was prepared according to modification of prior report.[3] To a solution of **4e** (332 mg, 2.05 mmol) in anhydrous MeOH (6.20 mL, 0.33 M) was added $NaBH_4$ (85.2 mg, 2.25 mmol) at 0 °C. The reaction was stirred at 0 °C for 2 hours. The reaction was quenched by addition of H_2O (10 mL), the mixture was transferred to a separatory funnel and extracted with CH_2Cl_2 (10 mL x 3), then the combined organic phases were washed with 25 mL of brine. The organic phase was separated, dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by reverse phase C18 chromatography on a gradient of MeCN:H_2O (1:9) to (9:1) to give **13** as a white solid (185 mg, 55%): ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 7.12 (d, *J* = 7.7 Hz, 1H), 7.00 (d, *J* = 7.3 Hz, 1H), 6.75 (t, *J* = 7.5 Hz, 1H), 5.27 (d, *J* = 4.8 Hz, 1H), 4.59 (q, *J* = 4.6 Hz, 1H), 4.25-4.17 (m, 2H), 2.09 (s, 3H), 2.00-1.83 (m, 2H). MS (ESI$^-$) *m/z* calcd for $C_{10}H_{12}O_2$ (M-H$^+$): 163.1, found: 163.1.

7-chlorochroman-4-ol (1)



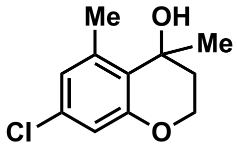
1 was prepared according to prior report.[3] To a solution of **4a** (535 mg, 2.93 mmol) in anhydrous MeOH (8.88 mL, 0.33 M) was added $NaBH_4$ (122 mg, 3.22 mmol) at 0 °C. The reaction was stirred at 0 °C for 2 hours. The reaction was quenched by addition of H_2O (10 mL), the mixture was transferred to a separatory funnel and extracted with CH_2Cl_2 (10 mL x 3), then the combined organic phases were washed with 25 mL of brine. The organic phase was separated, dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **1** as a white solid (408 mg, 75%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.3 Hz, 1H), 6.90 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.86 (d, *J* = 2.2 Hz, 1H), 4.77 (q, *J* = 4.6 Hz, 1H), 4.30-4.25 (m, 2H), 2.15-2.00 (m, 2H), 1.78 (d, *J* = 5.1 Hz, 1H). MS (ESI$^-$) *m/z* calcd for $C_{10}H_{12}O_2$ (M-H$^+$): 183.0, found: 183.0.

7-chloro-5-methylchroman-4-ol (14)



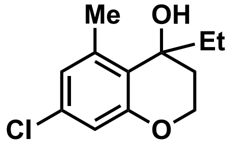
To a solution of **4f** (44.8 mg, 228 μmol) in anhydrous MeOH (691 μL, 0.33 M) was added $NaBH_4$ (9.48 mg, 251 μmol) at 0 °C. The reaction was stirred at 0 °C for 2 hours. The reaction was quenched by addition of H_2O (5 mL), the mixture was transferred to a separatory funnel and extracted with CH_2Cl_2 (10 mL x 3), then the combined organic phases were washed with 25 mL of brine. The organic phase was separated, dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by reverse phase C18 chromatography on a gradient of MeCN:H_2O (1:9) to (9:1) to give **14** as a white solid (34.5 mg, 76%): ^{1}H NMR (500 MHz, DMSO-*d₆*) *δ* 6.79 (d, *J* = 2.9 Hz, 1H), 6.68 (d, *J* = 2.5 Hz, 1H), 5.18 (s, 1H), 4.63 (s, 1H), 4.23-4.08 (m, 2H), 2.33 (s, 3H), 1.92-1.82 (m, 2H). ^{13}C NMR (500 MHz, DMSO-*d₆*) *δ* 155.2, 141.1, 132.1, 122.2, 121.4, 113.8, 60.8, 57.8, 31.0, 17.8. HRMS (ESI$^-$) *m/z* calcd for $C_{10}H_{11}O_2Cl$ (M-H$^+$): 197.0375, found: 197.0372.

7-chloro-4,5-dimethylchroman-4-ol (15)



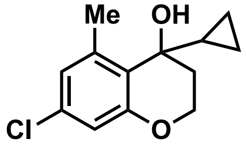
To a solution of **4f** (102 mg, 519 μmol) in anhydrous THF (2.59 mL, 0.20 M) was added MeMgBr (929 μL of 1.4 M solution in (1:3) THF:Toluene) at 0 °C under argon gas. The reaction was allowed to warm to room temperature as it stirred for 24 hours. The reaction was cooled to 0 °C and quenched with sat. aq. NH_4Cl solution (5 mL). The mixture was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **15** as a white solid (78.4 mg, 71%): ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 6.71 (s, 1H), 6.67 (s, 1H), 4.20-4.10 (m, 2H), 2.57 (s, 3H), 2.17-2.05 (m, 2H), 1.69 (s, 1H), 1.66 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) *δ* 155.5, 140.3, 133.4, 125.4, 124.7, 115.3, 68.6, 63.9, 41.5, 28.5, 21.7. HRMS (ESI$^-$) *m/z* calcd for $C_{11}H_{13}O_2Cl$ (M-H$^+$): 211.0531, found: 211.0532.

7-chloro-4-ethyl-5-methylchroman-4-ol (16)



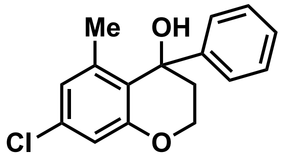
To a solution of **4f** (105 mg, 534 μmol) in anhydrous THF (2.67 mL, 0.20 M) was added EtMgBr (1.33 mL of 1.0 M solution in THF) at 0 °C under argon gas. The reaction was allowed to warm to room temperature as it stirred for 24 hours. The reaction was cooled to 0 °C and quenched with sat. aq. NH_4Cl solution (5 mL). The mixture was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **16** as a white solid (58.9 mg, 49%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.72 (s, 1H), 6.69 (s, 1H), 4.17-4.05 (m, 2H), 2.54 (s, 3H), 2.29-2.24 (m, 1H), 2.06-1.89 (m, 3H), 1.71 (s, 1H), 0.86 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 156.2, 140.4, 133.4, 124.9, 124.7, 115.4, 70.8, 63.3, 37.1, 32.2, 21.8, 8.20. HRMS (ESI$^-$) *m/z* calcd for $C_{12}H_{15}O_2Cl$ (M-H$^+$): 225.0688, found: 225.0685.

7-chloro-4-cyclopropyl-5-methylchroman-4-ol (17)



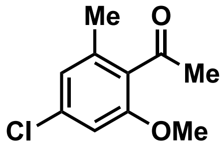
To a solution of **4f** (87.2 mg, 443 μmol) in anhydrous THF (2.22 mL, 0.20 M) was added *cyclo*PrMgBr (887 μL of 1.0 M solution in 2-methyl THF) at 0 °C under argon gas. The reaction was transferred to an oil bath and was refluxed under argon gas for 24 hours. The reaction was cooled to 0 °C and quenched with sat. aq. NH_4Cl solution (5 mL). The mixture was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by reverse phase C18 chromatography on a gradient of MeCN:H_2O (1:9) to (9:1) to give **17** as a white solid (72.3 mg, 68%): ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 6.71 (d, *J* = 2.5 Hz, 1H), 6.65 (d, *J* = 2.4 Hz, 1H), 4.93 (s, 1H), 4.25-4.14 (m, 2H), 2.53 (s, 3H), 2.02-1.93 (m, 2H), 1.15-1.10 (tt, *J* = 8.4, 5.5 Hz, 1H), 0.81-0.76 (dtd, *J* = 9.5, 5.7, 4.0 Hz, 1H), 0.59-0.53 (tdd, *J* = 8.8, 6.1, 4.0 Hz, 1H), 0.36-0.31 (tdd, *J* = 8.9, 5.8, 4.0 Hz, 1H), 0.27-0.22 (dtd, *J* = 9.2, 6.0, 4.0 Hz, 1H). ^{13}C NMR (500 MHz, DMSO-*d*$_6$) δ 154.3, 140.2, 130.1, 126.0, 122.0, 112.9, 65.6, 62.0, 37.6, 21.1, 19.8, 3.54, -0.50. HRMS (ESI$^-$) *m/z* calcd for $C_{13}H_{15}O_2Cl$ (M-H$^+$): 237.0688, found: 237.0684.

7-chloro-5-methyl-4-phenylchroman-4-ol (18)



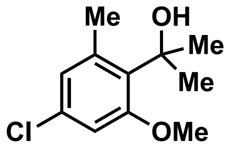
To a solution of **4f** (88.3 mg, 449 μmol) in anhydrous THF (2.25 mL, 0.20 M) was added PhMgBr (898 μL of 1.0 M solution in THF) at 0 °C under argon gas. The reaction was allowed to warm to room temperature as it stirred for 24 hours. The reaction was cooled to 0 °C and quenched with sat. aq. NH_4Cl solution (5 mL). The mixture was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by reverse phase C18 chromatography on a gradient of MeCN:H_2O (1:9) to (9:1) to give **18** as a white solid (83.1 mg, 67%): ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 7.31-7.20 (m, 5H), 6.80 (d, *J* = 2.5 Hz, 1H), 6.72 (d, *J* = 2.5 Hz, 1H), 5.93 (s, 1H), 4.20-4.15 (ddd, *J* = 11.2, 9.3, 2.6 Hz, 1H), 4.06-4.02 (ddd, *J* = 10.9, 6.2, 3.4 Hz, 1H), 2.20-2.15 (ddd, *J* = 14.2, 6.1, 2.6 Hz, 1H), 2.06-2.00 (ddd, *J* = 14.0, 9.5, 3.4 Hz, 1H), 1.78 (s, 3H). ^{13}C NMR (500 MHz, DMSO-*d*$_6$) δ 156.8, 149.0, 142.2, 132.5, 128.4, 126.8, 126.3, 125.7, 123.8, 114.7, 70.6, 62.9, 43.5, 21.3. HRMS (ESI$^-$) *m/z* calcd for $C_{16}H_{15}O_2Cl$ (M-H$^+$): 273.0688, found: 273.0685.

1-(4-chloro-2-methoxy-6-methylphenyl)ethan-1-one (19)



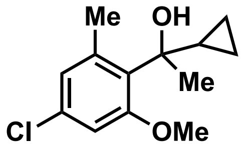
3g (496 mg, 2.69 mmol) was dissolved in anhydrous DMF (2 mL) and was added to a suspension of K_2CO_3 (408 mg, 2.96 mmol) in anhydrous DMF (3 mL) at room temperature under argon gas. The reaction was stirred for 30 minutes. Methyl iodide (419 mg, 2.96 mmol) was slowly added at room temperature under argon gas and the reaction was stirred overnight. The reaction was quenched with crushed ice, the mixture was partitioned between EtOAc (100 mL) and sat. aq. $NaHCO_3$ solution (75 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc/Hexanes (1:20) to (1:1) to give **19** as a white solid (465 mg, 87%): ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 7.03 (s, 1H), 6.94 (s, 1H), 3.81 (s, 3H), 2.40 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (500 MHz, DMSO-*d*$_6$) δ 203.6, 156.7, 136.5, 134.1, 129.6, 122.3, 109.6, 56.3, 31.9, 18.3. HRMS (ESI$^+$) *m/z* calcd for $C_{10}H_{11}O_2Cl$ (M+H$^+$): 199.0521, found: 199.0497.

2-(4-chloro-2-methoxy-6-methylphenyl)propan-2-ol (20)



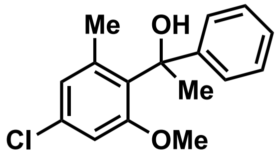
To a solution of **19** (121 mg, 609 µmol) in anhydrous THF (3.05 mL, 0.20 M) was added MeMgBr (1.09 mL of 1.4 M solution in (1:3) THF:Toluene) at 0 °C under argon gas. The reaction was allowed to warm to room temperature as it stirred for 24 hours. The reaction was cooled to 0 °C and quenched with sat. aq. NH_4Cl solution (5 mL). The mixture was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **20** as a white solid (98.3 mg, 75%): ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 6.87 (d, *J* = 2.4 Hz, 1H), 6.74 (d, *J* = 2.5 Hz, 1H), 4.73 (s, 1H), 3.75 (s, 3H), 2.53 (s, 3H), 1.52 (s, 6H). ^{13}C NMR (500 MHz, DMSO-*d*$_6$) δ 157.7, 139.3, 134.5, 130.4, 124.6, 110.3, 73.5, 55.9, 30.8, 24.1. HRMS (ESI$^+$) *m/z* calcd for $C_{11}H_{15}O_2Cl$ (M+H$^+$): 215.0834, found: 215.0470.

1-(4-chloro-2-methoxy-6-methylphenyl)-1-cyclopropylethan-1-ol (21)



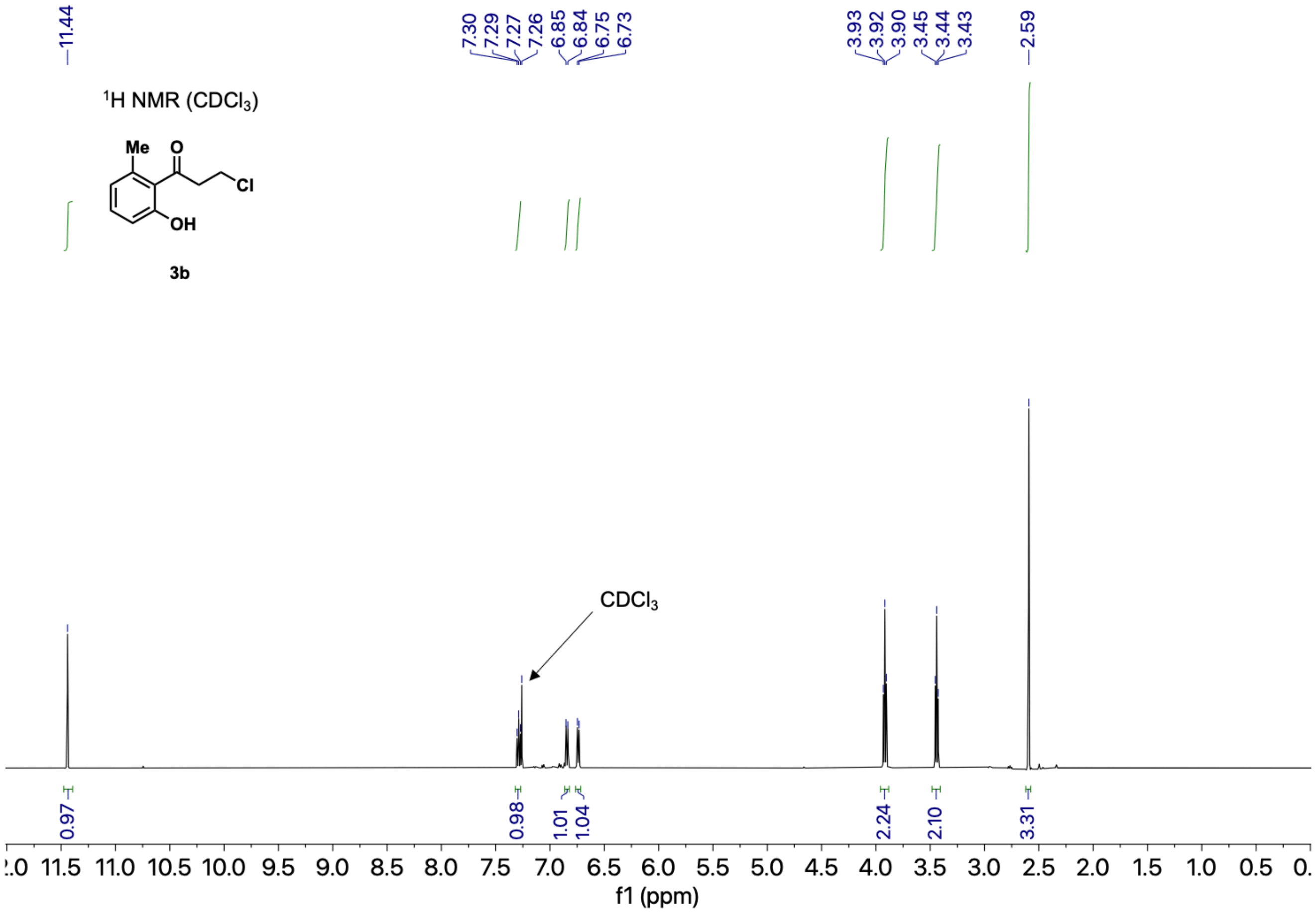
To a solution of **19** (119 mg, 599 µmol) in anhydrous THF (3.00 mL, 0.20 M) was added *cyclo*PrMgBr (1.20 mL of 1.0 M solution in 2-methyl THF) at 0 °C under argon gas. The reaction was transferred to an oil bath and was refluxed under argon gas for 24 hours. The reaction was cooled to 0 °C and quenched with sat. aq. NH_4Cl solution (5 mL). The mixture was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **21** as a white solid (87.3 mg, 61%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.79 (s, 2H), 4.47 (s, 1H), 3.85 (s, 3H), 2.52 (s, 3H), 1.59 (s, 3H), 1.44-1.39 (tt, *J* = 8.3, 5.7 Hz, 1H), 0.60-0.39 (m, 4H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 158.3, 138.3, 133.0, 131.9, 125.8, 110.7, 75.6, 56.1, 28.8, 24.6, 22.2, 2.47, 2.06. HRMS (ESI$^+$) *m/z* calcd for $C_{13}H_{17}O_2Cl$ (M+H$^+$): 241.0990, found: 241.0959.

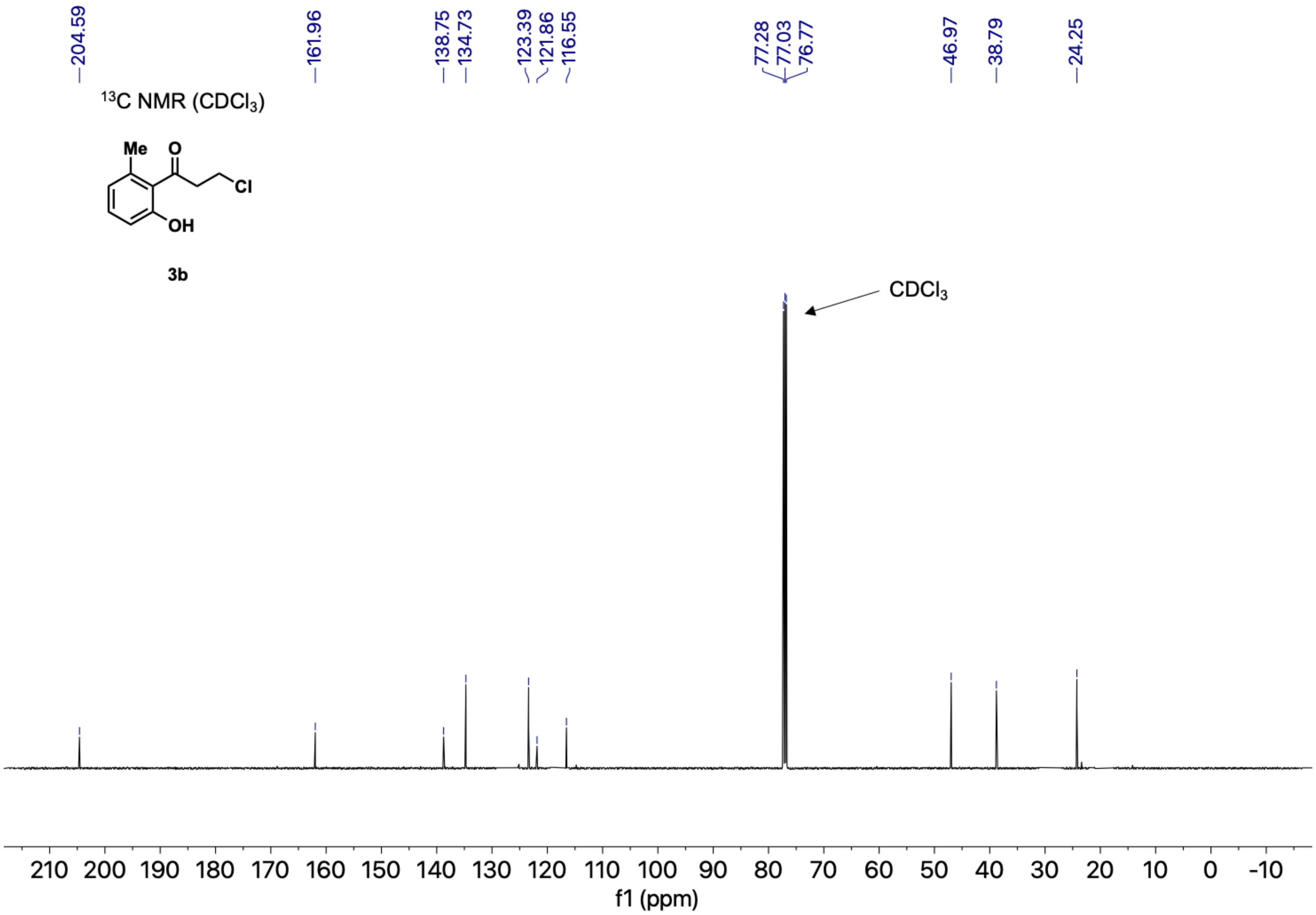
1-(4-chloro-2-methoxy-6-methylphenyl)-1-phenylethan-1-ol (22)

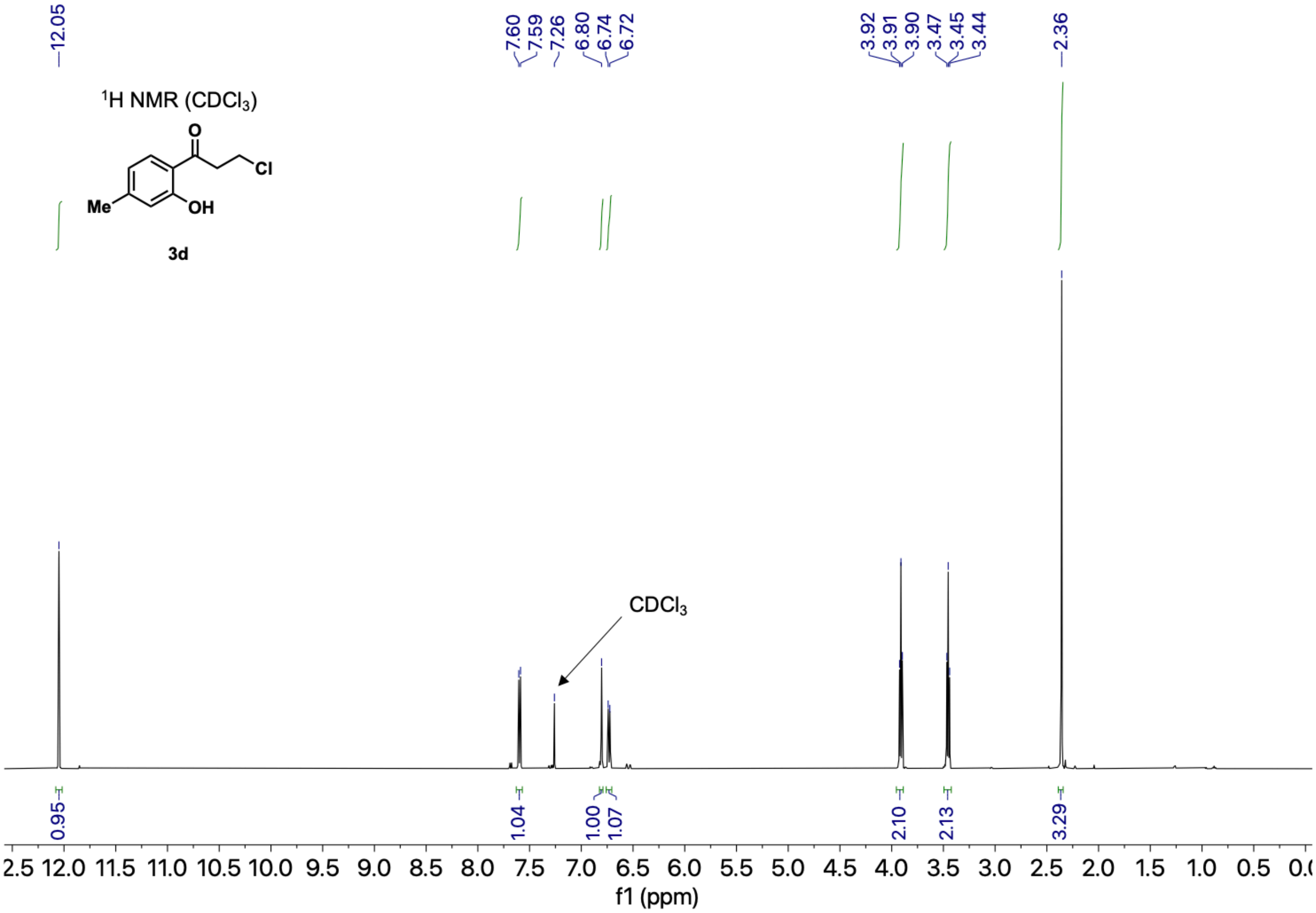


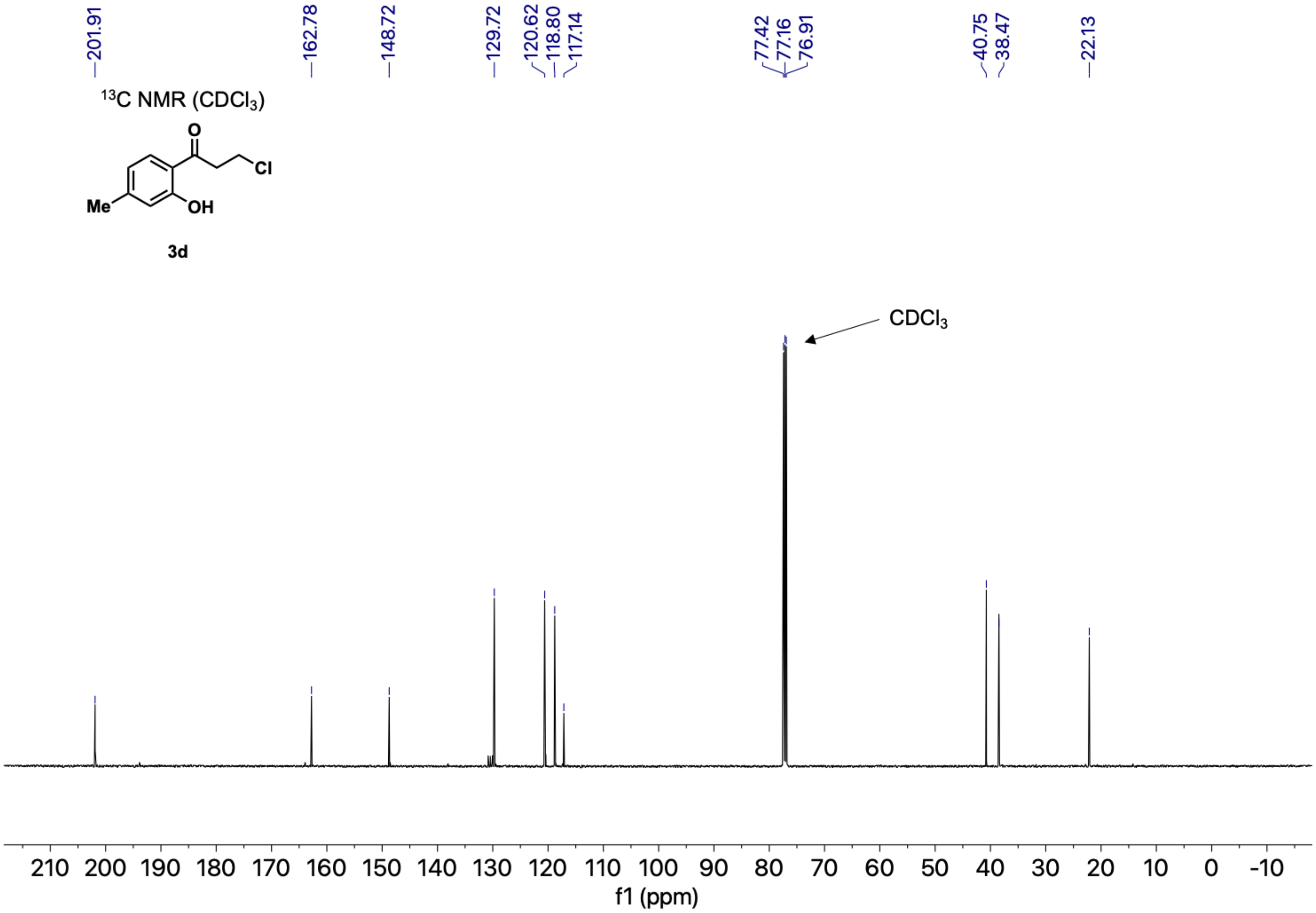
To a solution of **19** (122 mg, 614 μmol) in anhydrous THF (3.07 mL, 0.20 M) was added PhMgBr (1.23 mL of 1.0 M solution in THF) at 0 °C under argon gas. The reaction was allowed to warm to room temperature as it stirred for 24 hours. The reaction was cooled to 0 °C and quenched with sat. aq. NH_4Cl solution (5 mL). The mixture was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL), the organic phase separated, washed with brine (25 mL), dried over anhydrous Na_2SO_4 and the filtrate was evaporated under reduced pressure. The crude material was purified by silica gel flash column chromatography on a gradient of EtOAc:Hexanes (1:20) to (1:1) to give **22** as a white solid (112 mg, 66%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.27 (m, 4H), 7.22-7.18 (m, 1H), 6.81 (d, J = 2.3 Hz, 1H), 6.77 (d, J = 2.4 Hz, 1H), 4.77 (s, 1H), 3.57 (s, 3H), 2.26 (s, 3H), 1.95 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 158.6, 150.0, 138.9, 133.2, 132.7, 128.0, 126.6, 125.8, 124.9, 111.5, 78.3, 56.3, 31.4, 24.0. HRMS (ESI$^-$) *m/z* calcd for $C_{16}H_{17}O_2Cl$ (M-H$^+$): 277.0990, found: 277.0949.

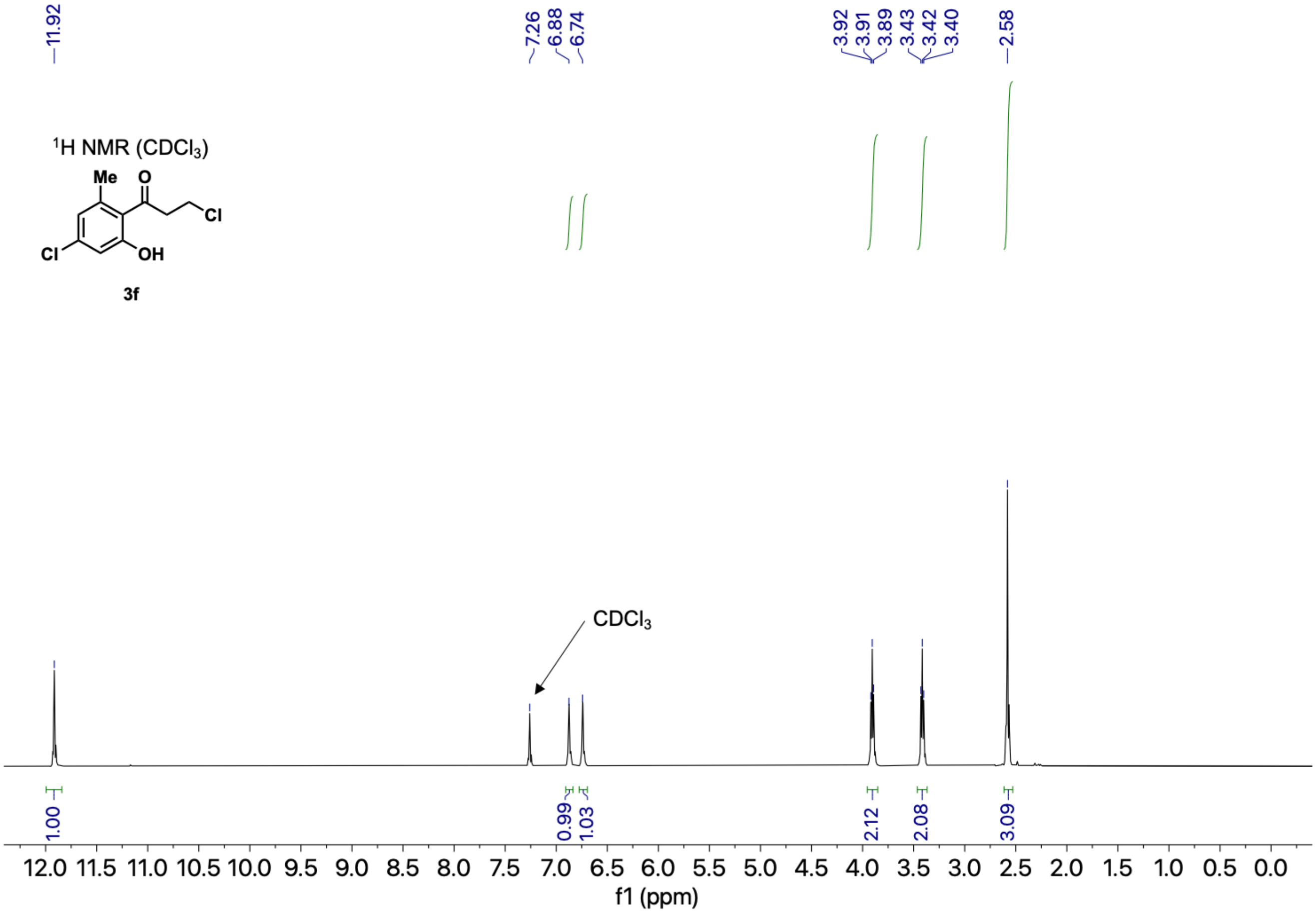
Chemistry – ^{1}H and ^{13}C NMR Spectra

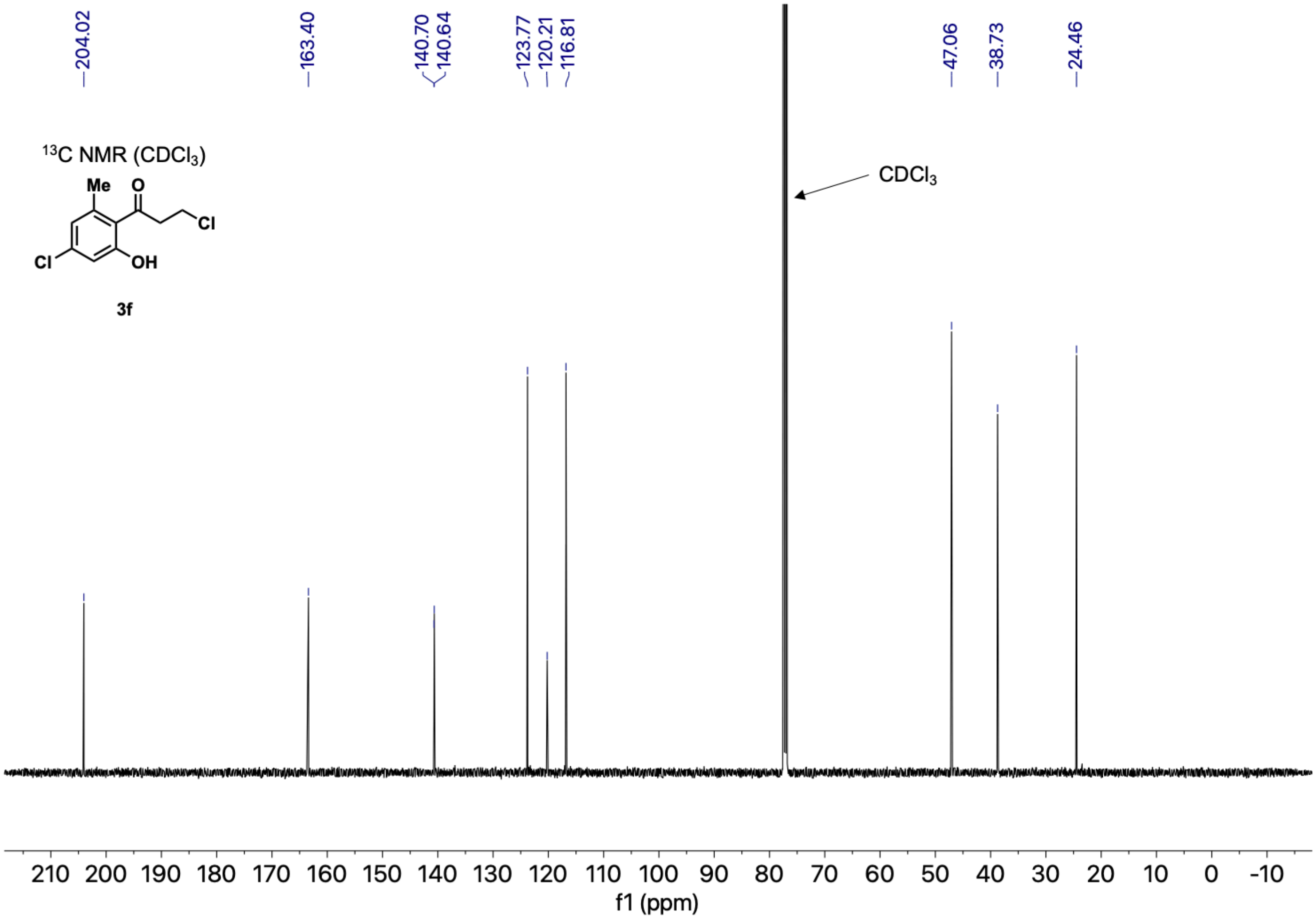



204.59
161.96
138.75
134.73
123.39
121.86
116.55
77.28
77.03
76.77
46.97
38.79
24.25
^{13}C NMR (CDCl$_3$)
Me
O
Cl
OH
3b
CDCl$_3$
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

12.05
7.60
7.59
7.26
6.80
6.74
6.72
3.92
3.91
3.90
3.47
3.45
3.44
2.36
^{1}H NMR (CDCl$_3$)
O
Cl
Me
OH
3d
CDCl$_3$
0.95
1.04
1.00
1.07
2.10
2.13
3.29
2.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)


201.91
162.78
148.72
129.72
120.62
118.80
117.14
77.42
77.16
76.91
40.75
38.47
22.13
^{13}C NMR (CDCl$_3$)
O
Cl
Me
OH
3d
CDCl$_3$
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


11.92
7.26
6.88
6.74
3.92
3.91
3.89
3.43
3.42
3.40
2.58
^{1}H NMR (CDCl$_3$)
Me
O
Cl
Cl
OH
3f
CDCl$_3$
1.00
0.99
1.03
2.12
2.08
3.09
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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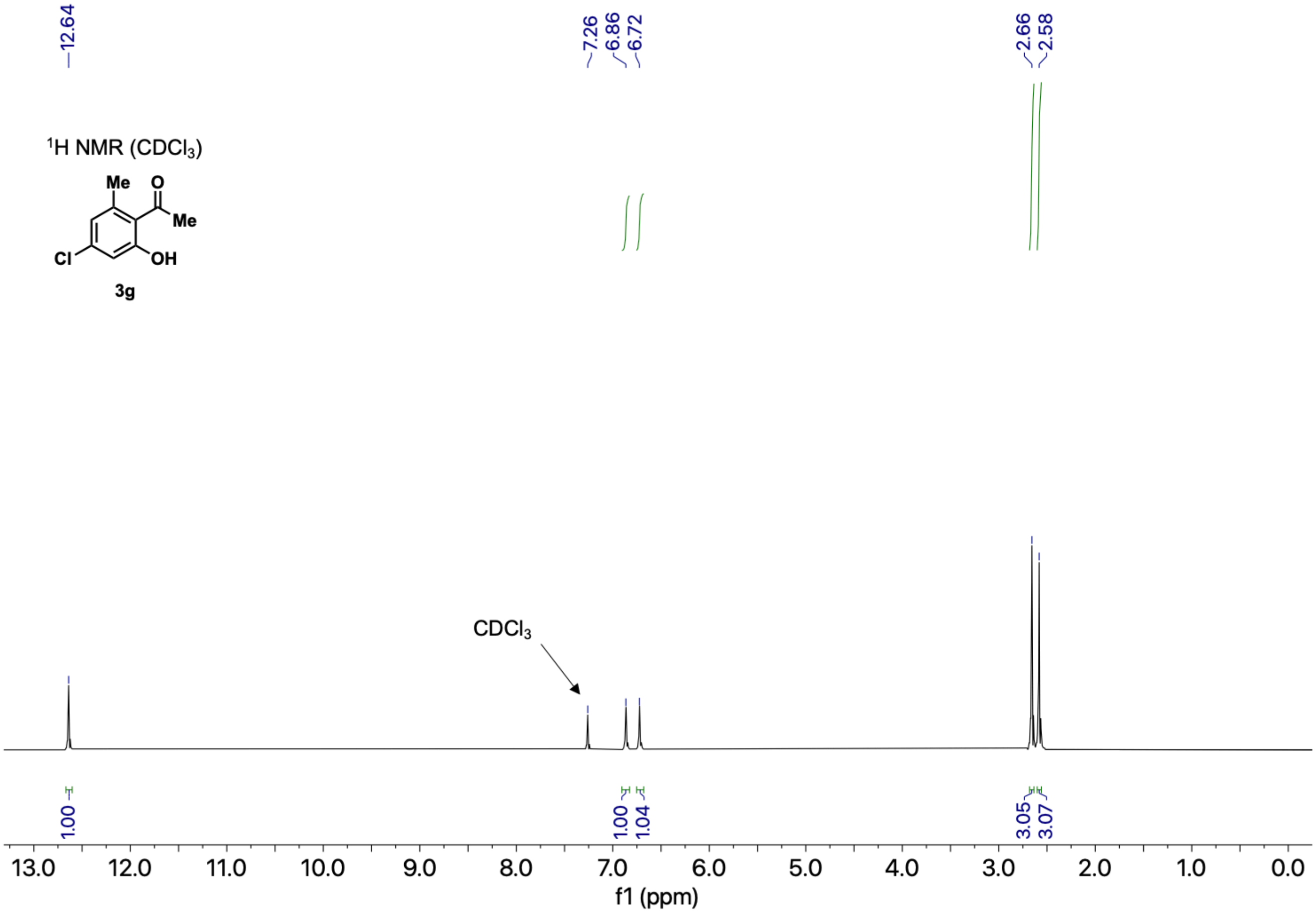
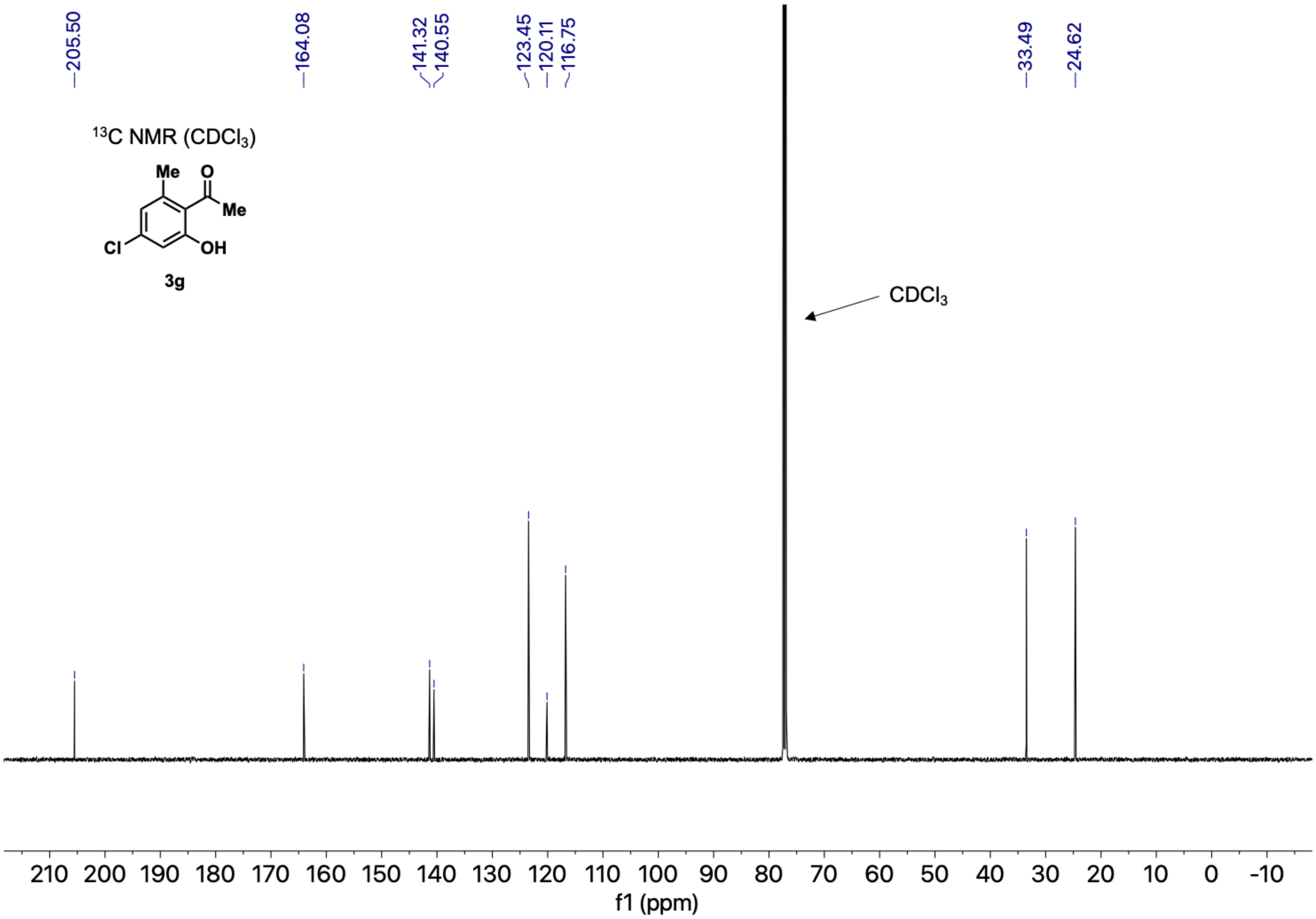
163.40
140.70
140.64
123.77
120.21
116.81
47.06
38.73
24.46
^{13}C NMR ($CDCl_3$)
Me
O
Cl
Cl
OH
3f
$CDCl_3$
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


^{1}H NMR (CDCl$_3$)
Me
O
Me
Cl
OH
3g
12.64
7.26
6.86
6.72
2.66
2.58
CDCl$_3$
1.00
1.00
1.04
3.05
3.07
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)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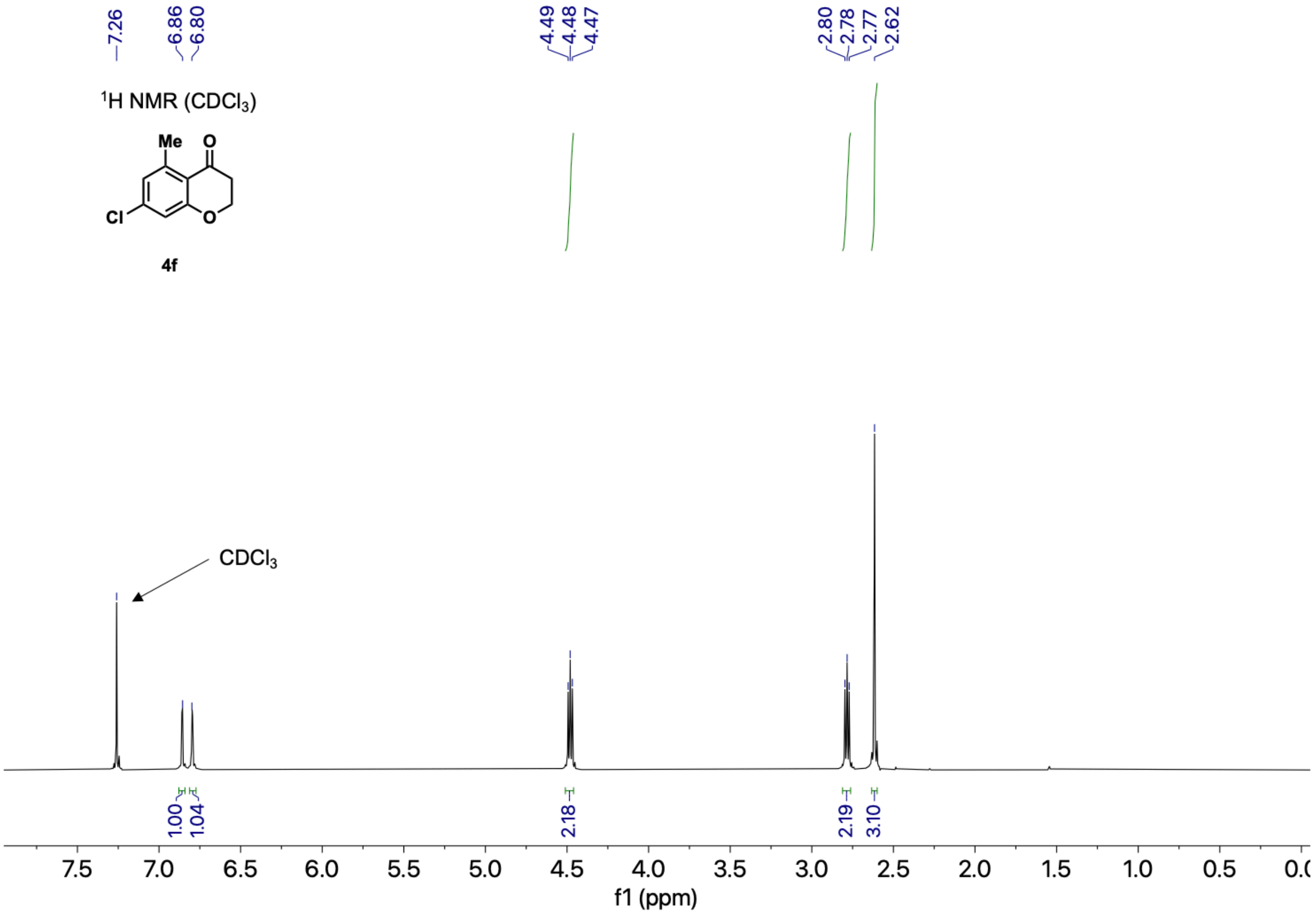
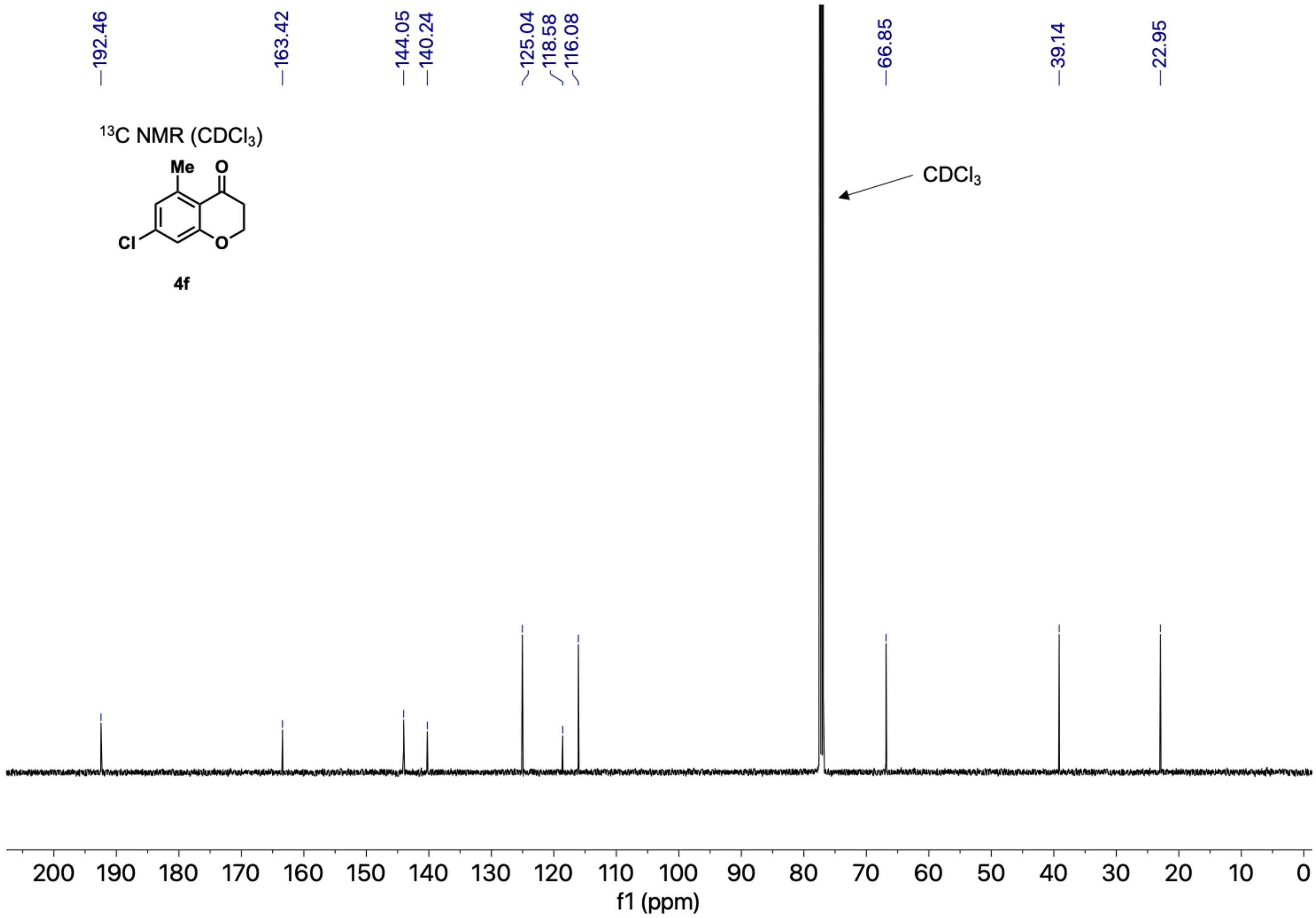
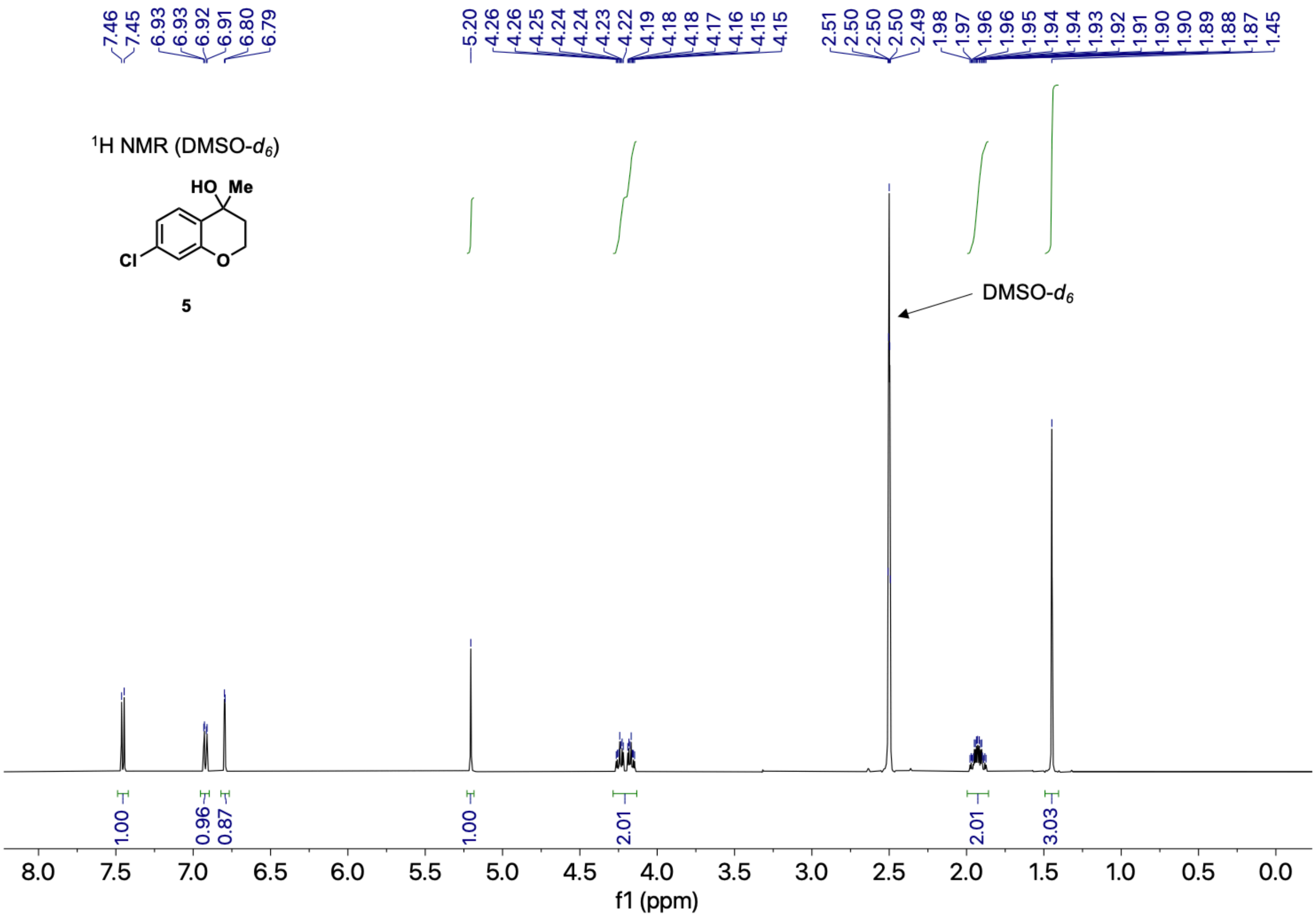
164.08
141.32
140.55
123.45
120.11
116.75
33.49
24.62
^{13}C NMR (CDCl$_3$)
Me
O
Me
Cl
OH
3g
CDCl$_3$
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


7.26
6.86
6.80
4.49
4.48
4.47
2.80
2.78
2.77
2.62
^{1}H NMR ($CDCl_3$)
Me
O
Cl
O
4f
$CDCl_3$
1.00
1.04
2.18
2.19
3.10
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


192.46
163.42
144.05
140.24
125.04
118.58
116.08
66.85
39.14
22.95
^{13}C NMR (CDCl$_3$)
Me
O
Cl
O
4f
CDCl$_3$
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7.45
6.93
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6.91
6.80
6.79
5.20
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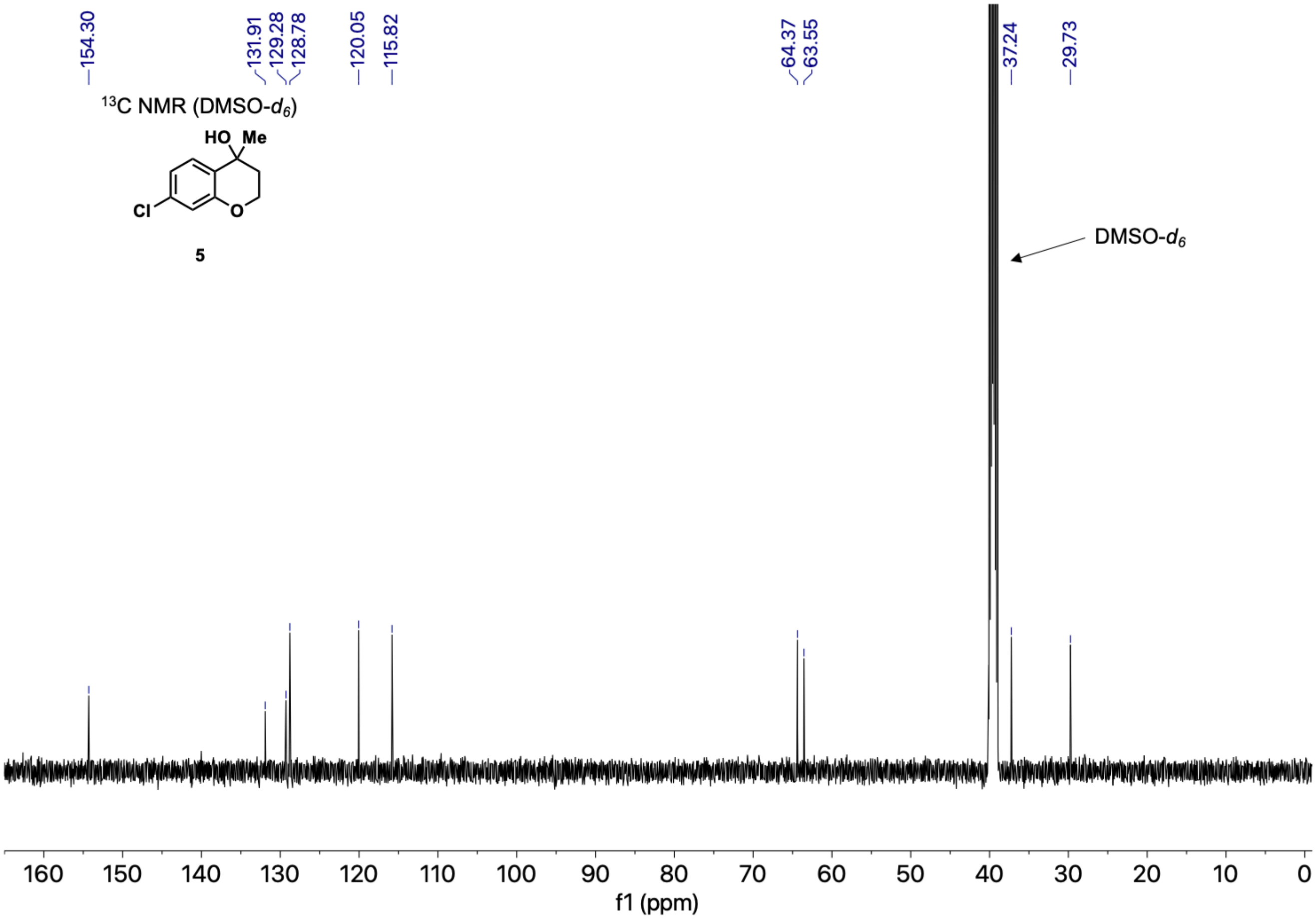
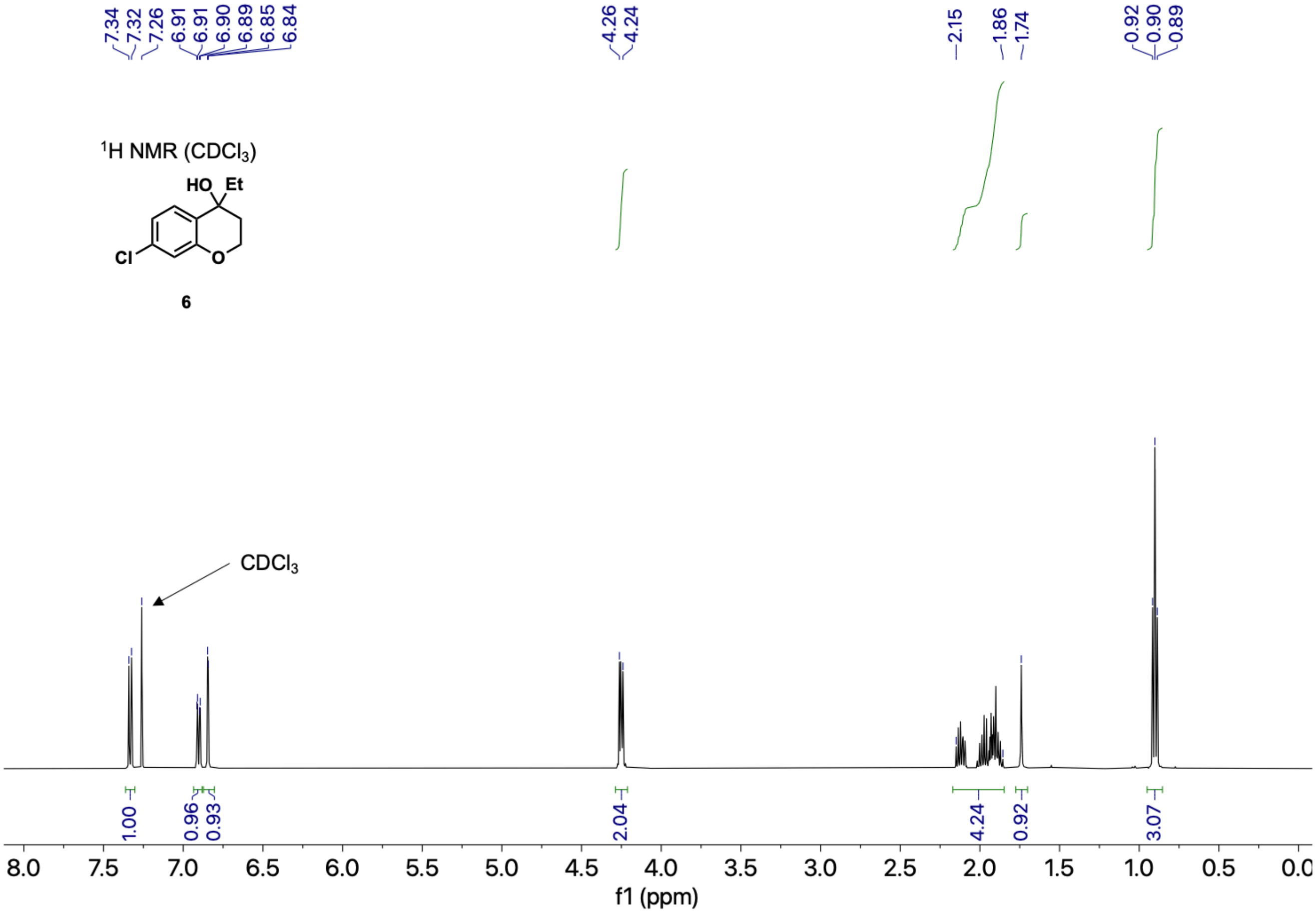
4.26
4.25
4.24
4.24
4.23
4.22
4.19
4.18
4.18
4.17
4.16
4.15
4.15
2.51
2.50
2.50
2.50
2.49
1.98
1.97
1.96
1.96
1.95
1.94
1.94
1.93
1.92
1.91
1.90
1.90
1.89
1.88
1.87
1.45
^{1}H NMR (DMSO-d_6)
HO
Me
Cl
O
DMSO-d_6
1.00
0.96
0.87
1.00
2.01
2.01
3.03
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

5



154.30
131.91
129.28
128.78
120.05
115.82
64.37
63.55
37.24
29.73
^{13}C NMR (DMSO-d_6)
HO
Me
Cl
O
5
DMSO-d_6
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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6.89
6.85
6.84
4.26
4.24
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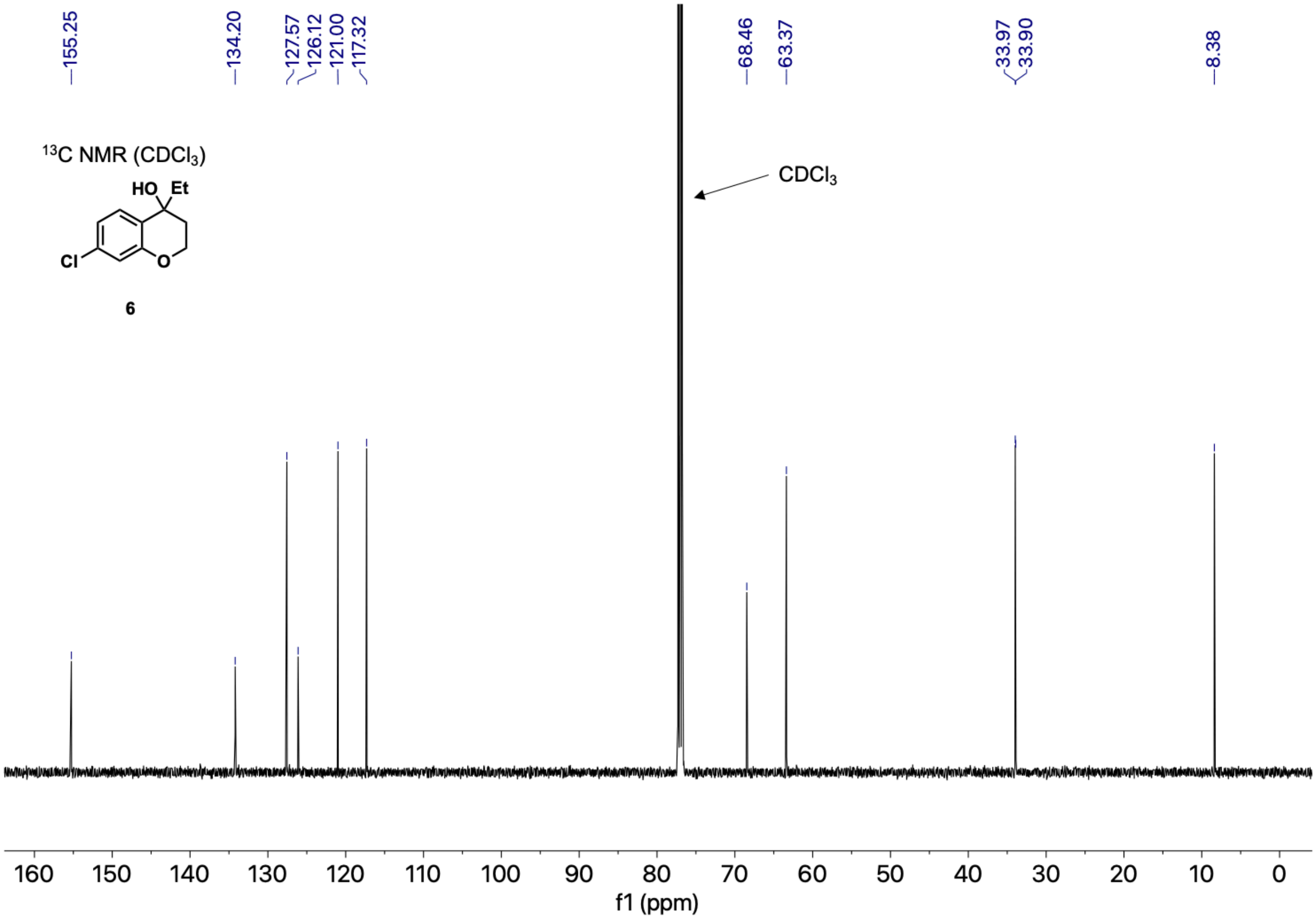
1.86
1.74
0.92
0.90
0.89
^{1}H NMR (CDCl$_3$)
HO
Et
Cl
O
6
CDCl$_3$
1.00
0.96
0.93
2.04
4.24
0.92
3.07
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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126.12
121.00
117.32
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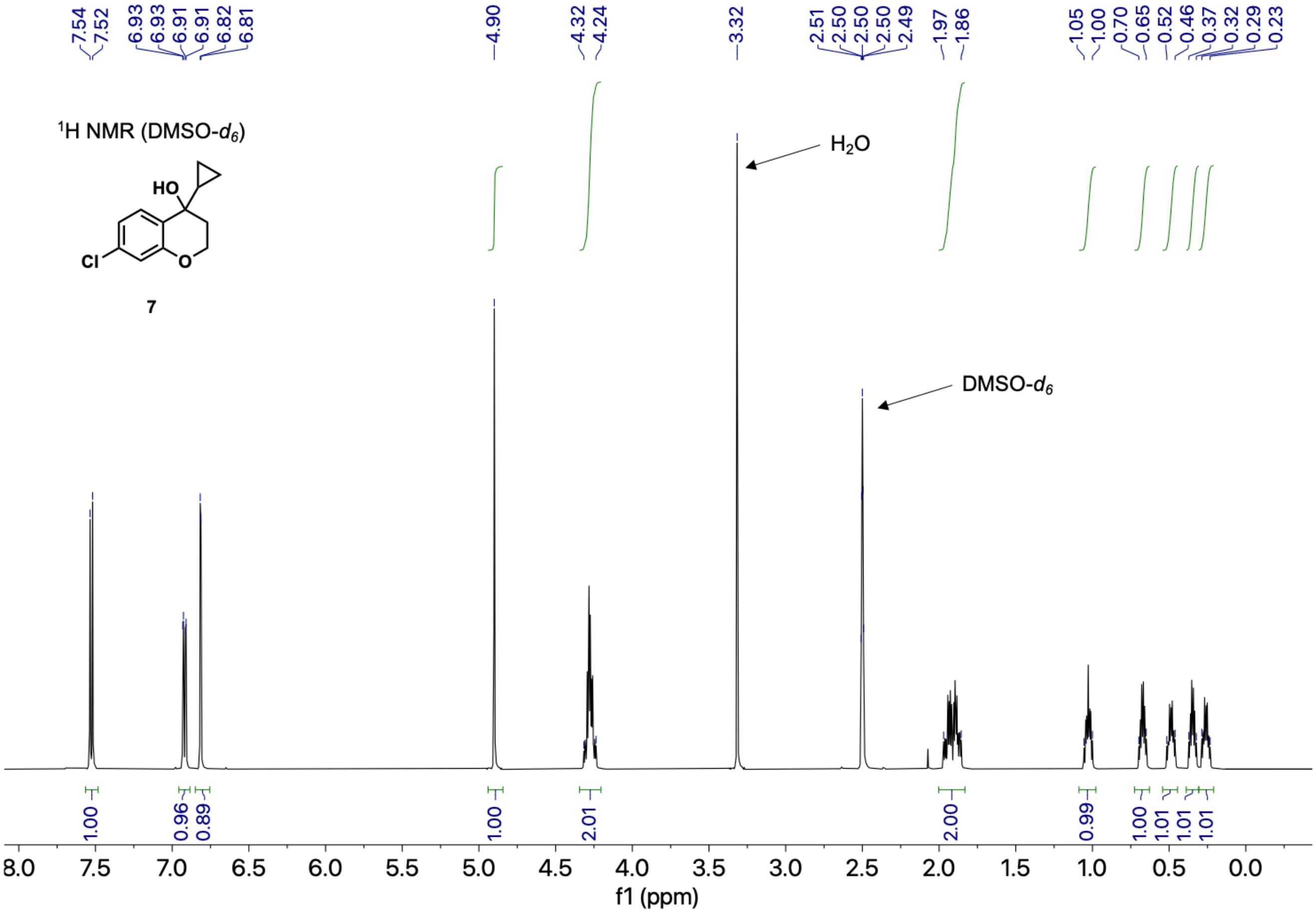
63.37
33.97
33.90
8.38
13C NMR (CDCl3)
HO
Et
Cl
O
6
CDCl3
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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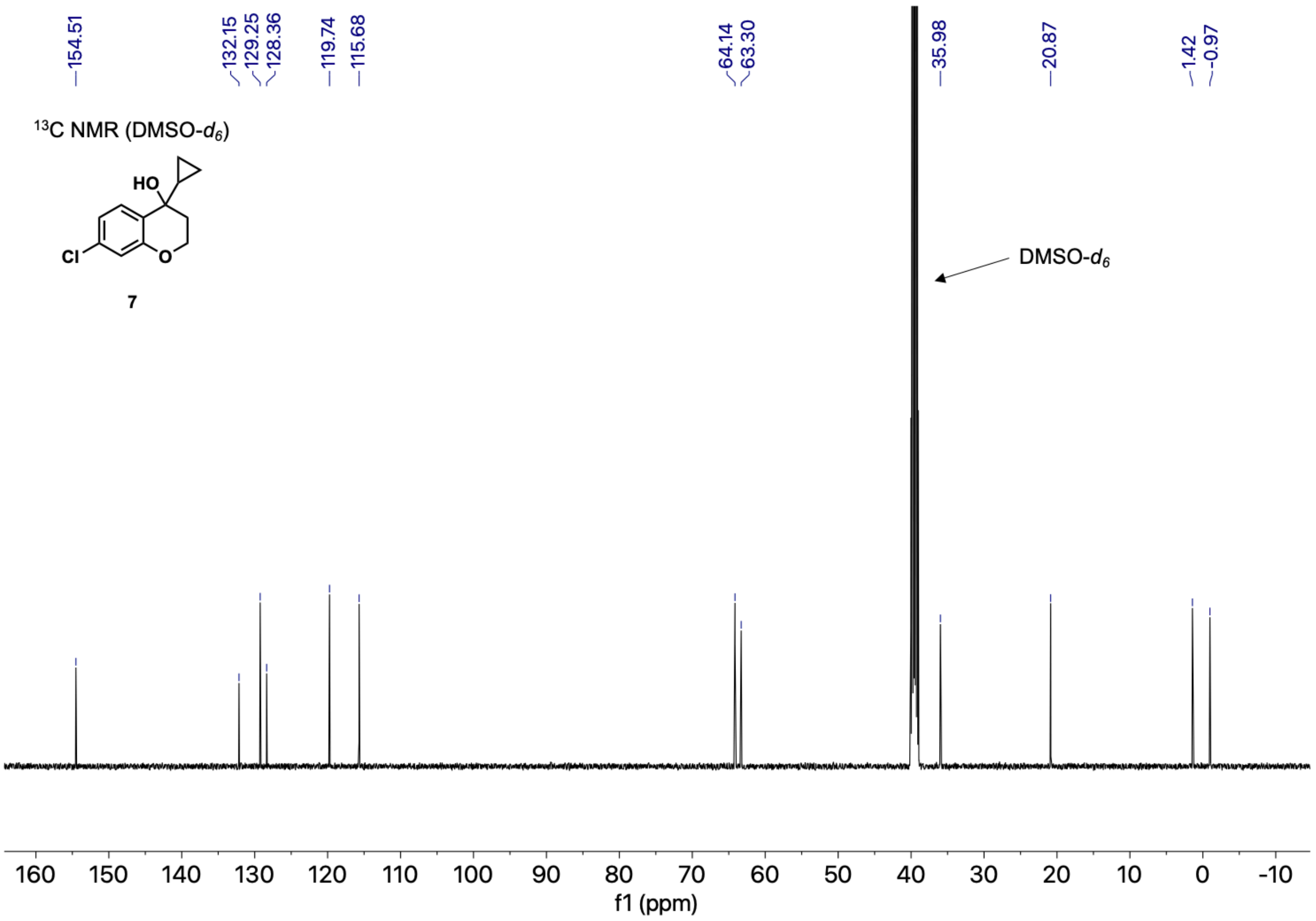
6.81
4.90
4.32
4.24
3.32
2.51
2.50
2.50
2.50
2.49
1.97
1.86
1.05
1.00
0.70
0.65
0.52
0.46
0.37
0.32
0.29
0.23
¹H NMR (DMSO-d_6)
HO
Cl
O
7
H_2O
DMSO-d_6
1.00
0.96
0.89
1.00
2.01
2.00
0.99
1.00
1.01
1.01
1.01
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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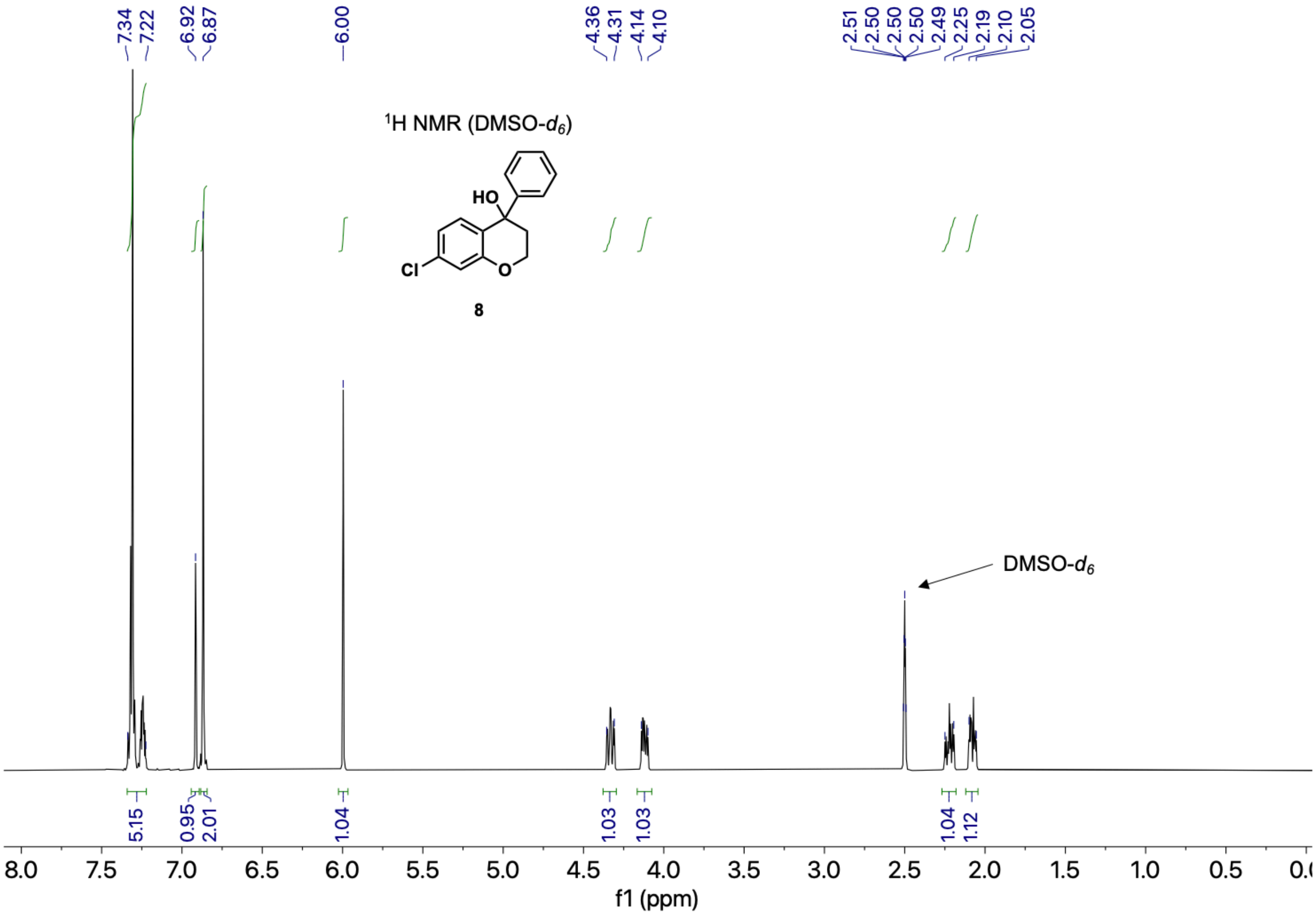
128.36
119.74
115.68
64.14
63.30
35.98
20.87
1.42
-0.97
^{13}C NMR (DMSO-d_6)
HO
Cl
O
7
DMSO-d_6
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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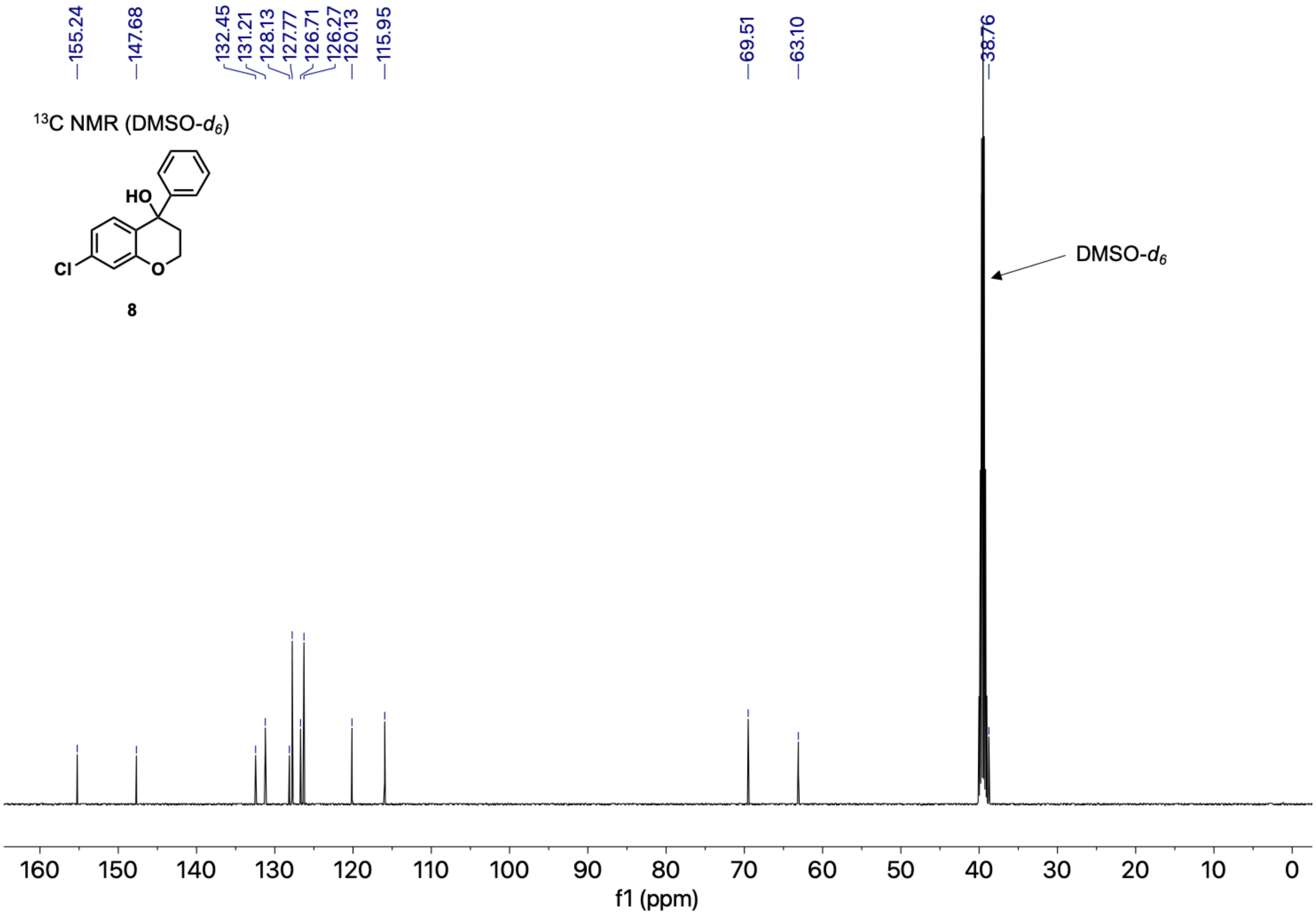
7.22
6.92
6.87
6.00
4.36
4.31
4.14
4.10
2.51
2.50
2.50
2.50
2.49
2.25
2.19
2.10
2.05
^{1}H NMR (DMSO-d_6)
HO
Cl
O
8
DMSO-d_6
5.15
0.95
2.01
1.04
1.03
1.03
1.04
1.12
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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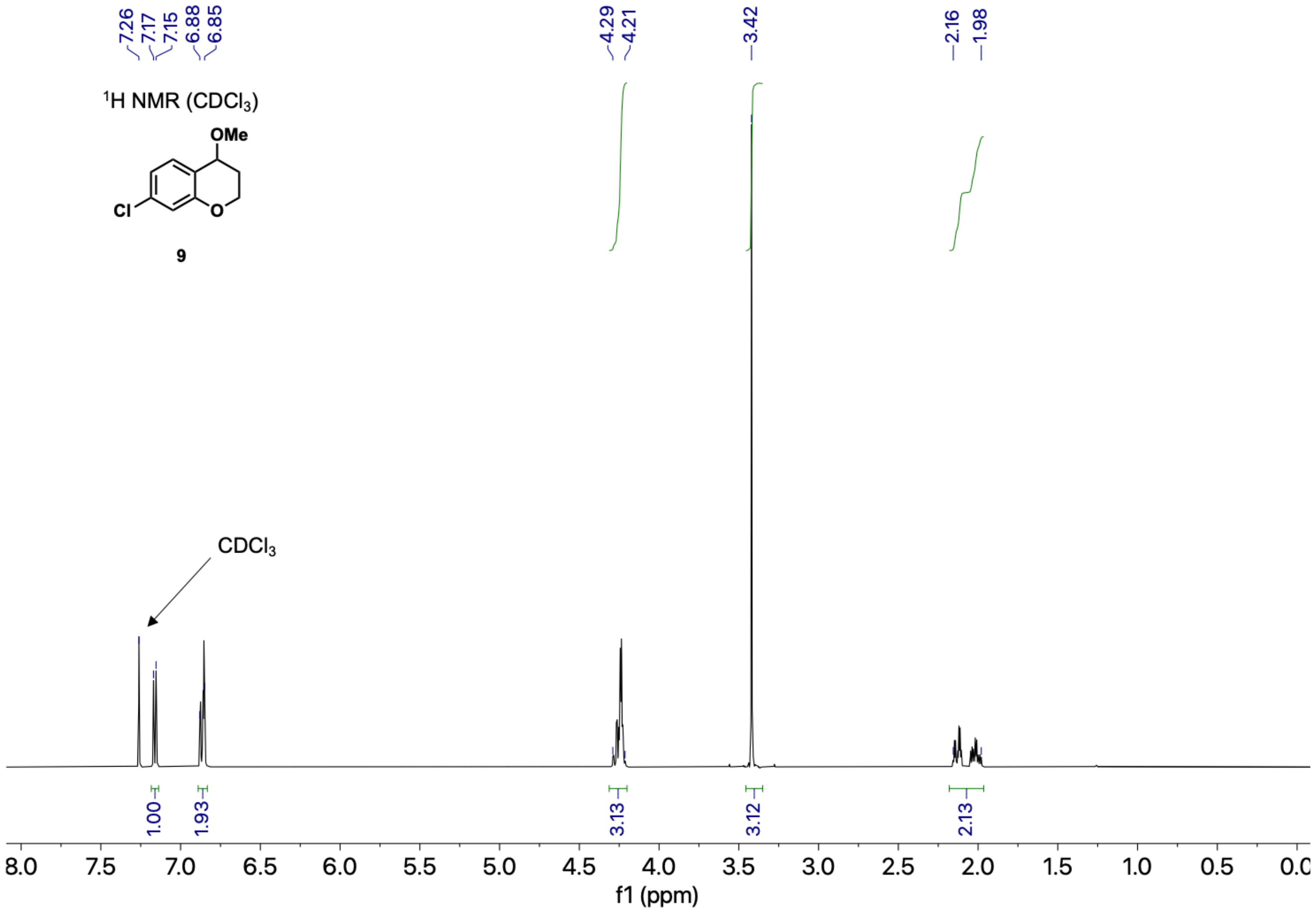
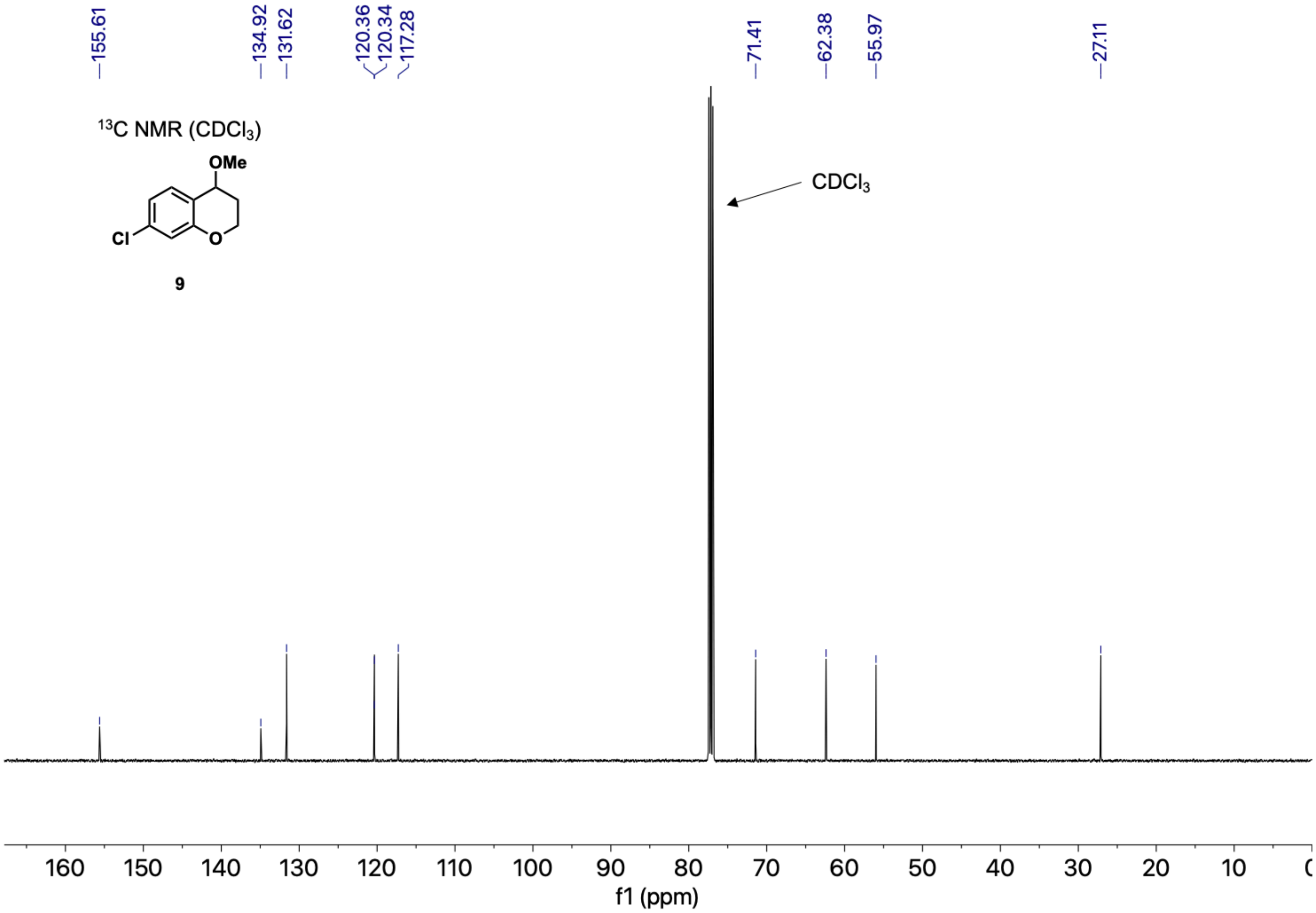
131.21
128.13
127.77
126.71
126.27
120.13
115.95
69.51
63.10
38.76
^{13}C NMR (DMSO-d_6)
HO
Cl
O
8
DMSO-d_6
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.26
7.17
7.15
6.88
6.85
4.29
4.21
3.42
2.16
1.98
^{1}H NMR (CDCl$_3$)
OMe
Cl
O
9
CDCl$_3$
1.00
1.93
3.13
3.12
2.13
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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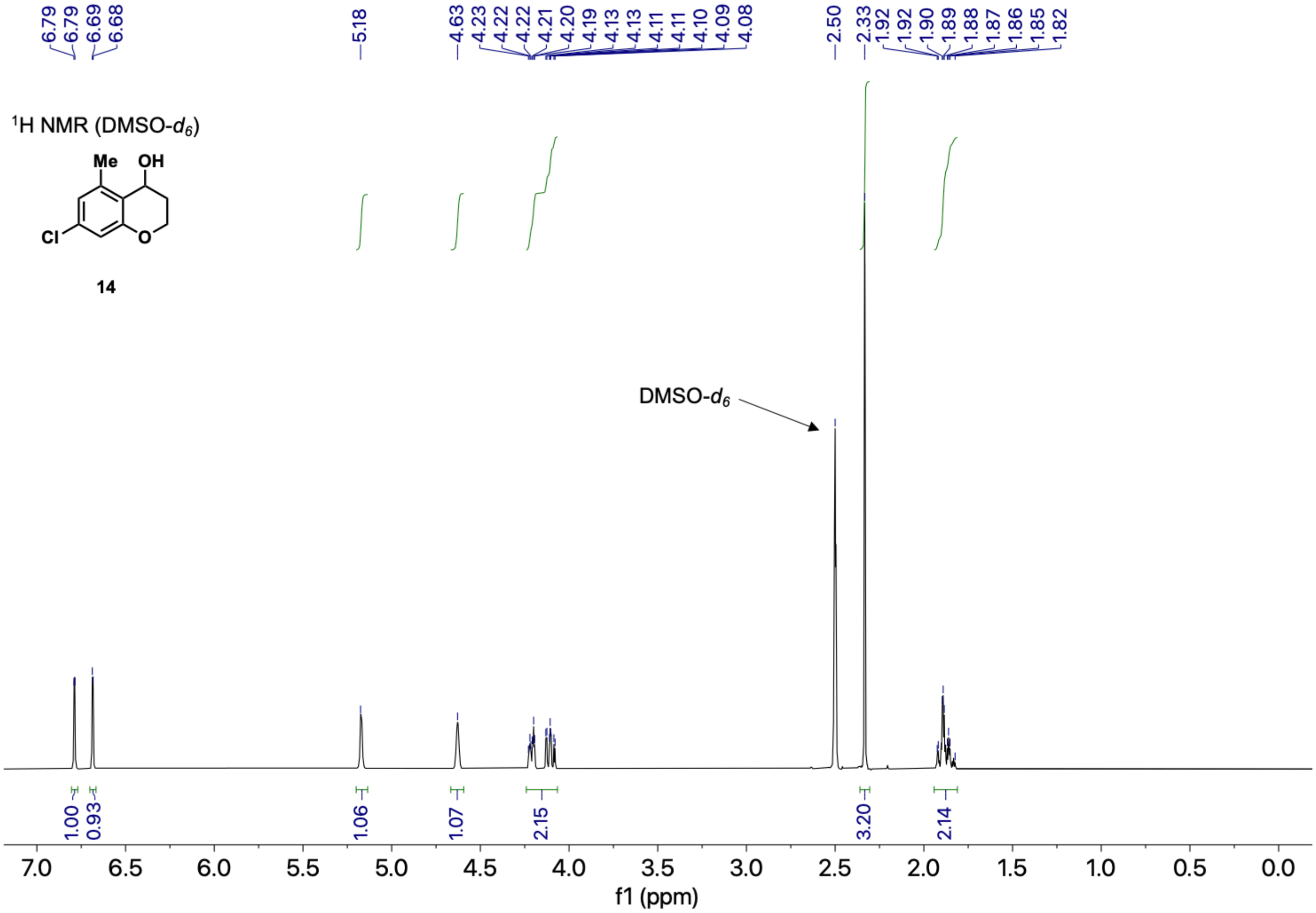
131.62
120.36
120.34
117.28
71.41
62.38
55.97
27.11
^{13}C NMR ($CDCl_3$)
OMe
Cl
O
9
$CDCl_3$
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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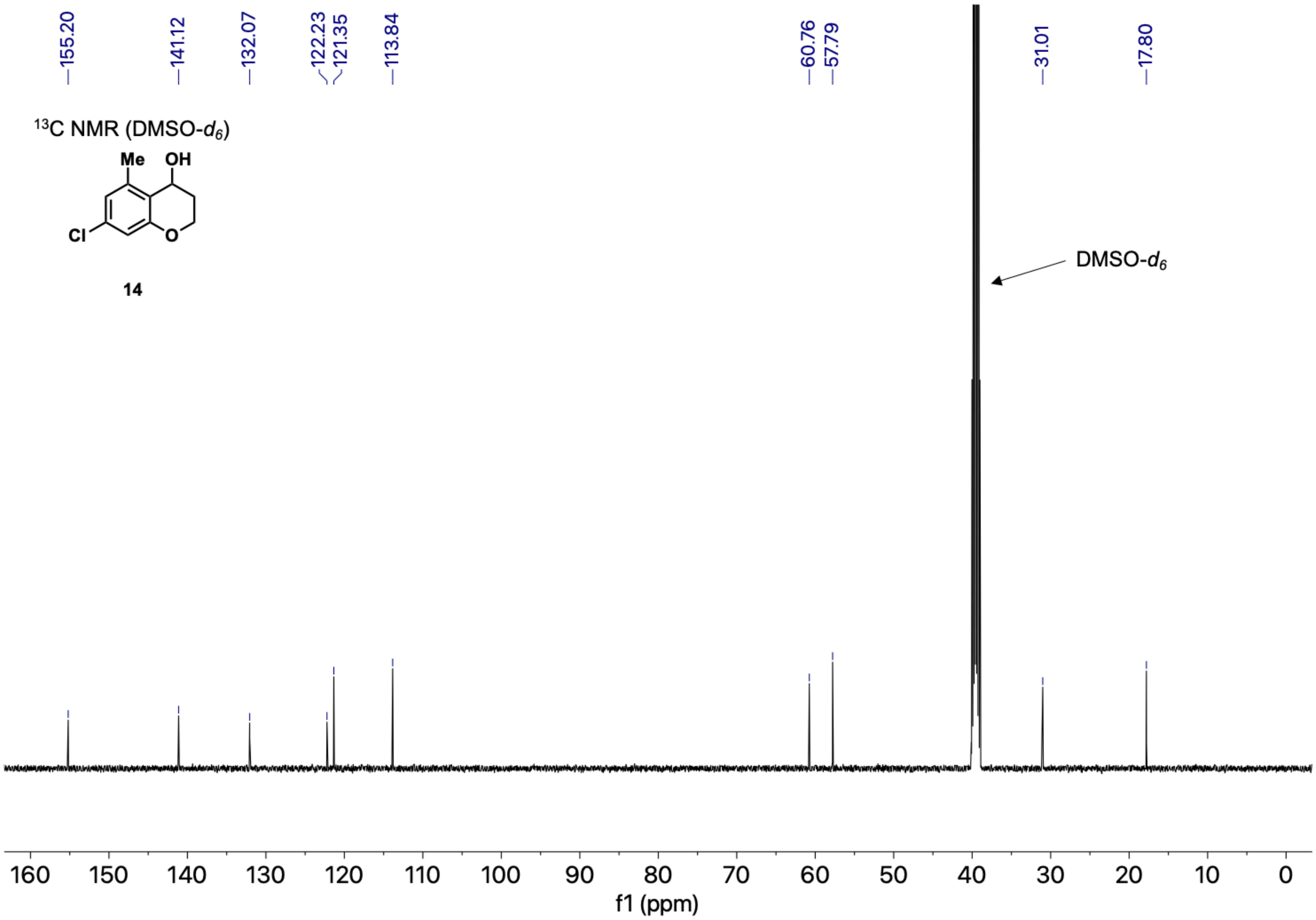
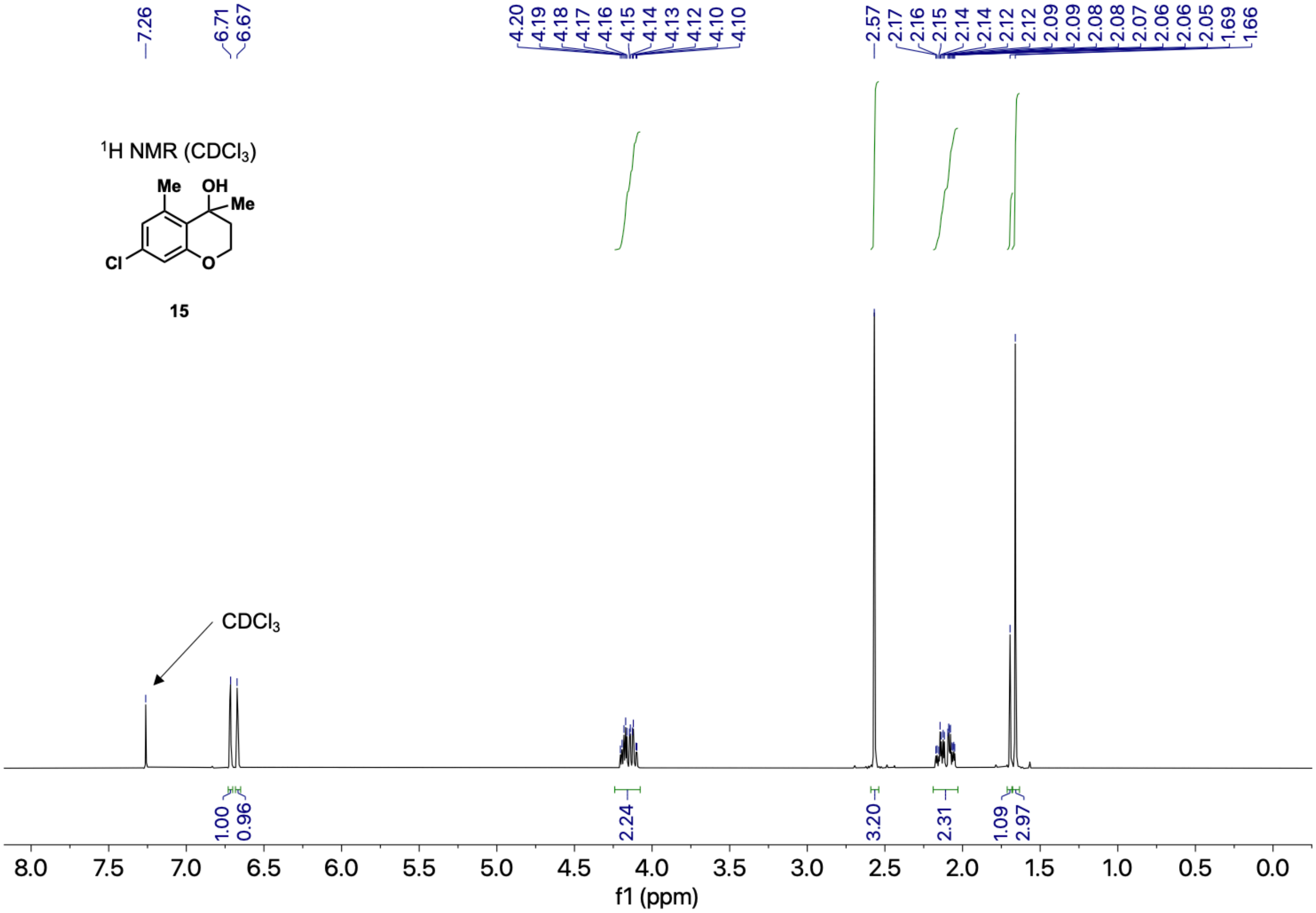
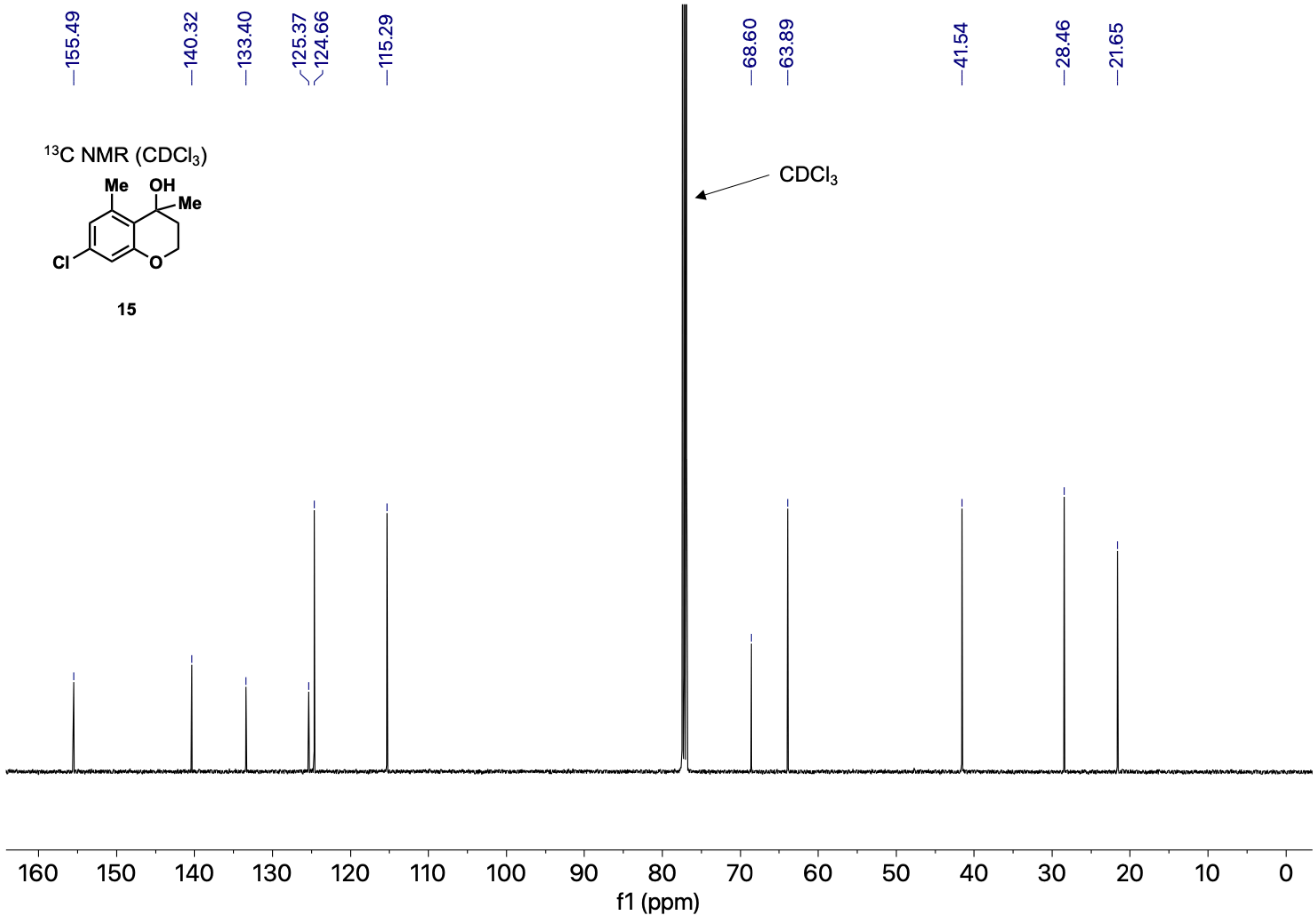
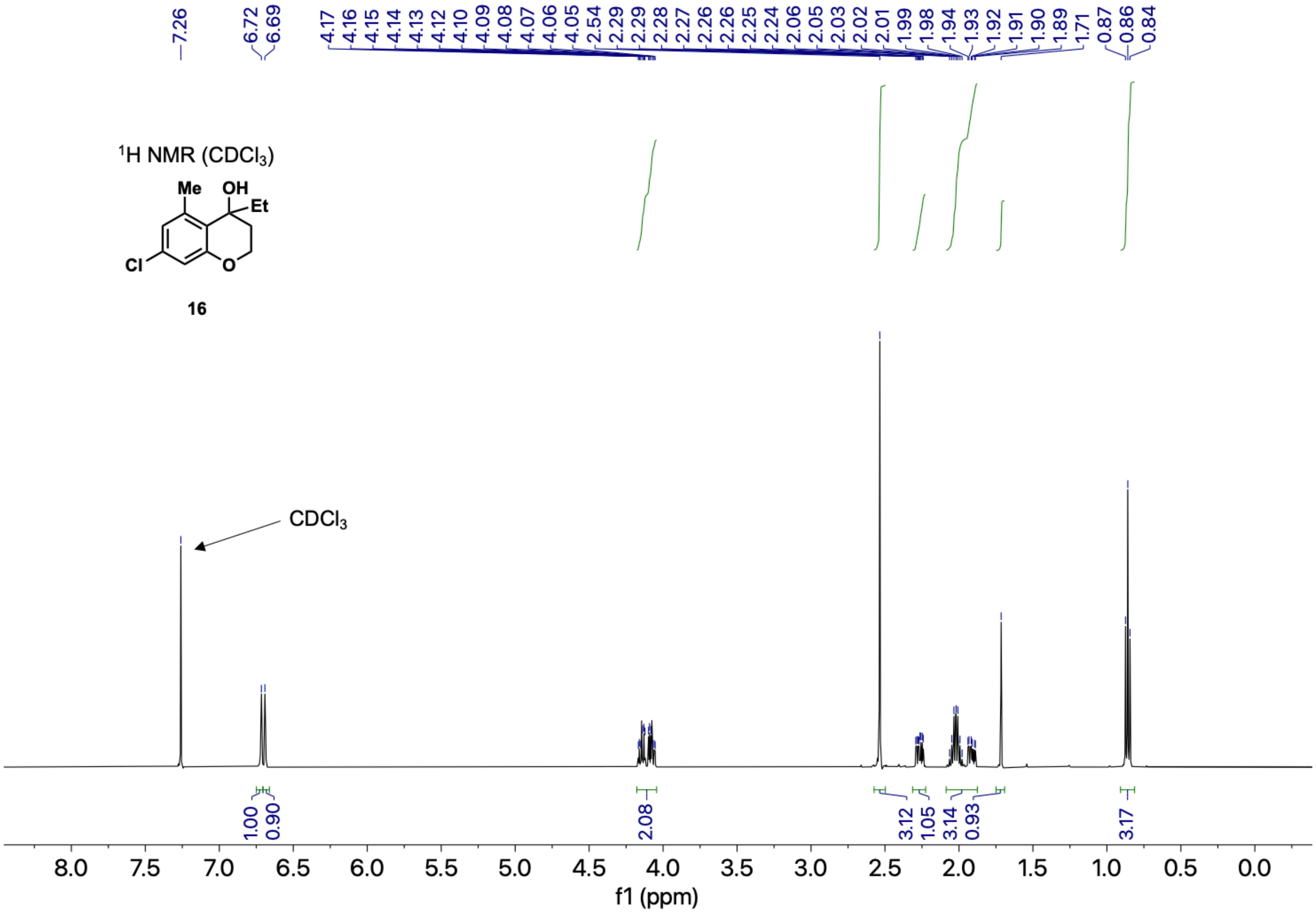
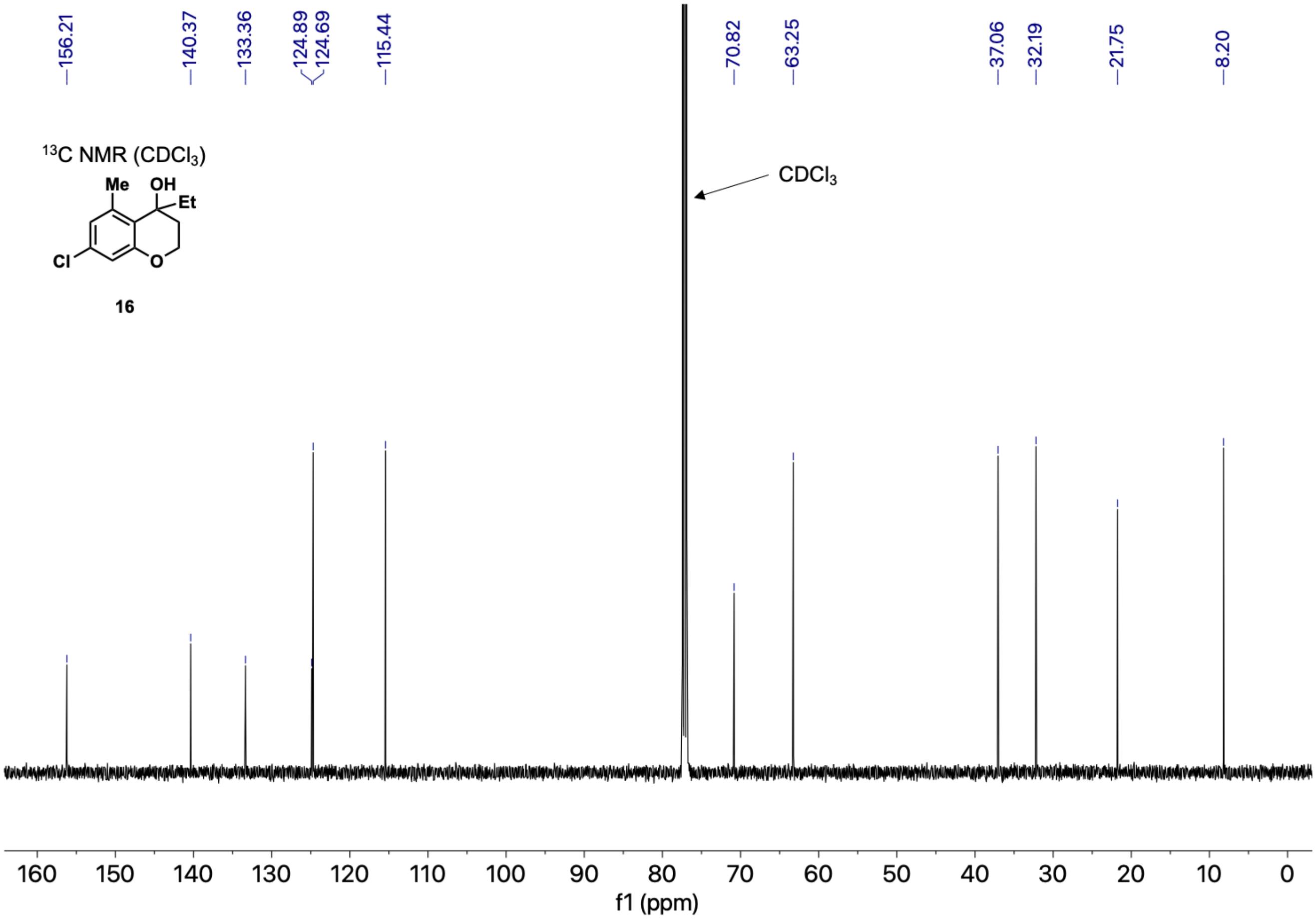
6.79
6.69
6.68
5.18
4.63
4.23
4.22
4.22
4.21
4.20
4.19
4.13
4.13
4.11
4.11
4.10
4.09
4.08
2.50
2.33
1.92
1.92
1.90
1.89
1.88
1.87
1.86
1.85
1.82
^{1}H NMR (DMSO-d_6)
Me
OH
Cl
O
14
DMSO-d_6
1.00
0.93
1.06
1.07
2.15
3.20
2.14
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


155.20
141.12
132.07
122.23
121.35
113.84
60.76
57.79
31.01
17.80
^{13}C NMR (DMSO-d_6)
Me
OH
Cl
O
14
DMSO-d_6
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

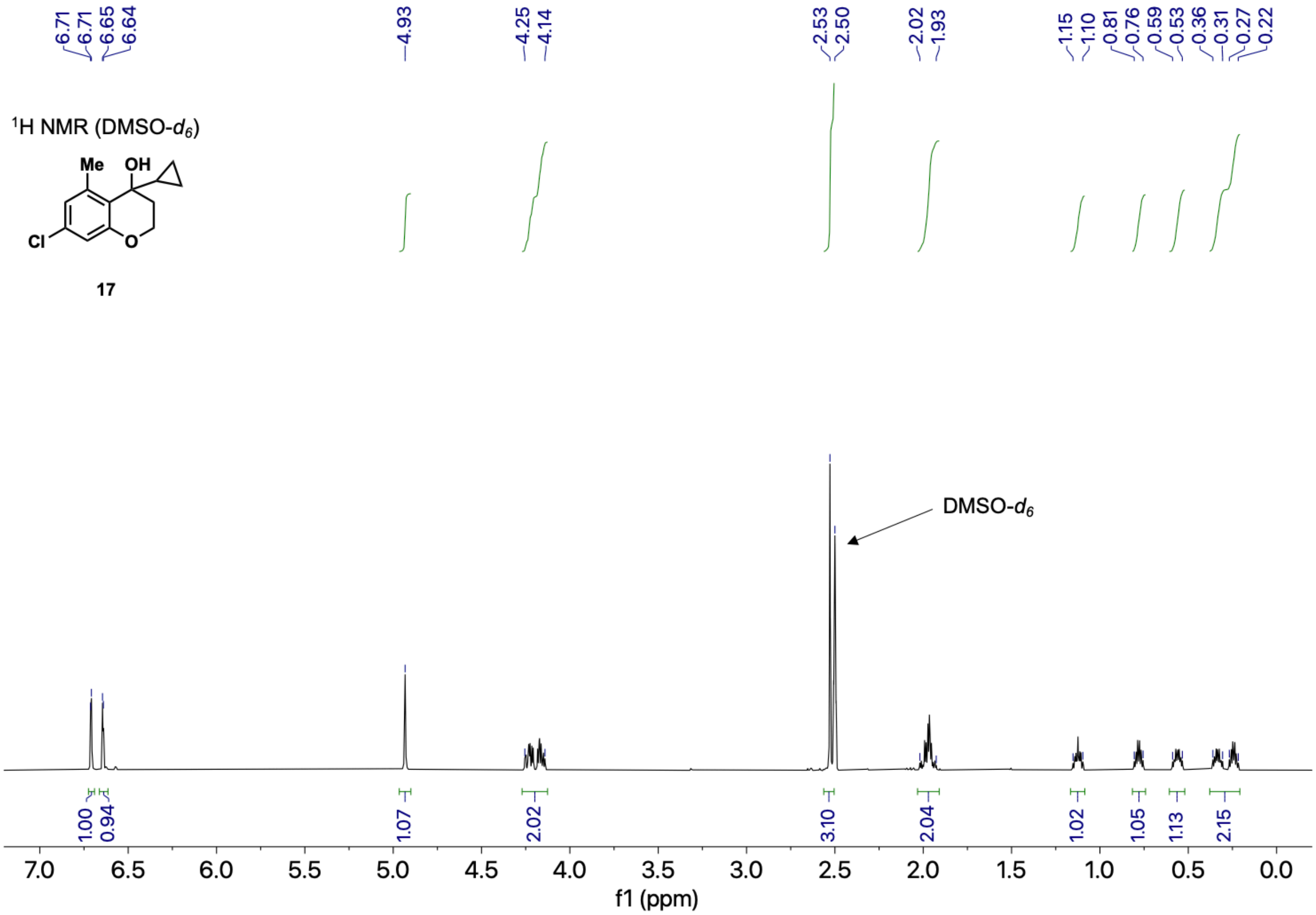

7.26
6.71
6.67
4.20
4.19
4.18
4.17
4.16
4.15
4.14
4.13
4.12
4.10
4.10
2.57
2.17
2.16
2.15
2.14
2.14
2.12
2.12
2.09
2.09
2.08
2.08
2.07
2.06
2.06
2.05
1.69
1.66
¹H NMR (CDCl₃)
Me
OH
Me
Cl
O
15
CDCl₃
1.00
0.96
2.24
3.20
2.31
1.09
2.97
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

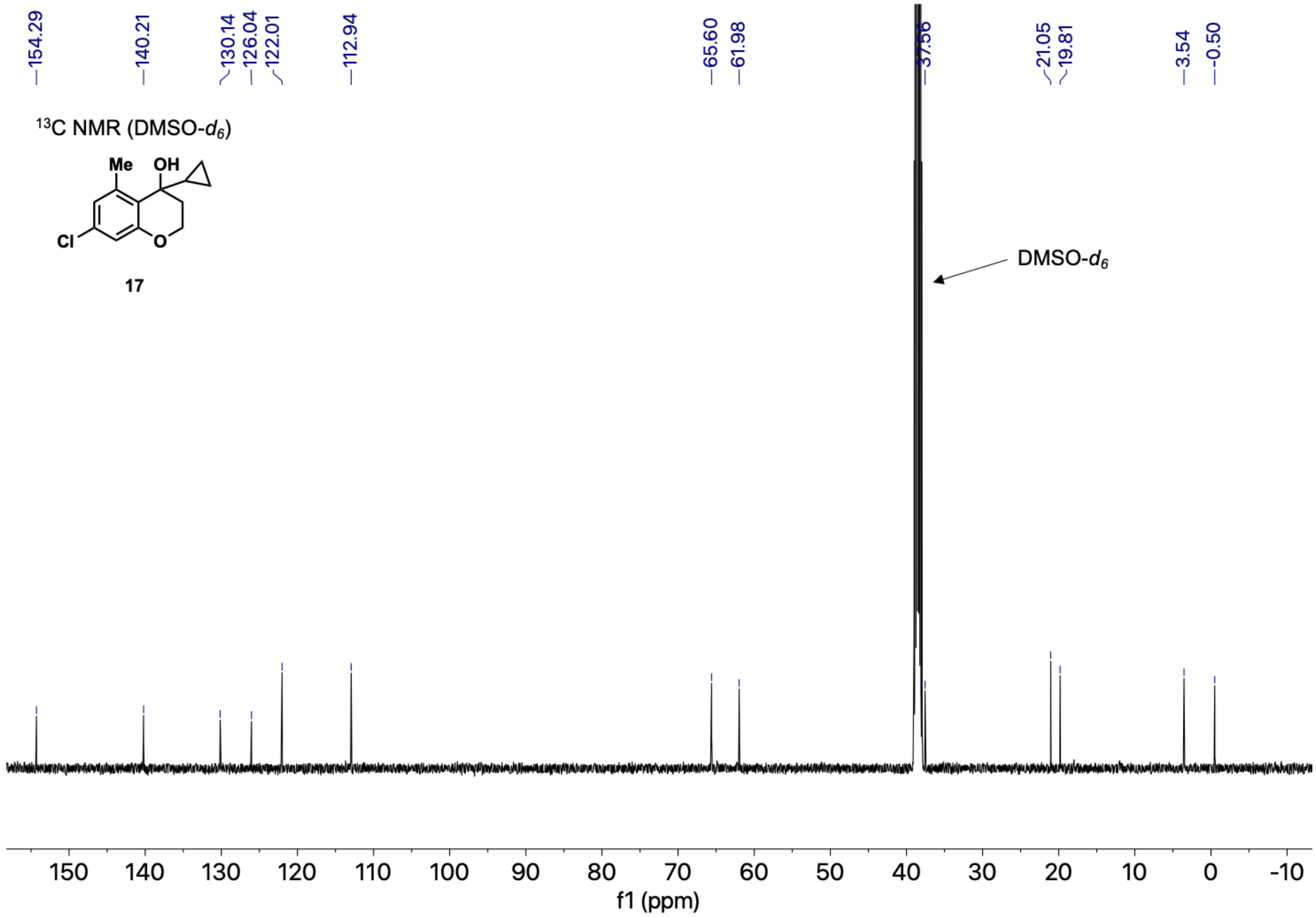

155.49
140.32
133.40
125.37
124.66
115.29
68.60
63.89
41.54
28.46
21.65
^{13}C NMR (CDCl$_3$)
Me
OH
Me
Cl
O
15
CDCl$_3$
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

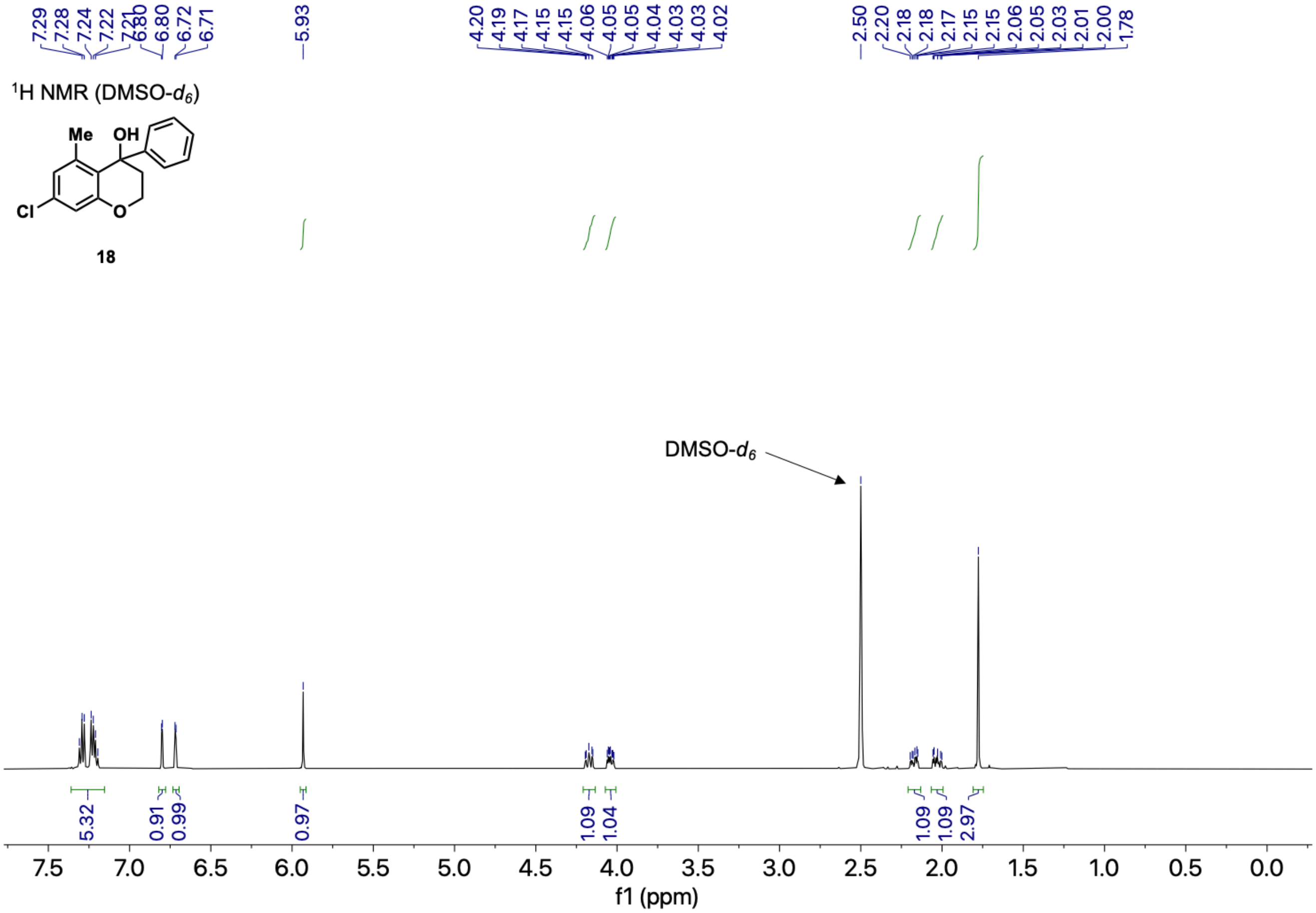

7.26
6.72
6.69
4.17
4.16
4.15
4.14
4.13
4.12
4.10
4.09
4.08
4.07
4.06
4.05
2.54
2.29
2.29
2.28
2.27
2.26
2.26
2.25
2.24
2.06
2.05
2.03
2.02
2.01
1.99
1.98
1.94
1.93
1.92
1.91
1.90
1.89
1.71
0.87
0.86
0.84
^{1}H NMR (CDCl$_3$)
Me
OH
Et
Cl
O
16
CDCl$_3$
1.00
0.90
2.08
3.12
1.05
3.14
0.93
3.17
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

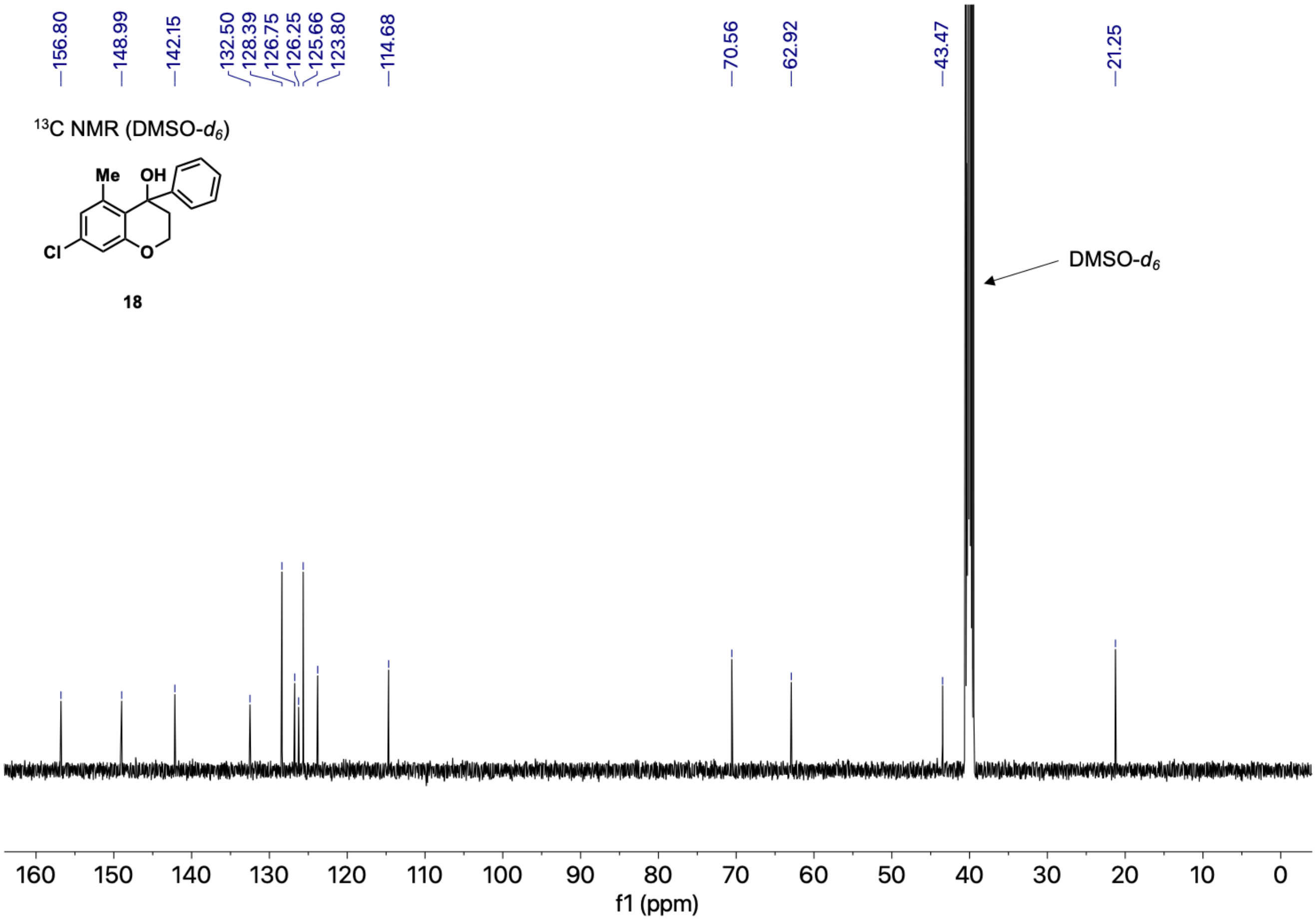


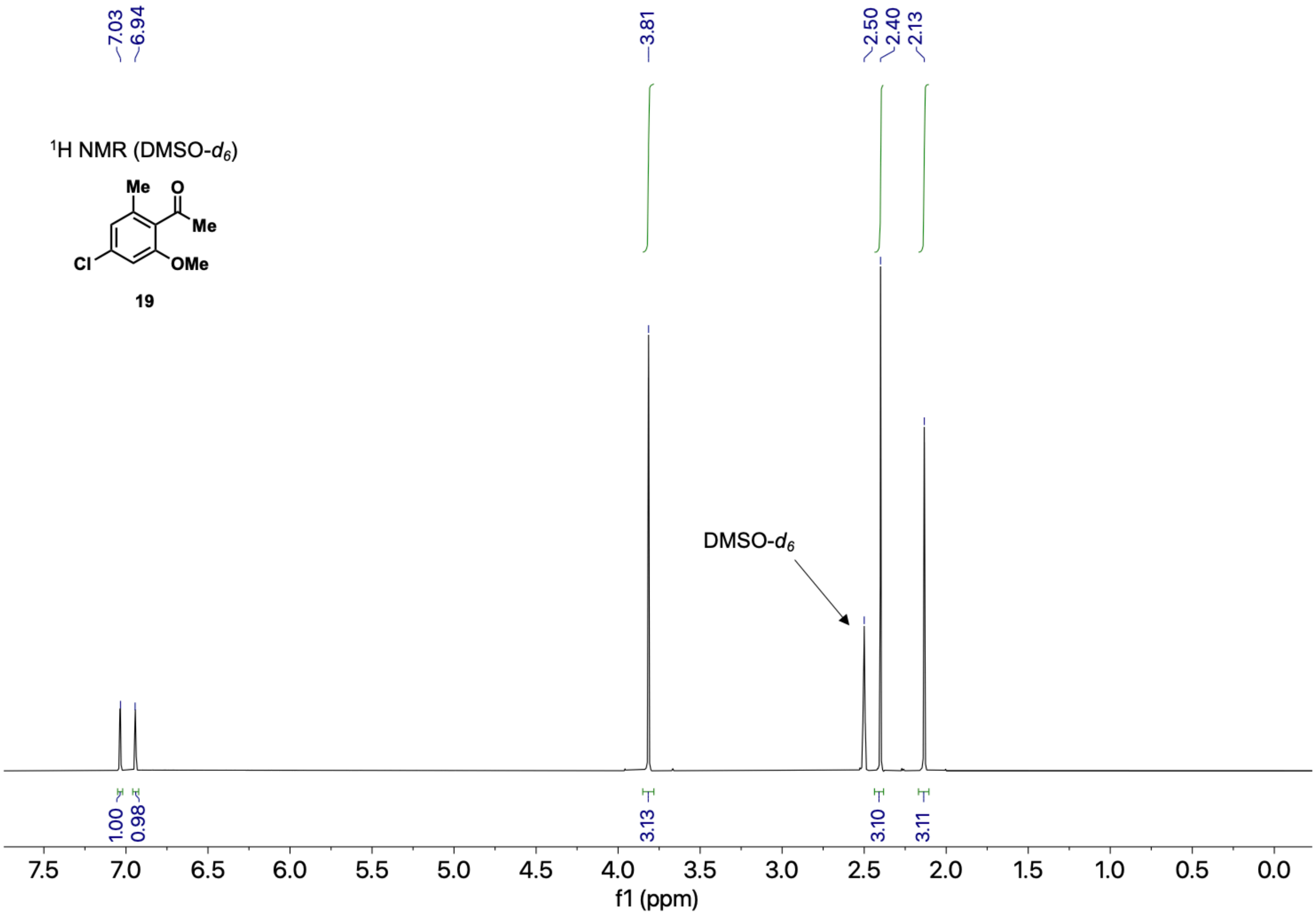
156.21
140.37
133.36
124.89
124.69
115.44
70.82
63.25
37.06
32.19
21.75
8.20
^{13}C NMR (CDCl$_3$)
Me
OH
Et
Cl
O
16
CDCl$_3$
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

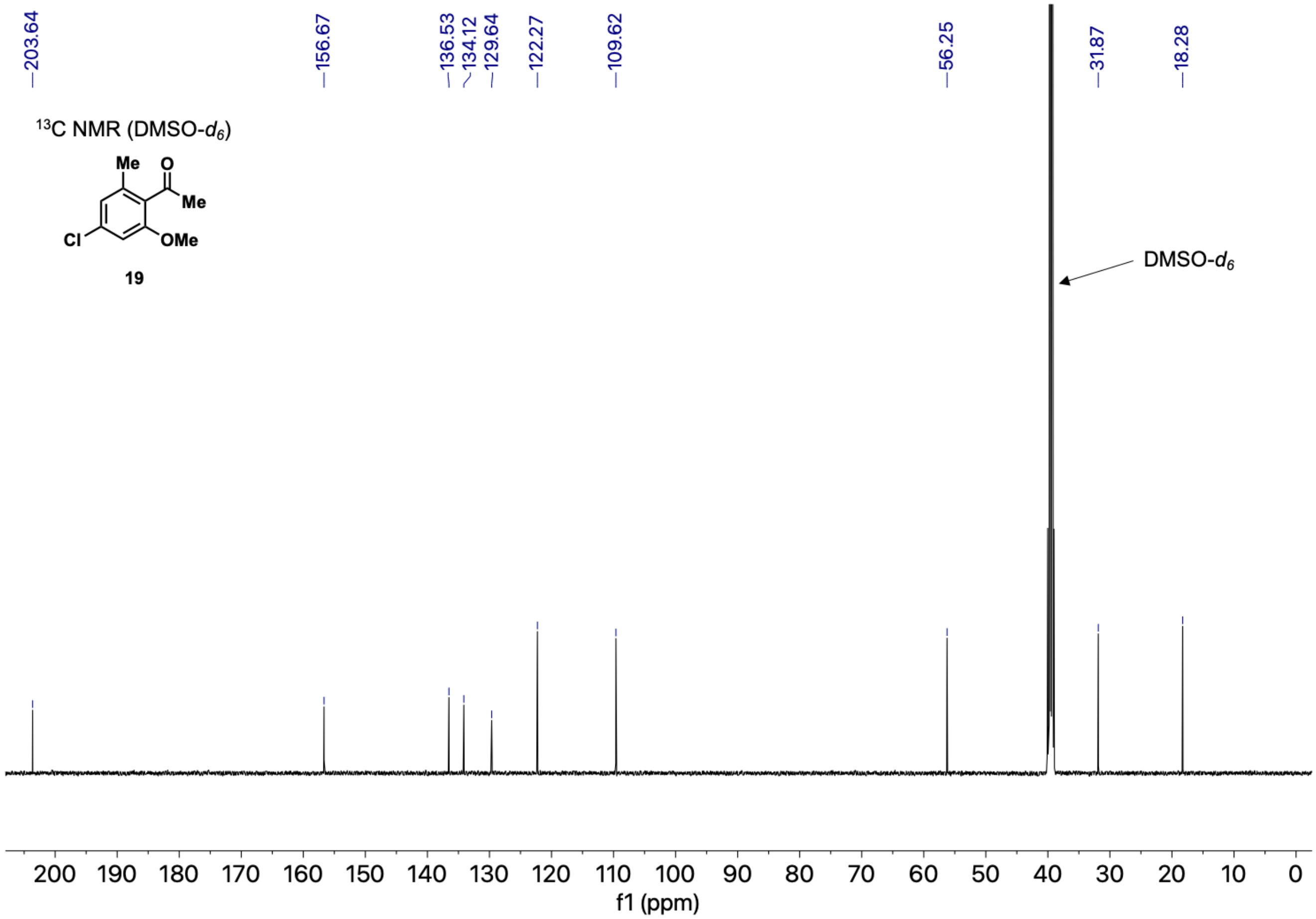

6.71
6.71
6.65
6.64
4.93
4.25
4.14
2.53
2.50
2.02
1.93
1.15
1.10
0.81
0.76
0.59
0.53
0.36
0.31
0.27
0.22
^{1}H NMR (DMSO-d_6)
Me
OH
Cl
O
17
DMSO-d_6
1.00
0.94
1.07
2.02
3.10
2.04
1.02
1.05
1.13
2.15
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

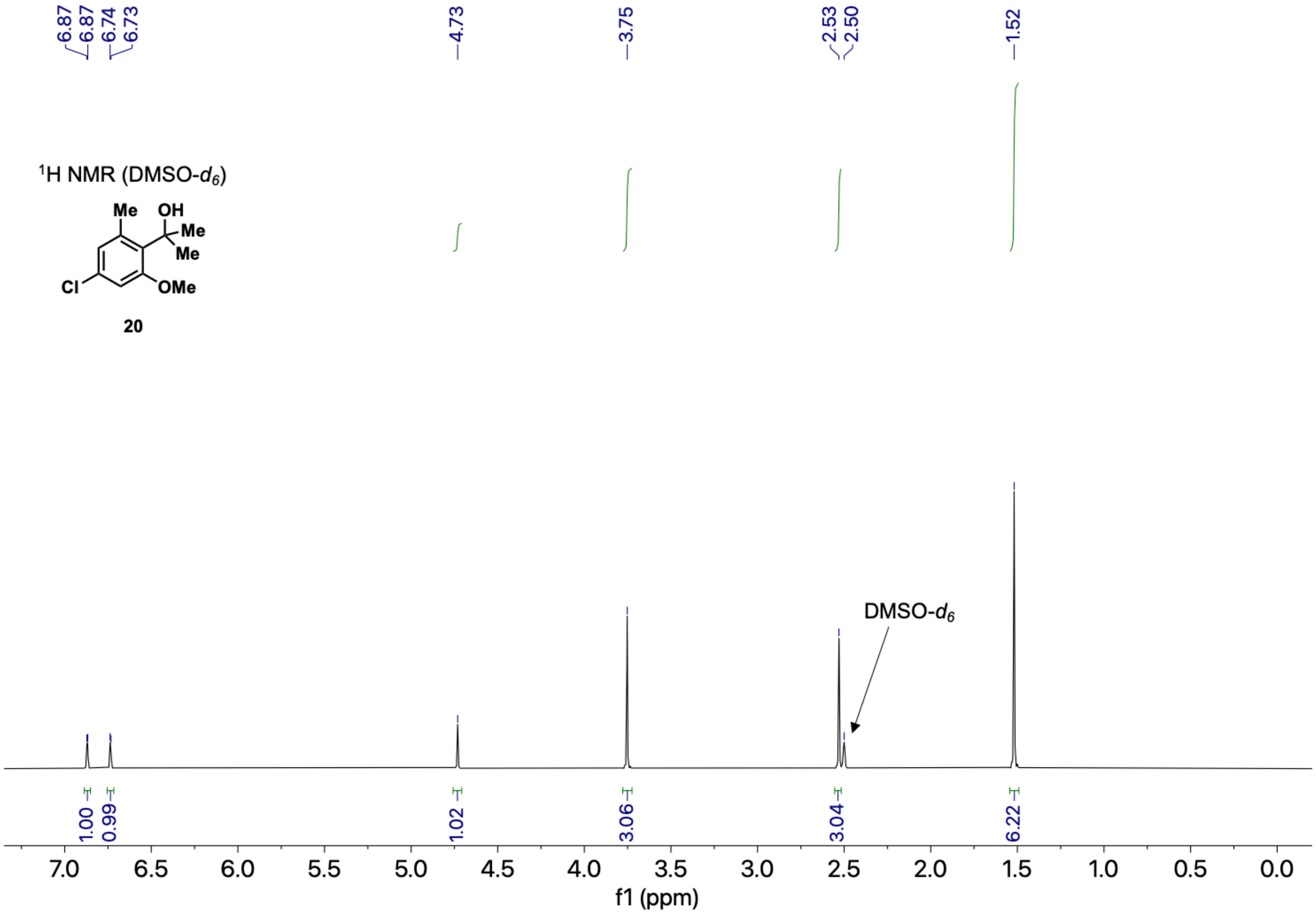

154.29
140.21
130.14
126.04
122.01
112.94
65.60
61.98
37.56
21.05
19.81
3.54
0.50
^{13}C NMR (DMSO-d_6)
Me
OH
Cl
O
17
DMSO-d_6
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

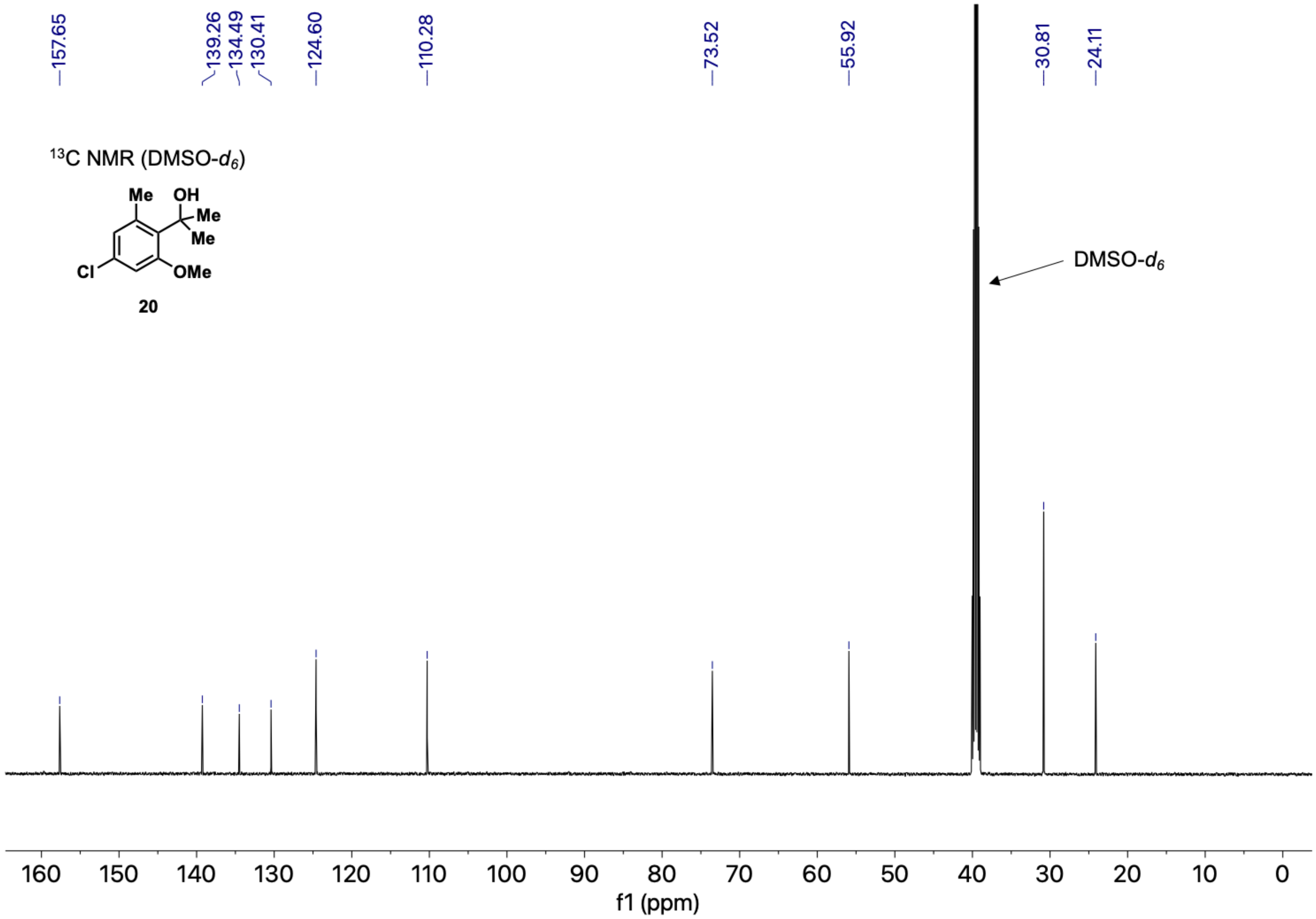

7.29
7.28
7.24
7.22
7.21
6.80
6.72
6.71
5.93
4.20
4.19
4.17
4.15
4.15
4.06
4.05
4.05
4.04
4.03
4.03
4.02
2.50
2.20
2.18
2.18
2.17
2.15
2.15
2.06
2.05
2.03
2.01
2.00
1.78
^{1}H NMR (DMSO-d_6)
Me
OH
Cl
O
18
DMSO-d_6
5.32
0.91
0.99
0.97
1.09
1.04
1.09
1.09
2.97
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

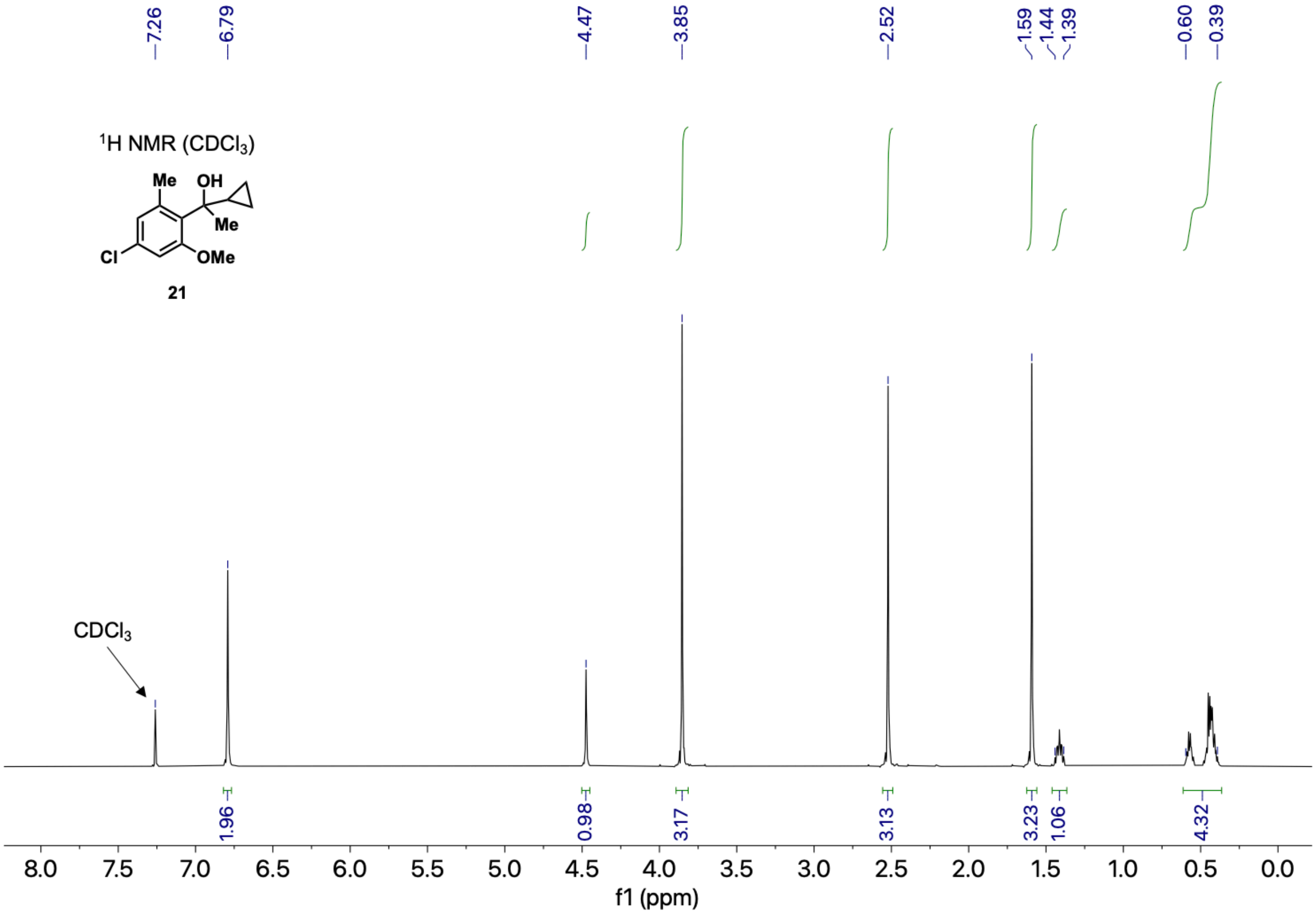

156.80
148.99
142.15
132.50
128.39
126.75
126.25
125.66
123.80
114.68
70.56
62.92
43.47
21.25
^{13}C NMR (DMSO-d_6)
Me
OH
Cl
O
18
DMSO-d_6
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.03
6.94
3.81
2.50
2.40
2.13
^{1}H NMR (DMSO-d_6)
Me
O
Me
Cl
OMe
19
DMSO-d_6
1.00
0.98
3.13
3.10
3.11
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

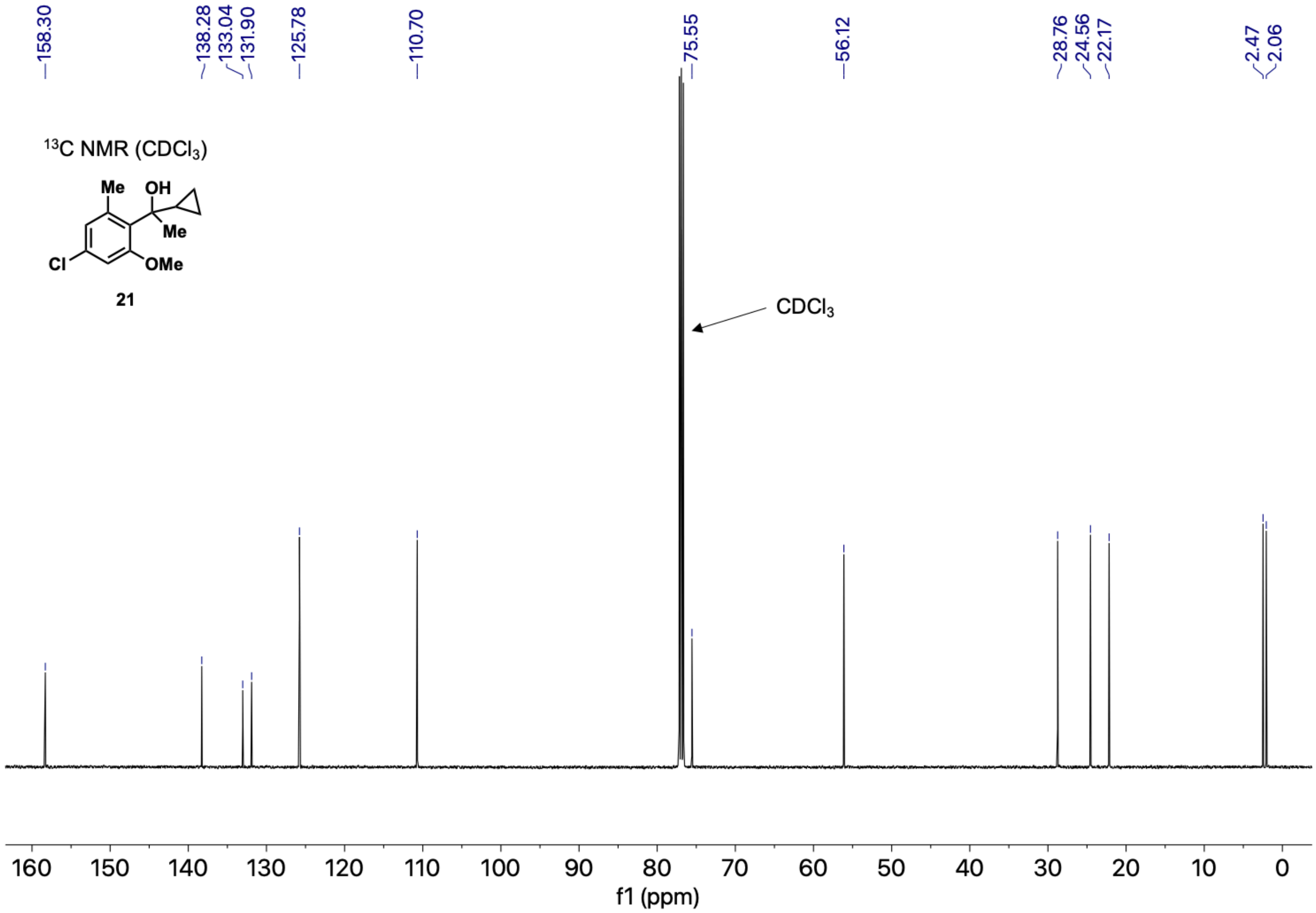

203.64
156.67
136.53
134.12
129.64
122.27
109.62
56.25
31.87
18.28
^{13}C NMR (DMSO-d_6)
Me
O
Me
Cl
OMe
19
DMSO-d_6
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

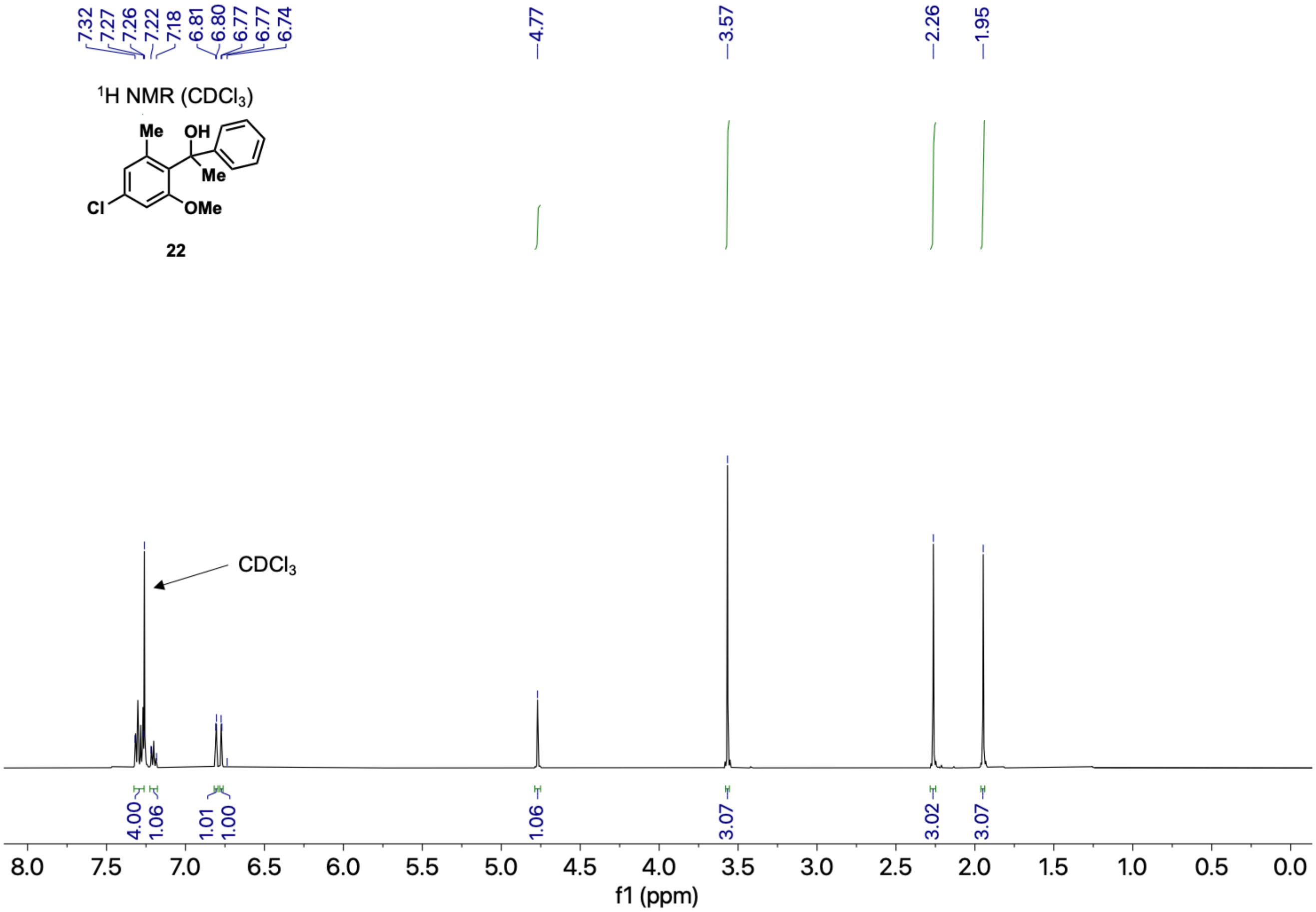

6.87
6.87
6.74
6.73
4.73
3.75
2.53
2.50
1.52
^{1}H NMR (DMSO-d_6)
Me
OH
Me
Me
Cl
OMe
20
DMSO-d_6
1.00
0.99
1.02
3.06
3.04
6.22
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

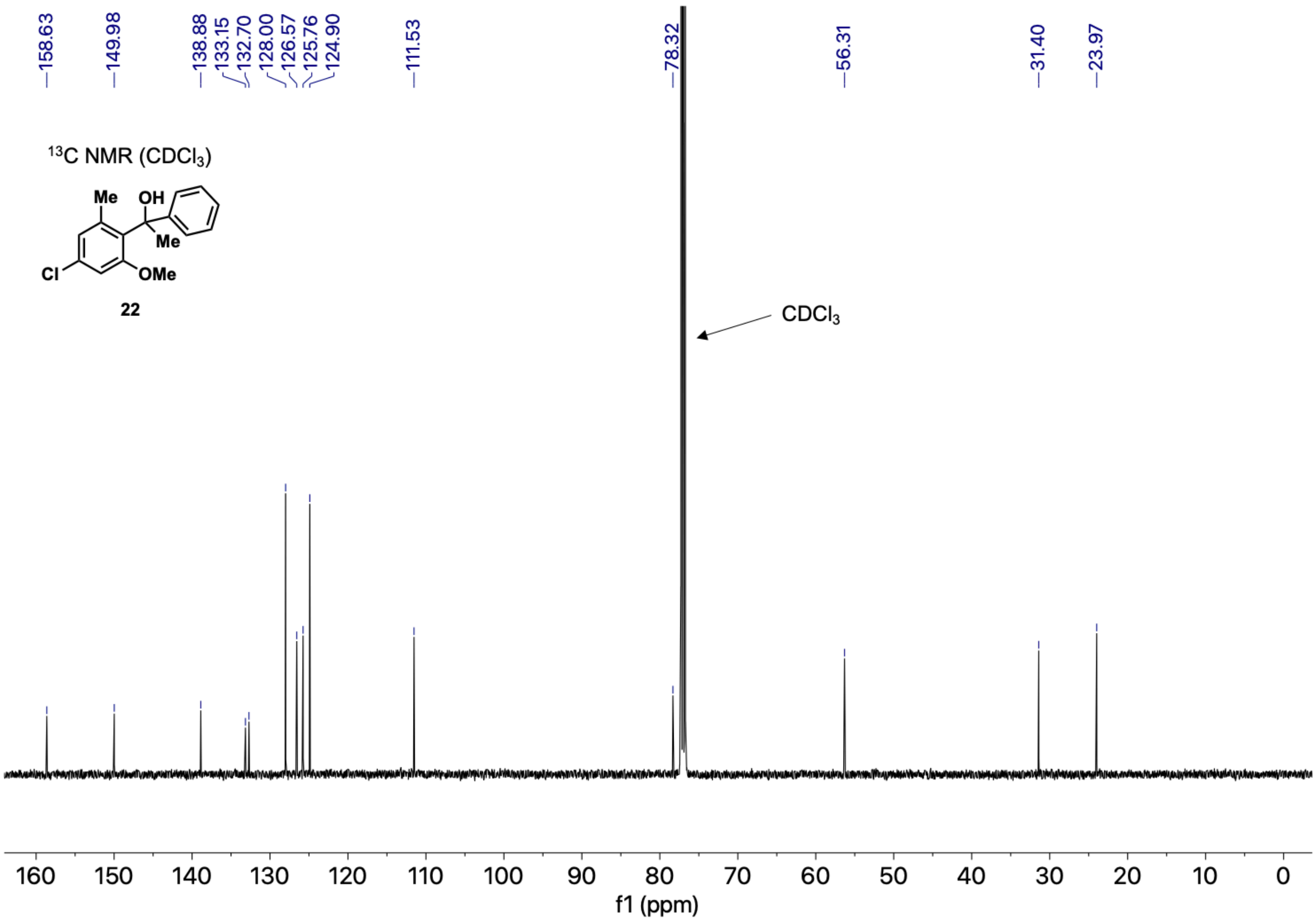

157.65
139.26
134.49
130.41
124.60
110.28
73.52
55.92
30.81
24.11
^{13}C NMR (DMSO-d_6)
Me
OH
Me
Me
Cl
OMe
20
DMSO-d_6
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



^{1}H NMR (CDCl$_3$)
Me
OH
Me
Cl
OMe
21
CDCl$_3$
7.26
6.79
4.47
3.85
2.52
1.59
1.44
1.39
0.60
0.39
1.96
0.98
3.17
3.13
3.23
1.06
4.32
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

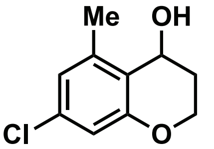
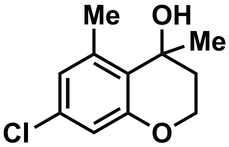
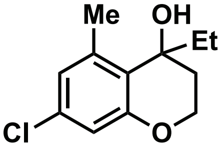
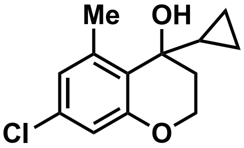
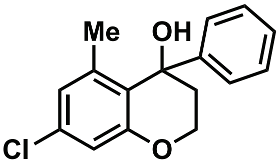

158.30
138.28
133.04
131.90
125.78
110.70
75.55
56.12
28.76
24.56
22.17
2.47
2.06
^{13}C NMR (CDCl$_3$)
Me
OH
Me
Cl
OMe
21
CDCl$_3$
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.32
7.27
7.26
7.22
7.18
6.81
6.80
6.77
6.77
6.74
4.77
3.57
2.26
1.95
^{1}H NMR (CDCl$_3$)
Me
OH
Me
Cl
OMe
22
CDCl$_3$
4.00
1.06
1.01
1.00
1.06
3.07
3.02
3.07
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


158.63
149.98
138.88
133.15
132.70
128.00
126.57
125.76
124.90
111.53
78.32
56.31
31.40
23.97
^{13}C NMR ($CDCl_3$)
Me
OH
Me
Cl
OMe
22
$CDCl_3$
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

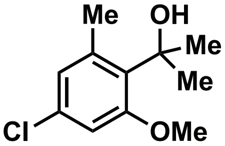
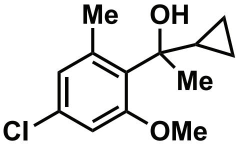
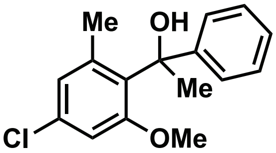
Supplementary Tables

Table S1. Affinity and LE of compounds 14-18

Compound	Structure	SPR K_d(μM) - AMP-PNP [a] SPR K_d(μM) + AMP-PNP	LE [b]
14		> 1 mM > 1 mM	—
15		> 1 mM > 1 mM	—
16		> 1 mM > 1 mM	—
17		> 1 mM > 1 mM	—
18		367 ± 23 717 ± 46	0.25

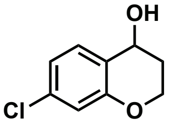
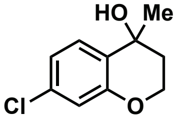
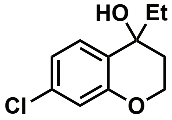
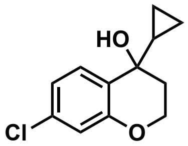
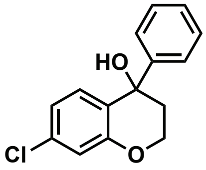
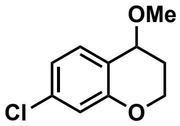
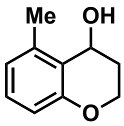
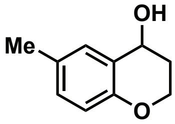
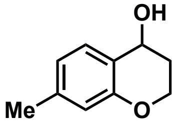
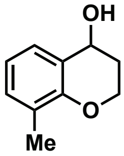
[a] K_d values shown represent the mean ± SEM of two determinations. [b] LE = 1.37pK_d/HA (heavy atom count).

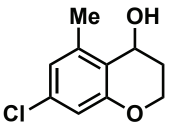
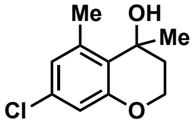
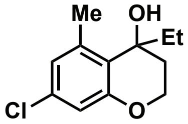
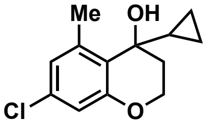
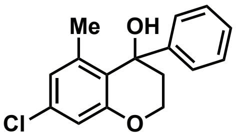
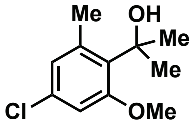
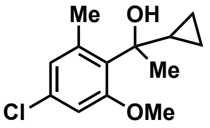
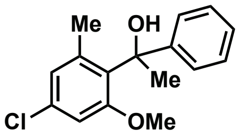
Table S2. Affinity and LE of compounds 20-22

Compound	Structure	SPR K_d(µM) - AMP-PNP [a] SPR K_d(µM) + AMP-PNP	LE [b]
20		> 1 mM > 1 mM	—
21		150 ± 53 143 ± 83	0.33
22		> 1 mM > 1 mM	—

[a]K_d values shown represent the mean ± SEM of two determinations. [b]LE = 1.37pK_d/HA (heavy atom count).

Table S3. Structures, HLB numbers, and SMILES of compounds 1, 5-18, 20-22

Compound	Structure	HLB Number	SMILES
1		HLB-0522184	OC2CCOc1cc(Cl)ccc12
5		HLB-0535152	CC2(O)CCOc1cc(Cl)ccc12
6		HLB-0535154	CCC2(O)CCOc1cc(Cl)ccc12
7		HLB-0535143	OC3(C1CC1)CCOc2cc(Cl)ccc23
8		HLB-0535139	OC3(c1ccccc1)CCOc2cc(Cl)ccc23
9		HLB-0535153	COC2CCOc1cc(Cl)ccc12
10		HLB-0535140	Cc1cccc2OCCC(O)c12
11		HLB-0535141	Cc2ccc1OCCC(O)c1c2
12		HLB-0535142	Cc1ccc2c(c1)OCCC2O
13		HLB-0535144	Cc1cccc2c1OCCC2O

Compound	Structure	HLB Number	SMILES
14	 	HLB-0535157	Cc1cc(Cl)cc2OCCC(O)c12
15	 	HLB-0535155	Cc1cc(Cl)cc2OCCC(C)(O)c12
16	 	HLB-0535156	CCC2(O)CCOc1cc(Cl)cc(C)c12
17	 	HLB-0535158	Cc2cc(Cl)cc3OCCC(O)(C1CC1)c23
18	 	HLB-0535159	Cc2cc(Cl)cc3OCCC(O)(c1ccccc1)c23
20	 	HLB-0535160	COc1cc(Cl)cc(C)c1C(C)(C)O
21	 	HLB-0535161	COc1cc(Cl)cc(C)c1C(C)(O)C2CC2
22	 	HLB-0535162	COc1cc(Cl)cc(C)c1C(C)(O)c2ccccc2

HLB (Harki Lab) numbers are unique molecular identifiers for molecules reported by the Harki group and can be referenced in material transfer requests.

Assay Procedures

Fragment Library

The fragment library was purchased from Life Chemicals high-solubility fragment collection and consisted of 1056 fragments. The fragments were dissolved at 200 mM in DMSO-*d₆* and dispensed into master plates. A portion of the 200 mM stocks was used to pool mixtures of 5 fragments at 40 mM in DMSO-*d₆* (fragments were pooled by chemical compatibility).

NIK Protein for Assays

Human NIK protein was purchased from GenScript at ≥ 80% purity (residues 336-686 with N-terminal 6xHis-tag and TEV cleavage site). The storage buffer for NIK protein consisted of 20 mM Tris (pH = 8.0), 300 mM NaCl, 40% glycerol, 0.25 mM TCEP. The 6xHis-tag was kept intact for all experiments. For CPMG NMR, the protein was directly diluted into buffer. For SPR, NIK was buffer exchanged into 40 mM KH_2PO_4/K_2HPO_4 (pH = 8), 100 mM KCl, 10 mM $MgCl_2$, 0.01% Tween-20 using Amicon Ultra-0.5 centrifugal filter units (MilliporeSigma, 3 kDa filter). NIK concentration was measured on a NanoDrop 2000 spectrophotometer (Thermo Scientific). EZ-Link NHS-PEG4-Biotin (Thermo Scientific) was dissolved in H_2O and added to the NIK protein at a 1:1 ratio. The mixture was incubated overnight at 4°C. The mixture was buffer exchanged into 40 mM KH_2PO_4/K_2HPO_4 (pH = 8.0), 100 mM KCl, 10 mM $MgCl_2$, 0.01% Tween-20 using Amicon Ultra-0.5 centrifugal filter units to remove the excess EZ-Link NHS-PEG4-Biotin reagent. The biotinylated NIK protein concentration was measured on a NanoDrop 2000 spectrophotometer and diluted into running buffer to be used for all SPR assays.

CPMG NMR – General Parameters

One-dimensional ^{1}H-NMR binding assays using standard CPMG pulse sequence with T_2 filtering[4] were run at the Minnesota NMR Center on a 700-MHz Bruker Avance I spectrometer equipped with a SampleJet autosampler. Assays were performed to measure the binding of fragments to the catalytic domain of human NIK. Buffer conditions used were 50 mM KH_2PO_4/K_2HPO_4 (pH = 8), 300 mM KCl, 10 mM $MgCl_2$, 10% D_2O in H_2O. All samples were made by diluting the fragments/pooled fragments (in DMSO-*d₆*) into buffer. The samples were run in 1.7 mm BioSpin NMR tubes (Bruker) at room temperature.

CPMG NMR – Screening Assay

While following the general parameters described above, in these experiments AMP-PNP (MilliporeSigma, 10102547001) was added to saturate the NIK orthosteric binding site. The autosampler was cooled to 4°C during the duration of the experiments to ensure protein stability while samples were waiting to be run. Two separate samples were made to record two spectra for each of the fragment mixtures. One sample contained five fragments at 100 μM concentration in buffer. The other sample contained five fragments at 100 μM, NIK protein at 10 μM, and AMP-PNP at 1 mM in buffer. Fragments were considered to be hits if binding was observed (approximately 50% signal attenuation or greater).

CPMG NMR – Competition Assay

While following the general parameters described above, in these experiments the fragments were run individually and three spectra were recorded for each fragment: 1) Fragment at 100 μM in buffer, 2) Fragment at 100 μM and NIK at 10 μM in buffer, 3) Fragment at 100 μM, NIK at 10 μM, and AMP-PNP at 1 mM in buffer. Fragments were considered hits if binding was observed, but competition with AMP-PNP was not observed (approximately 50% signal attenuation or greater for both 2 and 3 and greater signal attenuation for 3 than 2).

SPR Screening Assay

Surface plasmon resonance (SPR) was performed at the UMN Institute of Therapeutic Drug Discovery and Development (ITDD) High-Throughput Screening Laboratory. A Series S NeutrAvidin-coated sensor chip (Cytiva, 29407997) was docked into a Biacore S200 instrument. The running buffer consisted of 10 mM HEPES (pH = 7.4), 150 mM NaCl, 10 mM $MgCl_2$, 3 mM DTT, 0.5 mg/mL γ-globulins, 0.005% (v/v) Tween20, 5% DMSO (when testing small molecules). Biotinylated human NIK protein at 100 μg/mL was injected over flow-cells 2 and 4 (flow-cells 1 and 3 were used for reference subtraction) with a contact time of 600 sec at a flow-rate of 5 μL/min. Observed immobilization levels were 5000-8000 response units (RU). To block the remaining NeutrAvidin sites, 100 μM solution of biocytin was injected over all flow-cells with a contact time of 300 sec at a flow-rate of 10 μL/min. Compounds were run at a top concentration of 500 μM. 2-fold serial dilutions were made from the top concentration to obtain 5-dose response series for screening and 7 or 9 dose-response series for confirming hits and testing analogues. 1 mM AMP-PNP was added to compound containing wells when determining affinities in the presence of AMP-PNP. The activity of the chip surface was monitored by including reference compound staurosporine (pan-kinase inhibitor) first and last in each run. The assay was stable for one day at room temperature. K_d values were determined using Biacore software with a steady-state fit. The mean ± SEM was calculated using Microsoft Excel (version 16.76).

qNMR Solubility Assay

Solubility assay was set up using a modification of a published protocol.[5] A sample of 1 mM of maleic acid and 5 mM of **5** was made in assay buffer consisting of 50 mM K_2HPO_4 (pH = 7.4) in D_2O with 5% DMSO-d_6. A qNMR pulse sequence was run where D1 was set to 60 s with 256 scans using a 90° pulse. The NMR spectra was worked up in MestReNova (version 14.2.2). Integration of the peaks correlated with **5** were compared to the known concentration standard of maleic acid and the concentration of **5** in solution was determined.

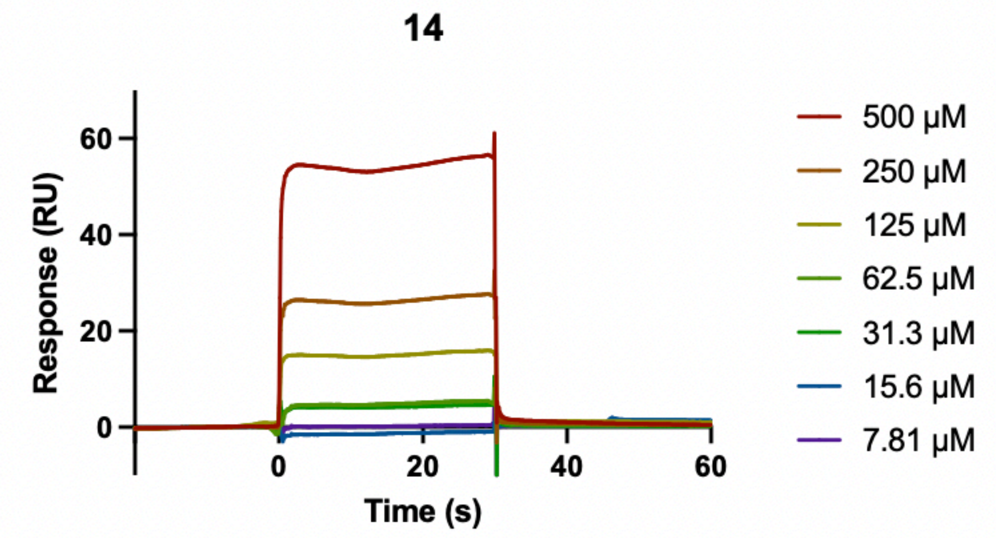
NMR Aggregation Assay

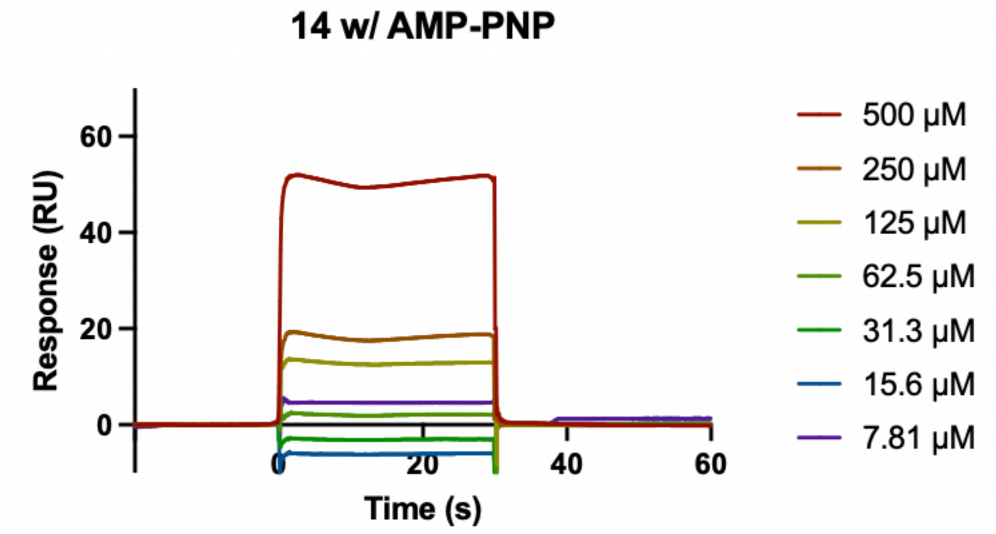
Aggregation assay was set up using a published protocol.[6] The data was worked up in MestReNova (version 14.2.2).

Resazurin Redox Assay

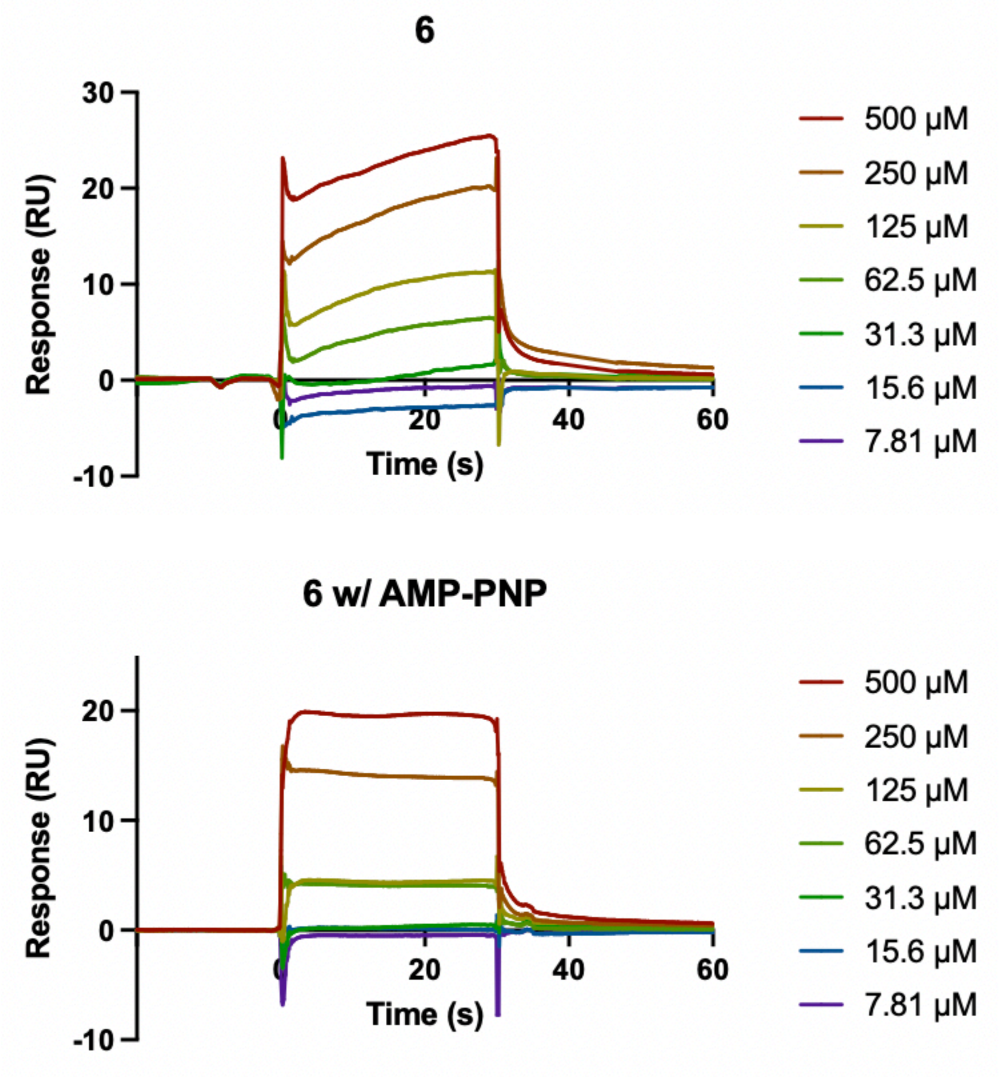
Resazurin redox assay was set up using a published protocol.[7] The data was worked up in Microsoft Excel (version 16.76) and graphed in Prism (version 9.3.1).

SPR Sensorgrams

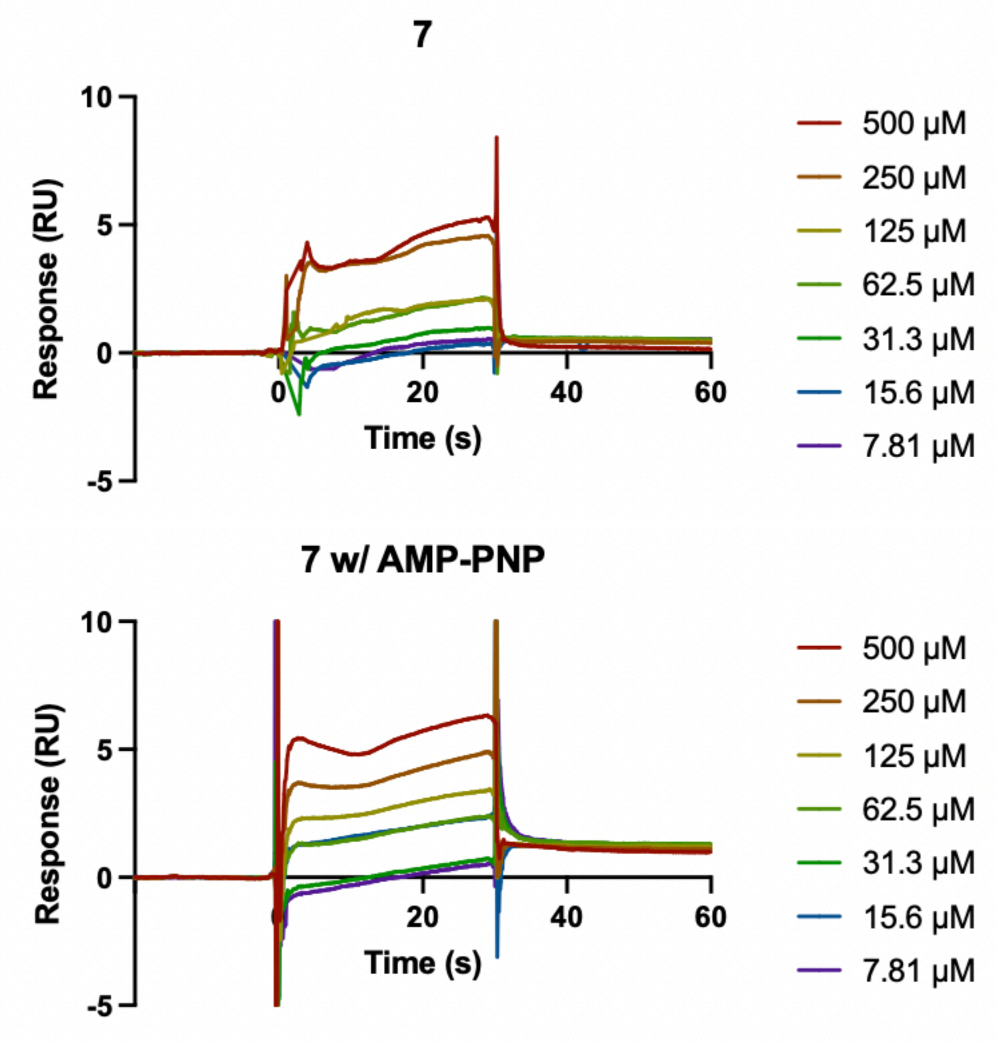

14


14 w/ AMP-PNP

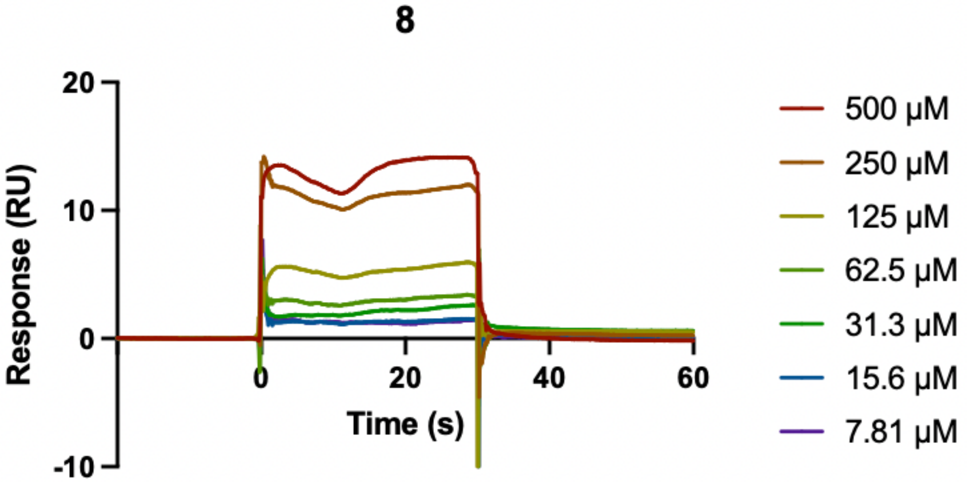
Representative sensorgrams of **14** with and without AMP-PNP (one replicate shown, but two replicates were generated). This sensorgram is representative of all compounds that did not generate a K_d of less than 1 mM.

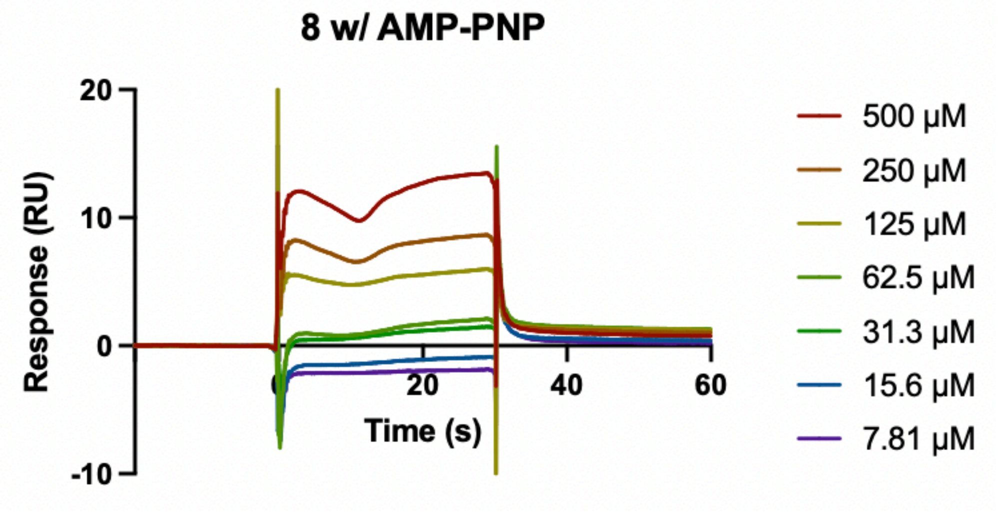


Representative sensorgrams of **6** with and without AMP-PNP (one replicate shown, but two replicates were generated).

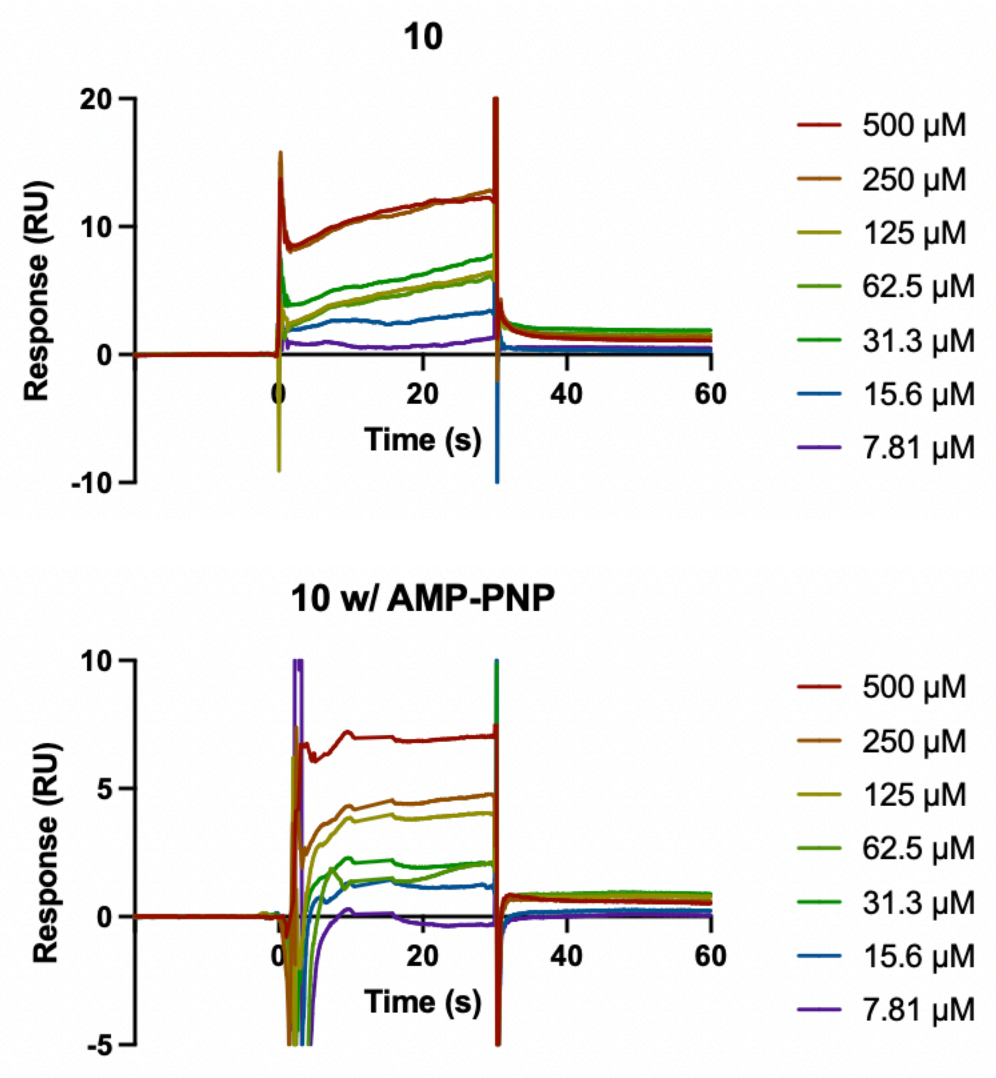


Representative sensorgrams of **7** with and without AMP-PNP (one replicate shown, but two replicates were generated).

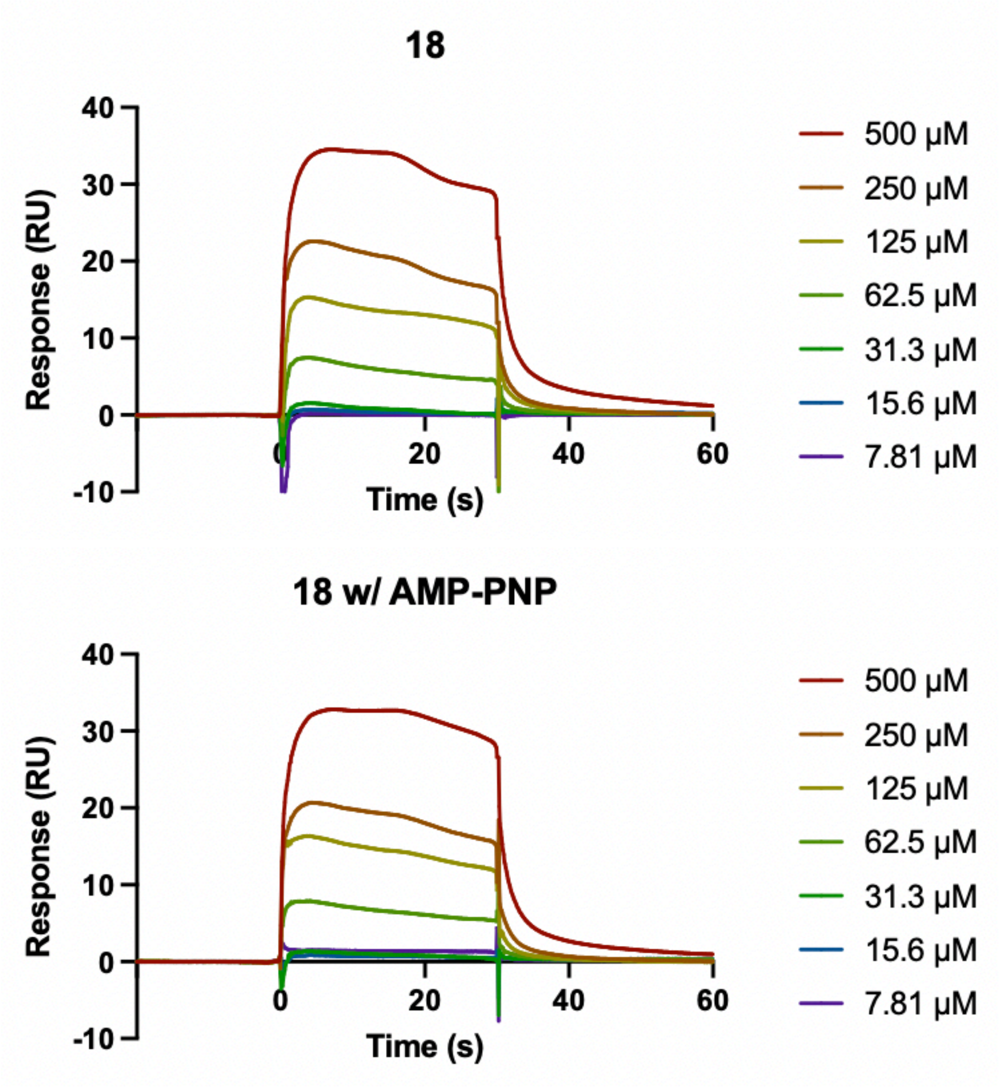




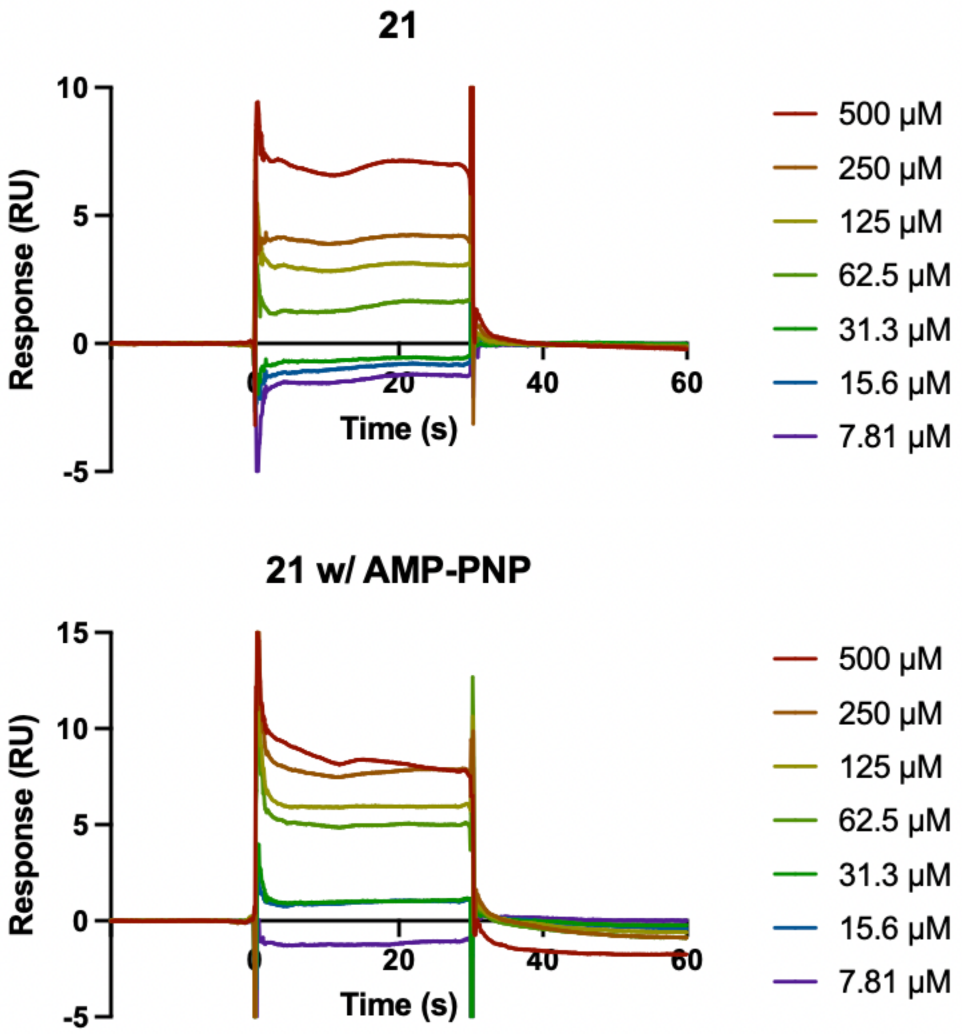
Representative sensorgrams of **8** with and without AMP-PNP (one replicate shown, but two replicates were generated).



Representative sensorgrams of **10** with and without AMP-PNP (one replicate shown, but two replicates were generated).



Representative sensorgrams of **18** with and without AMP-PNP (one replicate shown, but two replicates were generated).



Representative sensorgrams of **21** with and without AMP-PNP (one replicate shown, but two replicates were generated).

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