

Substituent Effects on the Sensitivity of a Quinoline Photoremovable Protecting Group to One- and Two-Photon Excitation

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Synthetic Procedures

General. All reagents and solvents were purchased and used without further purification. Moisture sensitive reactions were carried out using flame or oven-dried glassware and inert atmospheres were maintained using gas bubblers. Na₂SO₄ was used to dry solvent extracts. Pyridine was distilled over CaH₂. Tetrahydrofuran (THF), benzene, and toluene were dried by passing the solvents through activated alumina under positive nitrogen pressure before use. ¹H NMR and ¹³C NMR spectra were recorded on a 400 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. HPLC analyses were performed using Microsorb C-18 reverse phase columns, an autosampler, and a diode array detector. Mass spectrometry was performed on a quadrupole mass spectrometer with an electrospray ionization source. Electrospray ionization was used for HRMS. KMOPS buffer consisted of 100 mM KCl and 10 mM MOPS solution titrated with KOH to pH 7.2, with additional DTT (5 mM) for thiol experiments. Thin layer and column chromatography were performed on precoated silica gel 60 F₂₅₄ plates and 230-400 mesh silica gel 60, respectively.

7-(Methoxymethoxy)-8-nitroquinaldine (8a). Triethylamine (174 μ L, 1.695 mmol) was added to a solution of **5a** (174 mg, 0.847 mmol) in dry THF (5 mL) under a nitrogen atmosphere. Chloromethyl methyl ether (128 μ L, 1.695 mmol) was added to the mixture dropwise and the reaction was stirred for 1 h. The reaction was diluted with chloroform, washed with water followed by brine. The organic layer was dried over Na₂SO₄, and the solvent was evaporated to afford **8a** (200 mg, 0.806 mmol, 95%) as a bright yellow solid: ¹H NMR (CDCl₃) δ 8.00 (1H, d, J = 8.4 Hz), 7.81 (1H, d, J = 9.2 Hz), 7.47 (1H, d, J = 8.8 Hz), 7.26 (1H, d, J = 8.4 Hz), 5.35 (2H, s), 3.52 (3H, s), 2.69 (3H, s); ¹³C NMR (CDCl₃) δ 162.5, 148.3, 140.3, 137.7, 135.7, 130.3, 122.2, 122.2, 115.4, 95.3, 57.0, 25.9; FTIR (neat) 2955, 1626, 1525, 1508, 1377, 1247, 1226, 1193, 1153, 1065, 991, 924, 835, 806, 656 cm⁻¹; MS (ESI) m/z calcd for (C₁₂H₁₂N₂O₄ + H)⁺ 249, found 249; HRMS (ESI) m/z calcd for (C₁₂H₁₂N₂O₄ + H)⁺ 249.0875, found 249.0871.

7-(Methoxymethoxy)-8-nitroquinolin-2-yl formaldehyde (9a). Under a nitrogen atmosphere, selenium dioxide (11 mg, 0.099 mmol) was added to a solution of **8a** (22.1 mg, 0.089 mmol) in dioxane (2 mL) at 85 °C. The reaction was stirred for 20 h at 85 °C, then cooled, diluted with methanol, and vacuum filtered. The filtrate was collected and concentrated, leaving a yellow solid, which was purified by column chromatography (4:6 EtOAc/hexane) to provide **9a** (13.7 mg, 0.0523 mmol, 59%) as a yellow solid: ¹H NMR (CDCl₃) δ 10.15 (1H, s), 8.35 (1H, d, *J* = 8.4 Hz), 8.02 (1H, d, *J* = 8 Hz), 8.00 (1H, d, *J* = 9.6 Hz), 7.76 (1H, d, *J* = 9.2 Hz), 5.43 (2H, s), 3.56 (3H, s); ¹³C NMR (CDCl₃) δ 193.25, 154.14, 149.28, 140.47, 137.83, 137.55, 130.64, 125.26, 119.33, 117.34, 95.45, 57.24; FTIR (neat) 2858, 1715, 1529, 1263, 1197, 1155, 1061, 935, 918, 849, 806, 771, 658 cm⁻¹; MS (ESI) *m/z* calcd for (C₁₂H₁₀N₂O₅ + H)⁺ 263, found 263; HRMS (ESI) *m/z* calcd for (C₁₂H₁₀N₂O₅ + H)⁺ 263.0668, found 263.0662.

7-(Methoxymethoxy)-8-nitroquinolin-2-yl methanol (10a). NaBH₄ was added to a solution of **9a** (18.1 mg, 0.0691 mmol) in absolute EtOH (5 mL). The reaction mixture was stirred for 15 min, diluted with chloroform, washed with water followed by brine. The organic layer was dried over Na₂SO₄ and the solvent was evaporated to afford **10a** (8.3 mg, 0.032 mmol, 45%) as a yellow solid: ¹H NMR (CDCl₃) δ 8.14 (1H, d, *J* = 8.8 Hz), 7.90 (1H, d, *J* = 9.2 Hz), 7.58 (1H, d, *J* = 9.2 Hz), 7.31 (1H, d, *J* = 8.4 Hz), 5.39 (2H, s), 4.91 (2H, s), 3.54 (3H, s); ¹³C NMR (CDCl₃) δ 162.4, 149.0, 139.1, 136.7, 131.9, 130.6, 123.1, 118.5, 116.3, 95.4, 64.5, 57.1; FTIR (neat) 3583, 2923, 1635, 1516, 1263, 1157, 1084, 1047, 931, 916, 804 cm⁻¹; MS (ESI) *m/z* calcd for (C₁₂H₁₂N₂O₅ + H)⁺ 265, found 265; HRMS (ESI) *m/z* calcd for (C₁₂H₁₂N₂O₅ + H)⁺ 265.0824, found 265.0814.

7-(Methoxymethoxy)-8-nitroquinolin-2-yl methyl acetate (11a). DMAP (10.2 mg, 0.083 mmol) and acetic anhydride (15 μL, 0.16 mmol) were added to a solution of **10a** (8.3 mg, 0.032 mmol) in chloroform (4 mL) and pyridine (1 mL) under a nitrogen atmosphere. The reaction was stirred for 18 h. The solvent was evaporated and the residue was purified by column chromatography (3:7 EtOAc/hexane) to afford **11a** (7.6 mg, 0.025 mmol, 78%) as a yellow solid: ¹H NMR (CDCl₃) δ 8.16 (1H, d, *J* = 8.4 Hz), 7.88 (1H, d, *J* = 9.2 Hz), 7.56 (1H, d, *J* = 9.2 Hz), 7.44 (1H, d, *J* = 8.4 Hz), 5.37 (2H, s), 5.36 (2H, s), 3.53 (3H, s), 2.20 (3H, s); ¹³C NMR (CDCl₃) δ 170.8, 159.4, 148.7, 140.0, 137.7, 136.7, 130.4, 123.0, 119.0, 116.6, 95.3, 67.0, 57.1, 21.1; FTIR (neat) 2932, 1747, 1654, 1543, 1386, 1218, 1073, 810 cm⁻¹; MS (ESI) *m/z* calcd for (C₁₄H₁₄N₂O₆ + H)⁺ 307, found 307; HRMS (ESI) *m/z* calcd for (C₁₄H₁₄N₂O₆ + H)⁺ 307.0930, found 307.0930.

7-Hydroxy-8-nitroquinolin-2-yl methyl acetate (NHQ-OAc, 1a). One drop of 12 N HCl was added to a solution of **11a** (7.0 mg, 0.023 mmol) in methanol (5 mL). The reaction was stirred for 10 min at room temperature followed by evaporation of the solvent. The resulting residue was taken up in chloroform, and the resulting clear solution was decanted and concentrated. The resulting residue was purified by column chromatography (1:1 EtOAc/hexane) to afford **NHQ-OAc** (3.0 mg, 0.0115 mmol, 50%) as a dark orange solid: ¹H NMR (DMSO-*d*₆) δ 8.42 (1H, d, *J* = 8.4 Hz), 8.04 (1H, d, *J* = 9.2 Hz), 7.48 (1H, d, *J* = 8.4 Hz), 7.41 (1H, d, *J* = 8.8 Hz), 5.27 (2H, s), 2.15 (3H, s); ¹³C NMR (DMSO-*d*₆) 170.1, 158.5, 149.8, 139.5, 137.1, 133.9, 130.7, 120.7, 118.9, 117.7, 66.0, 20.5; FTIR (neat) 3076, 2922, 1740, 1637, 1527, 1506, 1450, 1375, 1342, 1304, 1213, 1140, 1063, 1028, 895, 844, 794, 656 cm⁻¹; MS (ESI) *m/z* calcd for (C₁₂H₁₀N₂O₅ + H)⁺ 263, found 263; HRMS (ESI) *m/z* calcd for (C₁₂H₁₀N₂O₅ + H)⁺ 263.0668, found 263.0651.

8-Chloro-7-methoxymethylquinoline (8c). Triethylamine (1.879 mL, 13.49 mmol) was added to a solution of **5c** (0.2612 g, 1.349 mmol) in THF (20 mL) under a nitrogen atmosphere. Chloromethyl methyl ether (0.241 mL, 3.17 mmol) was added to the mixture dropwise. After stirring for 2 h, the solution was diluted with EtOAc and washed successively with water (×2) and brine. The organic layer was dried over MgSO₄, filtered, and evaporated. The resulting residue was purified by flash chromatography (3:7 EtOAc/hexane) to afford **8c** (0.2298 g, 0.9668 mmol, 72%) as a yellow solid (mp 78–79 °C): ¹H NMR (CDCl₃) δ 8.01 (1H, d, *J* = 8.8 Hz), 7.67 (1H, d, *J* = 8.8 Hz), 7.47 (1H, d, *J* = 9.2 Hz), 7.26 (1H, d, *J* = 8.8 Hz), 5.40 (2H, s), 3.58 (3H, s), 2.82 (3H, s); ¹³C NMR (CDCl₃) δ 160.9, 153.8, 145.4, 136.6, 126.9, 123.5, 121.4, 116.7, 105.0, 95.7, 56.8, 26.0; FTIR (neat) 2919, 1611, 1508, 1259, 1011, 833, 783 cm⁻¹; MS (ESI) *m/z* calcd for (C₁₂H₁₂ClNO₂ + H)⁺ 238 (³⁵Cl) and 240 (³⁷Cl), found 238 (³⁵Cl) and 240 (³⁷Cl); HRMS (ESI) *m/z* calcd for (C₁₂H₁₂ClNO₂ + H)⁺ 238.0635 (³⁵Cl) and 240.0605 (³⁷Cl), found 238.0623 (³⁵Cl) and 240.0593 (³⁷Cl).

8-Chloro-2-formyl-7-methoxymethylquinoline (9c). Under a nitrogen atmosphere, selenium dioxide (0.0604 g, 0.544 mmol) was added to a solution of **8c** (0.1232 g, 0.5184 mmol) in *p*-dioxane (3 mL), and the resulting orange solution was stirred in a sealed flask for 2 h at 85 °C. The black precipitate was removed by vacuum filtration and the filtrate was evaporated, leaving a residue, which was purified by column chromatography (2:8 EtOAc/hexane) to afford **9c** (0.0724 g, 0.288 mmol, 56%) as a pale yellow solid (mp 118–121 °C): ¹H NMR (CDCl₃) δ 10.30 (1H, s), 8.30 (1H, d, *J* = 8.0 Hz), 7.99 (1H, d, *J* = 8.0 Hz), 7.81 (1H, d, *J* = 9.6 Hz), 7.70 (1H, d, *J* = 8.8 Hz), 5.45 (2H, s), 3.60 (3H, s); ¹³C NMR (CDCl₃) δ 193.9, 154.7, 153.4, 145.6, 138.0, 127.2, 127.0, 121.3, 120.2, 116.5, 95.7, 57.0; FTIR (neat) 2931, 2835, 1713, 1615, 1303, 1159, 1043, 920, 775 cm⁻¹; MS (ESI) *m/z* calcd for (C₁₂H₁₀ClNO₃ + H)⁺ 252 (³⁵Cl) and 254 (³⁷Cl), found 252 (³⁵Cl) and 254 (³⁷Cl); HRMS (ESI) *m/z* calcd for (C₁₂H₁₀ClNO₃ + H)⁺ 252.0427 (³⁵Cl) and 254.0398 (³⁷Cl), found 252.0418 (³⁵Cl) and 254.0390 (³⁷Cl).

8-Chloro-2-hydroxymethyl-7-methoxymethylquinoline (10c). NaBH₄ (0.0028 g, 0.074 mmol) was added to a solution of **9c** (0.0397 g, 0.158 mmol) in ethanol (4 mL). The mixture was stirred for 20 min. The solvent was evaporated, and the resulting solid residue was dissolved in chloroform, then washed with water (×2) followed by brine, dried over MgSO₄, and concentrated to afford **10c** (0.0396 g, 0.156 mmol, 99%) as a white, crystalline solid (mp 84–87 °C): ¹H NMR (CDCl₃) δ 8.09 (1H, d, *J* = 8.4 Hz), 7.71 (1H, d, *J* = 8.8 Hz), 7.52 (1H, d, *J* = 9.2 Hz), 7.24 (1H, d, *J* = 8.4 Hz), 5.41 (2H, s), 4.94 (2H, s), 3.58 (3H, s); ¹³C NMR (CDCl₃) δ 160.3, 154.2, 144.1, 137.2, 127.0, 124.4, 120.0, 117.4, 117.2, 95.7, 64.4, 56.8; FTIR (neat) 3342, 2925, 1615, 1155, 1005, 835, 781 cm⁻¹; MS (ESI) *m/z* calcd for (C₁₂H₁₂ClNO₃ + H)⁺ 254 (³⁵Cl) and 256 (³⁷Cl), found 254 (³⁵Cl) and 256 (³⁷Cl); HRMS (ESI) *m/z* calcd for (C₁₂H₁₂ClNO₃ + H)⁺ 254.0584 (³⁵Cl) and 256.0554 (³⁷Cl), found 254.0577 (³⁵Cl) and 256.0546 (³⁷Cl).

(8-Chloro-7-(methoxymethoxy)quinolin-2-yl)methyl acetate (11c). Under a nitrogen atmosphere, DMAP (0.0079 g), pyridine (1.0 mL), and acetic anhydride (0.121 mL, 1.28 mmol) were added to a solution of **10c** (0.0630 g, 0.2483 mmol) in chloroform (4 mL). After stirring for 2 h, the solvents were evaporated and the remaining residue was purified by column chromatography (1:3 EtOAc/hexane) to afford **11c** (0.0640 g, 0.216 mmol, 87%) as a white solid (mp 114–117 °C): ¹H NMR (CDCl₃) δ 8.14 (1H, d, *J* = 8.8 Hz), 7.71 (1H, d, *J* = 9.2 Hz), 7.54 (1H, d, *J* = 8.8 Hz), 7.42 (1H, d, *J* = 8.0), 5.47 (2H, s), 5.41 (2H, s), 3.58 (3H, s), 2.23 (3H, s);

^{13}C NMR (CDCl_3) δ 170.9, 157.8, 154.2, 145.2, 137.3, 127.0, 124.5, 120.4, 118.4, 117.8, 95.8, 67.6, 56.9, 21.2; FTIR (neat) 2937, 1745, 1619, 1514, 1379, 1245, 1058, 839, 783 cm^{-1} ; MS (ESI) m/z calcd for $(\text{C}_{12}\text{H}_{12}\text{ClNO}_3 + \text{H})^+$ 254 (^{35}Cl) and 256 (^{37}Cl), found 254 (^{35}Cl) and 256 (^{37}Cl); HRMS (ESI) m/z calcd for $(\text{C}_{12}\text{H}_{12}\text{ClNO}_3 + \text{H})^+$ 254.0584 (^{35}Cl) and 256.0554 (^{37}Cl), found 254.0577 (^{35}Cl) and 256.0546 (^{37}Cl).

(8-Chloro-7-hydroxyquinolin-2-yl)methyl acetate (CHQ-OAc, 1c). 12N HCl (1 drop) was added to a solution of **11c** (0.0140 g, 0.0473 mmol) in methanol (3 mL), and the mixture stirred for 10 min. The solvent was evaporated, and the residue was purified by column chromatography (3:7 EtOAc/hexane) to afford **CHQ-OAc** (0.0042 g, 0.017 mmol, 35%) as a white solid (mp 126–127 °C dec): ^1H NMR (CDCl_3) δ 8.13 (1H, d, J = 8.5 Hz), 7.68 (1H, d, J = 8.5 Hz), 7.40 (1H, d, J = 8.5 Hz), 7.34 (1H, d, J = 9.0 Hz), 5.46 (2H, s), 2.23 (3H, s); ^{13}C NMR (CDCl_3) δ 171.0, 157.7, 153.2, 144.7, 137.4, 127.6, 123.8, 118.2, 117.8, 115.1, 67.5, 21.2; FTIR (neat) 2928, 1737, 1629, 1511, 1343, 1251, 850, 777 cm^{-1} ; MS (ESI) m/z calcd for $(\text{C}_{12}\text{H}_{10}\text{ClNO}_3 + \text{H})^+$ 252 (^{35}Cl) and 254 (^{37}Cl), found 252 (^{35}Cl) and 254 (^{37}Cl); HRMS (ESI) m/z calcd for $(\text{C}_{12}\text{H}_{10}\text{ClNO}_3 + \text{H})^+$ 252.0427 (^{35}Cl) and 254.0398 (^{37}Cl), found 252.0420 (^{35}Cl) and 254.0390 (^{37}Cl).

4-Chloro-7-(dimethylamino)quinoline-2-carbaldehyde (14b). 4-Chloro-*N,N*,2-trimethylquinolin-7-amine¹ (**13b**, 100 mg, 0.453 mmol) was dissolved in *m*-xylene (5 mL). Selenium dioxide (75 mg, 0.68 mmol) was added and the mixture was heated to 90 °C under nitrogen overnight. The hot reaction mixture was filtered through cotton and the filtrate was evaporated. The residue was dissolved in chloroform, adsorbed onto silica gel, and purified by column chromatography (chloroform) to provide **14b** (83.1 mg, 0.35 mmol, 78%) as a yellow-orange solid: ^1H NMR (CDCl_3) δ 10.11 (1H, s), 8.12 (1H, d, J = 9.6 Hz), 7.77 (1H, s), 7.37 (1H, dd, J = 8.8, 2.4 Hz), 7.23 (1H, d, J = 2.8 Hz), 3.17 (6H, s); ^{13}C NMR (CDCl_3) δ 193.5, 153.0, 152.4, 151.1, 143.9, 125.3, 120.5, 119.6, 114.0, 107.2, 40.7, 30.0; FTIR (neat) 2926, 2856, 1697, 1616, 1514, 1363, 1296, 1124, 846, 775 cm^{-1} ; MS (ESI) m/z calcd for $(\text{C}_{12}\text{H}_{11}\text{ClN}_2\text{O} + \text{H})^+$ 235, found 235; HRMS (ESI) m/z calcd for $(\text{C}_{12}\text{H}_{11}\text{ClN}_2\text{O} + \text{H})^+$ 235.0638, found 235.0637.

(4-Chloro-7-(dimethylamino)quinolin-2-yl)methanol (15b). Compound **14b** (50 mg, 0.213 mmol) was dissolved in absolute ethanol (2 mL). NaBH_4 (8 mg, 0.213 mmol) was added to the solution and the mixture was stirred for 30 min. The solvent was evaporated and the residue dissolved in methanol and filtered. The filtrate was adsorbed onto silica gel and purified by column chromatography (7:3 EtOAc/hexane) to obtain **15b** (30 mg, 0.126 mmol, 60%). ^1H NMR (CDCl_3) δ 8.02 (1H, d, J = 8.8 Hz), 7.21 (1H, dd, J = 9.2, 2.8 Hz), 7.11 (1H, d, J = 2.8 Hz), 7.07 (1H, s), 4.81 (2H, s), 3.14, (6H, s); ^{13}C NMR (CDCl_3) δ 159.4, 152.1, 149.6, 143.1, 125.1, 117.9, 116.5, 114.3, 106.3, 64.2, 40.6; FTIR (neat) 2918, 1622, 1535, 1508, 1394, 1284, 1207, 1174, 1134; MS (ESI) m/z calcd for $(\text{C}_{12}\text{H}_{13}\text{ClN}_2\text{O} + \text{H})^+$ 237, found 237; HRMS (ESI) m/z calcd for $(\text{C}_{12}\text{H}_{13}\text{ClN}_2\text{O} + \text{H})^+$ 237.0838, found 237.0832.

(4-Chloro-7-(dimethylamino)quinolin-2-yl)methyl acetate (DMAQ-Cl-OAc, 2b). Pyridine (0.6 mL) was added to **15b** (30 mg, 0.126 mmol) and the solution was stirred under nitrogen for a few minutes before acetic anhydride (70 μL) was added dropwise. The solution was stirred under nitrogen for 17 h before the solvent was evaporated. The resulting residue was purified by column chromatography (3:7 EtOAc/hexane) to give **DMAQ-Cl-OAc** (20.0 mg, 71 μmol , 57%):

^1H NMR (CDCl_3) δ 8.03 (1H, d, $J = 9.2$ Hz), 7.23 (1H, d, $J = 9.2$ Hz), 7.11 (1H, s), 7.14 (1H, s), 5.30 (2H, s), 3.14 (6H, s), 2.23 (3H, s); ^{13}C NMR (CDCl_3) δ 170.6, 156.3, 151.9, 150.4, 143.0, 124.7, 117.9, 116.9, 115.4, 106.4, 67.1, 40.4, 21.0; FTIR (neat) 2992, 1744, 1616, 1514, 1425, 1373, 1225, 1132, 1053, 862, 845, 810, 756, 677 cm^{-1} ; MS (ESI) m/z calcd for $(\text{C}_{12}\text{H}_{15}\text{ClN}_2\text{O} + \text{H})^+$ 279, found 279; HRMS (ESI) m/z calcd for $(\text{C}_{12}\text{H}_{15}\text{ClN}_2\text{O} + \text{H})^+$ 279.0900, found, 279.0934.

Detailed Procedures for Photochemical Experiments

Determination of the uncaging quantum efficiency (Q_u). These experiments were carried out using a previously described method.²⁻⁵ KMOPS-buffered solutions (3 mL) of **1a–c**, **2**, and **3** (100 μM) in quartz cuvettes were irradiated with 365-nm light from a mercury lamp (Spectroline SB-100P; Spectronics, Westbury, NY) with a set of light filters. The spectral output of the lamp after the glass filters (CS0-52 and CS7-60, Ace Glass, Vineland, NJ) is 365 ± 15 nm. Aliquots (20 μL) of the reaction solution were removed for analysis by HPLC at periodic intervals. An external standard method was used to determine the concentration of the caged compound. The analytes were eluted isocratically (flow rate of 1 mL/min) with acetonitrile and water containing 0.1% TFA.

Compound	t_R (min)	λ_{obs} (nm)*	Mobile Phase
NHQ-OAc (1a)	7.9	320	65:35 acetonitrile/water (0.1% TFA)
CyHQ-OAc (1b)	6.4	320	35:65 acetonitrile/water (0.1% TFA)
CHQ-OAc (1c)	5.9	310	40:60 acetonitrile/water (0.1% TFA)
DMAQ-OAc (2a)	6.8	438	40:60 acetonitrile/water (0.1% TFA)
DMAQ-Cl-OAc (2b)	6.2	445	50:50 acetonitrile/water (0.1% TFA)
TQ-OAc (3)	5.8	330	35:65 acetonitrile/water (0.1% TFA)

*Detector observation wavelength

The uncaging quantum efficiency (Q_u) of each chromophore was calculated using equation (1),^{6,7} where I is the radiant power in $\text{einstein}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$, σ is the decadic extinction coefficient (10^3 times ϵ , molar extinction coefficient) in $\text{cm}^2\cdot\text{mol}^{-1}$, and $t_{90\%}$ is the irradiation time in s for 90% conversion to product. The UV intensity of the lamp I was measured by potassium ferrioxalate actinometry⁸ in the same setup.

$$Q_u = (I\sigma t_{90\%})^{-1} \quad (1)$$

Determination of the dark hydrolysis rate (τ_{dark}). Substrates were dissolved in KMOPS and stored in the dark at room temperature. HPLC analysis was carried out periodically to determine the extent of decay as described for determining the uncaging quantum efficiency.

Determination of the 2-photon uncaging action cross-section (δ_u). These experiments were carried out using a previously described method.²⁻⁵ Measurements were performed in microcuvettes (10×1×1 mm illuminated dimensions) using light from a fs-pulsed and mode-locked Ti:sapphire laser focused on the center of the cuvette chamber with a 25-mm focal length

lens optimized for IR lasers (06LXP003/076, Melles-Griot, Irvine, CA). The laser beam was attenuated by passing the beam through an electro-optical modulator or a ½-waveplate and polarizing beamsplitter cube so that the average power exiting the apparatus was 300–350 mW. Successive aliquots (25 µL) of each caged compound in KMOPS were placed into the cuvette and irradiated for 0, 5, 10, 20, and 40 min. Each aliquot was analyzed by HPLC to determine the concentration of the remaining caged compound as described for determining the uncaging quantum efficiency. For compounds **1c**, **2a**, **2b**, and **3** ($\tau_{\text{dark}} < 50$ h), a correction was made for background dark hydrolysis that occurred while the irradiated samples were waiting for HPLC analysis. The fraction of the decay from hydrolysis in the dark was calculated using the measured dark hydrolysis rate and the waiting time from the end of the experiment until the HPLC injection. This value was subtracted from the total decay (hydrolysis + photolysis) measured by HPLC. For compounds with $\tau_{\text{dark}} > 50$ h, dark hydrolysis did not measurably impact the concentration determination.

The two-photon uncaging action cross-section, δ_u , was estimated by referencing to fluorescein, a compound with a known fluorescence quantum yield ($Q_f = 0.9$) and absorbance cross-section ($\delta_a = 30$ GM at 740 nm),^{9,10} using equation (2):²

$$\delta_u = \frac{N_p \phi Q_f \delta_a C_F}{\langle F(t) \rangle C_S} \quad (2)$$

N_p is the number of molecules photolyzed per unit time (molecules/s, determined by HPLC analysis of the reaction); ϕ is the collection efficiency of the detector (SED033 on an IL-1700, International Light, Newburyport, MA) used to measure the fluorescence of fluorescein emitted at a right angle to the beam and passed through a 535/45 nm bandpass filter; C_F is the concentration of the fluorescein standard (mol/L); $\langle F(t) \rangle$ is the time averaged fluorescent photon flux (photons/s) of the fluorescein standard collected by the detector; and C_S is the initial concentration of the caged compound (mol/L).

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- (8) Hatchard, C. G.; Parker, C. A. *Proc. R. Soc. London, Ser. A* **1956**, *235*, 518-536.
- (9) Xu, C.; Guild, J.; Webb, W. W.; Denk, W. *Optics Lett.* **1995**, *20*, 2372-2374.
- (10) Xu, C.; Webb, W. W. *J. Opt. Soc. Am. B* **1996**, *13*, 481-491.

Additional Characterization Data for New Compounds

NHQ-OAc (1a)

NHQ-OAc

Automation directory: /home/walkup/vnmr/sys/data/auto_2006.12.11
Sample id : s_20061211_017
Sample : NHQ-OAc

Pulse Sequence: s2pu1

Solvent: dmsd

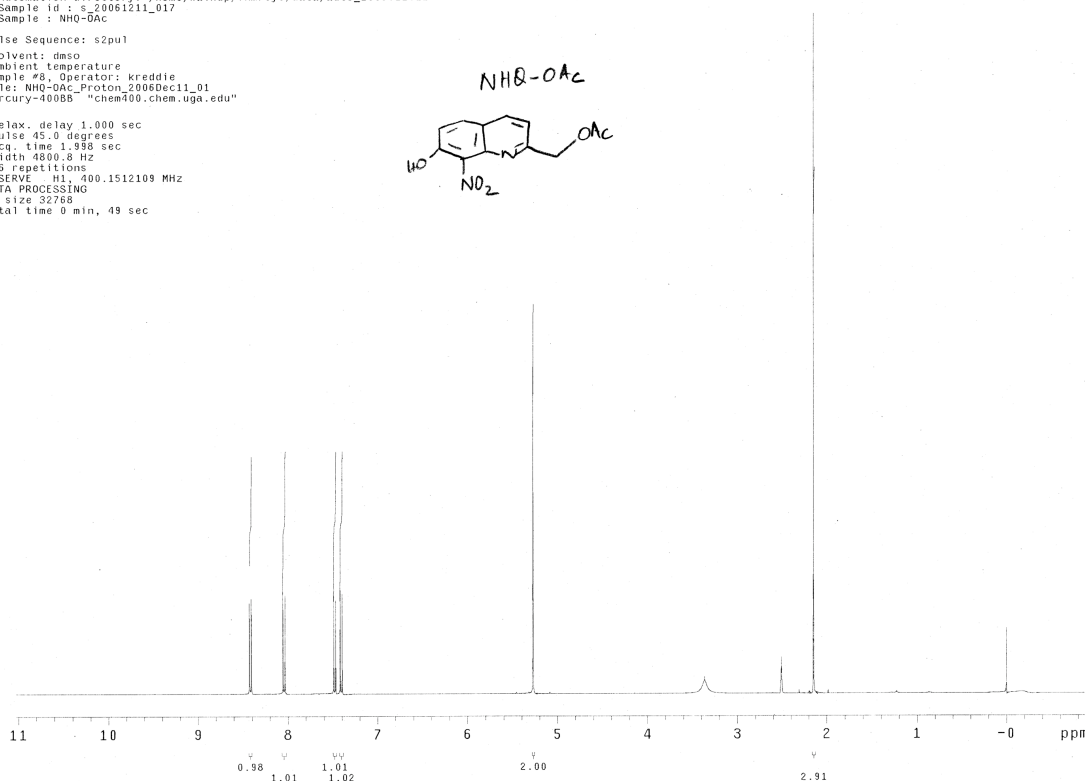
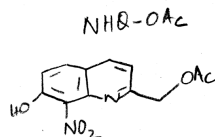
Ambient temperature

Sample #8, Operator: Kreddie

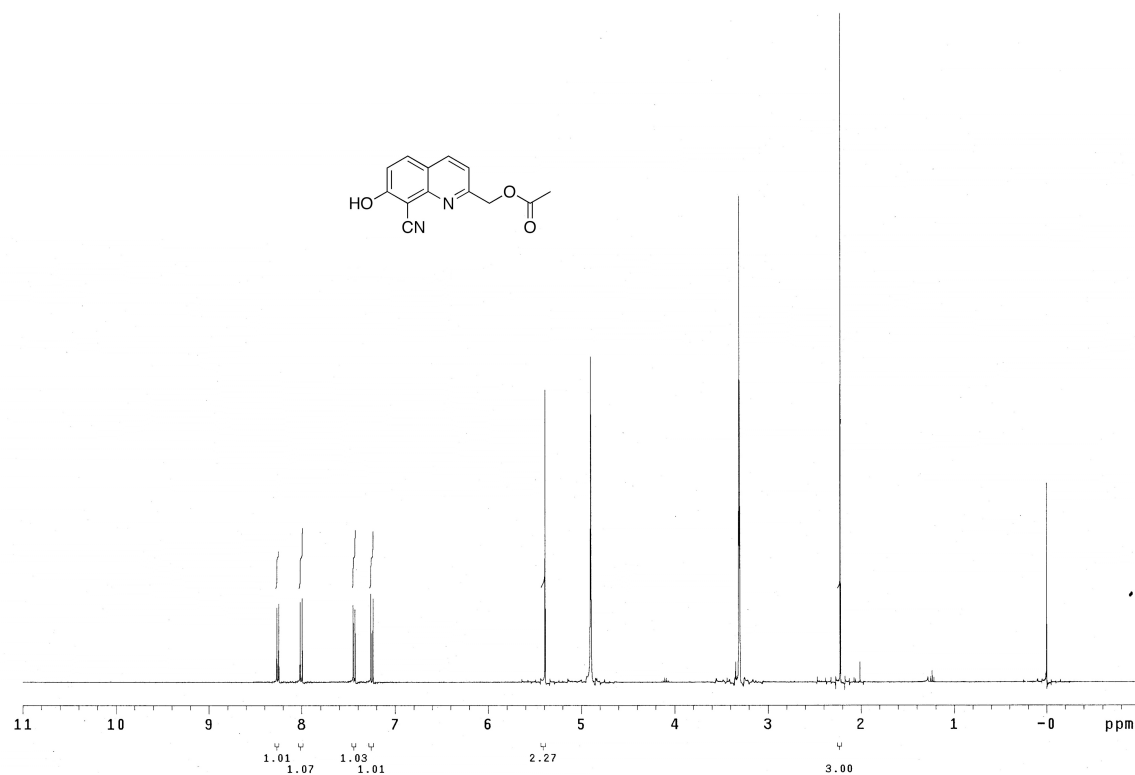
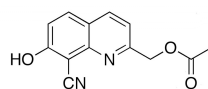
File: NHQ-OAc_Proton_2006Dec11_01

Mercury-400BB "chem400.chem.uga.edu"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 4890.8 Hz
16 repetitions
OBSERVE H1, 400.1512109 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 49 sec

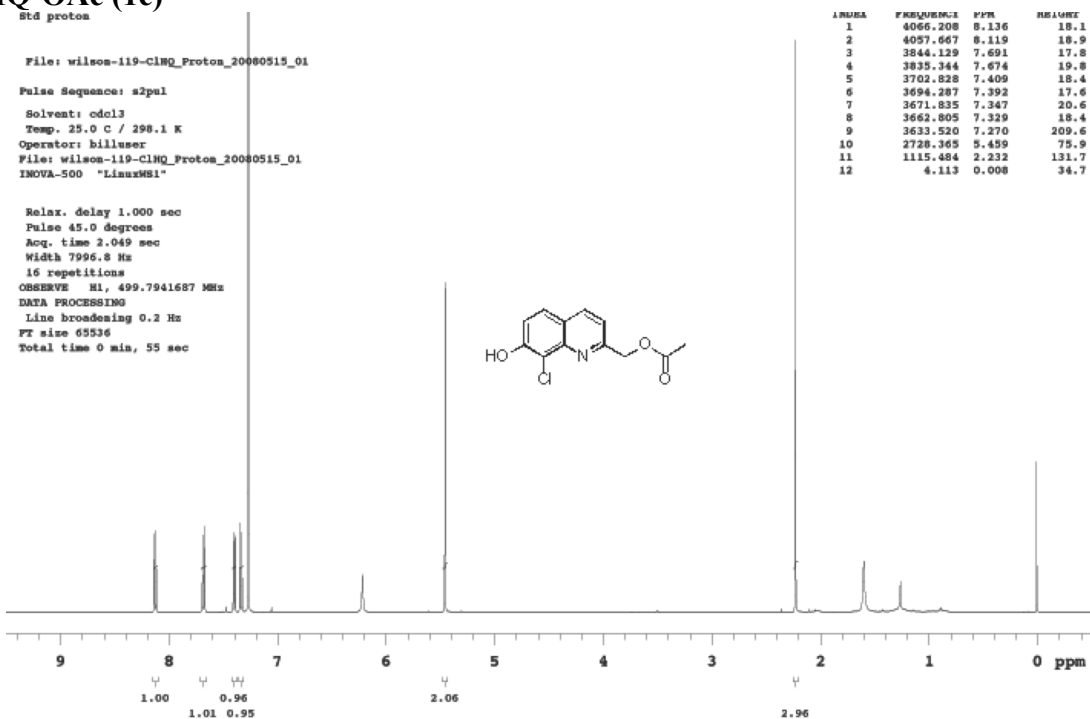


CyHQ-OAc (1b)

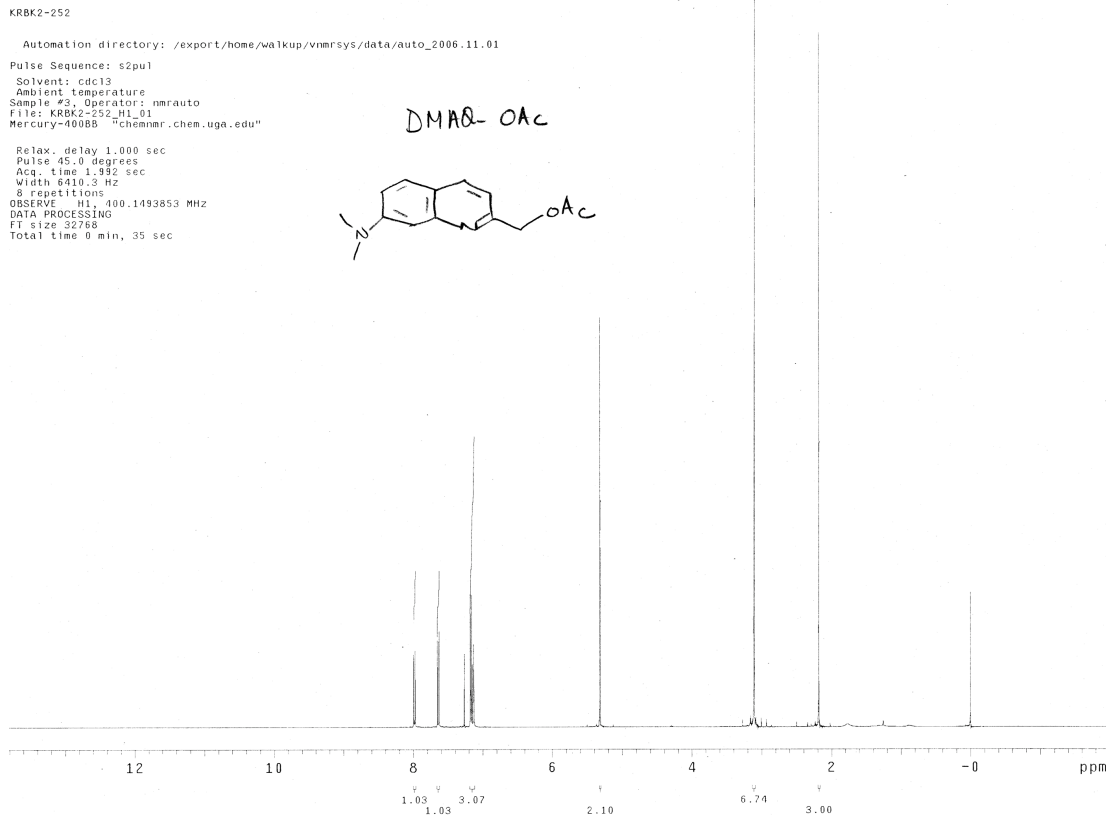


2PE-Sensitive Photoremovable Protecting Groups

CHQ-OAc (1c)



DMAQ-OAc (2a)



2PE-Sensitive Photoremovable Protecting Groups

DMAQ-Cl-OAc (2b)

Automation directory: /export/home/walkup/vnmr/chem/data/auto_2006.11.01
Sample id : s_20060802_12
Sample : KR8K4-114

Pulse Sequence: s2pu1

Solvent: cdcl3

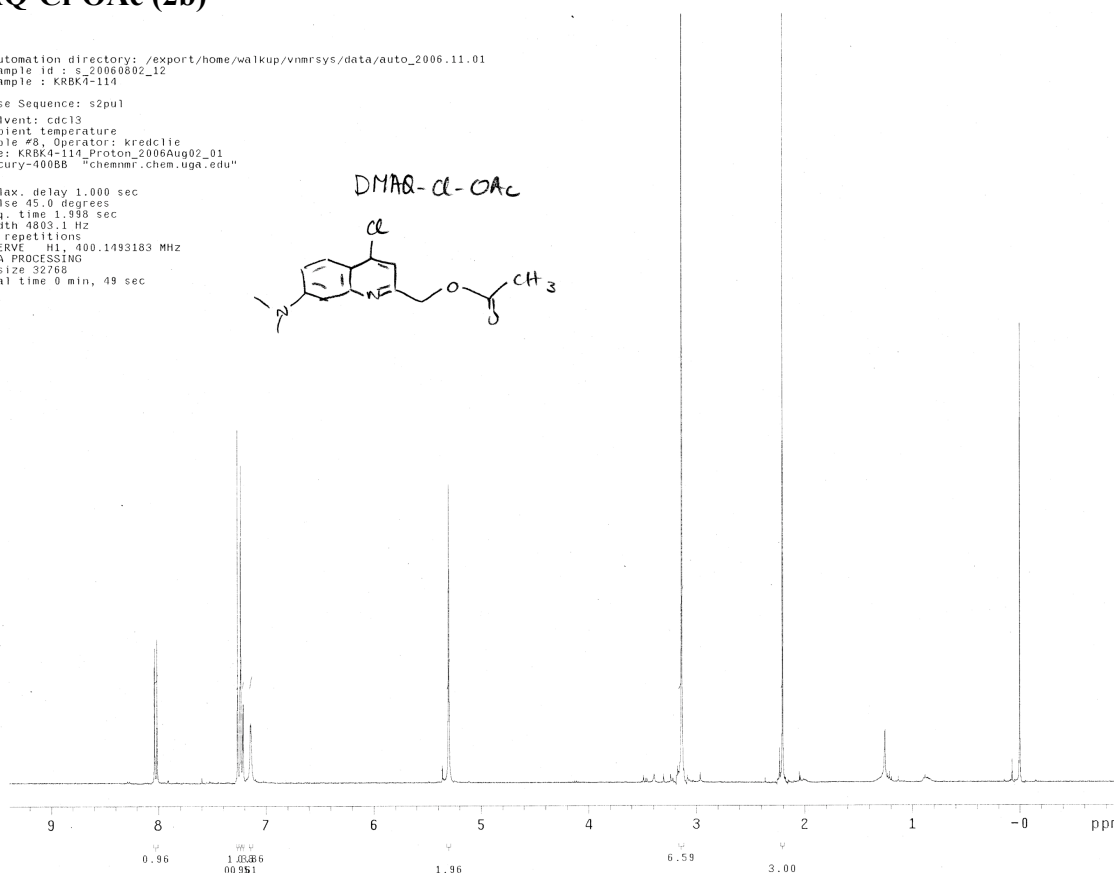
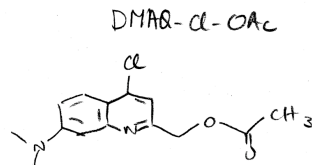
Ambient temperature

Sample #8, Operator: kredclie

File: KR8K4-114_Proton_2006Aug02_01

Mercury-400BB "chemmr.chem.uga.edu"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 4803.1 Hz
16 repetitions
OBSERVE H1, 400.1493183 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 49 sec



7-Hydroxy-8-nitroquinoline (5a)

Automation directory: /export/home/walkup/vnmr/chem/data/auto_2006.11.01
Sample id : s_20060919_01
Sample : NHQ

Pulse Sequence: s2pu1

Solvent: cd3od

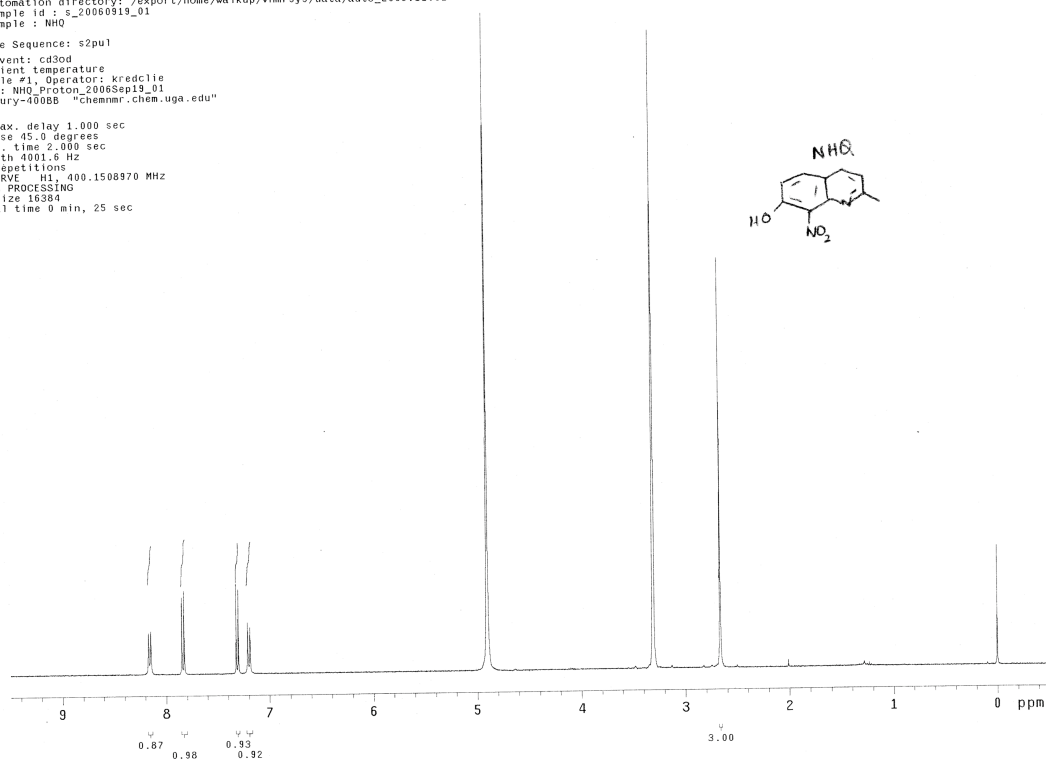
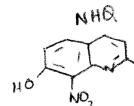
Ambient temperature

Sample #1, Operator: kredclie

File: NHQ_Proton_2006Sep19_01

Mercury-400BB "chemmr.chem.uga.edu"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.000 sec
Width 4001.6 Hz
8 repetitions
OBSERVE H1, 400.1508970 MHz
DATA PROCESSING
FT size 16384
Total time 0 min, 25 sec



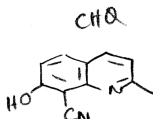
7-Hydroxy-2-methylquinoline-8-carbonitrile (5b)

CHQ-A

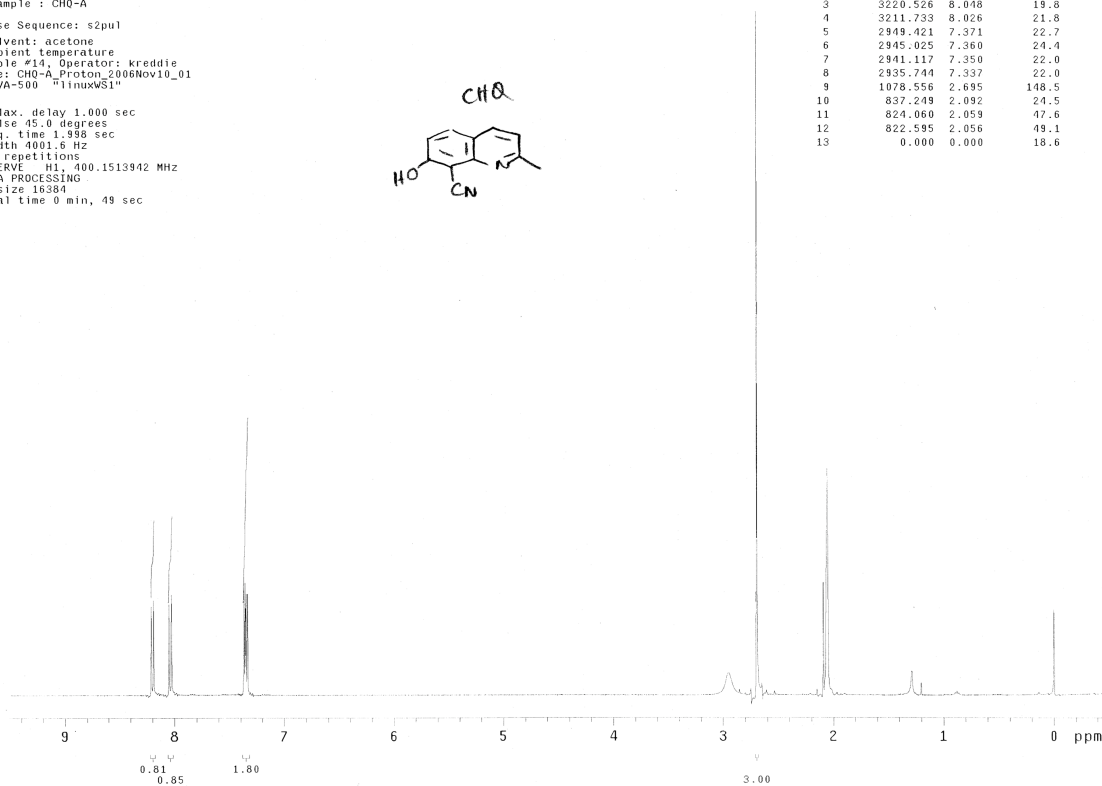
Automation directory:
Sample ID : s_20061110_018
Sample : CHQ-A

Pulse Sequence: s2pul
Solvent: acetone
Ambient temperature
Sample #14, Operator: kreddie
File: CHQ-A_Proton_2006Nov10_01
INOVA-500 "linuxWS1"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 4001.6 Hz
16 repetitions
OBSERVE H1, 400.1513942 MHz
DATA PROCESSING
FT size 16384
Total time 0 min, 49 sec



INDEX	FREQUENCY	PPM	HEIGHT
1	3285.005	8.209	19.2
2	3276.701	8.189	20.5
3	3220.526	8.048	19.8
4	3211.733	8.026	21.8
5	2949.421	7.371	22.7
6	2945.025	7.360	24.4
7	2941.117	7.350	22.0
8	2935.744	7.337	22.0
9	1078.556	2.695	148.5
10	837.249	2.092	24.5
11	824.060	2.059	47.6
12	822.595	2.056	49.1
13	0.000	0.000	18.6



8-Chloro-7-hydroxyquinoline (5c)

File: Wilson57-chlorideF37_Proton_2008Feb09_01

Pulse Sequence: s2pul

Solvent: cdcl3

Ambient temperature

Sample #39, Operator: hunter

File: Wilson57-chlorideF37_Proton_2008Feb09_01

INOVA-500 "LinuxWS1"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.998 sec

Width 6402.0 Hz

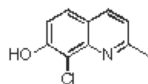
8 repetitions

OBSERVE H1, 400.1493213 MHz

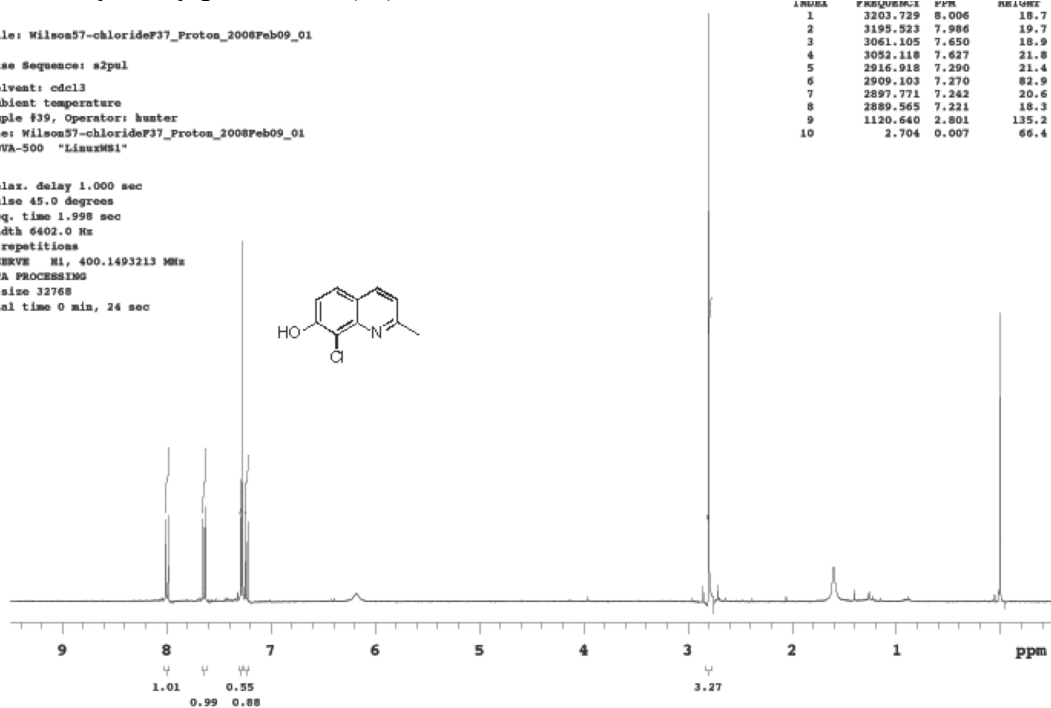
DATA PROCESSING

FT size 32768

Total time 0 min, 24 sec



INDEX	FREQUENCY	PPM	HEIGHT
1	3203.729	8.006	18.7
2	3195.523	7.986	19.7
3	3061.105	7.650	18.9
4	3052.118	7.627	21.8
5	2916.918	7.290	21.4
6	2909.103	7.270	82.9
7	2897.771	7.242	20.6
8	2889.565	7.221	18.3
9	1120.640	2.801	135.2
10	2.704	0.007	66.4

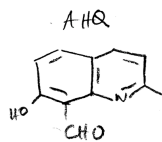


7-Hydroxy-2-methylquinoline-8-carbaldehyde (6)

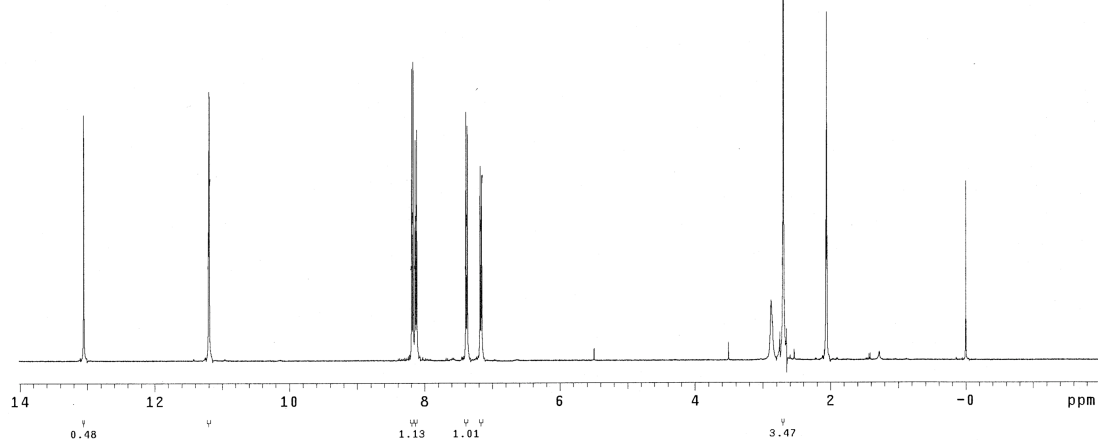
Automation directory: /export/home/walkup/auto_2006.09.25
 Sample id : s_20060925_01
 Sample : AHQ

Pulse Sequence: s2pu1
 Solvent: acetone
 Ambient temperature
 Sample #1, Operator: kredclie
 File: AHQ_Proton_2006Sep25_01
 Mercury-400BB "Chem400"

Relax. delay 1.000 sec
 Pulse 45.0 degrees
 Acq. time 1.998 sec
 Width 6410.3 Hz
 8 repetitions
 OBSERVE H1, 400.1513957 MHz
 DATA PROCESSING
 FT size 32768
 Total time 0 min, 25 sec



INDEX	FREQUENCY	PPM	HEIGHT
1	5223.201	13.053	54.3
2	4482.171	11.201	46.4
3	4478.650	11.192	59.5
4	3277.901	8.192	64.6
5	3269.685	8.171	66.2
6	3257.165	8.140	48.7
7	3248.166	8.117	51.1
8	2956.684	7.389	55.1
9	2948.468	7.368	51.9
10	2872.956	7.180	43.0
11	2863.957	7.157	41.0
12	1151.452	2.878	13.3
13	1142.844	2.856	8.5
14	1100.198	2.749	6.2
15	1079.853	2.699	398.0
16	1061.464	2.653	6.9
17	828.278	2.070	31.0
18	826.322	2.065	50.9
19	823.975	2.059	77.1
20	821.627	2.053	52.4
21	819.671	2.048	27.5
22	-0.000	-0.000	39.5

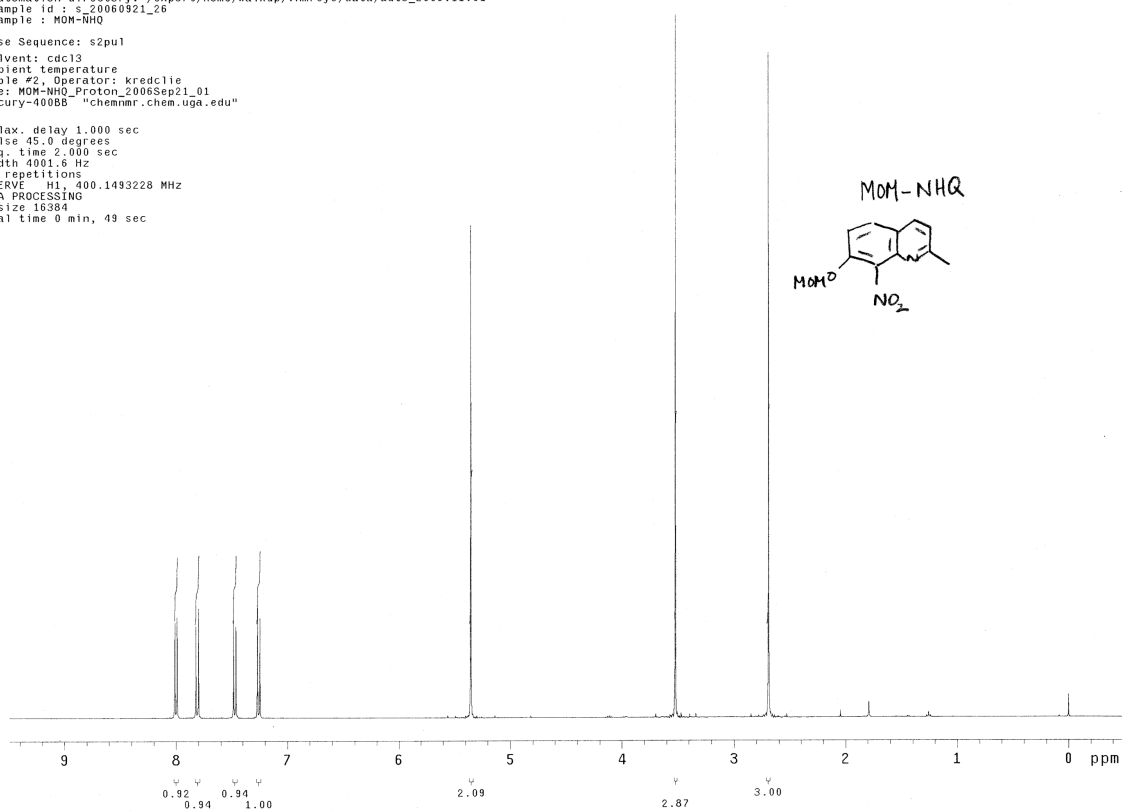
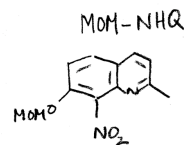


7-(Methoxymethoxy)-8-nitroquinoline (8a)

Automation directory: /export/home/walkup/vnmrsys/data/auto_2006.11.01
 Sample id : s_20060921_26
 Sample : MOM-NHQ

Pulse Sequence: s2pu1
 Solvent: cdcl3
 Ambient temperature
 Sample #2, Operator: kredclie
 File: MOM-NHQ_Proton_2006Sep21_01
 Mercury-400BB "chemnmr.chem.uga.edu"

Relax. delay 1.000 sec
 Pulse 45.0 degrees
 Acq. time 2.000 sec
 Width 4001.6 Hz
 16 repetitions
 OBSERVE H1, 400.1493228 MHz
 DATA PROCESSING
 FT size 16384
 Total time 0 min, 49 sec



7-(Methoxymethoxy)-2-methylquinoline-8-carbonitrile (8b)

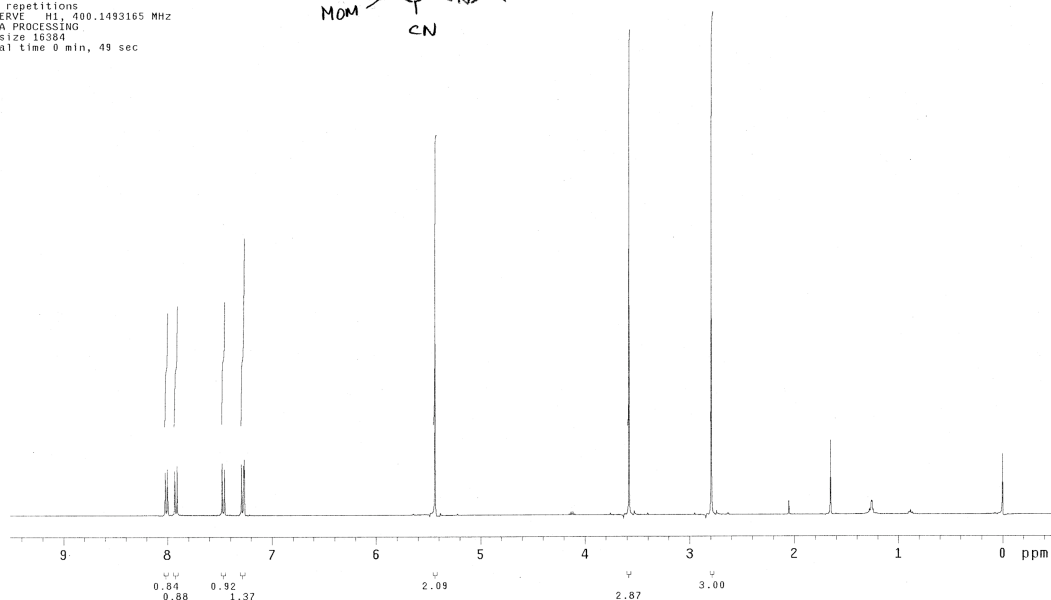
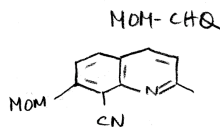
MM-CHQ

Automation directory: /home/walkup/vnmrsys/data/auto_2006.11.08_01
Sample id : s_20061110_002
Sample : MM-CHQ

Pulse Sequence: s2pul

Solvent: cdcl3
Ambient temperature
Sample #3, Operator: kreddie
File: MM-CHQ_Proton_2006Nov10_01
Mercury-400B5 "chem400.chem.uga.edu"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 4001.6 Hz
16 repetitions
OBSERVE M1, 400.1493165 MHz
DATA PROCESSING
FT size 16384
Total time 0 min, 49 sec



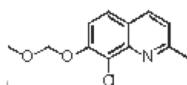
8-Chloro-7-methoxymethylquinoline (8c)

File: Wilson71-77-MM-Proton_2008Apr08_01

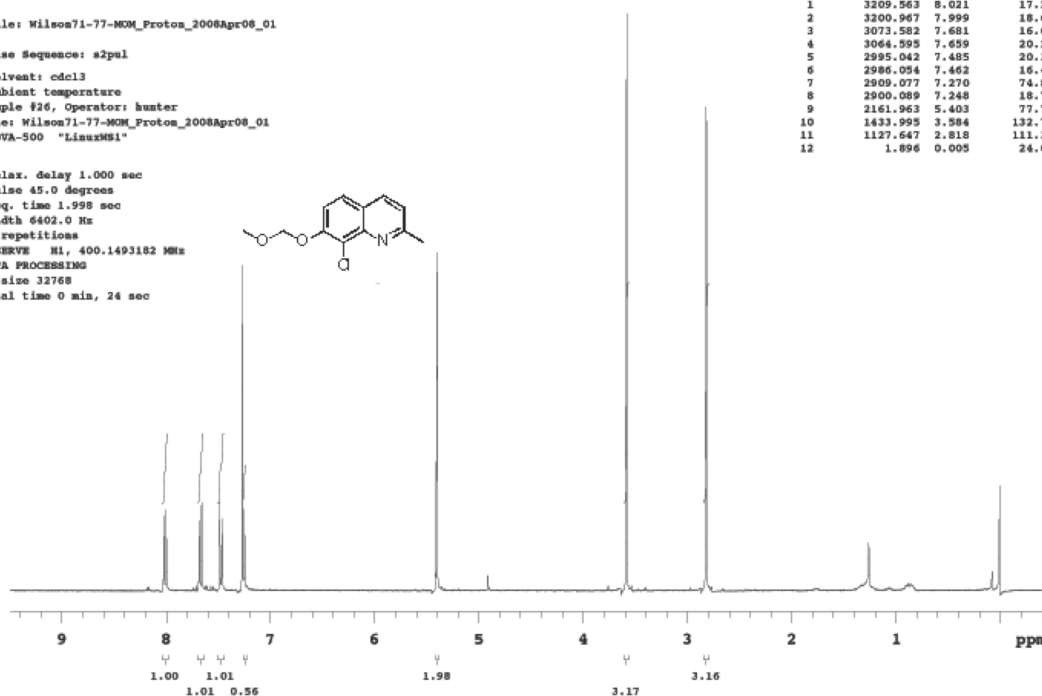
Pulse Sequence: s2pul

Solvent: cdcl3
Ambient temperature
Sample #26, Operator: hunter
File: Wilson71-77-MM-Proton_2008Apr08_01
INOVA-500 "LinuxMSI"

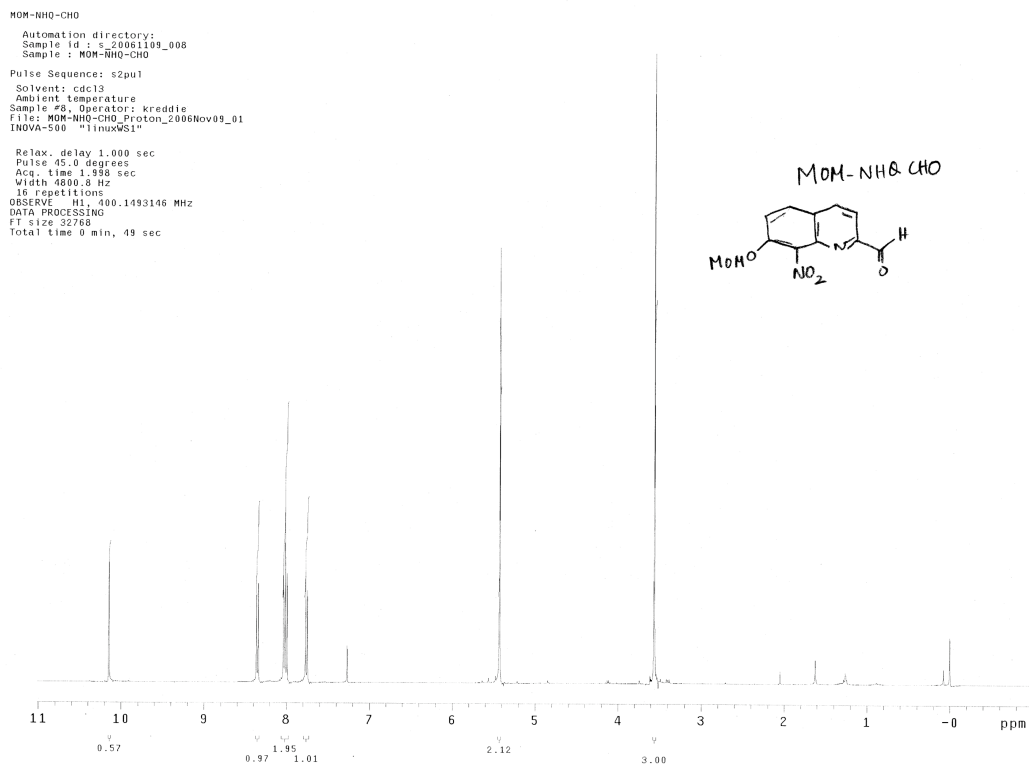
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.998 sec
Width 6402.0 Hz
8 repetitions
OBSERVE M1, 400.1493182 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 24 sec



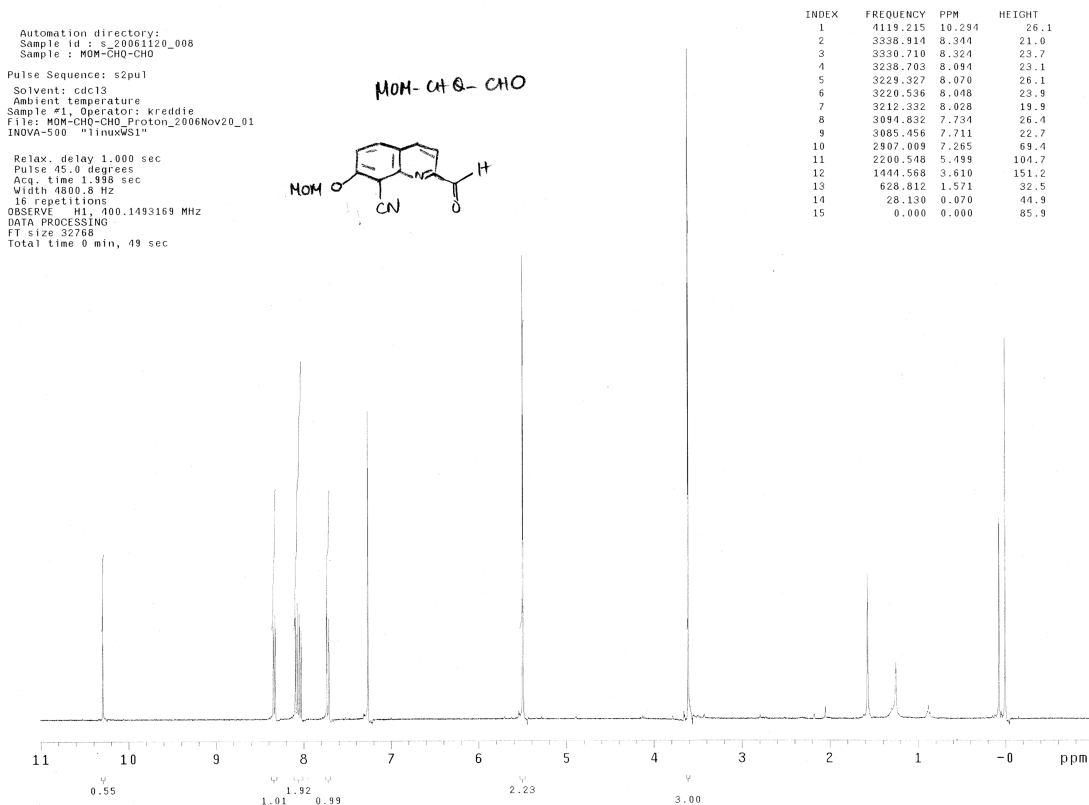
AREA	FREQUENCY	PPM	RELATIVE
1	3209.563	8.021	17.2
2	3200.967	7.999	18.6
3	3073.582	7.681	16.0
4	3064.595	7.659	20.2
5	2995.042	7.485	20.3
6	2986.054	7.462	16.4
7	2909.077	7.270	74.8
8	2900.089	7.248	18.7
9	2161.963	5.403	77.7
10	1433.995	3.594	132.7
11	1127.647	2.818	111.3
12	1.896	0.005	24.0



7-(Methoxymethoxy)-8-nitroquinolin-2-yl formaldehyde (9a)



2-Formyl-7-(methoxymethoxy)quinoline-8-carbonitrile (9b)



8-Chloro-2-formyl-7-methoxymethylquinoline (9c)

File: Wilson97-aldehyde_Proton_20080421_01

Pulse Sequence: s2pul

Solvent: cdcl3

Ambient temperature

Operator: hunter

File: Wilson97-aldehyde_Proton_20080421_01

INOVA-500 "LinuxMSI"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.998 sec

Width 6402.0 Hz

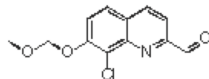
8 repetitions

OBSERVE H1, 400.1493068 MHz

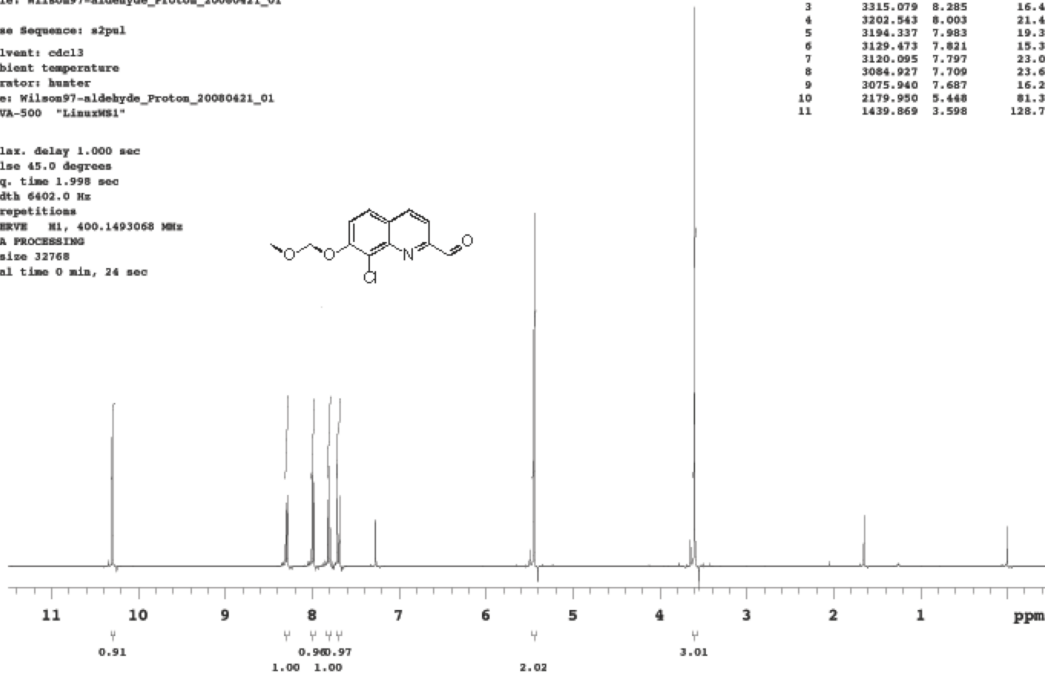
DATA PROCESSING

FT size 32768

Total time 0 min, 24 sec



AREA	FREQUENCY	PPM	INTEGR
1	4121.587	10.300	29.7
2	3323.285	8.305	14.5
3	3315.079	8.285	16.4
4	3202.543	8.003	21.4
5	3194.337	7.983	19.3
6	3129.473	7.821	15.3
7	3120.095	7.797	23.0
8	3084.927	7.709	23.6
9	3075.940	7.687	16.2
10	2179.950	5.448	81.3
11	1439.869	3.598	128.7



7-(Methoxymethoxy)-8-nitroquinolin-2-yl methanol (10a)

Automation directory: /home/walkup/vnmrsws/data/auto_2006.11.28

Sample id: s_20061129_025

Sample: MOM-NHQ-CH2OH

Pulse Sequence: s2pul

Solvent: cdcl3

Ambient temperature

Sample #14, Operator: kraddie

Files: MOM-NHQ-CH2OH_Proton_2006Nov29_01

Mercury-400BB "chem400.chm.uga.edu"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.998 sec

Width 4001.6 Hz

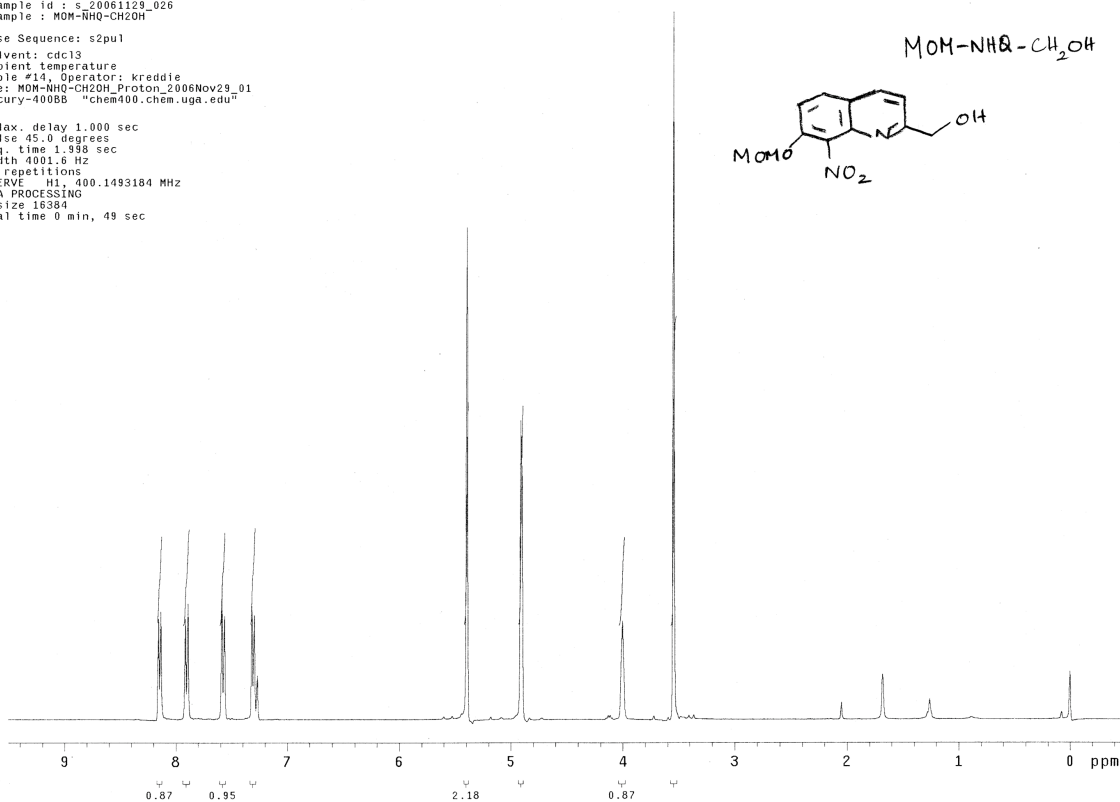
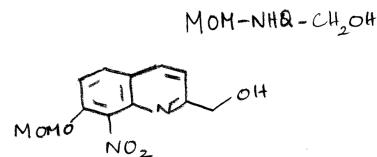
16 repetitions

OBSERVE H1, 400.1493184 MHz

DATA PROCESSING

FT size 16384

Total time 0 min, 49 sec



2-(Hydroxymethyl)-7-(methoxymethoxy)quinoline-8-carbonitrile (10b)

MOM-CH₂-OH

Automation directory: /export/home/walkup/vnmrsys/data/auto_2006.11.01
Sample id : S_20060728_11
Sample : CHK-I-64C

Pulse Sequence: s2pul

Solvent: cdcl₃

Temp: 25.0 C / 298.1 K

Sample #31, Operator: dore

File: CHK-I-64C_Proton_2006Jul28_01

Mercury-400BB "chemmr.chem.uga.edu"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.938 sec

Width 4893.1 Hz

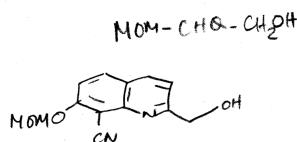
64 repetitions

OBSERVE H1, 400.1493183 MHz

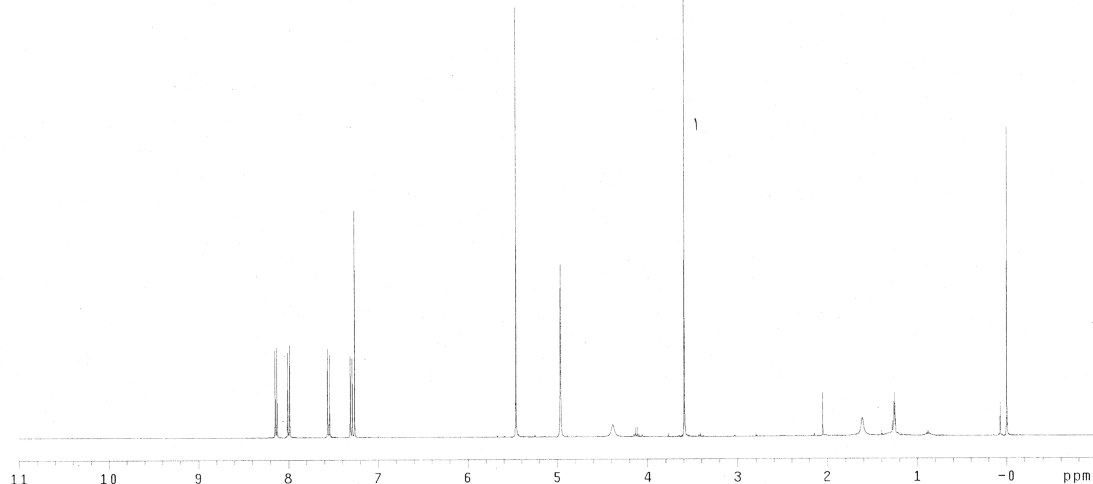
DATA PROCESSING

FT size 32768

Total time 3 min, 19 sec



INDEX	FREQUENCY	PPM	HEIGHT
1	3260.189	8.147	19.3
2	3251.981	8.127	20.0
3	3204.490	8.008	18.9
4	3195.402	7.986	20.5
5	3027.423	7.566	19.6
6	3018.042	7.542	18.3
7	2926.284	7.313	18.0
8	2917.783	7.292	17.6
9	2907.229	7.265	50.3
10	2186.652	5.465	95.6
11	1987.305	4.966	38.3
12	1437.638	3.593	151.2
13	-0.000	-0.000	68.5



8-Chloro-2-hydroxymethyl-7-methoxymethylquinoline (10c)

File: Wilson105-alcohol_Proton_20080430_03

Pulse Sequence: s2pul

Solvent: cdcl₃

Ambient temperature

Operator: hunter

File: Wilson105-alcohol_Proton_20080430_03

INOVA-500 "LinuxMSI"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.998 sec

Width 6402.0 Hz

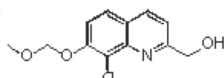
8 repetitions

OBSERVE H1, 400.1493134 MHz

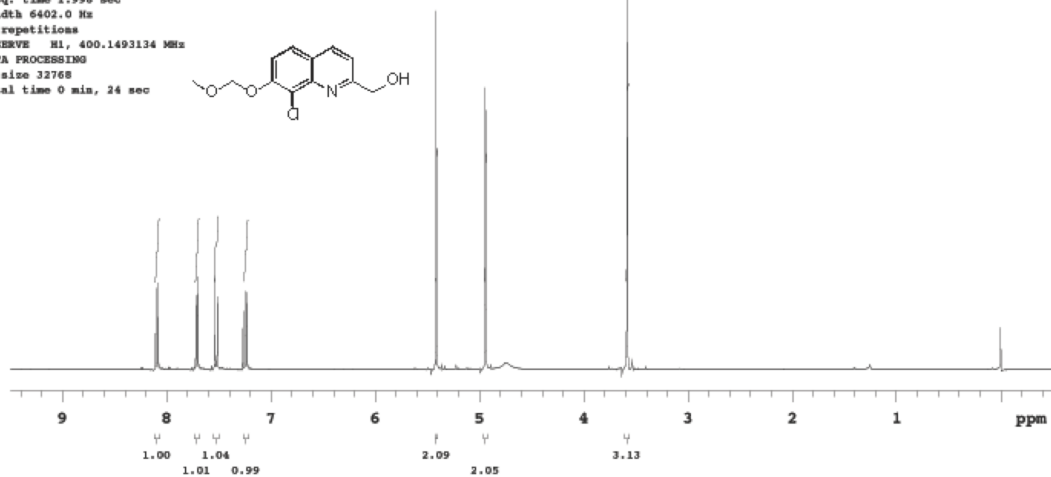
DATA PROCESSING

FT size 32768

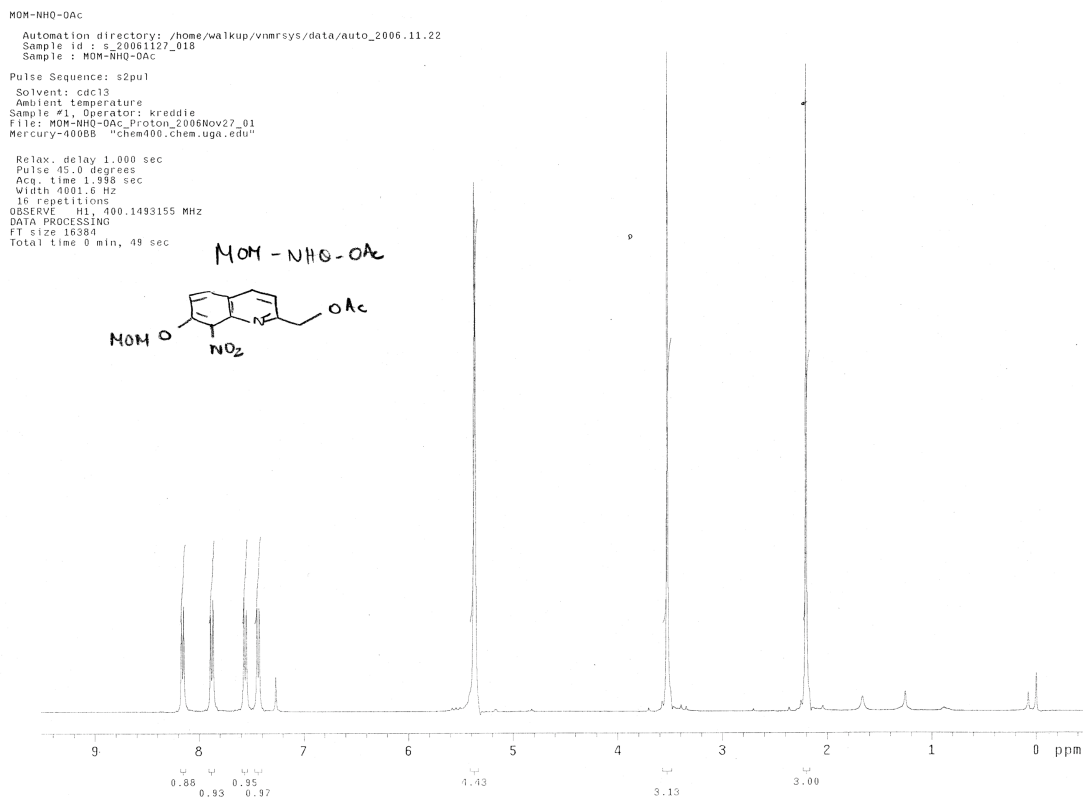
Total time 0 min, 24 sec



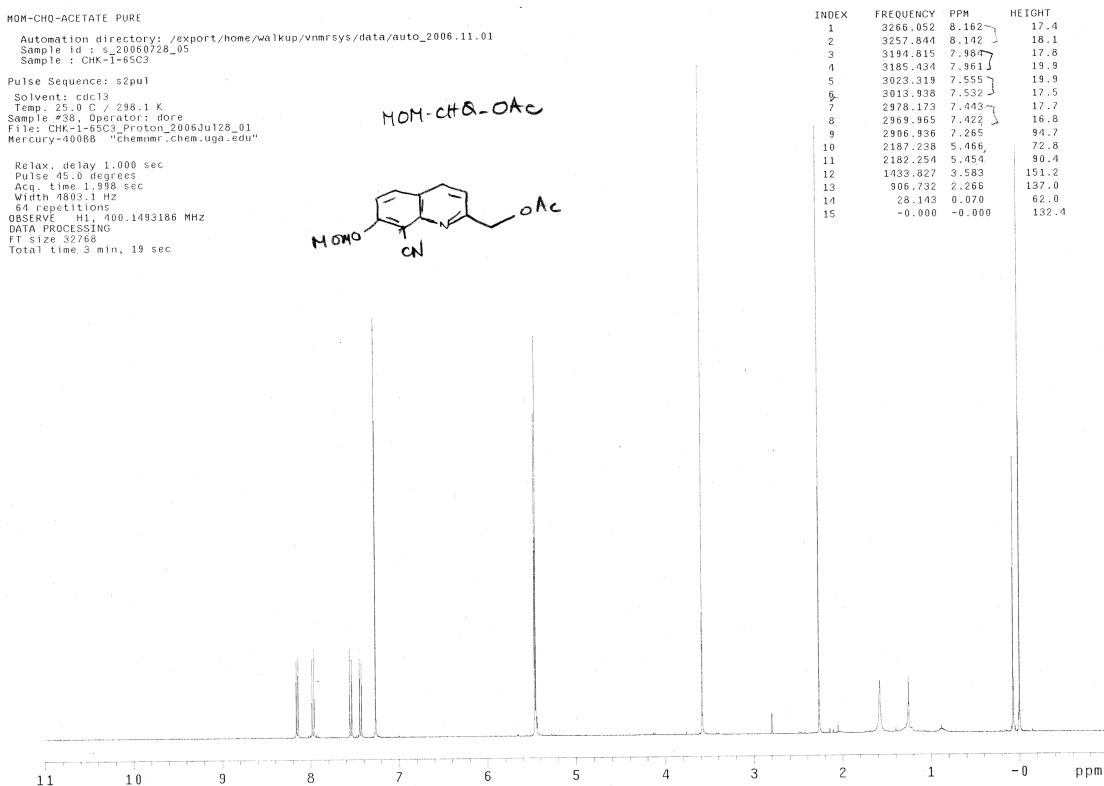
AREA	FREQUENCY	PPM	HEIGHT
1	3242.806	8.104	18.7
2	3234.209	8.083	19.9
3	3089.632	7.721	17.0
4	3080.645	7.699	21.4
5	3014.999	7.535	21.1
6	3006.011	7.512	16.8
7	2908.714	7.269	9.4
8	2900.899	7.250	18.0
9	2892.694	7.229	17.7
10	2165.899	5.413	82.5
11	1977.557	4.942	64.8
12	1434.024	3.584	127.4
13	-0.029	-0.000	9.6



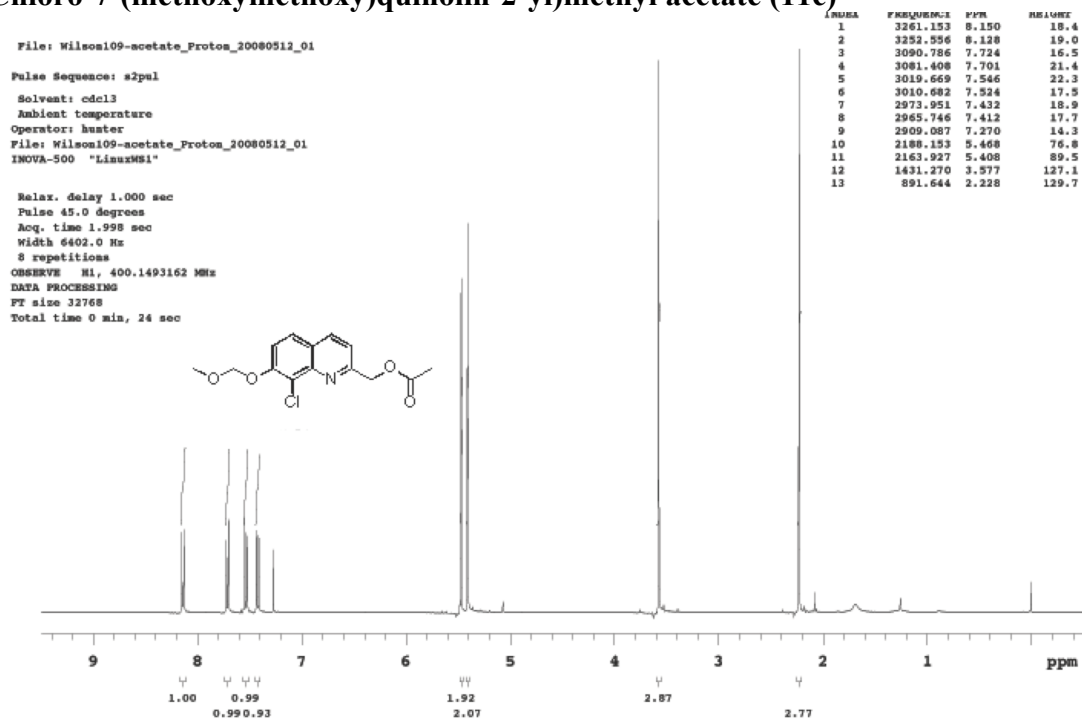
7-(Methoxymethoxy)-8-nitroquinolin-2-yl methyl acetate (11a)



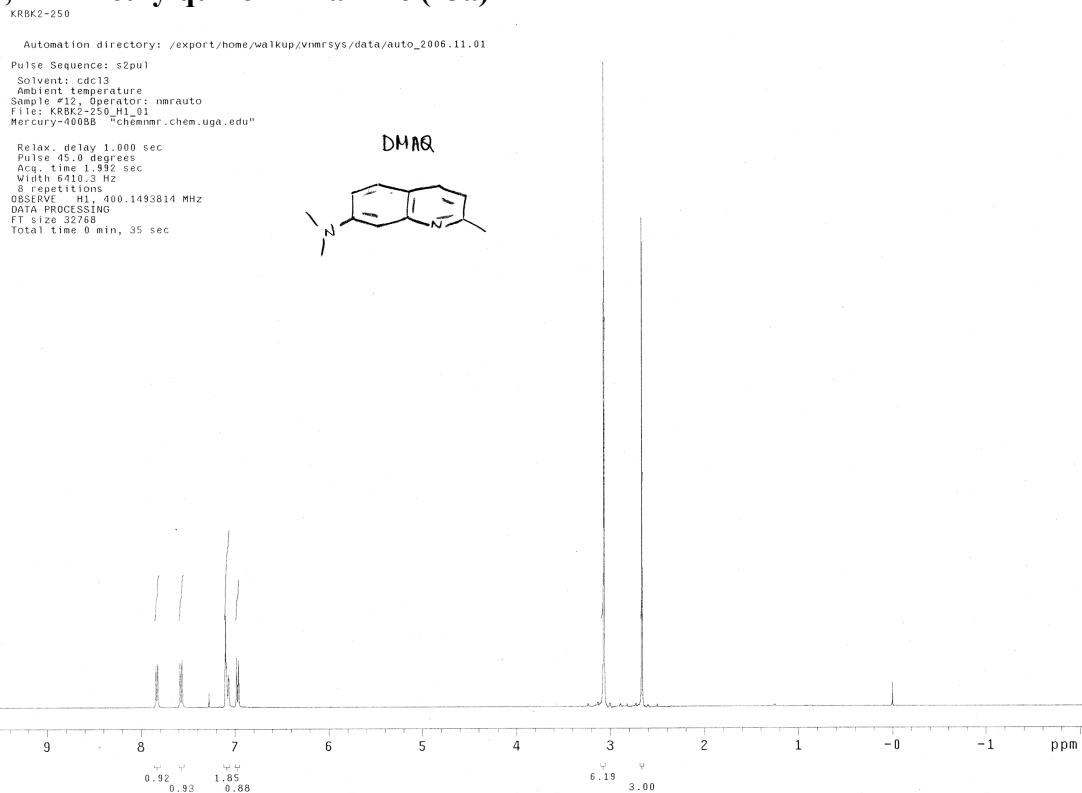
(8-Cyano-7-(methoxymethoxy)quinolin-2-yl)methyl acetate (11b)



(8-Chloro-7-(methoxymethoxy)quinolin-2-yl)methyl acetate (11c)



N,N,2-Trimethylquinolin-7-amine (13a)



7-(Dimethylamino)quinoline-2-carbaldehyde (14a)

KRBK2-251

Automation directory: /export/home/walkup/vnmrsys/data/auto_2006.11.01

Pulse Sequence: s2pul

Solvent: cdcl3

Ambient temperature

Sample #2, Operator: nmrauto

File: KRBK2-251_H1_01

Mercury-400BB "chemmr.chem.uga.edu"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.932 sec

Width 6410.3 Hz

8 repetitions

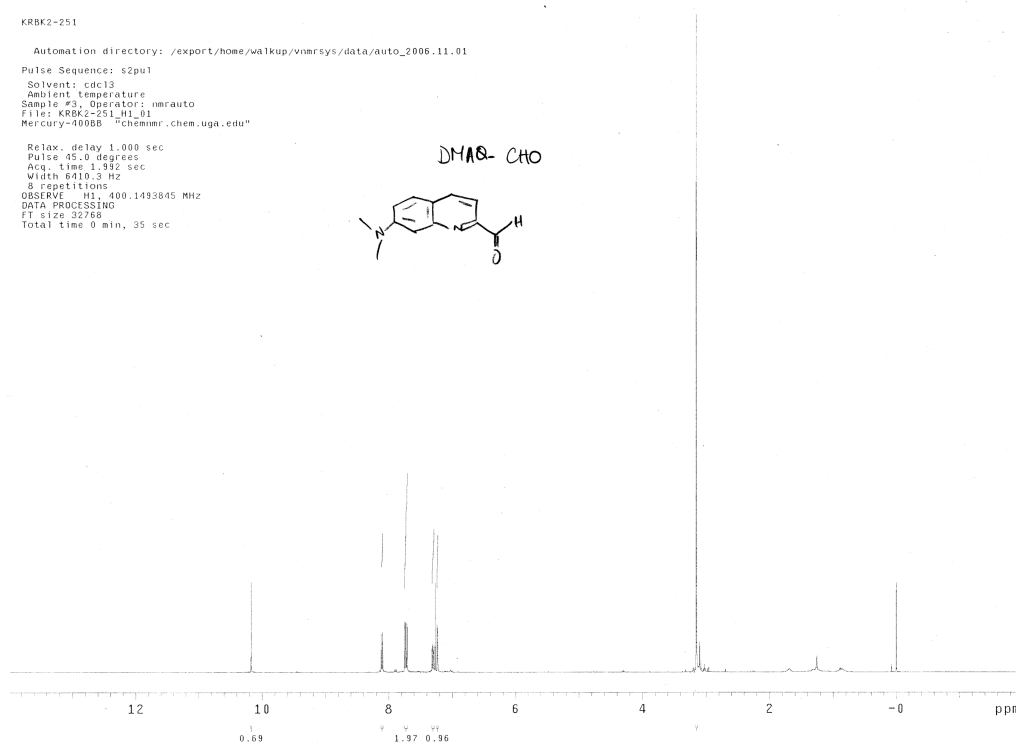
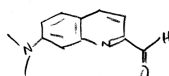
OBSERVE H1, 400.1493845 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 35 sec

DMAQ-CHO



4-Chloro-7-(dimethylamino)quinoline-2-carbaldehyde (14b)

Automation directory: /export/home/walkup/vnmrsys/data/auto_2006.11.01

Sample id: s_20060405_04

Sample: KRBK3-248c

Pulse Sequence: s2pul

Solvent: cdcl3

Ambient temperature

Sample #7, Operator: kredclie

File: KRBK3-248c_Proton_2006Apr05_01

Mercury-400BB "chemmr.chem.uga.edu"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.998 sec

Width 4803.1 Hz

16 repetitions

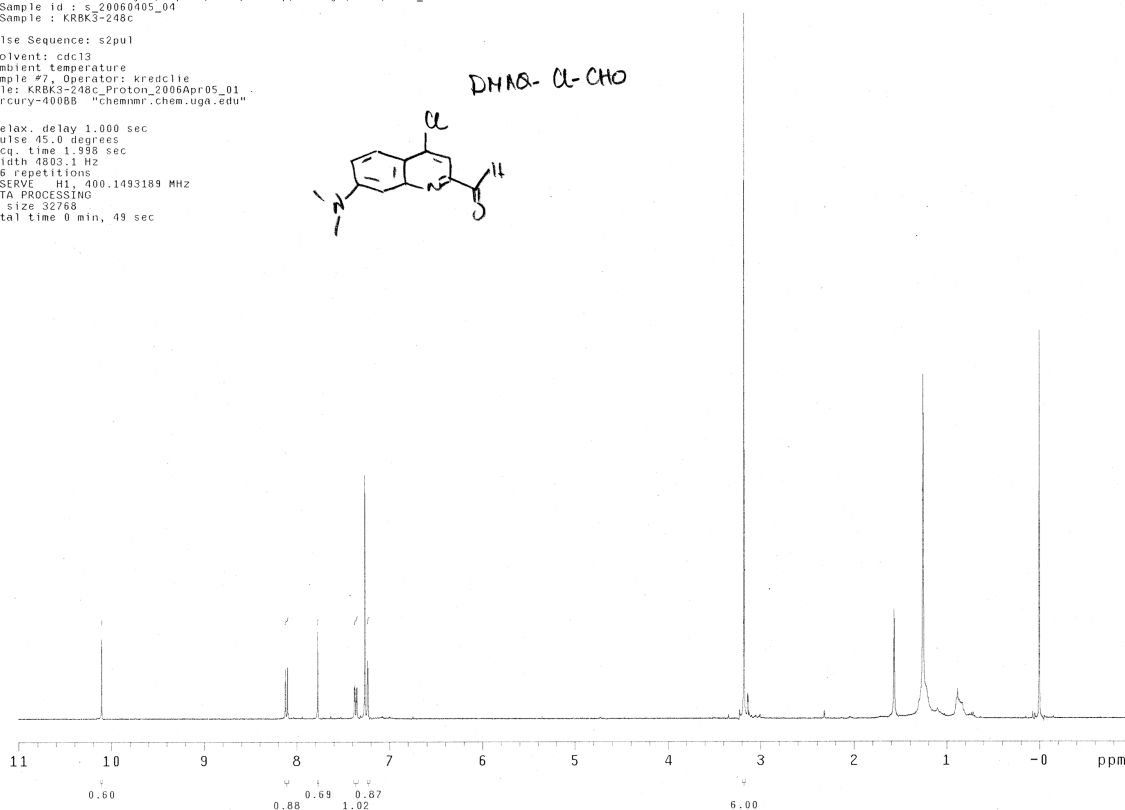
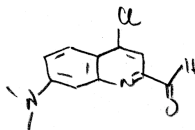
OBSERVE H1, 400.1493189 MHz

DATA PROCESSING

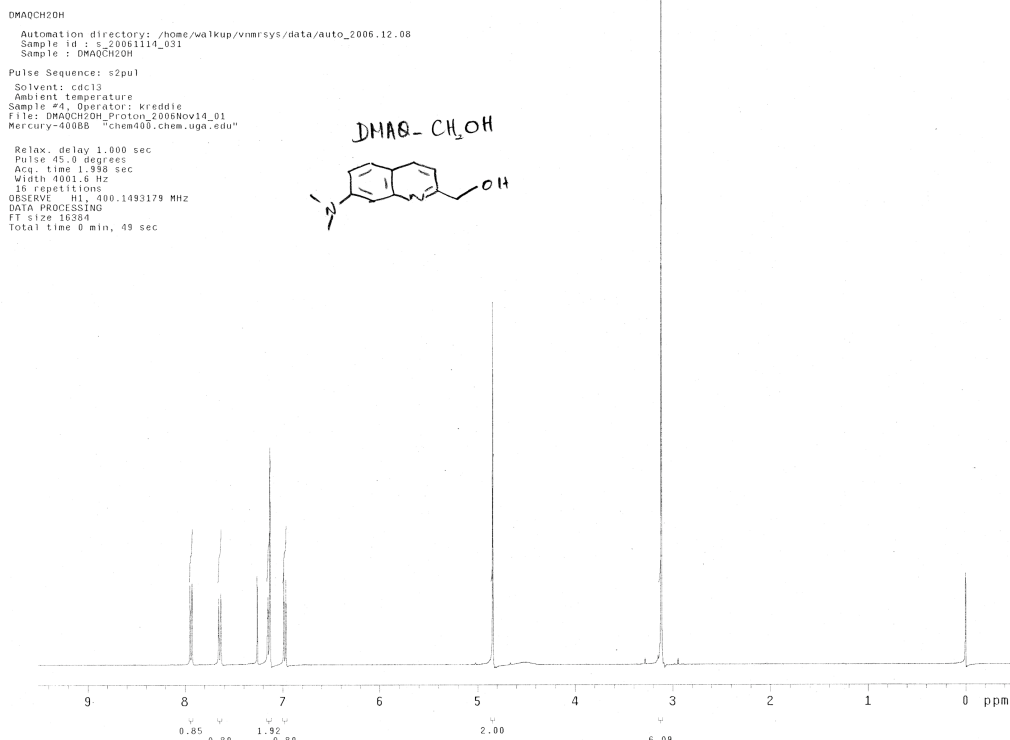
FT size 32768

Total time 0 min, 49 sec

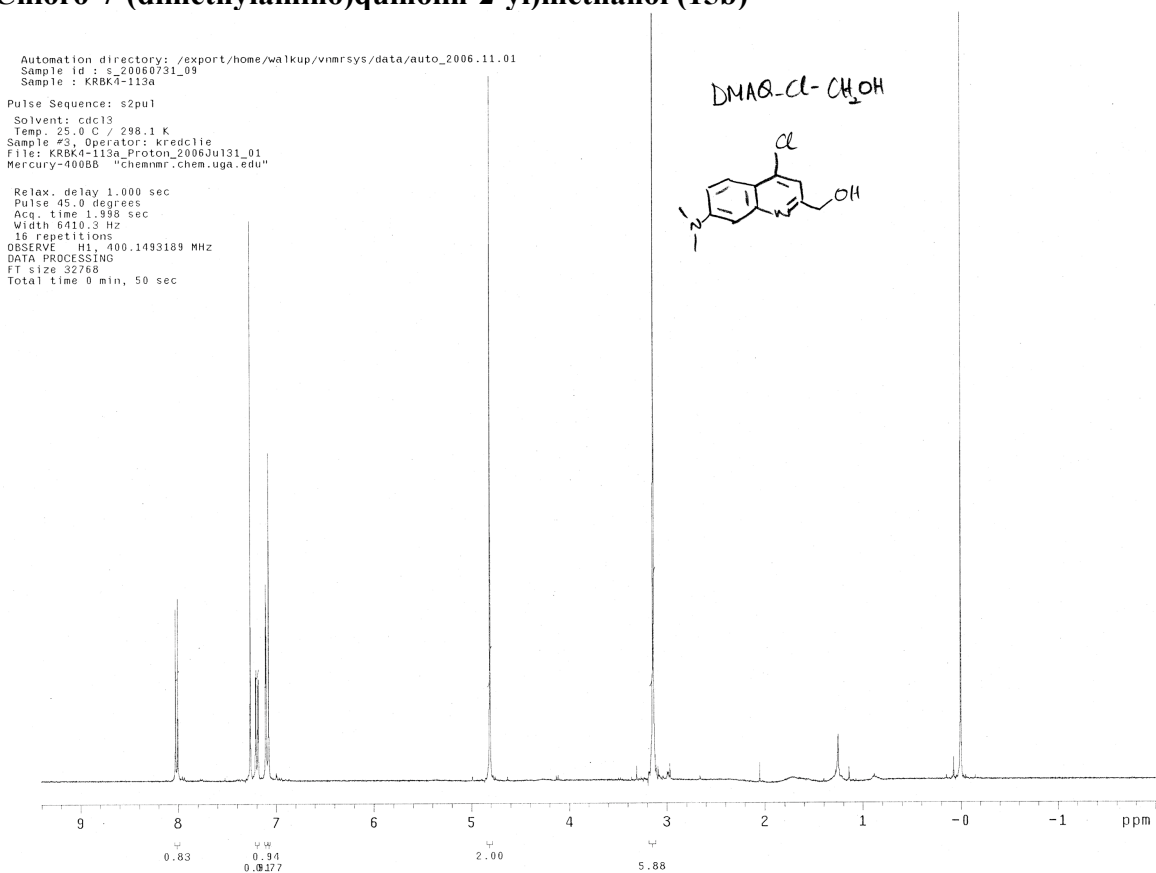
DMAQ-Cl-CHO



7-(Dimethylamino)quinolin-2-yl)methanol (15a)



(4-Chloro-7-(dimethylamino)quinolin-2-yl)methanol (15b)



O-2-Methylquinolin-7-yl dimethylcarbamothioate (16)

Automation directory: /export/home/walkup/auto_2006.09.18
Sample id: s_20060914_08
Sample: KRBK4-143b

Pulse Sequence: s2pul

Solvent: cdcl3

Ambient temperature

Sample #6, Operator: kredclie

File: KRBK4-143b_Proton_2006Sep14_01

Mercury-400BB "chem400"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 2.000 sec

Width 4001.6 Hz

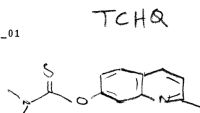
32 repetitions

OBSERVE H1, 400.1493179 MHz

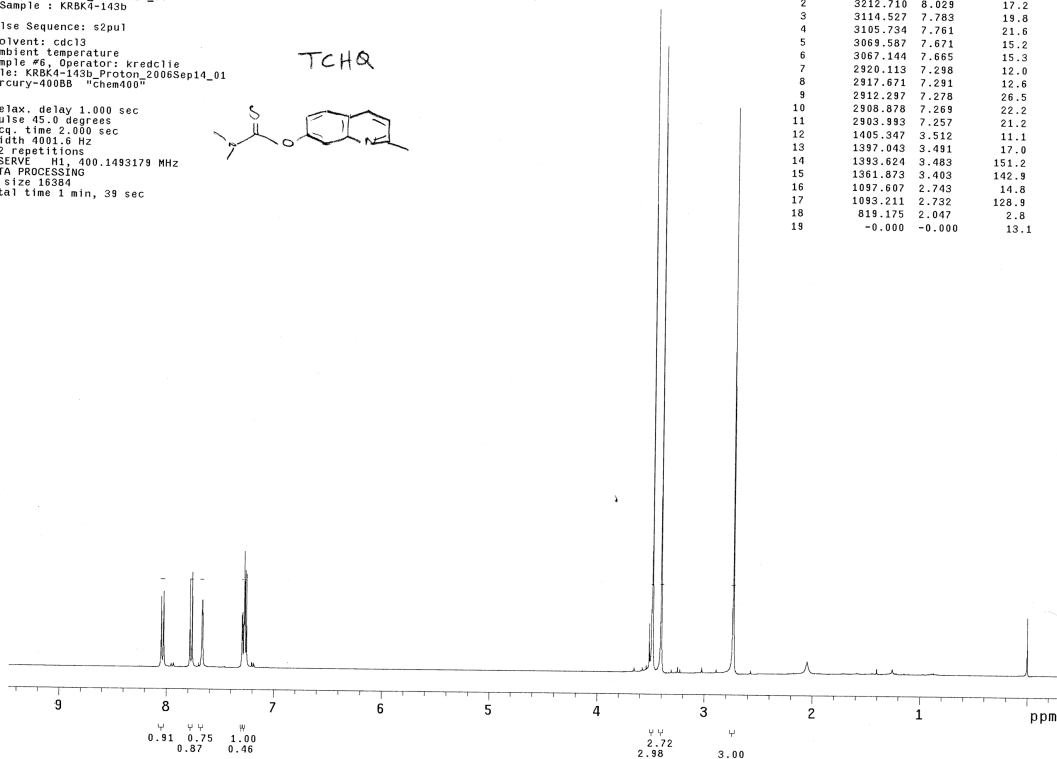
DATA PROCESSING

FT size 16384

Total time 1 min, 39 sec



INDEX	FREQUENCY	PPM	HEIGHT
1	3221.014	8.050	16.0
2	3212.710	8.029	17.2
3	3114.527	7.783	19.8
4	3105.734	7.761	21.6
5	3069.587	7.671	15.2
6	3067.144	7.665	15.3
7	2920.113	7.298	12.0
8	2917.671	7.291	12.6
9	2912.297	7.278	26.5
10	2908.878	7.269	22.2
11	2903.993	7.257	21.2
12	1405.347	5.512	11.1
13	1397.043	5.491	17.0
14	1393.624	5.483	151.2
15	1361.873	5.403	142.9
16	1087.607	2.743	14.8
17	1093.211	2.732	128.9
18	819.175	2.047	2.8
19	-0.000	-0.000	13.1



S-2-Methylquinolin-7-yl dimethylcarbamothioate (17)

Automation directory: /export/home/walkup/auto_2006.09.19
Sample id: s_20060919_19
Sample: KRBK4-153

Pulse Sequence: s2pul

Solvent: cdcl3

Ambient temperature

Sample #2, Operator: kredclie

File: KRBK4-153_Proton_2006Sep19_01

Mercury-400BB "chem400"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.998 sec

Width 6410.3 Hz

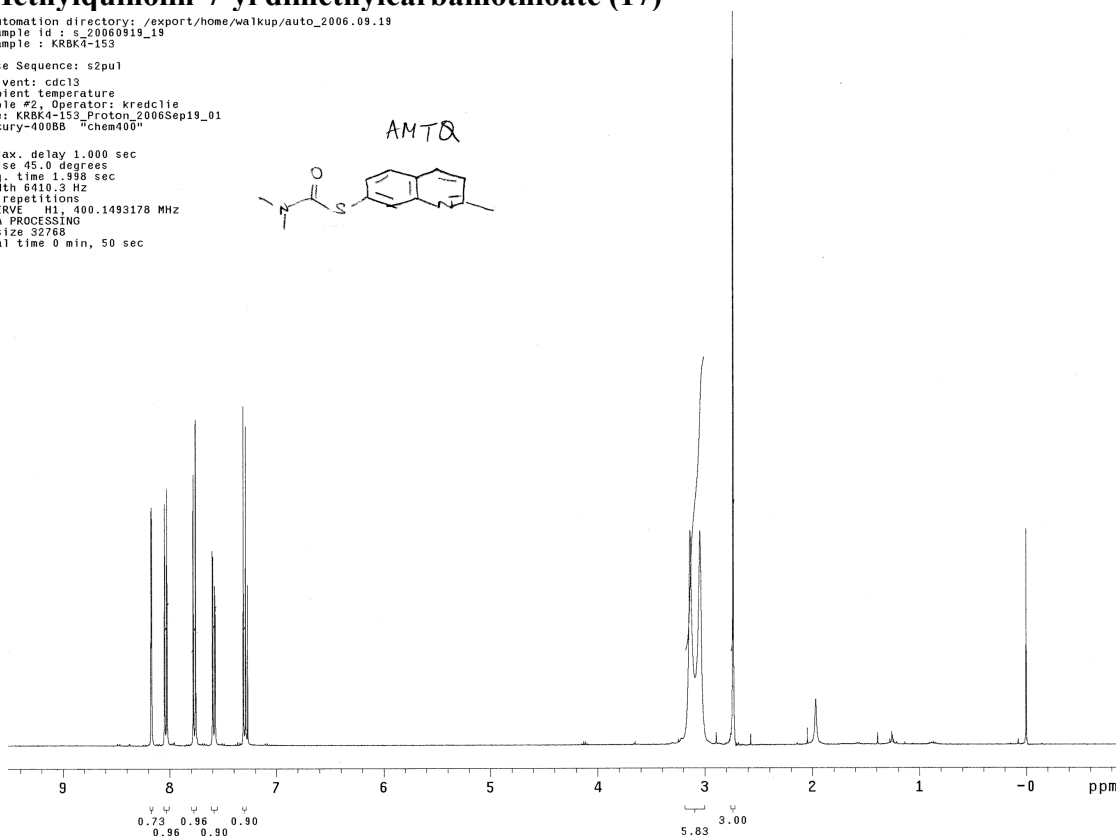
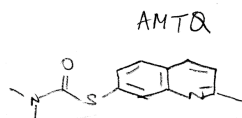
16 repetitions

OBSERVE H1, 400.1493178 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 50 sec



S-2-Formylquinolin-7-yl dimethylcarbamothioate (18)

Automation directory: /export/home/walkup/auto_2006.09.25
Sample id: s_20060925_16
Sample: KRBK4-158

Pulse Sequence: s2pul

Solvent: cdcl3

Ambient temperature

Sample #2, Operator: kredclie

File: KRBK4-158_Proton_2006Sep25_01

Mercury-400BB "Chem400"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.995 sec

Width 6410.3 Hz

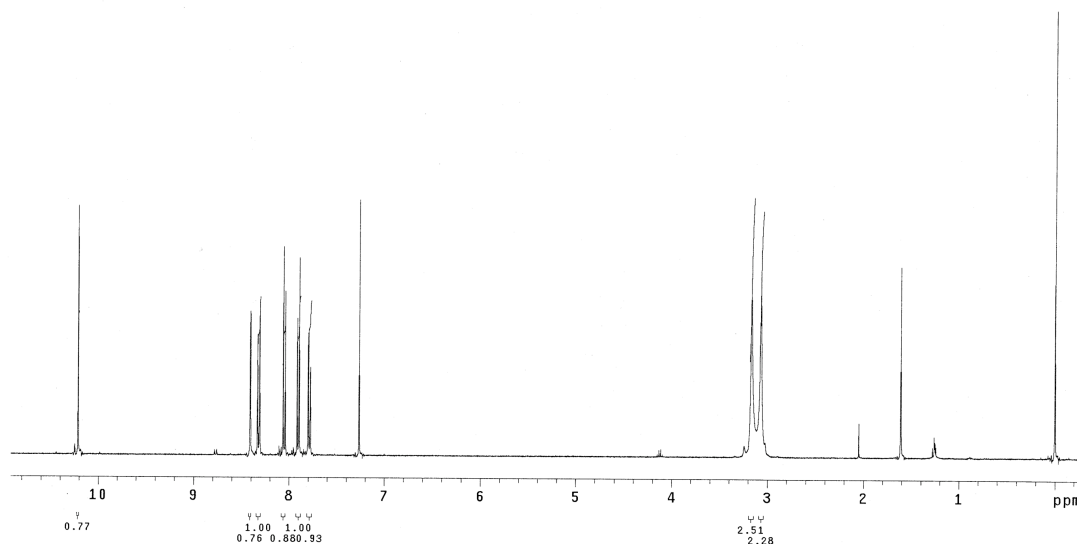
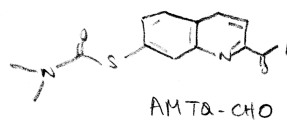
8 repetitions

OBSERVE H1, 400.1493201 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 25 sec



(7,7'-Disulfanediylbis(quinoline-7, 2-diyl))dimethanol (20)

TQdimerCH2OH

Automation directory: /home/walkup/vnmr/sys/data/auto_2006.11.22
Sample id: s_20061127_022
Sample: KRBK4-204A

Pulse Sequence: s2pul

Solvent: cdcl3

Ambient temperature

Sample #1, Operator: kredclie

File: KRBK4-204A_Proton_2006Nov27_01

Mercury-400BB "Chem400.Chem.uga.edu"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.998 sec

Width 4001.6 Hz

16 repetitions

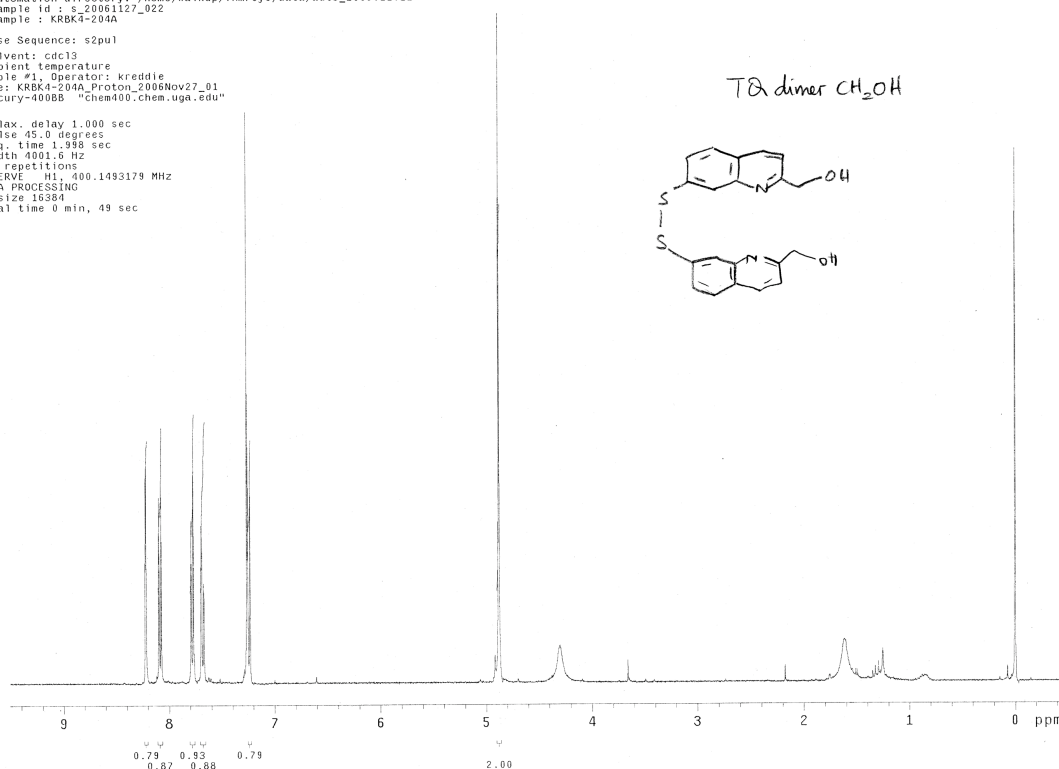
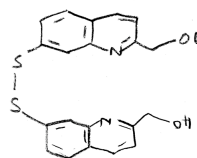
OBSERVE H1, 400.1493179 MHz

DATA PROCESSING

FT size 16384

Total time 0 min, 49 sec

TQ dimer CH₂OH



(7,7'-Disulfanediylbis(quinoline-7,2-diyl))bis(methylene) diacetate (21)

