Characterization of a Reductively-Activated Elimination Pathway Relevant to the Biological Chemistry of the Kinamycins and Lomaiviticins

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Chemical Science

Supporting Information

Index

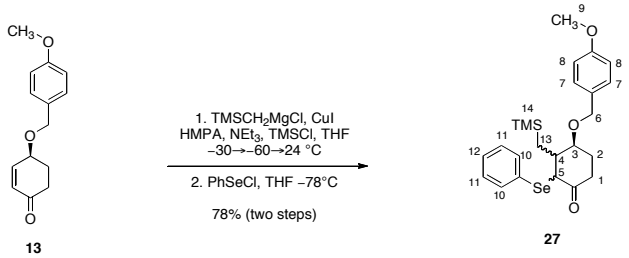
General Experimental Procedures. All reactions were performed in single-neck, flame-dried, round-bottomed flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula, or were handled in a nitrogen-filled drybox (working oxygen level <1 ppm). Organic solutions were concentrated by rotary evaporation at 30–33 °C. Flash-column chromatography was performed as described by Still et al.,[1] employing silica gel (60 Å, 40–63 μm particle size) purchased from Sorbent Technologies (Atlanta, GA). Analytical thin-layered chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore size) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV) and/or submersion in aqueous ceric ammonium molybdate solution (CAM) or acidic *p*-anisaldehyde solution (PAA), followed by brief heating on a hot plate (120 °C, 10–15 s). Preparative thin-layered chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore size) in a glass chamber equilibrated with eluent. Preparative TLC plates were visualized by exposure to UV light.

Materials. Commercial solvents and reagents were used as received with the following exceptions. Benzene, dichloromethane, and toluene were purified according to the method of Pangborn et al.[2] Acetonitrile, *N,N*-diisopropylethylamine, and triethylamine were distilled from calcium hydride under an atmosphere of nitrogen immediately before use. Tetrahydrofuran was distilled from sodium/benzophenone under an atmosphere of nitrogen immediately before use. Methanol was distilled from magnesium methoxide under an atmosphere of nitrogen immediately before use. Hexamethylphosphoramide and trimethylsilylchloride were distilled from calcium hydride and stored under nitrogen. Polymer-bound triphenylphosphine (styrene-divinylbenzene copolymer, 20% cross-linked, 3.00 mmol triphenylphophine/g resin) was obtained from Strem Chemicals (Newburyport, MA) and was stored in a nitrogen-filled drybox. Tris(diethylamino)sulfonium difluorotrimethylsilicate [TASF(Et)] was prepared by a modification[3] of the procedure described by Middleton,[4] and was stored in a nitrogen-filled drybox at –20 °C. 4-(*S*)-[(4-methoxybenzyl)oxy]-cyclohex-2-en-1-one (**13**),[5,6] 2,3-dibromo-5,6-dimethoxynaphthoquinone (**28**),[7] 2,3-dibromonaphthazarin (**29**),[7] the *ortho*-quinone methide **33**,[8] the monomeric lomaiviticin aglycon (**24**),[9] and 4,4-dimethylcyclohex-2-en-1-one (**35**)[10] were prepared according to published procedures. Solutions of trifluoromethanesulfonyl azide in hexanes were prepared according to the method of Charette and co-workers[11] immediately before use. Solutions of trifluoromethanesulfonyl azide in hexanes were standardized by ^{19}F NMR analysis using 2,2,2-trifluoromethylacetophenone[12] as an internal standard (TfN_3 δ F = –72.1 ppm; $PhCOCF_3$ δ F = –76.3 ppm; hexanes).

Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 400 or 500 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br = broad, app = apparent), integration, coupling constant in Hertz, and assignment. Proton-decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 100 or 125 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent ($CDCl_3$, δ 77.16). Distortionless enhancement by polarization transfer spectra [DEPT (135)] were recorded at 100 or 125 MHz at 24 °C, unless otherwise noted. ^{13}C NMR and DEPT (135) data are combined and represented as follows: chemical shift, carbon type [obtained from DEPT (135) experiments]. Proton-decoupled fluorine nuclear magnetic resonance spectra (^{19}F NMR) were recorded at 376 MHz at 24 °C in hexanes. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were obtained using a Thermo Electron Corporation Nicolet 6700

ATR-FTIR spectrometer referenced to a polystyrene standard. Data are represented as follows: frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). Circular dichroism (CD) spectra were obtained using a Jasco spectropolarimeter scanning over wavelengths of 200–600 nm with a bandwidth of 0.5 nm, as a compilation of 10 scans at 24 °C. CD spectra are represented as follows: wavelength at local maximum or minimum (nm), relative intensity. Analytical ultra high-performance liquid chromatography-mass spectrometry (UPLC/MS) was performed on a Waters UPLC/MS instrument equipped with a dual atmospheric pressure chemical ionization (API)/electrospray (ESI) mass spectrometry detector and photodiode array detector. Unless otherwise noted, samples were eluted over a reverse-phase C_{18} column (1.7 μm particle size, 2.1 × 50 mm) with a linear gradient of 20% acetonitrile–water→100% acetonitrile containing 0.1% formic acid over 3 min, followed by 0.1% formic acid–acetonitrile for 1 min, at a flow rate of 800 μL/min. Analytical UPLC/MS data are represented as follows: retention time (t_R) in min. High-resolution mass spectrometry (HRMS) data were obtained at the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University.

Synthetic Procedures.[13]

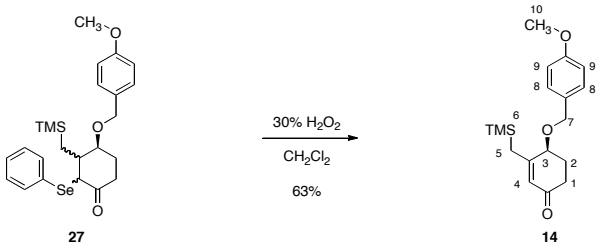


*Copper-Mediated Addition of Trimethylsilylmethylmagnesium Chloride to 4-(S)-[(4-Methoxybenzyl)oxy]-cyclohex-2-en-1-one (**13**) and Phenylselenylation of the Resulting Enoxysilane:*

A solution of trimethylsilylmethylmagnesium chloride in ether (1.00 M, 27.0 mL, 27.0 mmol, 3.00 equiv) was added to a suspension of cuprous iodide (171 mg, 900 μmol, 0.10 equiv) in tetrahydrofuran (11 mL) at 24 °C. The resulting suspension was immediately cooled to −30 °C. A solution of 4-(*S*)-[(4-methoxybenzyl)oxy]-cyclohex-2-en-1-one [**13**, 2.09 g, 9.01 mmol, 1 equiv; dried by azeotropic distillation with benzene (2 × 1 mL)] in tetrahydrofuran (11 mL) was then added dropwise via cannula over 2 min. The resulting pale yellow solution was stirred for 10 min at −30 °C, and then was cooled to −60 °C. The reaction mixture was stirred for 30 min at −60 °C and then was treated with hexamethylphosphoramide (4.70 mL, 27.0 mmol, 3.00 equiv), triethylamine (3.76 mL, 27.0 mmol, 3.00 equiv) and trimethylsilyl chloride (2.25 mL, 22.5 mmol, 2.50 equiv) in sequence. Upon completion of the addition, the solution was stirred for 30 min at −60 °C. The reaction mixture was then warmed over 10 min to 24 °C and was stirred at this temperature for 1 h. The product mixture was poured into a separatory funnel that had been charged with an ice-cold solution of 0.1 M aqueous sodium phosphate buffer (pH 7, 100 mL). The layers that formed were separated, and the aqueous layer was extracted with ether (4 × 200 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was dissolved in tetrahydrofuran (80 mL) and the resulting solution was cooled to −78 °C. A solution of phenylselenyl chloride (2.07 g, 10.8 mmol, 1.20 equiv) in tetrahydrofuran (10 mL) was then added dropwise via cannula over 5 min to the cooled mixture. The resulting orange solution was stirred for 10 min at −78 °C and then was warmed to 24 °C. The warmed solution was stirred for 20 min at 24 °C. The product mixture was diluted with saturated aqueous ammonium chloride solution (100 mL) and the diluted mixture was transferred to a separatory funnel. The layers that formed were separated, and the aqueous layer was extracted with dichloromethane (3 × 150 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with 5% ethyl acetate–hexanes initially, grading to 15% ethyl acetate–hexanes, two steps) to afford the α-selenide **27** as a viscous yellow oil (3.35 g, 78%; 5:1 mixture of diastereomers, stereochemistry not assigned).

R_f = 0.59 (25% ethyl acetate–hexanes; UV, PAA). ^{1}H NMR (500 MHz, $CDCl_3$, major diastereomer only): δ 7.55–7.53 (m, 2H, H_{11}), 7.39 (d, J = 8.7 Hz, 2H, H_7), 7.27–7.25 (m, 3H, H_{10}/H_{12}), 6.92 (d, J = 8.7 Hz, 2H, H_8), 4.71 (d, J = 11.7 Hz, 1H, H_6), 4.60 (d, J = 11.7 Hz, 1H, H_6), 3.81 (s, 3H, H_9), 3.58 (td, J = 14.4, 6.7, 1H, H_3), 3.50 (br, 1H, H_2), 3.39 (br, 1H, H_5), 2.92 (t, J = 6.1 Hz, 1H, H_4), 2.13 (ddd, J = 14.3, 5.4, 2.6 Hz, 2H, H_1/H_2), 2.07–1.96 (m, 1H, H_1/H_2), 0.60 (dd, J = 15.0, 8.2 Hz, 1H, H_{13}), 0.48 (dd, J = 15.0, 6.5 Hz, 1H, H_{13}), –0.05 (s, 9H, H_{14}). ^{13}C NMR (125

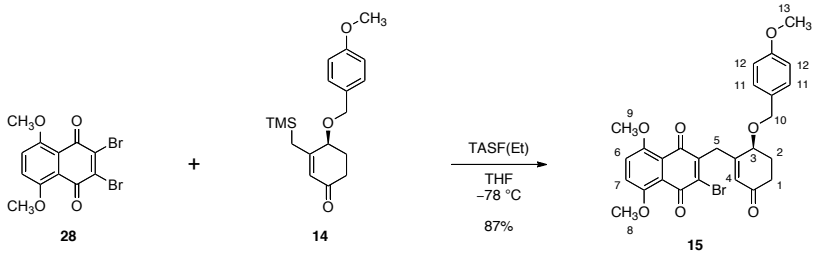
MHz, $CDCl_3$, major diastereomer only): δ 208.4 (C), 159.3 (C), 133.6 (CH), 132.6 (C), 130.5 (C), 129.2 (CH), 129.2 (CH), 127.8 (CH), 113.9 (CH), 76.5 (CH), 70.5 (CH_2), 55.4 (CH_3), 54.0 (CH), 43.1 (CH), 31.9 (CH_2), 26.3 (CH_2), 22.0 (CH_2), –1.0 (CH_3). IR (ATR-FTIR), cm^{-1}: 2950 (w), 1697 (s), 1512 (s), 1246 (s), 834 (s). LC/MS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{24}H_{33}O_3SeSi$, 477.1360; found, 477.1363.



*Oxidation of the α-Selenide **27**:*

A solution of aqueous hydrogen peroxide (30%, 754 μL, 6.63 mmol, 1.50 equiv) was added dropwise via syringe to a stirred solution of the α-selenide **27** (2.10 g, 4.42 mmol, 1 equiv) in dichloromethane (22 mL) at 24 °C. The resulting biphasic mixture was vigorously stirred for 40 min at 24 °C. The product mixture was diluted with dichloromethane (20 mL) and the diluted mixture was transferred to a separatory funnel. The layers that formed were separated, and the organic layer was washed with saturated aqueous sodium bicarbonate solution (10 mL). The washed organic layer was dried over sodium sulfate. The dried solution was eluted over silica gel (7.8 × 5.0 cm) and the filtrate was collected. The silica gel was washed with ethyl acetate (300 mL) and the filtrates were combined. The combined filtrates were concentrated and the residue obtained was purified by flash-column chromatography (eluting with 10% ethyl acetate–hexanes initially, grading to 50% ethyl acetate–hexanes, three steps) to afford the enone **14** as a light brown, viscous oil (885 mg, 63%).

R_f = 0.29 (25% ethyl acetate–hexanes; UV, PAA). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (d, J = 8.5 Hz, 2H, H_8), 6.89 (d, J = 8.7 Hz, 2H, H_9), 5.71 (s, 1H, H_4), 4.65 (d, J = 11.3 Hz, 1H, H_7), 4.43 (d, J = 11.3 Hz, 1H, H_7), 3.96 (br, 1H, H_3), 3.79 (s, 3H, H_{10}), 2.62–2.51 (m, 1H, H_1/H_2), 2.30–2.23 (m, 1H, H_1/H_2), 2.20–2.15 (m, 1H, H_1/H_2), 2.08–2.03 (m, 1H, H_1/H_2), 1.98 (d, J = 11.8 Hz, 1H, H_5), 1.77 (d, J = 11.8 Hz, 1H, H_5), 0.00 (s, 9H, H_6). ^{13}C NMR (125 MHz, $CDCl_3$) δ 198.1 (C), 165.6 (C), 159.4 (C), 129.9 (C), 129.6 (CH), 124.9 (CH), 113.9 (CH), 74.4 (CH), 71.1 (CH_2), 55.3 (CH_3), 34.0 (CH_2), 27.2 (CH_2), 26.5 (CH_2), –1.1 (CH_3). IR (ATR-FTIR), cm^{-1}: 2953 (w), 1663 (m), 1611 (m), 1512 (m), 1245 (s), 839 (s). LC/MS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{18}H_{27}O_3Si$, 319.1729; found, 319.23.

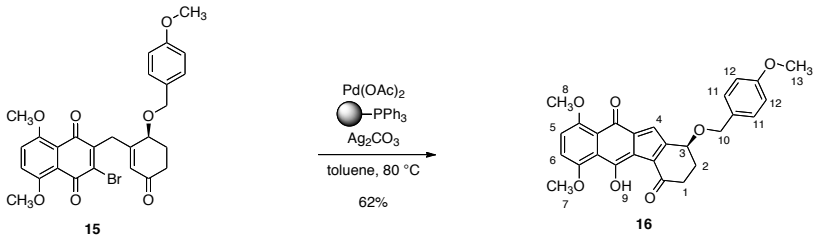


28 14 15

*Coupling of 2,3-Dibromo-5,6-dimethoxynaphthoquinone (**28**) and the β-Trimethylsilylmethyl Enone **14**:*

2,3-Dibromo-5,6-dimethoxynaphthoquinone (**28**) was dried under vacuum overnight at 45 °C before use. The β-trimethylsilylmethyl enone **14** was dried under vacuum overnight at 24 °C before use. A mixture of 2,3-dibromo-5,6-dimethoxynaphthoquinone (**28**, 355 mg, 943 μmol, 6.00 equiv) and the β-trimethylsilylmethyl enone **14** (50.0 mg, 157 μmol, 1 equiv) was dried by azeotropic distillation from 33% benzene–dichloromethane (2 × 7.5 mL). The resulting red residue was dissolved in tetrahydrofuran (15 mL) and the solution that formed was cooled to −78 °C. A solution of TASF(Et) (43.0 mg, 157 μmol, 1.00 equiv) in tetrahydrofuran (1 mL) was added dropwise over 30 min via syringe pump to the cold solution. The reaction mixture was stirred for 1 h at −78 °C. The cold product solution was diluted with 0.1 M aqueous sodium phosphate buffer solution (pH 7, 20 mL). The diluted solution was warmed over 10 min to 24 °C. The warmed biphasic mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with dichloromethane (3 × the coupled product **15** as an orange foam (80.0 mg, 87%).

R_f = 0.23 (75% ethyl acetate–hexanes; UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (app s, 2H, H_6/H_7), 7.24 (d, J = 8.5 Hz, 2H, H_{11}), 6.84 (d, J = 8.7 Hz, 2H, H_{12}), 5.68 (s, 1H, H_4), 4.67 (d, J = 11.3 Hz, 1H, H_{10}), 4.48 (d, J = 11.3 Hz, 1H, H_{10}), 4.28 (br, 1H, H_3), 3.99 (d, J = 15.5 Hz, 1H, H_5), 3.99 (s, 3 H, H_8/H_9), 3.91 (s, 3H, H_8/H_9), 3.81 (s, 3H, H_{13}), 3.64 (d, J = 16.4 Hz, 1H, H_5), 2.64–2.52 (m, 1H, H_1/H_2), 2.37–2.20 (m, 2H, H_1/H_2), 2.16–2.10 (m, 1H, H_1/H_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.4 (C), 180.4 (C), 176.3 (C), 159.3 (C), 159.2 (C), 154.1 (C), 154.0 (C), 147.1 (C), 140.1 (C), 129.7 (C), 129.2 (CH), 126.8 (CH), 120.5 (CH), 120.5 (CH), 120.0 (C), 120.0 (C), 113.8 (CH), 74.3 (CH), 71.3 (CH_2), 56.9 (CH_3), 56.8 (CH_3), 55.2 (CH_3), 35.7 (CH_2), 34.2 (CH_2), 27.3 (CH_2). IR (ATR-FTIR), cm^{-1}: 2937 (w), 1660 (s), 1513 (m), 1264 (s), 1209 (s), 1030 (s). HRMS-CI (*m/z*): $[M + H]^+$ calcd for $C_{27}H_{26}{}^{79/81}BrO_7$, 541.0862/543.0841; found, 541.0856/543.0842.



*Cyclization of the Coupled Product **15**:*

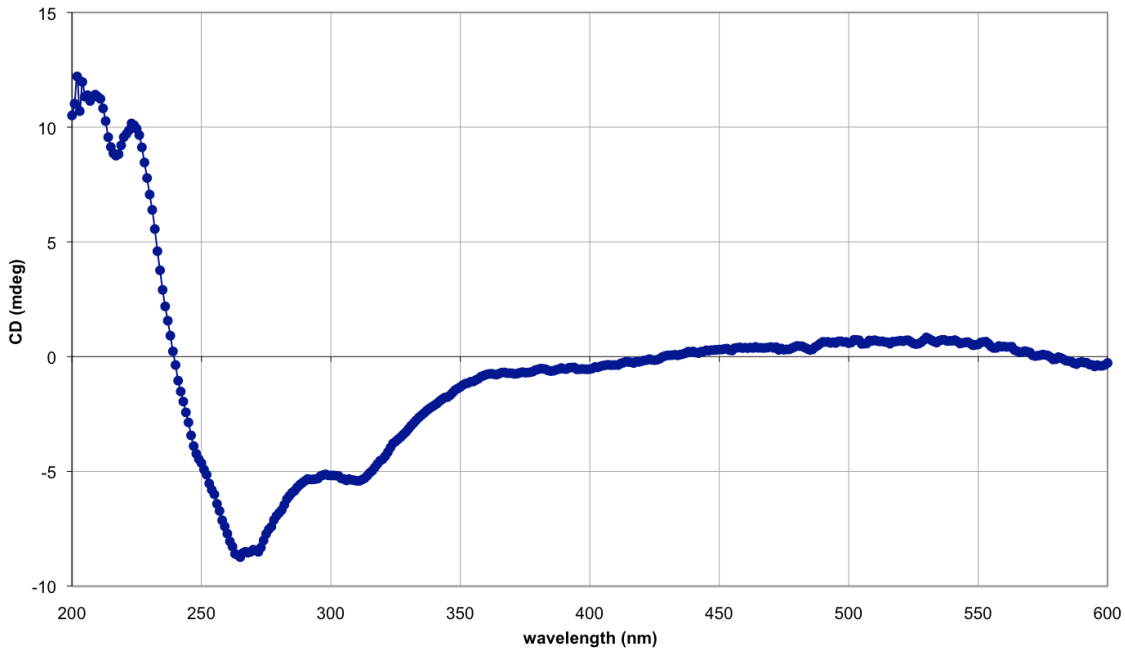
A 1-dram vial equipped with a magnetic stirbar and fitted with a rubber septum was charged with the coupled product **15** (67.0 mg, 123 μmol, 1 equiv). The coupled product **15** was dried by azeotropic distillation with benzene (3 × 500 μL). The vial was then sealed and transferred to a nitrogen-filled drybox. In the drybox, the vial was charged with palladium(II) acetate (27.7 mg, 123 μmol, 1.00 equiv) and polymer-bound triphenylphosphine (41.3 mg, 123 μmol triphenylphosphine, 1.00 equiv). The vial was removed from the drybox and then silver carbonate (68.3 mg, 248 μmol, 2.00 equiv) was added. The mixture was dried by azeotropic distillation with benzene (500 μL). Toluene (830 μL) was then added and the vial was sealed with a Teflon-lined cap. The sealed vial was placed in an oil bath that had been preheated to 80 °C. The reaction mixture was stirred and heated for 1.5 h at 80 °C. The product solution was cooled over 5 min to 24 °C and the cooled solution was filtered through Celite. The inside of the reaction vial was washed with dichloromethane (5 × 3 mL) and the washes were filtered over the Celite pad. The Celite pad was rinsed with acetone (5 mL). The filtrates were combined and the combined filtrates were concentrated to dryness. The residue obtained was purified by preparative thin-layer chromatography (eluting with 35% acetone–toluene). A red band (R_f = 0.45) was isolated and extracted from the silica gel with acetone (100 mL), followed by filtration of the silica gel. The filtrate was concentrated and the residue obtained was purified by preparative thin-layer chromatography (three elutions, 30% acetone–hexanes, followed by 50% acetone–hexanes, followed by 75% acetone–hexanes). A red band (R_f = 0.55) was isolated and extracted from the silica gel with acetone (100 mL), followed by filtration of the silica gel. The filtrate was concentrated to afford the *ortho*-quinone methide **16** as a purple film (35.5 mg, 62%).

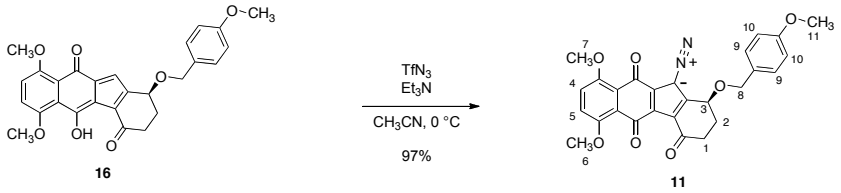
NOTE: ^{1}H NMR and LC/MS analysis of the crude product mixture revealed that the desired product formed as the only major species (est. purity >80%). However, we have found that the *ortho*-quinone methide **16** is unstable to purification and should be handled with care. For example, the *ortho*-quinone methide **16** undergoes solvolysis readily upon exposure to protic solvents (eg., methanol, ethanol, etc.; see manuscript). Flash-column chromatography results in extensive decomposition, irrespective of any conditions we have examined. Samples of **16** should be stored as solids at –20 °C to ensure compound integrity.

R_f = 0.18 (two elutions: 25% acetone–hexanes; UV, CAM). t_R = 1.79. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31 (d, J = 9.5 Hz, 1H, H_5/H_6), 7.29 (d, J = 8.7 Hz, 2H, H_{11}), 7.26 (d, J = 9.5 Hz, 1H, H_5/H_6), 6.91 (s, 1H, H_4), 6.88 (d, J = 8.5 Hz, 2H, H_{12}), 4.62 (dd, J = 5.0, 4.0 Hz, 1H, H_3), 4.58 (d, J = 12.0 Hz, 1H, H_{10}), 4.54 (d, J = 11.5 Hz, 1H, H_{10}), 4.00 (s, 3H, H_7/H_8), 3.97 (s, 3H, H_7/H_8), 3.81 (s, 3H, H_{13}), 3.07 (ddd, J = 18.0, 9.1, 5.0 Hz, 1H, H_1/H_2), 2.65 (dt, J = 18.0, 5.1 Hz, 1H, H_1/H_2), 2.37–2.23 (m, 2H, H_1/H_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.7 (C), 181.7 (C), 177.1 (C), 159.4 (C), 156.0 (C), 155.9 (C), 147.9 (C), 137.4 (C), 130.5 (C), 129.4 (CH), 123.6 (C), 123.4 (C), 122.6 (CH), 121.7 (C), 120.8 (CH) 119.8 (CH), 114.1 (CH), 70.3 (CH_2), 69.7 (CH), 57.5 (CH_3), 57.4 (CH_3), 55.5 (CH_3), 31.0 (CH_2), 30.6 (CH_2). IR (ATR-FTIR), cm^{-1}: 2931 (w),

1593 (m), 1405 (m), 1262 (m), 1186 (m). CD [1 mM, CH_3CN, λ (mdeg)] = 223 (10), 265 (–9), 310 (–5). HRMS-CI (*m/z*): $[M + H]^+$ calcd for $C_{27}H_{25}O_7$, 460.1595; found, 461.1600.

Graph S1. CD spectrum of **16** (1 mM in acetonitrile).





16 11

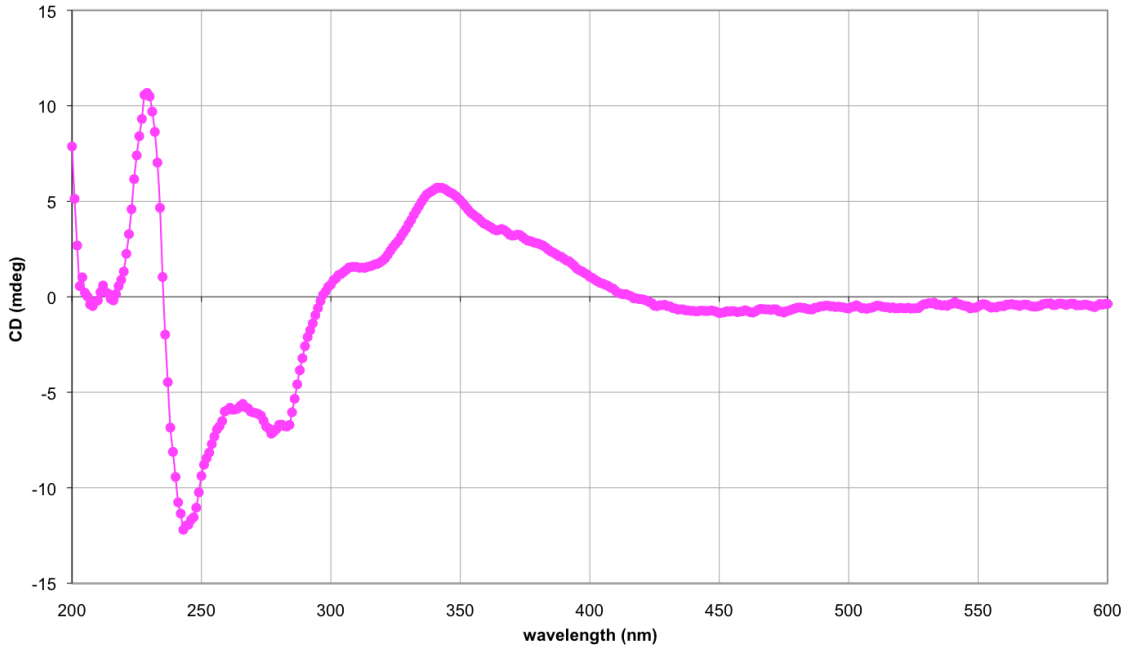
*Diazotransfer to the Ortho-Quinone Methide **16**:*

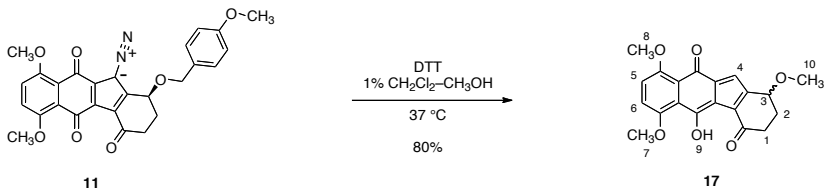
The *ortho*-quinone methide **16** (6.00 mg, 13.0 μmol, 1 equiv) was dissolved in acetonitrile (260 μL), to give a deep red solution. The solution was cooled to 0 °C and the cooled solution was treated with triethylamine (27.0 μL, 196 μmol, 15.0 equiv). The resulting yellow-orange mixture was stirred for 5 min at 0 °C. A solution of trifluoromethanesulfonyl azide in hexanes (228 mM, 147 μL, 32.0 μmol, 2.50 equiv) was added dropwise via syringe and the resulting brown solution was stirred for 5 min at 0 °C, with protection from light (aluminum foil). The product mixture was diluted with dichloromethane (20 mL) and the diluted mixture was transferred to a separatory funnel. An aqueous solution of cuprous sulfate (1.0 M, 20 mL) was added to the separatory funnel. The separatory funnel was stoppered and the stoppered funnel was vigorously shaken. The layers that formed were separated and the aqueous layer was extracted with dichloromethane (20 mL). The organic layers were combined and the combined organic layers were washed with distilled water (2 × 10 mL). The washed organic layer was dried over sodium sulfate

NOTE: The diazofluorene **11** is a very reactive molecule and poses several safety hazards. The diazofluorene **11** is potentially shock sensitive and may be an explosive hazard. Exposure to reducing agents should be avoided. Furthermore, the diazofluorene **11** is cytotoxic and all synthetic manipulations should be carried out with due diligence to personal safety. Samples of **11** should be stored as solids at –20 °C with protection from light.

R_f = 0.18 (100% ethyl acetate; UV, CAM). t_R = 2.04. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.30 (d, J = 9.5 Hz, 1H, H_4/H_5), 7.28 (d, J = 8.5 Hz, 2H, H_9), 7.22 (d, J = 9.5 Hz, 1H, H_4/H_5), 6.93 (d, J = 8.7 Hz, 2H, H_{10}), 4.93 (dd, J = 10.2, 4.7 Hz, 1H, H_3), 4.76 (d, J = 11.0 Hz, 1H, H_8), 4.55 (d, J = 11.0 Hz, 1H, H_8), 3.97 (s, 3H, H_6/H_7), 3.93 (s, 3H, H_6/H_7), 3.83 (s, 3H, H_{11}), 2.76 (dt, J = 16.8, 4.0 Hz, 1H, H_1/H_2), 2.61–2.55 (m, 1H, H_1/H_2), 2.55–2.46 (m, 1H, H_1/H_2), 2.17–2.04 (m, 1H, H_1/H_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.0 (C), 182.4 (C), 178.6 (C), 160.0 (C), 158.6 (C), 158.1 (C), 151.4 (C), 131.6 (C), 130.5 (CH), 130.2 (CH), 129.0 (C), 128.5 (CH), 128.4 (C), 124.9 (C), 114.4 (CH), 113.3 (C), 112.3 (C), 73.0 (CH), 71.1 (CH_2), 71.1 (C), 57.5 (C), 56.9 (C), 55.5 (CH_3), 37.6 (CH_2), 29.8 (CH_2). IR (ATR-FTIR), cm^{-1}: 3529 (br), 2935 (w), 2145 (s), 1679 (s), 1437 (s), 1239 (s). CD [1 mM, CH_3CN, λ (mdeg)] = 229 (11), 246 (–12), 277 (–7), 341 (6). HRMS-CI (*m/z*): $[M + H]^+$ calcd for $C_{27}H_{23}N_2O_7$, 487.1500; found, 487.1505.

Graph S2. CD spectrum of **11** (1 mM in acetonitrile).


15
10
5
0
-5
-10
-15
CD (mdeg)
200
250
300
350
400
450
500
550
600
wavelength (nm)

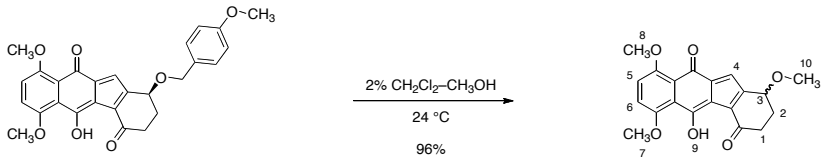


11 17

*Reduction–Solvolysis of the Diazofluorene **11**:*

The diazofluorene **11** (10.0 mg, 20.6 μmol, 1 equiv) was dissolved in 1% dichloromethane–methanol [v/v, 1.0 mL; each solvent was deoxygenated separately by sparging with dry argon (30 min) before use] and treated with dithiothreitol (3.20 mg, 20.6 μmol, 1.00 equiv). The reaction mixture was warmed to 37 °C and stirred at this temperature for 24 h. The purple product solution was diluted with dichloromethane (30 mL) and the diluted solution was transferred to a separatory funnel. The organic layer was washed with distilled water (10 mL). The washed organic layer was dried over sodium sulfate and the dried solution was filtered. The filtrate was concentrated and the residue obtained was purified by preparative thin-layer chromatography (eluting with 30% acetone–toluene). A yellow band (R_f = 0.20) was isolated and extracted from the silica gel with acetone (100 mL), followed by filtration of the silica gel. The filtrate was concentrated to afford the *ortho*-quinone methide **17** as a purple film (5.8 mg, 80%).

R_f = 0.50 (50% acetone–hexanes; UV, CAM). t_R = 1.91. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32 (d, J = 9.5 Hz, 1H, H_5/H_6), 7.26 (d, J = 9.5 Hz, 1H, H_5/H_6), 6.90 (s, 1H, H_4), 4.43 (t, J = 4.4 Hz, 1H, H_3), 4.00 (s, 3H, H_7/H_8), 3.97 (s, 3H, H_7/H_8), 3.42 (s, 3H, H_{10}), 3.03 (ddd, J = 18.1, 8.7, 5.6 Hz, 1H, H_1/H_2), 2.65 (dt, J = 18.1, 5.0 Hz, 1H, H_1/H_2), 2.34–2.25 (m, 2H, H_1/H_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.5 (C), 181.7 (C), 177.4 (C), 156.0 (C), 155.9 (C), 147.3 (C), 137.4 (C), 123.7 (C), 123.4 (C), 122.6 (CH), 121.8 (C), 121.4 (C), 120.8 (CH), 119.8 (CH), 72.3 (CH), 57.5 (CH_3), 57.3 (CH_3), 56.6 (CH_3), 30.7 (CH_2), 30.4 (CH_2). IR (ATR-FTIR), cm^{-1}: 2928 (m), 1594 (s), 1561 (s), 1406 (s), 1265 (s). HRMS-CI (*m/z*): $[M + H]^+$ calcd for $C_{20}H_{19}O_6$, 355.1176; found, 355.1176.

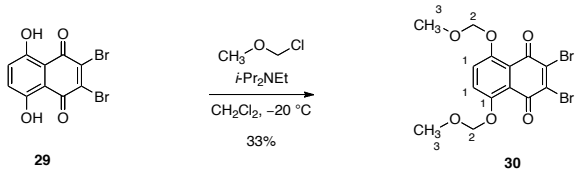


16 17

*Solvolysis of the Ortho-Quinone Methide **16**:*

A solution of the *ortho*-quinone methide **16** (13.3 mg, 28.9 mmol, 1 equiv) in 2% dichloromethane–methanol (v/v, 2.8 mL) was stirred for 72 h at 24 °C. The product mixture was concentrated to dryness and the residue obtained was purified by preparative thin-layer chromatography (two elutions, 30% acetone–toluene). A purple band (R_f = 0.35) was isolated and extracted from the silica gel with acetone (100 mL), followed by filtration of the silica gel. The filtrate was concentrated to afford the methanolysis product **17** as a purple film (9.9 mg, 96%).

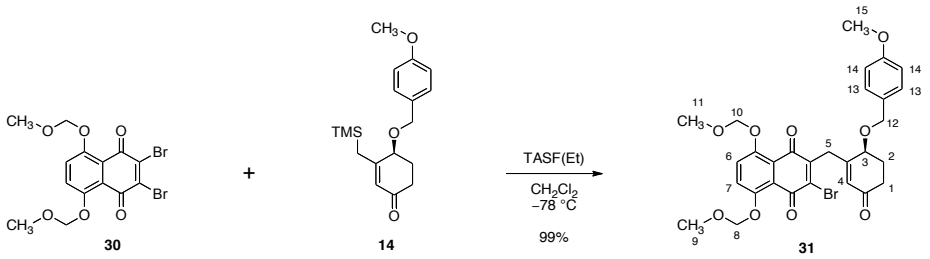
R_f = 0.50 (50% acetone–hexanes). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32 (d, J = 9.5 Hz, 1H, H_5/H_6), 7.26 (d, J = 9.5 Hz, 1H, H_5/H_6), 6.90 (s, 1H, H_4), 4.43 (t, J = 4.4 Hz, 1H, H_3), 4.00 (s, 3H, H_7/H_8), 3.97 (s, 3H, H_7/H_8), 3.42 (s, 3H, H_{10}), 3.03 (ddd, J = 18.1, 8.7, 5.6 Hz, 1H, H_1/H_2), 2.65 (dt, J = 18.1, 5.0 Hz, 1H, H_1/H_2), 2.34–2.25 (m, 2H, H_1/H_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.5 (C), 181.7 (C), 177.4 (C), 156.0 (C), 155.9 (C), 147.3 (C), 137.4 (C), 123.7 (C), 123.4 (C), 122.6 (CH), 121.8 (C), 121.4 (C), 120.8 (CH), 119.8 (CH), 72.3 (CH), 57.5 (CH_3), 57.3 (CH_3), 56.6 (CH_3), 30.7 (CH_2), 30.4 (CH_2). IR (ATR-FTIR), cm^{-1}: 2928 (m), 1594 (s), 1561 (s), 1406 (s), 1265 (s). HRMS-CI (*m/z*): $[M + H]^+$ calcd for $C_{20}H_{19}O_6$, 355.1176; found, 355.1176.



*Synthesis of 2,3-Dibromo-5,8-di(methoxymethyloxy)naphthoquinone **30**:*

Chloromethyl methyl ether (7.50 mL, 101 mmol, 4.83 equiv) was added to a solution of 2,3-dibromonaphthazarin (**29**) in dichloromethane (287 mL) at –20 °C. *N,N*-Diisopropylethylamine (25.4 mL, 144 mmol, 7.00 eq) was added dropwise via syringe pump over 4 h to the cold mixture. Upon completion of the addition, the reaction mixture was stirred for 8 h at –20 °C. The product mixture was diluted with saturated aqueous sodium bicarbonate solution (200 mL) and the diluted solution was warmed over 20 min to 24 °C. The warmed biphasic mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with dichloromethane (2 × 50 mL) and the organic layers were combined. The combined organic layers were dried over sodium sulfate and the dried solution was filtered. The filtrate was concentrated and the residue obtained was purified by flash-column chromatography, eluting with 30% ethyl acetate–hexanes initially, grading to 7% ethyl acetate–dichloromethane, one step) to afford the quinone **30** as a red solid (2.90 g, 33%).

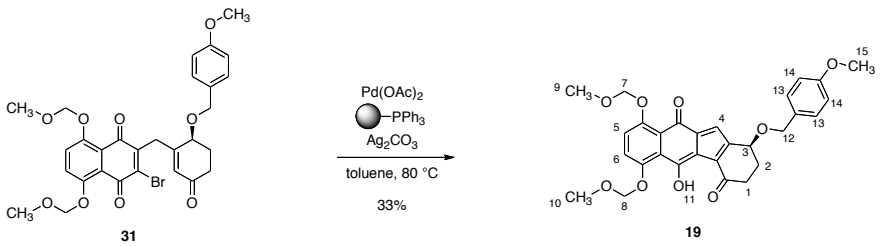
R_f = 0.74 (40% ethyl acetate–hexanes; UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.52 (app s, 2H, H_1/H_2), 5.29 (s, 4H, H_2), 3.54 (s, 6H, H_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.7 (C), 153.0 (C), 141.8 (C), 125.1 (CH), 120.7 (C), 95.8 (CH_2), 56.7 (CH_3). IR (ATR-FTIR), cm^{-1}: 2936 (w), 1665 (m), 1144 (m), 896 (m). HRMS-CI (*m/z*): $[M + H]^+$ calcd for $C_{14}H_{13}{}^{79/79;79/81;81/81}Br_2O_6$, 438.9038/436.9058/434.9079; found, 438.9020/436.9042/434.9071.



*Coupling of 2,3-Dibromo-5,8-di(methoxymethyloxy)naphthoquinone (**30**) and the β-Trimethylsilylmethyl Enone **14**:*

2,3-Dibromo-5,8-di(methoxymethyloxy)naphthoquinone (**30**) was dried under vacuum for 30 min at 45 °C before use. The β-trimethylsilylmethyl enone **14** was dried under vacuum for 30 min at 24 °C before use. A mixture of 2,3-dibromo-5,6-di(methoxymethyloxy)naphthoquinone (**30**, 972 mg, 2.22 mmol, 3.85 equiv) and the β-trimethylsilylmethyl enone **14** (184 mg, 580 μmol, 1 equiv) were combined in one flask and dried by azeotropic distillation from 33% benzene–dichloromethane (2 × 7.5 mL). The dried solid was heated under vacuum for 30 min at 45 °C. The red residue obtained was dissolved in dichloromethane (144 mL) and the resulting solution was cooled to −78 °C. A solution of TASF(Et) (159 mg, 580 μmol, 1.00 equiv) in dichloromethane (1 mL) was added dropwise over 3 min via syringe to the cold solution. The reaction mixture was stirred for 30 min at −78 °C and then was warmed to 24 °C. The warmed solution was stirred for 1 h at 24 °C. The product mixture was transferred to a separatory funnel that had been charged with 0.1 M aqueous sodium phosphate buffer solution (pH 7, 50 mL). The layers that formed were separated and the aqueous layer was extracted with dichloromethane (2 × 100 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography on deactivated silica gel (6% w/w distilled water; eluting initially with 10% ethyl acetate–hexanes, grading to 50% ethyl acetate–hexanes, two steps) to afford the coupled product **31** as an orange foam (346 mg, 99%).

R_f = 0.18 (50% ethyl acetate–hexanes; UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46 (app s, 2H, H_6/H_7), 7.23 (d, J = 8.5 Hz, 2H, H_{13}), 6.83 (d, J = 8.5 Hz, 2H, H_{14}), 5.67 (s, 1H, H_4), 5.27 (s, 2H, H_8/H_{10}), 5.22 (s, 2H, H_8/H_{10}), 4.67 (d, J = 11.3 Hz, 1H, H_{12}), 4.47 (d, J = 11.3 Hz, 1H, H_{12}), 4.26 (dd, J = 6.1, 4.2 Hz, 1H, H_3), 3.96 (d, J = 16.4 Hz, 1H, H_5), 3.78 (s, 3H, H_{15}), 3.63 (d, J = 16.0 Hz, 1H, H_5), 3.54 (s, 3H, H_9/H_{11}), 3.49 (s, 3H, H_9/H_{11}), 2.64–2.51 (m, 1H, H_1/H_2), 2.33–2.27 (m, 1H, H_1/H_2), 2.27–2.20 (m, 2H, H_1/H_2), 2.17–2.08 (m, 1H, H_1/H_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 198.5 (C), 180.4 (C), 176.3 (C), 159.4 (C), 159.3 (C), 152.7 (C), 152.6 (C), 147.4 (C), 140.4 (C), 129.8 (C), 129.4 (CH), 127.0 (CH), 125.1 (CH), 124.9 (CH), 121.4 (C), 121.3 (C), 114.0 (CH), 96.0 (CH_2), 96.0 (CH_2), 74.4 (CH), 71.5 (CH_2), 56.8 (CH_3), 56.7 (CH_3), 55.4 (CH_3), 35.9 (CH_2), 34.3 (CH_2), 27.4 (CH_2). IR (ATR-FTIR), cm^{-1}: 2906 (w), 1733 (m), 1664 (s), 1246 (m), 1144 (s). LC/MS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{29}H_{30}{}^{79/81}BrO_9$, 601.1073/603.1053; found, 601.35/603.21.



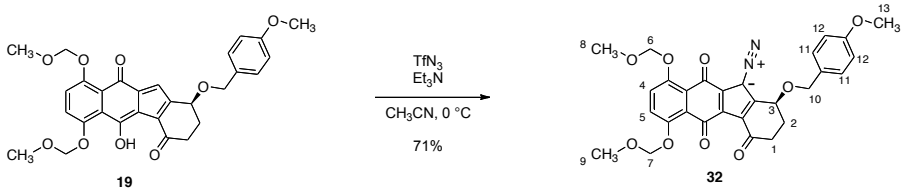
31 19

*Cyclization of the Coupled Product **31**:*

A 1-dram vial equipped with a magnetic stirbar and fitted with a rubber septum was charged with the coupled product **31** (98.0 mg, 163 μmol, 1 equiv). The coupled product **31** was dried by azeotropic distillation with benzene (2 × 1 mL). The vial was sealed and the sealed vial was transferred to a nitrogen-filled drybox. In the drybox, the vial was charged with palladium(II) acetate (36.5 mg, 163 μmol, 1.00 equiv) and polymer-bound triphenylphosphine (54.4 mg, 163 μmol triphenylphosphine, 1.00 equiv). The vial was removed from the drybox and then silver carbonate (89.7 mg, 326 μmol, 2.00 equiv) was added. The mixture obtained was dried by azeotropic distillation with benzene (500 μL). Toluene (1.1 mL) was then added and the vial was sealed with a Teflon-lined cap. The sealed vial was placed in an oil bath that had been preheated to 80 °C. The reaction mixture was stirred and heated for 1.5 h at 80 °C. The product solution was cooled over 5 min to 24 °C and the cooled solution was filtered through Celite. The inside of the reaction vial was washed with dichloromethane (3 × 5 mL) and the washes were filtered over the Celite pad. The filtrates were combined and the combined filtrates were concentrated to dryness. The resulting brown residue was purified by preparative thin-layer chromatography (two elutions, 35% acetone–hexanes, followed by 50% acetone–hexanes). A red band (R_f = 0.50) was isolated and extracted from the silica gel with acetone (100 mL), followed by filtration of the silica gel. The filtrate was concentrated and the residue obtained was purified by preparative thin-layer chromatography (eluting with 30% acetone–toluene). A red band (R_f = 0.55) was isolated and extracted from the silica gel with acetone (100 mL), followed by filtration of the silica gel. The filtrate was concentrated to afford the *ortho*-quinone methide **19** as a purple film (27.8 mg, 33%).

NOTE: ^{1}H NMR and LC/MS analysis of the crude product mixture revealed that the desired product formed as the only major species prior to purification (est. purity >80%). However, we have found that *ortho*-quinone methide **19** is unstable to purification and should be handled with care. For example, the *ortho*-quinone methide **19** undergoes solvolysis readily upon exposure to protic solvents (eg., methanol, ethanol, etc.; see manuscript). Flash-column chromatography results in extensive decomposition, irrespective of any conditions we have examined. Samples of **19** should be stored as solids at –20 °C to ensure compound integrity.

R_f = 0.43 (50% ethyl acetate–hexanes). t_R = 2.82. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43 (d, J = 9.3 Hz, 1H, H_5/H_6), 7.38 (d, J = 9.5 Hz, 1H, H_5/H_6), 7.28 (d, J = 8.7 Hz, 2H, H_{13}), 6.92 (s, 1H, H_4), 6.88 (d, J = 8.5 Hz, 2H, H_{14}), 5.30 (s, 2H, H_7/H_8), 5.27 (s, 2H, H_7/H_8), 4.62 (m, 1H, H_3), 4.57 (d, J = 11.7 Hz, 1H, H_{12}), 4.54 (d, J = 11.5 Hz, 1H, H_{12}), 3.80 (s, 3H, H_{15}), 3.57 (s, 3H, H_9/H_{10}), 3.57 (s, 3H, H_9/H_{10}), 3.08 (ddd, J = 17.9, 8.8, 5.0 Hz, 1H, H_1/H_2), 2.65 (dt, J = 18.1, 4.8 Hz, 1H, H_1/H_2), 2.34–2.27 (m, 2H, H_1/H_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 192.3 (C), 181.3 (C), 176.4 (C), 159.4 (C), 154.5 (C), 154.3 (C), 148.3 (C), 137.1 (C), 130.4 (C), 129.4 (CH), 127.4 (CH), 125.5 (CH), 124.8 (C), 123.3 (C), 123.0 (C), 122.1 (C), 120.1 (CH), 114.0 (CH), 96.7 (CH_2), 96.6 (CH_2), 70.3 (CH_2), 69.6 (CH), 56.9 (CH_3), 56.8 (CH_3), 55.4 (CH_3), 31.0 (CH_2), 30.8 (CH_2). IR (ATR-FTIR), cm^{-1}: 2934 (br), 1595 (s), 1247 (s), 1182 (s), 1151 (s), 1075 (s). LC/MS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{29}H_{29}O_9$, 521.1812; found, 521.33.



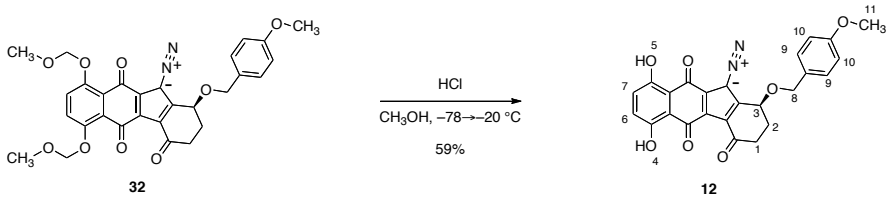
19 32

*Diazotransfer to the Ortho-Quinone Methide **19**:*

The *ortho*-quinone methide **19** (9.00 mg, 17.0 μmol, 1 equiv) was dissolved in acetonitrile (350 μL) to give a deep red solution. The red solution was cooled to 0 °C and the cooled solution was treated with triethylamine (36.0 μL, 259 μmol, 15.0 equiv). The resulting orange mixture was stirred for 5 min at 0 °C. A solution of trifluoromethanesulfonyl azide in hexanes (228 mM, 190 μL, 43.0 μmol, 2.50 equiv) was added dropwise via syringe and the resulting brown solution was stirred for 5 min at 0 °C, with protection from light (aluminum foil). The product mixture was diluted with dichloromethane (20 mL) and the diluted mixture was transferred to a separatory funnel. A 1.0 M aqueous solution of cuprous sulfate (10 mL) was added. The separatory funnel was stoppered and the stoppered funnel was vigorously shaken. The layers that formed were separated and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The organic layers were combined and the combined organic layers were washed with distilled water (2 × 20 mL). The washed organic layer was dried over sodium sulfate □□ as a yellow film (6.7 mg, 71%).

NOTE: The diazofluorene **32** is a very reactive molecule and poses several safety hazards. The diazofluorene **32** is potentially shock sensitive and may be an explosive hazard. Exposure to reducing agents should be avoided. Furthermore, the diazofluorene **32** is very cytotoxic and all synthetic manipulations should be carried out with due diligence to personal safety. Samples of **32** should be stored as solids at –20 °C with protection from light.

R_f = 0.20 (100% ethyl acetate; UV, CAM). t_R = 1.96. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (d, J = 9.3 Hz, 1H, H_4/H_5), 7.40 (d, J = 9.3 Hz, 1H, H_4/H_5), 7.28 (d, J = 8.7 Hz, 2H, H_{11}), 6.93 (d, J = 8.4 Hz, 2H, H_{12}), 5.30 (s, 2H, H_6/H_7), 5.25 (s, 2H, H_6/H_7), 4.93 (dd, J = 10.2, 4.7 Hz, 1H, H_3), 4.76 (d, J = 11.1 Hz, 1H, H_{10}), 4.56 (d, J = 11.1 Hz, 1H, H_{10}), 3.83 (s, 3H, H_{13}), 3.56 (s, 3H, H_8/H_9), 3.54 (s, 3H, H_8/H_9), 2.77 (dt, J = 7.7, 3.6 Hz, 1H, H_1/H_2), 2.62–2.42 (m, 2H, H_1/H_2), 2.15–2.05 (m, 1H, H_1/H_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 190.1 (C), 179.6 (C), 178.3 (C), 159.9 (C), 153.1 (C), 152.9 (C), 150.7 (C), 132.7 (C), 130.6 (C), 130.2 (CH), 128.7 (C), 127.4 (CH), 126.5 (C), 124.1 (C), 123.4 (CH), 122.3 (C), 114.3 (CH), 97.0 (CH_2), 95.9 (CH_2), 72.7 (CH), 70.9 (CH_2), 56.8 (CH_3), 56.7 (CH_3), 55.5 (CH_3), 37.3 (CH_2), 29.9 (CH_2). IR (ATR-FTIR), cm^{-1}: 2918 (br), 2146 (s), 1679 (s), 1514 (s), 1439 (s), 1239 (s), 1150 (s). LC/MS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{29}H_{27}N_2O_9$, 547.1717; found, 547.33.



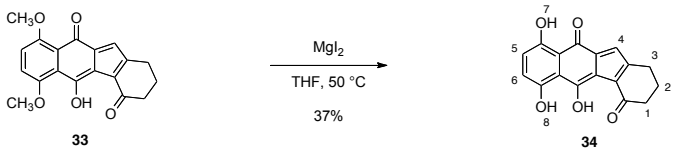
32 12

*Deprotection of the Diazofluorene **32***:

The diazofluorene **32** [17.0 mg, 31.0 μmol, 1 equiv; dried by azeotropic distillation from benzene (500 μL)] was dissolved in 25% dichloromethane–methanol (v/v, 2.8 mL). The resulting orange solution was cooled to −78 °C. A solution of hydrochloric acid in methanol (2.5 M, 1.25 mL, 3.11 mmol, 100 equiv) was added dropwise via syringe over 5 min to the cold solution. The resulting red reaction mixture was stirred for 10 min at −78 °C and then was warmed to −20 °C. The reaction mixture was stirred for 1.5 h at −20 °C. The product mixture was poured into a separatory funnel that had been charged with dichloromethane (30 mL). The organic layer was washed with distilled water (2 × 20 mL). The washed organic layer was dried over sodium sulfate and the dried solution was filtered. The filtrate was concentrated and the residue obtained was purified by preparative thin-layer chromatography (two elutions, 2% methanol–dichloromethane). A red band (R_f = 0.35) was isolated and extracted from the silica gel with acetone (100 mL), followed by filtration of the silica gel. The filtrate was concentrated to afford the diazofluorene **12** as a red film (8.5 mg, 59%).

NOTE: The diazofluorene **12** is a very reactive molecule and poses several safety hazards. The diazofluorene **12** is potentially shock sensitive and may be an explosive hazard. Exposure to reducing agents should be avoided. Furthermore, the diazofluorene **12** is very cytotoxic and all synthetic manipulations should be carried out with due diligence to personal safety. Samples of **12** should be stored as solids at –20 °C with protection from light.

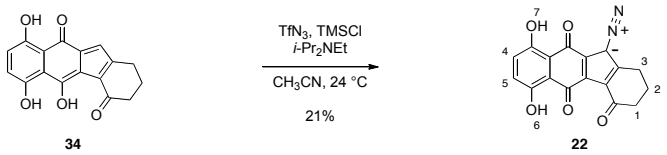
R_f = 0.22 (2% methanol–dichloromethane; UV, CAM). t_R = 2.15. ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.24 (s, 1H, H_4/H_5), 12.38 (s, 1H, H_4/H_5), 7.30 (d, J = 8.5 Hz, 2H, H_9), 7.20 (d, J = 9.5 Hz, 1H, H_6/H_7), 7.13 (d, J = 9.3 Hz, 1H, H_6/H_7), 6.94 (d, J = 8.5 Hz, 2H, H_{10}), 4.99 (dd, J = 10.5, 4.7 Hz, 1H, H_3), 4.79 (d, J = 11.0 Hz, 1H, H_8), 4.58 (d, J = 10.9 Hz, 1H, H_8), 3.84 (s, 3H, H_{11}), 2.78 (dt, J = 7.3, 3.3 Hz, 1H, H_1/H_2), 2.67–2.50 (m, 2H, H_1/H_2), 2.14–2.04 (m, 1H, H_1/H_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.0 (C), 182.4 (C), 160.0 (C), 158.6 (C), 158.1 (C), 151.4 (C), 131.6 (C), 130.5 (CH), 130.2 (CH), 129.0 (C), 128.5 (C), 128.4 (CH), 124.9 (C), 114.4 (CH), 113.3 (C), 112.3 (C), 73.0 (CH), 71.1 (CH_2), 55.5 (CH_3), 37.6 (CH_2), 29.8 (CH_2). IR (ATR-FTIR), cm^{-1}: 2951 (br), 2158 (s), 1683 (s), 1607 (s), 1443 (s), 1250 (s). LC/MS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{25}H_{19}N_2O_7$, 459.1192; found, 459.16.



*Deprotection of the Ortho-Quinone Methide **33**:*

The *ortho*-quinone methide **33** [89.0 mg, 275 μmol, 1 equiv; dried by azeotropic distillation with benzene (750 μL)] was dissolved in tetrahydrofuran (6.7 mL) in a 50-mL round-bottomed flask that had been fused to a Teflon-coated valve. A freshly-prepared solution of magnesium(II) iodide in ether (100 mM, 13.7 mL, 1.37 mmol, 5.00 equiv) was added dropwise via syringe at 24 °C. The flask was sealed and the purple reaction mixture was placed in an oil bath that had been preheated to 50 °C. The reaction mixture was stirred and heated for 2 h at 50 °C. The product solution was cooled over 5 min to 24 °C and the cooled solution was diluted sequentially with ethyl acetate (10 mL) and aqueous sulfuric acid solution (1 N, 10 mL). The resulting biphasic mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with dichloromethane (2 × 10 mL) and the organic layers were combined. The combined organic layers were dried over sodium sulfate and the dried solution was filtered. The filtrate was concentrated and the residue obtained was purified by flash-column chromatography (eluting with 0.5% acetic acid–dichloromethane) to afford the triol **34** as a purple solid (30.0 mg, 37%).

R_f = 0.43 (1% methanol–hexanes; UV, CAM). t_R = 2.42. ^{1}H NMR (500 MHz, $CDCl_3$): δ 13.34 (s, 1H, H_7/H_8), 11.69 (s, 1H, H_7/H_8), 7.16 (d, 1H, J = 9.0 Hz, H_5/H_6), 7.12 (d, 1H, J = 9.5 Hz, H_5/H_6), 6.73 (s, 1H, H_4), 2.83 (t, 2H, J = 6.5 Hz, H_1/H_3), 2.78 (t, 2H, J = 6.5 Hz, H_1/H_3), 2.20–2.14 (m, 2H, H_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 189.0 (C), 185.6 (C), 178.6 (C), 159.2 (C), 157.5 (C), 151.0 (C), 139.4 (C), 129.6 (CH), 128.9 (CH), 124.9 (C), 120.8 (C), 117.5 (CH), 113.4 (C), 112.6 (C), 32.1 (CH_2), 24.7 (CH_2), 24.4 (CH_2). IR (ATR-FTIR), cm^{-1}: 2945 (m), 1581 (s), 1450 (s). HRMS-CI (*m/z*): $[M + H]^+$ calcd for $C_{17}H_{13}O_5$, 297.0758; found, 297.0758.

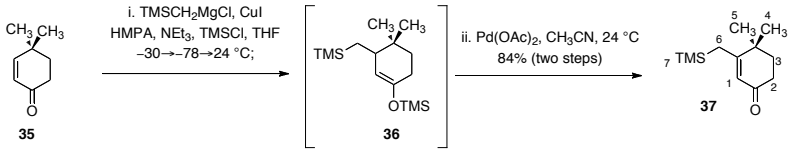


34 **22**

*Diazotransfer to the Triol **34**:*

N,N-diisopropylethylamine (38.9 μL, 223 μmol, 6.00 equiv) and trimethylsilyl chloride (11.1 μL, 111 μmol, 3.00 equiv) were added in sequence to a stirred solution of the triol **34** [11.0 mg, 37.0 μmol, 1 equiv; dried by azeotropic distillation with benzene (300 μL)] in acetonitrile (1.9 mL) at 24 °C. A solution of trifluoromethanesulfonyl azide in hexanes (220 mM, 338 μL, 74.0 μmol, 2.00 equiv) was added dropwise via syringe. The reaction mixture was stirred for 15 min at 24 °C. The product mixture was diluted sequentially with dichloromethane (30 mL) and aqueous sulfuric acid solution (1 N, 10 mL). The resulting biphasic solution was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with dichloromethane (2 × 5 mL) and the organic layers were combined. The combined organic layers were dried over sodium sulfate and the dried solution was filtered. The filtrate was concentrated and the residue obtained was purified by flash-column chromatography (eluting with 1% methanol–dichloromethane) to afford the diazofluorene **22** as a magenta solid (2.5 mg, 21%).

R_f = 0.10 (1% methanol–hexanes; UV, CAM). t_R = 1.47. ^{1}H NMR (500 MHz, $CDCl_3$): δ 13.27 (s, 1H, H_6/H_7), 12.46 (s, 1H, H_6/H_7), 7.23 (d, 1H, J = 9.0 Hz, H_4/H_5), 7.17 (d, 1H, J = 9.5 Hz, H_4/H_5), 2.96 (t, 2H, J = 6.0 Hz, H_3), 2.67 (t, 2H, J = 6.5, H_1), 2.27–2.23 (m, 2H, H_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.8 (C), 159.7 (C, determined indirectly by HMBC), 158.5 (C, determined indirectly by HMBC), 151.9 (C), 131.0 (C), 130.6 (CH), 130.4 (CH), 128.3 (C), 126.8 (C), 113.2 (C), 112.2 (C), 76.7 (C, determined indirectly by HMBC), 39.5 (CH_2), 23.4 (CH_2), 23.3 (CH_2). IR (ATR-FTIR), cm^{-1}: 2928 (m), 2143 (m), 1657 (s), 1444 (s). HRMS-CI (*m/z*): $[M + H]^+$ calcd for $C_{17}H_{11}N_2O_5$, 323.0662; found, 323.0663.

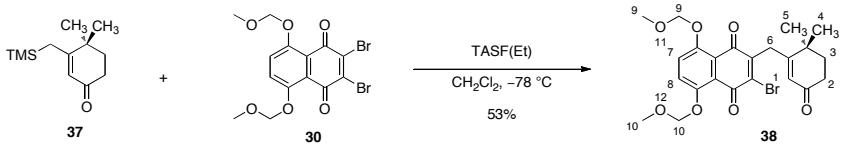


*Synthesis of the β-(Trimethylsilylmethyl)-α,β-unsaturated Ketone **37**:*

Cuprous iodide (153 mg, 805 μmol, 0.10 equiv) was added to a stirred solution of trimethylsilylmethylmagnesium chloride in ether (1.00 M, 8.86 mL, 8.86 mmol, 1.10 equiv) at 24 °C. The resulting solution was cooled to −30 °C, to afford a cloudy suspension. A solution of the cyclohexenone **35** (1.00 g, 8.05 mmol, 1 equiv) in tetrahydrofuran (2.5 mL) was then added dropwise via cannula. The flask containing the cyclohexenone **35** was rinsed with tetrahydrofuran (2 × 1.5 mL) and the rinses were added to the reaction vessel via cannula. The reaction mixture was cooled to −50 °C and then was stirred at this temperature for 30 min. The mixture was then was cooled to −60 °C and hexamethylphosphoramide (1.54 mL, 8.86 mmol, 1.10 equiv), triethylamine (2.23 mL, 16.1 mmol, 2.00 equiv), and trimethylsilyl chloride (2.05 mL, 16.1 mmol, 2.00 equiv) were added in sequence to the cooled solution. The resulting mixture was further cooled to −78 °C, and was stirred at this temperature for 1 h. The product mixture was warmed over 10 min to 24 °C, and the warmed solution was stirred for 10 min at 24 °C. The product mixture was diluted with ether (50 mL) and the diluted solution was poured into a separatory funnel that had been charged with saturated aqueous sodium bicarbonate solution (50 mL). The layers that formed were separated, and the aqueous layer was extracted with ether (50 mL). Each organic layer was washed separately with saturated aqueous sodium bicarbonate solution (2 × 30 mL) and saturated aqueous sodium chloride solution (30 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was dissolved in ether (20 mL) and the resulting solution was eluted over a pad of silica gel (2 × 5 cm). The pad of silica gel was washed with ether (3 × 30 mL), and the filtrates were combined. The combined filtrates were concentrated to afford the crude enoxysilane **36** as a clear, colorless oil (2.28 g).

Palladium(II) acetate (1.98 g, 8.82 mmol, 1.10 equiv) was added to a stirred solution of the enoxysilane **36** (2.28 g, 8.02 mmol, 1 equiv) in acetonitrile (80 mL) at 24 °C. The resulting black mixture was stirred for 12 h at 24 °C. The product mixture was diluted with ether (50 mL) and the diluted solution was filtered through a pad of celite (5 × 5 cm). The celite pad was washed with ether (3 × 25 mL) and the filtrates were combined. The combined filtrates were concentrated to dryness, and the residue obtained was purified by flash-column chromatography (eluting with 10% ether–hexanes) to afford the β-(trimethylsilylmethyl)-α,β-unsaturated ketone **37** as a clear, colorless oil (1.42 g, 84%).

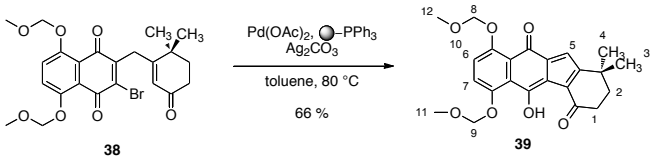
R_f = 0.35 (30% ethyl acetate–hexanes; CAM). t_R = 0.79. ^{1}H NMR (500 MHz, $CDCl_3$), δ 5.68 (s, 1H, H_1), 2.41 (t, 2H, J = 6.5 Hz, H_2), 1.82 (t, 2H, J = 7.0 Hz, H_3), 1.77 (s, 2H, H_6), 1.13 (s, 6H, H_5/H_4), 0.09 (s, 9H, H_7). ^{13}C NMR (125 MHz, $CDCl_3$), δ 198.5 (C), 173.7 (C), 124.6 (CH), 37.5 (CH_2), 35.9 (C), 34.1 (CH_2), 26.6 (2 × CH_3), 23.9 (CH_2), 0.00 (3 × CH_3). IR (ATR-FTIR): 2958 (w), 1659 (s), 1595 (m), 1242 (s). HRMS-CI(m/z): $[M+H]^+$ calcd for $C_{12}H_{23}OSi$, 211.1413; found, 211.1412.



*Coupling of 2,3-Dibromo-5,8-di(methoxymethyloxy)naphthoquinone (**30**) And The β-(Trimethylsilylmethyl)-α,β-unsaturated Ketone **37**:*

A neat mixture of 2,3-dibromo-5,8-di(methoxymethyloxy)naphthoquinone (**30**, 2.87 g, 6.58 mmol, 3.50 equiv) and the β-(trimethylsilylmethyl)-α,β-unsaturated ketone **37** (494 mg, 1.88 mmol, 1 equiv) was prepared in a 1-L round-bottomed flask. The mixture was dried by azeotropic distillation with benzene (2 × 10 mL). The dried mixture was dissolved in dichloromethane (380 mL) and was stirred at 24 °C until homogenous (ca. 10 min). The resulting red, homogenous mixture was cooled to –78 °C. A solution of TASF(Et) (1.18 g, 3.29 mmol, 1.75 equiv) in dichloromethane (10.0 mL) was added dropwise via syringe to the cold, stirred solution. The resulting black mixture was stirred for 15 min at –78 °C. The cold product mixture was diluted with 0.1 M aqueous sodium phosphate buffer solution (pH 7, 100 mL). The diluted solution was warmed over 30 min to 24 °C. The resulting biphasic mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with dichloromethane (3 × 50 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with 5% ethyl acetate–dichloromethane initially, grading to 40% ethyl acetate–dichloromethane, one step) to afford the coupled product **38** as a red solid (615 mg, 53%).

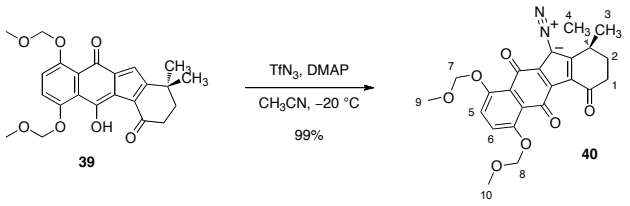
R_f = 0.52 (20% ethyl acetate–dichloromethane; CAM). t_R = 0.71. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.51 (d, 1H, J = 8.0 Hz, H_7/H_8), 7.48 (d, 1H, J = 8.0 Hz, H_7/H_8), 5.38 (app t, 1H, J = 1.6 Hz, H_1), 5.28 (s, 2H, H_9/H_{10}), 5.24 (s, 2H, H_9/H_{10}), 3.71 (app d, 2H, J = 2.0 Hz, H_6), 3.54 (s, 3H, H_{11}/H_{12}), 3.48 (s, 3H, H_{11}/H_{12}), 2.43 (t, 2H, J = 6.8 Hz, H_2), 1.91 (t, 2H, J = 6.4 Hz, H_3), 1.33 (s, 6H, H_4/H_5). ^{13}C NMR (125 MHz, $CDCl_3$), δ 198.8 (C), 180.0 (C), 175.98 (C), 166.87 (C), 152.63 (C), 152.56 (C), 147.13 (C), 141.0 (C), 125.0 (CH), 124.9 (CH), 123.9 (CH), 121.2 (C), 95.8 (CH_2), 95.7 (CH_2), 56.6 (CH_3), 56.5 (CH_3), 35.9 (CH_2), 34.2 (CH_2), 34.1 (CH_2), 26.4 (2 × CH_3). IR (ATR-FTIR): 2964 (w), 1663 (s), 1616 (m), 1469 (m). HRMS-CI(m/z): $[M+H]^+$ calcd for $C_{23}H_{26}BrO_7$, 493.0856; found, 493.0852/495.0780.



*Cyclization of the Coupled Product **38**:*

A mixture of palladium(II) acetate (123 mg, 549 μmol, 1.00 equiv), polymer-supported triphenylphosphine (549 mg, 549 μmol triphenylphosphine, 1.00 equiv], and silver(I) carbonate (302 mg, 1.10 mmol, 2.00 equiv) were added to a stirred solution of the coupled product **38** [253 mg, 549 μmol, 1 equiv; dried by azeotropic distillation with benzene (1 mL)] in toluene 5.5 mL at 24 °C. The resulting mixture was placed in an oil bath that had been preheated to 80 °C. The reaction mixture was stirred and heated for 1.5 h at 80 °C. The product solution was cooled over 10 min to 24 °C and the cooled solution was filtered over a celite pad (6 × 5 cm). The celite pad was washed with dichloromethane (4 × 20 mL). The filtrates were combined and the combined filtrates were concentrated to dryness. The residue obtained was purified by flash-column chromatography (eluting with 2% methanol–ether initially, grading to 20% methanol–ether, one step) to afford the cyclized product **39** as a dark purple solid (140 mg, 66%).

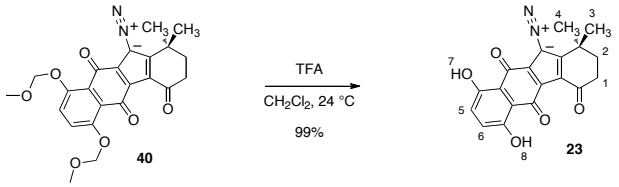
R_f = 0.34 (1% methanol–dichloromethane; CAM). t_R = 0.82. ^{1}H NMR (500 MHz, $CDCl_3$), δ 7.34 (d, 1H, J = 9.0 Hz, H_6/H_7), 7.32 (d, 1H, J = 9.5 Hz, H_6/H_7), 6.77 (s, 1H, H_5), 5.24 (s, 2H, H_8/H_9), 5.22 (s, 2H, H_8/H_9), 3.54 (s, 3H, H_{10}/H_{11}), 3.53 (s, 3H, H_{10}/H_{11}), 2.75 (t, 2H, J = 6.5 Hz, H_1), 1.93 (t, 2H, J = 6.5 Hz, H_2), 1.29 (s, 6H, H_3/H_4). ^{13}C NMR (125 MHz, $CDCl_3$), δ 192.0 (C), 181.0 (C), 173.9 (C), 160.7 (C), 154.1 (C), 153.9 (C), 137.4 (C), 126.7 (CH), 125.6 (CH), 124.4 (C), 123.1 (C), 121.8 (C), 121.7 (C), 117.9 (CH), 96.5 (CH_2), 96.4 (CH_2), 56.5 (2 × CH_3), 38.3 (CH_2), 32.2 (C), 31.4 (CH_2), 28.1 (2 × CH_3). IR (ATR-FTIR): 2959 (m), 1648 (s), 1561 (s), 1404 (s). HRMS-CI (m/z): $[M+H]^+$ calcd for $C_{23}H_{25}O_7$, 413.1600; found, 413.1595.



*Diazotransfer to the Ortho-Quinone Methide **39**:*

Triethylamine (334 μL, 2.40 mmol, 15.0 equiv) was added to a stirred solution of the cyclized product **39** [60.0 mg, 160 μmol, 1 equiv; dried by azeotropic distillation with benzene (500 μL)] in acetonitrile (8.0 mL) at 0 °C. A solution of trifluoromethanesulfonyl azide in hexanes (270 mM, 1.48 mL, 400 μmol, 2.50 equiv) was added dropwise via syringe and the resulting brown solution was stirred for 10 min at 0 °C. The product mixture was diluted with dichloromethane (20 mL) and the diluted mixture was transferred to a separatory funnel. An aqueous solution of cuprous sulfate (1.0 M, 10 mL) was added. The separatory funnel was stoppered and the stoppered funnel was vigorously shaken. The layers that formed were separated and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The organic layers were combined and the combined organic layers were washed with distilled water (2 × 20 mL). The washed organic layer was dried over sodium sulfate and the dried solution was filtered. The filtrate was concentrated and the residue obtained was purified by flash-column chromatography (eluting with 1% methanol–dichloromethane initially, grading to 3% methanol–dichloromethane, one step) to afford the diazofluorene **40** as yellow solid (64.0 mg, >99%).

R_f = 0.43 (100% ethyl acetate; CAM). t_R = 0.60. ^{1}H NMR (500 MHz, $CDCl_3$), δ 7.44 (d, 1H, J = 9.5 Hz, H_5/H_6), 7.40 (d, 1H, J = 9.5 Hz, H_5/H_6), 5.30 (s, 2H, H_7/H_8), 5.25 (s, 2H, H_7/H_8), 3.56 (s, 3H, H_9/H_{10}), 3.55 (s, 3H, H_9/H_{10}), 2.64 (t, 2H, J = 6.5 Hz, H_1), 2.04 (t, 2H, J = 6.5 Hz, H_2), 1.47 (s, 6H, H_3/H_4). ^{13}C NMR (125 MHz, $CDCl_3$), δ 190.9 (C), 179.4 (C), 178.3 (C), 158.0 (C), 152.9 (C), 152.6 (C), 132.7 (C), 131.6 (C), 127.1 (CH), 126.7 (C), 124.1 (C), 123.2 (CH), 122.2 (C), 96.9 (CH_2), 95.8 (CH_2), 73.9 (C), 56.6 (CH_3), 56.5 (CH_3), 39.0 (CH_2), 36.0 (CH_2), 33.9 (C), 27.6 (2 × CH_3). IR (ATR-FTIR): 2961 (w), 2136 (s), 1682 (s), 1466 (m), 1153 (s). HRMS-CI(m/z): $[M+H]^+$ calcd for $C_{23}H_{23}N_2O_7$, 439.1500; found, 439.1503



*Deprotection of the Diazofluorene **40**:*

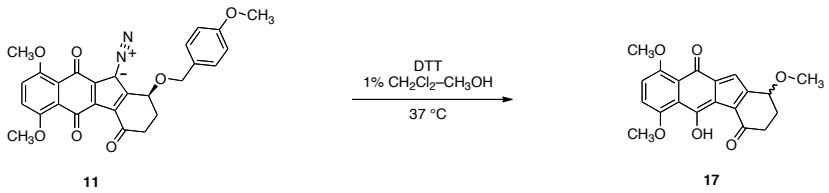
Trifluoroacetic acid (30 μL, 392 μmol, 8.60 equiv) was added dropwise to a stirred solution of the quinone methide [20.0 mg, 45.6 μmol, 1 equiv; dried by azeotropic distillation with benzene (500 μL)] in dichloromethane (5.0 mL) at 24 °C. The resulting purple solution was stirred for 10 min at 24 °C. The product mixture was concentrated to dryness, and the residue obtained was purified by flash-column chromatography (eluting with 3% methanol–dichloromethane) to afford the diazofluorene **23** as purple solid (16.0 mg, >99%).

R_f = 0.20 (40% ethyl acetate–hexanes; CAM). t_R = 0.70. ^{1}H NMR (500 MHz, $CDCl_3$), δ 13.22 (s, 1H, H_7/H_8), 12.38 (s, 1H, H_7/H_8), 7.21 (d, 1H, J = 9.5 Hz, H_5/H_6), 7.13 (d, 1H, J = 9.0 Hz, H_5/H_6), 2.68 (t, 2H, J = 6.5 Hz, H_1), 2.08 (t, 2H, J = 6.5 Hz, H_2), 1.53 (s, 6H, H_3/H_4). ^{13}C NMR (125 MHz, $CDCl_3$), δ 191.0 (C), 182.2 (C), 182.0 (C), 158.4 (2 × C), 131.8 (C), 130.5 (C), 129.8 (C), 128.2 (C), 125.1 (C), 113.1 (CH), 112.1 (CH), 75.3 (C), 38.8 (CH_2), 36.4 (CH_2), 34.1 (C), 27.8 (2 × CH_3). IR (ATR-FTIR): 2950 (w), 2136 (s), 1683 (s), 1340 (s). HRMS-CI(m/z): $[M+H]^+$ calcd for $C_{19}H_{15}N_2O_5$, 351.0976; found, 351.0978.

Reactivity Experiments.

*Determination of the Relative Extinction Coefficients of the Diazofluorene **11** and the Ortho-Quinone Methide **17**:*

Solutions of the diazofluorene **11** and the *ortho*-quinone methide **17** in dichloromethane (20 mM) were prepared separately. An appropriate amount of each stock solution was used to form mixtures of the following compositions: **11**:**17** = 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10. Each mixture was analyzed separately by LC/MS, with UV detection at 254 nm. The relative extinction coefficients (254 nm) of **11** and **17** was determined to be 5.1:1, respectively.

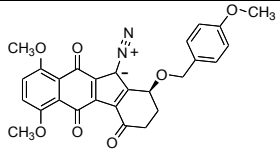
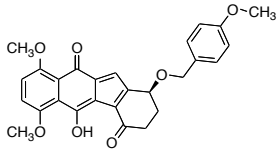
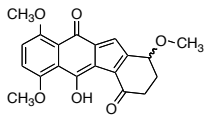


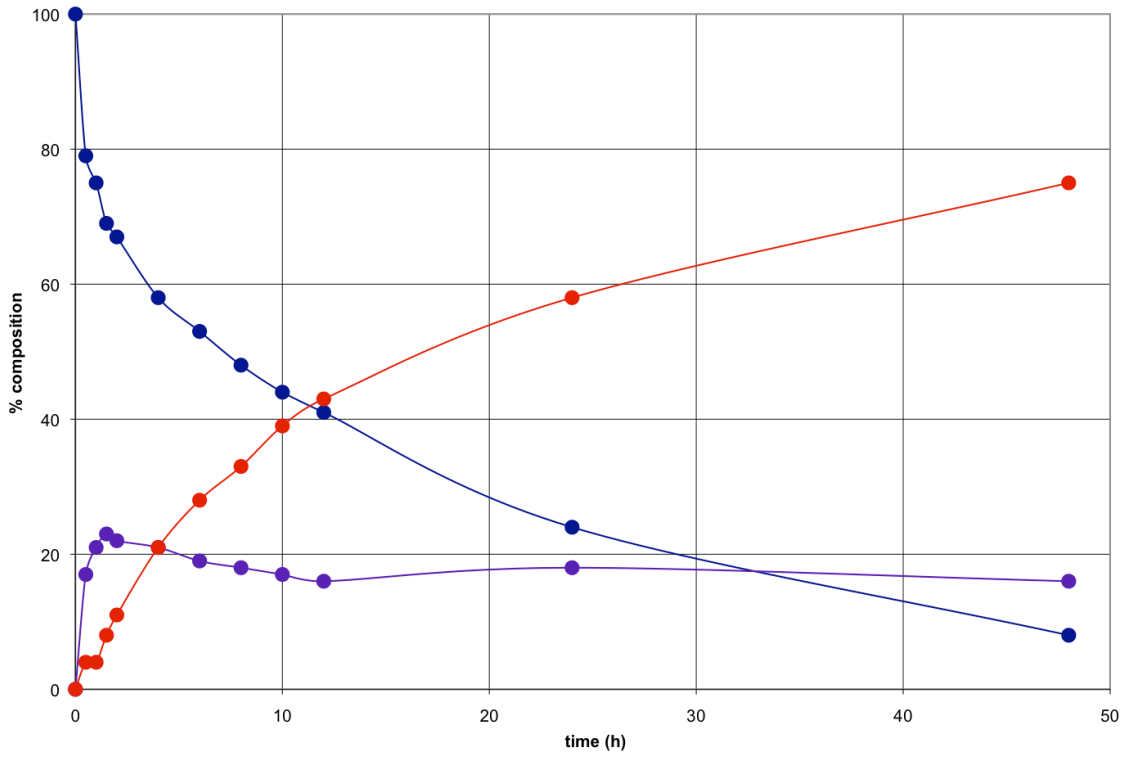
11 **17**

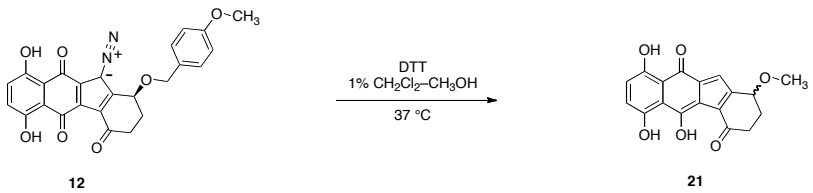
*Reduction–Solvolysis of the Diazofluorene **11**:*

Methanol and dichloromethane were separately sparged with argon for 30 min before use. A solution of the diazofluorene **11** in dichloromethane (20 mM, 10.0 μL, 200 nmol, 1 equiv) was diluted with methanol (980 μL) in an LC/MS vial. A freshly prepared solution of dithiothreitol in methanol (DTT, 20 mM, 10.0 μL, 200 nmol, 1.00 equiv) was added. The resulting solution was warmed to 37 °C. The reduction–solvolysis reaction was monitored by LC/MS (for conditions, please see the *Instrumentation* section above).

Graph S3. Reduction–Solvolysis of the Diazofluorene **11**. Navy – **11**; Purple – **16**; Red – **17**; Conditions: **11** (200 μM), DTT (200 μM), 1% dichloromethane–methanol, 37 °C.

Compound	t_R (min)
 **11**	2.04
 **16**	2.51
 **17**	1.91


100
80
60
40
20
0
% composition
0
10
20
30
40
50
time (h)



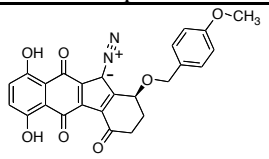
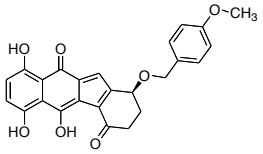
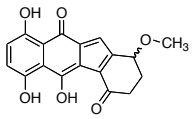
12 21

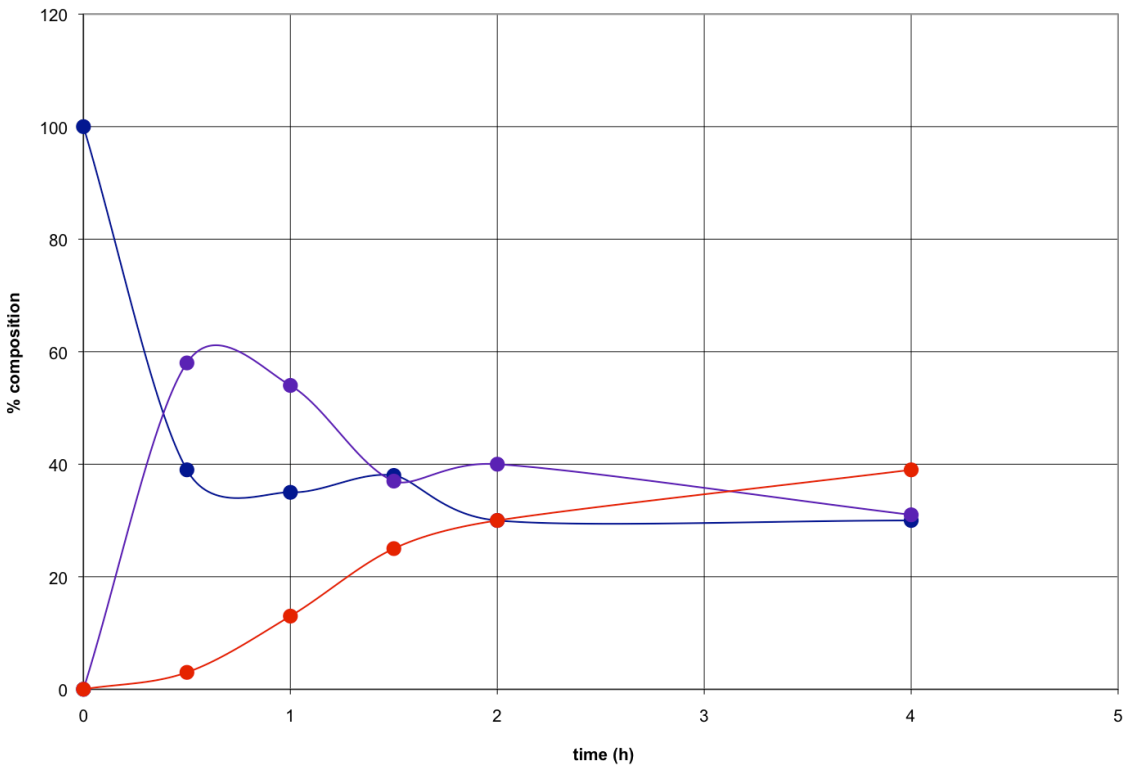
*Reduction–Solvolysis of the Diazofluorene **12**:*

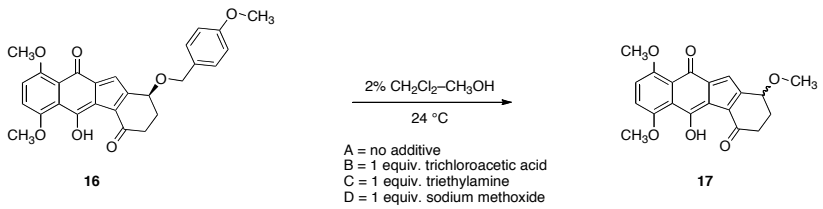
Methanol and dichloromethane were separately sparged with argon for 30 min before use. A solution of the diazofluorene **12** in dichloromethane (20 mM, 10.0 μL, 200 nmol, 1 equiv) was diluted with methanol (980 μL) in an LC/MS vial. A freshly prepared solution of dithiothreitol in methanol (DTT, 20 mM, 10.0 μL, 200 nmol, 1.00 equiv) was added. The resulting solution was warmed to 37 °C. The reduction–solvolysis reaction was monitored by LC/MS by elution over a reverse-phase C_{18} column (1.7 μm particle size, 2.1 × 50 mm) with a linear gradient of 20% acetonitrile–water→100% acetonitrile containing 0.1% formic acid over 7 min, followed by 0.1% formic acid–acetonitrile for 1 min, at a flow rate of 800 μL/min.

Note: Efforts to prepare the *ortho*-quinone methide **20**, derived from the diazofluorene **12**, were unsuccessful, presumably due to the instability of this compound. Thus, it is assumed that the relative extinction coefficients of these substrates is the same as the methyl ethers **11** and **17**.

Graph S4. Reduction and Solvolysis of the Diazofluorene **12**. Navy – **12**; Purple – **20**; Red – **21**; Conditions: **12** (200 μM), DTT (200 μM), 1% dichloromethane–methanol, 37 °C.

Compound	t_R (min)
 **12**	4.62
 **20**	5.80
 **21**	4.72


120
100
80
60
40
20
0
% composition
0
1
2
3
4
5
time (h)

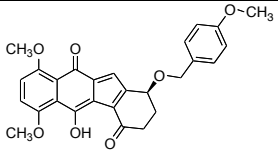
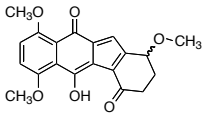


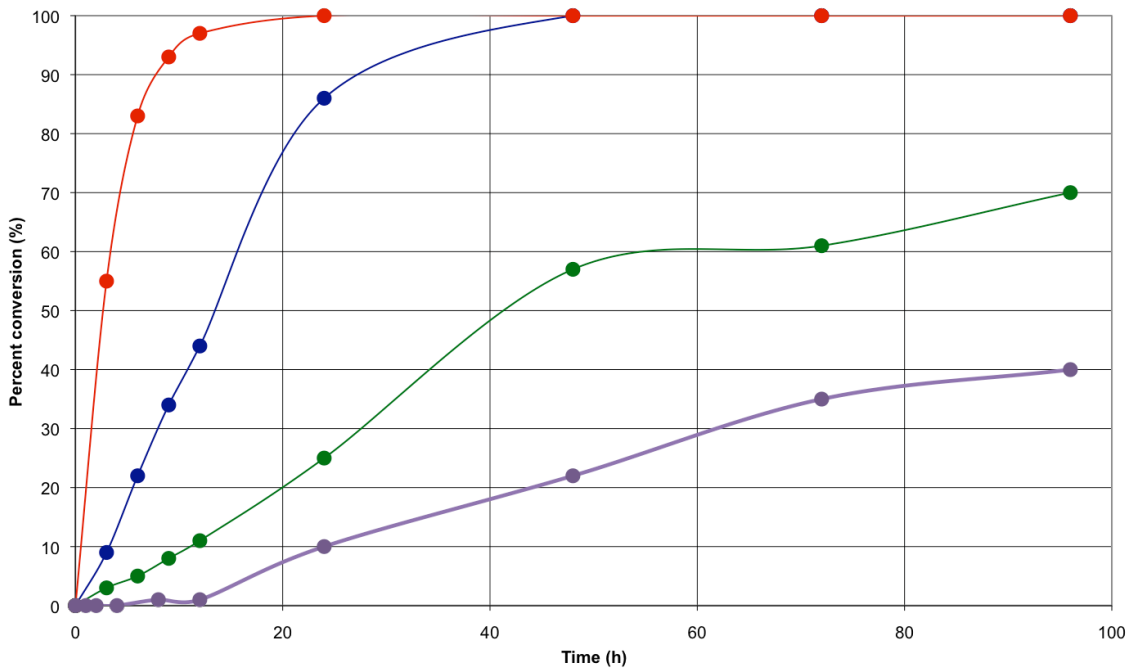
16 17

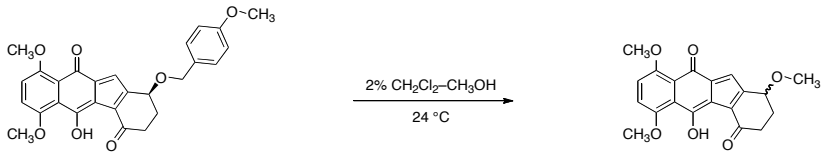
*Solvolysis of the Ortho-Quinone Methide **16** Under Neutral, Acidic, and Basic Conditions:*

Methanol and dichloromethane were separately sparged with argon for 30 min before use. Three aliquots of the *ortho*-quinone methide **16** in dichloromethane (20 mM, 10.0 μL, 200 nmol, 1 equiv) were added to four separate LC/MS vials (A, B, C, and D). Vial A was diluted with methanol (990 μL). A solution of trichloroacetic acid in methanol (20 mM, 10.0 μL, 200 nmol, 1.00 equiv) was added to vial B. The resulting mixture was diluted with methanol (980 μL). A solution of triethylamine in methanol (20 mM, 10.0 μL, 200 nmol, 1.00 equiv) was added to vial C. A solution of sodium methoxide in methanol (20 mM, 10.0 μL, 200 nmol, 1.00 equiv) was added to vial D. The resulting solution was diluted with methanol (980 μL). Vials A, B, C, and D were sealed with a plastic cap and mixed by manual agitation. The mixed solutions were warmed to 37 °C, and the solvolysis reaction was monitored by LC/MS.

Graph S5. Solvolysis of the *Ortho*-Quinone Methide **16** Under Neutral, Acidic, and Basic Conditions. Navy – Vial A (neutral); Red – Vial B (1 equiv trichloroacetic acid); Green – Vial C (1 equiv triethylamine); Purple – Vial D (1 equiv sodium methoxide); Conditions: **16** (200 μM), acid/base (200 μM), 1% dichloromethane–methanol, 37 °C.

Compound	t_R (min)
 **16**	1.80
 **17**	1.21


Percent conversion (%)
Time (h)
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100

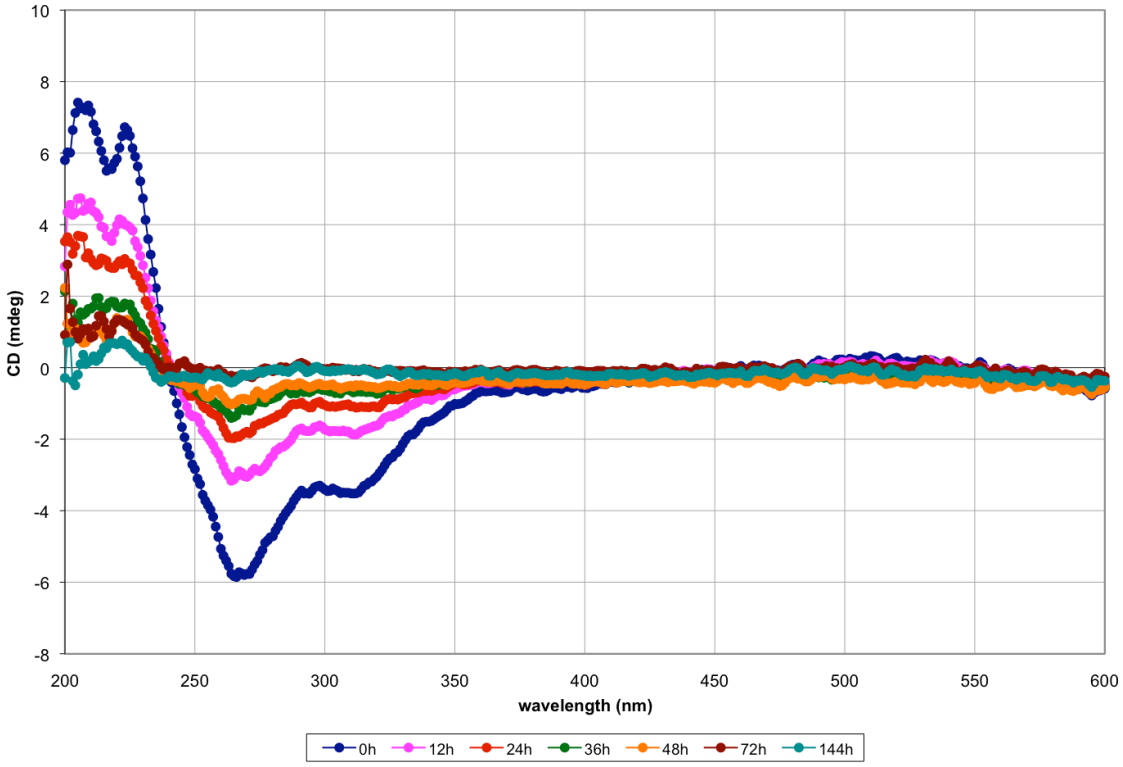


16 17

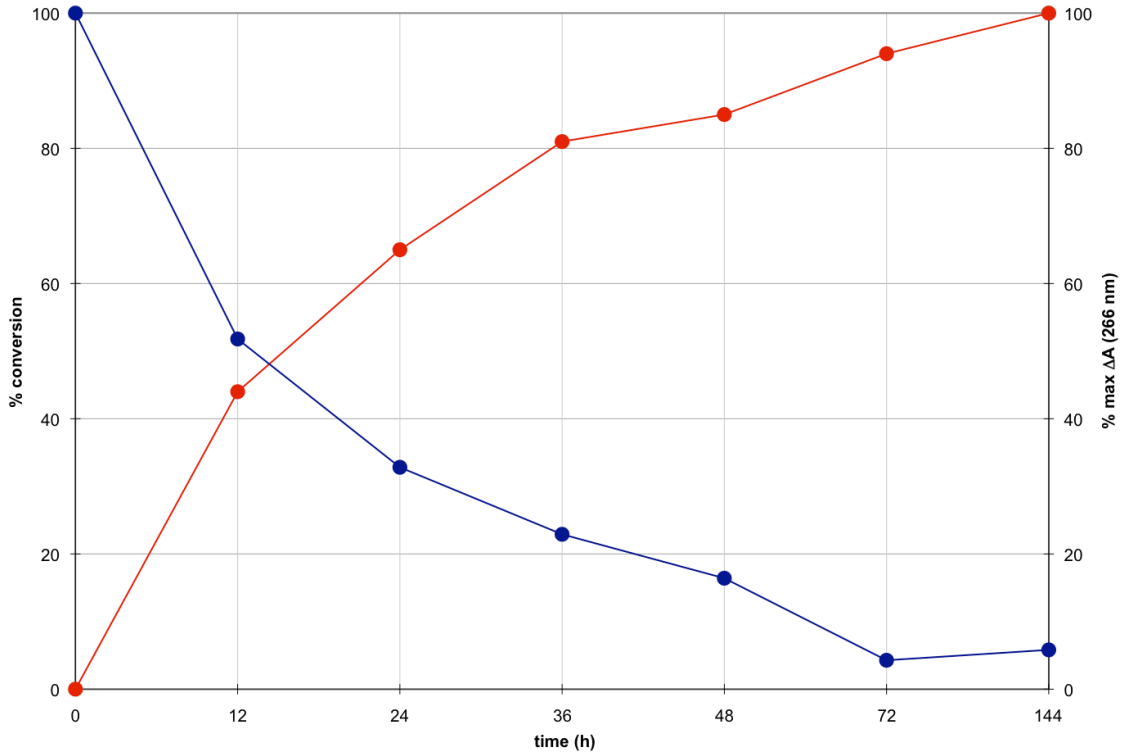
*Circular Dichroism-LC/MS Monitoring of the Solvolysis of the Ortho-Quinone Methide **16**.*

A solution of the *ortho*-quinone methide **16** in acetonitrile (100 mM, 60.0 μL, 6 μmol, 1 equiv) was added to an LC/MS vial. Methanol (540 μL) was added. The resulting solution was allowed to stand at 24 °C. At the appropriate time intervals, an aliquot of this solution (60 μL, 600 nmol) was removed and diluted with acetonitrile (540 μL) in a 1 mm quartz cuvette. The resulting solution was analyzed by circular dichroism and LC/MS (for conditions, please see the *Instrumentation* section above).

Graph S6. CD Analysis of the Solvolysis of the *Ortho*-Quinone Methide **16** as a Function of Time.



Graph S7. CD–LC/MS Analysis of the Solvolysis of the *Ortho*-Quinone Methide **16** as a Function of Time. Red – % conversion (LC/MS, left axis); Navy – % max ΔA, 266 nm (CD, right axis).



*Stability of the Ortho-Quinone Methide **16**:*

LnCAP cells grown to confluency were harvested and counted (*vide infra*). A 2 mL aliquot of cells in cell culture media (700,000 cells/mL) were treated with Triton X-100 (30 μL). The suspension of cells was vortexed and incubated for 12 h at 4 °C. The suspension of cells was centrifuged at 5000 rpm and decanted. The supernatant (300 μL) was combined with cell culture media (300 μL) to make a 50% cell lysate solution. A solution of the *ortho*-quinone methide **16** in acetone (100 mM, 2.00 μL, 200 nmol, 1 equiv) and a solution of *N,N*-dimethylbenzamide in acetone (100 mM, 2.00 μL, 200 nmol, 1.00 equiv) were diluted with 50% cell lysate solution (596 μL) in an LC/MS vial. The resulting solution was warmed to 37 °C. The decomposition reaction was monitored by LC/MS by elution over a reverse-phase C_{18} column (1.7 μm particle size, 2.1 × 50 mm) with a linear gradient of 20% acetonitrile–water→100% acetonitrile containing 0.1% formic acid over 7 min, followed by 0.1% formic acid–acetonitrile for 1 min, at a flow rate of 800 μL/min. The relative extinction coefficients (254 nm) of **16** and *N,N*-dimethylbenzamide was determined to be 7.1:1, respectively.

Cell Viability Assays. All tissue culture experiments were conducted in a biological safety cabinet in the dark. HeLa cells were obtained from Professor Craig M. Crews (Yale University). HCT-116 cells were obtained from Professor Peter M. Glazer (Yale University). K562 and LnCAP cells were obtained from Professor David Spiegel (Yale University). All cell culture reagents were obtained from Invitrogen. HeLa and HCT-116 cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin. K562 and LnCAP cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin. All cell lines were maintained at 37 ºC under an atmosphere of 5% CO_2 and constant humidity.

Kinamycin C (**4**, positive control) and analogs **11**–**12**, **16**, and **22**–**24** were prepared as 10 mM stock solutions in dimethylsulfoxide (DMSO) and stored with protection from light (aluminum foil) at –20 °C. Compounds were diluted two-fold serially in DMSO from 10 mM to 1.2 μM and arranged in quadruplicate in 2 μL volumes in 384-well low volume plates (Matrical). Vehicle control wells containing DMSO were included on each plate.

HeLa, HCT-116, and LnCAP cells grown to 70% confluency, were washed with phosphate-buffered saline (PBS), and then were trypsinized for 5 min at 37 ºC. The cells were harvested, centrifuged for 5 min at 1000 rpm, resuspended in 10 mL fresh growth medium and counted using a hemacytometer. K562 cells were pipetted before counting in a hemacytometer. The counted cells were plated into sterile white, clear bottom tissue culture-treated 384-well plates (Corning) at a concentration of 400 cells/well (20 μL total volume) using a MultiDrop (Thermo Fisher). Assay plates were centrifuged at 500 rpm for 2 s and incubated overnight at 37 °C in a humidified 5% CO_2 incubator. After incubation, 20 nL of compound solution was transferred from the compound source plate to the cell assay plate using an Aquarius (Tecan) with a 20 nL 384-well pin tool (V&P Scientific). One microliter (1 μL) of 210 μM kinamycin C diluted in media from a 10 mM stock solution in DMSO was added to the positive control wells on each plate by hand resulting in a final concentration of 10 μM. The final concentration of compounds ranged from 10 μM to 1.2 nM, and the final DMSO concentration was 0.1%. Assay plates were centrifuged at 500 rpm for 2 seconds and incubated for 72 hours at 37 °C in a humidified 5% CO_2 incubator. CellTiter-Glo (Promega) was used to measure cell viability in the assay wells.[14] It was prepared according to the manufacturer's instructions and 20 μL/well was added to the assay plates using a MultiDrop. Luminescence was read on an Envision plate reader (PerkinElmer) with 0.3 second sampling time per well after a 10 min room temperature incubation in the dark. Toxic effects have low luminescence signal relative to the vehicle control. Raw data (luminescence counts per second) was normalized to % effect by the formula [(DMSO vehicle – 10 μM kinamycin) /(DMSO vehicle – sample)] * 100. Ten μM kinamycin C was used to show 100% effect (toxicity), and DMSO vehicle was used to show 0% effect (no toxicity). Twenty four (24) wells of each control were run on every plate. Data was plotted in GraphPad Prism using a variable slope 4-parameter fit. The top of the curve was constrained to less than or equal 101%.

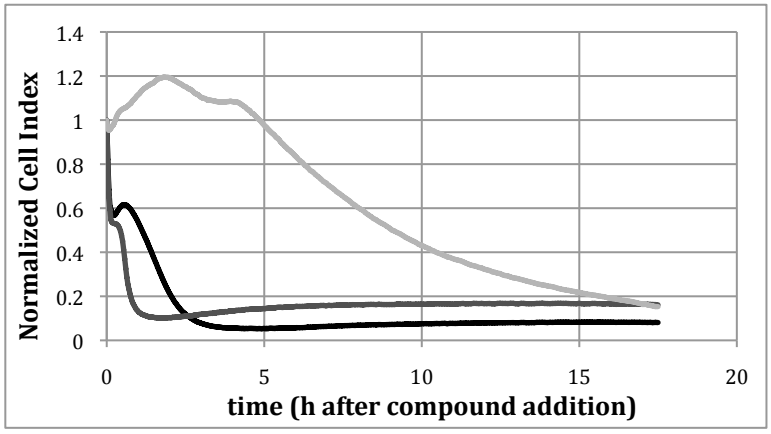
Time-Dependent Cell Response Profile (TCRP) Assay. A172 human glioblastoma cells were purchased from ATCC (#CRL-1620) and grown in T-flasks with Dulbecco's modified Eagle's medium supplemented with 10% HI-FBS. One day prior to the experiment, adherent cells were aspirated, washed with DPBS (5 mL), and treated with EDTA detachment solution (5 mL). The flask was incubated for 15 min, and the cells were fully detached by gently rinsing the solution over the bottom of the flask. The cell suspension was counted, aspirated, and diluted in FBS ADCC (to a final concentration of 50,000 cells/mL). Into each well of an E-plate (16-well E-plate-16, Roche #05469830001) was added 200 μL of the cell suspension (10,000 cells). The plate was allowed to stand at ambient temperature (30 min) and maintained in an incubator (37 °C, 12 h).

Growth curves were obtained on the Roche xCelligence system model RTCA-DP. The xCelligence system was maintained inside an incubator (37 °C). An E-plate containing 100 μL FBS ADCC media per well was used for obtaining background measurements. The seeded E-plate was placed in the port, and cell index readings were obtained (every 2 min for 30 min) to confirm that the cells had adhered properly.

Kinamycin C (**4**) and analogs **23** and **24** were prepared as 10 mM stock solutions in dimethylsulfoxide (DMSO) and stored with protection from light (aluminum foil) at –20 °C. Compounds were diluted to 1 mM, and 2 μL volumes were added in triplicate to the E-plate after incubation for a final concentration of 10 μM compound per well. Vehicle control wells containing DMSO were included. The E-plate was returned to the port, and cell index readings were obtained (every 2 min for 24 h, 37 °C).

Data analysis was performed using RTCA software v1.2. Cell index readings were normalized at the timepoint immediately after addition of the compounds. Graph S8 shows these data. Cytotoxicity (%) was calculated using equation below.[15] Normal cell growth was defined as that shown by vehicle control wells treated with DMSO.

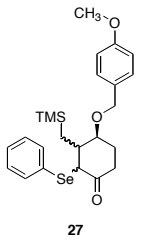
$$\text{Cytotoxicity (\%)} = 100 - (\text{cell index}_{\text{molecule}} / \text{cell index}_{\text{DMSO}}) \times 100$$



Graph S8. Compound-dependent cytotoxicity of A172 glioblastoma caner cells on treatment with kinamycin C (**4**, black), analog **23** (dark grey), or analog **24** (light grey). Cell index values were normalized immediately after compound addition, and presented as the triplicate average.

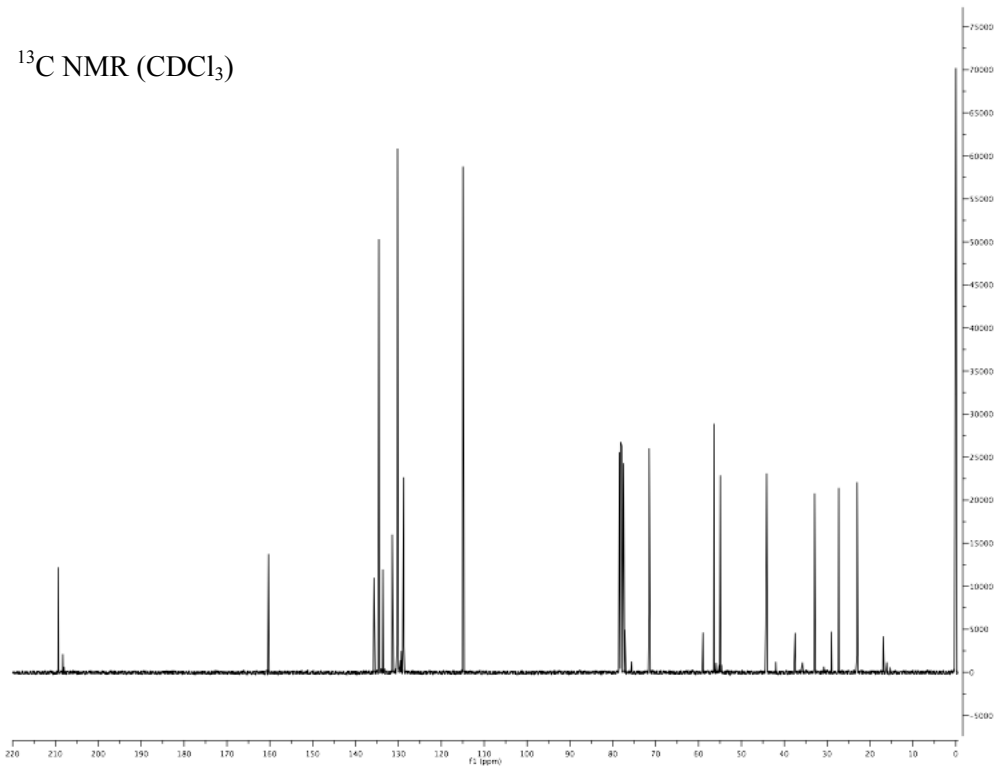
Catalog of Nuclear Magnetic Resonance and Infrared Spectra.

^{1}H NMR ($CDCl_3$)

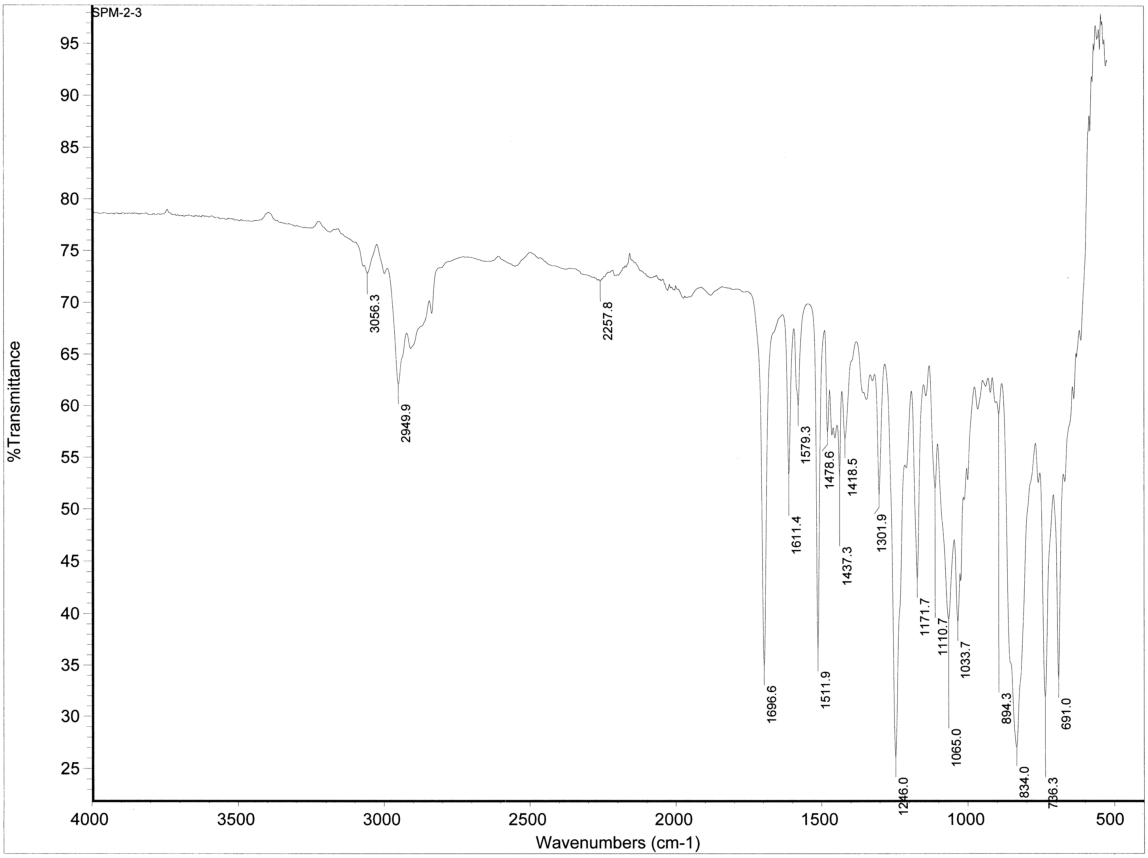


27

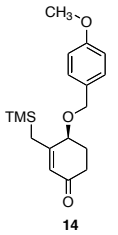
^{13}C NMR ($CDCl_3$)



IR

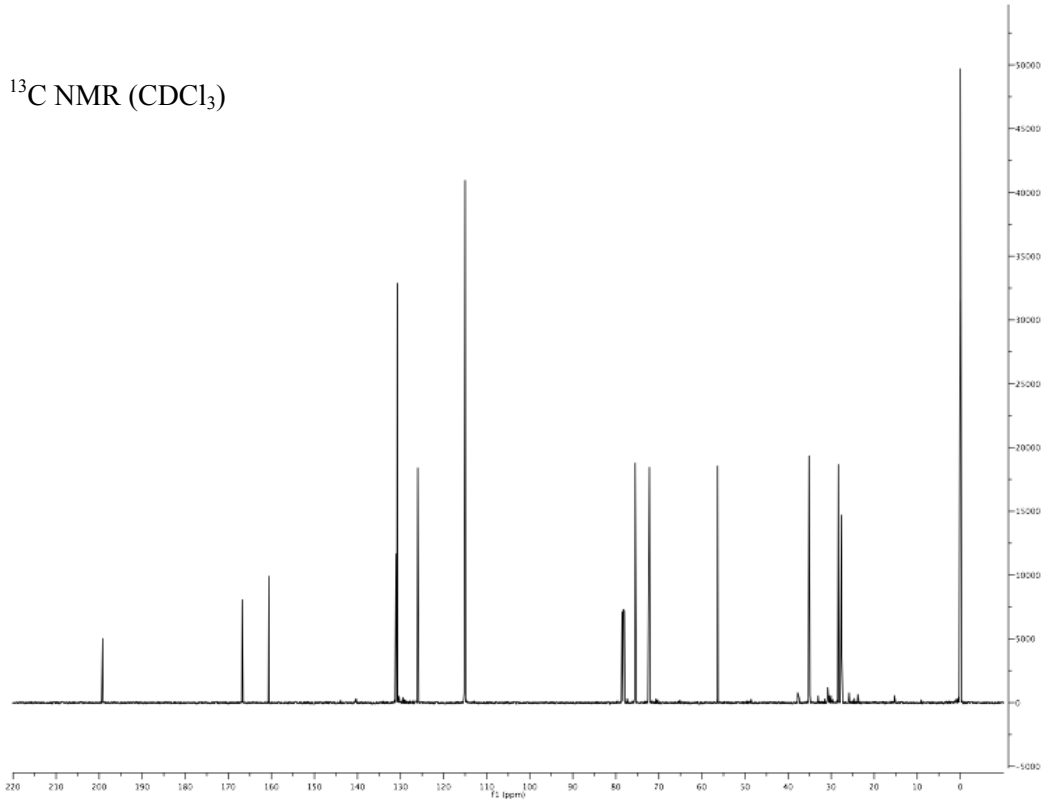

SPM-2-3
%Transmittance
Wavenumbers (cm-1)
3056.3
2949.9
2257.8
1696.6
1611.4
1579.3
1511.9
1478.6
1437.3
1418.5
1301.9
1246.0
1171.7
1110.7
1065.0
1033.7
894.3
834.0
736.3
691.0

^{1}H NMR ($CDCl_3$)

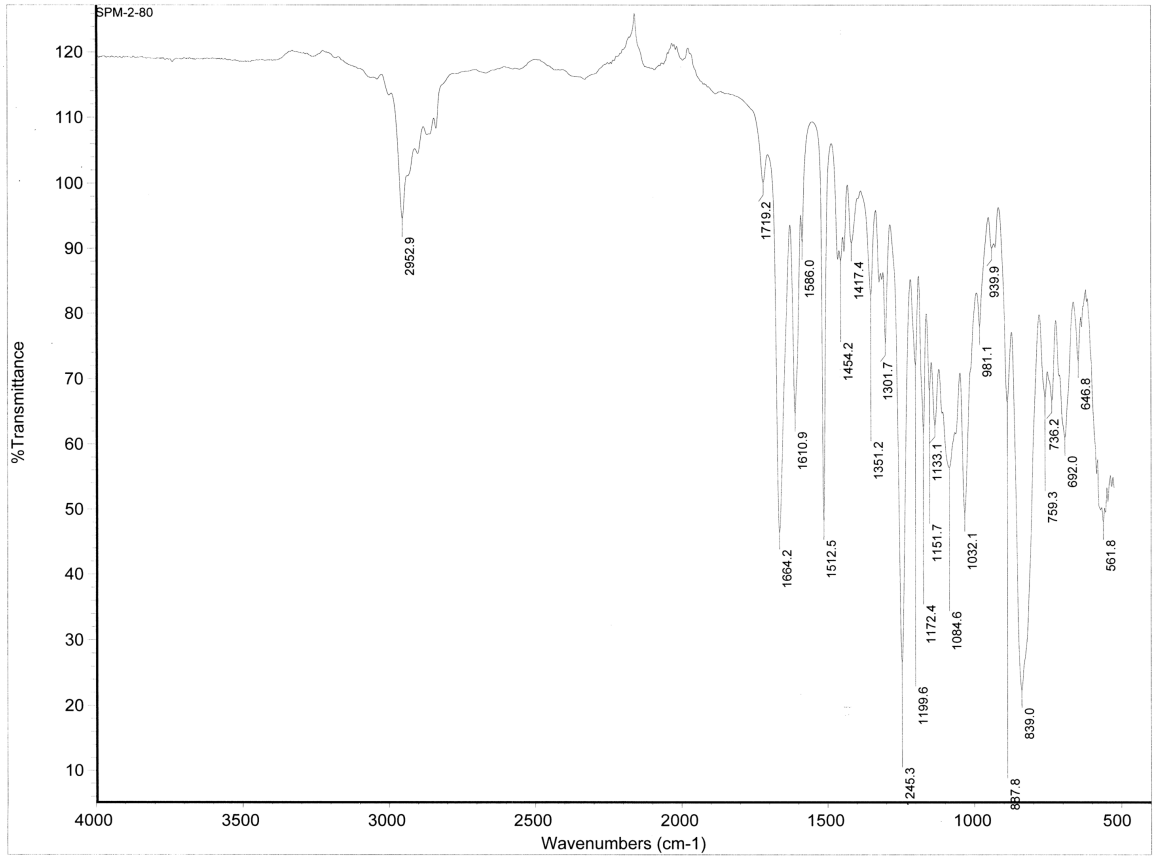


14

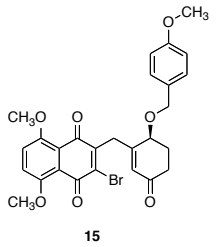
^{13}C NMR ($CDCl_3$)



IR

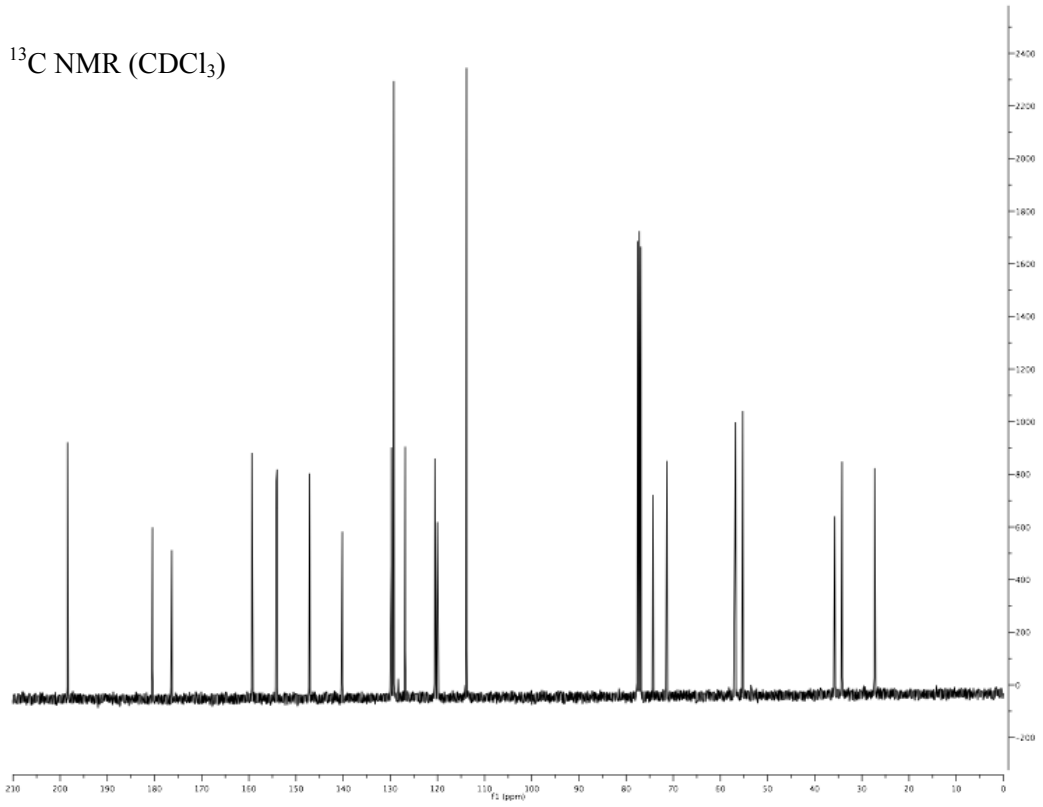

SPM-2-80
%Transmittance
Wavenumbers (cm-1)
120
110
100
90
80
70
60
50
40
30
20
10
4000
3500
3000
2500
2000
1500
1000
500
2952.9
1719.2
1664.2
1610.9
1586.0
1512.5
1454.2
1417.4
1351.2
1301.7
1199.6
1172.4
1151.7
1133.1
1084.6
1032.1
981.1
939.9
887.8
839.0
759.3
736.2
692.0
646.8
561.8
245.3

^{1}H NMR ($CDCl_3$)

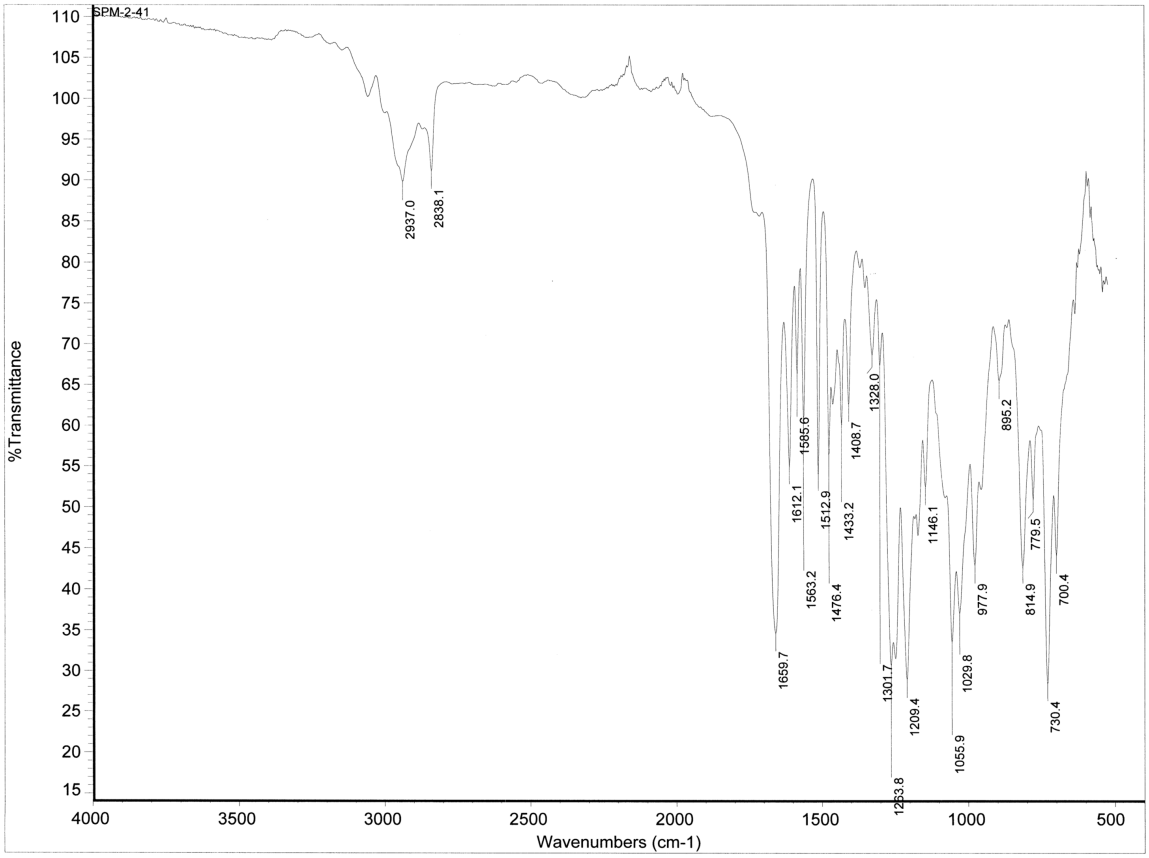


15

^{13}C NMR ($CDCl_3$)



IR


SPM-2-41
%Transmittance
Wavenumbers (cm-1)
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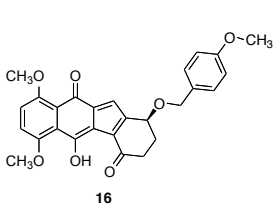
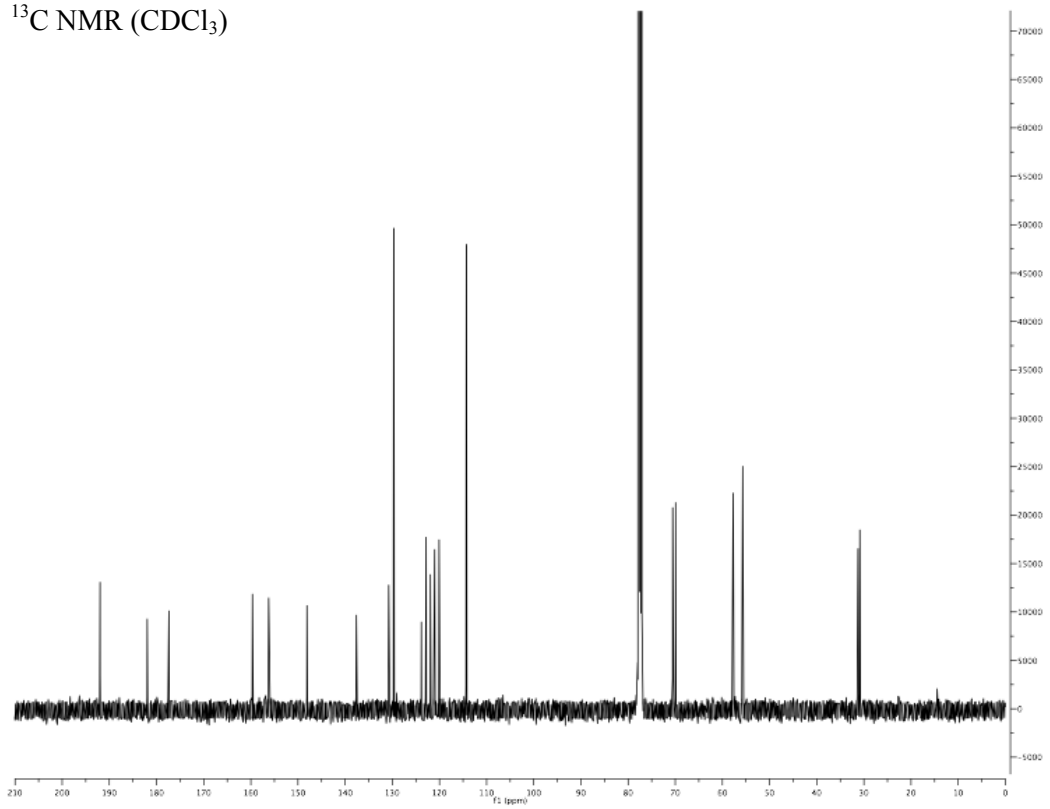
1612.1
1585.6
1563.2
1512.9
1476.4
1433.2
1408.7
1328.0
1301.7
1263.8
1209.4
1146.1
1055.9
1029.8
977.9
895.2
814.9
779.5
730.4
700.4

^{1}H NMR ($CDCl_3$)

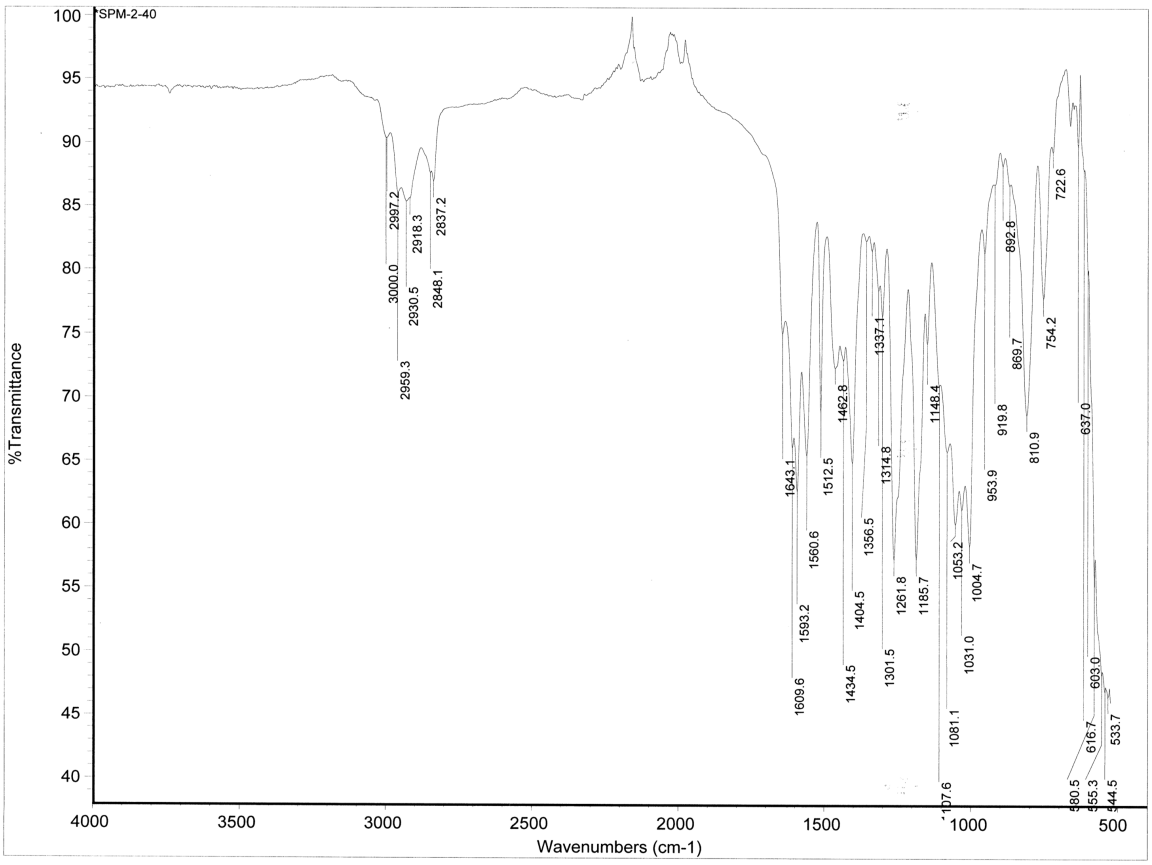


16

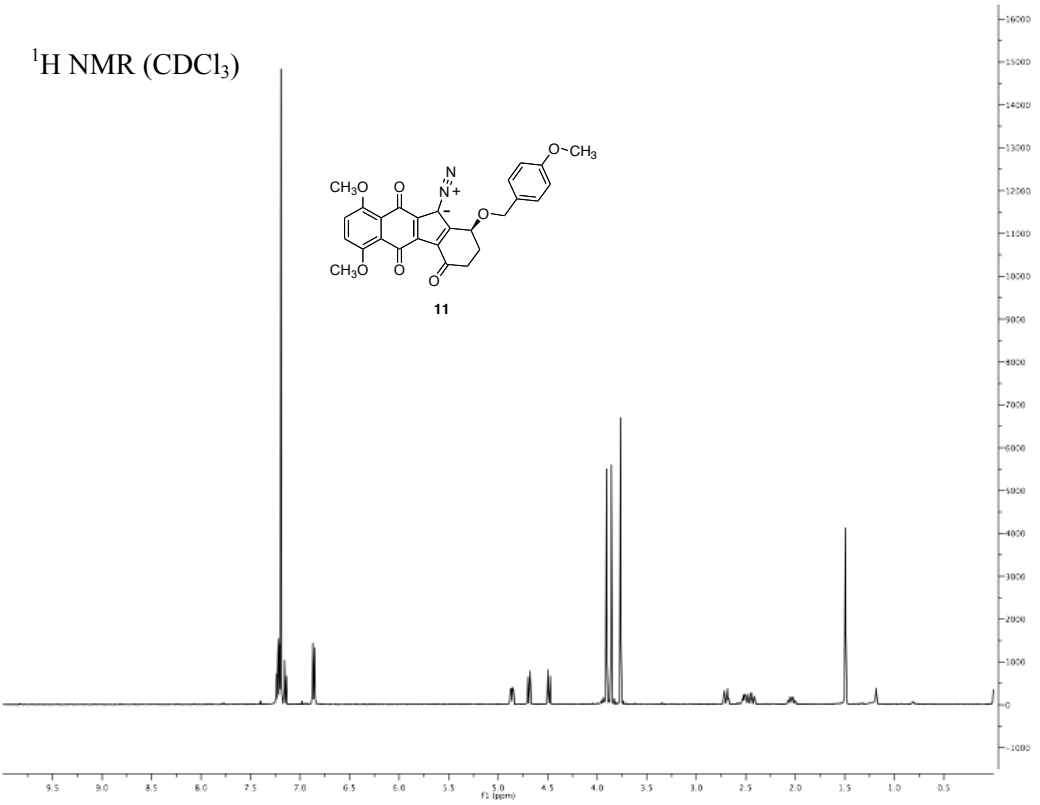
^{13}C NMR ($CDCl_3$)



IR

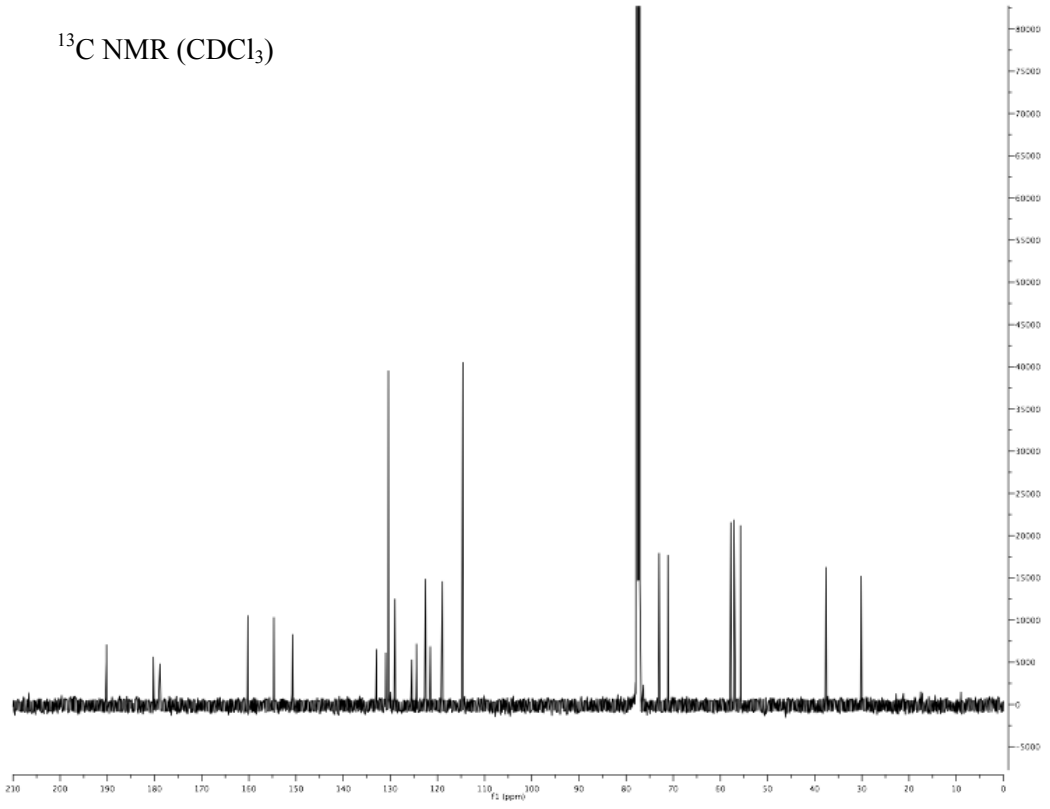

SPM-2-40
%Transmittance
Wavenumbers (cm-1)
100
95
90
85
80
75
70
65
60
55
50
45
40
4000
3500
3000
2500
2000
1500
1000
500
3000.0
2997.2
2959.3
2930.5
2918.3
2848.1
2837.2
1643.1
1609.6
1593.2
1560.6
1512.5
1462.8
1434.5
1404.5
1356.5
1337.1
1314.8
1301.5
1261.8
1185.7
1148.4
1107.6
1081.1
1053.2
1031.0
1004.7
953.9
919.8
892.8
869.7
810.9
754.2
722.6
637.0
603.0
616.7
533.7
480.5
455.3
444.5

^{1}H NMR ($CDCl_3$)

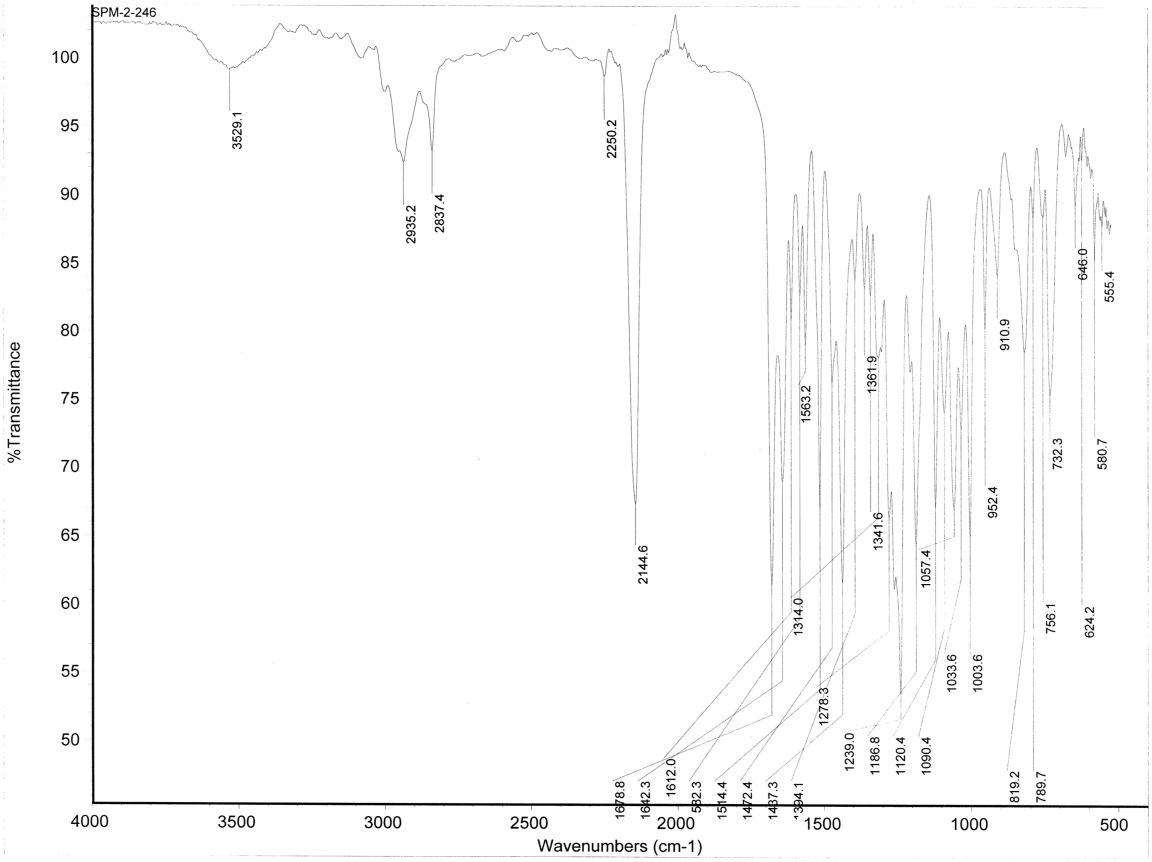


11

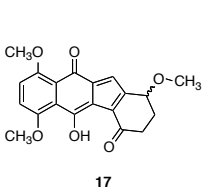
^{13}C NMR ($CDCl_3$)



IR

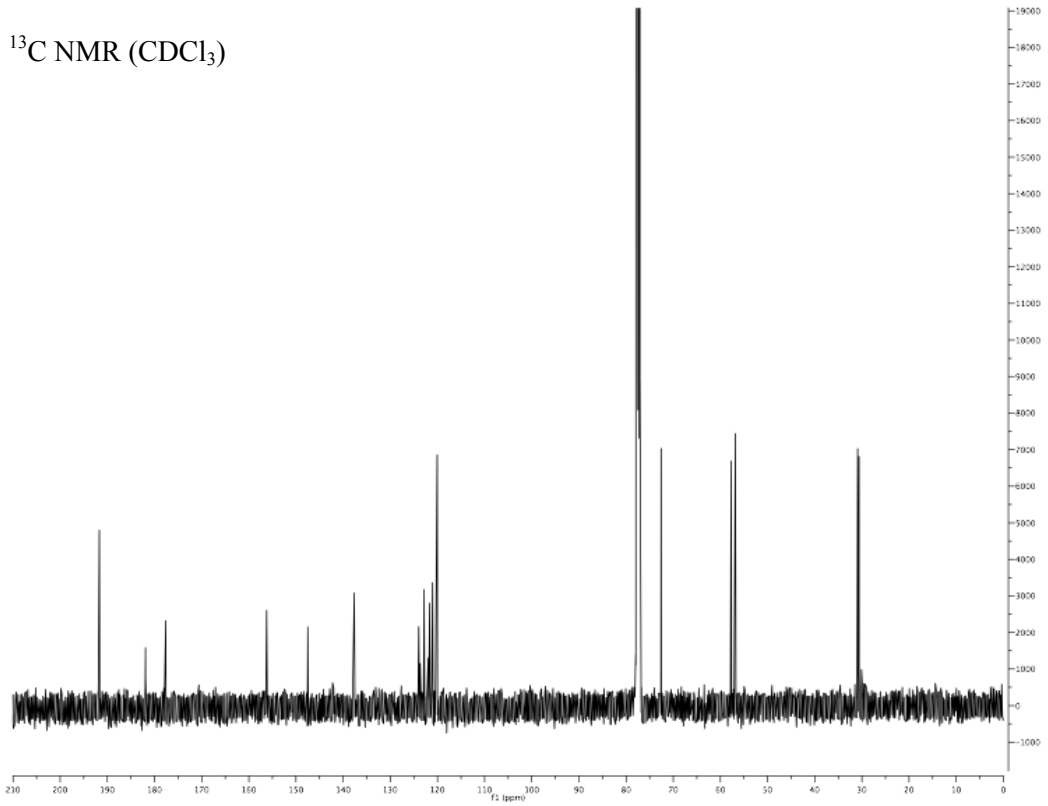

SPM-2-246
%Transmittance
Wavenumbers (cm-1)
3529.1
2935.2
2837.4
2250.2
2144.6
1678.8
1642.3
1612.0
1582.3
1514.4
1472.4
1437.3
1384.1
1341.6
1314.0
1278.3
1239.0
1186.8
1120.4
1090.4
1057.4
1033.6
1003.6
952.4
910.9
819.2
789.7
756.1
732.3
646.0
624.2
580.7
555.4
1563.2
1361.9

^{1}H NMR ($CDCl_3$)

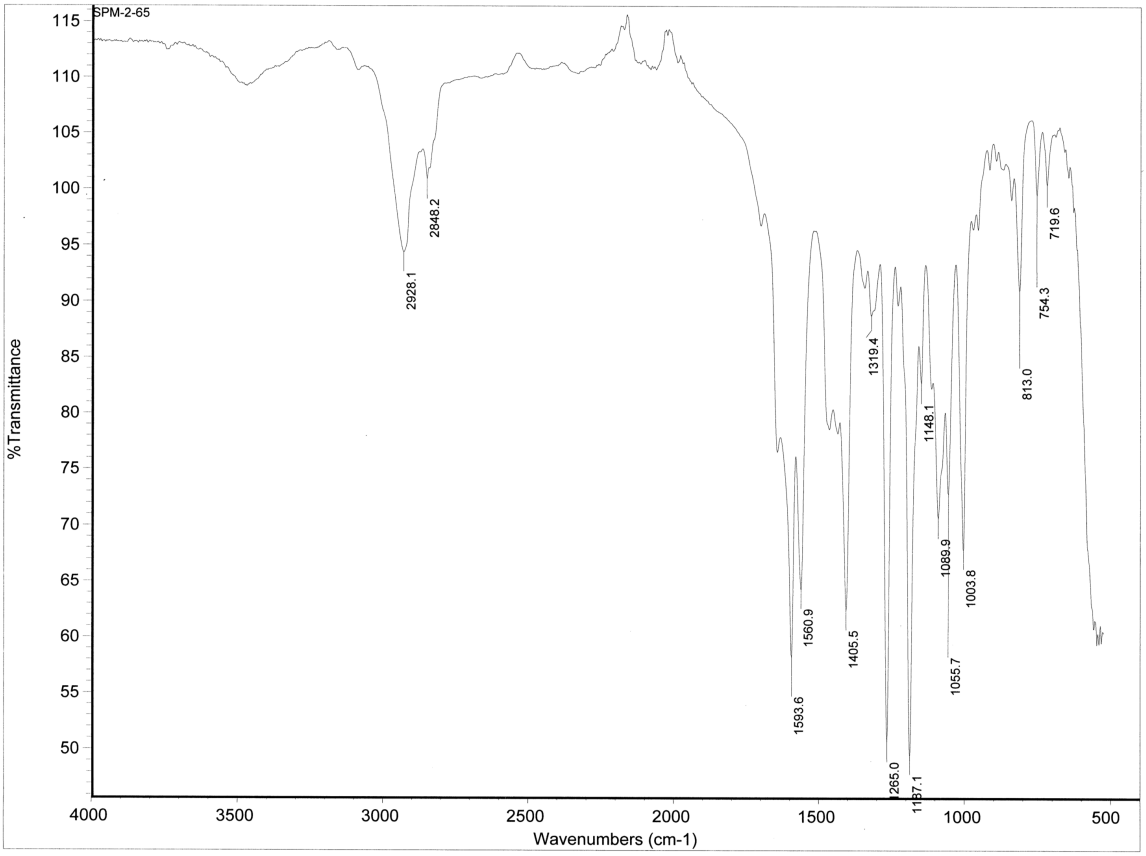


17

^{13}C NMR ($CDCl_3$)



IR


SPM-2-65
%Transmittance
Wavenumbers (cm-1)
2928.1
2848.2
1593.6
1560.9
1405.5
1319.4
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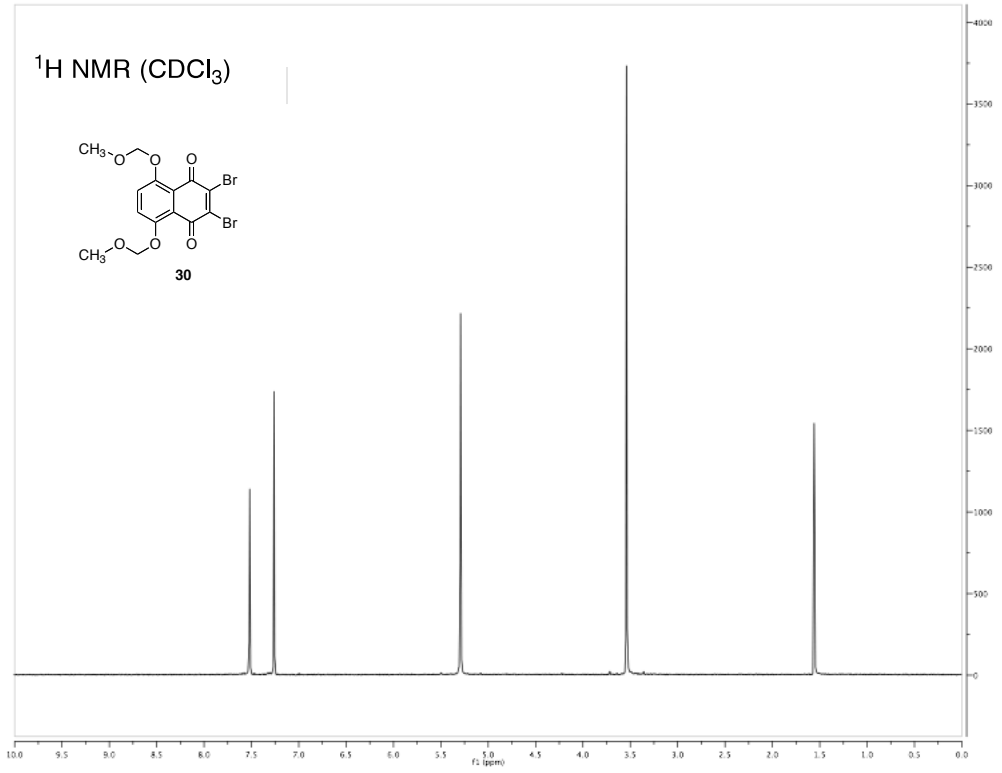
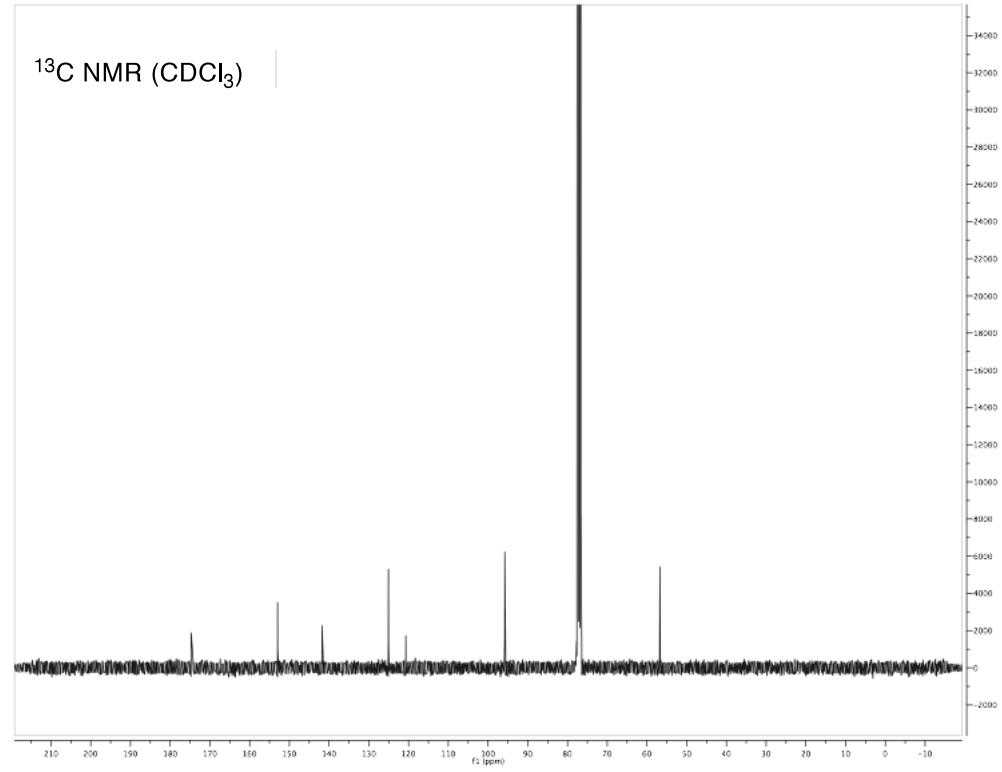
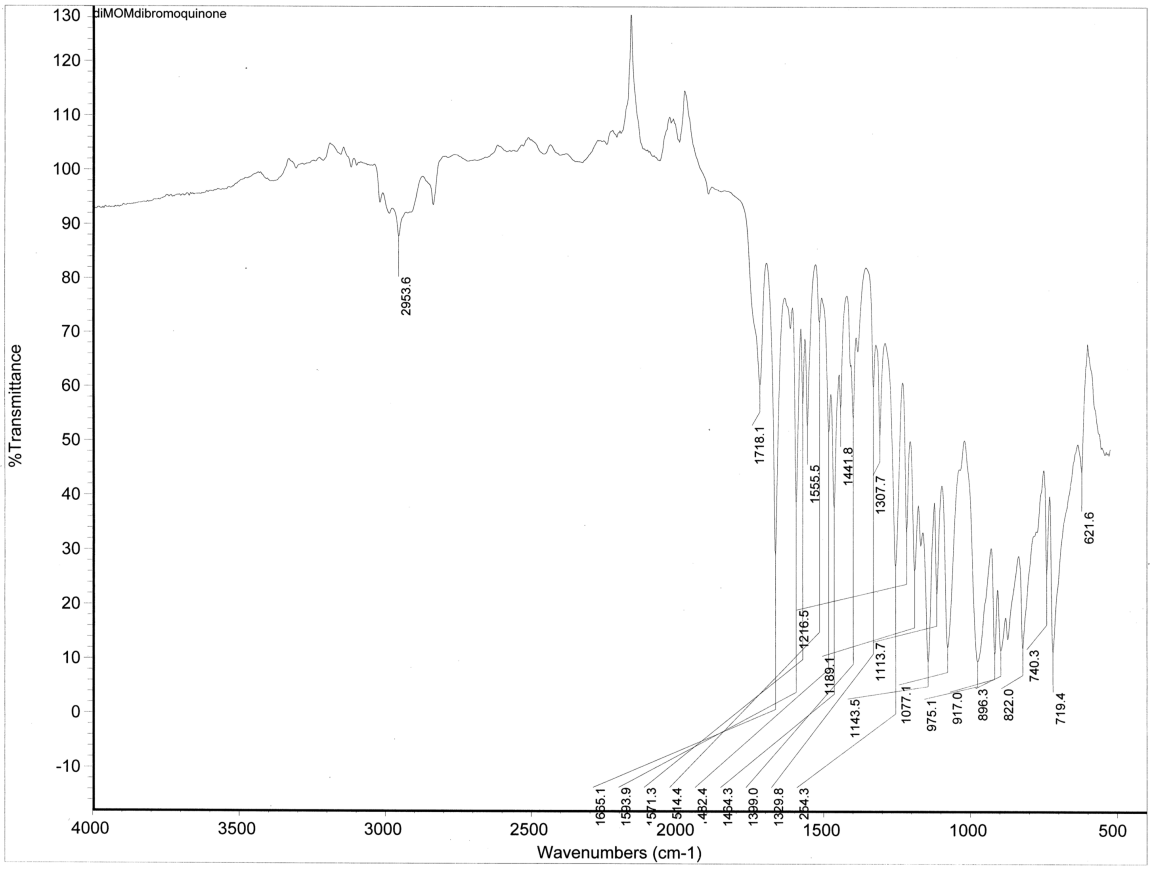
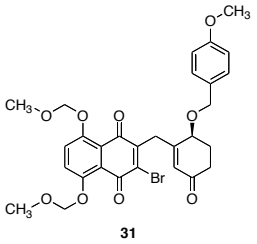
1187.1
1148.1
1089.9
1055.7
1003.8
813.0
754.3
719.6


^{1}H NMR ($CDCl_3$)
CH3
O
O
O
Br
Br
O
O
CH3
O
O
30


^{13}C NMR ($CDCl_3$)

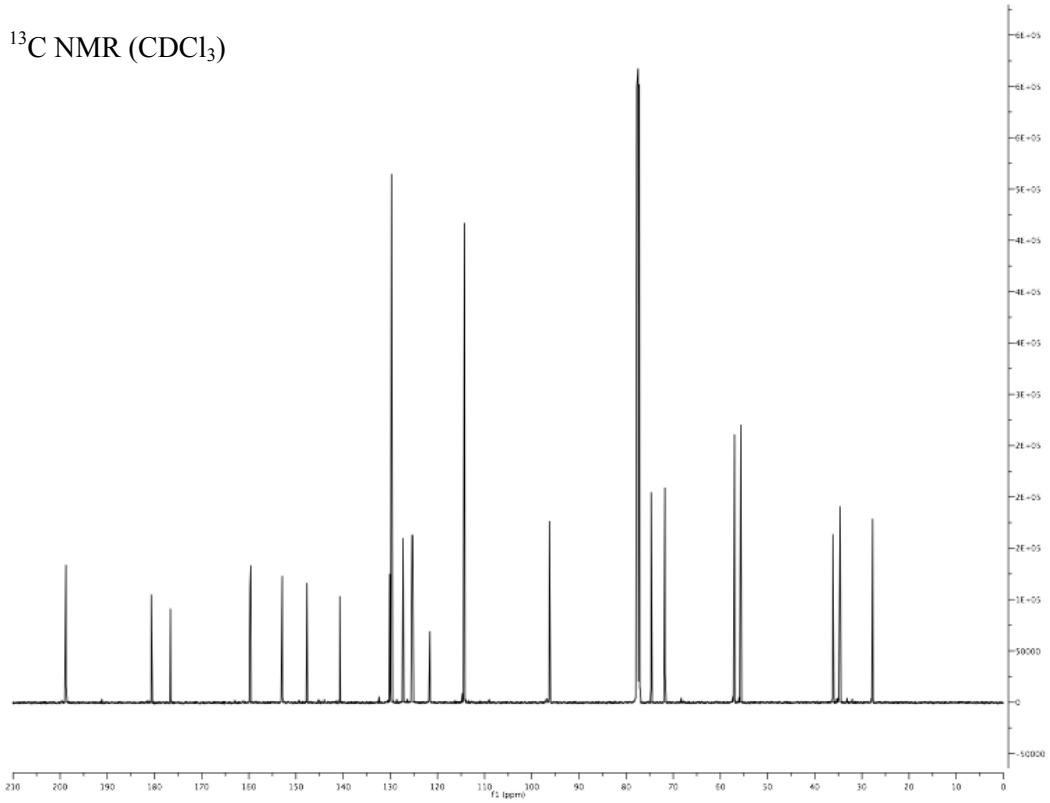

diMOMdibromoquinone
%Transmittance
Wavenumbers (cm-1)
2953.6
1718.1
1555.5
1441.8
1307.7
1216.5
1189.1
1143.5
1113.7
1077.1
975.1
917.0
896.3
822.0
740.3
719.4
621.6
1665.1
1593.9
1571.3
1514.4
1482.4
1454.3
1399.0
1329.8
1254.3

^{1}H NMR ($CDCl_3$)

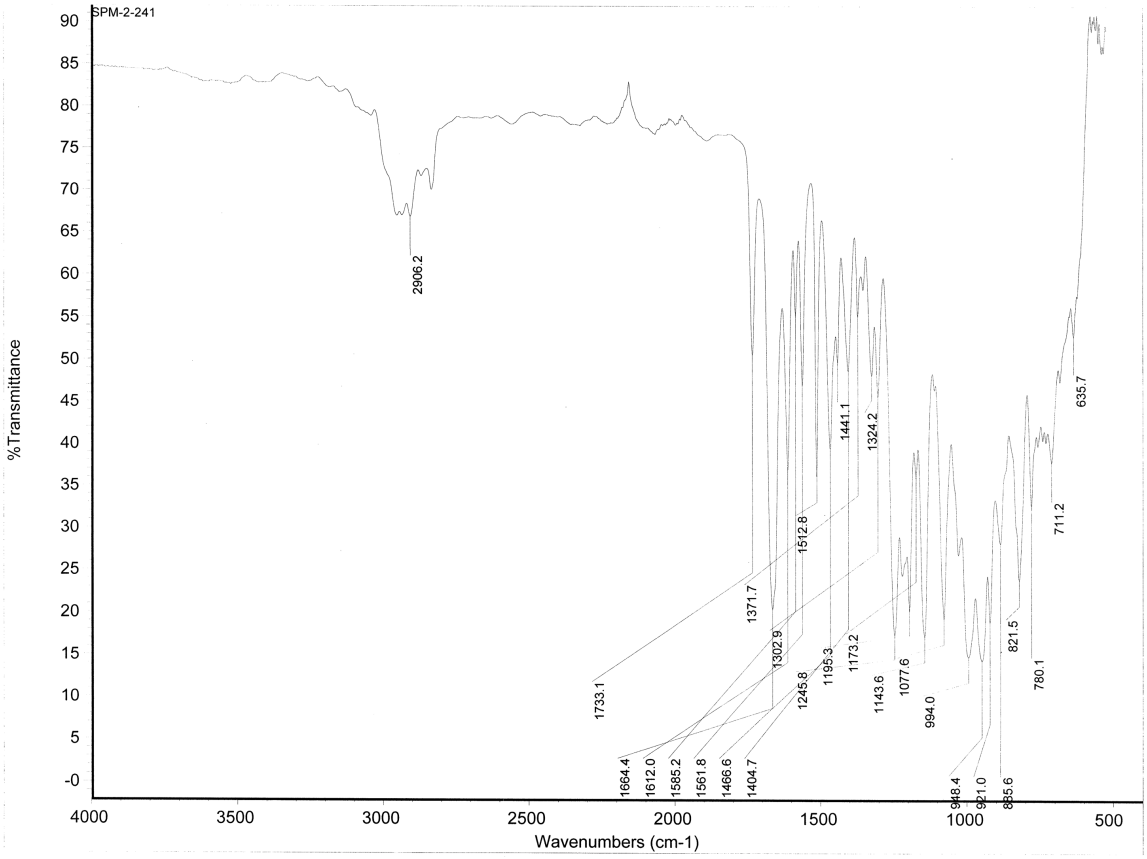


31

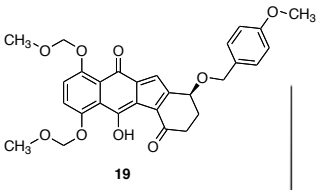
^{13}C NMR ($CDCl_3$)



IR

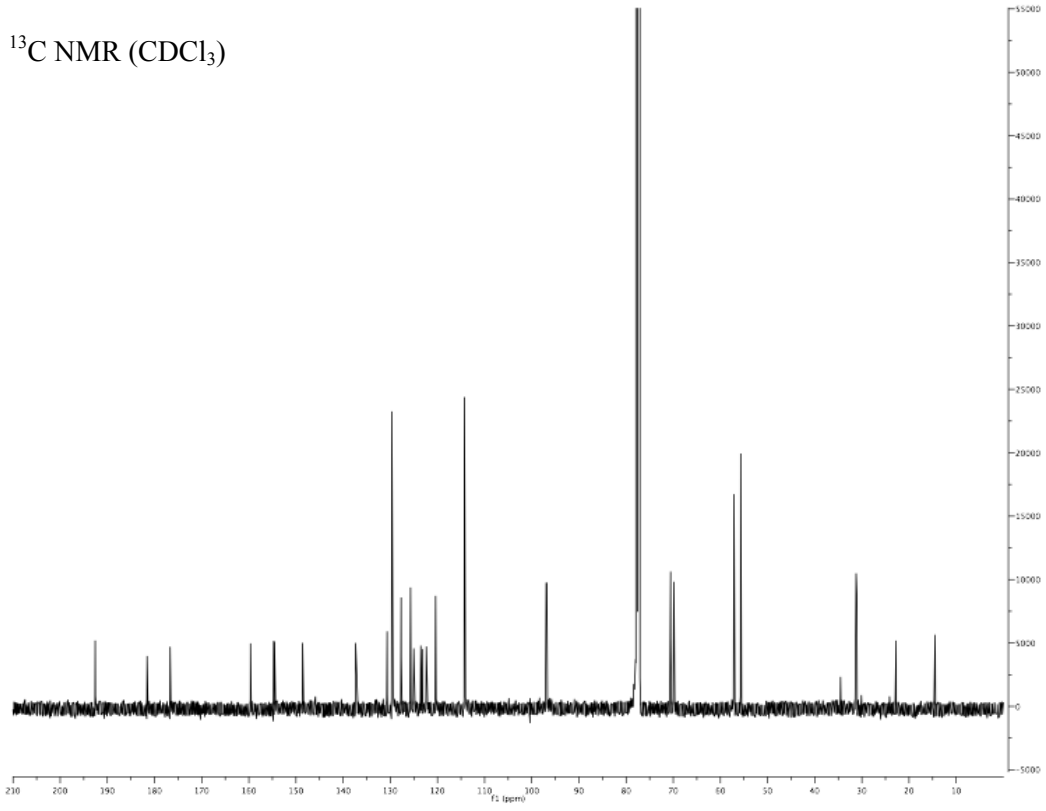

SPM-2-241
%Transmittance
Wavenumbers (cm-1)
2906.2
1733.1
1664.4
1612.0
1585.2
1561.8
1466.6
1404.7
1371.7
1302.9
1512.8
1441.1
1324.2
1245.8
1195.3
1173.2
1143.6
1077.6
994.0
948.4
921.0
885.6
821.5
780.1
711.2
635.7

^{1}H NMR ($CDCl_3$)

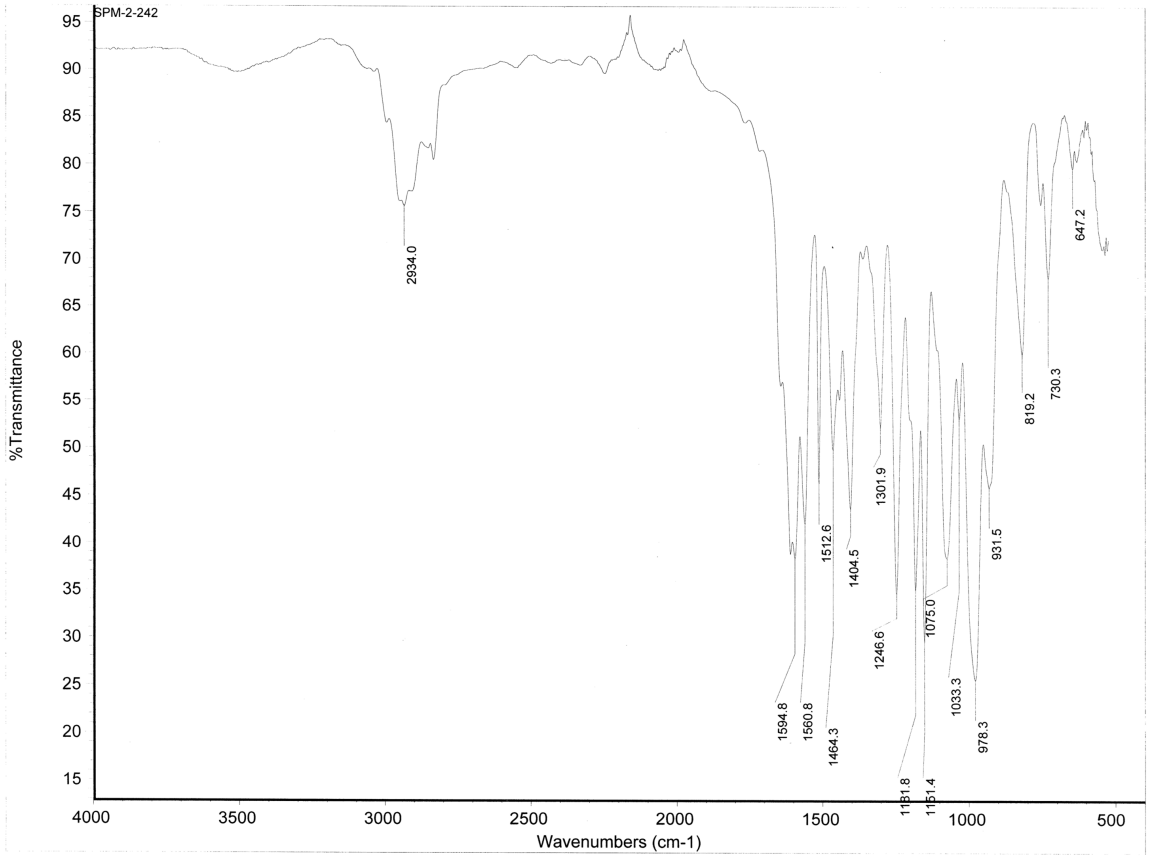


19

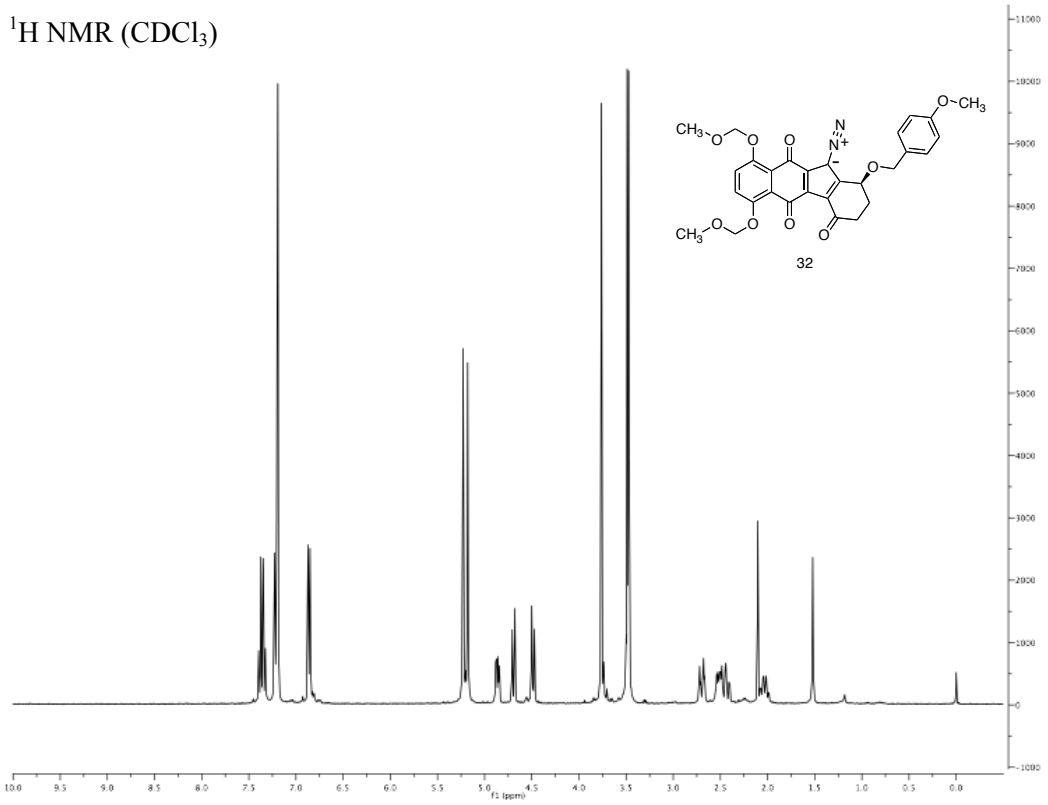
^{13}C NMR ($CDCl_3$)



IR

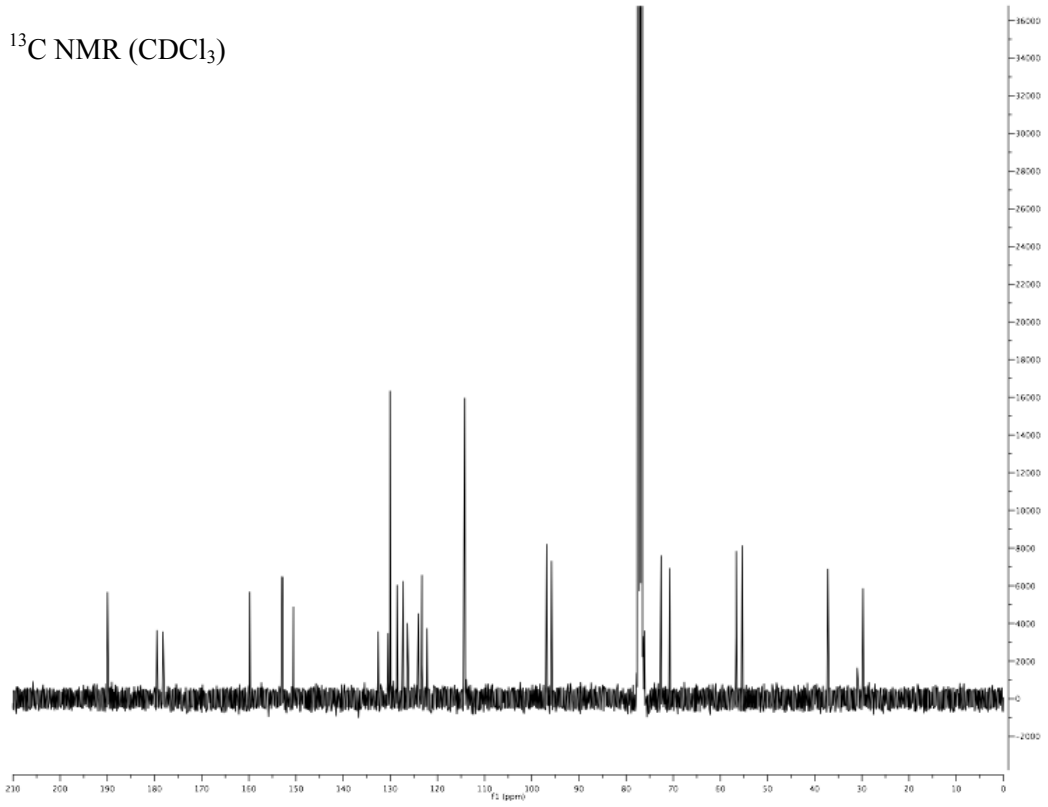

SPM-2-242
%Transmittance
Wavenumbers (cm-1)
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
4000
3500
3000
2500
2000
1500
1000
500
2934.0
1594.8
1560.8
1512.6
1464.3
1404.5
1301.9
1246.6
1151.8
1151.4
1075.0
1033.3
978.3
931.5
819.2
730.3
647.2

^{1}H NMR ($CDCl_3$)

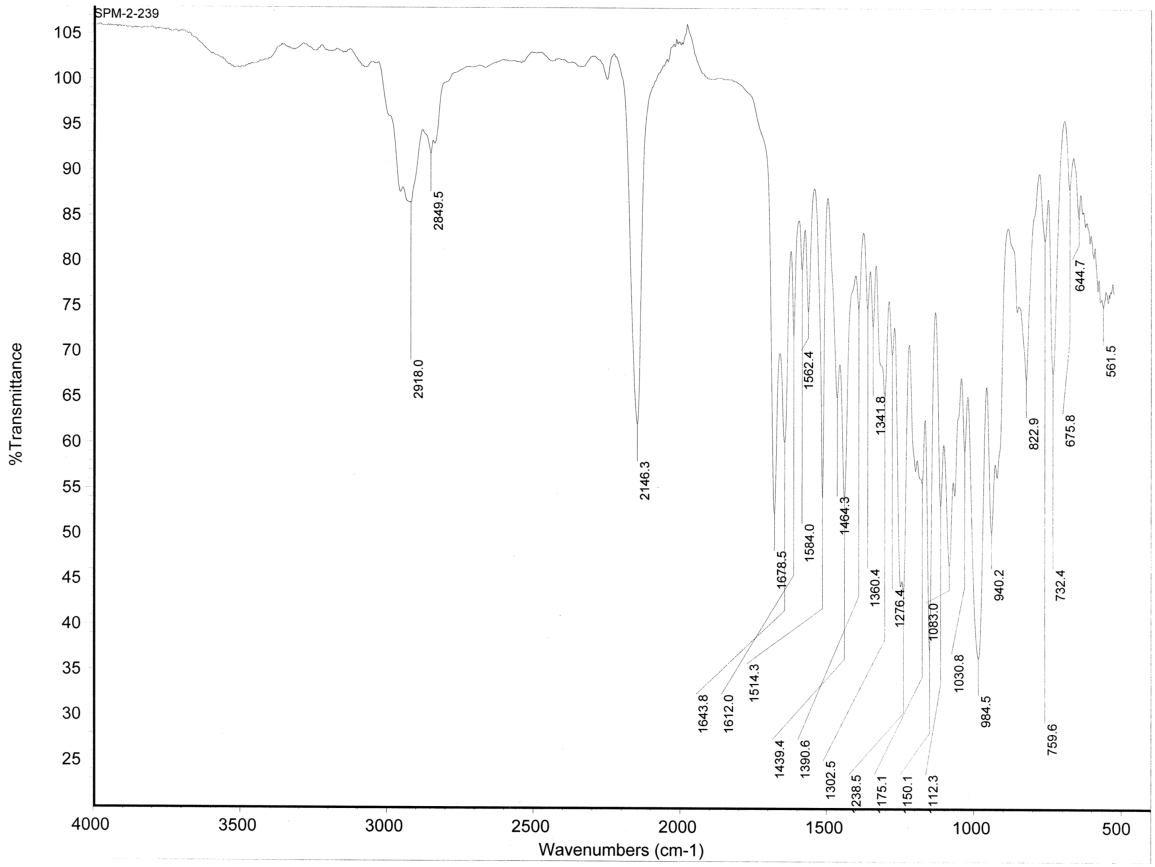


32

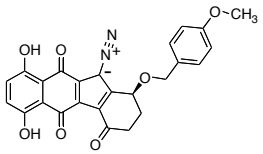
^{13}C NMR ($CDCl_3$)



IR

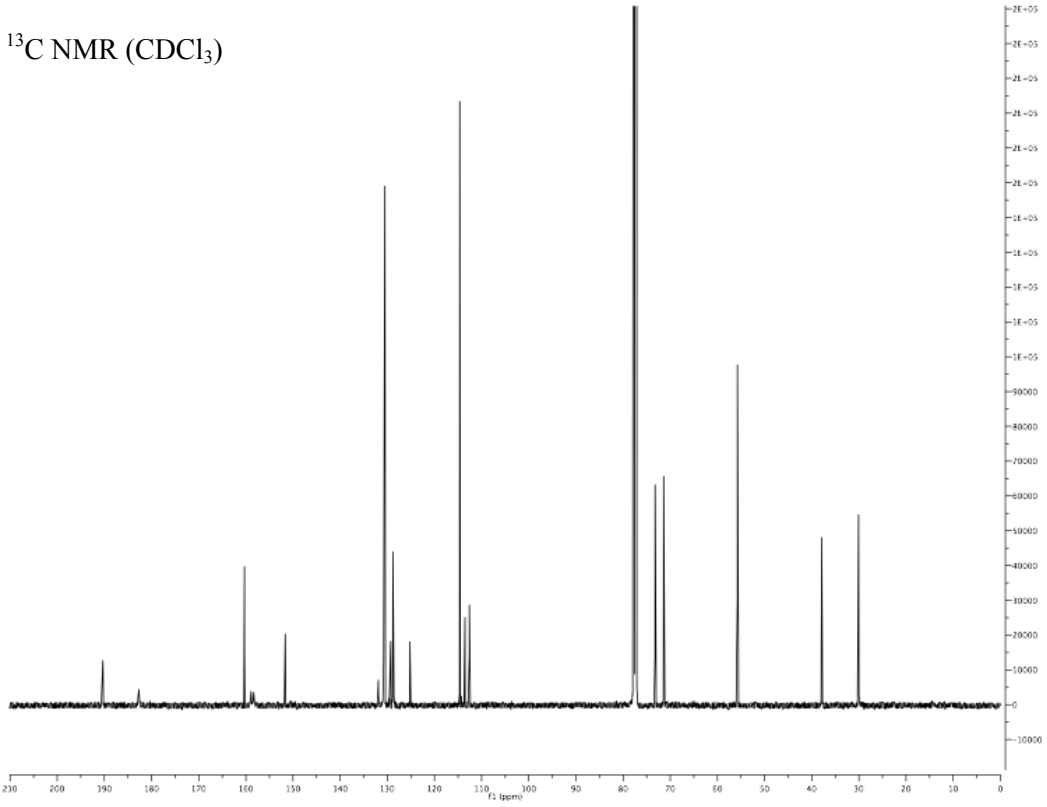

SPM-2-239
%Transmittance
Wavenumbers (cm-1)
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
4000
3500
3000
2500
2000
1500
1000
500
2918.0
2849.5
2146.3
1643.8
1612.0
1514.3
1678.5
1584.0
1562.4
1464.3
1439.4
1390.6
1360.4
1341.8
1302.5
1276.4
1238.5
1175.1
1150.1
1112.3
1083.0
1030.8
984.5
940.2
822.9
759.6
732.4
675.8
644.7
561.5

^{1}H NMR ($CDCl_3$)

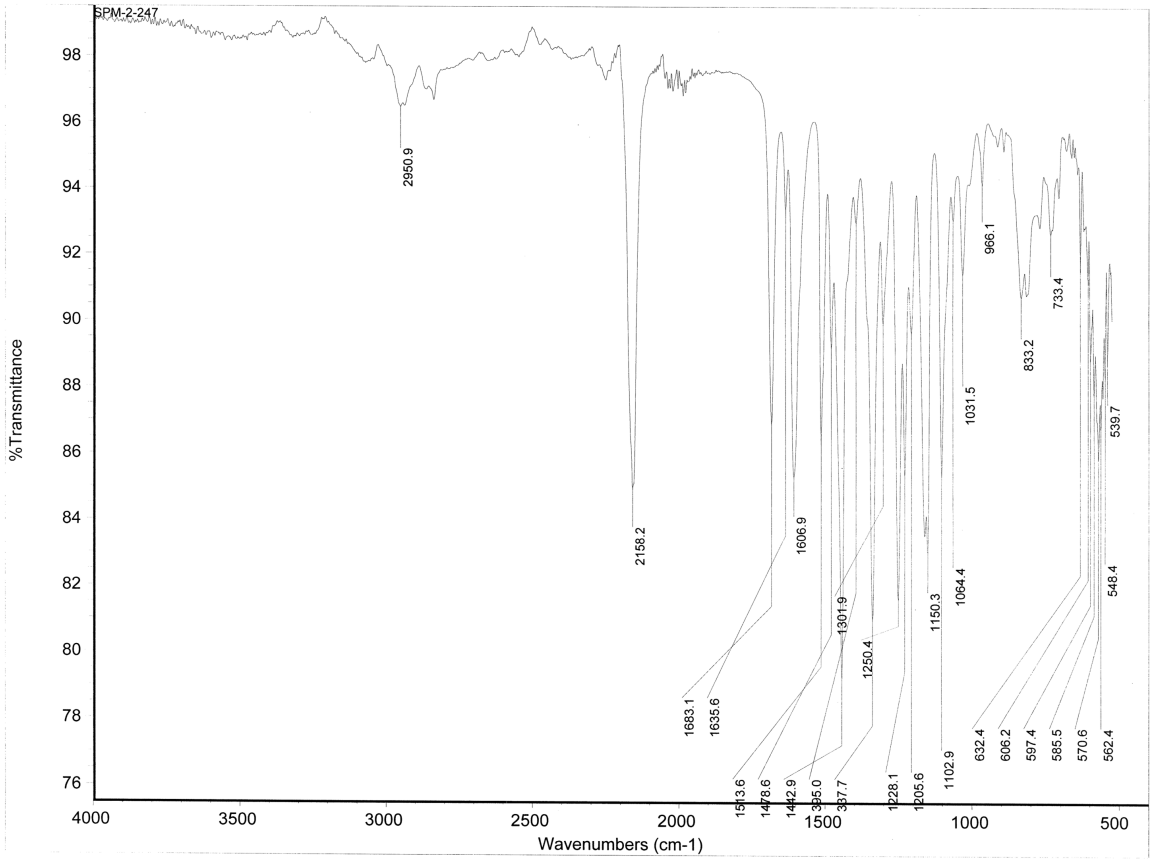


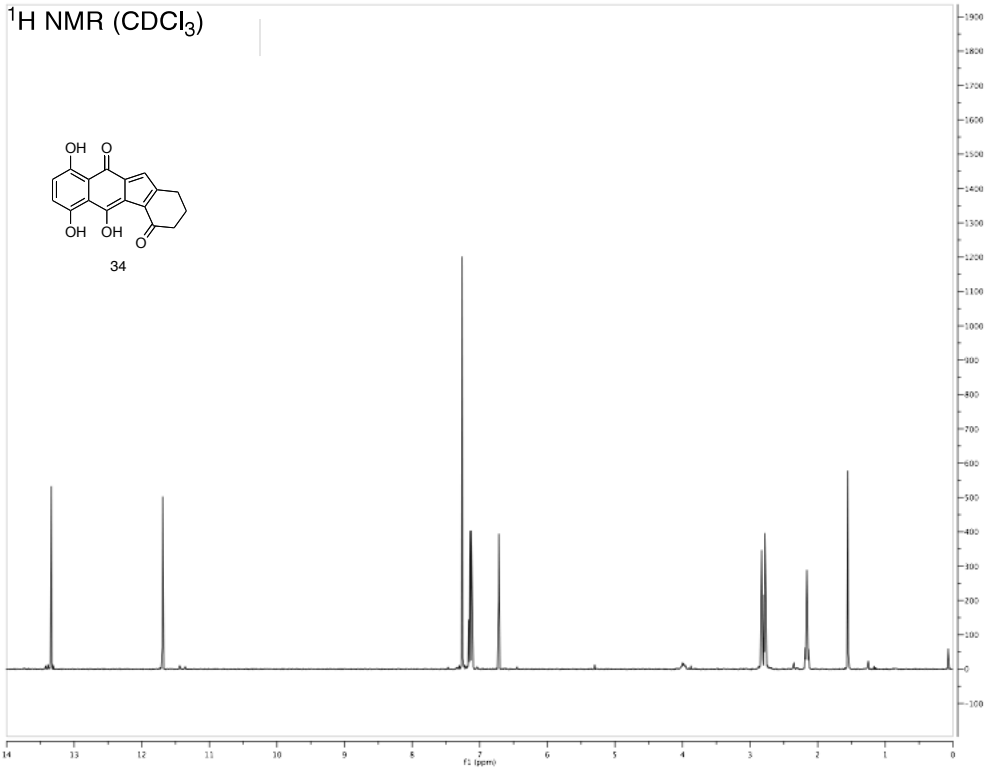
12

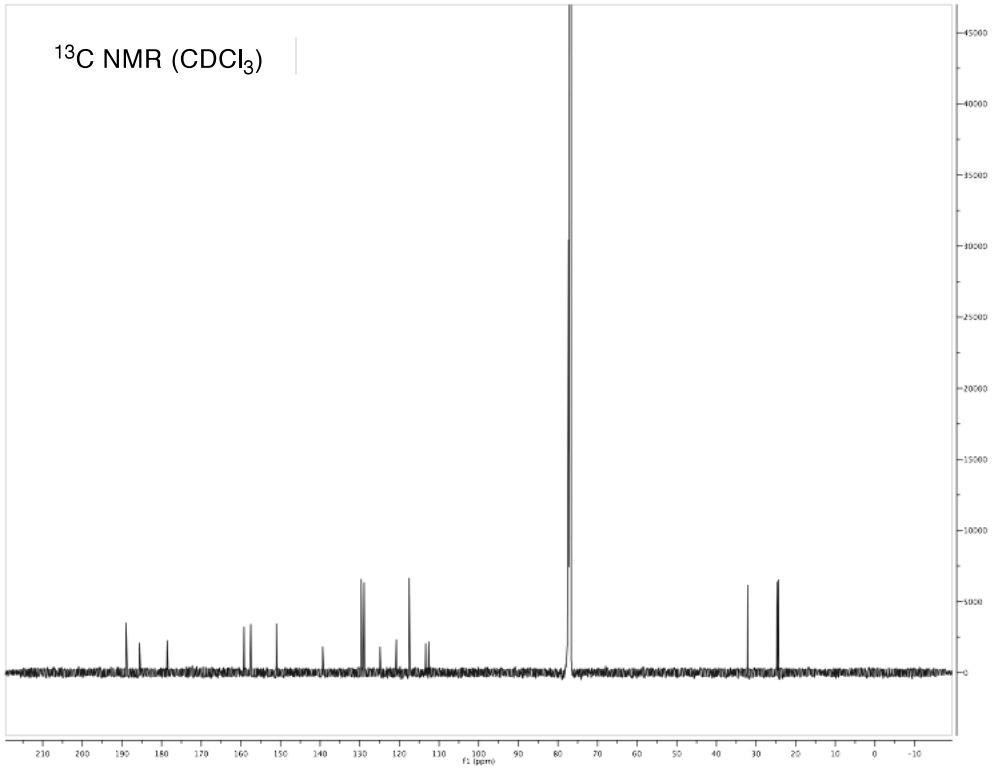
^{13}C NMR ($CDCl_3$)



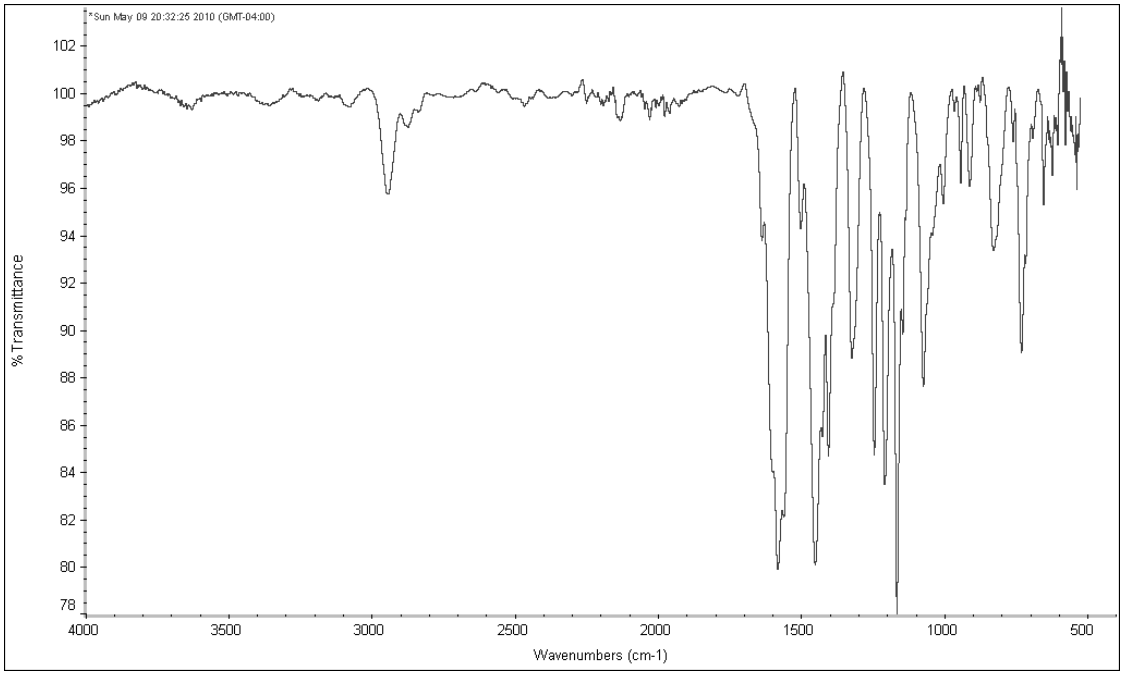
IR

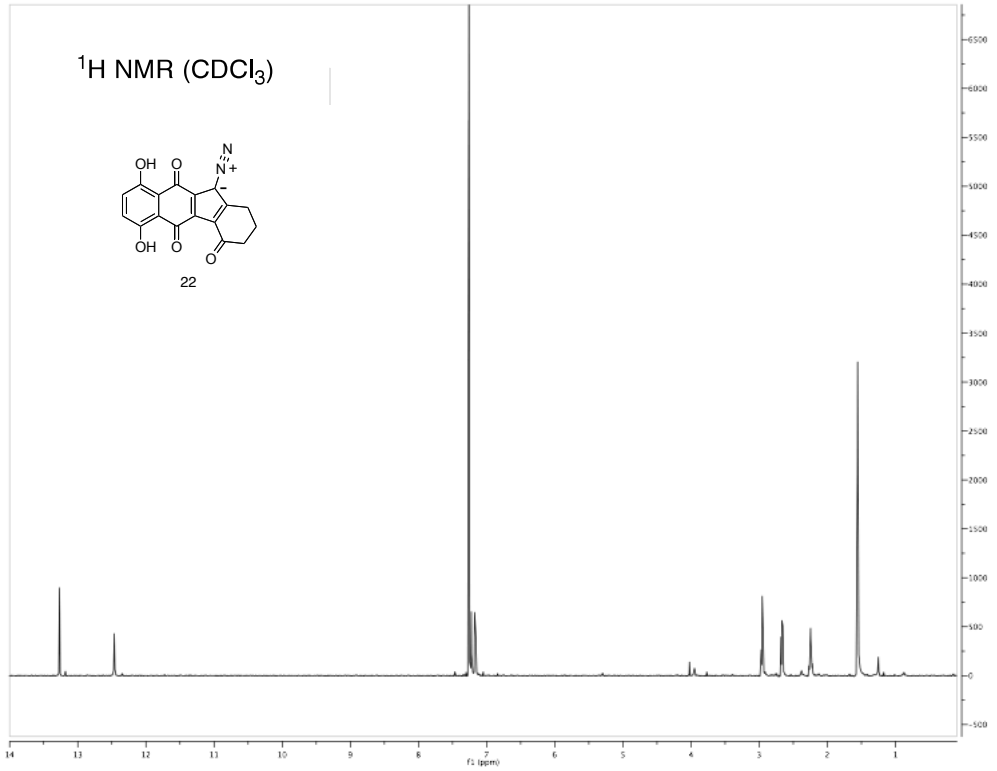

SPM-2-247
%Transmittance
Wavenumbers (cm-1)
98
96
94
92
90
88
86
84
82
80
78
76
4000
3500
3000
2500
2000
1500
1000
500
2950.9
2158.2
1683.1
1635.6
1606.9
1513.6
1478.6
1442.9
1395.0
1387.7
1301.9
1250.4
1228.1
1205.6
1150.3
1102.9
1064.4
1031.5
966.1
833.2
733.4
632.4
606.2
597.4
585.5
570.6
562.4
548.4
539.7

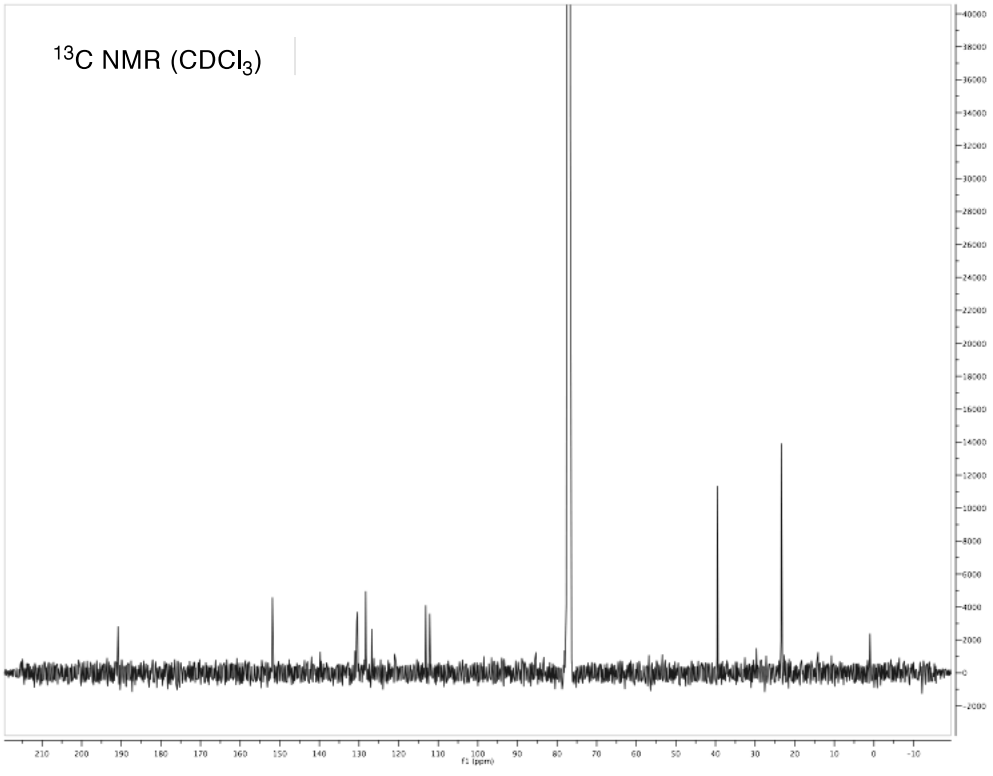

^{1}H NMR ($CDCl_3$)
OH
O
OH
OH
O
34


^{13}C NMR ($CDCl_3$)

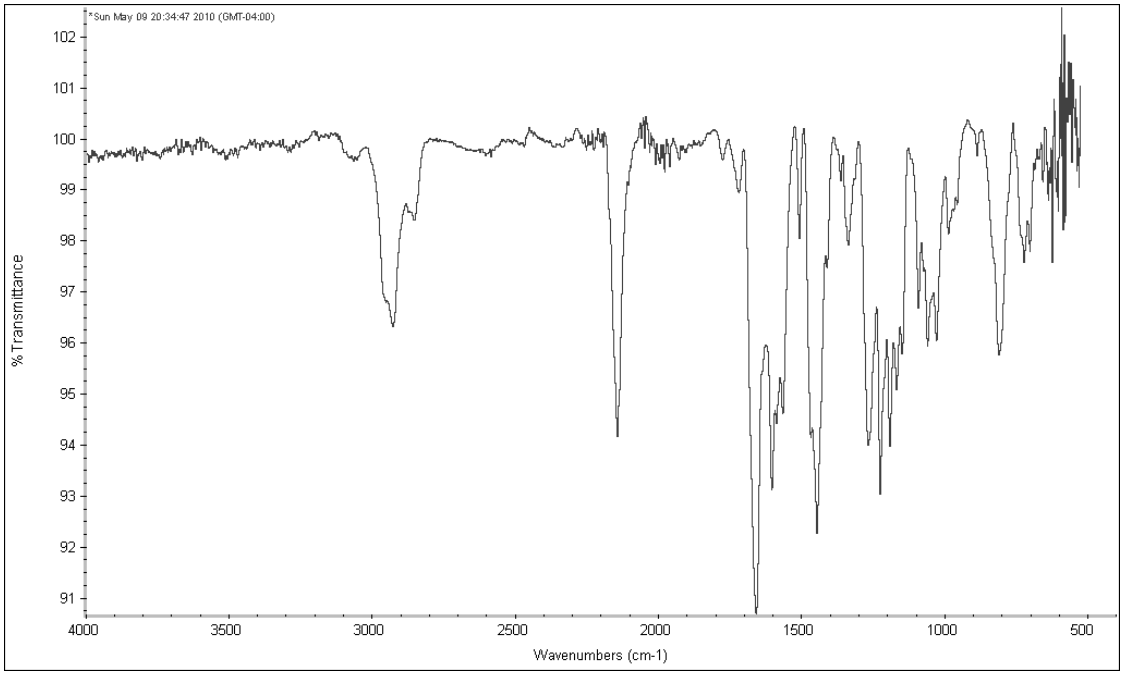
IR

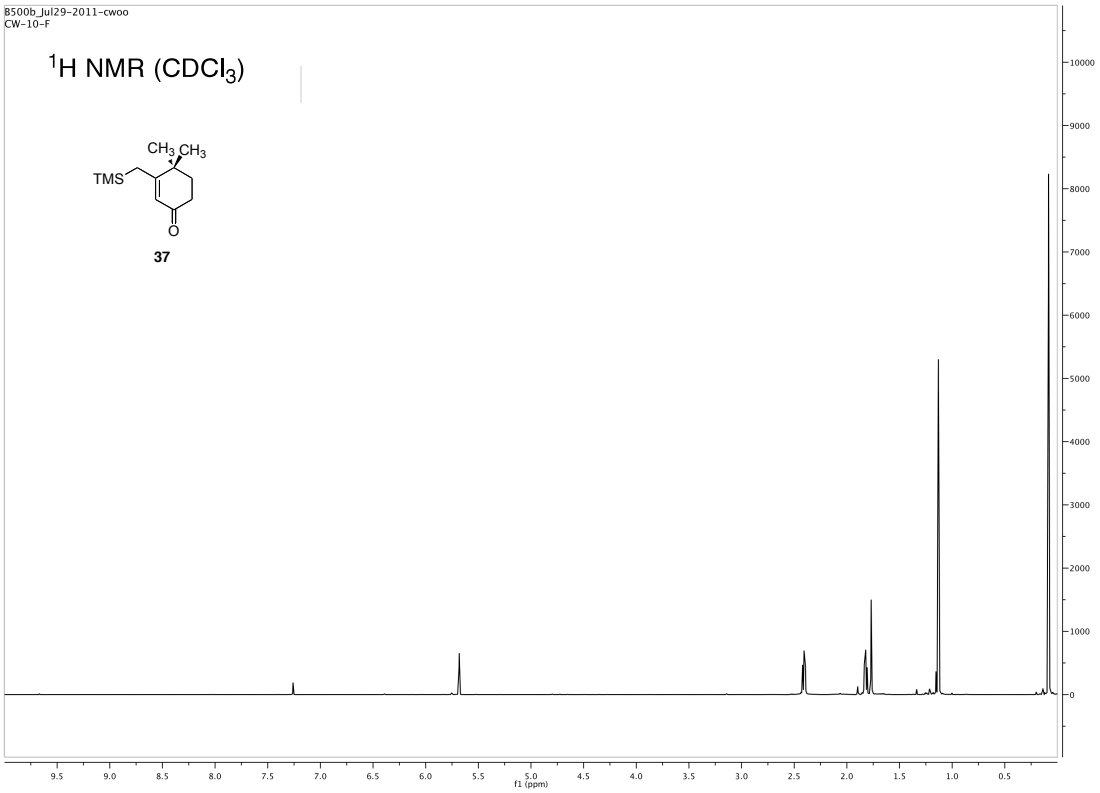

Sun May 09 20:32:25 2010 (GMT-04:00)
% Transmittance
102
100
98
96
94
92
90
88
86
84
82
80
78
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

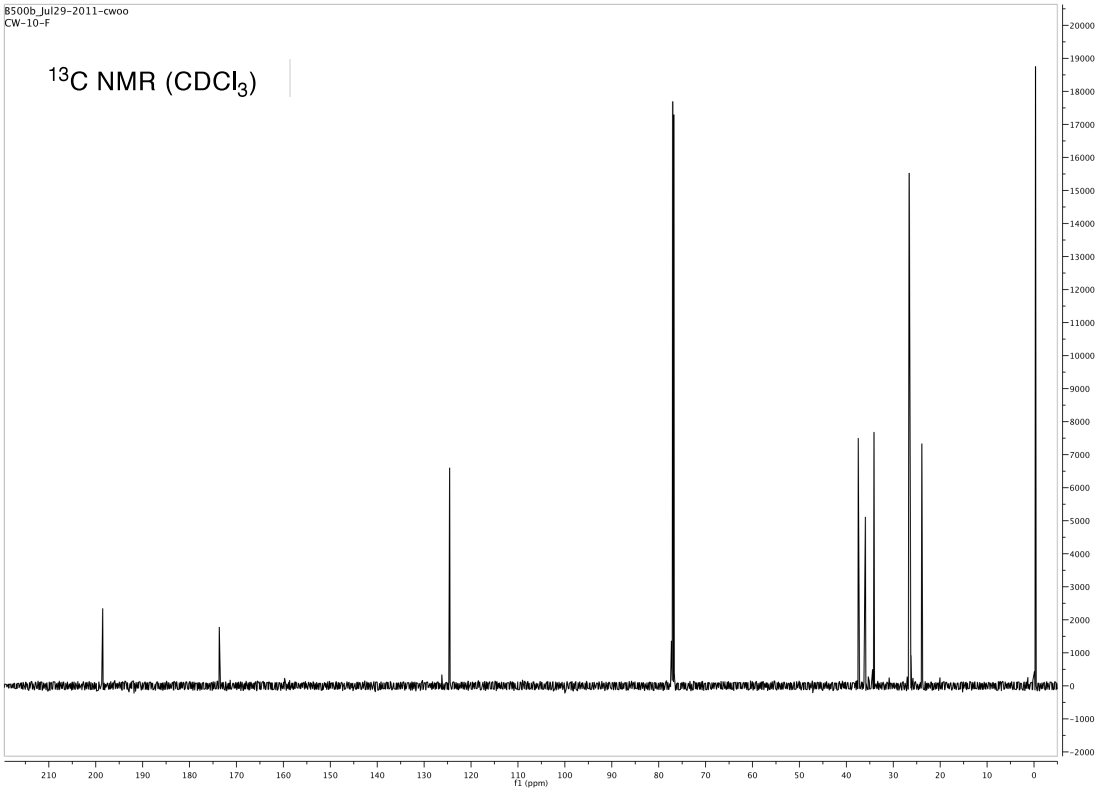

^{1}H NMR ($CDCl_3$)
OH
O
N
N+
OH
O
O
22


^{13}C NMR ($CDCl_3$)

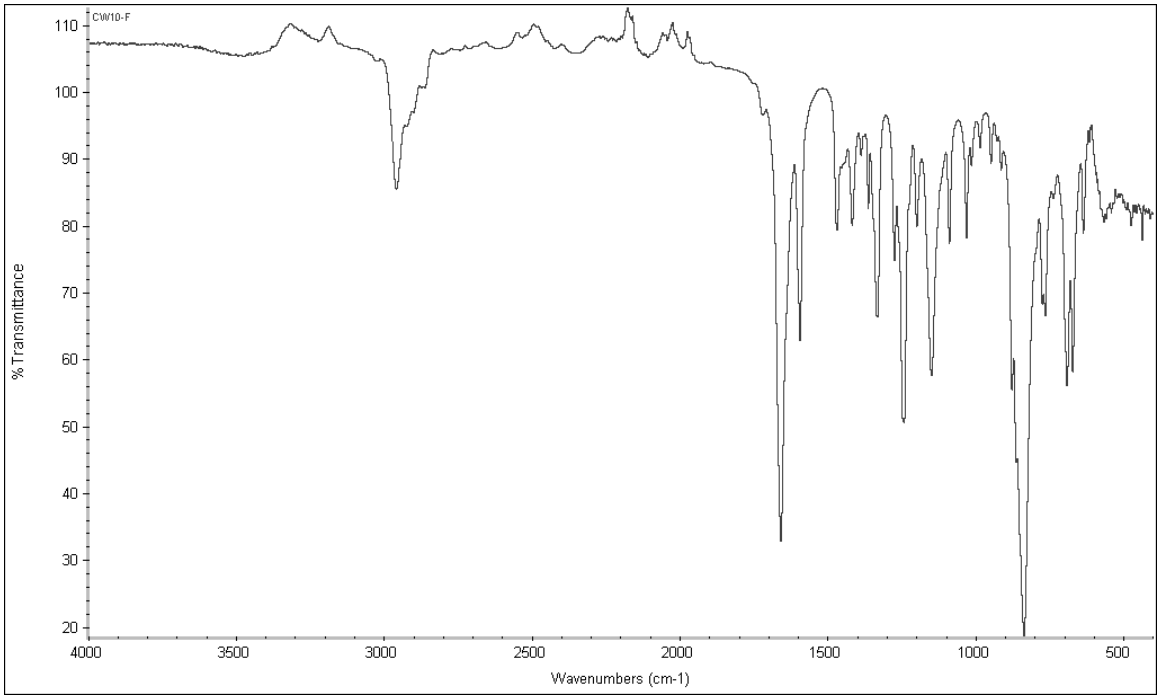
IR

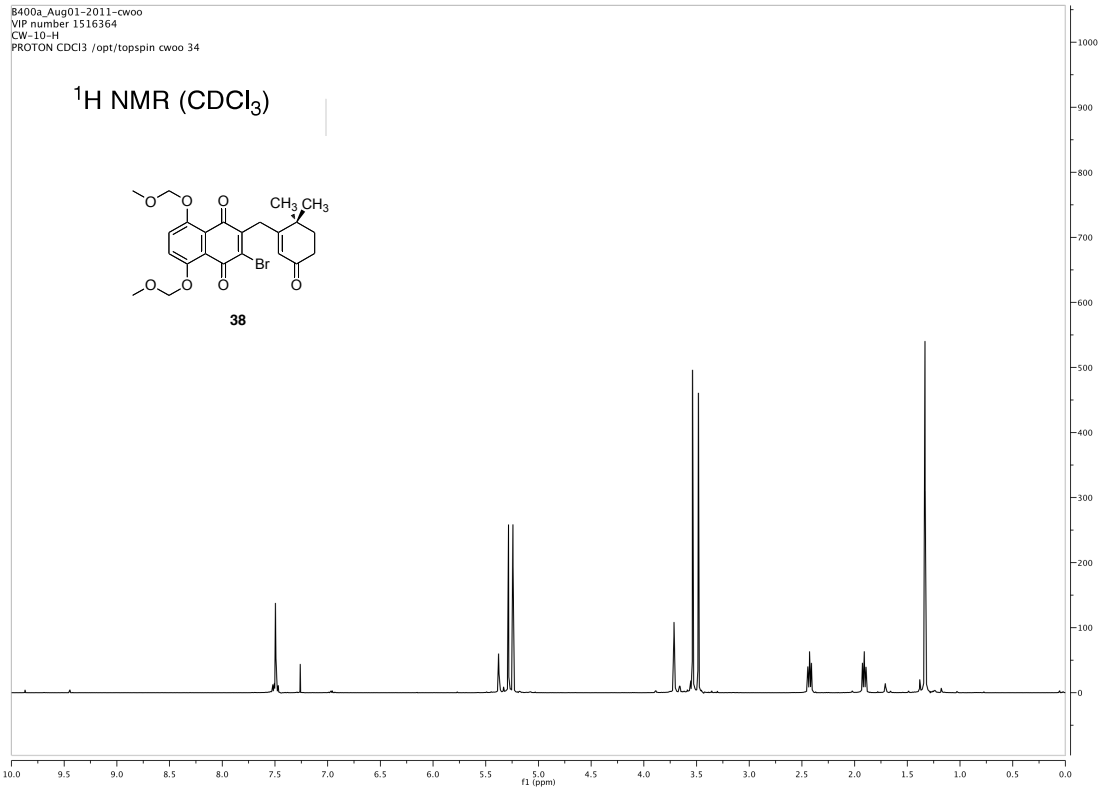

*Sun May 09 20:34:47 2010 (GMT-04:00)
% Transmittance
102
101
100
99
98
97
96
95
94
93
92
91
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

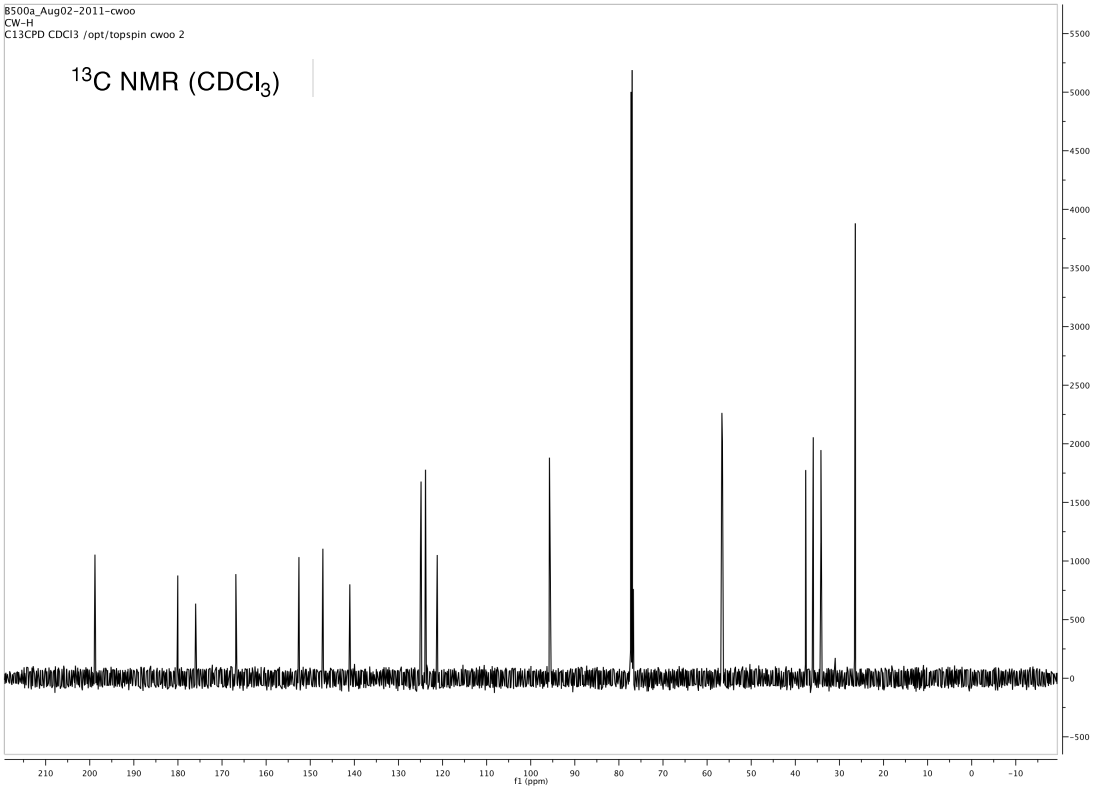

B500b_Jul29-2011-cwoo
CW-10-F
^{1}H NMR ($CDCl_3$)
CH_3 CH_3
TMS
O
37
f1 (ppm)


B500b_Jul29-2011-cwoo
CW-10-F
^{13}C NMR ($CDCl_3$)
f1 (ppm)

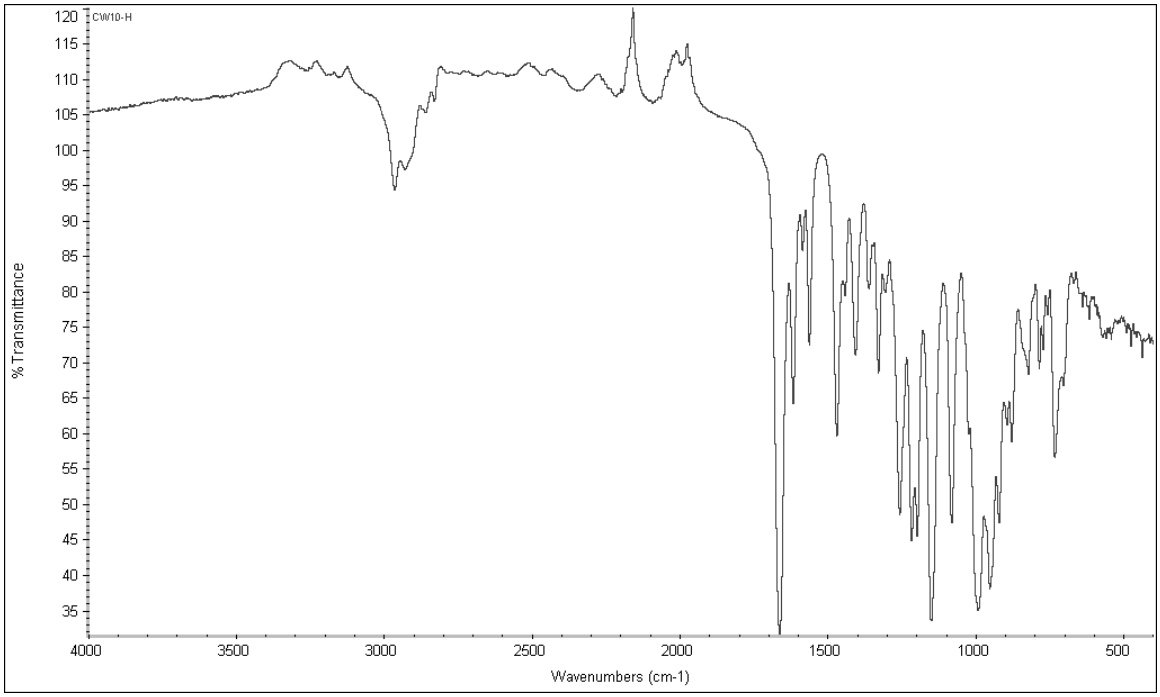
IR

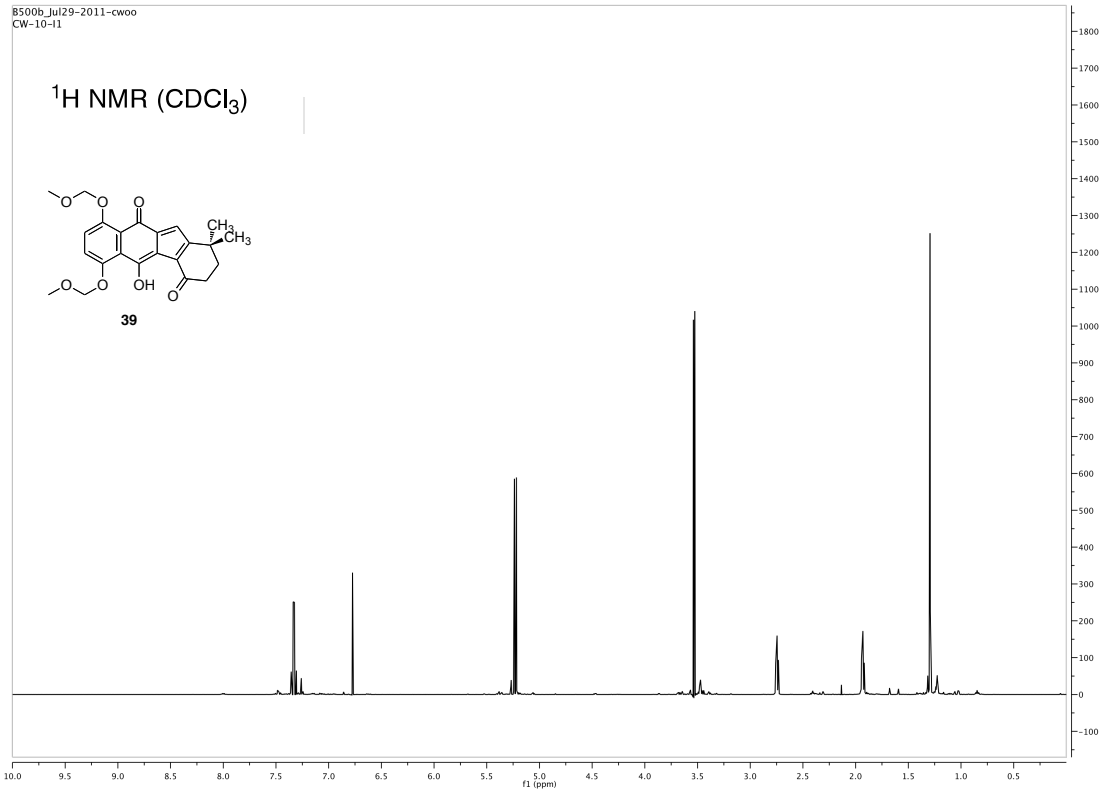

CW10-F
110
100
90
80
70
60
50
40
30
20
% Transmittance
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

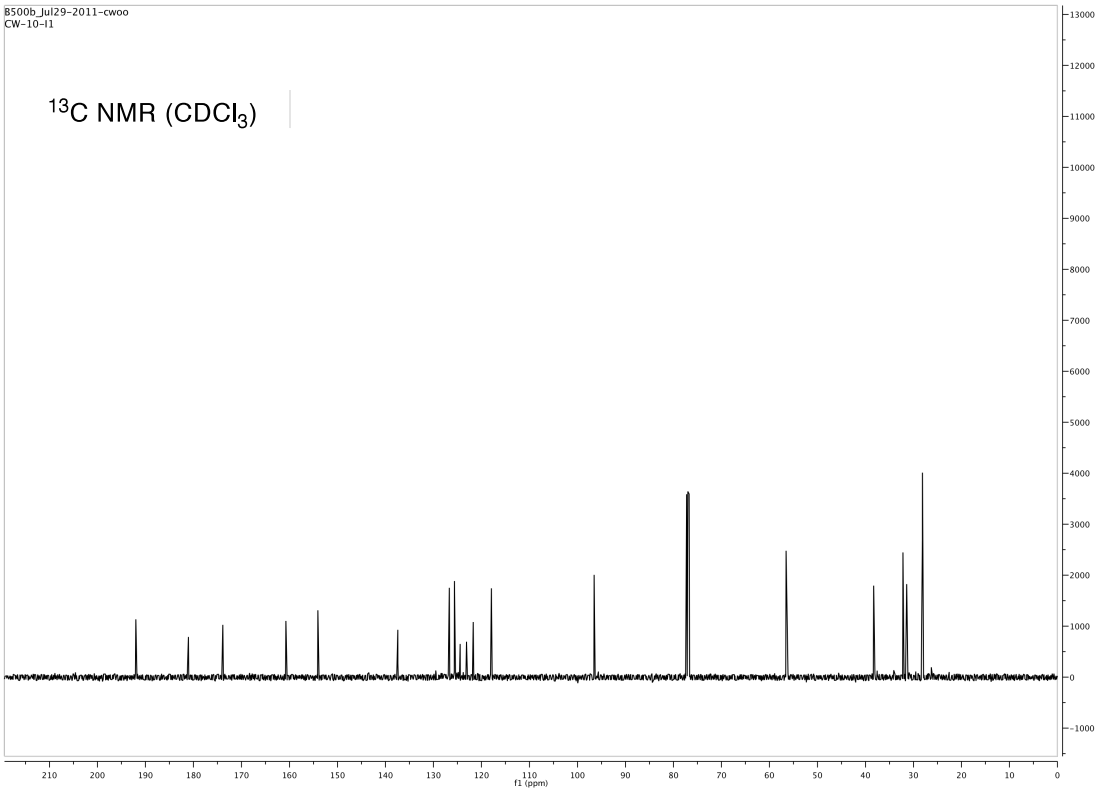

B400a_Aug01-2011-cwoo
VIP number 1516364
CW-10-H
PROTON CDCl3 /opt/topspin cwoo 34
^{1}H NMR ($CDCl_3$)
CH_3 CH_3
Br
38
f1 (ppm)


B500a_Aug02-2011-cwoo
CW-H
C13CPD CDCl3 /opt/topspin cwoo 2
^{13}C NMR ($CDCl_3$)
f1 (ppm)

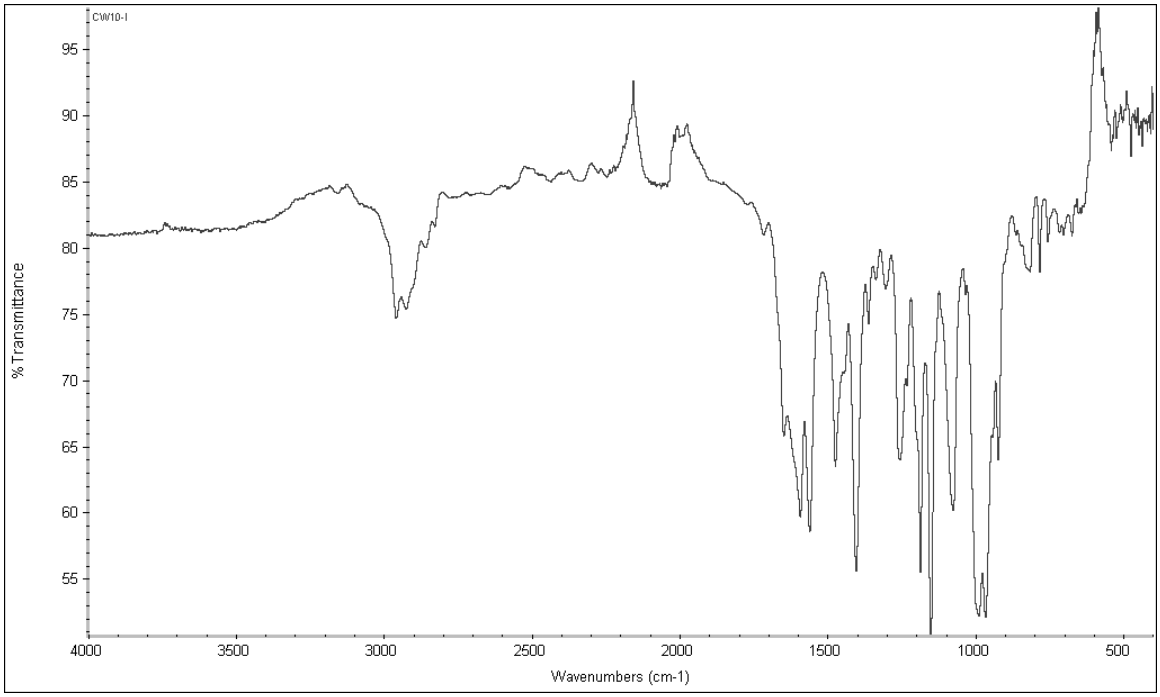
IR

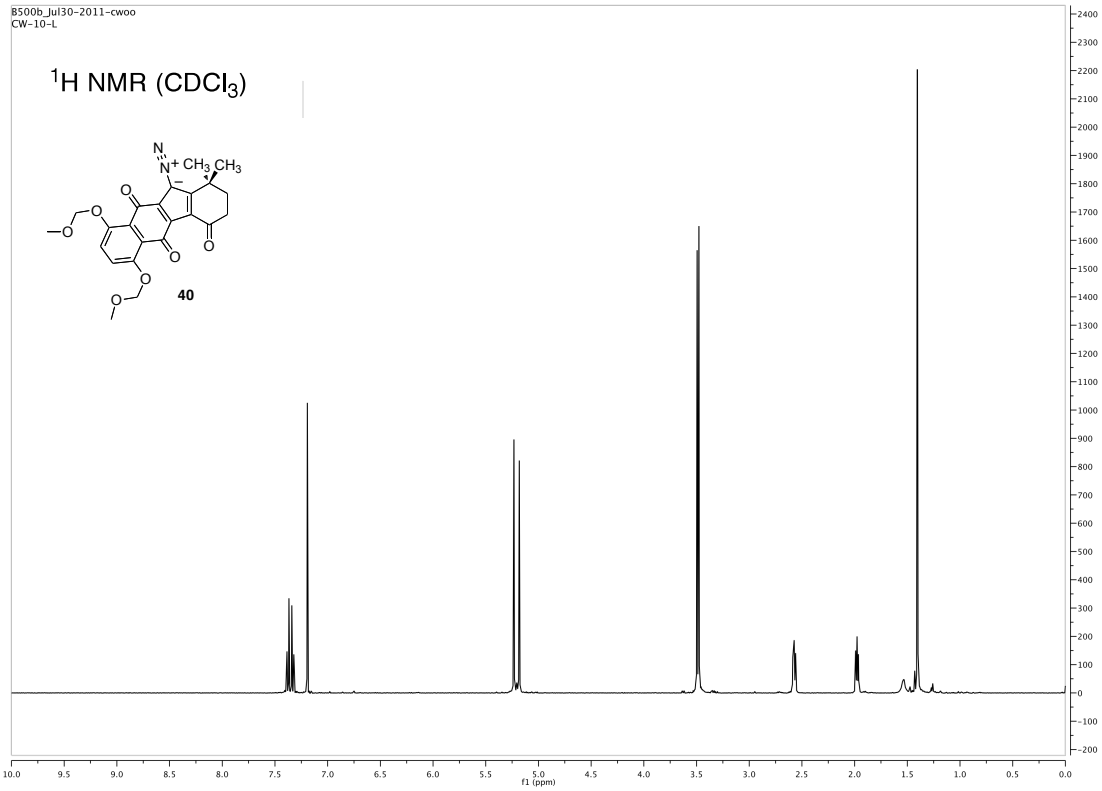


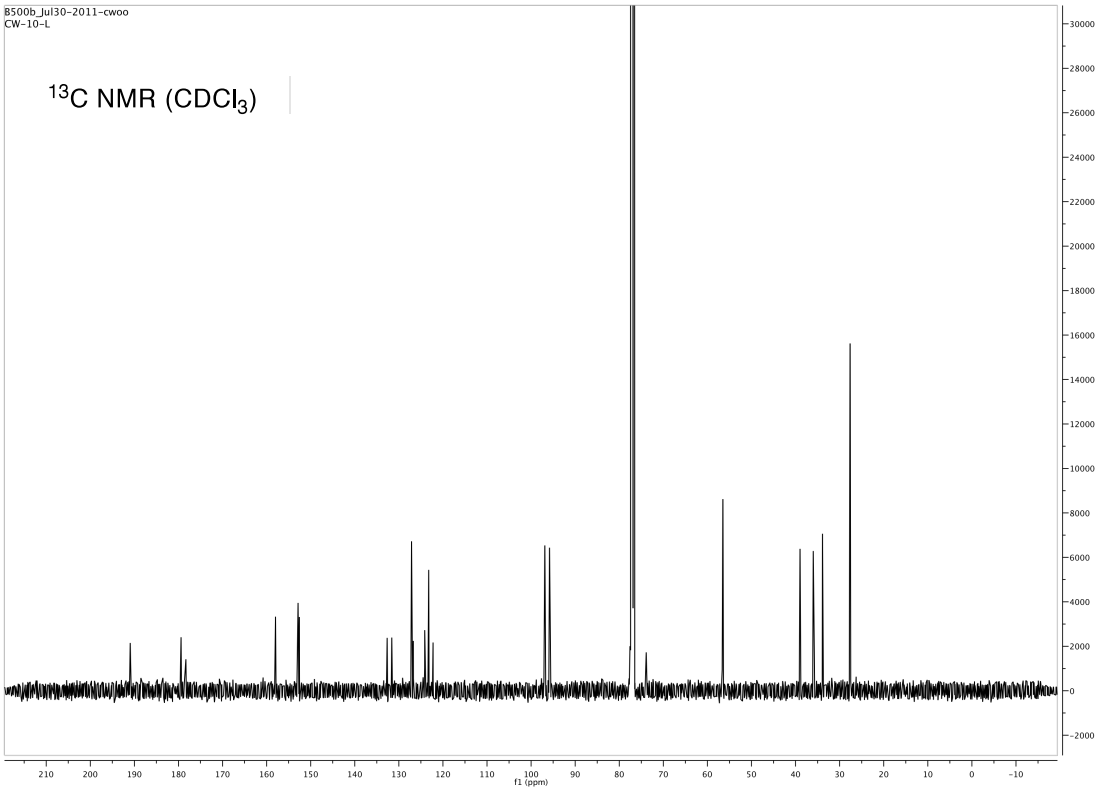

B500b_Jul29-2011-cwoo
CW-10-I1
^{1}H NMR ($CDCl_3$)
39
f1 (ppm)


B500b_Jul29-2011-cwoo
CW-10-I1
^{13}C NMR ($CDCl_3$)
f1 (ppm)

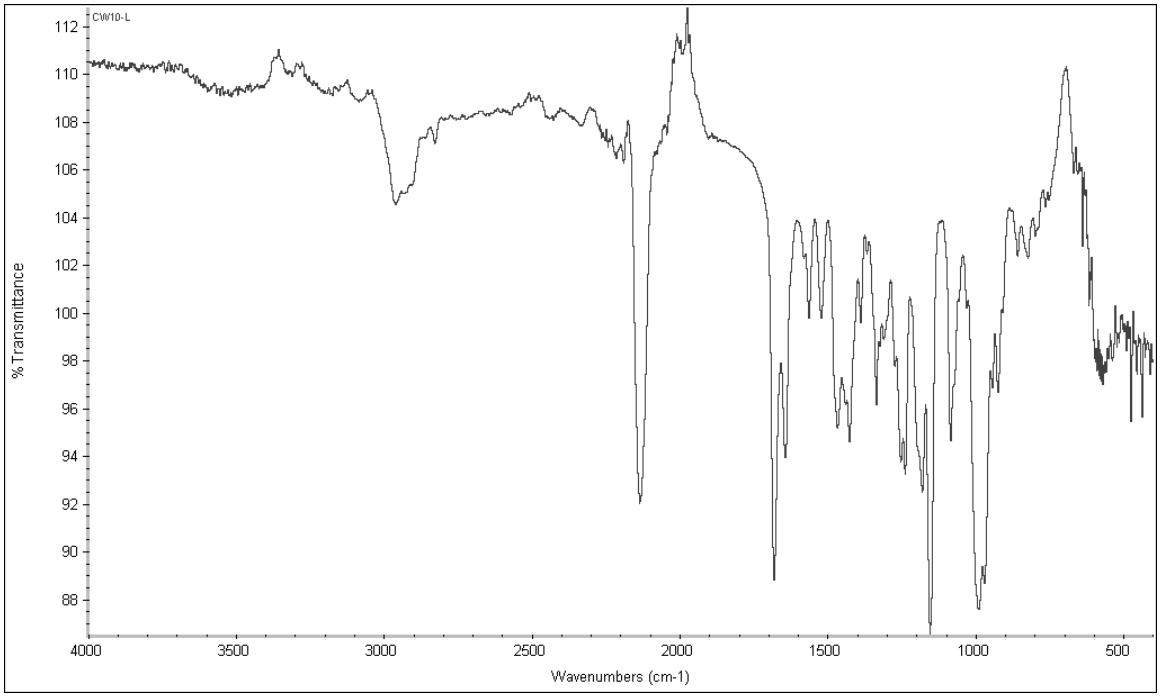
IR

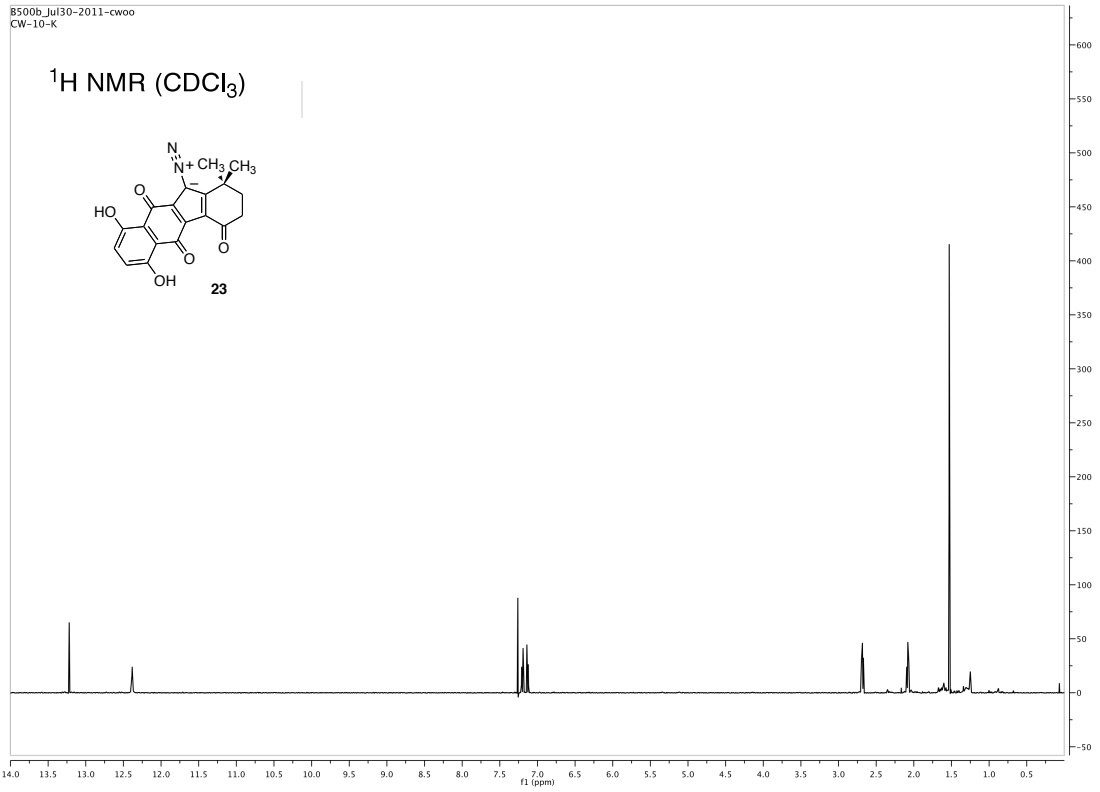

CW10-I
% Transmittance
95
90
85
80
75
70
65
60
55
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

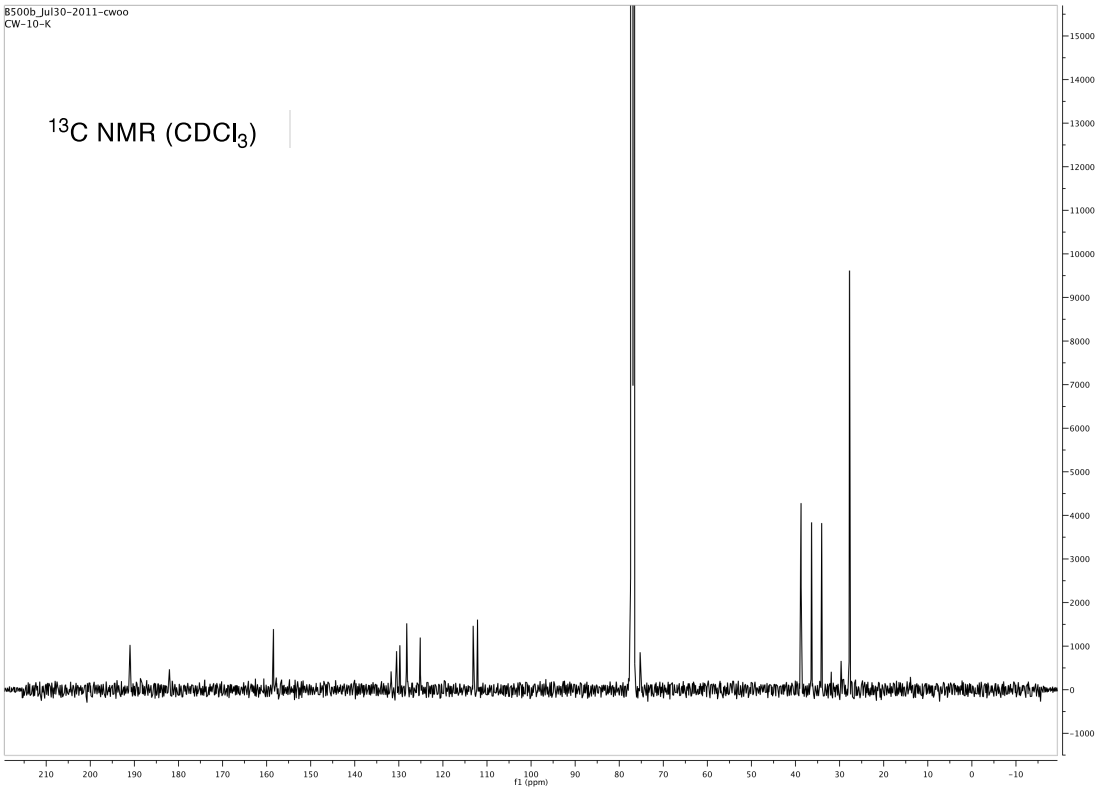

B500b_Jul30-2011-cwoo
CW-10-L
^{1}H NMR ($CDCl_3$)
40
f1 (ppm)


B500b_Jul30-2011-cwoo
CW-10-L
^{13}C NMR ($CDCl_3$)
f1 (ppm)

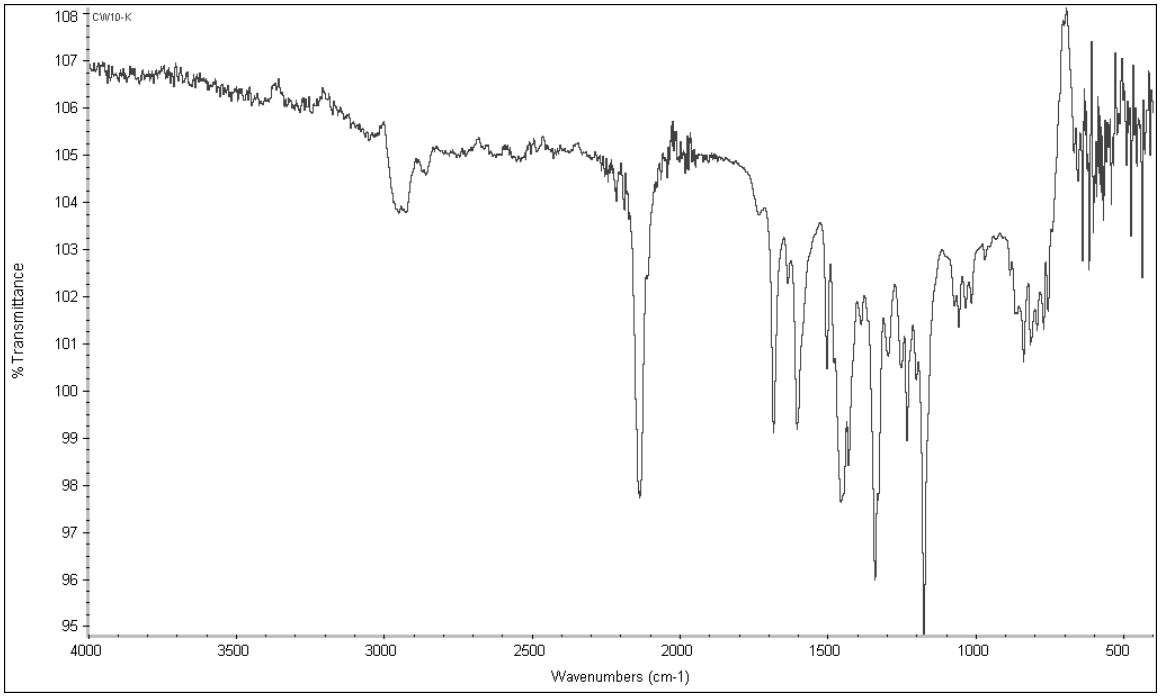
IR


CW10-L
% Transmittance
112
110
108
106
104
102
100
98
96
94
92
90
88
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)


B500b_Jul30-2011-cwoo
CW-10-K
^{1}H NMR ($CDCl_3$)
N
N+ CH3 CH3
HO
O
O
O
OH
23
f1 (ppm)


B500b_Jul30-2011-cwoo
CW-10-K
^{13}C NMR ($CDCl_3$)
f1 (ppm)

IR


CW10-K
% Transmittance
108
107
106
105
104
103
102
101
100
99
98
97
96
95
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

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