A Novel Fluorogenic Transformation:
Development of an Optical Probe for Coenzyme Q

Supporting Information

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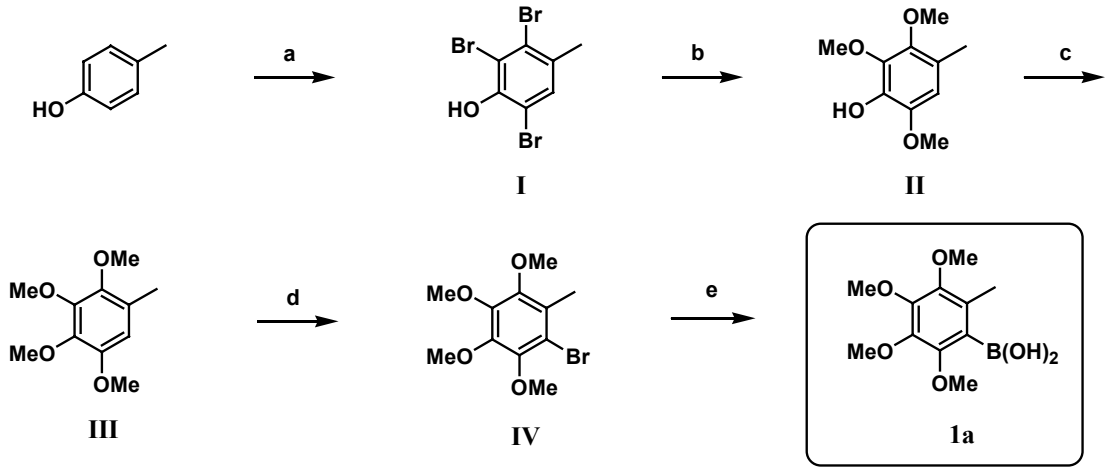
General

All manipulations of air and/or water sensitive compounds were performed using standard Schlenk techniques. Nuclear Magnetic Resonance spectra were recorded at 300 K (unless otherwise noted) on Bruker 300 or 400 or 500 Fourier transform NMR spectrometers. Unless otherwise noted, spectra were taken at 300K in MeOH-d_4 with the proton (δ 3.30) or carbon (δ 49.0) as the reference, in $CHCl_3$-d_1 with the proton (δ 7.26) or carbon (δ 77.0) as the reference, or in DMSO-d_6 with the proton (δ 2.49) or carbon (δ 39.5) as the reference. Flash chromatography was performed on SILICYCLE silica gel (230-400 mesh). Low resolution mass spectra were recorded on a JEOL LCmate (ionization mode: APCI+); high resolution mass spectra were obtained on a JMS_HX110 HF mass spectrometer (ionization mode: FAB+). All chemicals were purchased from Sigma-Aldrich, Acros, or Strem and used as received unless otherwise noted. All solvents were passed through a column of alumina under an argon atmosphere and used without further purification. Ultraviolet spectra were measured on a Molecular Devices SPECTRAmax Plus 384 UV-Visible spectrophotometer operated through a Dell Pentium PC by SOFTmax software. Fluorescence measurements were taken on a Jobin Yvon Fluorolog fluorescence spectrofluorometer. HPLC was performed on an Xterra RP_{18} 5μm column (4.6 x 150mm) with a Waters 600 Controller; fractions were detected with a Waters 2487 Dual λ Absorbance Detector and data was analyzed using OpenLynx software.

Part I: Synthetic Methods



a. Br_2 (3 eq.), Fe^0, $CHCl_3$, RT, 4hrs, 88%. **b.** NaOMe, CuCN, DME/MeOH, Me_2CO_3, 80°C, 24hrs, 66%. **c.** MeI, K_2CO_3, acetone, RT, 3hrs. **d.** N-bromosuccinimde, CH_2Cl_2, RT, 57% for two steps. **e.** i) *n*-BuLi, hexanes/THF, -78°C, 1.5hrs ii) $B(OMe)_3$ (neat), -78°C to RT, 20hrs.

Scheme S1. Synthesis of boronic acid **1a**.

2,3,6-tribromo-4-methylphenol (I).[1]

To a solution of 4-methylphenol [Aldrich] (10.80g, 100mmol, 1 eq.) and Fe powder (0.400g, 7mmol, 0.07eq.) in $CHCl_3$ (70mL) under argon was added Br_2 (16mL, 312 mmol, 3.12 eq.) slowly over 2 hours via syringe. The dark red mixture was allowed to stir for 4 hours at RT, at which time it was washed three times with a saturated aqueous Na_2SO_3 solution, washed once with brine, and dried with $MgSO_4$. The crude light yellow solid was recrystallized from hexanes to give the title compound as white needles (30.65g, 88%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38 (s, 1H), 5.88 (s, 1H), 2.41 (s, 3H); MS (HR-FAB+): Calc'd for $C_7H_5Br_3O$ 341.7890, measured 341.7890.

2,3,6-trimethoxy-4-methylphenol (II).[1]

To a stirred solution of NaOMe (18.9g, 350mmol, 10 eq.) in dry MeOH (30mL) under argon at RT was added DME (90mL), Me_2CO_3 (17.4mL, 193mmol, 5.5 eq.), and CuCN (5.0g, 56mmol, 1.6 eq.). The mixture was heated to 60°C and a solution of 2,3,6-tribromo-4-methylphenol (12.0 g, 35mmol, 1 eq.) in DME (30mL) was added via cannula. The green/blue heterogeneous mixture was heated at 80°C in the dark. The reaction progress was monitored by ^{1}H NMR, and additional NaOMe, CuCN, and Me_2CO_3 were added as necessary. The reaction was complete after 5 days, at which time the mixture was cooled to RT, quenched with aqueous 10% citric acid (150mL), diluted with EtOAc, and filtered. The organic phase was separated and dried with $MgSO_4$. The crude oil was purified by flash chromatography (10% EtOAc/hexanes) to give the title

[1] Keinan, E., Eren, D. *J. Org. Chem.* **1987**, *52*, 3872 – 3875.

compound as a light yellow oil (4.57g, 66%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.42 (s, 1H), 5.59 (s, 1H), 3.93 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (75 MHz): δ 145.2, 143.2, 140.4, 136.9, 121.0, 107.8, 60.7, 60.4, 56.2, 15.4. MS (LR-APCI): Calc'd for $C_{10}H_{14}O_4$ 198.09, measured 198.21.

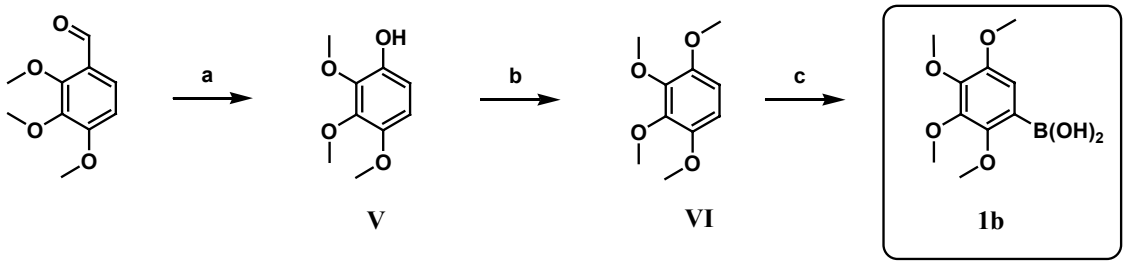
1,2,3,4-Tetramethoxy-5-methylbenzene (III).
To a stirred solution of 2,3,6-trimethoxy-4-methylphenol (2.45g, 12.4mmol, 1eq.) and K_2CO_3 (5.12g, 37.1mmol, 3 eq.) in acetone (40mL) was added MeI (4.6mL, 74.2mmol, 6eq.). The mixture was stirred at reflux for 6hrs, after which it was cooled to RT and filtered. The acetone was removed by rotary evaporation, and the crude material was redissolved in CH_2Cl_2, filtered, and dried with $MgSO_4$. The crude oil was used without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.42 (s, 1H), 3.91 (s, 3H), 3.86 (s, 3H), 3.79 (s, 3H), 3.76 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (75 MHz): δ 149.0, 146.9, 145.3, 140.7, 125.7, 108.2, 61.0, 60.5, 55.9, 15.7. MS (LR-APCI): Calc'd for $C_{11}H_{16}O_4$ 212.10, measured 212.08.

1-Bromo-2,3,4,5-tetramethoxy-6-methylbenzene (IV).[2]
To a stirred solution of 2,3,4,5-tetramethoxytoluene (2.6g, 12.4 mmol, 1 eq.) in CH_2Cl_2 (30mL) was added solid N-bromosuccinimide (NBS)(2.2g, 12.4mmol, 1 eq.) . The reaction was stirred at RT under argon for 2hrs, after which time it was washed with 50% NaCl/H_2O and dried with $MgSO_4$. The crude oil was purified by flash chromatography (10% EtOAc/hexanes) to give the title compound (2.05g, 57% for two steps). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.89 (s, 6H), 3.82 (s, 3H), 3.77 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (75 MHz): δ 148.3, 147.2, 146.3, 145.5, 127.2, 114.2, 61.3, 61.0, 60.7, 15.9. MS (LR-APCI): Calc'd for $C_{11}H_{15}Br\,O_4$ 290.02 / 292.01, measured 290.08 / 292.08.

(2,3,4,5-tetramethoxy-6-methyl)phenylboronic acid (1a).[3]
To a stirred solution of 1-Bromo-2,3,4,5-tetramethoxy-6-methyl-benzene (1.0g, 3.45mmol, 1 eq.) in THF (9mL) at –78^{o}C was added *n*BuLi (1.6M in hexanes, 2.35mL, 3.80mL, 1.1 eq.) dropwise. The mixture was stirred at –78^{o}C for 1.5 hours, at which time $B(OMe)_3$ (0.64mL, 5.2 mmol, 1.5 eq) was added at via syringe. The mixture was allowed to warm to RT and stirred for an additional 12 hours, after which time the reaction was cooled to 0^{o}C, quenched with 1N HCl (5mL), and extracted into CH_2Cl_2. After evaporation, the crude oil was used immediately without further purification for the synthesis of **2a**.

[2] Jung, Y-S., Joe, B-Y, Seong, C-M., Park, N-S. *Bull. Korean Chem. Soc.* **2000**, *21*, 463 – 464.

[3] a) Fukuyama, Y., Kiriyama, Y., Kodama, M. *Tetrahedron Lett.* **1993**, *34*, 7637 – 7638.



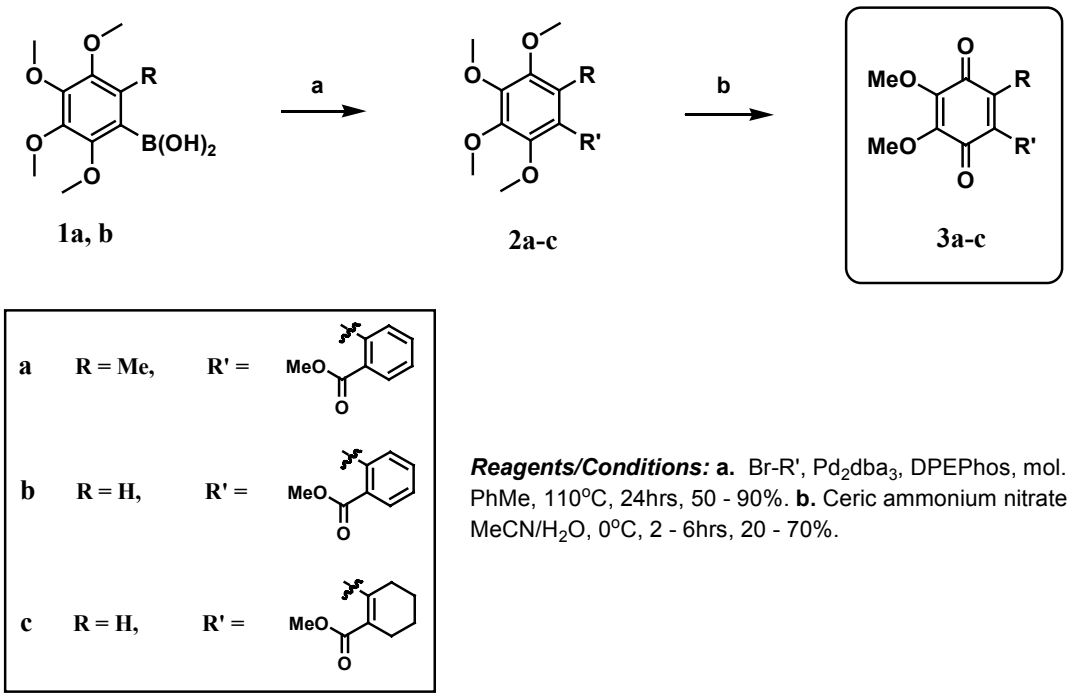
a. H_2O_2, MeOH/H_2SO_4, RT, 1hr, 74%. **b.** MeI, K_2CO_3, acetone, RT, 6hrs, 89%. **c.** i) *n*-BuLi, hexanes/THF, RT, 1.5hrs ii) $B(OMe)_3$ (neat), -78°C to RT, 2hrs iii) 1N HCl, 0°C, 20min.

Scheme S2. Synthesis of boronic acid **1b**.

2,3,4-trimethoxyphenol (V). To a solution of 2,3,4-trimethoxy-benzaldehyde [Aldrich] (5.88g, 30mmol, 1.0 eq) in H_2SO_4 / MeOH (1:100 v/v, 60mL) at 0°C under an atmosphere of argon was added 30% aqueous H_2O_2 (4.80mL, 38.4mmol, 1.15eq). After 1hr, the reaction was diluted with Et_2O (150mL), washed successively with H_2O (75mL) and saturated aqueous NaCl (75mL), dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude brown oil was purified by flash chromatography (silica, 10% EtOAc / hexanes) to give the title compound as a clear oil (4.10g, 74%). ^{1}H NMR ($CDCl_3$, 300MHz): δ 6.63 (d, *J* = 8.9Hz, 1H), 6.55 (d, *J* = 9.0Hz, 1H), 5.40 (s, 1H), 3.96 (s, 3H), 3.90 (s, 3H), 3.82 (s, 3H). MS (LR-APCI): Calc'd for $C_9H_{12}O_4$ 184.07, measured 184.02.

Tetramethoxybenzene (VI). To a stirred solution of **6** (4.10g, 22.3mmol, 1.0 eq.) and K_2CO_3 (9.2g, 66.9mmol, 3.0 eq.) in acetone (50mL) was added MeI (8.3mL, 134mmol, 6.0 eq.). The mixture was stirred at reflux for 6hrs, after which it was cooled to RT and filtered. The acetone was removed by rotary evaporation, and the crude material was redissolved in CH_2Cl_2, filtered, and dried with $MgSO_4$. The crude white solid was recrystallized from hexanes to give long colorless needles (3.95g, 89%). ^{1}H NMR ($CDCl_3$, 300MHz): δ 6.57 (s, 2H), 3.90 (s, 3H), 3.81 (s, 3H). ^{13}C NMR ($CDCl_3$, 75MHz): δ 147.4, 143.1, 106.2, 61.1, 56.3. MS (LR-APCI): Calc'd for $C_{10}H_{14}O_4$ 198.09, measured 198.15.

2,3,4,5-tetramethoxyphenylboronic acid (1b). To a stirred solution of **7** (500mg, 2.5mmol, 1.0 eq) in THF (5mL) at RT under positive pressure of argon, *n*-BuLi (1.6M in hexane; 1.74mL, 2.78mmol, 1.1 eq) was added dropwise (light yellow precipitate after complete addition). The mixture was stirred for 15 minutes at RT, then cooled to –78°C under positive pressure of argon. $B(OMe)_3$ (1.67mL, 15mmol, 6 eq) was added and the cloudy yellow solution became cloudy white, and, after warming slowly to RT, became clear and colorless. After 3hrs at RT, the solution was cooled to 0°C and aqueous HCl (1.0 M, 10mL) was added and the reaction was allowed to warm to RT. After 2hrs, the solution was diluted with Et_2O (50mL), washed successively with aqueous HCl (1.0 M, 20mL), saturated aqueous NaCl (20mL), dried over $MgSO_4$, filtered, and evaporated and the clear oil was used immediately without further purification for the synthesis of **2b,c**.



Reagents/Conditions: **a.** Br-R', Pd_2dba_3, DPEPhos, mol. sieves, PhMe, 110°C, 24hrs, 50 - 90%. **b.** Ceric ammonium nitrate (CAN), $MeCN/H_2O$, 0°C, 2 - 6hrs, 20 - 70%.

Scheme S3. Synthesis of Quinone Probes **3a-c**.

2',3',4',5'-Tetramethoxy-6'methyl-biphenyl-2-carboxylic acid methyl ester (2a)
The crude boronic acid (3.45mmol, 2 eq.) was combined with K_3PO_4 (1.09g, 10.4mmol, 3 eq.), Pd_2dba_3 (155mg, 0.35mmol, 0.1 eq.), DPEPhos (112mg, 0.35mmol, 0.1 eq.), and 4Å molecular sieves in a flame dried Schlenk flask, which was purged and backfilled with argon. PhMe (8.6mL) and methyl 2-bromo-benzoate (0.259mL, 1.73mmol, 1 eq.) were then added and the mixture was stirred at 110°C for 24 hours. The crude reaction mixture was cooled to RT, diluted with Et_2O and filtered through a plug of silica gel. The crude oil was purified by flash chromatography (5% EtOAc/hexanes) to give the title compound as a pale yellow oil (0.395g, 66%): . ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.98 (d, *J* = 7.7Hz, 1H), 7.51 (t, *J* = 7.1Hz, 1H), 7.40 (t, *J* = 7.4Hz, 1H), 7.19 (d, *J* = 7.9Hz, 1H), 3.98 (s, 3H), 3.91 (s, 3H), 3.82 (s, 3H), 3.64 (s, 3H), 3,51 (s, 3H), 1.85 (s, 3H). ^{13}C NMR (75 MHz): δ 167.6, 147.5, 146.4, 145.9, 144.3, 138.2, 131.4, 130.8, 130.1, 129.4, 128.0, 127.1, 124.5, 61.1, 60.6, 60.5, 51.7, 12.9. MS (LR-APCI): Calc'd for $C_{19}H_{22}O_6$ 346.14, measured 346.36.

2',3',4',5'-Tetramethoxy-biphenyl-2-carboxylic acid methyl ester (2b).
This compound was prepared from crude boronic acid **1b** using the same conditions as those used for the preparation of **2a**. Yellow oil (68%): ^{1}H NMR ($CDCl_3$, 300MHz): δ 7.86 (d, *J* = 7.7Hz, 1H), 7.52 (t, *J* = 7.5, 1H), 7.40 (t, *J* = 7.5Hz, 1H), 7.35 (d, *J* = 8.0Hz, 1H), 6.53 (s, 1H), 3.94 (s, 6H), 3.84 (s, 3H), 3.68 (s, 3H), 3.45 (s, 3H). ^{13}C NMR ($CDCl_3$, 125MHz): δ 168.5, 149.1, 146.7, 144.4, 142.3, 138.2, 131.48, 131.2, 131.1, 129.7, 129.6, 127.2, 107.6, 61.4, 61.0, 60.6, 56.1, 51.8. MS (LR-APCI): Calc'd for $C_{18}H_{20}O_6$ 332.13, measured 331.89.

2-(2,3,4,5-Tetramethoxy-phenyl)-cyclohex-1-enecarboxylic acid methyl ester (2c).
This compound was prepared from crude boronic acid **1b** using the same conditions as those used for the preparation of **2a** except that 2-bromo-cyclohex-1-enecarboxylic acid methyl ester[4] was used in place of methyl 2-bromobenzoate. ^{1}H NMR ($CDCl_3$, 400MHz): δ 6.23 (s, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.74 (s, 3H), 3.71 (s, 3H), 3.41 (s, H), 2.40 (bs, 2H), 2.35 (bs, 2H), 1.70 (s, 4H). ^{13}C NMR ($CDCl_3$, 100MHz): δ 169.0, 148.7, 146.5, 143.7, 143.4, 141.5, 131.7, 127.9, 106.3, 61.1, 61.0, 56.3, 56.2, 51.0, 32.4, 26.5, 22.5, 22.1. MS (LR-APCI): Calc'd for $C_{18}H_{24}O_6$ 336.16, measured 336.39.

2-(4,5-Dimethoxy-2-methyl-3,6-dioxo-cyclohexa-1,4-dienyl)-benzoic acid methyl ester (3a).[5]
To a stirred solution of 2',3',4',5'-Tetramethoxy-6'-methyl-biphenyl-2-carboxylic acid methyl ester (128mg, 0.37mmol, 1 eq.) in MeCN/H_2O (7:3)(5mL) at 0^{o}C under argon was added cerric ammonium nitrate (CAN)(507mg, 0.93mmol, 2.5 eq.) in MeCN/H_2O (1:1)(1mL) dropwise. The mixture was allowed to warm to RT and stirred for 1 hour, after which it was diluted with Et_2O, washed with brine, and dried with $MgSO_4$. The crude oil was purified by flash chromatography (10% EtOAc/hexanes) to give the title compound as a yellow solid (80mg, 68%): ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.09 (d, *J* = 7.9Hz, 1H), 7.57 (t, *J* = 7.4Hz, 1H), 7.45 (t, *J* = 7.7Hz, 1H), 7.12 (d, *J* = 7.1Hz, 1H), 4.06 (s, 3H), 3.97 (s, 3H), 3.77 (s, 3H), 1.80 (s, 3H). ^{13}C NMR (75 MHz): δ 184.5, 183.2, 166.2, 144.9, 144.4, 143.7, 136.6, 135.0, 132.4, 130.6, 130.2, 129.2, 128.7, 61.1, 61.0, 52.1, 13.2. MS (LR-APCI): Calc'd for $C_{17}H_{16}O_6$ 316.09, measured 316.16.

2-(4,5-Dimethoxy-3,6-dioxo-cyclohexa-1,4-dienyl)-benzoic acid methyl ester (3b).
This compound was prepared from **2b** using the same conditions as those used for the preparation of **3a**. ^{1}H NMR ($CDCl_3$, 300MHz): δ 8.06 (dd, *J* = 7.6Hz, 1.2Hz, 1H), 7.57 (dt, *J* = 7.4Hz, 1.4Hz, 1H), 7.53 (dt, *J* = 7.7Hz, 1.4Hz, 1H), 7.26 (dd, *J* = 7.0Hz, 1.3Hz, 1H), 6.51 (s, 1H), 4.10 (s, 3H), 4.00 (s, 3H), 3.81 (s, 3H). ^{13}C NMR (75 MHz): δ 185.4, 183.9, 166.8, 144.3, 143.9, 143.3, 136.2, 135.3, 132.1, 131.2, 129.9, 128.6, 128.8, 61.1, 61.0, 52.1. MS (LR-APCI): Calc'd for $C_{16}H_{14}O_6$ 302.08, measured 301.68.

2-(4,5-dimethoxy-3.6-dioxo-cyclohexa-1,4-dienyl)-cyclohex-1-enecarboxylic acid methyl ester (3c).
This compound was prepared from **2c** using the same conditions as those used for the preparation of **3a**. ^{1}H NMR ($CDCl_3$, 300MHz): δ 6.25 (s, 1H), 4.19 (s, 3H), 3.77 (s, 3H), 3.24 (s, 3H), 2.40 (m, 2H), 2.22 (m, 2H), 1.71 (m, 4H). ^{13}C NMR (75 MHz): δ 183.4, 181.2, 164.8, 145.1, 144.8, 144.5, 143.5, 135.9, 128.7, 61.2, 61.0, 51.6, 39.0, 34.2, 22.2, 21.8. MS (LR-APCI): Calc'd for $C_{16}H_{18}O_6$ 306.11, measured 305.57.

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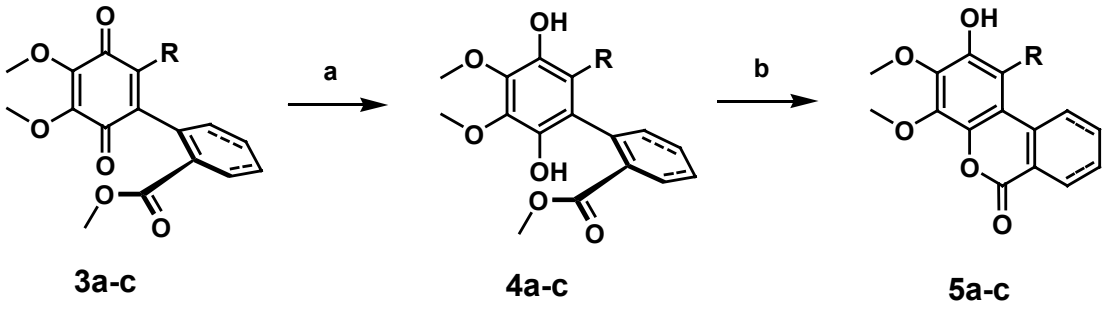
[4] Prepared in three steps from cyclohexanone: bromo/formylation [*Organometallics* **2000**, *19*, 5525 – 5528]; oxidation [*J. Org. Chem.* **1979**, *44*, 1022-1024]; esterification with ethereal diazomethane.
[5] Ohshima, M., Miyoshi, H., Sakamoto, K., Takegami, K., Iwata, J., Kuwabara, K., Iwamura, H., Yagi, T. *Biochemistry* **1998***, 37*, 6436 – 6445.

Synthesis of Quinols 4a-c and Coumarins 5a-c.



a R = Me, benzo-

b R = H, benzo

c R = H, cyclohexo-

Reagents/Conditions: **a.** H_2, 10% Pd-C, $CDCl_3$, ~3min, <95%.
b. $CDCl_3$, various temperatures, various times (see Part II).

Scheme S4. Synthesis of Quinols **4a-c** and Coumarins **5a-c**.

2',5'-Dihydroxy-3',4'-dimethoxy-6'methyl-biphenyl-2-carboxylic acid methyl ester (4a). Quinone **3a** (31mg, 0.1mmol) was dissolved in $CDCl_3$ and briefly degassed and refilled with argon. A few grains of 10% Pd-C were added and the solution was stirred and placed under an atmosphere of H_2. After 1-3 minutes, the characteristic red color of the quinone had dissipated and the solution was colorless, at which time it was immediately filtered through celite with $CDCl_3$ as eluent and stored under argon. ^{1}H NMR showed quantitative conversion to the quinol **4a**. Reduction could also be achieved by titrating a methanolic solution of the quinone with saturated aqueous $Na_2S_2O_4$, followed by extraction into ether. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.00 (d, *J* = 8.0Hz, 1H), 7.57 (t, *J* = 7.6Hz, 1H), 7.44 (t, *J* = 8.0Hz, 1H), 7.23 (d, *J* = 8.0Hz, 1H), 5.42 (s, 1H), 5.10 (s, 1H), 3.99 (s, 3H), 3.92 (s, 3H), 3.68 (s, 3H), 1.87 (s, 3H).

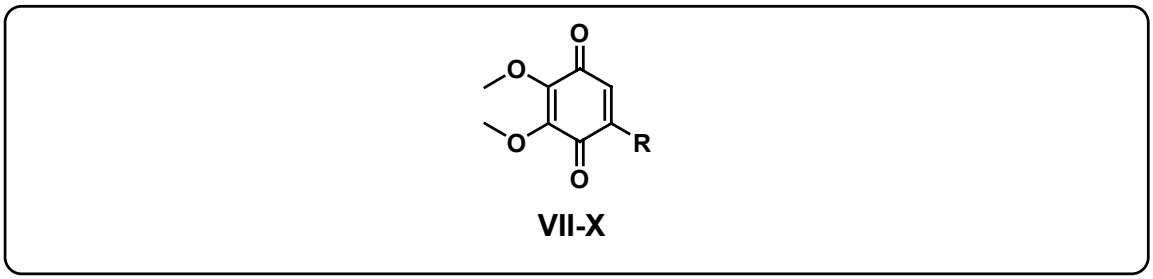
2',5'-Dihydroxy-3',4'-dimethoxy -biphenyl-2-carboxylic acid methyl ester (4b).
This compound was prepared from **3b** using the same conditions as those used for the preparation of **4a.** . ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.88 (dd, *J* = 7.5Hz, *J* = 1.2Hz, 1H), 7.54 (dt, *J* = 7.5Hz, *J* = 1.6Hz, 1H), 7.40 (dt, *J* = 7.8Hz, *J* = 1.5Hz, 1H), 7.33 (dd, *J* = 7.5Hz, *J* = 1.7Hz,1H), 6.53 (s, 1H), 5.38 (s, 1H), 5.30 (s, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 3.72 (s, 3H).

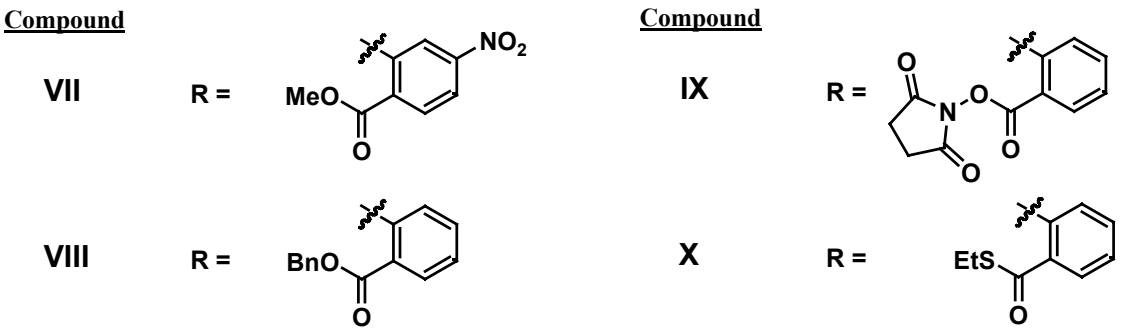
2-(2,5-Dihydroxy-3,4-dimethoxy-phenyl)-cyclohex-1-enecarboxylic acid methyl ester (4c).
This compound was prepared from **3c** using the same conditions as those used for the preparation of **4a**. ^{1}H NMR ($CDCl_3$, 400MHz): δ 6.36 (s, 1H), 5.35 (s, 1H), 5.19 (s, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 3.51 (s, 3H), 2.43 (t, *J* = 2.4Hz, 2H), 2.33 (t, *J* = 2.1Hz, 2H), 1.73 (m, 4H).

2-Hydroxy-3,4-dimethoxy-1-methyl-benzo[c]chromen-6-one (5a). Crude quinol **4a** in $CDCl_3$ was heated to 100°C in a sealed screwcap vial under argon. The reaction was periodically cooled to RT and the cyclization reaction was monitored by ^{1}H NMR (see Part II). After 7 days, the starting material was consumed and the crude reaction was purified by flash chromatography (10% EtOAc/hexanes) to give coumarin **5a** as a white solid (24mg, 84%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.46 (dd, *J* = 8.0Hz, *J* = 1.2Hz, 1H), 8.34 (d, *J* = 8.4Hz, 1H), 7.57 (dt, *J* = 7.6Hz, *J* = 1.6Hz, 1H), 7.56 (t, *J* = 7.6Hz, 1H), 5.99 (s, 1H), 4.10 (s, 3H), 4.03 (s, 3H), 2.70 (s, 3H). ^{13}C NMR (100 MHz): δ 160.5, 143.7, 140.2, 139.7, 137.7, 136.1, 133.8, 130.5, 127.5, 125.9, 121.5, 115.1, 113.9, 61.8, 61.5, 15.8. MS (LR-APCI): Calc'd for $C_{16}H_{14}O_5$ 286.08, measured 286.00.

2-Hydroxy-3,4-dimethoxy-benzo[c]chromen-6-one (5b). Crude quinol **4b** was cyclized as described above for the conversion of **4a** to **5a**, except for the reaction temperature and time. After 6 days at RT the crude reaction was purified by flash chromatography (10% EtOAc/hexanes) to give coumarin **5b** as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.40 (d, *J* = 7.8Hz, 1H), 7.96 (d, *J* = 8.1Hz, 1H), 7.80 (t, *J* = 7.2Hz, 1H), 7.56 (t, *J* = 7.2Hz, 1H), 7.35 (s, 1H), 5.76 (s, 1H), 4.10 (s, 3H), 4.08 (s, 3H).). ^{13}C NMR (100 MHz): δ 162.3, 145.5, 141.2, 139.4, 137.1, 136.0, 134.1, 131.2, 128.2, 127.4, 124.2, 115.0, 100.7, 61.9, 61.4. MS (LR-APCI): Calc'd for $C_{15}H_{12}O_5$ 272.07, measured 272.16.

2-Hydroxy-3,4-dimethoxy-7,8,9,10-tetrahydro-benzo[c]chromen-6-one (5c). Crude quinol **5b** was cyclized as described above for the conversion of **4a** to **5a**, except for the reaction temperature and time. After 58hrs at RT the crude reaction was purified by flash chromatography (10% EtOAc/hexanes) to give coumarin **5b** as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.86 (s, 1H), 5.68 (s, 1H), 4.07 (s, 3H), 4.03 (s, 3H), 2.69 (m, 2H), 2.58 (m, 2H), 1.82 (m, 4H). ^{13}C NMR ($CDCl_3$, 100MHz): δ 165, 161, 146.8, 144.9, 122.7, 116.3, 102.1, 99.5, 61.8, 61.6, 25.6, 24.2, 21.8, 21.6. MS (LR-APCI): Calc'd for $C_{15}H_{16}O_5$ 276.10, measured 272.16.





Scheme S5. Other variations of **3b,c** that were prepared (see Part II).

Compounds VII, VIII, IX, X (described in Part II) were prepared from boronic acid **1b** using the same two step procedure as detailed above for the synthesis of **3b**, using the corresponding bromides in place of methyl 2-bromobenzoate: methyl 2-bromo-4-nitrobenzoate[6] (**VII**), benzyl 2-bromobenzoate[7] (**VIII**). Compounds **IX** and **X** were prepared by hydrogenolysis[8] of **VIII**, followed by esterification with N-hydroxysuccinimide (**IX**) or ethanethiol (**X**) using standard coupling conditions.[9] All compounds had ^{1}H / ^{13}C NMR and MS that were consistent with their structures.

[6] Prepared from 2-bromo-4-nitrotoluene by oxidation [*Bioorg. Med. Chem.* **1999**, 3011], followed by esterification with ethereal diazomethane.
[7] Prepared by esterification of 2-bromobenzoic acid with benzyl alcohol (1.1 eq), DCC (1.1 eq), DMAP (0.1 eq), CH_2Cl_2, RT, 4hrs.
[8] 10% Pd-C, H_2 (1 atm), MeOH, RT, 2hrs.
[9] N-hydroxysuccinimide (or ethanethiol) (1.1 eq), DCC (1.1 eq), DMAP (0.1 eq), CH_2Cl_2, RT, 4hrs.

Part II: Lactonization

Protocol

Lactonization in $CDCl_3$. After reduction of quinones **3a-c** (see Part I)[10], ^1H NMR spectra were taken periodically to monitor the transformation of quinols **4a-c** to coumarins **5a-c**. The reactions were carried out in screw cap vials at RT or 100°C under argon, and spectra were taken at RT under argon (reactions at 100°C were cooled to RT before being transferred to the NMR tube).

Lactonization in $CDCl_3$/buffer. After reduction of quinone **3c** (see Part I), the $CDCl_3$ solution was combined with an equal volume of 50mM phosphate buffer (pH 7.4) and stirred vigorously at RT. Periodically, stirring was halted and a portion of the $CDCl_3$ layer was removed and analyzed by ^1H NMR.

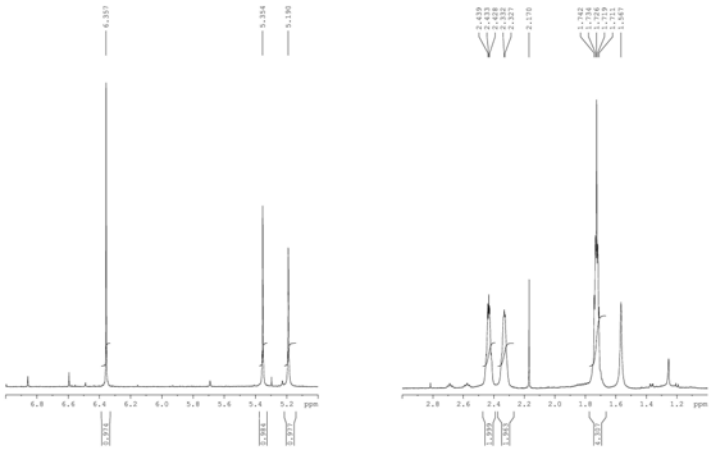
Lactonization in buffer. After reduction of quinone **3c** (see Part I), the $CDCl_3$ was removed by rotary evaporation and residual solvent was removed under high vacuum to give a clear oil which was kept under argon and diluted in DMSO-d_6. ^1H NMR of the DMSO-d_6 solution confirmed the presence of the quinol **4c**. An aliquot (10μL) of the DMSO-d_6 solution was added to 50mM phosphate buffer (pH 7.4) (990μL). Absorption and emission of this solution were immediately measured; UV-Vis and fluorescence spectra of this solution were consistent with formation of the coumarin, and simultaneous HPLC analysis corroborated its quantitative formation.

Data Analysis

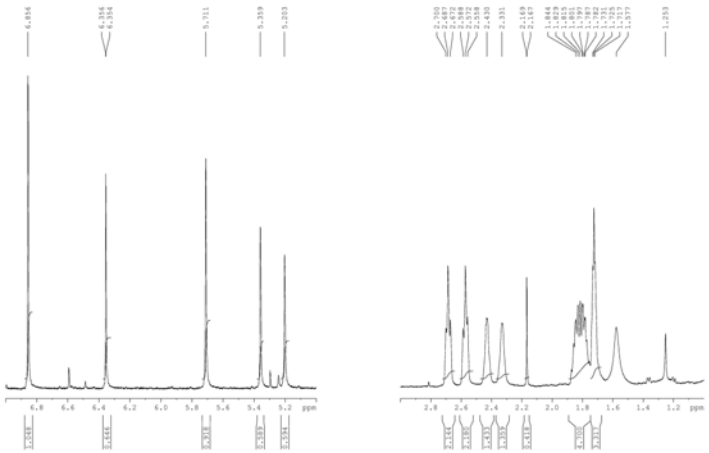
Lactonization Rate Determination. Integration of ^1H NMR peaks corresponding to the quinols **4a-c** and coumarins **5a-c** was used to determine the rate of lactonization. In all cases, the conversion of quinol to coumarin was quantitative by the detection limits of ^1H NMR and no internal standard was used. Figure S1 shows three time-elapsed spectra from the cyclization rate determination of **4c**.

[10] it is important that the palladium catalyst is removed immediately after reduction is complete (red to colorless transition); prolonged exposure to palladium catalyst enhanced the rate of cyclization by about one order of magnitude.

0 hrs [immediately after reduction]



10hrs [60% conversion]



19hrs [77% conversion]

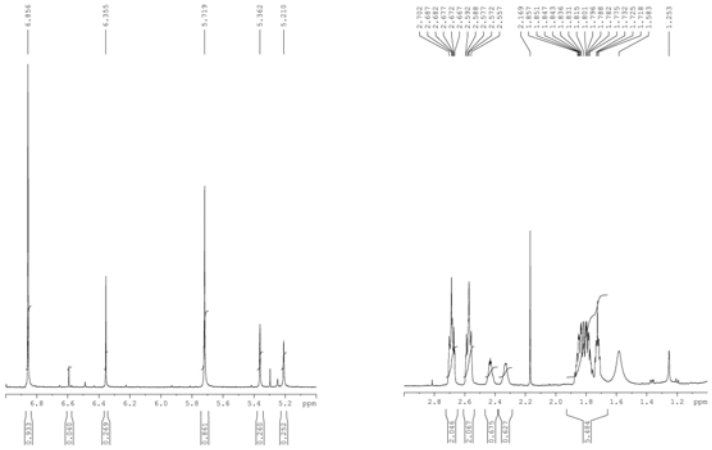


Figure S1. Time-elapsed ^{1}H NMR spectra of the conversion of **4c** to **5c**.

Since ^{1}H NMR showed quantitative conversion of **4** to **5**, with no side reactions, the sum of the relative integrations of **4** and **5** was used as an internal standard to measure the decrease of **[4]**. The rate of lactonization obeyed first order kinetics, since a plot of **ln[4]** vs. **time** was linear for each substrate with good correlation coefficients.

Substrate	**T** (^{o}C)	***k*** (10^{-3}min^{-1})	$\mathbf{R^2}$
4a	**100**	**0.29**	**0.96**
4b	**100**	**24.1**	**1.00**
4b	**25**	**0.17**	**0.99**
4c	**25**	**1.28**	**0.99**
VII	**25**	**0.07**	**0.99**
IX	**25**	**2.03**	**0.99**
X	**25**	**0.10**	**0.89**

Table S1. Cyclization in $CDCl_3$ of Various Quinol Derivatives.

Solvent	***k*** (10^{-3}min^{-1})	$\mathbf{R^2}$
$CDCl_3$	**1.28**	**0.99**
$CDCl_3$ / buffer	**36.6**	**0.97**
buffer	**$>10^{4*}$**	**-----**

Table S2. Cyclization of **4c** Various Solvent Systems.
*not determined by ^{1}H NMR; approximated by assuming 90% conversion at 15 seconds.

Part III: Reduction Assays

Materials

NADH was purchased from Sigma and stored at 4°C; a fresh stock solution (10mM) in buffer was prepared prior to use and kept at 0°C throughout the assay (no longer than 4hrs). Dithiothreitol (DTT) and glutathione (GSH) were from Sigma and were kept at 4°C; stock solutions (10mM) in buffer were prepared prior to use. $FeCl_2$ from was used to prepare an aqueous stock solution of Fe^{II} (10mM). Riboflavin and dopamine were purchased from Aldrich and fresh stock solutions were prepared in DMSO (1mM and 10mM respectively). Menaquinol, duroquinol, and ubiquinol were prepared fresh from the corresponding quinones (purchased from Aldrich) by catalytic hydrogenation in chloroform; the chloroform was removed and stock solutions in DMSO (10mM) were prepared before each assay. Unless otherwise noted, the buffer used contained 50mM phosphate, 10μM EDTA, 0.1mg/ml Triton-X100, adjusted to pH 7.4.

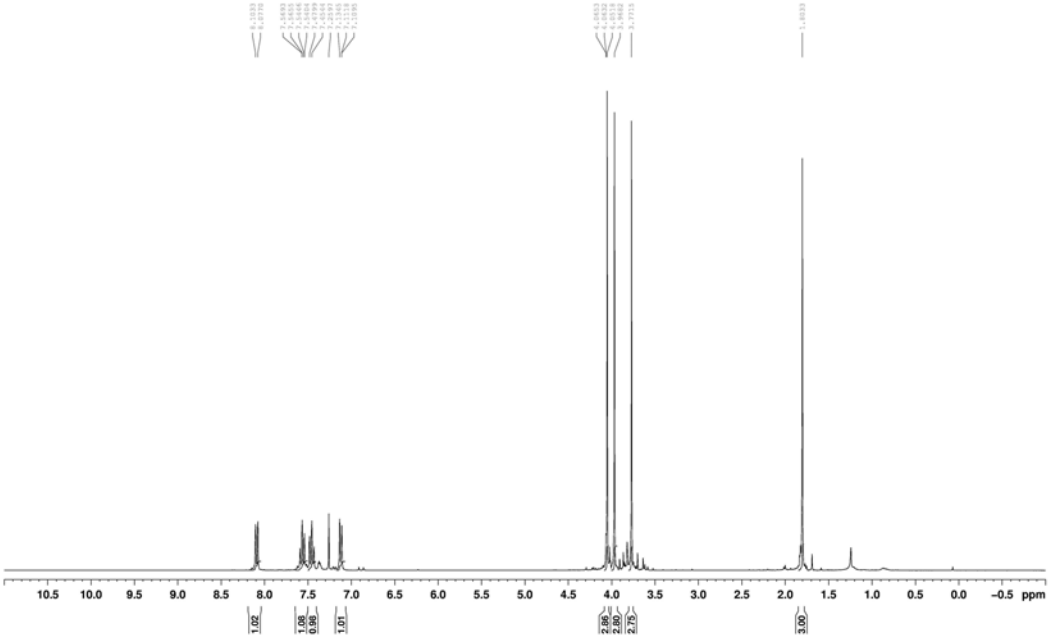
Protocol

Typical reactions were carried out with a final concentration of 100μM probe and 100μM/equivalent reductant, although it could be performed at concentrations as low as 30μM probe. An aliquot (10μL) of a stock solution of **3c** (10mM in DMSO) was diluted in buffer to a final volume of 1mL (final concentration = 10μM) in a disposable plastic cuvette. An initial absorption spectrum was recorded. An aliquot (10μL per equivalent) of a stock solution of the reductant was then added and the solution was agitated with a 1mL pipet. Initial (t = <30s) and final (t = 10 min) absorbance spectra were then measured with no further agitation; in some cases, time-elapsed spectra were recorded. All reactions were carried out at RT in open air. All reductants showed no significant oxidation by air well beyond the time frame of the assay (at least 30min). [analogous assays carried out using fluorescence detection (λ_{exc} = 340nm, λ_{em} = 550nm) at typical concentrations of 50μM probe, or as low as 10μM probe]

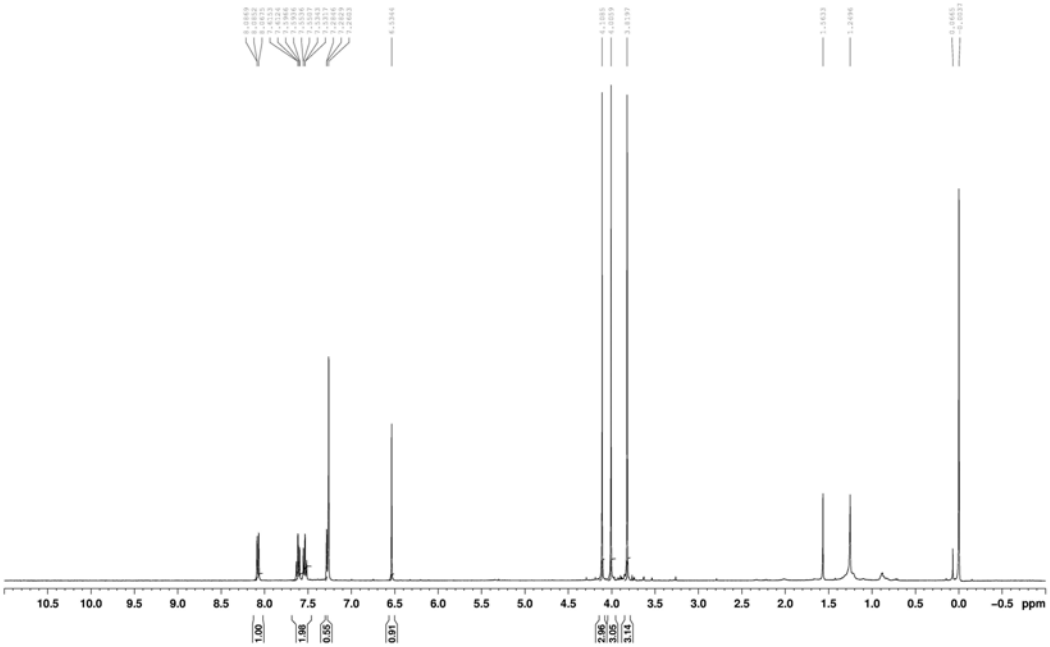
Data Analysis

Approximate rates were determined by measuring the increase in absorbance at 330nm (formation of **5c**), except in the cases of NADH (decrease in absorbance at 340nm), and menaquinol (increase in absorbance at 270nm). The magnitude of the change in absorbance intensity after a 10 min incubation time was used to assign a qualitative extent of reduction (see Figure 3, text). For selected reductants, the reaction was monitored at time points (see Figure 4, text).

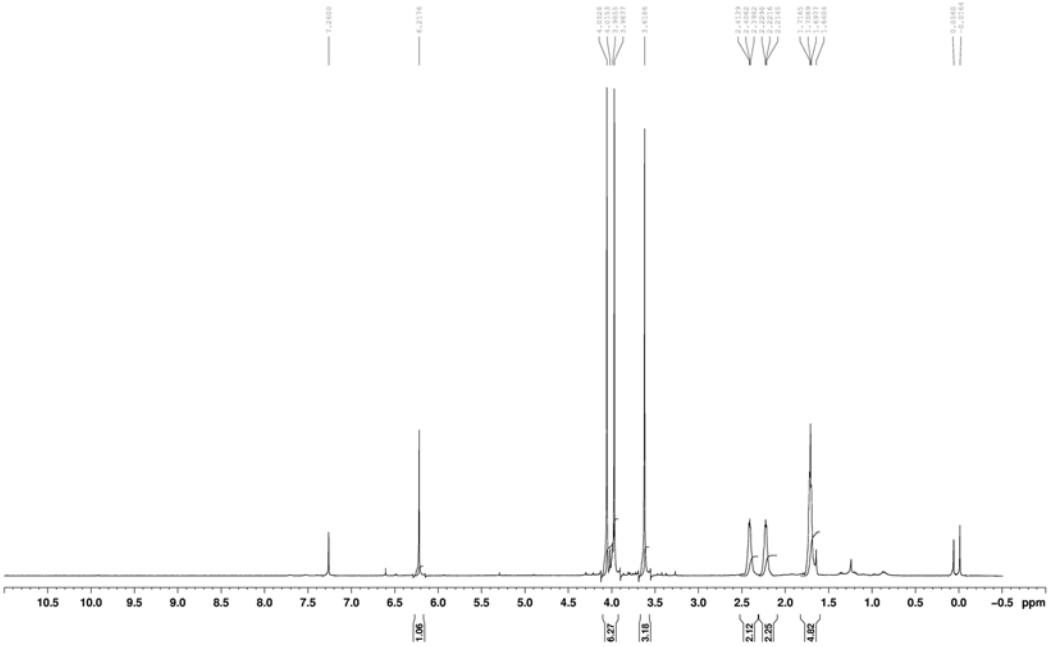
Part IV: ^{1}H NMR for 3a-c, 4a-c, 5a-c.



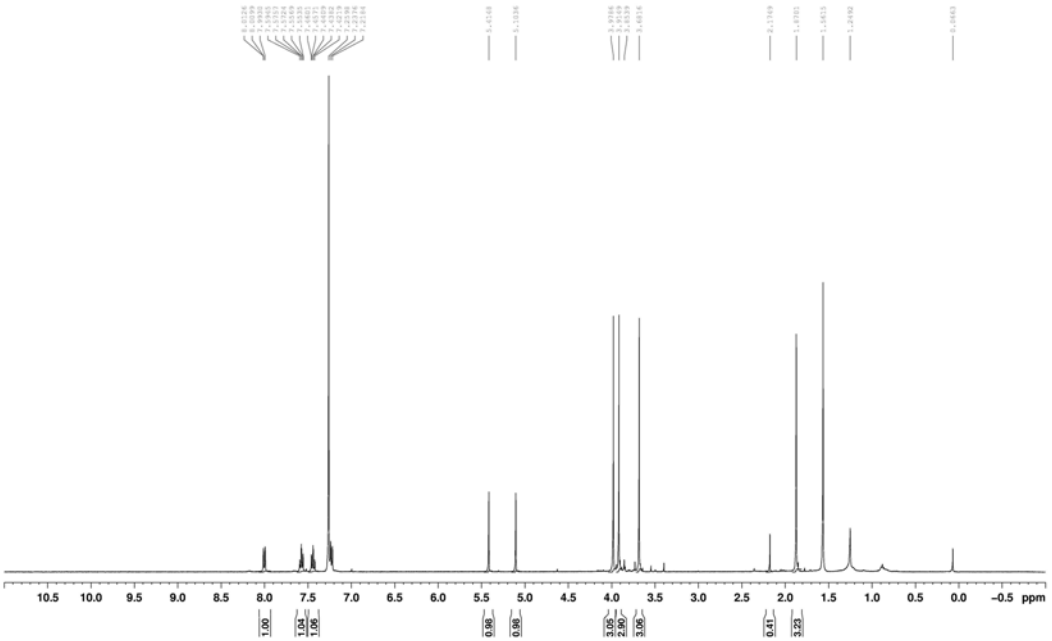
Quinone **3a**



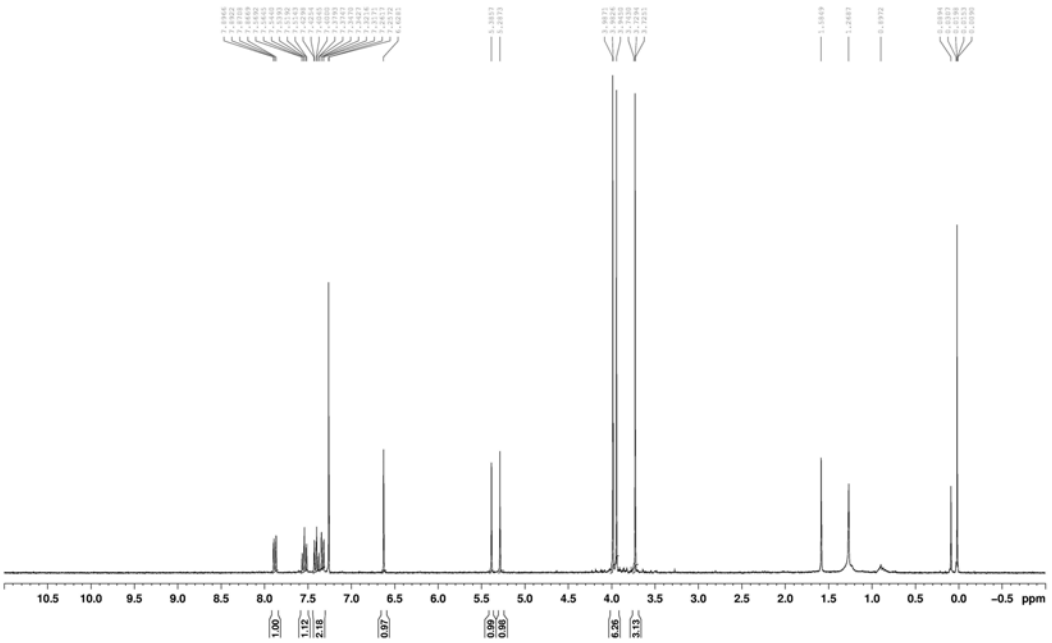
Quinone **3b**



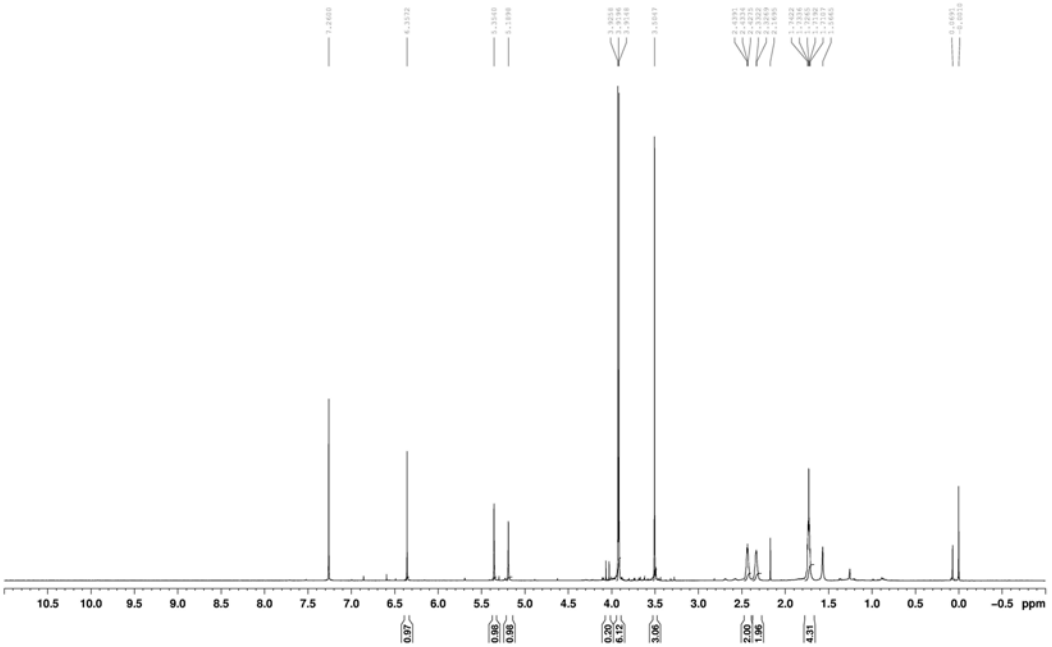
Quinone **3c**



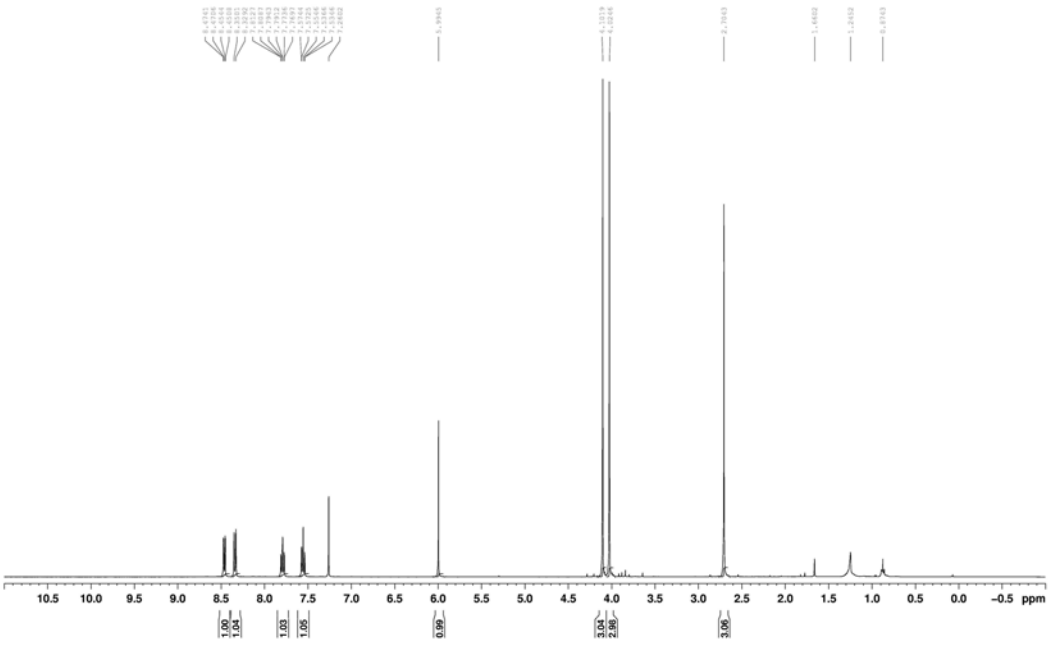
Quinol **4a**



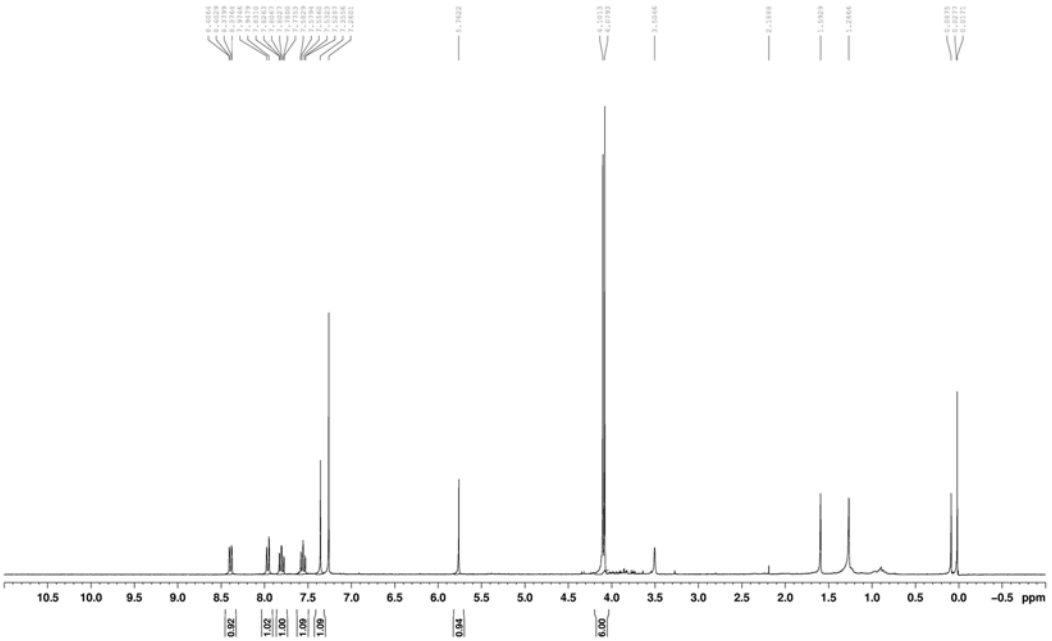
Quinol **4b**



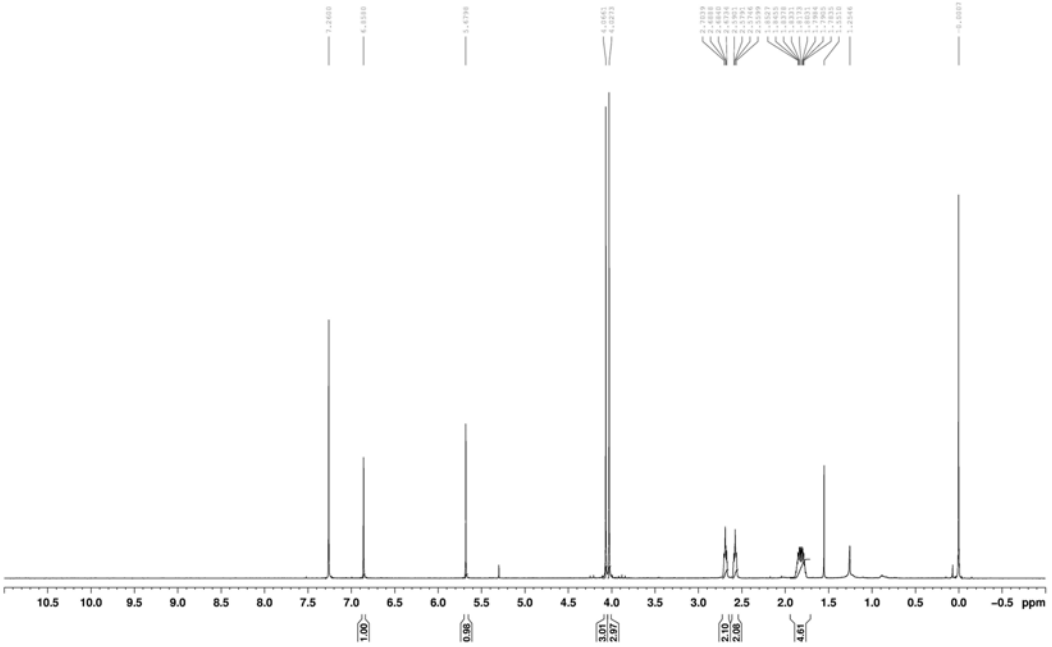
Quinol **4c**



Coumarin **5a**



Coumarin **5b**

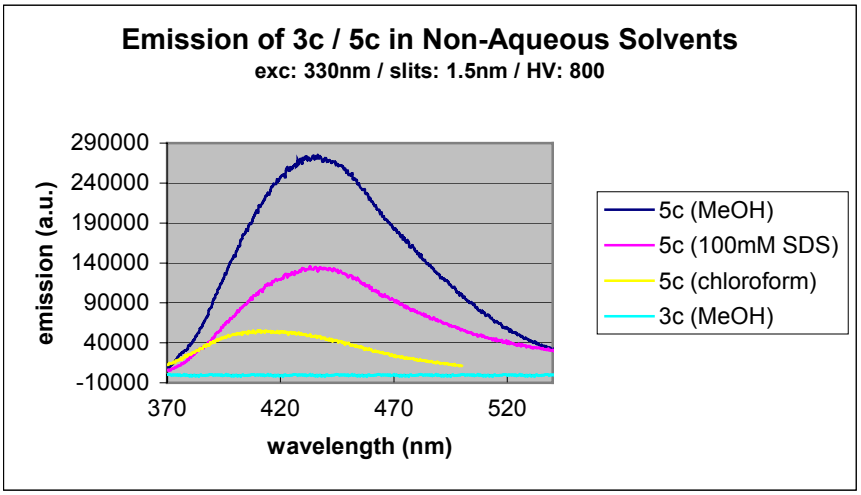


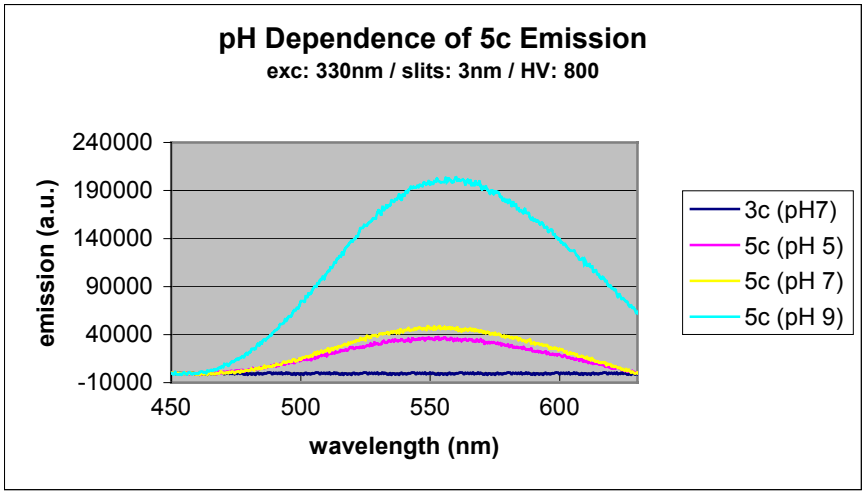
Coumarin **5c**

Part V: Selected Photophysical Data for Coumarins 5a-c

General

As noted in the text, coumarins **5a-c** were weakly fluorescent compounds. When compared to the commonly referenced standard diphenylanthracene (DPA), these compounds had <1% quantum yield. For our purposes, this finding deemed the accurate measurement of the quantum yield difficult and not useful. As expected, emission of coumarin **5c** showed some sensitivity to solvent polarity and pH. Quinone **3c** showed no emission. Below are selected spectra for compounds **3c** and **5c**. [all spectra taken at 100 μM; SDS = sodium dodecyl sulfate; pH 5 and 7 50mM phosphate; pH 9 50mM glycine].




pH Dependence of 5c Emission
exc: 330nm / slits: 3nm / HV: 800
240000
190000
140000
90000
40000
-10000
emission (a.u.)
450
500
550
600
wavelength (nm)
3c (pH7)
5c (pH 5)
5c (pH 7)
5c (pH 9)