Electronic Supporting Information

Fluorescent mimics of 5-hydroxytryptamine based on N-alkylated derivatives of 6-hydroxycarbostyril

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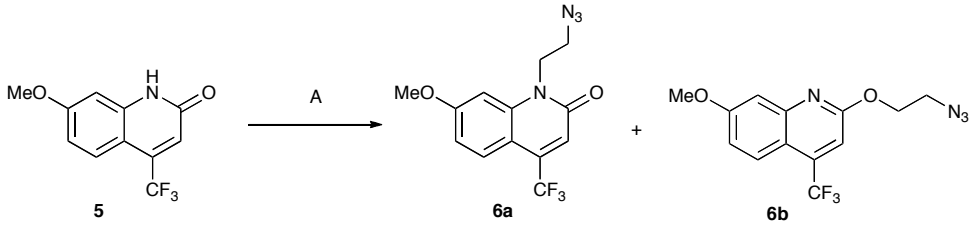


1-(2-azidoethyl)-7-methoxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (6a). Scheme **A**: Carbostyril **5** (5.0 g, 20.5 mmol) was placed in a round bottom flask charged with a stirbar, 2-azidoethyl tosylate[1] (5.0 g, 20.7 mmol) and oven dried K_2CO_3 (4.1 g, 30.0 mmol). DMF, a sufficient to allow the slurry to stir (~15 mL), was added then the reaction mixture was stirred at 130°C for 3h. After cooling, the reaction was poured into water and extracted three times with ~50 mL of EtOAc. TLC indicated the presence of both N-alkylated (**6a)** and O-alkylated (**6b)** intermediates in approximately 4:1 ratio in the organic layer. These were separated on a Biotage Isolera (25 g silica column, 3 column volumes of CH_2Cl_2, transitioning to EtOAc over 5 column volumes, followed by 3 column volumes of EtOAc). Yield (**6a** only): 558 mg, 9%

6a: ^{1}H NMR (500MHz, $CDCl_3$) δ 7.79 (dd, *J* = 9.0 Hz, *J* = 1.5 Hz, 1H, 6-H), 6.97 (d, *J* = 2.5 Hz, 1H, 8-H), 6.94-6.92 (m, 2H, 3-H, 5-H), 4.46 (t, *J* = 6.4 Hz, 2H, 1 N-CH_2), 3.95 (s, 3H, -OMe), 3.75 (t, *J* = 6.4 Hz, 2H, CH_2-N_3).

6a: ^{13}C NMR (125.75 MHz, $CDCl_3$) δ 162.38, 161.05, 141.89, 137.79-137.04 (q, *J* = 31.4 Hz, **C**-CF_3), 127.63-127.57 (q, *J* = 2.5 Hz, **C**-C-CF_3), 125.73-119.17 (q, *J* = 274.1 Hz, **C**F_3), 117.55-117.41 (q, *J* = 6.3 Hz, **C**-C-CF_3), 110.50, 109.4, 99.57, 55.75 (O-**C**H_3), 48.51, 42.12 (**C**H_2-N_3).

6b: ^{1}H NMR (500MHz, $CDCl_3$) δ 7.92 (dd, *J*=8.0 Hz, *J*=1.5 Hz, 1H, 6-H), 7.27 (d, *J*=2.5 Hz, 1H, 8-H), 7.17-7.15 (m, 2H, 3-H, 5-H), 4.69 (t, *J*=2.5 Hz, 2H, 1 N-CH_2), 3.96 (s, 3H, -OMe), 3.71 (t, *J*=5 Hz, 2H, CH_2-N_3)

6b: ^{13}C NMR (125.75 MHz, $CDCl_3$) δ 161.41, 160.80, 149.4, 137.64 (q, *J*=31.4 Hz, **C**-CF_3), 126.33 (q, *J*=275.4 Hz, **C**F_3), 125.12 (q, *J*=1.3 Hz, **C**-C-CF_3), 117.81, 114.64, 108.48 (q, *J*=6.3 Hz **C**-C-CF_3), 107.24, 64.85 (O-**C**H_3), 55.52 (N-**C**H_2), 49.99 (**C**H_2-N_3).

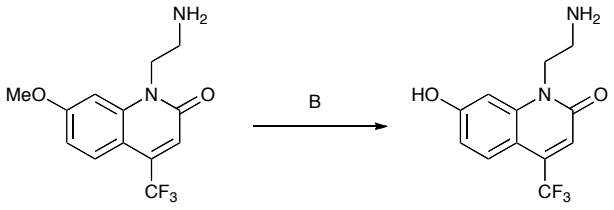
MS (FAB+) Calculated: 312, Found: 313 ($M + H^+$).

1-(2-aminoethyl)-7-methoxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (7a). A round bottom flask was charged with **6a** (525 mg, 1.7 mmol) and a stirbar. It was evacuated and filled with argon then capped with a septum. Argon purged THF (~ 5 mL) was added and the reactant was allowed to dissolve. A 1.0 M solution of PMe_3 (2.5 mL) was slowly added and vigorous bubbling was observed. Argon purged H_2O (1 mL) was then added and the reaction was allowed to proceed for 2 h. Solvent, excess PMe_3, H_2O and $POMe_3$ were removed by vacuum distillation. The column purification was performed on a Biotage Isolera (10 g silica column, 3 column volumes of CH_2Cl_2, transitioning to EtOAc over 5 column volumes, transitioning to MeOH:EtOAc, 1:1 over 5 column volumes). Yield: 340 mg, 70%

^{1}H NMR (500MHz, $CDCl_3$) δ 7.79 (dd, *J* = 9.0 Hz, *J* = 1.7 Hz, 1H, 6-H), 6.96 (d, *J* = 2.3 Hz, 1H, 8-H), 6.93 (s, 1H, 3-H), 6.91 (dd, *J* = 9.0 Hz, *J* = 2.3 Hz, 1H, 5-H), 4.38 (t, *J* = 7.0 Hz, 2H, 1 N-CH_2), 3.93 (s, 3H, -OMe), 3.11 (t, *J* = 7.0 Hz, 2H, C**H**$_2$-NH_2).

^{13}C NMR (125.75 MHz, $CDCl_3$) δ 162.28, 161.33, 141.81, 137.30-136.54 (q, *J* = 32.7 Hz, **C**-CF_3), 127.51-127.45 (q, *J* = 2.5 Hz, **C**-C-CF_3), 125.78-119.22 (q, *J* = 275.4 Hz, **C**F_3), 117.74-117.60 (q, *J* = 6.3 Hz, **C**-C-CF_3), 110.08, 109.4, 99.71, 55.70 (O-**C**H_3), 45.47 (N-**C**H_2), 39.50 (**C**H_2-NH_2).

MS (FAB+) Calculated: 286, Found:287 ($M + H^+$).



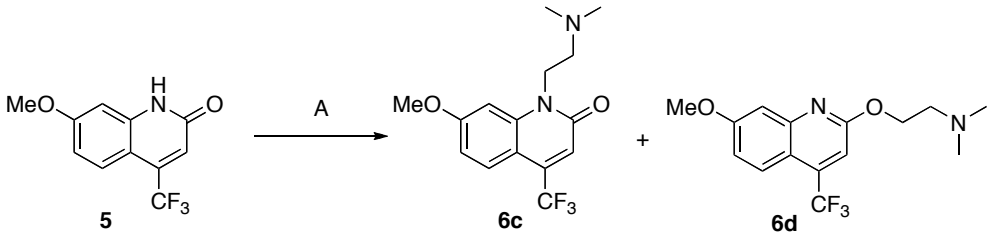
1-(2-aminoethyl)-7-hydroxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (1). Scheme **B**: An oven dried round bottom flask was charged with **7a** (324 mg, 1.13 mmol), a stirbar, a catalytic amount of oven dried K_2CO_3 (~ 5 mg), evacuated, filled with argon, then capped with a septum. Dry NMP (~ 3 mL) was added via syringe, followed by thiophenol (140 μL, 1.36 mmol). The reaction was heated to 150 °C and allowed to react for 45 min by which time it appeared deep yellow in color. NMP and excess was thiophenol removed by distillation. The reaction mixture was made into a slurry by addition of 1 mL of CH2Cl2 and 1 mL EtOAc, loaded onto a 10 g silica column and purified on a Biotage Isolera (1 column volume of CH_2Cl_2, transitioning to EtOAc over 3 column volumes, transitioning to MeOH over 5 column volumes, followed by 5 column volumes of MeOH). Yield: 187 mg, 61%

^{1}H NMR (500MHz, MeOD-D_4) δ 7.65 (d, *J* = 9.0 Hz, 1H, 6-H), 6.89 (d, *J* = 2.5 Hz, 1H, 4-H), 6.82 (dd, *J* = 9.0 Hz, *J* = 2.0 Hz, 1H, 5-H), 6.72 (s, 1H, 3-H), 4.46 (t, *J* = 7.0 Hz, 2H, 1 N-CH_2), 3.10 (t, *J* = 7.0 Hz, 2H, C**H**$_2$-NH_2).

^{13}C NMR (125.75 MHz, MeOD-D_4) δ 166.38, 162.30, 142.20 (139.33-138.59 (q, *J* = 30.2 Hz, **C**-CF_3), 128.19, 127.53-120.98 (q, *J* = 274.1 Hz, **C**F_3), 116.28, 114.26-114.13 (q, *J* = 6.3 Hz, **C**-C-CF_3), 108.06, 102.57, 44.58 (N-**C**H_2), 39.49 (**C**H_2-NH_2).

MS (direct probe) Calculated: 272, Found: 272.

HRMS (direct probe) Calculated: 272.0773, Found: 272.0769

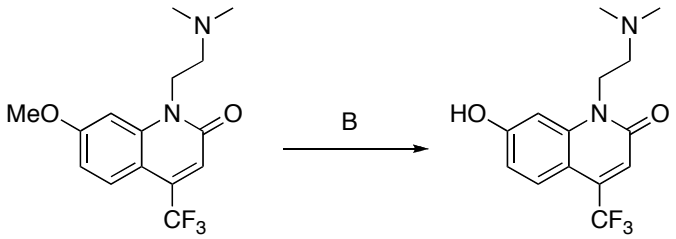


1-(2-(dimethylamino)ethyl)-7-methoxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (6c). Following Scheme **A** above, **5** (1 g, 4.12 mmol), 2-(Dimethylamino)-ethyl chloride hydrochloride (1.15 g, 7.99 mmol) were reacted to produce a mixture of **6c** and **6d**. TLC revealed the products to be inseparable and the mixture was carried forward without additional purification. Yield: 798 mg, 2.54 mmol, 61%

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (d, *J* = 9.0 Hz, 1H, 6-H), 6.96 (s, 1H, 8-H), 6.77 (s, 1H, 3-H), 6.68 (d, 2.2 Hz, 1H, 5-H), 4.20 (t, *J* = 7.3 Hz, 2H, 1 N-CH_2), 2.45 (t, *J* = 7.4 Hz, 2H, -OMe), 2.21 (s, 6H, -NMe_2), peaks listed **6c** only.

^{13}C NMR: Not obtained.

MS (FAB+) Calculated: 314, Found: 315 ($M + H^+$).



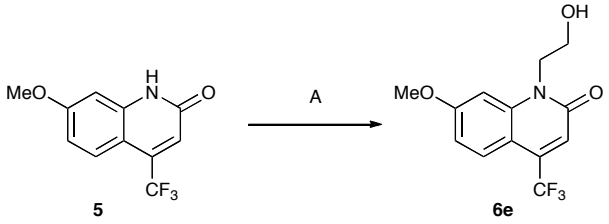
1-(2-(dimethylamino)ethyl)-7-hydroxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (2). Following Scheme **B** above, a mixture of **6c** and **6d** (400 mg, 1.27 mmol) was reacted with thiophenol (0.26 mL, 2.55 mmol). Under the reaction conditions, **6d** was found to decompose and neither the starting material, nor the demethylated product was isolated. Instead, we were able to isolate a small quantity of **5** and demethylated product as well. The desired product was obtained by dissolving the reaction mixture in a minimal amount of a MeOH/CH_2Cl_2 (1:10) then precipitating **2** from toluene as MeOH and CH_2Cl_2 were removed under reduced pressure. Yield: 54.3mg, 0.18 mmol, 14%

^{1}H NMR (300MHz, DMSO) δ 10.87 (s, 1H, -OH), 7.63 (d, *J* = 7.3 Hz, 1H, 6-H), 6.95 (s, 1H, 8-H), 6.89 (d, *J* = 8.65 Hz, 1H, 5-H), 6.79 (s, 1H, 3-H), 4.28 (t, *J* = 6.2 Hz, 2H, 1 N-CH_2), 2.48 (t, *J* = 7.7 Hz, 2H, -C**H**$_2$-NMe_2), 2.24 (s, 6H, -NMe_2).

^{13}C NMR (125.75 MHz, MeOD) δ 161.20, 159.63, 141.75, 135.71 (q, *J* = 30.7 Hz, **C**-CF_3), 126.60, 125.83 (q, *J* = 273.6 Hz, **C**F_3), 115.94, 112.99 (q, *J* = 32.4 Hz, **C**-C-CF_3), 106.73, 100.74, 55.33, 40.22.

MS (FAB+) Calculated: 300, Found: 301 ($M + H^+$).

HRMS (ESI+) Calculated: 301.1164 ($M + H^+$), Found: 301.1158 ($M + H^+$).



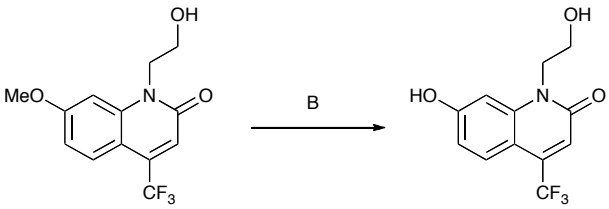
5 6e

1-(2-hydroxyethyl)-7-methoxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (6e). Following Scheme **A** above, **5** (1 g, 4.12 mmol) was reacted with 2-bromoethanol (0.34 mL, 4.94 mmol) to produce **6e**. Yield: 260 mg, 0.91 mmol, 22% yield

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (dd, *J* = 9.2 Hz, *J* = 1.8 Hz, 1H), 7.05 (d, *J* = 2.3 Hz, 1H), 6.90 (d, *J* = 2.7 Hz, 1H), 6.88 (d, *J* = 2.3 Hz, 1H), 6.86 (s, 1H), 4.46 (t, *J* = 6.0, 2H), 4.00 (t, *J* = 4.6 Hz, 2H), 3.90 (s, 3H), 3.56 (s, 1H).

^{13}C NMR (125.75 MHz, MeOD): δ 163.13, 162.06, 142.82, 137.97-137.04 (q, *J* = 31.5 Hz, **C**-CF_3), 126.98, 127.12-118.92 (q, *J* = 274.4 Hz, **C**F_3), 116.7-116.6 (q, *J* = 5.9 Hz, **C**-C-CF_3), 111.74, 109.36, 100.25, 58.98, 55.32, 45.36.

MS (FAB+) Calculated: 287, Found: 288 ($M + H^+$).



6e 3

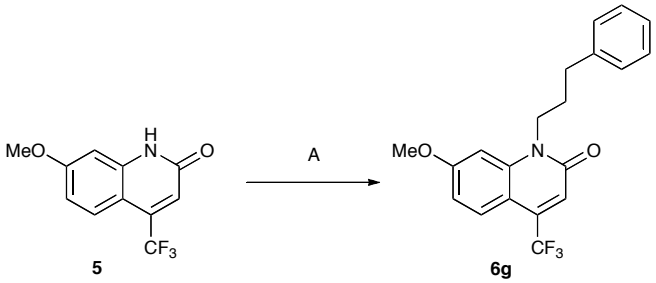
7-hydroxy-1-(2-hydroxyethyl)-4-(trifluoromethyl)quinolin-2(1*H*)-one (3). Following Scheme B above, **6e** (120 mg, 0.42 mmol) was reacted with thiophenol (0.09 mL, 0.84 mmol). However reaction began turning brown after 15 min so the reaction was stopped early to prevent further decomposition of the product. Yield: 56 mg, 48.8%

^{1}H NMR (400 MHz, MeOD): δ 7.72 (dd, *J* = 9.0 Hz and *J* = 1.9 Hz, 1H), 7.14 (d, *J* = 2.1 Hz, 1H), 6.89 (dd, *J* = 9.0 Hz and *J* = 2.2 Hz, 1H), 6.82 (s, 1H), 4.45 (t, *J* = 6.3 Hz, 2H), 3.87 (t, *J* = 6.2, 2H).

^{13}C NMR (125.75 MHz, DMSO): δ 161.29, 160.42, 142.98, 135.78 (q, *J* = 30.0 Hz, **C**-CF_3), 126.41, 125.91 (q, 272.5 Hz, **C**F_3), 116.27 (q, 5.0 Hz, **C**-C-CF_3), 112.81, 107.43, 101.96, 58.05, 45.07

MS (direct probe) Calculated: 273, Found: 273.

HRMS (direct probe) Calculated: 273.0613, Found: 273.0613.

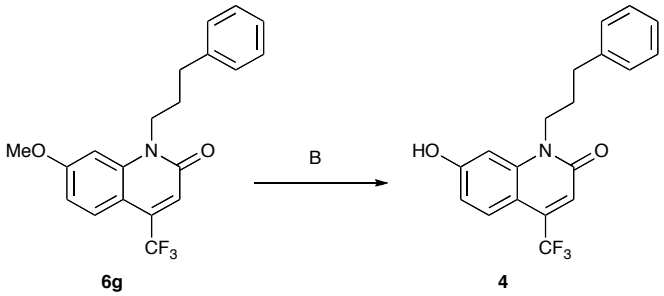


7-methoxy-1-(3-phenylpropyl)-4-(trifluoromethyl)quinolin-2(1*H*)-one (6g). Following Scheme A, above, **5** (500 mg, 2.06 mmol) was reacted with 3-phenylpropylbromide (0.46 mL, 4.12 mmol) to produce **6g**. Yield (**6g**): 200 mg, 0.55 mmol, 26.9%

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, *J*=8.9 Hz, 1H), 7.33 (bm, 5H), 6.91 (s, 1H), 6.85 (d, *J*=7.4 Hz, 1H), δ 6.73 (s, 1H), 4.26 (t, *J*=7.4 Hz, 2H), 3.70 (s, 3H), 2.81 (t, *J* = 6.9 Hz, 2H), 2.10 (bm, 2H).

^{13}C NMR (100.58 MHz, $CDCl_3$): δ 162.07, 161.77, 150.25, 142.02, 137.73-136.78 (q, *J* = 31.9 Hz, **C**-CF_3), 128.91, 128.89, 127.85-119.67, (q, *J* = 274.5 Hz, **C**F_3), 126.44, 125.48, 117.81, 114.80, 109.27-109.11 (q, *J* = 5.6 Hz, **C**-C-CF_3), 107.79, 66.11, 55.80, 32.81, 30.97.

MS (FAB+) Calculated: 361, Found: 362 ($M + H^+$).



7-hydroxy-1-(3-phenylpropyl)-4-(trifluoromethyl)quinolin-2(1*H*)-one (6g). Following Following Scheme **B** above, **6g** (100 mg, 0.28 mmol) was reacted with thiophenol (0.039mL, 0.42 mmol) to produce **4**. Yield: 79mg, 0.23 mmol, 81%

^{1}H NMR (400 MHz, $CDCl_3$/MeOD): δ 7.68 (d, *J*=7.7 Hz, 1H), 7.25 (bm, 5H), 6.85 (s, 2H), 6.77 (s, 1H), 4.27 (t, *J* = 7.1 Hz, 2H), 2.76 (t, *J* = 7.5 Hz, 2H), 2.06 (q, *J* = 7.3 Hz, 2H).

^{13}C NMR (125.75 MHz, $CDCl_3$/MeOD): δ 162.70, 162.52, 142.87, 141.96, 138.79-138.04 (q, *J* = 31.25 Hz, **C**-CF_3), 129.31, 129.13, 128.24, 127.09-120.50 (q, *J* = 275.4 Hz, **C**F_3), 126.96, 116.60-116.45 (q, *J* = 6.25 Hz, **C**-C-CF_3), 113.07, 109.49, 101.85, 43.70, 33.96, 29.43.

MS (direct probe) Calculated 347, Found 347.

HRMS (direct probe) Calculated: 347.1133, Found: 347.1137.

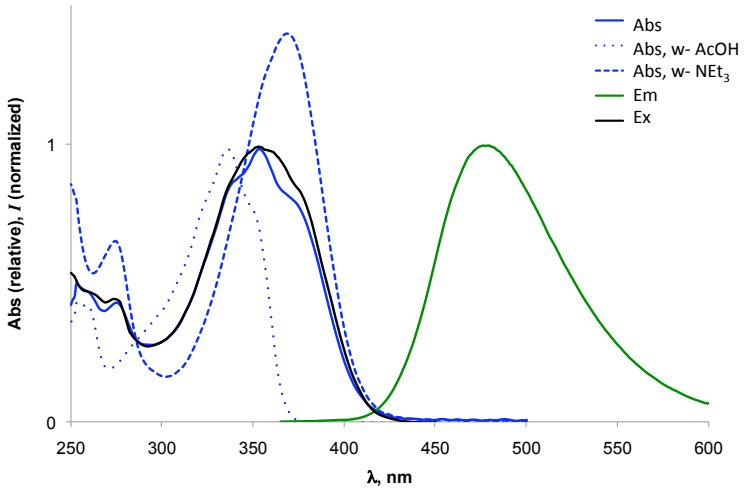


Figure S1: Optical spectra of **1**, ε = 12,000, Φ = 0.32 (DPBS)

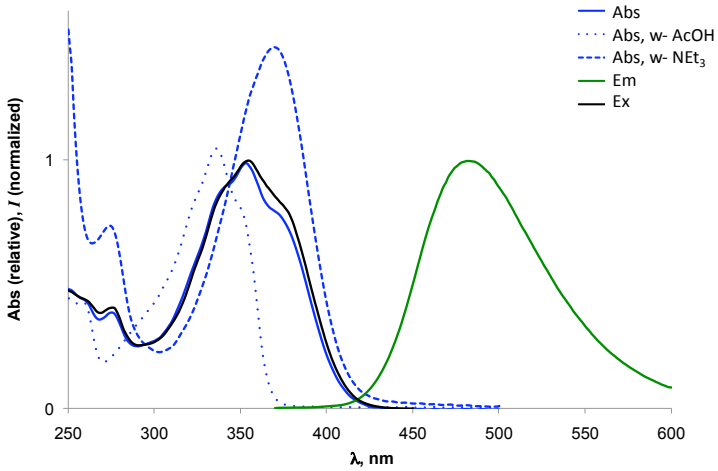


Figure S2: Optical spectra of **2**, ε = 12,000, Φ = 0.33 (DPBS)

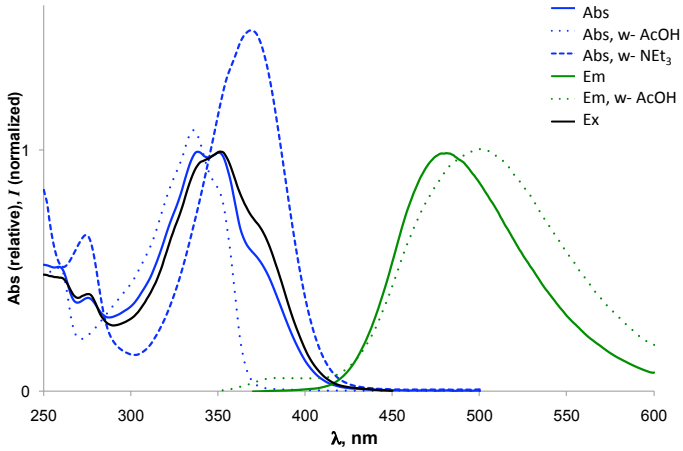


Figure S3: Optical spectra of **3**, ε = 12,000, Φ = 0.30 (DPBS)

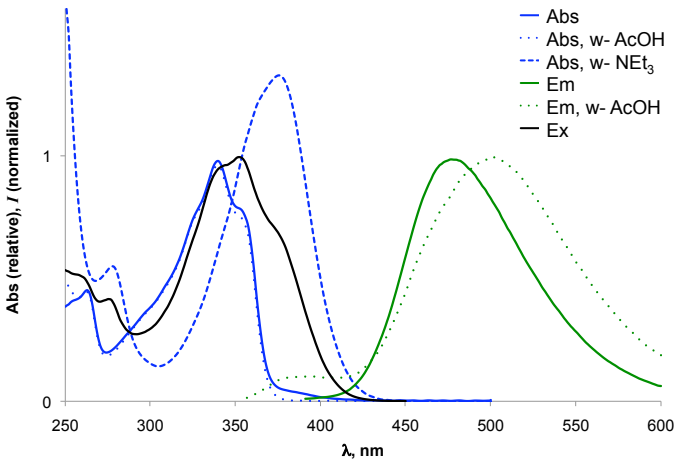


Figure S4: Optical spectra of **4**, ε = 13,000, Φ = 0.22 (DPBS)

Quantum yields taken relative to quinine sulfate (in 0.1 M H_2SO_4) and perylene (in cyclohexane).[2,3]

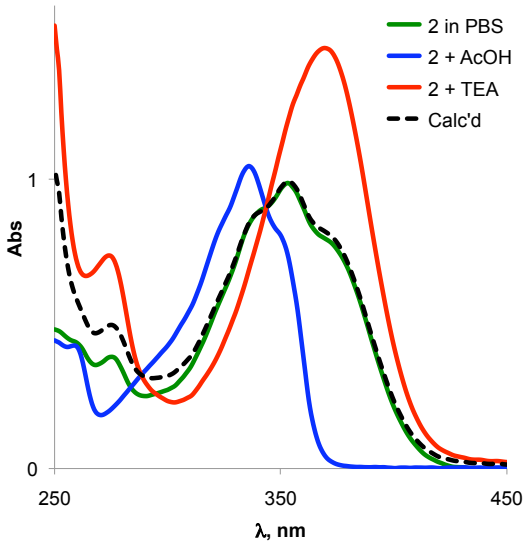


Figure S5: Absorbance of **2** with excess acid and excess base. The linear combination of high and low pH nearly perfectly overlaps with the measured absorbance in PBS.

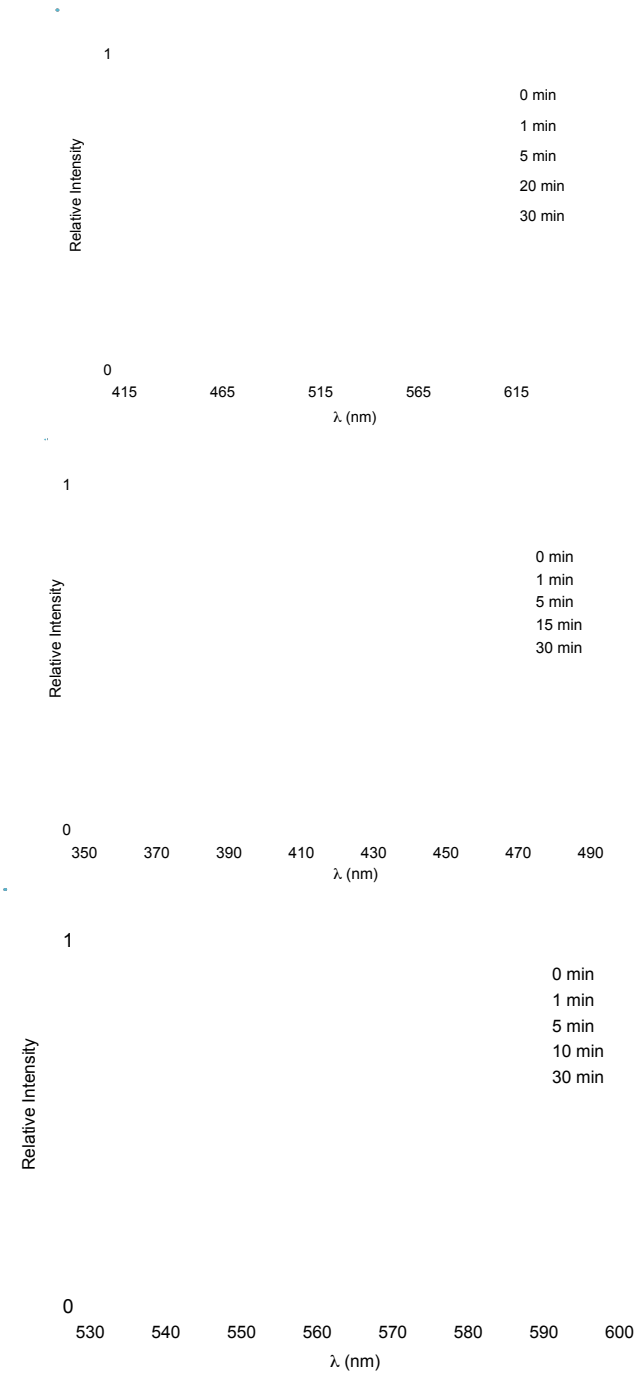


Figure S6. Photostability of **1** (top) in relation to fluorescence standards quinine sulfate (middle) and rhodamine 6G (bottom) taken at identical optical densities (0.01 a.u.) under illumination of a 9.9 W xenon lamp (slit width = 2.5 nm). Fluorescein was examined under similar conditions and found to not bleach appreciably.

Brainstem isolation and cell culture

Brainstems were isolated from chick embryos at day 14. Eggs were chilled and brainstems were identified and isolated under a light microsope and placed into Leibovitz media (L-15, Mediatech #45000-372) on ice. Tissue was washed 2 times with L15 media under sterile conditions and 15 ml trypsin/Hanks' Balanced Salt Solution (1X trypsin, Mediatech #45000-666; HBSS, Mediatech #MT21-021-CV) was added. At this time, tissue was homogenized using scissors and forceps and placed into a 37°C incubator for 30 min with continuous light agitation. Tissue was triturated with a 5ml pipet tip and 15ml trypsin neutralizing solution (TNS, Sciencell 0113) was added. Tissue was centrifuged for 5 min 1000 rpm. Samples were again triturated with fire blown glass pipet tips in HBSS (+ 1X Penicillin/Streptomycin, Sciencell 0503) and washed and centrifuged 2 more times. Cell number and viability was assessed using 0.4% trypan blue solution (Sciencell 0203). Cells were resuspended in neuronal media (NM, Sciencell 1521 containing basal medium, neuronal growth supplement, and penicillin/streptomycin supplement) at a final concentration of 10^6 cells/ml.

For confocal imaging, coverslip dishes were made as follows: coverslips (Gold Seal 1.5mm) were boiled in nitric acid for 12 hr, rinsed with Barnstead ultrafiltered water, and air dryed on parchment paper in a covered box overnight. Coverslips were handled with forceps. 1.54 cm^2 holes were punched into 35mm petri dishes (Falcon #1008). Silastic type A medical adhesive (#A-100, Factor II, Inc, Lakeside, AZ) was applied to the dish and the coverslip pressed on. The dishes were airdryed upside down for 48hr at room temperature and sterilized in 95% ethanol and dryed in a laminar flow hood and stored in a covered box until use.

Sterile coverslip dishes and 96 well plates (Biogreiner #353948 and 655936) were coated with poly-l-lysine (PLL, Sigma Chemicals #6282) as follows: PLL was dissolved in sterile H_2O to a concentration of 5mg/ml. Slides and cover slips were coated with 19.5$\mu g/cm^2$ PLL, incubated overnight at 37°C, and rinsed twice with sterile H_2O before use. Following PLL coating, slides and cover slips were coated with 13 $\mu g/cm^2$ natural mouse laminin (Culturex 3400-010-01) in cold Dulbecco's Phosphate Buffered Saline (DPBS, Mediatech #21-031-CV), incubated for 1hr at room temperature, and washed twice with PBS immediately before use.

Cells were plated onto slides and plates at a density of 10^4 cells/cm^2 and incubated at 37°C 5% CO_2 for 72-96 hr. Prior to use, slides and plates were washed 2 times with media to remove cell debris and non-adherent cells.

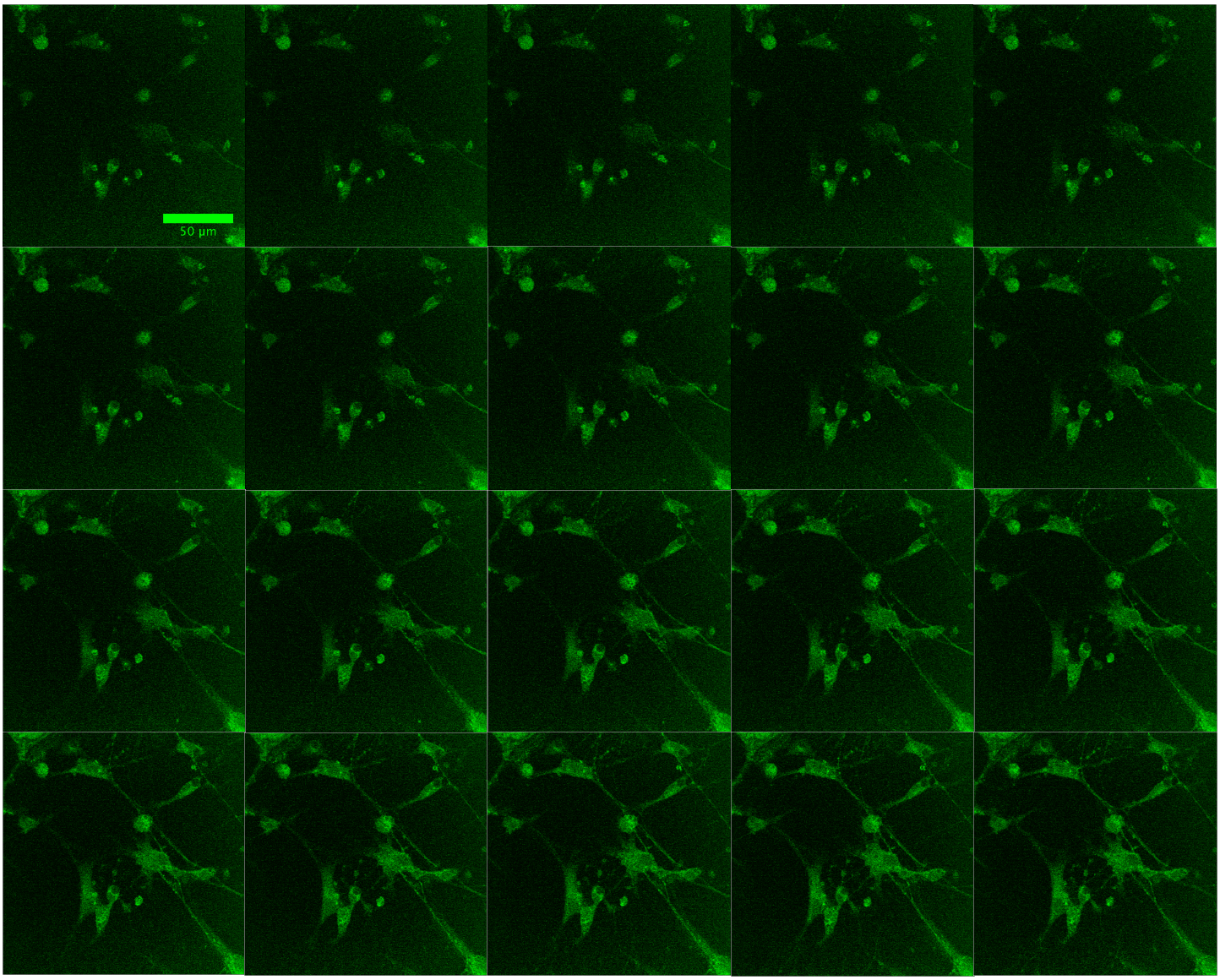
Microwell plate assay procedure

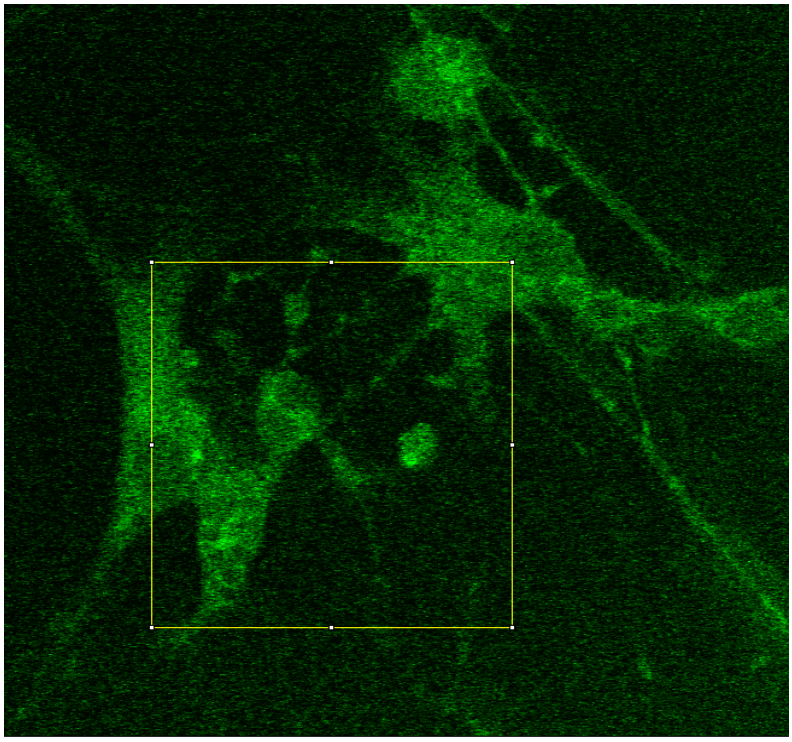
Cells were plated on appropriately (please see above) coated 96 well plates in 200 μL growth medium (see cell densities above). Fluorescent probes were added in 200 μL NM producing a final concentration of 5 μM in each well. Control wells consisted of cells only, media only, and compound only. Plates were incubated at room temp for 10 min. Plates were then gently washed two times with Dulbecco's Phosphate-Buffered Solution (DPBS, Mediatech #21-031-CV) to remove any residual compound and non-attached cells and 200 μL NM was added and left in each well. Fluorescence was measured in a Fluoroscan II microplate reader at combinations of 355 nm excitation and 444 nm emission; samples were run with at least seven replicates. Emission intensity is expressed in arbitrary units as a mean. All samples were corrected by subtracting residual fluorescence (compound only) and compared against a control (cells only).

Confocal microscopy procedure

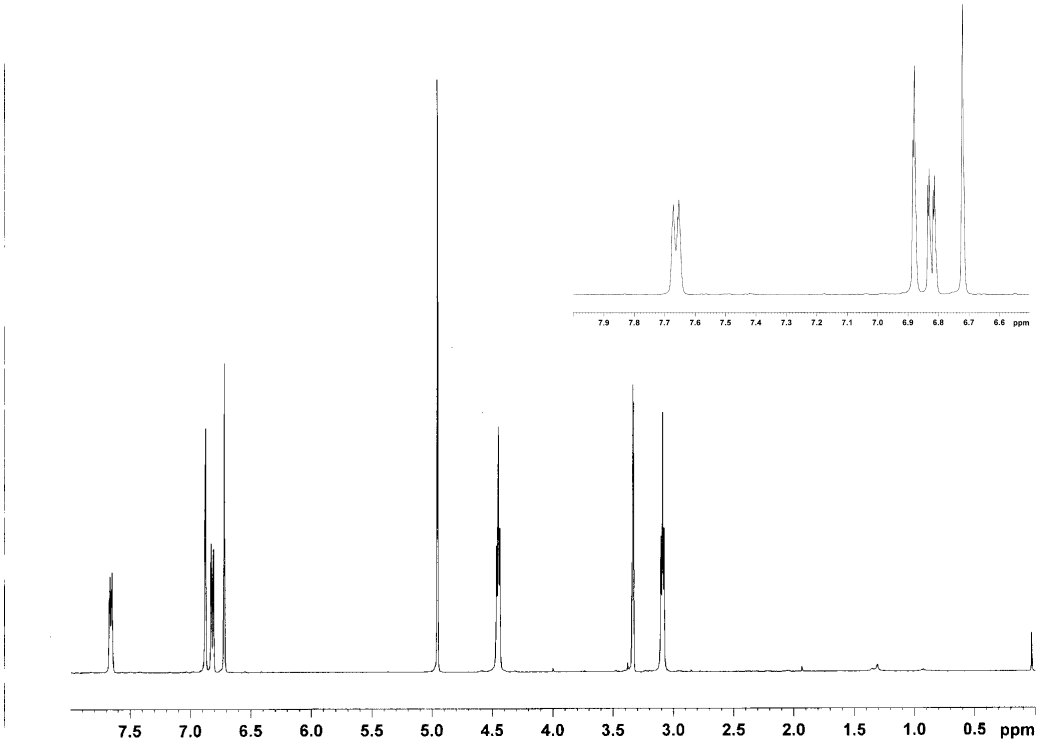
For confocal imaging, coverslip dishes were made as follows: coverslips (Gold Seal 1.5mm) were boiled in nitric acid for 12 hr, rinsed with Barnstead ultrafiltered water, and air dryed on parchment paper in a covered box overnight. Coverslips were handled with forceps. 1.54 cm^2 holes were punched into 35mm petri dishes (Falcon #1008). Silastic type A medical adhesive (#A-100, Factor II, Inc, Lakeside, AZ) was applied to the dish and the coverslip pressed on. The dishes were airdryed upside down for 48hr at room temperature and sterilized in 95% ethanol and dryed in a laminar flow hood and stored in a covered box until use. Cells were cultured on coverslip dishes as described above in Brainstem isolation and cell culture (S8).

Imaging was performed on a Leica SP5 confocal microscope housed within the UM Biology Imaging Core Facility. A 405 nm diode laser was used as the excitation source. XYZ-Scans were collected with 1 μm sections. Images were analyzed using ImageJ 1.41 software (NIH, USA). Images were taken immediately before and during the addition of 20 μL of a 1.3 mmol solution of **1** to cells in 200 mL of media (final concentration = 120 μM).

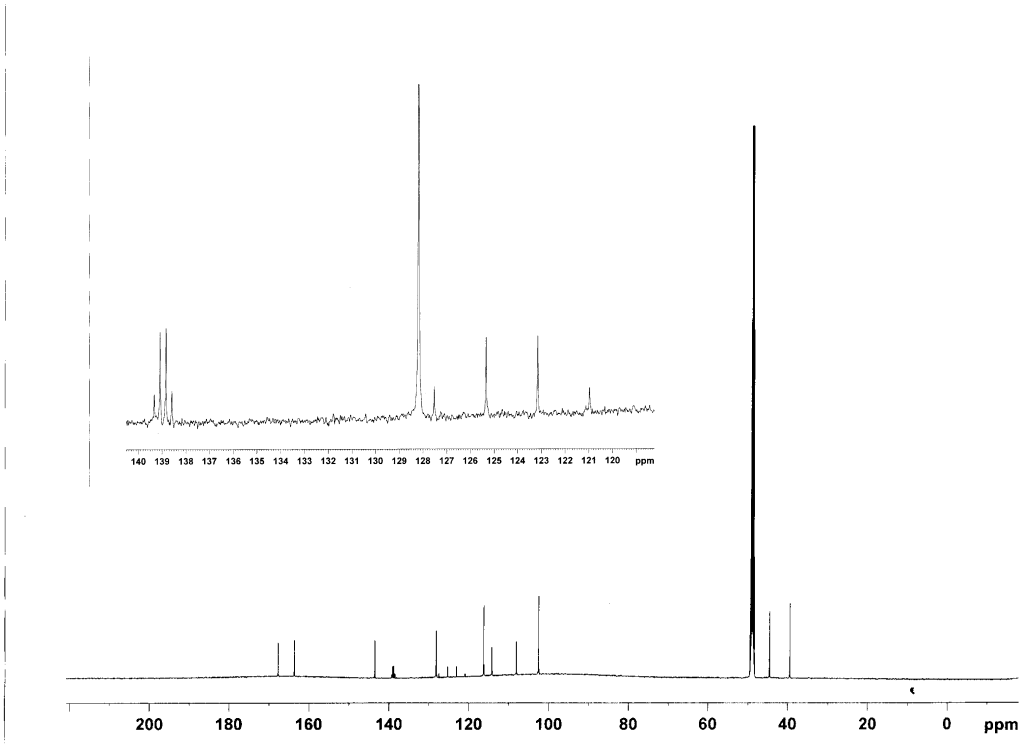




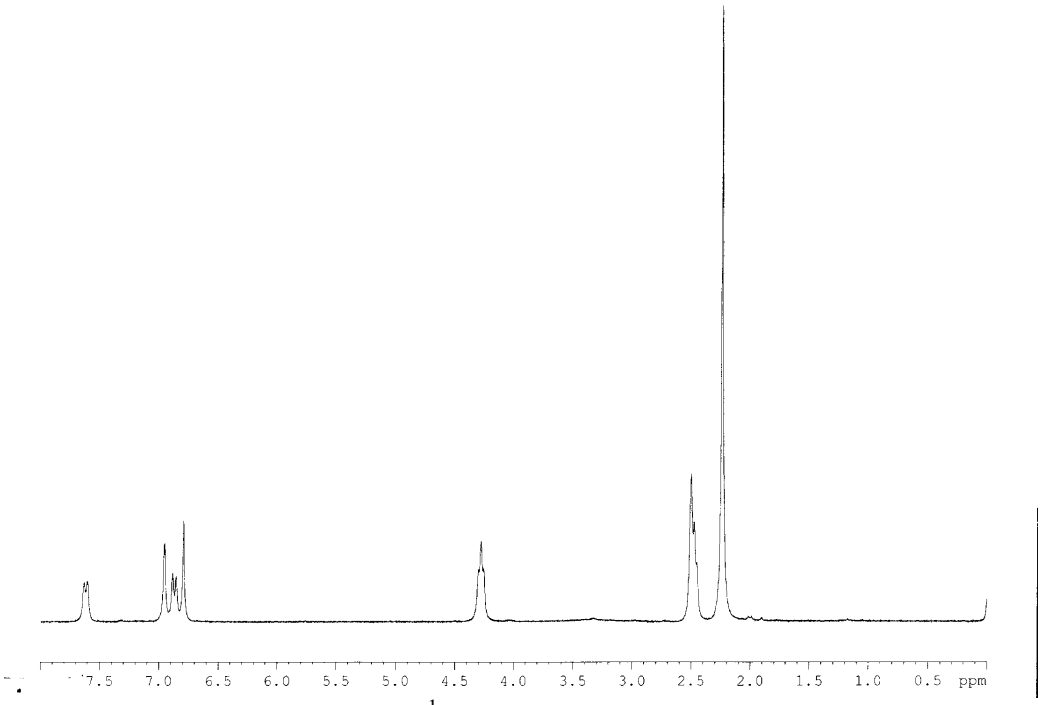
The highlighted area was used to generate a 3D rotation around the X and Y axes for the .avi movie clips (viewable in Quicktime) that are available as separate files.



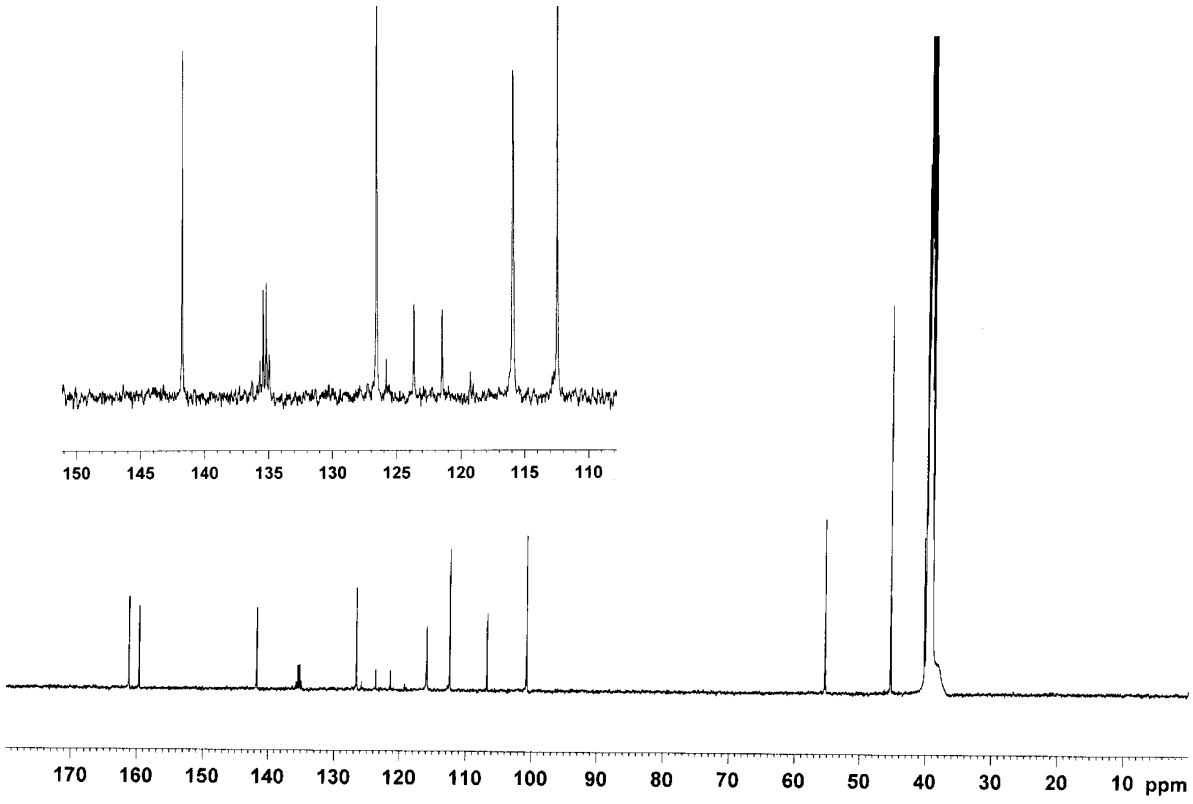
^{1}H NMR of **1**. Inset: Expansion of aromatic region.



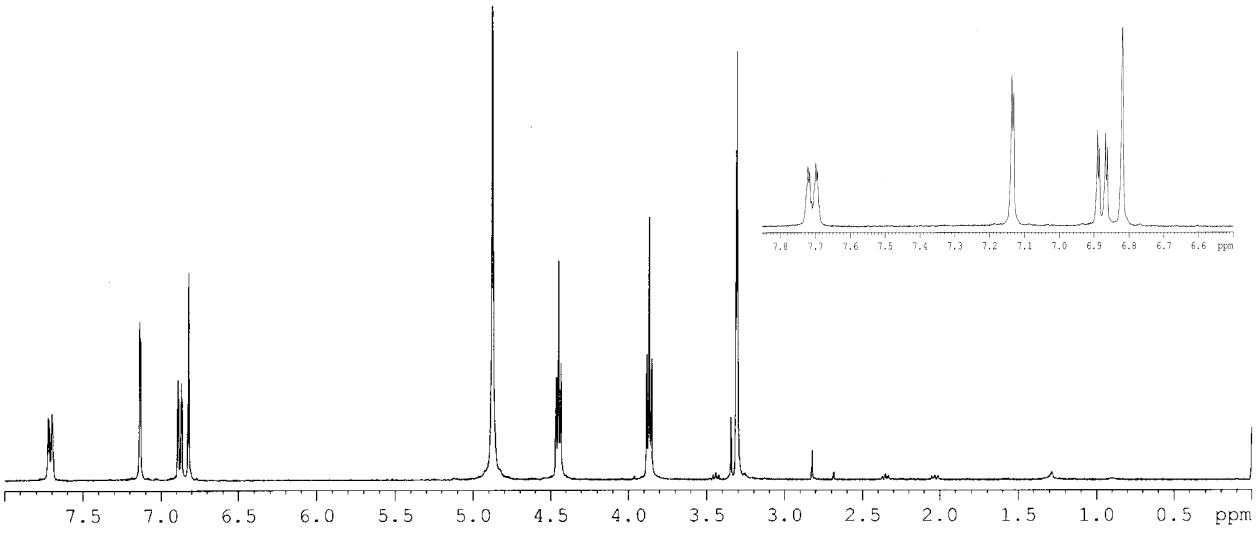
^{13}C NMR of **1**. Inset: Expansion of splitting due to ^{19}F coupling.



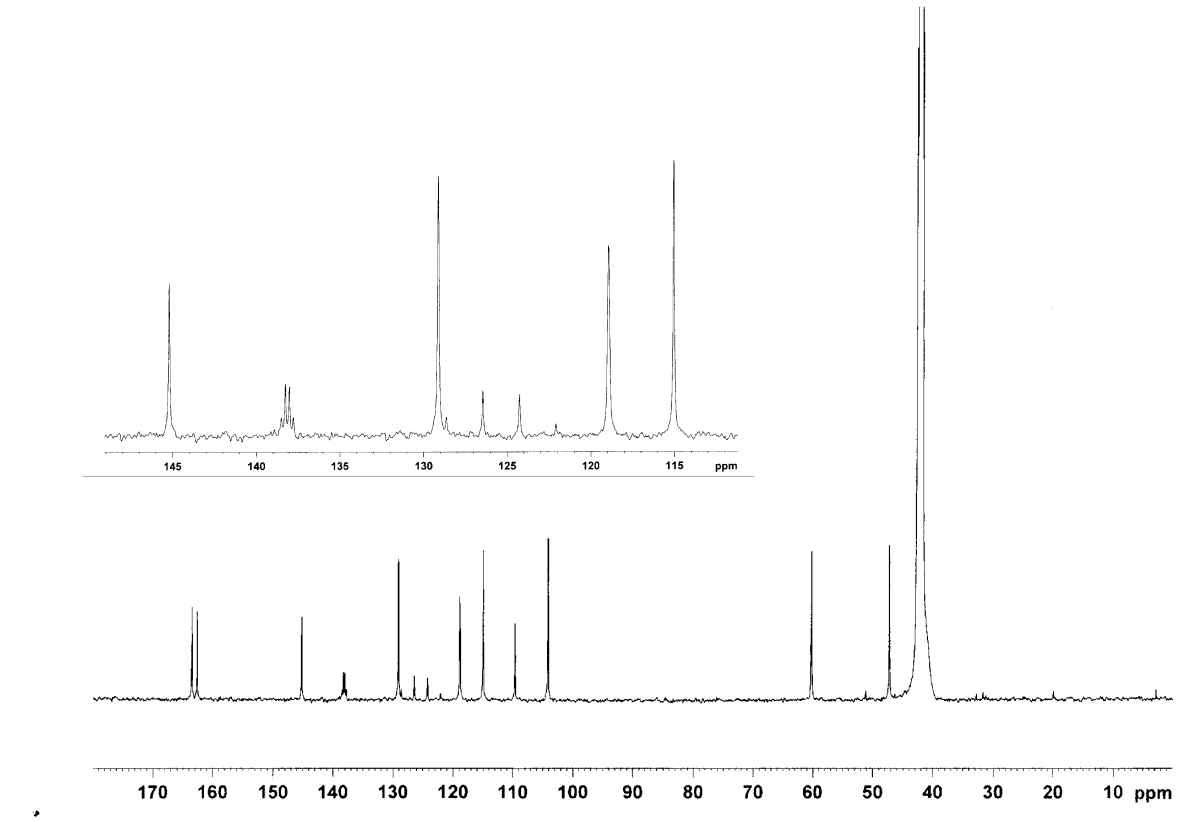
^{1}H NMR of **2**.



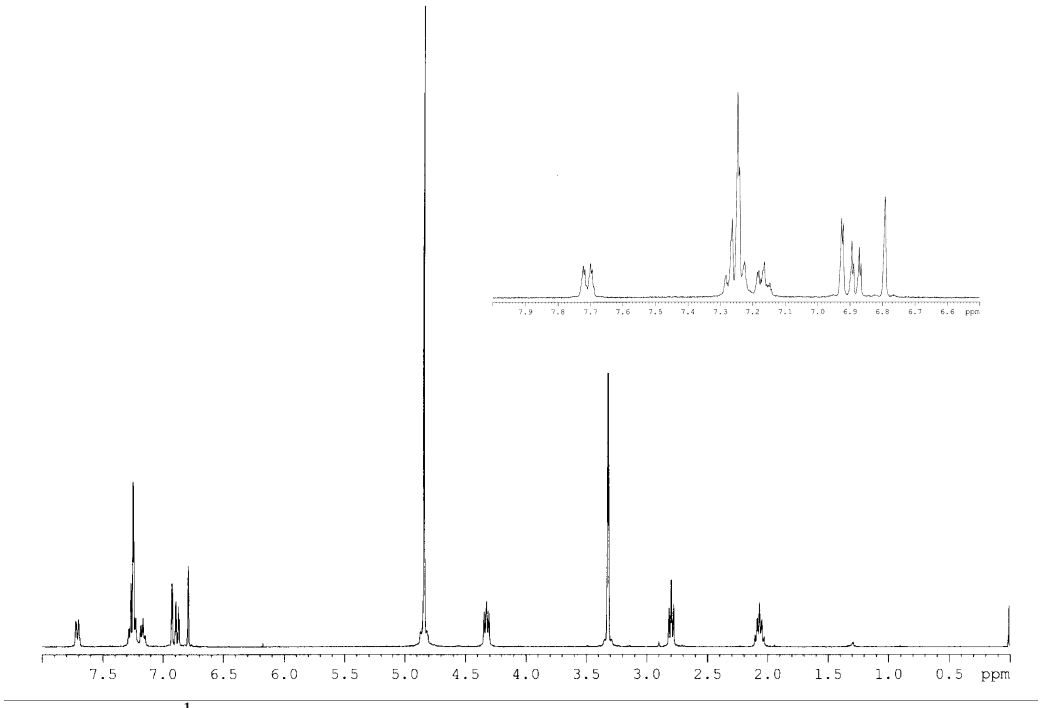
^{13}C NMR of **2**. Inset: Expansion of splitting due to ^{19}F coupling.



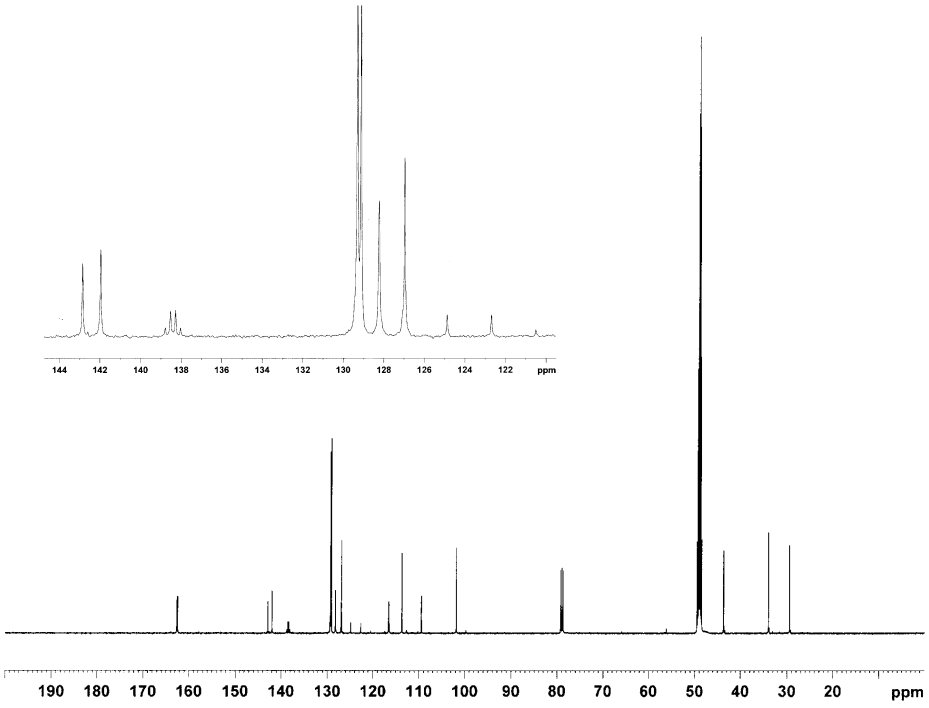
^{1}H NMR of **3**. Inset: Expansion of aromatic region.



^{13}C NMR of **3**. Inset: Expansion of splitting due to ^{19}F coupling.



^{1}H NMR of **4**. Inset: Expansion of aromatic region.



^{13}C NMR of **4**. Inset: Expansion of splitting due to ^{19}F coupling.

References

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2. D. F. Eaton, *Pure Appl. Chem.*, 1988, **60**, 1107.
3. I. B. Berlman, Handbook of fluorescence spectra of aromatic molecules, Academic Press, New York, 1965.