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SUPPLEMENTARY MATERIAL

CAPRYDAA, an anthracene dye analog to LAURDAN: a comparative study using cuvette and microscopy

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Organic Synthesis

The synthetic procedures to obtain compounds (2), (3), (4), (5) and final compound (6), are detailed below:

2,6-Dibromo-9,10-anthraquinone (2). To a mixture of **1** (0.10 g, 0.42 mmol) and $CuBr_2$ (0.23 mg, 1.04 mmol) in CH_3CN (5 mL) was added dropwise to *tert*-butyl nitrite (0.12 mL, 1.04 mmol). The mixture was kept at 90 °C for 24 h. The reaction was quenched by adding HCl (aq) 20% (six drops). The precipitate formed was filtered, washed with cold CH_3CN to afford **2** (145 mg, 94% yield) as a pale brown solid. MP: > 300 °C (Mp Lit. >300 °C)[2]. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.34 (d, *J* = 2.8 Hz, 2H), 8.16 (d, *J* = 8.9 Hz, 2H), 7.94 (dd, *J* = 9.0, 2.9 Hz, 2H).

2-Bromo-6-dimethylamino-9,10-anthraquinone (3). A mixture of **2** (1.50 g, 4.1 mmol) and anhydrous CsF (0.80 g, 5.3 mmol) in 60 mL of anhydrous DMSO was heated with magnetic stirring at 170 °C under a nitrogen atmosphere for 5 h. The mixture was cooled to room temperature, an aqueous solution (40%) of dimethylamine (0.62 mL, 4.9 mmol) and K_2CO_3 (0.68 g, 4.9 mmol) was added, and then all was stirred at 70 °C for 17 h. The mixture was poured into 50 mL of water and the precipitate formed was collected by filtration. The solid residue was separated by chromatography with $CHCl_3$ as eluent, to afford **3** (476 mg, 35% yield) as a red solid. Mp: 223 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.21 (d, *J* = 2.5 Hz, 1H, ArH), 8.5 (m, 3H, ArH), 7.31 (d, *J* = 2.5 Hz, 1H,

ArH), 7.14 (dd, *J* = 8.9, 2.6 Hz, 1H, ArH), 3.15 (s, 6H, CH_3). ^{13}C NMR (100 Hz, DMSO-d_6): δ 183.12, 179.45, 154.50, 136.66, 135.50, 134.49, 132.48, 129.88, 129.33, 129.26, 120.86, 116.77, 108.11, 99.94. 40.25.

2-Bromo-6-(*N*,*N*-dimethylamino)anthracene (4). To a suspension of **3** (1.60 g, 4.83 mmol) in *i*-PrOH (180 mL) was added $NaBH_4$ (8.0 g, 0.212 mol) at room temperature. The reaction mixture was stirred for 12 h and then heated under reflux for 10 h. After cooling to room temperature, the reaction was neutralized with 6 M HCl until bubbling ceased and was then heated under reflux for 2 h. The precipitate formed was discarded and the solvent was removed by evaporation. The residue was dissolved in *i*-PrOH (120 mL) and treated with another portion of $NaBH_4$ (5.5 g, 0.146 mol). The reaction mixture was refluxed for 12 h. After cooling, the reaction mixture was neutralized with 6 M HCl until bubbling ceased. The precipitate was again discarded, and the solvent removed. The solid residue was separated by chromatography with $CHCl_3$ as eluent, to afford **4** (978 mg, 67% yield) as a yellow solid. Mp: 200 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 8.31 (m, 2H, ArH) 2.0 Hz, 1H), 8.14 (s, 1H, ArH), 7.90 (m, 2H, ArH), 7.42 (d, *J* = 8.8 Hz, 1H, ArH), 7.33 (d, *J* = 9.3 Hz, 1H, ArH), 6.95 (s, 1H, ArH), 3.14 (s, 3H, CH_3). ^{13}C NMR (100 Hz, $CDCl_3$): ^{13}C NMR (100 Hz, $CDCl_3$): δ 145.31, 135.48, 132.57, 132.46, 129.97, 128.80, 128.33, 128.17, 125.19, 122.64, 121.54, 119.60, 108.58, 100.12, 43.76 (CH_3).

2-Cyano-6-(*N*,*N*-dimethylamino)anthracene (5). A mixture of **4** (200 mg, 0.66 mmol) and CuCN (354 mg, 3.96 mmol) in 20 mL of anhydrous DMF was heated under reflux in a nitrogen atmosphere for 12 h. The reaction mixture was cooled to room temperature and treated with 1.0 mL of ethylenediamine and 10 mL of water at 50 °C and stirred for a half hour. Water (30 mL) was added, and the precipitate formed was collected by filtration. The solid residue was separated by chromatography with ethyl acetate/hexane (1:3) as eluent to afford **5** (50 mg, 30% yield) as a yellow solid. Mp: 209 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 8.27 (m, 2H, ArH) 2.0 Hz, 1H), 8.10 (s, 1H, ArH), 7.87 (m, 2H, ArH), 7.39 (d, *J* = 8.7 Hz, 1H, ArH), 7.31 (d, *J* = 9.3 Hz, 1H, ArH), 6.92 (s, 1H, ArH), 3.13 (s, 6H, CH_3). ^{13}C NMR (100 Hz, $CDCl_3$): δ 148.98, 135.92, 135.68, 132.45, 129.64, 128.76, 127.65, 127.50, 127.38, 124.47, 122.95, 120.18, 118.95, 106.27, 103.43, 40.6 (CH_3).

1-(6-(Dimethylamino)anthracene-2-yl)nonan-1-one (6). To a mixture of **5** (80 mg, 0.32 mmol) and a trace of CuBr in anhydrous THF (60 mL) was added an excess of octylmagnesium bromide solution in anhydrous THF at room temperature under a nitrogen atmosphere. The reaction flask was fully covered with aluminum foil to avoid possible photoreactions and the solution was stirred

for 1 h at room temperature. The reaction mixture was treated with 6 M HCl until pH 2 was reached and was heated under reflux for 2 h. It was then cooled and neutralized with a saturated solution of $NaHCO_3$. Water (10 mL) was added, and the resulting mixture was extracted with dichloromethane. The organic layer was dried (Na_2SO_4), concentrated, and the solid residue was separated by chromatography with hexane/$CHCl_3$ (2:1) as eluent to afford **6** (21 mg, 19% yield) as an orange solid. Mp: 126 °C. Elemental Analysis: Calculated (experimental) values are: C, 83.06 (83.32); H, 8.64 (8.42); N, 3.87 (3.64) % and O, 4.43 (4.62) %. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.57 (s, 1H, ArH), 8.40 (s, 1H, ArH), 8.14 (s, 1H, ArH), 7.91 (m, 3H, ArH), 7.30 (d, *J* = 8.7 Hz, 1H, ArH), 6.96 (s, 1H, ArH), 3.13 (s, 6H, CH_3), 3.10 (t, *J* = 7.8 Hz, 2H), 1.81 (m, 2H), 1.29 (m, 10H), 0.89 (m, 3H). ^{13}C NMR (100 Hz, $CDCl_3$): δ 200.44, 148.68, 135.34, 133.57, 132.18, 131.44, 129.50, 128.75. 128.06, 127.81, 127.05, 122.87, 122.53, 118.36, 103.78, 40.66, 38.45, 31.89, 29.72, 29.52, 29.24, 24.85, 22.69, 14.14.

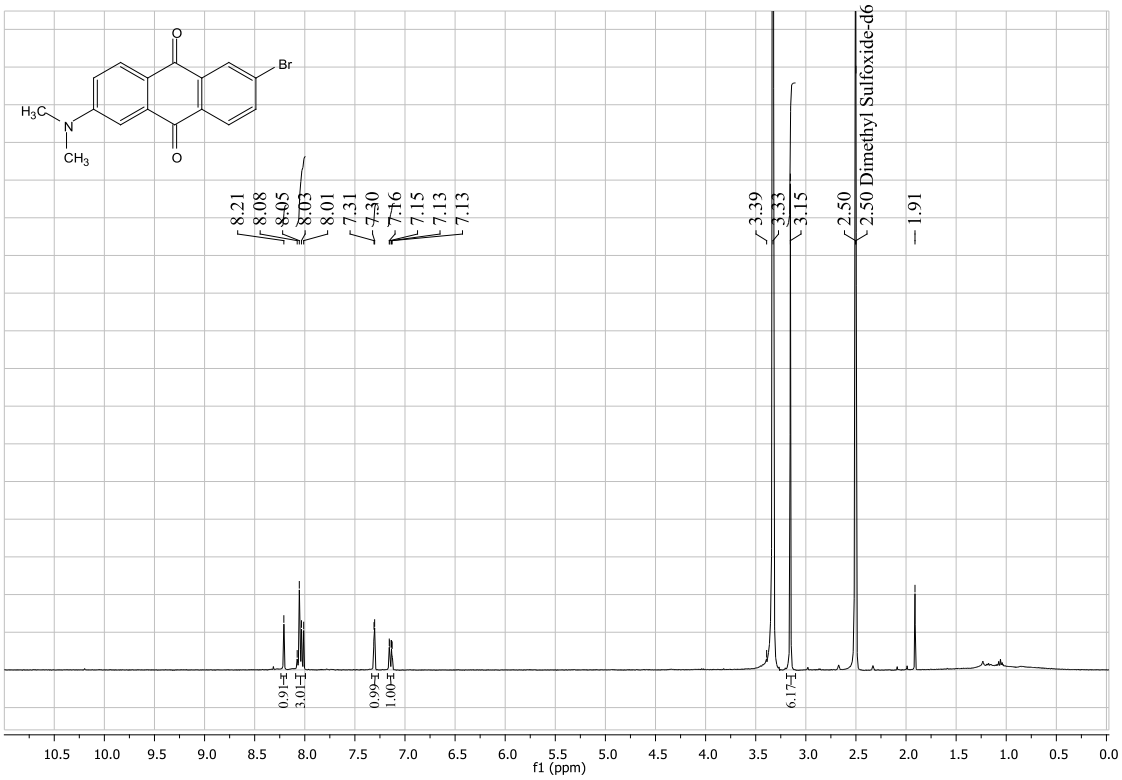

Figure S1 ^{1}H-NMR of 2-Bromo-6-dimethylamino-9,10-anthraquinone (3).

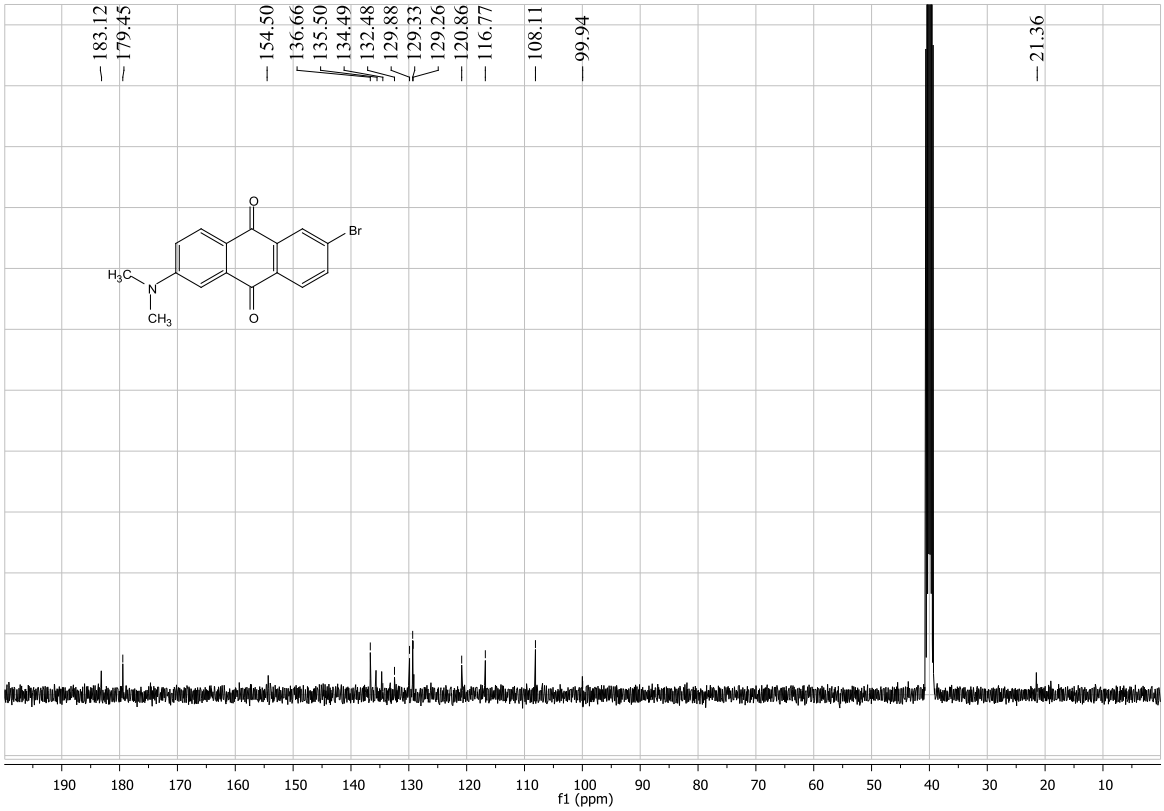

Figure S2 ^{13}C-NMR of 2-Bromo-6-dimethylamino-9,10-anthraquinone (3).

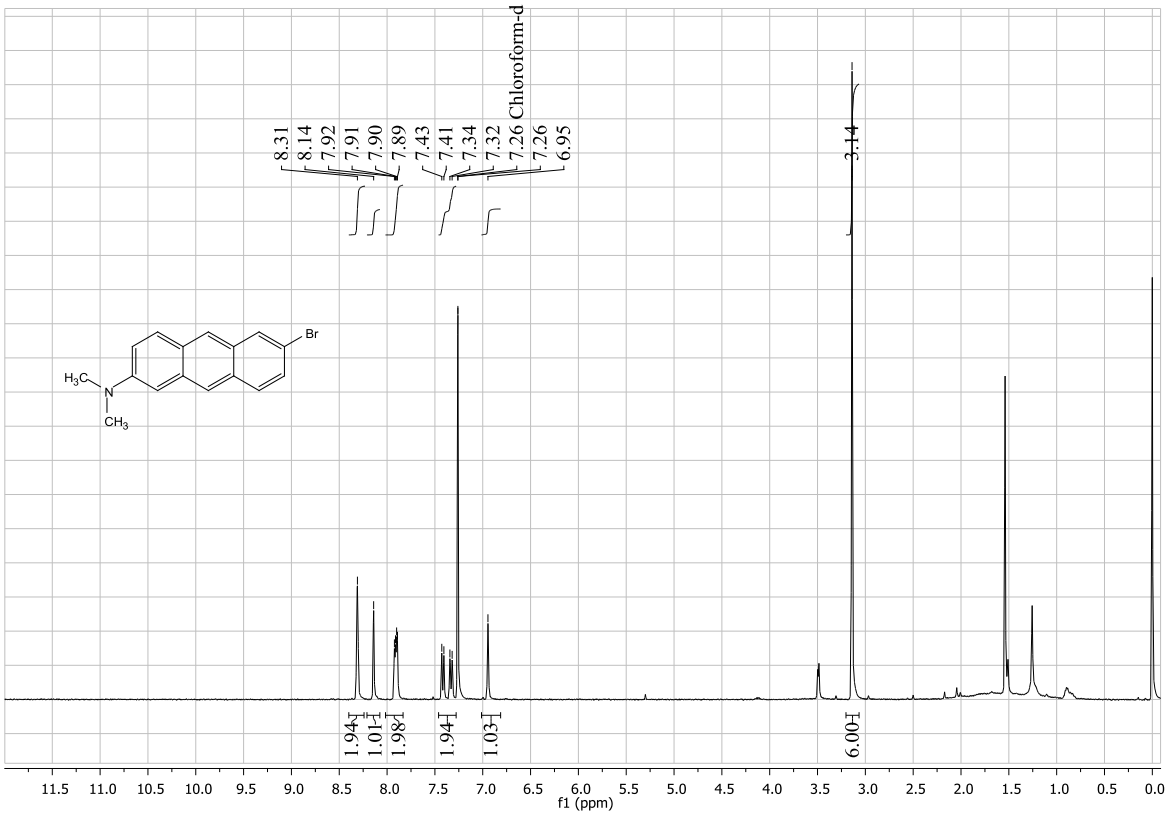

Figure S3 ^{1}H-NMR of 2-Bromo-6-(*N*,*N*-dimethylamino)anthracene (4).

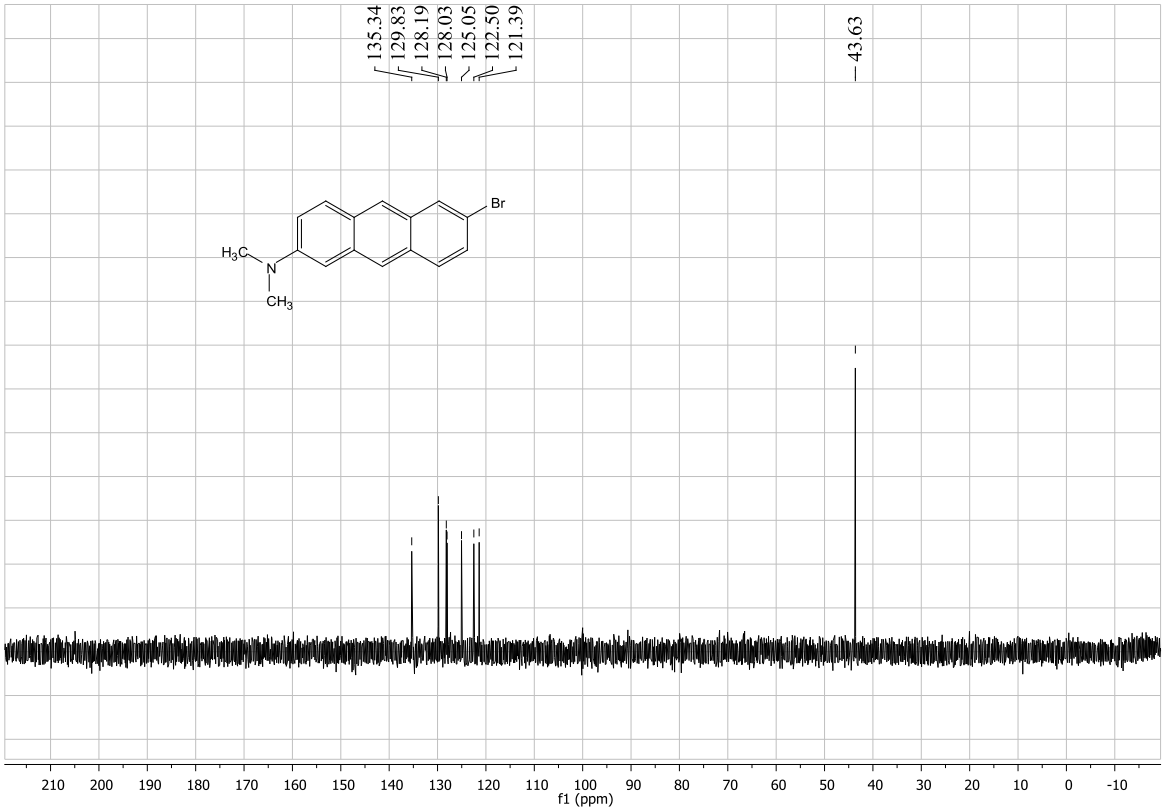

Figure S4 ^{13}C-NMR of 2-Bromo-6-(*N*,*N*-dimethylamino)anthracene (4).

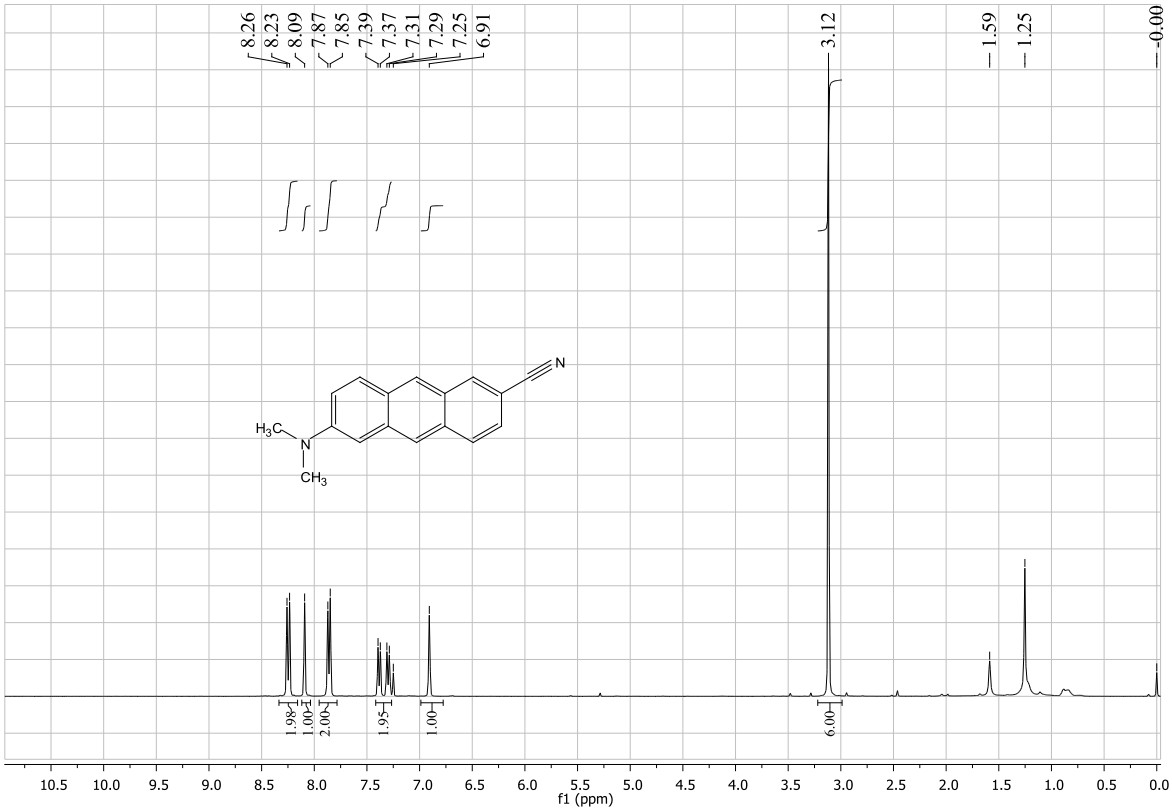

Figure S5 ^{1}H-NMR of 2-Cyano-6-(*N*,*N*-dimethylamino)anthracene (5).

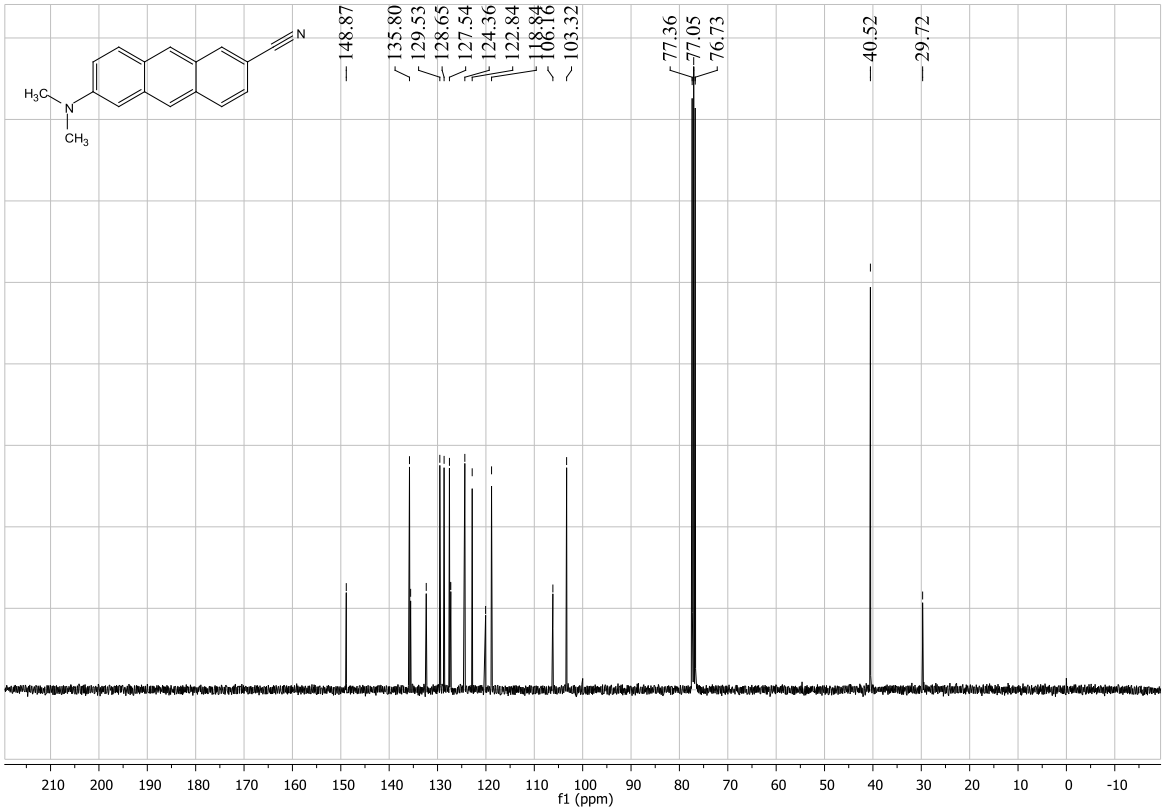

Figure S6 ^{13}C-NMR of 2-Cyano-6-(*N*,*N*-dimethylamino)anthracene (5).

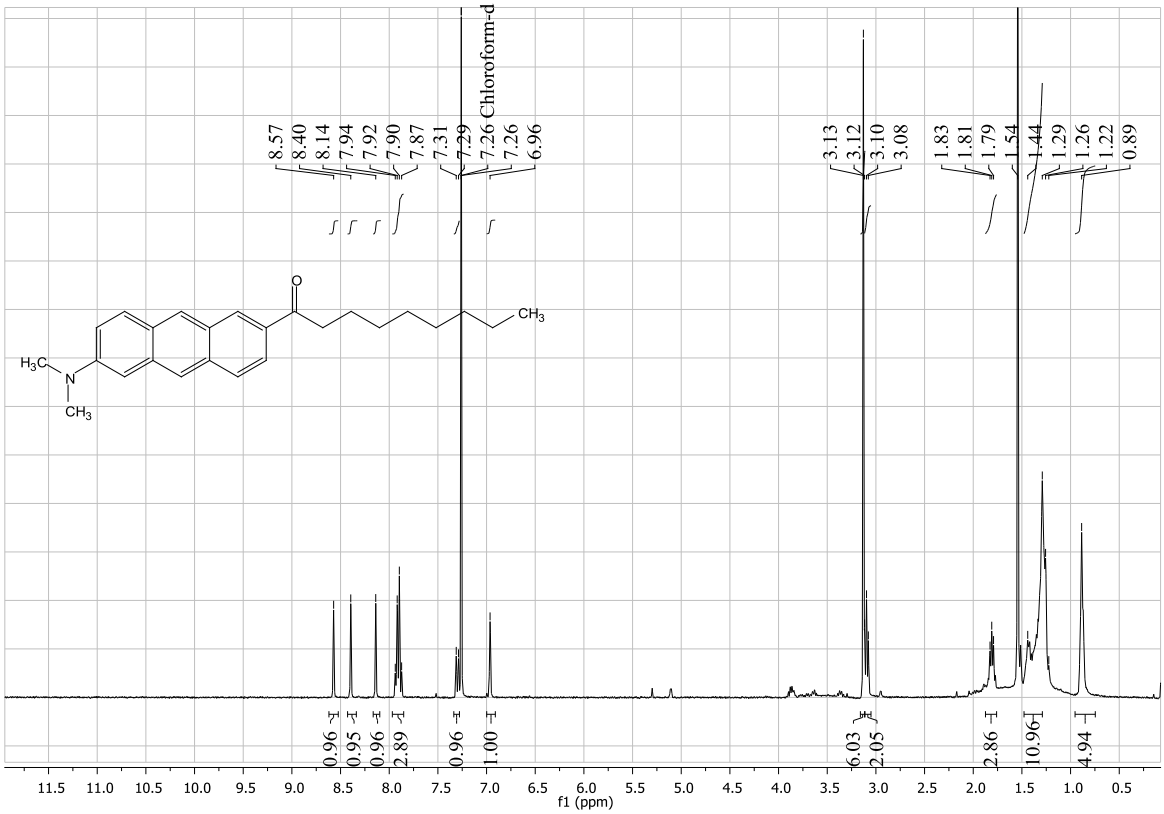

Figure S7 ^{1}H-NMR 1-(6-(Dimethylamino)anthracene-2-yl)nonan-1-one (6).

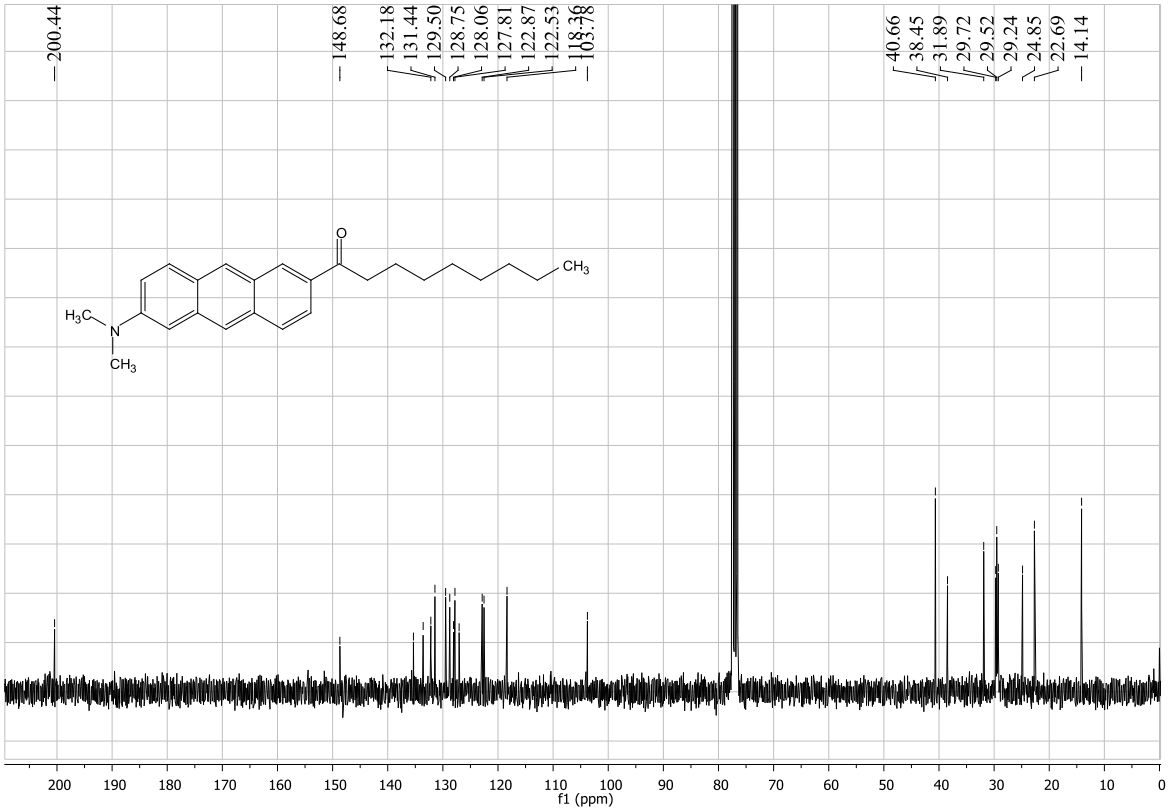

Figure S8 ^{13}C-NMR 1-(6-(Dimethylamino)anthracene-2-yl)nonan-1-one (6).

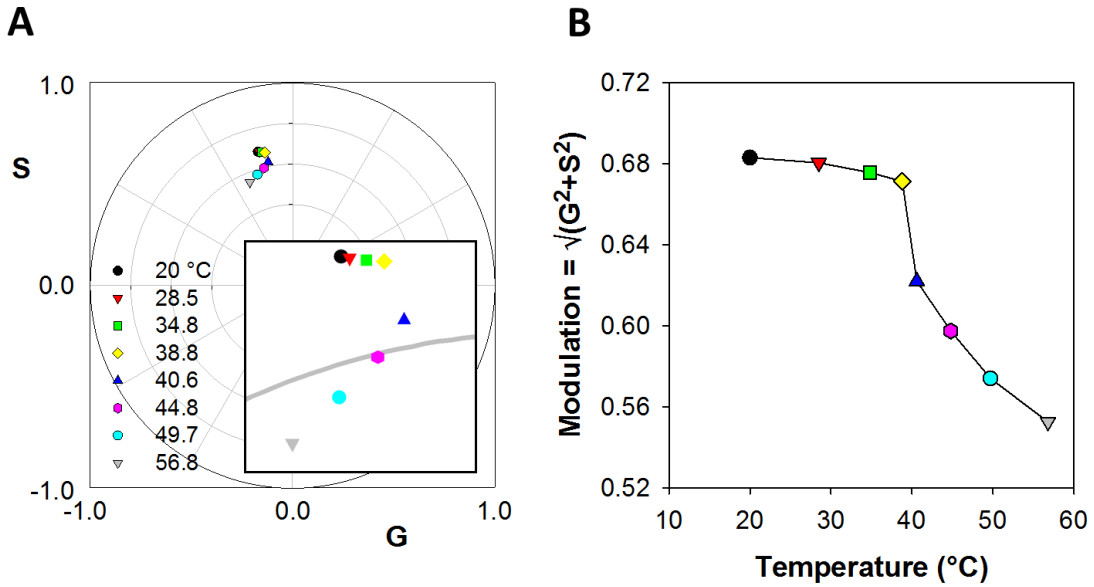

Figure S9 CAPRYDAA emission response to temperature in DPPC SUVs.

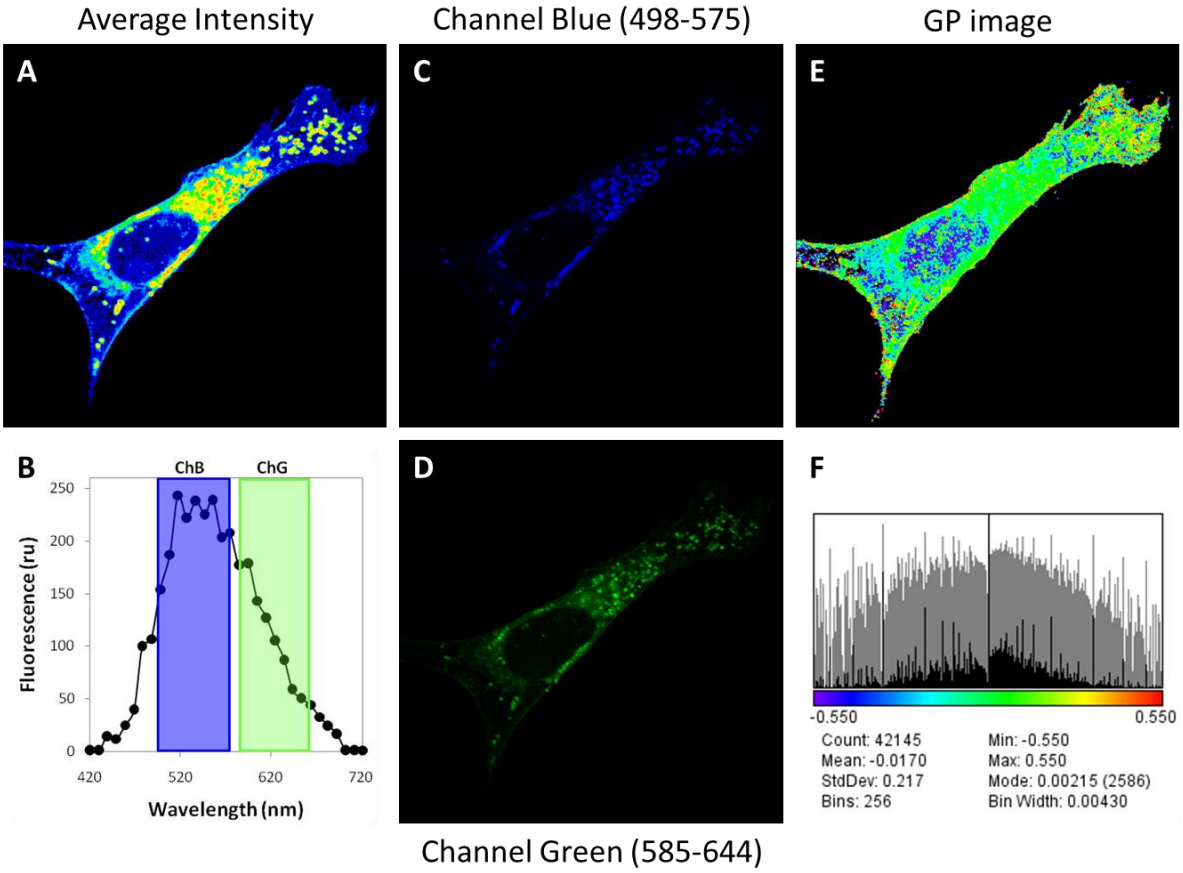

Figure S10: GP analysis of the CAPRYDAA fluorescence in NIH-3T3 cells. A) Intensity image obtained as average of the hysperspectral image in figure 8. B) Average spectra of the figure A where the blue and green channels used for the GP calculation are shown. The intensity on these channels was averaged to obtain the blue and green channels shows in C and D. The GP image (E) was calculated using an ImageJ plugin develop by Bob Dougherty and Jesper Søndergaard Hansen (https://www.optinav.info/Generalized_Polarization_Analysis.htm). F) The GP histogram shows the distribution of GP (in gray log scale was used).

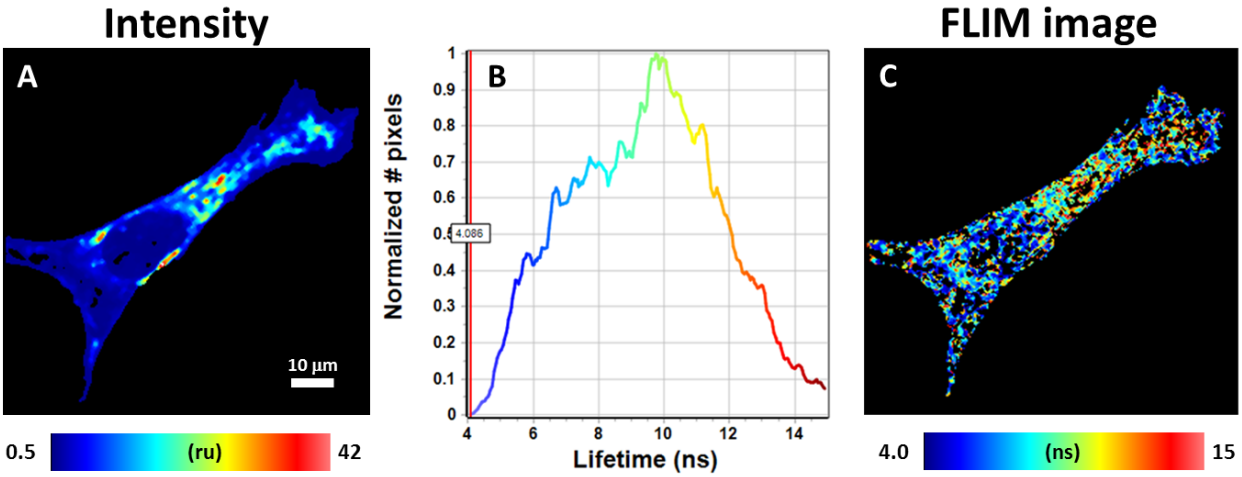

Figure S11: “Classic” FLIM analysis of CAPRYDAA fluorescence in NIH-T3T cells. A) Intensity image of CAPRYDAA fluorescence. B) Histogram of tau modulation. Notice that the color scale in the histogram trace was used for the FLIM image in C.