

Supplementary Information

Improving the Fluorescent Probe Acridonylalanine Through a Combination of Theory and Experiment

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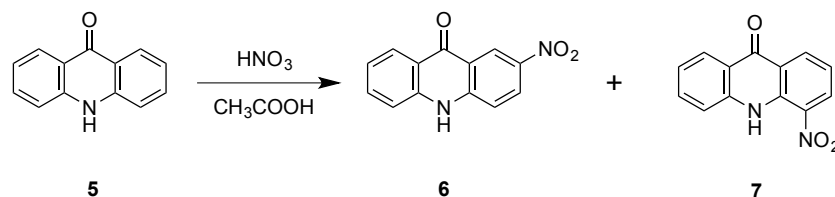
General Information

Materials. L-Tyrosine, thionyl chloride, di-*tert*-butyl dicarbonate (Boc₂O), methyl 2-amino benzoate, and phenylsepharose CL-4B resin were purchased from Sigma-Aldrich (St. Louis, MO, USA). *N*-Phenyl-bis(trifluoromethane sulfonimide) was purchased from Oakwood Chemical (West Columbia, SC, USA). Acridin-9(10*H*)-one, 2-aminoacridin-9(10*H*)-one, 2-methoxyacridin-9(10*H*)-one, and 3-Methyl-2-aminonaphthoic acid were purchased from Sigma-Aldrich. *N*^α-Fmoc-7-methoxycoumarin-4-yl-alanine (Mcm) was purchased from Bachem (Torrence, CA, USA). All other Fmoc protected amino acids and peptide coupling reagents, as well as Rink amide resin, were purchased from EMD Millipore (Billerica, MA, USA). Piperidine was purchased from American Bioanalytical (Natick, MA, USA). Milli-Q filtered (18 MΩ) water was used for all solutions (Millipore; Billerica, MA, USA). All other reagents and solvents were purchased from Fisher Scientific or Sigma-Aldrich unless otherwise specified.

Instruments. Low resolution electrospray ionization mass spectra (ESI-LRMS) were obtained on a Waters Acquity Ultra Performance LC connected to a single quadrupole detector (SQD) mass spectrometer (Milford, MA, USA). Accurate mass measurement analyses were conducted on either a Waters GCT Premier, time-of-flight, GCMS with electron ionization (EI), or an LCT Premier XE, time-of-flight, LCMS with electrospray ionization (ESI). The signals were measured against an internal lock mass reference of perfluorotributylamine (PFTBA) for EI-GCMS, and leucine enkephalin for ESI-LCMS. UV-Vis absorption spectra were acquired on a Hewlett-Packard 8452A diode array spectrophotometer (currently Agilent Technologies; Santa Clara, CA, USA). Fluorescence spectra were acquired on a Photon Technologies International (PTI) QuantaMaster40 fluorometer (currently Horiba Scientific, Edison, NJ, USA). Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker DRX 500 MHz instrument (Billerica, MA, USA). Analytical HPLC was performed on an Agilent 1100 Series HPLC system. Preparative HPLC was performed on a Varian Prostar HPLC system (currently Agilent Technologies). HPLC columns were purchased from W. R. Grace & Company (Columbia, MD, USA). Matrix-assisted laser desorption/ionization mass spectra (MALDI-MS) were collected with a Bruker Ultraflex III MALDI-TOF/TOF mass spectrometer (Billerica, MA). Stopped flow fluorescence measurements were made on a KinTek AutoSF-120 instrument (Snow Shoe, PA, USA).

Chemical Synthesis

2-Nitroacridin-9(10H)-one (**6**) and 4-nitroacridin-9(10H)-one (**7**)^[1]



Acridone (**5**, 50 mg, 0.25 mmol) in 36% acetic acid (250 μ L) was stirred vigorously at 50 $^{\circ}$ C. Then glacial acetic acid (500 μ L) and 70 % nitric acid (250 μ L) were added (Table S1, entry 2). The reaction mixture was stirred for 1 h at 50 $^{\circ}$ C. The mixture was then poured into ice-water (10 mL), neutralized with K₂CO₃, and extracted with EtOAc three times, resulting in a yellow precipitate in the aqueous layer. The yellow solid in the aqueous layer was filtered off, washed thoroughly with water and EtOAc, and dried overnight. Dried solid was then recrystallized in boiling ethanol to give **6** (30 mg, 49% isolated yield). The organic layer was extracted, dried with anhydrous Na₂SO₄, and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography to give **7** (6 mg, 10% isolated yield).

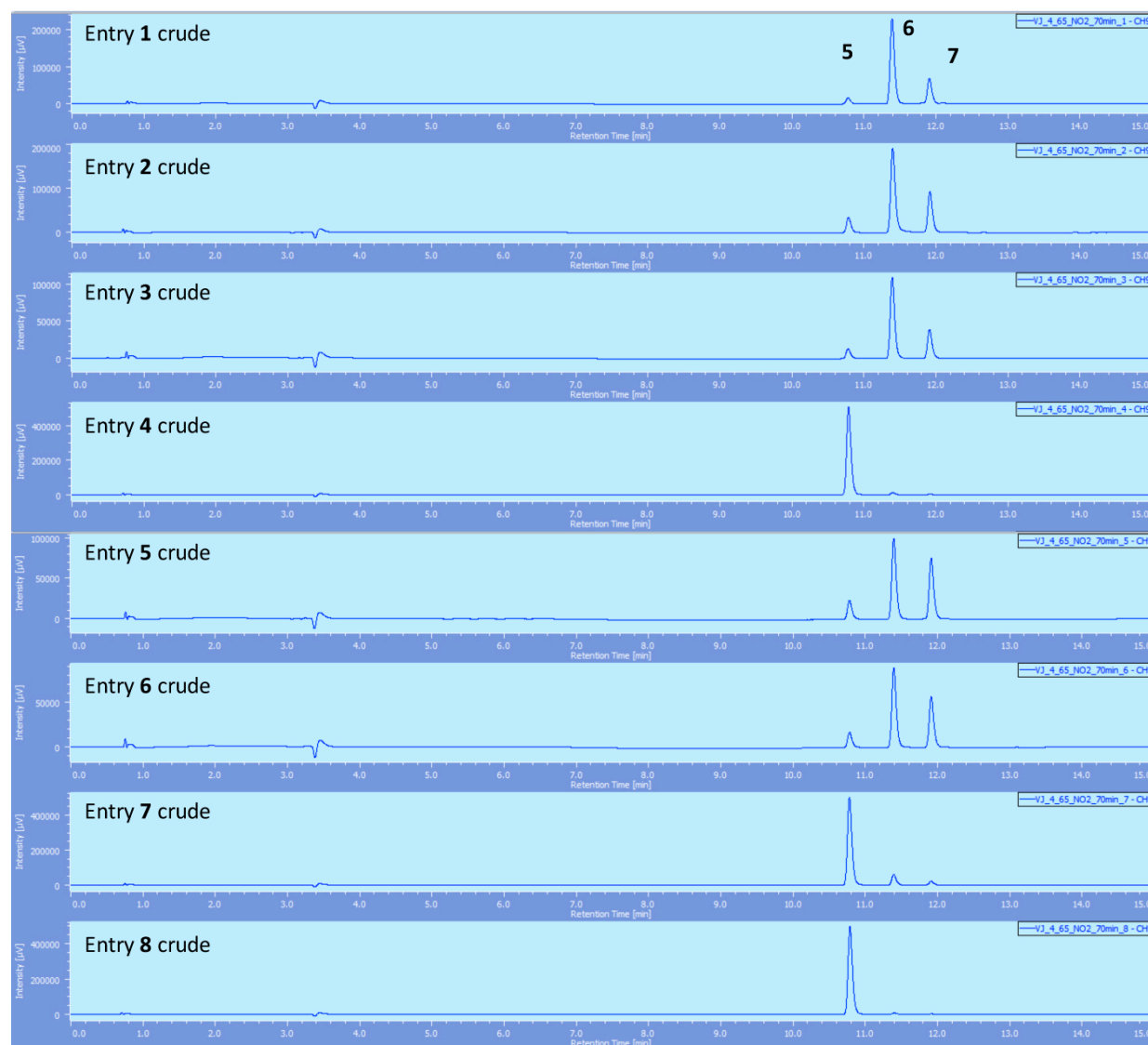
2-Nitroacridin-9(10H)-one (6) HRMS (ESI) calculated for C₁₃H₉N₂O₃⁺ is 241.0613, [M+H]⁺ found 241.0627. ¹H NMR (500 MHz, DMSO-*d*₆) δ_{H} : 12.34 (s, 1H), 8.98 (d, 1H, *J* = 2.6 Hz), 8.42 (dd, 1H, *J* = 9.5, 2.7 Hz), 8.23 (d, 1H, *J* = 8.2 Hz), 7.79 (t, 1H, *J* = 7.5 Hz), 7.60 (dd, 2H, *J* = 25.3, 9.0 Hz), 7.34 (t, 1H, *J* = 7.3 Hz) ¹³C NMR (100 MHz, DMSO-*d*₆) δ_{C} : 177.1, 145.7, 141.8, 141.1, 135.0, 127.8, 126.7, 123.7, 123.4, 121.5, 120.0, 119.8, 119.2

4-Nitroacridin-9(10H)-one (7) HRMS (ESI) calculated for C₁₃H₉N₂O₃⁺ is 241.0613 [M+H]⁺ found 241.0625. ¹H NMR (500 MHz, DMSO-*d*₆) δ_{H} : 11.47 (s, 1H), 8.69 – 8.66 (m, 2H), 8.22 (dd, 1H, *J* = 8.1, 1.5 Hz), 8.07 (dt, 1H, *J* = 8.4, 0.7 Hz), 7.81 (ddd, 1H, *J* = 8.5, 7.0, 1.6 Hz), 7.44 – 7.37 (m, 2H). ¹³C NMR (500 MHz, DMSO-*d*₆) δ_{C} : 176.5, 140.8, 136.0, 135.5, 135.3, 135.1, 132.2, 126.4, 123.9, 123.7, 121.3, 120.6, 119.9

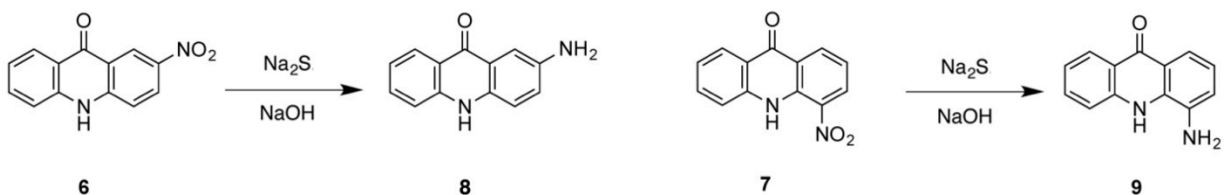
Table S1. Screening of Acridone Nitration Conditions

Entry	AcOH (%)	HNO ₃ (%)	5 (%)	6 (%)	7 (%)
1	75	17.5	5	74	21
2	59	17.5	10	61	29
3	43	17.5	7	68	25
4	27	17.5	98	2	0
5	75	12.5	11	50	38
6	59	12.5	10	55	35
7	43	12.5	87	10	3
8	27	12.5	98	1.5	0.5

Each reaction was performed on 50 mg (0.25 mmol) **5** following the general experimental procedure described above. Yields calculated based on the area of corresponding HPLC chromatogram peak (100% = area of **5** + **6** + **7**).

**Figure S1.** Screening of Acridone Nitration Conditions.

2-Aminoacridin-9(10H)-one (**8**) and 4-aminoacridin-9(10H)-one (**9**)



Reduction of 2-nitroacridone (**6**) to form 2-aminoacridone (**8**) was performed to test conditions for the reduction of nitroacridonylalanine (**S7**) to form aminoacridonylalanine (**4**).

Condition 1 (without NaOH):^[1] To a stirred suspension of 2-nitroacridin-9(10H)-one (**6**, 4.0 mg, 0.016 mmol) in ethanol (1.3 mL), a solution of sodium sulfide nonahydrate (110 mg, 0.46 mmol) in water (2.0 mL) was added. The reaction was refluxed for 20 h. Resulting analytical HPLC data of the crude, showed that there was no reaction.

Condition 2 (with NaOH):^[2] Subsequent reduction to 2-aminoacridin-9(10H)-one (**8**) was tried under alkaline condition (condition 2). To a stirred suspension of 2-nitroacridin-9(10H)-one (**6**), a premixed solution of sodium sulfide nanhydrate (110 mg, 0.46 mmol) and sodium hydroxide (26 mg, 0.65 mmol) in water (2.0 mL) was added. After refluxing the reaction mixture for 20 h, 2-nitroacridone (**6**) was almost completely reduced to 2-aminoacridone (**8**, 84% HPLC yield) with a trace amount of starting material.

4-nitroacridin-9(10H)-one (**7**, 4.0 mg, 0.016 mmol) was also reduced under basic solution (condition 2) to give 4-aminoacridin-9(10H)-one (**9**, 80% HPLC yield). HPLC yields were obtained by using 2-aminoacridone or 4-aminoacridone as a standard.

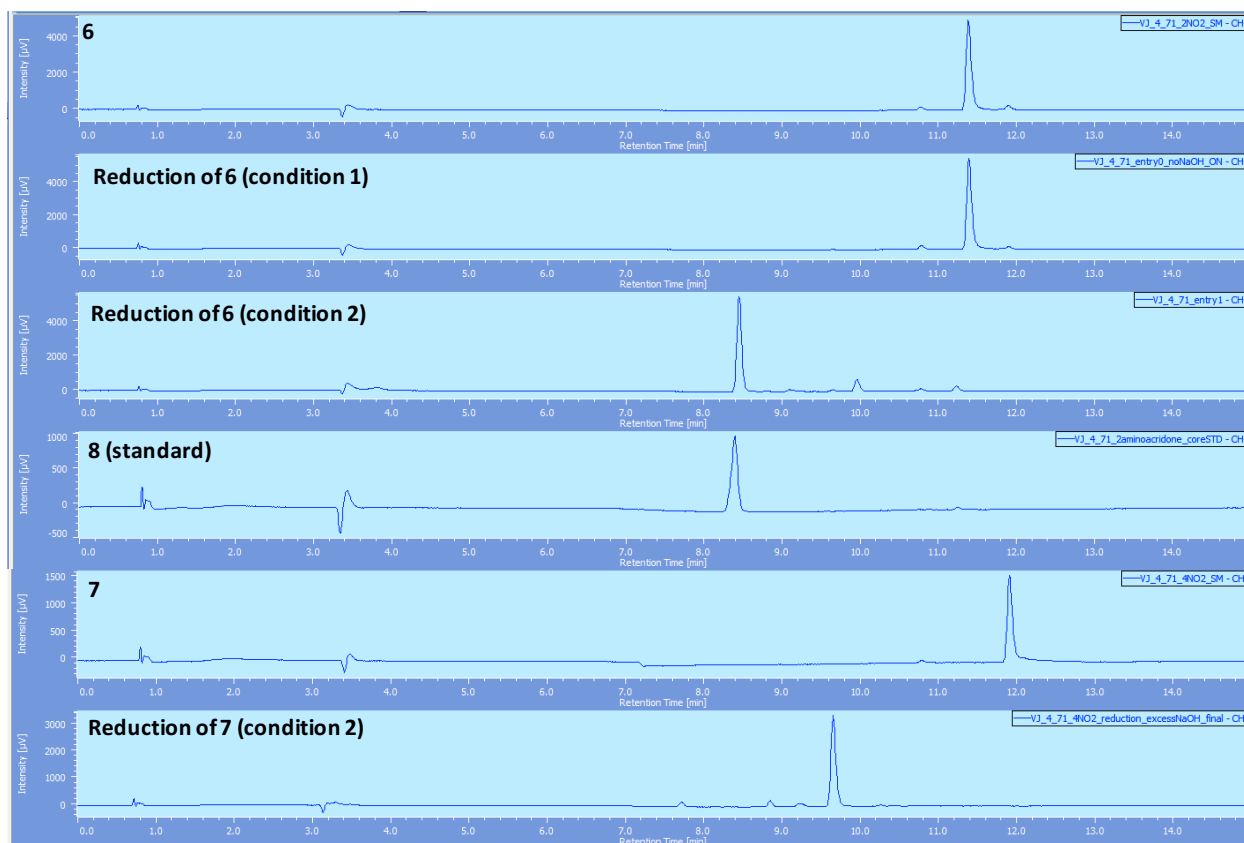
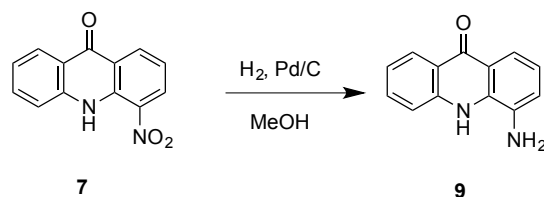


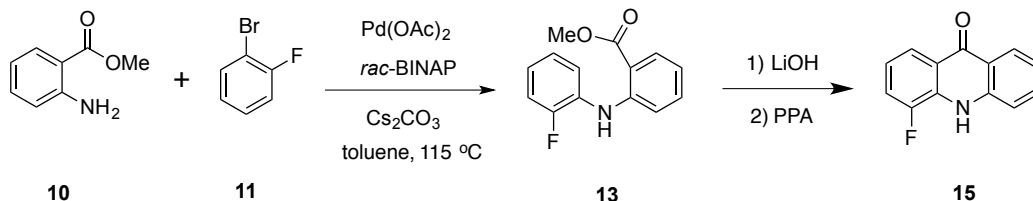
Figure S2. Screening of 2- and 4-Nitroacridone Reduction Conditions.

4-Aminoacridin-9(10H)-one (**9**)



4-Nitroacridin-9(10H)-one (**7**, 0.040 g) was dissolved in 10 mL MeOH and 5 mL CH₂Cl₂. Pd/C (20 mg) was added to the flask and it was capped with rubber septum. The reaction flask was evacuated and back-filled with H₂ gas from a balloon three times. The reaction was stirred at room temperature overnight. Then, the crude reaction was filtered through Celite to remove the Pd/C. The Celite was washed with CH₂Cl₂. The filtrate was concentrated and purified by flash column chromatography. (5:4:1 CH₂Cl₂/EtOAc/MeOH). 4-aminoacridin-9(10H)-one (**9**) was obtained in 29% isolated yield (10 mg). HRMS (ESI) *m/z* calculated for C₁₃H₃₀N₂O⁺ [M+H]⁺ is 211.0871, [M+H]⁺ found 211.0861. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H: 10.69 (1 H, s), 8.16 (1 H, d, *J* 8.1), 7.69 – 7.63 (2 H, m), 7.50 (1 H, p, *J* 3.6), 7.19 (1 H, tt, *J* 6.7, 2.9), 6.98 – 6.93 (2 H, m), 5.56 (2 H, s). ¹³C NMR (126 MHz, DMSO-*d*₆) δ_C: 177.36, 141.03, 137.26, 133.35, 130.04, 126.27, 121.85, 121.80, 121.22, 120.38, 117.95, 116.23, 113.81.

4-Fluoroacridin-9(10H)-one (**15**)



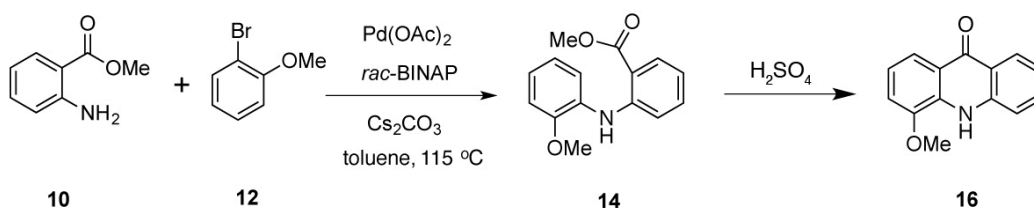
1-Bromo-2-fluorobenzene (**11**, 219 μL, 2.0 mmol) was added to 10 mL degassed toluene in a dried round-bottom flask, followed by methyl 2-aminobenzoate (311 μL, 2.4 mmol). The solution was degassed with Ar for 5 min. Then palladium(II) acetate (0.018 g, 0.08 mmol), and racemic 2,2' - bis(diphenyl-phosphino)-1,1' -binaphthyl (*rac*-BINAP, 0.100 g, 0.16 mmol) were added to the flask. Cesium carbonate (0.912 g, 2.80 mmol) was ground and added to the flask. The flask was then fitted with a reflux condenser and heated to 115 °C for 24 h. After the solution was allowed to cool to ambient temperature, the contents were filtered through a short plug of silica gel using CH₂Cl₂ to transfer the material to the silica (50 mL), and then ethyl acetate (100 mL) was used to elute the product. The clarified solution was then concentrated under reduced pressure and further

purified by flash column chromatography (9:1 Hexane/EtOAc) to afford 0.4211 g (86%) of methyl 2-((2-fluorophenyl)amino)benzoate (**13**).

Methyl 2-((2-fluorophenyl)amino)benzoate (**13**) was dissolved in 4 mL THF in a flask and then a LiOH solution (0.206 g in H₂O 2 mL) was added. The reaction mixture was refluxed at 80 °C for 1 h and then cooled to room temperature. The organic solvent was evaporated and the pH of the aqueous phase was adjusted to 6.0 with 6 M HCl. The precipitate was collected and dried under vacuum overnight giving 2-((2-fluorophenyl)amino)benzoic acid (0.2056 g, 52%).

7.0 g of polyphosphoric acid (PPA) was added to a round-bottom flask with a stir bar and heated to 135 °C in an oil bath. 2-((2-Fluorophenyl)amino)benzoic acid (0.2056 g, 0.89 mmol) was added to the flask and stirred for 16 h. Then, 10 mL water was slowly added and the reaction allowed to cool to 60 °C. After stirring for 1 h at 60 °C, the reaction was cooled to ambient temperature. Insoluble impurities were removed by vacuum filtration and the pH of the clarified solution was adjusted to 6.5 by addition of 8 M NaOH. Then, the solution was cooled to 4 °C for precipitation for about 4 h. The yellow precipitate was collected by vacuum filtration and dried overnight, affording 0.1758 g of 4-fluoroacridin-9(10*H*)-one (**15**, 93%). HRMS (ESI) *m/z* calculated for C₁₃H₈FNO is 213.0590, [M+H]⁺ found 213.0585. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H: 11.64 (1 H, s), 8.20 (1 H, dd, *J* 8.2, 1.5), 8.01 (1 H, dd, *J* 8.2, 1.3), 7.78 (1 H, d, *J* 8.2), 7.72 (1 H, ddd, *J* 8.4, 6.8, 1.6), 7.64 (1 H, ddd, *J* 11.6, 7.9, 1.4), 7.29 – 7.24 (1 H, m), 7.19 (1 H, td, *J* 7.9, 4.8). ¹³C NMR (126 MHz, DMSO-*d*₆) δ_C: 176.49 (d, *J* 2.8), 151.52 (d, *J* 246.4), 141.11, 134.10, 130.71 (d, *J* 13.3), 126.31, 122.85 (d, *J* 1.9), 123.62 – 120.56 (m), 120.97, 120.62 (d, *J* 6.9), 119.92 – 116.55 (m).

4-Methoxyacridin-9(10*H*)-one (**16**)

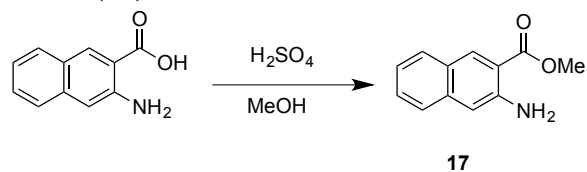


Degassed toluene (10 mL) was added to 2-bromoanisole (**12**, 249 μL, 2.0 mmol) in a dried round-bottom flask, followed by methyl 2-aminobenzoate (311 μL, 2.4 mmol). The solution was degassed with Ar for 5 min. Then, palladium(II) acetate (0.018 g, 0.08 mmol), and *rac*-BINAP (0.100 g, 0.16 mmol) were added to the flask. Cesium carbonate (0.912 g, 2.80 mmol) was ground

and added to the flask. The flask was then fitted with a reflux condenser and heated to 115 °C for 24 h. After the solution was allowed to cool to ambient temperature, the contents were filtered through a short plug of silica gel using CH₂Cl₂ to transfer the material to the silica (50 mL), and then ethyl acetate (100 mL) was used to elute the product. The clarified solution was then concentrated under reduced pressure and further purified by flash column chromatography (9:1 Hexane/EtOAc) afforded methyl 2-((2-methoxyphenyl)amino) benzoate (**14**, 0.400 g, 78%).

A solution of 13.5 M sulfuric acid (5 mL) was added to a flask containing methyl 2-((2-methoxyphenyl)amino) benzoate (**14**, 0.40 g, 1.55 mmol). The flask was then fitted with a reflux condenser and heated to 115 °C for 3 h in an oil bath. 15 mL water was then added to the flask and allowed to stir for 15 min. The reaction was then removed from the hot oil bath and allowed to cool down. The precipitate formed was then collected with a Buchner funnel. The filtrate was extracted with CH₂Cl₂ (3 x 40 mL). The organic phase was dried with MgSO₄ and evaporated. The crude product was then purified by flash column chromatography (99.5:0.5 CH₂Cl₂/EtOH) to afford 0.334 g (96%) of 4-methoxyacridin-9(10*H*)-one (**16**). HRMS (ESI) calculated for C₁₄H₁₁NO₂ is 225.0790, [M+H]⁺ found 225.0808. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H: 11.18 (1 H, s), 8.18 (1 H, dd, *J* 8.1, 1.7), 7.90 (1 H, d, *J* 8.5), 7.77 (1 H, dd, *J* 8.2, 1.3), 7.67 (1 H, ddd, *J* 8.5, 6.8, 1.7), 7.30 (1 H, dd, *J* 7.9, 1.4), 7.22 (1 H, ddq, *J* 8.1, 6.9, 1.1), 7.16 (1 H, td, *J* 8.0, 2.1), 4.01 (3 H, d, *J* 2.1). ¹³C NMR (126 MHz, DMSO-*d*₆) δ_C: 176.96, 148.21, 141.04, 133.47, 132.23, 126.11, 121.63, 121.49, 120.92, 118.68, 117.51, 112.74, 56.62.

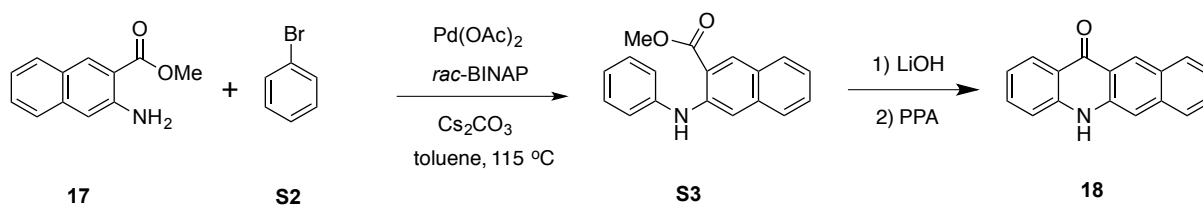
Methyl 3-amino-2-naphthoate (**17**)^[3]



3-Amino-2-naphthoic acid 1.0010 g (5.35 mmol) was added to an oven-dried flask and dissolved in methanol (15 mL). Concentrated H₂SO₄ 5 mL was then gradually added. The flask was connected to a condenser, and the reaction was refluxed at 100 °C overnight. After cooling the reaction, saturated NaCO₃ was added to the crude mixture, and extracted with CH₂Cl₂ (3 x 50mL). The combined organic layer was dried with MgSO₄, filtered, evaporated, and dried under vacuum overnight. Methyl 3-amino-2-naphthoate (**17**) was obtained as a white solid (0.8085 g, 75%). HRMS (ESI) calculated for C₁₂H₁₁NO₂⁺ is 201.0790, [M+H]⁺ found 201.0785. ¹H NMR (500 MHz,

CDCl₃) δ_H: 8.48 (1 H, d, *J* 2.3), 7.72 – 7.66 (1 H, m), 7.53 – 7.46 (1 H, m), 7.38 (1 H, ddt, *J* 8.6, 5.2, 1.7), 7.20 – 7.13 (1 H, m), 6.94 (1 H, d, *J* 2.3), 5.55 (2 H, s), 3.93 (3 H, s). ¹³C NMR (126 MHz, CDCl₃) δ_C: 168.20, 145.91, 140.97, 137.28, 133.36, 129.14, 128.69, 125.97, 125.08, 122.42, 114.67, 109.89, 51.83.

Benzo[*b*]acridin-12(5*H*)-one (18)



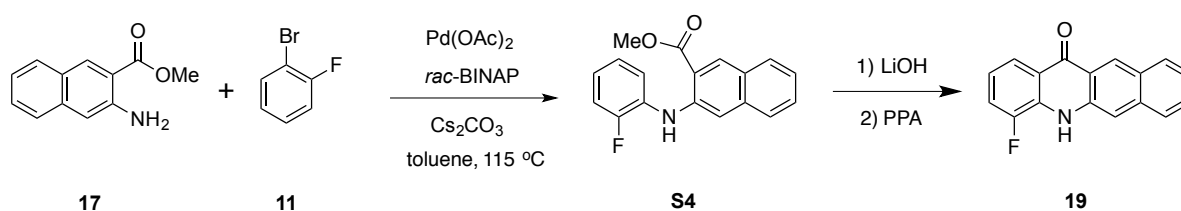
Methyl 3-amino-2-naphthoate (**17**) (0.241 g, 1.20 mmol) was dissolved in 10 mL degassed toluene in a dried sealed tube and bromobenzene (**S2**, 105 μL, 1.0 mmol) was added. Then palladium(II) acetate (0.009 g, 0.04 mmol), and *rac*-BINAP (0.050 g, 0.08 mmol) were added to the tube. Cesium carbonate (0.456 g, 1.40 mmol) was added to the tube. The reaction mixture was capped and heated to 115 °C for 24 h. After the solution was allowed to cool to ambient temperature, the contents were filtered through a short plug of silica gel using CH₂Cl₂ to transfer the material to the silica and then ethyl acetate (100 mL) was used to elute the product. The clarified solution was then concentrated under reduced pressure and further purified by flash column chromatography (9:1 Hexane/EtOAc) to afford 0.2311 g (83%) of methyl 3-(phenylamino)-2-naphthoate (**S3**).

Methyl 3-(phenylamino)-2-naphthoate was dissolved in THF (4 mL) and MeOH (2 mL) in a 25 mL flask and then a LiOH solution (0.0527 g in 2 mL H₂O) was added. The reaction mixture was refluxed at 90 °C for 1 h and then cooled to room temperature. The organic solvent was evaporated, and the pH of the aqueous phase was adjusted to 6.0 with 6 M HCl. The precipitate was collected and dried under vacuum overnight, giving 3-(phenylamino)-2-naphthoic acid (0.1131 g, 98%).

6.56 g PPA was added to a 25 mL round-bottom flask with a stir bar. The flask was heated to 125 °C in an oil bath. Then 3-(phenylamino)-2-naphthoic acid (0.1131 g, 0.43 mmol) was added to the flask and it was stirred for 2 h. Water (10 mL) was slowly added and the reaction allowed to cool to 60 °C. After stirring for 1 h, the reaction was cooled to ambient temperature. Insoluble impurities were removed by vacuum filtration and the pH of the clarified solution was adjusted to 7.0 by addition of 10 M NaOH. The yellow precipitate was collected by vacuum filtration and

dried overnight affording 0.090 g of benzo[*b*]acridin-12(5*H*)-one (**18**, 85%). HRMS (ESI) calculated for C₁₇H₁₁NO⁺ is 245.0841, [M+H]⁺ found 245.0863. ¹H NMR (500 MHz, Methanol-*d*₄) δ_H: 8.97 (1 H, s), 8.34 (1 H, dd, *J* 8.1, 1.5), 8.05 (1 H, d, *J* 8.5), 7.91 – 7.88 (2 H, m), 7.72 (1 H, ddd, *J* 8.6, 7.0, 1.6), 7.55 (1 H, ddd, *J* 8.3, 6.5, 1.2), 7.48 (1 H, d, *J* 8.3), 7.40 (1 H, ddd, *J* 8.0, 6.6, 1.2), 7.22 (1 H, ddd, *J* 8.1, 6.9, 1.1). ¹³C NMR (126 MHz, DMSO-*d*₆) δ_C: 178.20, 142.18, 138.90, 135.86, 134.21, 129.77, 128.53, 127.85, 127.37, 126.72, 126.56, 124.84, 121.42, 120.28, 118.4, 117.2, 112.5.

4-Fluorobenzo[*b*]acridin-12(5*H*)-one (**19**)



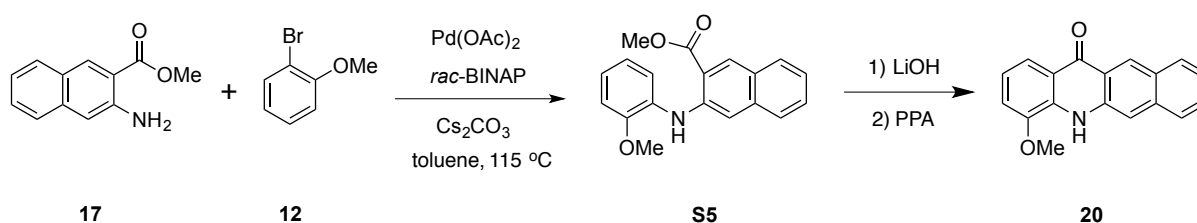
Methyl 3-amino-2-naphthoate (**17**) (0.241 g, 1.20 mmol) was dissolved in 10 mL degassed toluene in a dried sealed tube, followed by addition of 1-bromo-2-fluorobenzene (**11**) (109 μ L, 1.0 mmol). Then palladium(II) acetate (0.009 g, 0.04 mmol), and *rac*-BINAP (0.050 g, 0.08 mmol) were added to the tube. Cesium carbonate (0.456 g, 1.40 mmol) was added to the tube. The reaction mixture was capped and heated to 115 $^\circ$ C for 24 h. After the solution was allowed to cool to ambient temperature, the contents were filtered through a short plug of silica gel using CH₂Cl₂ to transfer the material to the silica and then ethyl acetate (100 mL) was used to elute the product. The clarified solution was then concentrated under reduced pressure and further purified by flash column chromatography (9:1 Hexane/EtOAc) to afford 0.2648 g (90%) of 3-((2-fluorophenyl)amino)-2-naphthoate (**S4**).

Methyl 3-(phenylamino)-2-naphthoate was dissolved in THF (4 mL) and MeOH (2 mL) in a 25 mL flask and then a LiOH solution was added (0.0527 g in 2 mL H₂O). The reaction mixture was refluxed at 90 $^\circ$ C for 1 h and then cooled to room temperature. The organic solvent was evaporated and the pH of the aqueous phase was adjusted to 6.0 with 6 M HCl. The precipitate was collected and dried under vacuum overnight giving 3-(phenylamino)-2-naphthoic acid 0.1131 g (98%).

PPA (6.56 g) was added to a 25 mL round-bottom flask with a stir bar. The flask was heated to 125 $^\circ$ C in an oil bath. 3-(phenylamino)-2-naphthoic acid 0.1131 g (0.43 mmol) was

added to the flask and stirred for 2 h. Then, 10 mL water was slowly added and the reaction allowed to cool to 60 °C. After stirring for 1 h, the reaction was cooled to ambient temperature. Insoluble impurities were removed by vacuum filtration and the pH of the clarified solution was adjusted to 7.0 by addition of 10 M NaOH. The yellow precipitate was collected by vacuum filtration and dried overnight affording 0.090 g of benzo[*b*]acridin-12(5*H*)-one (**19**, 85 %). HRMS (EI) calculated for C₁₇H₁₀FNO is 263.0746, found 263.0753. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H: 11.52 (1 H, s), 8.91 (1 H, s), 8.18 (1 H, s), 8.14 (1 H, d, *J* 8.3), 8.04 (1 H, d, *J* 8.1), 7.93 (1 H, d, *J* 8.4), 7.66 (2 H, dd, *J* 11.6, 7.7), 7.58 (1 H, dd, *J* 8.5, 6.6), 7.43 (1 H, q, *J* 7.4, 6.2), 7.15 (1 H, td, *J* 7.9, 4.8). ¹³C NMR (126 MHz, DMSO-*d*₆) δ_C: 177.84, 152.18, 150.23, 137.86, 136.21, 131.77, 130.05, 129.05, 128.37, 127.72, 126.96, 124.84, 122.42, 121.48, 119.73, 118.94, 113.46.

4-Methoxybenzo[*b*]acridin-12(5*H*)-one (**20**)



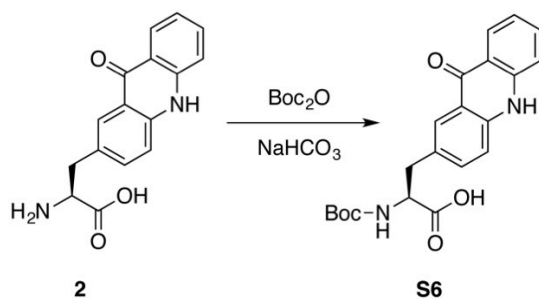
Methyl 3-amino-2-naphthoate (**17**, 0.241 g, 1.20 mmol) was dissolved in 10 mL degassed toluene in a dried sealed tube, followed by adding 2-bromoanisole (**12**, 125 μ L, 1.0 mmol). Then palladium(II) acetate (0.009 g, 0.04 mmol), and *rac*-BINAP (0.050 g, 0.08 mmol) were added to the tube. Cesium carbonate (0.456 g, 1.40 mmol) was added to the tube. The reaction mixture was capped and heated to 115 °C for 24 h. After the solution was allowed to cool to ambient temperature, the contents were filtered through a short plug of silica gel using CH₂Cl₂ to transfer the material to the silica and then ethyl acetate (100 mL) was used to elute the product. The clarified solution was then concentrated under reduced pressure and further purified by flash column chromatography (90:10 Hexane/EtOAc) to afford 0.2094 g (68%) of methyl 3-((2-methoxyphenyl)amino)-2-naphthoate (**S5**).

Methyl 3-((2-methoxyphenyl)amino)-2-naphthoate (**S5**, 0.1943 g, 0.632 mmol) was dissolved in THF (5 mL) and MeOH (3 mL) in a 25 mL flask and then a LiOH solution was added (0.0757 g in 3 mL H₂O). The reaction mixture was refluxed at 90 °C for 1 h and then cooled to room temperature. The organic solvent was evaporated and the pH of the aqueous phase was

adjusted to 6.0 with 6 M HCl. The precipitate was collected and dried under vacuum overnight giving 3-((2-methoxyphenyl)amino)-2-naphthoic acid 0.1623 g (98%).

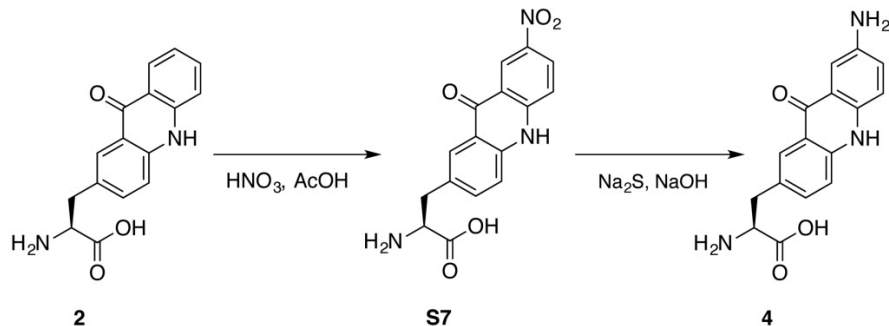
PPA (6.90 g) was added to a 25 mL round-bottom flask with a stir bar. The round-bottom flask was heated to 125 °C in an oil bath. 3-((2-methoxyphenyl)amino)-2-naphthoic acid 0.1513 g (0.516 mmol) was added to the flask and stirred for 2 h. Then 10 mL water was slowly added and the reaction allowed to cool to 60 °C. After stirring for 1 h at 60 °C, the reaction was cooled to ambient temperature. Insoluble impurities were removed by vacuum filtration and the pH of the clarified solution was adjusted to 7.0 by addition of 10 M NaOH. The yellow precipitate was collected by vacuum filtration and dried overnight affording 0.1261 g of 4-methoxybenzo[b]acridin-12(5*H*)-one (**20**, 89%). HRMS (EI) calculated for C₁₈H₁₃NO₂ is 275.0946, found 275.0948. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H: 11.06 (1 H, s), 8.88 (1 H, s), 8.32 (1 H, s), 8.12 (1 H, d, *J* 8.3), 7.89 (1 H, d, *J* 8.4), 7.80 (1 H, d, *J* 8.0), 7.58 – 7.51 (1 H, m), 7.43 – 7.36 (1 H, m), 7.34 – 7.28 (1 H, m), 7.12 (1 H, t, *J* 7.9), 4.03 (3 H, s). ¹³C NMR (126 MHz, DMSO-*d*₆) δ_C: 178.33, 147.85, 138.00, 135.99, 133.39, 129.99, 128.73, 128.18, 127.30, 126.96, 124.56, 121.58, 120.05, 119.82, 117.84, 113.50, 56.64.

(*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(9-oxo-9,10-dihydroacridin-2-yl)propanoic acid (S6**)**



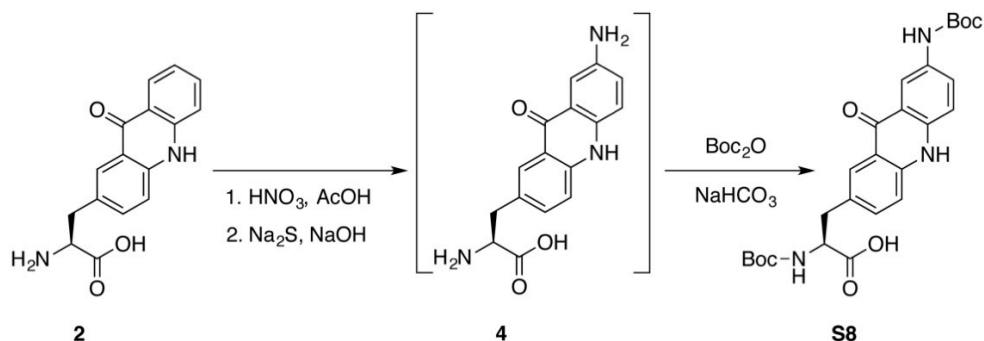
(*S*)-2-amino-3-(9-oxo-9,10-dihydroacridin-2-yl) propanoic acid (Acid, **2**, 0.050 g, 0.18 mmol), synthesized as previously described, was dissolved in EtOH (15 mL).^[4] Na₂CO₃ (0.029 g, 0.27 mmol) and di-*tert*-butyl dicarbonate (Boc₂O, 0.059 g, 0.27 mmol) was added to the flask. The reaction was stirred at room temperature for 16 h. The reaction mixture was evaporated and quenched with 0.1 M HCl. The crude product was extracted with CH₂Cl₂ three times. The combined organic layers were washed with saturated NaCl solution, dried with MgSO₄, and evaporated *in vacuo*. (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(9-oxo-9,10-dihydroacridin-2-yl) propanoic acid, Boc-Acid, **S6**) was obtained (0.064 g, 93%).

(S)-2-amino-3-(7-amino-9-oxo-9,10-dihydroacridin-2-yl)propanoic acid (4)



To 180 mg (0.64 mmol) of Acid (**2**) in a 40 mL scintillation vial, glacial acetic acid (3 mL) and 70% nitric acid (1 mL) were added. The reaction mixture was stirred for 2 h at 50 °C. The mixture was cooled to room temperature and cold brine (20 mL) was added to precipitate (S)-2-amino-3-(7-nitro-9-oxo-9,10-dihydroacridin-2-yl)propanoic acid (**S7**). The crude yellow precipitate (81 mg, 39% isolated yield; >90% conversion based on integration of the HPLC chromatogram, but isolation of pure material was limiting) was reduced without further purification. 81 mg **S7** (0.247 mmol, 1 equiv) was dissolved in 2 mL ethanol and 1 mL water in a 20 mL scintillation vial. In a separate vial, NaOH (333 mg, 8.3 mmol, 33 equiv) and sodium sulfide nonahydrate (500 mg, 2.08 mmol, 8.4 equiv) were mixed in 2 mL of water. The premixed solution of NaOH and sodium sulfide was added to the reaction vial and stirred overnight at 80 °C. After the reaction was complete, the crude solution was purified by ion-exchanged resin and lyophilized to give (S)-2-amino-3-(7-amino-9-oxo-9,10-dihydroacridin-2-yl)propanoic acid (Aad, **4**) (63 mg, 86 % yield). HRMS (ESI) calculated for C₁₆H₁₆N₃O₃⁺ is 298.1192, found 298.1191. ¹H NMR (500 MHz, D₂O) δ_H: 7.31 (1 H, d, *J* 2.1), 6.99 (1 H, dd, *J* 8.5, 2.0), 6.84 – 6.75 (2 H, m), 6.61 (2 H, dd, *J* 19.7, 8.6), 4.72 (1 H, d, *J* 1.1), 3.65 (1 H, dd, *J* 7.6, 5.6), 2.84 (1 H, dd, *J* 14.5, 5.6), 2.72 (1 H, dd, *J* 14.5, 7.6). ¹³C NMR (126 MHz, D₂O) δ_C: 176.34, 160.89, 140.29, 137.79, 133.85, 133.49, 128.21, 125.25, 124.46, 119.41, 117.96, 117.55, 117.43, 107.71, 56.19, 37.25.

(S)-2-((tert-butoxycarbonyl)amino)-3-(7-((tert-butoxycarbonyl)amino)-9-oxo-9,10-dihydroacridin-2-yl)propanoic acid (Boc₂-Aad, S8)



To 90 mg (0.32 mmol, 1 equiv) Acid (**2**) in a 40 mL scintillation vial, glacial acetic acid (1.5 mL) and 70% nitric acid (0.5 mL) were added. The reaction mixture was stirred for 2 h at 50 °C. The mixture was cooled down room temperature and cold water (5 mL) was added to quench the nitration reaction. For reduction, ethanol (5 mL) was added to the crude material followed by addition of premixed NaOH (512 mg, 12.8 mmol, 40 equiv) and sodium sulfide nonahydrate (769 mg, 3.2 mmol, 10 equiv) in water (2 mL). The reaction vial was stirred overnight at 80 °C. After the reaction was judged complete by chromatography analysis, the crude solution was cooled to room temperature and quenched by adding saturated NaHCO₃ (5 mL). The crude mixture was transferred to a 100 mL round bottom flask. NaHCO₃ (900 mg) was added to the reaction mixture and it was stirred at room temperature. Once the pH of the reaction mixture reached pH 8-9, Boc₂O (2.1 g, 9.6 mmol, 30 equiv) was added, and the reaction stirred at room temperature overnight. The mixture was then slightly acidified with a saturated NH₄Cl solution. The aqueous mixture was extracted with ethyl acetate three times, then the organic layer was collected and dried with Na₂SO₄. The organic layer was concentrated *in vacuo* and purified via silica column chromatography (0%-20% MeOH in CH₂Cl₂) to remove excess *t*-butanol. The product was obtained and lyophilized to give Boc₂-Aad (**S8**, 79 mg, 50% yield over three steps). HRMS (ESI) calculated for C₂₆H₃₂N₃O₇⁺ is 498.2240, found 498.2230. ¹H NMR (500 MHz, MeOD) δ_H: 8.28 (1 H, s), 8.16 (1 H, s), 7.83 (1 H, d, *J* 8.4 Hz), 7.61 (1 H, d, *J* 9.1 Hz), 7.45-7.41 (2 H, m), 4.39 (1 H, br. s), 3.28 (1 H, m), 3.04 (1 H, br. s), 1.55 (9 H, s), 1.33 (9 H, s), 1.40 (*t*BuOH residual peak), 3.35 (MeOH solvent residual peak) ¹³C NMR (126 MHz, MeOD) δ_C: 179.94, 158.19, 155.92, 141.52, 138.92, 136.86, 135.30, 132.84, 128.37, 122.33, 127.87, 122.33, 121.49, 119.38, 118.82, 115.67, 80.91, 58.07, 39.33, 29.25 (31.22 residual *t*BuOH). See **Figure S3**.

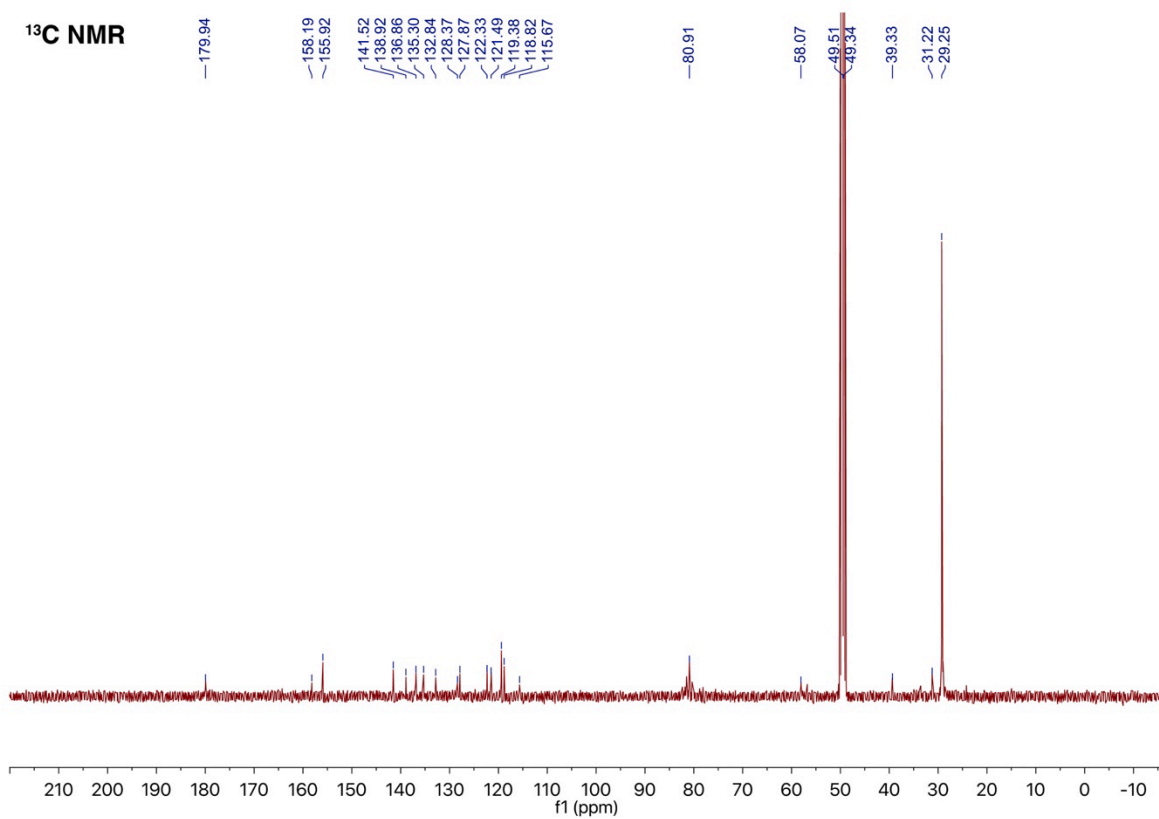
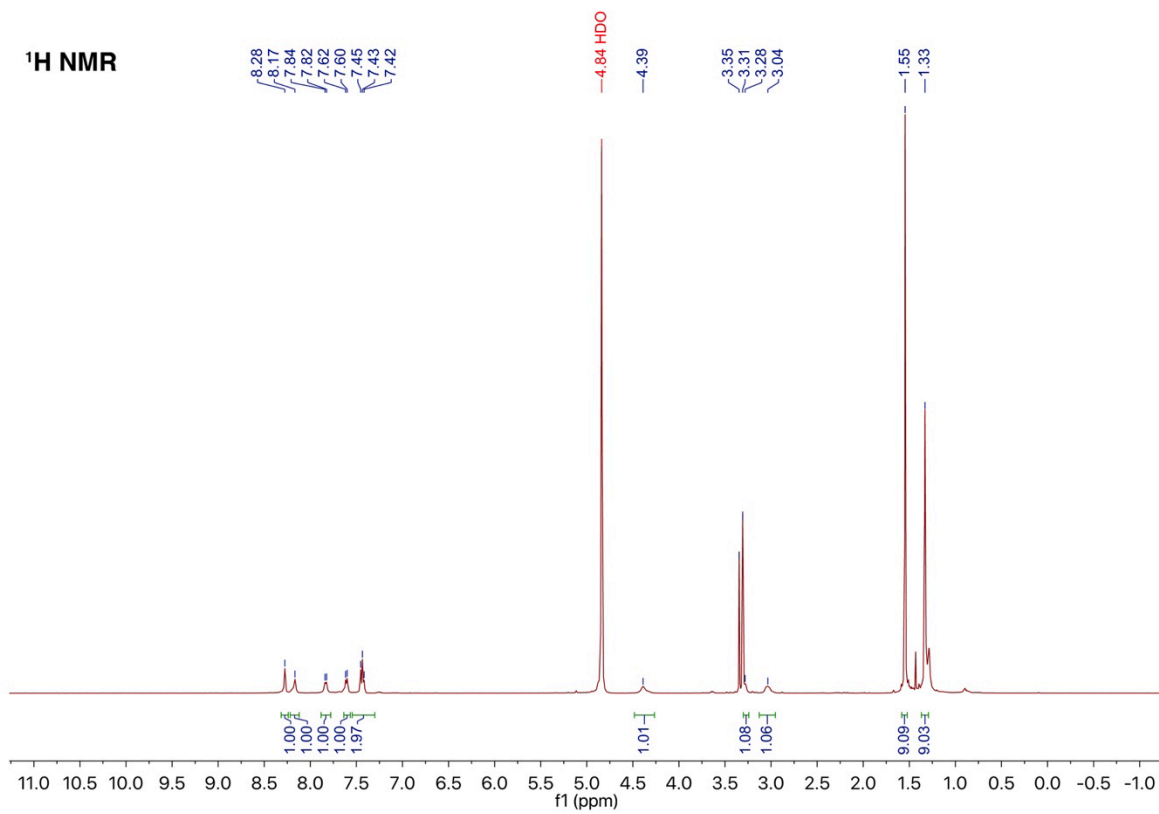


Figure 3. Boc₂-Aad (**S8**) ¹H and ¹³C NMR Spectra in MeOD.

Spectroscopic Characterization of Substituted Acridones

Prior to spectroscopic characterization, stocks of all acridone and benzoacridone compounds were prepared in acetonitrile at a concentration of 200 or 300 μM . All absorbance and fluorescence measurements were taken in 1:1 CH_3CN /buffer. Buffers include citrate buffer (89.1 mM citric acid 21.8 mM Na_2HPO_4) pH 2.6, phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 , 2 mM KH_2PO_4) pH 7.4, and 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer, pH 10.0. The absorbance and emission spectra of all compounds were acquired in 1:1 CH_3CN /PBS. The sensitivity of the absorbance and emission to pH was assessed for the parent acridone core as well as the 4- NH_2 and 2- NH_2 derivatives in 1:1 CH_3CN /citrate and 1:1 CH_3CN /CAPS. Absorbance measurements were acquired at concentrations of 75 and 7.5 μM for proper visualization of the spectral profile above and below ~ 300 nm (4-aminoacridone absorbance measurements were taken at 7.5 and 0.75 μM).

Fluorescence measurements were acquired under identical solution conditions at concentrations of 7.5 μM for all compounds, except for the 4- NH_2 , 2- NO_2 , and 4- NO_2 derivatives of Acridone which were obtained at 75 μM due to a lack of brightness. Spectra were collected using excitation wavelengths matching the maximum absorbance wavelength for each analog measured. The collection window started 15 nm from the excitation wavelength and extended to 650 nm. This window was truncated to no less than a total range of 150 nm for more blue shifted chromophores. All slit widths were set to 1.5 nm and spectra were acquired with an integration time of 0.25 sec/nm. Higher signal-to-noise spectra for the 4- NH_2 , 2- NO_2 , and 4- NO_2 derivatives were collected keeping all other settings the same but adjusting all slit widths to 3 nm.

Extinction coefficients were calculated from absorbance measurements collected on a Tecan M1000 plate reader (Mannedorf, Switzerland). Samples containing 100, 80, 60, 40 and 20 μM chromophore were prepared in 1:1 CH_3CN /PBS solution with a total sample volume of 100 μL . Following brief vortexing, samples were loaded into a Corning CoStar black, clear bottom, 96-well plate.

Absorbance and emission spectra are reported in **Figures S4-S9**. Emission spectra are shown normalized to the acridone emission at 412 nm to approximate quantum yields. The most prominent peak positions are reported along with extinction coefficients and these relative emission intensities in **Table S2**. Calculated spectra (described below) are shown for comparison.

Table S2. Calculated and Observed Photophysical Parameters of Acridone Derivatives.

Compound	Calculated		Observed	
	$\lambda_{\text{ex}}/\epsilon^{\text{a}}$	$\lambda_{\text{em}}/\text{Int.}^{\text{b}}$	$\lambda_{\text{ex}}/\epsilon^{\text{a}}$	$\lambda_{\text{em}}/\text{Int.}^{\text{b}}$
Acid 5	253/11.4	411/1.00	254/5.22	412/1.00
	364/1.51	434/0.45	382/0.72	435/0.83
	383/2.43		398/0.70	
2-NO ₂ 6	235/5.32	-	230/3.33	-
	282/2.53		296/0.87	
	341/1.03		356/0.76	
	408/1.87		402/0.37	
4-NO ₂ 7	243/6.62	-	244/2.81	-
	292/2.56		266/0.94	
	331/1.27		328/0.58	
	464/2.06		434/0.60	
2-NH ₂ 8	252/4.93	509/0.21	256/3.38	527/0.16
	278/2.96		278/2.46	
	419/0.47		420/0.52	
4-NH ₂ 9	252/5.74	492/0.59	260/4.19	540/0.01
	299/1.20		314/0.38	
	400/0.61		400/0.59	
4-F 15	248/12.8	397/0.48	252/4.44	412/0.65
	365/1.19		378/0.51	
2-OMe S1	251/10.0	444/0.47	252/5.32	447/0.63
	271/3.61		268/5.38	
	400/0.95		396/0.82	
4-OMe 16	249/6.62	421/0.35	256/6.30	431/0.37
	375/0.62		384/0.69	
Bz 18	234/1.38	495/0.31	222/1.78	507/0.42
	269/21.7		272/12.7	
	302/1.40		296/2.07	
	446/0.81		438/0.58	
4-F-Bz 19	268/15.3	496/0.24	268/9.96	508/0.21
	446/0.61		448/0.54	
4-OMe-Bz 20	272/14.0	512/0.25	272/9.42	512/0.14
	456/0.67		454/0.61	

^aExtinction coefficients (ϵ) reported as $10^4 \text{ M}^{-1} \text{ cm}^{-1}$.

^bEmission intensity (Int.) normalized the intensity of the highest emission peak of acridone for both calculated and observed spectra.

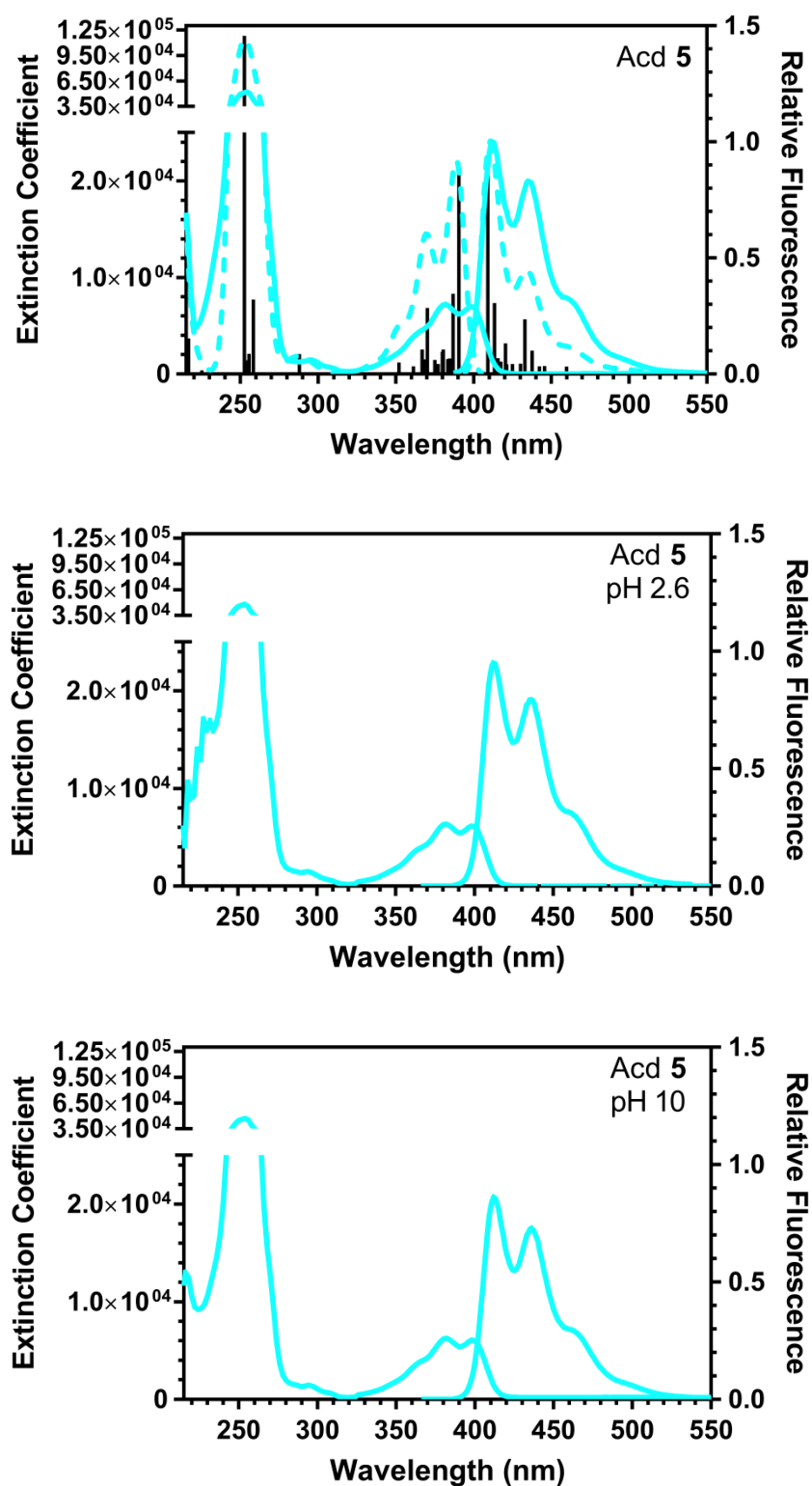


Figure S4. Acridone (5) Spectra. Experimental (solid lines) and calculated (dashed lines) spectra and with vibronic transitions to/from lowest excited state (black bars) of 5 at pH 7.4, and experimental spectra at pH 2.6 and 10.0.

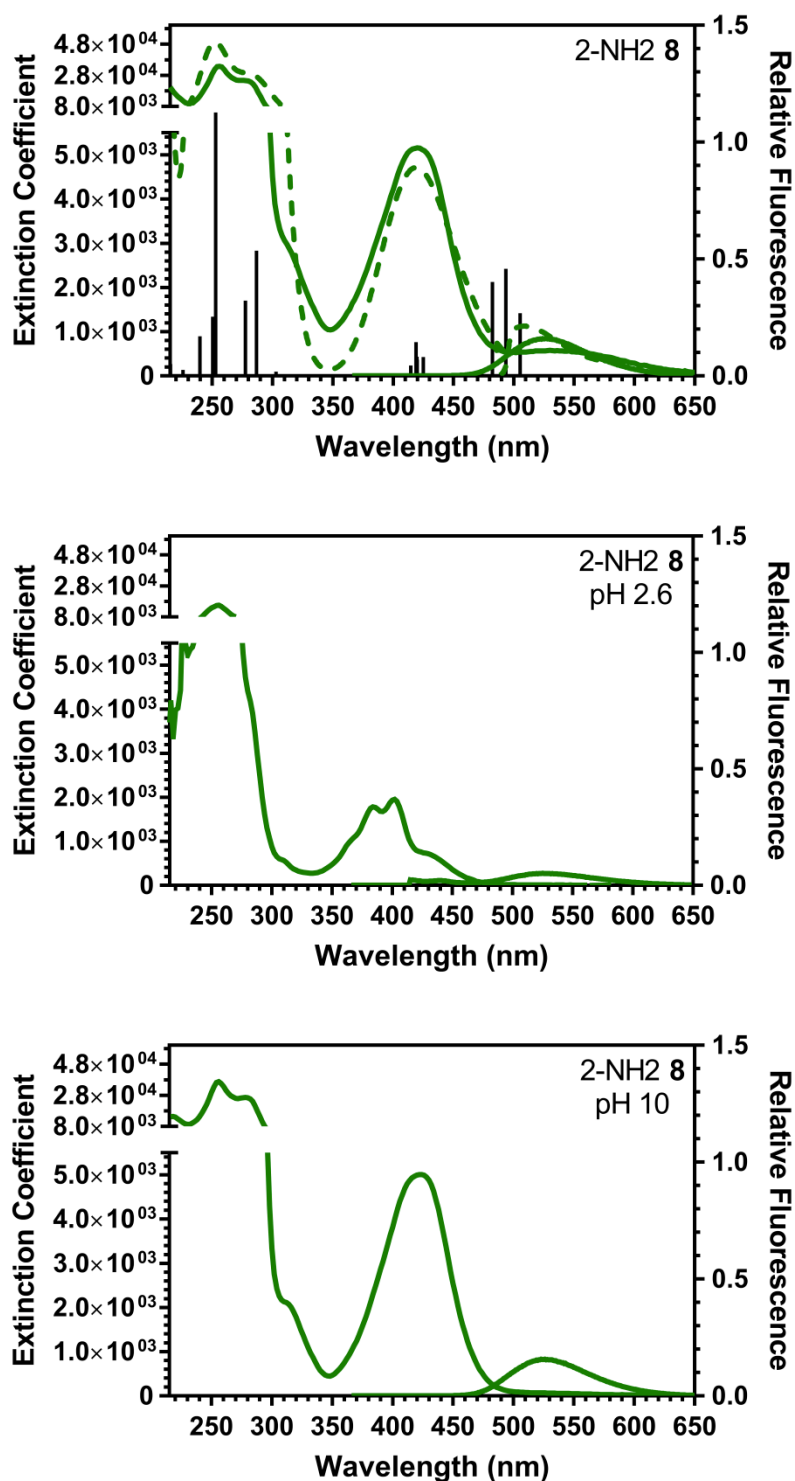


Figure S5. 2-Aminoacridone (**8**) Spectra. Experimental (solid lines) and calculated (dashed lines) spectra and with vibronic transitions to/from lowest excited state (black bars) of **8** at pH 7.4, and experimental spectra at pH 2.6 and 10.0.

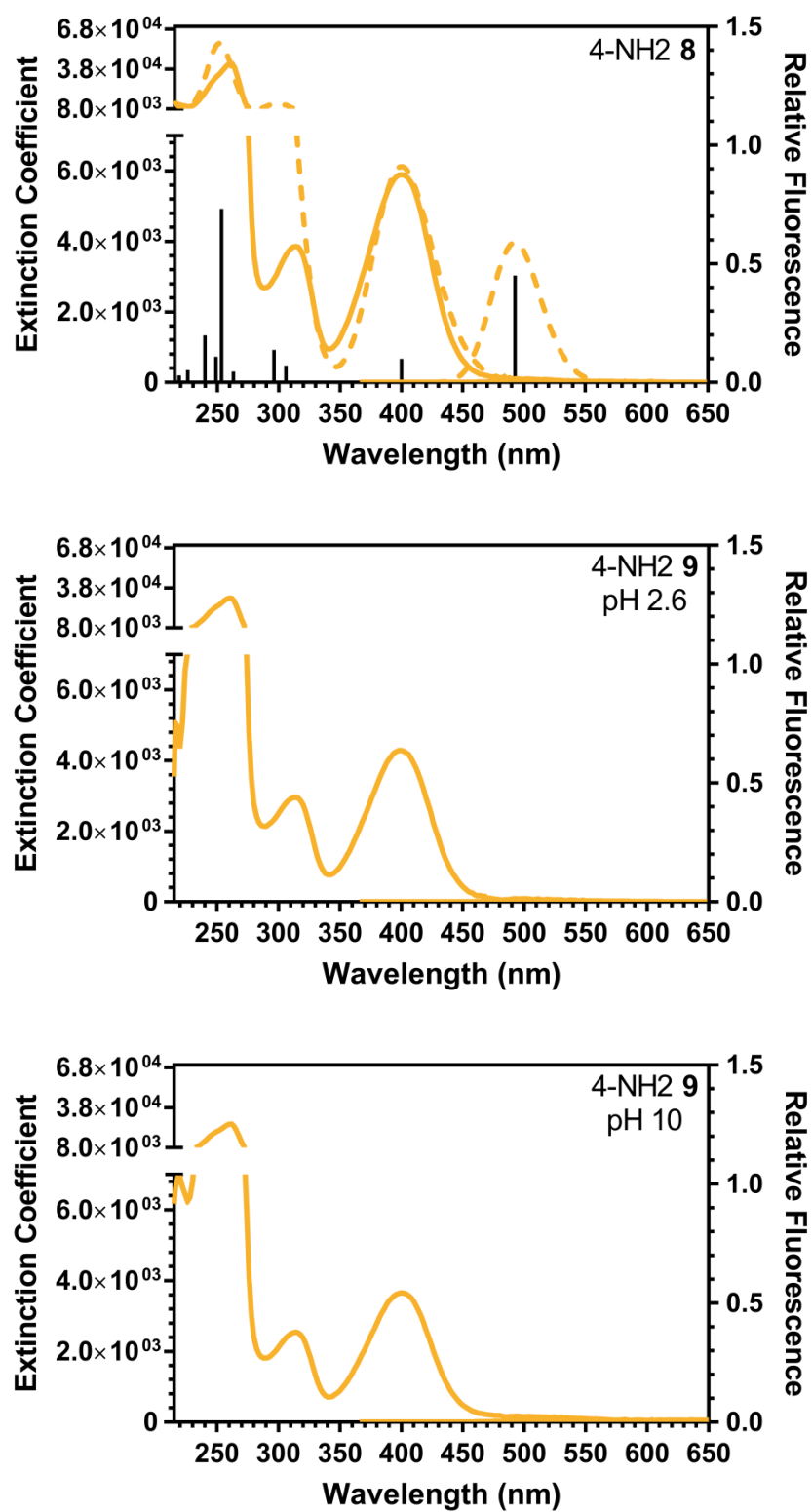


Figure S6. 4-Aminoacridone (**9**) Spectra. Experimental (solid lines) and calculated (dashed lines) spectra and vertical transitions (black bars) of **9** at pH 7.4, and experimental spectra at pH 2.6 and 10.0.

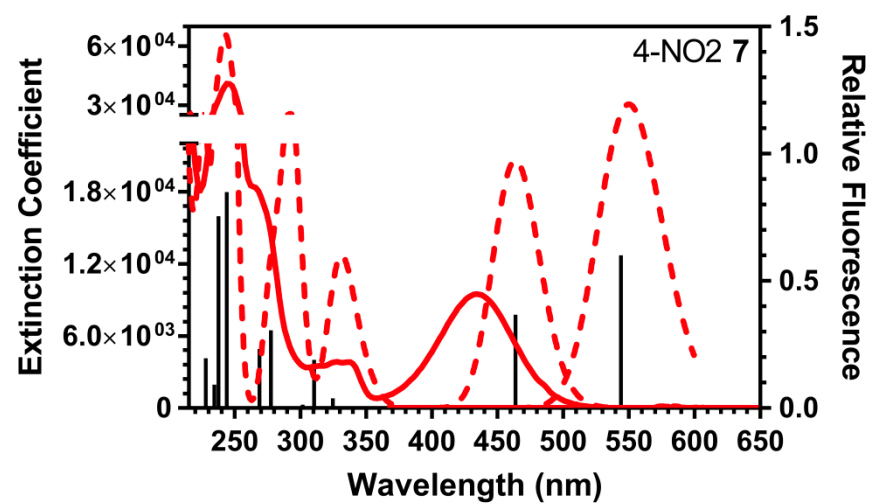
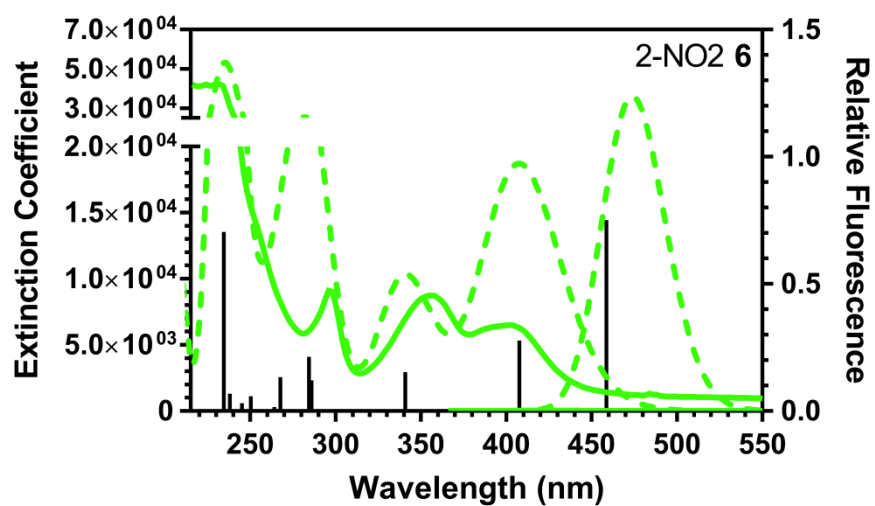


Figure S7. 2-Nitroacridone (6) and 4-Nitroacridone (7) Spectra. Experimental (solid lines) and calculated (dashed lines) spectra and vertical transitions (black bars) of 6 and 7 at pH 7.4.

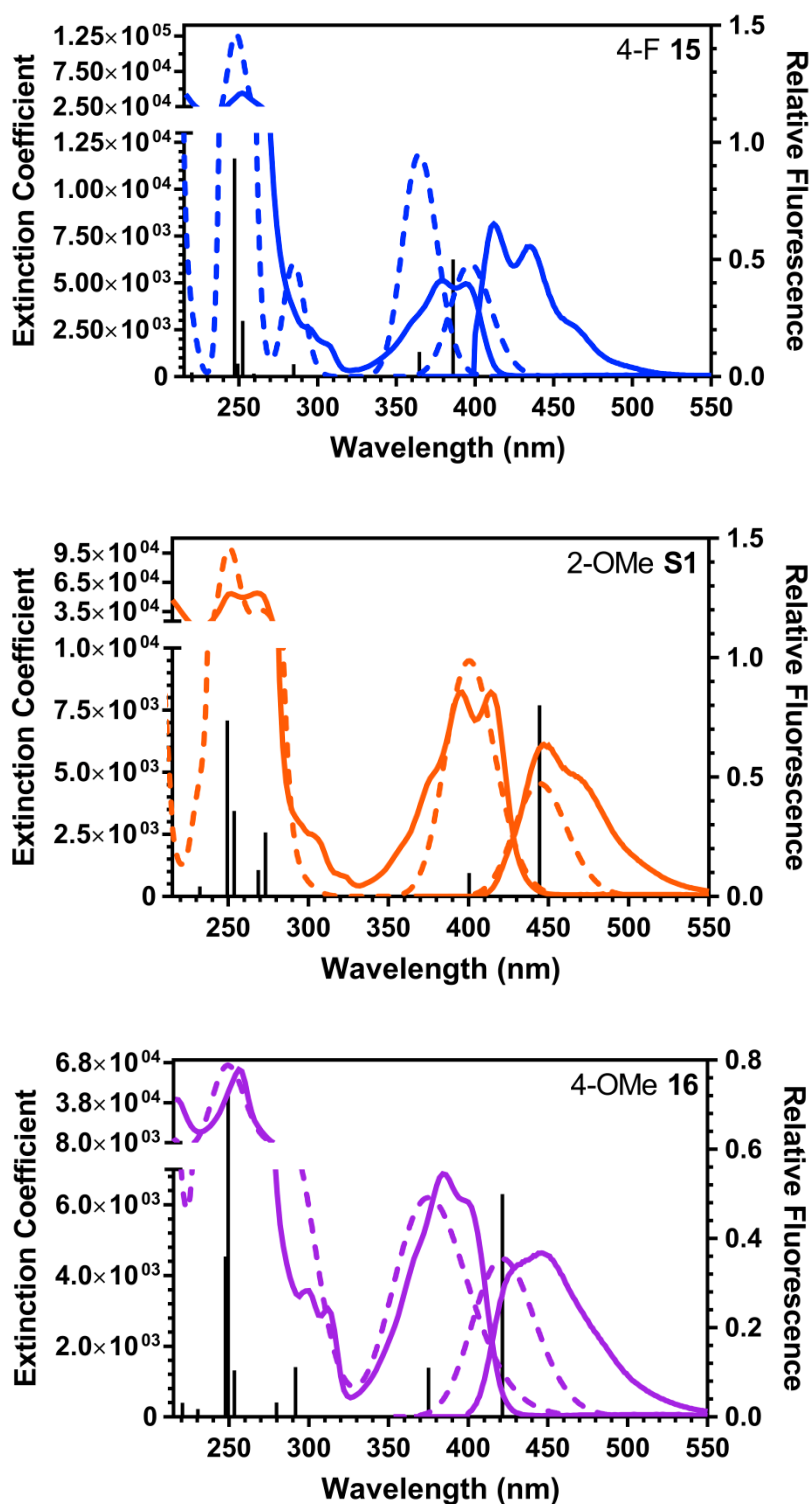


Figure S8. 2-Fluoroacridone (**15**), 2-Methoxyacridone (**S1**), and 4-Methoxyacridone (**16**) Spectra. Experimental (solid lines) and calculated (dashed lines) spectra and vertical transitions (black bars) of **15**, **S1**, and **16** at pH 7.4.

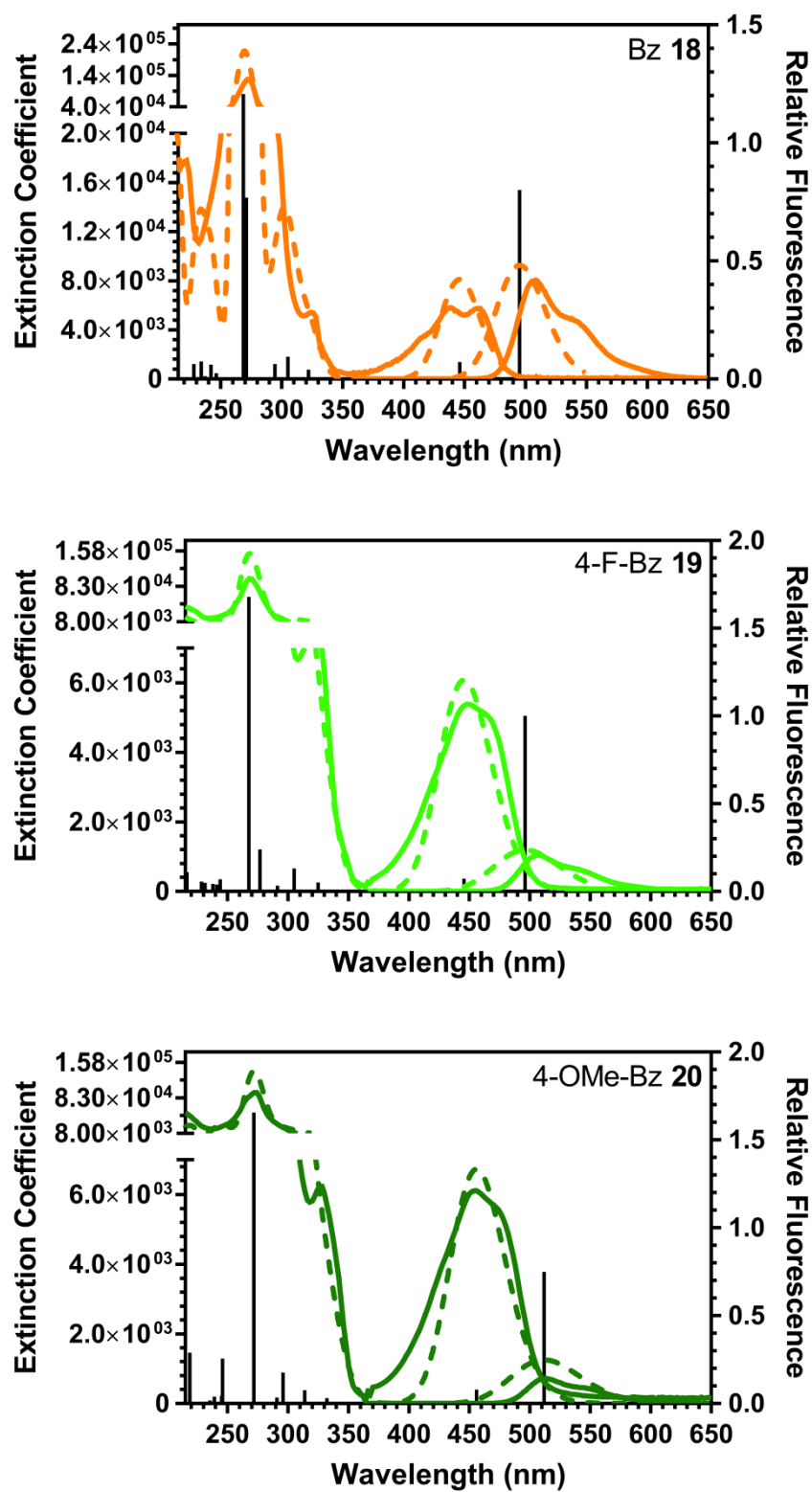


Figure S9. 4-Benzoacridone (**18**), 4-Fluorobenzoacridone (**19**), and 4-Methoxybenzoacridone (**20**) Spectra. Experimental (solid lines) and calculated (dashed lines) spectra and vertical transitions (black bars) of **18**, **19**, and **20** at pH 7.4.

Electronic Structure Analysis

Quantum Mechanical Calculations. *Ab initio* electronic structure calculations of the absorption and emission spectra employed the APF-D density functional as implemented in the Gaussian16TM suite of programs with the 6-311+G(2d,p) basis set which has been recommended for calculations of fluorescence spectra.^[5] The resulting absorption and emission spectra, as well as the vertical transitions, are shown in **Figures S4-S9** along with the corresponding experimental spectra. The line widths of the calculated spectra were adjusted to match the experimental spectra. The APF-D/6-311+G(2d,p) optimized geometry input files for these calculations are provided on the following pages.

For acridone, we combined Franck-Condon integral calculations with vibrational calculations of the ground and first excited states [Gaussian16 keyword(options) Freq=(ReadFC,FranckCondon,ReadFCHT)] as described in Reference 26, pages 364 to 371, to generate vibronic spectra representing acridone absorption and emission spectra in aqueous solution. We included the differential solvation of the ground state and first excited state as shown in Figure 3 by employing the procedure described in Reference 26, pages 371 to 379.

For acridone, we combined Franck-Condon integral calculations with vibrational calculations of the ground and first excited states [Gaussian16 keyword(options) Freq=(ReadFC,FranckCondon,ReadFCHT)] as described in Reference 26, pages 364 to 371, to generate vibronic spectra representing acridone absorption and emission spectra in aqueous solution. We included the differential solvation of the ground state and first excited state as shown in **Figure 3** by employing the procedure described in Foresman and Frisch.^[5b]

Acridone (5)

1-A1 Ground State in H2O

0 1
C, 0.0000000000, 3.6306927990,-0.9186812479
C, 0.0000000000, 2.4146763162,-1.5826017167
C, 0.0000000000, 1.2107638701,-0.8425503866
C, 0.0000000000, 1.2482568538, 0.5741490032
C, 0.0000000000, 2.5049249264, 1.2198424573
C, 0.0000000000, 3.6838400671, 0.4923858616
C, 0.0000000000, 0.0000000000, 1.3549238189
C, 0.0000000000,-1.2482568538, 0.5741490032
C, 0.0000000000,-1.2107638701,-0.8425503866
C, 0.0000000000,-2.4146763162,-1.5826017167
H, 0.0000000000,-2.3766350297,-2.6722037402
C, 0.0000000000,-3.6306927990,-0.9186812479
C, 0.0000000000,-3.6838400671, 0.4923858616
C, 0.0000000000,-2.5049249264, 1.2198424573
H, 0.0000000000, 0.0000000000,-2.5083812081
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H, 0.0000000000, 2.3766350297,-2.6722037402
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H, 0.0000000000, 4.6471295043, 1.0013567378
H, 0.0000000000,-4.5552582318,-1.4964701979
H, 0.0000000000,-4.6471295043, 1.0013567378
H, 0.0000000000,-2.5181803933, 2.3085515414
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N, 0.0000000000, 0.0000000000,-1.4960793388

Acridone (5)

1-A1 Excited State in H2O

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C, 0.0000000000, 1.2521151746, 0.6039370107
C, 0.0000000000, 2.5091592920, 1.2226613634
C, 0.0000000000, 3.7021384181, 0.4561747704
C, 0.0000000000, 0.0000000000, 1.3705139410
C, 0.0000000000,-1.2521151746, 0.6039370107
C, 0.0000000000,-1.2259581311,-0.8239702224
C, 0.0000000000,-2.4037172859,-1.5867102547
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C, 0.0000000000,-3.6569172585,-0.9354902191
C, 0.0000000000,-3.7021384181, 0.4561747704
C, 0.0000000000,-2.5091592920, 1.2226613634
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H, 0.0000000000, 4.6644697277, 0.9686122385
H, 0.0000000000,-4.5741985605,-1.5218267850
H, 0.0000000000,-4.6644697277, 0.9686122385
H, 0.0000000000,-2.5527930114, 2.3098531342
O, 0.0000000000, 0.0000000000, 2.6351450063
N, 0.0000000000, 0.0000000000,-1.4541792985

2-Nitroacridone (6)
1-A' Ground State in H2O

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C, 0.0000000000, 1.1083692115,-1.0794584082
C, 0.0000000000, 1.2091001297, 0.3223347870
C, 0.0000000000, 2.4787908123, 0.9140239669
C, 0.0000000000, 3.6179811896, 0.1403542559
C, 0.0000000000, 0.0049769978, 1.1534767246
C, 0.0000000000,-1.2683195809, 0.4220262587
C, 0.0000000000,-1.2925785479,-0.9889797358
C, 0.0000000000,-2.5248895912,-1.6731025512
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C, 0.0000000000,-3.6612814345, 0.4330673050
C, 0.0000000000,-2.4697512973, 1.1225490789
H, 0.0000000000,-0.1658062674,-2.6911524639
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H, 0.0000000000, 2.1845226655,-2.9484424806
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N, 0.0000000000,-4.9056821167, 1.1706642467
O, 0.0000000000,-4.8574463402, 2.3925484675
O, 0.0000000000,-5.9508515269, 0.5345008221

2-Nitroacridone (6)
1-A' Excited State in H2O

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C, 0.0000000000, 1.1120526987,-1.0821632228
C, 0.0000000000, 1.2107124239, 0.3408943131
C, 0.0000000000, 2.4732033400, 0.9100917489
C, 0.0000000000, 3.6120860972, 0.1104474259
C, 0.0000000000, 0.0014893701, 1.1703205309
C, 0.0000000000,-1.2777839418, 0.4534194984
C, 0.0000000000,-1.2888209536,-0.9467915619
C, 0.0000000000,-2.5004461031,-1.6538109294
H, 0.0000000000,-2.5017268257,-2.7383234907
C, 0.0000000000,-3.6894910544,-0.9583425658
C, 0.0000000000,-3.6885340585, 0.4428189248
C, 0.0000000000,-2.4831152601, 1.1510357784
H, 0.0000000000,-0.1487664074,-2.6649920778
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H, 0.0000000000, 2.1768501375,-2.9727768102
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H, 0.0000000000,-4.6362123199,-1.4821111153
H, 0.0000000000,-2.4788278170, 2.2311153250
O, 0.0000000000, 0.0608190520, 2.3968383478
N, 0.0000000000,-0.0965405775,-1.6518066875
N, 0.0000000000,-4.9208076004, 1.1324826396
O, 0.0000000000,-4.8868139541, 2.3988903221
O, 0.0000000000,-5.9991583178, 0.4651647643

4-Nitroacridone (7)
1-A' Ground State in H2O

0 1
C, 4.2706791532,-1.6770995227, 0.0000000000
C, 3.5358233972,-0.5111240329, 0.0000000000
C, 2.1362933155,-0.5779999317, 0.0000000000
C, 1.4908875446,-1.8234809855, 0.0000000000
C, 2.2616773103,-2.9930644058, 0.0000000000
C, 3.6372724251,-2.9280720643, 0.0000000000
C, 0.0315964372,-1.8907218710, 0.0000000000
C,-0.6719578221,-0.5984906583, 0.0000000000
C, 0.0541781771, 0.6161099439, 0.0000000000
C,-0.6971313185, 1.8226752602, 0.0000000000
C,-2.0851556742, 1.8000462506, 0.0000000000
C,-2.7769125555, 0.6009639073, 0.0000000000
C,-2.0632037550,-0.5845526096, 0.0000000000
H, 1.8703537116, 1.4842611253, 0.0000000000
H, 5.3541458670,-1.6214776564, 0.0000000000
H, 4.0294824796, 0.4551292924, 0.0000000000
H, 1.7412425006,-3.9440696552, 0.0000000000
H, 4.2301450662,-3.8357590404, 0.0000000000
H,-3.8598200482, 0.5988126314, 0.0000000000
H,-2.5700853780,-1.5432297620, 0.0000000000
O,-0.5792606748,-2.9574506488, 0.0000000000
N, 1.4037226082, 0.5836246598, 0.0000000000
H,-2.6119490387, 2.7453097156, 0.0000000000
N,-0.0664970336, 3.1170788075, 0.0000000000
O,-0.7675499054, 4.1148904064, 0.0000000000
O, 1.1658221404, 3.1779281042, 0.0000000000

4-Nitroacridone (7)
1-A' Excited State in H2O

0 1
C, 4.2799351667,-1.6273160480, 0.0000000000
C, 3.5350917752,-0.4755011641, 0.0000000000
C, 2.1195109878,-0.5686035137, 0.0000000000
C, 1.4740574698,-1.8346574337, 0.0000000000
C, 2.2565567903,-2.9767550906, 0.0000000000
C, 3.6459187605,-2.8804238608, 0.0000000000
C, 0.0088215042,-1.9056381204, 0.0000000000
C,-0.7125008075,-0.6294958322, 0.0000000000
C, 0.0073263866, 0.5655894407, 0.0000000000
C,-0.6732704176, 1.8141074822, 0.0000000000
C,-2.0620254147, 1.8064694885, 0.0000000000
C,-2.7759429898, 0.5982786218, 0.0000000000
C,-2.1235221587,-0.6124987259, 0.0000000000
H, 1.7804145426, 1.5070613990, 0.0000000000
H, 5.3620227416,-1.5709390056, 0.0000000000
H, 4.0046989514, 0.5015867754, 0.0000000000
H, 1.7659151846,-3.9430253303, 0.0000000000
H, 4.2452886589,-3.7834325767, 0.0000000000
H,-3.8597895630, 0.6340157373, 0.0000000000
H,-2.6544684390,-1.5552380271, 0.0000000000
O,-0.5852008237,-2.9811127796, 0.0000000000
N, 1.3819976160, 0.5441352008, 0.0000000000
H,-2.5811480533, 2.7548418567, 0.0000000000
N, 0.0081403382, 3.0532112916, 0.0000000000
O,-0.6732233391, 4.1039638031, 0.0000000000
O, 1.2891940616, 3.0916136716, 0.0000000000

2-Aminoacridone (8)
1-A Ground State in H2O

0 1
C, 0.0167353616, 3.5151422340,-1.2517512459
C, 0.0127791406, 2.2823725699,-1.8707319056
C, 0.0025984523, 1.1108120767,-1.0947657336
C,-0.0032170640, 1.2013453238, 0.3101776402
C, 0.0009878325, 2.4681178305, 0.9105128141
C, 0.0108313659, 3.6175009261, 0.1482536569
C,-0.0138061398,-0.0076404236, 1.1446320270
C,-0.0221870653,-1.2844596991, 0.4124943248
C,-0.0132118104,-1.3017504474,-0.9905989214
C,-0.0157807667,-2.5347547100,-1.6568322742
H,-0.0088828533,-2.5637297885,-2.7427704546
C,-0.0304795311,-3.7119780030,-0.9397066456
C,-0.0367125169,-3.7109672385, 0.4693433690
C,-0.0325722826,-2.4889494769, 1.1222959908
H, 0.0026430615,-0.1609416714,-2.7044738954
H, 0.0243870767, 4.4144039083,-1.8594191936
H, 0.0172317721, 2.2096779315,-2.9546828667
H,-0.0039242472, 2.5010377517, 1.9945605488
H, 0.0137833424, 4.5927947101, 0.6218455149
H,-0.0439005078,-4.6586727278,-1.4722660210
H,-0.0394708828,-2.4232585886, 2.2058309988
O,-0.0149444611, 0.0405894055, 2.3708696823
N,-0.0011128906,-0.1189596588,-1.6972990330
N,-0.1139093420,-4.9161342900, 1.1692497307
H, 0.1834344173,-4.8582060700, 2.1323976612
H, 0.3087005387,-5.7055980645, 0.7028691014

2-Aminoacridone (8)
1-A Excited State in H2O

0 1
C,-0.0104065555, 3.5252733155,-1.2577840895
C,-0.0081064624, 2.2544530622,-1.8610602733
C,-0.0011581820, 1.1171331190,-1.0693944234
C, 0.0037197042, 1.2032649274, 0.3418207680
C, 0.0012986288, 2.4805088441, 0.9181593988
C,-0.0057115741, 3.6305130137, 0.1221418550
C, 0.0109826836, 0.0007789266, 1.1554907611
C, 0.0128854227,-1.2708182034, 0.4381964257
C, 0.0078024010,-1.3110842988,-0.9829495816
C, 0.0096523352,-2.5374968588,-1.6681292440
H, 0.0056488758,-2.5365689233,-2.7538416787
C, 0.0164659317,-3.7367139445,-0.9797380688
C, 0.0215834103,-3.7144302309, 0.4303684076
C, 0.0196899607,-2.4731069491, 1.1204123696
H,-0.0024661770,-0.1722705367,-2.6713835317
H,-0.0158479925, 4.4133537364,-1.8800768827
H,-0.0117372548, 2.1586904816,-2.9426450824
H, 0.0050219377, 2.5507667968, 1.9995273752
H,-0.0074618600, 4.6089512197, 0.5922672454
H, 0.0178785074,-4.6817497971,-1.5101369475
H, 0.0236117660,-2.4528621014, 2.2050729762
O, 0.0154515741, 0.0391928399, 2.4119976460
N, 0.0011244607,-0.1373735397,-1.6609832900
N, 0.0282846859,-4.8548512812, 1.1424338744
H, 0.0319396467,-4.8404797706, 2.1497097126
H, 0.0298541260,-5.7552800375, 0.6905591486

4-Aminoacridone (9)

1-A + 1 H2O Ground State in H2O

0 1
 C, 3.6535204282,-1.1615434873, 0.0717195073
 C, 2.3690680852,-1.6525967306, 0.1023067207
 C, 1.2799405917,-0.7627287715, 0.0558779988
 C, 1.5114244882, 0.6222451947,-0.0238743375
 C, 2.8336918743, 1.0921139342,-0.0520820085
 C, 3.8969227206, 0.2205206108,-0.0052170763
 C, 0.3887085383, 1.5543188891,-0.0783954446
 C,-0.9475568647, 0.9569520985,-0.0437878697
 C,-1.0983362507,-0.4398724978, 0.0369610893
 C,-2.3972906354,-1.0145573463, 0.0733013547
 C,-3.4871963614,-0.1597800249, 0.0247315766
 C,-3.3355504253, 1.2293674581,-0.0508672748
 C,-2.0789321358, 1.7860710257,-0.0861613829
 H,-0.0943945810,-2.2604685687, 0.0981240582
 H, 4.4883275668,-1.8541361700, 0.1080294342
 H, 2.1812352819,-2.7196400620, 0.1616877894
 H, 2.9859683706, 2.1640694433,-0.1125964280
 H, 4.915555342, 0.5917738643,-0.0276897134
 H,-4.2187842311, 1.8584482971,-0.0857969149
 H,-1.9279559754, 2.8569747103,-0.1472199036
 O, 0.5575004023, 2.7765851860,-0.1493213054
 N, 0.0058424588,-1.2482566325, 0.0898121199
 H,-4.4847506320,-0.5894346974, 0.0506091534
 N,-2.5625845781,-2.3816233562, 0.2389957479
 H,-3.5031733867,-2.7027197974, 0.0615844145
 H,-1.8833396701,-3.0003386060,-0.1894667105
 O,-0.1748670891,-4.1161119674,-0.3090480280
 H, 0.2331620686,-4.4423983208,-1.1177709298
 H,-0.0358822329,-4.8045198655, 0.3496144930

4-Aminoacridone (9)

1-A + 1 H2O Excited State in H2O

0 1
 C, 3.7205841205,-1.1331287684,-0.0240058087
 C, 2.3996843407,-1.6264007562,-0.0345187026
 C, 1.3333914501,-0.7425021624,-0.0294509209
 C, 1.5318752674, 0.6600213826,-0.0138228057
 C, 2.8589947481, 1.1264875698,-0.0035416165
 C, 3.9359567476, 0.2351380342,-0.0086545239
 C, 0.4051665306, 1.5609591544,-0.0088577484
 C,-0.9255602394, 0.9469438110,-0.0206628738
 C,-1.0755015670,-0.4561486877,-0.0359978101
 C,-2.3831378514,-1.0309352510,-0.0471282107
 C,-3.5241906161,-0.1738989664,-0.0429079038
 C,-3.3638994541, 1.1810407686,-0.0281155073
 C,-2.0661947277, 1.7383236970,-0.0169848018
 H,-0.0476839256,-2.2531612822,-0.0509012223
 H, 4.5543154594,-1.8263283542,-0.0280049059
 H, 2.2093406390,-2.6950948640,-0.0465754253
 H, 3.0207813567, 2.1979898976, 0.0084508500
 H, 4.9504357944, 0.6228979768,-0.0004751702
 H,-4.2297228464, 1.8331599693,-0.0248269509
 H,-1.9309944013, 2.8137653097,-0.0051713225
 O, 0.5425087152, 2.8178686125, 0.0049160003
 N, 0.0336244225,-1.2382025835,-0.0399139389
 H,-4.5102238525,-0.6255541500,-0.0516056504
 N,-2.5818785810,-2.3573492221,-0.0615967736
 H,-3.5259875664,-2.7105382241,-0.0689725352
 H,-1.8389682804,-3.0523296699,-0.0649381496
 O,-0.2943229595,-4.1171128381,-0.0688756121
 H,-0.0603076006,-4.6433115877,-0.8410142287
 H,-0.0678117629,-4.6538850056, 0.6982143995

4-Fluoroacridone (15)
1-A Ground State in H2O

0 1
C, 3.6435061362,-1.2189516356,-0.0224901372
C, 2.3568605109,-1.7086873792,-0.0368817128
C, 1.2736611078,-0.8165437716,-0.0284415506
C, 1.5044743147, 0.5714132707,-0.0053708235
C, 2.8267710280, 1.0384626745, 0.0088477479
C, 3.8884099481, 0.1627801319, 0.0005542374
C, 0.3879133330, 1.5186017089, 0.0037671675
C,-0.9522250881, 0.9263777739,-0.0125177716
C,-1.0926958304,-0.4705739795,-0.0352634161
C,-2.3891030759,-1.0059820572,-0.0504264569
C,-3.5067929905,-0.2157169037,-0.0437697519
C,-3.3570193371, 1.1791226266,-0.0210096080
C,-2.0975897327, 1.7342415665,-0.0057262430
H,-0.1615737701,-2.2928952415,-0.0591521865
H, 4.4764144192,-1.9143207808,-0.0291275098
H, 2.1711211354,-2.7778374290,-0.0546934754
H, 2.9816267258, 2.1114928857, 0.0265609873
H, 4.9073532588, 0.5331468732, 0.0116755136
H,-4.2389894457, 1.8088558368,-0.0156595031
H,-1.9576535028, 2.8088697987, 0.0119360909
O, 0.5606260853, 2.7380599670, 0.0238829168
N,-0.0086443863,-1.2948675003,-0.0426313117
H,-4.4856821954,-0.6817573669,-0.0561940297
F,-2.4986036481,-2.3536420690,-0.0722391735

4-Fluoroacridone (15)
1-A Excited State in H2O

0 1
C, 3.6674039326,-1.2408023091,-0.0227113168
C, 2.3457745488,-1.7104622079,-0.0369686898
C, 1.2920240843,-0.7982063687,-0.0280573543
C, 1.5125149909, 0.6035201445,-0.0048221782
C, 2.8307167081, 1.0406251481, 0.0089045240
C, 3.9008830467, 0.1226279386,-0.0000123159
C, 0.3886228722, 1.5372731537, 0.0040639507
C,-0.9472446199, 0.9574462314,-0.0120037881
C,-1.1044290181,-0.4513021307,-0.0350211342
C,-2.3787866597,-1.0104736613,-0.0504439451
C,-3.5381143074,-0.2305422221,-0.0441654368
C,-3.3799778410, 1.1419669071,-0.0217142274
C,-2.1001161148, 1.7326096951,-0.0057671128
H,-0.1505239212,-2.2554867248,-0.0585069557
H, 4.4911855841,-1.9444684141,-0.0295281521
H, 2.1345430559,-2.7748590429,-0.0548394359
H, 3.0181463607, 2.1076533019, 0.0266934007
H, 4.9190437954, 0.4973050003, 0.0111741111
H,-4.2578190793, 1.7786506702,-0.0162333364
H,-1.9946732816, 2.8106814092, 0.0117704355
O, 0.5673659927, 2.7786135440, 0.0245551700
N,-0.0004278657,-1.2529976143,-0.0419291546
H,-4.5101242514,-0.7069582043,-0.0567155284
F,-2.4738230122,-2.3527652441,-0.0720915087

2-Methoxyacridone (S1)
1-A Ground State in H2O

0 1
C, -0.0657763583, 4.4482156266, -0.7142891871
C, -0.0206814037, 3.2949676294, -1.4628555235
C, 0.0133456185, 2.0450301425, -0.8198218541
C, 0.0010597147, 1.9809332855, 0.5875243604
C, -0.0452593191, 3.1764378474, 1.3216161070
C, -0.0784712479, 4.3977341367, 0.6897671437
C, 0.0358710777, 0.6926068022, 1.2758981663
C, 0.0830977021, -0.4903331590, 0.4124016229
C, 0.0928356294, -0.3503249571, -0.9869441385
C, 0.1382493444, -1.4972666268, -1.7875123078
H, 0.1461487492, -1.4007035893, -2.8686144553
C, 0.1731254332, -2.7511023660, -1.2156720908
C, 0.1635849115, -2.8973235709, 0.1842216423
C, 0.1187999465, -1.7672717694, 0.9768555426
H, 0.0660139523, 0.9701297300, -2.5613531507
H, -0.0918012859, 5.4083646882, -1.2193650219
H, -0.0110432324, 3.3391813392, -2.5474571517
H, -0.0538781899, 3.1017701469, 2.4033927618
H, -0.1141630012, 5.3157127696, 1.2657034856
H, 0.2078968155, -3.6184623020, -1.8630418468
H, 0.1107411382, -1.8586454433, 2.0570465466
O, 0.0262320165, 0.6023721602, 2.5067537299
N, 0.0581201128, 0.8973110020, -1.5544833741
O, 0.1957045553, -4.0903659771, 0.8328279016
C, 0.2421042266, -5.2748488314, 0.0554856442
H, 0.2615850181, -6.0982103639, 0.7664079337
H, -0.6431071892, -5.3677311374, -0.5807941320
H, 1.1442670547, -5.3096880827, -0.5625489843

2-Methoxyacridone (S1)
1-A Excited State in H2O

0 1
C, -0.0664467861, 4.4712300992, -0.7205170178
C, -0.0200771432, 3.2749955481, -1.4586124398
C, 0.0125934378, 2.0592677498, -0.7925668071
C, 0.0005905418, 1.9846026947, 0.6205669975
C, -0.0456848817, 3.1868008411, 1.3304180475
C, -0.0788671849, 4.4191013802, 0.6619694413
C, 0.0355463657, 0.6958723062, 1.2941139734
C, 0.0827434115, -0.4886999602, 0.4401432195
C, 0.0931853232, -0.3643407270, -0.9781135769
C, 0.1383384086, -1.4954843291, -1.8026477719
H, 0.1452195888, -1.3685089558, -2.8806208997
C, 0.1743897547, -2.7766740549, -1.2581380417
C, 0.1643836925, -2.9059441141, 0.1332601395
C, 0.1186550292, -1.7613668821, 0.9711959612
H, 0.0660376472, 0.9579715512, -2.5247672236
H, -0.0920286377, 5.4224445936, -1.2396454518
H, -0.0096950250, 3.2964958678, -2.5440333842
H, -0.0552764276, 3.1417691207, 2.4130269942
H, -0.1145800127, 5.3377389517, 1.2388327975
H, 0.2090477601, -3.6391899196, -1.9090911497
H, 0.1119972430, -1.8944463921, 2.0465208333
O, 0.0260799420, 0.5954033391, 2.5447527971
N, 0.0582603842, 0.8800978711, -1.5154031984
O, 0.1952610041, -4.0640299169, 0.7929859237
C, 0.2423576356, -5.2826597280, 0.0567218857
H, 0.2605118101, -6.0752033684, 0.7998729278
H, -0.6436854616, -5.3834367237, -0.5736720385
H, 1.1457443702, -5.3253177126, -0.5554035681

4-Methoxyacridone (16)
1-A' Ground State in H2O

0 1
C, 3.5921883436,-1.2323770657, 0.0000000000
C, 2.3007502043,-1.7080985996, 0.0000000000
C, 1.2259249316,-0.8037500313, 0.0000000000
C, 1.4724260588, 0.5820388618, 0.0000000000
C, 2.8001544953, 1.0347157703, 0.0000000000
C, 3.8521890085, 0.1476283938, 0.0000000000
C, 0.3646662965, 1.5385922950, 0.0000000000
C,-0.9796355755, 0.9610779757, 0.0000000000
C,-1.1388422928,-0.4303324752, 0.0000000000
C,-2.4363291509,-0.9965758695, 0.0000000000
C,-3.5380535955,-0.1676550151, 0.0000000000
C,-3.3725626856, 1.2288738145, 0.0000000000
C,-2.1173868007, 1.7853242020, 0.0000000000
H,-0.2353635852,-2.2590197489, 0.0000000000
H, 4.4178610385,-1.9365024112, 0.0000000000
H, 2.1027940370,-2.7752114763, 0.0000000000
H, 2.9661639849, 2.1063632020, 0.0000000000
H, 4.8753396299, 0.5066720613, 0.0000000000
H,-4.2537042670, 1.8611083687, 0.0000000000
H,-1.9714791618, 2.8589192177, 0.0000000000
O, 0.5524748229, 2.7582763007, 0.0000000000
N,-0.0595896828,-1.2639428366, 0.0000000000
H,-4.5367231693,-0.5866599046, 0.0000000000
O,-2.4488445024,-2.3498297369, 0.0000000000
C,-3.7077899194,-3.0053188609, 0.0000000000
H,-3.4904051664,-4.0707902141, 0.0000000000
H,-4.2809525910,-2.7470360613, 0.8944223908
H,-4.2809525910,-2.7470360613,-0.8944223908

4-Methoxyacridone (16)
1-A' Excited State in H2O

0 1
C, 3.6192841081,-1.2463427124, 0.0000000000
C, 2.2860751328,-1.7027227802, 0.0000000000
C, 1.2475085789,-0.7852091933, 0.0000000000
C, 1.4815302391, 0.6133690661, 0.0000000000
C, 2.8141092279, 1.0397428460, 0.0000000000
C, 3.8692902982, 0.1140541595, 0.0000000000
C, 0.3757008989, 1.5526028878, 0.0000000000
C,-0.9722392650, 0.9804150984, 0.0000000000
C,-1.1484132605,-0.4218228357, 0.0000000000
C,-2.4421632186,-0.9968027735, 0.0000000000
C,-3.5819772097,-0.1652221198, 0.0000000000
C,-3.4017015617, 1.1975545439, 0.0000000000
C,-2.1074985162, 1.7699583767, 0.0000000000
H,-0.2297240568,-2.2297274923, 0.0000000000
H, 4.4338163960,-1.9612719005, 0.0000000000
H, 2.0658122566,-2.7656062941, 0.0000000000
H, 3.0089755415, 2.1056740864, 0.0000000000
H, 4.8929040939, 0.4756350648, 0.0000000000
H,-4.2679460024, 1.8496613838, 0.0000000000
H,-1.9854404193, 2.8467110404, 0.0000000000
O, 0.5541041840, 2.7982600415, 0.0000000000
N,-0.0632415488,-1.2301839619, 0.0000000000
H,-4.5754517712,-0.5925737996, 0.0000000000
O,-2.4602710045,-2.3304332585, 0.0000000000
C,-3.7109805539,-3.0165185920, 0.0000000000
H,-3.4631375326,-4.0743057872, 0.0000000000
H,-4.2823034603,-2.7677205000, 0.8961208205
H,-4.2823034603,-2.7677205000,-0.8961208205

Benzoacridone (18)
1-A Ground State in H2O

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C,-6.8371509765,-1.3828079078,-0.0031005771
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C,-5.5895140154, 1.1082038078,-0.0398043608
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C,-7.6148741697,-0.2146471985,-0.0002249736
H,-2.9328827293,-0.8664411697,-0.0644484150
H,-7.3223075106,-2.3534372831, 0.0111865734
H,-4.8678148058,-2.2281232513,-0.0259138668
C,-4.9405156346, 2.4183359521,-0.0590025609
H,-7.5565154292, 1.9342218771,-0.0168833966
H,-8.6968572092,-0.2812997608, 0.0162136481
C,-2.7548531326, 1.1767647656,-0.0820920015
C,-1.3711305472, 1.1773360904,-0.1024929042
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H,-0.8285385922, 0.2363797487,-0.1035724436
C, 0.7624186962, 2.4161588446,-0.1430505298
C,-0.6420599455, 4.8447266483,-0.1402035796
H,-3.3331575985, 4.5241840470,-0.0981788864
C, 0.7247339469, 4.8391732491,-0.1603097234
C, 1.4307450181, 3.6093740839,-0.1616747019
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H, 1.2763357797, 5.7731507856,-0.1753073609
H, 2.5159284787, 3.6200297971,-0.1777489501
N,-3.4549701615,-0.0024231848,-0.0631012526
O,-5.5821047608, 3.4693677550,-0.0572722513

Benzoacridone (18)
1-A Excited State in H2O

0 1
C,-6.8312244429,-1.4174315396,-0.0029341986
C,-5.4407799116,-1.3178121976,-0.0241606877
C,-4.8433468807,-0.0609519444,-0.0422064432
C,-5.6113533078, 1.1250276634,-0.0396054525
C,-6.9968170451, 0.9968178670,-0.0182422207
C,-7.6028914090,-0.2614325648,-0.0000589807
H,-2.9333678806,-0.8134419949,-0.0648274214
H,-7.3012968656,-2.3939742262, 0.0111735075
H,-4.8225290550,-2.2100909550,-0.0267135823
C,-4.9606150553, 2.4381617804,-0.0588500217
H,-7.5923012541, 1.9025123444,-0.0161233747
H,-8.6852853157,-0.3344497860, 0.0164315629
C,-2.7839953626, 1.2153333021,-0.0819446184
C,-1.3901787585, 1.1931869788,-0.1023277001
C,-0.6474055285, 2.3950843648,-0.1221136712
C,-1.3599183747, 3.6364107641,-0.1207372333
C,-2.7814303041, 3.6193585997,-0.0996578000
C,-3.5140729435, 2.4424984476,-0.0802052927
H, 1.2869161337, 1.4564636857,-0.1437275207
H,-0.8681700453, 0.2407886374,-0.1030203751
C, 0.7552942083, 2.4031173537,-0.1428505923
C,-0.6268513592, 4.8257696682,-0.1402876619
H,-3.3240390087, 4.5587564406,-0.0985661873
C, 0.7713929600, 4.8099034951,-0.1607827852
C, 1.4635569752, 3.6038488894,-0.1621193063
H,-1.1564339853, 5.7734040163,-0.1394483567
H, 1.3157066575, 5.7483093079,-0.1757059914
H, 2.5476181207, 3.5950340749,-0.1780351824
N,-3.4696364252, 0.0459014935,-0.0632403044
O,-5.6328843665, 3.4953047538,-0.0567169101

4-Fluorobenzacridone (19)
1-A Ground State in H2O

0 1
C, 4.7307084433,-1.0091575903,-0.0000036772
C, 3.4957678571,-1.6009554336,-0.000001487
C, 2.3060583395,-0.8576485262, 0.0000032146
C, 2.4055428556, 0.5434248262, 0.0000038659
C, 3.6695042203, 1.1481026307,-0.0000004418
C, 4.8189853373, 0.3905428098,-0.0000041031
H, 1.0926653131,-2.5020404870, 0.0000047402
H, 5.6165919768,-1.6343762527,-0.0000064341
C, 1.1853447602, 1.3532857392, 0.0000110626
H, 3.7110345471, 2.2310207729, 0.0000001396
H, 5.7941143925, 0.8632860561,-0.0000069496
C,-0.0932607135,-0.8158408355, 0.0000046696
C,-1.2929986293,-1.5032432007, 0.0000028525
C,-2.5118577860,-0.8115808006, 0.0000009933
C,-2.5113301693, 0.6208411115, 0.0000008535
C,-1.2831858753, 1.2916388496, 0.0000029301
C,-0.0825178263, 0.6085000139, 0.0000054225
H,-3.7664630285,-2.5753149412,-0.0000011655
H,-1.2964573443,-2.5890880966, 0.0000022311
C,-3.7596286041,-1.4896886479,-0.0000011463
C,-3.7489960418, 1.3161712570,-0.0000011567
H,-1.2545852178, 2.3763591352, 0.0000036524
C,-4.9318875958, 0.6311780623,-0.0000031362
C,-4.9329479517,-0.7868052063,-0.0000031169
H,-3.7358872062, 2.4018512557,-0.0000011269
H,-5.8751398778, 1.1669171899,-0.0000047267
H,-5.8798255374,-1.3172075051,-0.0000047092
N, 1.1018018749,-1.4924715559, 0.0000041903
O, 1.2191079316, 2.5824650496,-0.0000029327
F, 3.3792922259,-2.9481740299,-0.0000007206

4-Fluorobenzacridone (19)
1-A Excited State in H2O

0 1
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C, 2.3216675210,-0.8404478372, 0.0000248005
C, 2.4134086196, 0.5680226006, 0.0000292746
C, 3.6820650537, 1.1391695596,-0.0000039611
C, 4.8315169643, 0.3459975992,-0.0000327559
H, 1.0645200927,-2.4590894125, 0.0000259891
H, 5.6212740727,-1.6761491000,-0.0000509689
C, 1.1943659051, 1.3786588806, 0.0000794316
H, 3.7546979031, 2.2201270789,-0.0000005146
H, 5.8088004668, 0.8161030246,-0.0000557838
C,-0.0871983669,-0.7706862036, 0.0000367420
C,-1.2867134905,-1.4814896032, 0.0000224635
C,-2.5269668563,-0.8067488107, 0.0000061197
C,-2.5248420472, 0.6237465986, 0.0000059656
C,-1.2816656465, 1.3144636504, 0.0000259453
C,-0.0621596344, 0.6572283183, 0.0000436269
H,-3.7404048786,-2.5824794347,-0.0000120798
H,-1.2676869063,-2.5671508950, 0.0000201170
C,-3.7490367049,-1.4968608516,-0.0000113999
C,-3.7506886543, 1.2925614985,-0.0000086736
H,-1.2757415017, 2.3993587873, 0.0000330697
C,-4.9571735940, 0.5844783533,-0.0000255326
C,-4.9594125319,-0.8059700301,-0.0000272549
H,-3.7614339759, 2.3779853834,-0.0000062523
H,-5.8955366461, 1.1288870405,-0.0000365138
H,-5.8962550933,-1.3516002919,-0.0000400011
N, 1.0891262692,-1.4463782818, 0.0000349400
O, 1.2461603330, 2.6304746415,-0.0000422355
F, 3.3408418923,-2.9512082238, 0.0000012594

4-Methoxybenzoacridone (20)
1-A Ground State in H2O

0 1
C, 4.7758683226,-0.9049034584,-0.0004139826
C, 3.5559539372,-1.5479003825,-0.0003454628
C, 2.3638511966,-0.7841174648,-0.0002149191
C, 2.4320337048, 0.6152656989,-0.0001522423
C, 3.6862876230, 1.2480988598,-0.0002208674
C, 4.8375301081, 0.4998584395,-0.0003411449
H, 1.1893600351,-2.4488688351,-0.0001998495
H, 5.6942947659,-1.4789496392,-0.0005391120
C, 1.1987983891, 1.4004778518, 0.0000020295
H, 3.7131377380, 2.3313182694,-0.0001780912
H, 5.8082096404, 0.9832501199,-0.0003995347
C,-0.0383146744,-0.7901568853,-0.0000685780
C,-1.2253895265,-1.5018313934,-0.0000492341
C,-2.4571097310,-0.8347830269, 0.0000198684
C,-2.4847397307, 0.5979627567, 0.0000709182
C,-1.2702991171, 1.2930022456, 0.0000526118
C,-0.0560468981, 0.6339367503,-0.0000096068
H,-3.6783260529,-2.6221511926,-0.0000040499
H,-1.2052053634,-2.5876052905,-0.0000942259
C,-3.6923974231,-1.5365800351, 0.0000359029
C,-3.7362113560, 1.2686359821, 0.0001382015
H,-1.2633768682, 2.3780510547, 0.0000984449
C,-4.9056638358, 0.5611103464, 0.0001521496
C,-4.8791072199,-0.8569509852, 0.0001001194
H,-3.7443311066, 2.3544825423, 0.0001774008
H,-5.8590938701, 1.0786054735, 0.0002024852
H,-5.8155033246,-1.4057234377, 0.0001131110
N, 1.1681911154,-1.4389040103,-0.0001628581
O, 1.2060547272, 2.6320893992,-0.0000739569
O, 3.3543428951,-2.8866733115,-0.0003849988
C, 4.4933205793,-3.7330549310, 0.0002579260
H, 4.1101183524,-4.7507555206, 0.0003556773
H, 5.1006351791,-3.5692063503,-0.8940175680
H, 5.1000429991,-3.5688033297, 0.8948602884

4-Methoxybenzoacridone (20)
1-A Excited State in H2O

0 1
C, 4.8037587398,-0.9219093100,-0.0001109775
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C, 2.3822562443,-0.7666170605,-0.0002414086
C, 2.4426648529, 0.6419796314,-0.0002764909
C, 3.6917394726, 1.2403678280,-0.0001967945
C, 4.8569783155, 0.4615230636,-0.0001177996
H, 1.1731402621,-2.4012774297,-0.0001734787
H, 5.7152734855,-1.5045429150,-0.0000583562
C, 1.2020125906, 1.4316678037,-0.0004358522
H, 3.7474041018, 2.3224422392,-0.0002200551
H, 5.8248589497, 0.9509775765,-0.0000694227
C,-0.0308571117,-0.7387231622,-0.0001890618
C,-1.2047895970,-1.4756947326,-0.0000830350
C,-2.4668553960,-0.8290008729, 0.0000220883
C,-2.4986542971, 0.6027871049, 0.0000099973
C,-1.2746674774, 1.3221702083,-0.0001182884
C,-0.0376971883, 0.6892029949,-0.0002325756
H,-3.6386209179,-2.6302198744, 0.0001479360
H,-1.1592573238,-2.5608720921,-0.0000690571
C,-3.6718948641,-1.5447478501, 0.0001383484
C,-3.7455291787, 1.2427510260, 0.0001111503
H,-1.2940900597, 2.4066573616,-0.0001491558
C,-4.9311756400, 0.5087845096, 0.0002248539
C,-4.8985516814,-0.8833980849, 0.0002386281
H,-3.7800985625, 2.3280797254, 0.0000977796
H,-5.8833888170, 1.0293715912, 0.0003002450
H,-5.8220443745,-1.4520956011, 0.0003254876
N, 1.1702479511,-1.3875323476,-0.0002240245
O, 1.2377598262, 2.6833605619,-0.0000078195
O, 3.3439637294,-2.8763111895,-0.0001819381
C, 4.4673959189,-3.7519554615, 0.0000717111
H, 4.0558391155,-4.757727827, 0.0001572974
H, 5.0732074520,-3.5994608477,-0.8955872654
H, 5.0729909854,-3.5992162211, 0.8958349276

Peptide Synthesis and FRET Assays

Solid Phase Peptide Synthesis and Purification. AcdLeuLeuLysAlaAlaAlaMcm and AadLeuLeuLysAlaAlaAlaMcm (Mcm = 7-methoxycoumarin-4-yl-alanine) were synthesized using standard protocols. Before peptide synthesis was started, the glass reaction vessel was coated with Sigmacote® (Sigma-Aldrich) and rinsed with CH₂Cl₂, followed by DMF. The vessel was allowed to dry for approximately 10 min before adding the 2-chlorotrityl resin and swelling with DMF for approximately 30 min. For Fmoc-7-methoxycoumarin-4-yl-alanine coupling, 2 equiv amino were used along with 4 equiv DIPEA (no HBTU was used as this was the first residue coupled to the 2-chlorotrityl resin). This coupling was performed twice. For all subsequent amino acids and Boc-Acd, coupling reactions were done with 5 equiv amino acid, 5 equiv HBTU, and 10 equiv DIPEA for 45 min. For deprotection, 20% piperidine in DMF was added to the reaction vessel, and the reaction was stirred at room temperature for 20 min. After each deprotection step, the resin was washed with DMF, CH₂Cl₂ twice, and DMF again. For Boc₂-Aad, 5 equiv amino acid was used along with 5 equiv HBTU and 10 equiv DIPEA. This coupling was done two times for 5 h and overnight, respectively.

For cleavage, the resin was treated with a cleavage cocktail for 45 minutes (35:55:5:5 v/v trifluoroacetic acid (TFA)/CH₂Cl₂/water/triisopropylsilane (TIPSH) for the Acd containing peptide or 90:5:5 v/v trifluoroacetic acid/water/TIPSH for the Aad containing peptide) while stirring at room temperature. After cleavage, the peptide solution was dried using rotary evaporation. The resulting oil was precipitated using cold ether, and the mixture was centrifuged at 3000 RPM for 5 minutes. The precipitate was collected and redissolved in CH₃CN/Water (1:1 v/v) for purification by HPLC. Peptides were purified using either a C18 preparative column (Vydac; 218TP1022) for the Acd containing peptide, or a C4 protein semi-preparative column (Vydac; 214TP1010) for the Aad containing peptide using the solvent gradients described in **Table S3**. [M+H]⁺ calculated for H₂N-AcdLeuLeuLysAlaAlaAlaMcm-CO₂H: 1095.55, found 1095.68; [M+H]⁺ calculated for H₂N-AadLeuLeuLysAlaAlaAlaMcm-CO₂H: 1110.56, found 1110.26. Purified peptides were dried on a lyophilizer (Labconco; Kansas City, MO, USA).

Table S3. HPLC Gradients for Peptide Purification (Peptide Retention Times).

Peptide	Time (min)	%B	Peptide	Time (min)	%B
Aad	0:00	2	Acid	0:00	2
(14.3 min)	5:00	2	(24.2 min)	5:00	2
	8:00	22		8:00	20
	28:00	35		28:00	40
	30:00	100		30:00	100
	33:00	100		33:00	100
	35:00	2		35:00	2
	40:00	2		40:00	2

Solvent A: 0.1 % TFA in water; Solvent B: 0.1 % TFA in acetonitrile.

Plate Reader Protease FRET Assay. In a typical trial, 5 μ M peptide was incubated in the presence or absence of the 250-fold diluted cathepsin B stock (Sigma C8571, 25UG) in 5.0 mM EDTA, 5.0 mM DTT, 20 sodium acetate, 50 mM NaCl, pH 5.0 at 27 °C, or in the presence or absence of the 25 μ g/mL trypsin in 67 mM sodium phosphate, pH 7.6 at 25 °C. The fluorescence was monitored as a function of time at 380 nm and 480 nm for the Acid containing peptide with an excitation wavelength of 325 nm on a Tecan InfinitePro M1000 plate reader. Three independent trials were performed for each assay to ensure reproducibility, and the ratio of 380 to 480 nm fluorescence was used to characterize proteolysis. An identical experiment was performed for the Aad peptide with monitoring at 380 nm and 530 nm.

Stopped-Flow Protease FRET Assay. In a typical trial, 200 μ L of 10 μ M peptide was rapidly mixed with 50 μ g/mL trypsin in 67 mM sodium phosphate, pH 7.6 at 25 °C. Fluorescence emission was recorded as a function of time at 390 (\pm 18) nm and 440 (\pm 40) nm for the Acid containing peptide or 390 (\pm 18) nm and 520 (\pm 36) nm for the Aad containing peptide, with excitation at 325 nm on a stopped-flow instrument (KinTek AutoSF-120; Snow Shoe, PA). At least three technical replicates were averaged together for each trial, and three separate trials were performed to ensure reproducibility. Proteolysis was characterized as a ratio of the fluorescence at 390 nm to 440 nm for the Acid peptide and 390/520 nm for the Aad containing peptide.

Pre- and Post-Cleavage Emission Measurements. Fluorescence spectra were acquired for Acd/Aad containing peptides in the absence (Pre) and presence (Post) of enzyme using a PTI Quantamaster40 fluorometer. Cleavage was allowed to proceed for >30 min at room temperature prior to measurement of the post-cleavage emission. For each peptide, measurements were acquired with excitation of 325 nm over an emission window spanning 335-550 nm (for Acd containing peptides) or 335-650 nm (for Aad containing peptides). Emission spectra from the direct excitation of Acd/Aad were acquired via excitation at 382 nm (Acd) or 420 nm (Aad) with scanning the emission from 392-550 nm or 430-650 nm, respectively. For all measurements, the step size and integration time were set to 1 nm and 0.25 seconds respectively and all slit widths were set to 3 nm.

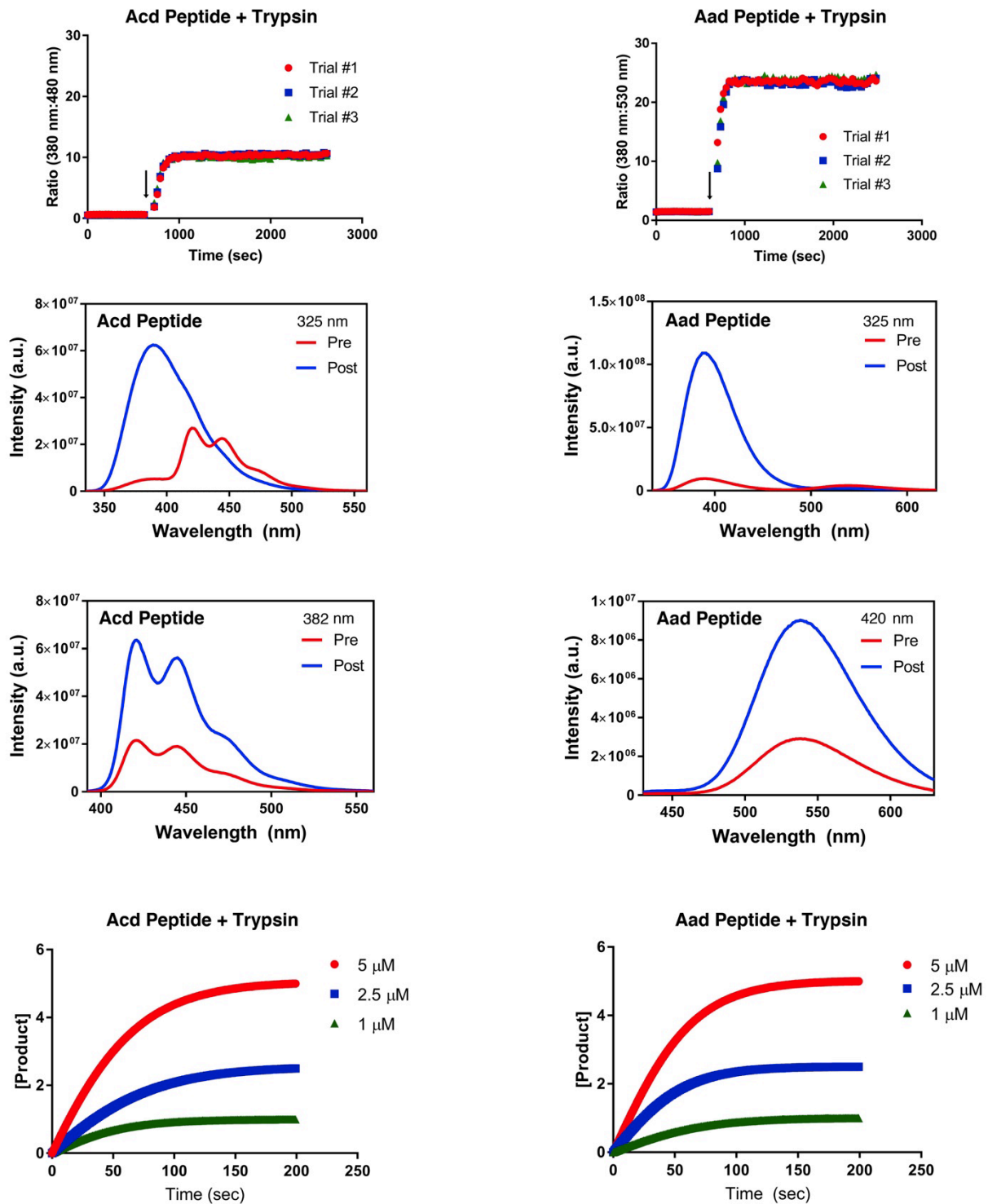


Figure S10. Trypsin FRET Assay. Fluorescence emission data for Acd (left) and Aad (right) peptides. Top: FRET ratios measured in a Tecan plate reader upon addition of trypsin (indicated by arrow). Three trials are shown to demonstrate reproducibility. Middle: Emission spectra of peptides pre- and post-cleavage with excitation at 325 nm or 382 nm (Acd) or 420 nm (Aad). Bottom: Stopped flow data shown for trials at varying peptide concentrations. Ratiometric FRET data were converted to product concentrations.

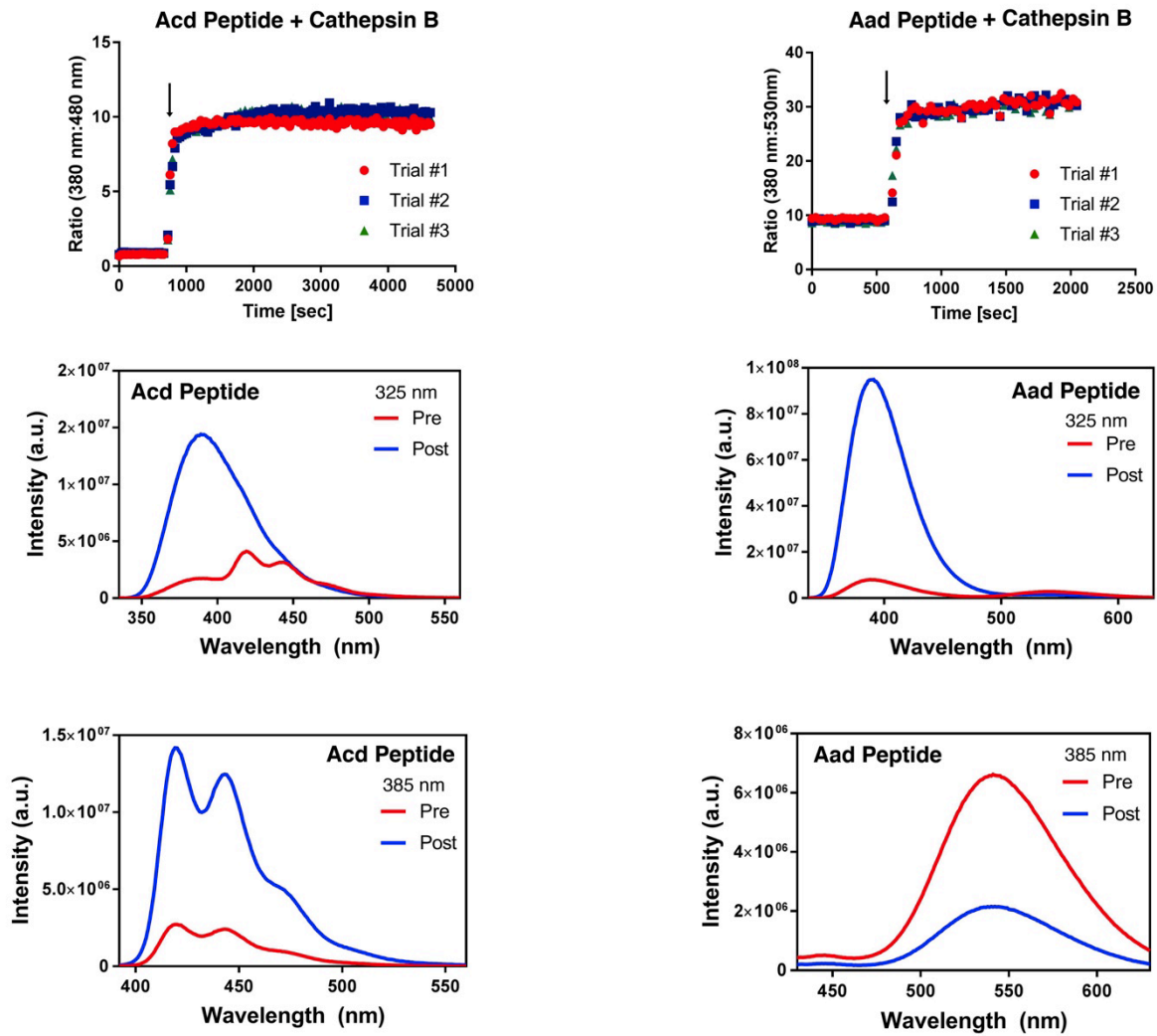


Figure S11. Cathepsin B FRET Assay. Fluorescence emission data for Acd (left) and Aad (right) peptides. Top: FRET ratios measured in a Tecan plate reader upon addition of cathepsin B (indicated by arrow). Three trials are shown to demonstrate reproducibility. Bottom: Emission spectra of peptides pre- and post-cleavage with excitation at 325 nm or 385 nm (Acd) or 420 nm (Aad).

Amino Acid Activation by Acd Aminoacyl tRNA Synthetase (AcdRS)

AcdRS Expression and Purification. We had previously generated recombinant Acd aminoacyl tRNA synthetase (AcdRS1) by overexpressing polyHis-tagged AcdRS1 from a pBAD construct in arabinose auto-induction media and purifying the enzyme using both cobalt and heparin affinity columns.^[6] For our amino acid activation assay, we used an enzyme aliquot from that AcdRS1 preparation, which we had exchanged into buffer (50 mM HEPES-KOH pH 7.5, 20 mM NaCl, 10 mM MgCl₂, 1 mM DTT, 50% glycerol) and stored at -80 °C.

AcdRS Activity Test Using a Malachite Green Assay. As previously described,^[6] we tested the activity of AcdRS1 with either Acd or Aad using an adapted form of an enzyme-coupled, colorimetric assay.^[7] Formation of inorganic pyrophosphate (PPi) during the amino acid activation step of the reaction is coupled to the activity of inorganic pyrophosphatase (PPiase), which splits PPi into two inorganic phosphate (Pi) molecules. Under acidic conditions, free Pi reacts with Malachite green to produce a colored Malachite green phosphomolybdate complex (absorbing at 600-660 nm). Since our AcdRS1 freezer stock only retained partial activity (data not shown), we chose conditions that drove the amino acid activation reaction toward completion. We prepared reactions in triplicate containing reaction buffer (50 mM HEPES-KOH pH 7.5, 20 mM NaCl, 10 mM MgCl₂, 1 mM DTT), 0.2 mM ATP, 2 U/mL PPiase (NEB), 250 μM amino acid substrate (Acd or Aad), and 4 μM AcdRS1 enzyme. To control for background phosphate signal in our reactions, we excluded AcdRS1 enzyme; to demonstrate the ATP-dependence of the amino acid activation reaction, we also prepared reactions excluding ATP. Reactions were incubated at 37 °C for 100 min, then quenched with three reaction volumes of ice-cold EDTA solution to a final concentration of 10 mM. Quenched reactions were developed with Malachite green per kit instructions (Malachite Green Phosphate Assay Kit, Sigma #MAK307) in a 96-well plate (Corning Costar). Absorbance at 620 nm was measured in an Infinite F200 plate reader (Tecan), and a Pi standard curve was used to calculate phosphate concentration in each reaction. Data were reported as mean and standard deviation (n=3) of amount of PPi formed after subtracting the background signal from the no enzyme control reactions.

Aad Docking Model. The structure of 2-aminoacridone was optimized in Gaussian at the AM1 level of theory with a 6-31G basis set. The optimized structure was aligned with the sidechain of

Acid docked into a homology model of AcdRS1 built using the crystal structure coordinates, as previously described.^[6,8] The 2-aminoacridone molecule was aligned to position the amino group as in Aad. Steric clashes with the sidechains of Val164 and Ala167 were observed.

References

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