Supporting Information

Design and Investigation of a Series of Rhodamine-Based Fluorescent Probes for Optical Measurements of pH

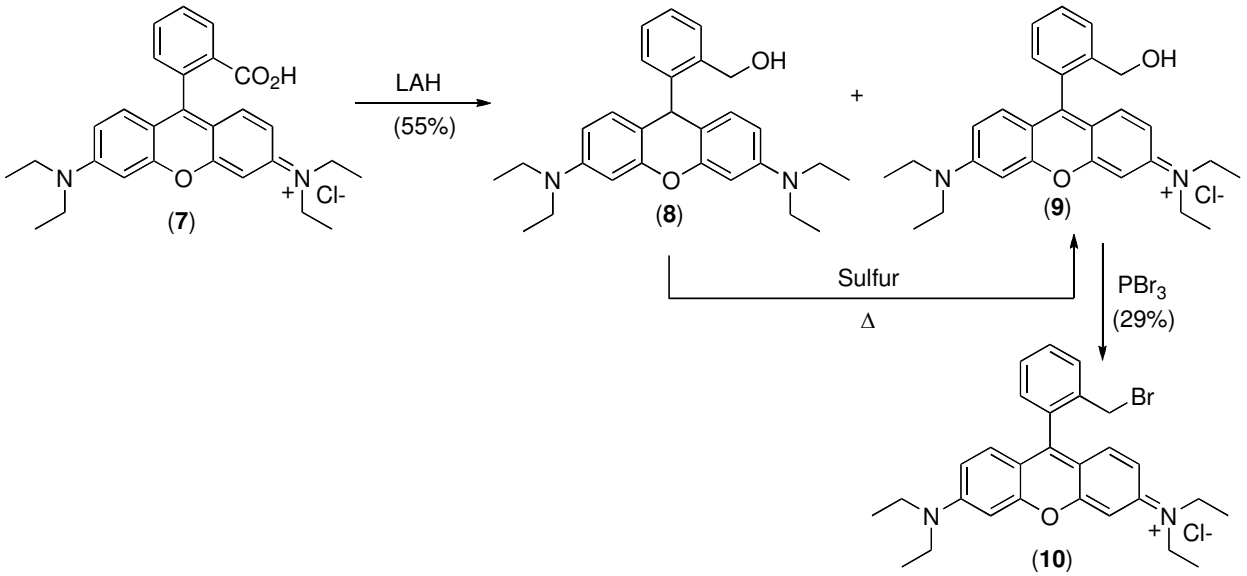
*Quinn A. Best; Ruisong Xu; Matthew E. McCarroll; Lichang Wang; and Daniel J. Dyer**

Department of Chemistry & Biochemistry, Southern Illinois University, Carbondale, IL 62901-4409, USA

Materials. All reagents were purchased from Acros, Aldrich, or Fisher Scientific and used as supplied unless otherwise noted. NMR solvents were purchased from Cambridge Isotopes. Column chromatography was performed with standard grade silica gel (63-200 Mesh) from Sorbent Technologies and TLC was performed on 250 μm silica gel 60 polyester backed plates with F_{254} fluorescent indicator from Whatman.

Equipment. NMR data were collected on a 400 MHz Mercury Varian NMR. Fluorescence spectra were recorded on a modular spectrofluorometer (Photon Technology International Inc.) by using FELIX software. Sample temperature was controlled and adjusted using a thermocirculator (NESLAB Instruments Inc.). The path length of the cell was 1 cm with a volume of 3.0 mL. Absorbance data was obtained using a Perkin-Elmer Lambda 25 UV-Vis

spectrometer. Mass spectra were measured with a Micromass VG 70-VSE (B) spectrometer, or an Applied Biosystmes Voyager-DE STR, or a Bruker Daltonics flexAnalysis, or a Micromass Q-Tof Ultima instrument at the Mass Spectrometry Laboratory of University of Illinois, Urbana Champaign.



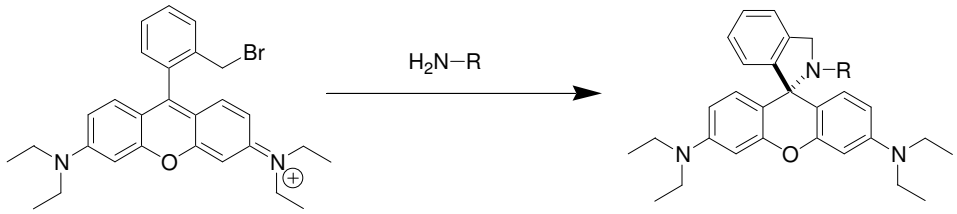
Scheme 1. Synthesis of rhodamine-based intermediate **10**.

(2-(3, 6-bis(diethylamino)-9H-xanthen-9-yl)phenyl)methanol (8). $LiAlH_4$ 95% (1.60 g, 40.1 mmol) was added to a solution of Rhodamine B (**7**) (10.0 g, 20.9 mmol) in 300 mL THF in a 500 mL round bottom flask; the mixture was stirred overnight under argon at room temperature. Water (100 mL) was then added dropwise and the mixture was extracted with $CHCl_3$ (3 × 150 mL). The combined organic layers was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness with rotovap to offer the crude product. Purification by column chromatography using graduated elutions from 70/30 (Hex/EtOAc) to 100% EtOAc yielded 4.1 g (46%) of a mixture of **8 and 9** as a red foam. Additional purification was carried out by a second column run using 100% EtOAc to obtain a pure sample of **8** for

analysis: R_F = 0.36 (Hex/EtOAc, 70/30); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 1H), 6.72 (m, 3H), 5.58 (d, *J* = 8.6 Hz, 2H), 6.43 (s, 2H), 6.29 (d, *J* = 10 Hz, 2H), 5.37 (s, 1H), 4.59 (s, 2H), 3.33 (q, *J* = 14.0 Hz, 8H), 1.17 (t, *J* = 7.0 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.94, 148.01, 145.10, 138.82, 131.61, 130.24, 129.69, 128.24, 127.10, 111.75, 107.72, 99.06, 63.21, 44.65, 40.11, 12.90; MS (TOF, ESI$^+$): *m/z* 430.3 (M^+), 429.3 $(M-H)^+$. HRMS (TOF, ESI$^+$): *m/z* calcd for $(M-H)^+$, 429.2542, found, 429.2545; Anal. Calcd for $C_{28}H_{34}N_2O_2$: C: 78.10; H: 7.96; N: 6.51, found: C: 77.11; H: 7.72; N: 6.46.

9-(2-hydroxylmethylphenyl)-3, 6-bis(diethylamino)- xanthylium chloride (9). The mixture of compounds **8** and **9** (1.64 g, 3.81 mmol) and elemental sulfur (0.9 g, 28.1 mmol) was heated to 160 ^{o}C under vacuum in a 50 mL pear shaped flask. After 30 minutes, the mixture was cooled to room temperature and 50 mL of ethanol was added, followed by addition of 4 mL of concentrated HCl. Water (100 mL) was then added and the resulting mixture was extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness to offer the crude product. This was purified by column chromatography with sequential elutions of 70/30 (Hexane/EtOAc) (to remove the starting materials) and 40/20 ($CHCl_3$/EtOH) for the product. Recrystallization from $CHCl_3$/Hexane offered 0.96 g (54%) of **9** as a purple crystals: R_F = 0.30 (CH_2Cl_2/EtOH, 90/10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 7.8 Hz, 1H), 7.58 (t, J = 7.8 Hz, 1H), 7.38 (t, J = 7.6 Hz, 1H), 7.25 (d, J = 7.7 Hz, 2H), 7.07 (d, J = 7.6 Hz, 1H), 6.85 (dd, J = 7.6 Hz, 2.2 Hz, 2H), 6.74 (s, 2H), 4.45 (s, 2H), 3.62 (q, J = 14.5 Hz, 8H), 1.33 (t, J = 7.05 Hz, 12H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 158.20, 155.86, 141.48, 132.79, 130.44, 129.90, 129.07, 128.25, 126.81, 114.34, 114.19, 96.30, 61.65, 46.28, 12.86; MS (MALDI): m/z 428.96 (M^+-Cl), calcd for (M^+-Cl), 429.25.

9-(2-bromomethylphenyl)-3, 6-bis(diethylamino)- xanthylium chloride (10). A solution of compound **9** (0.6 g, 1.14 mmol) in 30 mL of $CHCl_3$ in a 100 mL round bottom flask was cooled to 0 ^oC with an ice bath. PBr_3 (0.4 g, 1.47 mmol) was added dropwise by syringe and the reaction mixture was stirred for 2 hours. The reaction was allowed to warm to room temperature and was stirred for another 2 hours. The reaction was quenched with aqueous 10% Na_2CO_3 and extracted by $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness to offer 0.2 g (29%) of **10** as a purple solid. No further purification was needed: R_F = 0.5 (CH_2Cl_2/EtOH, 90/10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 7.8 Hz, 1H), 7.49 (t, *J* = 7.2 Hz, 1H), 7.42 (t, *J* = 7.3 Hz, 1H), 7.07 (d, *J* = 7.1 Hz, 1H), 7.00 (d, *J* = 7.9 Hz, 2H), 6.85 (dd, *J* = 9.2 Hz, 2H), 6.69 (s, 2H), 4.18 (s, 2H), 3.54 (q, *J* = 15 Hz, 8H), 1.19 (t, *J* = 7.01 Hz, 12H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 157.86, 155.83, 155.03, 135.73, 131.80, 131.68, 130.94, 130.82, 129.62, 114.59, 113.74, 96.69, 46.36, 43.48, 12.76; MS (TOF, ESI$^+$): *m/z* 491.2 (M^+), 493.2 (M^+); HRMS (TOF, ESI$^+$): *m/z* calcd for (M^+), 491.1698, found, 491.1714; Anal. Calcd for $C_{28}H_{32}BrClN_2O$: C: 63.70; H: 6.11; N: 5.31, found: C: 62.62; H: 6.16; N: 5.33.



10

1) R=H (85%)
2) R=Me (81%)
3) R=Benzyl (75%)

Scheme 2. Reaction between intermediate **10** and primary amines.

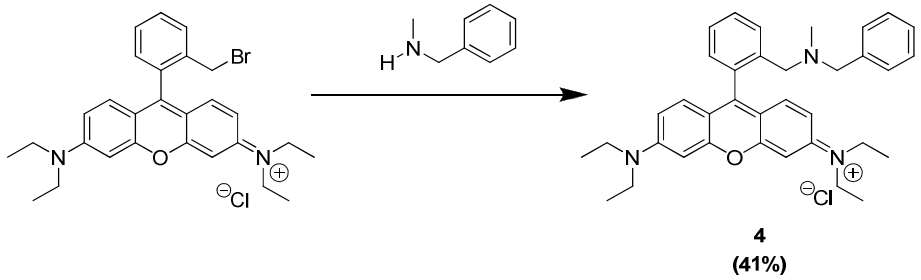
2,3-dihydrospiro[isoindole-1,9'-xanthene]-3',6'-diamine (1). In a dry 50 mL round bottom flask, 300 mg of compound **10** (0.57 mmol) and 15 mL of a 2.0 M solution of ammonia in

ethanol was added. The solution was stirred for 36 hours at room temperature. Evaporation of the solvent offered the crude product. Purification by column chromatography with graduated elutions from 70/30 (Hex/EtOAc) to 100% EtOAc yielded 207 mg (85%) of **1** as a colorless oil, which later formed a light pink solid: R_F = 0.31 (50/50 Hex/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 7.43 Hz, 1H), 7.26 (t, *J* = 7.23 Hz, 1H), 7.18 (t, *J* = 7.62 Hz, 1H), 6.94 (d, *J* = 7.42 Hz, 1H), 6.72 (d, *J* = 8.60 Hz, 2H), 6.36 (s, 2H), 6.31 (d, *J* = 8.79 Hz, 2H), 4.43 (s, 2H), 3.31 (q, *J* = 7.03 Hz, 8H), 2.03 (s, 1H), 1.32 (t, *J* = 7.03 Hz, 12H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 152.03, 148.15, 141.13, 129.44, 127.90, 127.30, 125.11, 122.20, 115.59, 108.16, 98.19, 65.77, 51.35, 44.67, 12.93; MS (MALDI): m/z 428.91 (M^+-H), calcd for (M^+-H), 427.26.

2-methyl-2,3-dihydrospiro[isoindole-1,9'-xanthene]-3',6'-diamine (2). In a dry 50 mL round bottom flask, 300 mg of compound **10** (0.57 mmol) and 25 mL of a 2.0 M solution of methylamine in ethanol was added. The solution was stirred for 36 hours at room temperature. Evaporation of the solvent offered the crude product. Purification by column chromatography with graduated elutions from 70/30 (Hex/EtOAc) to 50/50 (Hex/EtOAc) yielded 204 mg (81%) of **2** as a colorless oil, which later formed a light pink solid: R_F = 0.54 (50/50 Hex/EtOAc);^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 7.43 Hz, 1H), 7.25 (t, *J* = 7.23 Hz, 1H), 7.18 (t, *J* = 7.62 Hz, 1H), 6.94 (d, *J* = 7.42 Hz, 1H), 6.72 (d, *J* = 8.60 Hz, 2H), 6.37 (s, 2H), 6.31 (d, *J* = 8.79 Hz, 2H), 4.05 (s, 2H), 3.31 (q, *J* = 7.03 Hz, 8H), 2.02 (s, 3H), 1.43 (t, *J* = 7.03 Hz, 12H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 153.71, 148.88, 148.18, 140.65, 130.33, 127.66, 127.10, 124.95, 121.94, 111.13, 107.81, 97.91, 68.38, 58.37, 44.62, 34.70, 12.97; MS (MALDI): m/z 440.94 (M^+-H), calcd for (M^+-H), 441.28.

2-benzyl-2,3-dihydrospiro[isoindole-1,9'-xanthene]-3',6'-diamine (3). A solution of compound **10** (300 mg, 0.57 mmol) in 20 mL of THF was added to 0.63 g of benzylamine (5.9

mmol) in a 50 mL round bottom flask. The reaction was stirred at room temperature overnight. Evaporation of the solvent offered the crude product. Purification by column chromatography with graduated elutions from 70/30 (Hex/EtOAc) to 50/50 (Hex/EtOAc) yielded 221 mg (75%) of **3** as a red solid: R_F = 0.56 (50/50 Hex/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.2 (m, 8H), 6.92 (d, *J* = 7.3 Hz, 1H), 6.80 (d, *J* = 9.2 Hz, 2H), 6.37 (m, *4*H), 4.00 (s, 2H), 3.39 (s, 2H), 3.34 (q, *J* = 15.5 Hz, 8H), 1.18 (t, *J* = 7.03 Hz, 12H); ^{13}C NMR (400 MHz, $CDCl_3$) 153.24, 149.90, 148.14, 140.83, 139.62, 130.81, 128.71, 128.14, 127.65, 126.59, 124.79, 122.09, 107.73, 97.77, 68.18, 55.92, 53.01, 44.55, 12.98; MS (MALDI): *m/z* 518.31 (M^+-H), calcd for (M^+-H), 518.38.



4
(41%)

Scheme 3. Reaction between intermediate **10** and N-methylbenzylamine.

9-(2-{[benzyl(methyl)amino]methyl}phenyl)-6-(diethylamino)-N,N-diethyl-3H-xanthen-3-iminium chloride (4). A solution of compound **10** (180 mg, 0.34 mmol) in 20 mL of dry dichloromethane was added to 0.42 g of N-methyl-benzylamine (3.4 mmol) in a 50 mL round bottom flask. The reaction was stirred at room temperature overnight. Evaporation of the solvent offered the crude product. Purification by column chromatography with graduated elutions from dichloromethane to 90/10 (DCM/MeOH) yielded 80 mg (41%) of **4** as a dark purple solid: R_F = 0.5 (80:20:2 DCM:MeOH:NH_4OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 7.6, 1H), 7.50 (t, *J* = 7.4 Hz, 1H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.06 (m, 6H), 6.75 (m, 6H), 3.60 (q, J = 7.2, 8H), 3.22 (s, 2H), 3.11 (s, 2H) 1.78 (s, 3H), 1.28 (t, J = 7.0 Hz, 12H); ^{13}C NMR (400

MHz, $CDCl_3$) δ 158.57, 157.95, 155.67, 138.39, 131.96, 130.85, 130.32, 129.74, 128.84, 128.66, 128.17, 127.90, 127.03, 114.16, 113.61, 96.52, 62.56, 60.21, 46.38, 41.95, 12.91; MS (MALDI): m/z 533.24 (M^+-Cl), calcd for (M^+-Cl), 532.33.

pH Measurements. The following procedure was used for the fluorimetric analysis of compounds **1-4**. In a 200 mL beaker, 50 mL of a 0.37 μM solution of the fluorescent probe, in 15% ethanol/water, was allowed to stir in open air for approximately 0.5 hour allowing the solution to become saturated with oxygen. Using a digital pH meter (Accumet, AB15) equipped with a glass electrode (Accumet, pH/ATC Calomel) the pH was monitored and adjusted to acidic or basic conditions using small aliquots (microliter pipette, Oxford) of a 2 M HCl or 2 M NaOH solution, respectively. The pH was allowed to stabilize for ~ 1 minute and then 3 mL of the adjusted solution was added to a quartz cuvette for fluorescent analysis and UV-vis. The fluorimeter was operated using an excitation slit width of 3 nm and an emission slit width of 3 nm. A 0.37 μM solution of rhodamine B in H_2O was used as a standard.

Figure 1 Shows excitation and emission spectra as a function of pH for compound **3** (a) and compound **4** (b). Measured using 0.37 μM solutions in 15% EtOH/H_2O (v/v).

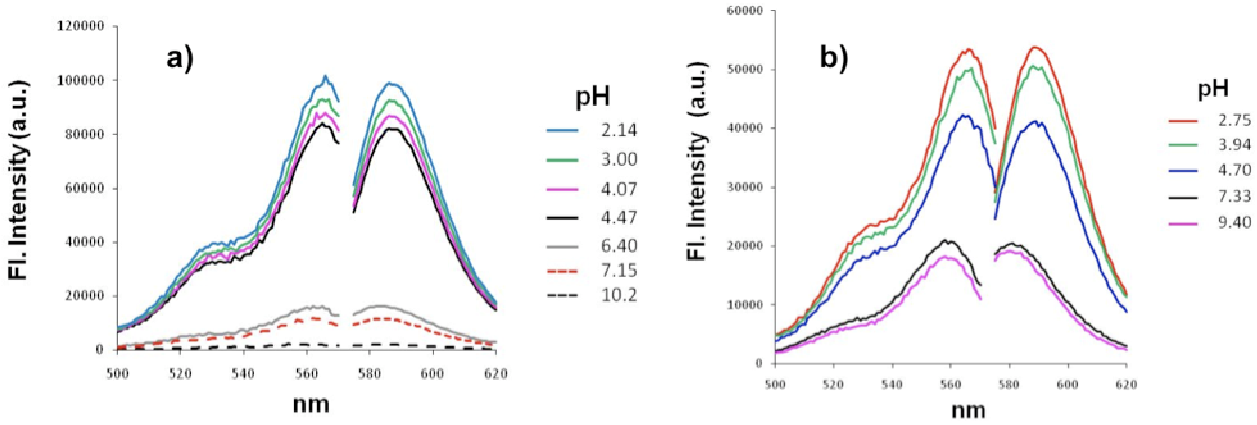


Figure 2 ^{1}H NMR of **1**

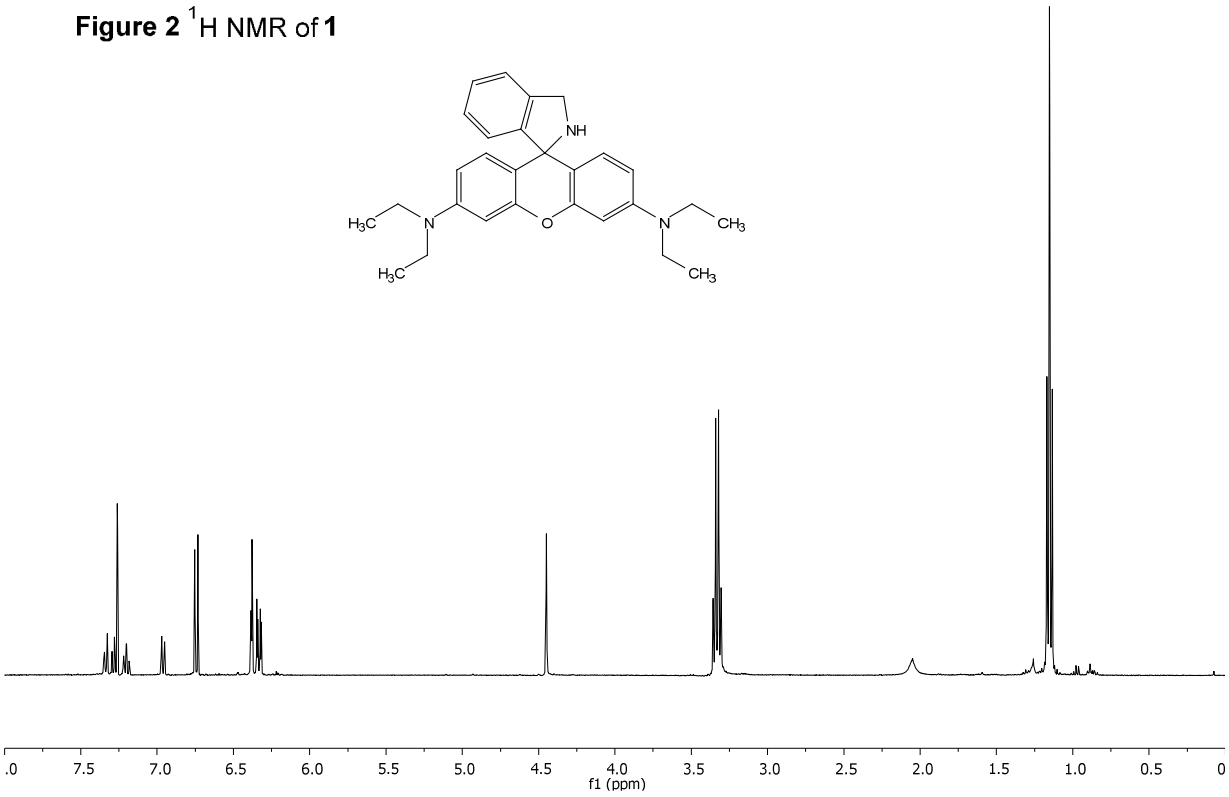


Figure 3 ^{1}H NMR of **2**

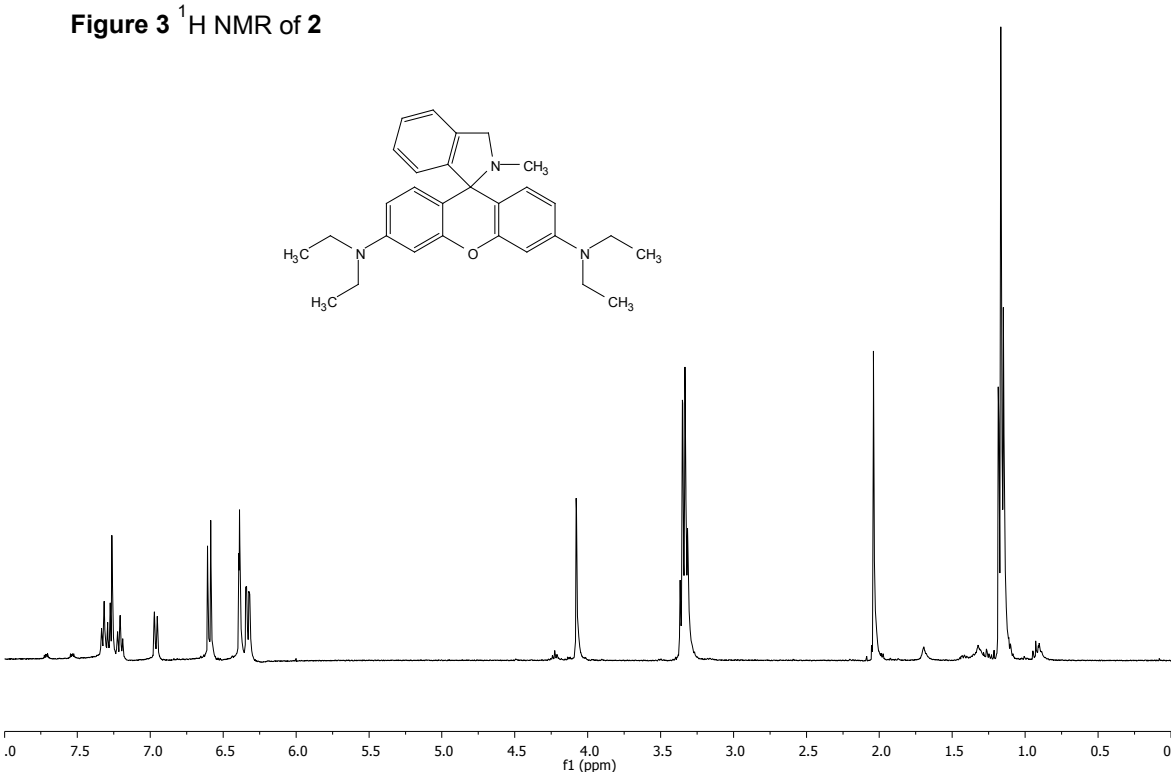


Figure 4 ^{1}H NMR of **3**

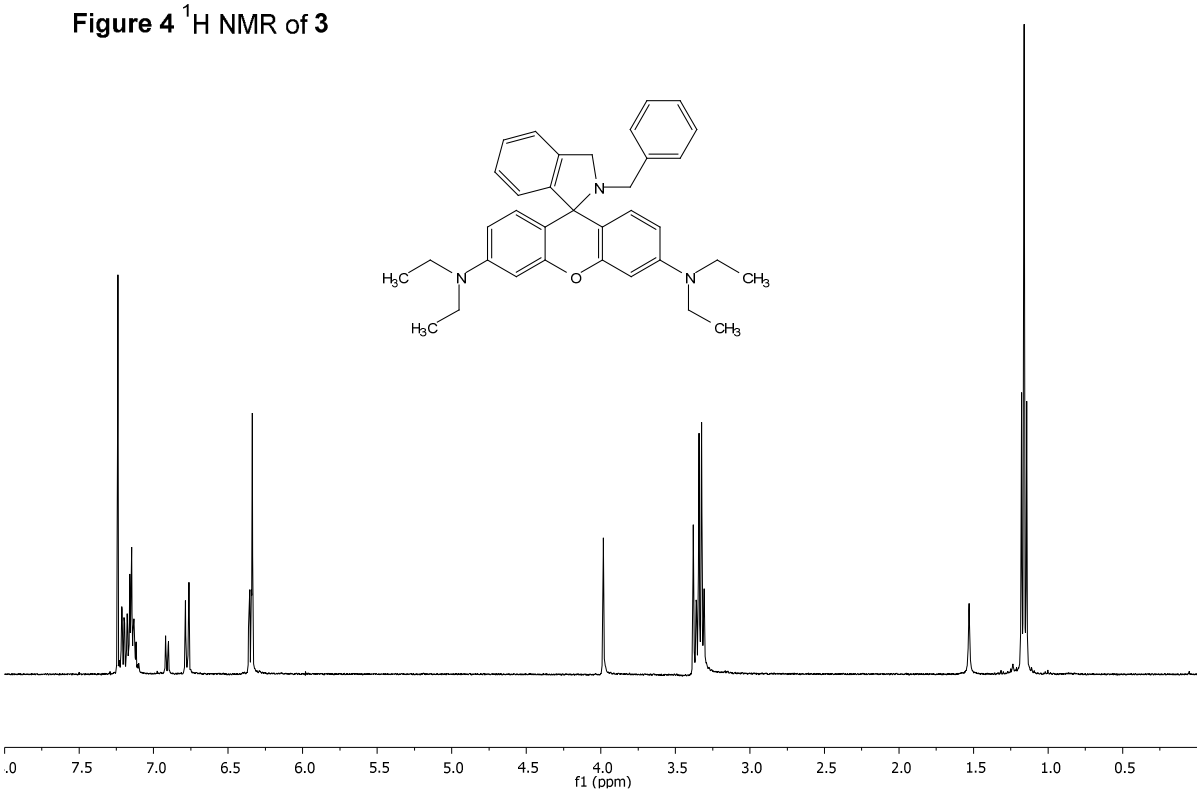


Figure 5 ^{1}H NMR of **4**

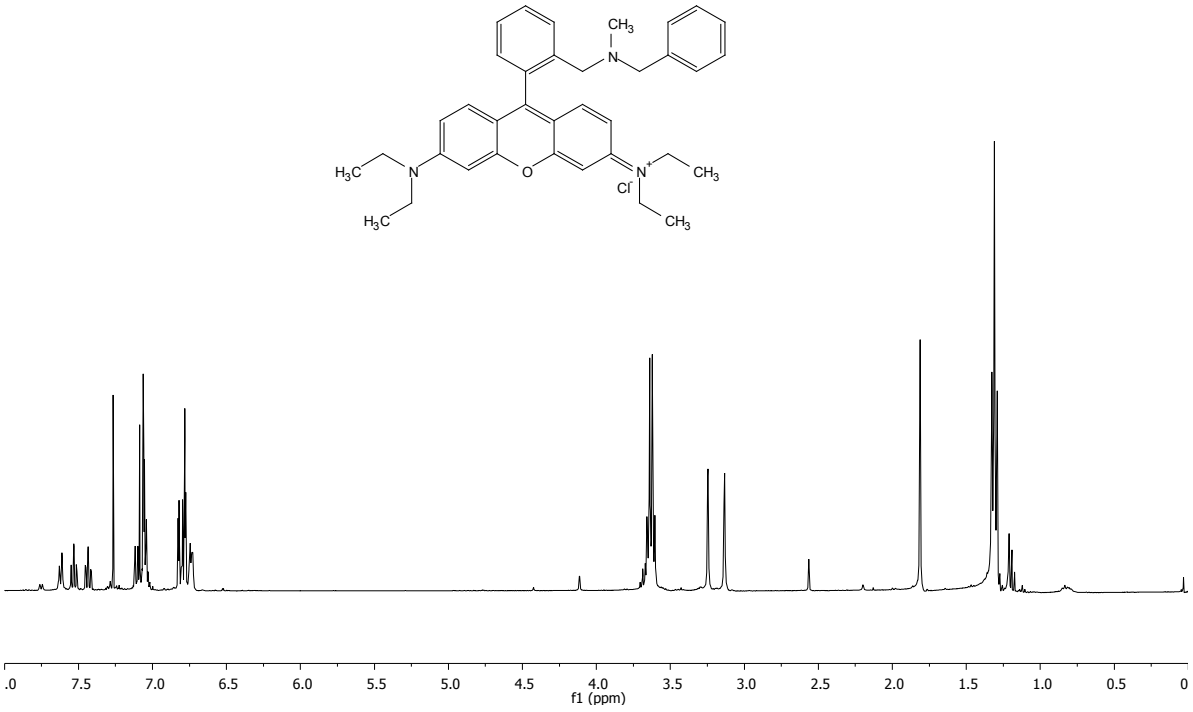
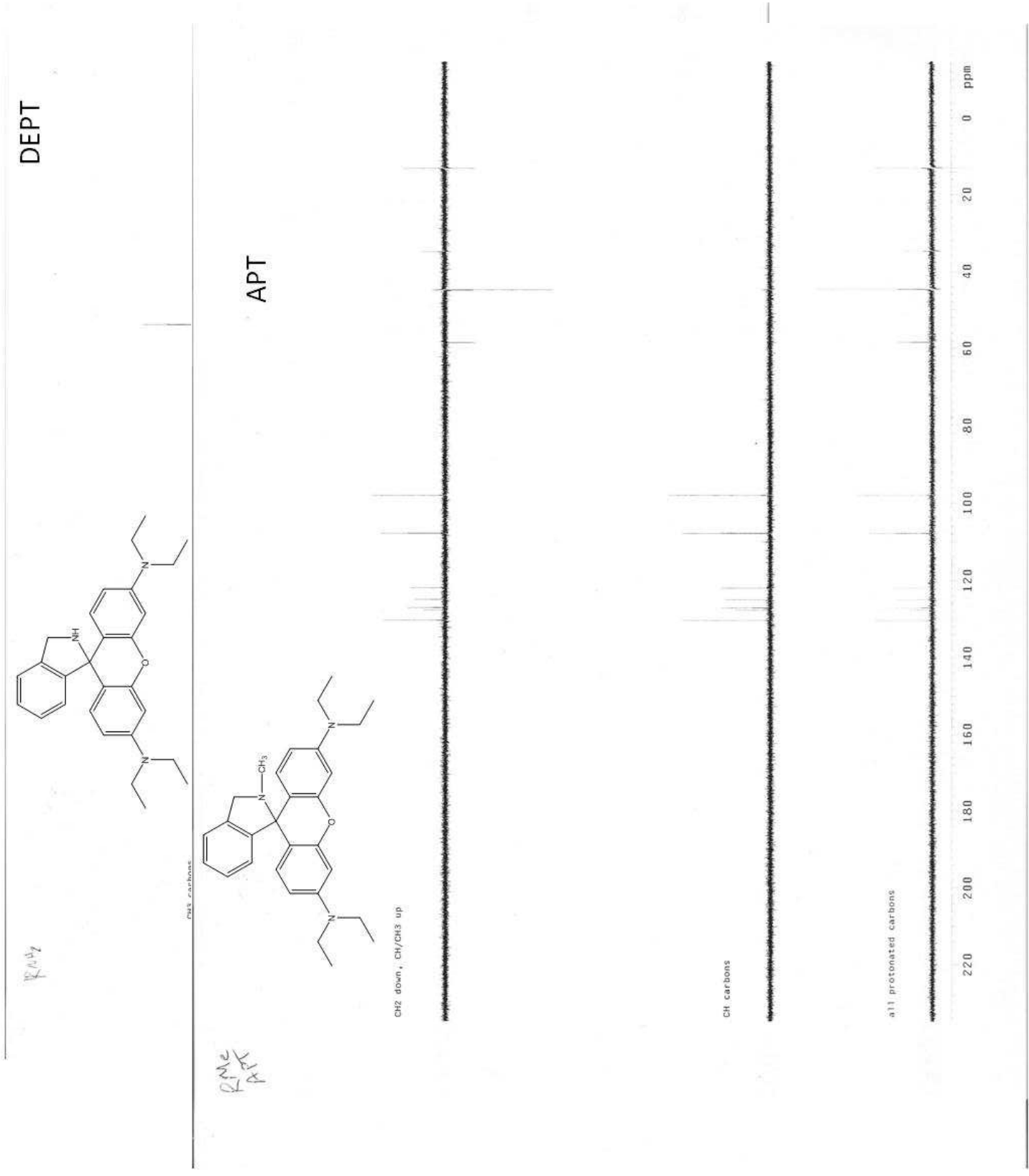


Figure 6 DEPT spectra of **1**

Figure 7



APT spectra of **2**

Figure 8 DEPT spectra of **3**

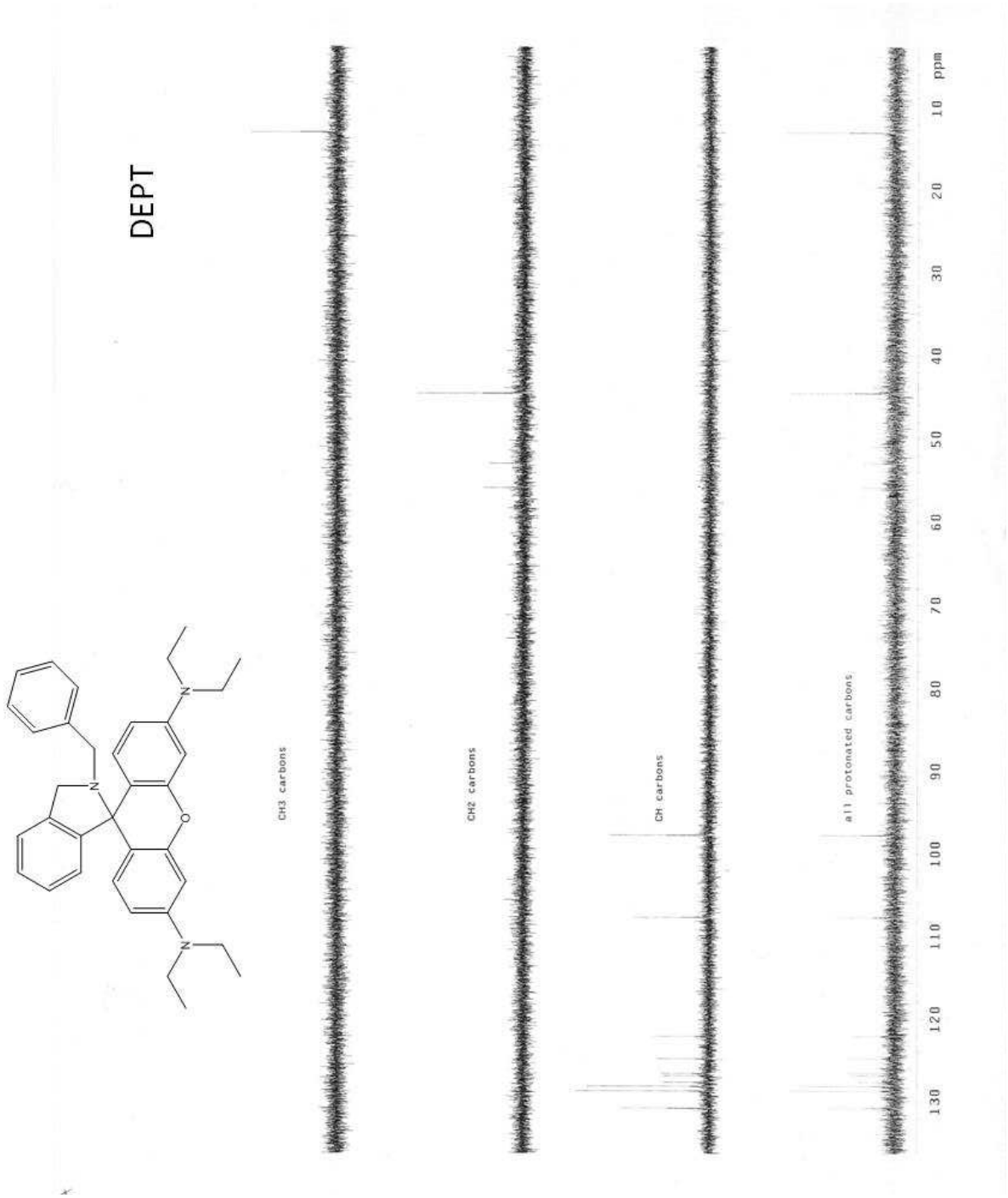


Figure 9 DEPT spectra of **4**