A Water Soluble Fluorene Derivative as Near-Neutral Ratiometric PH Fluorescent Indicator

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Experimental Section

2-Nitro-7-iodofluorene **2** was synthesized according to a literature procedure.¹ ¹H and ¹³C NMR spectra were recorded in CDCl₃ on a Varian 300 or Varian 500 NMR spectrometer (300 MHz or 500 MHz for ¹H, referenced to tetramethylsilane at δ = 0.0 ppm and 75 MHz or 125 MHz for ¹³C, referenced to CDCl₃ at δ = 77.0 ppm). Elemental analyses were performed by Atlantic Microlab. The HRMS analyses were carried out on a Finnigan MAT 95Q hybrid-sector mass spectrometer (ThermoFinnigan, San Jose, CA).

Preparation of 7-nitro-9H-fluorene-2-carbonitrile 3. 2-Nitro-7-iodo-9H-fluorene **2** (56.30 g, 0.167 mol) and CuCN (15.70 g, 0.175 mol) in dry DMF (100 mL) were refluxed for 1.5 h under N₂. Upon cooling, a solution of FeCl₃·6H₂O (66.8 g) in 36% HCl (16.6 mL) and water (100 mL) was added and the mixture was heated at 60–70 °C for 1h, and allowed to cool to room temperature with stirring. The precipitate was collected by filtration, then washed with water and 95% ethanol. The crude product was recrystallized

from DMSO/toluene to afford 23.4 g of pale yellow solid (59% yd). m.p. 280.5–282 °C. (lit. 281–282 °C).² ¹H NMR (300 MHz, CDCl₃-d₆) δ 8.45(s, 1H, Ph-H), 8.34(d, *J* = 9.0 Hz, 1H, Ph-H), 7.96-7.89(m, 3H, Ph-H), 7.75(d, *J* = 9.0 Hz, 1H), 4.10(s, 2H, CH₂).

Preparation of 7-nitro-9H-fluorene-2-carboxylic acid 4. 7-Nitro-9H-fluorene-2-carbonitrile **3** (21.43 g, 0.091 mol) in 70% H₂SO₄ (83 mL) and acetic acid (83 mL) was refluxed for 48 h. Upon cooling, the precipitate was collected by filtration and recrystallized from DMSO twice to afford 13.4 g of product (58% yd). m.p. 338-340°C (lit. 334-335 °C). ¹H NMR (300 MHz, CDCl₃) δ 8.48 (s, 1H, Ph-H), 8.31 (d, *J* = 8.4 Hz, 1H, Ph-H), 8.22 (s+d, 2H, Ph-H), 8.17 (d, *J* = 7.8 Hz, 1H, Ph-H), 8.03 (d, *J* = 8.1 Hz, 1H, Ph-H), 4.15 (s, 2H, CH₂). ¹³C NMR (75 MHz, CDCl₃) 167.73, 147.39, 146.92, 145.97, 145.68, 143.52, 131.28, 129.14, 126.92, 123.53, 122.29, 122.23, 121.17, 37.59. HRMS-ESI theoretical *m/z* [M+Na]⁺=278.0424, found 278.0441.

Preparation of 2-(7-nitro-9H-fluoren-2-yl)-benzothiazole 5. 7-Nitro-9H-fluorene-2-carboxylic acid **4** (8.0 g, 0.0313 mol) and thionyl chloride (20 mL) were refluxed for 4 h under N₂. Excess thionyl chloride was distilled, and the residue was dried *in vacuo*. The dried residue was mixed with 2-aminobenzenethiol (3.92 g, 0.0313 mol) in NMP (50 mL). The mixture was heated at 100–110 °C for 15 h. Upon cooling, 95% ethanol was added, and the precipitate was collected by filtration, washed with ethanol and hexane, and dried *in vacuo*. The crude product was recrystallized from DMSO to afford 8.0 g of **5** (74% yd). m.p. 298-299 °C, ¹H NMR (300 MHz, DMF-d₇) δ 8.72(s, 1H, Ph-H), 8.63(s, 1H, Ph-H), 8.57-8.43(m, 4H, Ph-H), 8.38(d, *J*=8.1 Hz, 1H, Ph-H), 8.29(d, *J*=9.0 Hz, 1H,

Ph-H), 7.77 (t, $J=8.1$ Hz, 1H, Ph-H), 7.68(t, $J=7.2$ Hz, 1H, Ph-H), 3.67(s, 2H, CH₂). HRMS-EI theoretical m/z $[M]^+=344.0620$, found 344.0620. Anal. Calcd. for (C₂₀H₁₂N₂O₂S): C, 69.75; H, 3.51; N, 8.13; S, 9.31. Found: C, 69.68; H, 3.53; N, 8.09; S, 9.39.

Preparation of 3-[2-benzothiazol-2-yl-9-(2-cyanoethyl)-7-nitro-9H-fluoren-9-yl]-propionitrile 6. 2-(7-Nitro-9H-fluoren-2-yl)-benzothiazole **5** (2.0 g, 5.8 mmol), acrylonitrile (0.92 g, 17.4 mmol), and triton B (0.12 g) in dioxane (50 mL) were heated at 60–70 °C for 48 h. Upon cooling, water was added, and the precipitate was collected by filtration. The crude product was purified by column chromatography using CH₂Cl₂/MeOH (99/1) as eluent to afford 2.51 g of yellow solid (91% yd). m.p. 236–237°C, ¹H NMR (500 MHz, CDCl₃) δ 8.43 (d, $J = 8.5$ Hz, 1H, Ph-H), 8.34 (s, 1H, Ph-H), 8.31 (s, 1H, Ph-H), 8.19 (d, $J = 7.5$ Hz, 1H, Ph-H), 8.13 (d, $J = 7.0$ Hz, 1H, Ph-H), 7.98 (m, 3H, Ph-H), 7.57 (t, $J = 7.5$ Hz, 1H, Ph-H), 7.47 (t, $J = 7.5$ Hz, 1H, Ph-H), 2.65 (m, 4H, CH₂), 1.73 (m, 2H, CH₂), 1.62 (m, 2H, CH₂). ¹³C NMR (75 MHz, DMSO-d₆) δ 167.58, 154.02, 149.06, 148.35, 148.19, 146.71, 142.02, 135.22, 134.61, 129.00, 127.41, 126.32, 125.18, 123.57, 123.48, 123.24, 123.03, 122.40, 120.68, 120.24, 55.14, 33.80, 12.49. HRMS-EI theoretical m/z $[M]^+=450.1150$, found 450.1156. Anal. Calcd. for C₂₆H₁₈N₄O₂S·0.38dioxane (determined by ¹H NMR) (475.99): C, 69.44; H, 4.46; N, 11.77. Found: C, 69.22; H, 4.56; N, 11.45.

Preparation of 3-[2-benzothiazol-2-yl-9-(2-cyanoethyl)-7-amino-9H-fluoren-9-yl]-propionitrile 7. 3-[2-Benzothiazol-2-yl-9-(2-cyanoethyl)-7-nitro-9H-fluoren-9-yl]-

propionitrile **6** (3.0 g, 6.66 mmol) in THF (70 mL) was degassed with N₂ for 20 min, then graphite (4.17 g) and hydriize hydrate (2.7 mL, 40 mmol) were added and further degassed for 30 min. The mixture was refluxed for 24 h to complete the reaction. The graphite was filtered off. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography using CH₂Cl₂/MeOH (98/2) as eluent to afford 2.7 g of yellow solid (82% yd). m.p. 90-91 °C ¹H NMR (500 MHz, CDCl₃) δ 8.12 (s, 1H, Ph-H), 8.09 (d, *J* = 8.0 Hz, 1H, Ph-H), 8.02 (d, *J* = 8.0 Hz, 1H, Ph-H), 7.93 (d, *J* = 8.0 Hz, 1H, Ph-H), 7.64 (d, *J* = 8.0 Hz, 1H, Ph-H), 7.56-7.51 (m, 2H, Ph-H), 7.41 (t, *J* = 7.8 Hz, 1H, Ph-H), 6.76 (d, *J* = 8.0 Hz, 1H, Ph-H), 6.68 (s, 1H, Ph-H), 4.04 (br. 2H, NH₂), 2.56 (m, 2H, CH₂), 2.38 (m, 2H, CH₂), 1.62 (m, 4H, CH₂). ¹³C NMR (75 MHz, CDCl₃) δ 167.18, 153.57, 147.80, 147.06, 144.20, 144.15, 134.43, 131.32, 130.22, 128.60, 126.12, 124.91, 122.65, 122.05, 121.27, 120.46, 119.11, 118.72, 115.42, 108.31, 53.29, 35.24, 12.11. Anal. Calcd. for C₂₆H₂₀N₄S·THF (determined by ¹H NMR) (492.64): C, 73.14; H, 5.73; N, 11.37; S, 6.51. Found: C, 72.80; H, 5.71; N, 11.31; S, 6.44.

Preparation of 3-[2-Benzothiazol-2-yl-9-(2-cyanoethyl)-7-diethylamino-9H-fluoren-9-yl]-propionitrile **8.** 3-[2-Benzothiazol-2-yl-9-(2-cyanoethyl)-7-amino-9H-fluoren-9-yl]-propionitrile **6** (0.77 g, 1.83 mmol) and triethylphosphate (3.3 g, 18.3 mmol) were refluxed under N₂ for 2 h. Excess triethylphosphate was distilled off under reduced pressure. A solution of sodium hydroxide (2 g) in water (12 mL) was added, and the mixture was refluxed for 1 h until the product solidified. The crude product was extracted with CH₂Cl₂ and purified by column chromatography using CH₂Cl₂/MeOH (100/2) to provide 0.84 g of product (96% yd). m.p. 190-191 °C ¹H NMR (500 MHz, CDCl₃) δ 8.10

(s, 1H, Ph-H), 8.07 (d, $J = 8.5$ Hz, 1H, Ph-H), 8.02 (d, $J = 8.0$ Hz, 1H, Ph-H), 7.97 (d, $J = 8.0$ Hz, 1H, Ph-H), 7.91 (d, $J = 8.0$ Hz, 1H, Ph-H), 7.57-7.48 (m, 3H, Ph-H), 7.38 (t, $J = 8.0$ Hz, 1H, Ph-H), 6.71 (d, $J = 9.0$ Hz, 1H, Ph-H), 6.56 (s, 1H, Ph-H), 3.44 (q, $J = 7.0$ Hz, 4H, NH₂), 2.54 (m, 2H, CH₂), 2.37 (m, 2H, CH₂), 1.62 (m, 4H, CH₂), 1.22 (t, $J = 7.0$ Hz, 6H, CH₃). ¹³C NMR (75 MHz, CDCl₃) δ 167.92, 154.16, 149.31, 147.63, 145.24, 144.48, 134.93, 131.08, 129.14, 127.54, 126.58, 125.30, 123.10, 122.57, 121.77, 120.86, 119.47, 119.09, 112.49, 104.60, 53.87, 45.12, 36.00, 13.02, 12.65. HRMS-ESI theoretical m/z [M+H]⁺=477.2107, found 477.2120, theoretical [M+Na]⁺=499.1927, found 499.1925. Anal. Calcd. for C₃₀H₂₈N₄S (476.64): C, 75.60; H, 5.92; N, 11.75; S, 6.73. Found: C, 75.27; H, 5.87; N, 11.40; S, 6.60.

Preparation of 3-[2-Benzothiazol-2-yl-9-(2-carboxy-ethyl)-7-diethylamino-9H-fluoren-9-yl]-propionic acid 1c. 3-[2-Benzothiazol-2-yl-9-(2-cyanoethyl)-7-diethylamino-9H-fluoren-9-yl]-propionitrile **7** (0.25 g, 0.52 mmol) in 50% H₂SO₄ (2 mL) and acetic acid (2 mL) was refluxed for 8 h. Upon cooling, the mixture was neutralized with powdered NaHCO₃ until the pH was around 4. After the water was evaporated under reduced pressure, the residue was dissolved in DMSO/acetone. Removal of the insoluble salt by suction filtration and the solvents by vacuum distillation gave a crude product. Crude **1c** was recrystallized from acetone/water/CH₃CN to afford 0.14 g crystals (52% yd). m.p. 184-185 °C. ¹H NMR (300 MHz, DMSO-d₆) δ 8.25-8.06 (m, 7H, Ph-H), 7.58-7.44 (m, 3H, Ph-H), 3.63 (b, 4H, CH₂), 2.54 (m, 4H, CH₂), 1.41 (m, 4H, CH₂), 1.07 (t, $J = 6.6$ Hz, 6H, CH₃). ¹³C NMR (75 MHz, DMSO-d₆) δ 174.09, 167.99, 154.05, 151.86,

149.76, 135.01, 128.12, 127.23, 126.03, 123.25, 122.80, 122.13, 54.86, 34.18, 29.72,
11.66. HRMS-ESI theoretical m/z $[M+H]^+=515.1999$, found 515.1995.

¹H-NMR of Compound 3.

¹H-NMR of Compound 4.

S423

¹³C-NMR of Compound 4.

nitrofluorene-9-carboxylic acid

exp1 s2pu1

SAMPLE		SPECIAL	
date	Dec 17 2004	time	not used
solvent	DMSO	gain	not used
File	exp	spin	not used
ACQUISITION		hst	0.008
sw	15822.8	ps99	19.000
at	1.818	alfa	20.000
np	57528	FLAGS	
fb	not used	fl	n
bs	64	lu	n
dl	1.000	dp	y
nt	1000	hs	nn
ct	1000	PROCESSING	
TRANSMITTER		lb	1.00
tn	C13	fn	not used
sfreq	75.408	DISPLAY	
tof	-380.2	sp	2479.7
tpwr	60	wp	10395.6
pw	9.500	rf	742.5
DECOUPLER		rfp	0
dn	H1	rp	-20.4
dcf	0	lp	-200.5
dm	yyy	PLOT	
dsm	w	wc	250
dpr	40	sc	0
dnt	12000	vs	70.1
		th	8
		el	no ph

S4203

O=[N+]([O-])c1ccc(cc1)-c2ccccc2

¹H-NMR of Compound 6.

Archive directory: /export/home/kschafer/vnmrsys/data
Sample directory: 6y207-3_09Nov2004
File: PROTON

Pulse Sequence: s2pu1
Solvent: CDCl3
Ambient temperature
UNITY-500 "unity"

Relax. delay 1.000 sec
Pulse delay 0.050 sec
Acq. time 0.000 sec
Width 5597.3 Hz
F2 300.136 MHz
OBSERVE 1 H 1.133.99 Hz
DATA PROCESSING
FT size 32768
Total time 1 min, 32 sec

¹³C-NMR of Compound 6.

¹H-NMR of Compound 7.

¹³C-NMR of Compound 7.

exp1 s2pul

SAMPLE		SPECIAL	
date	Dec 14 2004	temp	not used
solvent	CDCl3	gain	not used
file		exp	not used
ACQUISITION		hst	0.006
sw	3802.3	pu50	14.200
at	1.988	qlfa	20.000
np	14362	FLAGS	
fb	not used	il	n
be	18	in	n
d1	1.000	dp	y
nt	32	hs	nm
ct	32	PROCESSING	
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	300.104	sp	-76.1
tdf	58.2	wp	2607.9
tpwr	55	rt1	305.0
pu	7.100	rpf	0.000
DECOUPLER		rp	0.000
dn	C13	lp	0.000
dof	0	ph	0.000
dm	0	0	0.000
dmm	0	0	0.000
dpr	0	0	0.000
dpr	0	0	0.000
dpr	0	0	0.000

Integration values (from left to right): 6.413, 10.04, 3.13, 10.05, 3.37, 3.37, 13.63, 13.89, 18.60, 21.04, 0.000.

¹³C-NMR of Compound 8.

¹H-NMR of Compound 1c.

Archive directory: /export/home/kschafer/nmr/sys/data
Sample directory: sy225_21Dec2006-17:28:39
File: PROTON

Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
UNITY-500 "unity"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.092 sec
Width 5997.9 Hz
32 repetitions
OBSERVE H1, 499.7965251 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 32 sec

¹³C-NMR of Compound 1c.

References

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- ² Vink, J. A. J.; Verheijdt, P. L.; Cornelisse, J.; Havinga, E. *Tetra.* **1972**, *28*(19), 5081-5087.