

## *Supporting Information for:*

### **Turn-On, Fluorescent Nuclear Stains with Live Cell Compatibility**

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**Materials.** Reagents and solvents for chemical synthesis were purchased from Sigma-Aldrich and used without further purification. ctDNA was purchased from Sigma-Aldrich.

**Quantum Chemical Calculations.** Quantum chemical calculations on **1** and **2** were carried out utilizing the Gaussian '09 suite of electronic structure modeling software.<sup>1</sup> Ground state geometries of the dyes with were optimized by DFT with the B3LYP/6-31G(d) method using Truhlar's SMD solvation model.<sup>2</sup> Vertical transition energies and associated transition dipoles were obtained by TD-DFT calculations with the B3LYP/6-31G(d) method. Molecular orbitals and transition dipoles were visualized using the GaussView 5 program. The coordinates of optimized geometries are provided in the Supporting Information.

**Absorption and Steady State Fluorescence Spectroscopy.** UV-vis absorption spectra were obtained on a Perkin-Elmer Lambda 35 UV-vis spectrometer using chromophore solutions of 10  $\mu\text{M}$  unless otherwise noted in the text; path lengths were 1 cm. Fluorescence studies were performed on a Perkin-Elmer LS55 Fluorometer using probe concentrations of 1  $\mu\text{M}$  or less. For determination of  $\Phi_{\text{em}}$ , solutions were prepared to an optical density of less than 0.05 in order to minimize inner filter effects. Perylene in cyclohexane was used as a reference for quantum yields.<sup>3</sup>

**Flow Aligned Linear Dichroism Spectroscopy.** LD spectra were measured on a Chirascan CD spectropolarimeter equipped with an LD.3 linear dichroism detector (Applied Photophysics), run in linear dichroism mode. Sample orientation is achieved by shear flow in an outer rotating cylinder quartz Couette cell. A non-oriented sample was used as baseline. LD spectra were recorded at 1 dye per 5 basepairs in 0.5x TBE buffer.

**Cell Culture.** HEK-293 cells were maintained in DMEM (Dulbecco's Modification of Eagle's Medium with 4.5 g/L glucose, without L-glutamine and sodium pyruvate) containing 10% dialyzed FBS, 2 mM glutamine, 100 units/ml penicillin, 100  $\mu\text{g/ml}$  streptomycin, and 250  $\mu\text{g/ml}$  Geneticin at 37°C and 5%  $\text{CO}_2$ . MCF-7 cells were maintained in RPMI-1640 (with L-glutamine) containing 10% dialyzed FBS and 1% penicillin/streptomycin. For imaging, cells were seeded at a density of  $10^5$

cell/cm<sup>2</sup> in 96 microwell plates or 35 mm imaging dishes and incubated for at least 48 hours until a visible monolayer was established. Prior to imaging, DMEM was removed and L-15 (Leibovitz's L-15 modified, pH 7.3) was added to each well. 100  $\mu$ M stock solutions of **1**, **2**, DAPI and Hoechst 33342 were prepared in L15 media with 5% FBS and added to imaging wells or dishes to achieve the appropriate final concentration. Cells maintained a normal morphology during the course of the experiments and remained adhered to the imaging surface. For cell viability and growth studies, cells were plated at a density of 10<sup>5</sup> cell/cm<sup>2</sup> in 96 microwell plates. Confluence was initially 50% with readings taken every 24 hours until 100% confluence was reached; six sample wells were used for each dye concentration (10, 5, 1, 0.5, 0.1  $\mu$ M) and the control cells.

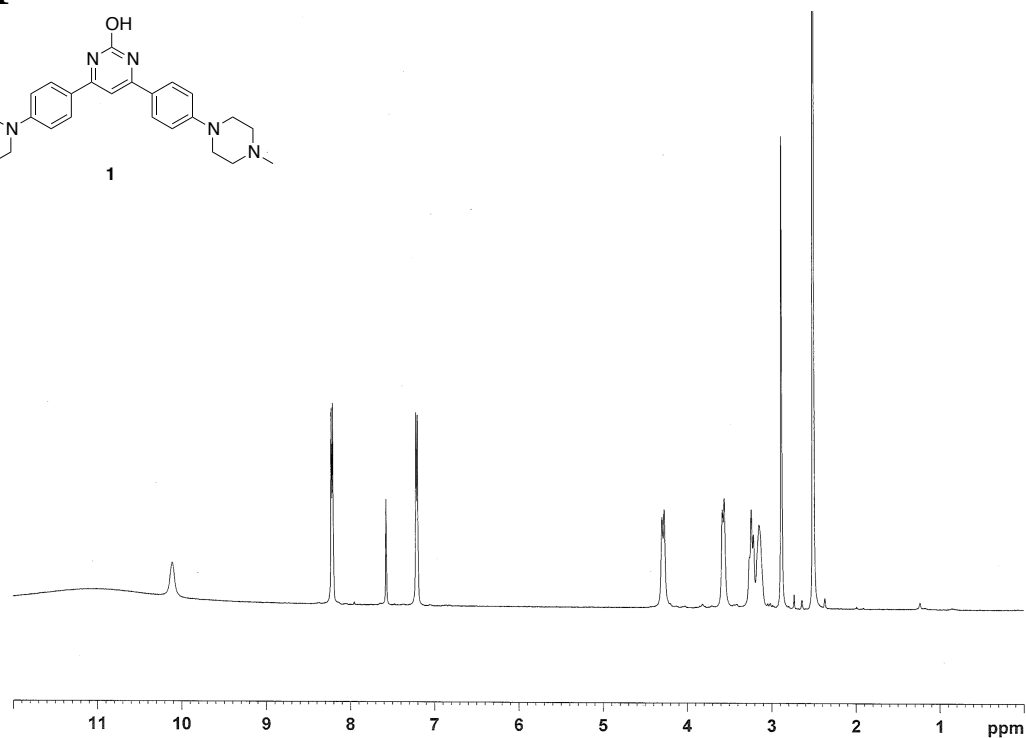
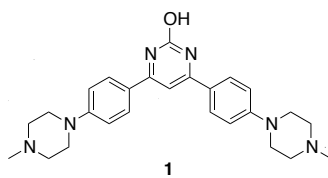
**Confocal Microscopy.** Imaging was performed on a Leica SP5 confocal microscope housed within the UM Biology Imaging Core Facility. Excitation was achieved using 405 nm (**1**, Hoechst 3342, DAPI), 514 nm (**2**) or 633 nm (CellMask DeepRed) lasers/laser lines. Time-course images were obtained in xyt mode with line averaging. Identical settings (photodiode gain, offset, etc.) were used when comparing brightness of the dyes. Images were analyzed using Fiji/ImageJA software (NIH, USA).

**Synthesis of 1.** 2.04 g (10.0 mmol) of 4-(4-methyl-1-piperazinyl)-benzaldehyde<sup>4</sup> was combined with 1.80 g (30.0 mmol) of urea and 35 mL of 10% HCl/ethanol in a pressure tube (65 mL) with a magnetic stirbar and stirred at room temperature. After 2 h, 2.18 g (10.0 mmol) of 1-(4-(4-methyl-1-piperazinyl)-phenyl)-ethanone<sup>5</sup> was added to the reaction mixture (3.0 mmol), which was capped heated at 100 °C for 24 h with vigorous stirring; after several hours, a yellow precipitate was observed. After 24 hours, the reaction mixture was cooled to room temperature; the precipitate was isolated by filtration and rinsed with saturated of NaHCO<sub>3</sub> solution. **1** was crystallized from isopropanol, isolated as yellow flakes by filtration and dried under vacuum. Yield: 960 mg, 22%; m.p; 210 °C (dec.); IR  $\nu_{\max}$  (cm<sup>-1</sup>): 789.18, 823.95, 887.56, 1227.9, 1580.45, 1623.71, 2930.72; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>),  $\delta$  (ppm): 2.88 (6H, s), 3.15 (4H, s), 3.21-3.24 (4H, d, *J* = 12.6 Hz), 3.56-3.58 (4H, d *J* = 10.9 Hz), 4.27-4.30 (4H, d *J* = 12.7

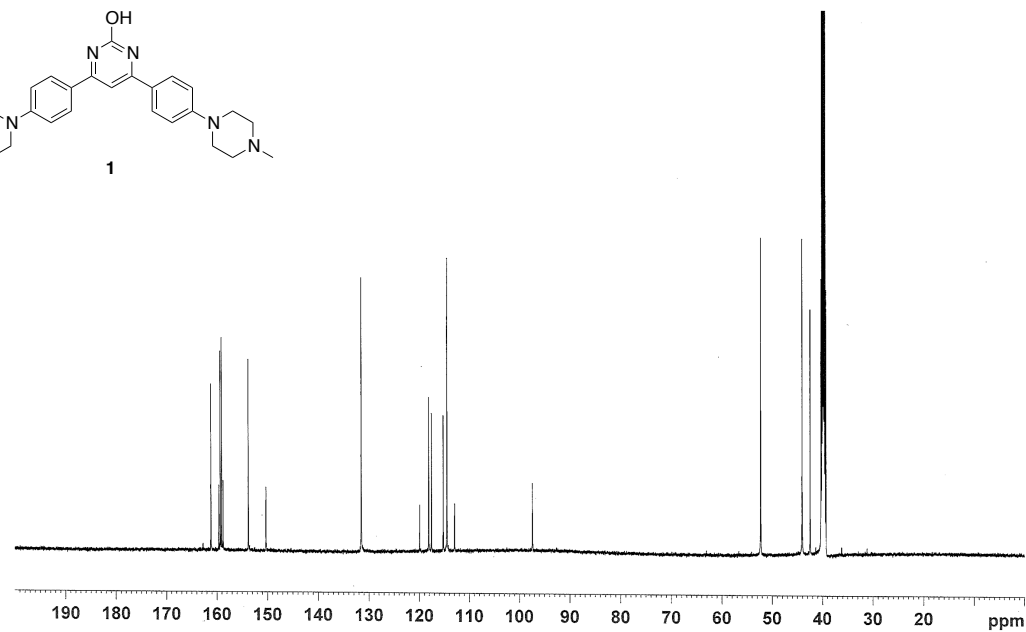
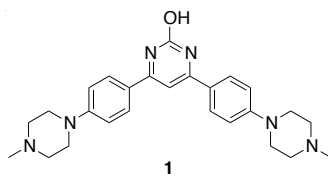
Hz), 7.20-7.22 (4H, d  $J = 8.7$  Hz), 7.57 (1H, s), 8.21-8.22 (4H, d  $J = 8.6$  Hz), 10.18 (2H, s);  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ , TFA),  $\delta$  (ppm): 42.46, 44.05, 52.27, 97.39, 114.49, 118.13, 131.54, 150.31, 153.80, 161.20; HRMS (ESI) calculated for  $\text{C}_{26}\text{H}_{33}\text{N}_6\text{O}^+$  ( $\text{M}+\text{H}^+$ ): 445.2710, found: 445.2718.

**Synthesis of 2.** 782 mg (3.0 mmol) of 1,3-bis(4-fluorophenyl)-1,3-propanedione was placed in an argon-purged 25 mL Schlenk flask charged with a stirbar. The flask was capped, cooled to 0 °C and 4.0 mL (32.0 mmol) of boron trifluoride diethyl etherate was added by syringe. The reaction was heated to 75 °C and stirred for 24 h. Excess boron trifluoride diethyl etherate was removed and the resultant solid was triturated with methanol to produce 1,3-bis(4-fluorophenyl)-1,3-propanedionato- $\kappa\text{O}$ ,  $\kappa\text{O}'$ ]difluoroborane as a precipitate that was collected by filtration, dried under vacuum and directly reacted with 1.0 mL of N-methylpiperazine at 120 °C for 24 h. The reaction mixture was cooled, directly loaded on a neutral alumina column and the product eluted using a 1:1 mixture of ethyl acetate and methanol. After removal of the solvents under reduced pressure, **2** was isolated as an orange-red powder. Yield: 124 mg (10.6%); m.p. 305 °C (dec.); IR  $\nu_{\text{max}}$ :  $\text{cm}^{-1}$  799.88, 1199.69, 1340.14, 1591.69, 1621.26, 2941.25, 3396.21;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ),  $\delta$  (ppm): 3.70 (6H, s), 4.36-4.38, (4H, d,  $J = 8.9$  Hz), 5.08-5.10 (4H, d,  $J = 10.0$  Hz), 7.97-7.99 (4H, d,  $J = 8.9$  Hz), 8.39 (1H, s), 9.03-9.05 (4H, d,  $J = 8.8$  Hz)  $^{13}\text{C}$  NMR (500 MHz,  $\text{CDCl}_3$ , TFA),  $\delta$  (ppm): 40.75, 44.03, 52.31, 91.55, 112.93, 121.10, 131.70, 154.33, 178.33. HRMS (ESI) calculated for  $\text{C}_{25}\text{H}_{32}\text{BF}_2\text{N}_4\text{O}_2^+$  ( $\text{M}+\text{H}^+$ ): 469.2581, found: 469.2540.

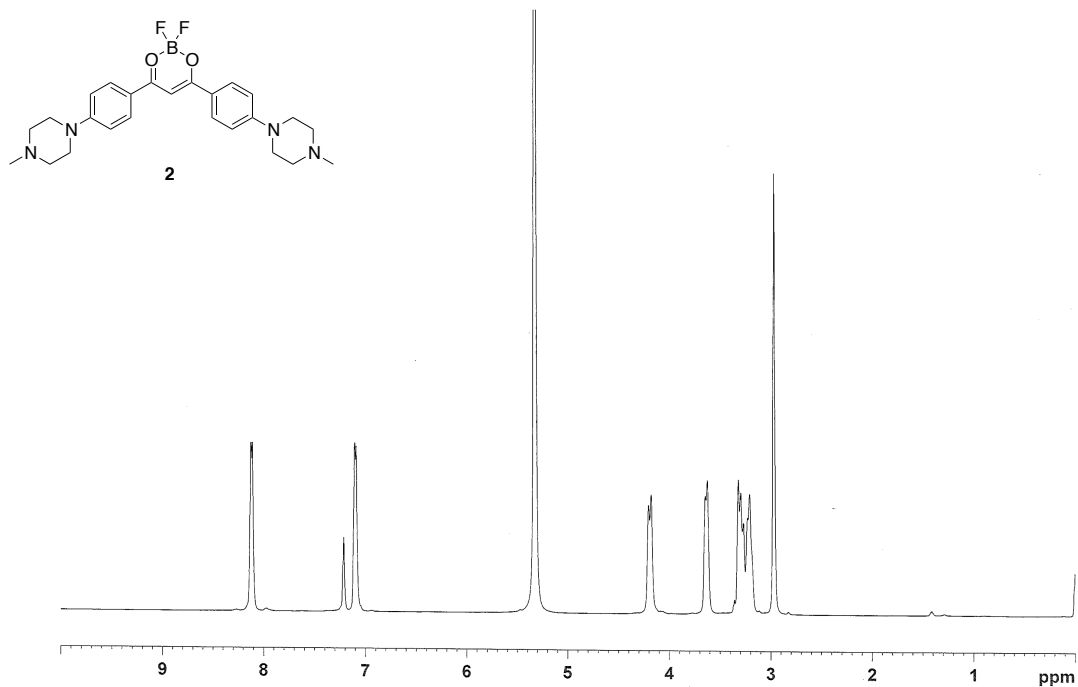
<sup>1</sup>H-NMR of **1**



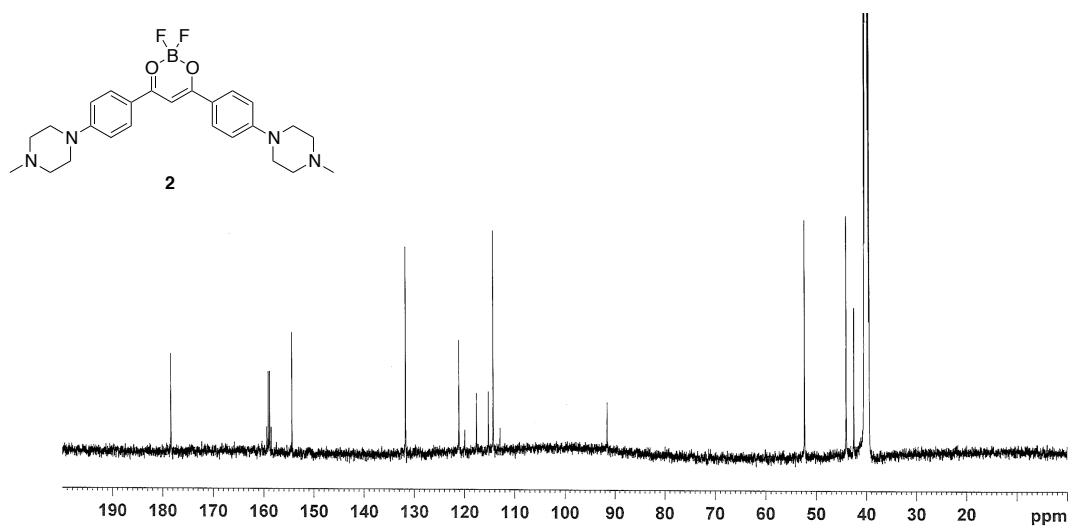
<sup>13</sup>C-NMR of **1**, the quartets centered at 159 ppm ( $J_{2C,F} = 35$  Hz) and between 119.9 ppm and 112.94 ppm ( $J_{1C,F} = 291$  Hz) correspond to TFA.



# <sup>1</sup>H-NMR of **2**



<sup>13</sup>C-NMR of **2**, the quartets centered at 159 ppm ( $J_{2C,F} = 35$  Hz) and between 119.9 ppm and 112.94 ppm ( $J_{1C,F} = 291$  Hz) correspond to TFA.



**Table S1:** Atomic Coordinates for ground state optimized **1** in MeOH<sup>a</sup>

H	-3.60525	2.19906	0.29849
C	-3.69403	1.12544	0.17199
C	-3.93005	-1.62307	-0.16121
C	-2.52246	0.37054	-0.02868
C	-4.94862	0.53908	0.21705
C	-5.10856	-0.86257	0.05726
C	-2.68318	-1.01775	-0.19844
H	-5.813	1.17188	0.37895
H	-1.82076	-1.65001	-0.38614
H	-3.99055	-2.6944	-0.30951
C	-1.20423	1.02854	-0.06221
C	1.21006	1.03568	-0.06323
N	-1.19371	2.38188	-0.1266
C	0.00441	0.32023	-0.01589
H	0.00697	-0.75536	0.08879
C	2.52928	0.37775	-0.03024
C	5.11716	-0.8521	0.05646
C	3.69937	1.13323	0.17657
C	2.69263	-1.0094	-0.20621
C	3.94041	-1.61316	-0.16887
C	4.95474	0.54853	0.22219
H	3.60761	2.20605	0.30733
H	1.83182	-1.64217	-0.39961
H	4.00284	-2.68361	-0.32268
H	5.8178	1.18181	0.38937
N	-6.34648	-1.45545	0.11108
C	-6.48192	-2.88883	-0.10866
H	-5.91049	-3.46423	0.63099
H	-6.13947	-3.18565	-1.10979
H	-7.53174	-3.16703	-0.01294
C	-7.54164	-0.63987	0.27779
H	-8.41553	-1.2907	0.31481
H	-7.67584	0.06749	-0.55248
H	-7.50812	-0.06508	1.21218
N	6.35595	-1.4435	0.11122
C	7.54954	-0.62656	0.282
H	7.51452	-0.05561	1.21871
H	7.68303	0.08449	-0.54524
H	8.42457	-1.276	0.31684
C	6.49406	-2.87566	-0.11432
H	7.54358	-3.15333	-0.01372
H	6.15758	-3.16857	-1.11869
H	5.91901	-3.45481	0.61954
N	1.20349	2.38866	-0.1271
C	0.005	2.96091	-0.14316
O	0.02293	4.31377	-0.19288
H	-0.90849	4.59874	-0.20346

<sup>a</sup>DFT 6-31G\*, SMD solvent model

**Table S2:** Atomic Coordinates for ground state optimized **2** in MeOH<sup>a</sup>

C	-2.52213	0.17379	-0.01965
C	-5.11595	-1.01741	0.01086
C	-3.68949	0.96056	0.01742
C	-2.68619	-1.22326	-0.05253
C	-3.94017	-1.80955	-0.03873
C	-4.94997	0.39068	0.03445
H	-3.58719	2.0397	0.03843
H	-1.81964	-1.87434	-0.1099
H	-4.00944	-2.88991	-0.07489
H	-5.81441	1.04244	0.06873
C	2.52212	0.17376	-0.01965
C	5.11593	-1.01745	0.01092
C	2.68618	-1.22329	-0.05259
C	3.68948	0.96053	0.01749
C	4.94996	0.39064	0.03455
C	3.94015	-1.80958	-0.03877
H	1.81962	-1.87436	-0.11005
H	3.58718	2.03966	0.03852
H	5.8144	1.0424	0.06887
H	4.00941	-2.88993	-0.075
N	-6.36585	-1.59255	0.03321
N	6.36584	-1.59255	0.03334
C	-7.55552	-0.75548	0.03703
H	-7.61127	-0.11862	-0.85679
H	-7.58943	-0.10484	0.9211
H	-8.44115	-1.39232	0.05434
C	-6.50616	-3.03871	-0.01536
H	-6.09739	-3.46158	-0.94424
H	-7.56458	-3.29812	0.03616
H	-5.9992	-3.52214	0.83045
C	6.50624	-3.0387	-0.01554
H	6.09763	-3.4614	-0.94457
H	5.99918	-3.52232	0.8301
H	7.56466	-3.29806	0.0361
C	7.55547	-0.75542	0.03706
H	7.61126	-0.11872	-0.85689
H	8.44113	-1.39221	0.05457
H	7.58927	-0.10461	0.921
C	-1.21004	0.82508	-0.0297
C	-0.00001	0.1361	0.1144
H	-0.00001	-0.92537	0.30594
C	1.21003	0.82506	-0.02974
O	1.2268	2.11743	-0.19818
O	-1.22678	2.11744	-0.19818
B	0.00001	2.97931	-0.01487
F	0.00002	3.93991	-0.9844
F	0.00002	3.47351	1.26617

<sup>a</sup>DFT 6-31G\*, SMD solvent model



## REFERENCES

1. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; et al. Gaussian 09, revision A.01; Gaussian, Inc: Wallingford, CT, 2009.
2. Marenich, A. V.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. B* 2009, 113, 6378–6396.
3. Berlman, I., *Handbook of Fluorescence Spectra of Aromatic Molecules*. 2nd ed.; Academic Press: New York, 1971.
4. Lieber, S.; Scheer, F.; Meissner, W.; Naruhn, S.; Adhikary, T.; Müller-Brüsselbach, S.; Diederich, W. E.; Müller R. *J. Med. Chem.* **2012**, 55, 2858-2868.
5. Yadav, N.; Dixit, S. K.; Bhattacharya, A.; Mishra, L. C.; Sharma, M.; Awasthi, S. K.; Bhasin, V. *K. Chem. Biol. Drug Des.* **2012**, 80, 340-347.