Supporting Information for

Polymer Dots and Glassy Organic Dots using Dibenzodipyridophenazine Dyes as Water-Dispersible TADF Probes for Cellular Imaging

By Don M. Mayder, [†] Cheyenne J. Christopherson, [†] William L. Primrose, Angela S.-M. Lin, and Zachary M. Hudson*

[†]These authors contributed equally to this work.

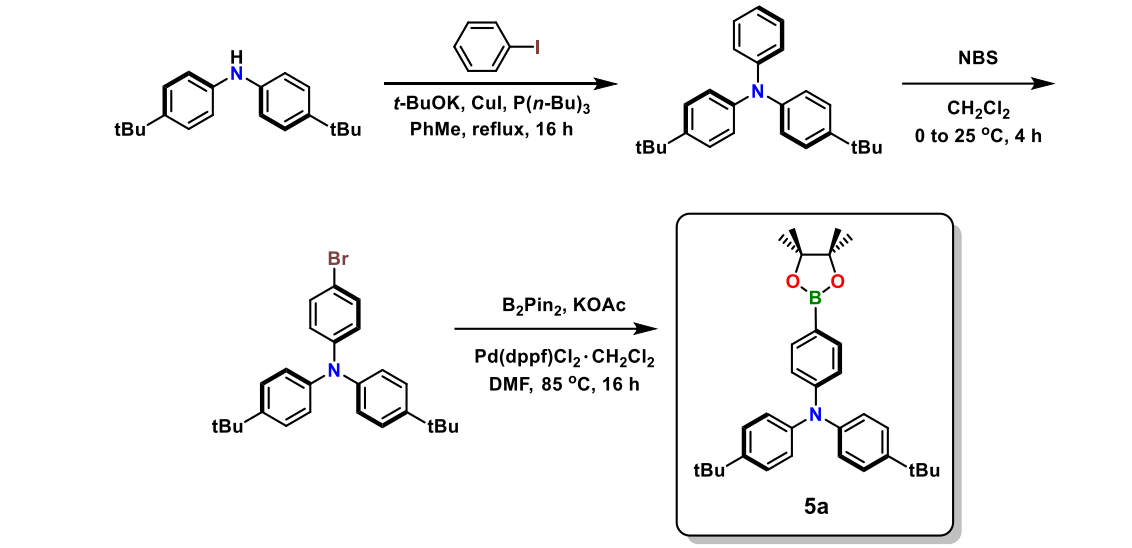
Department of Chemistry, The University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, Canada, V6T 1Z1.

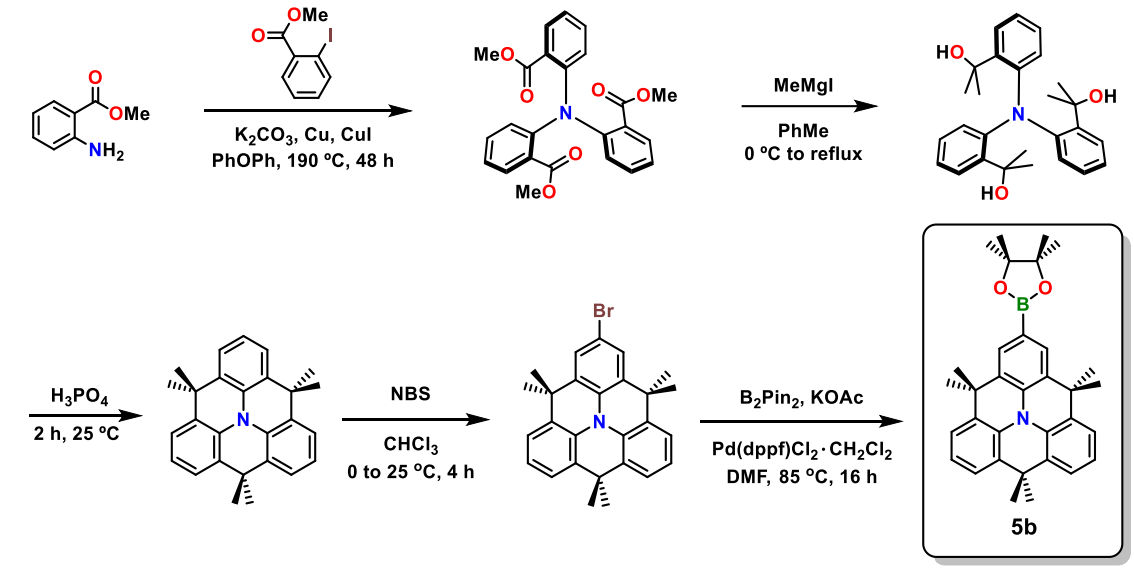
Tel: +1-604-822-3266; Fax: +1-604-822-2847; e-mail: zhudson@chem.ubc.ca.

Table of Contents

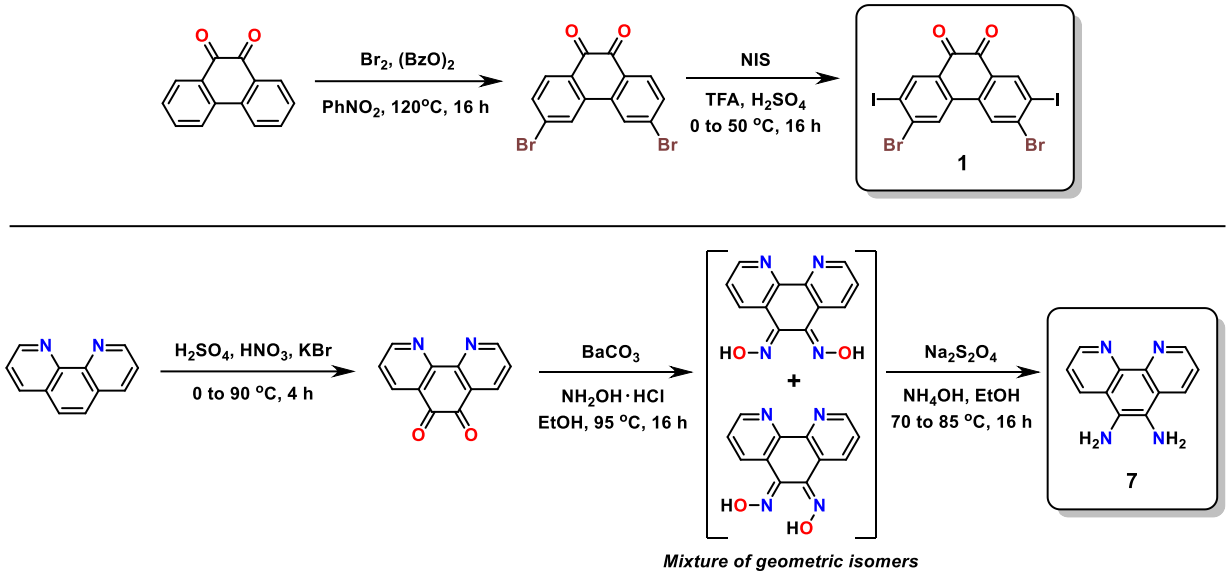
Synthetic Schemes S2
Experimental Details S5
Synthetic Procedures S9
^{1}H and ^{13}C{^{1}H} NMR Spectra S17
Photophysical Characterization S26
Thermal and Electrochemical Characterization S29
Density Functional Theory S30
Pdot Synthesis and Cellular Uptake S34
g-Odot Cellular Uptake S39
References S42

Synthetic Schemes

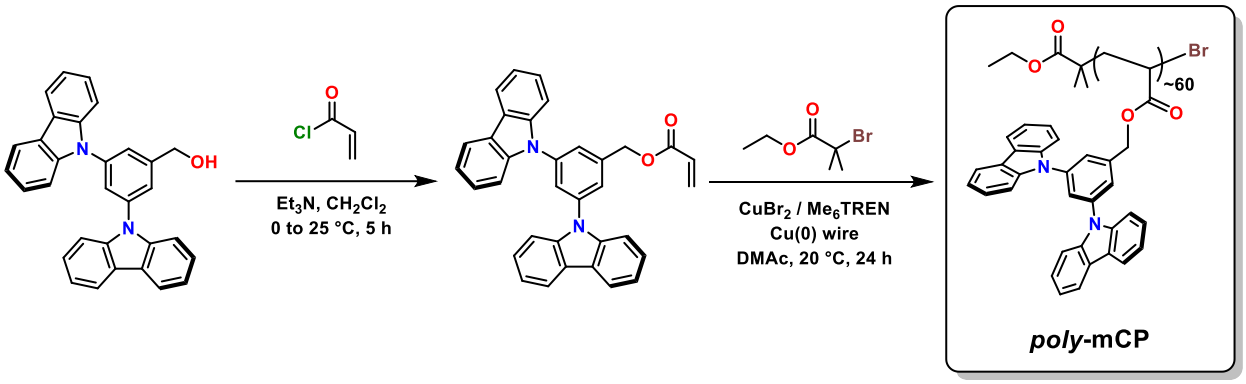




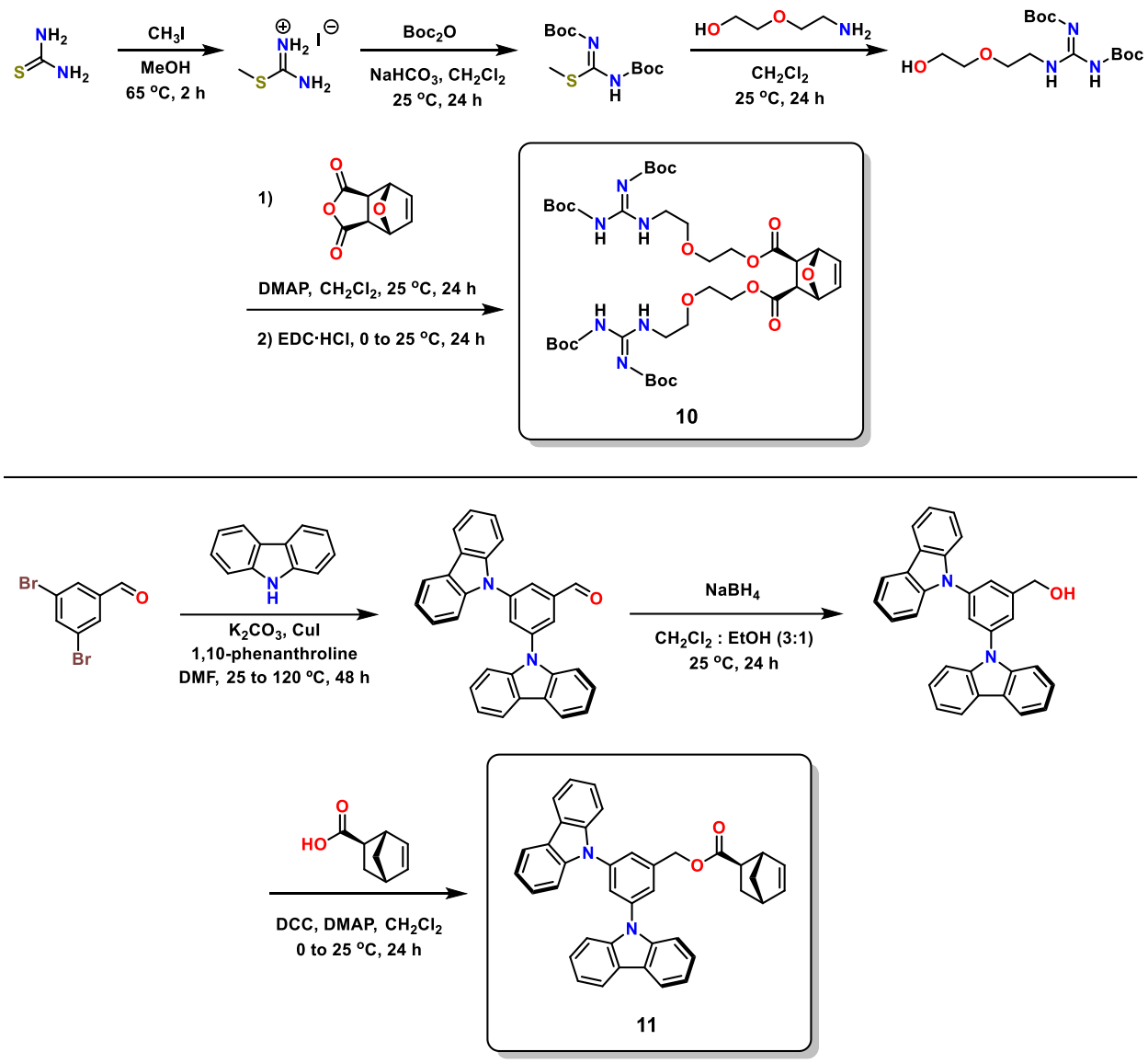
Scheme S1. Synthesis of pinacol esters **5a-b**.



Scheme S2. Synthesis of dione **1** and diamine **7**.



Scheme S3. Synthesis of poly-mCP used for doped thin film characterization.



Scheme S4. Synthesis of oxonorbornene (**10**) and norbornene (**11**) monomers.

Experimental Details

General Considerations. All reactions and manipulations were carried out under a nitrogen atmosphere using standard Schlenk or glove box techniques unless otherwise stated. Dry and degassed toluene and tetrahydrofuran was obtained from Caledon Laboratories, dried using an Innovative Technologies Inc. solvent purification system. *N*,*N*-Dimethylformamide was dried over 4 Å molecular sieves. Dichloromethane was distilled from P_2O_5, and collected onto 4 Å molecular sieves. All reagents were obtained from Sigma-Aldrich, Alfa Aesar, or Oakwood Chemical, and used as received unless otherwise stated. Human serum was purchased frozen from Sigma-Aldrich. Water (>18.2 MΩ cm^{-1}) was from a Milli-Q Synthesis Water Purification System from Millipore (Burlington, MA). 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (ammonium salt) (**DSPE-PEG2k**) was purchased from Avanti Polar Lipids through Sigma-Aldrich. Boronic esters **5a** and **5b**,[1] dione **1**,[2] diamine **7**,[3] oxonorbornene monomer **10**,[4] norbornene monomer **11**,[5] **mCP**,[6] and poly-mCP[7] were prepared according to literature procedures. The ^1H and $^{13}C\{^1H\}$ nuclear magnetic resonance (NMR) spectra were measured on a Bruker AV III HD 400 MHz spectrometer with chloroform-*d* ($CDCl_3$), tetrahydrofuran-d_8 (THF-d_8), or dichloromethane-d_2 (CD_2Cl_2) as the solvent. Mass spectra were recorded on a Kratos Concept IIHQ instrument using field desorption (FD) ionization, or on a Bruker HCTultra PTM Discovery System using electrospray ionization (ESI). Microwave syntheses of g-Odots were performed in a Biotage Initiator+ Microwave Synthesizer.

General Photophysical Characterization. Absorbance measurements were made on a Cary 60 spectrometer and fluorescence measurements were made on an Edinburgh Instruments FS5 spectrofluorometer or Edinburgh Instruments FLS1000 spectrofluorometer. Concentrations of 0.01 mg mL^{-1} were used unless otherwise stated. Absolute photoluminescence quantum yields were determined using an Edinburgh Instruments SC–30 Integrating Sphere Module, with optical densities less than 0.1. Lifetimes were obtained using an EPLED (λ_{exc} = 380 nm or 313 nm) coupled with TCSPC, or a Xe μF lamp coupled with MCS; emission maxima in each case were used for the determination of all lifetimes.

Photodestructive Quantum Yield Experiment: Adapted from the work of Rivera.[8] A mounted LED (ThorLabs 365 nm, 190 mW, model M365L2) was calibrated using chemical actinometry: a cuvette containing potassium ferrioxalate in an aqueous solution was stirred at room temperature and subsequently exposed to collimated radiation from a 365 nm excitation source for time intervals between 0 to 300 seconds. In concurrence with a standard curve for Fe^{2+}, the rate of incident photon radiation (I_0) on the experimental setup was determined using the equation below, where $N_{Fe}{}^{2+}$ is the number of Fe^{2+} ions formed in a given time interval (t):

$$I_0 = \frac{N_{Fe^{2+}}}{t[\Phi_{Fe^{2+}}]}$$

[Quantum yield of formation $\Phi_{Fe}{}^{2+}$ = 1.24]

Concentrations of each emitter in toluene solution were adjusted whereby their initial $A_{\lambda max}$ at t = 0 would lie within an absorbance range of 0.5-1.0 (Figure S19A). The solution was then exposed to a 365 nm LED, using the identical setup used for the actinometry calibration. The total light absorbed (I_a) for each sample was determined by the equation below:

$$I_a = \frac{I_0(1 - 10^{A_{365}})t}{V}$$

For a given time interval (t), while considering the irradiated sample volume (V) and the average absorbance at excitation wavelength (A_{365}), I_a was summed over the course of the experiment. Plotting the diminishing $A_{\lambda max}$, as a function of I_a, yields a linear regression (Figure S19B), with the equation as shown below:

$$y = \left[\frac{dA_{\lambda_{max}}}{dI_a}\right]x + b$$

Finally, the photodestructive quantum yield Φ_d (in units of 'molecules photon^{-1}') can be extracted from the above equation, in conjunction with Avogadro's number (N_A), the molar absorptivity of the sample (ε), and the path length of the cuvette (l) as shown below:

$$\Phi_d = \frac{N_A\left[-\frac{dA_{\lambda_{max}}}{dI_a}\right]}{\varepsilon l}$$

Electrochemical Methods. Cyclic voltammograms were recorded using a BASi Epsilon Eclipse potentiostat at room temperature using a standard three-electrode configuration (working electrode: 3 mm diameter glassy carbon; reference electrode: Ag/Ag pseudoreference electrode, referenced externally to ferrocene/ferrocenium, counter electrode: Pt wire) in 0.2 M tetrabutylammonium hexafluorophosphate in *o*-difluorobenzene. Experiments were run at a scan rate of 20 mV s^{-1} in degassed electrolyte solution with 2 μmol mL^{-1} of analyte.

Density Functional Theory. Quantum mechanical calculations were performed using the Gaussian 16 Rev. B.01 computational package via default settings unless otherwise stated. All geometries were optimized to a minimum, and frequency calculations were performed at the same level of theory to verify the absence of imaginary frequencies. Initial geometry optimizations were conducted at the ωB97XD/def2-TZVP level. The optimal range separation values (ω) were determined based at the ωB97XD/def2-TZVP level and are listed in Table S1. To optimize ω, the same procedure outlined by Paisley et al. was followed.[9] A second geometry optimization at the ωB97XD*/def2-TZVP level was conducted. Vertical excitation (absorption) energies of the lowest singlet and triplet excited states were calculated by the Tamm−Dancoff approximation (TDA) scheme of time-dependent DFT (TD-DFT) at the ωB97XD*/pc-1 level in toluene using the polarizable continuum model (PCM).

Nanoparticle Tracking Analysis. g-Odot and Pdot size distributions were determined by NTA (NanoSight NS300, Malvern Panalytical, Malvern, UK). Aqueous stock suspensions of g-Odot samples (1 mL) were diluted 100-fold into pre-filtered (0.22 μm pore size) ultrapure water prior to measurements. Aqueous Pdot samples (as prepared) were similarly diluted 50-100x for an appropriate measurement. Measurements were recorded in scattering mode using a 488 nm laser, as well as in fluorescence mode employing a 500 nm long-pass emission filter. Raw data were acquired in triplicate, and fit with a log-normal distribution.

Cell Culture. Both HeLa and HepG2 cell lines were cultured in a humidified incubator with 95% air / 5% CO_2 at 37 °C, sub-cultured once ~80% confluency was reached in each case. HeLa cells were cultured in DMEM with 10% fetal bovine serum (FBS) and 1% strep./pen. antibiotics. HepG2 cells were cultured in MEM-alpha with 10% FBS and 1% strep./pen. antibiotics.

For imaging experiments, 18 mm sterile, circular glass slips were placed into 12-well tissue culture-treated plates. The glass slips were coated with gelatin solution (0.1%, 220 μL), allowed to rest for 10 minutes, with residual gelatin aspirated off and the substrate allowed to dry for 15 minutes.

For Pdot-incubated HeLa and HepG2 cells: Cells were then seeded into each well (1 mL, 5 × 10^4 cells mL^{-1}), and incubated for a further 24 hours. A sterile aqueous Pdot solution was then added (~110 μL of stock Pdot solution, final concentration = 20 μg mL^{-1}) to the cells, followed by incubation for 30 min at 37 °C in 95% air / 5% CO_2.

For g-Odot-incubated HeLa cells: Cells were then seeded into each well (1 mL, 1 × 10^5 cells mL^{-1}), and incubated for a further 24 hours. Old medium was then removed from each well and replaced with fresh medium containing suspended g-Odots (1 mL, 1.7 × 10^9 particles mL^{-1}) prepared from a pelletized g-Odot sample (stored in 0.22 μm filtered MilliQ water or lyophilized), followed by a one-hour incubation period at 37 °C in 95% air / 5% CO_2.

Cell Fixation. Fixation of HeLa and HepG2 cells incubated with Pdots and/or g-Odots was performed for samples prepared on gelatin-coated glass cover slips in 12-well plates. First, all medium containing suspended nanoparticles was removed, and the samples were washed gently with 1X PBS buffer (3 × 1 mL, pH 7.4, 1.06 mM KH_2PO_4, 2.97 mM Na_2HPO_4, 155 mM NaCl; Gibco, Thermo Fisher Scientific). A volume of paraformaldehyde (600 μL, 4% w/v, prepared in 1X PBS) was added to each sample, followed by incubation at room temperature for 10 min in the dark. The paraformaldehyde solution was removed, and the samples were further washed with 1X PBS (2 × 1 mL). The glass slips were mounted and sealed onto glass microscope slides and stored in the dark until imaged.

Cell Fluorescence Microscopy. Images were collected using an Olympus FV1000 Laser Confocal Microscope with either 20X, 40X, or 60X objective lenses, or a PerkinElmer Ultraview VoX Spinning Disk Confocal Microscope using a 63X 1.3/glycerol objective lens. g-Odot/P-dot-incubated HeLa cells as ensemble experiments were excited with 405 nm laser light (λ_{em} = 495 – 595 nm for g-Odots, λ_{em} = 545 – 645 nm for Pdots) using the laser confocal microscope. HeLa/HepG2 cells incubated with Pdots were excited with 405 nm laser light (λ_{em} = 500 – 550 nm coupled with λ_{em} = 580 – 700 nm to account for broad Pdot emission spectra). SNR and SBR were calculated according to the Equations 2 and 3 below where I = intensity, bg = background, and σ = standard deviation.

$$SBR = \frac{I_{cell} - I_{bg}}{I_{bg}} \qquad \textbf{(Eq. 2)}$$

$$SNR = \frac{I_{cell} - I_{bg}}{\sigma(I_{bg})} \qquad \textbf{(Eq. 3)}$$

Time-Gated Serum/R640/g-Odot Measurements. *R640/g-Odot Samples:* A solution of rhodamine 640 perchlorate (R640, 0.25 mg mL^{-1}) in MilliQ water was prepared. A volume of stock **HMAT** g-Odots in MilliQ water (35 μL, 3.0×10^8 particles mL^{-1}), R640 (25 μL, 0.25 mg mL^{-1}) were combined in a cuvette containing additional water (3 mL). The mixed solution was then subjected to time-gated measurements using excitation at 313 nm with a Xe μF lamp on an Edinburgh Instruments FLS1000 spectrofluorometer. *Human Serum/g-Odot Samples:* A stock solution of **HMAT** g-Odots in MilliQ water (3.0×10^8 particles mL^{-1}) was diluted 4x using HEPES buffer (1 M HEPES, pH 7.5; 4x g-Odot dilution = 7.5×10^7 particles mL^{-1}). To a quartz cuvette, 4x diluted g-Odots in HEPES (2750 μL) and human serum (250 μL) was added and mixed. The combined solution was then subjected to time-gated emission measurements using excitation at 295 nm with a Xe μF lamp on an Edinburgh Instruments FLS1000 spectrofluorometer.

Transmission Electron Microscopy. TEM images were collected at the UBC Bioimaging Facility on a FEI Tecnai Spirit TEM, equipped with a DVC1500M side-mounted camera controlled by AMT software and a high-resolution FEI Eagle 4K bottom-mounted camera for capturing digital images, at an accelerating voltage of 80 kV. Samples for TEM were prepared by drop-casting 10 μL of aqueous nanoparticle suspension (~7.0×10^{10} nanoparticles mL^{-1}) onto a Formvar/Carbon 300 mesh copper grid (Ted Pella Inc.) placed on a piece of parafilm in a clean glass petri dish; the dish was then loosely covered and the samples were allowed to dry overnight in air.

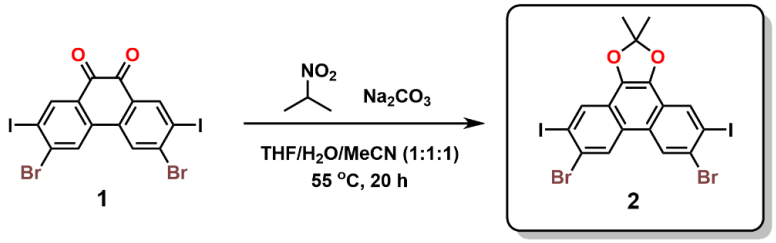
Size Exclusion Chromatography. SEC experiments were conducted in chromatography-grade THF at concentrations of 0.5 – 2.0 mg mL^{-1} using a Malvern OMNISEC GPC instrument equipped with a Viscotek TGuard column (CLM3008), and Viscotek T3000 (CLM3003) and T6000 (CLM3006) GPC columns packed with porous poly(styrene-*co*-divinylbenzene) particles regulated at 35 °C. Signal response was measured using a differential viscometer, differential refractive index, photodiode array, and light-scattering (90° and 7°) detectors. The interdetector volume was calibrated using a single polystyrene standard with M_n = 101,000 and $Đ$ = 1.04. Molecular weights for the Boc-protected polymers **P1**, **P2** and **P3** were determined by triple detection for the 1st block (d*n*/d*c* = 0.0805 in THF), and ^{1}H NMR for the second block.

Cell Viability Assay. Cell viability was measured in triplicate using a standard MTT assay. Cells were seeded in a 96-well plate (1.0×10^4 cells $well^{-1}$) in 100 μL of growth medium and incubated for 24 hours at 37 °C in 5% CO_2 to allow for attachment. For g-Odots, a sample of pelletized g-Odots with MilliQ water decanted off was reconstituted in medium and diluted to the desired concentrations in growth medium in serial dilution (such that each sample was half as concentrated as the sample preceding it). For Pdots, appropriate volumes of Pdot solutions were added, and diluted serially as described above. 100 μL of each dilution were added to the required wells before incubation for 1 or 24 hours. Then, 50 μL of MTT (2.5 mg mL^{-1} in PBS) were added to each well, followed by incubation for 3 hours. The solution was directly removed from all wells, and 100 μL DMSO was added. The absorbance at 570 nm of each well was measured on a Molecular Devices FilterMax F5 Multi-Mode Microplate Reader. All absorbance values were corrected for baseline absorbance. Cell viability was determined using Equation 4 below.

$$Cell\ viability\ (\%) = \frac{Mean\ abs.incubated\ cells}{Mean\ abs.control\ cells} \times 100\ \% \qquad \textbf{(Eq. 4)}$$

Synthetic Procedures

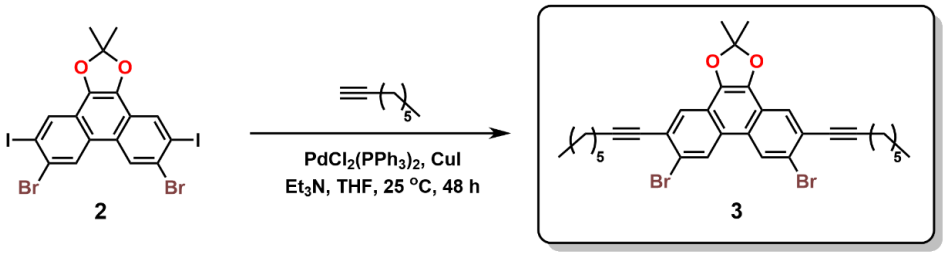
Compound **2**



Into a 500 mL round bottom flask, dione **1** (4.44 g, 7.19 mmol, 1.0 eq), Na_2CO_3 (6.17 g, 58.1 mmol, 8.1 eq), and nitropropane (6.5 mL, 73 mmol, 10 eq) were combined with a 1:1:1 mixture of THF:H_2O:MeCN (220 mL total volume). The mixture was stirred under nitrogen atmosphere, and heated using an oil bath at 55 °C for a total of 20 hours. The resulting suspension was then cooled to room temperature, and vacuum filtered to collect the solids. The solids were washed with H_2O (5 x 30 mL), followed by a wash with methanol (1 x 10 mL) to promote drying of the product. The pale-yellow solids were dried *in vacuo*, and did not require further purification as verified by

^{1}H NMR. Yield: 4.46 g (94%). ^{1}H NMR (300 MHz, Chloroform-*d*): δ 8.71 (s, 2H), 8.38 (s, 2H), 1.84 (s, 6H). ^{13}C{^{1}H} NMR (75 MHz, Chloroform-*d*): δ 136.8, 132.0, 126.9, 126.5, 125.6, 121.9, 121.0, 100.6, 26.2. HRMS (FD) *m/z*: $[M]^{+\bullet}$ calcd for $[C_{17}H_{11}Br_2I_2O_2]^{+\bullet}$, 658.7218; found, 658.7215; difference: +0.46 ppm.

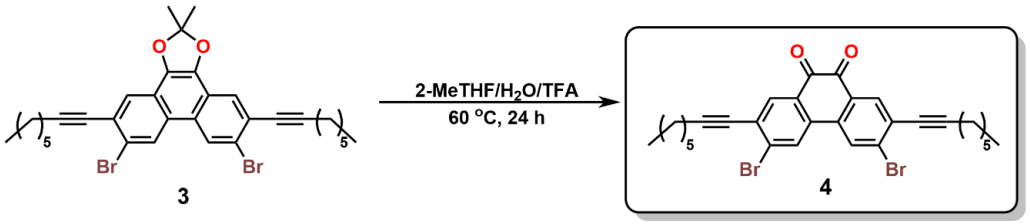
Compound **3**



A flame-dried 25 mL Schlenk flask was charged with dioxole **2** (0.35 g, 0.53 mmol, 1.0 eq), CuI (10 mg, 0.05 mmol, 0.1 eq), and $PdCl_2(PPh_3)_2$ (19 mg, 0.03 mmol, 0.05 eq). A solution of anhydrous Et_3N (0.3 mL, 2.2 mmol, 4.0 eq) in anhydrous THF (3 mL) was sparged with N_2 for five minutes, and added to the reaction flask; the mixture was stirred at 25 °C for 10 minutes. A solution of 1-octyne (0.32 mL, 2.2 mmol, 4.0 eq) in anhydrous THF (4 mL) was sparged for five minutes, and added to the flask. The mixture was stirred for 48 hours at 25 °C, after which time the solvent was removed *in vacuo*. The crude mixture was purified over silica (95:5 to 90:10 hexanes : CH_2Cl_2) to yield a yellow solid. Yield: 232 mg (70%).

^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.67 (s, 1H), 7.93 (s, 1H), 2.52 (t, *J* = 7.0 Hz, 2H), 1.82 (s, 3H), 1.67 (p, *J* = 7.0 Hz, 2H), 1.56 – 1.51 (m, 2H), 1.43 – 1.27 (m, 4H), 0.92 (t, *J* = 6.6 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, Chloroform-*d*): δ 137.1, 126.9, 125.5, 124.9, 124.8, 121.3, 120.6, 120.2, 96.8, 79.4, 31.4, 28.6, 28.6, 26.0, 22.6, 19.7, 14.1. HRMS (ESI) *m/z*: $[M]^{+\bullet}$ calcd for $[C_{33}H_{36}Br_2O_2]^{+\bullet}$, 622.1082; found, 622.1088; difference: +0.96 ppm.

Compound **4**



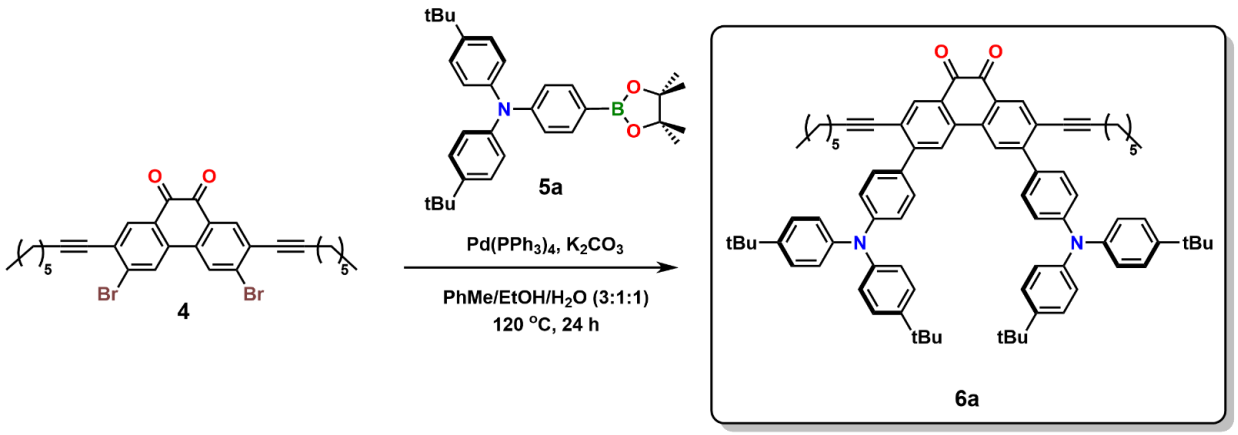
To a 250 mL round bottom flask, dioxole **3** (0.94 g, 1.5 mmol, 1.0 eq) was dissolved in 2-MeTHF (30 mL). A solution of TFA (40 mL) and H_2O (20 mL) was added to the flask, which was stirred open to air at 60 °C. After 24 hours, additional TFA (20 mL) was added, followed by a further 24 hours of stirring at 60 °C. Upon completion as monitored by TLC, the mixture was concentrated *in vacuo* to remove 2-MeTHF, followed by a slow addition of saturated $NaHCO_3$ to neutralize the solution, and extraction with CH_2Cl_2 (3 x 50 mL). The combined organics were washed with brine (1 x 30 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude material was purified over silica (1:1 to 7:3 CH_2Cl_2 : hexanes) to yield an orange solid. Yield 742 mg (85%).

^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.18 (s, 1H), 8.12 (s, 1H), 2.50 (t, *J* = 7.0 Hz, 2H), 1.66 (p, *J* = 7.1 Hz, 2H), 1.54 – 1.43 (m, 2H), 1.36 –1.32 (m, 4H), 0.92 (t, *J* = 6.9 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, Chloroform-*d*): δ 178.5, 134.9, 134.6, 133.5, 129.7, 128.5, 128.2, 100.3, 78.3, 31.5, 28.7, 28.4, 22.7, 19.9, 14.2. HRMS (ESI) *m/z*: $[M]^{+\bullet}$ calcd for $[C_{30}H_{30}Br_2O_2]^{+\bullet}$, 580.0613; found, 580.0613; difference: +0.13 ppm.

General Procedure A: Suzuki coupling for the preparation of diones **6a-b**.

To a 3-neck round bottom flask, dione **4** (0.45g, 0.78 mmol, 1.0 eq), pinacol ester **5a** or **5b** (1.7 mmol, 2.2 eq), K_2CO_3 (1.2 g, 8.5 mmol, 11 eq), and $Pd(PPh_3)_4$ (54 mg, 0.05 mmol, 0.06 eq) were combined. An N_2-sparged solution of PhMe:EtOH:H_2O (3:1:1, total volume 30 mL) was added to the flask, and the mixture was refluxed under N_2 at 120 °C using an oil bath until the reaction was complete as monitored by TLC (24-96 hours). The mixture was cooled, and extracted with toluene (3 x 30 mL). The combined organics were washed with brine (1 x 30 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude material was purified over silica.

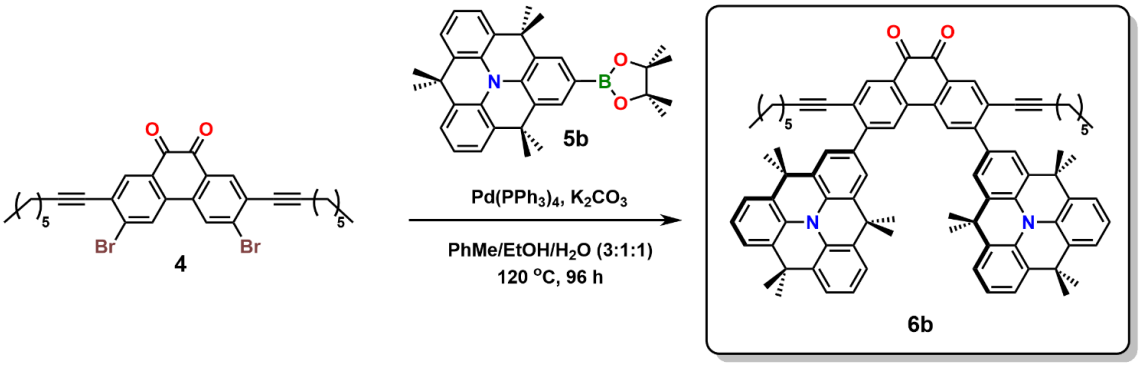
Compound **6a**



Synthesized according to General Procedure A. The reaction was complete after 24 hours, and the crude material was purified over silica (1:1 CH_2Cl_2:hexanes) to yield a dark purple solid. Yield: 514 mg (58%).

^{1}H NMR (400 MHz, THF-d_8): δ 8.20 (s, 1H), 8.16 (s, 1H), 7.61 (d, J = 8.7 Hz, 2H), 7.33 (d, J = 8.7 Hz, 4H), 7.06 (d, J = 8.7 Hz, 4H), 7.04 (d, J = 8.8 Hz, 2H), 2.40 (t, J = 7.0 Hz, 2H), 1.55 (p, J = 6.6 Hz, 3H), 1.47 – 1.36 (m, 2H), 1.33 (s, 18H), 1.31 – 1.29 (m, 4H), 0.87 (t, J = 7.0 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, Chloroform-d): δ 179.5, 150.2, 148.9, 146.6, 144.6, 135.7, 134.2, 131.5, 130.0, 129.0, 126.3, 124.9, 124.7, 123.8, 120.9, 97.0, 79.2, 34.5, 31.6, 31.4, 28.8, 28.4, 22.7, 19.8, 14.2. HRMS (ESI) m/z: $[M]^{+\bullet}$ calcd for $[C_{82}H_{90}N_2O_2]^{+\bullet}$, 1134.7002; found, 1134.7013; difference: +0.98 ppm.

Compound **6b**



Synthesized according to General Procedure A. The reaction was complete after 96 hours, and the crude material was purified over silica (5.5:4.5 hexanes : CH_2Cl_2) to yield a dark purple solid. Yield: 88 mg (21%).

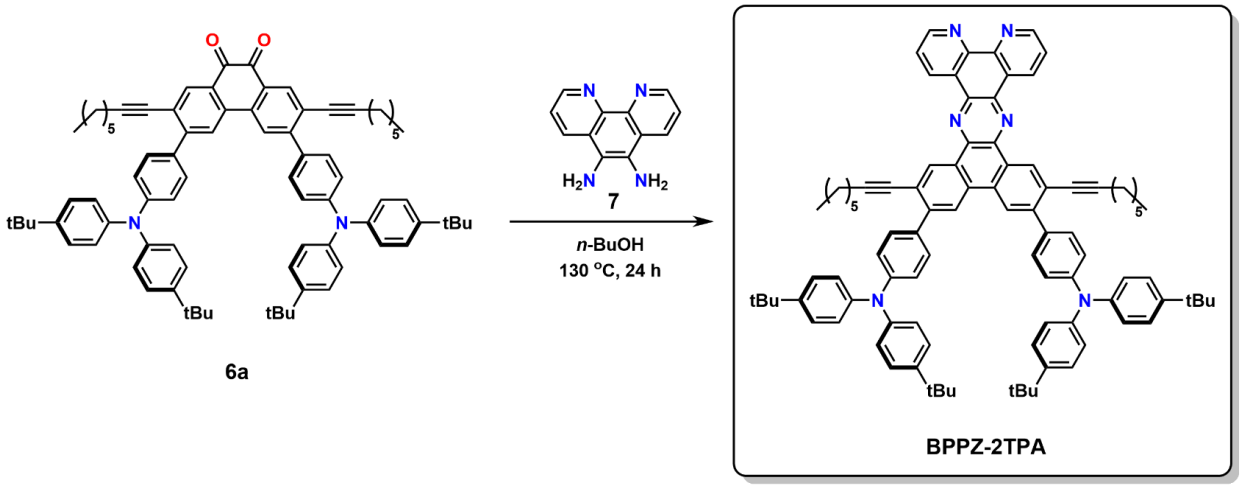
^{1}H NMR (400 MHz, Chloroform-d): δ 8.39 (s, 1H), 8.08 (s, 1H), 7.79 (s, 2H), 7.42 – 7.39 (m, 4H), 7.15 (t, J = 7.6 Hz, 2H), 2.37 (t, J = 7.1 Hz, 2H), 1.70 (s, 12H), 1.66 (s, 6H), 1.55 – 1.51 (m, 2H), 1.38 – 1.31 (m, 2H), 1.28 – 1.18 (m, 4H), 0.83 (t, J = 6.5 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, Chloroform-d): δ 179.6, 150.1, 136.4, 134.2, 133.1, 132.5, 131.6, 130.2, 129.9, 129.8, 129.1, 124.7, 124.6, 123.9, 123.8, 123.4, 97.0, 79.4, 35.8, 35.7, 33.7, 33.5, 31.4, 28.9, 28.7, 22.6, 20.1,

14.2. HRMS (ESI) *m/z*: $[M]^{+\bullet}$ calcd for $[C_{84}H_{82}N_2O_2]^{+\bullet}$, 1150.6376; found, 1150.6382; difference: +0.49 ppm.

General Procedure B: Condensation for the preparation of **BPPZ-2TPA and BPPZ-2HMAT**

To a 100 mL bottom flask, dione **6a**, **6b**, or **6c** (0.26 mmol, 1.0 eq), and diamine **7** (0.29 mmol, 1.1 eq) were combined with *n*-butanol (65 mL). The mixture was sparged with N_2 for 10 minutes, followed by heating the mixture to reflux under N_2 for 24 hours using an oil bath. The mixture was concentrated *in vacuo* to remove solvent, and purified over silica to yield **BPPZ-2TPA** and **BPPZ-2HMAT**.

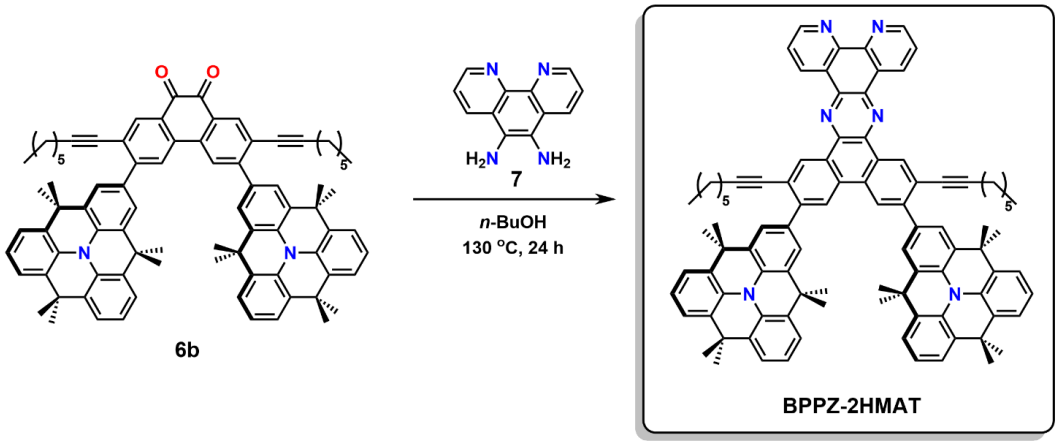
BPPZ-2TPA



Synthesized according to General Procedure B. The reaction was complete after 24 hours, and the crude material was purified over silica (99:1 $CHCl_3$:MeOH) to yield an orange solid. Yield: 220 mg (64%).

^{1}H NMR (400 MHz, Methylene Chloride-d_2): δ 9.56 (d, J = 8.4 Hz, 1H), 9.22 (s, 2H), 8.42 (s, 1H), 7.77 (m, 1H), 7.67 (d, J = 8.5 Hz, 2H), 7.35 (d, J = 8.7 Hz, 4H), 7.12 (d, J = 8.6 Hz, 6H), 2.46 (t, J = 7.0 Hz, 2H), 1.70 – 1.56 (m, 6H), 1.47 (m, 4H), 1.35 (s, 18H), 0.90 (t, J = 6.9 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, Methylene Chloride-d_2): δ152.0, 148.4, 147.9, 146.7, 145.5, 144.5, 140.2, 139.0, 133.4, 133.2, 130.9, 130.7, 130.6, 128.0, 127.4, 126.8, 125.1, 124.0, 123.7, 122.3, 121.8, 95.6, 81.1, 34.8, 32.0, 31.9, 29.4, 29.1, 23.3, 20.3, 14.6. HRMS (FD) *m/z*: $[M]^{+\bullet}$ calcd for $[C_{94}H_{96}N_6]^{+\bullet}$, 1308.7696; found, 1308.7684; difference: -0.98 ppm.

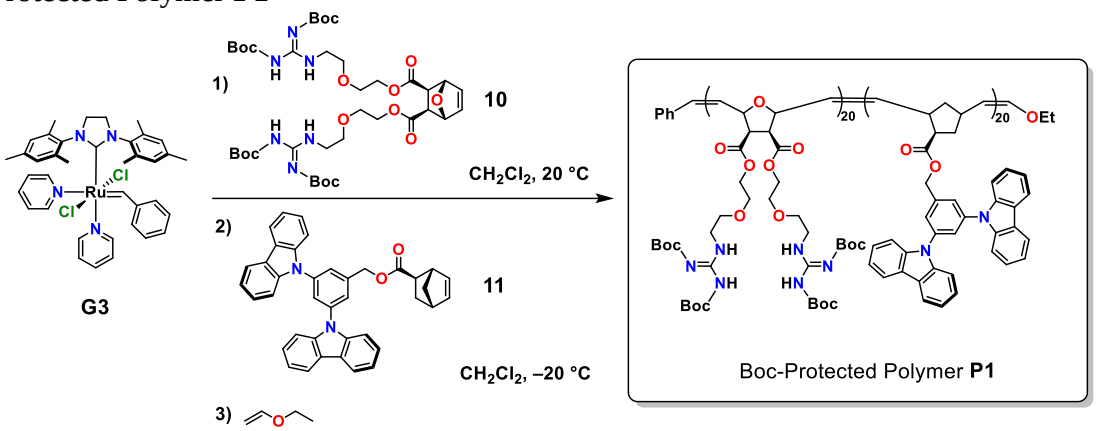
BPPZ-2HMAT



Synthesized according to General Procedure B. The reaction was complete after 24 hours, and the crude material was purified over silica (96:4 $CHCl_3$:MeOH) to yield a yellow solid. Yield: 109 mg (83%).

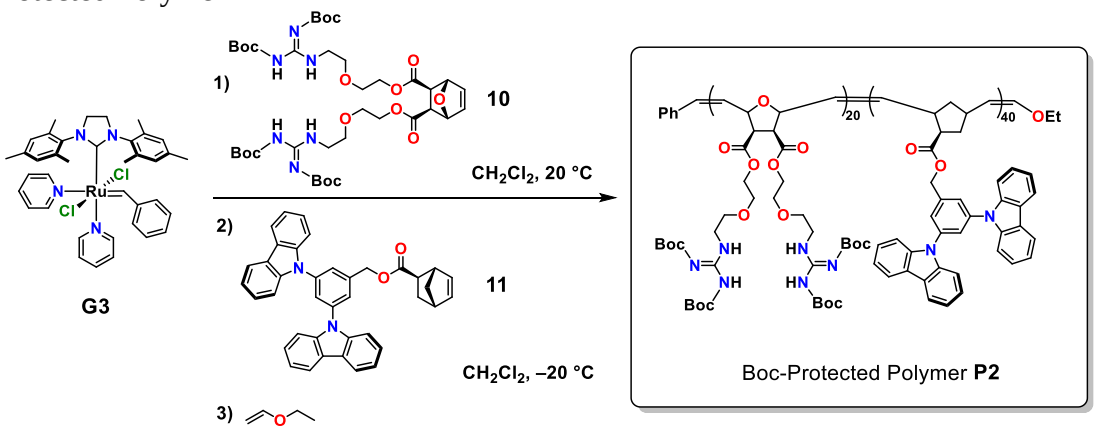
^{1}H NMR (400 MHz, Methylene Chloride-d_2): δ 9.87 (d, J = 8.1, 1H), 9.67 (s, 1H), 9.29 (d, J = 4.0 Hz, 1H), 8.75 (s, 1H), 7.97 (s, 2H), 7.91 (dd, J = 8.0, 4.4 Hz, 1H), 7.43 (dd, J = 7.7, 1.8 Hz, 4H), 7.16 (t, J = 7.7 Hz, 2H), 2.51 (t, J = 7.2 Hz, 2H), 1.75 (s, 12H), 1.65 (s, 6H), 1.52 – 1.43 (m, 2H), 1.28 – 1.26 (m, 4H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, Chloroform-d): δ 151.9, 146.8, 145.0, 141.1, 138.9, 134.4, 134.3, 131.8, 131.5, 131.0, 130.1, 129.9, 129.7, 128.2, 127.7, 125.0, 124.5, 123.9, 123.8, 123.7, 123.6, 123.2, 122.5, 95.5, 80.9, 35.8, 35.7, 33.8, 33.3, 31.5, 29.0, 28.9_8, 22.7, 20.3, 14.2. HRMS (FD) m/z: $[M]^{+\bullet}$ calcd for $[C_{96}H_{89}N_6]^{+\bullet}$, 1325.7149; found, 1325.7177; difference: +2.14 ppm.

Boc-Protected Polymer **P1**



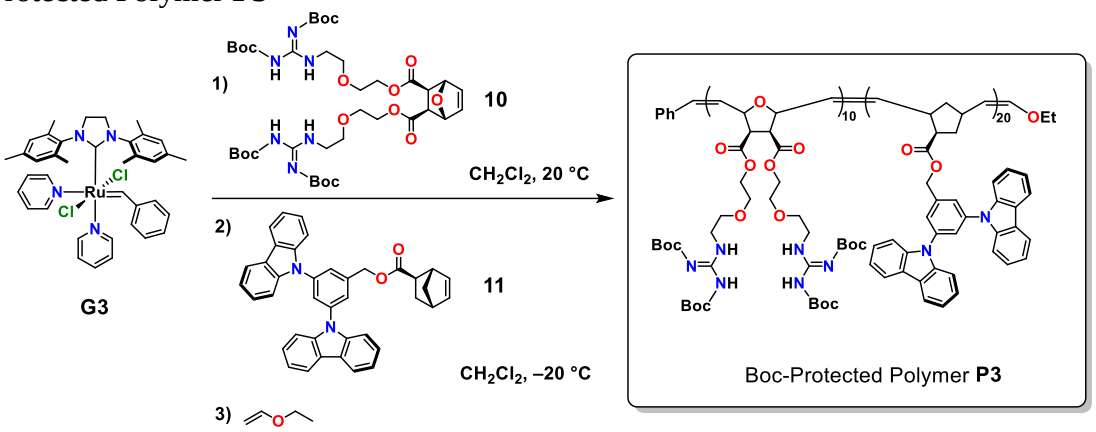
10 (50.0 mg, 59.3 mmol, 20 equiv.) was weighed into a 4 mL Teflon-capped vial equipped with a magnetic stir bar. In the glovebox, 172 µL of CH_2Cl_2 was added. Separately, a solution of Grubbs' 3rd gen. catalyst (**G3**) was dissolved in CH_2Cl_2 to a final concentration of 10 mg mL^{-1}. **G3** solution (263 µL, 1 equiv.) was added to the reaction vial, and the reaction was stirred at room temperature. After 12 hours, the solution was removed from the glovebox and cooled to –20 °C. Separately, **11** (33.1 mg, 59.3 mmol, 20 equiv.) was dissolved in 115 µL of CH_2Cl_2, before being added to the reaction vial under N_2. The polymerization proceeded for 24 hours at –20 °C, before quenching with ethyl vinyl ether. The polymer was purified by preparatory SEC, and isolated by precipitation from CH_2Cl_2 into hexanes. Yield: 43.4 mg (51%).

Boc-Protected Polymer **P2**



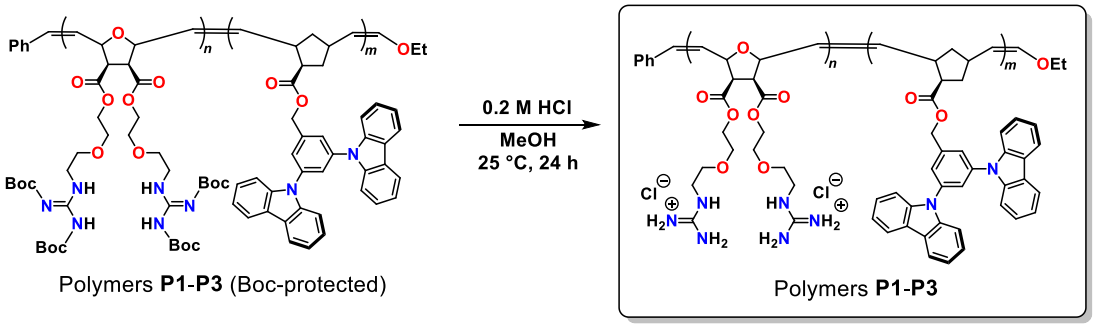
10 (50.0 mg, 59.3 mmol, 20 equiv.) was weighed into a 4 mL Teflon-capped vial equipped with a magnetic stir bar. In the glovebox, 220 µL of CH_2Cl_2 was added. Separately, a solution of Grubbs' 3rd gen. catalyst (**G3**) was dissolved in CH_2Cl_2 to a final concentration of 10 mg mL^{-1}. **G3** solution (263 µL, 1 equiv.) was added to the reaction vial, and the reaction was stirred at room temperature. After 12 hours, the solution was removed from the glovebox and cooled to –20 °C. Separately, **11** (66.3 mg, 119 mmol, 40 equiv.) was dissolved in 292 µL of CH_2Cl_2, before being added to the reaction vial under N_2. The polymerization proceeded for 24 hours at –20 °C, before quenching with ethyl vinyl ether. The polymer was purified by preparatory SEC, and isolated by precipitation from CH_2Cl_2 into hexanes. Yield: 61.4 mg (52%).

Boc-Protected Polymer **P3**



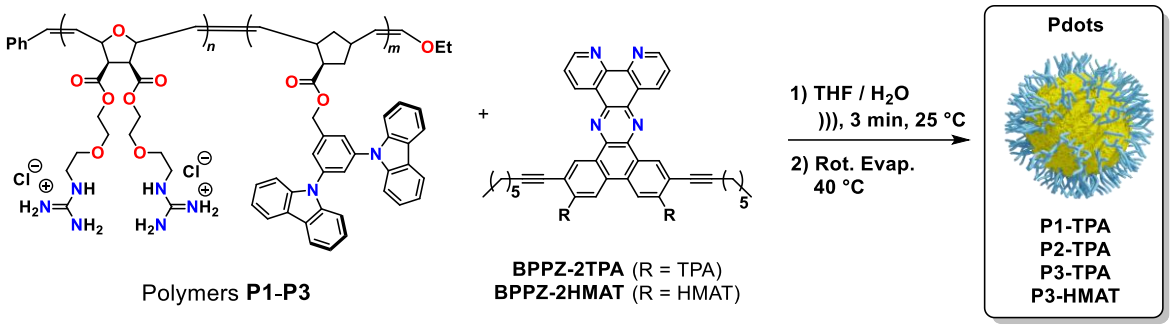
10 (50.0 mg, 59.3 mmol, 10 equiv.) was weighed into a 4 mL Teflon-capped vial equipped with a magnetic stir bar. In the glovebox, 100 µL of CH_2Cl_2 was added. Separately, a solution of Grubbs' 3rd gen. catalyst (**G3**) was dissolved in CH_2Cl_2 to a final concentration of 10 mg mL^{-1}. **G3** solution (525 µL, 1 equiv.) was added to the reaction vial, and the reaction was stirred at room temperature. After 12 hours, the solution was removed from the glovebox and cooled to –20 °C. Separately, **11** (66.3 mg, 119 mmol, 20 equiv.) was dissolved in 250 µL of CH_2Cl_2, before being added to the reaction vial under N_2. The polymerization proceeded for 24 hours at –20 °C, before quenching with ethyl vinyl ether. The polymer was purified by preparatory SEC, and isolated by precipitation from CH_2Cl_2 into hexanes. Yield: 80.0 mg (66%).

General Procedure C: Acidic deprotection of boc-protected polymers **P1**-**P3**



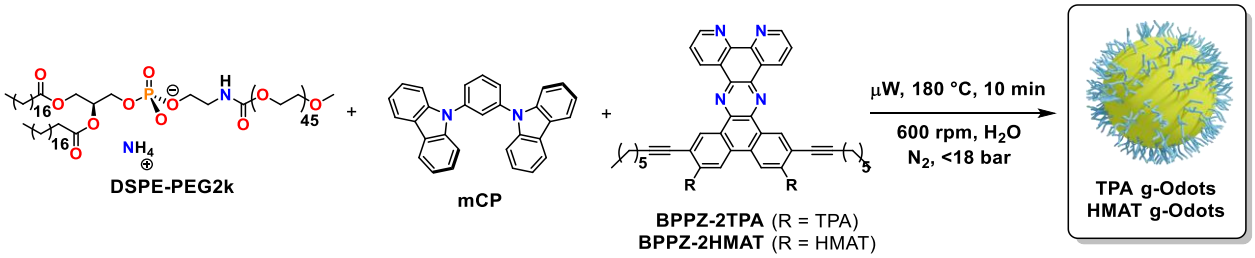
The removal of the boc protection groups was performed according to literature.[10] The boc-protected polymers were stirred in an excess (5 mL) of 1:1 MeOH and HCl (0.2 M $_{(aq)}$) at 25 °C for 24 hours. This solution was then transferred into a dialysis sac (Spectra/Por molecularporous membrane tubing, MWCO = 3.5 kDa), and placed in Milli-Q water (1 L) to remove any small molecule impurities. The polymers were dialyzed for 3 days, and the water was changed once a day. Finally, the polymer inside the dialysis tubing was transferred to a vial and lyophilized overnight to produce deprotected polymers as powders. Characterization of the deprotected polymers was only performed in Pdot form due to the differing solubility of the blocks.

General Procedure D: Preparation of Pdots



Polymer dot solutions were prepared by dissolving deprotected polymers **P1**, **P2**, or **P3** in THF at a concentration of 1 mg mL^{-1}. Separately, **BPPZ-2TPA** or **BPPZ-2HMAT** were dissolved in THF at a concentration of 2 mg mL^{-1}. 100 µL of TADF solution and 1 mL of polymer solution were combined and added to 10 mL of Milli-Q water under vigorous sonication for three minutes. The THF was then fully removed under reduced pressure, such that the final concentration of Pdots was 0.24 mg mL^{-1} in water. Polymer dot solutions were stored at 4 °C until use. These solutions were diluted with growth medium for cell studies.

General Procedure E: Preparation of g-Odots



Into Teflon-capped 4 mL vials, individual chloroform stock solutions were prepared of **mCP** (12.3 mmol L^{-1}), **DSPE-PEG2k** (10.6 mmol L^{-1}), and BPPZ dopant (1.27 mmol L^{-1}). To a microwave reaction vial (Biotage, 20 mL), each stock solution was added such that a 10:1 **mCP**:**DSPE-PEG2k** molar ratio was used, along with a BPPZ dopant amount of 5 wt%: **mCP** stock (862 µL, 4.33 mg, 10.6 µmol), **DSPE-PEG2k** stock (100 µL, 2.97 mg, 1.06 µmol), and BPPZ dopant stock (129-130 µL, contingent upon dopant molecular weight, 0.22 mg). The combined chloroform solutions were evaporated under a stream of nitrogen in the microwave vial, yielding a film that was further dried *in vacuo* at 60 °C for one hour. A magnetic stir bar and filtered MilliQ water (20 mL, 0.22 µm pore filtration) was added, and the vial was sealed with a Reseal™ cap. The vial contents were vigorously sparged with nitrogen gas for 30 minutes with rapid magnetic stirring. Each sample was heated to 180 °C for 10 minutes (<20 bar) with stirring at 600 rpm, and then rapidly cooled over 7 minutes using a stream of compressed air. The contents were centrifuged to remove larger particles (2 x 1500 rpm), collecting the supernatant in each case. The supernatant was then centrifuged to pelletize the suspended nanoparticles (3 x 6000 rpm), washing and resuspending the contents in new volumes of filtered MilliQ water (3 x 1.5 mL). The final pellet was resuspended in MilliQ water (1.0 mL), and stored at 4 °C until further use, or lyophilized as a solid material stored at 4 °C.

^{1}H and ^{13}C{^{1}H} NMR Spectra

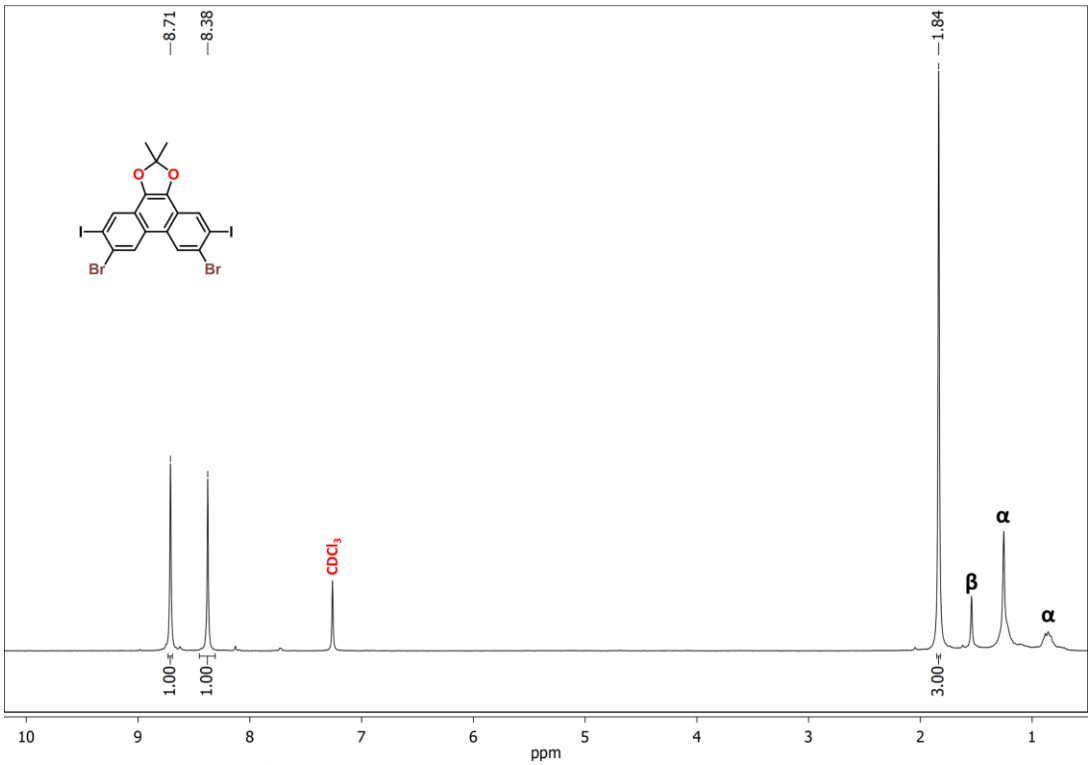


Figure S1. ^{1}H NMR (400 MHz) of **2** in $CDCl_3$ (**α**: grease, **β**: H_2O).

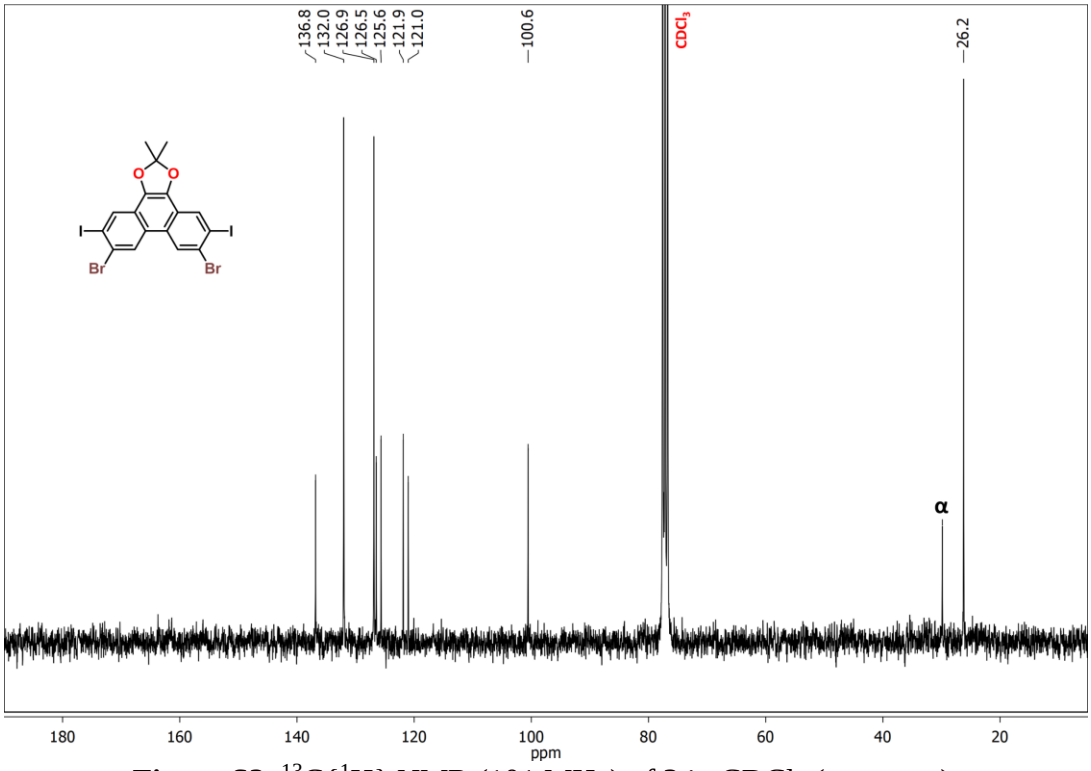


Figure S2. ^{13}C{^{1}H} NMR (101 MHz) of **2** in $CDCl_3$ (**α**: grease).

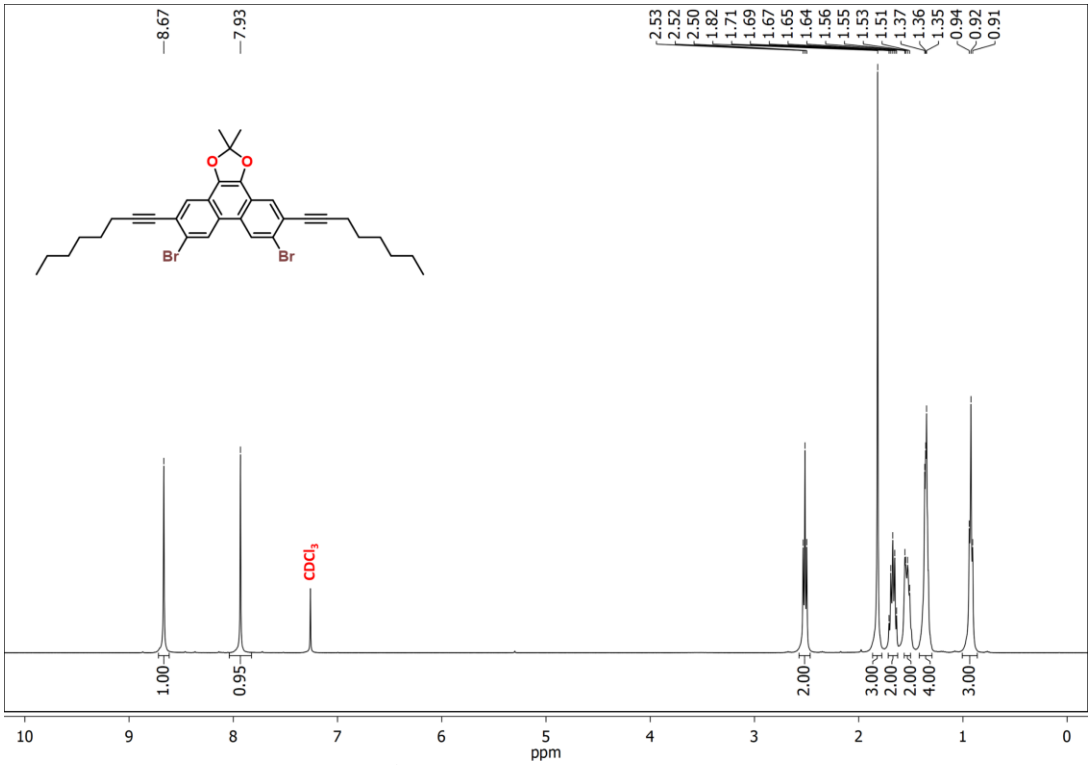


Figure S3. ^{1}H NMR (400 MHz) of **3** in $CDCl_3$.

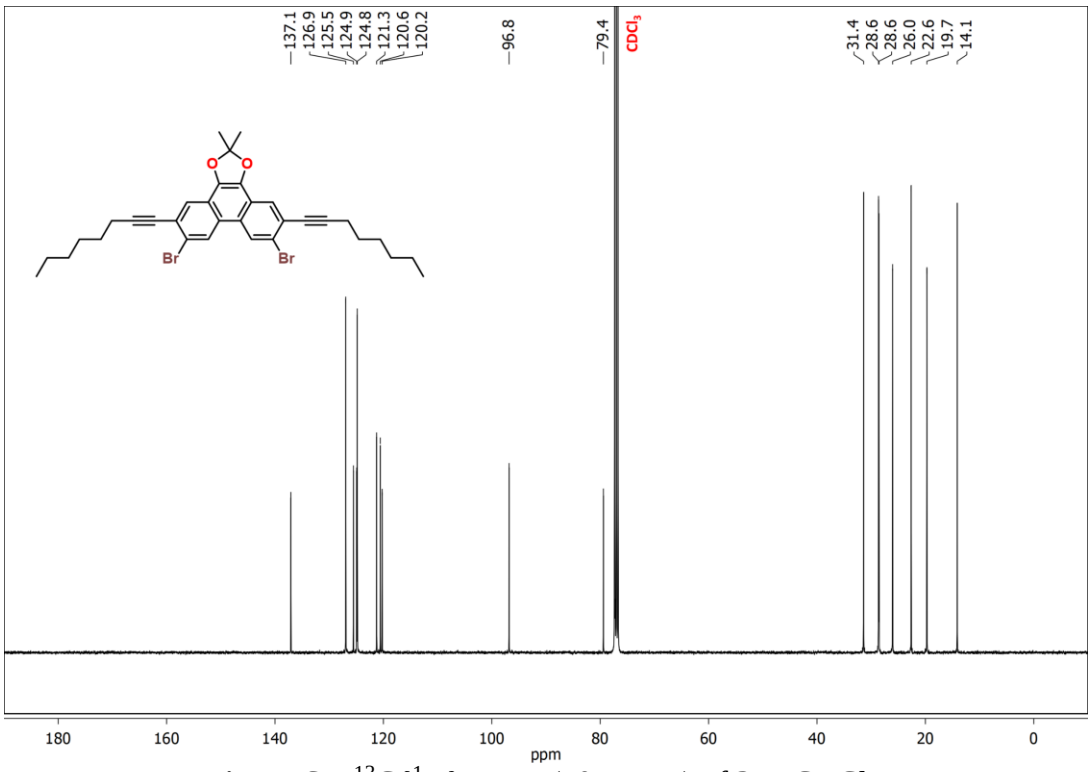


Figure S4. ^{13}C{^{1}H} NMR (101 MHz) of **3** in $CDCl_3$.

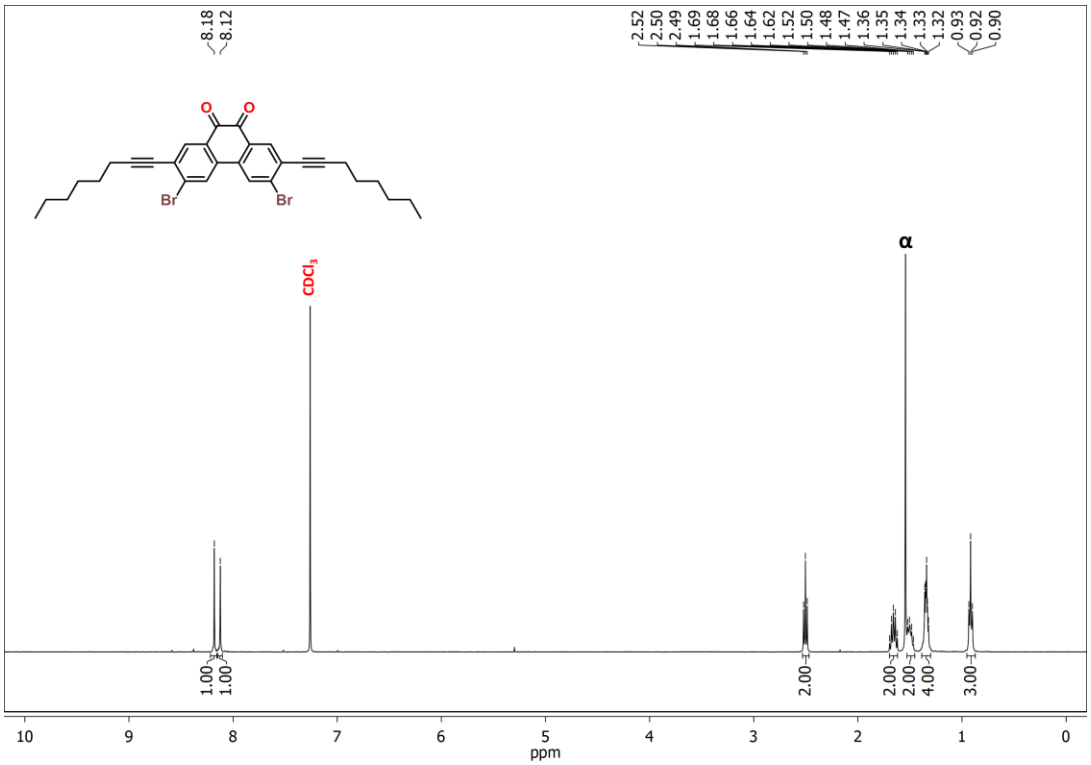


Figure S5. ^{1}H NMR (400 MHz) of **4** in $CDCl_3$ (**α**: H_2O).

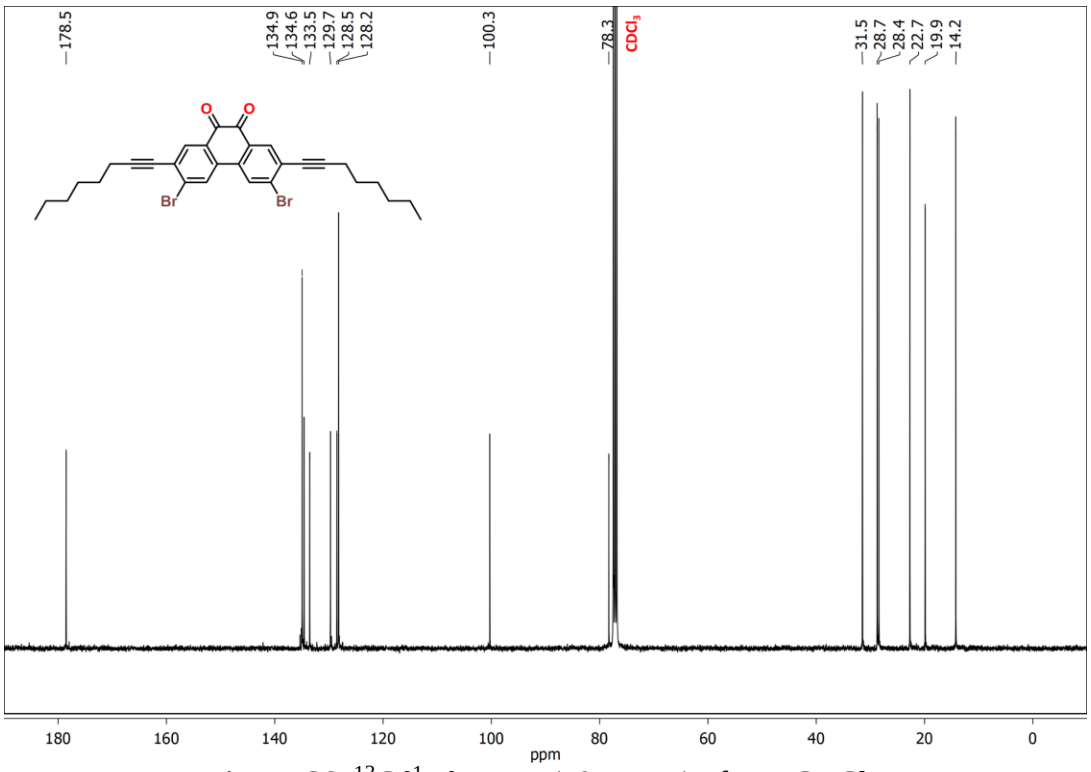


Figure S6. $^{13}C\{^{1}H\}$ NMR (101 MHz) of **4** in $CDCl_3$.

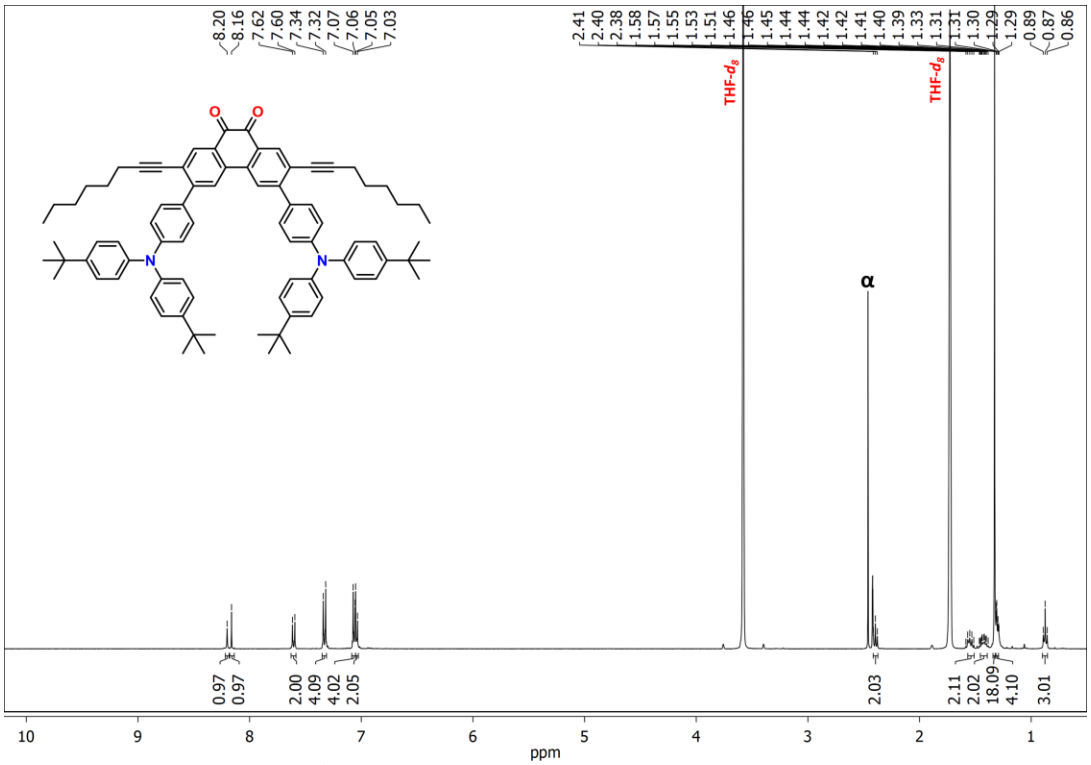


Figure S7. ^{1}H NMR (400 MHz) of **6a** in THF-d_8 (**α**: H_2O).

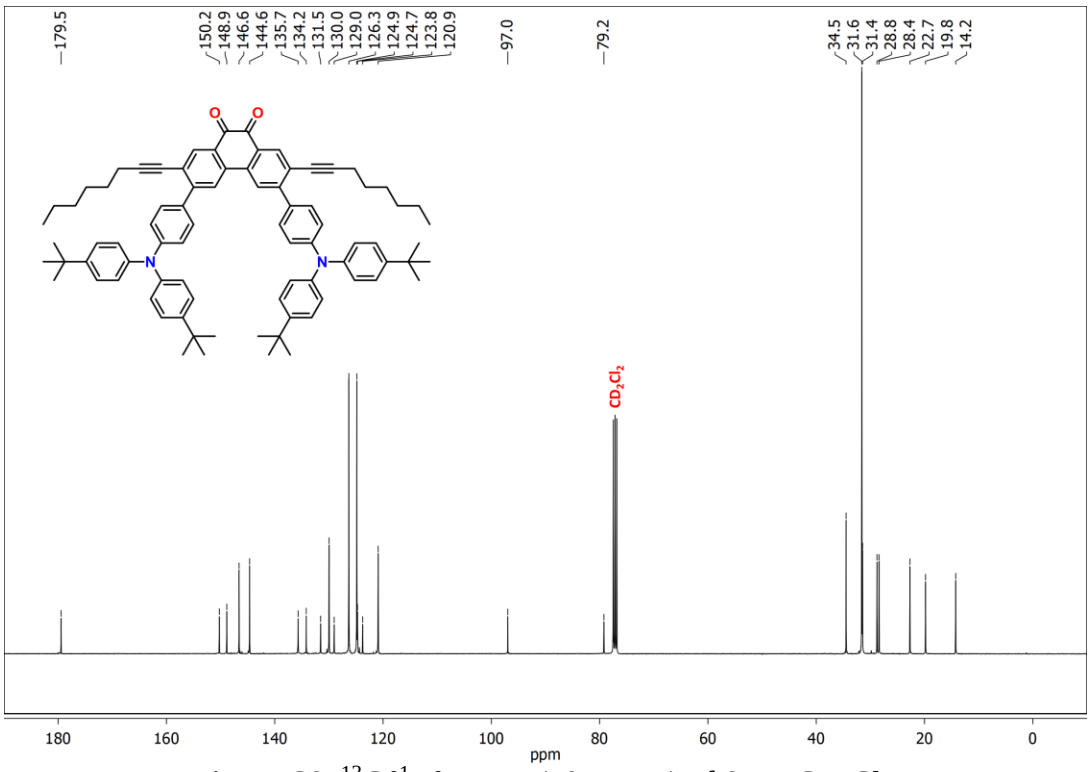


Figure S8. ^{13}C{^{1}H} NMR (101 MHz) of **6a** in CD_2Cl_2.

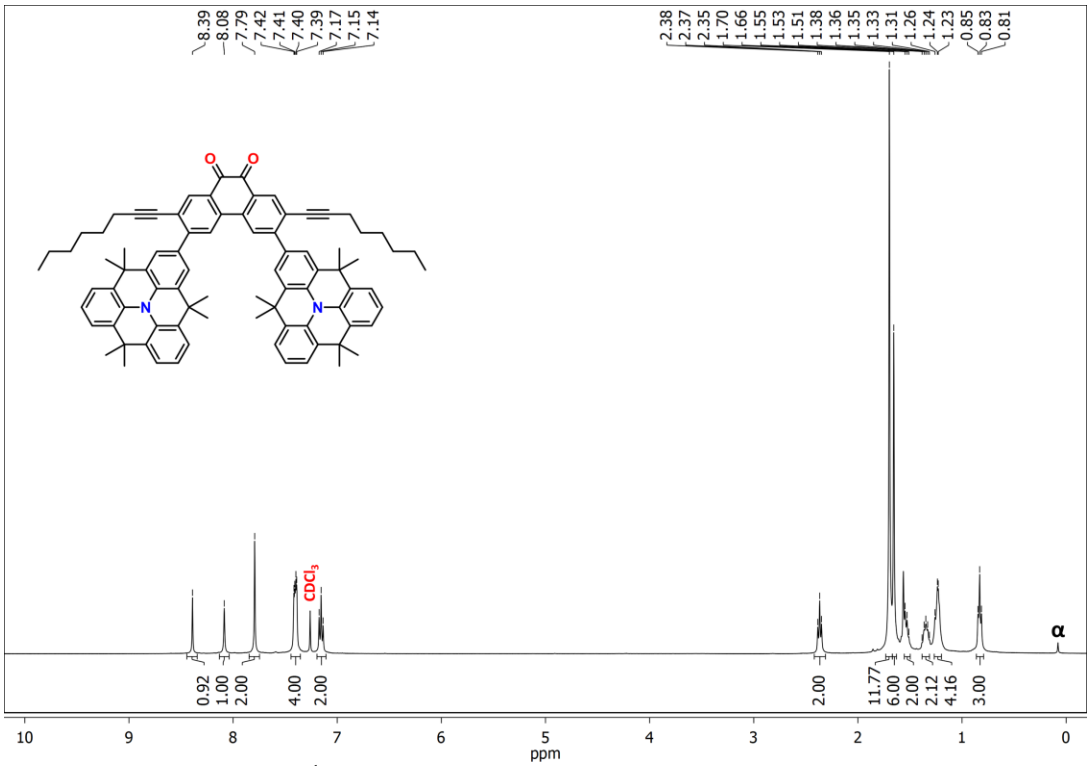


Figure S9. ^{1}H NMR (400 MHz) of **6b** in $CDCl_3$ (**α**: grease).

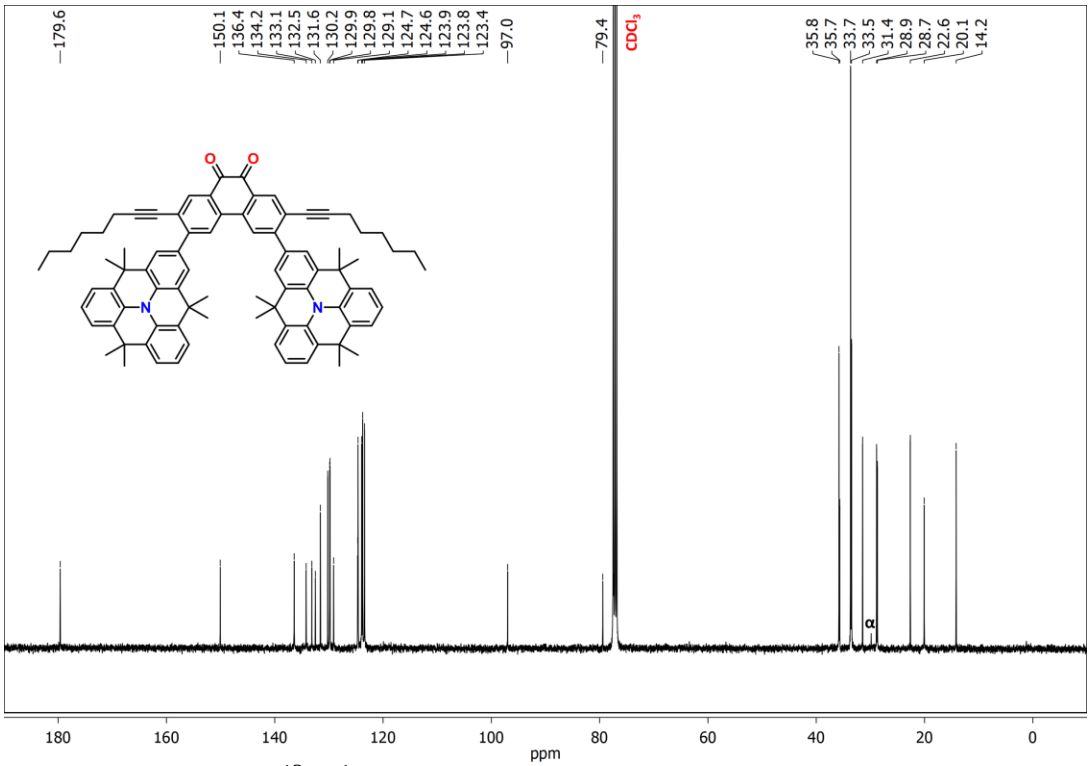


Figure S10. $^{13}C\{^{1}H\}$ NMR (101 MHz) of **6b** in $CDCl_3$ (**α**: grease).

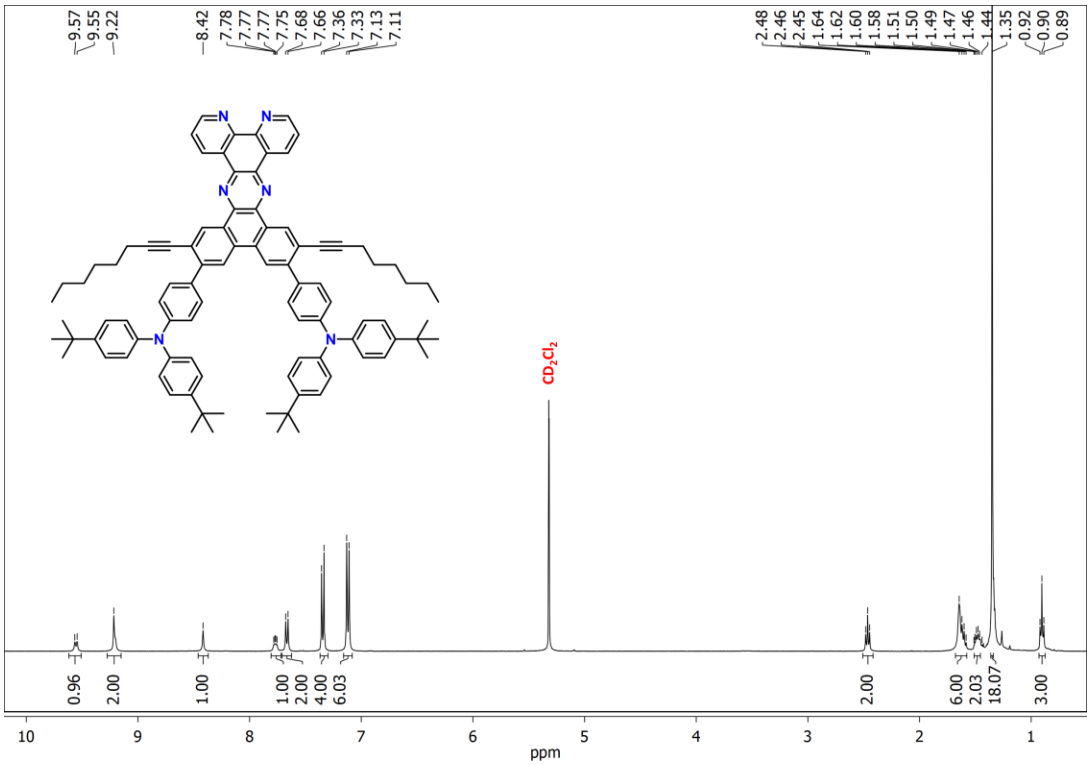


Figure S11. ^{1}H NMR (400 MHz) of **BPPZ-2TPA** in CD_2Cl_2 (**α**: H_2O).

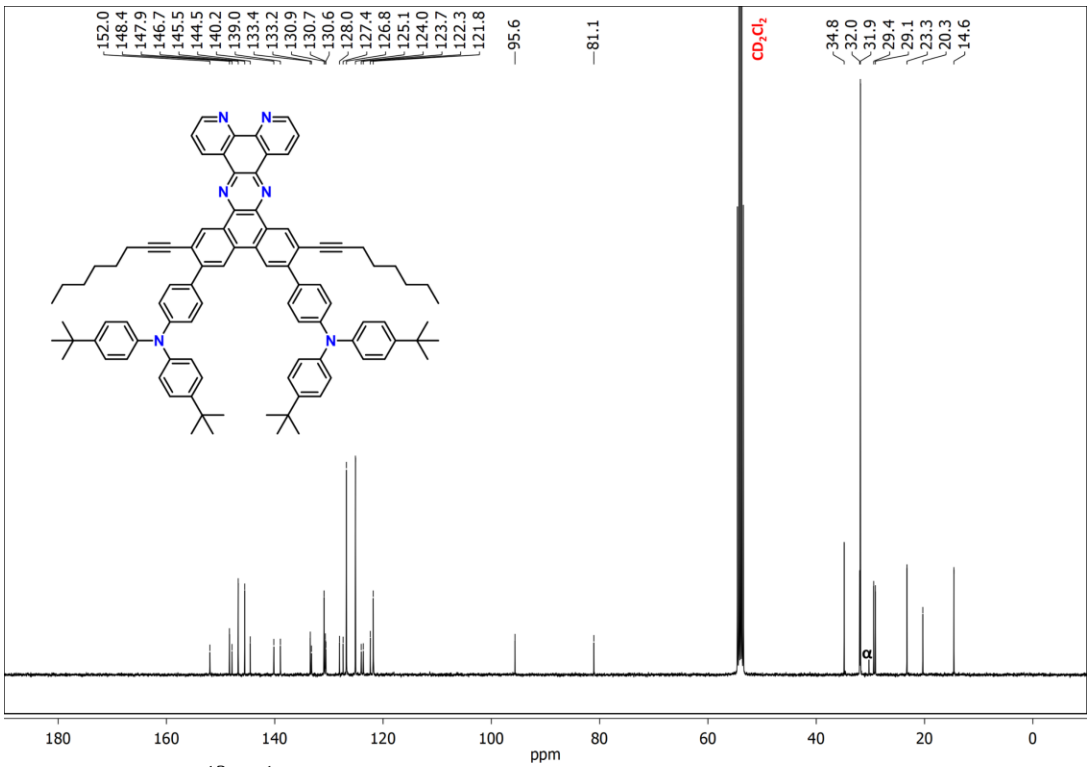


Figure S12. ^{13}C{^{1}H} NMR (101 MHz) of **BPPZ-2TPA** in CD_2Cl_2 (**α**: grease).

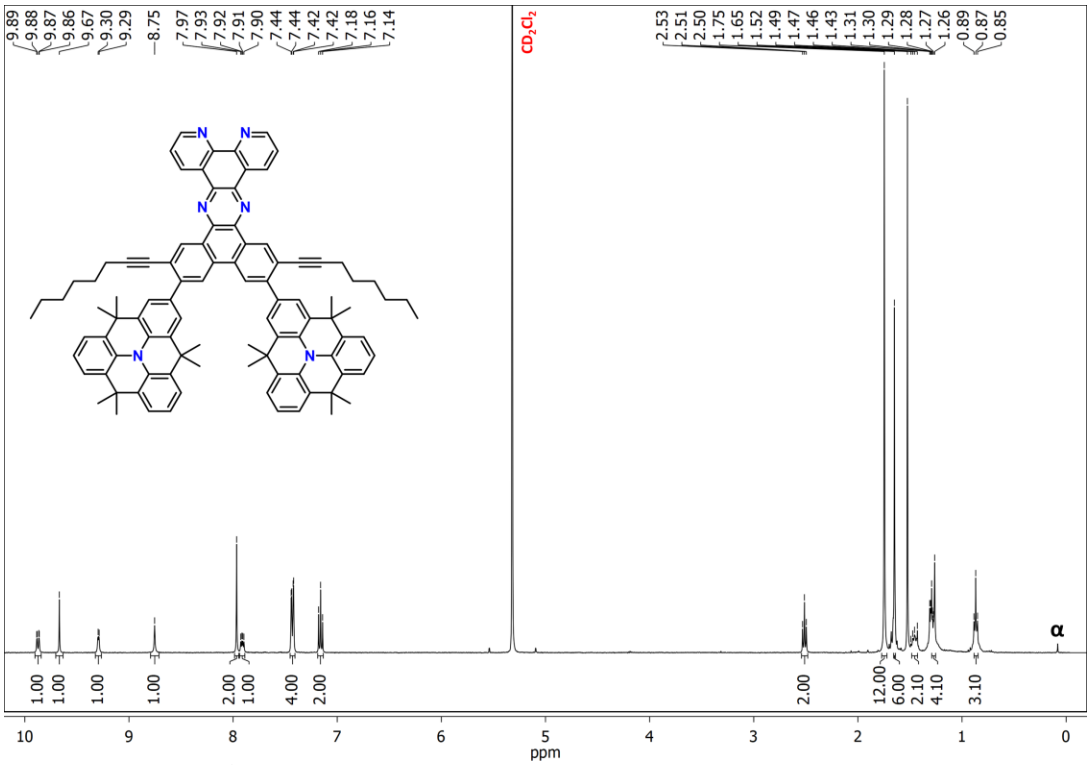


Figure S13. ^{1}H NMR (400 MHz) of **BPPZ-2HMAT** in CD_2Cl_2 (***α***: grease).

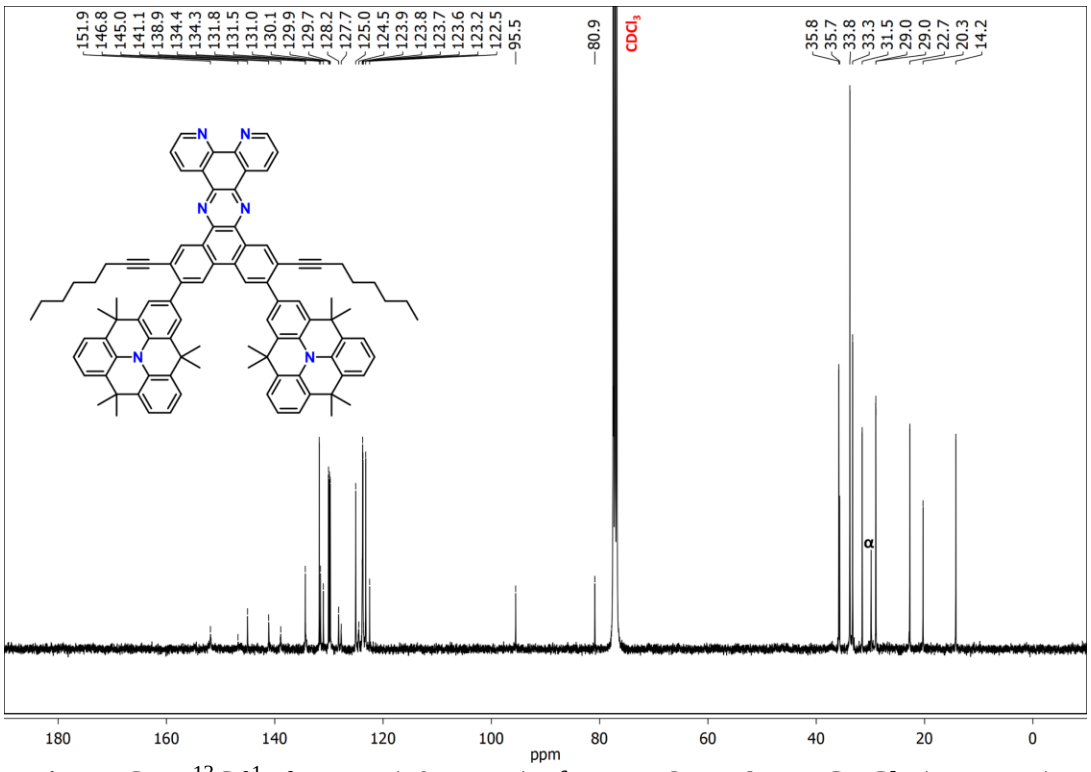


Figure S14. $^{13}C\{^{1}H\}$ NMR (101 MHz) of **BPPZ-2HMAT** in $CDCl_3$ (***α***: grease).

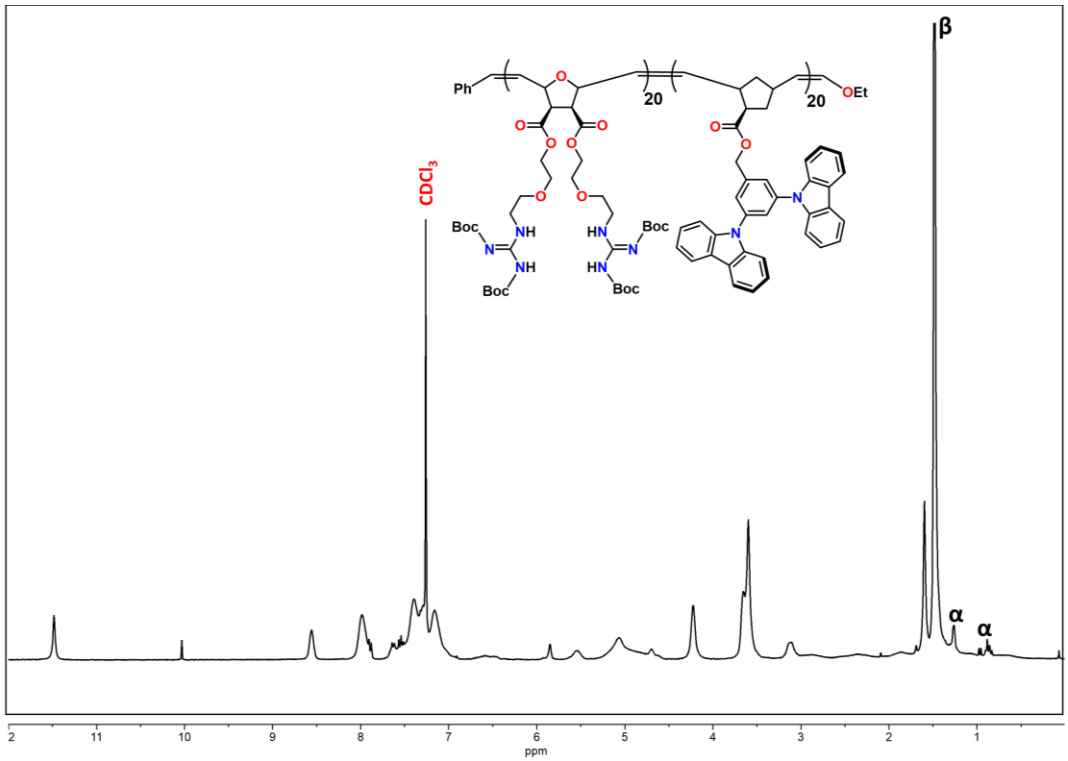


Figure S15. ^{1}H NMR (400 MHz) of Boc-protected **polymer P1** in $CDCl_3$ (**α**: grease, **β**: H_2O).

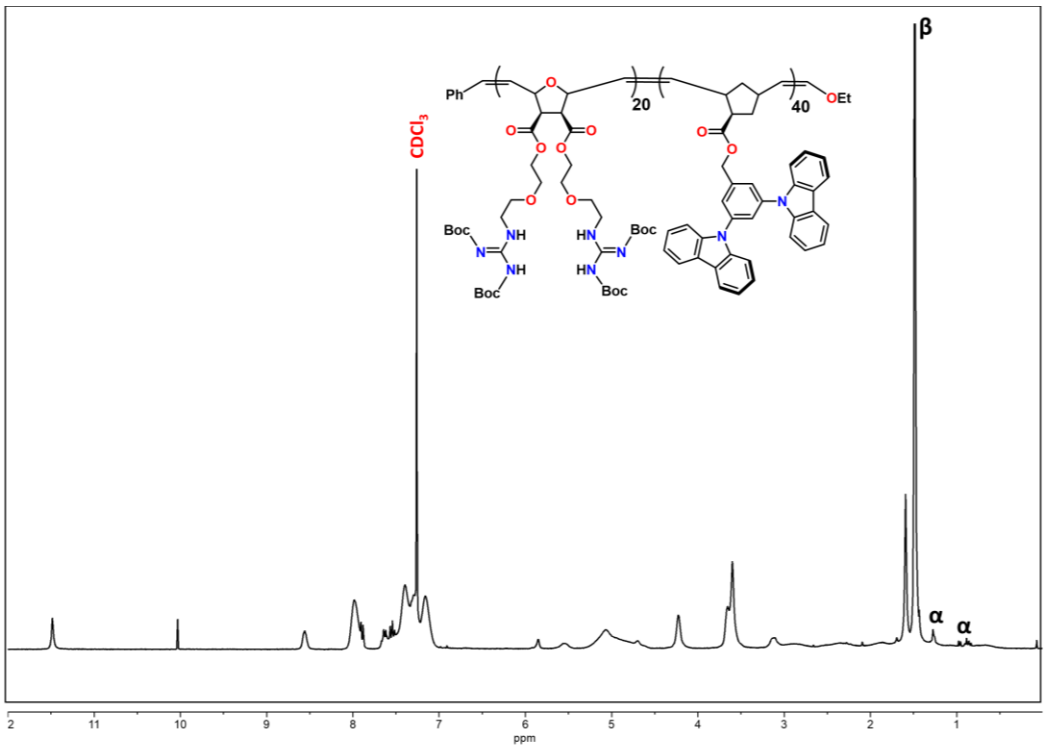


Figure S16. ^{1}H NMR (400 MHz) of Boc-protected polymer **P2** in $CDCl_3$ (**α**: grease, **β**: H_2O).

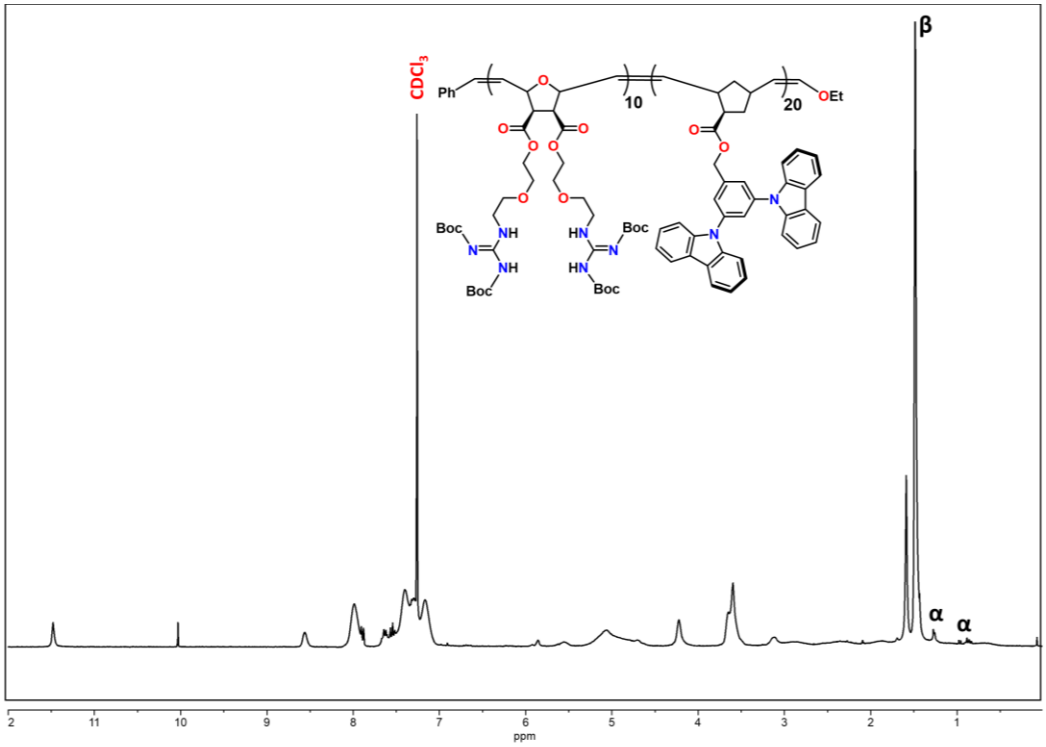


Figure S17. ^{1}H NMR (400 MHz) of Boc-protected polymer **P3** in $CDCl_3$ (**α**: grease, **β**: H_2O).

Photophysical Characterization

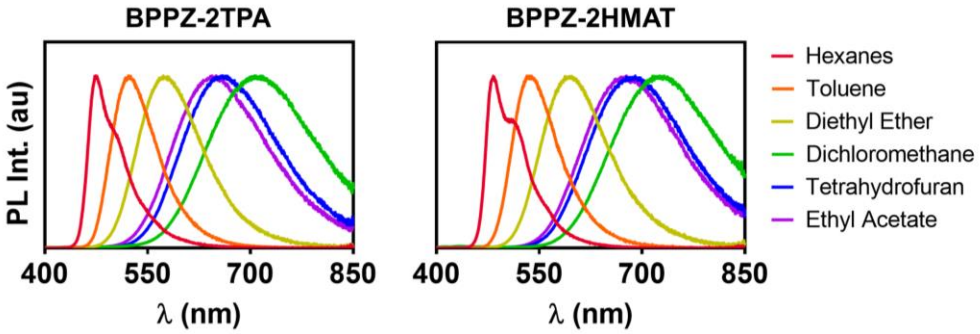


Figure S18. Normalized emission spectra showing solvatochromic, measured at concentrations of 1 x 10^{-3} mg mL^{-1}, and excited at 380 nm.

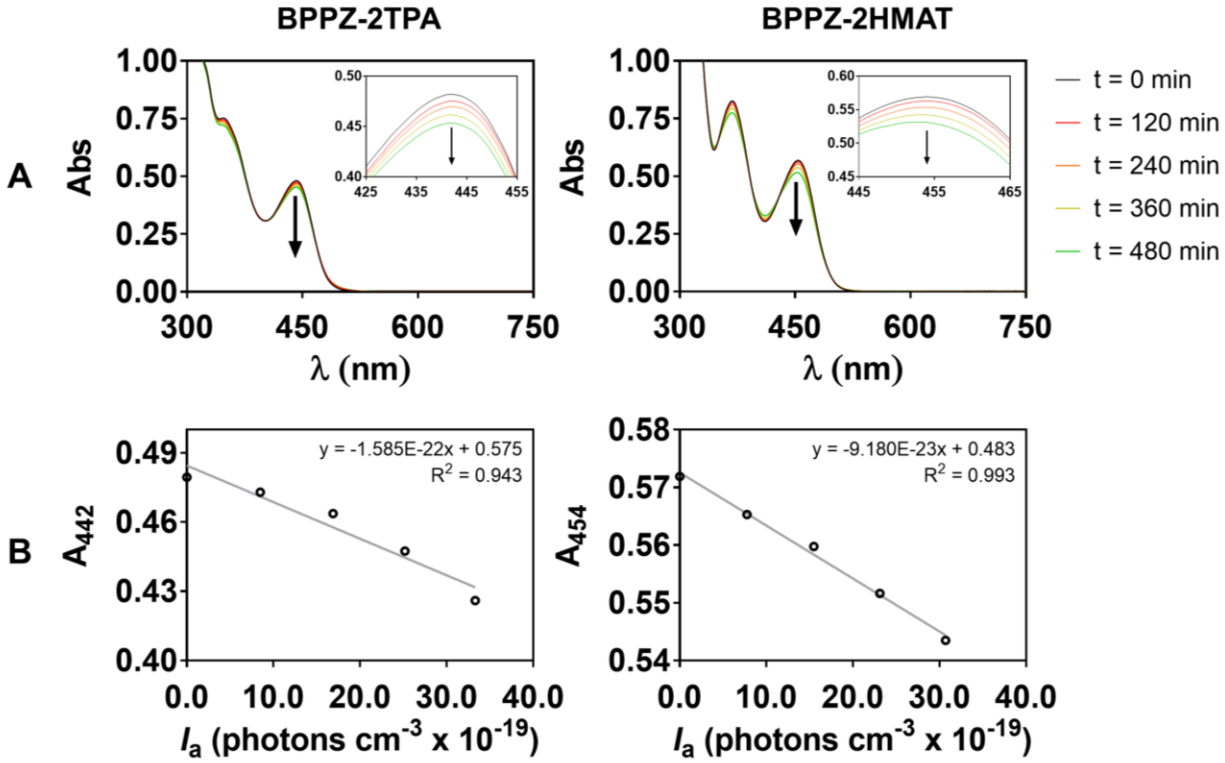


Figure S19. Photodestructive quantum yield data. (A) The change in absorption of a dilute toluene solution of each emitter upon exposure to an LED light source (λ_{ex} = 365 nm), with an inset zoomed in region shown. (B) A plot comparing the change in absorption (at λ_{max}) as a function of the number of photons absorbed for a given time interval. A linear regression line and corresponding equation (inset) are also shown.

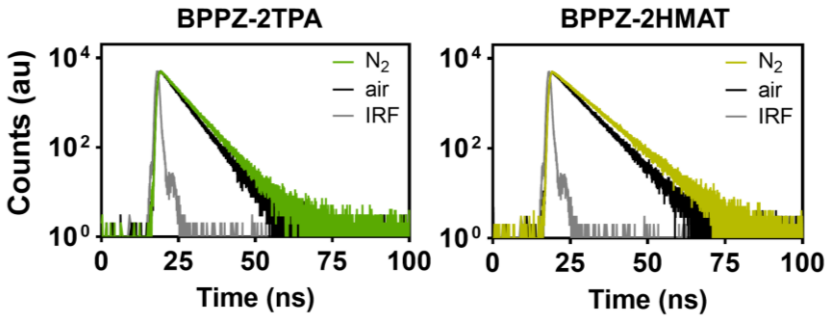


Figure S20. PL decays for toluene solutions at 1.0 x 10^{-3} mg mL^{-1} under air (black trace) or N_2 (coloured trace). All measurements were performed using TCSPC and a 380 nm EPLED source.

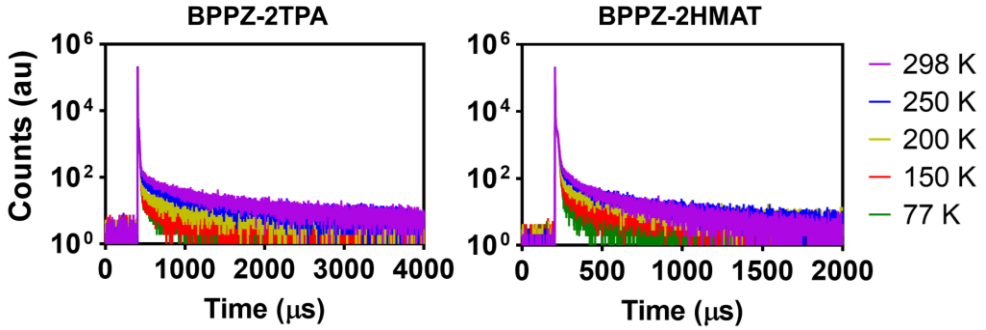


Figure S21. PL decays for 5 wt%-doped poly-mCP films, with temperatures ranging from 77 to 298 K, performed using MCS with a Xe μF source (λ_{exc} = 380 nm).

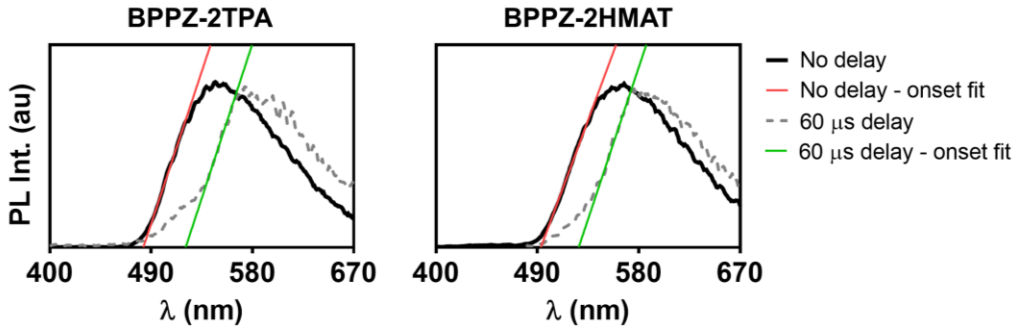


Figure S22. Time-gated 77 K fluorescence (black) and phosphorescence (gray, dashed) spectra, with corresponding linear onset fits. Samples were measured at 1.0 x 10^{-3} mg mL in 2-methyltetrahydrofuran (λ_{exc} = 380 nm).

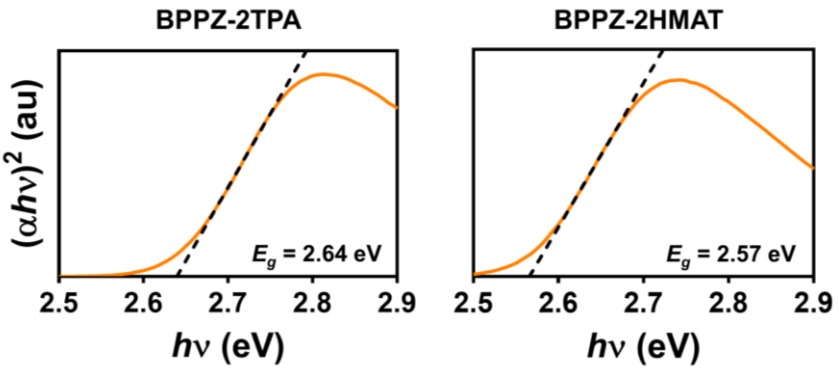


Figure S23. Tauc plots of each material, determined using UV-vis absorption spectra measured in toluene, with calculated optical gaps (E_g) displayed inset.

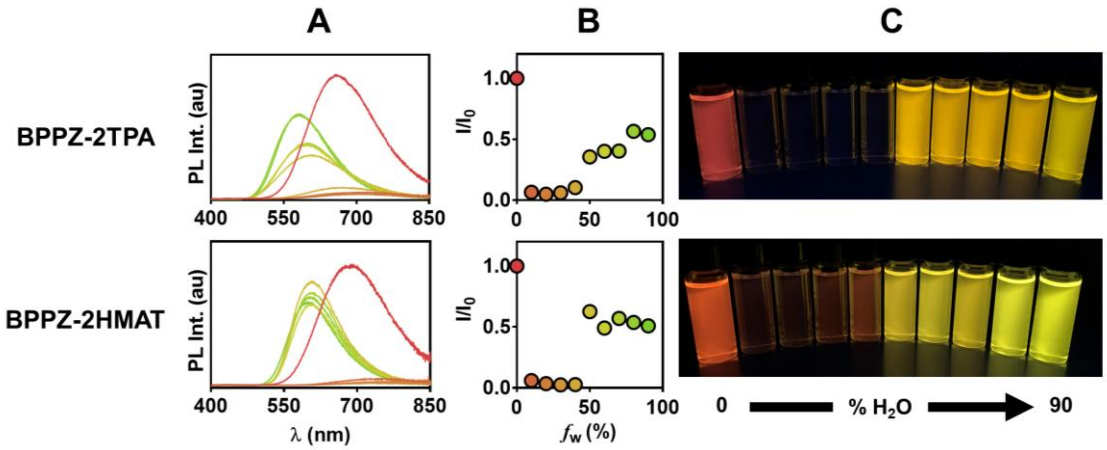


Figure S24. Emission spectra (A), and relative emission intensity (I/I_0) as a function of water fraction f_w (B) for solutions of **BPPZ-2TPA** and **BPPZ-2HMAT** in THF/water solutions ranging from 0% (red) to 90% (green) water. Samples were prepared as 2 x 10^{-2} mg mL^{-1} solutions, and were excited at 380 nm.

Thermal and Electrochemical Characterization

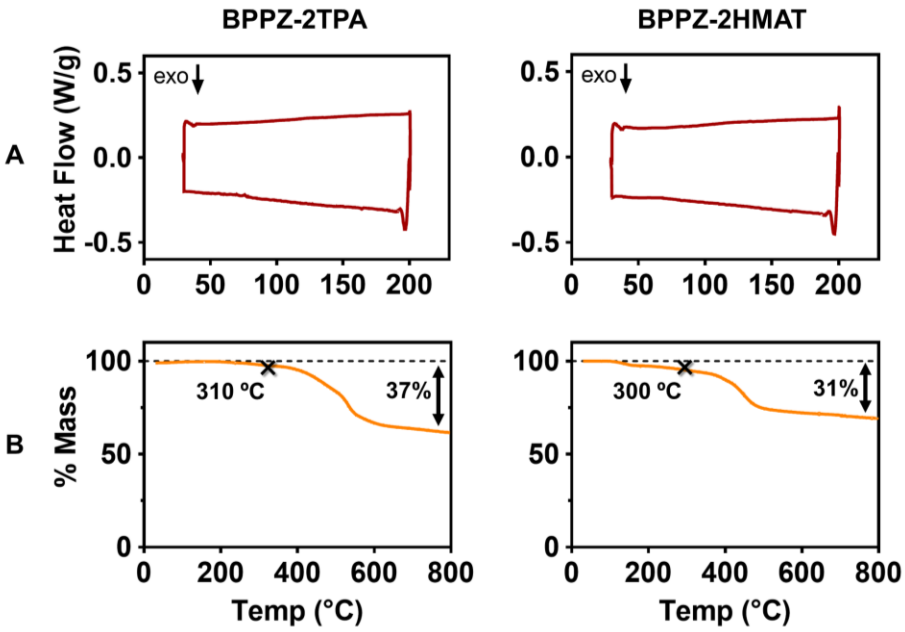


Figure S25. (A) Thermogravimetric analysis (TGA) performed at a rate of 10 °C min^{-1}, under a 50 mL min^{-1} flow of nitrogen gas, from 30 to 800 °C; (B) Differential scanning calorimetry (DSC) traces acquired at a rate of 10 °C min^{-1}, under a 50 mL min^{-1} flow of nitrogen gas. Two consecutive heating and cooling cycles were performed, with the second cycle shown in each case.

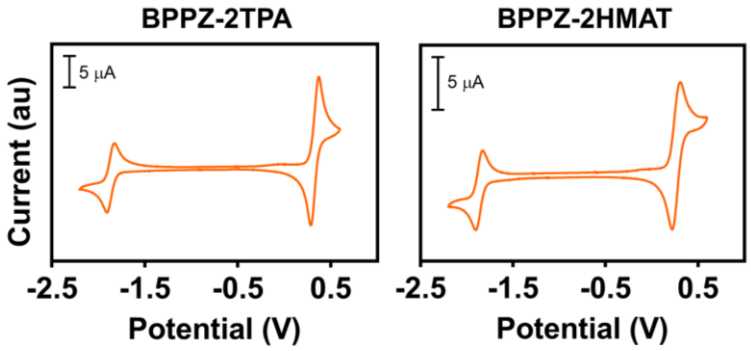


Figure S26. Cyclic voltammograms, with analyte measured at 2 µmol mL^{-1} in degassed *o*-difluorobenzene relative to $Fc^{0/+}$, with 0.2 M [*n*-Bu_4N^+][PF_6^-] at 20 mV s^{-1}. Each sample was run with four cycles, with the fourth cycle shown in each case.

Density Functional Theory

Table S1. Results of DFT and TDA-DFT calculations for **BPPZ-2TPA** and **BPPZ-2HMAT**.

Entry	ω (Bohr^{-1})	HOMO[a] (eV)	LUMO[a] (eV)	E_{gap} (eV)	E_{S1}[b] (eV)	f[b] ($S_0 \rightarrow S_1$)	# Imag. Freq.	E_{Total} (10^3 Hartrees)
BPPZ-2TPA	0.1011	-6.24	-1.48	4.76	3.12	1.154	0	-3.97
BPPZ-2HMAT	0.1006	-6.18	-1.52	4.66	3.05	0.721	0	-4.04

[a] Calculated at the ωB97XD/DefTZVP level. [b] Calculated using TDA-DFT at the ωB97XD/pc-1 level in toluene using PCM.

Table S2. Cartesian coordinates [Å] of the optimized structure for **BPPZ-2TPA.**

	X	Y	Z		X	Y	Z		X	Y	Z
C	4.31047	3.34126	0.08072	C	0.55999	11.22013	0.65720	C	-8.51473	-1.02990	-3.86591
C	4.55772	1.97549	0.06488	C	1.70082	12.02659	0.67415	C	-7.13500	-0.90147	-3.99663
C	3.45834	1.08050	0.05393	N	2.92948	11.56160	0.59486	C	-1.41156	-7.15966	1.24727
C	2.18144	1.60535	0.03065	N	5.47764	10.60969	0.42768	C	8.52610	-7.58201	-0.47475
C	1.91773	2.97581	0.03922	C	6.71015	10.15516	0.34797	C	-6.65784	-4.89109	3.53630
C	3.01302	3.85625	0.08190	C	7.03545	8.80074	0.23895	C	-9.45961	-1.11659	-5.05894
C	0.55750	3.48912	0.03984	C	6.01090	7.88655	0.21324	C	-8.71651	-1.05208	-6.39024
C	0.31877	4.86780	0.17578	C	2.82624	-1.10640	1.02016	C	-10.23017	-2.44071	-5.00210
C	1.44458	5.78331	0.22777	C	2.86046	-2.48521	1.05984	C	-10.45220	0.05062	-5.00511
C	2.77309	5.28645	0.15717	C	3.65431	-3.19699	0.16159	C	-6.66657	-4.35434	4.96471
C	-0.54554	2.64145	-0.08401	C	4.43821	-2.48456	-0.74723	C	-7.97692	-5.63293	3.29282
C	-1.85029	3.08659	-0.02039	C	4.41711	-1.10194	-0.76245	C	-5.48907	-5.87391	3.39884
C	-2.08034	4.47333	0.19218	C	-4.04114	2.29810	-0.97455	C	8.33007	-8.92513	-1.17201
C	-0.99520	5.33330	0.26163	C	-5.04376	1.35476	-1.08232	C	9.49829	-6.74349	-1.31074
C	5.88922	1.48077	0.11769	C	-4.97834	0.15186	-0.36940	C	9.13912	-7.84261	0.90578
C	-3.41260	4.92984	0.38525	C	-3.86173	-0.06580	0.44226	C	-2.57898	-6.52972	0.49157
C	6.99492	1.00434	0.17550	C	-2.87478	0.89458	0.55376	C	-1.34011	-8.64398	0.87130
C	8.29028	0.35058	0.19945	N	3.63951	-4.60133	0.17191	C	-1.68933	-7.03026	2.74958
C	8.39256	-0.78461	-0.82227	C	4.82554	-5.33344	0.00653	H	5.14121	4.03334	0.11844
C	9.73712	-1.48985	-0.77658	C	4.82293	-6.56592	-0.63917	H	1.36195	0.90060	0.00765
C	9.91285	-2.51631	-1.88367	C	2.31811	-6.33206	1.28504	H	-0.39344	1.58585	-0.26224
C	11.26369	-3.21412	-1.84881	C	2.41250	-5.25963	0.40001	H	-1.16035	6.39260	0.40706
C	11.49508	-4.12317	-3.04366	C	1.09606	-6.93142	1.52755	H	9.07975	1.08710	0.01130
C	-4.58704	5.13722	0.56255	C	-0.08163	-6.48903	0.92047	H	8.47496	-0.04372	1.20614
C	-6.02985	5.12125	0.72937	C	0.03292	-5.42461	0.03148	H	7.58522	-1.50232	-0.64352
C	-6.46643	3.80694	1.39437	C	1.25431	-4.82573	-0.23511	H	8.22379	-0.37303	-1.82305
C	-7.91656	3.43214	1.14383	C	6.04216	-4.84907	0.49171	H	10.54016	-0.74536	-0.85035
C	-8.25278	2.07038	1.73071	C	7.20444	-5.57487	0.31982	H	9.86391	-1.97468	0.19978
C	-9.64555	1.56754	1.38971	C	7.21718	-6.81773	-0.31657	H	9.11206	-3.26449	-1.82542
C	-9.95876	0.23233	2.04497	C	5.99584	-7.29113	-0.78507	H	9.79036	-2.01876	-2.85407
C	3.59670	-0.38499	0.10745	C	-6.31175	-3.12144	0.17379	H	12.05660	-2.45806	-1.80600

C	-2.93836	2.10021	-0.14375	C	-6.17361	-1.78233	0.53252	H	11.34904	-3.79136	-0.92040
N	1.21602	7.08576	0.32886	N	-5.99900	-0.79609	-0.46383	H	12.46063	-4.63054	-2.98227
C	2.25317	7.91320	0.35539	C	-6.82733	-0.86480	-1.60635	H	10.72034	-4.89057	-3.11183
C	3.57344	7.41979	0.27413	C	-6.30046	-0.82911	-2.89087	H	11.47706	-3.55313	-3.97685
N	3.80667	6.11678	0.17919	C	-6.21725	-1.45154	1.88128	H	-6.37884	5.98756	1.30010
C	2.02100	9.34003	0.46707	C	-8.20638	-0.99181	-1.45727	H	-6.49440	5.19964	-0.26182
C	3.10249	10.23925	0.49226	C	-6.47641	-4.09214	1.14315	H	-5.83041	3.00583	1.00959
C	4.47265	9.72737	0.40337	C	-6.50155	-3.78063	2.50382	H	-6.26684	3.86585	2.46917
C	4.69198	8.34197	0.29628	C	-6.37076	-2.43735	2.84454	H	-8.59171	4.19426	1.55211
C	0.72670	9.86098	0.55215	C	-9.02480	-1.07805	-2.56677	H	-8.09727	3.40943	0.06145
H	-7.51799	1.34146	1.36726	H	-10.81828	-2.52704	-4.08629	H	5.94043	-8.24566	-1.29282
H	-8.13277	2.10337	2.82115	H	-9.54484	-3.29093	-5.04159	H	-6.28839	-3.39516	-0.87473
H	-10.39043	2.31218	1.69378	H	-9.92808	1.00848	-5.04878	H	-5.22889	-0.73625	-3.02469
H	-9.73355	1.47609	0.30062	H	-11.14319	-0.00019	-5.85133	H	-6.11737	-0.41475	2.17997
H	-10.94146	-0.14345	1.75030	H	-11.04517	0.03479	-4.08843	H	-8.63152	-1.02704	-0.46105
H	-9.21464	-0.52425	1.77829	H	-6.77950	-5.18394	5.66672	H	-6.57246	-5.12420	0.82442
H	-9.94957	0.31944	3.13527	H	-5.73447	-3.83889	5.20952	H	-6.39598	-2.13321	3.88306
H	-0.11404	9.17889	0.53277	H	-7.49684	-3.66373	5.13268	H	-10.09353	-1.17382	-2.40991
H	-0.42593	11.66390	0.72558	H	-8.09935	-6.43797	4.02295	H	-6.68160	-0.86819	-4.97897
H	1.60281	13.10657	0.75659	H	-8.01246	-6.07766	2.29631	H	-9.43301	-1.11691	-7.21258
H	7.49833	10.90416	0.37105	H	-8.82755	-4.95349	3.38690	H	-8.16799	-0.11358	-6.50320
H	8.07121	8.48923	0.17694	H	-4.53417	-5.37048	3.56756	H	-8.01078	-1.87924	-6.50092
H	6.19245	6.82232	0.13007	H	-5.58361	-6.68171	4.13023	H	-10.91764	-2.51563	-5.84939
H	2.20802	-0.56994	1.73170	H	-5.45564	-6.32463	2.40492	H	-2.70863	-5.47467	0.74590
H	2.26193	-3.02416	1.78411	H	9.29179	-9.43656	-1.25884	H	-2.45263	-6.61108	-0.59095
H	5.05667	-3.02534	-1.45391	H	7.65699	-9.57786	-0.61037	H	-2.28465	-9.14165	1.10920
H	5.01907	-0.56927	-1.48831	H	7.92867	-8.80300	-2.18117	H	-0.54499	-9.15964	1.41355
H	-4.12165	3.21579	-1.54372	H	10.45093	-7.26679	-1.43380	H	-1.14932	-8.76487	-0.19787
H	-5.89213	1.55020	-1.72593	H	9.70536	-5.77965	-0.84132	H	-1.74913	-5.97939	3.04339
H	-3.77277	-0.98921	0.99947	H	9.08397	-6.54956	-2.30321	H	-2.63928	-7.50952	3.00279
H	-2.03499	0.70656	1.21408	H	8.46488	-8.44105	1.52325	H	-0.90646	-7.50127	3.34740
H	3.89214	-6.96291	-1.02662	H	10.08363	-8.38494	0.80518	H	1.30960	-3.99631	-0.93050
H	3.20988	-6.68896	1.78705	H	9.34439	-6.91244	1.43944	H	6.07107	-3.89581	1.00598
H	1.06205	-7.75739	2.22964	H	-3.50480	-7.04654	0.75585				
H	-0.84235	-5.04399	-0.47929	H	8.12736	-5.16024	0.71129				

Table S3. Cartesian coordinates [Å] of the optimized structure for **BPPZ-2HMAT.**

	X	**Y**	**Z**		**X**	**Y**	**Z**		**X**	**Y**	**Z**
C	1.216729	4.627618	0.089979	C	-6.81978	7.96266	-0.66655	C	-3.67615	-8.37311	-0.16557
C	2.312933	3.787929	0.18587	C	-6.51273	9.325584	-0.6484	C	-4.66575	-7.45861	0.515118
C	2.101796	2.385436	0.242367	N	-5.291	9.801064	-0.53291	C	-0.56246	-6.3325	-2.2502
C	0.816349	1.898415	0.151198	N	-2.76579	10.79673	-0.29336	C	-0.60067	-7.70901	-2.23149
C	-0.30067	2.734014	0.028722	C	-1.5483	11.28305	-0.17775	C	-1.62715	-8.31375	-1.54083
C	-0.0837	4.124471	0.016801	C	-0.40199	10.49315	-0.05856	C	-5.70772	-8.06676	1.202903

C	-1.64998	2.202806	-0.09742	C	-0.55056	9.127862	-0.06222	C	-6.66946	-7.341	1.869887
C	-2.74573	3.075619	-0.22874	C	3.516313	0.514152	-0.60788	C	-6.57153	-5.96754	1.84298
C	-2.52917	4.510111	-0.21846	C	4.614069	-0.33341	-0.54219	C	0.01736	-3.64978	-1.14749
C	-1.2154	5.027801	-0.09651	C	5.471189	-0.28328	0.568682	C	-1.44833	-3.65436	-3.19245
C	-1.9079	0.830342	-0.10371	C	5.18015	0.645691	1.583313	C	-5.4384	-3.357	2.697614
C	-3.17394	0.293338	-0.25557	C	4.092754	1.492896	1.452586	C	-6.87646	-3.27896	0.635505
C	-4.26809	1.184451	-0.40162	C	-2.39358	-1.96159	-0.93878	C	-3.01875	-9.27596	0.894352
C	-4.02851	2.550861	-0.37596	C	-2.43797	-3.34542	-0.93525	C	-4.42109	-9.24131	-1.19613
C	3.650964	4.264682	0.132692	C	-3.4593	-4.00941	-0.23556	C	6.570903	2.22716	2.900529
C	-5.59682	0.726214	-0.61977	C	-4.4072	-3.22332	0.441608	C	5.105291	0.539695	4.05916
C	4.833693	4.463692	0.008795	C	-4.31493	-1.8412	0.411368	C	4.94904	-0.42249	-3.00097
C	6.269572	4.424554	-0.20454	N	6.594762	-1.12777	0.66054	C	3.614843	-2.20618	-1.82873
C	6.620698	3.124741	-0.93986	C	7.42574	-1.07543	1.800618	C	10.38447	-2.59587	0.400052
C	8.101194	2.795812	-0.99246	C	7.156426	-0.18023	2.849951	C	8.901697	-4.33333	1.456594
C	8.346908	1.495246	-1.74138	C	6.005942	0.796488	2.837628	H	1.360529	5.698691	0.042128
C	9.788594	1.017883	-1.72872	C	4.824243	-1.26046	-1.71483	H	0.691787	0.824237	0.189191
C	9.987888	-0.23068	-2.5722	C	6.078978	-2.08212	-1.5359	H	-1.0928	0.133734	0.035515
C	-6.72822	0.364708	-0.82711	C	6.89467	-2.01686	-0.39263	H	-4.85637	3.237477	-0.49188
C	-8.08639	-0.10059	-1.03866	C	6.40917	-2.94946	-2.56874	H	6.611817	5.298216	-0.76879
C	-8.97039	0.030142	0.203148	C	7.51828	-3.7643	-2.5171	H	6.78432	4.45775	0.762918
C	-10.3588	-0.54506	-0.01481	C	8.305621	-3.70726	-1.38868	H	6.090952	2.303698	-0.44863
C	-11.2495	-0.43724	1.211427	C	8.019196	-2.85733	-0.32847	H	6.213043	3.178504	-1.95477
C	-12.6381	-1.01993	1.002701	C	8.950138	-2.91504	0.858995	H	8.660896	3.610864	-1.4676
C	-13.5189	-0.9101	2.235608	C	8.542628	-1.91971	1.91811	H	8.494825	2.708546	0.028285
C	3.238935	1.453268	0.365935	C	9.33545	-1.86042	3.056757	H	7.71269	0.710341	-1.3114
C	-3.31429	-1.17143	-0.27034	C	9.072307	-0.98885	4.089944	H	8.014888	1.612863	-2.78097
N	-3.57007	5.325395	-0.3265	C	7.982445	-0.15627	3.965917	H	10.4447	1.818906	-2.08913
C	-3.35463	6.634355	-0.31683	C	-1.35658	-4.05912	-1.7099	H	10.09017	0.82198	-0.69256
C	-2.04578	7.150408	-0.19402	C	-1.51212	-5.5569	-1.59852	H	11.01692	-0.59469	-2.52105
N	-1.00307	6.336941	-0.08694	C	-2.55538	-6.17903	-0.89076	H	9.330423	-1.03976	-2.24174
C	-4.48029	7.540712	-0.43413	N	-3.5301	-5.41428	-0.21454	H	9.757426	-0.03133	-3.62264
C	-4.27914	8.932892	-0.42641	C	-4.57394	-6.05693	0.484936	H	-8.53802	0.454116	-1.86931
C	-2.92107	9.468326	-0.29788	C	-5.54935	-5.31177	1.169785	H	-8.05415	-1.15028	-1.35342
C	-1.83264	8.584492	-0.18409	C	-5.55677	-3.80358	1.229149	H	-8.48711	-0.48056	1.042353
C	-5.78798	7.062831	-0.55782	C	-2.60241	-7.58324	-0.87459	H	-9.03857	1.085637	0.485646
H	-10.8384	-0.03508	-0.85981	H	-6.90255	-2.18888	0.657246	H	7.760294	0.542197	4.762991
H	-10.2711	-1.5991	-0.3076	H	-6.98129	-3.60096	-0.4027	H	0.233025	-5.83575	-2.79158
H	-10.7666	-0.94536	2.055837	H	-2.48697	-8.67207	1.632237	H	9.703078	-0.95754	4.96991
H	-11.3386	0.616105	1.505594	H	-3.76547	-9.87424	1.418838	H	0.15064	-8.29658	-2.74483
H	-13.1188	-0.51082	0.159177	H	-2.30409	-9.96263	0.437922	H	-1.67853	-9.39512	-1.51601
H	-12.546	-2.07172	0.707421	H	-5.1984	-9.8396	-0.71811	H	-5.7629	-9.14815	1.21461
H	-14.51	-1.33521	2.061924	H	-4.89302	-8.61251	-1.95369	H	-7.47466	-7.83429	2.400545
H	-13.0746	-1.43782	3.084449	H	-3.73776	-9.92641	-1.70041	H	-7.31378	-5.37662	2.365149
H	-13.6521	0.134249	2.531987	H	7.243051	2.403337	2.058569	H	0.09839	-3.92769	-0.09483
H	-5.95544	5.99307	-0.56475	H	5.773029	2.970692	2.861319	H	0.828737	-4.13599	-1.69109

H	-7.84738	7.633802	-0.76431	H	7.134133	2.386316	3.821485	H	0.169766	-2.57209	-1.22721
H	-7.30698	10.0637	-0.73262	H	5.662733	0.649495	4.990525	H	-1.33015	-2.57607	-3.31326
H	-1.46458	12.36734	-0.17836	H	4.272044	1.243513	4.086629	H	-0.67045	-4.14345	-3.78128
H	0.574022	10.9541	0.033869	H	4.695827	-0.47176	4.024311	H	-2.41793	-3.93824	-3.60615
H	0.295485	8.458042	0.027065	H	5.803235	0.25325	-2.93283	H	-4.50211	-3.71472	3.13042
H	2.869318	0.468068	-1.47546	H	5.088308	-1.0601	-3.87499	H	-5.45786	-2.269	2.77967
H	3.899602	2.221259	2.229694	H	4.053722	0.177157	-3.17004	H	-6.26181	-3.75118	3.295516
H	-1.61286	-1.4718	-1.50757	H	3.727009	-2.88692	-2.67464	H	-7.73771	-3.64672	1.196032
H	-5.04564	-1.25995	0.95632	H	3.508656	-2.80444	-0.92153	H	10.71705	-3.29091	-0.37219
H	5.769368	-2.98717	-3.44151	H	2.691142	-1.64295	-1.97414	H	9.568518	-4.4199	2.316161
H	7.760194	-4.43234	-3.33458	H	10.43912	-1.58521	-0.00757	H	7.888897	-4.57418	1.785626
H	9.176915	-4.3467	-1.3227	H	11.08717	-2.66588	1.23148	H	9.206681	-5.07911	0.720441
H	10.18867	-2.52286	3.132163								

Pdot Synthesis and Cellular Uptake

Table S4. Characterization of boc-protected polymers **P1**-**P3**.

Polymer	M_n of 1st block, poly-boc**10** (kDa)		1st block $Đ$ [b]	M_n of poly-boc**10**-*b*-**11** (kDa)		poly-boc**10**-*b*-**11**		
	Theor. [a]	Expt. [b]		Theor. [a]	Expt. [c]	$Đ$ [d] (before prep. SEC)	DP_n of monomer **10** (theor.)	DP_n of monomer **11** (theor.)
P1	16.8	16.1	1.08	28.9	26.7	1.31 (1.51)	19 (20)	19 (20)
P2	16.1	16.8	1.05	40.1	39.0	1.19 (1.39)	19 (20)	41 (40)
P3	8.0	8.4	1.12	20.4	19.2	1.20 (1.57)	9 (10)	20 (20)

[a] Calculated from feed ratio of monomers. [b] Calculated from SEC using triple detection. [c] Calculated from ^{1}H NMR.
[d] Calculated after preparatory SEC.

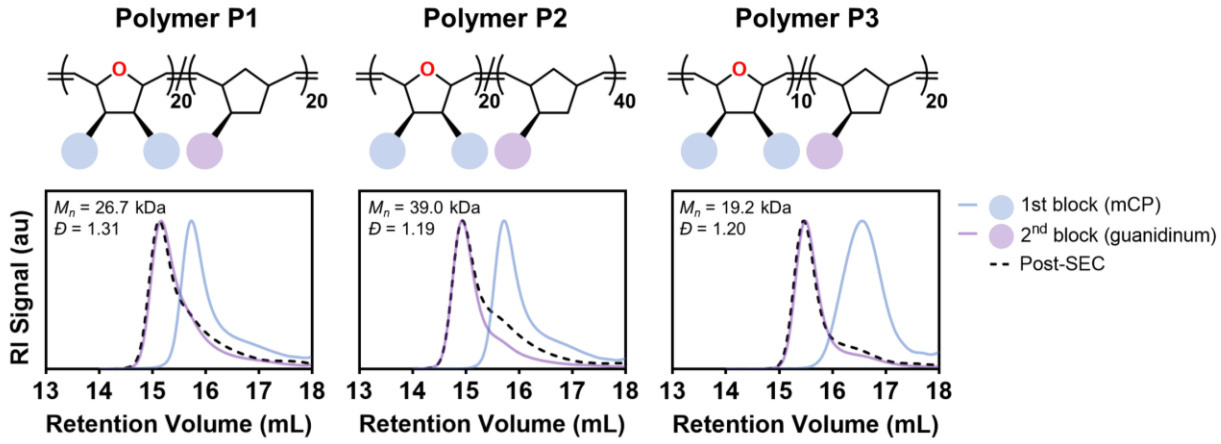


Figure S27. SEC RI traces during the preparation of **P1**-**P3**, showing the first (poly-guanidinium, boc-protected) block and second (poly-mCP) block. Successful chain extension is observed from first to second blocks, with significant tailing due to the interaction of the hydrophilic side chains with the stationary phase of the SEC column. Each boc-protected polymer sample was purified using preparatory SEC, revealing similar tailing between purified and unpurified samples; this point suggests tailing is not a result of failed chain extension.

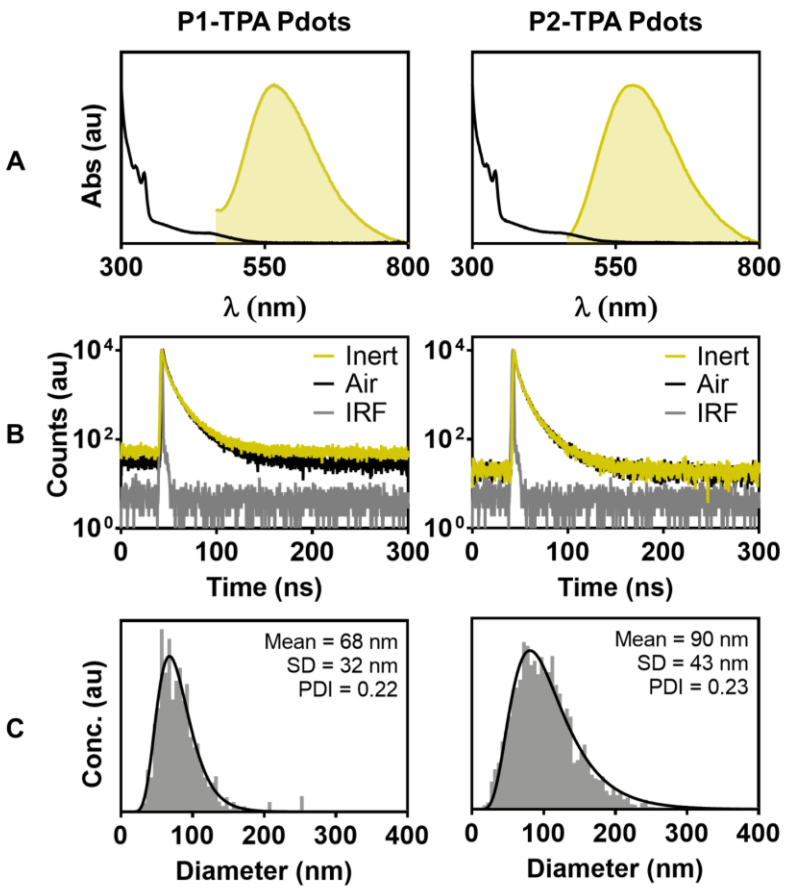


Figure S28. (A) Absorption (black trace) and photoluminescence (coloured trace, λ_{exc} = 313 nm) for neat aqueous Pdot samples. (B) PL decays performed under air or inert (N_2) atmosphere, with excitation using an EPLED at 313 nm and TCSPC detection. (C) Nanoparticle tracking analysis for Pdot samples, fitted with a lognormal distribution.

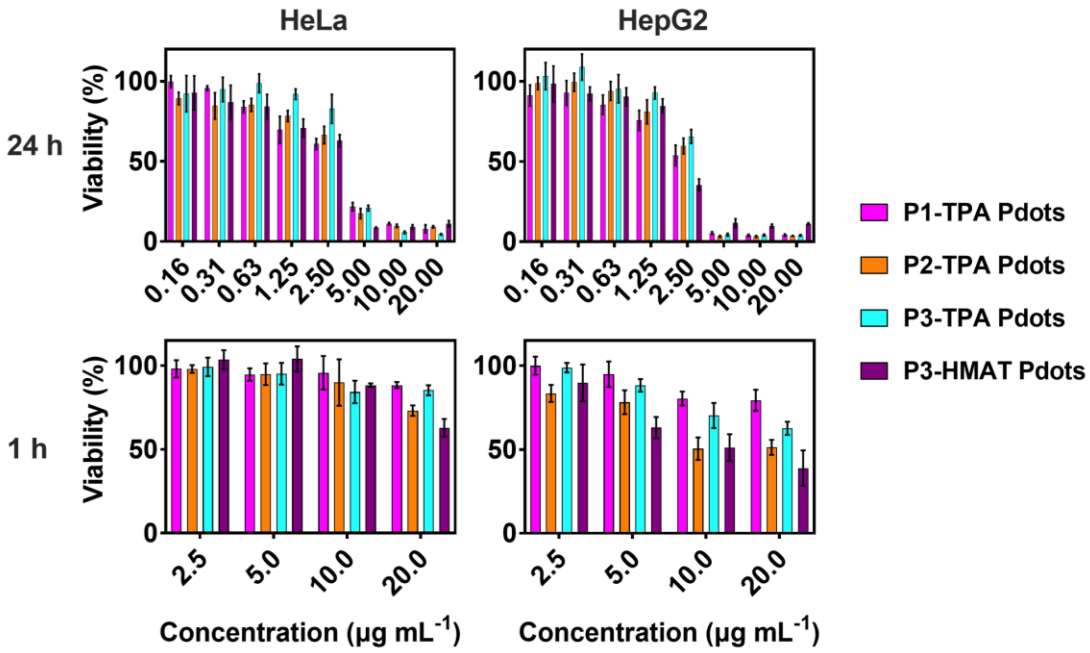


Figure S29. Cell viability of HeLa and HepG2 cells after incubation with Pdots at varying concentrations for 1 or 24 hours. Concentrations presented are based on the assumed amount of material (by mass) present in a bulk Pdot sample, and are calculated based on the amount of surfactant and dopant used in the synthesis of each sample.

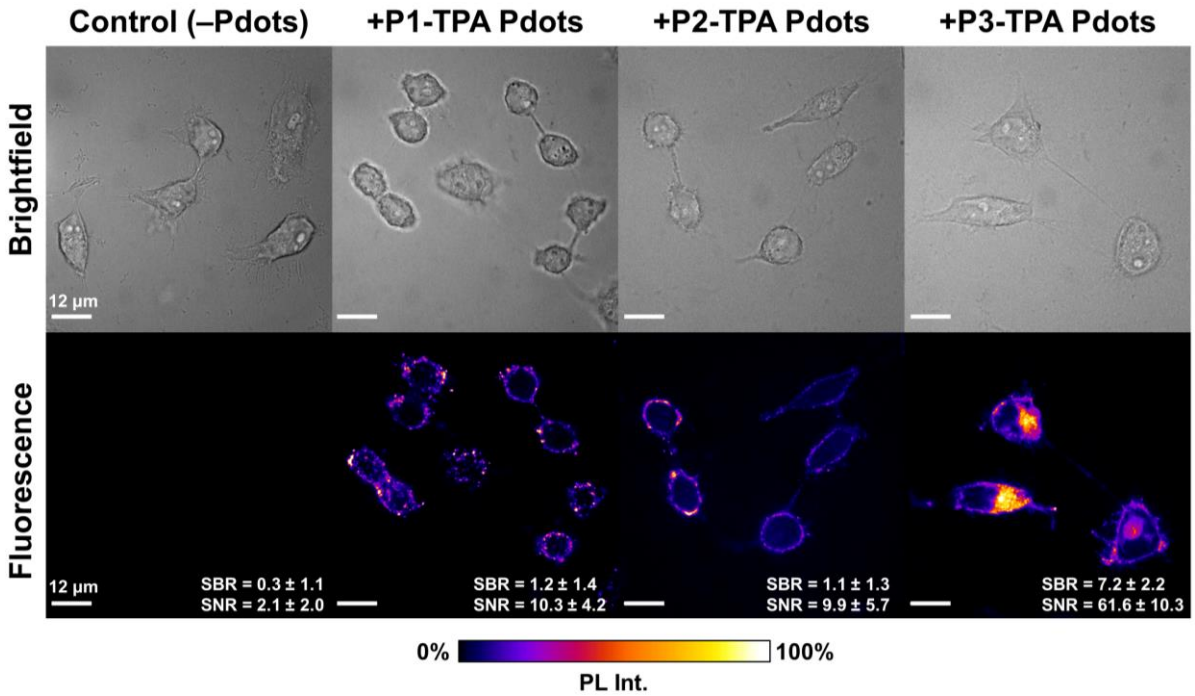


Figure S30. Confocal fluorescence and brightfield images of HeLa cells showing a control sample, and samples incubated with either **P1-TPA**, **P2-TPA**, or **P3-TPA** Pdots for one hour. Images obtained with 405 nm excitation, and emission detected with 500 – 550 nm / 580 – 700 nm combined filters.

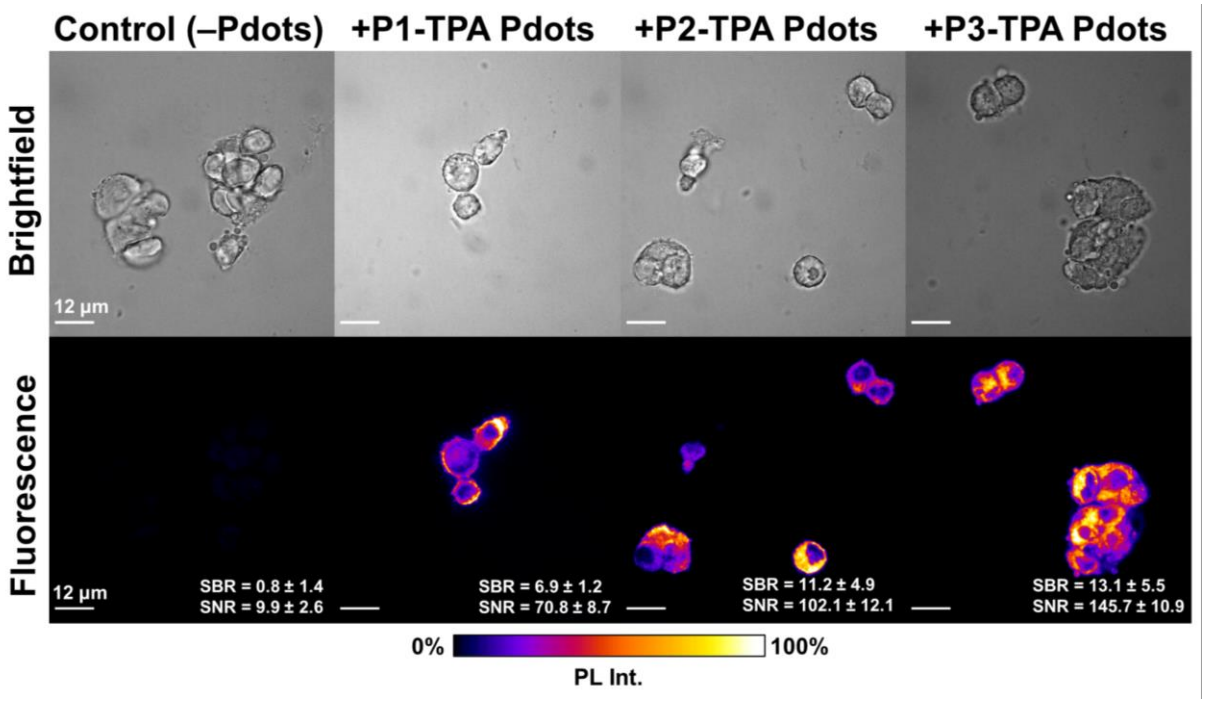


Figure S31. Confocal fluorescence and brightfield images of HepG2 cells showing a control sample, and samples incubated with either **P1-TPA**, **P2-TPA**, or **P3-TPA** Pdots for one hour. Images obtained with 405 nm excitation, and emission detected with 500 – 550 nm / 580 – 700 nm combined filters.

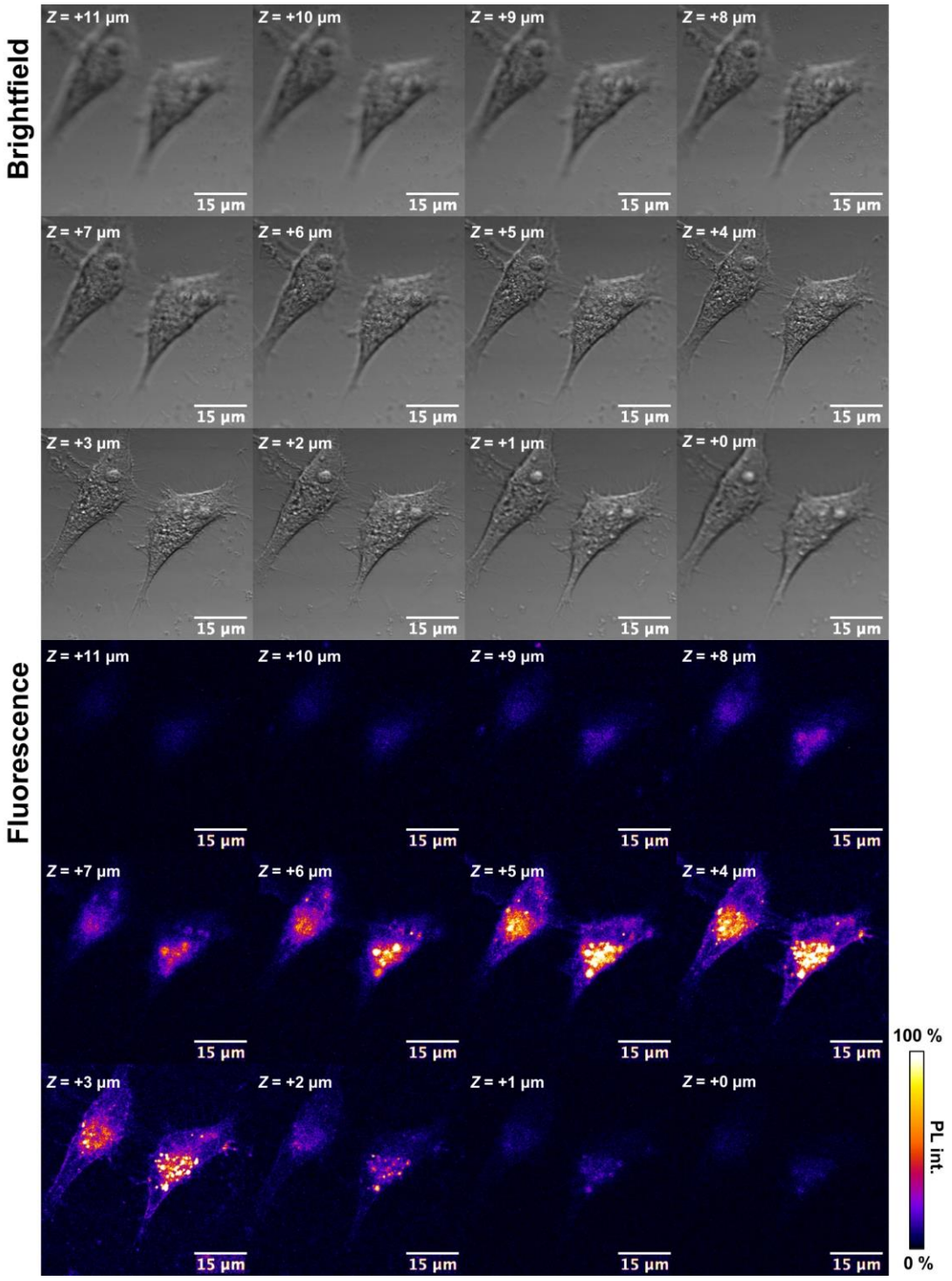


Figure S32. Selected *Z*-stack slices of HeLa cells incubated for one hour with **P3-HMAT Pdots**, with excitation at 405 nm, and varying indicated depths relative to the highest focal plane (Z = 0 µm) focused above the sample. Sample emission was captured in the range of 545 to 645 nm.

g-Odot Cellular Uptake

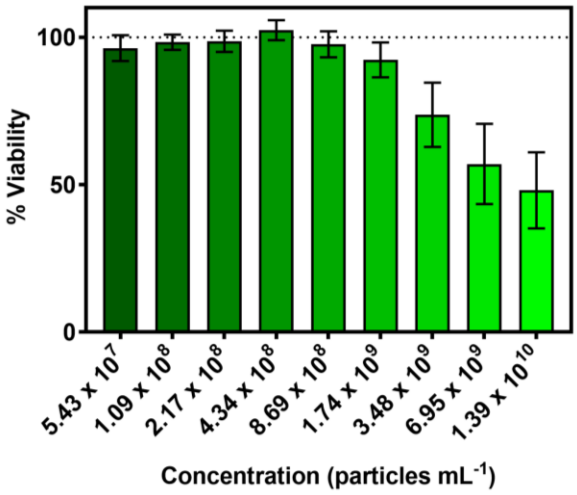


Figure S33. Results of a cell viability MTT assay for HeLa cells incubated with varying concentrations of **HMAT** g-Odots for 24 hours. Measurements were performed in triplicate, with cells (10^4 cells well^{-1}) incubated at each concentration with 200 μL of total g-Odot/cell medium suspension. Concentrations of nanoparticles used are based on NTA characterization results from scattering-based measurements of the bulk nanoparticle sample used for the cytotoxicity assay.

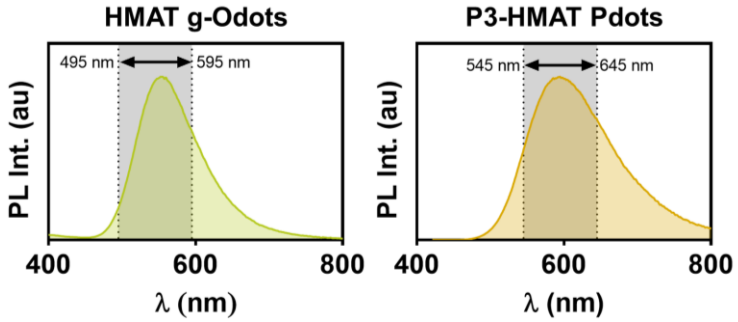


Figure S34. Emission spectra of each nanoparticle with the corresponding emission detection windows used for microscopy (shaded grey regions) for images presented in Figures 7, S32, and S35. All images were obtained with excitation at 405 nm.

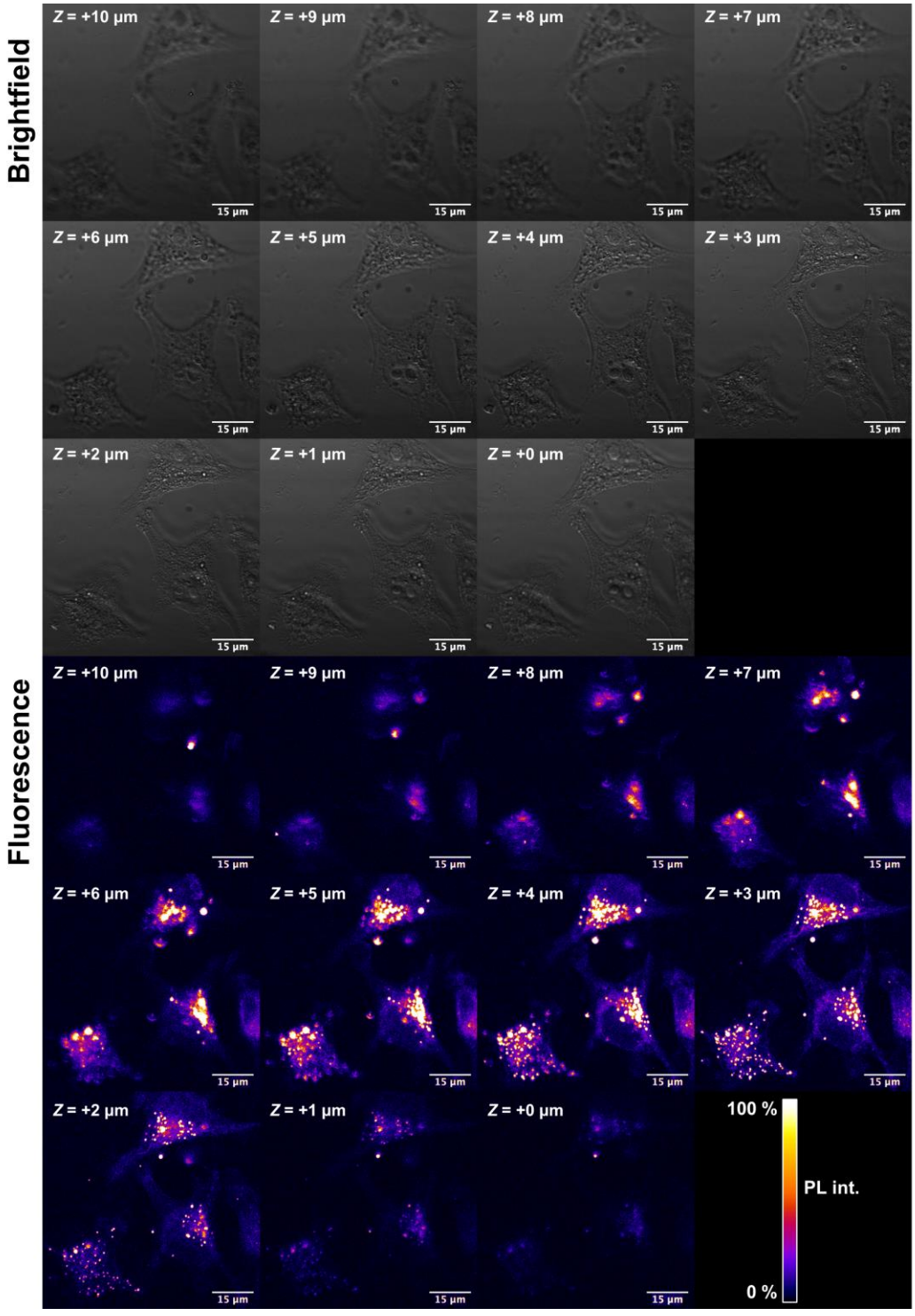


Figure S35. Selected *Z*-stack slices of HeLa cells incubated for 1 hour with **HMAT g-Odots**, with excitation at 405 nm, and varying indicated depths relative to the highest focal plane ($Z = 0$ µm) focused above the sample. Sample emission was captured in the range of 495 to 595 nm.

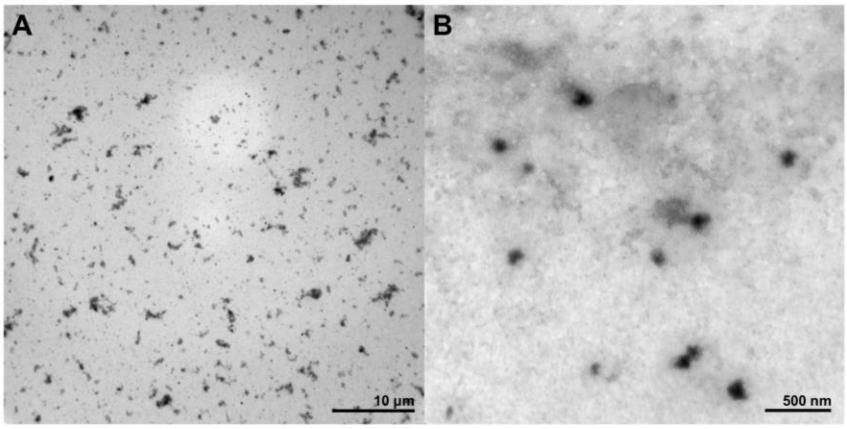


Figure S36. TEM images of **P3-HMAT** Pdots at two different levels of magnification.

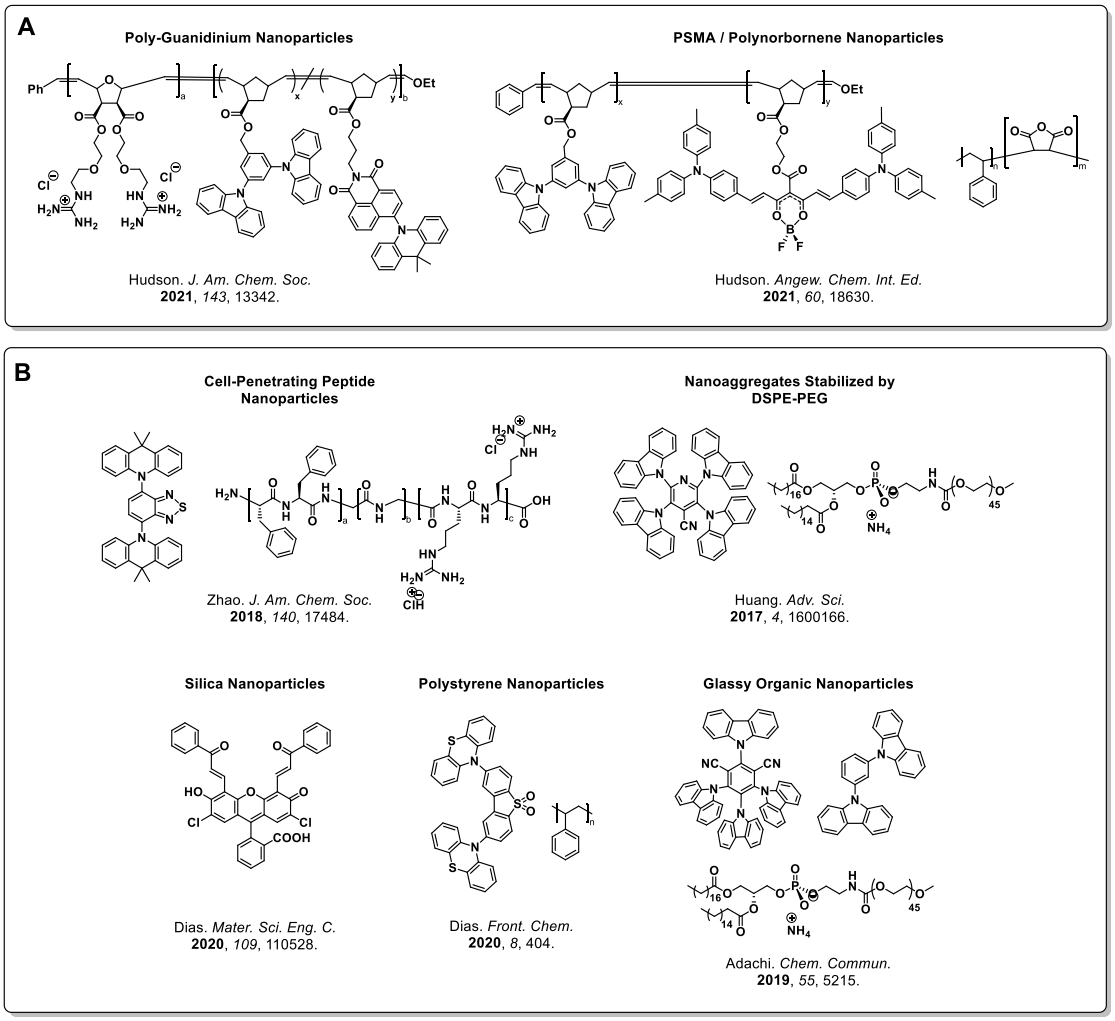


Figure S37. Examples of water-dispersible TADF nanoparticles formed through (A) covalently-bound polymeric host/emitter systems, and (B) nanoaggregation or nanoencapsulation of small molecule TADF emitters.

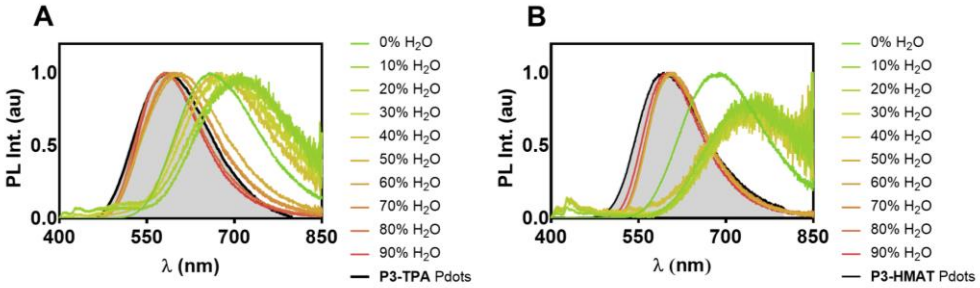


Figure S38. A comparison of normalized emission spectra (green to red traces) obtained from AIE experiments (THF/H_2O mixtures) for (A) **BPPZ-2TPA** and (B) **BPPZ-2HMAT**, with emission spectra of **P3** Pdots overlaid (black, grey shaded area) for comparison.

References

1 F. Schlütter, F. Rossel, M. Kivala, V. Enkelmann, J.-P. Gisselbrecht, P. Ruffieux, R. Fasel and K. Müllen, π-Conjugated Heterotriangulene Macrocycles by Solution and Surface-supported Synthesis toward Honeycomb Networks, *J. Am. Chem. Soc.*, 2013, **135**, 4550–4557.

2 Y. Shirai, A. J. Osgood, Y. Zhao, Y. Yao, L. Saudan, H. Yang, C. Yu-Hung, L. B. Alemany, T. Sasaki, J.-F. Morin, J. M. Guerrero, K. F. Kelly and J. M. Tour, Surface-Rolling Molecules, *J. Am. Chem. Soc.*, 2006, **128**, 4854–4864.

3 P. Comba, R. Krämer, A. Mokhir, K. Naing and E. Schatz, Synthesis of New Phenanthroline-Based Heteroditopic Ligands – Highly Efficient and Selective Fluorescence Sensors for Copper(II) Ions, *Eur. J. Inorg. Chem.*, 2006, **2006**, 4442–4448.

4 C. J. Christopherson, N. R. Paisley, Z. Xiao, W. R. Algar and Z. M. Hudson, Red-Emissive Cell-Penetrating Polymer Dots Exhibiting Thermally Activated Delayed Fluorescence for Cellular Imaging, *J. Am. Chem. Soc.*, 2021, **143**, 13342–13349.

5 N. R. Paisley, S. V. Halldorson, M. V. Tran, R. Gupta, S. Kamal, W. R. Algar and Z. M. Hudson, Near-Infrared-Emitting Boron-Difluoride-Curcuminoid-Based Polymers Exhibiting Thermally Activated Delayed Fluorescence as Biological Imaging Probes, *Angew. Chem. Int. Ed.*, 2021, **60**, 18630–18638.

6 T. Y.-H. Lee, Q. Wang, J. U. Wallace and S. H. Chen, Temporal stability of blue phosphorescent organic light-emitting diodes affected by thermal annealing of emitting layers, *J. Mater. Chem.*, 2012, **22**, 23175–23180.

7 C. J. Christopherson, D. M. Mayder, J. Poisson, N. R. Paisley, C. M. Tonge and Z. M. Hudson, 1,8-Naphthalimide-Based Polymers Exhibiting Deep-Red Thermally Activated Delayed Fluorescence and Their Application in Ratiometric Temperature Sensing, *ACS Appl. Mater. Interfaces*, 2020, **12**, 20000–20011.

8 M. Rivera, Photodestruction quantum yield of adenine in aqueous solution: A physical chemistry experiment, *J. Chem. Educ.*, 1989, **66**, 1049–1051.

9 N. R. Paisley, C. M. Tonge, D. M. Mayder, K. A. Thompson and Z. M. Hudson, Tunable benzothiadiazole-based donor–acceptor materials for two-photon excited fluorescence, *Mater. Chem. Front.*, 2020, **4**, 555–566.

10 C. J. Christopherson, N. R. Paisley, Z. Xiao, W. R. Algar and Z. M. Hudson, Red-Emissive Cell-Penetrating Polymer Dots Exhibiting Thermally Activated Delayed Fluorescence for Cellular Imaging, *J. Am. Chem. Soc.*, 2021, **143**, 13342–13349.