*Supporting Information*

# Amphoteric Tetrazole-Substituted Eleven-Ring-Fused Acene Derivatives with Multiple Fluorescent Protonation States

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**Contents**

*Materials and Methods*

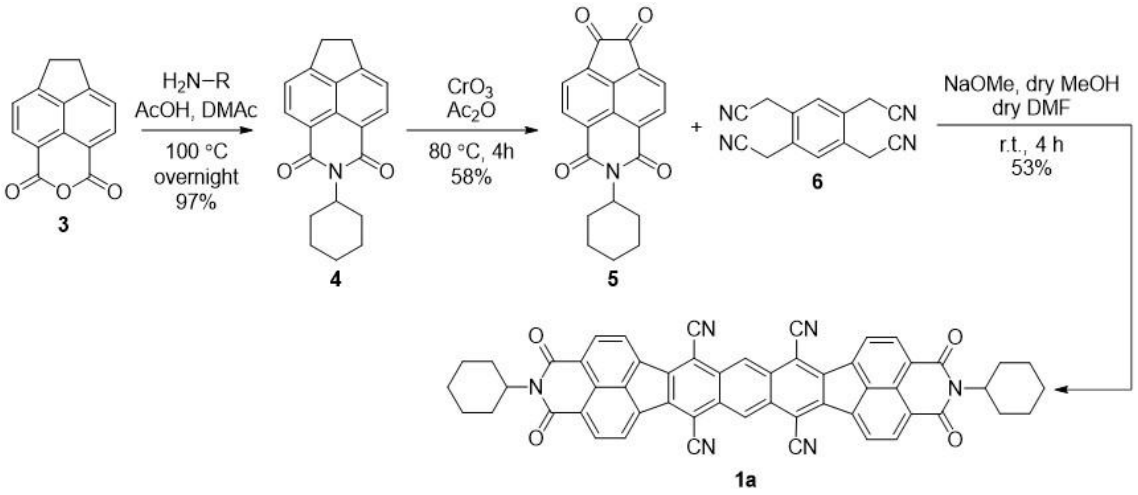
$^{1}H$ and $^{13}C$ NMR spectra were recorded on Bruker AVIIIHD spectrometers operating at 400 MHz and 500 MHz, respectively. Due to the extremely low solubility of **2** in common solvent (chloroform, DMSO, and various mixed solvents), one drop of triethylamine (dried with molecular sieves) was added to facilitate dissolution of **2** in MeOD-*d*4.

UV-Vis absorption spectra were recorded on a Jasco V-670 spectrophotometer using acetonitrile solutions, with or without one drop of the indicated acid/base, in quartz cuvettes. Emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer using the solution samples prepared under identical conditions as the absorption spectra. Absolute photoluminescence quantum yield (PLQY) was recorded on a Fluorolog 3 fluorometer from Horiba Jobin-Yvon and Quanta-Phi integrating sphere using the solution samples prepared under identical conditions as the absorption spectra. Time-correlated single photon counting (TCSPC) measurements were performed using an Edinburgh Instruments mini-tau lifetime spectrophotometer equipped with an EPL 405 pulsed diode laser. For UV-vis monitored titrations, the degree of (de)protonation was evaluated by fitting each intermediate spectrum as a linear combination of the initial spectrum (assumed to be excess majority neutral compound) and final spectrum (assumed to be excess majority dication/dianion). The corresponding linear coefficient of the final spectrum was taken as the portion of analyte converted to the ionic form (i.e. $[\mathbf{2a}^{2\pm}]/[\mathbf{2a}]_0$).

DFT calculations were performed using B3LYP functional with 6-31G(d) basis set, with the empirical (DG3BJ) dispersion correction, in a polarized continuum model to account for solvation effects (IEFPCM, MeCN), as implemented in Gaussian 16. The alkyl chains were replaced by a $CH_3$ group and the molecules were calculated in all charge states in the C2 symmetry point group.

*Synthesis and Characterization*

Acenaphthene, *N*-bromosuccinimide (NBS), dimethylcarbamoyl chloride, dimethylformamide (DMF), dimethylacetamide (DMAc), 97% benzoyl peroxide (BPO), and sodium azide ($NaN_3$) were purchased from Sigma Aldrich. Aluminum chloride ($AlCl_3$), durene, tetraethylammonium bromide (TEAB), glacial acetic acid, and cyclohexylamine were purchased from Fisher Scientific. *N*-methyl-2-pyrrolidone was purchased from VWR International. Chromium trioxide ($CrO_3$) was purchased from Merck KGaA. Sodium methoxide (NaOMe) was purchased from TCI Chemicals. Acidic anhydride ($Ac_2O$) was purchased from ACP Chemicals. All chemicals were used as received. The synthesis of **1a** was modified based on our previous work of **1b**.[1] Microwave-assisted reactions of **2** were performed in an Anton Paar microwave reactor Monowave 400.




2-Cyclohexyl-6,7-dihydro-1*H*-indeno[6,7,1-*def*]isoquinoline-1,3(2*H*)-dione (**4**).

Modified based on a literature procedure.[2] Under nitrogen atmosphere, a suspension of **3** (1.000 g, 4.46 mmol) in 8 mL of DMAc was stirred at room temperature, followed by addition of a solution of cyclohexylamine (0.77 mL, 6.7 mmol) in 8 mL of DMAc. Subsequently, 0.6 mL of acetic acid was introduced to the reaction mixture, which was then heated at 100 °C overnight. After cooling down to room temperature, the reaction was quenched by adding water. The mixture was extracted with $CH_2Cl_2$ and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The product was purified by silica chromatography (hexanes:$CH_2Cl_2$ 4:1 v/v) to obtain **4** as a light brown solid (1.322 g, 97%). NMR data was in accordance with literature.[3] $^1$H-NMR (400 MHz, $CDCl_3$): δ = 8.45 (d, 2H, *J* = 7.4 Hz), 7.53 (d, 2H, *J* = 7.4 Hz,), 5.05 (m, 1H), 3.55 (s, 4H), 2.58 (m, 2H), 1.88 (m, 2H), 1.72 (m, 3H), 1.50–1.22 (m, 3H) ppm.

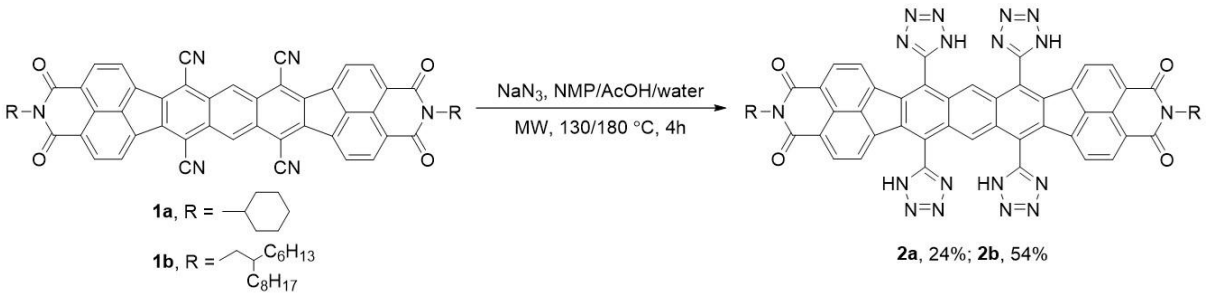
2-Cyclohexyl-1*H*-indeno[6,7,1-*def*]isoquinoline-1,3,6,7(2*H*)-tetraone (**5**).

To a solution of **4** (1.500 g, 4.91 mmol) in 24 mL of acidic anhydride was dropwise added a solution of $CrO_3$ (2.455 g, 24.55 mmol) in 24 mL of acidic anhydride. The reaction mixture was heated at 80 °C for 4 hours. After cooling down to room temperature, the reaction was poured into ice-water with 2M aq. HCl solution. The mixture was extracted with $CH_2Cl_2$ and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The product was purified by silica chromatography (hexanes:$CH_2Cl_2$ 1:4 v/v) to obtain orange **5** (0.949 g, 58%). $^1$H-NMR (500 MHz, $CDCl_3$): δ = 8.77 (d, 2H, *J* = 7.3 Hz), 8.33 (d, 2H, *J* = 7.3 Hz), 5.05 (m, 1H), 2.54 (m, 2H), 1.92 (d, 2H), 1.76 (m, 3H), 1.50–1.24 (m, 3H) ppm. $^{13}$C-NMR (125 MHz, $CDCl_3$) δ = 186.30, 162.99, 131.98, 131.75, 127.30, 122.91, 54.67, 29.05, 26.51, 25.34 ppm. APCI-HRMS (*m/z*): found 334.1086 for $[M+H]^+$ (Calculated 334.1074).

Di-*N*-(2-cyclohexyl)-tetracyanoanthracenediacenaphthalimides (**1a**).

A suspension of **5** (178 mg, 0.534 mmol) and **6** (50 mg, 0.213 mmol) in 10 mL of dry DMF

was stirred under $N_2$ atmosphere, followed by dropwise addition of a solution of NaOMe (58 mg, 1.07 mmol) in 10 mL of dry methanol. The reaction mixture was stirred at room temperature for 4 hours under $N_2$ atmosphere. The reaction was quenched by pouring into ice-water, followed by addition of 2M aq. HCl solution. The precipitate was collected by filtration, washed sequentially with water and $CHCl_3$, and dried in vacuum overnight to give **1a** as a dark red solid (93 mg, 53%). The product was used without further purification. APCI-HRMS (*m/z*): found 829.2580 for $[M+H]^+$ (Calculated 829.2558). The extremely low solubility of **1a** in common solvent (chloroform, DMSO, and various mixed solvents) precluded the NMR characterization.




CAUTION: Hydrazoic acid and its salts are toxic. Hydrazoic acid and many of its heavy metal salts are prone to spontaneous detonation. The production of hydrazoic acid gas during reactions can lead to elevated pressures within the reaction vessel. Therefore, we limited the reaction mixture volume to a maximum of 1.5 mL in a 10 mL microwave reaction tube.

Di-*N*-(2-cyclohexyl)-tetra(1*H*-tetrazole)diacenaphthoanthracenediimides (**2a**).
**1a** (60 mg, 0.072 mmol), $NaN_3$ (94 mg, 1.448 mmol), and a mixture of NMP, AcOH, and water (1.5 mL, 7:2:1 *v*/*v*) were placed in a 10 mL microwave tube. The reaction mixture was heated at 180 °C for 4 hours in a microwave reactor. After cooling down to room temperature, the reaction mixture was diluted with methanol, followed by the addition of triethylamine to dissolve the tetrazoles in methanol. The undissolved materials (30 mg) were removed by filtration. The methanol filtrate was acidified by adding 2M aq. HCl, resulting in precipitation. The precipitate was collected by the filtration, washed with methanol, and then purified by silica chromatography ([MeCN + 1%TEA]:MeOH 5:1 v/v) with an $R_f$ value of ~0.5. The main fraction was concentrated to ~30 mL, followed by the addition of 2M aq. HCl (~1 mL) to induce precipitation. The precipitate was collected by the filtration, washed with methanol and dried in vacuum overnight to give **2a** as a red solid (17 mg, 24%).
$^1$H-NMR of $\mathbf{2a}^{2-}$ (400 MHz, MeOD-*d*4 with triethylamine): $\delta$ = 8.20 (d, 4H, $J$ = 7.6 Hz), 8.04 (s, 2H), 6.56 (d, 4H, $J$ = 7.6 Hz), 4.99–4.93 (m, 2H), 1.88 (m, 6H), 1.74–1.66 (m, 8H), 1.45–1.41 (m, 6H) ppm. $^{13}$C NMR of $\mathbf{2a}^{2-}$ (125 MHz, MeOD-*d*4 with triethylamine) $\delta$ = 164.1, 156.9, 140.6, 137.0, 134.8, 132.5, 131.6, 128.8, 127.0, 125.3, 122.32, 122.03, 53.4, 28.9, 26.4, 25.3

ppm. ESI-HRMS (*m/z*): found 499.1507 for $[M - 2H]^{2-}$ (Calculated 499.1511); found 332.4328 for $[M - 3H]^{3-}$ (Calculated 332.4311).

Di-*N*-(2-hexyldecyl)-tetra(1*H*-tetrazole)diacenaphthoanthracenediimides (**2b**).
**1b** (40 mg, 0.036 mmol), $NaN_3$ (47 mg, 0.718 mmol), and a mixture of NMP, AcOH and water (0.7 mL, 7:2:1 *v*/*v*) were placed in a 10 mL microwave tube. The reaction mixture was heated at 130 °C for 4 hours in a microwave reactor. After cooling down to room temperature, the reaction mixture was diluted with methanol, followed by the addition of 2M aq. HCl to induce precipitation. The precipitate was collected by the filtration, washed with methanol, and then purified by silica chromatography ([MeCN + 1%TEA]:MeOH 5:1 v/v) with an $R_f$ value of ~0.5, to obtain purple **2b** (24 mg, 52%). The main fraction was collected and concentrated under vacuum to ~30 mL, followed by the addition of 2M aq. HCl (~1 mL) to induce precipitation. The precipitate was collected by the filtration, washed with methanol and dried in vacuum overnight to give **2b** as a red solid (24 mg, 52%).
$^1$H-NMR of $\mathbf{2b}^{2-}$ (400 MHz, MeOD-*d*4 with triethylamine): $\delta$ = 8.24 (d, 4H, $J$ = 7.6 Hz), 8.04 (s, 2H), 6.58 (d, 4H, $J$ = 7.6 Hz), 4.04 (d, 4H, $J$ = 7.3 Hz), 1.95 (m, 2H), 1.39–1.16 (m, 48H), 0.92–0.80 (m, 12H) ppm. $^{13}$C NMR of $\mathbf{2b}^{2-}$ (125 MHz, MeOD-*d*4 with triethylamine) $\delta$ = 165.0, 157.8, 141.9, 137.9, 135.9, 133.4, 132.7, 129.9, 127.9, 126.3, 123.2, 122.4, 44.7, 37.1, 32.49, 32.39, 32.34, 32.29, 30.39, 30.13, 30.01, 29.8, 26.98, 26.94, 23.17, 23.13, 13.93, 13.89 ppm. ESI-HRMS (*m/z*): found 641.3261 for $[M - 2H]^{2-}$ (Calculated 641.3232); found 427.2143 for $[M - 3H]^{3-}$ (Calculated 427.2131).

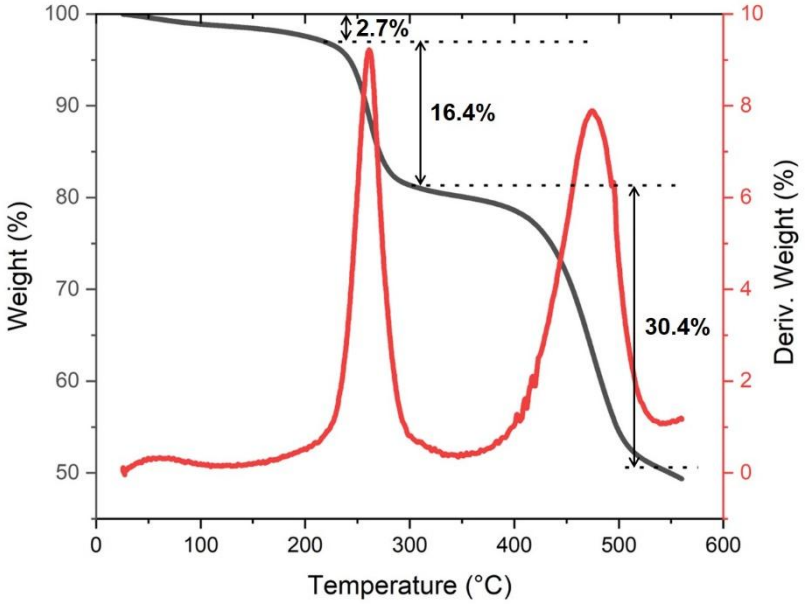



Figure S1. Thermogravimetric analysis (TGA) of **2b**. The first decomposition step at ~250 °C is accompanied by the mass loss of 16.4% which likely correspond to the release of dinitrogen from Tz moieties (-8$N_2$, 17.4%).

*Additional Optical Analysis*

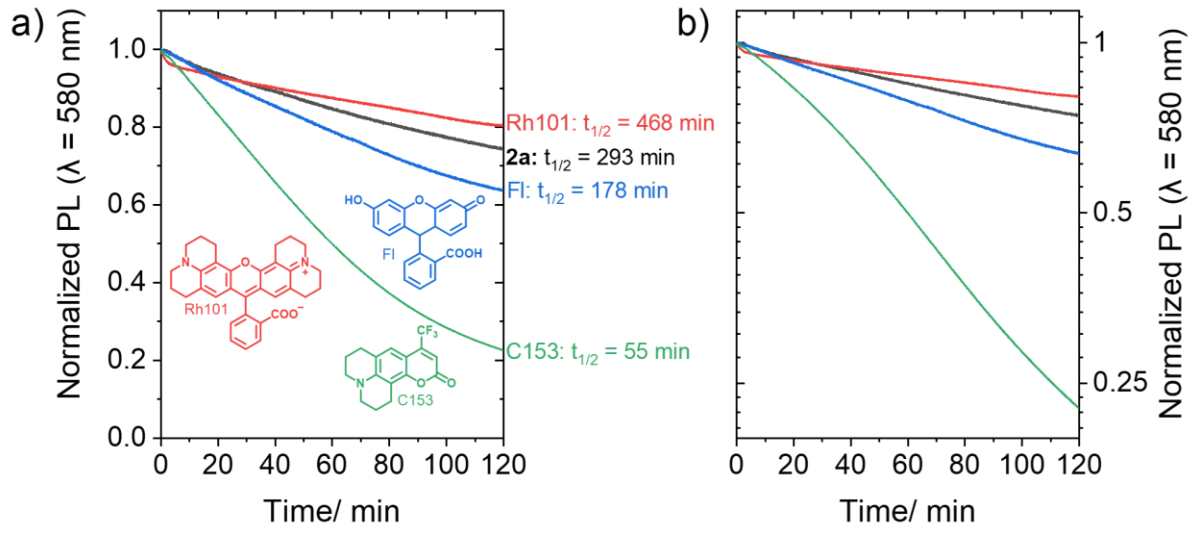



Figure S2. a) Photostability tracking of acetonitrile solutions of **2a**, rhodamine 101 (Rh101), fluorescein (Fl), and coumarin 153 (C153) under irradiation from 450 W Xe arc lamp, monochromated to 390 nm with 14.7 nm bandpass (net incident power 6.5 W). Emission intensity monitored at 580 nm and normalized to initial value. b) Logarithmic (base-2) plot from which $t_{1/2}$ values were derived.

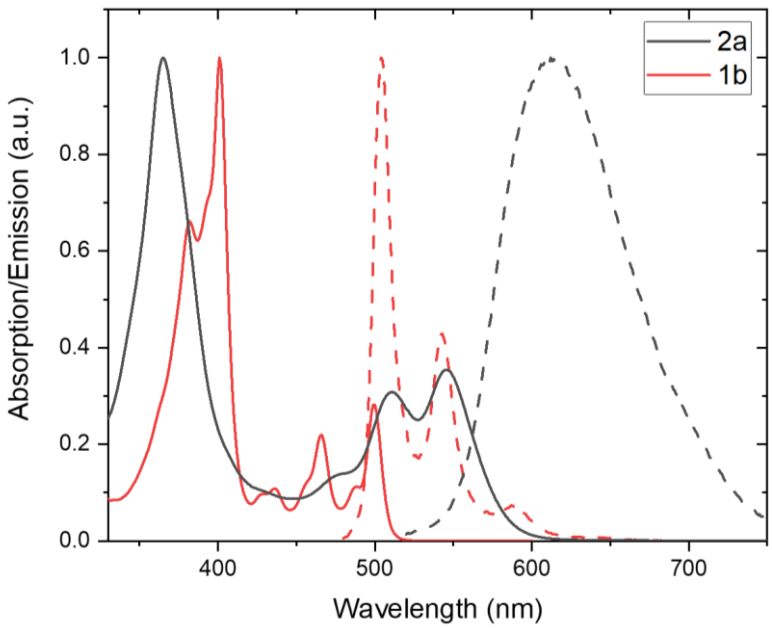



Figure S3. Absorption (solid) and emission (dashed) spectra of **2a** in acetonitrile and **1b** in chloroform.

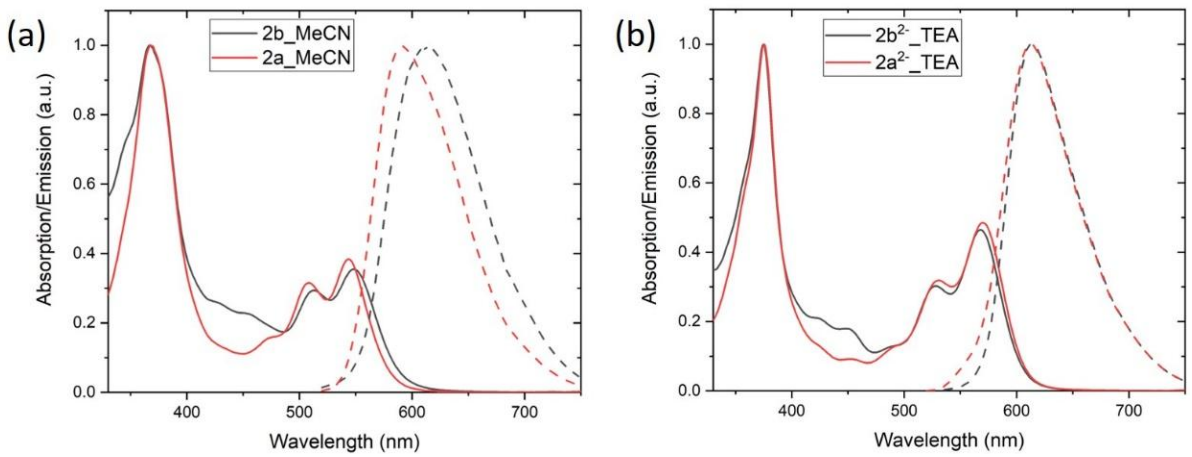



Figure S4. Absorption (solid) and emission (dash) spectra of (a) **2a** and **2b** in pure acetonitrile and (b) after addition of TEA (formation of dianions).

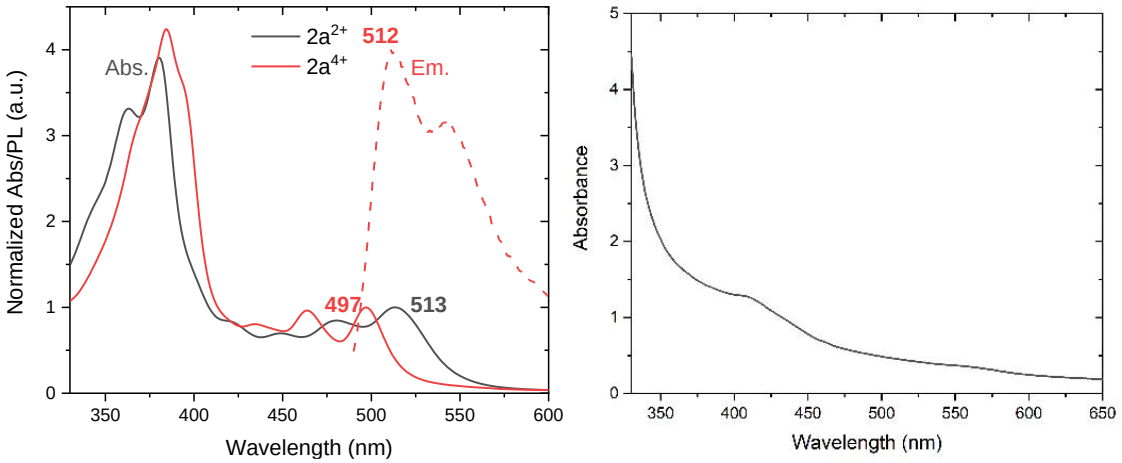



Figure S5. (left) Absorption and emission spectra of **2a** in pure TFA solution (corresponding to deprotonated $\mathbf{2a}^{2+}$) and after addition of TFSA (presumably, tetraprotonated $\mathbf{2a}^{4+}$). We could not obtain clear evidence of the reversibility of $\mathbf{2a}^{4+}$ (possibly, partial imide hydrolysis and/or tetrazole decomposition in very strong acids). (right) Absorption spectrum of **2a** in THF after addition of LDA. The characteristic acene absorption features are gone and the original absorption of **2a** is not restored after addition of AcOH, suggesting an immediate decomposition of $\mathbf{2a}^{4-}$.

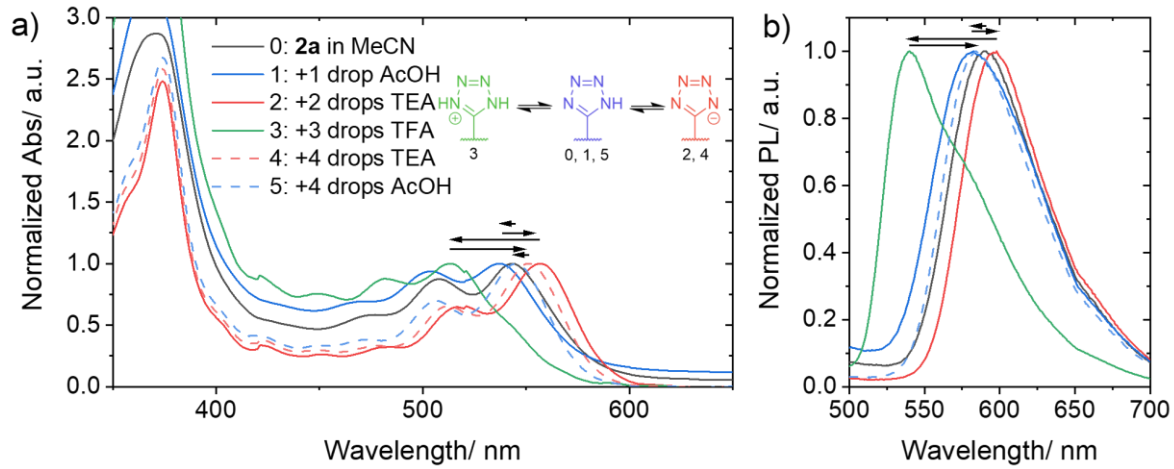



Figure S6. (a) Absorption and (b) emission spectra of acetonitrile solution of **2a** ($10^{-6}$ M) upon sequential deprotonation with TEA, protonation with TFA, and restoration of neutral state with AcOH, showing reversibility of the generation of the ionic states. Absorption spectra normalized to $S_1 \leftarrow S_0$ transition maxima, emission spectra normalized to maxima.

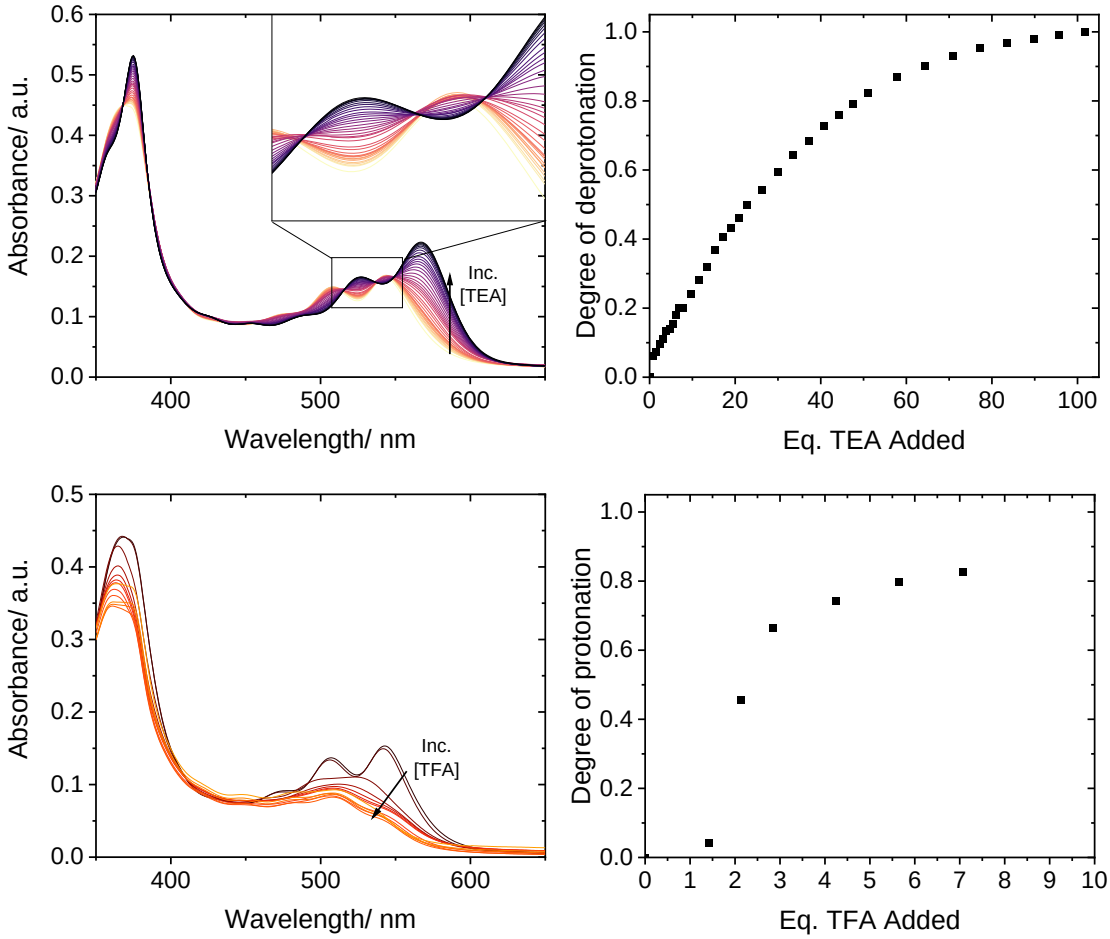



Figure S7. Titration of **2a** ($9\times10^{-6}$ M) in acetonitrile with (top) TEA and (bottom) TFA, monitored by UV-vis absorbance. Degrees of protonation/deprotonation computed from least squares fitting of intermediate spectra as linear combinations of starting and ending spectra.

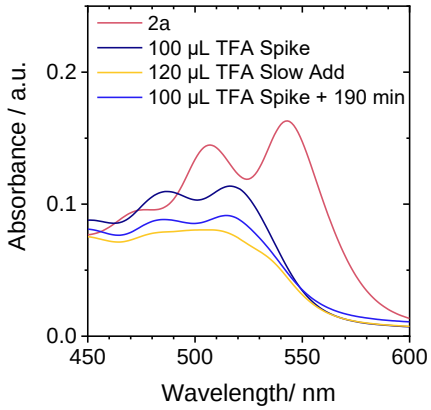



Figure S8. Acetonitrile solution of **2a** ($9\times10^{-5}$ M) protonated by gradual addition of TFA as in Fig. S7 (yellow) and rapid addition upon spiking (violet), and after 190 mins of aging time in the dark (blue). The difference between the fast and slow addition suggests aggregation of the deprotonated species in the latter experiment.

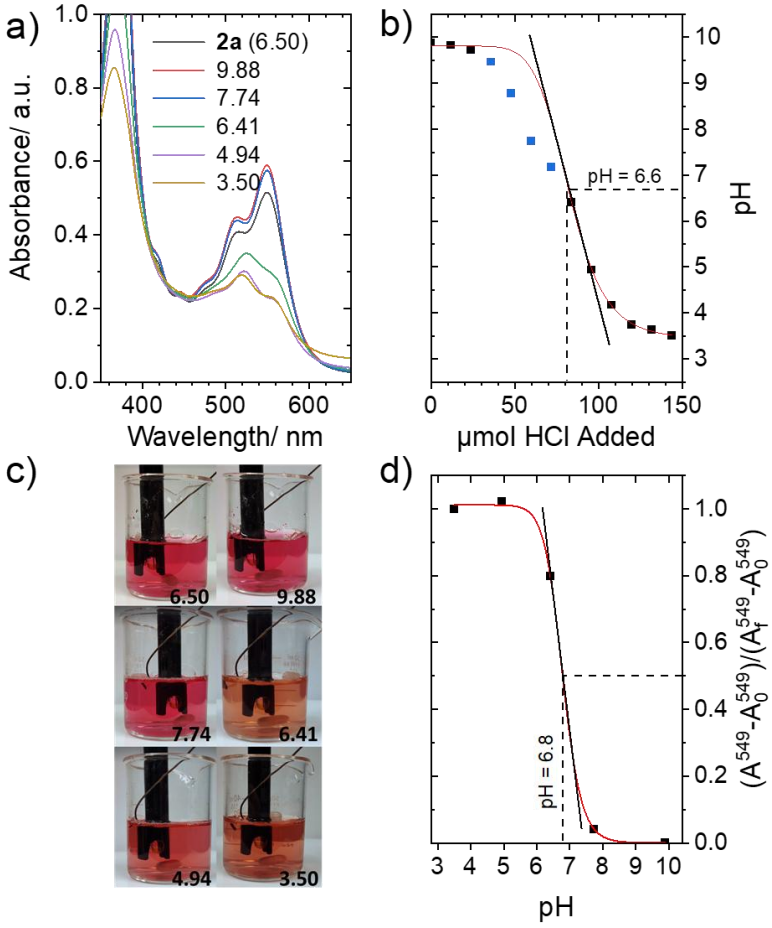



Figure S9. Aqueous titration of **2a** with 1% DMSO monitored by (a) UV-vis and (b) pH, early titration (blue points, ca. 7 < pH < 9.5) affected by protonation of residual $HCO_3^-$ ($pK_a$ = 7.7) from atmospheric $CO_2$ and excluded from logistic fitting. Initial 30 µM solution of **2a** (pH = 6.50) basified with 300 µmol NaOH (to pH = 9.88), then titrated with HCl. (c) Photographs of titration solution at various pH's. (d) Evolution of absorbance at 549 nm versus pH

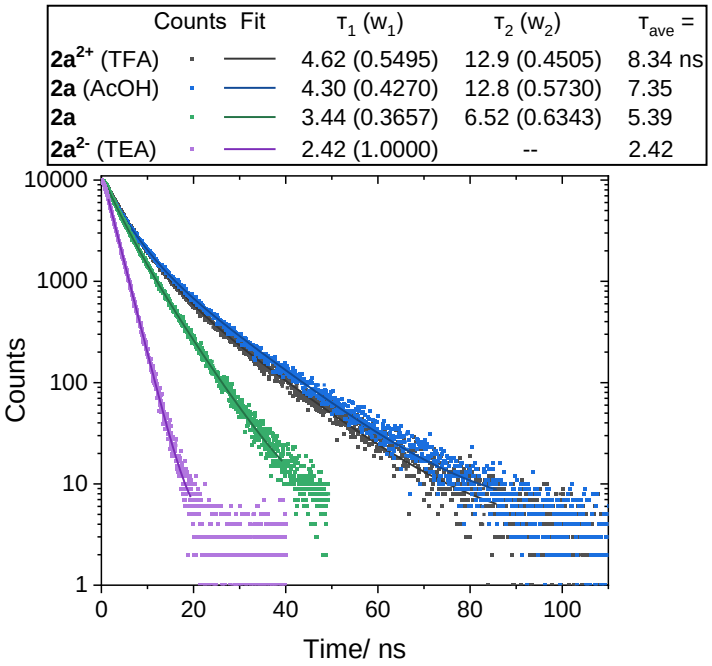



Figure S10. Time-correlated single photon count (TCSPC) decay traces of **2a**$^{2+}$, **2a**, and **2a**$^{2-}$ in acetonitrile solutions with TFA, AcOH, and TEA additives, with mono and biexponential fitting parameters ($\tau_i$ = component lifetime, $w_i$ = relative component weight, $\tau_{ave}$ = composited average lifetime).

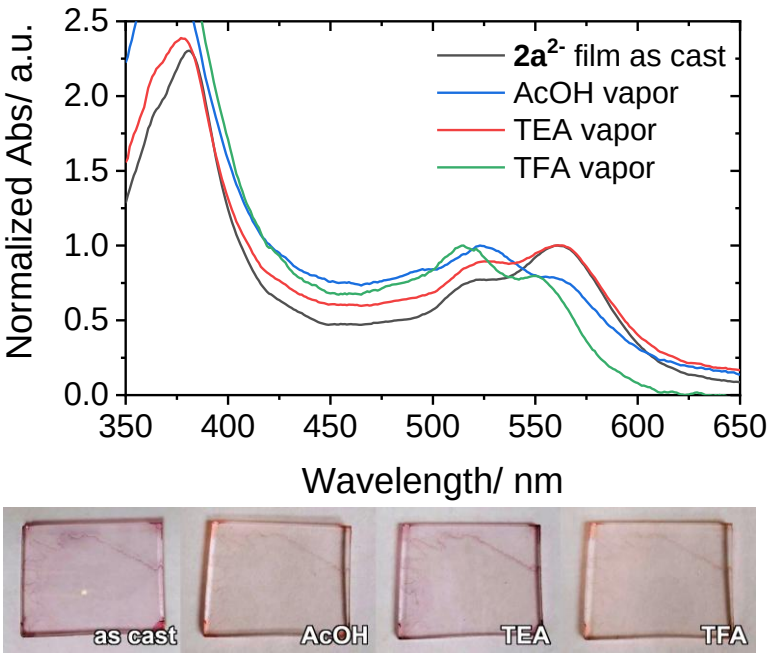



Figure S11. Absorption spectra and photographs of the **2a** film on quartz spin-coated from DCM with TEA. The spectral shifts suggest the predominantly anionic state in the as-cast film, neutralization in AcOH and further protonation to the cationic state in TFA vapor.

*Density Functional Theory (DFT) Calculations*

The ground-state geometry of neutral **2** (the alkyl substituents were replaced by methyl groups to simplify the calculations) and ionic species ($\mathbf{2}^{4+}$, $\mathbf{2}^{2+}$, $\mathbf{2}^{2-}$) were optimized using DFT (B3LYP/6-31G+(d)) with IEFPCM solvation model for acetonitrile in Gaussian 16. The GD3BJ dispersion correction was applied to account for weak intermolecular interactions due to the proximity of tetrazole substituents. Excited-state ($S_n$) geometries were obtained from the optimized ground-state structure *via* time-dependent (TD-DFT) calculations.

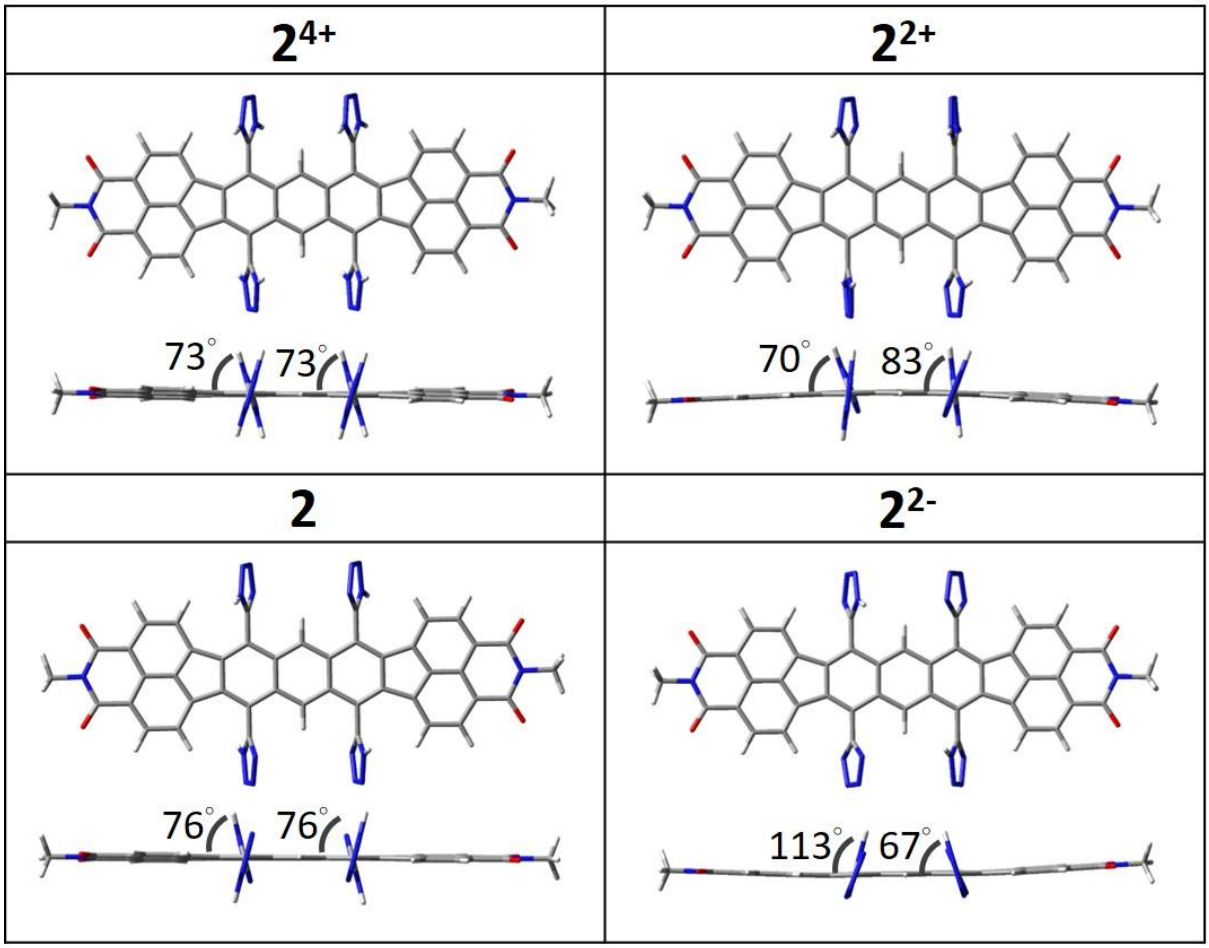



Figure S12. Geometry optimizations of $\mathbf{2}^{4+}$, $\mathbf{2}^{2+}$, **2**, $\mathbf{2}^{2-}$, using B3LYP/6-31G+(d) with IEFPCM solvation model of acetonitrile and GD3BJ dispersion correction. The torsion angle is defined as the angle between the average plane of the Tz substituent and its attached benzene ring.

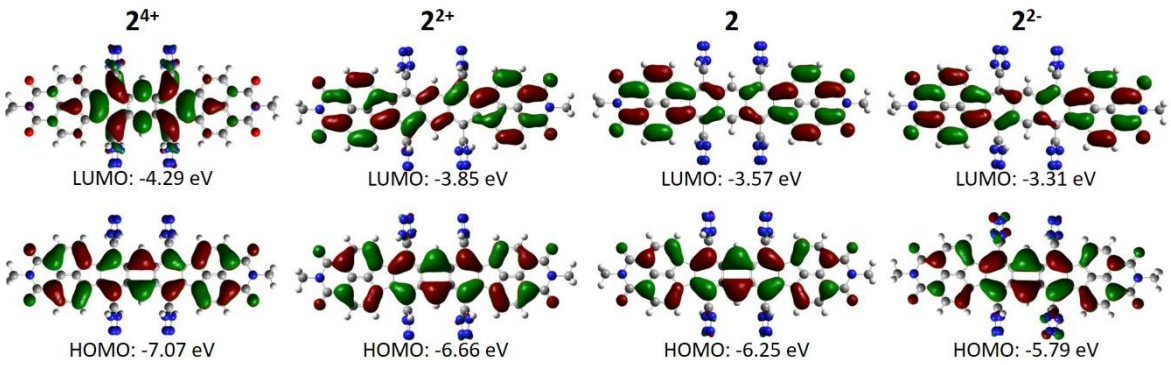



Figure S13. Calculated HOMO (bottom) and LUMO (top) energies and distributions for $\mathbf{2}^{4+}$, $\mathbf{2}^{2+}$, **2**, $\mathbf{2}^{2-}$, using B3LYP/6-31G+(d) with IEFPCM solvation model of acetonitrile and GD3BJ dispersion correction.

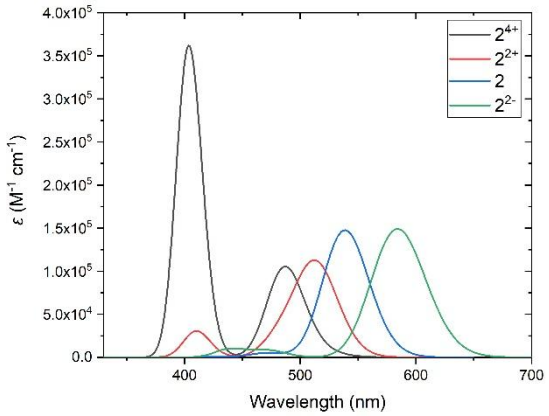



Figure S14. TD-DFT predicted UV-vis spectrum of $\mathbf{2}^{4+}$, $\mathbf{2}^{2+}$, **2**, $\mathbf{2}^{2-}$, using B3LYP/6-31G+(d) with IEFPCM solvation model of acetonitrile and GD3BJ dispersion correction.

Table S1. TD-DFT calculations of excited states of $\mathbf{2}^{4+}$, using B3LYP/6-31G+(d) with IEFPCM solvation model of acetonitrile and GD3BJ dispersion correction.

| State | Energy (eV) | λ (nm) | *f* | Electron Configuration |
|---|---|---|---|---|
| S1 | 2.38 | 522 | 0.0286 | H → L, 49.1% |
| S2 | 2.55 | 487 | 0.7777 | H–2 → L, 3.5%<br>H → L+1, 45.6% |
| S3 | 2.80 | 443 | 0.0000 | H-1 → L, 48.2% |
| S4 | 2.93 | 423 | 0.0000 | H → L+2, 47.7% |
| S5 | 3.07 | 404 | 2.6818 | H–8 → L+1, 1.4%<br>H–2 → L, 42.7%<br>H–1 → L+2, 1.4%<br>H → L+1, 3.5% |
| S6 | 3.10 | 400 | 0.0015 | H–7 → L+2, 1.4%<br>H–2 → L+1, 47.0% |

Table S2. TD-DFT calculations of excited states of $\mathbf{2}^{2+}$, using B3LYP/6-31G+(d) with IEFPCM solvation model of acetonitrile and GD3BJ dispersion correction.

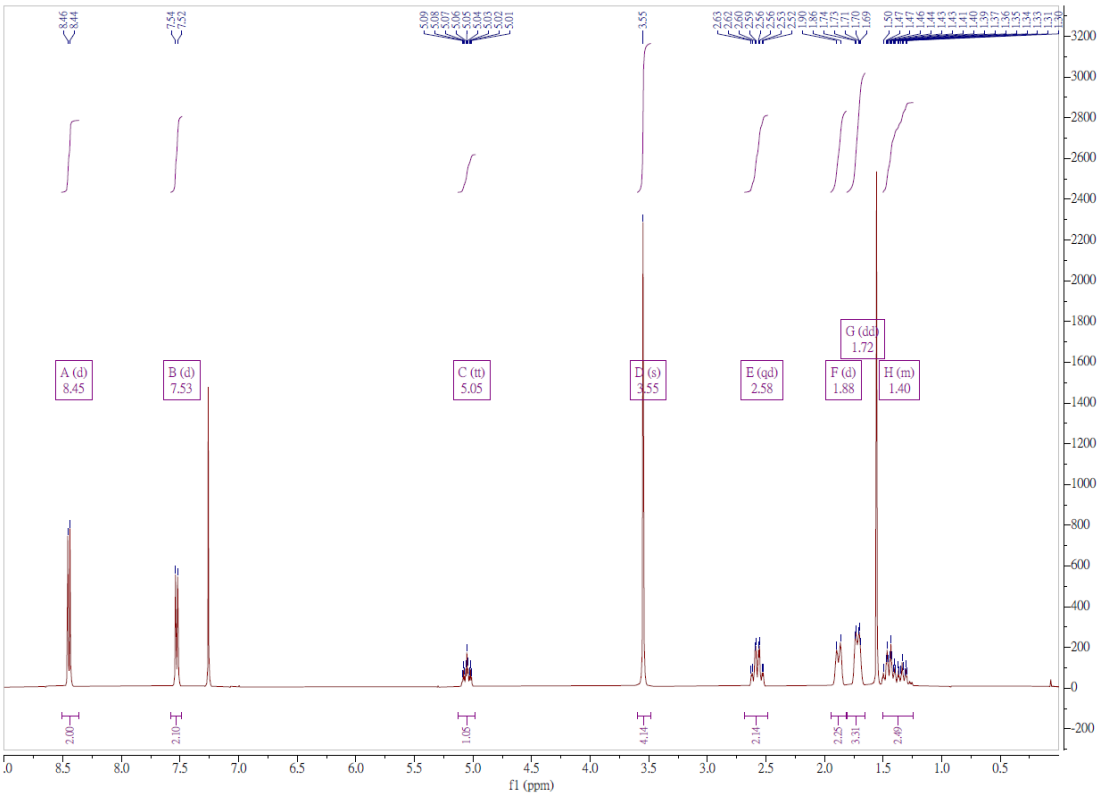
| State | Energy (eV) | λ (nm) | *f* | Electron Configuration |
|---|---|---|---|---|
| S1 | 2.41 | 514 | 0.7863 | H-2 → L+1, 1.3%<br>H → L, 47.9% |
| S2 | 2.56 | 485 | 0.2069 | H–2 → L, 1.4%<br>H → L+1, 48.0% |
| S3 | 2.80 | 443 | 0.0007 | H → L+2, 48.3% |
| S4 | 3.02 | 410 | 0.2259 | H–8 → L+2, 1.2%<br>H–2 → L, 44.5%<br>H–2 → L+1, 2.0%<br>H → L+1, 1.2% |
| S5 | 3.04 | 408 | 0.0001 | H–1 → L, 29.9%<br>H-1 → L+1, 18.7% |
| S6 | 3.16 | 393 | 0.0006 | H–2 → L+2, 2.4%<br>H–1 → L, 17.1%<br>H–1 → L+1, 27.9% |

Table S3. TD-DFT calculations of excited states of **2**, using B3LYP/6-31G+(d) with IEFPCM solvation model of acetonitrile and GD3BJ dispersion correction.

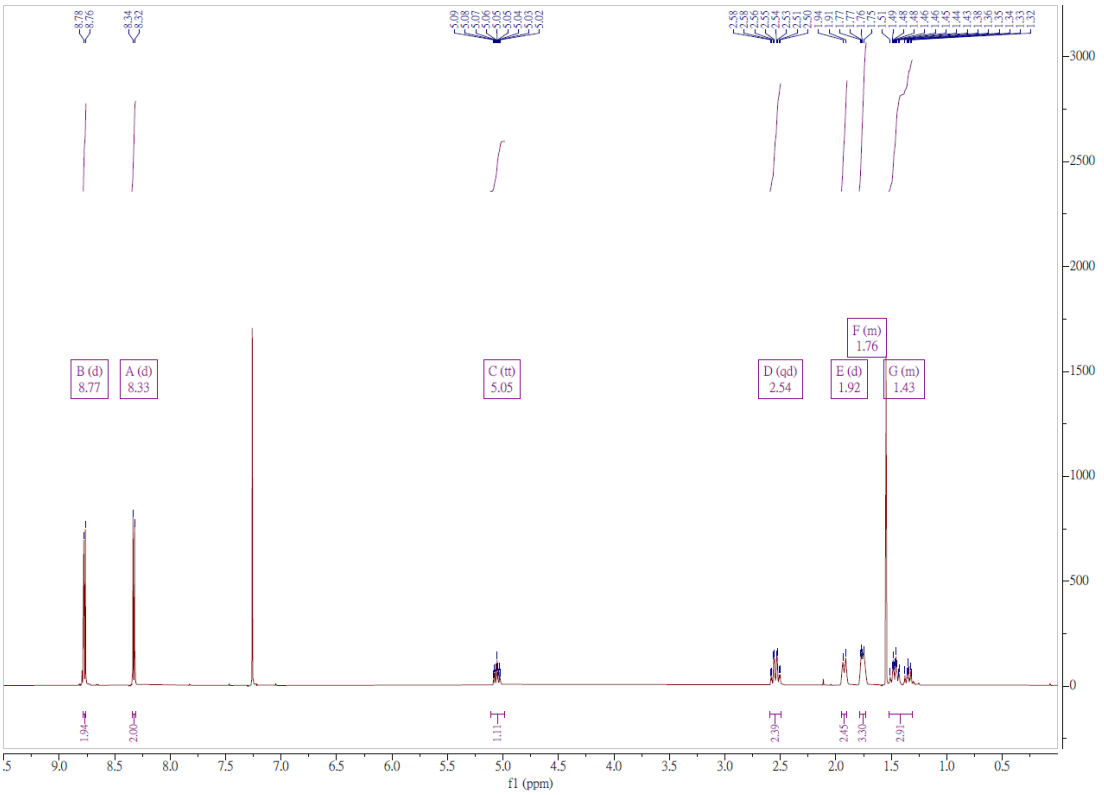
| State | Energy (eV) | λ (nm) | *f* | Electron Configuration |
|---|---|---|---|---|
| S1 | 2.30 | 539 | 1.0942 | H-2 → L+2, 1.3%<br>H → L, 48.3% |
| S2 | 2.63 | 472 | 0.0391 | H–2 → L, 1.8%<br>H → L+2, 47.7% |
| S3 | 2.67 | 465 | 0.0000 | H → L+1, 49.0% |
| S4 | 2.93 | 424 | 0.0040 | H–8 → L+1, 1.1%<br>H–2 → L, 46.1%<br>H–1 → L+2, 1.8% |
| S5 | 3.10 | 399 | 0.0000 | H-1 → L, 48.4% |
| S6 | 3.16 | 392 | 0.0000 | H–8 → L, 3.1%<br>H–4 → L, 1.5%<br>H–2 → L+1, 44.3% |

Table S4. TD-DFT calculations of excited states of $\mathbf{2}^{2-}$, using B3LYP/6-31G+(d) with IEFPCM solvation model of acetonitrile and GD3BJ dispersion correction.

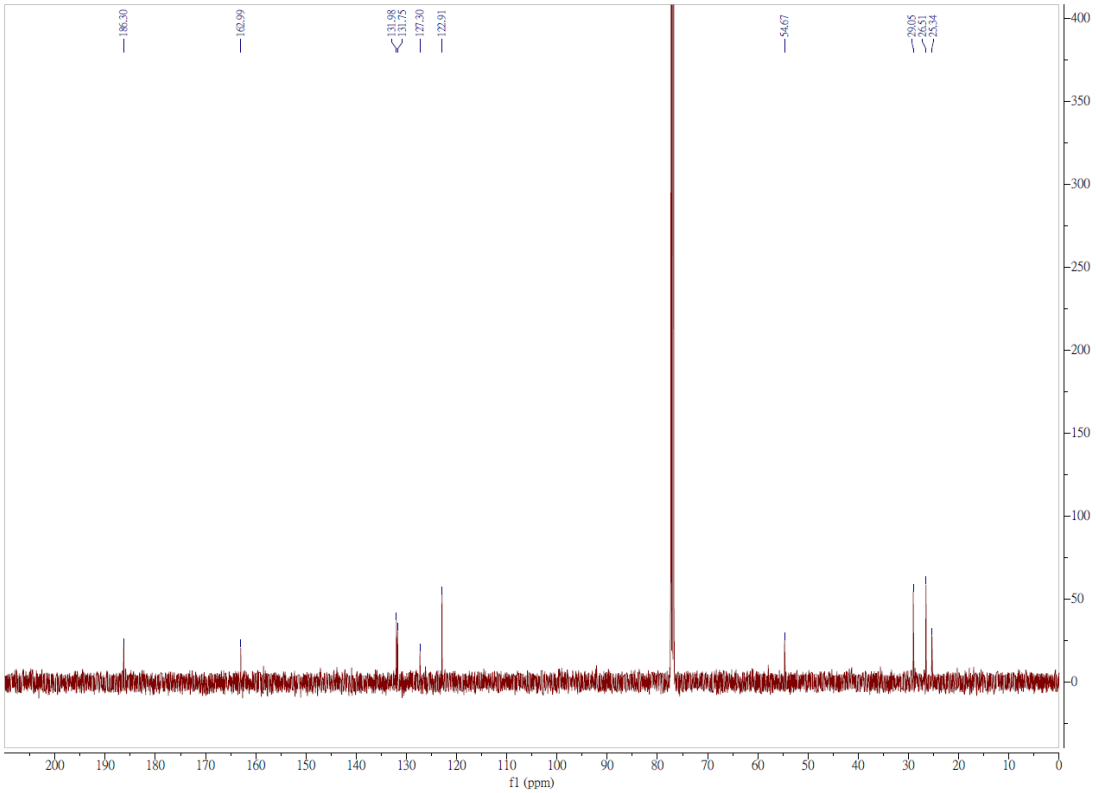
| State | Energy (eV) | λ (nm) | *f* | Electron Configuration |
|---|---|---|---|---|
| S1 | 2.12 | 584 | 1.1056 | H → L, 48.7% |
| S2 | 2.48 | 500 | 0.0005 | H → L+1, 49.0% |
| S3 | 2.63 | 471 | 0.0602 | H-2 → L, 4.1%<br>H → L+2, 45.4% |
| S4 | 2.82 | 439 | 0.0722 | H-2 → L, 43.5%<br>H → L+2, 4.1% |
| S5 | 3.07 | 428 | 0.0003 | H–3 → L, 1.2%<br>H-2 → L+1, 3.2%<br>H-1 → L, 44.3% |
| S6 | 3.07 | 403 | 0.0002 | H–3 → L, 1.7%<br>H–2 → L+1, 40.7%<br>H–1 → L, 4.1% |



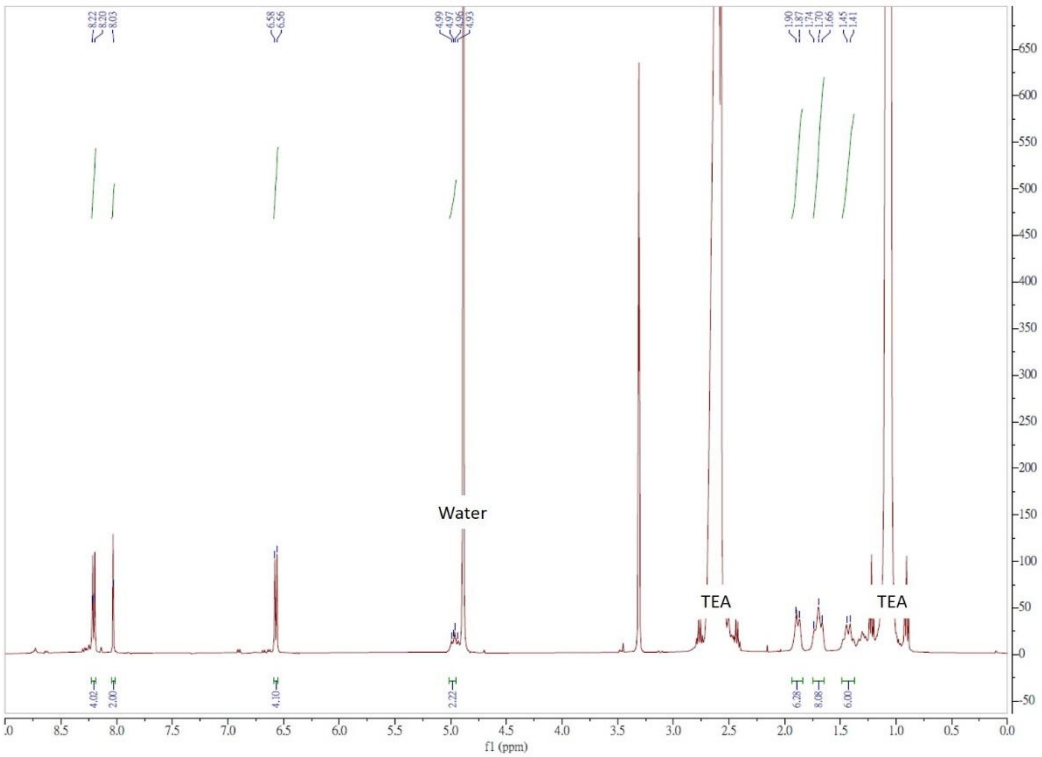

$^{1}$H-NMR of 2-cyclohexyl-6,7-dihydro-1*H*-indeno[6,7,1-*def*]isoquinoline-1,3(2*H*)-dione (**4**) ($CDCl_3$, 25 °C).



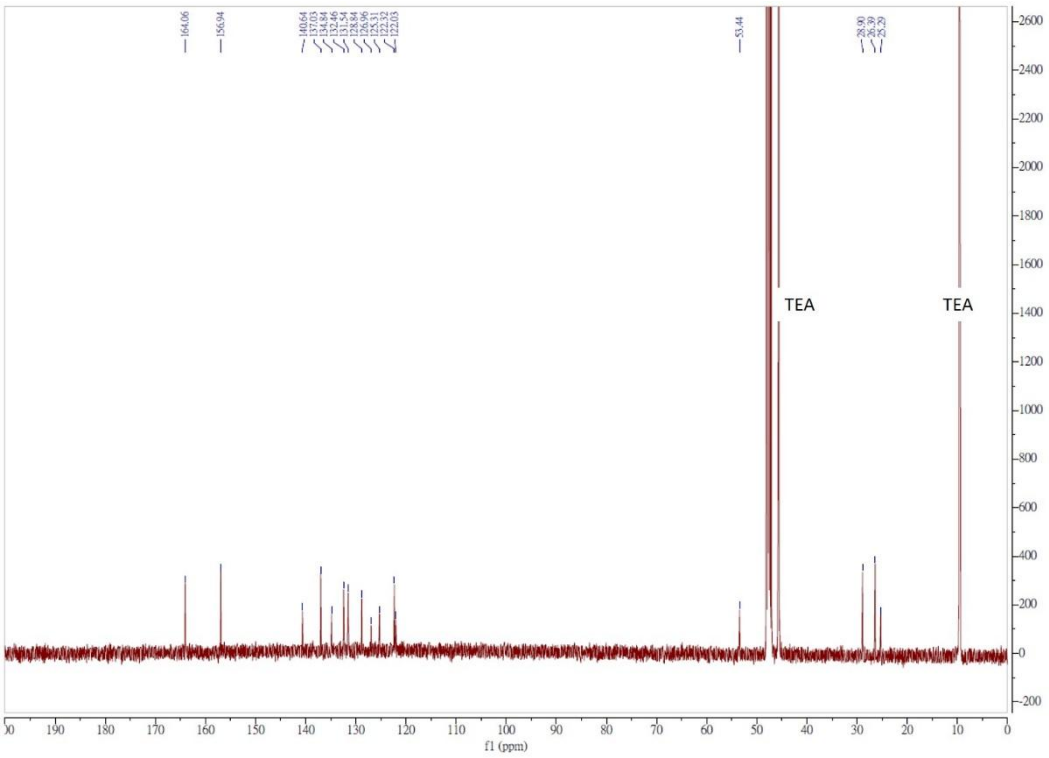

$^{1}$H-NMR of 2-cyclohexyl-1*H*-indeno[6,7,1-*def*]isoquinoline-1,3,6,7(2*H*)-tetraone (**5**) ($CDCl_3$, 25 °C).



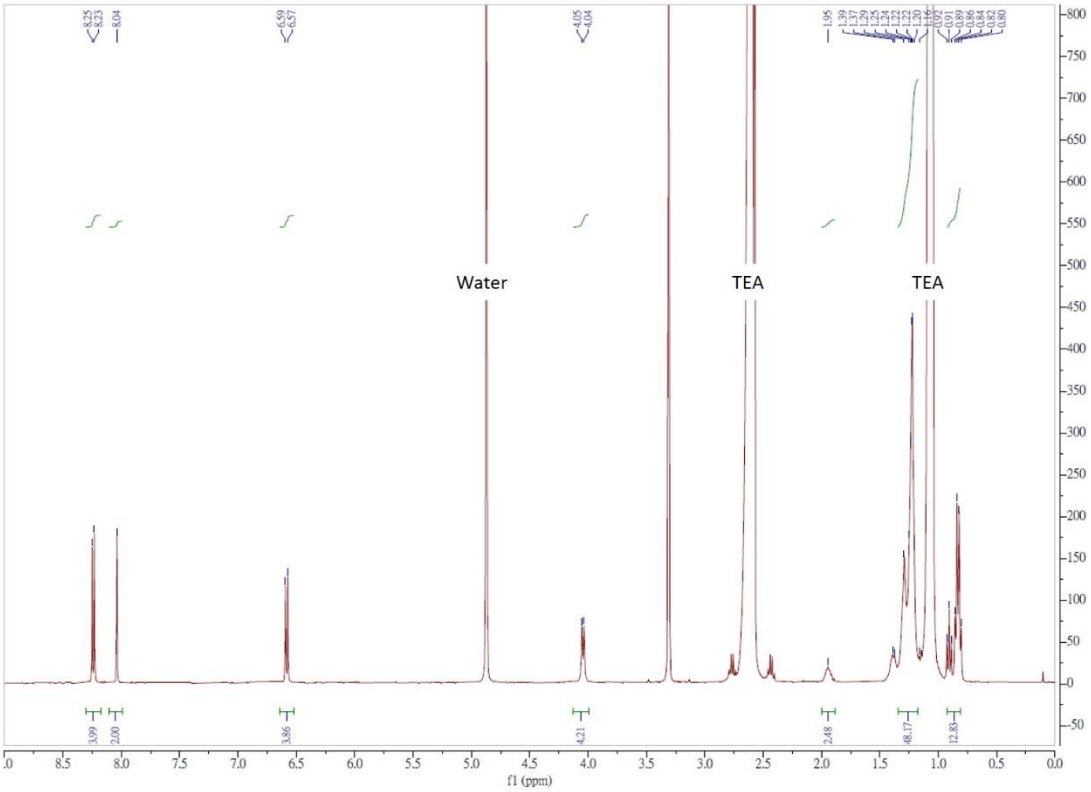

$^{13}$C-NMR of 2-cyclohexyl-1*H*-indeno[6,7,1-*def*]isoquinoline-1,3,6,7(2*H*)-tetraone (**5**) ($CDCl_3$, 25 °C).



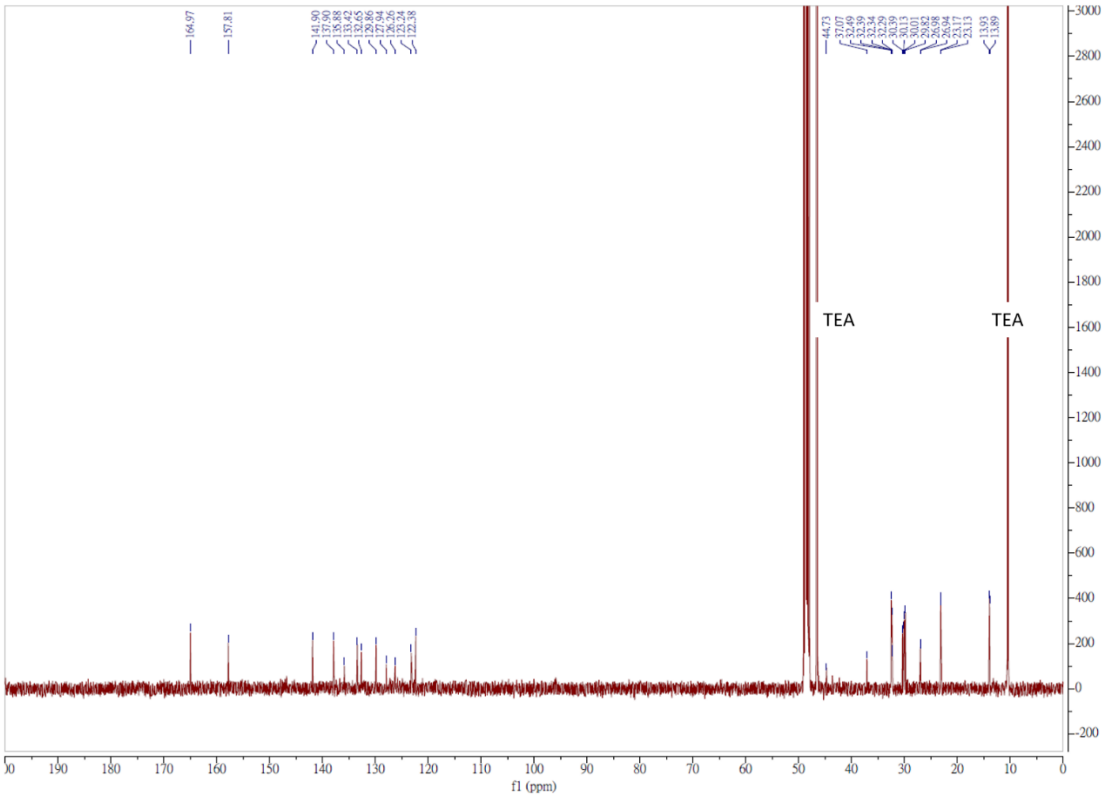

$^{1}$H-NMR of **2a**$^{2-}$ (MeOD-*d*4 with triethylamine, 25 °C).




$^{13}$C-NMR of **2a**$^{2-}$ (MeOD-*d*4 with triethylamine, 25 °C).




$^{1}$H-NMR of **2b**$^{2-}$ (MeOD-*d*4 with triethylamine, 25 °C).




$^{13}$C-NMR of **2b**$^{2-}$ (MeOD-*d*4 with triethylamine, 25 °C).

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