

Tracking the formation of supramolecular G-Quadruplexes via self-assembly enhanced emission

Diana Silva-Brenes, Loruhamá Delgado, & José M. Rivera*

Department of Chemistry and Molecular Sciences Research Center, University of Puerto Rico at Río Piedras, San Juan, PR, 00926. E-mail: riveralab.upr@gmail.com

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1. General Experimental Procedures

^1H NMR and ^{13}C NMR spectra were recorded on Bruker AV-400 spectrometer, with nominal frequencies of 400 MHz for proton and 101 MHz for carbon. ^1H NMR and ^{13}C NMR chemical shifts are reported in parts per million relative to the residual undeuterated solvent as an internal reference. All NMR experiments were performed at 298.2 K. The following abbreviations are used to explain the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiple; b, broad. Unless otherwise noted, all compounds were purified by flash chromatography on silica gel 60, 0.04-0.063 mm, and TLC (Sorbent Technologies). Visualization of spots was effected with UV light. High-resolution electrospray ionization mass spectrometry (ESI-MS) was recorded on a Q-ToF Ultima Global mass spectrometer (Micromass) equipped with a Z-spray source. Electrospray ionization was achieved in the positive mode by 3 kV on the needle. For self-assembly studies, 5 mM solutions of monomer in acetonitrile with 0.5 equivalents of the corresponding metal cation, at room temperature, were directly and continuously infused at a flow rate of 5 $\mu\text{L}/\text{min}$ with a syringe pump. The source block temperature was maintained at 60 $^\circ\text{C}$ and the desolvation gas was heated to 80 $^\circ\text{C}$. Argon was used as the collision gas and the cone voltage was set to 35 V. The mass spectrometer was operated in the mass range 0-5000 amu. FT-IR was performed on a Bruker Tensor 27 equipped with Helios Attenuated Total Reflectance (ATR) with a diamond crystal. UV/Vis absorption spectra were recorded with a Varian Cary 1E UV-visible spectrophotometer, in quartz cells. Fluorescent experiments were performed in a Varian Cary Eclipse fluorescence spectrophotometer. Data was processed using Origin (v. 7), and Microsoft[®] Excel[®] for Mac 2011, version 14.1.0. Molecular models were built and minimized using: *AMBER** (*MacroModel*), Version 9.5, Maestro 9.1.207; Schrödinger, LLC: New York, 2007, representing chloroform as a continuum solvent.

2. Synthesis and characterization

General procedure for the Claisen-Schmidt condensation for preparation of 8-chalcone-2'-deoxyguanosine derivatives

Aqueous NaOH (30%) (0.6 mL, 4.5 mmol) and 8-(m-acetylphenyl)-20-deoxyguanosine (100 mg, 0.26 mmol) were placed in an amber vial and stirred in 3 mL MeOH for 2 min until completely soluble. The corresponding aldehyde (0.65 mmol) was added and reaction was monitored with TLC (20% methanol: 80% dichloromethane) until full conversion was observed. Reaction mixture was diluted with ca. 5 mL water and neutralized with HCl (10%) until pH ~7. Product was filtered and re-suspended in ether to remove excess aldehyde. Product was decanted and dried in *vacuo* to give product as a solid.

General method for esterification and purification of derivatives 1 and 2

Precursor compounds **1a** or **2a** (0.260 mmol), were pre-dried by suspension in acetonitrile and solvent evaporation (3x), and finally suspended in anhydrous acetonitrile. To this suspension, TEA (0.571 mmol), acetic anhydride (0.571 mmol) and DMAP (0.026 mmol) were added. The reaction mixture was left stirring overnight. TLC (CH₂Cl₂:MeOH) showed complete conversion of starting material. The reaction mixture was quenched by adding excess of MeOH followed by solvent evaporation. The resulting solid material was dissolved in EtOAc and washed with 10% NaHCO₃ (2 x 15 ml) and brine (1 x 15 ml). The organic phase was separated, dried over MgSO₄ and evaporated into silica gel. Dry loading of the silica column followed by flash chromatography (CH₂Cl₂/MeOH; 95:5) affording the target compounds.

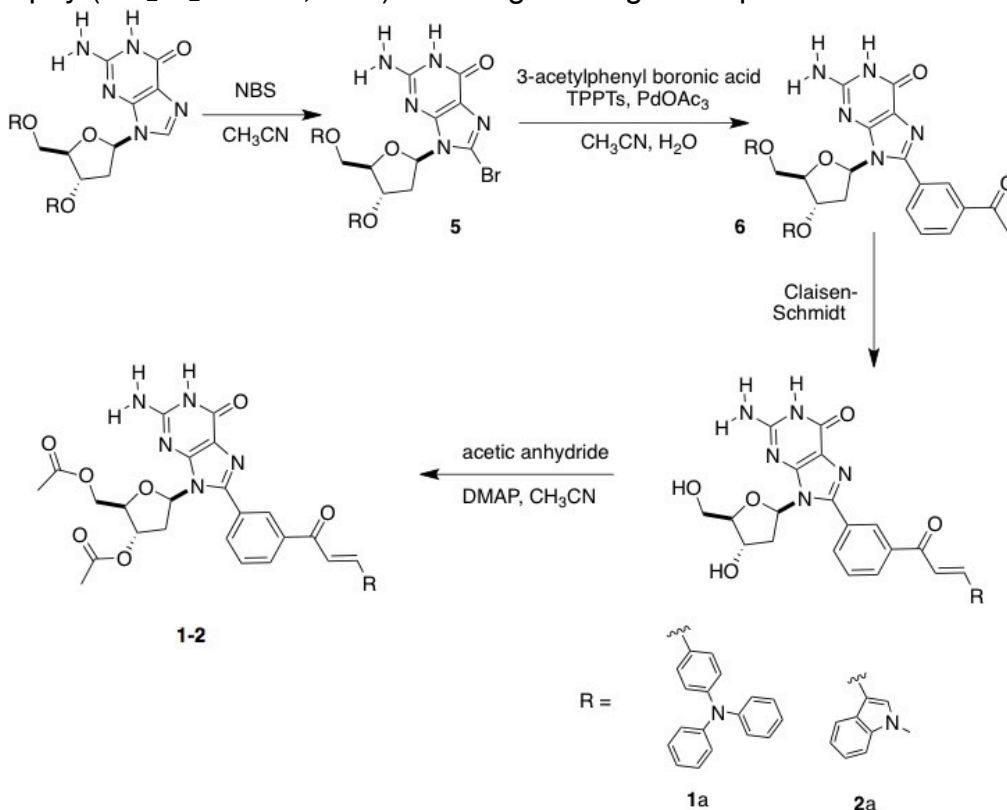
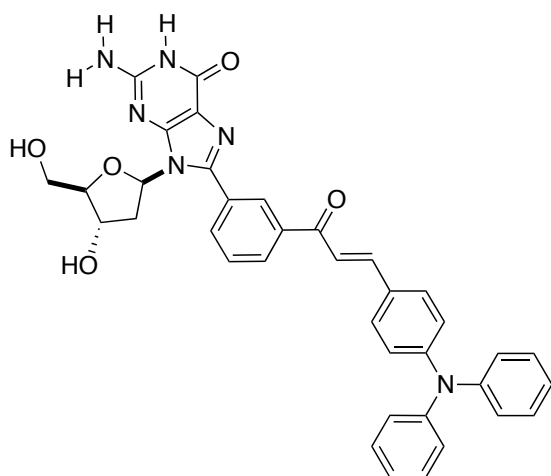


Figure S1. General synthetic scheme for the preparation of derivatives **1** and **2**.



Chemical Formula: C₃₇H₃₂N₆O₅
 Exact Mass: 640.2434
 Molecular Weight: 640.6872

(1a)

Bright orange powder, yield 93%. Dec. temp.: 190-193 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.87 (s, 1H), 8.33 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.83 - 7.69 (m, 5H), 7.37 (dd, *J* = 8.3, 7.5 Hz, 4H), 7.16 (d, *J* = 7.4 Hz, 2H), 7.12 (d, *J* = 7.5 Hz, 5H), 6.91 (d, *J* = 8.8 Hz, 2H), 6.49 (s, 2H), 6.09 (t, *J* = 7.3 Hz, 1H), 5.15 (d, *J* = 3.2 Hz, 1H), 4.96 (s, 1H), 4.32 (d, *J* = 2.8 Hz, 1H), 3.80 (td, *J* = 5.3, 3.2 Hz, 1H), 3.67 - 3.58 (m, 1H), 3.58 - 3.50 (m, 1H), 3.23 - 3.12 (m, 1H), 2.05 (ddd, *J* = 12.8, 6.5, 2.5 Hz, 1H). ¹³C NMR (101 MHz, DMSO) δ 188.42, 156.71, 153.20, 152.08, 149.75, 146.30, 146.24, 144.45, 138.22, 133.22, 130.93, 130.57, 129.81, 129.15, 128.90, 127.47, 125.34, 124.43, 120.62, 119.08, 117.25, 87.95, 84.60, 71.16, 62.08, 36.61. IR (ν_{max}): 3116, 2928, 1678, 1567, 1504, 1487, 1282 cm⁻¹. HRMS (*m/z*): [M + Na]⁺ calcd for C₃₇H₃₂N₆O₅Na, 663.2332; found, 663.2327.

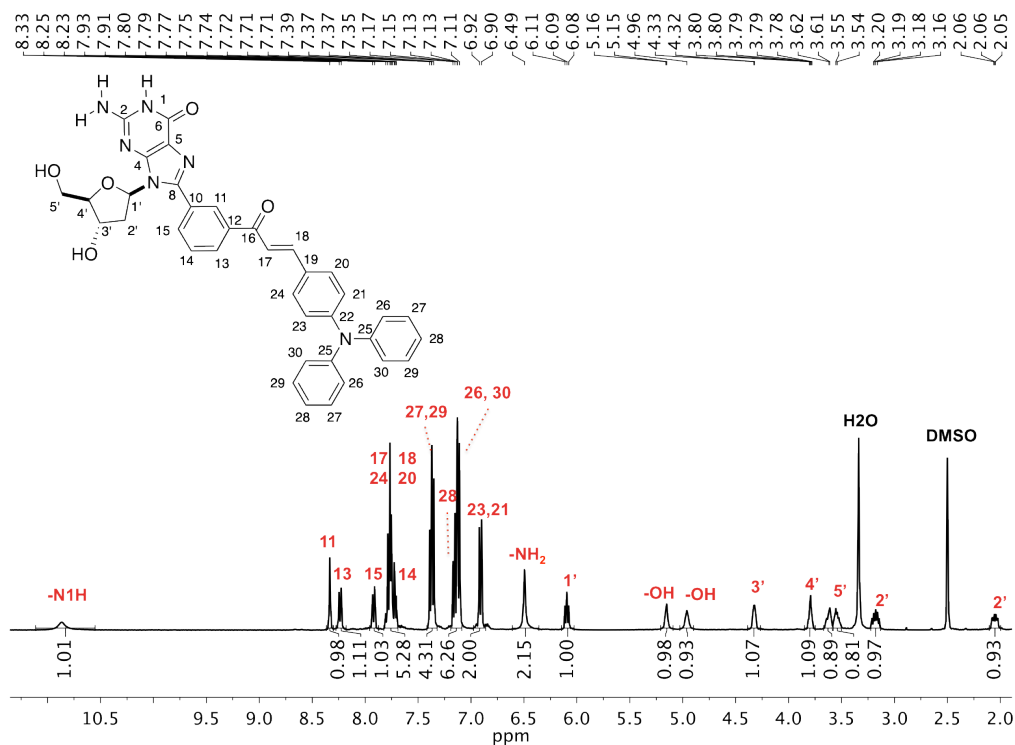


Figure S2. ¹H NMR (400 MHz, DMSO-*d*₆, 298 K) of 1a

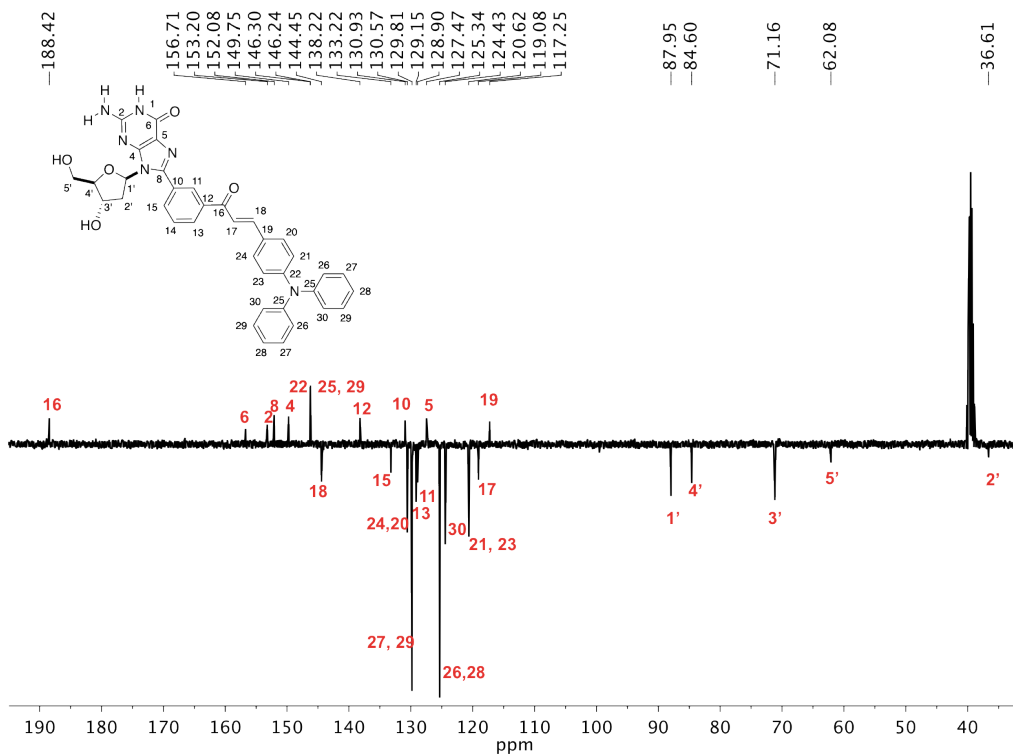
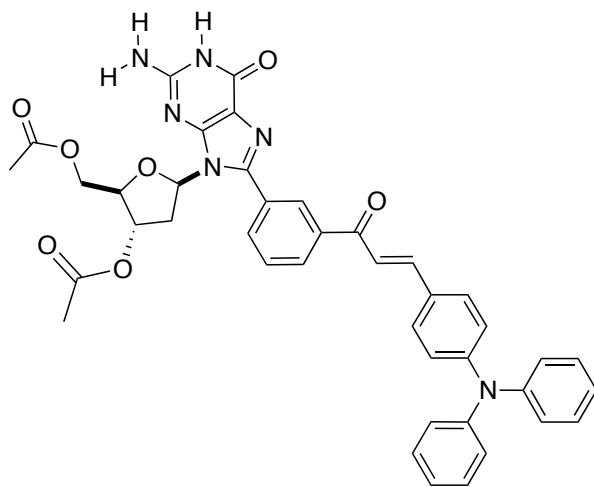


Figure S3. ^{13}C (APT) NMR (100 MHz, $\text{DMSO-}d_6$, 298 K) of **1a**



Chemical Formula: $\text{C}_{41}\text{H}_{36}\text{N}_6\text{O}_7$
 Exact Mass: 724.2645
 Molecular Weight: 724.7605

(1)

Bright orange powder, yield 99%. Dec. temp.: 145-147 °C. ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 10.85 (s, 1H), 8.33 (s, 1H), 8.23 (d, $J = 7.9$ Hz, 1H), 7.90 (d, $J = 7.8$ Hz, 1H), 7.83 - 7.62 (m, 5H), 7.37 (t, $J = 7.9$ Hz, 4H), 7.16 (d, $J = 7.4$ Hz, 2H), 7.12 (d, $J = 7.5$ Hz, 4H), 6.91 (d, $J = 8.7$ Hz, 2H), 6.53 (s, 2H), 6.14 (t, $J = 7.0$ Hz, 1H), 5.42 (dt, $J = 7.1, 3.5$ Hz, 1H), 4.44 (dd, $J = 11.5, 4.6$ Hz, 1H), 4.25 (dd, $J = 11.5, 7.3$ Hz, 1H), 4.19 - 4.13 (m, 1H), 3.59 - 3.41 (m, 1H), 2.39 (ddd, $J = 14.0, 7.3, 3.5$ Hz, 1H), 1.98 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$): δ 188.49, 170.14, 169.98, 156.65, 153.17, 151.99, 149.76, 146.22, 146.17, 144.42, 138.27, 133.06, 130.58, 130.48, 129.81, 129.28, 129.14, 128.94, 127.45, 125.35, 124.44, 120.58, 119.17, 117.17, 84.78, 81.79, 74.82, 63.73, 39.94, 33.87, 20.57. IR (ν_{max}): 3119, 1679, 1570, 1488, 1216 cm^{-1} . HRMS (m/z): $[\text{M} + \text{K}]^+$ calcd for $\text{C}_{41}\text{H}_{36}\text{N}_6\text{O}_7\text{K}$, 763.2283; found, 763.2288.

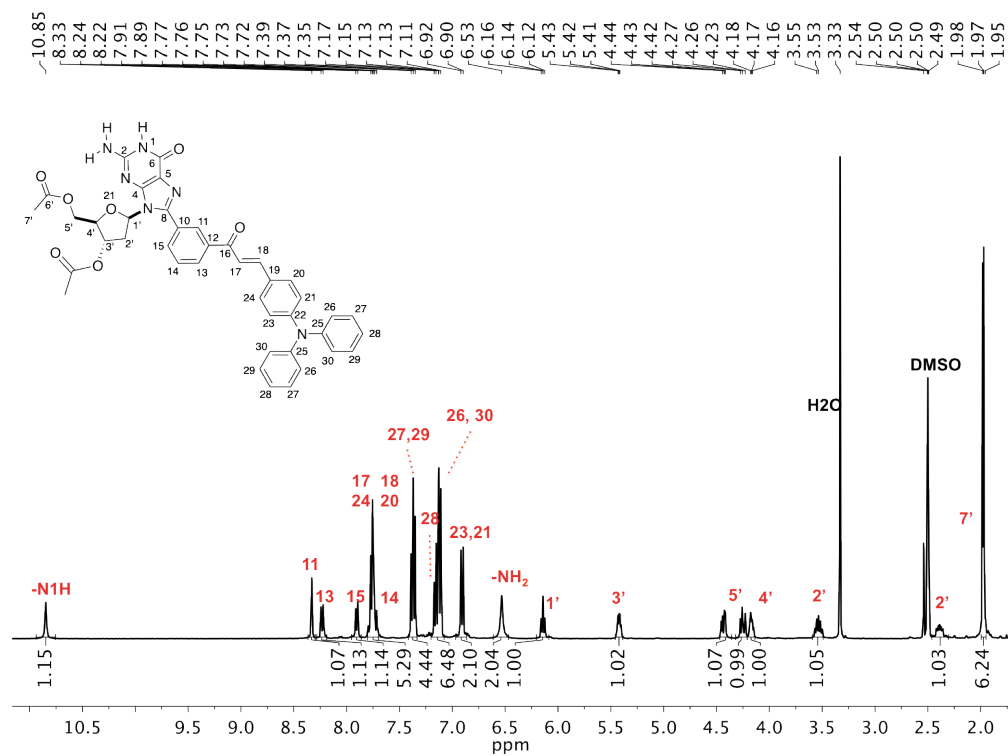


Figure S4. ^1H NMR (400 MHz, $\text{DMSO-}d_6$, 298 K) of **1**

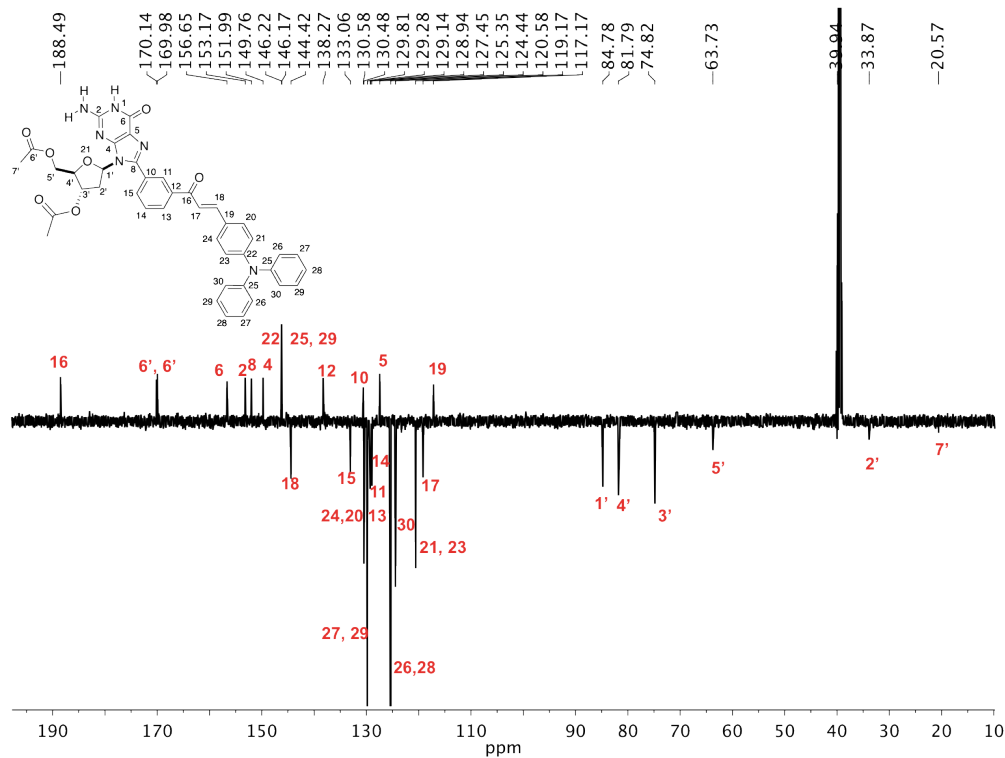


Figure S5. ^{13}C (APT) NMR (100 MHz, $\text{DMSO-}d_6$, 298 K) of **1**

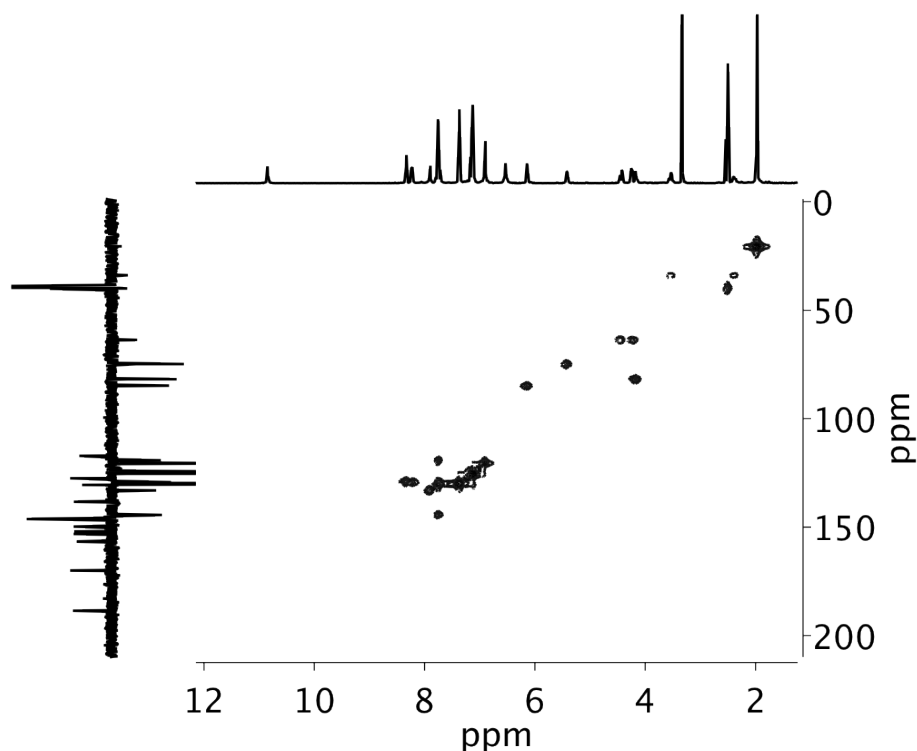
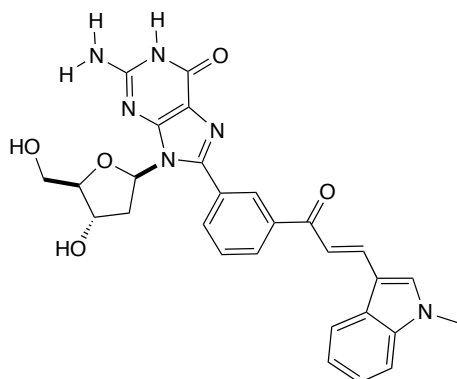


Figure S6. HMQC (DMSO- d_6 , 298 K) of **1**



Chemical Formula: $C_{28}H_{26}N_6O_5$
 Exact Mass: 526.1965
 Molecular Weight: 526.5432

(2a)

Bright yellow powder, yield 59%. Dec. temp.: 167-170 °C. 1H NMR (500 MHz, DMSO- d_6): δ 10.78 (s, 1H), 8.32 (s, 1H), 8.26 (d, $J = 7.8$ Hz, 1H), 8.16 (s, 1H), 8.10 (d, $J = 7.7$ Hz, 1H), 8.08 (d, $J = 15.4$ Hz, 1H), 7.91 (d, $J = 7.7$ Hz, 1H), 7.74 (t, $J = 7.7$ Hz, 1H), 7.67 (d, $J = 15.4$ Hz, 1H), 7.57 (d, $J = 8.0$ Hz, 1H), 7.31 (dd, $J = 8.0$ Hz, 7.1 Hz, 1H), 6.44 (s, 2H), 6.13 (t, $J = 7.3$ Hz, 1H), 5.15 (d, $J = 4.4$ Hz, 1H), 4.95 (t, $J = 5.8$ Hz, 1H), 4.35 (m, 1H), 3.87 (s, 3H), 3.83 (m, 1H), 3.65 (m, 1H), 3.57 (m, 1H), 3.22 (m, 1H), 2.06 (m, 1H). ^{13}C NMR (101 MHz, DMSO- d_6): δ 188.19, 156.84, 153.23, 152.15, 146.58, 139.05, 138.80, 138.08, 136.91, 132.91, 130.83, 129.23, 129.08, 128.59, 125.73, 122.93, 121.66, 120.48, 117.33, 114.99, 111.85, 110.97, 88.07, 84.80, 71.32, 62.18, 36.66, 33.14. IR (ν_{max}): 3304, 2927, 1673, 1637, 1558, 1523, 1373, 1282 cm^{-1} . HRMS (m/z): $[M + Na]^+$ calcd for $C_{28}H_{26}N_6O_5Na$, 549.1862; found, 549.1845.

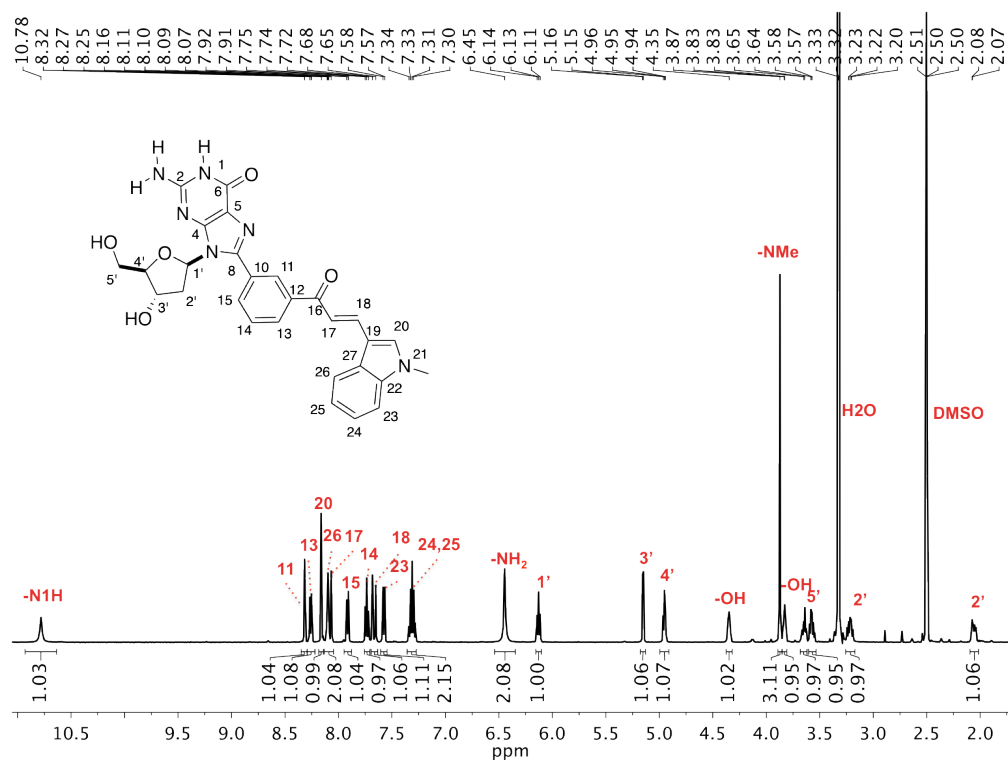


Figure S7. ^1H NMR (500 MHz, $\text{DMSO-}d_6$, 298 K) of 2a.

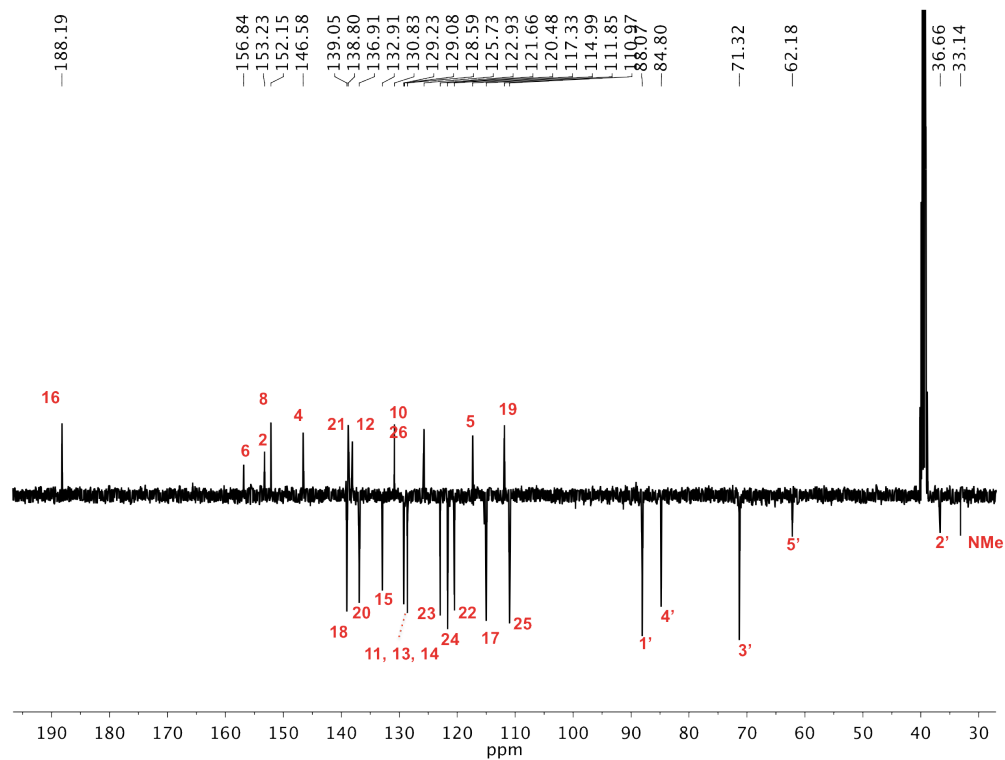
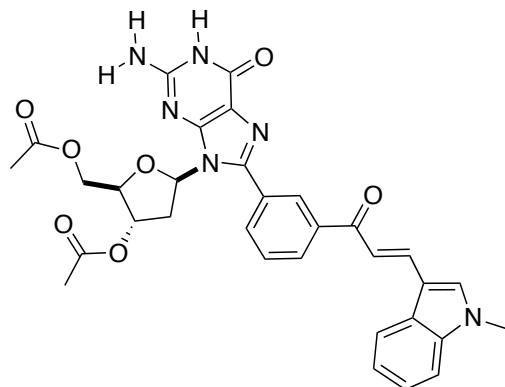


Figure S8. ^{13}C (APT) NMR (100 MHz, $\text{DMSO-}d_6$, 298 K) of 2a.



Chemical Formula: $C_{32}H_{30}N_6O_7$
 Exact Mass: 610.2176
 Molecular Weight: 610.6166

(2)

Bright yellow powder, yield 36%. Dec. temp.: 140-144 °C. 1H NMR (400 MHz, $DMSO-d_6$): δ 10.89 (s, 1H), 8.33 (s, 1H), 8.26 (d, $J = 7.8$ Hz, 1H), 8.15 (s, 1H), 8.11 (d, $J = 6.1$ Hz, 1H), 8.08 (d, $J = 15.2$ Hz, 2H), 7.91 (d, $J = 7.8$ Hz, 1H), 7.75 (t, $J = 7.7$ Hz, 1H), 7.67 (d, $J = 15.4$ Hz, 1H), 7.58 (d, $J = 8.0$ Hz, 1H), 7.33 (t, $J = 7.5$ Hz, 1H), 7.28 (t, $J = 7.9$ Hz, 1H), 6.56 (s, 2H), 6.19 (t, $J = 6.9$ Hz, 1H), 5.50 - 5.41 (m, 1H), 4.46 (dd, $J = 11.5, 4.6$ Hz, 1H), 4.28 (dd, $J = 11.4, 7.3$ Hz, 1H), 4.21 (dd, $J = 7.2, 3.6$ Hz, 1H), 3.87 (s, 3H), 3.55 (dd, $J = 17.5, 10.6$ Hz, 1H), 2.46 - 2.37 (m, 1H), 1.99 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (101 MHz, $DMSO-d_6$): δ 188.13, 170.15, 170.00, 156.71, 153.19, 151.98, 146.32, 139.00, 138.82, 138.04, 136.98, 132.71, 130.50, 129.23, 129.01, 128.54, 125.60, 122.84, 121.48, 120.44, 117.19, 114.99, 111.81, 110.92, 84.81, 81.85, 74.87, 63.72, 33.96, 33.08, 20.69. IR (ν_{max}): 3109, 1678, 1561, 1524, 1216 cm^{-1} . HRMS (m/z): $[M + K]^+$ calcd for $C_{32}H_{30}N_6O_7K$, 649.1813; found, 649.1889.

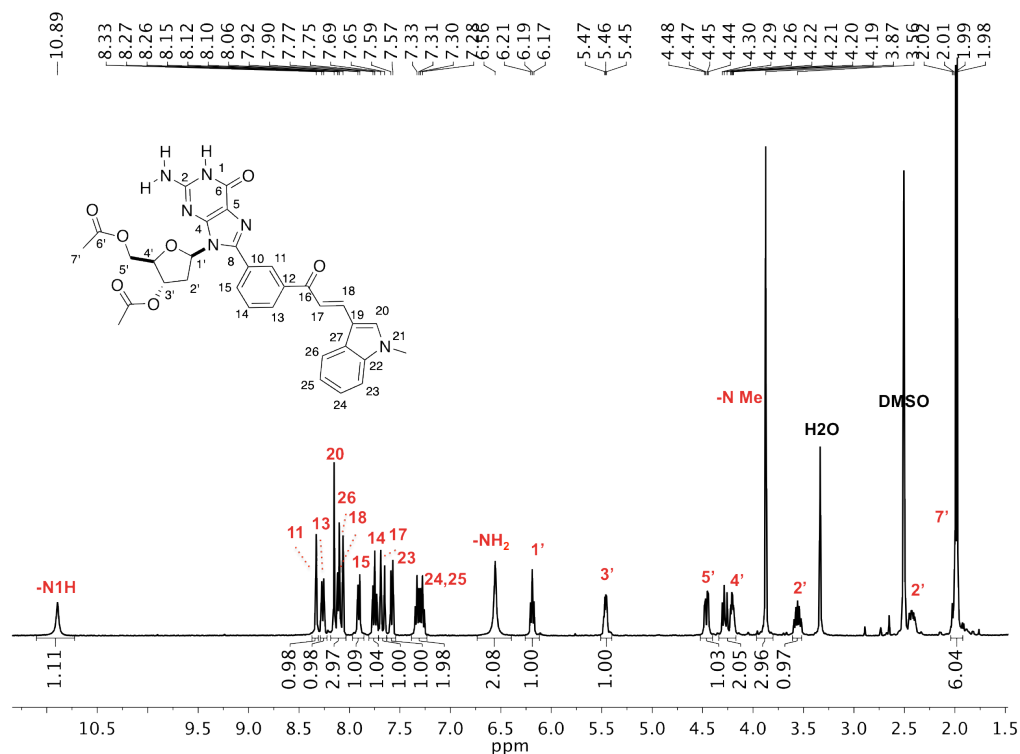


Figure S9. 1H NMR (500 MHz, $DMSO-d_6$, 298 K) of 2.

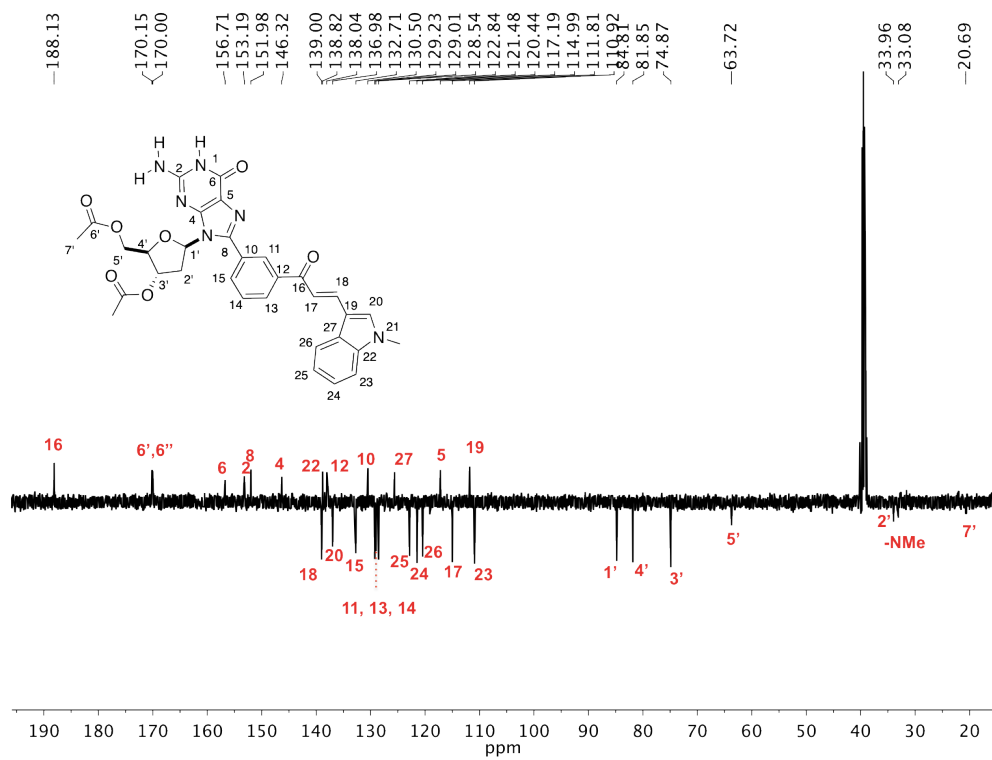


Figure S10. ^{13}C (APT) NMR (100 MHz, $\text{DMSO}-d_6$, 298 K) of 2.

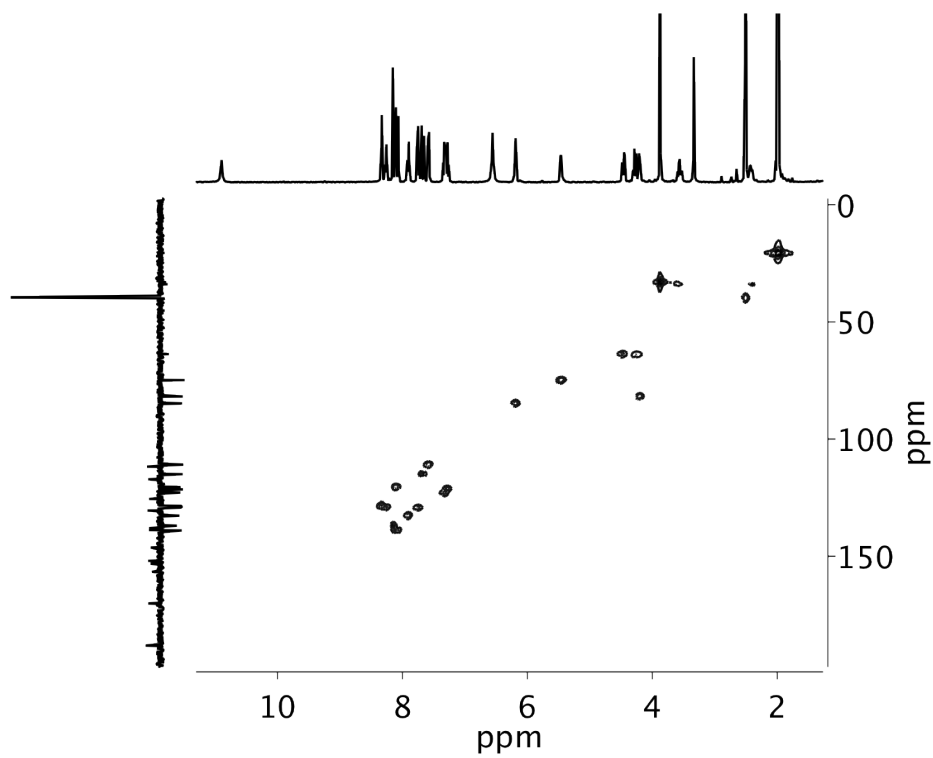


Figure S11. HMQC ($\text{DMSO}-d_6$, 298 K) of 2.

3. Photophysical characterization

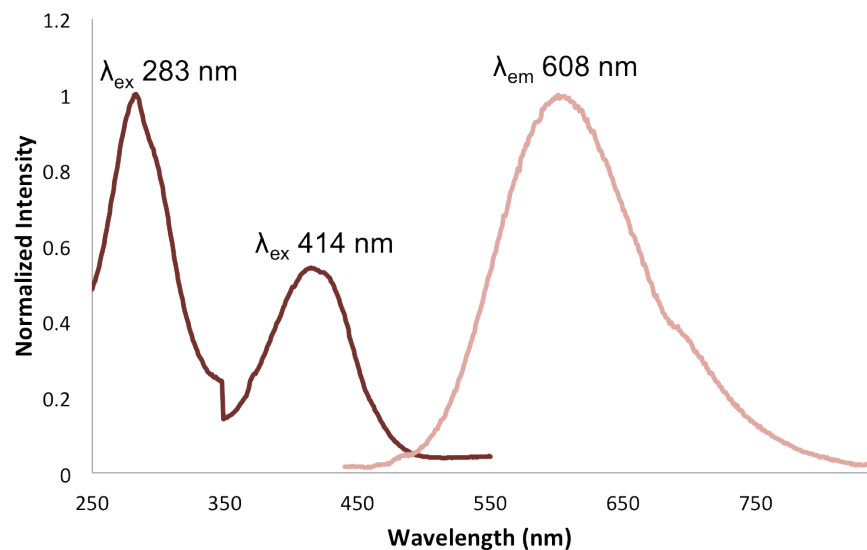


Figure S12. Normalized absorption and emission spectra (2.2 μ M CH₃CN, degasified) of **1**, showing excitation and emission maxima.

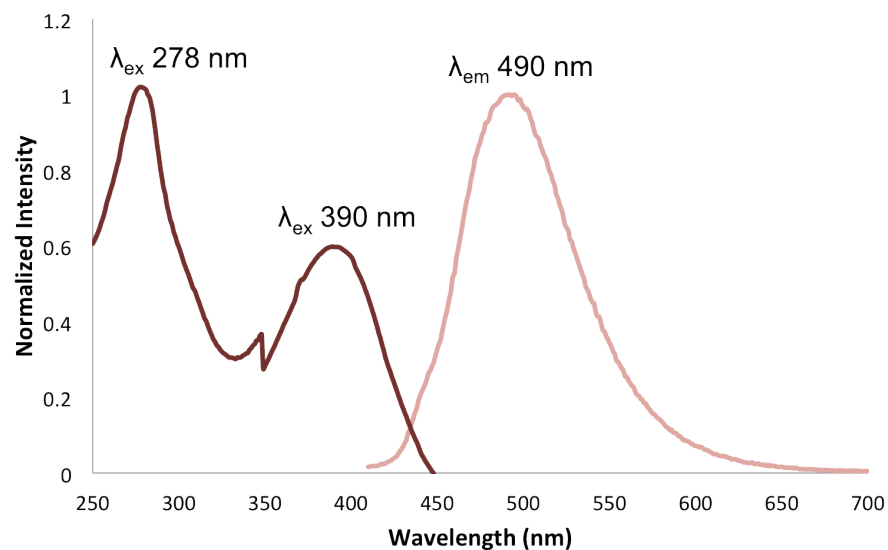


Figure S13. Normalized absorption and emission spectra (6.4 μ M CH₃CN; degasified) of **2**, showing excitation and emission maxima.

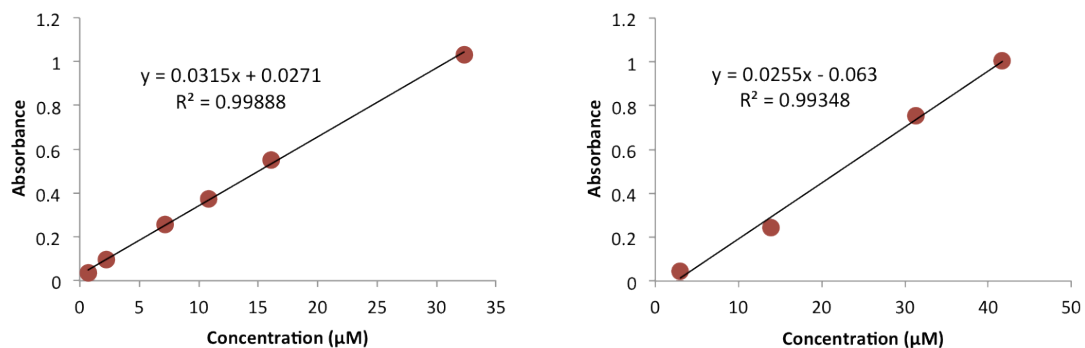


Figure S14. Beer-Lambert plot (absorption vs. concentration) for **1** (left) and **2** (right).

A neutral density filter was used when measuring fluorescence intensity of the standard to match its intensity with that of the samples.¹ This allowed measuring the standard (perylene) under the same conditions (slit sizes, PMT voltage) for the sample and the standard. The filter absorbance was measured and the intensity determined for the standard was corrected using the following equation:

$$I_r = I_f / (T)$$

Where I_r is the real emission intensity, I_f is the integrated emission intensity measured using the filter and T is the transmittance of the filter. The values obtained for I_r were used to produce the graphs for perylene shown in Figures S16 and S17.

Emission spectra of the solutions showing absorbance with less than 0.1 (to avoid inner filter effects), at the excitation wavelength for **1** (420 nm) and **2** (390 nm), in acetonitrile ($\eta = 1.3441$) were recorded. Perylene², $\Phi = 0.92$ in ethanol ($\eta_{ST} = 1.361$) was used as the standard and measurements for the standard were taken at both 390 nm and 420 nm. Plots of the integrated fluorescence intensity as a function of the absorbance were done, the straight-line intercept was set to 0 and the slope (Figures S15 and S16) was used to determine the relative fluorescence quantum yield using the following equation:

$$\Phi_x = \Phi_{ST} (\text{slope}_x / \text{slope}_{ST}) (\eta_x^2 / \eta_{ST}^2)$$

¹ Arce, R.; Pino, E. F.; Valle, C.; Ágreda, J., "Photophysics and Photochemistry of 1-Nitropyrene." *J. Phys. Chem. A* **2008**, 112, 10294-10304.

² Brouwer, A. Standards for photoluminescence quantum yield measurements in solution (IUPAC Technical Report). *Pure Appl. Chem.*, **2011**, 83, 2213-2228.

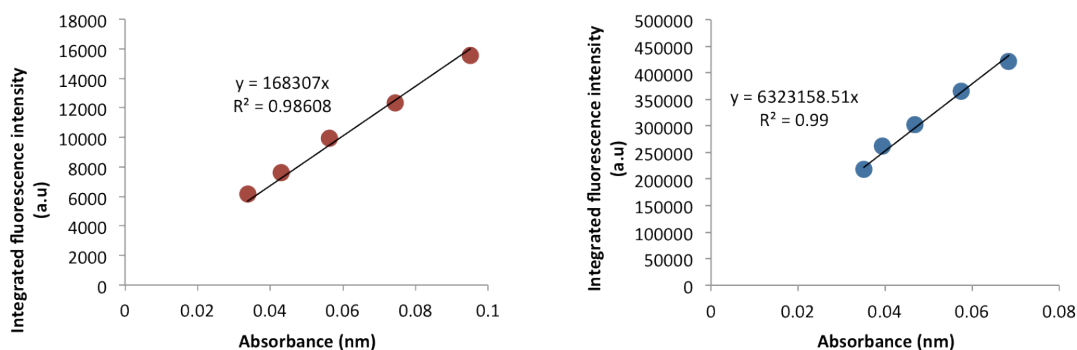


Figure S15. Plots of the integrated fluorescence emission intensity vs absorbance used to determine quantum yield of **1** (left) and perylene standard (right) (excitation wavelength 420 nm; excitation slit 5/emission slit 10; PMT voltage 650 V).

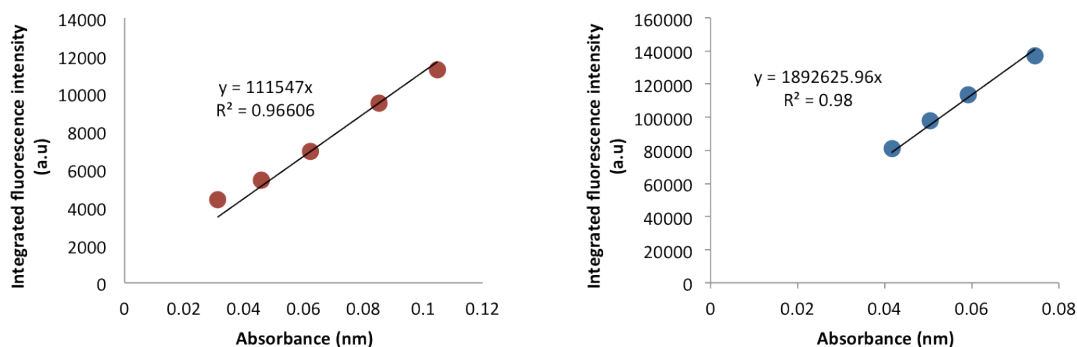


Figure S16. Plots of the integrated fluorescence emission intensity as a function of absorbance used to determine quantum yield of **2** (left) and perylene standard (right) (excitation wavelength 390 nm; excitation slit 5/emission slit 10; PMT voltage 650 V).

Compound	Molar absorptivity (M^{-1}, cm^{-1})	Stokes shift (cm^{-1})	Stokes shift (nm)	Quantum yield
1	31,500	7,707	194	0.029
2	25,500	5,233	100	0.053

4. Self-Assembly studies: NMR

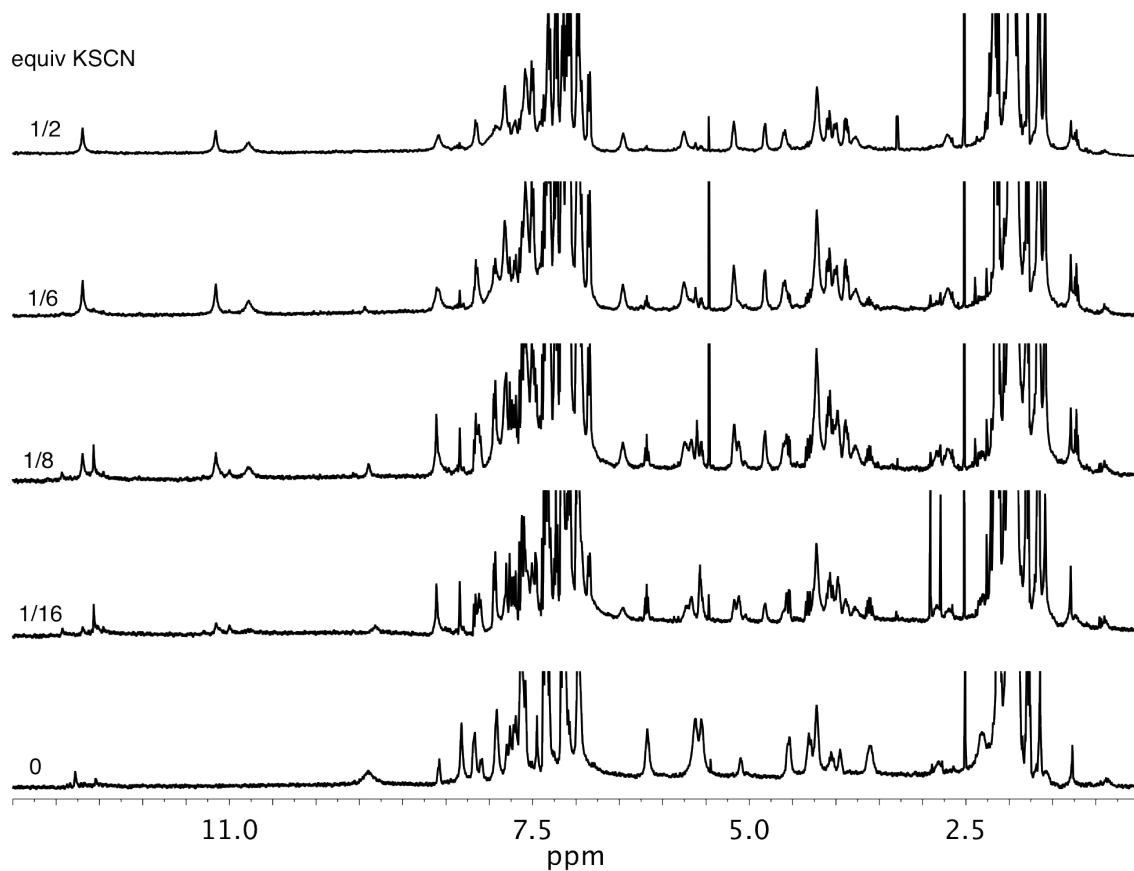


Figure S17. ¹H NMR (400 MHz, CD₃CN, 298 K) titration of **1** (5 mM) with increasing amounts of KSCN. (1) no KSCN, loosely bound aggregates (LBA), ~4% octamer; (2) 0.63 mM KSCN, 38% octamer, 34% hexadecamer; (3) 1.25 mM KSCN, 21% octamer, 61% hexadecamer; (4) 1.7 mM KSCN, 89% hexadecamer; and 5) 5.0 mM KSCN, 98% hexadecamer.

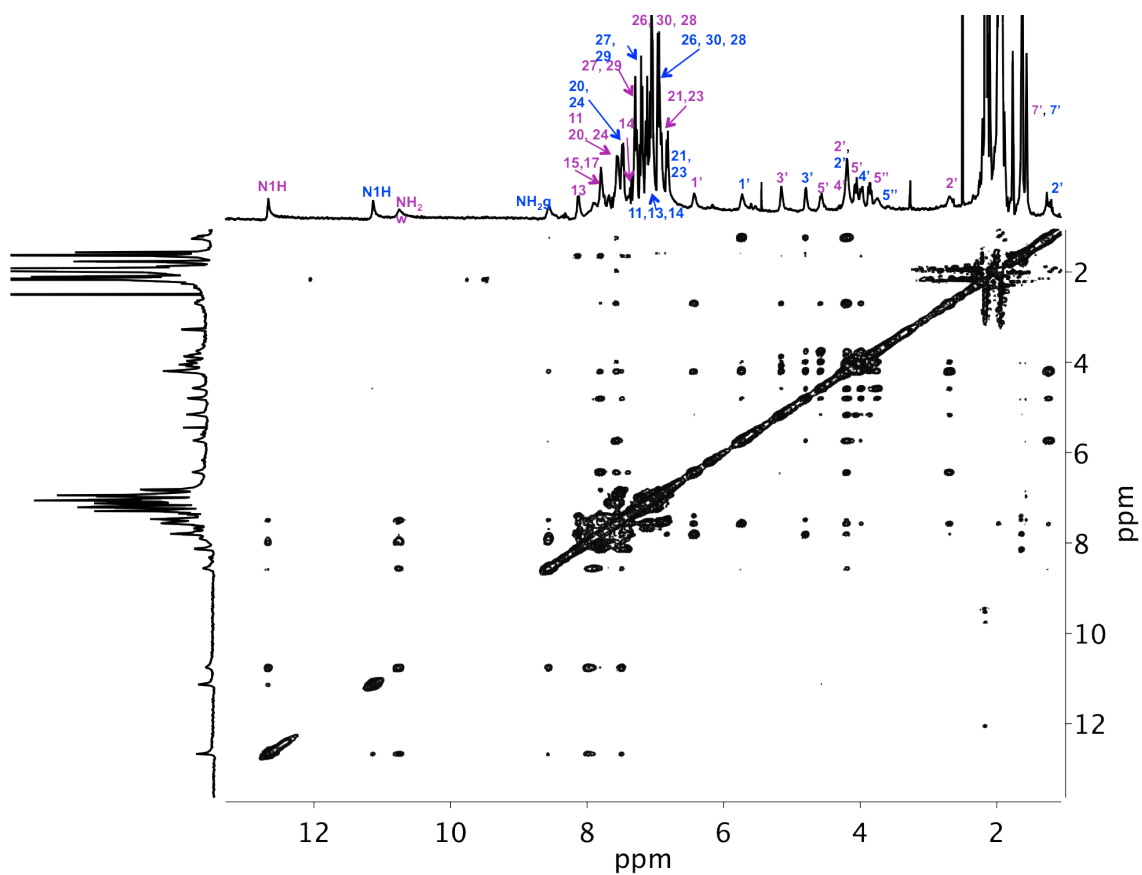


Figure S18. Full 2D NOESY spectrum (400 MHz, CD₃CN, 0.5 equiv KSCN) for **1**₁₆ (5 mM in **1**). Labels in magenta for the outer tetrads and in blue for the inner tetrads.

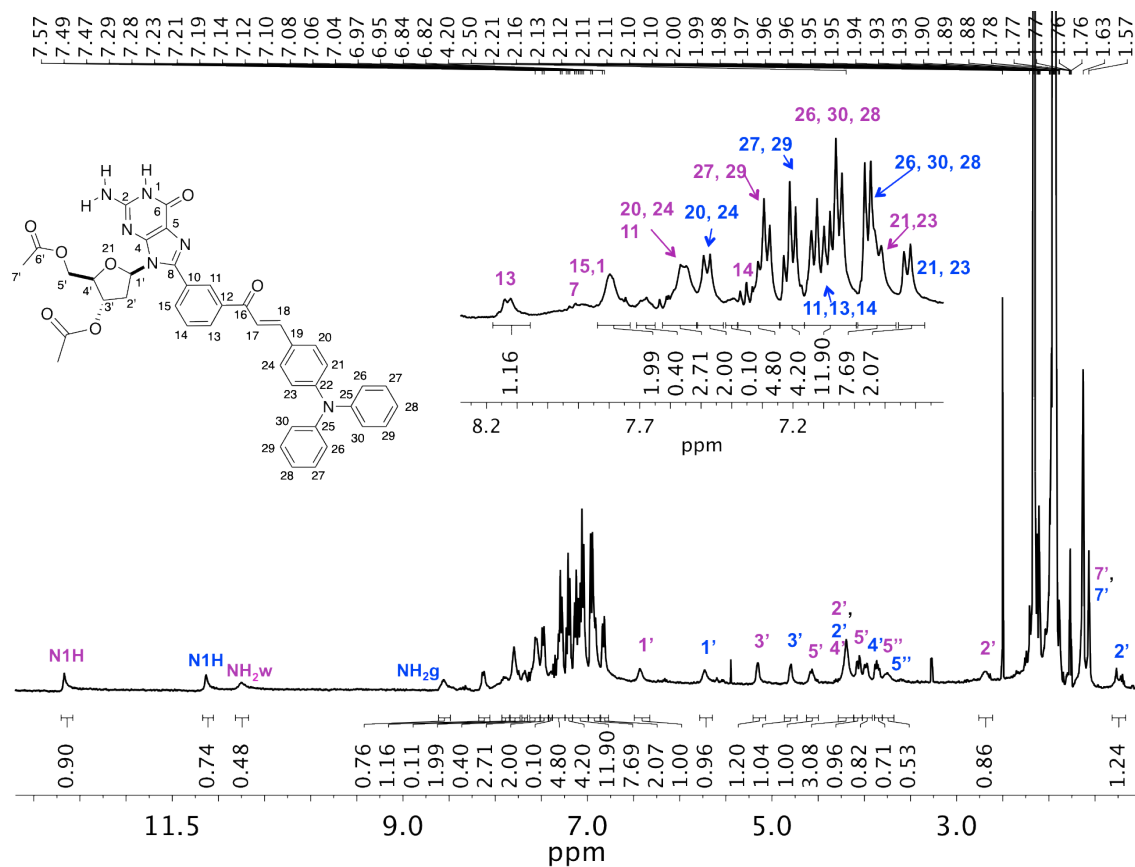


Figure S19. ^1H NMR (400 MHz, CD_3CN , 0.5 equiv KSCN) for **16** (5 mM in **1**). Labels in magenta for the outer tetrads and in blue for the inner tetrads. Signals assigned based on integrations, 2D NOESY spectrum, proton coupling and our own experience. The 6-9 ppm region of the spectrum allows different interpretations.

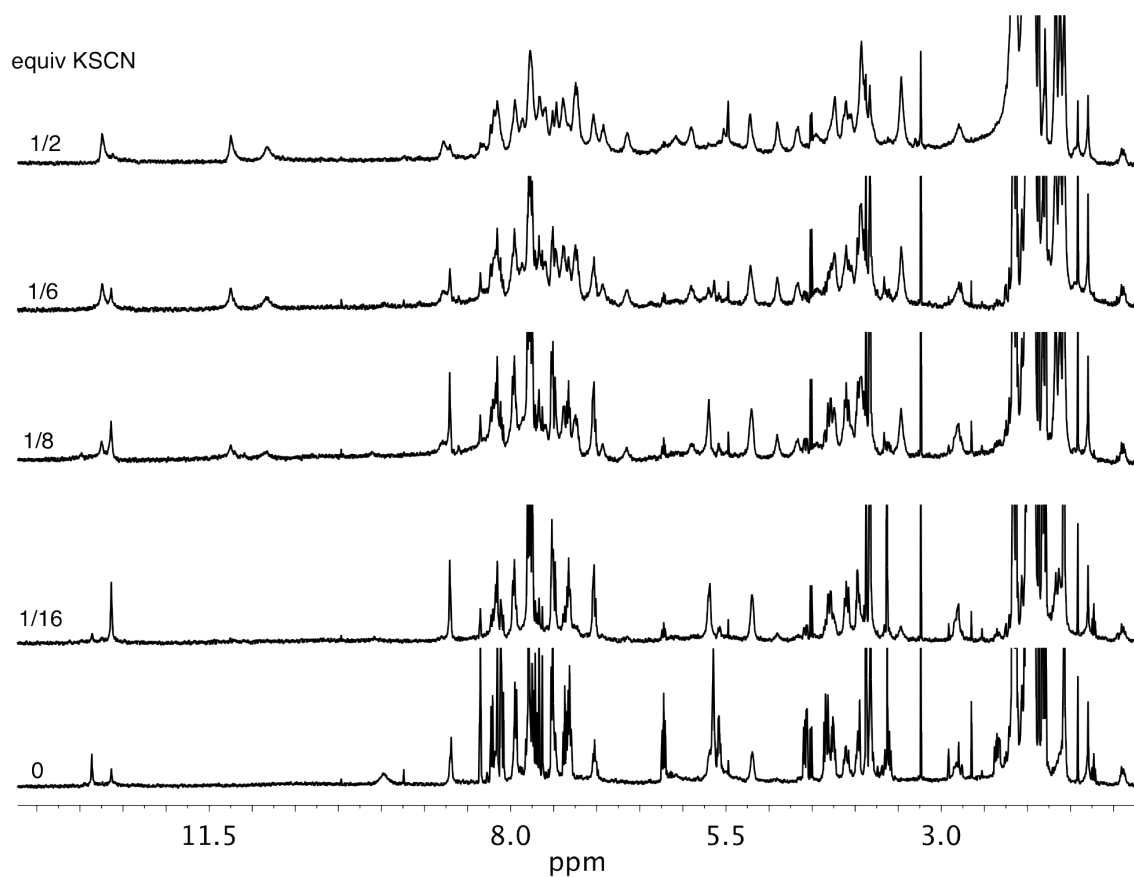


Figure S20. ¹H NMR (400 MHz, CD₃CN, 298 K) titration of **2** (5 mM) with increasing amounts of KSCN. (1) no KSCN, loosely bound aggregates (LBA), ~6% octamer; (2) 0.63 mM KSCN, 91% octamer; (3) 1.25 mM KSCN, 36% octamer, 53% hexadecamer; (4) 1.7 mM KSCN, 26% octamer, 74% hexadecamer; and 5) 5.0 mM KSCN, 96% hexadecamer.

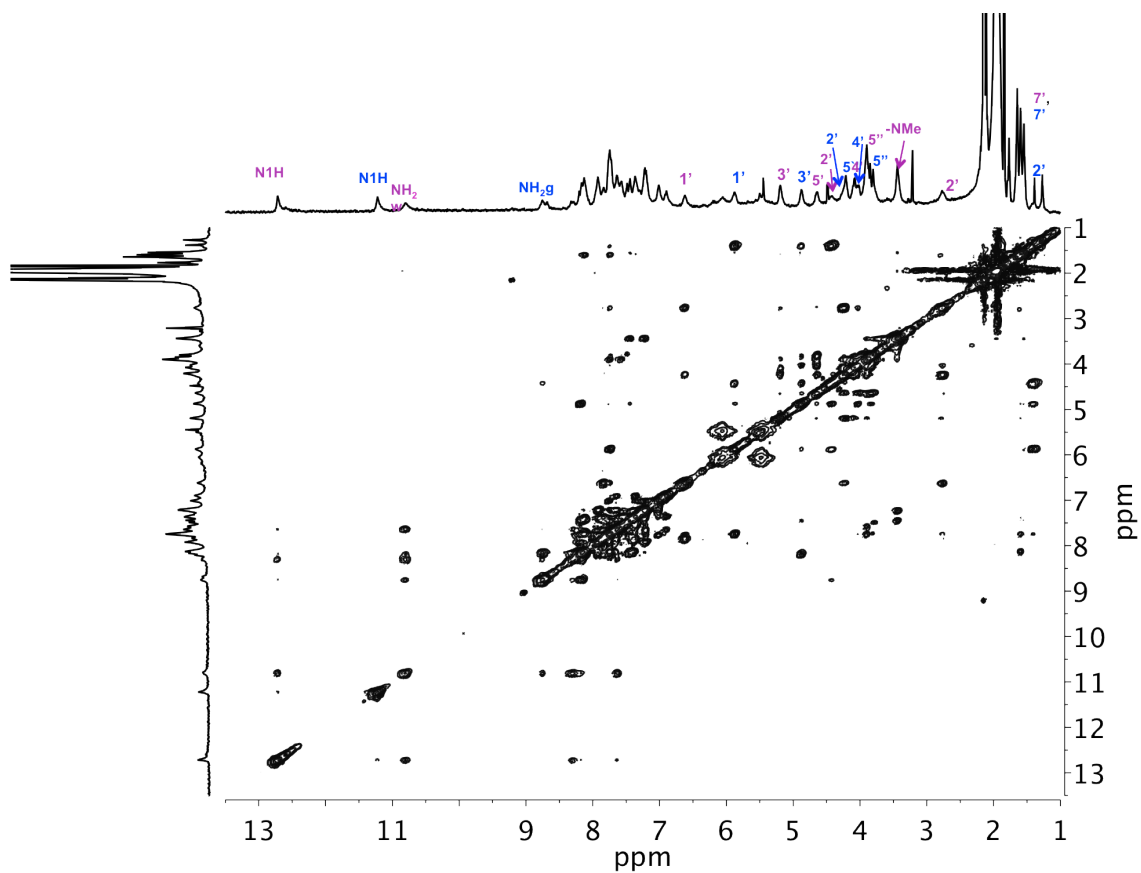


Figure S21. Full 2D NOESY spectrum (400 MHz, CD₃CN, 0.5 equiv KSCN) for **2**₁₆ (5 mM in **2**). Labels in magenta for the outer tetrads and in blue for the inner tetrads.

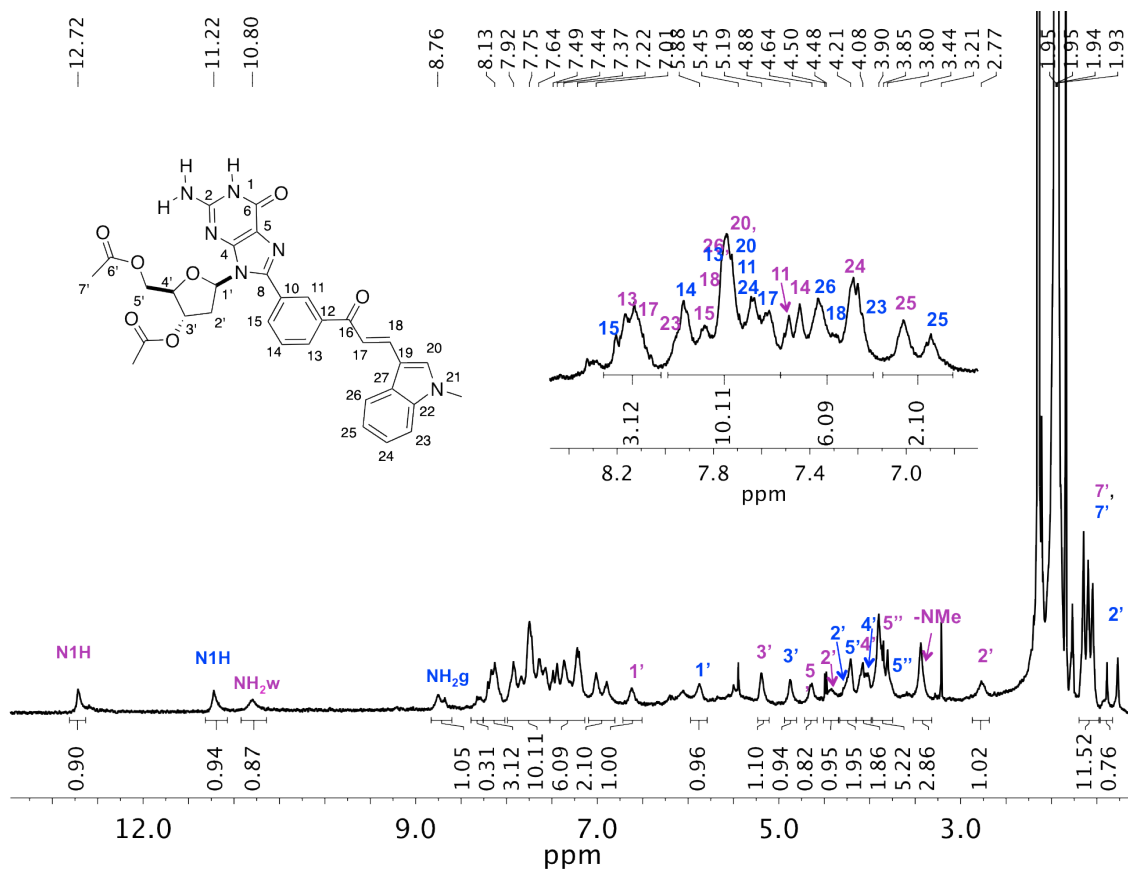


Figure S22. ^1H NMR (400 MHz, CD_3CN , 0.5 equiv KSCN) for **2**₁₆ (5 mM in **2**). Labels in magenta for the outer tetrads and in blue for the inner tetrads. Signals assigned based on integrations, 2D NOESY spectrum, proton coupling and our own experience. The 6-9 ppm region of the spectrum allows different interpretations.

5. Self-Assembly studies: ESI-Mass spectroscopy

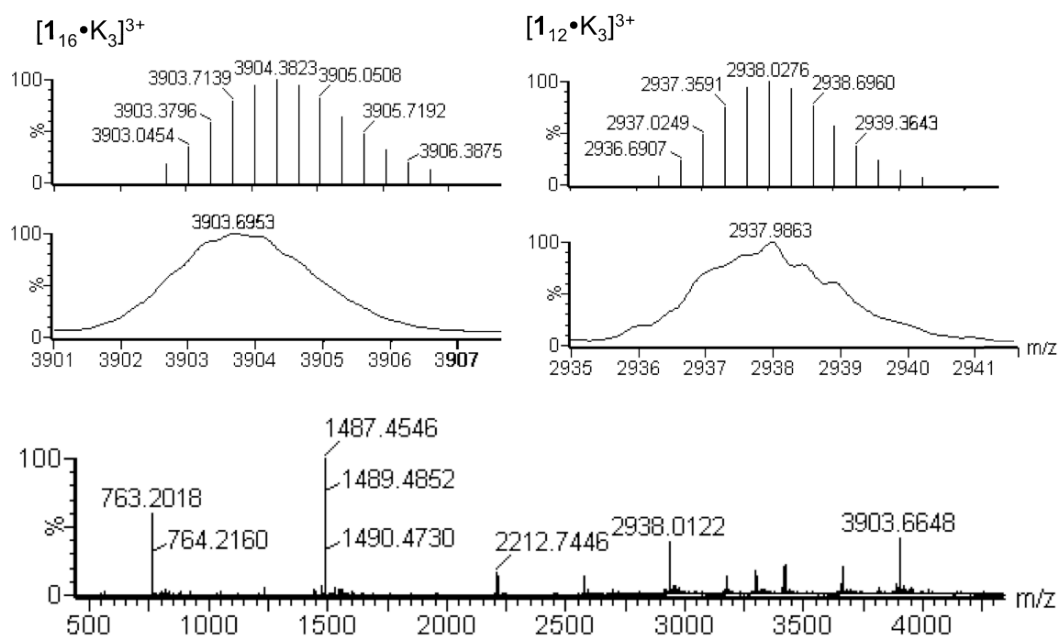


Figure S23. ESI-Mass spectrum (5 mM; CH₃CN, 0.5 equiv KSCN) of **1**₁₆. Top: Calculated isotopic patterns and experimental spectra for each of the mayor peaks in the spectrum. Bottom: Full mass spectrum for **1**₁₆.

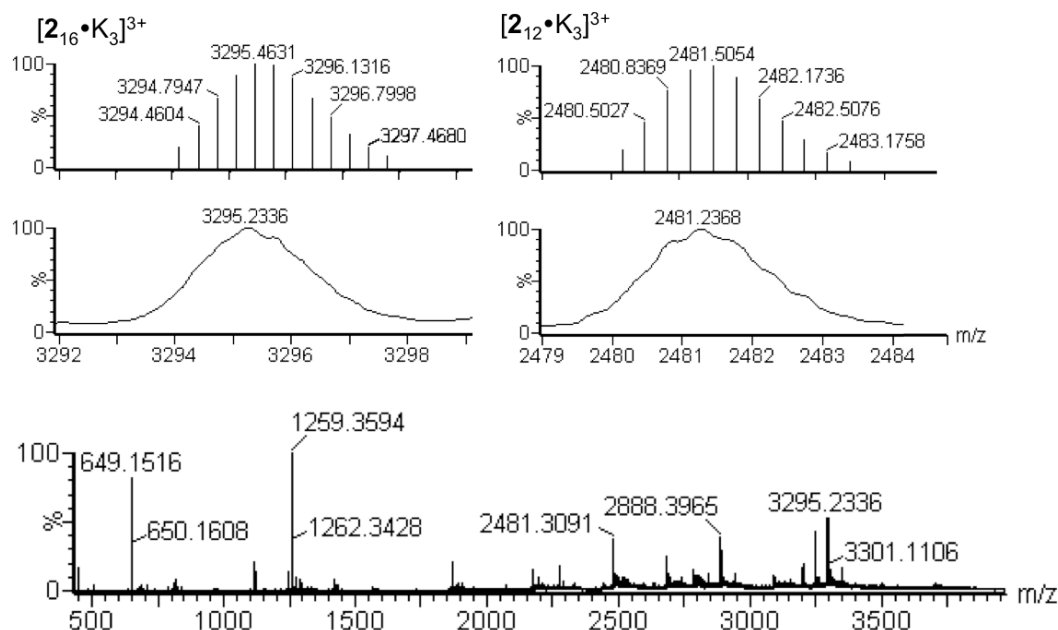


Figure S24. ESI-Mass spectrum (5 mM; CH₃CN, 0.5 equiv KSCN) of **2**₁₆. Top: Calculated isotopic patterns and experimental spectra for each of the mayor peaks in the spectrum. Bottom: Full mass spectrum for **2**₁₆.

6. Self-Assembly studies: Fluorescence spectroscopy

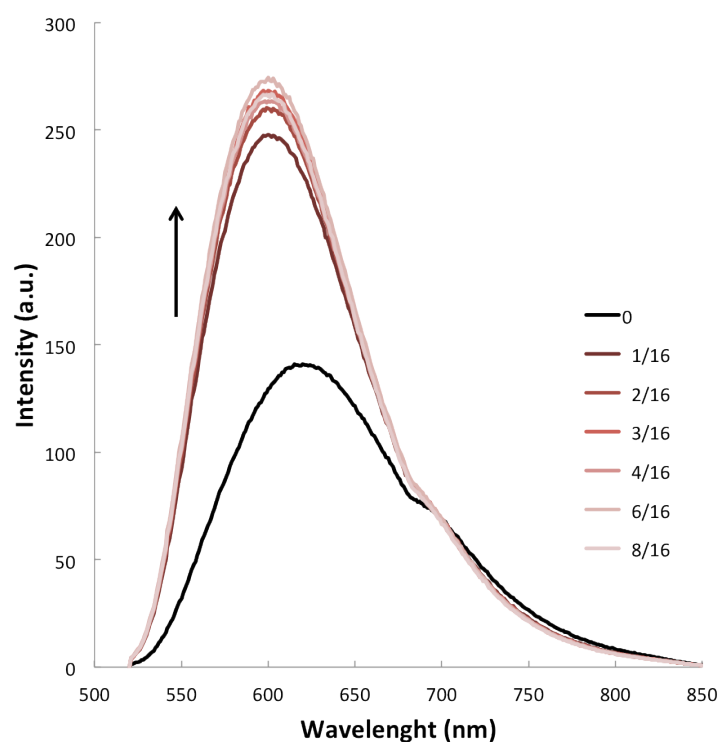


Figure S25. Emission spectra of **1** (ex. wavelength 500 nm, em/ex slit 5, 600V, CH₃CN, 5 mM) with increasing amounts of KSCN.

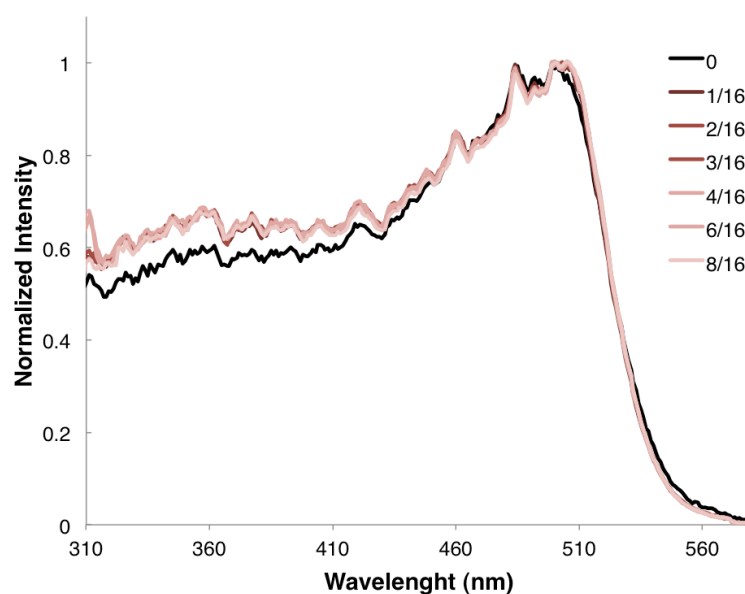


Figure S26. Excitation spectra of **1** (em. wavelength 620 nm, em/ex slit 5, 600V, CH₃CN, 5 mM) with increasing amounts of KSCN. At these concentrations (5mM), the derivatives tend to aggregate, resulting in a spectrum which is slightly different from the absorption spectrum measured at 2.2 μ M shown in Figure S12.

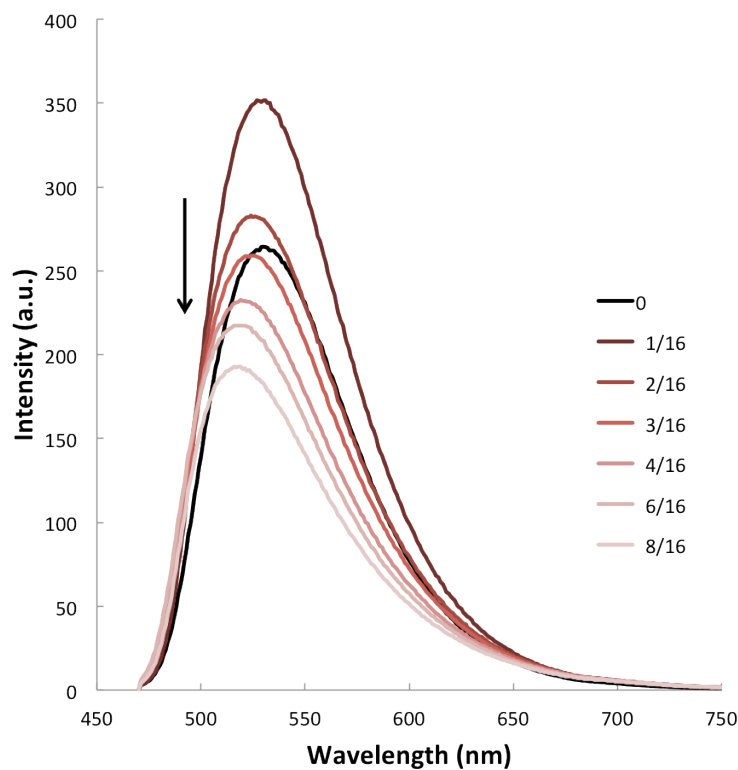


Figure S27. Emission spectra of **2** (ex. wavelength 450 nm, em/ex slit 5, 600V, CH₃CN, 5 mM) with increasing amounts of KSCN.

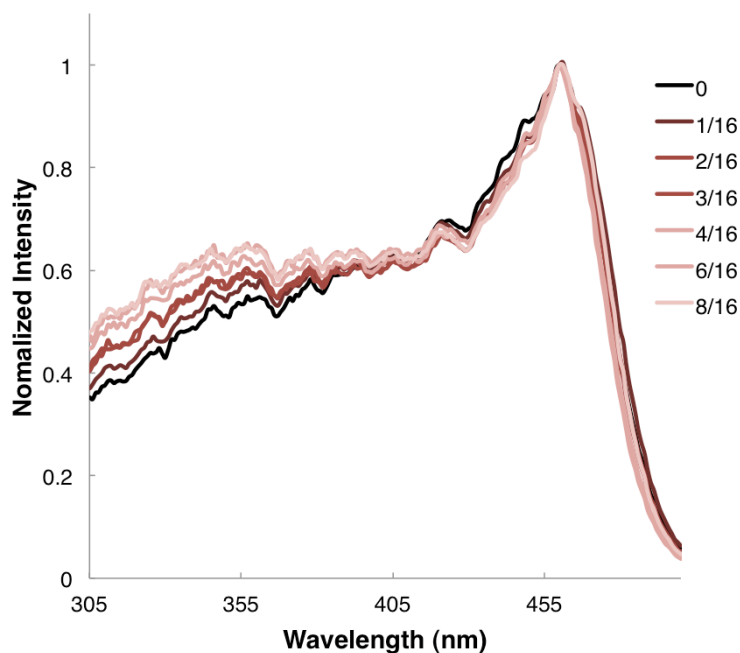


Figure S28. Excitation spectra of **2** (em. wavelength 520 nm, em/ex slit 5, 600V, CH₃CN, 5 mM) with increasing amounts of KSCN. At this concentration (5mM), the derivatives tend to aggregate, resulting in a spectrum which is slightly different from the absorption spectrum measured at 6.4 μ M shown in Figure S13.