

## Rational Design of an Acridine-derived Click Chemistry-based Artificial Metallo-Nuclease

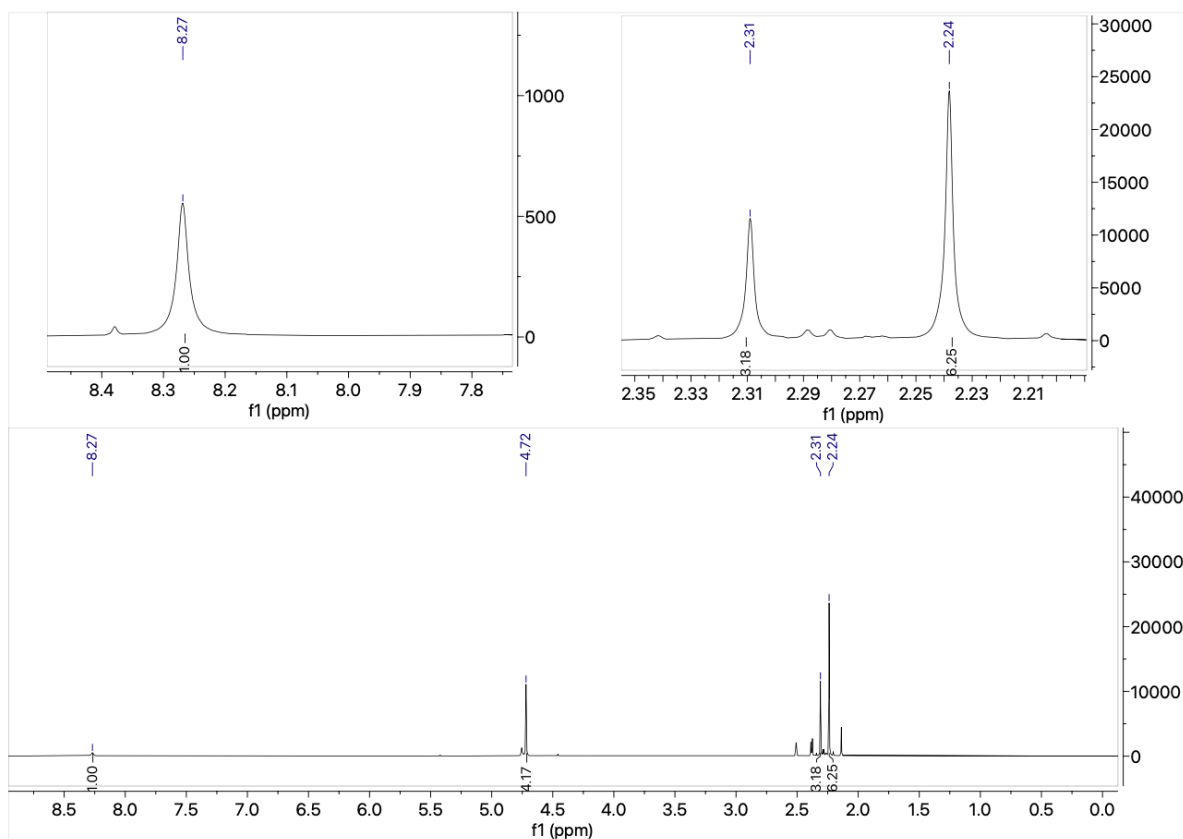
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### Supplementary Figures and Data



**Figure S1:** <sup>1</sup>H NMR spectrum of dibromide (1) in DMSO-d<sub>6</sub>

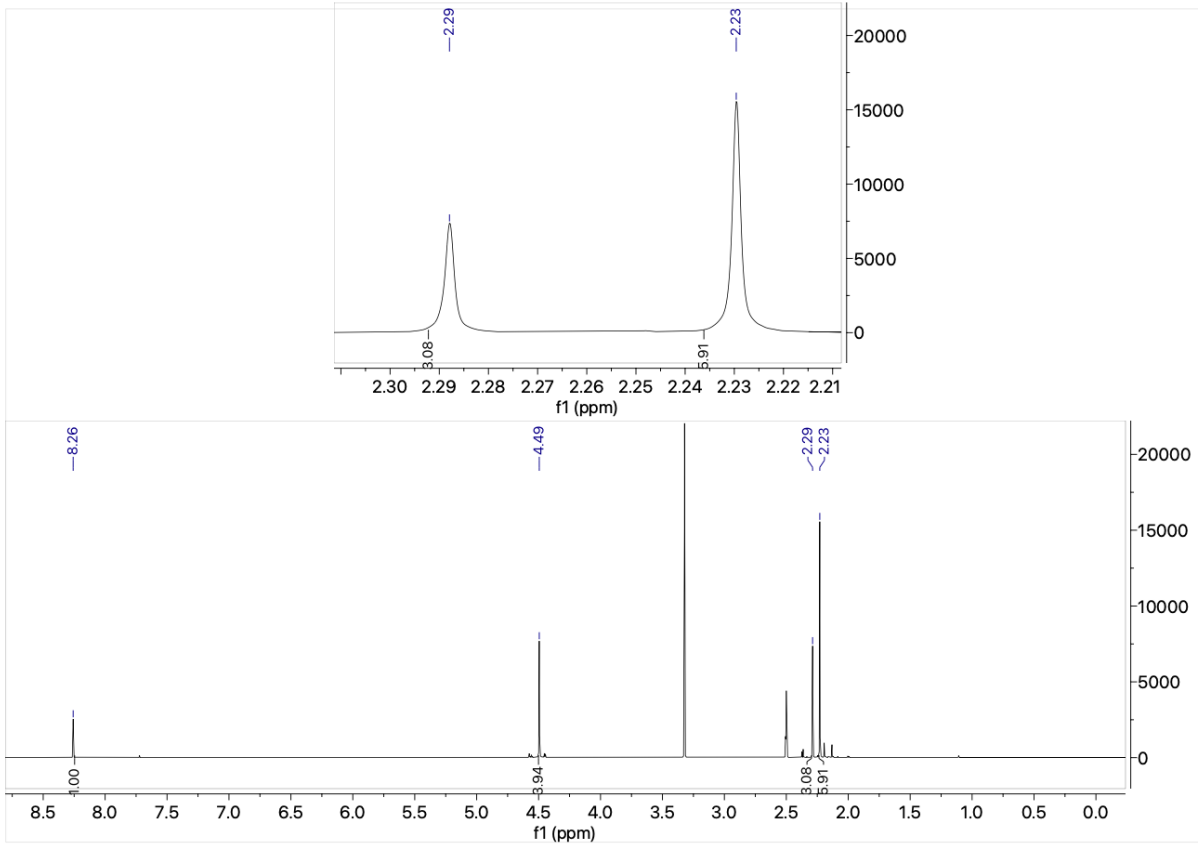


Figure S2: <sup>1</sup>H NMR spectrum of diazide (2) in DMSO-d<sub>6</sub>

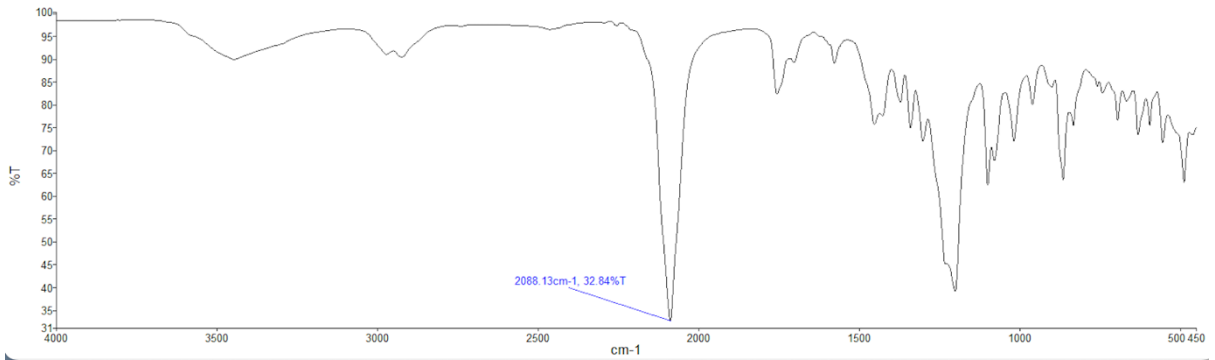


Figure S3: FTIR spectrum of 2 showing azide peak at 2088 cm<sup>-1</sup>

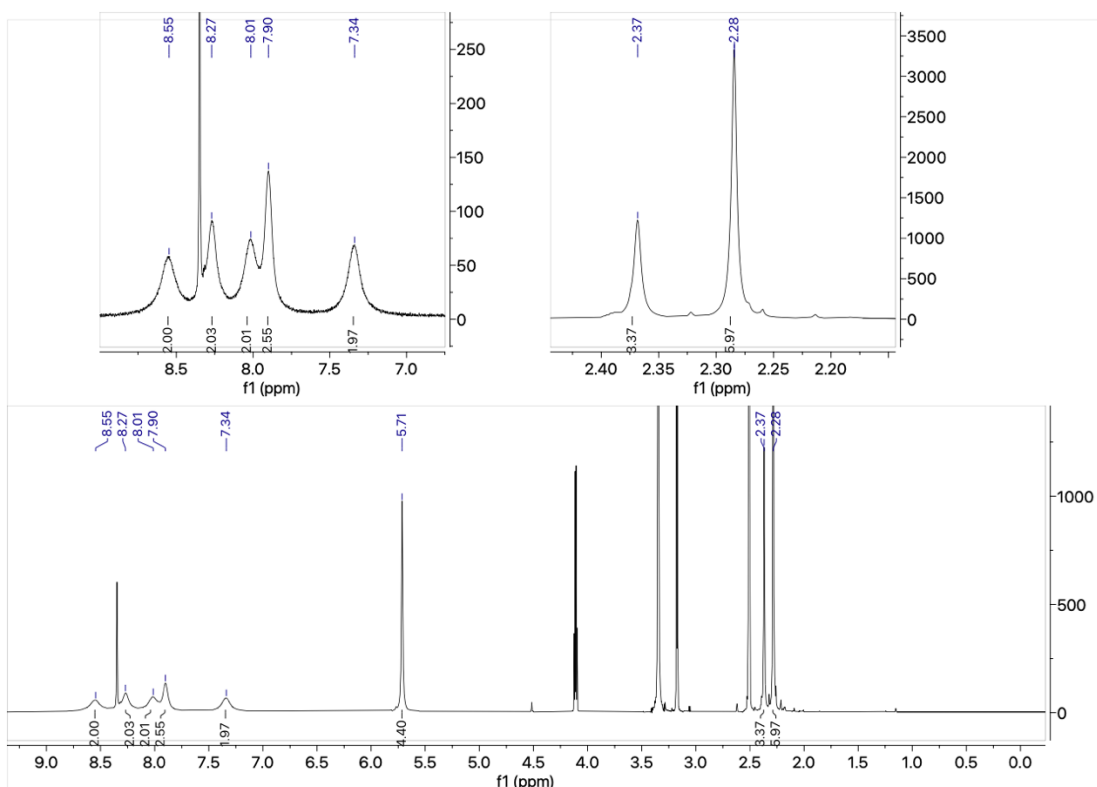


Figure S4:  $^1\text{H}$  NMR spectrum of DC-Py (**3**) in  $\text{DMSO-d}_6$

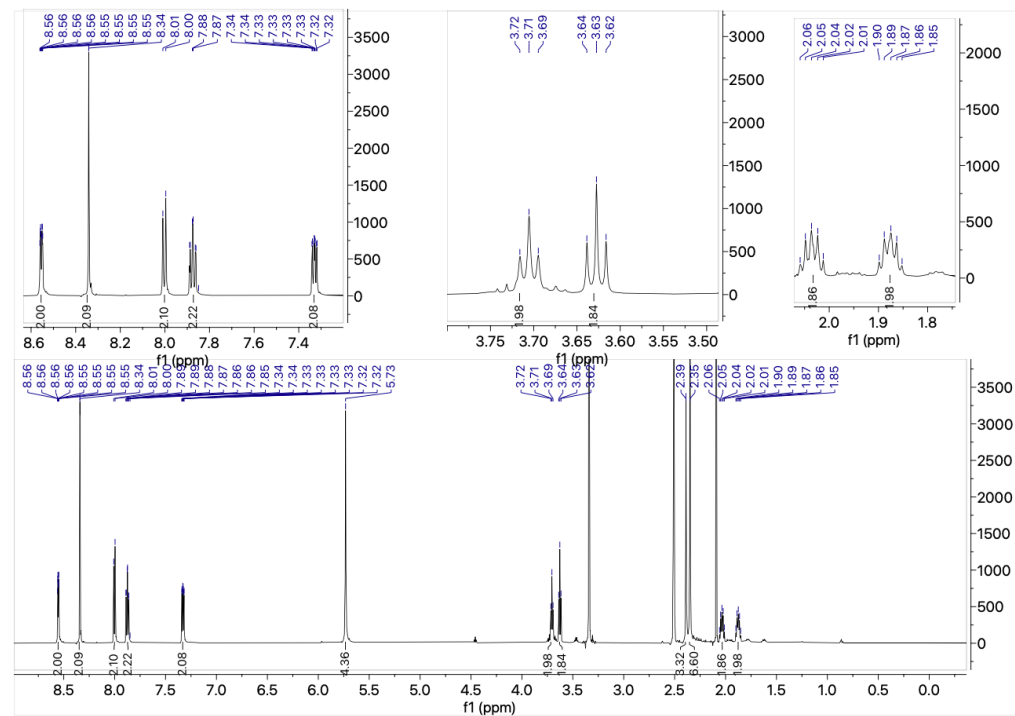
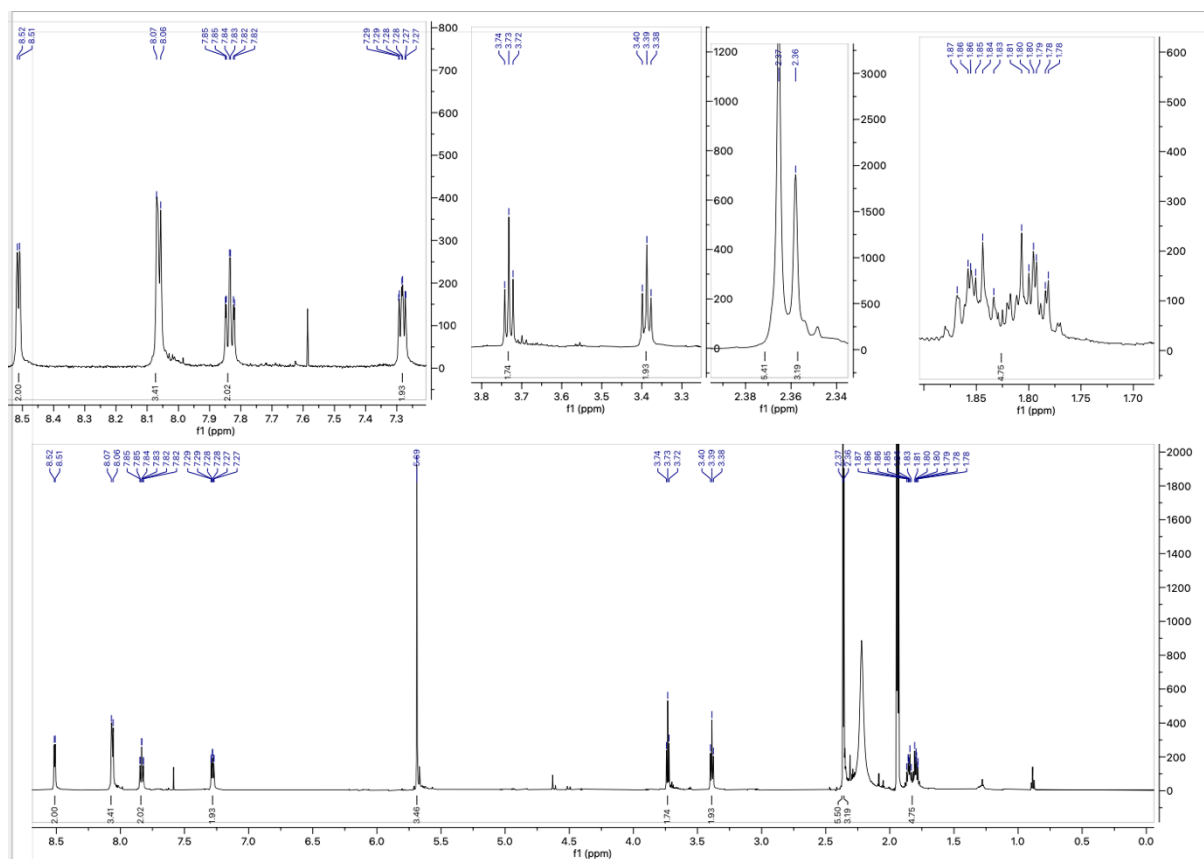


Figure S5:  $^1\text{H}$  NMR spectrum of DC-PyBr (**4**) in  $\text{DMSO-d}_6$



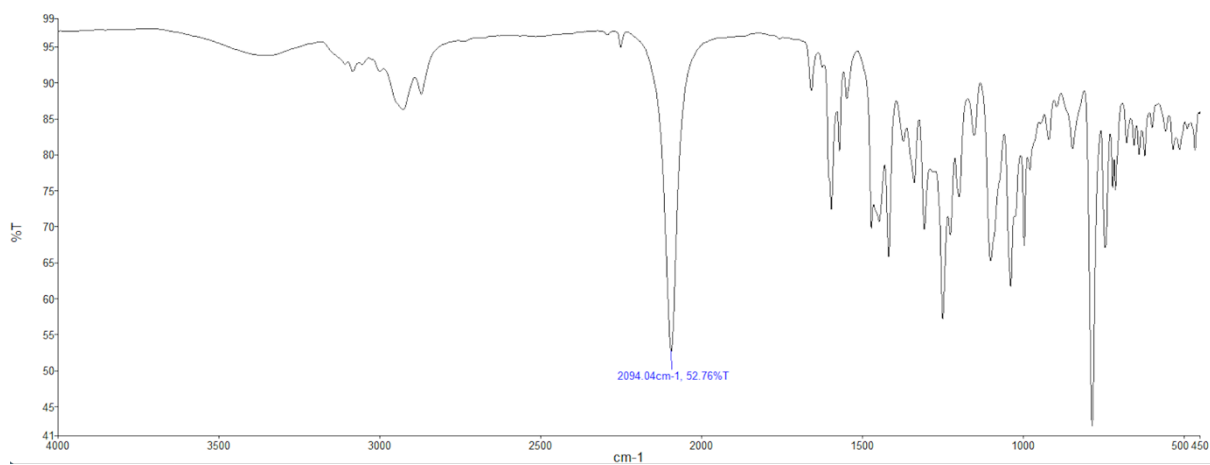


Figure S8: FTIR spectrum of 5, showing azide signal at 2094 cm<sup>-1</sup>.

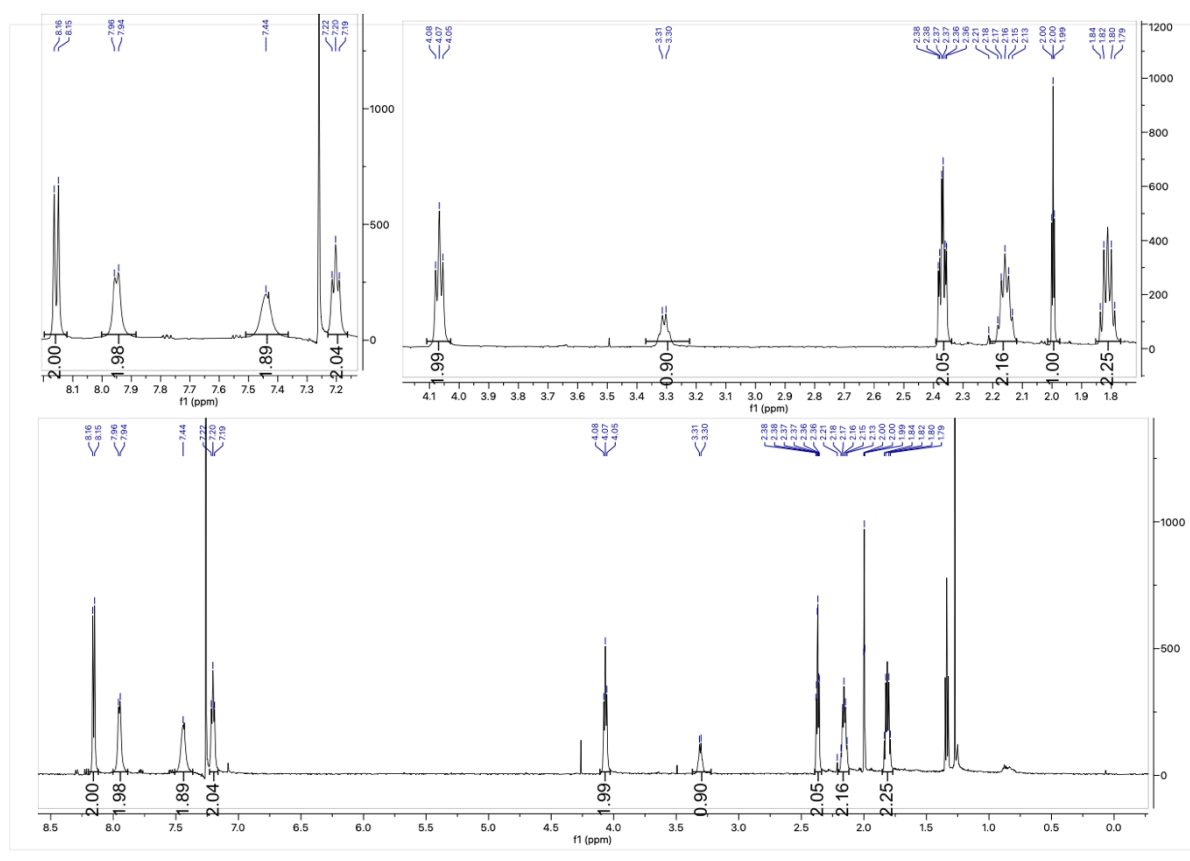


Figure S9: <sup>1</sup>H NMR spectrum of Hexynyl acridine (6) in CDCl<sub>3</sub>.



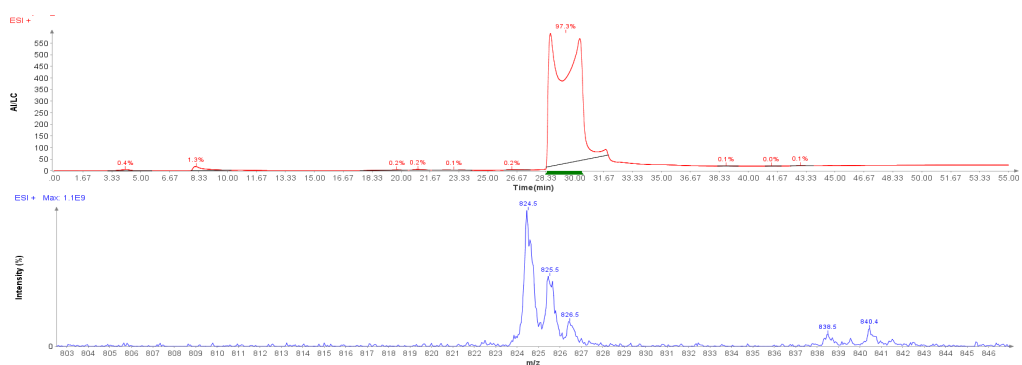


Figure S12: LC-MS and ESI-MS of DC-PyA (7).

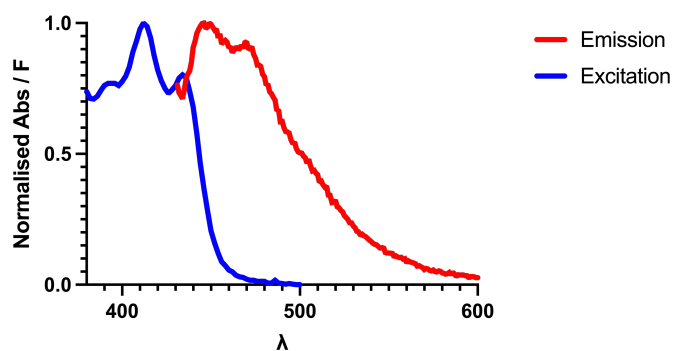


Figure S13: Excitation and emission scans of  $\text{Cu}_2\text{-DC-PyA}$  in 80mM HEPES, 25mM NaCl.

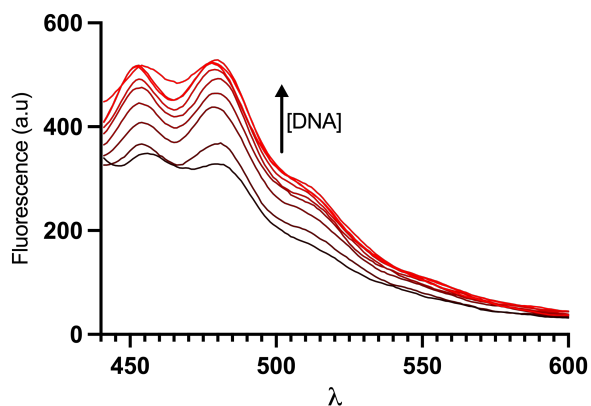
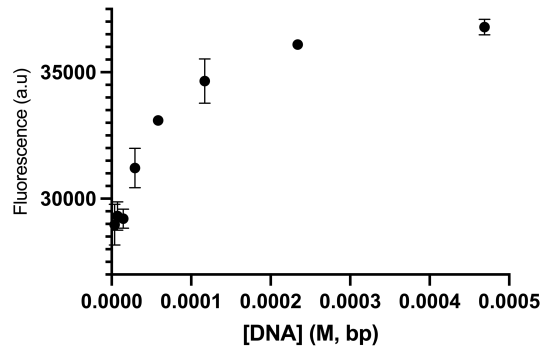
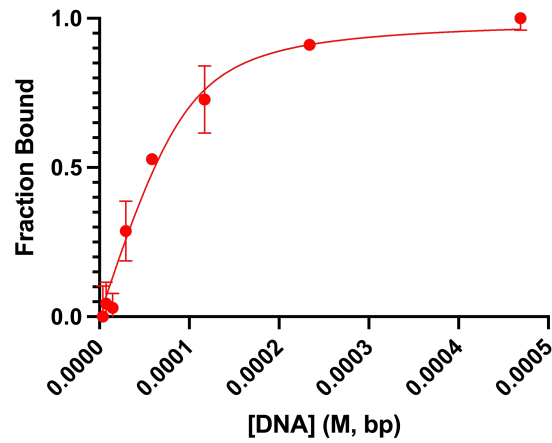


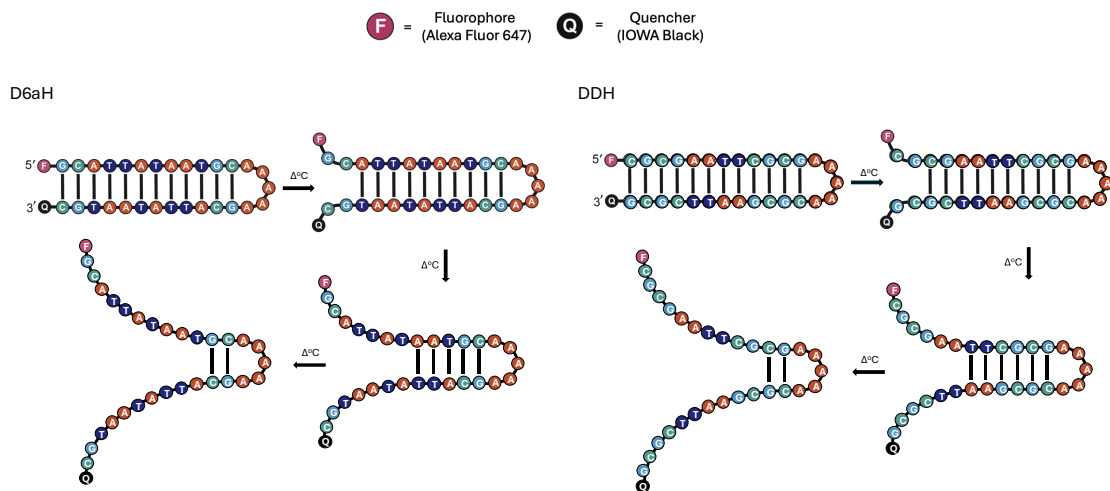
Figure S14: Fluorescence scans with a fixed ( $10\ \mu\text{M}$ ) concentration of  $\text{Cu}_2\text{-DC-PyA}$  and increasing ctDNA concentrations of ctDNA (excitation 415 nm).



**Figure S15:** Raw fluorescence values of 10  $\mu\text{M}$   $\text{Cu}_2\text{-DC-PyA}$  with increasing ctDNA concentrations (excitation 415 nm, emission 455 nm).



**Figure S16:** Binding curve and Bard fit of fraction bound of  $\text{Cu}_2\text{-DC-PyA}$  bound vs ctDNA concentration. Bard model was fit using equations S1 and S2.



**Figure S17:** Cartoon representation of FRET melting using DNA hairpins DDH and D6aH.

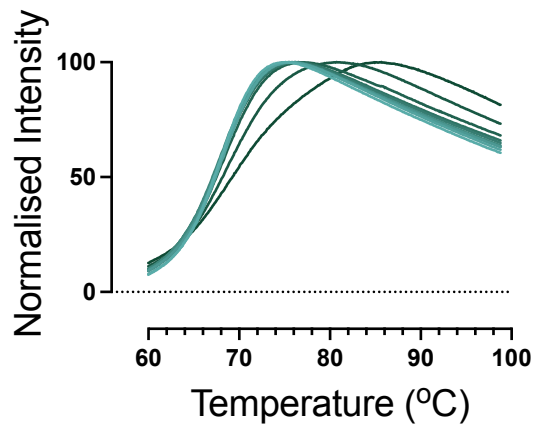


Figure S18: FRET melting curve of D6aH with Cu<sub>2</sub>-DC-PyA.

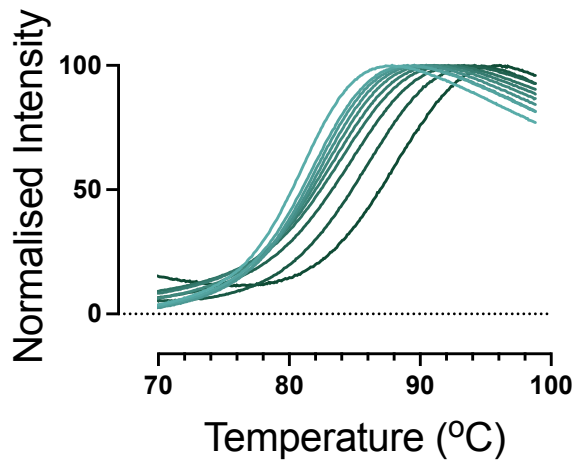


Figure S19: FRET melting curves of DDH with Cu<sub>2</sub>-DC-PyA.

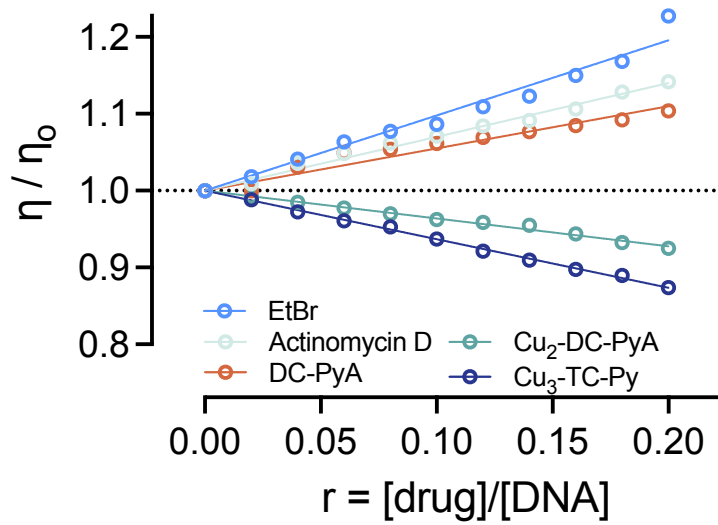
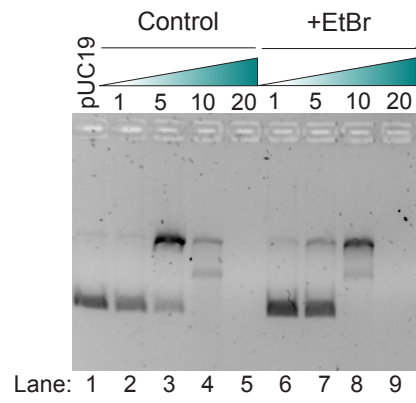


Figure S20: Plot of relative viscosity versus drug loading ratio for control and test compounds.



**Figure S21.** pUC19 cleavage analysis via EMSA, comparing a control concentration ramp containing 1, 5, 10 and 20  $\mu\text{M}$  Cu<sub>2</sub>-DC-PyA (lanes 2-5) to the same experiment in the presence of 8  $\mu\text{M}$  EtBr.