Electronic Supplementary Material (ESI) for Molecular BioSystems.


# Supplementary Information

## Diminazene or berenil, a classic duplex minor groove binder, binds to G-quadruplexes with low nanomolar dissociation cosntants and the amidine groups are also critical for G-quadruplex binding.

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**Displacement assays with the G-quadruplex binder, NMM**

In these assays, the triazene ligand was used at various concentrations: 0, 5, 10, 20, 50, 100, and 200 μM; and the DNA concentration was kept at 10 μM, whereas the concentration of NMM was 1 μM. The experiments were performed in 50 mM Tris-HCl buffer (pH 7.5) in the presence of 50 mM KCl. The excitation wavelength used for NMM was 400 nm and the emission was monitored between 550 and 700 nm (slit width = 5 nm, scan speed = 600 nm/min, averaging time = 0.1 sec, data interval = 1 nm, PMT detector voltage = 600 V, measurement temperature = 20 °C). The sample was initially heated to 95 °C and kept at this temperature for 5 min without ligand or NMM and then cooled down to room temperature for 15 min. Subsequently, the triazene ligand was added to the DNA and incubated for ~12 h.

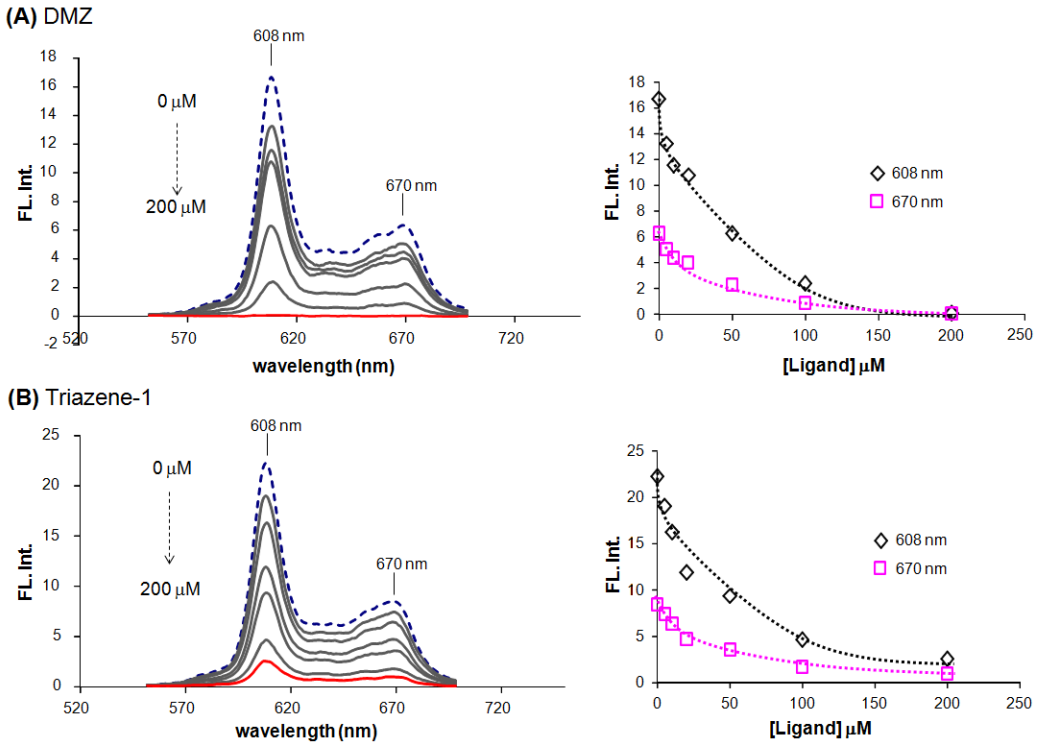



**Figure S1**. Displacement of G4 ligand, NMM (A and B), by triazine ligands. (Left) Displacement of NMM (1 μM) upon titration of triazene ligands (A) DMZ, (B) Triazene-1 on *c-kit1* (10 μM). The concentration of ligand is 0, 5, 10, 20, 50, 100, 200 μM. In the figure, 200 μM ligand concentration is emphasized with red line. Buffer = 50 mM Tris-HCl (pH 7.5), [KCl] = 50 mM. Fluorescence was measured with excitation

wavelength = 400 nm for NMM, emission wavelength = 550-700 nm. (Right) Fluorescence emission intensity at 608 nm (◇) and 670 nm (□) against ligand (A) DMZ, (B) Triazene-1concentrations. The concentrations of ligand are 0, 5, 10, 20, 50, 100, 200 µM.

## UV spectroscopy studies of DMZ binding to G quadruplexes and duplex DNA

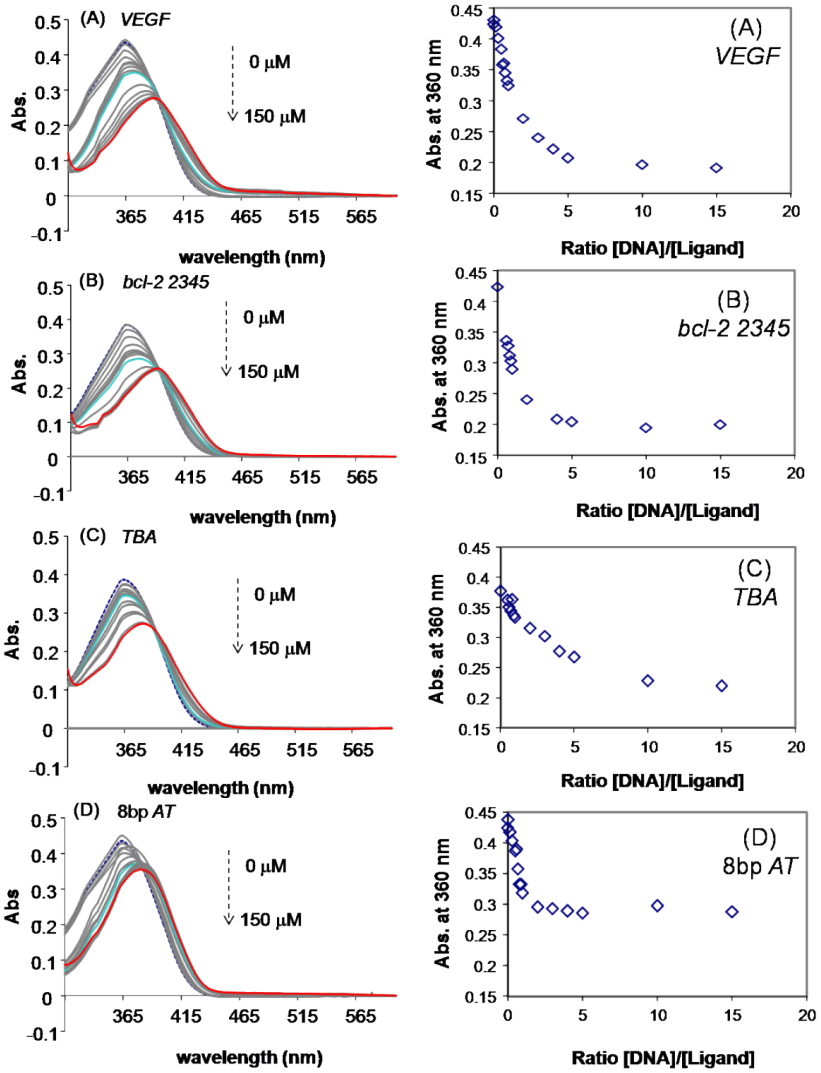



**Figure S2**. UV-titration studies showing binding of DMZ with DNA. (Left) Absorption sprectra of DMZ (10 µM) upon titration with (A) *VEGF*, (B) *bcl-2 2345*, (C) *TBA*, (D) 8bp *AT*. The concentrations of DNA are 0, 0.25, 1.5, 3, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 150 µM. In the graph, 10 µM and 150 µM DNA concentrations are specifically emphasized as cyan and red line respectively. [KCl] = 250 mM, Buffer = 50 mM Tris-HCl (pH 7.5). UV was measured at 20 °C. (Right) Plot of absorbance at 360 nm against concentration ratio of DNA and Ligand (DMZ). Ligand concentration is 10 µM, DNA = (A) *VEGF*, (B)

*bcl-2 2345*, (C) *TBA* and (D) 8bp *AT*. The concentrations of DNA are 0, 0.25, 1.5, 3, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 150 μM.

## UV spectroscopy studies of Triazene-1 binding to G quadruplexes and duplex DNA

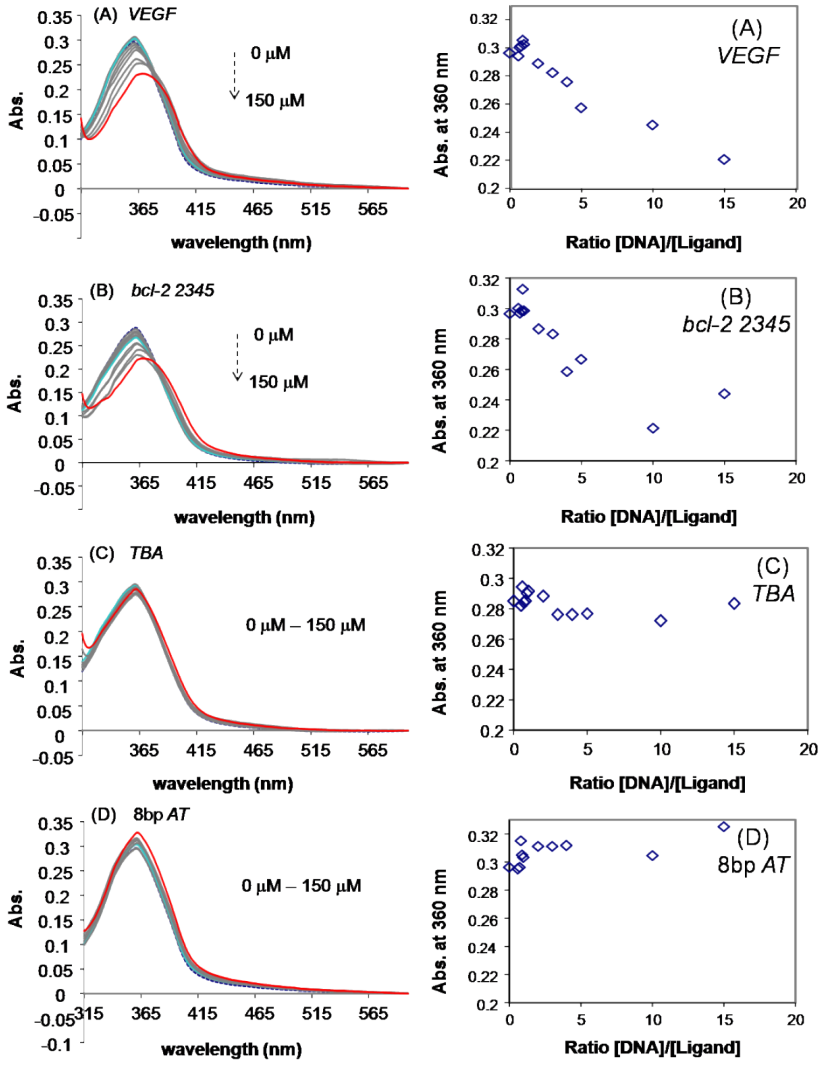



**Figure S3**. UV-titration studies of binding of Triazine-1 with DNA. (Left) Absorption spectra of Triazene-1 (10 μM) upon titration with (A) *VEGF*, (B) *bcl-2 2345*, (C) *TBA*, (D) 8bp *AT*. The concentrations of DNA are 0, 0.25, 1.5, 3, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 150 μM. In the graph, 10 μM and 150 μM DNA concentration is specifically emphasized as cyan and red line. [KCl] = 250 mM, Buffer = 50 mM Tris-HCl (pH 7.5). UV was measured at 20 °C. (Right) Plot of absorbance at 360 nm against concentration ratio of DNA and Ligand (Triazene-1). Ligand concentration is 10 μM, DNA = (A) *VEGF*, (B) *bcl-2 2345*, (C) *TBA*, (D) 8bp *AT*. The concentration of DNA is 0, 0.25, 1.5, 3, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 150 μM.

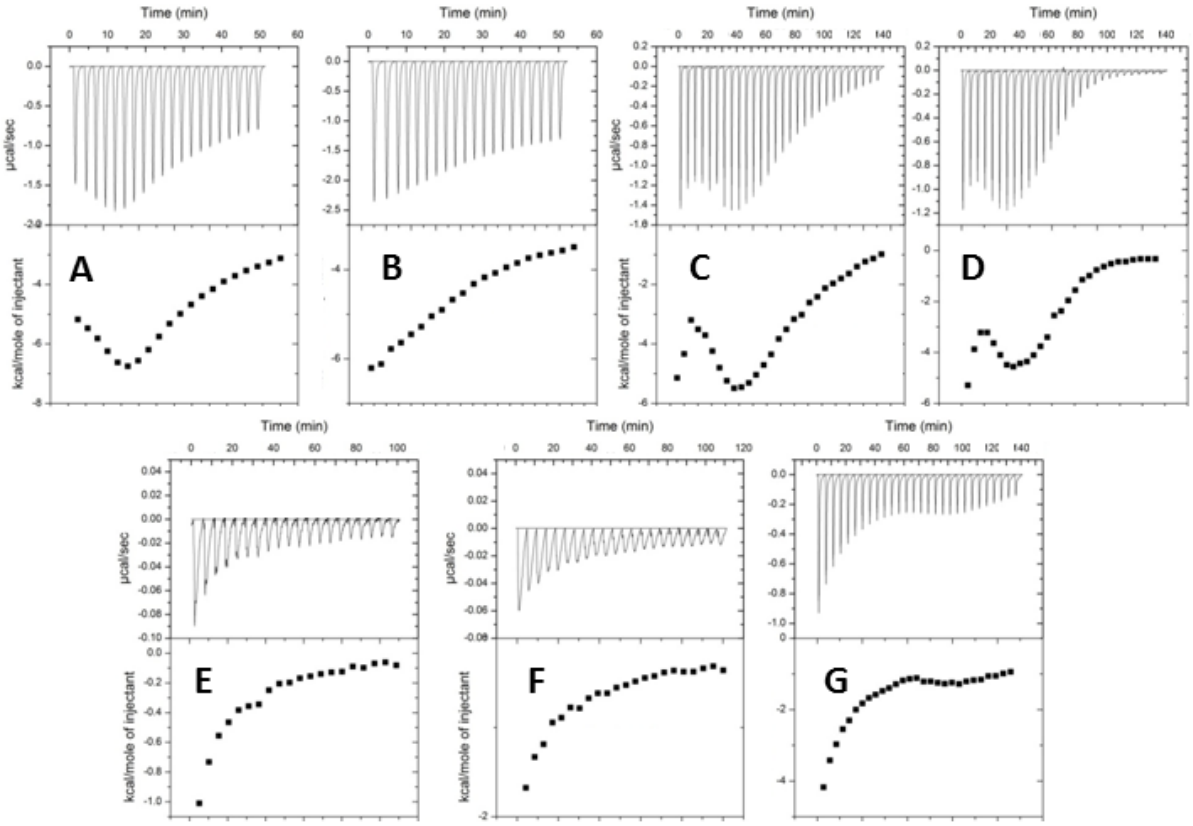



**Figure S4**: Heat data obtained from ITC titration experiments for the interactions between DMZ and **A**) 7bp *HP*•*AT*, **B**) 7 bp *HP*, **C**) 27mer *bcl-2*, **D**) 24mer *c-myc*, **E**) Tris buffer, **F**) 22mer *hTel* $Na^+$, and **G**) 22mer *hTel* $K^+$.

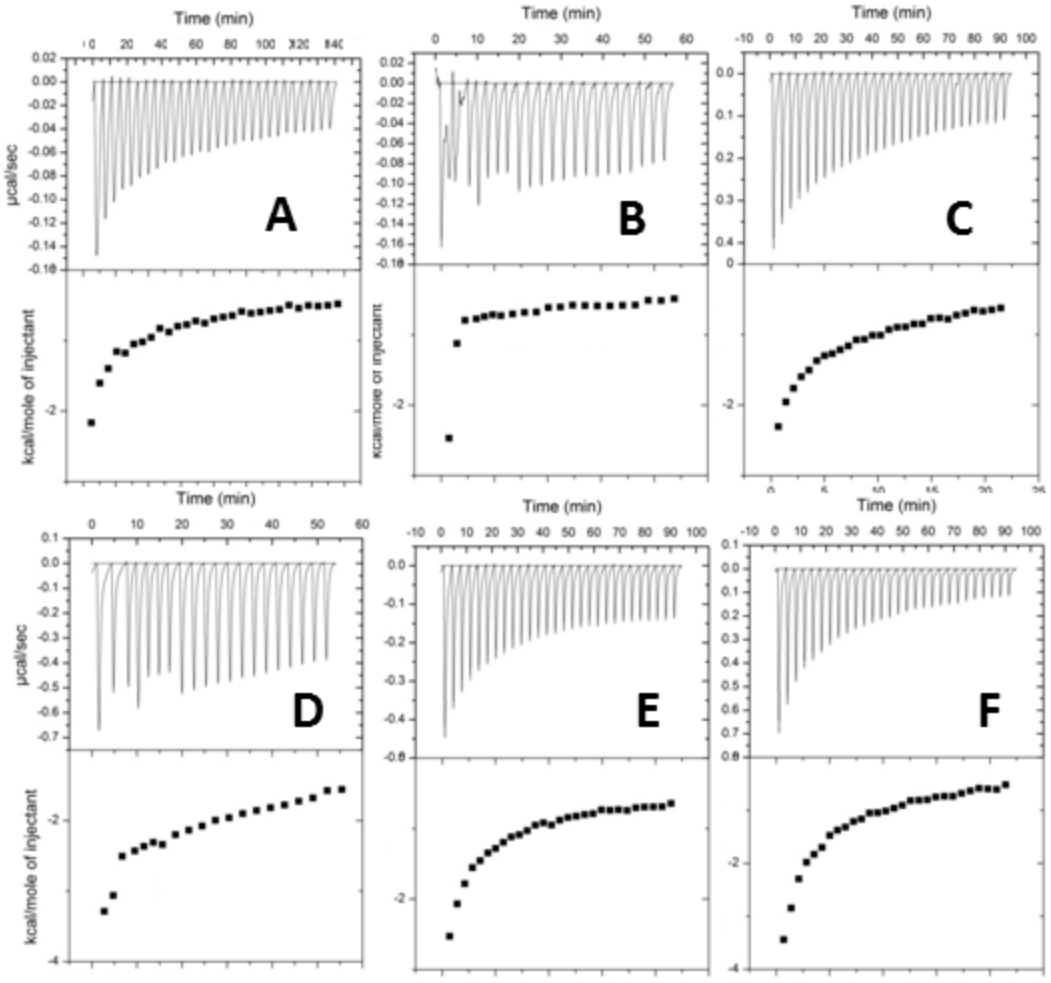



**Figure S5**: Heat data obtained from ITC titration experiments for the interactions between Triazene-1 and **A)** with Tris buffer, **B**) 22mer *hTel* $Na^+$ , **C**) 22mer *hTel* $K^+$, **D**) 7bp *HP*•*AT*, **E**) 27mer *bcl-2*, and **F**) 24mer *c-myc.*

**CD experiments for binding of ligands with duplex DNA (AT-rich)**

CD titration experiments, monitoring in the wavelength region corresponding to the bound ligand, were performed with different concentrations of 8bp *AT* (0-50 μM) added to a fixed concentration of either DMZ or Triazene-1 (40 μM). No induced CD was observed for Triazene-1 but a large positive induced CD was observed upon incubating 8bp *AT* (0-50 μM) with DMZ (compare Figures S1A and S1B). The resulting large positive signal in the CD spectrum of DMZ, upon addition of AT-rich duplex DNA, suggests a groove-binding mode of DMZ with duplex DNA,

consistent with literature (1). The lack of induced CD of Triazene-1 suggests that Triazene-1 does not bind to the minor groove of AT-rich DNA.

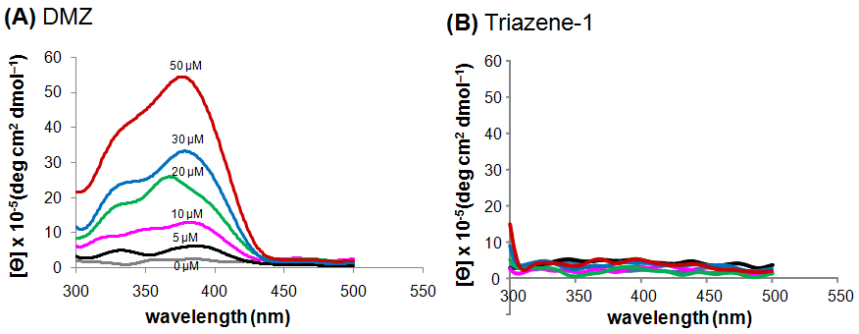
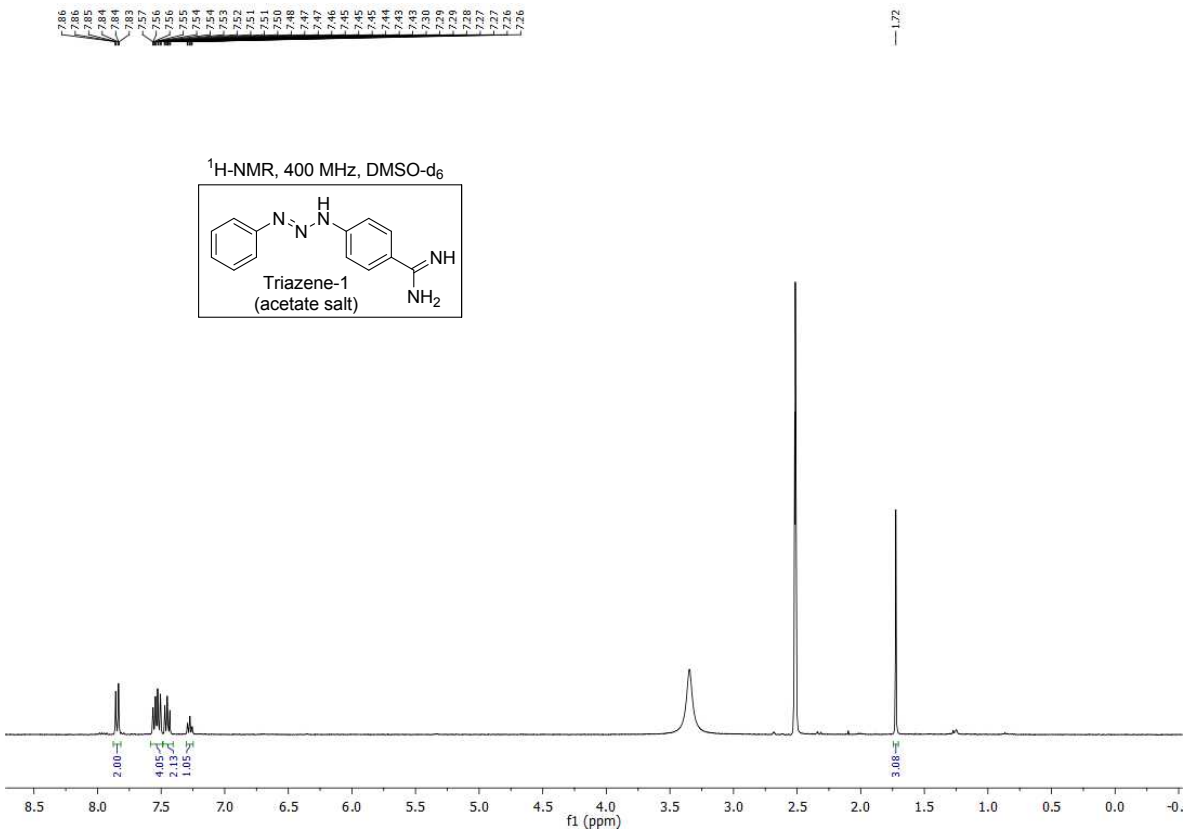
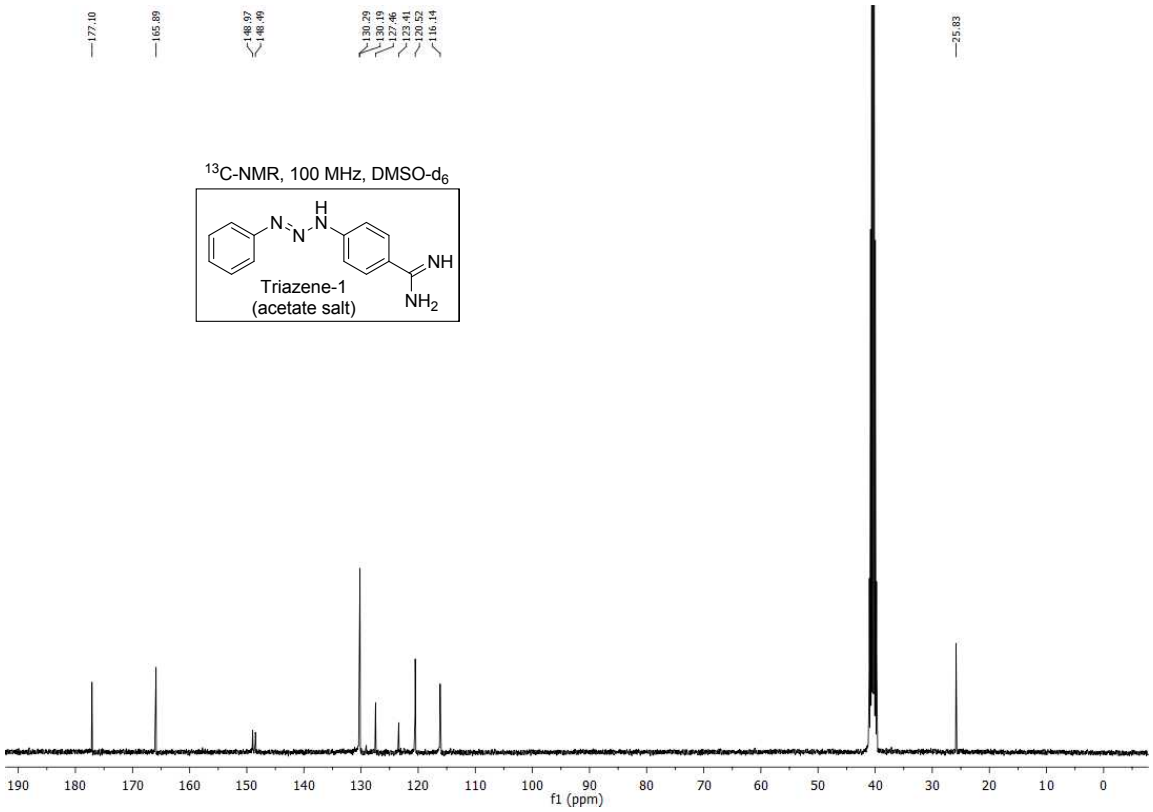



**Figure S6.** CD titration studies. Increasing concentrations of duplex DNA were added to (A) DMZ (40 μM), (B) Triazene-1 (40 μM). The concentrations of added duplex DNA were 0, 5, 10, 15, 20, 25, 30, 50 μM. Buffer = 50 mM Tris-HCl (pH 7.5), [KCl] = 250 mM. CD measurement was done at 20 °C.



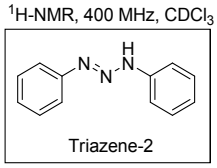



$^{13}$C-NMR, 100 MHz, DMSO-$d_6$

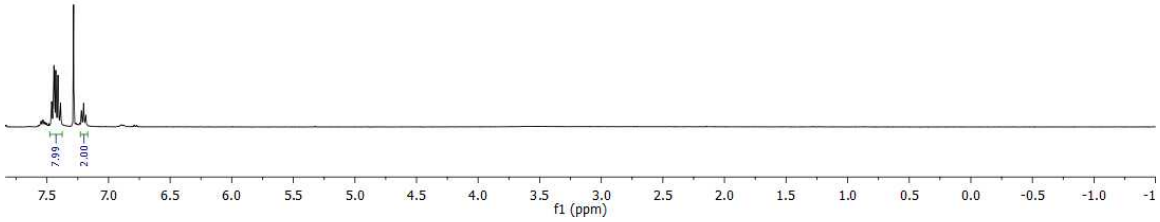


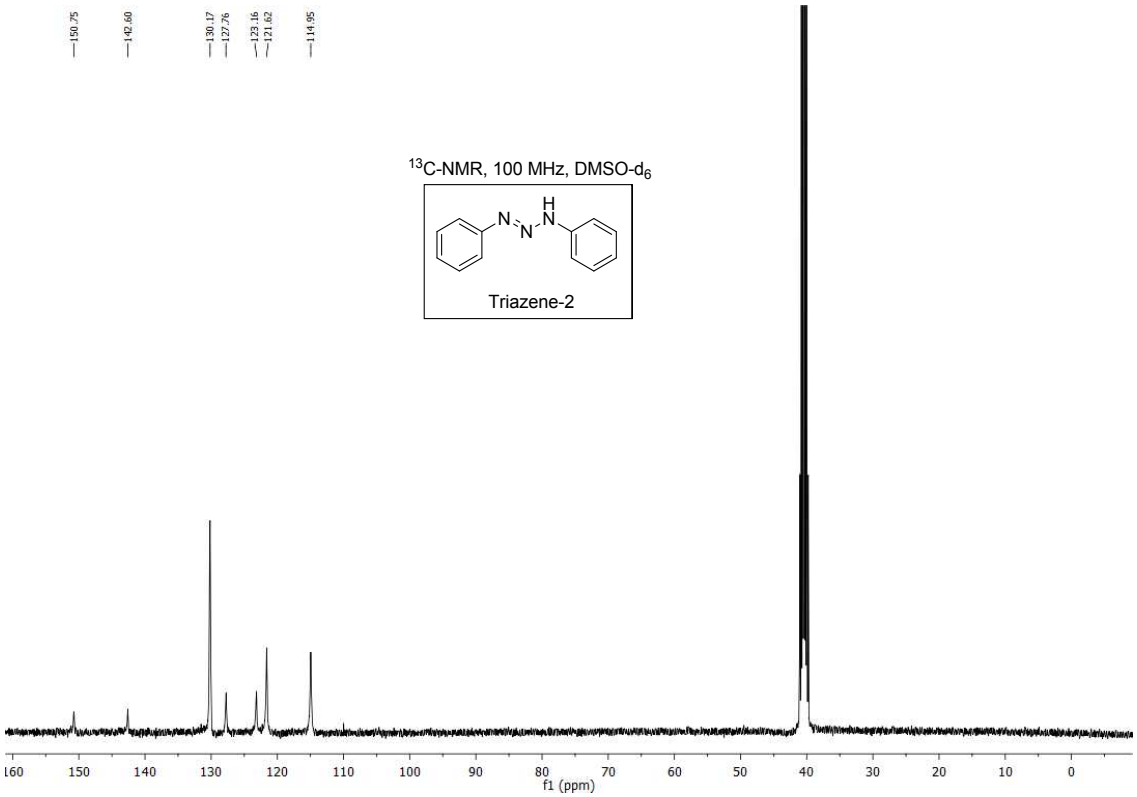

Triazene-1
(acetate salt)


$^{1}$H-NMR, 400 MHz, $CDCl_3$




Triazene-2






1. Yang, Q., Xiang, J., Yang, S., Li, Q., Zhou, Q., Guan, A., Zhang, X., Zhang, H., Tang, Y. and Xu, G. (2010) Verification of specific G-quadruplex structure by using a novel cyanine dye supramolecular assembly: II. The binding characterization with specific intramolecular G-quadruplex and the recognizing mechanism. *Nucleic Acids Res.*, **38**, 1022-1033.