Supporting Information

A Tetraphenylethene Derivative That Discriminates Parallel G-Quadruplexes.

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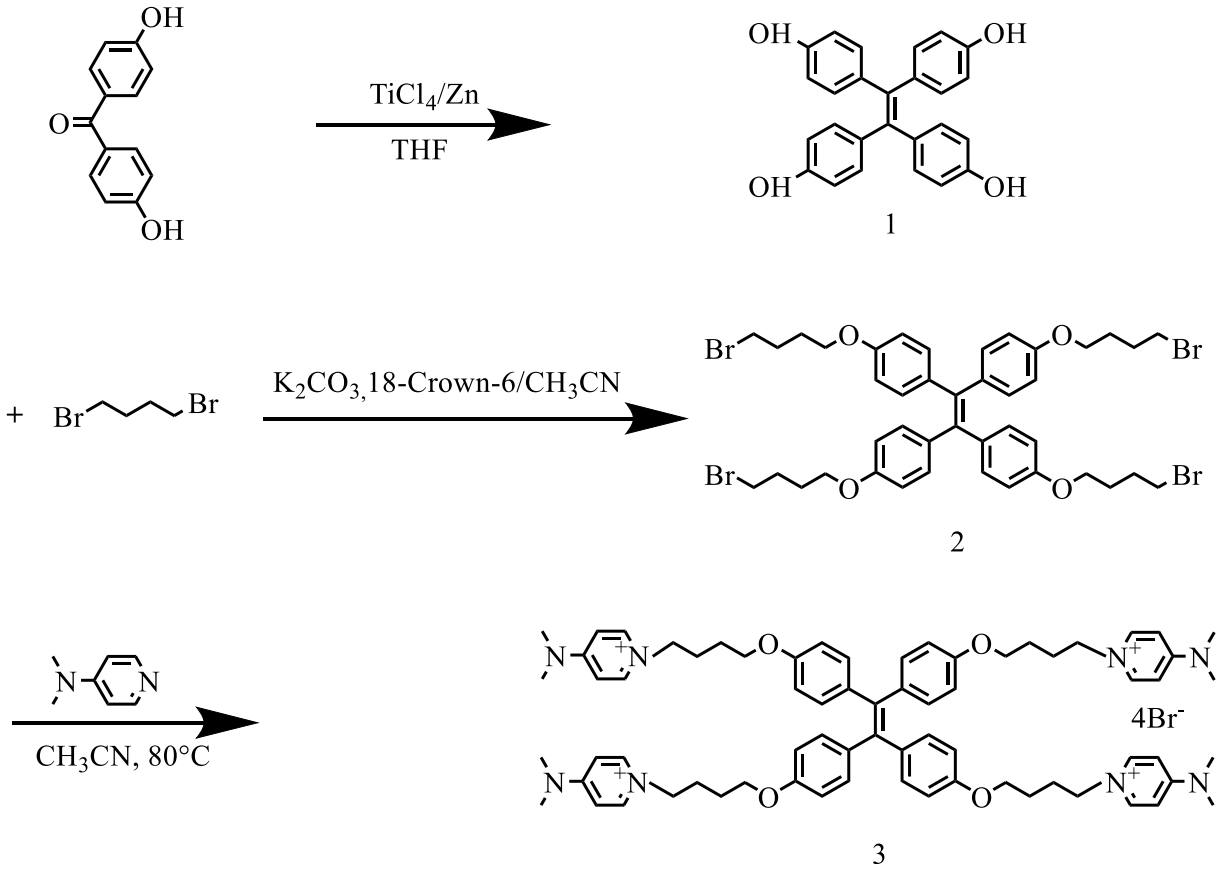
General.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL JNM-ECZ400s magnetic resonance spectrometer. DMSO-d_6 and D_2O were used as the solvents. ^{1}H spectra chemical shifts (δ) are reported in parts per million (ppm) referenced to residual protonated solvent peak (DMSO-d_6, δ = 2.50, D_2O, δ = 4.67). Coupling constants (*J*) values are given in hertz (Hz). Signal patterns are indicated as s (singlet), d (doublet), t (triplet), m (multiplet). All reagents were purchased from Aladdin. Silica gel (Wakogel® C-300, 200-325 mesh) was used for column chromatography. Mass spectra were recorded by an Agilent G6545 A UHD Accurate-Mass Q-TOF (California, USA). Fluorescence emission spectra were recorded on a VARIAN Cary Eclipse spectrofluorometer (Varian, Inc., Palo Alto, CA, USA) and VARIOSKAN LUX (Thermo Fisher Scientific, USA).

The synthetic route of TPE-B



Scheme S1. Synthesis of TPE-B.

Synthesis of compound 1. $TiCl_4$ (1.71 g, 9.0 mmol), Zinc powder (1.18 g, 18.0 mmol) and 4,4-dihydroxybenzophenone (1.61 g, 7.5 mmol) were slowly added to 350 mL of THF at 0 ℃ sequentially to prepare the reaction mixture. The mixture was refluxed overnight under N_2 atmosphere. After filtration and solvent evaporation, the product was purified by silica gel column (PE/EA). **Compound 1** is a light brown-red solid with a yield of 89 % (2.14 g). ^{1}H NMR (400 MHz, DMSO), δ (TMS, ppm): 9.23 (s, 4H), 6.65(d, 8H) and 6.43(d, 8H).

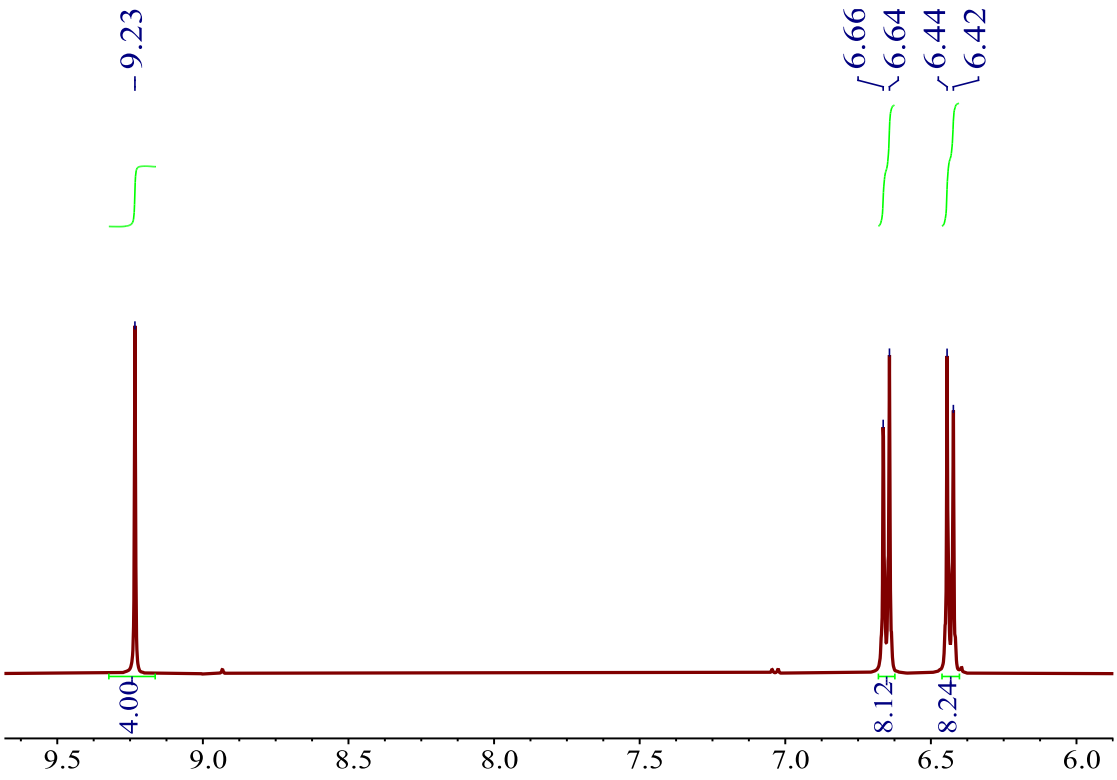


Figure S1. The ^{1}H NMR of **compound 1** recorded in DMSO at 25 ℃.

Synthesis of compound 2. Compound 1 (1.12 g, 2.7 mmol), 18-crown-6 (0.15 g, 0.55 mmol) and K_2CO_3 (3.84 g, 0.028 mol) were dissolved in 500 mL of acetonitrile. Then added 0.055 mol of 1,4-dibromobutane in to the reaction mixture, and stirred the mixture overnight at 65 ℃. The reaction mixture was filtered and evaporated to produce crude product. **Compound 2** was further purified by a silica gel column (DCM/PE) to obtain a yellow solid with 45 % yield (1.13 g). ^{1}H NMR (400 MHz, DMSO), δ (TMS, ppm): 6.78 (d, 8H), 6.64 (d, 8H), 3.87 (t, 8H), 3.55 (t, 8H), 1.89 (t, 8H) and 1.75 (t, 8H).

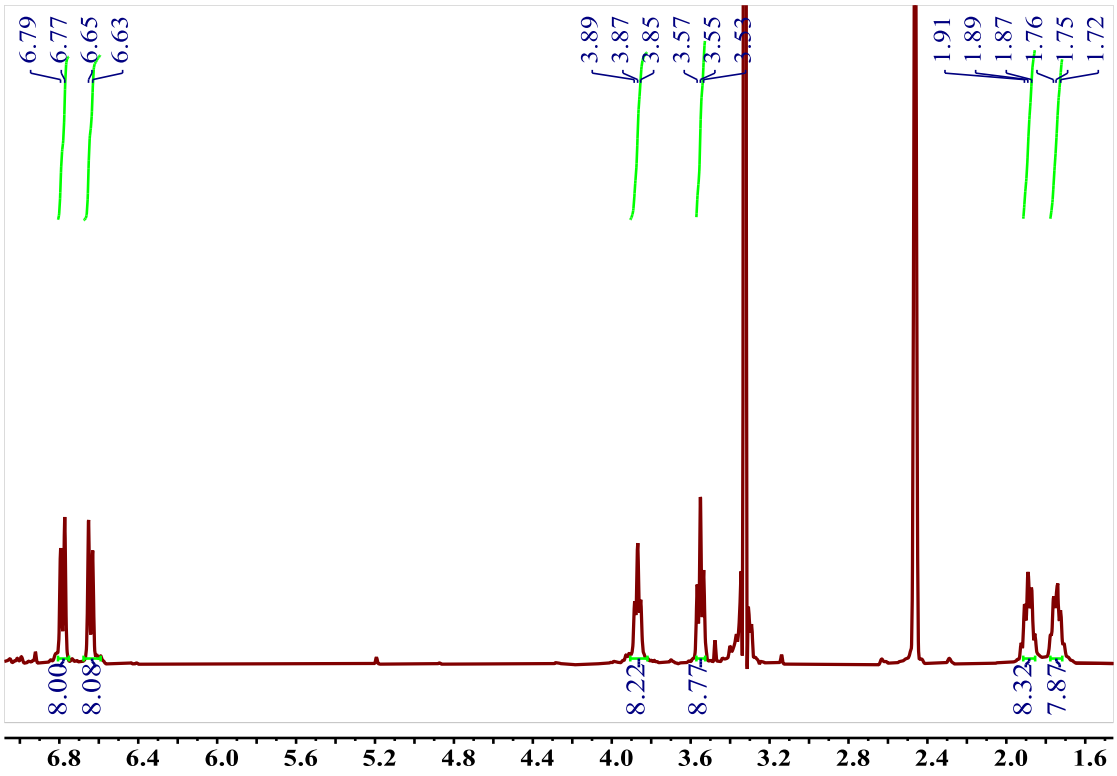


Figure S2. The ^{1}H NMR of compound 2 recorded in DMSO at 25 ℃.

Synthesis of compound 3. Compound 2 (0.52 g, 5.31 mmol) and 4-dimethylaminopyridine (0.64 g, 53.1 mmol) were dissolved in 50 mL of acetonitrile and heated at 80 °C for 8 hours. The reaction mixture was filtered and washed with acetonitrile to give the purified **compound 3** (TPE-B), which was a yellow solid with a yield of 45 % (0.88 g). ^{1}H NMR (400 MHz, D_2O), δ (TMS, ppm):7.78 (d, 8H), 6.70 (t, 24H), 3.80 (t, 16H), 2.99 (s, 24H), 1.72 (s, 8H) and 1.52 (s, 8H). MASS: m/z=570.222 corresponds to $[TPE\text{-}B\text{-}2H_2O]^{2+}$.

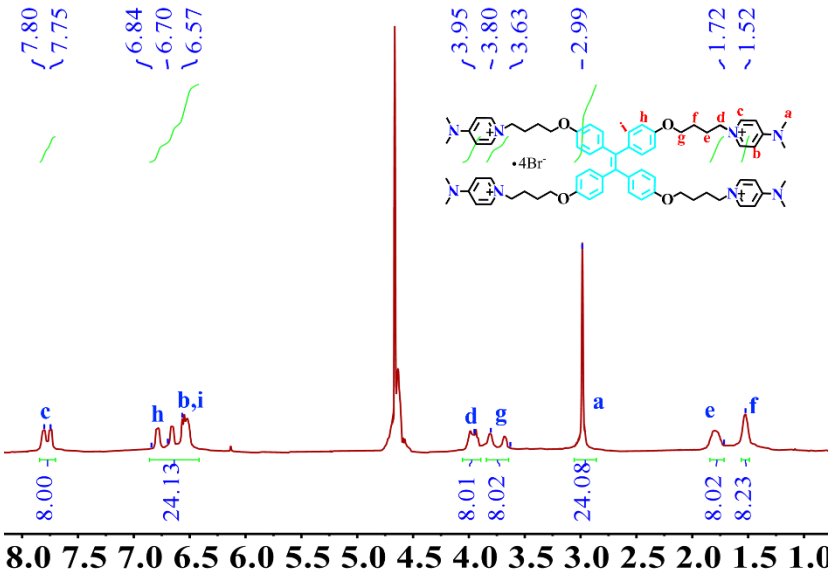


Figure S3. ^{1}H NMR spectrum of **compound 3** (**TPE-B**) recorded in D_2O at 25 ℃.

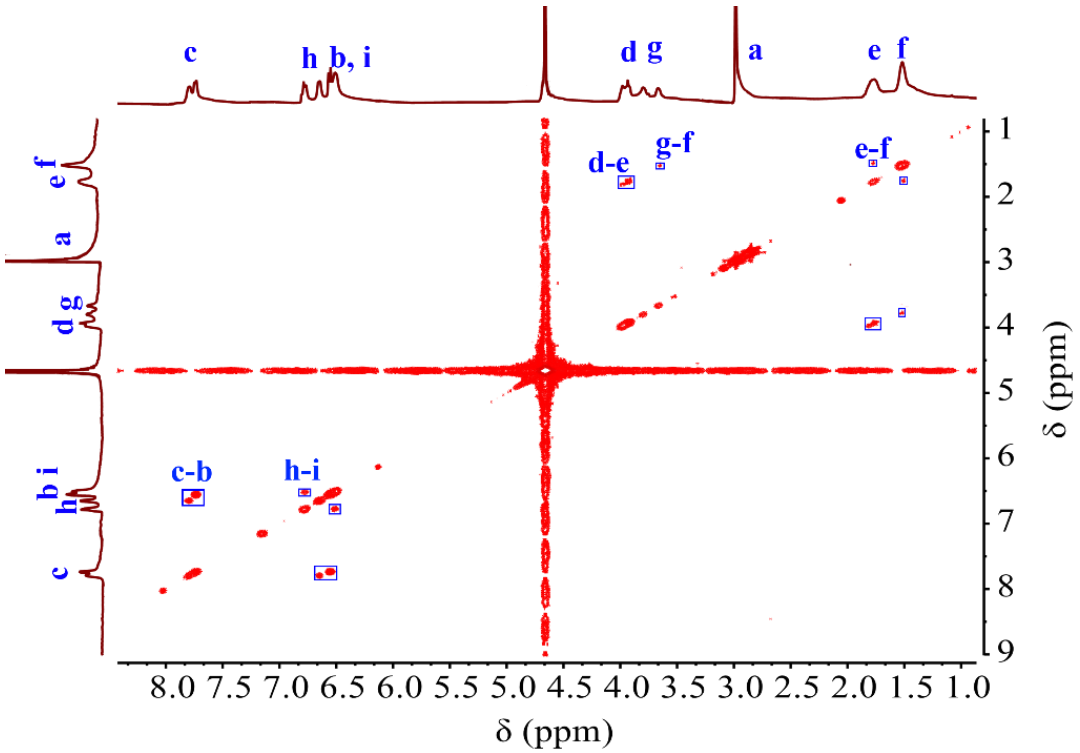


Figure S4. Partial COSY spectrum of the **TPE-B** recorded in D_2O at 25 ℃.

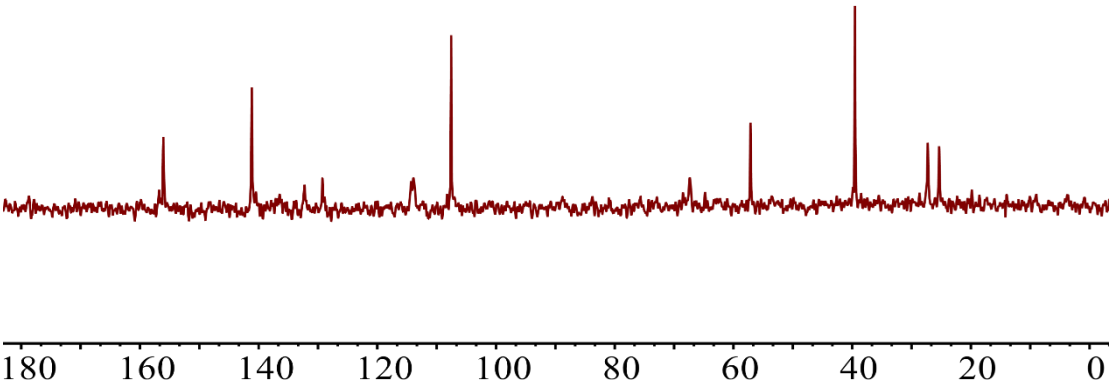


Figure S5. ^{13}C NMR spectra of TPE-B recorded in D_2O at 25 ℃.

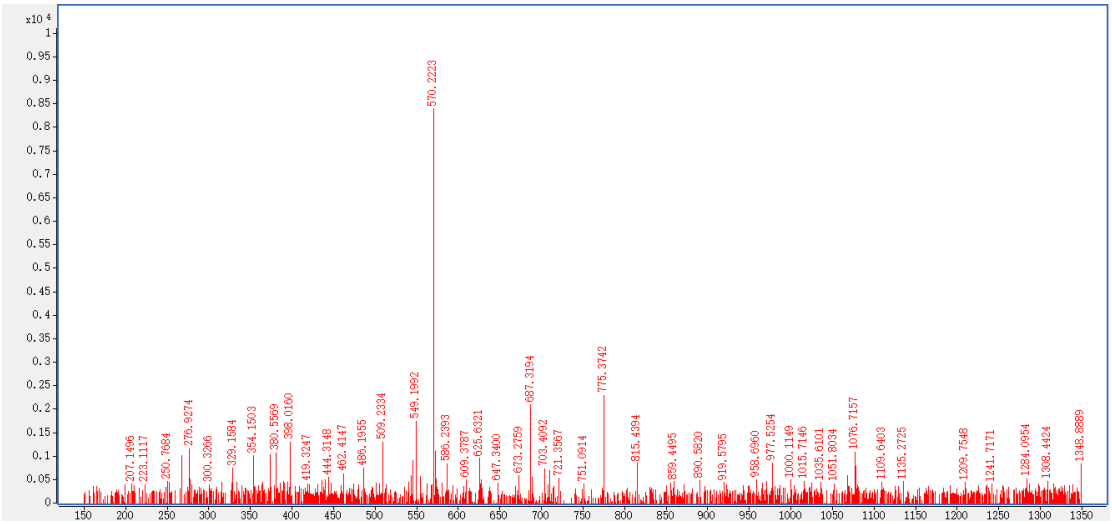
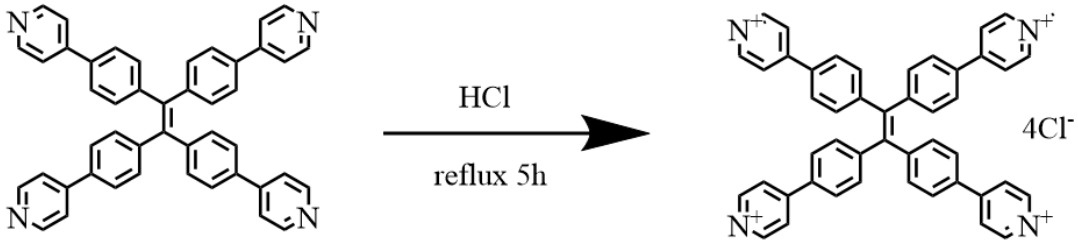


Figure S6. Mass spectra (ESI-MS) of TPE-B.

The strongest peak found at m/z=570.222 corresponds to $[TPE\text{-}B\text{-}2H_2O]^{2+}$.

Synthesis of TPPE



Scheme S2. Synthesis of TPPE.The purchased Tetra-(4-pyridylphenyl)ethylene (0.05 g) was protonated with 3 M hydrochloric acid, heated to reflux, concentrated and dried to obtain the desired yellow compound **TPPE**.

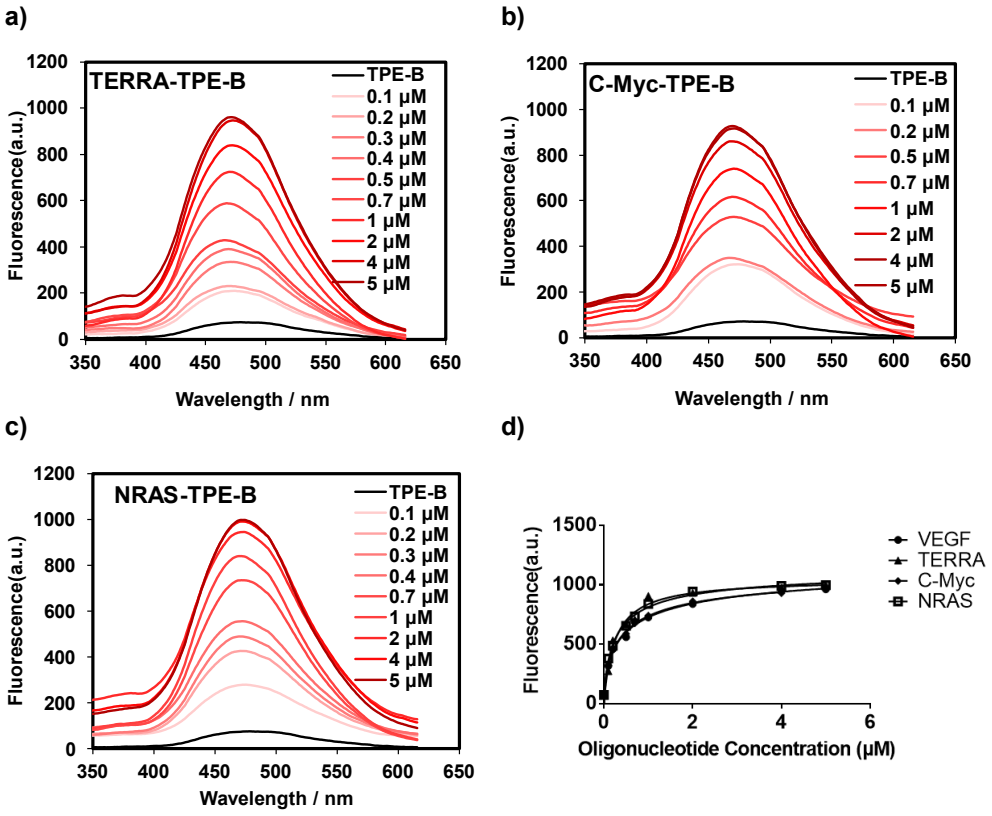


Figure S7. Fluorescence titration spectra of TPE-B with parallel G4s at different concentrations. a) with TERRA. b) with C-Myc. c) with NRAS. d): Fitting of the fluorescence titration data.

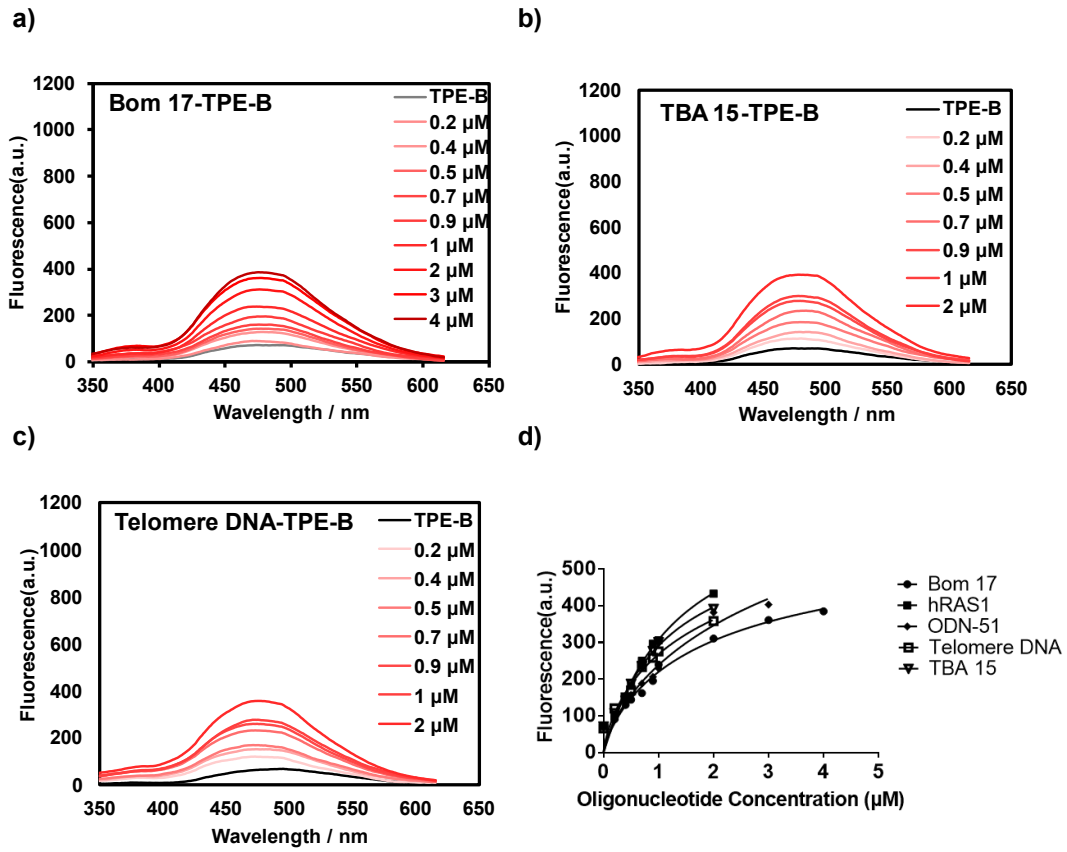


Figure S8. Fluorescence titration spectra of TPE-B with anti-parallel G4s at different concentrations. a) with Bom 17 b), with TBA 15 c), with Telomere DNA. d) Fitting of the fluorescence titration data.

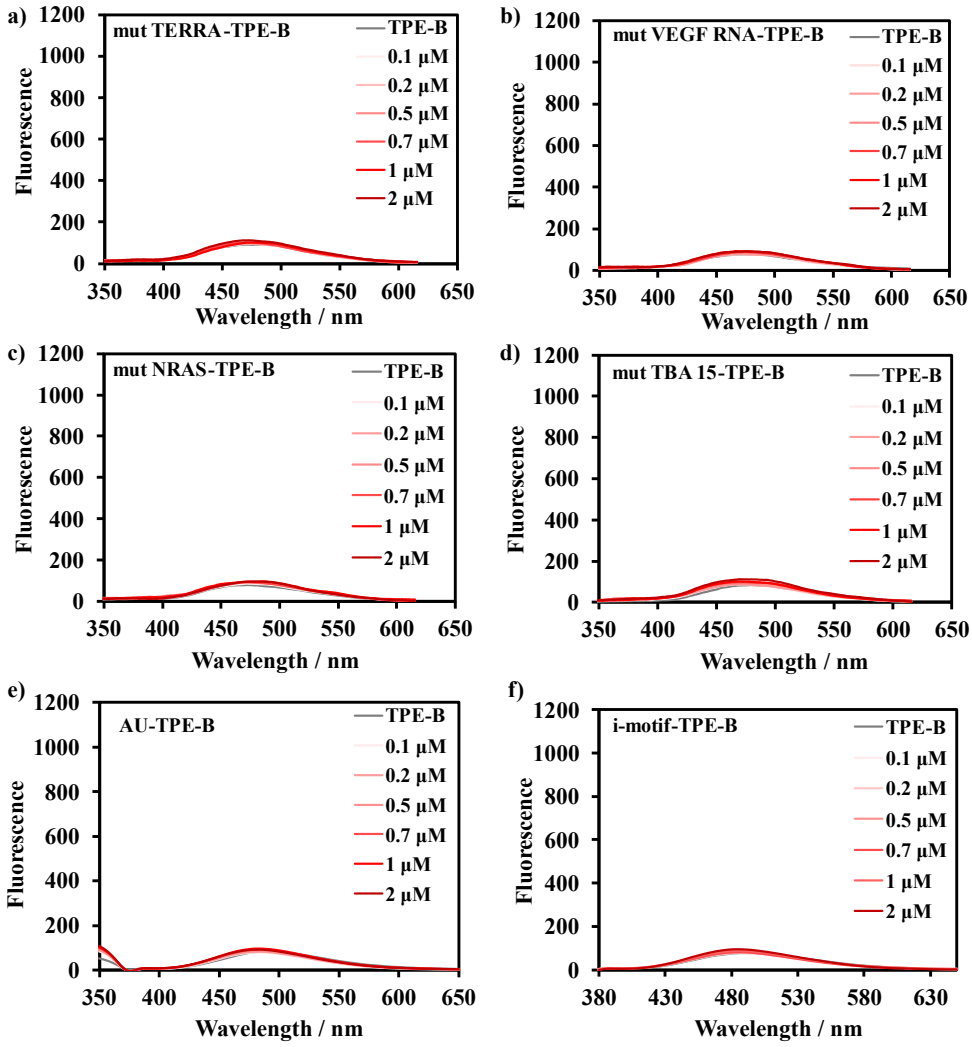


Figure S9. Fluorescence titration spectra of TPE-B with single-strand DNAs/RNAs and I-motif. a) with mut TERRA. b) with mut NRAS. c) with mut VEGF RNA. d) with mut TBA 15. e) with AU. f) with i-motif

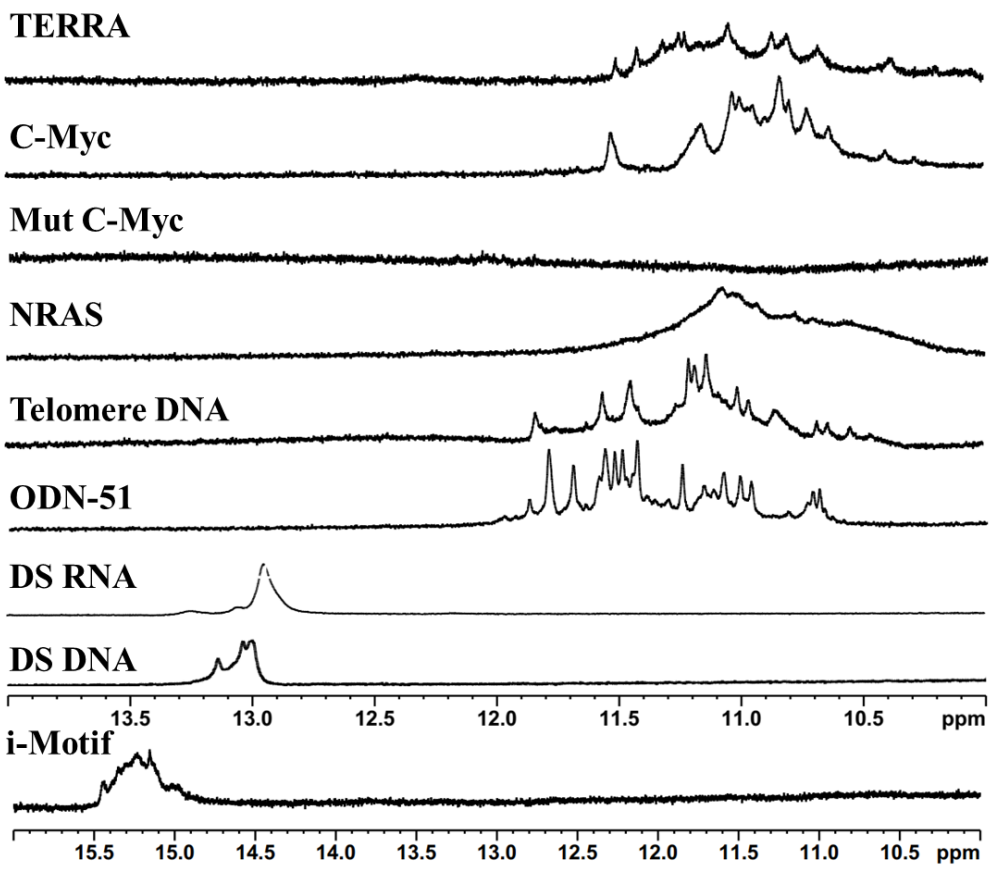


Figure S10. ^{1}H-NMR spectra of different sequences. G4 sequences including TERRA, C-Myc, NRAS, Telomere DNA, ODN-51 showed well-defined signals were observed between 10.0 and 12 ppm which are disappeared in spectra of mutant sequence. Double strands including DS DNA (AT), DS RNA (AU) showed signals between 12.5-14 ppm and i-motif showed signals between 15-16 ppm.

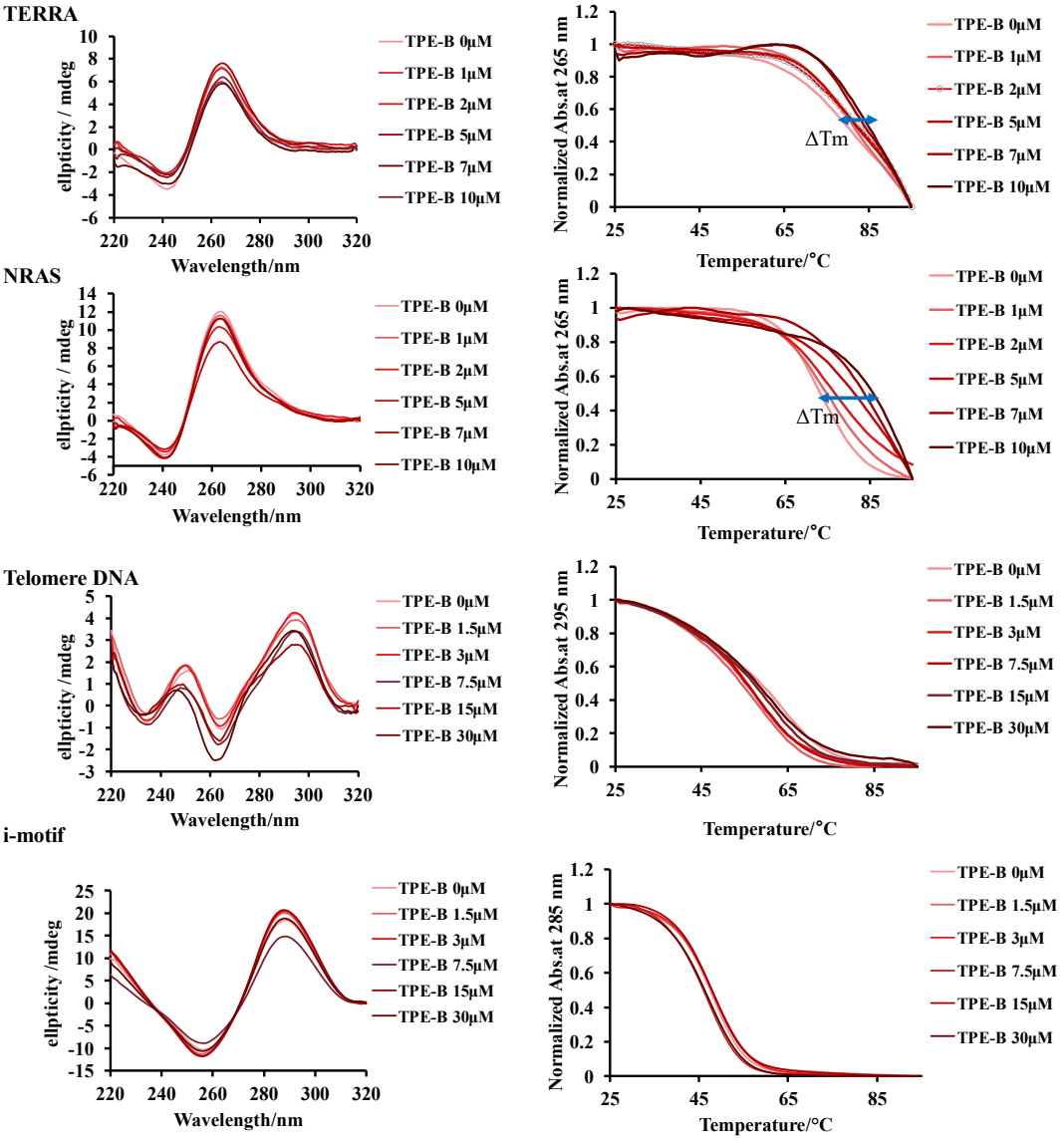


Figure S11. Left: Effect of TPE-B on CD spectra of G4s in the presence of 100 mM KCl. For Telomere DNA, the experiment was performed under 100 mM NaCl buffer conditions at 25 °C. Right: Melting curves of different G4s with TPE-B at different concentrations. TERRA G4 (parallel, 5 μM), NRAS G4 (parallel, 5 μM), Telomere DNA (anti-parallel, 15 μM), i-motif (i-motif, 15 μM).

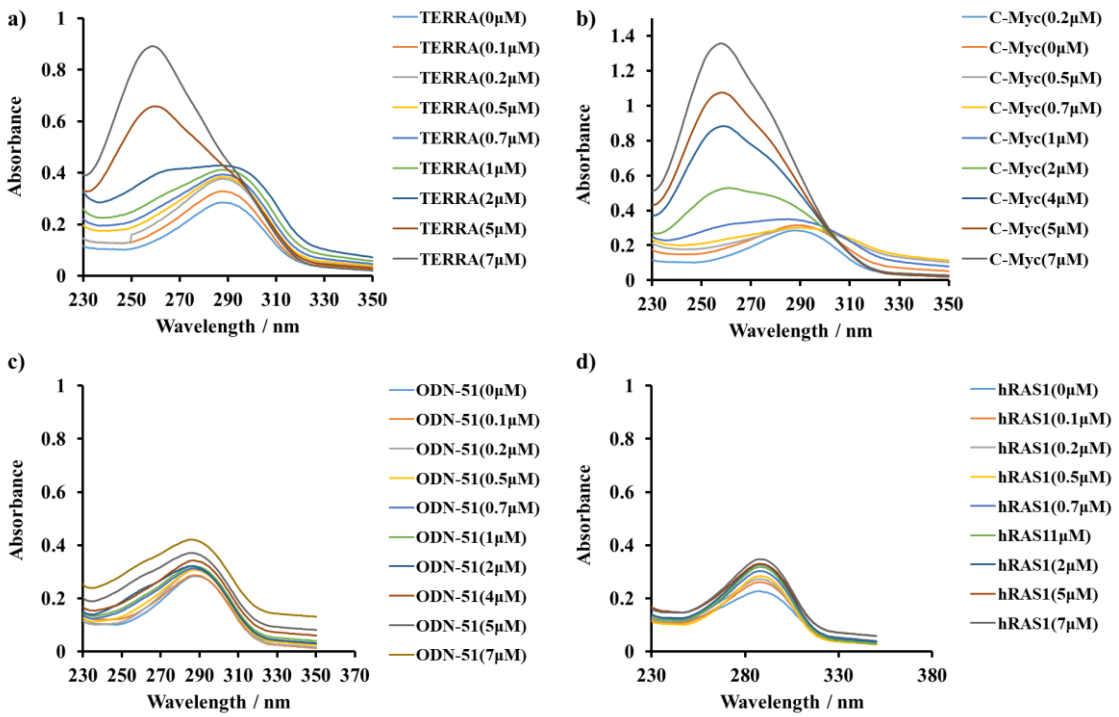


Figure S12. Absorption spectra of TPE-B upon addition of up to 1.25 equiv of G4s

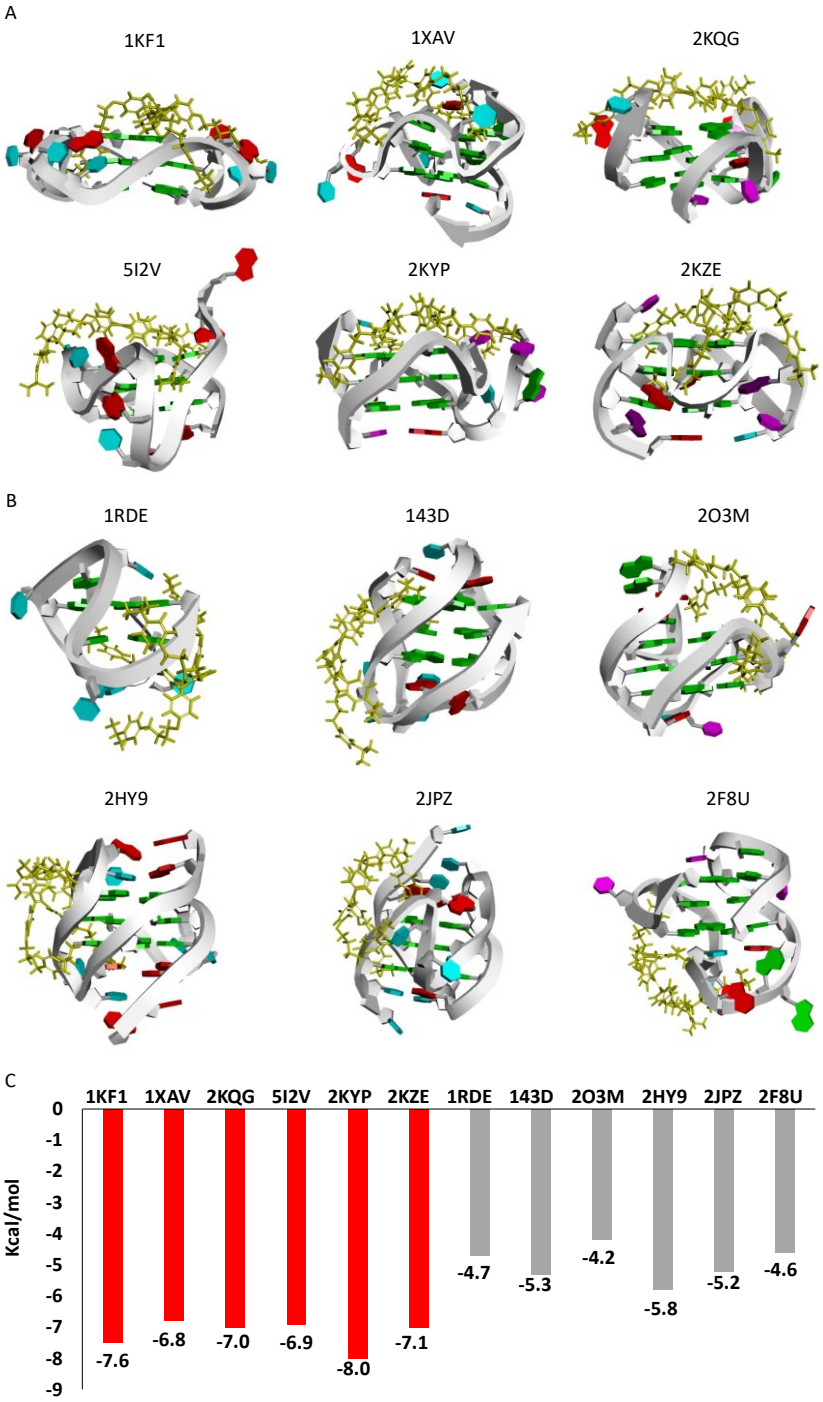


Figure S13. Best score binding modes of TPE-B with different G4s. a) TPE-B with parallel G4s.PDB ID of G4s were labelled including 1KF1(Parkinson et al., 2002), 1XAV(Ambrus et al., 2005), 2KQG(Hsu et al., 2009), 2KYP(Kuryavyi et al., 2010), 2KZE(Lim et al., 2010), 5I2V(Kerkour et al., 2017). b) TPE-B with anti-parallel/hybrid G4s including 1RDE(Mao et al., 2004), 2F8U(Dai et al., 2006), 2HY9(Dai et al., 2007b), 2JPZ(Dai et al., 2007a), 2O3M(Phan et al., 2007), 143D(Wang and Patel, 1993). c) Interaction energy (as the scoring function of binding) of the best docking of TPE-B with different G4s.

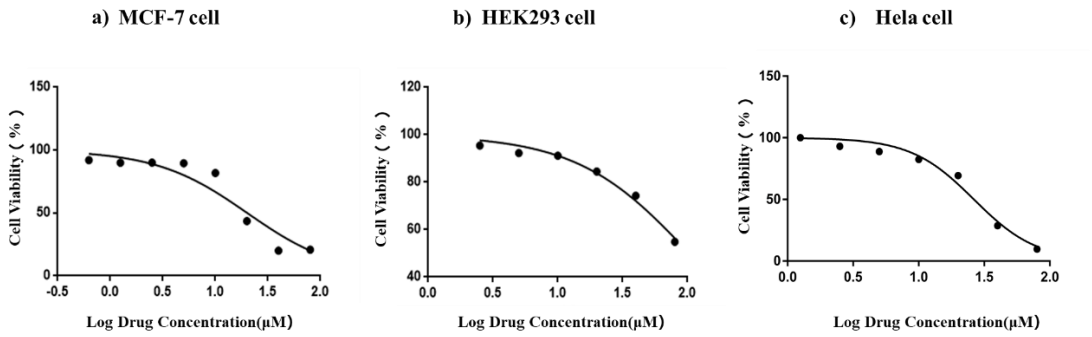


Figure S14. In vitro cytotoxicity test. Growth of a) MCF-7 cell, b) HEK293 cell and c) HeLa cells in the presence of different concentrations of TPE-B after 24 h incubation.

Table S1 Binding affinity between TPE-B and oligo strands.

	Kd [μM]
sequence	TPE-B
NRAS	0.21
TERRA	0.29
VEGF RNA	0.24
C-Myc	0.40
TBA 15	2.23
Bom 17	3.13
hRAS1	2.46
Telomere DNA	2.02
ODN-51	3.54
AT	undetected
mut NRAS	undetected
mut VEGF RNA	undetected
mut TERRA	undetected
mut TBA 15	undetected

Table S2 Fluorescence quantum yields of TPE-B with different sequences.

Sample	Φ_F
TPE-B $_{Tris}$	0.046
TPE-B $_{C\text{-}Myc}$	0.66
TPE-B $_{ODN\text{-}51}$	0.136
TPE-B $_{TBA\ 15}$	0.188
TPE-B $_{AT}$	0.054
TPE-B $_{mut\ TERRA}$	0.058

Table S3 Change of melting temperature of TPE-B combined with different G-quadruplexes.

Sequence	*Tm* value without TPE-B	*Tm* value with TPE-B(1:1 molecular ratio)	*ΔTm*[a]
TERRA	85.00 °C	88.60 °C	+3.6 °C
VEGF RNA	87.5 °C	above 95 °C	≥7.5 °C
NRAS	72.2 °C	87.01 °C	+13.51 °C
hRAS1	66.94 °C	67.01 °C	+0.07 °C
Telomere DNA	63.40 °C	62.73 °C	-0.67 °C
ODN-51	68.9 °C	67.4 °C	-1.50 °C
i-motif	47.66 °C	47.4 °C	-0.26 °C

[a] *ΔTm* values were calculated by subtracting Tm of G4 alone from the TPE-B-stabilized value.

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