

Supporting Information

Studying DNA G-quadruplex aptamer by ^{19}F NMR

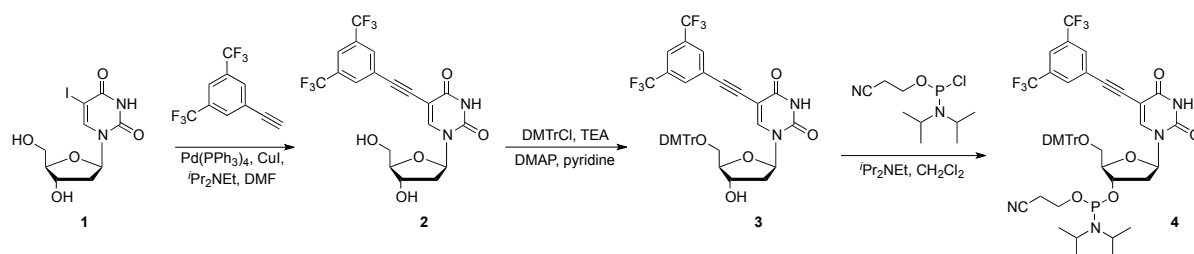
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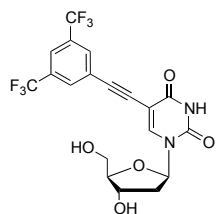
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General.

^1H -NMR, ^{13}C -NMR, ^{19}F -NMR and ^{31}P -NMR spectra were recorded on a BRUKER (AV-400M) magnetic resonance spectrometer. DMSO- d_6 and CDCl_3 were used as the solvents. Coupling constants (J) values are given in Hz and are correct to within 0.5 Hz. Signal patterns are indicated as br, broad; s, singlet; d, doublet; t, triplet; m, multiplet. All reagents were purchased from Aldrich, TCI (Tokyo Chemical Industry Co., Ltd.) or Wako (Wako Pure Chemical Industries, Ltd.). Thin layer chromatography was performed using TLC Silica gel 60 F₂₅₄ (Merck). Compounds were visualized by staining with a potassium permanganate solution. High-resolution mass spectra (HRMS) and electrospray ionization mass spectra (ESI-MS) were recorded on a Thermo Scientific Q Exactive instrument.



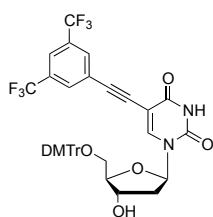
Scheme S1 Synthetic scheme of compound **4**.



5-ethynyl-3,5-bis(trifluoromethyl)phenyl-2'-deoxyuridine (2) 5-Iodo-2'-deoxyuridine **1** (1.75 g, 4.9 mmol) was co-evaporated with pyridine. To a solution of **1**, tetrakis(triphenylphosphine)palladium (571 mg, 0.49 mmol), CuI (188 mg, 0.99 mmol), and *N,N*-diisopropylethylamine (1.72 ml, 9.9 mmol) in DMF (15 ml) was added 1-ethynyl-3,5-bis(trifluoromethyl)benzene (1.48 ml, 8.4 mmol). The reaction was stirred

at room temperature for 12 h. The resulting mixture was filtered through Celite, which was washed with methanol. The filtrate was concentrated *in vacuo* and methanol was added to the residue. The precipitate was removed by filtration and the filtrate was concentrated *in vacuo*. CH_2Cl_2 -MeOH (1 : 1) mixture solution (100 ml) was added to the residue, and then the product was filtered and dried. This operation was performed two times to

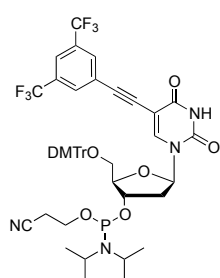
give compound **2** (1.6 g, 70%) as a white solid. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 11.78 (s, 1H), 8.53 (s, 1H), 8.14 (s, 1H), 8.13 (s, 2H), 6.12 (t, $J = 6.4$ Hz, 1H), 5.28 (d, $J = 4.4$ Hz, 1H), 5.20 (t, $J = 4.8$ Hz, 1H), 4.27 (m, 1H), 3.83 (q, $J = 3.4$ Hz, 1H), 3.68 (ddd, $J = 3.5, 4.9, 11.9$ Hz, 1H), 3.60 (ddd, $J = 3.5, 4.9, 11.9$ Hz, 1H), 2.19 (dd, $J = 5.4, 6.2$ Hz, 1H). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$) δ 161.20, 149.36, 145.38, 131.46, 131.06, 130.73, 130.40, 126.96, 125.10, 124.24, 121.89, 121.53, 96.97, 88.80, 87.62, 86.54, 85.08, 69.73, 60.67. $^{19}\text{F-NMR}$ (376 MHz, $\text{DMSO-}d_6$) δ -61.52. HRMS (ESI) for $\text{C}_{19}\text{H}_{13}\text{O}_5\text{N}_2\text{F}_6$ $[\text{M-H}]^-$: Calcd. 463.0723; Found. 463.0738.



5'-O-dimethoxytrityl-5-[(3,5-bis(trifluoromethyl)phenyl)ethynyl]-2'-deoxyuridine

(3) To a compound **2** (1.6 g, 3.5 mmol) dried three times by co-evaporation of pyridine and dissolved in dry pyridine (20 ml) was added 4,4'-dimethoxytritylchloride (1.75 g, 5.2 mmol), triethylamine (432 μL , 3.1 mmol) and 4-(dimethylamino)pyridine (16.8 mg, 0.14 mmol). After 2 h, the solution was evaporated *in vacuo*, and the residue was dissolved in

dichloromethane and added saturated NaHCO_3 aq. solution. The mixture was extracted two times with dichloromethane. The organic layer was dried over Na_2SO_4 , and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2 : $\text{MeOH} = 10 : 1$) to give the compound **3** (2.4 g, 90%) as a solid. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 11.85 (s, 1H), 8.35 (s, 1H), 8.06 (s, 1H), 7.55 (s, 2H), 7.41-7.10 (m, 9H), 6.83-6.78 (m, 4H), 6.16 (t, $J = 6.0$ Hz, 1H), 5.37 (d, $J = 4.4$ Hz, 1H), 4.37-4.30 (m, 1H), 3.96 (q, $J = 3.4$ Hz, 1H), 3.62 (s, 6H), 3.24 (m, 2H), 2.39-2.26 (m, 2H). HRMS (ESI) for $\text{C}_{40}\text{H}_{31}\text{O}_7\text{N}_2\text{F}_6$ $[\text{M-H}]^-$: Calcd. 765.2030; Found. 765.2048.



3'-O-[(2-cyanoethoxy)(diisopropylamino)phosphino]-5'-O-dimethoxytrityl-5-[(3,5-

bis(trifluoromethyl)phenyl)ethynyl]-2'-deoxyuridine (4) The compound **3** (1.15 g, 1.5 mmol) was treated with dry *N,N*-diisopropylethylamine (1.05 ml, 6.0 mmol) and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (1.0 ml, 4.5 mmol) in dry dichloromethane (5 ml) and stirred at room temperature for 4 h. After addition of dichloromethane, the reaction was stopped by adding an aqueous 5%- NaHCO_3 solution.

The aqueous layer was extracted two times with dichloromethane. The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hex : $\text{AcOEt} = 1 : 2$) to give the compound **4** (1.26 g, 87%) as a solid. $^{31}\text{P-NMR}$ (161 MHz, CDCl_3) δ 149.03, 148.58. HRMS (ESI) for $\text{C}_{49}\text{H}_{48}\text{O}_8\text{N}_4\text{F}_6\text{P}$ $[\text{M-H}]^-$: Calcd. 965.3108; Found. 965.3116.

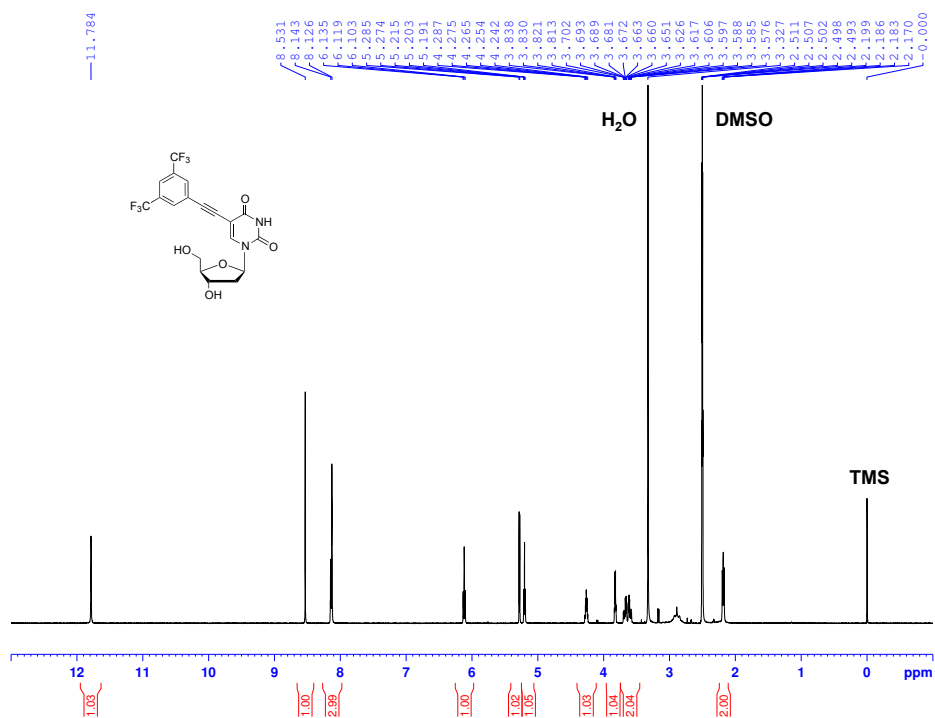


Figure S1. ¹H NMR spectrum of compound 2

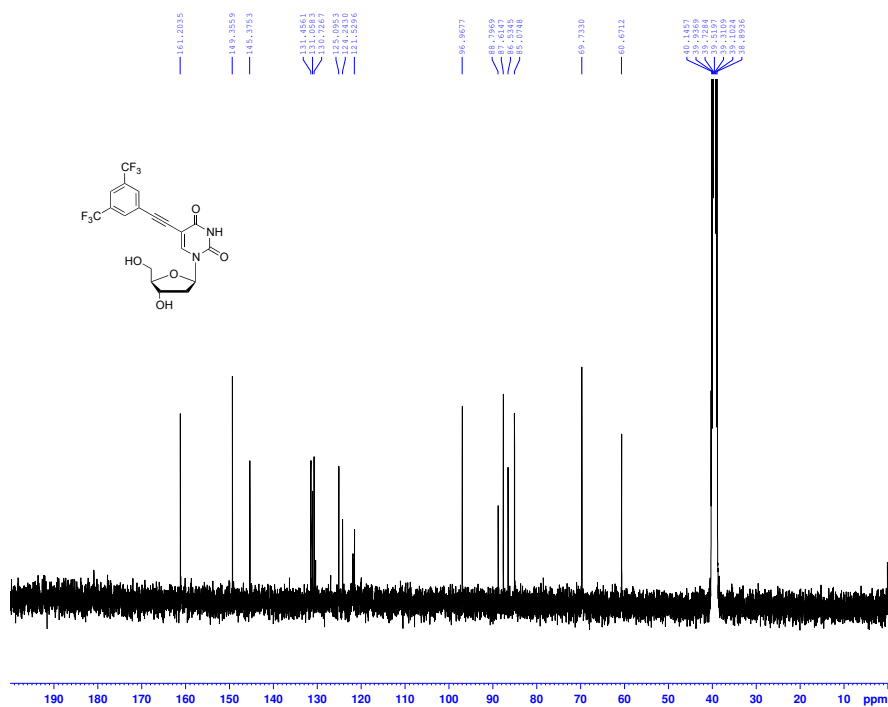


Figure S2. ¹³C NMR spectrum of compound 2

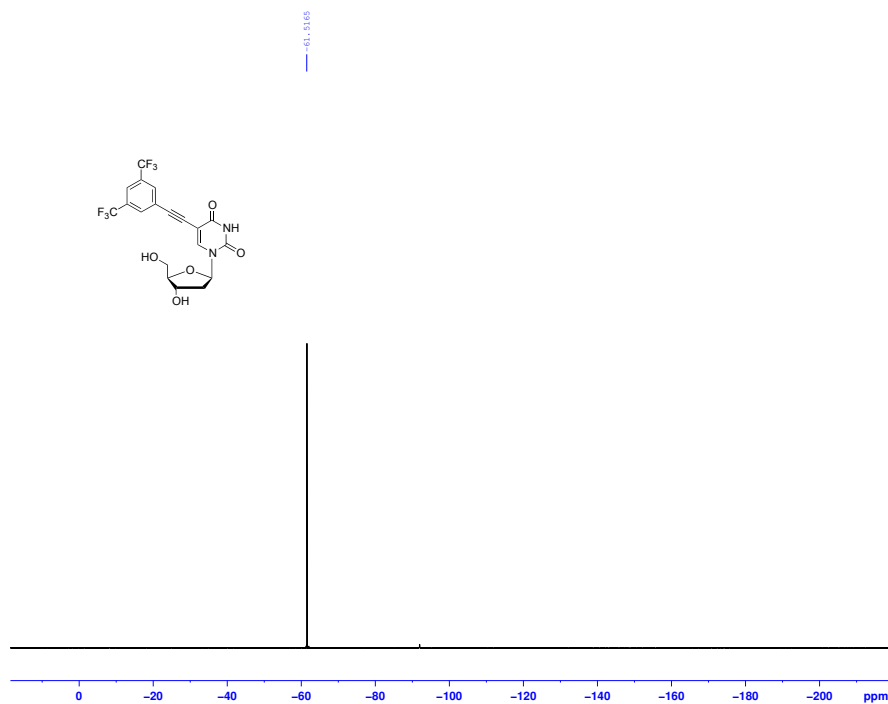


Figure S3. ^{19}F NMR spectrum of compound 2

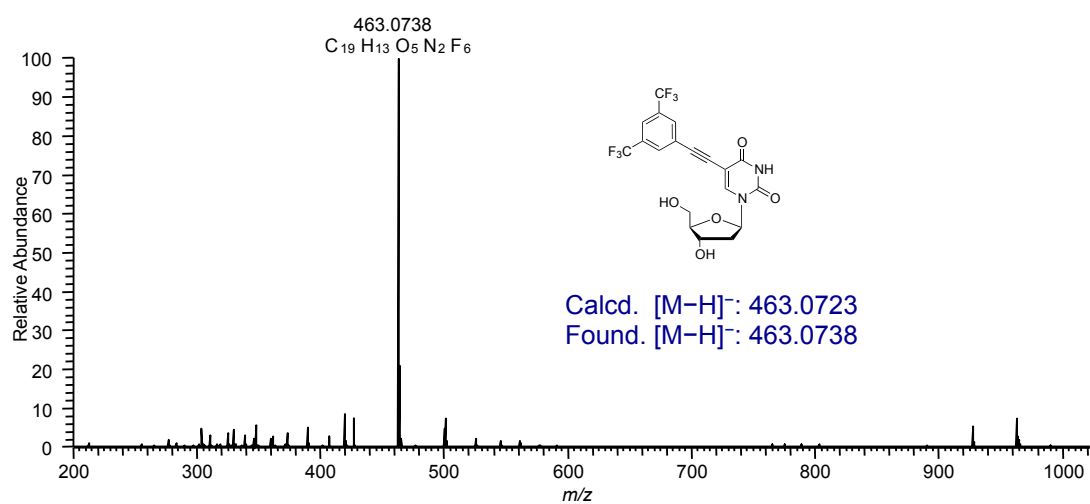


Figure S4. HRMS spectrum of compound 2

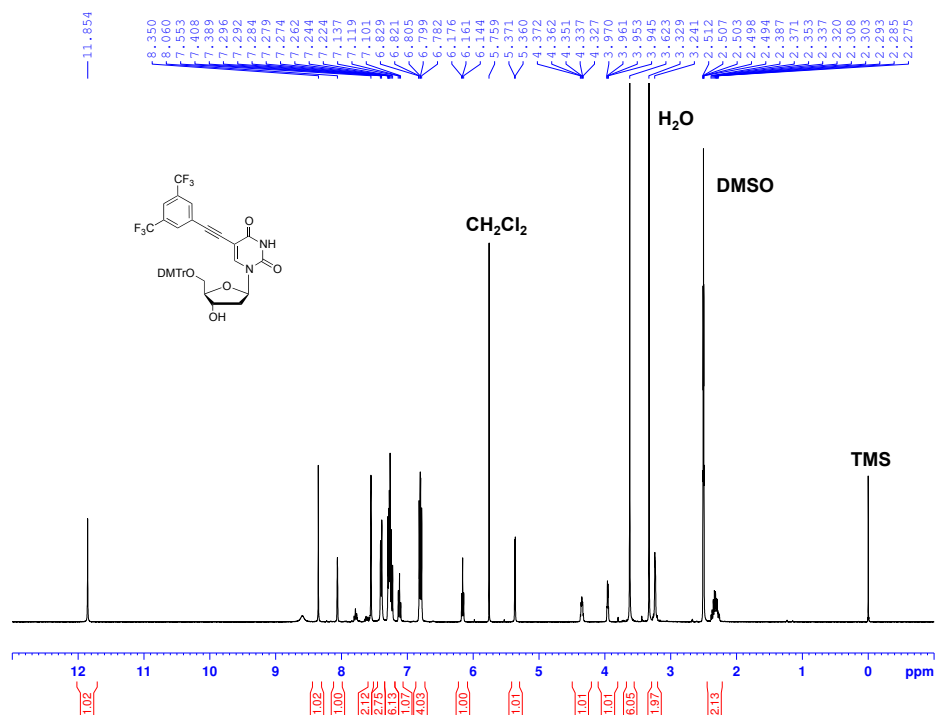


Figure S5. ¹H NMR spectrum of compound 3

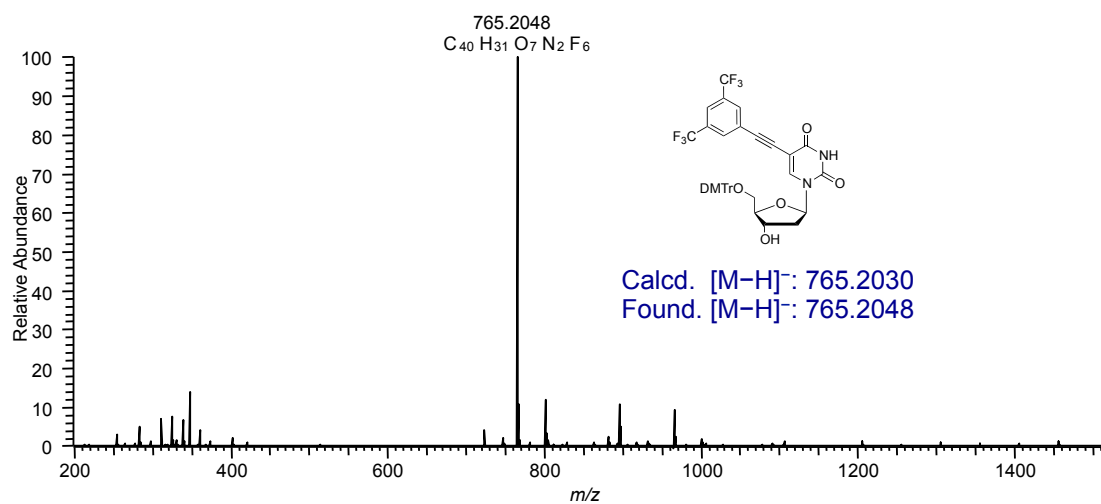


Figure S6. HRMS spectrum of compound 3

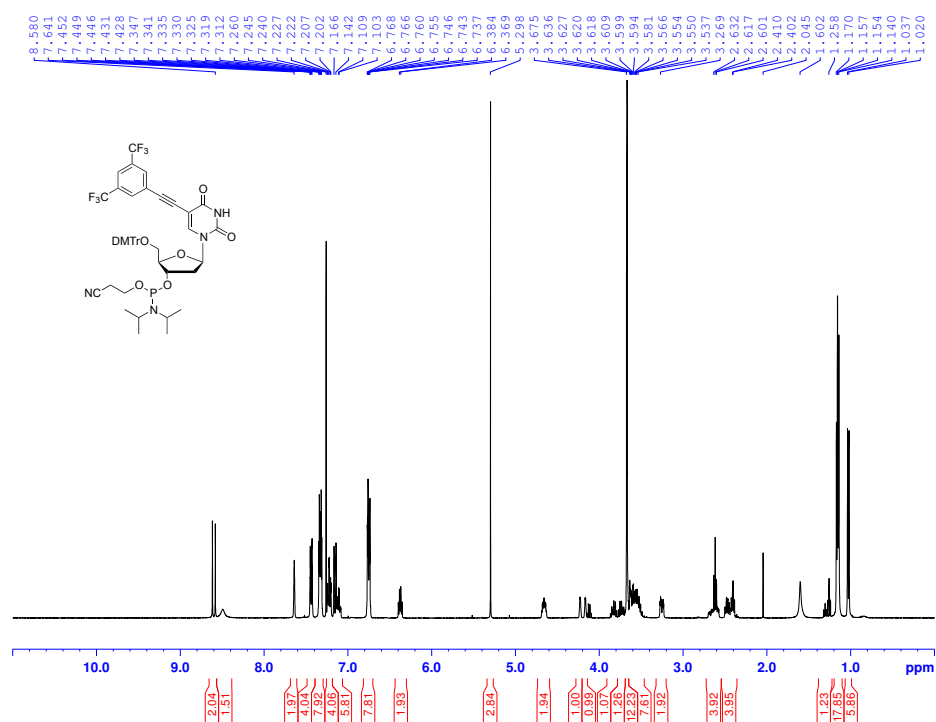


Figure S7. ¹H NMR spectrum of compound 4

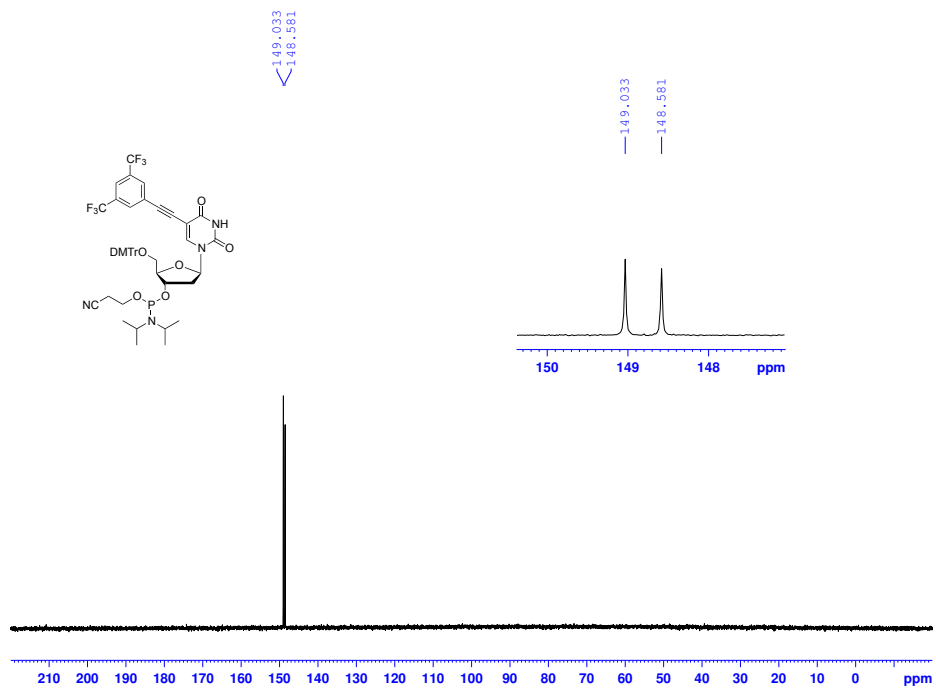


Figure S8. ³¹P NMR spectrum of compound 4

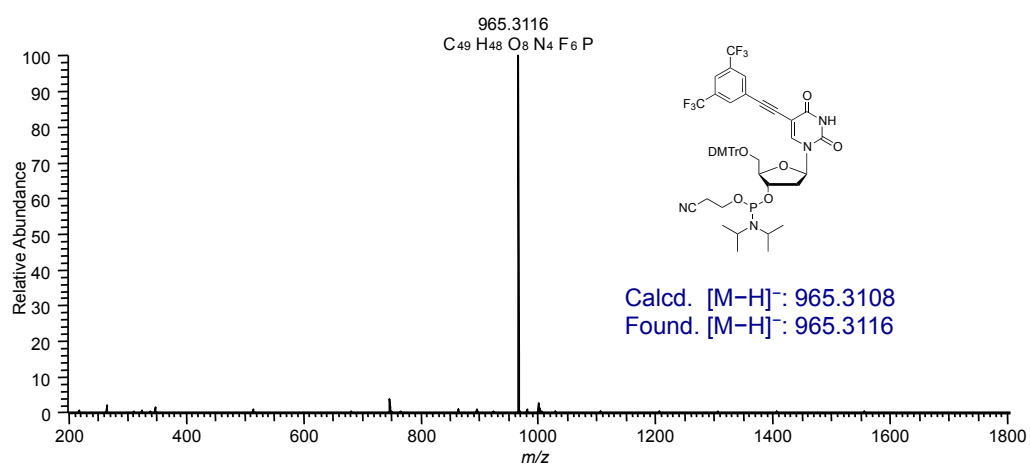


Figure S9. HRMS spectrum of compound 4

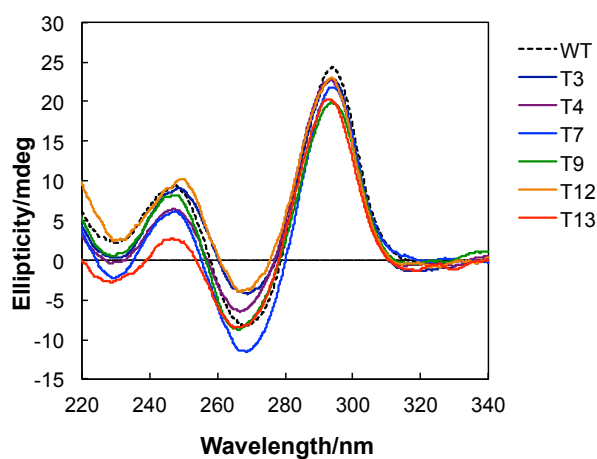


Figure S10. CD spectra of with and without ¹⁹F-labeled oligonucleotides. Condition: [DNA] = 5 μM, [KCl] = 100 mM, [Tris-HCl (pH7.0)] = 10 mM.

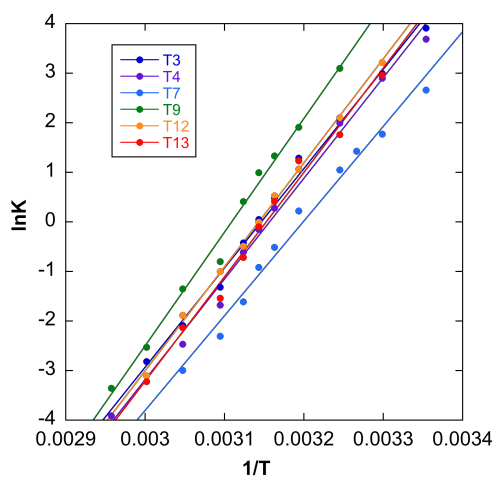


Figure S11. van't Hoff plots by plotting the ¹⁹F-NMR peak areas.

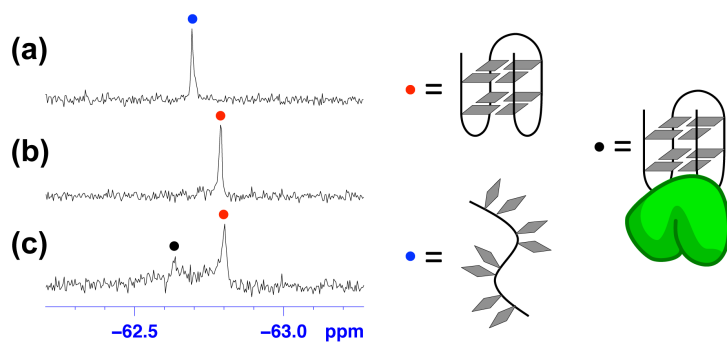


Figure S12. Discrimination of G-quadruplex structure and G-quadruplex-protein complex. ^{19}F NMR spectra of ^{19}F -labeled TBA oligonucleotide (T9) with 0 mM KCl (a), with 100 mM KCl (b) and after addition of thrombin (c). Condition: [DNA] = 100 μM , [KCl] = 0 or 100 mM KCl, [Tris-HCl buffer (pH 7.0)] = 10 mM, [thrombin] = 10 μM .

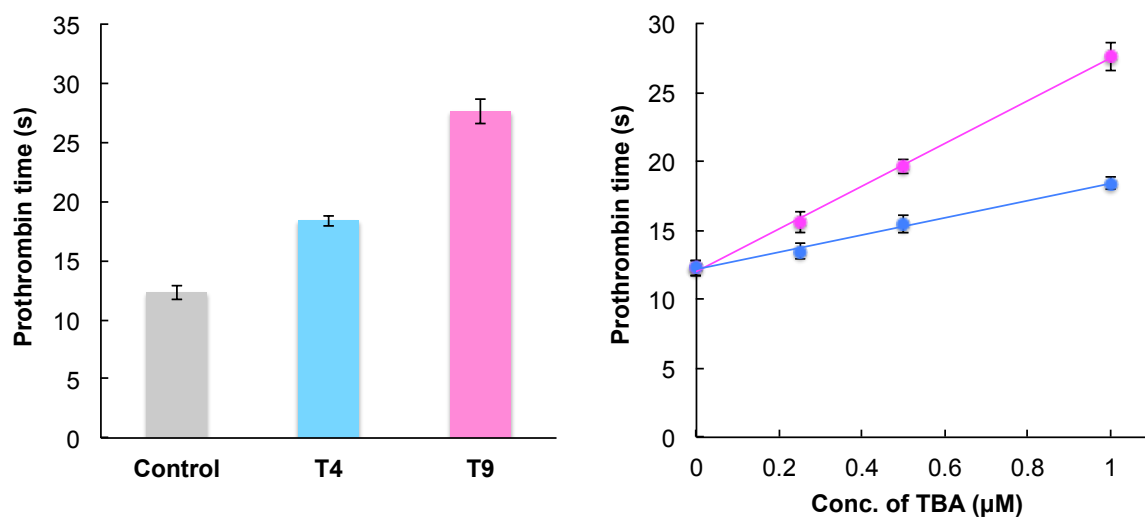


Figure S13. Prothrombin times measured in the presence of ^{19}F -labeled TBA oligonucleotides.

Table S1 MALDI-TOF MS of all oligonucleotides used in this study. **T** = 5-[{3,5-bis(trifluoromethyl)phenyl} ethynyl]-2'-deoxyuridine moieties

Name	Sequences	Calc. MS	Found MS
WT	5'-GGTTGGTGTGGTTGG-3'	4726.11	4725.89
T3	5'-GG T TGGTGTGGTTGG-3'	4947.19	4947.33
T4	5'-GGT T TGGTGTGGTTGG-3'	4947.19	4946.52
T7	5'-GGTTGG T GTGGTTGG-3'	4947.19	4946.54
T9	5'-GGTTGGTGT T GGTTGG-3'	4947.19	4947.21
T12	5'-GGTTGGTGTGG T TGG-3'	4947.19	4946.67
T13	5'-GGTTGGTGTGGT T GG-3'	4947.19	4946.62