Supporting information

Unexpectedly stable homopurine parallel triplex of SNA:RNA*SNA and L-*a*TNA:RNA*L-*a*TNA

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Experimental Section

Materials

All the oligonucleotides were HPLC grade and were purchased from Hokkaido System Science Co. Ltd. All the amidite monomers of SNA and L-*a*TNA were supplied by NICCA CHEMICAL Co. Ltd. except for the amidite monomer with Dabcyl on the SNA scaffold (compound **3**, see Scheme S1).

^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a BRUKER Ascend™ 500. Chemical shift values are expressed in δ values (ppm) relative to residual $CHCl_3$ (7.26 ppm) for ^{1}H NMR and to chloroform-*d*1 (77.16 ppm) for ^{13}C{^{1}H} NMR. For ^{31}P NMR, δ values (ppm) were not standardized. Multiplicities are described as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). HRMS of compounds were measured on a JMS-700 MStation.

Preparation of the solutions of oligonucleotides

All the oligonucleotides were dissolved in TBM buffer (100 μL, 90 mM Tris-borate buffer, 10 mM $MgCl_2$, pH 7.0) to a final concentration of 4 μM. Before T_m measurements, spectroscopic analyses, or PAGE, each strand was heated to 95 °C for 1 min and immediately cooled to 4 °C. Strands were then mixed and kept for 2 h at room temperature before measurement unless otherwise noted.

Fluorescence spectra measurements

Fluorescent spectra were measured on a JASCO model FP-8500 equipped with a programmable temperature controller at 4 °C unless otherwise noted. Excitation wavelength for FAM-labelled oligonucleotides was 495 nm with bandwidth 2.5 nm, response time 0.1 s, and medium sensitivity.

T_m measurements

T_m values of fluorophore-modified oligonucleotides were determined from fluorescence melting curves on a JASCO model FP-8500 or FP-8550 equipped with a programmable temperature controller by exciting FAM at 495 nm and monitoring fluorescence at 523 nm. The temperature ramp was 1.0 °C/min from 4 °C to 90 °C. T_m profiles were obtained by dividing the original fluorescence intensity of triplex by that of the hairpin at each temperature to compensate for the temperature effect on FAM intensity. T_m values of duplexes without fluorophores were obtained with a Shimadzu UV-1800 by measuring the change of absorbance at 260 nm versus temperature. The change of absorbance at 295 nm versus temperature of SNA-TFO and L-*a*TNA-TFO were monitored to evaluate the formation of G-quadruplex structure by Shimadzu UV-

1900i.

CD measurements

CD spectra were measured on a JASCO model J-820 using 10-mm quartz cells. The sample solutions contained 90 mM Tris-borate buffer, 10 mM $MgCl_2$, pH 7.0, 5.0 μM P-SNA-TFO or P-L-*a*TNA-TFO.

Fluorescent titration measurements

To 500 μL of buffer solution containing 2 μM S:R-HP, S:D-HP, or LT:R-HP was added 5 μL of 20 μM TFO at room temperature. After 3 min at room temperature, fluorescent measurement was performed at 4 °C. These measurements were repeated 20 times until final solution volume became 600 μL.

Analysis of triplex formation by native PAGE

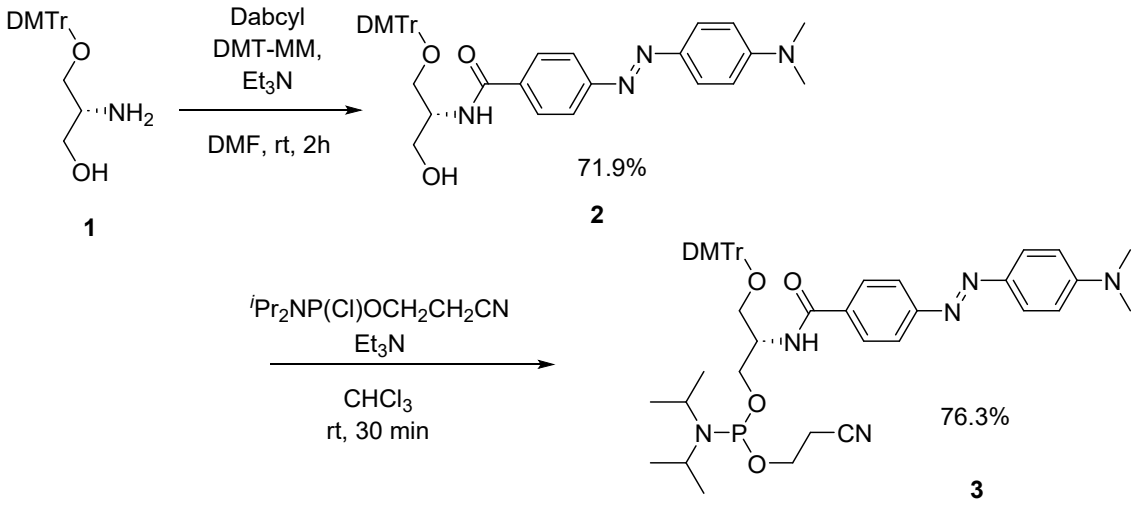
S:R-HP, Non-S:R, or S:D-HP was mixed with appropriate TFO to a final concentration of 0.5 μM each strand, and 20 μL of the solution was added to 4 μL of loading buffer containing 60% glycerol and 0.05% bromophenol blue. An aliquot of 6 μL was subjected to electrophoresis on a 15% polyacrylamide gel with 10% glycerol (0.5X TB buffer) at 750 CV for 3 h. During the electrophoresis, temperature was kept at 4 °C using a water-bath holder equipped with a circulator. Gels were analyzed with a Typhoon FLA 9500 (GE Healthcare) by excitation of TAMRA and FAM with 532 and 473 nm light, respectively.

RNase If experiments

RNase If (50,000 unit/ml, New England Biolabs Japan) was diluted 400 fold with TBM buffer to 125 units/ml). To 8 μL of a solution of S:R-HP or Non-S:R and SNA-TFO (0.5 μM each) was added 2 μL of diluted RNase If. After incubation at 4 °C for 4 h, 2 μL of loading buffer containing 60% glycerol and 0.05% bromophenol blue was added. An aliquot of 6 μL of the sample solution was subjected to electrophoresis on a 15% polyacrylamide gel with 10% glycerol (0.5X TB buffer) at 750 CV for 3 h. During the electrophoresis, the temperature was kept at 4 °C using a water-bath holder equipped with a circulator. Gels were analyzed with a Typhoon FLA 9500 (GE Healthcare) by excitation of TAMRA and FAM with 532 and 473 nm light, respectively.

Computer modeling

Maestro Version 12.6 (Schrödinger) was used to obtain optimized geometries and energy minimized structures. The initial triplex structure was prepared from C:G*G Watson-Crick and Hoogsteen pairs observed in the X-ray crystal structure of an SNA:RNA duplex.[1)] The OPLS_2005 force field was employed for the energy minimization.



Scheme S1. Scheme for synthesis of amidite monomers of Dabcyl on the SNA scaffold.

Synthesis of compound 2

Dimethoxytritylated serinol (compound **1**) was synthesized as previously reported.[2)] 4-Dimethylaminoazobenzene-4'-carboxylic acid (Dabcyl, Tokyo Chemical Industry Co., Ltd. >97.0%, 3.23 g, 12.0 mmol, 1.2 eq) was suspended in DMF (70 ml) and triethylamine (7.0 ml, 51 mmol, 5.0 eq). Then a solution of compound 1 (0.72 M, 13.9 ml, 10.0 mmol) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM) *n*-hydrate (14.8 mmol, 1.5 eq) in DMF was added. After stirring at room temperature for 2 h, $CHCl_3$ (270 ml) was added to quench the DMT-MM. The organic phase was washed with sat. aq. $NaHCO_3$ (300 ml) x 2, sat. aq. NaCl(300 ml) x 2, and then evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane:AcOEt, 1:5 (v/v) with 1% Et_3N) to afford compound **2** as a red solid (4.64 g, 7.19 mmol, 71.9%).

Synthesis of compound 3

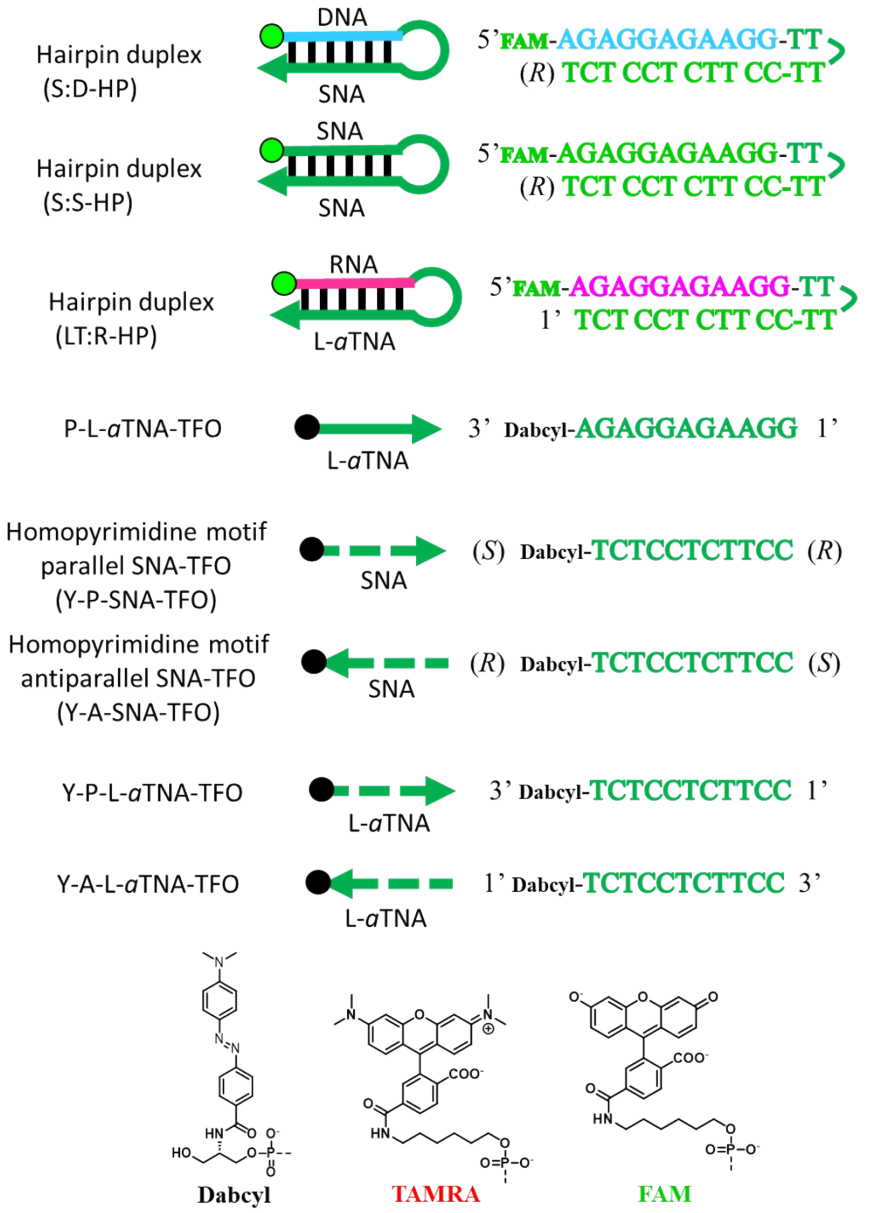
2-Cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite (Fujifilm Wako Pure Chemical Industries, Ltd. >90%, 2.40 ml, 10.25 mmol, 1.5 eq) was added to a solution of compound **2** (4.64 g, 7.19 mmol) and triethylamine (5.0 ml, 36.12 mmol, 5.0 eq) in dry $CHCl_3$ (30 ml) at 0 °C under a N_2 atmosphere. After stirring at room temperature for 30 min, the mixture was concentrated, and the residue was purified using silica gel column chromatography (*n*-hexane:AcOEt, 1:2 (v/v) including 3% Et_3N) to afford compound **3** as a red solid (4.64 g, 5.49 mmol, 76.3%). ^{1}H NMR [$CDCl_3$, 500 MHz] δ 7.83-7.71 (m, 6H), 7.37 (d, 2H), 7.28-7.24 (m, 3H), 7.20 (t, 2H), 7.11 (t, 1H), 6.76-6.68 (m, 4H), 6.65 (d, 2H), 6.48 (dd, 1H), 4.53-4.35 (m, 1H), 4.01-3.79 (m, 2H), 3.77-3.55 (m, 9H), 3.55-3.45 (m, 2H) , 3.45-3.35 (m, 1H), 3.24-3.15 (m, 1H), 2.98 (s, 6H), 2.47-2.37

(m, 2H), 1.08 (t, 6H), 1.04 (dd, 6H) (Fig. S7). $^{13}C\{^{1}H\}$ NMR [$CDCl_3$, 125 MHz] δ 166.4, 166.3, 158.4, 155.0, 152.7, 144.72, 144.69, 143.5, 135.9, 135.8, 134.44, 134.37, 130.0, 128.1, 127.8, 125.31, 125.29, 122.11, 122.08, 117.5, 113.1, 111.4, 86.0, 62.4, 62.3, 62.1, 61.6, 58.32, 58.30, 55.1, 50.22, 50.17, 50.16, 43.14, 43.10, 43.04, 43.00, 40.2, 24.6, 24.54, 24.49, 20.3 (Fig. S8). ^{31}P NMR [DMSO-*d*6, 202 MHz] δ 147.0, 146.8 (Fig. S9). HRMS (FAB): Calcd for $C_{48}H_{58}N_6O_6P$ $[M + H]^+$, 845.4150; found, 845.4179.

References

1) Kamiya, Y. *et al.*, Intrastrand backbone-nucleobase interactions stabilize unwound right-handed helical structures of heteroduplexes of L-*a*TNA/RNA and SNA/RNA. *Commun. Chem*., 2020, **3**, 156

2) Benhida, R. *et al.* Incorporation of serinol derived acyclic nucleoside analogues into oligonucleotides: Influence on duplex and triplex formation. *Tetrahedron Lett.* 1998, **39**, 6167.



Scheme S2. Schematics and sequences of oligonucleotides and chemical structures of dyes used in this study.

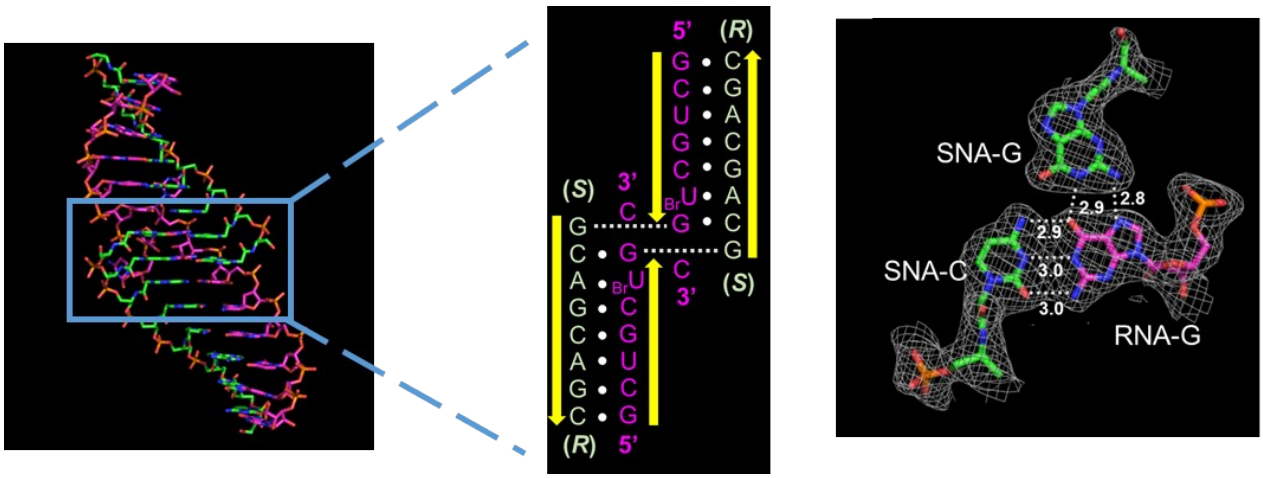


Figure S1. Canonical Watson-Crick and parallel-type Hoogsteen base pairs between SNA and RNA observed in the X-ray crystallographic structure of an RNA:SNA heteroduplex [1)]

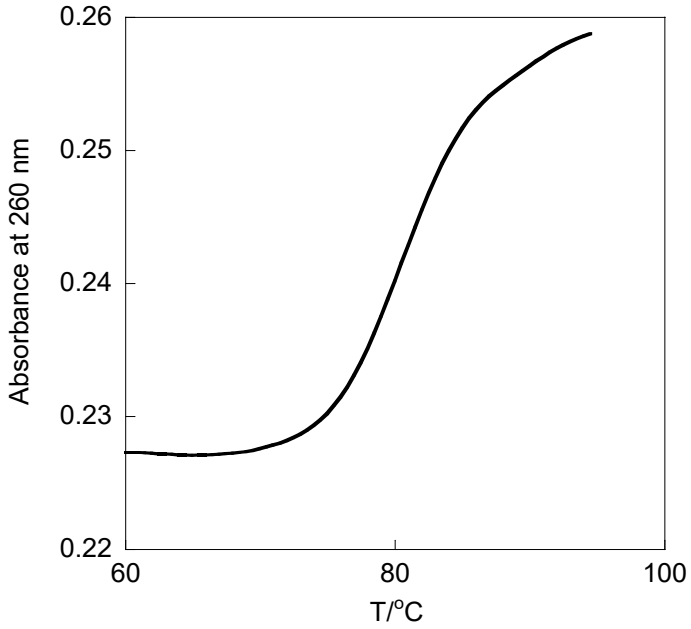


Figure S2. Melting profile of SNA:RNA-HP. [S:R-HP] = 2.0 μM, 90 mM Tris-borate buffer, 10 mM $MgCl_2$, pH 7.0.

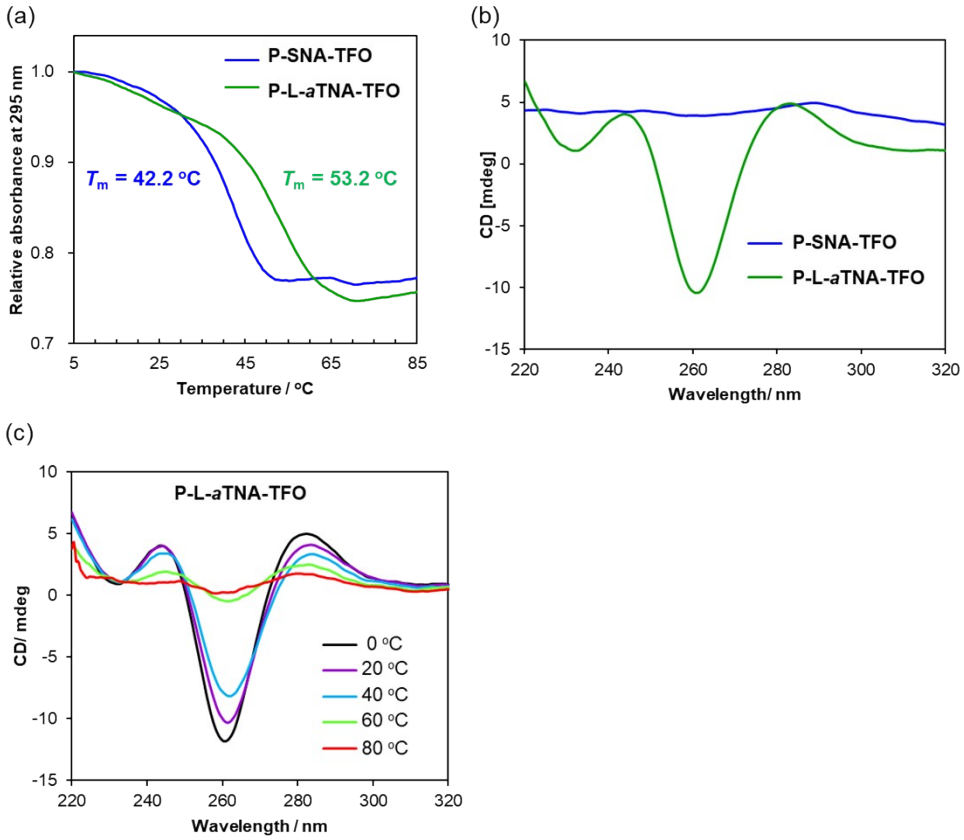


Figure S3. (a)Melting profile of SNA-TFO and L-*a*TNA-TFO obtained with a Shimadzu UV-1900i by measurement of the change in absorbance at 295 nm versus temperature. The temperature ramp was 0.5 °C min^{-1}. (b)CD profile of SNA-TFO and L-*a*TNA-TFO recorded with a JASCO J-820 at 4 °C, and (c) 80, 60, 40, 20, and 0 °C. [P-SNA-TFO] = [P-L-*a*TNA-TFO] = 5.0 μM, 90 mM Tris-borate buffer, 10 mM $MgCl_2$, pH 7.0.

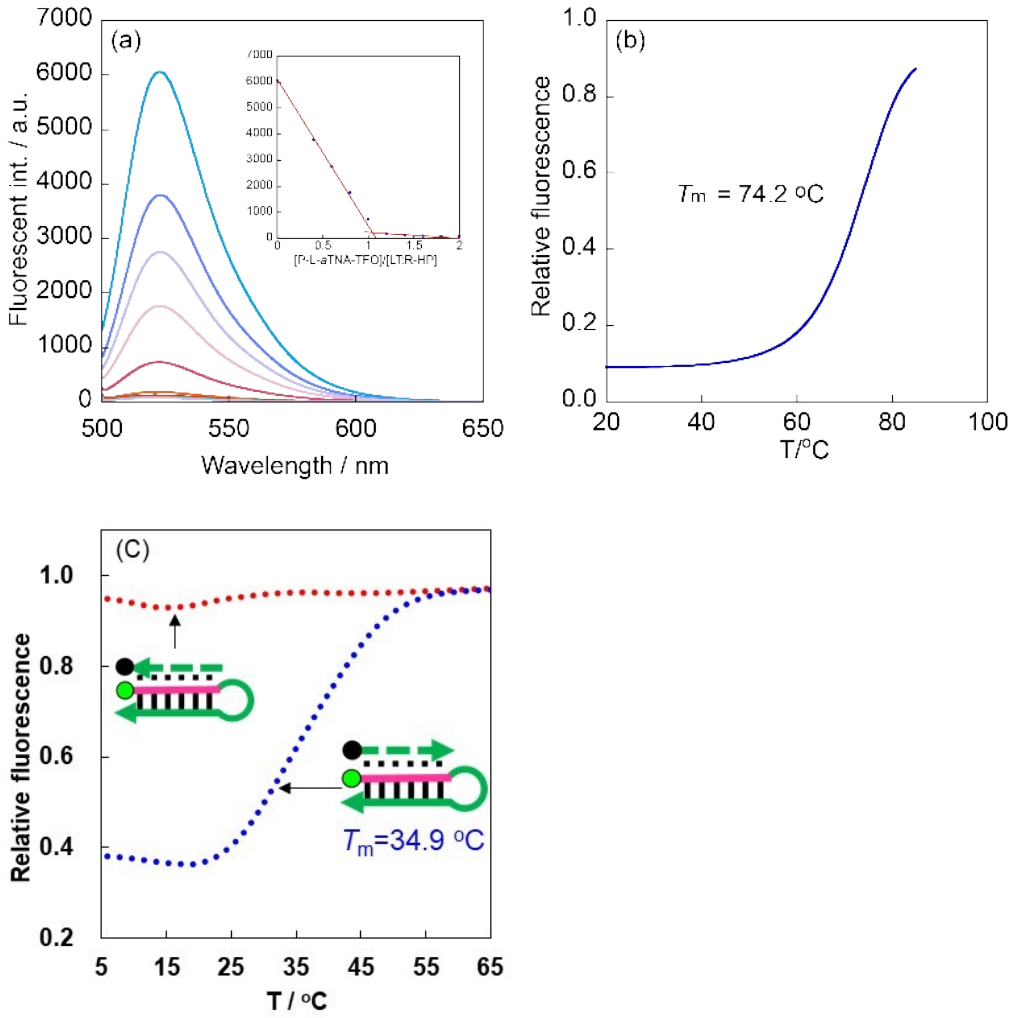


Figure S4. (a) Fluorescence of FAM-LT:R-HP in the presence of a range of concentrations of Dabcyl-P-L-*a*TNA-TFO. Inset shows fluorescence as a function of the ratio of [P-L-*a*TNA-TFO] to LT:R-HP. Melting profile of (b) LT:R-HP and P-L-*a*TNA-TFO triplex, (c) LT:R-HP and Y-P-L-*a*TNA-TFO or Y-A-L-*a*TNA-TFO triplex. 2.0 μM each oligonucleotide solved in 90 mM Tris-borate buffer, 10 mM $MgCl_2$, pH 7.0 was used. Excitation wavelength was 495 nm, and fluorescence intensity was monitored at 523 nm.

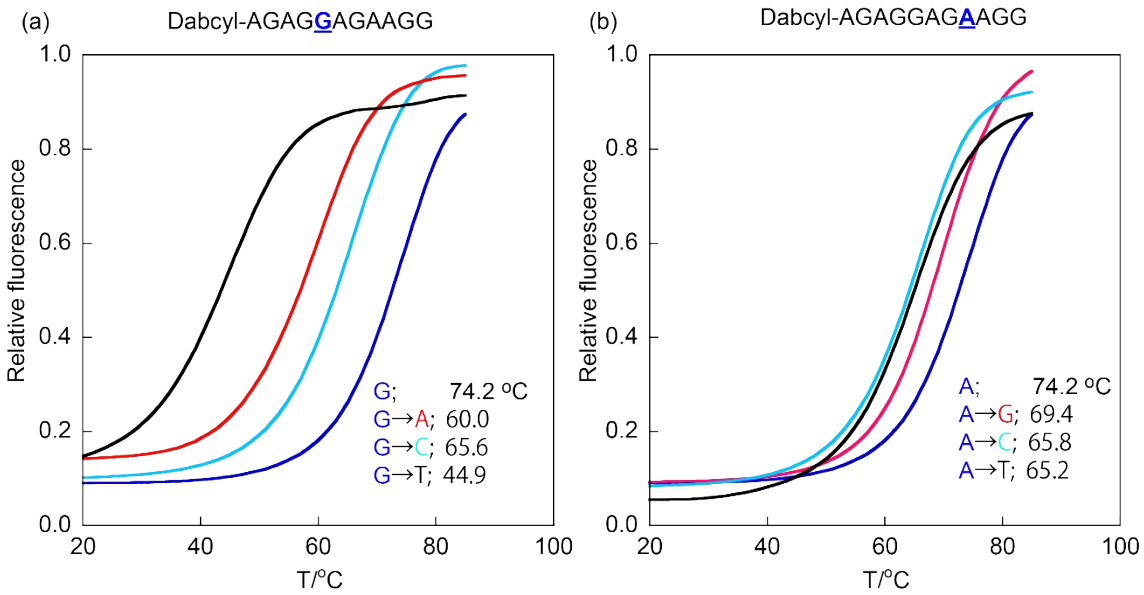


Figure S5. Effect of mismatches on T_m of parallel triplex containing L-*a*TNA strands. [P-L-*a*TNA-TFO] = [LT:R-HP] = 2.0 μM, 90 mM Tris-borate buffer, 10 mM $MgCl_2$, pH 7.0.

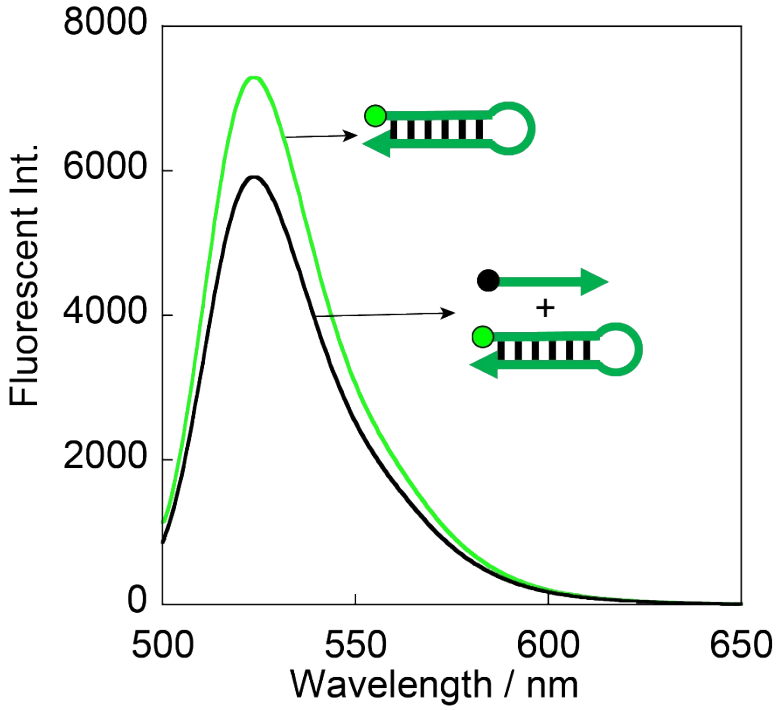


Figure S6. Fluorescence spectra of Fam-S:S-HP in the presence (black line) or absence (green line) of Dabcyl-P-SNA-TFO. [P-SNA-TFO] = [S:S-HP] = 2.0 μM, 90 mM Tris-borate buffer, 10 mM $MgCl_2$, pH 7.0.

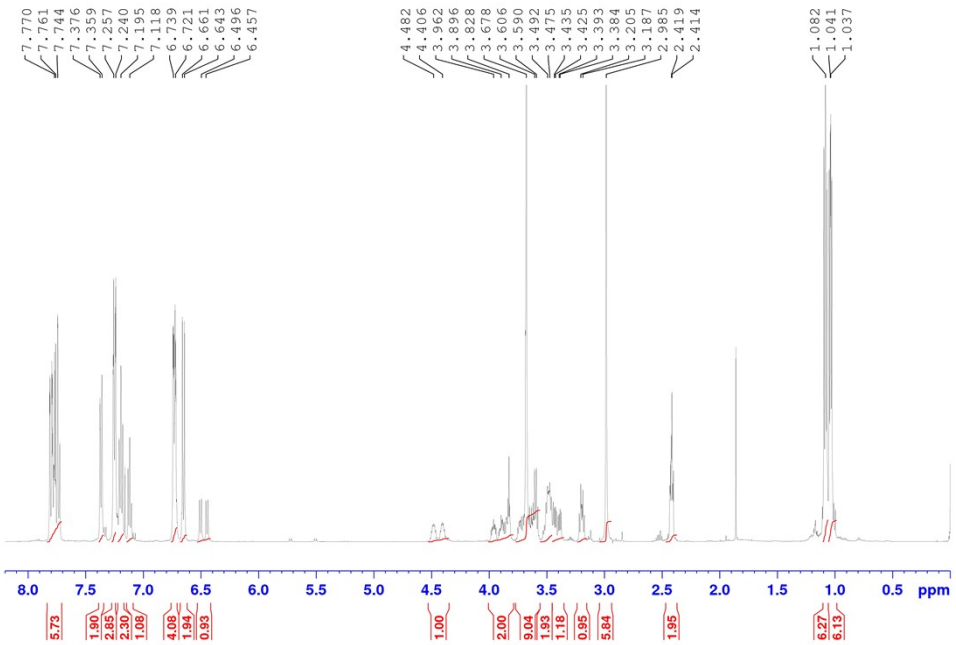


Figure S7. ^{1}H NMR (500 MHz) spectrum of compound **3** in $CDCl_3$

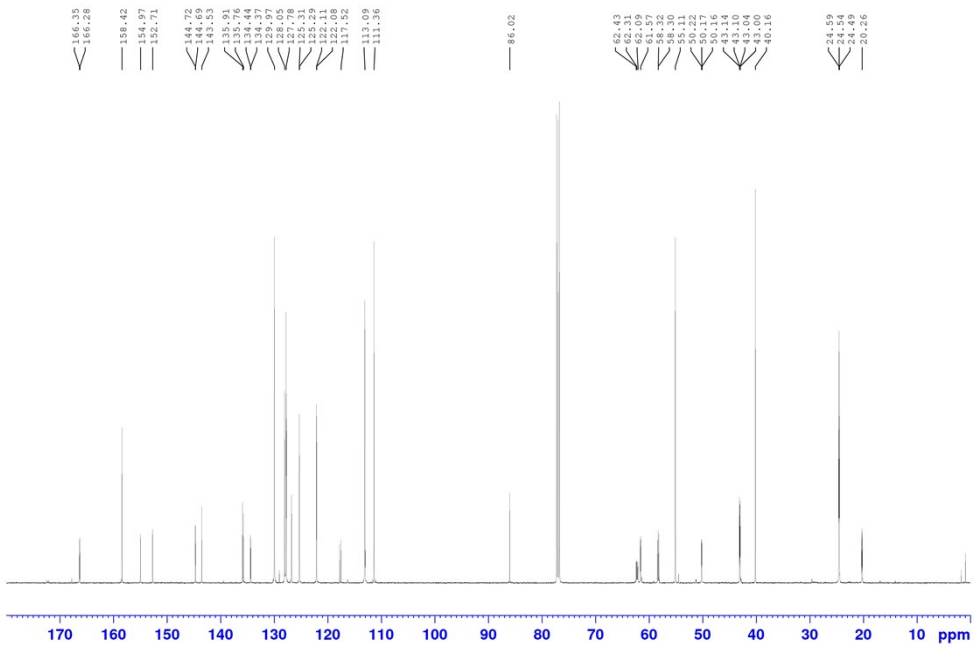


Figure S8. $^{13}C\{^{1}H\}$ NMR (125 MHz) spectrum of compound **3** in $CDCl_3$

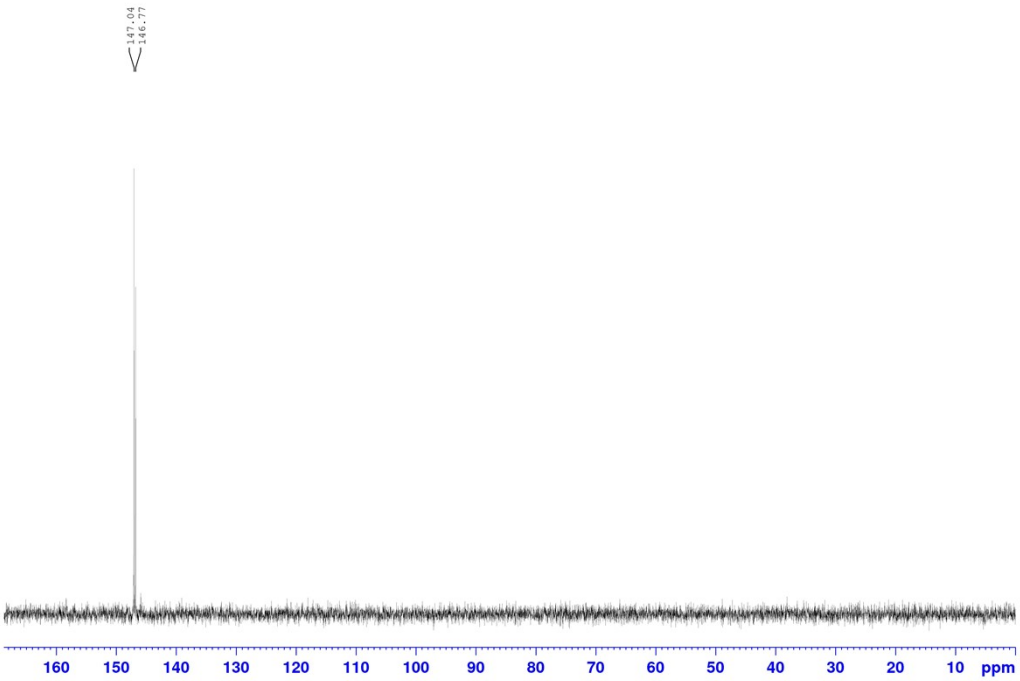


Figure S9. ^{31}P NMR (202 MHz) spectrum of compound **3** in DMSO-*d*6