Electronic Supplementary Material (ESI) for ChemComm.

Experimental Procedures

Methods

RNA Synthesis and Purification. The synthesis of 8-bromoguanosine phosphoramadite was following the previous report[1]. The synthesis of the RNAs were carried out by phosphoramidite chemistry. All RNAs were synthesized on the 1 μmol scale with an automatic DNA/RNA synthesizer (Nihon Techno Service Co., LTD.). After automated synthesis, the oligonucleotides were detached from the support and deprotected according to the manufacturer's protocol. All oligonucleotides were purified by Reverse phase-HPLC (JASCO). RNA sequences are Table S1.

CD Measurements and Analysis of CD Melting Profile. CD spectra were measured using a Jasco model J-810 CD spectrophotometer. Samples were prepared by heating the oligonucleotides at 90 °C for 5 min and gradually cooling them to room temperature. The melting curves were obtained by monitoring at 265 nm CD band. Solutions for CD spectra were prepared as 0.3 mL samples at 1 – 10 μM concentrations in the presence of 100 mM NaCl, 10 mM Tris buffer (pH 6.8).

Fluorescence measurements. Fluorescence spectra were obtained on a Spectrofluorometer FP-8200 (JASCO) with an excitation wavelength of 492 nm in 50 mM Tric-HCl buffer (pH 6.8) containing 100 mM NaCl. Emission spectra of FAM- and TAMRA-attached ORN-14 (5'-FAM-AGGGUUAGGGUUAGGGUUAGGG- TAMRA-3') (2.5 μM strand concentration) were measured from 500 nm to 700 nm with deceasing tempurture from 60 °C to 25 °C.

DMS footprint assay. Add 2 μl of carrier tRNA (5 mg/ml) to 5 μl of 3' end-labeled RNA. Mix and chill to 0°C. Then add 0.5 μl of dimethyl sulfate, mix and centrifuge quickly, and incubate at 90°C for 0.5-1 min. Chill on ice immediately and add 75 μl of cold 1.0 M Tris acetate, pH 7.5/1.0 M 2-mercaptoethanol/1.5 M sodium acetate and 900 μl of cold ethanol. Chill and pellet. Reprecipitate from 200 Ml of 0.3 M sodium acetate, then rinse with ethanol and dry. Redissolve the RNA in 10 μl of 1.0 M Tris.HCl (pH 8.2) and 10 μl of fresh 0.2 M $NaBH_4$, Incubate on ice, in the dark, for 30 min. Add 200 μl of cold 0.6 M sodium acetate/0.6 M acetic acid (pH 4.5) containing 0.025 mg of carrier tRNA per ml. Mix, and then add 600 μl of cold ethanol. Chill, pellet, rinse with ethanol, and dry. Dissolve the chemically modified RNA in 20 Ml of 1.0 M aniline/acetate buffer, pH 4.5, and incubate in the dark at 60 °C for 20 min. Terminate the reaction by freezing at -70°C and lyophilize. Redissolve the sample in 20 Ml of water, freeze, and lyophilize to dryness. Repeat the last step; then dissolve the sample in

2-3 μl of 8 M urea/20 mM Tris-HCl, pH 7.4/1 mM EDTA/0.05% xylene cyanol/0.05% bromphenol blue. Heat the samples at 90°C for 30 sec, chill them on ice immediately, and then layer them on a sequencing gel. [2]

Rnase T1 footprint assay. The Rnase T1 was purchased from ambion®, and the RNA structure analysis proceed was followed the product manual.

NMR Experiments. NMR experiments were performed a BRUKER AVANCE 600 MHz spectrometer. A special Micro Tube that is designed for use with reduced sample volumes was used (Shigemi NMR tube, Shigemi Co. Ltd., Tokyo, Japan). Spectra were recorded at 25-60 °C. RNA samples (1.1 mM) were dissolved in 0.30 mL of 90% H_2O/10% D_2O, 10 mM sodium phosphate buffer, pH 6.8, 100 mM NaCl.

Gel Electrophoresis. Denaturing gel electrophoresis experiment was performed on 10 M urea/20% polyacrylamide gel in 1 × TBE buffer.

Molecular Modeling. The model of G-quadruplex structure were manually generated in two general steps: (i) In vacuum, models were generated based on the reported G-quadruplex structure (PDB code 2aqy) using the BIOVIA Discovery Studio 2.5.

Then molecular dynamics simulations were performed by the standard dynamics cascade in BIOVIA Discovery Studio 4.5 with some modifications. the structure was heating from 50 K to 300 K over 4 ps and equilibration at 300 K with 100 ps simulation time. The save results interval in the production step was 2 ps during 100 ps simulation time at 300 K. 10 best conformations generated by simulation were further energy minimized. The conformation with lowest energy was selected as shown in Figure 4.

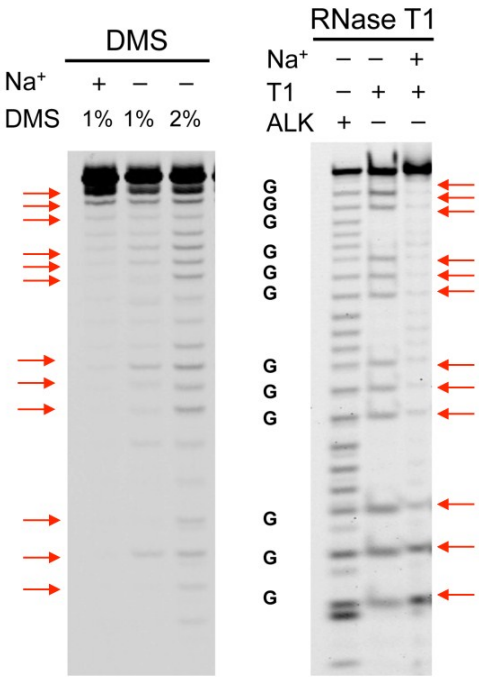

Figure S1. RNase T1 and dimethyl sulfate (DMS) footprinting assays for analysis of G-quadruplex formation. FAM-labeled ORN-N in the presence or absence of Na^+-containing binding buffer was treated with RNase T1 and DMS. ALK indicated base hydrolysis ladders. rG residues that involved in G-quadruplex are indicated by arrows.

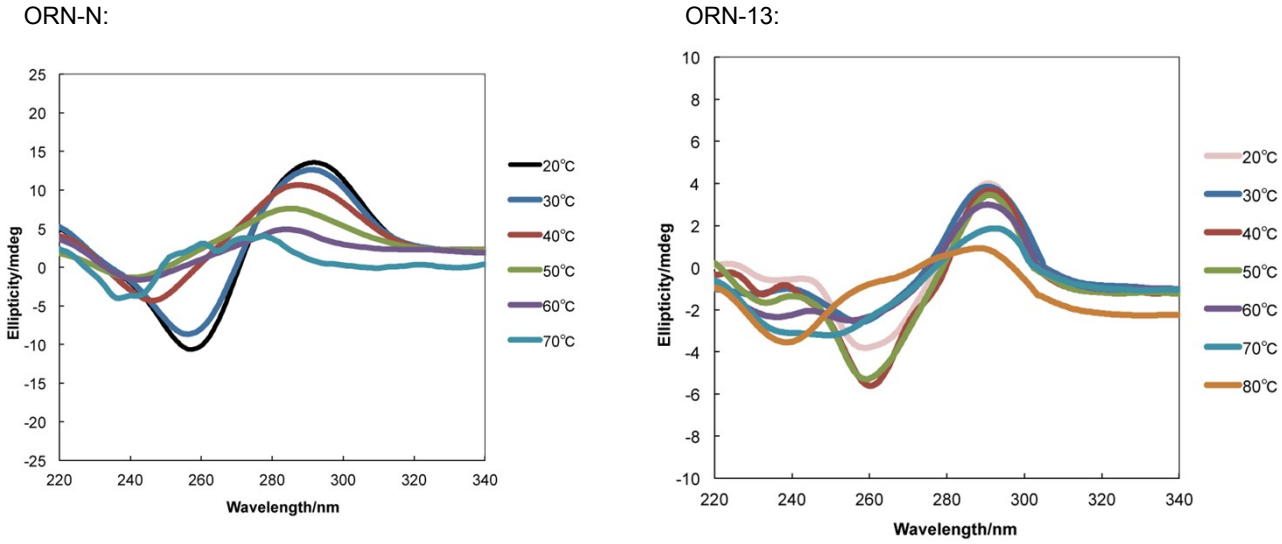

Figure S2. CD spectra of ORN-N and ORN-13 at different temperatures in the presence of 100 mM NaCl.

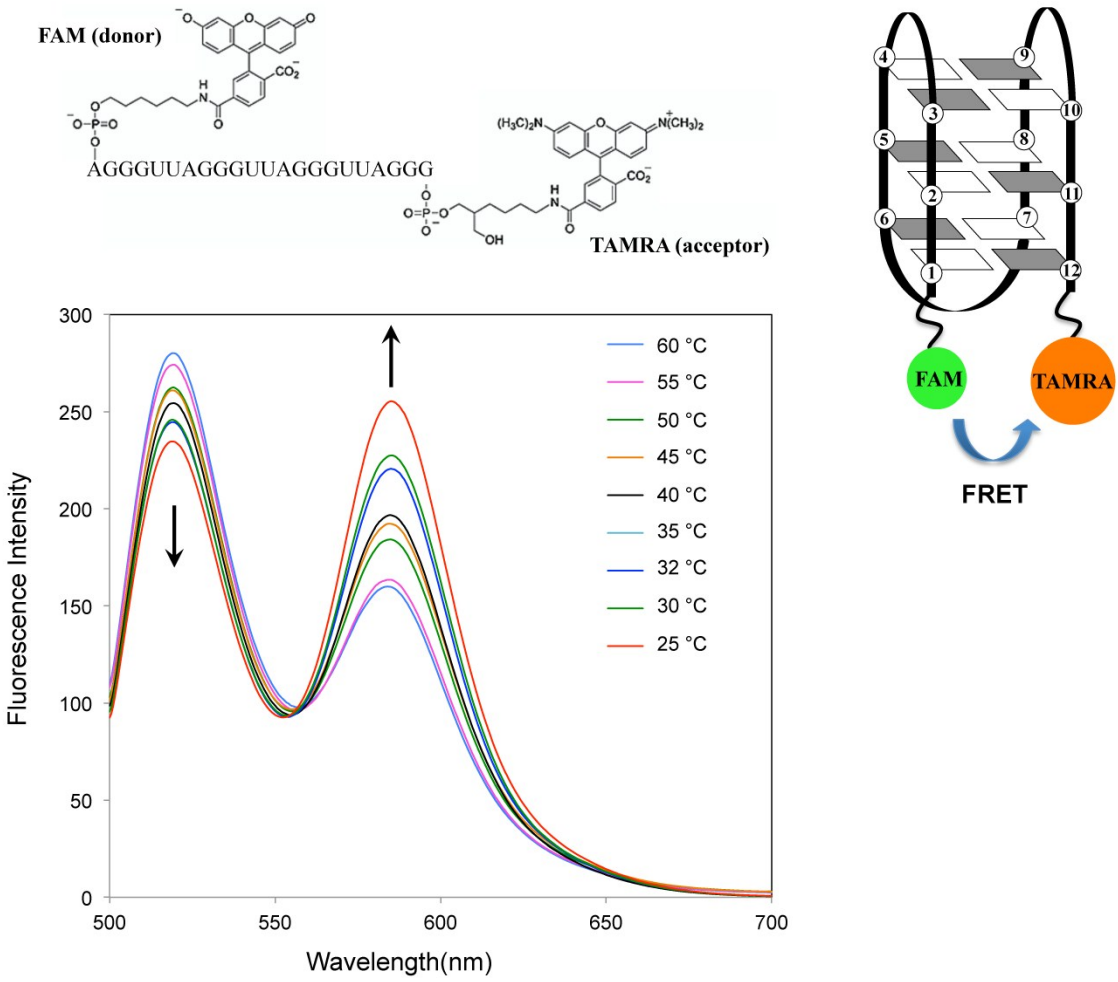

Figure S3. Chemical structure of FAM- and TAMRA-attached ORN-14 and its schematic antiparallel G-quadruplex resulting in a FRET change. Fluorescence change of FAM- and TAMRA-attached ORN-14 with deceasing tempurture from from 60 °C to 25 °C. λex = 490 nm.

Table S1. RNA sequences used in this study. The substituted 8^{Br}rG is colored in red.

Name	Sequence	rG position
ORN-N	AGGGUUAGGGUUAGGGUUAGGG	
ORN-1	A8^{Br}rGGGUUAGGGUUAGGGUUAGGG	①
ORN-2	AG8^{Br}rGGUUAGGGUUAGGGUUAGGG	②
ORN-3	AGG8^{Br}rGUUAGGGUUAGGGUUAGGG	③
ORN-4	AGGGUUA8^{Br}rGGGUUAGGGUUAGGG	④
ORN-5	AGGGUUAG8^{Br}rGGUUAGGGUUAGGG	⑤
ORN-6	AGGGUUAGG8^{Br}rGUUAGGGUUAGGG	⑥
ORN-7	AGGGUUAGGGUUA8^{Br}rGGGUUAGGG	⑦
ORN-8	AGGGUUAGGGUUAG8^{Br}rGGUUAGGG	⑧
ORN-9	AGGGUUAGGGUUAGG8^{Br}rGUUAGGG	⑨
ORN-10	AGGGUUAGGGUUAGGGUUA8^{Br}rGGG	⑩
ORN-11	AGGGUUAGGGUUAGGGUUAG8^{Br}rGG	⑪
ORN-12	AGGGUUAGGGUUAGGGUUAGG8^{Br}rG	⑫
ORN-13	A8^{Br}rG8^{Br}rGGUUA8^{Br}rGGGUUA8^{Br}rG8^{Br}rGGUUA8^{Br}rGGG	
ORN-14	FAM-AGGGUUAGGGUUAGGGUUAGGG-TAMRA	

References

1. Xiao CD.; Ishizuka T.; Xu Y., Antiparallel RNA G-quadruplex Formed by Human Telomere RNA Containing 8-Bromoguanosine. *Sci Rep*. 2017, *7*, 6695.

2. Peattie, D.A., Direct chemical method for sequencing RNA. *Proc Natl Acad Sci U S A*. 1979, *76*, 1760.