

Supporting information

Stabilization of telomeric G-quadruplex by ligand binding increases susceptibility to S1 nuclease

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Materials and methods

Stock solution of **1-3**: **1-3** was dissolved in DMSO (10 mM). Further dilution was carried out from this solution. All experiments were performed with < 0.2% DMSO. All buffers used in this study were described as below.

Tris-KCl buffer (pH 7.4)	: 50 mM Tris-HCl, 100 mM KCl (pH 7.4).
Cacodylate-KCl buffer	: 10 mM cacodylic acid-KOH, 100 mM KCl (pH 7.4).
S1 buffer	: 50 mM potassium acetate, 280 mM KCl, 4.5 mM ZnSO ₄ (pH 4.5).
DNase 1 buffer	: 40 mM Tris-HCl, 10 mM KCl, 6 mM MgCl ₂ , 1 mM CaCl ₂ (pH 7.9).
TBE buffer	: 90 mM Tris-borate, 2 mM EDTA.

Non-labelled oligonucleotides were purchased from Eurofin and 2-aminopurine modified oligonucleotides from IDT with HPLC grade and were used without further purification. S1 nuclease (Promega), and DNase 1 (Takara Bio) were purchased and used without purification. **1-3** were synthesized as previously reported.^[1,2] All other reagents were obtained in the molecular biology grades from Sigma-Aldrich, Wako chemicals, or TCI. S1 nuclease digests were separated by HPLC (column, temperature, methods) prior to MALDI-MS measurement. PAGE analysis was carried out with Mini-Protean Gel system (Bio-Rad). PAGE images were acquired on ChemiDoc XRS (Bio-Rad). Image analysis was performed using ImageJ 1.47c (National Institutes of Health, USA). CD spectra were recorded on a J-720 spectropolarimeter (JASCO) using a quartz cell of 1 mm optical path length and an instrument scanning speed of 500 nm min⁻¹ with a response time of 1 s, and over a wavelength range of 230–320 nm. The fluorescent measurement was performed with an excitation wavelength of 300 nm and a detection wavelength of 320–500 nm using the FP-8600 (JASCO). UV spectra were recorded with a V-630 spectropolarimeter (JASCO) using a quartz cell with an optical path length of 10 mm (scanning speed: 100 nm min⁻¹, wavelength range: 230–330 nm). The molar mass of the samples was detected by MALDI-TOF MS measurement (Autoflex speed, Bruker Daltonics). The measurement was performed in positive mode. 3-hydroxypicolinic acid was used as the matrix.

S1 Nuclease digestion

(a) HT24, HT19 and ss24 (8 μ M, 5 μ L) pre-annealed in Tris-KCl buffer (pH 7.4) were incubated with L2H2-6OTDs (**1-3**) (20 or 100 μ M, 4 μ L in Tris-KCl (pH 7.4)) and incubated for 1 h at 37 °C. The pre-treated oligonucleotides with the L2H2-6OTDs were subjected to S1 nuclease reaction in S1 buffer (0.8 U/ μ L S1 nuclease, 20 mM Tris-KCl, 320 mM KCl, 50 mM potassium acetate, 4.5 mM ZnSO₄ (pH 5.1) in 20 μ L) and incubated for 1–60 min at 37 °C. The reaction was stopped by the addition of EDTA (50 mM EDTA, 6 μ L).

For PAGE analysis: The reaction mixture (2 μ L) and urea (10 M, 8 μ L) was mixed and heated to 80 °C for 5 min then immediately loaded onto a denaturing polyacrylamide gel (7 M urea, 18% acrylamide, 2% bisacrylamide gel in TBE buffer) and the gels were run at 60 °C. The gels were stained with SYBR-gold to visualize the undigested oligonucleotides.

For MALDI-MS analysis: To the reaction mixture (8 μ L), the same amount of MeOH was added and centrifuged at 15,000 G for 10 min to remove proteins, then the supernatant was transfer to a new 0.5 mL tube. The mixture was lyophilized and dissolve in deionized water. The sample was subjected to HPLC (YMC-Triart ODS, 4.6 x 50 mm; A: 400 mM HFIP-TEA (pH 7.0) buffer, B: MeOH; 0–3 min: 7% B; 3–23 min 7–35% B; 23–23.5 35-100% B; 23.5–28 min 100% B) to purify the degraded oligonucleotides. The sample was lyophilized and dissolved in deionized water to subject to MALDI-MS.

(b) The solution of HT24 and HT24-rev (each 4 μ M, 5 μ L) were annealed in Tris-KCl buffer (pH 7.4) to form dsDNA. The dsDNA solution was incubated with 3,3-6OTD (**1**) (100 μ M, 4 μ L in Tris-KCl (pH 7.4)) and incubated for 1 h at 37 °C. The pre-treated oligonucleotides with **1** was subjected to S1 nuclease reaction in S1 buffer (0.8 U/ μ L S1 nuclease, 20 mM Tris-KCl, 320 mM KCl, 50 mM potassium acetate, 4.5 mM ZnSO₄ (pH 5.1) in 20 μ L) and incubated for 1–60 min at 37 °C. The reaction was stopped by the addition of EDTA (50 mM EDTA, 6 μ L). The reaction mixture (4 μ L) and ficoll 400 (100 mg/mL, 1 μ L) was mixed and loaded onto native polyacrylamide gel (12% acrylamide, 0.32% bisacrylamide gel in TBE buffer) and the gels were run at 4 °C. The gels were stained with ethidium bromide to visualize the dsDNA.

Circular dichroism (CD) spectrometry

CD spectra were recorded on a JASCO-810 spectropolarimeter (Jasco, Easton, MD) using a quartz cell of 1 mm optical path length and an instrument scanning speed of 100 nm/min with a response time of 1 s, and over a wavelength range of 230-320 nm. CD spectra are representative of ten averaged scans taken at 37 °C. The nucleotide, HT24 was dissolved in MilliQ water as a

100 μM stock solution to be used without further purification. Further dilution of the nucleotide was 100 mM KCl, 50 mM Tris-HCl buffer (pH 7.2) and CD spectra were performed with a 10 μM oligonucleotide solution. The solution was annealed by heating at 95 $^{\circ}\text{C}$ for 5 min, then slowly cooled to room temperature. 190 μL of the nucleotide solution and 10 μL of the compound solution (in 1% DMSO) were mixed and injected into a quartz cell.

2-AP titration analysis

The 2-aminopurine quenching fluorescence analysis was performed with an excitation wavelength of 300 nm and a detection wavelength of 320-520 nm using the FP-8600 (JASCO, Japan). The labeled oligonucleotides (Table S1) were dissolved in MilliQ water as stock solutions (100 μM) to be used without further purification. The oligonucleotides were denatured at 95 $^{\circ}\text{C}$ for 5 min and annealed in Tris-KCl buffer. The solution of 3,3-6OTD (**1**) (400 μM in Tris-KCl buffer, 4% DMSO) was titrated (1-40 μM) to the oligonucleotide solution (2 μM , 1 mL). The solution was incubated at 25 $^{\circ}\text{C}$ for 30 min at each concentration. The fluorescent spectrum of 2-aminopurine was taken three times at 25 $^{\circ}\text{C}$, and the spectra are average of three scans.

DNase 1 digestion

Oligonucleotides HT24 and SS24 (8 μM , 5 μL) pre-annealed in Tris-KCl buffer were incubated with (100 μM , 4 μL) of 3,3-6OTD (**1**) and incubated for 1 h at 37 $^{\circ}\text{C}$. To the mixture, DNase 1 diluted by DNase 1 buffer (0.9 U/ μL , 11 μL) was added and incubated for 1-60 min at 37 $^{\circ}\text{C}$. The reaction was stopped by heating at 80 $^{\circ}\text{C}$ for 15 min. The reaction mixture (2 μL) and urea (10 M, 8 μL) was mixed and heated to 80 $^{\circ}\text{C}$ for 5 min then immediately loaded onto a denaturing polyacrylamide gel (7 M urea, 18% acrylamide, 2% bisacrylamide gel in TBE buffer) and the gels were run at 60 $^{\circ}\text{C}$. The gels were stained with SYBR-gold to visualize the resulted oligonucleotides.

UV measurement

In the UV experiment, oligonucleotides were dissolved in Cacodylate-KCl buffer (pH 7.4) to give a final concentration of 5 μM , respectively. The samples were annealed at 95 $^{\circ}\text{C}$ for 5 min followed by slow cooling to 25 $^{\circ}\text{C}$. UV spectra were recorded with a V-630 spectropolarimeter (JASCO) using a quartz cell with an optical path length of 10 mm (scanning speed: 100 nm min^{-1} , wavelength range: 230-330 nm). The melting profiles at 295 nm were recorded from 15 to 95 $^{\circ}\text{C}$ at a scan rate of 1.0 $^{\circ}\text{C}/\text{min}$. Each data point was normalized to the absorbance which corresponds

to HT 24 or HT19 without ligands at 95 °C where all quadruplex and triplex were unfolded. The T_m values correspond to $\theta = 0.5$.

Oligonucleotide Sequences

Table S1. Oligonucleotide sequences used in this study.

Oligonucleotides	Sequence
HT24	d[TTG GGT TAG GGT TAG GGT TAG GGA]
HT24-8 ^{AP}	d[TTG GGT T ^{AP} G GGT TAG GGT TAG GGA]
HT24-14 ^{AP}	d[TTG GGT TAG GGT T ^{AP} G GGT TAG GGA]
HT24-20 ^{AP}	d[TTG GGT TAG GGT TAG GGT T ^{AP} G GGA]
HT11	d[TTG GGT TAG GG]
HT13	d[TTG GGT TAG GGT T]
HT19	d[TTG GGT TAG GGT TAG GGT T]
SS24	d[TTA GAG TTA GAG TTA GAG TTA GAG]
HT24-rev	d[TCC CTA ACC CTA ACC CTA ACC CAA]

^{AP} stands for 2-aminopurine substitution.

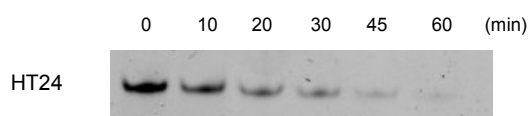


Fig. S1 Denaturing PAGE analysis for S1 treatment of HT24 (2 μ M) in the absence of ligands at each reaction time point.

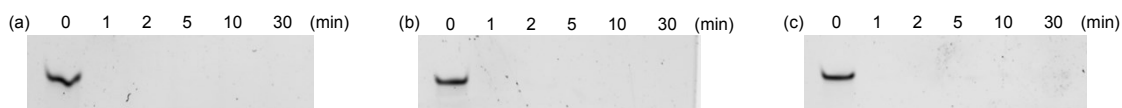


Fig. S2 Denaturing PAGE analysis for S1 treatment of SS24 (2 μ M) in the absence or presence of ligands **1-3** (a: **1**, b: **2**, c: **3**) at each reaction time point.

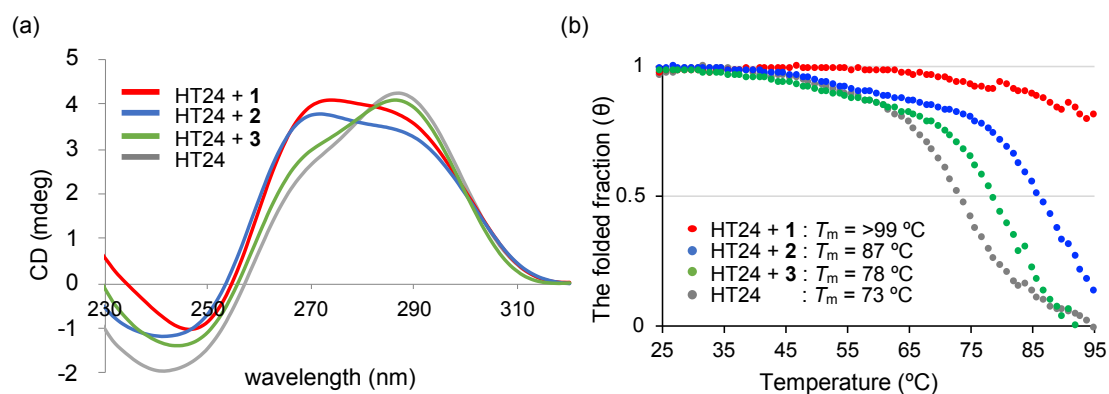


Fig. S3 (a) CD spectrometry and (b) UV-melting (295 nm) of HT24 under the acidic condition (pH 5.1) in which S1 nuclease reaction was carried out (20 mM Tris-KCl, 320 mM KCl, 50 mM potassium acetate, 4.5 mM ZnSO₄ (pH 5.1)) in the presence of **1–3**.

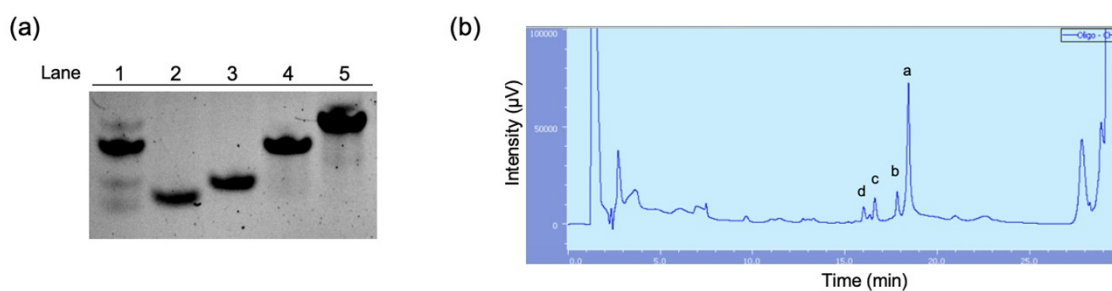


Fig. S4 (a) Denaturing PAGE of S1 nuclease digests of HT24 with 3,3-6OTD (20 μM). Lane 1: HT24 treated by S1 nuclease with **1** for 60 min and lane 2–5: HT11, 13, 19, and 24 as markers. (b) HPLC analysis of S1 nuclease digests of HT24.

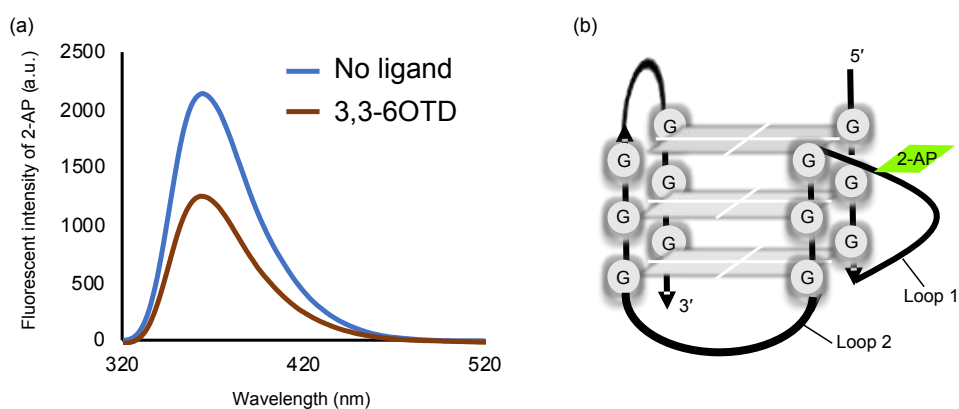


Fig. S5 (a) Fluorescent spectrometry of 2-aminopurine in HT24-8^{AP} (2 μM in Tris-KCl

buffer) in the presence of 3,3-6OTD (**1**, 20 μM). (b) Schematic structure of HT24 to show the position of 2-aminopurine substitution in HT24-8^{AP}.

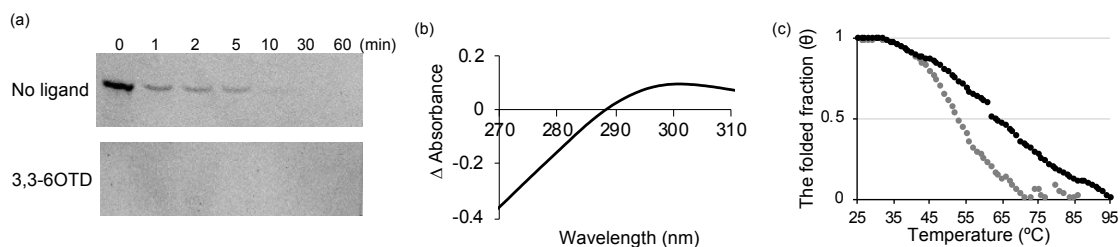


Fig. S6 (a) Denaturing PAGE analysis for S1 treatment of HT19 (2 μM) in the absence or presence of 3,3-6OTD (10 μM) at each reaction time point. (b) The isothermal difference spectra (IDS) of HT19 in the presence or absence of 3,3-6OTD (**1**, 25 μM) in cacodylate-KCl buffer (pH 5.1). (c) UV melting at 295 nm (gray dots: no ligand, black dots: 25 μM 3,3-6OTD (**1**)) in cacodylate-KCl buffer.

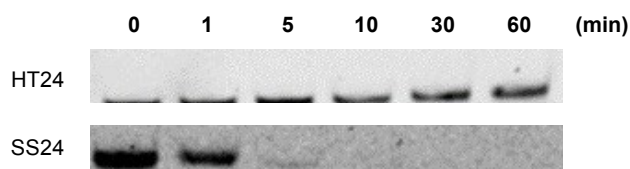


Fig. S7 Denaturing PAGE analysis for DNase 1 treatment of HT24 (2 μM) or SS24 (2 μM) in the presence of 3,3-6OTD (**1**, 20 μM).

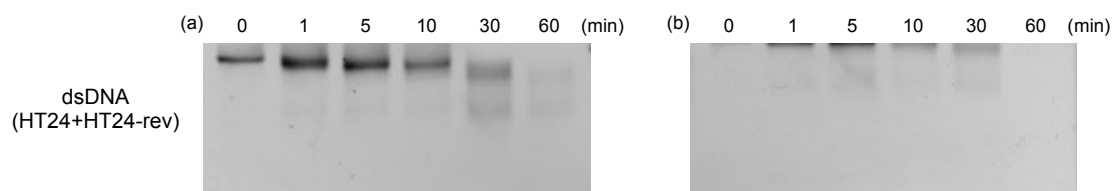


Fig. S8 Native PAGE analysis for S1 treatment of dsDNA (HT24+HT24-rev; 2 μM) in the absence (a) or presence (b) of 3,3-6OTD (**1**, 20 μM) at each reaction time point.

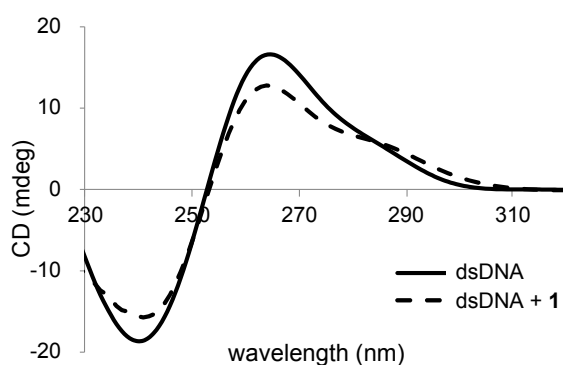


Fig. S9 CD spectrometry of dsDNA (HT24+HT24-rev; 2 μ M) under the acidic condition (pH 5.1) in which S1 nuclease reaction was carried out (20 mM Tris-KCl, 320 mM KCl, 50 mM potassium acetate, 4.5 mM ZnSO₄ (pH 5.1)) in the presence (dashed line) or absence (solid line) of **1**.

Supplementary Reference

- [1] M. Sakuma, Y. Ma, Y. Tsushima, K. Iida, T. Hirokawa, K. Nagasawa, *Org. Biomol. Chem.*, **2016**, *14*, 5109-5116.
- [2] M. Tera, H. Ishizuka, M. Takagi, M. Suganuma, K. Shin-ya and K. Nagasawa, *Angew. Chem. Int. Ed.*, **2008**, *47*, 5557-5560.