Supporting Information

Selective alkylation of parallel G-quadruplex structure

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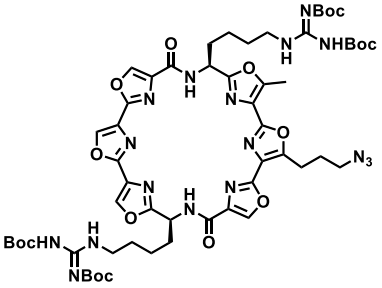
Materials and methods

The general chemicals were purchased from FUJIFILM Wako Pure Chemical, the Tokyo Chemical Industry, Kanto Chemical or Aldrich. The target DNAs and RNAs were purchased from JBioS (Japan). The ^{1}H NMR spectra (400 or 600 MHz) were recorded by a JEOL JNM-ECX 400 or Bruker AVANCE III 600 spectrometer. The ^{13}C NMR spectra (125 or 151 MHz) were recorded by a JNM-ECA 500 or Bruker AVANCE III 600 spectrometer. The high resolution electrospray mass analysis was performed by a JEOL JMS-T100LC or Bruker MicrOTOF-Q II. The HPLC purification was performed by a JASCO HPLC System (PU-2089Plus, UV-2075Plus and CO-2065Plus). A reverse-phase C_{18} column (COSMOSIL 5C_{18}-AR-II, Nacalai tesque, 4.6×250 mm or 10×250 mm). MALDI-TOF MS measurements were performed by a Bruker Autoflex speed instrument using a 3-hydroxypicolinic acid/diammonium hydrogen citrate matrix.

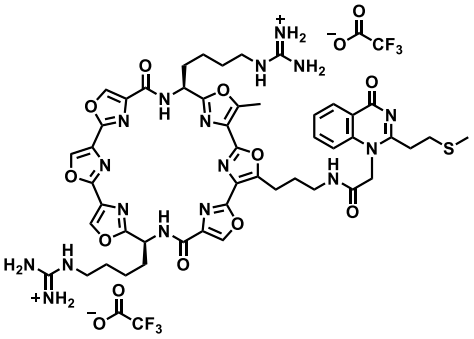
Experimental procedures

L2G2-6OTD-1M1PA-N_3 (1)



^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 11.4 (2H, br), 9.11 (1H, s), 9.07 (1H, s), 8.90 (1H, s) 8.85-8.83 (1H, m), 8.36-8.30 (2H, m), 8.24 (2H, t, J = 5.6 Hz), 5.46 (1H, dt, J = 12.4, 7.3 Hz), 5.36 (1H, dt, J = 12.4, 7.3 Hz), 3.48 (2H, t, J = 6.4 Hz), 3.24-3.19 (6H, m), 2.71 (3H, s), 2.08-1.88 (6H, m), 1.54-1.30 (44H, m); ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm)164.5, 163.1, 162.3, 162.1, 158.9, 158.7, 155.6, 155.4, 155.1, 154.5, 153.2, 152.0, 151.4, 142.4, 142.2, 141.6, 140.9, 136.1, 135.9, 129.8, 128.5, 124.7, 123.7, 82.7, 79.2, 78.0, 59.7, 49.8, 47.3, 47.2, 35.8, 33.3, 30.7, 28.1, 27.9, 27.5, 26.5, 14.1, 11.5. ESI-HRMS (m/z): $[M+Na]^+$ calcd for $C_{56}H_{73}N_{17}O_{16}Na^+$, 1262.5319; found 1262.5284.

Synthesis of L2G2-6OTD-1M1PA-VQ-SMe (6OTD-VQ-SMe)



To a solution of the **compound (1)** (22 mg, 17.7 μmol) in THF-MeOH (1:1, 0.5 mL) was added $Pd(OH)_2$/C (10 mg, 50 wt%), and the reaction mixture was stirred at room temperature under hydrogen atmosphere. After stirred for 2 h, the reaction mixture was filtered through a pad of Celite® and eluted with $CHCl_3$-MeOH (9:1). The solution was concentrated *in vacuo* to give amine, which was used without further purification. To a mixture of the amine (4.3 mg, 3.5 μmol) and **(2)** (1.5 mg, 5.4 μmol) in DMF (0.3 mL) were added DIPEA (6 μL, 3.5 μmol), HOBt (0.7 mg, 5.2 μmol), HBTU (2.0 mg, 5.2 μmol), and the reaction mixture was stirred at room temperature. After stirred for 4 h, the reaction mixture was diluted with EtOAc (10 mL), and then washed with water (10 mL), sat. aq. NH_4Cl (10 mL) and brine (10 mL). The organic layer was separated and dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography ($CHCl_3$: MeOH= 10:1) to afford compound **3-SMe** as a yellow solid (4.6 mg). This solid was dissolved in DCM (0.1 mL) and to this solution were added triethylsilane (2.5 μL, 15.6 μmol) and TFA (0.9 mL). The reaction mixture was stirred at room temperature for 1h. The reaction mixture was then concentrated and co-evaporated with acetonitrile five times. The residue was purified by reverse phased HPLC by a liner gradient of 15-60%/40min acetonitrile in 0.1% TFA buffer at a flow rate of 4 mL/min at 40 °C, and monitored by UV detection at λ = 254 nm to afford the desired product as a white solid. The concentration of **6OTD-VQ-SMe** solution (280 μL of DMSO) was determined by quantitative ^{1}H-NMR using maleic acid as an internal standard (6.63 mM, 28%).

^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 9.08 (1H, s), 9.07 (1H, s), 8.88 (1H, s), 8.82 (1H, s) 8.64 (1H, t, J = 6.0 Hz), 8.29 (1H, d, J = 7.2 Hz), 8.25 (1H, d, J = 7.2 Hz), 8.07 (1H, d, J = 7.8 Hz), 7.75 (1H, t, J = 7.8 Hz), 7.49-7.46 (4H, m), 7.20 (8H, br), 5.42 (1H, q, J = 7.2 Hz), 5.32 (1H, q, J = 7.2 Hz), 4.98 (2H, s), 3.25 (2H, m), 3.16 (2H, m), 3.10-3.02 (4H, m), 2.86 (2H, t, J = 7.2 Hz), 2.63 (3H, s), 2.06 (3H, s), 2.05 (4H, m), 1.95-1.89 (4H, m), 1.5-1.3 (6H, m), 1.20-1.15 (2H, m). ^{13}C NMR (DMSO-d_6, 151 MHz) δ (ppm) 166.4, 164.8, 163.5, 162.4, 159.2, 159.1, 156.9, 155.9, 155.8, 154.9, 154.1, 151.9, 142.8, 142.6, 142.0, 141.4, 136.3, 136.1, 134.5, 130.0, 128.8, 127.7, 126.2, 124.9, 124.0, 119.6, 117.8, 115.9, 49.2, 47.6, 47.5, 40.9, 34.6, 33.8, 30.2, 28.4, 27.3, 23.1, 21.5, 21.4, 15.1, 11.8, 1.43. ESI-HRMS (*m/z*): $[M+2H]^{2+}$ calcd for $C_{49}H_{57}N_{17}O_{10}S^{2+}$, 537.7092; found 537.7081.

Synthesis of L2G2-6OTD-1M1PA-VQ-S(O)Me (6OTD-VQ-S(O)Me)

To a solution of **6OTD-VQ-SMe** (1.25 nmol) in DMSO (0.25 μL) was added a solution of MMPP (5 nmol) in water (4.75 μL) and the mixture was rest at room temperature for 1 min to afford compound **6OTD-VQ-S(O)Me**. This was used for alkylation reactions without further purification. For ESI-MS measurement, the **6OTD-VQ-S(O)Me** was purified by reverse phased HPLC. ESI-HRMS (*m/z*): $[M+2H]^{2+}$ calcd for $C_{49}H_{57}N_{17}O_{11}S^{2+}$, 545.7067; found 545.7066.

Annealing procedure

DNA and/or RNA (50 μM) were diluted in these following 1x buffers (50 μL).
K^+ buffer: 100 mM KCl, 10 mM K_2HPO_4/KH_2PO_4 (pH 7.0), 1 mM K_2EDTA.
Na^+ buffer: 100 mM NaCl, 10 mM Na_2HPO_4/NaH_2PO_4 (pH 7.0), 1 mM Na_2EDTA.
To perform the thermal annealing of DNA and/or RNA, the DNA solution was heated at 90 °C for 10 min and cooled from 90 °C to 20 °C at a rate of 0.5 °C/min by a thermal cycler.

Alkylation of targets.

A solution (10 μL) of **6OTD-VQ-S(O)Me** (0.5 μM), and DNA or RNA (0.25 μM) in phosphate buffer (pH 7.0, K^+ or Na^+) containing 2% DMSO was incubated at 37 °C. Aliquots (1.5 μL) of the reaction mixture was collected at various point of time and quenched by addition of loading buffer (3.0 μL, 95% formamide, 50 mM EDTA pH 8.0, 0.05% bromophenol blue (BPB), 0.05% xylene cyanol FF) and cooled the mixture to 0 °C. PAGE was performed on a 16% polyacrylamide gel electrophoresis containing 20% formamide with 1× TBE and 6.0 M urea at 500V for 90 min for G-4. The fluorescence labelled targets were visualized and quantified with FLA-5100 (Fujifilm Co., Tokyo, Japan).

Purification of the alkylated HT-G4-RNA

The separation of the alkylated HT-G4-RNA was performed by PAGE. The alkylated gel bands were cut and crushed, and the RNAs were extracted by an elution buffer (200 mM NaCl and 10 mM EDTA (pH 8)). The extracted RNAs were desalted by Sep-Pak (Waters) and the alkylated RNA products were confirmed by MALDI-TOF mass spectroscopy measurement (Fig. S1).
Mono-alkylated RNA (*) $[M-H]^-$: calcd. 9098.4 found 9097.4.
Di-alkylated RNA (**)$[M-H]^-$: calcd. 10123.8, found 10121.2.

CD measurements

A solution (100 μL) of G4-DNA or RNA (1.0 μM) and **6OTD-VQ-SMe** (0 to 10 μM) in 1× K^+ or Na^+ buffer (containing 2% DMSO, pH 7.0) were transferred to a cylindrical micro cell with a 1-cm path length. The measurement was performed at 25 °C by a J-720WI (JASCO Co., Hachioji, Japan) equipped with a Peltier temperature controller. For the melting temperature measurement, CD changes at the fixed wavelength were recorded from 25 °C to 90 °C using G4-DNA or RNA (1.0 μM) and **6OTD-VQ-SMe** (0 or 2.0 μM) at an interval of 1 or 2 °C, with temperature increase at a rate of 1 °C/ min. The measurements were repeated three times.

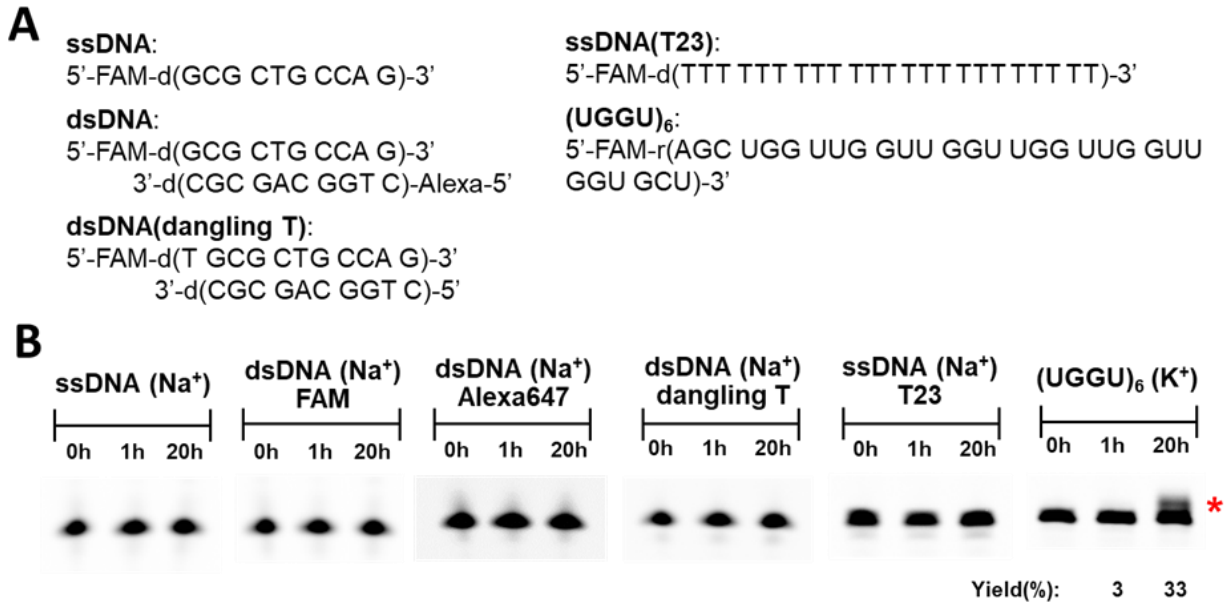


Figure S1. Alkylation screening using **6OTD-VQ-S(O)Me**. (A) Sequences used for alkylation screening. (B) PAGE analysis. The alkylation was carried out using **6OTD-VQ-S(O)Me** (0.50 μM) and target DNA or RNA (0.25 μM) in phosphate buffer (pH 7.0) at 37 ºC. The electrophoresis was performed on a 16% denaturing polyacrylamide gel containing 20% formamide. The asterisk indicates the alkylated product.

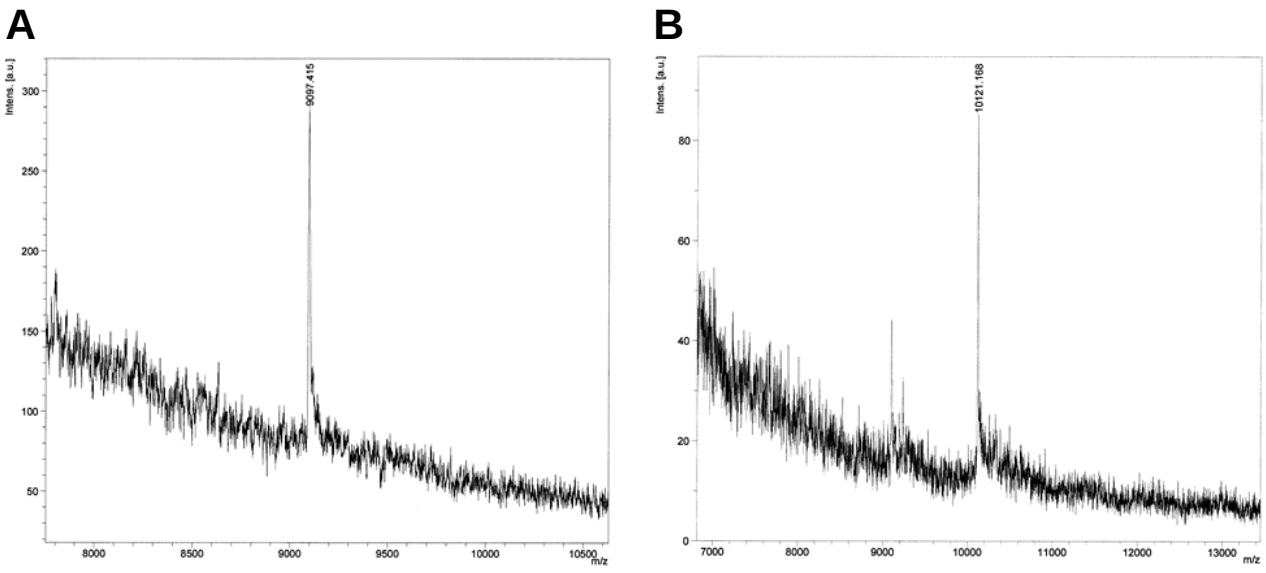


Figure S2. MALDI-TOF mass spectra of the alkylated HT-G4-RNA. (A) The mono-alkylated RNA (*). (B) The di-alkylated RNA (**).

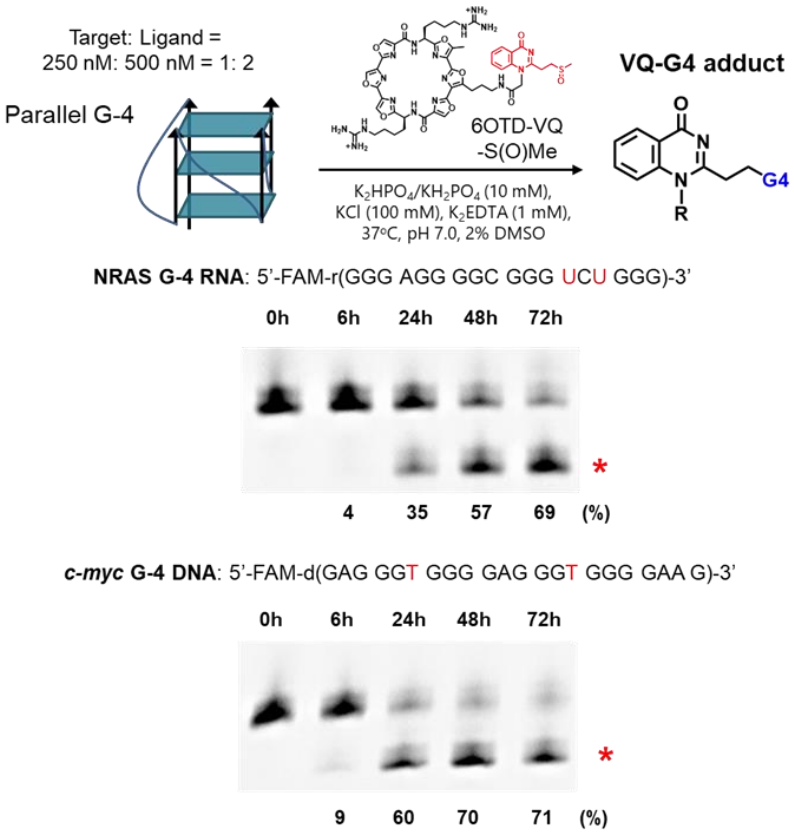


Figure S3. Parallel G-4 alkylation reactions. The ligand (500 nM) was incubated with G-quadruplex (G-4) (250 nM) at 37 °C in K^+ phosphate buffer solution (100 mM KCl, 10 mM K_2HPO_4/KH_2PO_4, 1 mM K_2EDTA, pH 7.0). The electrophoresis was performed on a 16% denaturing polyacrylamide gel containing 20% formamide.

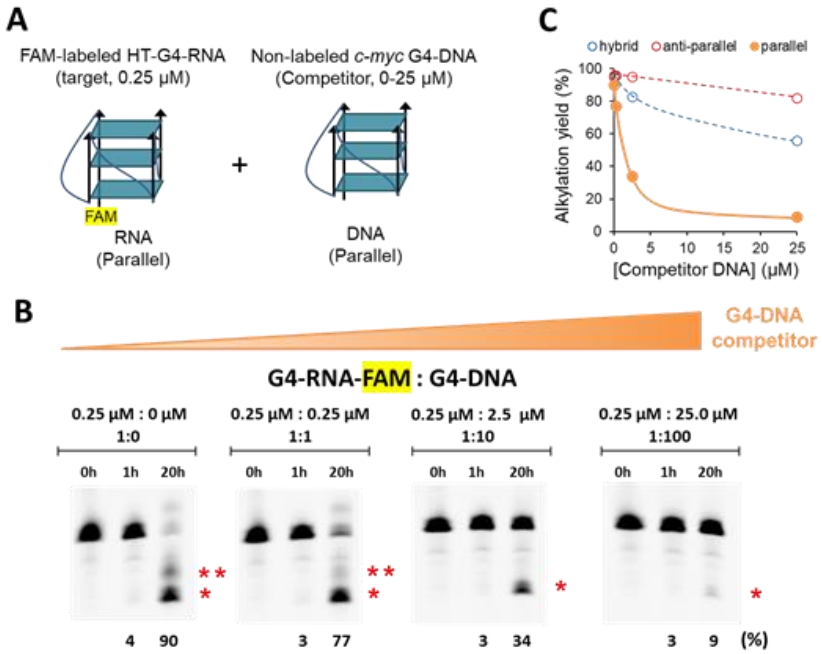


Figure S4. Competitive alkylation assay using **6OTD-VQ-S(O)Me**, FAM-labelled HT-G4-RNA and non-labelled *c-myc* G-4 DNA. (A) G-4 topologies used for the assay. (B) PAGE analysis. The alkylation was carried out using **6OTD-VQ-S(O)Me** (1.25 μM), HT-G4-RNA (0.25 μM) and *c-myc* G-4 DNA (0-25 μM) in K^+ phosphate buffer (pH 7.0). (C) The alkylation yield changes in the presence of increasing concentrations of the non-labelled G4-DNA (0-25 μM).

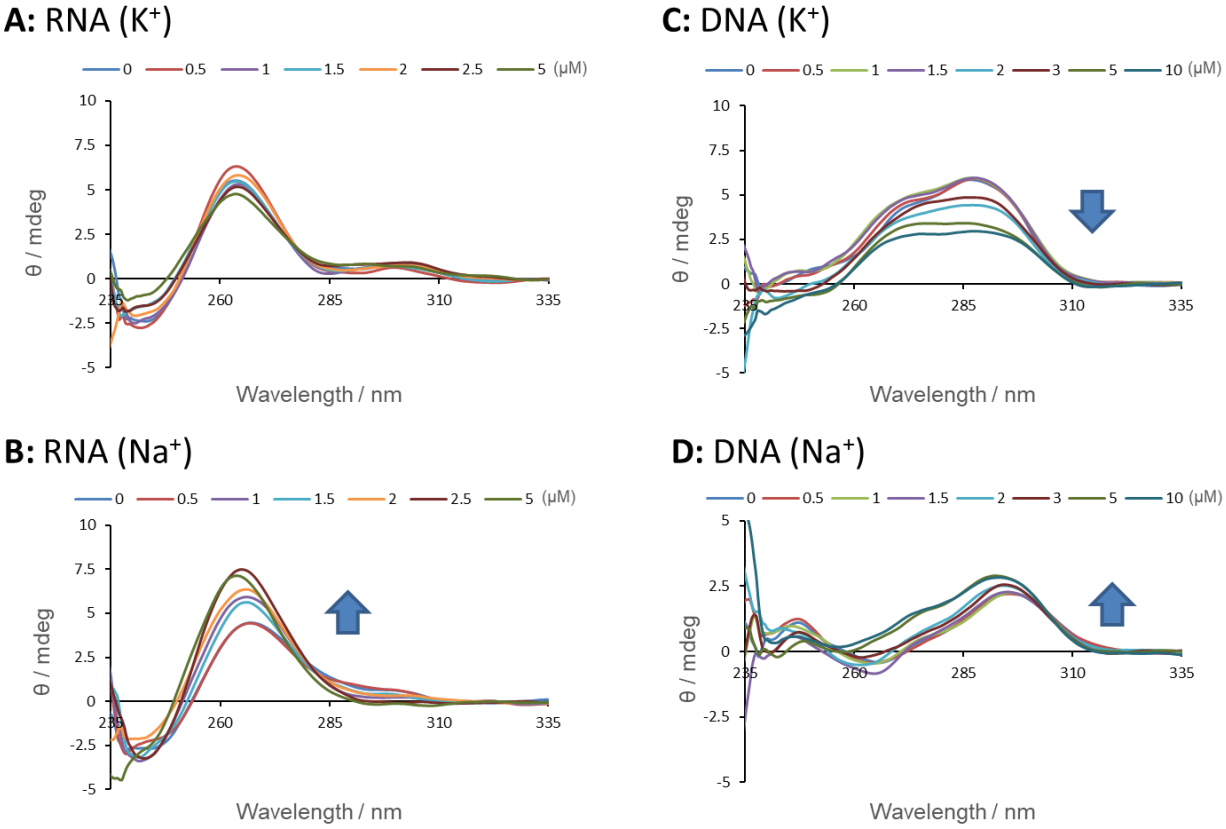


Figure S5. CD spectra of HT-G4-RNA and DNA with **6OTD-VQ-SMe**. (A) RNA in potassium phosphate buffer (K^+), (B) in sodium phosphate buffer (Na^+), (C) DNA in potassium phosphate buffer (K^+), (D) in sodium phosphate buffer (Na^+). CD spectra were measured using G-4 (1.0 μM) and **6OTD-VQ-SMe** (0 to 5.0 or 10 μM) in 1× K^+ or Na^+ buffer (containing 2% DMSO, pH 7.0) at 25 °C.

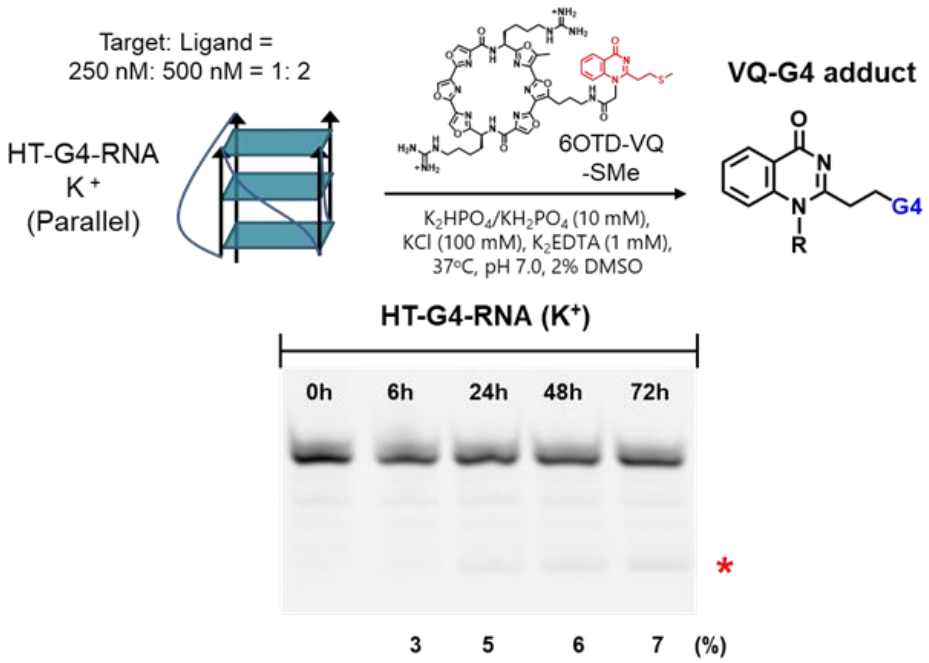


Figure S6. G-4 alkylation with **6OTD-VQ-SMe**. The ligand (500 nM) was incubated with HT-G4-RNA (250 nM) at 37 °C in the K^+ phosphate buffer solution (100 mM KCl, 10 mM K_2HPO_4/KH_2PO_4, 1 mM K_2EDTA, pH 7.0). The electrophoresis was performed on a 16% denaturing polyacrylamide gel containing 20% formamide.

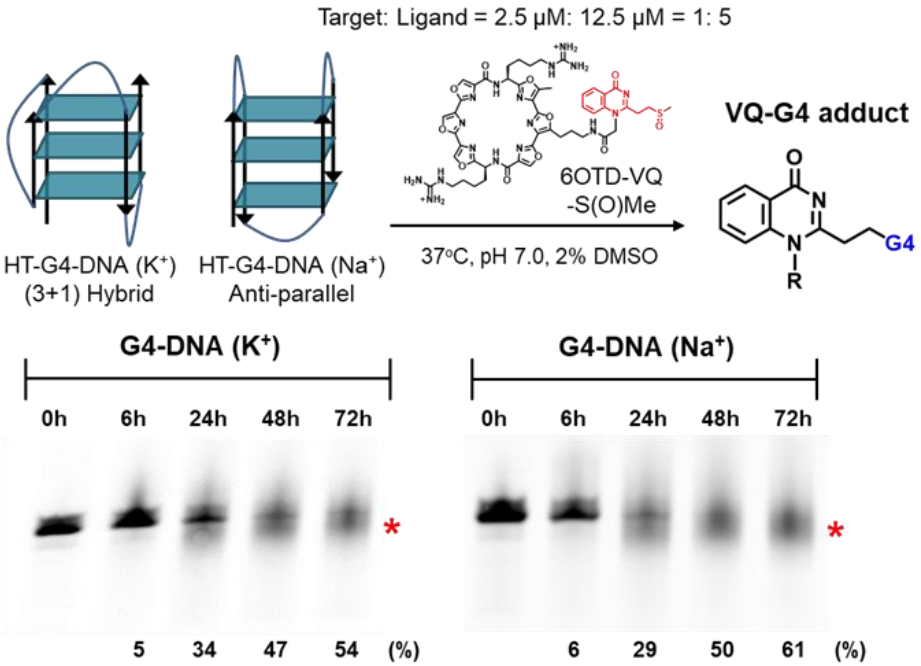


Figure S7. G-4 alkylation with a high concentration of **6OTD-VQ-S(O)Me**. The ligand (12.5 μM) was incubated with HT-G4-RNA (2.5 μM) at 37 °C in K^+ or Na^+ phosphate buffer (pH 7.0). The electrophoresis was performed on a 16% denaturing polyacrylamide gel containing 20% formamide.

Table S1. T_m values of G-4 structures with **6OTD-VQ-SMe**. [a)]

	w/o ligand (°C)	with ligand (°C)	ΔT_m
DNA (K^+), 286 nm	69	70	+1
DNA (Na^+), 295 nm	61	62	+1
RNA (K^+), 264 nm	81	85	+4
RNA (Na^+), 266 nm	54	57	+3

a) T_m values was measured by CD changes using G4-DNA or RNA (1.0 μM) and 6OTD-VQ-SMe (0 or 2.0 μM) in K^+ or Na^+ phosphate buffer (pH 7.0).

Table S2. T_m values of the alkylated G-4 structures. [a)]

	SMe (°C)	S(O)Me, 24 h (°C)	ΔT_m
RNA (K^+), 6OTD, 264 nm	85	85	0
RNA (Na^+), 6OTD 266 nm	57	58	+1

a) The alkylated G4 was prepared using **6OTD-VQ-S(O)Me** (2.0 μM) and HT-G4-RNA (1.0 μM) in K^+ or Na^+ phosphate buffer (pH 7.0) at 37 °C for 24 h, and the solution was directly used for T_m measurements.

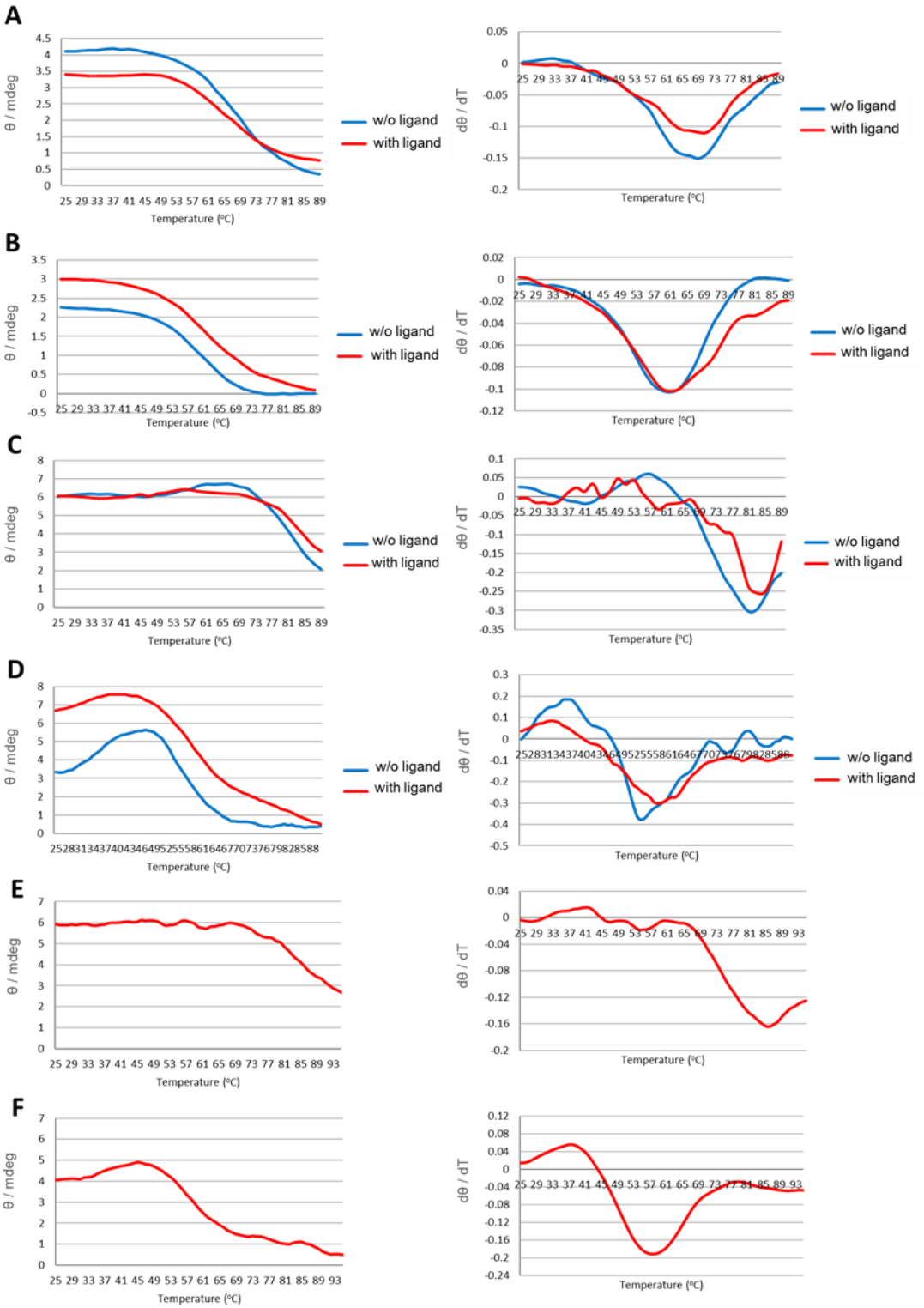


Figure S8. T_m measurements of G-4 structures with **6OTD-VQ-SMe** and alkylated G4 structures. The CD melting curves (left) and determination of T_m values (right). (A) HT-G4-DNA and **6OTD-VQ-SMe** in K^+ phosphate buffer, (B) HT-G4-DNA and **6OTD-VQ-SMe** in Na^+ phosphate buffer, (C) HT-G4-RNA and **6OTD-VQ-SMe** in K^+ phosphate buffer, (D) HT-G4-RNA and **6OTD-VQ-SMe** in Na^+ phosphate buffer, (E) the alkylated HT-G4-RNA with **6OTD-VQ-S(O)Me** in K^+ phosphate buffer, (F) the alkylated HT-G4-RNA with **6OTD-VQ-S(O)Me** in Na^+ phosphate buffer. The alkylated G4 was prepared using **6OTD-VQ-S(O)Me** (2.0 μM) and HT-G4-RNA (1.0 μM) in phosphate buffer (pH 7.0) at 37 ºC for 24 h, and the solution was directly used for T_m measurements.

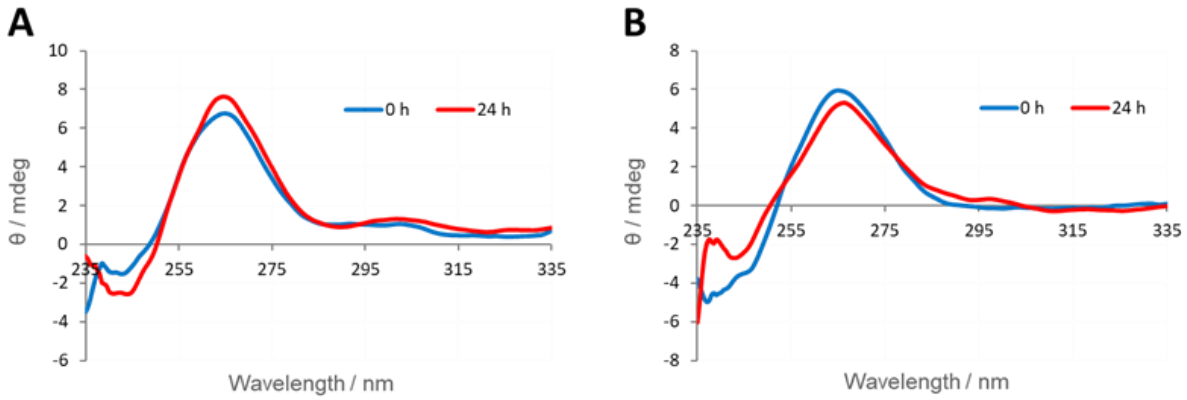
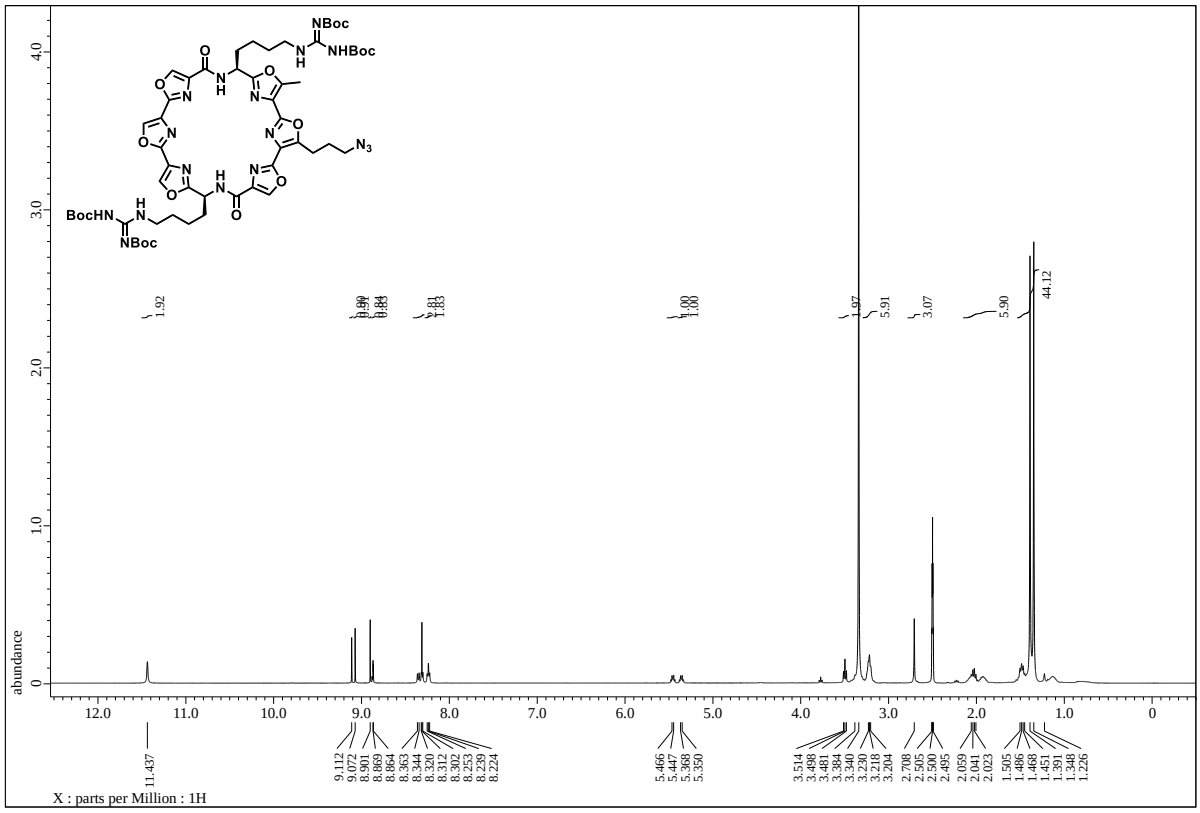


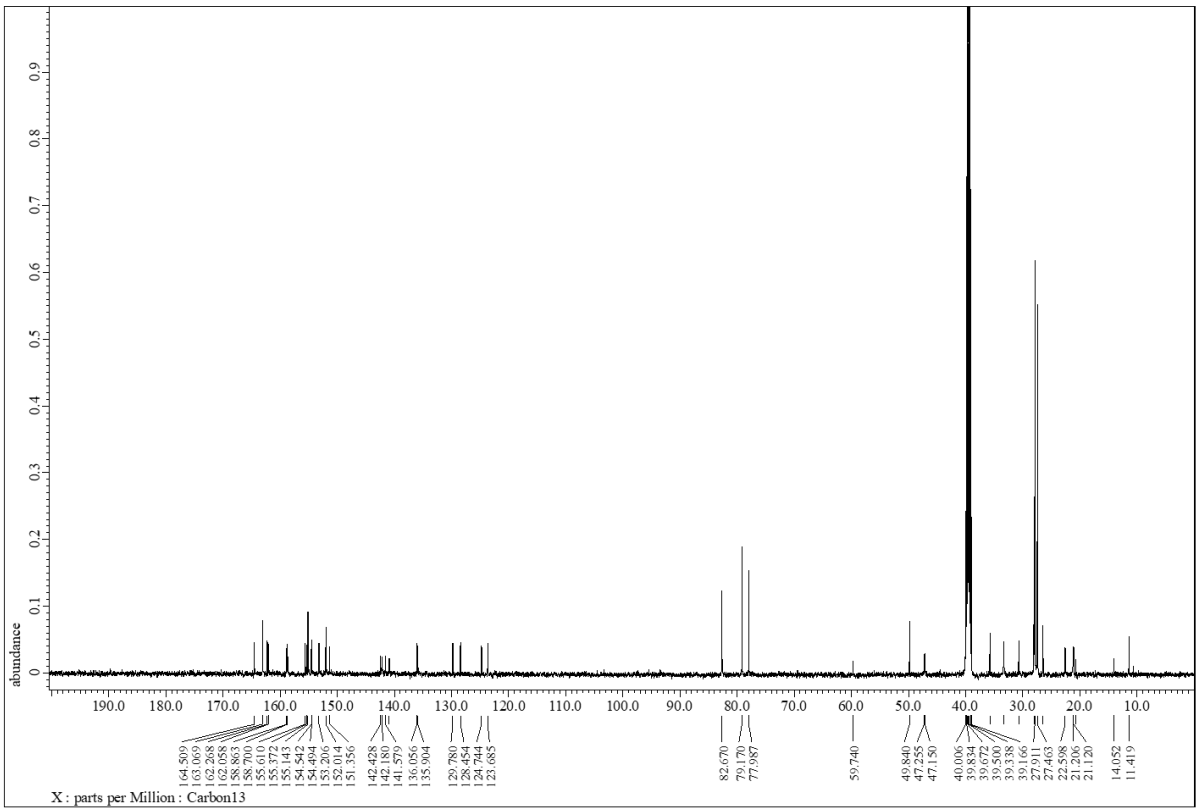
Figure S9. CD spectra of HT-G4-RNA before and after the alkylation with **6OTD-VQ-S(O)Me**. The alkylated G4 was prepared using **6OTD-VQ-S(O)Me** (2.0 μM) and HT-G4-RNA (1.0 μM) in phosphate buffer (pH 7.0) at 37 °C for 24 h, and the solution was directly used for CD measurements. CD spectra were measured using HT-G4-RNA (1.0 μM) and **6OTD-VQ-S(O)Me** (2.0 μM) in (A) K^+ or (B) Na^+ phosphate buffer (containing 2% DMSO, pH 7.0) at 25 °C.

NMR Data

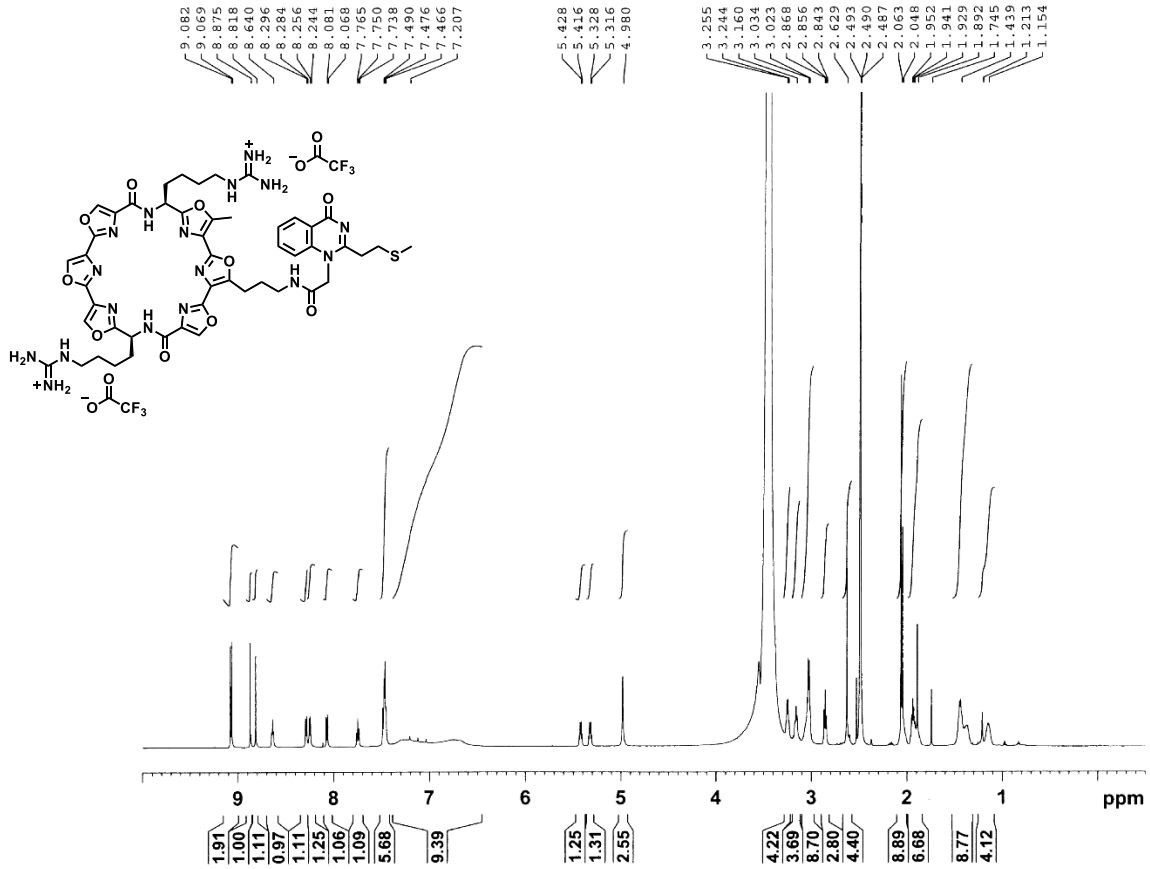
L2G2-6OTD-1M1PA-N_3 (1), ^{1}H NMR (DMSO-d_6, 400 MHz)



^{13}C NMR (DMSO-d_6, 125 MHz)



6OTD-VQ-SMe, ^{1}H NMR (DMSO-d_6, 600 MHz)



^{13}C NMR (DMSO-d_6, 151 MHz)