Light-driven conformational regulation of human telomeric G-quadruplex DNA in physiological conditions

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Supplementary Information

Experimental Section

Materials and Methods

The oligomers used in this study were purchased from Invitrogen (China). All reagents used in this study were purchased from Shanghai Medical .Com and were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300 and 600 spectrometers NMR spectra were recorded on a Varian Mercury VX-300MHz spectrometer, operating at 300 MHz for ^{1}H Chemical shifts in the ^{1}H NMR spectra are reported in ppm relative to residual hydrogens in the deuterated solvents: δ=2.50 and 7.25, for d_6-DMSO, $CDCl_3$.Coupling constants J are reported in Hz. Mass spectroscopy was performed on a Brucker APEX IV (7.0 T) and APEX II FT-ICR mass spectrometer.

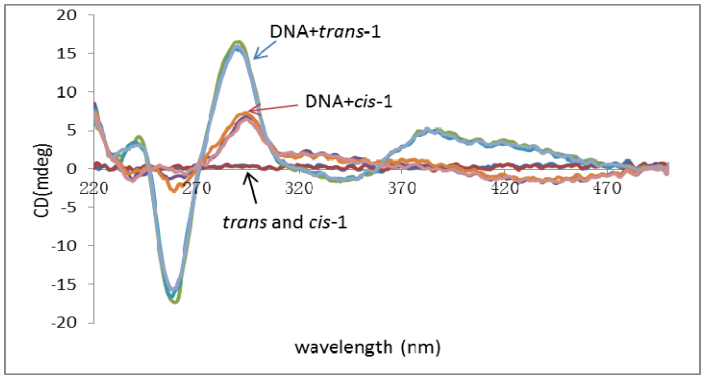
Photoisomerization and UV/Vis Analysis

UV spectra were recorded on a Shimadzu 2550 UV–Vis double-beam spectrophotometer at 25 °C. The photoisomerization was measured with 25 μM ligands 2-4 in 10 mM Tris-HCl and 1 mM EDTA buffer solution at pH 7.4. In the sample-irradiation experiments with UV light at 350 nm, the sample was irradiated under a 50 W high-pressure mercury lamp, and a filter was employed to extract light of wavelength 350 nm with a 15 nm peak width at half height. In the sample-irradiation experiments with visible light (>400 nm), an incandescent lamp (Philips) with an 11 W E27 cool day light bulb was used as the light source. UV or visible light was focused on the sample through an aperture at a distance of 3 cm. The experiment was carried out at the SHIMADZU -UV-2550 spectrophotometer.

Circular Dichroism Spectroscopy

The samples for CD spectroscopy were recorded on a Chirascan CD spectrometer (Applied Photophysics, UK) equipped with a Peltier temperature controller (TC125) at 25 °C with a quartz cell with an optical path length of 1 mm.All CD spectra were baseline-corrected for signal contributions of the buffer and were collected from 230 to 320 nm and a scanning speed of 100nm/min. The oligomer $d(TTAGGG)_4$ at a final concentration of 20 μM was resuspended in 0.01 M Tris-HCl buffer (1 mM EDTA, pH 7.4). For the K^+ rich conditions, 50 mM KCl was added and 50 mM NaCl was added for the Na^+ rich conditions.

a）



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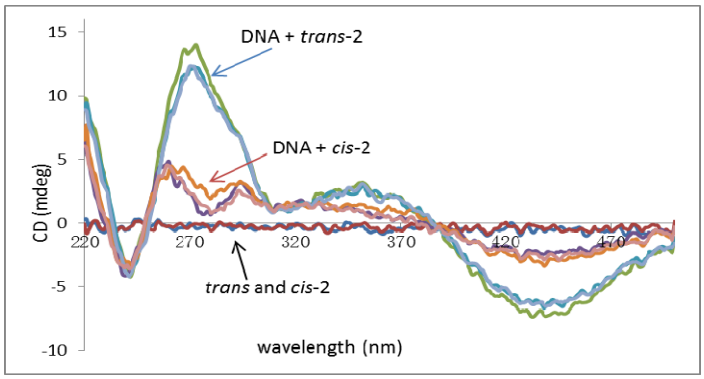

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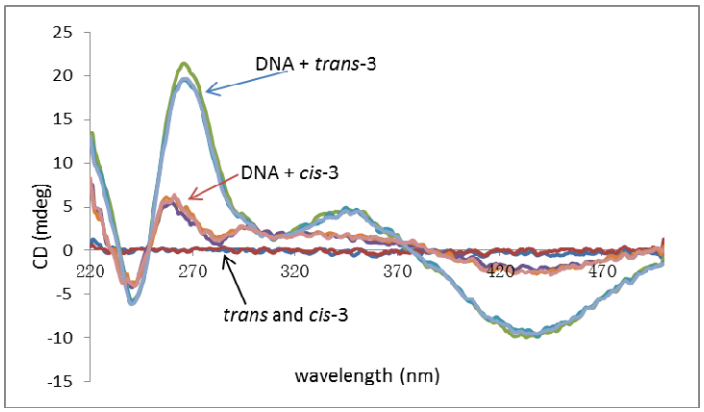


Figure S1. Induced CD signals of these compouds (200 μM) in 10 mM Tris/HCl buffer with EDTA (1 mM) at pH 7.4 in the absence of cation. a) UV/Vis spectra of compound **1** upon UV irradiation at 350 nm prior to irradiation at various reaction times (300 s,middle lines), under visible light at various reaction times (300 s,top lines) followed by the UV irradiation at 350 nm, and only compound **1** without or with photoirradiation at various reaction times (bottom lines); b) for the same situation of compound **2**; c) for compound **3**.

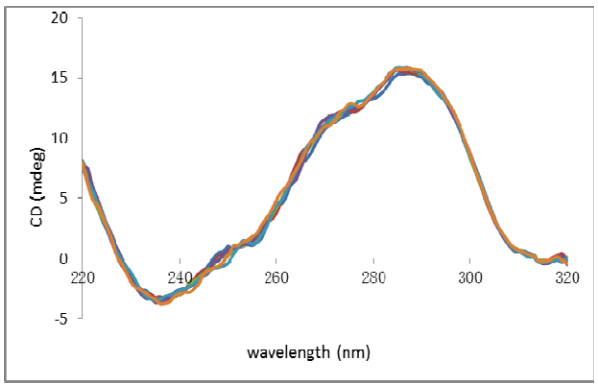


Figure S2. DNA photostability. CD studies of telomere DNA (20 μM) in 10 mM Tris/HCl buffer with EDTA (1 mM) at pH 7.4 in the presence of K^+(50 mM) without or with photoirradiation at various reaction times.

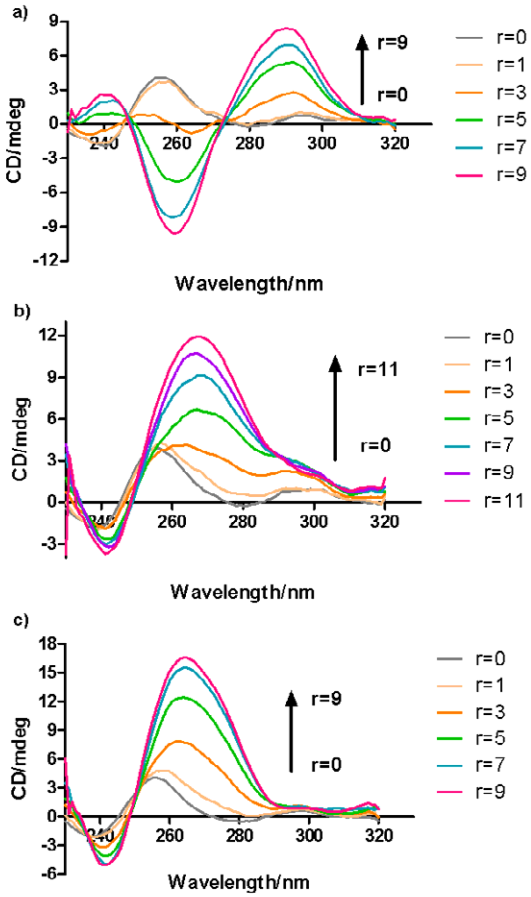


Figure S3. In the presence of Li^+ (50 mM), CD studies of telomere DNA (20 μM) in 10 mm Tris/HCl buffer with EDTA (1 mm) at pH 7.4. a) Titration of *trans*-**1**; b) Titration of *trans*-**2**; c) Titration of *trans*-**3**

Fluorescence experiment

The series of the whole detect of the G-rich sequence with 2-aminopurime（Ap）substiturtions at different adenine residues in 21G can give the information of the G-quadruplex conformation.Fluorescence spectra of the solutions with 1 μM oligonucleotide ,0.01 M Tris-HCl buffer (1 mM EDTA, pH 7.4),50 mM K^+, which were annealing from 95 °C to 4 °C over 4 h, were collected from 325 nm to 440 nm with excitation at 305 nm.This experiment was conducted at the LS55 PerkinElmer fluorescence experiment.

The sequences of the DNA:

Ap-6: GGGTTApGGGTTAGGGTTAGGG

Ap-12: GGGTTAGGGTTApGGGTTAGGG

Ap-18: GGGTTAGGGTTAGGGTTApGGG

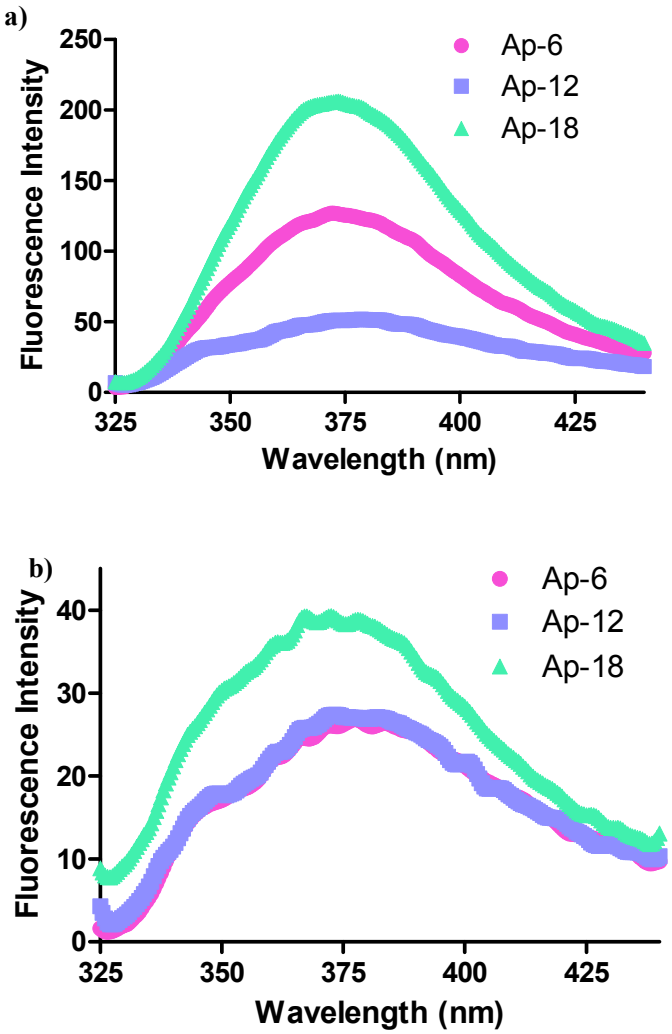


Figure S4. Fluorescent emissions of 2-aminopurine (Ap) substitute at different positions in the 21G (1 μM). Samples treatment before emission measurement: (a) heated at 95°C for 5 min in the presence of 50 mM K^+ and 0.01 M Tris-HCl buffer (1 mM EDTA, pH 7.4),then slowly cooled down to 25°C over 4 h; (b) heated at 95°C for 5 min in the presence of 150 mM K^+ , 20 μM compound and 0.01 M Tris-HCl buffer (1 mM EDTA, pH 7.4),then cooled to 25°C over 4 h.

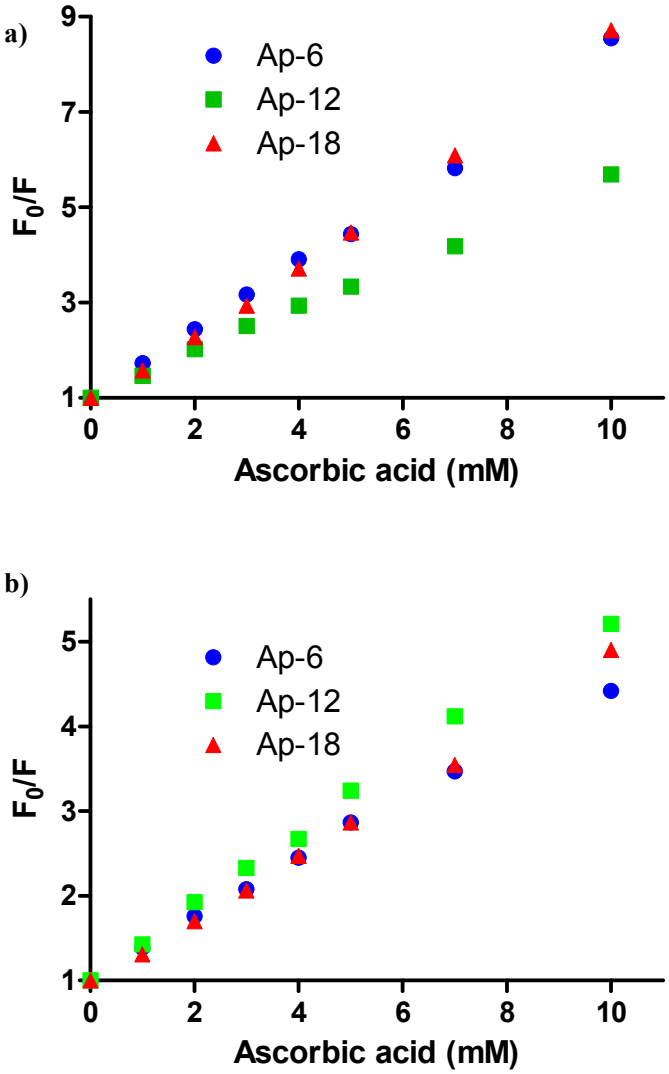


Figure S5. Fluorescence quenching experiment titrated with ascorbic acid. (a) heated at 95°C for 5 min in the presence of 50 mM K^+ and 0.01 M Tris-HCl buffer (1 mM EDTA, pH 7.4), then slowly cooled down to 25°C over 4 h; (b) heated at 95°C for 5 min in the presence of 150 mM K^+ ,20 μM compound and 0.01 M Tris-HCl buffer (1 mM EDTA, pH 7.4), then cooled to 25°C over 4 h.

Native-gel electrophoresis

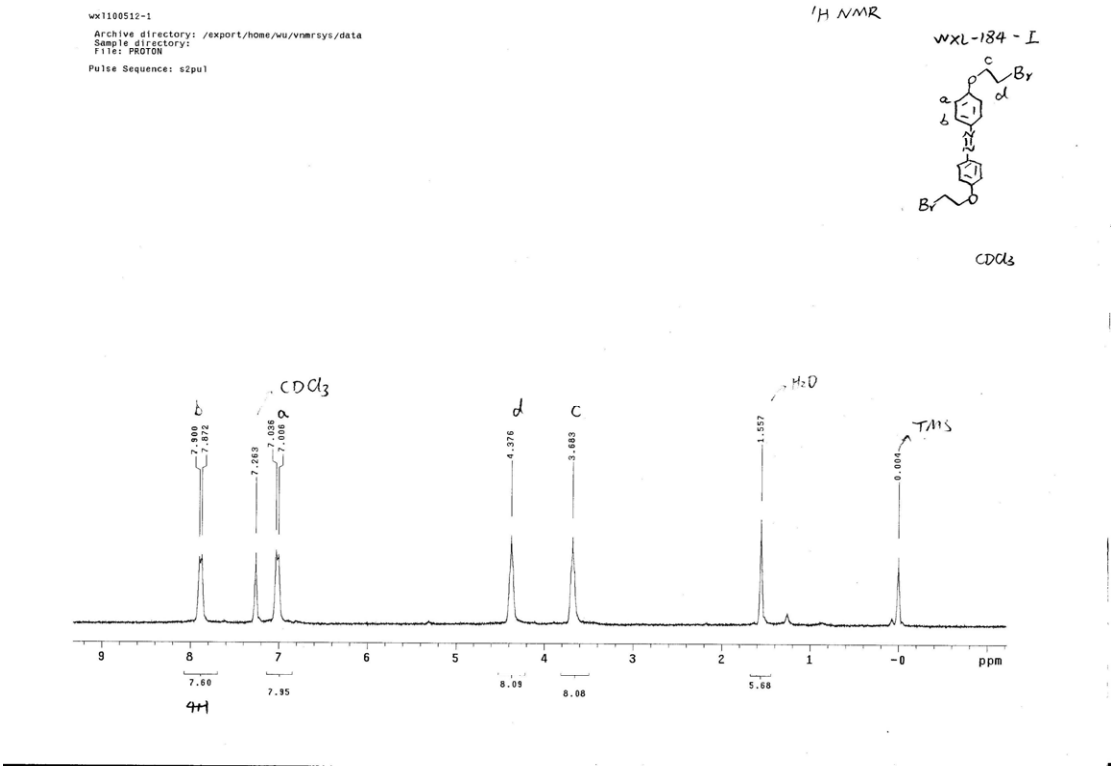
The native gel electrophoresis was run on 20% polyacrylamide gel containing 50 mM KCl at 4°C,27V/cm in 1×TBE buffer containing 50 mM KCl.The oligonucleotids in the sample were all labeled with FAM and inmaged under irradiation of UV light. The mixture containing 1 μM FAM labeled DNA,50 mM KCl , 0.01 M Tris-HCl buffer (1 mM EDTA, pH=7.4) were heated to 94°C,and then slowly cooleddown to 4°C with different concentration of compound.

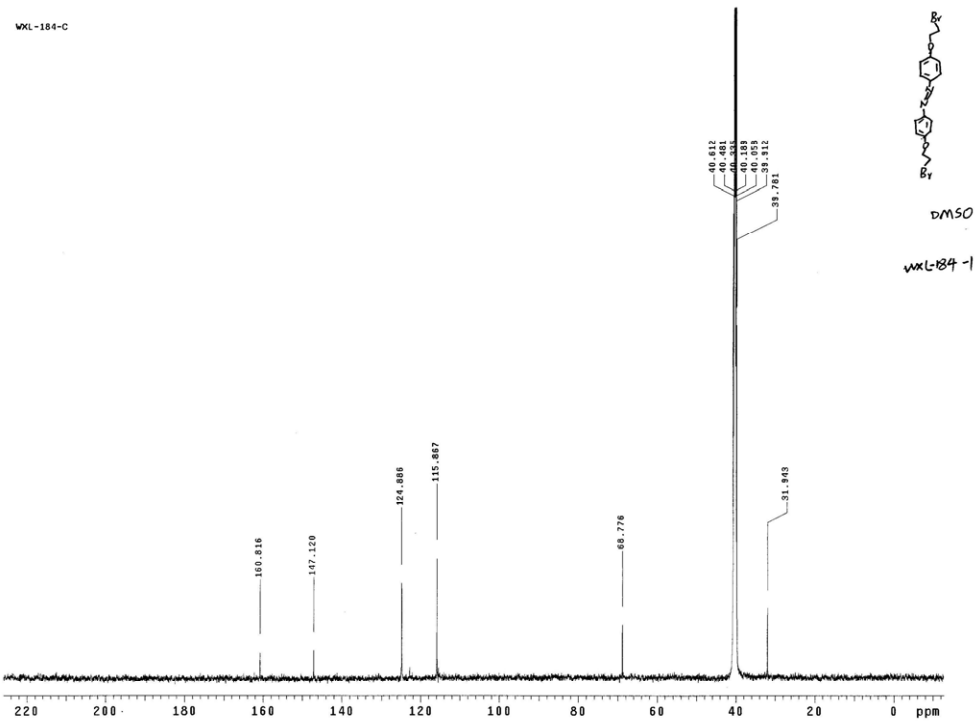
Synthesis

Compound 4.

4, 4'-Dihydroxyazobenzene (1.070 g, 5 mmol) was refluxed with 1, 2-dibromoethane (5.583 g, 30 mmol) in dry acetone in presence of anhydrous K_2CO_3 (1.500 g, 10 mmol) as base. After stirring

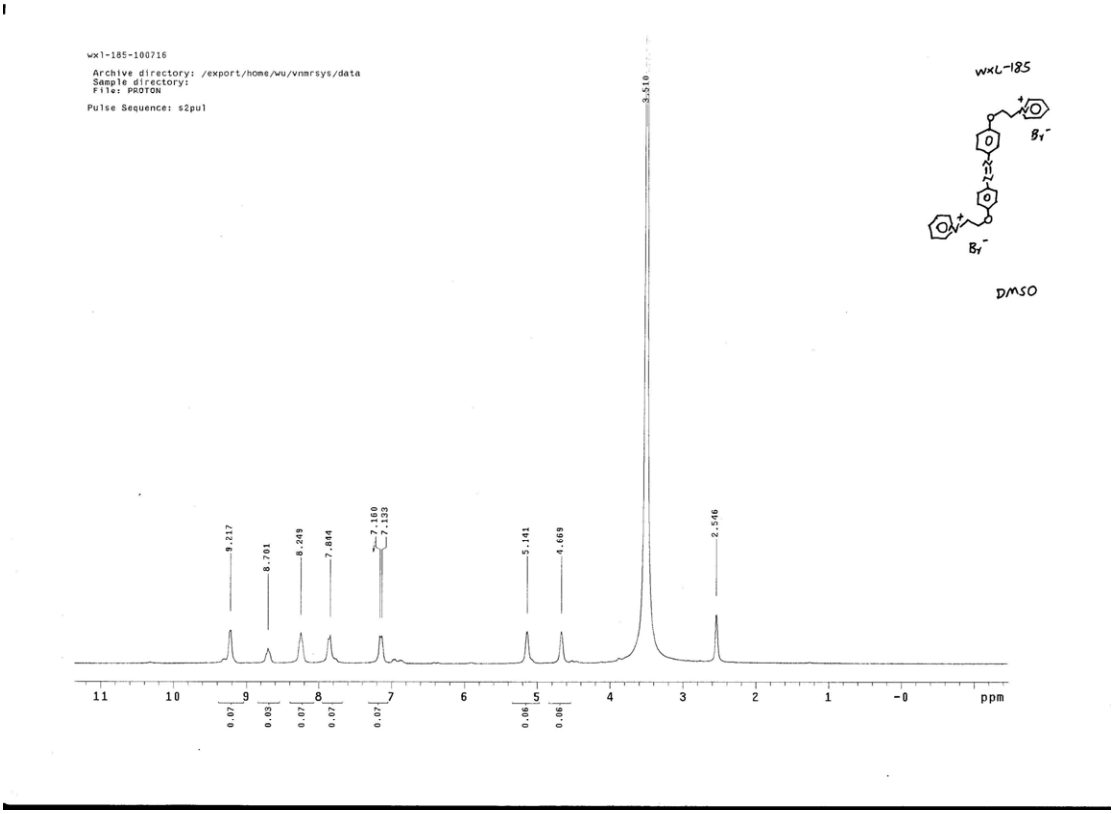
for 48 hr, the reaction mixture was cooled down and filtered. Then acetone was removed under reduced pressure, and the resulting orange solid was dissolved in $CHCl_3$. The solution was washed with water for several times, then dried with Na_2SO_4, and finally concentrated to dryness. The crude product was purified by silica gel column chromatography (chloroform) to afford yellow solid (0.342 g, 16.1% yield). ^{1}H NMR ($CDCl_3$): δ=3.68 (br, 4H), 4.38 (br, 4H), 7.02 (d, J = 9.0 Hz, 4H), 7.89 ppm (d, J = 9.0 Hz, 4H); ^{13}C NMR (d_6-DMSO): δ = 31.94, 68.78, 115.87, 124.89, 147.12, 160.82 ppm. HRMS-ESI for $C_{16}H_{16}Br_2N_2O_2$: calcd. $(M+H^+)^+$: 426.9651, found: 426.9638.

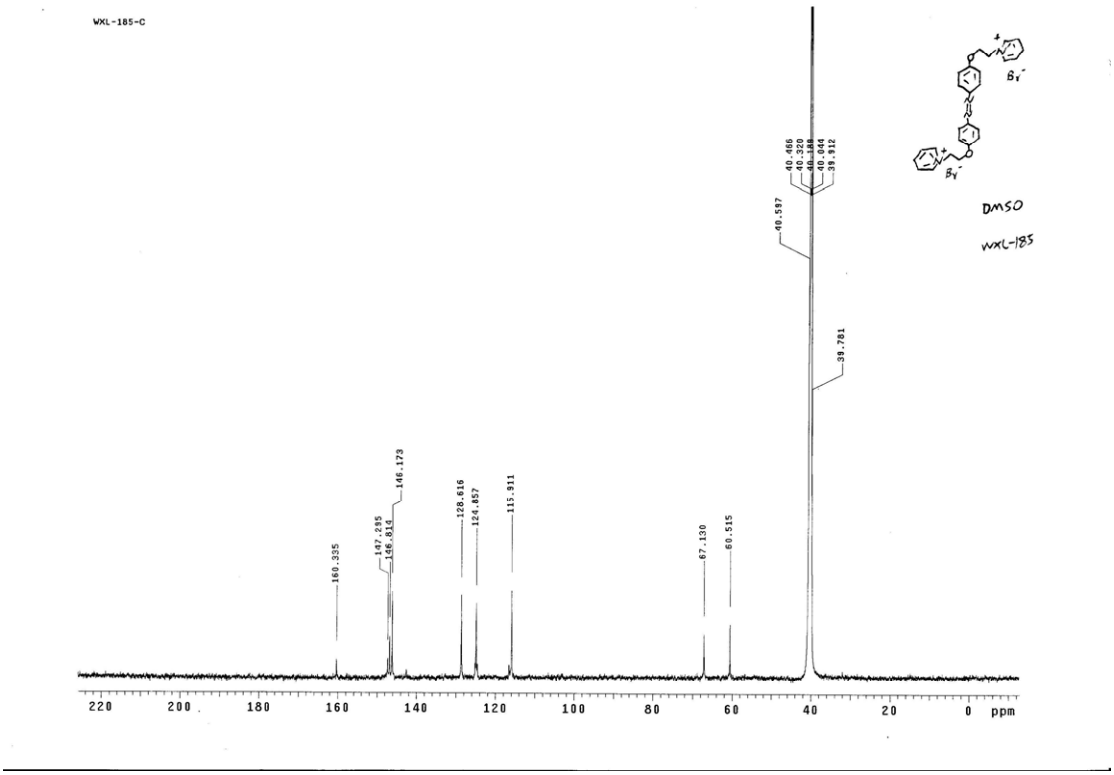




Compound 1.

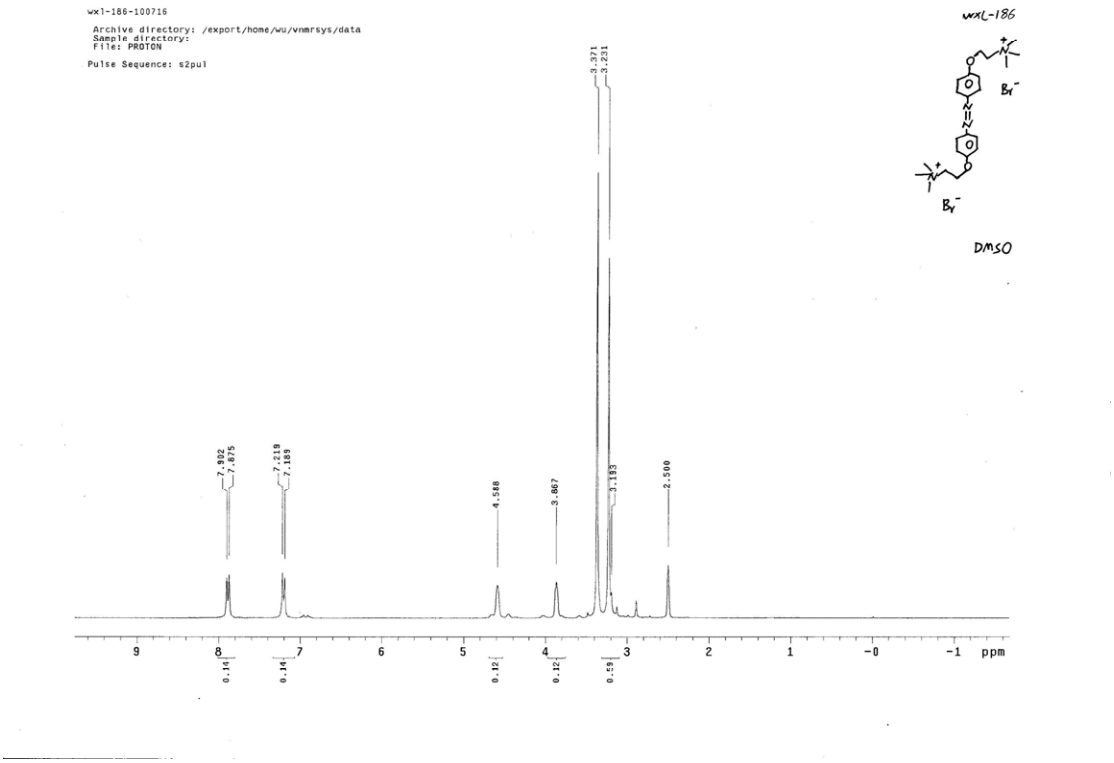
Under Ar atmosphere, pyridine (2 ml, 33 mmol) was added to a solution of 4 (0.050 g, 0.117 mmol) in ethanol (5 ml). After stirring for 12 hr at 80 □, the reaction mixture was cooled down, and the excess pyridine and ethanol were removed under reduced pressure. The collected yellow solid was recrystallized with methanol/ diethyl ether (1:5) to afford the desired product as a orange solid (0.460 g, 67.3% yield). UV-Visable spectrum (10mM Tris/HCl aqueous buffer with EDTA (1 microM) at pH 7.4.): 354 nm (peak), 279 nm (valley); ^{1}H NMR (d_6-DMSO): δ=4.67 (br, 4H), 5.14 (br, 4H), 7.15 (d, 4H), 7.84 (br, 4H), 8.25 (br, 4H), 8.70 (br, 2H), 9.22 ppm (br, 4H); ^{13}C NMR (d_6-DMSO): δ = 60.52, 67.13, 115.91, 124.86, 128.62, 146.17, 146.81, 147.30, 160.34 ppm. ESI: 213.4 ([M-2Br]/2). HRMS-ESI for $C_{26}H_{26}Br_2N_4O_2$: calcd. $(M\text{-}C_5H_5N\text{-}Br^{□})^+$: 426.2000, found: 426.0805

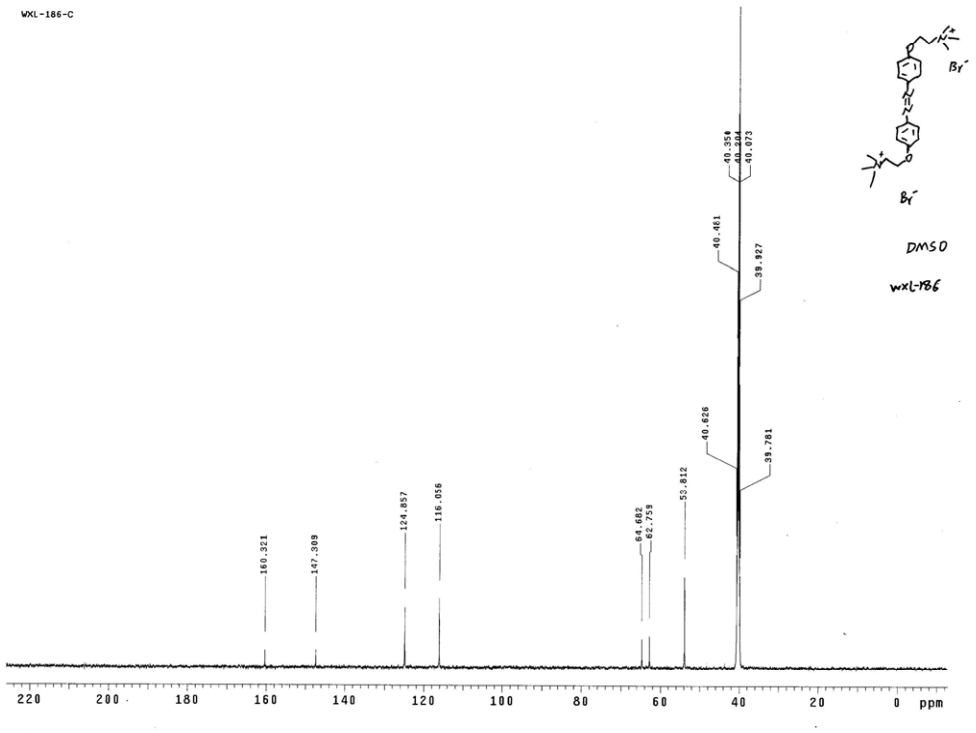




Compound 2.

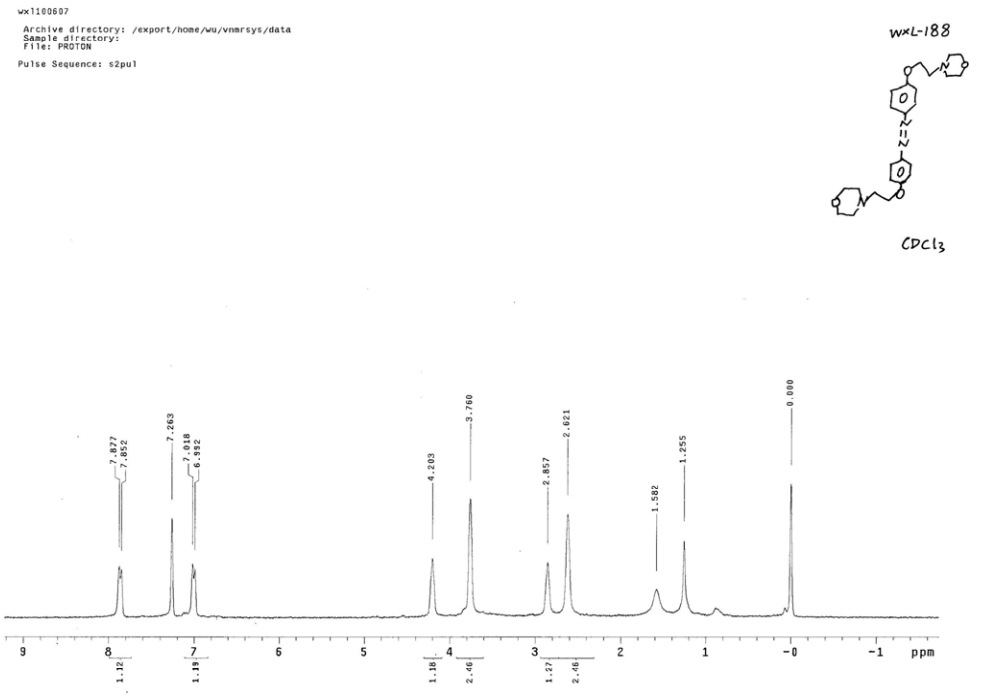
Compound 4 (0.050 g, 0.117 mmol) was added to trimethylamine (5ml 33% ethanol solution) under Ar atmosphere. After stirring for 12 hr at 80□, the reaction mixture was cooled down and the excess trimethylamine and ethanol were removed under reduced pressure. The collected yellow solid was recrystallized with methanol/ diethyl ether (1:5) to afford the desired product as a yellow solid (0.420 g, 66.0% yield).UV-Visable spectrum (10mM Tris/HCl aqueous buffer with EDTA (1 microM) at pH 7.4.): 346 nm (peak), 277 nm (valley); ^{1}H NMR (d_6-DMSO): δ=3.21 (s, 18H), 3.87 (br, 4H), 4.59 (br, 4H), 7.20 (d, J = 9.0 Hz, 4H), 7.89 ppm (d, J = 9.0 Hz, 4H); ^{13}C NMR (d_6-DMSO): δ = 53.81, 62.76, 64.68, 116.06, 124.86, 147.31, 160.32 ppm. ESI: 193.4 ([M-2Br]/2). HRMS-ESI for $C_{22}H_{34}Br_2N_4O_2$: calcd. $(M\text{-}Br^-)^+$: 465.1865, found: 465.1849.

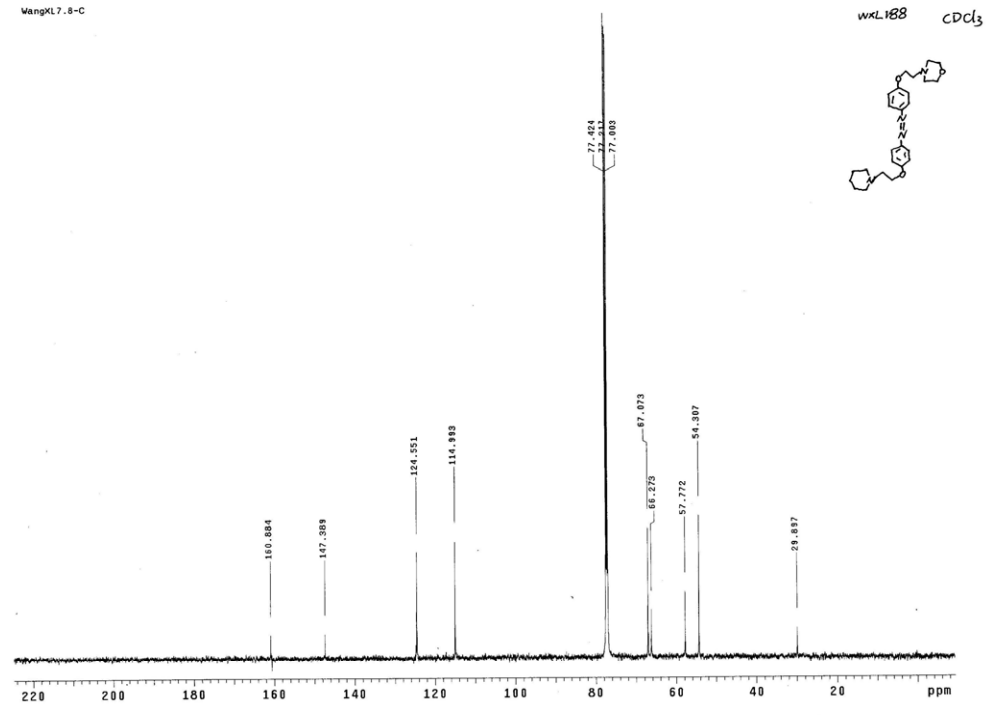




Compound 5.

Morpholine (1 ml, 12 mmol) was added to a solution of 4 (0.050 g, 0.117 mmol) in ethanol(5 ml) in presence of anhydrous K_2CO_3 (1.500 g, 10 mmol) as base under Ar atmosphere. After stirring for 24 hr at 80□, the reaction mixture was cooled down and filtered. Then the excess morpholine and ethanol were removed under reduced pressure. The crude product was purified by aluminium oxide active basic for column chromatography (dichloromethane) to afford yellow solid (0.046 g, 89.3% yield). ^{1}H NMR ($CDCl_3$): δ=2.62 (br, 8H), 2.86 (br, 4H), 3.76 (br, 8H), 4.20 (br, 4H), 7.00 (d, J = 9.0 Hz, 4H), 7.86 ppm (d, J = 9.0 Hz, 4H); ^{13}C NMR ($CDCl_3$): δ = 54.31, 57.78, 66.27, 67.07, 114.99, 124.55, 147.39, 160.88 ppm. HRMS-ESI for $C_{24}H_{32}N_4O_4$: calcd. ($M+H^+$) : 441.2496 , found: 441.2510 .





Compound 3.

CH_3I (2 ml, 39 mmol) was added to a solution of 5 (0.030 g, 0.068 mmol) in $CHCl_3$(2 ml). After stirring for 24 hr at 45□, the reaction mixture was filtered. The collected yellow solid was

recrystallized with methanol/ diethyl ether (1:5) to afford the desired product as an orange solid (0.030 g, 61.2% yield).UV-Visable spectrum (10mM Tris/HCl aqueous buffer with EDTA (1 microM) at pH 7.4.): 353 nm (peak), 287 nm (valley); ^{1}H NMR (d_6-DMSO): δ=3.32 (s, 6H), 3.58 (br, 8H), 4.00 (br, 12H), 4.62 (br, 4H), 7.20 (d, J = 9.0 Hz, 4H), 7.89 ppm (d, J = 9.0 Hz, 4H); ^{13}C NMR (d_6-DMSO): δ = 48.00, 60.47, 60.52, 62.11, 63.03, 116.08, 124.84, 147.33, 160.26 ppm. ESI: 235.3 ([M-2I]/2), 597.2 (M-I). HRMS-ESI for $C_{28}H_{38}N_4O_2I_2$: calcd. $(M\text{-}I^-)^+$: 597.1932, found:597.1925.