Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Informations

Contents

1. Experimental Section

General

Reagents and solvents were purchased from standard suppliers and used without further puridication. A series of buffers of various pH was purchased from Tokyo Chemical Industry Co., Ltd. (TCI). Reactions were monitored with TLC plates precoated with Merck silica gel 60 F_{254}. Spots were visualized with UV light, phosphomolybdic acid or anisaldehyde. Wakogel C-200 were used for silica gel flash chromatography. NMR spectra were measured at 600 MHz for ^{1}H and 150 MHz for ^{13}C by JEOL JNM-ECA600. The chemical shifts are expressed in ppm relative to residual solvent as an internal standard. Coupling constant (*J* values) are represented in hertz. High resolution mass spectra were recorded on a JEOL AccuTOF JMS-T 100N mass spectrometer. Absorption spectra were recorded on a BECKMAN COULTER spectrophotometer DU 800. The thermal denaturation profile was recorded on a Shimadzu UV-Vis spectrophotometer UV-2700 equipped with a Shimadzu TMSPC-8 temperature controller. Fluorescence spectra were recorded on a JASCO spectrofluorometer FP-8500 at a temperature of 25 °C controlled by JASCO ETC-815 Peltier thermostatted single cell holder. SPR was measured using BIACORE T200.

Boc-azaDANP (2)

A solution of 3,6-dichloropyrido[2,3-*b*]pyrazine **1** (20.6 mg, 0.103 mmol) in 1,3-diaminopropane (1 mL) was stirred at room temperature for 16 h. The solvent was removed under reduced pressure. Obtained crude product was dissolved in 1:1 mixture of 1,4-dioxane and 10% aqueous solution of $NaHCO_3$, and was added $(Boc)_2O$ (109 mg, 0.515 mmol). The mixture was stirred at room temperature for 1d. The organic materials were extracted with chloroform and washed with brine, and dried over anhydrous sodium sulfate. The solvents were evaporated under reduced pressure, and the crude compound was purified by silica gel column chromatography (AcOEt) to give **2** (29.5 mg, 0.062 mmol, 60%) as yellow solids: ^{1}H NMR (CD_3OD): δ = 7.85 (s, 1H), 7.69 (d, *J* = 8.9 Hz, 1H), 6.61 (d, *J* = 8.9 Hz, 1H), 3.58-3.55 (4H), 3.22-3.18 (4H), 1.89-1.83 (4H), 1.48-1.46 (18H); ^{13}C-NMR ($CDCl_3$): δ = 161.5, 158.9, 156.9, 153.8, 138.1, 133.8, 126.5, 111.3, 80.2, 39.8, 39.4, 39.3, 31.0, 30.8, 29.1; HR-MS (ESI): Calcd. for $C_{23}H_{38}N_7O_4^+$ $[M+H]^+$, 476.2980; found, 476.2977.

AzaDANP (N^1,$N^{1'}$-(pyrido[2,3-*b*]pyrazine-3,6-diyl)bis(propane-1,3-diamine)) as a HCl salt

To a $CHCl_3$ (1 mL) solution of **2** (29.5 mg, 0.062 mmol) was added ethyl acetate containing 4 N HCl (2.5 mL). The mixture was stirred at room temperature for 30 min. Solvent was evaporated to give azaDANP (quantitative) as yellow solids: ^{1}H NMR (D_2O:CD_3OD=1:1): δ = 8.31-7.99 (2H), 6.96 (m, 1H), 3.71-3.68 (4H), 3.21 (m, 2H), 3.14 (m, 2H), 2.19 (m, 2H), 2.12 (m, 2H); ^{13}C-NMR (D_2O:CD_3OD=1:1): δ = 156.8, 155.2, 146.5, 142.8, 137.6, 124.3, 111.4, 41.0, 38.8, 38.5, 38.3, 27.9, 27.3; HR-MS (ESI): Calcd. for $C_{13}H_{22}N_7^+$ $[M+H]^+$, 276.1931; found, 276.1934.

2. UV-vis and Fluorescent Spectra

Absorption and fluorescence spectra were recorded on a BECKMAN COULTER spectrophotometer DU 800 at room temperatures and a JASCO spectrofluorometer FP-8500 at a temperature of 25 °C controlled by JASCO ETC-815 Peltier thermostatted single cell holder in a 1×0.2 cm path length quartz cuvette.

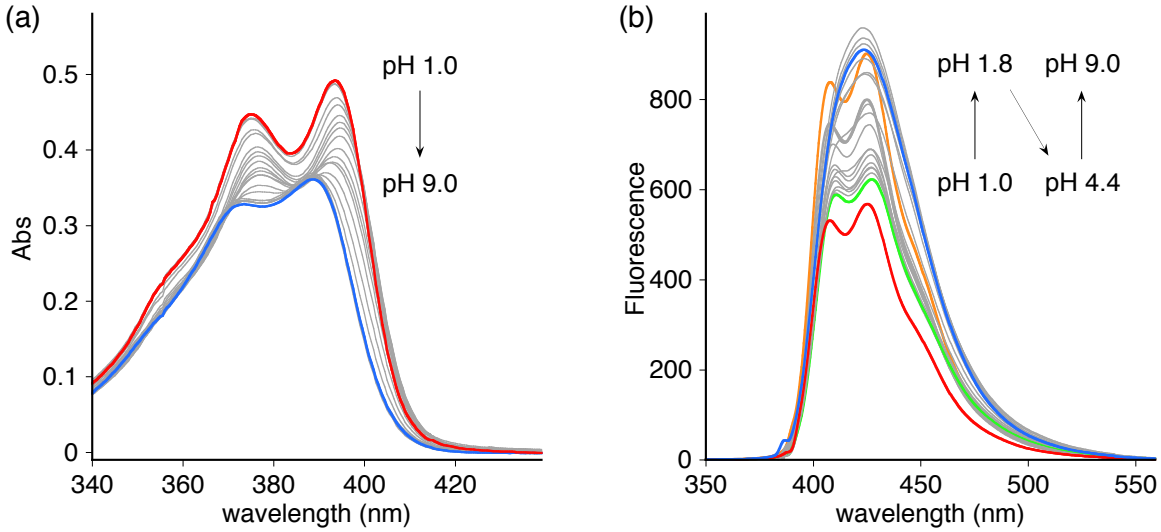


Figure S1. (a) Absorption and (b) fluorescent spectra of azaDANP (20 μM) at various pHs in the solutions of buffer (20 mM) and NaCl (100 mM). Excitation wavelength was at the absorption maxima. Fluorescent spectra at 1.0 (red line), 1.8 (orange line), 4.4 (green line), and 9.0 (blue line) were shown colored.

3. Absorption and Fluorescent Spectra with 5'-CNC-3'/5'-GG-3' Bulge DNA

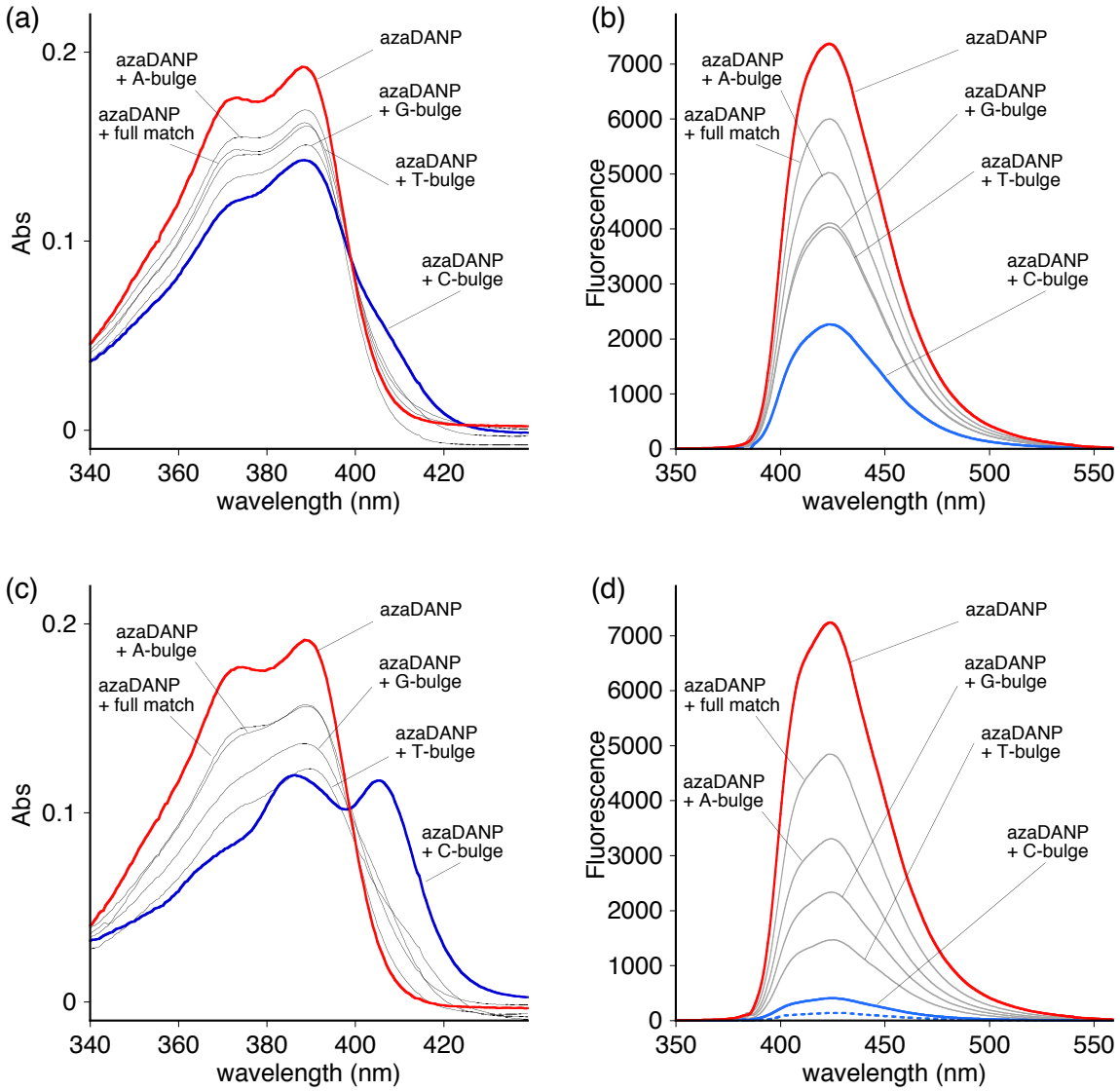


Figure S2. Absorption (a and c) and fluorescent (b and d) spectra of azaDANP (10 μM) (red line) with bulged DNA (30 μM) 5'-d(GTTGC*N*CTGGA)-3'/3'd(CAACGGACCT)-5', where *N* is A, T, G, C(blue line), or full match at pH 7.0 (a and b) or 5.5 (c and d) (10 mM sodium cacodylate, 100 mM NaCl). Excitation wavelength was at the absorption maxima. Blue dotted line in (d) showed fluorescent spectra excited at 405 nm.

4. Tm Measurement (bulge DNA)

AzaDANP or DANP (100 μM) were dissolved in a sodium cacodylate (10 mM, pH 7.0, 6.0, or 5.5) containing bulge duplex of sequences of 5'-d(GTTGC*N*CTGGA)-3'/3'-d(CAACGGACCT)-5' (5 μM) and NaCl (100 mM). The thermal denaturation profile was recorded on a Shimadzu UV-Vis spectrophotometer UV-2700 equipped with a Shimadzu TMSPC-8 temperature controller. The absorbance of the sample was monitored at 260 nm from 2 °C to 85 °C with a sample heating rate of 1 °C min^{-1}.

azaDANP

pH 7.0	Tm(-)	Tm(+)	ΔTm
dA	31.9 (1.47)	32.8 (0.50)	0.9 (0.97)
dT	29.7 (0.48)	33.0 (0.47)	3.3 (0.01)
dG	31.6 (0.35)	33.2 (0.55)	1.6 (0.56)
dC	33.2 (0.65)	35.7 (0.40)	2.5 (0.30)
full	44.6 (0.28)	45.8 (0.37)	1.2 (0.65)

pH 6.0	Tm(-)	Tm(+)	ΔTm
dA	31.8 (0.35)	32.9 (0.56)	1.2 (0.21)
dT	30.1 (0.58)	33.2 (0.39)	3.1 (0.46)
dG	31.9 (0.20)	33.3 (0.55)	1.4 (0.74)
dC	32.9 (0.62)	38.3 (0.31)	5.4 (0.70)
full	45.3 (0.20)	45.1 (0.46)	–0.2 (0.64)

pH 5.5	Tm(-)	Tm(+)	ΔTm
dA	30.7 (1.06)	31.4 (0.74)	0.7 (0.78)
dT	29.4 (0.56)	33.0 (0.20)	3.6 (0.64)
dG	30.1 (0.43)	32.8 (0.62)	2.7 (0.40)
dC	32.5 (0.35)	38.9 (0.43)	6.4 (0.55)
full	44.0 (0.31)	43.0 (0.80)	–1.0 (0.66)

DANP

pH 7.0	Tm(-)	Tm(+)	ΔTm
dA	31.7 (0.17)	36.3 (0.39)	4.7 (0.56)
dT	30.3 (0.58)	39.9 (0.33)	9.6 (0.33)
dG	31.8 (0.66)	37.0 (0.38)	5.3 (0.60)
dC	33.8 (0.45)	42.4 (0.29)	8.7 (0.73)
full	45.6 (0.42)	46.3 (0.28)	0.8 (0.50)

pH 6.0	Tm(-)	Tm(+)	ΔTm
dA	31.3 (0.21)	38.3 (0.29)	7.0 (3.98)
dT	29.8 (0.56)	42.4 (0.42)	12.7 (7.51)
dG	31.7 (0.43)	39.1 (0.92)	7.5 (1.10)
dC	32.7 (0.53)	45.0 (0.55)	12.3 (0.77)
full	44.9 (0.51)	46.8 (0.54)	1.9 (0.09)

pH 5.5	Tm(-)	Tm(+)	ΔTm
dA	30.0 (0.38)	37.8 (0.47)	7.8 (0.11)
dT	28.8 (0.71)	41.8 (0.68)	13.0 (1.17)
dG	29.6 (0.27)	37.8 (0.65)	8.2 (0.41)
dC	31.6 (0.38)	44.4 (0.32)	12.8 (0.65)
full	43.9 (0.75)	46.4 (0.27)	2.5 (0.97)

Table S1. Summary of melting temperature of bulge DNA duplexes (5 μM) with (Tm(+)) or without (Tm(-)) addition of azaDANP or DANP (100 μM). Standard deviations were provided in parentheses.

5. Calculated Energy of Complexes and Their Components

Calculation of energy of complexes and their components were performed by Gaussian 09 using parameter of B3LYP/6-311++(d,p). The energy of complex formation (ΔE) was calculated by the following equation:

$$\Delta E = E(\text{complex}) - [E(\text{nucleobase}) + E(\text{protonated azaDANP})]$$

compound	E/kcal mol^{-1}	
cytosine	E(C)	−2.72573E+05
thymine	E(T)	−3.09735E+05
azaDANP1	E(aD1)	−3.91511E+05
azaDANP2	E(aD2)	−3.91514E+05

complex	E/kcal mol^{-1}		ΔE/kcal mol^{-1}	
complex I	E(aD1_C)	−6.64118E+05	E(aD1_C)−[E(aD1)+E(C)]	−3.43086E+01
complex II	E(aD2_C)	−6.64120E+05	E(aD2_C)−[E(aD2)+E(C)]	−3.28291E+01
complex III	E(aD1_T1)	−7.01273E+05	E(aD1_T1)−[E(aD1)+E(T)]	−2.74709E+01
complex IV	E(aD2_T1)	−7.01276E+05	E(aD2_T1)−[E(aD2)+E(T)]	−2.68781E+01
complex V	E(aD1_T2)	−7.01275E+05	E(aD1_T2)−[E(aD1)+E(T)]	−2.87669E+01
complex VI	E(aD2_T2)	−7.01277E+05	E(aD2_T2)−[E(aD2)+E(T)]	−2.80870E+01

Table S2. Summary of energies of complexes and their components.

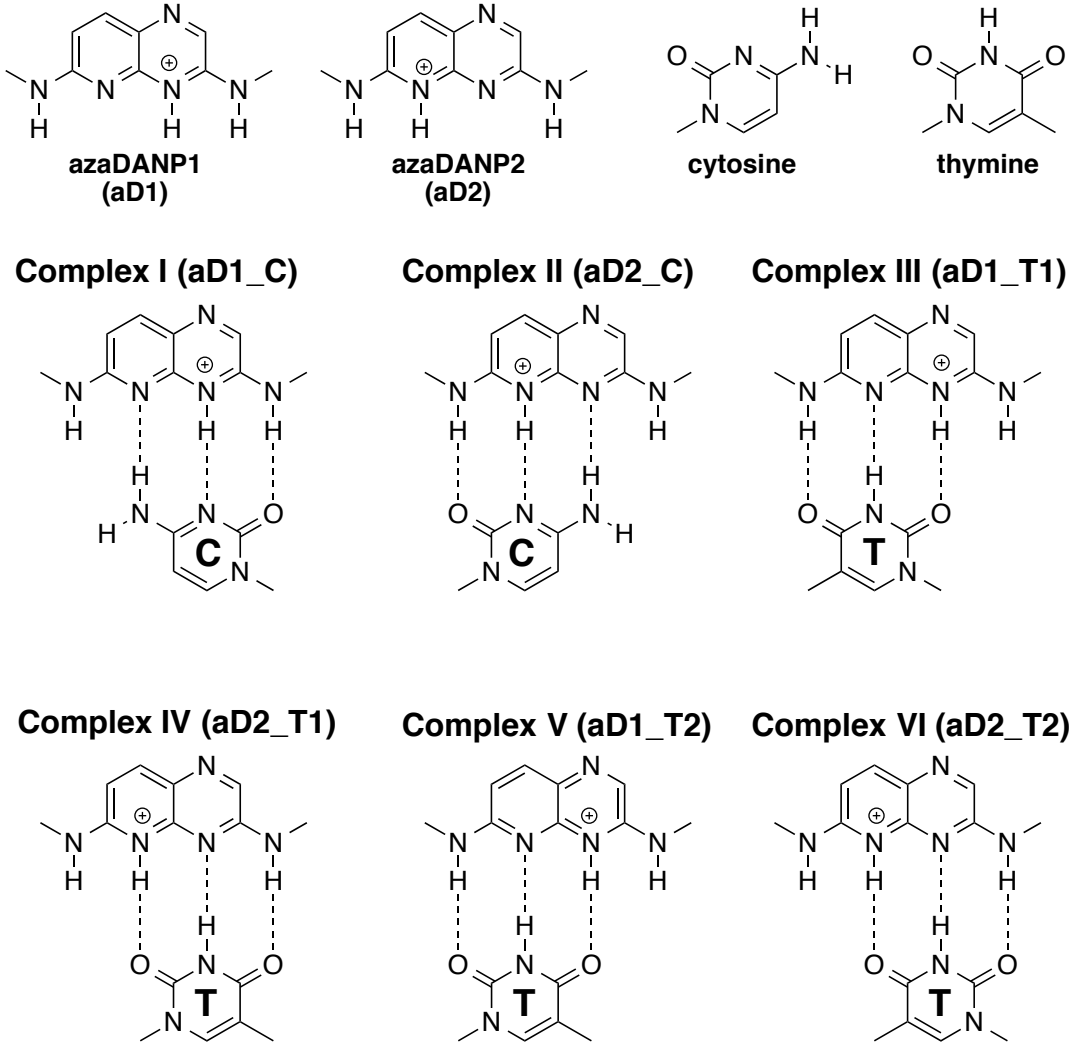


Figure S3. Structures of complexes and their components.

6. Titration with 5'-XCX-3'/5'-YY-3' Bulged DNA

Absorption titration of azaDANP was investigated with a C-bulge DNA having various flanking base pairs (5'-d(GTTG*X*C*X*TGGA)-3'/3'-d(CAAC*YY*ACCT)-5', where *X*/*Y* is A/T, T/A, G/C, or CG) at 10 °C. The solutions of DNA were titrated into solutions of the compound at fixed concentration (1 μM) in 10 mM sodium cacodylate buffer (pH 5.5) and 100 mM NaCl. The concentrations of DNA were 0, 0.025, 0.05, 0.1, 0.2, 0.55, 1.0, 2.0, 4.0, and 8.0 μM.

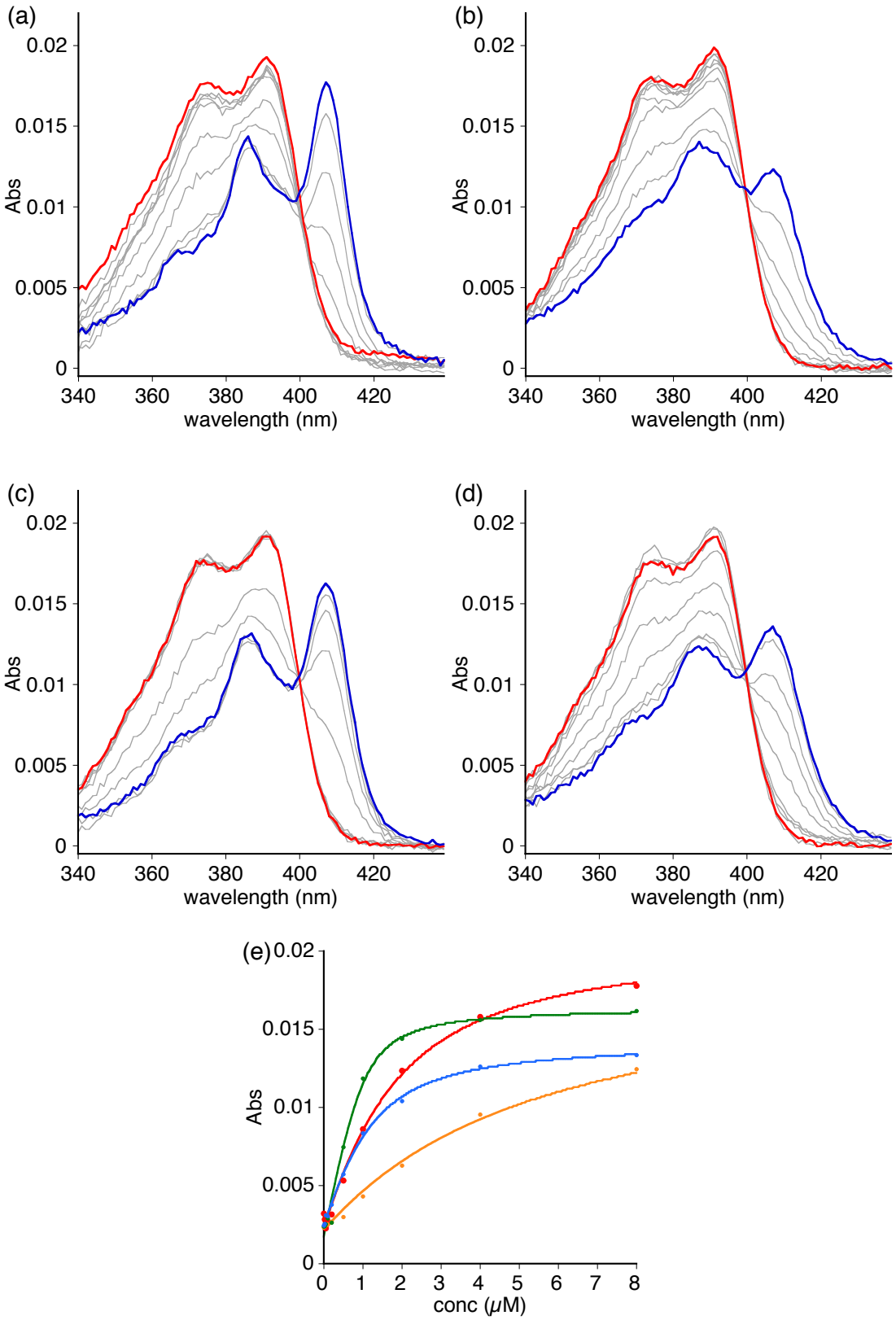
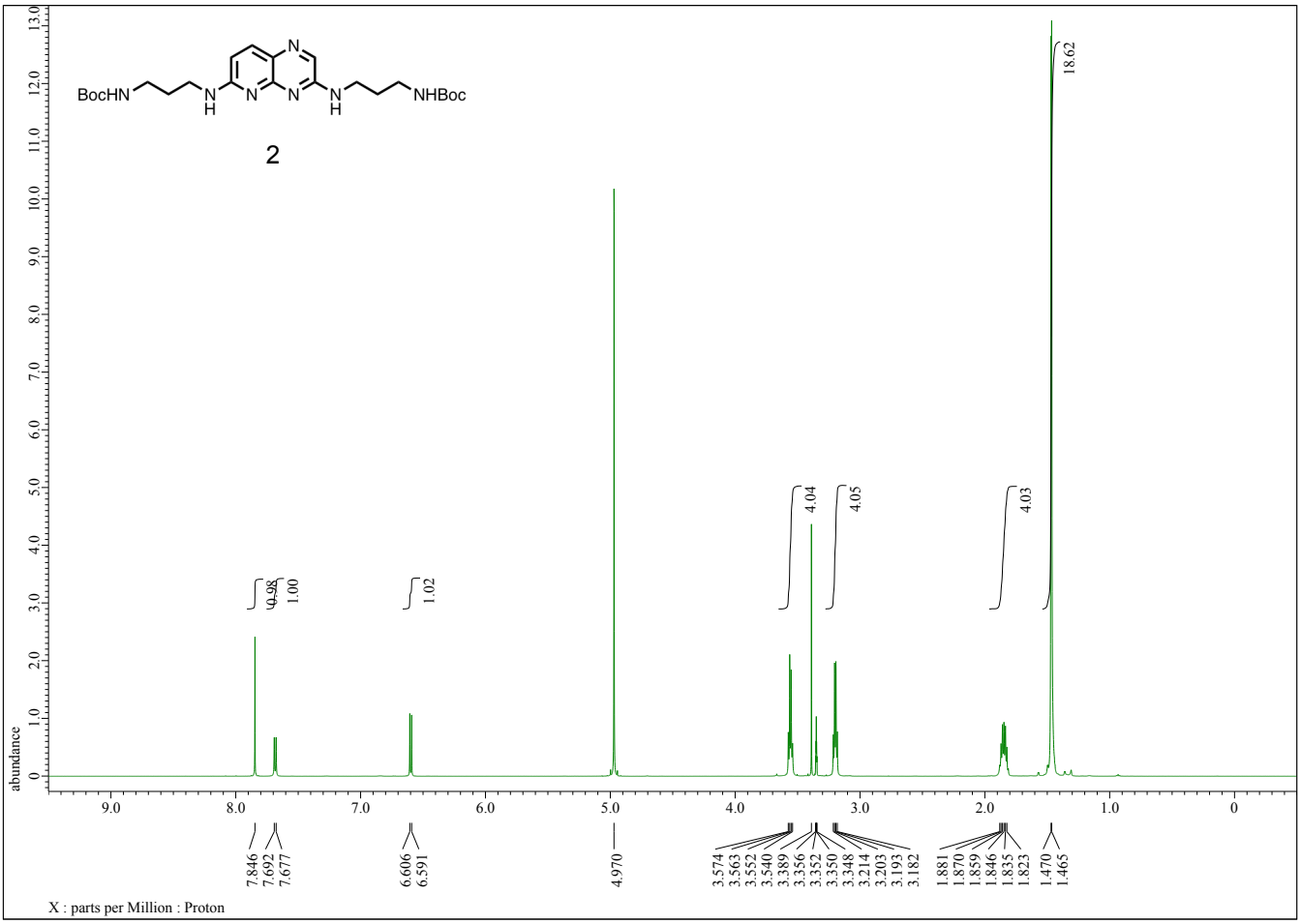
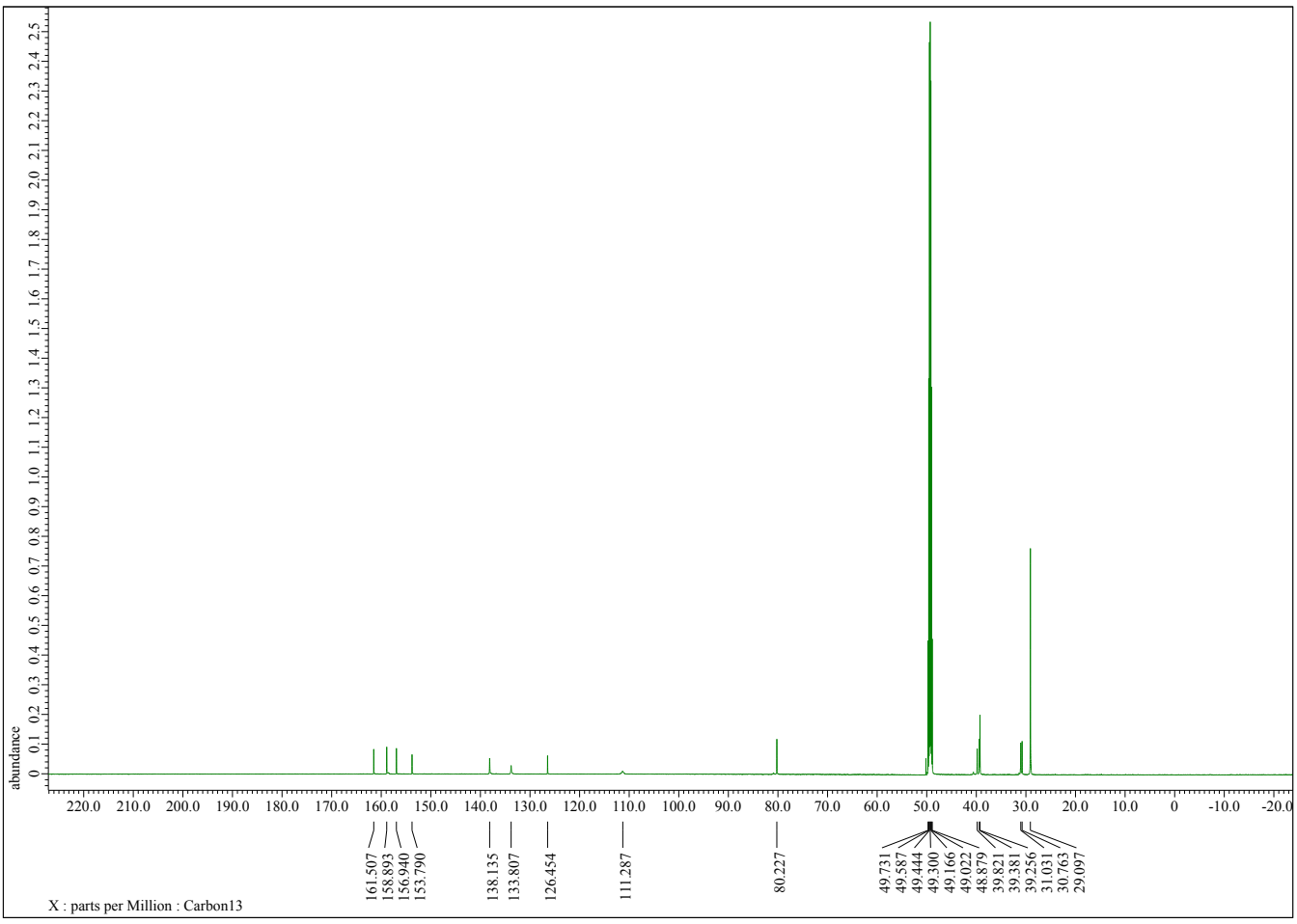


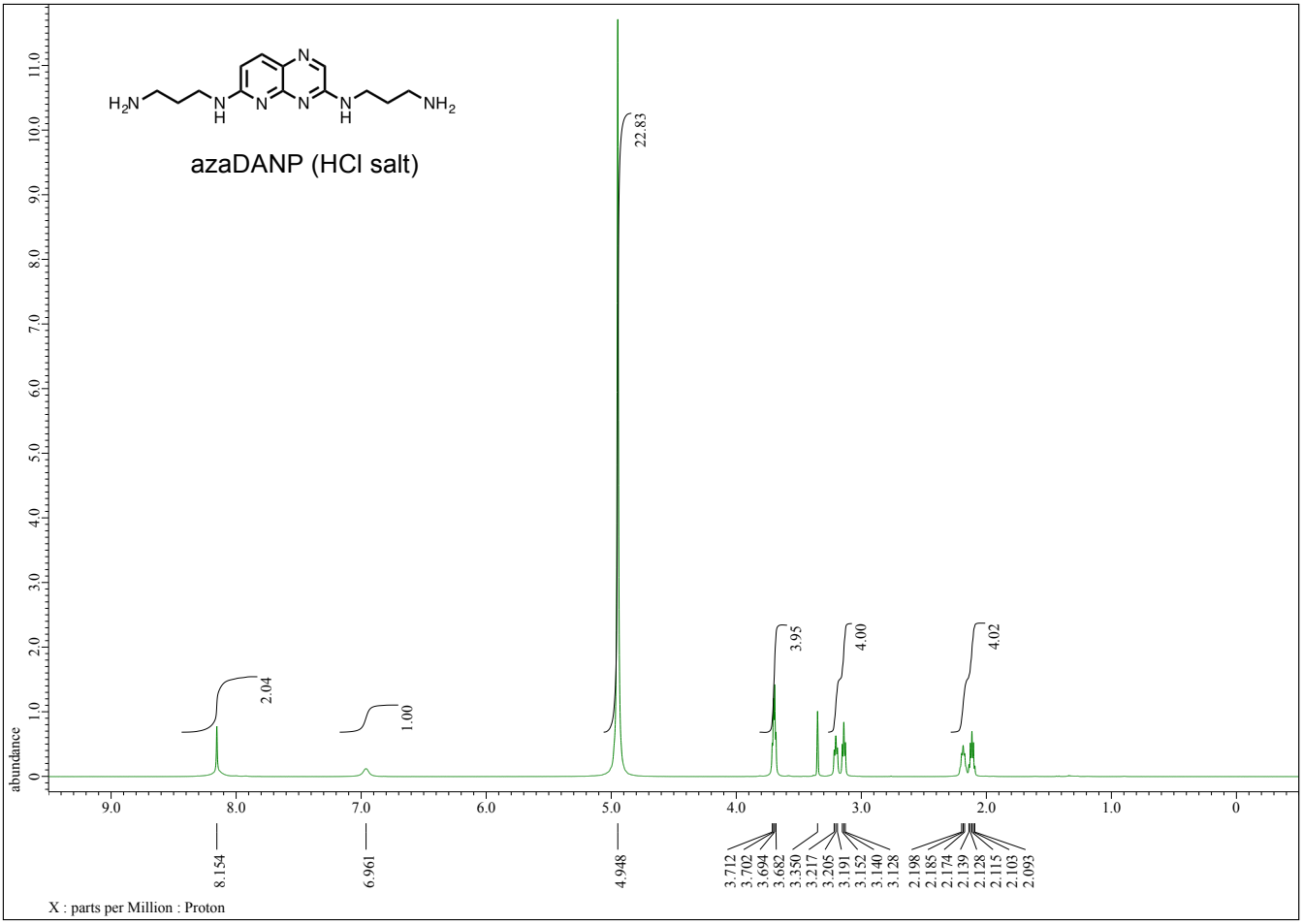
Figure S4. Titration of azaDANP (10 μM) with XCX/YY bulged DNA (5'-d(GTTG*X*C*X*TGGA)-3'/3'-d(CAAC*YY*ACCT)-5') (0(red line), 0.025, 0.05, 0.1, 0.2, 0.55, 1.0, 2.0, 4.0, and 8.0 (blue line) μM) at pH 5.5 (10 mM sodium cacodylate, 100 mM NaCl) at 10 °C, where XCX/YY is (a) ACA/TT, (b) TCT/AA, (c)

GCG/CC, or (d) CCC/GG, and (e) the plots of absorbance at 407 nm of azaDANP with ACA/TT (red), TCT/AA (orange), GCG/CC (green), and CCC/GG (blue) bulged DNA.

7. ^{1}H and ^{13}C NMR spectra






azaDANP (HCl salt)
abundance
X : parts per Million : Proton
2.04
1.00
22.83
3.95
4.00
4.02
8.154
6.961
4.948
3.712
3.702
3.694
3.682
3.350
3.217
3.205
3.191
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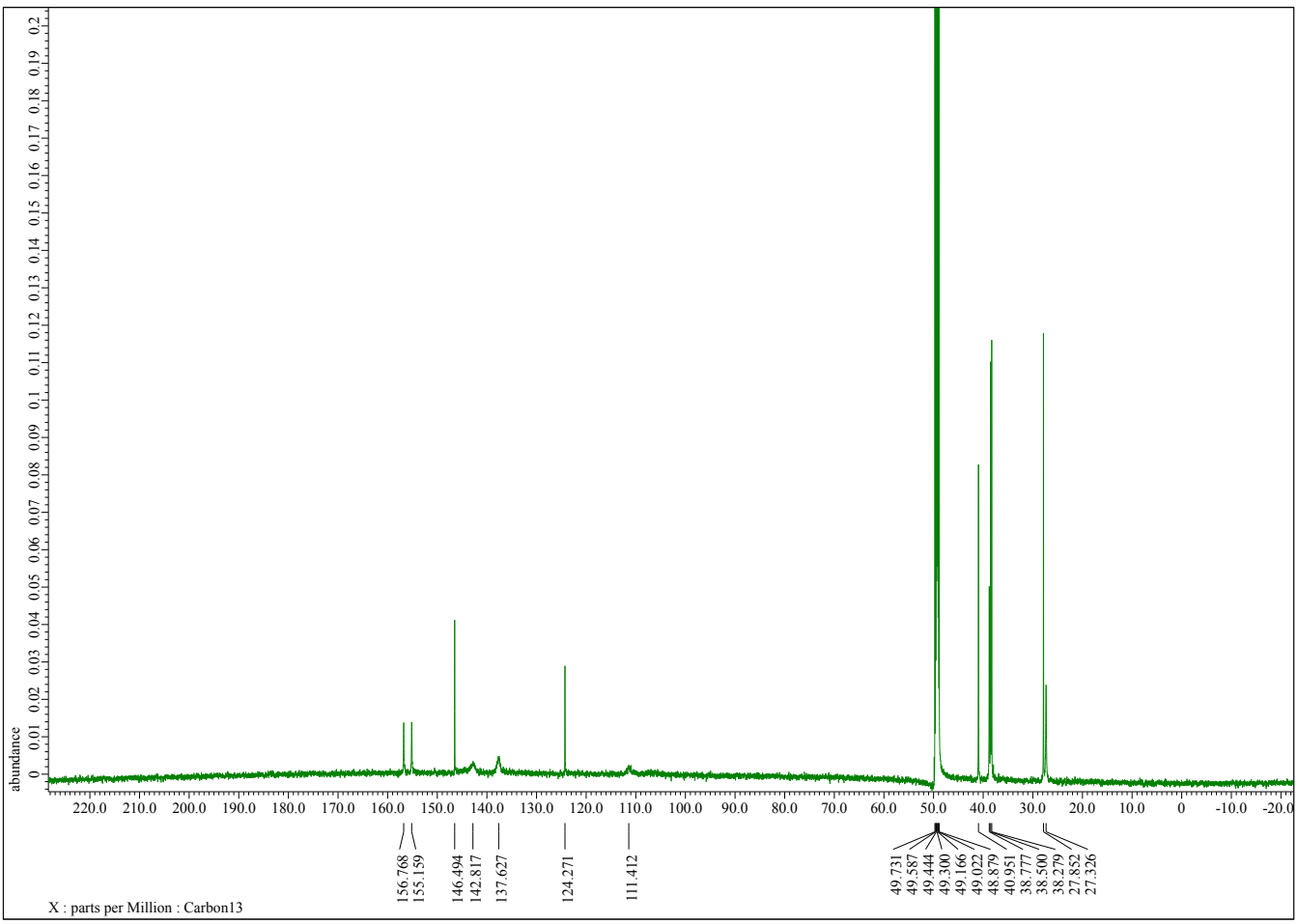
2.174
2.139
2.128
2.115
2.103
2.093


abundance
X : parts per Million : Carbon13
156.768
155.159
146.494
142.817
137.627
124.271
111.412
49.731
49.587
49.444
49.300
49.166
49.022
48.879
40.951
38.777
38.500
38.279
27.852
27.326