Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Site-selective DNA Hydrolysis Induced by a Metal-free Peptide Nucleic Acid-Cyclen Conjugate

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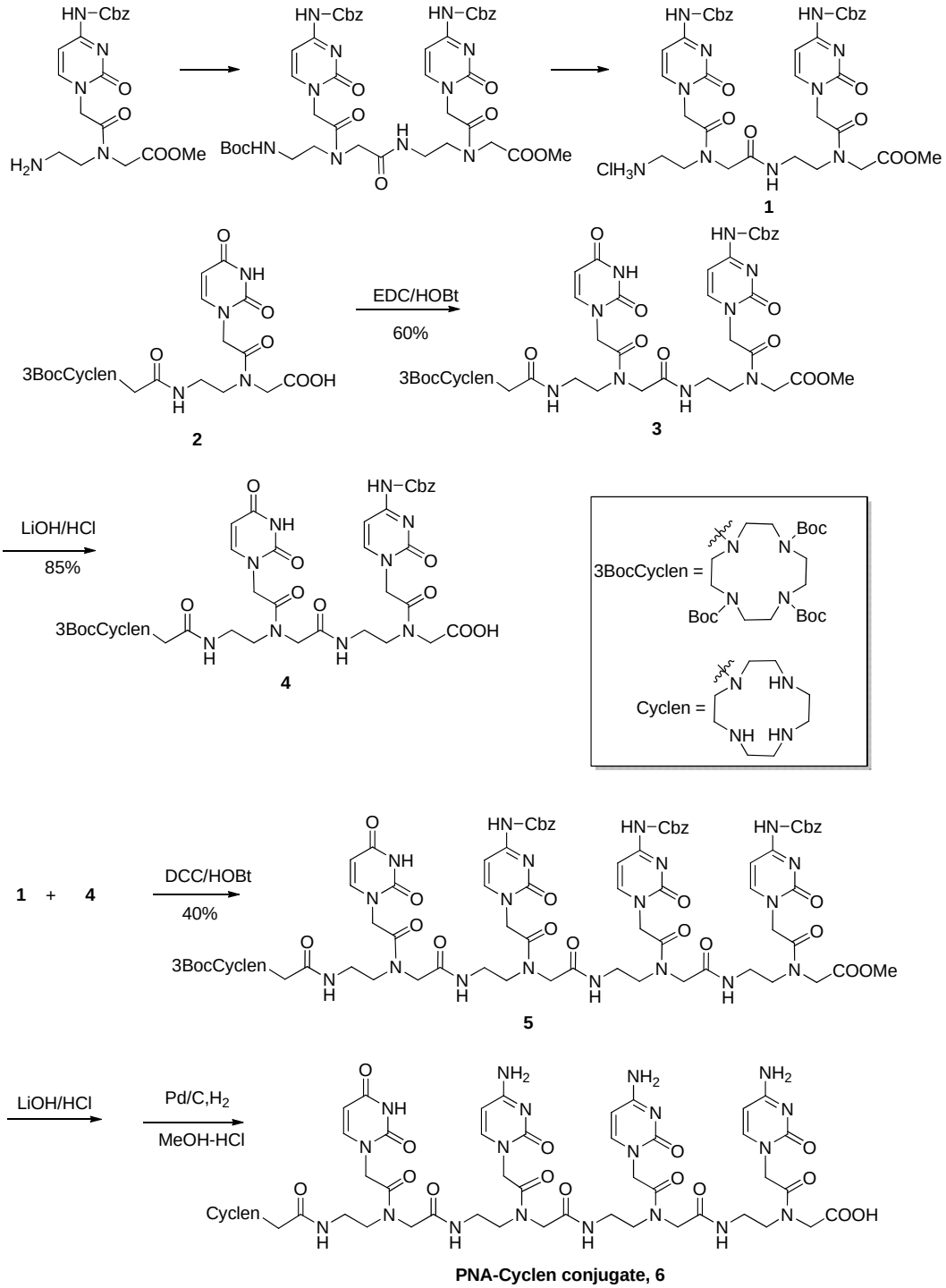
S-1: Synthesis and characterization of PNA-Cyclen conjugate



TFA.H-[$C^{(Z)}C^{(Z)}$]-OMe ***(1):*** This compound was prepared according to the literature method [1].

3BocCyclen-[$UC^{(Z)}$]-OMe ***(3)*:** N-[2-(N-uracil) acetyl)-N-[2-[4, 7, 10-tris (tert-butoxy-carbonyl)-1, 4, 7, 10-tetraazacyclododecan-1-yl-acetyl]-aminoethyl)glycine **2** (1.2 g, 1.53 mmol) synthesized as reported [2] was dissolved in DCM at 0 °C. followed by addition HOBt (0.28 g, 1.84 mmol) and EDC·HCl (0.35 g, 1.84 mmol), the reaction mixture was stirred for 5 min and then a solution of TFA.H-[PNA-$C^{(Z)}$]-OMe (0.79 g, 1.53 mmol) and DIEA (0.25 mL, 1.53 mmol)

in DCM (50 mL) was poured into the reaction mixture. The mixture was stirred for 1 h at 0 °C and 11 h at ambient temperature, then washed with saturated aqueous $NaHCO_3$ (3×50 mL) and brine (50 mL). The organic layer was dried with anhydrous Na_2SO_4. After the solvent was removed, the residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH=10:1, Rf =0.5) to afford **3** (0.91 g, 0.77 mmol) as a white solid: yield 50.9%. ^{1}H NMR (400 MHz, DMSO-d_6) 8.10 (d, 1H, J = 6 Hz, cytosin-5-C<u>H</u>), 7.34-7.46 (m, 6H, Ar-<u>H</u>, uracil-5-C<u>H</u>), 6.99-7.03 (m, 1H, cytosin-6-C<u>H</u>), 5.52 (d, 1H, J = 8 Hz, uracil-6-C<u>H</u>), 5.18 (s, 2H, Ar-C<u>H</u>$_2$), 4.81-4,86 (m, 2H, 3BoccyclenC<u>H</u>$_2$CO), 4.62-4.71 (m, 4H, COC<u>H</u>$_2$-cytosin), 4.08-4.10 (m, 2H, COC<u>H</u>$_2$-uracil), 3.98 (s, 2H, NC<u>H</u>$_2$COOCH$_3$), 3.62 (s, 3H, COOC<u>H</u>$_3$), 3.14–3.46 (m, 24H, cyclen-C<u>H</u>$_2$, C<u>H</u>$_2$N, NHC<u>H</u>$_2$), 1.36-1.39 (br, 27H, 3 Boc-C(C<u>H</u>$_3$)$_3$); ESI-MS: m/z calcd for $C_{54}H_{80}N_{13}O_{17}$ $[M+H]^+$: 1182.5795, Found: 1182.5792;

*3BocCyclen-[UC$^{(Z)}$]-OH (**4**)*: Compound **3** (0.91 g, 0.77 mmol) was dissolved in MeOH (50 mL) and cooled to 0 °C, then LiOH aqueous solution (1 M, 8.0 mL) was added. After stirring for 2 h at room temperature and monitored by TLC, the solvent MeOH was removed under reduced pressure. The pH was adjusted to 5 by 1 M aqueous HCl solution. The product was extracted with CH_2Cl_2 (3×100 mL) and the organic layer was dried with anhydrous Na_2SO_4. The solvent was removed to afford the title compound **4** as a white solid (0.78 g, 0.67 mmol): yield 87.2%. ^{1}H NMR (400 MHz, DMSO-d_6) 8.97 (s, 1H, COO<u>H</u>), 8.12 (d, 1H, J = 7.2 Hz, cytosin-5-C<u>H</u>), 7.34-7.43 (m, 6H, Ar-<u>H</u>, uracil-5-C<u>H</u>) 6.97-6.99 (d, 1H, J = 7.2 Hz, cytosin-6-C<u>H</u>), 5.51 (d, 1H, J = 8 Hz, uracil-6-C<u>H</u>), 5.18 (s, 2H, Ar-C<u>H</u>$_2$), 4.91 (s, 2H, 3BoccyclenC<u>H</u>$_2$CO), 4.52-4.61 (m, 4H, COC<u>H</u>$_2$-cytosin), 3.16–3.51 (m, 24H, cyclen-C<u>H</u>$_2$, C<u>H</u>$_2$N, NHC<u>H</u>$_2$), 1.36-1.39 (br, 27H, 3 Boc-C(C<u>H</u>$_3$)$_3$); ESI-MS: m/z calcd for $C_{53}H_{78}N_{13}O_{17}$ $[M+H]^+$: 1168.5639, Found: 1168.5587;

*3BocCyclen-[UC$^{(Z)}$C$^{(Z)}$C$^{(Z)}$]-OMe (**5**)*: A solution of carboxylic acid **4** (0.78 g, 0.67 mmol), TFA.H-[C$^{(Z)}$C($^{Z)}$]-OMe **1** (0.52 g, 0.67 mmol) and Et_3N (0.14 g, 1.34 mmol) in DMF (100 mL) was cooled to 0 °C. Then HOBt (0.12 g, 0.8 mmol), DCC (0.17 g, 0.8 mmol) were added for stirring 1 h at 0 °C. After that, the reaction was stirred overnight at room temperature. The solvent was evaporated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and freezed for 4 h. The suspension was filtered to remove the precipitated DCU. The solution was washed with saturated aqueous $NaHCO_3$ (2×50 mL) and saturated brine (50 mL). The organic layer was dried with anhydrous Na_2SO_4. After the solvent was removed under vacuum, the residue was

purified by column chromatography on silica gel (CH_2Cl_2:MeOH=5:1, Rf =0.4) to afford the title compound **5** as a white solid (0.49 g, 0.25 mmol): yield 37.4 %. HPLC at 260 nm, CH_3CN:H_2O=95:5 (v:v), t_R=4.10 min, >96 %. ^{1}H NMR (400 MHz, DMSO-d_6) 8.07-8.18 (m, 3H, cytosin-5-C<u>H</u>), 7.32-7.39 (m, 16H, Ar-<u>H</u>, uracil-5-C<u>H</u>), 6.97-6.99 (d, 3H, *J* =7.2 Hz, cytosin-6-C<u>H</u>), 5.52-5.56 (m, 1H, uracil-6-C<u>H</u>), 5.17 (t, 6H, *J* =8.8 Hz, Ar-C<u>H_2</u>), 3.22–3.40 (m, 32H, cyclen-C<u>H_2</u>, C<u>H_2</u>N, NHC<u>H_2</u>), 1.36-1.39 (br, 27H, 3 Boc-C(C<u>H_3</u>)$_3$); ESI-MS: m/z calcd for $C_{90}H_{118}N_{23}O_{27}$ $[M+H]^+$: 1952.8568, Found: 1952.8575.

PNA-Cyclen conjugate ***6*****:** Compound **5** (0.49 g, 0.25 mmol) was dissolved in MeOH (50 mL) and cooled to 0 °C, then LiOH aqueous solution (1 M, 3.0 mL) was added. After stirring for 2 h at room temperature and monitored by TLC, the solvent MeOH was removed under reduced pressure. The pH was adjusted to 5 by 1 M aqueous HCl solution. The product was extracted with CH_2Cl_2 (3×100 mL) and the organic layer was dried with anhydrous Na_2SO_4. The solvent was removed to afford the carboxylic acid product as a white solid.

Then the product was dissolved with MeOH (25 mL) in three-neck bottle, Pd/C was added in three-neck bottle and removed the atmosphere. The mixture was stirred under H_2 at room temperature overnight. Then Pd/C was filtrated. The solution was evaporated under vacuum. Then excessive amount of TFA solution was added. It was stirred for 3 h, and monitored by TLC. After reaction, the solvent was removed under vacuum to afford a white solid. Then, the solid was dissolved in twice distilled water, and a suitable amount of strongly alkaline styrene anion exchanged resin 201×7 was added to the solution, it was stirred until it turned to be basic. The solution was filtrated, evaporated, dried over P_2O_5 and gained a white solid. (0.15 g, 0.12 mmol): yield 48.5 %. The product was analyzed by reverse-phase HPLC at 260 nm, CH_3CN:H_2O=70:30 (v:v), t_R=6.20 min, >95%. MALDI-TOF-MS: m/z calcd for $C_{50}H_{74}N_{23}O_{15}$ $[M+H]^+$ 1236.573, Found: 1236.763.

S-2: Experimental Materials for the binding and cleavage experiments

General information: High Resolution Mass Spectrometer (HRMS) and MALDI-TOF-MS spectra data were respectively recorded on a Bruker Daltonics Bio TOF mass spectrometer and a Voyager-DE PRO mass spectrometer. The ^{1}H NMR spectra measured on a Bruker AV II spectrometer and the δ scale in ppm referenced to residual solvent peaks or internal tetramethylsilane (TMS). High Performance Liquid Chromatography (HPLC) date recorded on LC-2010AHT. The electrophoresis apparatus was a Biomeans stack II-electrophoresis system, PPSV-010. All reagents and chemicals such as KI, NaN_3, DMSO, and t-BuOH were of analytical grade and used without further purifications. CT-DNA (stored at 4 °C and used for not more than 4 days) which was purchased from Sigma Aldrich was directly dissolved in water at a concentration of 1 mg/mL. Its concentration was determined according to absorption intensity at 260 nm with a molar extinction coefficient value of 6600 $M^{-1}cm^{-1}$.The ONDs were purchased in a PAGE purified form Sangon Biotech (Shanghai), Co.,Ltd. Plasmid DNA (pUC 19) were purchased from Takara Biotechnology Company. [^{32}P] ATP [γ-P] was purchased from Furui. T4 polynucleotide kinase and T4 ligase were purchased from Fermentas. Storage solutions of PNA-Cyclen conjugate **6** were prepared by dissolving in deionized water and stored at 4 °C.

DNA melting experiments: Experiments were performed on a Hitachi U1900 spectrophotometer with a polyscience temperature controller system (±0.1 °C) and a cell path length of 1 cm. The absorption of CT-DNA (0.134 mM) was monitored at 260 nm in 2.5 mL phosphate (10 mM, pH 7.0) buffer in the absence and presence of the 26.8 μM of **6**. Temperature of the cell containing the cuvette increased from 25 to 95 °C and the absorbance at 260 nm was measured every 1–2 °C. All the T_m values derived from the derivative curves by using microcal origin 7.5.

Fluorescence spectroscopic studies: Fluorescence spectra measured at the room temperature in air by a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer and corrected for the system response**.** Fluorescent spectral were performed by the measurement of the emission intensity of ethidium bromide (EB). The experiments were carried out in 10 mM phosphate buffer (pH 7.0) by adding different concentrations of **6** to the EB-bound CT-DNA (0.1 mM) solution. The fluorescence spectra of EB were measured using excitation wavelength at 480 nm and the emission range was set between 520 and 750 nm. The relative binding propensity of the compound to CT-DNA was established by the classical Stern–Volmer equation as described previously.[3] The apparent binding

constant *Kapp* was also calculated from the equation $K_{app} = (K_{EB} \times [EB])/[Q]_{50}$, where $K_{EB} = 1 \times 10^7$ M^{-1}, [EB] = 1.3×10^{-6} M, and $[Q]_{50}$ was the concentration of where 50% reduction in fluorescence is achieved [4].

DNA cleavage experiments: Plasmid DNA (pUC 19) cleavage activity was monitored by using agarose gel electrophoresis. Supercoiled plasmid DNA (pUC 19) (5 mL, 0.025 g/L) in Tris-HCl buffer (50 mM, pH 7.4) was treated with **6** and diluted to a total volume of 17.5 μL. The samples were analyzed by 1% agarose gel containing 1.0 g/ml ethidium bromide. Electrophoresis was carried out at 40 V for 30 min in TAE buffer. Bands were visualized by UV light and photographed followed by the estimation of the intensity of the DNA bands using a Gel Documentation System. In the electropherogram, the fastest migration will be observed for the intact supercoiled form (Form I). If single-strand breaks, the supercoiled form will produce a slower moving relaxed form (Form II). If double-strands are cleaved, a linear form (Form III) will be generated that migrates between Form I and Form II [5].

The 24-mer DNA was [5'-^{32}P]-end-labeled with 5'-[γ-^{32}P] ATP and T4 polynucleotide kinase and purified by 20 % polyacrylamide gel electrophoresis. The DNA cleavage reaction containing the 5'-^{32}P-labeled DNA was carried out in 10 μL (total volume) of 50 mM Tris-HCl buffer, pH 7.4, containing 0.57 mM of **6**. Reaction mixture was incubated at 37 °C for 48 h, and then dissolved in a gel loading buffer (98 % formamide, 2 mM EDTA, 0.025 % xylene FF, and 0.025 % bromophenol blue)and then analyzed by 15% denaturing polyacrylamide gel electrophoresis at 15 w for 4 h. The gel was analyzed using phosphorimager.

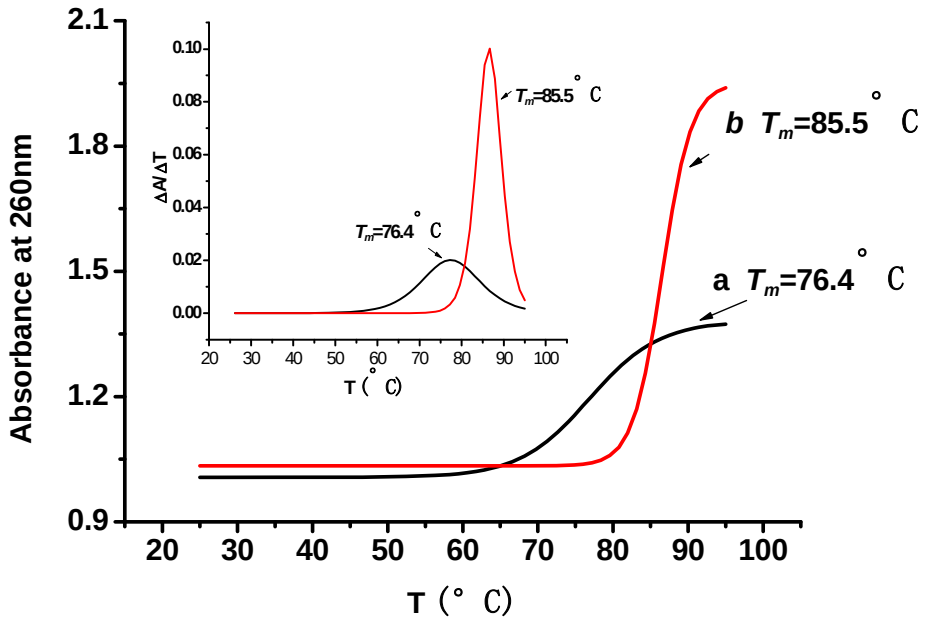


Fig. S1. Thermal denaturation curves (T_m) for the CT-DNA in the absence (a, T_m=76.4 °C) and presence (b, T_m=85.5 °C) of **6** in 10 mM NaH_2PO_4/Na_2HPO_4 buffer (errors in measurements approximately ±0.1 ℃). The inset shows the first derivatives of the melting curves.

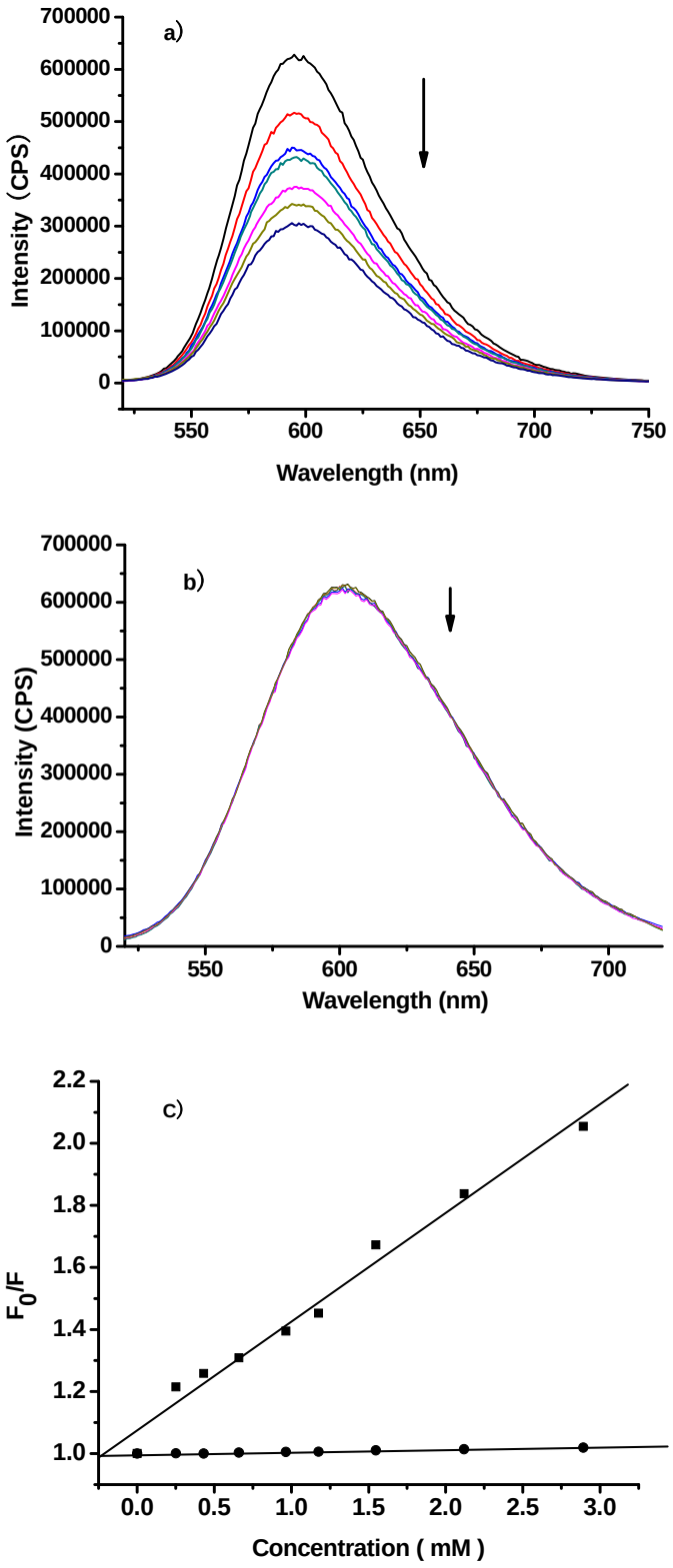


Fig. S2. Emission spectra of EB bound to CT DNA at increasing amounts (0-2.89 mM) of the compounds in 10 mM Tris-HCl buffer (pH 7.4) at 25 °C. a) PNA-Cyclen conjugate **6**. b) Cyclen. c) The plots of F_0/F vs the compounds. **6** (■) and cyclen (●).

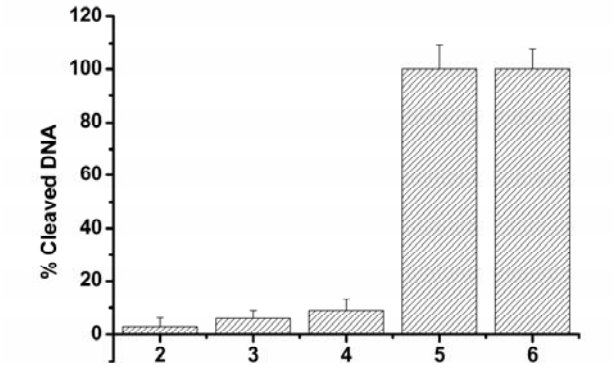


Fig. S3. Quantitation of % plasmid relaxation relative to plasmid DNA per lane of Fig. 2 in the manuscript.

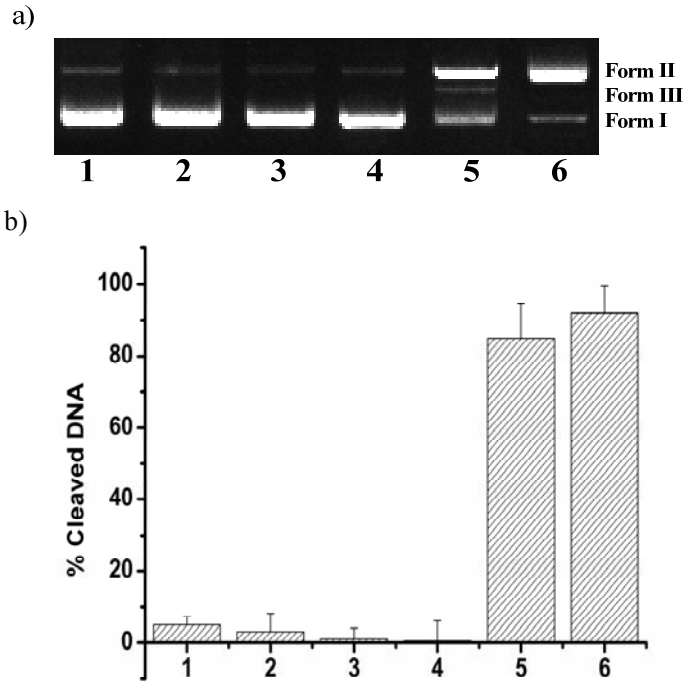


Fig. S4. Control experiments on DNA cleavage. a) Agarose gel of pUC 19 (7 μg/mL) incubated in Tris-HCl buffer (50 mM, pH 7.4) at 37 °C in the dark for 24 h. Lane 1, DNA control; Lane 2, 6 mM of EDTA; Lane 3, 0.57 mM of 3BocCyclen-[UCCC]-OH (demethylation product of **5**) in a H_2O/MeOH (6:1 v/v) solution; Lane 4, 0.57 mM of 3BocCyclen-[UCCC]-OH + 6 mM EDTA in a H_2O/MeOH (6:1 v/v) solution; Lane 5, 0.57 mM of **6** + 6 mM EDTA; Line 6, 0.57 mM of **6**. b) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

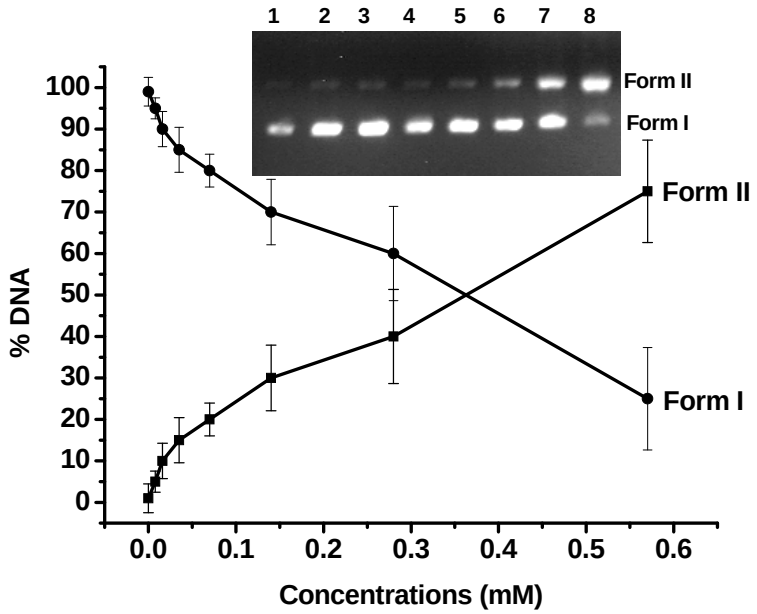


Fig. S5. Plot of percent cleaved DNA vs different concentrations of **6**. The inset is the agarose gel of the concentration-variable reactions incubated in Tris-HCl buffer (50 mM, pH 7.4) for 24 h at 37 °C: lane 1, DNA control; and lanes 2–8, concentrations of compound of 0.008, 0.016, 0.035, 0.071, 0.142, 0.285, and 0.570 mM, respectively.

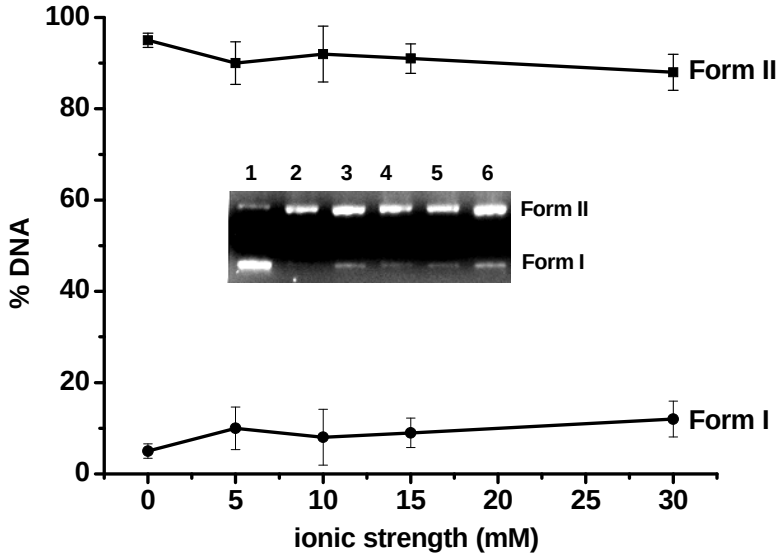


Fig. S6. Ionic strength-dependent profile for DNA cleavage promoted by **6** (0.57 mM).The inset is the agarose gel of the DNA cleavage reactions in Tris-HCl (50mM, pH 7.4) at 37 °C for 24 h under different ionic strength conditions: lane 1, DNA control; lane 2, no NaCl; and lanes 3–6, ionic strengths of 5, 10, 15, 30 mM, respectively.

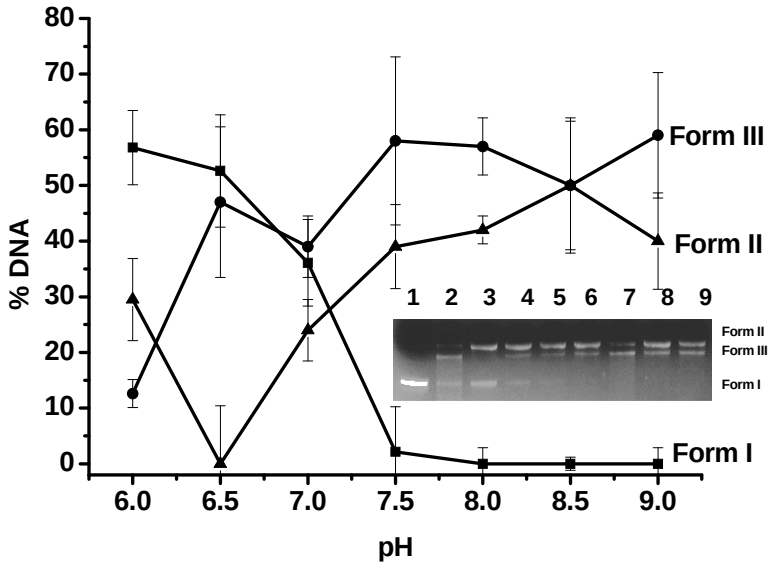


Fig. S7. pH-dependent profile for DNA cleavage promoted by **6** (0.57 mM). The inset is the agarose gel of the DNA cleavage reactions in Tris-HCl (50mM, pH 7.4) at 37 °C for 24 h under different pH conditions: lane 1, DNA control; lane 2–9, pH of 6.0, 6.5, 7.0, 7.4, 7.5, 8.0, 8.5, 9.0, respectively.

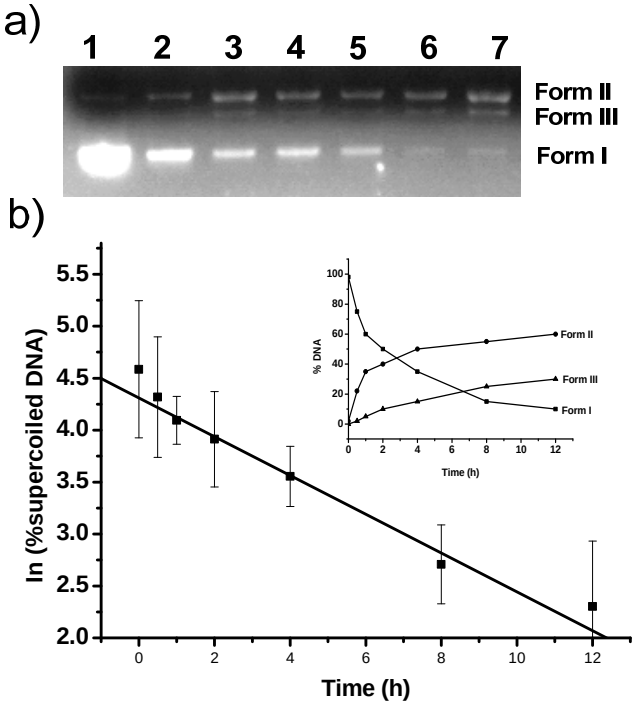


Fig. S8. Time course of pUC 19 DNA cleavage promoted by **6** (0.57 mM). (a) Agarose gel of the time-variable reaction products: lanes 1–8, reaction times of 0, 0.5, 1.0, 2.0, 4.0, 8.0, and 12.0 h, respectively. (b) Plot of ln (% supercoiled DNA) vs reaction time. The inset of is the plot of percent DNA vs time.

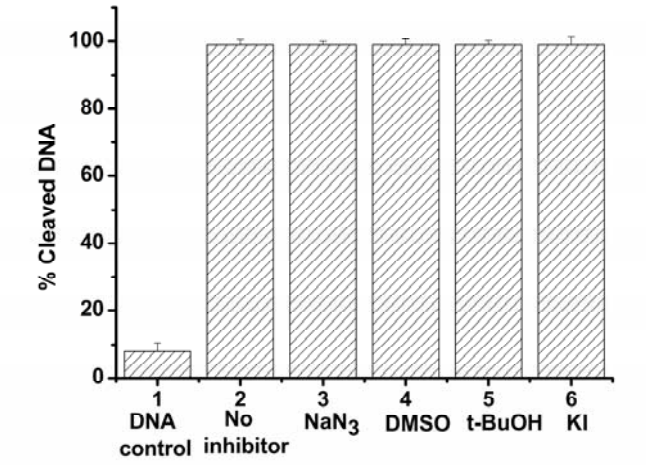


Fig. S9. Quantitation of % plasmid relaxation relative to plasmid DNA per lane of Fig. 3a.

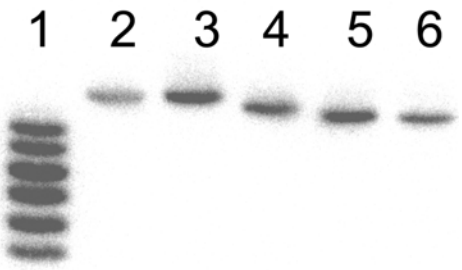


Fig. S10. Sequence dependence of cleavage reactions. The same procedures as those for preparing samples loaded in Lane 3 in Fig. 4 were used except that oligonucleotide was replaced. Line 1, 16-21 nt makers; line 2, a 24-nt (5'-^{32}P-AAAAAAAAAAAACCCTATATAGGG-3') oligonucleotide alone; line 3, reaction of oligonucleotide (5'-^{32}P-AAAAAAAAAAAAGCCTATATAGGC-3'); line 4, reaction of oligonucleotide (5'-^{32}P-AAAAAAAAAAAACGCTATATAGCG-3'); line 5, reaction of oligonucleotide (5'-^{32}P-AAAAAAAAAAAACCGTATATACGG-3'); line 6, reaction of oligonucleotide (5'-^{32}P-AAAAAAAAAAAACCCAATATTGGG-3');

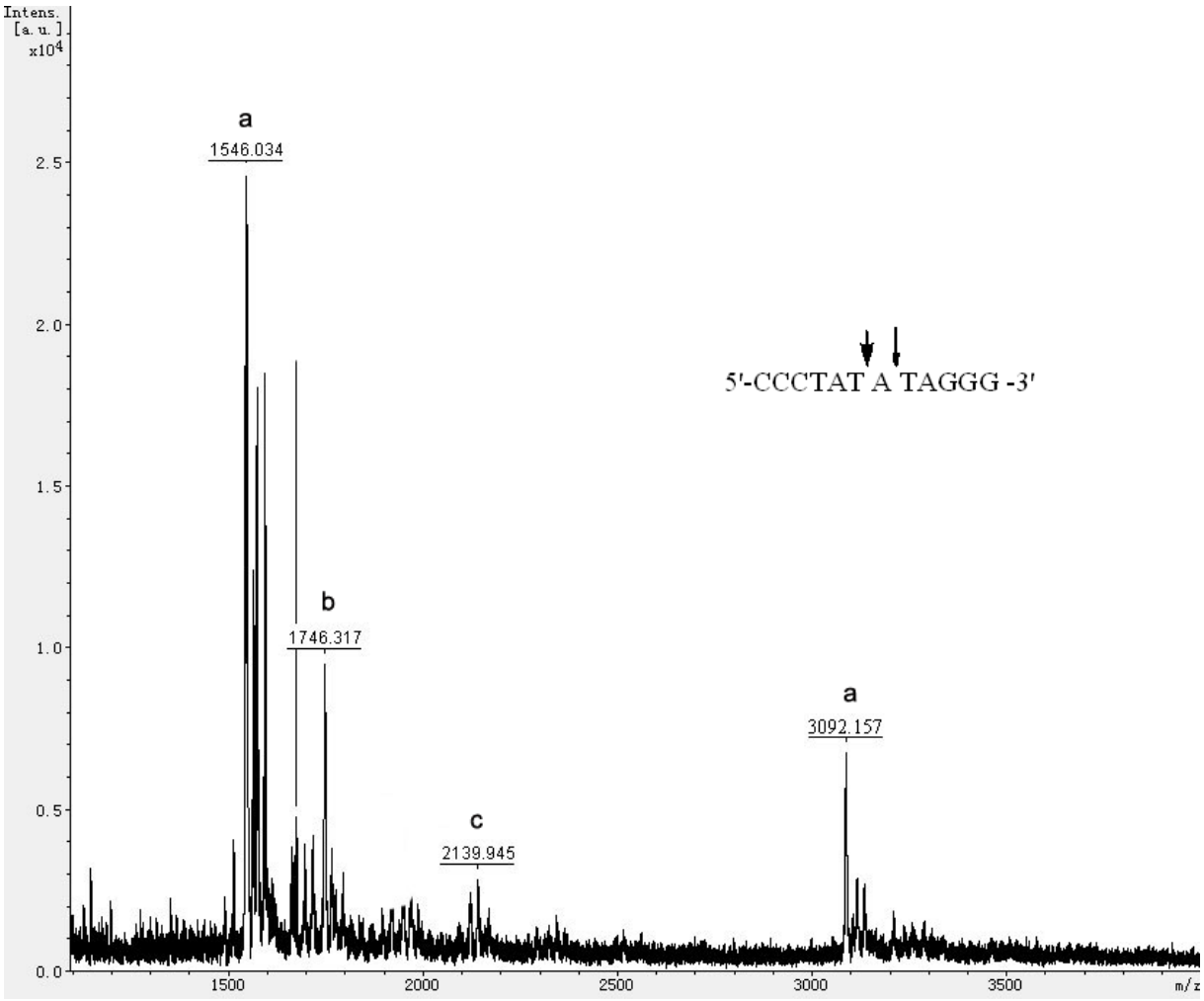
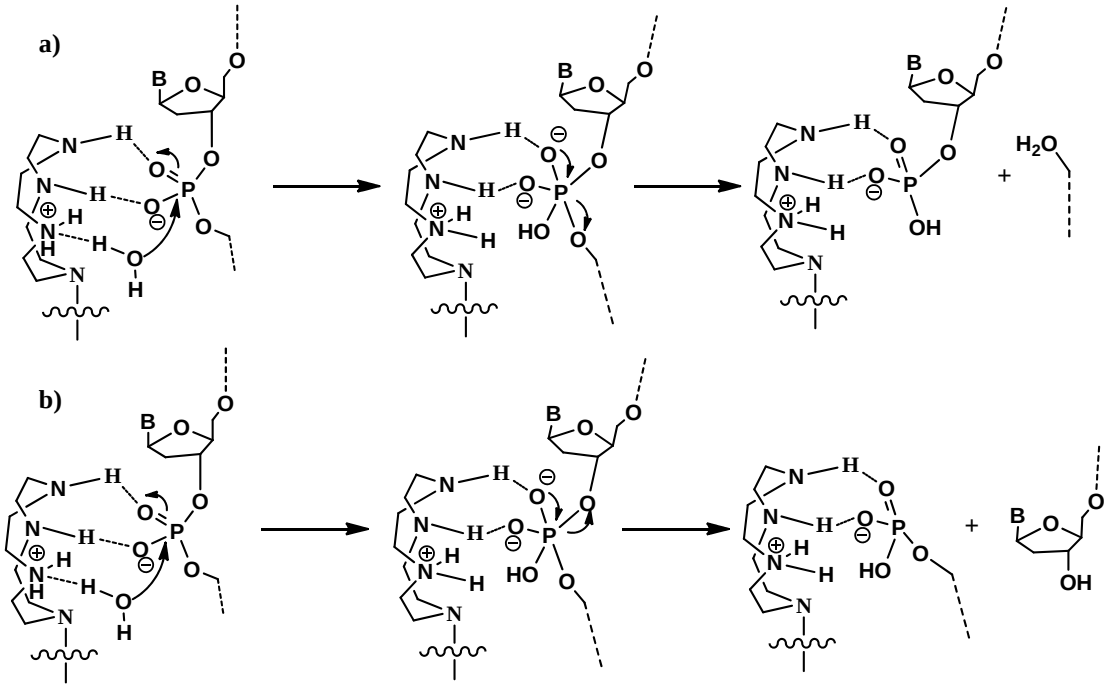


Fig. S11. MALDI-TOF mass spectra obtained after cleavage reaction of 12-nt oligonucleotide in Tris-HCl buffer (50 mM, pH 7.4) at 37 °C for 48 h by adding 0.57 mM of **6**. The inset is DNA cleavage sites obtained by mass analysis.

Table S1. MALDI-TOF MS characterization of **6**-induced cleavage products of 12-nt oligonucleotide.

Fragments	Sequence	Calculated m/z	Found m/z	Charge state
a	5'-OH-ATAGGG-3'-OH	3092.940	3092.157	$[M+H]^+$
	Cyclen-UCCC	1546.974	1546.034	$[M+2H]^{2+}$
b	5'-OH-CCCTAT-3'-OH	1745.354	1746.317	$[M+H_3O]^+$
c	5'-OH-CCCTATA -3'-phosphate	2138.375	2139.945	$[M+H_3O]^+$



Scheme S1. Proposed plausible Mechanism for DNA Cleavage Promoted by **6**. DNA cleavage promoted by cyclen motif was that in which H_2O molecules activated by protonated cyclen motif. Then, the activated phosphorus atom is nucleophilically attacked by the hydroxy, Finally, one of the P-O ester bonds was broken by the intramolecular charge delivery to achieve the DNA cleavage.

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