Supporting Information

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PPG Peptide Nucleic Acids that Promote DNA Guanine Quadruplexes


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Experimental Procedures

General

Reactions under anhydrous conditions were carried out under an atmosphere of nitrogen. Commercial-grade reagents and solvents were used without further purification except as indicated. TLC was run on silica 60 F254 glass sheets. 1H NMR spectra were recorded at 400 MHz and 13C NMR spectra at 100 MHz (δH: CDCl3 7.26 ppm, DMSO-d6 2.50; δC: CDCl3 77.1 ppm, DMSO-d6 39.5). Chemical shifts are reported in ppm relative to either tetramethylsilane or the deuterated solvent as an internal standard for 1H NMR and 13C NMR. Coupling constants (J values) are given in Hz. High-resolution mass spectra (HRMS) were obtained on a LC/MSD TOF (Agilent Technologies, Santa Clara, CA, USA). DNA oligomers were purchased from Integrated DNA Technologies, Inc. (Coralville, IA, USA) and used without further purification. UV quantification of PNA and DNA was performed using an Agilent 8453 UV-Vis Spectrophotometer.

Scheme S1. Reagents and conditions: a) TEA, THF, 100 °C, 91%; b) triphosgene, TEA, benzyl alcohol, 100 °C, 77%; c) NaOH, THF, RT, 99% (2 steps); d) EDC, DHBT, DMF, 40 °C, 88%; e) LiOH.H₂O, THF, 99%; f) EDC, DHBT, DMF, 40 °C, 87%; g) LiOH.H₂O, THF, 89%. TEA = triethyl amine, EDC = N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide, DHBT = 3-hydroxy-1,2,3-benzotriazine- 4(3H)-one, DMF = dimethyl formamide, THF = tetrahydrofuran.
Description of Synthesis:
Condensation of 2-amino-4,6-dichloropyrimidine-5-carboxaldehyde (3) with ethyl hydrazinoacetate salt (4) under basic conditions at 100 °C produced 5 in 91% yield without the need for further purification. The exocyclic amine of 5 was converted to the carbamate via treatment with triphosgene, followed by benzyl alcohol to trap the reactive intermediate. After column chromatography, compound 6 was isolated in 77% yield as a white powder. The hydrolysis of 6 was accomplished by treatment with finely ground NaOH suspended in anhydrous THF. When aqueous conditions were employed, removal of the Cbz-protecting group on the exocyclic amine drastically reduced overall yield of the reaction. After 15 hours, HPLC analysis showed consumption of starting material leaving two intermediates and the desired product 7. The reaction was then quenched and the contents isolated. The combined mixture was then subjected to another treatment with NaOH in anhydrous THF. After 48 hours, HPLC analysis showed only desired product and the reaction was quenched once more to afford the PPG-acetic acid 7 in 99% yield. The nucleobase acetic acid could then be employed to make different types of PNA monomers.

Ethyl 2-(6-amino-4-chloro-1H-pyrazolo[3,4-d]pyrimidin-1-yl)acetate (5). 2-Amino-4,6-dichloropyrimidine-5-carboxaldehyde 3 (1.981 g, 10.31 mmols, Aldrich) was dissolved in 165 mL of dry THF in an oven dried round bottom flask. Ethyl hydrazinoacetate hydrochloride (3.175 g, 20.62 mmol, 2.0 equivalents, Aldrich) and triethylamine (3.01 mL, 21.66 mmol, 2.1 equivalents, Aldrich) were added to the stirring solution and the resulting suspension was heated at 100 °C (reflux) for 30 minutes. TLC showed complete consumption of starting material. The yellow suspension was filtered via Buckner funnel and the retinate was washed with ethyl acetate. The filtrate was diluted with 600 mL of ethyl acetate and the solution was washed with water (3 X 200mL). The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure to afford 2.4 g (~91% yield) of ethyl 2-(6-amino-4-chloro-1H-pyrazolo[3,4-d]pyrimidin-1-yl)acetate (5) as an off-white powder. ¹H NMR (400 MHz, CDCl₃) δ 1.27 (t, J = 6.9 Hz, 3H), 4.23 (q, J = 6.9 Hz, 2H), 5.03 (s, 2H), 5.39 (s, 2H), 7.94 (s, 1H); ¹³C NMR (100MHz, CDCl₃) δ 14.12, 48.05, 62.00, 108.00, 133.59, 155.37, 156.41, 160.90, 167.44; HRMS (EI, m/z) Calculated for C₉H₁₀ClN₅O₂ (M + 1)⁺: 256.0601, found: 256.0607.
2-(6-(((benzyloxy)carbonyl)amino)-4-chloro-1H-pyrazolo[3,4-d]pyrimidin-1-yl)acetate (6). Ethyl 2-(6-amino-4-chloro-1H-pyrazolo[3,4-d]pyrimidin-1-yl)acetate 5 (1.075 g, 4.20 mmol) was dissolved in 50 mL of dry THR in a 150 mL round bottom flask. Triphosgene (1.87 g, 6.31 mmol, 1.5 equivalents, Aldrich) was dissolved in 10 mL of dry THF and added to the stirring solution slowly. Triethylamine (1.75 mL, 12.6 mmol, 3.0 equivalents, Aldrich) was then added (solution went from bright yellow, clear to cloudy, dark yellow) and the solution was heated at 100 °C (reflux) for 1.5 hours. Benzyl alcohol (1.31 mL, 12.6 mmol, 3.0 equivalents, Aldrich) was then added, and the solution was refluxed for an additional 30 minutes. The solution was cooled and filtered via Buckner funnel. The retinate was washed with ~ 100 mL of ethyl acetate and the combined organic layers were concentrated under reduced pressure to afford reddish oil. Column chromatography (4:1 hexanes to ethyl acetate) produced a 1.27 g (~77% yield) of ethyl 2-(6-(((benzyloxy)carbonyl)amino)-4-chloro-1H-pyrazolo[3,4-d]pyrimidin-1-yl)acetate (6) as a white powder.

2-(6-(((benzyloxy)carbonyl)amino)-4-oxo-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-1-yl)acetic acid (7). Ethyl 2-(6-(((benzyloxy)carbonyl)amino)-4-chloro-1H-pyrazolo[3,4-d]pyrimidin-1-yl)acetate 6 (1.10 g, 2.82 mmol) was dissolved in 131 mL of dry THF. Finely ground sodium hydroxide (1.13 g, 28.2 mmol, 10.0 equivalents) was suspended in the solution and the resulting suspension was left to stir with the round bottom flask sealed. HPLC analysis showed complete consumption of starting material after 15 hours leaving three peaks corresponding to final product and two possible intermediates. The reaction was quenched with 1 M HCl (~300 mL) and the solution was washed with ethyl acetate (3 X 200 mL). The combined organic layers were concentrated under reduced pressure and re-suspended in 131 mL of dry THF and finely ground NaOH (1.13 g, 28.2 mmol, 10.0 equivalents) and stirred in a sealed round bottom flask. Reaction was monitored via HPLC until only 1 peak (product) remained (~48 hours). The suspension was quenched with 1 M HCl (200 mL) and washed with ethyl acetate (3 X 200 mL). The combined organic layers were evaporated under reduced pressure to afford 960 mg (~99% yield) of 2-(6-(((benzyloxy)carbonyl)amino)-4-oxo-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-1-yl)acetic acid (7) as a flocculent white solid. Premature quenching of the reaction and resubmission to NaOH was found to be the most effective means of avoiding side products which was arduous to remove from the final product. $^1$H NMR (400
MHz, CDCl$_3$ $\delta$ 4.95 (s, 2H), 5.26 (s, 2H), 7.40 (m, 5H), 8.03 (s, 1H), 11.26 (s, 1H), 11.65 (s, H1), 13.24 (s, 1H); $^{13}$C NMR (100MHz, CDCl$_3$ $\delta$ 48.41, 67.41, 102.19, 128.00, 128.29, 128.48, 135.02, 135.43, 149.37, 152.96, 154.55, 155.95, 168.98.

**aegPNA PPG(Z) monomer ethyl ester (10).** 2-(6-(((benzyloxy)carbonyl)amino)-4-oxo-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-1-yl)acetic acid 7 (480 mg, 1.77 mmol), EDC (275 mg, 1.77 mmol) and DHBT (283 mg, 1.73 mmol) were dissolved in 40 mL of dry DMF and heated at 40 °C for 5 minutes (bright yellow solution). aegPNA backbone 8 (480 mg, 1.95 mmol) was dissolved in 2 mL of dry DMF and added via syringe to the stirring solution. The reaction was allowed to run over night (12 hours). The solution was then added to 150 mL of cold water (forming an off white suspension) and extracted with EtOAc (4 X 50 mL). The combined organic layers were washed with 1 M HCl (3 X 50 mL), sat. NaHCO$_3$ solution (3 X 50 mL), water (3 X 50 mL) and sat. NaCl solution (3 X 50 mL). The organic layer was dried of Na$_2$SO$_4$ and evaporated under reduced pressure to afford 890 mg (88 % yield) of aegPNA PPG(Z) monomer ethyl ester 10 as a flocculent off-white solid. $^1$H NMR (400 MHz, DMSO) $\delta$ 1.16 (t, $J$ = 7.1 Hz, 3H), 1.37 (s, 9H), 3.8 (m, 2H), 3.49 (t, $J$ = 6.5, 2H), 4.03 (s, 2H), 4.07 (q, $J$ = 7.2 1H), 5.18 (s, 2H), 5.26 (s, 2H), 6.96 (br, 1H), 7.40 (m, 5H), 8.002 (s, 1H), 11.24 (s, 1H), 11.63 (s, 1H); Minor Rotomer: 1.25 (t, $J$ = 7.1 Hz, 3H), 1.36 (s, 9H), 3.00 (m, 2H), 3.30 (t, $J$ = 7.2, 2H), 4.19 (q, $J$ = 7.2 1H), 4.4 (s, 2H), 5.05 (s, 2H), 6.76 (br, 1H), 7.996 (s, 1H), 11.26 (s, 1H), 11.68 (s, 1H); HRMS (EI, m/z) Calculated for C$_{26}$H$_{33}$N$_7$O$_8$ (M + 1)$^+$: 572.2469, found: 272.2460.

**aegPNA PPG(Z) monomer (1).** aegPNA PPG-(Z) Monomer Ethyl Ester 10 (79 mg, 0.14 mmol) was dissolved in THF (5.7 mL) and cooled to 0 °C via ice bath. NaOH (22 mg, 0.55 mmol) was dissolved in 5 mL H$_2$O and was added slowly to the stirring solution. The ice bath was then removed and the reaction was stirred under N$_2$. The reaction was monitored via HPLC, showing the reaction completed with no side products within 2 hours. The solution was diluted with cold H$_2$O (6 mL) and washed with ethyl ether (2 x 6 mL). The pH of the aqueous layer was lowered to ~2 with 3M HCl (drop wise) forming a white precipitate. The solution was washed with EtOAc (4 x 15 mL) and the organic layers were dried over Na$_2$SO$_4$. The solution was evaporated under reduced pressure to afford 74 mg (~99% yield) of aegPNA PPG-(Z) monomer 1 as a flocculent, off-white solid. This compound was used in PNA synthesis without further
purification. $^1\text{H}$ NMR (MeOD, 400 Hz, TMS reference) $\delta$ 11.67 (br s, 1H), 11.26 (s, 1H), 7.99 (s, 1H), 7.44-7.34 (m, 6H), 6.96 (s, 1H), 6.76 (s, 1H), 5.25 (s, 2H), 5.17 (s, 1H), 5.04 (s, 1H), 4.30 (s, 1H), 3.96 (s, 2H), 3.48 (br s, 2H), 3.29 (s, 1H), 3.19 (t, $J$ = 5.7 Hz, 1H), 3.02 (br s, 1H), 1.37 (m, 9H) $^{13}\text{C}$ NMR (100 MHz, MeOD): $\delta$ 171.4, 170.9, 167.2, 166.7, 156.5, 156.2, 156.0, 155.1, 155.0, 153.8, 153.7, 149.5, 135.8, 135.2, 128.9, 128.7, 128.4, 102.5, 78.5, 78.2, 67.7, 49.7, 48.7, 48.4, 47.8, 47.2, 38.7, 38.1, 28.6, 28.6. ESI-MS found $m/z$ 544.2 [M+H]$^+$, calculated for C$_{24}$H$_{30}$N$_7$O$_8$ 544.2.

tcypPNA PPG(Z) monomer ethyl ester (11). EDC (22 mg, 0.115 mmol) was added to a stirred solution of 9 (15 mg, 0.055 mmol), 2-(6-(((benzyloxy)carbonyl)amino)-4-oxo-4,5-dihydro-1$H$-pyrazol[3,4-d]pyrimidin-1-yl)acetic acid (40 mg, 0.116 mmol), and 3-hydroxy-1,2,3-benzotriazine-4(3H)-one (18 mg, 0.110 mmol) in DMF (2 mL) at 40 °C. After stirring overnight at 40°C, H$_2$O (3 mL) was added and the product was extracted with EtOAc (3 × 6 mL). The combined organic layers were washed with 1N HCl (6 mL), saturated aqueous NaHCO$_3$ (6 mL), H$_2$O (6 mL), and brine (6 mL). The organic layer was dried over Na$_2$SO$_4$ and evaporated under reduced pressure and the compound was purified by silica gel column chromatography using 0-4% MeOH in CH$_2$Cl$_2$ as eluents to afford 11 as a white solid material (29 mg, 87%). $^1\text{H}$ NMR (CDCl$_3$, 400 Hz, TMS reference) $\delta$ 11.19 (br s, 1H), 10.89 (br s, 1H), 7.95 (s, 1H), 7.34 (s, 5H), 5.61 (br s, 2H), 5.24 (m, 2H), 4.94 (s, 2H), 4.18 (m, 2H), 3.64 (s, 3H), 2.61 (s, 1H), 2.15 (s, 1H), 1.72 (m, 4H), 1.36 (s, 9H). $^{13}\text{C}$ NMR (100 MHz, CDCl$_3$): $\delta$ 167.8, 167.0, 156.8, 156.5, 135.6, 134.6, 129.0, 128.7, 128.6, 128.3, 102.7, 69.5, 68.8, 68.2, 61.9, 54.5, 53.8, 52.2, 50.6, 48.2, 28.3. ESI-MS found $m/z$ 598.2 [M+H]$^+$, calculated for C$_{28}$H$_{36}$N$_7$O$_8$ 598.2.

tcypPNA PPG(Z) monomer (2). Compound 11 (28 mg, 0.046 mmol) was dissolved in THF (0.7 mL) and cooled to 0 °C followed by the dropwise addition of a solution of LiOH (26 mg, 0.622 mmol) in H$_2$O (0.6 mL). Resulting reaction mixture was stirred at RT for 5h and than diluted with H$_2$O (1 mL), extracted with ethyl ether (3 x 2 mL). Aq. layer was acidified with aq. 3N HCl to pH1 and than the solution was extracted with ethyl acetate (5 x 3 mL). Combined organic phase were dried over Na$_2$SO$_4$ and evaporated under reduced pressure to afford 2 as a white solid material (24 mg, 89%). This compound was used in PNA synthesis without further purification. $^1\text{H}$ NMR (MeOD, 400 Hz, TMS reference) $\delta$ 7.96 (s, 1H), 7.95 (s, 1H), 7.34 (m,
6H), 5.23 (br s, 2H), 4.58 (s, 2H), 3.87 (m, 2H), 1.92 (s, 2H), 1.67 (br s, 2H), 1.39 (m, 11H) \(^{13}\)C NMR (100 MHz, MeOD): \(\delta 173.8, 171.3, 171.2, 171.1, 169.1, 168.7, 168.2, 167.8, 159.6, 157.7, 156.6, 155.9, 155.8, 154.8, 154.7, 153.8, 149.2, 149.1, 141.2, 135.1, 134.9, 128.3, 128.1, 127.9, 126.8, 126.6, 102.0, 99.5, 79.0, 78.7, 67.9, 63.8, 62.3, 62.2, 61.3, 61.1, 53.4, 52.8, 45.0, 43.4, 45.0, 43.4, 29.4, 28.2, 27.56, 26.2, 252.7, 22.3, 19.5, 19.0, 13.1. ESI-MS found \(m/z\) 584.2 [M+H]\(^+\), calculated for \(\text{C}_{27}\text{H}_{34}\text{N}_{7}\text{O}_{5}\) 584.2.

**Preparation of PNA Oligomers.** PNA oligomer synthesis was performed on an Applied BioSystems 433A Automated Peptide Synthesizer. Boc-mPEG was purchased from Peptides International (Louisville, KY, USA). MBHA Resin (0.3 mmol/g) was prepared by swelling in DCM and downloading the resin with Boc protected \(N,N\)-dimethyl-L-lysine to 0.1 mmol/g capacity. Boc-mPEG was purchased from Peptides International (Louisville, KY, USA). PNA oligomers were made via solid-phase peptide synthesis on 5 \(\mu\)mol scale.

**General Resin Cleavage.** Upon completion of PNA synthesis or solid phase coupling, the PNA-bound resin was transferred to a glass reaction vessel and washed with DCM, then TFA. The resin was swelled in TFA. The solvent was removed and a solution of \(m\)-cresol (150 \(\mu\)L), thioanisole (150 \(\mu\)L), TfOH (300 \(\mu\)L), and TFA (900 \(\mu\)L) was added and allowed to sit on the resin for 60 min. The solution was drained into a scintillation vial. This was repeated for a total of 3 washes, each time collecting the eluent in the scintillation vial. The pooled solution was concentrated, transferred to microfuge tubes, and precipitated using diethyl ether at a ratio of 1:10. The resulting flaky off-white solid was washed 3 times with diethyl ether and dried under vacuum. The resulting residue was diluted with 2:1 water:ACN and further purified on reversed phase HPLC. Purification of PNA oligomers was carried out using a X-Bridge Prep BEH 130 C18 5\(\mu\)m (10 x 250 mm) column on an Agilent 1200s HPLC. The typical flow rate was 4 mL/min. HPLC solvents consisted of HPLC grade acetonitrile:MilliQ water (9:1) and 0.10% aqueous TFA. PNAs were purified by RP-HPLC on Xbridge Prep BEH130 C-18 column (5 \(\mu\)m, 10 mm \(\times\) 150 mm) at 35 °C eluting with a linear gradient of acetonitrile:water (9:1) in water containing 0.1 \% of TFA, over 35 min. Wavelengths 220 nm, 260 nm, and 315 nm were monitored, and the fraction containing the major peak was collected, lyophilized to dryness to
afford pure PNA samples. The molecular weight of the synthesized PNAs was confirmed by mass spectrometry.

**Quantification of PNA Oligomer Conjugates.**
Lyophilized PNA oligomers were dissolved in water. The absorbance of an aliquot was determined by UV-VIS spectroscopy after heating the sample for 5 min at 90°C. This was performed in triplicate. Using the extinction coefficient of the analogous DNA oligomer obtained from Applied Biosystems (Life Technologies, Grand Island, NY), the concentration was determined.

**Table S1.** List of synthesized PNA oligomers.

<table>
<thead>
<tr>
<th>PNA</th>
<th>Sequence[a]</th>
<th>Predicted Mass</th>
<th>Observed Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H$_2$N - G - G - A - G - G - C - G - G - G - Lys</td>
<td>2710.57</td>
<td>2710.6</td>
</tr>
<tr>
<td>2</td>
<td>H$_2$N - Glu - G - G - A - G - G - C - G - G - Glu · Lys</td>
<td>2968.80</td>
<td>2968.4</td>
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<tr>
<td>3</td>
<td>H$_2$N - G - G - C - G - A - G - G - Lys</td>
<td>2710.57</td>
<td>2710.8</td>
</tr>
<tr>
<td>4</td>
<td>H$_2$N - Glu - G - G - G - A - G - G - Glu · Lys</td>
<td>2968.80</td>
<td>2968.3</td>
</tr>
<tr>
<td>5</td>
<td>H$_2$N - G - G - A - X - G - C - G - X - G - Lys</td>
<td>2710.57</td>
<td>2711.0</td>
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<tr>
<td>7</td>
<td>H$_2$N - Glu - G - G - A - X - G - C - G - X - G - Glu · Lys</td>
<td>2968.80</td>
<td>2969.0</td>
</tr>
<tr>
<td>8</td>
<td>H$_2$N - Glu - G - X - G - C - G - X - A - G - Glu · Lys</td>
<td>2968.80</td>
<td>2968.5</td>
</tr>
<tr>
<td>9</td>
<td>H$_2$N - Glu - G - X - G - C - G - X - A - G - Glu · Lys</td>
<td>3048.93</td>
<td>3048.2</td>
</tr>
</tbody>
</table>

[a] PNA oligomers are written from N terminus to C terminus. Lys = lysine, Glu = glutamic acid, X = PPG residue, X = (S,S)-trans cyclopentane PPG residue.

**General Annealing Condition for Formation of PNA:DNA or PNA:PNA Duplexes and quadruplexes.**
RNA/DNAase free microfuge tubes, PNA, DNA and PBS buffer containing 10 mM sodium phosphate, 0.1 mM EDTA, and 150 mM NaCl (pH 7.0) were combined at room temperature. The solution was heated to 90°C, held for 5 min, then slowly allowed to cool down to 25°C over a period of 3 h.
DEPC probing assay.
The DEPC probing assay was performed as described previously. Briefly, after incubation with PNAs for 20 hours at room temperature plasmids were probed with 2 μl diethylpyrocarbonate (DEPC) for 5 min at room temperature. Modified plasmids were cut with restriction enzymes (Fermentas) and end-labeled with [α-32P]-dCTP (Perkin Elmer) and Klenow fragment of DNA polymerase I (Fermentas). The sites of DEPC modification were revealed by incubation in 10% piperidine at 95 °C for 20 min. Samples were analyzed by electrophoresis on 12% denaturing polyacrylamide gels. To measure the intensity of the individual bands, the intensity profile of each lane was generated from the digitized gel image using Image Gauge software (FUJI Medical Systems USA). Intensity of the band corresponding to A in the PNA binding site was normalized on the intensity of whole lane with background subtraction.

S1 nuclease cleavage assay.
pCRBcl2 plasmid (1 μg) was incubated with 20 pmol PNA in 20 μL total volume in TEK buffer (1 mM Tris*HCl, pH 7.4, 0.1 mM EDTA, 50 mM KCl) at room temperature for 20 hours. Ten μL of the sample was subject to S1 nuclease assay while the other 10 μl were used for the HpyF10VI protection assay. S1 nuclease cleavage was performed in 20 μl final volume in S1 nuclease buffer (30 mM NaAc pH 4.6, 10 mM ZnAc) with 10 units of S1 nuclease (Invitrogen) on ice for 2 minutes. Reactions were stopped with EDTA (25 mM final concentration), extracted with equal volume (20 μl) of Phenol:Chloroform:Isoamyl Alcohol (25:24:1, Sigma) and purified on G50 Microspin columns (GE Healthcare). The purified samples were digested with Dra1 restriction endonuclease (Thermo Scientific) and analyzed in 1% Agarose gels. Gels were stained in 1 μg/ml ethidium bromide and scanned with a FluorImager 595 instrument. Intensity of the bands was measured by ImageQuant Software.

HpyF10VI protection assay.
Samples were digested with 1 unit FD HpyF10VI and 1 unit FD EcoR1 restriction endonucleases (Thermo Scientific) in FD buffer at 37C for 10 minutes. Then 1 μCi of [α-32P]-dATP and 1 u of Klenow Fragment (Thermo Scientific) were added and incubation continued at room temperature for 2 minutes. Reaction was stopped with 20 μl formamide with bromophenol blue followed by boiling for 2 minutes in water. 5 μL of the samples were analyzed in 8%
polyacrylamide denaturing gels (National Diagnostics). Autoradiography of the gels was done with FUJI Bas 2500 BioImager, and intensity of the bands were measured by ImageGauge software (FUJI Imaging).

**A**

\[GGAGGC\text{CGGG} \text{[aligned]}\]

5′-ACCCGACCGCCCCCTCCGCCGCCCTGGGCGG-3′

3′-TGGGCTGCGGGGGGCCGGGGCCGGAGGACC-5′

**B**

Figure S1. (A) Target sequence in the pCRBcl2 plasmid and PNA (bold). The adenine (A) that became open after PNA invasion in the target sequence is marked with arrow. (B) Intensity of the adenine modification after invasion of the panel of PNAs.

Figure S2. PNA invasion measured by S1 nuclease cleavage assay. Percent of cleavage was calculated from the ratio of intensities of the 2683 bp fragment appeared after S1 nuclease cleavage at the PNA invasion site to the intensity of the linear plasmid in the same lane of 1% agarose gel.
Reference