Supporting Information


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1.0 General procedures: All chemicals used for reactions were analytical and laboratory grade. For each reaction, the solvents used were dried or distilled. Reactions were monitored by thin-layer chromatography (TLC). TLC was carried out on pre-coated silica gel GF<sub>254</sub> sheets (Merck 5554). TLC plates were visualized under an UV lamp by spraying with ninhydrin solution, followed by heating of the plate. Column chromatographic separations were performed using silica gel (60-120 or 100-200 mesh).

All <sup>1</sup>H and <sup>13</sup>C NMR were recorded using Bruker AC-400 (400 MHz) or JEOL 400 MHz NMR spectrometers in CDCl<sub>3</sub> and DMSO-d<sub>6</sub>. Chemical shifts are reported in parts per million (ppm, δ scale). UV-Visible spectrophotometry (Perkin Elmer Lambda 45 double beam UV-Vis spectrophotometer) data were collected for compound/peptide characterization. Mass spectra for reaction intermediates were obtained by HRMS and the integrity of PNA oligomers were analyzed by using MALDI-TOF-MS. PNA oligomers were purified by reverse phase HPLC system using a semipreparative Phenomenex C<sub>18</sub> (10 X 250 mm) column. DNA oligonucleotides were obtained commercially from Integrated DNA Technologies (IDT). Reagents used in buffer preparation such as sodium cacodylate and salts potassium chloride were obtained from Sigma-Aldrich.
2.0 Synthesis of $bm$-PNA (C/T) monomers and N9-propargyl guanine:

The monomers $bm$-PNA-C 1 and $bm$-PNA-T 2 were synthesized, characterized and purified according to previously reported protocols.\(^1\)

![Monomer structures](image)

Synthesis of N9-propargyl guanine

N9-Propargyl guanine synthesized according to standard literature protocols.\(^2\)

![Synthesis reaction](image)

3. Solid phase syntheses of $bm$-PNA-G5 oligomer\(^1\)

The synthesis of $bm$-PNA-G5 (C-GTCTTC-A) oligomer was done from C terminus to N-terminus direction by solid phase synthesis using L-lysine derivatized MBHA functionalized resin (3) with 0.22 mmol/g loading. N-Boc group was deprotected with 50% TFA in DCM to get 4, which was neutralised to get resin 5 with free amino group. This was then coupled with $aeg$-PNA-G monomer using HOBT/HATU as coupling agent to yield 6. The deprotection - coupling cycle was repeated using $bm$-PNA-(C/T) monomers (1 and 2) in the order (TCTTC) as per desired sequence to give 7. This was followed by last coupling with $aeg$-PNA-A monomer to yield resin bound $bm$-PNA oligomer 8 having five ethyl azide sidechains. In the subsequent step, a global click reaction of ethyl azido group on solid phase with N9-propynyl guanine provided MBHA-$bm$-PNA-G5 9, which was further subjected to reaction with TFA/TFMSA. This lead to free $bm$-PNA-G5 oligomer 10.
**Figure 1** Solid phase synthesis protocol of bm-PNA (N-ACTTCTG) by Boc strategy.

3a. Solid phase synthesis of iso-PNA-G5: This was done by a similar procedure as above, using appropriate iso-PNA monomers as reported previously.
3b. Solid phase synthesis of bm-PNA-G5-Cf: The resin bound bm-PNA oligomer 9 was deprotected, followed by coupling with carboxy fluorescein or cyanine -3-carboxylic acid. The resulting conjugates were cleaved from the resin and deprotected to yield fluorescent bm-PNA-G5-Cf and bm-PNA-G5-Cy3 for experiments with FRET and cell uptake.
3c. Solid phase synthesis of bm-PNA-G5-Cy3

A. i. 50% TFA:DCM
   ii. 10% DIPEA

B. HOBr, HBTU, DIPEA,
   Cyanine-3-Carboxylic acid

Resin cleavage
TFA
TFMSA
Thioanisole
3d. Solid phase synthesis of aeg-PNA-G5-Cf: This was prepared by a similar procedure as above using aeg-PNA-G5.
**3f. Solid phase synthesis of mix-aeg-PNA-Cf**: This was prepared by a similar procedure as above using mix-aeg-PNA-G5.
4.0 RP-HPLC Chromatograms and MALDI-TOF of PNA Oligomers

The purification of PNA oligomers done by RP-HPLC and characterization by MALDI-TOF

4a. RP-HPLC and MALDI of aeg-PNA-G5

![RP-HPLC and MALDI of aeg-PNA-G5](image)

RT-11.3 min  
Calcd mass:[M+2H]⁺ 1603.58  
Obsd mass:[M+2H]⁺ 1603.21

4b. RP-HPLC and MALDI of iso-PNA-G5

![RP-HPLC and MALDI of iso-PNA-G5](image)

RT-13.1 min  
Calcd mass:[M+K]⁺ 2176.38  
Obsd mass:[M+K]⁺ 2176.46
4c. RP-HPLC and MALDI of \( bm \)-PNA-G5

\[
\text{RP-HPLC}
\]

\[
\text{MALDI-TOF}
\]

- 13.6 min
- \([M+K]^+ 3343.91\)
- Calcd mass: \([M+K]^+ 3343.34\)
- Obsd mass: \([M+K]^+ 3343.91\)

4d. RP-HPLC and MALDI of \( mix-aeg \)-PNA

\[
\text{RP-HPLC}
\]

\[
\text{MALDI-TOF}
\]

- 12.3 min
- \([M+Na]^+ 2036\)
- Calcd. Mass \([M+Na]^+ 2036.00\)
- Obsd. Mass \([M+Na]^+ 2036.00\)
4e. RP-HPLC and MALDI of \( bm \)-PNA-G5-Cf

\[
\text{MALDI-TOF}
\]

\[
\text{Caled mass } [M+2H]^+ 3664.56
\]

\[
\text{Obsd mass } [M+2H]^+ 3664.07
\]

4f. RP-HPLC and MALDI of \( bm \)-PNA-G5-Cy3

\[
\text{MALDI-TOF}
\]

\[
\text{Caled mass } [M-3H-Cy3]^+ 3396.37
\]

\[
\text{Obsd mass } [M-3H-Cy3]^+ 3396.12
\]
4g. RP-HPLC and MALDI of *aeg*-PNA-G$_5$-Cf

4h. RP-HPLC and MALDI of mix-*aeg*-PNA-Cf
Table 1. MALDI-TOF analysis of PNA oligomers

<table>
<thead>
<tr>
<th>Entry</th>
<th>PNA</th>
<th>Mol. formulae</th>
<th>Calcd. Mass</th>
<th>Obsd. Mass</th>
</tr>
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<tr>
<td>1</td>
<td>aeg-PNA-G5</td>
<td>C\textsubscript{62}H\textsubscript{32}N\textsubscript{38}O\textsubscript{16}</td>
<td>[M+2H]\textsuperscript{+} 1603.58</td>
<td>[M+2H]\textsuperscript{+} 1603.21</td>
</tr>
<tr>
<td>2</td>
<td>iso-PNA-G5</td>
<td>C\textsubscript{84}H\textsubscript{121}K\textsubscript{57}O\textsubscript{13}</td>
<td>[M+K]\textsuperscript{+} 2176.38</td>
<td>[M+K]\textsuperscript{+} 2176.46</td>
</tr>
<tr>
<td>3</td>
<td>bm-PNA-G5</td>
<td>C\textsubscript{131}H\textsubscript{159}K\textsubscript{79}O\textsubscript{29}</td>
<td>[M+K]\textsuperscript{+} 3343.34</td>
<td>[M+K]\textsuperscript{+} 3343.91</td>
</tr>
<tr>
<td>4</td>
<td>mix-aeg-PNA</td>
<td>C\textsubscript{81}H\textsubscript{109}N\textsubscript{39}NaO\textsubscript{24}</td>
<td>[M+Na]\textsuperscript{+} 2036.00</td>
<td>[M+Na]\textsuperscript{+} 2036.00</td>
</tr>
<tr>
<td>5</td>
<td>bm-PNA-G5-Cf</td>
<td>C\textsubscript{152}H\textsubscript{171}N\textsubscript{79}O\textsubscript{35}</td>
<td>[M+2H]\textsuperscript{+} 3664.56</td>
<td>[M+2H]\textsuperscript{+} 3664.07</td>
</tr>
<tr>
<td>6</td>
<td>bm-PNA-G5-Cy3</td>
<td>C\textsubscript{161}H\textsubscript{196}ClN\textsubscript{81}O\textsubscript{30}</td>
<td>[M+2H]\textsuperscript{+} 3664.56</td>
<td>[M+2H]\textsuperscript{+} 3664.56</td>
</tr>
<tr>
<td>7</td>
<td>aeg-PNA-G5-Cf</td>
<td>C\textsubscript{82}H\textsubscript{92}N\textsubscript{38}O\textsubscript{22}</td>
<td>[M+H]\textsuperscript{+} 1960.87</td>
<td>[M+H]\textsuperscript{+} 1961.30</td>
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<tr>
<td>8</td>
<td>mix-aeg-PNA-Cf</td>
<td>C\textsubscript{102}H\textsubscript{119}N\textsubscript{39}O\textsubscript{30}</td>
<td>[M]\textsuperscript{*} 2371.92</td>
<td>[M]\textsuperscript{*} 2371.32</td>
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6. DNA oligonucleotides used for biophysical studies

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<tr>
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<tr>
<td>1</td>
<td>DNA 1</td>
<td>CAGAAGT</td>
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7. UV-\(T_m\) of PNA variants at 260 nm:

A. \(dTG_5\)

60.6

B. \(aeg\)-PNA-\(G_5\)

71

C. \(iso\)-PNA-\(G_5\)

58.2

D. \(bm\)-PNA-\(G_5\)

52.2

8. Full LC profile of \(bm\)-PNA-G5 tetraplex:

Retention time : 0.63 min

The PNA was diluted in 50% ACN (1 ul of KCL was added) and separated on C4 column by using isocratic solution of 50% ACN.
9. NIH-3T3 cell_24h uptake @4 µM concentration of PNA

Confocal microscopy images of NIH-3T3 cells treated with (A–E) bm-PNA-G5-Cf, (F–J) aeg-PNA-G5-Cf and (K–O) mix-aeg-PNA-Cf. (A), (F), and (K) are images of Hoechst. (B), (G), and (L) show signals from DIC. (C), (H), and (M) show the images of cf-PNAs. (D), (I), and (N) show the images of Hoechst + PNAs-Cf and (E), (J), and (O) show the merged images.
10. NMR and HRMS spectra:

**1H NMR**

![1H NMR spectrum]

**13C NMR**

![13C NMR spectrum]
11. References