**Supporting Information**

# Supramolecular Polyplexes from Janus Peptide Nucleic Acids (*bm*-PNA-G5): Self assembled *bm*-PNA G-Quadruplex and its Tetraduplex with DNA

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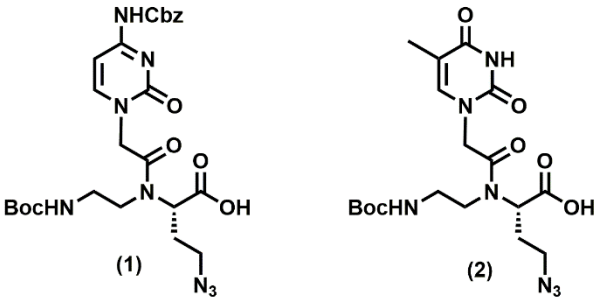
**1.0 General procedures:** All chemicals used for reactions were analytical and laboratory grade. For each reaction, the solvents used were dried or distilled. Reactions were monitored by thin-layer chromatography (TLC). TLC was carried out on pre-coated silica gel $GF_{254}$ sheets (Merck 5554). TLC 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 $^{1}H$ and $^{13}C$ NMR were recorded using Bruker AC-400 (400 MHz) or JEOL 400 MHz NMR spectrometers in $CDCl_3$ and DMSO-$d_6$. Chemical shifts are reported in parts per million (ppm, $\delta$ 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 C18 (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.

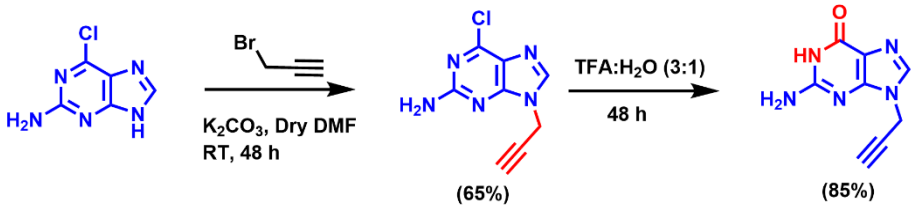
**2.0 Synthesis of *bm*-PNA (C/T) monomers and N9-propargyl guanine:**

The monomers *bm*-PNA-C **1** and *bm*-PNA-T **2** were synthesized, characterized and purified according to previously reported protocols.[1]




**Synthesis of N9-propargyl guanine**

N9-Propargyl guanine synthesized according to standard literature protocols.[2]




**3. Solid phase syntheses of *bm*-PNA-G5 oligomer[1]**

The synthesis of *bm*-PNA-G5 (*C*-GTCTTCA-*N*) 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**.

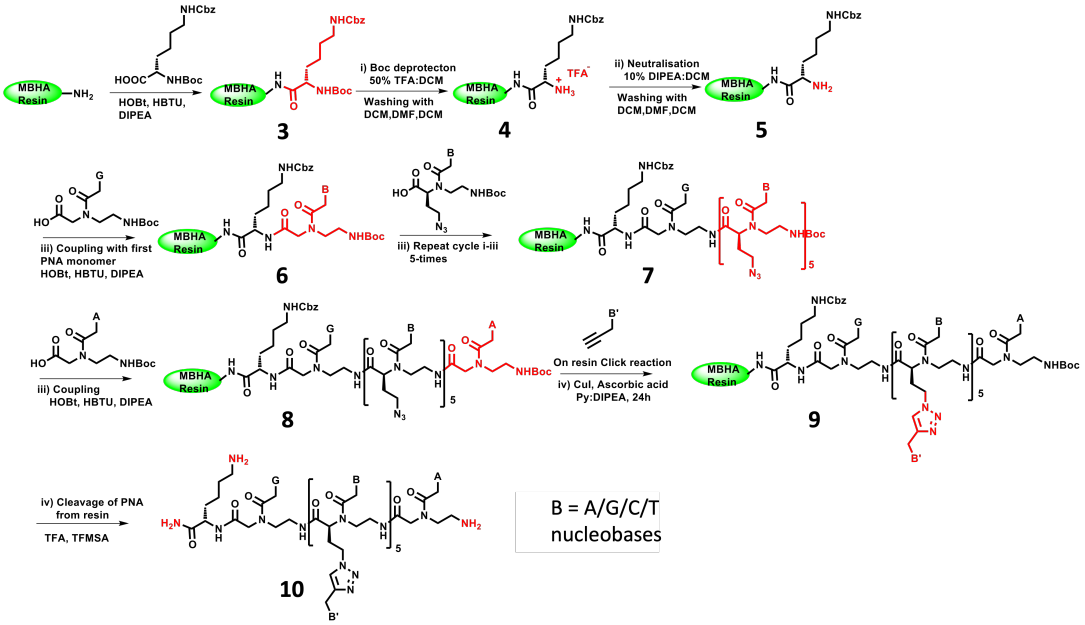
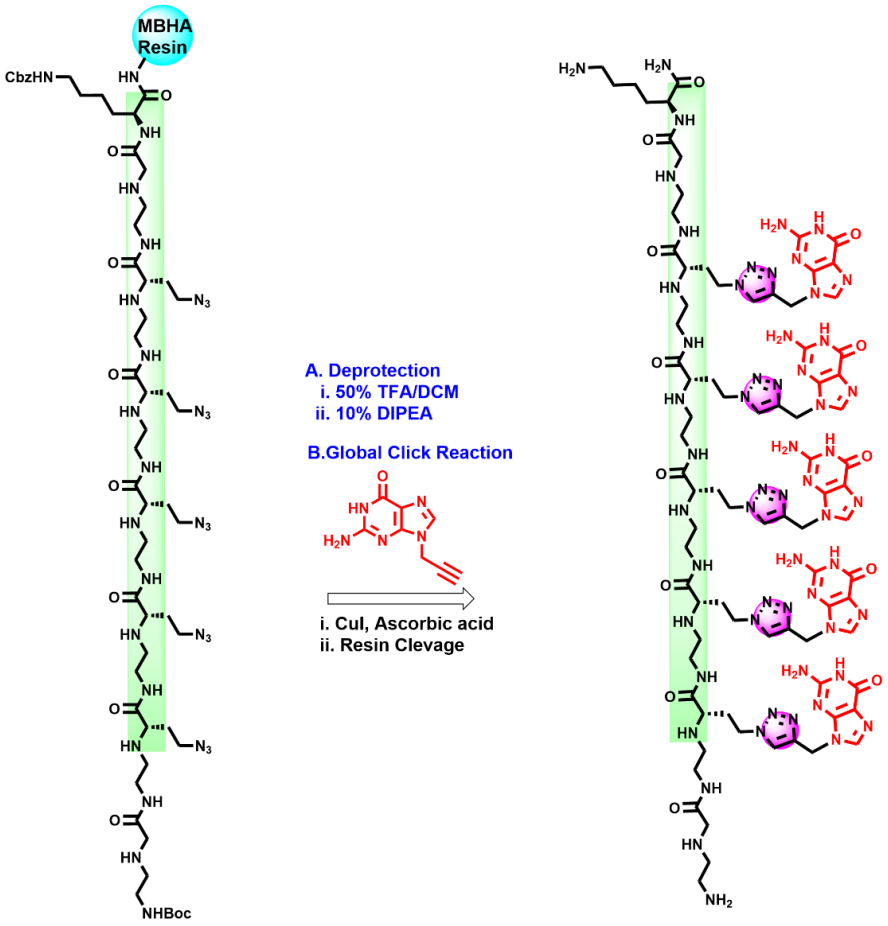
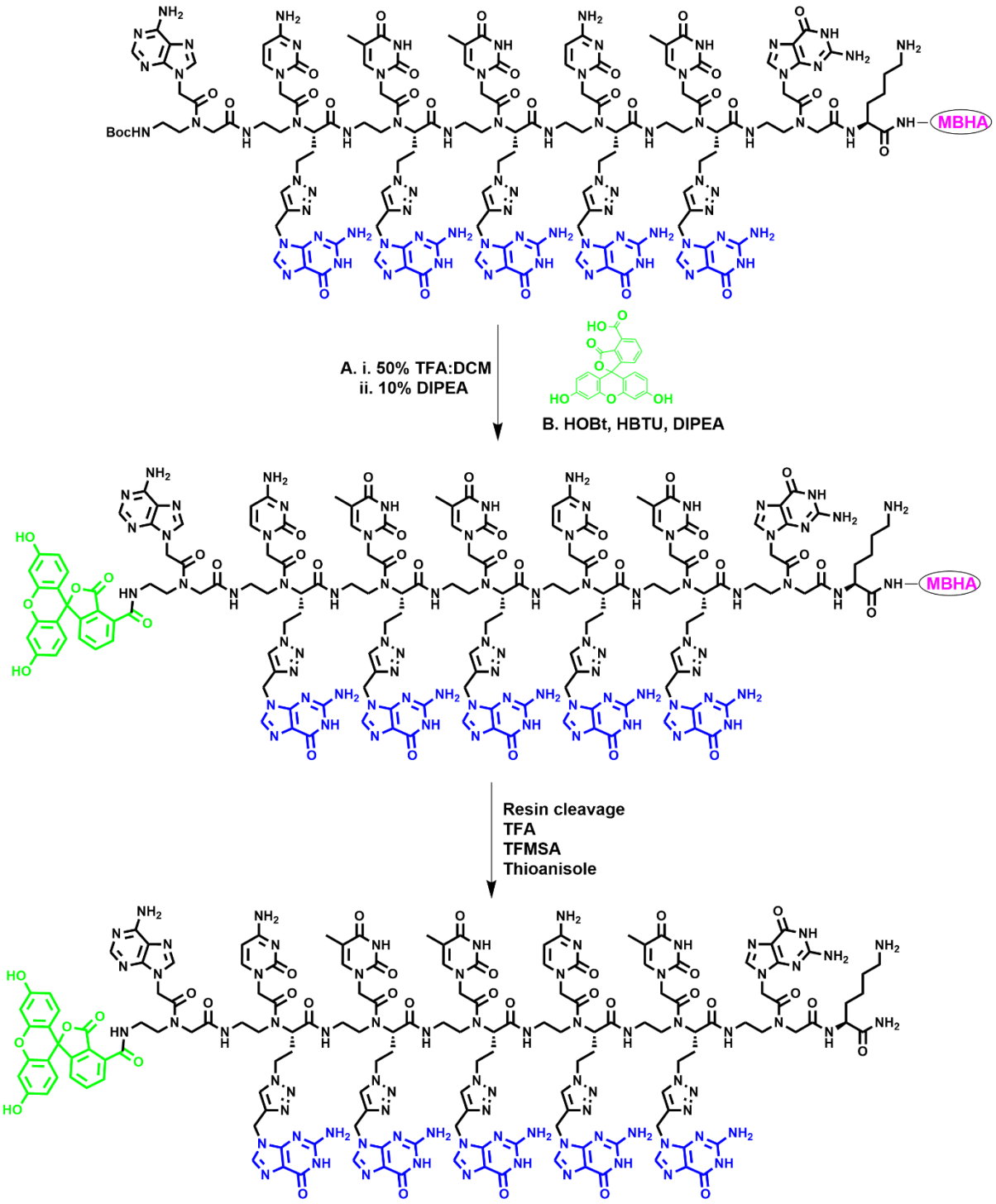



**Figure 1** Solid phase synthesis protocol of *bm*- PNA (*N*-ACTTCTG) by *Boc* strategy.

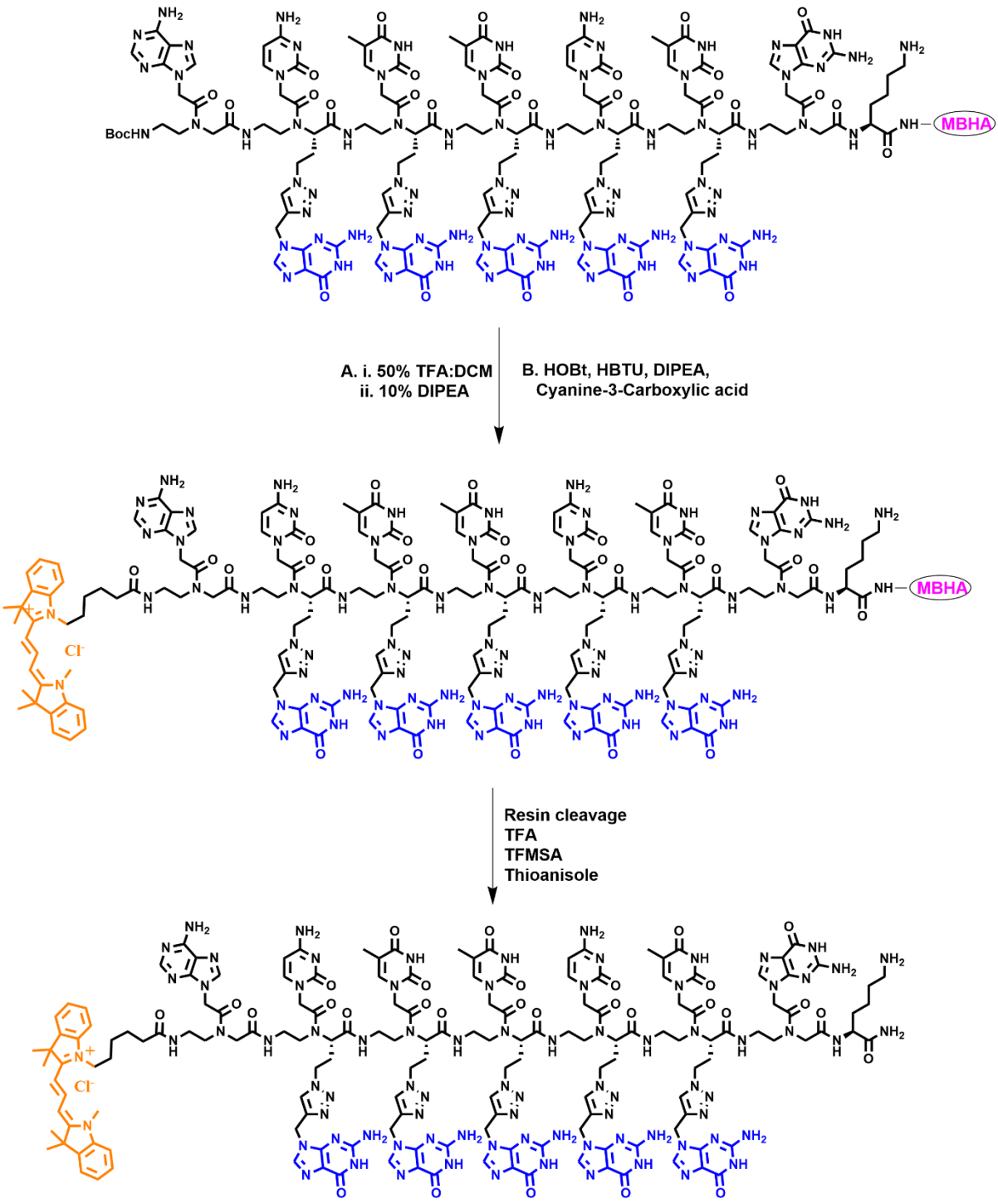
**3a. Solid phase synthesis of *iso*-PNA-G$_5$:** This was done by a similar procedure as above, using appropriate iso-PNA monomers as reported previously.



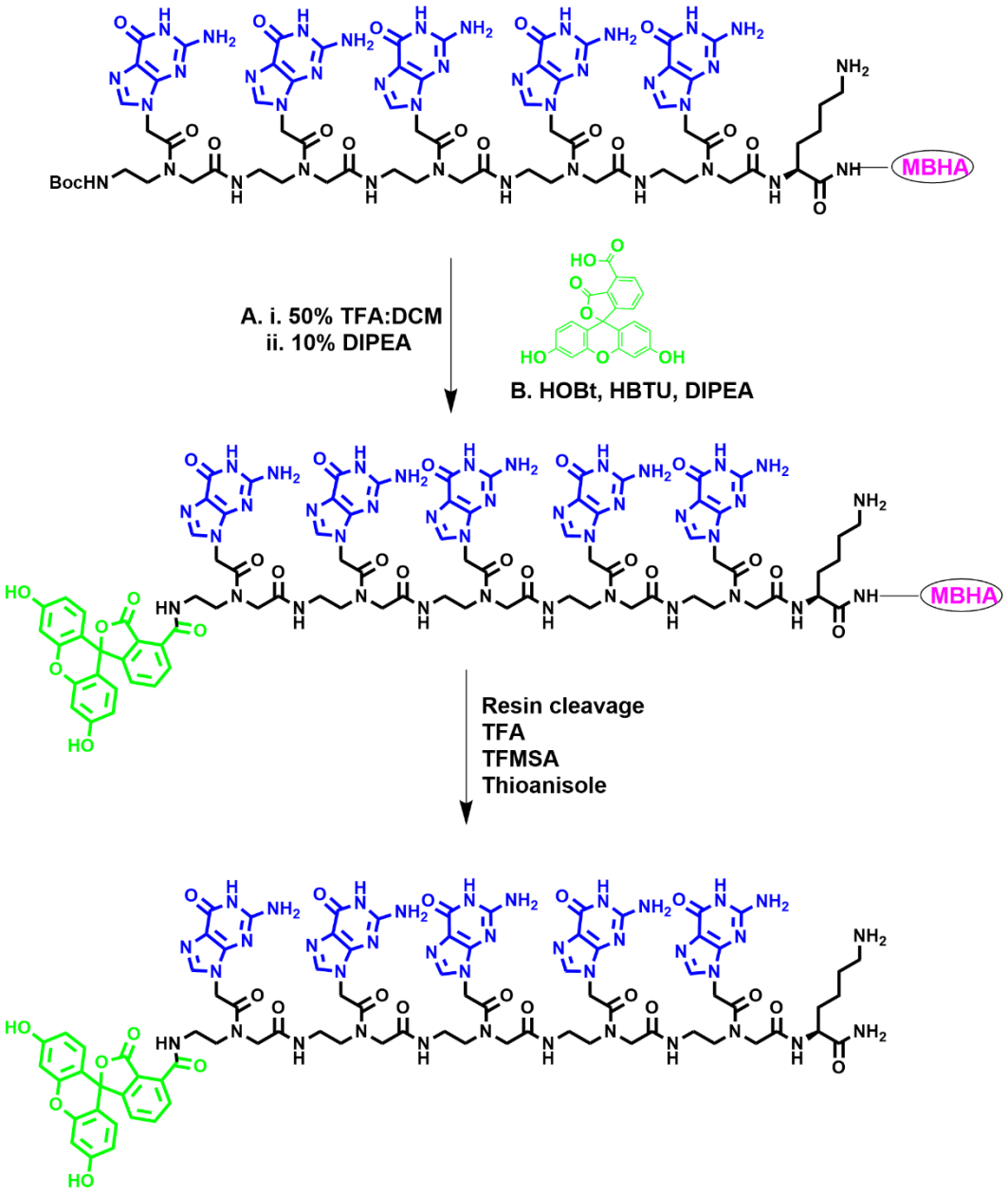
**3b. Solid phase synthesis of *bm*-PNA-G5-*Cf:*** The resin bound *bm*-PNA oligomer **9** was deproteted, followed by coupling with carboxy fluorescein or cyanine -3-carboxylic acid. The resulting conjugates were cleaved from the resin and deprotected to yield fluorescent bm-PNA-G5-*Cf* and *bm*-PNA-G5-*Cy3* for experiments with FRET and cell uptake.



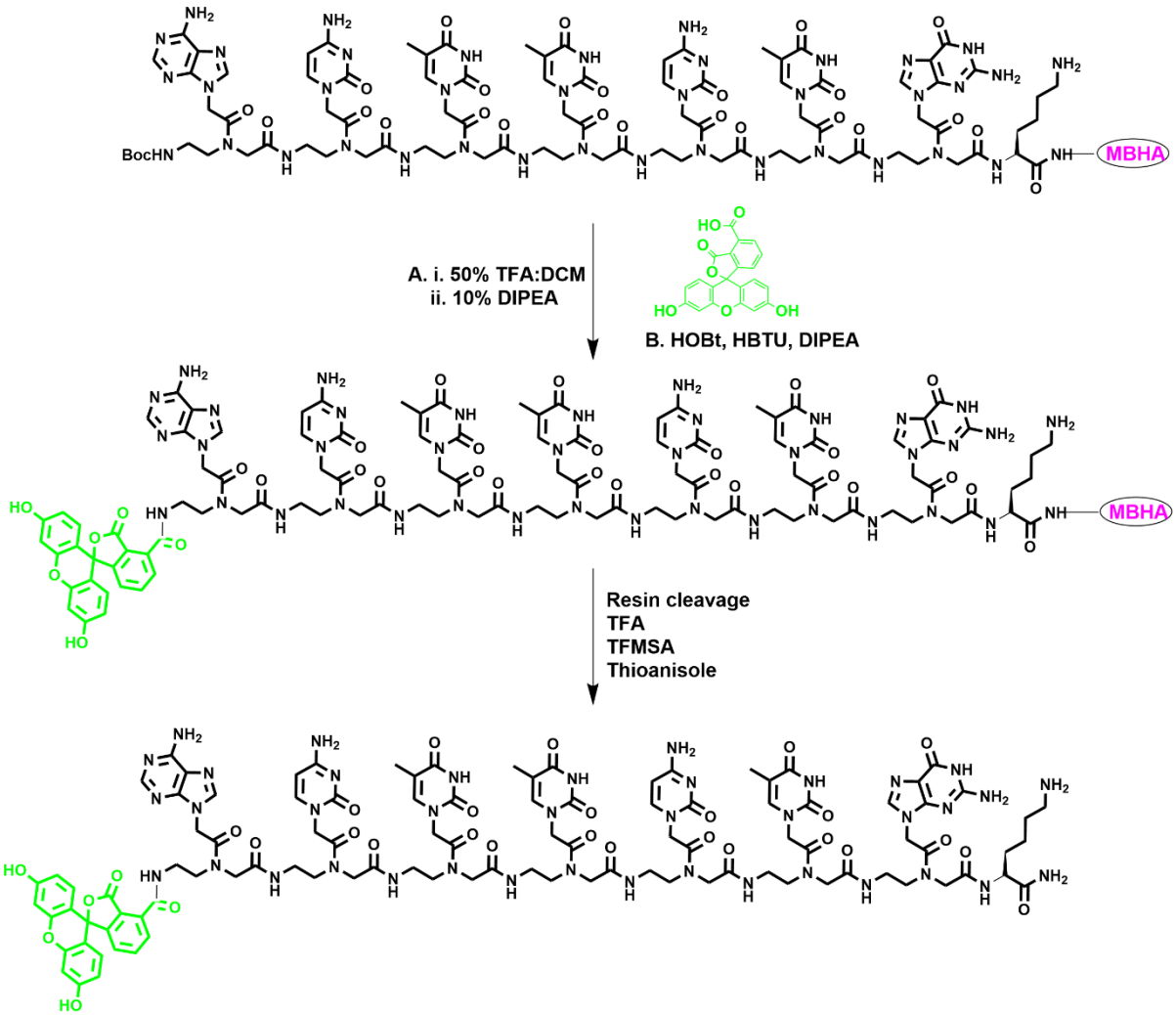
### 3c. Solid phase synthesis of *bm*-PNA-G5-*Cy3*



**3d. Solid phase synthesis of *aeg*-PNA-G5-*Cf* :** This was prepared by a similar procedure as above using *aeg*-PNA-G5



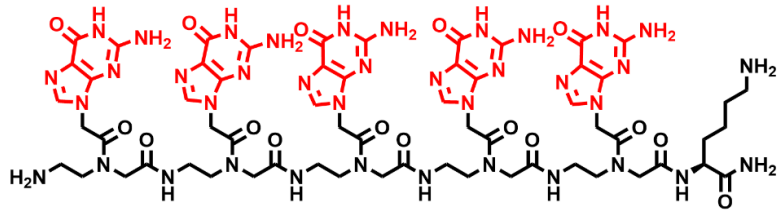
**3f. Solid phase synthesis of mix-aeg-PNA-*Cf:*** This was prepared by a similar procedure as above using mix-*aeg*-PNA-G5.

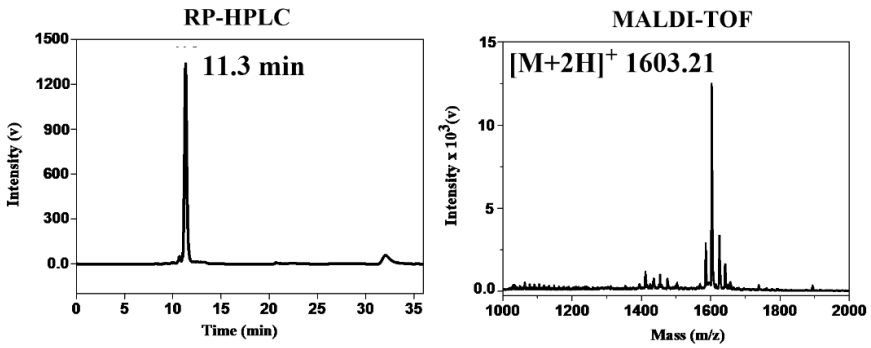


## 4.0 RP-HPLC Chromatograms and MALDI-TOF of PNA Oligomers

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

### 4a. RP-HPLC and MALDI of *aeg*-PNA-$G_5$



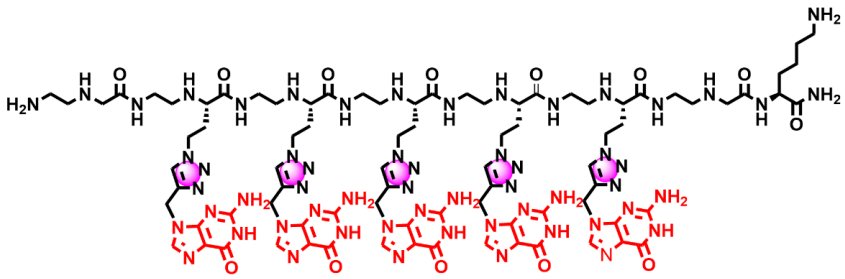


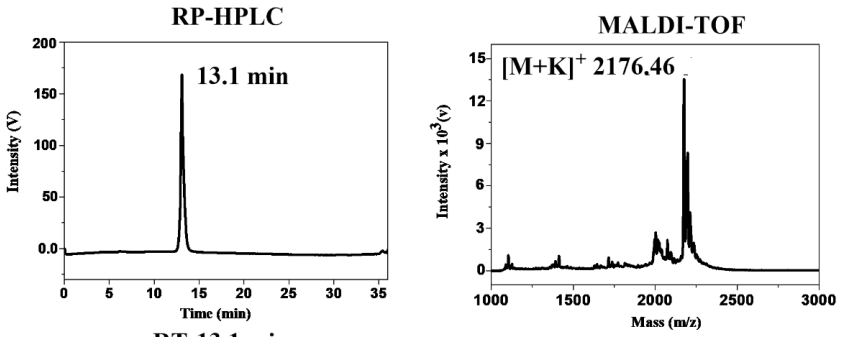

RT-11.3 min

Calcd mass:$[M+2H]^+$ 1603.58

Obsd mass:$[M+2H]^+$ 1603.21

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



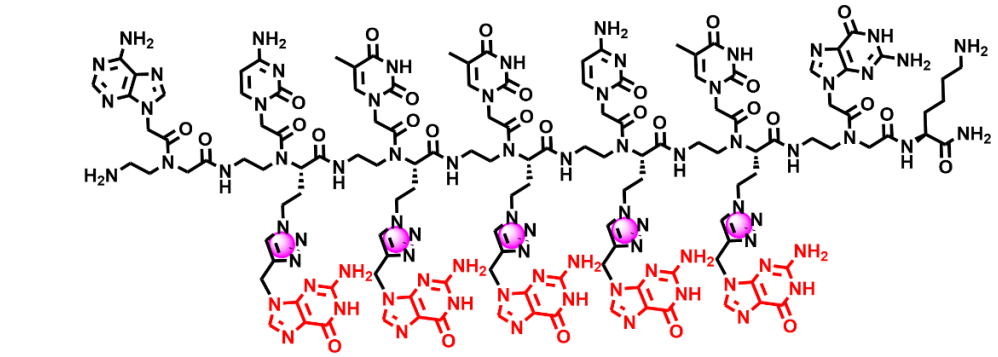


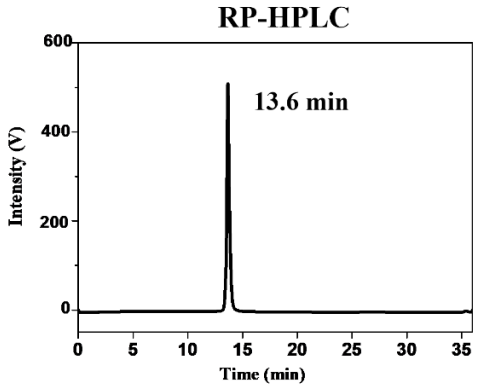

RT-13.1 min

Calcd mass:$[M+K]^+$ 2176.38

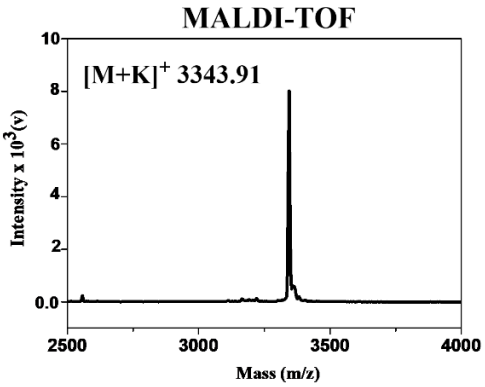
Obsd mass:$[M+K]^+$ 2176.46

## 4c. RP-HPLC and MALDI of *bm*-PNA-$G_5$





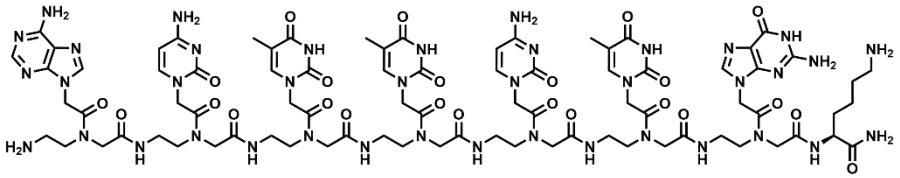

RT-13.6 min

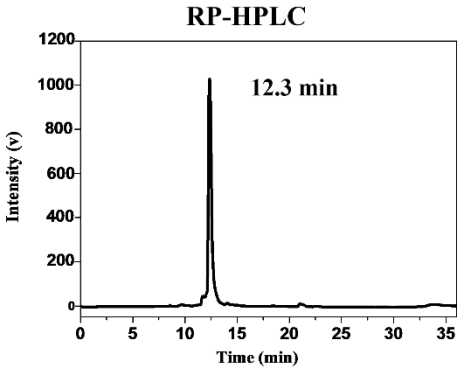


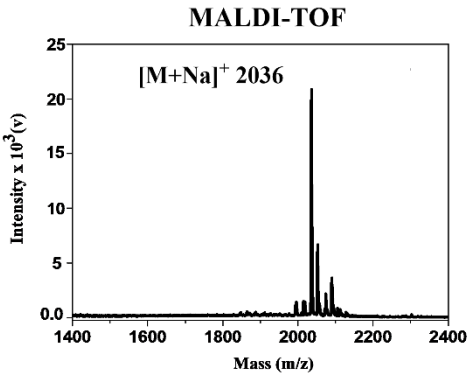

Calcd mass:$[M+K]^+$ 3343.34

Obsd mass:$[M+K]^+$ 3343.91

## 4d. RP-HPLC and MALDI of mix-*aeg*-PNA



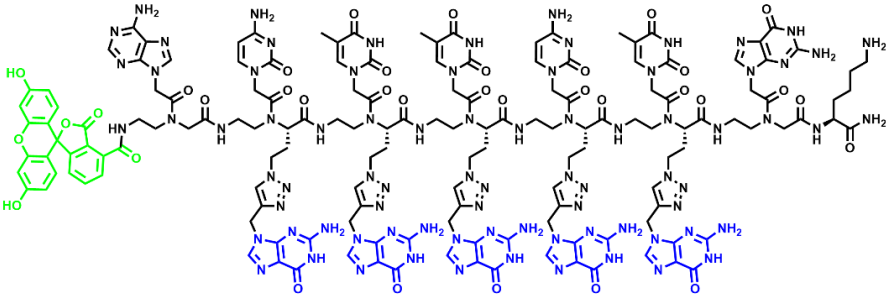


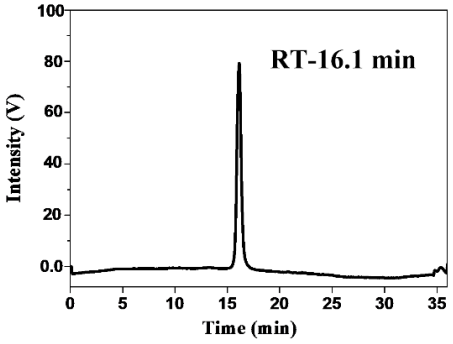

Calcd. Mass $[M+Na]^+$ 2036.00

Obsd. Mass $[M+Na]^+$ 2036.00

## 4e. RP-HPLC and MALDI of *bm*-PNA-$G_5$-Cf

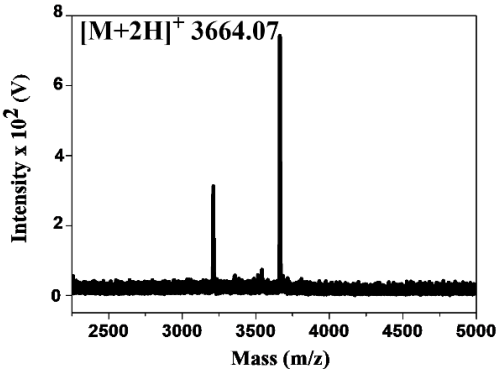


RP-HPLC



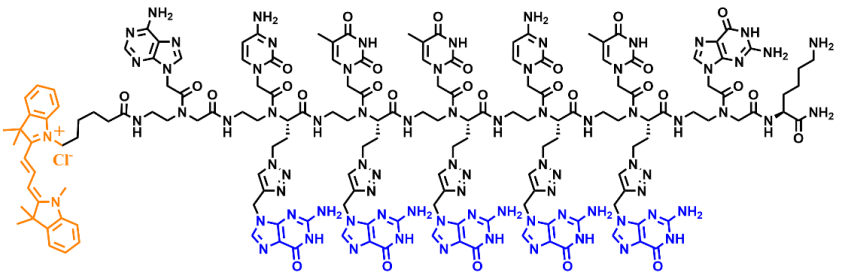

RT-16.1 min

MALDI-TOF

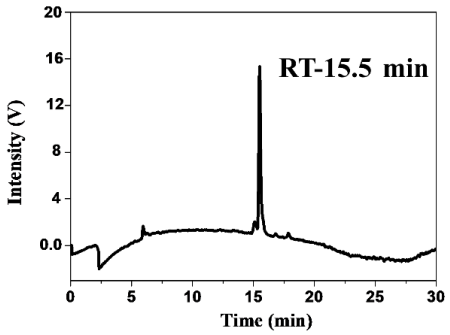



Calcd mass [M+2H] 3664.56
Obsd mass [M+2H] 3664.07

## 4f. RP-HPLC and MALDI of *bm*-PNA-$G_5$-Cy3

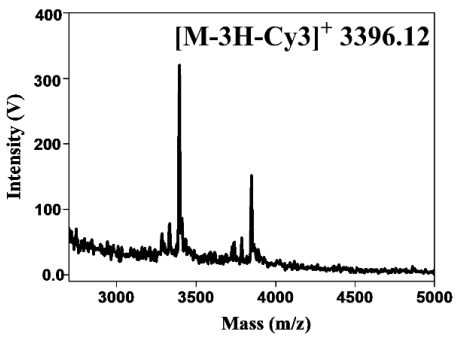


RP-HPLC



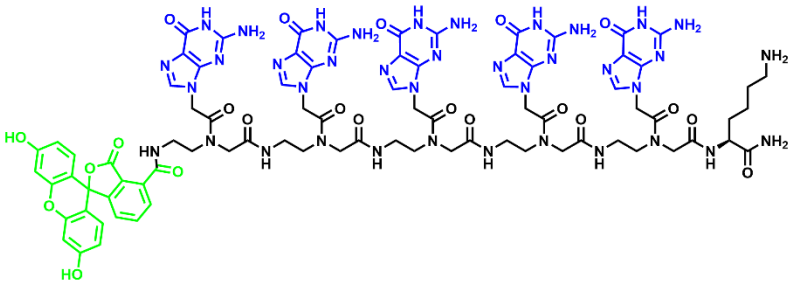

RT-15.5 min

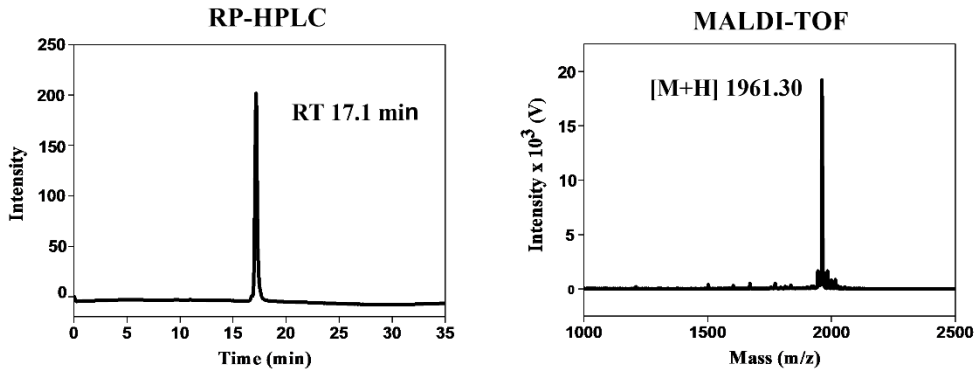
MALDI-TOF




Calcd mass [M-3H-Cy3] 3396.37
Obsd mass [M-3H-Cy3] 3396.12

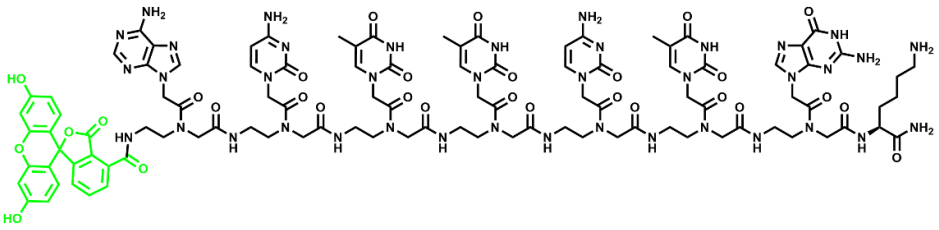
## 4g. RP-HPLC and MALDI of *aeg*-PNA-G$_5$-*Cf*

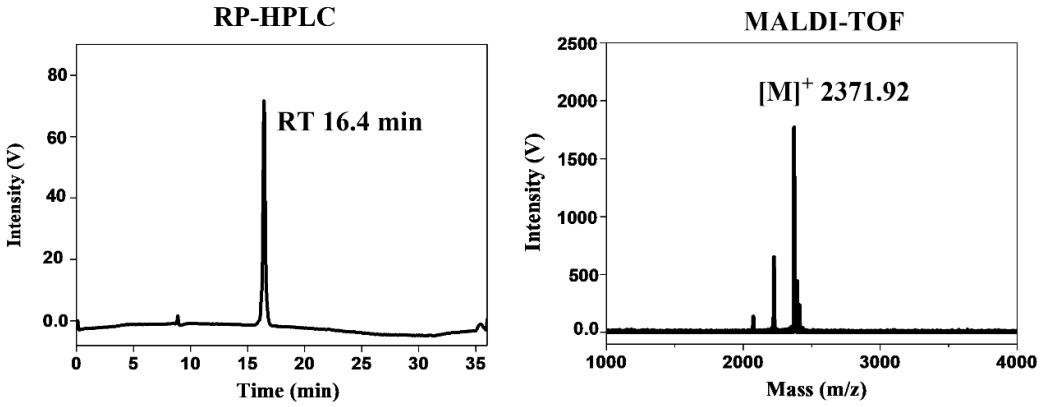





Calcd. Mass [M+H] 1960.87
Obsd. Mass [M+H] 1961.30

## 4h. RP-HPLC and MALDI of mix-*aeg*-PNA-*Cf*






RT 16.4 min

Calcd Mass [M]$^+$ 2371.32
Obsd Mass [M]$^+$ 2371.92

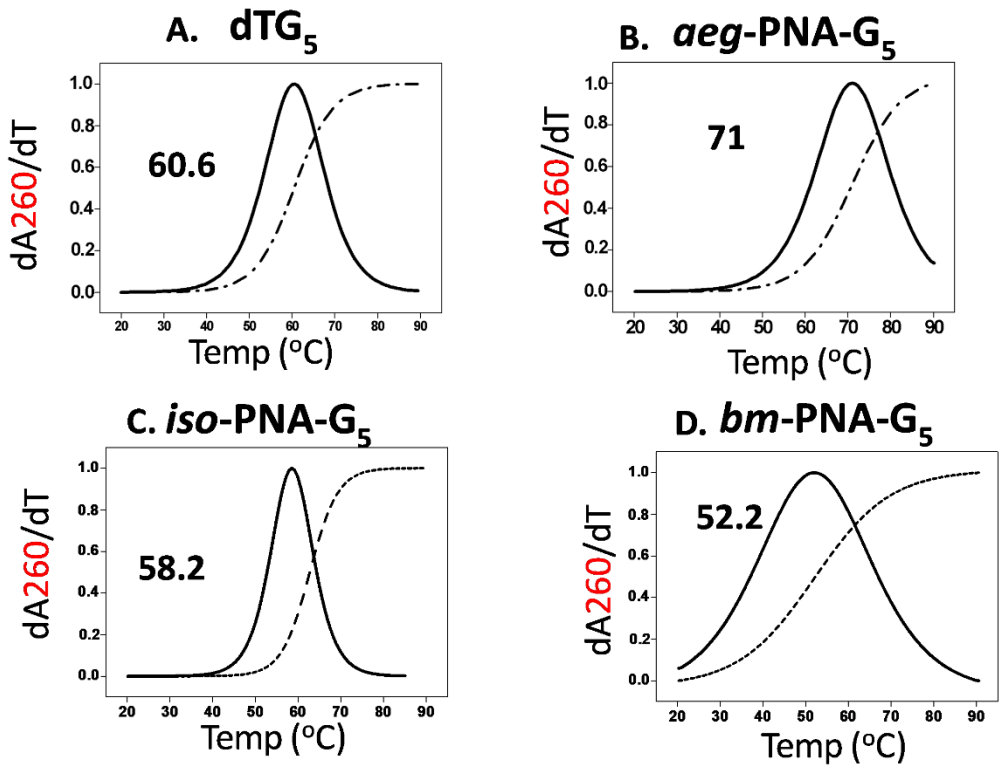
**5. Table 1. MALDI-TOF analysis of PNA oligomers**

| Entry | PNA | Mol. formulae | Calcd. Mass | Obsd. Mass |
|---|---|---|---|---|
| **1** | *aeg*-PNA-$G_5$ | $C_{61}H_{82}N_{38}O_{16}$ | $[M+2H]^+$ 1603.58 | $[M+2H]^+$ 1603.21 |
| **2** | *iso*-PNA-$G_5$ | $C_{84}H_{121}KN_{57}O_{13}$ | $[M+K]^+$ 2176.38 | $[M+K]^+$ 2176.46 |
| **3** | *bm*-PNA-$G_5$ | $C_{131}H_{159}KN_{79}O_{29}$ | $[M+K]^+$ 3343.34 | $[M+K]^+$ 3343.91 |
| **4** | *mix-aeg*-PNA | $C_{81}H_{109}N_{39}NaO_{24}$ | $[M+Na]^+$ 2036.00 | $[M+Na]^+$ 2036.00 |
| **5** | *bm*-PNA-$G_5$-Cf | $C_{152}H_{171}N_{79}O_{35}$ | $[M+2H]^+$ 3664.56 | $[M+2H]^+$ 3664.07 |
| **6** | *bm*-PNA-$G_5$-Cy3 | $C_{161}H_{196}ClN_{81}O_{30}$ | $[M+2H]^+$ 3664.56 | $[M+2H]^+$ 3664.56 |
| **7** | *aeg*-PNA-$G_5$-Cf | $C_{82}H_{91}N_{38}O_{22}$ | $[M+H]^+$ 1960.87 | $[M+H]^+$ 1961.30 |
| **8** | *mix-aeg*-PNA-Cf | $C_{102}H_{119}N_{39}O_{30}$ | $[M]^+$ 2371.92 | $[M]^+$ 2371.32 |

**6. DNA oligonucleotides used for biophysical studies**

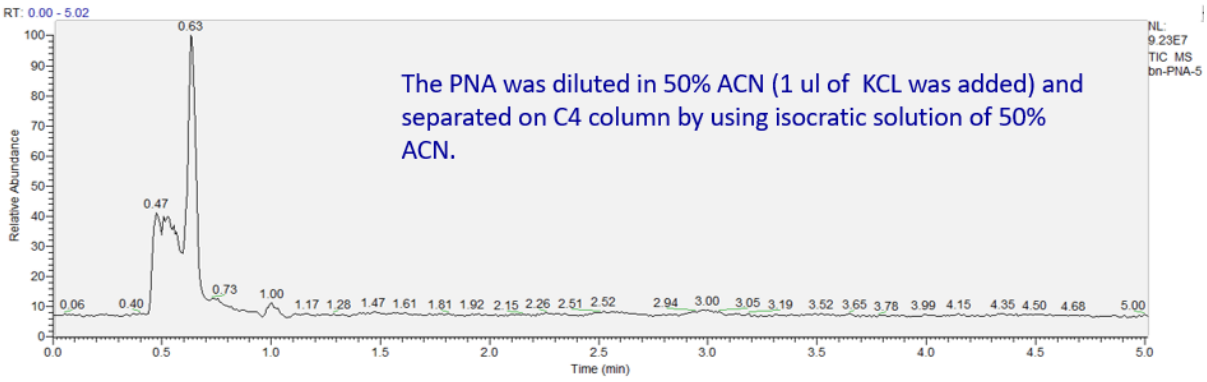
| Entry | DNA | Sequence (5' to 3') |
|---|---|---|
| **1** | DNA **1** | CAGAAGT |

**7. UV-$T_m$ of PNA variants at 260 nm:**

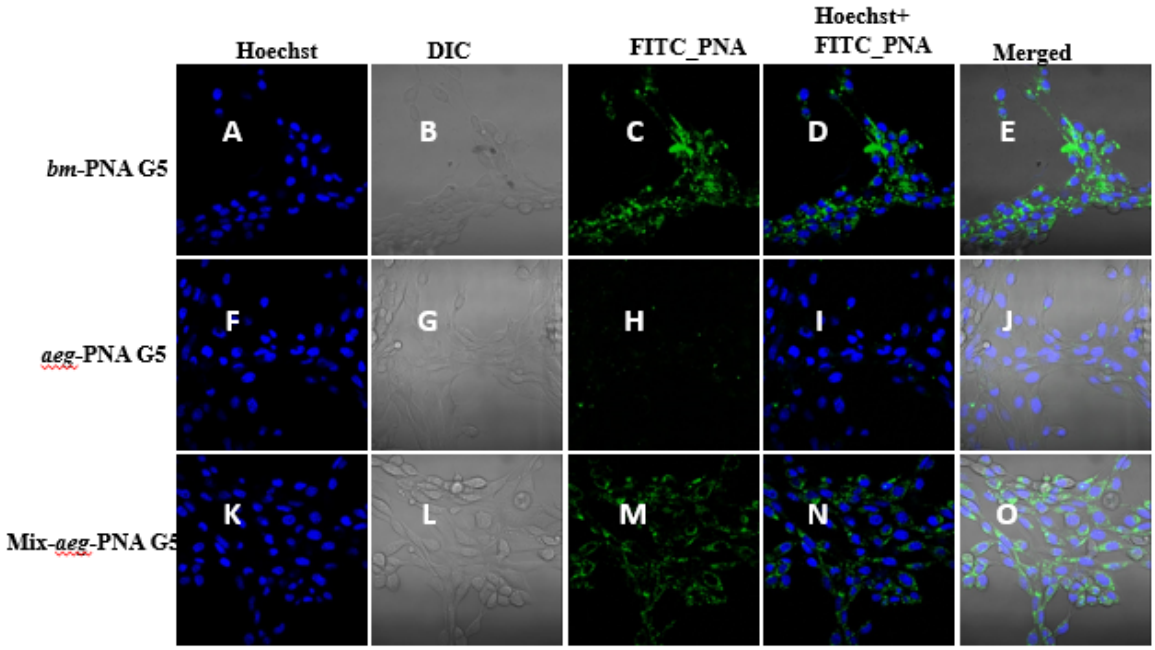



**8. Full LC profile of bm-PNA-G5 tetraplex:**

**Retention time : 0.63 min**



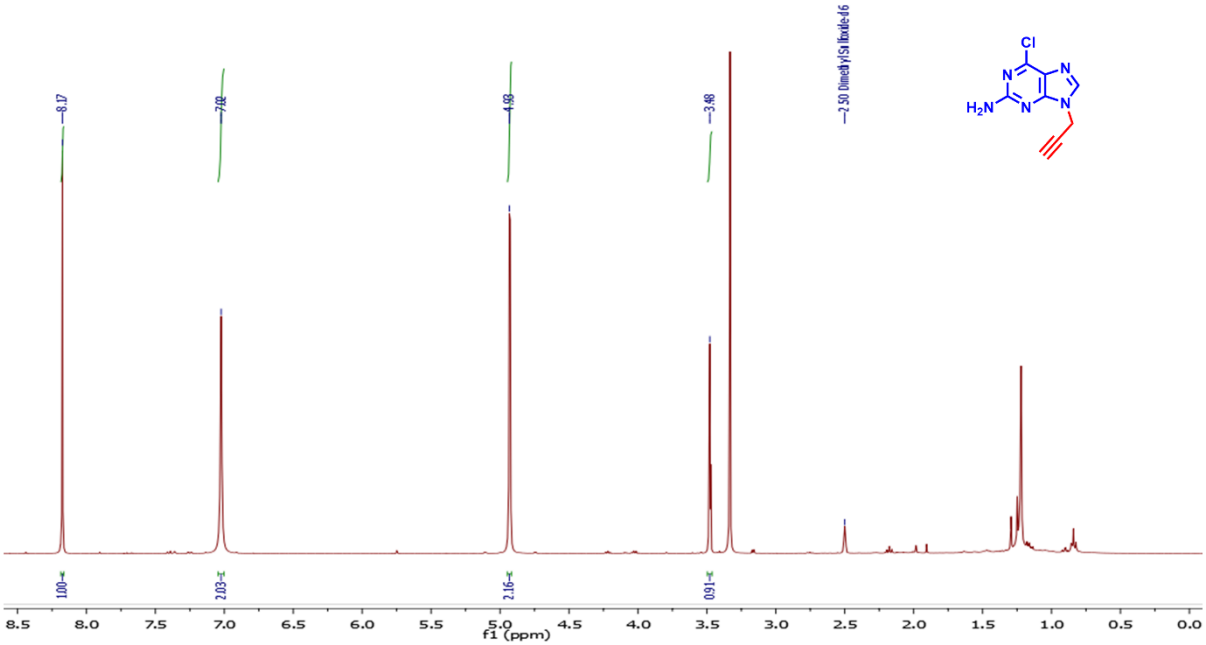
**9 . NIH-3T3 cell_24h uptake @4 µM concentration of PNA**



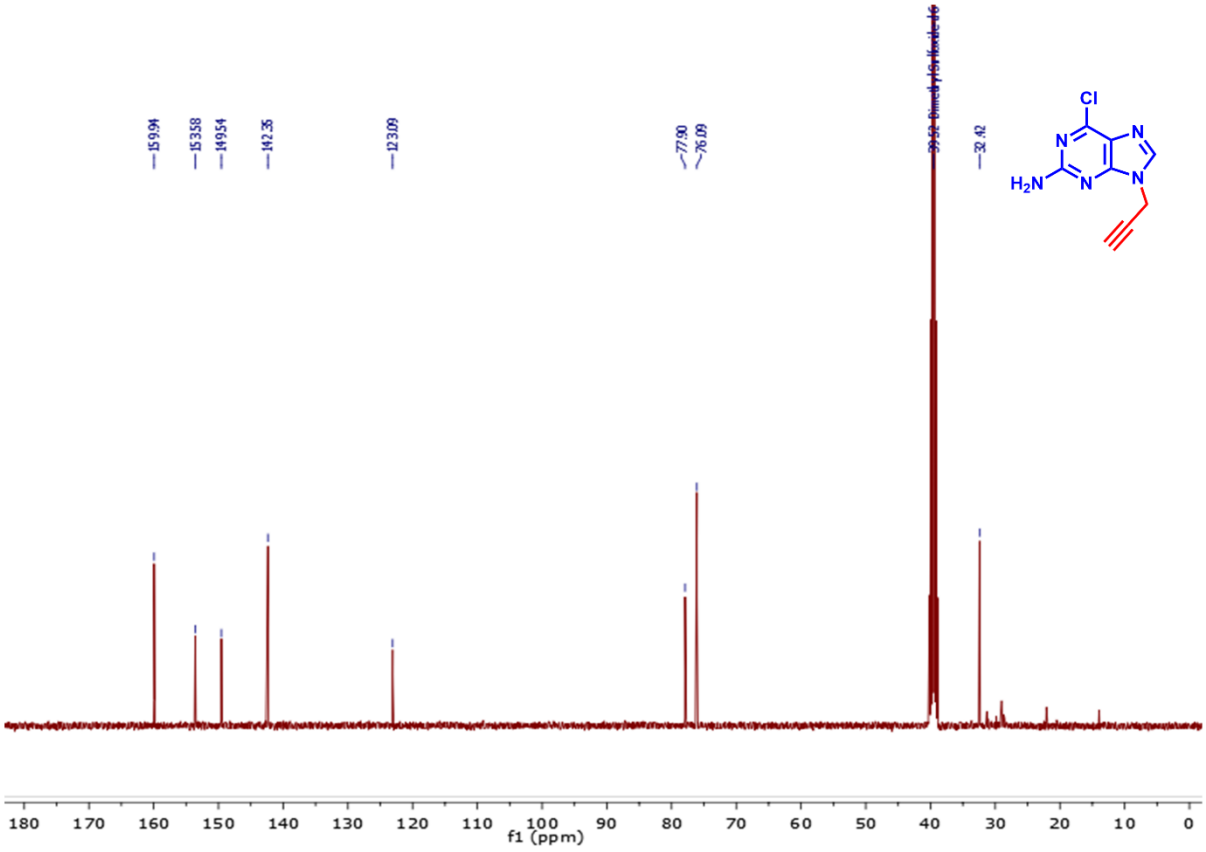

Confocal microscopy images of NIH-3T3 cells treated with (A–E) *bm*-PNA-G5-*Cf*, (F-J) *aeg*-PNA-G5-*Cf* and (K-O) mix-*aeg*-PNA-*Cf* (A), (F), and (K) are images of Hoechst (B), (G), and (L) show signals from DIC. (C), (H), and (M) show the images of *cf*-PNAs. (D), (I), and (N) show the images of Hoechst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

**10. NMR and HRMS spectra:**

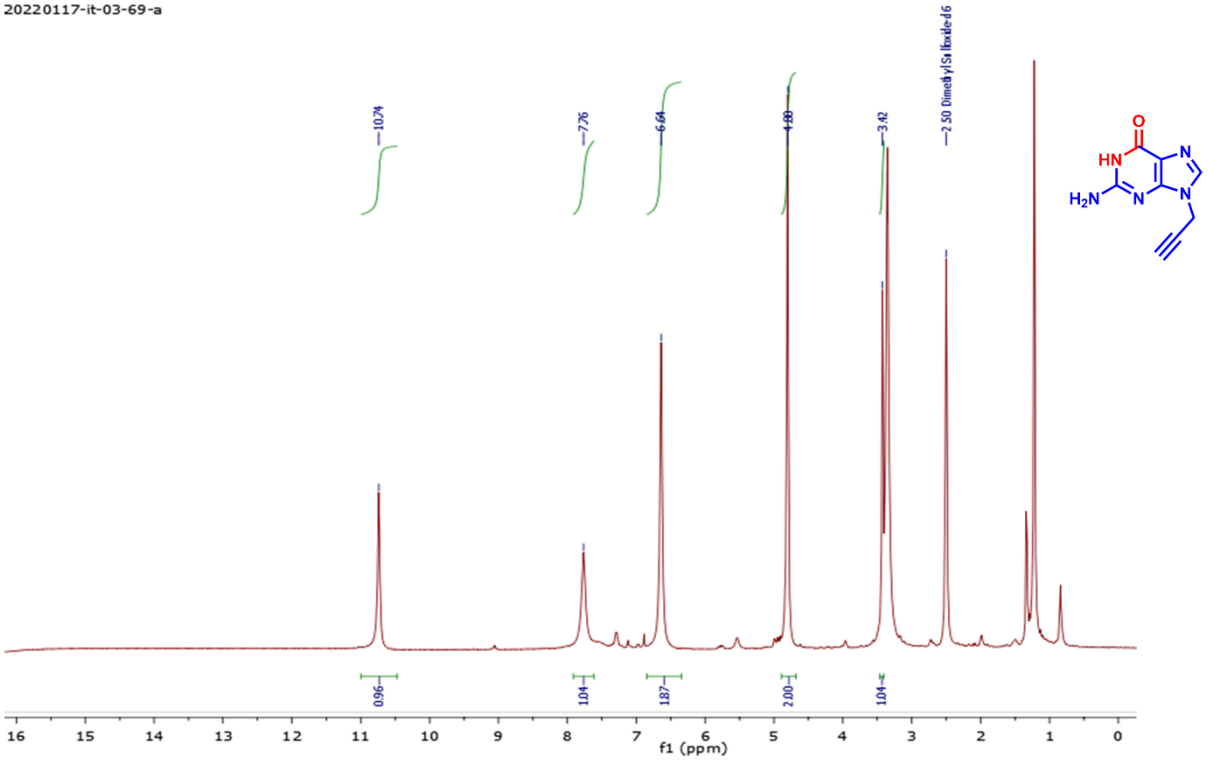
**1H NMR**



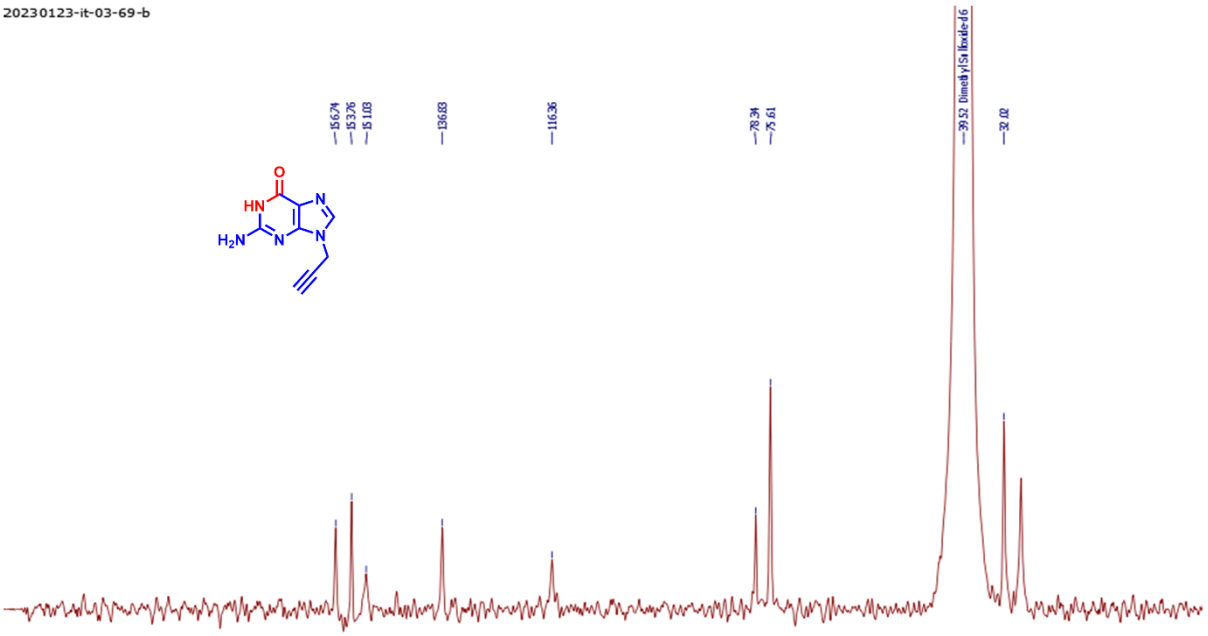

**13C NMR**



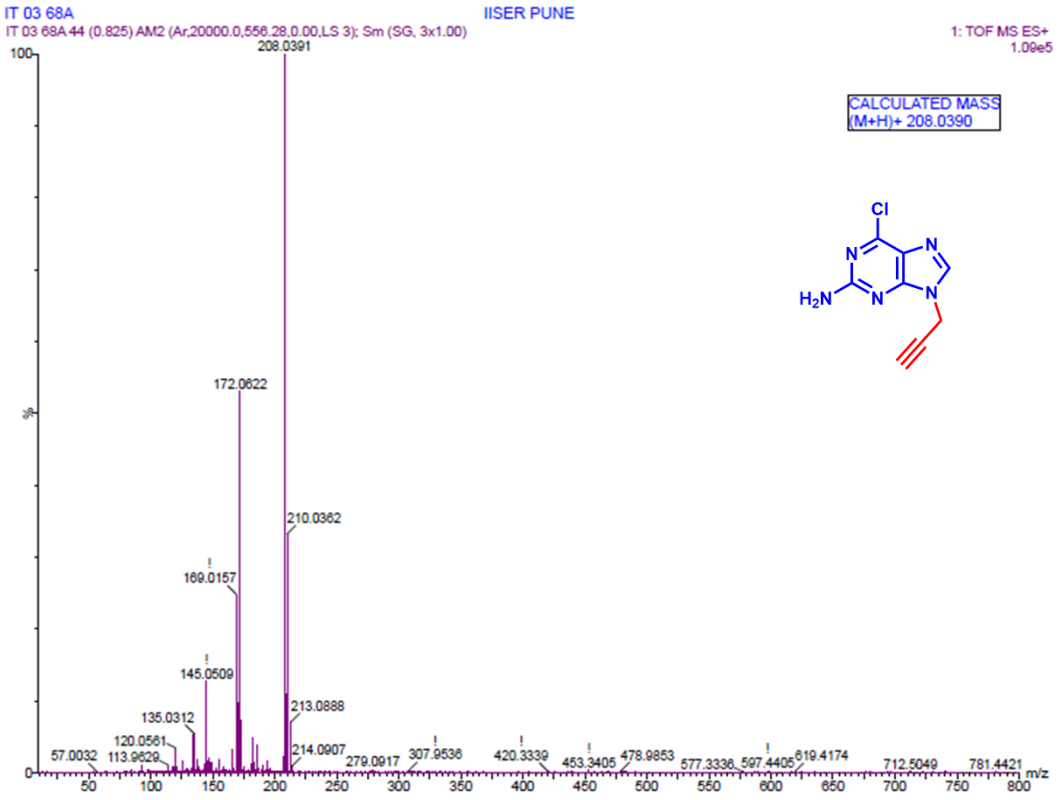
## 1H NMR




## 13C NMR



## HRMS


IT 03 68A
IT 03 68A 44 (0.825) AM2 (Ar,20000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
IISER PUNE
1: TOF MS ES+
1.09e5
CALCULATED MASS
(M+H)+ 208.0390
208.0391
172.0622
210.0362
169.0157
145.0509
213.0888
135.0312
120.0561
214.0907
57.0032
113.9629
279.0917
307.9536
420.3339
453.3405
478.9853
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597.4405
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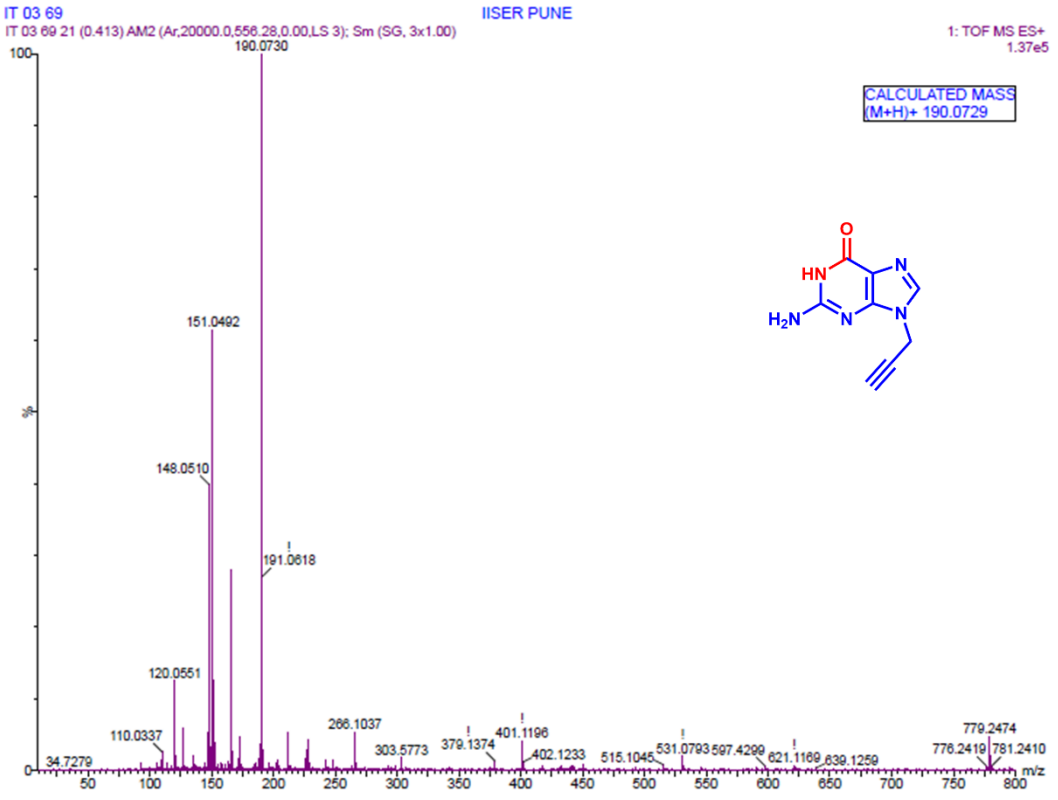
712.5049
781.4421
m/z

## HRMS


IT 03 69
IT 03 69 21 (0.413) AM2 (Ar,20000.0,556.28,0.00,LS 3); Sm (SG, 3x1.00)
IISER PUNE
1: TOF MS ES+
1.37e5
CALCULATED MASS
(M+H)+ 190.0729
190.0730
151.0492
148.0510
191.0618
120.0551
110.0337
266.1037
303.5773
379.1374
401.1196
402.1233
515.1045
531.0793
597.4299
621.1169
639.1259
779.2474
776.2419
781.2410
34.7279
m/z

## 11. References


1. M. K. Gupta, B. R. Madhanagopal, D. Datta, K. N. Ganesh, *Org. Lett.* 2020**, 22**, 5255.
2. N. Nagapradeep, S. Verma, *Chemical Commun.* 2011**, 47**, 1755