JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

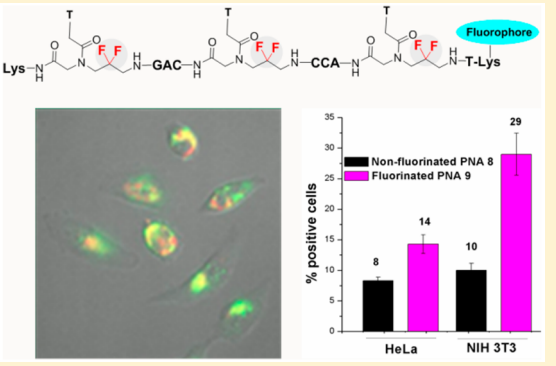
Fluorous Peptide Nucleic Acids: PNA Analogues with Fluorine in Backbone (γ-CF_2-*apg*-PNA) Enhance Cellular Uptake

Satheesh Ellipilli and Krishna N. Ganesh*

Chemical Biology Unit, Indian Institute of Science Education and Research (IISER), Dr. Bhabha Road, Pune 411008, Maharashtra India

Supporting Information

ABSTRACT: Fluorous PNA analogues possessing fluorine as inherent part of aminopropylglycine (*apg*) backbone (γ-CF_2-*apg* PNA) have been synthesized and evaluated for biophysical and cell penetrating properties. These form duplexes of higher thermal stability with cRNA than cDNA, although destabilized compared to duplexes of standard *aeg*-PNA. Cellular uptake of the fluorinated γ-CF_2-*apg* PNAs in NIH 3T3 and HeLa cells was 2−3-fold higher compared to that of nonfluorinated *apg* PNA, with NIH 3T3 cells showing better permeability compared to HeLa cells. The backbone fluorinated PNAs, which are first in this class, when combined with other chemical modifications may have potential for future PNA-based antisense agents.



INTRODUCTION

Peptide nucleic acids (PNAs) are a class of DNA analogues synthesized by replacing the sugar-phosphate backbone of DNA with a neutral and achiral polyamide backbone consisting of *N*-(2-aminoethyl)glycine units (Figure 1a).[1−3]

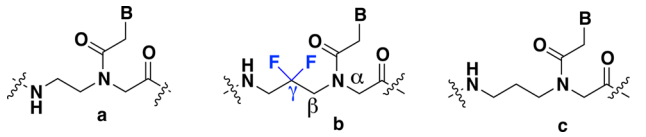


Figure 1. Chemical structures of (a) *aeg*-PNA, (b) γ-CF_2-*apg*-PNA and (c) *apg*-PNA; B = A/G/C/T.

PNA readily forms Watson−Crick base-pairing with complementary DNA and RNA with stronger affinity than the iso-sequential DNA/DNA or DNA/RNA duplexes due to a higher flexibility and neutral charge of the backbone.[4,5] In spite of their superior DNA/RNA complementation properties and resistance to cellular enzymes such as nucleases and proteases, their application as an antisense/antigene drug is inadequate due to poor aqueous solubility and inefficient cellular uptake.[5−8] To overcome these limitations, PNA has been chemically modified in many ways, some of which have shown improvement in desired attributes.[8−10]

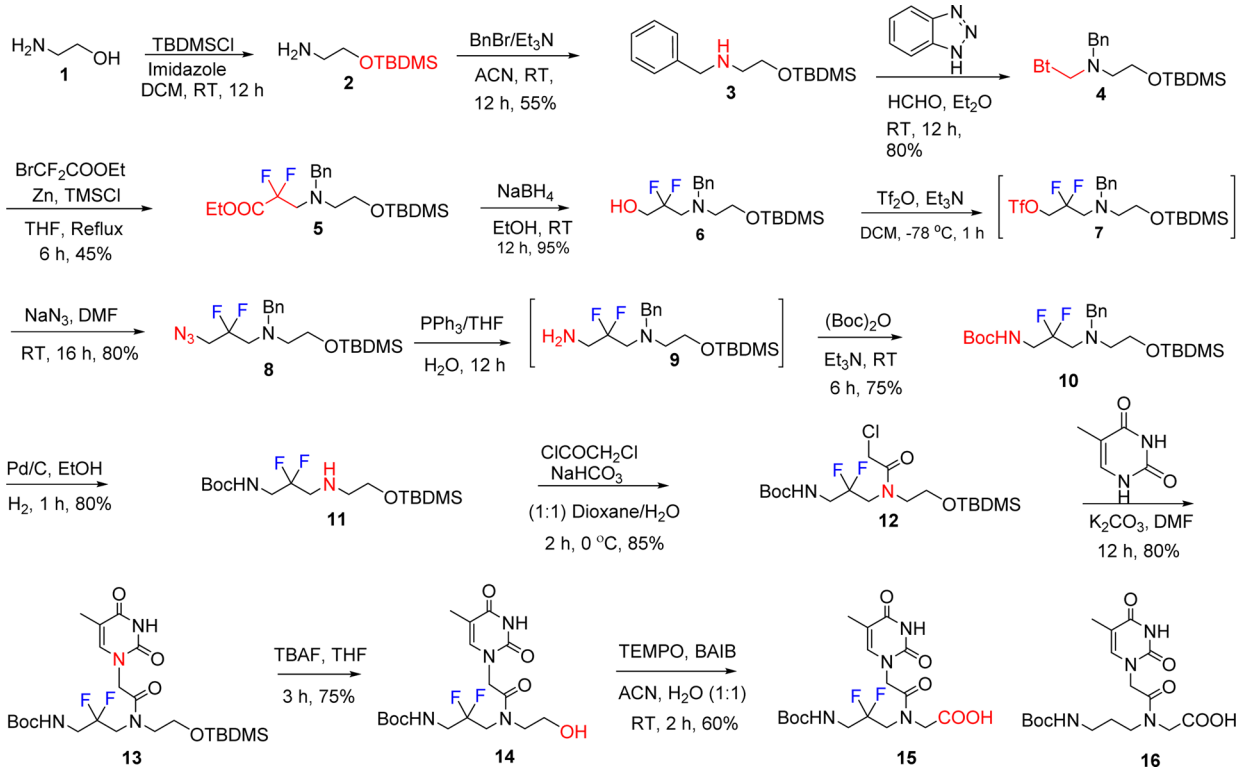
Fluorine, an isostere of hydrogen, is small in size with higher electronegativity and low polarizability. Substitution of hydrogen with fluorine in molecules is well-known to affect physiochemical properties like lipophilicity, volatility, solubility, acidity, basicity, hydrogen bonding, etc.[11] The polar hydrophobicity of fluorine enhances the affinity to natural receptors and increases its metabolic stability leading to a higher availability of the drug.[12] Fluorine has played an important role in medicinal chemistry, with several known examples of drugs incorporating fluorine,[13] including fluorinated oligonucleotides for potential antisense activity.[14,15] Herein, we describe new analogues of PNA that have fluorine in the backbone, which show an increase in the cell permeability compared to standard PNAs, without seriously altering the strength and fidelity of hybridization with complementary DNA/RNA.

The first fluorinated PNA analogue reported was olefinic PNA in which the tertiary rotameric amide bond was replaced with a more rigid fluoro vinyl moiety to demonstrate the important role of amide in stabilizing the PNA duplex with cDNA.[16] In a recent report,[17] the thymine base in PNA side chain was replaced by 5-CF_3/$C(CF_3)_3$-uracil in order to follow hybridization using ^{19}F NMR. However, there are no reports on the effect of fluorine substitution of PNA backbone on comparative hybridization with cDNA/RNA and importantly on their cell permeability. We now report the synthesis of C_γ-difluoro aminopropyl glycine peptide nucleic acid (γ-CF_2-*apg* PNA, Figure 1b) derived from substituting the central methylene group in aminopropyl moiety of *apg* PNA (Figure 1c) with a difluoromethylene group and studied their relative hybridization properties with antiparallel complementary DNA/RNA and cell uptake ability. The results indicate that fluorinated PNAs show significant enhancements in cell permeability, with minimal effects on the stability of derived PNA:DNA/RNA duplexes, retaining the fidelity of base pairing. The fluorinated γ-CF_2-*apg* PNAs also translocate into

Received: July 13, 2015

DOI: 10.1021/acs.joc.5b01614
J. Org. Chem. XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of γ-CF_2 *apg* PNA-T Monomer[a]



[a]Compounds **7** and **9** not isolated; yields of **8** and **10** mentioned are overall yields for two steps

Table 1. UV–T_m (°C) Values of PNA:DNA and PNA:RNA Duplexes

PNA	PNA sequence	cDNA **1**	cRNA **1**	ΔT_m^1 (RNA**1**-DNA**1**)	DNA **2** (mismatch)	ΔT_m^2 (DNA**2**-DNA**1**)	RNA **2** (mismatch)	ΔT_m^3 (RNA**2**-RNA**1**)
PNA **1**	H-TTACC*T*CAGT-Lys	42.6	53.1	+10.5	31.1	-11.5	34.0	-19.1
PNA **2**	H-TTACCT*CAGT-Lys	38.2 (-4.4)	51.1(-2.0)	+12.9	35.2	-3.0	34.8	-16.3
PNA **3**	H-T*T*ACC*T*CAGT-Lys	42.1	49.1	+7.0	27.6	-14.5	28.1	-21.0
PNA **4**	H-TT*ACCT*CAGT-Lys	39.9 (-2.2)	48.3 (0.8)	+8.4	33.4	-6.5	33.4	-14.9
PNA **5**	H-T*T*ACC*T*CAG*T*-Lys	38.8	48.3	+9.5	27.8	-11.0	36.2	-12.1
PNA **6**	H-TT*ACCT*CAGT*-Lys	38.4 (-0.4)	47.9 (-0.4)	+9.5	33.2	-5.2	38.4	-9.5
PNA **7**	H-TTACCTCAGT-Lys	48.6	60.6	+12.0	28.6	-20.0	40.5	-20.1

[a] T* = γ-*CF_2 apg*-T; *T* = *apg*-T; ΔT_m indicates the difference in T_m of PNA with cDNA **1** and mismatch DNA **2**; The values are accurate to ± 0.5 °C. cDNA **1** = 3'AATGGAGTCA5'; DNA **2** = 3'AATGGCGTCA5'; cRNA 1 = 3'AATGGAGTCA5'; RNA **2** = 3'AAUGGCGUCA5'

cytoplasm and localize in endoplasmic reticulum (ER) better than the nonfluorinated *apg* PNA.

RESULTS AND DISCUSSION

Synthesis of γ-CF_2-*apg* PNA-T Monomer 15. The synthesis of fluorinated γ-CF_2-*apg* PNA-T monomer **15** (Scheme 1) was started from ethanolamine **1**, which was O-protected by reaction with TBDMSCl to obtain compound **2**.[18a] This was N- alkylated with benzyl bromide (1 equiv) to get compound **3**, followed by reaction with formalin and benzotriazole extending the backbone by one carbon to yield **4**. This was subjected to Reformatsky reaction with ethyl-bromodifluoroacetate that resulted in the difluoromethylene compound **5**. The ester group in compound **5** was reduced to alcohol **6**, followed by its conversion to corresponding azide via the triflate **7**. The azide was reduced to the amine **9** through Staudinger reaction and protected as the Boc derivative **10**. The *N*-benzyl group in **10** was hydrogenatively deprotected to the secondary amine **11** followed by N-acylation with chloroacetyl chloride to obtain **12**. This was coupled with thymine nucleobase under standard conditions to obtain compound **13**. The deprotection of O-TBDMS using TBAF to the alcohol **14** and subsequent oxidation to acid using TEMPO/BAIB gave the desired monomer **15**. All intermediates and final compound **15** were characterized by spectroscopic techniques (^{1}H, ^{13}C, and ^{19}F NMR) and mass spectrometry (Supporting Information). The control *apg*-PNA monomer **16** was synthesized by following standard procedure[18b] used for synthesis of *aeg*-PNA, starting from 1,3-diamino propane **17**, which was monoprotected to the N1-Boc derivative **18**, and *N*-alkylated with ethylbromo acetate to **19**. This was N-acylated with chloroacetyl chloride to **20**, followed by coupling with thymine

DOI: 10.1021/acs.joc.5b01614
J. Org. Chem. XXXX, XXX, XXX−XXX

to get the apg-PNA-T ethylester **21** that was hydrolyzed to the required acid monomer **16** (Supporting Information).

Synthesis of *apg* and γ-CF_2-*apg* PNA Oligomers. The modified *apg*-PNA monomers **15** and **16** were incorporated site specifically into a 10-mer *aeg*-PNA sequence (part of antisense sequence ON-705 corresponding to aberrant splice site of luciferase gene pLuc/705)[19] at different sites by manual solid-phase peptide synthesis on L-lysine-derivatized MBHA resin using standard Boc-chemistry protocol. The couplings were performed using HBTU-HOBt under microwave conditions (25 W at 75 °C). The synthesized PNA oligomers were cleaved from the resin using TFA:TFMSA reagent that also effected deprotection of Boc and Cbz groups. For cellular uptake studies, the PNA oligomers were tagged with 5(6)-carboxy fluorescein at the N-terminus through a lysine linker using *N*,*N*′-diisopropylcarbodiimide (DIPCDI) as coupling agent in pyridine on solid support. All the synthesized PNAs **1**−**9** (Table 1) were purified by RP HPLC and characterized by MALDI-TOF mass spectrometry (Supporting Information).

PNA:DNA/RNA Hybridization Studies by UV and CD Spectroscopy. The effect of substituting γ-CH_2 by γ-CF_2 in PNA backbone on the thermal stability of PNA duplexes with complementary DNA **1** and RNA **1** were examined by temperature-dependent UV absorbance and the comparative T_m values are shown in Table 1. The centrally modified γ-CF_2-*apg* PNA **2** destabilized the duplex with cDNA **1** by −4.4 °C as compared to the T_m of control *apg*-PNA **1**:DNA **1** duplex. The doubly modified γ-CF_2-*apg* PNA **4** (N-terminus and middle) destabilized the derived duplex with cDNA PNA **4**:DNA **1** by 2.2 °C compared to the corresponding control duplex *apg*-PNA **3**:DNA **1**. The triple modified (C, N, and middle) γ-CF_2-*apg* PNA **6** showed duplex thermal stability almost same as that of the corresponding nonfluorinated *apg* PNA **5** (Table 1).

The analogous duplexes with cRNA **1** constituted from the PNAs **1**−**6** showed T_ms significantly higher than the corresponding duplexes with cDNA **1** (ΔT_m^1 ∼ +7.0−13 °C, Table 1). The centrally modified γ-CF_2-*apg* PNA **2** destabilized the duplex with cRNA **1** by 2.0 °C compared to that of control *apg* PNA **1**. The RNA duplexes from the double modified PNA **4** (N-terminus and middle) and triple modified PNA **6** with γ-CF_2-*apg* units have almost the same stability as duplex from the corresponding control PNAs **3** and **5**. The DNA/RNA duplex stabilities of all *apg*-PNAs (PNAs **1**−**6**) were lower than the reference *aeg*-PNA (PNA **7**), and the magnitude of thermal stability of PNA:RNA and PNA:DNA duplexes decreased slightly with the increasing degree of *apg*-substitution consistent with previous reports.[20] This arises from the fact that the *apg*-PNAs reported here have a hybrid *aeg*-*apg* backbone, leading to different base spacings at *aeg* and *apg* steps, leading to a nonuniform backbone, unlike *aeg*-PNA **7** with uniform base spacing on backbone. To determine the fidelity of sequence specificity, the fluorinated PNAs were hybridized with DNA **2**/RNA **2** having a single mismatch base at the middle of the sequence (Table 1). The γ-CF_2-*apg* PNAs (PNA **2**, **4**, and **6**) showed a lower mismatch tolerance (ΔT_m^2 = −3.0 to −6.5 °C) for duplex with DNA **1** compared to that of corresponding unmodified *apg* PNAs (**1**, **3**, and **5**) (ΔT_m^2 = −11.5 to −14.5 °C). In contrast, all PNAs showed much higher discrimination for mismatch RNA **2** (ΔT_m^2 = 12−21 °C), and the triple modified γ-CF_2-*apg* PNA **6** had ΔT_m^2 of 9 °C.

The CD spectra of duplexes of γ-CF_2-*apg* PNAs with cDNA **1** showed a positive band at ∼266 nm and negative band at ∼243 nm, and for duplexes with cRNA, a positive band at ∼261 nm and negative bands at ∼291 and ∼239 nm. These CD signatures are not different from that of unmodified duplexes PNA **1**:DNA **1** and PNA **1**:RNA **1** (Supporting Information), suggesting similar conformations for both fluorinated and nonfluorinated *apg* PNAs.

Cell Uptake Studies. The fluorinated PNAs were examined for their cell-uptake ability and intracellular localization site using NIH 3T3 and HeLa cell lines. The fluorinated PNAs **5** and **6** on solid support were coupled with 5(6)-carboxyfluorescein at the N-terminus through a lysine unit (Supporting Information) to obtain the corresponding fluorescent PNAs (*apg* PNA **8**-Flu and γ-CF_2-*apg* PNA **9**-Flu). The cell permeability, localization, and uptake studies of these PNAs were carried out in two different cell lines, NIH 3T3 and HeLa, by confocal microscopy and FACS analysis. The cells were incubated with the fluorescently labeled PNAs (PNA **8**-Flu and PNA **9**-Flu; 2 μM) for 24 h followed by nuclear stain (Hoechst 33342, blue) and ER-tracker dye (ER-Red). The live cell confocal images for the PNA **8**-Flu and PNA **9**-Flu in NIH 3T3 and HeLa cells (Figure 2) indicate that both PNAs were taken

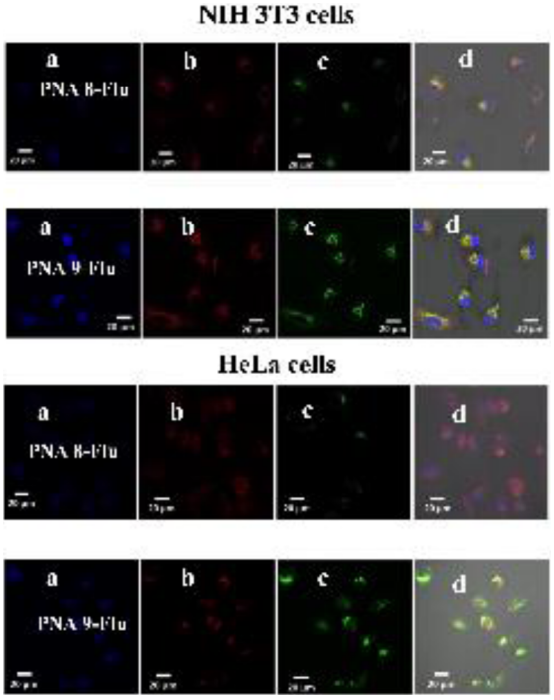


Figure 2. Confocal images of PNAs **8-Flu** and **9-Flu** in NIH 3T3 cells and HeLa cells: (a) nuclear stain, Hoechst 33442; (b) red fluorescence image from ER-red; (c) green fluorescence image from PNA; and (d) superimposed image of A−C and bright-field image.

by the cells and localized in cytoplasm. Further, the presence of PNAs in ER was inferred from the superimposed images of ER-red dye and PNA green fluorescence that gave yellow color resulting from colocalization of red and green spots in NIH 3T3 cells and HeLa cells (Figure 2d).

The quantitative estimation of the cell-penetrating abilities of the PNA oligomers was done by fluorescence activated cell sorter (FACS) analysis (Figure 3). The mean fluorescence intensities were found to be slightly more for fluorinated γ-CF_2-*apg* PNA (PNA **9**-Flu) as compared to *apg* nonfluorinated PNA (PNA **8**-Flu). The percent positive cells (proportion of cells uptaking PNA) were computed by instrument software after excluding auto fluorescence from overall gated cells (Figure 4).

DOI: 10.1021/acs.joc.5b01614
J. Org. Chem. XXXX, XXX, XXX−XXX

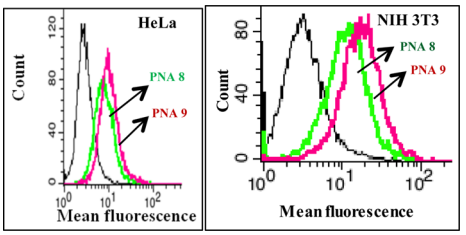


Figure 3. Mean fluorescence of PNA cell permeation in HeLa and NIH 3T3 cells (black is cells without dye).

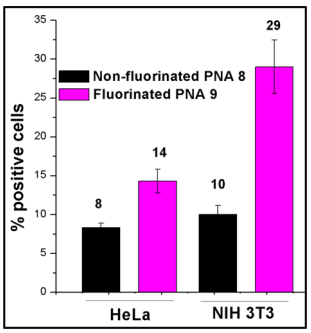


Figure 4. Percentage count of positive cells of fluorinated and nonfluorinated PNAs in HeLa and NIH 3T3 cells.

The data indicated that the fluorinated γ-CF_2-*apg* PNA (PNA **9**-Flu) is taken up by ~14% and ~29% in HeLa and NIH 3T3 cells, respectively, about 2- and 3-fold higher than the nonfluorinated PNA (PNA **8**-Flu).

The backbone fluorinated PNAs (PNAs **2**, **4**, and **6**), in which the γ-CH_2 of *apg*-PNA-T unit is replaced by γ-CF_2 moiety, form a duplex with complementary RNA **1** better than that with cDNA **1**[ΔT_m (RNA − DNA) ~ +8.4 to +12.9 °C]. The duplex T_ms depend on the site of incorporation of fluorinated units and decrease slightly with increasing degree of substitutions. However, the DNA/RNA duplexes of fluorinated PNAs (except the center modified PNA) showed about the same stability as that of corresponding nonfluorinated *apg* PNAs with cDNA/RNA (Table 1). The confocal images of cell line experiments involving blue nuclear stain, Hoechst 33442, and ER-red dye tracker illustrate that the fluorinated PNA **9**-Flu is taken by both the cell lines into cytoplasm and localized in ER. Quantitative estimation of cell uptake measured using FACS indicates that the tri-substituted γ-CF_2-*apg* PNA **9**-Flu exhibits 2−3-fold greater cell permeating ability in HeLa and NIH 3T3 cells compared to *apg* PNA **8**-Flu. The data in Figure 4 suggest that the cell permeability and localization are better in NIH 3T3 cells than HeLa cells.

CONCLUSION

Introduction of fluorine that has medicinally relevant atomic attributes of small size, higher electronegativity, and increased lipophilicity into PNA backbone in the form of a γ-CF_2-*apg* unit has led to the first examples of fluorous PNAs. The synthetic route is amenable for extension to synthesis of monomers with other nucleobases as well, with potential for the synthesis of fully modified γ-CF_2-*apg* PNAs in the context of future work. The study of their hybridiziation properties indicated that the thermal stabilities of duplexes from fluorinated PNAs with cDNA and cRNA do not change very much; both *apg* and γ-CF_2-*apg* PNAs are biased to bind RNA more than DNA. Significantly, the efficiency of cellular entry of fluorinated γ-CF_2-*apg* PNAs is better than its corresponding nonfluorinated *apg* PNA analogues, which get localized in the ER of NIH 3T3 and HeLa cells. The conceptual demonstration that the fluorinated PNAs are better cell-permeable will add a new repertoire for growing tactics to improve PNAs in antisense applications.[21] This inherent modification of backbone neither introduces chirality nor alters the conformational features of PNAs. It can be used in combination with other modifications (cationic,[22] conjugating hydrophobic chains,[15] etc.), including fluoro nucleobases (5-F-U, 5-CF_3-U) to increase the fluorine content to further enhance the biochemical attributes. Such work on exploration of fluorous PNAs in conjunction with other modifications is currently in progress.

EXPERIMENTAL SECTION

All commercial solvents were distilled prior to use: THF was distilled over sodium/benzophenone under nitrogen atmosphere, CH_2Cl_2 over CaH_2, and DMF over P_2O_5. Thin-layer chromatography (TLC) was performed on precoated silica gel GF254 plates. Column chromatography was performed using silica gel (100−200 μm). ^{1}H, ^{13}C, and ^{19}F NMR were recorded at 400, 100, and 376.6 MHz, respectively, in $CDCl_3$ or DMSO-d_6. The compounds **2** and **16** were prepared as per reported procedures.[18] The chemical shift values are in delta scale. For ^{19}F NMR, hexafluorobenzene was used as internal reference. IR spectra were recorded with neat samples. MALDI-TOF mass spectra of PNA oligomers were recorded using DHB as the matrix. HRMS data were recorded using ESI positive mode. PNA oligomers were purified by HPLC system using semipreparative C18 (10 × 250 mm) column, using gradient elution with water and increasing amounts of ACN (5%−50%).

Synthesis of *N*-Benzyl-2-((*tert*-butyldimethylsilyl)oxy)-ethanolamine 3. To the solution of compound **2** (10 g, 57.3 mmol) in ACN (100 mL), bromobenzene (5.0 mL, 40.1 mmol) and Et_3N (19.4 mL, 28.6 mmol) were added, and stirring continued at RT for 12 h. After completion of the reaction, water was added, and the product was extracted with ethyl acetate (3 × 50 mL). The dried organic layer was concentrated under reduced pressure, and product was purified by column chromatography, eluting with petroleum ether/EtOAc (50:50) to obtain the compound **3** (27.6 g, yield 55%). IR (neat) 3061, 3029, 2929, 2857, 1496, 1392, 1253, 1099, 938, 833, 777; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.06 (s, 6H), 0.90 (s, 9H), 1.92 (bs, 1H), 2.75 (t, 2H), 3.76 (t, 2H), 3.83 (s, 2H), 7.26−7.34 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.7, 18.0, 25.6, 50.8, 53.3, 61.9, 76.4, 76.7, 77.0, 126.6, 127.8, 128.1, 140.0; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{15}H_{28}NOSi$ 266.1940; Found 266.1944.

Synthesis of *N*-((1H-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-*N*-benzyl-2-((*tert*-butyldimethylsilyl) oxy)ethanolamine 4. To a solution of compound **3** (5 g, 18.8 mmol) in diethyl ether (50.0 mL), benzotriazole (3.4 g, 28.3 mmol) and formalin (2 mL of 35% formalin, 22.6 mmol) were added at 0 °C, and stirring continued at RT for 12 h. After completion of the reaction, the reaction mixture was filtered, and filtrate was concentrated. The product was purified by column chromatography, eluting with petroleum ether/EtOAc (70:30) to obtain the compound **4** (5.9 g, 80% yield): IR (neat) 3062, 2856,1495, 1253, 115, 1001, 834, 777, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.02 (min.) 0.05 (maj.) (s, 6H), 0.87 (min.) 0.89 (maj.) (s, 9H), 2.84 (t, J = 4.0 Hz, 2H), 3.77 (t, J = 8.0 Hz, 2H), 3.82 (s, 2H), 5.59 (s, 2H), 7.24−8.06 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.2, 26.0, 53.5, 53.7, 56.8, 62.4, 66.7, 110.4, 118.4, 119.8, 123.8, 126.2, 127.2, 127.4, 128.4, 128.8, 129.0, 133.8, 138.4, 145.9; HRMS (ESI-TOF) m/z: [M − CH_2Bt + H]$^+$ Calcd for $C_{15}H_{28}NOSi$ 266.1940; Found 266.1938.

Synthesis of Ethyl 3-(Benzyl(2-((*tert*-butyldimethylsilyl)oxy)-ethyl)amino)-2,2-difluoropropanoate 5. Zinc (4.1 g, 63.1 mmol) and TMSCl (1.6 mL, 12.6 mmol) were added to freshly distilled THF (25 mL), and the mixture was stirred vigorously at 50 °C for 15 min. Ethyl bromodifluoroacetate (6.4 mL, 50.5 mmol) was added and stirring continued for 20 min, followed by addition of compound **4** (5 g, 12.6 mmol) in THF (10 mL) and stirring at RT for 6 h. The

DOI: 10.1021/acs.joc.5b01614
J. Org. Chem. XXXX, XXX, XXX−XXX

reaction mixture was filtered through Celite pad, and filtrate was concentrated under vacuo. The crude product was purified by column chromatography, eluting with petroleum ether/EtOAc (80:20) to obtain pure compound **5** (2.2 g, 45% yield): IR (neat) 2958, 2859, 1743, 1452, 1370, 1254, 1209, 1101 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.00 (s, 6H), 0.86 (s, 9H), 1.30 (t, J = 8.0 Hz, 3H), 2.68 (t, J = 8 Hz, 2H), 3.30 (t, J = 12.0 Hz, 2H), 3.62 (t, J = 8.0 Hz, 2H), 3.80 (s, 2H), 4.27 (q, J = 8.0 Hz, 2H), 7.22−7.28 (m, 5H); ^{19}F-NMR (376.6 MHz, $CDCl_3$, C_6F_6 as internal reference) δ: −109.15(s, 2F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.3, 14.0 25.9, 55.6, 57.3(t, J_{C-F} = 27.0 Hz), 59.4, 61.1, 62.7, 127.1, 128.3, 128.8, 138.8; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{20}H_{34}F_2NO_3Si$ 402.2277; Found 402.2273.

Synthesis of 3-(Benzyl(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-amino)-2,2-difluoropropan-1-ol (6). To the solution of ester **5** (2.25 g, 5.6 mmol) in ethanol (20.0 mL), sodium borohydride (0.85 g, 22.4 mmol) was added at 0 °C and stirred at RT 12 h. After completion of reaction, water was added and extracted with ethyl acetate (3 × 25 mL). The dried organic layer was concentrated, and the product was purified by column chromatography, eluting with petroleum ether/EtOAc (70:30) to obtain the compound **6** (1.91 g, 95% yield): ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.04 (s, 6H), 0.90 (s, 9H), 2.66 (t, J = 8.0 Hz, 2H). 3.07 (t, J = 12.0 Hz, 2H), 3.73 (t, J = 8.0 Hz, 2H), 3.74 (s, 2H), 3.83 (t, J = 12.0 Hz, 2H), 4.02 (maj.) 4.12 (min.) (t, J = 8.0 Hz, 1H), 7.30 (s, 5H); ^{19}F-NMR (376.6 MHz, $CDCl_3$, C_6F_6 as internal reference) δ: −107.28(s, 2F) ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.4, 18.3, 25.9, 55.4 (t, J_{C-C-F} = 32.0 Hz), 56.2, 60.1, 61.3, 63.5 (t, J_{C-C-F} = 32.0 Hz), 123.0 (t, J_{C-F} = 244.0 Hz) 127.45, 128.5, 129.0, 138.2; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{18}H_{32}F_2NO_2Si$ $[M + H]^+$ 360.2170; Found 360.2168.

Synthesis of 3-Azido-*N*-benzyl-*N*-(2-((*tert*-butyldimethyl-silyl)oxy)ethyl)-2,2-difluoropropan-1-amine (8). To a solution of compound **6** (1.9 g, 5.3 mmol) in DCM, triethylamine (0.8 mL, 5.8 mmol) and triflic anhydride (10 mL, 5.8 mmol) were added at −78 °C and stirred for 1 h. After completion of reaction, reaction was quenched with water and extracted with DCM, and the dried organic layer upon concentration gave triflate **7** which was used further without purification. The triflate **7** (2.6 g, 5.3 mmol) in DMF (5.0 mL) was treated with sodium azide (1.3 g, 21.2 mmol) and stirred at RT for 12 h. Aqueous work up, extraction with ethyl acetate (3 × 25 mL), and concentration of organic layer yielded product that was purified by column chromatography, eluting with petroleum ether/EtOAc (75:25) to obtain the compound **8** (1.62 g, overall 80% yield from **6**): IR (neat) 3658, 2939, 2398, 2109, 1660, 1474, 1284, 1180, 1078, 958, 759, 684 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.06 (s, 6H), 0.91 (s, 9H), 2.73 (t, J = 8.0 Hz, 2H), 3.07 (t, J = 12.0 Hz, 2H), 3.58 (t, J = 12.0 Hz, 2H), 3.65 (t, J = 8.0 Hz, 2H), 3.80 (s, 2H), 7.31−7.34 (m, 5H); ^{19}F-NMR (376.6 MHz, $CDCl_3$, C_6F_6 as internal reference) δ: −106.36 (s, 2F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.3, 18.3, 25.9, 52.2 (t, J_{C-C-F} = 28.0 Hz), 56.2 (t, J_{C-C-F} = 27.0 Hz), 56.5, 56.5, 60.1, 61.5, 123.1 (t, J_{C-F} = 243.0 Hz), 127.3, 128.4, 128.8, 138.9; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{18}H_{31}F_2N_4OSi$ 385.2235; Found 385.2247.

Synthesis of *tert*-Butyl(3-(benzyl(2-((*tert*-butyldimethylsilyl)-oxy)ethyl)amino)-2,2-difluoropropyl) carbamate (10). The azide **8** (1.6 g, 4.1 mmol) in THF (15 mL) containing triphenylphosphine (1.1 g, 4.2 mmol) was stirred at RT for 12 h. Water (5 mL) was added to the reaction mixture and warmed to 45 °C for 4 h, after which THF was evaporated and product was extracted with ethyl acetate (3 × 15 mL). The dried organic layer was concentrated to obtain crude compound **9**, which was used for further reaction without purification. To the solution of compound **9** (1.5 g, 4.2 mmol) in ACN (10.0 mL), triethylamine (0.6 mL, 4.6 mmol) and Boc-anhydride (1.0 g, 4.6 mmol) were added. The reaction mixture was stirred at RT for 6 h, followed by aqueous work up, extraction with ethyl acetate (3 × 20 mL), and purification of crude product by column chromatography, eluting with petroleum ether/EtOAc (80:20) gave compound **10** (1.4 g, overall 75% yield from **8**): IR (neat) 2954, 2859, 1722, 1513, 1461, 1390, 1368, 1252, 1167, 1095, 1004 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.04 (s, 6H), 0.89 (s, 9H), 1.44 (s, 9H), 2.70 (t, J = 8.0 Hz, 2H), 2.97 (t, J = 12.0 Hz, 2H), 3.56−3.64 (m, 2H), 3.72 (t, J = 8.0 Hz, 2H), 3.76 (s, 2H), 5.04 (t, J = 8.0 Hz, 1H), 7.32−7.34 (m, 5H); ^{19}F-NMR (376.6 MHz, $CDCl_3$, C_6F_6 as internal reference) δ: −106. 57 (s, 2F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.3, 18.3, 26.0, 28.4, 43.4 (t, J_{C-C-F} = 29.0 Hz), 55.98 (t, J_{C-C-F} = 27.0 Hz), 56.5, 60.3, 61.5, 79.6, 122.9 (t, J_{C-F} = 244.0 Hz), 127.3, 128.5, 129.0, 129.1 138.8, 155.9; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{23}H_{41}F_2N_2O_3Si$ 459.2855; Found 459.2855.

Synthesis of *tert*-Butyl(3-((2-((*tert*-butyldimethylsilyl)oxy)-ethyl)amino)-2,2-difluoropropyl) carbamate (11). The compound **10** (1.4 g, 3.1 mmol) in ethanol (10.0 mL) was hydrogenated using Pd/C (0.05 g, 10% mmol) as catalyst and hydrogen balloon (∼14.0 psi) at RT for 1 h. The reaction mixture was filtered through Celite pad and the filtrate was concentrated and the residue purified by column chromatography. Elution with petroleum ether/EtOAc (60:40) yielded compound **11** (0.9 g, 80% yield): IR (neat) 3010, 2969, 1740, 1436, 1369, 1222, 901 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.07 (s, 6H), 0.90 (s, 9H), 1.45 (s, 9H), 1.84 (bs, 1H), 2.77(t, J = 4.0 Hz, 2H), 3.01 (t, J = 12.0 Hz, 2H), 3.56−3.65 (m, 2H), 3.70 (t, J = 4.0 Hz, 2H), 5.03 (bs, 1H); ^{19}F-NMR($CDCl_3$, 376.6 MHz) δ: −109.55 (s, 2F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.2, 18.3, 25.9, 28.3, 29.7, 43.5 (t, J_{C-C-F} = 30.0 Hz), 51.2, 51.5, 51.8, 62.3, 80.0, 122.0 (t, J_{C-F} = 242.0 Hz), 155.8; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{16}H_{35}F_2N_2O_3Si$ 369.2385; Found 369.2383.

Synthesis of *tert*-Butyl(3-(*N*-(2-((*tert*-butyldimethylsilyl)oxy)-ethyl)-2-chloroacetamido)-2,2-difluoropropyl) carbamate (12). To a solution of compound **11** (0.9 g, 2.5 mmol) in 1:1 water, dioxane (5 mL), sodium bicarbonate (0.5 g, 5.4 mmol), and chloroacetyl chloride (0.2 mL, 2.7 mmol) were added at 0 °C and stirred for 2 h, monitoring the completion of reaction by TLC. After completion of the reaction, water was added and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and product was purified by column chromatography, eluting with petroleum ether/EtOAc (70:30) to obtain the compound **12** (1.1 g, 85% yield): IR (neat) 3004, 2965, 1737, 1660, 1512, 1426, 1368, 1221, 924 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.05 (s, 6H), 0.88 (s, 9H), 1.45 (s, 9H), 3.46−3.51 (m, 2H), 3.62−3.67 (m, 2H), 3.76−3.86 (m, 2H), 4.09 (min.) 4.12 (maj.) (s, 1H), 4.34 (maj.) 4.37 (min.) (s,1H), 5.45 (min.) 5.63 (maj.) (bs, 1H); ^{19}F-NMR (376.6 MHz, $CDCl_3$, C_6F_6 as internal reference) δ: −107.31 (s, 2F); ^{13}C NMR $CDCl_3$, 100 MHz) δ: −5.5, 18.2, 25.8, 28.4, 29.7, 41.3, 46.6, 50.8, 59.8, 79.9, 121.4, 156.0, 169.7; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{35}ClF_2N_2NaO_4Si$ 467.1920; Found 467.1920.

Synthesis of *tert*-Butyl(3-(*N*-(2-((*tert*-butyldimethylsilyl)oxy)-ethyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-acetamido)-2,2-difluoropropyl)carbamate (13). To a solution of compound **12** (1.1 g, 2.4 mmol) in DMF, thymine (0.31 g, 2.4 mmol) and K_2CO_3 (0.3 g, 2.4 mmol) were added at 0 °C and stirred at RT for 12 h. The reaction was quenched with aq. sat. $KHSO_4$ solution and extracted with ethyl acetate (3 × 20 mL). The dried organic layer was concentrated under reduced pressure, and the residue was purified by column chromatography. Elution with petroleum ether/EtOAc (50:50) to obtain the compound **13** (1.0 g, 80% yield): IR: 3007, 2968, 1739, 1444, 1368, 1222 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.09 (s, 6H), 0.09 (s, 9H), 1.43 (s, 9H), 1.93 (s, 3H), 3.41−3.49 (m, 2H), 3.63 (t, J = 8.0 Hz, 2H), 3.75−3.84 (m, 4H), 4.51 (min.) 4.68 (maj.) (s, 2H), 5.41 (t, J = 8.0 Hz, 1H), 6.91 (s, 1H), 8.86 (bs, 1H); ^{19}F-NMR (376.6 MHz, $CDCl_3$, C_6F_6 as internal reference) δ: −107.52 (maj.) −108.69 (min.) (s, 2F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.3, 12.5, 18.4, 26.0, 28.3, 29.7, 42.3 (t, J = 30 Hz), 46.9 (t, J = 31 Hz), 48.7, 50.3, 60.2, 79.9, 110.9, 121.49, 140.8, 151.0, 155.8, 164.1, 169.0; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{23}H_{40}F_2N_4NaO_6Si$ 557.2583; Found 557.2572.

Synthesis of *tert*-Butyl(2,2-difluoro-3-(N-(2-hydroxyethyl)-2-(5-methyl-2,4-dioxo-3,4-dihydro pyrimidin1(2H)-yl)acet-amido)propyl)carbamate (14). To a solution of compound **13** (1.0 g, 2.0 mmol) in THF (1.0 mL), TBAF (1.6 mL of 1.0 M, 6.0 mmol) was added at 0 °C and stirred at RT for 3 h. THF was evaporated followed by aqueous work up and extraction with ethyl

DOI: 10.1021/acs.joc.5b01614
J. Org. Chem. XXXX, XXX, XXX−XXX

acetate (4 × 25 mL), and concentration of the dried organic layer gave crude product. This was purified by column chromatography, eluting with DCM/MeOH (90:10) to obtain the compound **14** (0.6 g, 75% yield): IR (neat) 3443, 2970, 1732, 1516, 1460, 1367, 1217 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 1.38, (s, 9H), 1.75 (s, 3H) 3.46−3.62 (m, 4H), 3.83 (maj.) 3.95 (min.) (t, J = 16.0 Hz, 2H), 4.53 (min.) 4.71 (maj.) (s, 2H), 5.01(t, J = 4.0 Hz, 1H), 7.05−7.08 (m, 1H), 7.35(bs, 1H); ^{19}F-NMR (376.6 MHz, DMSO-d_6, C_6F_6 as internal reference) δ: −106.20 (maj.) −107.56 (min.) (s, 2F) ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.4, 28.6, 43.0, 48.4, 49.8, 50.2, 65.4, 78.9, 108.8, 122.3, 142.5, 151.5, 156.3, 164.9, 168.8, 169.5, 170.2, 170.7; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{17}H_{26}F_2N_4NaO_6$ 443.1718; Found 443.1710.

Synthesis of 2-(*N*-(3-((*tert*-Butoxycarbonyl)amino)-2,2-difluoropropyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)acetic acid (15). To a solution of compound **14** (0.6 g, 1.5 mmol) in water:ACN (1:1), TEMPO (25 mg, 0.15 mmol) and BAIB (0.5 g, 1.6 mmol) were added at RT, stirred for 2 h followed up by standard work up, and washed with diethyl ether (2 × 5 mL) to remove by-products. Addition of aq. sat. $KHSO_4$ followed by extraction with ethyl acetate and solvent evaporation afforded the difluoro PNA monomer **15** (0.38 g, 60% yield). IR (neat) 3359, 2976, 2927, 1676, 1518, 1468, 1418, 1393, 1249, 1165, 1109 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 1.38 (maj.) 1.40 (min.) (s, 9H), 1.75 (s, 3H), 3.47−3.55 (m, 2H), 3.77−3.96 (m, 2H), 4.01 (maj.) 4.29 (min.) (s, 2H), 4.56 (maj.) 4.63 (min.) (s, 2H), 7.08 (t, J = 8.0 Hz, 1H), 7.33−7.40 (m, 1H), 11.31 (bs, 1H); ^{19}F-NMR (376.6 MHz, DMSO-d_6, C_6F_6 as internal reference) δ: −106.70 (min.) −107.31 (maj.) (s, 2F); ^{13}C NMR (100 MHz, DMSO-d_6) δ: −12.4, 28.6, 43.0, 48.4, 49.8, 50.2, 78.9, 108.6, 119.6, 122.3, 124.5, 142.5, 151.4, 156.1, 164.9, 168.8, 169.5, 170.2, 170.7; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{17}H_{24}F_2N_4NaO_7$ 457.1511; Found 457.1509.

Synthesis of Ethyl 2-((3-((*tert*-Butoxycarbonyl)amino)-propyl)amino)acetate (19).[18c] To a solution of excess propylene 1,3-diamine **17** (10 g, 135 mmol) in dichloromethane (250 mL), Boc-anhydride (15 mL, dissolved in 50 mL DCM, 135 mmol) was added at 0 °C dropwise and stirred at RT for 12 h. After completion of reaction, water was added and extracted with dichloromethane (3 × 50 mL). The organic layer was washed with aq. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and concentrated to obtain the crude compound **18**, which was used for next reaction without any purification. To the solution of compound **18** (15 g, 86.2 mmol) in ACN (50 mL), triethylamine (23.3 mL, 86.2 mmol) and ethyl bromoacetate (9.6 mL, dissolved in 20 mL ACN, 86.2 mmol) were added at 0 °C dropwise, and stirring continued at RT for 12 h. The reaction was monitored by TLC, and after completion of the reaction, ACN was evaporated, and water was added and extracted with EtOAc (3 × 50 mL). The organic layer was washed with brine solution, dried over anhydrous Na_2SO_4, and concentrated to obtain the product **19**. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.28 (t, J = 6 Hz, 3H), 1.60−1.73 (m, 2H), 1.79 (bs, 1H), 2.67 (t, J = 6 Hz, 2H), 3.16−3.29 (m, 2H), 3.39 (s, 2H), 4.20 (q, J = 8 Hz, 2H), 4.99 (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.2, 28.4, 29.9, 38.8, 47.2, 50.8, 60.7, 79.0, 156.0, 172.4.

Ethyl 2-(*N*-(3-((*tert*-Butoxycarbonyl)amino)propyl)-2-chloroacetamido)acetate (20). To a solution of the compound **19** (20 g, 76.9 mmol) in water:dioxane (1:1, 50 mL), $NaHCO_3$ (14.2 g, 169.2 mmol) was added, and after stirring for 5 min, chloroacetyl chloride (6.8 mL, 84.6 mmol) was added at 0 °C dropwise, and stirring continued for 5 h. Water was added, product extracted with EtOAc (3 × 50 mL), and the dried organic layer was concentrated. The product was purified by column chromatography, eluting with petroleum ether/EtOAc (1:1) to obtain the compound **20**. (Yield 20.16 g, 78%). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.21−1.27 (m, 3H) 1.38 (maj) 1.39 (min) (s, 9H), 1.59−1.83(m, 2H), 3.03−3.14 (m, 2H), 3.42 (q, 2H, J = 8 Hz), 4.00 (maj) 4.01 (min) (s, 2H), 4.09−4.22 (m, 4H), 4.91 (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1, 28.4, 29.1, 37.0, 37.9, 40.8, 41.1, 45.3, 47.2, 48.0, 49.9, 53.5, 61.4, 62.1, 76.8, 77.1, 77.5, 79.0, 79.5; HRMS (ESI-TOF) m/z: $[M + K]^+$ Calcd for $C_{14}H_{25}ClKN_2O_5$ 375.1089; Found 375.1090.

Synthesis of Ethyl 2-(*N*-(3-((*tert*-Butoxycarbonyl)amino)-propyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-acetamido)acetate (21). To a solution of the compound **20** (10 g, 29.8 mmol) in DMF (20 mL), K_2CO_3 (4.1 g, 29.8 mmol) and thymine (3.75 g, 29.8 mmol) were added and stirred at RT for 12 h. The reaction was quenched with aq. $KHSO_4$, extracted with EtOAC (3 × 25 mL), and the dried organic layer was concentrated. The residual product was purified by column chromatography, eluting with petroleum ether/EtOAc (1:1) to obtain the compound **20**. (Yield 9.5 g, 75%). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.2−1.27 (m, 3H), 1.37 (s, 9H), 1.84(s, 3H), 2.82−3.41 (m, 8H), 4.0−4.57 (m, 4H), 5.25 (bs, 1H), 7.04 (s, 1H), 9.92 (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.4, 14.1, 28.4, 31.5, 36.5, 46.1, 47.6, 47.9, 48.0, 49.5, 61.4, 62.2, 79.0, 110.6, 141.4, 151.5, 156.5, 162.7, 164.6, 167.1,168.9, 169.3; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{19}H_{30}N_4NaO_7$ 449.2012; Found 449.2003.

Synthesis of 2-(*N*-(3-((*tert*-Butoxycarbonyl)amino)propyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)acetic acid (16). The ester **21** (9.0 g, 21.12 mmol) in THF was saponified with 10% aq. LiOH at 0 °C for 3 h. The mixture was acidified with sat. aq. $KHSO_4$, the acid compound was extracted with EtOAc, dried over Na_2SO_4, and evaporated to obtain the desired monomer **16** (Yield 6.20 g, 77%). ^1H NMR (400 MHz, DMSO-d_6) δ: 1.36 (min) 1.37 (maj) (s, 9H), 1.74 (s, 3H), 1.49−1.72 (m, 2H) 2.86−3.01 (m, 2H), 3.23−3.35 (m, 2H), 3.95 (maj) 4.18 (min) (s, 2H), 4.45(min) 4.60 (maj) (s, 2H), 6.82 (bs, 1H), 7.30 (min) 7.39 (maj) (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.4, 27.8, 28.7, 37.7, 45.7, 47.8, 48.2, 78.0, 108.4, 142.7, 151.4, 156.0, 164.9, 167.3, 167.8, 170.8, 171.3; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{17}H_{27}N_4O_7$ 399.1880; Found 399.1873.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01614.

^1H NMR, ^{13}C NMR, and ^{19}F NMR of compounds **3**−**15**, HPLC of PNA oligomers **1**−**8**, MALDI-TOF mass spectra PNA oligomers **1**−**8**, UV−T_m profiles of duplexes of PNA oligomers **1**−**6** with DNA **1**, **2** and RNA **1**, **2**. CD spectra of PNA oligomers **1**−**6** and their duplexes with DNA **1** and RNA **1** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: kn.ganesh@iiserpune.ac.in.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

S.E. thanks CSIR (New Delhi) for research fellowship. K.N.G. is a recipient of JC Bose Fellowship and a Honorary Professor at JNCASR, Bengaluru.

■ REFERENCES

(1) Nielsen, P. E. *Annu. Rev. Biophys. Biomol. Struct.* **1995**, *24*, 167−183.
(2) Hyrup, B.; Nielsen, P. E. *Bioorg. Med. Chem.* **1996**, *4*, 5−23.
(3) (a) Good, L.; Nielsen, P. E. *Antisense Nucleic Acid Drug Dev.* **1997**, *7*, 431−437. (b) Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 2796−2823.
(4) Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. *Nature* **1993**, *365*, 566−568.
(5) Jensen, K. K.; Qrum, H.; Nielsen, P. E.; Norden, B. *Biochemistry* **1997**, *36*, 5072−5077.
(6) Nielsen, P. E. *Acc. Chem. Res.* **1999**, *32*, 624−630.
(7) Nielsen, P. E. *Mol. Biotechnol.* **2004**, *26*, 233−248.

(8) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Anti-Cancer Drug Des.* **1993**, *8*, 53−63.
(9) Kumar, V. A.; Ganesh, K. N. *Acc. Chem. Res.* **2005**, *38*, 404−412.
(10) Kumar, V. A.; Ganesh, K. N. *Curr. Top. Med. Chem.* **2007**, *7*, 715−726.
(11) Smart, B. E. *J. Fluorine Chem.* **2001**, *109*, 3−11.
(12) (a) Biffinger, J. C.; Kim, H. W.; DiMagno, S. G. *ChemBioChem* **2004**, *5*, 622−627. (b) Bohm, H.-J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Muller, K.; Obst-Sander, U.; Stahl, M. *ChemBioChem* **2004**, *5*, 637−643.
(13) Wang, J.; Sánchez-Roselló, M.; Acena, J. L.; del Pozo, C.; Sorochinsky, A. E.; Fustero, S.; Soloshonok, V. A.; Liu, H. *Chem. Rev.* **2014**, *114*, 2432−2506.
(14) Doi, Y.; Katafuchi, A.; Fujiwara, Y.; Hitomi, K.; Tainer, J. A.; Ide, H.; Iwai, S. *Nucleic Acids Res.* **2006**, *34*, 1540−1551.
(15) Dolain, C.; Patwa, A.; Godeau, G.; Barthélémy, P. *Appl. Sci.* **2012**, *2*, 245−259.
(16) Hollenstein, M.; Leumann, C. J. *Org. Lett.* **2003**, *5*, 1987−1990.
(17) Kiviniemi, A.; Murtola, M.; Ingman, P.; Virta, P. *J. Org. Chem.* **2013**, *78*, 5153−5159.
(18) (a) Compound 2: Inman, M.; Moody, C. J. *J. Org. Chem.* **2010**, *75*, 6023−6026. (b) Meltzer, P. C.; Liang, A. Y.; Matsudiara, P. *J. Org. Chem.* **1995**, *60*, 4305−4308. (c) Shibata, N.; das, B. K.; Honjo, H.; Takeuchi, Y. *JCS Perkin I* **2001**, 1605−1611.
(19) Kang, S. H.; Cho, M. J.; Kole, R. *Biochemistry* **1998**, *37*, 6235−6239.
(20) Hyrup, B.; Egholm, M.; Rolland, M.; Nielsen, P. E.; Berg, R. H.; Buchardt, O. *J. Chem. Soc., Chem. Commun.* **1993**, *6*, 518−519.
(21) (a) Hatamoto, M.; Ohashi, A.; Imachi, H. *Appl. Microbiol. Biotechnol.* **2010**, *86*, 397−402. (b) Gambari, R. *Expert Opin. Ther. Pat.* **2014**, *24*, 267−294.
(22) (a) Jain, D. R.; Anandi, L. V.; Lahiri, M.; Ganesh, K. N. *J. Org. Chem.* **2014**, *79*, 9567−9577. (b) Sahu, B.; Chenna, V.; Lathrop, K. L.; Thomas, S. M.; Zon, G.; Livak, K. J.; Ly, D. H. *J. Org. Chem.* **2009**, *74*, 1509−1516.

DOI: 10.1021/acs.joc.5b01614
J. Org. Chem. XXXX, XXX, XXX−XXX