JOC The Journal of Organic Chemistry

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Clickable C^{γ}-Azido(methylene/butylene) Peptide Nucleic Acids and Their Clicked Fluorescent Derivatives: Synthesis, DNA Hybridization Properties, and Cell Penetration Studies

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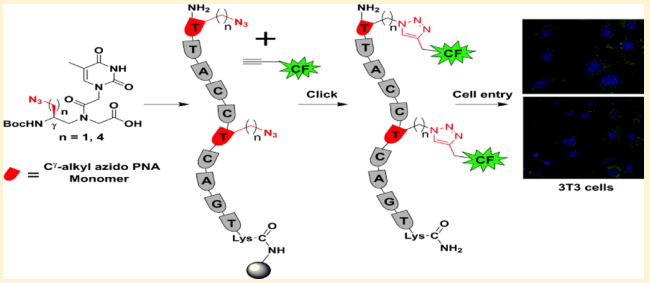
Supporting Information

ABSTRACT: Synthesis, characterization, and DNA complementation studies of clickable C^{γ}-substituted methylene (*azm*)/butylene (*azb*) azido PNAs show that these analogues enhance the stability of the derived PNA:DNA duplexes. The fluorescent PNA oligomers synthesized by their click reaction with propyne carboxyfluorescein are seen to accumulate around the nuclear membrane in 3T3 cells.



Peptide nucleic acids are effective DNA analogues in which the sugar phosphate backbone is replaced with a pseudopeptide backbone in the form of achiral 2-(aminoethyl)-glycine (*aeg*) linkage (Figure 1a). Nucleobases (A/T/G/C) are attached through a methylene carbonyl linker to this backbone at the amino nitrogen.[1,2] PNA hybridizes with complementary DNA/RNA via Watson−Crick base pairing with higher avidity compared to DNA:DNA or DNA:RNA duplexes which may arise from a high flexibility and the absence of charge in the backbone. PNAs are resistant to both proteases and nucleases and consequently have a longer life span in the cellular environment.[3] The simplicity of chemical structure, ease of synthesi,s and superior binding properties have made PNA an attractive gene regulatory agent.[4−6] However, these suffer from poor aqueous solubility, cell penetrability, and equal affinity for binding to cDNA and RNA, which reduces their target specificity by half.[7−9] Many PNA modifications have been rationally designed to address these concerns. They include modifications in the backbone to preorganize PNA conformation to tune with that of cDNA or RNA, introduction of charged moieties to enhance solubility, and conjugation with transfer molecules to improve cell penetrability.[10−12] Another approach is to make them inherently cationic and chiral to simultaneously address these issues.[13] Employing click chemistry, PNAs carrying N-terminus azide function can be conjugated with application specific functional ligands such as ferrocene (electrochemical detection),[14a] 2,2′-dipicolylamine (^{99m}Tc-labeling, biodistribution),[14b] quinoline (Re complex, bioimaging),[14c] or fluorophores (cell penetration[13] and molecular beacons[15]) or use in addressable PNA microarrays.[16,17] Site-specific labeling via click chemistry has been achieved via either azide bearing nucleobase[18] or at pyrrolidine ring within the backbone.[19] PNA monomers bearing azide moiety have been employed to synthesize triazolyl PNA oligomers by click reaction on solid phase.[20]

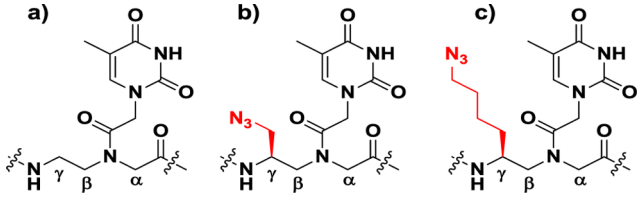


Figure 1. Chemical structures of (a) *aeg* PNA, (b) $\gamma(R)$-*azm aeg* PNA, and (c) $\gamma(S)$-*azb aeg* PNA.

Here we describe an approach to synthesize site-specific azido PNAs (Figure 1b,c) wherein the clickable azido moiety is placed on the PNA chain, separated from backbone by methylene or buytlene chain to examine the spacer dependent effects on the DNA complementation. Employing click chemistry, fluorophore carboxyfluorescein is conjugated to these PNAs. The synthesis of target azido PNA monomers (**11a** and **11b**), their insertion at specific sites into a PNA sequence to give azido PNAs, hybridization with complementary and mismatch DNA, and click attachment of carboxyfluorescein PNAs for live cell imaging are reported. It is shown that both methylene- and butylene-linked azido PNAs show improved efficiency of hybridization with cDNA compared to unmodified PNA and the butylene-linked azido PNAs perform better than the methylene PNAs.

Received: April 22, 2014
Published: June 18, 2014

dx.doi.org/10.1021/jo500834u | *J. Org. Chem.* 2014, 79, 6708−6714

Scheme 1. Synthesis of *γ-azm aeg* PNA and *γ-azb aeg* PNA Monomers

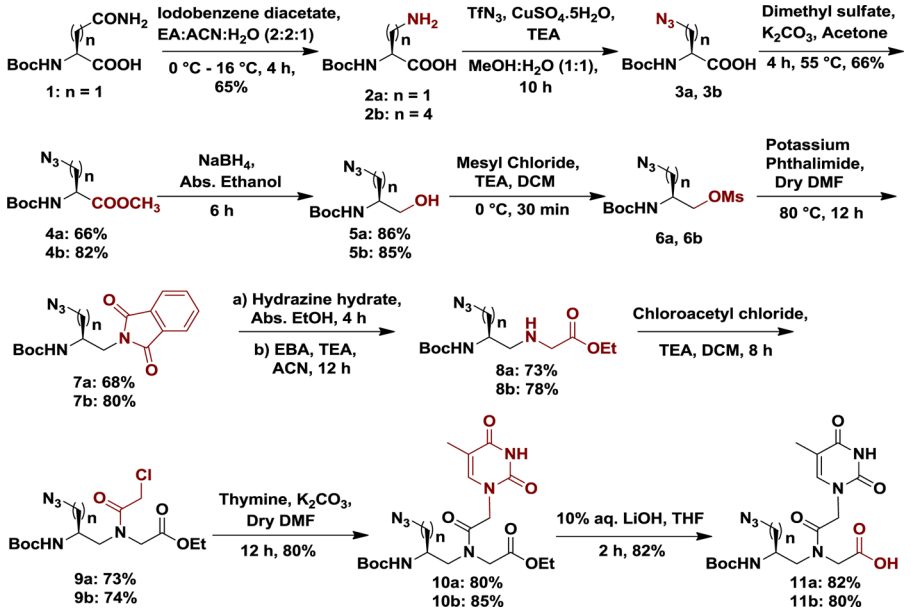


Table 1. PNA Sequences and UV–T_m of PNA/DNA Duplexes[a]

Entry	PNA Oligomers 12 34 5 67 89 10		T_m	
			DNA 1 (ap)	DNA 2 (mm)
1	*aeg*PNA**1**	H-TTACCTCAGT-LysNH$_2$	43.4	37.4
2	*azm*-T$_2$PNA **2**	H-T**T**ACCTCAGT-LysNH$_2$	43.9	33.8
3	*azm*-T$_6$PNA **3**	H-TTACC**T**CAGT-LysNH$_2$	49.7	35.3
4	*azm*-T$_{10}$PNA **4**	H-TTACCTCAG**T**-LysNH$_2$	45.8	35.8
5	*azm*-T$_{2,6}$ PNA **5**	H-T**T**ACC**T**CAGT-LysNH$_2$	43.6	33.2
6	*azb*-$\underline{\boldsymbol{T}}_2$PNA **6**	H-T$\underline{\boldsymbol{T}}$ACCTCAGT-LysNH$_2$	44.9	37.2
7	*azb*-$\underline{\boldsymbol{T}}_6$PNA **7**	H-TTACC$\underline{\boldsymbol{T}}$CAGT-LysNH$_2$	49.2	35.9
8	*azb*-$\underline{\boldsymbol{T}}_{10}$PNA **8**	H-TTACCTCAG$\underline{\boldsymbol{T}}$-LysNH$_2$	50.9	36.3
9	*azb*-$\underline{\boldsymbol{T}}_{2,6}$PNA **9**	H-T$\underline{\boldsymbol{T}}ACC\underline{\boldsymbol{T}}$CAGT-LysNH$_2$	50.6	37.9
10	*azm*-$\underline{\mathbf{T}^f}_2$ PNA **10**	H-T$\underline{\mathbf{T}^f}$ACCTCAGT-LysNH$_2$	43.8	31.7
11	*azm*-$\underline{\mathbf{T}^f}_6$PNA **11**	H-TTACC$\underline{\mathbf{T}^f}$CAGT-LysNH$_2$	42.0	32.8
12	*azm*-$\underline{\mathbf{T}^f}_{10}$PNA **12**	H-TTACCTCAG$\underline{\mathbf{T}^f}$-LysNH$_2$	42.0	33.2
13	*azm*-$\underline{\mathbf{T}^f}_{2,6}$ PNA **13**	H-T$\underline{\mathbf{T}^f}$ACC$\underline{\mathbf{T}^f}$CAGT-LysNH$_2$	40.0	31.0
14	*azb*-$\underline{\boldsymbol{T}^f}_6$ PNA **14**	H-TTACC$\underline{\boldsymbol{T}^f}$CAGT-LysNH$_2$	46.4	32.8
15	*azb*-$\underline{\boldsymbol{T}^f}_{10}$ PNA **15**	H-TTACCTAG$\underline{\boldsymbol{T}^f}$-LysNH$_2$	47.8	32.9
16	*azb*-$\underline{\boldsymbol{T}^f}_{2,6}$ PNA **16**	H-T$\underline{\boldsymbol{T}^f}ACC\underline{\boldsymbol{T}^f}$CAGT-LysNH$_2$	40.0	nb

[a]See the Supporting Information for mass spectra; $\underline{\mathbf{T}}$ = azidomethylene (*azm*) PNA unit and $\underline{\boldsymbol{T}}$ = azidobutylene (*azb*) PNA unit; $\underline{\mathbf{T}^f}$ and $\underline{\boldsymbol{T}^f}$ are the carboxyfluorescein derivatives; DNA **1**, 5′ACTGAGGTAA3′(ap, antiparallel); DNA **2**, 5′ACTG**C**GGTAA3′ (mm, mismatch); [PNA/DNA] = 2 μM each strand. Buffer: sodium phosphate (10 mM), NaCl (10 mM) and EDTA (0.1 mM); nb = not binding.

SYNTHESIS OF *Cγ*-AZIDOALKYLIDENE PNA MONOMERS

The synthesis of desired *Cγ*-azidomethylene (**11a**) and *Cγ*-azidobutylene (**11b**) PNA-T monomers containing thymine nucleobase was started from the commercially available *N*-*α*-Boc-L-asparagine (**1**) (Scheme 1) for methylene spacer and *N*-*α*-Boc-L-lysine (**2b**) for butylene spacer. The side chain amido group in compound **1** was converted to the amino function via Hofmann rearrangement which yielded 3-amino-2-(Boc-amino)propanoic acid (**2a**).[21] This was subjected to diazo transfer reaction for the conversion of primary amine to azide derivative. Triflyl azide synthesized as per known method[22] was reacted with amine **2a** in the presence of $CuSO_4{\cdot}5H_2O$ and triethylamine to achieve azo transfer to the azide **3a**. This was esterified using dimethyl sulfate/activated K_2CO_3 to give the methyl ester **4a** that was reduced with $NaBH_4$ to the primary alcohol **5a**. It was treated with mesyl chloride to give the mesylate **6a** that was immediately reacted with potassium phthalimide to obtain the N-protected compound **7a**. Deprotection of phthalimide with hydrazine hydrate gave the free amine which was in situ *N*-alkylated with ethyl bromoacetate to yield product **8a**. The N-acylation of this compound with chloroacetyl chloride led to the chloromethyl compound **9a**, and subsequent reaction with nucleobase thymine gave *γ*-(azidomethylene)aminoethylglycyl ethyl ester **10a** in quantitative yield. The *γ-azm aeg* PNA ester **10a** was hydrolyzed using aqueous lithium hydroxide to obtain the *γ-azm aeg* PNA acid **11a**.

The synthesis of *γ-azb aeg* PNA-T monomer was achieved starting from *N*-*α*-Boc-L-lysine (**2b**) that was converted to the azido derivative **3b** and then following a similar reaction sequence as for **11a** gave the *γ-azidobutylene aeg* PNA acid **11b**. All the intermediate new compounds and the final monomers were characterized by analytical and spectroscopic data (Supporting Information).

dx.doi.org/10.1021/jo500834u | J. Org. Chem. 2014, 79, 6708–6714

SOLID-PHASE SYNTHESIS OF PNA OLIGOMERS AND THEIR CHARACTERIZATION

The PNA oligomers were synthesized by manual solid-phase peptide synthesis on L-lysine-derivatized MBHA resin using the *Boc*-chemistry protocol[23] and standard HOBt−HBTU coupling reagents. The PNA oligomers (Table 1) incorporating azidoalkylidene units at chosen sites in the unmodified PNA sequence **1** were obtained by coupling with the monomers **11a** and **11b** at the desired positions of the PNA sequence. The azido PNAs (PNA **2**−**9**) were transformed to the fluorescently labeled PNA oligomers (PNA **10**−**16**) by on-resin click reaction with alkyne derivative of 5(6)-carboxyfluorescein[24] in the presence of copper (Supporting Information). This enabled attachment of a fluorophore at any desired position in the sequence. At the end of the assembly, the PNA oligomers were cleaved from the resin by TFA−TFMSA.[25] at which time the Cbz-protection on exocyclic amine of nucleobases was also simultaneously removed to give crude PNAs. All PNA oligomers were purified by reversed-phase HPLC and characterized by MALDI-TOF spectrometry (Supporting Information). The various modified and unmodified *aeg* PNA oligomers which were synthesized are listed in Table 1. The fluorescent PNAs *azm*-T(cf) PNA **10**−**13** and *azb*-*T*(cf) PNA **14**−**16** obtained from click reactions were characterized by their fluorescence emission band at 520 nm upon excitation at 484 nm arising from 5(6)-carboxyfluorescein moiety (Supporting Information).

THERMAL STABILITY OF PNA:DNA HYBRIDS

To evaluate the effect of the chiral, charge-neutral azide group linked at C$^{\gamma}$-position through methylene/butylene chains on the thermal stability of PNA:DNA duplexes, the melting temperatures (T_m) of duplexes with complementary DNA **1** were measured by temperature-dependent UV absorbance. The various PNAs were annealed individually with stoichiometric amounts of antiparallel cDNA strand (DNA **1**) to constitute PNA:DNA duplexes. The sequence specific recognition of modified PNA oligomers with cDNA was also assessed from their binding studies with DNA **2** having a single base mismatch. The T_m results for all PNA:DNA duplexes are shown in Table 1.

Incorporation of neutral azide function in the C$^{\gamma}$-side chain of *aeg* PNA backbone affects the thermal stability of the derived PNA:DNA hybrids (Table 1, Figure 2). In the case of azidomethylene (*azm*) PNAs, the PNA:DNA duplex was enhanced by 0.2−6.3 °C depending on the site of modification. Substitution at the C-terminus (PNA **4**, ΔT_m = 2.4 °C) was found to be better than that at the N-terminus (PNA **2**, ΔT_m = 0.5 °C) and even better when the *azm* modification is in the middle of the sequence (PNA **3**, ΔT_m = 6.3 °C). However, when these two modifications were combined, the doubly modified PNA (PNA **5**) showed stability similar to that of *aeg* PNA **1**. In the case of longer side chain azidobutylene (*azb*) PNAs, single modification at the C-terminus (PNA **8**) gave higher stability (ΔT_m = 7.5 °C) than the N-terminus modified (PNA **6**) (ΔT_m = 1.5 °C) similar to the shorter chain *azm* modification. However, the middle modification exhibited a stabilization (PNA **7**; ΔT_m = 5.8 °C) in between that of the N-terminus (PNA **6**) and C-terminus (PNA **8**) modifications unlike the case of *azm*, where it was the highest. The *azb* PNA **9** carrying double modification at the C-terminus and middle showed significant stabilization of 7.2 °C. These results on

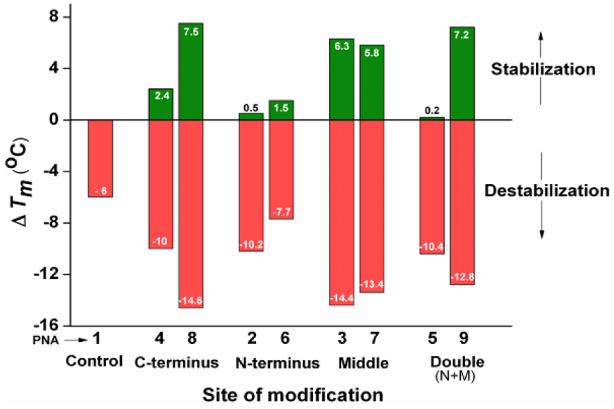


Figure 2. Comparative ΔT_mvalues for PNA:DNA duplexes of *γ-azm*(PNA **2**−**5**) and *γ-azb*(PNA **6**−**9**) with cDNA **1** and mismatch DNA **2**. ΔT_m (green bars):stabilization compared to *aeg*-PNA **1**:DNA **1** duplex. ΔT_m(red bars):destabilization of PNA:DNA **2** duplexes compared to PNA:DNA **1** duplexes.

thermal stability of azidoalkylidene PNA:DNA hybrids suggest that (i) the extent of stability depends on the number of methylenes in the side chain, with PNAs having longer butyl chain showing better stability than those shorter methylene chains and (ii) the stabilizing factors are site dependent, but not additive in nature.

Fluorescently labeled PNAs generated from online click reaction of various azido PNAs with propyne carboxyfluorescein were examined for their abilities to form duplexes with cDNA **1** and mismatch DNA **2**. The extent of relative stabilization/destabilization compared to their azido analogues are shown in Table 1 (see the Supporting Information). The *azm* PNAs (PNA **10**−**13**) showed destabilization (ΔT_m= −3.4 to +0.4 °C) at almost all sites, while the *azb* analogues were slightly better stabilized with modifications at the C-terminus (PNA **15**) or in the middle (PNA **14**) (ΔT_m= 3.0−4.4 °C). Thus, the bulky carboxyfluorescein is tolerated only when placed away from the PNA backbone.

To determine whether the increased affinity is at the cost of sequence specificity, the T_m's of modified PNAs hybridized with DNA**2** having single base mismatch in the middle of the sequence were determined (Table 1, Figure 2). The introduction of one base-pair mismatch significantly lowered the T_m of the azido PNA:DNA **2** duplexes compared to that with corresponding azido PNA:DNA **1** duplexes with cDNA. The relative destabilization for the *azm* and *azb*-PNA:DNA **2** complexes (ΔT_m= −10 to −14.6 °C) was much higher than that for the *aeg*-PNA:DNA **2** duplex (ΔT_m= −6 °C). These results clearly suggest that chiral, azide-modified PNAs not only have higher binding affinity with cDNA but also have a better mismatch discrimination in their specificity for target cDNA. The fluorescently labeled PNAs also show better mismatch discrimination compared to control *aeg* PNA (Supporting Information).

The effect of *γ*-side-chain substitution on the conformation of PNA:DNA duplexes was examined by CD spectroscopy. Single strand PNAs have weak or nonexistent CD signals, while the PNA:DNA complexes exhibit characteristic CD signatures due to the helicity induced by the chiral DNA in the hybrid. However, the CD profiles for duplexes of azide modified PNAs were similar to that of the unmodified PNA:DNA duplex (Supporting Information).

dx.doi.org/10.1021/jo500834u | J. Org. Chem. 2014, 79, 6708−6714

The relative binding affinities of azide-bearing PNAs with cDNA was examined by their ability to invade DNA:DNA duplex, bind to cDNA, and displace the isosequential second DNA strand. This reaction was monitored by an ethidium bromide (EtBr) displacement assay[26] wherein the fluorescence from the dye intercalated in the DNA duplex is decreased as PNAs invade the duplex to form PNA:DNA duplex that cannot bind the dye.[27] The relative dye displacement efficiencies followed by monitoring the decrease in fluorescence at 610 nm (see the Supporting Information) for *aeg* (**1**), *azb* (**8**), and *azm* (**4**) PNAs were 84%, 89%, and 63%, respectively, at the same DNA duplex concentrations. These suggest that the *azb*-PNA (**8**) which forms the most stable duplex is also the best in displacement of the intercalated dye.

CELL PENETRATION STUDIES OF FLUORESCENTLY LABELED PNA OLIGOMERS

The cell penetration abilities and the intracellular distribution of PNAs were investigated in NIH 3T3 cell lines by confocal microscopy employing standard cellular uptake assay, incubating the live cells with fluorescently labeled PNAs. Figure 3

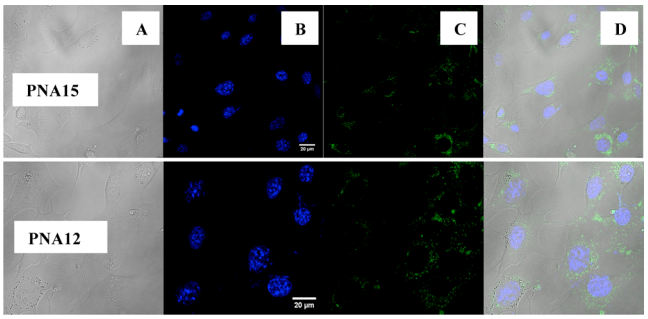


Figure 3. Distribution of various PNAs inside NIH 3T3 cells: (A) bright field image (B) Hoechst 33342 stained image, (C) green fluorescent image, and (D) superimposed image of A–C.

shows live cell images for cell penetration of PNAs *azm*-$\mathbf{T}^{\mathbf{f}}$ PNA **12** and *azb*-T^f PNA **15** in NIH 3T3 cells, bright field image (Figure 3A), Hoechst 33342 stained image (Figure 3B), the green fluorescent image (Figure 3C), and the superimposed image (Figure 3D). In comparison with control PNAs (Supporting Information), these suggest that the cells accumulate the modified PNAs in the cytoplasm in the vicinity of the nucleus.

In conclusion, it is demonstrated that the C^{γ}-substituted methylene/butylene azido PNAs enable the attachment of fluorophore through click reactions at desired sites in the PNA. PNA analogues with a C^{γ}-butylene spacer chain show higher stabilization of DNA hybrids than that from a methylene spacer chain. The fluorescent PNA oligomers accumulate around the nuclear membrane as seen by live cell imaging in 3T3 cells. Since azido function does not need protection, the side-chain conjugation strategy reported here permits multisite labeling of same ligand in a single step to enhance the sensitivity of detection. The outcome of the work has potential for future utilization of azido PNAs to conjugate different ligands for multipurpose probing various biofunctions.

EXPERIMENTAL SECTION

See the Supporting Information for general information on the source of materials and methods.

3-Amino-2-(Boc-amino)propanoic Acid (2a).[21] A slurry of compound *N*-Boc-L-asparagine (**1**) (5 g, 21.5 mmol), EtOAc (24 mL), CH_3CN (24 mL), H_2O (12 mL), and iodobenzene diacetate (8.3 g, 25.8 mmol) was cooled and stirred at 16 °C for 30 min The temperature was allowed to reach 20 °C, and the reaction mixture was stirred for 4 h. It was cooled to 0 °C and filtered under vacuum. The solid residue was washed with EtOAc and dried in vacuum to obtain compound **2a** (2.87 g, 65% yield): mp = 210–212 °C; R_f = 0.25 EtOAc/MeOH (50:50); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (bs, 3H), 7.24–7.22 (d, J = 8 Hz, 1H), 4.24–4.19 (m, 1H), 3.22–3.17 (m, 1H), 3.02–2.98 (m, 1H), 1.39 (s, 9H); HRMS (ESI-MS) m/z calcd for $C_8H_{16}N_2O_4$ $[M + H]^+$ 205.1188, found 205.1198.

3-Azido-2-(Boc-amino)propanoic Acid (3a). To a stirred solution of compound **2a** (1.2 g, 5.9 mmol) in MeOH/H_2O (1:1) were added Et_3N (2.5 mL, 17.9 mmol) and $CuSO_4{\cdot}5H_2O$ (145 mg, 0.58 mmol) in H_2O (0.5 mL). To this solution was added triflyl azide in DCM through a dropping funnel, and the reaction mixture was stirred for 10 h. The solvent was evaporated under vacuum, and saturated aq $NaHCO_3$ was added to the residue and extracted with EtOAc (2 × 20 mL). The aqueous layer was acidified to pH 2–3 by slow addition of saturated aq $KHSO_4$ solution and extracted with EtOAc (3 × 30 mL). The combined EtOAc extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated to give compound **3a** as yellow sticky liquid and used for next step without further purification: R_f = 0.2 EtOAc; IR (neat) 3187, 2983, 2937, 2109, 1691, 1513 cm^{-1}.

6-Azido-2-(Boc-amino)hexanoic Acid (3b). By following a similar procedure as above starting from *N*-Boc-L-lysine (**2b**) (246 mg, 1.0 mmol) in CH_3OH/H_2O (1:1), and employing Et_3N (0.3 g, 0.42 mL, 3 mmol) and aq $CuSO_4{\cdot}5H_2O$ (12.5 mg, 0.05 mmol) (0.5 mL), triflyl azide in DCM, followed by usual workup gave compound **3b**, which was used in the next step without further purification: R_f = 0.22 EtOAc; IR (neat) 3207, 2982, 2106, 1691, 1613 cm^{-1}.

Methyl 3-Azido-2-(Boc-amino)propanoate (4a). To a stirred solution of compound **3a** (1 g, 4.34 mmol) in acetone (15 mL) was added K_2CO_3 (1.8 g, 13 mmol) followed by dimethyl sulfate (0.65 g; 0.5 mL, 5.2 mmol). The reaction mixture was heated to 55 °C for 4 h under reflux condenser. Acetone was evaporated completely, and water (30 mL) was added to the concentrate, which was then extracted with EtOAc (3 × 40 mL). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified on silica gel (60–120 mesh) using petroleum ether and EtOAc to give compound **4a** as colorless liquid (0.7 g, 66% yield): R_f = 0.55 petroleum ether/EtOAc (75:25); $[\alpha]^{25}_D$ +5.8 (c 1, Methanol); IR (neat) 3361, 2925, 2855, 2105, 1711, 1506 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.38 (app d, J = 6 Hz, 1H), 4.50–4.43 (m, 1H), 3.79 (s, 3H), 3.72 (app d, J = 2 Hz, 2H), 1.45 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.2, 155.0, 80.4, 53.4, 52.7, 52.5, 28.1; HRMS (ESI-MS) m/z calcd for $C_9H_{16}N_4O_4$ $[M + Na]^+$ 267.1069, found 267.1075.

Methyl 6-Azido-2-(Boc-amino)hexanoate (4b). Using the same procedure as above with compound **3b** (4.4 g, 16.2 mmol) in acetone (80 mL), K_2CO_3 (5.6 g, 40.4 mmol), and dimethyl sulfate (2.44 g; 1.88 mL, 19.4 mmol), and workup followed by column purification over silica gel (60–120 mesh) and elution with petroleum ether and EtOAc gave compound **4b** as a yellowish liquid (3.8 g, 82% yield): R_f = 0.8 petroleum ether/EtOAc (60:40); $[\alpha]^{25}_D$ −16.0 (c 1, CH_3OH); IR (neat) 2925, 2856, 2095, 1707, 1512 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.06 (d, J = 8 Hz, 1H), 4.33–4.23 (m, 1H), 3.72 (s, 3H), 3.25 (t, J = 7 Hz, 2H), 1.76–1.53 (m, 6H), 1.42 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.1, 155.3, 80.0, 53.1, 52.3, 51.1, 32.2, 28.3, 28.2, 22.5; MS (MALDI-TOF) m/z calcd for $C_{12}H_{22}N_4O_4$ $[M + K]^+$ 325.1278, found 325.1252.

2-[(Azidomethylene)-*N*-Boc-amino]ethanolamine (5a). To a stirred solution of compound **4a** (3.8 g, 15.6 mmol) in absolute EtOH (50 mL) was added $NaBH_4$ (1.78 g, 46.8 mmol), and the reaction mixture was stirred for 6 h under nitrogen atmosphere at room temperature. EtOH was evaporated completely, and water (100 mL) was added to the concentrate which was extracted with EtOAc (3 × 75 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was

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purified on silica gel (60−120 mesh) using petroleum ether and EtOAc to give compound **5a** as a colorless liquid (2.9 g, 86% yield): R_f = 0.35 petroleum ether/EtOAc (70:30); $[\alpha]^{25}_D$ +13.6 (*c* 1, CH_3OH); IR (neat) 3331, 2977, 2933, 2097, 1687, 1514 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.06 (d, *J* = 6 Hz, 1H), 3.81−3.70 (m, 3H), 3.52−3.43 (m, 2H), 2.66 (br, 1H), 1.44 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.7, 80.2, 62.2, 51.5, 28.3; HRMS (ESI-MS) *m*/*z* calcd for $C_8H_{16}N_4O_3$ $[M + Na]^+$ 239.1119, found 239.1127.

***tert*-Butyl (6-Azido-1-hydroxyhexan-2-yl)carbamate (5b).** Using a similar procedure as above starting from compound **4b** (1.6 g, 5.6 mmol) in absolute EtOH (30 mL), $NaBH_4$ (0.64 g, 16.8 mmol), workup, and column chromatography purification gave compound **5b** as a colorless liquid (1.2 g, 85% yield): R_f = 0.5 petroleum ether/EtOAc (50:50); $[\alpha]^{25}_D$ −12.9 (*c* 1, methanol); IR (neat) 3341, 2937, 2868, 2093, 1684, 1511 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.84 (d, *J* = 4 Hz, 1H), 3.56−3.47 (m, 2H), 3.49−3.47 (m, 1H), 3.24 (t, *J* = 6 Hz, 2H), 3.12 (br, 1H), 1.72−1.52 (m, 6H), 1.40 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 156.3, 79.5, 65.2, 52.3, 51.2, 31.0, 28.6, 28.3, 23.1; HRMS (ESI-MS) *m*/*z* calcd for $C_{11}H_{22}N_4O_3$ $[M + Na]^+$ 281.1589, found 281.1602.

3-Azido-2-(Boc-amino)propyl Methanesulfonate (6a). To an ice-cold solution of compound **5a** (2.1 g, 9.72 mmol) and Et_3N (2.44 g, 3.38 mL, 24.3 mmol) in dry DCM (40 mL) was added mesyl chloride (1.48 g, 1 mL, 12.63 mmol), and the reaction mixture was stirred for 30 min at 0 °C under nitrogen atmosphere. To the reaction mixture was added DCM (20 mL), which was washed with water (20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to give compound **6a** (2.29 g, 80% crude yield): R_f = 0.69 petroleum ether/EtOAc (50:50) which was used for next step without further purification.

6-Azido-2-(Boc-amino)hexyl Methanesulfonate (6b). Compound **5b** (3.25 g, 12.6 mmol), dry DCM (60 mL), Et_3N (3.19 g, 4.4 mL, 31.5 mmol), and mesyl chloride (1.87 g, 1.27 mL, 16.38 mmol) under similar reaction conditions, workup, and purification gave compound **6b** (3.5 g, 83% crude yield): R_f = 0.62 petroleum ether/EtOAc (50:50) which was used for next step without further purification.

***tert*-Butyl (1-Azido-3-(1,3-dioxoisoindolin-2-yl)propan-2-yl)-carbamate (7a).** To a stirred solution of compound **6a** (2.25 g, 7.65 mmol) in dry DMF (30 mL) were added K_2CO_3 (2.11 g, 15.3 mmol) and potassium phthalimide (5.66 g, 30.6 mmol), and the reaction mixture was heated to 80 °C for 12 h. To this was added water (60 mL) and the solution extracted with EtOAc (3 × 50 mL). The organic layer was washed with water (50 mL) and brine (30 mL), and the dried organic layer on concentration gave a residue which was purified on silica gel (100−200 mesh) using petroleum ether and EtOAc to give compound **7a** as a white solid (1.8 g, 68% yield): mp = 114−117 °C; R_f = 0.72 petroleum ether/EtOAc (50:50); $[\alpha]^{25}_D$ +50.0 (*c* 0.5, methanol); IR (neat) 3370, 2977, 2936, 2102, 1773, 1711, 1512 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.88−7.86 (m, 2H), 7.74−7.72 (m, 2H), 5.04 (app d, *J* = 8 Hz, 1H), 4.12 (br, 1H), 3.89−3.73 (m, 2H), 3.58−3.55 (m, 2H), 1.30 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 155.3, 134.1, 131.9, 123.5, 79.9, 52.7, 49.4, 39.7, 28.1; MS (MALDI-TOF) *m*/*z* calcd for $C_{16}H_{19}N_5O_4$ $[M + K]^+$ 384.1074, found 384.1098.

***tert*-Butyl (6-Azido-1-(1,3-dioxoisoindolin-2-yl)hexan-2-yl)-carbamate (7b).** Compound **6b** (3.5 g, 10.4 mmol) in dry DMF (50 mL), K_2CO_3 (2.87 g, 20.8 mmol) and potassium phthalimide (7.7 g, 41.6 mmol) under similar conditions as above, workup, and column purification gave compound **7b** as a white solid (3.2 g, 80% yield): mp = 94−96 °C; R_f = 0.79 petroleum ether/EtOAc (60:40); $[\alpha]^{25}_D$ +20.6 (*c* 0.5, methanol); IR (neat) 3371, 2936, 2867, 2093, 1772, 1704, 1614, 1511 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.84−7.82 (m, 2H), 7.70−7.68 (m, 2H), 4.62 (app d, *J* = 12 Hz, 1H), 3.97−3.93 (m, 1H), 3.70−3.60 (m, 2H), 3.27 (t, *J* = 8 Hz, 2H), 1.64−1.43 (m, 6H), 1.21 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 155.7, 133.9, 132.0, 123.2, 79.2, 51.2, 49.6, 42.2, 32.3, 28.5, 28.0, 23.0; MS (MALDI-TOF) *m*/*z* calcd for $C_{19}H_{25}N_5O_4$ $[M + K]^+$ 426.1544, found 426.1518.

Ethyl 2-((3-Azido-2-(Boc-amino)propyl)amino)acetate (8a). To a stirred solution of compound **7a** (1.1 g, 3.18 mmol) in absolute EtOH (20 mL) was added hydrazine hydrate (3.09 g, 3 mL, 63.6 mmol), and the reaction mixture was stirred for 4 h at room temperature. After completion of reaction, solvent was evaporated completely, water (30 mL) was added, and the solution extracted with EtOAc (3 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give free amine (0.68 g), which was taken in CH_3CN (20 mL). To this, Et_3N (0.8 g, 1.1 mL, 7.9 mmol) was added, the mixture was stirred at 0 °C for 10 min, ethyl bromoacetate (0.46 g, 0.31 mL, 2.86 mmol) was added dropwise, and stirring was continued for 12 h at room temperature. Aqueous workup and extraction with EtOAc (3 × 40 mL) and concentration gave a residue which was purified on silica gel (100−200 mesh), eluting with petroleum ether, and EtOAc to give compound **8a** as a yellow sticky oil (0.7 g, 73% yield): R_f = 0.79 EtOAc; $[\alpha]^{25}_D$+ 8.0 (*c* 1, methanol); IR (neat) 3337, 2975, 2927, 2857, 2099, 1737, 1702, 1514 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.09 (br, 1H), 4.18 (q, *J* = 8 Hz, 2H), 3.76 (br, 1H), 3.49−3.33 (m, 4H), 2.81−2.67 (m, 2H), 2.24 (br, 1H), 1.44 (s, 9H), 1.27 (t, *J* = 8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 155.4, 79.8, 60.9, 52.5, 50.7, 50.0, 29.6, 28.4, 14.1; HRMS (ESI-MS) *m*/*z* calcd for $C_{12}H_{23}N_5O_4$ $[M + Na]^+$ 324.1647, found 324.1653.

Ethyl 2-((6-Azido-2-(Boc-amino)hexyl)amino)acetate (8b). Using a similar procedure with compound **7b** (0.8 g, 2.06 mmol) in absolute EtOH (20 mL), hydrazine hydrate (2.06 g, 2 mL, 41.2 mmol), Et_3N (0.5 g, 0.7 mL, 5 mmol), and ethyl bromoacetate (0.3 g, 0.2 mL, 1.8 mmol), aqueous workup and column chromatography purification gave compound **8b** as an oil (0.55 g, 78% yield): R_f = 0.5 EtOAc; $[\alpha]^{25}_D$ +10.6 (*c* 1, methanol); IR (neat) 2926, 2859, 2094, 1741, 1672, 1517 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.74−4.71 (app d, *J* = 6 Hz, 1H), 4.24−4.13 (q, *J* = 8 Hz, 2H), 3.65−3.63 (m, 1H), 3.42−3.38 (d, *J* = 8 Hz, 2H), 3.30−3.24 (t, *J* = 6 Hz, 2H), 2.74−2.60 (m, 2H), 1.93 (br, 1H), 1.64−1.52 (m, 6H), 1.44 (s, 9H), 1.31−1.24 (t, *J* = 7 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.4, 155.8, 79.2, 60.8, 52.9, 51.3, 50.9, 32.8, 28.7, 28.4, 23.1, 14.2; MS (MALDI-TOF) *m*/*z* calcd for $C_{15}H_{29}N_5O_4$ $[M + K]^+$ 382.1857, found 382.1859.

Ethyl 2-(*N*-(3-Azido-2-(Boc-amino)propyl)-2-chloro-acetamido)acetate (9a). To an ice-cold solution of compound **8a** (1.1 g, 3.65 mmol) in dry DCM (20 mL) and Et_3N (1.1 g; 1.5 mL, 10.9 mmol) was added chloroacetyl chloride (0.45 g; 0.32 mL, 4 mmol), and the reaction mixture was stirred for 8 h. The reaction mixture was diluted with DCM (20 mL) and washed with water (40 mL) and brine (40 mL). The dried organic layer was concentrated to obtain a residue which was purified on silica gel (100−200 mesh) eluting with petroleum ether and EtOAc to give compound **9a** as pale yellow sticky oil (1 g, 73% yield): R_f = 0.65 petroleum ether/EtOAc (50:50); $[\alpha]^{25}_D$ +5.2 (*c* 1, Methanol); IR (neat) 3340, 2977, 2928, 2860, 2102, 1742, 1704, 1659, 1515 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.14−5.12 (maj) 5.06−5.05 (min) (app d, *J* = 8 Hz, 4 Hz, 1H), 4.27−4.18 (m, 4H), 4.02 (s, 2H), 3.89−3.65 (m, 2H), 3.59−3.41 (m, 3H), 1.44 (min) 1.43 (maj) (s, 9H), 1.33−1.30 (maj) 1.29−1.27 (min) (t, *J* = 6 Hz, 4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 168.4, 167.6, 80.0, 62.2, 52.3, 51.3, 50.4, 49.1, 48.7, 40.9, 28.3, 14.1; HRMS (ESI-MS) *m*/*z* calcd for $C_{14}H_{24}ClN_5O_5$ $[M + Na]^+$ 400.1363, found 400.1371.

Ethyl 2-(*N*-(6-Azido-2-(Boc-amino)hexyl)-2-chloro-acetamido)acetate (9b). Compound **8b** (1 g, 2.9 mmol) in dry DCM (20 mL) and Et_3N (0.88 g; 1.21 mL, 8.7 mmol) and chloroacetyl chloride (0.326 g; 0.23 mL, 2.9 mmol) under a procedure similar to that above gave compound **9b** as a yellowish sticky oil (0.9 g, 74% yield): R_f = 0.67 petroleum ether/EtOAc (50:50); $[\alpha]^{25}_D$ −1.2 (*c* 1, methanol); IR (neat) 3333, 2977, 2938, 2868, 2095, 1742, 1694, 1655, 1515 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.76−4.72 (min) and 4.62−4.5 (maj) (d, *J* = 8 Hz, 6 Hz, 1H), 4.29−4.08 (m, 4H), 4.01 (s, 2H), 3.91−3.60 (m, 2H), 3.53−3.13 (m, 3H), 1.74−1.49 (m, 6H), 1.43 (maj) and 1.42 (min) (s, 9H), 1.34−1.25 (comp, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.7, 167.3, 155.5, 79.1, 61.8, 53.1, 51.0, 49.5, 48.7, 40.9, 32.0, 28.4, 28.1, 23.2, 13.9; MS (MALDI-TOF) *m*/*z* calcd for $C_{17}H_{30}ClN_5O_5$ $[M + K]^+$ 458.1573, found 458.1588.

Ethyl 2-(*N*-(3-Azido-2-(Boc-amino)propyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)acetate (10a). A solution of compound **9a** (1 g, 2.65 mmol) in dry DMF (15 mL)

dx.doi.org/10.1021/jo500834u | *J. Org. Chem.* 2014, 79, 6708−6714

containing K_2CO_3 (0.44 g, 3.18 mmol) and thymine (0.4 g, 3.18 mmol) was stirred at room temperature for 12 h. To the reaction mixture was added water (40 mL), the solution was extracted with EtOAc (3 × 50 mL), and the organic layer was washed with water (50 mL) and brine (30 mL). The dried organic extractupon concentration and coulmn chromatography purification gave compound **10a** as white solid (1 g, 80% yield): R_f = 0.6 EtOAc; mp = 102−105 °C; $[\alpha]^{25}_D$ +9.4 (*c* 1, methanol); IR (neat) 3331, 2977, 2101, 1737, 1686, 1518, 1467, 1417 cm^{-1};^{1}H NMR (400 MHz, CD_3OD) δ 7.30−7.28 (comp, 1H), 4.79−4.50 (m, 2H), 4.4−4.1 (m, 4H), 4.1−3.8 (m, 2H), 3.75−3,55 (m, 2H), 3.45−3.35 (m, 1H), 3.31−3.24 (m, 1H), 1.88 (s, 3H), 1.45 (s, 9H), 1.34−1.31 (min) 1.29−1.27 (maj) (t, *J* = 6 Hz, 4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 171.0, 170.3, 167.4, 158.2, 153.3, 144.0, 117.2, 111.3, 81.1, 62.9, 53.2, 51.4, 51.0, 50.4, 29.1, 14.8, 12.6; HRMS (ESI-MS) *m*/*z* calcd for $C_{19}H_{29}N_7O_7$ $[M + Na]^+$ 490.2026, found 490.2029.

Ethyl 2-(*N*-(6-Azido-2-(Boc-amino)hexyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)acetate (10b). Compound **9b** (1 g, 2.38 mmol) in dry DMF (15 mL), K_2CO_3 (0.4 g, 2.9 mmol), and thymine (0.36 g, 2.9 mmol) under a similar procedure gave compound **10b** as a white sticky solid (1 g, 85% yield): R_f = 0.63 EtOAc; $[\alpha]^{25}_D$ +1.7 (*c* 1, methanol); IR (neat) 2936, 2354, 2317, 2097, 1792, 1769, 1740, 1705, 1677, 1658 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.52 (maj) 9.32 (min) (br, 1H), 7.09, 7.03 (s, 1H); 5.33−5.29 (d, *J* = 8 Hz, 1H), 5.02−4.71 (comp, 1H), 4.54−4.12 (m, 5H), 3.97−3.77 (comp, 1H), 3.56−3.22 (m, 5H), 1.91 (min) 1.90 (maj) (s, 3H), 1.62−1.48 (m, 6H), 1.43 (maj) 1.39 (min) (s, 9H), 1.31−1.23 (comp, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.8,169.1, 167.4, 164.5, 155.9, 151.2, 141.3, 141.0, 110.6, 79.5, 61.4, 52.6, 51.1, 49.3, 47.6, 31.7, 28.5, 28.3, 23.3, 14.1, 12.3; HRMS (ESI-MS) *m*/*z* calcd for $C_{22}H_{35}N_7O_7$ $[M + Na]^+$ 532.2496, found 532.2491.

2-(*N*-(3-Azido-2-(Boc-amino)propyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)acetic Acid (11a). To a stirred solution of compound **10a** (1 g, 2.14 mmol) in THF was added a solution of 10% LiOH in water, and the reaction mixture was stirred for 2 h. Solvent removal, aqueous workup with acidified saturated $KHSO_4$ at pH 3−4, and extraction with diethyl ether afforded acid monomer **11a** as a white solid (0.85 g, 90% yield): mp = 150−152 °C; R_f = 0.5 EtOAc/MeOH (50:50); $[\alpha]^{25}_D$ +11.2 (*c* 0.5, methanol); IR (neat) 3338, 2975, 2822, 2371, 2317, 2099, 1733, 1682, 1670, 1525 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (maj) and 11.28 (min) (s, 1H), 7.27−7.26 (comp, 1H), 7.15−7.13 (maj) and 7.01−6.98 (min) (d, *J* = 8 Hz, 12 Hz, 1H), 4.73−4.41 (m, 2H), 4.27−4.21 (m, 1H), 4.05−3.86 (m, 2H), 3.60−3.40 (m, 3H), 3.23−3.0 (m, 1H), 1.75 (s, 3H), 1.39 (min) and 1.38 (maj) (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.3, 167.4, 164.4, 155.2, 151.0, 141.9, 108.2, 78.4, 51.2, 49.4, 49.2, 48.5, 47.7, 39.5, 28.2, 11.9; HRMS (ESI-MS) *m*/*z* calcd for $C_{17}H_{25}N_7O_7$ $[M + Na]^+$ 462.1713, found 462.1710.

2-(*N*-(6-azido-2-(*t*-Boc-amino)hexyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)acetic Acid (11b). A similar reaction with **10b** (0.5 g, 0.98 mmol) in THF and 10% aq LiOH gave the acid monomer **11b** as white solid (0.4 g, 84% yield): mp = 167−169 °C; R_f = 0.48 EtOAc/MeOH (50:50); $[\alpha]^{25}_D$ +1.4 (*c* 1, methanol); IR (neat) 3361, 2937, 2318, 2097, 1677, 1530 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (maj) and 11.27 (min) (br s, 1H), 7.22 (s, 1H), 6.82 (maj) and 6.67 (min) (d, *J* = 8 Hz, 1H), 4.75−4.45 (m, 2H), 4.21−3.86 (m, 3H), 3.69−3.52 (m, 2H), 3.2−2.96 (m, 2H), 1.75 (s, 3H), 1.57−1.42 (m, 6H) 1.38 (min) and 1.36 (maj) (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.4, 167.3, 164.4, 155.7, 151.0, 141.9, 115.7, 108.1, 78.0, 51.5, 50.9, 50.6, 49.2, 48.5, 47.7, 30.8, 28.2, 28.0, 22.9, 12.0; HRMS (ESI-MS) *m*/*z* calcd for $C_{20}H_{31}N_7O_7$ $[M + Na]^+$ 504.2183, found 504.2179.

Solid-Phase Synthesis Protocol. The modified and unmodified PNA monomers were incorporated into 10-mer PNA sequence by solid phase synthesis on L-lysine derivatized MBHA resin having 0.35 mmol/g loading value. The deprotection of *N*-*t*-Boc group from the resin bound lysine with 50% TFA in DCM (3 × 15 min) was followed by washing with DCM and DMF (3 × 10 mL) to give a TFA salt of amine which was neutralized using 10% DIPEA in DCM (3 × 10 min) to liberate free amine. After washing with DCM and DMF (3 × 10 mL), the free amine was coupled with carboxylic acid of incoming monomer (A/T/G/C; 3 equiv) in DMF (500 μL) using HOBt (3 equiv), HBTU (3 equiv), and DIPEA (3 equiv). The reagents were then removed by filtration, and the resin was washed with DMF.

Synthesis of Fluorescent PNA Oligomers on Solid Support by Click Reaction. The resin-bound azido PNA oligomers (10 mg, 0.35 mmol/g) in DMF:pyridine (1:2) were reacted with the propyne 5(6)-carboxyfluorescein (8.66 mg, 6 equiv) in the presence of CuI (12 mg, 18 equiv), ascorbic acid (3.1 mg, 5 equiv), and DIPEA (15 μL, 24 equiv) for 24 h at room temperature. The reagents were removed by filtration and the resin was washed with DMF and subjected to cleavage reaction.

Purification of PNA Oligomers by Reversed-Phase HPLC. PNA purification was carried out on Dionex ICS 3000 HPLC system. For the purification of peptides, semipreparative BEH130 C18 (10 × 250 mm) column was used. Purification of PNA oligomers was performed with gradient elution method: A to 100% B in 20 min; A = 0.1% TFA in CH_3CN/H_2O (5:95); B = 0.1% TFA in CH_3CN/H_2O (1:1) with flow rate of 3 mL/min. Control *aeg*, *azm*, and *azb* modified PNA oligomers were monitored at 254 nm wavelength while all fluorescent PNA oligomers were monitored at both 254 and 490 nm wavelengths.

UV−T_m Measurements. UV−melting experiments were carried out on Varian Cary 300 UV spectrophotometer equipped with a Peltier attachment. The samples for T_m measurement were prepared by mixing the calculated amounts of respective oligonucleotides in stoichiometric ratio (1:1, for duplex) in sodium phosphate buffer (10 mM) containing EDTA (0.1 mM) and NaCl (10 mM); pH 7.2 to achieve a final strand concentration of 2 μM for each strand. The samples were annealed by heating at 90 °C for 3 min followed by slow cooling to room temperature for at least 6−8 h and then refrigerated for at least 4 to 5 h. The samples (500 μL) were transferred to quartz cell and equilibrated at the starting temperature for 2 min. The OD at 260 nm was recorded in steps from 20 to 85 °C with temperature increment of 1 °C/min. Each melting experiment was repeated at least twice. The normalized absorbance at 260 nm was plotted as a function of the temperature. The T_m was determined from the first derivative of normalized absorbance with respect to temperature and is accurate to ±0.5 °C. The data were processed using Microcal Origin 8.5. [The concentration of all oligonucleotides were calculated on the basis of absorbance from the molar extinction coefficients of the corresponding nucleobases, i.e., T = 8.8 $cm^2/\mu mol$; C = 7.3 $cm^2/\mu mol$; G = 11.7 $cm^2/\mu mol$, and A = 15.4 $cm^2/\mu mol$.]

Circular Dichroism. CD spectra were recorded on JASCO J-815 spectropolarimeter connected with a Peltier. The calculated amounts for PNA oligomers, and the complementary DNA**1** were mixed together in stoichiometric ratio (1:1, for duplex) in sodium phosphate buffer (10 mM) containing EDTA (0.1 mM) and NaCl (10 mM), pH 7.2, to achieve a final strand concentration of 5 μM for each strand. The samples were annealed by heating at 90 °C for 3 min followed by slow cooling to room temperature for at least 6−8 h. The cooled samples were kept at 4 °C for 4 h. The CD spectra of PNA:DNA duplexes and single-stranded PNAs were recorded at 10 °C with an accumulation of five scans from 300 to 190 nm using 2 mm cell, a resolution of 0.1 nm, bandwidth of 1 nm, sensitivity of 2 m deg, response of 2 s, and a scan speed of 50 nm/min.

Ethidium Bromide Displacement Assay from *ds*DNA. Calculated amounts of DNA strands were annealed to get a DNA duplex (2 μM, 400 μL) in the above said buffer system. The duplex in buffer was titrated with EtBr (250 μM) with incremental addition of 1 μL of solution and the fluorescence intensity at 610 nm was recorded till it was saturated. The resulting EtBr−*ds*DNA complex was titrated individually against various PNA oligomers in the aliquots of 0.4 μL and change in fluorescence intensity at 610 nm was recorded. The percent change observed in fluorescence intensity (I/I_0) upon displacement of EtBr was plotted against the concentration of PNA oligomers using Microcal Origin 8.5.

Cellular Uptake Experiment Using Confocal Microscopy. NIH 3T3 cells were plated in 8-well chambered coverglass in 200 μL of Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal

dx.doi.org/10.1021/jo500834u | J. Org. Chem. 2014, 79, 6708−6714

bovine serum (FBS) at the concentration of 8000 cells per well. The cells were grown by maintaining at 37 °C in a humidified atmosphere containing 5% CO_2 for 16 h. The required amounts of fluorescent PNA stock solutions were added to the corresponding wells to achieve the desired final concentration of 1 μM. The cells incubated with fluorescent PNA oligomers were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. After the incubation period was over, the medium was aspirated and cells were rinsed or washed three times with 1 medium-volume equivalent of ice-cold PBS. The cells were then replenished with 400 μL of OPTIMEM medium containing Hoechst 33342 at a dilution of 1/1000 of the supplied stock solution and left for 30 min at 37 °C. After incubation for 30 min, the medium containing nuclear stain Hoechst 33342 was removed and fresh OPTIMEM medium was added to the cells. The cells were then immediately visualized using 40× objective of Zeiss LSM 710 laser scanning confocal microscope. The well of cells without incubation of any PNA oligomer was also visualized as control experiment.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR and mass spectra of compounds **4a**−**11a** and **4b**−**11b**; ^{1}H NMR spectrum of compound **2a**; IR spectra of compounds **3a** and **3b**; HPLC and MALDI-TOF of PNA **1**−**16**; UV−melting profiles of various modified PNA:DNA duplexes; CD and fluorescence emission spectra of various PNA:DNA duplexes; cell uptake studies on control PNAs. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

D.R.J. acknowledges an IISER Pune for award of a research fellowship. K.N.G. is a recipient of a JC Bose Fellowship, Department of Science and Technology, New Delhi. We thank Dr. Mayurika Lahiri, IISER Pune, for assistance and helpful discussions regarding cell permeability studies.

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