1,4-linked 1,2,3-Triazole des-peptidic analogues of PNA (TzNA)

Synthesis of TzNA oligomers by “click” reaction on solid phase and stabilization of derived triplexes with DNA

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1,2,3-triazole analogues of PNA (TzNA) in which the amide link in backbone is replaced by triazole ring is synthesized on solid phase by ‘click’ chemistry and such triazolothymine PNA chimeric oligomers are shown to significantly stabilize the derived PNA₂:DNA triplexes. With increasing number of triazole units in the backbone, single stranded PNA oligomers exhibit enhanced self-ordering.

Introduction

Peptide Nucleic acid (PNA) is one of the most successful examples of nucleic acid analogues that exhibit strong hybridization with cDNA/RNA.¹ PNAs are neutral, unnatural analogues of nucleic acids that have high chemical and enzymatic stability and greater sequence discriminating ability.² There have been several modifications of PNA backbone aimed at enhancing the selectivity of hybridization (parallel/antiparallel, DNA/RNA hybridization preferences) and improving solubility, but most of them have retained the polyamide backbone.³⁻⁵ There has been a long-standing interest in non-peptidic links that adopt well-defined conformations as alternative to peptide bond in design of peptidomimetic drugs.⁶ Recently, replacement of amide bonds by cyclic rings incorporating 1,2,3-triazole or 1,2,3,4-tetrazole moieties have provided interesting examples of locking the peptide bond in cis-form as compared to the generally preferred trans-form.⁷ Moreover, such azole structures can be easily accessed synthetically through copper catalyzed version of Huisgen (3 + 2) cycloaddition reaction (“click” reaction).⁸⁻¹⁰ Triazoles have been shown to be good isosteres of peptide bond and hence used to design backbone of cyclotetrapeptide mimics,¹¹ peptoid oligomers¹² and even to replace the phosphate linkage in DNA.^{13,14} Such triazole based backbone oligomers have been shown to display protein-like secondary structural motifs, inducing turns and β -strands.^{15,16} In oligonucleotides, they can be used to crosslink the two termini to give rise to dumbbell structures¹⁷ and linear triazolamers form pharmacophores recognizing tetraplex structures.¹⁸ Triazole rings generated by click chemistry have also found structural applications to link side chains to main backbone as

in oligopeptoids¹⁹ and side-chain ferrocene linked PNA.²⁰ These examples illustrate the versatility of triazole moiety in replacing the amide links, with fair retention of conformational properties. The unique attribute of triazole ring is a large dipole moment (~ 5 D) causing favorable dipole-dipole interactions and torsional effects, leading to defined conformations.²¹

In the above context of literature, we have explored the replacement of the amide functions in polyamide backbone of PNA with the triazole rings to examine its effect on PNA hybridization properties. The triazole link in **C** was generated from click reaction of the polymer supported azido component **A** with the acetylenic component **B** in solution. It is demonstrated here that 1,2,3-triazole analogues of PNA (TzNA) wherein the amide link in backbone is replaced by triazole (**C**) can be synthesized on solid phase by ‘click’ chemistry and the corresponding mixed chimeric (*aeg*-triazole) T-PNA significantly stabilize the derived PNA₂:DNA triplexes. The completely modified homotriazolylthymine oligomer (TzNA-T) is strongly pre-organized and fails to form DNA hybrids.

Results and Discussion

Synthesis of N1-(t-Boc)-N2-(prop-2-ynyl)-N2-(thyminyl)-N1-acetyl-1,2-diaminoethane (5). The synthesis of the N-alkyne functionalized compound **5** (Scheme 1) was accomplished starting from the commercially available 1,2-diaminoethane **1**. The mono N-Boc protection of ethylenediamine **1** was done selectively by using one equivalent of tert-butyl pyrocarbonate following reported procedure²² to obtain the product **2**. This was mono N-alkylated at free amino terminal with propargyl bromide to

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Scheme 1. Synthesis of TzNA by click reaction and synthesis of monomer 5.

yield N1-(*tert*-Boc)-N2-(prop-2-ynyl) diaminoethane **3**. This was acylated at N2 with chloroacetyl chloride to afford N1-(*tert*-Boc)-N2-(2-chloroacetyl)-N2-(prop-2-ynyl) diaminoethane **4**. Thymine was reacted with compound **4** in presence of K_2CO_3 in DMF to yield the desired product **5**. The amino ethyl glycol (*aeg*) thymine monomer for initial coupling was synthesized by the reported procedure.²² All new compounds were characterized by 1H , ^{13}C NMR, IR and mass spectral data.

Synthesis of triazole based PNA oligomers on solid support. The synthesis of PNA analogues in which the amide bond is replaced by 1,2,3-triazole unit was achieved on solid phase using the Huisgen 1,3-dipolar cycloaddition reaction.⁸⁻¹⁰ The standard *aeg*-thymine PNA monomer (Scheme 2) was first linked to solid phase (4-methylbenzhydryl)amine (MBHA) resin derivatized

with L-lysine **6** as per literature²³ using HBTU/HOBt/DIEA in DMF as the coupling reagent to obtain **7**. In the second step, the Boc group was removed by treatment with 50% TFA in DCM, followed by the conversion of the free amine to the azide **8** by azido transfer protocol²⁴ using triflyl azide and copper sulphate in DCM. The completion of the reaction was monitored by Kaiser test, which indicated the absence of amino function on the resin.

The alkyne-functionalized component **5** was reacted with the azide **8** bound to the solid phase using copper (I) iodide in DIEA to introduce the 1,2,3-triazole moiety by click chemistry in the desired site on the PNA backbone. The deprotection of Boc group and condensation (with *aeg*-T monomer or **5**) was repeated in cycles to obtain the target mixed backbone PNA oligomers having 1,2,3-triazole unit in backbone at positions indicated by

Scheme 2. Solid phase synthesis of TzNA oligomer: Complete synthetic cycle.

an asterisk (*) in Table 1. After the completion of synthesis, the oligomers were cleaved from MBHA resin using TFA-TFMSA, followed by RP-HPLC purification on C18 column. All synthesized oligomers were characterized by HPLC retention times (column 4) and the molecular weight data (columns 6 and 7) obtained from mass spectrometry (MALDI-TOF) given in Table 1. It is noticed that for the same length of oligomers, the HPLC retention times increase with increase in the number of modifications, suggesting incremental increase in overall hydrophobicity resulting from replacing amide bond with triazole units.

Stoichiometry of -TzNA:DNA hybrids. Doubly modified triazole *aeg*-TzNA 8 was mixed with complementary DNA 1 having A_8 sandwiched between CG sequences at termini (to prevent slippage), in relatively variable stoichiometric ratios. Upon increasing additions of DNA 21, a broad positive signal emerged at 265 nm in CD, with increased intensity of the negative signal at 250 nm (Fig. 1A). The positive band at 220 nm and negative band at 205 nm also increased progressively, with increasing addition of DNA 21. The formation of complex is indicated by isobestic points. The binding stoichiometry for *aeg*-TzNA

Table 1. Sequences of synthesized *ae*g-TzNA oligomers*

Entry	PNA	Sequence	R _t (min)	Mol. formula	Calc. MW	Obs. MW
1	11	H-TTT*T- Lys NH ₂	7.4	C ₅₁ H ₇₁ N ₂₁ O ₁₆	1234.1	1234.8
2	12	H-T*TT*T- Lys NH ₂	7.8	C ₅₂ H ₇₁ N ₂₃ O ₁₅	1258.2	1258.8
3	13	H-TT*TT- Lys NH ₂	7.4	C ₅₁ H ₇₁ N ₂₁ O ₁₆	1234.2	1235.8
4	14	H-T*T*T*T- Lys NH ₂	8.0	C ₅₃ H ₇₁ N ₂₅ O ₁₄	1282.3	1282.8
5	15	H-TTTTT- Lys NH ₂	7.9	C ₇₂ H ₉₉ N ₂₇ O ₂₅	1742.7	1742.3
6	16	H-TTTTT*T- Lys NH ₂	8.1	C ₇₃ H ₉₉ N ₂₉ O ₂₄	1766.7	1767.1
7	17	H-T*TTTT- Lys NH ₂	8.1	C ₇₃ H ₉₉ N ₂₉ O ₂₄	1766.7	1767.2
8	18	H-TT*TT*TT- Lys NH ₂	8.6	C ₇₄ H ₉₉ N ₃₁ O ₂₃	1790.0	1791.1
9	19	H-T*TT*TT*T- Lys NH ₂	8.8	C ₇₅ H ₉₉ N ₃₃ O ₂₂	1814.8	1816.2
10	20	H-T*T*T*T*T*T- Lys NH ₂	9.3	C ₇₇ H ₉₉ N ₃₇ O ₂₀	1862.8	1863.2

*For experimental details on HPLC and MALDI-TOF, please see Supplemental Material. DNA **21**: CGA AAA AAG C.

Figure 1. UV and CD Job's plot for *ae*g-TzNA 18 with different stoichiometric ratios of DNA21 [(a) 100:0 (b) 90:10 (c) 80:20 (d) 70:30 (e) 60:40 (f) 50:50 (g) 40:60 (h) 30:70 (i) 20:80 (j) 10:90 and (k) 0:100].

Figure 2. Derivative curves of temperature dependent UV absorbance experiment of (A) *aeg*-TzNA 15–18 with complementary DNA 21 (B) single stranded *aeg*-TzNA 19 and TzNA 20.

Table 2. UV- T_m of triazolyl *aeg*-TzNA:DNA 21 hybrids*

<i>aeg</i> -TzNA	Sequence	pH 7.4			pH 5.8	
		UV- T_m single strand	UV- T_m (°C)	$\Delta T_m^{\#}$ (°C)	UV- T_m (°C)	$\Delta T_m^{\$}$ (°C)
15	H-TTTTTT-LysNH ₂	-	20.5	-	-	-
16	H-TTTTTT*-LysNH ₂	-	29.8	+8.4	26.0	-3.8
17	H-T*TTTTT-LysNH ₂	-	31.9 (24.0) [†]	+11.4	ND	ND
18	H-TT*TT*TT-LysNH ₂	-	34.3	+13.8	45.4	+11.1
19	H-T*TT*TT*TT-LysNH ₂	42.9	-	-	48.6	+5.7
TzNA20	H-T*TT*TT*TT*TT-LysNH ₂	48.1	-	-	47.4	+0.4

Complementary DNA 21 is 5'-CGA₈GC-3'; [†]with respect to difference with control PNA 15; [§]with respect to difference in T_m at pH 7.4; ND indicates not detected; [†]Mismatch DNA 22 is 5'-CGA AAT AAA AGC-3'.

18:DNA 21 complex was established by Job's plot using CD ellipticity data at 256 nm (Fig. 1B) and UV absorbance data at 256 nm (Fig. 1C), both indicating 2:1 ratio as expected for *aeg*-TzNA₂:DNA triplex.

UV- T_m and CD studies of single stranded *aeg*-TzNA and TzNA:DNA hybrids. The single stranded *aeg*-TzNA oligomers were subjected to temperature dependent UV absorbance measurement. Single modified *aeg*-TzNA 16, *aeg*-TzNA 17 and doubly modified *aeg*-TzNA 18 oligomers do not show self-melting as indicated by only linear change in absorption with temperature (Sup. Material). However, the triple modified *aeg*-TzNA 19 and the fully modified TzNA 20 show sigmoidal melting curves, with melting temperatures of 42.9°C and 48.1°C respectively, suggesting self-organization of single strands.

The hybridization of different triazolyl PNAs with complementary DNA 21 was studied by temperature dependent UV absorbance measurements (Fig. 2 and additional data in Sup. Material). The thermal stabilities (T_m , Table 2) of triplexes of the *aeg*-TzNA hexamers with complementary DNA 21 at pH 7.4 and 5.8 were determined from the first derivative data. At pH 7.4, the *aeg*PNA- T_6 hexamer PNA 15 shows UV- T_m of 20.5°C with the complementary DNA 21. The modification of the peptide

bond in the *aeg*PNA backbone with 1,2,3-triazole moiety results in increase in the UV- T_m with increase in the number of modifications in the oligomers. *aeg*-TzNA 16 and *aeg*-TzNA 17 having single end-modification show increase in T_m by 8.4°C and 11.4°C respectively (Table 2, entry 2 and 3), with N-terminal modification more stable than the C-terminal modification. *aeg*-TzNA 18 carrying two triazole modifications at alternate sites, enhanced the T_m of corresponding triplex by 13.8°C (Table 2, entry 4) compared to that of unmodified triplex. The increase in T_m perhaps arises from a better inter strand base stacking in triplex, due to structural alterations in backbone induced by triazole links. This may result in a conformational pre-organization of PNA backbone favorable towards DNA hybridization. However, triple (*aeg*-TzNA 19) and complete (five) modifications in the TzNA 20 oligomer failed to show any binding with DNA (no discontinuity in Job's plot and no discernible changes in CD profiles), but resulted in enormous increase of T_m as seen by sigmoidal melting curves of corresponding single strands. The increase in T_m was by 23.1°C and 27.4°C (Table 2, entry 5 and 6) respectively compared to that of unmodified PNA 15. No change in T_m was seen in the hybridized DNA complexes compared to that of single strands. This clearly suggested that oligomers with higher

Figure 3. CD titration spectra for (A) PNA 17:DNA 21, (B) PNA 18:DNA 21, (C) PNA 19:DNA 21, (D) TzNA 20:DNA 21 complex at pH 7.4, 10 mM phosphate buffer (100 mM NaCl) [F1, F2, F3, F4, F5, F6, F7, F8, F9, F10 indicates complex of 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0 μ M PNA respectively with 2.0 μ M DNA 21].

number of triazole modifications do not form complexes with complementary DNA.

In order to unambiguously confirm the formation of DNA complexes by PNAs 17 and 18, CD spectra were recorded by keeping the concentration of DNA 21 constant and adding stoichiometric amounts of individual PNAs 17 and 18. The resultant changes in CD profile of DNA 21 are shown in **Figure 3A and B**. It is seen that the ellipticity of bands at 260 nm and 272 nm are systematically enhanced with the incremental addition of PNAs and at PNA:DNA ratio of 2:1, the bands reached maximum intensity and this profile is characteristic of PNA₂:DNA triplexes. In contrast such changes were absent when PNA 19 and TzNA 20 were added to DNA 21 and CD spectral changes were uniform throughout without any changes in specific bands (**Fig. 3C and D**). Further, temperature dependent CD spectra were also recorded for complexes of DNA with different triazole PNAs. Upon increase of temperature, the PNA 17:DNA 21 and PNA 18:DNA 21 complexes showed significant and specific decrease in intensity of CD peaks at 260 and 272 nm, in contrast to DNA complexes with PNA 19 and TzNA 20, which exhibited a monotonic decrease in all CD bands (**Sup. Material**). A UV- T_m

experiment was also carried out with the 2:1 complex of PNA 17 with DNA 22 having one base mismatch site and it was observed that the enhancement in T_m with respect to unmodified complex was only by 3.1° (**Table 2**), in comparison to 11.4° seen for full complementary complex PNA 17:DNA 21. Since triazole rings are basic, temperature dependent UV absorbance measurements for the complexes of PNA 18–20 were done at a slightly acidic pH 5.8. For PNA 18 that forms a complex with DNA 21, an enhancement in T_m of 11° was seen, while for PNAs 19 and 20 which do not form complexes, the increase in T_m was less significant by 5.7° and 0.4° respectively.

All the above CD and UV- T_m results together unambiguously prove the formation of complexes of single and double modified triazole PNAs (PNA 17 and PNA 18) with DNA 21 with enhanced stability compared to unmodified PNA and the failure of higher modified (PNA 19) and all-modified triazole PNA (PNA 20) to form similar complexes with DNA 21. The exact origin of stability seen at pH 5.8 in PNA:DNA complex is not clear (combination of electrostatic and conformational) at present. The overall results presented here suggest that the triazole backbone modified PNAs become progressively pre-organized with increasing

degree of modifications, to form ordered self-coiled structures (enhanced single strand T_m) in such a way that after 2 modifications, they fail to form complexes with DNA.²⁵ Although it could not be quantified, compared to unmodified oligo-T PNAs which are poorly soluble, the triazole oligo-T PNAs had a better solubility (lack of precipitation upon cooling), perhaps a consequence of increased self-order and the protonated triazole backbone. The helical structures are perhaps a consequence of higher dipole moment of triazole rings compared to amide bonds.

Experimental Section

N1-(tert-butyloxycarbonyl)-N2-(prop-2-ynyl)-1,2-diaminoethane (3). Propargyl bromide (2.5 ml, 28.1 mmol) was added slowly to a cooled solution of tert-butyl 2-aminoethylcarbamate **2** (5 g, 31.2 mmol) in dry CH_3CN (70 mL) containing K_2CO_3 (6.5 g, 46.9 mmol). The reaction mixture was slowly allowed to attain room temperature and stirred for 8 hrs after which CH_3CN was removed under vacuo. The residue was dissolved in water and was extracted with ethyl acetate (3 x 40 ml) and the combined organic layer was washed with saturated aqueous NaHCO_3 and brine and dried over Na_2SO_4 . The organic layer upon evaporation in vacuo gave a yellow liquid which was purified by column chromatography (3.5 g, 56%). ^1H NMR (200 MHz, CDCl_3) δ 1.45 (9H, s), 2.24 (1H, t), 2.79 (2H, t), 3.23 (2H, q), 3.42 (2H, d), 5.0 (1H, bs); ^{13}C NMR (50 MHz, CDCl_3) δ 28.3, 37.6, 39.9, 47.8, 71.5, 79.2, 81.8, 156.1; IR (neat) $\nu(\text{cm}^{-1})$ 3,305, 2,977, 2,931, 2,107, 1,699 and 648. MS (EI) m/z 198. Found: 199.25 $[\text{M} + \text{H}^+]$, 221.24 $[\text{M} + \text{Na}^+]$.

N1-(tert-butyloxycarbonyl)-N2-(2-chloroacetyl)-N2-(prop-2-ynyl)-1,2-diaminoethane (4). To a stirred solution of the amine **3** (3.5 g, 17.7 mmol) and Et_3N (9.8 ml, 70.8 mmol) in dry CH_2Cl_2 (35 mL) cooled to 0°C , chloroacetyl chloride (2.8 ml, 35.4 mmol) was added drop wise, followed by diluting with DCM (5 mL). After 30 min the reaction mixture was washed with water followed by saturated NaHCO_3 and brine. DCM was removed under vacuo and the residue was purified by column chromatography to afford the chloro compound **4** as a yellow liquid (3.9 g, 81%). ^1H NMR (200 MHz, CDCl_3) δ , 1.44 (9H, s), 2.29 (0.47H, t) and 2.38 (0.39H, t) - $\text{NHCO}-\text{CH}_2-\text{Cl}$ cis/trans isomers, 3.33–3.41 (2H, m), 3.62 (2H, t), 4.15–4.25 (4H, m), 4.9 (1H, brs); ^{13}C NMR (50 MHz, CDCl_3) δ 28.3, 35.1, 38.3, 38.6, 41.0, 41.3, 46.7, 47.2, 72.6, 73.7, 78.4, 79.4, 80.0, 156.1, 166.8; IR (neat) $\nu(\text{cm}^{-1})$ 3,307, 2,979, 2,934, 2,120, 1,659, 663 cm^{-1} . MS (EI) m/z 274. Found: 275.26 $[\text{M} + \text{H}^+]$, 297.28 $[\text{M} + \text{Na}^+]$.

N1-(tert-butyloxycarbonyl)-N2-(2-(3,4-dihydro-5-methyl-2,4-dioxypyrimidin-1(2H)-yl)-N2-(prop-2-ynyl)-1,2-diaminoethane (5). A mixture of the chloro compound **4** (3.9 g, 14.2 mmol), thymine (1.8 g, 14.2 mmol) and anhydrous K_2CO_3 (2.4 g, 17.04 mmol) in dry DMF (20 ml) was stirred at RT for overnight under inert atmosphere. The mixture was concentrated under reduced pressure and the residue was extracted with ethyl acetate followed by washing with brine and dried over Na_2SO_4 . The organic layer was evaporated and the crude product was purified by column chromatography to obtain a colorless solid (4.3 g, 83%). mp $147.8\text{--}150^\circ\text{C}$, ^1H NMR (200 MHz, CDCl_3) δ

1.44 (9H, s), 1.92 (3H, s), 2.29 (0.53H, t), 3.31–3.37 (2H, m), 3.59 (2H, q), 4.17–4.27 (2H, dd, $J = 2.27$ Hz, $J = 2.53$ Hz) 4.58 (2H, d, $J = 10.86$ Hz), 5.08 (0.41H, br m) and 5.3 (0.54H, br m) - $\text{NHCO}-\text{CH}_2$ cis/trans isomers, 7.01 (1H, s), 9.33 (1H, s). ^{13}C NMR (200 MHz, CDCl_3) δ 12.3, 28.3, 35.2, 38.0, 38.5, 46.3, 47.4, 48.2, 72.8, 74.0, 78.3, 79.9, 110.6, 141.0, 141.3, 151.4, 156.2, 164.6, 166.7, 167.1; IR (CHCl_3) $\nu(\text{cm}^{-1})$ 3306.7, 3019.5, 2400.5, 1683.2, 1506.1, 1470.4, 668.7; MS (EI) 364. Found 365.41 $[\text{M} + \text{H}]$, 387.44 $[\text{M} + \text{Na}^+]$.

Synthesis of azide and 1,2,3-triazole on solid support. The free amine was converted to azide by reacting the resin (100 mg, Loading value 0.35 meqg^{-1}) with freshly prepared TfN_3 . A mixture of Et_3N (0.2 μL 3.5 eq), catalytic amount of CuSO_4 and freshly prepared TfN_3 in DCM was added to the resin bound amine. This mixture was brought to homogeneity by adding MeOH and the conversion to azide was completed within 2–4 hrs. as monitored by Kaiser test. The resin bound azide was then reacted with the alkyne-functionalized monomer **5** to generate the 1,2,3-triazole link. To the solid supported azide, a mixture of alkyne **5** (9 mg, 7 eq), CuI (15 mg, 13 eq) and DIPEA (10 μL , 17 eq) in DMF:pyridine (5:3) was added. The reaction was complete within 24 hrs with more than 90% purity in all the steps as confirmed by RP-HPLC.

Synthesis of aeg-TzNA oligomers. The modified PNA monomers were incorporated into PNA oligomers by solid phase synthesis using standard procedure on L-lysine derivatized (4-methylbenzhydryl) amine (MBHA) resin (initial loading 0.35 meqg^{-1}) with HBTU/HOBt/DIEA in DMF as the coupling reagents. The triazole unit was incorporated into solid phase by reacting the resin bound azide with the alkyne functionalized monomer **5** in presence of CuI/DIEA in DMF/pyridine as the solvent system.⁶ The oligomers were cleaved from the resin with TFMSA and purified by RP-HPLC (C-18 column, UV-detector, 254 nm) and characterized by MALDI-TOF mass spectrometry. The overall yield of the crude oligomers was found to be more than 90%.

UV- T_m measurements. The concentration of aeg-TzNA and DNA oligomers were calculated on the basis of UV absorbance using molar extinction coefficient of the corresponding nucleobases. The hybridized complexes were constituted in sodium phosphate buffer (pH 7.4, 10 mM), containing NaCl (100 mM) and by annealing the samples at 85°C for 2 min followed by slow cooling to 4°C over 7–8 hrs. The absorbance versus temperature profiles were obtained by monitoring UV absorbance at 260 nm with Perkin-Elmer Lambda 35 UV-vis spectrometer equipped with peltier heating in the range 10°C to 85°C with a ramping rate of 0.5°C per minute. The data were processed using Microcal Origin 6.1 and the T_m values were obtained from the derivative curves (Table 2).

Conclusions

It is demonstrated here that the amide links in the PNA backbone can be replaced by the isosteric 1,2,3-triazole link, which is efficiently synthesized in-situ on the resin using “click” reaction on solid phase. The azide component was synthesized on the

resin from the amino function and reacted with the propylene 5 to create the 1,2,3-triazole link. The oligomers with single and double modification with 1,2,3-triazole unit show an increase in melting temperature of the DNA complex. The triple modified triazolo oligothymine sequence *aeg*-TzNA 19 and all-modified TzNA 20 form highly self-coiled structures in single stranded form. There have been great interests in triazolo oligomers. Further work on the influence of triazole link in backbone on duplex formation in mixed sequences and investigation of self-ordered phenomena in all-modified triazolyl PNA are currently in progress, which would be interesting in generation of new oligomers for DNA/RNA hybridization based applications and for new materials.

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Note Added in Proof

While this manuscript was in revision, a report on a triazole modified PNA appeared in literature.²⁵ This paper reports the synthesis of triazoles by click reaction on solid phase using slightly different conditions, incorporation of only 1 and 2 thymine-triazole modifications in a mixed base sequence environment and their hybridization properties done by ESI-MS technique.

Note

Supplemental material can be found at:

www.landesbioscience.com/journals/artificialdna/article/13185

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