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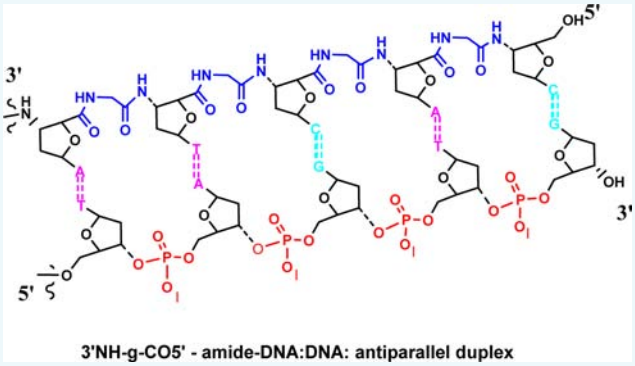
Glycine-Linked Nucleoside-β-Amino Acids: Polyamide Analogues of Nucleic Acids

Anjan Banerjee, Seema Bagmare, Manojkumar Varada, and Vaijayanti A. Kumar*

Division of Organic Chemistry, CSIR-National Chemical Laboratory, Pune, India 411008

Supporting Information

ABSTRACT: 3′-5′-Deoxyribose-sugar-phoshate backbone in DNA is completely replaced by 2′-deoxyribonucleoside-based β-amino acids interlinked by glycine to create uncharged polyamide DNA with 3′-5′-directionality. These oligomers as conjugates of α-amino acids and nucleoside-β-amino acids bind strongly and sequence-specifically only to the antiparallel complementary RNA and DNA.



INTRODUCTION

In 1978, Zamecnik and Stephenson discovered that the sequence specific recognition of mRNA by oligonucleotides (ON) allows them to act as antisense agents.[1] The natural sugar-phosphodiester linkages in DNA are susceptible for hydrolysis with various nucleases and this limits their use for various biological applications, unless chemically modified.[2,3] This opened a vast new area of research interest in the chemistry of nucleic acid. To meet the needs of synthetic oligonucleotides as therapeutic agents, over the last couple of decades, several synthetic oligonucleotides were developed which led to the first generation (phosphorothioate),[4] second generation (2′-modifications),[5] and even third generation (e.g., LNA,[6] PNA,[7,8] PMO[9]) of synthetic oligonucleotides. Peptide nucleic acid[7,8] (PNA, Figure 1) is by far the most promising mimic of DNA in which sugar-phosphodiester linkages are completely replaced by uncharged aminoethyl glycyl repeating units. The achiral and acyclic backbones with neutral amide linkages lead to a completely uncharged backbone of PNA.[7,8] The strong pairing of uncharged PNA with high sequence fidelity is attributed to the absence of charge−charge repulsions observed in DNA duplexes and specific W−C base recognition. Due to their strong sequence specific binding to natural DNA and RNA, PNAs have found extensive application in hybridization based techniques such as probes with enhanced specificity for the detection of nucleic acids, as biomolecular tool and also in antisense/antigene technologies.[10] PNAs bind very strongly and sequence specifically to cDNA/RNA, but they also bind in both parallel and antiparallel orientations.[10] The sense of antiparallel 3′ → 5′ directionality of DNA duplex is compromised in PNA in the absence of chiral sugars in the backbone, which is a very important aspect of natural DNA. A plethora of chiral PNA modifications were then studied and are known in the literature that enhance the discrimination of parallel/antiparallel binding, DNA versus RNA binding, and also improve other properties of PNA such as lower aqueous solubility and cellular uptake.[11−17] In most of these studies the chiral functionalization of PNA is achieved by introducing chiral amino acids within the PNA scaffold[18−20] or by synthesizing PNA−DNA conjugates.[21] The positively charged chiral PNA analogues containing amino- and guanidino-functionalities[20,22] and the negatively charged PNA analogues containing carboxy[23] and sulfate[24] groups have been studied which enhance PNA properties depending on the charges on the backbone and experimental conditions. The prolyl-(ACPC) PNA[25,26] (Figure 1) and its homologues[27] are the unique examples in which an entirely new backbone comprising alternating α- and β-amino acids exhibited strong preference of binding with complementary antiparallel DNA and thus

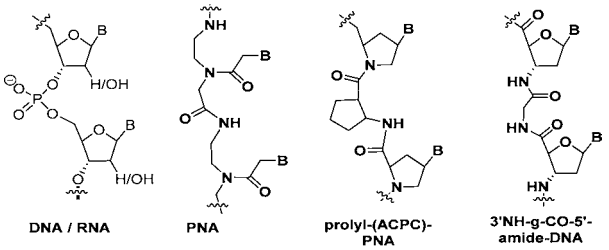


Figure 1. DNA analogues and proposed backbone of nucleoside-β-amino acids.

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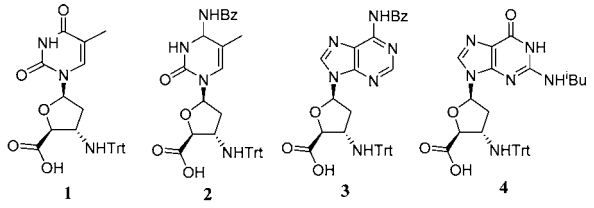
indicated optimized internucleoside geometries to bind antiparallel cDNA but comparatively less so, with complementary RNA. In this article, we present for the first time the synthesis of mixed base sequences comprising the 3′-NH-*g*-CO-5′ linked oligonucleosides backbone that may maintain the 3′-5′-directionality of DNA/RNA. Here, we present the parallel/antiparallel orientation selectivity in duplex and triplex mode, DNA versus RNA binding selectivity, and mismatch tolerance of this novel homogeneous sugar-amide backbone.

RESULTS AND DISCUSSION

In our initial studies, with this concept, we reported thyminyl-sequences comprising (thyminyl-β-amino acid + α-L-amino acids) where the 5-atom amide linkers (3′-NH-aa-CO-5′) were placed instead of the anionic 4-atom phosphodiester (3′-O-P-O-CH_2-) linkages to sequentially join the thymine nucleosides.[28,29] The amide linked-octathyminyl-oligomers with different α-amino acids, formed highly stable complexes with cDNA/RNA in 2:1 stoichiometry but due to the sequence limitation, the directionality of binding could not be ascertained. For the synthesis of amide-linked-octathyminyl sequences, we used Fmoc protected 3′-deoxy-3′-aminothymidin-4′-carboxylic as nucleoside-β-amino acid monomer and rink-amide resin was used as the solid support.[28] This strategy could not be extended for the synthesis of mixed base sequences due to the requirement of orthogonal protection of exocyclic amino groups. For the synthesis of mixed base sequences containing purine nucleosides, the use of TFA/TFA:TFMSA also needs to be avoided due to likely depurination in strongly acidic medium. With an aim to achieve complete replacement of phophodiester linkages in mixed pu/py sequences by the charge-neutral amide linkages in sugar-amide backbone, we previously converted nucleosides into NH-trityl protected β-amino acid derivatives[30,31] (**1**−**4**, Figure 2). In the present studies, we extend this strategy for the synthesis of oligomer sequences containing only py (C and T) (TRT1) and a mixed pu/py (TRT2) that would give us insight into the orientation selective DNA/RNA binding properties of amide-DNA. The sequence TRT1 is an unsymmetrical pyrimidine sequence containing T and C nucleoside-β-amino acids would maintain directionality of binding unlike homothymidine sequences[28] reported by us earlier. The sequence TRT2 is a purine and pyrimidine mixed base sequence containing T, A, G, and C nucleoside-β-amino acids. TRT2 is part of the biologically important miR-29b sequence.[32] The succinate ester at the 5′-end as a linker for attachment of the first 3′-amino-nucleoside via succinamide linker to the solid support again proved to be a good strategy, as the succinate ester could be cleaved concomitantly with the deprotection of exocyclic amino groups, using aqueous ammonia at the end of the synthesis, leaving free 5′-OH group on the oligomer. The synthesis was done from the 5′ → 3′ direction. By using 3% TCA in DCM for deprotection of the trityl group and the TBTU/HOBt activation strategy for coupling of monomers, the amide-DNA sequence was synthesized. We decided to avoid the capping step (except after initial nucleoside loading on the resin) after each coupling in the present study to get longer sequences, as we observed that the 3′-NH-trityl functionality of the growing chain was partially acylated resulting in termination of the synthesis after 6−7mer synthesis. The acylation of the 3′NH-trityl group was also observed in solution-phase synthesis of the monomers even with isobutyric anhydride.[30,33] The synthesis of 10mer and 12mer mixed base purine/pyrimidine amide-linked oligomers comprising alternate nucleoside-β-amino acids (Figure 2, **1**−**4**) and glycine as -α-amino acid was accomplished, and the synthesized sequences are shown in Table 1 (couplings monitored by trityl UV absorbance at 410 nm, SI Experimental, S2−S3). As the capping step after coupling was avoided to get longer sequences, the synthetic purity of the crude oligomers was affected. In the case of the TRT2 sequence, we have a single guanine amino acid in the sequence, and the trityl absorbance (SI S3) showed that the yield of this coupling was much less than that of the other amino acids. It may be necessary to protect the O6 of guanine to improve the yield, as the solubility of guanine amino acid also will be improved. In addition, unprotected O6 of guanine residues could take part in coupling reactions, thus complicating the synthesis further. Nevertheless, the sequences were cleaved from the resin and deprotected by aqueous NH_3 treatment, were purified by HPLC on a PepRP column, and were further characterized by MALDI-TOF mass spectrometry (SI Figures S4−S5; SI Table 1 S6). The sequences TRT1 and TRT2 (Table 1) were used to determine their strength of binding with antiparallel complementary DNA, RNA; antiparallel mismatched DNA, RNA; and parallel complementary DNA, RNA in comparison with corresponding DNA and PNA sequences. The parallel/antiparallel and mismatch DNA/RNA/PNA sequences used in the UV−T_m experiments are listed in Supporting Information (SI, Table 2, S6).



1: 2', 3'-Deoxy-3'-tritylaminothymidine-4'-carboxylic acid
2:2',3'Dideoxy-3'-tritylamino-N^4-benzoyl-5-methylcytidin-4'-carboxylic acid
3:2',3'-Dideoxy-3'-tritylamino-N^6-benzoyl-adenin-4'-carboxylic acid
4.2',3'-Dideoxy-3'-tritylamino-N^2-*iso*butyryl-guanosin-4'-carboxylic acid

Figure 2. Suitably protected β-amino acids from all four natural Nucleosides.

The polyamide−DNA sequences were examined for their binding affinity with cDNA/cRNA sequences by employing UV-thermal denaturation studies. Job's plot[34] suggested 1:1 complexation between the TRT1:cDNA1 at pH 7.2 (see SI Figure S7). The modified oligomers TRT1 and TRT2 were annealed with corresponding antiparallel (*ap*) complementary DNA/RNA (cDNA/cRNA) in 1:1 stoichiometry and were subjected to temperature dependent UV studies at 260 nm. The T_m values were obtained by the first derivative of these curves. The results are tabulated in Table 1. The isosequential unmodified DNA1/PNA1 and DNA2/PNA2 were used for comparison in these experiments. At 10 mM NaCl, the DNA:DNA and DNA:RNA duplexes showed no transition (for DNA1) or very low UV−T_m (for DNA2); therefore, the UV−T_m values for DNA:DNA and RNA:DNA duplexes were studied at 100 mM salt concentrations. At 10 mM NaCl, complexes of PNA1 and PNA2 in the UV−T_m plots show a single sigmoidal transition, characteristic of PNA:cDNA/cRNA duplex melting (SI Figure S8). The amide-DNA oligomers TRT1 and TRT2 were also able to form duplexes with both

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Table 1. Temperature Dependent Melting of Duplexes at 260 nm (UV−T_m in °C)

code	sequences 5′→ 3′	*ap*-cDNA1[b]	*ap*-cRNA1[b]	mm-*ap*-DNA5[c]	mm-*ap*-RNA5[c]	*p*-cDNA3[d]	*p*-cRNA3[d]
DNA1[a]	CTTCTTCCTT-3′	nt (**27.8**)	nt (**36.1**)	(**18.6**), (−9.2)	(**29.4**), (−6.7)	nt	nt
PNA1	H-cttcttcctt-K-NH_2	47.3(+19.5)	51.3(+15.2)	37.0(−10.3)	41.2(−10.1)	32.9(−14.4)	38.5(−13.8)
TRT1	$C_gT_gT_gC_gT_gT_gC_gC_gT_gT$-3′	39.7(+11.9)	48.3(+12.2)	30.8(−8.9)	38.3(−10.1)	nt	nt
		ap-cDNA2	*ap*-cRNA2	mm-*ap*-DNA6	mm-*ap*-RNA6	*p*-cDNA4	*p*-cRNA4
DNA2[a]	CACTGATTTCAA-3′	>10 (**35.9**)	>10 (**44.7**)	(**29.4**), (−6.5)	(**32.8**), (−11.9)	nt	nt
PNA2	H-cactgatttcaa-K-NH_2	53.8(+17.9)	60.6(+15.9)	45.7(−8.1)	48.6(−12.0)	40.4(−13.4)	48.6(−12.0)
TRT2	$C_gA_gC_gT_gG_gA_gT_gT_gT_gC_gA_gA$-3′	45.3(+9.4)	54.0(+9.3)	37.5(−7.8)	43.2(−10.8)	nt	nt

[a]For DNA:DNA/RNA duplex melting studies, values in parentheses and bold type correspond to the T_m when the salt concentration used was 100 mM NaCl in sodium phosphate buffer at pH 7.2. The remaining results are for the experiments carried out at 10 mM NaCl in sodium phosphate buffer at pH 7.2. Subscript 'g' between the bases denotes the glycine linker in the backbone of polyamide DNA. Lower case letters denote PNA sequence. [b]Values in parentheses indicate ΔT_m = modified DNA- control DNA/RNA. [c]Values in parentheses indicate ΔT_m = *ap*-cDNA/*ap*-cRNA-mm-*ap*-cDNA/mm-*ap*-cRNA. [d]Values in parentheses indicate ΔT_m = (*ap*-*p* DNA/RNA). nt denotes no sigmoidal transition. The symbol 'C' in TRT1 and TRT2 sequences is for 5-methylcytosine derivative.

cDNA and cRNA in the antiparallel orientation. The stability of these complexes as measured by UV−T_m was found to be in between the PNA:cDNA/cRNA (at 10 mM NaCl) and DNA:cDNA/cRNA (at 100 mM NaCl). We also did the experiments at higher salt concentrations (100 mM NaCl) for PNA1/PNA2 and amide-DNA sequences TRT1/TRT2. For PNA:DNA duplexes the duplex stability did not show any salt dependence, and was almost (ΔT_m = 1−2 °C) the same at 10 mM and 100 mM salt concentrations as reported earlier.[10,23] In the case of amide-DNA sequences, however, we found that as the salt concentration is increased the absorbance of the solution drastically decreased upon annealing the sample. The amide-DNA TRT1 and TRT2 are devoid of any charged moiety unlike PNA1 and PNA2, which have a lysine at C-terminus. We suspect some aggregation/precipitation of the oligomer at higher salt concentrations. Such aggregation was also observed earlier even for uncharged PNA.[21] Although we have not done such studies for this particular case, it is known in the literature that the aggregation properties of uncharged DNA analogues such as PNA can be improved by the conjugation with positively/negatively charged amino acids, oligonucleotides, or with PEG.[10,21,23,35] Similar fully modified PNA analogues such as POM−PNA with and without charged amino acids at C/N-termini also exhibited differential behavior in melting studies.[36] The specificity of this sequence recognition was further examined by studying their complexes with complementary sequences in parallel (*p*) orientation and also using single base mismatch targets in *ap* orientation. The results are summarized in Table 1.

The results presented in Table 1 clearly point out the specificity of the amide-DNA oligomers TRT1 and TRT2 to bind to cDNA/cRNA only in antiparallel orientation. Similar to DNA1/DNA2, in the case of TRT1 and TRT2, no sigmoidal transition was observed for the experiments where the DNA/RNA sequences used were complementary in parallel orientation (*p*-DNA3,4/*p*-RNA3,4) (SI Figure S9) . The achiral PNA1/PNA2 were able to form complexes with parallel cDNA/RNA. The orientation specificity of binding of natural nucleic acids is thus maintained in the present amide-DNA. The achiral and acyclic PNA backbone is known to bind to DNA/RNA in either parallel or antiparallel orientation, and in this particular sequence context, PNAs also formed complexes with parallel cDNA/cRNA albeit with lower stability than in antiparallel orientation. We then studied mismatch (mm) tolerance of these amide-DNA oligomers by measuring the UV-T_m using mm-*ap*-DNA5 and mm-*ap*-RNA5 for TRT1 and mm-*ap*-DNA6 and mm-*ap*-RNA6 for TRT2 in comparison with the mismatch tolerance of natural DNA and PNA (SI Figure S9). The observations are summarized in Table 1. It is seen that the modified DNA backbone, being devoid of any positive or negative charges,[35,36] is as sensitive to single base mismatch and the stability of duplexes is compromised by 7−10 °C as compared to the fully complementary targets.

To study the conformational properties of the antiparallel duplexes and to confirm the preferred helical handedness in antiparallel orientation, a CD-spectroscopic analysis of TRT2:*ap*-cDNA2 and TRT2:*ap*-cRNA2 was undertaken. The right-handed helical formation by DNA:DNA/RNA/PNA duplexes shows a characteristic positive CD band in the region 280−260 nm depending on B or A form of the duplex.[36]

The results are depicted in Figure 3. The positive CD band at 280 nm for both TRT2:*ap*-cDNA2 and TRT2:*ap*-cRNA2 duplexes indicates a right-handed helical geometry similar to B-form DNA:DNA duplexes.[37]

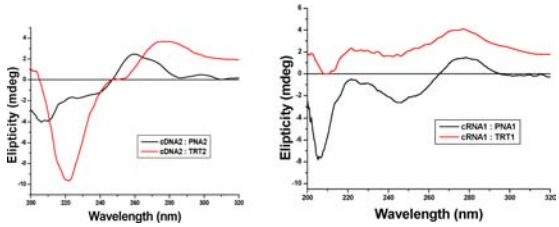

Figure 3. CD spectral analysis of TRT2:*ap*-cDNA2 and TRT2:*ap*-cRNA2duplexes.

Further, we wanted to study the possibility of binding of the TRT1 homopyrimidine sequence in triplex binding mode. The homopyrimidine PNA sequences are known to bind to cDNA in either parallel or antiparallel mode, albeit with some preference for parallel mode of binding where both PNA strands could be either parallel or antiparallel to the central purine strand, thus losing directional selectivity even in the triplex mode.[10] In our earlier studies, the homothyminyl sequences indeed showed triplex structures similar to 2PNA:DNA triplexes, but in that case, the directionality of binding was irrelevant.[28] For the triplex mode of binding for the mixed cytosine and thymine containing polypyrimidine sequences, the formation of Hoogsteen hydrogen bonds requires protonation of cytosine residues in the sequence.[10] In the present studies, the cytosine derivatives used are substituted 5-methylcytosine derivatives which can be proto-

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nated easily at lower pH.[21] Indeed, the Job's plot[34] suggested a shift toward 2:1 complexation between the TRT1:*ap*-cDNA1 and PNA1:*ap*-cDNA1 at pH 5.8, suggesting possible triplex mode of binding at pH 5.8 (SI Figure S7). We choose *ap*-cDNA1 for these studies as the W−C duplex formation for TRT1 sequence was observed only with *ap*-cDNA1. As expected, the 2PNA1:cDNA1 at pH 5.8 showed a single transition for the triplex at 52 °C with a shift in T_m of about +5 °C compared to that observed at pH 7.2. The TRT1:cDNA1 complex at acidic pH, however, showed two separate transitions in the melting graph, at 14 and 37 °C (Figure 4). The third

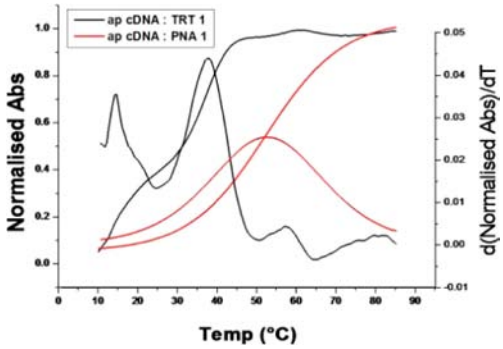

Figure 4. Triplex melting of TRT1:ap-cDNA1 and PNA1:ap-cDNA1 at pH 5.8, and at 10 mM NaCl, 10 mM sodium phosphate buffer.

strand binding in antiparallel orientation for the TRT1 sequence to the central purine strand of the TRT1:*ap*-cDNA1 duplex seems to be very weak compared to the one observed in 2PNA1:cDNA1 triplex. The results are shown Figure 4. We also observe marginal destabilization of the antiparallel duplex in this case at acidic pH (ΔT_m = −2.7 °C).

Over the past decade, we and others have been working toward the synthesis of such chimeric four- and five-atom charge-neutral amide linked nucleic acid analogues by partial replacement of four-atom phosphodiester linkages at selected positions, for achieving RNA selective binding. Several such chimeric deoxyribo/ribo ON analogues[38−47] are known in the literature. Such chimeric ONs recognized and formed duplexes with complementary RNA (cRNA) with a moderate RNA binding selectivity but with diminished affinity, in antiparallel orientation. Egli's group carried out a systematic study for RNA binding affinity of five-atom amide-linked DNA analogues.[43,44] The five-atom amide-linked oligonucleotides containing thymidine−thymidine and thymidine−cytosine and cytosine−cytosine dimers were reported by our group where thioacetamido backbone (TANA/isoTANA) exhibited discrimination in binding affinity toward DNA/RNA.[45−47] In all these studies, only the partial replacement of phophodiester linkages was selected by synthesizing dimer block phosphoramidites in solution phase which were used in automated DNA synthesizer to get the chimeric ONs. Our own studies[48] and also those of Rozners' group[49,50] recently demonstrated the usefulness of such chimeric modified ONs in biological studies, but in these studies mostly only TT/UU/CT dimer blocks were synthesized and replaced in the longer sequences, and therefore have sequence limitations. A straightforward synthetic strategy to synthesize a mixed pu/py sequence with a homogeneous amide-DNA backbone could not be achieved so far due to the unavailability of all four nucleoside-amino acids. The present study is the first example in the literature that brings about complete replacement of sugar-phosphodiester linkages by neutral sugar-amide linkages in mixed pu/py sequences without any sequence limitation.

In conclusion, synergistic use of peptide chemistry (use of α-amino acid as internucleoside linker, amide bond formation, and MBHA resin) and DNA synthesis chemistry (use of trityl and benzoyl/isobutyryl orthogonal protecting groups during solid phase synthesis and deprotection/cleavage from support with ammonia treatment) resulted in the challenging synthesis of uncharged sugar-amide-DNA. The amide-DNA exhibited sequence-specific binding with cDNA/cRNA following W−C base pairing. The amide-DNA behaved as a close cousin of natural DNA having very similar properties, with special features being better binding with cRNA compared to cDNA, effective mismatch discrimination, and directional specificity. In addition, it binds to both cDNA/cRNA with better strength in the absence of the electrostatic repulsions arising from the phosphate negative charges of natural DNA/RNA duplexes. This interesting sugar-amide backbone has large scope of further development using different natural-L-/D-amino acids.[29] The ability of these amide-DNAs to cross the cell membrane and the stability of these amide-DNAs in biological systems needs to be explored. It will also be possible to conjugate cationic cell-penetrating peptides to amide-DNA oligomers to improve their aggregation and cell-uptake properties.[51,52]

EXPERIMENTAL SECTION

Solid Phase Oligomer Synthesis of Amide-DNA Sequences TRT1 and TRT2. MBHA resin (60 mg, 1.75 mmol/g, 0.1 mmol) was washed and swelled in DCM for 1 h in a solid phase reaction vessel (5 mL). The solvent was drained and the resin washed with DMF. To a solution of *N*-4-benzoyl-5-methyl 3′-tritylamino-2′,3′-dideoxycytidine-5′-succinylate (16.19 mg, 0.024 mmol) in 500 μL DMF, DIPEA (12.47 μL, 0.072 mmol) and TBTU (9.63 mg, 0.028 mmol) was added. To this solution HOBt (3.24 mg, 0.024 mmol) in 100 μL of DMF was added. This activated monomer solution was added to the resin and the suspension was kept for 12 h with occasional gentle shaking. Solvent was drained and the resin was washed with DMF (2 × 1 mL), DCM (2 × 1 mL), and pyridine (2 × 1 mL). Unreacted amino groups were capped with 10% acetic anhydride in pyridine for 1 h. The nucleoside loading on solid support was determined by spectrophotometric determination of the concentration of trityl cation at 410 nm released after detritylation using 3% TCA in DCM. The calculated loading value for resin using molar extinction coefficient 35 300 M^{-1} cm^{-1} at 410 nm for trityl cation was 35.2 μmol/g, which was good enough for further oligomer synthesis. Synthesis of polyamide DNA sequences was then undertaken using protected nucleoside-β-amino acid monomeric units shown in Figure 2 and N-trityl-glycine on preloaded MBHA resin. The following synthetic condition were used, deprotection of trityl group using 3% trichloroacetic acid (3 min × 3). Neutralization using 5% DIPEA in DCM (3 min × 2) followed by coupling using 3 equiv of the monomer, 9 equiv DIPEA, 3 equiv TBTU, and 1.5 equiv of HOBt as activator with respect to loading value of resin. All are premixed in DMF prior to addition to resin. This suspension was added to the resin. Reaction time was kept to be 6 h with occasional swirling. The couplings were monitored each time by spectrophotometric determination of the concentration of trityl cation at 410 nm released after detritylation using 3% TCA in DCM (SI Table1−2).

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In a typical cleavage reaction, the resin bound oligomers (5 mg) were treated with aqueous methanolic ammonia (1.5 mL) at 55 °C for 8 h. After that, the resin was filtered, followed by evaporation of the filtrate under vacuum. The 3′-terminal trityl group was deprotected before cleavage using 3% TCA in DCM. Purification of the modified oligomers was carried out by reverse phase HPLC on RP-C18 column using 5% acetonitrile in 0.1 M TEAA buffer as eluent system and monitored at 260 nm, and were characterized by MALDI-TOF mass spectrometry. The spectra were acquired in linear mode and the matrix used for analysis was THAP (2′,4′,6′-trihydroxyacetophenone)/ammonium citrate (2:1).

UV−T_m Measurements. The concentrations of the oligomers were calculated on the basis of absorbance from the molar extinction coefficients of the corresponding nucleobases of DNA/RNA/PNA. The experiments were performed at 1 μM concentration of each strand. The complexes were prepared in 10 mM sodium phosphate buffer, pH 7.2, containing 10 mM NaCl (100 NaCl for DNA:DNA/RNA) and were annealed by keeping the samples at 90 °C for 5 min followed by slow cooling to room temperature and then to 0 °C. Absorbance versus temperature profiles were obtained by monitoring at 260 nm with Varian Cary 300 spectrophotometer scanning from 10 to 85 °C at a ramp rate of 0.5 °C per minute. Experiments were repeated at least thrice and the data were processed using Origin software 6.1. T_m (°C) values were derived from the first derivative curves and are accurate to within ±0.3 °C.

CD Analysis of the Oligomers. The complexes were prepared in 10 mM sodium phosphate buffer, pH 7.2, containing NaCl (10 mM) and were annealed by keeping the samples at 90 °C for 5 min followed by slow cooling to room temperature. The experiments were performed at 1 μM concentrations of each strand. All the CD spectra were recorded at room temperature. All spectra represent an average of at least 8 scans recorded from 320 to 210 nm at a rate of 100 nm per minute in a 1 cm path length cuvette. All spectra were processed using Origin software 6.1, baseline subtracted, and smoothed using a 5 point adjacent averaging algorithm.

■ ASSOCIATED CONTENT

Supporting Information

HPLC and MALDI-ToF mass spectra of TRT1 and TRT2. A Table showing PNA1, PNA2, DNA1, DNA2 and all the parallel/antiparallel complementary, mismatched DNA/RNA sequences, Job's plot analysis for binding stoichiometry of TRT1 and PNA1 with DNA1 at pH 7.2 and 5.8 respectively. UV−T_m experiments. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.5b00296.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: va.kumar@ncl.res.in. Tel: +912025902340.

Notes

The authors declare no competing financial interest.

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